

OPTIMIZATION OF PCR FOR MICROFLUIDIC AMPLIFICATION OF STR LOCI

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STR analysis for human identification is a traditionally lengthy process, requiring 8-10 hours to complete under routine conditions. The largest contributor to this analysis time is a PCR amplification requiring >3 hours. By translating sample processing to a microscale format, faster, more cost-effective analysis can be achieved. Integration and automation are also advantages realized through microfluidic analysis, as well as the ability to perform multiplex sample processing on a single device. The challenge for transitioning to microscale PCR is to continue to meet the quality standards for STR analysis set by the forensic community while concurrently reducing sample and reagent volume and amplification time.

The use of infrared (IR)-mediated heating has been pioneered by the Landers group for PCR amplification on a microfluidic device, while different methods have been demonstrated by other groups [1]. Non-contact IR-mediated heating allows for a dramatic decrease in amplification time, but multiplexed analysis has not yet been demonstrated with previous work limited to amplification of a single sample at a time. In the work presented here, we demonstrate the successful transition from the use of a broadband lamp for IR-mediated heating [2,3] to an IR laser. Transitioning to an IR laser allows for amplification of multiple samples simultaneously on a single polymeric microdevice due to the smaller size of the laser compared to the broadband lamp.

The IR laser is used in combination with a non-contact temperature sensing method for thermal cycling on a disposable, polymeric microdevice containing multiple PCR domains. DNA is first purified from buccal swabs obtained from various donors, mixed with PCR reagents for STR amplification and then loaded into PCR chambers on the device. Amplification of all STR loci was observed after microchip PCR through use of conventional separation and detection instrumentation. A time reduction up to ~6-fold was observed compared to conventional PCR analysis time, with resulting STR profiles achieving intracolor, intercolor and heterozygote peak balance required by the forensic community. Simultaneous amplifications were performed, demonstrating that replicate analysis or analysis of multiple samples can be performed simultaneously using the IR laser system and a polymeric microdevice. Microchip PCR using the IR laser system can be further multiplexed in the future, allowing greater sample throughput, and can also be integrated with microchip electrophoresis on a single microdevice. Integration and automation of a microfluidic STR analysis system would result in at least 5-fold reduction in the time required for forensic sample processing while greatly streamlining the process of forensic STR analysis by removing sample transfer steps.

References:

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