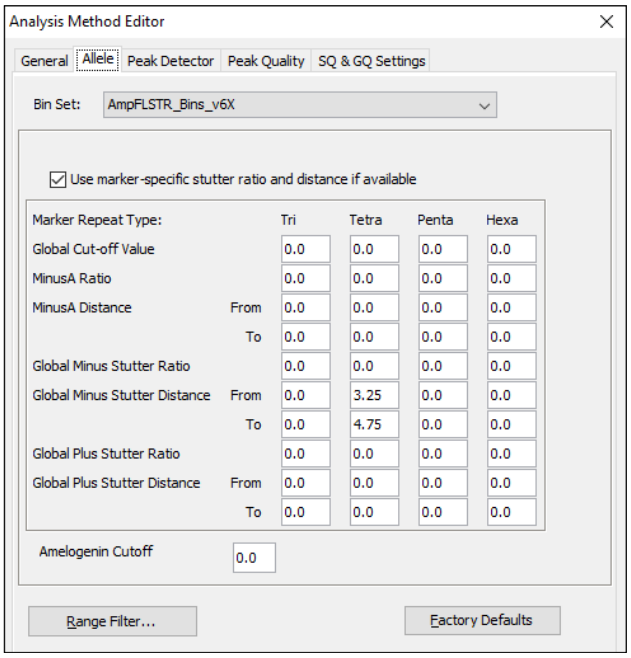
1. Open the GeneMapper ID-X version 1.6 software and login with the appropriate user name and password.

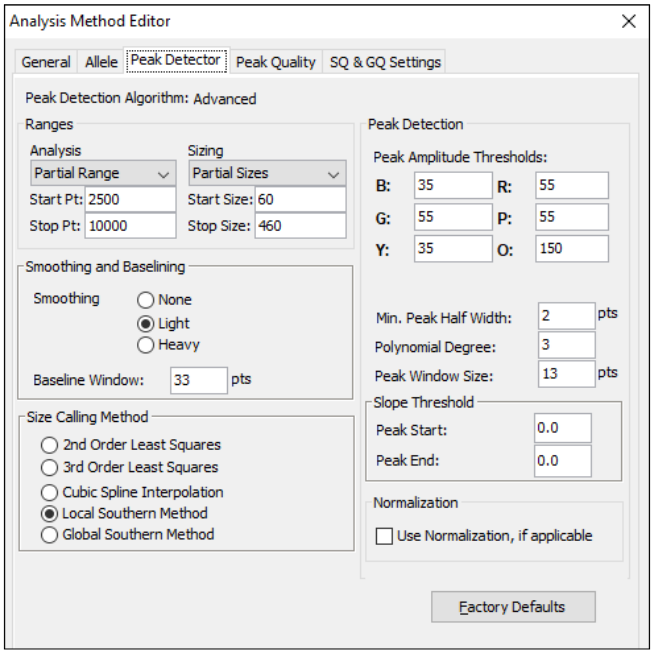
NOTE: The instructions below describe specific ways to accomplish tasks or select options in the software, but recognize these descriptions are not exhaustive. Often, there are multiple pathways of carrying out the same function, e.g. using the icon menu, text menu, or shortcut keys. Please see the GeneMapper® ID-X Software Version 1.5 Reference Guide, GeneMapper™ *ID-X* Software v1.6 User Bulletin, and the software menus for alternative options.

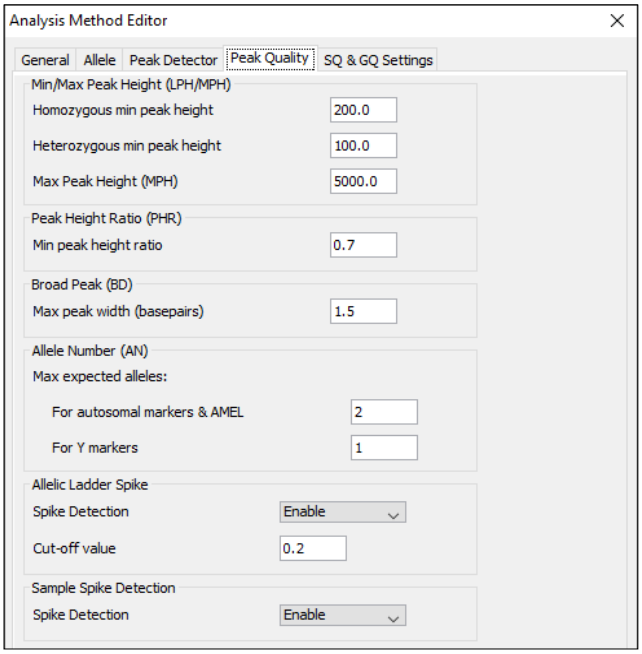
To verify setup:

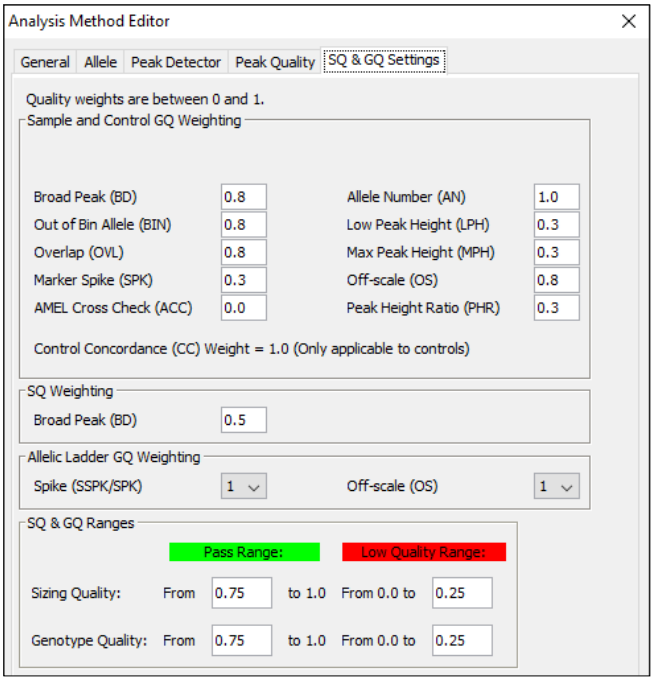
1. Select the 'Tools' menu, then select 'Panel Manager' to verify that the panels folder 'AmpFLSTR_Panels_v6X' is present in the window in the upper-left under the 'Panel Manager' icon. Then select the 'AmpFLSTR_Panels_v6X' folder to verify that the 'AmpFLSTR_Bins_v6X' bin set is loaded in the panel. If the panel and bin set are not present, select the 'Panel Manager' icon in the upper-left window, then from the menu select 'File' and 'Import Panels'. Select 'AmpFLSTR_Panels_v6X' from the Panels folder list and select the 'Import' button. Then select the newly imported 'AmpFLSTR_Panels_v6X' folder in the upper-left window. From the menu, select 'File' and 'Import Bins'. Select 'AmpFLSTR_Bins_V6X' from the Panels folder list and select the 'Import' button. Then select 'Apply' and then 'OK'.

2. Select the 'Tools' menu, then select GeneMapper ID-X Manager. Select the 'Analysis Methods' tab. Verify the method 'YFP_CTDSS' is listed. If not, import the method from the 'Panels_Bins' folder on the U: Drive. To verify the settings of the method, select it from the list of 'Analysis Methods' and select 'Open'. Successively, select the 'Allele', 'Peak Detector', 'Peak Quality', and 'SQ & GQ Settings' tabs and verify the settings match the screen shots to the right and on the next page. If any of the settings do not match, correct them and select 'Save'. Note: the start point for the analysis partial range is listed as 2500 scans, but should be adjusted for each set of data to avoid including the amplification primer front in the analysis range so the software's auto-scaling of the Y-axis feature will adjust the sample's allele peaks to the appropriate scale.









3. Select the 'Table Settings' tab and verify that 'CT-DSS Table' is listed. If not, import the method from the 'Panels_Bins' folder on the U: Drive. To verify the settings of the table, select it from the list of 'Table Settings' and select 'Open'. Select the 'Samples' tab and verify that the only 'Column Settings' selected to show are 'Sample File', 'Sample Name', 'Sample Type', 'Analysis Method', 'Panel', 'Size Standard', 'Sizing Quality Overridden (SQO)', and 'Sizing Quality (SQ)'. Sort by 'Sample File', 'Ascending', then by 'None', then by 'None'. Select the 'Genotypes' tab and verify that the only 'Column Settings' selected to show are 'Sample File', 'Marker', 'Allele', 'Size', and 'Height'. Sort by 'Sample File', 'Ascending', then by 'Marker',

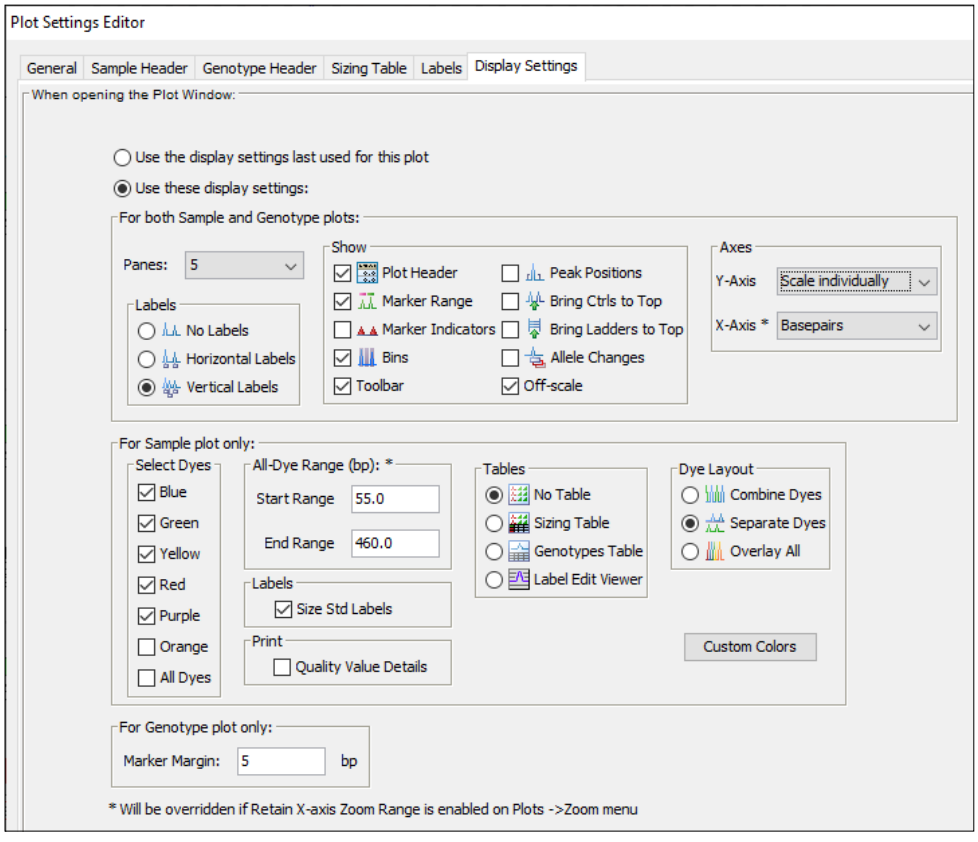
'Ascending', then by 'None'. If any of the settings do not match those below, correct them and select 'OK'.

To Analyze Data:

1. If another project is already open, clear it by selecting 'New Project' icon from the menu header. To import data files, select the 'Add Samples to Project' icon in the menu header and navigate to the data folder. Select the data folder and then select 'Add To List >>' on the lower-left and the folder will appear in the 'Samples To Add:' list on the right. Select more than one data folder if needed. Select the 'Add' button on the lower-right to add the data to your project. The samples will appear in the 'Samples' tab list.
2. Verify that the 'Table Setting:' in the menu header is set to 'CT-DSS Table'. If not, use the drop down menu to select it.
3. In the 'Sample Type' column, verify that your ladders have the 'Allelic Ladder' type and all other samples have the 'Sample' type selected. Adjust if needed.
4. For samples amplified by the Yfiler™ Plus Kit, select 'YFP_CTDSS', 'Yfiler_Plus_Panel_v4.1X-Allele-Specific, and 'GS600_LIZ_(60-460)' for the Analysis Method, Panel, and Size Standard, respectively. Tip: Select the method, panel, and size standard for the first sample in the list. Then select the 'Analysis Method' cell in the header and while holding the left mouse button drag the selection across to the 'Panel' and 'Size Standard' cells to select all three columns. Then select 'Ctrl+D' on the computer keyboard to fill down these choices to all the sample and ladders.
5. Select the green play button icon in the menu header to analyze the data with these settings. The 'Save Project' window will pop up asking for a project name. Name the project 'YFP-3500#-Batch ID-Modifier#-Any other details', e.g. YFP-2-MAP102219-2-JES. The modifier number and other details are optional. Select 'OK'. The project will be analyzed and saved to the oracle database of the software.
6. When analysis of the samples is complete, select the 'Samples' tab. The 'SQ' column will indicate the sizing quality of the ILS for each sample. A green square indicates passing, a yellow triangle indicates caution and that there may be a problem with the ILS, and a red stop sign indicates ILS failure. To view the ILS for the samples, use the left mouse button to select the cell containing '1' to the left of the first sample in the list and while holding down the left mouse button drag down the entire list of samples. Select the 'Size Match Editor' icon in the menu header and the editor will open. Selecting a sample from the list on the left will display the ILS peaks detected for that sample. A passing ILS will typically have a score of 1.0, but any value ≥ 0.75 will pass as determined by the Analysis Method's 'SQ & GQ Settings' (see screen shot above). Occasionally, spikes, spectral pullup, or other artifacts in the orange dye channel may be identified by the software as an ILS peak altering the detection of one or more of the true ILS peaks. To modify the detection of a peak, first select

the peak with the left mouse button such that it fills with a dark blue color. Then select this blue highlighted peak with the right mouse button and select 'Add…', 'Delete', or 'Change…' as appropriate. Select the 'Apply' and then 'OK' buttons and reanalyze the sample by selecting the green play button icon in the menu header. If the 'Size Match Editor' has been used to modify the ILS for a sample, a green check will appear in the 'SQO' column for that sample. Alternatively, reinject, or replate and reinject the sample(s).

7. To view electropherograms (egram) for the allelic ladders and samples, select the sample(s) with the left mouse button and select the 'Display Plots' icon in the menu header. To alter display plot settings, select 'Tools' from the menu, then 'Plot Settings'. Select the 'Sample Header' tab and verify that 'Sample File' is the only attribute to show on the egram. Select the 'Labels' tab and verify that 'Label 1:' is 'Allele Call' for 'Assigned Allele', 'Custom Allele', and 'Allelic Ladder' and is 'Artifact label' for 'Artifact'. Verify that 'Label 2:' is 'Height' for 'Assigned Allele' and 'Custom Allele' and all other labels are 'NONE'. Verify that in the 'When opening the Plot Window' box all items are unchecked and 'Label Color:' is 'Dye Color-Border'. Select the 'Display Settings' tab and verify that it matches the screen shot below. These display settings may be modified to aid in sample analysis and editing, but the below settings should be restored prior to egram printing with the exception of the left and right margins. The margins can be narrowed to print as little of the spaces to the left and right of the marker ranges as possible. To do this, place the mouse cursor on the left side of the egram below the loci headers of any dye, but above the profile window such that the mouse cursor changes to a magnifying glass. Locate the magnifying glass to the desired left-hand margin and then press and hold the left mouse button and drag across to the



desired right-hand margin and then release the mouse button. To undo these narrowed margins, position the mouse cursor to change it to a magnifying glass, then double click the left mouse button. For editing, it is advantageous to keep the margins wider in case there are variant alleles significantly outside the marker ranges for the smallest and largest loci of each dye color.

8. Allelic ladders that injected poorly or are missing allele calls for other reasons can be disabled by changing the 'Sample Type' for that ladder to 'Sample'.

9. Interpret and edit the egrams of the samples and controls as warranted (see DNA SOP-40). To delete or rename an allele/peak call, place the mouse cursor in the allele/peak label and then click the right mouse button. Choose 'Delete label(s)' or 'Rename Allele Label' as appropriate. If deleting, the software will ask you to confirm. After selecting 'Yes', the software window will prompt you to input a 'Reason(s). Type in the appropriate abbreviation, e.g. 'pu' for pull-up. Click 'OK'. If renaming, select 'Custom Allele Label' or 'Custom Artifact label', enter the new name and then click 'OK'. When project editing is complete, select 'File' from the menu and then select 'Save Project'.

10. To print the egrams for the controls and samples, first select the samples from the list, as previously described, that you want to print and select the 'Display Plots' icon from the menu header. Samples can be printed one at a time or as a batch. Set the desired left and right-hand margins as described above. Select 'File' from the menu and then 'Page Setup'. Select the 'Plot' tab and then select 'Small', 'Medium', or 'Large' as desired. Click 'OK'. Select 'File' from the menu and then 'Print Preview'. Based on the 'Print Preview', make adjustments to 'Page Setup' as desired and then select 'File' from the menu and then select 'Print'. Select the appropriate printer and properties and then select 'Print'. Close the egram by selecting the 'X' in the upper right-hand corner. Peak heights in the printed egram label are not required for positive controls or knowns. Access 'Plot Settings' in the 'Tools' menu as previously described and change 'Label 2:' for 'Assigned Allele' to 'NONE' to print without peak height in the label.

11. To print the sample edits, first select all the samples in the sample list for which you want to print edits. Then select 'View' from the menu, then select 'Label Edit Viewer'. A window opens with a list of the edits for each sample. Click the 'Export' button and navigate to the folder location for the raw data. Export as a 'Tab-delimited text (.txt)' file. Click 'Export'. By default, the exported file name will (and should) contain the project name. Open the exported file with DNA QR-330 and click 'Print'.

12. To export the completed project to the U: Drive, select 'Tools' from the menu header, then select 'GeneMapper™ ID-X Manager'. Select the 'Projects' tab and then select the project from the list. Click on the 'Export' button. Navigate to the folder location for the raw data and click 'Save'.

13. To export a file for import into the Concordance checker (DNA QR-37), select the 'Genotypes' tab. Select 'File' from the menu header, then select 'Export Table'. Export the file as 'Tab-delimited text (.txt)'. Navigate to the appropriate folder on the U: Drive, then click 'Export'.