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Rapid and High-Throughput Forensic Short Tandem Repeat Typing Using a 96-Lane Microfabricated Capillary Array Electrophoresis Microdevice*

ABSTRACT: A 96-channel microfabricated capillary array electrophoresis (μCAE) device was evaluated for forensic short tandem repeat (STR) typing using PowerPlex 16® and AmpFℓSTR® Profiler Plus® multiplex PCR systems. The high-throughput μCAE system produced high-speed <30-min parallel sample separations with single-base resolution. Forty-eight previously analyzed single-source samples were accurately typed, as confirmed on an ABI Prism 310 and/or the Hitachi FMBIO II. Minor alleles in 3:1 mixture samples containing female and male DNA were reliably typed as well. The instrument produced full profiles from sample DNA down to 0.17 ng, a threshold similar to that found for the ABI 310. Seventeen nonprobative samples from various evidentiary biological stains were also correctly typed. The successful application of the μCAE device to actual forensic STR typing samples is a significant step toward the development of a completely integrated STR analysis microdevice.

KEYWORDS: forensic science, short tandem repeat typing, forensic identification, capillary electrophoresis, capillary array electrophoresis, PowerPlex, AmpFℓSTR® Profiler Plus®, microfabrication

Short tandem repeat (STR) typing is a powerful tool for forensic identification due to the unique profile it generates for an individual as a result of the highly polymorphic nature of these markers (1–4). Polymerase chain reaction (PCR)-based STR analysis followed by electrophoretic separation of the amplified products allows simultaneous amplification and analysis of multiple loci and is the gold standard for human identification. The significant role of DNA profiling in criminal investigations has resulted in an increased backlog of casework DNA samples in forensic laboratories both nationally and internationally. Unfortunately, the current STR analysis process is time-consuming and laborious and the technologies do not provide the capability for fast, large-scale, low-volume integrated sample processing at low cost. To address these obstacles, there is an immediate need for next-generation genetic analysis devices that can perform high-speed, high-throughput, and sensitive STR analyses.

Microfabricated capillary electrophoresis (CE) devices provide many advantages that can benefit forensic STR analyses. In these devices, photolithographic masking and wet chemical etching are used to fabricate CE channels in glass wafer sandwiches (5). The precise, nanoliter sample-plug size defined by the dimensions of a microfabricated CE channel together with the high electrophoretic field provide rapid, high-quality separations. Fast separations of STR amplicons for human identification have been demonstrated on microchip CE devices with up to 16 lanes (6). High-speed DNA sequencing and fragment sizing have been demonstrated on microfabricated capillary array electrophoresis (μCAE) devices containing from 96 to 384 lanes (7–10). In addition, nanoliter reactors can be readily integrated with these devices for PCR (11–13), sample extraction, and cleanup. (14,15). While these microtechnologies have been primarily developed for genome sequencing and diagnostics, they should be well suited for forensic STR typing as well.

In this study, we explore the use of a 96-channel μCAE device coupled to a four-color confocal fluorescence scanning system for high-performance STR typing using commercially available multiplex systems. The STR amplicons are separated by the μCAE system in less than 30 min with single-base resolution on 96 CE channels simultaneously. The allele calls obtained on the μCAE system for single-source samples are examined for concordance with the expected results and with those performed in the ABI Prism 310. The μCAE system is also tested to determine the minimum amount of genomic DNA required for full typing and to explore its ability to identify minor alleles in mixtures. Finally, 17 nonprobative samples from various sources, including blood, semen, and saliva stains, were examined for verification on real-world samples. This successful study demonstrates the power of μCAE technology for high-performance forensic STR human identification.

Materials and Methods

DNA Sample Preparation

All DNA extracts were prepared for amplification at the Palm Beach County Sheriff's Office (PBSO) Serology/DNA Section. The stock DNA extracts used for the single-source population, sensitivity, and mixture ratio studies were generously provided by the National Institute of Standards and Technology (NIST). PBSO

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used the BioMek2000 robotic workstation (Beckman-Coulter, Fullerton, CA) to perform reconstitution of all the NIST lyophilized DNA samples. The BioMek2000 was also used to perform dilution series for the sensitivity studies. *Sensitivity study*: GT37778 stock DNA, a Hispanic database sample obtained from NIST Population Study database, was serially diluted (22, 11, 5.5, 2.75, 1.38, 0.69, 0.34, 0.17, 0.08, 0.043, 0.021, 0.011, and 0.0054 ng/μL) in a specially formulated TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) buffer.

Mixture and single-source study—Lyophilized female:male mixture samples at ratios of 10:0, 9:1, 3:1, 3:2, 2:3, 1:3, 1:9, and 0:10 were reconstituted to a total concentration of 2 ng/μL with sterile water. Previously characterized population DNA extracts were re-quantified from lyophilized samples to an average of 1 ng/μL (0.75–1.5 ng/μL) for single source studies.

Nonprobative samples—PBSO DNA extracts from adjudicated forensic casework previously analyzed using the Hitachi FMBIO II allele detection instrument (MiraiBio, Alameda, CA) were analyzed on the μCAE system using the same DNA template concentration as in the original casework analysis. Sample sources from five cases included one semen (both sperm and nonsperm fractions), nine saliva, four blood, and two mixed blood stains previously extracted with either organic extraction or DNA IQ™ (Promega Corporation, Madison, WI). All samples were quantified using Quantiblot (Applied Biosystems, Foster City, CA) with Hitachi CCDBIO (Hitachi, Alameda, CA) signal detection, as reported previously (16).

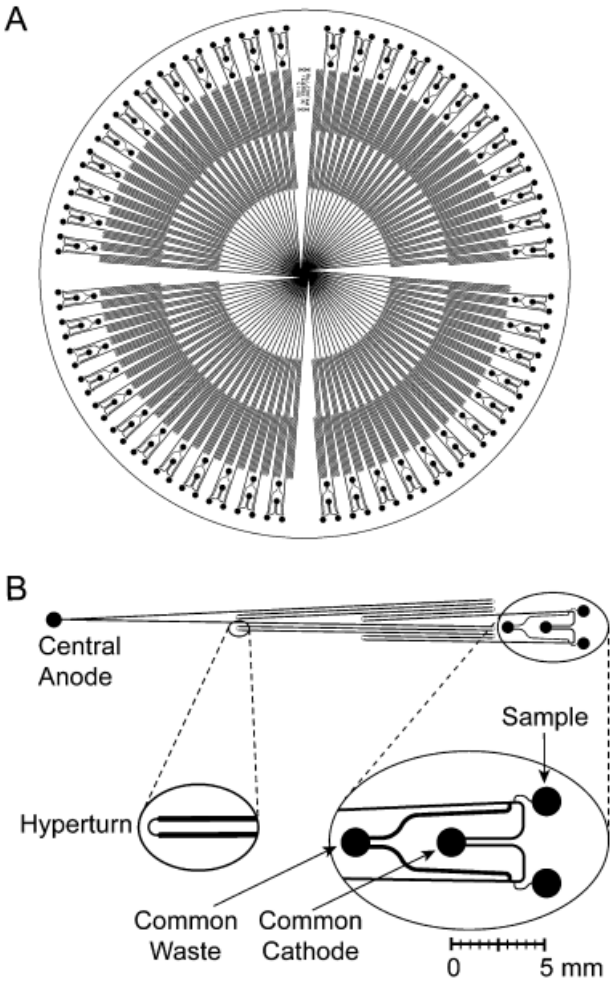

FIG. 1—*Design of the 96-channel microfabricated capillary array electrophoresis chip. The 96 adjacent lanes are grouped into 48 doublet structures on a 150-mm diameter glass wafer. (B) Each doublet structure includes an injector with two sample wells that share common cathode and waste wells. The sample plug is formed at the intersection of the sample arm and waste arm and migrates down the gel-filled serpentine channel through the hyper-turns toward the central anode.*

PCR Amplification

All PCR samples were prepared and amplified at PBSO. DNA samples were amplified using the GenePrint PowerPlex® 16 System (Promega Corporation) and the Profiler Plus® STR (Applied Biosystems) following the manufacturer's recommendations (16–18). DNA from 0.5 to 1 ng was used in the amplification reactions except for the sensitivity and mixture samples, which used 1 μL of samples at the previously noted concentrations. Aliquots of the amplified products were shipped overnight to University of California, Berkeley laboratory and the Virginia Department of Forensic Science (VDFS) for electrophoresis on the μCAE device and the ABI 310, respectively.

μCAE Fabrication and Design

The microdevices were fabricated on 150-mm diameter Borofloat glass wafers (Schott, Yonkers, NY) using published procedures (19). The design of the device is presented in Fig. 1 (8). Briefly, the 96 adjacent lanes are paired into a doublet structure and arranged radially around the central anode. Each doublet contains two sample wells, which share common cathode and waste wells. After photolithography, all features were isotropically etched to a depth of 25 μm with hydrofluoric acid. The postetch width is 85 μm for the arm from the sample to the separation

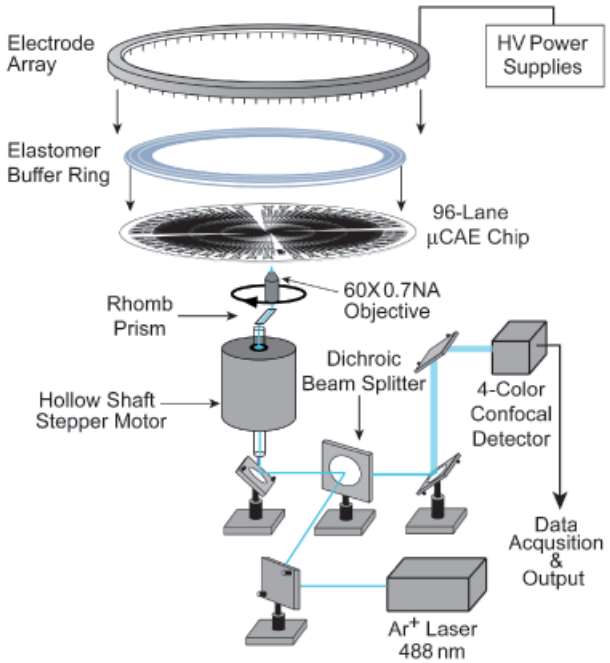

FIG. 2—*The Berkeley rotary confocal fluorescence scanning system. A 488-nm argon ion laser beam is passed through a dichroic beam splitter and directed up the hollow shaft of a stepper motor. The beam is deflected 1 cm off the axis of rotation by a rhomb prism and focused by a 60 × objective on the microfabricated capillary array electrophoresis (μCAE) channels. The collected fluorescence travels back along the same path into a four-color confocal detector. A polydimethlysiloxane (PDMS) elastomer ring on top of the μCAE plate is used to create a continuous buffer reservoir for the cathode and waste wells. An electrode array is used to supply voltage to various reservoirs.*

channel, 300 μm from the waste to the separation channel, and 200 μm for the separation channel connecting the cathode and the central anode. The fluidic wells were diamond-drilled into the etched wafers using a CNC mill. The wafer was then cleaned and thermally bonded to a blank wafer to create a closed channel sandwich structure. The microchannels were coated according to a modified Hjerten procedure (20).

μCAE Analysis

Each PowerPlex® 16 amplified PCR sample (1 μL) was mixed with 1 μL of Internal Lane Standard (ILS) 600 in 6 μL of 50% formamide (Sigma, St. Louis, MO) (1:1 deionized formamide solution in distilled deionized water). The PowerPlex® 16 allelic ladder (1.5 μL) was mixed with 1 μL of the ILS 600 in 4.5 μL of 50% formamide. For the Profiler Plus® amplified samples, 1 μL of sample was mixed with 0.8 μL of GeneScan-500 ROX and 6.2 μL of 50% formamide while 4.5 μL of the allelic ladder was mixed with 1.2 μL of GeneScan 500 Rox and 3.3 μL of 50% formamide. The mixed samples were denatured at 95°C for 3 min and quickly chilled on ice before loading. Each sample was loaded into two or three lanes for each run, and at least two–three allelic ladder samples were run in parallel lanes.

μCAE Operation

The 96 lanes of the μCAE device were first filled with Long Read linear polyacrylamide (Amersham Biosciences, Piscataway, NJ) simultaneously from the central anode well until the gel fills the sample, cathode, and waste wells using a high-pressure filling station (21). The excessive gel was evacuated from the sample wells and replaced with sample solutions. An electrode-array ring was placed in the sample wells to supply voltage. A polydimethlysiloxane (PDMS) elastomer ring was secured on top of the cathode and waste wells to create continuous buffer reservoirs. These reservoirs and the central anode well were filled with 5× TTE (250 mM Tris/250 mM TAPS/5 mM EDTA, pH 8.3). The assembly was placed on the Berkeley rotary confocal fluorescence scanner for detection. The preparation time was approximately 20 min.

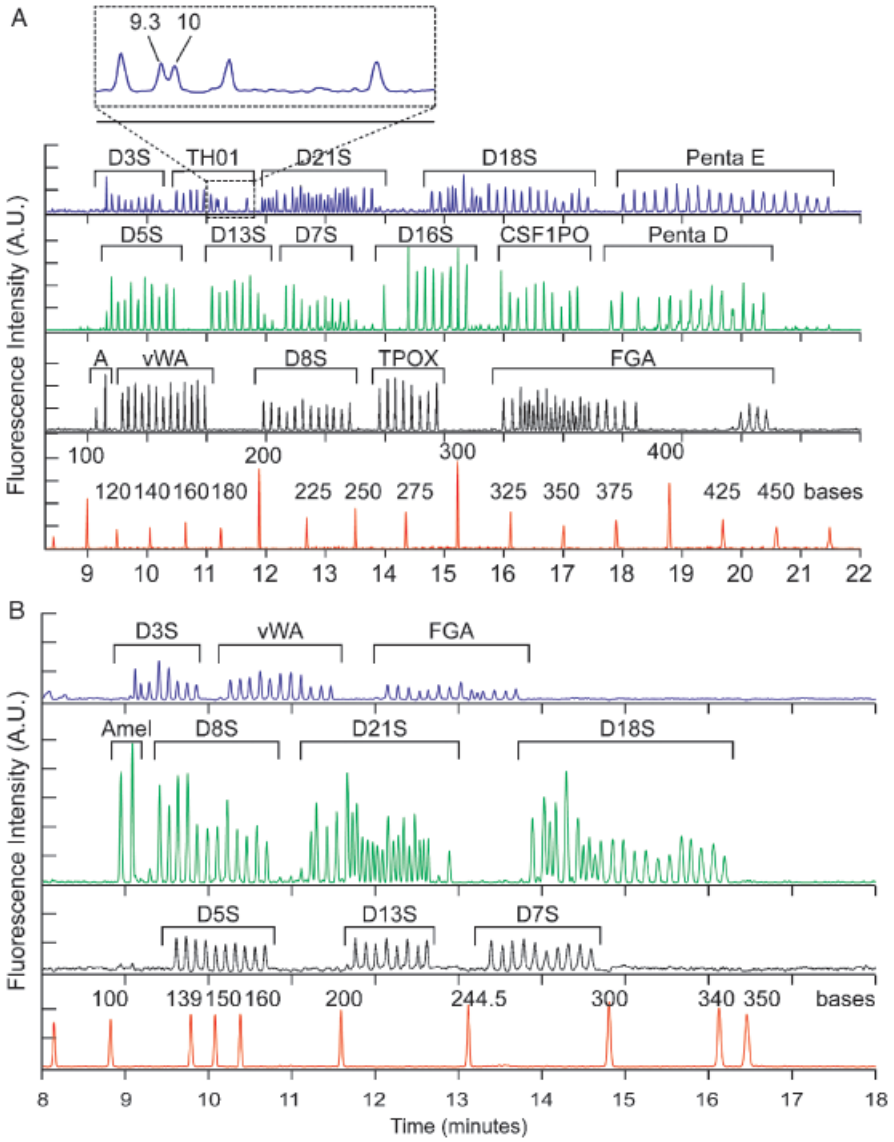

FIG. 3—*Processed traces of (A) the PowerPlex® 16 allelic ladder mix and (B) the ABI AmpFℓSTR® Profiler Plus® allelic ladder mix obtained on the microfabricated capillary array electrophoresis (μCAE) system.*

The Berkeley four-color rotary confocal scanner has previously been described in detail (22). A schematic of the system is presented in Fig. 2. Briefly, an Ar^+ laser beam is first passed through a dichroic beam splitter and directed up the hollow shaft of a stepper motor. The beam is displaced 1 cm off the axis of rotation by a rhomb prism and focused on the microchannels by a 60 × objective. The collected fluorescence travels back along the same path, passes through the dichroic beam splitter, and enters a four-color confocal detector consisting of a series of beamsplitters, filters, and four photo-multiplier tube (PMT) detectors.

To perform a separation, the stage was heated up to 67°C and equilibrated for 2 min. Electrophoresis was initiated with electrokinetic injection of the samples toward the waste wells at 170 V while grounding the sample wells and floating the cathode and the central anode wells. The PowerPlex® 16 samples were injected for 65 sec and the Profiler Plus® samples for 45 sec. Separation was achieved by grounding the cathode, applying 2500 V to the anode, 180 V to the sample, and 200 V to the waste. The resulting electric field is 150 V/cm in the main separation channel. The 1 nL-injected sample plug travels down the 15.9 cm serpentine channel, which contains four hyperturns to minimize band broadening (23). After each run, the sieving matrix was cleared from the microchannels and washed with deionized water using the high-pressure loading apparatus.

Data Acquisition and Analysis

The four-color fluorescence data were first converted to binary format and appended with proper header information by a custom LabView program (National Instruments, Austin, TX). The pre-processed data files were then input for allele calling using the MegaBACE Fragment Profiler 1.2 (Amersham Biosciences) where they underwent baseline fitting and color cross-talk correction. Independent data analysis was performed at the VDFS and PBSO in order to validate the μCAE generated Fragment Profiler data and allele calls.

ABI 310 Analysis at VDFS

All amplified samples were covered in foil and stored at − 20°C until use. Samples amplified using the PowerPlex® 16 System and the AmpFℓSTR® Profiler Plus® were prepared for CE following the manufacturer's recommendations (16,17) with minor modifications. For PowerPlex 16® samples, 1.0 μL of the amplified reaction was mixed with 1.0 μL ILS 600 and 12 μL of HiDi Formamide. For the Profiler Plus® samples, 1.5 μL of amplified reaction was mixed with 1.0 μL GeneScan-500 ROX and 24 μL of HiDi Formamide. The samples were heated at 95°C for 2–3 min and snap-cooled on ice for 2–3 min before injection. All

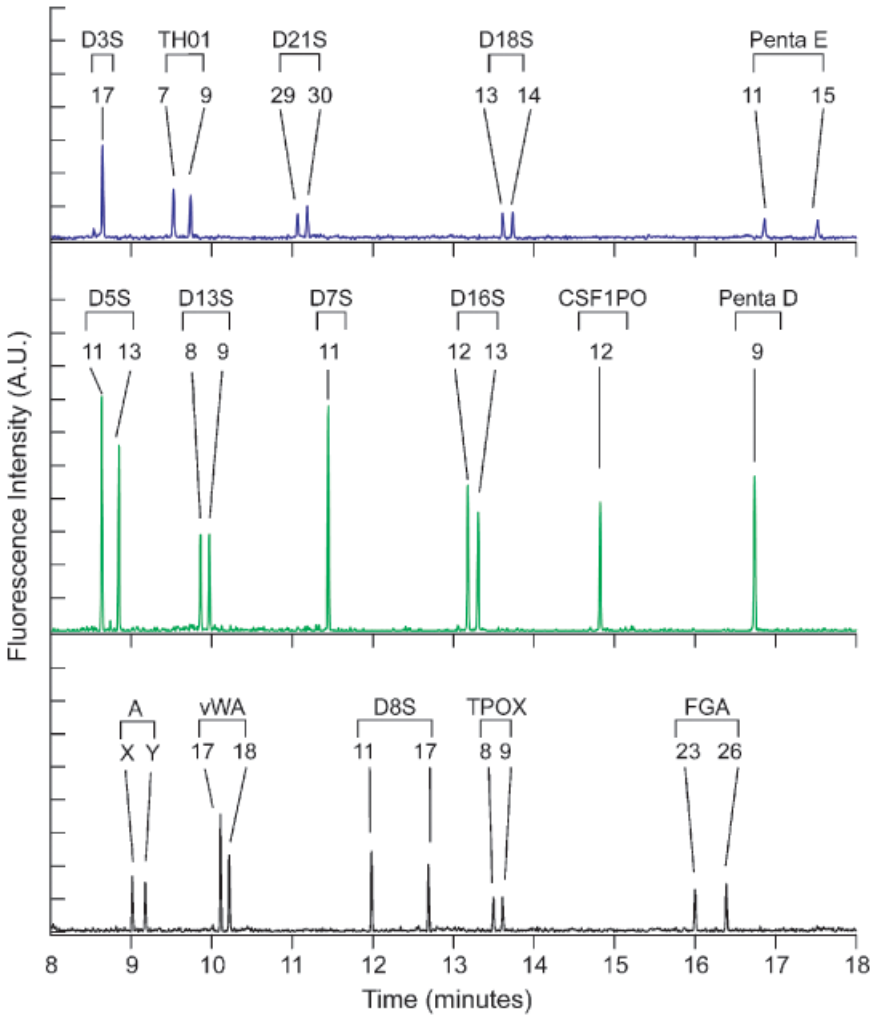

FIG. 4—*Processed PowerPlex® 16 profile of a National Institute of Standards and Technology (NIST) population sample OT06795 obtained on the microfabricated capillary array electrophoresis (μCAE) system using 1 ng of DNA template.*

the injection parameters followed exactly the respective manufacturer's recommendation, except the Profiler Plus® samples, which underwent a 5-sec injection. The electrophoresis was complete in 34 min for the PowerPlex® 16 samples and in 30 min for the Profiler Plus® samples.

Data collection was performed using the GeneScan® Data Collection v. 2.1 and Analysis v. 3.1 software programs with the recommended parameters and the Light Smooth Options. Allele typing was performed with the Genotyper® software program utilizing the PowerTyper™ 16 Macro for the PowerPlex® 16 samples and the AmpFℓSTR® Profiler Plus® Macro for the Profiler Plus® samples.

Results and Discussion

We explore here the capability of a 96-lane μCAE microdevice for forensic identification. To perform forensic genotyping, the μCAE device must accurately and reproducibly distinguish alleles that differ by a single base. The device also must discern the alleles of minor contributor(s) in a mixed DNA sample, detect amplicons from low-level DNA samples, and reliably execute STR typing with actual casework samples. We have assessed the ability of the μCAE to meet each of these criteria using the PowerPlex® 16 and Profiler Plus® multiplex systems on a variety of typical samples encountered in working forensic laboratories.

Resolution, Speed, and Concordance

Figure 3 illustrates electrophoretic separations of the PowerPlex® 16 and Profiler Plus® allelic ladders, which were achieved in 28 and 20 min, respectively, on the 96-channel μCAE device. The TH01 9.3 allele is a common microvariant that is 1 bp smaller than the TH01 10 allele. The resolution obtained based on these two alleles is 0.76 (Rb = 1.3), which favorably compares with resolutions reported using commercial STR typing kits with the ABI 310 (24). The 2-bp D18S51 allelic ladder microvariants, as well as those in other loci, were also successfully resolved. These results indicate the high resolution with which STR alleles can be

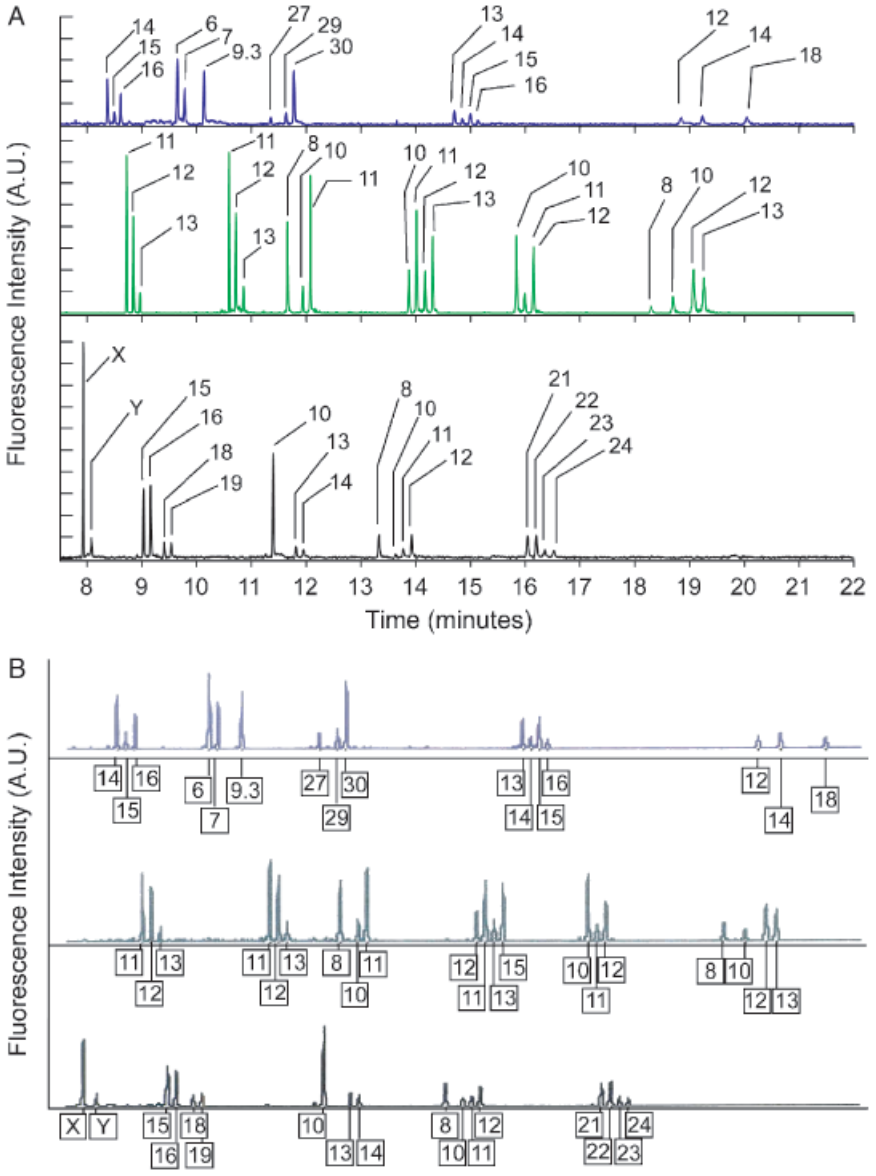

FIG. 5—*(A) PowerPlex® 16 profile of a National Institute of Standards and Technology (NIST) 3:1 female-to-male mixture sample obtained with a total DNA concentration 2 ng/μL. (B) PowerPlex® 16 profile of the same mixture sample obtained on the ABI PRISM 310 Genetic Analyzer.*

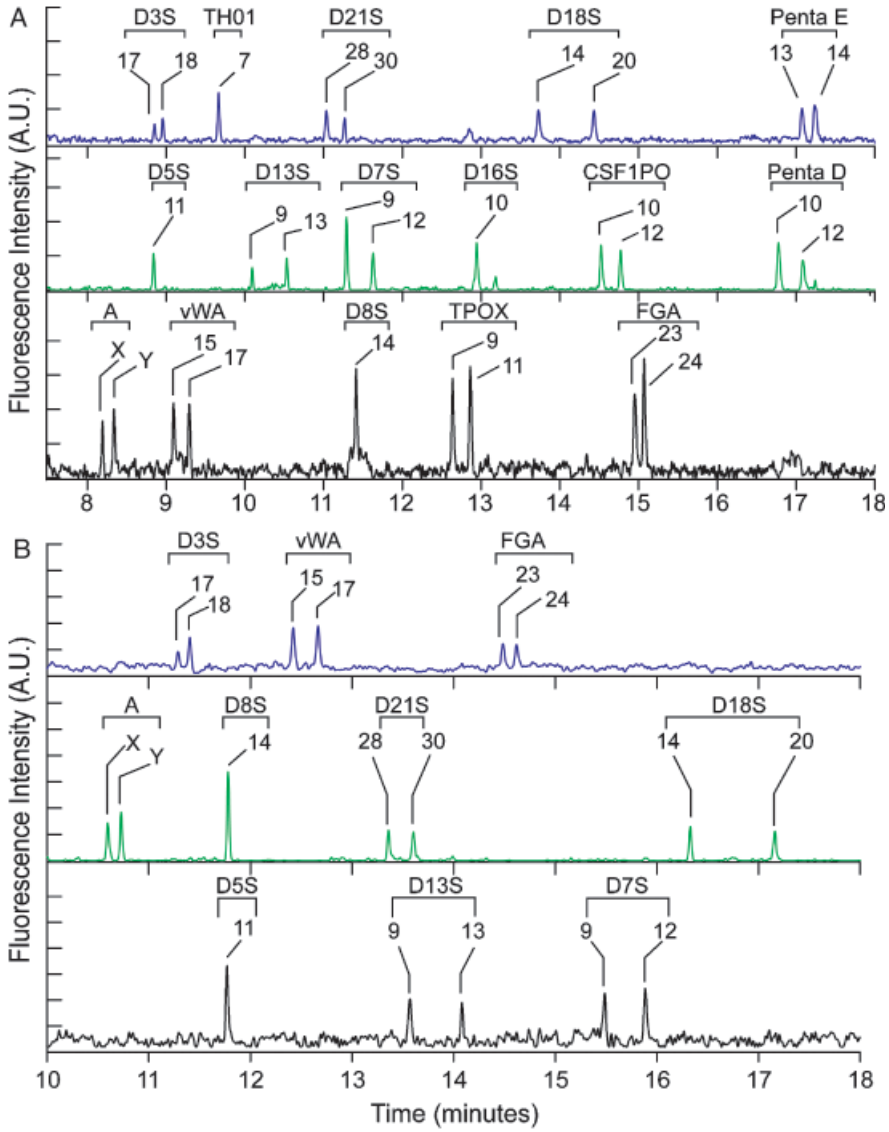

FIG. 6—*Profile from a 0.17 ng sample amplified using (A) A PowerPlex® 16 and (B) Profiler Plus® on the microfabricated capillary array electrophoresis (μCAE) system.*

resolved and sized using the μCAE system. This 96-sample separation system is at least 10 min faster than the single-capillary ABI 310 and 30 min faster than the 16-capillary 3100 instruments. Single-source population samples (28 Hispanic, 3 Asian, and 17 African American) amplified with the PowerPlex® 16 system were analyzed on the μCAE system and showed consistent allele calls for all microsatellites and microvariants compared with the expected results on Short Tandem Repeat DNA Internet Database (STRbase) (25) and the ABI 310 (26). Figure 4 shows processed DNA data as an example of a typical single-source sample DNA profile that will commonly be seen in database work. This analysis demonstrates the capability of the μCAE system to perform high-volume, parallel STR sample analyses on a rapid time scale.

Mixture Study

It is not unusual to detect a mixture of DNA profiles in an evidentiary biological stain during forensic casework typing. The performance and reliability of the μCAE system in resolving mixtures were evaluated and compared with the ABI 310 with PowerPlex® 16 samples consisting of female and male DNA at ratios of 10:0, 9:1, 3:1, 3:2, 2:3, 1:3, 1:9, and 0:10. The 3:1 and 1:3 samples are the lowest ratios in which all minor components were successfully detected and typed. An example of a 3:1 mixture profile is shown in Fig. 5*A*. The μCAE analysis presents very similar allele signal ratios as compared with the profile obtained on the ABI 310 (Fig. 5*B*). This study demonstrates that the μCAE system has a similar capacity to detect the minor alleles as the currently available commercial CE instruments for forensic typing.

Sensitivity Study

The sensitivity of the μCAE system was assessed using PowerPlex® 16 and Profiler Plus® samples amplified with serially diluted DNA templates (22, 11, 5.5, 2.75, 1.38, 0.69, 0.34, 0.17, 0.08, 0.043, 0.021, 0.011, and 0.0054 ng). Figure 6 presents traces showing the successful detection of all the STR alleles from the 0.17 ng amplified DNA samples using the μCAE system. The signal-to-noise (*S*/*N*) ratios are above the limit of detection (defined as $S/N \geq 3$) for all loci and all allele calls are correct. The *S*/*N* ratios of the PowerPlex® 16 and Profiler Plus® loci for the 0.17 ng sample are shown in Figs. 7*A* and 8*A*, calculated as the averaged

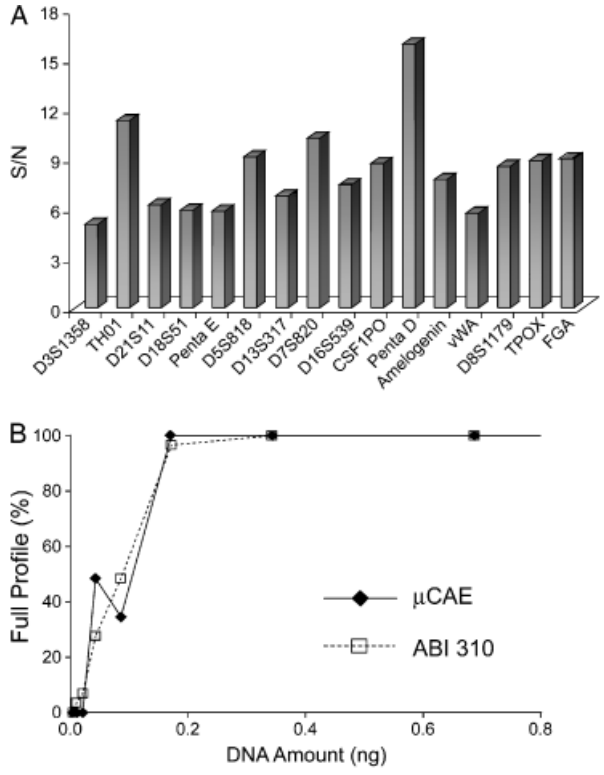

FIG. 7—*(A) Signal-to-noise (S/N) ratios for the PowerPlex® 16 loci obtained for the 0.17 ng sample. (B) Percent of full PowerPlex® 16 profile obtained on both the microfabricated capillary array electrophoresis (μCAE) system and the ABI 310 as a function of input genomic DNA.*

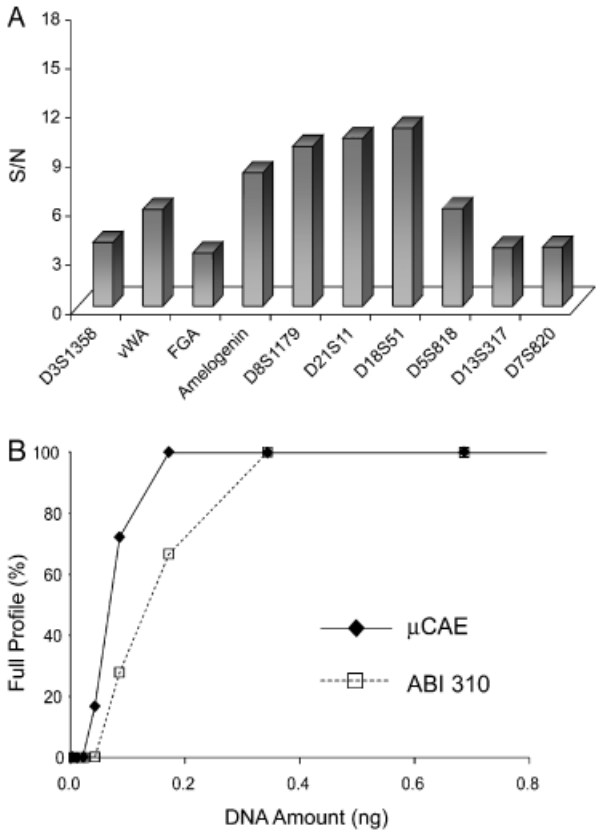

FIG. 8—*(A) Signal-to-noise (S/N) ratios for the Profiler Plus® loci obtained for the 0.17-ng sample. (B) Percent of full Profiler Plus® profile obtained on both the microfabricated capillary array electrophoresis (μCAE) system and the ABI 310 as a function of input genomic DNA.*

S/N of the two alleles for the heterozygous loci with $S/N \geq 3$ for both alleles. The μCAE system allele call results agreed with the STRbase results and those obtained on the ABI 310. In addition, full profiles for both multiplex systems were generated by the μCAE system. Figures 7*B* and 8*B* show that the μCAE was able to yield 100% profiles for the PowerPlex® 16 and the Profiler Plus® samples down to 0.17 ng of DNA, respectively. At 0.17 ng of DNA, 96% of the PowerPlex® 16 profiles and 67% of Profiler Plus® profiles were fully typed by the ABI 310. These observations were made applying a 150-rfu threshold to the allele calling process on the ABI 310; at lower thresholds, additional alleles would be labeled. There is no threshold yet defined for the prototype μCAE instrument in forensic science; thus the *S/N* ratio is the only standard that can be applied. Although the signal threshold standards applied to the μCAE and ABI 310 were different, the μCAE exhibited a sensitivity similar to the ABI 310.

Nonprobative Study

The μCAE system must also be able to perform analyses on real-world forensic casework samples. We have explored these capabilities of the μCAE device by typing 17 nonprobative DNA samples from case evidence previously processed and analyzed by PBSO using both the PowerPlex 16® and Profiler Plus® systems. The DNA samples were extracted from a variety of common sources encountered in forensic analysis, including semen, saliva, single, and mixed blood stains from sexual assault, paternity, burglary, armed robbery as well as homicide cases. The DNA profile results generated on the μCAE system and ABI 310 were compared with the originally reported DNA profiles generated using the Hitachi FMBIO II allele detection system.

The DNA data obtained using the μCAE system for the less complicated paternity and single-source bloodstain case samples were compared with those generated on the ABI 310 and those originally reported. Of the three cases examined, all produced full profiles that matched with the previously reported case results (26). More complicated DNA samples from sexual assault and bloodstain mixtures were also analyzed on the μCAE system. For the sperm-fraction obtained from the vaginal-swab semen in a sexual assault, the original report analysis indicated that the DNA profile did not match the suspect's DNA profile. Both the μCAE and ABI 310 analyses resulted in the same conclusion. However, the μCAE system was able to discern additional alleles in the nonsperm fraction of the semen stain, consistent with the semen contributor that were not callable previously using the FMBIO II. No alleles were detected that were foreign to either the victim or the suspect by the μCAE system or ABI 310 analysis. The higher molecular weight fragments such as Penta E and Penta D, however, were much weaker than the original results due most likely to the degradation following the initial analyses in 2002.

In a second case, DNA results from a bloodstain mixture collected from a knife blade and another from a sandal in a homicide indicated the presence of at least two individuals. The detection of DNA mixtures in such forensic samples is usually one of the most challenging types of STR data to interpret (27). The original report stated that a DNA profile was obtained for all 16 genetic markers, with both the victim and the suspect included as possible contributors to these mixtures. The μCAE system analyses generated data that were identical to the original report as well as to the ABI 310 analyses even at loci with imbalanced peak heights, indicating major and minor contributors. The μCAE system likewise showed

these peak height variations. The full concordance of the μCAE data with the previously analyzed casework data, and the ability to detect minor alleles in complex casework samples showcase the ability of the μCAE system to perform analyses on a variety of probative samples.

In summary, the combination of the μCAE genetic analysis system and the high discriminating power of STR typing presents a powerful new tool for human identification. With the increasing number of samples submitted for DNA testing in addition to the escalating casework backlogs, the ability of the μCAE device to process rapidly a collection of samples in parallel is particularly important to forensic throughput. Furthermore, the μCAE device is amenable to integration with other small-volume sample preparation steps. As the final volume of the amplicons analyzed is only on the scale of 1 nL, representing only 1/250,000 of the amplified PCR products used, the incorporation of a small-volume PCR reactor and sample cleanup functionalities should lead to high-throughput integrated STR analysis systems that are more cost-effective, efficient, and reliable with reduced labor and manual processing and reagent use (28). While the focus of this work has been on the development of a high-throughput automated analyzer for forensic lab use, the full utilization of microfabrication technology should also lead to fully integrated portable STR analysis devices (29,30) for on-site crime scene, mass disaster, and real-time forensic identification.

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References

1. Hammond HA, Jin L, Zhong Y, Caskey CT, Chakraborty R. Evaluation of 13 short tandem repeat loci for use in personal identification applications. Am J Hum Genet 1994;55:175–89.
2. Andersen JF, Greenhalgh MJ, Butler HR, Kilpatrick SR, Piercy RC, Way KA, et al. Further validation of a multiplex STR system for use in routine forensic identity testing. Forensic Sci Int 1996;78:47–64.
3. Chakraborty R, Stivers DN, Su B, Zhong YX, Budowle B. The utility of short tandem repeat loci beyond human identification: implications for development of new DNA typing systems. Electrophoresis 1999;20:1682–96.
4. Butler JM. Forensic STR typing: biology, technology and genetics of STR markers. 2nd ed. New York: Elsevier; 2005.
5. Woolley AT, Sensabaugh GF, Mathies RA. High-speed DNA genotyping using microfabricated capillary array electrophoresis chips. Anal Chem 1997;69:2181–6.
6. Goedecke N, McKenna B, El-Difrawy S, Carey L, Matsudaira P, Ehrlich D. A high-performance multilane microdevice system designed for the DNA forensics laboratory. Electrophoresis 2004;25:1678–86.
7. Simpson PC, Roach D, Woolley AT, Thorsen T, Johnston R, Sensabaugh GF, et al. High-throughput genetic analysis using microfabricated 96-sample capillary array electrophoresis microplates. Proc Natl Acad Sci USA 1998;95:2256–61.
8. Paegel BM, Emrich CA, Weyemayer GJ, Scherer JR, Mathies RA. High throughput DNA sequencing with a microfabricated 96-lane capillary array electrophoresis bioprocessor. Proc Natl Acad Sci USA 2002;99:574–9.
9. Medintz IL, Berti L, Emrich CA, Tom J, Scherer JR, Mathies RA. Genotyping energy-transfer-cassette-labeled short-tandem-repeat amplicons with capillary array electrophoresis microchannel plates. Clin Chem 2001;47:1614–21.
10. Emrich CA, Tian HJ, Medintz IL, Mathies RA. Microfabricated 384-lane capillary array electrophoresis bioanalyzer for ultrahigh-throughput genetic analysis. Anal Chem 2002;74:5076–83.
11. Burns MA, Johnson BN, Brahmasandra SN, Handique K, Webster JR, Krishnan M, et al. An integrated nanoliter DNA analysis device. Science 1998;282:484–7.
12. Lagally ET, Simpson PC, Mathies RA. Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system. Sensors Actuator B-Chem 2000;63:138–46.
13. Roper MG, Easley CJ, Landers JP. Advances in polymerase chain reaction on microfluidic chips. Anal Chem 2005;77:3887–94.
14. Wolfe KA, Breadmore MC, Ferrance JP, Power ME, Conroy JF, Norris PM, et al. Toward a microchip-based solid-phase extraction method for isolation of nucleic acids. Electrophoresis 2002;23:727–33.
15. Paegel BM, Yeung SHI, Mathies RA. Microchip bioprocessor for integrated nanovolume sample purification and DNA sequencing. Anal Chem 2002;74:5092–8.
16. Applied Biosystems. AmpFℓSTR® Profiler Plus® PCR amplification kit user's manual. Foster City, CA: Applied Biosystems; 2000.
17. Promega Corporation. PowerPlex® 16 System technical manual No. D012. Madison, WI: Promega; 2002.
18. Krenke BE, Tereba A, Anderson SJ, Buel E, Culhane S, Finis CJ, et al. Validation of a 16-locus fluorescent multiplex system. J Forensic Sci 2002;47:773–85.
19. Simpson PC, Wolley AT, Mathies RA. Microfabrication technology for the reduction of capillary array electrophoresis chip. Biomed Microdevices 1998;1:7–26.
20. Hjerten S. High-performance electrophoresis—elimination of electroendosmosis and solute adsorption. J Chromatogr 1985;347:191–8.
21. Scherer JR, Paegel BM, Wedemayer GJ, Emrich CA, Lo J, Medintz IL, et al. High-pressure gel loader for capillary array electrophoresis microchannel plates. Biotechniques 2001;31:1150–4.
22. Shi YN, Simpson PC, Scherer JR, Wexler D, Skibola C, Smith MT, et al. Radial capillary array electrophoresis microplate and scanner for high-performance nucleic acid analysis. Anal Chem 1999;71:5354–61.
23. Paegel BM, Hutt LD, Simpson PC, Mathies RA. Turn geometry for minimizing band broadening in microfabricated capillary electrophoresis channels. Anal Chem 2000;72:3030–7.
24. Buel E, LaFountain M, Schwartz M, Walkinshaw M. Evaluation of capillary electrophoresis performance through resolution measurements. J Forensic Sci 2001;46:341–5.
25. http://www.cstl.nist.gov/biotech/strbase
26. http://www.cchem.berkeley.edu/ramgrp/str_sup
27. Kline MC, Duewer DL, Redman JW, Butler JM. NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. Anal Chem 2003;75:2463–9.
28. Paegel BM, Blazej RG, Mathies RA. Microfluidic devices for DNA sequencing: sample preparation and electrophoretic analysis. Curr Opin Biotechnol 2003;14:42–50.
29. Lagally ET, Scherer JR, Blazej RG, Toriello NM, Diep BA, Ramchandani M, et al. Integrated portable genetic analysis microsystem for pathogen/infectious disease detection. Anal Chem 2004;76:3162–70.
30. Skelley AM, Scherer JR, Aubrey AD, Grover WH, Ivester RHC, Ehrenfreund P, et al. Development and evaluation of a microdevice for amino acid biomarker detection and analysis on Mars. Proc Natl Acad Sci USA 2005;102:1041–6.

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