A MULTISAMPLE PLATFORM FOR AUTOMATED GENETIC ANALYSIS FOR HUMAN ID

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Short tandem repeat (STR) analysis is the current, well-established method for human identification. Although conventional techniques for genetic profiling via STR analysis are highly successful and reliable, analysis times are lengthy, averaging 8-11 hours and leading to lengthy sample turn-around times. With the development of microfluidic devices, recent efforts have been made to reduce the time and cost of forensic genetic analysis by transitioning each sample processing step to the microscale. Miniaturization allows for automation and integration of the processes which, in turn, results in an expedited and cost-effective analysis.

Microfluidic integration of multiple analysis steps has been proven effective for pathogen detection [1], combining DNA extraction, PCR amplification, electrophoretic separation and detection on a single microdevice. However, human identification via STR analysis presents unique challenges for integrated systems due to the importance of achieving forensic-quality profiles. Progress toward a fully-integrated microfluidic sample processing and analysis system for STR typing has been presented by our group [2,3]; we have demonstrated the reduction of the conventional 8-11 hour forensic STR analysis to less than 60 minutes [3] using a single glass microdevice. Further work presented here exhibits strides made toward overcoming the challenges of integrated forensic genetic analysis, including achieving efficient DNA isolation, microscale PCR amplification of the core STR loci with commercially-available reagents (that were designed for large volume (25 µL) amplification), precise fluidic control, rapid separation of the amplified target fragments with single base resolution, and five-color fluorescence detection.

This work will demonstrate the use of a plastic, multichannel microdevice interfaced with a single instrument for simultaneousfour sample analysis of some combination of unknowns/controls. This includes integrated PCR amplification, separation and detection of an 18-plex STR profile from four samples simultaneously. The PCR amplification uniquely utilizes an IR laser for non-contact heating in combination with a non-contact temperature sensing system greatly reduces total amplification times. This non-contact approach greatly reduces the complexity of both the microdevice, and the interface between the microdevice and the instrument. Using all four channels, balanced microchip PCR amplifications are achieved with DNA from buccal swabs of both shedders and non-shedders using optimized chemistry and conditions. Integration of PCR and separation is achieved as the amplified PCR products are mobilized by the instrument to a sample reservoir for microchip separation. Using a polymer optimized for plastic microchips, the separation can be completed in a 7 cm effective length in less than 12 minutes. The data from the multicolor detection system is processed through automated software to produce a forensic profile. Allele calling is achieved using commercially available software packages. Seamless integration of these analytical methods onto a single disposable device under automated computer control provides a method for increasing sample throughput with a multiplexed device, thus reducing manual manipulation during sample processing.

REFERENCES:

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