B104 Differentiation of Individual Contributors in Contact Epidermal Cell Mixtures Using Fluorescently Labeled Antibody Probes, High Resolution Microscopy, and Flow Cytometry

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After attending this presentation, attendees will understand the biochemical variation within epidermal cells across individuals and how this diversity can be coupled to antibody-based tagging procedures to differentially label contributors in a biological mixture. Attendees will also learn how flow cytometry and Fluorescence Activated Cell Sorting (FACS) may be used to rapidly characterize complex cell populations and physically isolate individual cell populations from a mixture sample.

This presentation will impact the forensic science community by introducing a new strategy for resolving touch mixtures that involves physical separation of cell populations before DNA analysis. This technique can assist forensic laboratories by providing an alternative to complex mixture interpretation procedures, thereby reducing analytical subjectivity and loss of evidence.

Analysis of contact or "touch" mixtures is a significant problem for DNA caseworking laboratories. The presence of cells from multiple contributors in an evidence sample can produce Short Tandem Repeat (STR) profiles that are difficult or impossible to interpret. Although many protocols for cell separation exist, most cannot be applied to mixture samples with only one cell type. New methods are needed that can differentiate epidermal cell populations from different contributors and allow them to be physically isolated prior to DNA profiling. One promising strategy for resolving complex cell mixtures is to target the diversity in protein structures in cells from different individuals. Differentially expressed proteins can be tagged with molecular antibody probes and used to label each contributor's cells in a mixture. Although this strategy is routinely used in biomedical applications, it has rarely been tested in a forensic context. Therefore, the goals of this study were to survey biochemical variation in epidermal cell populations and to identify specific protein targets that may be used to differentially label and sort individual cell populations from a touch mixture.

"Touch" epidermal cells were collected from ~20 individuals and characterized using high-resolution microscopy and flow cytometry. Analysis of unstained samples showed that the touch samples were composed of two distinct fractions: one containing keratinocytes ~30µm-50µm and the other composed of cell fragments/debris <10µm. Keratinocyte populations from each donor were isolated and hybridized to antibody probes targeting the Human Leukocyte Antigen (HLA) complex and cytokeratin filaments within the cell. Hybridization of epidermal cells with allele-specific HLA probes showed no discernable increase in fluorescence compared to unstained controls and subsequent experiments utilizing pan-HLA probes exhibited similar trends. This suggests that, unlike other cells types (e.g., White Blood Cell (WBC) and buccal cell), HLA antigens are either inadequately expressed or insufficiently reactive on epidermal cell surfaces to be a suitable target for cell labeling.

Next, epidermal cells were hybridized with cytokeratin probes AE1 and AE3 which target different sets of filament proteins. Fluorescence levels of AE1-hybridized cell populations showed significant differences across donors with median fluorescence ranging from ~700 to ~11,000 Relative Fluorescence Units (RFUs) and some donors showing as much as a two-fold increase in intensity over other individuals. Hybridization with AE3 probe showed similar trends with median fluorescence of cell population from different donors ranging from ~200 to ~600 RFUs. This indicates that AE1/AE3 antibody probes may be a useful tool for capturing biochemical variation between donor populations and differentially labeling cells in a mixture.

To test whether these biochemical differences could be used to separate cell populations in a forensic sample, touch mixture samples were labeled with AE1 probe and processed using FACS. Isolated cell populations were then subjected to STR profiling. Results showed that touch epidermal cells from different contributors could be labeled and then sorted, intact, with high efficiency into separate reservoirs (~95% of input cells captured). While partial to complete 23-locus STR profiles were obtained from the majority of sorted cell populations, DNA yields from these fractions vis-à-vis cell count suggest that intracellular keratinocyte DNA is highly degraded

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and that a significant portion of amplifiable DNA from contact epidermal samples may be extracellular. Thus, flow cytometry-based strategies for sorting the most challenging complex mixtures (i.e., mostly epidermal) may need to be coupled to sensitive DNA profiling techniques as well as workflows for processing the extracellular fraction of the mixture sample, which can easily be isolated during FACS for direct amplification.

Mixture Interpretation, STR Profiling, Flow Cytometry