B80 The Development and Automation of a Swab In-DNA Out Platform Using Dynamic Solid Phase Extraction (SPE)

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Learning Overview: After attending this presentation, attendees will have been introduced to a novel, automated microfluidic method for DNA extraction and purification from forensic samples based on dynamic SPE. Additionally, a novel microfluidic lysis protocol and a novel fluidic control strategy will be introduced and applied to forensic genetic sample preparation.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by describing a cost-effective, automated microfluidic technique to lessen sample preparation demands placed on forensic laboratories upstream of genetic analysis.

As the amount of DNA evidence collected in criminal investigations increases, demand for forensic genetic testing continues to grow. The influx of samples to forensic laboratories often outpaces their processing capabilities, leading to a backlog of untested samples. Silica-based SPE techniques are widely accepted forensically, but are time and labor intensive. Although SPE can be automated using liquid-handling robots to increase throughput and reduce contamination risk, these instruments are limited to use in a centralized laboratory. A portable, automated, and multiplexed swab in-DNA out microfluidic platform is proposed for the purification of DNA from buccal swabs using silica-based dynamic SPE (dSPE). The device contains four identical domains to allow for multiple extractions simultaneously. The DNA obtained from this device is amenable to direct amplification by Polymerase Chain Reaction (PCR) and subsequent Short Tandem Repeat (STR) profiling, which represents a significant step toward reducing sample preparation demands placed on forensic laboratories.

Previous work demonstrated semi-automated microfluidic dynamic solid phase extraction of PCR-ready DNA from whole blood, replacing pipetting steps with rotationally controlled fluid flow and magnetic mixing.[1] However, some fluidic control steps required user intervention, preventing complete assay automation. In the work presented, a novel laser-based valving strategy supplants the manual valves, introducing the possibility for complete assay automation. A single laser can be used to both open and close microvalves. This capability is essential for the sequential delivery of the wash and elution buffers required for DNA purification. The dSPE protocol initially utilized three washes to remove both contaminants and PCR inhibitors, including a chaotrope used to facilitate DNA-silica binding and Isopropanol (IPA), which is used to remove cellular components loosely adsorbed to the silica surface. To remove the residual IPA, a Tris/EDTA (TE) wash was implemented. However, as this was the same buffer used to elute the purified DNA from the beads, an appreciable amount of DNA was lost during this step. To prevent this, the TE wash was removed and a novel strategy for IPA removal was implemented. A gas-permeable membrane was incorporated onto the back of the elution chamber to allow for IPA evaporation without sample loss. DNA yields following this modified on-disc protocol (0.43 ± 0.01ng/µL) are comparable to a control reaction done in-tube (0.41 ± 0.16ng/µL), but with the added benefit of heightened reproducibility The generation of full, 18-plex STR profiles following off-disc amplification and electrophoretic separation of amplicons demonstrates successful inhibitor removal and biocompatibility of assay reagents and device materials. Significant progress has been made toward complete automation of a microfluidic protocol for dSPE-based purification of DNA directly from buccal swabs. This includes integration of enzymatic lysis direct from buccal swab cuttings where the required volume of lysate for dSPE can be delivered directly to the purification architecture through device rotation and valving. Samples lysed and purified on the microfluidic device resulted in STR profiles with 100% correlation to control samples that were prepared in parallel via a conventional, in-tube protocol.

Adopting a microfluidic approach decreases the requirements for reagents and consumables relative to conventional SPE approaches and provides enhanced portability not possible with current forensic biorobotic protocols. Automation and multiplexing represent an important step toward a sample in-DNA out format, ultimately lessening the sample preparation demands exerted on forensic laboratories and increasing throughput.

Reference(s):

1. Kimberly Jackson, Juliane Borba, Marlina Meija, Daniel Mills, Doris Haverstick, Katherine Olson, Roman Aranda, Gavin Garner, Emanuel Carrilho, and James Landers. DNA purification using dynamic solid-phase extraction on a rotationally-driven polyethylene-terephthalate microdevice. *Analytica chimica acta* 937 (September, 2016): 1-10.

Forensic DNA, Dynamic Solid Phase Extraction, Microfluidics

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