HORMONE-SPECIFIC MOLECULAR PROBES FOR SCREENING AND SEPARATION OF TRACE MIXTURE EVIDENCE

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With the rapid increase of trace DNA evidence samples processed in crime laboratories, new screening methods are needed to identify contributor cell populations and separate them prior to DNA profiling. To address this, we have developed a novel method that utilizes sex-specific hormones within the cell as a marker to presumptively differentiate and enhance either male or female epithelial cell populations derived from touch/trace mixture samples. First, hormone molecule targets such as testosterone, dihydrotestosterone, and estradiol are tagged within the cells using fluorescent antibody probes. Presumptive identification of male and/or female cells is then accomplished by quantifying the abundance and distribution of fluorescently-labeled cells in an automated, non-destructive, and high-throughput manner using flow cytometry. Additionally, certain patterns of intracellular fluorescence following probe hybridization may be used to differentiate individual cells originating from separate individuals. Ultimately, labelled cell populations were used as the basis for a front end cell separation workflow for isolating male from female cell populations. We tested this workflow on mock casework samples consisting of two-person trace epithelial cell mixtures. Antibody staining of mixtures in which the donor cell ratio was approximately 1:1 produced cell populations that were successfully differentiated based on fluorescence histogram profiles. Sorting parameters were then created for isolating each cell population. DNA profiling of pre- and post-sorted fractions using PowerPlex® Fusion combined with TrueAllele® Casework Probabilistic Modeling demonstrated that the contributor cell populations from the 1:1 mixtures were enriched in each sorted fraction and that donor profiles could be associated with the original mixture with high statistical support. Certain contributor cell populations could also be resolved from mixtures with more imbalanced donor ratios in the original sample, indicating that this may be a useful approach for many types of touch/trace biological samples, one of the most challenging types of forensic evidence.