

VALIDATION OF MASTR™ SOFTWARE (MIXTURE ANALYSIS FROM STRS): A FULLY-CONTINUOUS PROBABILISTIC MIXTURE ANALYSIS VALIDATION USING POWERPLEX®FUSION 2 – 5 CONTRIBUTOR MIXTURES

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Probabilistic genotyping (PG) for evaluation of mixtures provides many advantages compared to historical (CPI, binary likelihood ratio) approaches. Fully continuous approaches utilize more of the information from the evidentiary profile; such as peak heights, stutter percentages, mixture ratio, probability of drop-in/out to provide weighted genotypes from the mixture profile. The weighted genotypes are used to calculate a likelihood ratio that a profile is included, or not included, in the mixture. Mixture profiles are compared to hypothetical profiles created based on these parameters and hypothetical combinations of genotypes present in the sample in order to calculate how probable it is that a given individual contributed to the mixture. Integration over a large number of interrelated parameters is required to calculate relative probabilities but doing so directly is not feasible. Markov Chain Monte Carlo (MCMC) is a widely used sampling method to approximate such complex integrals with high accuracy.^{1,3} Statistically sound, rigorous software tools are essential for forensic interpretation of complex mixtures that often have multiple contributors, low-level DNA of one or more of the contributors, shared alleles, minor contributor alleles in stutter positions to major contributors and drop-out. Thorough validation of methodologies is required for forensic laboratories. Conclusions of a preliminary evaluation of MaSTR software indicated MaSTR software provides the rigor of a fully continuous probabilistic approach¹ in a straight-forward software program. A full validation was proposed as the results of the preliminary study were concordant with the known mixture contributors. The validation study of MaSTR software provides the forensic community with a detailed report of the capabilities and reliability of this newly available tool to assist the forensic analyst in applying their expertise to evaluate mixture profiles.

The validation study was designed in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDM) and Organization of Scientific Area Committees (OSAC) guidelines. Purified, de-identified DNA from 40 donors was obtained from the Nebraska Biobank (University of Nebraska Medical Center), quantified using the Quantifiler Human DNA Quantification kit (Thermo Fisher), amplified with PowerPlex®Fusion (Promega Corporation) and analyzed on an ABI PRISM® 3130 Genetic Analyzer (Thermo Fisher). Replicates of single source samples were genotyped (GeneMarker®HID software). Files were selected to provide a range of allele calls and stutter information. Results of these replicates and their dilutions were imported into MaSTR software to calculate the variance coefficient and stutter ratios required for the protocol data set. The protocol data set provides the software with context of expected peak height variation over the range of potential RFU values and stutter during the evaluation of mixture profiles. Mixtures ranged from simple, two-contributors with major/minor components with few shared alleles to complex four and five-contributor mixtures with a range of mixture ratios and shared alleles. Mixture samples were also diluted to examine low-template effects across the range of contributors.

The study utilized the standard model within the software. This model utilizes peak heights, drop-in/out, degradation, stutter, co-ancestry (NRC I and NRC II) and allele frequencies from USA populations.⁵ Tests included evaluation of mixtures with: 1) a known contributor profile and

no person of interest (POI) profile(s), 2) a known contributor profile and POI profile(s), 3) no known and no POI profiles(s) to evaluate deconvolution capabilities 4) contributor and non-contributor samples to evaluate exclusion capabilities. Additional parameters included use of the elimination database, containing some of the known contributor genotypes, to mimic detection of staff profiles in a mixture in order to evaluate MaSTR's capability to detect contamination. The number of MCMC iterations and chains required increased with complexity of the mixtures. Results were consistent with known contributors and exclusions. Methodology and tabulated results will be presented.

¹SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems, 2015 and 2016 NIST draft on guidelines for validating probabilistic genotyping software.

²Organization of Scientific Area Committees (OSAC) 2016 Validation Standards for Probabilistic Genotyping Systems Draft

³Taylor D., Bright J.-A., Buckleton J.S. 2013. The interpretation of single source and mixed DNA profiles, *Forensic Sci. Int.:Genetics* 7:516-528

⁴Gill P., Gusmão L., Haned H., Mayr W.R., Morling N., Parson W., et al., (2012) DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods, *Forensic Sci. Int:Genetics* 6:679-688

⁵Hill C.R., Dueder, D.L., Kline, M.C., Coble, M.D., Butler, J.M. *Forensic Science International: Genetics* 7 (2013)