SEPARATION OF COMPLEX BIOLOGICAL MIXTURES USING HUMAN LEUKOCYTE ANTIGEN PROBES AND FLUORESCENCE ACTIVATED CELL SORTING

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Analysis of evidence samples containing complex biological mixtures of two or more individuals is an ongoing issue for forensic laboratories. Although techniques have been developed to separate cells by size and/or morphology, few can be applied to mixtures of the same cell type, in particular blood or epithelial cells, which are often encountered in forensic casework. DNA profiles resulting from these mixtures are difficult to interpret, often leading to inconclusive results and, potentially, loss of evidence. New techniques are needed that can physically separate the cells prior to DNA analysis for the generation of unambiguous STR profiles.

In this research, we present a technique for physically isolating individual cell populations from a mixture using fluorescently-labeled antibody probes hybridized to the cell surface. Each probe is specific for an antigen allele within the Human Leukocyte Antigen (HLA) complex, a highly polymorphic set of genes with well-characterized population frequencies. Because of the natural diversity of HLA alleles, these antibody probes can be used to selectively label cells of a specific genotype within a mixture. Next, fluorescently-labeled cells are separated from the mixture using Fluorescence Activated Cell Sorting (FACS), a high-throughput, non-destructive technique. Isolated cell populations were then subjected to DNA profiling. As a proof-of-concept experiment, we tested this technique on two- and four-person whole blood mixtures using a single fluorescent antibody probe specific for the A02 allele. For each type of mixture, flow cytometry analysis of the hybridized mixture showed two cell populations, each with a distinct fluorescent profile--one corresponding to the contributor carrying the A02 allele, and the other corresponding to the contributor(s) negative for the same allele. Once isolated by FACS, STR profiles of the labeled populations were compared against STR profiles of the reference samples from each contributor. Results showed that the STR profile of the labeled population within each type of mixture was identical to its single source profile for the A02 positive contributor(s) across all six STR loci tested. Furthermore, no evidence of allelic contributions from the A02-negative contributor(s) was seen. These results show that fluorescent HLA antibody probes coupled to FACS may be a promising technique to generate DNA profiles of individual contributors in a complex whole blood mixture.

Epithelial cell mixtures were also hybridized with the A02 allelic probe and processed using FACS. Unlike white blood cells, the epithelial cell types tested (buccal, dermal) showed variable levels of HLA expression as well as high levels of auto-fluorescence depending on the tissue source. Shifts in fluorescence intensity for A02-negative buccal samples suggest that the probe was cross-reactive with other antigen alleles. However, dermal cells did not exhibit the same levels of cross-reactivity. Various parameters of the hybridization assay are being optimized to facilitate more specific antibody interactions. These include using alternate blocking agents in the hybridization buffer, and varying the structure of primary binding site of the antibody probe. Current efforts are also underway to implement novel cell-sorting strategies to overcome some of the technical limitations of antibody labelling and FACS.