DIFFERENTIATION OF INDIVIDUAL CELL POPULATIONS IN A TOUCH MIXTURE USING ANTIBODY PROBES AND FLOW CYTOMETRY

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Mixture interpretation continues to be a challenge for the forensic community. While methods exist to separate cells from different contributors prior to DNA extraction, most can only be used on mixtures of different cell types. Yet, an increasing proportion of evidentiary samples submitted for casework are contact mixtures in which two or more individuals have contributed epidermal cells. Resolving these mixtures is more problematic due to the consistent morphology and size of epidermal cells across individuals. Protein biochemistry offers one promising avenue for differentiating contributors in a mixture since individuals may vary in the abundance of certain structural alleles within epidermal cells. The objective of this research was to survey biochemical profiles of contact epidermal cells across individuals by labeling them with allele-specific antibodies. Observed differences in the protein abundance and/or diversity would then be used to separate individual cell populations from the mixture using flow cytometry.

Two and three person touch mixtures were generated from ~20 individual donors by holding or rubbing the same substrate. Intact epidermal cells ~30-50 µm were then collected, labeled with antibody probes targeting the Human Leukocyte Antigen (HLA) Complex and Cytokeratin (CK) filaments within the cell, and analyzed with flow cytometry. Epidermal cells hybridized with HLA probes showed no discernable increase in cellular fluorescence, suggesting that their HLA-receptors on the cell surface are non-reactive. However, epidermal cells from different donors showed consistently different interactions with CK-specific probe AE1 suggesting that the abundance of allele targets within the cell may be a useful signature for differentiating and sorting individual contributors within a mixture.

In order to examine the forensic utility of this approach, we used AE1 probe to label two person mixtures, sorted cell populations based on their fluorescence profiles, and performed STR profiling on each fraction. Although fluorescence differences were consistently observed and cells were successfully separated intact with ~95% efficiency, the amplifiable DNA content of sorted cells were significantly lower than expected, <100pg per $1x10^6$ cells which may be due to the dissociation of extracellular DNA from cell surfaces during sorting. Current efforts are focusing on coupling this approach with ultrasensitive DNA profiling platforms for forensic applications.