

GeneMarker Manual		Document ID: 6311
Status: Published		
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 1 OF 33

# GeneMarker Manual

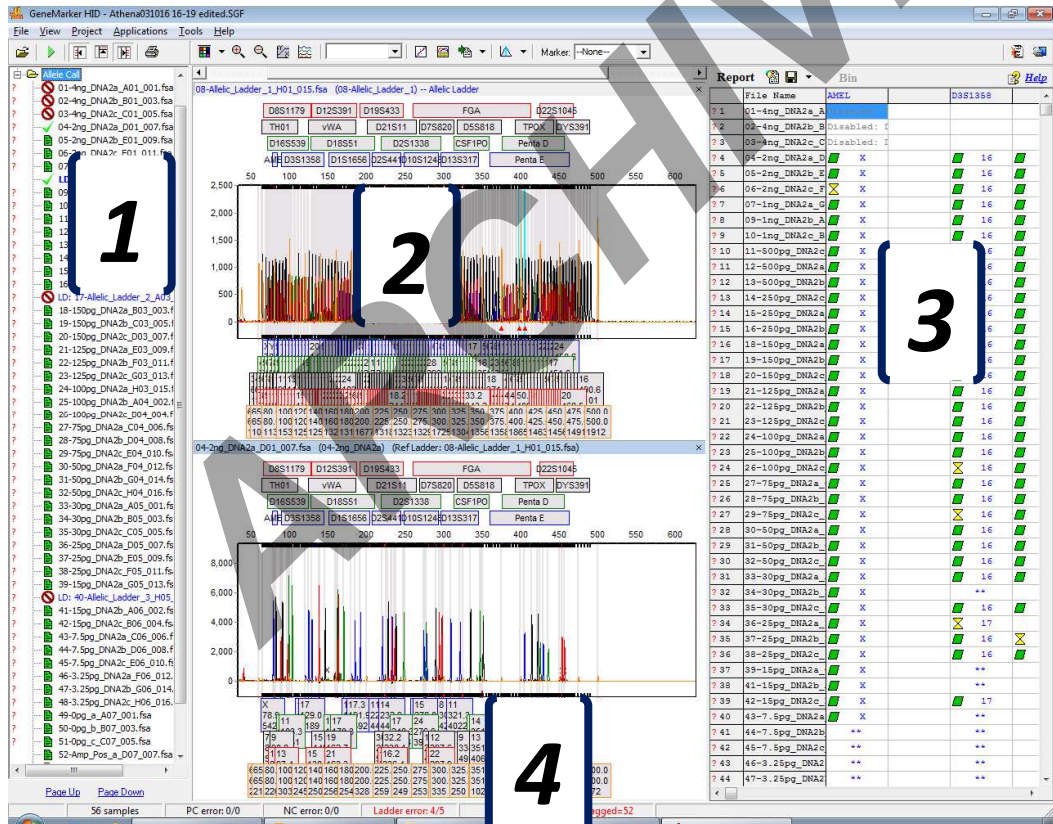
## I. General Overview

### Main Analysis Window

There are four major displays in the main analysis window.

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

See **Helpful Icons Index** for further details.



Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.




## FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

<b>GeneMarker Manual</b>		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 2 OF 33

### Navigator; Sample File Tree

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**

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

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

### Project Summary

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

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

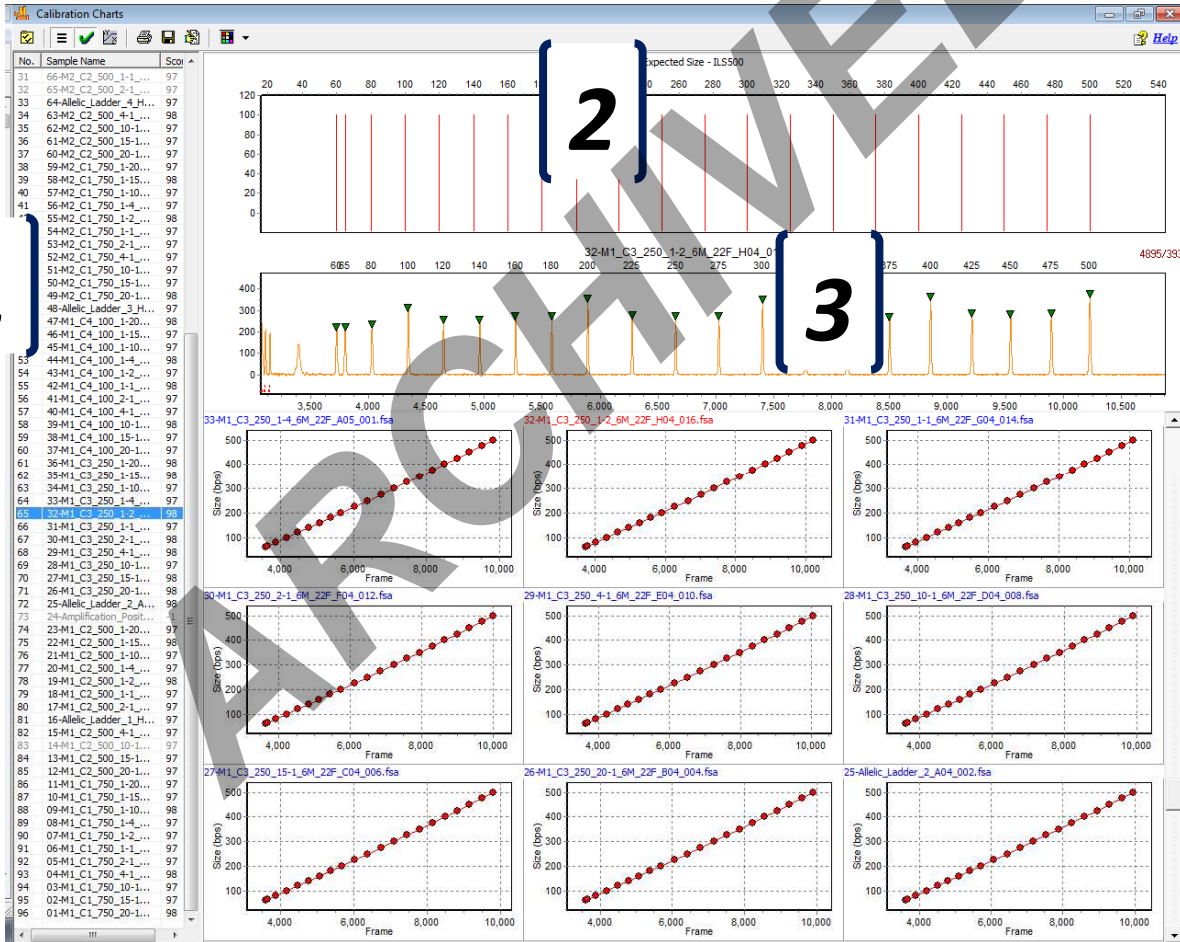
**NOTE: Flagging features are an indication of potential failures. The ultimate decision of passing/failure is made by the analyst after further evaluation.**

<b>GeneMarker Manual</b>		
Status: Published	Document ID: 6311	
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 3 OF 33

### Calibration Chart Window

There are three major displays in the calibration chart window.

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



### Sample List

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.

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
<b>DATE EFFECTIVE</b> 04/20/2017	<b>APPROVED BY</b> Nuclear DNA Technical Leader	<b>PAGE</b> 4 OF 33

The sample may fail automatically if the size standard is bad enough, or may have to be failed manually by the analyst.

**Size Standard Template**

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

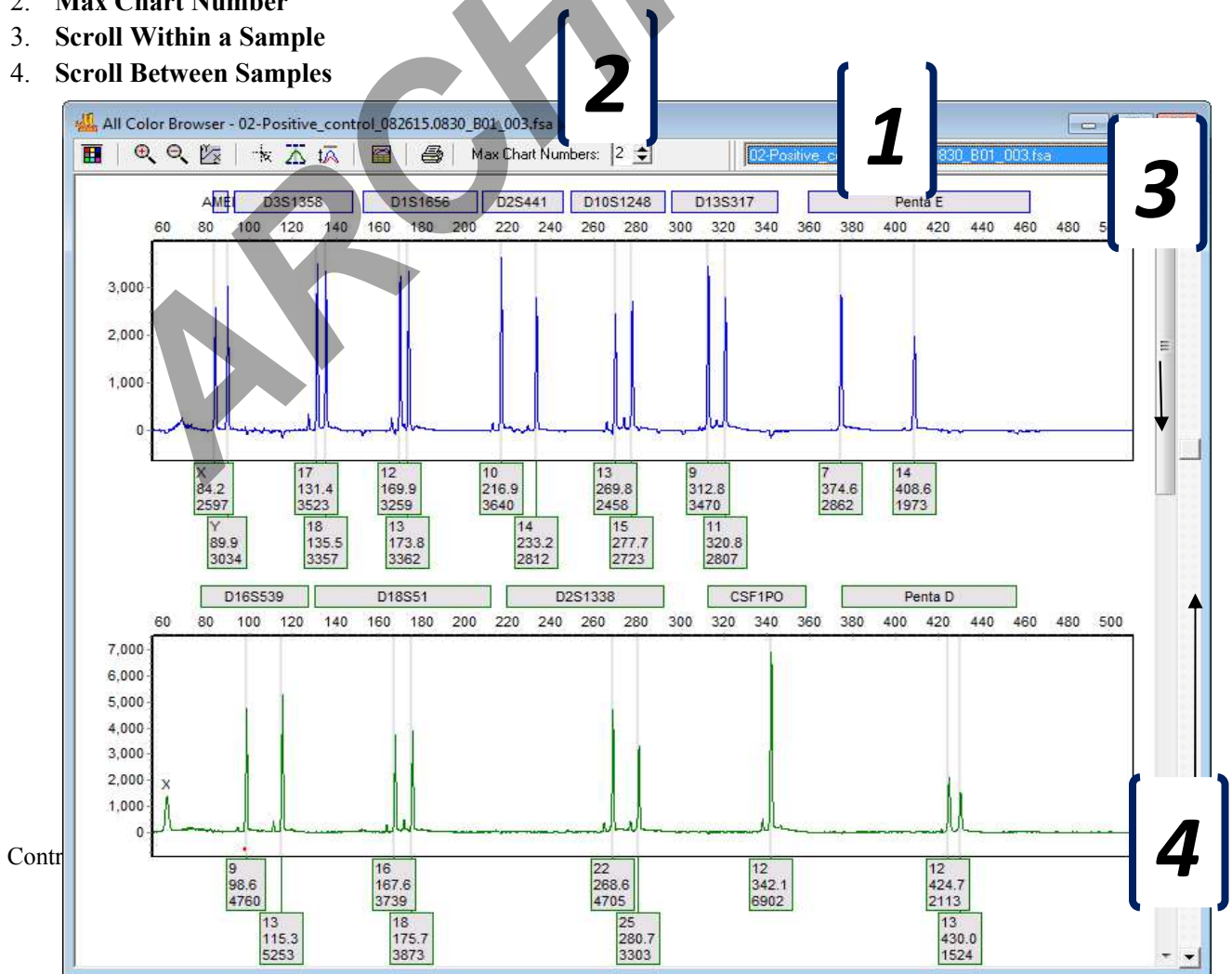
**Samples ILS**

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.

**Browse by All Color Window**

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**



<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 5 OF 33

### Sample Drop-Down

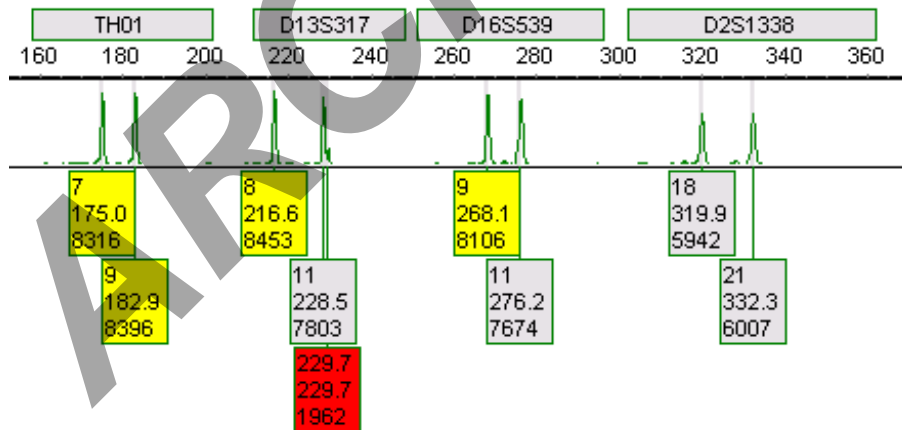
The sample drop-down lists all the samples within a project. A user can move between samples by clicking on the sample name instead of scrolling through all electropherograms.

### Max Chart Number

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

### Allele Labels

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




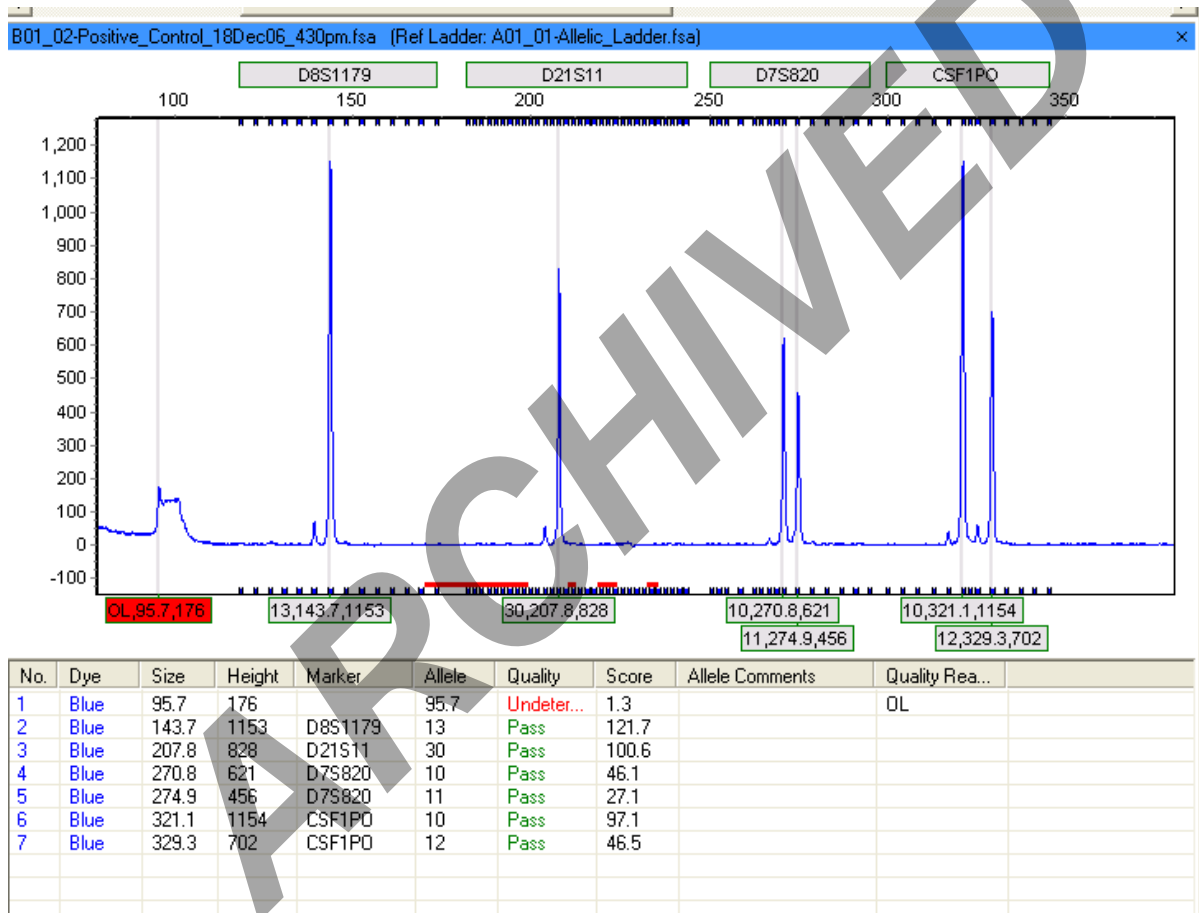
This indicates that one or more quality criteria have not been met in the software. See **Quality Reasons Index** for more details.

**NOTE:** Flagger 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.

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 6 OF 33

## Peak Table

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 icon 

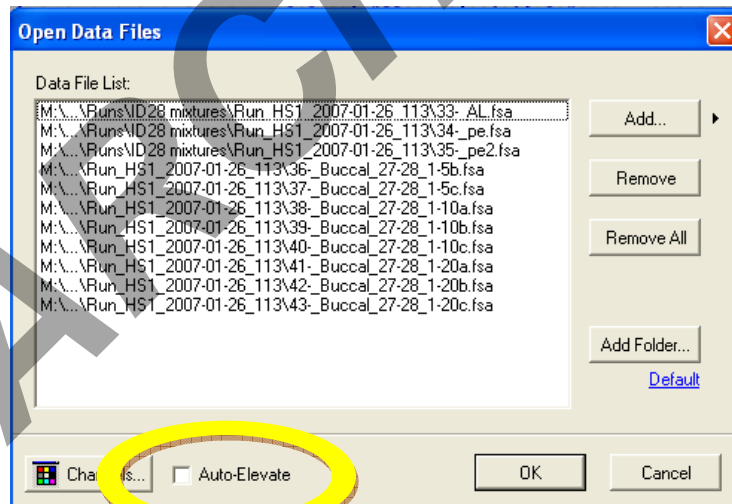


This table will indicate to an analyst which alleles were flagged and which quality reason was triggered. See **Peak Table Options Index** for further details.

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 7 OF 33

## II. Load Data

1. To start your project, go to **File** → **Open Data** → **Add**
  - i. Navigate to the network folder containing the data files (i.e. the .fsa files) to be analyzed.
  - ii. Select files to add (**include only one ladder per project**)
    - i. Hold **Ctrl** key to select multiple files
    - ii. If analyzing all files hold **Ctrl** and **A** keys
  - iii. Select **Open**
    - i. Ensure correct files are added to list
  - iv. **Deselect** Auto-Elevate



- v. Select **OK**
2. Ensure that prefixes **LD**, **PC** and **NC** are labeling the **allelic ladder**, **positive control** and **negative control**, respectively, in the **Sample File Tree**.
    - i. If they are not, **right click** on the sample name in the **Sample File Tree** → **Set Sample Type** → **Select appropriate sample type**

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 8 OF 33

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



### III. Run Wizard

1. In **Template Selection** window, select the appropriate existing template.

*a. PowerPlex Fusion® LIZ*

Template Name: Fusion

Panel: Fusion\_OCME\_no filter

Size Standard: ILS500

Standard Color: ORANGE

Analysis Type: HID

*b. Yfiler® LIZ*

Template Name: Yfiler

Panel: Yfiler\_OCME

Size Standard: LIZ\_Yfiler

Standard Color: ORANGE

Analysis Type: HID



# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

<h2>GeneMarker Manual</h2>		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 9 OF 33

2. **Click Next**

3. In the **Data Process** window, ensure the following settings are used based on amplification kit.

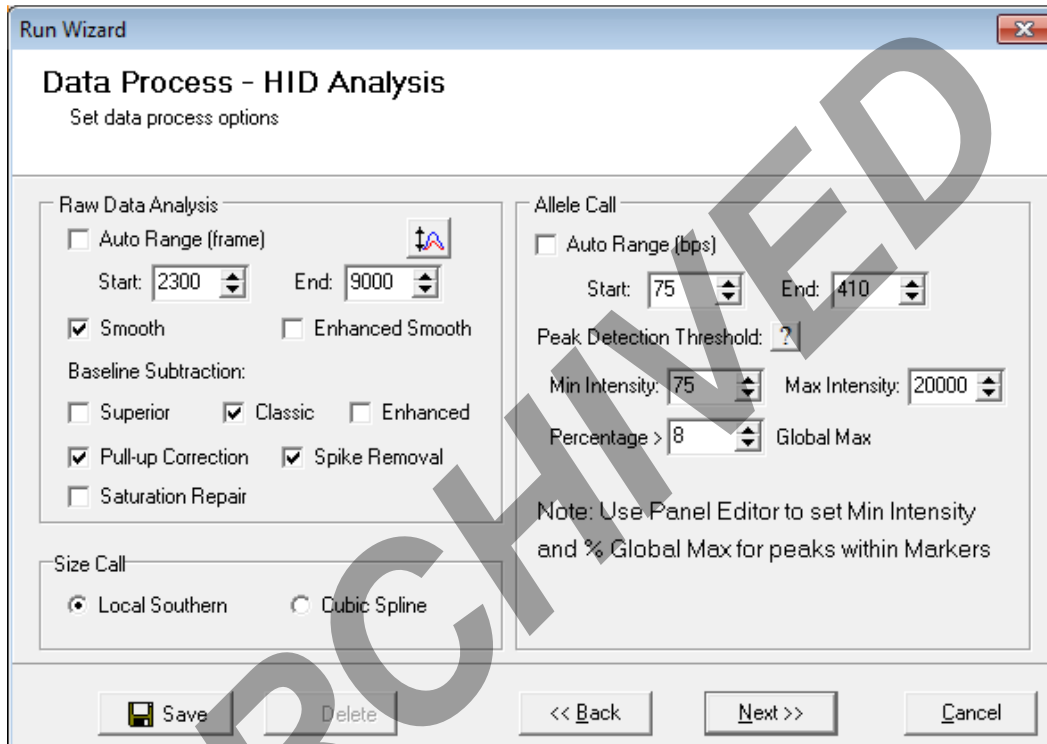
a. *PowerPlex Fusion® / PowerPlex Fusion®\_STRmix™*

The screenshot shows the 'Run Wizard' dialog box for 'Data Process - HID Analysis'. The window title is 'Run Wizard' and the subtitle is 'Data Process - HID Analysis'. Below the subtitle is the text 'Set data process options'. The main area is divided into three sections: 'Raw Data Analysis', 'Allele Call', and 'Size Call'. In the 'Raw Data Analysis' section, 'Auto Range (frame)' is checked with a start of 0 and end of 10000. 'Smooth' is checked, and 'Enhanced Smooth' is unchecked. Under 'Baseline Subtraction', 'Superior' is checked, while 'Classic' and 'Enhanced' are unchecked. 'Pull-up Correction' is unchecked, 'Spike Removal' is checked, and 'Saturation Repair' is unchecked. In the 'Allele Call' section, 'Auto Range (bps)' is unchecked with a start of 55 and end of 510. 'Peak Detection Threshold' is set to '?'. 'Min Intensity' is 50 and 'Max Intensity' is 32760. 'Percentage >' is 0 and 'Global Max' is unchecked. A note below this section reads: 'Note: Use Panel Editor to set Min Intensity and % Global Max for peaks within Markers'. In the 'Size Call' section, 'Local Southern' is selected with a radio button, and 'Cubic Spline' is unselected. At the bottom, there are buttons for 'Save', 'Delete', '<< Back', 'Next >>', and 'Cancel'. The 'Next >>' button is highlighted with a dashed border.

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 10 OF 33

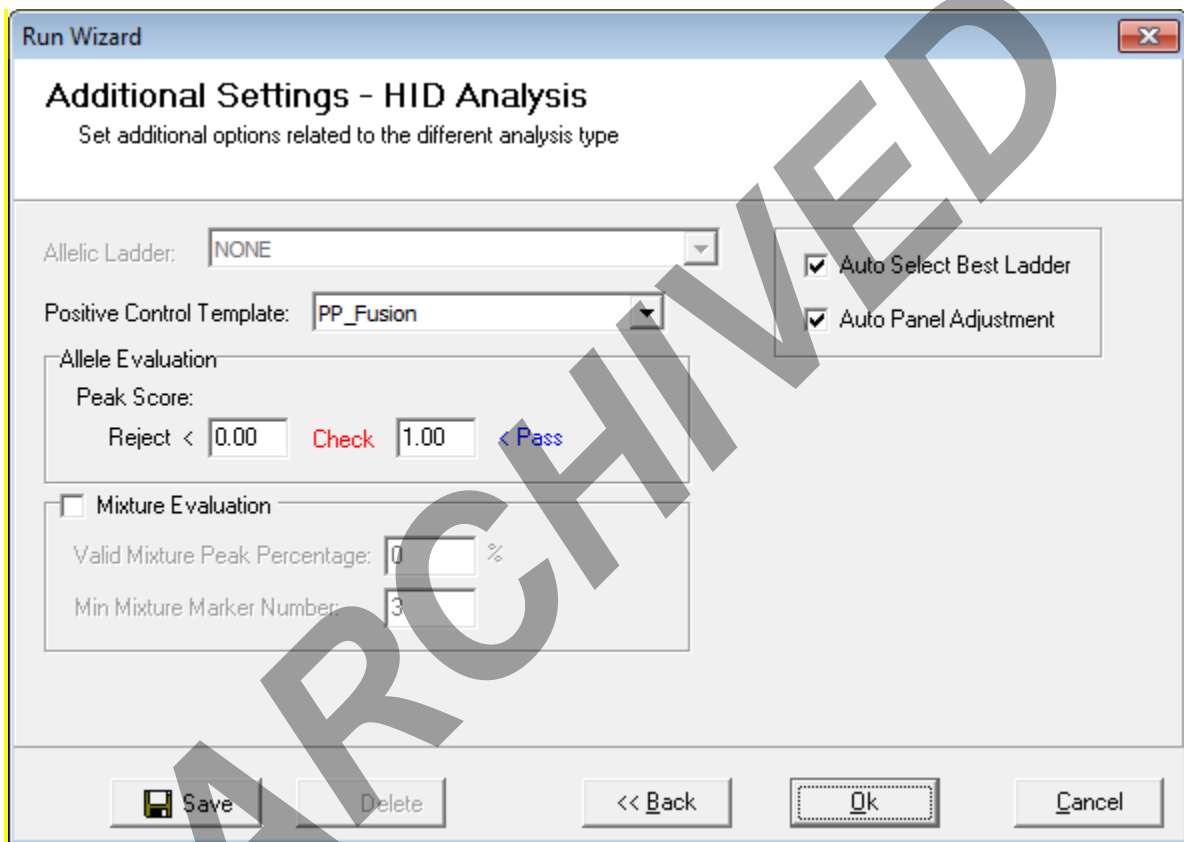
*b. Yfiler®*



4. **Click Next**
5. In the **Additional Settings** window, ensure the following settings are used:

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 11 OF 33

*a. PowerPlex Fusion®/ PowerPlex Fusion®\_STRmix™*



- i. **Allelic Ladder:**
  - a. NONE is selected (grayed out, change not possible)
- ii. **Auto Select Best Ladder:**
  - a. Box is **Checked**
- iii. **Auto Panel Adjustment:**
  - a. Box is **Checked**
- iv. **Select the Positive Control Template** using the Positive Control dropdown:
  - a. For PowerPlex Fusion® - **PP\_Fusion**
- v. **Allele Evaluation Peak Score:**
  - a. **Reject 0.00**

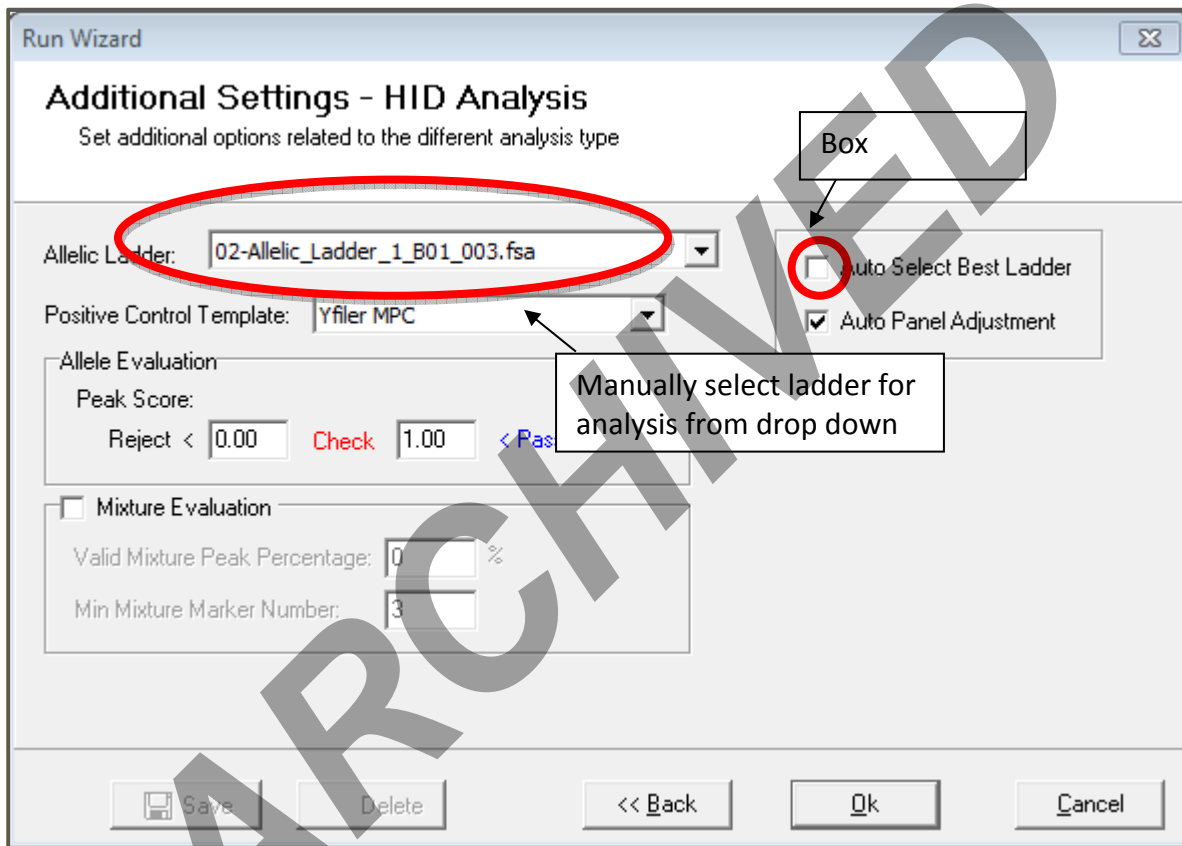
**FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS**

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 12 OF 33

**b. Pass 1.00**

**vi. Deselect Mixture Evaluation**

**b. Yfiler®**



**i. Allelic Ladder:**

a. **Select appropriate ladder** for analysis from the drop down box.

**ii. Auto Select Best Ladder:**

a. **Box is Unchecked**

**iii. Auto Panel Adjustment:**

a. **Box is Checked**

**iv. Select the Positive Control Template** using the Positive Control dropdown:

a. **For Yfiler® - Yfiler MPC**

**v. Allele Evaluation Peak Score:**

a. **Reject 0.00**

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 13 OF 33


**b. Pass 1.00**

**vi. Deselect Mixture Evaluation**



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

6. **Click OK** → Once data has finished processing **Click OK**
7. 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)

#### IV. Checking the Allelic Ladder




1. There are two areas in the **Main Analysis** window that indicate if the allelic ladder used for analysis has passed.
  - a. In the **Sample File Tree**, a red question mark next to the allelic ladder would indicate a potentially failed ladder.
  - b. In the **Project Summary**, a ladder error would indicate a potential failed ladder.
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.
3. If the ladder used for analysis failed, 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 Section II. Load Data, step 2 and proceed.

#### V. Checking the Size Standard

1. Located on the top toolbar in the main analysis window, **Click Size Calibration** 
2. In the **Calibration Charts** window, Click **Chart Synchronize** 

## FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 14 OF 33


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 as per the amplification kit.
  - a. If the peak is recognized by the software, a green inverted triangle appears at the top of the peak. 
  - b. If the size standard needs manual editing by adding or deleting a peak; **Right Click on the desired peak** → **Select Delete/Add Peak** → **Right Click Again** → **Select Update Calibration**
  - c. Close **Calibration Charts** window.
    - i. If any sample 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**
    - ii. If any sample has failing or poor size standard, and was not automatically marked as disabled by the software, any peak labels present must be deleted. 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. **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. In the **Main Analysis** window, **Click on the Down Arrow** next to the Show Color  icon located in the top toolbar. → **Click Hide All**
5. In the **Sample File Tree**, **Right Click on Allele Call** → **Click Select Max**
6. In the **Main Analysis** window, **Click on the Down Arrow** next to the Show Color  icon located in the top toolbar. → **Click ORANGE**



Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

## FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 15 OF 33

### 7. Adjust axis to view size standard depending on amplification kit

- a. On the top toolbar, **Click Set Axis icon** 
- b. **Click Fixed 'X'** → enter X axis range
  - i. PowerPlex Fusion® = 55-510
  - ii. Yfiler® = 75-410
- c. **Click Set Axis** again → **Select Auto Fit Y**

**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 the 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.

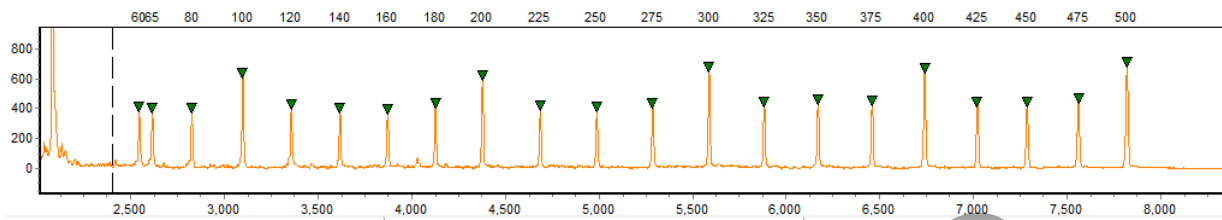
8. Review size fragments by ensuring that all required peaks are labeled within +/- 0.5bp of the fragment size.
  - a. If a peak is not labeled, then the size standard fails.
    - i. **Right click** on sample in Sample File Tree within Main Analysis Window → Click **Edit Comments** → Enter **rerun code** in Comments field → Click **OK**
    - ii. If any sample with failing or poor size standard has peaks called, the peak labels of those peaks will need to be deleted. 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

## Size Standard

### PowerPlex Fusion®

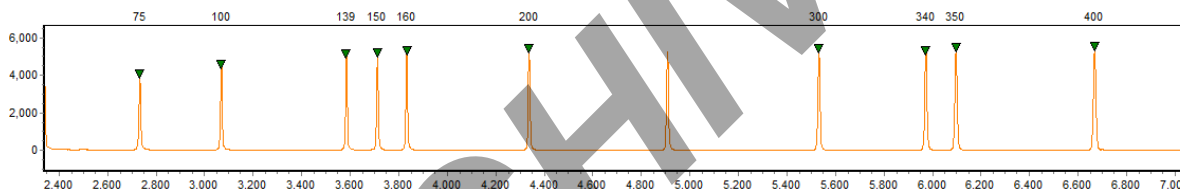
- i. PowerPlex Fusion® samples are run with the WEN ILS500. ILS500 has a range of 60-500bp (Fragments: 60, 65,80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500). All size fragments must be labeled in the calibration chart and the orange dye lane.

<b>GeneMarker Manual</b>		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 16 OF 33




**Yfiler®**



- i. Yfiler® samples are run with LIZ 500. The LIZ\_Yfiler size template for LIZ 500 does not have the 250 bp sizing peak labeled as it is not utilized for data sizing. This peak should not be labeled within the Calibration Chart window. All other size calls in the range of 75-400 bp peaks must be properly match scored/sized
- ii. When viewing the size standard in the orange dye lane in the **Browse by All Color**, the 250bp will be labeled. This peak is not being used for sizing.



**VI. Analyzing Samples**

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

1. Adjust axis to view samples depending on amplification kit
  - a. On the top toolbar, **Click Set Axis icon** 
  - b. **Click Fixed 'X'** → enter X axis range
    - i. PowerPlex Fusion® = 55-510
    - ii. Yfiler® = **75-410**
  - c. **Click Set Axis** again → **Select Auto Fit Y**



**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



## FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

<b>GeneMarker Manual</b>		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 17 OF 33

and hold while dragging the mouse in the direction you want to scroll.

2. If using the **Browse by All Color** window, **adjust Max Chart Number to 2**
3. Analyst may choose to use the Peak Table  during analysis. Click the  icon to turn the chart/table on and off.
4. Editing a peak
  - a. **Click on Peak to Highlight → Right Click → Click Edit Comments → Select Edit From List or Type New Edit (refer to edit code list) → Click OK.**
  - b. To select multiple peaks for editing, **Hold the Ctrl key + left mouse click → Drag the mouse to highlight intermittent peaks**
  - c. Once the correct Edit is selected, **highlight the peak and press Delete key** to remove the label from the peak.
  - d. To check peak history → **Right click on peak → Click view history**
    - i. 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.

**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)

**Note:** Peaks will only be considered stutter by the software if they are within 0.5bp from the parent peak. If a stutter peak is >0.5bp from the parent peak the software will not automatically filter this out as stutter, even if is under the stutter filter percentage for that location. +/- 4 stutter should not be manually edited from **evidence** samples in Fusion. Stutter < 30% of the parent peak can be edited from Fusion positive control samples and exemplar samples..

5. Scheduling Re-Runs
  - a. If a sample **Failed** (over saturation, bad size standard, no data, 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
    - i. All peak labels must be removed from **failed samples**. 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

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

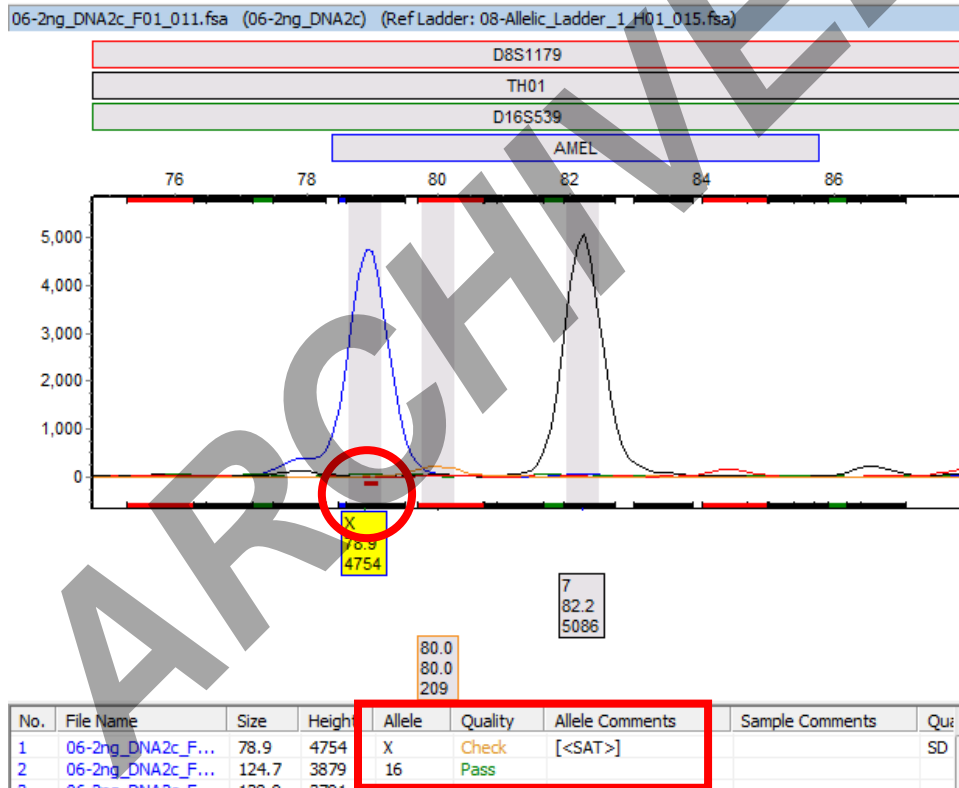
**FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS**

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 18 OF 33

- b. 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

6. Over- Saturation

- a. **Check for saturated samples** → 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. If the sample has excessively saturated peaks (>10,000 RFU or split peaks) this sample must be rerun at a dilution.




- 7. 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**  
You must save over the existing project every time you save

**VII. Exporting for LIMS (all systems and sample types)**

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

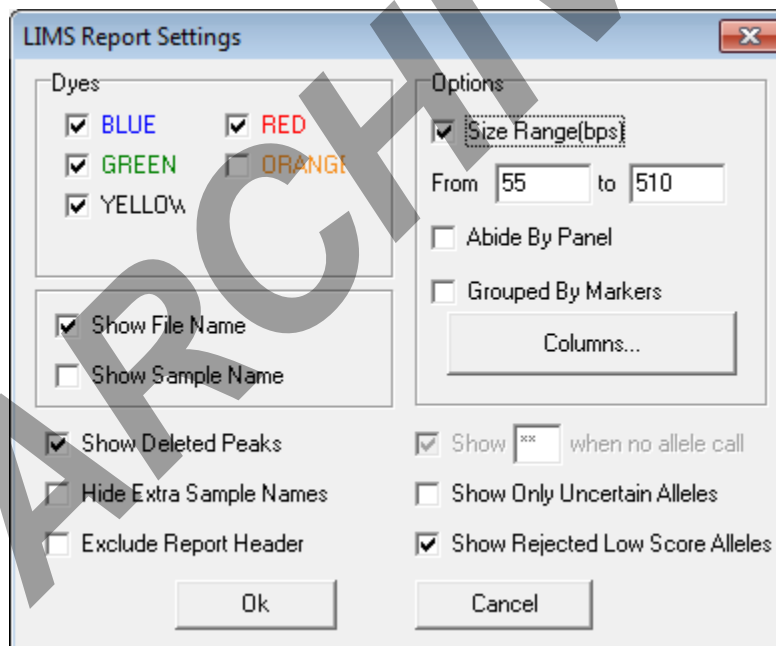
## FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

<b>GeneMarker Manual</b>		
Status:Published	Document ID: 6311	
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 19 OF 33

1. 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
2. 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.
  - a. In the LIMS report settings window that pops up, ensure the settings are as follows:

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

90-340 for Yfiler and 55-510 for Fusion.



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

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 20 OF 33




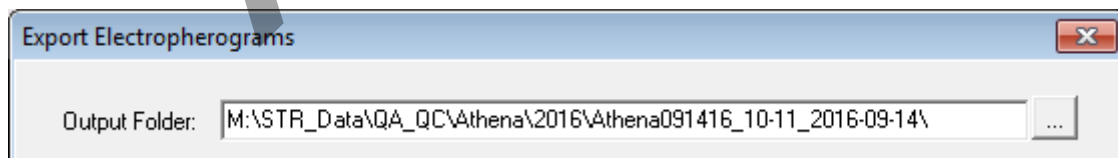
***This is crucial to proper LIMS importation.***

2. Click **OK**; Click OK again in the Allele Report Settings window. Save the file as type “Excel File (\*.xlsx;\*.xls)” under the appropriate run name (**make sure to delete suffix “\_AlleleReport”**). 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.

### **VIII. Exporting Electropherograms (all system and sample types)**

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:
    - a. 55-510 for Fusion
    - b. 90-340 for Yfiler
  - **Select Auto Fit Y**
1. Click Tools → Export Electropherogram
  2. **Set Output Folder to desired location for project PDFs by clicking  icon**



3. Ensure settings match below: Make sure to select PDF as export format



GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 21 OF 33

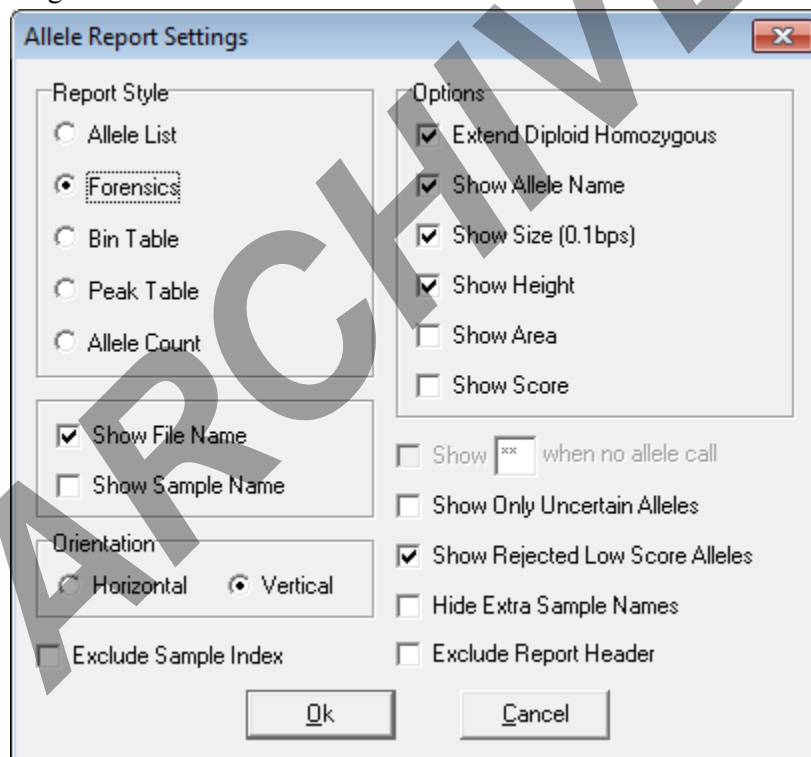
The screenshot shows a software window titled 'Select Dyes'. It contains a list of color options with checkboxes: BLUE (checked), GREEN (checked), YELLOW (checked), RED (checked), and ORANGE (unchecked). Below this is a 'Dye Image Size' section with input fields for 'Width: 1024 pixels' and 'Height: 700 pixels'. There are three checkboxes: 'One image per sample' (unchecked), 'Auto Scale Size Range' (checked), and 'Auto Scale Marker Intensity' (unchecked). At the bottom, there is an 'Export Format:' dropdown menu set to 'PDF file'.


4. In the suffix field enter the run name with a “\_” before it. (Ex: \_Newton061316 88-89U A)
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**
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**
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.
8. If analyzing **Evidence**: Wait to import PDFs into LIMS until after the completion of STRmix analysis.
9. If analyzing **Exemplars**: Import all PDFs into LIMS.

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 22 OF 33

## IX. Fusion Exemplars Only - Exporting EXEMPLAR table for STRmix™ input (for evidence table instructions see section XII)

- In Main Analysis Window Click  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:





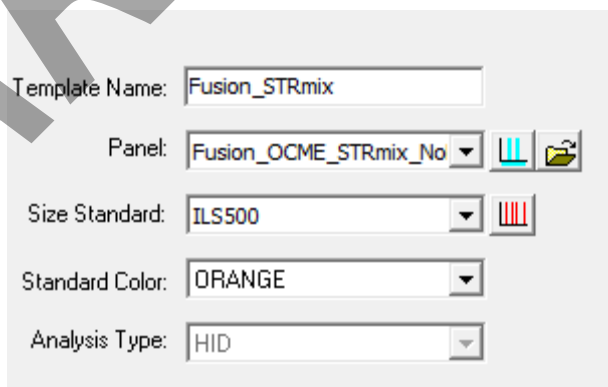
- In the report display section of the main analysis screen, click on the down arrow next to the Save Report icon . Ensure the “Save Report” is selected.
- Navigate to appropriate folder and save file as “Run name\_SM”

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 23 OF 33

4. For proper STRmix™ import the GeneMarker® to STRmix™ macro needs to be run.
  - a. Open the exported project excel file from Step 1 above.
  - b. Open GeneMarker® to STRmix™ macro.
  - c. Follow the instructions on the “instructions” tab.
  - d. After macro has run click “save as” within the GeneMarker® to STRmix™ macro file.
  - h. Change the file type to “.txt” (text, tab delimited)
  - i. Navigate to folder containing your project data and save file there.

## **X. Fusion Evidence Only - STRmix™ Analysis (stutter filters off)**

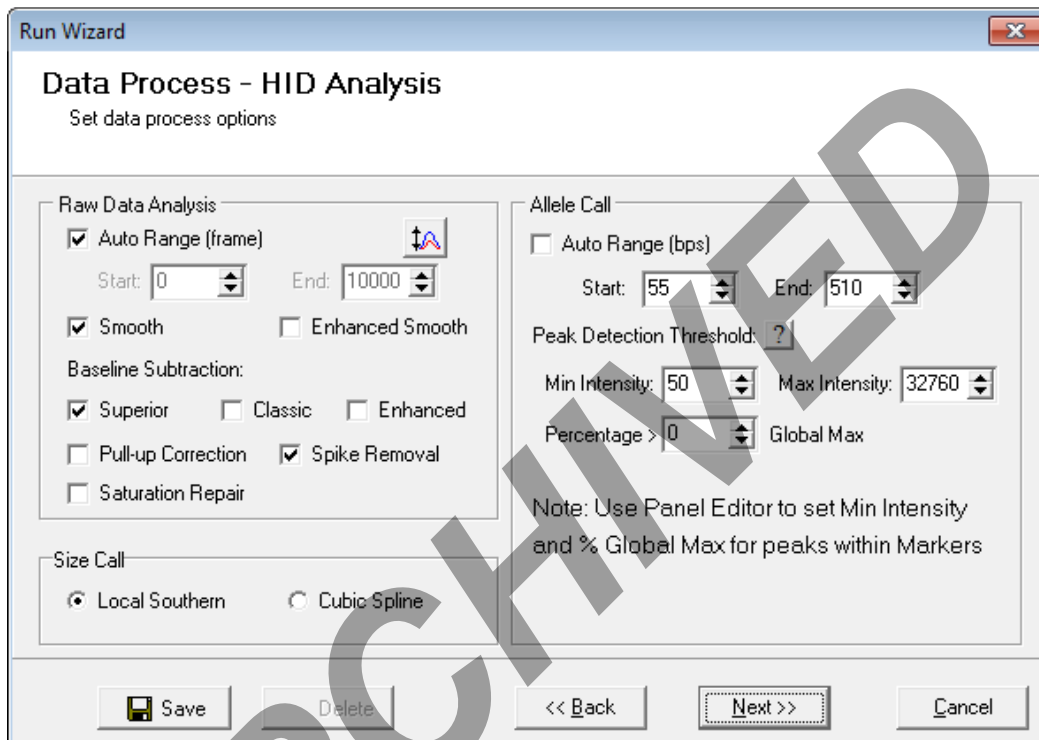
1. Begin by importing the data from the initial analysis into LIMS to generate the edit sheet. Have the edit sheet open in LIMS as you perform the second analysis with stutter filters off for STRmix™ to refer to and ensure the same edits are being made the second time.
2. If Project is still open → **Click Run Project**  located in the top toolbar.
  - a. If project is not open → Click **File** → **Open Project** → **Navigate to run** → **select project** → **click open** → **Click Run Project**  located in the top toolbar.
3. In **Template Selection** window, select the appropriate existing template.



4. **Click Next**
  - a. In the **Data Process** window, ensure the following settings are used based on amplification kit.

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 24 OF 33

i. *PowerPlex Fusion®\_STRmix™*



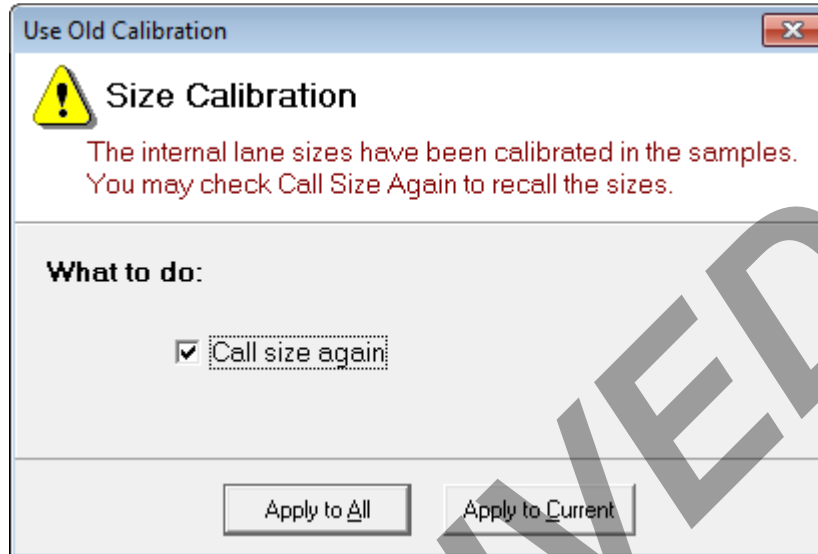
5. Click Next
6. In the **Additional Settings** window, ensure the following settings are used.
  - a. **Make sure Auto Select Best Ladder is selected**
  - b. **Auto Panel Adjustment:** Box is checked
  - c. **Select the Positive Control Template** using the Positive Control drop-down.
    - i. For PowerPlex Fusion® **PP\_Fusion**
  - d. Under Allele Evaluation Peak Score, **Reject 0.00 and Pass 1.00**
  - e. **Deselect Mixture Evaluation**

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

7. Click OK → A window will pop up:



<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 25 OF 33




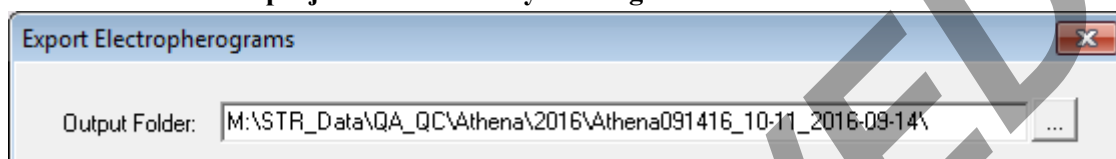
Check “Call size again” and Click Apply to All → Allow data to process → Click OK

8. On the top left corner of the screen, Click File → Click Save Project → Save Project with suffix \_SM
9. Perform a second analysis of this run
  - a. Do not recheck or edit Ladder, Size Standard or Controls. Passing controls and size standard are determined by the initial analysis.
  - b. In Sample Tree → Right click and disable every control in the project except the Ladder. Enter Comment “Not Used”
  - c. Ensure any samples that failed initial analysis are disabled.
  - d. Remove the labels from the same peaks that were edited in the initial analysis. It is not necessary to enter edit codes again.
  - e. If a sample had a rerun code during the initial analysis it is NOT necessary to enter the rerun code again.
10. Once the entire run has been reviewed, and labels have been removed from all artifact peaks, Click File → Click Save Project

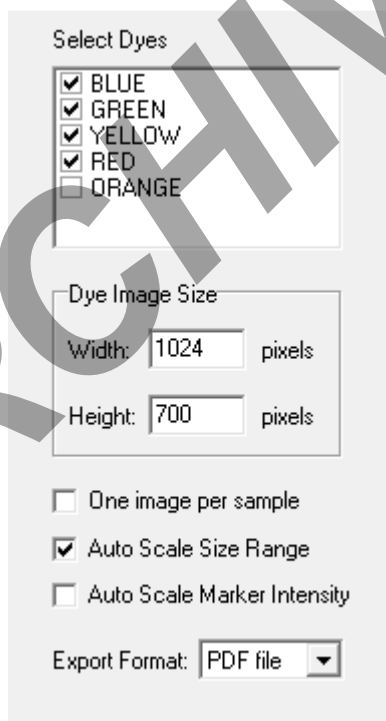
GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 26 OF 33

## XI. Fusion Evidence Only - Exporting Electropherograms after STRmix™ Analysis (samples only)

1. In the main analysis window, set the fixed X axis to 55-510 and set the Y axis to Auto Fit Y.
2. Click Tools → Export Electropherogram
3. Set Output Folder to the same location where the PDFs for the initial Fusion analysis of the same project were saved by clicking  icon



4. Ensure settings match below:



5. 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.
6. Printing the samples only: Right click in the sample list → click Select All → Uncheck the box for all ladders and controls in the run and any samples that were disabled or failed, **leaving only the passing samples** → clear the prefix field → click **Export**. This will create 4 PDFs for every sample, one for each dye.

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 27 OF 33

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

## XII. Fusion Evidence Only - Exporting EVIDENCE table for STRmix™ input

1. In the report display section of the main analysis screen, click on the report settings icon



Make sure settings match below:

**Allele Report Settings**

Report Style

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

Options

- Extend Diploid Homozygous
- Show Allele Name
- Show Size (0.1bps)
- Show Height
- Show Area
- Show Score

Show File Name

Show Sample Name

Orientation

- Horizontal
- Vertical

Exclude Sample Index

Show \*\*\* when no allele call

Show Only Uncertain Alleles

Show Rejected Low Score Alleles

Hide Extra Sample Names

Exclude Report Header

Ok Cancel

5. Click **OK**
6. In the report display section of the main analysis screen, click on the **arrow** of the Save Report icon .
7. Click **Save Report**.
8. Navigate to appropriate folder and save file as type “Excel File (\*.xlsx;\*.xls)” under the appropriate run name (**make sure to delete suffix “\_AlleleReport”**). Click **Save**.
9. For proper STRmix™ import the GeneMarker® to STRmix™ macro needs to be run.
  - a. Open the exported project excel file from Step 1 above.
  - b. Open GeneMarker® to STRmix™ macro.

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
<b>DATE EFFECTIVE</b> 04/20/2017	<b>APPROVED BY</b> Nuclear DNA Technical Leader	<b>PAGE</b> 28 OF 33

- c. Follow the instructions on the “instructions” tab.
- d. After macro has run click “**save as**” within the GeneMarker® to STRmix™ macro file.
- h. Change the file type to “.txt” (Text, tab delimited)
- i. Navigate to folder containing your project data and save file there.

### **XIII. Reporting Analyst/Case Tech Reviewer Sample Review**

1. Verify all STR runs associated with case file samples checking for:
  - i. Passing Allelic Ladder was used to analyze run
  - ii. Passing controls were present
  - iii. Passing Size Standard for relevant case file samples
  - iv. Appropriate edits were made for relevant case file samples and labels were removed from edited peaks
  - v. Any relevant samples needing re-run were scheduled and/or disabled
2. Determine if sample is suitable for comparison/STRmix™ analysis
3. If a true OL/OB was detected in a sample the allele must be assigned a call for STRmix™
  - i. Open STRmix™ .txt input file and replace OL/OB call with true call







ARCHIVED

**FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS**

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
<b>DATE EFFECTIVE</b> 04/20/2017	<b>APPROVED BY</b> Nuclear DNA Technical Leader	<b>PAGE</b> 29 OF 33

**HELPFUL ICONS INDEX**








**Main Toolbar Icons**

<b>Icon</b>	<b>Name</b>	<b>Function</b>
	<b>Run Project</b>	Opens Run Wizard for processing the data.
	<b>Show/Hide Toggles</b>	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.
	<b>Show Color</b>	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)
	<b>Zoom In/Zoom Out</b>	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.
	<b>Set Axis</b>	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.
	<b>Browse by All Colors</b>	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.

**FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS**

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 30 OF 33

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






Icon	Name	Function
	<b>Size Calibration</b>	Displays calibration charts for linearity of lane analysis.
	<b>Show Chart/Table</b>	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> .
	<b>Save Peak Table</b>	Allows user to export the <i>Peak Table</i> as an Excel(.xls) file or a tab-delimited Text (.txt) file.
	<b>Call Allele</b>	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).
	<b>Marker Drop-down Menu</b>	Allows user to select a marker to view. Available after the samples have been compared to a Panel.
	<b>Event Log</b>	Displays the processing success/failure of each dye lane.
	<b>Magic Wizard</b>	Activates the <i>Start Your Project</i> , <i>Run</i> and/or <i>Report</i> dialog boxes.

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.


**FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS**

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 31 OF 33

**Browse By All Colors Icons**

Icon	Name	Function
	<b>Zoom In/Zoom Out</b>	Same use as in the <i>Main Analysis</i> Screen.
	<b>Show/Hide Mouse Cross Lines</b>	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.
	<b>Show/Hide Bin Ranges</b>	Allows user the option to show/hide the Bin brackets at the top and bottom of the electropherogram.
	<b>Auto Scale Markers</b>	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).
	<b>Print</b>	Opens the <i>Print Report</i> settings box. Also accessible in the <i>Main Analysis</i> screen.



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

Icon	Name	Description
	<b>Report Settings</b>	Allows user to customize <i>Report Table</i> display settings.

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

**FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS**

<b>GeneMarker Manual</b>		
Status:Published		Document ID: 6311
<b>DATE EFFECTIVE</b> 04/20/2017	<b>APPROVED BY</b> Nuclear DNA Technical Leader	<b>PAGE</b> 32 OF 33

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

**PEAK TABLE OPTIONS INDEX**

<b>Dye</b>	dye lane location of peak
<b>Size</b>	peak basepair size (x-axis)
<b>Height</b>	value given as relative frequency units (RFU) of the peak (y-axis)
<b>Height Ratio</b>	peak height divided by height of highest peak in the dye lane or Marker
<b>Area</b>	indicates the area under the curve of the peak; calculated based on x-axis Start/End column settings
<b>Area Ratio</b>	peak area divided by area of highest peak in the dye lane or Marker
<b>Marker</b>	locus location of the peak
<b>Allele</b>	bin location of the peak (based on kit/system panel and ladder analysis of project)
<b>Difference</b>	absolute value of distance between the peak center and <i>Bin</i> center in basepairs
<b>Quality</b>	assigns <b>Pass/Check/Undetermined</b> quality ranking for each peak relative to the Allele Evaluation <i>Peak Score</i> settings in the Run Wizard (see Additional Settings)
<b>Score</b>	an exponential curve based evaluation of the peak; calculation of the value is based on signal-to-noise ratio and peak shape (or morphology)
<b>Start/End</b>	beginning and finish basepairs of the peak's Area calculation
<b>Allele Comments</b>	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 <b>Edit Comments Index</b> )
<b>Sample Comments</b>	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 <b>Sample Comments Index</b> )
<b>Quality</b>	letter code abbreviation(s) of reason(s) for a peak's <i>Quality</i>

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.



**FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS**

<b>GeneMarker Manual</b>		
Status: Published		Document ID: 6311
DATE EFFECTIVE 04/20/2017	APPROVED BY Nuclear DNA Technical Leader	PAGE 33 OF 33

<b>Reasons</b>	assignment if ranked <b>Check/Undetermined</b>
----------------	--

ARCHIVED