B13 Separation of Epithelial Cell Mixtures Using Fluorescently Labeled Antibodies and Flow Cytometry

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The goal of this presentation is to introduce attendees to a novel molecular technique that utilizes fluorescent antibody probes that target antigens within the Major Histocompatability Complex (MHC) on epithelial cell surfaces. Attendees will also learn how antibody hybridization can be coupled to flow cytometry to provide rapid separation of targeted cell populations from a forensic mixture.

This presentation will impact the forensic science community by introducing a high throughput, non-destructive method for generating single-source DNA profiles corresponding to each contributor of an epithelial cell mixture. The technique can assist forensic caseworking units by providing an alternative to complex profile interpretation procedures, thereby reducing analytical bottlenecks and loss of evidence.

Previous research has shown the application of flow cytometry coupled to antibody probes targeting the Human Leukocyte Antigen (HLA) system for selectively isolating individual cell populations from a blood mixture to generate single-source Short Tandem Repeat (STR) profiles. The goal of this research was to test this technique against various types of epithelial cell mixtures for resolving individual contributor profiles. Due to its efficacy in earlier studies, an antibody probe complementary to the A*02 allele was chosen for all experiments. Hybridization experiments were conducted on both buccal cell mixtures and contact epithelial cell mixtures. Results from buccal cell mixture hybridization demonstrated that this cell type has intrinsically high levels of fluorescence which can interfere with the signal originating from specific probe-antigen interactions on the cell surface; however, detailed statistical analysis of fluorescence histograms did indicate greater shifts in cell fluorescence when donor cell populations were either heterozygous or homozygous for the A02 allele. This suggests that separation of labeled cell populations is still possible even if antibody reactivity is not strictly a function of HLA genotype.

In the second experiment, hybridization of the A02 probe was tested against contact epithelial cells. Cells were sampled from the stratum corneum of the epidermal layer from four different individuals representing a range of HLA types. Hybridization results showed little to no interaction with the cell surface across all donors tested. Preliminary experiments using enzymatic digestion of cell surface prior to probe hybridization, as well as novel types of surface antibodies, indicate that these may be effective strategies for enhancing differential labeling and separation of individual cell populations from contact epithelial mixtures. Because epidermal cells and buccal cells showed drastically different reactivity to A02 probe, mixtures containing these two cell types were analyzed. Two-person mixtures containing buccal cells from one contributor and epidermal cells from a second contributor produced two distinct cell populations after antibody hybridization that were easily resolved by flow cytometry. When the labeled cell population (i.e., buccal cells) was separated by Fluorescence Activated Cell Sorting (FACS), the STR profile was identical to its single-source profile across all six STR loci tested. This suggests that HLA antibody probes have the potential to resolve complex mixtures containing different sources of epithelial cells (buccal cells from skin) which may aid in the analysis of certain types of forensic cases.

Mixture, Flow Cytometry, STR Profiling

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