

USING SEX AS A WEAPON: SEXUALLY DIMORPHIC MARKERS FOR CELL SEPARATION AND SCREENING

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With the rapid increase of “touch” 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 identify and enhance either male or female epithelial cell populations derived from touch/trace mixture samples. First, testosterone and dihydrotestosterone 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 probe binding within the cell in an automated and high-throughput manner using either flow cytometry or conventional microscopic imaging. These sex-specific fluorescence signatures can also be used as the basis for a front end cell separation workflow that physically isolates male from female cell populations. We tested this workflow on mock casework samples consisting of two-person trace epithelial cell mixtures generated from male and female donors. Antibody staining of cell mixtures successfully differentiated the two cell populations. The fluorescence profiles were used to create novel sorting parameters for separating male and female cells. DNA profiling of pre- and post-sorted fractions using PowerPlex® Fusion combined with TrueAllele® Casework probabilistic modeling demonstrated that the contributor cell populations were separated by sex and donor profiles could be associated with the original mixture with high statistical support. Therefore, this workflow has the potential to be utilized in cell screening and separation techniques for touch/trace samples, one of the most challenging types of forensic evidence.