A28 Obtaining STR Profiles From Low Copy Number Biological Materials Utilizing Laser Microdissection and Optimized Collection Procedures

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After attending this presentation, attendees will learn about the collection of low copy number samples using of laser microdissection for STR analysis.

This presentation will impact the forensic community by demonstrating that using laser microdissection with low volume amplifications, full STR profiles can be obtained in as little as five cells. Being able to precisely collect an exact number of cells or separate mixtures using laser microdissection and obtain full STR profiles may impact how many low copy number samples are processed.

Processing low copy number (LCN) samples for STR analysis is a challenging endeavor. In cases where low amounts of biological material are present and standard collection/elution methods are implemented, it is difficult to determine how many cells are recovered upon collection. In some cases, low levels of differing cellular types can be overlooked with standard screening methods resulting in STR mixtures. Commercially available quantification systems have difficulties with LCN samples due to low level sensitivity limitations which can lead to poor amplification results. With the use of laser microdissection (LM), we can visually confirm the presence/type of biological material, collect an exact amount of cells, and separate cells of differing morphologies to resolve mixtures.

Prior to utilizing LM, different techniques of cellular collection were investigated to determine the best collection method for the recovery of cells from paper, steel, and cotton substrates. Previous studies have shown that the enzymatic digestion of cotton swabs with cellulase improves elution of biological material. Based on these studies, we implemented and optimized the addition of *Aspergillus niger* cellulase and found improved elution of LCN biological material with no detrimental effects.

The collection of biological materials was performed on two separate laser microdissection systems. The first system utilizes laser energy and caps comprised of a thin thermoplastic film to remove tissues or individual cells. After the cell of interest is targeted with the laser, the laser is fired, melting the thermoplastic film on the base of the cap to the targeted material. The second system uses a high energy UV laser to transfer cells from glass slides into collection vessels via non-contact cellular catapulting. The laser utilized in this system first makes direct contact with target cells and then pressure catapults them into collection caps. Both of these systems allow analysts to collect an exact number of cells with extreme precision to carry through DNA extraction.

Several commercially available DNA extraction kits were evaluated for extraction efficiency from laser microdissected tissues. DNA extracts were concentrated to 3µl to allow for maximum template input for low volume amplifications. Amplifications were performed at 30-32 cycles using autosomal, mini-STR, and Y-STR multiplex amplification kits.

Using the optimized techniques listed above, we have achieved full STR profiles from 5 to 25 laser microdissected white blood cells, epithelial cells, and spermatozoa taken from paper, steel, and cotton surfaces. Single source profiles have also been obtained from two person mixtures from cells of different morphologies in as little as five cells. The use of LM has allowed us to determine the exact number of cells needed obtain full STR profiles with various kits which eliminates setting up PCR reactions based on quantifications with LCN sensitivity limitations. The success of this study has shown that laser microdissection can be a powerful forensic tool for the precise collection and processing of low copy number biological evidence.

Laser Microdissection, Low Copy Number DNA, Short Tandem Repeat DNA Typing

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