MICROCHIP

Microchip Capillary Electrophoresis: Progress Toward an Integrated Forensic Analysis System

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The integration of PCR with capillary electrophoresis on a microchip is undeniably a significant step toward a total integrated forensic analysis system.

INTRODUCTION

Integration of capillary electrophoresis (CE) onto a microchip for forensic short tandem repeat (STR) analysis is the first critical step to produce a fully integrated and automated STR analysis system. Microchip capillary array electrophoresis (µCAE) analyzers provide rapid high-throughput separation of forensic samples and can increase workflow and reduce costs (1). The microchip CE format is also important because it facilitates electrophoretic analysis of submicroliter to nanoliter sample volumes. This low-volume analysis capability facilitates integration of PCR on the microchip, which will further increase automation, improve reliability and reduce operator intervention (2,3). Ultimately such PCR-CE technology also should be integrated with DNA extraction, STR sample cleanup and desalting (4) to make a fully integrated forensic analysis system for both high-throughput work and point-of-analysis applications. The goal of this review is to describe the current capabilities of microchip CE technology and point the way to the future.

MICROCHIP CAPILLARY ELECTROPHORESIS

The advent of microchip-based CE separations of DNA can be traced back more than a decade to a number of laboratories engaged in the effort (5,6). These microchips consist of a glass wafer that has been chemically etched through a photolithographic pattern to define the injection and separation channels. The etched wafer is then bonded to a second wafer containing drilled holes to provide fluidic access to the channels (Figure 1). The transition from conventional glass capillary systems used in DNA sequencing to an etched glass plate demanded that obstacles be surmounted, including the development of 1) reliable cross-injection designs and methods on capillary chips, 2) new separation matrices that provide single-base resolution and are easily pumped into the microchip channels, and 3) novel turn geometries to increase capillary length with no loss in resolution. Such improvements allowed the development of dense microfluidic circuitry while keeping the microchip similar in size to a compact disc (1).

Work in the Mathies' lab at the University of California, Berkeley, and at the Virginia Department of Forensic Science (VDFS) has demonstrated that this microchip system, together with the rotary confocal scanner developed by Scherer *et al.* at Berkeley, produces rapid reliable state-of-the-art forensic analyses. The fast heat dissipation enabled by the high surface-to-volume ratios of microCE (µCE) channels allows high-voltage separation of the nanoliter DNA sample plug. By pairing this virtue with a high-performance sieving matrix, such as linear polyacrylamide (LPA), rapid 20-minute CE with single-base resolution can be achieved. Yeung *et al.* (7) accurately profiled nonprobative and mock forensic samples in <30 minutes using a 96-capillary µCAE device (Figure 2). Data generated by Yeung *et al.* were comparable in quality to commercial CE systems. Moreover, a similar system was successfully

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implemented at the VDFS as a collaborative effort between the Mathies' lab at UC Berkeley, VDFS and Palm Beach County Sheriff's Office (manuscript submitted).

MICROCHIP PCR

The versatility of PCR in genetic analysis has attracted interest in miniaturization and integration with microchip CE analysis for applications ranging from genotyping for disease diagnostics to forensic DNA profiling. Standalone microchip PCR reactors were initially demonstrated in stationary fluid cycling (8,9) and continuous-flow systems (10,11). To fully realize the potential of microchip PCR, however, it must be integrated with other upstream or downstream analysis steps, such as CE. The first demonstration of coupling microchip PCR with µCE analysis was performed in the Mathies' lab in 1996 (2). This was followed by incorporation of polydimethylsiloxane (PDMS) membrane pneumatically actuated valves and vents for fluidic control and a 280 nl PCR chamber to achieve 20 PCR cycles in only 10 minutes (3; Figure 3, Panel A). This integrated format was recently scaled to multiple reactors on the same chip and applied to genotyping, infectious disease detection and expression monitoring (12). The capabilities of rapid thermal cycling and electrophoresis as a result of fast heat dissipation were critical to shortening analysis time. The precise positioning of tiny heating elements and sensors on a microchip makes temperature control and monitoring more accurate. More importantly, the nanoliter PCR reactor reduces the consumption of expensive PCR reagents, decreasing cost while minimizing pipetting errors between the two steps. The integration of PCR with CE is undeniably a significant step toward a total integrated forensic analysis system.

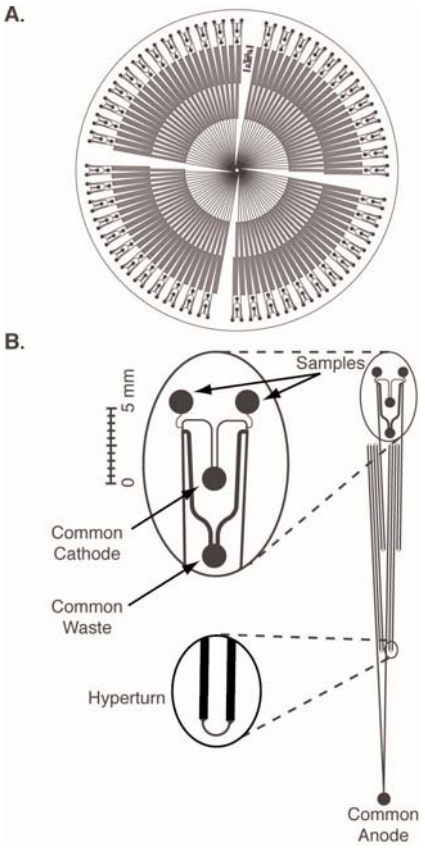

Figure 1. Panel A. Schematic of the 96-channel microfabricated capillary array electrophoresis chip. **Panel B.** Each doublet includes an injector with two sample wells that share common cathode and waste wells. The sample plug is formed at the intersection of the sample and waste arms and electrophoretically migrates toward the central anode, where it is detected by the confocal fluorescence scanner.

PORTABLE ANALYSIS SYSTEMS

There is increasing interest in portable point-of-analysis forensic STR typing systems for military, antiterrorism and mass disaster applications as well as limited crime scene processing (13,14). Toward this end, Liu and co-workers demonstrated STR typing of forensic DNA samples on a portable briefcase-sized device (Figure 3, Panels B and C) that integrates PCR, CE, and fluorescence excitation and detection (13). This system produces a multiplex Y-STR DNA profile from a sample in only 1.5 hours. Figure 4 presents STR results from a real-time demonstration of integrated microchip PCR-CE on a benchtop detection unit at the National Institute of Justice's Grantees' conference in July of 2006, where a DNA sample was profiled during the poster session (15).

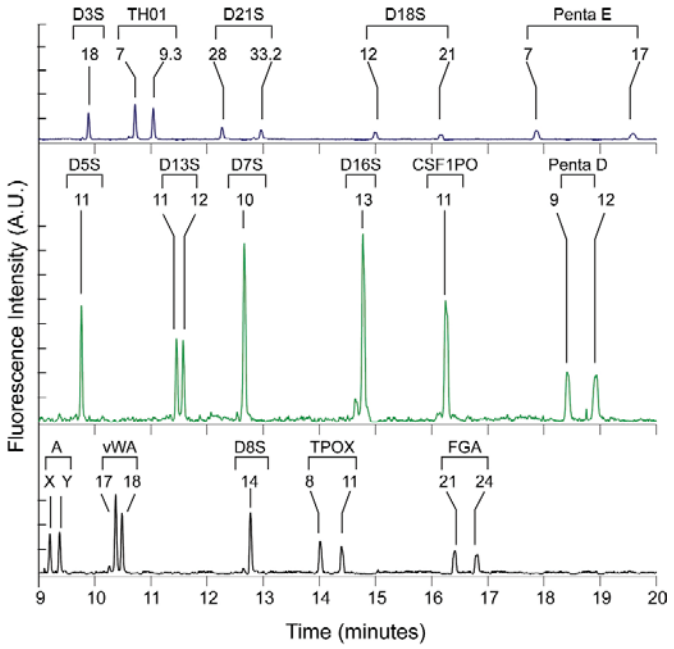

Figure 2. Nonprobative sexual assault casework sample profile generated with the µCAE. PowerPlex® 16 electropherogram showing results from the sperm fraction.

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FUTURE DIRECTIONS

What do these technological advances mean for the future of forensic DNA profiling? Rapid, integrated sample analysis systems may create a flexible, dynamic and much more active role for the forensic laboratory. Reaping the greatest benefit requires the concomitant pairing of microchip technologies and expert systems for extremely rapid sample profiling.

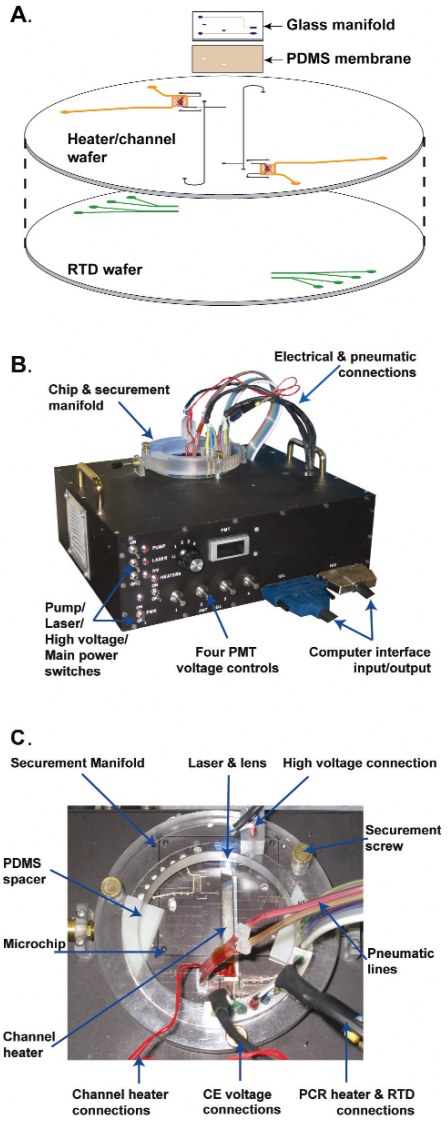

Figure 3. Panel A. Design of the integrated PCR-CE microchip. **Panel B.** Photograph of the portable PCR-CE system. The analysis system has dimensions of 12 × 10 × 4 inches. **Panel C.** Close-up of the microchip and manifold.

Ultimately we envision a microchip system that incorporates DNA extraction from a raw sample, as well as improved processes for STR product cleanup and concentration normalization. Development is in progress to integrate microchip CE with affinity gel capture-based PCR sample cleanup, as recently demonstrated for sequencing (16). This may facilitate extremely sensitive, low-copy-number (LCN) profiling, eliminating the need for increased PCR cycle number while reducing the incidence of contamination, leading to higher rates of success for samples with <100 pg of DNA (17). Moreover, research is also being conducted on STR profiling using plastic chips, furthering efforts to produce commercially viable microchip systems (18). New separation polymers, such as the thermally controlled "viscosity switching" polymers, may facilitate mobile microchip systems by removing the requirement for high-pressure loading while providing a viscous medium for high-resolution fragment separation (19).

CONCLUSION

While an integrated or modular microchip system capable of rapid DNA extraction, amplification, normalization, fragment separation and data analysis will not relieve the ever-present bottlenecks of evidence examination, presumptive testing, report writing and peer review for complex samples, it may produce an automated system that can seamlessly and rapidly perform DNA analysis tasks. This will greatly reduce turnaround times and backlogs, and enhance forensic capacity without increasing cost and staff requirements.

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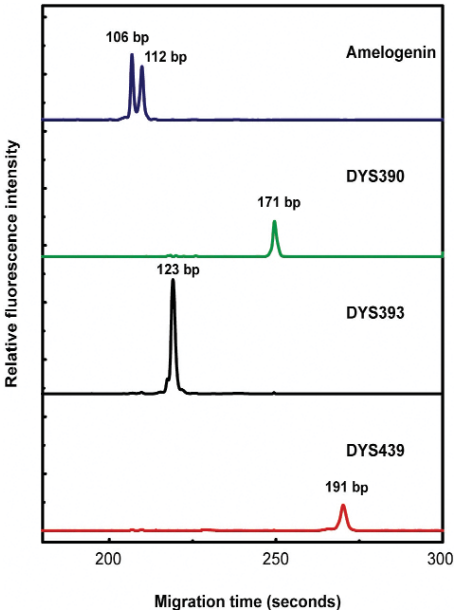

Figure 4. A full 9948 male standard DNA profile obtained from 50 template copies with 35 PCR cycles on the portable PCR-CE integrated system. Presented at the Seventh Annual DNA Grantees' Workshop in Washington, D.C., on June 27, 2006.

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