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GeneMarker® v3.0.0 Operation Manual

1 Guiding Principles and Scope

- 1.1 This method contains the steps for the analysis of **PowerPlex® Fusion 5C** (Fusion) and **PowerPlex® Y23** (PPY23) STR runs performed on **3500xL** Genetic Analyzers and analysis of **PowerPlex® Fusion 5C** (Fusion) and **AmpF/STR® Yfiler** (Yfiler) STR runs performed on **3130xL** Genetic Analyzers using **GeneMarker® HID v3.0.0**.
- 1.2 For more information on the use of GeneMarker® HID v3.0.0 see Section 19 General Overview
- 1.3 For first time GeneMarker® use, for a new installation, or for new computer hardware see [QC355- GeneMarker® setup](#).
- 1.4 This manual cannot cover all situations that may arise during the analysis of an STR run. If an analyst encounters something that is not covered by this manual, they should seek their supervisor for further guidance.

2 Load Data

- 2.1 To start your project, go to **File** → **Open Data** → **Add**.
- 2.2 Navigate to the network folder containing the **.hid** (3500 data), or **.fsa** files (3130 data) data files to be analyzed → Select files to add.
- 2.3 Include only one ladder per project.
- 2.4 Hold Ctrl key to select multiple files. If analyzing all files, hold Ctrl, click **A** → Select **Open**.
- 2.5 Ensure correct files are added to list → Select **Ok**.
- 2.6 Ensure that prefixes **LD**, **PC1** and **NC** are labeling the **allelic ladder**, **positive control**, and **negative control**, respectively, in the **Sample File Tree**.
 - 2.6.1 If they are not, right click on the sample name in the **Sample File Tree** → go to **Set Sample Type** → Select the appropriate sample type. Check to ensure the prefix has been included.
- 2.7 Click **Run Project**  located in the top toolbar.

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3 Run Wizard

- 3.1 In the **Template Selection** window, on the left side, select the appropriate existing template. The following is a list of the available Wizard Templates. The screenshots for each window in the **Run Wizard** specific to each template listed are displayed below.

| Wizard Template | Instrument | Used for Analysis of |
|-----------------------|------------|------------------------------------------------------|
| Fusion 3500 | 3500 | evidence (with stutter filters) |
| Fusion 3500 STRmix | | evidence for STRmix import (without stutter filters) |
| Fusion 3500 Exemplars | | exemplars |
| Fusion | 3130 | evidence (with stutter filters) |
| Fusion STRmix | | evidence for STRmix import (without stutter filters) |
| Fusion Exemplars | | exemplars |
| PPY23 3500 | 3500 | evidence |
| PPY23 3500 Exemplars | | exemplars |
| Yfiler | 3130 | evidence and exemplars |

Note: Do NOT click Save on any of the following windows in the Run Wizards. This will overwrite the template for all analysts.

- 3.2 Check the **Template Selection** window against the corresponding screenshots below for initial analysis. If “Last Template” is displayed in the Template Name field, ensure that the appropriate Panel is listed for your analysis. For Wizard Templates for STRmix™ analysis, see Section 15. Select **Next** twice to move through the **Data Process** and **Analysis Settings** windows and check all settings against the corresponding screenshots.

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3.3 Fusion on 3500 Run Wizard Screenshots

Fusion on 3500 (Evidence)

Run Wizard ×

Template Selection
Set the template of the project

Select an existing template or create one

- Fusion
- Fusion Exemplars
- Fusion_3500
- Fusion_3500_Exemplars
- Fusion_3500_STRmix
- Fusion_STRmix
- PPY23_3500
- PPY23_3500_Exemplars
- YFiler

Use last template

Template Name: Fusion_3500

Panel: Fusion_3500

Size Standard: ILS500

Standard Color: Orange

Fusion on 3500 (Exemplars)

Run Wizard ×

Template Selection
Set the template of the project

Select an existing template or create one

- Fusion
- Fusion Exemplars
- Fusion_3500
- Fusion_3500_Exemplars
- Fusion_3500_STRmix
- Fusion_STRmix
- PPY23_3500
- PPY23_3500_Exemplars
- YFiler

Use last template

Template Name: Fusion_3500_Exemplars

Panel: Fusion_3500_Exemplars

Size Standard: ILS500

Standard Color: Orange

Data Process - HID Analysis

Run Wizard ×

Data Process - HID Analysis
Set data process options

Raw Data Analysis

Auto Range (frame)
Start: 0 End: 10000

Smooth Enhanced Smooth

Baseline Subtraction:
 Superior Classic Enhanced

Pull-up Correction Spike Removal

Saturation Detection Saturation Repair

Allele Call

Auto Range (bps)
Start: 55 End: 510

Max Intensity: 32500

Peak Detection Threshold: ? Dye Specific

| | Min RFU | Min RFU% |
|--------|---------|----------|
| Blue | 85 | 0 |
| Green | 120 | 0 |
| Yellow | 130 | 0 |
| Red | 160 | 0 |
| Orange | 85 | 0 |

Size Call
 Local Southern Cubic Spline

Data Process - HID Analysis

Run Wizard ×

Data Process - HID Analysis
Set data process options

Raw Data Analysis

Auto Range (frame)
Start: 0 End: 10000

Smooth Enhanced Smooth

Baseline Subtraction:
 Superior Classic Enhanced

Pull-up Correction Spike Removal

Saturation Detection Saturation Repair

Allele Call

Auto Range (bps)
Start: 55 End: 510

Max Intensity: 32500

Peak Detection Threshold: ? Dye Specific

| | Min RFU | Min RFU% |
|--------|---------|----------|
| Blue | 85 | 10 |
| Green | 120 | 10 |
| Yellow | 130 | 10 |
| Red | 160 | 10 |
| Orange | 85 | 10 |

Size Call
 Local Southern Cubic Spline

Additional Settings - HID Analysis

Run Wizard ×

Additional Settings - HID Analysis
Set additional options related to the different analysis type

Allelic Ladder: NONE

P.C. Template 1: PP_Fusion

P.C. Template 2: NONE

Allele Evaluation
Peak Score: Reject < 0.00 Check 1.00 < Pass

Auto Select Best Ladder
Allow Match # Variance: 0
Max Average Size Diff: 0.20
 Use Ladder Library
Min Heterozygosity: 0.50

Auto Panel Adjustment

Mixture Evaluation
Valid Mixture Peak Percentage: 0 %
Min Mixture Marker Number: 3

Sample Quality Sensor
'Q' Allele: _____
'S' Allele: _____

Additional Settings - HID Analysis

Run Wizard ×

Additional Settings - HID Analysis
Set additional options related to the different analysis type

Allelic Ladder: NONE

P.C. Template 1: PP_Fusion

P.C. Template 2: NONE

Allele Evaluation
Peak Score: Reject < 0.00 Check 1.00 < Pass

Auto Select Best Ladder
Allow Match # Variance: 0
Max Average Size Diff: 0.20
 Use Ladder Library
Min Heterozygosity: 0.50

Auto Panel Adjustment

Mixture Evaluation
Valid Mixture Peak Percentage: 0 %
Min Mixture Marker Number: 3

Sample Quality Sensor
'Q' Allele: _____
'S' Allele: _____

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3.4 Fusion on 3130 Run Wizard Screenshots

Fusion on 3130 (Evidence)

Run Wizard
Template Selection
Set the template of the project

Select an existing template or create one

Template Name: Fusion

Panel: Fusion_OCME_no filter

Size Standard: ILS500

Standard Color: Orange

Use last template

Save Delete << Back Next >> Cancel

Fusion on 3130 (Exemplars)

Run Wizard
Template Selection
Set the template of the project

Select an existing template or create one

Template Name: Fusion Exemplars

Panel: Fusion_OCME_10%

Size Standard: ILS500

Standard Color: Orange

Use last template

Save Delete << Back Next >> Cancel

Data Process - HID Analysis

Run Wizard
Data Process - HID Analysis
Set data process options

Raw Data Analysis

Auto Range (frame) Start: 0 End: 10000

Smooth Enhanced Smooth

Baseline Subtraction: Superior Classic Enhanced

Pull-up Correction Spike Removal

Saturation Detection Saturation Repair

Allele Call

Auto Range (bps) Start: 55 End: 510

Max Intensity: 32500

Peak Detection Threshold: ? Dye Specific

Min Intensity: 50

Percentage > 0 Global Max

Note: Use Panel Editor to set Min Intensity and % Global Max for peaks within Markers

Size Call

Local Southern Cubic Spline

Save Delete << Back Next >> Cancel

Additional Settings - HID Analysis

Run Wizard
Additional Settings - HID Analysis
Set additional options related to the different analysis type

Allelic Ladder: NONE

P.C. Template 1: PP_Fusion

P.C. Template 2: NONE

Allele Evaluation

Peak Score: Reject < 0.00 Check 1.00 < Pass

Auto Select Best Ladder

Allow Match # Variance: 0

Max Average Size Diff: 0.20

Use Ladder Library

Min Heterozygosity: 0.50

Auto Panel Adjustment

Mixture Evaluation

Valid Mixture Peak Percentage: 0 %

Min Mixture Marker Number: 3

Sample Quality Sensor

'Q' Allele: _____

'S' Allele: _____

Save Delete << Back Ok Cancel

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3.5 PPY23 on 3500 Run Wizard Screenshots

PPY23 on 3500 (Evidence)

Run Wizard

Template Selection
Set the template of the project

Select an existing template or create one

- Fusion
- Fusion Exemplars
- Fusion_3500
- Fusion_3500_Exemplars
- Fusion_3500_STRmix
- Fusion_STRmix
- PPY23_3500**
- PPY23_3500_Exemplars
- Yfiler

Template Name: PPY23_3500
Panel: PPY23_3500
Size Standard: ILS500
Standard Color: Orange

Save Delete << Back Next >> Cancel

Run Wizard

Data Process - HID Analysis
Set data process options

Raw Data Analysis

Auto Range (frame)
Start: 0 End: 10000
 Smooth Enhanced Smooth

Baseline Subtraction:
 Superior Classic Enhanced
 Pull-up Correction Spike Removal
 Saturation Detection Saturation Repair

Allele Call

Auto Range (bps)
Start: 55 End: 510
Max Intensity: 32500
Peak Detection Threshold: ? Dye Specific

| | Min RFU | Min RFU% |
|--------|---------|----------|
| Blue | 60 | 0 |
| Green | 70 | 0 |
| Yellow | 90 | 0 |
| Red | 120 | 0 |
| Orange | 60 | 10 |

Size Call
 Local Southern Cubic Spline

Save Delete << Back Next >> Cancel

Run Wizard

Additional Settings - HID Analysis
Set additional options related to the different analysis type

Allelic Ladder: NONE

P.C. Template 1: PPY23
P.C. Template 2: NONE

Allele Evaluation
Peak Score:
Reject < 0.00 Check 1.00 < Pass

Auto Select Best Ladder
Allow Match # Variance: 0
Max Average Size Diff: 0.20
 Use Ladder Library
Min Heterozygosity: 0.50
 Auto Panel Adjustment

Mixture Evaluation
Valid Mixture Peak Percentage: 0 %
Min Mixture Marker Number: 3

Sample Quality Sensor
'Q' Allele: _____
'S' Allele: _____

Save Delete << Back Ok Cancel

PPY23 on 3500 (Exemplars)

Run Wizard

Template Selection
Set the template of the project

Select an existing template or create one

- Fusion
- Fusion Exemplars
- Fusion_3500
- Fusion_3500_Exemplars
- Fusion_3500_STRmix
- Fusion_STRmix
- PPY23_3500**
- PPY23_3500_Exemplars**
- Yfiler

Template Name: PPY23_3500_Exemplars
Panel: PPY23_3500_Exemplars
Size Standard: ILS500
Standard Color: Orange

Save Delete << Back Next >> Cancel

Run Wizard

Data Process - HID Analysis
Set data process options

Raw Data Analysis

Auto Range (frame)
Start: 0 End: 10000
 Smooth Enhanced Smooth

Baseline Subtraction:
 Superior Classic Enhanced
 Pull-up Correction Spike Removal
 Saturation Detection Saturation Repair

Allele Call

Auto Range (bps)
Start: 55 End: 510
Max Intensity: 32500
Peak Detection Threshold: ? Dye Specific

| | Min RFU | Min RFU% |
|--------|---------|----------|
| Blue | 60 | 10 |
| Green | 70 | 10 |
| Yellow | 90 | 10 |
| Red | 120 | 10 |
| Orange | 60 | 10 |

Size Call
 Local Southern Cubic Spline

Save Delete << Back Next >> Cancel

Run Wizard

Additional Settings - HID Analysis
Set additional options related to the different analysis type

Allelic Ladder: NONE

P.C. Template 1: PPY23
P.C. Template 2: NONE

Allele Evaluation
Peak Score:
Reject < 0.00 Check 1.00 < Pass

Auto Select Best Ladder
Allow Match # Variance: 0
Max Average Size Diff: 0.20
 Use Ladder Library
Min Heterozygosity: 0.50
 Auto Panel Adjustment

Mixture Evaluation
Valid Mixture Peak Percentage: 0 %
Min Mixture Marker Number: 3

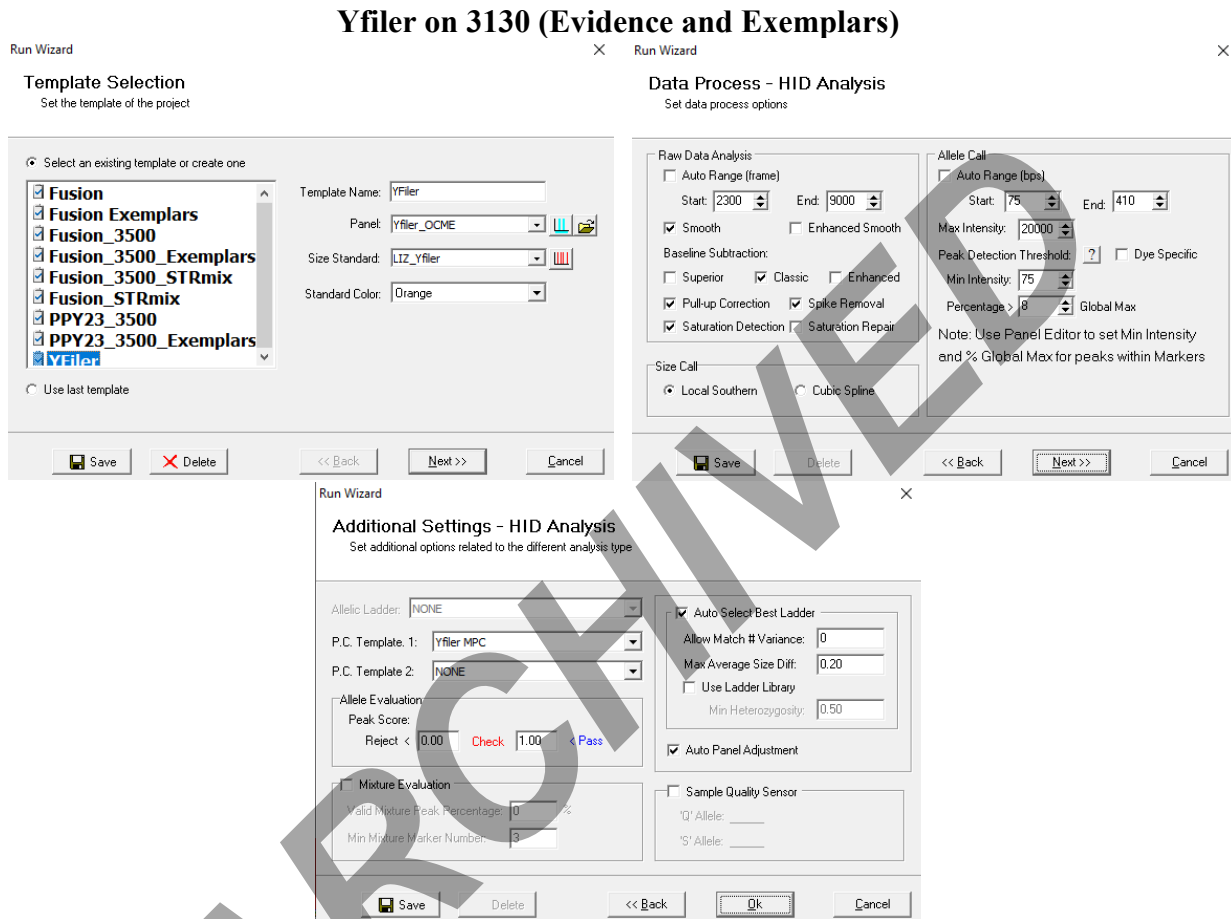
Sample Quality Sensor
'Q' Allele: _____
'S' Allele: _____

Save Delete << Back Ok Cancel

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3.6 **Yfiler on 3130 Run Wizard Screenshots**



3.7 If **NONE** is listed under **P.C. Template 1** in the **Analysis Settings Window**, select the appropriate positive control template using the dropdown:

- Fusion on 3500 or 3130: **PP_Fusion**
- PPY23 on 3500: **PPY23**
- Yfiler on 3130: **Yfiler MPC**

Note: DO NOT click Save on any of the windows in the Run Wizards. This will overwrite the template for all analysts.

3.8 Click **Ok** → Once data has finished processing, click **Ok** again.

3.9 On the top left corner of the screen, click **File** → **Save Project** → navigate to desired folder and save project using the naming system based on the **instrument, date, injection number(s), kit**

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(‘U’ for Fusion, ‘P’ for PPY23, ‘M’ for Yfiler), and **analysis set** (i.e. Carmody090619 8-11U A or Avogadro020321 113P B).

3.9.1 You must save over the existing project every time you save.

4 Checking the Size Standard

- 4.1 The size standard needs to be checked in two locations: in the **Calibration Charts**  and in the Main Analysis Window (or in the **Browse by All Color**  analysis window).
- 4.2 Check the size standard for the **Allelic Ladder** first before checking the positive control, the negative controls, and then the samples. If the size standard of the **Allelic Ladder** fails, replace, and check other ladders in the analysis set. Refer to the [Analysis on 3500s Fusion and PPY23 manual](#) or [STR Results Interpretation – PowerPlex® Fusion & STRmix™](#) for more information on failing ladders and controls.
- 4.2.1 In the case of a **failed ladder**, to switch out the ladder, click **File** from the menu bar → click **Open Data** → highlight the failed ladder from the **Data File List** → click **Remove** → click **Add...** → navigate to the **.hid** files → select a different ladder in the same run for analysis → click **Open** → click **Ok** → return to [Section 2: Load Data](#) above and proceed.
- 4.3 If any sample or control is automatically marked as disabled by the software, review the size standard in the **Calibration Charts**  window to ensure this was due to failing or poor size standard. If it is confirmed that the sample has a failing or poor size standard, a [Rerun Code](#) must be applied to that sample.
- 4.3.1 Right click on the sample in **Sample File Tree** within Main Analysis Window → click **Edit Comments** → enter rerun code in **Comments** field → click **Ok**.
- 4.4 To check the calibration charts, click **Size Calibration**  located on the top toolbar in the main analysis window.
- 4.4.1 In the Calibration Charts window, click Chart Synchronize .
- 4.4.2 Click the **Sample Name** header to sort samples in order. In the **Sample List**, either click sample by sample or navigate using the Up/Down Arrows.
- 4.4.3 If the peak is recognized by the software, a green inverted triangle appears at the top of the peak - . Check the size standards for each sample, starting with the allelic ladder, to ensure each peak is recognized.

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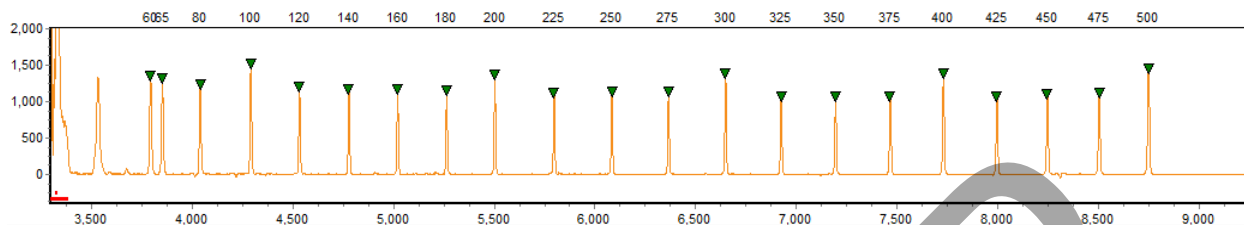
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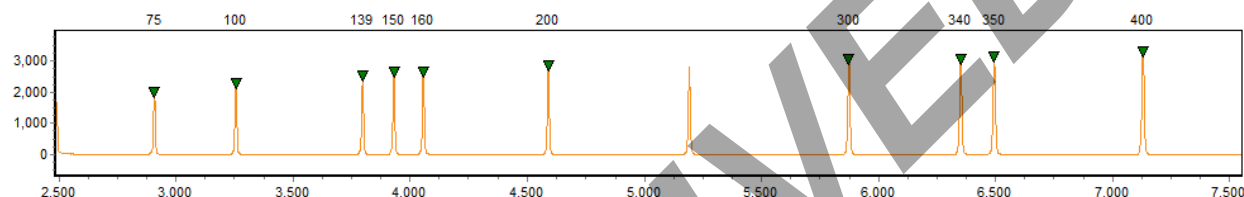
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4.4.4 Fusion and PPY23 ILS



4.4.5 Yfiler ILS




4.4.6 If a sample contains a size standard peak without a green triangle present, the size standard for that sample fails. Refer to the [Analysis on 3500s Fusion and PPY23 manual](#) or [STR Results Interpretation – PowerPlex® Fusion & STRmix™](#) for further information.


4.4.7 Close Calibration Charts window.

4.5 To check the size standard in the second location, in the Main Analysis window, click on the Down Arrow next to the **Show Color**  icon located in the top toolbar. → click **Hide All** → click the down arrow next to the **Show Color**  icon again → click **ORANGE**.

4.5.1 In the **Sample File Tree**, Right click → click **Select Max**.

4.5.2 Adjust axis to view size standard by selecting the **Set Axis** icon  on the top toolbar.

4.5.3 Click Fixed X axis → enter X axis range 55-510 for Fusion/PPY23 or 75-410 for Yfiler.

4.5.4 Click **Set Axis**  again → Select **Auto Fit Y**.

4.5.5 Review size fragments by ensuring that all required peaks are labeled within +/- **0.5bp** of the fragment size. See 4.5.6 and 4.5.7 below for comparison. (Screenshots can also be viewed in the [Fusion Ladder, PE and SS appendix](#), the [References - Allelic Ladders, Controls, and Size Standards](#), or the [Manual Appendix for PowerPlex® Y23](#) documents).

4.5.5.1 If a peak is not labeled or not within +/-0.5bp, then the size standard fails. Refer to the [Analysis on 3500s Fusion and PPY23 manual](#) or [STR Results Interpretation – PowerPlex® Fusion & STRmix™](#) for more information on failing ladders and controls.

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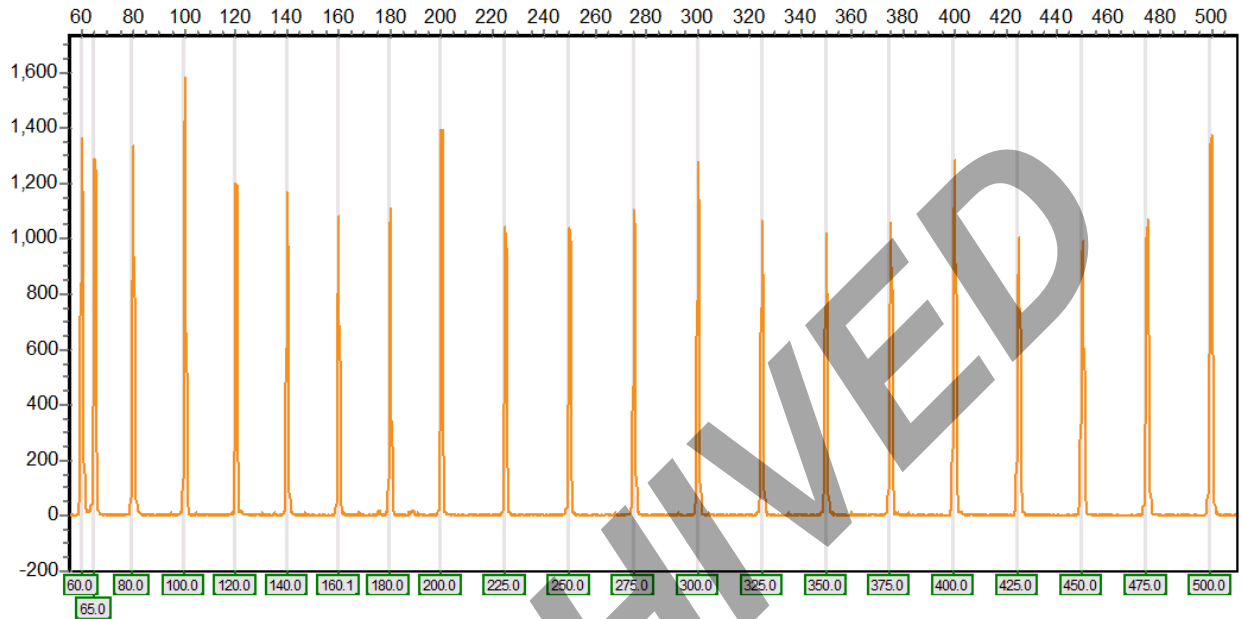
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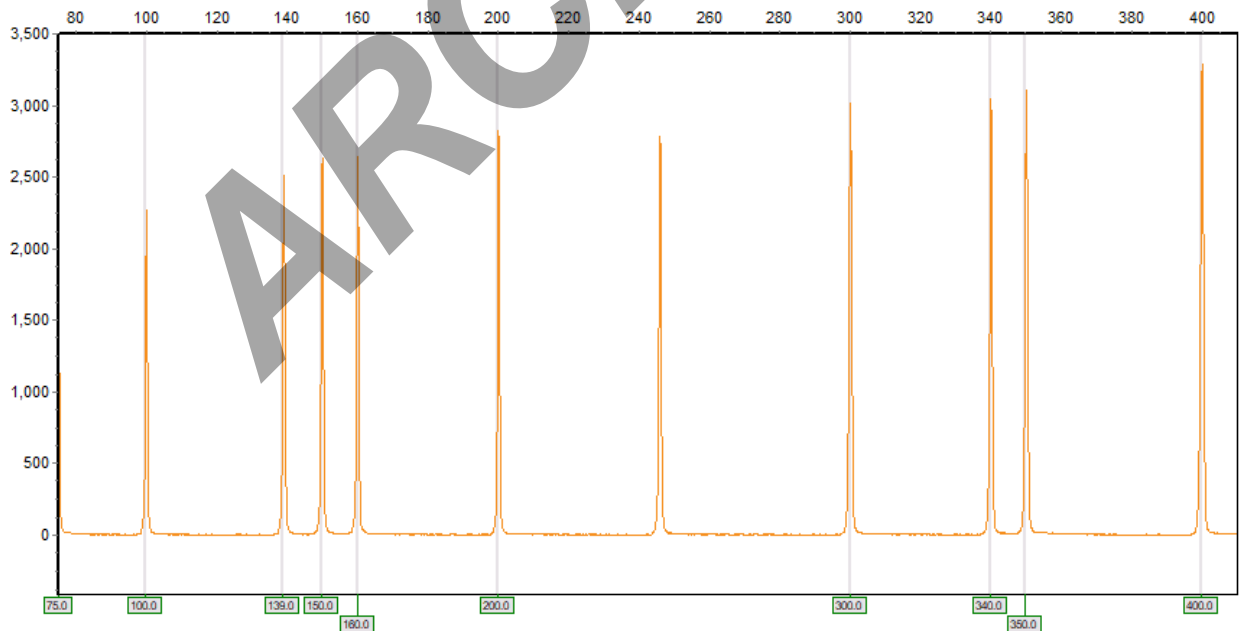
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4.5.6 Fusion and PPY23 size standard.



4.5.7 Yfiler size standard.




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4.5.8 If any sample with a failing or poor size standard has peaks called, the peak labels on peaks in all other channels (blue, green, yellow, and red) will need to be deleted.

4.5.8.1 Open the **Browse by All Color**  analysis window → navigate to the relevant sample(s) → highlight all peaks in one dye by holding down Ctrl + Left click and dragging around any peaks present in that dye → click **Delete** to remove the peak labels → **repeat for each dye**.

4.6 For any sample with a failing or poor size standard, right click on the sample in the **Sample File Tree** within the Main Analysis Window → click **Edit Comments** → enter [Rerun Code](#) in **Comments** field → click **Ok**.

4.7 Oversaturated samples can also cause the size standard to fail. In this case, the sample should be diluted for rerun if possible. If it is a Fusion Direct exemplar, it should be scheduled for extraction and quantitation.


4.7.1 Refer to the [Analysis on 3500s Fusion and PPY23 manual or STR Results Interpretation – PowerPlex® Fusion & STRmix™](#) for what constitutes an oversaturated sample.

5 Checking the Allelic Ladder

5.1 There are two areas in the Main Analysis window that indicate if the allelic ladder used for analysis has passed.

5.1.1 In the **Sample File Tree**, a red question mark next to the allelic ladder would indicate a potentially failed ladder.

5.1.2 In the **Project Summary**, a ladder error would indicate a potential failed ladder.

5.2 To visually confirm expected alleles in the ladder, right click in the **Sample File Tree** → click **Select Max** → open the **Browse by All Color** icon . See below ladders for comparison. (Screenshots can also be viewed in the [Fusion Ladder, PE and SS appendix](#), the [References - Allelic Ladders, Controls, and Size Standards](#), or the [Manual Appendix for PowerPlex Y23](#) documents.)

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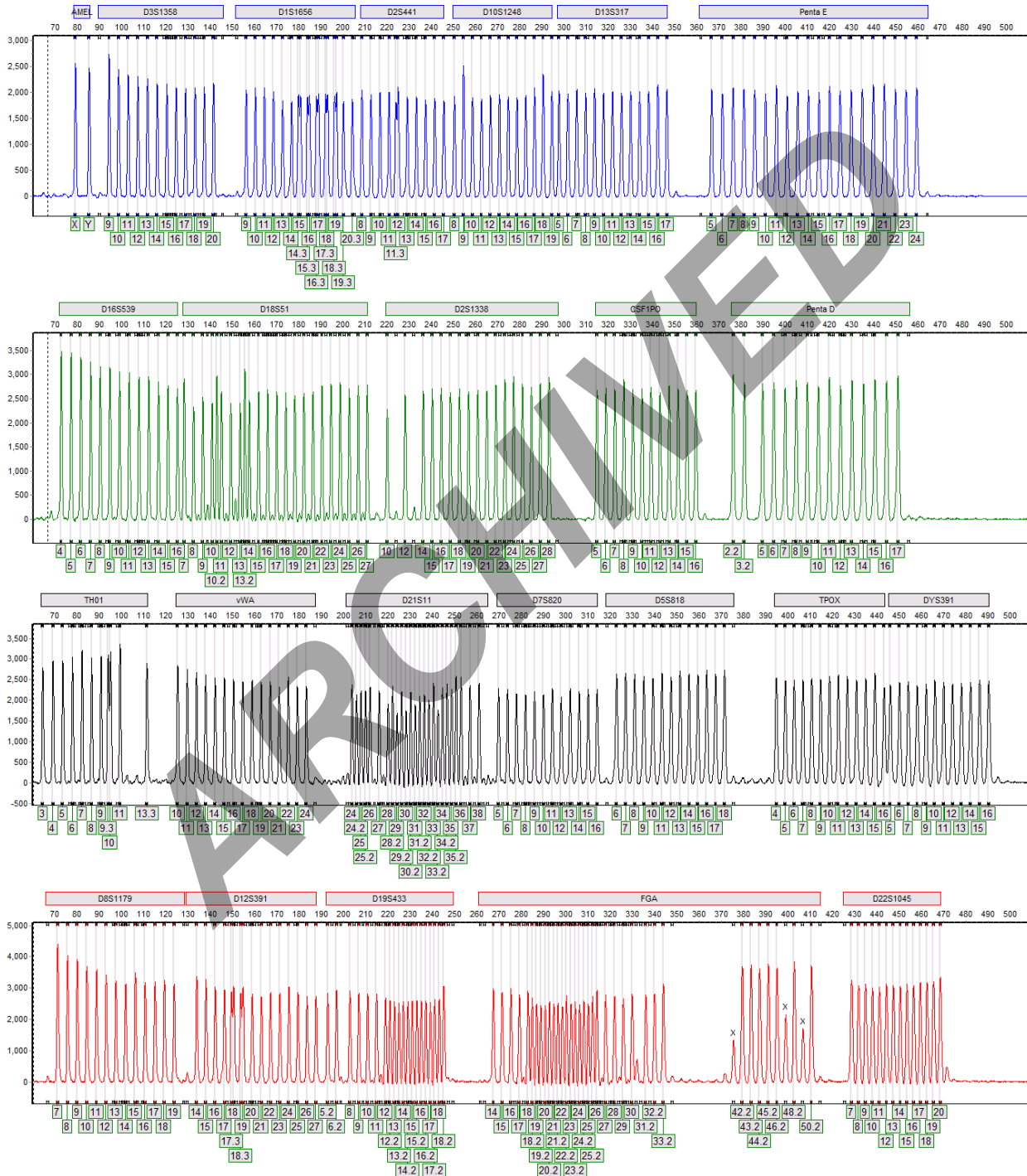
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5.2.1 Fusion Ladder



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5.2.2 PPY23 Ladder



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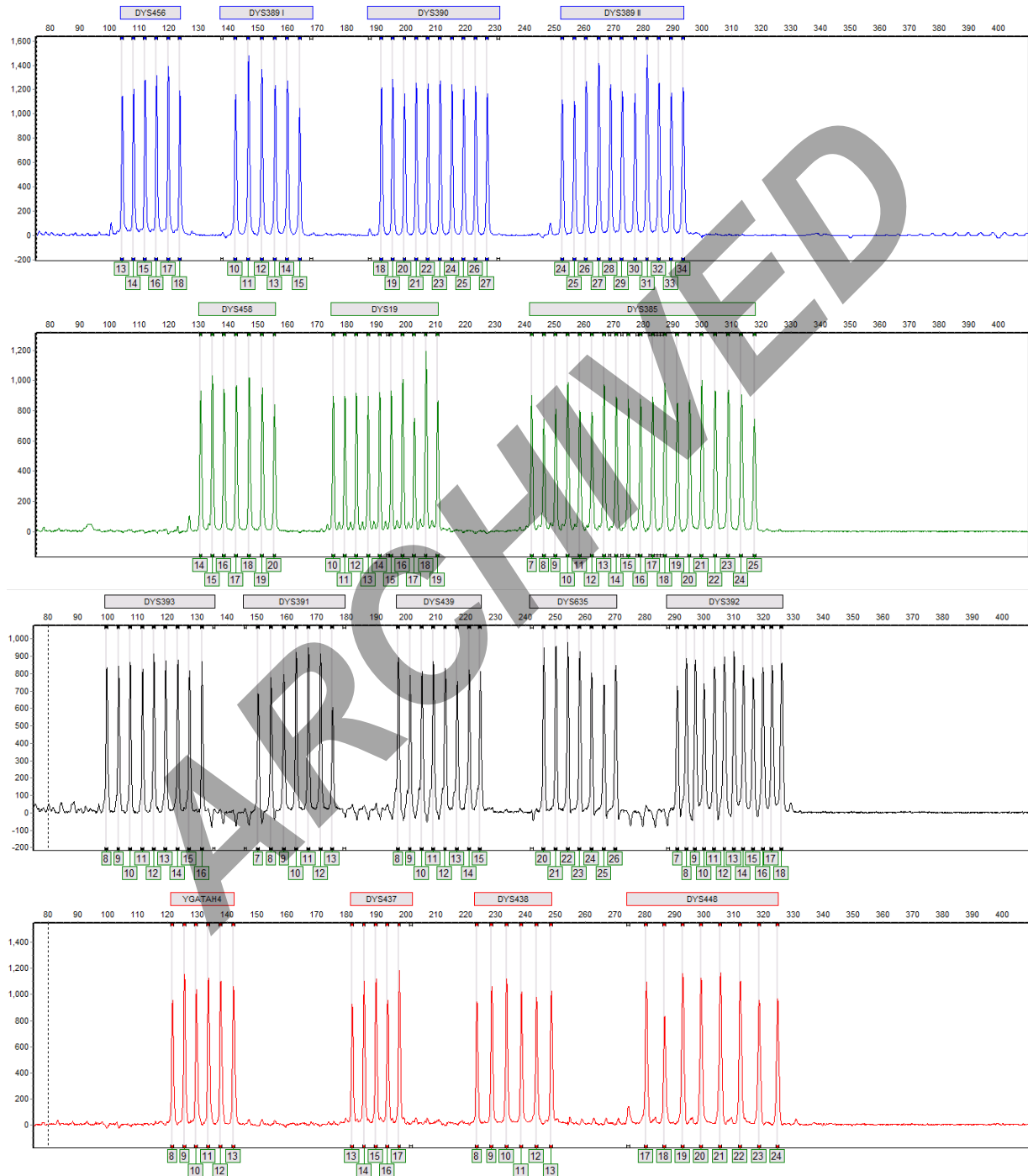
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5.2.3 Yfiler Ladder



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5.3 If the ladder used for the initial analysis failed for presence of unexpected alleles (see Section 4 for size standard-related failures) and there is another ladder in the analysis set that could be used, switch out the ladder for analysis.

5.3.1 Click **File** from the menu bar → click **Open Data** → highlight the failed ladder from the **Data File** List → click **Remove** → click **Add...** → navigate to the **.hid/.fsa** files → Select a different ladder for analysis → click **Open** → click **Ok** → return to Section 2: Load Data and proceed.

5.4 If there are no other passing allelic ladders in the analysis set refer to the [Analysis on 3500s Fusion and PPY23 Manual](#) or [STR Results Interpretation – PowerPlex® Fusion & STRmix™](#) for Electrophoresis Run Failures.


6 Checking the Positive Control

6.1 For any STR plate, there must be at least one amplification positive control present.

6.2 The amplification positive control may be run at a different dilution than the corresponding samples and the amplification set can pass.

6.3 A positive control may be edited for amplification or electrophoresis artifacts (see [Analysis on 3500s Fusion and PPY23](#) or [STR Results Interpretation – PowerPlex® Fusion & STRmix™](#)).

6.4 Analysis of the data can be performed in the Main Analysis window and/or the **Browse by All Color**  window.

6.5 Adjust the axis to view size standard depending on amplification kit by selecting the **Set Axis** icon  on the top toolbar.

6.5.1 Click **Fixed X** → enter X axis range

- **Fusion:** 60-510
- **PPY23:** 65-450
- **Yfiler:** 90-340

6.5.2 Click **Set Axis** again → select **Auto Fit Y**

6.6 Visually confirm all expected alleles in the positive control according to the amplification kit used below. (Screenshots can also be viewed in the [Fusion Ladder, PE and SS appendix](#), the [References - Allelic Ladders, Controls, and Size Standards](#), or the [Manual Appendix for PowerPlex Y23](#) documents).

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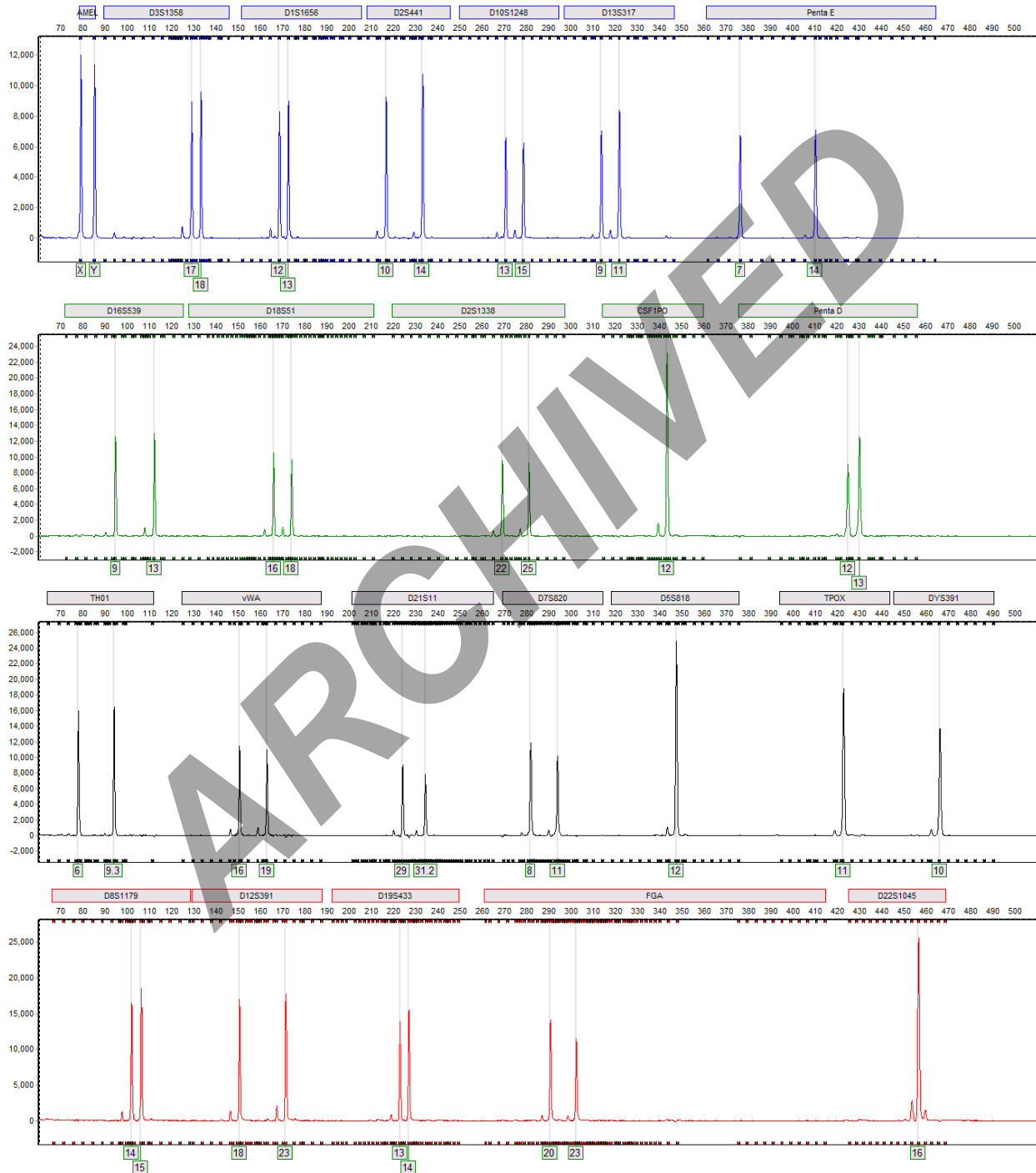
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6.6.1 Fusion Positive Control



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6.6.2 PPY23 Positive Control



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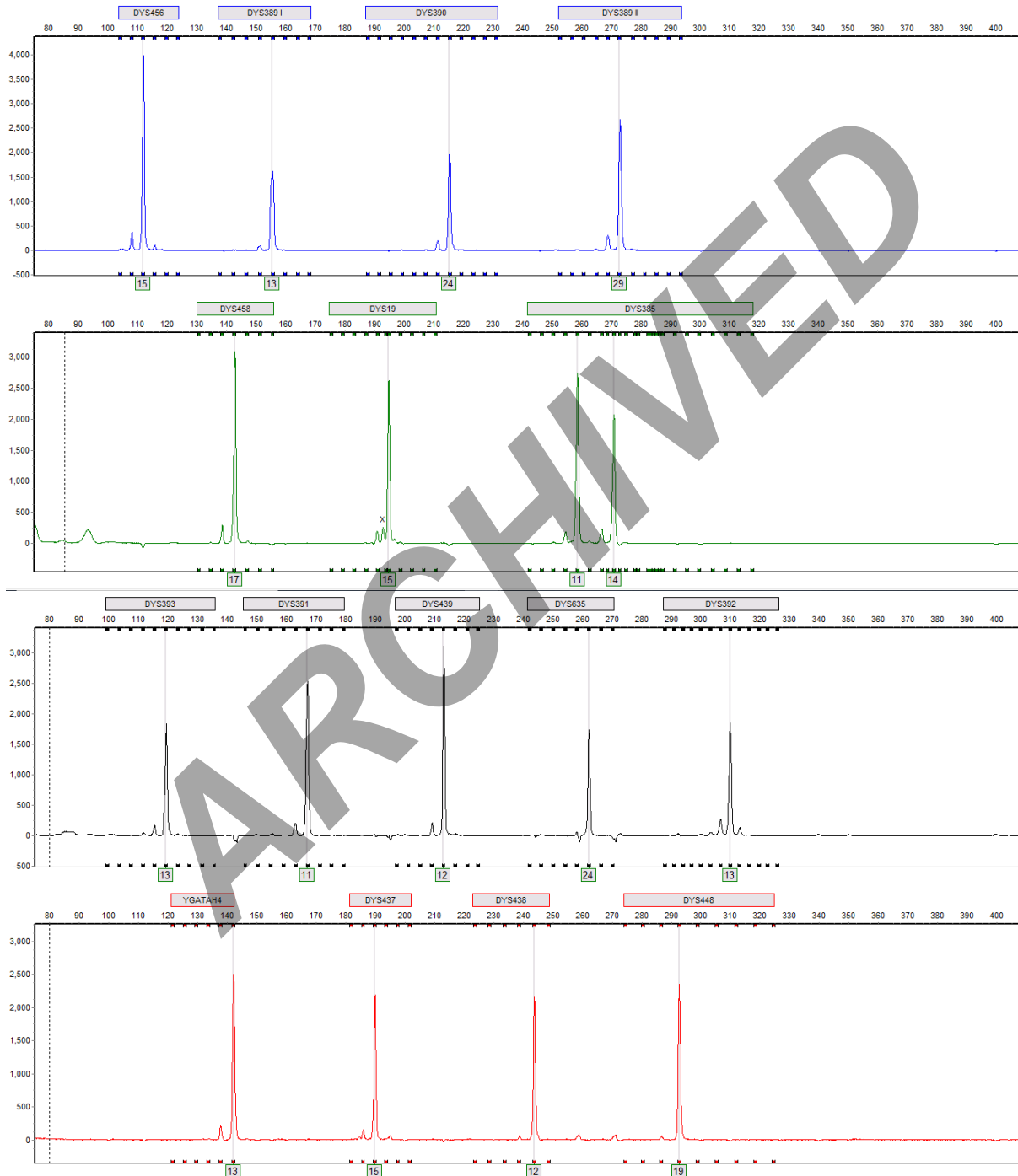
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6.6.3 Yfiler Positive Control



6.7 A positive control that does not generate a complete genotype or gives an incorrect

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genotype will be indicated as failing. For analysis, editing, evaluating, and retesting guidelines refer to the [Analysis on 3500s Fusion and PPY23](#) manual and the [STR Results Interpretation - PowerPlex® Fusion & STRmix™](#) manual.

7 Checking Negative Controls

- 7.1 Evaluate the extraction and/or amplification negative control for expected results. If peaks attributed to DNA are detected, refer to the [Analysis on 3500s Fusion and PPY23](#) manual and [STR Results Interpretation - PowerPlex® Fusion & STRmix™](#) manual.
- 7.2 Some artifacts can be edited in a negative control if they are by-products of the STR/amplification products. Refer to the [Analysis on 3500s Fusion and PPY23](#) or [STR Results Interpretation – PowerPlex® Fusion & STRmix™](#) manual.
- 7.3 If a negative control with no peaks present contains misshapen size standard peaks that are correctly called, the control passes.

8 Analyzing Samples

- 8.1 Analysis of the data can be performed in the Main Analysis window and/or the **Browse by All Color**  window.
- 8.2 Adjust the axis to view size standard depending on amplification kit by selecting the **Set Axis** icon  on the top toolbar.
 - 8.2.1 Click **Fixed X** → enter X axis range
 - **Fusion:** 60-510
 - **PPY23:** 65-450
 - **Yfiler:** 90-340
 - 8.2.2 Click **Set Axis** again → select **Auto Fit Y**

9 Editing a Peak

- 9.1 If a labeled peak is determined to be an artifact, click on the peak to highlight it → right click → click **Edit Comments** → Enter code 'a' for artifact (refer to [Analysis on 3500s Fusion and PPY23](#) and [STR Results Interpretation - PowerPlex® Fusion & STRmix™](#)) → click **Ok**.
- 9.2 Once the edit is recorded, highlight the peak and press delete to remove the label from the peak.
 - 9.2.1 To select multiple peaks for deleting, hold the Ctrl key + Left mouse button → Drag the mouse to highlight all consecutive peaks.

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- 9.3 If a label has been removed from a peak and needs to be restored, click on the peak to highlight it → right click → **Undelete**.
- 9.4 If an edit needs to be removed from a peak → right click on peak → click **Edit Comments** → remove text from **Comments** box so the text field is blank → click **Ok** (the 'E' will still be visible but the edit comment will be removed).
- 9.5 To check peak history → right click on peak → click **View History**.
- 9.5.1 Any changes made to the peak will be listed in order from most recent down. Click on any line to view specific change details in the Current/Old Values window. Scroll all the way to the right to view the Edit Code applied to the peak in Allele Comments column.

10 Oversaturation

- 10.1 Check for saturation detection. Software indicators of saturation will be in the following locations:
- 10.1.1 In the Main Analysis Window, a red line under one or more peaks present in an electropherogram will indicate the possible saturation of said peak(s). This red line will be seen in the same area along the x-axis for all dye lanes of the electropherogram.
- 10.1.2 In the **Peak Table**, **<SAT>** will be in the **Allele Comments** field and/or **SD** will be in the **Quality Reasons** field for said peak(s).
- 10.2 Refer to the [Analysis on 3500s Fusion and PPY23](#) or [STR Results Interpretation – PowerPlex® Fusion & STRmix™](#) manual for more information on saturation limits and how to handle oversaturated samples.

11 Scheduling Re-Runs

- 11.1 If a sample failed (over-saturation, bad size standard, no data, instrument issue, etc.) → right click on the Sample in the **Sample Tree** within the Main Analysis Window → click **Edit Comments** → type [Re-Run Code](#) in the **Comments** box → click **Ok**.
- 11.1.1 [Re-run Codes](#) cover the majority of, but not all, situations. If one does not exist which specifically describes the issue, please enter a reason in LIMS under the sample status column as to why the sample failed– ex. possible injection issue.
- 11.1.2 Fusion Direct plates should not be realiquoted. Fusion Direct samples should be reinjected, reamplified, reextracted, or recut if a rerun is necessary.
- 11.1.3 If a sample failed, **check that the sample has not already been rerun before scheduling it for rerun again**. If it has already been rerun, check for reasons it may have failed and consider options other than reinjecting or re-aliquoting such as reamplifying or allowing the

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reporting analyst of the case to make a case-specific decision.

11.2 All peak labels must be removed from failed samples.

11.2.1 Open the **Browse by All Color** analysis window → navigate to the relevant sample(s) → highlight all peaks in one dye by holding down Ctrl + Left mouse button and dragging around any peaks present in that dye → click **Delete** to remove the peak labels → repeat for each dye.

11.3 If a sample did not fail but re-run is desired, do not delete the peak labels → right click on the Sample in the **Sample Tree** within the Main Analysis Window → click **Edit Comments** → type **Re-Run Code** in the **Comments** box → click **Ok**.

12 Exporting for LIMS (All Systems and Sample Types)

12.1 Once the entire run has been reviewed, all edits have been made, and labels have been removed from all edited peaks, on the top left corner of the screen, click **File** → click **Save Project**.

12.2 You must save over the existing project every time you save.


12.3 In the Main Analysis Window → click the Down Arrow next to the **Show All Color** icon  → click **Show All** to turn on all colors before exporting.

12.4 In the report display section of the main analysis screen, click on the down arrow next to the Save Report icon . Ensure that **Save LIMS report** is selected.

12.5 In the LIMS report settings window that pops up, ensure the settings are as follows:

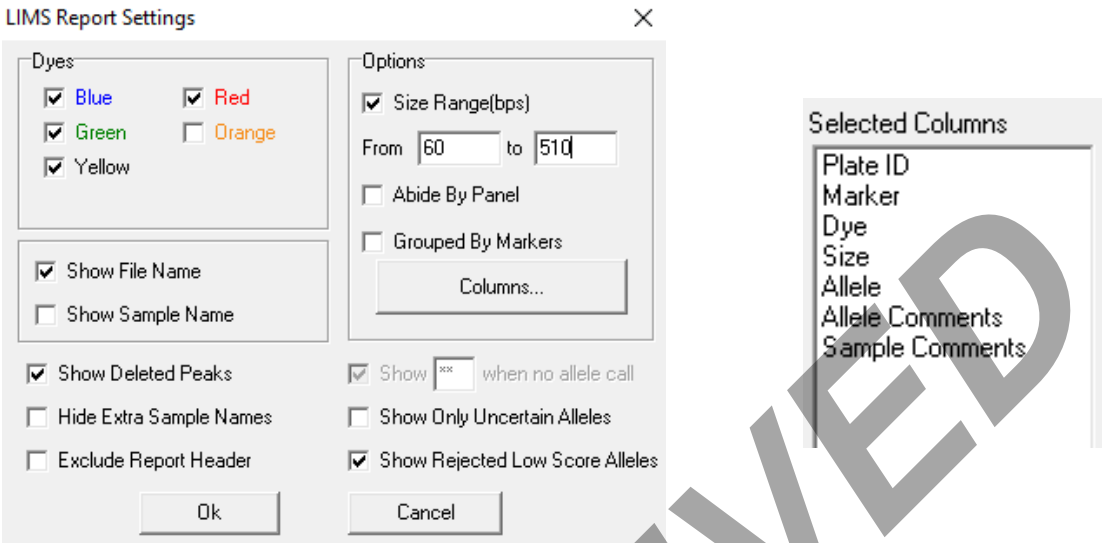
12.5.1 The **Size Range** will be the same as the X-axis range for analysis of samples:

- **Fusion:** 60-510
- **PPY23:** 65-450
- **Yfiler:** 90-340

12.5.2 Under **Options**, click  → use the **Add/Remove** buttons to ensure the Selected Columns are present and listed in the order that follows. **This is crucial to proper LIMS importation.**

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- 12.6 Click **Ok** → click **Ok** again in the **Allele Report Settings** window.
- 12.7 Save the file as type “Excel File (*.xlsx;*.xls)” under the appropriate run name (delete the suffix “_AlleleReport”).
- 12.8 Prior to import into LIMS, the **Plate ID** needs to be added to the excel file created.
 - 12.8.1 Navigate to the appropriate folder → open the saved excel file → under **Plate ID** type in the plate name (i.e. Avogadro021519 19-21 NOT Avogadro021519 19-21*UA*) and **fill down for all samples** → click **Save**.
 - 12.8.2 **NOTE:** This is necessary for import into LIMS. Be careful not to change anything else in the file.
- 12.9 Navigate to the data entry screen for the corresponding test batch in LIMS → click **Import Instrument Data** → import the excel file for the project. Refer to the [Test Batch Analysis, Review and Approval](#) manual for importing instructions.
- 12.10 Fill out the data entry screen keeping in mind the following definitions:
 - **Loaded** – the data from GeneMarker® has been imported
 - **Not Loaded** –the sample or control was not imported/loaded into GeneMarker®
 - **Pass** – the control has passed
 - **Fail** – the control has not passed
 - **No Data** – the control has not been analyzed (for example, if the run fails due to a failed ladder, the remaining controls cannot be analyzed)
 - **Not Used** – the control was not used in analysis of the STR run, typically reserved for additional allelic ladders PPY23: 65-450

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12.11 If a control needs to be rerun, a comment (reason as to why the sample failed) and resolution must be entered into the **STRReRun** column in LIMS. See Section 11: Scheduling Re-Runs.

13 Printing Initial Analysis Electropherograms (All Systems and Sample Types)

13.1 In the **Sample File Tree** → right click → select **Max**.

13.2 Double-click to **DESELECT** the failed samples that are scheduled for rerun due to “no or poor size standard”. The .pdf of the electropherogram is not necessary since the sample status is indicated on the rerun table.

13.2.1 Include all samples that failed for reasons other than “no or poor size standard.” The .pdfs of the electropherograms with all peak labels removed are included within the casefile.

13.3 Click **View** → **Preferences** → **Others** tab → ensure box for **Enable Sample Grouping** is checked → click **Ok**.

13.4 In Main Analysis Window click **Project** → **Apply Sample Grouping** (if grayed out return to step 13.1) → click **Group By Order** on the bottom left → change **Group Size to 1** → click **Match** on the bottom right → click **Ok**.


13.5 In the main analysis window → click **Set Axis** → click **Fixed X axis**. The Size Range will be the same as the X- axis range for analysis:

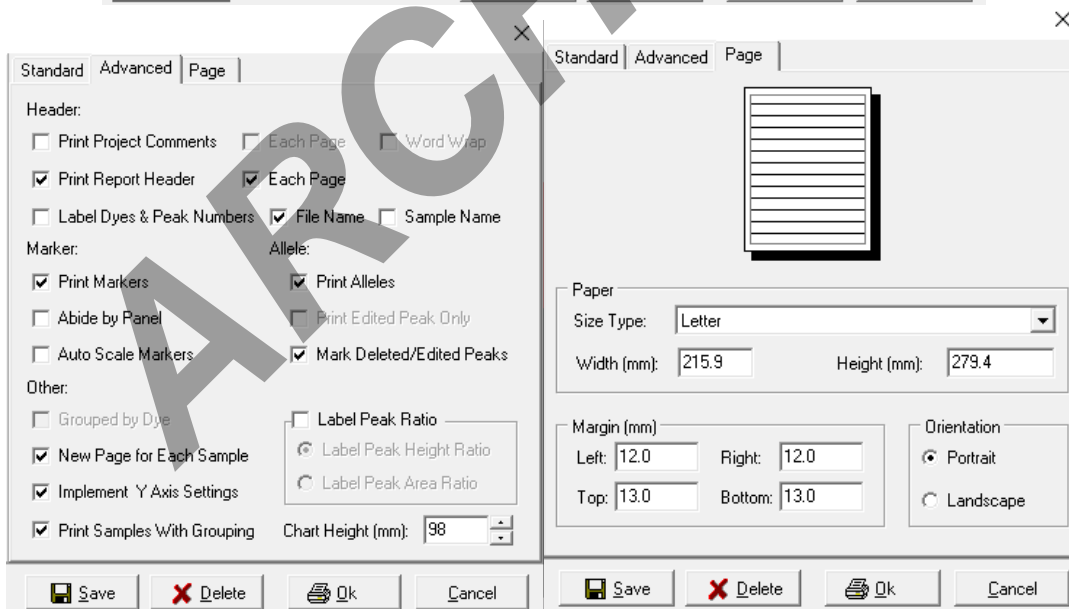
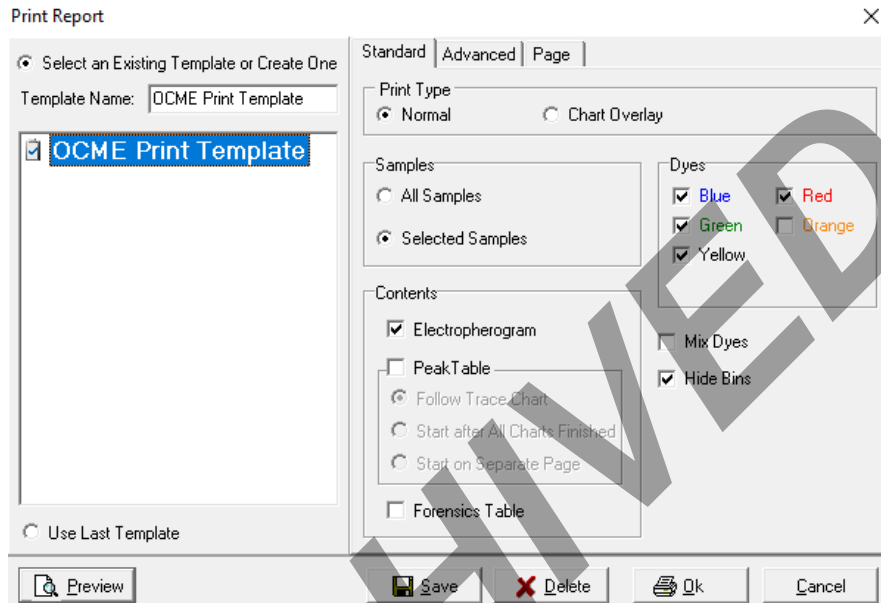
- **Fusion:** 60-510
- **PPY23:** 65-450
- **Yfiler:** 90-340

13.6 Click **Set Axis** again → Select **Auto Fit Y**.

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- 13.7 In the Main Analysis window → click **Print Icon**  → Select **OCME Print Template** on left and ensure the settings match below in each of the three tabs (Standard, Advanced, Page):

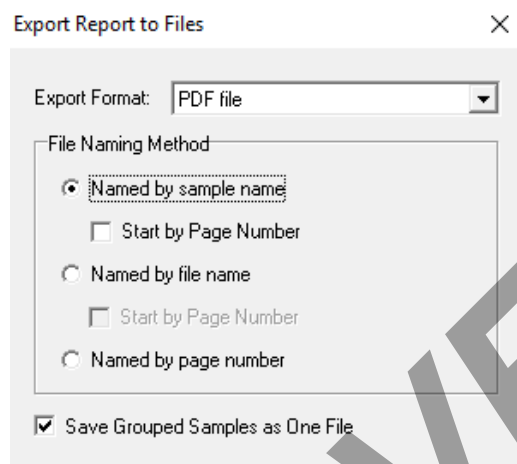


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13.8 Click **Preview** → click **Export to File** icon . Ensure settings match below:





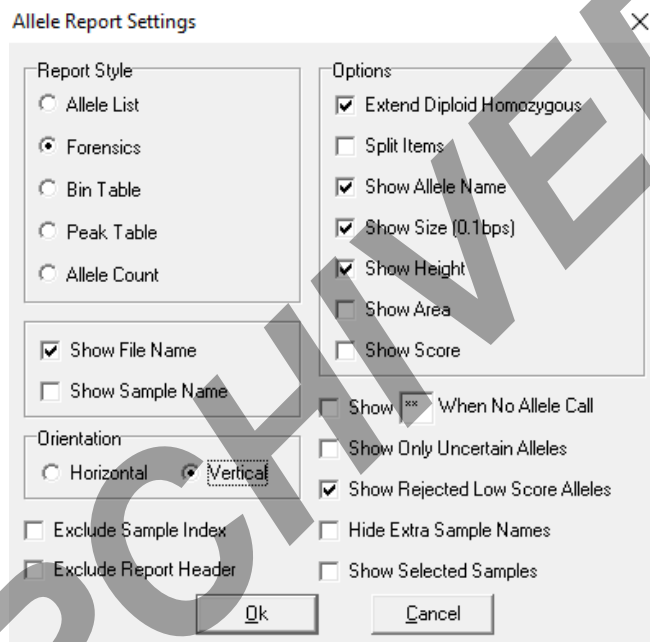
- 13.9 Click on the “...” icon to choose the appropriate export directory for the PDF files → click **Ok** → the **Export Report to PDFs Events** window will appear → Once complete, click **Ok**.
- 13.10 If analyzing **Fusion Evidence**, wait to import all PDFs into LIMS until after the completion of STRmix analysis in Section 17.
- 13.11 If analyzing exemplars or **PPY23/Yfiler evidence** → Import all PDFs into LIMS.


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14 Fusion Exemplars Only - Exporting EXEMPLAR Table for STRmix™ Input (for Evidence Table Instructions see Section 17)

- 14.1 In the Main Analysis Window click the **Show All Color**  icon → select **Show All** → click on the report settings icon  (report display section of the main analysis screen) → ensure the settings match below:




- 14.2 Click **Ok** → click on the Down Arrow next to the **Save Report** icon  (in the report display section of the main analysis screen) → ensure that **Save Report** is selected.
- 14.3 Navigate to the appropriate folder and save file as a .csv file named as **RunName_SM** (i.e. Pavlov090619 1-3U A_SM).
- 14.4 For proper STRmix™ import, the GeneMarker® to STRmix™ macro needs to be run:
- 14.4.1 Open the exported project excel file from 14.3
 - 14.4.2 Open [GeneMarker® to STRmix™ macro](#) → follow the “instructions” tab.
 - 14.4.3 After the macro has run click **Save As** within the GeneMarker® to STRmix™ macro file.
 - 14.4.4 Change the file type to “.txt” (text, tab delimited).
 - 14.4.5 Navigate to the folder containing your project data and save the file there.

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15 Fusion Evidence Only - STRmix™ Analysis (Stutter Filters Off)

- 15.1 Begin by importing the data from the initial analysis into LIMS to generate the edit sheet. Have the edit sheet open in LIMS as you perform the second analysis with stutter filters off for STRmix™ to refer to and ensure the same edits are being made.
- 15.2 If the project is still open → click **File** → click **Save Project** → Save as a new project with suffix “_SM”.
- 15.3 If project is not open → click **File** → **Open Project** → navigate to run → select project → click **Open** → click **File** → click **Save Project** → Save as a new project with suffix “_SM”.
- 15.4 Click **Run Project**  located in the top toolbar.
- 15.5 In the **Template Selection** window on the left side, select one of the following according to the CE instrument:
 - **3500 Data:** Fusion_3500_STRmix
 - **3130 Data:** Fusion_STRmix

Note: Do NOT click Save on any of the following windows in the Run Wizard. This will overwrite the template for all analysts.

- 15.6 Check the right side of the **Template Selection** window against the corresponding screenshots below. Select **Next** twice to move through the **Data Process** and **Analysis Settings** windows and check all settings against the corresponding screenshots.

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15.7 Fusion evidence STRmix™ Run Wizard Screenshots:

Fusion on 3500 (Evidence for STRmix™ Import)

Run Wizard

Template Selection
Set the template of the project

Select an existing template or create one

- Fusion
- Fusion Exemplars
- Fusion_3500
- Fusion_3500_Exemplars
- Fusion_3500_STRmix
- Fusion_STRmix
- PPY23_3500
- PPY23_3500_Exemplars
- VFile

Use last template

Template Name: Fusion_3500_STRmix
Panel: Fusion_3500_STRmix
Size Standard: IL5500
Standard Color: Orange

Save Delete << Back Next >> Cancel

Run Wizard

Data Process - HID Analysis
Set data process options

Raw Data Analysis

Auto Range (frame)
Start: 0 End: 10000
 Smooth Enhanced Smooth

Baseline Subtraction:
 Superior Classic Enhanced
 Pull-up Correction Spike Removal
 Saturation Detection Saturation Repair

Size Call
 Local Southern Cubic Spline

Allele Call
 Auto Range (bps)
Start: 55 End: 510
Max Intensity: 32500
Peak Detection Threshold: ? Dye Specific

| | Min RFU | Min RFU% |
|--------|---------|----------|
| Blue | 85 | 0 |
| Green | 120 | 0 |
| Yellow | 130 | 0 |
| Red | 160 | 0 |
| Orange | 85 | 0 |

Save Delete << Back Next >> Cancel

Run Wizard

Additional Settings - HID Analysis
Set additional options related to the different analysis type

Allelic Ladder: NONE
P.C. Template 1: PP_Fusion
P.C. Template 2: NONE

Allele Evaluation
Peak Score: Reject < 0.00 Check 1.00 < Pass

Mixture Evaluation
Valid Mixture Peak Percentage: 0 %
Min Mixture Marker Number: 3

Auto Select Best Ladder
Allow Match # Variance: 0
Max Average Size Diff: 0.20
 Use Ladder Library
Min Heterozygosity: 0.50
 Auto Panel Adjustment

Sample Quality Sensor
'Q' Allele: _____
'S' Allele: _____

Save Delete << Back Ok Cancel

Fusion on 3130 (Evidence for STRmix™ Import)

Run Wizard

Template Selection
Set the template of the project

Select an existing template or create one

- Fusion
- Fusion Exemplars
- Fusion_3500
- Fusion_3500_Exemplars
- Fusion_3500_STRmix
- Fusion_STRmix
- PPY23_3500
- PPY23_3500_Exemplars
- VFile

Use last template

Template Name: Fusion_STRmix
Panel: Fusion_OCME_STRmix_Not
Size Standard: IL5500
Standard Color: Orange

Save Delete << Back Next >> Cancel

Run Wizard

Data Process - HID Analysis
Set data process options

Raw Data Analysis

Auto Range (frame)
Start: 0 End: 10000
 Smooth Enhanced Smooth

Baseline Subtraction:
 Superior Classic Enhanced
 Pull-up Correction Spike Removal
 Saturation Detection Saturation Repair

Size Call
 Local Southern Cubic Spline

Allele Call
 Auto Range (bps)
Start: 55 End: 510
Max Intensity: 32500
Peak Detection Threshold: ? Dye Specific

Min Intensity: 50
Percentage > 0 Global Max

Note: Use Panel Editor to set Min Intensity and % Global Max for peaks within Markers

Save Delete << Back Next >> Cancel

Run Wizard

Additional Settings - HID Analysis
Set additional options related to the different analysis type

Allelic Ladder: NONE
P.C. Template 1: PP_Fusion
P.C. Template 2: NONE

Allele Evaluation
Peak Score: Reject < 0.00 Check 1.00 < Pass

Mixture Evaluation
Valid Mixture Peak Percentage: 0 %
Min Mixture Marker Number: 3

Auto Select Best Ladder
Allow Match # Variance: 0
Max Average Size Diff: 0.20
 Use Ladder Library
Min Heterozygosity: 0.50
 Auto Panel Adjustment

Sample Quality Sensor
'Q' Allele: _____
'S' Allele: _____

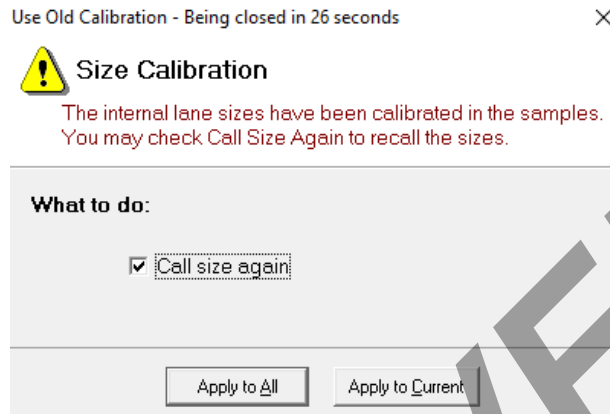
Save Delete << Back Ok Cancel

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15.8 When you click **Ok** after the template wizard, a window will pop up:



15.9 Check **Call size again** → click **Apply to All** → Allow the data to process → click **Ok**.

15.10 Perform a second analysis of this run:

15.10.1 Do not recheck or edit Ladder, Size Standard, or Controls. Passing controls and size standard are determined by the initial analysis.

15.10.2 Leave all controls and failed samples **ENABLED**.

15.10.3 Remove the labels from the same peaks that were edited in the initial analysis. It is NOT necessary to enter edit codes again.

15.10.4 If an artifact (excluding stutter modeled by STRmix™) was previously filtered out in the original analysis (ex. a pull-up that was adjacent to a stutter bin) but is present in the STRmix™ analysis, the label must be removed from this analysis set.

15.10.4.1 This edit must be recorded directly into the LIMS data entry screen.

15.10.5 If a sample had a rerun code during the initial analysis, it is NOT necessary to enter the rerun code again.

15.11 Once the entire run has been reviewed, and labels have been removed from all artifact peaks, click **File** → click **Save Project**.

15.12 You must save over the existing project every time you save.

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16 Fusion Evidence Only - Printing Electropherograms after STRmix™ Analysis (EVIDENCE Samples Only)

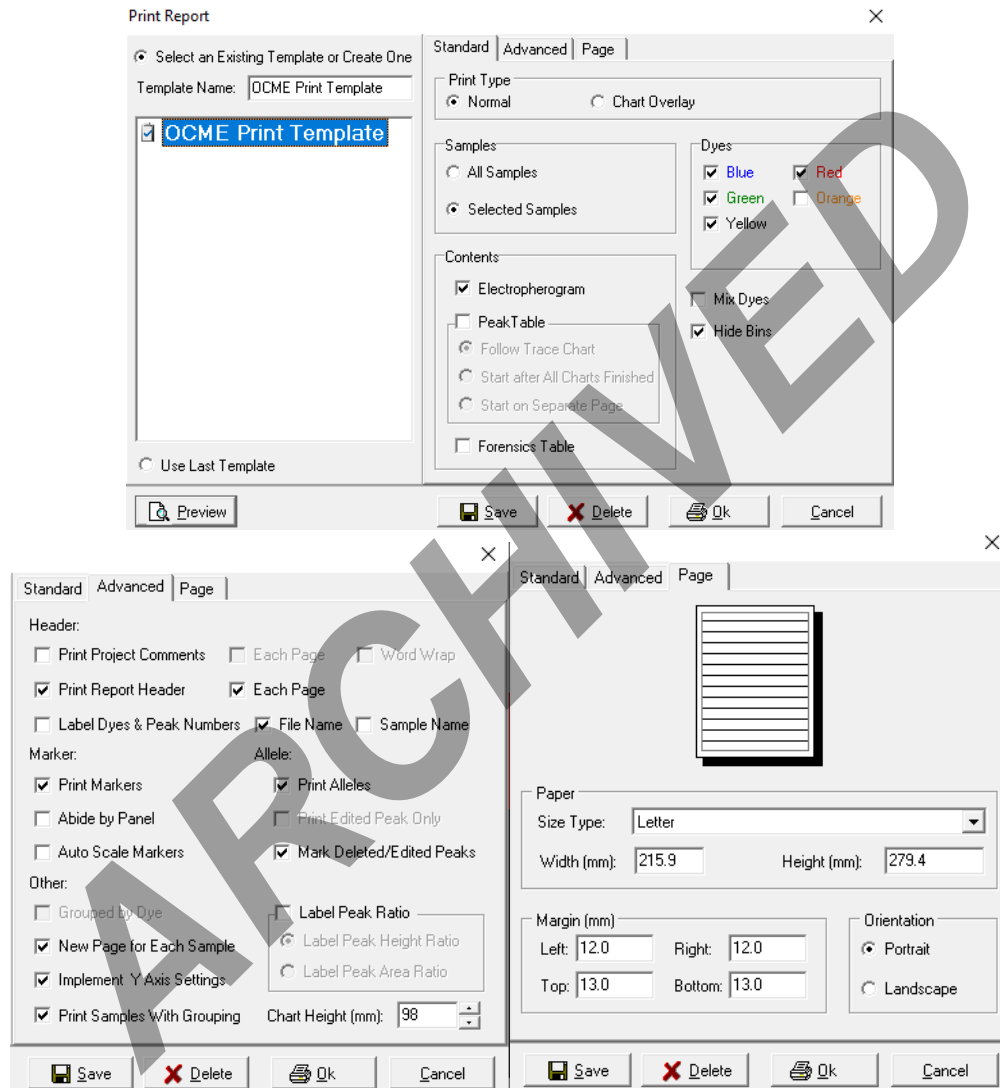
Note: If printing or exporting a PDF of an electropherogram from a run that was analyzed in GeneMarker® v 2.8.2, please refer to section 18.

- 16.1 In the **Sample File Tree** → right click → select **Max**
- 16.2 Double click on all controls (Ladder, PC, NC, Eneg, Mneg) in order to **DESELECT**.
- 16.3 Right-click on any failed sample (for poor size standard or otherwise) and choose **DISABLE**.
- 16.4 Click **View** → **Preferences** → **Others** tab → ensure box for **Enable Sample Grouping** is checked → click **Ok**.
- 16.5 In Main Analysis Window click **Project** → **Apply Sample Grouping** (if grayed out return to step 13.1) → click **Group By Order** on the bottom left → change **Group Size to 1** → click **Match** on the bottom right → click **Ok**.
- 16.6 In the main analysis window → click **Set Axis** → click **Fixed X axis**. The Size Range will be the same as the X-axis range for analysis:
 - **Fusion: 60-510**
- 16.7 Click **Set Axis** again → Select **Auto Fit Y**.

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- 16.8 In the Main Analysis window → click **Print Icon**  → Select **OCME Print Template** on left and ensure the settings match below in each of the three tabs (Standard, Advanced, Page):

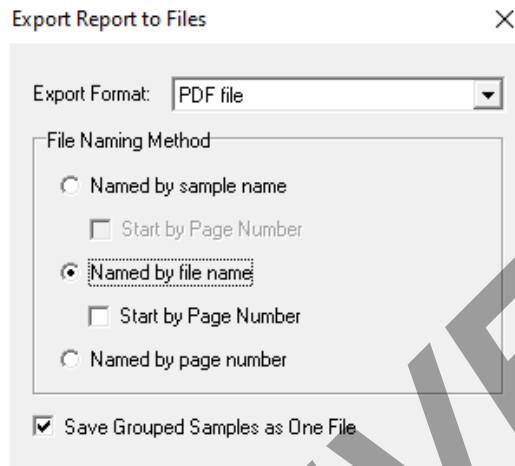


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16.9 Click **Preview** → click **Export to File** icon . Ensure settings match below:



Export Report to Files

Export Format: PDF file

File Naming Method

- Named by sample name
 - Start by Page Number
- Named by file name
 - Start by Page Number
- Named by page number

Save Grouped Samples as One File



16.10 Click on the “...” icon to choose the appropriate export directory for the PDF files → click **Ok** → the **Export Report to PDFs Events** window will appear → once complete, click **Ok**.

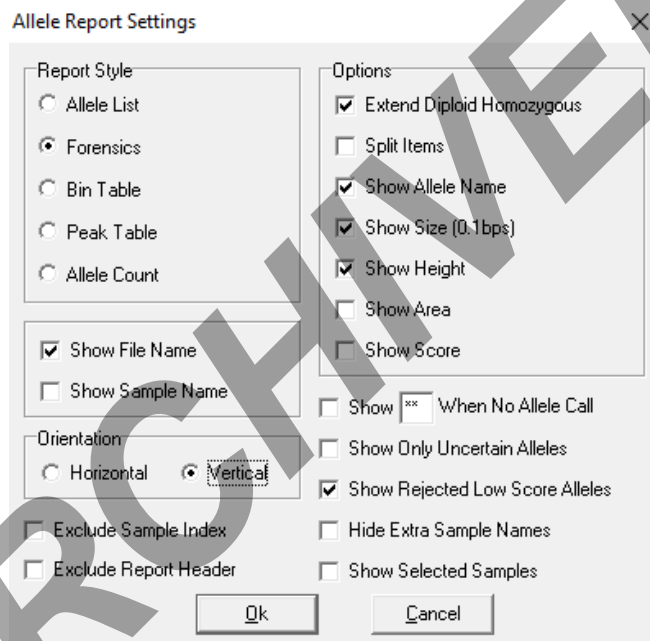
16.11 Import all PDFs (initial Fusion analysis and STRmix™ analysis) at the same time into LIMS.

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17 Fusion Evidence Only - Exporting EVIDENCE Table for STRmix™ Input

- 17.1 In the Main Analysis Window click the **Show By All Color**  icon → select **Show All**.
Ensure that all failed samples are DISABLED.
- 17.2 Click on the report settings icon  (report display section of the main analysis screen) → make sure the settings match below:



Allele Report Settings

Report Style

- Allele List
- Forensics
- Bin Table
- Peak Table
- Allele Count

Show File Name

Show Sample Name

Orientation

- Horizontal
- Vertical


Exclude Sample Index

Exclude Report Header

Options

- Extend Diploid Homozygous
- Split Items
- Show Allele Name
- Show Size (0.1bps)
- Show Height
- Show Area
- Show Score
- Show *** When No Allele Call
- Show Only Uncertain Alleles
- Show Rejected Low Score Alleles
- Hide Extra Sample Names
- Show Selected Samples

Ok Cancel

- 17.3 Click **Ok** → click on the Down Arrow next to the **Save Report** icon  (in the report display section of the main analysis screen) → ensure that **Save Report** is selected.
- 17.4 Navigate to the appropriate folder and save file as a .csv file named as **RunName_SM** (ex. Newton090615 8-11U A_SM).
- 17.5 For proper STRmix™ import, the GeneMarker® to STRmix™ macro needs to be run:
- 17.5.1 Open the exported project excel file from Step 17.3 above.
 - 17.5.2 Open [GeneMarker® to STRmix™ macro](#) → follow the “instructions” tab.
 - 17.5.3 After the macro has run click **Save As** within the GeneMarker® to STRmix™ macro file.
 - 17.5.4 Change the file type to “.txt” (text, tab delimited).

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17.5.5 Navigate to the folder containing your project data and save the file there.

18 Re-Printing/Exporting a PDF for a Sample from a Run that was Analyzed in previous versions of GeneMarker®

18.1 For re-printing/exporting a PDF for a run analyzed in version 2.9.0, use sections 13 and 16.

18.2 For re-printing/exporting a PDF for a run analyzed in version 2.8.2, use the following:

18.3 In the Main Analysis window,

18.3.1 Click **Set Axis** → Click **Fixed X** axis

18.3.2 The size range will be the same as the X-axis range for analysis:

- **Fusion:** 60-510
- **Yfiler:** 90-340

18.3.3 Select Auto Fit Y.

18.4 Click Tools → Click Export Electropherogram...

18.5 Set **Output Folder** to desired location for project PDFs by clicking the “...” icon.

18.6 Ensure the setting match the following:

Select Dyes

- Blue
- Green
- Yellow
- Red
- Orange

Dye Image Size

Width: 1024 pixels

Height: 700 pixels

One image per sample

Auto Scale Size Range

Auto Scale Marker Intensity

Export Format: PDF file

18.6.1 For **exemplar or non-SM analysis**: In the suffix field, enter the run name with a “_” before it. (i.e. _Newton061316 88-89U A)

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- 18.6.2 For **SM analysis**: In the suffix field, enter the run name with a “_” before it followed by “_SM” (Ex: _Newton061316 88-89U A_SM) to indicate STRmix™ analysis.
- 18.7 **Printing the ladder** - Right click in the Select Samples list → click Deselect All → Check the box for the ladder → In the Prefix field enter “ladders_” → click Export.
- 18.8 **Printing the controls** - Uncheck the box for the ladder and check the box for all controls in the run → In the Prefix field enter “controls_” → click Export.
- 18.9 **Printing the samples** - Right click in the Select Samples list → click Select All → Uncheck the box for all ladders and controls in the run, leaving all samples selected → clear the Prefix field → click Export. This will create 4 PDFs for every sample, one for each dye.
- 18.10 Import all PDFs (initial Fusion analysis and STRmix™ analysis, as applicable) at the same time into LIMS.

19 General Overview

19.1 Main Analysis Window

19.1.1 There are four major displays in the main analysis window:

- 1 Navigator; Sample File Tree
- 2 Electropherograms
- 3 Report; Report Table
- 4 Project Summary

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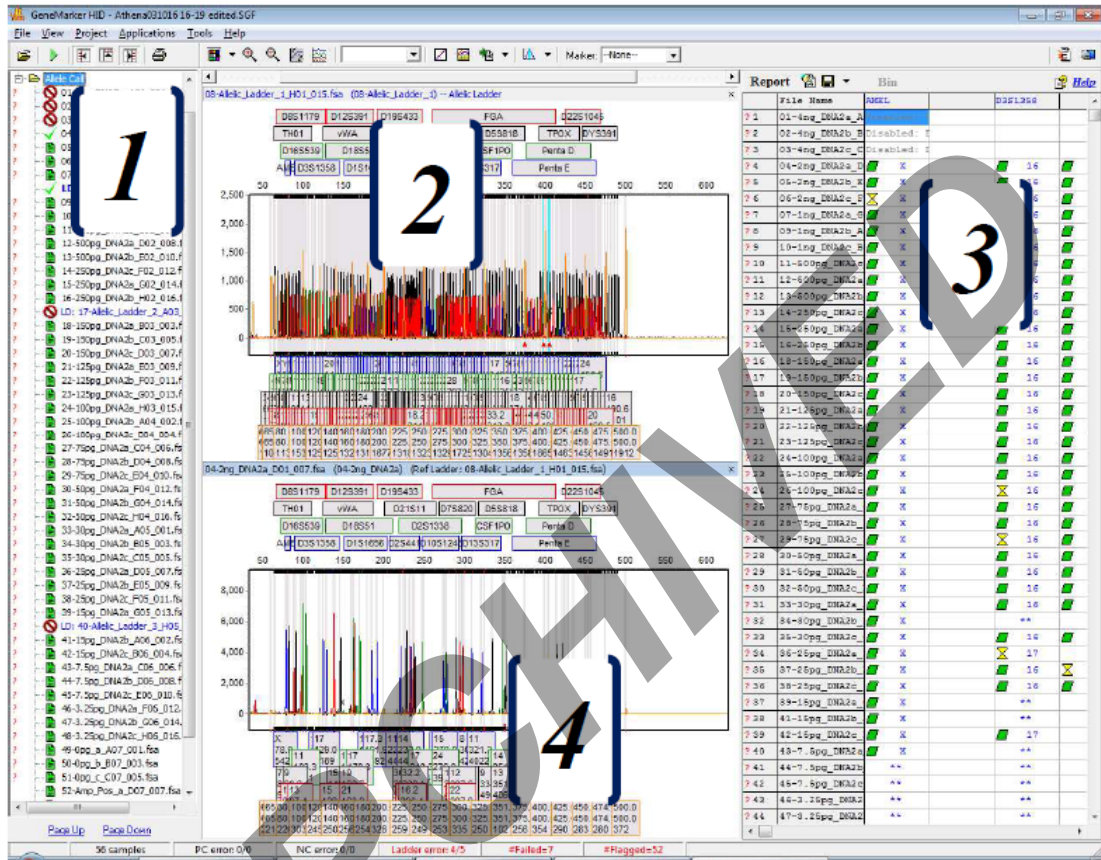
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NOTE: See [Helpful Icons Index](#) for further details.



19.2 Navigator; Sample File Tree

19.2.1 This view lists all the samples that are included in the project. Prefixes **LD**, **PC1** and **NC** are labeling the **allelic ladder**, **positive control**, and **negative control**

19.2.2 The sheet icon to the left of the sample name indicates the quality of the internal lane standard (ILS) with the following color/code:

- **Green** = High Lane Quality; Passing Size Standard
- **Yellow** = Requires Verification
- **Red strike through** = No Sizing Occurrence; failed to call size
- **Red Question Mark** = one or more quality criteria are not met based on the analysis parameters;
- See [Quality Reasons Index](#) for more details.

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19.3 Project Summary

19.3.1 This bar is located at the bottom of the main analysis screen and summarizes the project as well as alerts the analyst to samples that did not pass analysis parameters.

| | | | | | | |
|-----|------------|---------------|---------------|-------------------|-----------|-------------|
| New | 22 samples | PC error: 1/1 | NC error: 3/3 | Ladder error: 0/1 | #Failed=1 | #Flagged=19 |
|-----|------------|---------------|---------------|-------------------|-----------|-------------|

- Column 1: **New** reflects that the data set is not a project that has been previously analyzed. If it reads **Modified**, the project was previously analyzed in the software.
- Column 2: This denotes the number of samples (including controls) contained within the data set.
- Columns 3-5: This indicates the number and types of controls in the data set that do not meet the quality criteria set in the software.
- Column 6: This indicates the number of samples that were disabled.
- Column 7: This indicates the overall number of samples, excluding the allelic ladder, that do not meet the quality criteria set in the software.
- **NOTE:** Flagging features are an indication of potential failures. The ultimate decision of passing/failure is made by the analyst after further evaluation.

19.4 Calibration Chart Window

19.4.1 There are three major displays in the calibration chart window:

- 1 Sample List
- 2 Size Standard Template
- 3 Sample ILS

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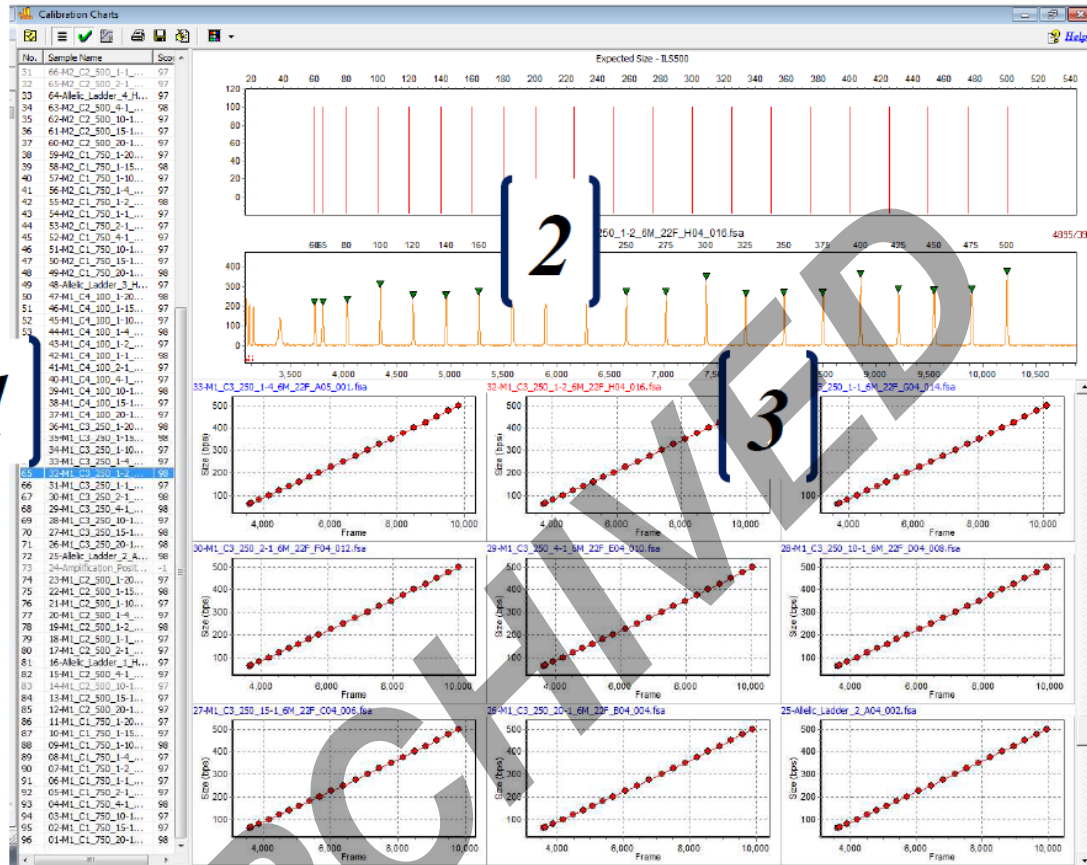
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19.4.2 Sample List

19.4.2.1 This view lists all the samples that were processed in the project. The **Score** column represents how well the size standard template and the sample ILS match. The closer the score is to 100, the better the match. The sample may fail automatically if the size standard is bad enough or may have to be failed manually by the analyst.

19.4.3 Size Standard Template

19.4.3.1 The template highlights all the peaks that should be labeled in the corresponding sample ILS for easier comparison.

19.4.4 Sample ILS

19.4.4.1 The sample ILS displays the peaks that were detected as well as the approximate start of the data analysis view, indicated by the dash line.

19.5 Browse by All Color Window

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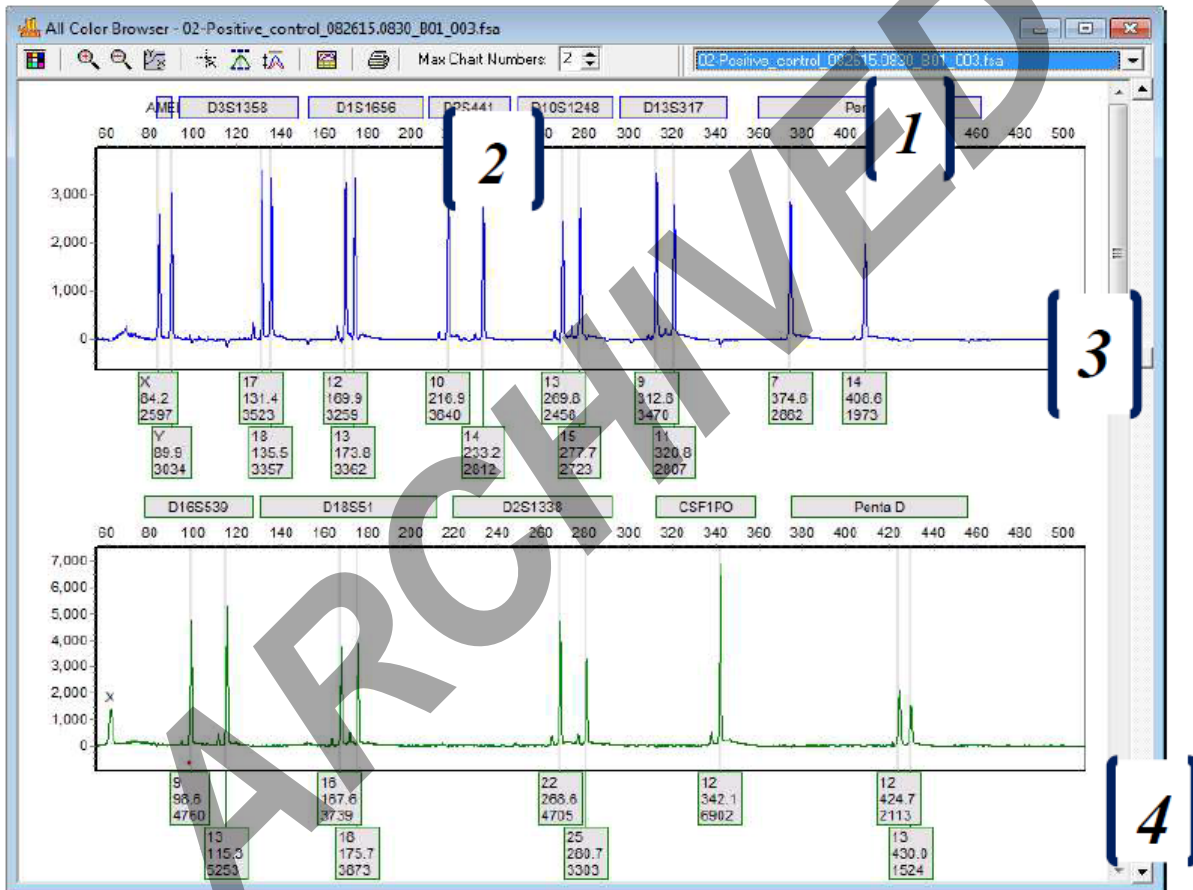
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19.5.1 There are four features in the **Browse by All Color** that allow for straightforward review of the sample:

- 1 Sample Drop-Down
- 2 Max Chart Number
- 3 Scroll Within a Sample
- 4 Scroll Between Samples



19.5.2 Sample Drop-Down

19.5.2.1 The sample drop-down lists all the samples within a project. A user can move between samples by clicking on the sample name instead of scrolling through all electropherograms.

NOTE: Analysts can zoom in/out by using the zoom icons  located at the top toolbar. As an additional option, hold down the left button on the mouse and drag the dotted box that appears from the upper left to the lower right (around the desired “zoom in” area). To zoom out, hold down the left button

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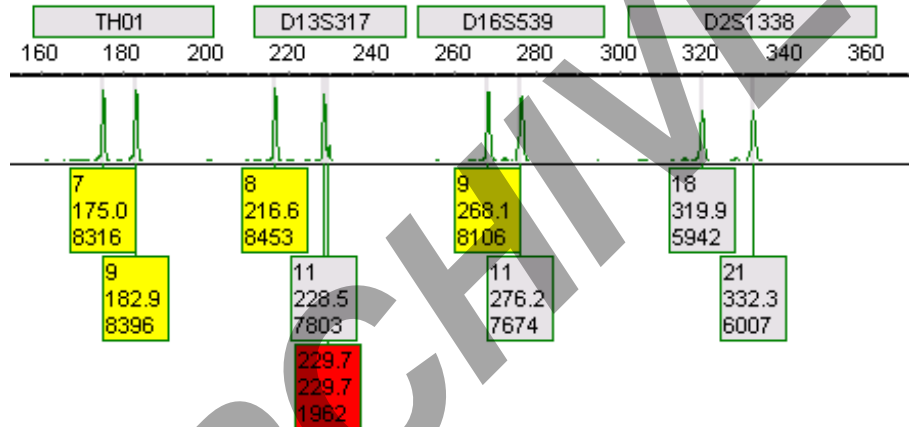
on the mouse again dragging box from lower right to upper left. To scroll while zoomed in, right click and hold while dragging the mouse in the direction you want to scroll.

19.5.3 Max Chart Number

19.5.3.1 Max chart number views each sample by the number of dye lanes as per the user's needs during sample analysis.

19.6 Allele Labels

19.6.1 When analyzing samples in the Main Analysis window or the **Browse by All Color** window, alleles may be colored yellow or red.



19.6.2 This indicates that one or more quality criteria have not been met in the software. See [Quality Reasons Index](#) for more details.

Note: Flagging features are an indication of potential artifacts and/or allelic imbalance and/or peak saturation. The ultimate decision of passing/failure is made by the analyst after further evaluation.

19.7 Peak Table

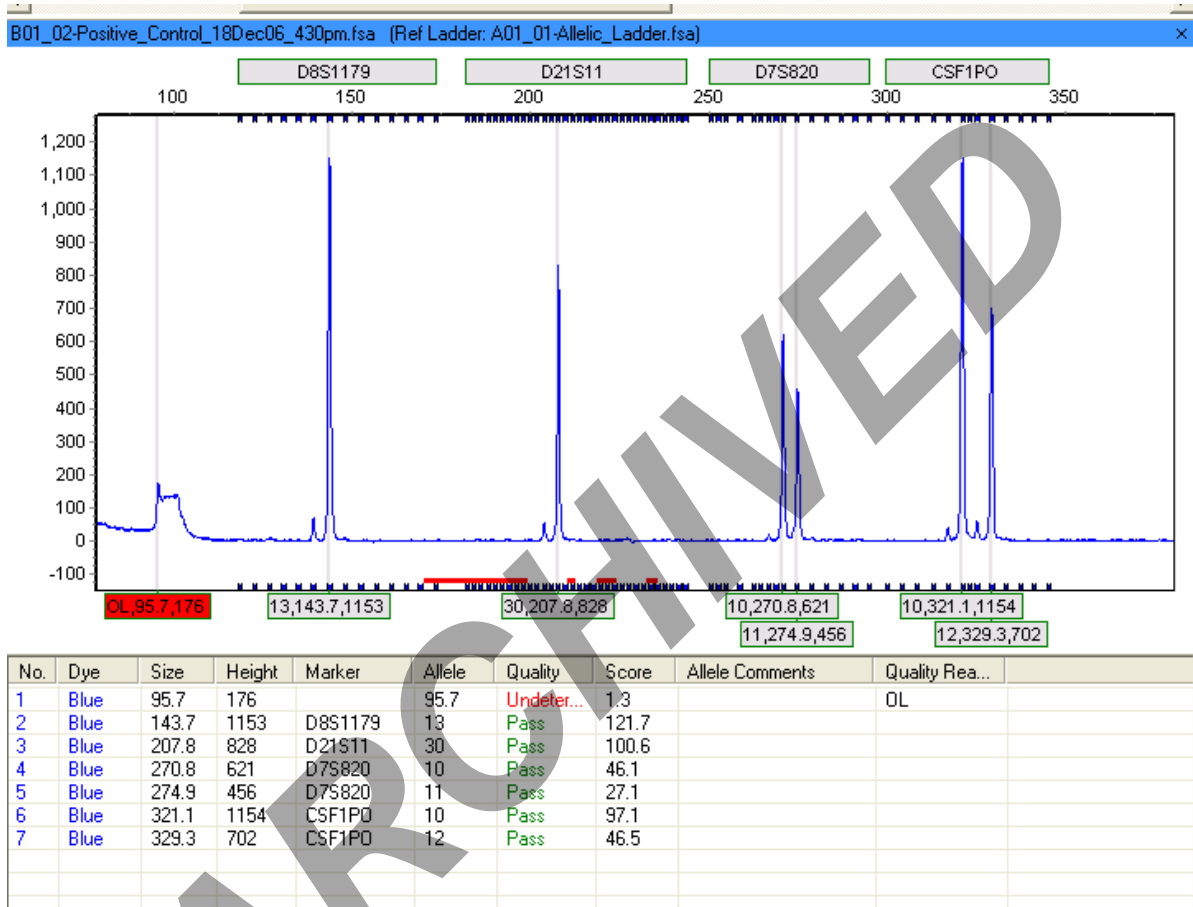
19.7.1 When analyzing samples in the Main Analysis window or the **Browse by All Color** window, a peak table may be added to supplement analysis by clicking **Show Chart/Table**



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19.7.2 This table will indicate to an analyst which alleles were flagged and which quality reason was triggered. See [Error! Reference source not found.](#) for further details.









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19.8 Helpful Icons Index





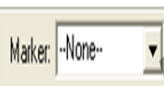


19.8.1 Main Toolbar Icons

| Icon | Name | Function |
|-------------------------------------------------------------------------------------|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | Run Project | Opens Run Wizard for processing the data. |
|  | Show/Hide Toggles | Allows user to show/hide the frames for the <i>Sample File Tree</i> , <i>Synthetic Gel Image</i> and <i>Report Table</i> , respectively. |
|  | Show Color | Allows user the choice of viewing/hiding all color lanes or single dye lane layer (with single left mouse click on the icon, obtain single dye view) |
|  | Zoom/ Zoom Out | Allows for more discriminate view of electropherogram. Alternately, hold down left mouse button and draw a box; dragging from top left corner to bottom right zooms in on image while dragging from bottom right corner to top left zooms out. |
|  | Set Axis | Default sets Y-axis by maximum peak intensity for the sample displayed. The other 2 options either auto-fit the Y-axis by the peak intensity of the alleles displayed, or allow for setting the X- and Y- axes ranges manually. |
|  | Browse by All Colors | Allows for comparative view display of sample electropherograms by dye color. Individual samples can be selected from the drop down menu in the upper right corner of the All Color Browser display window. |

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19.8.2 Allele Call Icons available after raw data processing only; Sample File Tree Allele Call folder must be selected.






| Icon | Name | Function |
|-------------------------------------------------------------------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | Size Calibration | Displays calibration charts for linearity of lane analysis. |
|  | Show Chart/Table | Allows user to toggle the display to show only the <i>Peak Table</i> , the <i>Peak Table</i> and the <i>Electropherogram</i> , or just the <i>Electropherogram</i> . |
|  | Save Peak Table | Allows user to export the <i>Peak Table</i> as an Excel(.xls) file or a tab-delimited Text (.txt) file. |
|  | Call Allele | Allows user to call alleles by sample(s), by marker or by dyes. Allows user to make some modifications to the threshold/filter settings without having to activate Run Wizard again (i.e. Peak Detection Threshold, Stutter Peak Filter and Peak Score Threshold). |
|  | Marker Drop-down Menu | Allows user to select a marker to view. Available after the samples have been compared to a Panel. |
|  | Event Log | Displays the processing success/failure of each dye lane. |
|  | Magic Wizard | Activates the <i>Start Your Project</i> , <i>Run</i> and/or <i>Report</i> dialog boxes. |

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19.8.3 Browse By All Colors Icons

| Icon | Name | Function |
|-------------------------------------------------------------------------------------|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | Zoom In/ Zoom Out | Same use as in the <i>Main Analysis</i> Screen. |
|  | Show/Hide Mouse Cross Lines | Allows user the option to show/hide X- and Y- axis grid lines that appear at the tip of the mouse cursor along with the basepair size and RFU value of the mouse cursor position. |
|  | Show/Hide Bin Ranges | Allows user the option to show/hide the Bin brackets at the top and bottom of the electropherogram. |
|  | Auto Scale Markers | Allows user the option to adjust the RFU intensities of low peaks to match the intensity of the highest peak in the dye color. When low peaks are increased, the intensity magnification factor is noted in the marker (can adjust from 2X to 8X). |
|  | Print | Opens the <i>Print Report</i> settings box. Also accessible in the <i>Main Analysis</i> screen. |

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19.8.4 Report Table Icons located directly above the *Report Table*.

| Icon | Name | Description |
|-----------------------------------------------------------------------------------|----------------------|---------------------------------------------------------------------------------------------------------------------------------|
|  | Report Settings | Allows user to customize <i>Report Table</i> display settings. |
|  | Save Report | Allows user to export the <i>Report Table</i> as an Excel (.xls) file or tab-delimited Text (.txt) file. |
|  | Customize Bin Column | Allows user to select which bins to include/exclude in the Report Table (check with TSL as this is not accessible at this time) |

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19.8.5 Peak Table Options Index

| | |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Dye | dye lane, location of peak |
| Size | peak basepair size (x-axis) |
| Height | value given as relative frequency units (RFU) of the peak (y-axis) |
| Height Ratio | peak height divided by height of highest peak in the dye lane or Marker |
| Area | indicates the area under the curve of the peak; calculated based on x-axis Start/End column settings |
| Area Ratio | peak area divided by area of highest peak in the dye lane or Marker |
| Marker | locus location of the peak |
| Allele | bin location of the peak (based on kit/system panel and ladder analysis of project) |
| Difference | absolute value of distance between the peak center and <i>Bin</i> center in basepairs |
| Quality | assigns Pass/Check/Undetermined quality ranking for each peak relative to the Allele Evaluation <i>Peak Score</i> settings in the Run Wizard (see Additional Settings) |
| Score | an exponential curve-based evaluation of the peak; calculation of the value is based on signal-to-noise ratio and peak shape (or morphology) |
| Start/End | beginning and finish basepairs of the peak's Area calculation |
| Allele Comments | software and user edited comments for the peak; right mouse click peak in chart to add peak edit comments or select from the dropdown list (more in Edit Comments Index) |
| Sample Comments | user added comments for the sample; right click sample name in the sample tree to add new sample edit comments or select from the dropdown list (more in Sample Comments Index) |
| Quality Reasons | letter code abbreviation(s) of reason(s) for a peak's <i>Quality</i> assignment if ranked Check/Undetermined |