B21 Microdevice Solid Phase Purification Utilizing Dual Pressure/ Electro-Elution for Concentration and Enhanced Recovery of DNA

Joan M. Bienvenue, MS, Natalie Duncalf, BS, James M. Karlinsey, BS, Jerome P. Ferrance, PhD, and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904*

After attending this presentation, the attendee can expect to learn about these advancements and enhancements to microchip solid phase extraction of DNA.

This presentation will impact the forensic community and/or humanity through the potential enhancement of DNA recovery by the inclusion of an electric field during the elution step of solid phase DNA extractions in microchip. This will improve the overall yields of these extractions and the potential increase for successful downstream analysis.

A shift from organic solvent-based extractions to solid-phase extrac- tions on silica (or ion exchange resins) for DNA purification has made the process more efficient, allowing for purification of DNA from samples from which successful isolation of DNA would not previously have been possible. In addition, these newer methods are more amenable to incorpo- ration into microchip-based devices. Glass microdevices packed with solid phases such as silica beads, sol-gel immobilized silica beads, or sol-gels alone have demonstrated utility as potentially low-cost alternatives for highly efficient and reproducible extraction of DNA. Solid phase extraction (SPE) in microdevices reduces the time, volume of reagents, and sample volumes necessary for successful purification of genomic DNA.

Currently, DNA extractions in microdevices are carried out in a pressure-driven mode, using a standard syringe pump to control flow through the device. Solid phase extractions provide the benefits of repro- ducibility and high extraction efficiency, while also yielding highly purified, PCR-ready DNA in a reasonably small volume. These SPE methods are not only effective for DNA purification, but in self-contained microdevices provide a tremendous advantage for forensic analysis by inherently removing many potential sources of contaminants. However, DNA is typically eluted from these devices in a volume of 15-35 µL. For evidentiary samples containing a relatively small amount of DNA, this volume reduces the overall concentration of DNA (per µL) that can be used in subsequent PCR reactions, compromising the ability to amplify DNA from these sources. In addition, as with all solid phase extraction protocols, a small amount of DNA is irretrievably lost to the solid phase, thus low- ering overall extraction efficiencies and reducing the ability to recover small amounts of DNA from low copy number samples. These problems are further exacerbated when integration of SPE with downstream microfluidic processes (such as PCR) is considered. With typical volumes of microchambers in PCR devices on the order of hundreds of nanoliters, the volume incompatibility between SPE and PCR becomes of critical concern.

This research demonstrates the use of an electric field during the DNA elution phase of the SPE to enhance recovery and provide a more concen- trated sample for downstream genetic analysis. A glass device designed with dual pressure/electro-elution capabilities is described, with results from preliminary testing detailed. The device, containing embedded elec- trodes, allows for continuous, syringe-driven flow to be accomplished, while a low voltage electric field is applied. Using this device, a typical solid phase extraction (sample load, protein wash, DNA elutions) using pressure-driven flow is accomplished, with the electric field imposed during the final elution step to both trap DNA as it exits the device as well as to enhance DNA recovery. The ability to trap and retain DNA at the anode during flow is a demonstration of precision DNA elution from the microdevice following termination of applied field. Retrieval and concen- tration of DNA from a silica-based solid phase using the dual purpose design is demonstrated and elution profiles both with and without the appli- cation of field are presented. The results of STR analysis using concen- trated vs. non-concentrated samples are depicted and the reduction in volume as it relates to current microchip PCR methods is discussed.

DNA, Extraction, Microchip

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