applied biosystems

Microsatellite Analysis Software

USER GUIDE

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For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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A.0	28 June 2018	New document. Material represents the Help content in the Microsatellite Analysis Software.

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About the Microsatellite Analysis Software

Microsatellite Analysis Software is a microsatellite genotyping module available on Thermo Fisher Cloud.

This software may be used to analyze a mixture of DNA fragments, separated by size, on any one of the supported capillary electrophoresis systems (see "Compatible instruments" on page 8). The analysis provides a profile of the separation, precisely calculates the sizes of the fragments, and determines the microsatellite alleles present in the sample.

The software allows you to view, edit, analyze, print, and export microsatellite marker data generated using Applied Biosystems™ genetic analyzers.

Compatible instruments

Capillary Electrophoresis System	Supported software version(s)	File extension
Applied Biosystems™ SeqStudio™ Genetic Analyzer	v1.0 or later	FSA
Applied Biosystems™ 3500/3500xL Genetic Analyzer		
Applied Biosystems™ 3130/3130*xl* Genetic Analyzer		
Applied Biosystems™ 3730/3730*xl* DNA Analyzer		

Computer requirements

Component	Recommended specification
Processor	2.4 GHz or higher
RAM	8 GB or higher
Display	1280 × 1024 or higher

Workflow

Microsatellite Analysis Software Workflow

Create a project and import samples (page 11)

▼

Review samples and perform initial sizing analysis in the Setup screen (page 15)

▼

Examine low-quality sizing results (page 21)

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(Optional) Review sizing information in the Results screen (page 65)

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Create a panel (page 29)

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Perform genotyping analysis in the Setup screen (page 51)

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Examine low-quality genotyping results (page 54)

▼

Review genotype information in the Results screen (page 82)

▼

Export results (page 100)

Microsatellite data analysis workflow

The workflow shown below is an example approach for data analysis.

Review samples and perform initial sizing analysis in the Setup screen (page 15)

Review samples after first import (page 15)

Perform initial sizing analysis using default analysis settings (page 17)

(If needed) Reanalyze samples using custom sizing analysis settings (page 18)

▼

(If needed) **Examine low-quality sizing results (page 21)**

View data collection settings (page 21)

Examine EPT and raw data (page 21)

Apply a different size standard to the project or a sample (page 26)

Modify the default analysis settings (page 25)

▼

Create a panel (page 29)

Create a new panel in Panel Manager (page 30)

Manually add markers to a panel (page 31)

Manually add bins to a marker (page 35)

▼

Perform genotyping analysis in the Setup screen (page 51)

Analyze project using default genotyping analysis settings (page 51)

(If needed) Reanalyze samples using custom genotyping analysis settings (page 51)

▼

(If needed) **Examine low-quality genotyping results (page 54)**

Modify the default analysis settings (page 25)

Review samples with Suspect and Low Quality GQ values (page 55)

Edit a panel in Panel Manager (page 43)

▼

Review genotype information in the Results screen (page 82)

▼

Review sizing information in the Results screen (page 65)

▼

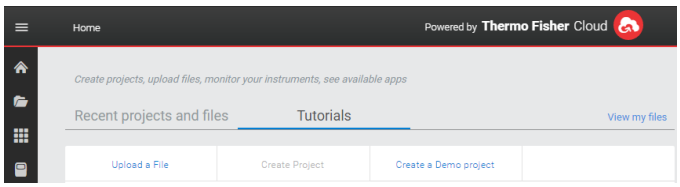
Export results (page 100)

Create a project and import samples

Create a Demo project

IMPORTANT! We recommend that new users create a Demo project to quickly learn how to use the module.

1. In the Thermo Fisher Cloud Dashboard, click **Tutorials**, then click **Create a Demo project**.



2. At the bottom of the **Tutorials** screen, click Create a Demo Project, then click **OK**.

3. In the **My Apps** pane, click MSA.

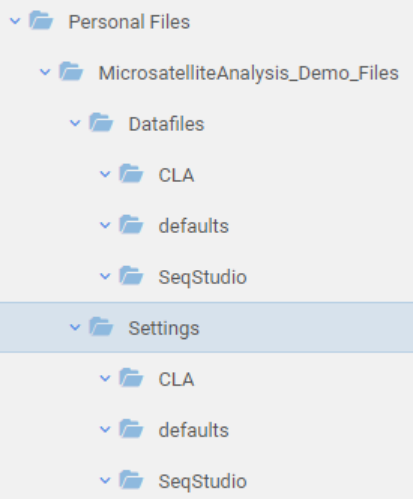
4. In the **Open Project** dialog box, select **Use Existing Project**, select **Microsatellite_DEMO_Project** from the list, then click **OK**.

   Note: It may take several minutes for the Demo project to appear in the **Personal Files** folder.

   When the Demo project is created, the following folders and files are added to your Personal folder:

   - **Microsatellite_DEMO_Project** that contains default data files created on a SeqStudio™ Genetic Analyzer
   - **MicrosatelliteAnalysis_Demo_Files** that contains two additional sets of sample data files and settings:
     - **CLA**—Example data files and analysis settings for Cell Line Authentication (CLA).
     - **SeqStudio**—These files are similar to the default data files, but the Settings file naming convention has been improved for easier identification and import.

If you want to use the CLA or SeqStudio data files, you have to import them into the Demo project.



5. (If needed) Review the **Getting Started** pop-up window, then click **OK**.
6. Proceed with "Review samples and perform initial sizing analysis in the Setup screen" on page 15.

Create a project in the Thermo Fisher Cloud Dashboard

You must have saved FSA files from an instrument run before you can create a project in the module. The FSA files can be analyzed or unanalyzed.

1. In the Thermo Fisher Cloud Dashboard, click **Create project**.

2. Enter a project name, select a save location, then click **Create**.
3. (If needed) Review the **Getting Started** pop-up window, then click **OK**.
4. In the **Manage Data Files** window, select an option to import FSA files:
    1. **Import From My Computer**—Imports FSA files from your computer.
    2. **Import from Thermo Fisher Cloud**—Imports FSA files from the Thermo Fisher Cloud. The Cloud contains Demo files from Thermo Fisher Scientific and any files that you have previously uploaded in Data Manager.
    3. **Import From Dropbox**—Imports FSA files from Dropbox.

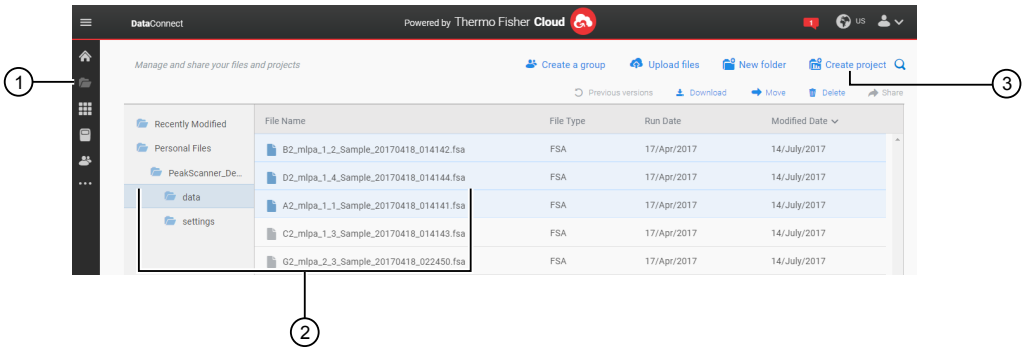
    The imported files are displayed in the table.
5. In the navigation bar, click the Microsatellite Analysis Software icon MSA.

Other ways to create projects

Create from the DataConnect window

1. In the Thermo Fisher Cloud Dashboard, click in the navigation bar to open the **DataConnect** screen.
2. Browse to and select the trace files of interest, then click Create project.
3. In the **Create Project** dialog box, enter a project name, select a save location, then click **Create**.
4. Proceed with step 3 in "Create a project in the Thermo Fisher Cloud Dashboard" on page 12.

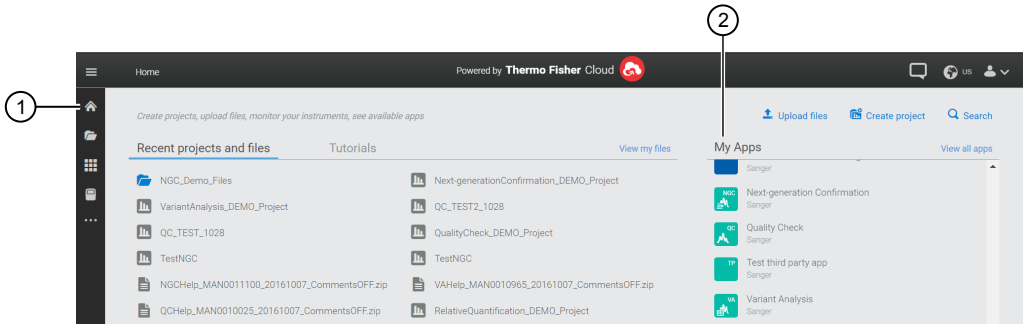
   You can import additional trace files, or skip "Create a project in the Thermo Fisher Cloud Dashboard" on page 12.



① Files icon—Click to open the **DataConnect** window
② Project folders and trace files
③ Create project link—Click to open the **Create Project** dialog box

Create from My Apps in the **Home** screen

1. In the Thermo Fisher Cloud Dashboard, click ⌂ in the navigation bar to open the **Home** screen.
2. In the **My Apps** pane, click MSA.
3. In the **Open Project** dialog box, select **Create New Project**, enter a project name, select a save location, then click **OK**.
4. Proceed with step 3 in "Create a project in the Thermo Fisher Cloud Dashboard" on page 12.

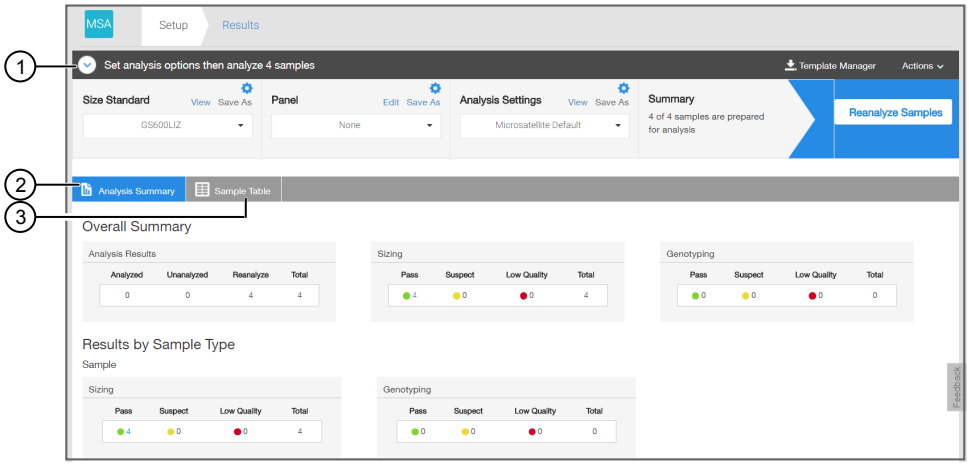


① Home icon—Click to open the **Home** window
② Module icon in the **My Apps** pane—Click to open the Microsatellite module

Review samples and perform initial sizing analysis in the **Setup** screen

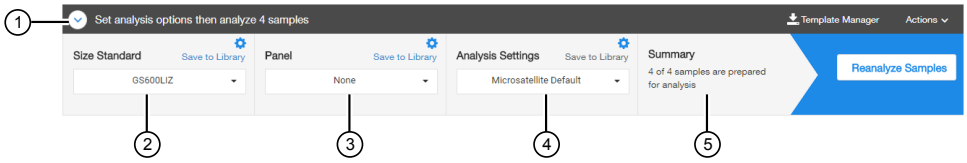
Review samples after first import

When you import samples, the **Setup** screen is displayed (see "Parts of the Setup screen" on page 106).



① Analysis Details
② Analysis Summary
③ Sample Table

1. Review the information that is imported for the project.



① Show/hide analysis options.
② **Size Standard**—A size standard is selected for the project (see "Size Standard selected by the software" on page 116).
③ **Panel**—The software does not include default panel information. You must create or import a panel for genotyping analysis (see "Create a panel" on page 29).
④ **Analysis Settings**—Microsatellite Default.
⑤ **Summary**—The number of samples that will be analyzed using the selected settings. View selected samples in the Sample Table.

2. Review the Analysis Summary for information that is imported for the project.
   - Overall Summary

   (1) Analysis Results—The overall analysis status for the samples in the project (see "Analysis status" on page 117).

   (2) Sizing analysis results—Overview of Sizing Quality (SQ) analysis status for the samples in the project based on the **Quality Flags** setting from the data collection analysis settings (see "Sizing Quality (SQ) status" on page 133).

   (3) Genotyping analysis results—Overview of Genotyping Quality (GQ) analysis status for the samples in the project based on the PQVs in the **Quality Flags** tab of the analysis method used to analyze the data (see "Genotype Quality (GQ)" on page 138).

   Genotyping analysis results are not available until you apply a panel to the project, then click **Reanalyze** (see "Analyze project using default genotyping analysis settings" on page 51).

   - Results by Sample Type—Overview of SQ analysis and GQ analysis results based on sample type as defined in the data collection analysis settings.

3. Click the **Sample Table** tab, then review the following information for the samples in the sample table (see "Sample Table fields" on page 106).

Parameter	Description
Status	• for SeqStudio™ (3200) and 3500 FSA files • for files analyzed using the current analysis settings. Analyze samples after the first import to apply current settings. • for 31xx and 3730/3730*xl* FSA files[1]
SQ	• , , or for SeqStudio™ and 3500 FSA files, based on the **Quality Flags** setting from the data collection analysis settings. • Blank for 31xx and 3730/3730*xl* FSA files[1]
GQ	Blank
OS	• or for SeqStudio™ and 3500 FSA files, based on the **Quality Flags** setting from the data collection analysis settings. • Blank for 31xx and 3730/3730*xl* FSA files[1]
Size Standard	See "Size Standard selected by the software" on page 116

[1] If 31xx or 3730/3730*xl* FSA files were previously analyzed using the Peak Scanner™ desktop software, the imported **Status**, **SQ** and **OS** parameters will be the same as those for SeqStudio™ (3200) and 3500 FSA files.

Note: This table describes the parameter fields following initial import. These parameters will update as the user applies modified analysis settings to the project.

4. As needed:
   - Click the **Sample Type** column header to sort and view control and allelic ladder samples as needed.
   - "Customize columns in the Sample, Sizing, or Genotype Table" on page 20.
   - "Search the Genotype Table" on page 52.

Perform initial sizing analysis using default analysis settings

Perform an initial analysis using default settings to size the data so that you have sample files available as reference data to create a panel for genotyping analysis.

1. In the Sample Table, select one or more samples to analyze.
2. Click **Reanalyze Samples**.
3. Review results in the **Setup** screen, then:
   - If the sample quality is 🟢, proceed to "Perform genotyping analysis in the Setup screen" on page 51.
   - If the sample quality is 🟡 or 🔴, or analysis fails, proceed to:
     - "Reanalyze samples using custom sizing analysis settings" on page 18
     - "Examine low-quality sizing results" on page 21

Create a new template from current settings

Use a template to apply a specific set of analysis options (Size Standard, Panel, Analysis Settings, Plot Settings and table settings) to a project.

To modify settings, see "Reanalyze samples using custom sizing analysis settings" on page 18.

1. In the **Setup** screen, click **Template Manager**.
2. Click **Create from current settings**.
3. Enter a template name and description, then click **Save**.

Analyze samples using a template

Use a template to apply a specific set of analysis options (Size Standard, Panel, Analysis Settings and table settings) to a project.

1. In the **Setup** screen, click **Template Manager**.
2. Select an option to apply a template:

Option	Action
Select existing template	1. Select an existing template from the list.
Import template (ZIP)	1. Click **Import**. 2. Enter a template name and description, Browse to and select a template file (ZIP), then click **Open**. 3. Select an option to import a template file (ZIP), then click **Import** : • **My Computer**—Imports the ZIP from your computer. • **Thermo Fisher Cloud**—Imports the ZIP file from the Thermo Fisher Cloud. The Cloud contains any files that you have previously uploaded in Data Manager. 4. Browse to and select the template file of interest, then click **Import**. 5. Select the template from the list.

3. Select **Auto analyze**, then click **Apply**.

Reanalyze samples using custom sizing analysis settings

1. Perform any of the following tasks as needed:
   - "Modify the default analysis settings" on page 25
   - "Edit sample name and type" on page 20
   - "Apply a different size standard to the project or a sample" on page 26
   - "Modify size matches" on page 22
   - "Adjust Size Quality Flag ranges" on page 26
2. Click **Reanalyze Samples**.

Add/remove samples

In the **Setup** screen, select the appropriate option:.

Option	Description
Add more files	1. Click **Actions** 2. Click **Add More Files** 3. Select the appropriate import option, then click MSA.
Remove files	1. Select the samples to remove in the Sample Table. 2. Click **Actions**. 3. Click **Remove Files**. **Note:** When you click **Remove Files**, no message is displayed. You can add the files back to the project, but will have to analyze again to display results.

Search the Sample Table

Search the Sample Table using one of the following options:

Option	Action
Search by term	1. Enter the search term in the field at the top right corner of the table, then click Q. **Note:** The ✓ icon indicates a filter is already applied.
Search by SQ, GQ, OS or Analysis status	1. Click ⌄ at the top right corner of the table. 2. Select a search parameter from the drop down menu. 3. Select a parameter status from the drop down menu. 4. Click **Search**.

Click ⌄, then click **Clear All** to clear previous search results.

Edit sample name and type

Edit a sample name and type in the Sample Table.

- **Edit the sample name**—Click the sample name.
- **Edit the sample type**—Click the sample type, then select an option from the dropdown list.
  - **Sample**
  - **Positive Control**
  - **Allelic Ladder**
  - **Primer Focus**
  - **Negative Control**

  Note: **Primer Focus** cannot be used for genotyping analysis.

  Note: Always reanalyze a project after changing the sample type to **Allelic Ladder**.

  Note: The user has the option to arrange control plots on top during review of sizing and genotype plots in the **Results** screen. See "Customize sizing plot data and views" on page 70 and "Customize genotype plot data and views" on page 89.

Customize columns in the Sample, Sizing, or Genotype Table

Customize the columns displayed in tables.

- **Sort the columns in the table**—Click a column header.
- **Edit the order of columns in the table**—Click a header, then drag and drop in the desired order.

  Note: The order of columns can only be edited in the Sample and Sizing Tables.
- **Show/Hide columns in the table**—Click ⚙ at the top right corner of the table, then select the columns to show or hide. Click **Apply**.
- **Edit Quality Metrics Display**—See "Edit Quality Metrics Display" on page 20.
- Click **Restore to default** to undo previous Show/Hide selections.

Edit Quality Metrics Display

Show Sizing Quality (SQ) and Genotyping Quality (GQ) metrics as symbols (default) or numbers in the Sample Table and Genotype Table.

1. Click ⚙ at the top right corner of the table.
2. Click **Quality Metrics Display**, then select the appropriate option.
3. Click **Apply**.

Examine low-quality sizing results

View data collection settings

1. In the **Setup** screen, open the **Sample Details** window using one of the following methods:
   - Right-click on the sample file in the Sample Table, then click **View Sample Details**.
   - Click **Actions** ▸ **View Sample Details** .
2. Click **Information** to review the data collection settings.
3. Click **Close**.

Examine EPT and raw data

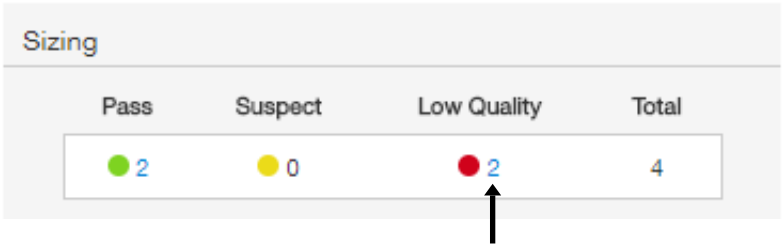
Review the EPT (ElectroPherogram Telemetry) plot to identify instrument performance issues that can affect size quality.

Review the raw data to evaluate anomalies, the causes of poor size-calling, and to determine the start and stop points for analysis. The start point for data analysis occurs after the primer peak and before the first sizing peak. The stop point for analysis occurs after the last sizing peak.

1. Open the sample details window using one of the following methods:
   - Right-click on the sample file in the Sample Table, then click **View Sample Details**.
   - Click **Actions** ▸ **View Sample Details**.
2. Click **EPT** to view the EPT plot.
3. Click **Raw Data** to view the raw data plot.
4. Click **Close**.

Review size matches

1. In the **Setup** screen, click the **Analysis summary** tab.
2. In the **Sizing** analysis results pane, click on the blue number next to 🟡 or 🔴 to review size matches for these samples in the **Size Matching** tab of the **Results** screen.



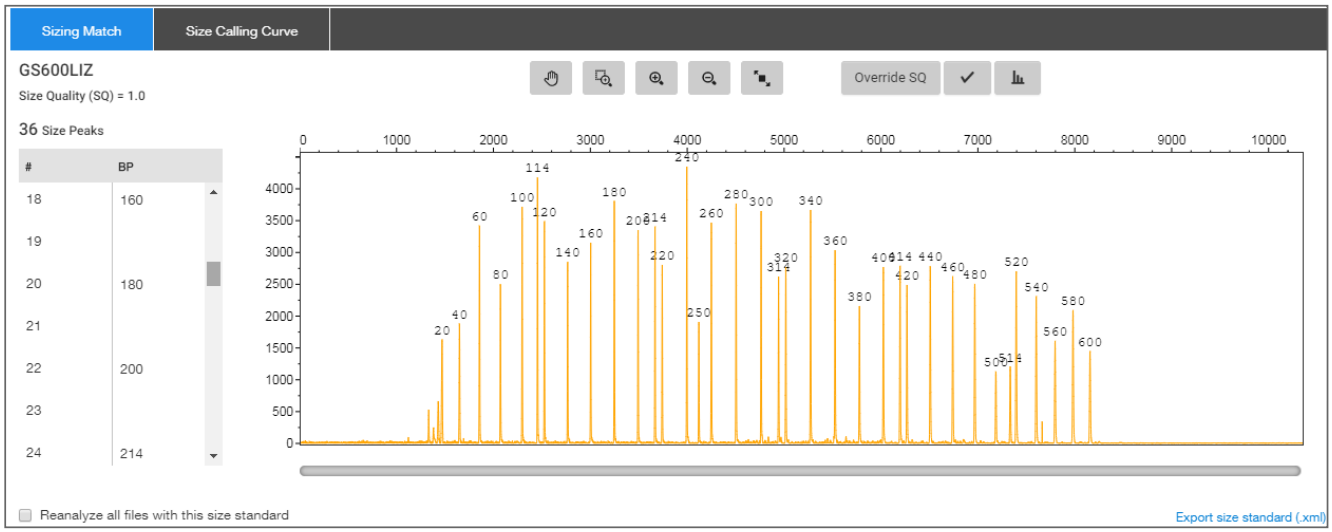
3. Edit the size matches as needed (see “Modify size matches“ on page 22).

Modify size matches

If the software does not correctly detect size standard peaks, you can modify the size matches, then apply the modified size standard definition to all selected samples in the project.

1. Select an option to view the peak assignments for the size standard peaks in the selected sample:

Option	Action
View in Size Matching tab	1. In the **Results** screen, click the **Size Matching** tab. 2. Select a sample from the sample list to view the peak assignments in the Sizing Match pane at the bottom of the screen.
View in Size Match Editor	1. In the **Results** screen, click the **Sizing** tab. 2. Select one or more samples from the sample list. 3. Click **Actions** ▸ **View Size Match Editor**.



2. Examine all size standard peaks to ensure that all peaks are present, peaks are labeled correctly, and the sizes match the fragment sizes in the size standard definition file that is used for the sample.

3. Change size values or delete peaks as needed, then auto-adjust size matches. This function is useful if the software incorrectly labels a peak.

Task	Action
Change a size value	1. Click the size value in the list, or click a peak or peak size label in the plot, then select a different value from the dropdown list. 2. Select the same peak, then click [auto adjust] (auto adjust). For example, changing the 110 peak to 100, then clicking [auto adjust] sequentially shifts the 110 size label to the current 100 peak, the 120 size label to the current 110 peak, and so on.
Delete a peak	1. Click the size value in the list, or click a peak or peak size label in the plot, then select **Delete** in the dropdown list. 2. Select the same peak, then click [auto adjust] For example, deleting the 110 peak, then clicking [auto adjust] sequentially shifts the 110 size label to the current 120 peak, the 120 size label to the current 130 peak, and so on.

4. Click [✓] to recalculate the SQ using the new sizes.

5. Click **Override SQ**.
   The SQI (Sizing Quality Invalid) indicator will be displayed
   - In the top right of the **Results** screen, in the **Sizing** tab, any sample that is analyzed with the size standard—Displays **SQI: Y**.

     Note: If **SQI** does not display in the **Sizing** tab, see "Customize the Plot View" on page 73.
   - In the **Setup** screen Sample Table —Displays ✔ in the SQI field.

6. *(Optional)* To create a new size standard definition for the project using the modified size matches, click **Reanalyze all files with this size standard**.

7. Click **Done**.

See “Sizing Quality (SQ) status“ on page 133.

Modify the default analysis settings

1. In the **Setup** screen, in the **Analysis Settings** pane, click ⚙.
2. Select the analysis setting from the list, then select an option:

Option	Action
Create new—Create new analysis settings from the default settings	1. Click **Create New**. 2. Enter the analysis settings name and description. 3. Click the Sizing Settings and Genotype Settings tabs, then modify the settings as needed (see "Analysis settings" on page 117). 4. Click **Save**.
Clone—Create new analysis settings by cloning a factory default setting, then editing existing analysis settings	1. Select an analysis setting from the list, then click **Clone**. 2. Enter the analysis settings name and description. 3. Click the Sizing Settings and Genotype Settings tabs, then modify the settings as needed (see "Analysis settings" on page 117). 4. Click **Save**.
Import—Import existing analysis settings	1. Click **Import**. 2. Select an option to import: • **Import From My Computer**—Imports files from your computer. • **Import from Thermo Fisher Cloud**—Imports files from the Thermo Fisher Cloud. The Cloud contains any files that you have previously uploaded in Data Manager. **Note:** Exported GeneMapper™ v5 or higher microsatellite analysis methods files (XML) can be imported into Thermo Fisher Cloud. 3. Browse to and select the analysis settings file (XML), then click **Open**.
Edit—Edit existing analysis settings	1. Select an analysis setting from the list, then click **Edit**. 2. Click the Sizing Settings and Genotype Settings tabs, then modify the settings as needed (see "Analysis settings" on page 117). 3. Click **Save**.

Note: Click **Restore all to defaults** to restore default analysis settings.

3. Select **Auto analyze**, then click **Apply**.

Adjust Size Quality Flag ranges

1. In the **Setup** screen, in the **Analysis Settings** pane, click ⚙.
2. Select an analysis setting, then click **Edit**.
3. Click the **Sizing Settings** tab.
4. Click **Quality Flags**.
5. Adjust the low, medium and high ranges as appropriate. See "Sizing Quality (SQ) status" on page 133.

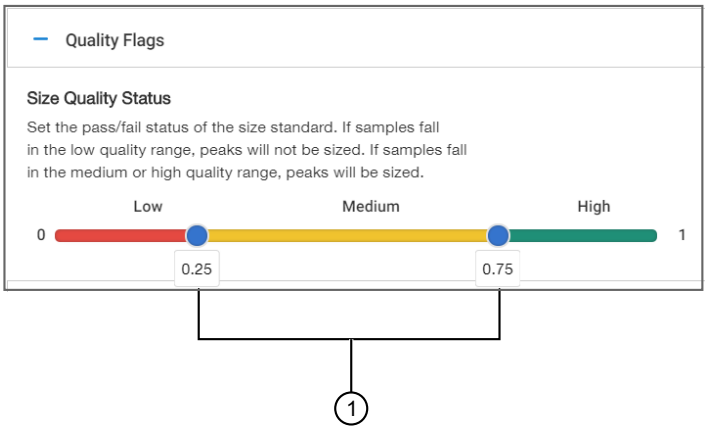


Figure 1 Quality Flag ranges

① Drag to adjust the quality flag ranges.

6. Click **Save**, select the **Auto analyze** checkbox, then click **Apply**.

Apply a different size standard to the project or a sample

You can do any of the following:

- "Select a single size standard for a project" on page 27
- "Select a size standard for individual samples" on page 27
- "Create a new size standard definition" on page 28

Select a single size standard for a project

See "Create a new size standard definition" on page 28 to create a custom size standard for your project.

1. In the **Setup** screen, select an option to apply a size standard:

Option	Action
Factory default Size Standard	1. In the **Size Standard** pane, select a factory default Size Standard from the dropdown list.
Import—Import a size standard file from your computer.	1. In the **Size Standard** pane, click ⚙. 2. Click **Import**. 3. Browse to and select a size standard, then click **Open**. 4. *(Optional)* Select **Auto analyze**. 5. Click **Apply**. **Note:** Imported size standards only appear in the project in which they were imported.

2. Click **Reanalyze Samples**.

Select a size standard for individual samples

The software allows you to apply different size standards to, then analyze individual samples in a project. When you analyze the project, only the size standard that is selected for the project is used, and size standards that are selected for individual samples are overwritten.

1. In the **Setup** screen, select the samples in the Sample Table to which you want to apply a specific size standard.
2. In the **Size Standard** pane, select a Size Standard from the dropdown list or click ⚙. Select the appropriate option to apply a size standard. See "Apply a different size standard to the project or a sample" on page 26.
3. Click **Apply**.

   Note: The new size standard is not displayed in the Size Standard column of the sample list until the samples are analyzed.
4. In the **Setup** screen, click **Reanalyze Samples**.

Create a new size standard definition

1. In the **Setup** screen, create a new size standard using one of the following options.

Option	Action
Create a customized Size Standard using the factory default Size Standard	1. In the Size Standard pane, click ⚙. 2. Select a factory default Size Standard from the list, then click **Clone**. 3. Edit the numeric values for the size standard definition. Separate size peak values with commas.
Manually create a customized Size Standard	1. In the Size Standard pane, click ⚙. 2. Click **Create new**. 3. Enter the following information: • Name • Dye Color • Description • Numeric values for the size standard definition. Separate size peak values with commas.
Create a customized Size Standard using an existing Size Standard	1. In the Size Standard pane, select a Size Standard from the dropdown list, then click **Save As**. 2. Enter the Size Standard name and description, then click **Save to Library**. 3. In the Size Standard pane, select the new Size Standard from the dropdown list, then click **Edit**. 4. Edit the numeric values for the size standard definition. Separate size peak values with commas. 5. Click **Save**. 6. In the Size Standard pane, click ⚙. 7. Select the new Size Standard from the list.

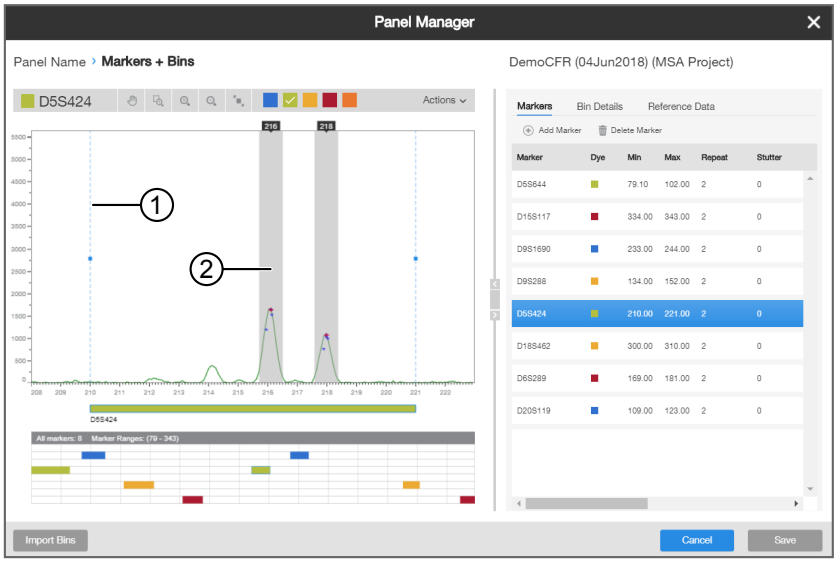
2. (*Optional*) Select **Auto analyze**.
3. Click **Apply**.

Create a panel

Overview

Genoytping analysis uses a panel consisting of markers and bins.

- **Panel**—A collection of markers
- **Marker**—A fragment size range (bp), dye color, and repeat length
- **Bin**—The fragment size (bp) or base pair range that defines an allele within a specific marker



① Marker range (dotted blue line)
② Bins (grey columns)

The software does not include default panel information. You must create a new panel (see "Create a new panel in Panel Manager" on page 30) or import a panel ("Import a panel in Panel Manager" on page 40) to your library to perform genotyping analysis.

Create a panel workflow

The workflow shown below is an example approach for creating a panel.

Create a new panel in Panel Manager (page 30) or Import a panel in Panel Manager (page 40) (Panels may be imported with or without markers/bins)

▼

Manually add markers to a panel (page 31) or Create markers using reference data (page 32)

▼

Manually add bins to a marker (page 35) or Create bins using reference data (page 36)

▼

Review markers and bins (page 42)

▼

Edit a panel in Panel Manager (page 43)

Create a new panel in Panel Manager

To import panels that contain marker and bin information, see “Import a panel in Panel Manager“ on page 40.

1. In the **Setup** screen, in the **Panel** pane, click ⚙, then click **Create New**.
2. Enter the panel name and description, then click **Next**.
3. Click **Save**.

Add markers to complete panel setup. You can do either of the following:

- “Manually add markers to a panel“ on page 31
- “Create markers using reference data“ on page 32

Manually add markers to a panel

1. Select an option to edit the panel.

Option	Action
Edit the library panel—Changes are saved to the original panel in the library.	1. In the **Setup** screen, in the **Panel** pane, click ⚙. 2. Select a panel from the list, then click **Edit**.
Edit the project panel—Changes are made to the panel applied to the project, not to the original panel in the library. The edited project panel can be saved to the library using the **Save As** option.	1. In the **Setup** screen, in the **Panel** pane, select a panel from the dropdown list to apply it to the project. **Note:** Once a panel is selected, the software automatically generates a project panel using the naming convention: Library Panel Name (Project Name). 2. Click **Edit**.

2. In the **Panel Manager** dialog box, click **Markers**.
3. Click **Add Marker**, then enter the following information:
   - Marker name
   - Dye color
   - Minimum size in basepairs (Min)
   - Maximum size in basepairs (Max)
   - *(Optional)* Stutter
   - Repeat
   - *(Optional)* Control alleles
   - *(Optional)* Ladder alleles
   - *(Optional)* Comments
4. Repeat for remaining markers.
5. Click **Save**, then click **Ok**.
6. Close the **Panel Manager** dialog box.

7. Select an option to reanalyze the samples.

Option	Action
Reanalyze using the edited library panel	1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list. 2. Click **Reanalyze Samples**.
Reanalyze using the edited project panel	Click **Reanalyze Samples**.

Add bins to markers to complete panel setup. You can do any of the following:

- "Manually add bins to a marker" on page 35
- "Create bins using reference data" on page 36
- "Create bins using project alleles" on page 39
- "Import bins in Panel Manager" on page 41

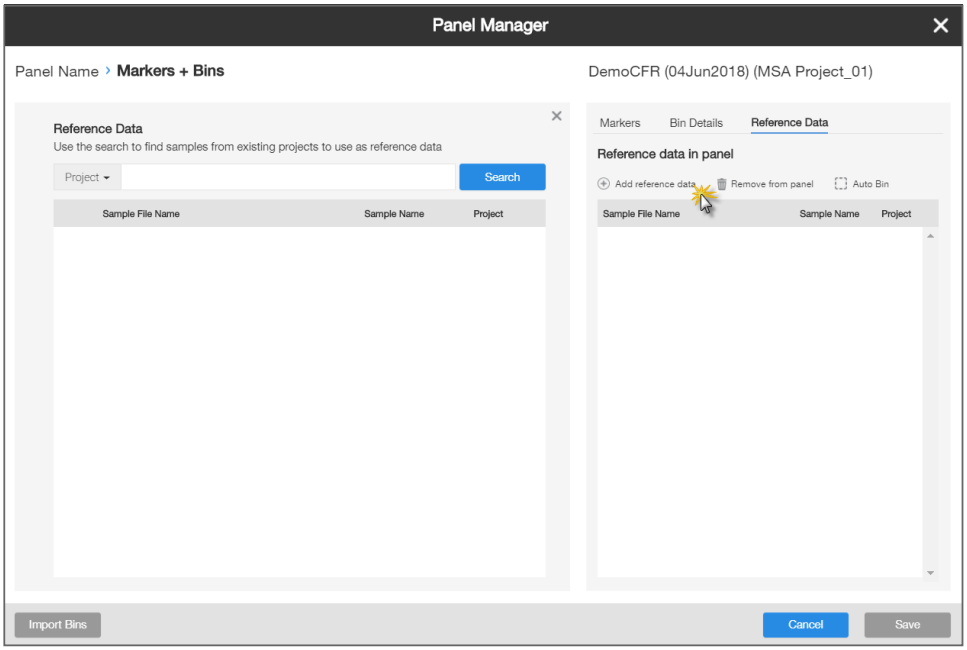
Create markers using reference data

Samples in the current project must be sized before they can be used as reference data to create markers in a panel (see "Perform initial sizing analysis using default analysis settings" on page 17).

1. Select an option to edit the panel.

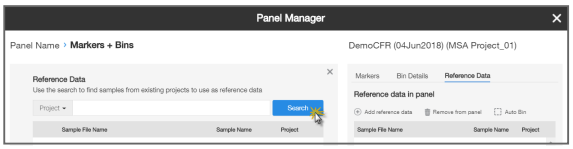
Option	Action
Edit the library panel—Changes are saved to the original panel in the library.	1. In the **Setup** screen, in the **Panel** pane, click ⚙. 2. Select a panel from the list, then click **Edit**.
Edit the project panel—Changes are made to the panel applied to the project, not to the original panel in the library. The edited project panel can be saved to the library using the **Save As** option.	1. In the **Setup** screen, in the **Panel** pane, select a panel from the dropdown list to apply it to the project. **Note:** Once a panel is selected, the software automatically generates a project panel using the naming convention: Library Panel Name (Project Name). 2. Click **Edit**.

2. In the **Panel Manager** dialog box, click **Reference Data**, then click **Add reference data** to open the search field.



3. Select a search option.

Note: Only project samples analyzed with the current panel will appear in the **Reference Data** pane.

Option	Action
Search for all samples in the current project	1. Leave the reference data search field blank, then click **Search**.  **Note:** If no files are listed in the **Reference Data** results list, ensure that the **Analysis status** of the files in the project is ✓. If the **Status** column displays ↻, click **Reanalyze Samples**.
Search for specific sample files	1. Select a search parameter from the dropdown list (**Project**, **Sample name**, or **Sample file name**). 2. Enter project or sample information in the reference data search field, then hit **Enter**. **Note:** The wildcard symbol (*) cannot be used in the search field.

4. In the **Reference Data** pane, click ⊕ to add the sample as reference data. Repeat for remaining reference data files.

   Note: You can add one or more sample files in a project as reference data.

   Note: A green check mark in the **REF** column of the Sample Table indicates samples used as reference data.

5. In the **Reference data in panel** pane, select a sample file to display the sizing information for that sample in the electropherogram.

6. In the **Markers** tab, use the reference data peak information to add markers to the project.
   a. Click **Add Marker**, then enter the following information:
      - Marker name
      - Dye color
      - Minimum size in basepairs (Min)
      - Maximum size in basepairs (Max)
      - *(Optional)* Stutter
      - Repeat
      - *(Optional)* Control alleles
      - *(Optional)* Ladder alleles
      - *(Optional)* Comments

   b. Ctrl+click (Windows™) or Command+click (Mac OS™) to deselect the newly created marker and view the electropherogram for the sample. Add markers for the remaining reference samples.

7. Click **Save**, then click **Ok**.

8. Close the **Panel Manager** dialog box.

9. Select an option to reanalyze the samples.

Option	Action
Reanalyze using the edited library panel	1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list. 2. Click **Reanalyze Samples**.
Reanalyze using the edited project panel	Click **Reanalyze Samples**.

Add bins to markers to complete panel setup. You can do any of the following:

- "Manually add bins to a marker" on page 35
- "Create bins using reference data" on page 36
- "Create bins using project alleles" on page 39
- "Import bins in Panel Manager" on page 41

Manually add bins to a marker

1. Select an option to edit the panel.

Option	Action
Edit the library panel—Changes are saved to the original panel in the library.	1. In the **Setup** screen, in the **Panel** pane, click ⚙. 2. Select a panel from the list, then click **Edit**.
Edit the project panel—Changes are made to the panel applied to the project, not to the original panel in the library. The edited project panel can be saved to the library using the **Save As** option.	1. In the **Setup** screen, in the **Panel** pane, select a panel from the dropdown list to apply it to the project. **Note:** Once a panel is selected, the software automatically generates a project panel using the naming convention: Library Panel Name (Project Name). 2. Click **Edit**.

2. In the **Panel Manager** dialog box, click **Bin Details**.

3. Select a marker from the list, then select an option to create a bin/bins:

Option	Action
Add a single bin	1. Click **Add Bin**. 2. Enter the bin name and information, then click **Add**. 3. Repeat for each bin associated with the selected marker.
	1. Right-click on the electropherogram, then click **New Bin**. 2. Click-drag the dotted blue line to define the left and right cutoffs of the bin. 3. Enter the bin name, then click **Add**.
Add multiple bins	**Note:** Selecting this option will delete any bin information associated with the selected marker. 1. Click **Add Multi Bin**. 2. Select the naming convention and the bin options, then click **Add**.

4. Repeat for the remaining markers.

5. Click **Save**.

6. Close the **Panel Manager** dialog box.

7. Select an option to reanalyze the samples.

Option	Action
Reanalyze using the edited library panel	1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list. 2. Click **Reanalyze Samples**.
Reanalyze using the edited project panel	Click **Reanalyze Samples**.

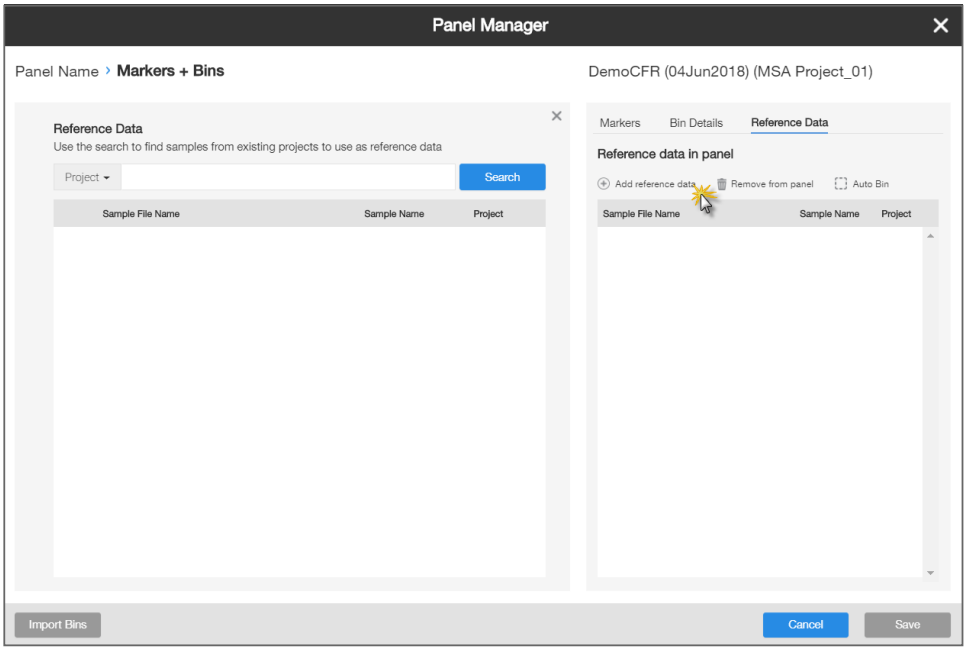
Create bins using reference data

Samples in the current project must be sized before they can be used as reference data to create markers in a panel (see "Perform initial sizing analysis using default analysis settings" on page 17).

1. Select an option to edit the panel.

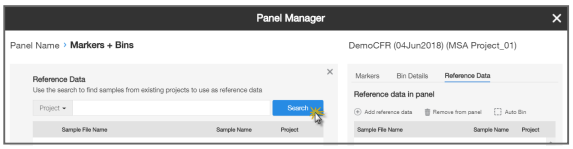
Option	Action
Edit the library panel—Changes are saved to the original panel in the library.	1. In the **Setup** screen, in the **Panel** pane, click ⚙. 2. Select a panel from the list, then click **Edit**.
Edit the project panel—Changes are made to the panel applied to the project, not to the original panel in the library. The edited project panel can be saved to the library using the **Save As** option.	1. In the **Setup** screen, in the **Panel** pane, select a panel from the dropdown list to apply it to the project. **Note:** Once a panel is selected, the software automatically generates a project panel using the naming convention: Library Panel Name (Project Name). 2. Click **Edit**.

2. In the **Panel Manager** dialog box, click **Reference Data**, then click **Add reference data** to open the search field.



3. Select a search option.

Note: Only project samples analyzed with the current panel will appear in the **Reference Data** pane.

Option	Action
Search for all samples in the current project	1. Leave the reference data search field blank, then click **Search**.  **Note:** If no files are listed in the **Reference Data** results list, ensure that the **Analysis status** of the files in the project is ✓. If the **Status** column displays ↻, click **Reanalyze Samples**.
Search for specific sample files	1. Select a search parameter from the dropdown list (**Project**, **Sample name**, or **Sample file name**). 2. Enter project or sample information in the reference data search field, then hit **Enter**. **Note:** The wildcard symbol (*) cannot be used in the search field.

4. In the **Reference Data** pane, click ⊕ to add the sample as reference data. Repeat for remaining reference data files.

   Note: You can add one or more sample files in a project as reference data.

   Note: A green check mark in the **REF** column of the Sample Table indicates samples used as reference data.

5. In the **Reference data in panel** pane, select a sample file to view the analyzed plot for that sample in the electropherogram.

6. In the **Bin Details** tab, select a marker from the list, then select an option to create a bin/bins using the reference data peak information.

Option	Action
Add a single bin	1. Click **Add Bin**. 2. Enter the bin name and information, then click **Add**. 3. Repeat for each bin associated with the selected marker.
	1. Right-click on the electropherogram, then click **New Bin**. 2. Click-drag the dotted blue line to define the center of the bin, then enter the left and right cutoff values. 3. Enter the bin name, then click **Add**.
Add multiple bins	**Note:** Selecting this option will delete any bin information associated with the selected marker. 1. Click **Add Multi Bin**. 2. Select the naming convention and the bin options, then click **Add**.

7. Repeat for the remaining markers.

8. Click **Save**.

9. Close the **Panel Manager** dialog box.

10. Select an option to reanalyze the samples.

Option	Action
Reanalyze using the edited library panel	1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list. 2. Click **Reanalyze Samples**.
Reanalyze using the edited project panel	Click **Reanalyze Samples**.

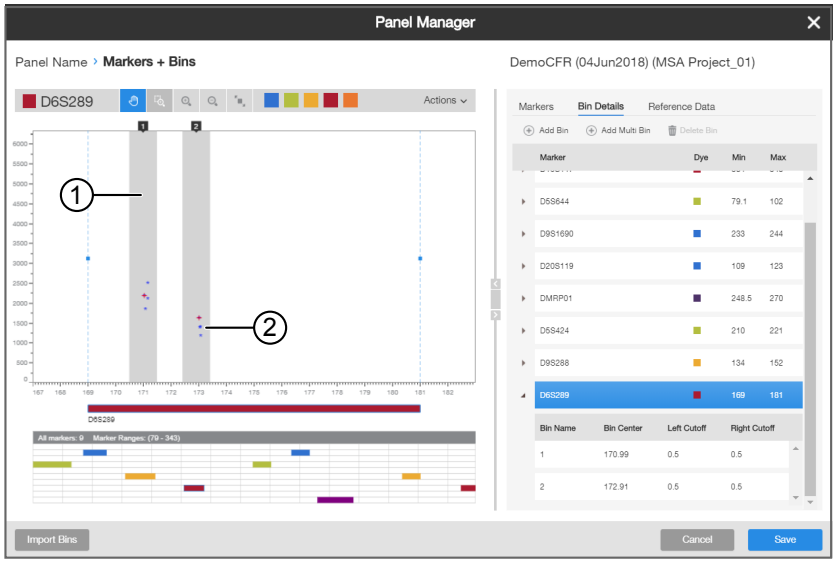
Create bins using project alleles

1. Select an option to edit the panel.

Option	Action
Edit the library panel—Changes are saved to the original panel in the library.	1. In the **Setup** screen, in the **Panel** pane, click ⚙. 2. Select a panel from the list, then click **Edit**.
Edit the project panel—Changes are made to the panel applied to the project, not to the original panel in the library. The edited project panel can be saved to the library using the **Save As** option.	1. In the **Setup** screen, in the **Panel** pane, select a panel from the dropdown list to apply it to the project. **Note:** Once a panel is selected, the software automatically generates a project panel using the naming convention: Library Panel Name (Project Name). 2. Click **Edit**.

2. In the **Panel Manager** dialog box, click **Bin Details**, then select a marker from the list.

   Note: Click **Actions** ▸ **Show Project Alleles** to view project alleles.



① Bins (grey columns)
② Project alleles (blue asterisks) for each bin

The project alleles (alleles detected in the sample data) appear as blue asterisks in each bin. The y-axis position of each indicates the GQ score for that marker and sample.

3. Select an option to create a bin/bins using project alleles.

Option	Action
Add a single bin	1. Click **Add Bin**. 2. Enter the bin name and information, then click **Add**. 3. Repeat for each bin associated with the selected marker.
	1. Right-click on the electropherogram, then click **New Bin**. 2. Click-drag the dotted blue line to define the center of the bin, then enter the left and right cutoff values. 3. Enter the bin name, then click **Add**.
Add multiple bins	**Note:** Selecting this option will delete any bin information associated with the selected marker. 1. Click **Add Multi Bin**. 2. Select the naming convention and the bin options, then click **Add**.

4. Repeat for the remaining markers.
5. Click **Save**.
6. Close the **Panel Manager** dialog box.
7. Select an option to reanalyze the samples.

Option	Action
Reanalyze using the edited library panel	1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list. 2. Click **Reanalyze Samples**.
Reanalyze using the edited project panel	Click **Reanalyze Samples**.

Import a panel in Panel Manager

You can import panels (TXT files) that contain marker information. For example, panel files are available in the GeneMapper™ Software (v4.1 and above) for some of the kits available from Thermo Fisher Scientific.

1. In the **Setup** screen, in the **Panel** pane, click ⚙.
2. Click **Import**.

3. Select panel files to import:
   - **GeneMapper** v5 or higher—Import both a TXT file for panel (marker information) and a TXT file for bin information.

     Note: Import only a TXT file for the panel if you want to create your own bins for the markers (see "Manually add bins to a marker" on page 35).

     Note: (*If needed*) Import bin information separately in the **Panel Manager** dialog box (see "Import bins in Panel Manager" on page 41).
   - **Microsatellite** —Import a ZIP file that contains both panel (marker) and bin information.
4. Select an import option:
   - **Import From My Computer**—Imports files from your computer.
   - **Import from Thermo Fisher Cloud**—Imports files from the Thermo Fisher Cloud. The Cloud contains any files that you have previously uploaded in Data Manager.
5. Browse to and select the panel file(s) (TXT or ZIP), then click **Open**.
6. Click **Import**.
7. Click **Save**.
8. Review the panel, then click **Back to Library**.
9. *(Optional)* Click **Apply**, then click **Reanalyze Samples**.
10. Proceed to "Analyze project using default genotyping analysis settings" on page 51.

Import bins in Panel Manager

1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list, then click **Edit**.
2. In the lower-left corner of the **Panel Manager** dialog box, click **Import Bins**.
3. Select an import option:
   - **Import From My Computer**—Imports files from your computer.
   - **Import from Thermo Fisher Cloud**—Imports files from the Thermo Fisher Cloud. The Cloud contains any files that you have previously uploaded in Data Manager.
4. Browse to and select the bin file(s) (TXT), then click **Open**.
5. Click **Import**.
6. Click **Save**.

7. Close the **Panel Manager** dialog box.

8. In the **Panel** pane, select the panel with the added bins from the dropdown list, then click **Reanalyze Samples**.

Review markers and bins

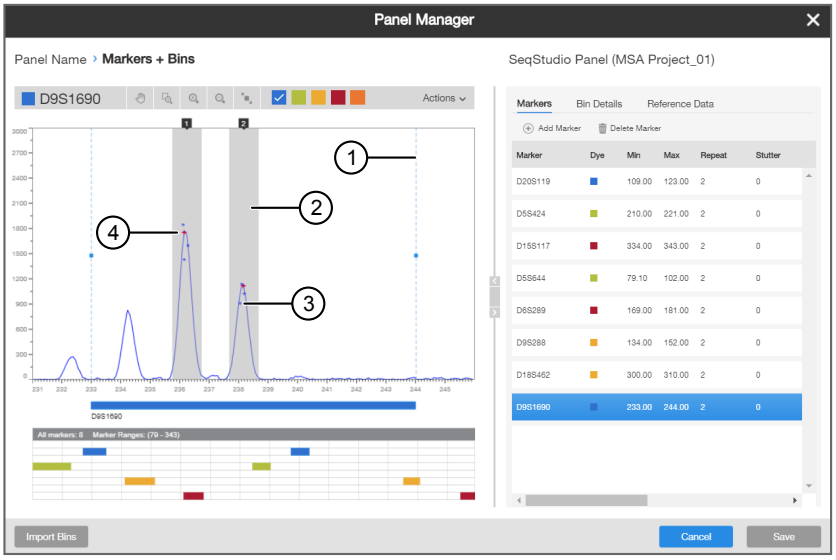
See “Parts of the Panel Manager dialog box“ on page 108.

1. In the **Reference Data** tab, select a reference sample to review the electropherogram peaks for the selected sample.

2. In the **Markers** tab, select a marker using one of the following options:
   - In the **Markers** tab, click on the marker name.
   - In the Marker overview pane, click on the marker.

3. Review electropherogram for the following information:

   Note: Click **Actions ▸ Show Project Alleles** to view project alleles.



① Marker range (dotted blue line)
② Bins (grey columns)
③ Project alleles (blue asterisks) for each bin
④ Reference alleles (red cross hatches) for each bin

4. Repeat for each marker to confirm that bins were created for it.

Edit a panel in Panel Manager

1. In the **Setup** screen, in the **Panel** pane, click ⚙.
2. Select an option to edit an existing panel in the library:

Option	Action
Clone—Create a new panel by cloning, then editing an existing panel	1. Select a panel from the list, then click **Clone**. 2. Enter the panel name and description, then click **Next**.
Edit—Edit an existing panel	1. Select a panel from the list, then click **Edit**.

3. Proceed with any of the following:
   - "Manually add markers to a panel" on page 31
   - "Edit or delete an existing marker in Panel Manager" on page 44
   - "Manually add bins to a marker" on page 35
   - "Edit or delete an existing bin in Panel Manager" on page 47
   - "Refine a panel using the Auto Bin procedure" on page 48

Manually add markers to a panel

1. Select an option to edit the panel.

Option	Action
Edit the library panel—Changes are saved to the original panel in the library.	1. In the **Setup** screen, in the **Panel** pane, click ⚙. 2. Select a panel from the list, then click **Edit**.
Edit the project panel—Changes are made to the panel applied to the project, not to the original panel in the library. The edited project panel can be saved to the library using the **Save As** option.	1. In the **Setup** screen, in the **Panel** pane, select a panel from the dropdown list to apply it to the project. **Note:** Once a panel is selected, the software automatically generates a project panel using the naming convention: Library Panel Name (Project Name). 2. Click **Edit**.

2. In the **Panel Manager** dialog box, click **Markers**.
3. Click **Add Marker**, then enter the following information:
   - Marker name
   - Dye color
   - Minimum size in basepairs (Min)
   - Maximum size in basepairs (Max)

- *(Optional)* Stutter
- Repeat
- *(Optional)* Control alleles
- *(Optional)* Ladder alleles
- *(Optional)* Comments

4. Repeat for remaining markers.
5. Click **Save**, then click **Ok**.
6. Close the **Panel Manager** dialog box.
7. Select an option to reanalyze the samples.

Option	Action
Reanalyze using the edited library panel	1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list. 2. Click **Reanalyze Samples**.
Reanalyze using the edited project panel	Click **Reanalyze Samples**.

Add bins to markers to complete panel setup. You can do any of the following:

- "Manually add bins to a marker" on page 35
- "Create bins using reference data" on page 36
- "Create bins using project alleles" on page 39
- "Import bins in Panel Manager" on page 41

Edit or delete an existing marker in Panel Manager

See "Parts of the Panel Manager dialog box" on page 108.

1. Select a marker using one of the following options:
   - In the **Markers** tab, click on the marker name.
   - In the Marker overview pane, click on the marker.
2. Edit marker using one of the following options:

Option	Action
Edit in the **Markers** tab	Click in a field in the table of markers to edit marker information.
Delete in the **Markers** tab	Click **Delete Marker.**
Edit in the electropherogram	In the electropherogram, click-drag the left or right handles that define the marker range (dotted blue lines).

3. Click **Save**, then click **Back to Library** to apply the updated panel to the project.

   Note: To undo any changes to the panel, click **Back to Library** without saving. All changes made to the panel will be lost.

Manually add bins to a marker

1. Select an option to edit the panel.

Option	Action
Edit the library panel—Changes are saved to the original panel in the library.	1. In the **Setup** screen, in the **Panel** pane, click ⚙. 2. Select a panel from the list, then click **Edit**.
Edit the project panel—Changes are made to the panel applied to the project, not to the original panel in the library. The edited project panel can be saved to the library using the **Save As** option.	1. In the **Setup** screen, in the **Panel** pane, select a panel from the dropdown list to apply it to the project. **Note:** Once a panel is selected, the software automatically generates a project panel using the naming convention: Library Panel Name (Project Name). 2. Click **Edit**.

2. In the **Panel Manager** dialog box, click **Bin Details**.

3. Select a marker from the list, then select an option to create a bin/bins:

Option	Action
Add a single bin	1. Click **Add Bin**. 2. Enter the bin name and information, then click **Add**. 3. Repeat for each bin associated with the selected marker.
	1. Right-click on the electropherogram, then click **New Bin**. 2. Click-drag the dotted blue line to define the left and right cutoffs of the bin. 3. Enter the bin name, then click **Add**.
Add multiple bins	**Note:** Selecting this option will delete any bin information associated with the selected marker. 1. Click **Add Multi Bin**. 2. Select the naming convention and the bin options, then click **Add**.

4. Repeat for the remaining markers.

5. Click **Save**.

6. Close the **Panel Manager** dialog box.

7. Select an option to reanalyze the samples.

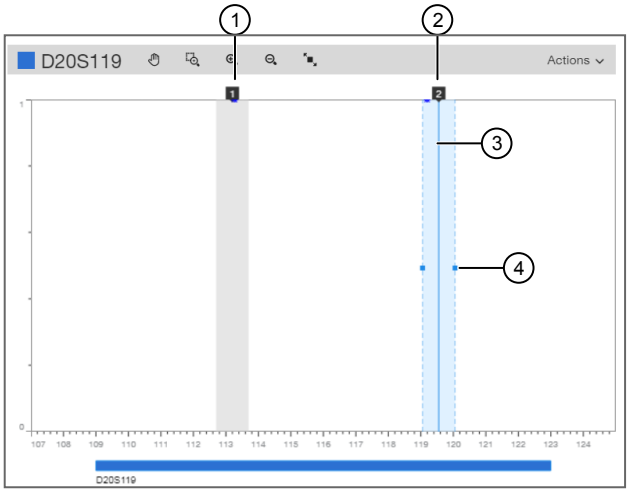
Option	Action
Reanalyze using the edited library panel	1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list. 2. Click **Reanalyze Samples**.
Reanalyze using the edited project panel	Click **Reanalyze Samples**.

Edit or delete an existing bin in Panel Manager

See "Parts of the Panel Manager dialog box" on page 108.

1. In the **Bin Details** tab, click on the marker name to view bins, then click on the bin name.

2. Edit bins using one of the following options:

Option	Action
Edit in the **Bin Details** tab	Click in a field in the table of markers to manually edit bin information.
Delete in the **Bin Details** tab	Click **Delete Bin**.
Shift all bins	Click **Actions** ▸ **Shift All Bins**, enter the number of basepairs and direction to shift, then click **Save**.
Edit in the electropherogram	In the electropherogram, edit bins using the following options: 1. **Edit bin center**—Click-drag the line that define the bin center (dotted blue lines). 2. **Edit bin range**—Click-drag the left or right handles that define the bin range (dotted blue lines). 3. **Edit bin information**—Right-click on the bin, click **Edit Bin**, enter bin information, then click **Update**.  ① Bin 1 ② Bin 2 ③ Bin center ④ Bin range
Delete in the electropherogram	In the electropherogram, right-click on the bin, then click **Delete Bin**.

3. Click **Save**, then click **Back to Library** to apply the updated panel to the project.

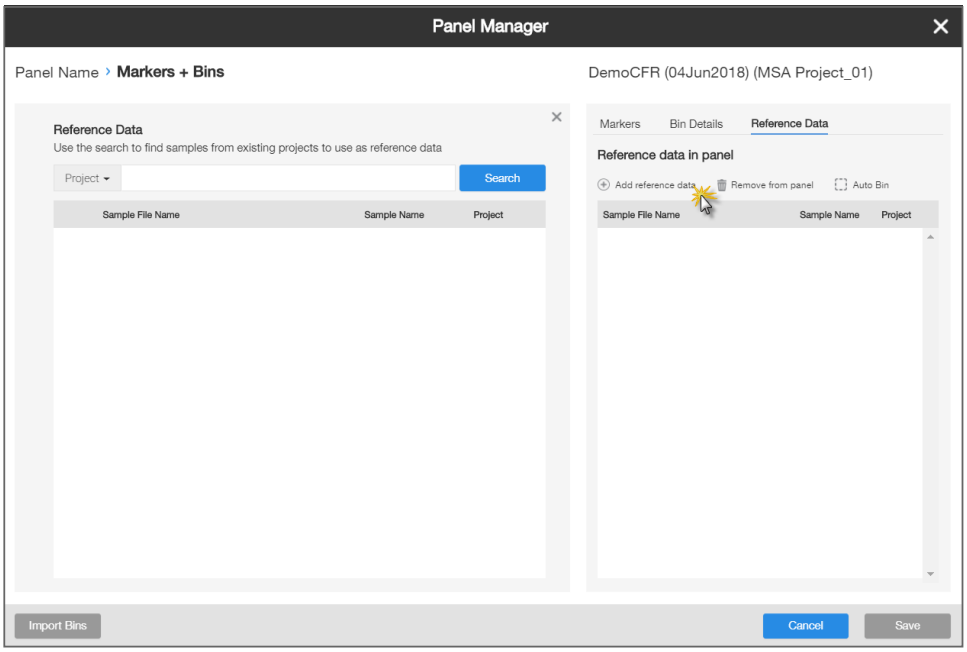
   Note: To undo any changes to the panel, click **Back to Library** without saving. All changes made to the panel will be lost.

Refine a panel using the Auto Bin procedure

The Auto Bin procedure can be used to enhance genotyping results using reference sample data files from another project analyzed with the same panel. The auto binning function adds incremental bins or replaces existing bins in the panel.

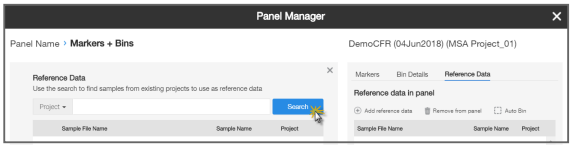
1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list, then click **Edit**.

2. In the **Panel Manager** dialog box, click **Reference Data**, then click **Add reference data** to open the search field.



3. Select a search option.

   Note: Only project samples analyzed with the current panel will appear in the **Reference Data** pane.

Option	Action
Search for all samples in the current project	1. Leave the reference data search field blank, then click **Search**.  **Note:** If no files are listed in the **Reference Data** results list, ensure that the **Analysis status** of the files in the project is ✓. If the **Status** column displays ↻, click **Reanalyze Samples**.
Search for specific sample files	1. Select a search parameter from the dropdown list (**Project**, **Sample name**, or **Sample file name**). 2. Enter project or sample information in the reference data search field, then hit **Enter**. **Note:** The wildcard symbol (*) cannot be used in the search field.

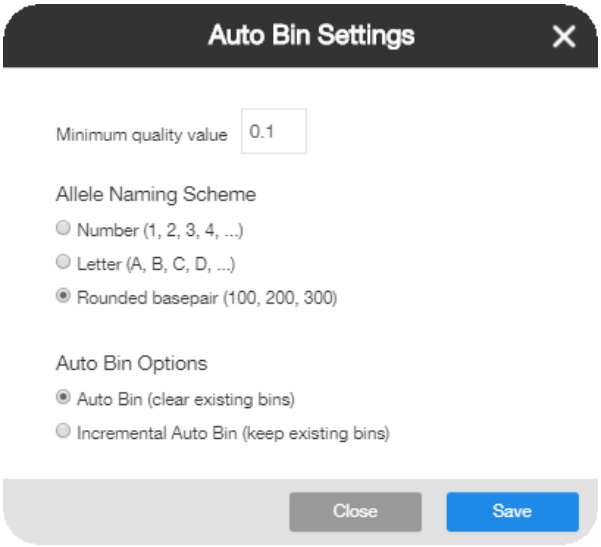
4. In the **Reference Data** pane, click ⊕ to add the sample as reference data. Repeat for remaining reference data files.

   Note: You can add one or more of the sample files in a project as reference data.

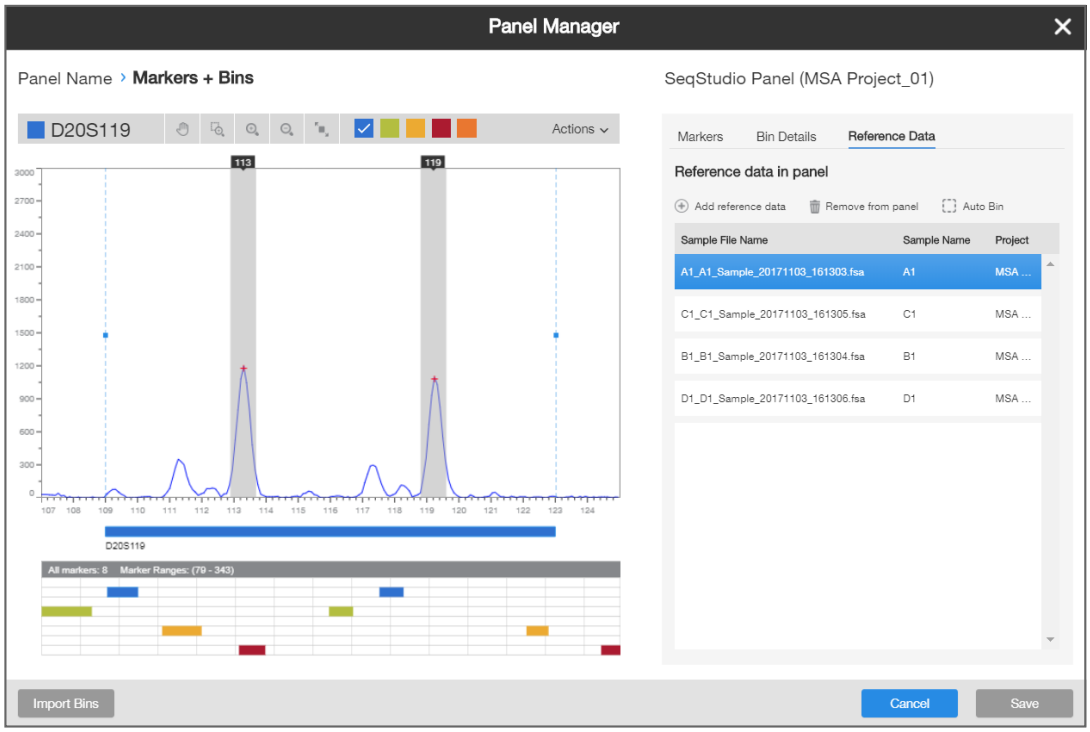
   Note: A green check mark in the **REF** column of the Sample Table indicates samples used as reference data.

5. Click **Auto Bin**.

6. In the **Auto Bin Settings** dialog box, select the appropriate settings, then click **Save**.
   - **Minimum quality value** (recommended value of 0.1)
   - **Allele Naming Scheme**
   - **Auto Bin Options**



7. In the **Panel Manager** dialog box, click **Save**.



8. Close the **Panel Manager** dialog box.

Perform genotyping analysis in the **Setup** screen

Analyze project using default genotyping analysis settings

1. In the **Setup** screen, in the **Panel** pane, click  .
2. Select a panel from the list.
3. Click **Reanalyze Samples**.
4. Review results in the **Setup** screen, then:
   - If the genotyping quality is  , proceed to "Review genotype information in the Results screen" on page 82.
   - If the sample quality is  or  , or analysis fails, proceed to:
     - "Reanalyze samples using custom genotyping analysis settings" on page 51
     - "Examine low-quality genotyping results" on page 54

Reanalyze samples using custom genotyping analysis settings

1. Perform any of the following tasks as needed:
   - "Modify the default analysis settings" on page 25
   - "Edit a panel in Panel Manager" on page 43
   - "Edit sample name and type" on page 20
   - "Adjust Genotype Quality Flag weights and ranges" on page 55
2. Click **Reanalyze Samples**.

Add/remove samples

In the **Setup** screen, select the appropriate option:.

Option	Description
Add more files	1. Click **Actions** 2. Click **Add More Files** 3. Select the appropriate import option, then click MSA.
Remove files	1. Select the samples to remove in the Sample Table. 2. Click **Actions**. 3. Click **Remove Files**. **Note:** When you click **Remove Files**, no message is displayed. You can add the files back to the project, but will have to analyze again to display results.

Search the Genotype Table

In the **Results** screen, search the **Genotype Table** using the following options:

Option	Action
Search by term	1. Enter the search term in the field at the top right corner of the table, then click Q. **Note:** The ✓ icon indicates a filter is already applied.
Search by dye color	1. Click ⌄ at the top right corner of the table. 2. Select Dye Color from the drop down menu, then click on a dye color to select. 3. Click **Search**.
Search by Process Quality Value	1. Click ⌄ at the top right corner of the table. 2. Select a PQV parameter from the drop down menu. 3. Select Quality Value from the drop down menu. 4. Click **Search**. See "Genotype Table fields" on page 113 for Quality Value descriptions.

Option	Action
Search by allele size, peak height or peak area	1. Click ⌄ at the top right corner of the table. 2. Select Size, Height or Peak Area from the drop down menu. 3. Enter a size or size range (bp) in the Value fields. 4. Click **Search**.

Click ⌄, then click **Clear All** to clear previous search results.

Customize columns in the Sample, Sizing, or Genotype Table

Customize the columns displayed in tables.

- **Sort the columns in the table**—Click a column header.
- **Edit the order of columns in the table**—Click a header, then drag and drop in the desired order.

  Note: The order of columns can only be edited in the Sample and Sizing Tables.
- **Show/Hide columns in the table**—Click ⚙ at the top right corner of the table, then select the columns to show or hide. Click **Apply**.
- **Edit Quality Metrics Display**—See "Edit Quality Metrics Display" on page 20.
- Click **Restore to default** to undo previous Show/Hide selections.

Edit Quality Metrics Display

Show Sizing Quality (SQ) and Genotyping Quality (GQ) metrics as symbols (default) or numbers in the Sample Table and Genotype Table.

1. Click ⚙ at the top right corner of the table.
2. Click **Quality Metrics Display**, then select the appropriate option.
3. Click **Apply**.

Create a template from current analysis options

Create a template from current analysis options (Size Standard, Panel, Analysis Settings and table settings). The template is stored in your library and can be imported for use in future projects (see "Analyze samples using a template" on page 18).

1. In the **Setup** screen, click **Template Manager**.
2. Click **Create from current settings**.
3. Enter the template name and description, then click **Save**.

Examine low-quality genotyping results

Modify the default analysis settings

1. In the **Setup** screen, in the **Analysis Settings** pane, click ⚙.
2. Select the analysis setting from the list, then select an option:

Option	Action
Create new—Create new analysis settings from the default settings	1. Click **Create New**. 2. Enter the analysis settings name and description. 3. Click the Sizing Settings and Genotype Settings tabs, then modify the settings as needed (see "Analysis settings" on page 117). 4. Click **Save**.
Clone—Create new analysis settings by cloning a factory default setting, then editing existing analysis settings	1. Select an analysis setting from the list, then click **Clone**. 2. Enter the analysis settings name and description. 3. Click the Sizing Settings and Genotype Settings tabs, then modify the settings as needed (see "Analysis settings" on page 117). 4. Click **Save**.
Import—Import existing analysis settings	1. Click **Import**. 2. Select an option to import: • **Import From My Computer**—Imports files from your computer. • **Import from Thermo Fisher Cloud**—Imports files from the Thermo Fisher Cloud. The Cloud contains any files that you have previously uploaded in Data Manager. **Note:** Exported GeneMapper™ v5 or higher microsatellite analysis methods files (XML) can be imported into Thermo Fisher Cloud. 3. Browse to and select the analysis settings file (XML), then click **Open**.
Edit—Edit existing analysis settings	1. Select an analysis setting from the list, then click **Edit**.

Option	Action
	2. Click the Sizing Settings and Genotype Settings tabs, then modify the settings as needed (see "Analysis settings" on page 117). 3. Click **Save**.

Note: Click **Restore all to defaults** to restore default analysis settings.

3. Select **Auto analyze**, then click **Apply**.

Review samples with Suspect and Low Quality GQ values

1. In the **Setup** screen, click the **Analysis summary** tab.
2. In the **Genotyping** analysis results pane, click on the blue number next to 🟡 or 🔴 to review allele calls and PQVs for these samples in the **Genotyping** tab of the **Results** screen.

3. Review PQV values and allele calls.
   - "Review PQVs of samples with Suspect and Low Quality GQ values" on page 85
   - "Review, edit, and add alleles" on page 85

Adjust Genotype Quality Flag weights and ranges

1. In the **Setup** screen, in the **Analysis Settings** pane, click ⚙, then click **Edit**.
2. In the **Genotype Settings** tab, click **Quality Flags**.

3. Adjust the PQV weights, and the low, medium and high Quality Flag ranges as appropriate. See "Sizing Quality (SQ) status" on page 133.

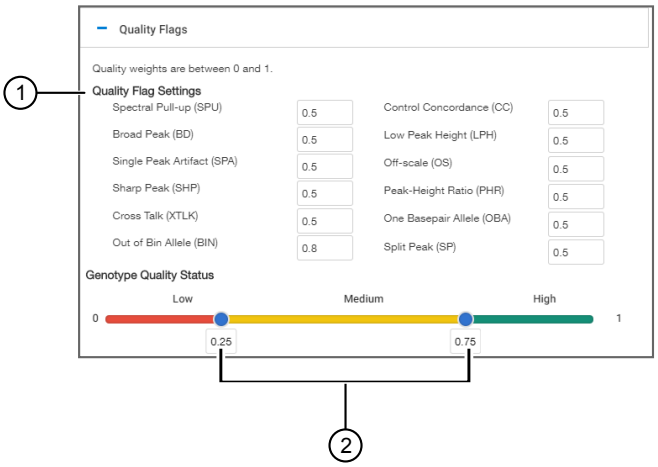


Figure 2 Quality Flag weights and ranges

① Enter value in field to adjust quality flag weights.
② Drag to adjust to quality flag ranges.

4. Click **Save**, select **Auto analyze**, then click **Apply**.

Edit a panel in Panel Manager

1. In the **Setup** screen, in the **Panel** pane, click ⚙.

2. Select an option to edit an existing panel in the library:

Option	Action
Clone—Create a new panel by cloning, then editing an existing panel	1. Select a panel from the list, then click **Clone**. 2. Enter the panel name and description, then click **Next**.
Edit—Edit an existing panel	1. Select a panel from the list, then click **Edit**.

3. Proceed with any of the following:
   - "Manually add markers to a panel" on page 31
   - "Edit or delete an existing marker in Panel Manager" on page 44
   - "Manually add bins to a marker" on page 35
   - "Edit or delete an existing bin in Panel Manager" on page 47
   - "Refine a panel using the Auto Bin procedure" on page 48

Manually add markers to a panel

1. Select an option to edit the panel.

Option	Action
Edit the library panel—Changes are saved to the original panel in the library.	1. In the **Setup** screen, in the **Panel** pane, click ⚙. 2. Select a panel from the list, then click **Edit**.
Edit the project panel—Changes are made to the panel applied to the project, not to the original panel in the library. The edited project panel can be saved to the library using the **Save As** option.	1. In the **Setup** screen, in the **Panel** pane, select a panel from the dropdown list to apply it to the project. **Note:** Once a panel is selected, the software automatically generates a project panel using the naming convention: Library Panel Name (Project Name). 2. Click **Edit**.

2. In the **Panel Manager** dialog box, click **Markers**.
3. Click **Add Marker**, then enter the following information:
   - Marker name
   - Dye color
   - Minimum size in basepairs (Min)
   - Maximum size in basepairs (Max)
   - *(Optional)* Stutter
   - Repeat
   - *(Optional)* Control alleles
   - *(Optional)* Ladder alleles
   - *(Optional)* Comments
4. Repeat for remaining markers.
5. Click **Save**, then click **Ok**.
6. Close the **Panel Manager** dialog box.
7. Select an option to reanalyze the samples.

Option	Action
Reanalyze using the edited library panel	1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list. 2. Click **Reanalyze Samples**.
Reanalyze using the edited project panel	Click **Reanalyze Samples**.

Add bins to markers to complete panel setup. You can do any of the following:

- "Manually add bins to a marker" on page 35
- "Create bins using reference data" on page 36
- "Create bins using project alleles" on page 39
- "Import bins in Panel Manager" on page 41

Edit or delete an existing marker in Panel Manager

See "Parts of the Panel Manager dialog box" on page 108.

1. Select a marker using one of the following options:
   - In the **Markers** tab, click on the marker name.
   - In the Marker overview pane, click on the marker.
2. Edit marker using one of the following options:

Option	Action
Edit in the **Markers** tab	Click in a field in the table of markers to edit marker information.
Delete in the **Markers** tab	Click **Delete Marker.**
Edit in the electropherogram	In the electropherogram, click-drag the left or right handles that define the marker range (dotted blue lines).

3. Click **Save**, then click **Back to Library** to apply the updated panel to the project.

   Note: To undo any changes to the panel, click **Back to Library** without saving. All changes made to the panel will be lost.

Manually add bins to a marker

1. Select an option to edit the panel.

Option	Action
Edit the library panel—Changes are saved to the original panel in the library.	1. In the **Setup** screen, in the **Panel** pane, click ⚙. 2. Select a panel from the list, then click **Edit**.
Edit the project panel—Changes are made to the panel applied to the project, not to the original panel in the library. The edited project panel can be saved to the library using the **Save As** option.	1. In the **Setup** screen, in the **Panel** pane, select a panel from the dropdown list to apply it to the project. **Note:** Once a panel is selected, the software automatically generates a project panel using the naming convention: Library Panel Name (Project Name). 2. Click **Edit**.

2. In the **Panel Manager** dialog box, click **Bin Details**.

3. Select a marker from the list, then select an option to create a bin/bins:

Option	Action
Add a single bin	1. Click **Add Bin**. 2. Enter the bin name and information, then click **Add**. 3. Repeat for each bin associated with the selected marker.
	1. Right-click on the electropherogram, then click **New Bin**. 2. Click-drag the dotted blue line to define the left and right cutoffs of the bin. 3. Enter the bin name, then click **Add**.
Add multiple bins	**Note:** Selecting this option will delete any bin information associated with the selected marker. 1. Click **Add Multi Bin**. 2. Select the naming convention and the bin options, then click **Add**.

4. Repeat for the remaining markers.

5. Click **Save**.

6. Close the **Panel Manager** dialog box.

7. Select an option to reanalyze the samples.

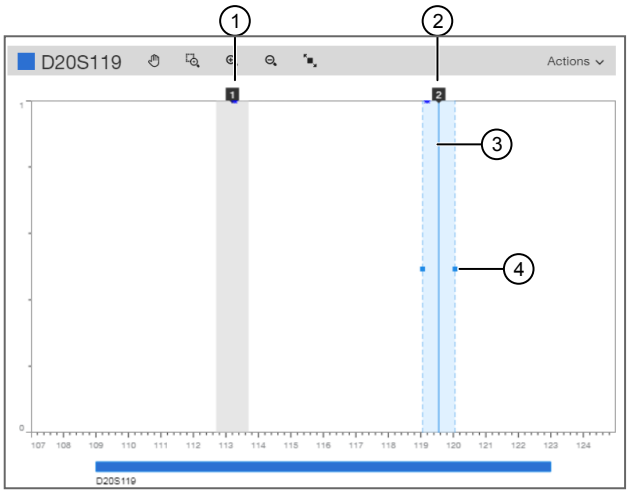
Option	Action
Reanalyze using the edited library panel	1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list. 2. Click **Reanalyze Samples**.
Reanalyze using the edited project panel	Click **Reanalyze Samples**.

Edit or delete an existing bin in Panel Manager

See "Parts of the Panel Manager dialog box" on page 108.

1. In the **Bin Details** tab, click on the marker name to view bins, then click on the bin name.

2. Edit bins using one of the following options:

Option	Action
Edit in the **Bin Details** tab	Click in a field in the table of markers to manually edit bin information.
Delete in the **Bin Details** tab	Click **Delete Bin**.
Shift all bins	Click **Actions** ▸ **Shift All Bins**, enter the number of basepairs and direction to shift, then click **Save**.
Edit in the electropherogram	In the electropherogram, edit bins using the following options: 1. **Edit bin center**—Click-drag the line that define the bin center (dotted blue lines). 2. **Edit bin range**—Click-drag the left or right handles that define the bin range (dotted blue lines). 3. **Edit bin information**—Right-click on the bin, click **Edit Bin**, enter bin information, then click **Update**.  ① Bin 1 ② Bin 2 ③ Bin center ④ Bin range
Delete in the electropherogram	In the electropherogram, right-click on the bin, then click **Delete Bin**.

3. Click **Save**, then click **Back to Library** to apply the updated panel to the project.

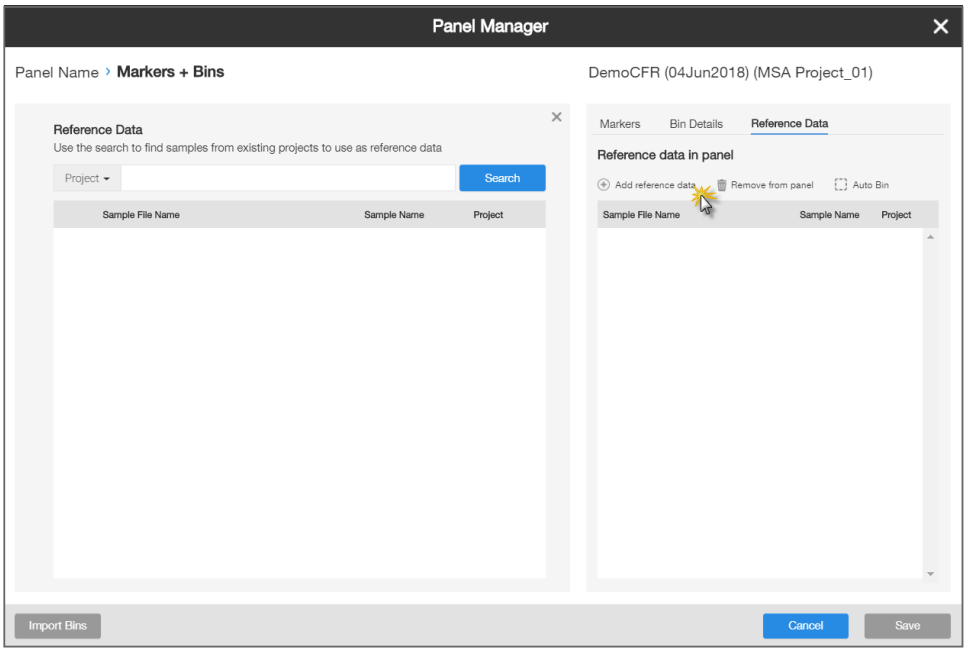
   Note: To undo any changes to the panel, click **Back to Library** without saving. All changes made to the panel will be lost.

Refine a panel using the Auto Bin procedure

The Auto Bin procedure can be used to enhance genotyping results using reference sample data files from another project analyzed with the same panel. The auto binning function adds incremental bins or replaces existing bins in the panel.

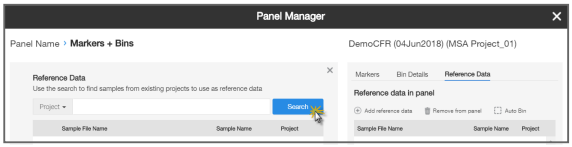
1. In the **Setup** screen, in the **Panel** pane, select the panel from the dropdown list, then click **Edit**.

2. In the **Panel Manager** dialog box, click **Reference Data**, then click **Add reference data** to open the search field.



3. Select a search option.

   Note: Only project samples analyzed with the current panel will appear in the **Reference Data** pane.

Option	Action
Search for all samples in the current project	1. Leave the reference data search field blank, then click **Search**.  **Note:** If no files are listed in the **Reference Data** results list, ensure that the **Analysis status** of the files in the project is ✓. If the **Status** column displays ↻, click **Reanalyze Samples**.
Search for specific sample files	1. Select a search parameter from the dropdown list (**Project**, **Sample name**, or **Sample file name**). 2. Enter project or sample information in the reference data search field, then hit **Enter**. **Note:** The wildcard symbol (*) cannot be used in the search field.

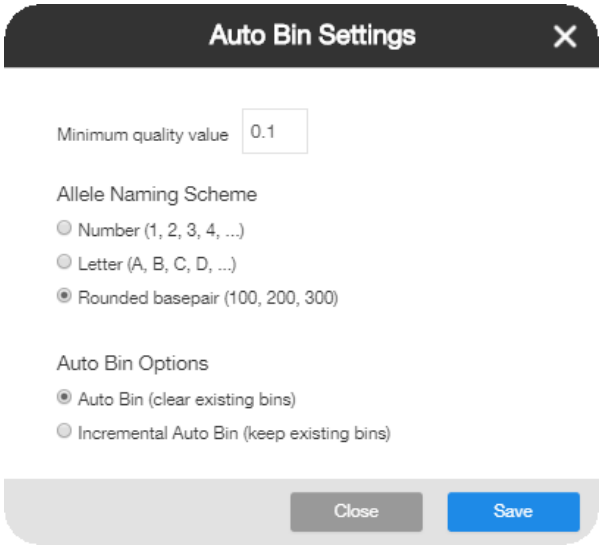
4. In the **Reference Data** pane, click ⊕ to add the sample as reference data. Repeat for remaining reference data files.

   Note: You can add one or more of the sample files in a project as reference data.

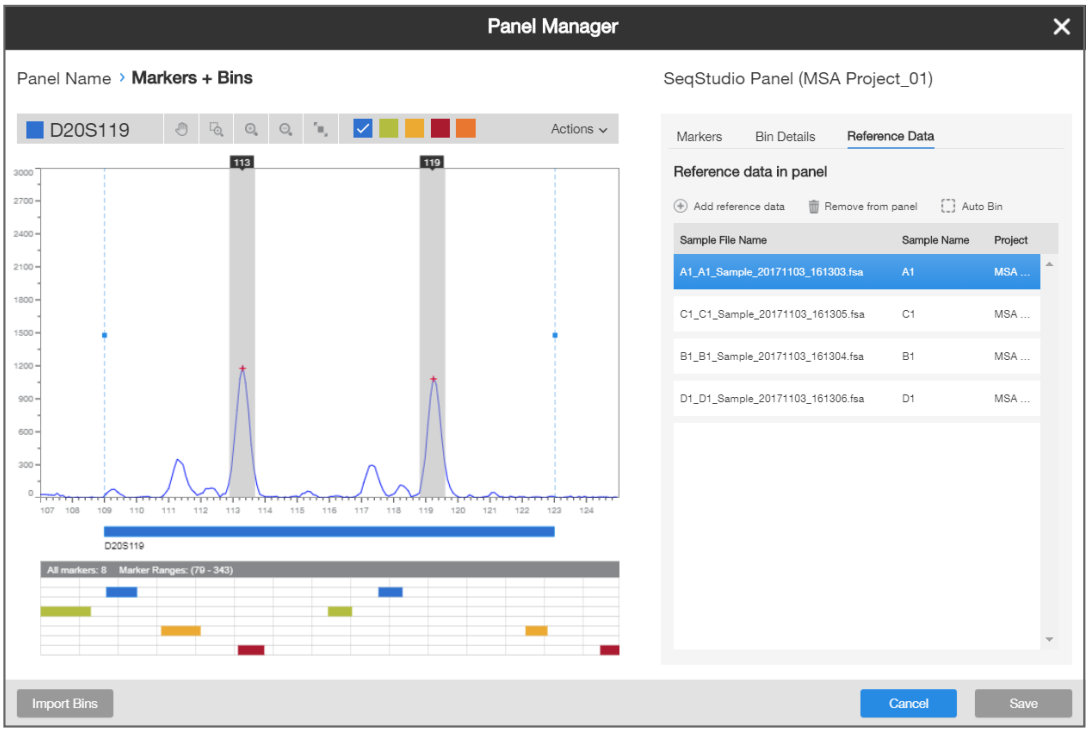
   Note: A green check mark in the **REF** column of the Sample Table indicates samples used as reference data.

5. Click **Auto Bin**.

6. In the **Auto Bin Settings** dialog box, select the appropriate settings, then click **Save**.
   - **Minimum quality value** (recommended value of 0.1)
   - **Allele Naming Scheme**
   - **Auto Bin Options**



7. In the **Panel Manager** dialog box, click **Save**.



8. Close the **Panel Manager** dialog box.

Troubleshooting

Observation	Possible cause	Action
SQ column is blank	The imported FSA file does not contain an SQ value.	Analyze samples.
SQ is ● or ●	The software is unable to properly assign size standard peaks.	See "Sizing Quality (SQ) status" on page 133.
All PQV values are greyed out	The allele was manually edited.	See "Genotyping Quality (GQ) status and Process Quality Values (PQVs)" on page 134.
Allele name is a ?	A bin is not defined for the allele.	See "Manually add bins to a marker" on page 35 or "Create bins using reference data" on page 36.
Genotyping tab is greyed out.	Genotyping analysis has not been performed.	Apply a panel to the project, then reanalyze the samples.
Reference data files are included in project, but are not displayed in reference data list in **Panel Manager**.	Analysis status of reference data is not analyzed.	Reanalyze.
	The panel name of the project does not match the new panel name.	Apply the new panel to the project, then reanalyze the samples.
PQV Flag Indicatior is ■	The sample or genotype passed the PQV test.	No further action required.
PQV Flag Indicatior is ▲	A possible problem exists for the sample or genotype.	See "Genotyping Quality (GQ) status and Process Quality Values (PQVs)" on page 134.
The Summary Report will not open.	A pop up blocker prevented the opening of the Summary Report in a new window.	Disable the pop up blocker.
The scale of the peaks for a custom dye color is incorrect.	Dye scale settings set in **Zooming + Scaling Options** are overwritten by the dye scale settings in **Custom Dyes**.	Change scale settings in **Custom Dyes**. See "Customize the dyes on the plot" on page 80.
		Turn **Custom Dyes** off.

Review sizing information in the **Results** screen

Review results

1. Click the **Results** tab at the top of the screen.
2. In the **Sizing** tab, perform any of the following tasks as needed:
   - "Examine low-quality sizing results" on page 21
   - "Customize labels on the plot" on page 77
   - "Zoom on the electropherogram" on page 67
   - "Edit peaks using Peak Editor" on page 66
   - "Edit peaks in the Sizing Table" on page 66
   - "Customize columns in the Sample, Sizing, or Genotype Table" on page 20
   - "Sort and filter peaks in the Sizing Table" on page 66

Review and edit peaks

Editing peaks allows you to redefine, add, delete, merge, and split peaks, which may be necessary with suboptimal peak resolution.

Search the Sizing Table

Search the Sizing Table using one of the following options:

Option	Action
Search by term	1. Enter the search term in the field at the top right corner of the table, then click 🔍.
Search by peak parameter	1. Click ⌄ at the top right corner of the table. 2. Select a peak parameter from the drop down menu. 3. Enter a size or size range (bp) in the Value fields. 4. Click **Search**.
Search by dye color	1. Click ⌄ at the top right corner of the table. 2. Select Dye Color from the drop down menu, then click on a dye color to select. 3. Click **Search**.

Click ⌄, then click **Clear All** to clear previous search results.

Sort and filter peaks in the Sizing Table

Task	Action
Sort	Click a dye color in the Peak overview panel to sort the Sizing Table by dye color.
Filter	Select **All Peaks**,**Selected Peaks**, or **Dye Selected Peaks** in the drop down menu in the upper-right corner of the table to filter table results.

Edit peaks in the Sizing Table

1. Select the peak or peaks to edit using one of the following methods:
   - In the electropherogram, place the pointer over a peak, then click to select it. Repeat to select additional peaks.
   - In the Sizing Table, click a row.
2. Select the appropriate action from the **Edit Peaks** dropdown list in the Sample Table header:

Option	Description
Delete peaks	Allows you to remove extraneous peaks that may result from dye-labeled primers, contaminants, or nonspecific PCR amplification. Information that is associated with a particular peak is deleted from the Sizing Table, but the peak is still displayed in the plot view.
Merge peaks	Allows two individual peaks to be treated as a single peak. The new "bin" is automatically determined. This function is typically used for offscale peaks, where overloading of sample results in peak splitting.
Split peak	Allows you to separate a single peak into two peaks. The new bins are automatically determined for both peaks by the software. This option is commonly used when two peaks are not sufficiently resolved.

Edit peaks using Peak Editor

1. In the **Results** screen, select a sample to review.
2. Click **Actions**.
3. Click **Turn on Peak Editor**.
4. In the Peak Editor window, select the dye color of the peaks you want to edit.
5. In the electropherogram, deselect all zoom controls, then select individual peak or peaks to edit using one of the following methods:
   - Place the pointer over a peak, then click to select it. Repeat to select additional peaks.
   - Click and drag the pointer to select a range of peaks.

6. Select then apply the appropriate action:

Option	Description
Delete peaks	Allows you to remove extraneous peaks that may result from dye-labeled primers, contaminants, or nonspecific PCR amplification. Information that is associated with a particular peak is deleted from the sizing table, but the peak is still displayed in the plot view.
Add missing peaks	Allows you to add and enter into the sizing table peak information that was not included in the original analysis.
Merge peaks	Allows two individual peaks to be treated as a single peak. The new "bin" is automatically determined. This function is typically used for offscale peaks, where overloading of sample results in peak splitting.
Split peak	Allows you to separate a single peak into two peaks. The new bins are automatically determined for both peaks by the software. This option is commonly used when two peaks are not sufficiently resolved.

7. Click ✕ to close the **Peak Editor** window and turn off the peak editing function.

Zoom on the electropherogram

To set zoom and scale defaults, see "Set plot Zooming and Scaling Options" on page 93.

Action	Icon	Description
Turn panning on/off		Pan over the plot. Click, then drag to move over the plot. Panning cannot be selected simultaneously with rectangular selection.
Turn rectangular selection on/off		Zoom in on a rectangular region of the plot. Click, then drag to select a rectangular region. Rectangular selection cannot be selected simultaneously with panning.
Zoom in x-axis		Click to zoom in on the x-axis. Alternatively, zoom in on the x-axis by clicking directly on the value in the axis.
Zoom out x-axis		Click to zoom out on the x-axis. Double-click on the x-axis to reset x-axis zoom.
Reset zoom to fit		Reset the plot view, panning and rectangular selection options to default settings.
Turn synchronized zoom on		Synchronize zooming when viewing multiple plots.

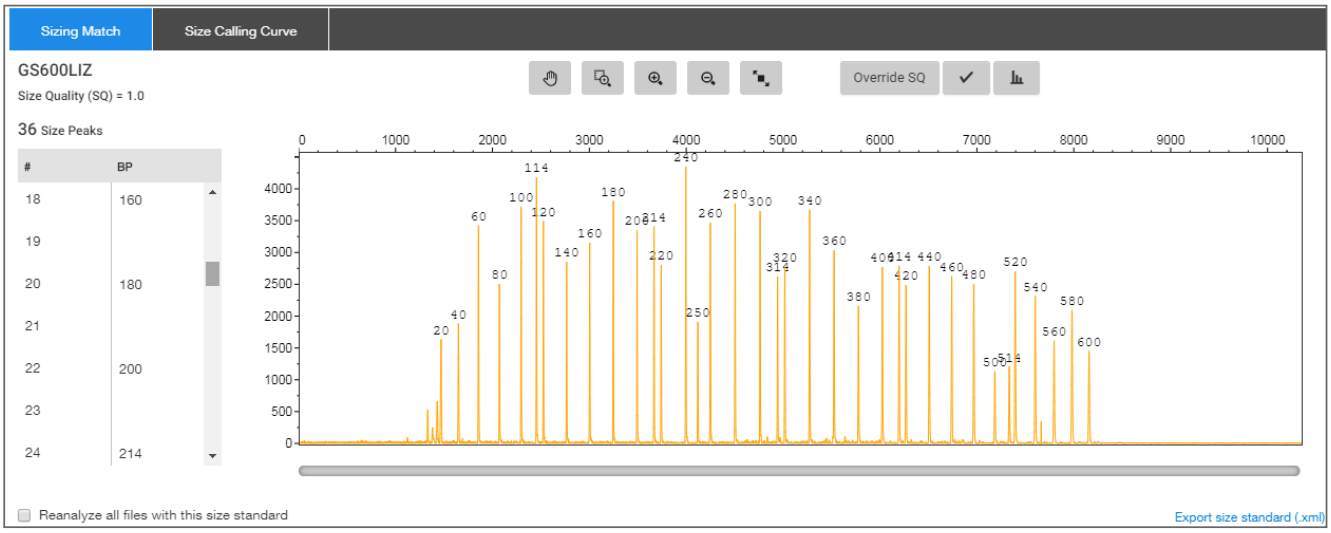
Action	Icon	Description
Turn synchronized zoom off		Turn off synchronize zooming when viewing multiple plots. Highlighted icon indicates synchronized zoom is currently turned on.
Zoom in y-axis	None	Zoom in on the y-axis by clicking directly on the value in the axis.
Zoom out y-axis	None	Double-click on the y-axis to reset y-axis zoom.
Show full screen		Click to show the plot in full screen.

Modify size matches

If the software does not correctly detect size standard peaks, you can modify the size matches, then apply the modified size standard definition to all selected samples in the project.

1. Select an option to view the peak assignments for the size standard peaks in the selected sample:

Option	Action
View in Size Matching tab	1. In the **Results** screen, click the **Size Matching** tab. 2. Select a sample from the sample list to view the peak assignments in the Sizing Match pane at the bottom of the screen.
View in Size Match Editor	1. In the **Results** screen, click the **Sizing** tab. 2. Select one or more samples from the sample list. 3. Click **Actions** ▸ **View Size Match Editor**.



2. Examine all size standard peaks to ensure that all peaks are present, peaks are labeled correctly, and the sizes match the fragment sizes in the size standard definition file that is used for the sample.

3. Change size values or delete peaks as needed, then auto-adjust size matches. This function is useful if the software incorrectly labels a peak.

Task	Action
Change a size value	1. Click the size value in the list, or click a peak or peak size label in the plot, then select a different value from the dropdown list. 2. Select the same peak, then click (auto adjust). For example, changing the 110 peak to 100, then clicking sequentially shifts the 110 size label to the current 100 peak, the 120 size label to the current 110 peak, and so on.
Delete a peak	1. Click the size value in the list, or click a peak or peak size label in the plot, then select **Delete** in the dropdown list. 2. Select the same peak, then click For example, deleting the 110 peak, then clicking sequentially shifts the 110 size label to the current 120 peak, the 120 size label to the current 130 peak, and so on.

4. Click to recalculate the SQ using the new sizes.

5. Click **Override SQ**.
   The SQI (Sizing Quality Invalid) indicator will be displayed
   - In the top right of the **Results** screen, in the **Sizing** tab, any sample that is analyzed with the size standard—Displays **SQI: Y**.

     Note: If **SQI** does not display in the **Sizing** tab, see "Customize the Plot View" on page 73.
   - In the **Setup** screen Sample Table —Displays ✔ in the SQI field.

6. *(Optional)* To create a new size standard definition for the project using the modified size matches, click **Reanalyze all files with this size standard**.

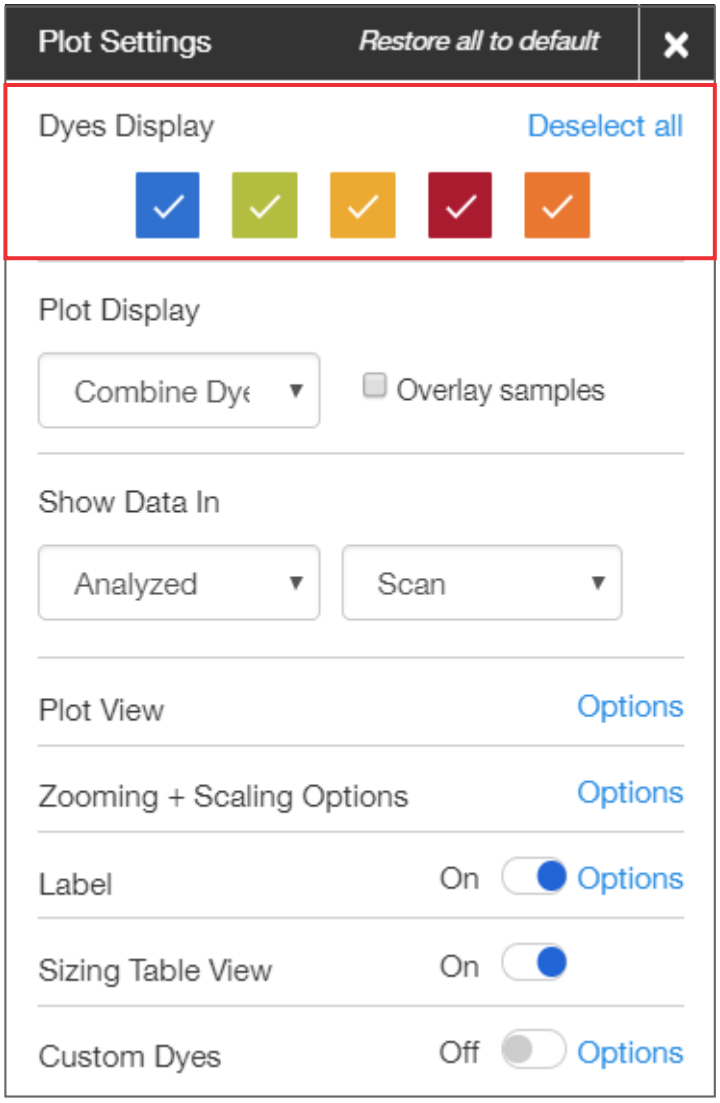
7. Click **Done**.

See "Sizing Quality (SQ) status" on page 133.

Customize sizing plot data and views

Select dye data to display

1. In the **Results** screen, in the **Sizing** tab, click **Plot Settings** to display the plot settings selector screen.

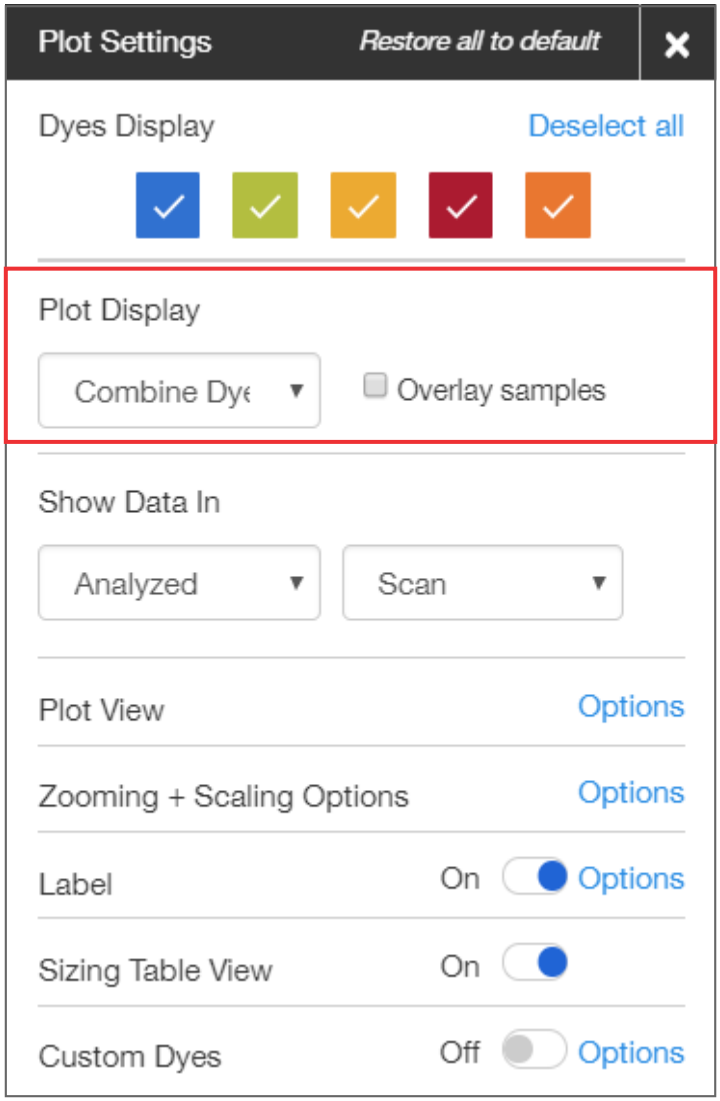


2. Review the default dye display settings and change as needed.

3. To display only selected dyes:
   - Click the square of the dye color to hide.
   - Click **Deselect all**, then click the square of the dye color to display.

View dyes in combined or separate plots

1. In the **Results** screen, click **Plot Settings** to display the plot settings selector screen.



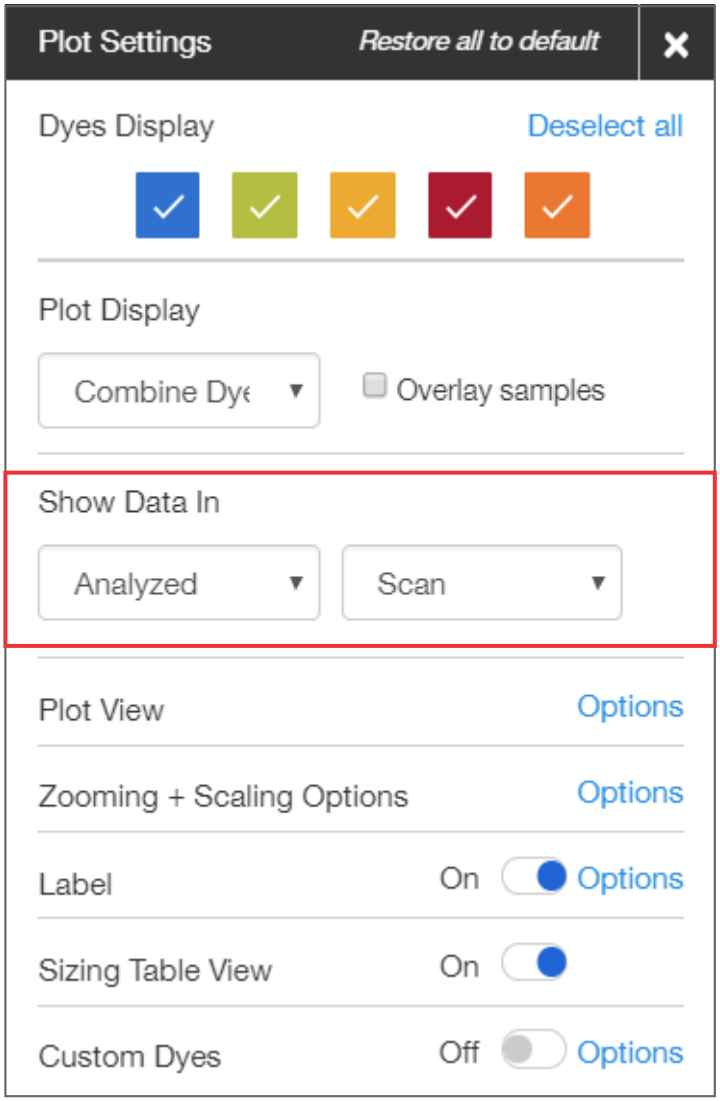
2. Select an option from the Plot Display dropdown list:

Option	Description
Combine Dyes (Default)	All dye data for the selected sample or samples is presented in one plot.
Separate Dyes	Data for each dye color for the selected sample or samples is presented in a separate plot.

3. Close the dialog window.

View raw and/or analyzed data in the sizing plot

1. In the **Results** screen, in the **Sizing** tab, click **Plot Settings** to display the plot settings selector screen.



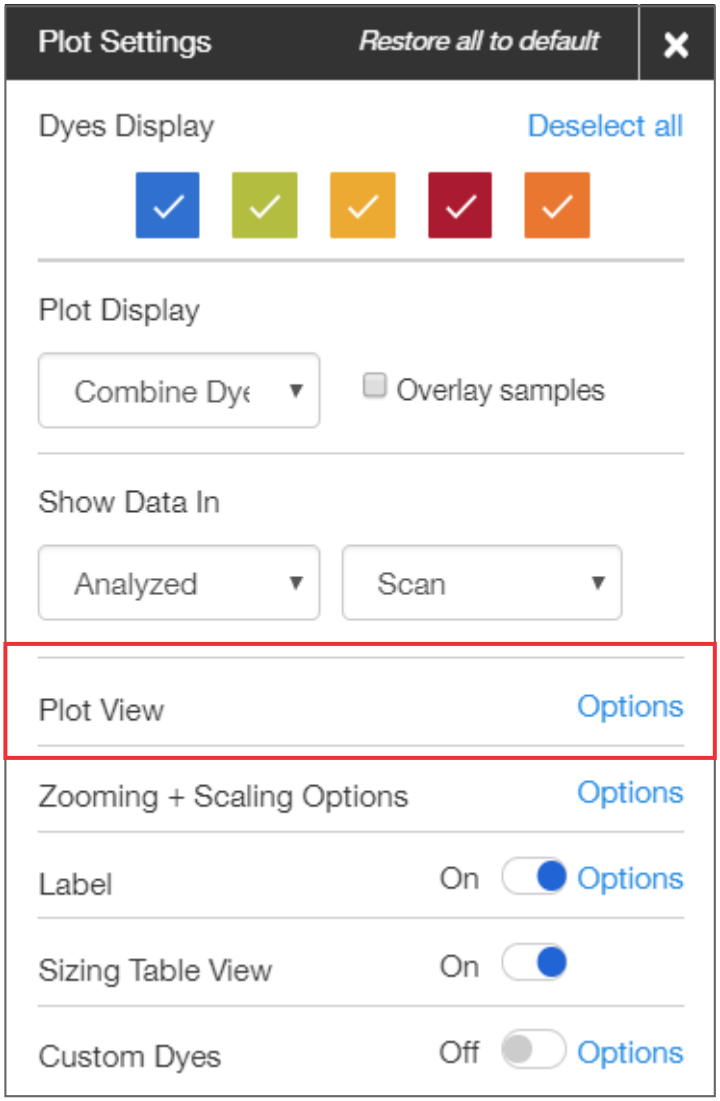
2. In **Show Data In**, select one of the following options:
   - **Analyzed** *(Default)*

     Note: Reset plot Zoom and Scaling if you change the view from Scan to Basepairs. See "Set plot Zooming and Scaling Options" on page 75.
   - **Raw**
   - **Analyzed + Raw**

3. Close the dialog window.

Customize the Plot View

1. In the **Results** screen, in the **Sizing** tab, click **Plot Settings** to display the plot settings selector screen.



2. In the **Plot View** section, click **Options**.

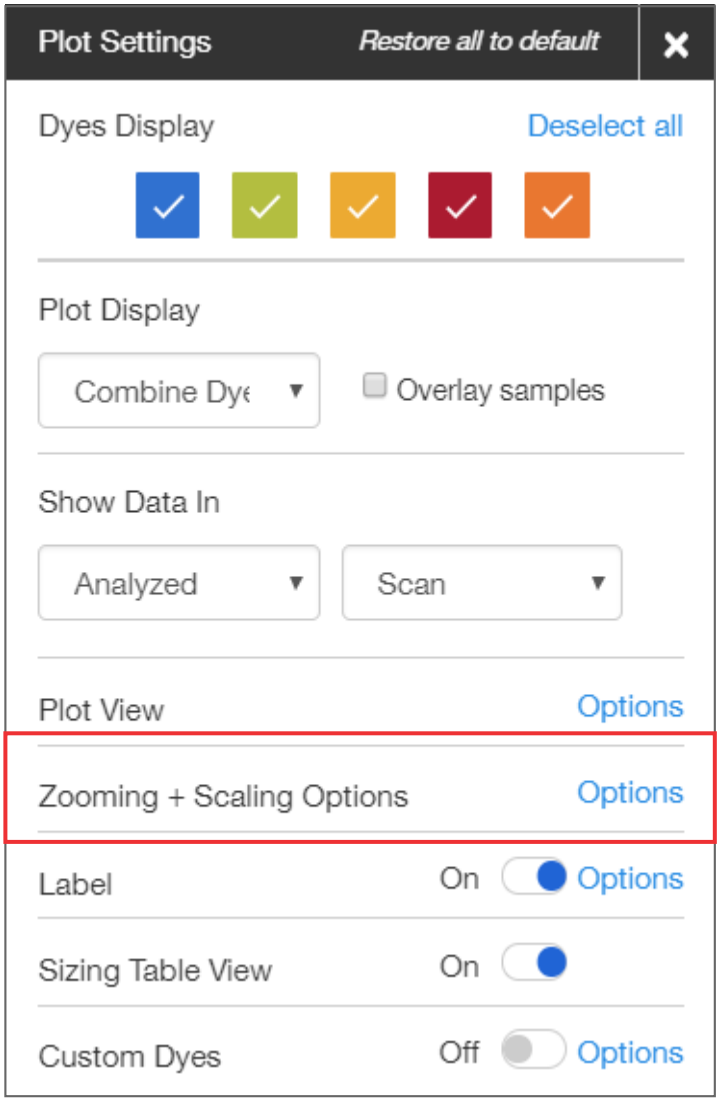
3. Customize the plot view using the following options:

Setting	Description
Plot Layout Options	Change plot layout options to view controls, samples or separate dye plots within the electropherogram window.
Plot Header Options	Select up to 3 parameters to display in the plot heading: • **Sample Name** • **File Name** *(Default)* • **Sample type** *(Default)* • **R^2 Value** • **Sizing Quality** *(Default)* • **Sizing Quality Invalidated (SQI) Flag**
Plot Display	Display the following: • **Sizing Curve** • **Peak Position** • **Offscale Indicator** *(Default)*

4. Click **Back** or close the dialog window.

Set plot Zooming and Scaling Options

1. In the **Results** screen, in the **Sizing** tab, click **Plot Settings** to display the plot settings selector screen.



2. In the **Zooming + Scaling Options** section, click **Options**.

3. Customize zooming and scaling using the following options:

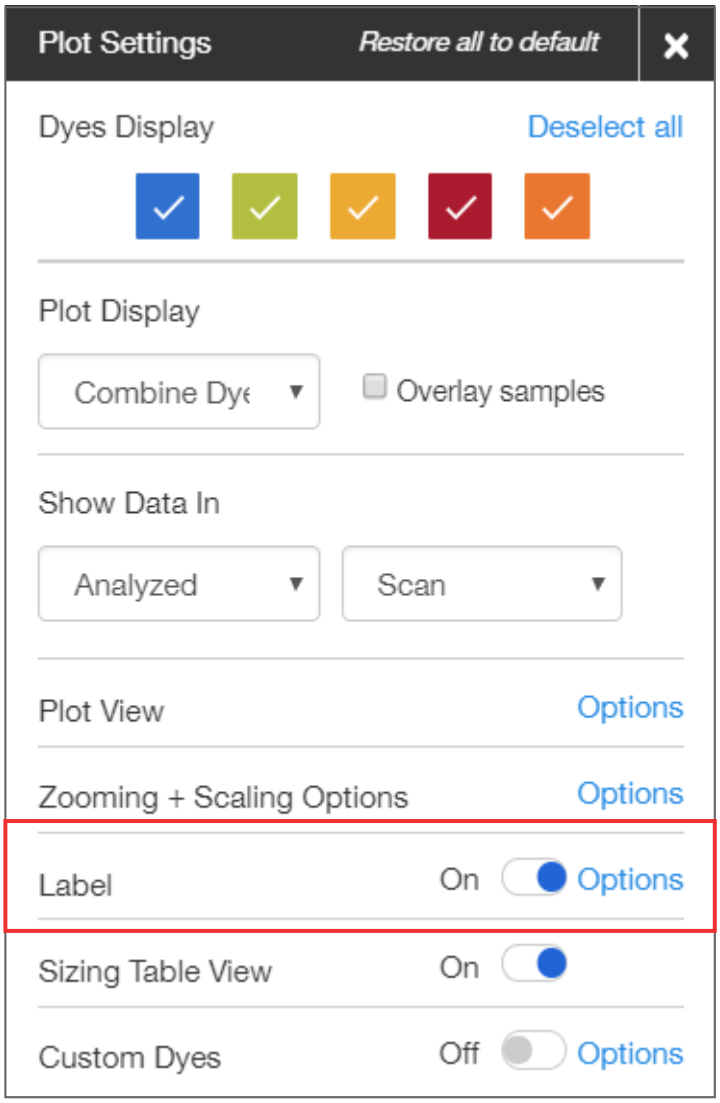
Setting	Description
Zoom Plots	Select the X- and y-axis displays start and stop point. Default settings are from 0–100,000.
Axis Settings	Scale the x-axis and y-axis. Possible parameters include: • **Individually** *(Default)* • **To Maximum Value** • **To Value**
Scale dyes	Select the scale for individual dyes.

4. Click **Back**.

Customize labels on the plot

Labels on the plot are turned on by default.

1. In the **Results** screen, in the **Sizing** tab, click **Plot Settings** to display the plot settings selector screen.



2. In the **Label** section, click **Options**.

3. Customize the labels on the plot using the following options:

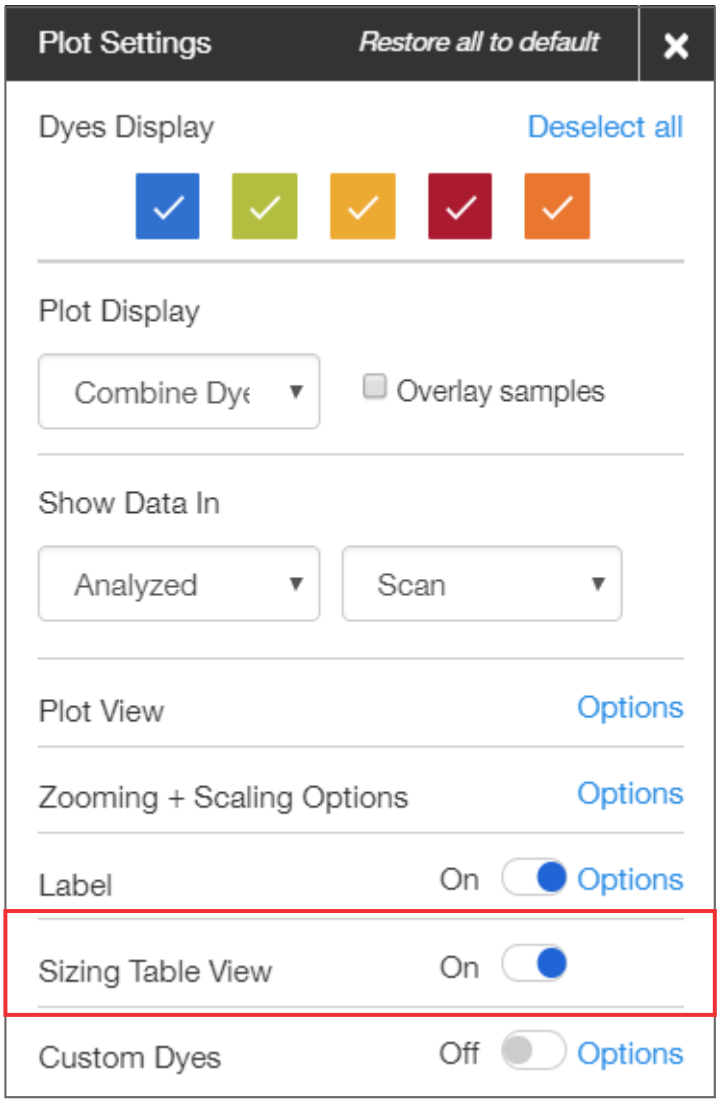
Setting	Description
Show Labels For	Select the type of peaks to label: • **Selected peak** *(Default)* • **All Peaks** • **Specified Range**
Label Display	Select parameters to display in the plot heading. Possible parameters include: • **Area (ar)** *(Default)* • **Height (ht)** *(Default)* • **Size (sz)** *(Default)* • **Data Point (dp)** *(Default)* • **Comments (c)** *(Optional) Select abbreviated label prefixes.*
Text size	Select the text size for the labels.
Visual Style	Select one of the following: • **Dye Color**—*(Default)* Label outline is the same color as the dye peak. • Black and White— Label outline is black.

4. Click **Back** or close the dialog window.

Show/Hide the Sizing Table

The Sizing Table is displayed by default in the **Results** screen.

1. In the **Results** screen, in the **Sizing** tab, click **Plot Settings** to display the plot settings selector screen.

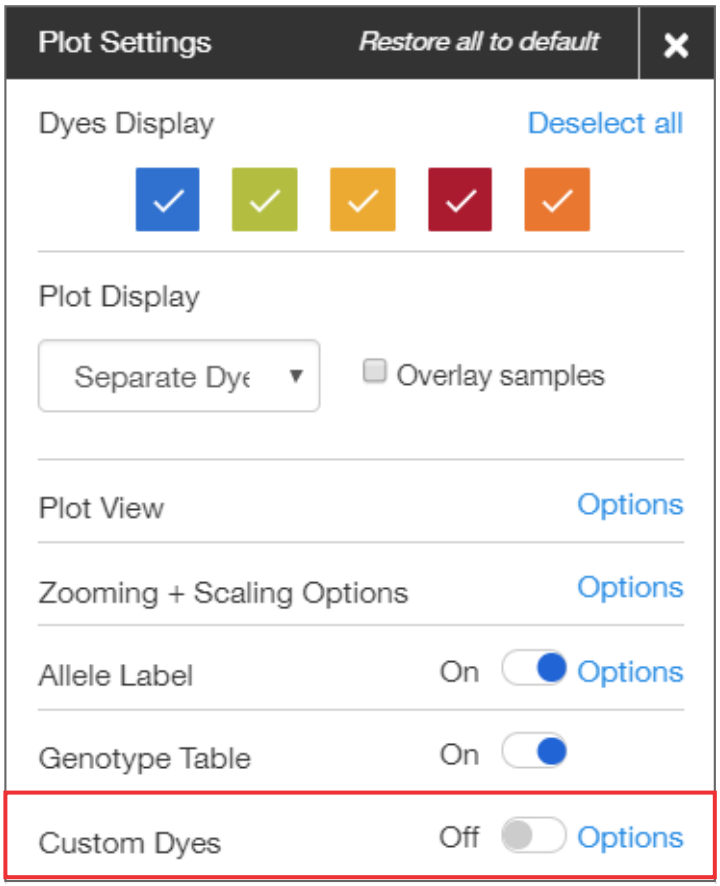


2. Toggle the **Sizing Table View** on or off.

Customize the dyes on the plot

Custom dyes on the plot are turned off by default.

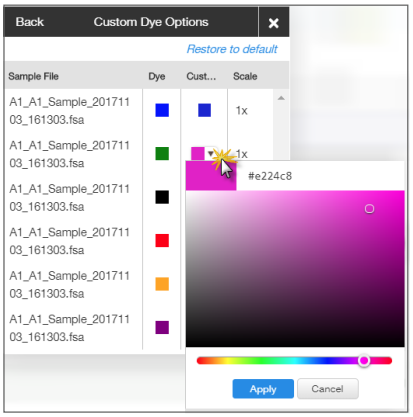
1. In the **Results** screen, click **Plot Settings** to display the plot settings selector screen.



2. In the **Custom Dyes** section, toggle the **Custom Dyes** feature on, then click **Options**.

3. Customize the dyes on the plot using the following options.

   Note: The dye colors in the **Dye** and **Custom** columns are the same by default.

Setting	Description
Select a custom dye	1. In the **Custom** column, click on the dye color, then select a new color from the dropdown palette.  2. Click **Apply**. **Note:** Custom dye settings are not saved as part of the project results. **Note:** Custom dye colors are not reflected in the Sizing Match plot.
Scale dyes	Select the scale for individual dyes.

4. Click **Back** or close the dialog window.

Show plots in full screen

In the **Results** screen above the plot, click the enlarge icon ⛶ to show the plots in full screen.

Review genotype information in the **Results** screen

Review genotyping results

1. Click the **Results** tab at the top of the screen.
2. In the **Genotyping** tab, review allele calls in the Genotype Table and perform any of the following tasks as needed:
   - "Customize columns in the Sample, Sizing, or Genotype Table" on page 20
   - "Search the Genotype Table" on page 52
   - "Sort and filter columns in the Genotype Table" on page 84
   - "Modify allele settings in the Genotype Table" on page 84
   - "Review PQVs of samples with Suspect and Low Quality GQ values" on page 85
3. Review the Genotype plot and perform any of the following tasks as needed:
   - "Review, edit, and add alleles" on page 85
   - "Zoom on the electropherogram" on page 67
   - "Customize genotype plot data and views" on page 89

Customize columns in the Sample, Sizing, or Genotype Table

Customize the columns displayed in tables.

- **Sort the columns in the table**—Click a column header.
- **Edit the order of columns in the table**—Click a header, then drag and drop in the desired order.

  Note: The order of columns can only be edited in the Sample and Sizing Tables.
- **Show/Hide columns in the table**—Click ⚙ at the top right corner of the table, then select the columns to show or hide. Click **Apply**.
- **Edit Quality Metrics Display**—See "Edit Quality Metrics Display" on page 20.
- Click **Restore to default** to undo previous Show/Hide selections.

Search the Genotype Table

In the **Results** screen, search the **Genotype Table** using the following options:

Option	Action
Search by term	1. Enter the search term in the field at the top right corner of the table, then click Q. **Note:** The ⊘ icon indicates a filter is already applied.
Search by dye color	1. Click ⌄ at the top right corner of the table. 2. Select Dye Color from the drop down menu, then click on a dye color to select. 3. Click **Search**.
Search by Process Quality Value	1. Click ⌄ at the top right corner of the table. 2. Select a PQV parameter from the drop down menu. 3. Select Quality Value from the drop down menu. 4. Click **Search**. See "Genotype Table fields" on page 113 for Quality Value descriptions.
Search by allele size, peak height or peak area	1. Click ⌄ at the top right corner of the table. 2. Select Size, Height or Peak Area from the drop down menu. 3. Enter a size or size range (bp) in the Value fields. 4. Click **Search**.

Click ⌄, then click **Clear All** to clear previous search results.

Sort and filter columns in the Genotype Table

Task	Action
Sort table by column field	1. Click ⚙ in the upper-right corner of the table. 2. In the **Sort Columns** tab, select up to 3 Genotype Table fields to use to sort the table (see "Genotype Table fields" on page 113). Delete any unused sort columns. 3. Click **Apply**. click **Restore to default** to clear previous sort settings.
Filter table selected sample(s)	Select an option from the dropdown menu in the upper-right corner of the table to filter results by samples selected in the Sample Table: 1. **All** — Shows the genotypes of selected samples. 2. **Selected** — Shows only the marker rows for selected alleles in the Sample plot.

Modify allele settings in the Genotype Table

Click ⚙ in the upper-right corner of the table, then select the **Allele settings** tab.

Option	Description
Show number of Alleles	Specify the maximum number (up to 20) of alleles shown.
Limit number of alleles shown to number of bins	Limit number of alleles shown to number of bins for a marker.
Keep Allele name, size, height, peak area, data point, mutation, comments together	Group individual allele information in the table.
Show only binned alleles in their respective bin positions	Number and display allele information based on bin number, not sequential order. For example, if a marker has alleles in bins 1 and 3, the alleles will be shown in bin locations 1 and 3. If this option is not selected, the bin positions would be sequential.
Duplicate homozygous alleles	Show 2 allele entries for homozygous alleles.

Click **Restore to default** to clear all previous sort settings.

Review PQVs of samples with Suspect and Low Quality GQ values

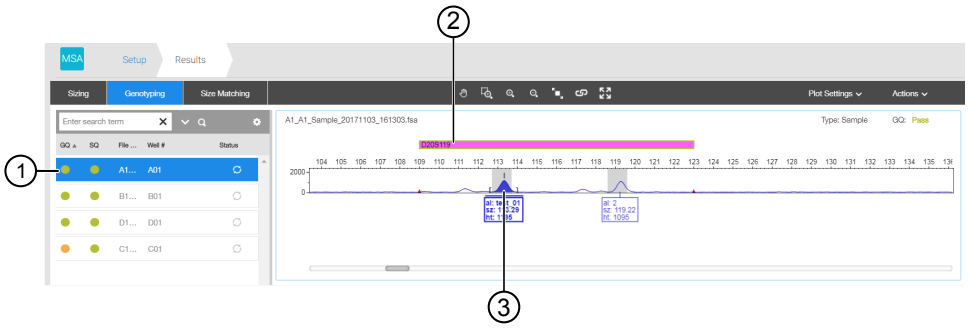
1. Filter the **Genotype Table** to display samples with Suspect and Low Quality GQ values.
2. For each sample, identify the PQV values that are contributing to the GQ failure.
3. Perform any of the following tasks as needed:
   - See “Genotyping Quality (GQ) status and Process Quality Values (PQVs) “ on page 134 for an overview and trouble shooting information about individual PQVs.
   - “Delete alleles“ on page 86
   - “Create, edit or delete a bin using Panel Editing“ on page 86

Review, edit, and add alleles

Add alleles

Ensure that **Marker Range**, **Bins**, and **Marker Indicators** are displayed on the electropherogram. See “Customize the Plot View“ on page 91.

1. In the **Results** screen, in the **Genotyping** tab, select a sample from the sample list to review.
2. In the electropherogram, select a marker, then place the pointer over an allele peak and click to select it.



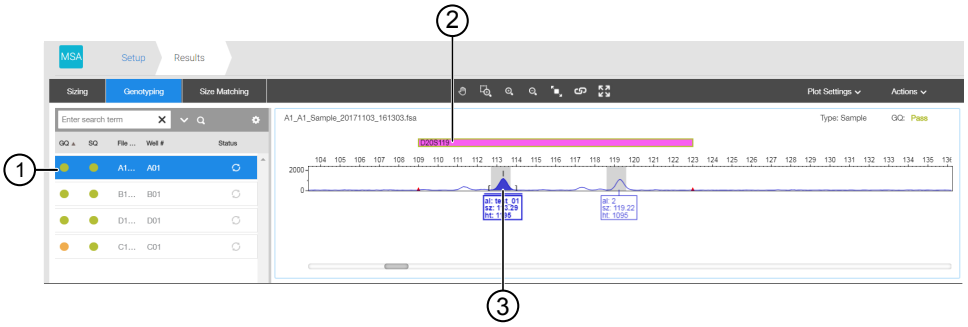
① Sample list.
② Selected marker (highlighted in pink).
③ Selected allele (highlighted peak).

3. Right click on the selected allele, then click **Add Allele Call**.
4. In the **Add Allele Call** dialog box, enter the allele name and comments, then click **Save**.

Rename alleles

Ensure that **Marker Range**, **Bins**, and **Marker Indicators** are displayed on the electropherogram. See "Customize the Plot View" on page 91.

1. In the **Results** screen, in the **Genotyping** tab, select a sample from the sample list to review.
2. In the electropherogram, select a marker, then place the pointer over an allele peak and click to select it.



① Sample list.
② Selected marker (highlighted in pink).
③ Selected allele (blue peak).

3. Right click on the selected allele, then click **Rename Allele**.
4. In the **Rename Allele** dialog box, enter the allele name and comments, then click **Save**.

Delete alleles

Ensure that **Marker Range**, **Bins**, and **Marker Indicators** are displayed on the electropherogram. See "Customize the Plot View" on page 91.

1. In the **Results** screen, select a sample in the Sample Table to review.
2. In the electropherogram, place the pointer over an allele peak, then click to select it. Repeat for additional allele peaks.
3. Right click on the selected allele, then click **Delete Allele**.
4. In the **Delete Allele** dialog box, enter comments, then click **Save**.

Create, edit or delete a bin using Panel Editing

Ensure that **Marker Range**, **Bins**, and **Marker Indicators** are displayed on the electropherogram. See "Customize the Plot View" on page 91.

1. In the **Results** screen, in the **Genotyping** tab, select a sample in the Sample list to review.
2. Click **Actions** ▸ **Turn on Panel Editing**.
3. In the electropherogram, click on the Marker Range label to select the marker (the selected marker will be highlighted in pink).
4. Right click on the selected Marker Range label, then click **Zoom into Marker**.

5. In the electropherogram, select a bin, then click and drag to define the new bin range.

Option	Action
Create a new bin	1. Right click in the electropherogram, then click **New Bin**. 2. Click-drag the left or right handles to define the bin range. 3. In the **New Bin** dialog box, enter the bin name and bin information, then click **Save**.
Edit an existing bin	1. Select a bin in the electropherogram. Right click on the bin, then click **Edit Bin**. 2. In the **Edit Bin** dialog box, enter the bin name and bin information, then click **Save**.
Delete an existing bin	1. Select a bin in the electropherogram. Right click on the bin, then click **Delete Bin**. 2. In the dialog box, click **OK** to confirm the deletion.

6. In the **Panel Editing On** dialog box, select one of the following options:

Option	Description
Save Sample Panel	Apply the panel changes, then automatically reanalyze selected sample.
Discard Panel Changes	Discard any changes made in the Panel Editing session.
Save Project Panel	Save changes as a new panel in the panel library. Select **Re-analyze project with the project panel** before clicking **Save** to immediately reanalyze project with the new panel.
Turn off Panel Editing	Close Panel Editing.

7. In the **Panel Editing On** dialog box, click **Turn off Panel Editing** when editing is complete.

Zoom on the electropherogram

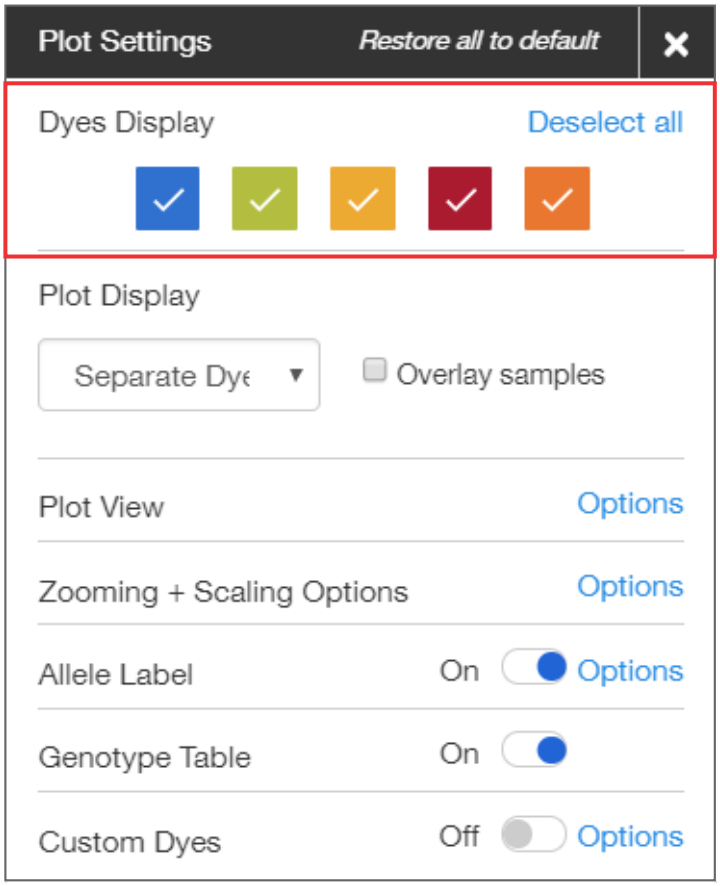
To set zoom and scale defaults, see "Set plot Zooming and Scaling Options" on page 93.

Action	Icon	Description
Turn panning on/off		Pan over the plot. Click, then drag to move over the plot. Panning cannot be selected simultaneously with rectangular selection.
Turn rectangular selection on/off		Zoom in on a rectangular region of the plot. Click, then drag to select a rectangular region. Rectangular selection cannot be selected simultaneously with panning.
Zoom in x-axis		Click to zoom in on the x-axis. Alternatively, zoom in on the x-axis by clicking directly on the value in the axis.
Zoom out x-axis		Click to zoom out on the x-axis. Double-click on the x-axis to reset x-axis zoom.
Reset zoom to fit		Reset the plot view, panning and rectangular selection options to default settings.
Turn synchronized zoom on		Synchronize zooming when viewing multiple plots.
Turn synchronized zoom off		Turn off synchronize zooming when viewing multiple plots. Highlighted icon indicates synchronized zoom is currently turned on.
Zoom in y-axis	None	Zoom in on the y-axis by clicking directly on the value in the axis.
Zoom out y-axis	None	Double-click on the y-axis to reset y-axis zoom.
Show full screen		Click to show the plot in full screen.

Customize genotype plot data and views

Select dye data to display

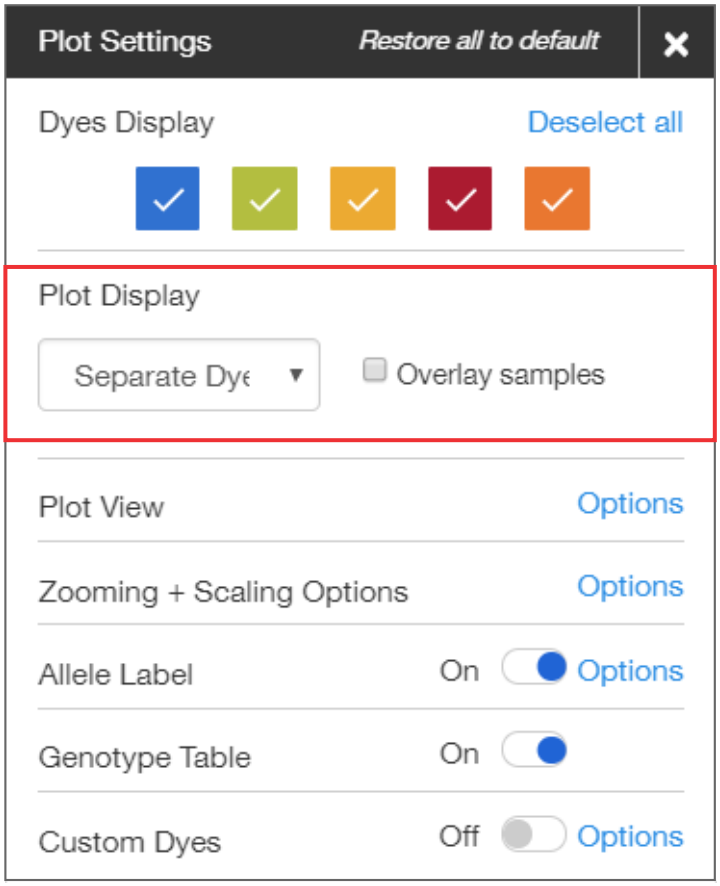
1. In the **Results** screen, in the **Genotyping** tab, click **Plot Settings** to display the plot settings selector screen.



2. Review the default dye display settings and change as needed.
3. To display only selected dyes:
   - Click the square of the dye color to hide.
   - Click **Deselect all**, then click the square of the dye color to display.

View dyes and samples in combined or separate plots

1. In the **Results** screen, in the **Genotyping** tab, click **Plot Settings** to display the plot settings selector screen.



2. Select an option from the Plot Display dropdown list:

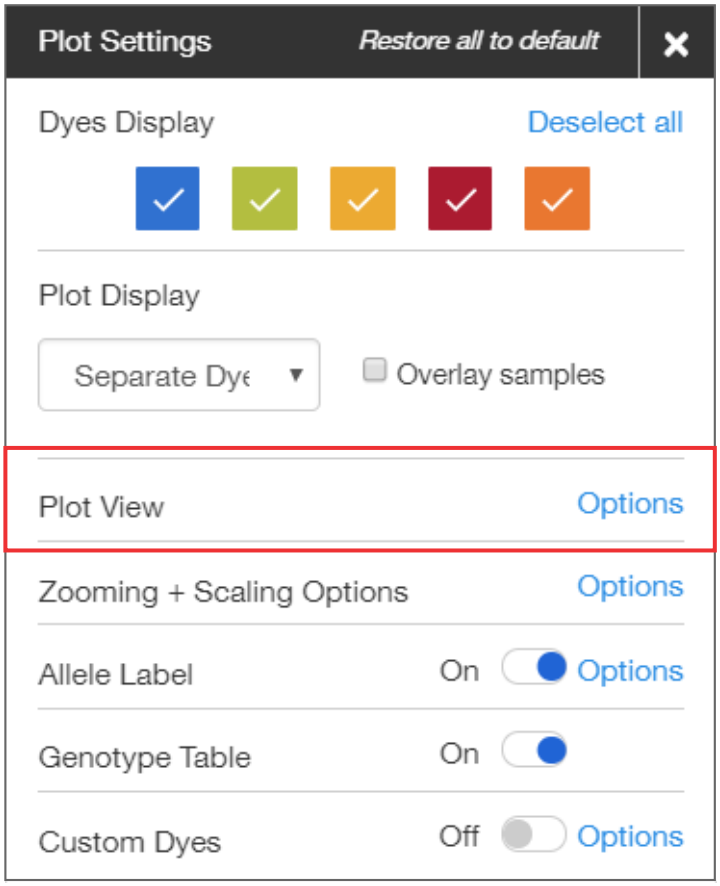
Option	Description
Separate Dyes (Default)	Data for each dye color for the selected sample or samples is presented in a separate plot.
Combine Dyes	All dye data for the selected sample or samples is presented in one plot.

3. (*Default*) Select **Overlay samples** to display selected samples in one plot.

4. Close the dialog window.

Customize the Plot View

1. In the **Results** screen, in the **Genotyping** tab, click **Plot Settings** to display the plot settings selector screen.



2. In the **Plot View** section, click **Options**.

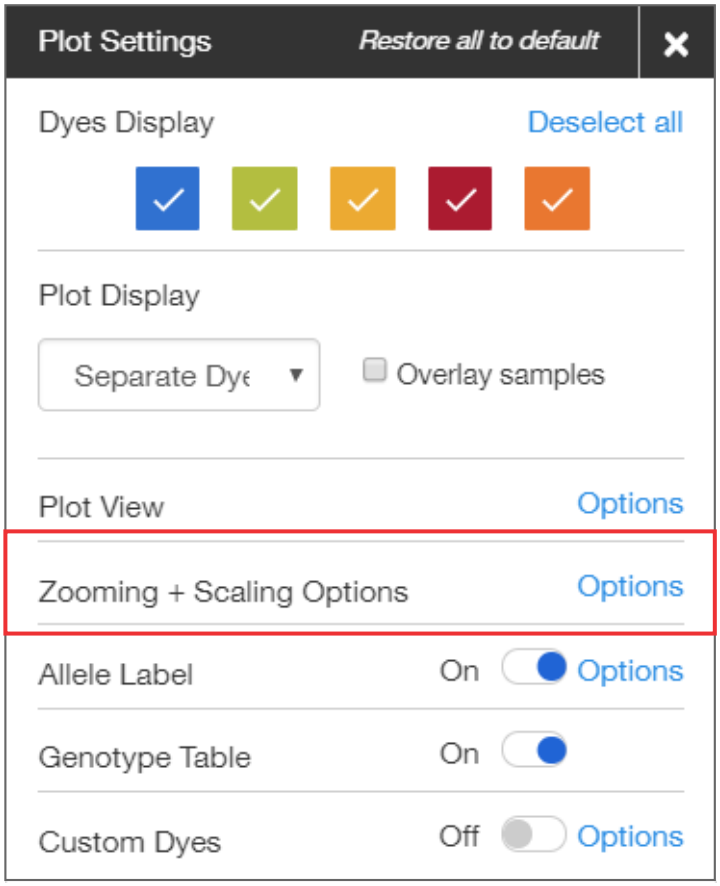
3. Customize the plot view using the following options:

Setting	Description
Plot Layout Options	Change plot layout options to view controls, samples or separate dye plots within the electropherogram window. Select **Lock on top** to display control plots at the top of the electropherogram window.
Plot Header Options	Select up to 3 parameters to display in the plot heading: • **Sample Name** • **File Name** *(Default)* • **Sample type** *(Default)* • **R^2 Value** • **Genotype Quality** *(Default)* • **Sizing Quality**
Plot Display	Display the following: • **Marker Range** *(Default)* • **Bins** *(Default)* • **Marker Indicators** *(Default)* • **Offscale Indicator** • **Peak Position**

4. Click **Back** or close the dialog window.

Set plot Zooming and Scaling Options

1. In the **Results** screen, in the **Genotyping** tab, click **Plot Settings** to display the plot settings selector screen.



2. In the **Zooming + Scaling Options** section, click **Options**.

3. Customize zooming and scaling using the following options:

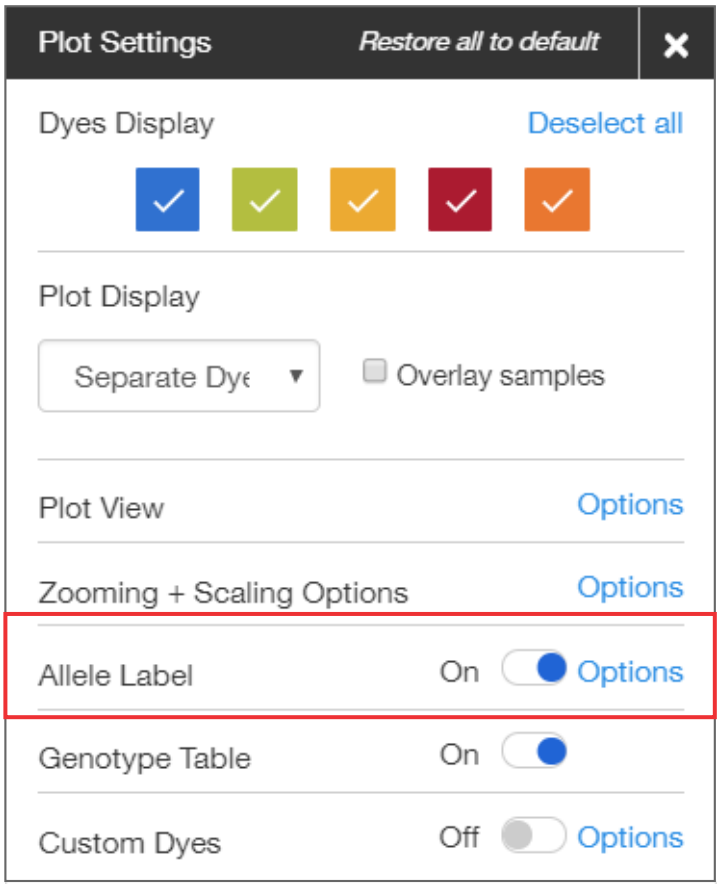
Setting	Description
Axis display	Select the x- and y-axis displays start and stop point. Default axis start and stop points are set to the following: • X-axis: 0–2,000 • Y-axis: 0–10,000
Axis Settings	Scale the x-axis and y-axis. Possible parameters include: • **Individually** *(Default)* • **To Maximum Value** • **To Value**
Scale dyes	Select the scale for individual dyes. **Note:** The dye scale selected here is only applied if **Custom Dyes** is turned off. Dye scale settings in **Custom Dyes** will override dye scale settings in **Zooming + Scaling Options**.

4. Click **Back**.

Customize allele labels on the plot

Allele labels on the plot are turned on by default.

1. In the **Results** screen, in the **Genotyping** tab, click **Plot Settings** to display the plot settings selector screen.



2. In the **Allele Label** section, click **Options**.

3. Customize the allele labels on the plot using the following options:

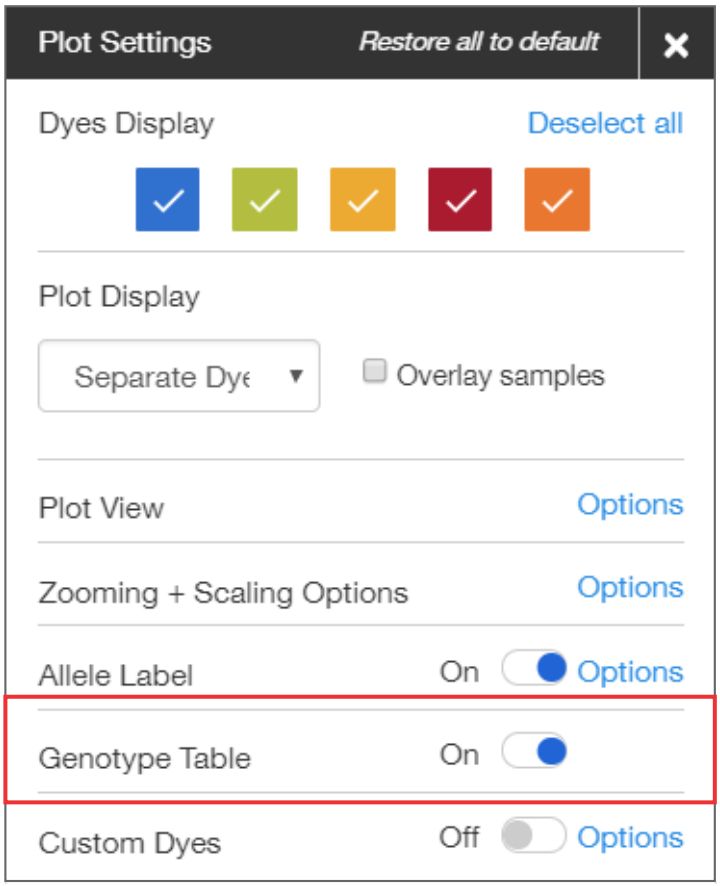
Setting	Description
Show Allele Labels	Select the style for the allele labels: • **Vertically** *(Default)* • **Horizontally** *(Optional) Select to* ***Show Allele Changes*** *(default),* ***Show Allele Edit Type****, and/or* ***Show Allele Edit Comment*** *(default).*
Label Display	Select parameters to display in the plot heading. Possible parameters include: • **Area (ar)** • **Height (ht)** *(Default)* • **Size (sz)** *(Default)* • **Allele Call (al)** *(Default)* • **Data Point (dp)** • **AE Comment (ae)** **Note:** Select this checkbox to allow users to enter a comment when renaming or deleting an allele. *(Optional) Select abbreviated label prefixes.*
Text size	Select the text size for the labels.
Visual Style	Select one of the following: • **Dye Color**—*(Default)* Label outline is the same color as the dye peak. • **Black and white**— Label outline is black.

4. Click **Back** or close the dialog window.

Show/Hide the Genotype Table

The Genotype Table is displayed by default in the **Results** screen.

1. In the **Results** screen, in the **Genotyping** tab, click **Plot Settings** to display the plot settings selector screen.

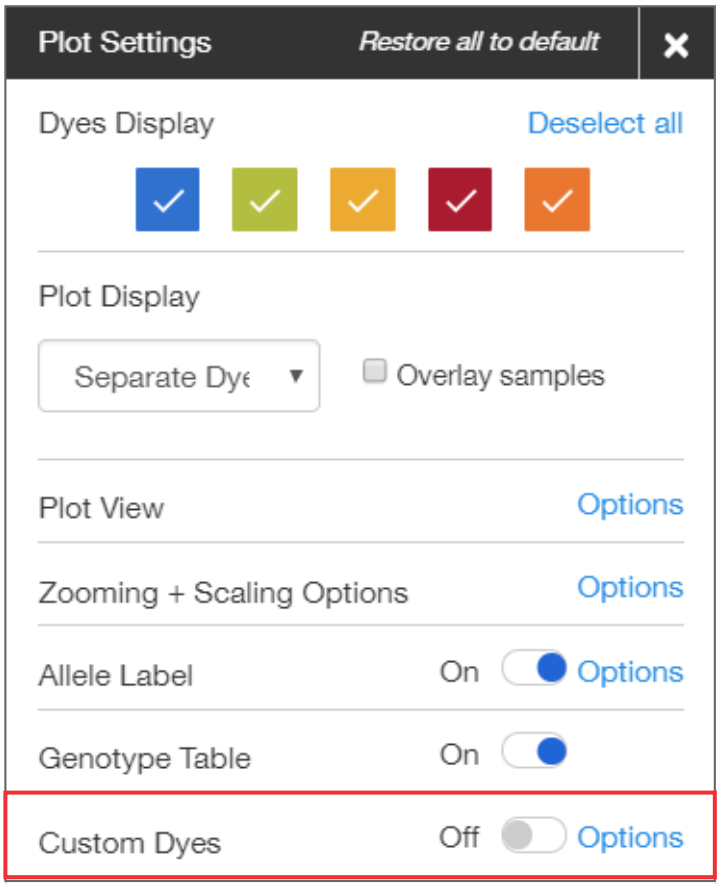


2. Toggle the **Genotype Table** display on or off.

Customize the dyes on the plot

Custom dyes on the plot are turned off by default.

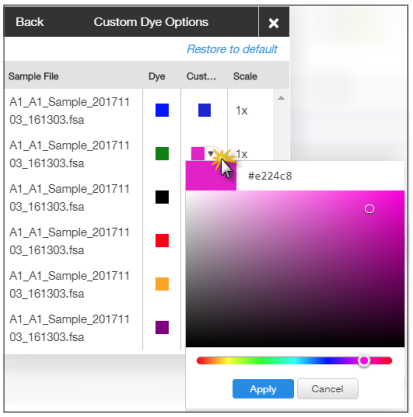
1. In the **Results** screen, click **Plot Settings** to display the plot settings selector screen.



2. In the **Custom Dyes** section, toggle the **Custom Dyes** feature on, then click **Options**.

3. Customize the dyes on the plot using the following options.

   Note: The dye colors in the **Dye** and **Custom** columns are the same by default.

Setting	Description
Select a custom dye	1. In the **Custom** column, click on the dye color, then select a new color from the dropdown palette.  2. Click **Apply**. **Note:** Custom dye settings are not saved as part of the project results. **Note:** Custom dye colors are not reflected in the Sizing Match plot.
Scale dyes	Select the scale for individual dyes.

4. Click **Back** or close the dialog window.

Show plots in full screen

In the **Results** screen above the plot, click the enlarge icon ⛶ to show the plots in full screen.

Export results

Export a size standard

1. In the **Setup** screen, in the **Size Standard** pane, click ⚙.
2. Select a size standard from the list, then click **Export**.
3. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
4. Click **Export**.

Export a panel

1. In the **Setup** screen, in the **Panel** pane, click ⚙.
2. Select a panel from the list, then click **Export**.
3. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
4. Click **Export**.

Export analysis settings

1. In the **Setup** screen, in the **Analysis Settings** pane, click ⚙.
2. Select analysis settings from the list, then click **Export**.
3. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
4. Click **Export**.

Export a template

1. In the **Setup** screen, click **Template Manager**.
2. Select a template from the list, then click **Export**.
3. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
4. Click **Export**.

Export FSA files

1. In the **Setup** screen, select samples in the Sample Table, then click **Actions** ▸ **Export .FSA files**.
2. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
3. Click **Export**.

The exported ZIP file only contains files for selected samples in the Sample Table.

Export the Sample Table

1. In the **Setup** screen, select samples in the sample table to export.
2. Select **Actions** ▸ **Export Sample Table**.
3. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
4. Click **Export**.

A table is generated only for selected samples in the Sample Table.

Export plot

1. In the **Results** screen, in the **Genotyping** or **Sizing** tab, click **Actions** ▸ **Export Plot**.
2. Select the following options:
   - PNG or JPEG image
   - Low, Medium, or High resolution
3. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
4. Click **Export**.

The exported ZIP file contains individual plot files for selected samples in the **Results** screen.

If **Separate Dyes** is selected for **Plot Display** in **Plot Settings**, one plot for each dye color selected displays in the **Results** screen.

Export Alleles

1. In the **Results** screen, in the **Genotyping** tab, click **Actions** ▸ **Export Alleles** to export an allele to a tab-delimited CSV file.
2. In the **Samples** pane, select one of the following:
   - **All Samples**
   - **Selected Samples**
3. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
4. Click **Export**.

Export the Genotype Table

1. In the **Results** screen, in the **Genotyping** tab, click **Actions** ▸ **Export Genotype Table** to export the sizing table to a tab-delimited CSV file.
2. In the **Samples** pane, select one of the following:
   - **All Samples**
   - **Selected Samples**

3. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
4. Click **Export**.

Export the Sizing Table

1. In the **Results** screen, in the **Sizing** tab, click **Actions** ▸ **Export Sizing Table** to export the sizing table to a tab-delimited CSV file.
2. In the **Samples** pane, select one of the following:
   - **All Samples**
   - **Selected Samples**
3. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
4. Click **Export**.

Export Summary Report

1. In the **Results** screen, select either the **Sizing** or **Genotyping** tab, then select samples in the Sample List.
2. Select **Actions** ▸ **Export Summary Report**
3. Select the samples and contents to include in the Summary Report.
4. In the **Export To** pane, click one of the following:
   - **My Computer**
   - **Thermo Fisher Cloud**
5. Click **Export**.
6. Wait until the Summary Report PDF is displayed before using the software again.

   Note: A Summary Report for a few samples can take several minutes to generate. The time increases for reports with many samples.

Troubleshooting

Observation	Possible cause	Action
SQ column is blank	The imported FSA file does not contain an SQ value.	Analyze samples.
SQ is ● or ●	The software is unable to properly assign size standard peaks.	See "Sizing Quality (SQ) status" on page 133.
All PQV values are greyed out	The allele was manually edited.	See "Genotyping Quality (GQ) status and Process Quality Values (PQVs)" on page 134.
Allele name is a ?	A bin is not defined for the allele.	See "Manually add bins to a marker" on page 35 or "Create bins using reference data" on page 36.
Genotyping tab is greyed out.	Genotyping analysis has not been performed.	Apply a panel to the project, then reanalyze the samples.
Reference data files are included in project, but are not displayed in reference data list in **Panel Manager**.	Analysis status of reference data is not analyzed.	Reanalyze.
	The panel name of the project does not match the new panel name.	Apply the new panel to the project, then reanalyze the samples.
PQV Flag Indicatior is ■	The sample or genotype passed the PQV test.	No further action required.
PQV Flag Indicatior is ▲	A possible problem exists for the sample or genotype.	See "Genotyping Quality (GQ) status and Process Quality Values (PQVs)" on page 134.
The Summary Report will not open.	A pop up blocker prevented the opening of the Summary Report in a new window.	Disable the pop up blocker.
The scale of the peaks for a custom dye color is incorrect.	Dye scale settings set in **Zooming + Scaling Options** are overwritten by the dye scale settings in **Custom Dyes**.	Change scale settings in **Custom Dyes**. See "Customize the dyes on the plot" on page 80.
		Turn **Custom Dyes** off.

Software screen descriptions

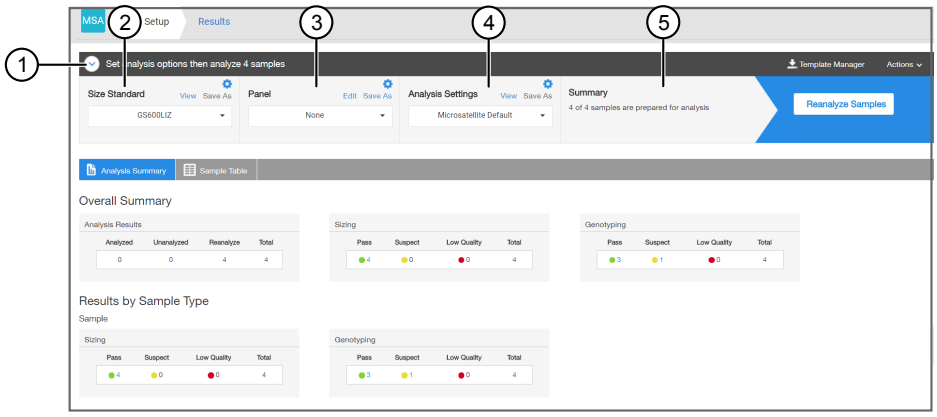
Setup

The **Setup** screen displays a list of all sample files in the project and provides QC flag, run, and data collection information for each file.

How to ...	Learn more about...
"Review samples after first import" on page 15	"Parts of the Setup screen" on page 106
"Perform initial sizing analysis using default analysis settings" on page 17	"Sample Table fields" on page 106
"Create a template from current analysis options" on page 53	"Search the Sample Table" on page 19
"Analyze samples using a template" on page 18	"Parts of the Panel Manager dialog box" on page 108
"Reanalyze samples using custom sizing analysis settings" on page 18	"Customize columns in the Sample, Sizing, or Genotype Table" on page 20
"Add/remove samples" on page 19	"Create a panel" on page 29
"Edit Quality Metrics Display" on page 20	"Examine low-quality sizing results" on page 21
"View data collection settings" on page 21	"Examine low-quality genotyping results" on page 54
"Review size matches" on page 22	"Sizing Quality (SQ) status" on page 133
"Modify the default analysis settings" on page 25	"Genotyping Quality (GQ) status and Process Quality Values (PQVs)" on page 134
"Apply a different size standard to the project or a sample" on page 26	"Troubleshooting" on page 64
"Create a new panel in Panel Manager" on page 30	"Export results" on page 100
"Import a panel in Panel Manager" on page 40	
"Edit a panel in Panel Manager" on page 43	
"Analyze project using default genotyping analysis settings" on page 51	
"Review samples with Suspect and Low Quality GQ values" on page 55	
"Adjust Genotype Quality Flag weights and ranges" on page 55	
"Adjust Size Quality Flag ranges" on page 26	

How to ...	Learn more about...

Parts of the Setup screen



① **Show Analysis Details**—Show/hide analysis options.

② **Size Standard**—A size standard is selected for the project (see "Size Standard selected by the software" on page 116).

③ **Panel**—The software does not include default panel information. You must create or import a panel for genotyping analysis (see "Create a panel" on page 29).

④ **Analysis Settings**—Microsatellite Default.

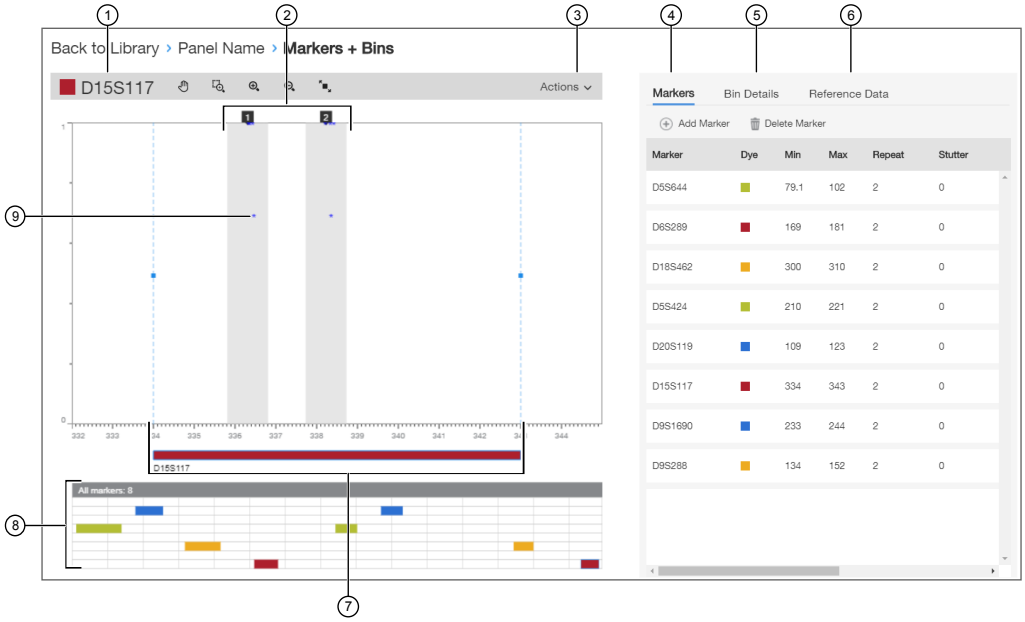
⑤ **Summary**—The number of samples that will be analyzed using the selected settings. View selected samples in the Sample Table.

Sample Table fields

Column name	Description
Status	Indicates the analysis status of individual samples. Possible states include: • Unanalyzed — The sample has not been analyzed. • Analyzed — The sample is analyzed using the current analysis settings. • Reanalyze — The sample needs to be reanalyzed using the current analysis settings.
Size Quality (SQ)	pass, suspect, or low quality. The sizing quality is defined in the **Quality Flags** tab in analysis settings and is user editable. To view the numerical value of the SQ flag, see "Edit Quality Metrics Display" on page 20. See "Sizing Quality (SQ) status" on page 133 and "Adjust Size Quality Flag ranges" on page 26.

Column name	Description
Genotyping Quality (GQ)	🟢 pass, 🟡 suspect, or 🔴 low quality based on the cumulative marker status of a sample. The genotyping quality is defined in the **Quality Flags** tab in analysis settings and is user editable. To view the numerical value of the GQ flag, see "Edit Quality Metrics Display" on page 20. See "Genotype Quality (GQ)" on page 138 and "Adjust Genotype Quality Flag weights and ranges" on page 55.
Sample File Name	The name of the sample file.
Sample Name	The name of the sample is derived from the sample name tag used in the sample file; user editable.
Sample Type	Indicates the sample type: • Sample • Positive Control • Allelic ladder • Primer Focus • Negative Control This field is user editable.
Plate ID	Indicates the plate ID associated with sample.
Well	Indicates the well number of the sample.
UD1-UD10	A user-defined field where you can make notes or comments about the samples.
Size Standard	Indicates the size standard applied to the current analysis settings.
Panel	Indicates the panel applied to the current analysis settings.
Offscale (OS)	🟢 pass, 🟡 suspect, or 🔴 low quality. To correct offscale data, adjust the amounts of labeled fragments. The software cannot correct for offscale data. Although offscale samples can still be analyzed, their peak sizes may not be accurate. See "Offscale (OS)" on page 139.
Reference sample (REF)	A green check mark indicates that the sample was used as a reference sample to create panel markers and/or bins.
Sizing Quality Invalidated (SQI)	✔ indicates that the Size Standard peaks definition for the sample was manually edited by the user and, therefore, the SQ automatically generated by the software is invalid.
Instrument Model	Indicates the instrument model on which the experimental run was performed.

Parts of the Panel Manager dialog box



1. Marker name—Name of the marker that is displayed in the electropherogram.
2. Bins—Basepair range (gray) for each bin in a marker.
3. Actions pull down menu—Show/Hide Project Alleles or Shift All Bins.
4. Markers tab—Table of markers in the panel. Click on marker name to view it in the electropherogram. Add, edit, or delete markers.
5. Bin Details tab—Table of bins for each marker in the panel. Click on bin name to view it in the electropherogram. Add, edit, or delete bins.
6. Reference Data tab—Search for reference data to add predefined bins, and view reference data in current panel.
7. Marker range—Basepair range (dotted blue lines) for the selected marker.
8. Marker overview—Dye color, location and size range for all markers in a panel. Click on a marker to view it in the electropherogram.
9. Project Alleles—Allele (blue star) for a sample in the project.

Results–Sizing

How to ...	Learn more about...
"Examine low-quality sizing results" on page 21	"Parts of the Results screen" on page 111
"Modify size matches" on page 22	"Parts of the electropherogram" on page 112
"Edit peaks in the Sizing Table" on page 66	"Sizing Table fields" on page 113
"Edit peaks using Peak Editor" on page 66	"Sizing overview" on page 126
"Search the Sizing Table" on page 65	
"Sort and filter peaks in the Sizing Table" on page 66	
"Zoom on the electropherogram" on page 67	
"Customize sizing plot data and views" on page 70	
"Customize columns in the Sample, Sizing, or Genotype Table" on page 20	
"Show plots in full screen" on page 81	
"Export plot" on page 102	
"Export the Sizing Table" on page 103	
"Export Summary Report" on page 103	

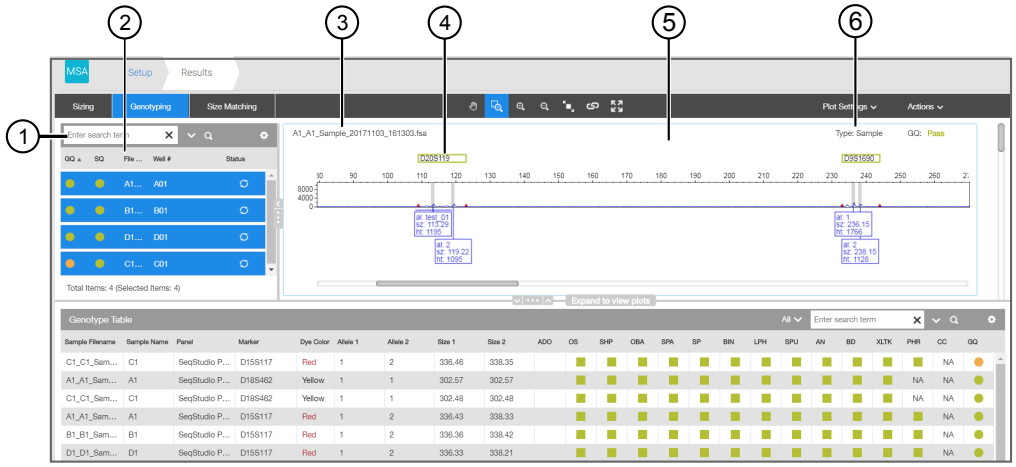
Results–Genotyping

How to ...	Learn more about...
"Examine low-quality genotyping results" on page 54	"Parts of the Results screen" on page 111
"Edit Quality Metrics Display" on page 20	"Parts of the electropherogram" on page 112
"Customize columns in the Sample, Sizing, or Genotype Table" on page 20	"Genotype Table fields" on page 113
"Search the Genotype Table" on page 52	"Genotyping Quality (GQ) status and Process Quality Values (PQVs)" on page 134
"Sort and filter columns in the Genotype Table" on page 84	
"Zoom on the electropherogram" on page 67	
"Modify allele settings in the Genotype Table" on page 84	
"Review PQVs of samples with Suspect and Low Quality GQ values" on page 85	
"Review, edit, and add alleles" on page 85	
"Customize genotype plot data and views" on page 89	
"Show plots in full screen" on page 81	
"Export plot" on page 102	
"Export Alleles" on page 102	
"Export the Genotype Table" on page 102	
"Export Summary Report" on page 103	

Results–Size Matching

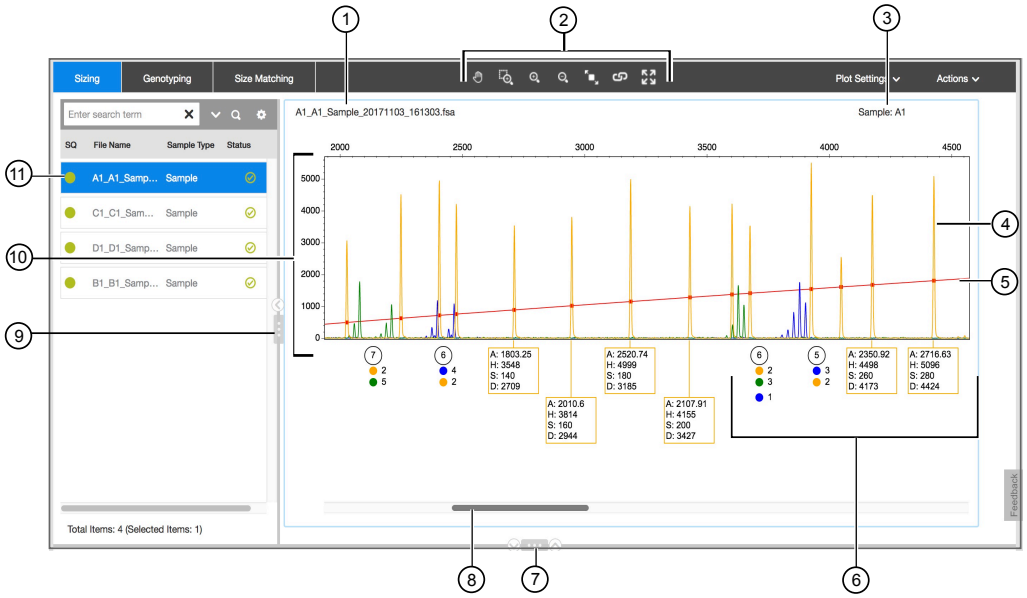
How to ...	Learn more about...
"Size matching" on page 126	"Sizing overview" on page 126
"Size-calling curve generation and size calling" on page 127	"Examine low-quality sizing results" on page 21
"Modify size matches" on page 22	"Apply a different size standard to the project or a sample" on page 26
"Zoom on the electropherogram" on page 67	"Sizing Quality (SQ) status" on page 133
	"Parts of the Results screen" on page 111

Parts of the Results screen



1. Search, sort, and filter samples displayed in the electropherogram.
2. Sample Table.
3. File name of the sample that is displayed in the electropherogram.
4. Marker Range label.
5. Electropherogram window.
6. Name of the sample that is displayed in the electropherogram.

Parts of the electropherogram



1. File name—File name of the sample that is displayed in the electropherogram.
2. Zoom controls—Controls zooming options for the electropherogram.
3. Sample name—Name of the sample that is displayed in the electropherogram.
4. Peak Position—Black markers indicate the peak start, peak end, and peak apex. Turn on peak position markers in **Plot Settings**.
5. Sizing Curve—Used to determine the size of unknown fragments in an experimental sample. Display the sizing curve in **Plot Settings**.
6. Labels on Plot—Labels display area (A), height (H), size (S), and data point (D) information for individual peaks. Customize labels on Plot in **Plot Settings**.

   Multiple peaks in the same region are indicated by circles under the x-axis. The numbered circle indicates the total number of peaks in the region. Color circles indicate the number of peaks of that dye color in the region. Zoom in on the x-axis to view individual peak labels.
7. Resize the electropherogram window - vertical—Click, then drag to vertically to resize the electropherogram window.
8. Scroll bar—Scroll back and forth to view the x-axis.
9. Resize the electropherogram window - horizontal—Click, then drag to horizontally to resize the electropherogram window.
10. Y-axis—Click directly on the axis to zoom.
11. Sample selection—Sample that is displayed in the electropherogram.

Sizing Table fields

Column	Description
Sample File Name	Indicates the sample file name.
Dye Color	Indicates the peak dye color. Possible states are blue, green, yellow, red, and orange.
Dye, Sample Peak	Assigns a name to each peak. The format is color, peak number.
Sample Name[1]	Indicates the sample name.
Size	Indicates the peak size based on base pairs.
Height	Indicates the peak height.
Area (Data Point)	Indicates the peak area based on scan number.
Area (Base Pairs)	Indicates the peak area based on base pairs.
Data Point	Indicates the peak data point or scan number.
Begin Point (Data Point)	Indicates the begin point of the peak based on the scan position.
End Point (Data Point)	Indicates the end point of the peak based on the scan position.
Begin Point (Base Pairs)	Indicates the begin point of the peak based on base pairs.
End Point (Base Pairs)	Indicates the end point of the peak based on based on base pairs.
Comments[1]	Displays user comments.

[1] To display this column, see "Customize columns in the Sample, Sizing, or Genotype Table" on page 20.

Genotype Table fields

Column name	Description
Sample File Name	The name of the sample file.
Sample Name	The name of the sample is derived from the sample name tag used in the sample file.
Panel	The name of panel used for the genotype analysis.
Marker	The name of the marker.
Dye Color	Indicates dye color of the marker.

Column name	Description
Allele 1	The name of the first allele identified for the marker. The table displays 2 alleles by default. See "Modify allele settings in the Genotype Table" on page 84.
Size 1	The size of the first allele identified for the marker. The table displays 2 alleles by default. See "Modify allele settings in the Genotype Table" on page 84.
Allele 2	The name of the second allele identified for the marker. The table displays 2 alleles by default. See "Modify allele settings in the Genotype Table" on page 84.
Size 2	The size of the second allele identified for the marker. The table displays 2 alleles by default. See "Modify allele settings in the Genotype Table" on page 84.
Allele Display Overflow (ADO)	A ✔ indicates that the associated sample contains a number of alleles at the specified marker that is greater than the user-defined limit in the **Allele Settings**. See "Allele Display Overflow (ADO)" on page 135.
Offscale (OS)	Displays OS PQV value. Possible states include ■ Pass or ▲ Suspect. See "Offscale (OS)" on page 139.
Sharp Peak (SHP)	Displays the SHP PQV value. Possible states include ■ Pass or ▲ Suspect. See "Sharp Peak (SHP)" on page 142.
One Basepair Allele (OBA)	Displays the OBA PQV value. Possible states include ■ Pass or ▲ Suspect. See "One Basepair Allele (OBA)" on page 140.
Single Peak Artifact (SPA)	Displays the SPA PQV value. Possible states include ■ Pass or ▲ Suspect. See "Single Peak Artifact (SPA)" on page 142.
Split Peak (SP)	Displays the SP PQV value. Possible states include ■ Pass or ▲ Suspect. See "Split Peak (SP)" on page 143.
Out of Bin Allele (BIN)	Displays the BIN PQV value. Possible states include ■ Pass or ▲ Suspect. See "Out of Bin Allele (BIN)" on page 140.
Low Peak Height (LPH)	Displays the LPH PQV value. Possible states include ■ Pass or ▲ Suspect. See "Low Peak Height (LPH)" on page 139.

Column name	Description
Spectral Pull-Up (SPU)	Displays the SPU PQV value. Possible states include ■ Pass or ▲ Suspect. See "Spectral Pull-Up (SPU)" on page 143.
Allele Number (AN)	Displays the AN PQV value. Possible states include ■ Pass or ▲ Suspect. See "Allele Number (AN)" on page 135
Broad Peak (BD)	Displays the BD PQV value. Possible states include ■ Pass or ▲ Suspect. See "Broad Peak (BD)" on page 136.
Cross Talk (XLTK)	Displays the XLTK PQV value. Possible states include ■ Pass or ▲ Suspect. See "Cross Talk (XLTK)" on page 137.
Peak Height Ratio (PHR)	Displays the PHR PQV value. Possible states include ■ Pass or ▲ Suspect. See "Peak Height Ratio (PHR)" on page 141.
Control Concordance (CC)	Displays the CC PQV value. Possible states include ■ Pass or ▲ Suspect. See "Control Concordance (CC)" on page 136.
Genotype Quality (GQ)	Displays the GQ PQV value. Possible states include ● (Pass), ● (Suspect), or ● (Low Quality) . See "Genotype Quality (GQ)" on page 138.
Genotype Comments[1]	Displays comments entered for genotype information.
Allele Edit (AE)[1]	Displays the AE PQV value. Possible states include ✔. See "Allele Edit (AE)" on page 136.
Deleted Allele Comment (DAC)[1]	Displays comments entered for deleted alleles. See "Deleted Allele Comment (DAC)" on page 137.
Height[1]	Indicates the peak height.
Peak Area[1]	Indicates the peak area.
Data Point[1]	Indicates the peak data point or scan number.
Comment[1]	Displays user comments.

[1] To display this column, see "Customize columns in the Sample, Sizing, or Genotype Table" on page 20.

A Reference information

Size Standard selected by the software

When samples are first imported, the software selects a size standard for the project based on the data imported in the FSA file.

Instrument	Size Standard data in FSA file	Size Standard selection
SeqStudio™ Genetic Analyzer	Name and values	**Factory default**—If a matching size standard name and value is found, the software selects it for the project. **Custom**—If a matching size standard name and value is not found, the software creates a custom size standard, then selects it for the project.
3500 Genetic Analyzer		
3130 Genetic Analyzer	Name[1]	**Factory default**—If a matching size standard name is found, the software selects it for the project. **GS500-250LIZ**—If a matching size standard name is not found, the software selects GS500-250LIZ (the first size standard in the factory default list) for the project.
3730/3730*xl* DNA Analyzer		

[1] Size Standard names are not included in all FSA files

Analysis status

Icon	Analysis status	Description
	Reanalyze	The sample has not been analyzed using the current analysis setting. Reanalyze to apply current settings. **Note:** SeqStudio™ (3200) and 3500 FSA files display after being imported because these files include the Size Standard, Size Quality, and peak information from the data collection software analysis.
	Unanalyzed	**Individual sample**—The sample has not been analyzed. **Note:** 3130/3130*xl* and 3730/3730*xl* FSA files display after being imported because these files do not include information from the data collection software analysis. **Project**—One or more of the samples in the project have not been analyzed using the current analysis settings.
	Analyzed	**Individual sample**—The sample has been analyzed using the current analysis settings. **Project**—All of the samples in the project have been analyzed using the current analysis settings.

Analysis settings

Analysis settings – sizing

Setting	Description
Analysis Range	• **Full Range**—*(default)* To analyze the entire scan region as collected by the genetic analysis instrument, including the primer peak. • **Specificed Range**—To analyze only data points within a specified range. Enter Start Point in data points after the primer peak and before the first required size standard peak. Enter a Stop Point after the last required size standard fragment. Start and Stop points may vary from instrument to instrument and platform to platform. View raw data to determine the appropriate analysis range. Data points outside the specified analysis range are ignored. **Note:** Ensure the **Analysis Range** contains all size standard fragments included in the **Sizing Range**.

Setting	Description
Sizing Range	The size range (in base pairs) appropriate for the kit you are using: • **Full Range** for the software to analyze fragments of all sizes in the **Analysis Range**. • **Partial Range** for the software to analyze only fragments within a specified range. Enter a **Start Size** and a **Stop Size** appropriate for the size standard used.
Peak Amplitude Threshold	The peak height threshold (RFU) for peak detection for each dye color. Peaks below the threshold are still displayed in the electropherogram plots but are not detected or labeled. **Note:** Use the same peak amplitude thresholds in Microsatellite Analysis Software.
Size Quality Status	The pass/fail range for the Size Quality Status. See "Sizing Quality (SQ) status" on page 133.
Linear Migration	The range in basepairs for fragments that migrate linearly.
Baseline Window	Specify a window to adjust the baseline signals of all detected dye colors to the same level for an improved comparison of relative signal intensity. See "Baseline Window size" on page 120. See "Effects of varying Baseline Window size" on page 121.
Minimum Peak Half Width	Specify the minimum full peak width at half maximum **Peak Height** required for peak detection. The range is 2 to 99 data points.
Polynomial Degree	**Polynomial Degree** cannot be greater than **Peak Window Size**. Adjust to affect the sensitivity of peak detection. You can adjust this parameter to detect a single base pair difference while minimizing the detection of shoulder effects and/or noise. See "Polynomial Degree and Peak Window Size" on page 122.
Peak Window Size	Enter a window width in data points for peak detection sensitivity. If more than one peak apex is within the window, all are labeled as a single peak. See "Polynomial Degree and Peak Window Size" on page 122.
Peak Slope Start	The peak starts when the first derivative (slope of the tangent) in the beginning of the peak signal before the inflection point becomes equal to or exceeds the **Peak Start** value. This threshold is set to 0 by default, which means that the peak will normally start at the leftmost point where the slope of the tangent is closest to 0° (horizontal line). A value other than 0 moves the peak start point toward its center. The value entered must be non-negative. See "Slope Thresholds for Peak Start/End parameters" on page 123.

Setting	Description
Peak Slope End	The peak ends when the first derivative (slope of the tangent) in the end of the peak signal after the inflection point becomes equal to or exceeds the **Peak End** value. This value is set to 0 by default, which means that the peak will normally end at the rightmost point where the slope of the tangent is closest to 0° (horizontal line). A value other than 0 moves the peak end point toward its center. The value entered in this field must be non-positive.
Peak Smoothing	Select an option to smooth the outline of peaks and reduce the number of false peaks detected: • **None** (default) to apply no smoothing. Best if the data display sharp, narrow peaks of interest. • **Light** to provide the best results for typical data. Light smoothing slightly reduces peak height. • **Heavy** for data with very sharp, narrow peaks of interest. Heavy smoothing can significantly reduce peak height. **Note:** The peak heights that are listed in the sizing table match the peak heights that are shown in the electropherogram when smoothing is applied. See "Smoothing" on page 122.
Size Calling Method	• **Local Southern**—*(default)* Determines the fragment sizes using the reciprocal relationship between fragment length and electrophoretic mobility. • **Global Southern**—Compensates for standard fragments with anomalous electrophoretic mobility (similar to least squares methods). • **Least Square Second Order**—Uses regression analysis to build a bestfit size calling curve. • **Least Square Third Order**—Uses regression analysis to build a bestfit size calling curve. • **Cubic Spline Interpolation**—Forces the sizing curve through all the known points of the selected size standard. See "Size Calling - Local Southern Method" on page 128. See "Size Calling – Global Southern Method" on page 129. See "Size Calling – Least Squares Method" on page 130. See "Size Calling – Cubic Spline Interpolation Method" on page 132.
Size Standard Normalization	For 3500 samples only, select to apply the normalization factor from the data collection software.

Analysis settings – genotyping

Setting	Description
Marker Repeat Type	• **Cut-off value**— Specify the minimum peak height ratio for a peak to be labeled as an allele. For example, a **Cut-off value** of 0.25 indicates the smaller peak must be at least 25% of the height of the larger peak to generate an allele call. • **PlusA ratio**—Specify the ratio of the height of PlusA peaks with the height of the true allele peak. • **PlusA distance**—Specify the distance between PlusA peaks and the true allele peak. • **Stutter ratio**—Specify the ratio of the combined heights of the stutter peaks with the height of the true allele peak. • **Stutter distance**—Specify the distance between stutter peaks and the true allele peak.
Range Filter	Remove labels from peaks in the designated size range.
Signal Level	Specify minimum peak heights for homozygous and heterozygous peaks.
Heterozygous Balance	Specify minimum peak height ratio.
Peak Morpholgy	Specify maximum peak width.
Pull-up Peak	Specify pull-up ratio and pull-up scan.
Allele Number	Specify the maximum number of alleles for a marker.
Cross-talk Peak	Specify the cross-talk ratio.
Quality Flag Settings	The applied weights of specific PQVs to the Genotype Quality value. See "Weights of PQVs" on page 135.
Genotype Quality Status	The pass/fail range for the Genotype Quality Status. See "Genotype Quality (GQ)" on page 138.

Peak detection

Baseline Window size

The Baseline Window size parameter controls baselining for a group of peaks.

The software determines a reference baseline value for each data point. In general, the software sets the reference baseline to be the lowest value that it detects in a specified window size (in data points) centered on each data point.

A small baseline window relative to the width of a cluster, or grouping of peaks spatially close to each other, can result in shorter peak heights.

Larger baseline windows relative to the peaks being detected can create an elevated baseline, resulting in peaks that are elevated or not resolved to the baseline.

Guidelines for the Baseline Window size parameter

Choose a value that encompasses the width in data points of the peaks being detected while preserving a qualitatively smooth baseline.

The trade-off for a smoother baseline that touches all peaks is a reduction in peak height.

Effects of varying Baseline Window size

The baseline window size determines the size of the window. Increasing the window size will decrease the baselining effect.

The following figure shows a sample with different reference baselines (zero in the analyzed electropherogram) that result from different baseline window size settings:

- The red trace shows a baseline derived from an extreme baseline window size value of 2801. At this setting, the reference baseline does not touch all peaks and elevates peak heights.
- The blue trace shows a baseline derived from the default value of 51 data points.
- The black trace shows a baseline that is derived from an extreme baseline window size value of 5 data points. At this setting, the reference baseline tracks the peaks, significantly reducing peak height.

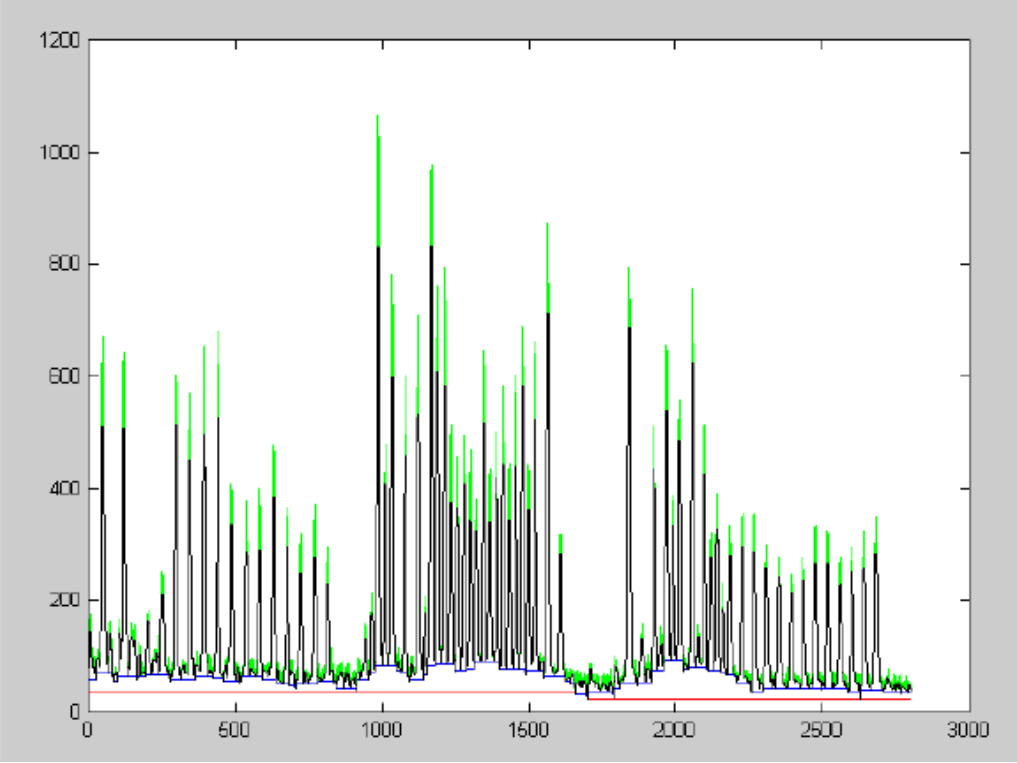


Figure 3 Baseline Window example

Smoothing

This parameter smooths the outline of peaks, and reduces the number of false peaks that are detected.

Smoothing is performed before peak detection and can be set to:

Option	Description
None	Applies no smoothing. Select for slower runs with very broad peaks, or to avoid the detection of sharp edges.
Light	Provides the best results for typical data. Light smoothing slightly reduces peak height.
Heavy	Select for data with very sharp, narrow peaks of interest. Heavy smoothing can significantly reduce peak height.

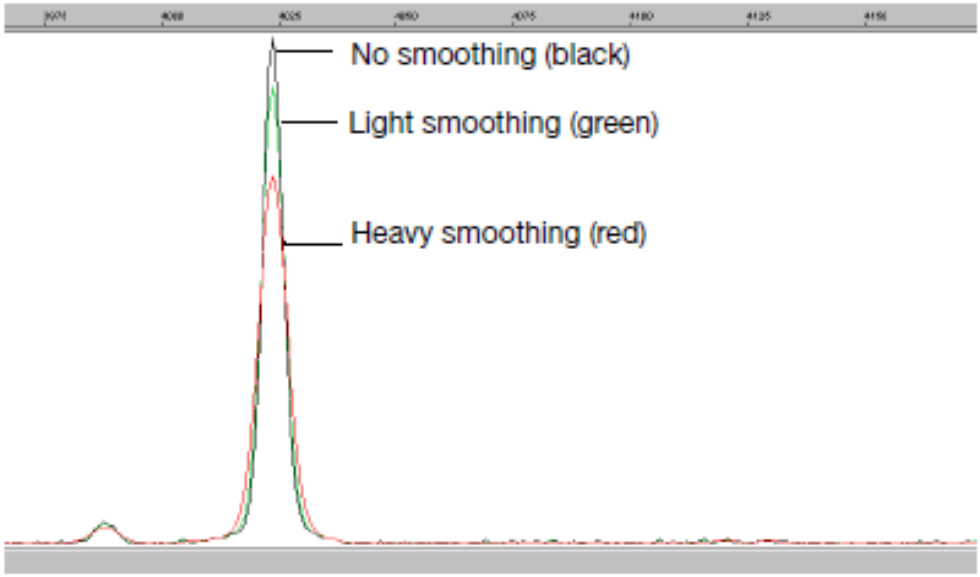


Figure 4 Smoothing example

Polynomial Degree and Peak Window Size

Polynomial Degree and Peak Window Size settings affect the peak detection sensitivity. You can adjust these parameters to detect a single base pair difference while minimizing the detection of shoulder effects and/or noise (see Table 1 .

The peak window size functions with the polynomial degree to set the sensitivity of peak detection. The peak detector calculates the first derivative of a polynomial curve fitted to the data within a window that is centered on each data point in the analysis range.

Using curves with larger polynomial degree values allows the curve to more closely approximate the signal and, therefore, the peak detector captures more of the peak structure in the electropherogram.

The peak window size sets the width (in data points) of the window to which the polynomial curve is fitted to data:

- Higher peak window size values smooth out the polynomial curve, which limits the structure being detected.
- Smaller window size values capture more of the peak structure.

Table 1 Change peak detection sensitivity using Polynomial Degree and Peak Window Size

Function	Polynomial Degree value	Peak Window Size value
Increase sensitivity	Higher	Lower
Decrease sensitivity	Lower	Higher

Slope Thresholds for Peak Start/End parameters

The Slope Threshold for Peak Start and Slope Threshold for Peak End parameters adjust the start and end points of a peak.

The values assigned to these parameters can be used to better position the start and end points of an asymmetrical peak, or a poorly resolved shouldering peak to more accurately reflect the peak position and area.

In general, from left to right, the slope of a peak increases from the baseline up to the apex. From the apex down to the baseline, the slope decreases negatively until it returns to zero at the baseline.

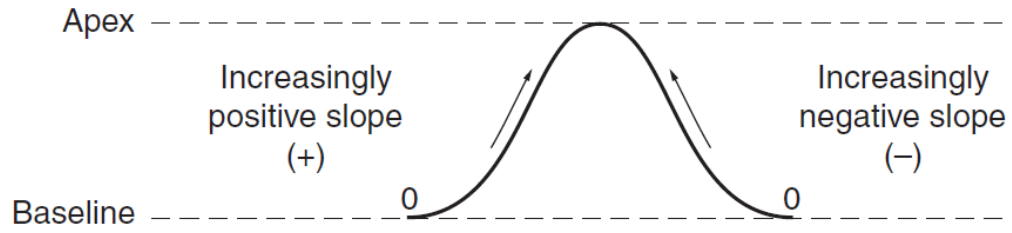


Figure 5 Peak slope

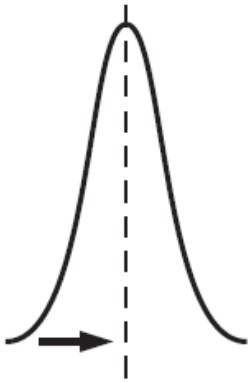
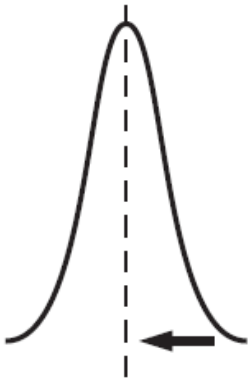
If either of the slope values you enter exceeds the slope of the peak being detected, the software overrides your value and reverts to zero.

Guidelines for Slope Threshold Peak Start and Peak End parameters

- For typical or symmetrical peaks, use a value of zero.
- For asymmetrical peaks, select values other than zero to better reflect the beginning and end points.
- A value of zero does not affect the sizing accuracy or precision of an asymmetrical peak.

Using Slope Threshold Peak Start and Peak End parameters

Note: The size of a detected peak is the calculated apex between the start and end points of a peak. Peak size does not change based on start and end settings.

To move the...	Then...	Example
Start point of a peak closer to its apex	Change the Slope Threshold for Peak Start value from zero to a positive number.	
End point of a peak closer to its apex	Change the Slope Threshold for Peak End value to a more negative number.	

Slope Threshold example — Asymmetrical peak

Figure 6 shows the initial electropherogram analyzed with value of 0 for Peak Start and Peak End. Note the asymmetrical peak with a noticeable tail on the right side.

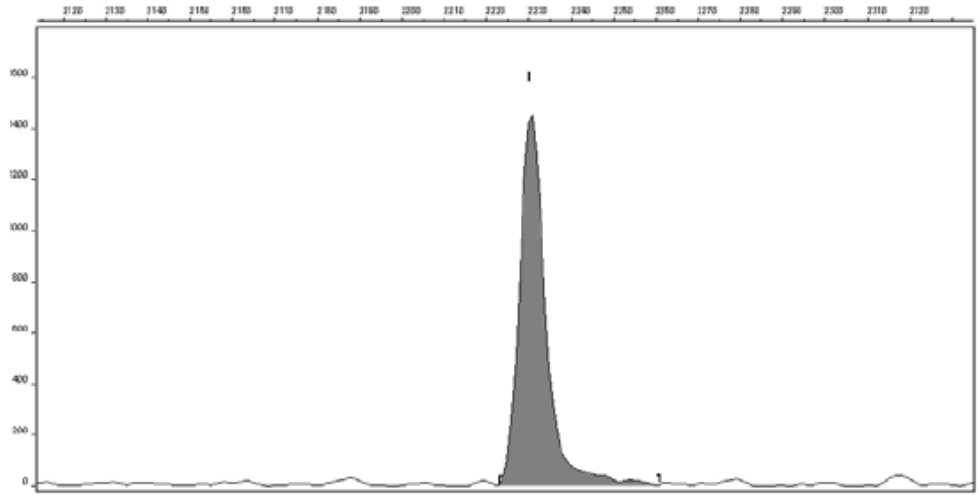


Figure 6 Electropherogram showing an asymmetrical peak

After reanalysis with a value of –35.0 for the Slope Threshold for Peak End, the end point that defines the peak moves closer to its apex, thereby removing the tail (Figure 7). Note that the only change to tabular data is the area (peak size and height are unchanged).

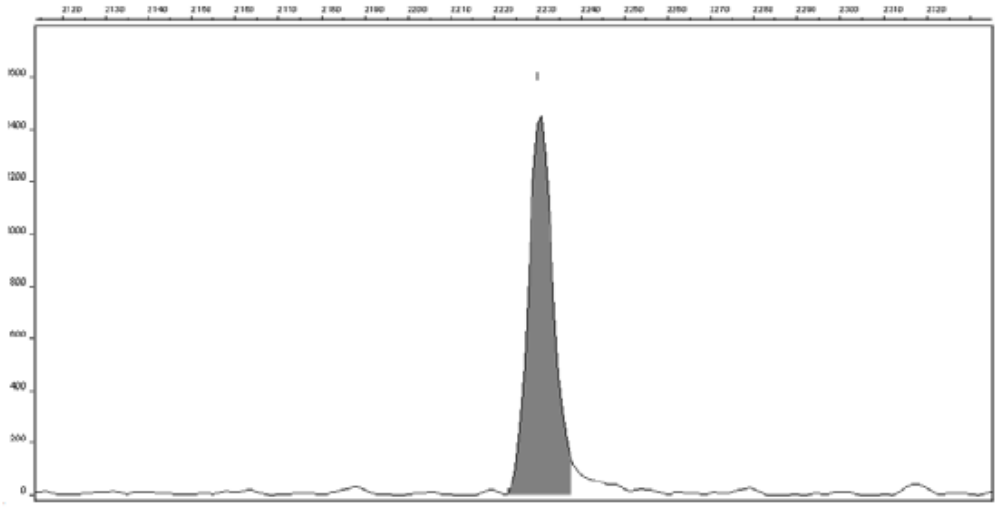


Figure 7 Electropherogram showing the effect of changing the slope threshold for peak end

Sizing overview

Size matching

During size matching, the software matches the size standard fragments from the electropherogram to the list of fragment sizes in the size standard definition specified in the software.

Size matching uses ratio matching, based on relative height and distance of neighboring peaks. It then derives quality values statistically by examining the similarity between the theoretical (from the size standard definition) and actual (observed) fragment patterns.

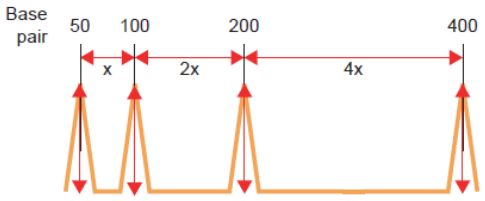
The software ignores anomalous peaks that do not match the expected patterns. The software constructs a best-fit curve using the data points of each size standard fragment detected. A comparison between the sizes calculated from the best-fit curve and the matched peaks from the size standard definition file using the array of numbers is performed. Size-matching (and subsequent size calling) fails if significant differences in peak patterns are found, if no match can be made based on the expected patterns, or if all peaks are not found.

Because the software uses ratio-matching (looks for the expected number of alleles and expected peak patterns instead of specific data points), it is not necessary to define new size-standard definitions due to migration shifts.

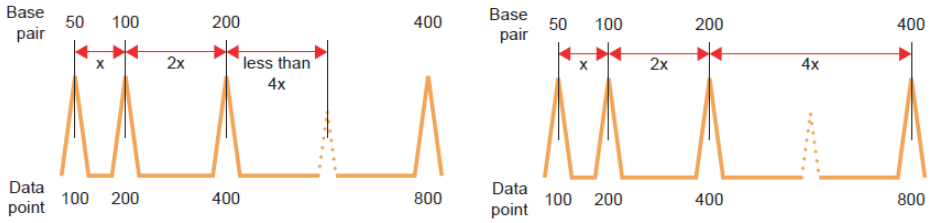
The size matching and size-calling curve generation algorithm:

- **Determines the expected peak sizing and height ratios** — Uses the list of sizes from the Size Standard definition file.

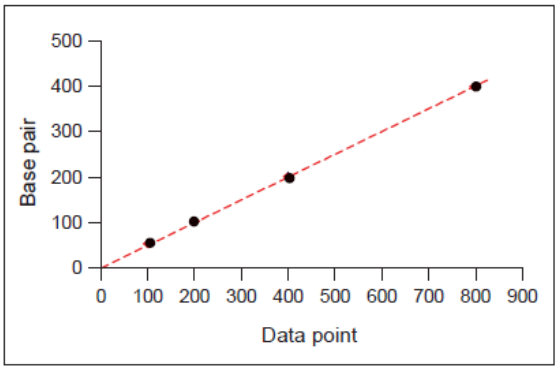
  Note: The values that are used are for example only and do not indicate typical size-standard values.



- **Evaluates peaks in the size standard data**— Ignores peaks that do not meet the expected pattern (dotted peak).



- **Plots the size–calling curve** — Uses peaks that meet the expected pattern.



Size-calling curve generation and size calling

To generate the size-calling curve, the software plots the actual data points of the size standard against the expected size of each size standard peak. The size-calling method determines how the size-calling curve is generated and used to size each sample.

During size matching and size calling:

- 2 size-calling curves are generated for each sample:
  - Black: A best-fit second order curve, regardless of the size-calling method that is selected.
  - Red: A curve based on the size-calling method that is selected in the analysis method.
- The data points of non-size-standard peaks are plotted against the size-calling curve.
- Peaks are sized according to the size-calling method that is selected in the analysis method.

Size-calling methods are:

- Local Southern
- Global Southern
- Least Squares (2nd Order or 3rd Order)
- Cubic Spline Interpolation

Size Calling - Local Southern Method

The Local Southern method determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility, as described by E. M. Southern (1979).

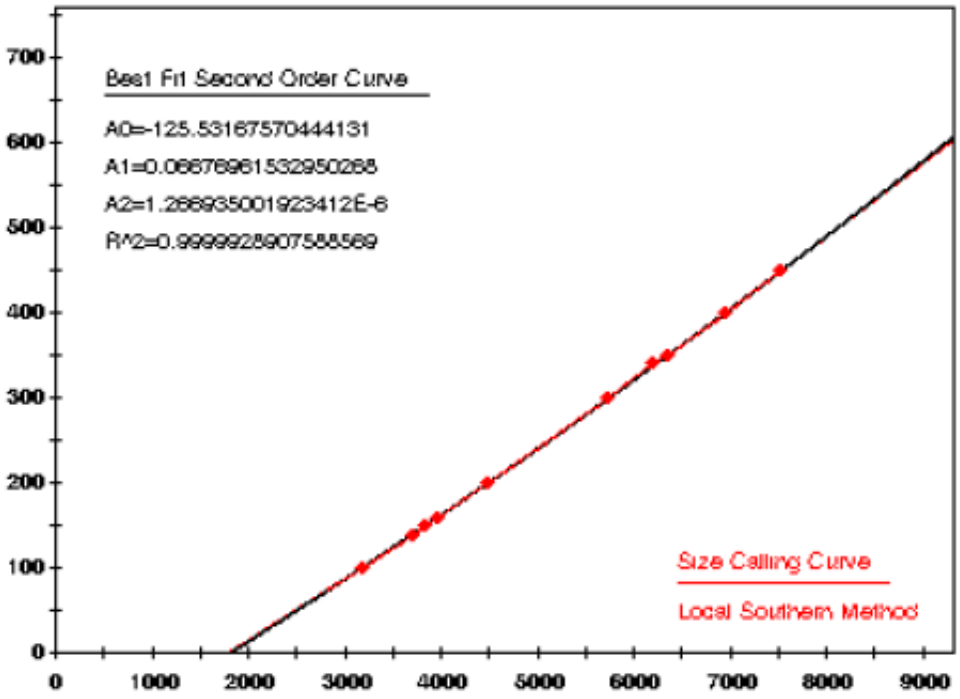


Figure 8 Local Southern method

Local Southern method equation

The equation attempts to describe the reciprocal relationship between the mobility, m, and the length, L0, of the standard fragments.

$L = [c/(m - m0)] + L0$

How the Local Southern method works

This method, which is similar to the Cubic Spline method, uses the four fragments closest in size to the unknown fragment to determine a best-fit line value. Only the region of the size standard near the fragment of unknown length is analyzed.

Note: Size estimates may be inaccurate if any of the standard fragments run anomalously.

In the Local Southern method:

1. The fitting constants of the curve are calculated for each group of three neighboring points on the standard.
2. A curve is then created using three standard points (two points below and one point above the fragment), then a fragment size is determined.
3. Another curve is created using an additional set of three points (one point below and two points above the fragment), to assign another value.
4. The two curves are averaged to determine the unknown fragment length.

Size Calling – Global Southern Method

The Global Southern method is similar to the Least Squares method in that it compensates for standard fragments that may run anomalously. The method creates a best-fit line through all the available points, and then uses values on that line to calculate the fragment values.

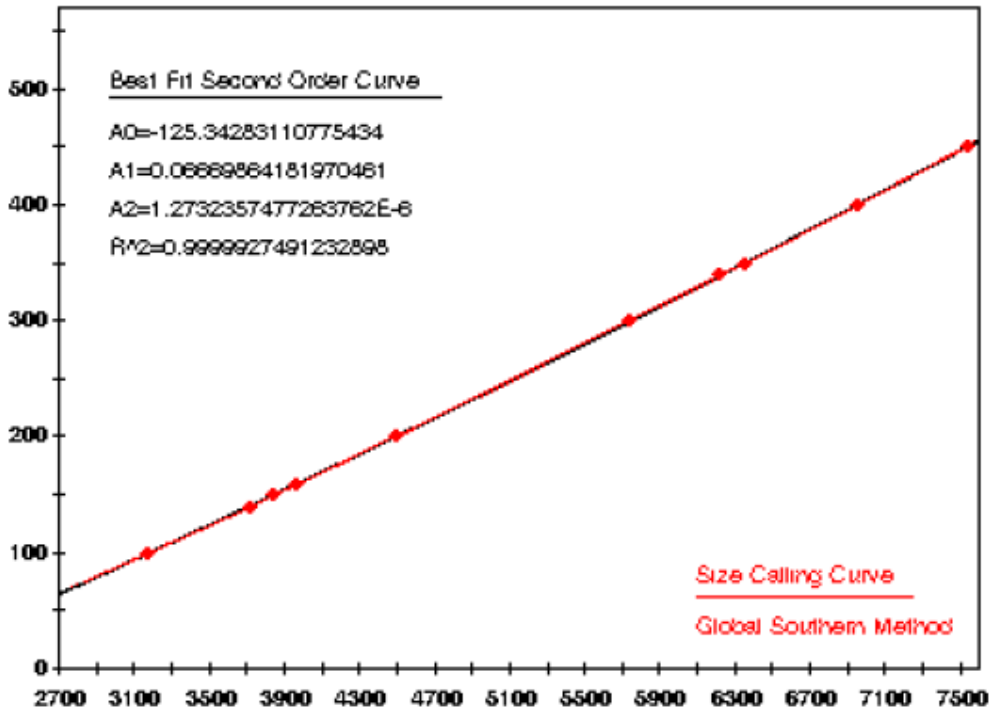


Figure 9 Global Southern method

Global Southern method equations

Table 2

Equation	Description
$L = [c/(m - m0)] + L0$	Attempts to describe the reciprocal relationship between the mobility, m, and the length, L0, of the standard fragments.
$\Sigma\{Li - [c/((mi - m0) + L0)]\}^2$	The fitting constants L0, m0, and c are calculated by a least-squares fit to minimize the left side quantity.

How the Global Southern method works

All points in the standard are weighted equally, and the curve is not constrained to go through any specific point. The software can analyze a large range of fragment sizes with this method. For best results, use a standard that brackets all the fragments of interest.

Size Calling – Least Squares Method

Both Least Squares methods (2nd-Order and 3rd-Order) use regression analysis to build a best-fit size-calling curve. This curve:

- Produces the minimum additive distance from the curve to the plotted data points.
- Compensates for any fragments that may run anomalously.

Consequently, this method typically results in the least amount of deviation for all the fragments, including the size standards and the samples.

Depending on whether you choose the 2nd- or 3rd-Order Least Squares Method in the Analysis Parameters dialog box, the resulting size curve is either a quadratic or a cubic function. The software uses the known standard fragments and the associated data points to produce a sizing curve based on Multiple Linear Regression.

How the Least Squares method works

The following figures show that in nearly all instances the mobility of an individual DNA fragment is coincident with the best curve fit of the entire data set. Stated differently, the mobility of most DNA fragments is strictly length dependent. This method automatically compensates for fragments that run anomalously.

To generate the Least Squares curve, the software:

- Plots the known fragment sizes (bp) versus data points.
- Generates a best-fit curve using regression analysis.
- To generate the Least Squares curve, the software:
  - Plots the known fragment sizes (bp) versus data points.
  - Generates a best-fit curve using regression analysis.
  - Applies the following calculation to determine the size in data points of the unknown fragments:

    $Y = Ax3 + Bx2 + Cx + D$

    where:
    - Y = size (bp)
    - x = datapoint
    - A = First order coefficient
    - B = Second order coefficient

– C = Third order coefficient
– D = Zeroth Coefficient or constant

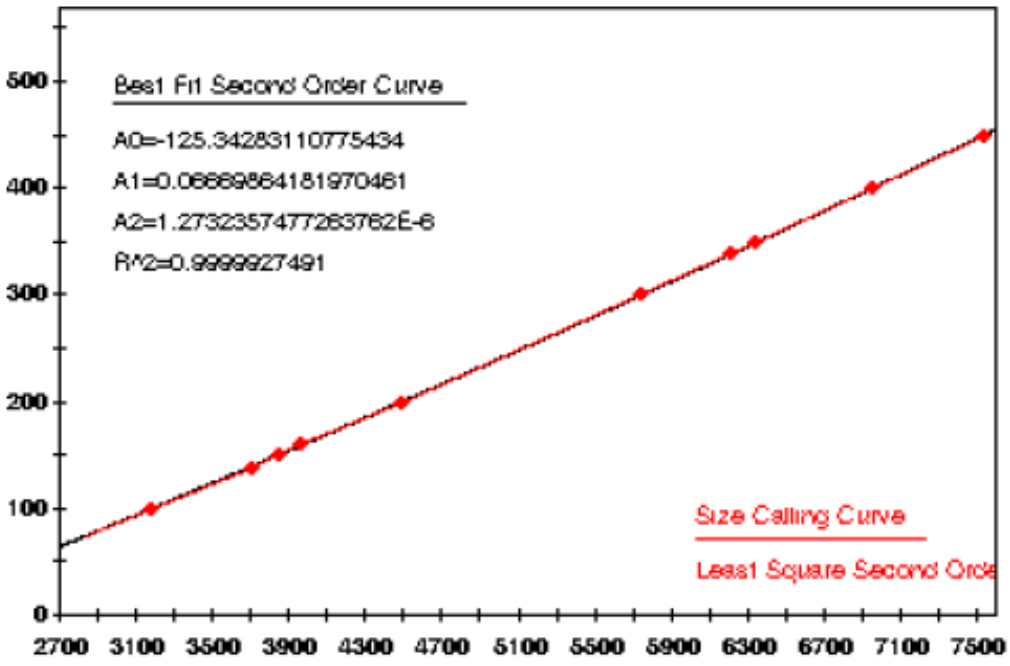


Figure 10 2nd-Order Least Squares size-calling curve (quadratic)

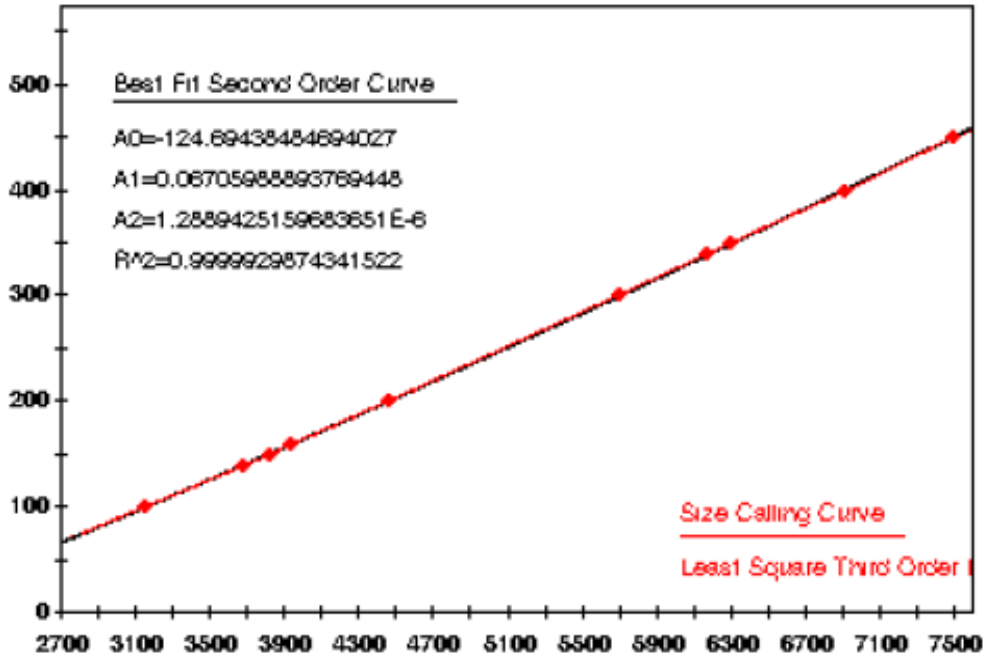


Figure 11 3rd-Order Least Squares size-calling curve (quadratic)

Size Calling – Cubic Spline Interpolation Method

The Cubic Spline method, which is similar to the Local Southern method, forces the sizing curve through all the known points of the selected size standard. Although this enforcement produces exact results for the values of the standards themselves, it does not compensate for standard fragments that may run anomalously.

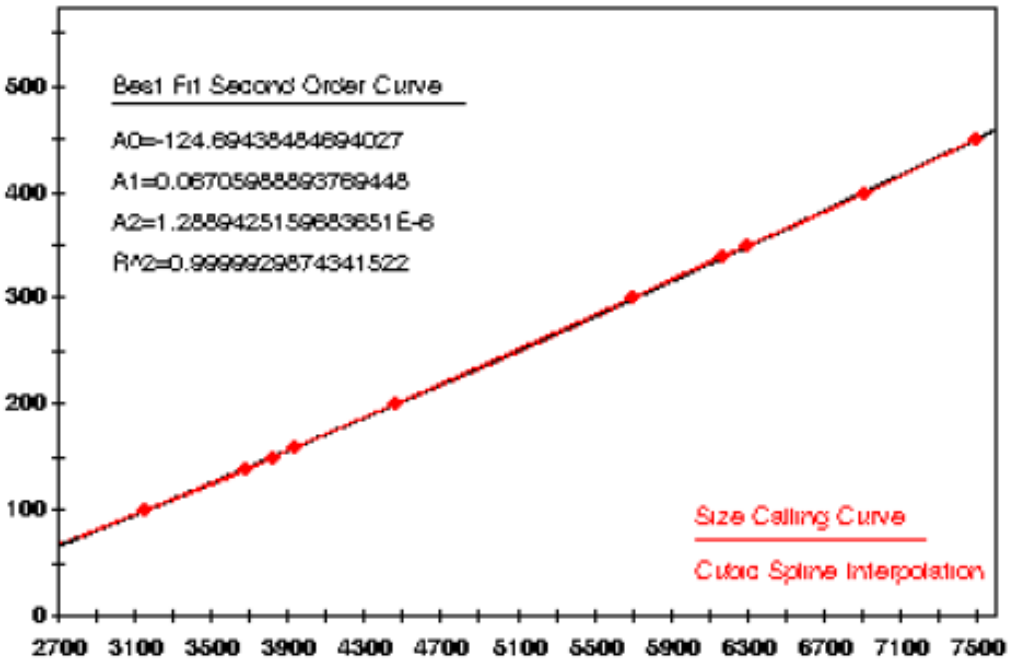


Figure 12 Cubic Spline Interpolation method

Possible local sizing inaccuracy

Mobility of any DNA fragment can be affected by its sequence, and by secondary and tertiary structure formation. If any internal size standard fragment has anomalous mobility, the Cubic Spline method may exhibit local sizing inaccuracy.

For example, assume that a standard fragment is close in molecular length to an unknown sample fragment. Assume further that the standard fragment runs anomalously. The Cubic Spline method assigns the official value to this standard fragment, even though it may be slightly incorrect. The size of the unknown fragment is then likely to be calculated incorrectly as well.

Note: This method does not determine the degree of sizing accuracy error.

Sizing Quality (SQ) status

The Sizing Quality assessment evaluates the quality of the size standard profile within each sample (SQ) and allows you to flag samples with size standards that have poor peak resolution. You can adjust the ranges that correspond to the High, Medium, and Low Quality ranges in the Analysis Settings. See "Adjust Size Quality Flag ranges" on page 26.

Acronym	Full name	Function/Rule(s)	Flag Indicator	
SQ	Sizing Quality	Evaluates the similarity between the fragment pattern for the size standard dye that is specified in the size standard definition and the actual distribution of size standard peaks in the sample.	🟢	SQ is within the user-defined High Quality range (Default = 0.75 to 1.0)
			🟡	SQ is within the user-defined Medium Quality range. (Default = 0.26 to 0.74)
			🔴	SQ is within the user-defined Low Quality range (Default = 0.0 to 0.25)

Genotyping overview

After samples are detected and sized, sizing quality is determined, then samples are genotyped based on the panel and bin information in the **Analysis Settings**.

If allelic ladders are used, the ladders are detected and sized, sizing quality is determined, then allelic ladders are genotyped. After allelic ladders are genotyped, samples are detected and sized, sizing quality is determined, then samples are genotyped.

During genotyping of samples, the software:

- Performs allele-calling and filtering.
- Assesses genotype quality.
- Assesses sample quality.

Allele calling and filtering of samples

OL (Off-Ladder) is called only when Allele Ladder samples are present in the project.

During allele calling of samples, the software:

- Labels allele peaks in unknown samples:
  - Compares sizes of the unknown peaks to the bins.
  - Assigns the relevant allele label if a peak falls within one of the defined bins.
  - Assigns an OL (Off-Ladder) label when a peak falls outside of the defined bins or within a bin overlap region
- Applies filters to eliminate peaks from consideration as allele peaks:
  - Analysis method settings (see "Analysis settings – genotyping" on page 120)
  - Marker-specific stutter ratios if defined in the panel

Genotyping Quality (GQ) status and Process Quality Values (PQVs)

Process Quality Values (PQVs) provide the basis for troubleshooting fragment analysis data using the software. PQVs are application-specific metrics, where each evaluates the data for a specific quality that is consistent with a problem associated with the type of analysis. In this way, the PQV system can alert you to potential problems and provide you with a starting point for investigation.

Each individual PQV displays the result of a unique algorithmic test that evaluates a specific property of the fragment analysis data. The software performs the PQV tests in a specific sequence during the analysis. With the exception of the Sizing Quality (SQ) PQV, the software completes the analysis of each sample in a project even if a sample fails one or more PQV tests.

The majority of PQV metrics yield numeric values between 0 and 1, where 1 indicates that the related sample data or genotype completely passed the associated test. Following the analysis, the software uses the upper and lower thresholds for each PQV to translate the numeric score into one of three symbols, displayed in the Samples Table and Genotype Table.

Note: The thresholds of certain PQVs can be customized, see "Adjust Genotype Quality Flag weights and ranges" on page 55.

Symbol	Definition	Default range
	Pass: The sample or genotype passed the PQV test.	0.75 to 1.0
	Suspect: A possible problem exists for the sample or genotype.	0.25 to 0.75
	Low Quality: There is a strong possibility that a problem exists for the sample or genotype.	0.0 to 0.25

Note: We recommend examining all samples with  or  values.

Note: The Allele Display Overflow (ADO) and Allele Edit (AE) PQVs of the Genotypes tab report their results as instead of the colored icons.

Note: The Microsatellite Analysis Software does not complete the analysis of samples that display (Low Quality) for the Sizing Quality (SQ) PQV test.

Threshold Settings of PQVs

Some PQV metrics include components that can be customized. In those cases, the user-defined parameters for the PQV appear in the Peak Quality tab of analysis methods for the applicable analysis type.

Weights of PQVs

The majority of PQV contribute to the Genotype Quality (GQ) PQV, a metric used to gauge the confidence of each genotype call. In those cases, some PQV contain user-defined weights that determine how significantly the associated PQV affect the GQ PQV calculation. For those PQV, the user-defined weights appear in the Quality Flag tab of analysis methods for the applicable analysis type. For more information on the calculation of the GQ PQV, see "Genotype Quality (GQ)" on page 138.

Allele Number (AN)

Function/Rule(s)

Indicates that the associated sample contains either:

- A number of alleles at the specified marker that exceeds the **Max expected alleles** setting in the **Peak Quality** section of the genotype analysis settings (see "Analysis settings – genotyping" on page 120)
- No alleles are present at the specified marker

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

- Modify the **Max expected alleles** setting in the **Peak Quality** section of the genotype analysis settings (see "Analysis settings – genotyping" on page 120)
- No alleles are present at the specified marker

Allele Display Overflow (ADO)

Function/Rule(s)

Indicates that the number of alleles called for the associated sample at the specified marker exceeds the **Allele Settings** in the Genotype Table. Because the software is configured to display fewer alleles than are present, the data for the additional allele is hidden from view.

Flag Indicator

A ✔ indicates that the associated sample contains a number of alleles at the specified marker that is greater than the user-defined limit.

Troubleshooting

Select the affected genotype, then review the affected sample for miscalled peaks. Modify **Allele Settings** in the Genotype Table as needed (see"Modify allele settings in the Genotype Table" on page 84).

Allele Edit (AE)

Function/Rule(s)

Indicates whether or not a user modified the allele call for the associated genotype.

Flag Indicator

A ✔ indicates that the associated genotype call has been edited.

Broad Peak (BD)

Function/Rule(s)

Indicates that the width of the peak for the associated genotype exceeds the Max peak width setting (in the Peak Quality tab of the analysis method).

Note: When the BD PQV is triggered, the software reduces the GQ PQV by 50% because the default multiplier is 0.5.

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Select the affected genotype, then review the associated peak in the genotype plot for irregularities.

Control Concordance (CC)

Function/Rule(s)

Indicates that the associated control sample does not exactly match the defined alleles for the related marker.

IMPORTANT! We recommend running the control sample at least once for every panel.

Note: The CC PQV serves primarily as an internal control for quality assurance.

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Select the affected control sample in the Sample Table, open the Panel Manager, then review the positions of the peaks relative to the bins.

Flag indicator	Possible cause	Solution
▲ (Suspect)	The allele calls of the sample defined as the Positive Control in the Samples tab do not match the Positive Control allele calls in the marker definition because the well contains the incorrect positive control sample.	Run the correct positive control and add the sample file to the project, then define the sample as the Positive Control (see "Edit sample name and type" on page 20).
	The allele calls of the sample defined as the Positive Control in the Samples tab do not match the positive control allele calls in the marker definition because the alleles were defined incorrectly.	Edit the Positive Control allele calls in the marker definition in the Panel Manager (see "Edit a panel in Panel Manager" on page 43).
	The sample defined as the Negative Control contains an "allele peak" due to the presence of a spike caused by dust or a gas bubble.	Rerun the negative control and add the sample file to the project, then define the sample as the Negative Control (see "Edit sample name and type" on page 20).

Cross Talk (XLTK)

Function/Rule(s)

Indicates that, at the peak position of the associated genotype, the ratio of the signals collected from the neighboring capillaries exceed the Cross-talk ratio setting in the Peak Quality section of the genotype analysis settings (see "Analysis settings – genotyping" on page 120).

Note: When the XTLK PQV is triggered, the software reduces the GQ PQV by 50% (the default multiplier is 0.5).

Flag Indicator

■ (Pass) or ▲ (Suspect)

Deleted Allele Comment (DAC)

Function/Rule(s)

Indicates that a comment was entered for a deleted allele.

Flag Indicator

The **DAC** column of the Genotype Table displays comments entered for deleted alleles.

Genotype Quality (GQ)

Function/Rule(s)

Provides a summary of the quality metrics for each genotype. The GQ value is a calculated combination of the relevant, weighted PQVs and the Marker Quality value for the genotype.

Calculation of the Genotype Quality (GQ) metric

The following general formula describes the genotype quality calculation:

$GQ = MQ \times ((1 - BD) \times (1 - OS) \times \ldots \times (1 - SPU))$

The Marker Quality (MQ) value is modified by the user-defined PQVs to generate the final GQ value, and the PQVs are weighted from 0 to 1. The actual value of each PQV in the equation is 1 minus the weight assigned in the Quality Flags tab of the analysis method used to analyze the data.

PQV weight	Net effect on GQ calculation
0	No effect on the GQ calculation. The initial value of 1 minus the weight of 0 yields a PQV of 1. When used in the GQ calculation, the PQV has no effect since 1 ∞ MQ = MQ.
1	Reduces the GQ value to 0 The initial value of 1 minus the weight of 1 yields a PQV of 0. When used in the GQ calculation, the PQV automatically causes the GQ to fail since 0 ∞ MQ = 0.
$0 \leqslant x \leqslant 1$	Reduces the GQ value to the fraction specified by the weight. The higher the value, the greater the effect on GQ.

IMPORTANT! The filtering of individual PQVs is controlled by the threshold settings in the Peak Quality tab of the genotype analysis settings.The PQVs remain fully functional regardless of the weights used.

Calculation of the Marker Quality (MQ) metric

The software generates a Marker Quality (MQ) value from sample peak data with assigned Allele Quality (AQ) values. AQ values are a function of quality value assignments for: sizing quality, allele calling quality, bin assignment quality, and bin quality.

Flag Indicator

● (Pass), ● (Suspect), or ● (Low Quality)

Note: The software assigns the GQ PQV flags based on the PQV threshold settings in the Quality Flags tab of the analysis method.

Troubleshooting

Review the PQV for the affected genotype to determine the metric that is causing the GQ PQV to fail (see "Review PQVs of samples with Suspect and Low Quality GQ values" on page 85).

Low Peak Height (LPH)

Function/Rule(s)

Indicates that the height of the peak for the associated genotype is lower than the associated heterozygous or homozygous height limit that is specified in the Peak Quality section of the genotype analysis settings.

Note: When the LPH PQV is triggered, the software reduces the GQ PQV by 50% (the default multiplier is 0.5).

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

- Select the affected genotype, then review the associated peak in the genotype plot for irregularities.
- Modify the homozygous and heterozygous height value settings (see "Modify the default analysis settings" on page 25)

Offscale (OS)

Function/Rule(s)

The OS PQV is displayed for both Sizing and Genotyping results , but the function of the OS PQV in each tab differs in the following way:

- **OS PQV for the Sizing results** – The signal associated with the size standard of the specified sample contains one or more peaks that exceed the maximum detectable range.
- **OS PQV for the Genotyping results** – The signals associated with the given sample contain one or more peaks that exceed the maximum detectable range.

Note: When the OS PQV Genotyping results is triggered, the software reduces the GQ PQV by 50% (the default multiplier is 0.5).

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Review the raw data for offscale peaks (see "Examine EPT and raw data" on page 21).

One Basepair Allele (OBA)

Function/Rule(s)

Indicates, for the associated genotype, that the apex of the associated peak is present at a position within 1 base pair of another peak.

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Select the affected genotype, then review the allele at the appropriate bin location in the genotype table for a microvariant peak or an invalid allele call.

Out of Bin Allele (BIN)

Function/Rule(s)

Indicates that the apex of the peak for the associated genotype is outside of the boundaries that define the associated bin.

Note: When the BIN PQV is triggered, the software reduces the GQ PQV by 80% because the default multiplier is 0.8.

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Select the affected genotype, then review the associated peak in the genotype plot for irregularities.

Flag indicator	Possible cause	Solution
▲ (Suspect)	After using Auto Bin to generate bins, the software did not create a bin for an allele peak because it considered the peak to be a single peak artifact. The SPA flag was triggered because the software did not detect any stutter peaks to the left of the allele peak; a result of the minimum fragment length for the marker being set too high	Correct the SPA flag by editing the marker minimum fragment length, then reanalyze and perform the Auto Bin again.
	The software detected an allele peak that did not fit into any of the defined bins because the bins were not calibrated to the allelic ladder; a result of a sample file containing an allelic ladder that is not designated as an allelic ladder in the Sample Table.	Set the Sample Type of the sample containing the allelic ladder to Allelic Ladder (see "Edit sample name and type" on page 20).
	You generated bins using the Auto Bin function but the GQ value for a marker was less than the Minimum Quality Value of 0.1 (as set in the Auto Bin dialog box).	View the allele peak(s) for the marker in the Genotypes Plot. Determine if the allele peaks(s) are valid. If so, manually create bin(s) for the peak(s) (see "Create, edit or delete a bin using Panel Editing" on page 86).

Peak Height Ratio (PHR)

Function/Rule(s)

Indicates that the apex of the peak for the associated genotype is:

- Present at a position within 1 base pair of another peak
- The ratio of the height of the lower peak to that of the higher peak is less than the Minimum Peak Height Ratio setting in the Peak Quality section of the genotype analysis settings.

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Select the affected genotype, then review the associated peak in the genotype plot for irregularities.

Flag indicator	Possible cause	Solution
▲ (Suspect)	The sample has undergone Loss of Heterozygosity (LOH). A difference in peak heights between alleles is expected.	Normal occurrence. No action necessary. Further evaluate the sample for LOH using the Report Settings Editor and Report Manager.

Sharp Peak (SHP)

Function/Rule(s)

Indicates that the peak for the associated genotype is part of a cluster of peaks with a large, narrow peak in the middle whose width is 50% less than either of the neighboring peaks.

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Select the affected genotype, then review the associated peak in the genotype plot for irregularities.

Single Peak Artifact (SPA)

Function/Rule(s)

Indicates that no peaks are present within a two-basepair range before the peak for the associated genotype. This PQV is designed to detect the absence of stutter peaks that accompany microsatellite peaks.

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Select the affected genotype, then review the associated peak in the genotype plot for irregularities.

Flag indicator	Possible cause	Solution
▲	The software did not detect any stutter peaks to the left of the allele peak because the minimum fragment length for the marker was set too high.	In the Panel Manager, edit the marker minimum fragment length, then reanalyze.

Spectral Pull-Up (SPU)

Function/Rule(s)

Indicates that the apex of the peak for the associated genotype is at a position where the marker signal contains pull-up peaks (also called bleed-through peaks). Pull-up peaks occur when the peak height of the called allele is less than X% of the larger peak that is within ±1 data point.

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Select the affected genotype, then review the associated peak in the genotype plot for irregularities.

Split Peak (SP)

Function/Rule(s)

Indicates that the peak for the associated genotype is part of a pair of overlapping peaks that are less than 0.25 base pairs apart (the horizontal distance between two peak apexes).

Flag Indicator

■ (Pass) or ▲ (Suspect)

Troubleshooting

Select the affected genotype, then review the associated peak in the genotype plot for irregularities.

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