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Separation of Blood Mixtures Using Fluorescently Labeled Antibodies

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Abstract

Identifying and analyzing biological mixture samples at a crime scene are of paramount concern for forensic scientists, especially if that type of evidence contains only one cell type. The presence of multiple contributors in a biological evidence sample reduces the probative value of DNA evidence and can sometimes lead to its eventual loss of value. As such, this study was performed in an attempt to examine and evaluate flow cytometry analysis as a means to separate blood mixture samples labeled with fluorescent antibodies. Fluorescein Isothiocyanate (FITC) antibodies were specifically targeted and bound to HLA (Human Leukocyte Antigens) markers present on nucleated cells in the blood, after which they were isolated from the blood mixture utilizing Fluorescent Activated Cell Sorting (FACS) - A high throughput technique that separates cell populations based on their optical activity, followed by STR analysis. This approach was tested on fresh blood mixtures containing two contributors, where one contributor possessed an HLA A*02 allele that was not shared with the other contributor. We hypothesize that HLA A*02 positive samples would exhibit fluorescence when bound with the fluorescently labeled antibodies while the HLA A*02 samples would not. As such, we would be able to separate both cell populations using FACS followed by STR analysis. Such a work flow is believed to yield discriminant STR profiles unique to each contributor thus increasing the probative value of the evidence at hand. Results supported our hypothesis and yielded discriminant STR profiles for both contributors, with minor peaks from the A*02 negative contributor being observed in A*02 positive contributor sample. We can then conclude that HLA A*02 antibodies coupled to FACS is a suitable method that can be utilized to evaluate and separate blood mixture samples in an attempt to yield discriminant STR profiles.

Keywords: FACS; Flow Cytometry Analysis; HLA A*02; Antibody Hybridization; Blood Analysis; FITC labeled; STR Profiling

Introduction

Forensic casework involving mixture samples are becoming ever more abundant and problematic for forensic laboratories. Many laboratory methods have been implemented into existing workflows to facilitate the separation of certain components of a biological mixture prior to PCR amplification and STR profiling. Some of these include differential lysis, flow cytometry, and laser capture microdissection. The use of these methods has subsequently allowed scientists to obtain DNA profiles from mixture samples containing two different cell types (e.g.. Sperm and epithelial cells). However, these techniques are incapable of separating and analyzing a mixture sample from multiple contributors containing the same tissue type.

Cellular immunochemistry offers a promising avenue for distinguishing similar cells from different sources in a forensic mixture before submitting the cells to STR typing. Proteins within the Human Leukocyte Antigen (HLA) Class I Complex, which are surface glycoproteins expressed on most nucleated cells, serve as self-recognition markers for cells within the immune system. For forensic analysis, the key attribute of HLA antigens is the wide range of molecular structures that result from genetic polymorphisms within the HLA coding region. The diversity of HLA alleles and their respective frequencies in a number of major population groups have been extensively documented in worldwide databases. The methods described here harness the variability of HLAs as expressed on the cell surface, leaving cells intact for downstream STR analysis. Cells that have bound fluorescent probes targeting almost any intracellular or extracellular protein can be detected and physically separated from unlabeled cells with a specialized type of flow cytometry called Fluorescence Activated Cell Sorting (FACS). Very few studies have explored FACS as a method for characterizing and separating cells in complex forensic mixtures containing one cell type. Therefore, the objective of our study was to test HLA antibody tagging coupled to FACS with the ultimate goal of obtaining distinct STR profiles of individual contributors in a blood mixture.

Methods
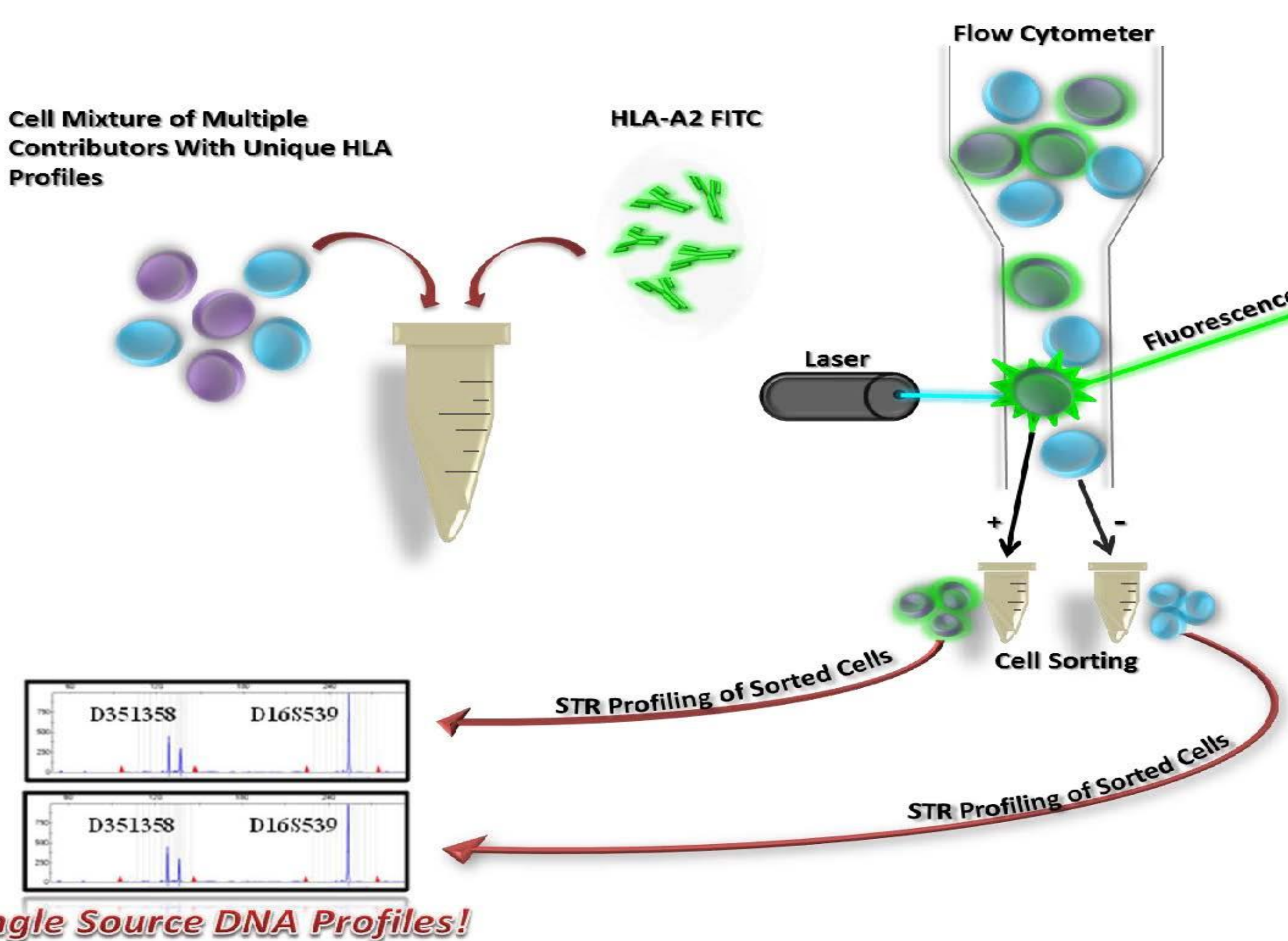
Figure 1. Individual contributors in a mixture sample are labeled with a FITC HLA-A*02 fluorescent antibody probe. Utilizing FACS, each cell is passed along a single file in a fluid stream through a light beam emitted by a laser. The cell's characteristics (i.e. size, granularity, morphology) influences how light scatters when it strikes the cell. Furthermore, light of specific wavelengths (i.e. 488-530/30 for FITC) interacts with the antibody-coupled fluorophores bound to the cell's surface producing a fluorescence of proportional intensity to the number of fluorophores present.

FACS also allows for physical isolation of cells that satisfy a certain set of criteria or 'gate'. Gating involves setting upper and lower limits for one or more parameters of interest, such as fluorescence intensity. At the same time that sensors are collecting a cell's light scatter and/or fluorescent characteristics, a computer determines whether those characteristics satisfy a defined gate. If they do, the cell is diverted toward one container through the use of electromagnetic deflector plates; if they do not, the cell is diverted toward another container.

Screening of HLA-A*02 Allele in FITC Labeled Blood Samples
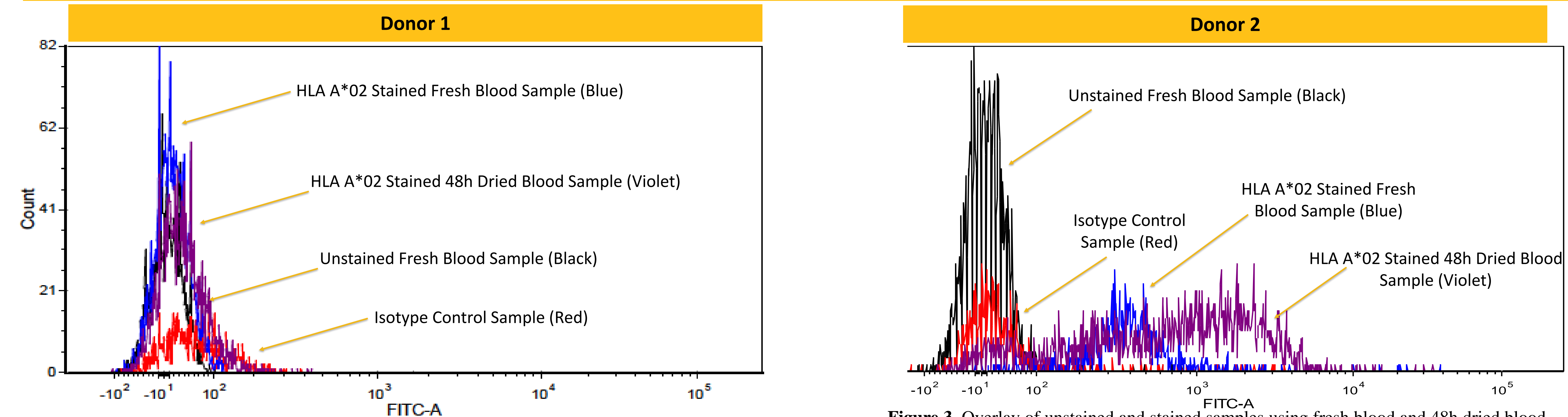
Donor 1

Donor 2

Figure 2. Overlay of unstained and stained samples using fresh blood and 48h dried blood from donor 1. A BD FACSCanto system was used to screen for the HLA*A02 allele in the FITC channel (excitation/emission 488/530nm). There was an overlap in histograms between Donor 1's unstained sample (Black), isotype control sample (Red), fresh blood HLA A*02 stained sample (Blue), and 48h stained dried Blood sample (Violet). Blood Samples from Donor 1 exhibited unremarkable fluorescence when excited by this laser leading us to conclude Donor 1 did not possess the HLA A*02 allele.

Figure 3. Overlay of unstained and stained samples using fresh blood and 48h dried blood from donor 2 .A BD FACSCanto system was used to screen for the HLA*A02 allele in the FITC channel (excitation/emission 488/530nm). There was an overlap in histograms between Donor 1's unstained sample (Black) and isotype control sample (Red). However, Donor 2's stained fresh blood sample (Blue), and 48h stained dried blood sample (Violet) showed a remarkable shift when excited by this laser. This lead us to conclude that Donor 2 possessed the HLA A*02 allele.

STR Analysis

	CSF1PO	D3S1358	TH01	D13S317	D2S1338	D18S51	AMEL
Contributor 134 (HLA –ve)	11	13,14	7,8	11,13	18,21	15,16	X, X
Contributor 135 (HLA +ve)	10,11	15,16	6,9.3	8,13	24	16,18	X, Y
2-Person Mixture Before Sorting	10,11	13,14,15,16	6,7,8,9.3	8,11,13	18,21,24	15,16,18	X, Y
A*02 + (sorted)	10,11	(13), (14),15,16	6, (7), (8),9.3	8, (11),13	(18), (21), 24	(15),16,18	X, Y
A*02 - (sorted)	11	13,14	7,8	11,13	21	16	X, X

Table 1. STR profiles developed from references and two person mixture separated by FACS.
(): Minor peaks

D3S1358

A) Two Person Mixture

D2S1338

B) Two Person Mixture
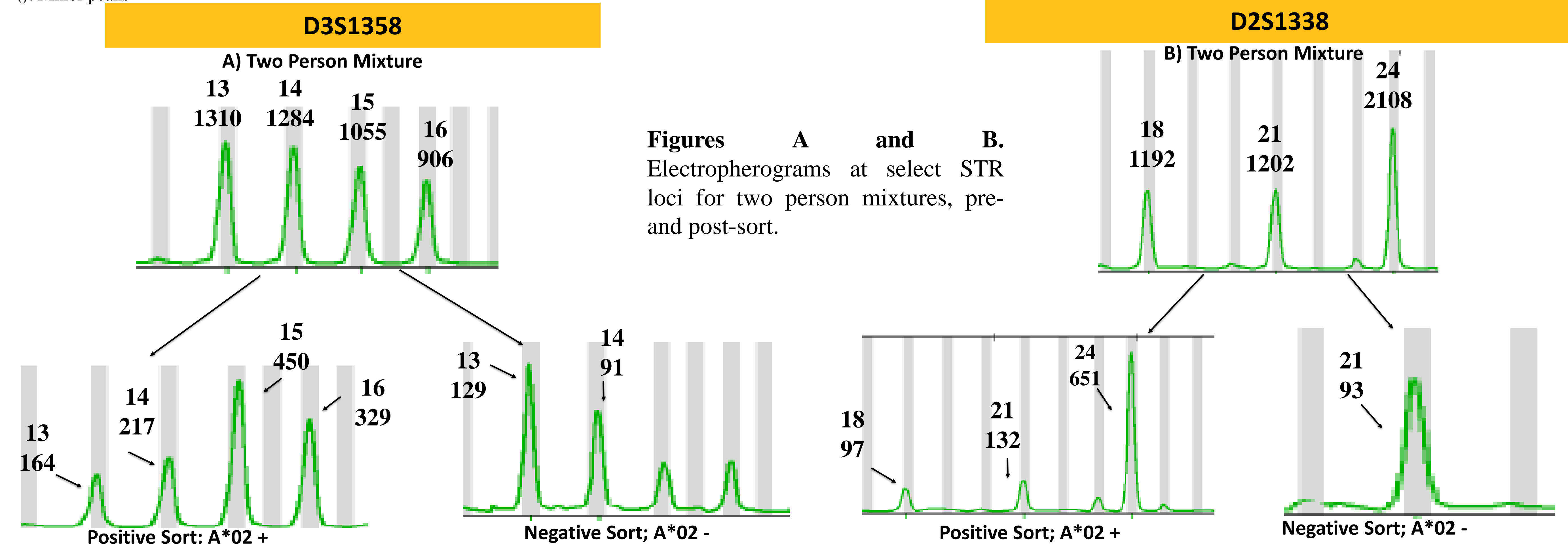
Figures A and B. Electropherograms at select STR loci for two person mixtures, pre- and post-sort.

Conclusion

- HLA-antibody probes can be used to differentiate individual contributors in an uncompromised and compromised complex cell mixture containing only one cell type.

- FACS is a promising technique to physically isolate antibody-labeled cells prior to forensic DNA typing, and can possibly be amended to other sample types such as touch cell mixtures.

- FACS is a non destructive technique that can be used to increase the probative value of uncompromised and compromised DNA evidence.

- The use of multiple antibody probes directed toward different HLA antigens and conjugated to different fluorophores may aid in resolving complex mixtures of three or more individuals, as well as aid in attempts to overcome signal loss due to auto fluorescence.

Acknowledgements