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PAPER

Integrated DNA purification, PCR, sample cleanup, and capillary electrophoresis microchip for forensic human identification

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A fully integrated microdevice and process for forensic short tandem repeat (STR) analysis has been developed that includes sequence-specific DNA template purification, polymerase chain reaction (PCR), post-PCR cleanup and inline injection, and capillary electrophoresis (CE). Fragmented genomic DNA is hybridized with biotin-labeled capture oligos and pumped through a fluidized bed of magnetically immobilized streptavidin-coated beads in microchannels where the target DNA is bound to the beads. The bead–DNA conjugates are then transferred into a 250 nL PCR reactor for autosomal STR amplification using one biotin and one fluorescence-labeled primer. The resulting biotin-labeled PCR products are electrophoretically injected through a streptavidin-modified capture gel where they are captured to form a concentrated and purified injection plug. The thermally released sample plug is injected into a 14 cm long CE column for fragment separation and detection. The DNA template capture efficiency provided by the on-chip sequence-specific template purification is determined to be 5.4% using K562 standard DNA. This system can produce full 9-plex STR profiles from 2.5 ng input standard DNA and obtain STR profiles from oral swabs in about 3 hours. This fully integrated microsystem with sample-in-answer-out capability is a significant advance in the development of rapid, sensitive, and reliable micro-total analysis systems for on-site human identification.

1. Introduction

Forensic scientists are always seeking techniques that can improve short tandem repeat (STR) analysis for better throughput, cost, sensitivity, and reliability.^{1–3} Microfabricated bioanalysis devices have the potential to address these challenges by providing lower reagent consumption, the ability to integrate multiple analytical steps on a single device, and facile scaling capability.^{4–6} Significant advances have been achieved toward developing microdevices on which individual STR typing steps, such as DNA extraction,^{7–9} polymerase chain reaction (PCR),^{10–12} or capillary electrophoresis (CE),^{13–15} can be carried out. Although these chip-based analyses provide improved performance, they are still primarily utilized by the academic research community. The lack of integration and their dependence on other off-chip processes makes the adoption of these new technologies less attractive to forensic laboratories.

The most valuable advantages provided by micro-total analysis systems (μ TASs) stem from their integration capability. Performing the entire DNA typing process in a single

microsystem decreases the reagent and time consumption, makes the process more automated and robust, and dramatically reduces sample handling by users, which eliminates the risk of sample mix-up and contamination. Landers group recently successfully integrated the solid-phase extraction of template DNA with on-chip PCR for STR typing from a complex biological sample, whole blood.¹⁶ However, the thermal cycling and the analysis of the PCR products still relied on conventional instruments. Our group has reported several studies towards the integration of sample processing steps, such as PCR with microfabricated electrophoresis systems for forensic STR typing. We developed an integrated PCR–CE microdevice as well as a portable detection instrument for on-site rapid human identification.¹⁷ Real-time DNA analyses at a mock crime scene were successfully conducted in collaboration with Palm Beach County Sheriff's Office.¹⁸ While this demonstration validates the feasibility of STR typing using integrated devices, this microsystem falls short of the ultimate vision because DNA template was purified off-chip. Landers' group successfully integrated a solid-phase extraction (SPE) column for DNA extraction with PCR and CE on a single device for pathogen detection.¹⁹ However, the delicate operation and low efficiency of the pressure-driven cross-injection for CE separation and the transfer of purified DNA from the SPE column to the PCR reactor might be problematic for analyzing forensic samples, which have a great disparity in sample quality and quantity. More recently, Hopwood and

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co-workers successfully developed an integrated microfluidic system that consists of a DNA extraction and amplification cartridge coupled with a CE microchip for rapid STR analysis from reference buccal samples.²⁰ However, the more conventional format required operation at the more microlitre scale.

To address the low efficiency associated with the conventional cross-injector for CE separation, researchers have developed various preconcentration and inline injection methods.^{21–23} Yeung *et al.* developed an integrated STR sample cleanup, concentration method that employs a photopolymerized streptavidin-gel capture chemistry coupled to a direct injector geometry to achieve ~19 fold higher fluorescence signals and improved sensitivity for STR typing.^{24,25} The integration of this structure into a fully integrated microsystem should significantly advance the system sensitivity, reliability, and the capability of quantitative analysis.

Sample transfer from the DNA extraction process to PCR is more challenging because the process involves both sample transportation and buffer exchange. Several commercial DNA extraction systems that make use of magnetic particles, such as ChargeSwitch® and DNA IQ™, have been used for forensic STR analysis, and could potentially be translated into chip formats.²⁶ However, the DNA binding in these systems require PCR-incompatible solutions, such as chaotropic salts. Magnetic beads modified by oligonucleotide probes are capable of capturing specific DNA templates from a complex cellular background.^{27–29} This approach is attractive in a fully integrated microsystem because no PCR inhibiting chemicals are employed during DNA binding and the release of the captured DNA from magnetic beads can be controlled by heating instead of buffer exchange. In addition, since only the DNA of interest is captured for the subsequent PCR, the PCR efficiency should be significantly improved. This method has been successfully utilized for the extraction of plasmid DNA and genomic DNA fragments from clinical samples, but it has not to our knowledge been applied to microchip forensic STR typing.^{29–31}

The work presented here explores integration of the novel sequence-specific DNA purification and the improved post-PCR cleanup with inline injection into our well-characterized PCR–CE microsystem to form a fully integrated microdevice for forensic STR analysis. Using the bead capture structure developed previously,^{32,33} a fluidized bed of streptavidin-coated magnetic beads captures the conjugates of biotin-labeled oligonucleotide probes and genomic DNA fragments containing the sequences of STR loci. After DNA capture, the bead–DNA conjugates are pumped to a PCR reactor for 9-plex STR amplification. The resulting biotin-labeled PCR products are electrophoretically driven through a streptavidin-modified capture gel where they are bound and concentrated into a narrow injection plug, followed by thermal release for CE separation. By integrating these components on a single chip, we automate the STR analysis process, improve the reliability, and minimize the risk of contamination during the sample analysis.

2. Experimental

2.1 Microdevice design

The microdevice shown in Fig. 1 contains two identical genetic analysis systems forming a symmetrical doublet on a 4 inch glass

wafer. The structure is similar to the device developed in our group previously,³³ but the design is modified to integrate the post-PCR cleanup and inline injection functions and to adapt to the newly developed scanner instrument. Each analytical system includes a poly(dimethylsiloxane) (PDMS) micropump and two PDMS microvalves³⁴ for fluidic control, a 4 cm long bead capture structure with a system of bifurcating channels for DNA template capture,³² a 250 nL PCR chamber with a micro-fabricated heater and a resistance temperature detector (RTD) for PCR thermal cycling, a 500 μ m long double-T channel junction with a tapered structure for post-PCR cleanup and inline injection,²⁴ and a 14 cm long channel for CE separation. These two systems share an anode, a cathode, and a waste well to reduce the number of reservoirs on the chip.

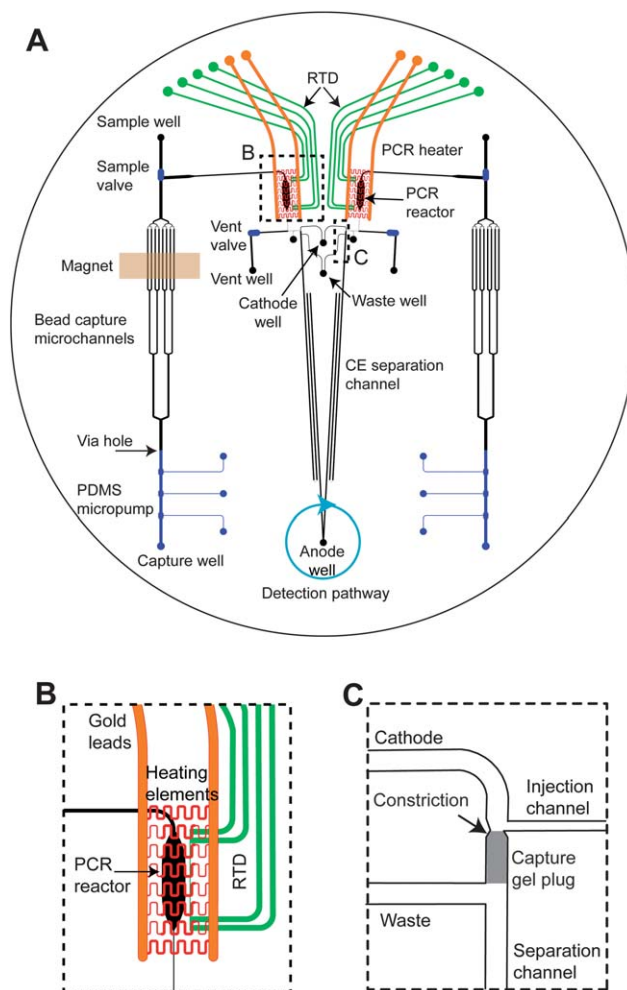


Fig. 1 Schematic of fully integrated microdevice for forensic STR analysis. (A) Microchip mask design for performing DNA template purification, PCR, post-PCR cleanup, inline injection and CE separation. The microchannels are indicated in black, the microfabricated RTD and electrodes are in green, the heater is shown in red, the gold leads of the heater are drawn in gold, and the PDMS micropumps and microvalves are in blue. (B) Expanded view of the PCR chamber and the micro-fabricated heater and RTD. (C) Expanded view of the affinity gel-based capture inline injector. Shaded area indicates the location of streptavidin capture gel.

The microdevice is constructed using a four-layer wafer stack consisting of (from top to bottom) glass manifolds, PDMS membranes, a glass fluidic wafer, and a glass RTD wafer. The microfabrication process has been previously described in details.^{17,33} Briefly, on the bottom side of the fluidic layer, the PCR and CE channels are etched to a depth of 40 μm while the bead capture structure is etched 30 μm deep. On the top side, the channels for the on-chip micropump are etched to a depth of 30 μm and the PCR heaters are microfabricated after the glass etching. The RTDs are fabricated on a 762 μm thick Borofloat glass wafer and then thermally bonded with the fluidic wafer in a vacuum furnace. The glass manifold is etched from a 700 μm Borofloat glass wafer. A glass microchip which only contains a PDMS micropump and bead capture microchannels was also fabricated for studying the DNA capture efficiency.

Prior to use, all the microstructures are coated with 0.25% polyDuramide (pDuramide) dynamic coating polymer to minimize DNA absorption to the channel walls and electroosmotic flow during electrophoresis. The coating procedure consists of 1 M HCl incubation for 15 min, DI water flush, and pDuramide incubation for one hour. After treatment, the chips are flushed with water again, and then dried with vacuum.

2.2 Scanner detection instrument

The instrument used to perform analyses has been described in details previously.³⁵ It contains a 488 nm diode laser (75 mW, Sapphire 488-75, Coherent, Santa Clara, CA), an optical system with a rotary objective for detecting four different fluorescence signals, pneumatics for controlling PDMS microvalves, electronics for PCR temperature control, and four high voltage power supplies for CE. The analysis system has dimensions 12 \times 12 \times 8 in., which can be used as an either bench-top or portable instrument.

During operation, the microdevice is placed onto a 6" heating stage of the instrument and held in place with a plexiglass manifold as well as vacuum supplied by the instrument. The manifold contains spring-loaded pins pressed against the electrical pads on the device, providing the connections for sensing the RTDs and powering the PCR heaters. The manifold also contains Pt electrodes that are positioned within the reservoirs on the microchip for the application of high voltages.

2.3 STR typing and DNA template capture probes

A 9-plex autosomal STR typing system was developed previously based on the primer sequences and fluorescence dye labeling scheme used in PowerPlex® 16 System (Promega, Madison, WI).^{17,24} To enable the post-PCR cleanup and inline injection, the unlabeled primers are replaced with biotin-labeled primers (IDT, Coralville, IA). The STR loci in the 9-plex system are amelogenin, D3S1358, TH01, D21S11, D5S818, D13S317, D7S820, vWA and D8S1179. Primer sets without any labeling are also synthesized for DNA quantitation using real-time PCR. The PCR mixture prepared for on-chip 9-plex STR typing is comprised of 1.5 \times Gold ST®R buffer (Promega, Madison, WI), primer mixture, 0.12 U μL^{-1} FastStart Taq DNA polymerase (Roche Applied Science, Indianapolis, IN), 0.1% bovine serum albumin (BSA), and deionized water (DI water).

The biotin-labeled primers in the 9-plex STR typing systems are also employed as capture probes for DNA template purification prior to PCR. The concentrations of the capture probes in a 10 \times capture probe mixture are listed as follows: amelogenin: 27 nM, D3S1358: 17 nM, TH01: 22 nM, D21S11: 43 nM, D5S818: 11 nM, D13S317: 13 nM, D7S820: 47 nM, vWA: 13 nM and D8S1179: 11 nM.

2.4 DNA sample preparation

Standard genomic DNA 9947A, 9948 and K562 are purchased from Promega. To determine the heating time to fragment genomic DNA, K562 DNA (0.4 μg μL^{-1}) with high molecular weight is subjected to 95 $^{\circ}\text{C}$ heat incubation for different times (from 10 to 40 min in 5 min intervals) in a PTC-200 thermocycler (MJ Research, Waltham, MA). DNA samples are then run on a 1.2% agarose gel with 100–4 kb and 1–4 kb sizing ladders (Lonza, Allendale, NJ). To prepare DNA samples for on-chip template capture, 20 μL DNA samples are first heated at 95 $^{\circ}\text{C}$ for 15 min in the thermocycler. After mixing the fragmented DNA with 25 μL of 20 \times sodium saline citrate (SSC) buffer and 5 μL of 10 \times biotin-labeled capture probe mixture, the solution is further heated to 95 $^{\circ}\text{C}$ for 5 min, followed by incubation at 50 $^{\circ}\text{C}$ for 20 min to allow DNA hybridization between the biotin-labeled capture probes and target DNA fragments.

2.5 Sequence-specific DNA template capture

A commercial streptavidin-coated magnetic bead system, Dynabeads M-280 (2.8 μm diameter, 6 \times 10⁵ beads per μL , Invitrogen, Carlsbad, CA), is used for on-chip sequence-specific DNA template capture following a modified protocol from Invitrogen. 10 μL beads are washed two times and resuspended in 25 μL 10 \times SSC solution for DNA template capture.

Evaluation of the efficiency of the DNA template capture is performed on the glass microchip which only contains bead capture microchannels with a PDMS micropump. The chip is first filled with a 1% w/v solution of BSA and incubated for 15 min to block non-specific bead adhesion to the channels. Next, acetonitrile (ACN) is drawn by vacuum into the channel to eliminate any bubbles in the system followed by rinse with DI water. 10 \times SSC is finally loaded into the bead capture channels to serve as a running buffer for the subsequent DNA capture. The DNA template capture process begins by introducing 5 μL of prepared Dynabeads solution (\sim 1.2 \times 10⁶ beads) into the capture well. The magnetic beads are driven into the capture structure using the on-chip micropump and immobilized in the microchannels using a nickelplated neodymium magnet (All Electronics, Van Nuys, CA, MAG-74). A multi-step loading procedure is employed to establish equal bead distributions across all parallel capture channels.³²

A 20 μL DNA solution containing 0.8 μg fragmented K562 DNA prepared using the protocol described as above is pumped through the bead bed using a 9-step pumping protocol with three "flutter" steps (200 ms per step for pumping and 100 ms per step for fluttering).^{32,33} The target DNA template hybridized with biotin-labeled capture probes is captured by magnetic beads *via* the strong streptavidin-biotin interaction. Following sample loading, 20 μL 1 \times Gold ST®R PCR buffer is rinsed through the

beads to eliminate the unbound material from the system. Finally, the entire bead bed is pumped out of the channels and collected for subsequent DNA quantitation. To quantify DNA capture efficiency, real-time PCR is performed on ABI 7300 instrument (Applied Biosystems, Foster City, CA) using an SYBR® Green PCR Master Mix (Applied Biosystems). Each locus in the 9-plex STR typing system is quantitated separately. Two μL of bead solution containing target DNA template is mixed with a PCR master mix with 2 μL primer sets and diluted to a final volume of 25 μL . The DNA quantity is determined by a calibration curve generated using serially diluted standard DNA with known concentrations. The measurements are repeated three times to determine the standard deviations.

2.6 Streptavidin capture gel preparation

A 500 μL streptavidin gel solution, containing 5% (v/v) bis-acrylamide (19 : 1, Bio-Rad, Hercules, CA), 8 M Urea, 1 \times TTE (500 mM Tris, 500 mM TAPS acid and 100 mM EDTA), 2 μg μL^{-1} streptavidin–acrylamide (Invitrogen), 0.0006% riboflavin (w/v) and 0.125% TEMED (v/v), is prepared following the method developed previously.²⁴ Using a UV exposure setup installed on a Nikon inverted microscope,^{24,36} a 500 μm capture gel plug is formed in the double-T channel junctions for post-PCR cleanup and inline injection.

2.7 Microchip operation

Following the photopolymerization of the capture gel plugs, a separation matrix (5% linear polyacrylamide (LPA) with 8 M Urea in 1 \times TTE) is loaded from the anode to the waste and from the cathode to the coinjection reservoir to form a matrix-capture-matrix gel sandwich structure in the capture inline injection regions. The tapered structure in the capture region ensures that the capture gel plug will be retained. After gel loading, the bead capture channels and the PCR chambers are coated with 1% BSA for 15 min followed by water rinsing. 10 \times SSC is then loaded into the bead capture channels while keeping the vent valve close to prevent SSC from contaminating the PCR reactors.

The bead loading and DNA template capture are performed following the procedure described above. An aliquot of 20 μL of prepared 9947A standard DNA is manually pipetted into the capture well for sample loading. It takes about 20 min to pump the sample through the bead bed with a flow rate of $\sim 1 \mu\text{L min}^{-1}$. During this process, the vent microvalve is closed and all waste flows out to the sample well, preventing any potential contamination of the PCR chamber. After DNA sample loading, 10 μL PCR master mix is used to wash the bead bed. The beads are then pumped into the PCR chamber by closing the sample valve, opening the vent valve, and manually placing the magnet above the reactor. Ten more microlitres PCR master mix is loaded into the capture well and pumped through the beads. After that, the PCR thermal cycling begins with all valves held closed at 20 kPa. The modified thermal cycling protocol starts with an initial activation of the Taq polymerase at 95 °C for 4 min. For the next 32 PCR cycles, the temperature is held at 94 °C for 10 s denaturing, then ramped to 58 °C for 20 s annealing, and to 70 °C for 30 s extension. Finally, a post extension step is performed at 70 °C for 5 min. Total PCR time is about 40 min.

To perform post-PCR purification, the sample valve is held open and the biotin-labeled PCR products are electrophoretically injected using an electrical field of 25 V cm^{-1} from the PCR chamber to the waste well through the capture gel plug. The products are bound *via* the biotin-streptavidin interaction to form a tightly concentrated plug in the capture gel. In the following washing step, unbound materials are washed away by applying an electrical field of 25 V cm^{-1} from the cathode to the waste well for 5 min. The fluorescently labeled DNA strands retained in the capture gel are then thermally released into the separation channel by heating the whole chip to 67 °C and applying an electrical field of 250 V cm^{-1} towards the anode. After each run, all the gels and solutions in the chip are removed out with water and the channels are cleaned using piranha (7 : 3 H_2SO_4 : H_2O_2) to prevent run-to-run carryover contamination.

2.8 Oral swab sample processing

Two oral swab samples provided by the Virginia Department of Forensic Science (VDSF) were shipped to Berkeley for on-chip STR analysis. Buccal cells are first released in 200 μL DI water by pressing the swab heads against the tube wall. After mixing 10 μL cells with 10 μL Microlysis-Plus (Gelcompany, San Francisco, CA), the mixture is heated in a PTC-200 thermal cycler at 65 °C for 15 min, followed by denaturation at 95 °C for 20 min. To capture the DNA templates, 25 μL of 20 \times SSC buffer and 5 μL of 10 \times capture probes are added into the mixture, and then further heated to 95 °C for 5 min, followed by incubation at 50 °C for 20 min. Finally, an aliquot of 20 μL of the processed mixture is manually pipetted into the microdevice for on-chip DNA typing.

3. Results and discussion

3.1 Microdevice integration

The integration of the entire STR analysis on a single microdevice is more than the simple combination of several microfabricated units.^{2,4} Not only must all units have high performance, the sample/product transport between each analytical step also needs to be convenient and efficient. These considerations are even more critical for forensic STR analysis, where high sensitivity and reliability are desired due to the great disparity in sample quality and quantity, as well as the probative value of these samples.

In a fully integrated microdevice designed for forensic STR typing, three sample transfer steps should be considered: macro-to-micro-interface, DNA extraction to PCR, and PCR to electrophoresis. The fully integrated microsystem presented here has successfully addressed each of these issues. First, the capture of DNA-probe conjugates using a magnetic bead bed immobilized in the microchannels can concentrate DNA samples with various volumes and concentrations into the microdevice, serving as an efficient macro-to-micro-interface for the microsystem. Second, to transfer the sample from the DNA extraction step to PCR, magnetic beads were employed as a medium to carry DNA into the PCR reactor. Following the introduction of the target solution into the microsystem, the magnetic beads can be conveniently and thoroughly washed with PCR solution to remove inhibitors. Third, a capture inline injection structure was

integrated into the system to replace the inefficient cross-injector for CE separation. These structures not only provide near 100% sample transfer efficiency in each step so that high-sensitivity analysis can be achieved, they also eliminate the need for delicate timing control and voltage balance characterization of electrophoretic and electroosmotic transport.

3.2 Genomic DNA fragmentation

To enable the sequence-specific DNA capture, genomic DNA with high molecular weight must be fragmented into an appropriate size range which can be captured by magnetic beads while still providing intact templates for subsequent STR amplification. Physical methods, including hydrodynamic shearing,^{37,38} nebulization,³⁹ and ultrasonication,⁴⁰ are the most extensively used methods for breaking chromosomes in DNA sequencing library construction. Restriction enzymatic digestion is another well-established method to fragment genomic DNA.^{41,42} DNA digestion by heating was chosen in this study because it can be rapidly performed in a conventional thermal cycler. Moreover, since the DNA extraction process already has a heating step for cell lysis, the heat digestion can be easily combined with the current procedure. As shown in Fig. 2, heating DNA samples at 95 °C for 20 min is sufficient to fragment genomic DNA into a size range of 1–4 kb, which is a suitable for the subsequent DNA capture. Breaking the DNA into smaller fragments will result in poor PCR efficiency, because it is more likely that DNA is broken inside the target sequences. On the other hand, fragments longer than 4 kb will decrease the DNA capture efficiency.

3.3 Sequence-specific DNA purification

The DNA template capture efficiency using streptavidin-coated magnetic beads was characterized on a microchip with only bead capture channels and a PDMS micropump. The capture channel design, pumping scheme, and flow control followed the process optimized previously.^{32,33} Since the capture probes have different capture efficiencies for each STR locus, the concentrations of these probes were iteratively adjusted to ensure balanced

template capture for all 9 STR loci. As shown in Fig. 3, the capture efficiencies calculated based on copy numbers for these 9 STR loci are in a range of 3.7–7.0% with an average of $5.4 \pm 1.3\%$. Although this efficiency is lower than those of many stand-alone DNA extraction microdevices developed previously,^{7,8} the 100% transfer of the captured DNA to the PCR reactor compensates for this drawback and makes the sequence-specific DNA capture suitable for on-chip STR typing.

An important advantage provided by sequence-specific DNA purification is the improved PCR efficiency due to the removal of background DNA sequences. Fig. 4 presents 9-plex STR profiles amplified from 1 ng of 9948 male whole genomic DNA (333 haploid copies) and from the same amount of DNA template purified from 20 ng whole genomic DNA (~5% purification efficiency). The PCR amplifications were performed in a conventional thermal cycler with a PCR protocol which includes denaturation at 94 °C for 10 s, annealing at 58 °C for 20 s, and extension at 70 °C for 30 s. Compared to the PCR protocol recommended by the manufacturer, over 1.5 h is saved. Using this shortened cycling, full STR profiles still can be obtained from the purified DNA. In contrast, the profiles from the whole genomic DNA experienced dropout of the TH01 9.3 allele. We did not observe a correlation between amplicon size and PCR efficiency and the allele drop-out is locus-sepecific.⁴³ This comparison demonstrates the effectiveness of sequence-specific DNA purification for improving STR amplification while using a shortened PCR protocol.

3.4 Standard DNA and limit-of-detection test

DNA template capture, 9-plex autosomal STR amplification, post-PCR cleanup, capture inline injection, and CE separation on the fully integrated microsystem were optimized and the limit-of-detection (LOD) was explored using serially diluted female standard 9947A DNA (10, 5, and 2.5 ng input DNA). As shown in Fig. 5, full DNA profiles can be obtained with as low as 2.5 ng input DNA. In our previous study, the limit of detection of the PCR–CE microdevice was determined to be 100 copies of DNA template (0.333 ng) in the 160 nL PCR reactor.¹⁸ However, in

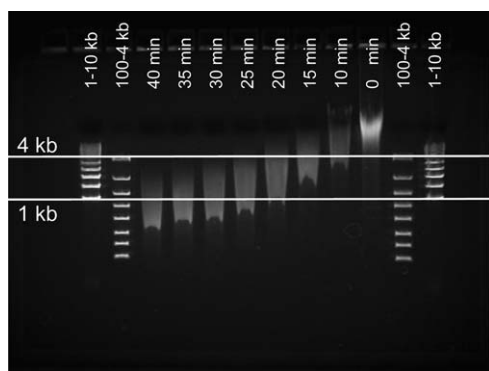


Fig. 2 Photograph of the slab gel separation of genomic DNA thermally fragmented for different lengths of time. K562 standard genomic DNA with high molecular weight was subjected to 95 °C heat incubation for the indicated times and run on a 1.2% agarose gel. As indicated by two white lines (the positions of 1 kb and 4 kb fragments), a 20 min heating is sufficient to fragment the genomic DNA into a size range of 1–4 kb.

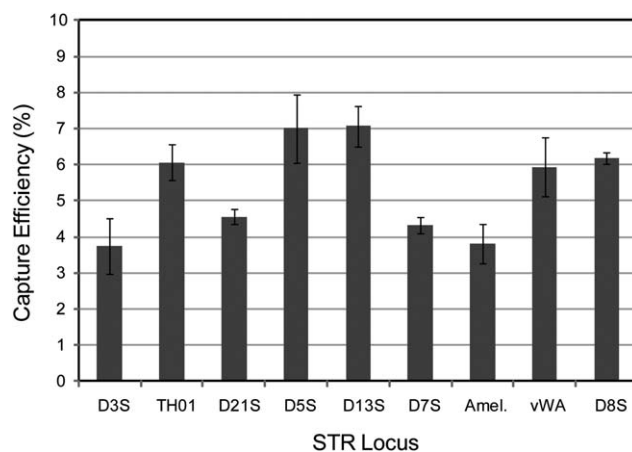


Fig. 3 The capture efficiencies for the sequence-specific DNA template purification for the Amelogenin and the 8 STR loci. By optimizing the capture probe concentrations in the probe mixture, capture efficiencies with an average of 5.4% were obtained for all 9 loci.

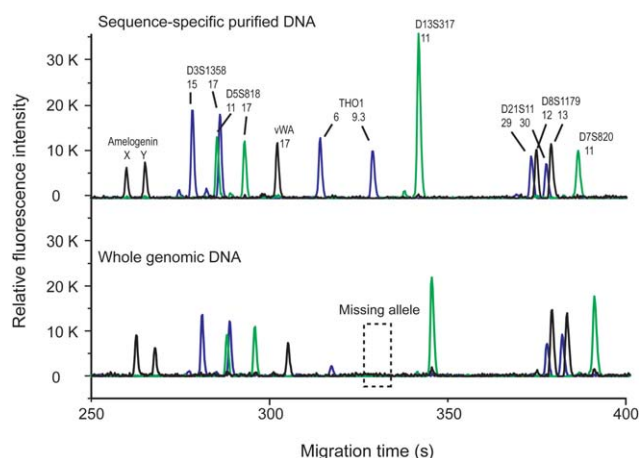


Fig. 4 Comparison of the 9-plex STR profiles amplified from whole genomic DNA and from bead purified DNA. A total of 1 ng whole genomic DNA (333 haploid copies) and the same amount of DNA template (~ 333 haploid copies) purified from the whole genomic DNA by sequence-specific DNA capture were amplified in a conventional PTC-200 thermocycler with a PCR protocol consisting of denaturation at 94 °C for 10 s, annealing at 58 °C for 20 s, and extension at 70 °C for 30 s. With this expedited PCR cycling protocol, balanced STR profiles were obtained from the DNA sample purified by sequence-specific DNA capture. In contrast, an allele drop-out in TH01 9.3 was observed in the profiles from the whole genomic DNA.

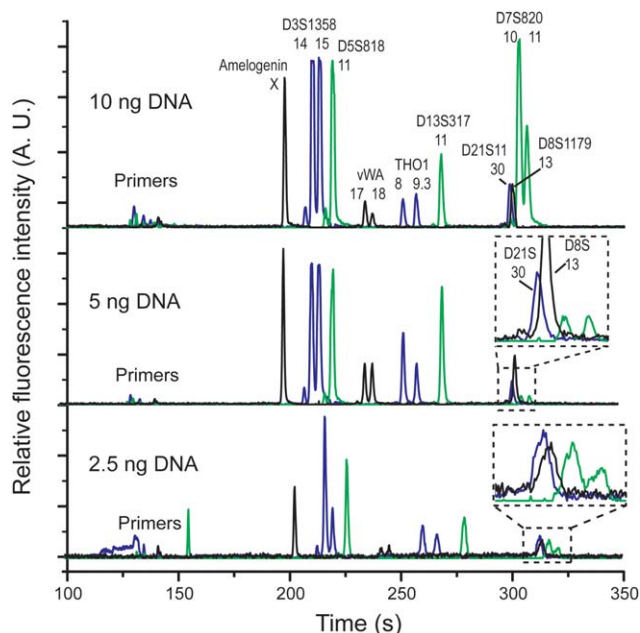


Fig. 5 Limit-of-detection test for the 9-plex STR analysis using serially diluted 9947A female standard genomic DNA performed on the micro-device. DNA template capture, STR amplification, post-PCR cleanup, capture inline injection, and CE separation were successfully conducted on the fully integrated microsystem. With only 2.5 ng of input genomic DNA, full 9-plex STR profiles was successfully obtained.

order to load 100 copies of DNA into such a small reactor, a 10 μ L PCR solution containing ~ 21 ng DNA is needed. By integrating the bead capture structure upstream from the PCR

reactor, the DNA template of interest can be selectively purified, concentrated, and transported into the PCR reactor. Therefore, the actual DNA required to produce full STR profiles was reduced ~ 8 fold. This result illustrates the importance of an efficient macro-to-micro-interface for the microsystem.

In STR amplifications, high annealing temperatures, long holding time and slow temperature ramping rate are usually employed in order to ensure balanced and highly stringent amplifications of all STR loci in a complex genomic background. For example, the Powerplex 16 STR kit recommends 60 °C for annealing and a temperature ramping rate of 0.5 °C s⁻¹ from denaturation to annealing, resulting in a PCR protocol longer than 3 hours. In our previous study of on-chip PCR–CE, we also found that the slow ramping rates are critical for generating reproducible and balanced STR profiles from genomic DNA.¹⁸ The on-chip PCR time was thus only shortened to 2 hours. Other methods, such as using fast PCR enzymes,⁴³ have demonstrated the possibility of rapid amplification of STR profiles with off-the-shelf STR typing kits. However, so far only purified DNA was tested. Sequence-specific DNA template capture is an effective way to overcome this hurdle for rapid STR amplification. The removal of unnecessary DNA sequences eliminates the potential for non-specific amplification, adding stringency prior to the PCR step. Therefore, it is feasible to use a lower annealing temperature allowing more efficient primer binding together with a faster ramp rate that accelerates the PCR process. As demonstrated in Fig. 5, full STR profiles can be successfully obtained using a 45 min PCR protocol, in which the annealing temperature is decreased to 58 °C. The over 1 hour reduction of time is important for forensic STR typing when turnaround time is critical.

The post-PCR cleanup and inline injection step also contributes to the improvement of the system performance. In this integrated microsystem, the streptavidin gel column was designed to provide sufficient capacity to capture all the PCR products amid unreacted biotin-labeled primers (data not shown). The sensitivity improvement by the capture inline injection can be estimated as follows: the LOD of the microsystem is 2.5 ng DNA. Considering the average 5.4% capture efficiency of the sequence-specific purification, about 45 copies of DNA were purified from the 2.5 ng input and loaded into the PCR chamber for the following amplification, post-PCR cleanup, inline injection and CE detection. As the LOD of the PCR–CE system with a conventional cross-injector is 100 copies of DNA template,¹⁸ the replacement of the cross-injection with the more efficient capture inline injection improved the sensitivity ~ 2 fold. While this improvement is still lower than that demonstrated in our previous work,^{24,25} due probably to the presence of magnetic beads during PCR, the detection limit of 45 copies for PCR amplification is already approaching the low-copy-number range (≤ 100 pg or 33 copies of each locus).⁴⁴ In addition, the capture inline injection reduces the probability for allele dropouts, simplifies the injection optimization, and eliminates the dominant primer peaks, which open up extra space to incorporate more loci with smaller sizes.

3.5 STR typing from oral swab samples

To demonstrate the capability of our fully integrated microsystem for rapid forensic human identification, STR analyses

from oral swab samples were performed on the fully integrated microdevice. Oral swab samples were chosen because buccal cell collection with a cotton oral swab has been extensively employed to collect reference samples from a person of interest in many situations.⁴⁵ Two oral swabs were prepared by the Virginia Department of Forensic Science and analyzed on our system in Berkeley. As shown in Fig. 6, these two samples were successfully extracted, amplified, and separated on the microsystem. The total analysis time of this assay is about 3 hours, which includes 15 min of cell lysis, 45 min of DNA digestion and hybridization, 40 min of DNA template capture and washing, 40 min of PCR amplification, and 30 min of post-PCR cleanup, capture inline injection and CE. Compared to conventional forensic STR typing (7–8 hours), a minimum of 3–4 hours can be saved. Moreover, the samples stay inside the microstructures during the process of purification, amplification, and separation, which could effectively minimize the risks of contamination and sample mix-up. Therefore, this microsystem can be utilized as an automated instrument for rapid forensic STR analysis in forensic

laboratories. In addition, since the control and detection instrument has a small footprint and can be battery-powered,³⁵ this fully integrated microsystem can be more effectively used as a portable system for on-site real-time human identification when supported by basic lab equipment.

Regulation of the amount of input DNA for STR amplification is essential to ensure the production of well-resolved and balanced STR profiles.¹ While the on-chip bead capture process helps to prevent DNA overload due to the limited capture capacity of the magnetic beads, no precise DNA quantification was incorporated into the current microsystem. Therefore, this system is best utilized as an automated and portable platform for rapid typing of reference samples, such as buccal swabs, where DNA quantity is in a predictable range. In addition, since only an aliquot of pretreated samples was loaded into the microsystem, further verification or generation of final STR profiles for database entry can be performed using conventional methods. The sequence-specific purification prior to PCR may also benefit the analysis of degraded DNA which can be captured directly without digestion. The concentration of the target STR sequences as well as the removal of background DNA could significantly improve the STR amplification.

4. Conclusions

A fully integrated microdevice capable of performing DNA template purification, PCR, post-PCR cleanup, and CE separation using a compact detection and control instrument was successfully developed for rapid forensic STR analysis. Efficient sample transfers between each function unit were achieved by the integration of sequence-specific DNA template capture using magnetic beads and post-PCR inline capture injection. The operation of this microdevice was optimized using standard DNA samples and the detection limit was determined to be 2.5 ng of total sample DNA. The successful STR typing from oral swab samples proved that this system can be used for rapid human identification. The success development of this proof-of-concept microsystem is a significant step towards a fully integrated and portable forensic analysis system for rapid real-time human identification at crime scenes, security check, or other point-of-analysis situations.

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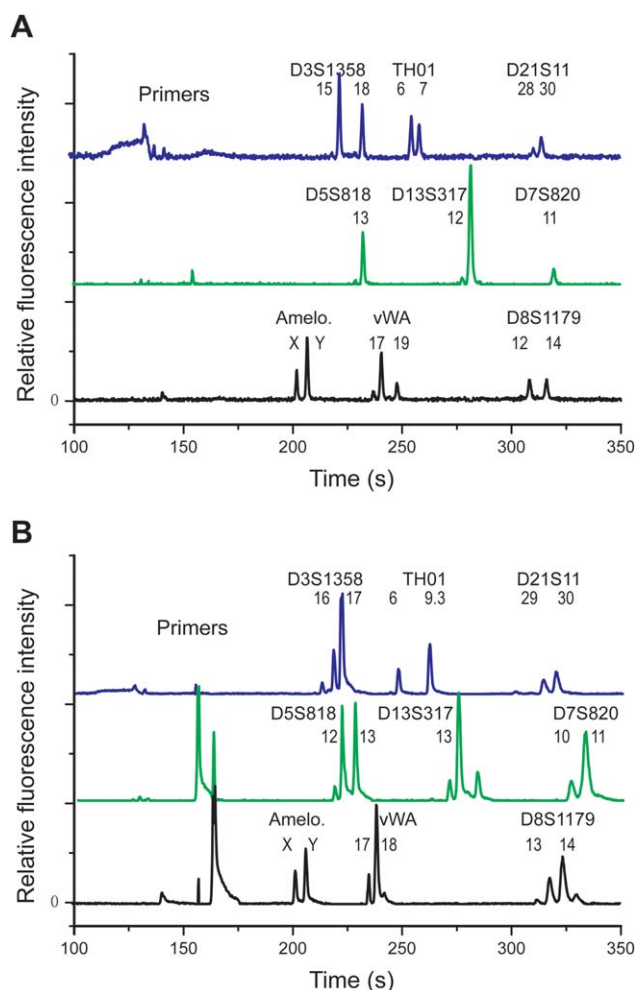


Fig. 6 Rapid STR analyses of two different oral swab samples performed on the microsystem. Full profiles were successfully obtained in about 3 hours, including 15 min of cell lysis, 45 min of DNA digestion and hybridization, 40 min of DNA template capture and washing, 45 min of PCR amplification, and 30 min of post-PCR cleanup, capture inline injection and CE.

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