B128 The Generation of Interpretable, Single-Source, Short Tandem Repeat (STR) Profiles From Mixed Samples Using Optical Tweezers

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Learning Overview: The goal of this presentation is to show the robust nature of the optical tweezer for separating out target cells from mixed samples. Furthermore, after attending this presentation, attendees will see how a full single source STR profile can be generated from as few as 39 sperm cells separated from a mixed sample using the optical tweezer.

Impact on the Forensic Science Community: This presentation will impact the forensic community by exemplifying the utility of the optical tweezer for separating out target cells from a mixed sample to generate an interpretable, single source, DNA profile. When compared to the traditional method of differential extraction, this technique does increase the amount of time the DNA analyst spends on each suspected mixture sample. However, by implementing this technique on the front-end of the DNA analysis workflow, the DNA analyst spends less time on interpretation as a single source DNA profile is generated.

Optical tweezers are an ideal tool for cell separation and numerous reviews have described the operational principles behind single-focus optical tweezers. Although highly technical in nature, it suffices to say that an optical tweezer is a tightly focused laser beam in a low index medium (i.e., water, n=1.33) that attracts dielectric particles having an optical index of refraction larger than the surrounding medium (i.e., cells, $n \approx 1.36$). These dielectric particles or cells will migrate towards the focal spot of the laser beam and remain trapped for long distance transport (i.e., millimeters). To focus the laser beam to a sufficiently tight spot ($D_{spot} \approx 1$ μm) the beam is sent through a high magnification (100x) immersion objective. This degree of magnification serves two purposes. The first is to create the highly-focused laser trap and the second is to enable microscopic cell identification by cell morphology or fluorescence. This technique provides a high degree of manipulation for cells trapped, since each cell can be moved in the x, y, and z direction. This property of the optical tweezer provides separation of the cells of interest from a mixed population.

Initially, optical tweezers were utilized to capture and remove defined numbers of sperm cells (5-60 cells) from neat semen samples. Quantification of the DNA resulting from these samples resulted in detectable, amplifiable DNA in qualitative agreement with expected results based on cell number. Full DNA profiles were developed in multiple replicates. Following the success of this preliminary work, three mixed populations of vaginal epithelial cells released from a cotton swab were mixed with diluted seminal fluid, and the optical tweezers were utilized to separate the spermatozoa from the mixed population.

The DNA was isolated from the tweezed sperm cells using the QIAamp DNA Investigator Kit (Qiagen™ NV), quantified with the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific), vacuum centrifuged and the entire extract was amplified using the AmpFLSTR® Identifiler® Plus Amplification Kit (Thermo Fisher Scientific). The amplicons were separated using the 3130*xl* Genetic Analyzer (Thermo Fisher Scientific) and all samples were analyzed with GeneMapper® Software Version 4.1 using a threshold of 50 RFU.

A total of nine samples ranging from 39 to 74 sperm cells were obtained via separation with the optical tweezer. Eight out of nine samples yielded full STR profiles, with only a single dropout allele in one sample.

In conclusion, this has shown that the optical tweezer is a viable instrument for target cell isolation and separation from mixed samples. Furthermore, the results of this research show the potential of the optical tweezer for generation of single source STR profiles from other types of mixed samples commonly found in forensic DNA casework.

Optical Tweezer, Cell Separation, DNA Workflow