

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 1 OF 38

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 20.
- 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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 2 OF 38

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)

A screenshot of a software window titled 'Fusion (Evidence)'. It contains four rows of settings, each with a label and a dropdown menu. The first row is 'Template Name:' with the value 'Fusion'. The second row is 'Panel:' with the value 'Fusion_OCME_no filter' and a small icon of a folder. The third row is 'Size Standard:' with the value 'ILS500' and a small icon of a DNA ladder. The fourth row is 'Standard Color:' with the value 'ORANGE'. A large, semi-transparent watermark 'ARCHIVED' is overlaid diagonally across the entire screenshot.

3.2.1 Click Next

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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual

Status:Published

Document ID: 6311

DATE EFFECTIVE
12/10/2019

APPROVED BY
Nuclear DNA Technical Leader

PAGE
3 OF 38

Run Wizard

Data Process - HID Analysis

Set data process options

Raw Data Analysis

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

Baseline Subtraction:

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

Allele Call

- Auto Range (bps)
Start: 55 End: 510
- Score Filter
- Peak Detection Threshold: ?
- Min Intensity: 50 Max Intensity: 32760
- Percentage > 0 Global Max

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

Size Call:
 Local Southern Cubic Spline

Save Delete << Back Next >> Cancel

3.2.3 Click Next

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

Run Wizard

Additional Settings - HID Analysis

Set additional options related to the different analysis type

Allelic Ladder: NONE

Positive Control Template: PP_Fusion

Allele Evaluation

Peak Score:
Reject < 0.00 Check 1.00 < Pass

Mixture Evaluation

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

Auto Select Best Ladder
 Auto Panel Adjustment

Save Delete << Back Ok Cancel

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual

Status:Published

Document ID: 6311

DATE EFFECTIVE
12/10/2019

APPROVED BY
Nuclear DNA Technical Leader

PAGE
4 OF 38

3.3 Fusion Exemplars

3.3.1 Click Next

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

Run Wizard
Data Process - HID Analysis
Set data process options

Raw Data Analysis
 Auto Range (frame)
Start: 0 End: 10000
 Smooth Enhanced Smooth
Baseline Subtraction:
 Superior Classic Enhanced
 Pull-up Correction Spike Removal
 Saturation Detection Saturation Repair

Allele Call
 Auto Range (bps)
Start: 55 End: 510
 Score Filter
Peak Detection Threshold: ?
Min Intensity: 50 Max Intensity: 32760
Percentage > 10 Global Max
Note: Use Panel Editor to set Min Intensity and % Global Max for peaks within Markers

Size Call
 Local Southern Cubic Spline

Save Delete << Back Next >> Cancel

3.3.3 Click Next

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

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

Allelic Ladder: NONE
Positive Control Template: PP_Fusion

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

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

Auto Select Best Ladder
 Auto Panel Adjustment

Save Delete << Back Ok Cancel

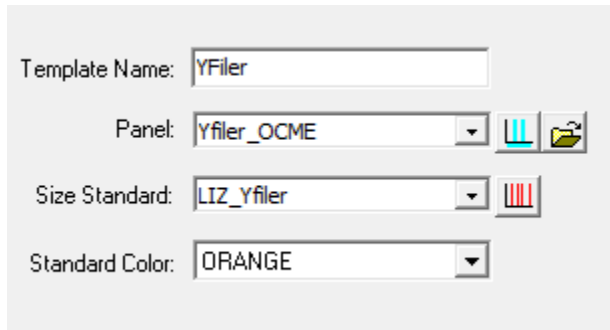
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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 5 OF 38

3.4 Yfiler® (Evidence or Exemplars)



Template Name: Yfiler

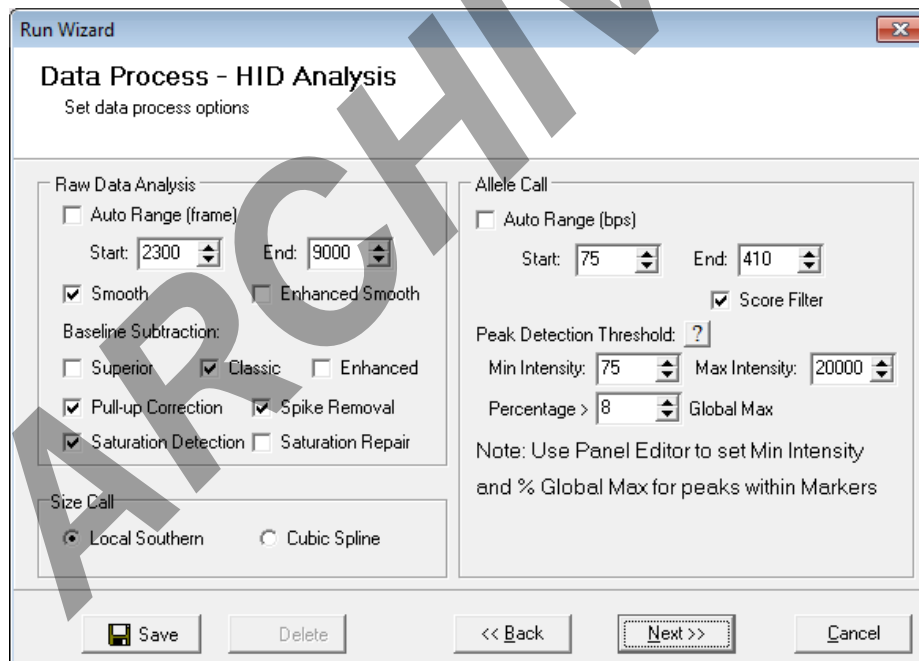
Panel: Yfiler_OCME

Size Standard: LIZ_Yfiler

Standard Color: ORANGE

3.4.1 Click Next

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



Run Wizard

Data Process - HID Analysis
Set data process options

Raw Data Analysis

- Auto Range (frame)
- Start: 2300 End: 9000
- Smooth Enhanced Smooth
- Baseline Subtraction:
 - Superior Classic Enhanced
 - Pull-up Correction Spike Removal
 - Saturation Detection Saturation Repair
- Size Call:
 - Local Southern Cubic Spline

Allele Call

- Auto Range (bps)
- Start: 75 End: 410
- Score Filter
- Peak Detection Threshold: ?
- Min Intensity: 75 Max Intensity: 20000
- Percentage > 8 Global Max
- Note: Use Panel Editor to set Min Intensity and % Global Max for peaks within Markers

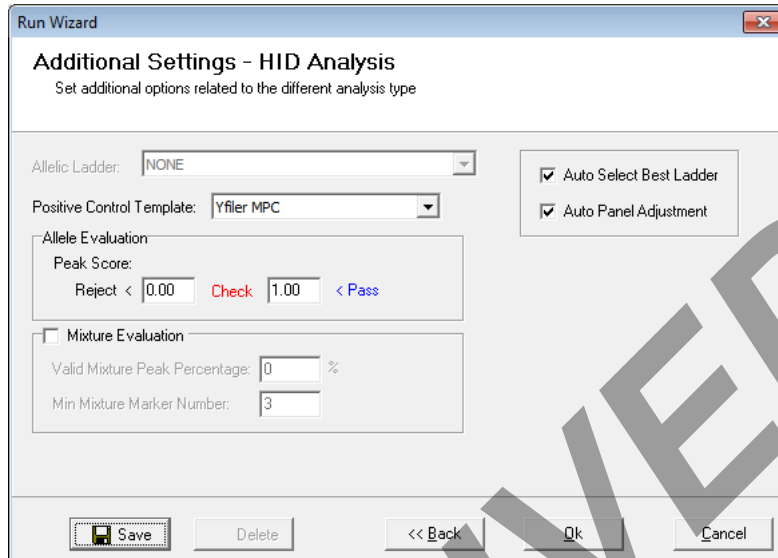
Buttons: Save, Delete, << Back, Next >>, Cancel

3.4.3 Click Next

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



FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 6 OF 38



- 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**

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual

Status:Published

Document ID: 6311

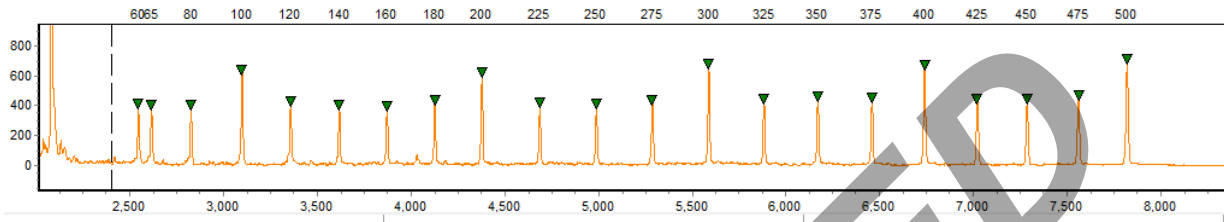
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12/10/2019

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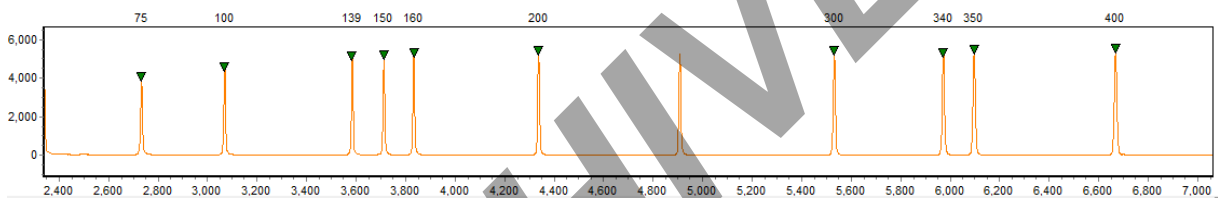
PAGE
7 OF 38


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 & STRmix™ 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

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 8 OF 38


- 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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 9 OF 38

- 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 & STRmix™ 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 & STRmix™ 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 & STRmix™ manual](#).

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 10 OF 38

- 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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 11 OF 38

- 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**.


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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 12 OF 38

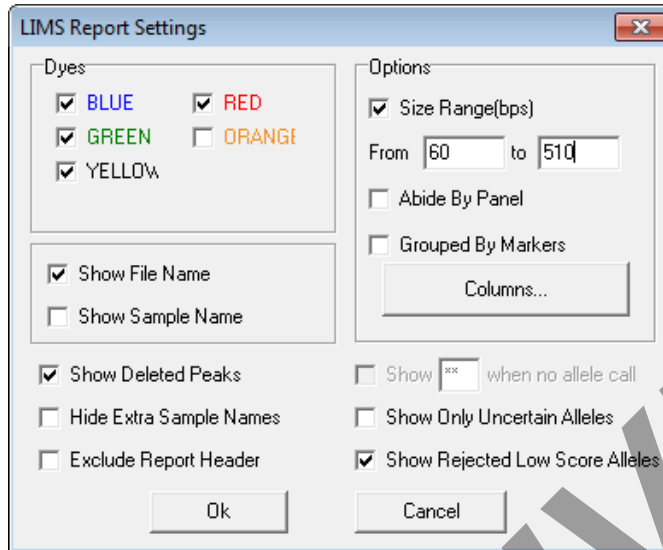
- 11.2.1 Open the Browse by All Color analysis window → navigate to the relevant sample(s) → highlight all peaks in one dye by holding down Ctrl, left click and dragging around any peaks present in that dye → click **Delete** to remove the peak labels → repeat for each dye.
- 11.3 If sample did not fail 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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 13 OF 38



- 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

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 14 OF 38

- 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 19.

- 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

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 15 OF 38

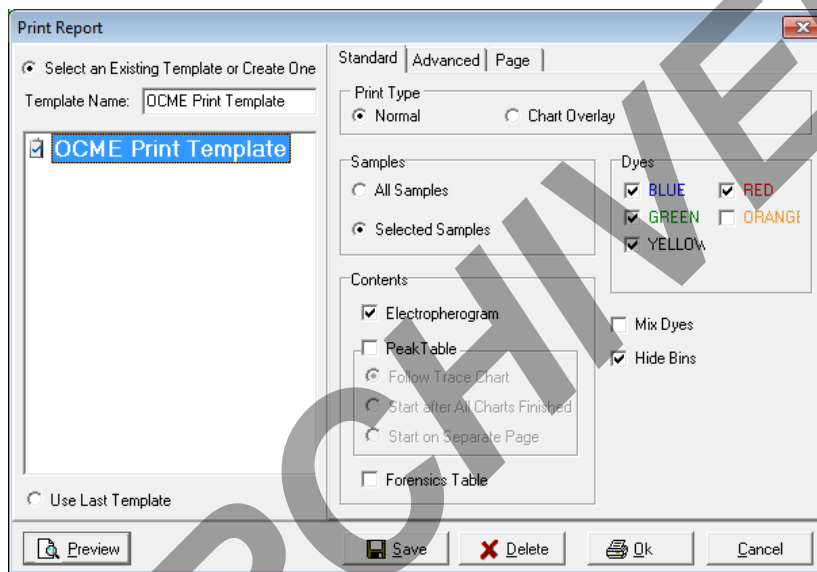
- 90-340 for Yfiler

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:

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual

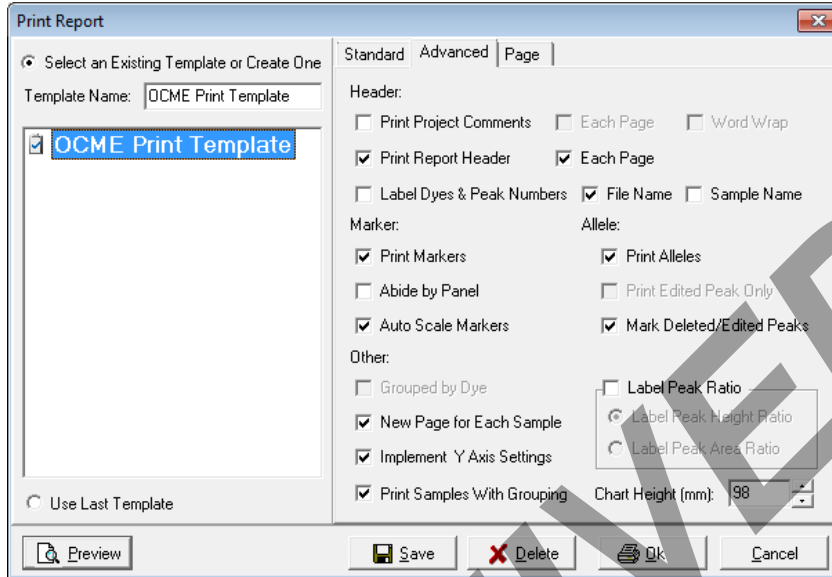
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DATE EFFECTIVE
12/10/2019

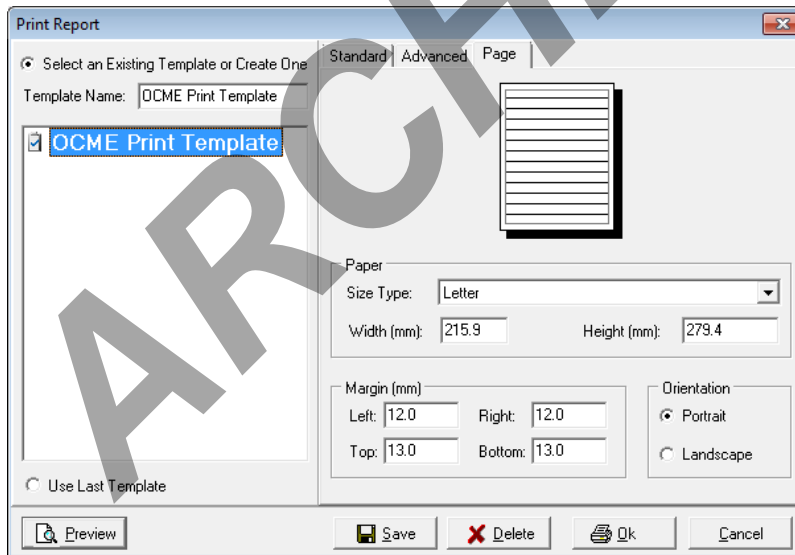
APPROVED BY
Nuclear DNA Technical Leader

PAGE
16 OF 38



13.6.1.3

Page Tab



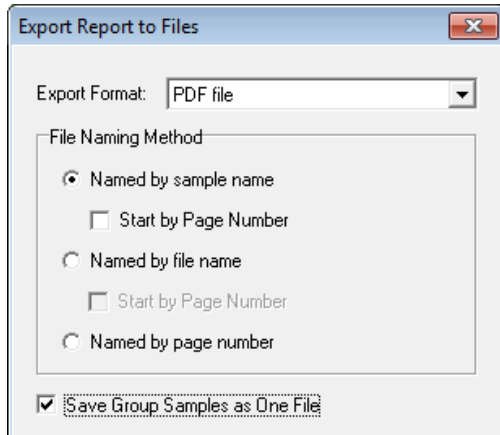
13.6.2 Click Preview → Click Export to File icon  Assure settings match below:

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
FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 17 OF 38



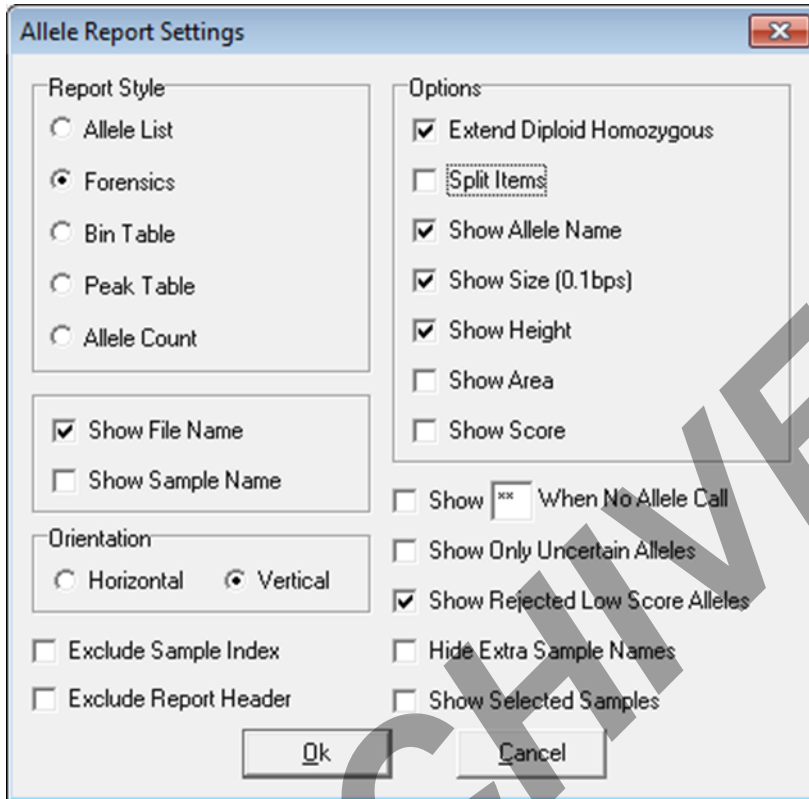
- 13.6.3 Click on the “...” icon to choose the appropriate export directory for the PDF files
- 13.6.4 Click OK → Export Report to PDFs Events window will appear. Once completed click OK
- 13.7 If analyzing Fusion **Evidence**: Wait to import PDFs into LIMS until after the completion of STRmix analysis, section [15](#).
- 13.8 If analyzing **Exemplars or Yfiler Evidence**: Import all PDFs into LIMS.


14 Fusion Exemplars Only - Exporting EXEMPLAR table for STRmix™ 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:

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 18 OF 38



- 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.

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 19 OF 38

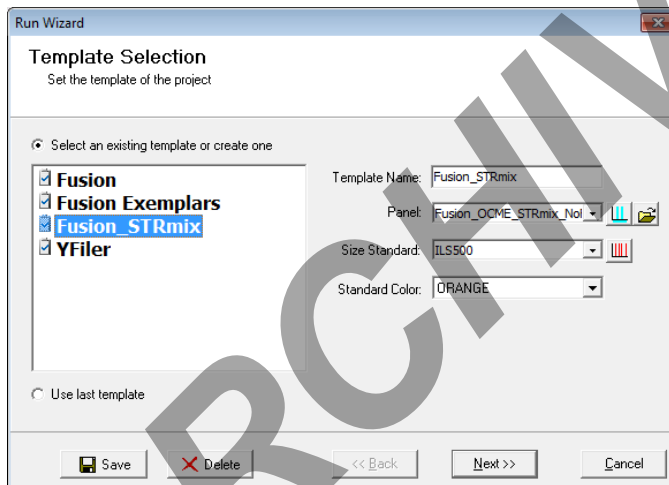
15 Fusion Evidence Only - STRmix™ Analysis (stutter filters off)

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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 20 OF 38

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

Run Wizard
Data Process - HID Analysis
Set data process options

Raw Data Analysis

Auto Range (frame)
Start: 0 End: 10000

Smooth Enhanced Smooth

Baseline Subtraction:

Superior Classic Enhanced

Pull-up Correction Spike Removal

Saturation Detection Saturation Repair

Size Call

Local Southern Cubic Spline

Allele Call

Auto Range (bps)
Start: 55 End: 510

Score Filter

Peak Detection Threshold: ?

Min Intensity: 50 Max Intensity: 32760

Percentage > 0 Global Max

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

Save Delete << Back Next >> Cancel

15.5 Click Next

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

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

Allelic Ladder: NONE

Positive Control Template: PP_Fusion

Allele Evaluation

Peak Score:
Reject < 0.00 Check 1.00 < Pass

Mixture Evaluation

Valid Mixture Peak Percentage: 0 %

Min Mixture Marker Number: 3

Save Delete << Back Ok Cancel

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

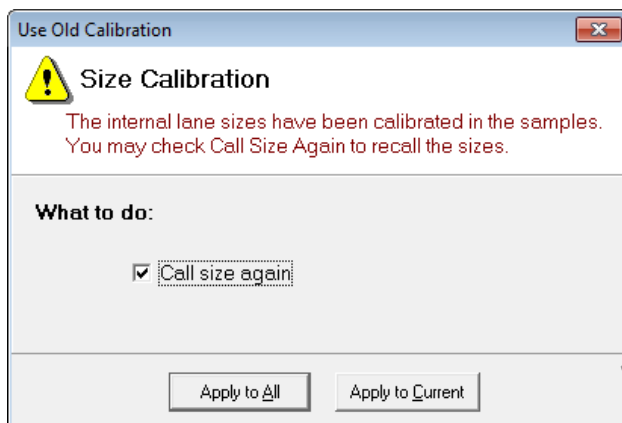
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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 21 OF 38

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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 22 OF 38

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 19

- 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:

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual

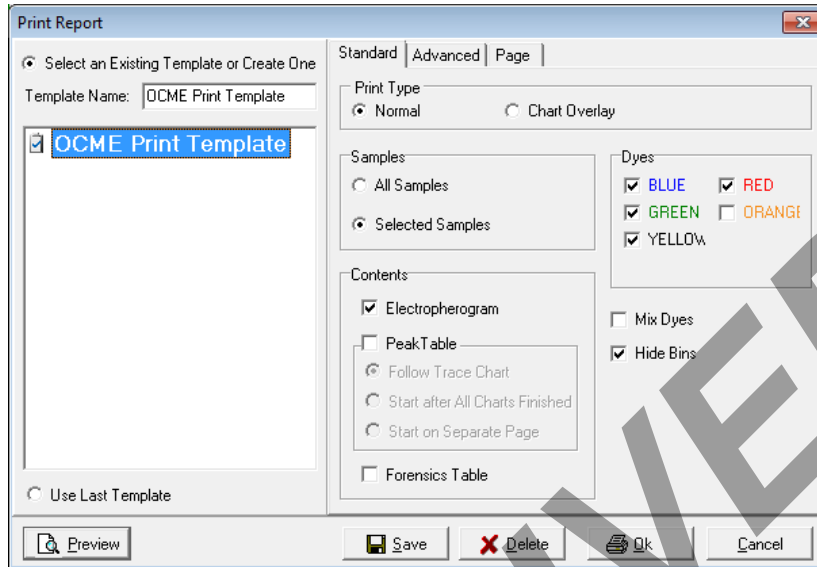
Status:Published

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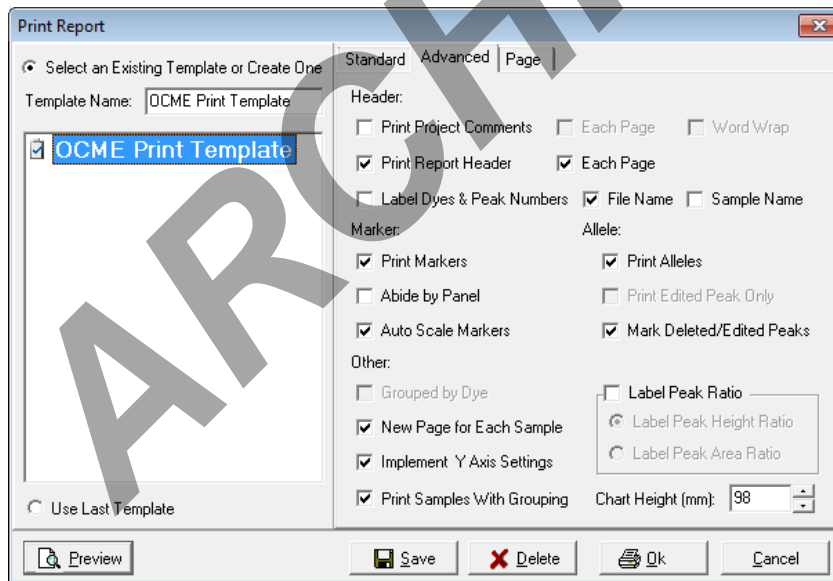
DATE EFFECTIVE
12/10/2019

APPROVED BY
Nuclear DNA Technical Leader

PAGE
23 OF 38



16.13.2 Advanced Tab:



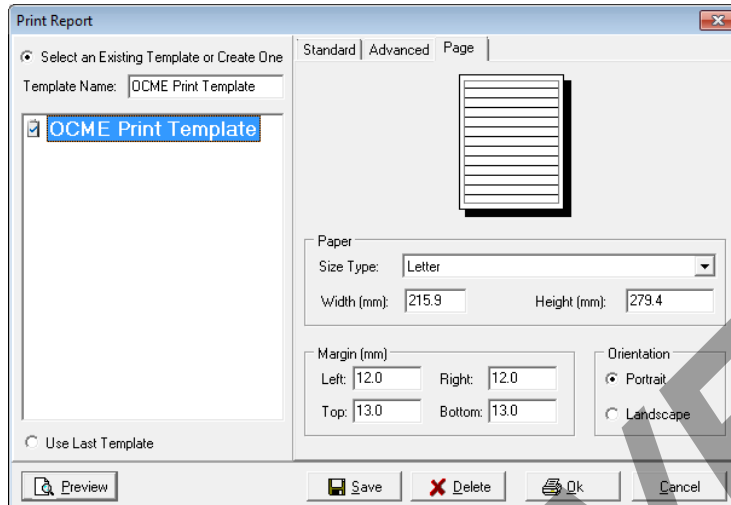
16.13.3 Page Tab

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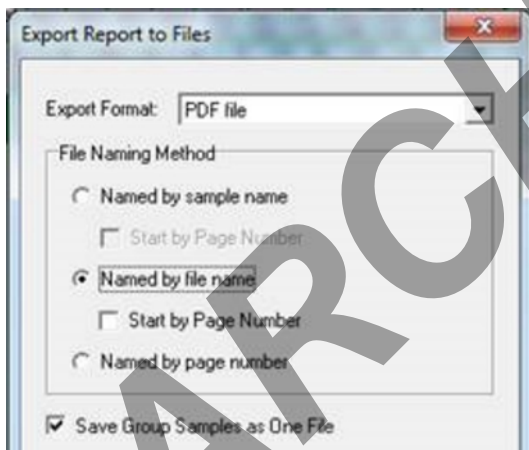
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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published	Document ID: 6311	
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 24 OF 38



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

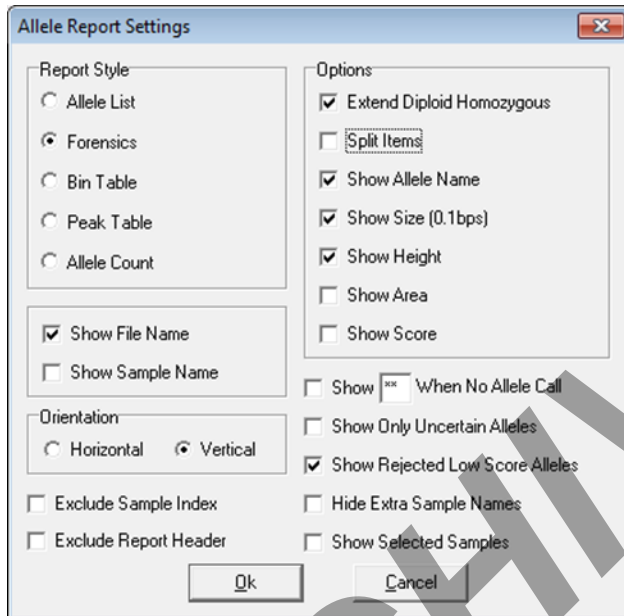
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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 25 OF 38

- 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 STRmix™ 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.


FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 26 OF 38

18 Reporting Analyst/Case Tech Reviewer Sample Review

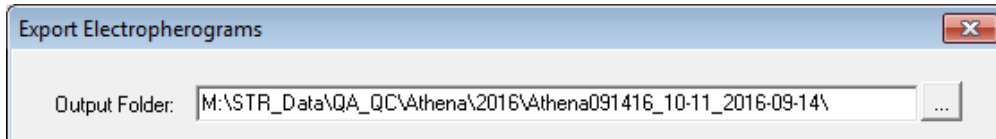
- 18.1 Verify all STR runs associated with case file samples checking for:
- 18.1.1 Passing Allelic Ladder was used to analyze run
 - 18.1.2 Passing controls were present
 - 18.1.3 Passing Size Standard for relevant case file samples
 - 18.1.4 Appropriate edits were made for relevant case file samples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

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

- 19.1 In the main analysis window,
- 19.1.1 Click Set Axis → Click Fixed X axis
 - 19.1.2 The Size Range will be the same as the X- axis range for analysis:
 - 60-510 for Fusion
 - 90-340 for Yfiler
 - 19.1.3 Select Auto Fit Y
- 19.2 Click Tools → Export Electropherogram
- 19.3 Set Output Folder to desired location for project PDFs by clicking  icon

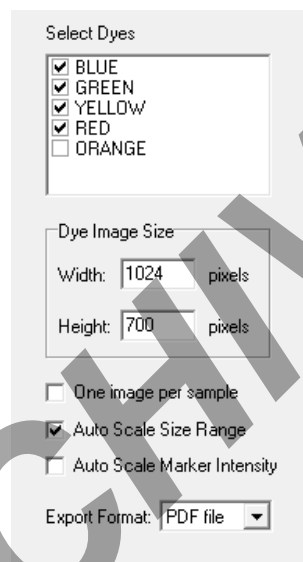
FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status: Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 27 OF 38



19.4 Ensure settings match below:

19.4.1 Make sure to select PDF as export format



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

19.4.3 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.

19.5 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**

19.6 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**

19.7 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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		Document ID: 6311
Status:Published	APPROVED BY	PAGE
DATE EFFECTIVE 12/10/2019	Nuclear DNA Technical Leader	28 OF 38

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

20 General Overview

20.1 Main Analysis Window

20.1.1 There are four major displays in the main analysis window.

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

20.1.2 See [HELPFUL ICONS INDEX](#) for further details.



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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 29 OF 38

20.2 Navigator; Sample File Tree

20.2.1 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**

20.2.2 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.

20.3 Project Summary

20.3.1 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	22 samples	PC error: 1/1	NC error: 3/3	Ladder error: 0/1	#Failed=1	#Flagged=19
-----	------------	---------------	---------------	-------------------	-----------	-------------

- 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.

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

20.4 Calibration Chart Window

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual

Status:Published

Document ID: 6311

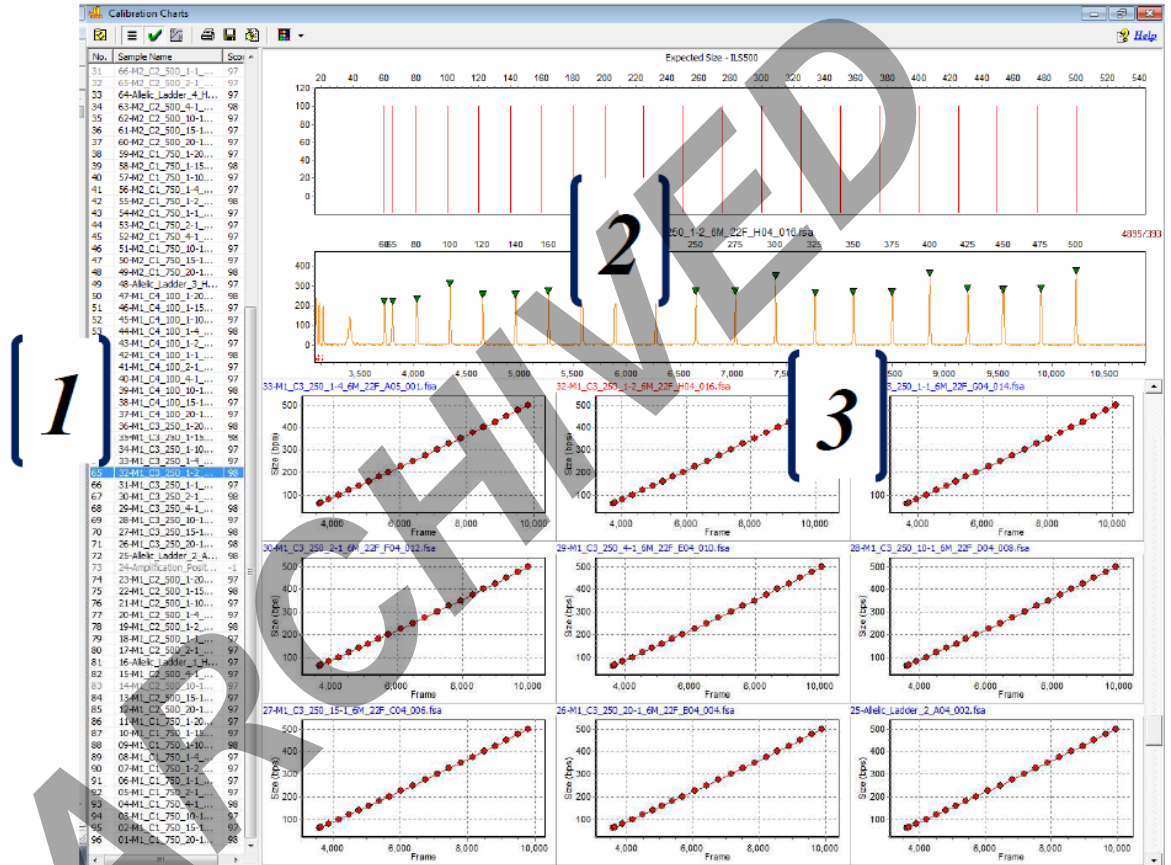
DATE EFFECTIVE
12/10/2019

APPROVED BY
Nuclear DNA Technical Leader

PAGE
30 OF 38

20.4.1 There are three major displays in the calibration chart window.

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



20.5 Sample List

20.5.1 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.

20.6 Size Standard Template

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

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 31 OF 38

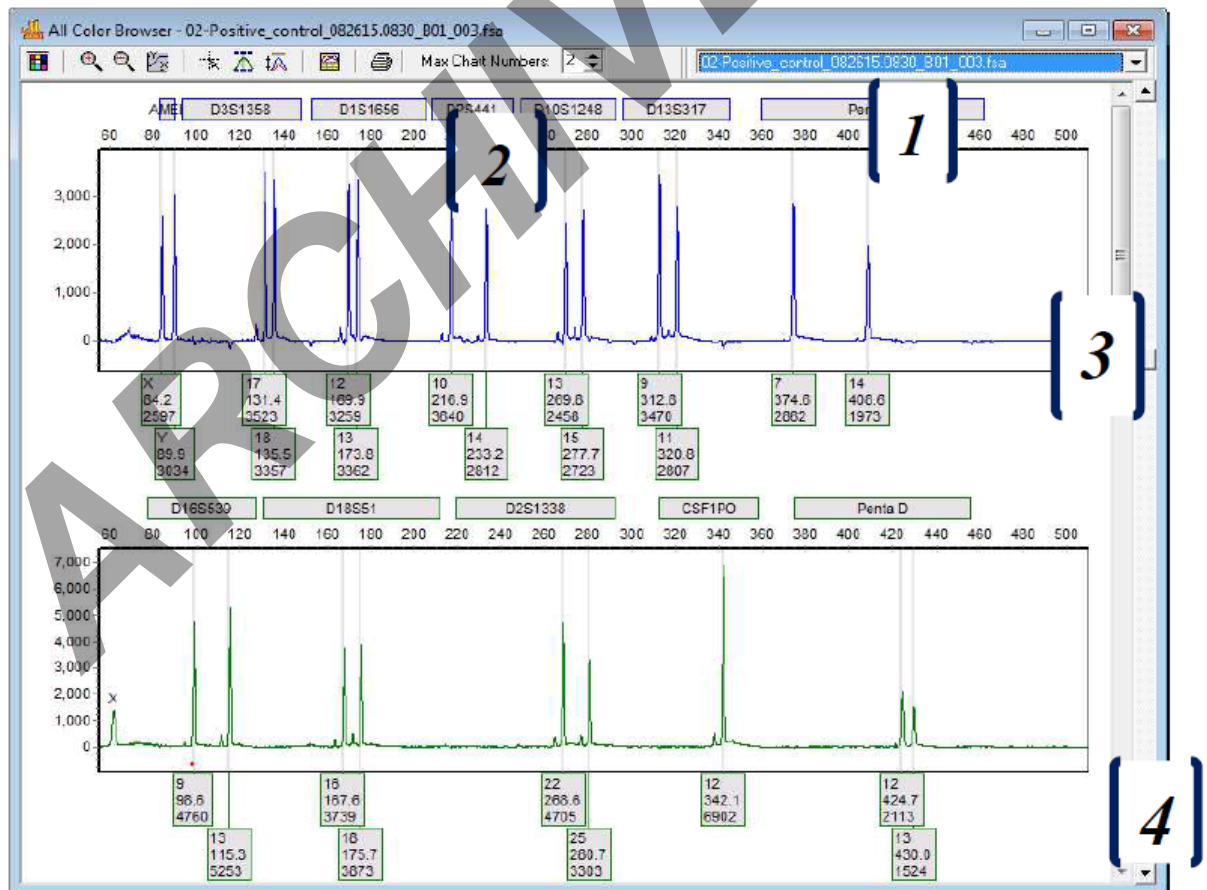
20.7 Samples ILS

20.7.1 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.

20.8 Browse by All Color Window

20.8.1 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




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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 32 OF 38

20.9 Sample Drop-Down

20.9.1 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.

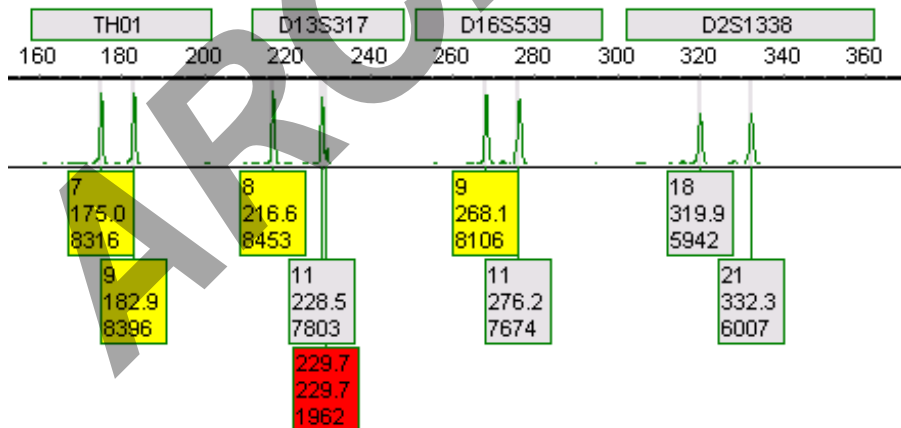
20.9.2 **NOTE:** Analysts can zoom in/out by using the zoom icons  located at the top toolbar. As an additional option, hold down the left button on the mouse and drag the dotted box that appears from the upper left to the lower right (around the desired “zoom in” area). To zoom out, hold down the left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

20.10 Max Chart Number

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

20.11 Allele Labels

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



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

20.11.3 **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.

20.12 Peak Table

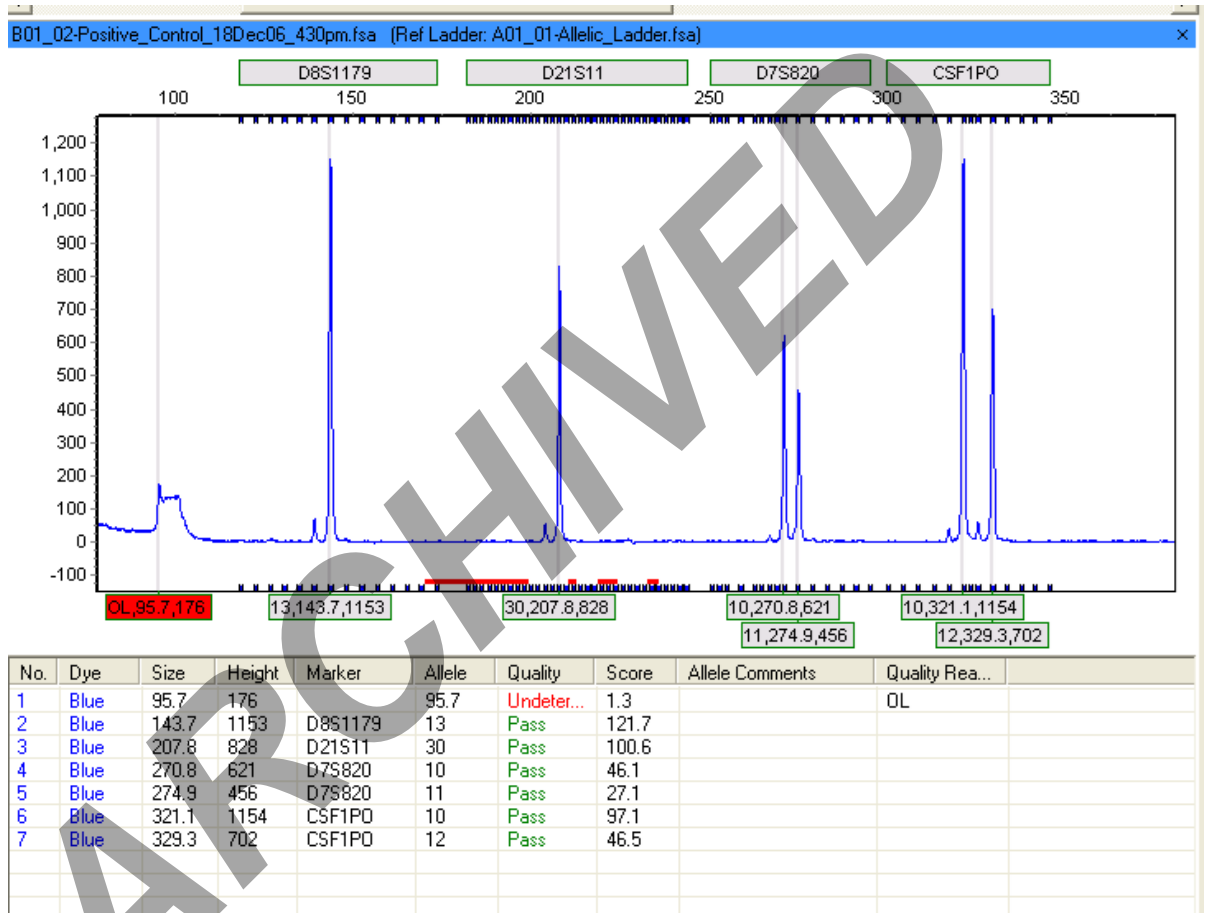
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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 33 OF 38

20.12.1 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









20.12.2 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.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 34 OF 38

21 HELPFUL ICONS INDEX





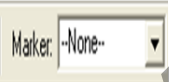


21.1 Main Toolbar Icons

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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 35 OF 38

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






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

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 36 OF 38




21.3 Browse By All Colors Icons

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

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
Status:Published		Document ID: 6311
DATE EFFECTIVE 12/10/2019	APPROVED BY Nuclear DNA Technical Leader	PAGE 37 OF 38

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

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

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FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

GeneMarker Manual		
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22 PEAK TABLE OPTIONS INDEX

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