## THE USE OF THE I-STREAM MIXTURE DECONVOLUTION SOFTWARE TOOL IN THE FSS-I<sup>3</sup>™ SUITE FOR THE ANALYSIS OF CASEWORK SAMPLES

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During Phase I of the NEST Project, FSS-i<sup>3</sup>TM Software (Promega Corporation, Madison, Wisconsin) and other software programs were evaluated for their many features as expert systems for the analysis of single-source samples. In Phase II, different mixture deconvolution software tools are being evaluated. Mixture results pose an additional challenge in case interpretation and can be quite time-consuming, even for the experienced forensic scientist. The use of a mixture deconvolution tool can aid the forensic scientist in consistent unbiased evaluation of the data. These software programs and tools are not expert systems, rather they are fancy genetic calculators designed to assist forensic scientists in mixture interpretation of casework STR data that include calculating mixing proportions and peak height ratios.

The focus of this presentation is the use of the i-STReam module in FSS-i³™ software to evaluate two-person mixtures. i-STReam has the ability to produce best-fit major and minor profiles. The i-STReam module calculates the mixing ratio of two donors in a mixture; it does not perform calculations for three or more contributors. The i-STReam module produces an overall mixing proportion for the samples and a list of possible candidate genotypes per locus. The overall mixing proportion is presented as a ratio, along with the mean, minimum, and maximum proportions for the combined loci in a sample lane. The mixture estimate provides guidance at each locus for the possible genotypes of each contributor. Based on the defined Preferential Amplification Rule and the defined Mixing Proportion Rule, i-STReam reports genotype combinations that should or should not be included in the overall analysis.

Controlled mixture studies were conducted to produce simulated casework data. The design of the mixed samples includes varying ratios of female and male DNA and varying input levels of DNA. These varying ratios and varying input quantities of DNA were amplified with PowerPlex® 16 System (Promega Corporation) and AmpFLSTR Identifiler®, Profiler Plus®, and COfiler® PCR Amplification Kits (Applied Biosystems, Foster City, California). All samples were run on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems).

The results of these studies demonstrate that the i-STReam module in the FSS-i<sup>3</sup>™ software can assist an analyst with challenging casework data. The peak height ratio calculations can be time-consuming when performed by hand and introduce the risk for human error or inconsistent calculations. An advantage to this new technology is that the peak height ratios for all combinations at each locus are calculated automatically and consistently. In addition, mixing proportions for the two contributors are calculated per locus as well as over the entire sample. As a result, the forensic analyst is freed to spend less time in performing mundane calculations and more time considering the various combinations produced by the given data.

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