

THE DEVELOPMENT OF INTEGRATED MICROFLUIDIC SYSTEMS FOR PORTABLE, RAPID, AND AUTOMATED STR ANALYSIS

Orion N. Scott¹, Ph.D., Jessica V. Norris¹, Ph.D., Brian Root¹, Ph.D., Peter Trost, Ph.D., Michael Egan, B.S., Doug South, M.S., Annelise Barron², Ph.D., Abby Mackness³, J.D., Ph.D., Paul Kinnon, B.Sc.¹, James P. Landers⁴, PhD, Joan M. Bienvenue³

¹ZyGEM/MicroLab Corporation, Dale Avenue, Charlottesville, VA, 22903; ²Stanford University, Department of Bioengineering, Palo Alto, CA; ³Lockheed Martin, 9221 Corporate Boulevard, Rockville, MD 22407; ⁴University of Virginia, Departments of Chemistry, Mechanical Engineering, and Pathology, McCormick Road, Charlottesville, VA 22904

STR typing has become the accepted gold standard for human identification over the past two decades, and is now successfully employed in forensic, civil, and military laboratories. Although highly successful and reliable, current methodologies require 8-10 hours to complete under routine conditions, use large sample volumes, costly reagents, and are labor-intensive. Additionally, samples are exposed to the environment at multiple points during sample processing, making them susceptible to contamination. A transition of these sample processing and analytical methods to the microscale format will permit automation, miniaturization, and integration that will provide the end user with a system that provides expedited, cost-effective analysis in a closed system that reduces sample handling and possible contamination.

Human identification using STR typing poses a number of new challenges for integrated systems, including: efficient miniaturized DNA purification, PCR amplification of the required thirteen core STR targets with commercial multiplexed kits, fine-tuning the use of commercial kits optimized for large volume amplification (>10 μ L) to function effectively at the microscale and, finally, rapidly separating the amplified target fragments with single base resolution and detection of 5-color fluorescence. Efforts towards the integration of these processes for forensic STR typing have been previously presented; however, the challenges highlighted above have remained a focal point of research in this area.

A system capable of fully-automated processing and analysis of STR loci directly from buccal swab samples (RapID™) will be presented. The system utilizes a single, integrated, and disposable microfluidic chip and includes liquid DNA extraction, PCR amplification, and electrophoretic separation of STR loci. Expedited liquid extraction of DNA from crude samples is performed in less than ten minutes, and the resultant purified DNA guided into a microfluidic PCR chamber for amplification. The PCR process can be completed in less than forty minutes, with efficient amplification of 16-18 loci in sub-microliter volumes using commercially produced reagents. Following amplification, separation is carried out using electrophoresis in a short channel, using an optimized polymer, with baseline resolution and with 5-color detection. With the RapID™ System, the multistep process that consumes 8-10 hours for conventional forensic STR analysis is carried out in less than 70 minutes. The functionality of the integrated instrument capable of accepting the microfluidic device will be highlighted, with data supporting the capability of the microfluidic system for rapid, automated, end-to-end genetic analysis for human identification.