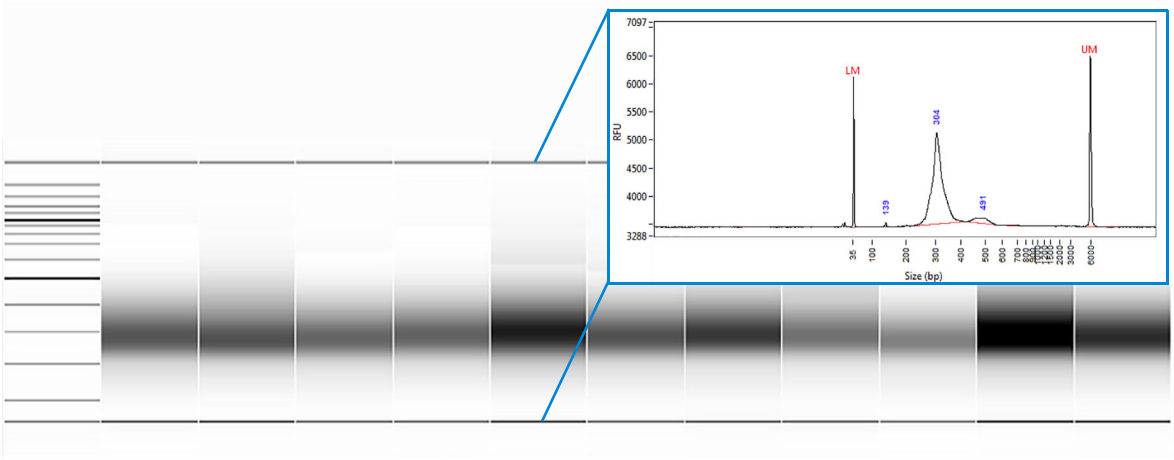
Agilent

ProSize Data Analysis Software

User Manual



Notices

Document Information

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In This Guide

Agilent has prepared this manual as a technical reference for the ProSize data analysis software for use with the Fragment Analyzer, ZAG DNA Analyzer and Femto Pulse parallel electrophoresis systems.

This document includes software system requirements, installation procedures, software operation, troubleshooting guide, and technical support information.

This document is intended for use by technical personnel that are proficient with analytical instrumentation operation. A certain level of training and expertise is assumed and fundamentals are not addressed herein. Information is presented in a section-by-section format using screen captures and written descriptions. If questions remain after reviewing a given topic, please contact your corresponding Agilent Sales/Service Representative.

1 ProSize System Overview

This chapter gives an overview about the ProSize data analysis software.

2 ProSize System Requirements and Installation

This chapter provides information on the requirements and installation instructions for the ProSize software.

3 ProSize Main Screen

This chapter provides an overview of the ProSize software main screen with the functions and menus available to the user. The file menu for opening and closing data files is also covered in this chapter.

4 ProSize Configuration

This chapter provides an overview of the Global Configuration dialog of the ProSize software. It also provides information on setting individual configuration parameters in opened data files.

5 ProSize Size Calibration Screen

This chapter describes the size calibration screen (also referred to as calibration curve screen). The calibration curve screen is used to select the conditions for calibrating the size of unknown sample peaks/smears.

6 ProSize Compare Files

This chapter gives an overview of the possible ways to compare samples in the ProSize software.

7 Exporting Data from ProSize

This chapter provides an overview of the options available for exporting processed data from the ProSize software. The following sections describe how to open the Export Data window; provide an outline of various data export options; describe how to perform data export; and give examples of exported data.

8 Generating Reports from ProSize

This chapter provides an overview of the options available for generating reports from the ProSize software. The following sections describe how to open the Generate Report window; provide an outline of various report generation options; describe how to perform the report generation; and give examples of reported data.

9 ProSize View Capillary Positions

This chapter briefly covers the tools and functions of the View Capillary Positions window.

10 ProSize Troubleshooting Guide

This chapter gives an overview of troubleshooting the ProSize software.

11 Batch Processing Using ProSize

This chapter explains how to perform a batch process and provides an outline of the various exporting options.

Content

1 ProSize System Overview

This chapter gives an overview about the ProSize data analysis software.

About the System

ProSize Data Analysis Software

Agilent's ProSize software is designed for analyzing the raw data from the parallel capillary electrophoresis instruments and reporting the size (in base pairs for dsDNA or nucleotides for RNA) and concentration (in ng/mL or nmol/L) of separated DNA or RNA fragments. The ProSize software performs the following general functions:

- Reads the raw data files generated from the instrument operational software.
- Provides calibration of DNA size (in bp) or RNA size (in nt) from standard mixtures and conversely measures DNA/RNA size of unknown fragments in a sample.
- Provides quantitative measurements of DNA/RNA concentrations present in a sample either from relative comparisons to an internal/external standard peak or by comparison to a calibration DNA/RNA ladder.
- Exports size and concentration data as well as raw electropherogram trace data (time vs. fluorescence intensity) in a generic comma separated values (.csv) file format; and digital electropherogram trace or gel view images in .jpg, .bmp, or .png image formats.
- Generates PDF format sample reports containing user specified information including measured DNA/RNA size, DNA/RNA concentration, digital gel images, experimental information, and/or other various calculated sample properties.

This manual serves as a guide to the ProSize software and will assist the user in taking advantage of the many benefits of the parallel capillary electrophoresis instruments.

2 ProSize System Requirements and Installation

This chapter provides information on the requirements and installation instructions for the ProSize software.

System Requirements

General Software Requirements

Report Review

A PDF viewer is required to read Adobe PDF formatted reports.

Install the most recent version of Adobe Reader. The software is available free of charge for download at www.adobe.com.

Data File Export

- The ProSize software exports data in a generic comma-separated values (.csv) file format, which can be read by most spreadsheet and database management programs. Install a spreadsheet program (for example, Excel, OpenOffice) to open and read exported .csv files.
- Electropherogram and digital gel images are exported in a jpg, .bmp, or .png image format. A suitable program for viewing these file formats should be installed.

Operating System

The ProSize software must be installed on a computer running a Windows operating system to function properly.

Table 1 Supported operating systems

Operating System	Details
Windows	Windows 10 or 11, 64-bit

PC Recommendation

The table provides the recommended hardware configuration for the ProSize software.

Table 2 Recommended hardware configuration

Item	Details
Processor speed (CPU)	Intel i5, or above
Physical memory (RAM)	4 GB
Hard disk	500 GB (for accommodation of raw data files)
Graphic resolution	1280 x 800 minimum screen resolution 1280 x 1024 recommended

ProSize Software Installation Instructions

To Install the Software

1 Download the appropriate installer from the Agilent website.

2 Navigate to the folder *ProSize data analysis software*.

3 Run the installer and follow the instructions and prompts provided by the installation wizard:

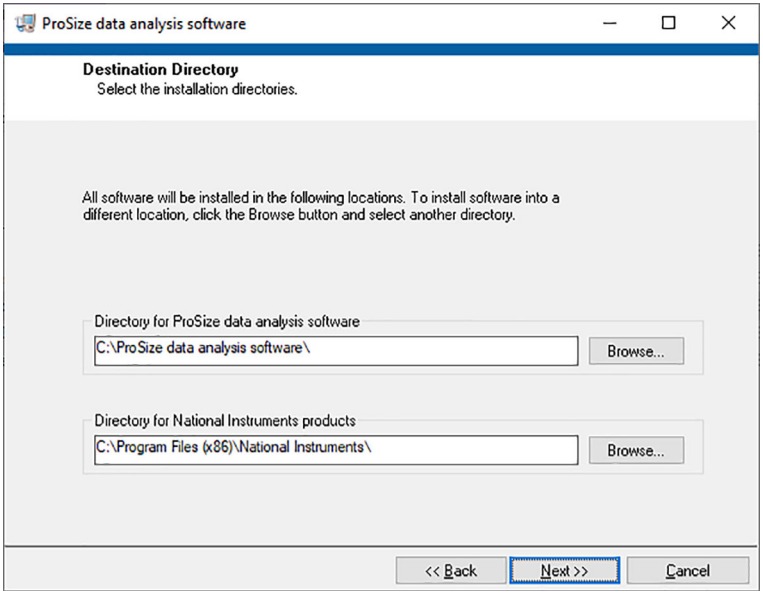


Figure 1 Default directory locations for the ProSize data analysis software and supporting National Instrument software

a Provide the installation directory. The default directory is C:\ProSize data analysis software\.

A shortcut of the ProSize software is created in the **Start** menu.

Supported software from National Instruments will also be installed under C:\Program Files\National Instruments\.

b Complete the following installation steps.

c Restart the computer to complete the installation process.

Upon successful installation of the ProSize software, several sub-folders will have been created within the C:\ProSize data analysis software\ directory (see **Figure 2**):

- **Configurations**: This folder contains ProSize global configuration setting files, optimized for different application kits for the parallel capillary electrophoresis instruments.
- **Ladders**: This folder contains ProSize sizing ladder information files (.txt format), for use with different application kits for the parallel capillary electrophoresis instruments.
- **Support**: This folder contains supporting files for ProSize software operation.
- **Training Test Runs**: This folder contains data files, for use with a set of tutorial videos which are loaded upon installation of the ProSize software.

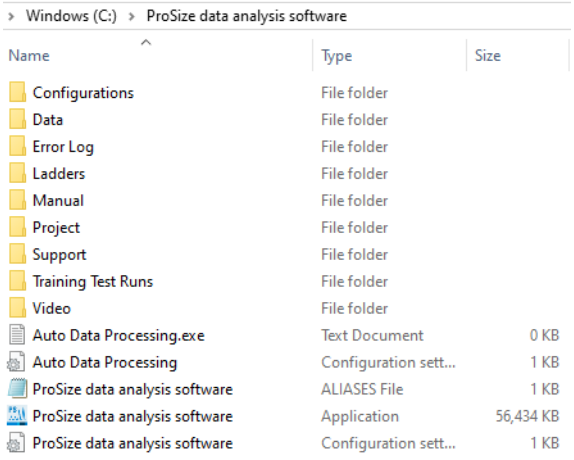


Figure 2 Sub-folders created within the C:\ProSize data analysis software\ directory

4 Update the Configurations, Ladders and Video folders within the C:\ProSize data analysis software\ directory to the latest versions. You can obtain the most recent version of software and files from Agilent support.

   To update the folder files:

   a Navigate to C:\ProSize data analysis software\.

   b Delete the contained folders **Configurations**, **Ladders**, and **Video**.

   c Paste the folders **Configurations**, **Ladders**, and **Video** supplied from Agilent into the same directory C:\ProSize data analysis software\.

NOTE

After installation of the ProSize 3.0 software, the system will install System.Data.SQLite. Please follow all screen instructions on this installation.

To properly read PDF reports:

1 Install the latest version of Adobe Reader on your computer. This software is available free of charge for download at www.adobe.com.

Starting the ProSize Program

1 In the Windows Start menu, navigate to **ProSize data analysis software**. Alternatively, create a shortcut from your desktop to launch the program.

A splash screen appears showing the software version (**Figure 3**).

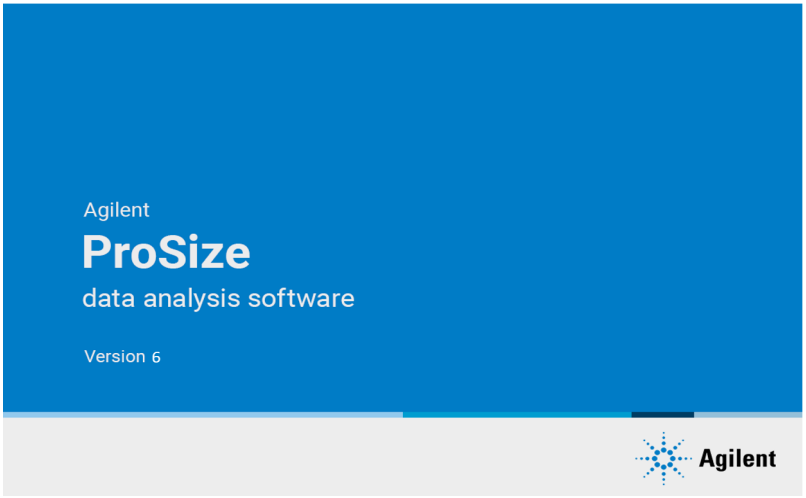


Figure 3 Splash screen displaying version information upon starting the ProSize software

When the software is open, a screen with a white background screen is displayed. At the upper part of the screen you see two active main menu options (see **Figure 4**):

- **File** > **Open File**: Use this drop-down menu to open data files. Alternatively, from the toolbar, select to open your data.
- **Help** > **About**: Shows information about the software version and Agilent contact information.

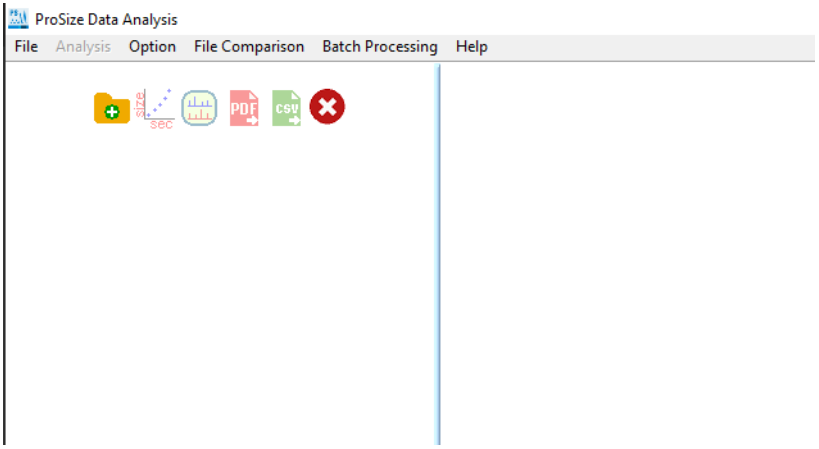


Figure 4 Main screen of the ProSize software

Exit the ProSize software

To close the ProSize software, select **Close** from the **File** menu, or press .

3 ProSize Main Screen

This chapter provides an overview of the ProSize software main screen with the functions and menus available to the user. The file menu for opening and closing data files is also covered in this chapter.

Main Screen (No Data File Open)

The functions and menu items available from the main screen of the ProSize software prior to opening a *.raw data file are summarized in **Table 3**.

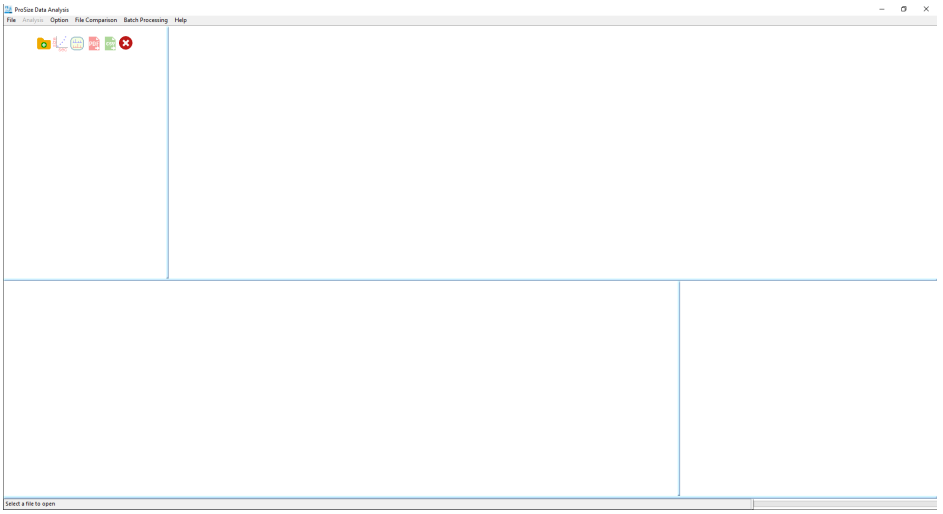


Figure 5 Main screen of ProSize software (no data file open).

Table 3 Main Menu screen menu items (active with no data file open)

Menu Command	Description
File	
Recent Files	Opens a drop-down list of recently viewed files.
Open File	Opens a **Select folder** dialog for locating and opening a raw data file (*.raw extension) generated by the parallel capillary electrophoresis instruments for data analysis (**Figure 7**). Note that if the configuration name (ProSize) and the method name (Fragment Analyzer controller software) do not exactly match, the first time a data file is opened in the ProSize software, the **Global Configuration** dialog is displayed. The screen allows you to select the appropriate predefined data configuration file (*.ini extension), or to customize a configuration file be selecting the data analysis mode (DNA, RNA, NGS, gDNA) and analysis settings (**Figure 9**).
Close	Closes the ProSize software. When closing, the data file, all settings and operations are automatically saved.

Table 3 Main Menu screen menu items (active with no data file open)

Menu Command	Description
Option > Option dialog settings	
Default Data Path	Allows you to set the default file path for accessing and saving data files. In the **Select folder** dialog, navigate to the desired directory and select the folder to be set to open data files (Figure 7). The default directory for opening *.raw data files is C:\Agilent\Data\.
Capillary Array Format	Allows you to arrange the data from left to right in the **Digital Gel Image** display by **Row** (A1, A2, A3, etc.) or by **Column** (A1, B1, C1, etc.).
Display Option	Allows you to select the view of the data by selecting **Scale to Sample** or the **AutoFit function**.
Molarity Calculation	Allows you to **Use Peak Maxima** or **Use Avg. Size** for all molarity calculations.
Concentration unit	Allows you to select the preferred concentration units, **ng/µL** ... **nmole/L**, or **pg/µL** ... **pmole/L**.
Image Resolution	Define the image resolution as **1X**, **2X**, **3X**, or **4X** used for exporting electropherograms or gel images.
Edit Configuration	Opens the **Global Configuration** dialog (Figure 9), where you can set parameters for data analysis mode, peak and marker integration, quantification, ladder size, etc.
File Comparison	
Load	Opens a **Select Folder** dialog for locating and opening a file comparison data file (*.proj extension) generated for data analysis.
Create	Allows you to open the **Compare Files** screen and create a file comparison by selecting different *.raw data files.
Batch Processing	
Batch Data Process	Allows you to process a set of *.raw files within a selected folder, and to define an output folder within which processed files are sent.
View Error Log	Allows you to view the error log associated with a particular batch data process.
Help	
User Manuals	Access all ProSize User manuals.
About	Displays software information, version information, copyright information and Agilent technical support contact information.
End User License Agreement (EULA)	Terms and restrictions of the ProSize data analysis software.
Create a Support Package	Creates a zip file to be shared with support when technical support is required.

The **Options** pop-up dialog is shown in **Figure 6**. The properties of the dialog are described in **Table 3** above.

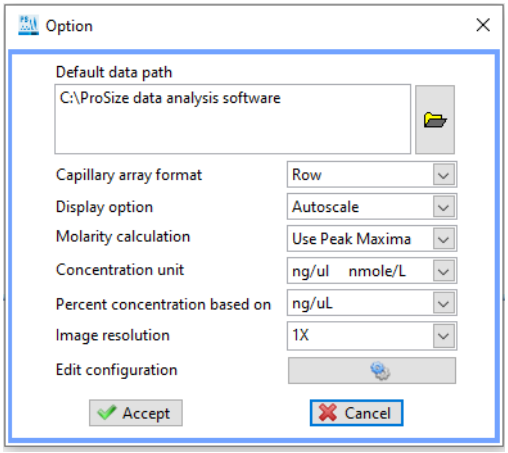


Figure 6 **Option** dialog

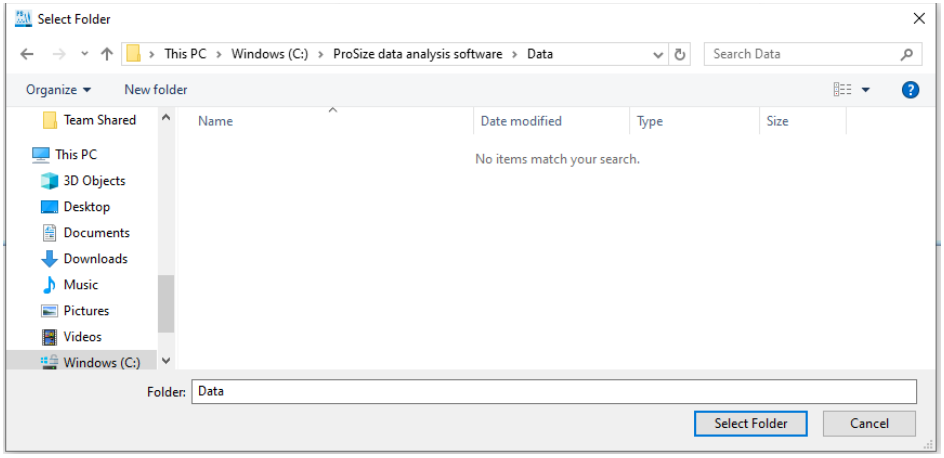


Figure 7 Set Default Data Path selection screen

Opening a Data File in ProSize Software

To Open a Data File

1 From the menu, select **File** > **Open File** (alternatively, select from the toolbar).

The **Select Folder** dialog opens (**Figure 7**).

2 Navigate to your raw data file (*.raw extension) for data analysis.

3 Select the folder with the raw data.

In the ProSize software, the **Open** screen is displayed, showing the selected file directory (**Figure 8**).

If you need to navigate to a higher-level directory, select the folder to the right of the file path and a standard file browser will open.

4 In the **Open** screen, click a data file.

A file previewer window will be displayed on the right side of the screen, containing a digital gel image of the data, a **Sample Information** list, any typed **Note**, and a summary of the experimental **Method** used to collect the data file (**Figure 8**). If the data file has not been previously processed in ProSize, the heading of the digital gel image will be labeled *Non-processed Data*. If the data file has been previously processed, no heading will be displayed on the digital gel image.

5 If the correct data file is verified, select **Open** to load the data file into ProSize. Select **Cancel** to close the **Open** screen without loading a file.

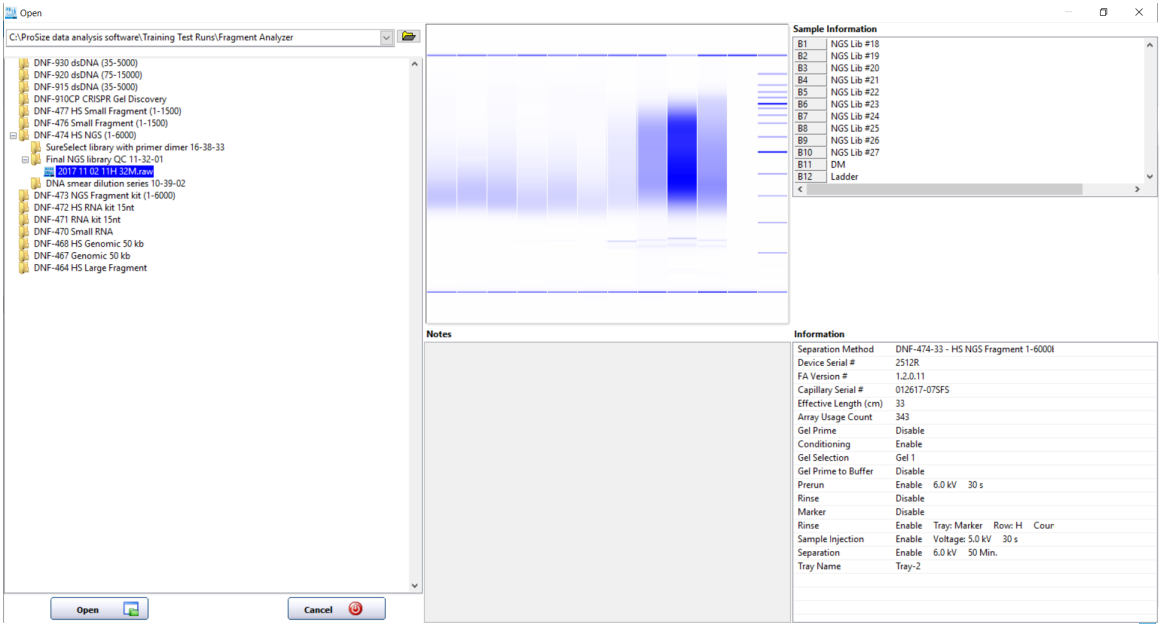


Figure 8 Open file data selection and previewer screen

Configuration Dialog

Upon selecting **Open**, the data file will be processed if the matching predefined data analysis configuration file is available (for example, **DNF-473-33 - SS NGS Fragment 1-6000bp.ini**). The first 15 characters of the configuration file name must match the first 15 characters of the separation method used to automatically process the data.

NOTE

A minimum of 15 characters must be used to name both the method (instrument) and configuration (ProSize).

Should there be no matching configuration file, the **Global Configuration** dialog will be loaded when opening a previously unprocessed data file. Should the wrong configuration file be chosen, the user can navigate to the **Option** window, and select **Edit Configuration** to load the correct configuration.

Upon opening, there are ten primary tabs in the **Global Configuration** dialog (**Figure 9**). The settings are discussed in detail in **Chapter 4**, "ProSize Configuration".

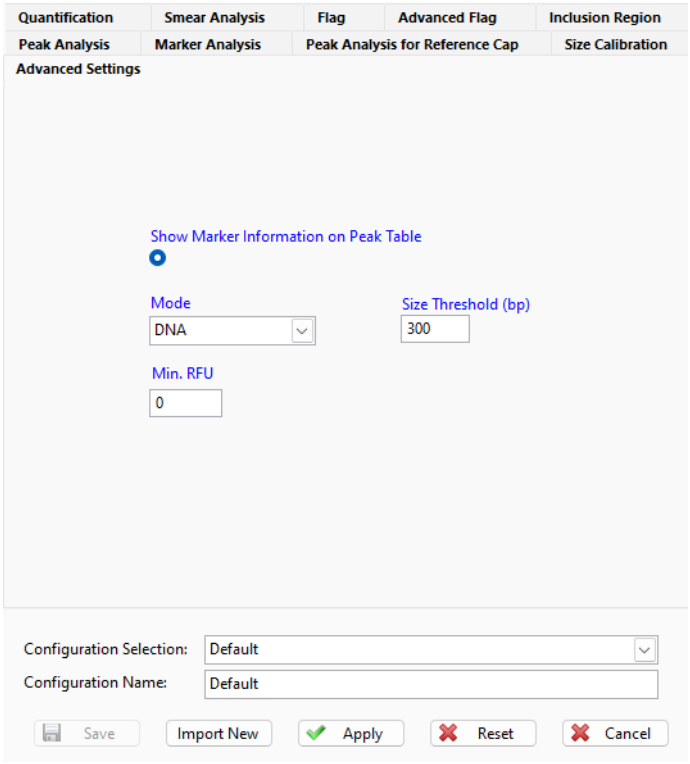


Figure 9 Global Configuration dialog (Advanced Settings tab is selected)

NOTE

You may create a customized configuration by selecting the proper data analysis mode (DNA, RNA, etc.) and analysis settings.

NOTE

In most cases, predefined, optimized global configuration files are available to load for each Agilent Reagent Kit. The configuration files are labeled according to the particular kit number, for example, **DNF-910-33 - DNA 35-1500bp.ini**.

After the configuration settings have been loaded, select **Apply** to open the data file in the main screen of the ProSize software.

Main Screen (Data File Open)

Once a data file is opened in ProSize, several additional main screen functions are enabled as summarized in **Table 4**. The opened data is displayed in the main screen as shown in **Figure 10** (12-capillary data) and **Figure 11** (96-capillary data).

Table 4 Additional Main screen menu items (active only with data file open)

Menu Option	Description
File	
Close File	Closes the currently opened data file and displays the main screen. All settings up to the point of closing the data file will be saved and reloaded when reopening the data file.
Generate Report or 	Opens a menu for configuring and generating an Adobe .pdf report, containing user specified information (electropherograms, gel images, peak information, etc.). For more information, see **Chapter 8**, "Generating Reports from ProSize".
Export Data or 	Opens a menu for configuring and exporting user specified data to a user specified folder. Types of data include gel images, electropherograms, peak tables, size calibration data, etc. For more information, see **Chapter 7**, "Exporting Data from ProSize".
Analysis	
Show Size Calibration or 	Opens the **Calibration Curve** screen, which contains input data associated with the size calibration ladder. For more information, see **Chapter 5**, "ProSize Size Calibration Screen".
File Comparison or 	Opens the **Compare Files** screen, where you can overlay gel images or electropherograms from individual lanes or different data files. For more information, see **Chapter 6**, "ProSize Compare Files".
View Capillary Positions	Opens the **View Capillary Positions** screen, which allows you to view and modify the alignment of the detector signal with the capillary positions. For more information, see **Chapter 9**, "ProSize View Capillary Positions".
Quick Display	Displays the **Quick Display** window, which shows non-normalized electropherograms for all 12 capillaries (or windows of 12 capillaries each for a 96-capillary array format) enabling quick review of the data.
Display Separation Parameters	Displays the **Separation Parameters** window, which allows the user to view the current, voltage, and pressure that occurred during the separation.

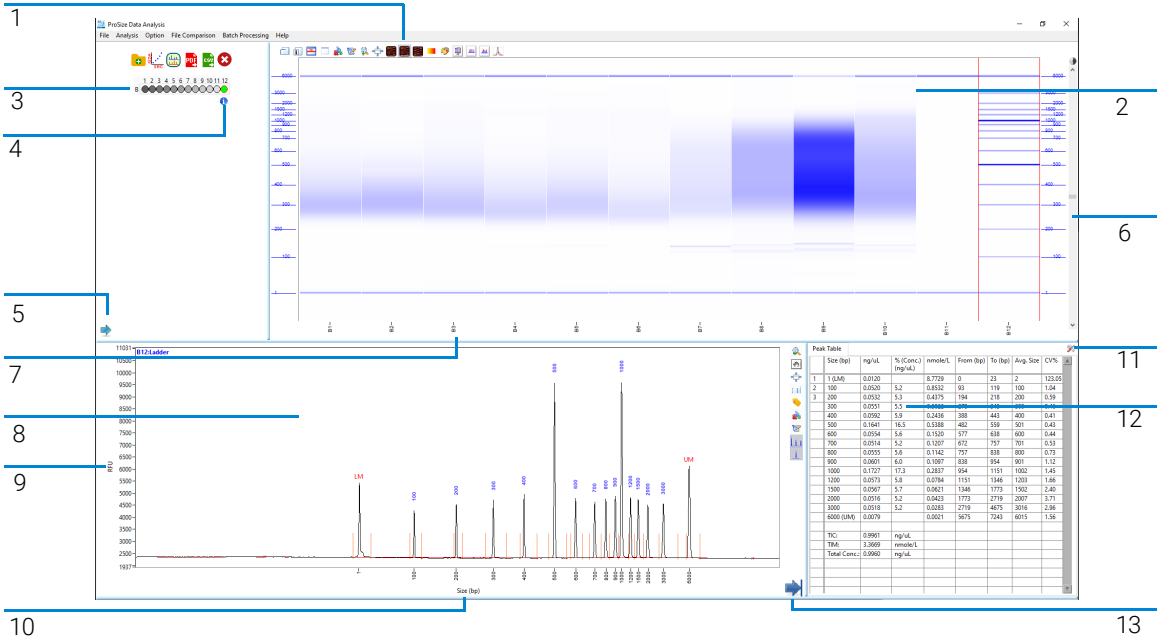


Figure 10 Main screen of ProSize software (12-capillary data file open)

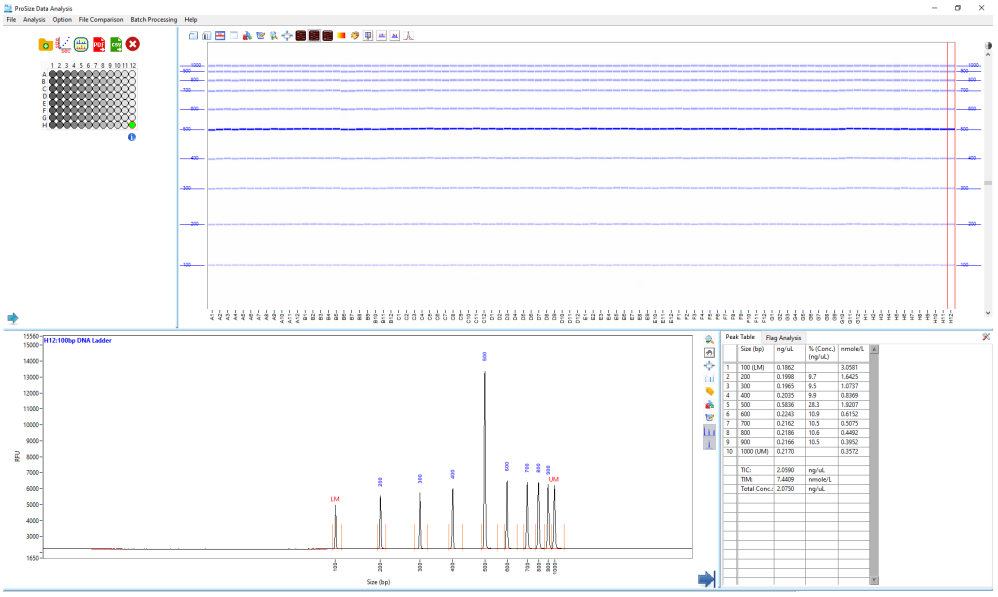


Figure 11 Main screen of ProSize software (96-capillary data file open)

The primary accessible functions from the main screen are shown in **Figure 10**, and are summarized in the legend table:

1	**Main screen toolbar**	
2	**Digital Gel image**	Digital Gel Image of the data set (see also **Figure 14**, **Table 6**). The sample wells are normally displayed in increasing order for each row. Starting on the left side starting with well A1 (A1, A2, A3-A12; followed by B1-B12, etc.). Change the view to ascending order of the well columns from left to right (A1, B1, C1, etc...). From the **Option** menu, select **Capillary Array Format Column**. To toggle back to a row-by-row format, select **Capillary Array Format Row.**
3	**Plate map**	Shows the currently selected well highlighted in green (A1 is the default location on opening the file). Click on the appropriate well by using the keyboard arrows, or double-clicking the appropriate lanes of the digital gel image. You can select any well of the plate for individual viewing. The functions available within the Plate Map menu are summarized in **Table 5**.
4	**Info**	Click to access experimental information regarding the method used to collect the data file.
5	**Sample ID navigation**	Enables Sample ID navigation. The Sample ID list as entered into the instrument software will be displayed, enabling you to navigate the samples by Sample ID and to edit sample names if desired. Close the Sample ID navigation view by right-clicking in the list, and select **Close**.
6	**Contrast**	Use the slider bar to change the intensity contrast of the gel image.
7	**Re-scale**	Re-scale the electropherogram image and gel image by sliding the bar between the two images.
8	**Electropherogram trace**	An Electropherogram trace of the currently selected well. The well position and sample name (if imported) is displayed in the upper left corner of the trace. Right-click the screen to process or annotate the data (**Table 7** and **Table 8**).
9	**Scaling y-axis**	Units for the y-axis can be changed by right clicking the axis title and selecting RFU or nmole/L.
10	**Scaling x-axis**	Units for the x-axis can be changed by right-clicking the axis title and selecting time scale or size scale.
11	**Set Individual Parameters**	Select to reveal the configuration window, for which most settings can be individually adjusted for each sample as desired (see **Figure 13**). A brief summary of the primary configuration functions are presented in this section. For more detailed information, refer to **Chapter 4**, "ProSize Configuration".
12	**Peak table**	Displays information about the selected and integrated sample peaks and markers (see also **Figure 18**). Depending on the configurations: • A **Flag Analysis** tab will be shown if the flag configuration function contains any flag conditions (**Figure 33**). • A **Smear Analysis** tab will be shown if the smear analysis configuration contains any entries (**Figure 32**). • An **Advanced Flag** tab will be displayed if any advanced flag functions are used (**Figure 35**).
13	**Expand Electropherogram trace**	Select to expand the Electropherogram trace across the bottom panel which provides you with more zoom options (see **Figure 12**).
	Filename	Upon opening a data file, the currently selected Filename/directory is displayed in the lower left toolbar.

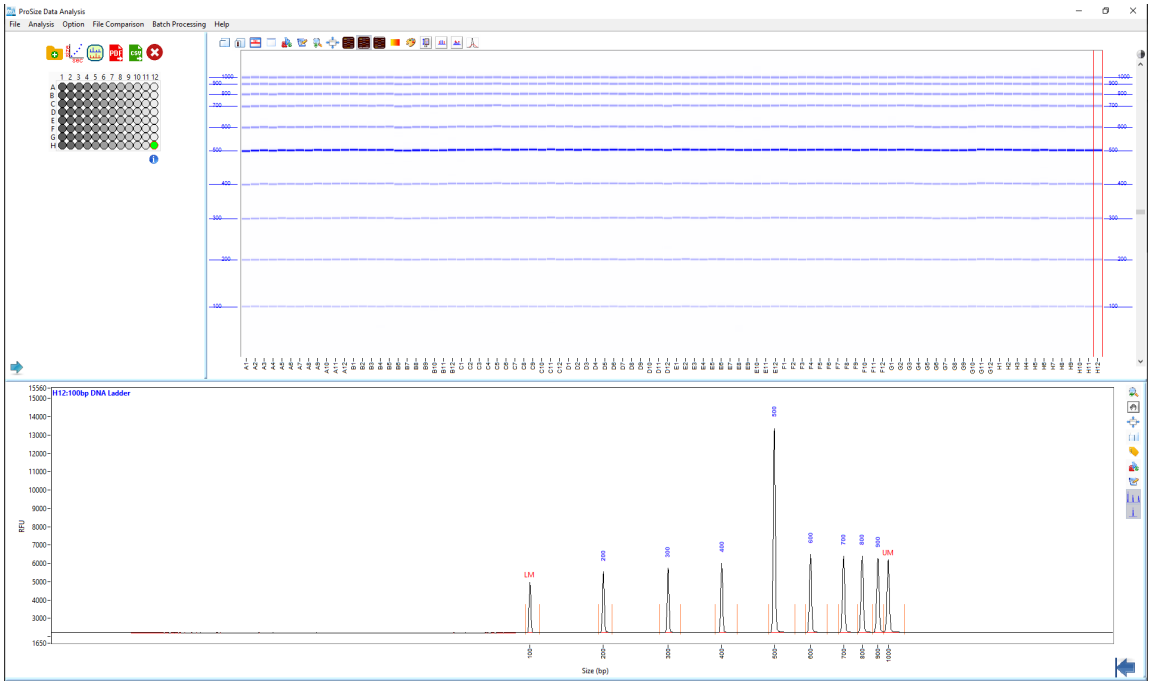


Figure 12 Expanded Electropherogram Trace

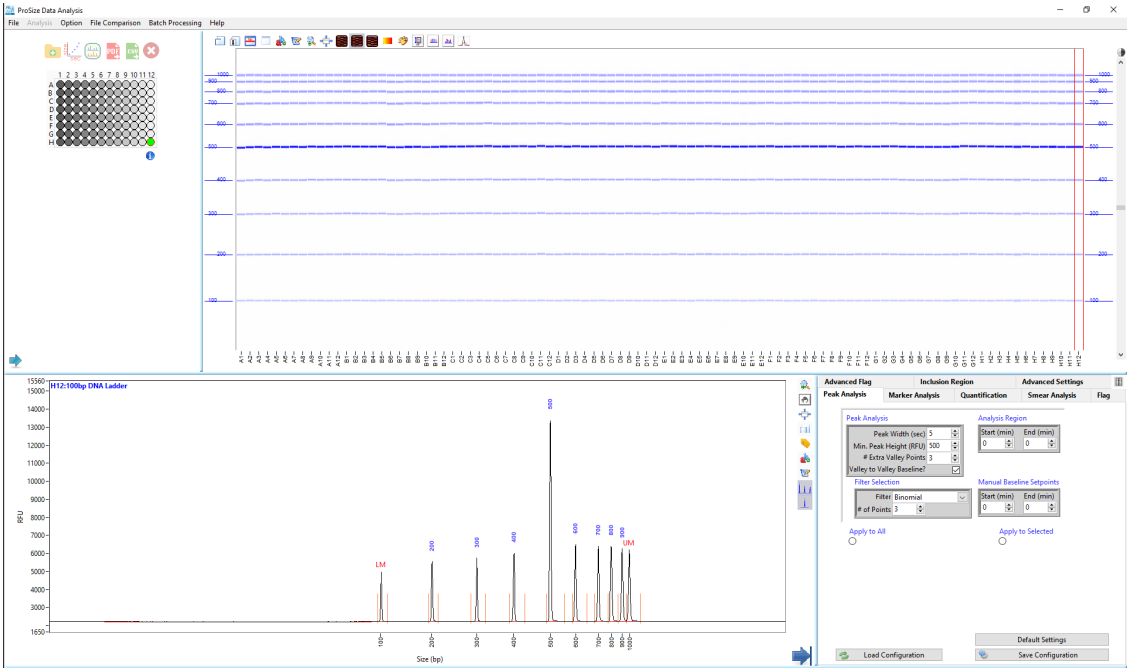


Figure 13 Main screen with data file open and open configuration dialog (Set Individual Parameters dialog)

Plate Map

The functions available within for the Plate Map are summarized in **Table 5**.

Table 5 Plate Map menu functions

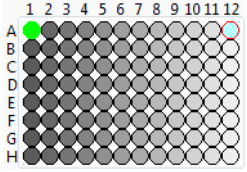
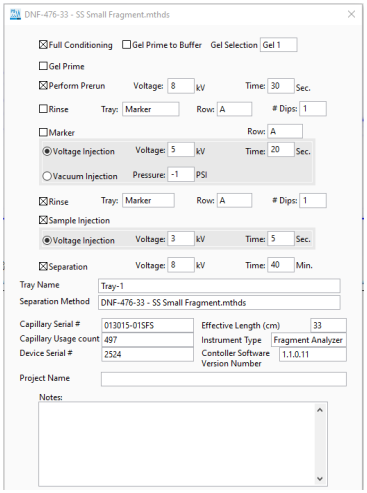
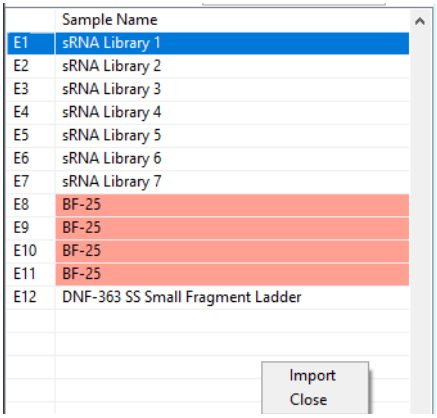
Menu Option	Description
Plate Map	Used to select a sample well for adjusting marker/peak integration, assigning flag or smear analysis conditions, viewing Peak Table information and Electropherogram Trace, or adjusting lane contrast in the Digital Gel Image. Possibilities to select a well: • Click the well. • Change the well selection via the keyboard arrows. • Double-click the lane in the digital gel image. • Right-click the sample wells to overlay them in the Electropherogram section of the screen. • Select the **Sample ID** in the Sample ID navigation pane.  To display a well/sample name, place the mouse cursor over the well.

Table 5 Plate Map menu functions

Menu Option	Description
Experimental Information	Shows the instrument system software user interface, containing the experimental information, Capillary Array information, instrument system information, and Notes fields for the collected data file. To close the window, click ×. 
Sample ID navigation	The Sample ID list will be displayed. In the list, you can navigate the samples by the Sample ID previously entered into the instrument system software. Double-click on a sample name allows you to edit the Sample ID information. Right-click the sample ID allows you to **Import** a .txt file. Select **Close** to exit the Sample ID list and return to the plate map view. 

Digital Gel Image Toolbar

The toolbar options above the digital gel image are summarized in **Table 6**. The gel image is annotated on both the left and right side with the sample sizing ladder in bp or nt. The currently selected lane is bracketed by vertical cursors (**Figure 14**).

Right-click on any sample lane will overlay the samples in the electropherogram view. Double-click on a sample lane to view a single sample well in the electropherogram view.

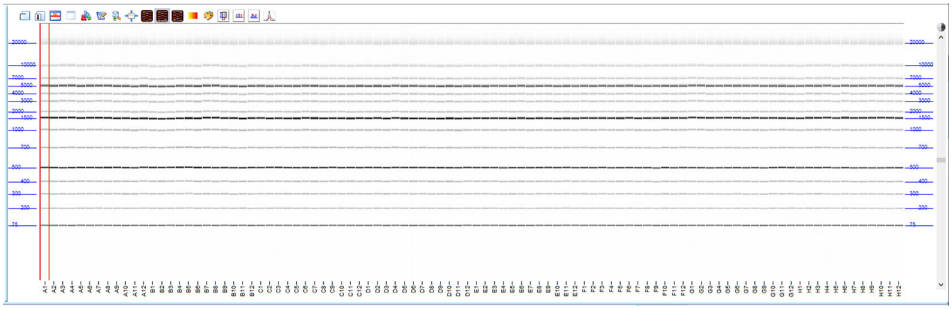


Figure 14 Main screen zoomed to the digital gel image and its toolbar menu

Table 6 Digital Gel Image toolbar

Toolbar Option	Description
Copy Full Gel Image	Copies an image of the full Digital Gel Image to the clipboard, for pasting in another program such as Microsoft PowerPoint. If zoomed into the gel image, it will copy the visible portion of the image. The left and right side of the gel image is annotated with the sizing ladder in the copied gel image.
Copy Selected Gel Lane Image	Copies an image of the currently selected gel lane to the clipboard, for pasting in another program such as Microsoft PowerPoint. If zoomed into the gel image, it will copy the visible portion of the gel lane image. The left side of the gel lane image is annotated with the sizing ladder in the copied image.

Table 6 Digital Gel Image toolbar

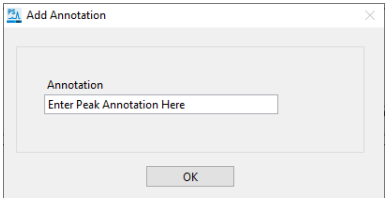
Toolbar Option	Description
Show Cursors	Displays a horizontal line cursor across the digital gel image, annotated by size (bp or nt) corresponding to the current cursor position. The cursor can be dragged to any position along the gel image by holding the mouse over the cursor and pressing and holding the left mouse button. When the cursor is active and displayed, a similar vertical cursor annotated by size is displayed in the Electropherogram Trace, whose movement corresponds to that in the gel image. Up to four cursors can be displayed at one time.
Hide Cursors	To deactivate the cursor, press the button a second time.
Create Annotation	To create customized annotations in the Digital Gel Image display. When selected, the **Add Annotation** window opens. You can type desired annotation into the field, and select **OK**. The annotation will be displayed in the Digital Gel Image window.  To move the annotation to the desired position on the trace: Click the annotation and drag it to the desired position. An arrow will appear at the opposite end of the annotation upon dragging. Multiple annotations can be created by repeatedly selecting **Create Annotation**. Any created annotations will be copied to the clipboard with the **Copy** function.

Table 6 Digital Gel Image toolbar

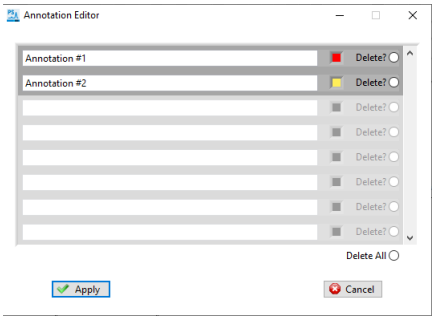
Toolbar Option	Description
Edit Annotation	Opens the **Annotation Editor** window. Allows you to: • Edit the annotation text by typing in the text field. • Change the color of the annotation by left clicking on the annotation color. • Delete one annotation by selecting **Delete** next to each annotation, or delete all annotations by selecting **Delete All**.  Select **Apply**, to confirm your settings.
Zoom	When selected, zooming to an area by clicking and dragging is enabled.
AutoFit	Autoscales the Digital Gel Image x-/y-axis view for all 12 or 96 lanes. The autoscaled image will display the gel from the selected lower marker to upper marker, or if only a lower marker only is used from the lower marker to the end of the separation window.
Auto Intensity	Autoscales the intensity of the Digital Gel Image view for all 12 or 96 lanes. The viewed intensity across the image will reflect the actual observed fluorescence signals from each lane.
Normalize Intensity to Lower Marker	Normalizes the intensity of the Digital Gel Image view for the lower marker to the same value for all 12 or 96 lanes, adjusting the relative intensities of all bands in each lane accordingly. The viewed intensity across the image will as a result reflect the fluorescence signals from each lane after normalization to the lower marker, as is done when calculating the concentration of samples using the lower marker.
Normalize Intensity to Upper Marker	Normalizes the intensity of the Digital Gel Image view for the upper marker to the same value for all 12 or 96 lanes, adjusting the relative intensities of all bands in each lane accordingly. The viewed intensity across the image will as a result reflect the fluorescence signals from each lane after normalization to the upper marker, as is done when calculating the concentration of samples using the upper marker.

Table 6 Digital Gel Image toolbar

Toolbar Option	Description
Enhanced Contrast	Increases the contrast of an individual lane while holding other lanes constant. When selected, the Lane Contrast Control window opens. Drag the scroll bar to adjust the contrast for the currently selected lane in the gel image. Once adjusted, click **OK** to accept changes, or select **Cancel** to discard your changes. Note: The enhanced contrast setting works on the currently selected lane only (marked with vertical cursors in gel image). The Auto Intensity, Normalize Intensity to Lower Marker, and Normalize Intensity to Upper Marker functions will undo any previous enhanced contrast operations.
Gel Image Color	Changes the color scheme for the **Digital Gel View**, the gel image in the **Compare Files** screen and any exported or printed report. Options for the gel image color: • White on Black (white bands on black background) • Black on White (black bands on white background) • Green on Black (green bands on black background) • Red on Black (red bands on black background) • Pseudo Color (green to red bands on blue background)
1:1 Gel Image	Displays the gel image in an adjustable window for changing the aspect ratio of the gel image (useful for producing figures for papers). The adjusted image can be copied to the clipboard.
Hide/Show Marker Peaks	Toggle to hide or show the marker peaks in the digital gel image screen. This is useful when comparing to a slab gel result which does not use lower/upper normalization markers.
Hide/Show non-integrated peaks	Toggle to hide or show non-integrated peaks in the digital gel image screen. This is useful for eliminating noise peaks from the gel image.
Gaussian Fit	Toggle to fit all peaks to a narrow Gaussian peak.

Electropherogram Trace Toolbar Menu

The options available in the Electropherogram Trace toolbar are summarized in **Table 7**.

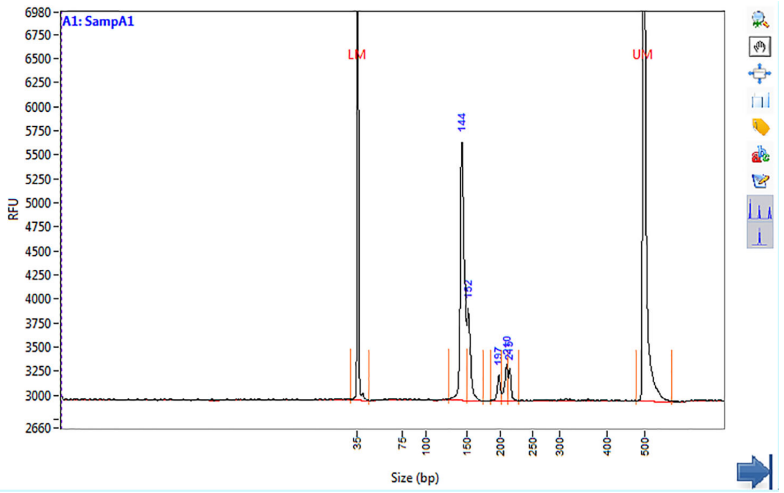


Figure 15 Main screen zoomed to the Electropherogram Trace and its toolbar menu. Note the y-axis scale is set to the sample peaks and not the marker peak heights.

NOTE

The Electropherogram Trace will automatically autoscale to the largest observed sample peak, regardless of the lower/upper marker peak heights. To view the entire y-axis scale including marker peaks, in the **Option** window, select **AutoFit** as the **Display Option** (see **Figure 6**).

Table 7 **Electropherogram Trace toolbar functions**

Toolbar Option	Description
Zoom	Enables zooming in the x- and y-axis of the Electropherogram Trace. To zoom-in: Place the mouse over the trace, click and drag it outward to expand a box area to define the zoom region. Release the mouse button to apply. The zoomed image can be copied to the clipboard. The zoom region will be preserved when viewing other samples in the Plate Map. To undo the zoom, use the **AutoFit** function.
Drag	Enables panning of the image. Move the image around with the mouse cursor.

Table 7 Electropherogram Trace toolbar functions

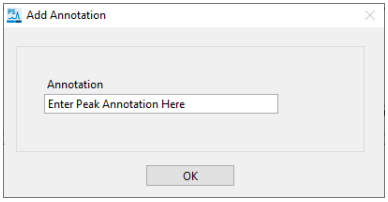
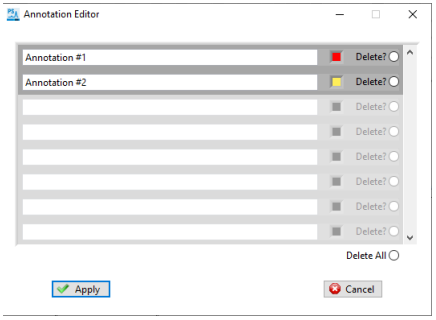
Toolbar Option	Description
AutoFit	To autoscale the Electropherogram Trace x-/y-axis display. The autoscaled image will display the full trace from the start to the end of the separation when using the **Time Scale** and **Size Scale** display modes; when using the **Uniform Size Scale** the autoscaled display will start at 0 bp. Note: the y-axis scale will automatically zoom to the highest detected sample peak, regardless of the marker peak heights. To view the entire y-axis scale including markers, go to **Option** window, and as **Display Option**, select **AutoFit**.
Copy	Copies an image of the current view of the Electropherogram Trace to the clipboard, for pasting in another program such as Microsoft PowerPoint. Any zoom, annotation, baseline and/or peak start/end point displayed will be copied in the image. The well ID and sample name will be copied in the top left of the trace, and the x-/y-axis will correspond to the currently selected view in the copied image.
Peak Label	Displays a menu for changing the peak annotation of the Electropherogram Trace. Only integrated peaks are annotated. Units for the peak annotation: • None • Peak ID (labels in order as 1, 2, etc.) • Migration Time (min:sec) raw migration time • Peak Height (in RFUs) • Corrected Peak Area (Peak Area/Migration Time) • Size (in bp or nt) • Average Size (in bp or nt) • Concentration (in ng/µL) • nmole/L
Create Annotation	To create customized annotation in the Electropherogram Trace display. In the **Add Annotation** dialog, the user can type desired annotation into the field. Click **OK** and the annotation will be displayed in the Electropherogram Trace window.  To move the annotation to the desired position on the trace: Click the annotation and drag it to the desired position. An arrow will appear at the opposite end of the annotation upon dragging. Multiple annotations can be created by repeatedly selecting **Create Annotation**. Any created annotations will be copied to the clipboard with the **Copy** function.

Table 7 Electropherogram Trace toolbar functions

Toolbar Option	Description
Edit Annotation	Opens the **Annotation Editor** window. Allows the user to: • Edit the annotation text by typing in the text field. • Change the color of the annotation by left clicking on the annotation color. • Delete one annotation by selecting **Delete** next to each annotation, or delete all annotations by selecting **Delete All**.  Select **Apply** to confirm your settings.
Show/Hide Baseline	Toggle to display or hide the baseline drawn for the peak integration, shown as an orange line. Displaying the baseline helps if adjustments need to be made to the baseline to better define the actual peak area via the **Peak Width (sec)**, **Valley to Valley**, or **Manual Baseline Setpoints** tools. Note: It is highly recommended to enable the display of the baseline, to ensure the correct baseline is being drawn to the data, especially with DNA/RNA smears.
Show/Hide Peak Start/End Point	Toggle to diplay or hide the start and end points used for peak integration, shown as vertical orange lines. Displaying the peak integration start/end points helps if adjustments need to be made to the peak integration to better define the actual peak area. The user can change the peak start/end points by adjusting the **Peak Width (sec)**, or by using the right click menu tools in the Electropherogram Trace such as **Split Peak** or **Move Peak Start/End Points** (see Table 8). The user can change the baseline drawn (and subsequently the peak start/end points) via the **Peak Width (sec)**, **Valley to Valley**, or **Manual Baseline Setpoints** tools. Note: It is highly recommended to enable the display of the baseline, to ensure the correct integration is being drawn to each peak, especially with DNA/RNA smears.
Expand Electropherogram Trace	Expands the Electropherogram Trace across the bottom panel covering the Peak Table. Provides more user control over zoom.

Electropherogram Trace Context Menu

The context menu of the trace, the y-axis, and the x-axis contains functions for adjusting marker peak assignment, peak integration and scaling the x-axis of the Electropherogram Trace (**Figure 16**). Functions are also provided for copying the trace and exporting the data, and are summarized in **Table 8**.

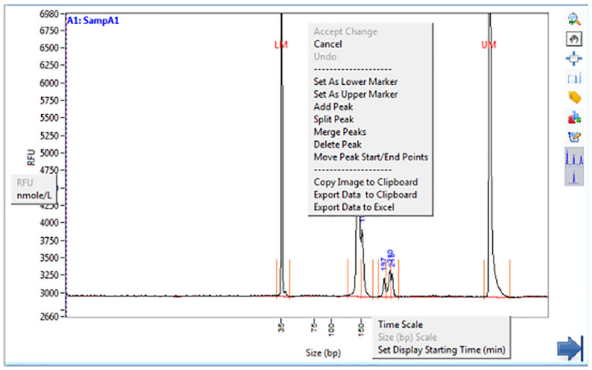


Figure 16 Main screen zoomed to the Electropherogram Trace and context menus. Note that the y-axis scale is set to the sample peaks and not the marker peak heights.

Table 8 Electropherogram Trace context menu commands

Menu commands	Description
Accept Change	Accepts any manual modification to the performed peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). After the manual modification has been made, select **Accept Change** to apply the changes to the trace.
Cancel	Cancels any manual modification to the peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). Select **Cancel** to discard the changes applied to the trace.
Undo	To undo any manual modification applied to the peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). Select **Undo** to revert back one step.
Set As Lower Marker/ Set As Upper Marker	To automatically set any integrated peak to the lower or upper marker, and correspondingly re-normalize the trace to the new marker. Right-click the peak to be reassigned as the marker. Select **Set As Upper (or Lower) Marker.** The peak will be set as the marker and renormalized.

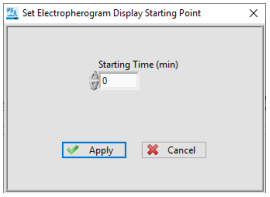
Table 8 Electropherogram Trace context menu commands

Menu commands	Description
Add Peak	To manually add a peak which has not been auto-integrated by the **Peak Analysis** settings and to define the start and end point for integration. Zoom-in to the region where the peak is to be added. Right-click this region and select **Add Peak**. Two red vertical cursors will appear; the left cursor defines the new peak start point and the right cursor the new peak end point. Drag each cursor to the desired position, then right-click, and select **Accept Change** to add the peak for integration. Note: The Add Peak function will not add a peak that is located outside the lower/upper marker window (or before lower marker if using only lower marker).
Split Peak	To manually split a peak which is currently integrated into two peaks and to define where the split occurs. Zoom-in to the region where the peak is to be split. Right-click this region, and select **Split Peak**. A red vertical cursor will appear. Drag the cursor to the desired location, then right-click, and select **Accept Change** to split the peak into two peaks for integration.
Merge Peaks	To merge any number of peaks and integrate as a single peak. Zoom-in to the region where the peak is to be split. Right-click this region, and select **Merge Peaks**. Two red vertical cursors will appear; the left cursor defines the left most peak to merge and right cursor defines the right most peak to merge. Drag the cursor within that peak's start/end point region to merge, then right click, and select **Accept Change** to merge the peaks into a single peak for integration.
Delete Peak	To manually delete a peak which has been integrated. Zoom-in to the region where the peak is to be deleted. Right-click this region, and select **Delete Peak**. A red vertical cursor will appear. Drag the cursor to the desired peak location, then right-click, and select **Accept Change** to delete the peak from the integration.
Move Peak Start/End Points	To change the currently positioned start/end integration points of a peak. Zoom-in to the region where the peak is located. Place the cursor between the start and end points of the integration. Right-click and select **Move Peak Start/End Points**. Two red vertical cursors will appear at the current start/end points. Drag the cursors to the desired locations, then right-click and select **Accept Change** to apply the new start/end point positions to reintegrate the peak. Note: This function will not change the baseline; only the start and end points are affected. To change the baseline, the user must adjust the via the **Peak Width (sec)**, **Valley to Valley,** or **Manual Baseline Setpoints** tools.

Table 8 Electropherogram Trace context menu commands

Menu commands	Description
Copy Image to Clipboard	Copies an image of the current view of the Electropherogram Trace to the clipboard, for pasting in another program, such as Microsoft PowerPoint. Any zoom, annotation, baseline and/or peak start/end point displayed will be copied in the image. The well ID and sample name will be copied in the top left of the trace, and the x-/y-axis will correspond to the currently selected view in the copied image.
Export Data to Clipboard	Copies the electropherogram x-data (time; in HH:MM:SS) and y-data (RFUs) in a .csv format that can be pasted directly into common spreadsheet programs such as Microsoft Excel. The x-data is copied into column 1, and the y-data into column 2 of the file upon pasting. To export, right-click and select **Export Data to Clipboard**. Open a spreadsheet, select the desired cell and paste the copied data. Note: In the **Time Scale** and **Size Scale** display modes, the exported data will be: • x = time, and • y = RFU. In the **Uniform Size Scale,** the data will be: • x = size, and • y = RFU.
Export Data to Excel	This function requires Microsoft Excel to be installed on the computer to function properly. Selection will automatically open Microsoft Excel and copy the electropherogram x (time; in HH:MM:SS) and y (RFUs) in a .csv format. The x-data is copied into column 1, and the y-data into column 2 of the file upon pasting. To export, right-click and select **Export Data to Excel**. The file can then be processed and saved as desired. Note: In the **Time Scale** and **Size Scale** display modes, the exported data will be: • x = time, and • y = RFU. In the **Uniform Size Scale**, the data will be: • x = size, and • y = RFU.
Time Scale	Changes the x-axis to units of migration time in hours, minutes and seconds (HH:MM:SS). To change, right-click and select **Time Scale**. The x-axis scale will change to time and the trace with AutoFit.
Size (bp) Scale	Changes the x-axis to units of size (bp in DNA, NGS, gDNA, Mutation Analysis or Restriction Digest modes; nt in RNA modes). The display will use underlying units of time and convert the time to size from the current **Size Calibration** settings. To change, right-click and select **Size Scale**. The x-axis scale will change to a size scale and the trace with AutoFit.

Table 8 Electropherogram Trace context menu commands

Menu commands	Description
Set Display Starting Time (min)	To set-up the displayed start time at the x-axis. Right-click the x-axis and select **Set Display Starting Time (min)**. In the dialog, enter a start time (in minutes), and select **Apply** to confirm your entry. 
Set nmol/L	To set-up the scaling for the y-axis. Allows you to change the scale from RFU to nmol/L. To change, right-click the y-axis, and select **Set nmol/L**. To change the y-axis scale back to RFU, right-click the y-axis, and select **RFU**.

Electropherogram Trace Context Menu – RNA Mode

In RNA (Eukaryotic), RNA (Prokaryotic), or RNA (Plant) analysis modes, additional commands are shown in the Electropherogram Trace context menu (see **Figure 17** and **Table 9**).

NOTE

The currently selected 16S/18S ribosomal RNA peak is highlighted pink, and the currently selected 23S/25S/28S ribosomal RNA peak is highlighted blue in the Electropherogram Trace when in these RNA analysis modes.

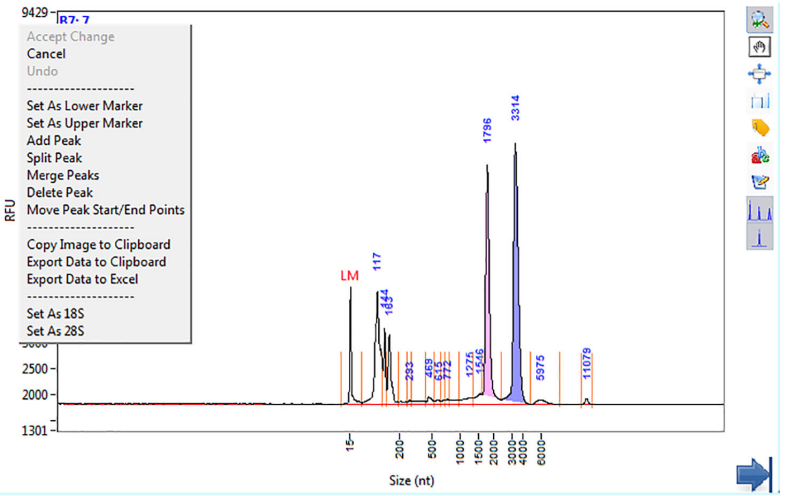


Figure 17 Zoomed-in Electropherogram Trace screen showing context menu in RNA (Eukaryotic) mode. Note that the ribosomal RNA peaks are shaded pink and blue, respectively.

Table 9 Electropherogram Trace context menu commands (only available in RNA mode)

Menu commands	Description
RNA (Eukaryotic) Mode	
Set As 18S	To manually assign a peak as the 18S ribosomal RNA peak for calculating the sample's RNA Quality Number (RQN). Place the mouse over the peak to be reassigned as the 18S peak. Right-click and select **Set As 18S**. The peak will be set as the 18S peak and the RQN recalculated.

Table 9 Electropherogram Trace context menu commands (only available in RNA mode)

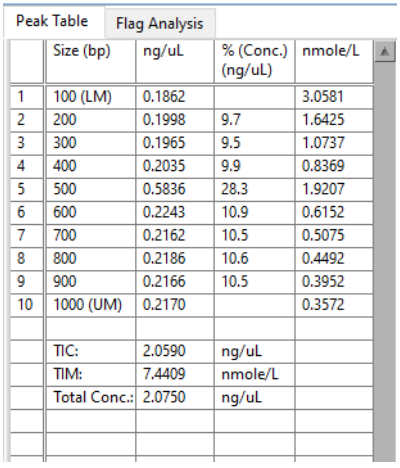
Menu commands	Description
Set As 28S	To manually assign a peak as the 28S ribosomal RNA peak for calculating the sample's RNA Quality Number (RQN). Place the mouse over the peak to be reassigned as the 28S peak. Right-click and select **Set As 28S**. The peak will be set as the 28S peak and the RQN recalculated.
RNA (Prokaryotic) Mode	
Set As 16S	To manually assign a peak as the 16S ribosomal RNA peak for calculating the sample's RNA Quality Number (RQN). Place the mouse over the peak to be reassigned as the 16S peak. Right-click and select **Set As 16S**. The peak will be set as the 16S peak and the RQN recalculated.
Set As 23S	To manually assign a peak as the 23S ribosomal RNA peak for calculating the sample's RNA Quality Number (RQN). Place the mouse over the peak to be reassigned as the 23S peak. Right-click and left click **Set As 23S**. The peak will be set as the 23S peak and the RQN recalculated.
RNA (Plant) Mode	
Set As 18S	To manually assign a peak as the 18S ribosomal RNA peak for calculating the sample's RNA Quality Number (RQN). Place the mouse over the peak to be reassigned as the 18S peak. Right-click and select **Set As 18S**. The peak will be set as the 18S peak and the RQN recalculated.
Set As 25S	To manually assign a peak as the 25S ribosomal RNA peak for calculating the sample's RNA Quality Number (RQN). Place the mouse over the peak to be reassigned as the 25S peak. Right-click and select **Set As 25S**. The peak will be set as the 25S peak and the RQN recalculated.

Peak Table – DNA Mode

The **Peak Table** displays information about the selected and integrated sample peaks and markers (**Figure 18**). The data in this table can be directly exported via its context menu. The fields of the **Peak Table** are summarized in **Table 10**.

NOTE

When the **Show Marker Information on Peak Table** setting is selected (**Global Configuration** dialog > **Advanced Settings** tab), entries for the lower and/or upper marker will also be visible in the table.



Peak Table | Flag Analysis

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L
1	100 (LM)	0.1862		3.0581
2	200	0.1998	9.7	1.6425
3	300	0.1965	9.5	1.0737
4	400	0.2035	9.9	0.8369
5	500	0.5836	28.3	1.9207
6	600	0.2243	10.9	0.6152
7	700	0.2162	10.5	0.5075
8	800	0.2186	10.6	0.4492
9	900	0.2166	10.5	0.3952
10	1000 (UM)	0.2170		0.3572
	TIC:	2.0590	ng/uL	
	TIM:	7.4409	nmole/L	
	Total Conc.:	2.0750	ng/uL	

Figure 18 Peak Table form on main screen (DNA mode)

Table 10 Peak Table functions (only available in DNA mode)

Item	Description
Size	Reports the calculated size in bp (DNA, NGS, gDNA modes) or nt (RNA modes) for each integrated peak.
Conc. (ng/uL)	Reports the calculated concentration in units of nanogram per microliter (ng/µL) for each integrated peak. The concentration is calculated from comparison to the lower or upper marker, or to the calibration sizing ladder referenced to the lower marker depending upon the current settings in the **Quantification** tab of the **Global configuration** dialog. Any factoring from dilution of the input sample is also calculated via the **Quantification** settings.
% (Conc.)	Reports the calculated % of total concentration (not including markers) for each integrated peak in the sample.

Table 10 Peak Table functions (only available in DNA mode)

Item	Description
nmole/L	Reports the calculated concentration in units of nanomoles per liter (nmole/L) for each integrated peak. The concentration is calculated from comparison to the lower or upper marker, or to the calibration sizing ladder referenced to the lower marker depending upon the current settings in the **Quantification** tab of the **Global configuration** dialog. Any factoring from dilution of the input sample is also calculated via the **Quantification** settings. Note: This calculation is only approximate for smear peaks, as the molecular weight of the DNA/RNA fragments constituting the smear is a mixture of many different length species.
TIC	Reports *Total Integrated Concentration (TIC)*, in ng/µL of all integrated peaks for the sample (not including markers). Note: The TIC does not factor in the molarity for peaks or smears that are not integrated in the sample result.
TIM	Reports *Total Integrated Molarity (TIM)*, in nmole/L of all integrated peaks for the sample (not including markers). Note: The TIM does not factor in the concentration for peaks or smears that are not integrated in the sample result.
Total Conc.:	Reports the calculated total concentration in ng/µL of all peaks for the sample (not including markers), regardless of whether the peaks are integrated or not. Note: The Total Conc. takes into account all of the total peak area above the baseline after the lower marker when using the lower marker only for normalization, or the total peak area above the baseline and between the lower and upper markers when using both markers for normalization. The Total Conc. does not include peak area below the lower marker or after the upper marker.
Export Data to Clipboard	Right-click in the table and select **Export Data to Clipboard**. The peak table information is exported to the clipboard for pasting directly into common spreadsheet programs such as Microsoft Excel. The full table is exported.

Peak Table – NGS Mode

In NGS analysis mode, additional information about selected peaks and/or smears is displayed in the **Peak Table** (Figure 19 and Table 11).

This additional information is useful when examining DNA smear characteristics for NGS applications.

Peak Table

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	35 (LM)	0.0437		1.9863	26	71	36	7.50
2	100	0.0525	5.3	0.8543	89	118	101	2.41
3	200	0.0564	5.7	0.4616	188	230	201	1.37
4	300	0.0567	5.7	0.3100	283	322	301	0.97
5	400	0.0602	6.1	0.2474	384	436	400	0.81
	500	0.1657	16.7	0.5432	467	558	502	0.91
	600	0.0510	5.1	0.1395	577	656	601	0.87
	700	0.0486	4.9	0.1141	682	732	701	0.77
	800	0.0478	4.8	0.0984	756	866	800	0.82
	900	0.0533	5.4	0.0976	866	950	900	1.04
	1000	0.1749	17.6	0.2872	950	1143	1002	1.74
	1200	0.0577	5.8	0.0791	1143	1425	1202	2.15
	1500	0.0544	5.5	0.0595	1425	1768	1505	2.67
	2000	0.0563	5.7	0.0459	1768	2756	2019	6.09
	3000	0.0557	5.6	0.0299	2756	3968	3064	5.58
	6000 (UM)	0.0470		0.0128	5516	8197	6028	2.65
	TIC:	0.9913	ng/uL					
	TIM:	3.3678	nmole/L					
	Total Conc.:	0.9960	ng/uL					

Figure 19 Peak Table form on main screen (NGS mode).

Table 11 Additional Peak Table functions (only available in NGS mode)

Item	Description
From (bp)	Reports the size (in bp) of the starting integration point of a DNA peak/smear (i.e., minimum size value).
To (bp)	Reports the size (in bp) of the ending integration point of a DNA peak/smear (i.e., maximum size value).
Avg. Size	Reports the mean size (in bp) of the integrated DNA peak/smear, accounting for the distribution of the concentration of the peak/smear. Therefore, the **Avg. Size** will reflect the size corresponding to 50% of the total peak/smear concentration being below and above this value.
CV%	Reports the distribution of concentration from the mean for a peak/smear, in terms of the coefficient of variation (CV), where CV is the ratio of the standard deviation (σ) to the mean (μ): CV(%) = σ / μ The value is reported as a percentage; smaller values indicate a tighter size distribution of the integrated peak/smear concentration.

Peak Table – gDNA Mode

In the gDNA analysis mode, additional information about the selected peaks and/or smears is displayed in the Peak Table (**Figure 20** and **Table 12**).

This additional information is useful when examining genomic DNA.

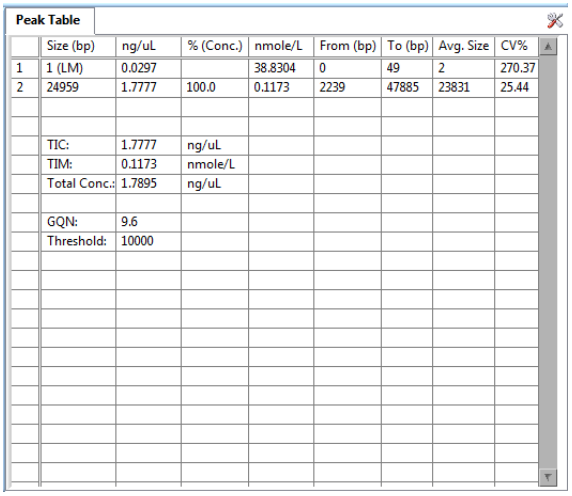


Figure 20 Peak Table form on main screen when in gDNA mode

Table 12 Additional Peak Table functions (only in gDNA mode)

Item	Description
GQN	Reports the sample's calculated *Genomic Quality Number (GQN)*. This value is based upon the concentration of sample above and below the User defined Size Threshold (bp) and is based on a scale of 1 (poorest) to 10 (highest). GQN is reflected as percent/10 above the user-defined threshold (defined in **Global Configuration** dialog > **Advanced Settings** tab).
Threshold	The user defined size (bp) used in the calculation of **GQN**.

Peak Table – Total RNA Mode

In the RNA (Eukaryotic), RNA (Prokaryotic), or RNA (Plant) analysis modes, additional information is displayed to the right of the Peak Table (**Figure 21** and **Table 13**).

This additional information is useful for examining RNA sample characteristics and for calculating a sample's RQN (RNA Quality Number).

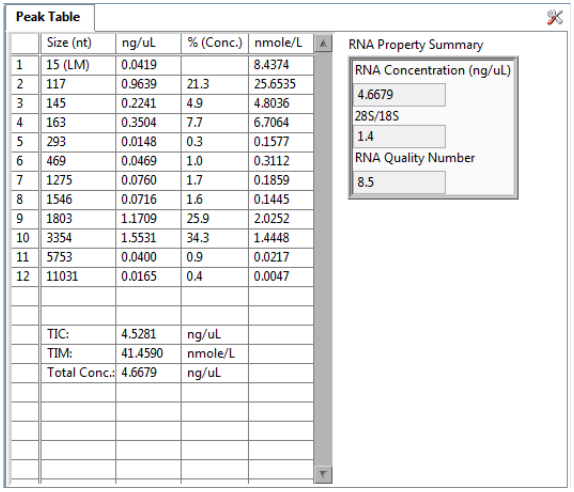


Figure 21 Peak Table form on main screen when in RNA modes

Table 13 Additional Peak Table functions (only available in RNA mode)

Item	Description
RNA Concentration (ng/µL)	Reports the calculated total concentration in ng/µL of all peaks for the sample (not including the markers), regardless of whether the peaks are integrated or not. Note: The RNA concentration takes into account all of the total peak area above the baseline after the lower marker when using the lower marker only for normalization. The RNA concentration does not include peak area below the lower marker.

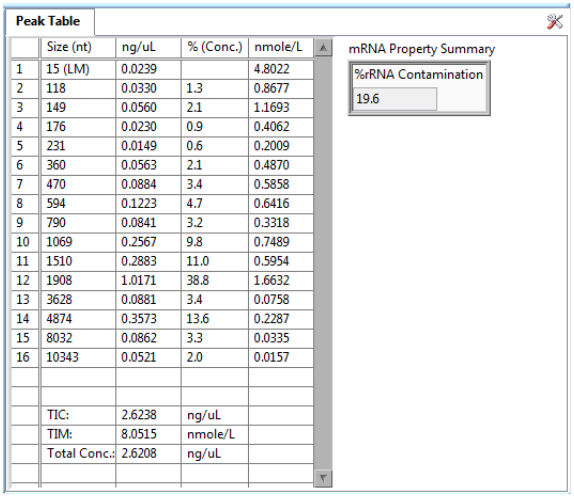
Table 13 Additional Peak Table functions (only available in RNA mode)

Item	Description
28S/18S (Eukaryotic), or 23S/16S (Prokaryotic), or 25S/18S (Plant)	Reports the calculated concentration ratio of the selected ribosomal RNA peaks. This ratio is an indication of the quality of the RNA sample and is used in calculating the sample's *RNA Quality Number (RQN)*.
RNA Quality Number	Reports the sample's calculated *RNA Quality Number (RQN)*. This value is based upon several factors including sample concentration, ribosomal RNA peak ratios and sample purity, and is based on a scale of 1 (poorest) to 10 (highest). Note: If the ribosomal peaks are not detected or fall below a threshold concentration level, **NaN** will be displayed and no RQN value will be reported.

Peak Table – mRNA Mode

In the mRNA analysis mode, additional information is displayed to the right of the Peak Table (**Figure 22** and **Table 14**).

This additional information is useful for examining RNA sample characteristics and for calculating a sample's % rRNA contamination.



	Size (nt)	ng/uL	% (Conc.)	nmole/L
1	15 (LM)	0.0239		4.8022
2	118	0.0330	1.3	0.8677
3	149	0.0560	2.1	1.1693
4	176	0.0230	0.9	0.4062
5	231	0.0149	0.6	0.2009
6	360	0.0563	2.1	0.4870
7	470	0.0884	3.4	0.5858
8	594	0.1223	4.7	0.6416
9	790	0.0841	3.2	0.3318
10	1069	0.2567	9.8	0.7489
11	1510	0.2883	11.0	0.5954
12	1908	1.0171	38.8	1.6632
13	3628	0.0881	3.4	0.0758
14	4874	0.3573	13.6	0.2287
15	8032	0.0862	3.3	0.0335
16	10343	0.0521	2.0	0.0157
	TIC:	2.6238	ng/uL	
	TIM:	8.0515	nmole/L	
	Total Conc.:	2.6208	ng/uL	

Figure 22 Peak Table form on main screen when in mRNA mode

Table 14 Additional Peak Table functions (only available in mRNA mode)

Item	Description
% rRNA Contamination	Reports the calculated ribosomal RNA contamination in the sample (not including the markers), regardless of whether the peaks are integrated or not.

Peak Table – Small RNA Mode

In the Small RNA analysis mode, additional information is displayed to the right of the Peak Table (**Figure 23** and **Table 15**).

This additional information is useful for examining Small RNA sample characteristics and for calculating a sample's % micro RNA.

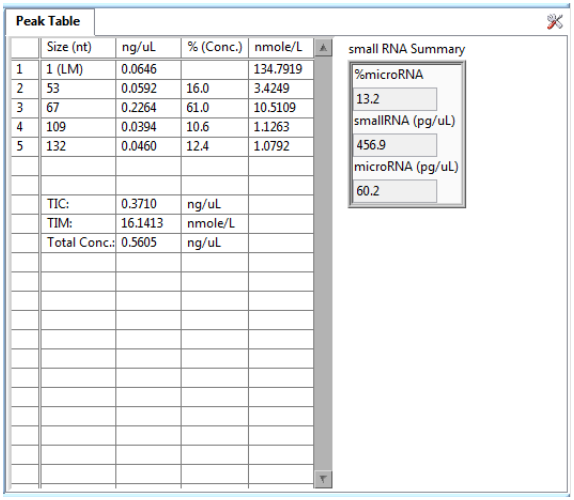


Figure 23 Peak Table on main screen when in small RNA mode

Table 15 Additional Peak Table functions (only available in small RNA mode)

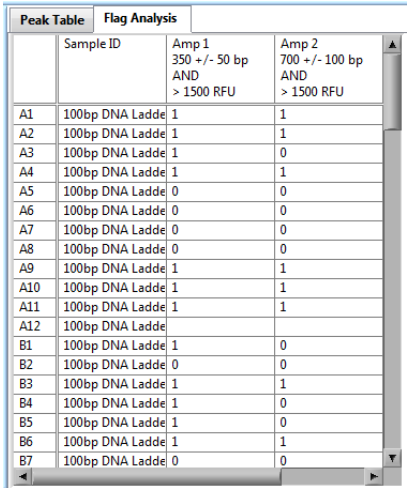
Item	Description
%microRNA	Reports the sample's calculated **%microRNA** within the small RNA defined region. This value is based upon several factors including sample concentration, Maximum miRNA (nt), and Maximum Small RNA (nt).
smallRNA (pg/µL)	Reports the concentration from the Lower Marker to the Maximum Small RNA (nt) setpoint.
microRNA (pg/µL)	Reports the concentration from the Lower Marker to the Maximum miRNA (nt) setpoint.

Flag Analysis Table

If the configuration contains any **Flag** settings, a **Flag Analysis** tab will also be visible as a separate tab with the **Peak Table** (Figure 24 and Table 16). The flagged results are displayed in rows, with each row corresponding to a sample well of the plate. The columns are titled by the flag tag which is the field used to name the particular flag condition. The results are output in a binary format with 0 = FALSE and 1 = TRUE.

NOTE

The information in the **Flag Analysis** tab can be exported to the clipboard for pasting into common spreadsheet programs by right clicking the mouse and left clicking **Export Data to Clipboard**. Further information on the flag functions can be found in Chapter 4, “ProSize Configuration”.



	Sample ID	Amp 1 350 +/- 50 bp AND > 1500 RFU	Amp 2 700 +/- 100 bp AND > 1500 RFU
A1	100bp DNA Ladde	1	1
A2	100bp DNA Ladde	1	1
A3	100bp DNA Ladde	1	0
A4	100bp DNA Ladde	1	1
A5	100bp DNA Ladde	0	0
A6	100bp DNA Ladde	0	0
A7	100bp DNA Ladde	0	0
A8	100bp DNA Ladde	0	0
A9	100bp DNA Ladde	1	1
A10	100bp DNA Ladde	1	1
A11	100bp DNA Ladde	1	1
A12	100bp DNA Ladde		
B1	100bp DNA Ladde	1	0
B2	100bp DNA Ladde	0	0
B3	100bp DNA Ladde	1	1
B4	100bp DNA Ladde	1	0
B5	100bp DNA Ladde	1	0
B6	100bp DNA Ladde	1	1
B7	100bp DNA Ladde	0	0

Figure 24 Flag Analysis tab on main screen

Table 16 Flag Analysis tab

Item	Description
Well/Sample Name	The first column labels the sample well; the second **Sample ID** column lists the entered sample name.
Columns	Each column is titled by the respective tag entry and flag conditions for each entry.

Table 16 Flag Analysis tab

Item	Description
Column/Row Data	Data is reported in binary format, following the specified criteria for each flag condition: 0 = FALSE (conditions not met) 1 = TRUE (conditions met)
Export Data to Clipboard	From the context menu of the table, select **Export Data to Clipboard**. The peak table information is exported to the clipboard for pasting directly into common spreadsheet programs such as Microsoft Excel. The full table is exported.

Smear Analysis Table

If the configuration of the **Smear Analysis** tab contains any filter settings, a **Smear Analysis** tab will also be visible as a separate tab with the **Peak Table** (Figure 25 and Table 17).

The calculated results table shows for each sample row the following columns (for details, see Table 17):

- the sample ID and well position
- the customized smear size **Range**
- the calculated concentration in **ng/µL** for the defined size range
- the **% Total**, which is the percent of total sample concentration in the smear range
- the **nmole/L** concentration of the defined smear range
- the **Avg. Size**
- **%CV** of the defined smear range

Smear Analysis ranges are color coded and a vertical dotted line will show on the electropherogram view showing the range and reporting the corresponding average size for the smears (see Figure 26).

If a sample well has not been selected for the **Smear Analysis**, its row will be left blank. If multiple size ranges are selected for a sample, multiple rows will be displayed. In Figure 25, all wells have two smear ranges selected: 190 bp to 500 bp, and 500 bp to 900 bp.

NOTE

The information in the **Smear Analysis** tab can be exported to the clipboard for pasting into common spreadsheet programs. Select **Export Data to Clipboard** from the context menu. Further information on the flag functions are discussed in Chapter 3, “ProSize Main Screen”.

Peak Table | Smear Analysis

ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
H1: Sample A1	100 bp to 2500 bp	4.5760	99.6	17.2809	436	35.11
	500 bp to 2500 bp	1.0569	23.0	2.7109	642	28.07
H2: NGS Lib Mix 1	100 bp to 2500 bp	6.0333	99.0	20.0585	495	46.56
	100 bp to 600 bp	4.9439	81.1	19.2688	422	20.95
H3: Adapter NGS Lil	100 bp to 2500 bp	3.1396	99.2	12.4723	414	34.61
	100 bp to 600 bp	2.8641	90.5	12.2740	384	23.55
H4: NGS Lib Master	100 bp to 2500 bp	3.8746	99.2	14.1503	451	43.91
	100 bp to 600 bp	3.3697	86.3	13.9386	398	22.77
H5: x	100 bp to 2500 bp	0.0018	29.6	0.0167	180	103.34
	100 bp to 600 bp	0.0018	29.4	0.0181	165	27.19
H6: x	100 bp to 2500 bp	0.0024	50.1	0.0068	584	140.33
	100 bp to 600 bp	0.0019	40.0	0.0188	168	25.44
H7: x	100 bp to 2500 bp	0.0017	20.2	0.0033	854	105.85
	100 bp to 600 bp	0.0011	13.5	0.0082	226	52.99
H8: x	100 bp to 2500 bp	0.0022	61.9	0.0244	147	23.56
	100 bp to 600 bp	0.0022	61.9	0.0244	147	23.56

Figure 25 Smear Analysis tab on main screen

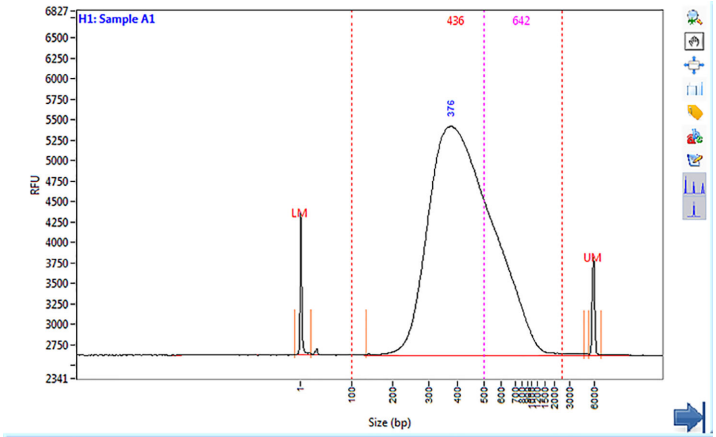


Figure 26 Electropherogram view when Smear Analysis tab selected on main screen

Table 17 Smear Analysis tab

Item	Description
ID	Lists the Sample Well: Sample Name.
Range	Lists the smear range, in size (bp). The smear range corresponds to the **Start Size** and **End Size** entered in the **Smear Analysis** tab (**Global Configuration** dialog). Note: If the **Apply** function has not been made to the respective well, it will be blank in the table.
ng/uL	Reports the concentration of the defined smear range in ng/µL.
% Total	Reports the % of the total concentration of the sample that resides in the defined smear range.
nmole/L	Reports in alternate concentration units of nmole/L for the defined smear range.
Average Size	Reports the mean size (in bp) of the defined smear range, accounting for the distribution of the concentration of the smear. Therefore, the Avg. Size will reflect the size corresponding to 50% of the total smear concentration being below and above this value.
% CV	Reports the distribution of concentration from the mean for the defined smear, in terms of the coefficient of variation (CV), where CV is the ratio of the standard deviation (σ) to the mean (µ): $CV(\%) = \sigma / \mu$ The value is reported as a percentage; smaller values indicate a tighter size distribution of the smear concentration.
Export Data to Clipboard	From the context menu of the table, select **Export Data to Clipboard**. The peak table information is exported to the clipboard for pasting directly into common spreadsheet programs such as Microsoft Excel. The full table is exported.

Advanced Flag Analysis Table

The **Advanced Flag** tab of the **Global Configuration** dialog offers flag settings. An **Advanced Flag Analysis** tab will be displayed as a separate tab with the **Peak Table** (Figure 27). The flagged results are displayed, with each page representing a single well.

The **Advanced Flag Analysis** tab reports the following results columns:

- size concentration
- % Concentration
- nmole/L
- RFU

To see results for all wells simultaneously, export the data to a .csv file using the **Export** button in the main menu.

Further information on the flag functions can be found in **Chapter 4**, “ProSize Configuration”.

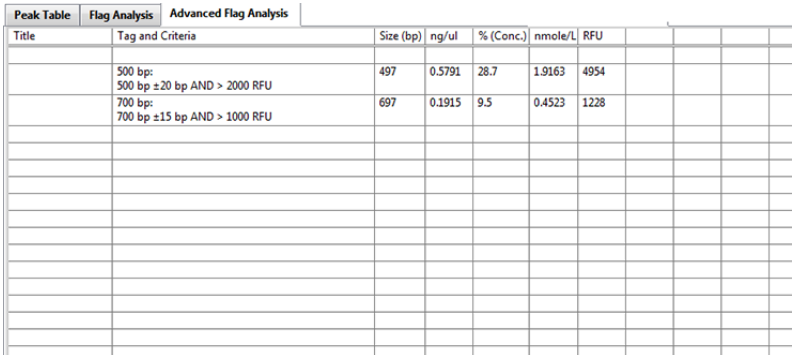


Figure 27 Advanced Flag Analysis tab on main screen

Set Individual Parameters

When a data file is initially loaded into the ProSize software, the settings for selection of lower/upper markers, sample peak integration, size calibration, quantification, and any flag/smear analysis are globally set for all samples in the **Global Configuration** dialog (for more information, see **Chapter 4**, "ProSize Configuration").

After the data is loaded, any of the common configuration parameters can be individually edited for each sample independently (local setting), or changes can be made and applied to all samples (global setting), or to a customized subset of samples.

To view and adjust the configuration settings for the currently viewed lane/sample, select **Set Individual Parameters** on the right side of the **Peak Table**. This will reveal the configuration dialog, for which most settings can be individually adjusted for each sample as desired (**Figure 28**). To toggle back to the **Peak Table**, select **Show Results and Save Settings**.

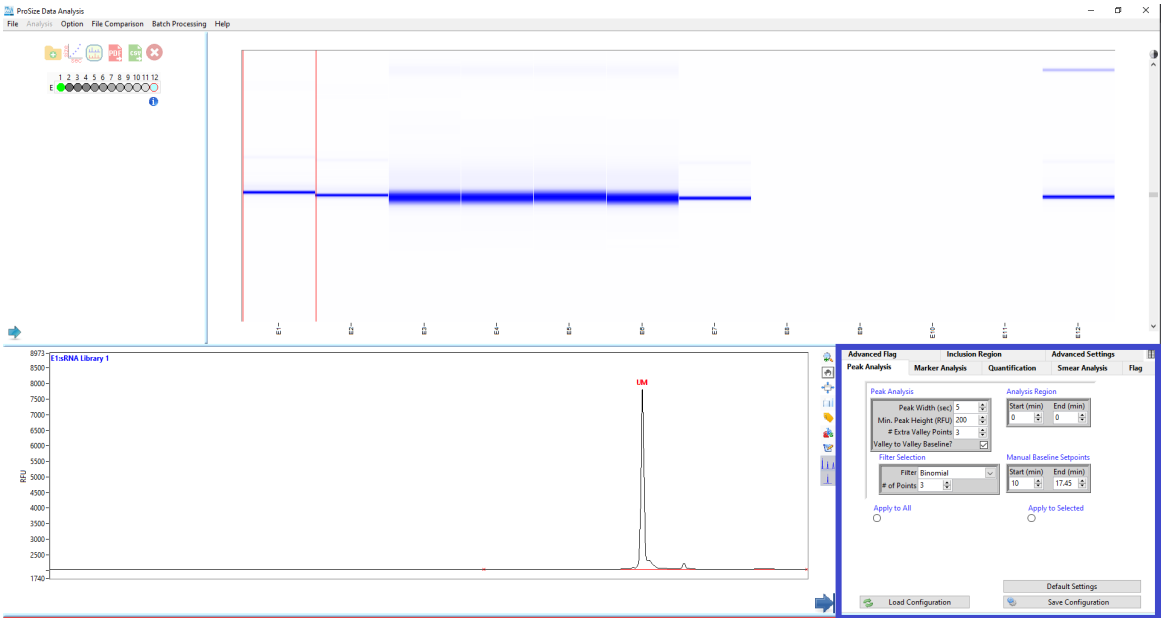


Figure 28 Main screen with data file open and configuration dialog open (clicked)

The individual settings within each tab are described in detail in **Chapter 4**, "ProSize Configuration". The following sections describe the settings that apply to individual configuration tabs.

Configuration – Peak Analysis

The **Peak Analysis** tab contains settings to define the sample peak integration, data analysis filter, analysis region, and manual baseline set points (**Figure 29**). These functions are described in detail in section **"Global Configuration - Peak Analysis"** on page 82.

New settings in the **Peak Analysis** tab are applied to the current selected sample well only, or to all samples of the sample plate (see **Table 18**).

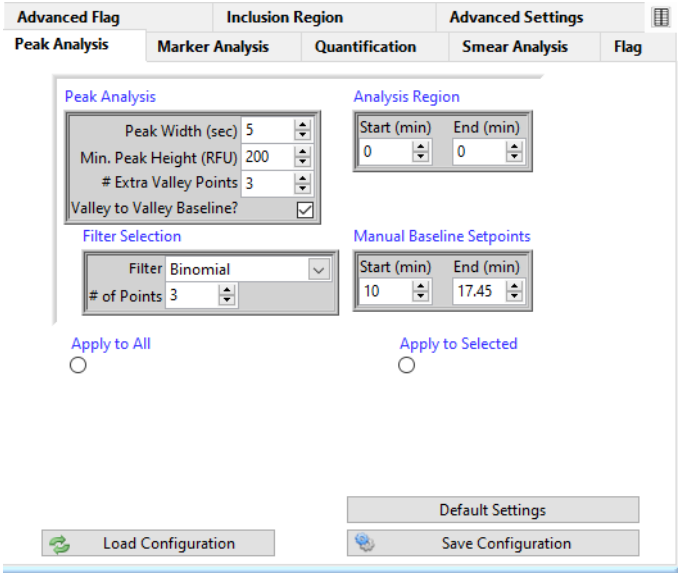
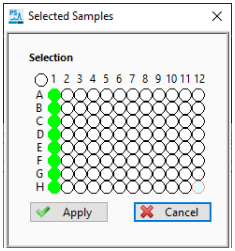


Figure 29 Peak Analysis tab in Set Individual Parameters dialog

Table 18 Additional Peak Analysis settings

Setting	Description
Apply to All	All current settings of the **Peak Analysis** tab will be applied to all samples of the loaded data file. This is therefore a global function. Note: Any previous individually tuned Peak Analysis tab settings for different samples of the data file will be overwritten when using this function.
Apply to Selected	All current settings of the **Peak Analysis** tab will be applied to the specified wells of the sample plate. Upon selecting this function, the **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the current settings to.  Click the desired wells, rows or columns, and click **OK** to apply; click **Cancel** to abort the operation. Note: Any previous individual setting of the Peak Analysis tab for the selected samples of the data file will be overwritten when using this function.

Configuration – Marker Analysis

The **Marker Analysis** tab contains settings to define the marker peak selection and marker peak integration (**Figure 30**). These settings are described in detail in **"Global Configuration - Marker Analysis"** on page 86.

New settings in the **Marker Analysis** tab can be applied to the current selected sample well only, or to all samples of the sample plate (see **Table 19**).

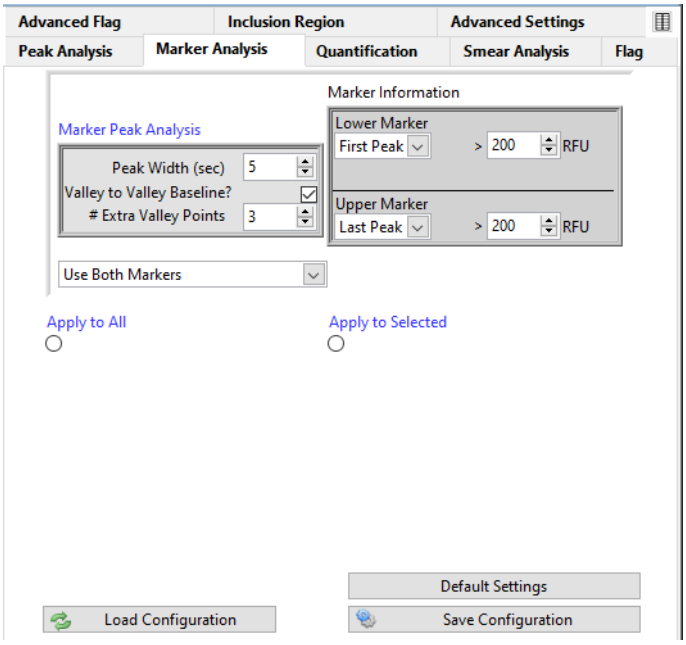
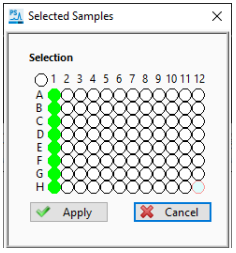


Figure 30 Marker Analysis tab Set Individual Parameters dialog

Table 19 Additional Marker Analysis settings

Setting	Description
Apply to All	All current settings of the **Marker Analysis** tab will be applied to all samples of the loaded data file. This is therefore a global function. Note: Any previous individually tuned Marker Analysis tab settings for different samples of the data file will be overwritten when using this function.
Apply to Selected	All current settings of the **Marker Analysis** tab will be applied to the specified wells of the sample plate. Upon selecting this function, the **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the current settings to.  Select the desired wells, rows or columns, and click **OK** to apply; click **Cancel** to abort the operation. Note: Any previous individual setting of the Marker Analysis tab for the selected samples of the data file will be overwritten when using this function.

Configuration – Quantification

The **Quantification** tab contains settings to assign a marker or ladder for quantification; its working concentration and any dilution factor applied to the sample prior to injection (**Figure 31**). These functions are described in detail in **"Global Configuration - Quantification"** on page 94.

New settings in the **Quantification** tab are applied to the current selected sample well only, or to all samples of the sample plate (see **Table 20**).

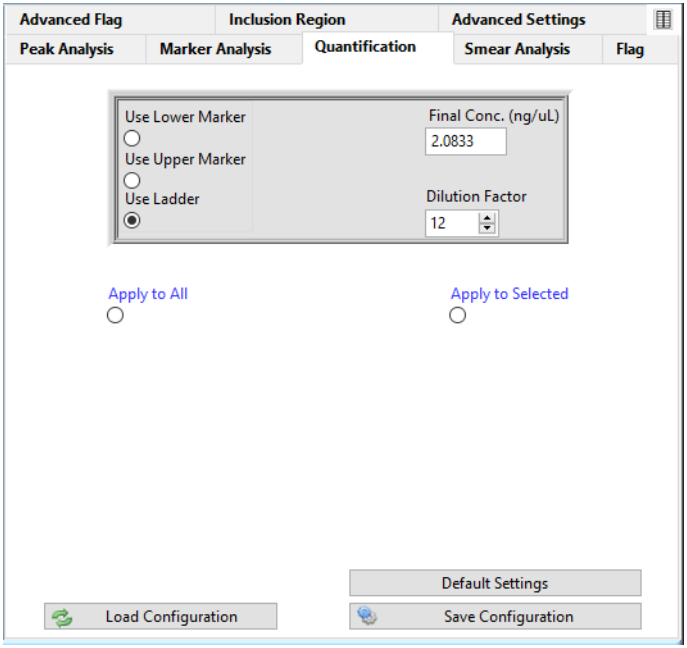
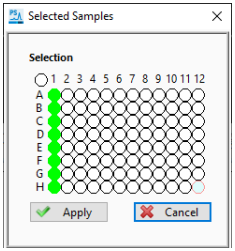


Figure 31 Quantification tab in Set Individual Parameters dialog

Table 20 Additional Quantification settings

Setting	Description
Apply to All	All current settings of the **Quantification** tab will be applied to all samples of the loaded data file. This is therefore a global function. Note: Any previous individually tuned Quantification tab settings for different samples of the data file will be overwritten when using this function.
Apply to Selected	All current settings of the **Quantification** tab will be applied to the specified wells of the sample plate. Upon selecting this function, the **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the current settings to.  Select the desired wells, rows or columns, and click **OK** to apply; click **Cancel** to abort the operation. Note: Any previous individual setting of the Quantification tab for the selected samples of the data file will be overwritten when using this function.

Configuration – Smear Analysis

The **Smear Analysis** tab contains settings to enable the user to specify a size range(s) for which to integrate and calculate the total concentration in ng/mL (**Figure 32**). These functions are described in detail in **"Global Configuration - Smear Analysis"** on page 96

New settings in the **Smear Analysis** tab are applied to the current selected sample well only, or to all samples of the sample plate (see **Table 21**).

NOTE

If **Display Smear Range** is enabled, two color coded vertical dotted cursors are displayed in the Electropherogram Trace screen. These cursors define the entered values for the **Start Size (bp)** and the **End Size (bp)** for the currently selected smear range. The colors can be changed by left clicking on the color next to the smear range. The start and end points of the smear range can be adjusted by clicking on a cursor peak, and dragging the respective cursor left or right; the smear analysis region will be automatically updated to the new cursor location.

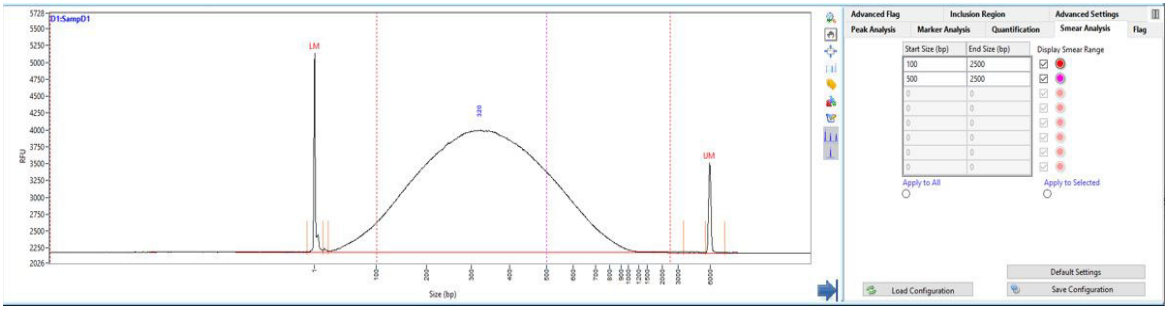
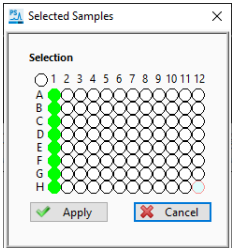


Figure 32 Smear Analysis tab in Set Individual Parameters dialog with Display Smear Range enabled

Table 21 Additional Smear Analysis settings

Setting	Description
Apply to All	All current settings of the **Smear Analysis** tab will be applied to all samples of the loaded data file. This is therefore a global function. Note: Any previous individually tuned Smear Analysis tab settings for different samples of the data file will be overwritten when using this function.
Apply to Selected	All current settings of the **Smear Analysis** tab will be applied to the specified wells of the sample plate. Upon selecting this function, the **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the current settings to.  Select the desired wells, rows or columns, and click **OK** to apply; click **Cancel** to abort the operation. Note: Any previous individual settings of the Smear Analysis tab for the selected samples of the data file will be overwritten when using this function.

Configuration – Flag Analysis

The **Flag Analysis** tab contains settings to enable the user to specify specific criteria (size or concentration range) to be met within the data, and generate a binary output (0 = false, 1 = true) of the results (**Figure 33**). These settings are described in detail in **"Global Configuration - Flag"** on page 98.

New settings in the **Flag Analysis** tab are applied to all samples of the sample plate. Some of these settings are described in **Table 22**.

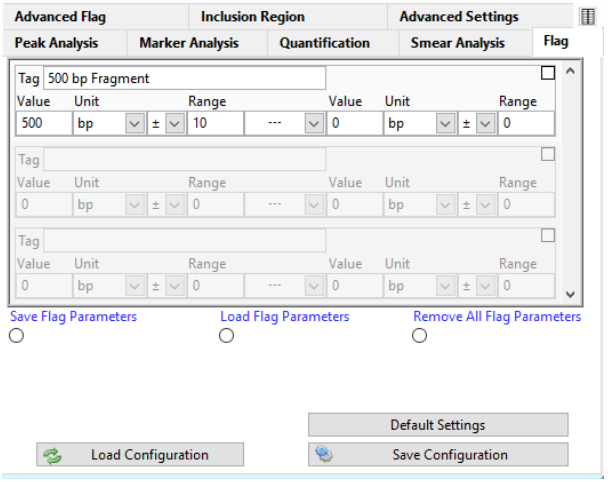


Figure 33 Flag Analysis tab in Set Individual Parameters dialog

Table 22 **Additional Flag Analysis settings**

Setting	Description
Save Flag Parameters	The current Flag Analysis settings will be saved as a file (*.Flag extension) that can be loaded into different data files. A file browser menu will open to navigate and save the desired filename and location; click **OK** to save the file, or **Cancel** to discard your changes.
Load Flag Parameters	The previously saved Flag Analysis settings will be loaded from a file (*.Flag extension). A file browser menu will open to navigate and load the desired filename and location; click **OK** to load the file, or **Cancel** to discard your changes.
Remove All Flag Parameters	All settings will be cleared from the tab.

Configuration – Advanced Settings

The **Advanced Settings** tab contains settings to assign the data analysis mode for processing data (**Figure 34**). All settings of this tab are described in detail in **"Global Configuration – Advanced Flag"** on page 100.

NOTE

The **Advanced Settings** parameters are applied to all samples in the data file. Individual samples cannot use different analysis modes. It is assumed all data is processed with a single analysis mode in a single experiment or data file.

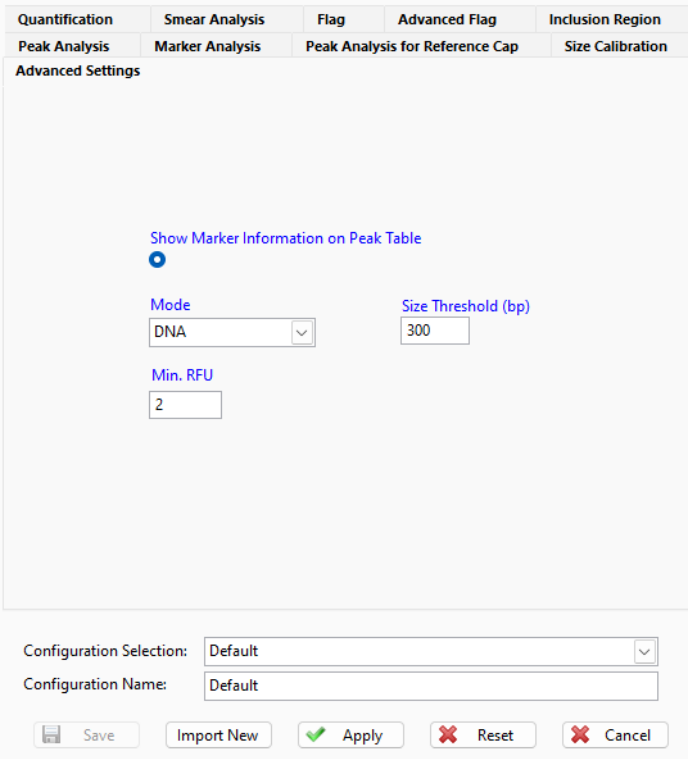


Figure 34 Advanced Settings tab in Set Individual Parameters dialog

Configuration – Advanced Flag Analysis

The **Advanced Flag Analysis** tab contains settings to specify specific criteria (size, concentration range, various logic criteria) to be met within the data, and generate a table with results (**Figure 35**). These settings are described in detail in **"Global Configuration – Advanced Flag"** on page 100.

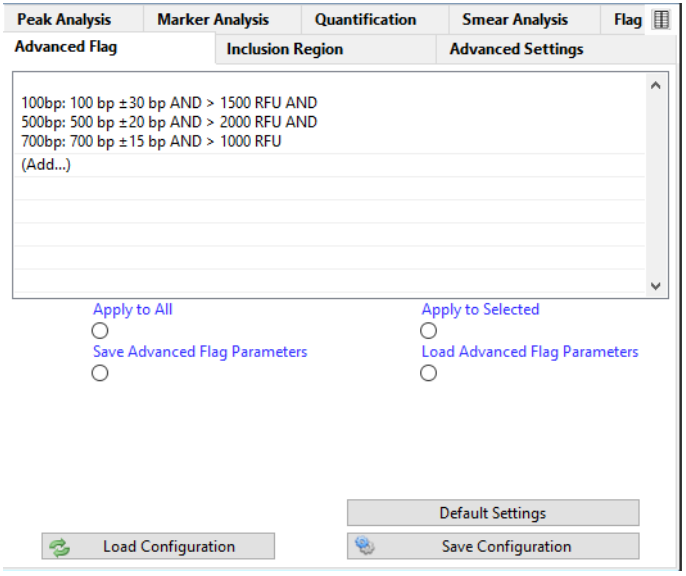


Figure 35 Advanced Flag tab in Set Individual Parameters dialog

4 ProSize Configuration

This chapter provides an overview of the **Global Configuration** dialog of the ProSize software. It also provides information on setting individual configuration parameters in opened data files.

About Configuration

The **Global Configuration** dialog (or **Set Individual Parameters** dialog for opened data files) is used to load, edit, or save the data analysis mode and settings for processing the data file, such as size calibration, marker/sample peak integration, quantification, smear analysis, and/or flag analysis criteria.

By using established method configurations for the different types of sample analysis on the instrument, the user can streamline data analysis with the ProSize software. This requires that the first 15 characters of the ProSize configuration file name matches the first 15 characters of the parallel capillary electrophoresis system.

NOTE

In most cases, predefined, optimized global configuration files are available to load when processing data collected from different Agilent Reagent Kits. The configuration files are labeled according to the particular kit number, e.g., **DNF-910-33 – 35-1500bp.ini**. These configuration files are available from Agilent upon request if needed. The configuration files preloaded upon software installation are located in the C:\ProSize data analysis software\Configurations folder.

Access Configuration Information in the ProSize Software

There are two ways to access configuration information in the ProSize software:

Global Configuration Dialog

This option is used to define how an unprocessed data file is first opened in the ProSize software. The user is automatically prompted with the **Global Configuration** dialog when opening an unprocessed data file if the corresponding separation method used does not match the naming configuration (**Figure 37**).

Alternatively, once a data file is opened, the user can modify at any time the configuration by selecting **Option**, and clicking **Edit Configurations**  .

NOTE

In most cases, predefined, optimized global configuration files are available to load when processing data collected from different Agilent Reagent Kits. The configuration files are labeled according to the particular kit number, for example, **DNF-910-33 – 35-1500bp.ini**. These configuration files are available from Agilent upon request if needed. The configuration files preloaded upon software installation are located in the C:\ProSize data analysis software\Configurations folder.

When making changes to a configuration, a new method name must be entered in the Configuration Name to enable the **Save** feature.

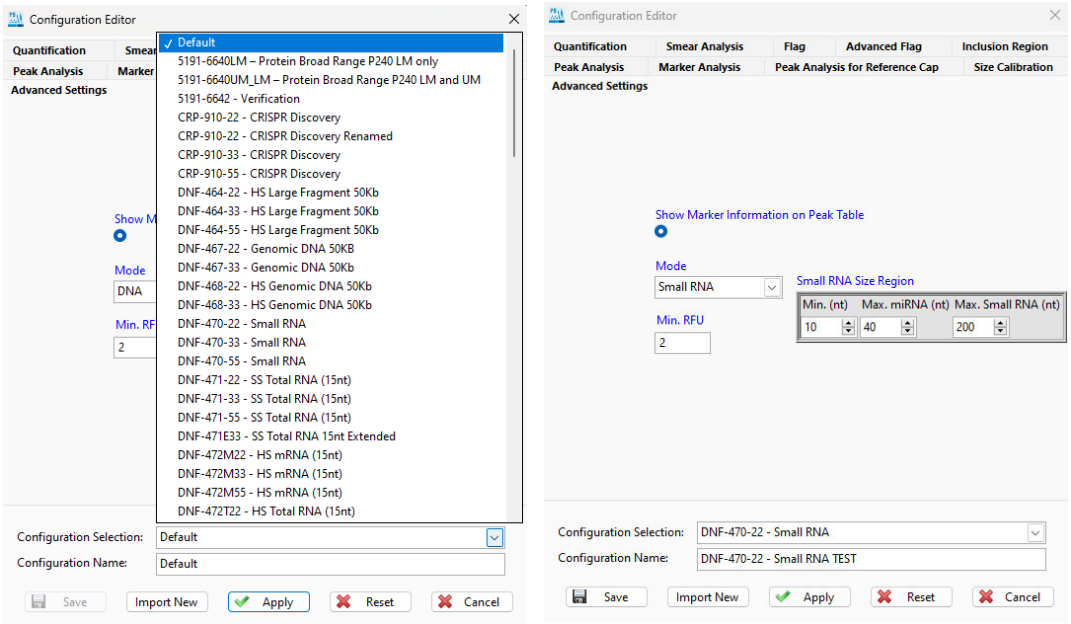


Figure 36 Global Configuration dialog - Configuration selection

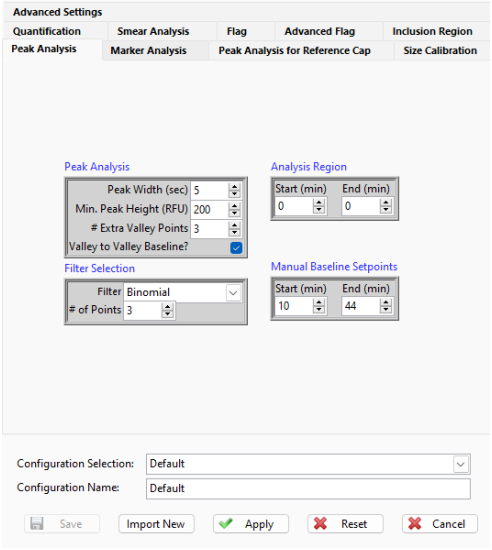


Figure 37 Global Configuration dialog

Set Individual Parameters Dialog

This dialog is used to modify or change configuration settings after a data file is opened. The **Set Individual Parameters** dialog can be accessed in an open data file by selecting on the right side of the main menu (**Figure 38**). Note that this icon toggles between the **Set Individual Parameters** and **Show Results and Save Settings** ( ) form, which displays the **Peak Table** and **Smear Analysis** or **Flag Analysis** tables.

NOTE

Using **Set Individual Parameters** dialog, the currently selected tab is applied independently from the other configuration tabs. In addition, if the user select [**Enter**] after changing a setting, the change is only applied to the currently selected sample; therefore this is a "local" setting. To apply the change made in the tab to all samples, click **Apply to All**; to apply the change only to selected samples, click **Apply to Selected**.

The settings within each tab of the **Global Configuration** and **Set Individual Parameters** dialog perform the same functions. Each tab of the configuration is discussed in further detail in the following sections of this chapter.

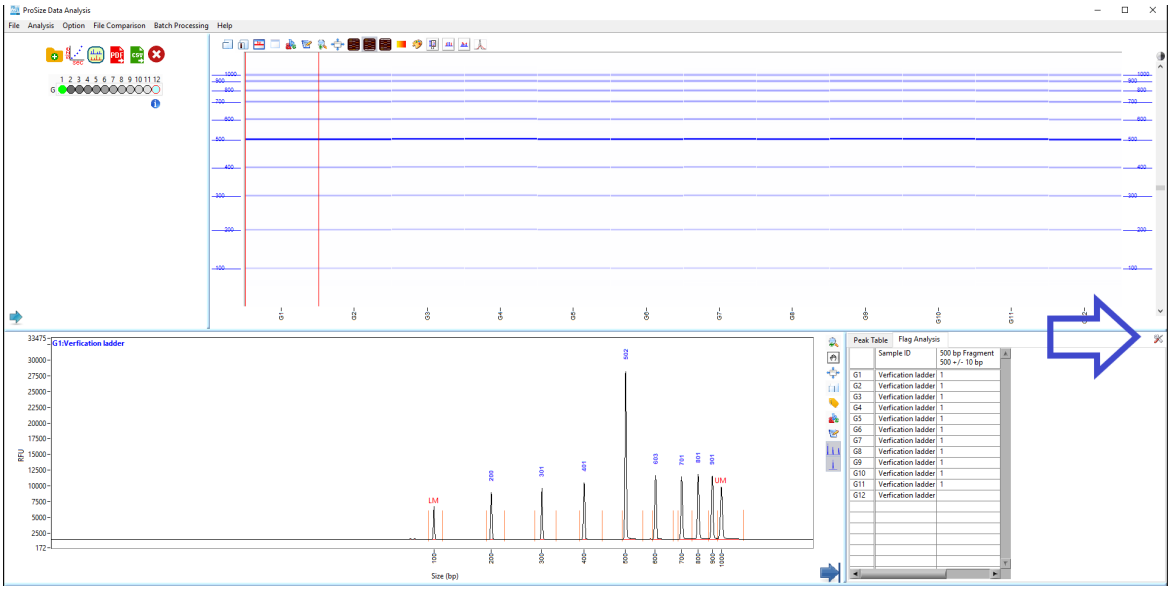


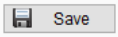
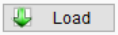
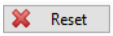
Figure 38 Set Individual Parameters icon for configuration settings after a data file is opened

Overview Global Configuration Dialog

There are ten tabs in the **Global Configuration** dialog to define the appropriate data analysis mode and settings for processing the data, as described in the following sections.

At the bottom of the **Global Configuration** dialog, the user is able to **Save**, **Load**, **Apply,** or **Reset** the configuration settings (see **Table 23**).

Table 23 Global Configuration dialog buttons

Button	Description
	Saves the global configuration as a user defined file (*.ini format). The settings from all tabs are saved, regardless of which tab is open when saving the file.
	Loads a predefined configuration file (*.ini format). Note than upon installation, predefined configuration files are installed for most Agilent Reagent Kits in the in the C:\ProSize data analysis software\Configurations folder.
	Applies the current global configuration settings to the data file. If the data file is unprocessed, pressing the **Apply** button will open the data file with the currently loaded settings. If the data file is already open, the new settings will be applied to the data, overwriting any previously defined individual configuration settings.
	Cancels all changes and closes the global Configuration window.
	Resets all parameters to the default values.

Global Configuration - Advanced Settings

The settings of the **Advanced Settings** tab allow the user to assign the data analysis mode for processing data (**Figure 39**). The settings are described in detail in **Table 24**.

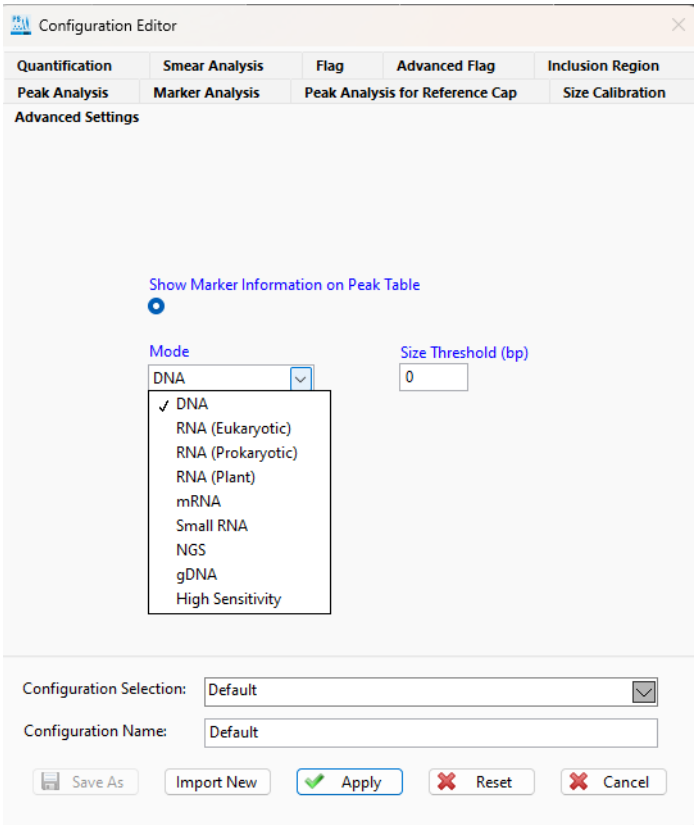


Figure 39 Advanced Settings tab in Global Configuration dialog

Table 24 Advanced Settings configuration tab

Settings	Description
Mode	
DNA	For analyzing standard dsDNA fragment (for example, PCR product) samples where discrete, Gaussian peaks are present. This mode displays size in basepairs (bp) using a DNA Standard Ladder for calibration. Quantification is achieved via comparison to either an upper or lower marker, or to the DNA Standard Ladder present at a known concentration. Note: The DNA Mode is not recommended for samples containing non-Gaussian peaks, smears or uneven baselines.
RNA (Eukaryotic)	For analyzing eukaryotic total RNA samples. Calculates size in nucleotides (nt) using a RNA Standard Ladder for calibration. Quantification is achieved via comparison of the lower marker to the RNA Ladder, present at a known concentration. The ratio of the 18S and 28S ribosomal RNA peaks is calculated, as well as the RNA Quality Number (RQN).
RNA (Prokaryotic)	For analyzing prokaryotic total RNA samples. Calculates size in nucleotides (nt) using a RNA Standard Ladder for calibration. Quantification is achieved via comparison of the lower marker to the RNA Ladder, present at a known concentration. The ratio of the 16S and 23S ribosomal RNA peaks is calculated, as well as the RNA Quality Number (RQN).
RNA (Plant)	For analyzing plant total RNA samples. Calculates size in nucleotides (nt) using a RNA Standard Ladder for calibration. Quantification is achieved via comparison of the lower marker to the RNA Ladder, present at a known concentration. The ratio of the 18S and 25S ribosomal RNA peaks is calculated, as well as the RNA Quality Number (RQN).
mRNA	For analyzing messenger RNA (mRNA) samples. Calculates size in nucleotides (nt) using a RNA Standard Ladder for calibration. Quantification is achieved via comparison of the lower marker to the RNA Ladder, present at a known concentration.
Small RNA	For analyzing mico RNA (miRNA) samples. Calculates %microRNA, small RNA (pg/µL), microRNA (pg/µL), and size in nucleotides (nt) using a RNA Standard Ladder for calibration. Quantification is achieved via comparison of the lower marker to the RNA Ladder, present at a known concentration.
NGS	For analyzing dsDNA samples containing peaks, smears, non-Gaussian peaks and/or uneven baselines. This mode is commonly used for Next Generation Sequencing (NGS) fragment library analysis. This mode displays size in basepairs (bp) using a DNA Standard Ladder for calibration. Quantification is achieved via comparison to either an upper or lower marker, or to the DNA Standard Ladder present at a known concentration. The **Peak Table** in this mode displays additional fields for size range, average size, and % CV.

Table 24 Advanced Settings configuration tab

Settings	Description
gDNA	For analyzing dsDNA samples containing peaks, smears, non-Gaussian peaks and/or uneven baselines. his mode is commonly used for Genomic DNA (gDNA) analysis. This mode displays size in basepairs (bp) using a DNA Standard Ladder for calibration. Quantification is achieved via comparison to a lower marker, or to the DNA Standard Ladder present at a known concentration. The **Peak Table** in this mode displays additional fields for size range, average size, % CV, and GQN.
High Sensitivity	Changes the display (export, .pdf, peak table) to pg/µL. For NGS analysis only (and only for very low concentrations).
Min. RFU	Allows you to designate the minimum RFU required for integration.
Show Marker Information on Peak Table	When enabled, the sample **Peak Table** will display size and concentration information for the lower/upper marker peaks along with all currently integrated sample peaks.

Global Configuration - Peak Analysis

The settings of the **Peak Analysis** tab allow the user to assign width and height thresholds for selecting and integrating sample peaks; setting data filtering; and selecting the time region to be analyzed (**Figure 40**). The settings are described in detail in **Table 25**.

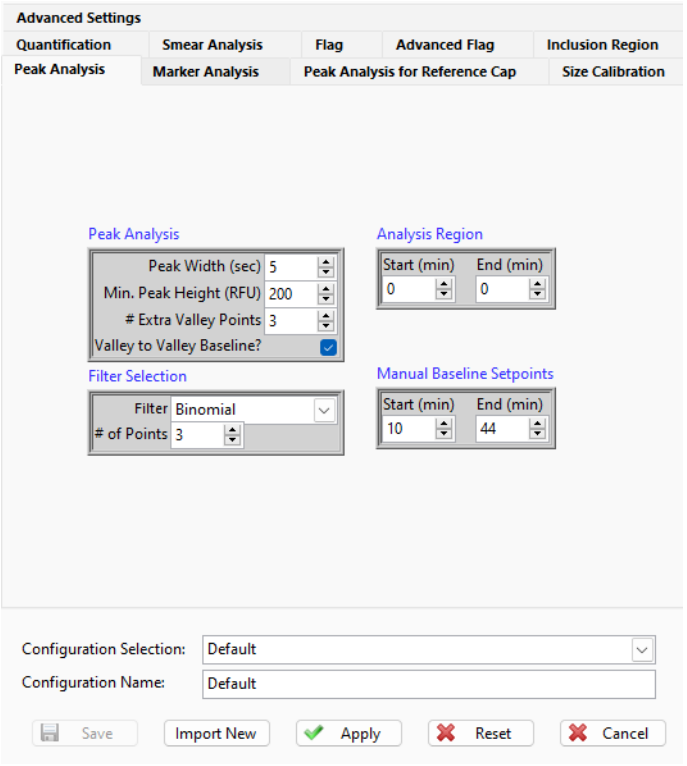


Figure 40 Peak Analysis tab in Global Configuration dialog

Table 25 Peak Analysis configuration settings

Settings	Description
Peak Analysis	
Peak Width (sec)	Defines the width threshold for peak detection in seconds. Higher settings better define wide peak start/end points; smaller settings better define sharp peak start/end points. Typical values range from 3-5 for sharp peaks or 25-50 for smears. The maximum value for this setting is 50.
Min. Peak Height (RFU)	Defines the minimum peak height threshold to select a peak for integration in RFUs. Peaks below the set value will not be selected for integration. Typical values for this setting are 20-100 RFU, depending upon the sample signal and application. Note the typical baseline peak-to-peak noise is less than 5 RFU.
# Extra Valley Points	This setting influences the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. Higher values draw a straighter baseline between the first peak start point and second peak end point; lower values draw the baseline more to the "valley" between the two unresolved peaks. Note: The **Valley to Valley Baseline?** setting needs to be enabled for this setting to be active.

Table 25 Peak Analysis configuration settings

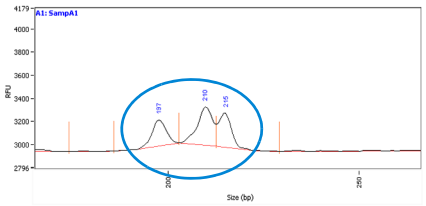
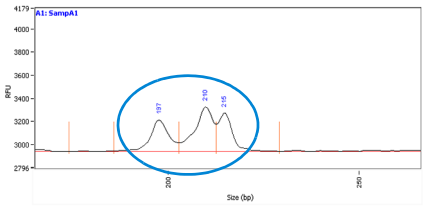
Settings	Description
Valley to Valley Baseline?	This setting influences the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. When disabled, will draw a straighter baseline between the first peak start point and second peak end point. When enabled, will draw the baseline more to the "valley" between the two unresolved peaks. In the example below, the Top trace shows the baseline drawn when Valley to Valley Baseline is enabled; the Bottom trace shows the baseline for the same sample when this setting is disabled.  Valley to Valley = Enabled  Valley to Valley = Disabled
Filter Selection	
Filter	Defines the algorithm type to use when filtering the raw data. There are five options for data filtering available to the user: • **None**: No filtering is performed on the data. • **Moving Average**: Averages points on either side of a point. • **Savitzky Golay**: A polynomial regression filter where the coefficients are calculated using Savitzky and Golay's algorithm. • **Binomial**:. A polynomial regression filter based on the binomial coefficients for implementing Gaussian filtering. • **Wavelets De-noising**: A discrete wavelet transform filter. Note: The recommend filter for most applications is a 3 to 20 point Binomial filter.

Table 25 Peak Analysis configuration settings

Settings	Description
# of Points	Defines the number of points to use in the selected data filter. Maximum points to use for each filter type: • Moving Average: 9 • Savitsky Golay: 9 • Binomial: 20 • Wavelets De-Noising: 4
Analysis Region	
Start (min)	Defines the start time in minutes for which to integrate and analyze the electropherogram data. This includes both marker and sample peaks. Note: If 0 is entered, will analyze data from start of the electropherogram at 0 min.
End (min)	Defines the end time in minutes for which to integrate and analyze the electropherogram data. This includes both marker and sample peaks. Note: If 0 is entered, will automatically analyze data to the full end time range of the electropherogram.
Manual Baseline Setpoints	Defines the baseline using time points outside the lower/upper marker region. This function is particularly useful for accurately measuring the total concentration of samples containing smears or uneven baselines.
Start (min)	Defines the start time in minutes for which to draw the start of the baseline when manually setting the baseline. The Y axis position of the baseline at the entered start point in minutes will be used to form the new baseline start point. Note: If 0 is entered for both the **Start (min)** and **End (min)**, this function is disabled. When entering values for this function, enter the ending time value in the **Start (min)** field first. When next entering the start time the end time value will automatically populate the **End (min)** field.
End (min)	Defines the end time in minutes for which to draw the end of the baseline when manually setting the baseline. The Y-axis position of the baseline at the entered end point in minutes will be used to form the new baseline end point. Note: If 0 is entered for both the **Start (min)** and **End (min)**, this function is disabled. When entering values for this function, enter the ending time value in the **Start (min)** field first. When next entering the start time, the end time value will automatically populate the **End (min)** field.

Global Configuration - Marker Analysis

The settings of the **Marker Analysis** tab allow the user to select the criteria for choosing the lower and/or upper peak, used to normalize each capillary by migration time and concentration (**Figure 41**). The settings are described in detail in **Table 26**.

In ProSize, the user can independently set different peak parameters for the lower/upper markers from those set for the sample peak parameters in **Peak Analysis** tab. This is useful if, for example, the sample contains broad smearing peaks as compared to markers, which are typically sharp Gaussian peak profiles.

The user can select a lower/upper marker pair (**Use Both Markers**), or use only a lower marker (**Use Lower Marker Only**) or upper marker (**Use Upper Marker Only**) for data normalization by selecting the appropriate drop-down option.

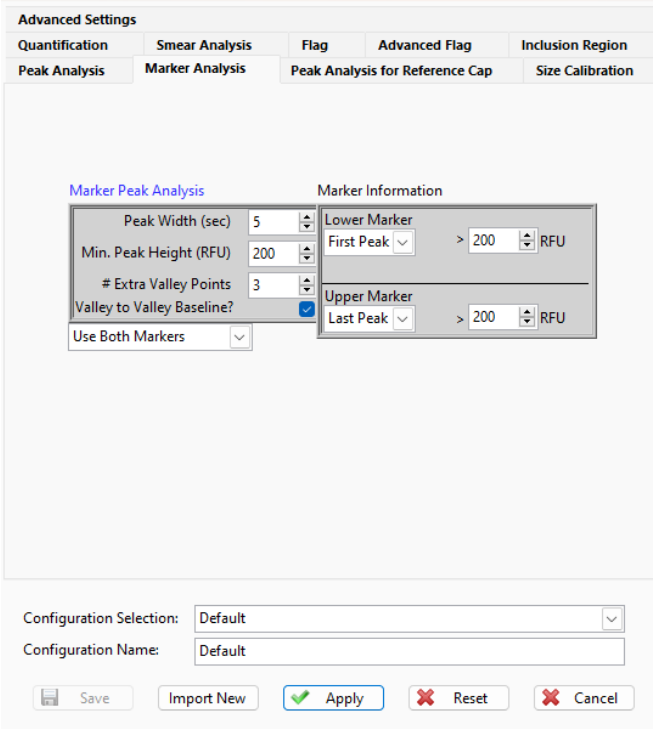


Figure 41 Marker Analysis tab in Global Configuration dialog

The markers can be selected based on peak width and peak height thresholds, and also by time range in the **Marker Information** section (Figure 42).

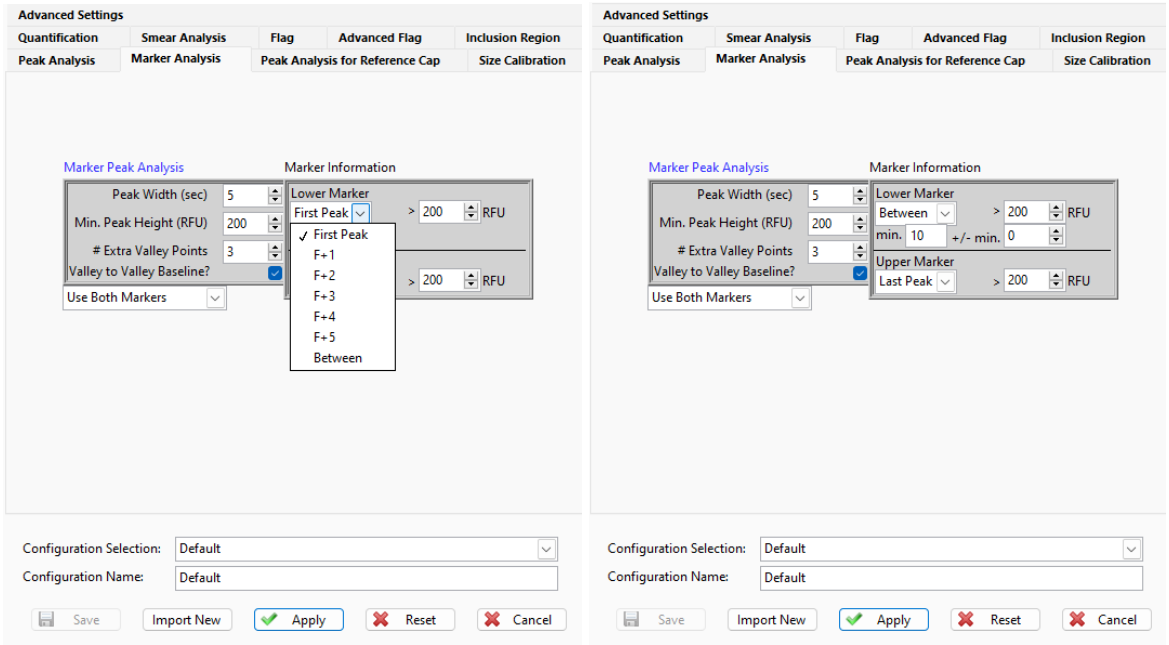


Figure 42 Marker Analysis tab showing marker selection options

The lower marker can be selected as the **First Peak**, or second peak (**F + 1**), third peak (**F + 2**), etc. up to the sixth peak above a threshold value in RFU units. Similarly, the upper marker can be selected as the **Last Peak**, or **L – 1**, **L – 2**, etc. Markers can also be selected based upon a time window, by selecting **Between** from the drop-down and entering a time in X min +/- Y min. For example, the right image of Figure 42 shows lower marker selection criteria of a peak > 200 RFUs between 8 – 12 min (10 min +/- 2 min).

NOTE

The **Min. Peak Height (RFU)** setting under **Marker Peak Analysis** sets the primary height threshold for the markers. The height threshold values for each individual marker shown under **Marker Information** must therefore be equal to or greater than the **Min. Peak Height (RFU)** value.

The settings of the **Marker Analysis** tab are described in Table 26.

Table 26 Marker Analysis configuration settings

Settings	Description
Marker Peak Analysis	
Peak Width (sec)	Defines the width threshold for peak detection and peak start/end point in seconds. Higher values better define wide peak start/end points; smaller settings better define sharp peak start/end points. Typical values are 3-5 for marker peaks.
Min. Peak Height (RFU)	Defines the minimum peak height threshold to select a marker peak in RFUs. Peaks below the set value will not be selected as markers.
# Extra Valley Points	This setting influences the start/end point of baseline integration of the marker peaks and the baseline. This setting most affects the baseline between two peaks that are not baseline resolved. Higher values draw a straighter baseline between the first peak start point and second peak end point; lower values draw the baseline more to the "valley" between the two unresolved peaks. Note: The **Valley to Valley Baseline?** setting needs to be enabled for this setting to be active.
Valley to Valley Baseline?	This setting influences the start/end point of baseline integration of the marker peaks and the baseline. This setting most affects the baseline between two peaks that are not baseline resolved. When disabled, will draw a straighter baseline between the first peak start point and second peak end point. When enabled, will draw the baseline more to the "valley" between the two unresolved peaks.
Use Both Markers	Normalizes and aligns the CE data to both a selected lower and upper size marker.
Use Lower Marker Only	Normalizes and aligns the CE data to only the selected lower marker.
Use Upper Marker Only	Normalizes and aligns the CE data to only the selected upper marker.
Marker Information	
Lower Marker	This setting determines how the lower marker is selected from the CE data, using the threshold value in RFU as the minimum peak height for selection. The lower marker is selectable from the drop-down control as: • First Peak (F) • F + 1, F + 2, F +3, F + 4, or F +5 • Between: Enter a specific time in X minutes +/- Y min
Upper Marker	This setting determines how the upper marker is selected from the CE data, using the threshold value in RFU as the minimum peak height for selection. The upper marker is selectable from the drop-down control as: • Last Peak (L) • L - 1, L - 2, L - 3, L - 4, or L - 5 • Between: Enter a specific time in X minutes +/- Y min

Global Configuration - Peak Analysis for Reference Cap

The settings of the **Peak Analysis for Reference Cap** tab allow the user to assign width and height thresholds for selecting and integrating peaks in the "Reference" capillary, which is the assigned well containing the DNA or RNA Ladder used to calibrate size (**Figure 43**). The settings are described in detail in **Table 27**.

This feature enables the user to define a higher threshold for the ladder peaks as compared to the sample peaks.

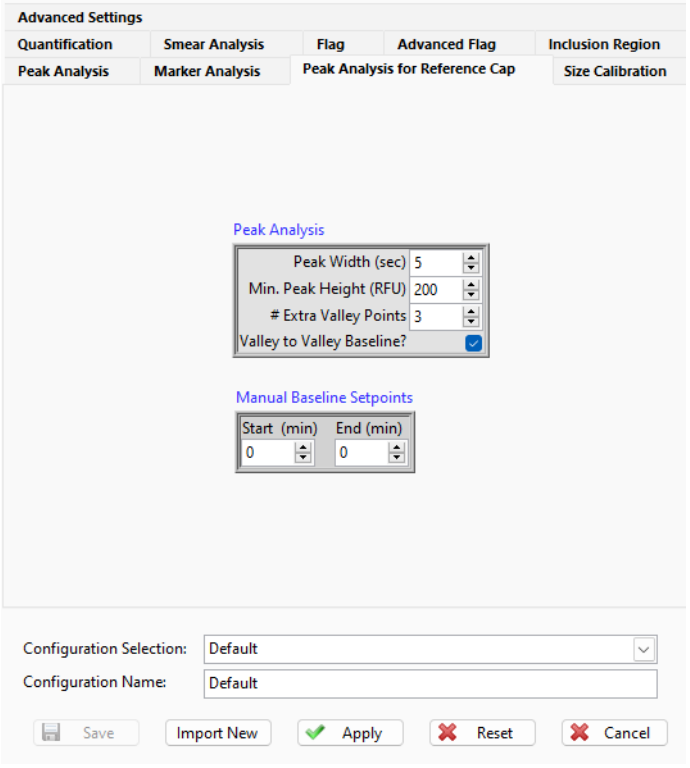


Figure 43 Peak Analysis for Reference Cap tab in Global Configuration dialog

Table 27 Peak Analysis for Reference Cap configuration settings

Settings	Description
Peak Analysis	
Peak Width (sec)	Defines the width threshold for peak detection in seconds. Higher settings better define wide peak start/end points; smaller settings better define sharp peak start/end points. Typical values range from 3-5 for sharp peaks.
Min. Peak Height (RFU)	Defines the minimum peak height threshold to select a peak for integration in RFUs. Peaks below the set value will not be selected for integration. Typical values for this setting when working with a DNA or RNA Ladder 100-200 RFU, depending upon the ladder concentration and application.
# Extra Valley Points	This setting influences the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. Higher values draw a straighter baseline between the first peak start point and second peak end point; lower values draw the baseline more to the "valley" between the two unresolved peaks. Note: The **Valley to Valley Baseline?** setting needs to be enabled for this setting to be active.
Valley to Valley Baseline?	This setting influences the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. When disabled, will draw a straighter baseline between the first peak start point and second peak end point. When enabled, will draw the baseline more to the "valley" between the two unresolved peaks.

Global Configuration - Size Calibration

The settings of the **Size Calibration** tab allow the user to select the proper "Reference" well containing a DNA or RNA Ladder for calibration of sample size; to enter in the proper size and number of fragments (including lower/upper markers) in the calibration ladder; and to apply a fit algorithm for plotting the size vs. migration time data from the ladder (**Figure 44**). The settings are described in detail in **Table 28**.

In ProSize, the user can calibrate the size of sample fragments either by running a standard DNA or RNA ladder in a well of the sample plate (recommended), or by importing a previously calculated and exported size calibration file (*.scal extension).

NOTE

If an imported size calibration is used in ProSize, the same Separation Gel, capillary array dimensions, injection/run voltage, and lower/upper markers should be used for both the analyzed data and the imported calibration to ensure maximum sizing accuracy.

To properly calibrate the size, the size ladder must be bracketed by the same lower/upper markers used with the samples. In some cases, the lower or upper marker may co-migrate with a ladder peak; this is acceptable for sizing calibration. In some application kits (for example, RNA and Genomic DNA kits), only a lower marker is used; the data is hence normalized using the lower marker only compared to the respective DNA or RNA Size Ladder.

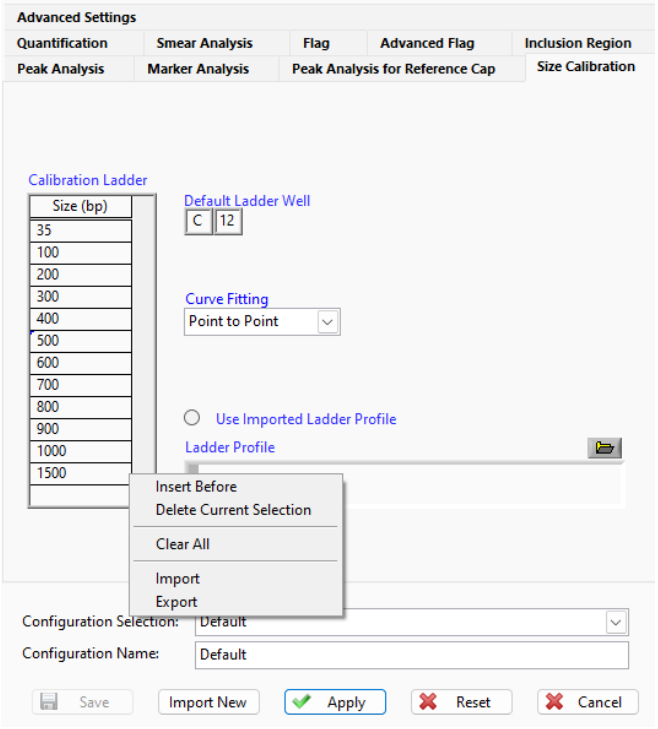


Figure 44 Size Calibration tab in Global Configuration dialog

It is important to ensure the sample containing the size calibration ladder ("Reference" well) has all ladder fragments integrated correctly with no additional peaks integrated prior to analyzing the remaining samples.

The size of each fragment to be included in the DNA/RNA Ladder (including lower/upper markers) is entered directly or imported into the **Calibration Ladder** field. To directly enter a value, click in the field and enter the size value.

A context menu is available when selecting the **Calibration Ladder** table. It offers options such as inserting or deleting fragments in the table; clearing all entries; and importing or exporting ladder information files (*.txt extension).

NOTE

In most cases, predefined ladder information files optimized for different Agilent reagent kits are available and are loaded as part of the **Global Configuration**. The ladder files are labeled according to the particular kit number, for example, **DNF-910 - 35-1500 100 bp ladder.txt**. These ladder files are available from Agilent upon request if needed. The preloaded ladder files are located in the **Ladders** folder under C:\ProSize data analysis software.

Table 28 Size Calibration configuration settings

Settings	Description
Calibration Ladder	Lists size of DNA/RNA fragments contained in calibration ladder in tabular format. The size of each fragment (include lower/upper markers) is entered directly into the table or imported by using the context menu (right-click in the **Calibration Ladder** table).
Calibration Ladder > Insert Before	Inserts a blank cell above the currently selected cell in the calibration ladder table. Note: Click in the respective table cell before using this option.
Calibration Ladder > Delete Current Selection	Deletes the currently selected cell in the calibration ladder table. Note: Click in the respective table cell before using this option.
Calibration Ladder > Clear All	Deletes all cells in the calibration ladder table.
Calibration Ladder > Import	Imports a previously exported and saved sizing ladder information file. When selected, a file browser menu will open to navigate and select the desired file (*.txt extension). Standard ladder information files are located in the C:\ProSize data analysis software\Ladders folder.
Calibration Ladder > Export	Exports the current sizing ladder table information (*.txt extension). When selected, a file browser menu will open to navigate and save the desired filename and location. When exporting ladder information files, it is highly recommended to save the created files to a common directory, located in the C:\ProSize data analysis software\Ladders folder.
Default Ladder Well	Defines the well of the sample plate, which contains the size calibration ladder. From the drop-down list, select the proper row (A-H) and column (1-12) location containing the size calibration ladder.
Curve Fitting	• Point to Point Fits the size calibration plot of Size (bp or nt) vs. Time (sec) with a Point to Point fit (recommended), drawing a straight line between each point of the curve. • Polynomial Fits the size calibration plot of Size (bp or nt) vs. Time (sec) with a polynomial fit. Enter the order value (from 3 to 5) in the **Order** field.
Use Imported Ladder Profile	Imports a previously exported size calibration file (*.scal extension) to use for size calibration.
Ladder Profile	Displays file path for the imported size calibration file. To load an imported file, click  . A file browser menu will open to navigate and select the desired file (*.scal extension). Note: When importing a size calibration file, the same conditions (separation gel, capillary array length, lower/upper markers, CE separation voltage) should be used. Differences between the analyzed and imported conditions will adversely affect the sizing accuracy.

Further information on the Size Calibration function is described in chapter **Chapter 5**, “ProSize Size Calibration Screen”.

Global Configuration - Quantification

The settings of the **Quantification** tab allow the user to assign how the sample is quantified in the ProSize software (by markers or ladder); to define the working concentration of the marker or ladder reference; and to apply a dilution factor for calculating the final sample concentration (**Figure 45**). The settings are described in detail in **Table 29**.

There are two different methods by which the sample concentration may be quantified in the ProSize software:

- **Use Lower/Upper Marker**: This method compares the sample peak or sample smear area to either the lower or upper marker peak area. The peak areas are taken as corrected peak area (area/migration time), with each data point collected being divided by its migration time. This method relies on a marker of known concentration, typically mixed directly with the sample.
- **Use Ladder**: This method compares the sample peak or sample smear area to that of a RNA or DNA ladder of known total concentration. The ratio of the RNA/DNA ladder to the lower marker is calculated; then the ratio of the lower marker to the sample is calculated to determine the final sample concentration. This method relies on a RNA/DNA ladder of known concentration, typically mixed directly with the sample.

  An advantage of the **Use Ladder** quantification method is that it can correct for any systematic errors in sample preparation. As long as the sample and ladder are prepared using the same diluent marker solution, any bias from pipetting or preparation is accounted for as long as the RNA/DNA Ladder is pipetted and mixed the same as the sample.

For the **RNA** modes, **Use Ladder** is the default **Quantification** method. For the various **DNA** modes, the user may select from either the **Use Lower/Upper Marker** or the **Use Ladder** method. Refer to the corresponding User Manual for each specific Agilent reagent kit for suggested settings.

NOTE

The most accurate means of quantification involves diluting the sample in a diluent containing marker of known concentration (internal standard). Approximate concentration or relative concentration differences between samples can also be obtained by diluting a sample in a diluent matrix (e.g., 1X TE buffer) that matches that of markers prepared in a separate marker plate, and injected under identical conditions (voltage/time).

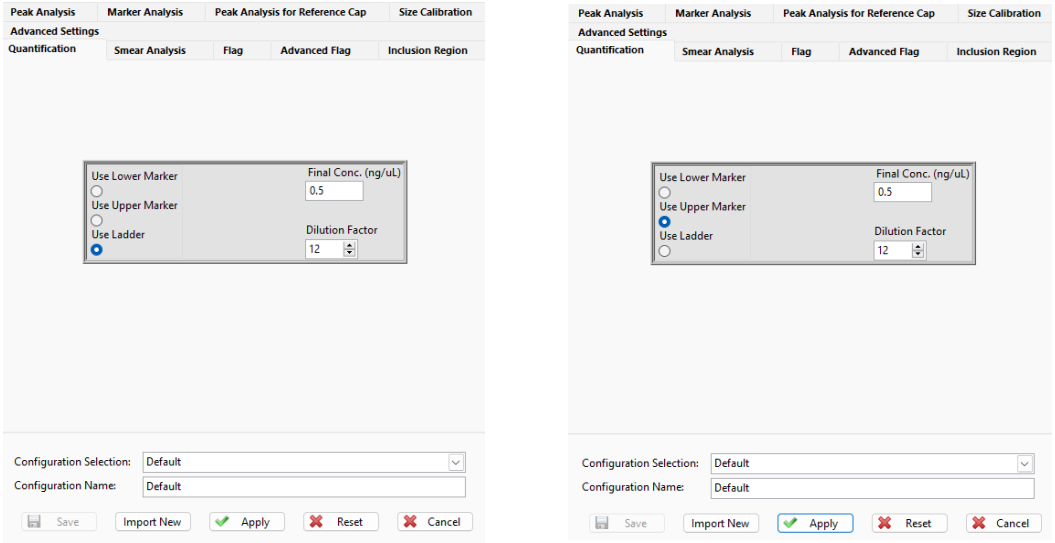


Figure 45 Quantification tab in Global Configuration dialog (Left: **Use Ladder** selected; Right: **Use Upper Marker** selected)

Table 29 Quantification configuration settings

Settings	Description
Use Lower Marker	Uses the ratio of the sample corrected peak area to the lower marker corrected peak area for calculation of sample peak/smear quantification.
Use Upper Marker	Uses the ratio of the sample corrected peak area to the upper marker corrected peak area for calculation of sample peak/smear quantification.
Use Ladder	Compares the total corrected peak area of the ladder to the lower marker. The result will be ratioed to the corrected peak area of sample compared to its respective lower marker peak.
Final Conc. (ng/uL)	The value of final working concentration in ng/mL of the marker or ladder to be used for quantification. Example: In the RNA Kit (DNF-471), the RNA Ladder is diluted 12X (2 mL Ladder + 22 mL diluent) and has a starting concentration of 96 ng/mL. Therefore, the **Final Conc. (ng/µL)** value is set to 8 ng/L and the **Dilution Factor** is set to 12.
Dilution Factor	The multiplication factor to be applied to the sample or ladder to account for any pre-dilution steps prior to analysis. Note: This value may differ between samples depending upon whether a sample has been pre-diluted prior to analysis. For example, in the HS NGS Fragment Kit, 2 mL of sample is diluted into 22 mL of diluent/marker solution; a dilution factor of 12 should therefore be used when using the **Use Upper Marker** quantification method for the ProSize software to accurately output the starting sample concentration. Note: A **Dilution Factor** of 1 indicates no dilution of the sample.

Global Configuration - Smear Analysis

The settings of the **Smear Analysis** tab allow the user to specify a size range(s) for which to integrate and calculate the total concentration in ng/mL. This is useful for example for DNA smears, where knowledge of the concentration of a specific size range within a smear is more important than that of the total smear size range (**Figure 46**). The settings are described in detail in **Table 30**.

Several different size ranges can be applied at once to a sample; the calculated total concentration within the specified range is presented in a tabular format that can be exported in a .csv format or printed as a hard copy PDF report.

If one or more selections are entered in the **Smear Analysis** tab, upon opening the data a **Smear Analysis** tab will displayed next to the **Peak Analysis** tab, with each individual result populating a separate line, showing the calculated concentrations for each range.

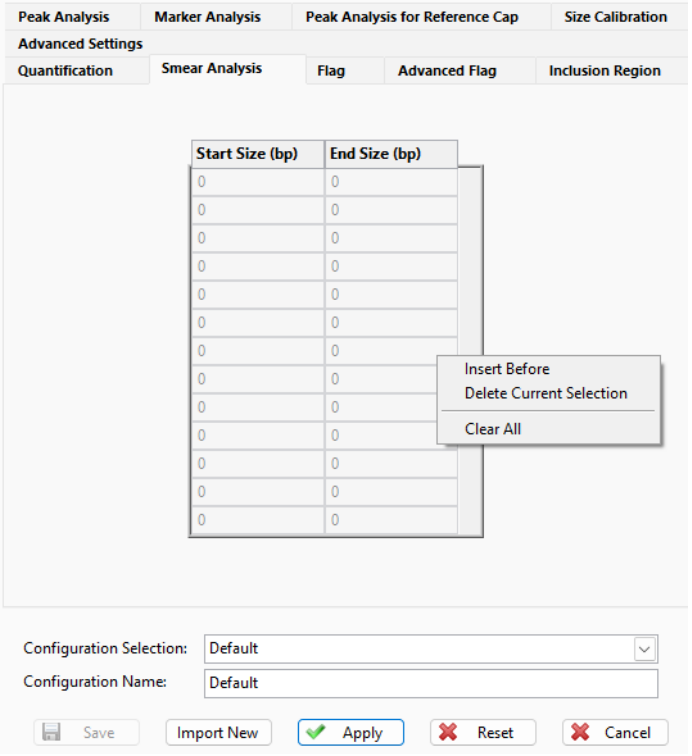


Figure 46 Smear Analysis tab in Global Configuration dialog

Table 30 Smear Analysis configuration settings

Settings	Description
Start Size (bp)	Defines the lower size threshold (in bp) to include in the calculation of total concentration. Note: If the entered size is in the middle of a peak/smear, the concentration is calculated from that size and higher and will not include the portion of the peak/smear below the size threshold.
End Size (bp)	Defines the upper size threshold (in bp) to include in the calculation of total concentration. Note: If the entered size is in the middle of a peak/smear, the concentration is calculated from that size and below and will not include the portion of the peak/smear above the size threshold.
Insert Before	Context menu option of the **Smear Analysis** table. Inserts a blank cell row above the currently selected cell in the table. Note: Click in the respective table cell before using this option.
Delete Current Selection	Context menu option of the **Smear Analysis** table. Deletes the currently selected cell row in the table. Note: Click in the respective table cell before using this option.
Clear All	Context menu option of the **Smear Analysis** table. Deletes all cells in the table.

Global Configuration - Flag

The settings of the **Flag** tab allow the user to specify specific criteria (size or concentration range) to be met within the data, and generate a binary output (0 = false, 1 = true) of the results. This is useful for applications (for example, genotyping) where the presence/absence of bands is to be scored (**Figure 47**). The settings are described in detail in **Table 31**.

Flag criteria can be set based on size, concentration, peak height and/or corrected peak area. Boolean logic is applied to the criteria for establishing various condition.

If one or more selections are entered in the **Flag** tab, upon opening the data an additional **Flag Analysis** tab will be displayed with the **Peak Table** for each individual results, displaying the flag results in a binary 1/0 format.

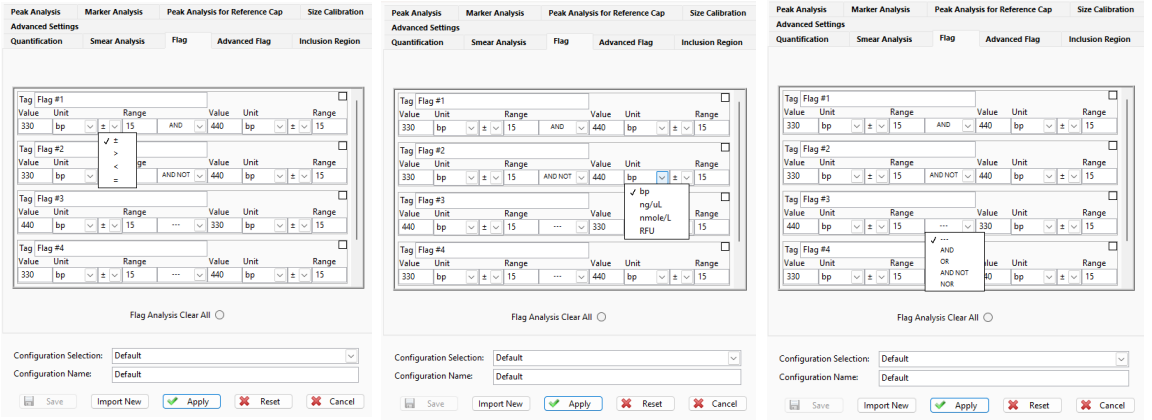


Figure 47 Flag tab in Global Configuration dialog

Table 31 Flag configuration settings

Settings	Description
Tag	Allows naming of the flag condition being used.
Value	Defines the numerical value of the unit for the flag condition.
Unit	Drop-down list which defines the units of the condition to flagged. The first **Unit** is always size (bp or nt) based; for the second **Unit** criteria there are four secondary flagging options: • bp (size) • ng/mL (concentration) • nmole/L (concentration) • RFU (Relative Fluorescence Units)
Range	Defines the numerical range to use for applying the flag condition. The drop-down to the left of this field determines the operation to apply: • **±** (will flag the value entered with a plus and minus range) • **>** (will flag when greater than the range) • **<** (will flag when less than the range) • **=** (will flag when equal to the range)
Boolean Operation	Drop-down list which defines the Boolean operation to apply to the flag condition. There are five options to apply: • **---**: No second flag condition is applied. • **AND**: Both the first and second flag condition must apply to be true • **OR**: First or second flag condition must apply to be true • **AND NOT**: The first flag condition must apply and the second must not apply to be true • **NOR**: Both the first and second flag condition must not apply to be true
Flag Analysis Clear All	Clears all existing flag conditions from the table.
Checkbox ☐	Clears the corresponding row flag condition entry.

Global Configuration – Advanced Flag

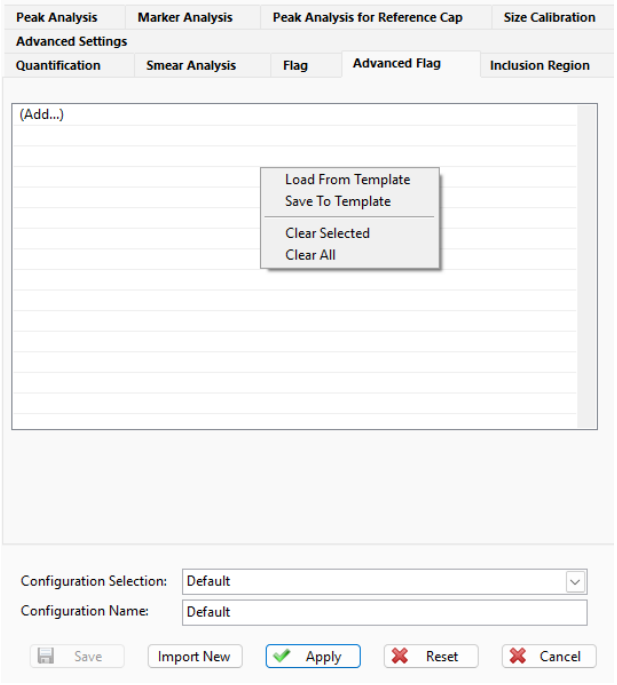
The **Advanced Flag** tab allows the user to define more complex flag conditions. However, only a single set of advanced flag conditions can be applied to all 96 capillaries.

In contrast, **Set Individual Parameters** dialog allows unique flag conditions for each individual well. A plate map of individual flag conditions can be defined, saved, and re-applied to new plates. An entire plate map of advanced flag conditions may be applied to all 96 capillaries, where each advanced flag condition is different for each capillary.

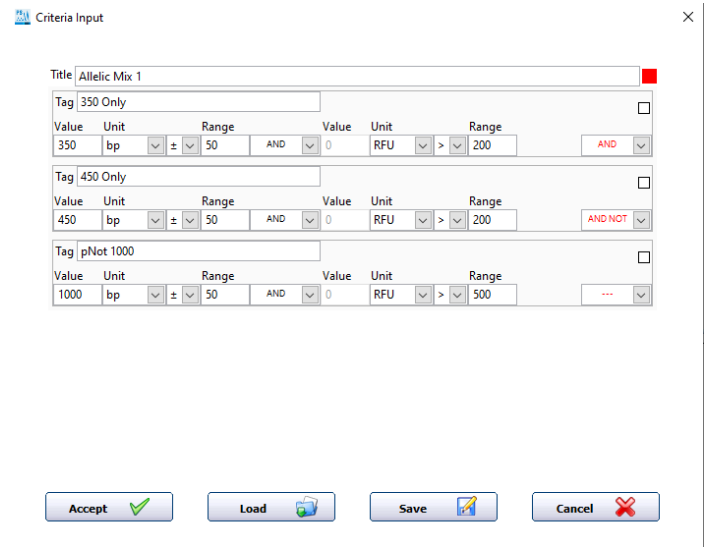
Add a Flag Criteria

You can define complex flag criteria with an unlimited number of conditions. A criteria can be set based on size, concentration, peak height, RFU and/or corrected peak area. Boolean logic is applied to the criteria for establishing various conditions. This is useful for applications (for example, genotyping) where a complex flag pattern analysis is required.

1 In the **Advanced Flag** tab, click **Add**.



The **Criteria Input** window opens. The settings are described in **Table 33**.



2 Enter the flag criteria (size, concentration range, RFU range, mixed fragment criteria, etc.) to be met within the data. This will be used for the result output table.

If one or more selections are entered in the **Advanced Flag** tab, upon opening the data an additional **Advanced Flag Analysis** tab will be displayed with the **Peak Table**.

Saving Formats for Flag Conditions

- If you want an individual flag condition to be applied as one of several flag conditions within a well, save the flag as .template.
- If you want a set of flag conditions to be applied to an individual well (which may contain one or more individual conditions), save the flag as .SCtmpt (where *SC* is abbreviated for single capillary).
- If you want a set of flag conditions to be applied to an entire plate, where each well may have different flag conditions (which can be applied only in the “Set Individual Parameters” discussed later in this chapter), save the flag as .ACtmpt (where “AC” stands for “all capillaries”).

Table 32 describes the settings of the **Advanced Flag** tab.

Table 32 Advanced Flag configuration settings

Settings	
Add.....	Double-click opens the **Criteria Input** window. Allows to enter a set of flag conditions.
Context menu	
Load From Template	Opens and applies a pre-defined set of flag condition (previously created and saved by user) for an individual well (.SCtmpt). The same set of conditions defined by the .SCtmpt file are applied to all 96 capillaries.
Save to Template	Saves the flag template as .SCtmpt for the single well—applied to all 96 capillaries (i.e., the same flag condition is applied to all 96 capillaries).
Clear Selected	Clears a selected flag condition (which are applied to all 96 capillaries).
Clear All	Clears all flag conditions (for all 96 capillaries).

Table 33 Criteria Input settings to define a flag condition

Settings	Description
Title	To name the set of conditions being used (which may include several tags).
Tag	To name an individual flag condition being used.
Value	Defines the numerical value of the unit for the flag condition.
Unit	Drop-down list which defines the units of the condition to flagged. The first **Unit** is always size (bp or nt) based; for the second **Unit** criteria there are four secondary flagging options: • bp (size) • ng/µL (concentration) • nmole/L (concentration) • RFU (Relative Fluorescence Units)
Range	Defines the numerical range to use for applying the flag condition. The drop-down to the left of this field determines the operation to apply: • **±** (will flag the value entered with a plus and minus range) • **>** (will flag when greater than the range) • **<** (will flag when less than the range) • **=** (will flag when equal to the range)

Table 33 Criteria Input settings to define a flag condition

Settings	Description
Boolean Operation	Drop-down list which defines the Boolean operation to apply to the flag condition. There are five options to apply: • **---**: No second flag condition is applied. • **AND**: Both the first and second flag condition must apply to be true • **OR**: First or second flag condition must apply to be true • **AND NOT**: The first flag condition must apply and the second must not apply to be true • **NOR**: Both the first and second flag condition must not apply to be true
Accept	To accept the flag conditions defined for the individual well
Load	To load an individual set of flag conditions (*.template). Note that multiple individual flag conditions can be applied to the same well. For example: 200 bp only; 300 bp AND 500 bp; 600 bp only.
Save	Saves an individual flag condition (*.template). Note that multiple individual flag conditions can be applied to the same well. For example: 200 bp only; 300 bp AND 500 bp; 600 bp only.
Cancel	Cancels any information entered and closes screen.

Global Configuration – Inclusion Region

The settings of the Inclusion Region tab allow the user to define up to four size inclusion ranges. Only peaks (excluding markers) that fall into the defined range(s) will be displayed. This will affect the screen display, PDF reports and exported data. The Inclusion Region tab is shown in with functions described in Table 34.

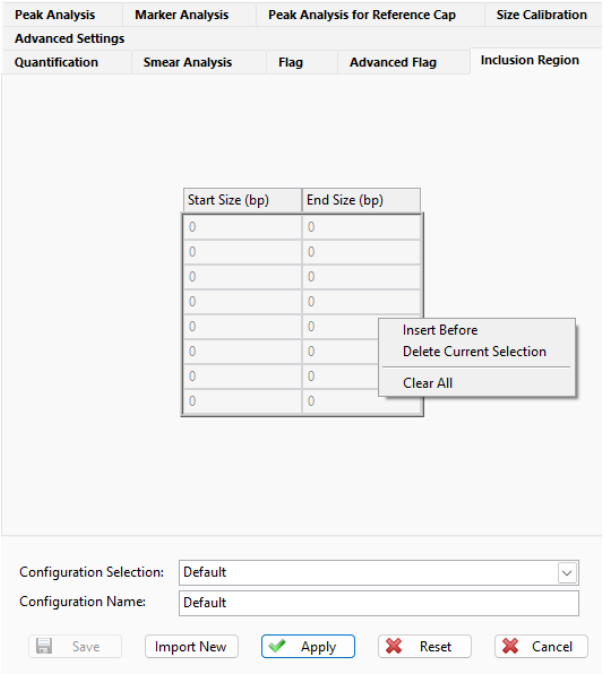


Figure 48 Inclusion Region tab in Global Configuration dialog

Table 34 Inclusion Region configuration settings

Settings	Description
Start Size (bp)	Defines the lower size boundary (in bp) of the Inclusion Region. Peaks (with the exception of the Lower Marker) smaller than the lower boundary will not be displayed.
End Size (bp)	Defines the upper size boundary (in bp) of the Inclusion Region. Peaks (with the exception of the Upper Marker) larger than the upper boundary will not be displayed.
Insert Before	Context menu option of the **Inclusion Region** table. Inserts a blank cell row above the currently selected cell in the table. Click in the respective table cell to use this option.
Delete Current Selection	Context menu option of the **Inclusion Region** table. Deletes the currently selected cell row in the table. Click in the respective table cell to use this option.
Clear All	Context menu option of the **Inclusion Region** table. Deletes all cells in the table.

Set Individual Parameters

After a data file is opened in the ProSize program, the configuration settings can be adjusted for individual samples by the **Set Individual Parameters** option.

The **Set Individual Parameters** dialog consist of main tabs that can be adjusted similar to those tabs in the **Global Configuration** dialog. RNA and Genomic modes also present an extra tab specific to those analyses in the **Set Individual Parameters**.

Some tabs can be applied to individual samples, while others can only be applied to all samples:

- **Advanced Settings**

  When a file is open and the analysis mode is changed, all samples are changed to the newly selected mode.

- **Peak Analysis**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Marker Analysis**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Quantification**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Smear Analysis**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Flag**

  When a file is open, all samples are changed to the current Flag settings and cannot be individually adjusted.

- **Advanced Flag**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Inclusion Region**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Total RNA Exclusion Region**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Small RNA Region**

  When a file is open, configuration settings of individual samples can be edited, or all samples may be changed at once.

- **Genomic Quality Number**

  When a file is open, all samples are changed to the current GQN settings and cannot be individually adjusted.

NOTE

When changing configuration settings in the **Set Individual Parameters** dialog, the reference ladder well is treated separately from the sample wells. Therefore, if **Apply to All** is performed on a sample well, the current reference ladder well will not be updated.

If the user chooses **Apply to All** from the reference ladder well, all sample wells including the ladder well will be updated.

At the bottom of the **Set Individual Parameters** dialog are two functions for setting and loading the configuration, as shown in **Figure 49** and summarized in **Table 35**.

Table 35 Set Individual Parameters buttons

Button	Description
Default Settings	Reverts setting to the default settings from the ProSize configuration file before any changes have been made.
Load Configuration	Opens a file browser menu for loading a previously created configuration file (*.ini extension). Navigate to the desired directory location containing the configuration file, and click **OK** to load.
Save Configuration	Saves the current configuration for use later. This is useful if you want to create a custom configuration for your data.

The settings of the tabs in the **Set Individual Parameters** dialog differ from those in the **Global Configuration** dialog. The tabs are summarized in the following sections.

Set Individual Parameters - Advanced Settings

The settings of the **Advanced Settings** tab allow the user to assign the data analysis mode for processing data (Figure 49). The **Minimum RFU for Signal Processing** is used to set a minimum RFU value for integration.

NOTE

When any change is made to the **Mode**, all samples are changed, including the reference ladder well.

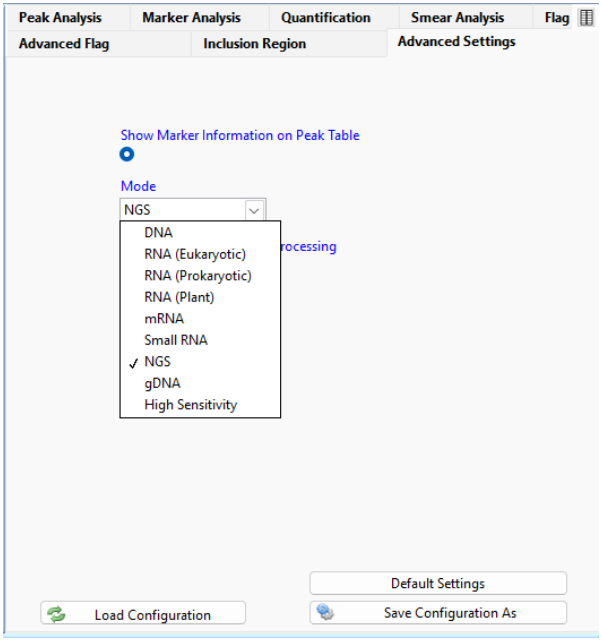


Figure 49 Advanced Settings tab in Set Individual Parameters dialog

Set Individual Parameters - Peak Analysis

Table 36 describes additional settings of the **Peak Analysis** tab not available in the **Global Configuration** dialog. If the user changes a field and/or presses [**Enter**], the new setting will be applied to the current selected sample well only. The Peak Analysis tab is shown in Figure 50.

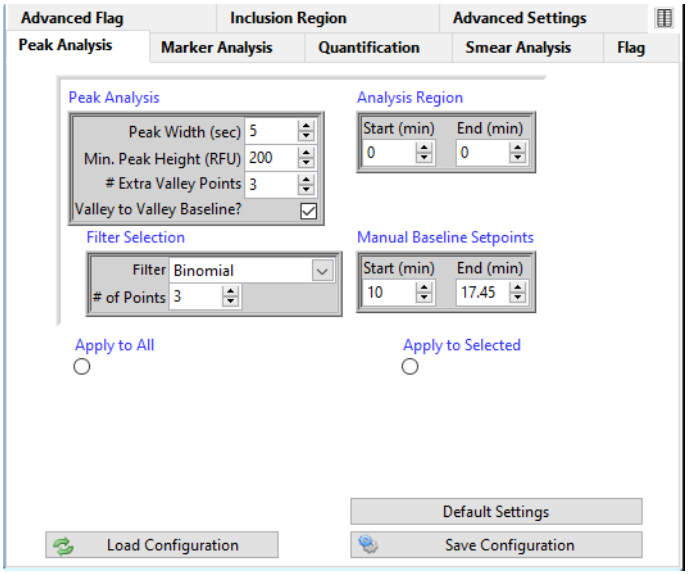
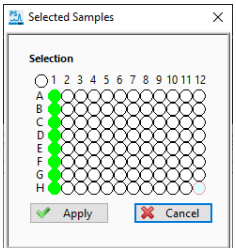


Figure 50 Peak Analysis tab in Set Individual Parameters dialog

Table 36 Additional settings in the Peak Analysis tab

Settings	Description
Apply to All	All current settings of just the **Peak Analysis** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Peak Analysis** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.
Apply to Selected Samples	Applies all current settings of just the **Peak Analysis** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; click **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Peak Analysis** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.

Set Individual Parameters – Marker Analysis

Table 37 describes additional settings of the **Marker Analysis** tab not available in the **Global Configuration**. If the user changes a field and/or presses [**Enter**], the new setting will be applied to the current selected sample well only. The Marker Analysis tab is shown in Figure 51.

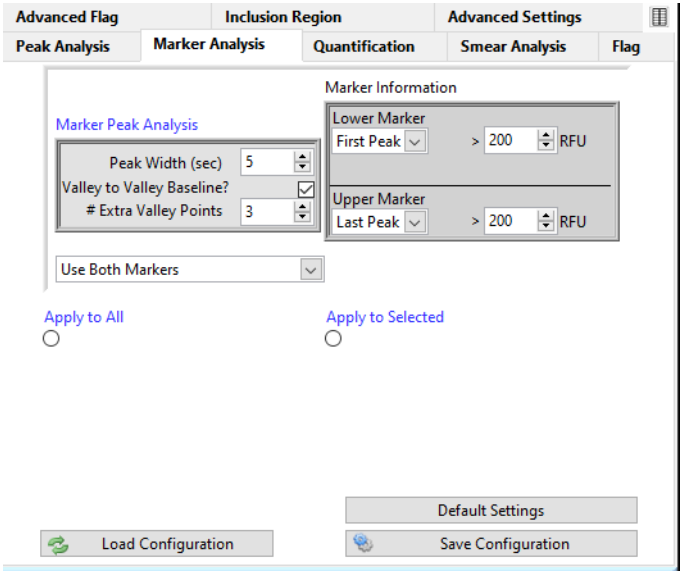
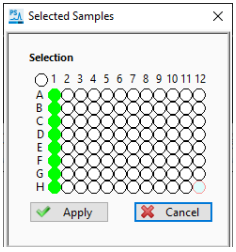


Figure 51 Marker Analysis tab in Set Individual Parameters dialog

Table 37 Additional settings of the Marker Analysis tab

Settings	Description
Apply to All	All current settings of just the **Marker Analysis** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Marker Analysis** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.
Apply to Selected Samples	Applies all current settings of just the **Marker Analysis** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; click **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Marker Analysis** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.

Set Individual Parameters – Quantification

Table 38 describes additional settings of the **Quantification** tab not available in the **Global Configuration**. If the user changes a field and/or presses [**Enter**], the new setting will be applied to the current selected sample well only. The Quantification tab is shown in Figure 52.

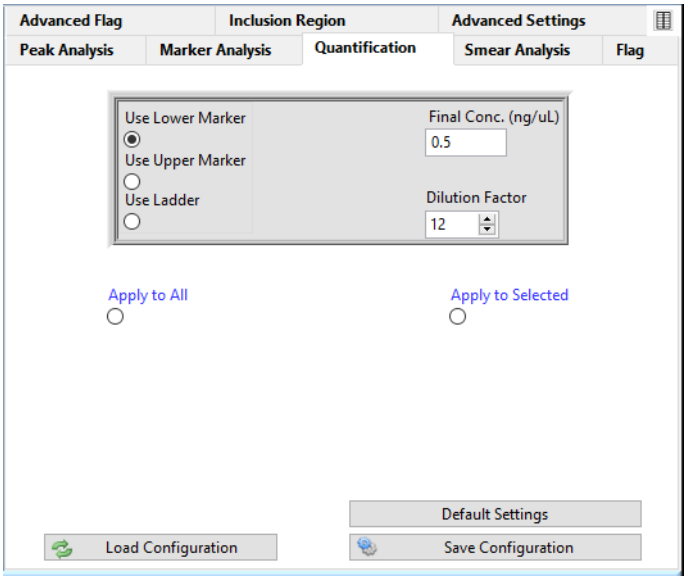
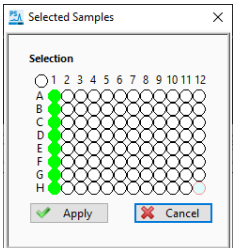


Figure 52 uantification tab in Set Individual Parameters dialog

Table 38 Additional settings in the Quantification tab

Settings	Description
Apply to All	All current settings of just the **Quantification** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Quantification** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.
Apply to Selected Samples	Applies all current settings of just the **Quantification** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; click **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Quantification** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.

Set Individual Parameters – Smear Analysis

Table 39 describes additional settings of the **Smear Analysis** tab not available in the **Global Configuration**. If the user changes a field and/or presses [**Enter**], the new setting will be applied to the current selected sample well only. The Smear Analysis tab is shown in Figure 53.

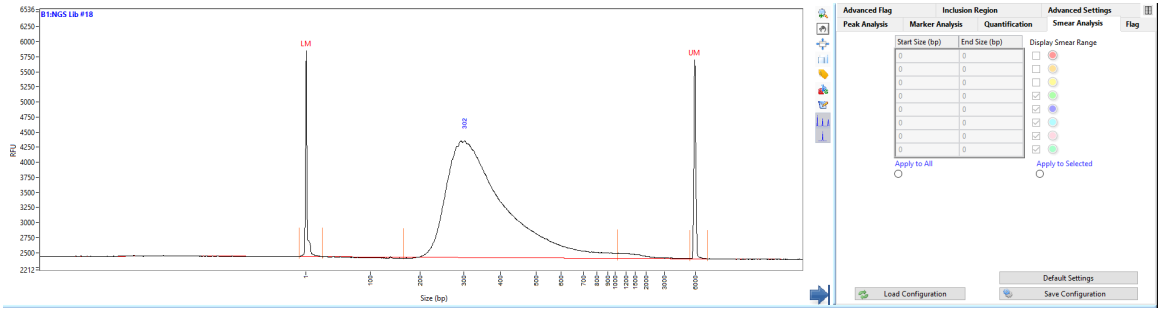


Figure 53 Smear Analysis tab in Set Individual Parameters dialog

Table 39 Additional settings in the Quantification tab

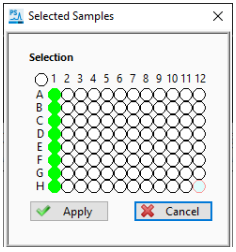
Settings	Description
Apply to All	All current settings of just the **Smear Analysis** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Smear Analysis** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.

Table 39 Additional settings in the Quantification tab

Settings	Description
Apply to Selected Samples	Applies all current settings of just the **Smear Analysis** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; click **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Smear Analysis** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.
Display Smear Range	When enabled, two vertical cursors are displayed in the Electropherogram Trace screen. These cursors define the entered values for the Start Size (bp) and the End Size (bp) for the currently selected smear range. The start and end points of the smear range can be adjusted by clicking on a cursor peak, and dragging the respective cursor left or right; the smear analysis region will be automatically updated to the new cursor location.

Set Individual Parameters – Flag Analysis

Table 40 describes additional settings of the **Flag Analysis** tab not available in the **Global Configuration**. If the user changes a field and/or presses [**Enter**], the new flag setting will be applied to all samples of the sample plate. The Flag Analysis tab is shown in Figure 54.

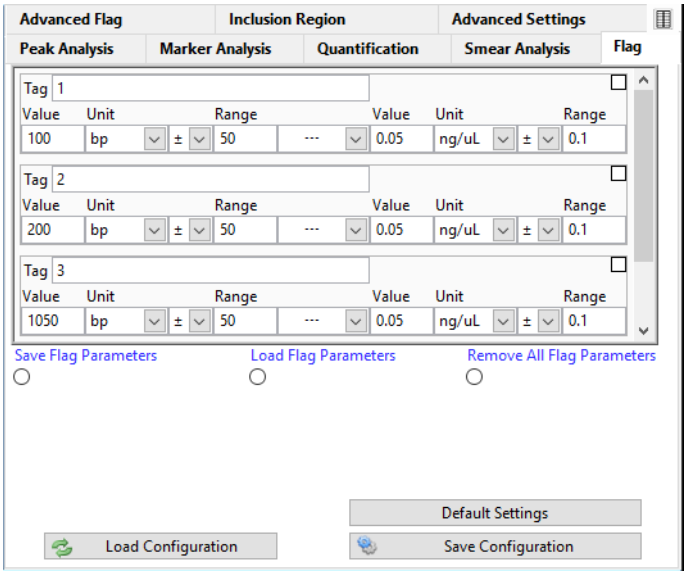


Figure 54 Flag tab in Set Individual Parameters dialog

Table 40 Additional Flag Analysis settings

Setting	Description
Save Flag Parameters	When selected, the current Flag Analysis settings will be saved as a file (*.Flag extension) that can be loaded into different data files. A file browser menu will open to navigate and save the desired filename and location; click **OK** to save the file, or **Cancel** to discard your changes.
Load Flag Parameters	When selected, the previously saved Flag Analysis settings will be loaded from a file (*.Flag extension). A file browser menu will open to navigate and load the desired filename and location; click **OK** to load the file, or **Cancel** to discard your changes.
Remove All Flag Parameters	When selected, all settings will be cleared from the tab.

Set Individual Parameters – Advanced Flag Analysis

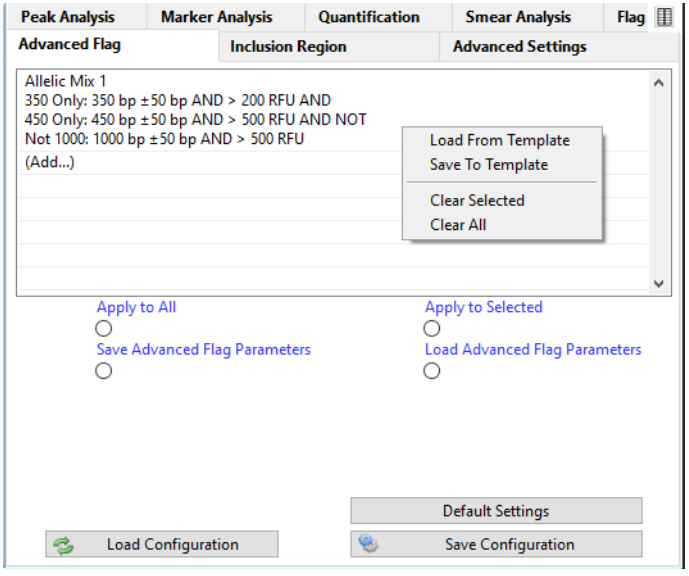
Advanced Flag allows the user to define more complex flag conditions.

The **Advanced Flag** tab in the **Global Configuration** dialog allows to apply only a single set of flag conditions to all 96 capillaries. However, here in the **Set Individual Parameters** dialog, unique flag conditions can be set up for each individual well, and a plate map of individual flag conditions can be defined, saved, and re-applied to new plates.

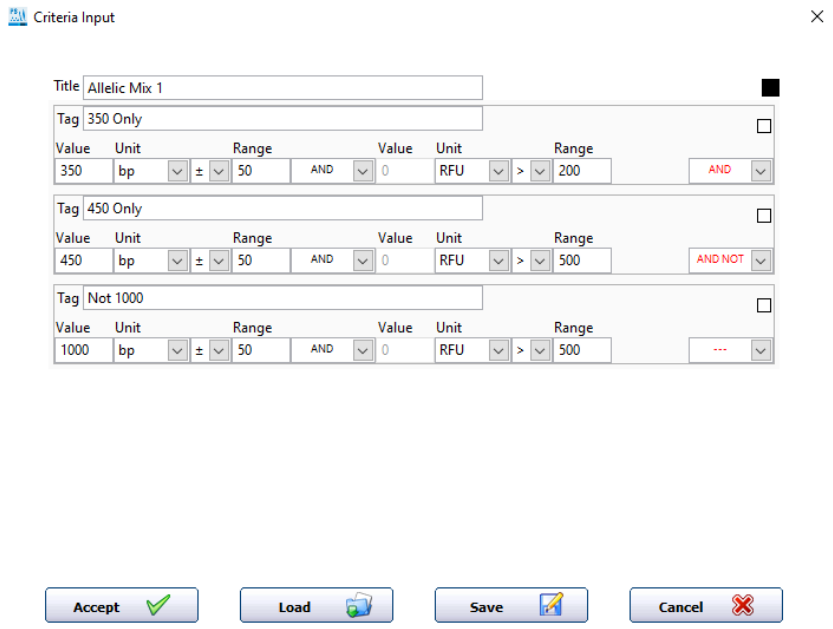
Add a Flag Criteria

Define complex flag criteria with an unlimited number of conditions. A criteria can be set based on size, concentration, peak height, RFU and/or corrected peak area. Boolean logic is applied to the criteria for establishing various conditions. This is useful for applications (for example, genotyping) where a complex flag pattern analysis is required.

1 In the **Advanced Flag** tab, double-click **Add** (the settings are described in Table 41).



The **Criteria Input** window opens.



2 Enter the flag criteria (size, concentration range, RFU range, mixed fragment criteria, etc.) to be met within the data. This will be used for the result output table. The settings are described in Table 42.

If one or more selections are entered in the **Advanced Flag** tab, upon opening the data an additional **Advanced Flag Analysis** tab will be displayed with the **Peak Table**.

If you changes a field and/or click [**Enter**], the new flag setting will be applied to only that well of the sample plate. To apply the conditions to all wells, select **Apply to all** in the tab.

Saving Formats for Flag Conditions

- If you want an individual flag condition to be applied as one of several flag conditions within a well, save the flag as .template.
- If you want a set of flag conditions to be applied to an individual well (which may contain one or more individual conditions), save the flag as .SCtmpt (where *SC* is abbreviated for single capillary).

- If you want a set of flag conditions to be applied to an entire plate, where each well may have different flag conditions (which can be applied only in the “Set Individual Parameters”tab), save the flag as .ACtmpt (where “AC” stands for “all capillaries”).

Table 41 Advanced Flag configuration settings

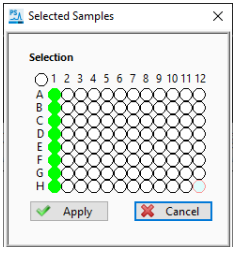
Settings	Description
Add.....	Double-click opens the **Criteria Input** window. Allows to enter a set of flag conditions.
Context menu	
Load From Template	Opens and applies a pre-defined set of flag condition (previously created and saved by user) for an individual well (.SCtmpt). The same set of conditions defined by the .SCtmpt file are applied to all 96 capillaries.
Save to Template	Saves the flag template as .SCtmpt for the single well—applied to all 96 capillaries (i.e., the same flag condition is applied to all 96 capillaries).
Clear Selected	Clears a selected flag condition (which are applied to all 96 capillaries).
Clear All	Clears all flag conditions (for all 96 capillaries).
Apply to All	All current settings of just the **Advanced Flag** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Advanced Flag** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.

Table 41 Advanced Flag configuration settings

Settings	Description
Apply to Selected Samples	Applies all current settings of just the **Advanced Flag** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; click **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Advanced Flag** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.
Save Advanced Flag Parameters	Saves the current **Advanced Flag Analysis** settings to a file (*.ACtempt extension) that can be loaded into different data files. A file browser menu will open to navigate and save the desired filename and location; click **OK** to save the file, or **Cancel** to abort operation. Note that this saves the entire plate flag map (which may have different flag conditions for each different well). Note: This option saves an entire plate map of different flag conditions to each well.
Load Advanced Flag Parameters	Loads previously saved **Advanced Flag Analysis** settings from a file (*.ACtempt extension). A file browser menu will open to navigate and load the desired filename and location; click **OK** to load the file, or **Cancel** to abort operation. Note that this applies an entire plate flag map. Note: This option applies an entire plate map of different flag conditions to each well.

Table 42 Criteria Input settings to define a flag condition

Settings	Description
Title	To name the set of conditions being used (which may include several tags).
Tag	To name an individual flag condition being used.
Value	Defines the numerical value of the unit for the flag condition.
Unit	Drop-down list which defines the units of the condition to flagged. The first **Unit** is always size (bp or nt) based; for the second **Unit** criteria there are four secondary flagging options: • bp (size) • ng/µL (concentration) • nmole/L (concentration) • RFU (Relative Fluorescence Units)

Table 42 Criteria Input settings to define a flag condition

Settings	Description
Range	Defines the numerical range to use for applying the flag condition. The drop-down to the left of this field determines the operation to apply: • **±** (will flag the value entered with a plus and minus range) • **>** (will flag when greater than the range) • **<** (will flag when less than the range) • **=** (will flag when equal to the range)
Boolean Operation	Drop-down list which defines the Boolean operation to apply to the flag condition. There are five options to apply: • **---**: No second flag condition is applied. • **AND**: Both the first and second flag condition must apply to be true • **OR**: First or second flag condition must apply to be true • **AND NOT**: The first flag condition must apply and the second must not apply to be true • **NOR**: Both the first and second flag condition must not apply to be true Adding a Boolean operator at the end of a flag condition allows you to enter a continuation of flag conditions-enabling complex combinations.
Accept	To accept the flag conditions defined for the individual well
Load	To load an individual set of flag conditions (*.template). Note that multiple individual flag conditions can be applied to the same well. For example: 200 bp only; 300 bp AND 500 bp; 600 bp only.
Save	Saves an individual flag condition (*.template). Note that multiple individual flag conditions can be applied to the same well. For example: 200 bp only; 300 bp AND 500 bp; 600 bp only.
Cancel	Cancels any information entered and closes the window.

Set Individual Parameters – Inclusion Region

The settings of the **Inclusion Region** tab allow the user to define up to four size inclusion ranges (**Figure 55**). Only peaks (excluding markers) that fall into the defined range(s) will be displayed. The Inclusion Region will affect the screen display, PDF reports and exported data. **Table 43** describes additional settings not displayed in **Global Configuration** dialog.

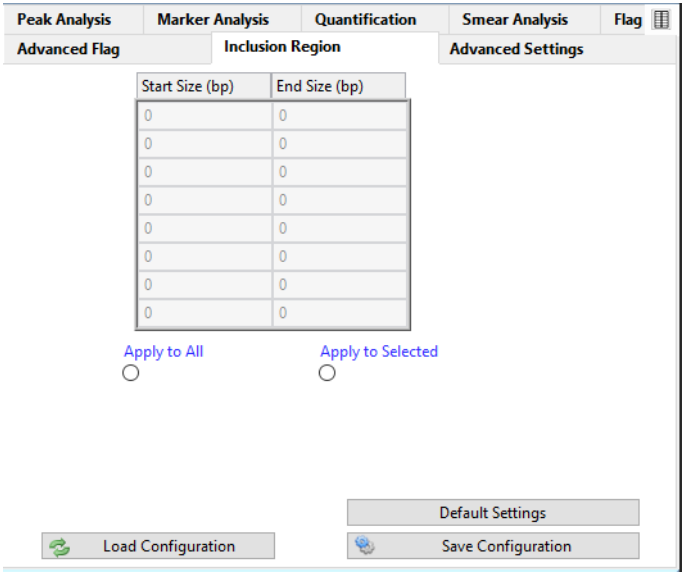
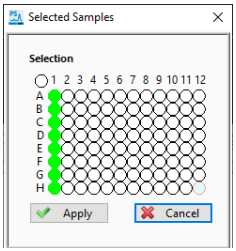


Figure 55 Inclusion Region tab in Set Individual Parameters dialog

Table 43 Additional settings in the Inclusion Region tab

Settings	Description
Apply to All	All current settings of just the **Inclusion Region** tab will be applied to all samples of the loaded data file. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated.  Note: You should only select this option if you wish to apply all settings of the **Inclusion Region** tab to all samples. Any previous changes made for the samples of the data file will be overwritten.
Apply to Selected Samples	Applies all current settings of just the **Inclusion Region** tab only to any user specified wells of the sample plate. The **Selected Samples** plate map menu will be displayed to select the wells of the sample plate to apply the settings to. Select desired wells, rows, or columns, and click **OK** to apply; press **Cancel** to abort the operation. If this is applied in the reference ladder well, all samples and the reference ladder will be updated; if this is applied in a sample well, all sample wells but not the reference ladder will be updated. Note: You should only select this option if you wish to apply all settings of the **Inclusion Region** tab to the selected set of samples. Any previous changes to the selected samples of the data file will be overwritten.

5 ProSize Size Calibration Screen

This chapter describes the size calibration screen (also referred to as calibration curve screen). The calibration curve screen is used to select the conditions for calibrating the size of unknown sample peaks/smears.

About Size Calibration

In the ProSize software, the user can calibrate the size of sample fragments either by running a standard DNA or RNA ladder in a well of the sample plate (recommended), or by importing a previously calculated and exported size calibration file (*.scal extension).

NOTE

If an imported size calibration is used in ProSize, the same separation gel, capillary array dimensions, injection/run voltage, and lower/upper markers should be used for both the analyzed data and the imported calibration to ensure maximum sizing accuracy.

To properly calibrate the size, the size ladder must be bracketed by the same lower/upper markers used with the samples. In some cases, the lower or upper marker may co-migrate with a ladder peak; this is acceptable for sizing calibration. In some application kits (for example, RNA and Genomic DNA kits), only a lower marker is used; the data is hence normalized using the lower marker only compared to the respective DNA or RNA Size Ladder.

It is important to ensure the sample containing the size calibration ladder has all ladder fragments integrated correctly with no additional peaks integrated prior to analyzing the remaining samples.

The following sections describe how to load, edit and import/export from the calibration curve screen, and how to identify and correct an improperly calibrated data file.

Loading the Size Calibration Curve

The calibration curve can be defined using two methods:

A: When an unprocessed data file is first opened and a matching configuration is not found, the **Global Configuration** dialog will appear which is shown in **Figure 56**. This is the most common way to define the calibration curve.

NOTE

In most cases, predefined, optimized global configuration files are available to load when processing data collected from different Agilent Reagent Kits. The configuration files are labeled according to the particular kit number, for example, **DNF-910 Kit - Fragment Analyzer.ini**. These configuration files are available from Agilent upon request if needed. The preloaded configuration files are located in the C:\ProSize data analysis software\Configurations folder.

B: At any time after a file is opened, you can view the **Calibration Curve** screen by selecting  from the main screen, or navigating to **Analysis** > **Show Size Calibration**.

The **Size Calibration** tab in the **Global Configuration** dialog is shown in **Figure 56**; the settings are described in **Table 44**.

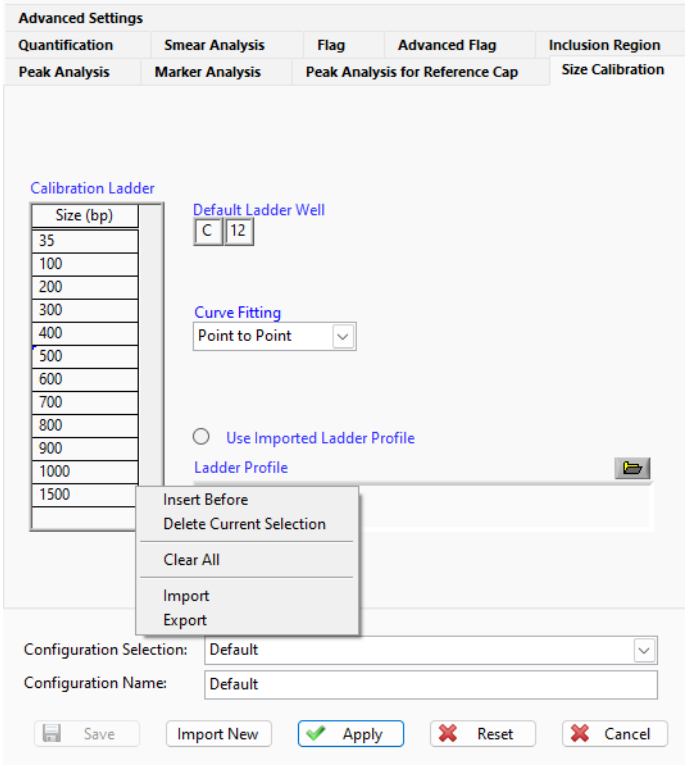


Figure 56 Size Calibration tab in the Global Configuration dialog

Table 44 Size Calibration settings

Settings	Description
Calibration Ladder	Lists size of DNA/RNA fragments contained in calibration ladder in tabular format. The size of each fragment (include lower/upper markers) is entered directly into the table or imported by using the context menu (right-click in the Calibration Ladder table).
Calibration Ladder > Insert Before	Inserts a blank cell above the currently selected cell in the calibration ladder table. Note: Click in the respective table cell to use this option.
Calibration Ladder > Delete Current Selection	Deletes the currently selected cell in the calibration ladder table. Note: Click in the respective table cell to use this option.
Calibration Ladder > Clear All	Deletes all cells in the calibration ladder table.

Table 44 Size Calibration settings

Settings	Description
Calibration Ladder > Import	Imports a previously exported and saved sizing ladder information file. When selected, a file browser menu will open to navigate and select the desired file (*.txt extension). Standard ladder information files are located in the C:\ProSize data analysis software\Ladders folder.
Calibration Ladder > Export	Exports the current sizing ladder table information (*.txt extension). When selected, a file browser menu will open to navigate and save the desired filename and location. When exporting ladder information files, it is highly recommended to save the created files to a common directory, located in the C:\ProSize data analysis software\Ladders folder.
Default Ladder Well	Defines the well of the sample plate, which contains the size calibration ladder. From the drop-down list, select the proper row (A-H) and column (1-12) location containing the size calibration ladder.
Curve Fitting	• Point to Point Fits the size calibration plot of Size (bp or nt) vs. Time (sec) with a Point to Point fit (recommended), drawing a straight line between each point of the curve. • Polynomial Fits the size calibration plot of Size (bp or nt) vs. Time (sec) with a polynomial fit. Enter the order value (from 3 to 5) in the **Order** field.
Use Imported Ladder Profile	Imports a previously exported size calibration file (*.scal extension) to use for size calibration.
Ladder Profile	Displays file path for the imported size calibration file. To load an imported file, click  . A file browser menu will open to navigate and select the desired file (*.scal extension). Note: When importing a size calibration file, the same conditions (separation gel, capillary array length, lower/upper markers, CE separation voltage) should be used. Differences between the analyzed and imported conditions will adversely affect the sizing accuracy.

Define Calibration Ladder

The size of each fragment to be included in the DNA/RNA Ladder (including lower/upper markers) is listed in the **Calibration Ladder** table (see **Figure 56**). You can enter directly a value into table or use the **Import** command of the context menu (right-click in the table). **Table 44** describes the commands of the context menu, which include inserting or deleting fragments, clearing all entries, and importing or exporting ladder information files (*.txt extension).

NOTE

In most cases, predefined ladder information files optimized for different Agilent Reagent Kits are available and are loaded as part of the global configuration. The ladder files are labeled according to the particular kit number, for example, **DNF-910-33 - 35-1500bp.txt**. These ladder files are available from Agilent upon request if needed. The preloaded ladder files are located in the C:\ProSize data analysis software\Ladders folder.

Define Default Ladder Well

If a size ladder is contained in the sample plate, the sample well for the ladder is displayed in the **Default Ladder Well** field (see **Figure 56**, **Table 44**). Click in the field, and from the drop-down list, select the proper well row (A, B, etc.) and well column (1, 2, 3 etc.) of the well containing the sizing ladder. When processing 12-capillary data, only the well column field (1-12) is selectable.

Define Curve Fitting

The **Curve Fitting** drop-down menu allows you to define how the size calibration curve is fitted - via a **Point to Point** or **Polynomial Fit** (3rd to 5th order) (see **Figure 56**, **Table 44**).

NOTE

It is highly recommended to use Point to Point when fitting the size calibration curve. All standard methods use this setting.

Use Imported Ladder

To use your previously exported size calibration settings, select **Use Imported Ladder** (see **Figure 56**). To import your file, select  . A file browser menu will open to navigate and select the desired file (*.scal extension) (**Figure 56**, **Table 44**).

Peak Analysis for Reference Cap

The default ladder well is considered to be the “Reference capillary“. When loading the configurations (for example, for an unprocessed file), note that a separate tab labeled **Peak Analysis for Reference Cap** is available (**Figure 57**). This tab enablesyou to enter independent peak settings to properly identify the DNA/RNA Ladder peaks as compared to the sample peaks, as outlined in **Table 45**.

Further information on these settings is available in **Chapter 4**, “ProSize Configuration”. When loading or editing the settings of the global configurations, it is important to set these parameters properly to ensure the ladder peaks are selected correctly for size calibration.

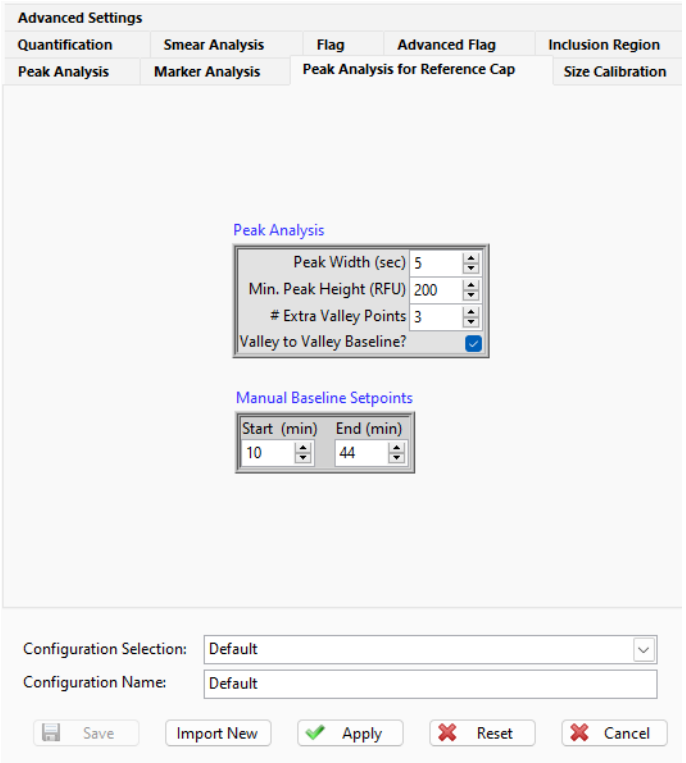


Figure 57 Peak Analysis for Reference Cap tab in the Global Configuration dialog

Table 45 Peak Analysis for Reference Cap configuration settings

Settings	Description
Peak Analysis	
Peak Width (sec)	Defines the width threshold for peak detection in seconds. Higher settings better define wide peak start/end points; smaller settings better define sharp peak start/end points. For DNA/RNA Ladders, the typical value ranges from 3-5.
Min. Peak Height (RFU)	Defines the minimum peak height threshold to select a peak for integration in RFUs. Peaks below the set value will not be selected for integration.
# Extra Valley Points	This setting affects the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. Higher values draw a straighter baseline between the first peak start point and second peak end point; lower values draw the baseline more to the "valley" between the two unresolved peaks. Note: The **Valley to Valley Baseline?** setting needs to be enabled for this function to be active. A typical setting for a DNA Ladder is 3; for a RNA Ladder this is typically set to 0.
Valley to Valley Baseline?	This setting affects the start/end point of baseline integration of peaks and the baseline drawn between peaks. This setting most affects the baseline between two peaks that are not baseline resolved. When disabled, will draw a straighter baseline between the first peak start point and second peak end point. When enabled, will draw the baseline more to the "valley" between the two unresolved peaks. This setting is typically enabled for a DNA Ladder and disabled for a RNA Ladder.

Once the proper **Calibration Ladder** fragments, **Default Ladder Well**, and **Curve Fitting** algorithm have been selected (or previous calibration imported) and the correct **Peak Analysis for Reference Cap** settings have been loaded, along with the other configuration settings, the file is loaded into the ProSize software.

Viewing and Exporting the Size Calibration (File Open)

Once a data file is loaded into the ProSize software, if the size calibration ladder is contained within the sample plate its well location will be marked with a light blue color (**Figure 58**, well H12 highlighted with a blue circle). To view the ladder well, click on the respective well in the Plate Map.

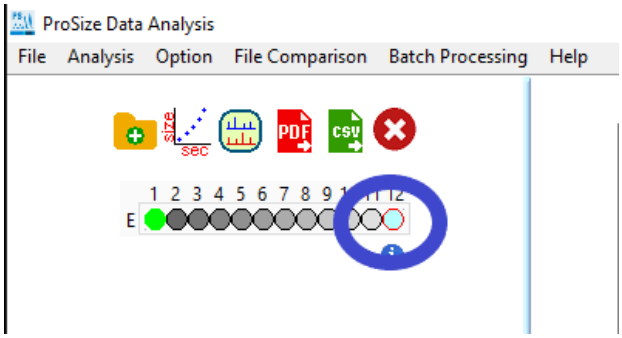


Figure 58 Main screen: The Size Calibration well H12 is marked in blue in the Plate Map (circled in blue to highlight position of reference capillary).

If the number of integrated peaks in the reference well matches the number of fragments in the **Calibration Ladder** table, the size calibration curve will be fitted with the selected **Curve Fitting** algorithm and the corresponding calculated sizes will be reported for each sample. The bottom task bar of the ProSize software will display the file path and no errors will be displayed (**Figure 58**).

NOTE

It is generally recommended to inspect the size calibration well as well as the calibration curve screen prior to processing or exporting data, to ensure the desired fragments/markers are selected properly.

To view the size calibration curve from the main screen of the ProSize software, click in the main screen, or navigate to **Analysis** > **Show Size Calibration**. The calibration curve screen will be displayed (**Figure 59**).

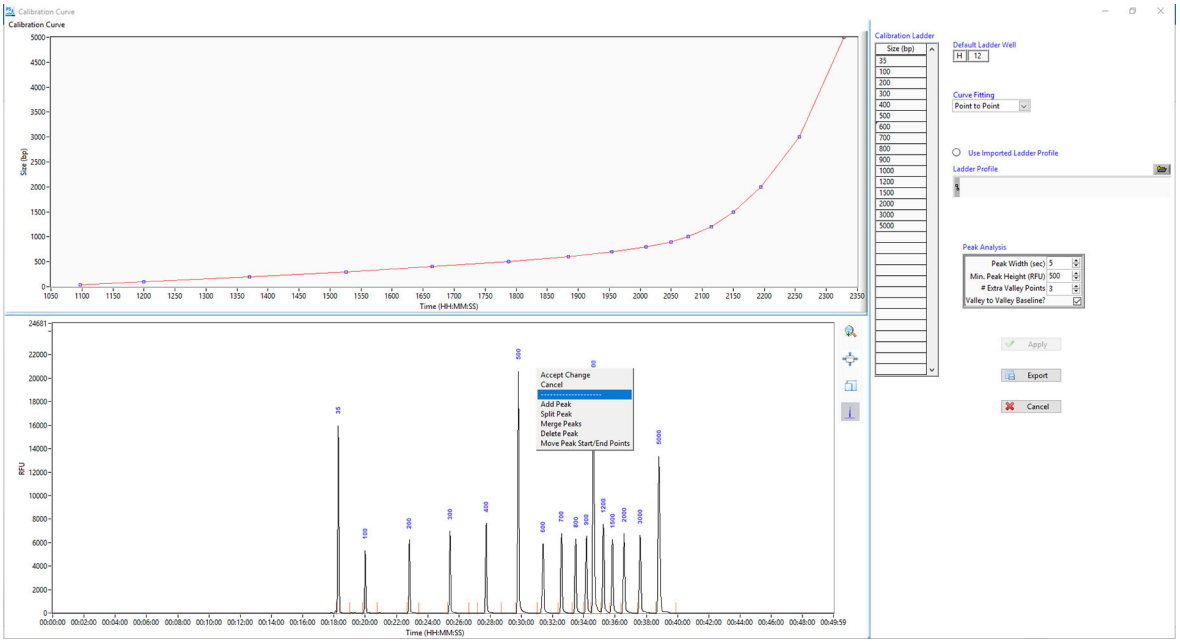


Figure 59 Figure Calibration Curve screen showing properly selected size calibration ladder

The size calibration curve is a plot of **Time** vs. **Size** in upper left corner. A proper fit is characterized by the red fit line passing through each blue data point as closely as possible. In most cases, the **Point to Point** algorithm provides the best fit to the data. However, in some situations the **Polynomial algorithm** may provide smoother, more continuous fit in the higher size region.

The functions in this screen are the same as in the **Size Calibration** and **Peak Analysis for Reference Cap** tabs in the **Global Configuration** dialog (**Figure 56** and **Figure 57**).

- The **Calibration Ladder** table and its context menu functions, and the **Default Ladder Well**, **Curve Fitting**, and **Use Imported Ladder Profile** functions are described in **Table 44**.
- The **Peak Analysis** panel enables adjustments to be made to the peak integration settings of calibration ladder; these functions are the same as those summarized in **Table 45**.

In the calibration curve screen also an Electropherogram Trace (bottom) of the calibration ladder well is displayed. The toolbar of the trace enables you to zoom, autoscale, copy or view the start/end points of each peak as summarized in **Table 46**. The functions of the Electropherogram Trace context menu enable you to modify and adjust the selected peaks in the selected reference well as needed to match those present in the **Calibration Ladder** table (**Table 46**).

You can export the currently viewed size calibration by selecting **Export**.

Table 46 Calibration Curve screen additional functions

Item	Description
Apply	Applies the current size calibration settings and exit to the main screen of the ProSize software. Any changes made from the initially opened Calibration Curve screen will be applied to the data.
Export	Exports the current size calibration settings and fitted electropherogram/plot, which can be imported to other similarly generated data files (*.scal extension). A file browser menu will open to navigate and save the desired filename and location.
Cancel	Closes the Calibration Curve screen and exit to the main screen of the ProSize software. Any changes made from the initially opened Calibration Curve screen will not be applied to the data.
Zoom 	Enables zooming-in on any portion of the Electropherogram Trace. To zoom-in, click the icon and position the mouse cursor over desired starting position; click and drag the cursor to the desired location, and release the mouse button.
AutoFit 	To autoscale the Electropherogram Trace x-/y-axis view.
Copy 	Copies an image of the Electropherogram Trace to the clipboard, for pasting in another program such as Microsoft PowerPoint.
Show/Hide Peak Start/End Edge 	Toggle to display or hide the start and end points used for peak integration, shown as vertical orange lines. Displaying the peak integration start/end points can aid in determining if adjustments need to be made to the peak integration to better define the actual peak area. The user can change the peak start/end points by adjusting the **Peak Width (sec)**, or by using the context menu of the Electropherogram Trace such as **Split Peak** or **Move Peak Start/End Points**.
Accept Change	Accepts any manual modification to the peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). After the manual modification has been made, right-click the Electropherogram trace, and select **Accept Change**.

Table 46 Calibration Curve screen additional functions

Item	Description
Cancel	Cancels any manual modification to the peak integration (**Add Peak**, **Split Peak**, **Merge Peaks**, **Delete Peak**, **Move Peak Start/End Points**). After the manual modification has been made, right-click the Electropherogram trace, and select **Cancel**.
Add Peak	To manually add a peak which has not been auto-integrated by the **Peak Analysis** settings and to define the start and end point for integration. Zoom-in to the region where the peak is to be added. Right-click this region and select **Add Peak**. Two red vertical cursors will appear; the left cursor defines the new peak start point and the right cursor the new peak end point. Drag each cursor to the desired position, then right-click, and select **Accept Change** to add the peak for integration. Note: The Add Peak function will not add a peak that is located outside the lower/upper marker window (or before lower marker if using only lower marker).
Split Peak	To manually split a peak which is currently integrated into two peaks and to define where the split occurs. Zoom-in to the region where the peak is to be split. Right-click this region, and select **Split Peak**. A red vertical cursor will appear. Drag the cursor to the desired location, then right-click, and select **Accept Change** to split the peak into two peaks for integration.
Merge Peaks	To merge any number of peaks and integrate as a single peak. Zoom-in to the region where the peak is to be split. Right-click this region, and select **Merge Peaks**. Two red vertical cursors will appear; the left cursor defines the left most peak to merge and right cursor defines the right most peak to merge. Drag the cursor within that peak's start/end point region to merge, then right click, and select **Accept Change** to merge the peaks into a single peak for integration.

Table 46 Calibration Curve screen additional functions

Item	Description
Delete Peak	To manually delete a peak which has been integrated. Zoom-in to the region where the peak is to be deleted. Right-click this region, and select **Delete Peak**. A red vertical cursor will appear. Drag the cursor to the desired peak location, then right-click, and select **Accept Change** to delete the peak from the integration.
Move Peak Start/End Points	To change the currently positioned start/end integration points of a peak. Zoom-in to the region where the peak is located. Place the cursor between the start and end points of the integration. Right-click and select **Move Peak Start/End Points**. Two red vertical cursors will appear at the current start/end points. Drag the cursors to the desired locations, then right-click and select **Accept Change** to apply the new start/end point positions to reintegrate the peak. Note: This function will not change the baseline; only the start and end points are affected. To change the baseline, the user must adjust the via the **Peak Width (sec)**, **Valley to Valley,** or **Manual Baseline Setpoints** tools.

Identifying and Correcting an Improper Size Calibration

When performing the size calibration, the ProSize software will attempt to fit the integrated peaks in the size calibration well to the fragments listed in the size **Calibration Ladder** table. If the number of integrated peaks in the ladder well does not match the number of fragments in the size Calibration Ladder table (either less or more peaks integrated), a red flashing error message will appear at the bottom of the main screen: **Warning: Mis-match between detected peaks and ladder assignment! No sizing calibration curve is established**.

An example of less integrated peaks than calibration fragments is shown in **Figure 60** and **Figure 61** an example of too many integrated peaks is shown in **Figure 62** and **Figure 63**. In each case, you need to adjust the **Peak Analysis** settings either in the main screen configuration tab, or in the calibration curve screen, to select the proper number of peaks in the size calibration ladder well (including lower/upper markers) to achieve the proper size calibration. Once the proper number of peaks is selected, click the **Apply** button. The bottom task bar of the ProSize software will then display the file path with no errors.

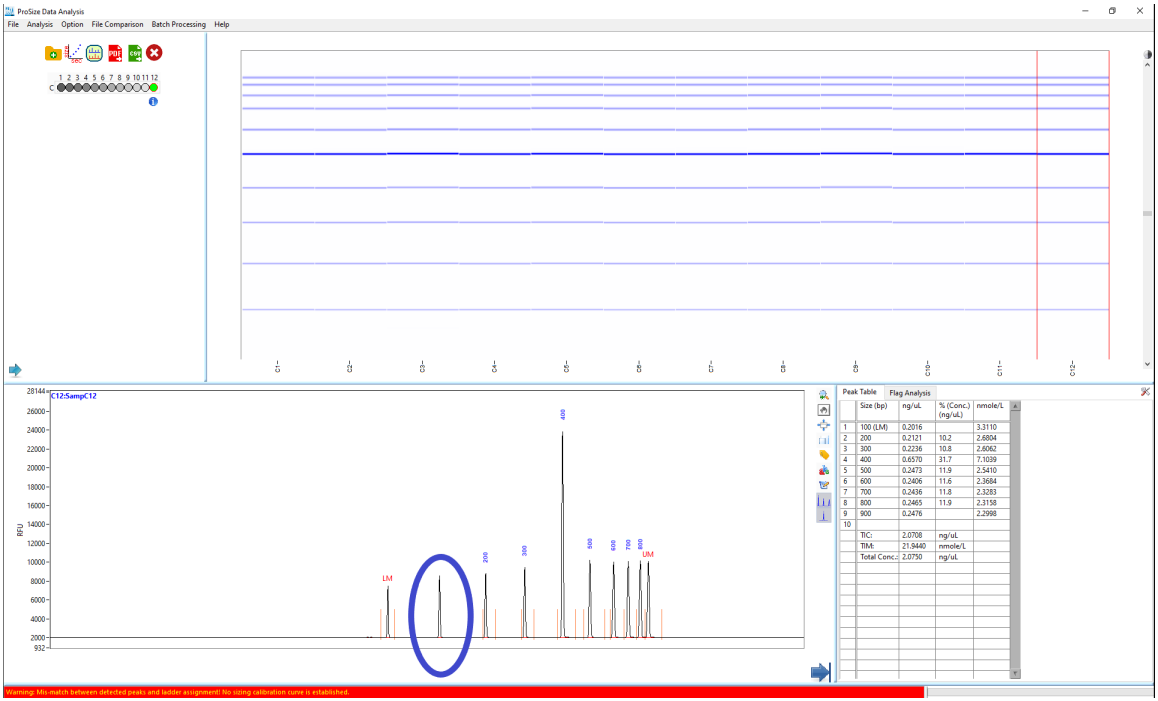


Figure 60 Main screen showing improperly fitted size calibration (not enough integrated peaks). Solution to correct: In the **Peak Analysis** table lower the Min. Peak Height (RFU) threshold to integrate missing peak (circled in blue).

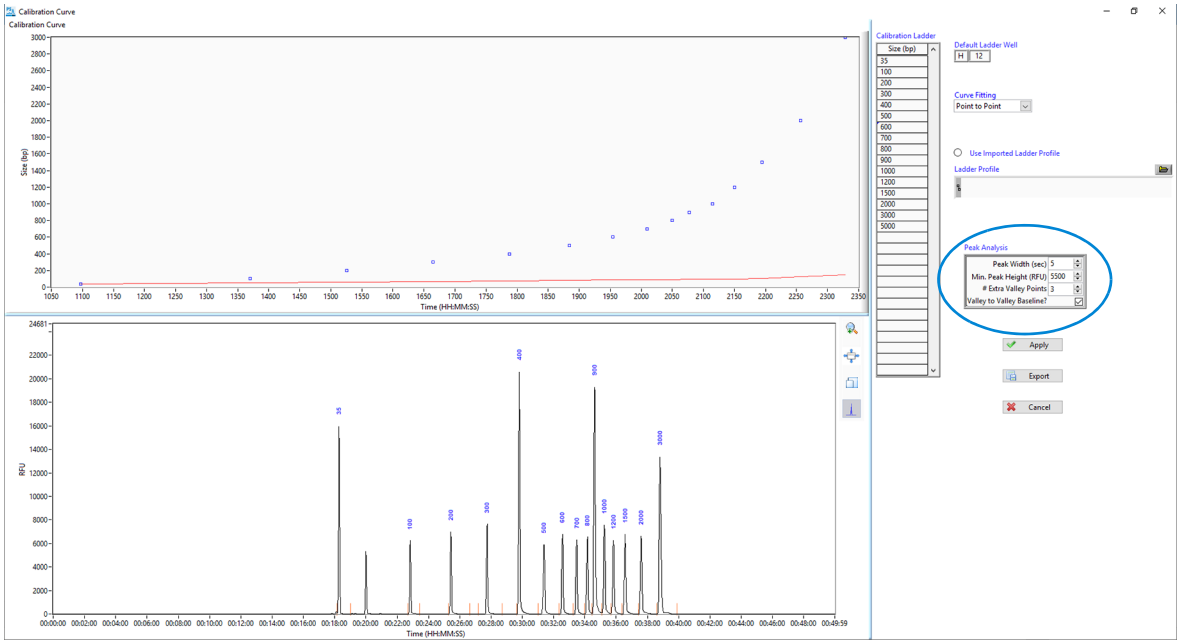


Figure 61 Calibration Curve screen showing improperly fit size calibration from **Figure 60**.

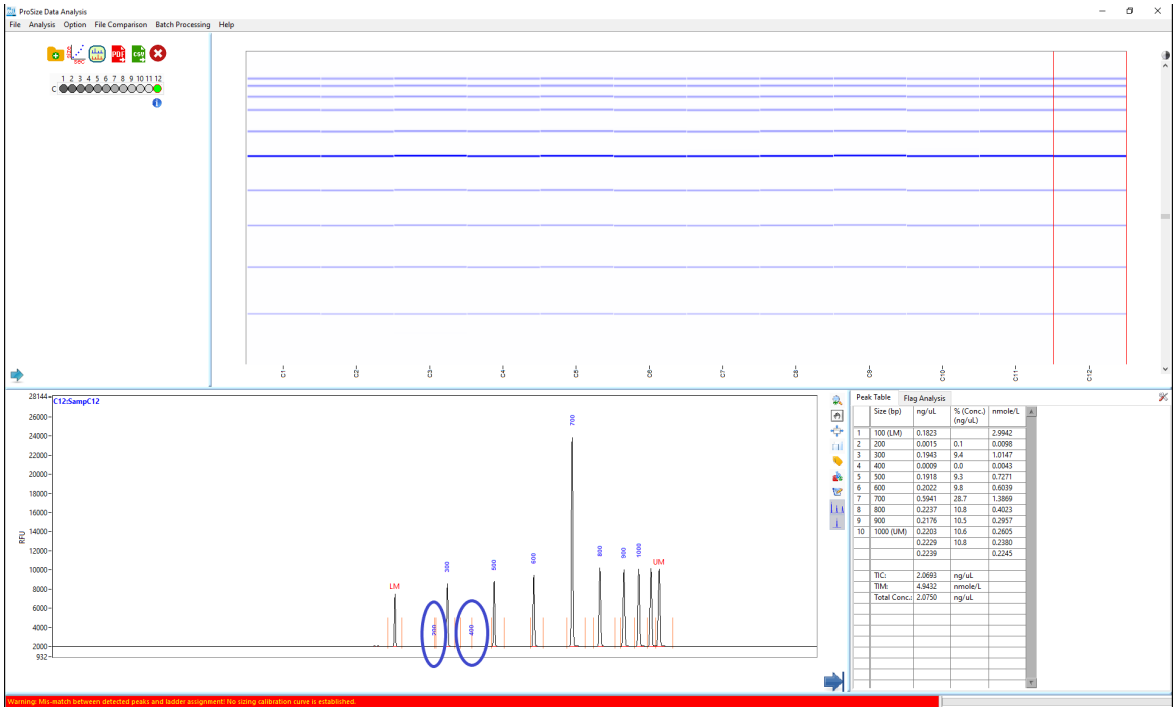


Figure 62 Main screen showing improperly fitted size calibration (too many integrated peaks, circled in blue). Solution to correct: increase the Min. Peak Height (RFU) threshold to no longer integrate extra peaks.

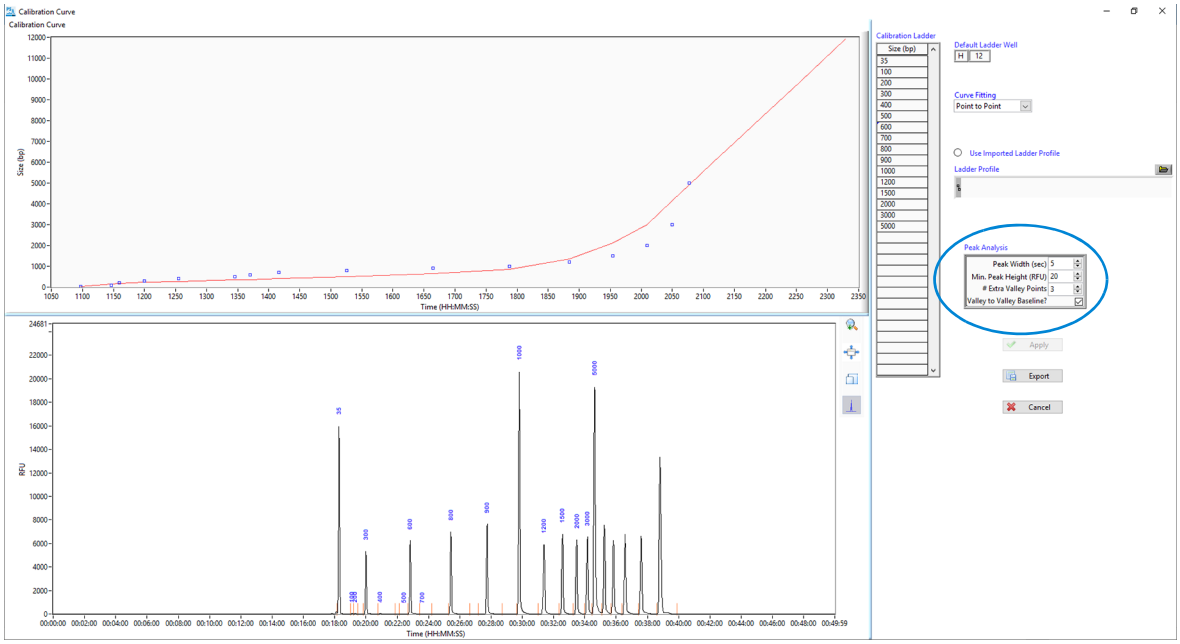


Figure 63 Calibration Curve screen showing improperly fit size calibration from **Figure 62**

6 ProSize Compare Files

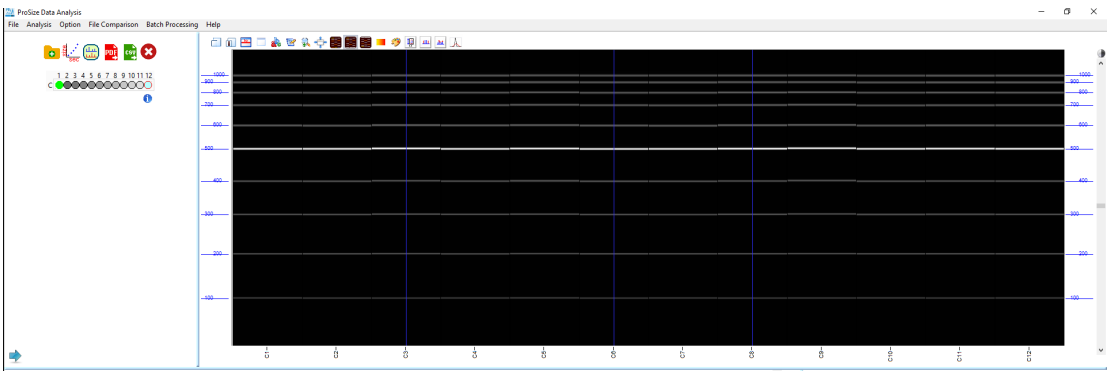
This chapter gives an overview of the possible ways to compare samples in the ProSize software.

Overlay Options

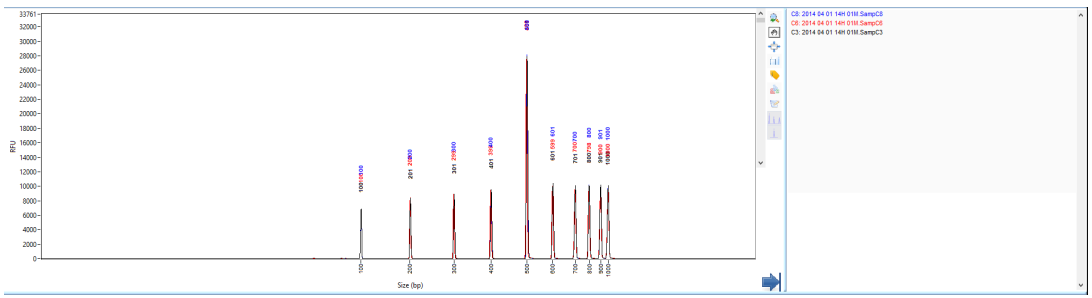
There are two options for comparing samples: The *Quick Overlay* and the *Compare Files* screen.

Quick Overlay

1 Right-click the sample on the gel image you want to overlay.



The overlay images appear in the electropherogram area.



2 Be sure to select the desired *Normalize* icon (for example, **Normalize to Lower Marker**) to get an accurate representation of how the samples compare with each other.

3 To end the *Quick Overlay* view, double-click on any lane of the gel image.

Compare Files Screen

The **Compare Files** screen is used to overlay and generate comparisons, in digital gel view and electropherogram view, of selected samples from the currently opened data file and optionally from different data files (when using the same markers and experimental conditions), and generate reports of the overlaid data.

Once the **Compare Files** screen is opened, all results are handled and saved as Projects. Before you can export data or generate a .pdf report, the results of the overlay plots must be saved as a Project.

The following sections describe how to open, select data to overlay, adjust/annotate overlaid data, open additional data files, and copy/export information from the **Compare Files** screen.

Opening the Compare Files Screen

1 To view the **Compare Files** screen from the main screen, select  or navigate to **Analysis** > **Overlay Samples**.

The **Compare Files** screen is displayed (**Figure 64**). **Table 47** summarizes the top menu bar functions.

Figure 64 **Compare Files** screen (no samples selected)

Table 47 **Compare Files top menu functions**

Menu Item	Description
Load	Opens a previously saved file comparison.
Save	Saves the current file comparison. Note: The user must save a file comparison before the export or "generate report" icons are active.
Save As	Saves an additional copy of the file comparison without replacing the original. Note: The user must save a file comparison before the export or "generate report" icons are active.
Create	Creates a new file comparison. This re-opens the file with a blank screen.
View Data File	To temporarily exit the file comparison screen to view the data file. To return to the **Compare Files** screen, select **File Comparison**.

Table 48 summarizes the available functions in the screen prior to opening any sample wells. A plate map menu is visible in the top left corner for selection of samples to overlay; the sample filename is listed at the top of the menu.

Table 48 Compare Files screen functions (no samples selected)

Menu Item	Description
Open	When selected, a file browser window opens for locating and opening an additional raw data file (*.raw extension) for data analysis comparison to the currently opened data file. Note: If multiple files are to be opened for comparison in the **Compare Files** screen, to generate a meaningful comparison, the additional opened files should: • Be previously processed in the ProSize software (i.e., markers selected, size calibrated). • The same lower/upper markers must be used. • Similar experimental conditions (separation gel, array length, separation voltage) must be used.
Exit **Compare Files**	Closes the **Compare Files** screen and returns to the main screen of the ProSize software.
Default Layout	Shows an "overlay of samples" using both the electropherogram and digital gel view in the same window)(**Figure 67**).
Image Layout	Shows an "overlay of samples" using a digital gel image window only (**Figure 68**).
Trace Layout	Shows an "overlay of samples" using an electropherogram trace window only (**Figure 69**).
PDF	Generates a PDF report of the **Compare Files** (see **Figure 70**). Each file will be color coated. Report options are discussed in detail in **Chapter 8**, "Generating Reports from ProSize". Data will be color coded per separation. Note: A project must be saved before the program allows you to generate a report.
Export Data	Exports data from the **Compare Files** (see **Figure 71**). Export options are discussed in detail in **Chapter 7**, "Exporting Data from ProSize". Data will be color coded per separation. Note: A file must be saved before the program allows you to export data.

Table 48 Compare Files screen functions (no samples selected)

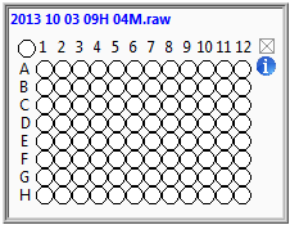
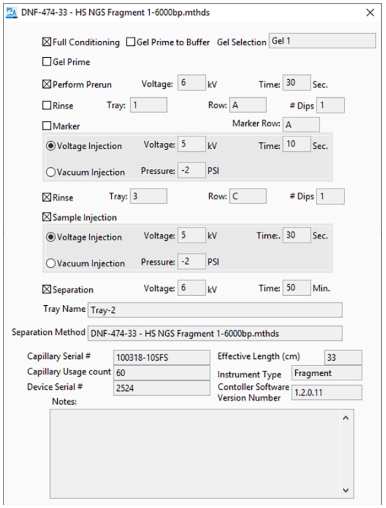
Menu Item	Description
Plate Map	Used to select a sample well(s) for comparison. To select well(s), click the well to add to the overlay; click a second time to remove (in any order). To add/remove a column, click the corresponding column number (**1**, **2**, etc.). To add/remove a row, click the corresponding row letter (**A**, **B**, etc.). To select/remove all wells of the plate, select the well in the upper left corner of the plate. The sample filename is displayed at the top of the Plate Map. 

Table 48 Compare Files screen functions (no samples selected)

Menu Item	Description
Sample Lists 	When selected, a **Sample ID** list will slide out (Below), enabling the User to select the samples for overlay by the Sample ID previously entered into the instrument software. To select a sample, click the sample name; that well will be highlighted. To remove a well, click it a second time. To clear all wells, right-click and select **Clear All**. To close the **Sample ID** navigation, click the arrow at the bottom right of the panel. 
Information 	When selected, a window opens displaying the instrument software user interface, containing experimental information and notes fields. 

Once sample wells are selected in the **Compare Files** screen, they are displayed in order of selection from left to right on the digital gel view and from bottom to top on the electropherogram trace view (**Figure 65**). If 24 samples are selected the Sample ID will be shown above the gel view, if more than 24 samples are selected the Sample ID will not be shown.

In the digital gel view, each sample/lane selected is annotated at the top by well ID and sample name; the currently opened data file is labeled with a (1) to signify it is the first file open. Additional data files if opened will have their samples/lanes labeled with (2), (3), etc. The side axes for the digital gel image are labeled by size, corresponding to the size calibration ladder. On the right side of the digital gel image, a slider bar is present allowing the adjustment of the intensity/contrast of the image by left clicking and dragging the bar.

In the electropherogram trace overlay, each sample is displayed in a different color with matching color annotation to the right of the overlay by well ID and sample name; the currently opened data file is labeled with a (1) to signify it is the first file open. Additional data files if opened will have their samples/lanes labeled with (2), (3), etc. The x-axis is labeled by **Size** and the y-axis by **RFU** in the trace overlay. On the right side of the electropherogram trace overlay, a slider bar is present to adjust the vertical spacing between traces by left clicking and dragging the bar.

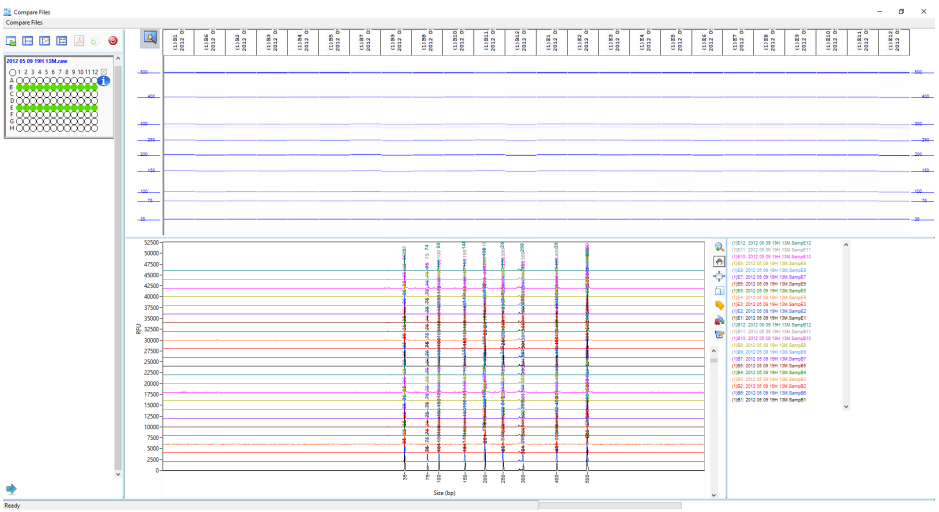


Figure 65 **Compare Files** screen (24 samples selected)

Display, Annotation and Export Options in the Compare Files Screen

Toolbar functions are available in the **Compare Files** screen for both the digital gel view and electropherogram trace overlays to adjust the display, annotate samples, and export results. These functions are summarized in **Table 49**.

To access the toolbar functions of the digital gel view overlay, click the corresponding icons (**Figure 66**). For the electropherogram trace overlay, in addition to the toolbar, a context menu is also available.

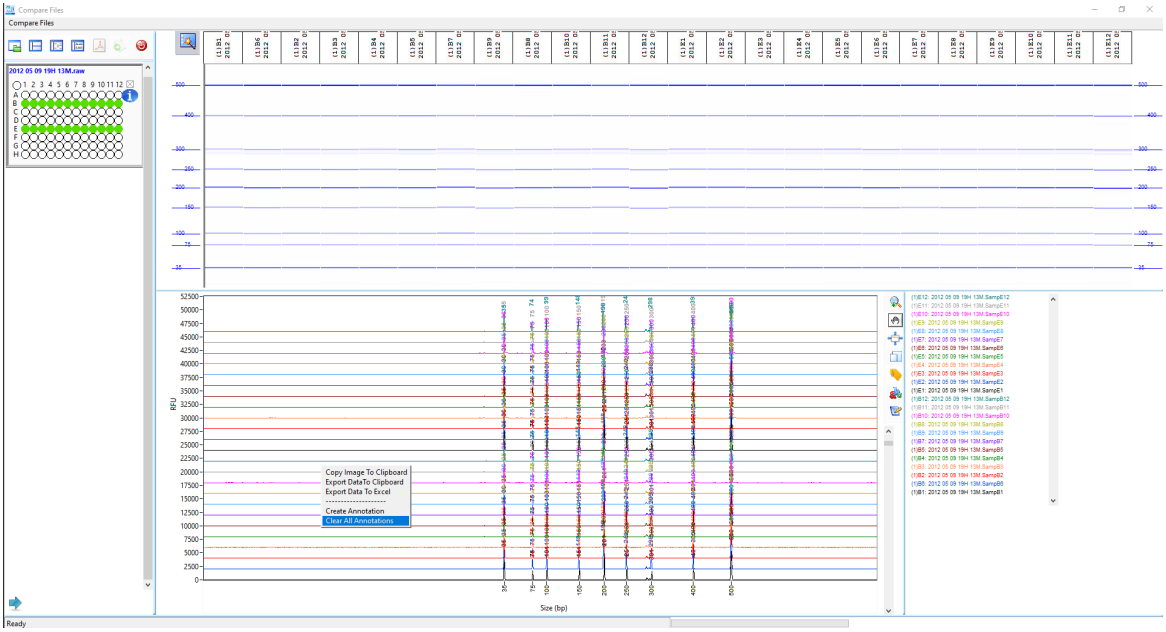


Figure 66 **Compare Files** screen (digital gel image toolbar and electropherogram trace with context menu)

Table 49 **Compare Files toolbar and context menu commands (samples selected)**

Menu Item	Description
Digital Gel Image Toolbar	
Copy Full Gel Image	Copies an image of the current 24 lane digital gel view overlay to the clipboard, for pasting in another program such as Microsoft PowerPoint. Any zoom, image normalization, or annotation will be copied in the image. The well ID and sample name will be copied on the top of each lane, and the y-axis will correspond to the currently selected size range obtained from the size calibration ladder in the copied image.

Table 49 Compare Files toolbar and context menu commands (samples selected)

Menu Item	Description
Show/Hide Cursor	Displays a horizontal line cursor across the digital gel image overlay, annotated by size (bp or nt) corresponding to the current position. The cursor can be dragged to any position along the gel image by clicking the cursor and holding the mouse button. When the cursor is active and displayed, a similar vertical cursor annotated by size is displayed in the electropherogram trace overlay, whose movement corresponds to that in the gel image. To deactivate the cursor, click the icon a second time.
Zoom	Enables zooming in the y-axis (Size) of the digital image overlay. To zoom-in: Place the mouse over the trace, click and drag it outward to expand a box area to define the zoom region. Release the mouse button to apply. The zoomed image can be copied to the clipboard.
AutoFit	To autoscale the digital gel image overlay y-axis view for all 24 currently viewed lanes. The autoscaled image will display the gel from the selected lower marker to upper marker, or if only a lower marker is used from the lower marker to the end of the separation window.
Auto Intensity	To autoscale the intensity of the digital gel image overlay. The viewed intensity across the image will reflect the actual observed fluorescence signals from each lane.
Normalize Intensity to Lower Marker	To normalize the intensity of the digital gel image overlay lower marker for each lane to the same value, adjusting the relative intensities of all bands in each lane accordingly. The viewed intensity across the image will as a result reflect the fluorescence signals from each lane after normalization to the lower marker, as is done when calculating the concentration of samples using the lower marker.
Normalize Intensity to Upper Marker	To normalize the intensity of the digital gel image overlay upper marker for each lane to the same value, adjusting the relative intensities of all bands in each lane accordingly. The viewed intensity across the image will as a result reflect the fluorescence signals from each lane after normalization to the upper marker, as is done when calculating the concentration of samples using the upper marker.
Gel Image Color	Changes the color scheme for the **Digital Gel View**, the gel image in the **Compare Files** screen and any exported or printed report. Options for the gel image color: • White on Black (white bands on black background) • Black on White (black bands on white background) • Green on Black (green bands on black background) • Red on Black (red bands on black background) • Pseudo Color (green to red bands on blue background)

Table 49 Compare Files toolbar and context menu commands (samples selected)

Menu Item	Description
Electropherogram Trace Overlay Toolbar	
Zoom	Enables zooming in the x-and y-axis of the Electropherogram Trace. To zoom-in: Place the mouse over the trace, click and drag it outward to expand a box area to define the zoom region. Release the mouse button to apply. The zoomed image can be copied to the clipboard. The zoom region will be preserved when viewing other samples in the Plate Map. To undo the zoom, use the **AutoFit** function.
Drag	Enables dragging in the x-and y-axis of the Electropherogram Trace. To drag: Place the mouse over the trace, click and drag it in any direction, and release the mouse.
AutoFit	To autoscale the Electropherogram Trace x-/y- axis display. The autoscaled image will display the full trace from the start to the end of the separation when using the **Time Scale** and **Size Scale** display modes; when using the **Uniform Size Scale**, the autoscaled display will start at 0 bp. Note: The y-axis scale will automatically zoom to the highest detected sample peak, regardless of the marker peak heights. To view the entire y-axis scale including markers, go to **Option,** and select **AutoFit** as the **Display Mode**.
Copy	Copies an image of the current view of the Electropherogram Trace to the clipboard, for pasting in another program, such as Microsoft PowerPoint. Any zoom, annotation, baseline and/or peak start/end point displayed will be copied in the image. The well ID and sample name will be copied in the top left of the trace, and the x-/y-axis will correspond to the currently selected view in the copied image.
Peak Label	Displays a menu for changing the peak annotation of the Electropherogram Trace. Only integrated peaks are annotated. Units for the peak annotation: • None • Peak ID (labels in order as 1, 2, etc.) • Migration Time (min:sec) raw migration time • Peak Height (in RFUs) • Corrected Peak Area (Peak Area/Migration Time) • Size (in bp or nt) • Average Size (in bp or nt) • Concentration (in ng/µL)

Table 49 Compare Files toolbar and context menu commands (samples selected)

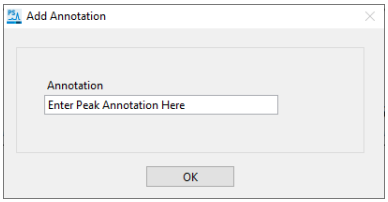
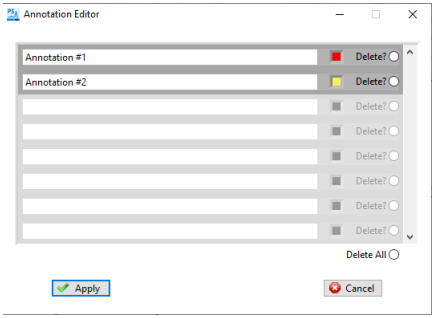
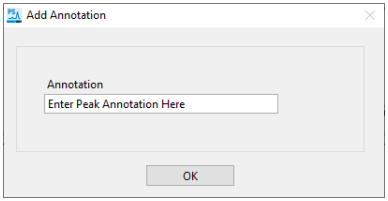
Menu Item	Description
Create Annotation	To create customized annotation in the Electropherogram Trace display. In the **Add Annotation** dialog, the user can type desired annotation into the field. Click **OK** and the annotation will be displayed in the Electropherogram Trace window.  To move the annotation to the desired position on the trace: Click the annotation and drag it to the desired position. An arrow will appear at the opposite end of the annotation upon dragging. Multiple annotations can be created by repeatedly selecting **Create Annotation**. Any created annotations will be copied to the clipboard with the **Copy** function.
Edit Annotation	Opens the **Annotation Editor** window. Allows the user to: • Edit the annotation text by typing in the text field. • Change the color of the annotation by left clicking on the annotation color. • Delete one annotation by selecting **Delete** next to each annotation, or delete all annotations by selecting **Delete All**.  Select **Apply** to confirm your settings.
Electropherogram Trace Overlay Context Menu	
Copy Image to Clipboard	Copies an image of the current view of the Electropherogram Trace to the clipboard, for pasting in another program, such as Microsoft PowerPoint. Any zoom, spacing, or annotation displayed will be copied in the image. This function operates similar to the **Copy** icon function.

Table 49 Compare Files toolbar and context menu commands (samples selected)

Menu Item	Description
Export Data to Clipboard	To copy the electropherogram x-data (time; in seconds, starting with the lower marker) and y-data (RFUs) in a .csv format that can be pasted directly into common spreadsheet programs such as Microsoft Excel. The x-data is copied into the first column, and the y-data into the second column of the file from left to right starting with the bottom- most sample upon pasting. The columns are annotated starting with Plot 0, 1, 2, etc. in order. To export, right-click to display the context menu and select **Export Data to Clipboard**. Then open spreadsheet, position in desired cell and paste into the program; process and save as desired.
Export Data to Excel	This function requires Microsoft Excel to be installed on the computer to function properly. Selection will automatically open Microsoft Excel and copy each overlaid electropherogram x-data (time; in seconds, starting with the lower marker) and y-data (RFUs) in a .csv file format. The x-data is copied into the first column, and the y-data into the second column of the file from left to right starting with the bottom- most sample upon pasting. The columns are annotated starting with Plot 0, 1, 2, etc. in order. To export, right-click to display the context menu, and select **Export Data to Excel**. The file can then be processed and saved as desired.
Create Annotation	To create customized annotation in the Electropherogram Trace display. In the **Add Annotation** dialog, the user can type desired annotation into the field. Click **OK** and the annotation will be displayed in the Electropherogram Trace window.  To move the annotation to the desired position on the trace: Click the annotation and drag it to the desired position. An arrow will appear at the opposite end of the annotation upon dragging. Multiple annotations can be created by repeatedly selecting **Create Annotation**. Any created annotations will be copied to the clipboard with the **Copy** function.
Clear All Annotations	Clears your created annotations from the electropherogram trace overlay display.

Displaying Multiple Files in the Compare Files Screen

Several different data files can be simultaneously opened and compared using the **Compare Files** screen in ProSize.

To compare different data sets, several criteria should be met when overlaying multiple files:

- The data must use the same size lower/upper marker combination. For RNA analysis, the same lower marker should be used.
- Any additional data files opened in the **Compare Files** screen should be previously processed, with the lower/upper markers correctly selected for the data and the size calibration correctly performed. No additional processing can be done on the opened comparison files; only adjustments to the intensity/spacing can be made.
- The experimental conditions should ideally be the same or similar for both sets of data, using the same separation gel, array length, separation voltage, and/or assay type (for example, NGS Kit).

With the currently open data file displayed in the **Compare Files** screen, select  to open a second data file. A file browser menu will be displayed for locating and opening the additional raw data file (*.raw extension). Once opened, a second **Plate Map** will be displayed for the second data file below the original file, labeled at the top with the filename (**Figure 67**).

All functions apply the same to the second opened data file compared the first; to close the second data file at any time, click ⊠ in the upper right corner of the Plate Map.

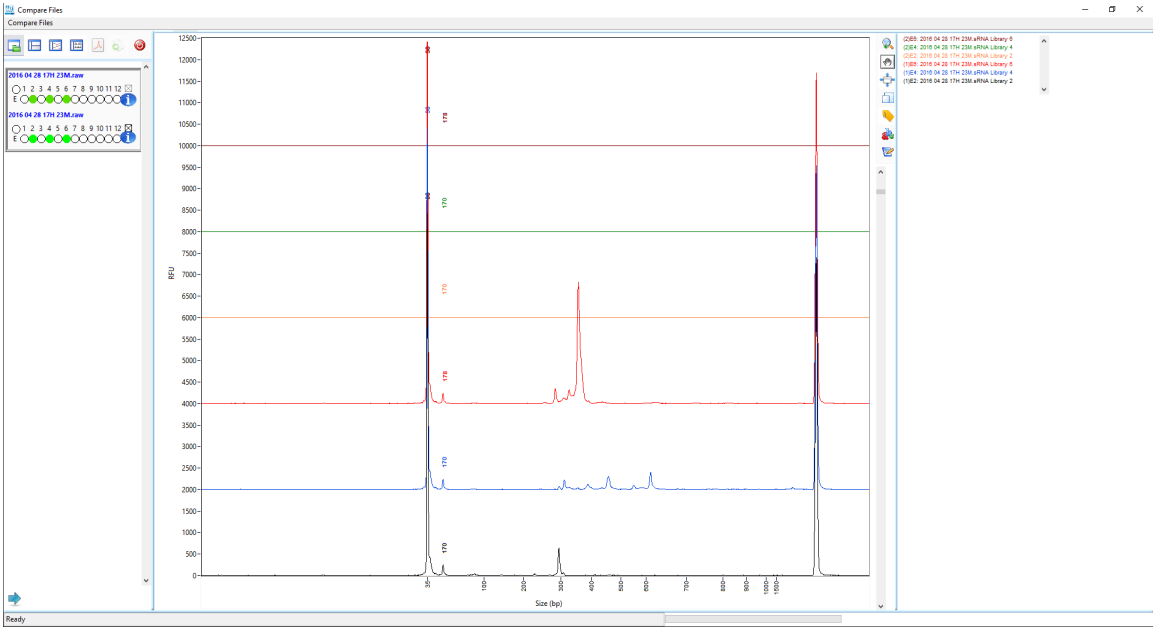


Figure 67 **Compare Files** screen displaying two different data files open

Alternate Views in the Compare Files Screen

Several different views can be displayed in the Compare Files screen as described in **Table 48**. **Figure 68** shows the Image Layout view; **Figure 69** shows the Trace Layout view. The functions in these screens are the same as those in the Default Layout view.

Figure 68 **Compare Files** screen displaying Image Layout view

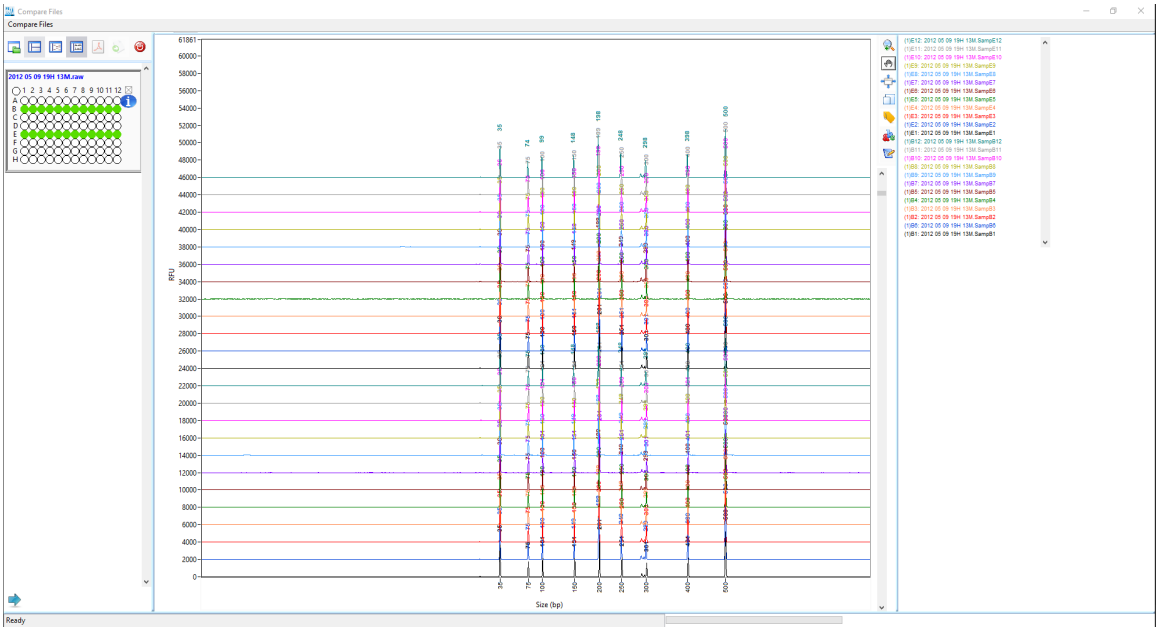


Figure 69 Compare Files screen displaying Trace Layout view

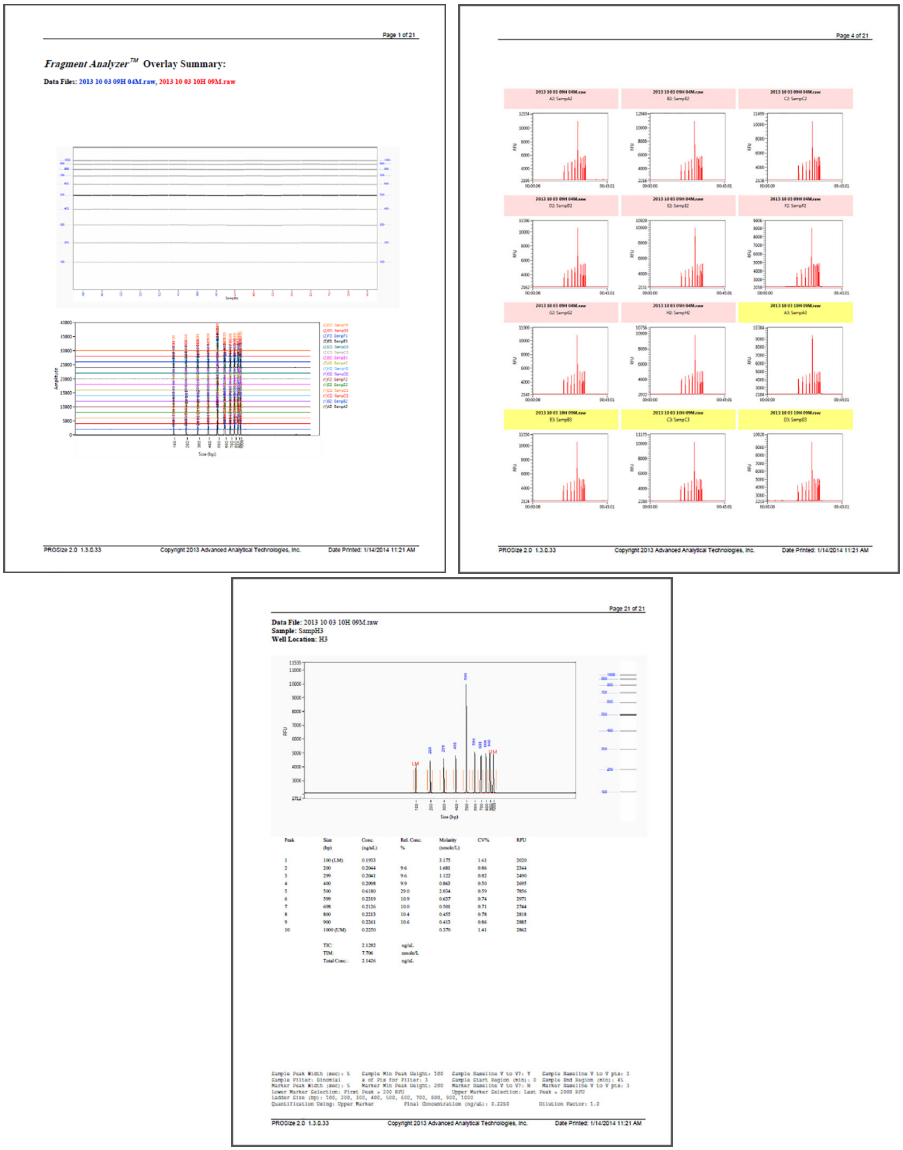


Figure 70 **Compare Files** PDF Report showing the first page of overlayed traces page, Traces Summary page, and Electropherogram/Peak Table page

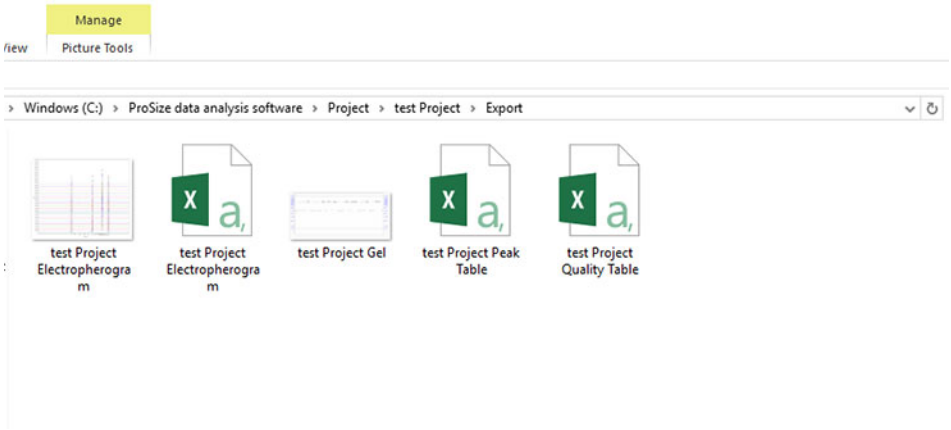


Figure 71 Export folder showing files generated from the **Compare Files** screen

7 Exporting Data from ProSize

This chapter provides an overview of the options available for exporting processed data from the ProSize software. The following sections describe how to open the Export Data window; provide an outline of various data export options; describe how to perform data export; and give examples of exported data.

Export Data Utility

Once the data is opened and processed within the ProSize program, the measured/calculated information can be exported in common formats for storage in a common database or for use in other programs.

Export Data is used to export information from the ProSize program.

Export Data Window Settings

To export sample results from the ProSize software:

1 From the main screen, select , or navigate to **File** > **Export Data**.

The **Export Data** window will be displayed (Figure 72).

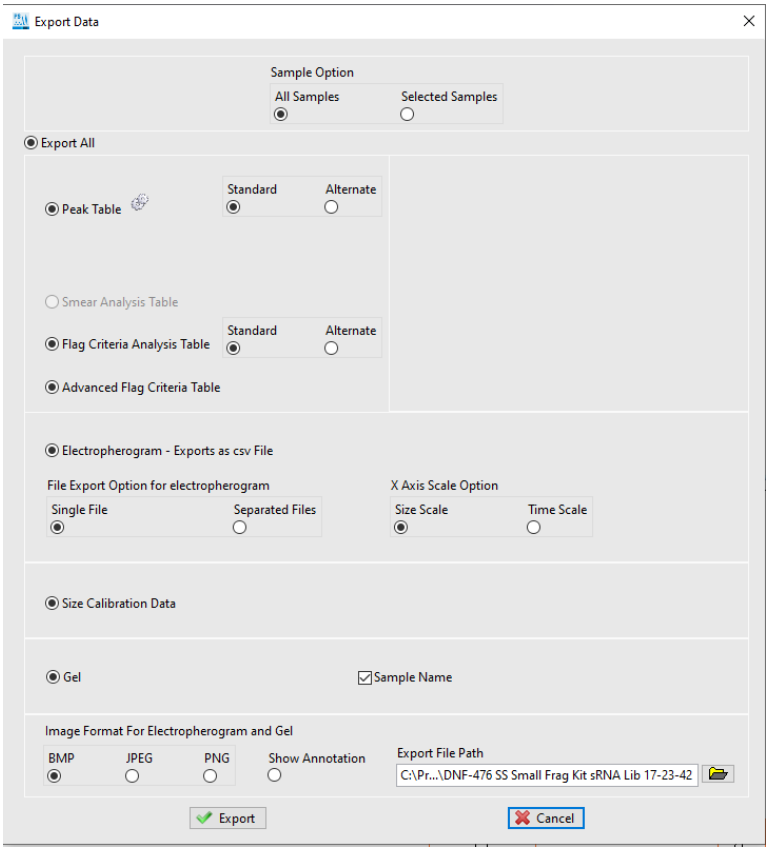


Figure 72 Export Data window

Table 50 summarizes the available settings in the **Export Data** window.

Table 50 Export Data settings

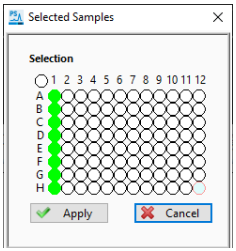
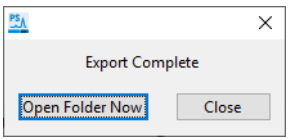
Settings	Description
Sample Option	Determines which samples of the data file will have information exported. There are two options: **All Samples** (results for all samples of data file exported); and **Selected Samples**. The Selected Samples option will open the **Selected Samples** window.  Select the respective wells, columns and/or rows of the sample plate, and click **Apply**. To abort the operation and export all samples, click **Cancel**.
Export All	Exports all fields of the Export Data utility for the data file.
Peak Table – Standard or Alternate	**Standard**: Exports the **Peak Table** for all selected samples as a single .csv file, listed in order of sample well (**Figure 74**). **Alternate**: Exports the **Peak Table** for all selected samples as a single .csv file, listed in rows (**Figure 75**).
Smear Analysis Table	Exports the **Smear Analysis** table for all selected samples as a single .csv file, listed in order of sample well (**Figure 76**). If no data is present in the **Smear Analysis** table, this function will be disabled.
Flag Analysis Criteria Table – Standard or Alternate	**Standard**: Exports the **Flag Analysis** table for all selected samples as a single .csv file, listed in order of sample well (**Figure 77**). The output will be in a binary 0/1 format, with each column labeled by the respective Flag condition. **Alternate**: Export the **Flag Analysis** table for all selected samples as a single .csv file, listed in order of sample well) (**Figure 78**). The output will list the values within the set criteria of the Flag analysis by row. If no data is present in the **Flag Analysis** table, this function will be disabled.
Advanced Flag Criteria Table	Exports the **Advanced Flag Analysis** table for all selected samples as a single .csv file, listed in order of sample well (**Figure 79**). The output will lists the values for the configurations of the **Advanced Flag analysis** by row.
Electropherogram – Exports as CSV File	Exports electropherogram data in a .csv file format.

Table 50 Export Data settings

Settings	Description
File Export Option	Determines how electropherogram .csv data is exported. When **Single File** is selected, will export all electropherograms together in a single file, with the x-axis in the first column and each sample result's y-axis data in separate columns increasing from left to right (**Figure 80**). When **Separated Files** is selected, will export each electropherogram as a separate file, with the x-axis in the first column and the y-xis data in the second column (**Figure 81**).
X Axis Scale Option	Determines how electropherogram .csv data is formatted in x-axis. When **Size Scale** is selected, will export x-axis data as size, starting with 0 (**Figure 80**). When **Time Scale** is selected, will export x-axis as time, starting with 0 seconds (**Figure 81**).
Size Calibration Data	Exports the **Size Calibration** information as a .csv file, with column 1 as **Ladder Size** and column 2 as **Time** (sec) (**Figure 82**).
Exports Selected Samples Digital Gel Image	Exports a digital gel image overlay of the selected samples as an image file, in the format specified (.bmp, .jpg, or .png).
Image Format for Electropherogram and Gel	Determines which image file format is used for exported electropherogram images and digital gel image overlay (**Figure 83**). There are three different image file format options: • BMP (.bmp extension) • JPEG (.jpg extension) • PNG (.png extension)
Show Annotation	Shows any annotation(s) made to the sample wells on Digital Gel Image.
Export File Path	Determines the file path for saving exported data. The default directory is the same folder that contains the .raw data file (recommended). To select an alternative directory, click the folder icon. In the file browser menu, navigate to the desired directory, and save the file.
Export	Exports the data file with the settings made. In the dialog **Export Complete**, select **Open Folder Now** to open the directory. Click **Close** to close without opening the export folder. 
Cancel	Cancels the export operation and returns to the main screen of the ProSize software.

Examples of Exported Data

When exporting data from ProSize, the exported files will be named by the .raw file name followed by an extension dependent upon the information exported (**Figure 73** and **Table 51**).

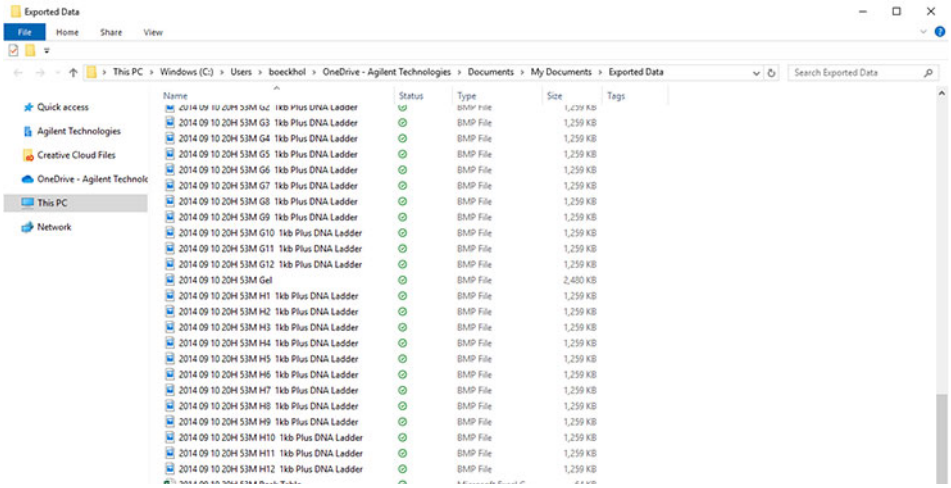


Figure 73 Exported data showing file name conventions

Table 51 **Exported data naming conventions**

Exported Item	Description
Peak Table	Filename **Peak Table** Example: *2013 07 15 18H 29M Peak Table*
Size Calibration	Filename **Size Calibration** Example: *2013 07 15 18H 29M Size Calibration*
Smear Analysis	Filename **Smear Analysis Result** Example: *2013 07 15 18H 29M Smear Analysis Result*
Flag Analysis Result	Filename **Flag Analysis Result** Example: *2013 07 15 18H 29M Flag Analysis Result*
Advanced Flag Analysis Result	Filename **Advanced Flag Analysis Result** Example: *2013 05 08 16H 02M Advanced Flag Analysis Result*
Selected Samples Gel Image	Filename **Gel** Example: *2013 07 15 18H 29M Gel*

Table 51 Exported data naming conventions

Exported Item	Description
Individual Electropherogram Gel Image	Filename <Well ID> <Sample Name> Example: *2013 07 15 18H 29M H1 SampH1*
Individual Electropherogram Data (CSV)	• If exported as one file: Filename **Electropherogram** Example: *2013 07 15 18H 29M Electropherogram* • If exported as individual files: Filename <Well ID> <Sample Name> Example: *2013 07 15 18H 29M H1 SampH1*

The following figures show examples of typical exported data formats:

- Peak Table

	A	B	C	D	E	F	G	H	I
1	C1	2014 04 01 14H 01M.SampC1							
2	Peak	Size (bp)	Concentra	Percent relative concentration	RFU	Corrected peak area	nmole/L	From (bp)	To (bp)
3	1	100 (LM)	0.1825		5097	19.982	2.9966	92	113
4	2	200	0.1891	9.6	5968	20.702	1.5543	194	228
5	3	299	0.1862	9.4	6124	20.391	1.0211	289	325
6	4	399	0.1945	9.8	6558	21.294	0.7999	389	426
7	5	499	0.5746	29.1	19572	62.915	1.8909	484	547
8	6	600	0.2116	10.7	7284	23.171	0.5804	588	656
9	7	700	0.2037	10.3	7054	22.304	0.4789	687	779
10	8	798	0.2052	10.4	6981	22.467	0.4226	779	874
11	9	900	0.2111	10.7	7210	23.113	0.386	874	961
12	10	1000 (UM)	0.2139		7108	23.426	0.3518	961	1180
13									
14		TIC:	1.9759	ng/uL					
15		TIM:	7.1341	nmole/L					
16		Total Con	1.984	ng/uL					
17									
18	C2	2014 04 01 14H 01M.SampC2							
19	Peak	Size (bp)	Concentra	Percent relative concentration	RFU	Corrected peak area	nmole/L	From (bp)	To (bp)
20	1	100 (LM)	0.1825		5508	22.225	2.9966	88	119
21	2	200	0.1934	9.8	6704	23.56	1.5904	195	229
22	3	299	0.1897	9.6	6944	23.102	1.0401	295	331
23	4	399	0.1972	10	7355	24.012	0.811	391	430
24	5	499	0.5742	29	20865	69.924	1.8895	483	545
25	6	599	0.2137	10.8	7795	26.029	0.5872	584	648
26	7	699	0.2044	10.3	7413	24.894	0.4806	685	771
27	8	798	0.2063	10.4	7472	25.123	0.4249	771	865
28	9	900	0.2023	10.2	7160	24.642	0.37	865	961
29	10	1000 (UM)	0.2067		7150	25.17	0.3402	961	1159
30									
31		TIC:	1.9812	ng/uL					
32		TIM:	7.1937	nmole/L					
33		Total Con	1.9893	ng/uL					

Figure 74 Exported Peak table - Standard file format

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Well	Sample ID	Peak	Size (bp)	Concentration (ng/uL)	Percent relative concentration	RFU	Corrected peak area	nmole/L	From (bp)	To (bp)	TIC	TIM	Total Conc.
2	C1	SampC1	1	100 (LM)	0.1825		5097	19.982	2.9966	92	113	1.9759	7.1341	1.984
3	C1	SampC1	2	200	0.1891	9.6	5968	20.702	1.5543	194	228	1.9759	7.1341	1.984
4	C1	SampC1	3	299	0.1862	9.4	6124	20.391	1.0211	289	325	1.9759	7.1341	1.984
5	C1	SampC1	4	399	0.1945	9.8	6558	21.294	0.7999	389	426	1.9759	7.1341	1.984
6	C1	SampC1	5	499	0.5746	29.1	19572	62.915	1.8909	484	547	1.9759	7.1341	1.984
7	C1	SampC1	6	600	0.2116	10.7	7284	23.171	0.5804	588	656	1.9759	7.1341	1.984
8	C1	SampC1	7	700	0.2037	10.3	7054	22.304	0.4789	687	779	1.9759	7.1341	1.984
9	C1	SampC1	8	798	0.2052	10.4	6981	22.467	0.4226	779	874	1.9759	7.1341	1.984
10	C1	SampC1	9	900	0.2111	10.7	7210	23.113	0.386	874	961	1.9759	7.1341	1.984
11	C1	SampC1	10	1000 (UM)	0.2139		7108	23.426	0.3518	961	1180	1.9759	7.1341	1.984
12	C1	SampC1										1.9759	7.1341	1.984
13	C2	SampC2	1	100 (LM)	0.1825		5508	22.225	2.9966	88	119	1.9812	7.1937	1.9893
14	C2	SampC2	2	200	0.1934	9.8	6704	23.56	1.5904	195	229	1.9812	7.1937	1.9893
15	C2	SampC2	3	299	0.1897	9.6	6944	23.102	1.0401	295	331	1.9812	7.1937	1.9893
16	C2	SampC2	4	399	0.1972	10	7355	24.012	0.811	391	430	1.9812	7.1937	1.9893
17	C2	SampC2	5	499	0.5742	29	20865	69.924	1.8895	483	545	1.9812	7.1937	1.9893
18	C2	SampC2	6	599	0.2137	10.8	7795	26.029	0.5872	584	648	1.9812	7.1937	1.9893
19	C2	SampC2	7	699	0.2044	10.3	7413	24.894	0.4806	685	771	1.9812	7.1937	1.9893
20	C2	SampC2	8	798	0.2063	10.4	7472	25.123	0.4249	771	865	1.9812	7.1937	1.9893
21	C2	SampC2	9	900	0.2023	10.2	7160	24.642	0.37	865	961	1.9812	7.1937	1.9893
22	C2	SampC2	10	1000 (UM)	0.2067		7150	25.17	0.3402	961	1159	1.9812	7.1937	1.9893
23	C2	SampC2										1.9812	7.1937	1.9893
24	C3	SampC3	1	100 (LM)	0.1825		5578	22.567	2.9966	85	118	2.0413	7.3364	2.0507

Figure 75 Exported Peak Table - Alternate file format

- Smear Analysis

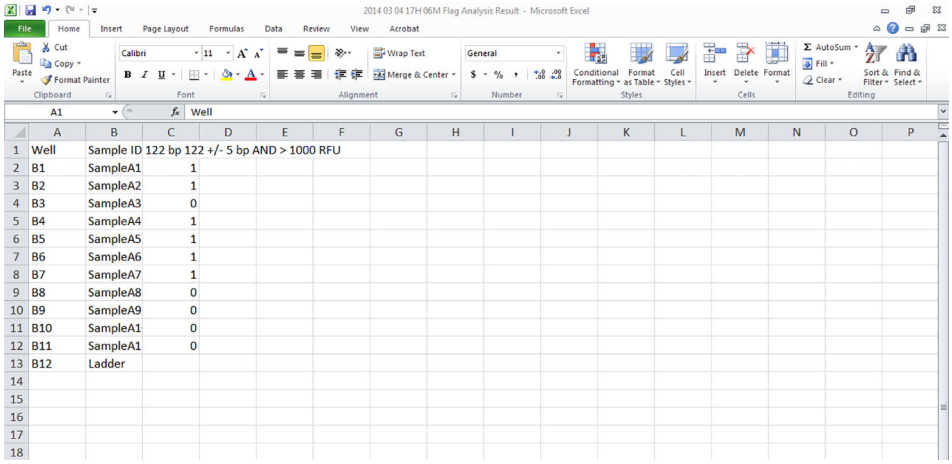
NOTE

When multiple Smear Analysis conditions are entered for a sample, separate lines will be created in the exported table. More information is available in **Chapter 4**, “ProSize Configuration”.

	A	B	C	D	E	F	G	H
1	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
2	E1	212 nt	160 nt to 313 nt	38.5423	83.8	6.5639	199	6.56
3	E2	410 nt	358 nt to 572 nt	21.7135	62.7	5.0154	425	5.02
4	E3	493 nt	398 nt to 595 nt	13.0394	48.1	5.529	476	5.53
5	E4	894 nt	694 nt to 1188 nt	35.7947	94.3	3.5555	908	3.56
6	E5	996 nt	866 nt to 1313 nt	24.0513	82.3	4.3096	1013	4.31
7	E6	1902 nt	1494 nt to 3453 nt	13.909	80.5	15.6505	1987	15.65

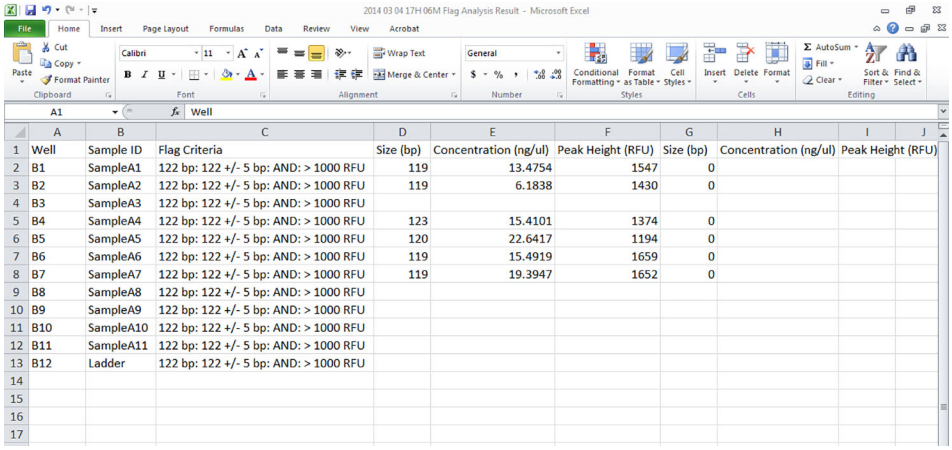
Figure 76 Exported smear Analysis file format

- Flag Analysis Criteria Table



	A	B	C
1	Well	Sample ID 122 bp 122 +/- 5 bp AND > 1000 RFU	
2	B1	SampleA1	1
3	B2	SampleA2	1
4	B3	SampleA3	0
5	B4	SampleA4	1
6	B5	SampleA5	1
7	B6	SampleA6	1
8	B7	SampleA7	1
9	B8	SampleA8	0
10	B9	SampleA9	0
11	B10	SampleA1	0
12	B11	SampleA1	0
13	B12	Ladder	

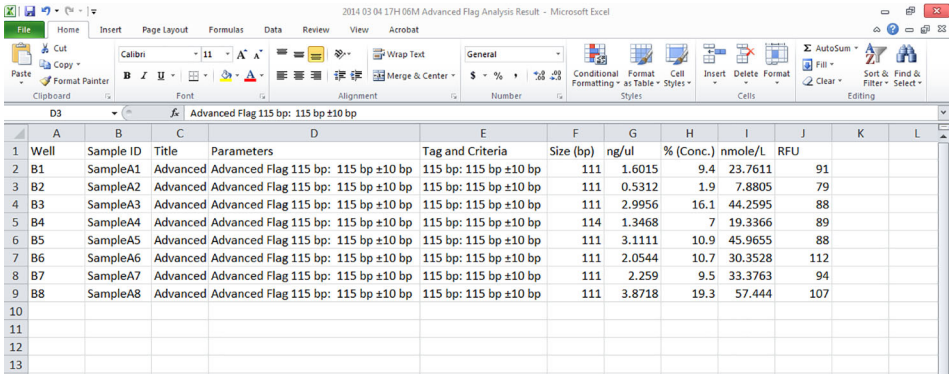
Figure 77 Exported Flag Analysis - Standard file format



	A	B	C	D	E	F	G	H	I	J
1	Well	Sample ID	Flag Criteria	Size (bp)	Concentration (ng/ul)	Peak Height (RFU)	Size (bp)	Concentration (ng/ul)	Peak Height (RFU)	
2	B1	SampleA1	122 bp: 122 +/- 5 bp: AND: > 1000 RFU	119	13.4754	1547	0			
3	B2	SampleA2	122 bp: 122 +/- 5 bp: AND: > 1000 RFU	119	6.1838	1430	0			
4	B3	SampleA3	122 bp: 122 +/- 5 bp: AND: > 1000 RFU							
5	B4	SampleA4	122 bp: 122 +/- 5 bp: AND: > 1000 RFU	123	15.4101	1374	0			
6	B5	SampleA5	122 bp: 122 +/- 5 bp: AND: > 1000 RFU	120	22.6417	1194	0			
7	B6	SampleA6	122 bp: 122 +/- 5 bp: AND: > 1000 RFU	119	15.4919	1659	0			
8	B7	SampleA7	122 bp: 122 +/- 5 bp: AND: > 1000 RFU	119	19.3947	1652	0			
9	B8	SampleA8	122 bp: 122 +/- 5 bp: AND: > 1000 RFU							
10	B9	SampleA9	122 bp: 122 +/- 5 bp: AND: > 1000 RFU							
11	B10	SampleA10	122 bp: 122 +/- 5 bp: AND: > 1000 RFU							
12	B11	SampleA11	122 bp: 122 +/- 5 bp: AND: > 1000 RFU							
13	B12	Ladder	122 bp: 122 +/- 5 bp: AND: > 1000 RFU							

Figure 78 Exported Flag Analysis - Alternate file format

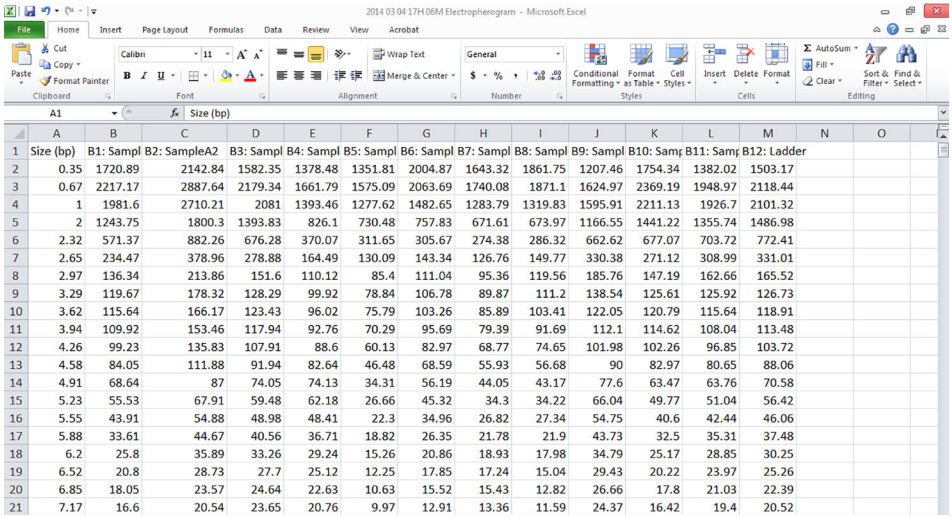
- Advanced Flag Analysis Criteria Table



	A	B	C	D	E	F	G	H	I	J	K	L
1	Well	Sample ID	Title	Parameters	Tag and Criteria	Size (bp)	ng/ul	% (Conc.)	nmole/L	RFU		
2	B1	SampleA1	Advanced	Advanced Flag 115 bp: 115 bp ±10 bp	115 bp: 115 bp ±10 bp	111	1.6015	9.4	23.7611	91		
3	B2	SampleA2	Advanced	Advanced Flag 115 bp: 115 bp ±10 bp	115 bp: 115 bp ±10 bp	111	0.5312	1.9	7.8805	79		
4	B3	SampleA3	Advanced	Advanced Flag 115 bp: 115 bp ±10 bp	115 bp: 115 bp ±10 bp	111	2.9956	16.1	44.2595	88		
5	B4	SampleA4	Advanced	Advanced Flag 115 bp: 115 bp ±10 bp	115 bp: 115 bp ±10 bp	114	1.3468	7	19.3366	89		
6	B5	SampleA5	Advanced	Advanced Flag 115 bp: 115 bp ±10 bp	115 bp: 115 bp ±10 bp	111	3.1111	10.9	45.9655	88		
7	B6	SampleA6	Advanced	Advanced Flag 115 bp: 115 bp ±10 bp	115 bp: 115 bp ±10 bp	111	2.0544	10.7	30.3528	112		
8	B7	SampleA7	Advanced	Advanced Flag 115 bp: 115 bp ±10 bp	115 bp: 115 bp ±10 bp	111	2.259	9.5	33.3763	94		
9	B8	SampleA8	Advanced	Advanced Flag 115 bp: 115 bp ±10 bp	115 bp: 115 bp ±10 bp	111	3.8718	19.3	57.444	107		
10												
11												
12												
13												

Figure 79 Exported Advanced Flag Analysis

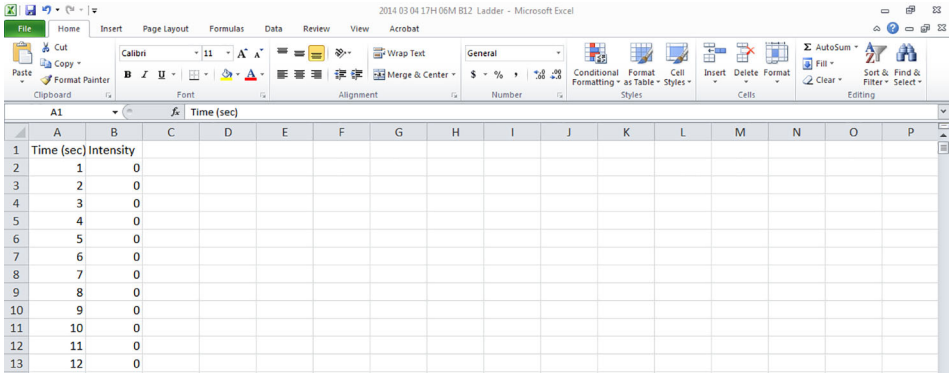
- Electropherogram CSV – Single File – Size Scale



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Size (bp)	B1: Sampl	B2: SampleA2	B3: Sampl	B4: Sampl	B5: Sampl	B6: Sampl	B7: Sampl	B8: Sampl	B9: Sampl	B10: Samp	B11: Samp	B12: Ladder		
2	0.35	1720.89	2142.84	1582.35	1378.48	1351.81	2004.87	1643.32	1861.75	1207.46	1754.34	1382.02	1503.17		
3	0.67	2217.17	2887.64	2179.34	1661.79	1575.09	2063.69	1740.08	1871.1	1624.97	2369.19	1948.97	2118.44		
4	1	1981.6	2710.21	2081	1393.46	1277.62	1482.65	1283.79	1319.83	1595.91	2211.13	1926.7	2101.32		
5	2	1243.75	1800.3	1393.83	826.1	730.48	757.83	671.61	673.97	1166.55	1441.22	1355.74	1486.98		
6	2.32	571.37	882.26	676.28	370.07	311.65	305.67	274.38	286.32	662.62	677.07	703.72	772.41		
7	2.65	234.47	378.96	278.88	164.49	130.09	143.34	126.76	149.77	330.38	271.12	308.99	331.01		
8	2.97	136.34	213.86	151.6	110.12	85.4	111.04	95.36	119.56	185.76	147.19	162.66	165.52		
9	3.29	119.67	178.32	128.29	99.92	78.84	106.78	89.87	111.2	138.54	125.61	125.92	126.73		
10	3.62	115.64	166.17	123.43	96.02	75.79	103.26	85.89	103.41	122.05	120.79	115.64	118.91		
11	3.94	109.92	153.46	117.94	92.76	70.29	95.69	79.39	91.69	112.1	114.62	108.04	113.48		
12	4.26	99.23	135.83	107.91	88.6	60.13	82.97	68.77	74.65	101.98	102.26	96.85	103.72		
13	4.58	84.05	111.88	91.94	82.64	46.48	68.59	55.93	56.68	90	82.97	80.65	88.06		
14	4.91	68.64	87	74.05	74.13	34.31	56.19	44.05	43.17	77.6	63.47	63.76	70.58		
15	5.23	55.53	67.91	59.48	62.18	26.66	45.32	34.3	34.22	66.04	49.77	51.04	56.42		
16	5.55	43.91	54.88	48.98	48.41	22.3	34.96	26.82	27.34	54.75	40.6	42.44	46.06		
17	5.88	33.61	44.67	40.56	36.71	18.82	26.35	21.78	21.9	43.73	32.5	35.31	37.48		
18	6.2	25.8	35.89	33.26	29.24	15.26	20.86	18.93	17.98	34.79	25.17	28.85	30.25		
19	6.52	20.8	28.73	27.7	25.12	12.25	17.85	17.24	15.04	29.43	20.22	23.97	25.26		
20	6.85	18.05	23.57	24.64	22.63	10.63	15.52	15.43	12.82	26.66	17.8	21.03	22.39		
21	7.17	16.6	20.54	23.65	20.76	9.97	12.91	13.36	11.59	24.37	16.42	19.4	20.52		

Figure 80 Exported Electropherogram CSV Single File – Size Scale file format

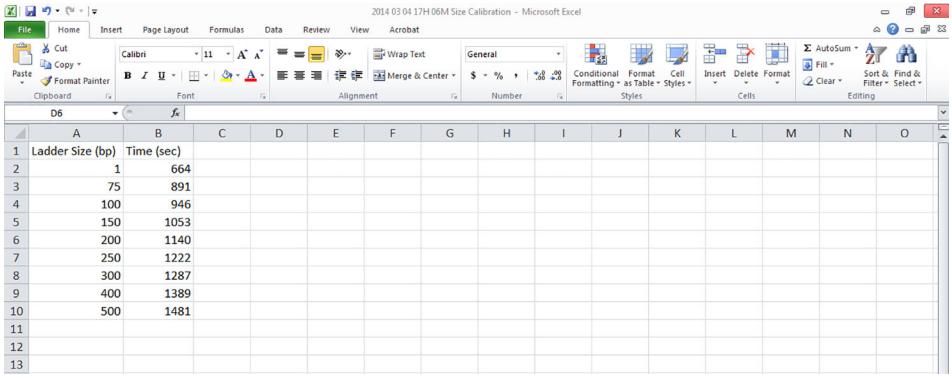
- Electropherogram CSV – Separated Files – Time Scale



	A	B
1	Time (sec)	Intensity
2	1	0
3	2	0
4	3	0
5	4	0
6	5	0
7	6	0
8	7	0
9	8	0
10	9	0
11	10	0
12	11	0
13	12	0

Figure 81 Exported Electropherogram CSV Separated Files – Time Scale file format

- Size Calibration



	A	B
1	Ladder Size (bp)	Time (sec)
2	1	664
3	75	891
4	100	946
5	150	1053
6	200	1140
7	250	1222
8	300	1287
9	400	1389
10	500	1481
11		
12		
13		

Figure 82 Exported Size Calibration file format

- Selected Samples Gel Image

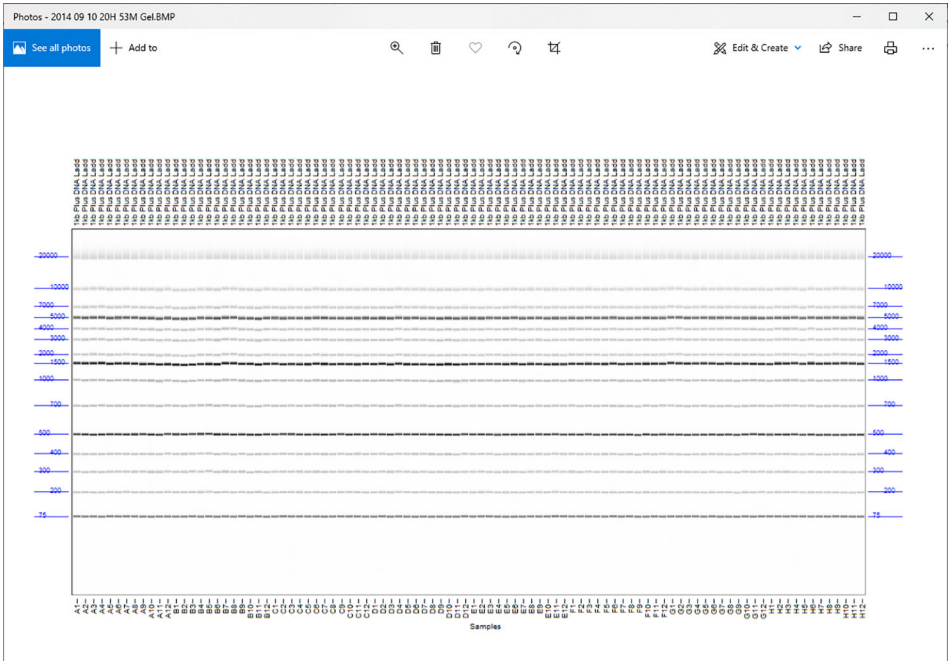


Figure 83 Exported Selected Samples Gel Image file format

- Individual Electropherogram Image

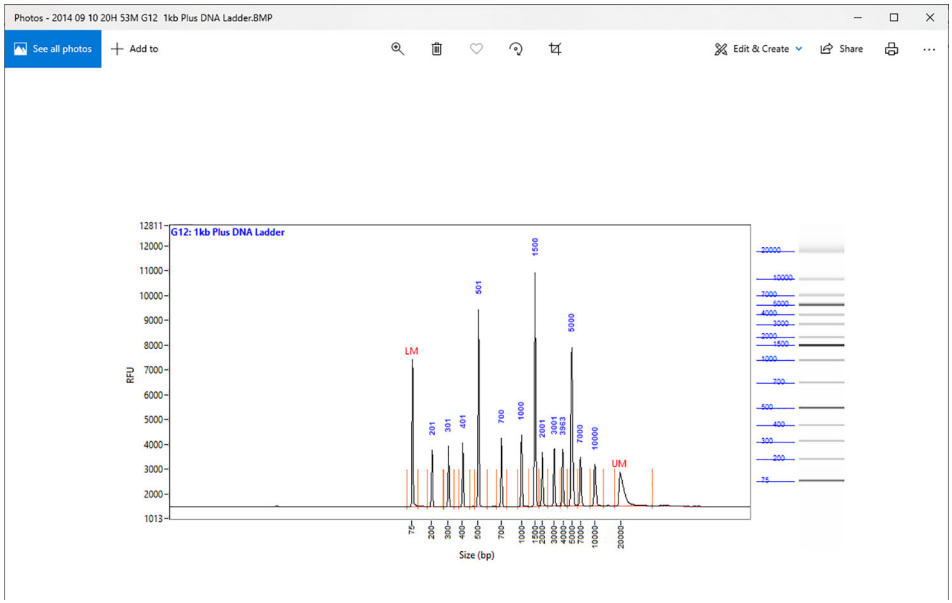


Figure 84 Exported Individual Electropherogram Image file format

8 Generating Reports from ProSize

This chapter provides an overview of the options available for generating reports from the ProSize software. The following sections describe how to open the **Generate Report** window; provide an outline of various report generation options; describe how to perform the report generation; and give examples of reported data.

Generate a Report

Generate a report of sample results

The ProSize software can generate PDF formatted reports for convenient viewing of processed data, reporting detailed information for each sample analysis. The **Generate Report** command is used to generate PDF formatted results.

1 From the main screen, select , or navigate to **File** > **Generate Report**.

The **Generate PDF** window opens (**Figure 85**). **Table 52** summarizes the available settings of this window.

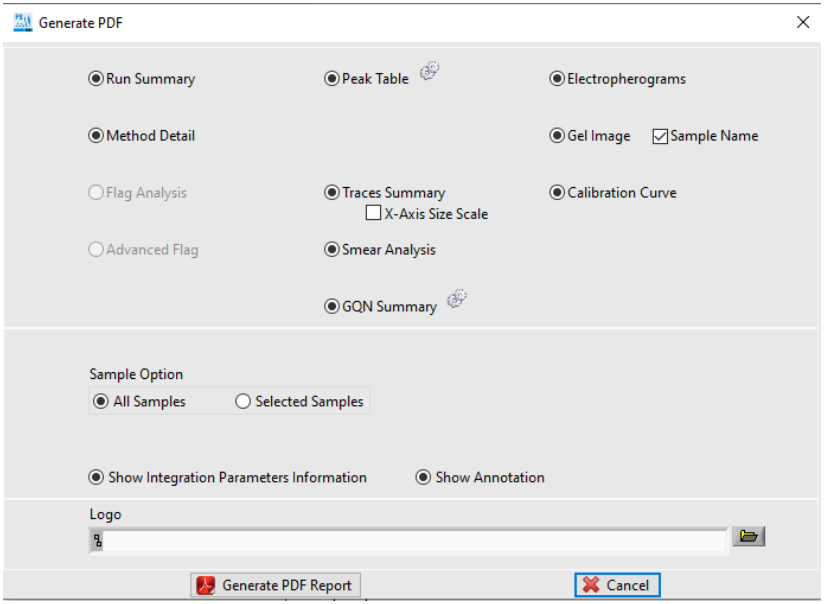


Figure 85 Generate PDF window

Table 52 Generate PDF settings

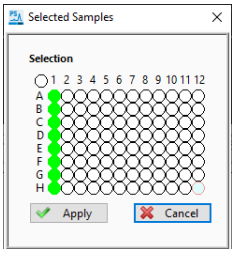
Settings	Description
Sample Option	Determines which samples of the data file will be included in the report. All selected samples will be saved in a single PDF report file; each sample will be printed on a separate page of the report. There are two options: **All Samples** (results for all samples of data file exported); and **Selected Samples**. The Selected Samples option will open the **Selected Samples** window.  Select the respective wells, columns and/or rows of the sample plate, and click **Apply**. To abort the operation and export all samples, click **Cancel**.
Run Summary	A summary of run information will be printed on the first page of PDF report including **Filename** and **Data Path**; date **Created**; **# of Capillaries**; **Array Serial #**; capillary **Effective Length**; **Array Usage Count**; **Version #** of software; **Device Serial #** (Figure 86). If this is not selected, these fields will be blank.
Method Detail	Details of the performed experimental method will be printed on the first page of the PDF report (from the user interface **Information** file (in ProSize Plate Map)). If not selected, these fields will be blank. An example for the Fragment Analyzer is shown below: **Method Information:** Method Name: DNF-493-33 – HS Large Fragment.mthds Gel Prime: No Full Conditioning: Yes Gel Prime to Bufer: Yes Gel Selection: Gel 1 Perform Prerun: 6.0 kV, 30 sec. Rinse: No Marker 1: No Rinse: Tray: 3, Row: H, # Dips: 1 Sample Injection: 5.0 kV, 30 sec. Separation: 6.0 kV, 50.0 min. Tray Name: Tray-1 Analysis Mode: NGS NOTE Testing

Table 52 Generate PDF settings

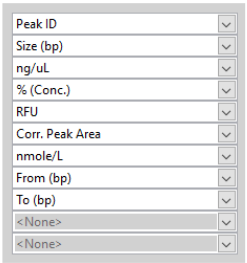
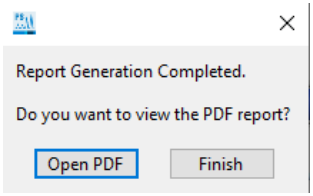
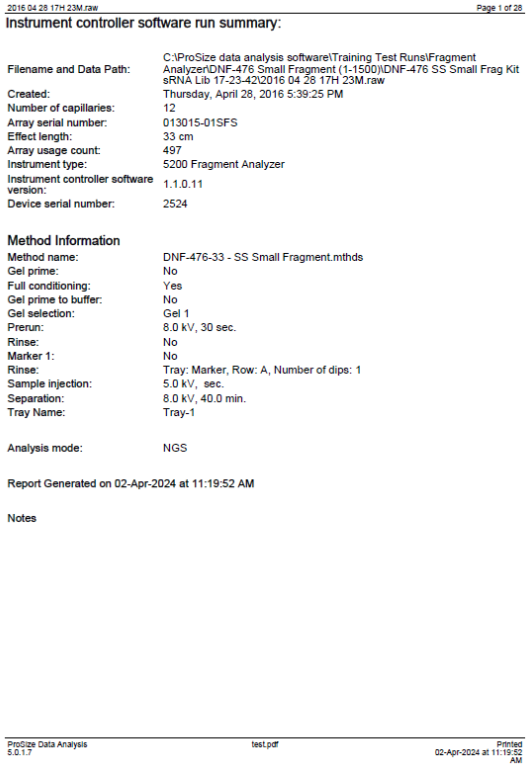
Settings	Description
Traces Summary	The **Trace Summary** page will be printed with up to 12 electropherograms per page (**Figure 87**). Traces will show the **Sample ID** information for each trace. If not selected, these field will be blank.
Peak Table	For all selected samples, the **Peak Table** will be printed on each sample result page in the PDF report (**Figure 88**). If not selected, this field will be blank. Select  to define the reported items and their order in the table. You can save these settings for future use. 
Electropherograms	For all selected samples, the Electropherogram Trace will be printed on each sample result page in the PDF report. Shown are the peaks with the currently selected annotation and x-axis scale in ProSize at the time of printing (**Figure 88**). The digital gel image will be displayed to the right, labeled by size. If not selected, this field will be blank.
Flag Analysis	For all selected samples, a **Flag Analysis** results table will be printed on a separate page in the PDF report, with one page per Flag condition (**Figure 89**). The output will be in a binary 0/1 format, with each page column labeled by the respective Flag condition. If no **Flag Analysis** table is present for the data file, this option will be disabled.
Advanced Flag Analysis	For each sample well defined, the **Advanced Flag Analysis** results will be printed on a separate page in the PDF report (**Figure 90**). The output will list the values within the set criteria of the **Advanced Flag** analysis.
Smear Analysis	For each selected sample, the **Smear Analysis** results will be printed underneath the Peak Table. The color-coded vertical smear range lines and Average Size (bp) will be also reported (**Figure 88**). If no **Smear Analysis** table is present for the data file, this option will be disabled.

Table 52 Generate PDF settings

Settings	Description
Gel Image	Prints a digital gel overlay image of the selected samples on the first page of the PDF report (**Figure 86**). If not selected, this field will be blank.
Calibration Curve	Prints a separate page at the end of the PDF report showing the **Size Calibration** curve including the well location and fit type used (**Figure 91**). If not selected, this page will be not printed.
Show Integration Parameters Information	Prints a detailed summary of the individual integration parameters for each selected sample at the bottom of each sample result page in the PDF report (**Figure 88**). If not selected, this field blank will be blank.
Show Annotation	Shows the annotation(s) made to individual sample electropherograms. If not selected, no annotation for samples will be shown.
Logo	Allows to import a custom logo (which will print at the top of each page). This is for customers that want their company logo to be printed on each page.
Generate PDF report	Generates the configured report. A file browser menu will open to navigate and save the file in the desired directory. The default directory is that containing the .raw data file. In the **Message** dialog, click **OK** to open the report; click **Cancel** to close without opening the report. 
Cancel	Abort the report generation and return the main screen of the software.

Examples of Generated Reports

Figure 86 through **Figure 91** below show examples of the PDF report pages generated by the ProSize software. The header of each page lists the .raw data file name and the page number; the footer contains software version and copyright information as well the date and time of report generation.



2016 04 28 17H 23M.raw Page 1 of 28

Instrument controller software run summary:

Filename and Data Path:	C:\ProSize data analysis software\Training Test Runs\Fragment Analyzer\DNF-476 Small Fragment (1-1500)\DNF-476 SS Small Frag Kit sRNA Lib 17-23-42\2016 04 28 17H 23M.raw
Created:	Thursday, April 28, 2016 5:39:25 PM
Number of capillaries:	12
Array serial number:	013015-01SFS
Effect length:	33 cm
Array usage count:	497
Instrument type:	5200 Fragment Analyzer
Instrument controller software version:	1.1.0.11
Device serial number:	2524

Method Information

Method name:	DNF-476-33 - SS Small Fragment.mthds
Gel prime:	No
Full conditioning:	Yes
Gel prime to buffer:	No
Gel selection:	Gel 1
Prerun:	8.0 kV, 30 sec.
Rinse:	No
Marker 1:	No
Rinse:	Tray: Marker, Row: A, Number of dips: 1
Sample injection:	5.0 kV, sec.
Separation:	8.0 kV, 40.0 min.
Tray Name:	Tray-1

Analysis mode: NGS

Report Generated on 02-Apr-2024 at 11:19:52 AM

Notes

ProSize Data Analysis 5.0.1.7 test.pdf Printed 02-Apr-2024 at 11:19:52 AM

Figure 86 Page 1 of PDF report (all fields shown; 12 samples were selected)

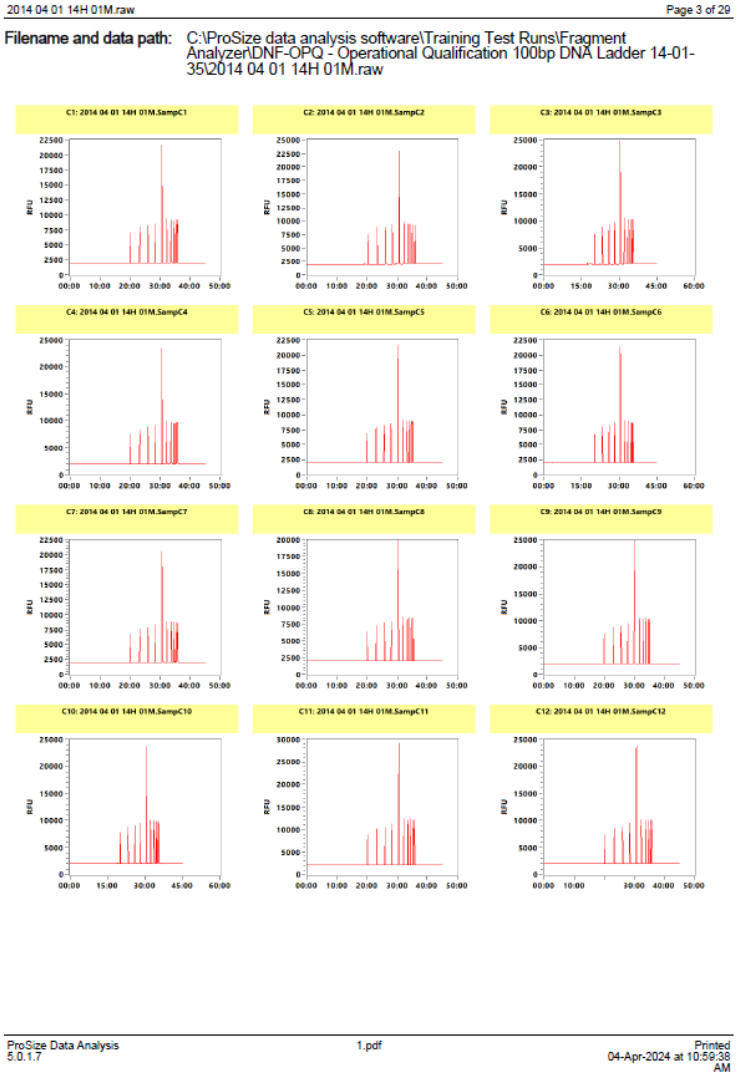
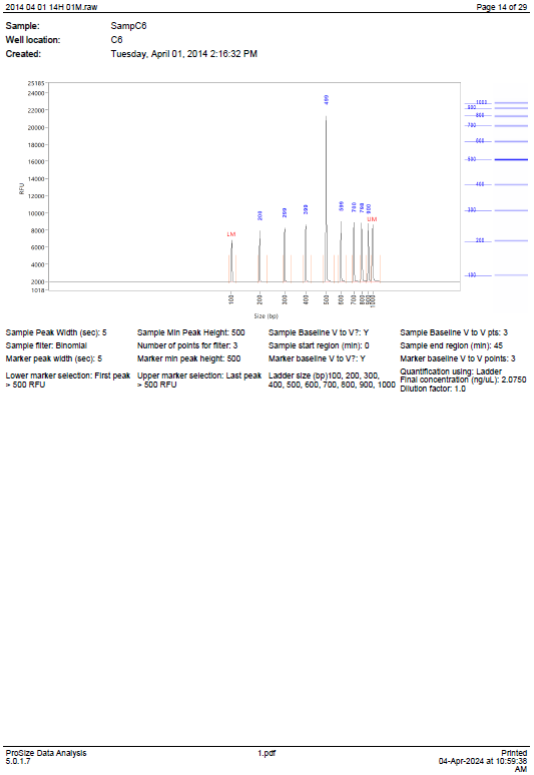


Figure 87 Trace Summary of PDF report (all fields shown; 12 samples were selected). Note: When reporting total RNA data, the RQN is reported.

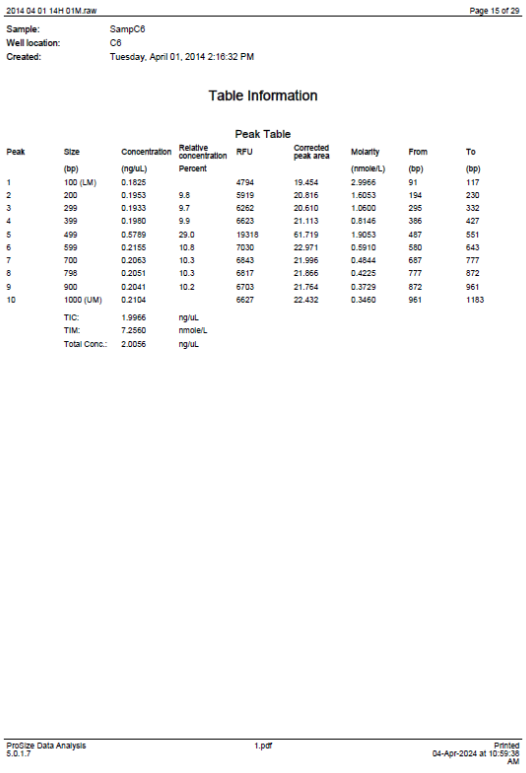


2014 04 01 14H 01M.raw Page 14 of 29

Sample: SampC6
Well location: C6
Created: Tuesday, April 01, 2014 2:16:32 PM

Sample Peak Width (sec): 5 | Sample Min Peak Height: 500 | Sample Baseline V to V?: Y | Sample Baseline V to V pts: 3
Sample filter: Binomial | Number of points for filter: 3 | Sample start region (min): 0 | Sample end region (min): 45
Marker peak width (sec): 5 | Marker min peak height: 500 | Marker baseline V to V?: Y | Marker baseline V to V points: 3
Lower marker selection: First peak > 500 RFU | Upper marker selection: Last peak > 500 RFU | Ladder size (bp)100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 | Quantification using: Ladder Final concentration (ng/uL): 2.0750 Dilution factor: 1.0

ProSize Data Analysis 5.0.1.7 1.pdf Printed 04-Apr-2024 at 10:59:38 AM



2014 04 01 14H 01M.raw Page 15 of 29

Sample: SampC6
Well location: C6
Created: Tuesday, April 01, 2014 2:16:32 PM

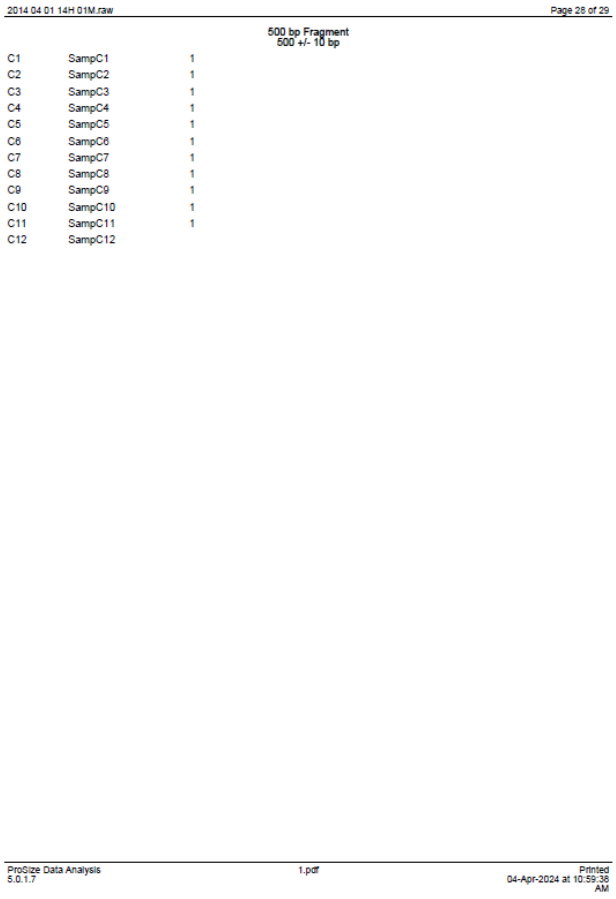
Table Information

Peak Table

Peak	Size (bp)	Concentration (ng/uL)	Relative concentration Percent	RFU	Corrected peak area	Molarity (nmole/L)	From (bp)	To (bp)
1	100 (LM)	0.1625		4794	19.454	2.9966	91	117
2	200	0.1953	9.8	5919	20.816	1.6053	194	230
3	299	0.1933	9.7	6262	20.610	1.0600	295	332
4	399	0.1980	9.9	6623	21.113	0.8146	386	427
5	499	0.5789	29.0	19318	61.719	1.9053	467	551
6	599	0.2155	10.8	7030	22.971	0.5910	580	643
7	700	0.2063	10.3	6843	21.996	0.4844	687	777
8	798	0.2051	10.3	6817	21.866	0.4225	777	872
9	900	0.2041	10.2	6703	21.764	0.3729	872	961
10	1000 (UM)	0.2104		6627	22.432	0.3460	961	1183
	TIC:	1.9966	ng/uL					
	TIM:	7.2560	nmole/L					
	Total Conc.:	2.0056	ng/uL					

ProSize Data Analysis 5.0.1.7 1.pdf Printed 04-Apr-2024 at 10:59:38 AM

Figure 88 Individual result page of PDF report (all fields shown). Note: When reporting total RNA data, the rRNA peaks are highlighted and the rRNA ratio and RQN are reported. Not shown: When reporting Genomic Data, the GQN will be reported.



2014 04 01 14H 01M.raw Page 28 of 29

500 bp Fragment
500 +/- 10 bp

C1	SampC1	1
C2	SampC2	1
C3	SampC3	1
C4	SampC4	1
C5	SampC5	1
C6	SampC6	1
C7	SampC7	1
C8	SampC8	1
C9	SampC9	1
C10	SampC10	1
C11	SampC11	1
C12	SampC12	

ProSize Data Analysis 5.0.1.7 1.pdf Printed 04-Apr-2024 at 10:59:38 AM

Figure 89 Example Flag Analysis table in PDF report

Parameters	Tag & Criteria	Size (bp)	ng/ul	RFU
200 bp only		199	0.1879	2976
:	200 bp ±50 bp AND 300 bp ±50 bp	298	0.1897	3247
200 bp ±50 bp AND 300 bp ±50 bp				

Figure 90 Example Advanced Flag Analysis table in PDF report

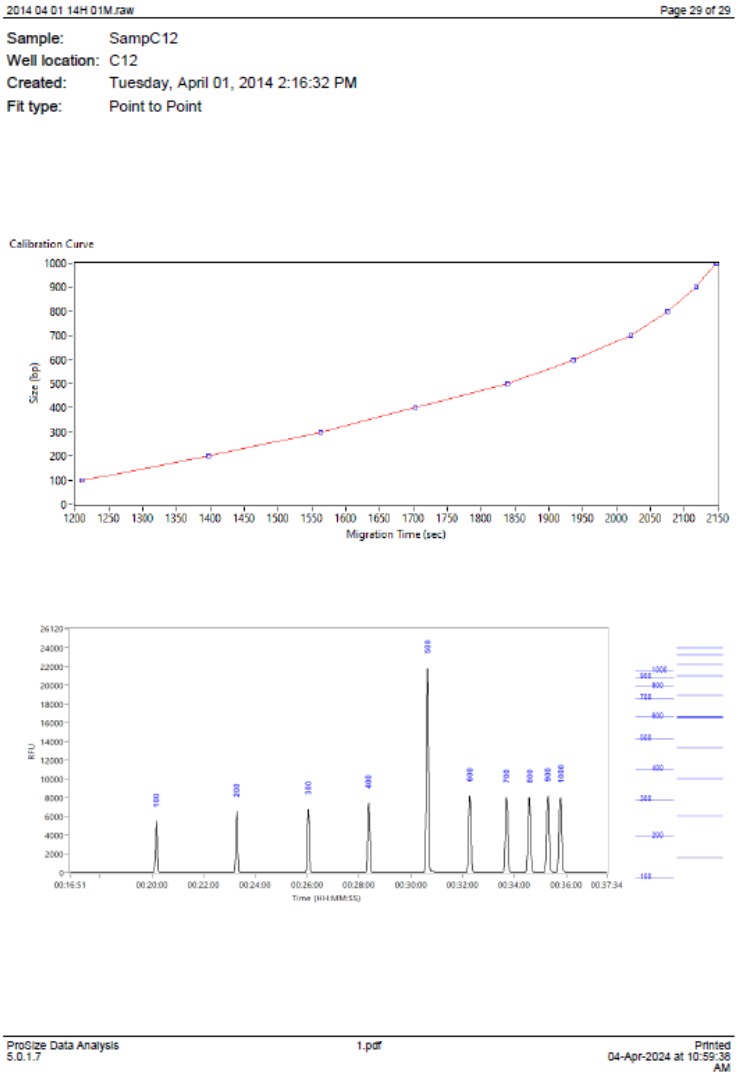


Figure 91 Example Size Calibration page in PDF report

9 ProSize View Capillary Positions

This chapter briefly covers the tools and functions of the View Capillary Positions window.

About View Capillary Positions

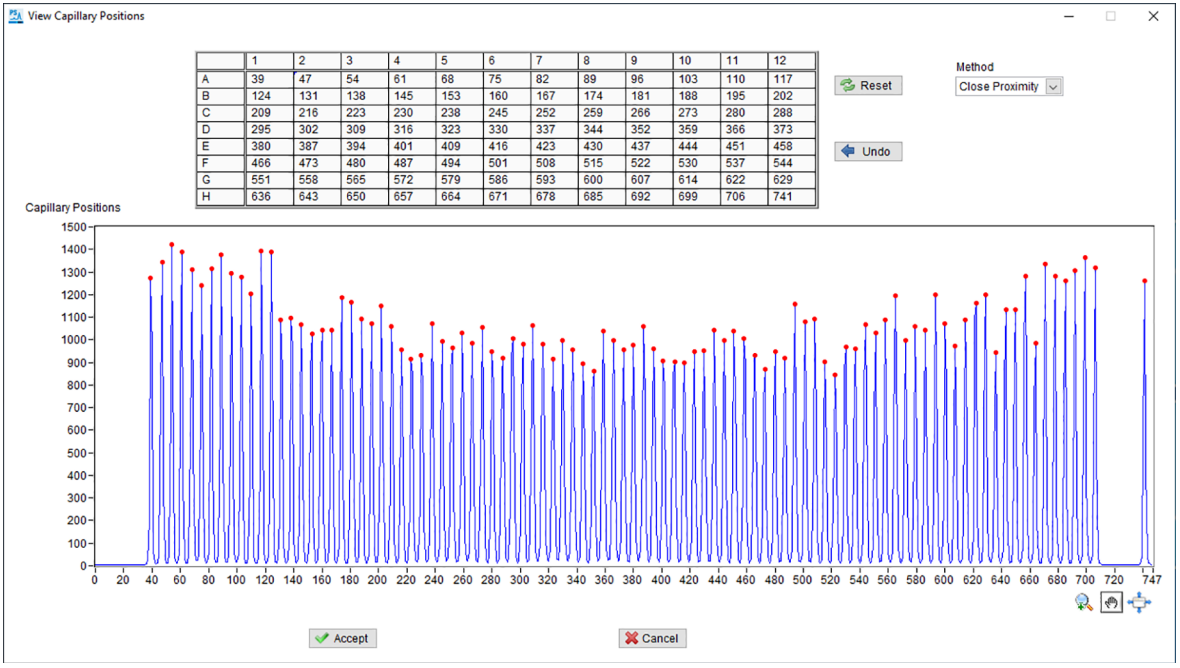
The ProSize software performs an automated capillary alignment procedure when reading in .raw data files. This ensures that the capillary locations on the CCD detector selected for data analysis are of maximum fluorescence intensity for providing the best possible signal to noise ratio.

View Capillary Positions is used to examine the capillary array alignment and the assigned locations used for data analysis. This option is typically not used in routine use since the locations are automatically assigned; it serves rather as a diagnostic/troubleshooting tool.

Open the View Capillary Positions Window

1 From the main screen of the ProSize software program, select **Analysis** > **View Capillary Positions**.

The **View Capillary Positions** window opens (**Figure 92**). **Table 53** summarizes the available functions in this window.



	1	2	3	4	5	6	7	8	9	10	11	12
A	39	47	54	61	68	75	82	89	96	103	110	117
B	124	131	138	145	153	160	167	174	181	188	195	202
C	209	216	223	230	238	245	252	259	266	273	280	288
D	295	302	309	316	323	330	337	344	352	359	366	373
E	380	387	394	401	409	416	423	430	437	444	451	458
F	466	473	480	487	494	501	508	515	522	530	537	544
G	551	558	565	572	579	586	593	600	607	614	622	629
H	636	643	650	657	664	671	678	685	692	699	706	741

Figure 92 View Capillary Positions utility

The **View Capillary Positions** window contains two sections:

- A plot of **Capillary Positions** (Pixels) vs. **Signal Distribution** (Intensity), which shows a summation of fluorescence intensity for each capillary.
- The **Capillary Positions** table listing the pixel location for each of the 12, 48, or 96 capillaries in the 12-capillary, 48-capillary, or 96-capillary array, respectively.

In **Figure 92**, a proper alignment is shown where the red **Current Positions** symbols are centered on the peaks corresponding to each capillary. The algorithm automatically detects the peak locations and sets the capillary positions. If no fluorescence is detected in a particular capillary, the algorithm will

fill the gap using the spacing between capillaries as a reference. In this way, the positions of all capillaries are indexed properly, even if no data was generated by some capillaries of the array.

NOTE

It is important when installing a new capillary array to perform a run with samples (for example, DNA Ladder or marker plate), followed by performing an optical alignment using the collected data file (i.e., Read Raw optical alignment function). This is to ensure that the assigned instrument capillary locations are close to actual positions. For further information, refer to the **Capillary Alignment** section of the System Manual.

During typical operation, no modifications should need to be made to the capillary positions. In rare occurrences, it may be necessary to adjust the positions slightly for one or two capillaries, or to manually adjust positions as described below. provides information on how to change a location and save the changes. Only changes necessary to center the red **Current Positions** symbols on any misaligned capillaries should be performed, to avoid introducing artifacts into the data.

View Capillary Positions Window Settings

Table 53 summarizes the settings of **View Capillary Positions** window.

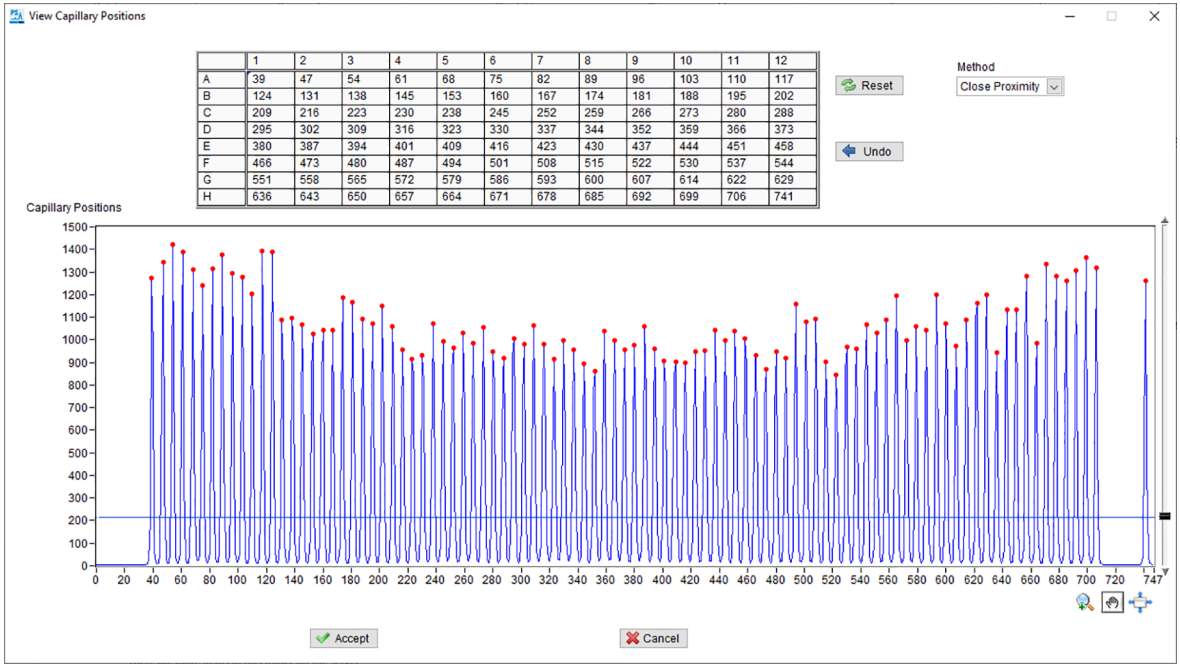
Table 53 Capillary Positions window functions

Menu Item	Description
Capillary Positions	Lists the pixel locations used on the CCD detector for data analysis. There are either 12, 48 or 96 cells, each corresponding to a capillary of the array. The table is labeled by row and well position. To view a particular capillary position, click the cell in the table, or click on a peak in the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity) to view the corresponding assigned well. A red vertical cursor will be displayed on the plot to indicate the detector position.
Reset	Performs an automatic alignment algorithm to reset the capillary locations. Any manual changes made to the pixel positions will be overwritten. A blue threshold line will appear (see **Figure 93**). This line can be moved up or down to select an appropriate threshold for automatically determining capillary location.
Method	Two methods for determining capillary location can be used: • **Close Proximity** (default): This setting will use the original instrument alignment file to determine where to locate the capillary positions, filling any gaps in signal as needed. • **Ignore Original**: If the Close Proximity method fails to locate the correct capillary locations, this will attempt to assign capillary positions based on the signal positions; press **Reset** and use the threshold cursor and **Peak Width** settings to adjust assignments.
Peak Width	Provides a width threshold for selecting peaks in the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity); a higher value selects wider peaks while a smaller value selects narrow peaks. The recommended value is 3.
Undo	To undo the last manual adjustment operation to the **Capillary Positions** table.

	1	2	3	4	5	6	7	8
A	39	47	54	61	68	75	82	89
B	124	131	138	145	153	160	167	174
C	209	216	223	230	238	245	252	259
D	295	302	309	316	323	330	337	344
E	380	387	394	401	409	416	423	430
F	466	473	480	487	494	501	508	515
G	551	558	565	572	579	586	593	600
H	636	643	650	657	664	671	678	685

Table 53 Capillary Positions window functions

Menu Item	Description
Zoom	Enables zooming in the x- and y-axis of the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity). To zoom-in: Place the mouse over the plot, click and drag it outward to expand a box area to define the zoom region. Release the mouse button to apply. . To undo the zoom, use the **AutoFit** function.
AutoFit	To autoscale the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity).
Drag	Enables panning of the plot of Capillary Positions (Pixels) vs. Signal Distribution (Intensity). Move the image around with the mouse cursor: Place the mouse over the trace and click; drag the cursor to shift the current view. This is most often used in combination with the **Zoom** function.
Accept	Accepts the current **Capillary Positions** table locations for the capillary array to use for data analysis and return to the main screen of the ProSize program.
Cancel	Cancels any manual modification to the **Capillary Positions** table locations for the capillary array. It closes the **View Capillary Positions** window and return to the main screen of the ProSize program.



	1	2	3	4	5	6	7	8	9	10	11	12
A	39	47	54	61	68	75	82	89	96	103	110	117
B	124	131	138	145	153	160	167	174	181	188	195	202
C	209	216	223	230	238	245	252	259	266	273	280	288
D	295	302	309	316	323	330	337	344	352	359	366	373
E	380	387	394	401	409	416	423	430	437	444	451	458
F	466	473	480	487	494	501	508	515	522	530	537	544
G	551	558	565	572	579	586	593	600	607	614	622	629
H	636	643	650	657	664	671	678	685	692	699	706	741

Figure 93 Capillary Positions window after pressing **Reset**. A blue horizontal cursor can be used to select the threshold for locating capillary positions.

Manually Resetting Capillary Positions

In very few situations, it may be necessary to manually relocate the capillary positions in the **View Capillary Positions** window. This is usually the result of a combination of the two following conditions:

- The instrument optical alignment (i.e., capillary positioning) is not set properly. When the set capillary locations are far from the actual locations, the ProSize software will have difficulty locating them automatically, especially for 96-capillary data. Therefore is it very important to ensure the capillary array locations have been reset and properly aligned after changing a capillary array.
- A capillary within the array has no detectable fluorescent signal, either due to no sample or marker being loaded into the particular sample well, or because the capillary is plugged.

Ensuring the capillary array is properly aligned will in most all cases enable automatic detection of capillary locations, even if some capillaries do not generate signal. In the event, it is necessary to manually adjust or reset a capillary location, the user should follow the steps outlined below.

Figure 94 shows the **View Capillary Positions** window for a file where a capillary signal is missing (118 pixels), and as a result of a slight misalignment of the instrument optical alignment the software has mistakenly assigned a capillary to pixel 495 and not assigned a location to the second capillary from the right (circled). In this instance, it is not possible to adjust the threshold to successfully select the proper capillaries.

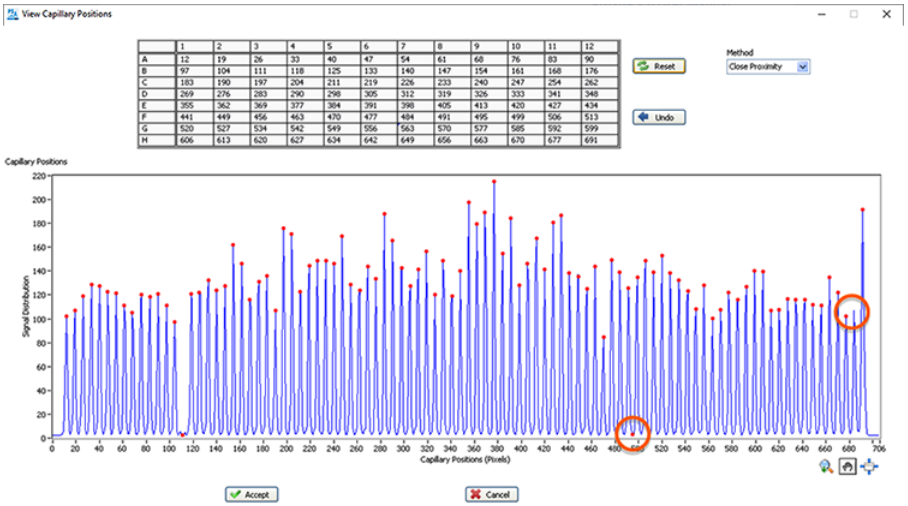


Figure 94 View Capillary Positions window where capillary is not assigned properly (circled in red)

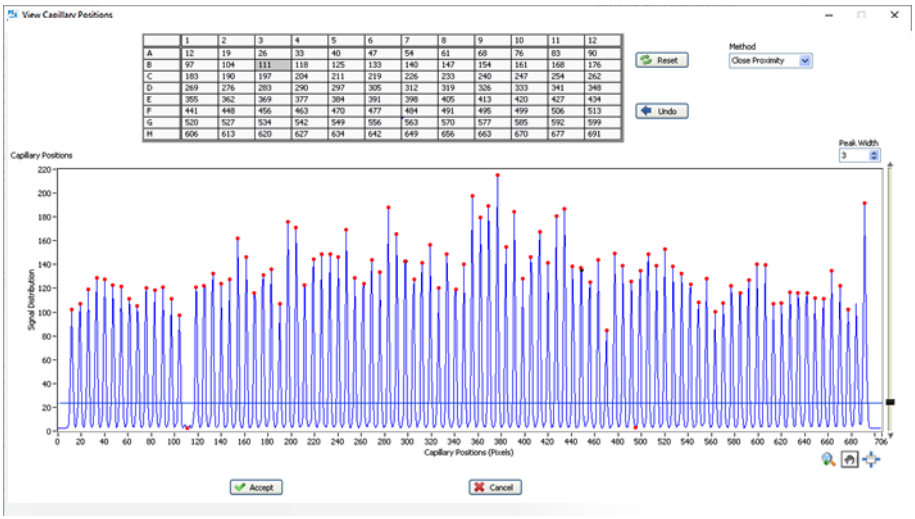


Figure 95 View Capillary Positions window showing misaligned capillary not reset by threshold

In **Figure 95** it is shown that clicking **Reset** and using the threshold will not correctly reassign the capillaries due to the low signal from pixel 118.

To manually delete the improper capillary position, click the mouse over the location, and click [**Delete**] on the keyboard (**Figure 96**).

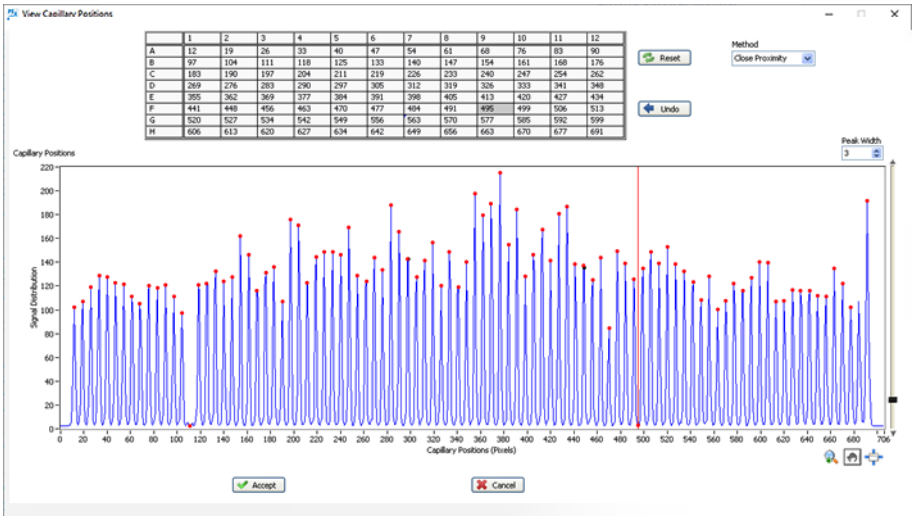


Figure 96 Manually deleting pixel 495 by selecting it, and click [**Delete**]

Note in that **Figure 97** pixel 495 has now been removed from the Capillary Positions table and a blank cell is present in H12.

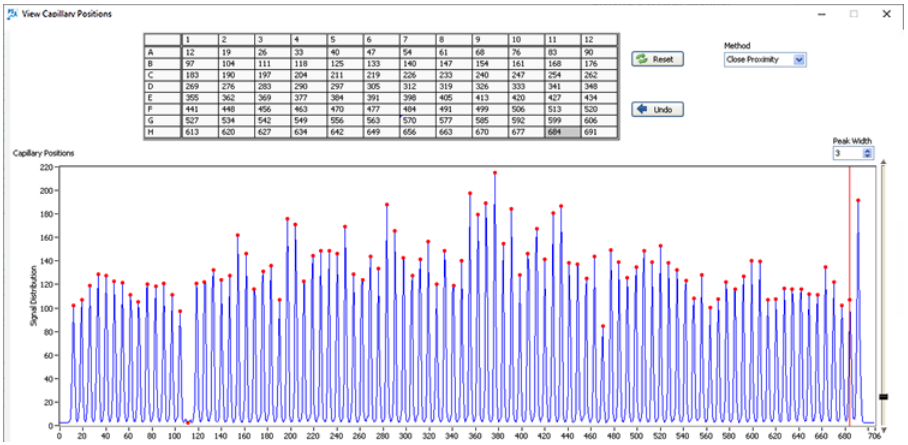


Figure 97 Alignment after manually deleting pixel 495

Next, to manually add a location, click on the adjacent capillary location to where the user wishes to add the capillary, and insert it by clicking [**Insert**] on the keyboard. Click the keyboard arrows to move the newly added vertical red cursor to the desired location, in this case pixel 684 (**Figure 98**).

Once the proper positions have been adjusted, press **Accept** to accept the current **Capillary Positions** table locations for the capillary array and return to the main screen of the ProSize program. All subsequent data analysis will use the newly saved capillary locations.

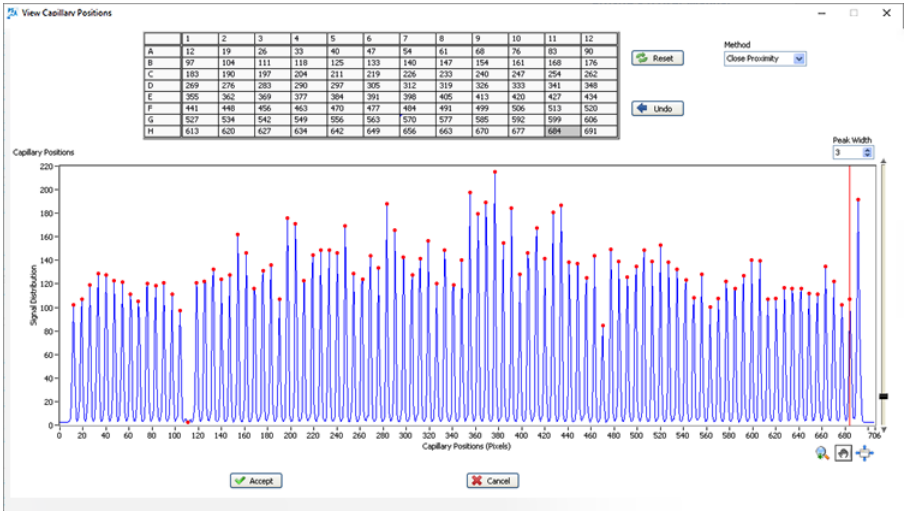


Figure 98 Alignment after clicking [**Insert**] at the rightmost position and using the keyboard arrow to move the vertical cursor to pixel 684. Capillary positions are now correctly located. Press **Accept** to save.

10 ProSize Troubleshooting Guide

This chapter gives an overview of troubleshooting the ProSize software.

ProSize Software Error Messages

The following table lists some potential error messages or issues that may be encountered when using the ProSize software and how to correct them.

- When multiple causes and corrective actions are listed, the items are presented from most likely to the least likely.
- If all corrective actions are employed and a problem still persists, contact your corresponding Agilent Service Representative for additional help.

For hardware related issues, please refer to the individual Reagent Kit User Manual, or the instrument System Manual.

Table 54 ProSize software error messages

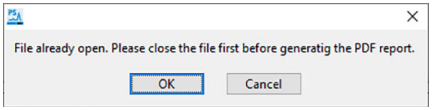
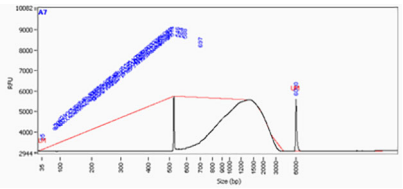
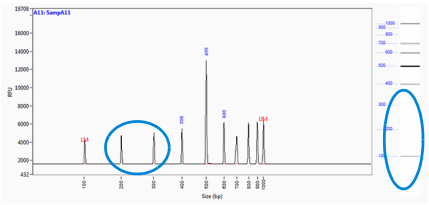
Problem	Cause	Corrective Action
When processing data in main screen, a red flashing message appears on the bottom toolbar: **Warning: Mis-match between detected peaks and ladder assignment! No sizing calibration curve is established.**	1 The number of peaks integrated in the sample well assigned for size calibration does not match the number of fragments in calibration ladder. 2 Wrong sample well selected for size calibration ladder.	1 Verify the correct number of peaks are integrated, adjust as necessary. Markers should be included in the calibration ladder table. 2 Verify correct sample well location of size calibration ladder, reassign in Size Calibration menu. For more information, refer to **Chapter 5**, "ProSize Size Calibration Screen".
When generating a PDF report, the following message is displayed: 	1 Another PDF report is open with the same name.	1 Close any additional open files and repeat the operation.
When attempting to integrate a smear, the baseline does not properly follow the trace. See example below: 	1 The **Peak Width (sec)** setting is too low.	1 Increase the **Peak Width (sec)** setting until baseline draws properly. 2 Use the **Manual Baseline Setpoints** function to set the baseline from outside of the lower/upper marker peak region. See **Table 25** for further information.

Table 54 ProSize software error messages

Problem	Cause	Corrective Action
Peaks are observed in the Electropherogram Trace view but no corresponding band is seen in the Digital Gel View. Example: 	1 The **Peak Width (sec)** setting is too high. 2 The function **Hide/Show Non-Integrated Peaks** on the Digital Gel Image toolbar is set to hide non-integrated peaks.	1 Decrease the **Peak Width (sec)** setting until peaks are integrated properly. 2 Toggle the icon to **Show Non-Integrated Peaks**.
In RNA mode, the RQN number is stated as **NaN**.	1 The concentration of the sample is too low for the software to accurately calculate the RQN number.	1 Increase the injection time of the sample method to inject more sample. 2 Increase the concentration of the sample added to the Diluent Marker.
The lower and/or upper markers do not properly line up between lanes in the Digital Gel Image display.	1 The **Min. Peak Height (RFU)** threshold is set too high, preventing the marker peaks from being selected. 2 Extra peaks (for example, primer/dimer) that are migrating close to the marker are being assigned as the lower marker.	1 Adjust/decrease the **Min. Peak Height (RFU)** threshold in the **Marker Analysis** tab of the **Set Individual Parameters** menu; apply to individual sample or all samples as needed. 2 Adjust/increase the **Min. Peak Height (RFU)** threshold in the **Marker Analysis tab of the Set Individual Parameters** menu; apply to individual sample or all samples as needed.
Peak Height above RFU threshold error given under Warning(s) tab.	1 The concentration of the sample has exceeded the detection limits of the system.	1 Dilute the sample concentration to within the specific kit specifications.

11 Batch Processing Using ProSize

This chapter explains how to perform a batch process and provides an outline of the various exporting options.

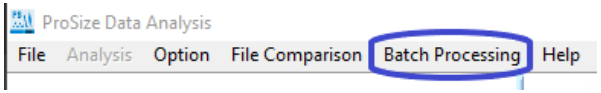
Batch Processing

The ProSize software can batch process a list of runs within a folder, and send the parameters/reports to an output folder. If an output folder is not designated, the data is exported and placed back into the original data folders.

To successfully apply the batch processing routine, the name of the Run Method in the instrument software must exactly match the configuration method in ProSize.

Perform a Batch

1 From the menu, select **Batch Processing** > **Batch Data Process**.



The **Batch Data Processing** window opens (**Figure 99**). **Table 55** summarizes the available options in this window.

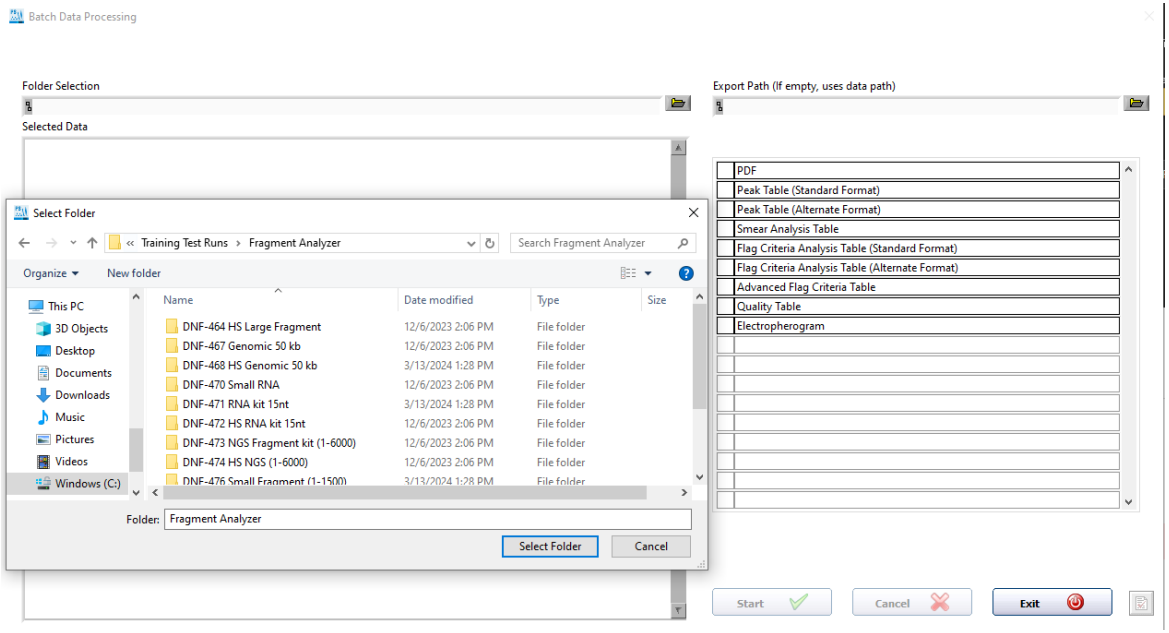


Figure 99 Batch Data Processing window. With open file selection browser to define folder.

Table 55 Batch Data Processing window options

Item	Description
Folder Selection	Allows you to select the folder location with data files to be batch processed. After locating the folder, select **Current Folder** to set the folder location.
Export Path (If empty, uses data path)	Allows you to select a folder location for exported data. After locating the folder, select **Current Folder** to set the folder location. Note: If this field is left empty, the program will export the data to the data folders in **Folder Selection** above.
Export File Options	Allows you to define an export option available in data export (PDF, peak table, flag analysis, etc.).
Start	Starts the batch processing routine.
Exit	Terminates the batch processing routine and closes the window.
View Error Log 	Opens an **Error Log** dialog which allows you to quickly determine errors associated with batch processing (**Figure 100**).

To initiate batch processing:

1 In the Batch Data Processing window, under **Folder Selection**, select  and navigate to your folder with the input files.

2 Click **Select Folder** to accept the folder location.

3 Under **Export Path**, select  and navigate to a folder location for the exported files.

4 Click **Select Folder** to accept the folder location.

NOTE

If no folder is selected, the program will export the data back into the original data folders.

5 Select **Start** to initiate the process.

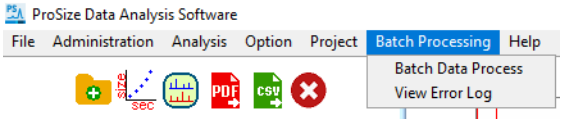
Once the batch processing is finished, the **Error Log** window shows the final processing status for each file.

To display the batch processing status of the files:

1 Select **View Error Log** within the **Batch Data Processing** window.

Or

From the menu, select **Batch Processing** > **View Error Log**.



The **Error Log** window opens (**Figure 100**). **Table 56** describes the output errors associated with this window.

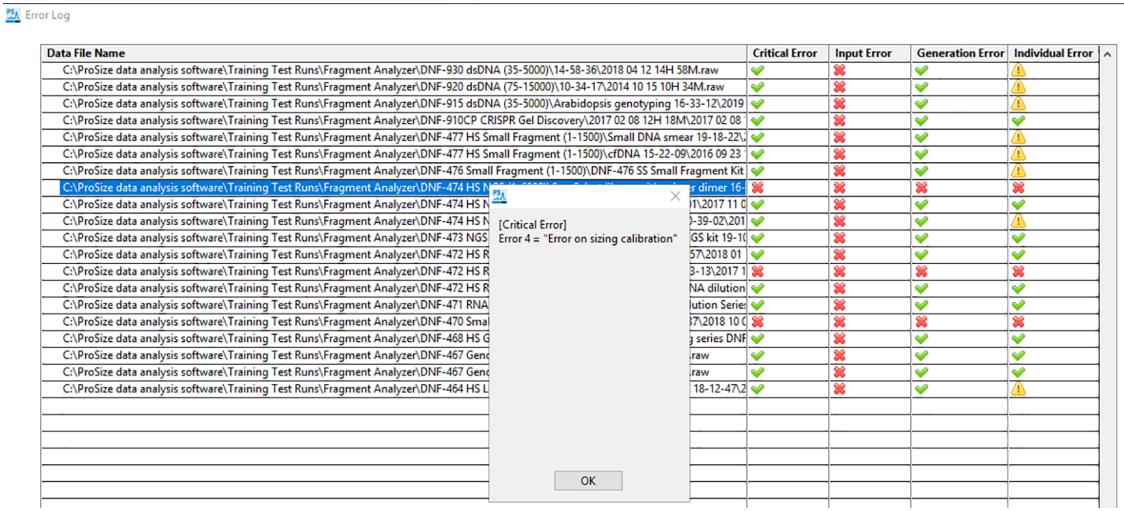


Figure 100 Error Log window

2 To view the specific error for a file, click the respective file.

A dialog opens and displays information about the error.

3 To open the file in ProSize, close the dialog and double-click on the file.

Detailed information for the error is provided.

A folder with the .txt files of all errors is generated and located under C:ProSize data analysis software/Error Log (**Figure 101**).

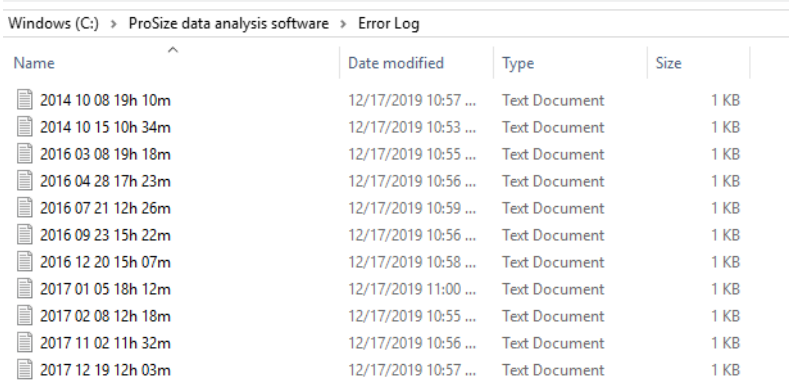


Figure 101 Generated error text files in C:ProSize data analysis software/Error Log

Table 56 Error messages generated by the batch process routine

Error class	Description
Critical Error	**1** ProSize cannot find the configuration file (there is no file with the exact same name as the method file). **2** A configuration file exists, but ProSize was not able to apply the sizing calibration.
Input Error	**1** The user has specified a table (such as **Flag Analysis** or **Smear Analysis**), but there are no parameters set up in ProSize to generate these reports. The user must go to the **Set Individual Parameters** screen and set up the appropriate features (Flag, Smear, Advanced Flag, etc.).
Generation Error	**1** The user has specified a table (such as **Flag Analysis** or **Smear Analysis**), but there are no parameters set up in ProSize to generate these reports. The user must go to the **Set Individual Parameters** screen and set up the appropriate features (Flag, Smear, Advanced Flag, etc.). **2** The user is using a mode such as DNA, and has requested a **Quality** table, which is only available in the RNA mode.
Individual Error	**1** The markers are too broad (FWHM is to large). **2** There are no peaks.

In This Book

This manual contains information about the ProSize data analysis software.

The manual describes the following:

- System overview,
- Requirements and installation instructions,
- Software main screen and menus,
- Data configuration,
- Comparing samples,
- Data export,
- Report generation,
- Troubleshooting,
- Batch processing.

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