B116 Laser Microdissection: Low Copy Number Analysis of Sperm From Mixtures

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After attending this presentation, attendees will learn about the use of laser microdissection for recovery of minute amounts sperm cells from a semen/epithelial cell mixture for low copy number analysis.

This presentation will impact the forensic community and/or humanity by demonstrating how the development of improved methods for cell separation and tools for the recovery of limited amounts of available sperm cells in evidentiary samples are necessary to overcome problems in genotype interpretation from mixtures with incomplete separation.

The goal of this presentation is to present the results of recent research in the area of mixture separation utilizing laser microdissection for recovery of minute amounts of sperm cells from an epithelial cell mixture. STR analysis has become a routine tool in identifying the source of bio- logical stains in the investigation of sexual assault crimes. Difficulties in analysis arise primarily in the interpretation of mixed specimens or when only a small number of target cells are available for analysis. Development of improved methods for cell separation and tools for the recovery of limited amounts of available sperm cells in evidentiary samples are nec- essary to overcome these current problems.

Laser microdissection technology (LMD) has emerged as a method to capture single cells or a group of cells of interest from heterogeneous tissue. This technology is typically employed on histological tissue cryosections to collect specimens for further DNA, RNA or protein analysis. The purpose of this research is to use LMD on biological smears to identify sperm and epithelial cells while selectively dissecting and recovering the cells of interest for forensic DNA analysis.

In the study presented, mixtures of male semen and female oral epithelial cells were prepared onto plastic-foiled glass microscope slides. Stained spermatozoa were identified, dissected, and recovered through the semi-automated Leica AS LMD instrument. Collections of 10, 20, 40, and 80 sperm cells were recovered in Lyse-N-Go™ reagent (Pierce) for DNA isolation, and amplified using the Ampf/STR® Profiler Plus kit (Applied Biosystems) with separation by capillary electrophoresis. DNA was sub- jected to PCR amplification at 34, 38 and 42 cycles.

The results of STR typing show pure genotypes from the male donor without carryover from the female donor. Although allelic drop-out from the haploid cells was observed, genotyping was achieved from as little as 10 sperm cells

The laser microdissection method presented physically dissects target cells without the contamination of adjacent foreign cells in a mixture then collects the target cells for direct DNA isolation and PCR. This bypasses the multi-step, high-manipulation process of a preferential lysis procedure and traditional human DNA quantification. Thus, LMD can facilitate the pure recovery of sperm cells for low copy number analysis.

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DNA Typing, Laser Microdissection, Cell Separation

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