A MULTI-CHANNEL MICRODEVICE FOR PCR AMPLIFICATION AND ELECTROPHORETIC SEPARATION OF DNA

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Current human identification laboratory methods require highly trained scientists to develop short tandem repeat (STR) profiles from even the most routine samples, such as buccal swabs. Individual processes have been automated; however, until recently, no one instrument could carry out these processes from beginning to end. Although these conventional methods are successful and reliable, there are advantages to a fully-integrated process. The most obvious advantage is speed, with analysis times dropping from ~8 hours to sub-90 minutes. Additionally, laboratories will gain efficiencies by validating one instrument in the place of multiple instruments and purchasing only one consumable. By reducing the level of skill required to process a buccal swab, these instruments will free analyst time for more complex casework. By translating each sample processing step to the microscale, these processes can be integrated into a single chemical cartridge and all the hardware condensed into a single instrument.

Microfluidic integration of multiple analysis steps has been proven effective for pathogen detection [1], combining DNA extraction, PCR amplification, electrophoretic separation/ 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 in the past [2,3]. We have shown the evolution of chip-based systems that reduce the conventional 8-11 hour forensic STR analysis time to less than 60 minutes [3] using a single glass microdevice for PCR and electrophoresis, but the DNA isolation was carried out off chip.

We present here, for the first time, the FULL integration of extraction, PCR, separation and detection on a single device – a 'swab in-peaks out' solution – for generating STR profiles with commercially-available reagents, precise fluid control, and separation of amplified target fragments using a single, plastic chip. As might be expected from microfluidic miniaturization, efforts have resulted in a plastic, multichannel microdevice that interfaces with a single instrument capable of performing all steps necessary for generating STR profiles from four buccal swabs. The microdevice (the cartridge) is the size of 96-well microtiter plate and, while only a few millimeters thick, contains all of the complex circuitry needed for on-board reagents to carry out sample preparation and separation/detection. The LE yields a predictable quantity of DNA (in <10 min) for PCR without a quantification step. PCR driven by an IR laser with remote temperature sensing, allows for amplification in <45 min. PCR is integrated with separation of DNA in a separation polymer optimized for plastic microchips. Using only a 7 cm microchannel (i.e., micro-capillary), separation is complete in <12 min. Following data processing through automated software, a forensic profile results with allele calling carried out automatically. By fully integrating all the laboratory processes for human DNA identification into a single, disposable microdevice with an automated platform, a sample-to-answer instrument system results; one that can reduce time, cost, and manual labor and, possibly, errors.

References:

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