RAPID PCR AMPLIFICATION OF STR LOCI USING MICROFLUIDIC DEVICES

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The proven utility of forensic DNA evidence has increased the demand for DNA analysis services. Although conventional analysis techniques are effective, they are time-consuming and laborious, which has contributed to an overwhelming backlog of forensic casework samples with possible biological evidence. Conventional DNA typing steps include DNA extraction, quantitation, PCR amplification of multiple STR loci, and electrophoretic separation of the resulting STR fragments; of these steps, PCR amplification is the most time consuming, taking two hours or more with standard thermal cycling protocols. Increasing the speed for PCR amplification of STR loci has the potential to not only help speed up the overall DNA typing process, but to improve the throughput of a crime laboratory. Previous expedited PCR studies have demonstrated rapid amplification of using a standard 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.[1]

Microchip technology offers the potential of a rapid, cost-effective alternative to conventional DNA analysis methods. Microdevices provide self- contained, closed systems for analysis procedures, diminishing the potential for contamination or loss of sample. Techniques performed on microchips are advantageous because they can be integrated with upstream or downstream analytical steps on a single microfluidic device in the form of a lab-on-a-chip. These integrated microfluidic systems, which incorporate all of the sample processing steps required for DNA analysis, will reduce analysis times, and therefore, the forensic casework backlog. PCR amplification on microfluidic devices is well-established and has been shown for variety of applications, including human identification and STR analysis.[2] One example of PCR on a microfluidic device, 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. In addition, microchip PCR offers the distinct advantage of significantly decreasing hold times during cycling by reducing the reaction volume by an order of magnitude or more when compared with conventional thermal cycling protocols.

The work presented will highlight the development of expedited PCR amplification of STR loci using microfluidic devices. Unique to others exploring microfluidic PCR for STR profiling, PCR amplification is accomplished in sub-microliter reaction volumes using commercially-developed STR kits and reagents. Results of separations of the STR fragments from DNA amplified from the microdevices are described. A rapid cycling protocol that amplifies 16 or more loci from STR typing kits in as little as 25 minutes is demonstrated. The presented work represents the development of rapid multiplex PCR assays using microfluidic devices – a major step towards the development of a fully-integrated microdevice capable of total DNA analysis, as well as a reduction in overall time required for STR analysis.

References:
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