Allele Specific Stutter Filters in GeneMarker®HID Software

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Introduction

"Stutter product amounts increase with the length of the allele." In a study on Y-STR stutter heights, Andersen *et al*. **"concluded the use of a single stutter threshold per locus is not optimal because stutter rates are allele –specific and differ based on the amount of template DNA. If high stutter values are used for software filters, then in many cases usable data is thrown away during DNA mixture interpretation."** [1]

The ability to apply allele specific stutter filters results in fewer analyst interventions, especially for the larger alleles at each locus. Laboratories that measure and apply allele specific stutter typically export the peak table results to a macro that applies the designated stutter values and removes allele calls attributed to stutter.

New tools within GeneMarkerHID software detect and apply the filters for allele specific stutter, additive stutter and peaks within the stochastic range, eliminating the vast majority of manual edits required for each DNA profile. The time saved by eliminating these manual edits, data transfer and the time needed to record/document those edits adds up quickly when analyzing large data sets; especially when the data sets contain mixture DNA profiles.

Procedure

The allele specific stutter settings provide greater precision in applying stutter filters (Table 1, Figure 1). The stutter filters are applied additively to alleles that are in a shared stutter position (Figure 2). Alleles that are themselves filtered because they fall within the stutter range are not used to impact the filtering of a peak that is in a stutter position (Figure 3). Peaks that are in the stochastic range are not used to impact the filtering of a peak in a stutter position (Figure 4). Allele specific stutter in GeneMarkerHID software is easily implemented in the panel editor (Figure 5).

Table 1 is an example of a typical variation in stutter ratios between smaller and larger repeat alleles. Alleles are named by the number of repeats at that allele and x=number of nucleotides in the repeat. This example includes only the major stutter position, one repeat smaller than the allele. If the analyst uses the median plus two or three standard deviations they will filter out peaks of the lower size alleles that could potentially have a minor contributor influencing peak height.

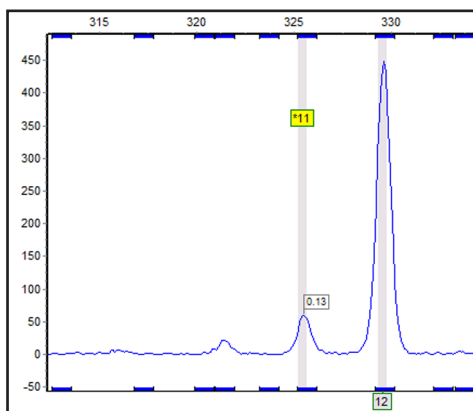| Allele	N-x %stutter
7	8
8	8
9	8
9.3	8
10	10
11	10
12	10
12.3	10
13	12
14	12
15	12
16	12
16.1	14
17	14
18	14
19	14
20	14
Ave. Stutter	11.17647
Std Dev	2.35147
Median	12

Table 1

Figure 1: In this example, the marker specific approach, (the median + 2 SD) would have an N-x stutter of 16.7%, and would result in allele 11 not being called. In contrast, by applying an allele specific stutter filter, the 11 allele is called because it is greater than 10% of allele 12 RFU value. The alternative allele label for allele 11 indicates it is in the stochastic range specified in the panel for this marker.
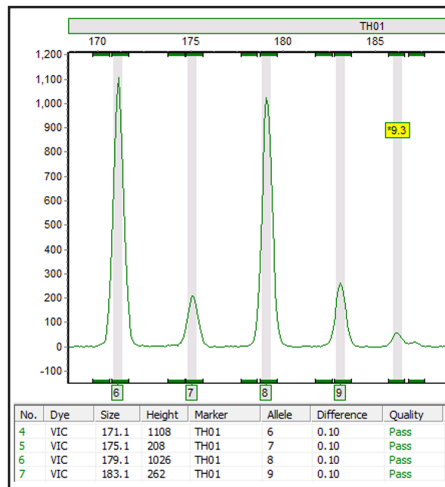
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No.	Dye	Size	Height	Marker	Allele	Difference	Quality
4	VIC	171.1	1108	TH01	6	0.10	Pass
5	VIC	175.1	208	TH01	7	0.10	Pass
6	VIC	179.1	1026	TH01	8	0.10	Pass
7	VIC	183.1	262	TH01	9	0.10	Pass

Figure 2: Allele specific stutter filters are applied additively. Allele 7 is in the shared stutter position for alleles 6 and 8. If Allele 7 ratio is greater than the N+X and N-X for alleles 6 and 8 respectively, then allele 7 is called. If allele 7 ratio is greater than the allele specific stutter for allele 8, but not the added stutter percentages for 8 and 6, then allele 7 would not be called.
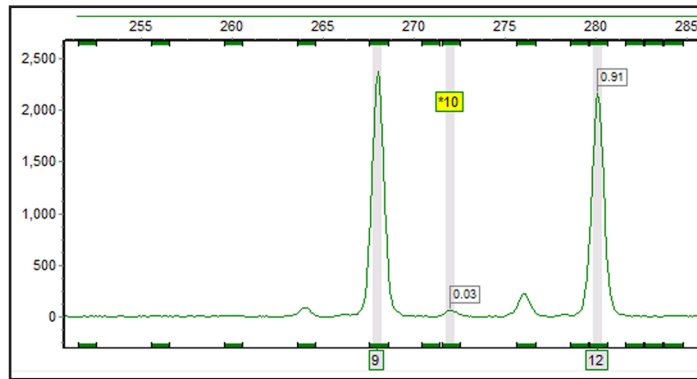

Figure 3: Peaks that are under the stutter filter of an allele do not impact the stutter allowance for other peaks. The peak at allele 11 is not called because it is filtered out by the N-x stutter filter for allele 12. The peak at allele 10 is not influence by the stutter filter for allele 11; allele 10 is only influenced by the N+x stutter filter for allele 9.
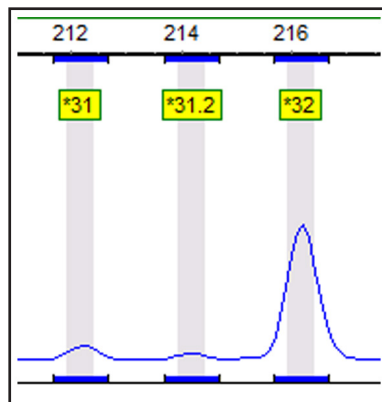

Figure 4: Peaks that are within the stochastic range do not impact the stutter allowance for other peaks. Allele 32 is in the specified stochastic range. It is not used to filter allele 31.
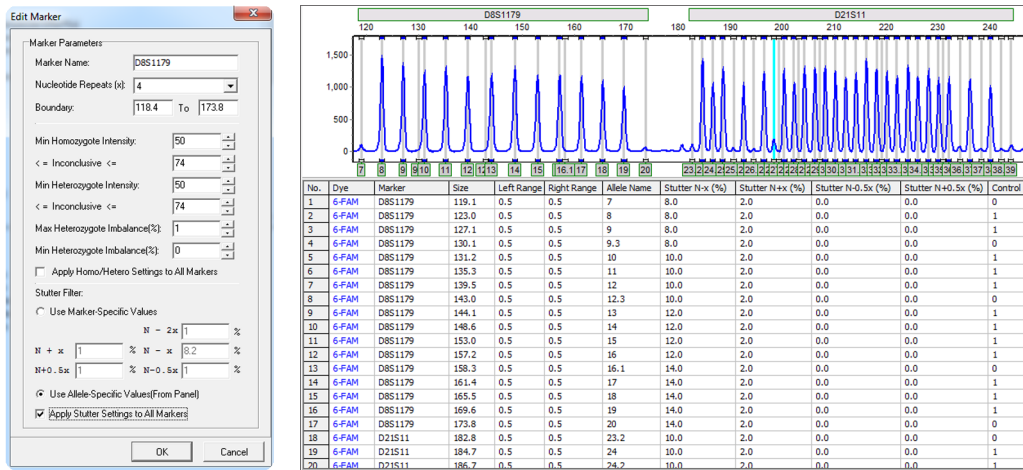
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Figure 5: Implementing Allele Specific Stutter: Select the *panel* from the *panel template list*. Right mouse click on the *Locus label* and select *Edit Marker*. At the bottom of the settings box select *Use Allele-Specific Values*. In the table enter the stutter values determined from the lab validation studies for each position. If there is no stutter associated with a position the value should be 0. Save the panel.

Discussion

Applying allele specific stutter avoids over-filtering smaller repeat alleles and under-filtering large repeat alleles. Including allele specific stutter filters in GeneMarkerHID software avoids the time-consuming and potentially error-prone process of exporting results to a macro for further analysis. GeneMarkerHID software is compatible with major CE and Rapid DNA Analysis platform data files, Windows® 7 – 10 OS and all major commercial human identity chemistries.

References

1. Butler, J.M (2014). Advanced Topics in Forensic DNA Typing: Interpretation. Pages 72,73 and 76. San Diego: Elsevier Academic Press.

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