FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

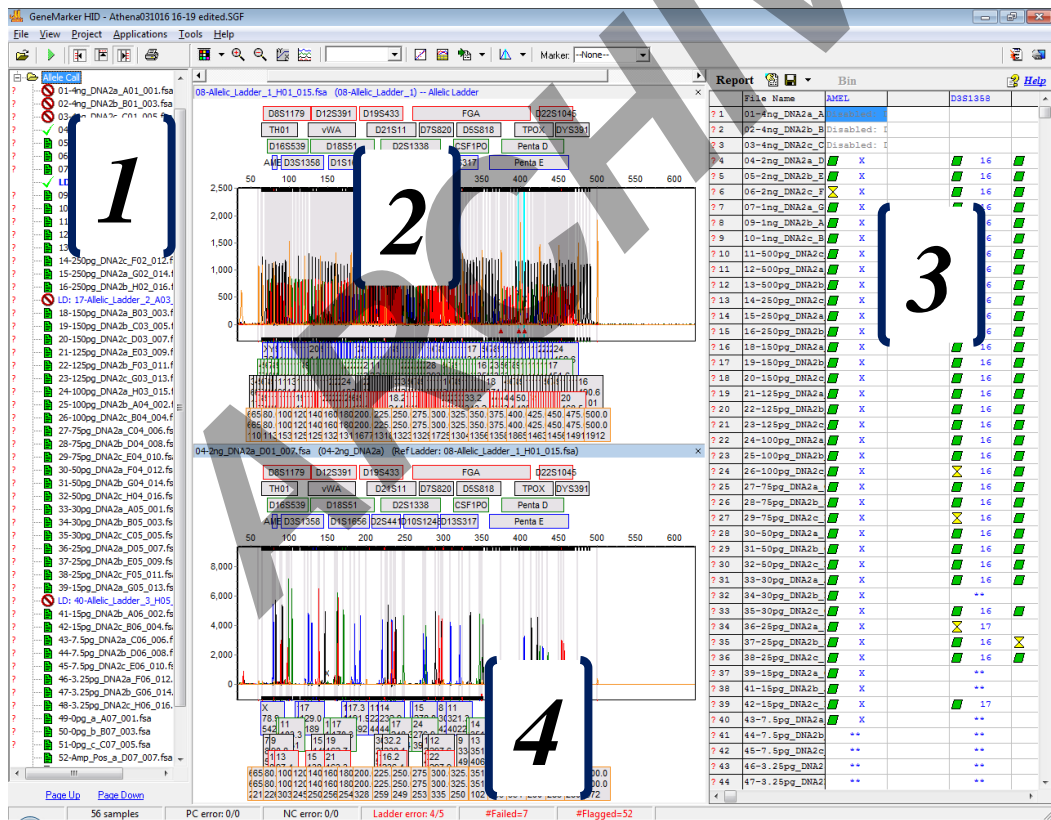|	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	**APPROVED BY** Nuclear DNA Technical Leader	**PAGE** 1 OF 29

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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 2 OF 29

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 Reason Index** for more details.

Project Summary

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

1.   **New** reflects the data set is not a project that has been previously analyzed.

    a. If the first column read **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. See **Quality Reason Index** for more details.

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. See **Quality Reason Index** for more details.

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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual

Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 3 OF 29

Calibration Chart Window

There are three major displays in the calibration chart window.

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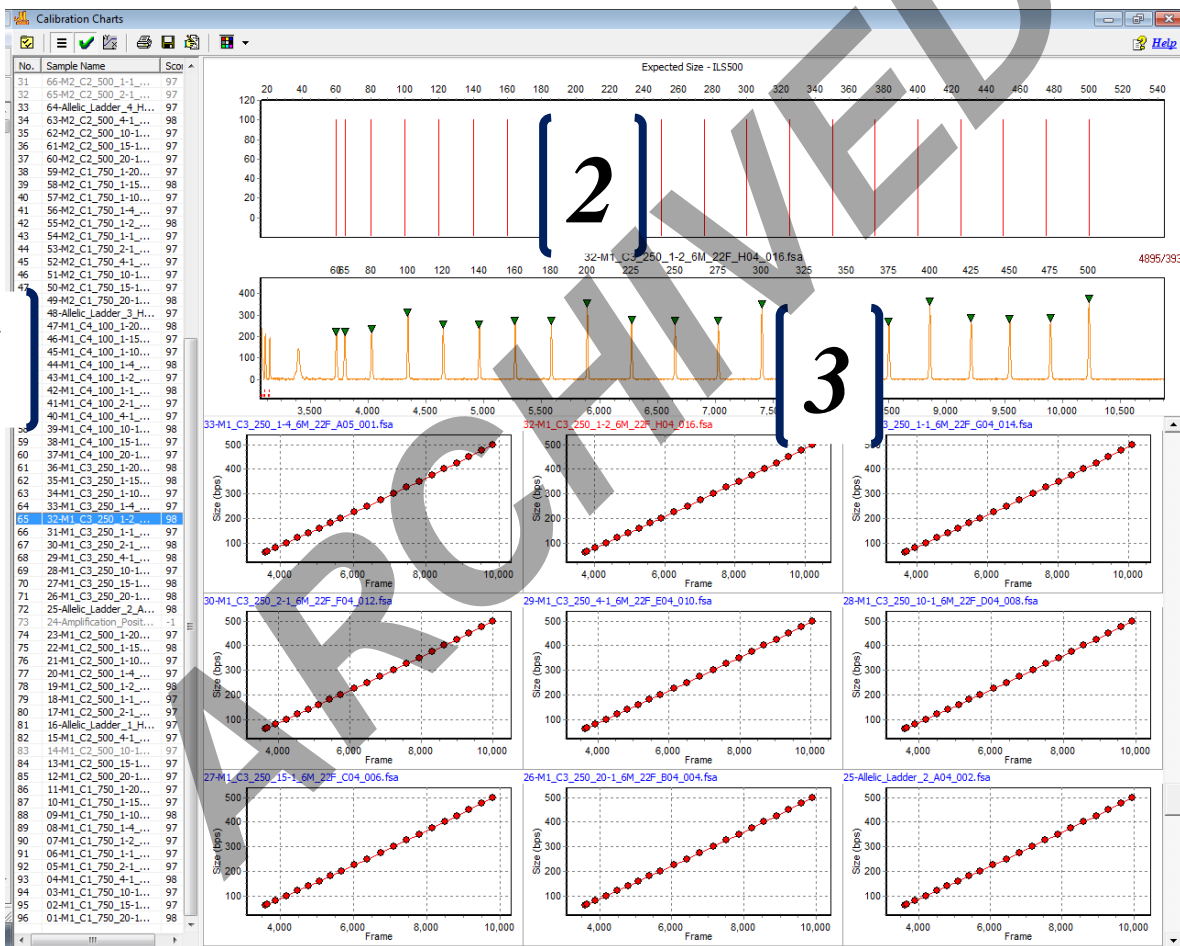
Sample List

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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 4 OF 29

Size Standard Template

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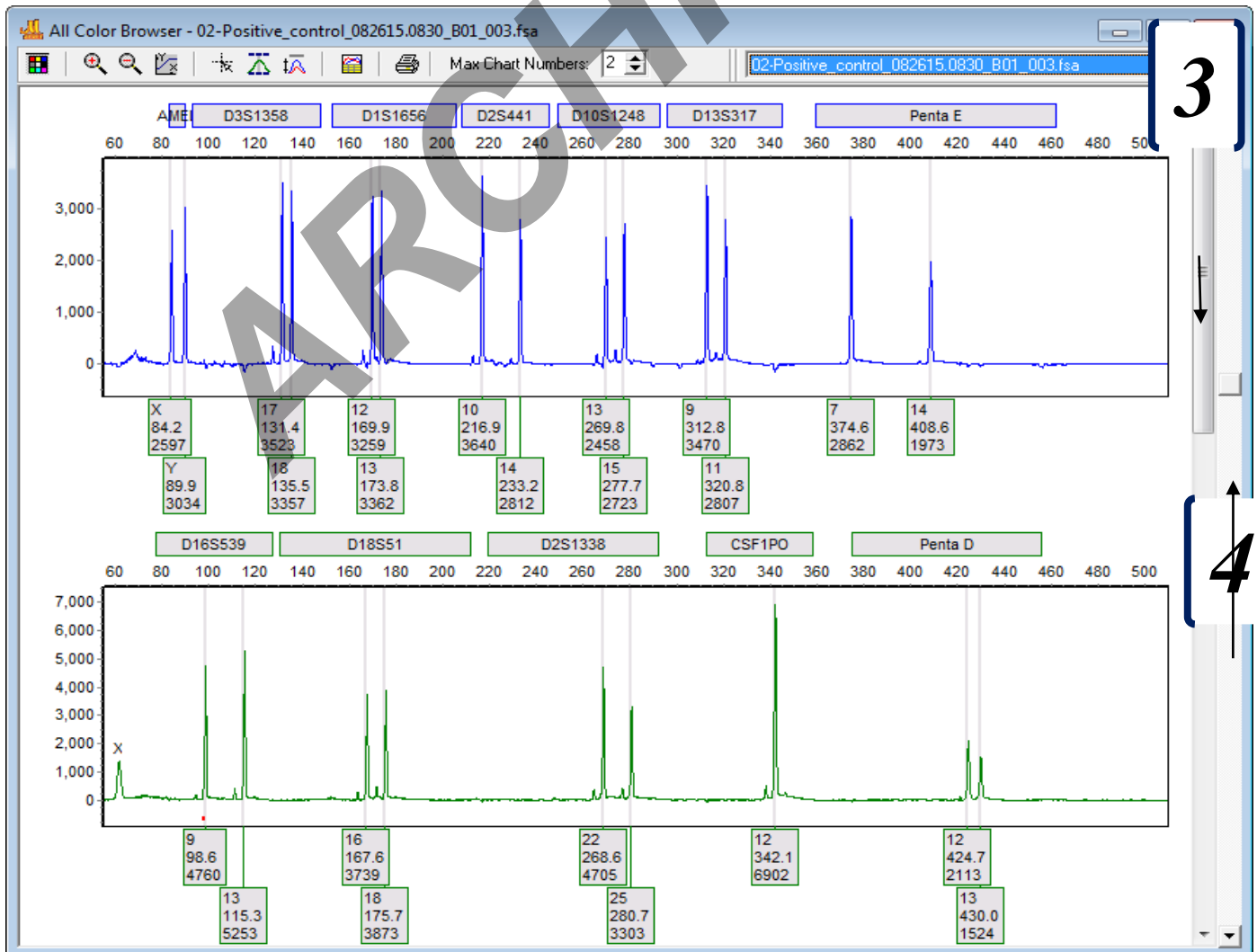
Samples ILS

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.

All Color Browser Window

There are four features in the All Color Browser 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**

| GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 5 OF 29

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 **All Color Brower** window, alleles maybe colored yellow or red.
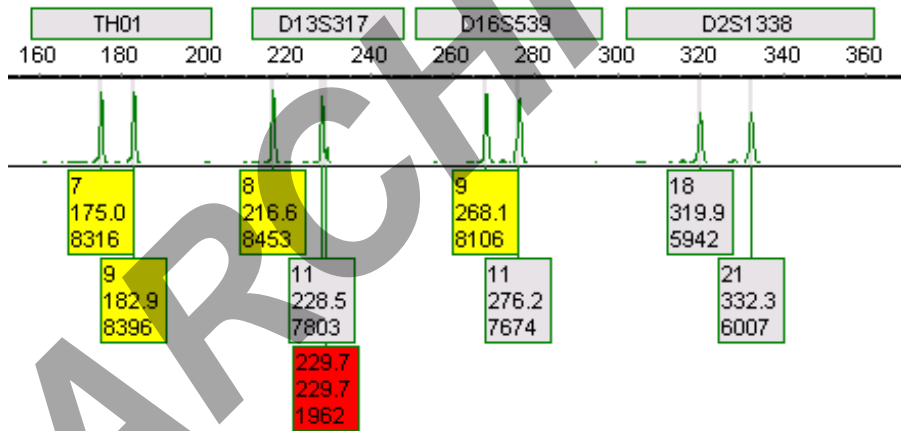
This indicates that one or more quality criteria have not been met in the software. See **Quality Reason Index** for more details.

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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 6 OF 29

Peak Table

When analyzing samples in the **Main Analysis** window or the **All Color Brower** window, a peak table maybe added to supplement analysis by clicking Show Chart/Table icon
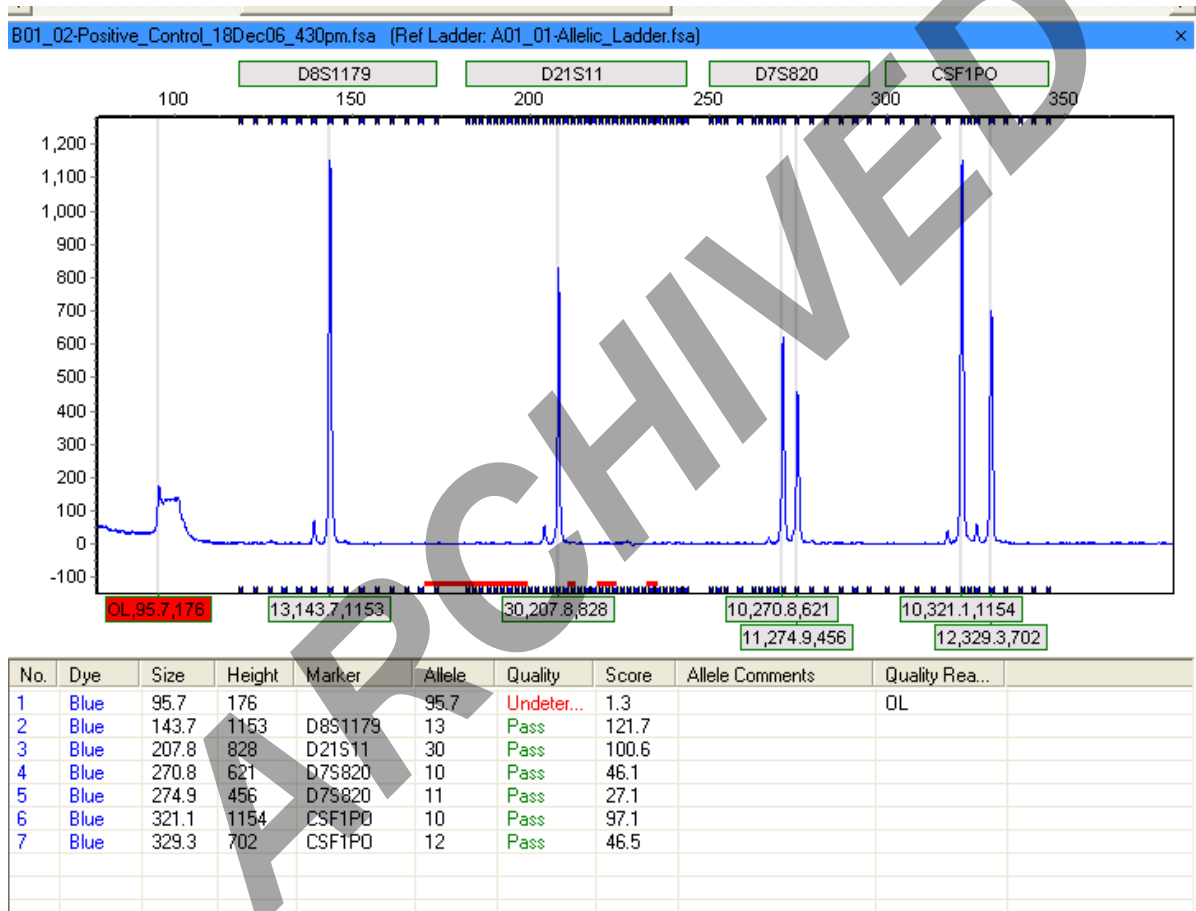

This table will indicate to an analyst which alleles were flagged and which quality reason was triggered. See **Peak Table Options Index** for further details.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 7 OF 29

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
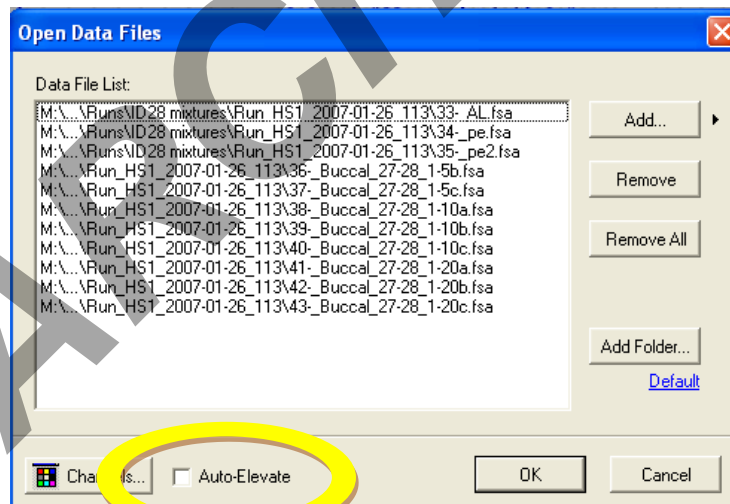
    v. Select **OK**

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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 8 OF 29

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

III.   Run Wizard

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

a. Yfiler® LIZ

b. PowerPlex Fusion® LIZ

2. **Click Next**

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 9 OF 29

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

a. Yfiler®

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 10 OF 29

b. PowerPlex Fusion® / PowerPlex Fusion®_STRmix^{TM}
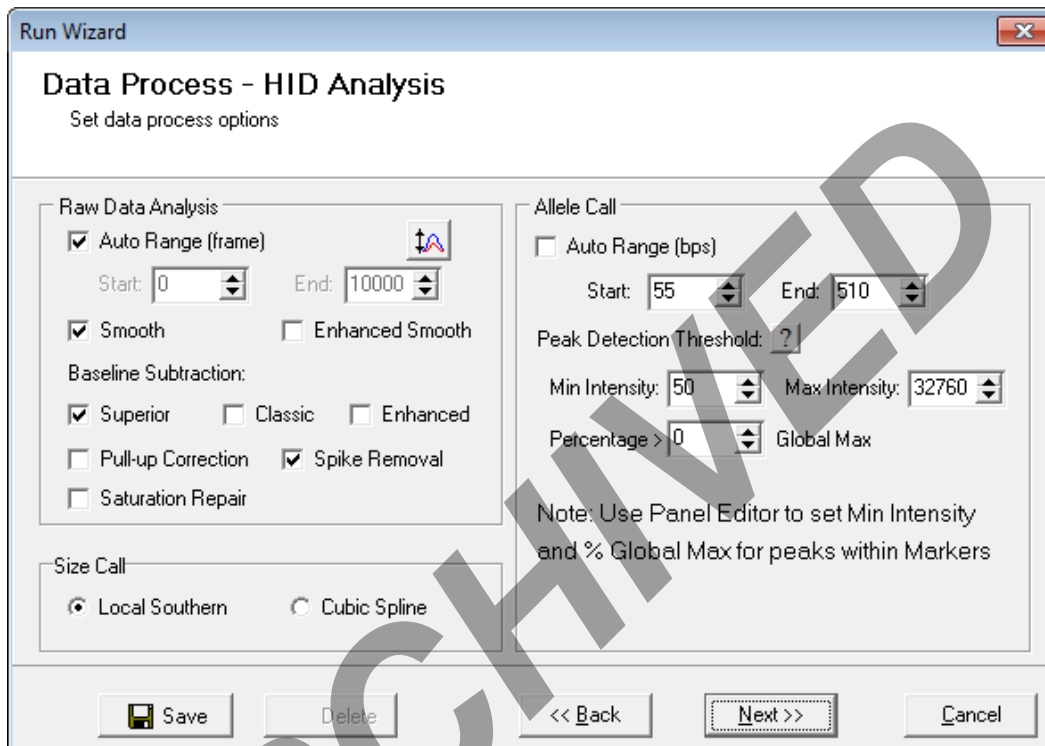
4. **Click Next**
5. In the **Additional Settings** window, ensure the following settings are used.
    i. **Allelic Ladder:**
        i. **Fusion** – Auto Select Best Ladder is Checked
        ii. **YFiler** – Auto Select Best Ladder is **Unchecked**. Select Ladder for analysis from drop down box.
    ii. **Auto Panel Adjustment:** Box is checked
    iii. **Select the Positive Control Template** using the Positive Control drop-down.
        i. For Yfiler® **Yfiler MPC**
        ii. For PowerPlex Fusion® **PP_Fusion**
    iv. Under Allele Evaluation Peak Score, **Reject 0.00 and Pass 1.00**
    v. **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**

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 11 OF 29

7. On the top left corner of the screen, **Click File → Click Save Project →** Navigate to desired folder and save project using current naming system (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. Visually confirm all expected alleles in the allelic ladder are present. This may be done in the Main Analysis Window or the All Color Brower.

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**

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. If a size call fails, in the **Sample List**, **Right Click on the Sample Name → Select Mark as Failed.**

    d. Close **Calibration Charts** window.

        i. If any sample was marked as failed a rerun code must be applied to that sample. **Right click** on 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**

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. Yfiler® = 75-410
        ii. PowerPlex Fusion® = 55-510

    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. In addition, hold down left button on 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 left button on 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.

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. Go back to the **Calibration Chart window.** In the **Sample List**, **Right Click on the Sample Name → Select Mark as Failed.**

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 13 OF 29

i. If any sample was marked as failed a rerun code must be applied to that sample. **Right click** on sample in Sample File Tree within Main Analysis Window → Click **Edit Comments** → **Enter rerun code** in Comments field → Click **OK**
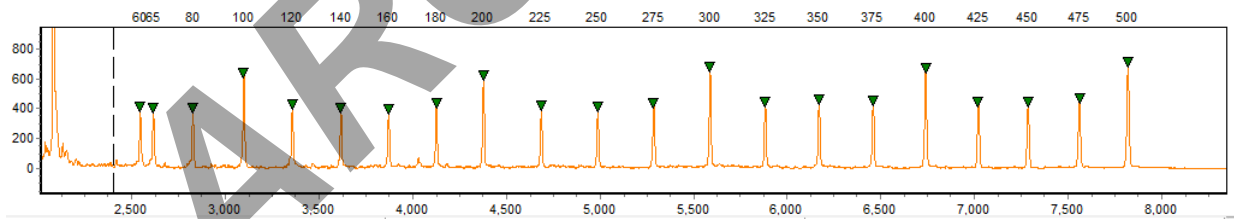
Size Standard

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. 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, the 250bp will be labeled. This peak is not being used for sizing.
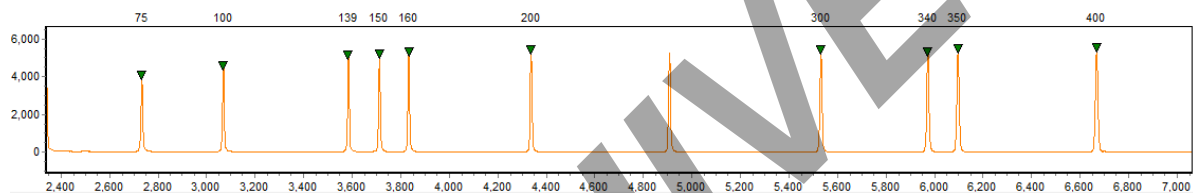
PowerPlex Fusion®

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.

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 14 OF 29

VI. Analyzing Samples

Analysis of the data can be performed in the **Main Analysis** window and/or the **All Color Browser** 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. Yfiler® = 90-340
      ii. PowerPlex Fusion® = 55-510
   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. In addition, hold down left button on 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 left button on 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.

2. If using the **All Color Brower** 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 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 peak is deleted and needs to be undeleted, **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: +/- 4 stutter should not be manually edited from **evidence** samples in Fusion. This edit is only to be used in positive control samples, exemplar samples, and Yfiler.

5. Scheduling Re-Runs
    a. If sample **Failed** (over saturation, bad size standard, etc..) → Right click on **sample** in **Sample Tree** within Main Analysis Window → Click **Disable** → Type Re-Run Code in the comment box → Click OK
    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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

|	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 16 OF 29

7. Once the entire run has been reviewed, all edits have been made and all edited peaks have been deleted, on the top left corner of the screen, **Click File → Click Save Project**
    a. You must save over the existing project every time you save

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

1. In the report display section of the main analysis screen, click on the 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.
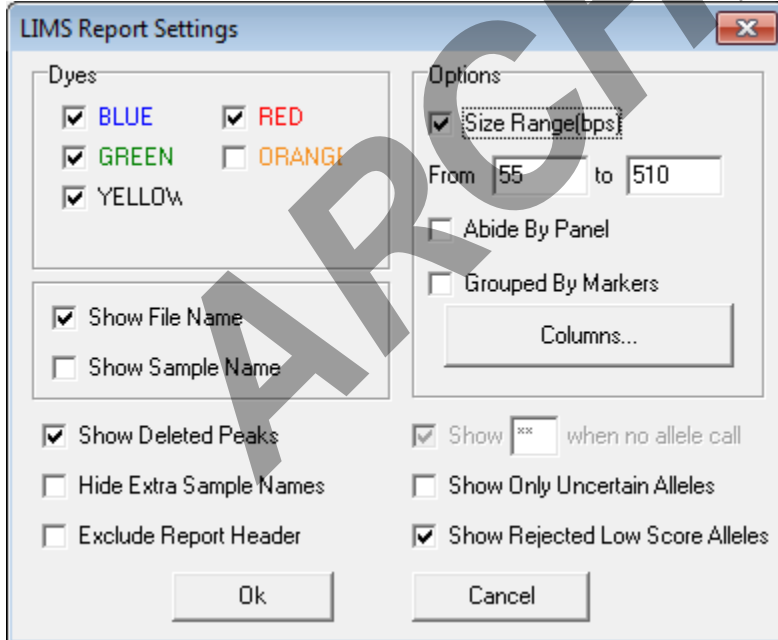
b. In **Options**, click on **"Columns..."**; use the Add/Remove buttons to ensure the Selected Columns are present and listed in the order that follows:

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 17 OF 29

Selected Columns

Plate ID
Marker
Dye
Size
Allele
Allele Comments
Sample Comments

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").**

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. 90-340 for Yfiler
    b. 55-510 for Fusion
- **Select Auto Fit Y**

1. Click Tools→ Export Electropherogram

2. **Set Output Folder to desired location for project PDFs by clicking** [...] **icon**

Export Electropherograms

Output Folder: M:\STR_Data\QA_QC\Athena\2016\Athena091416_10-11_2016-09-14\   ...
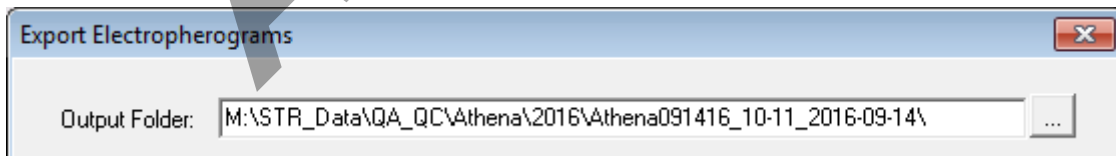
3. Ensure settings match below: Make sure to select PDF as export format

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 18 OF 29

4. In the suffix field enter the run name with a "_" before it. (Ex: _Newton061316 88-89)

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 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.

8. Import all PDFs into LIMS.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 19 OF 29

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

1. In Main Analysis Window **Click** [icon] **icon** → Select **Show All**. In the report display section of the main analysis screen, click on the report settings icon [icon]. Make sure settings match below:
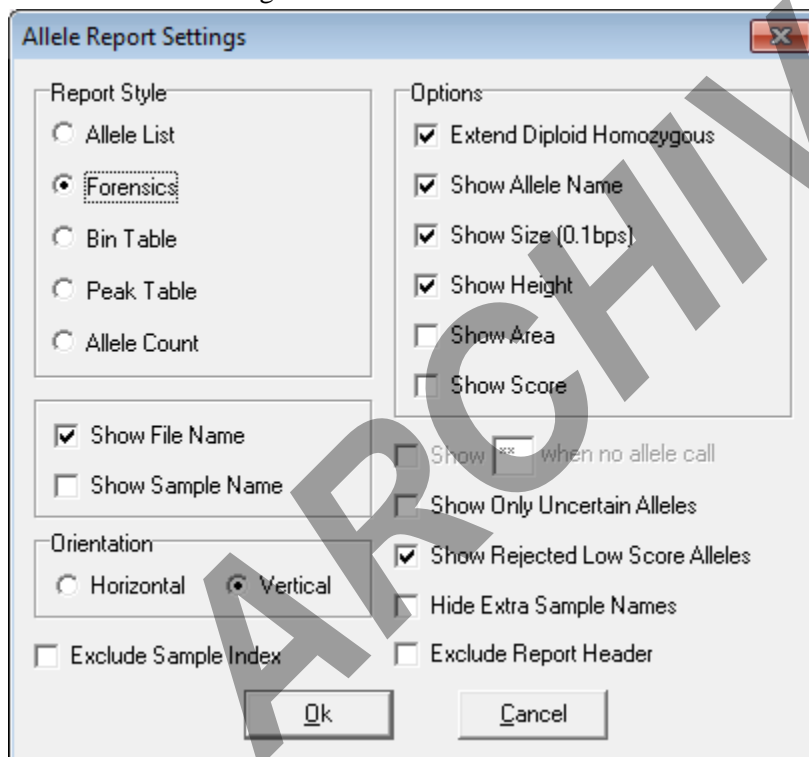
2. In the report display section of the main analysis screen, click on the down arrow next to the Save Report icon [icon]. Ensure the "Save Report" is selected.

3. Navigate to appropriate folder and save file as "Run name_SM"

4. For proper STRmix^{TM} import the GeneMarker® to STRmix^{TM} macro needs to be run.

a. Open the exported project excel file from Step 1 above.

b. Open GeneMarker® to STRmix^{TM} macro.
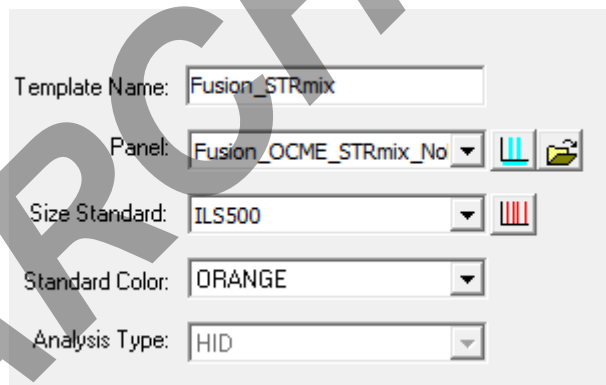
FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 20 OF 29

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** [icon] located in the top toolbar**.**

    a. If project is not open → Click **File → Open Project → Navigate to run → select project → click open → Click Run** [icon] **ect** 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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 21 OF 29

### i. *PowerPlex Fusion®_STRmix^{TM}*

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 Evalution 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:**

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 22 OF 29
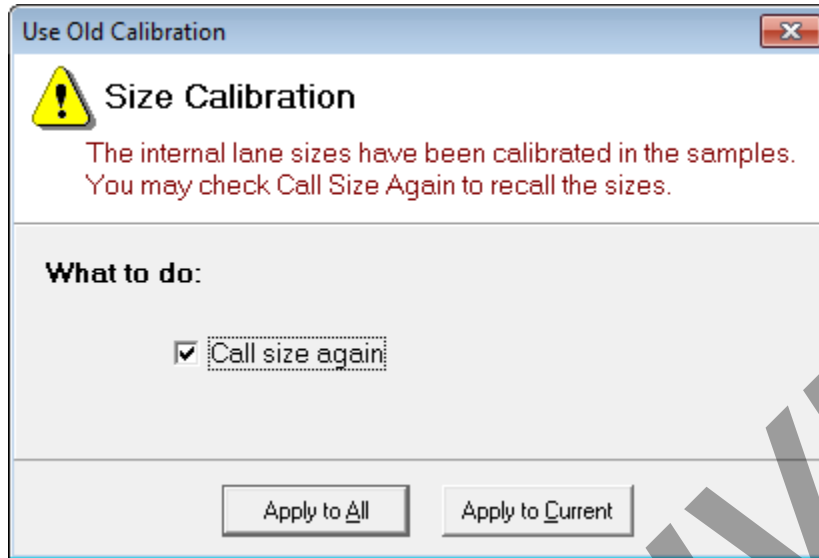
Check "Call size again" and Click Apply to All → Allow data to process → Click OK

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. **Delete the same peaks that were edited and deleted from the initial analysis. It is not necessary to enter edit codes again.**
    d. **Ensure any sample that failed initial analysis is disabled.**
    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 all artifact peaks have been deleted, **Click File → Click Save Project**

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

|	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 23 OF 29

XI. Fusion Evidence Only - Exporting Electropherograms after STRmix^{TM} 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 desired location for project PDFs by clicking [...] icon**
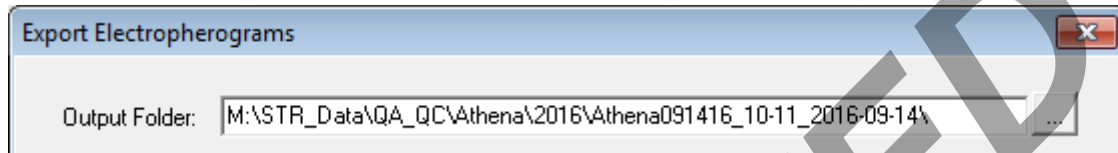
4. Ensure settings match below:
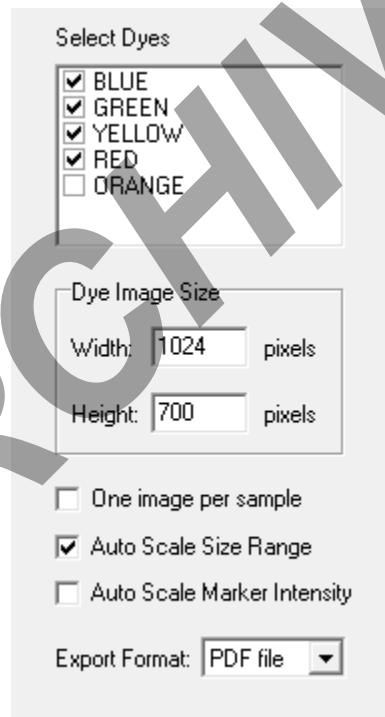
5. In the suffix field enter the run name with a "_" before it followed by **_SM** (Ex: _Newton061316 88-89_SM) to indicate STRmix^{TM} analysis.
6. Printing the samples only: Right click in the sample list → click Select All → Uncheck the box for all ladders and controls in the run and any samples that 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.
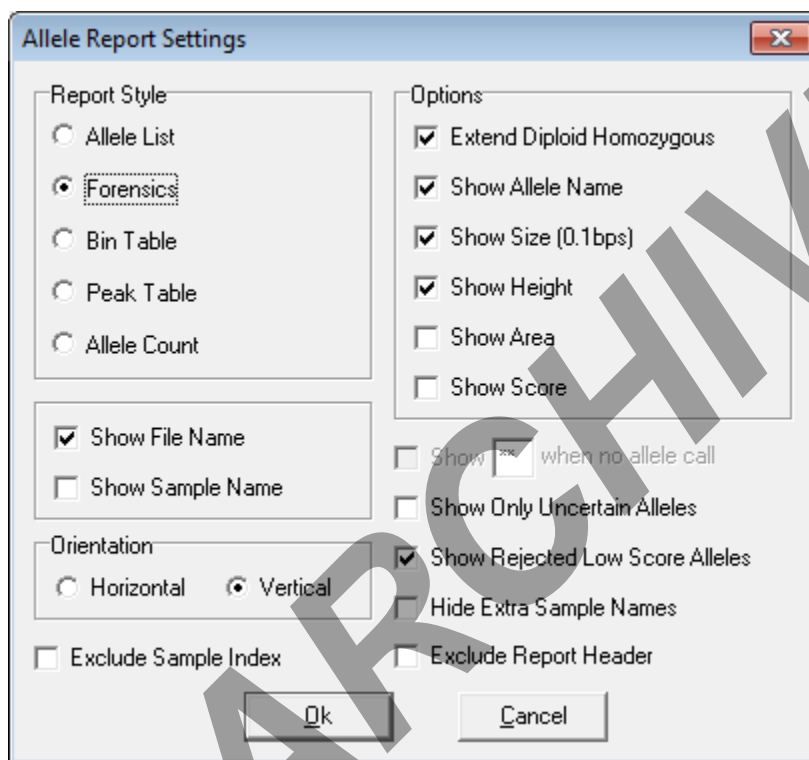7. Import PDFs into LIMS.

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 24 OF 29

XII. Fusion Evidence Only - Exporting EVIDENCE table for STRmix^{TM} input

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

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^{TM} import the GeneMarker® to STRmix^{TM} macro needs to be run.

a. Open the exported project excel file from Step 1 above.
b. Open GeneMarker® to STRmix^{TM} macro.
c. Follow the instructions on the "instructions" tab.
d. After macro has run click "**save as**" within the GeneMarker® to STRmix^{TM} macro file.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual	
Status:Published	Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 25 OF 29

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. Review 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 edited peaks were deleted
    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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

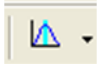|	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 26 OF 29

HELPFUL ICONS INDEX

Main ToolbarIcons

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 In/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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 27 OF 29

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: --None--	**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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 28 OF 29

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.

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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

	GeneMarker Manual	
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/13/2016	APPROVED BY Nuclear DNA Technical Leader	PAGE 29 OF 29

Bin	**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)

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