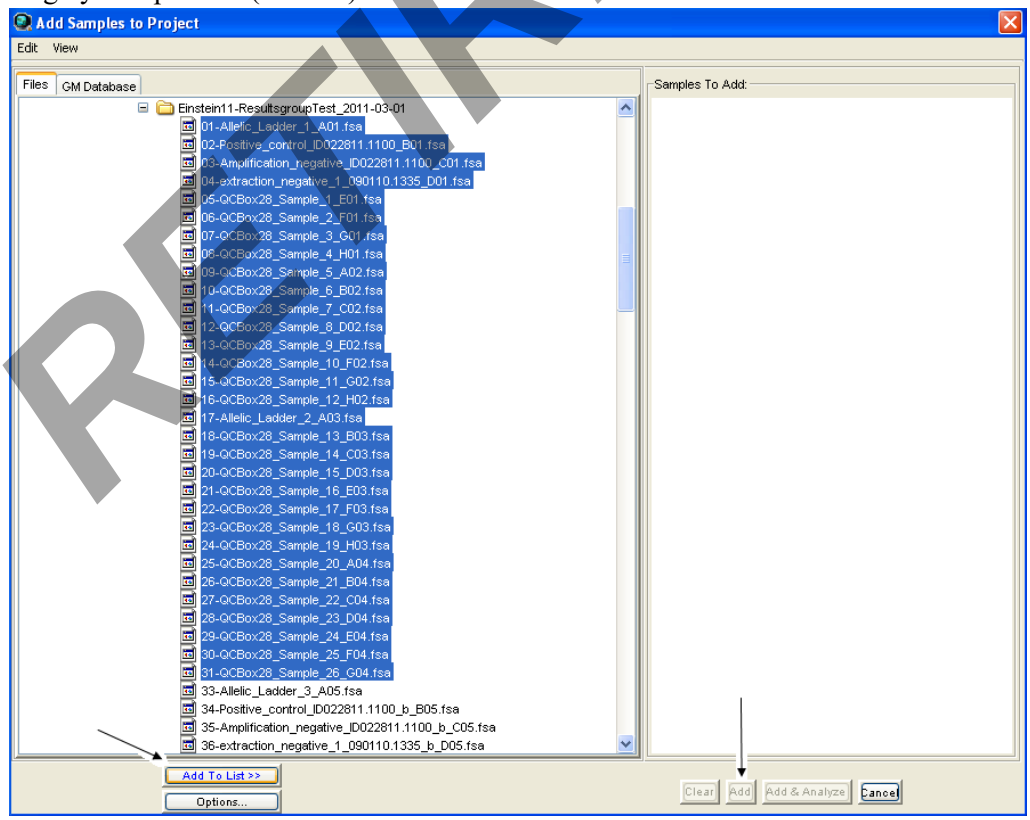
Genemapper ID Analysis

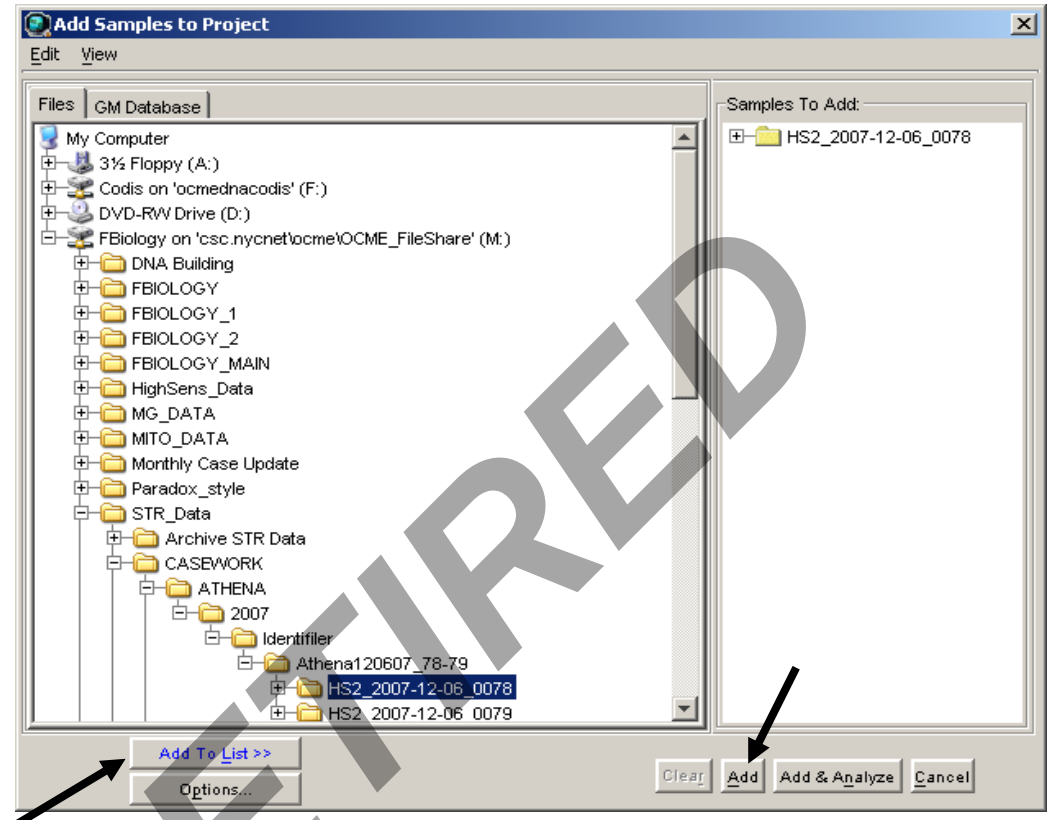
A. CREATING A NEW PROJECT

1. Double click on the GeneMapper ID v3.2.1 icon on the analysis station desktop.
2. When prompted, enter your username and password.
3. The program will automatically open a new (blank) project. This main window is called the **"Project Window"**.
4. Click on **File→Add Samples to Project…**or **Ctrl+K**. A new window will open, listing the drives or folders from which to add the samples on the left.
5. Navigate to the proper drive, and choose the folder that contains the run folders or samples that need to be analyzed. Select the run folder(s) or samples and click on **Add to List**.
6. On the bottom right Click **Add**. The chosen samples will now populate the project.

Adding by Sample File (.fsa file):



Adding by Run Folder:

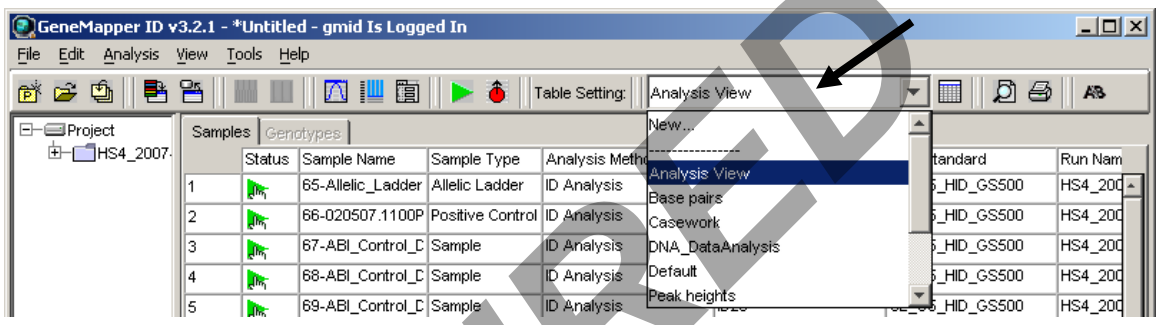


B. ANALYSIS SETTINGS

1. All defined settings must be used and can be referenced in *Appendix D. Analysis Method Editor* and *Appendix G. Default Table and Plot Settings*.

2. From the **"Table Setting"** drop-down menu in the toolbar, select "**Analysis View**".

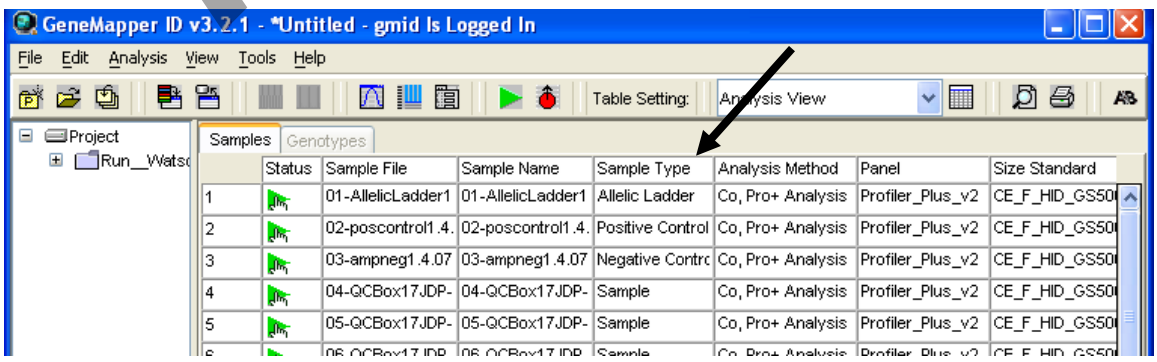
Project Window:



3. If the ladders, positive control, and negative control have not yet been designated, do so now under "***Sample Type***".

4. When there is more than one ladder in a project, designate one of the ladders as "Allelic Ladder" in the ***Sample Type*** column. Additional allelic ladders within the project should be designated as "Sample". If the allelic ladder analyzes correctly the additional ladders should be deleted from the project. If the allelic ladder does not analyze correctly, another allelic ladder in the project or folder may be designated as "Allelic Ladder" and the failed ladder deleted.

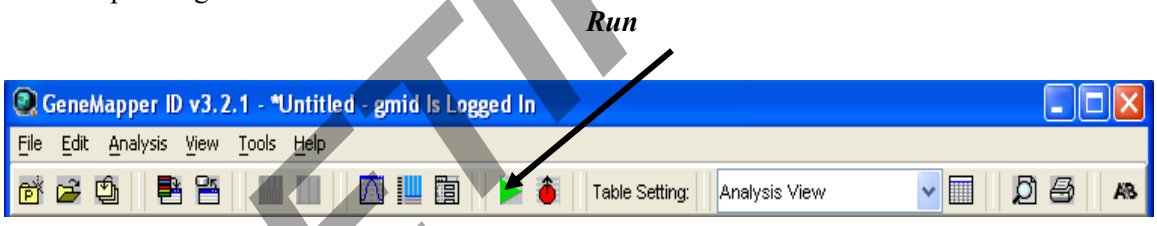
Project Window:



5. Fill in the correct analysis method, panel, and size standard following the table below. Once the analysis method, panel, and size standard have been chosen for the first sample, you can fill down the same information by selecting all three columns. Do this by selecting the title row of the columns and then while holding down the left mouse button drag across the three columns, the selected columns will be highlighted blue. Next, click on **Edit → Fill Down** or **Ctrl+D**.

System	Analysis Method	Panel	Size Standard
Identifiler 28 Cycles	ID Analysis	ID28	LIZ-250-340
Identifiler 31 Cycles	ID Analysis	ID31	LIZ-250-340
MiniFiler	MiniFiler Analysis	MiniFiler_GS500_v1	LIZ-250-340
YFiler	YFiler	YFiler_v2	LIZ-YFiler

6. A green arrow in the **Status** column of each sample means that the data is ready to be analyzed. Click on the **green arrow** in the **toolbar**. A "save project" prompt will pop-up asking for the run to be named.



7. Name the project with the same name of the run followed by the analysis parameter and the analysis set (i.e., "Newton062514 32-33IR A or Serena06141451-53M B"). Click **OK** to start analysis.

8. The progress of the analysis can be seen at the bottom of the project window in the progress status bar. Once analysis is finished the blue progress bar will stop, and the bottom left corner of the screen will read "Analysis Completed."

C. VIEWING ANALYZED DATA

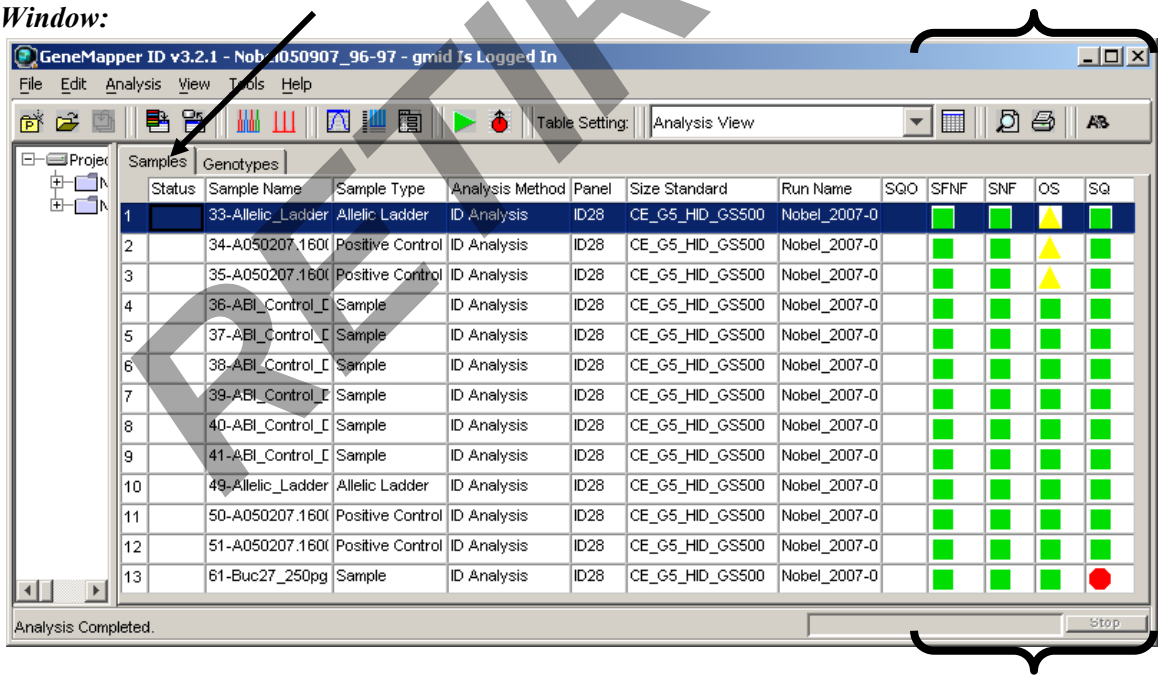
Samples View – Overall Sample Quality Flags

1. In the ***Project Window*** under the ***Samples*** tab, the columns to the right side with colored shapes are Process Quality Value (PQV) flags. These flags do not replace our method for editing samples. Each sample must still be viewed and edited. The flags are simply a tool to draw your attention to samples that have analysis problems therefore assisting you with initial analysis, and editing.

2. The **Pass** (green square) symbol indicates that no problem exists. 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. Whether a problem is flagged or not, proceed to the sizing section of the manual to individually check each size standard.

Samples tab ***Quality Flags***

Project Window:

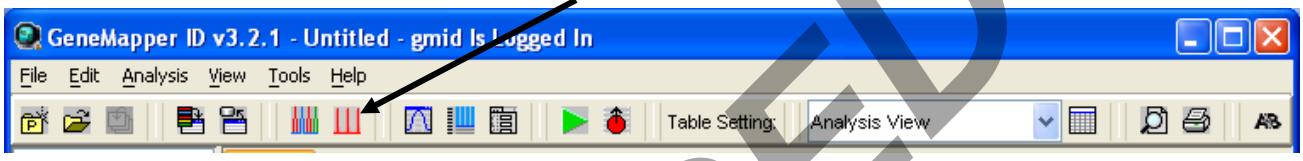


D. SIZING

1. Select all of the samples in the ***Samples*** tab by clicking on **Edit→ Select All**.

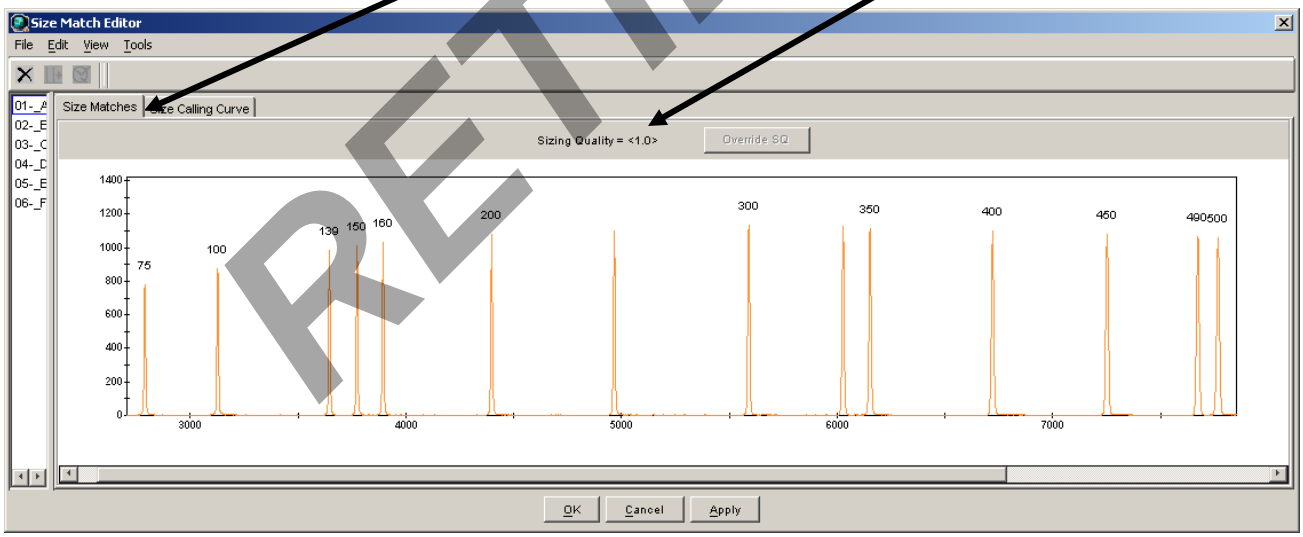
2. Next, click on the ***Sizing*** icon and the ***Size Match Editor*** window will open.

Sizing icon



Size Matches tab ***Sizing Quality value***

Size Match Editor window:



3. Using the arrow keys, scroll through the samples on the left column and check the sizing for each sample in the ***Size Matches*** tab. The sizing is displayed as a plot with the base pairs displayed above each peak. See Appendix F for a reference of size standards.

a. Identifiler samples are run with LIZ 500 and should not have the 250 bp or 340 bp size standard labeled. At least the 100bp to 450bp peaks must be present for proper sizing.

b. MiniFiler samples are run with LIZ 500 and should not have the 250 bp or 340 bp size standard labeled. At least the 75bp to 400bp peaks must be present for proper sizing.

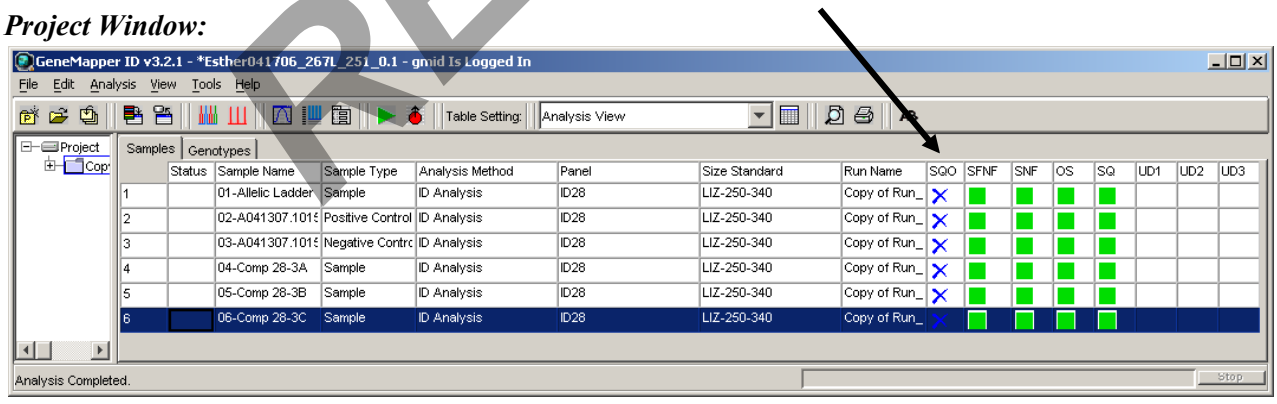
c. Yfiler™ samples are run with LIZ 500 (LIZ-YFiler) and should not have the 250 bp size standard labeled. At least the 75 – 400 bp peaks must be present for proper sizing.

4. **Red octagon symbol in the SQ column of the project window**:

In some cases you may still be able to use this data by redefining the size standard for that sample. For instructions on how to re-label peaks which have been incorrectly labeled, see the Appendix E – Troubleshooting section of this manual.

5. While still in the Size Match Editor window document that each sample size standard has been inspected by selecting **Edit → "Override All SQ"** or **Ctrl+Shift+O**; Click **Apply** and then **OK**. The Size Match Editor window will then automatically close. A blue "X" will appear in the sizing quality check box (SQO) for each sample, signaling that the size standard for each sample has been reviewed.

Project Window:



6. If a green triangle appears in the status column for any of the samples after you applied the SQO, press the green analyze button in the toolbar to finish the sizing quality override.

E. PLOT VIEWS

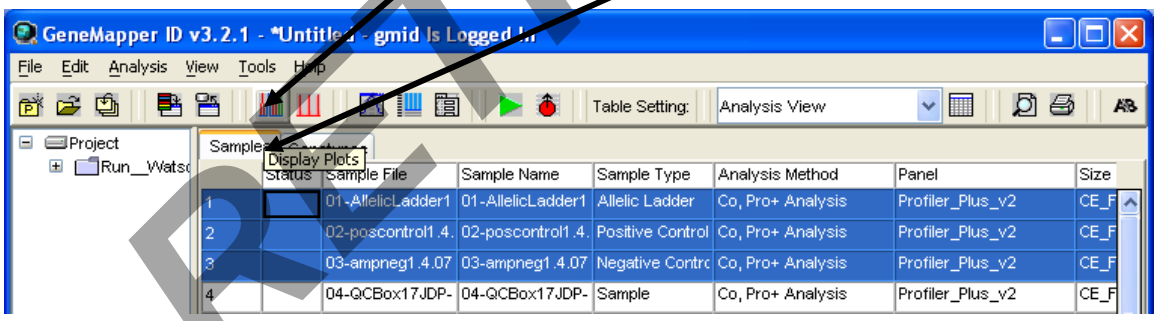
Samples Plot – Reviewing Ladders, Controls, and Samples

1. First, check the ladders and controls in the project using the following steps. If a project contains more than one allelic ladder, each ladder must be reviewed and pass analysis. Then repeat the steps for the samples. See Appendix F for a reference of allelic ladders and positive controls.

2. If there are two positive controls of the same date and time (i.e. high and normal), you can remove one by selecting it in the ***Samples*** tab of the ***Project Window***, then from the pull down menu select Edit → Delete from Project → OK.

3. In the ***Samples*** tab of the ***Project Window***, select the sample rows you want to view (i.e. ladders, controls, or samples) then click the plot button to display the plots (Analysis → Display Plots or Ctrl+L). Use the shift key or the ctrl key to select multiple samples.

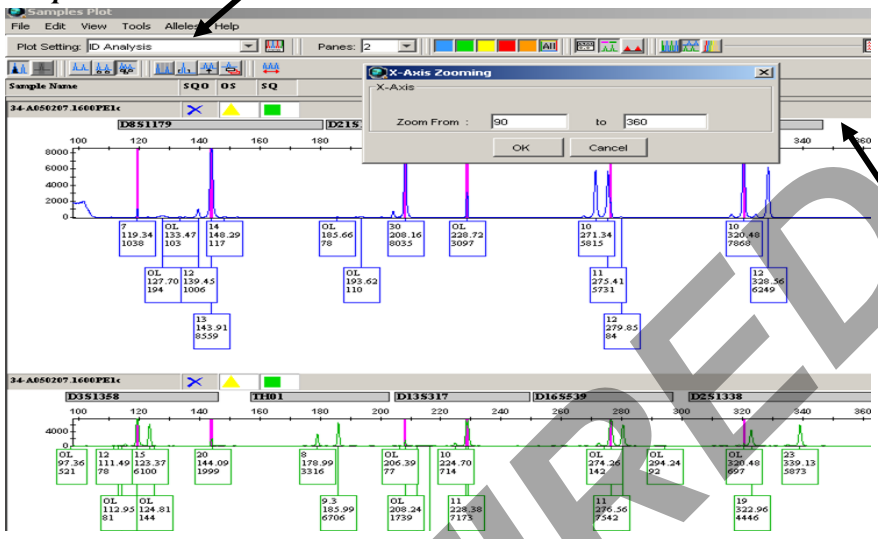
Project Window:



4. In the "*Samples Plot*" window toolbar there is a **Plot Setting dropdown list**. For Identifiler and YFiler, select *"Analysis View."* For Minifiler, select *"Mini Analysis."* This will label the peaks with base pairs, RFUs and allele name.

"Plot Setting" dropdown list

Samples Plot window:



Right click in this area for x-axis zooming

5. Adjust the window zoom by right clicking above the plot pane and using the X Axis Zooming dialog box to zoom into a specific range. Alternatively, hover the mouse above the panel; it will change into a magnifying glass that can be used to draw a box around a selected area to zoom in.

6. If you still have "no room for labels", for example when you have many alleles per locus such as the Allelic Ladder, it may be easier to review the sample in the "Genotypes Plot" as described in *Appendix E – Troubleshooting Guide, 3. Genotypes Plot – Locus Specific Quality Flags*. The Genotypes Plot is an alternate view option showing each locus in a separate pane. The locus specific quality flags can only be viewed in the ***Genotypes Plot*** window.

   NOTE: Refer to the Appendix A – "Quality Flags" for a description of the flags and the problems they identify.

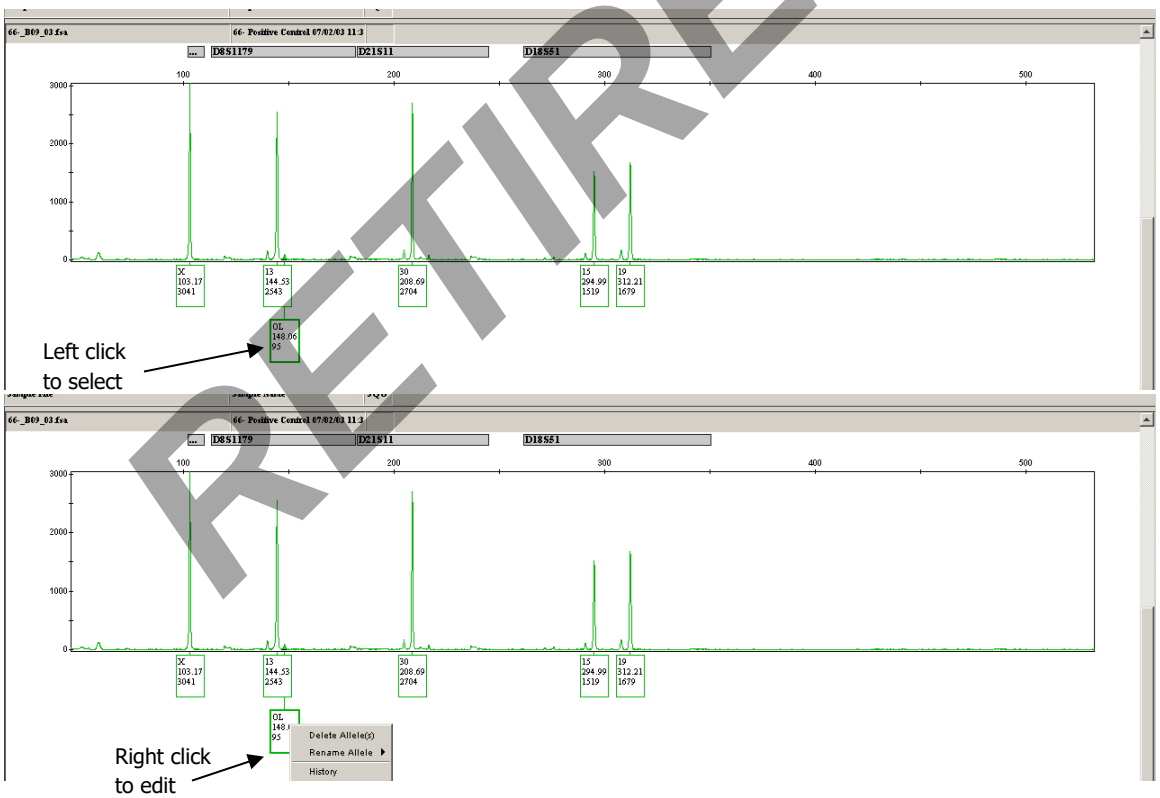
F. EDITING

Electronic Editing – First Analysis

1. You can view the sample in the ***Samples Plot*** window or the ***Genotypes Plot*** window or minimize back and forth between these views to facilitate analysis. Just ensure that you are using the correct view settings ("Analysis View" or "Mini Analysis.")

2. Left click on the allele in question to select it.

3. To edit the allele you must right click on it while it is highlighted and you will see a list of three choices – Delete Allele(s); Rename Allele; History.



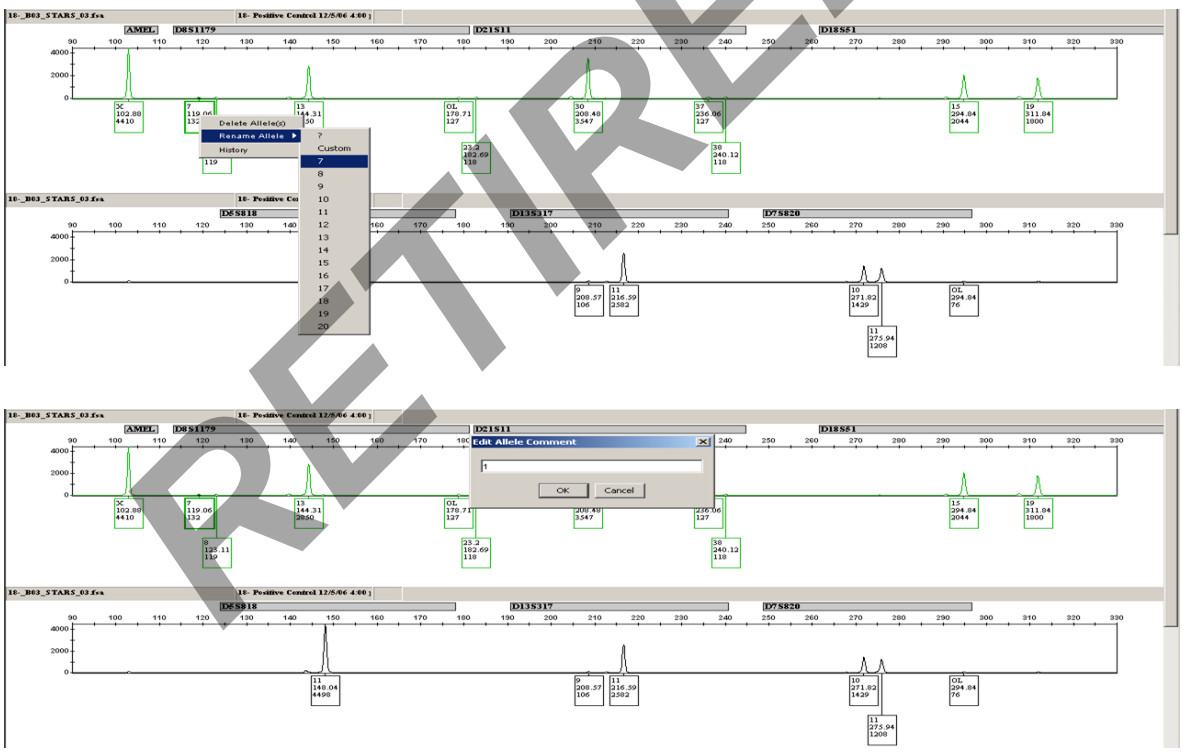
4. Select ***Rename Allele***; another drop down menu will appear listing all of the possible choices for alleles at that locus including ***"?"*** and ***Custom***.

5. If the sample has been labeled an Off Ladder (OL), choose "?". If the peak has been given an allele call, chose that same allele call from the drop-down list.

   For example, if a pull-up peak has been labeled a 7, highlight the 7 then right click and rename the allele 7 from the drop-down menu. This is done so that the reviewer can see what the allele was originally called.

6. A dialog box will then prompt you for an Edit Allele Comment. In the box enter the code for the allele edit (see Appendix B for a list of editing codes).

7. Click OK.



8. You will notice on the electropherogram that the peak has been labeled as follows: "changed", the allele call, base pair, and RFU, followed by the corresponding edit code.

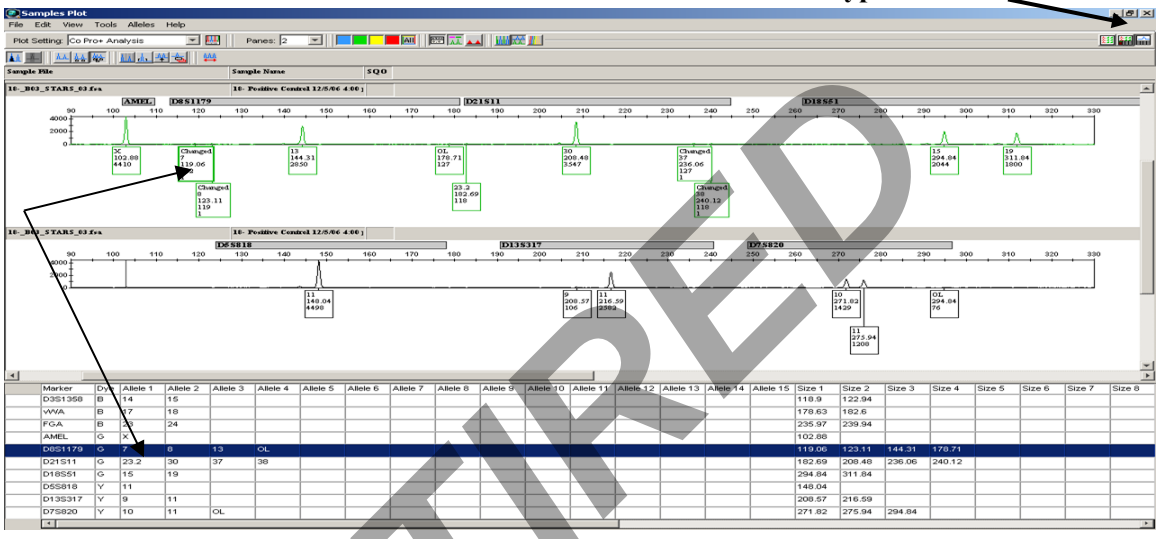
9. If you are removing all the labels from peaks in the entire sample because it needs to be rerun, for example, when a sample is completely overblown, then you can delete all the labels from peaks together without renaming each peak. The rerun is documented in column UD1.
   a. To delete a range of labels from peaks, select the first peak of the range, and while the first peak is still highlighted, drag a box across the range of peaks to select everything. Right click on the selection and click Delete Allele(s). When doing so, a box may pop-up with a message that more than one allele will be deleted. Click OK then enter the edit type in the allele comment box.
   b. If the removed labels from peaks need to be put back in, highlight the necessary samples from the ***Samples*** tab in the project window. From the ***Analysis*** drop down menu, select ***"Analyze Selected Samples."*** A pop up window will ask for confirmation and state the action cannot be undone. Click OK. Edit the sample(s) appropriately. If this action is done as a change to the original project, there is no need to change the project name. Create new tables and re-export the project.

10. If you mistakenly delete a label from a peak peak instead of renaming it first try to undo by selecting ***edit*** from the drop down menu then select ***undo***. You can undo as many changes as you made while that plot window was open, but if you close and re-open the plot window you will not be able to undo.

11. To revert a deleted peak back to the original allele call, select the peak, right click, then choose ***add allele call*** when prompted for an ***add allele comment*** leave it blank.
   a. The original allele call will be added to the peak but the word "changed" will still appear in the label.
   b. The word "changed" will not appear in the printed electropherogram, but it will appear in the electronic editing sheet as a sample entry with no edit comment.
   c. When the editing sheet is generated, scan through the sheet for any sample entries without edit comments these are the peak labels that were added back in. **Manually remove them from the worksheet before you print.**

12. Once editing has been completed you can view the edits in the Genotypes table. This table contains all of the alleles, sizes, and edits for all of the samples. Up to 15 edits can be captured per locus.

Genotypes Table

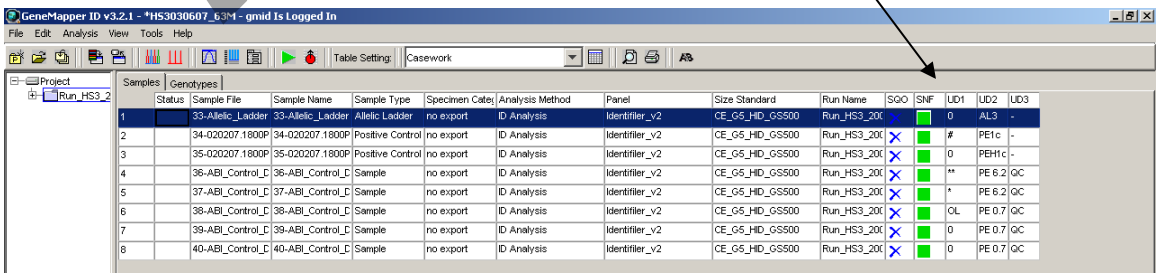


Electronic Rerun Sheet

1. If a sample needs to be rerun, this too is electronically noted. Close the ***Sample Plots*** window and return to the ***Samples*** tab in the ***Project Window***.

enter rerun code in UD1

Project Window:



2. Each sample scheduled for rerun must contain a code in column UD1. The first figure of the code stands for the **sample status**, the second figure stands for the **multiplex system** of the sample, and the third figure stands for the **rerun parameter**. The following are a few examples:
   a. A sample was overblown and all labels from peaks were removed. It should be rerun at a 1/10 dilution in Identifiler. Rerun Code: **ID
   b. An ID28 sample contained an off-ladder allele and needs to be rerun normal in Identifiler. Rerun Code: ^I.
   c. An ID31 sample has a poor size standard and needs to be rerun at the normal parameter. Rerun Code: #IN
   d. A sample has already been rerun once and the second time still produces an off ladder allele, therefore it will **not** be rerun. Rerun code: ^N/A
   e. A ID31 sample needs to be rerun at two separate parameters: one rerun at normal parameter for a range of labels on peaks removed and another to confirm an off-ladder using rerun high. List both parameters separated by a comma. Rerun code: *IN, ^IH

3. After entering a code, click outside of the cell for the data to export properly.

4. See the Appendixes B and C for a complete list of edit, system, and rerun codes.

Exporting Data for LIMS

Any case documentation developed outside of the LIMS should be scanned to a PDF document and attached to the appropriate electronic case record

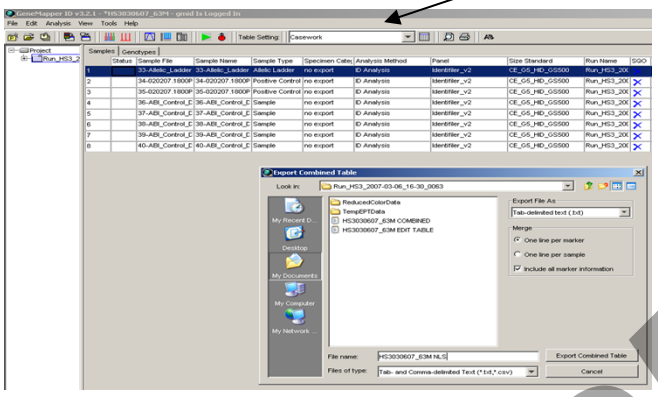
1. To export this information for use in the **LIMS**:
   a. First, in the ***Project Window***, make sure the table setting drop down menu is set to **"Casework"**. In this view you will notice an additional category column "Specimen Category" this column should be set to "no export" for all the samples.
   b. Then, Go to ***File → Export Combined Table***. This table combines the rerun information from the ***Samples*** table and the editing information from the ***Genotypes*** table.

2. Select the appropriate run folder and check the run name contains the initials of the person analyzing the run.

3. The file must be exported as Text-tab delimited (.txt). Ensure this is selected and click "Export Combined Table."

Casework table setting

Project Window:



To make the data available for review, the project needs to be exported from the Oracle database and placed on the network. Once on the network, the reviewer will have to re-import the project into a local Genemapper station before being able to review.

Exporting a Project

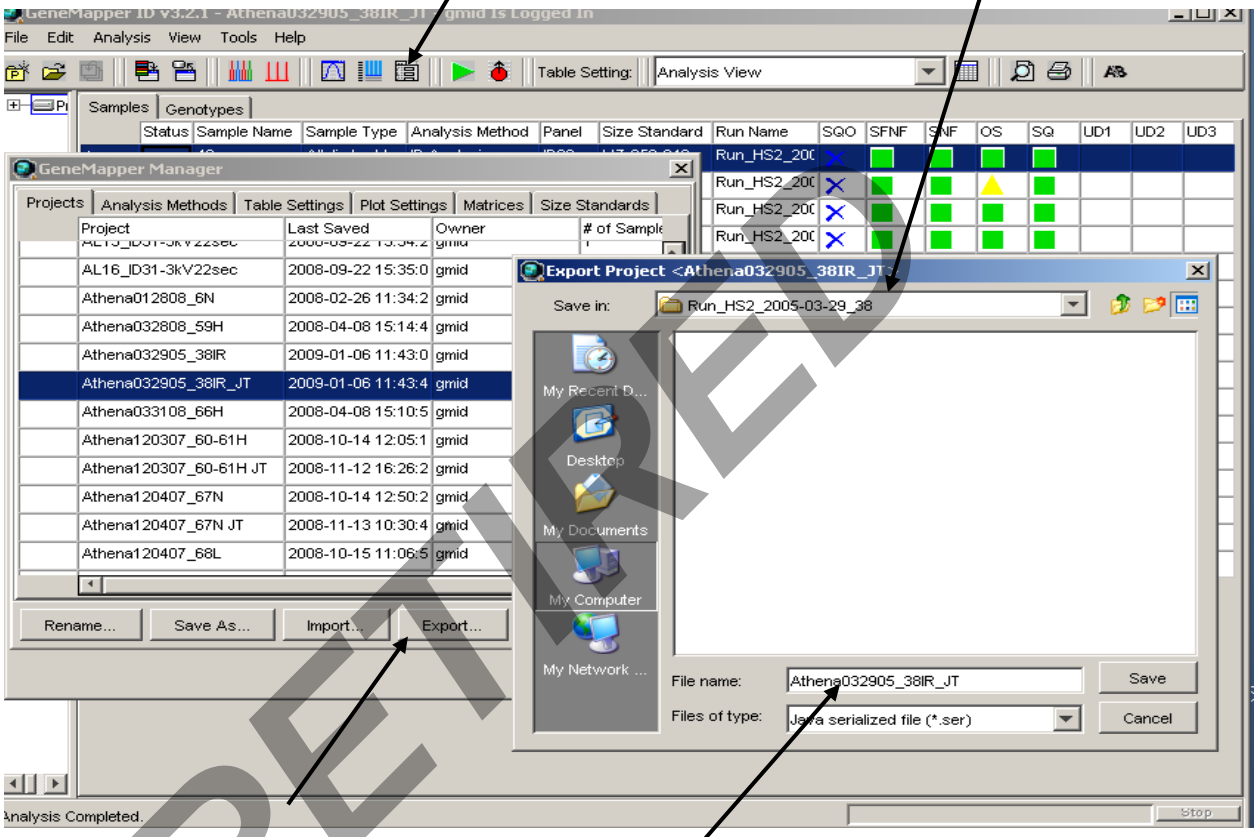
1. Click on Tools → GeneMapper Manager (Ctrl+M) or click on the GeneMapper Manager icon.

Select the project to export and click the **"Export"** button. A new window will open. Navigate to the 3130x*l* run folder through the "Save in" drop down box. In the *"File name"* box type in the name of the run. The *"Files of type"* box should be defaulted to Java serialized file (*.ser).

GeneMapper Manager icon

Save in your 3130xl run folder

Project Window:



Export

Type in the name of your run

G. EDITING - REVIEWER

Importing a Project

1. To import the project, open the GeneMapper Manager and click Import.

2. A new window will open asking for the file name. Navigate to the appropriate run folder, select the project and click **Import**. The project will be imported into GeneMapper.

3. To open the project you just imported, click *File → Open Project (Ctrl + O).* Select your project and click **Open**.

Electronic Editing - Reviewer

1. The reviewer should check the edits on the editing documentation against the electronic data.

2. To display the sample plots, highlight all samples and click the "Plot View" button or click "Analysis à Display Plots". For more detailed information, refer to Section E *"Plot Views"*.

3. The software always keeps the original allele assignments and a list of all the changes made. If desired, the allele history can be viewed. See *"Appendix E – Troubleshooting Guide, 6. Allele History"* for instructions.

4. To change, revert, or add an edit into the documentation, the reviewer should make the correction in the edit table.

5. In the GMID project, to revert an edited peak back to the original allele call, left click on the allele to select it, then right click to ***Rename Allele***; another drop down menu will appear listing all of the possible choices for alleles at that locus. Select the correct allele assignment to re-label the peak. This change will still be added to the history of that allele.

   NOTE: Peaks can be selected and labels deleted together. For example when a sample is overblown, and you need to remove labels from many peaks in a range, simply select the first peak of the range, and while the first peak is still highlighted, drag a box across the range of peaks to select all. Press the delete key.

   If the reviewing analyst disagrees with the removal of labels from all peaks made during the first analysis, the reviewer should not complete the review. Have the analyzing analyst go back to the project and reanalyze the affected sample(s), re-export the data and create new allele, edit and rerun tables and re-submit for review. The reviewer should then review the entire project again.

6. Once the reviewer approves all the edits, the peaks that are slated to be removed should be deleted by selecting the peaks individually and using the Delete key.

7. A "Delete Allele Comment" box will pop-up. This can be left blank if you agree with the edit. If you made a change to the edit on the editing table, enter the new edit code. Click OK.

8. Once the changed allele labels are deleted, the electronic editing sheet cannot be recreated. Therefore, **Re-Save the project as the run name with "Reviewed"** so the original edited project is not lost.

9. Generate the electropherograms using the instructions in the next section, Section H *Printing and Electropherogram Generation* .

9. Export the new project to the run folder on the network as described in the previous section.

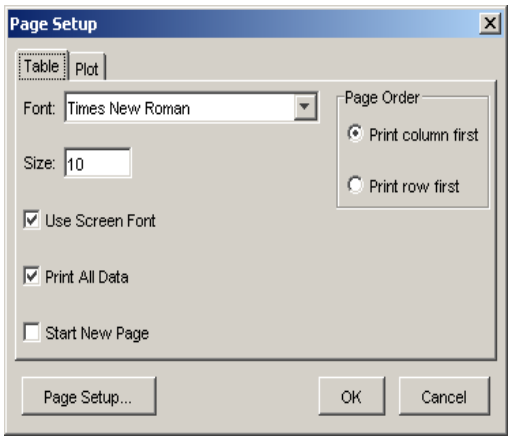
10. Once the project is exported, delete it from the project window in the GeneMapper Manager.

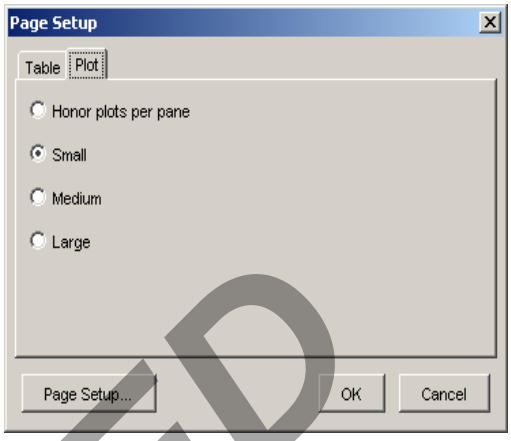
11. Changes to any reviewed project can be saved under the same "reviewed" name. However, the affected pages must be hand initialed by the analyst making the changes.

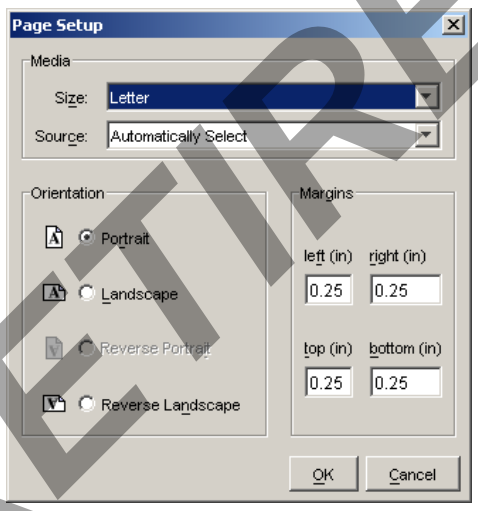
H. PRINTING AND ELECTROPHEROGRAM GENERATION

The following are the page settings for the printer that can be checked by selecting *File* from the drop down menu, then *Page Setup* while in the *Samples Plot* view.

Table Tab ***Plot Tab***







Printing: ID28, YFiler, and MiniFiler

1. Printing is done separately for the allelic ladders, controls, and samples. All allelic ladders in a project must be printed.

2. In the ***Project Window*** under the ***Samples*** tab, select only the rows you want to print.

3. Click the plots button.

4. In the Samples Plot window, select the plot setting from the drop down list according to the system and sample type you need:

Print - ID Allelic Ladder	Print - ID Controls	Print - ID 28 Samples
Print - YFiler Allelic Ladder	Print - YFiler Controls	Print - ID 31 PE and Samples
Print - Mini Allelic Ladder	Print - Mini Controls	Print – YFiler Samples
	Print – ID31 Negative Controls	Print - Mini Samples

5. Notice that the font size is reduced to accommodate the print setting. This setting will add the appropriate labels to each peak for printing.

6. Zoom to the appropriate range by using the X-Axis Zooming dialog box to set the plot to the correct range listed in the table below:

X-Axis Zooming:

Identifiler	Zoom from 90 to 370
YFiler	Zoom from 90 to 340
MiniFiler	Zoom from 68 to 300

7. Select *File* from the drop down menu, and then *print* (ctrl+P). Print to PDF format for LIMS. Save the PDF into the same directory as the analysis project. For the ladder, save the file as "Ladders". For the controls, save the file as "Controls". For the samples, save the file as the "[sample number]" on the plate. For example, if the sample was run as sample #23 on the plate, then the PDF will be saved as "23.pdf".

8. If the peaks appear unusually small against the baseline in the printed electropherogram, follow the additional instructions in *Appendix E - Troubleshooting, 4. Printing*, and re-print the affected pages.

Printing: ID31Positive Control (PE) and Samples

1. For ID31 Allelic Ladders and Negative Controls, use the associated ID print views. Continue below for printing the Positive Control and Samples.

2. In the ***Project Window*** under the ***Samples*** tab, select the replicates of one sample and its corresponding pooled sample (i.e. "trigger_swab_a", "trigger_swab_b", "trigger_swab_c", and "trigger_swab_abc").

3. Click the plots button.

4. In the Samples Plot window, select the plot setting from the drop down list titled "Print – ID31 PE and Samples".

5. Notice that in the Samples Plot tool bar only the blue dye is selected. This is because one color will be printed at a time for these sample replicates.

6. Using the X-Axis Zooming dialog box, set the plot to zoom from 90 to 370.

7. Select *File* from the drop down menu, and then *print* (ctrl+P). Print to PDF format for LIMS. Save the PDF into the same directory as the analysis project.

8. If the peaks appear unusually small against the baseline in the printed electropherogram, follow the additional instructions in *Appendix E. Troubleshooting Guide, 4. Printing*, and re-print the affected pages.

9. In the Samples Plot tool bar, unselect the blue dye by clicking it, and select the green dye. With only the green dye selected repeat steps 6 and 7 for the green dye. Then repeat steps 6 and 7 for the yellow dye and red dyes individually.

10. After all colors have been printed for one triplicate sample, repeat steps 1 through 7 for the next sample in the injection until all samples in that run have been printed.