INTEGRATED PORTABLE MICROCHIP SYSTEM FOR RAPID FORENSIC STR TYPING

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Microfabrication technology enables the production of dense microfluidic circuits on a single wafer, allowing rapid, low-volume, and reliable capillary electrophoretic (CE) analyses in a highly parallel fashion. Combining microfabricated capillary electrophoresis (μCAE) technology with the high discriminatory power of DNA short tandem repeat (STR) fingerprinting is poised to advance the field of forensic human identification. We have demonstrated successful STR typing using a 96-lane μCAE system on samples conventionally prepared using the PowerPlex® 16 and AmpFℓSTR® Profiler Plus® kits.[1] The μCAE system can also be integrated with on-chip sample preparation steps such as PCR[2] and sample pre-concentration[3] to develop completely integrated STR sample preparation and analysis systems. Such fully integrated and fast STR typing devices would be particularly useful for point-of-care clinical diagnostics as well as criminal investigation where rapid on-site identification is demanded.

To address this goal, we have developed a portable forensic analysis system consisting of a microfluidic device for amplification and separation of STR fragments together with an instrument that contains 4-color confocal laser fluorescence detection and all the necessary optical and electronic components for chip operation. The integrated microchip includes 160-nL PCR reactors, pneumatic valve control for fluidic manipulation, microfabricated heaters and resistance temperature detectors (RTDs), and 7-cm long microchannels for CE separation. We have explored the forensic applications of this portable system by performing 4-plex mini-Y chromosome STR typing consisting of a sex-typing marker, amelogenin, and three Y-STR loci (DYS390, DYS393 and DYS439) with a size range of 106–191 bp. The integrated PCR-CE microchip produced rapid thermal cycling and DNA separation in only 90 min. We determined the minimum amount of genomic DNA required for full typing using 0, 20, 30 and 50 copies of male standard DNA. All the amplicons can be detected down to 20 copies of template DNA. We also evaluated the performance of the system in resolving male alleles in the presence of female DNA using standard male and female DNA mixed at ratios 1:1, 1:5 and 1:10. A balanced profile can be obtained for all four loci at a ratio of 1:10. To demonstrate the capabilities of the PCR-CE system to perform real-world forensic analysis we also successfully typed human bone and oral swab extracts from case evidence previously processed and analyzed by the Palm Beach County Sheriff's Office.[4] Our demonstration of successful STR analyses performed on this portable PCR-

CE system validates the concept of point-of-analysis DNA typing in a mass disaster or at a security check point.

We are also working on the development of improved reagents and methods for forensic identification. A multiplex PCR system using energy-transfer (ET) cassette labeled primers[5] for the loci used in the PowerPlex® 16 kit is being constructed. The expected sensitivity improvements range from 2-fold for the FAM-labeled loci to 6-fold for the TAMRA-labeled loci; these improvements should enable amplification from lower copy number (LCN) samples or with fewer PCR cycles. Even more advanced, integrated sample cleanup systems are also in development employing a linear acrylamide gel conjugated with oligos designed to capture specific STR products in an amplified sample.[3] This gel-phase capture approach permits the allelic products to be normalized and desalted for effective electrophoretic analysis. We expect this approach to be particularly useful for LCN typing.

The application of improved reagents and methods together with the integrated PCR-CE system further accelerates the development of fully automated low-volume sample preparation systems for low-cost, high-throughput and reliable forensic DNA analysis.

References

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