Genemapper ID-Troubleshooting Guide

1. **REDEFINING THE SIZE STANDARD**

    1.1. **PROBLEM: "No Sizing Data" message; red octagon in SQ column**

    a. Select the flagged sample in the *Samples* tab of the *Project Window* as shown in the picture below.

    b. From the *View* drop down menu, select *Raw Data* **-** this will show what the sample looks like. If raw data is visible, and after analysis there is "No Sizing Data", most likely the size standard is mislabeled. If no raw data is visible, the injection for that capillary failed or no sample was loaded in to the well.

Raw data view shows usable data:
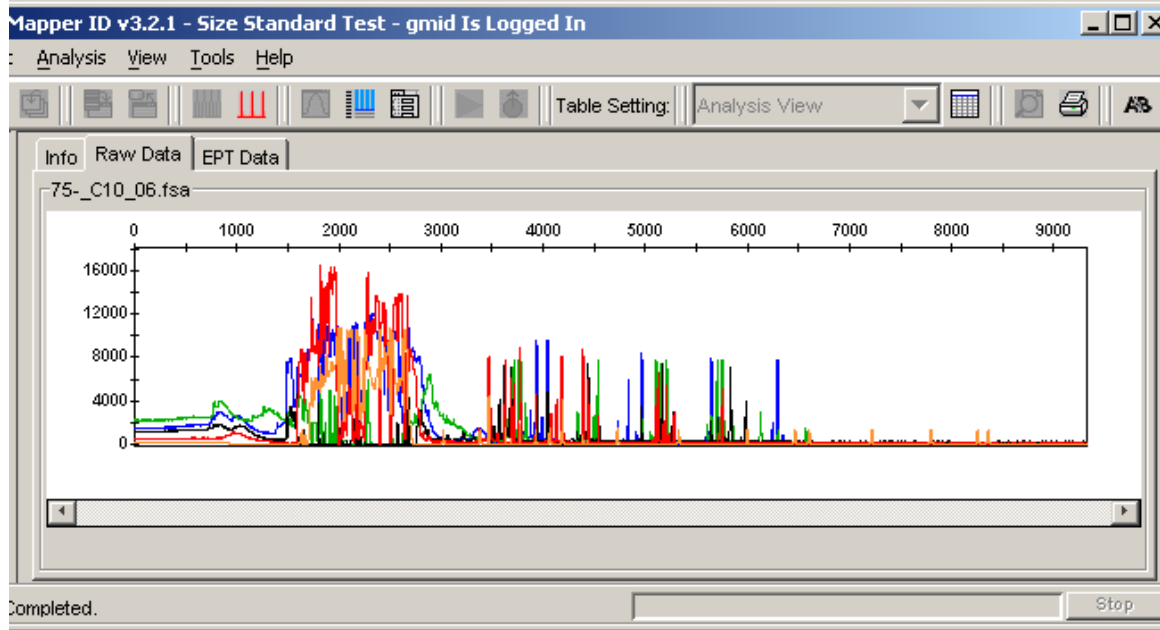
Raw data shows poor quality injection, this injection fails:

    c.   Click on the Size Match Editor icon ⊔⊔⊔ in the toolbar to open the sizing window. Here you can see the labels that the macro assigned to each peak in the size standard for that sample.

    d.   Using the magnifying tool, zoom in on the area that appears to be mislabeled.
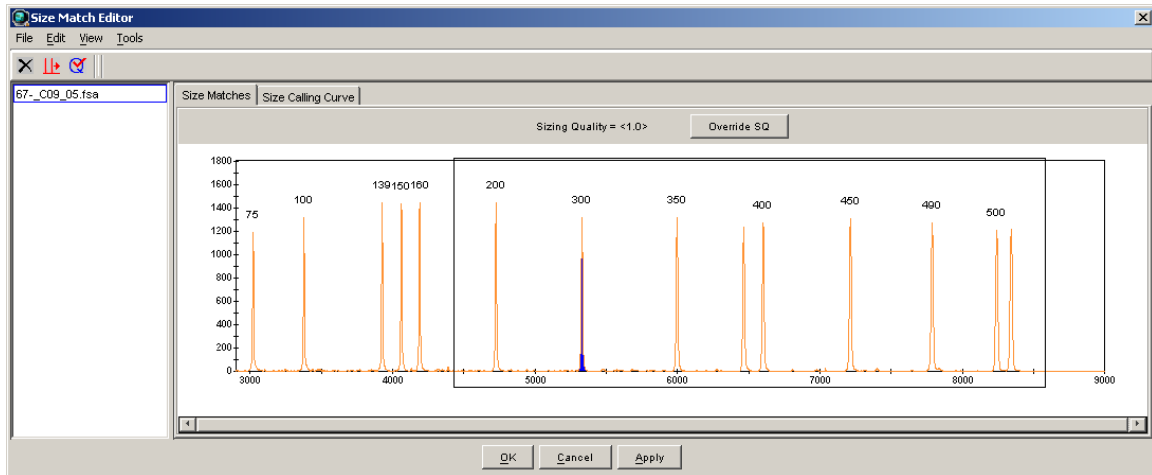
    e.   Left click to select the peak that needs to be changed. The peak will be highlighted in blue.

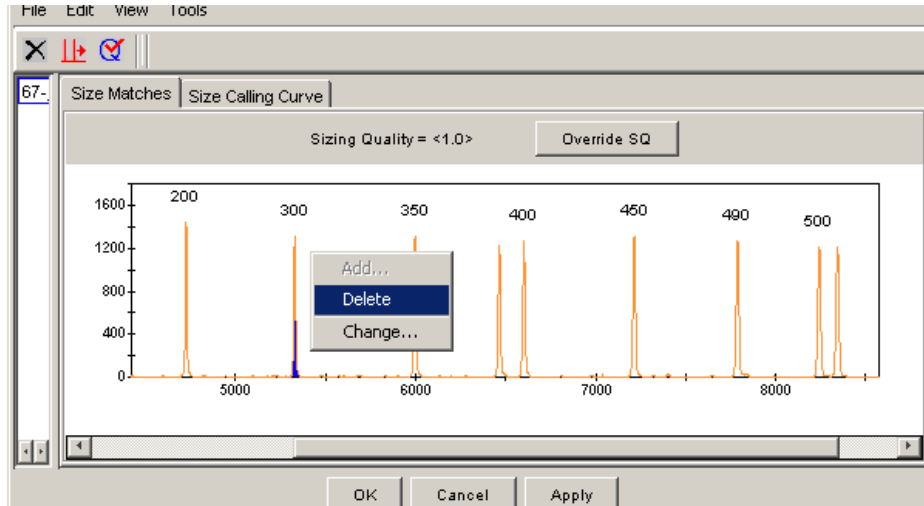   f.   Right click on the peak which is mislabeled, a menu pops up, with add, delete or change.

g.   If a peak is labeled which is not supposed to be (the 250 or 340 peaks), select delete and the peak is unlabeled.

h.   To re-label a peak correctly, select *change*, a dropdown list appears with the choices for that size standard. Choose the correct one. The peak will be re-labeled.
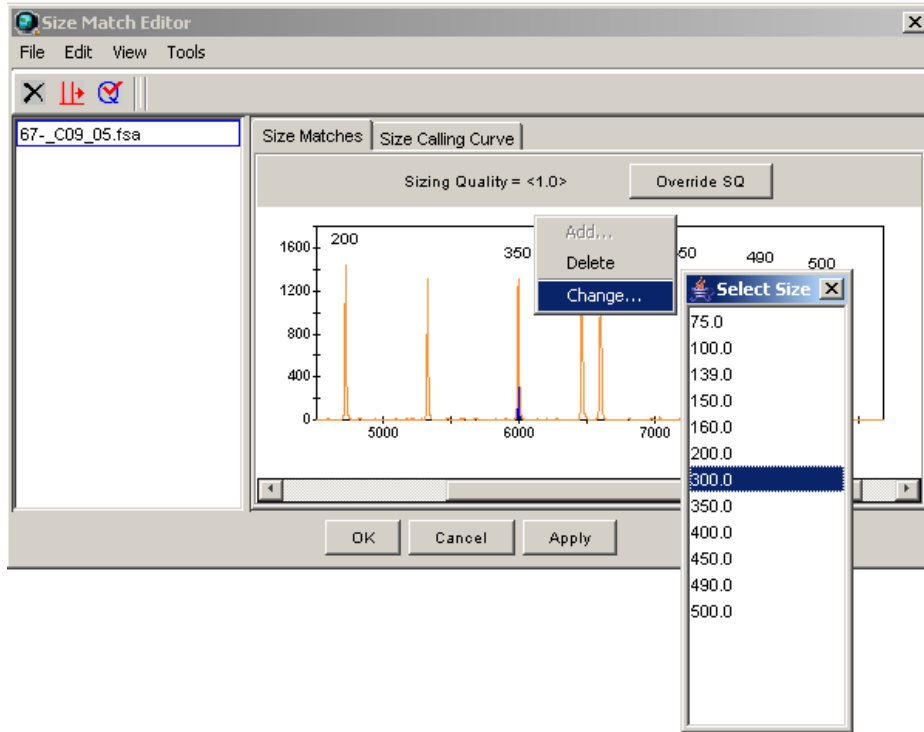
i.   Once all the changes are made, click on *Apply* to apply the changes. And then *Ok* to close the window.

j.   From the *View* drop down menu, select *Samples* to return to the *Samples* tab. In the *Analysis View* table setting, notice that the SQO box for that sample has a blue "X", the SQ box has turned to a green square, and the status box for that sample has a green arrow. The green arrow indicates that a setting (in this case it's the size standard) has been modified and it needs to be re-analyzed.
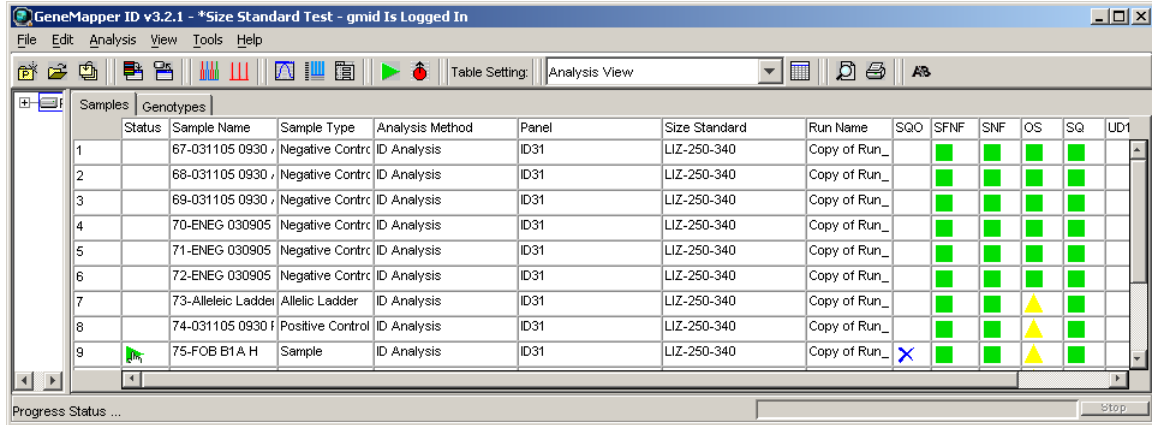
FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR TYPING

GENEMAPPER ID – TROUBLESHOOTING GUIDE		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 7 OF 16

    k.   Click on the green analyze button ▶ in the toolbar to re-analyze that sample with the redefined size standard.

2. ADJUSTING THE ANALYSIS DATA START POINT AND STOP POINT RANGE

2.1. **PROBLEM: The data is too far to the left or right of the injection scan range, or the size standard is cut out of the analysis range and therefore labeled incorrectly.**

    a.   From the *View* drop down menu, select *Raw Data.*

    b.   In the raw data view, choose a *start point* between the dye blob region that appears at the beginning of every injection, and the first required peak of the size standard by hovering the mouse pointer over that peak on the x-axis. At the bottom of the screen you will see that the data point and RFU is displayed for the area you are hovering with the mouse. Try not to include any of the blobs in the beginning of the run as they tend to be very high RFUs and the software uses the highest signal in each color to determine the Y axis cut-off in the plot view.

    c.   Choose a *stop point* anywhere after the last peak in the size standard.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR TYPING

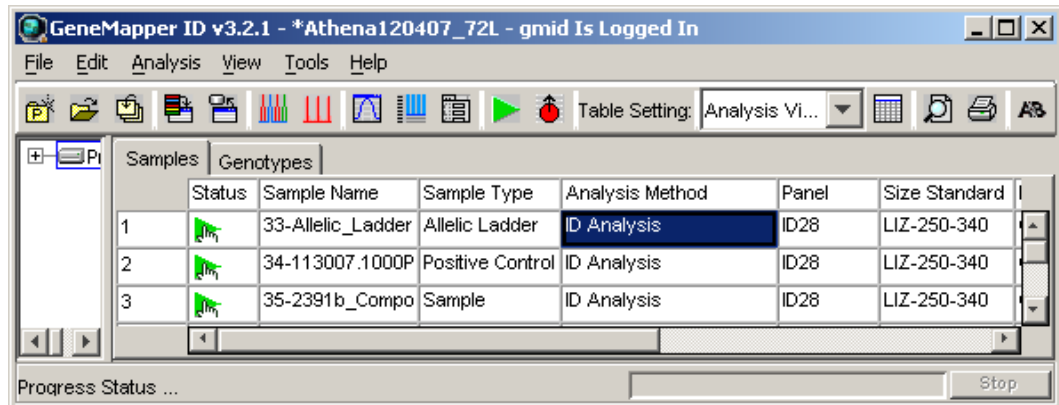|		
	GENEMAPPER ID – TROUBLESHOOTING GUIDE	
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 8 OF 16

d. At a minimum the following size standard peaks must be present for proper analysis:
- For Identifiler, 100bp to 450bp minus the 250bp and 340bp peaks.
- For Minifiler, 75bp to 400bp minus the 250bp and 340bp peaks. (The Analysis Methods peak detector tab must start at 65bp and not 75bp in order to properly size peaks.   This is because the 3rd Order Least Squares is the size calling method used.)
- For Yfiler, 75bp to 400bp minus the 250bp

NOTE: If the data in an Identifiler run is too far to the right and the last two peaks of the size standard (490 bp and 500 bp) are cut out of the visible range (as seen in the raw data view), the run can still be analyzed by selecting the size standard named "LIZ-250-340-490-500". In this case your *stop point* for the analysis range should be set to 10,000. Additionally, QC should be notified to inspect the instrument as this occurrence is usually indicative of a polymer leak.

e. From the *View* drop down menu, select *Samples* to return to the *samples* tab.

f. Select the analysis method in the project window to highlight it blue, and then double click to open it.

g. The *Analysis Method Editor* window will automatically open to the *Peak Detector* tab.
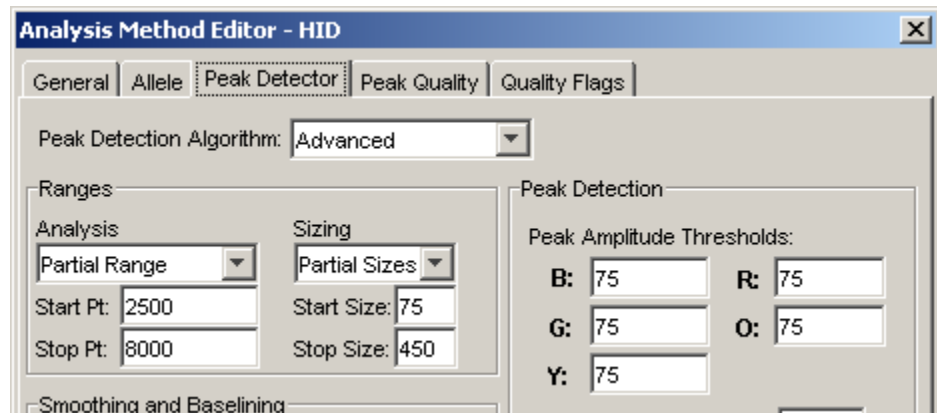
h. In the *Ranges* section, change the *start point* and *stop point* as necessary. The only other setting that can be changed in this window is the *Peak Amplitude Thresholds* for the color of the size standard. If the size standard produced a low RFU signal this setting can be lowered to 25 RFU only in orange for Identifiler, MiniFiler and Yfiler.

i. Click *OK*.

j. When you return to the *samples* tab, you will see that the samples have a green arrow in the status column signaling that a setting has been modified and it needs to be re-analyzed.

k. Click on the green analyze button [▶] in the toolbar to re-analyze with the modified setting.

3. Genotypes Plot – Locus Specific Quality Flags

3.1. PROBLEM: You see "no room for labels" in the panes of the *Samples Plot* window.

a. In the *Project Window* select the *Genotypes* tab, and then click the plot button (Analysis → Display Plots or Ctrl+L). This plot window displays each locus in a separate pane; this is called the "*Genotypes Plot*". Here you can clearly view each locus with its relevant quality flags. Once you are in the plot view you can toggle between the *Samples Plot* and the *Genotypes Plot* by going to the *Project Window* and selecting the *Samples* tab or *Genotypes* tab.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR TYPING

GENEMAPPER ID – TROUBLESHOOTING GUIDE		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 10 OF 16

    b.   If a locus contains a peak that exceeds the saturation threshold of the 3130x*l* a pink line will indicate the affected basepair range in every color, and draw attention to areas where the off-scale peaks have created pull-up.

    c.   These pink lines can be turned on or off from the plot window by selecting View → "Off-scale peak indicator" from the pull down menu. Ensure that the off scale peak indication is checked on.

Samples tab Genotypes tab

Plot button

Project Window:

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR TYPING

	GENEMAPPER ID – TROUBLESHOOTING GUIDE	
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 11 OF 16

Locus Specific Quality Flags
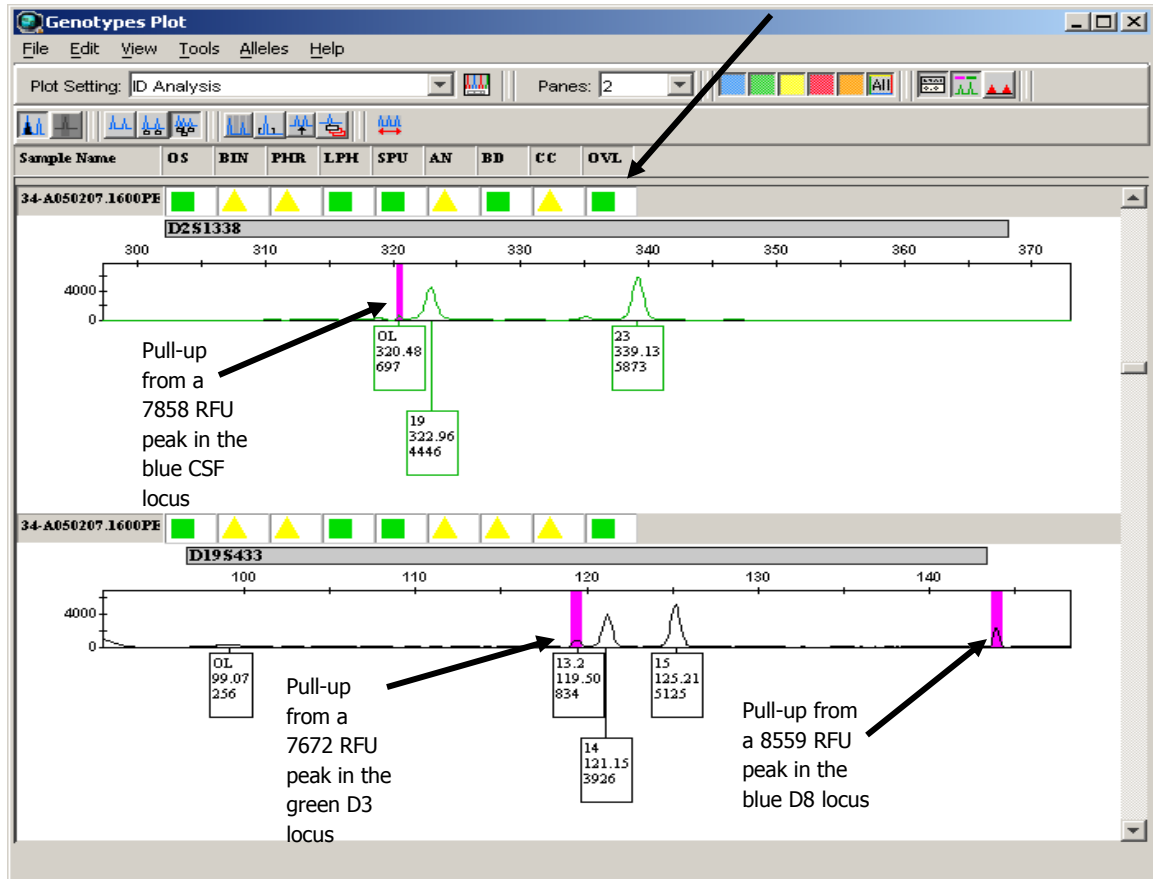
d. Regardless of peak height, if the pink off scale indicator is not triggered, the sample does not need to be rerun.

e. If the pink off-scale indicator is triggered, do one of the following (may be team specific):

   i. Remove all peaks in the sample and run at a dilution (oversaturated single source samples with plateau shaped or misshaped peaks or mixtures)

    ii. Remove all peaks in loci containing pink saturation lines and in all other loci within that base pair range. These other loci will also be easily identifiable because they have the pink line indicating where the overblown peak from the other color has interrupted that entire base pair range. Rerun at a lower parameter (if applicable) or with a dilution.

    f. The quality flags in the *Genotypes* window indicate locus specific problems. If a yellow "check" flag, or a red "low quality" flag result in any of the columns, refer to the appendix A – "Quality Flags" for a description of the flags and the problems they identify.

NOTE: The locus specific quality flags can only be viewed in the *Genotypes Plot* window.
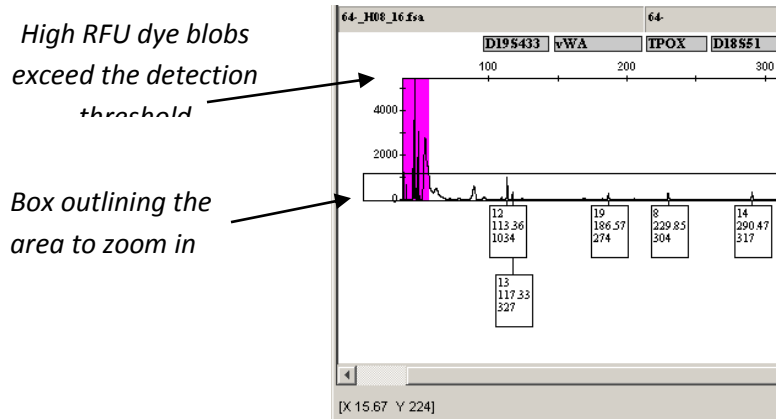
4. PRINTING

4.1. PROBLEM: The peaks in the printed electropherogram appear unusually small.

    a. The maximum RFU signal in each color is used to calculate the Y axis cut-off value for the plot display.

    b. When the analysis range includes too much of the dye blob region that appears at the beginning of each run, the Y axis cut-off will be very high because the blobs in the beginning of the run generally have high RFUs. As a result, the true peaks will appear really small in the plot display.

    c. To adjust the Y axis cut-off, move the mouse pointer over the numbers on the Y axis. Notice that the pointer will turn into a magnifying glass. While holding the left mouse button down you can move the magnifying glass up and down the Y axis and a box will form outlining the area to be zoomed in. Choose a level directly above the tallest peak. When you release the left mouse button, the area will automatically zoom in.

    d. If you need to zoom back out to the full range, double click on the Y axis while the mouse pointer is in the magnifying glass form.

High RFU dye blobs exceed the detection threshold

Box outlining the area to zoom in

e. Do this individually for each color where the peak display is affected by the high RFU blob region.

f. Print the electropherogram as described in section H. *Printing*.

5. ALLELIC LADDER

5.1. PROBLEM: All of the peaks in the ladders and my samples are labeled "OL".

Make sure that only the allelic ladders are designated as "Allelic Ladder" in the *Sample Type* column in the project window and rerun the analysis.
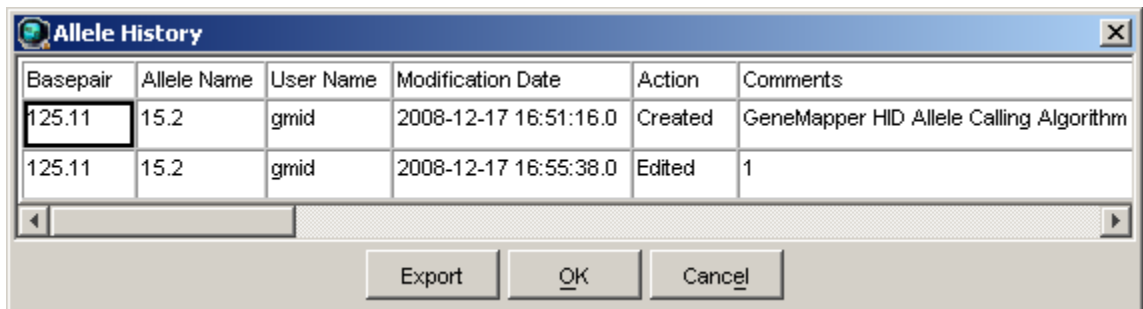
    5.2.   **PROBLEM: There is a confirmed off-ladder in my sample, how do I determine the closest allele call?**

        a.   Select the ladder with your sample and view the plot by clicking on the ***Display Plots*** button in the toolbar.

        b.   Turn off all colors except the color in which the OL appears using the quick select color buttons in the toolbar.

        c.   Turn the bins on by clicking on the ***Show Bins*** button in the toolbar.

        d.   Zoom in to the locus where the OL appears. The bins for that locus will be shaded in grey and you can determine what the true allele would be.

6.   **ALLELE HISTORY**

    6.1.   **PROBLEM: How do I know the history of an allele that was edited?**

        a.   Double click on the allele and a window opens with the allele history of that peak. When an allele is created by the macro, it will read "GeneMapper HID Allele Calling Algorithm" in the comments section. The rest of the table describes the action taken on that peak. In this example allele 15.2 was edited as pull-up. The action column describes what was done to the peak and the comments column contains the editing code.

Basepair	Allele Name	User Name	Modification Date	Action	Comments
125.11	15.2	gmid	2008-12-17 16:51:16.0	Created	GeneMapper HID Allele Calling Algorithm
125.11	15.2	gmid	2008-12-17 16:55:38.0	Edited	1

b.   If when you double click on a peak, a button pops up that reads "add allele call", it means that the peak was not labeled by the GeneMapper macro.

6.2.   PROBLEM: How do I view all deleted peak calls in a project?

Select all the samples in the *samples* tab of the *project window*.   Click the Samples Plot button to view the electropherogram.   In the *View* dropdown menu, select *Allele Changes*.   Any peak that was called and subsequently deleted will appear with a strike out as depicted below.
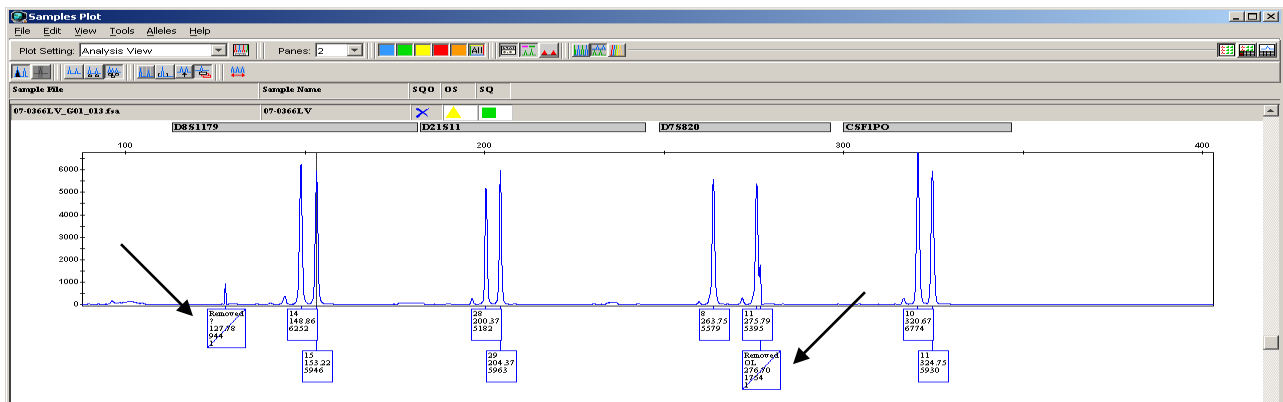
7.   SAMPLE HISTORY

7.1.   PROBLEM: How can I see the run log for a sample to determine how the run was injected and analyzed?

a.   In the *project window* under the *samples* tab, select the sample(s) of interest.

b.   From the *View* drop down menu, select *Sample Info*

c.   This view contains all of the information pertaining to the sample including error messages, current settings, run information, data collection settings, and capillary information.

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR TYPING

| GENEMAPPER ID – TROUBLESHOOTING GUIDE		
DATE EFFECTIVE	APPROVED BY	PAGE
06-20-2016	NUCLEAR DNA TECHNICAL LEADER	16 OF 16

8. TYPOGRAPHICAL ERROR IN SAMPLE

8.1. PROBLEM: There is a typo in the sample name.

In the *project window* under the *Samples* tab, click on the sample name in the *Sample Name* column and correct the error.

9. TABLE ERRORS

9.1. PROBLEM: An error message occurs when making the allele table.

If you get an error message, this means that you have exported the combined table while still in "Analysis View".
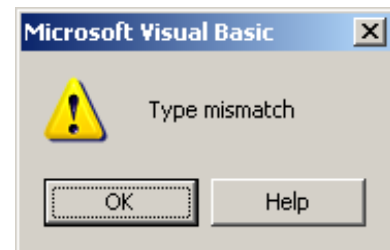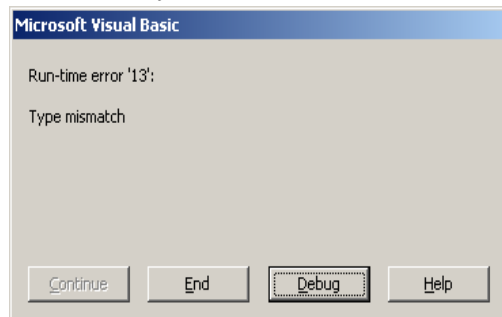
Click "End" or "OK" to close the error window, and close the excel worksheet without saving. Go back to your project in GeneMapper® *ID*. In the *Project Window* change the table setting drop down menu to **"Casework"**. Re-export the combined tables, then re-import into a new excel worksheet.