

MaSTR™: AN EFFECTIVE PROBABILISTIC GENOTYPING TOOL FOR INTERPRETATION OF TWO-PERSON STR MIXTURES ASSOCIATED WITH DIFFERENTIALLY DEGRADED DNA

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The interpretation of STR mixtures has advanced significantly with the development of software that allows for probabilistic genotyping. As a result, more information in a mixture is considered, and consistency in the final steps of interpretation is established; i.e., assessment of genotype combinations in comparison to reference and evidentiary profiles, and statistical weight estimates of resulting matches or exclusions. One aspect of the software-based interpretation process that has been poorly evaluated is the assessment of differentially degraded mixtures. We used MaSTR™, a recently developed software package from SoftGenetics, Inc., to interpret two-person mixtures of differentially degraded DNA; pristine samples mixed with artificially degraded sources of DNA with an average template size of 150 or 250 base pairs (bps), along with combinations of degraded DNA.

MaSTR™ uses the Markov Chain Monte Carlo method to provide fully continuous probabilistic genotyping for up to five-person mixtures. As expected, the software considers quantitative peak height data and several other biological parameters such as ratio of contributors (mixture weight), stutter, drop in, drop out, and sample degradation. The processing unit of MaSTR™ (server/database) can receive analysis jobs from multiple client computers (workstations), with jobs entered into a queue and processed automatically two at a time in the order they were added, and with an average time of less than three minutes per analysis job for two-person mixtures. This approach significantly streamlines the analysis process.

Mixtures were prepared from two pairs of donors with known Fusion 6C STR profiles. Ratios considered were 1:1, 1:3, 1:6, and 1:10, with combinations of pristine and degraded sources of DNA rotated between major and minor contributors. Data was analyzed using GeneMarker™ HID using a validated analytical threshold of 60 RFU. Results were exported to a text file which was imported into MaSTR™ and the analysis performed using a model panel established through in-house Fusion 6C data. Likelihood ratios (LRs) were generated for contributor mixtures using two sets of parameters; 10,000 versus 40,000 iterations per chain (eight chains total, with a burn-in of 2,000), and with or without a known contributor. LRs were consistent with expectations for all mixture types, including the most differentially degraded samples. In addition, there was no difference in LRs when comparing 10,000 and 40,000 iterations per chain. As expected, LRs were lower when a known contributor was not provided, especially for samples containing degraded DNA. Overall, MaSTR™ proved to be a reliable tool for the analysis of two-person mixtures of differentially degraded DNA.