ASSESSING THE IMPACT OF MASKING STUTTER IN MIXTURES USING A SEMI-CONTINUOUS PROBABILISTIC GENOTYPING (PG) SYSTEM

Annette Ortiz, M.S.
Denver PD Crime Laboratory

Stutter is a well-characterized by-product of STR PCR amplification and is typically observed in a reverse (n-4) or forward (n+4) position relative to the parent allele. Discrete probabilistic genotyping systems (i.e., Lab Retriever) assess the absence or presence of an allele and apply estimated probabilities and allele frequency data to that information; stutter and other biological factors are not modeled. Although it is well understood, stutter can complicate the interpretation of a DNA mixture, particularly when the minor contributor(s) is present at the same level as filtered stutter peaks. Uncertainty in allele designation arises when these minor alleles are masked by either a major peak or by stutter. Peaks in stutter positions can be a combination of allelic and stutter product or can result from stutter product only.

Lab Retriever (SCIEG) can accommodate for this uncertainty through the use of an “assumed” or a “masking” profile. Peaks that are indistinguishable from stutter are included in the evidence profile *and* entered in the software as an assumed profile. The masking profile is considered in both the numerator and the denominator of the likelihood ratio (LR); the drop-out model is not applied to the assumed profile. When allelic peaks from a minor contributor are filtered as stutter, this is not true drop-out and may result in misleading estimates of probability of drop-out, which then impacts the resulting LR.

PowerPlex® Fusion internal validation mixture data (n=6) was analyzed with stutter filters on and off to evaluate the effect of potential stutter masking on probability of drop-out rates and on the overall LR value. Because the contributors to the mixtures were known, the benchmark drop-out rates of the minor contributors with the stutter filters on and off were compared to the estimated drop-out rates using an internally validated logistic regression curve. The drop-out rates with the stutter filters off were closer to the estimated drop-out rates 67% of the time. Furthermore, the LRs calculated accounting for potential stutter masking resulted in higher values than the standard approach. One sample resulted in no change to the order of magnitude of the LR, while the most extreme example changed by 9 orders of magnitude. Accounting for potential stutter masking can have a significant impact on resulting casework LR analysis and therefore should be evaluated in validation studies of semi-continuous software systems.