GeneMarker v2.9.0 Manual

1 Guiding Principles and Scope

1.1 This method contains the steps for the analysis of PowerPlex Fusion and Yfiler STR runs from the 3130 using GeneMarker HID v2.9.0.

1.2 For more information on the use of GeneMarker HID v2.9.0 see General Overview Section 19.

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 could arise during the analysis of an STR run. If an analyst encounters something that isn't 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**

2.1.1 Navigate to the network folder containing the data files (i.e. the .fsa files) to be analyzed.

2.1.2 Select files to add (include only one ladder per project)

2.1.3 Hold **Ctrl** key to select multiple files

2.1.4 If analyzing all files hold **Ctrl** and **A** keys

2.1.5 Select **Open**

2.1.6 Ensure correct files are added to list

2.1.7 Ensure Auto-Elevate is not selected.

2.1.8 Select OK

2.2 Ensure that prefixes **LD**, **PC** and **NC** are labeling the **allelic ladder**, **positive control** and **negative control**, respectively, in the **Sample File Tree.**

2.2.1 If they are not, right click on the sample name in the Sample File Tree → Set Sample Type → Select appropriate sample type

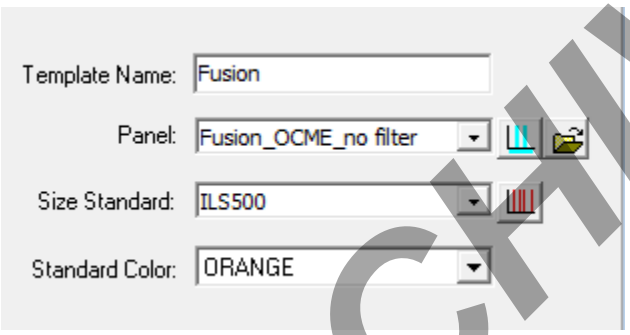
2.2.2 **Click Run Project** located in the top toolbar**.** 

3 Run Wizard

3.1 In Template Selection window, select the appropriate existing template; Fusion (Evidence), Fusion Exemplars, or Yfiler® (Evidence or Exemplars). If "Last Template" is displayed in Template Name field, ensure that the appropriate Panel is listed for your analysis.

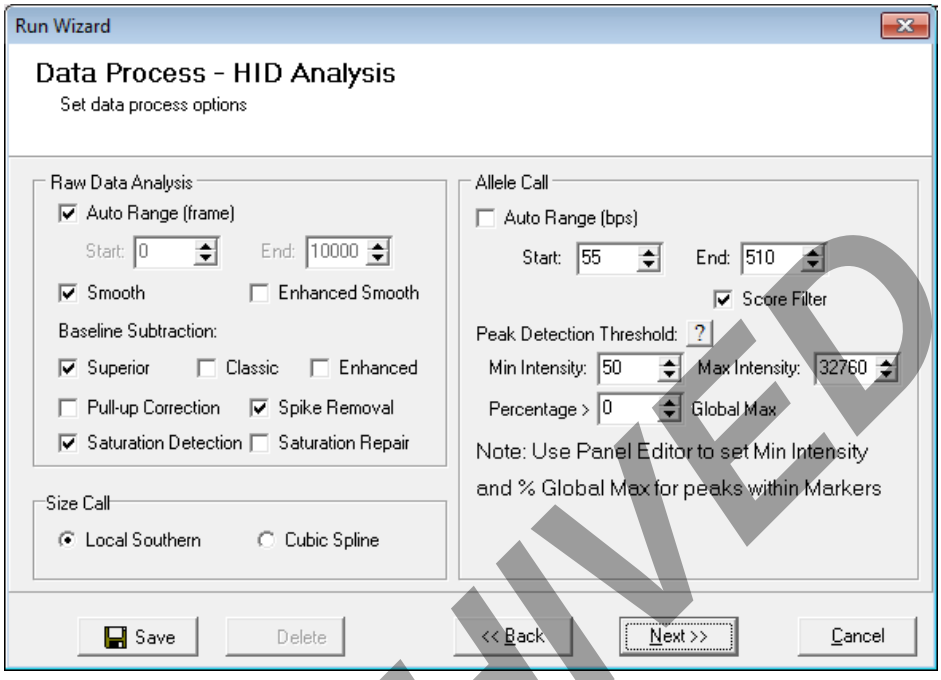
3.1.1 **Note**: Do NOT click Save on any of the following windows. This will overwrite the template for all analysts.

3.2 Fusion (Evidence)



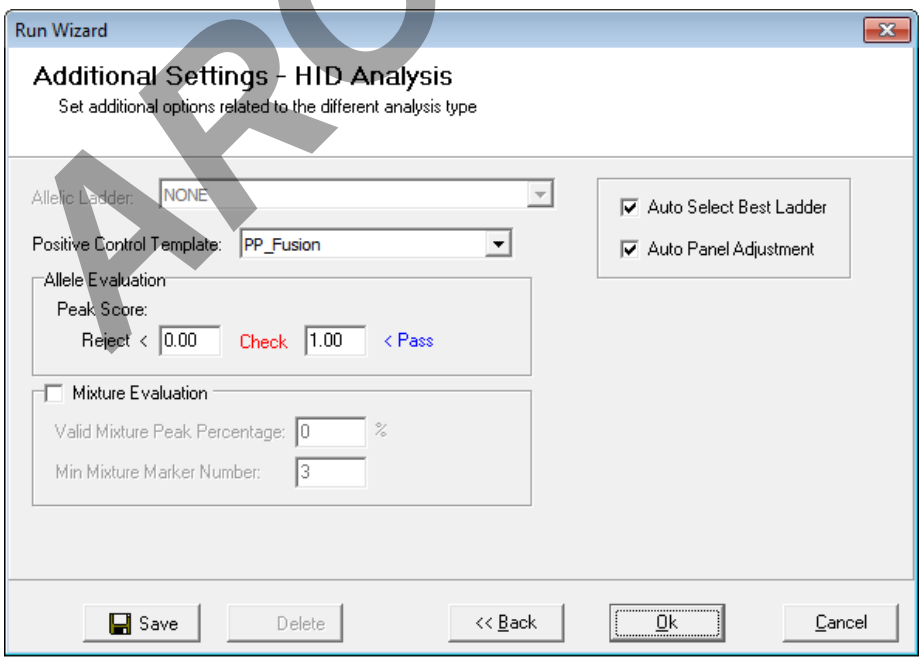
3.2.1 Click Next

3.2.2 In the Data Process window, ensure the following settings are used:



3.2.3 Click Next

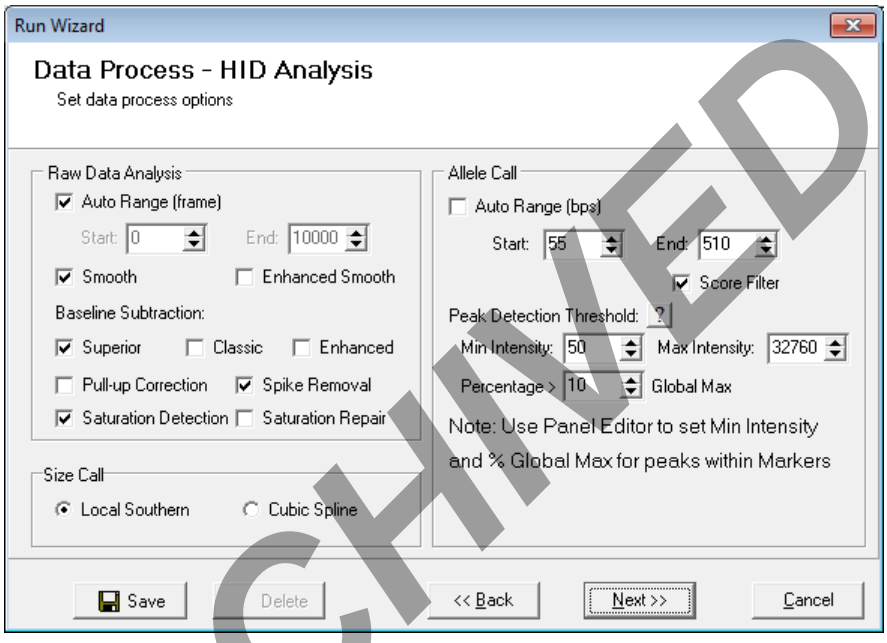
3.2.4 In the Additional Settings window, ensure the following settings are used:



3.3 Fusion Exemplars

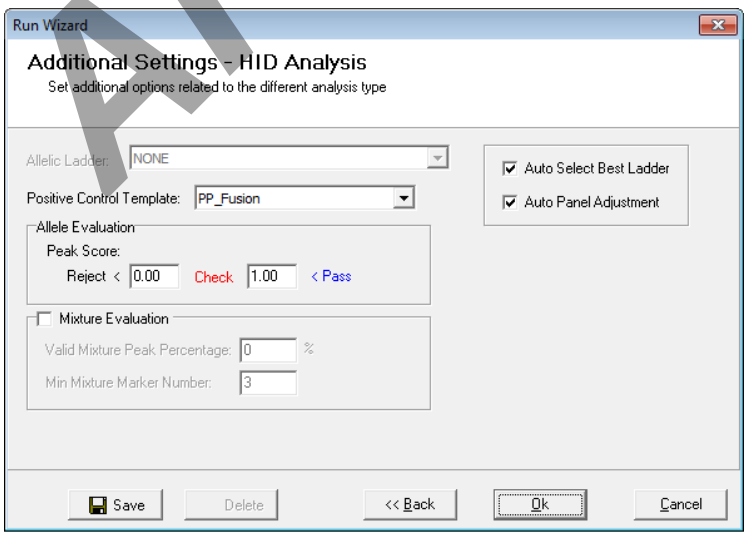
3.3.1 Click Next

3.3.2 In the Data Process window, ensure the following settings are used:

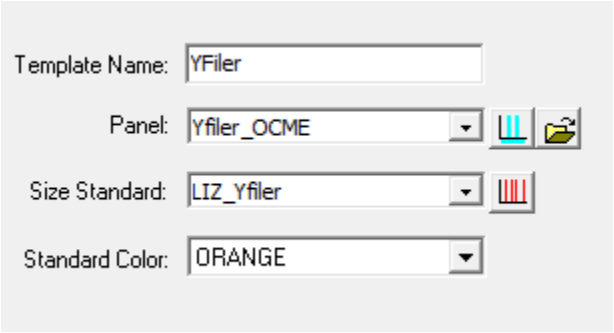


3.3.3 Click Next

3.3.4 In the Additional Settings window, ensure the following settings are used:

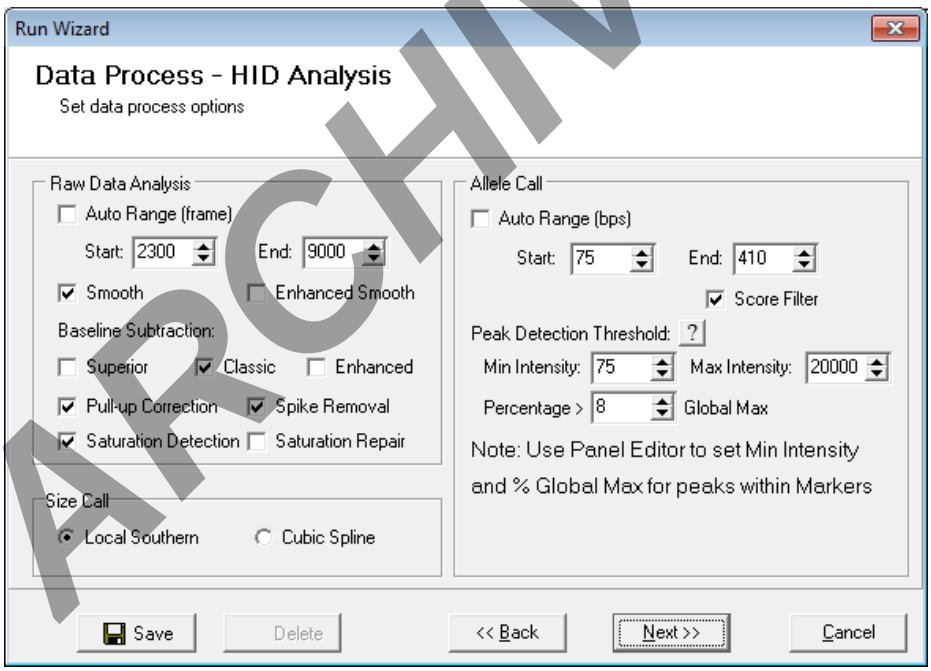


3.4 Yfiler® (Evidence or Exemplars)



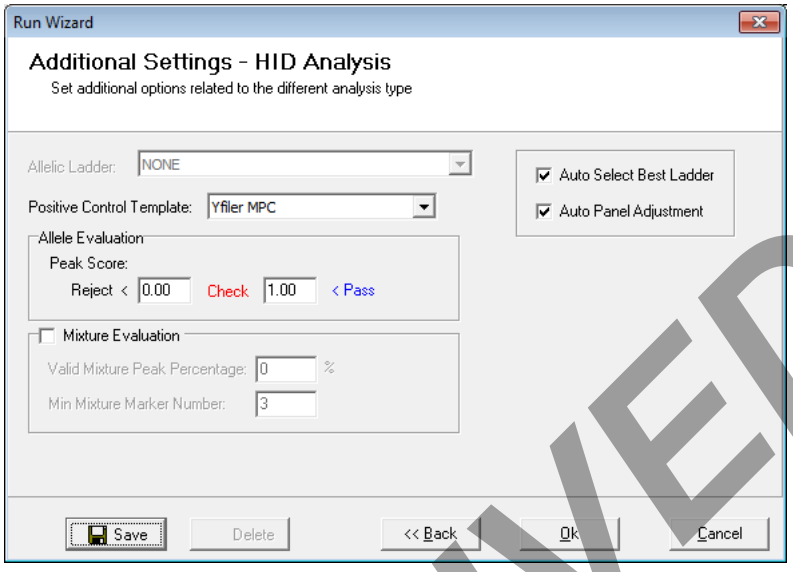
3.4.1 Click Next

3.4.2 In the Data Process window, ensure the following settings are used:



3.4.3 Click Next

3.4.4 In the Additional Settings window, ensure the following settings are used:



3.5 If **NONE** is listed under Positive control template, select the Positive Control Template using the dropdown:

- For PowerPlex Fusion® (all templates) - PP_Fusion
- For Yfiler® - Yfiler MPC

3.6 **Note:** Do NOT click Save within the Data Process Window. This will overwrite the template for all analysts.

3.7 **Click OK** → Once data has finished processing **Click OK**

3.8 On the top left corner of the screen, **Click File → Click Save Project** → Navigate to desired folder and save project using the naming system based on the instrument, date, injection number(s), kit and analysis set (i.e. Newton090615 8-11U A)

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

4 Checking the Size Standard

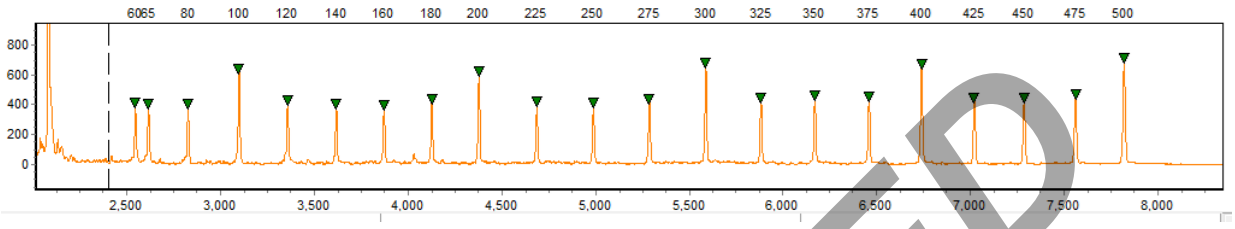
4.1 Located on the top toolbar in the main analysis window, **Click Size Calibration** 

4.2 In the Calibration Charts window, Click Chart Synchronize 

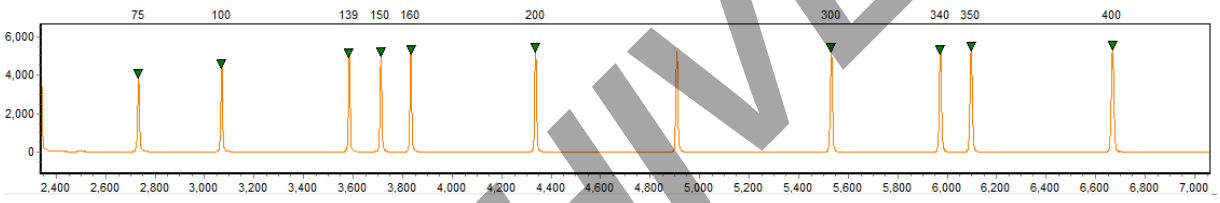
4.3 Click **Sample Name** header to sort samples in order. In the **Sample List,** either **Click on a**

Sample or **Navigate using the Up/Down Arrows**, check the size standard for each sample and control as per the amplification kit.

4.3.1 PowerPlex Fusion®



4.3.2 Yfiler ®



4.3.3 If the peak is recognized by the software, a green inverted triangle appears at the top of the peak.

4.3.4 If a sample contains a size standard peak without a green triangle present the size standard for that sample fails. Refer to STR Results Interpretation-PowerPlex Fusion & STRmixTM manual.

4.4 Close Calibration Charts window.

4.4.1 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. **Right click** on the sample in Sample File Tree within Main Analysis Window → Click **Edit Comments** → **Enter rerun code** in Comments field → Click **OK**

4.4.2 In the Main Analysis window, Click on the Down Arrow next to the Show Color icon located in the top toolbar. → Click Hide All

4.4.3 In the Sample File Tree, Right Click on Allele Call →Click Select Max

4.4.4 In the Main Analysis window, Click on the Down Arrow next to the Show Color  icon located in the top toolbar. → Click ORANGE

4.4.5 Note: The size standard can also be viewed in the Browse by All Color Window

4.5 Adjust axis to view size standard depending on amplification kit

4.5.1 On the top toolbar, Click Set Axis icon 

4.5.2 Click Fixed 'X ' → enter X axis range

- **PowerPlex Fusion® = 55-510**
- **Yfiler® = 75-410**

4.5.3 Click Set Axis again → Select Auto Fit Y

4.6 Review size fragments by ensuring that all required peaks are labeled within +/- 0.5bp of the fragment size. See the Fusion Ladder, PE and SS appendix or for Yfiler, the References - Allelic Ladders, Controls, and Size Standards documents.

4.6.1 If a peak is not labeled or not within +/-0.5bp, then the size standard fails. Refer to STR Results Interpretation-PowerPlex Fusion & STRmixTM manual.

4.7 If any sample with failing or poor size standard has peaks called, the peak labels of those peaks will need to be deleted.

4.7.1 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

4.8 For any sample with failing or poor size standard, right click on sample in Sample File Tree within Main Analysis Window →Click Edit Comments→Enter rerun code in Comments field→Click OK

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 Right click in the Sample File Tree → Click Select Max. Open the Browse by All Color Window icon  . Visually confirm all expected alleles in the allelic ladder are present. Refer to the Fusion Ladder, PE and SS appendix or for Yfiler, the References - Allelic Ladders, Controls, and Size Standards documents.

5.3 If the ladder used for analysis failed, try another ladder present on the same analysis set if one is available. 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 .fsa files → Select a different ladder for analysis → Click **Open** → Click **OK**. Return to Load Data, Section 2.2 and proceed.

5.4 If there are no other passing allelic ladders in the analysis set refer to Refer to STR Results Interpretation-PowerPlex Fusion & STRmixTM manual.

6 Amplification positive control (Fusion/YFiler)

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 including (but not limited to) pull-up, stutter and elevated baseline.

6.4 Visually confirm all the expected alleles in the positive control according to the amplification kit used. Refer to the Fusion Ladder, PE and SS appendix or for Yfiler, the References - Allelic Ladders, Controls, and Size Standards documents..

6.4.1 A positive control that does not generate a complete genotype or gives an incorrect genotype will be indicated as failing. Refer to STR Results Interpretation-PowerPlex Fusion & STRmixTM manual for re-testing strategies

7 Negative Controls (Fusion/YFiler)

7.1 Evaluate the extraction and/or amplification negative control for expected results. If peaks attributed to DNA are detected refer to STR Results Interpretation-PowerPlex Fusion & STRmixTM manual.

7.2 Some artifacts can be edited in a negative control if they are by-products of the STR/amplification process. These include artifacts such as dye blobs, spikes, and primer front. Only re-test if artifacts are so abundant that amplified DNA might be masked.

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 axis to view samples depending on amplification kit

8.2.1 On the top toolbar, Click Set Axis icon

8.2.2 Click Fixed 'X ' → enter X axis range

- PowerPlex Fusion® = 60-510
- Yfiler® = 90-340

8.2.3 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 → Right Click→ Click Edit Comments→ Enter code 'a' for artifact (refer to the editing codes document) → Click OK.

9.1.1 To select multiple peaks for editing, Hold the Ctrl key + left mouse click → Drag the mouse to highlight all consecutive peaks

9.2 Once the Edit is selected, highlight the peak and press Delete key to remove the label from the peak.

9.2.1 **Note:** If a label has been removed from a peak and needs to be restored, **Click on Peak** to Highlight → **Right Click →Undelete.** 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)

9.3 To check peak history → Right click on peak → Click view history

9.3.1 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 edit code applied to peak in Allele Comments column.

9.4 +/- one repeat stutter should not be manually edited from evidence in Fusion. Stutter can be edited from Fusion positive control samples and exemplar samples.

Note: Peaks will only be considered stutter by the software if they are within the bin of the stutter peak. If a peak is outside of the stutter bin of the parent peak the software will not automatically filter this out as stutter, even if is under the stutter filter percentage for that location.

10 Over- Saturation

10.1 Check for saturation detection

10.1.1 In the Main Analysis Window electropherogram a pink line will indicate the possible saturation of peak. This indication will also be noted in the Peak Table as <SAT> for that allele in the Allele Comment field.

10.1.2 If an evidence sample has excessively saturated peaks (>10,000 RFU or split peaks) this sample must be rerun at a dilution. All peak labels must be removed. Enter the appropriate re-run code and dilution factor.

10.1.3 If an evidence sample has labeled peaks above 7,000 RFU, with minimal artifacts, editing may be performed. If there are many instances of pull-up, pull-down, and/or elevated baseline, the sample may be rerun at a dilution. All peak labels must be removed. Enter the appropriate re-run code and dilution factor.

10.1.4 Exemplars or positive controls with saturated peaks may be interpreted if artifacts are easily recognizable and can be edited. With the exception of Fusion Direct samples, exemplars may be rerun at a dilution if needed.

11 Scheduling Re-Runs

11.1 If a sample **Failed** (over saturation, bad size standard, no data, instrument issue, etc.) → Right click on **sample** in **Sample Tree** within Main Analysis Window → Click **Edit Comments** → Type Re-Run Code in the comment 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 as to why the sample failed under the sample status column – ex. possible injection issue.

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 click and dragging around any peaks present in that dye → click **Delete** to remove the peak labels→ repeat for each dye.

11.3 If sample did not fail but re-run is desired → Right click on **sample** in **Sample Tree** within Main Analysis Window → Click **Edit Comments** → Type Re-Run Code in the comment 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.

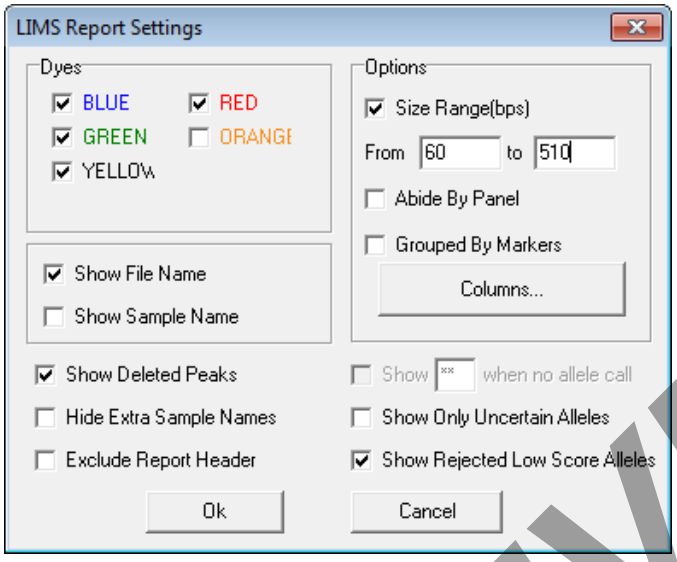
12.3 In the Main Analysis Window → Click the down arrow next to the Show All Color icon → Click **Show All** to turn on all colors before exporting.

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

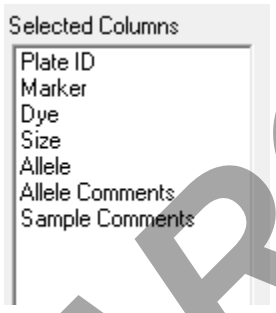
12.5 In the LIMS report settings window that pops up, ensure the settings are as follows:

12.5.1 The Size Range will be the same as the X- axis range for analysis

- 90-340 for Yfiler
- 60-510 for Fusion/Fusion Exemplars



12.5.2 In **Options**, click on **"Columns..."**; use the Add/Remove buttons to ensure the Selected Columns are present and listed in the order that follows:



Note: This is crucial to proper LIMS importation.

12.5.3 Click OK; Click OK again in the Allele Report Settings window.

12.6 Save the file as type "Excel File (*.xlsx;*.xls)" under the appropriate run name (delete suffix "_AlleleReport").

12.7 Navigate to the data entry screen for the corresponding test batch in LIMS. Click Import Instrument Data and import the excel file for the project.

12.8 Fill out the data entry screen keeping in mind the following definitions:

12.8.1 Loaded – the data from GeneMarker has been imported

12.8.2 Not loaded –the sample or control was not imported/loaded into GeneMarker

12.8.3 Pass – the control has passed

12.8.4 Fail – the control has not passed

12.8.5 No Data – the control has not been analyzed

12.8.6 Not Used – the control was not used in analysis of the STR run, typically reserved for additional allelic ladders

12.9 If a control needs to be rerun, a comment (reason as to why the sample failed) and resolution must be entered into the LIMS data entry screen.

13 Printing Initial Analysis Electropherograms (all system and sample types)

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

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

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

13.1.2 Include all samples that failed for reasons other than "no or poor size standard." The .pdfs of the electropherograms with all peak labels removed must be included within the casefile.

13.2 Click View→ Preferences → Others tab

13.2.1 Ensure box for "Enable Sample Grouping" is checked → Click OK

13.3 In Main Analysis Window click Project → Apply Sample Grouping (if grayed out return to step 13.1)

13.3.1 Click Group By Order on the bottom left

13.3.2 Change Group Size to 1

13.3.3 Click **Match** on the bottom right → Click OK

13.4 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:

- 60-510 for Fusion

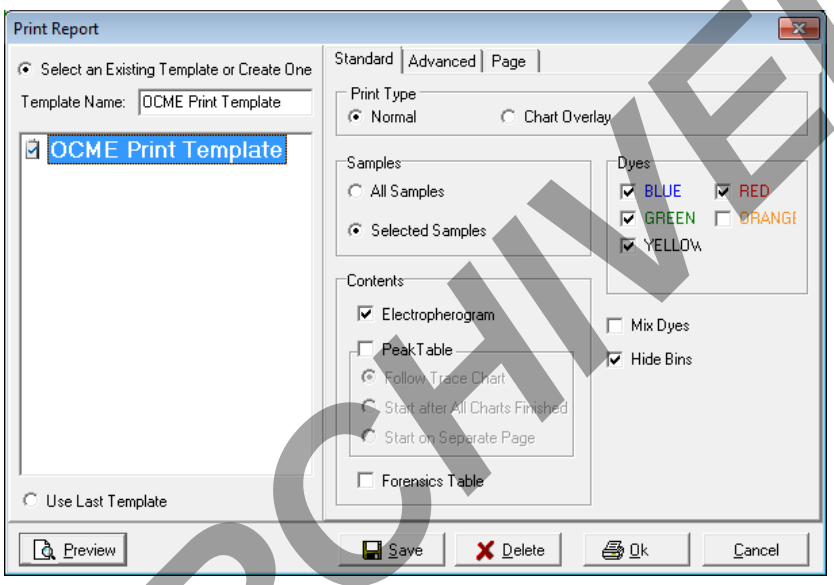
- 90-340 for Yfiler

13.5 Click Set Axis again → Select Auto Fit Y 

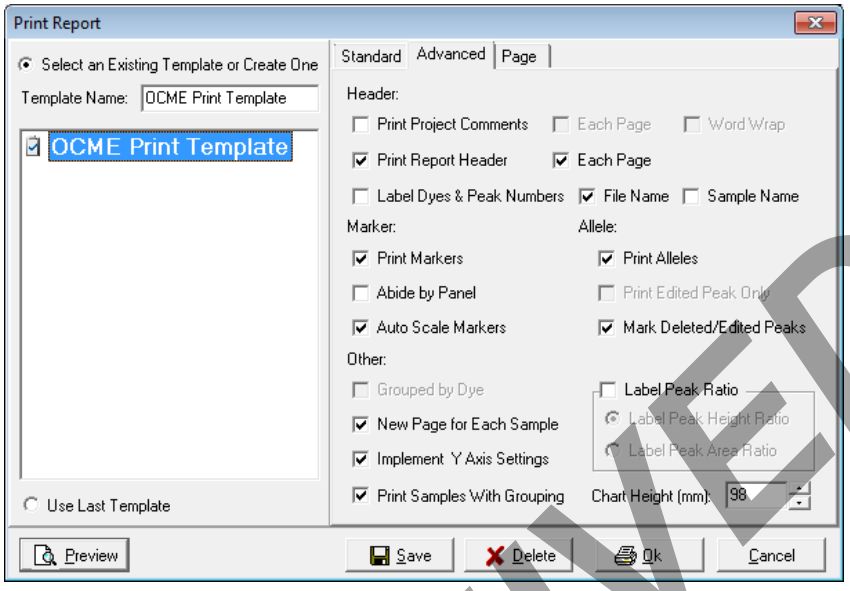
13.6 In Main Analysis window → Click Print Icon

13.6.1 Select OCME Print Template on left and ensure the settings match below:

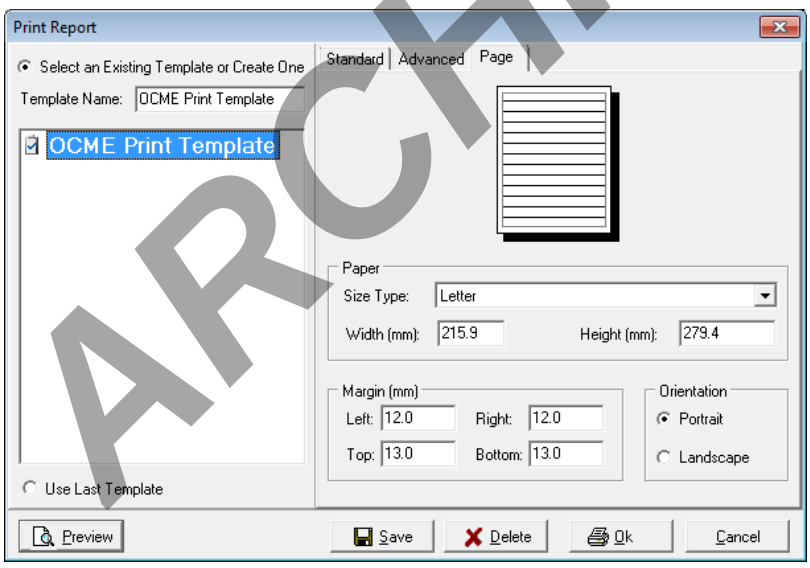
13.6.1.1 Standard Tab:



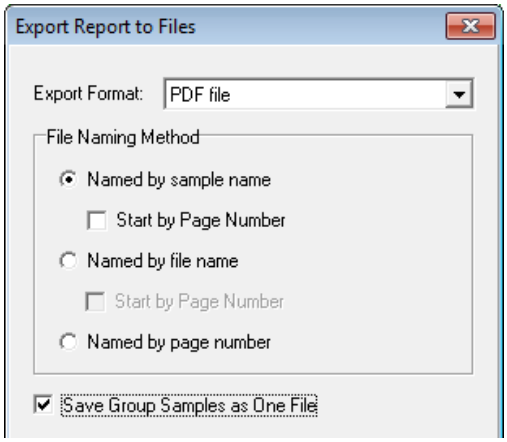
13.6.1.2 Advanced Tab:



13.6.1.3 Page Tab



13.6.2 Click Preview → Click Export to File icon Assure settings match below:



13.6.3 Click on the "…" icon to choose the appropriate export directory for the PDF files

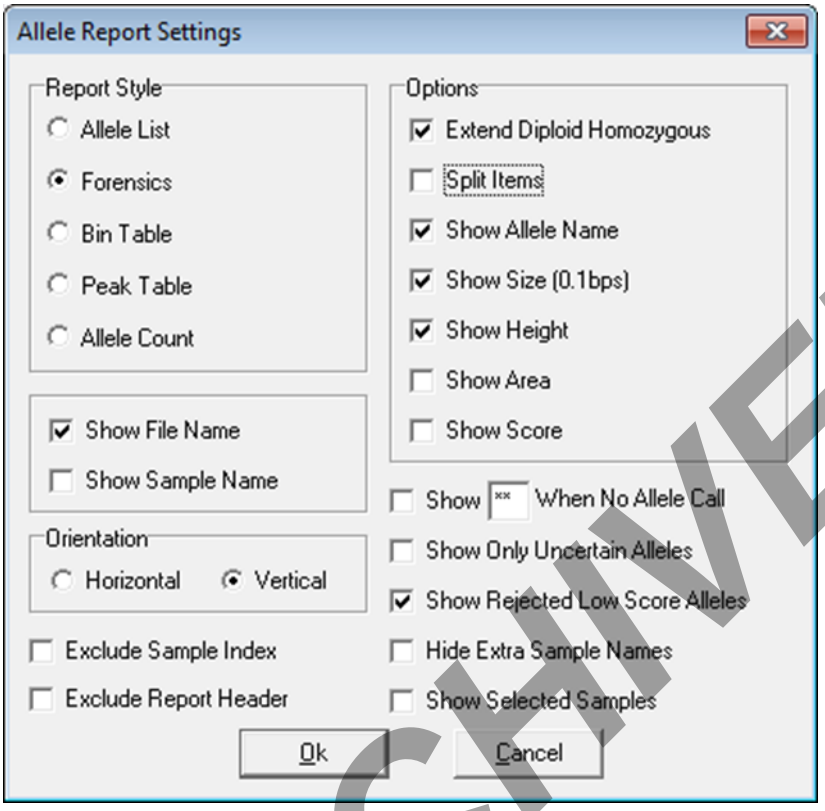
13.6.4 Click OK → Export Report to PDFs Events window will appear. Once completed click OK

13.7 If analyzing Fusion **Evidence**: Wait to import PDFs into LIMS until after the completion of STRmix analysis, section 15.

13.8 If analyzing **Exemplars or Yfiler Evidence**: Import all PDFs into LIMS.

14 Fusion Exemplars Only - Exporting EXEMPLAR table for STRmixTM input (for evidence table instructions see Section 17)

14.1 In Main Analysis Window **Click the**  **icon** → Select **Show All**. In the report display section of the main analysis screen, click on the report settings icon  . Make sure settings match below:



14.2 Click OK → In the report display section of the main analysis screen, click on the down arrow next to the Save Report icon _____. Ensure the "Save Report" is selected.

14.3 Navigate to appropriate folder and save file as an excel file (*.xlsx;*.xls)"named as "Run name_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 Step 14.3 above.

14.4.2 Open GeneMarker® to STRmix™ macro.

14.4.3 Follow the instructions on the "instructions" tab.

14.5 After macro has run click "**save as**" within the GeneMarker® to STRmix™ macro file.

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

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

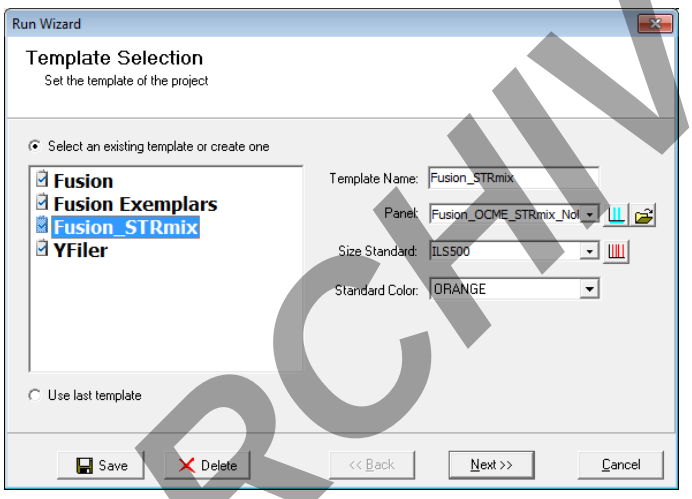
15 Fusion Evidence Only - STRmix™ Analysis (stutter filters off)

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 the second time.

15.1.1 If Project is still open → **Click Run Project**  located in the top toolbar**.**

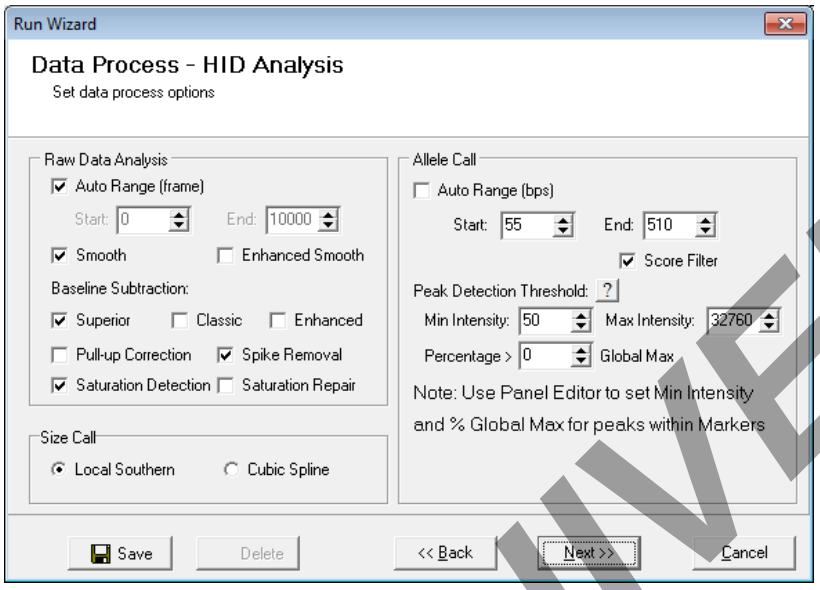
15.1.2 If project is not open → Click File → Open Project → Navigate to run → select project → click open → Click Run Project  located in the top toolbar.

15.2 In Template Selection window, select the appropriate existing template.



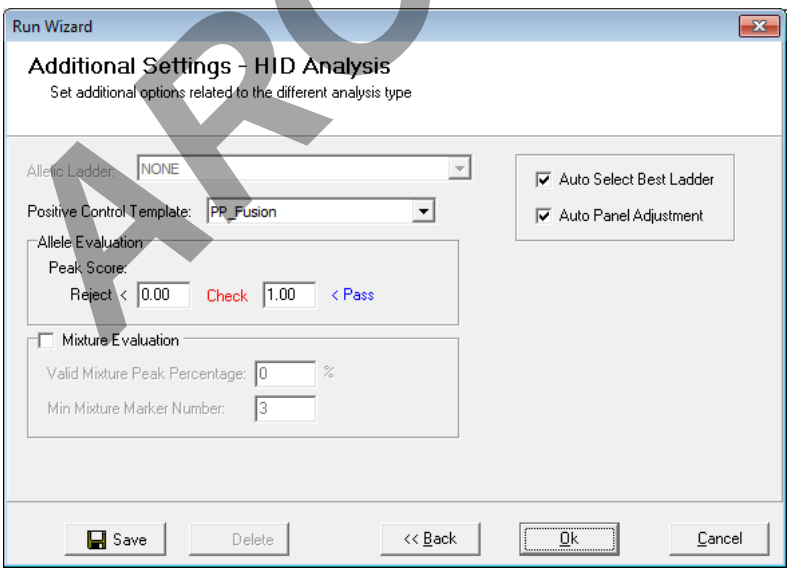
15.3 Click Next

15.4 In the Data Process window, ensure the following settings are used:



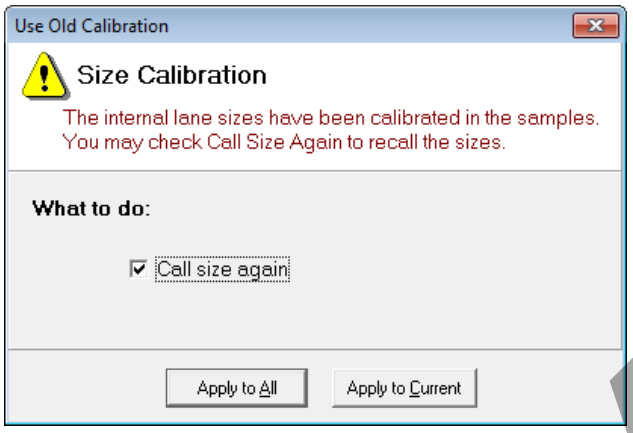
15.5 Click Next

15.6 In the Additional Settings window, ensure the following settings are used:



15.6.1 **Note**: Do NOT click Save within the Data Process Window. This will overwrite the template for all analysts.

15.7 Click OK → A window will pop up:



15.7.1 Check "Call size again" and Click Apply to All → Allow data to process → Click OK

15.8 On the top left corner of the screen, Click File → Click Save Project → Save Project with suffix _SM

15.9 Perform a second analysis of this run

15.9.1 Do not recheck or edit Ladder, Size Standard or Controls. Passing controls and size standard are determined by the initial analysis. **Leave all controls and failed samples ENABLED**

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

15.9.2.1 If an artifact (excluding stutter) 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. This edit must be recorded directly into the LIMS data entry screen.

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

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

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 Sample File Tree → right click → Select Max

16.2 Double click on sample to **DESELECT**:

- All Controls (Ladder, PC, NC, Eneg, Mneg)
- Any failed sample

16.3 Click View→ Preferences → Others tab

16.4 Ensure the 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 16.1)

16.6 Click Group By Order on the bottom left

16.7 Change Group Size to 1

16.8 Click Match on the bottom right → Click OK

16.9 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:

- 60-510 for Fusion

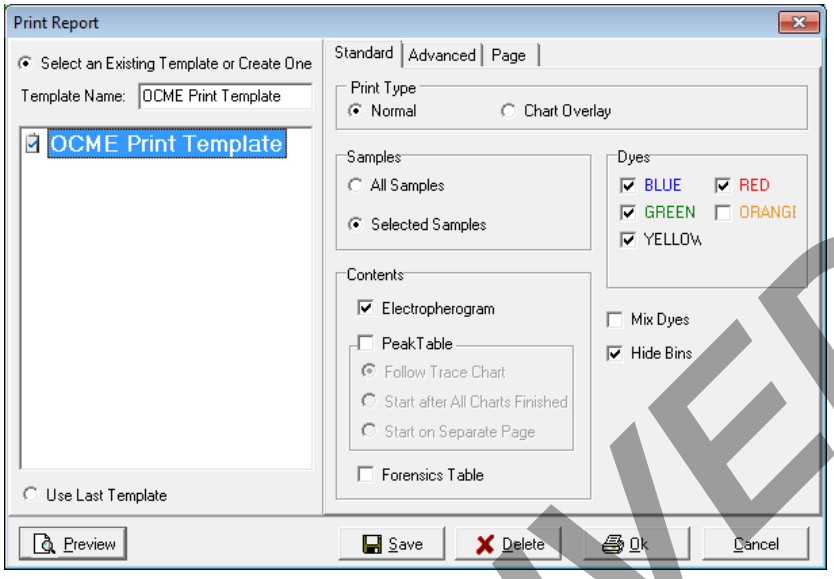
16.10 Click Set Axis again → Select Auto Fit Y

16.11 In Main Analysis window → Click Print Icon

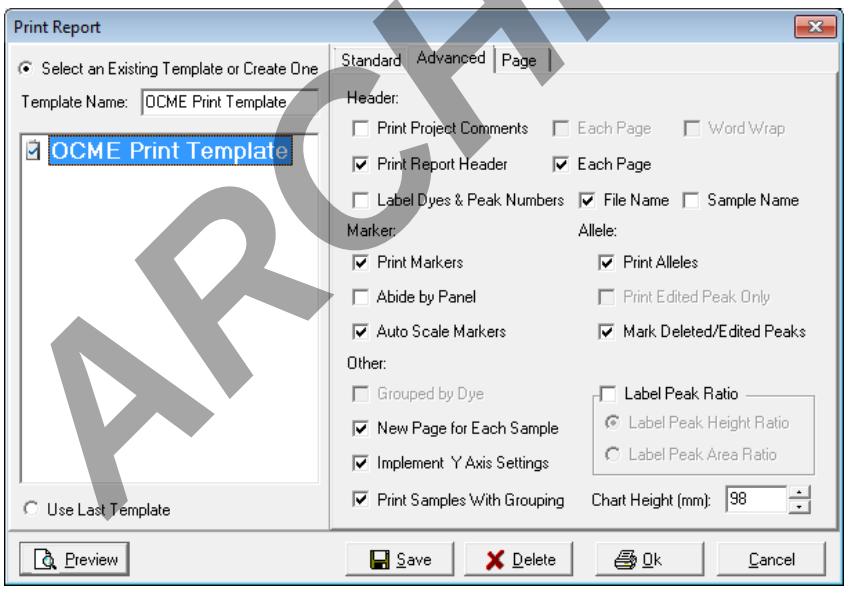
16.12 Select OCME Print Template on left

16.13 Ensure the settings match below:

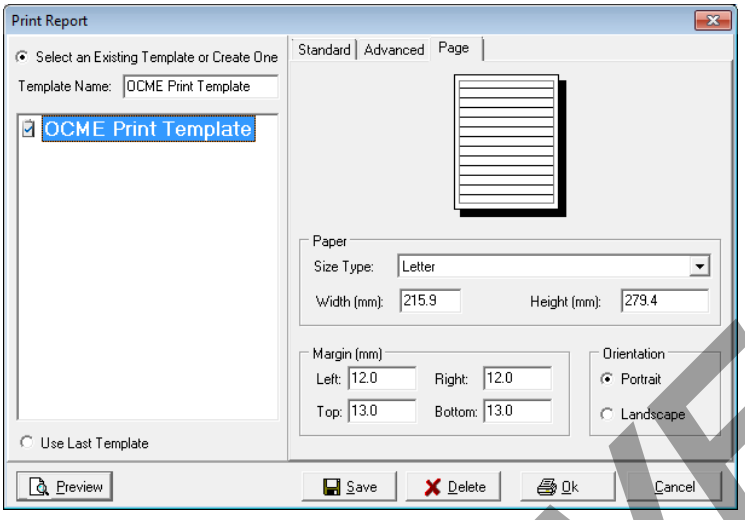
16.13.1 Standard Tab:



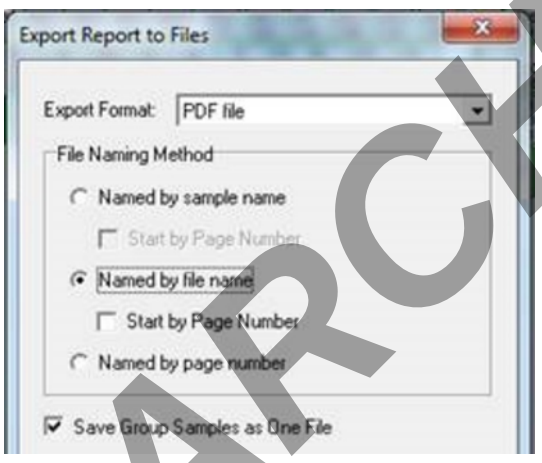
16.13.2 Advanced Tab:



16.13.3 Page Tab



16.14 Click Preview → Click Export to File icon Assure settings match below:



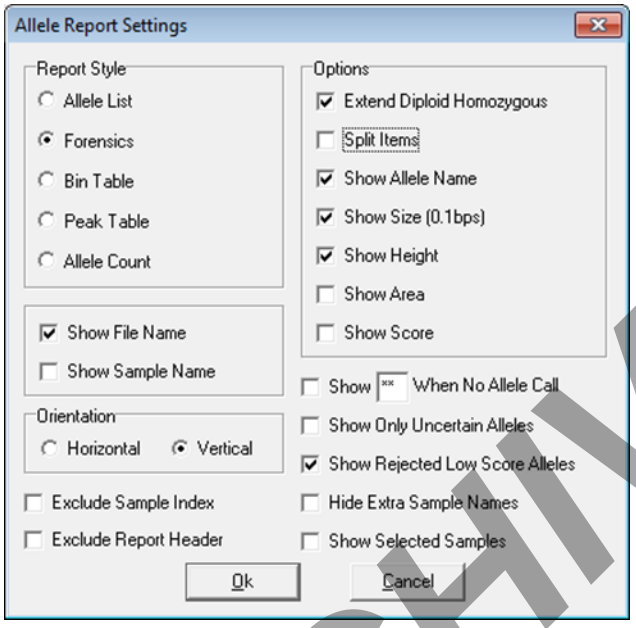
16.15 Click on the "…" icon to choose the appropriate export directory for the PDF files

16.16 Click OK → Export Report to PDFs Events window will appear. Once completed click OK

16.17 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 report display section of the main analysis screen, click on the report settings icon . Make sure settings match below:



17.2 Click **OK**

17.3 In the report display section of the main analysis screen, click on the **arrow** of the Save Report icon . Click "Save Report"

17.4 Navigate to appropriate folder and save file as "Excel File (*.xlsx;*.xls)" named as "Run name_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 17.4 above.

17.5.2 Open GeneMarker® to STRmixTM macro.

17.5.3 Follow the instructions on the "instructions" tab.

17.5.4 After macro has run click "**save as**" within the GeneMarker® to STRmix™ macro file.

17.5.5 Change the file type to "**.txt**" (Text, tab delimited)

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

17.5.7

18 Re-printing/exporting a PDF for a sample from a run that was analyzed in GeneMarker v 2.8.2

18.1 In the main analysis window,

18.1.1 Click Set Axis → Click Fixed X axis

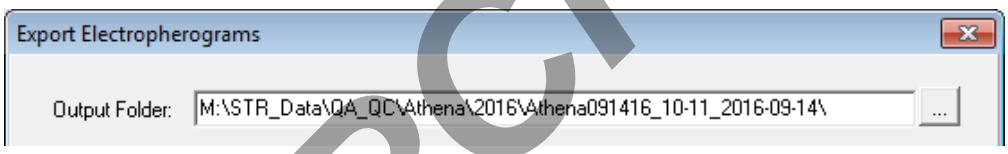
18.1.2 The Size Range will be the same as the X- axis range for analysis:

- 60-510 for Fusion
- 90-340 for Yfiler

18.1.3 Select Auto Fit Y

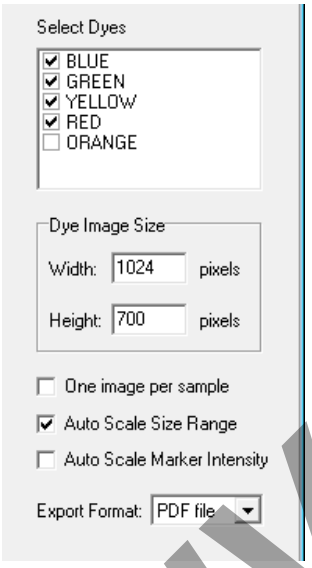
18.2 Click Tools→ Export Electropherogram

18.3 Set Output Folder to desired location for project PDFs by clicking  icon



18.4 Ensure settings match below:

18.4.1 Make sure to select PDF as export format



18.4.2 For exemplar or non-SM analysis: In the suffix field enter the run name with a "_" before it. (Ex: _Newton061316 88-89U A)

18.4.3 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.

18.5 Printing the **ladder**: Right click in the sample list → click Deselect All → Check the box for the ladder → In prefix field enter **ladders_** → click **Export**

18.6 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**

18.7 Printing the **samples**: Right click in the sample 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.

18.8 Import all PDFs (initial Fusion analysis and STRmix analysis, as applicable) at the same time into LIMS.

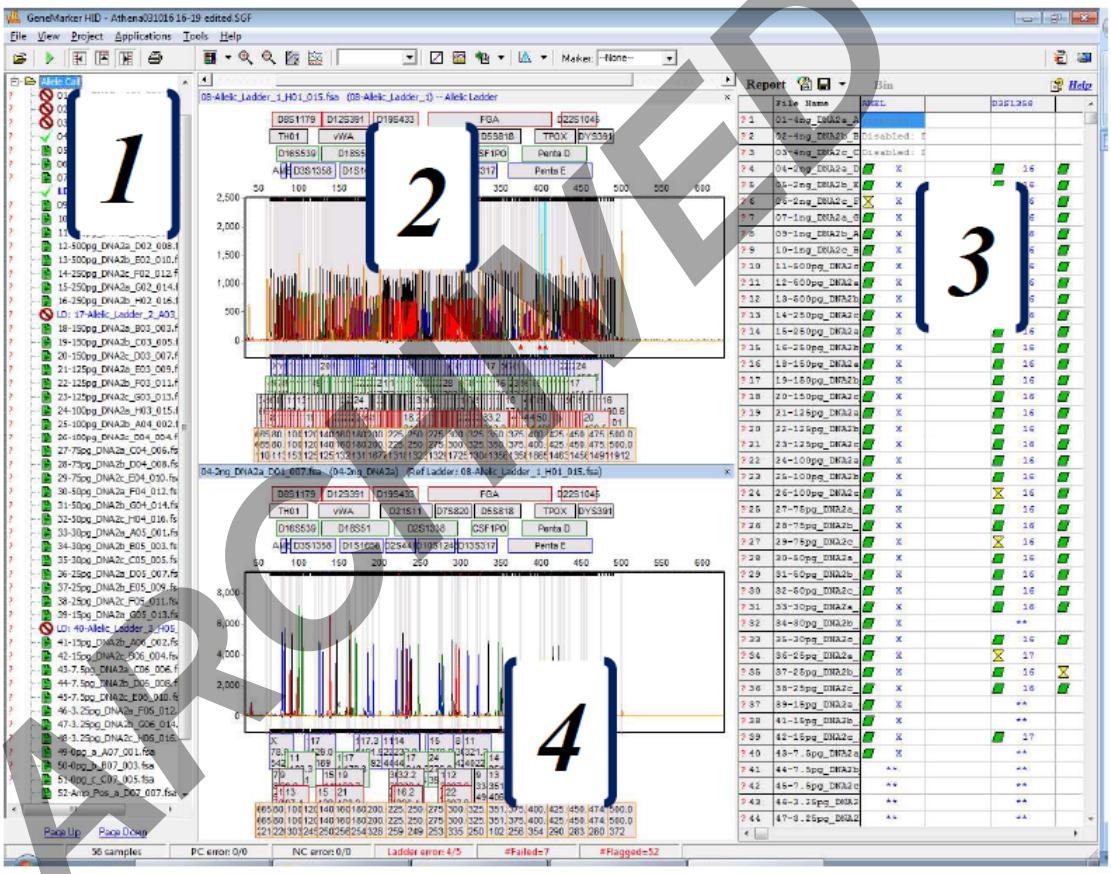
19 General Overview

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**

19.1.2 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**, **PC** and **NC** are labeling the **allelic ladder**, **positive control** and **negative control**

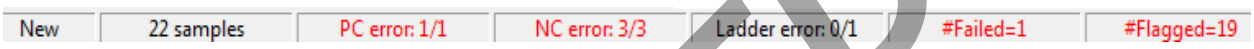
19.2.2 The sheet icon to the left of the sample name indicated the quality of the internal lane standard (ILS) with the following color/code:

- 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 located at the bottom of the main analysis screen summarizes the project as well as alerts the analyst to samples that did not pass analysis parameters.



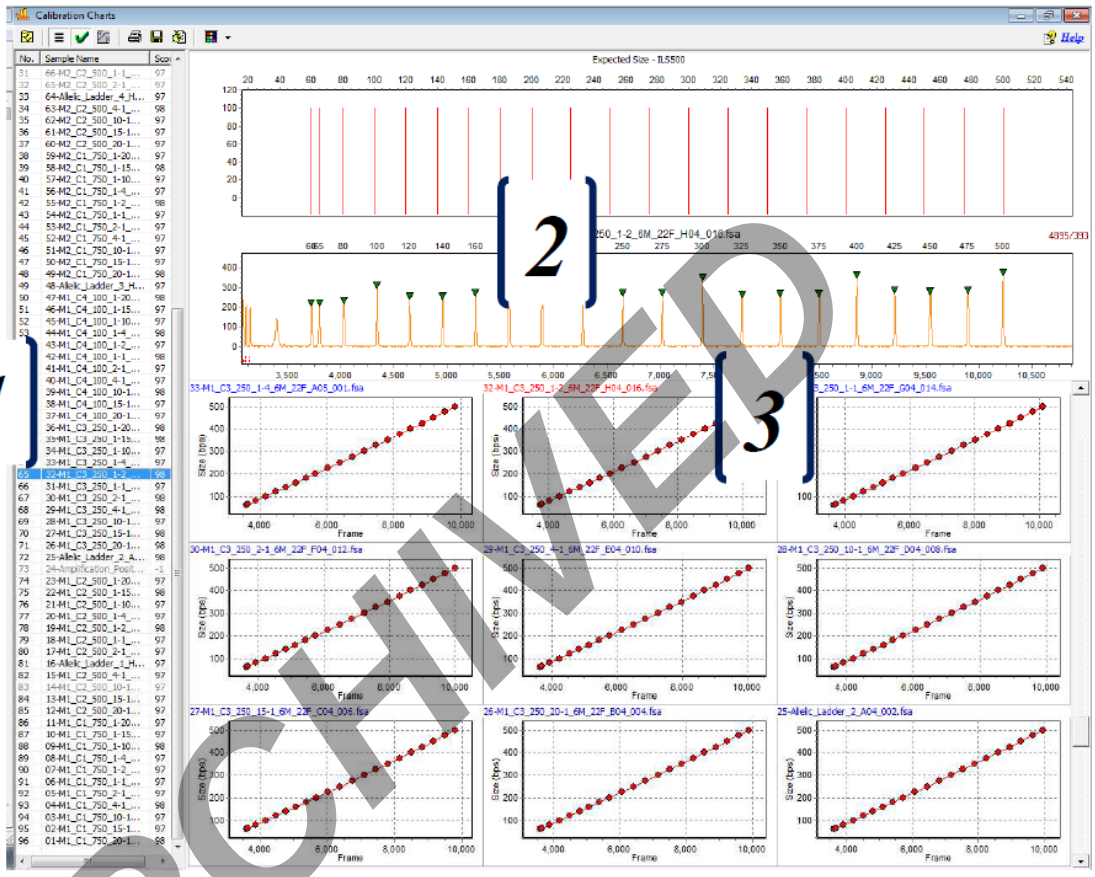
- New reflects the data set is not a project that has been previously analyzed.
- If the first column reads Modified, the project was previously analyzed in the software.
- The second column denotes the number of samples (including controls) contained in the data set.
- Columns 3-5 indicate the number of controls in the data set that do not meet the quality criteria set in the software.
- Column 6 denotes the number of samples that were disabled.
- Column 7 denotes the overall number of samples, except the allelic ladder, that do not meet the quality criteria set in the software.

19.3.2 **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



19.5 Sample List

19.5.1 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.

19.6 Size Standard Template

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

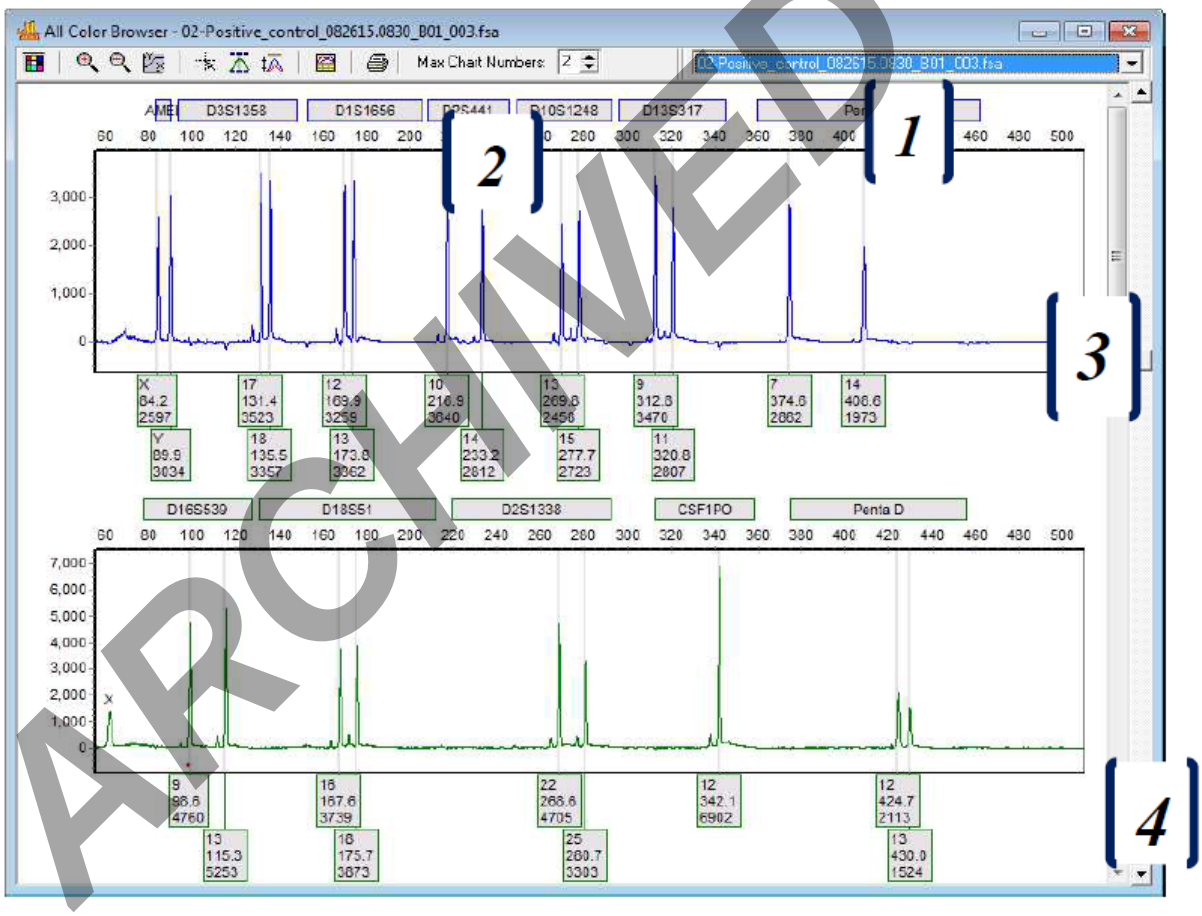
19.7 Samples ILS

19.7.1 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.

19.8 Browse by All Color Window

19.8.1 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



19.9 Sample Drop-Down

19.9.1 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.

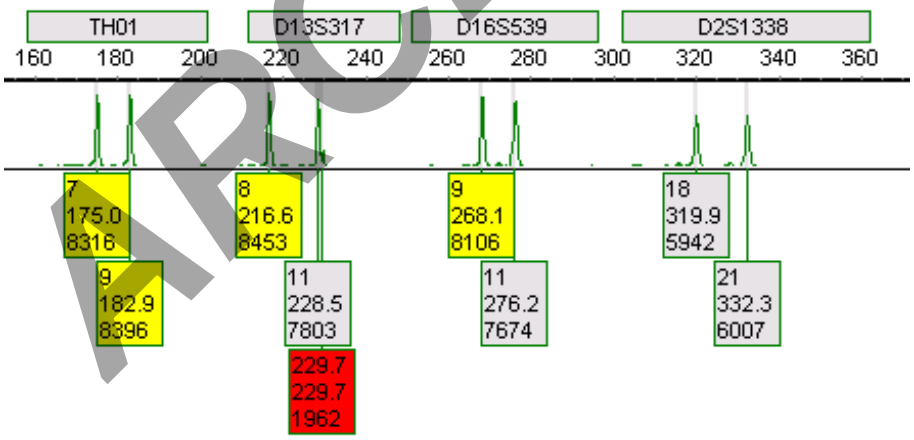
19.9.2 **NOTE:** Analysts can zoom in/out by using the zoom icons  located at the top toolbar. As an additional option, hold down the left button on the mouse and drag the dotted box that appears from the upper left to the lower right (around the desired "zoom in" area). To zoom out, hold down the left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

19.10 Max Chart Number

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

19.11 Allele Labels

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

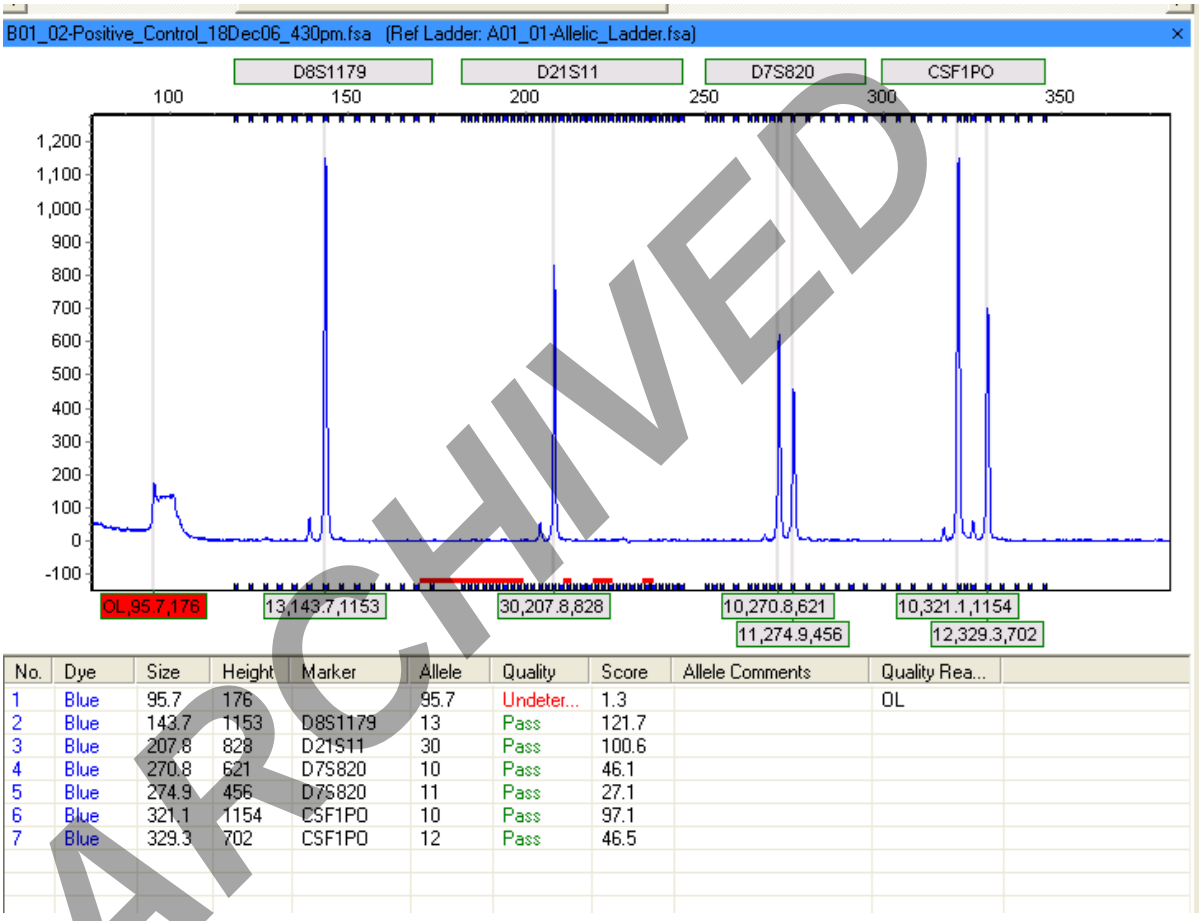


19.11.2 This indicates that one or more quality criteria have not been met in the software. See **Quality Reasons Index** for more details.

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

19.12 Peak Table

19.12.1 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 



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		

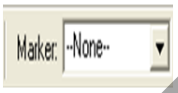
19.12.2 This table will indicate to an analyst which alleles were flagged and which quality reason was triggered. See PEAK TABLE OPTIONS INDEX for further details.

20 HELPFUL ICONS INDEX

20.1 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.**

20.2 **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.**

20.3 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.**

20.4 **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)**

21 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 drop down list (more in Edit Comments Index)**
Sample Comments	**user added comments for the sample; right mouse 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**