Anal. Chem. **2009,** *81,* 210–217

Integrated Sample Cleanup−Capillary Electrophoresis Microchip for High-Performance Short Tandem Repeat Genetic Analysis

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An integrated PCR sample cleanup and preconcentration process is developed for forensic short tandem repeat (STR) analysis using a streptavidin-modified photopolymerized capture gel injector for microchip capillary electrophoresis (μCE). PCR samples generated with one biotinylated primer and one fluorescent primer provide the input to the streptavidin-based affinity capture-μCE device. Monoplex PCR samples processed by the device exhibited ~10- to 50-fold increased fluorescence intensities, and DNA profiles generated using 9-plex STR samples displayed ~14- to 19-fold higher signal intensities compared to those analyzed using traditional cross injection. Complete STR profiles were obtained with as few as 25 copies of DNA template using the capture-μCE device. Four DNA samples with various degrees of degradation were also tested. Samples analyzed using the capture-μCE device resulted in a significant increase of successful allele detection. The ability of our capture-μCE device and method to remove contaminating ions, to concentrate the sample injection plug, and to eliminate electrokinetic injection bias provides a powerful approach for integrating sample cleanup with DNA separation.

Microfabricated bioanalysis devices hold great promise for delivering rapid, high-throughput, low-cost, and even portable genetic analyses because of the small processing volume, facile scaling capability, and the ability to integrate multiple functions on a single device. These benefits are especially pertinent to the field of forensic DNA profiling where the demand for DNA evidence analysis is continuously increasing and contamination-free analysis is key to successful profile interpretation. The gold standard for DNA typing is the separation of PCR amplified short tandem repeat (STR) markers. Microchip-based capillary electrophoresis (μCE) systems have been demonstrated for fast and high-throughput STR typing [1−3] and on-chip integration with PCR has proven useful for real-time forensic DNA typing.[4−7] These microchip devices have typically employed a cross injector geometry,[8] which is a valuable approach for the analysis of nanoliter volume samples defined by the microchannel geometry. However, the injected sample in this geometry is only a tiny fraction (~1%) of the total sample produced, and the injection suffers from electrokinetic injection bias toward the small, high electrophoretic mobility ions and excess primers present in PCR reactions.[9,10] Although sample desalting methods, such as spin columns and ethanol precipitation, have been shown to improve DNA injection efficiency and hence peak signal strength and quality,[11−14] these methods are not amenable to integration within microfluidic structures.

A variety of on-chip concentration and separation methods have been explored for sensitivity enhancement,[15−17] including an oligonucleotide-based gel capture method for DNA sequencing [18,19] and genotyping.[20] Although these and similar approaches have laid the technological foundation for integrated DNA analysis, in the previous work the capture gel was synthesized off-chip,

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(1) Greenspoon, S. A.; Yeung, S. H. I.; Johnson, K.; Chu, E. M.; Rhee, H. N.; McGuckian, A. B.; Crouse, C. A.; Chiesl, T. N. *J. Forensic. Sci* **2008**, *53*, 1–10.

(2) Yeung, S. H. I.; Greenspoon, S. A.; McGuckian, A.; Crouse, C. A.; Emrich, C. A.; Ban, J.; Mathies, R. A. *J. Forensic Sci.* **2006**, *51*, 740–747.

(3) Goedecke, N.; McKenna, B.; El-Difrawy, S.; Carey, L.; Matsudaira, P.; Ehrlich, D. *Electrophoresis* **2004**, *25*, 1678–1686.

(4) Liu, P.; Yeung, S. H. I.; Crenshaw, K. A.; Crouse, C. A.; Scherer, J. R.; Matheis, R. A. *Forensic Sci. Int: Genet.* **2008**, *2*, 301–309.

(5) Liu, P.; Seo, T. S.; Beyor, N.; Shin, K. J.; Scherer, J. R.; Mathies, R. A. *Anal. Chem.* **2007**, *79*, 1881–1889.

(6) Horsman, K. M.; Bienvenue, J. M.; Blasier, K. R.; Landers, J. P. *J. Forensic Sci.* **2007**, *52*, 784–799.

(7) Easley, C. J.; Karlinsey, J. M.; Bienvenue, J. M.; Legendre, L. A.; Roper, M. G.; Feldman, S. H.; Hughes, M. A.; Hewlett, E. L. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 19272–19277.

(8) Harrison, D. J.; Fluri, K.; Seiler, K.; Fan, Z.; Effenhauser, C. S.; Manz, A. *Science* **1993**, *261*, 895–897.

(9) Huang, X.; Gordon, M. J.; Zare, R. N. *Anal. Chem.* **1988**, *60*, 375–377.

(10) Mohamadi, M. R.; Kataoka, M.; Mahmoudian, L.; Jabasini, M.; Shinohara, Y.; Baba, Y. *Chromatographia* **2005**, *61*, 339.

(11) Devaney, J. M.; Marino, M. A. *Capillary Electrophoresis of Nucleic Acids: Practical Applications of Capillary Electrophoresis*; Humana Press: NJ, 2001.

(12) McCord, B. R.; Jung, J. M.; Holleran, E. A. *J. Liq. Chrom. Relat. Technol.* **1993**, *16*, 1963–1981.

(13) Salas-Solano, O.; Ruiz-Martinez, M. C.; Carrilho, E.; Kotler, L.; Karger, B. L. *Anal. Chem.* **1998**, *70*, 1528–1535.

(14) Almira, E. C.; Panayotova, N.; Farmerie, W. G. *J Biomol. Technol.* **2003**, *14*, 270–277.

(15) Breadmore, M. C. *Electrophoresis* **2007**, *28*, 254–281.

(16) Guzman, N. A.; Blanc, T.; Phillips, T. M. *Electrophoresis* **2008**, *29*, 3259–3278.

(17) Chiesl, T. N.; Shi, W.; Barron, A. E. *Anal. Chem.* **2005**, *77*, 772–779.

10.1021/ac8018685 CCC: $40.75
Published on Web 12/05/2008

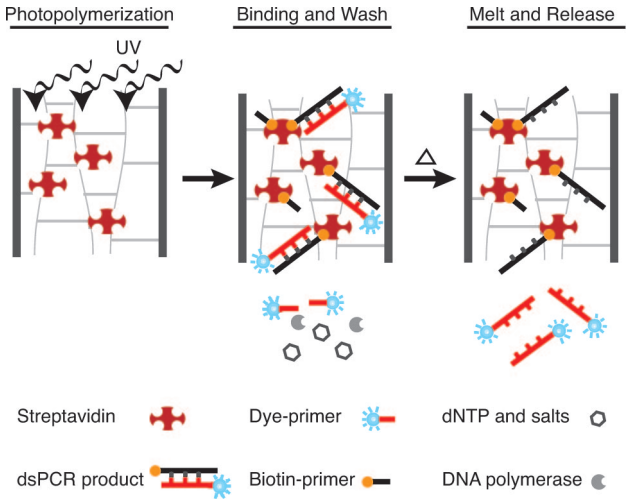

Figure 1. Schematic of the streptavidin-gel capture method for purifying and concentrating forensic PCR products. STR samples are generated with one primer labeled with fluorescent dye and one with biotin. The PCR reaction is electrophoresed through the photopolymerized cross-linked polyacrylamide gel network where the ds-DNA products bind strongly to the streptavidin-modified gel. Unbound PCR reactants are washed off, effecting purification, concentration, and desalting. The fluorescently labeled ss-DNA is then released for electrophoresis by melting the duplex DNA.

necessitating meticulous microchip design and gel loading procedures.[19] In addition, DNA concentration using the oligonucleotide-capture method relies on the hybridization between a known DNA sequence in the sample and the oligo immobilized in a gel matrix. Extension of this approach to the high-order multiplex PCR reactions used in STR typing can be complex because of the different amplicon sequences in the STR loci; moreover, a point mutation at the oligonucleotide binding site could result in the loss of that allele.

To address these issues, we have developed a simple and efficient affinity-based injector for integrated PCR sample cleanup, concentration, and injection which is based on photopolymerizable streptavidin-modified gel technology. Photoinitiated polymerization in conjunction with photolithographic patterning has been explored for a variety of microchip applications such as peptide separations.[21−23] Because of the precise definition produced by lithographic patterning, this approach makes it possible to create small, spatially confined affinity gels in desired channel regions. Furthermore, by taking advantage of the strong interaction between streptavidin and biotin ($K_D \approx 10^{-15}$ M), biotinylated DNA can be efficiently trapped by a streptavidin-modified gel in a manner that is independent of DNA sequence.[24] As illustrated in Figure 1, a cross-linked streptavidin-capture gel is immobilized in a microchannel utilizing a photoinitiated polymerization process. Biotin-labeled double-stranded (ds) DNA products generated by PCR using one biotin and one fluorescence-labeled primer are electrophoresed through the capture gel bed where they are bound efficiently via the biotin−streptavidin interaction. Unbound materials are washed off and the concentrated biotinylated dsDNA products are retained in the capture gel. The bound dsDNA is then melted, releasing the fluorescently labeled ssDNA for a high quality, quantitative electrophoretic injection.

In this study, we demonstrate that the streptavidin-capture process can be integrated with microchip CE to concentrate, cleanup, and desalt PCR products, followed by 100% efficient injection and electrophoresis. To evaluate the injection efficiency and the potential scalability of this capture method for forensic DNA profiling, we performed multiplex STR analyses at nine commonly used STR loci employed in the Federal Bureau of Investigation's Combined DNA Index System (CODIS) national database. The fluorescence signals of the DNA samples analyzed using the capture-μCE device were compared to those using cross injection. The limit of detection of the integrated method was also assessed for forensic applications. Degraded DNA analysis is one of the most challenging tasks in forensic STR typing because allelic dropout is prevalent and locus dropout is often observed for the larger STR markers. We tested the ability of the capture-μCE device to process and improve analyses of four degraded DNA samples. This study demonstrates the successful use of the integrated streptavidin-μCE process to eliminate the distortion from size-bias injection and to inject sample quantitatively for dramatic STR signal enhancement. This methodology addresses one of the most difficult problems associated with the direct CE analysis of low concentration forensic PCR products.

MATERIALS AND METHODS

DNA Sample Preparation. DNA standard samples 9947A, K562, and 9948 were purchased from a commercial vendor (Promega Corp., Madison, WI) and diluted to 0.2 ng/μL for all experiments except the sensitivity study, in which 9948 DNA was serially diluted (0.2, 0.13, 0.07, 0.03, 0.01 ng/μL) in deionized water. The Virginia Department of Forensic Science (VDFS) provided laboratory prepared degraded DNA samples extracted from bloodstains exposed to 56 or 80 °C for three months. These samples had been subsequently stored at room temperature in the dark for approximately 6 years prior to DNA extraction for this study. The blood stain extracts were prepared from 0.6-cm diameter punches using the Biomek 2000 Automation Workstation (Beckman Coulter, Fullerton, CA) with a Promega DNA IQ digestion, according the VDFS standard protocol.[25,26] The extracted DNA was quantified using the Plexor HY System (Promega) on the Stratagene Mx3005P (Stratagene, La Jolla, CA), according the manufacturer's protocol, except that the plate was set up on the Biomek 2000 Automation Workstation. DNA concentration information for these samples is listed in Table 1.

Short Tandem Repeat Typing. A FAM-labeled TH01 monoplex (10×), FAM-labeled TH01/D13 duplex (10×), and a 9-plex primer mix (10×) were prepared based on the primer sequences and fluorescence dye labeling scheme used in PowerPlex 16

(18) Paegel, B. M.; Blazej, R. G.; Mathies, R. A. *Curr. Opin. Biotechnol.* **2003**, *14*, 42–50.
(19) Blazej, R. G.; Kumaresan, P.; Cronier, S. A.; Mathies, R. A. *Anal. Chem.* **2007**, *79*, 4499–4506.
(20) Toriello, N. M.; Liu, C. N.; Mathies, R. A. *Anal. Chem.* **2006**, *78*, 7997–8003.
(21) Hatch, A. V.; Herr, A. E.; Throckmorton, D. J.; Brennan, J. S.; Singh, A. K. *Anal. Chem.* **2006**, *78*, 4976–4984.
(22) Throckmorton, D. J.; Shepodd, T. J.; Singh, A. K. *Anal. Chem.* **2002**, *74*, 784–789.
(23) Yu, C.; Svec, F.; Fréchet, J. M. J. *Electrophoresis* **2000**, *21*, 120–127.
(24) Dimsoski, P.; Woo, S. L. *Croat. Med. J.* **2005**, *46*, 619–621.
(25) Virginia Department of Forensic Science. *Forensic Science Manuals*; Available at: http://www.dfs.virginia.gov/manuals/manuals.cfm?id=5, 2008.
(26) Greenspoon, S. A.; Sykes, K. L. V.; Ban, J. D.; Pollard, A.; Baisden, M.; Farr, M.; Graham, N.; Collins, B. L. *Forensic Sci. Int.* **2006**, *164*, 240–248.

Table 1. Simulated Degraded DNA Samples[a]

DNA sample	degradation conditions (°C)	DNA extract (ng/μL)	input DNA (μL)	input DNA Amount (ng)	cross injection percent profile (%)	capture-μCE percent profile (%)
A1	56	0.14	5.0	0.700	46.7	100.0
A2			2.5	0.350	0.0	33.3
A3	80	0.00975	5.0	0.049	0.0	33.3
A4			2.5	0.024	0.0	6.7
B1	56	0.114	5.0	0.570	82.4	100.0
B2	80	0.261	5.0	1.305	82.4	100.0
B3			2.5	0.653	11.8	82.4

[a] Blood stains were incubated for 3 months in an oven at the temperatures indicated and kept at room temperature in the dark for 6 years prior to use for DNA extraction.

System (Promega) [27,28] except that unlabeled primers were replaced with biotin-labeled primers (Integrated DNA Technology, Coralville, IA). All primer mixes contained equimolar concentrations of the biotin and fluorescently labeled primers for each STR locus. The STR loci included in the automsomal 9-plex system are amelogenin for sex typing, vWA and D8S1179, D3S1358, TH01, D21S11, D5S818, D13S317, and D7S820.

For the resolution study, K562 DNA (0.5 ng) was amplified using the FAM-duplex to obtain 179 bp and 180 bp fragments. Measurements of resolution (R_s) were obtained using $R_s = [2(\ln 2)]^{1/2}(\Delta X)/(W_{h1} + W_{h2})$ where ΔX is the peak-to-peak distance, W_{h1} is the width at half-height of peak 1, and W_{h2} of peak 2. Valley value (Vv) was also obtained by dividing the height of the peak with greater signal by that of the valley (the point at which the two peaks merge) as described in Buel et al.[29] For the multiplex construction and cross-injection comparison, 9948 and 9947A DNA (0.5 ng) were used in the amplification reactions. For the sensitivity study, 2.5 μL of 9948 DNA was used at the previously noted concentrations to achieve input DNA amounts of 100, 75, 50, 25, and 10 copies (assuming 3 pg per copy). Monoplex and duplex PCR samples (25-μL) were prepared and amplified following the manufacturer recommendations for the PowerPlex 16 Monoplex (Promega) and the 9-plex samples following that for the PowerPlex 16 Multiplex Systems (Promega). Each degraded DNA sample was amplified using the 9-plex PCR primer mix with both 2.5 and 5 μL of input DNA at the concentration indicated in Table 1 in half the manufacturer's recommended volume for the PowerPlex16 kit.[26] All PCR samples were amplified using a PTC-200 thermocycler (MJ Research, Waltham, MA) according to the manufacturer recommended protocol for the PowerPlex16 kit.

All streptavidin-μCE studies used 3 μL of undiluted PCR reaction products. To compare with cross injection, diluted PCR samples (8-fold) were also prepared in 50% Hi-Di formamide/deionized water (Applied Biosystems, Foster City, CA) for runs on both the capture-μCE and the cross-injection devices. For cross injection, a PowerPlex 16 ILS 600 sizing standard (1 μL) was also included in the diluted samples[2] and heat denatured at 95 °C for 3 min prior to loading. Each sample was loaded into two or three independent microchannels for parallel analysis within the same run on the Berkeley four-color rotary scanner.[30] The allele patterns obtained using cross injection for all standard DNA samples and the intact templates for the laboratory prepared degraded samples were compared to the corresponding results in the capture-μCE studies to ensure accurate genotyping.

Microchip Fabrication and Design. The design of the 4-channel capture-μCE device is presented in Figure 2A. The capture device contains a 1-mm offset for the affinity gel while the offset in the cross injection device is reduced to 100 μm. Fabrication of the glass sandwich structures followed procedures previously described.[31] All features were isotropically etched to a depth of 40 μm and a width of 160 μm. The DNA plug generated in both devices travels down a separation channel consisting of two hyperturns[32] with a 10-cm effective separation length. Each processor can be operated individually or in parallel with the adjacent processor by electrically connecting their anode wells. To prevent electroosmotic flow (EOF), the microchannel surface was first coated with a modified Hjerten coating.[33]

Streptavidin Capture Gel Preparation. A 500-μL solution of 5% (v/v) acrylamide containing bis-acrylamide (19:1, Bio-Rad, Hercules, CA) in 1× TTE (500 mM Tris, 500 mM TAPS acid, and 100 mM EDTA) was sparged under N_2 gas for 10 min at room temperature in an opaque 2-mL scintillation vial with Teflon closure (National Scientific, Rockwood, TN). A solution containing 30 μL of 0.01% riboflavin (w/v), 1.25 μL of 50% TEMED (v/v), and 2 μg/μL of streptavidin-acrylamide (Invitrogen, Carlsbad, CA) reconstituted in deionized water (50 μL) was added into the sparged acrylamide solution using a needle. To form a capture gel, each microchannel was first aligned with a 900 μm square exposure window patterned on a 0.70-mm chrome photomask (Nanofilm, Westlake Village, CA) and secured on a vacuum stage set up on a Nikon inverted microscope (TE2000U). The prepared capture gel solution was loaded into the microchannels via capillary action. Excess solution was evacuated from each reservoir and replaced with a 2% hydroxypropomethyl cellulose solution (~10 μL) prepared in 1× TTE to prevent hydrodynamic flow. Polymerization was initiated by exposing the unmasked micro-

(27) Krenke, B. E.; Tereba, A.; Anderson, S. J.; Buel, E.; Culhane, S.; Finis, C. J.; Tomsey, C. S.; Zachetti, J. M. *J. Forensic Sci.* **2002**, *47*, 773–785.

(28) Yeung, S. H. I.; Seo, T. S.; Crouse, C. A.; Greenspoon, S. A.; Chiesl, T. N.; Ban, J. D.; Mathies, R. A. *Electrophoresis* **2008**, *29*, 2251–2259.

(29) Buel, E.; LaFountain, M.; Schwartz, M.; Walkinshaw, M. *J. Forensic Sci.* **2001**, *46*, 341–345.

(30) Shi, Y. N.; Simpson, P. C.; Scherer, J. R.; Wexler, D.; Skibola, C.; Smith, M. T.; Mathies, R. A. *Anal. Chem.* **1999**, *71*, 5354–5361.

(31) Simpson, P. C.; Woolley, A. T.; Mathies, R. A. *Biomed. Microdevices* **1998**, *1*, 7–26.

(32) Paegel, B. M.; Hutt, L. D.; Simpson, P. C.; Mathies, R. A. *Anal. Chem.* **2000**, *72*, 3030–3037.

(33) Hjerten, S. *J. Chromatogr.* **1985**, *347*, 191–198.

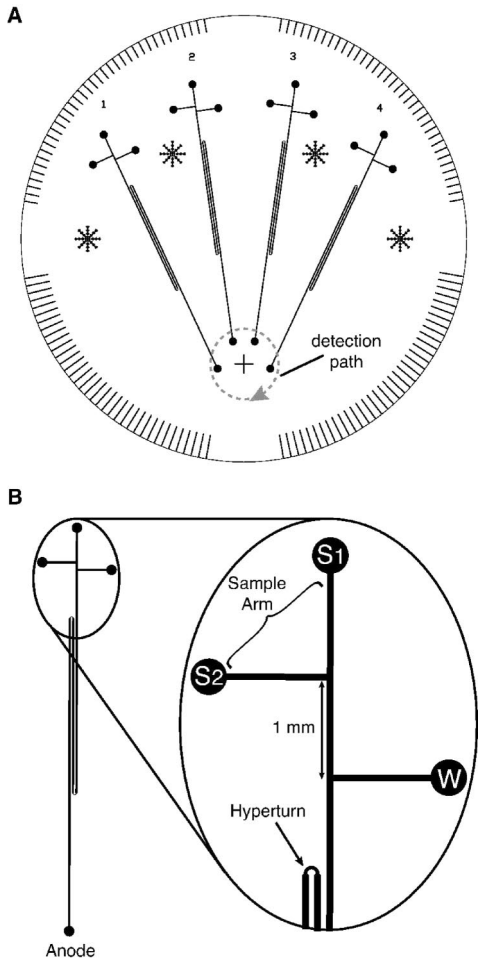

Figure 2. (A) Layout of the 4-channel capture-μCE processor array on a 100-mm diameter wafer. (B) Expanded view of one affinity capture-μCE processor, which contains two sample wells (S_1 and S_2), a waste well (W), and a 1-mm offset capture zone, in which a streptavidin-gel plug ($\sim$ 900 μm in length) is formed above a folded microchannel with a 10-cm effective separation length. A similar device with a 100-μm offset was used for the cross injection experiments.

channel regions with ultraviolet radiation (10 mW/cm^2) provided by a Hg arc lamp at 365 nm for 10 min. Unreacted solution was evacuated out of the channel and replaced with 1× TTE buffer.

Capture-μCE Device Operation. Following the formation of the capture gel plug, a 5% linear polyacrylamide (LPA) with 6 M urea DNA separation matrix was loaded from the anode well using a custom-built high-pressure loading station[34] until the gel filled the waste well. Buffer (1× TTE) was removed from both sample wells without emptying the sample arm and replaced manually with 1.5 μL of unpurified PCR sample, which made contact with the buffer in the sample arm and filled the entire arm without trapping air. Buffer (1× TTE) was loaded into the anode and waste wells. The loaded microdevice was equilibrated at 30 °C. An electrode-array ring was placed in sample well 1 (S_1) and waste well to supply voltage. Streptavidin capture was carried out by applying 25 V/cm between S_1 and the waste well for 10 min. A small electric field ($\sim$ 5 V/cm) was also applied between the anode and the waste wells to keep unbound materials from entering the separation column. Once complete, the sample was evacuated from S_1, S_2, and the sample arm and replaced with fresh 1× TTE. The bound product was electrokinetically washed for 5 min to remove any unbound materials in the capture gel using the same conditions as the capture step. The waste well, S_1, S_2, and sample arm were then replaced with Hi-Di formamide (Applied Biosystems, Foster City, CA). An electric field of 270 V/cm was applied between the S_1 and the waste well, and the denaturant moves into the capture region by diffusion. This step is performed to lower the thermal release temperature from 80 to 65 °C to avoid melting of separation matrix, and the induced ion depletion because of the applied electric field creates stacking of the DNA by isotachophoresis. The separation channel was washed to move any unbound materials toward the waste by replacing the contents of anode and waste wells with fresh 1× TTE and applying an electric field of 25 V/cm for 3.5 min. The region above the capture plug, the waste well, and anode were all replaced with 1× TTE, and the microdevice was equilibrated at 65 °C for $\sim$1 min to denature the fluorescently labeled DNA strands from their complementary captured strands. Separation was initiated by applying 250 V/cm between S_1 and the anode. The entire analysis process from DNA capture to separation required $\sim$40 min to complete. The microdevice was cleaned by pushing deionized water into the waste well to clear the capture gel plug followed by the removal of the separation matrix with deionized water using the same high-pressure apparatus. The microdevice was then ready for a new run without recoating. The physical removal of the capture gel plug, followed by the formation of a fresh new plug was designed to avoid carry over contaminations. The device lifetime is determined by physical failure and was empirically over one hundred usages.

Cross-Injection Device Operation. To set up cross-injection analysis, each μCE processor was first filled with a 5% LPA (6 M urea) separation matrix from the anode well until the gel filled all the wells using the high-pressure filling station. After evacuation of excess LPA, sample was loaded into S_2, and 1× TTE was loaded into S_1, waste, and anode wells. An electrode ring array was placed in the S_1, S_2, and waste wells. To perform a separation, the stage was heated up to 65 °C and equilibrated for 1 min. Electrophoresis was initiated with electrokinetic injection of the samples toward the waste wells at 170 V for 60 s, while grounding S_2 and floating S_1 and the anode well. Separation was achieved by grounding S_1, and applying 2700 V to the anode, 220 V to both S_2 and the waste to achieve an electric field of 250 V/cm. After each run, the sieving matrix was cleared from the microchannels and the microdevice was washed with deionized water using the high-pressure station.

In all cases, the four-color fluorescence data first underwent baseline and color crosstalk corrections using BaseFinder 6.1.16.[35] The processed data were then used to determine the peak properties with GRAMS/AI Spectroscopy Software Suite (Thermo Fisher Scientific Inc., Waltham, MA).

RESULTS AND DISCUSSION

The purification of PCR amplicons from the high concentration of buffer ions, unused nucleotide triphosphates, and primers can be readily accomplished by exploiting the strong interaction between biotin and streptavidin. The progress of a typical

(34) Scherer, J. R.; Paegel, B. M.; Wedemayer, G. J.; Emrich, C. A.; Lo, J.; Medintz, I. L.; Mathies, R. A. *BioTechniques* **2001**, *31*, 1150–1154.

(35) Giddings, M. C.; Severin, J.; Westphall, M.; Wu, J. Z.; Smith, L. M. *Genome Res.* **1998**, *8*, 644–665.

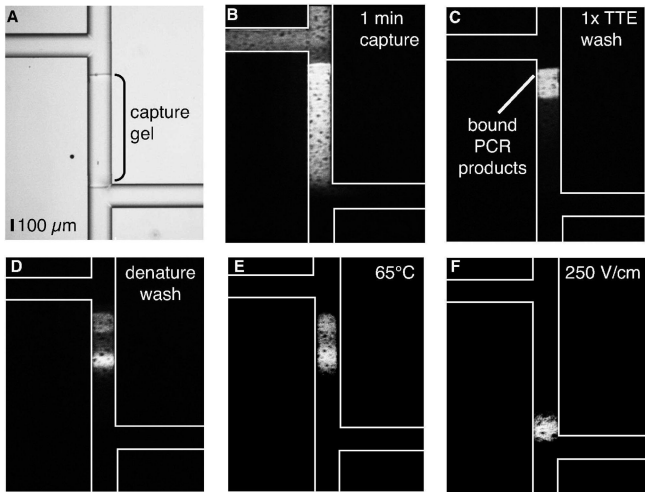

Figure 3. (A) Fluorescence images showing the progress of a typical streptavidin affinity capture-μCE operation. (B) The biotin-PCR products bind to streptavidin and concentrate at the entrance of the capture gel within one minute. (C) Unbound PCR products are washed off with 1x TTE. (D) After the denaturant wash, the captured DNA band disperses across the capture gel. (E) The device is then heated up to 65 °C to release the products and (F) electrophoresed at 250 V/cm toward the anode. The stacking that results from the lower ionic strength inside the capture gel focuses the products to a ~ 200 μm band for injection.

streptavidin capture gel operation is shown in Figure 3. The unpurified PCR reaction is placed in the two sample wells located above and left of the capture zone, filling the entire sample arm. PCR materials are driven toward the waste well at 25 V/cm and 30 °C. As the capture proceeds, the fluorescence-dye/biotin labeled dsDNA products bind to the streptavidin embedded in the capture gel, while the excess fluorescent primers and salts in the PCR reaction pass through the capture gel network to waste. After the captured zone was electrokinetically washed with fresh buffer, a discrete concentrated band is revealed in the first 200 μm of the capture gel. The release of the fluorescent ssDNA from its complementary strand requires a relatively high temperature (~80 °C), which causes the loss of mechanical stability of the separation and capture gel matrices. To avoid this problem, deionized formamide is introduced into the capture gel region as a denaturant to enable release of the fluorescent strand at 65 °C. While the denaturant wash results in dispersion of the captured DNA band, this broadening is reversed in the final release step because of isotachophoretic stacking of the DNA band into a ~200-μm (~1.5 nL) plug for injection.

Using the integrated capture-μCE procedure, the buffer ions, salts, and excess primers that impede electrokinetic injection of the PCR amplicons are completely removed. Some excess biotinylated primers are also retained in the capture gel but not released as confirmed by capturing FAM-biotin conjugates in separate experiments (data not shown). The high concentration of streptavidin in the capture gel ensures sufficient capacity for capturing the amplicons in the presence of these unreacted primers. The entire purification process takes ~25 min from the capture to the release step. Compared to cross injection, the fluorescence intensity of the concentrated DNA band, as shown in Figure 4A, is enhanced by at least 10-fold compared to cross injection for a diluted PCR sample as prepared in previous cross-

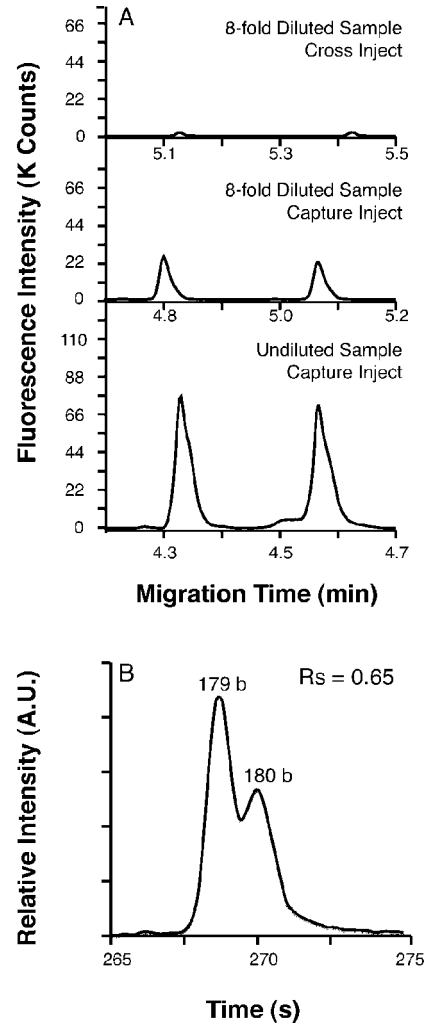

Figure 4. (A) Electropherograms of a biotin-modified monoplex PCR sample amplified from 0.5 ng of 9948 DNA. The top trace was obtained by cross injection of an 8-fold diluted PCR sample. The middle trace was obtained by using the affinity capture-μCE device for the same diluted sample. The bottom trace was obtained by the affinity capture-μCE device with the undiluted PCR reaction. (B) Electropherogram obtained on the affinity capture-μCE device for a PCR sample containing DNA fragments 179 and 180 bases in length, demonstrating a resolution of 0.65.

injection studies of STR samples.[2] When an undiluted PCR sample is used, the signal intensity is enhanced by ~ 50 fold. In addition, this injected zone is very sharp, leading to little injection band broadening. DNA fragments that differ by a single base can be differentiated with an average resolution (R_s) of 0.65 (σ = 0.1) and an average valley value of (V_v) of 50% (σ = 3%) as shown in Figure 4B. This resolution is comparable to that obtained for high-quality DNA genotyping performed on both microchip and commercial CE platforms,[1,29] demonstrating that the inline affinity capture produced a high quality CE injection method.

Integrated Streptavidin-μCE Analysis of STR Samples. To evaluate the capability of the capture-μCE device and method to process complex forensic PCR reactions, a biotin-modified 9-plex autosomal STR typing kit was constructed and balanced using primer sequences and fluorescent dye-labels found in the commercial genetic identity typing kit, PowerPlex 16. A parallel system for ABI typing kits could not be explored because their primer sequences are not readily accessible. This nine-STR typing system

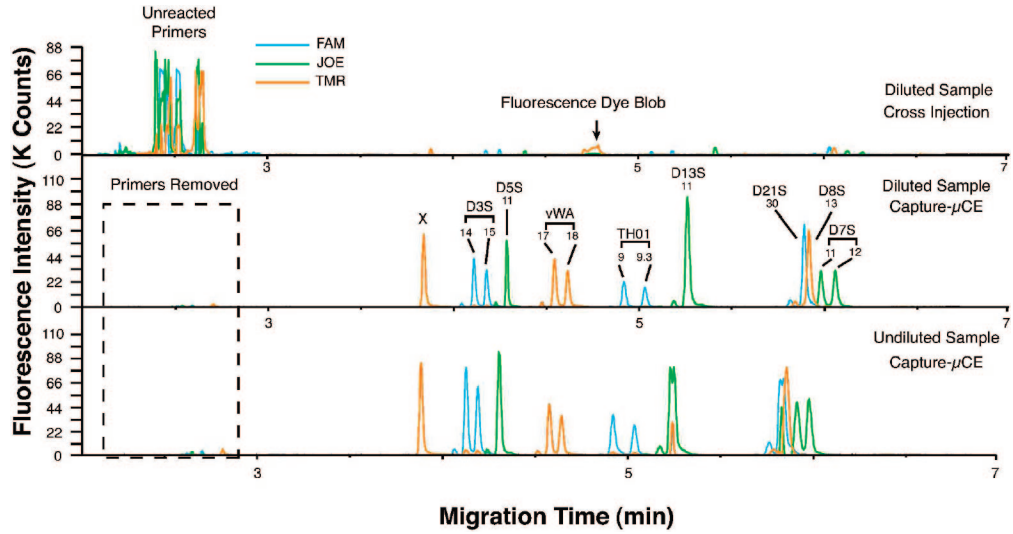

Figure 5. DNA profiles amplified using the biotin-modified 9-plex kit from 0.5 ng of 9947A DNA. The top trace was obtained by cross injection of a diluted PCR sample. Intense primer peaks dominated the electropherogram and a TMR dye blob overlapped with the vWA alleles. The middle trace was obtained using the capture-μCE device for the same diluted PCR sample. The bottom trace was obtained with the capture-μCE device for the undiluted PCR sample. Primer peaks are dramatically reduced in the affinity purified samples.

Table 2. Affinity Captured Alleles in Degraded DNA Samples

sample	degradation conditions (°C)	D3	TH01	D21	D5	D13	D7	Amel	vWA	D8	
	sample A expected alleles:	15, 18	6, 7	28, 30	13	12	11	X, Y	17, 19	12, 14	percent profile (%)
A1	56	●●	●●	●●	●	●	●	●●	●●	●●	100.0
A2		●●			●				●●		33.3
A3	80	●●			●				●	●	33.3
A4		●									6.7
	sample B expected alleles:	16, 17	6, 9.3	29, 30	12, 13	13	10, 11	X, Y	17, 18	13, 14	percent profile (%)
B1	56	●●	●●	●●	●●	●	●●	●●	●●	●●	100.0
B2	80	●●	●●	●●	●●	●	●●	●●	●●	●●	100.0
B3		●●	●●		●●	●	●●	●	●●	●●	82.4

• represents an allele above limit of detection.

interrogates the sex-typing locus amelogenin and eight of the FBI's 13 CODIS STR loci with an amplicon size range between 106−259 bases.[4] PCR samples amplified using these kits were analyzed and compared to separations obtained with cross injection.

As shown in Figure 5, a 14-fold increase in fluorescence intensity was observed for the diluted 9-plex reactions amplified from 0.5 ng of standard human genomic DNA using the streptavidin-μCE analyses compared to separations with the cross-injector device with the same detection settings. Similar peak balance across the DNA profile was maintained for both analysis methods and was observed for different DNA quantities and samples (data not shown). This result indicates that the integrated capture-μCE treatment does not exhibit DNA size or sequence bias. When raw amplified PCR samples were used, the fluorescence intensity was increased by ~19×. The excess primers and the TMR dye peak observed in the cross-injection experiments were also completely removed by the purification process. The fluorescence intensities of the D13 and D8 allele peaks in the undiluted sample exceeded the range of our analog/digital converter, producing the split-peak appearances with pull-up TMR and JOE signals, respectively, after color-crosstalk correction. This problem can be easily resolved by extending the analog/digital converter dynamic range and modest dilution. The signal strength of the multiplex samples was not enhanced to the same magnitude as the monoplex samples simply because of the higher number of alleles of different sizes that have to be captured by the same amount of streptavidin in the gel. These results demonstrate the capability of the streptavidin capture method to purify and concentrate simple and high-order multiplex PCR reactions to achieve higher fluorescent signal intensities and to eliminate dye label interference. The complete removal of primer peaks will be especially beneficial for miniSTR analyses[36] by opening up space in the electropherogram for smaller molecular weight marker sets.

Limit of Detection. A sensitivity study was carried out to evaluate the detection limit of the streptavidin capture-μCE method by using biotin-modified 9-plex PCR reactions amplified from serially diluted 9948 standard human genomic DNA. Figure 6A plots the percent allele detection as a function of DNA template copy number. All 15 expected STR alleles were successfully and reproducibly detected (defined as $S/N \geq 3$) with as few as 25

(36) Butler, J. M.; Shen, Y.; McCord, B. R. *J. Forensic Sci.* **2003**, *48*, 1054–1064.

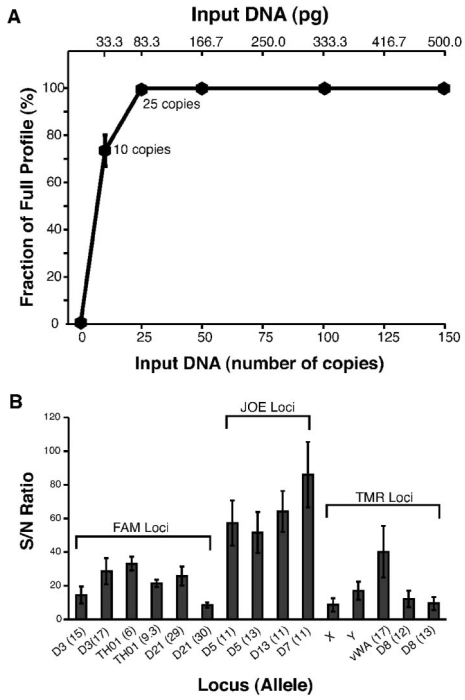

Figure 6. (A) Percentage of full STR profiles obtained using the capture-μCE device for 9948 DNA samples amplified with the biotin 9-plex as a function of input DNA copy number. Full STR profiles were reliably obtained from as low 25 copies of DNA template. (B) Average allele signal-to-noise (S/N) ratios for the 25-copy DNA sample on the capture-μCE device. The homozygous D13, D7, and vWA loci produced single allele peaks with about twice the height of the heterogyzous allele peaks.

copies of DNA input. We were still able to detect ~73% of the expected profile (~3−5 alleles dropped out at the three FAM loci, Amelogenin, and D8) with only 10 copies of template. This is half the input DNA typically required for forensic multiplex typing of a similar number of STR loci on commercial CE instruments[37] and for cross-injection based microchip platforms.[1,2] The average S/N ratios of each allele for the 25-copy sample are shown in Figure 6B. Since 9948 DNA is homozygous at the D13, D7 and vWA loci, the peak heights at these loci are about twice of the heterozygote allele peaks. Stochastic effects are usually observed for samples with DNA input <100 pg (low copy number (LCN) typing), producing heterozygous allele imbalance.[38] This effect was also observed for the 25-copy samples. The detection limit here is limited by PCR rather than the detection system. However, the enhanced fluorescence intensities achieved using the capture-μCE device should make the minor alleles more readily detectable in situations where one of the sister alleles has a disproportionately smaller peak height.

Degraded DNA Typing. DNA samples collected from crime scenes may have been exposed to harsh environmental conditions such as heat, direct sunlight, and moisture that break down the DNA polymer into smaller pieces. Analyses of degraded or compromised DNA samples often lead to allelic dropout at the larger loci, resulting in poor profiles. To maintain high discrimination power, it is crucial that the longer genetic markers be successfully amplified and injected for analyses.

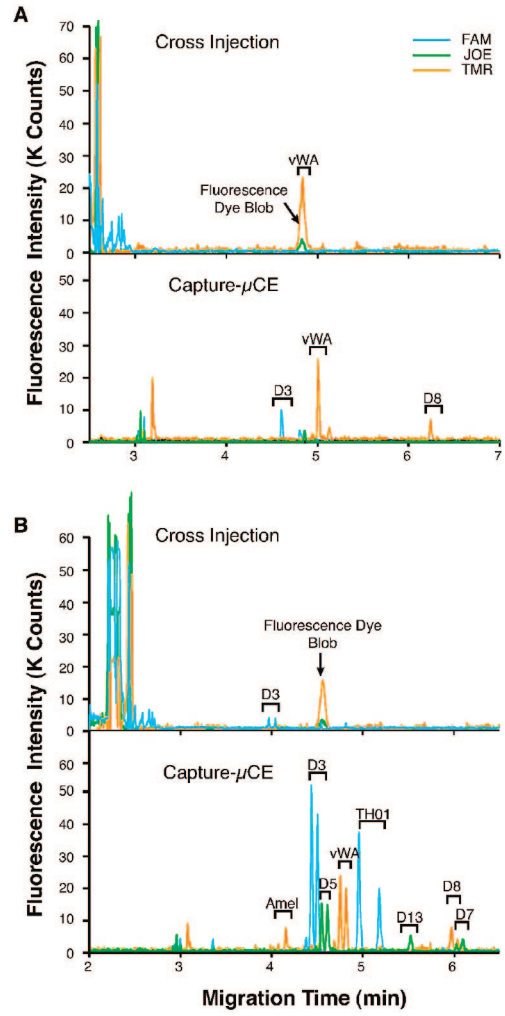

Figure 7. Analyses of PCR reactions amplified from two degraded DNA samples. (A) For sample A3, no alleles were detected using the cross injection method and a dye blob was observed overlapping with a putative vWA peak. Analysis of the same sample with the capture-μCE device and method produced three alleles at D3, vWA, and D8 loci with fluorescent counts of 10K, 30K and 5K counts. (B) For sample B3, two D3 alleles were detected using the cross injection method and a dye blob observed overlapping with the putative vWA alleles. Analysis of the same sample with the capture-μCE process yielded ~10X higher fluorescent intensity for the two D3 alleles and 10 additional alleles with signals from 5 to 30K fluorescent counts.

To determine the extent to which our improved processing and injection might benefit analysis of degraded DNA, four degraded DNA samples were prepared and provided by the VDFS. Blood samples collected from two individuals were each exposed to 56 and 80 °C for 3 months and afterward stored at room temperature for six years to produce DNA degradation. The extracted DNA samples were amplified using the 9-plex biotin system at two DNA input amounts (Table 1). Only one amplification was prepared for sample B1 because of DNA scarcity. Each amplified sample was processed by the capture-μCE device and compared to results obtained using the cross injection. DNA profiles generated with the capture-μCE device have overall higher fluorescence intensity and a complete absence of primer interference and dye blobs. More alleles were detected with the capture-μCE device than the corresponding profiles obtained using cross

(37) Tomsey, C. S.; Kurtz, M.; Kist, F.; Hockensmith, M.; Cal, P. *Croat. Med. J.* **2001**, *42*, 239–243.
(38) Gill, P. *Croat. Med. J.* **2001**, *42*, 229–232.

injection as summarized in Table 1. Three full DNA profiles were obtained using the capture-μCE device, while none of the profiles obtained using cross injection yielded all the expected alleles. These full profiles displayed 4−10× higher fluorescence intensities for the larger alleles; these alleles were below the limit of detection in profiles obtained with cross injection. For DNA samples that resulted in no alleles (Figure 7A) or a few alleles (Figure 7B) with cross injection, ~ 33% and ~71% more allelic markers were obtained, respectively, using the streptavidin capture-μCE method. Although preferential amplifications of the smaller DNA fragments and drop-out of the larger alleles were still observed for these low-integrity samples, more of the higher molecular weight fragments were detected because of the higher fluorescence signals associated with the affinity-capture injection. Table 2 lists the alleles detected for the affinity captured samples. The ability to gain additional genetic information from degraded DNA samples unequivocally demonstrates the advantages of this streptavidin-μCE method for higher fidelity genetic analyses of compromised samples.

CONCLUSION

We have successfully demonstrated a photopolymerizable streptavidin-gel capture process and a simple injector geometry for rapid purification and electrophoretic separation of biotin-modified PCR amplicons in an integrated microchip system. The streptavidin-based affinity capture method is applicable to both simple and multiplex PCR samples, making this approach transferable to a wide variety of genetic analyses.[39] The entire process from sample immobilization to the completion of separation required no sample transfer and took only ~40 min. This process is twice as fast as conventional protocols, reduces reagent usage, and can be easily scaled for high-throughput analyses. Detection of the concentrated sample plug inline with the separation column results in at least 10-fold increase in signal intensity, allowing the detection of STR alleles from half the input DNA normally required for electrokinetic injection of impure samples. This enhanced sensitivity enabled the capture of additional allele data from degraded DNA samples. The ability to precisely define the size and position of the capture gel can help improve forensic laboratory efficiency by dramatically increasing the fluorescence signals for low-level DNA samples, or by normalizing the DNA signals when the capture gel is a limiting reagent. Finally, this inline capture injection process can also be integrated with on-chip PCR methods,[4,5] leading to the realization of rapid, contamination-free, highly sensitive and low-cost portable genetic analysis systems for a variety of applications.

(39) Rautio, J. J.; Kataja, K.; Satokari, R.; Penttila, M.; Soderlund, H.; Saloheimo, M. *J. Microbiol. Methods* **2006**, *65*, 404–416.

ACKNOWLEDGMENT

S.H.I.Y. and P.L. contributed equally to this work. We thank Eric Chu for microdevice fabrication, Thomas Chiesl for discussions on isotachophoresis, and Promega Corp. for providing PowerPlex 16 information for multiplex construction. This project was supported by Grant 2007-DN-BX-K142 to R.A.M. and by a Graduate Research Fellowship to S.H.I.Y. awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the U.S. Department of Justice. R.A.M. discloses a financial interest in Microchip Biotechnologies Inc. (Dublin, CA) that is commercially developing aspects of the technologies presented here.

Received for review September 3, 2008. Accepted October 14, 2008.

AC8018685