FULLY-INTEGRATED, MULTIPLEXED STR-BASED HUMAN IDENTIFICATION USING A SINGLE MICROFLUIDIC CHIP AND AUTOMATED INSTRUMENT

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There is little doubt that STR typing has become the accepted gold standard for human identification over the past two decades, now successfully employed in paternity testing, criminal casework, and missing person cases, as well as for databasing efforts. 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 open to the environment at multiple points during processing, making them susceptible to contamination. A translation 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.

Examples of fully-integrated microfluidic sample processing and analysis systems have begun to evolve (e.g., Easley et al).[1] Although integration of purification, PCR amplification, and electrophoretic separation/detection has been successfully demonstrated for pathogen detection, human identification using STR typing poses a number of new challenges for integrated systems, including: highly efficient miniaturized DNA purification, PCR amplification of the required 13 core STR targets with commercial multiplexed kits, fine-tuning the use of commercial kits optimized for large volume amplification (25 µL) to function effectively at the microscale, precision fluidic control, and, finally, rapid separation of the amplified target fragments with single base resolution and detection of 5-color fluorescence with a miniaturized optical system. These elements, along with high throughput, portability, and policy considerations, have made the introduction of integrated microfluidic systems for human identification challenging. Forensic human identification remains, however, one of the first applications in which fully engineered microfludic systems capable of carrying out sample-in, answer-out analysis are being developed.

A microfluidic forensic genetic analysis system is currently in joint development between the Lockheed Martin Corporation and MicroLab Diagnostics. This system is capable of the rapid, fully-automated processing of buccal swab samples and maintains full data analysis capabilities. Utilizing a single, integrated and disposable microfluidic chip, the multi-step sample processing and analysis that consumes 8-10 hours for conventional forensic STR analysis, can be carried out in less than 45 minutes. With this system, a novel liquid DNA purification technology is utilized to purify DNA from crude sample in less than 15 minutes and the resultant purified sample guided into a chamber for STR PCR amplification using IR-mediated thermocycling using conventional kit-based reagents. The PCR process, alone requiring ~3 hrs with conventional thermocycling, can be completed in less than 25 minutes due to the excellent thermal properties of the microchip and the use of IR as a heat source, with efficient amplification of all 16 STR loci. Separation is then carried out using electrophoresis in a short channel (6 cm), using an optimized polymer, with baseline resolution and with 5-color detection based on acousto-optic filtering. Seamless integration of these methods on a single disposable microdevice provides a means long sought after for performing sample processing and fluidic manipulation entirely in sub-microliter volumes regime. Additionally, a stand alone, fully-engineered, and integrated system, capable of controlling all of the processing, data handling, and sample handling associated with the sample analysis, that allows for the microfluidic device to be inserted and end-to-end sample processing to occur has been built. A first in microfluidics, a fully-integrated instrument and microfluidic device has been designed, built and functionalized to perform total, rapid, end-to-end genetic analysis for human identification.

1. Easley, C. J., Karlinsey, J. M., Bienvenue, J. M., Legendre, L. A., Roper, M. G., Feldman, S. H., Hughes, M. A., Hewlett, E. L., Merkel, T. J. Ferrance, J. P. Landers, J. P. *PNAS* **2006**, *103***,** 19272-19277.