B39 The Generation and Comparison of Various Short Tandem Repeat (STR) Stutter Positions and Longest Uninterrupted Stretch (LUS) Stutter Settings for a Probabilistic Genotyping Software Following Various Electrophoretic Protocols

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Learning Overview: After attending this presentation, attendees will be more familiar with the capability of the MaSTR™ software by SoftGenetics, LLC, to incorporate traditional and non-traditional stutter parameters for probabilistic genotyping and how appropriate stutter filters can affect likelihood ratio calculations.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by illustrating the increased capability of the MaSTR™ software to perform probabilistic genotyping on complex DNA mixtures. This presentation will illustrate the importance of using LUS as well as non-traditional stutter positions in the interpretation of forensic STR profiles containing contributions from multiple donors.

Stutter is a common DNA interpretation artifact that occurs as a result of strand slippage of polymerase during the elongation phase of Polymerase Chain Reaction (PCR). The two most commonly observed stutter artifacts are back stutter and forward stutter. Back stutter is well defined in literature, which allows filters to be applied during Short Tandem Repeat (STR) analysis. Forward stutter peaks are less common and occur at a lower frequency than backward stutter peaks.[1,2] Some of the features that affect the amount of stutter observed include Longest Uninterrupted Stretch (LUS), STR locus, total number of base pairs within the repeat, and the total number of repeats of the allele.[3] Studies have shown that LUS is a better predictor of expected stutter percentage compared to the total number of repeats in an allele.[2] Research has also shown that a longer LUS often leads to increased stutter and reduced amplification (lower peak height).[2,3] The ratio of the stutter peak height to that of the main allele peak is used to calculate the percentage stutter observed at a particular locus and can sometimes be used to differentiate stutter peaks from true allelic peaks of minor contributors in a mixed DNA.[5] DNA samples submitted to forensic labs for analysis are often composed of more than one contributor and each contributor may make up varying proportions of the sample. This makes analysis difficult and requires the use of statistical analysis and interpretation methods that are proficiently applied in continuous models of probabilistic genotyping methods.

Continuous probabilistic genotyping methods involve the use of weighted genotypes in the calculation of likelihood ratios. This requires the use of extensive information from the evidence sample such as variability of peak heights in heterozygote and homozygote alleles, stutter ratio and percentages, and probability of allele drop in and drop out.[3-5] MaSTR™ software is a fully continuous probabilistic genotyping software that uses Markov Chain Monte Carlo (MCMC) algorithms to generate weighted genotypes that are used in the calculation of likelihood ratios. The MaSTR™ software has been validated for use in forensic labs, using Scientific Working Group on DNA Analysis Methods (SWGDAM) and Organization of Scientific Area Committees (OSAC) guidelines, and it has been shown to give accurate results when tested on sample mixtures with two to five DNA contributors.[5]

This study focuses on identifying locus-specific, non-traditional stutter products, developing new stutter filters based on these non-traditional stutter positions, and applying these filters to the new version of the MaSTR™ software. Large data sets (30+ single source profiles) from multiple PCR amplification kits were collected and analyzed to locate traditional and non-traditional stutter products. Appropriate allelic stutter filters for non-traditional stutter locations including LUS stutter amplicon products were calculated. Once the stutter percentages were calculated, they were imported into MaSTR™ software. With the release of a new version of MaSTR™ this fall, the new calculated stutter positions will be included. A performance check will be performed following SWGDAM guidelines. The performance check will utilize a set of mixtures known to be affected by stutter, and the genotype weight effects of the new stutter filters will be evaluated. Preliminary examination of the datasets indicates the prevalence of n-2 and n-8 non-traditional stutter products, with n-2 stutter being prevalent in marker D1S156 and n-8 stutter positions being prevalent in a majority of targeted markers. The percentage of the non-traditional stutter positions in the different STR markers will be calculated once the datasets have been examined in detail. The new stutter filters generated from this project and applied to the new version of the MaSTR™ software can be adopted by forensic labs for interpretation of mixed STR profiles.

Reference(s):

1. Butler, John M. Advanced Topics in Forensic DNA Typing. Academic Press.
2. Aponte, Rachael A. et al. Sequence-Based Analysis of Stutter at STR Loci. Forensic Science International: Genetics Supplement Series. 5. 10.1016/j.fsigss.2015.09.181.
3. Bright, Jo-Anne et al.Characterizing the STR Locus D6S1043 and Examination of Its Effect on Stutter Rates. Forensic Sci Int Genet. 2014 Jan;8(1):20-3. doi: 10.1016/j.fsigen.2013.06.012.
4. Bright, Jo-Anne et al. Developing Allelic and Stutter Peak Height Models for a Continuous Method of DNA Interpretation. Forensic Sci Int Genet. 2013 Feb;7(2):296-304. doi: 10.1016/j.fsigen.2012.11.013.
5. Adamowicz, Michael et al. Validation of MaSTR™ Software. Forensic Science International: Genetics (FSI:G) Supplement Series.

Stutter, Probabilistic Genotyping, Forensic Science

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