Rapid Determination of Monozygous Twinning with a Microfabricated Capillary Array Electrophoresis Genetic-Analysis Device†

Stephanie H. I. Yeung,1‡ Igor L. Medintz,2‡ Susan A. Greenspoon,3 and Richard A. Mathies1,4*  
1 UCSF/UCB Joint Graduate Group in Bioengineering, University of California, Berkeley, CA; 2 Center for Bio/Molecular Science and Engineering, Code 6900, US Naval Research Laboratory, Washington, DC; 3 Virginia Department of Forensic Science, DNA Unit, Richmond, VA; 4 Department of Chemistry, University of California, Berkeley, CA; * address correspondence to this author at: Department of Chemistry, University of California, Berkeley, CA 94720. e-mail Rich@zinc.cchem.berkeley.edu. † Points of view expressed in this document are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. ‡ S.H.I.Y. and I.L.M. contributed equally to this work.

BACKGROUND: Microfabricated genetic-analysis devices have great potential for delivering complex clinical diagnostic technology to the point of care. As a demonstration of the potential of these devices, we used a microfabricated capillary array electrophoresis (µCAE) instrument to rapidly characterize the family and genotypic relationship of twins who had been assigned fraternal (dizygous) status at birth.

METHODS: We extracted the genomic DNA from buccal samples collected from the twin sons, the parents, another sibling, and an unrelated control individual. We then carried out multiplex PCR amplification of sequences at 16 short tandem repeat loci commonly used in forensic identity testing. We simultaneously separated the amplicons from all of the individuals on a µCAE device and fluorescently detected the amplicons with single-base resolution in <30 min.

RESULTS: The genotypic analysis confirmed the identical status of the twins and revealed, in conjunction with the medical data, that their twin status arose from the rarer dichorionic, diamniotic process.

CONCLUSIONS: The ability to rapidly analyze complex genetic samples with µCAE devices demonstrates that this approach can help meet the growing need for rapid genetics-based diagnostics.

Lab-on-a-chip devices have the potential to improve healthcare across the globe by bringing rapid, specific diagnostic capabilities to remote areas (1) and by helping to substantially reduce costs and speed up diagnostics at the point of care (2). These benefits are attributable to the characteristics of such devices, which include drastically reduced instrumental costs, assay costs, and reagent usage; disposability; the elimination of sample-collection, transportation, and storage issues; automation; simplified sample processing; and dramatically increased assay speed and sample throughput (3, 4). All of these features can empower both the patient and the healthcare provider in even the most remote locations (1). Other applications envisioned for these devices include real-time forensic analysis at security and crime scenes (2), monitoring of microbial contagions in food and water (5), and analyses in highly challenging environments, such as in planetary exploration (6). Although the reliability of the microfluidics and integration characteristics of these devices requires further development, it is highly likely that electrophoretic separation of PCR-based DNA products will play a substantial role in such applications (3, 7). This is because, in the case of genetic analysis, many samples consist of DNA oligonucleotides with highly repetitive sequences and because the time-consuming (i.e., >24 h) and instrument-intensive multistep processes are required when chip-based hybridization arrays are used for analyses. Examples of repetitive sequences not easily analyzed with microarrays include short tandem repeats (STRs) and the numerous differentially labeled amplicons produced in a multiplex PCR analysis.

Microfabricated capillary array electrophoresis (µCAE) analytical devices provide inherent advantages for many types of complex separation-based analyses. These advantages include high-throughput DNA sequencing and capabilities for analyzing single base pairs, multicolor fluorescence detection, requirements for only minute quantities of sample and reagents, and a small device footprint. The capabilities of µCAE devices have been demonstrated in rapid (approximately 30 min) high-quality genomic and mitochondrial DNA sequencing analyses (1-bp resolution with 99% accuracy) of 96 samples simultaneously (8, 9). The facile scaling capability of these devices was illustrated in the typing of single-stranded conformation polymorphisms for 384 bladder cancer samples and the mutational screening of 384 samples for hereditary hemochromatosis–related single-nucleotide polymorphisms in <6 min (>1 sample/s throughput) (10). The demonstration of the advantages of µCAE devices for high-speed/high-throughput STR typing (11) has led to the adoption of this approach to improve DNA-typing efficiency and forensic identification in crime laboratories (4). In the present study, we have used µCAE analysis with a commercial STR DNA
fragment–typing system to rapidly characterize the genetic status of twin siblings who were thought at birth to be fraternal. The large amount of data we generated in this study epitomizes the complex types of rapid genetic-analysis and diagnostic capabilities of these devices when fully implemented.

At the Naval Research Laboratory, we collected buccal cells on sterile cotton swabs from the twin siblings, a daughter, the parents, and an unrelated individual. All samples were collected with informed consent, and all applicable institutional regulations on human samples were observed. DNA was extracted and quantified with the DNA IQ System and the AluQuant Human DNA Quantification System (Promega), respectively, by the Virginia Department of Forensic Science. Sample DNA was amplified with the GenePrint PowerPlex® 16 System (Promega) in accordance with the manufacturer’s instructions. The PCR reaction mixture (1 μL) was mixed with 1 μL of Promega Internal Lane Standard 600 in 6 μL of 500 mL/L formamide in deionized water (Applied Biosystems), denatured at 95 °C for 3 min, and loaded onto 3 parallel microchannel lanes.

The design and fabrication of the μCAE device (see Fig. 1 in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol54/issue6) have been described extensively (8, 11). The 96 adjacent

Fig. 1. Separation of PCR amplicons with the μCAE device and analysis of results.
Presented are 16-locus profiles for the father (A), the mother (B), and the twin siblings (C, D) generated on the 96-channel μCAE system.
lanes are grouped into 48 doublets and arranged radially around the central anode on a Borofloat glass wafer (Schott) 150 mm in diameter. Each doublet contains 2 sample wells that share common cathode and waste wells. Microchannels were coated with poly-N-hydroxyethylacrylamide (polyDuramide) in a dynamic coating procedure (12). A polydimethylsiloxane-elastomer ring was secured on top of the cathode and waste wells to create continuous buffer reservoirs. Microchannels were simultaneously filled with Long Read Matrix (GE Healthcare/Amersham Biosciences) from the central anode via a high-pressure filling station (13). Reservoirs and the central anode wells were filled with 5× TTE buffer (250 mmol/L Tris, 250 mmol/L TAPS, 5 mmol/L EDTA, pH 8.3). An electrode-array ring placed in the sample wells supplied the voltage. The assembly was placed on the stage of a Berkeley rotary confocal fluorescence scanner (14), with assembly and stage preheated to 67 °C for separation and detection. Samples were electrokinetically injected for 45 s at 170 V and separated in an electric field of approximately 150 V/cm. The 1 nL of injected sample plug traveled down the 15.9-cm serpentine channel, and the scanner interrogated the sample via laser-induced fluorescence.

We used a custom LabVIEW program (National Instruments) to convert 4-color fluorescence data appended with appropriate header information to binary format and sized the alleles with the MegaBACE Fragment Profiler 1.2 (GE Healthcare/Amersham Biosciences). The data were corrected for baseline and color cross-talk with the aid of a matrix generated with BaseFinder 6.1.16 (the source of free software can be found at http://bioinfo.unc.edu/glabsoftware/BaseFinder/index.html).

At birth, the twin siblings had presented with 2 placentae (chorions) and 2 amniotic sacs, which led to the immediate medical characterization of the twins as dizygotic and thus fraternal. As the 2 boys grew older, however, their almost indistinguishable appearance strongly suggested that they might be identical, or monozygous, twins. We used the commercial PowerPlex 16 STR-analysis kit used to establish sibling status. This method interrogates the sex-typing loci AMELY [amelogenin (amelogenesis imperfecta 1, X-linked)] and AMELX (amelogenin, Y-linked), 2 pentanucleotide-repeat loci (Penta D and Penta E), and the Federal Bureau of Investigation’s 13 Combined DNA Index System (CODIS) STR loci. When applied to identity testing, the probability of a random match with this method is approximately $1 \times 10^{-12}$ (15). For determining paternity with the same loci, the combined paternity index and the power of exclusion exceed 500 000 and 0.999998, respectively (16), indicating the informative power of using these loci

---

**Table 1. Genotypes for the 16 tested amelogenin and STR loci.**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Father</th>
<th>Mother</th>
<th>Son</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>D8S1179</td>
<td>15.2</td>
<td>16.1</td>
<td>16.1</td>
<td>11.2</td>
</tr>
<tr>
<td>D7S820</td>
<td>12.1</td>
<td>14.1</td>
<td>14.1</td>
<td>11.1</td>
</tr>
<tr>
<td>D13S317</td>
<td>13.1</td>
<td>15.1</td>
<td>15.1</td>
<td>12.1</td>
</tr>
<tr>
<td>D5S818</td>
<td>14.1</td>
<td>16.1</td>
<td>16.1</td>
<td>13.1</td>
</tr>
<tr>
<td>D16S539</td>
<td>15.1</td>
<td>17.1</td>
<td>17.1</td>
<td>14.1</td>
</tr>
<tr>
<td>D7S820</td>
<td>16.1</td>
<td>18.1</td>
<td>18.1</td>
<td>15.1</td>
</tr>
<tr>
<td>D13S317</td>
<td>17.1</td>
<td>19.1</td>
<td>19.1</td>
<td>16.1</td>
</tr>
<tr>
<td>D5S818</td>
<td>18.1</td>
<td>20.1</td>
<td>20.1</td>
<td>17.1</td>
</tr>
<tr>
<td>D16S539</td>
<td>19.1</td>
<td>21.1</td>
<td>21.1</td>
<td>18.1</td>
</tr>
</tbody>
</table>

---

*Chromosomal locations are given in parentheses.*
concurrently. Our analysis combines aspects of both forensic identity testing and paternity determination.

Fig. 1 shows the 16-locus profiles obtained for the father (Fig. 1A), the mother (Fig. 1B), and the twin siblings (Fig. 1, C and D). The profiles for the 2 siblings are matched at all 16 loci, indicating unequivocally that they are identical twins; the profiles also demonstrate perfect Mendelian inheritance from the parents (Table 1). The genotyping results also confirm the sibling relationship of the twins and their sister and the sister’s Mendelian inheritance from the parents. The derived sibling index (likelihood ratio of siblingship) for the twins and the daughter of >1500 confirms this relationship. The profile for the sample from the unrelated control individual clearly excludes any familial relationship with the other individuals. The high probabilities generated from testing the 15 unlinked STR loci make the results indisputable. The presence of 2 chorions and amnions at birth confirm that the twins are dichorionic/diamniotic identical twins arising from a single zygote, a relatively rare gestation process (occurring only in approximately 20% of identical twins). Most identical twins share a chorion but have distinct amnions, as opposed to the third, much rarer monochorionic/monoamniotic process, which occurs only in approximately 1% of cases of identical twins (17). True dizygous (fraternal) twins would still reflect inheritance of their parents’ genes but would not be matched across most loci, let alone be matched at these particular 16 loci simultaneously.

The demand for genetic testing and disease screening will continue to grow with our knowledge of more complex genetic phenomena (chimerism and multigene disorders), with our understanding of the genetic factors that underlie many diseases (including cancer and rheumatic diseases), and with the development of specific pharmacogenomic medications. The present analysis is primarily intended to demonstrate what can be accomplished with μCAE systems. The 6 profiles, which contain genetic information from 16 different loci and >100 ampiclons, were obtained simultaneously after <30 min of separation and approximately 10 min of postseparation analysis. The dramatic increases in sample throughput and the substantial decreases in analysis times that are possible with the full use of μCAE systems with 96 or 384 separation channels can permit the analysis of ≥1 locus/s, as in this 16-locus example. Clearly, the ability of the μCAE system to perform high-speed, highly parallel genetic analyses may be useful in growing numbers of applications in various areas, including forensics, medical diagnostics, and pathogen identification.

The fact that the reagents used in this analysis are derived from a commercially available multiplex STR-typing system commonly deployed in forensic identification obviates the development of a specific assay. The performance of this microfabricated separation system, including its precision, resolution and concordance, has been demonstrated to be similar to that of commercial capillary instruments in both routine and forensic DNA analyses (4). Other assays can be readily adapted to this technology to permit similarly rapid analyses (5, 18). Examples include screening for disease-related mutations (19), screening for pathogens in patient samples (18), performing multivariant analyses to identify pathogenic microorganisms (1), and monitoring cancer-related susceptibility markers (10). Furthermore, recent advances in microfluidics have showcased the possibility of integrating other nanoscale upstream sample-processing steps into a single lab-on-a-chip device, which would eliminate the potential for manual-transfer errors between steps and make DNA testing more streamlined, more reliable, far less expensive, and, ultimately, routinely available (5, 7, 20).

Grant/Funding Support: This study was supported by a grant (2004-DN-BX-K216) and a Graduate Research Fellowship from the National Institute of Justice, Office of Justice Programs, US Department of Justice, and by a Graduate Research and Education in Adaptive Bio-Technology Training (GREAT) award from the University of California Biotechnology Research and Education Program.

Financial Disclosures: R.A.M. has a financial interest in Microchip Biotechnologies, Inc., which is commercially developing aspects of the technologies described.


DOI: 10.1373/clinchem.2007.102319