

RAPID STR SEPARATIONS ON POLYMERIC MULTICHANNEL MICROFLUIDIC DEVICES

Carmen R. Reedy, Ph.D.¹, Brian Root, Ph.D.¹, Peter Trost, Ph.D.³, Orion N. Scott, Ph.D.¹, Annalise Barron, Ph.D.², Paul Kinnon, B.Sc.¹, Joan M. Bienvenue³, Ph.D., James P. Landers, Ph.D.¹

¹ZyGEM-MicroLab Diagnostics, Charlottesville, VA 22903

²Stanford University, Stanford, CA 94305

³Lockheed Martin Corporation, Rockville, MD 20850

Forensic DNA analysis, conventionally, is time-consuming and laborious, requiring 8-10 hours for analysis. This lengthy processing time and the increased demand for forensic DNA analysis, has significantly contributed to the increasing backlog of forensic casework samples. Therefore, the demand for new analytical techniques that can reduce time and throughput has increased substantially. Typically, conventional STR analysis requires extraction and quantitation of the genomic DNA, multiplexed PCR amplification of the STR loci, and electrophoretic separation of the amplified STR fragments. Currently, separation of STR PCR product requires 30 minutes or more to complete and is performed on a large capillary electrophoresis instrument. By reducing the time requirements for analysis, the throughput of a crime laboratory can be increased.

Recently, efforts have focused on the translation of forensic DNA analysis to the microscale platform to potentially address both of these issues, significantly impacting the forensic community. Microdevices would permit automation, integration of multiple sample processing steps, and miniaturization, all providing a reduced analysis time, footprint, and potentially in a more cost-effective way. Reduced analysis time, in comparison to conventional CE methods, for forensic STR separations can be simply achieved by the transition to a microfluidic chip. Furthermore, as most forensic laboratories require the simultaneous analysis of positive and negative controls for quality control purposes, a multichannel microfluidic device, capable of analysis of multiple samples, would further reduce time. In addition to the time benefits associated with microfluidics, amount of reagents can be significantly reduced by fabrication of a cost-efficient, single-use substrate (i.e., plastic).

The work presented here describes development of a method for rapid STR separations, using five-color laser-induced fluorescence (LIF) detection in 10 minutes, on a plastic multichannel microdevice with single-base resolution. A robust separation and detection system with associated hardware and software is necessary and demonstrated through analysis of multiple donors with accurate allele calling of forensic quality data. This rapid (4.5-fold time reduction) separation method provides the steps necessary in the development of a seamlessly, integrated device for multiple sample analysis on a single, disposable microdevice, reducing overall analysis time and increasing sample throughput, essential factors in forensic DNA analysis.