CHARACTERIZATION AND SEPARATION OF TOUCH MIXTURES USING ENDOGENOUS AND EXOGENOUS PROPERTIES OF COMPONENT CELL POPULATIONS

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Analysis of complex cell mixtures -- which comprise a growing proportion of casework samples particularly with the rise in “touch” sampling -- is a significant challenge for DNA caseworking units. Currently, interpretation of STR profiles containing multiple contributors requires time-consuming and frequently subjective procedures that may produce results of limited (or no) probative value. Our group and others have developed methods for isolating cells from a mixture by taking advantage of either morphological or immunochemical differences between donor cell populations. However, these methods have yet to be demonstrated on “touch” samples which are primarily comprised of sloughed epidermal cells and thus have vastly different biological and structural properties from other forensically relevant cell types (e.g., vaginal, buccal, white blood cells). These differences may make conventional immunochemical staining and separation methods less effective on “touch” samples.

Here, we present a survey of biochemical and optical variation in touch epithelial cell populations and, for the first time, present a new candidate signature for separating cell populations in touch mixture samples based on red autofluorescence (650-670nm). Cell characterizations initially focused on two different protein systems, Human Leukocyte Antigen (HLA) complex and cytokeratin (CK) filaments. Hybridization experiments using pan and allele-specific HLA antibody probes showed that surface antigens on cells transferred from the palmar surface of volunteers are largely unreactive. Cytokeratin expression was surveyed in touch samples by hybridizing cell populations with a pan-CK probe AE1, which targets CK proteins 10, 14, 15, 16 and 19. In contrast to HLA probes, cells readily bound AE1, with fluorescence levels varying slightly across donors (mean fluorescence intensities ranging from 417 to 663 relative fluorescence units (RFUs)), as well as across sampling days.

Next, we examined variation in red autofluorescence (650-670nm) as a potential discriminating signature in touch samples. Clear differences were observed with mean fluorescence intensities ranging from ~200 RFU to 1,200 RFU between donor cell populations. As with CK surveys, the intensity of red autofluorescence varied between cell populations from the same contributor sampled on different days. Initially, it was unclear whether these differences might be linked to endogenous molecules or exogenous fluorescent compounds. However, we subsequently found that autofluorescence in touch samples is influenced by a donor’s contact with specific material prior to handling the substrate from which cells were collected for this study. For example, direct handling of laboratory gloves, plant material, or certain types of marker ink imparted red fluorescence signal onto the cells that could be easily visualized microscopically or using flow cytometry. Further, the red fluorescence signal could still be detected after donors washed their hands.

To test whether these observed optical differences could potentially be used as the basis for a cell separation workflow, controlled two-person touch mixtures were separated into two fractions via Fluorescence Activated Cell Sorting (FACS) using gating criteria based on intensity of 650-670nm emissions, and then subjected to DNA analysis. STR typing of the sorted fractions provided partial profiles that were consistent with separation of individual contributors from the mixture. Overall, these results suggest that when an individual transfers cells by touch, these

cells carry with them exogenous signatures that may be harnessed to isolate that individual's contribution to a mixture and thus facilitate single source typing.