B68 Integrated DNA Extraction and PCR Amplification of STRs: Interfacing Microfluidic Devices With Current Methodologies and Conventional Instrumentation

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The goal of this research project is to integrate DNA extraction and amplification for forensic genetic analysis in microfluidic systems in a manner that interfaces with conventional methods and laboratory instrumentation.

This presentation will impact the forensic community and/or humanity by detailing the interfacing of microfluidic technology with conventional techniques and instrumentation for forensic genetic analysis, a possible stepping stone towards the inclusion of totally automated microfluidic systems in forensic casework analysis.

Solid-phase extraction (SPE), PCR amplification, and high- resolution separations of PCR-amplified DNA from a variety of clinical, biohazardous, and forensically-significant samples are now readily carried out in microfluidic systems. With successful microchip adaptation of these individual processes becoming more commonplace and now being evaluated in forensic labs, research focus has shifted towards integration of these methods and the creation of multi-process, stand-alone devices with full-genetic profiling capabilities[1]. Integrated microfluidic systems offer an analysis platform capable of reducing the time, reagents, and sample necessary to perform many forensic and clinical analyses; however, there are many challenges associated with integrating multiple analysis steps on-chip for forensic analysis. Of particular concern is the difficulty of integrating solid phase DNA purification with PCR amplification, due primarily to the inherent incompatibility of the SPE reagents (guanidine and isopropanol) with the amplification reaction[2]. This is complicated by the need to carry out complex, multiplexed PCR amplifications using established commercially-available reagents and protocols. In addition, as the community looks to incorporate new microfluidic technology in crime labs, the transition from current bench-top systems towards microchip platforms could prove problematic, as the cost of this shift to unvalidated methods and instrumentation may quickly become prohibitive. In order to facilitate this transition, the fusing of already validated and established techniques/instrumentation with microfluidic systems provides a stepping stone towards the inclusion of stand-alone microfluidic total analysis systems in forensic casework analysis. By improving the timeliness and cost-effectiveness of analysis without requiring a prohibitively expensive overhaul of equipment and instrumentation, hybrid systems that effectively utilize existing technology, while exploiting microfluidic components become an attractive solution to this problem.

The research presented here describes an advancement that allows for integrated sample preparation for forensic applications to be carried out in a valveless microfluidic device using conventional bench-top instrumentation. With a focus on the fabrication and implementation of integrated glass microdevices for extraction and PCR amplification of STRs, these valveless devices are interfaced with conventional technology. DNA extraction is accomplished using a simple syringe pump and microchip-contained silica solid phase, followed by on-chip PCR amplification using a common bench-top thermocycler to accomplish amplification of STRs in ~500 nL with standard commercially-available amplification kits. Sample removed from the device is demonstrated to be amenable to analysis on standard capillary electrophoresis instrumentation. Methods for integrated DNA extraction and PCR amplification of STRs from forensically-relevant samples, with this commonly-available instrumentation are discussed. The work reported here highlights the feasibility of using a microdevice for sequential DNA extraction and PCR amplification on the same device, by interfacing the low-volume, closed-system, cost-effective micro- sample processor with currently-available benchtop instrumentation, circumventing the expense associated with the instrumentation that would be needed for new analysis platform. This work highlights the development of an integrated microfluidic extraction and amplification device that could be seamlessly assimilated into crime laboratories without the addition of costly instrumentation, improving forensic genetic analysis and providing a more facile transition to fully- automated genetic analysis systems.

References:

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DNA Extraction, PCR, Microchip

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