A152 Liquid DNA Extraction and Expedited PCR for the Analysis of Forensic Biological Samples

Jenny A. Lounsbury, MSFS, and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904*

After attending this presentation, attendees will have gained an understanding of a phase-less DNA purification method to prepare biological samples for direct transfer to an expedited DNA amplification reaction.

This presentation will impact the forensic science community by describing a simplistic and faster method for DNA purification, as well as a more rapid method for DNA amplification. These improvements have the potential to greatly decrease overall analysis time and, therefore, increase sample throughput in forensic laboratories.

Solid phase extraction (SPE) is a widely used DNA extraction method, which typically utilizes a silica-based solid phase to reversibly bind DNA, followed by a series of washes to remove cellular debris, proteins, and PCR inhibitors. The bound DNA can then be eluted from the solid phase by using a low ionic strength buffer. SPE methods have been successfully adapted for use on a microdevice, providing purified DNA in a concentrated, small volume.[1] However, success with microfluidic-based SPE can be hindered by several problems that may arise, such as uneven packing of the solid phase within the microchannel and high backpressure, if the method is not well-optimized. Many of the issues with microscale SPE methodologies can be resolved by moving to a phase-less, liquid purification method, thus eliminating the need for a solid phase and simplifying microscale DNA extraction by making it less time consuming and more automatable.

Microfluidic devices provide a rapid, cost-effective alternative to conventional DNA analysis techniques and provide a platform for integration of multiple techniques on a single device. Along with SPE, PCR can readily be adapted to a microdevice where amplification can be performed in nanoliter reaction chambers. A non-contact heating method, such as infrared (IR)-mediated PCR, provides faster heating and cooling rates than can be achieved in a conventional thermal cycler, resulting in more rapid thermal cycling times.[2]

Previous expedited PCR studies have demonstrated the successful amplification of DNA in ~36 minutes using a commercially available STR amplification kit, commercially available polymerases that have been modified to have faster extension rates and improved processivity over traditional polymerases, and a conventional thermal cycler.[3] While a substantial advancement for the forensic community, improvements could broaden the use of this method to compromised and/or degraded samples with the use of a mini-STR amplification kit as well as more rapid thermal cycling technology. Using faster polymerases and the mini- STR amplification kit, in combination with IR-mediated heating on a microfluidic device, could lead to even faster amplification times and therefore, decrease the overall analysis time.

The current work presented here focuses on the evaluation of a DNA purification process without the use of a solid phase that requires only a 20 min incubation to obtain PCR-amplifiable DNA from crude samples. The sample, either whole blood or epithelial cells eluted from a buccal swab, is added directly to the purification reaction mix that consists of buffer and enzyme. Several purification reaction recipes were evaluated to maximize the amount of DNA recovered. Once purified, the DNA was added to an expedited PCR mixture, was loaded onto a microdevice, and PCR was performed using an IR-mediated thermal cycling method. The results indicate that the phase-less DNA purification method is able to produce amplifiable DNA, yielding full (nine of nine loci) mini-STR profiles. This method will demonstrate the first example of a phase-less DNA purification method in combination with IR-mediated PCR on a microfluidic device in ~ 1 hr, a reduction of three hours in analysis time in comparison to conventional methodology.

References:

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3 Vallone, PM, Hill, CR, Butler, JM. FSI: Genetics 2008;3(1):42-45.

PCR, DNA Extraction, STR Analysis

 ** Presenting Author*