

PROBABILISTIC MODELING COUPLED TO CELL SEPARATION PLATFORMS CAN PROVIDE A QUANTITATIVE ASSESSMENT OF MIXTURE SIMPLIFICATION AND IMPROVE INTERPRETATION OF COMPLEX SAMPLES

Susan A. Greenspoon, PhD, Nancy A. Stokes, MS², Cristina E. Stanciu, MS², Christopher J. Ehrhardt, PhD²

¹ Virginia Department of Forensic Science

² Virginia Commonwealth University, Department of Forensic Science

STR profiles generated from forensic evidence comprised of multiple individuals can be complicated to interpret. Fluorescence Activated Cell Sorting (FACS) has been demonstrated to enhance a population of cells for a donor exhibiting a specific cell surface antigen, but different from other contributors to the mixture.¹ FACS utilizes flow cytometry to separate cells based on optical scatter and fluorescence. The resultant mixture simplification can be qualitatively evaluated, but without a quantitative assessment, the magnitude of that simplification may be hard to grasp. For a quantitative assessment of mixture simplification, TrueAllele[®] Casework (TA) was utilized for probabilistic modeling.

Blood from three, four and five different individuals was mixed together and then dried to create mock evidence to be separated by FACS and evaluated both pre- and post-cell sorting. Contributor cells were separated by targeting the HLA-A02 antigen allele using a fluorescently labeled antibody probe. DNA from the original mixture (presorted cells), as well as the sorted cell populations was purified, quantified and then amplified using Promega's PowerPlex[®] Fusion System (PPF). Amplified DNA was separated on the 3500x1 Genetic Analyzer and the .hid files for the samples uploaded for mixture analysis by TA.

One of the contributors was successfully separated into an enriched cell fraction (P2) for the three, four and five person mixture samples such that the single source profile could be easily determined, while the other sorted cell fraction (P3) contained a mixture of cells from all of the other non-target contributors (i.e., HLA-A02 negative cells). We quantified the mixture simplification by TA analysis of the PPF profiles generated from both pre- and post-FACS sorted samples. TA analysis of these samples demonstrated enhanced statistical power by as much as 12 orders of magnitude. For e.g, pre-sorted samples for a three person mixture of individuals 105, 106, and 107 had log likelihood ratio (log(LR)) values for each contributor of 8.85, 11.36, and 8.85, respectively. TA analysis of fraction P2 (post-cell sorting) produced a slightly higher value for contributor 106 (12.34) and excluded 105 and 107 (negative log(LR)s generated). TA analysis of fraction P3 produced a slightly higher value for 105 and nearly 12 orders of magnitude higher value for 107, respectively (11.59, 20.57) and excluded 106. TA analyses thus confirmed and quantified observations by manual genotyping that enrichment for individual contributors and increased statistical power may be accomplished with the use of FACS for cell sorting for mixtures containing as many as five contributors that would otherwise be uninterpretable through traditional methods.

¹Dean L, Kwon YJ, Philpott MK, Stanciu CE. *Forensic Sci Int Genet.* 2015 Jul;17:8-16. doi: 10.1016/j.fsigen.2015.03.003. Epub 2015 Mar 12.