B105 Cell Separation of Multiple Contributor Samples to Facilitate DNA Mixture Analysis

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After attending this presentation, attendees will better understand a possible new solution for simplifying Short Tandem Repeat (STR) genotyping of samples derived from multiple contributors. This technique involves separation of cells by the process of Fluorescence Activated Cell Sorting (FACS) prior to DNA extraction and amplification, thus enriching for contributors the resulting fractions and enhancing statistical strength of the genotyping analysis.

This presentation will impact the forensic science community by illustrating that forensic DNA evidence samples comprised of multiple individuals can significantly complicate interpretation of the resulting STR profiles and decrease the strength of the statistical analyses. In order to separate DNA sources within a mixture sample prior to STR analysis, FACS was utilized to segregate donor cells, based upon cellular characteristics of the contributors within the sample. The resulting separate fractions have reduced complexity, thereby facilitating STR analysis of the mixture sample.

FACS was utilized to separate contributor cells within Human Leukocyte Antigen (HLA) -labeled blood mock evidence mixture samples and mock touch evidence mixture samples based upon cell morphology. DNA was obtained from the mixture (presorted) and FACS sorted samples by the DNA IQ™ system. STR amplification was performed with the PowerPlex® Fusion System and resulting profiles were compared for the presorted and sorted samples. Genotyping was conducted manually for all profiles and by the probabilistic modeling program TrueAllele® Casework (TA) for selected profiles.

Presorted blood mock evidence mixture samples from three and four contributors yielded complex STR profiles. One of the sorted blood fractions from both three and four contributor mixtures showed enrichment to the level of one or two individuals. The other fraction was still a mixture but was less complex (i.e., at least one contributor was almost completely selected out). TA analysis of these presorted and sorted samples demonstrated enhanced statistical power post-sorting. Presorted samples for mixtures of individuals 105, 106, and 107 had similar log Likelihood Ratio (log(LR)) values for each contributor (9.86, 11.40, and 9.65, respectively). For the two FACS-sorted cell populations, TA analysis of fraction P2 produced a slightly higher value for contributor 106 (12.34) and excluded 105 and 107 (negative log(LRs) generated). TA analysis of fraction P3 produced a slightly higher value for 105 and nearly 11 orders of magnitude higher value for 107, respectively (10.13, 20.56) and excluded 106. Moreover, TA analysis of the sorted mixture from four individuals produced a log(LR) value to approximately single-source level (28.18) for contributor 103 in fraction P2. TA analyses thus confirmed and quantified observations by analyst genotyping that enrichment for individual contributors and increased statistical power may be accomplished with the use of FACS for cell sorting.

Mock epithelial touch evidence samples were analyzed as to whether the source of DNA was extracellular (free DNA), intracellular, or both. Intracellular DNA was isolated from pelleted and washed presorted cells and free DNA was collected from the supernatant of the washed cells and subjected to Microcon-100 concentration. Sorted cells yielded only intracellular DNA as the washes and sorting process removed free DNA from the sample. Polymerase Chain Reaction (PCR) quantification of presorted DNA showed that intracellular DNA was present, but the ratio of free DNA to intracellular DNA was approximately 2:1, though genotyping data were generated from both DNA sources. Epithelial samples were FACS sorted based on cell morphology (>20µm or <20µm). Six epithelial touch mock evidence mixture samples, each comprised of two or three contributors, were FACS sorted. The >20µm fraction yielded partial profiles for a single donor from two samples, inconclusive results from two samples, and no results from two samples. The free DNA yielded a full STR profile for a single donor from one of the samples, a partial single-source profile from four samples, and one sample failed to yield any results. These results demonstrate that FACS separation may be performed on touch evidence based upon cell morphology after additional protocol development and that at least a portion of the DNA from touch evidence is intracellular in origin.

Cell Sorting, Mixture Samples, Probabilistic Genotyping

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