

- 5.3 Ensure that all selections are properly designated, as indicated in Figure 11. The size calling method used for the PowerPlex® 16 STR kit is the Local Southern method.

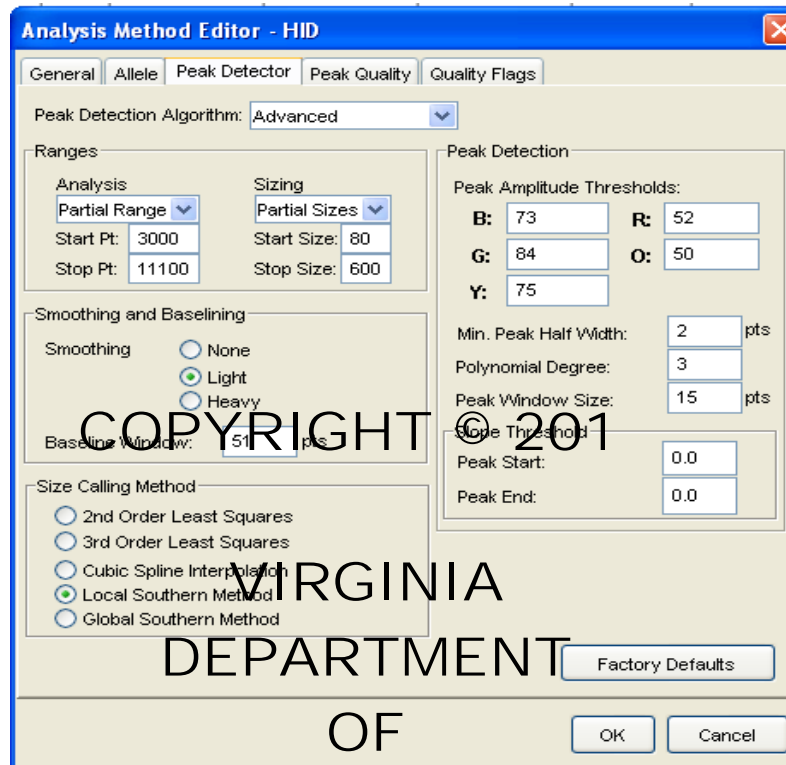


Figure 11. Peak Detector tab of the Analysis Method Editor. Only the analysis range and threshold for the red channel may be altered during data analysis.

- 5.3.1 The analysis range starting and stopping points may be altered on a run-by-run basis to accommodate migration times that may vary between injections. Typically, a starting point of 3000 and stopping point of 10,000 is appropriate.
- 5.3.2 The values for the peak amplitude thresholds are based upon empirical data derived from the limit of detection analysis performed during the validation work and specific for each 3130*x**l* instrument. While the ILS 600 (which uses the red dye) will have its default peak amplitude threshold set based on the LOD analysis, the threshold may be adjusted upward, high enough to prevent detection of a known artifact present in the Promega ILS 600 size standard (Figure 4), or reduced when the ILS 600 peaks may fall below the default threshold (this can occasionally occur with the 2 second injection). It is important to note that ONLY the Red dye (ILS 600 size standard) may be adjusted if such conditions are present.

5.4 The settings under the “Allele” tab should remain as shown in Figure 12. The Bin Set is defined as “Promega_Bins_ID3.2.0” and the marker specific stutter ratio is used.

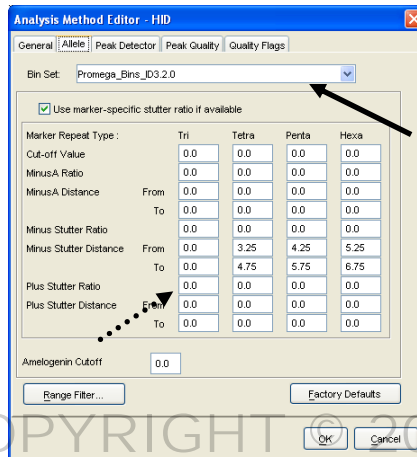


Figure 12. Analysis Method Editor – Allele Tab. The solid arrow points to where the bin set is defined. The dashed arrow points to where the stutter distances are defined.

5.5 The default settings for the Peak Quality and Quality Flags tabs are used, as shown in Figures 13 and 14.

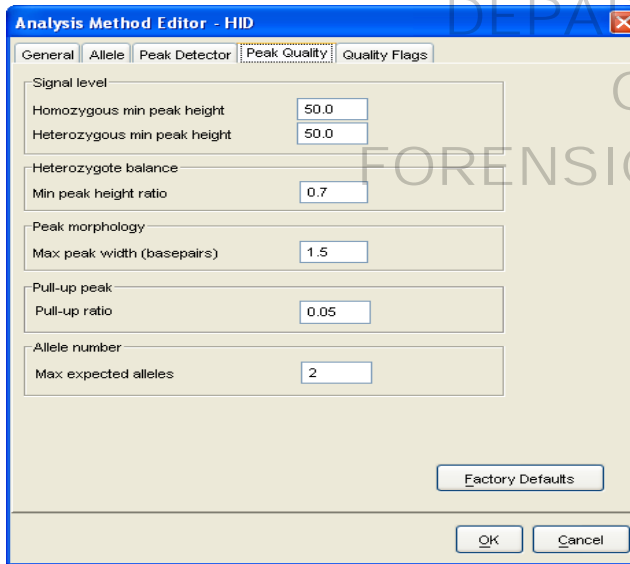


Figure 13. Analysis Method Editor – Peak Quality Tab

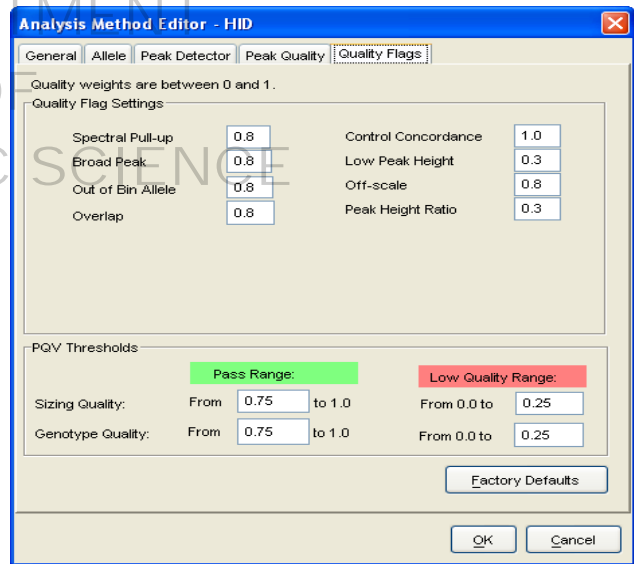


Figure 14. Analysis Method Editor –Quality Flags Tab

5.5.1 Verify that the settings are the same as those shown in Figures 11 and 12, then click OK.

6 Panels and Bins

- 6.1 Panels and bins must be created for allele calling of PowerPlex 16 data. In the GeneMapper® ID software, navigate to “Tools” > “Panel Manager”. In the tree pane view on the left side of the dialog box (shown in Figure 15), choose “Panel Manager”.

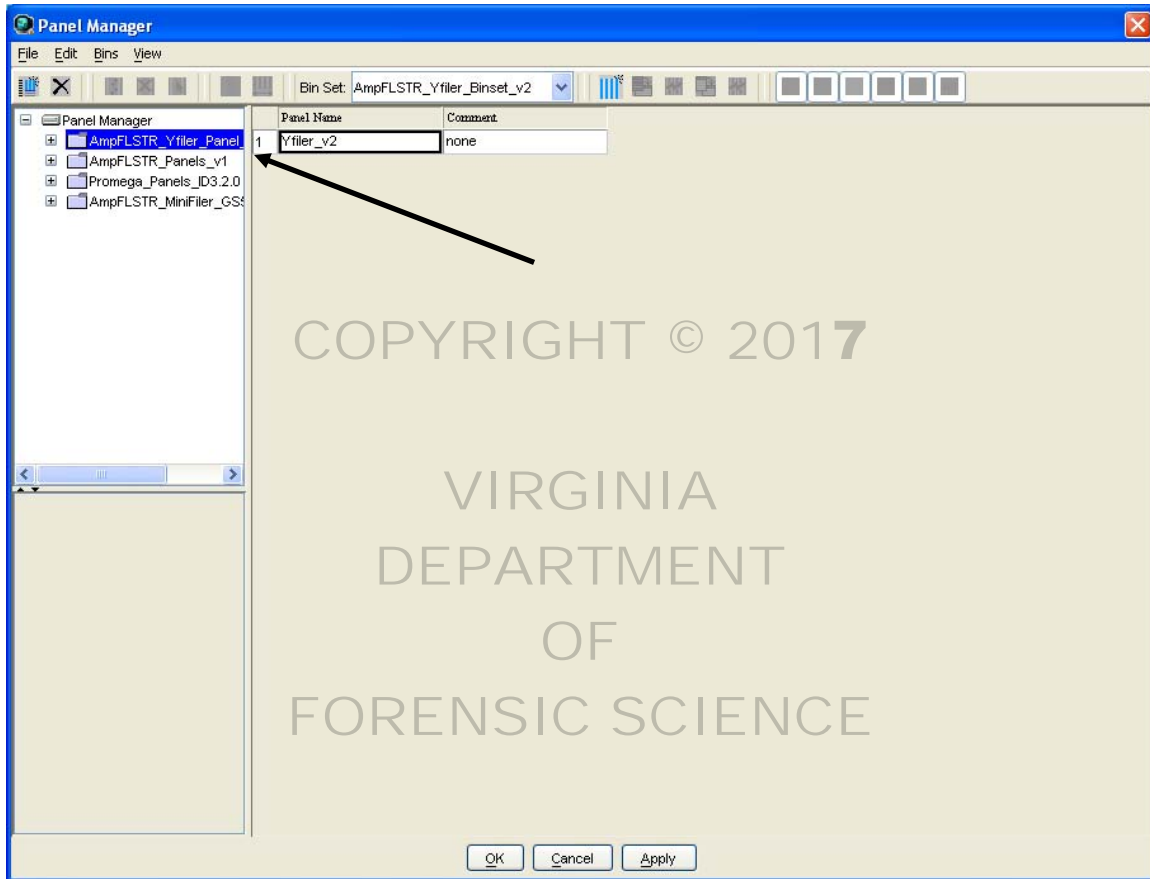


Figure 15. Panel Manager.

- 6.2 Choose “File”>”Import Panels” and browse to the file on CD containing the panels for the PowerPlex 16 kit. Note: the panels need to be obtained from Promega, as they are not installed on the computer by Applied Biosystems.
- 6.3 Navigate to “Promega_Panels_ID3.2.0” of the treepane view (at the left side of the screen), choose “File”>”Import Bin Set” and browse to the Promega bins file, typically entitled “Promega_Bins_ID3.2.0”.

- 6.4 Navigate to “Promega_Panels_IS3.2.0”> “PowerPlex_16_ID3.2.0” of the treepane view (at the left side of the screen). The resulting PowerPlex® 16 panel/bin set is shown in Figure 16. The marker specific stutter ratios must be updated to reflect the validation data obtained at VDFS. The new stutter ratios should be edited to reflect those shown in Figure 16. Click OK after completing.

The screenshot shows the 'Panel Manager' window with a tree view on the left and a data table on the right. The tree view shows 'Promega_Panels_IS3.2.0' > 'PowerPlex_16_ID3.2.0'. The table contains 16 rows of marker data. An arrow points to the 'Stutter Ratio' column, which contains values such as 0.13, 0.05, 0.14, 0.14, 0.08, 0.11, 0.11, 0.1, 0.11, 0.04, 0.18, 0.11, 0.06, and 0.13.

Marker Name	Dye Color	Min. Size	Max. Size	Control Alleles	Marker 1	Marker 2	Comments	Ladder Alleles
1 D3S1358	blue	94.0	151.0	14,15	4	0.13	none	12,13,14,15,16,17,18,19,20
2 TH01	blue	152.0	195.0	8,9,3	4	0.05	none	4,5,6,7,8,9,3,10,11,13,3
3 D21S11	blue	196.0	262.0	30	4	0.14	none	24,24,2,25,25,2,26,27,28,28
4 D18S51	blue	279.0	364.0	15,19	4	0.14	none	8,9,10,10,2,11,12,13,13,2,1
5 Penta_E	blue	370.0	480.0	12,13	5	0.08	none	5,6,7,8,9,10,11,12,13,14,15,
6 D5S818	green	112.0	158.0	11	4	0.11	none	7,8,9,10,11,12,13,14,15,16
7 D13S317	green	166.0	208.0	11	4	0.11	none	7,8,9,10,11,12,13,14,15
8 D7S820	green	210.0	252.0	10,11	4	0.1	none	6,7,8,9,10,11,12,13,14
9 D16S539	green	260.0	310.0	11,12	4	0.11	none	5,8,9,10,11,12,13,14,15
10 CSF1PO	green	312.0	361.0	10,12	4	0.11	none	6,7,8,9,10,11,12,13,14,15
11 Penta_D	green	365.0	449.0	12	5	0.04	none	2,2,3,2,5,7,8,9,10,11,12,13,
12 AMEL	yellow	102.0	112.0	x	9	0.0	none	X,Y
13 vWA	yellow	120.0	184.0	17,18	4	0.18	none	10,11,12,13,14,15,16,17,18,
14 D8S1179	yellow	200.0	252.0	13	4	0.11	none	7,8,9,10,11,12,13,14,15,16,
15 TPOX	yellow	255.0	303.0	8	4	0.06	none	6,7,8,9,10,11,12,13
16 FGA	yellow	305.0	466.0	23,24	4	0.13	none	16,17,18,18,2,19,19,2,20,20

Figure 16. Panel/bin set for PowerPlex® 16. An arrow indicated the column of marker specific stutter ratios that must be edited to reflect the validation data obtained at VDFS. The marker-specific stutter ratios shown here are the values obtained at VDFS.

7 Plot Settings

- 7.1 In GeneMapper® ID, navigate to “Tools”>”GeneMapper Manager”. In the tabs near the top of the GeneMapper Manager dialog box, choose “Plot Settings” to create custom plot settings. Two different custom plot settings will be created: “Samples” and “Allelic Ladders”.
- 7.2 Create a new plot setting by clicking “New” and the “Plot Settings Editor” will appear. In the “General” tab, type a name for the plot settings. In this instance, choose “Samples”.

7.3 Figures 17 – 21 show the various tabs of the plot settings editor. Verify that the plot settings match those in the Figures below.

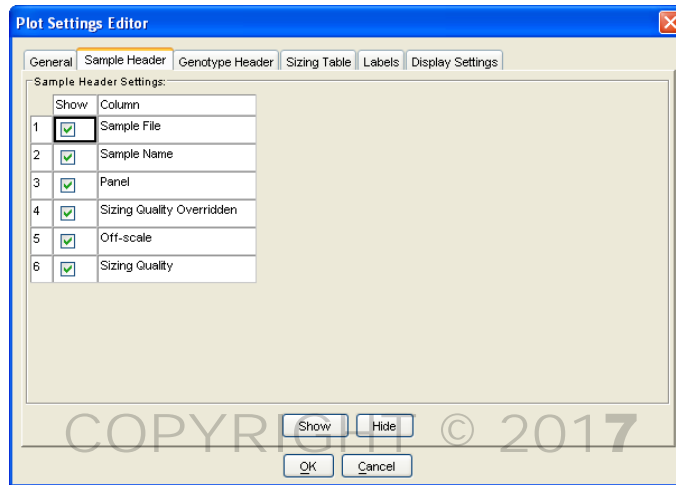
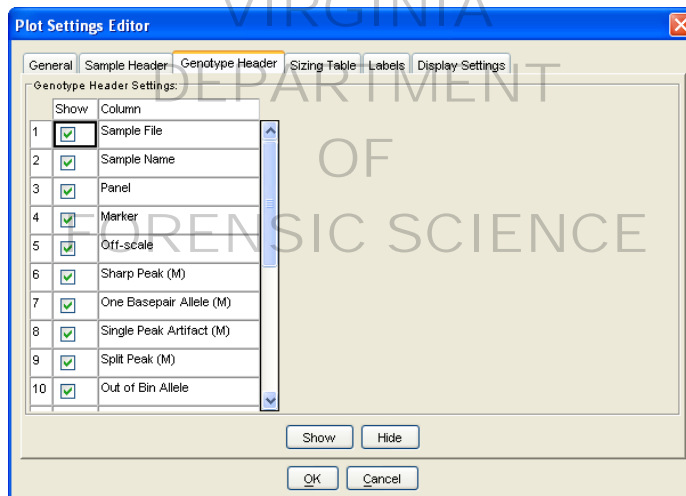


Figure 17. Plot Settings Editor: Sample Header.

A



B

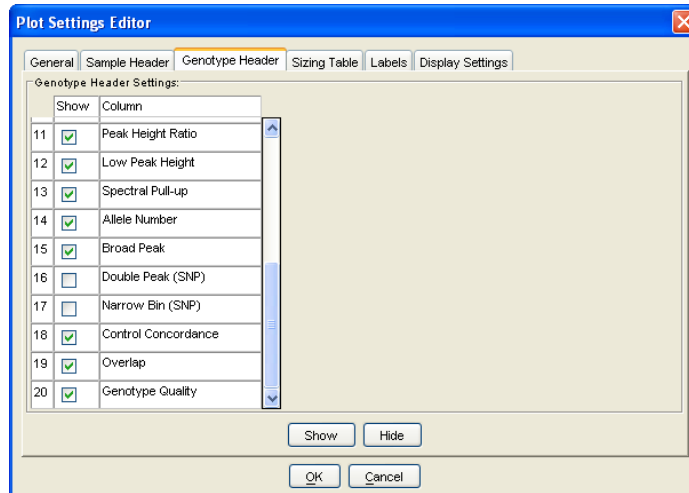


Figure 18. Plot Settings Editor: Genotype Header.

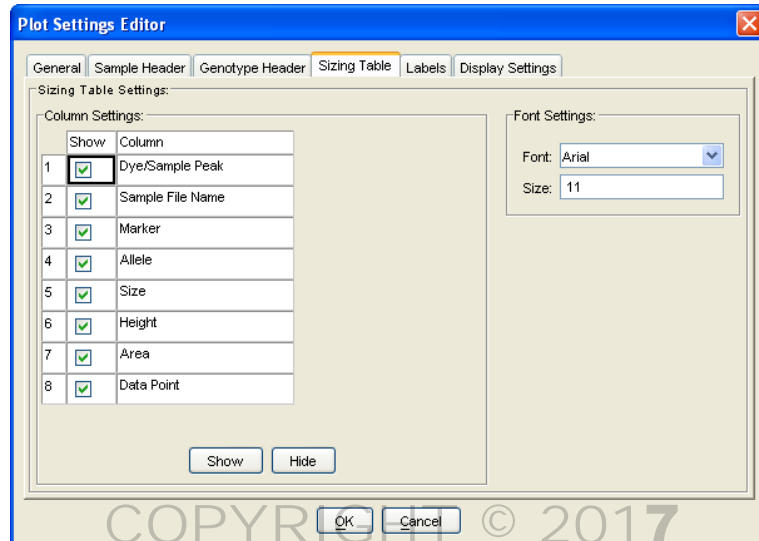


Figure 19. Plot Settings Editor: Sizing Table.

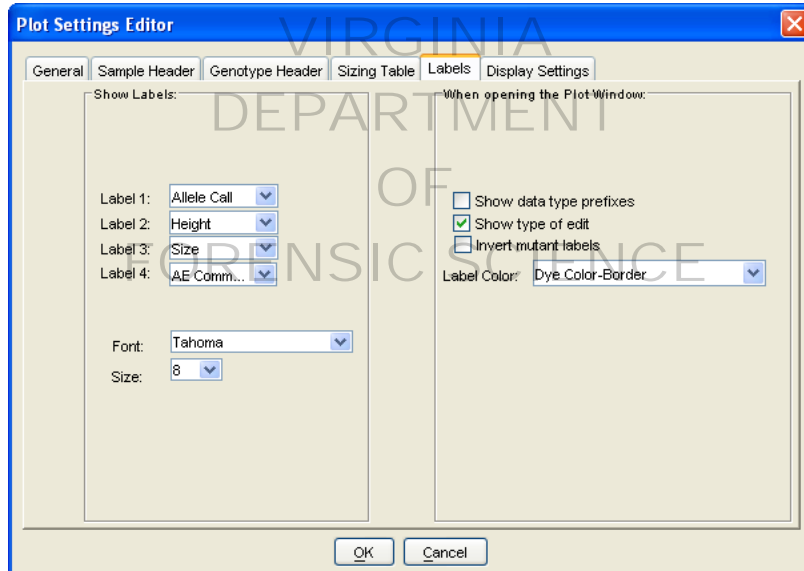


Figure 20. Plot Settings Editor: Labels. Note: these label settings are specific to the “Samples” plot settings. A second plot settings file will be created for allelic ladders in which the allele labels are different.

NOTE: Labels may be adjusted by an examiner based on his or her preferences.

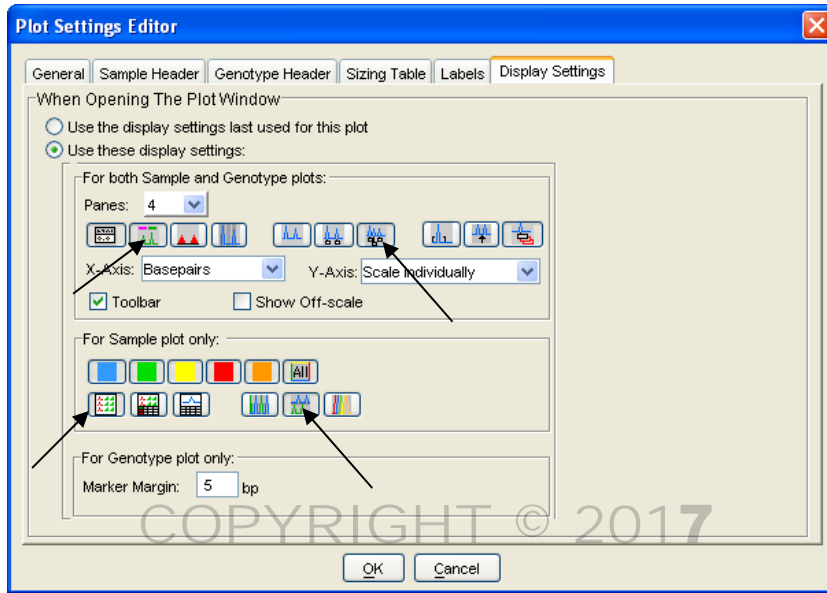


Figure 21. Plot Settings Editor: Display Settings. The arrows indicate the icons that must be clicked.

- 7.3.1 Note that many of the settings in Figure 21 are difficult to discern from the figure because they are toggle switches. The icons to which an arrow is pointing must be clicked (once) for the appropriate settings. The number of panes may be set at the analyst’s discretion, but this is typically 2.
- 7.4 Click OK.
- 7.5 A second Plot Setting may be created for ease of printing allelic ladders. Create a new plot setting by clicking “New” and the “Plot Settings Editor” will appear. In the “General” tab, type a name for the plot settings. In this instance, choose “Allelic Ladders”.
- 7.6 Figures 17 – 21 show the various tabs of the plot settings editor. Verify that the plot settings match those in the Figures. The only difference between the allelic ladder and samples plot settings is the allele label. For allelic ladders, only the allele call is used, as indicated in Figure 22.
- 7.7 Choose OK to close and save the plot settings and Done to close GeneMapper Manager.

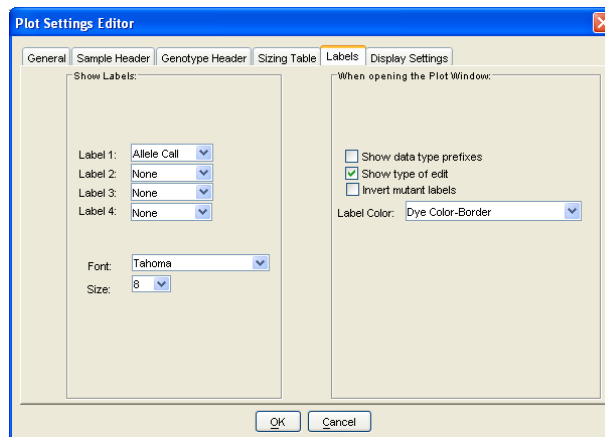


Figure 22. Plot Settings Editor: Labels. Note that these label settings are specific to the “Allelic Ladders” plot settings.