SDPD Forensic Science Section – Forensic Biology Unit

Modification of the change in peak detector parameters in the Analysis Method Editor

Baseline Window and Peak Window Size changes

Purpose

The transition of the MiniFiler STR kit to the 3500 Genetic Analyzer, with the empirically derived 100 analytical threshold, resulted in the increased observation of pull-down (aka: bridge pull up) peaks. Such spectral artifact peaks are due to neighboring heterozygous peaks that are separated by one repeat or less. True STR allele peaks exhibit specific characteristics such as a morphology that is neither too narrow or too broad[1]. GeneMapper ID-X Software applies a variety of parameters to identify peaks of the proper morphology or flag peaks that are inconsistent with true STR allele peaks. Since the implementation of MiniFiler STR kit, 3500 Capillary Electrophoresis instruments, and GeneMapper ID-X v1.4, the San Diego Police Department's settings for the employed Analysis Method have been 51 pts for the *Baseline Window* and 15 pts for the *Peak Window Size* (will be referred to as the 51/15 parameters throughout the rest of this write-up), which are the default parameters for analysis. However, Applied Biosystems recommended these values be changed to 33 pts for the *Baseline Window* and 13 pts for the *Peak Window Size* (will be referred to as the 33/13 parameters throughout the rest of this write-up) in order to reduce the number of observed bridge pull up peaks[2*]. The smaller baseline window size in these settings is known to reduce peak heights of all detected peaks. Smaller baseline window sizes cause the baseline to move vertically into the peaks resulting in shorter peaks in the analyzed data. Current and recommended settings can be observed in Figure 1. The purpose of this study was to determine whether the new recommended settings reduced the presence of bridge pull up peaks and to what effect peak heights of true STR allele peaks were reduced.

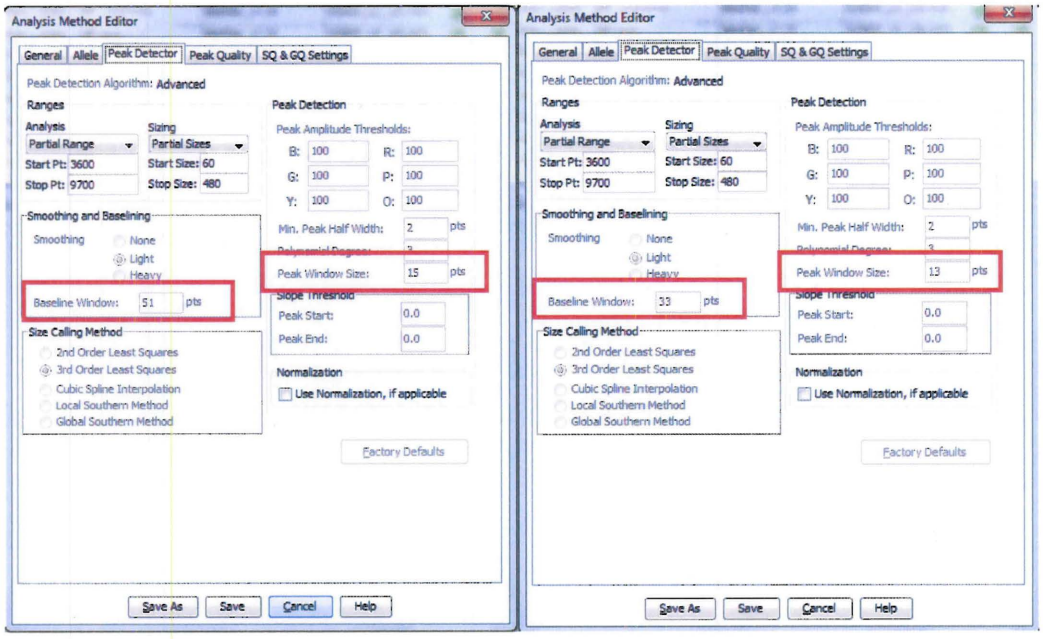

Figure 1. Implemented settings (above left) and recommended settings (above right).

*These settings were previously implemented with the GlobalFiler testing kit.

Materials and Method

One known reference sample and two 007 positive controls (for a total of three samples) were amplified with a target input of approximately 0.4ng of DNA using the MiniFiler STR kit. The samples were imported twice into GeneMapper ID-X v1.4 and analyzed once with the 51/15 parameters and secondly with the 33/13 parameters. The occurrences of bridge pull up (designated as pull-down) peaks were recorded. The allele size tables were exported and a side by side comparison was performed to evaluate the change in RFU between the two analysis methods. The comparison was reported as the percent decrease between implemented and recommended settings. The average percent RFU decrease was calculated for each locus among the three samples.

Results

Five instances of flagged bridge pull up peaks were observed in the three samples using the 51/15 parameters. No instances of such flagged bridge pull up peaks were observed in the same samples analyzed using the 33/13 parameters. The new analysis method also reduced instances of artifact pull ups.

Allele peak heights decreased with the 33/13 parameters; however, the average percent decrease in RFU's was less than 1%. Figure 2 shows the average percent RFU decrease between the 51/15 and 33/13 parameters across all MiniFiler loci.

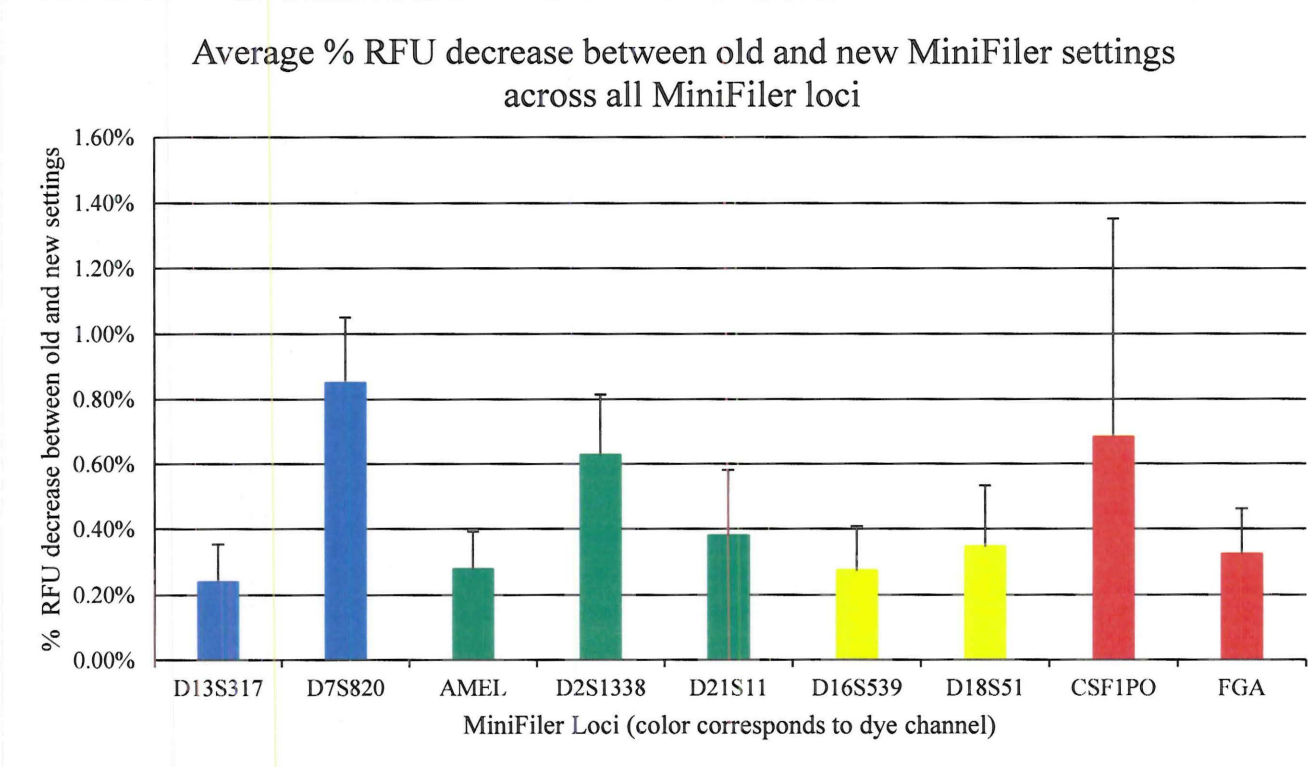

Figure 2. Average percent RFU decrease of alleles between the 51/15 and 33/13 parameters. The different column colors correspond to the dye channels in which each locus is represented.

Conclusions

Based on the recommendation from Applied Biosystems technical support in addition to the results obtain during this comparison study, a value of 33 pts for the *Baseline Window* and 13 pts for the *Peak Window Size* will be implemented into our current analysis methods for evidence and reference samples.

Reference

1. Applied Biosystems. *Advanced Topics: Peak Morphology and Detection in GeneMapper ID-X Software for Human Identification (HID) Laboratories*. Technical Note. August 3, 2013.
2. Communication with Ellen Crone, Senior Technical Applications Scientist for Thermo Fischer Scientific HID Technical Support.

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