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.

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** 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.

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.

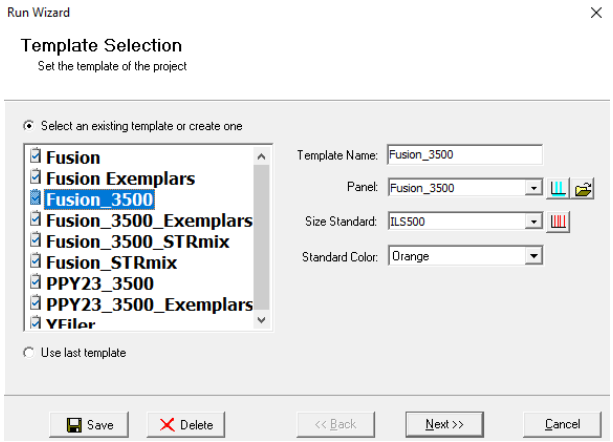
Wizard Template	Instrument	Used for Analysis of
Fusion_3500	3500	evidence (with stutter filters)
Fusion_3500_STRmix		evidence for STRmix import (without stutter filters)
Fusion_3500_Exemplars		exemplars
PPY23_3500	3500	evidence
PPY23_3500_Exemplars		exemplars

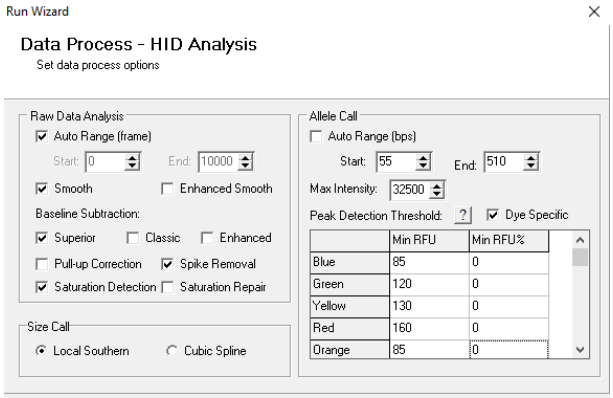
Note: **Do NOT click Save** on any of the following windows in the Run Wizards. This will overwrite the template for all analysts.

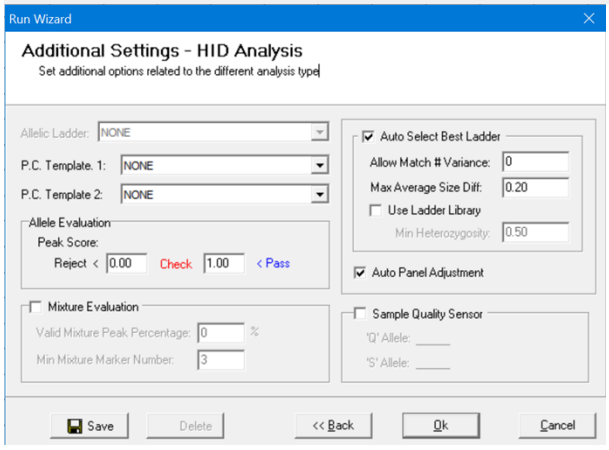
3.2 Check the **Template Selection** window against the corresponding screenshots below for initial analysis. If "Last Template" is displayed in the Template Name field, ensure that the appropriate Panel is listed for your analysis. For Wizard Templates for STRmix™ analysis, see Section 15. Select **Next**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

3.3 **Fusion on 3500** Run Wizard Screenshots

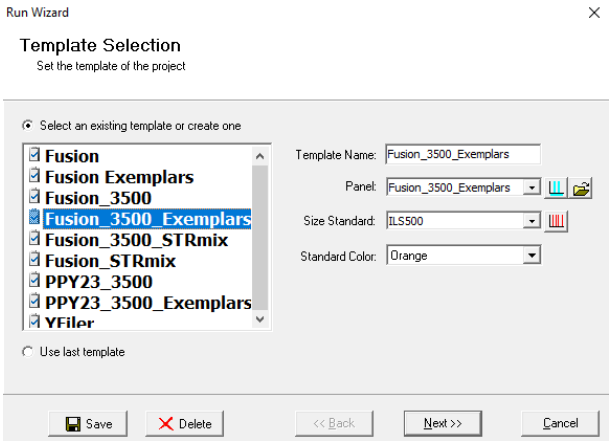
Fusion on 3500 (Evidence)

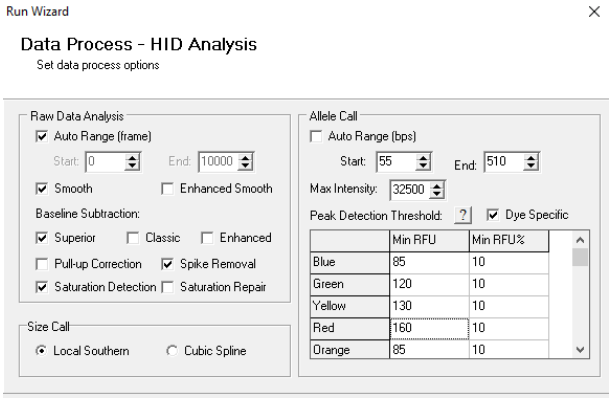


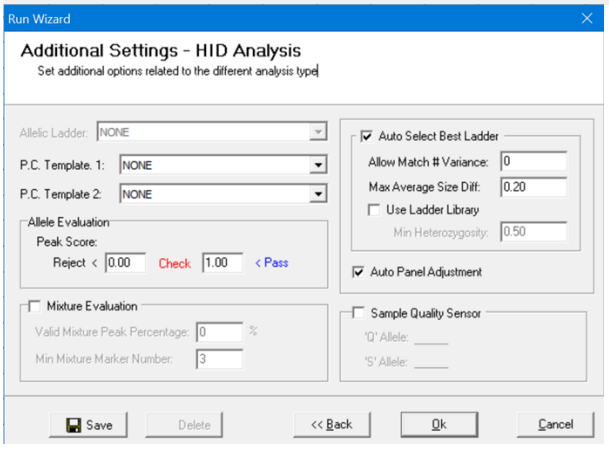




Fusion on 3500 (Exemplars)

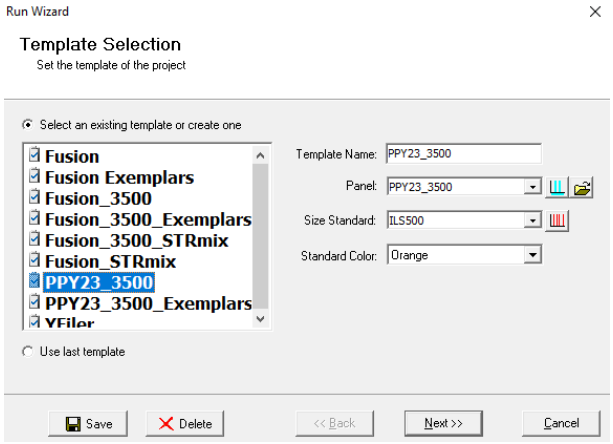


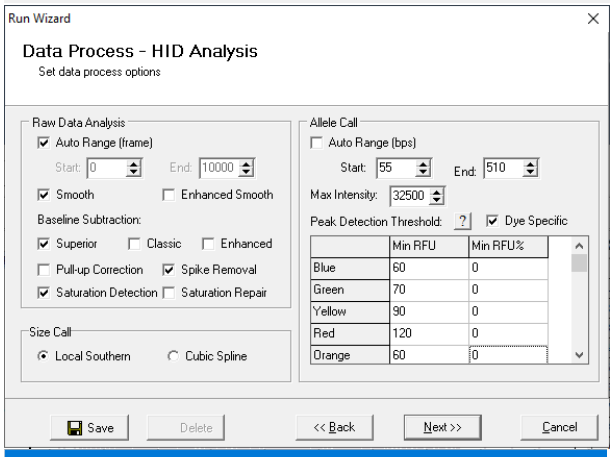


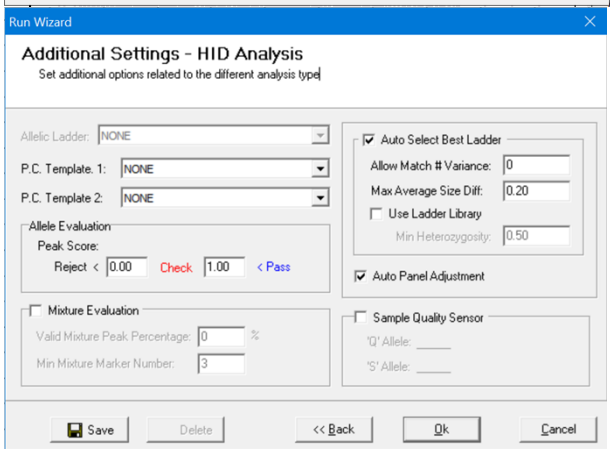


3.4 **PPY23 on 3500** Run Wizard Screenshots

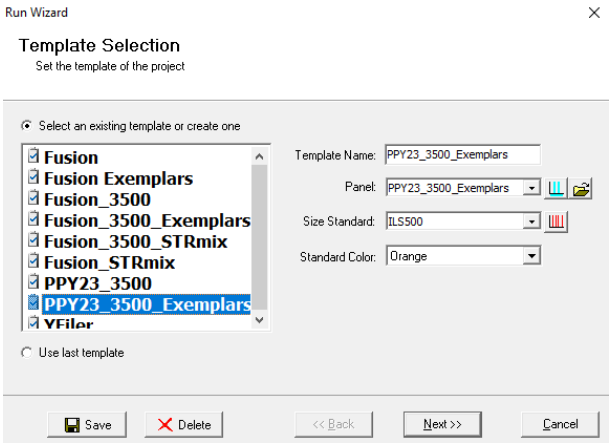
PPY23 on 3500 (Evidence)

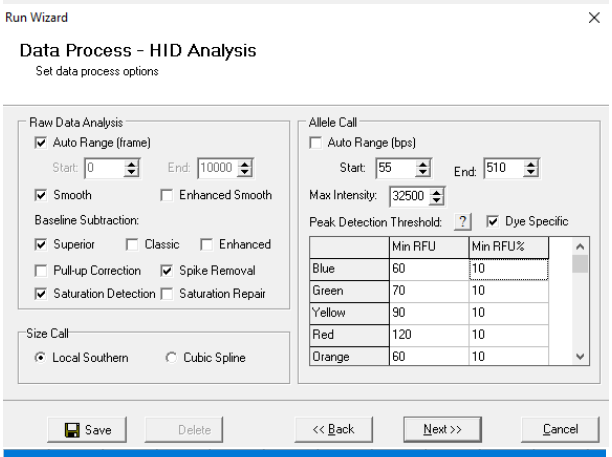


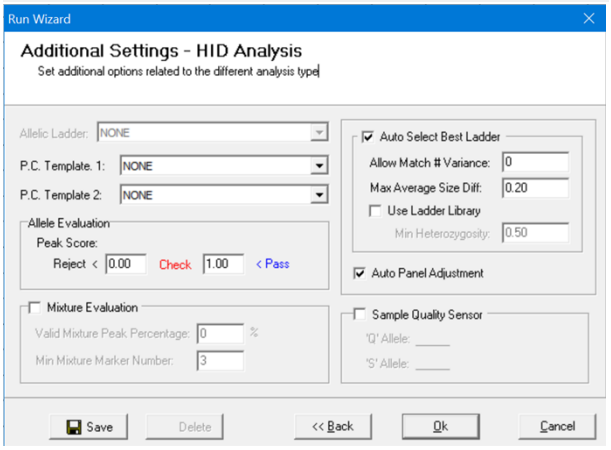




PPY23 on 3500 (Exemplars)







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

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

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

3.6.1 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 STR Analysis on 3500xL Genetic Analyzers manual and the 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**.

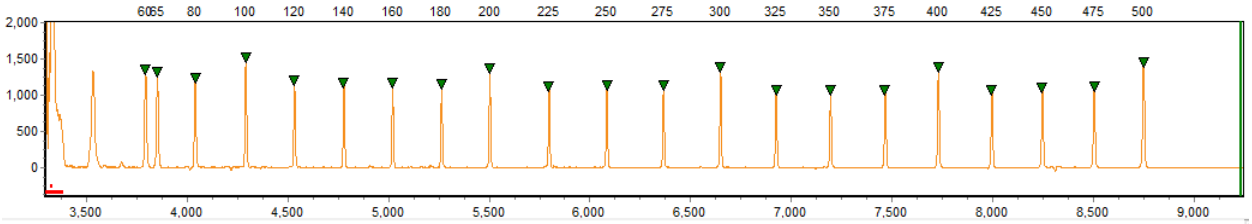
4.4 To check the calibration charts, click **Size Calibration** located on the top toolbar in the main analysis window.

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.

4.4.4 Fusion and PPY23 ILS



4.4.5 If a sample contains a size standard peak without a green triangle present, the size standard for that sample fails. Refer to the STR Analysis on 3500xL Genetic Analyzers manual and the STR Results Interpretation – PowerPlex® Fusion & STRmix™ for further information.

4.4.6 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 to view the entire range of the ILS.

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. (ILS screenshots can be viewed in the Fusion Ladder, PE and SS appendix or the Manual Appendix for PowerPlex® Y23 documents for reference).

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

4.5.6 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.

4.5.6.1 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 theSTR Analysis on 3500xL Genetic Analyzers manual and the 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 . (Ladder screenshots can be viewed in the Fusion Ladder, PE and SS appendix or the Manual Appendix for PowerPlex Y23 documents for reference.)

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** 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 STR Analysis on 3500xL Genetic Analyzers manual and the 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 STR Analysis on 3500xL Genetic Analyzers manual and the 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

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. (Positive control screenshots can be viewed in the Fusion Ladder, PE and SS appendix or the Manual Appendix for PowerPlex Y23 documents for reference).

6.7 A positive control that does not generate a complete genotype or gives an incorrect genotype will be indicated as failing. For analysis, editing, evaluating, and retesting guidelines refer to the STR Analysis on 3500xL Genetic Analyzers 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 STR Analysis on 3500xL Genetic Analyzers 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 STR Analysis on 3500xL Genetic Analyzers manual and 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

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 STR Analysis on 3500xL Genetic Analyzers manual and STR Results Interpretation – PowerPlex® Fusion & STRmix™) → click **Ok**. (See Editing Codes)

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.

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 STR Analysis on 3500xL Genetic Analyzers manual and the 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 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.

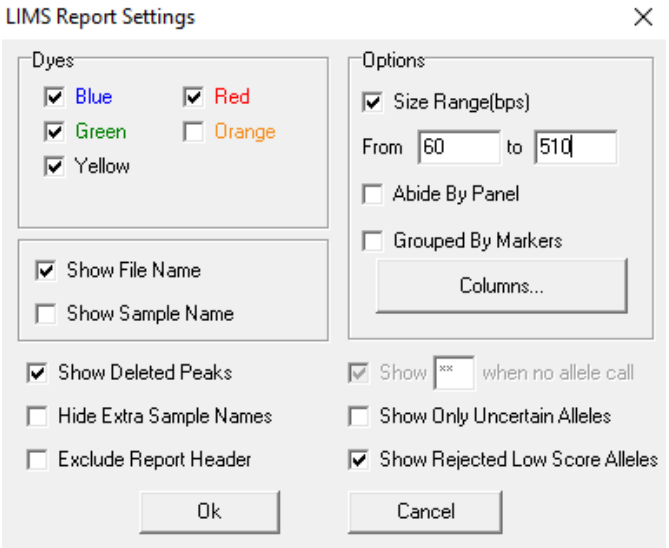
12.4 In the report display section of the main analysis screen, click on the down arrow next to the Save Report icon. Ensure that **Save LIMS report** is selected.

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

12.5.2 Under **Options**, click Columns... → 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.**



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

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**.

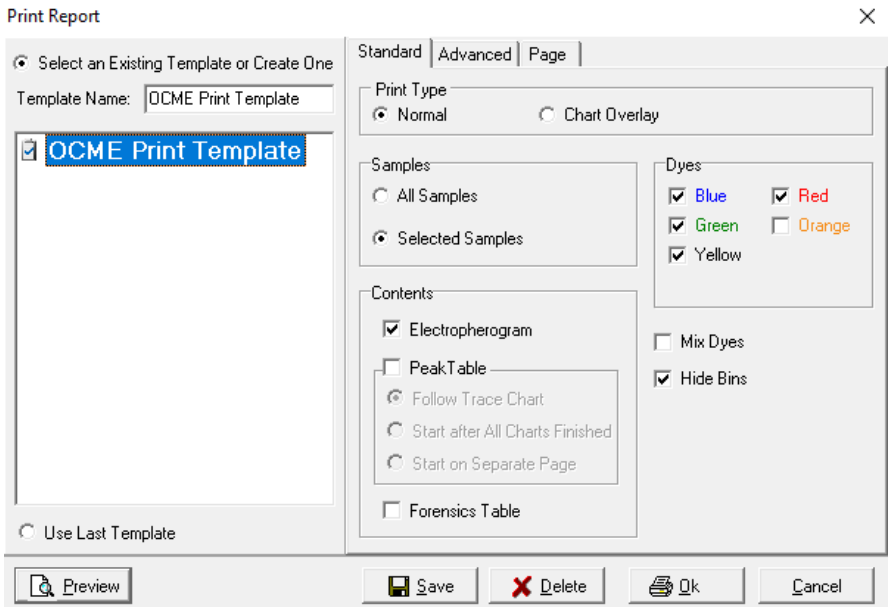
13.4 In Main Analysis Window click **Project** → **Apply Sample Grouping** (if grayed out return to step 13.1) → click **Group By Order** on the bottom left → change **Group Size to 1** → click **Match** on the bottom right → click **Ok**.

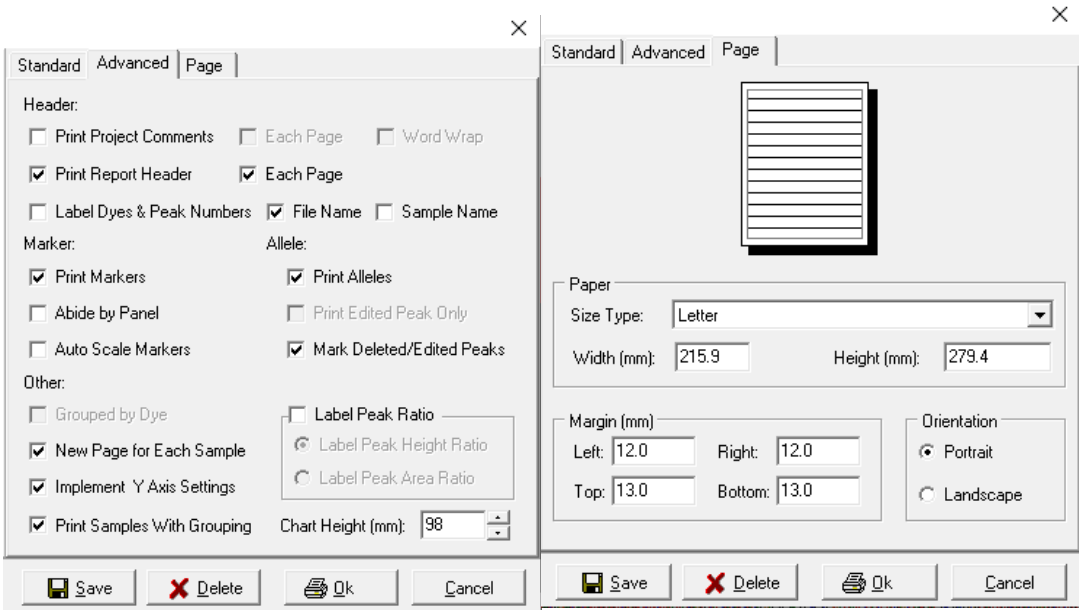
13.5 In the main analysis window → click **Set Axis** → click **Fixed X axis**. The Size Range will be the same as the X- axis range for analysis:

- **Fusion**: 60-510
- **PPY23**: 65-450

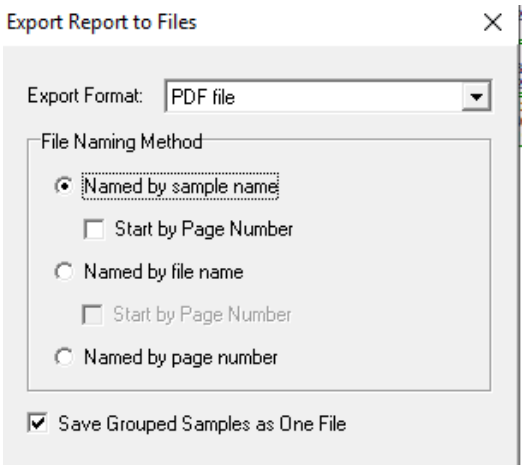
13.6 Click **Set Axis** again → Select **Auto Fit Y**.

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):





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**.

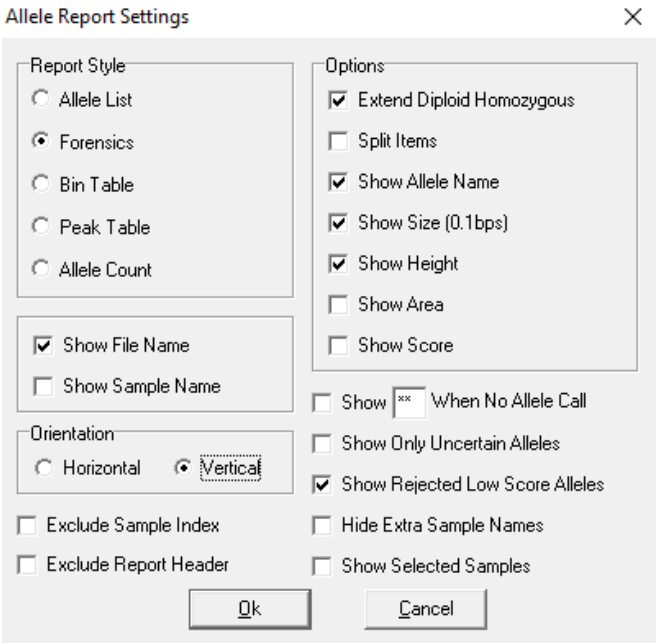
13.10 If analyzing **Fusion Evidence**, wait to import all PDFs into LIMS until after the completion of STRmix analysis in Section 17.

13.11 If analyzing exemplars or **PPY23 evidence** → Import all PDFs into LIMS.

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.** Ensure that all failed samples are DISABLED.

14.2 Click on the report settings icon (report display section of the main analysis screen) → ensure the settings match below:



14.3 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.

14.4 Navigate to the appropriate folder and save file as a .csv file named as **RunName_SM** (i.e., Pavlov090619 1-3U A_SM).

14.5 For proper STRmix™ import, the GeneMarker® to STRmix™ macro needs to be run:

14.5.1 Open the exported project excel file from 14.4

14.5.2 Open GeneMarker® to STRmix™ macro → follow the "instructions" tab.

14.5.3 After the macro has run click **Save As** within the GeneMarker® to STRmix™ macro file.

14.5.4 Change the file type to ".txt" (text, tab delimited).

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

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:

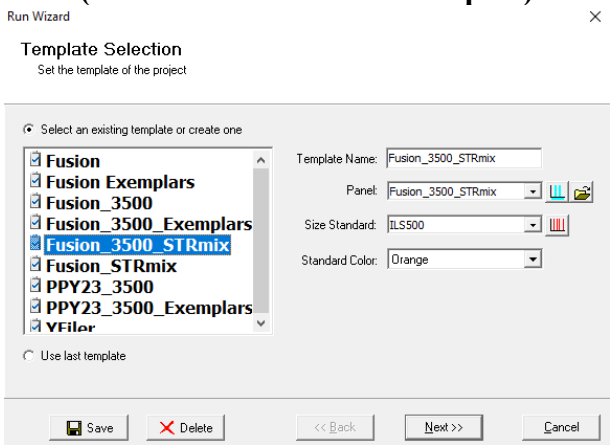
- **3500 Data:** Fusion_3500_STRmix

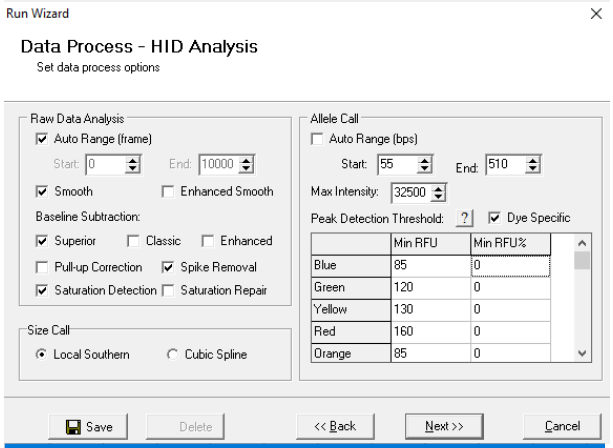
Note: **Do NOT click Save** on any of the following windows in the Run Wizard. This will overwrite the template for all analysts.

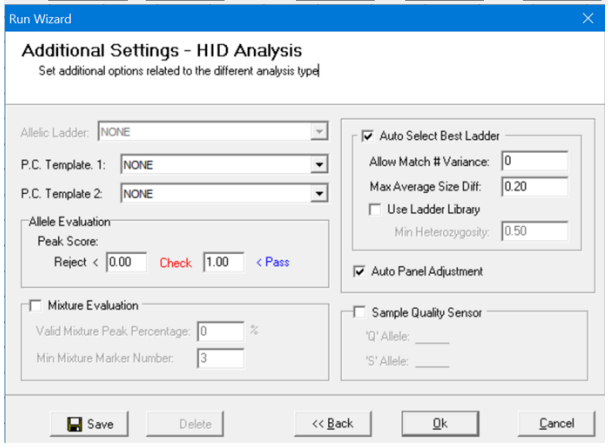
15.6 Check the right side of the **Template Selection** window against the corresponding screenshots below. Select **Next** twice to move through the **Data Process** and **Analysis Settings** windows and check all settings against the corresponding screenshots.

15.7 **Fusion evidence STRmix™** Run Wizard Screenshots:

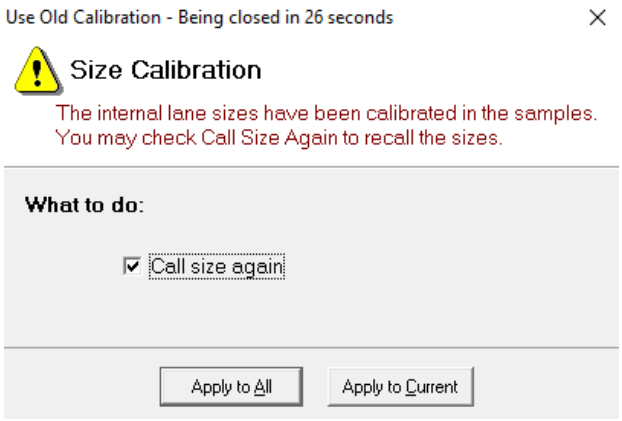
Fusion on 3500
(Evidence for STRmix™ Import)







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.

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.

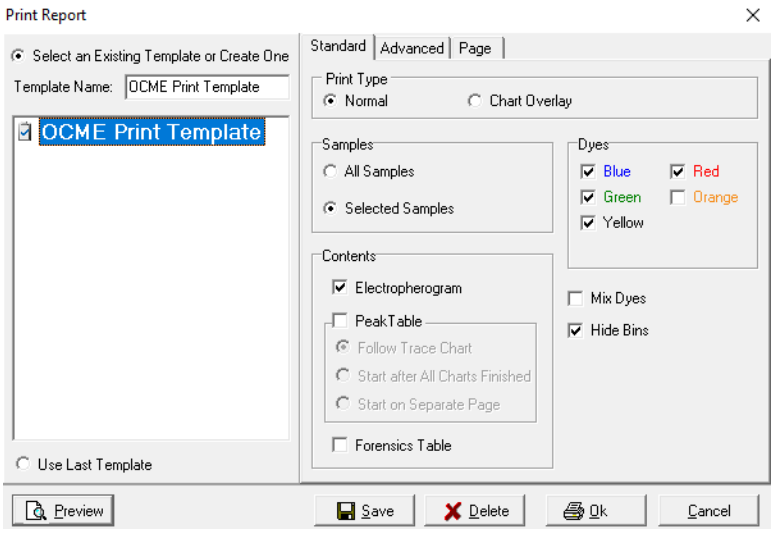
16.5 In Main Analysis Window click **Project** → **Apply Sample Grouping** (if grayed out return to step 13.1) → click **Group By Order** on the bottom left → change **Group Size to 1** → click **Match** on the bottom right → click **Ok**.

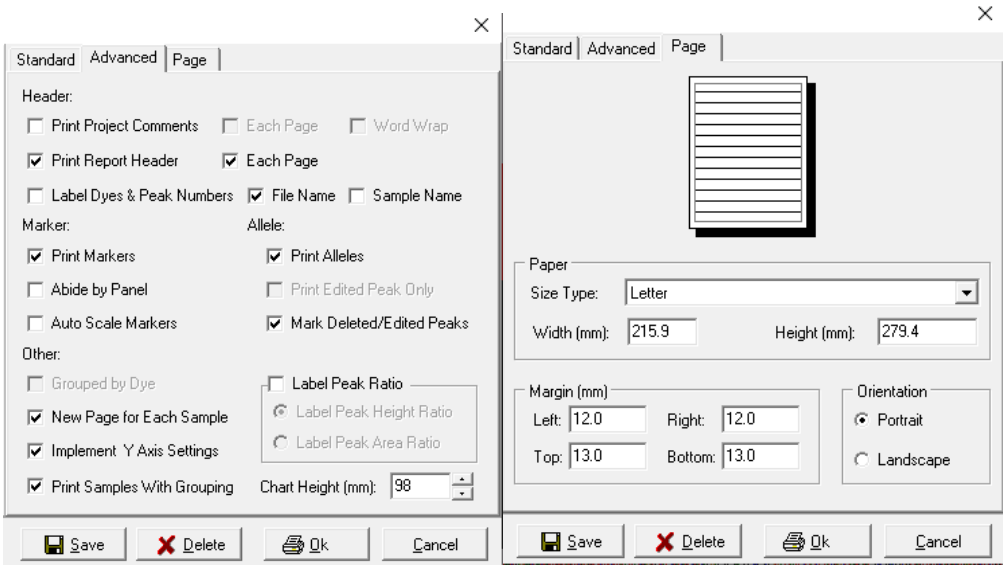
16.6 In the main analysis window → click **Set Axis** → click **Fixed X axis**. The Size Range will be the same as the X-axis range for analysis:

- **Fusion**: 60-510

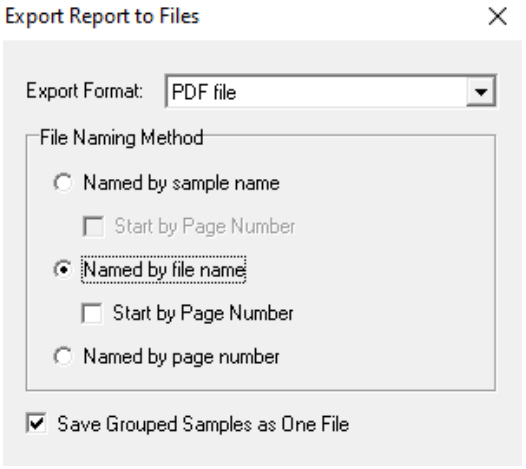
16.7 Click **Set Axis** again → Select **Auto Fit Y**.

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):





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



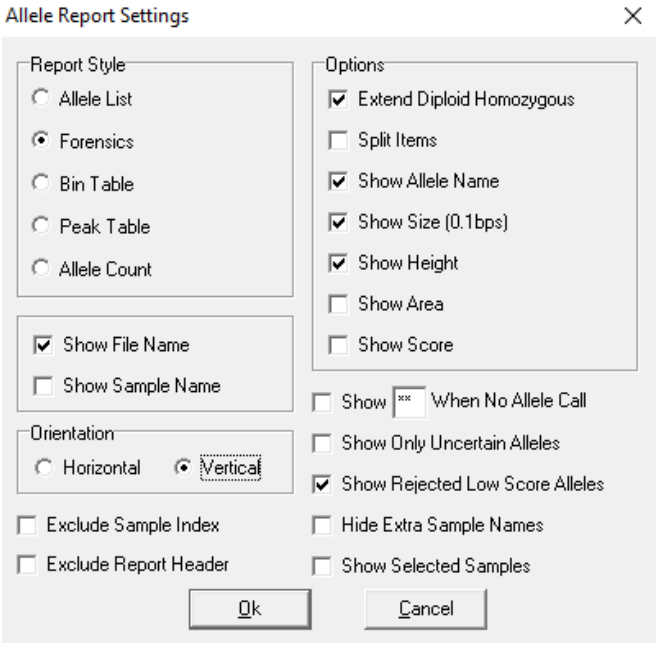
16.10 Click on the "**…**" icon to choose the appropriate export directory for the PDF files → click **Ok** → the **Export Report to PDFs Events** window will appear → once complete, click **Ok**.

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

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:



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 17.3above.

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).

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:

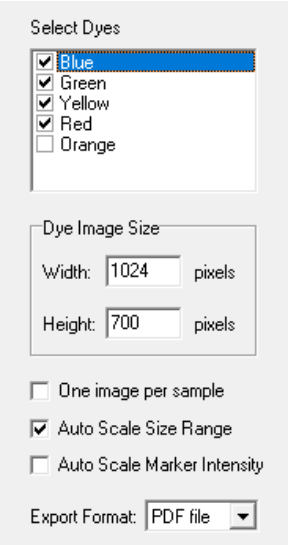
- **Fusion**: 60-510

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:



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)

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
NOTE: See Helpful Icons Index for further details.

19.2 Navigator; Sample File Tree

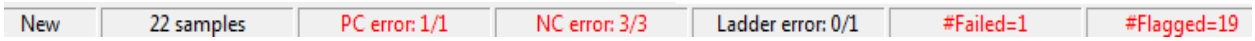
19.2.1 This view lists all the samples that are included in the project. Prefixes LD, PC1 and NC are labeling the allelic ladder, positive control, and negative control

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.

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.

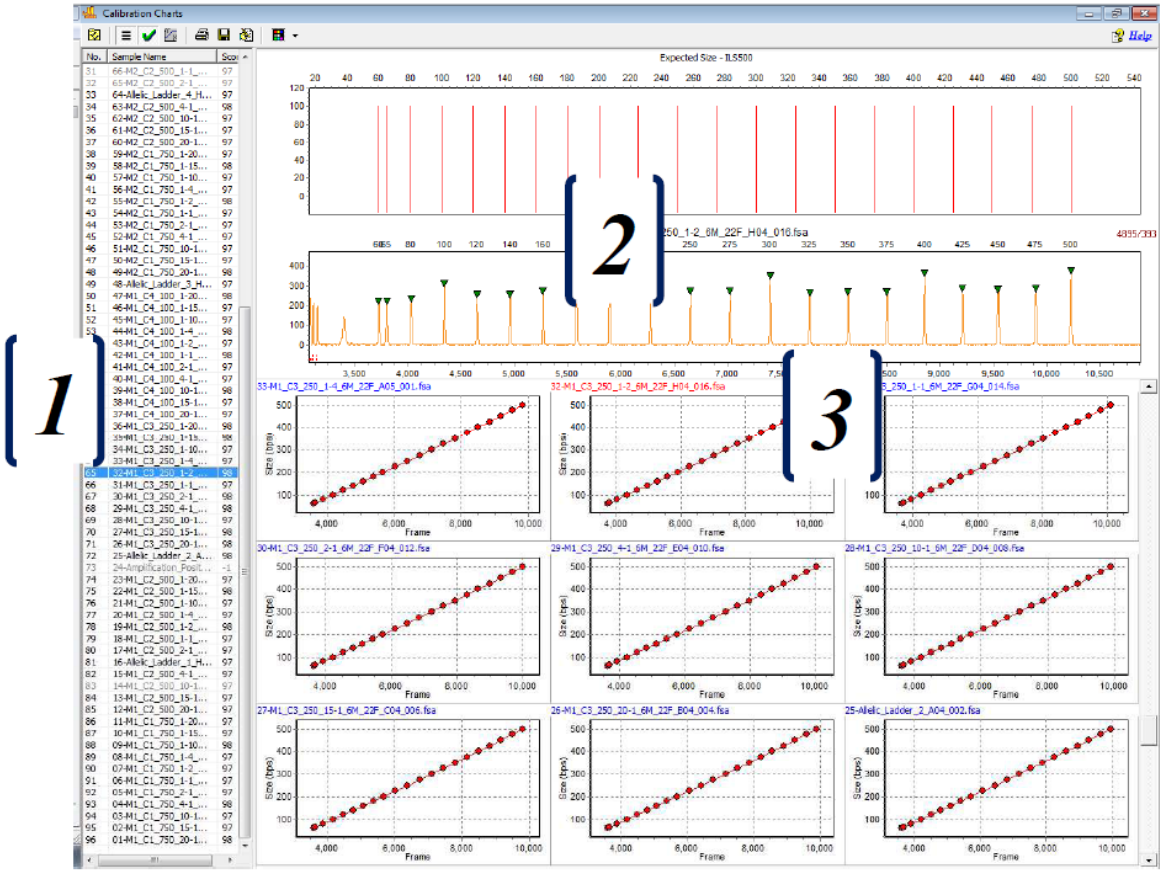


- Column 1: **New** reflects that the data set is not a project that has been previously analyzed. If it reads **Modified**, the project was previously analyzed in the software.
- Column 2: This denotes the number of samples (including controls) contained within the data set.
- Columns 3-5: This indicates 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 Sample Size Standard Template
3 Sample ILS



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.

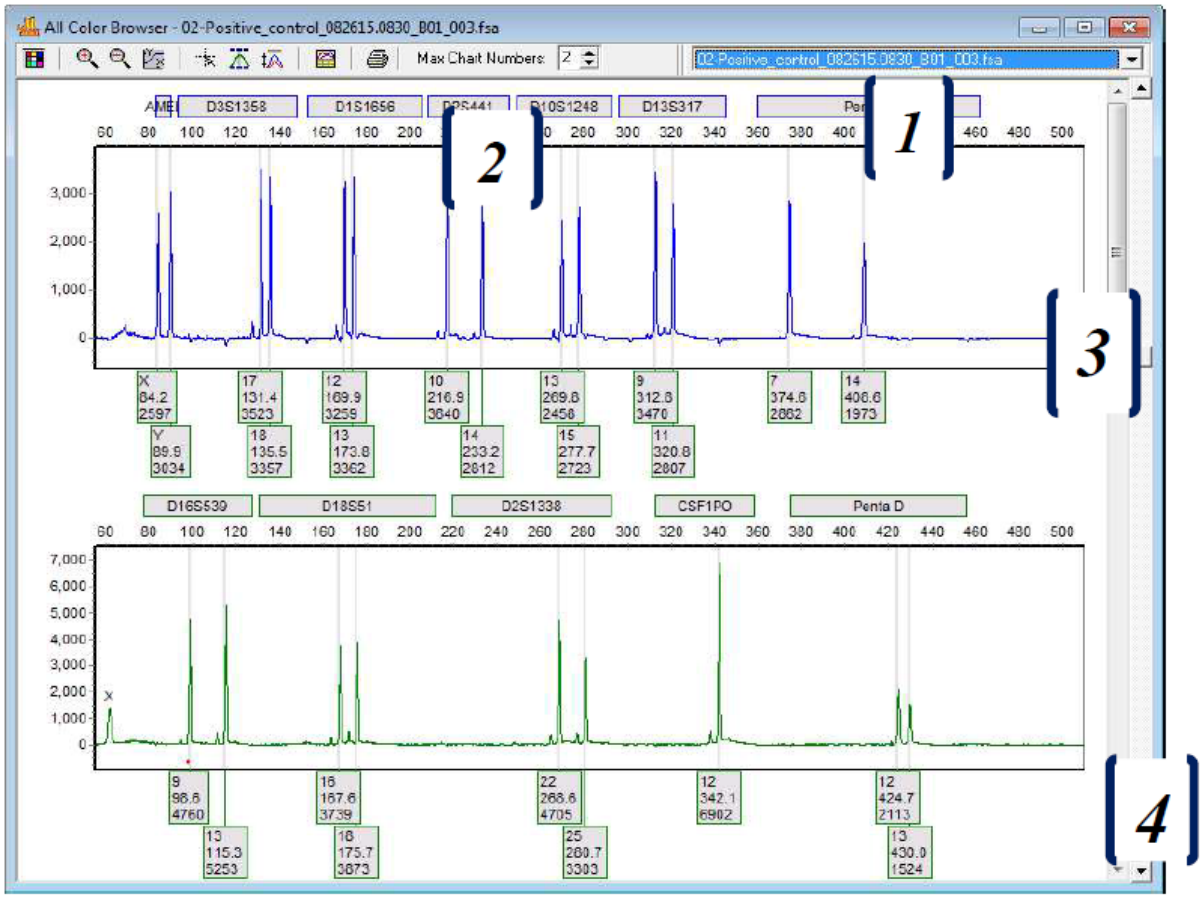
19.4.4 Sample ILS

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

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.

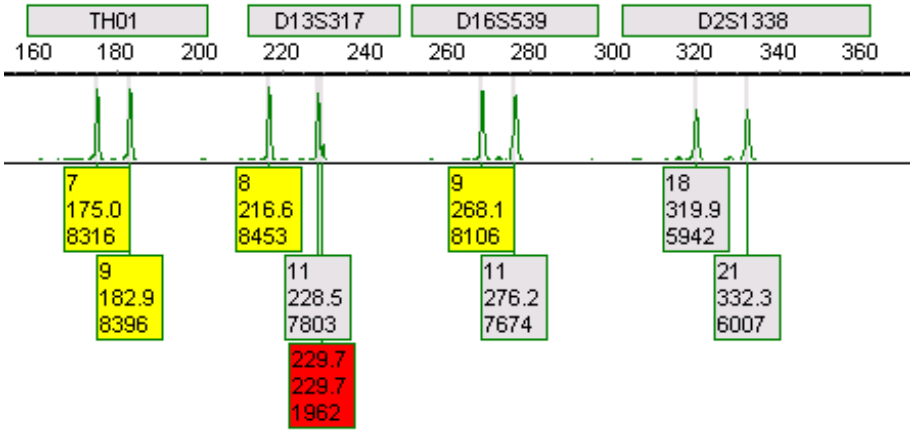
NOTE: Analysts can zoom in/out by using the zoom icons located at the top toolbar. As an additional option, hold down the left button on the mouse and drag the dotted box that appears from the upper left to the lower right (around the desired "zoom in" area). To zoom out, hold down the left button on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



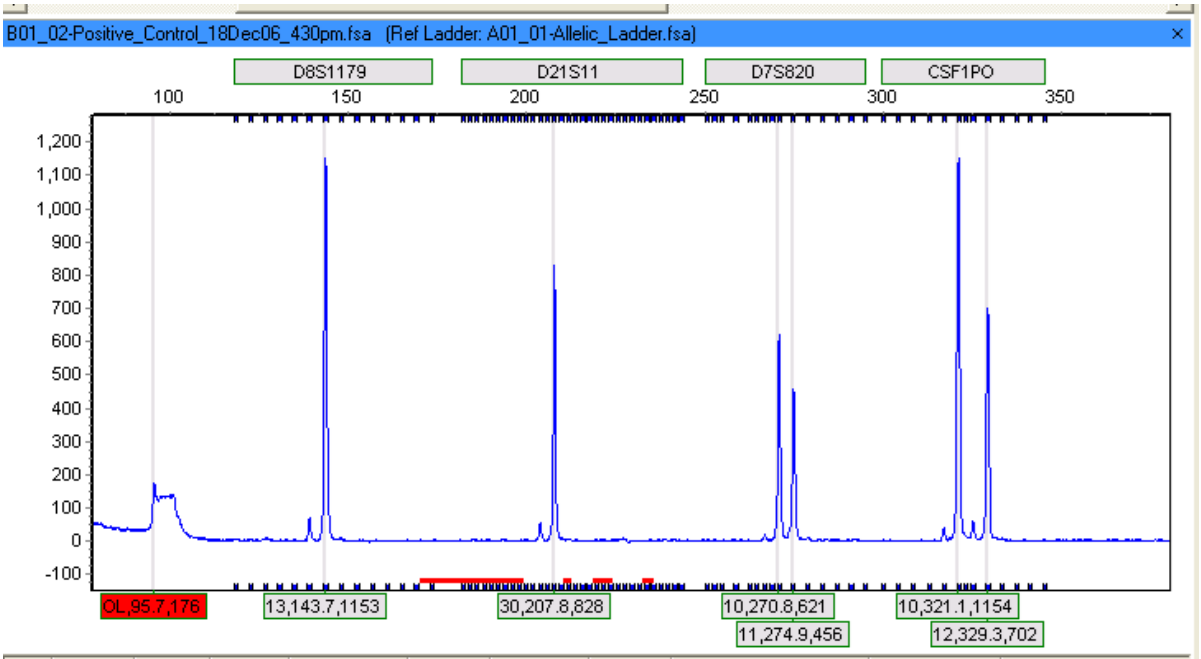
19.6.2 This indicates that one or more quality criteria have not been met in the software. See Quality Reasons Index for more details.

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 maybe added to supplement analysis by clicking **Show Chart/Table** icon 

19.7.2 This table will indicate to an analyst which alleles were flagged and which quality reason was triggered.



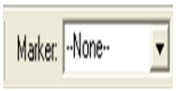
No.	Dye	Size	Height	Marker	Allele	Quality	Score	Allele Comments	Quality Rea...
1	Blue	95.7	176		95.7	Undeter...	1.3		OL
2	Blue	143.7	1153	D8S1179	13	Pass	121.7		
3	Blue	207.8	828	D21S11	30	Pass	100.6		
4	Blue	270.8	621	D7S820	10	Pass	46.1		
5	Blue	274.9	456	D7S820	11	Pass	27.1		
6	Blue	321.1	1154	CSF1PO	10	Pass	97.1		
7	Blue	329.3	702	CSF1PO	12	Pass	46.5		

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 *Sample File Tree*, *Synthetic Gel Image* and *Report Table*, 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.

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 *Peak Table*, the *Peak Table* and the *Electropherogram*, or just the *Electropherogram*.
	Save Peak Table	Allows user to export the *Peak Table* 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 *Start Your Project*, *Run* and/or *Report* dialog boxes.

19.8.3 Browse By All Colors Icons

Icon	Name	Function
	Zoom In/ Zoom Out	Same use as in the *Main Analysis* 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 *Print Report* settings box. Also accessible in the Main Analysis screen.

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

Icon	Name	Description
	Report Settings	Allows user to customize *Report Table* display settings.
	Save Report	Allows user to export the *Report Table* 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)

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 *Bin* center in basepairs
Quality	assigns Pass/Check/Undetermined quality ranking for each peak relative to the Allele Evaluation *Peak Score* 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 drop down list (more in Sample Comments Index)
Quality Reasons	letter code abbreviation(s) of reason(s) for a peak 's *Quality* assignment if ranked Check/Undetermined