

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/45638866>

Integrated Microfluidic System for Rapid Forensic DNA Analysis: Sample Collection to DNA Profile

ARTICLE in ANALYTICAL CHEMISTRY · AUGUST 2010

Impact Factor: 5.64 · DOI: 10.1021/ac101355r · Source: PubMed

CITATIONS

54

READS

193

18 AUTHORS, INCLUDING:



[Cedric Hurth](#)

29 PUBLICATIONS 324 CITATIONS

[SEE PROFILE](#)



[Jianing Yang](#)

The University of Arizona

26 PUBLICATIONS 1,307 CITATIONS

[SEE PROFILE](#)



[Alan R Nordquist](#)

The University of Arizona

10 PUBLICATIONS 85 CITATIONS

[SEE PROFILE](#)

Integrated Microfluidic System for Rapid Forensic DNA Analysis: Sample Collection to DNA Profile

Andrew J. Hopwood,^{*,†} Cedric Hurth,[‡] Jianing Yang,[‡] Zhi Cai,[‡] Nina Moran,[†] John G. Lee-Edghill,[†] Alan Nordquist,[‡] Ralf Lenigk,[‡] Matthew D. Estes,[‡] John P. Haley,[†] Colin R. McAlister,[†] Xiaoja Chen,[‡] Carla Brooks,[‡] Stan Smith,[‡] Keith Elliott,[†] Pieris Koumi,[†] Frederic Zenhausern,^{*,‡} and Gillian Tully[†]

Research and Development, Forensic Science Service, Trident Court 2960 Solihull Parkway, Birmingham Business Park, Birmingham UK B37 7YN, and Center for Applied NanoBioscience and Medicine, The University of Arizona College of Medicine, 425 N. Fifth Street, Phoenix, Arizona 85004

We demonstrate a conduit for the delivery of a step change in the DNA analysis process: A fully integrated instrument for the analysis of multiplex short tandem repeat DNA profiles from reference buccal samples is described and is suitable for the processing of such samples within a forensic environment such as a police custody suite or booking office. The instrument is loaded with a DNA processing cartridge which incorporates on-board pumps and valves which direct the delivery of sample and reagents to the various reaction chambers to allow DNA purification, amplification of the DNA by PCR, and collection of the amplified product for delivery to an integral CE chip. The fluorescently labeled product is separated using micro capillary electrophoresis with a resolution of 1.2 base pairs (bp) allowing laser induced fluorescence-based detection of the amplified short tandem repeat fragments and subsequent analysis of data to produce a DNA profile which is compatible with the data format of the UK DNA database. The entire process from taking the sample from a suspect, to database compatible DNA profile production can currently be achieved in less than 4 h. By integrating such an instrument and microfluidic cartridge with the forensic process, we believe it will be possible in the near future to process a DNA sample taken from an individual in police custody and compare the profile with the DNA profiles held on a DNA Database in as little as 3 h.

DNA analysis in a forensic context relies on the extraction of DNA from a sample; quantification and normalization of the DNA; concurrent PCR-based amplification of a number of specific short tandem repeat loci and separation/detection of the PCR products, usually by capillary electrophoresis (CE); thereby allowing precise sizing of each fragment and accurate typing of the alleles in the DNA profile. This forensic process allows for the routine analysis of DNA samples from controls such as buccal swabs in 24–72 h

from receipt of the sample.¹ Such processes are laboratory-based and require that the sample, once taken from a suspect in custody is transported to a laboratory for processing. For convenience, samples are usually stored and batched by the police force prior to dispatch to the lab, a process that can take between a few hours to a few weeks. Even with samples that are dispatched to the laboratory immediately, the suspect is highly likely to have been released from custody while the sample is processed. A sample taken from a suspect in the UK has a 2.3% chance of matching with a crime sample held on the National DNA Database (NDNAD) of the UK.² Furthermore, evidence suggests that individuals released on police bail have a high incidence of offending while on bail.³ It would therefore be beneficial to the arresting agency for the individual to remain in custody while the DNA sample is processed and compared against the NDNAD. This would require the availability of both rapid DNA processing and real-time access to the national database.

Within the laboratories of the FSS, current process allows for reference samples in urgent cases to be prioritized and, once delivered to the laboratory, these can be processed manually in as little as 8 h. However, this is a labor intensive and therefore relatively expensive process. The implementation of a rapid system whereby a reference sample can be processed within the police custody area would be of value to the law enforcement community: a suspect's DNA sample could be processed and compared to a database of crime sample DNA profiles while the individual remains in custody, eliminating the need to locate and rearrest an individual in response to a match on the database. Rapid elimination of an individual from an investigation could also be achieved, freeing up resources for the investigation of alternative leads in a criminal case.

A number of publications have reported approaches to the rapid analysis of DNA for forensic science and various methods have been described including the acceleration of specific parts

* To whom correspondence should be addressed. E-mail: andy.hopwood@fss.pnn.police.uk (A.H.); Frederic.Zenhausern@arizona.edu (F.Z.).

[†] Forensic Science Service.

[‡] The University of Arizona College of Medicine.

- (1) Hedman, J.; Albinsson, L.; Ansell, C.; Tapper, H.; Hansson, O.; Holgersson, S.; Ansell, R. *Forensic Sci. Int.: Genet.* **2008**, 2, 184–189.
- (2) Annual Report of The National DNA Database 2007–2009. <http://www.npia.police.uk/en/docs/NDNAD07-09-LR.pdf> (Accessed March 2010).
- (3) Morgan, P. M.; Henderson, P. F. Home Office Report 184 1998 ISBN 1 84082 074 8 <http://www.homeoffice.gov.uk/rds/pdfs/hors184.pdf> (Accessed Feb. 2010).

of the process such as PCR^{4–6} or the optimization of the whole process which requires the intensive use of highly skilled technicians.⁷ The application of Hybeacon technology which allows for a rapid analysis of STR loci from a crude lysate of buccal cells without the need for physical separation of the alleles^{8–10} has shown some promise but has the drawback that the resolution of microvariant alleles is difficult and the analysis of all required alleles in single tube is not currently achievable. The development of integrated microfluidic systems, so-called micro total analysis systems, or microTAS for DNA analysis has been discussed for many years and a number of groups have demonstrated successful modules for DNA extraction,^{11–14} PCR amplification,^{15–18} and CE.^{19–23} Integrated systems for extraction and PCR^{24–26} or PCR and CE^{27–29} have also been reported and illustrate that a fully integrated DNA analysis system should be feasible for forensic application. Indeed, full integration of all the constituent parts required for genetic analysis including the application of capillary

- (4) Vallone, P. M.; Hill, C. R.; Podini, D.; Butler, J. M. *Forensic Sci. Int.: Genet. Suppl.* **2009**, *2*, 111–112.
- (5) Vallone, P. M.; Hill, C. R.; Butler, J. M. *Forensic Sci. Int.: Genet.* **2008**, *3*, 42–45.
- (6) Wang, D. Y.; Chang, C. W.; Hennessy, L. K. *Forensic Sci. Int.: Genet. Suppl.* **2009**, *2*, 115–116.
- (7) Hopwood, A.; Fox, R.; Round, C.; Tsang, C.; Watson, S.; Rowlands, E.; Titmus, A.; Lee-Edghill, J.; Cursiter, L.; Proudlock, J.; McTernan, C.; Grigg, K.; Thornton, L.; Kimpton, C. *Int. Congr. Ser.* **2006**, *1288*, 639–641.
- (8) French, D. J.; McDowell, D. G.; Thomson, J. A.; Brown, T.; Debenham, P. G. *Int. Congr. Ser.* **2006**, *1288*, 707–709.
- (9) Gale, N.; French, D. J.; Howard, R. L.; McDowell, D. G.; Debenham, P. G.; Brown, T. *Org. Biomol. Chem.* **2000**, *6*, 4553–4559.
- (10) French, D. J.; Howard, R. L.; Gale, N.; Brown, T.; McDowell, D. G.; Debenham, P. G. *Forensic Sci. Int.: Genet.* **2008**, *2*, 333–339.
- (11) Ji, H. M.; Samper, V.; Chen, Y.; Hui, W. C.; Lye, H. J.; Mustafa, F. B.; Lee, A. C.; Cong, L.; Heng, C. K.; Lim, T. M. *Sens. Actuators, A Phys.* **2007**, *139*, 139–144.
- (12) Wolfe, K. A.; Breadmore, M. C.; Ferrance, J. P.; Power, M. E.; Conroy, J. F.; Norris, P. M.; Landers, J. P. *Electrophoresis* **2002**, *23*, 727–733.
- (13) Breadmore, M. C.; Wolfe, K. A.; Arcibal, I. G.; Leung, W. K.; Dickson, D.; Giordano, B. C.; Power, M. E.; Ferrance, J. P.; Feldman, S. H.; Norris, P. M.; Landers, J. P. *Anal. Chem.* **2003**, *75*, 1880–1886.
- (14) Oakley, J. A.; Shaw, K. J.; Docker, P. T.; Dyer, C. E.; Greenman, J.; Greenway, G. M.; Haswell, S. J. *Lab Chip* **2009**, *9*, 1596–1600.
- (15) Obeid, P. J.; Christopoulos, T. K.; Crabtree, H. J.; Backhouse, C. J. *Anal. Chem.* **2003**, *75*, 288–295.
- (16) Yang, J.; Liu, Y.; Rauch, C.; Stevens, R. L.; Liu, R. H.; Lenigk, R.; Grodzinski, P. *Lab Chip* **2002**, *2*, 179–187.
- (17) Giordano, B. C.; Ferrance, J.; Swedberg, S.; Hühmer, A. F. R.; Landers, J. P. *Anal. Biochem.* **2001**, *291*, 124–132.
- (18) Simpson, P. C.; Roach, D.; Woolley, A. T.; Thorsen, T.; Johnston, R.; Sensabaugh, G. F.; Mathies, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2256–2261.
- (19) Medintz, I. L.; Paegel, B. M.; Mathies, R. A. *J. Chromatogr., A* **2001**, *924*, 265–270.
- (20) Shi, Y.; Simpson, P. C.; Scherer, J. R.; Wexler, D.; Skibola, C.; Smith, M. T.; Mathies, R. A. *Anal. Chem.* **1999**, *71*, 5354–5361.
- (21) Shi, Y. *Electrophoresis* **2006**, *27*, 3703–3711.
- (22) Zheng, J.; Webster, J. R.; Mastrangelo, C. H.; Ugaz, V.; Burns, M. A.; Burke, D. T. *Sens. Actuators, B* **2007**, *125*, 343–351.
- (23) Goedecke, N.; McKenna, B.; El-Difrawy, S.; Carey, L.; Matsudaira, P.; Erlich, D. *Electrophoresis* **2004**, *25*, 1678–1686.
- (24) Lee, C. Y.; Lee, G. B.; Lin, J. L.; Huang, F. C.; Liao, C. S. *J. Micromech. Microeng.* **2005**, *15*, 1215–1223.
- (25) Legendre, L. A.; Bienvenue, J. M.; Roper, M. G.; Ferrance, J. P.; Landers, J. P. *Anal. Chem.* **2006**, *78*, 1444–1451.
- (26) Bienvenue, J. M.; Legendre, L. A.; Ferrance, J. P.; Landers, J. P. *Forensic Sci. Int.: Genet.* **2010**, *4*, 178–186.
- (27) Woolley, A. T.; Hadley, D.; Landre, P.; deMello, A. J.; Mathies, R. A.; Northrup, M. A. *Anal. Chem.* **1996**, *68*, 4081–4086.
- (28) Lagally, E. T.; Medintz, I.; Mathies, R. A. *Anal. Chem.* **2001**, *73*, 565–570.
- (29) Liu, P.; Yeung, S. H. I.; Crenshaw, K. A.; Crouse, C. A.; Scherer, J. A.; Mathies, R. A. *Forensic Sci. Int. Gen.* **2008**, *2*, 301–309.

electrophoresis has been demonstrated for the detection of *Bacillus anthracis* from whole blood³⁰ but to date we are unaware of any system which has been used to routinely produce an STR profile from DNA extraction to DNA profile with no operator intervention. Integrated microfluidic systems and their application to high-performance genetic analysis has been recently reviewed³¹ and will not be further discussed here.

Within the UK, 75% of individuals arrested are processed and released from custody within 6 h and about 95% are processed within 24 h.³² We defined our target for the process to be capable of processing the sample to give a DNA profile and returning any match from a database within 2 h such that the sample could be processed comfortably, and duplicated if required, within the 6 h window that a suspect is present in the custody suite.

The fluidic cartridge-based system described here is built using the principle of closed architecture, which minimizes any opportunity for contamination of the sample—a critical requirement for the forensic application—and performs DNA extraction, DNA amplification, resolution of the STR alleles by CE and detection using laser induced fluorescence (LIF). The polycarbonate cartridge for DNA extraction, PCR and post PCR manipulation is prefilled with the reagents required for the entire process and simply clips into an adapter which also holds the glass micro capillary electrophoresis (μ CE) chip used to resolve and detect the dye-labeled amplicons. The extraction-PCR cartridge is held in contact with an electronic circuit board which provides the functional control, allowing routine use by an unskilled technician with the minimum amount of training. Fluidic movement is fully automated and controlled using simple electro-chemical pumps and single use thermally activated valves as previously described,^{33,34} rather than more complex reusable thermally activated valves³⁵ avoiding the need for complex fittings and fixings to integrate the cartridge with the instrumentation, and eliminating the potential for sample contamination between the different functions of the cartridge. A servo-controlled magnet is activated as required for the collection of magnetic particles for DNA purification. Embedded resistive heaters are used to activate valves as previously described,³⁴ and Peltier devices employed for thermal cycling are programmed to activate in a specific sequence to facilitate processing. A miniaturized high voltage power supply is integrated to the hardware and allows for the separation of the STR amplicons through a matrix of polyvinylpyrrolidone (PVP) and hydroxyethylcellulose (HEC). Data collected from the μ CE is processed manually using commercially available software and the DNA profile is recorded in a format compatible with the data requirement for submission to the National DNA Database. The whole system is designed to allow simple loading of a crude cell lysate from a buccal scrape to the cartridge and robust walk-away

- (30) Easley, C.; Karlinsey, K.; Bienvenue, J.; Legendre, L.; Roper, M.; Feldman, S.; Hughes, M.; Hewlett, E.; Merkel, T.; Ferrance, J. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 19272–19277, Landers.
- (31) Liu, P.; Mathies, R. A. *Trends Biotechnol.* **2009**, *27*, 572–581.
- (32) Philips C. Her Majesty's Stationery Office, 1981; ISBN 0101809212 ASIN: B002K6GPAQ.
- (33) Liu, R. H.; Bonanno, J.; Yang, J.; Lenigk, R.; Grodzinski, P. *Sens. Actuators, B* **2004**, *98*, 328–336.
- (34) Liu, R. H.; Yang, J.; Lenigk, R.; Bonanno, J.; Grodzinski, P. *Anal. Chem.* **2004**, *76*, 1824–1831.
- (35) Pal, R.; Yang, M.; Johnson, B. N.; Burke, D. T.; Burns, M. A. *Anal. Chem.* **2004**, *76*, 3740–3748.

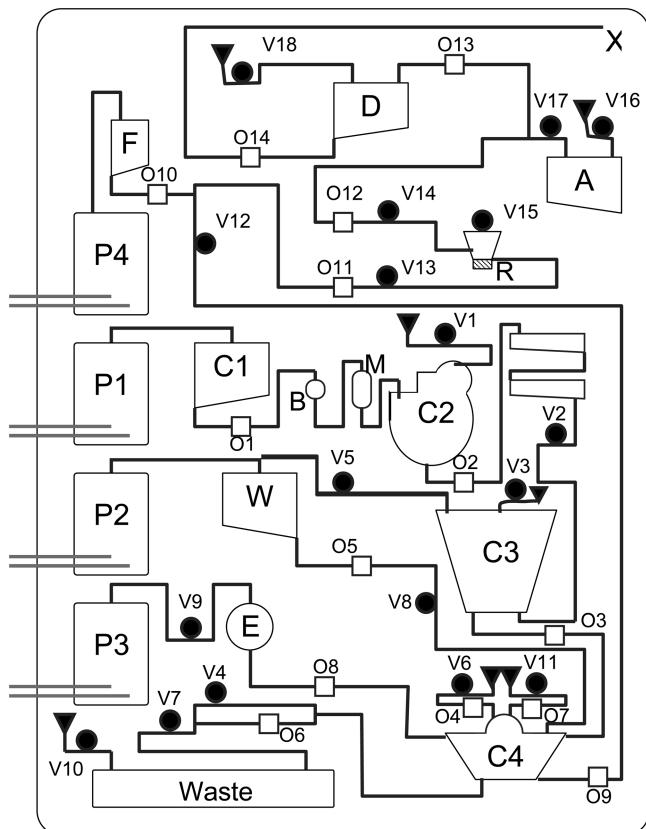


Figure 1. Representation of the Cartridge for DNA extraction, amplification and post PCR denaturation showing P1–P4 = electrochemical pumps; C1 = lysate input chamber; C2 = bead chamber; C3 = mixing/incubation chamber; C4 = washing and elution chamber; R = PCR chamber; A = DNA extract archive chamber; D = denaturation chamber; M = bead storage chamber; B = Binding buffer chamber; W = wash solution storage chamber; E = elution buffer storage chamber; F = Formamide/ILS storage chamber; X = output to CE. A closing valve is represented by ●; an opening valve by □; and a vent by ▼.

processing of the sample culminating in an STR profile which is compatible with The National DNA Database of the UK.

EXPERIMENTAL SECTION

Samples. Buccal samples were collected from consenting individuals using an OMNISwab (Whatman, Maidstone, UK) and processed immediately using the method described below.

Cartridge Design and Fabrication. The cartridge device for DNA extraction, amplification and post PCR manipulation (Figure 1) was made from 3 mm computed numerically controlled (CNC) machined polycarbonate (PC) plate stock and comprised a number of chambers for the storage of reagents and sample manipulation. The architecture of the fluidic system was designed such that outlets from one chamber to the next were typically on the bottom side of the chamber, thus taking advantage of gravity to ensure that any bubbles generated were removed from the fluid within the system. The channels linking the chambers were fitted with opening valves (O) and closing valves (V) formed from paraffin wax with a melting point of 65 °C (Sigma-Aldrich, Poole, UK) as previously described³³ which were hot dispensed into their respective positions. The exception to this was valve V15 which comprised two chambers connected vertically which were filled with different paraffin waxes; the lower chamber, closest to the

Table 1. Reagents and Volumes Required for Cartridge Operation. All Reagents Were Added Prior to Loading the Cartridge into the Instrument

chamber	reagent	volume required
pump 1–3	0.25 M NaCl	600 μL
pump 4	0.5 M NaCl	600 μL
M	ChargeSwitch (CS) beads	15 μL
	water	10 μL
B	CS purification buffer	30 μL
W	CS wash buffer	200 μL
E	CS elution buffer	150 μL
C1	Buccal lysate	150 μL
R	PowerPlex ESI 16 multimix in ReaX beads	two beads
F	Hi-Di formamide	45 μL
	ILS 500 CC5	5 μL

Peltier was filled with H1 Sasol wax with a nominal melting temperature of 105 °C (Sasol Wax North America Corp., Hayward, CA), the second chamber contained the standard paraffin wax. The H1 wax prevented V15 firing prematurely from exposure to the heat generated during the thermal cycling of the Peltiers. Aluminum wire electrodes were installed into the pump chambers, sealed using Dymax 1180-M UV glue and cured using a Dymax Bluewave 200 UV Curing System (Dymax, Torrington, CT) in accordance with the manufacturer's instructions. The PCR multimix was formulated into a ReaX bead (Q Chip Ltd., Cardiff, UK) and added to chamber R prior to binding the 0.5 mm PC cover to the 3 mm cartridge using a two sided pressure sensitive adhesive (90106 PSA, Adhesive Research, Glen Rock, PA) and pressing the assembly at 122 psi for 20 s and then 245 psi for 20 s. The ReaX bead-packaged multimix reagents are stable for at least 16 weeks when stored at 4 °C (data not shown). The assembled cartridge was stored at 4 °C until required. Other reagents required for sample processing were added to the appropriate reservoirs immediately prior to use as detailed (Table 1). The single-use cartridge was inserted into a holder in a vertical position and the 140 mm reusable glass microCE chip was positioned in a horizontal plane (Figure 2A). The two components were connected via a 100 mm length of Teflon tubing with an internal diameter of 200 μm which was manually connected to the output channel of the plastic cartridge at X in Figure 1, and to the sample well of the adapter on the electrophoresis chip (Figure 2B) by sliding over short sections of PEEK tubing (IDEX Corporation, Northbrook, IL) with an outside diameter of 0.78 mm (Figure 2C) prior to loading into the instrument. The Teflon tubing was renewed for each sample.

Electronic Control. The printed circuit board comprises embedded resistive heaters and control circuitry for managing the different operations required of the cartridge.

The instrument houses the electronic control systems, the optical excitation and detection equipment and holds the printed circuit board to which the cartridge is precisely aligned and clamped, to allow robust connection to the electrodes for the electrochemical pumps and surface contact for valve firing by thermal transfer. The operation of the cartridge is as follows. The cells on the buccal swab sample were directly lysed by adding 1 mL ChargeSwitch (Life Technologies, Inchinnan, UK) Lysis Buffer and 10 μL ChargeSwitch Proteinase K solution (20 mg/mL in 50

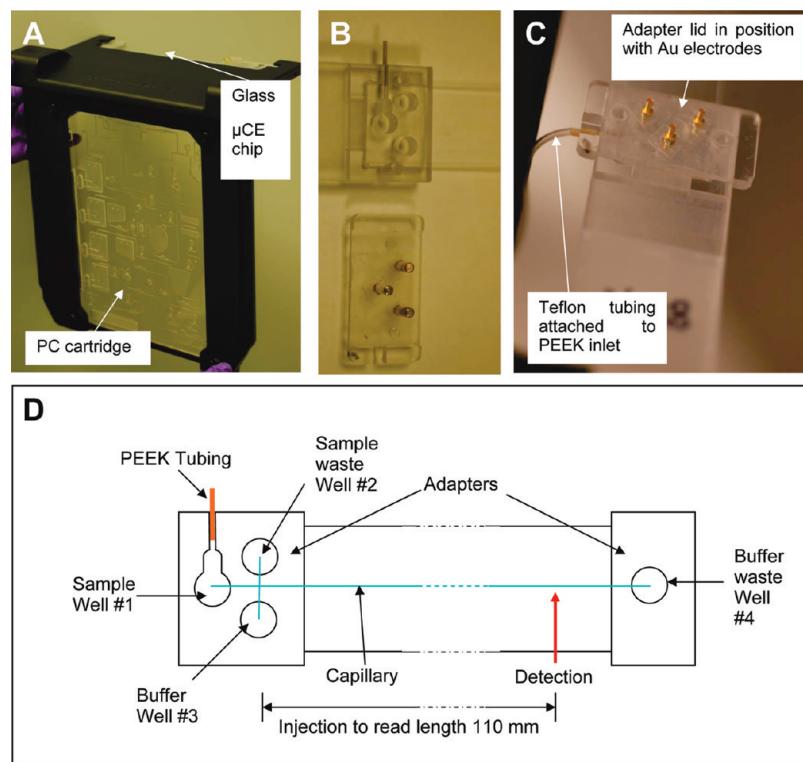


Figure 2. Assembly of the polycarbonate sample preparation cartridge and the borosilicate glass microCE chip. (A) The PC cartridge is positioned in a vertical plane, the glass CE chip in the horizontal plane. (B) Top image shows the adapter with wells for the electrodes. The PEEK tubing inlet can be clearly seen entering the elongated well of the sample well no. 1. Bottom image shows the adapter lid with gold-plated electrodes. (C) The adapter lid in position. The attached Teflon tubing can be seen attached to the PEEK inlet tube. (D) Representation of the adapters fitted to the CE chip and layout of the CE channel in relation to the wells.

mM Tris-HCl, pH 8.5, 5 mM CaCl₂, 50% glycerol) in a microcentrifuge tube and incubating at 60 °C for 15 min.

An aliquot of 150 µL of the sample lysate was introduced into chamber C1 in the analysis cartridge using a manual pipet. Pump P1 was fired to pressurize the system and valve O1 was opened to direct the lysate via the binding buffer chamber B containing 30 µL ChargeSwitch purification buffer, through the paramagnetic bead storage chamber M containing 25 µL of ChargeSwitch magnetic beads (1.87 mg/mL in 6 mM MES, pH 5.0, 6 mM NaCl) and into chamber C2, where the initial sample mixing occurred. The sample was then passed through two expansion chambers into chamber C3, where complete sample mixing was ensured by supplementary activation of the pump to produce a stream of bubbles which passed through the sample. The sample was incubated in this chamber for 3 min to facilitate DNA binding. The sample was then directed into chamber C4 where the ChargeSwitch magnetic beads were captured by a magnetic field of 450 ± 100 gauss generated via a servo activated magnet positioned behind the PCB. The supernatant liquid containing any unbound DNA and other lysis products was directed to the Waste chamber. The DNA-bound ChargeSwitch beads were then washed by flushing the beads with 200 µL of ChargeSwitch wash buffer from chamber W by activation of pump P2. One hundred and fifty µL of ChargeSwitch elution buffer (E5) was then directed to chamber C4 where the purified DNA was released from the beads by incubating at 60 °C for 3 min with the magnetic field removed. The beads were then recaptured by activation of the magnetic field and the eluted DNA solution was directed to the archive chamber A via the PCR chamber R by activation of pump P3.

The PCR chamber was designed to capture a 10 µL total volume of DNA solution and was preloaded with PowerPlex ESI 16 PCR amplification multimix (Promega, Madison, WI) packaged in ReaX reagent beads. The PCR chamber was sealed from the rest of the cartridge prior to amplification by firing valves V13 and V14.

Amplification of the 16 loci multiplex PCR system was performed as follows: Activation of the PCR mix at 96 °C for 2 min followed by 27 cycles of 94 °C for 30 s, 59 °C for 120 s, 72 °C for 90 s, and a final incubation at 60 °C for 45 min.

The valves V13 and V14 were then reopened by heating and the liquid wax pushed through to the paraffin trap chamber O12, and valve V15 activated to close the PCR chamber bypass channel. The PCR solution was then collected by flowing 45 µL Hi-Di formamide (Applied Biosystems, Warrington, UK) containing 5 µL internal size standard (ILS 500 CC5, Promega) from the formamide reservoir F using pump P4. The formamide/ILS/sample was collected in the denaturation chamber and incubated at 95 °C for 3 min prior to pumping to the CE chip.

The CE microchip was custom-made from Schott Borofloat borosilicate glass (Micronit Microfluidics BV, Enschede, The Netherlands). The electrophoresis channel had a semielliptic cross-section and measured 50 µm wide and 20 µm deep. A machined polycarbonate adapter was interfaced with the powder-blasted access holes on the microchip using UV-curable glue to provide buffer wells that could contain a maximum volume of 35 µL, and a PEEK attachment point for the Teflon tubing from the cartridge performing DNA extraction and amplification. A pair of polycarbonate plates, one with three 1 mm diameter gold-coated

Table 2. Capillary Electrophoresis Injection and Separation Conditions. The sample is Presented to the Sample Well No. 1 and Injected into the Separation Column by a Gated Injection

	sample sample well no. 1 (V)	waste well no. 2 (V)	buffer buffer well no. 3 (V)	waste well no. 4 (V)	buffer time (s)
loading	ground	800	ground	600	40
injection	ground	400	350	2000	2
separation	ground	400	ground	2700	1000

pins (Figure 2B), one with a single pin were designed as adapter lids to clip onto the adapters to close the fluidic system (Figure 2C) and connect to the control system. The reusable CE chip was prepared by flushing with water to remove the polymer from the previous run, followed by 1 M hydrochloric acid for 10 min and rinsing with water for 10 min to remove the acid prior to loading the polymer. The polymer, 3.5% w/v PVP/HEC in a 20/80 ratio in 1 × ABI 310 running buffer (Applied Biosystems) acted as both sieving and coating matrix³⁶ and was loaded for 25 min at 50 °C. Owing to the design of the cartridge system, the risk of contamination of the subsequent sample with PCR products from the CE chip was minimized by loading the reagents for the plastic cartridge before connecting it to the CE chip. A volume of 25 μL of 1 × ABI 310 running buffer was added to each of the wells of the electrophoresis chip prior to clipping the adapter lids in place. The sample was delivered into the sample well and injected into the separation channel using a gated injection scheme³⁷ prior to separation under the conditions described in Table 2 and the labeled fragments were detected at a point 110 mm from injection. The electrophoresis chip was maintained at a temperature of 50 ± 0.15 °C through direct contact with a heater plate.

Laser-Induced Fluorescence (LIF) Detection. LIF detection was achieved with a modified confocal fluorescence setup. The excitation source, a diode-pumped solid-state (DPSS) laser (Calypto, Cobolt AB, Solna, Sweden) was cleaned-up using a 480–520 nm band-pass filter (Omega Optical, Brattleboro, VT) and aligned onto a high-reflectivity elliptic mirror centered on a Raman laser reflector. The laser output was adjusted to provide 21 mW of power at 491 nm to the CE microchip and the incident light was focused onto the CE microchip using a ×40 objective (LucPLFLN, Olympus, Center Valley, PA), such that a circular 60 μm Gaussian beam hit the microchannel at a point 110 mm from the injection point. Alignment and focusing of the chip and detection system was achieved by a push-button activated servo motor and a micrometer screw respectively, on a 3 mm translation stage (Thorlabs, Newton, NJ).

The emission wavelengths of the fluorescently labeled DNA fragments were collected onto a cooled charge-coupled device (CCD) camera (Newton 920, Andor, South Windsor, CT) mounted onto a high-throughput spectrometer (CP140-1605, Horiba Scientific, Newton, NJ) after a long-pass emission filter (cut-on: 520 nm). The diffraction grating in the spectrometer disperses the different wavelengths in the emission spectrum over the length of the CCD, allowing a quantitative measurement of each color

(36) Boulos, S.; Cabrices, O.; Blas, M.; McCord, B. R. *Electrophoresis* 2008, 29, 4695–4708.

(37) Ermakov, S. V.; Jacobson, S. C.; Ramsey, J. M. *Anal. Chem.* 2000, 72, 3512–3517.

in the sample over time. The instrument design is further described in Hurth et al.³⁸

The raw spectral data from the CCD was deconvoluted to provide peak information for each of the five colors used in the multiplex analysis using NanoIdentity v 1.12 (SoftGenetics, State College, PA) and the data were further analyzed to provide genotype information using GeneMarker HID V 1.76 (Soft-Genetics).

During optimization and testing of protocols, DNA quantification of the DNA extracted from the samples was performed using the Quantifiler Human DNA Quantification Kit (Applied Biosystems) in accordance with the manufacturer's instructions.

Electrophoresis of control samples was executed using the Applied Biosystems 3130xl in accordance with the PowerPlex ESI 16 kit instructions.

RESULTS AND DISCUSSION

The aim of the process is to generate a DNA profile with all 16 loci present in the electropherogram. To ensure this happens, a number of variables were identified as critical to the efficiency of the process: Sample collection; DNA extraction; PCR efficiency; PCR reaction recovery and robustness of the CE detection instrumentation. Each of these variables have been investigated and stabilized as far as possible.

The sample input is the major cause of variation. Different samples taken from different individuals will have different quantities of cells, releasing different quantities of DNA upon lysis. For example, van Wieren-de Wijer et al observed total DNA yield ranging from 0.08 to 1078.0 μg (median 54.3 μg; mean 82.2 μg).³⁹ Since there is no simple way to normalize the DNA concentration on the cartridge by dilution, it was essential to minimize this variation in DNA concentration extracted from the buccal swab by some means. The ChargeSwitch gDNA Normalized Buccal Cell Kit is claimed to produce a normalized yield of genomic DNA at a concentration of 1–3 ng/μL in 150 μL under standard conditions.⁴⁰ Compared with other available DNA extraction chemistries the ChargeSwitch protocol is comparatively simple and the reagents were considered less likely to cause inhibition of the downstream processes. In our experience, with samples of freshly collected swabs extracted on cartridge as described above, a DNA concentration of between 0.47 and 1.92 ng/μL of DNA with a mean of 0.86 ± 0.41 ng/μL (*n* = 27) was routinely attained.

The amount of DNA produced by PCR amplification varied with a number of factors including the amount of DNA in the reaction, the total volume of the PCR and the number of cycles of PCR. The volume of DNA extract added to the reaction was governed by the size of the PCR chamber and the volume of the ReaX beads therein. The PCR chamber (Figure 3) was designed such that it is filled by the DNA solution traveling from the chamber C4 to the archive chamber A and the volume of the

(38) Hurth, C.; Smith, S.; Nordquist, A.; Lenigk, R.; Surve, A.; Hopwood, A.; Haley, J.; Chen, X.; Estes, M.; Yang, J.; Cai, Z.; Lee-Edghill, J.; Moran, N.; Elliott, K.; Tully, G.; Zenhausern, F. *Rev. Sci. Instrum.* 2010, Submitted.

(39) van Wieren-de Wijer, D. B. M. A.; Maitland-van der Zee, A. H.; de Boer, A.; Belitsier, S. V.; Kroon, A. A.; de Leeuw, P. W.; Schiffrers, P.; Janssen, R. G. J. H.; van Duijn, C. M.; Stricker, B. H. C. H.; Klungel, O. H. *Eur. J. Epidemiol.* 2009, 24, 677–682.

(40) ChargeSwitch® gDNA Buccal Cell Kits. Instruction Manual. Invitrogen Life Technologies. http://tools.invitrogen.com/content/sfs/manuals/chargeswitch_gdna_buccal_man.pdf (Accessed March 2010).

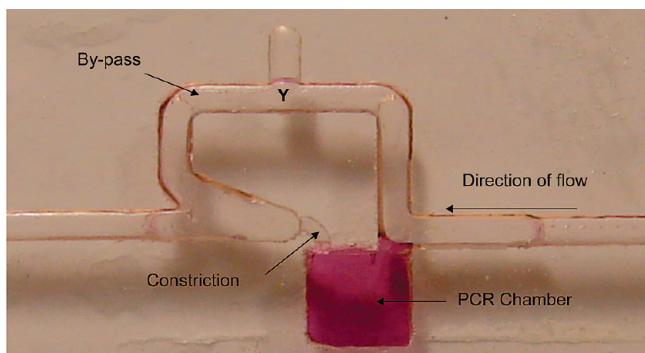


Figure 3. Design of the PCR chamber fluidic system. The DNA solution enters the chamber from the right and fills the chamber. When the liquid level reaches the constriction channel in the top left-hand side of the chamber, the pressure required to pass through this constriction is much greater than that required for the solution to traverse the bypass. The DNA solution therefore passes over the bridge, leaving a precise volume of DNA in the PCR chamber (chamber highlighted by red solution). Following PCR amplification, the reaction volume is recovered by closing the bridge at Y using valve V15 and flushing the formamide through the chamber, collecting the PCR product.

chamber was filled until the solution reached the restriction channel. The pressure required to breach this channel was greater than that required for the solution to flow over the bypass and consequently the flow took that direction, leaving a precisely measured volume of PCR reaction ($9.96 \pm 0.21 \mu\text{L}$ ($n = 20$)) in the PCR chamber. The amount of DNA in the $10 \mu\text{L}$ PCR chamber therefore approximated $5.2 \pm 2.5 \text{ ng}$, based upon the ReaX bead volume of $4 \mu\text{L}$.

Samples recovered from the PCR chamber and run on the AB 3130xl gave similar peak heights to the control samples amplified in tube (data not shown).

Following amplification, V15 is closed and the whole of the PCR sample is recovered from the PCR chamber and delivered with ILS 500 CC5 in formamide to the loading well of the electrophoresis chip. We rely on surface effects to smoothly remove the vast majority of the PCR reaction from the chamber. As the formamide is driven from its storage chamber, the air in the channel in front of the formamide is pushed through the PCR chamber and removes the bulk (80–90%) of the PCR reaction through the constriction. The formamide then fills the chamber, taking the remaining PCR solution with it. The surface tension at this small scale ensures that the bulk of the formamide is also flushed from the chamber leaving just one or two microlitres in the 90° corners of PCR chamber. The volume delivered to chamber D varied between cartridges with a mean volume of $25.1 \pm 4.8 \mu\text{L}$ ($n = 25$) with a range of 15 – $35 \mu\text{L}$. The variation in volume was due to three things: (1) The wax in the valves 13 and 14 is molten as the liquid flows by, allowing some liquid to mix with the wax. This liquid is trapped in the system as the wax solidifies. (2) Liquid is occasionally lost in the valve structures of V13 and/or V14. (3) Our channels are machined rather than injection molded, creating a rough surface that can retain 1–2 μL per inch of channel. The volume of sample released from PCR, denatured in chamber D and delivered to the μCE is a variable that could have a significant effect on the final result: If only half of the PCR volume of a given PCR product was recovered we would expect to observe lower peak heights in the profile. The

system was therefore designed to push the PCR mixture out of the PCR chamber prior to flushing the chamber with formamide and we believe that the majority of the PCR product is recovered, minimizing any variability from this function of the cartridge. Sample loss in the channels, wax and valve housings in the circuit before the PCR chamber will reduce the amount of formamide and size standard reaching the denaturation chamber while the inefficiencies in the circuitry post PCR chamber would reduce the volume of PCR product and formamide in proportion to their volumetric ratios. Current laboratory practice is that the sample is heat denatured at 95°C for 3 min and snap-cooled on ice prior to loading onto the 3130xl platform for injection to the CE. While the sample was easily heat denatured on the cartridge, no facility for snap cooling was available and the sample was loaded directly into the CE. Evaluation of the profiles demonstrated that this process was acceptable because the DNA profiles produced with no snap cooling were found to be comparable to those that had been snap cooled. The electropherogram from the internal lane standard (GeneScan 500 LIZ) diluted to a volume ratio of 9:1 with Hi-Di formamide (Applied Biosystems) and heat-denatured at 95°C for 5 min was used to assess the separation efficiency of the μCE module. The chromatographic resolution R was calculated by fitting the peaks with a Gaussian curve to obtain the migration time, t_i , and the full-width at half-maximum, w_i for peak i according to the following equations:⁴¹

$$R = (2 \ln 2)^{1/2} \frac{t_2 - t_1}{w_1 + w_2}$$

And subsequently: $R_{\text{bp}} = \Delta \text{bp}/R$ expresses the resolution in base pairs, that is, the minimum base pair separation of two peaks that would still appear separated at the baseline in the electropherogram. Figure 4A displays an example of an electropherogram which represents the “binned” data prior to deconvolution into the target wavelengths, that is, the electropherogram represents the sum of photon counts over time, and represents the whole of the signal collected by the CCD by time. Figure 4B shows the evolution of the resolution R_{bp} on the μCE system as a function of the known peak size (red trace) and the comparison with an electropherogram obtained on a commercial ABI 310 capillary electrophoresis analyzer (Applied Biosystems) using the same polymer matrix at 65°C with a cathode potential of 10.3 kV. The resolution obtained on the μCE module is very close to that of a commercial CE apparatus.

Typically, the resolution values obtained for a GeneScan 500 LIZ varied between 1.02 and 1.24 bp for peaks within the 140–160 bp range. Ongoing work aims at further improving the resolution by (1) using a pinched injection and (2) tailoring the composition of the HEC/PVP mixture for use in a microchip rather than a capillary.

A series of allelic ladder runs was recorded on the CE subsystem using a CC5-labeled ILS 500 size standard (Promega) to estimate the run-to-run stability of the system. Consecutive runs were acquired on three different microchips to determine the variability between microchips. Figure 5 shows a typical sized run processed with GeneMarker HID 1.76. Inserts B though E are

(41) Manabe, T.; Chen, N.; Terabe, S.; Yohda, M.; Endo, I. *Anal. Chem.* **1994**, 66, 4243–4252.

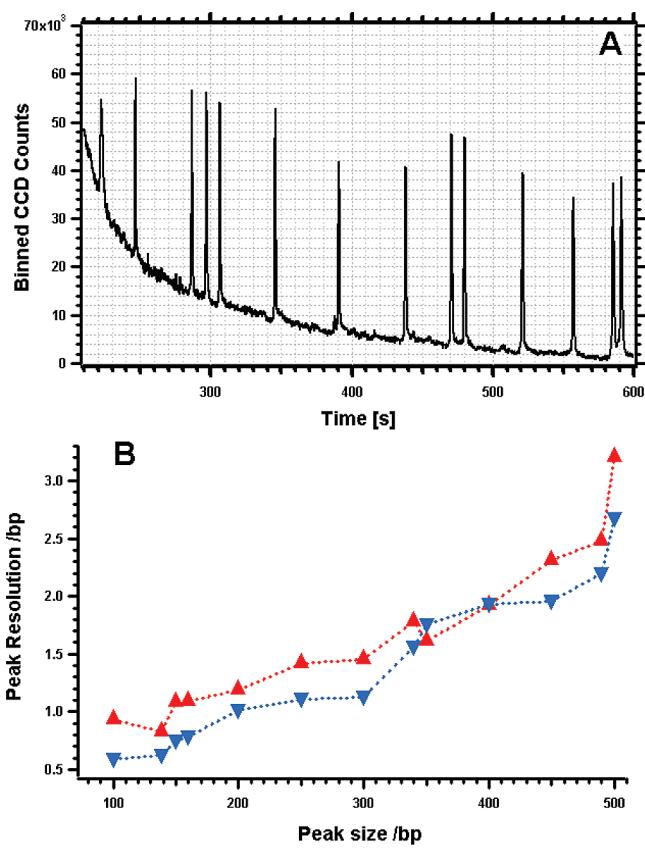


Figure 4. (A) Typical raw electropherogram obtained for a GeneScan 500 LIZ run on the CE subsystem using a 140 mm microchip at 50 °C. (B) Comparison of the resolution (in base pair) R_{bp} of the μ CE system (red trace; \blacktriangle) to that obtained on a 310 apparatus (Applied Biosystems) at 65 °C in a 36 cm capillary (blue trace; \blacktriangledown).

close-ups of the [7–9] allele region (304–310 bp) on the D22S1045 marker, the [10–11] region (146–150 bp) on the D18S51 marker,

the [9–11] region (97–105 bp) on the TH01 marker, and the [21.2–26] region (177–195 bp) on the FGA marker respectively. Table 3 summarizes the observed shifts in base pair sizing for five different allelic ladder runs using a number of alleles from Figure 5B–E. The maximum observed variation around the tabulated value in the positioning of an allele is only about 0.2 bp on the TH01 marker, 0.7 bp on the D18S51 marker, 0.4 bp on the FGA marker, and 0.7 bp on the D22S1045 marker. The variability increases with the fragment size as expected from the absolute chromatographic resolution of the CE subsystem given in Figure 4B. The allele sizing data gained from running a minimum of five allelic ladders of known designations was used to define the expected sizes of the unknown alleles in the amplified DNA samples.

Integrated Sample Run. We were able to obtain STR profiles from lysed cells from buccal swabs using a disposable plastic cartridge attached to a reusable glass microchip device without any manual interruption of the programmed DNA extraction, purification, amplification, transfer and CE analysis process. A typical electropherogram including the ILS is shown in Figure 6 after applying GeneMarker HID 1.76 to size the migrated DNA fragments. The DNA profiles obtained as an integrated automated run were always similar to those obtained on an Applied Biosystems ABI 3130xl analyzer in terms of the number and position of the peaks and their relative intensities. Currently we are unable to routinely label all the peaks with a definitive allele designation as the migration of the peaks of some samples has given out of window peaks. We believe this may be due to a difference in the time base of the instrumentation and the NanoIdentity software: The software assumes an equal time base per data point while the instrumentation collects data on a variable time base which averages 0.21 s with a maximum of 0.35 s and a minimum of 0.07 s. An updated

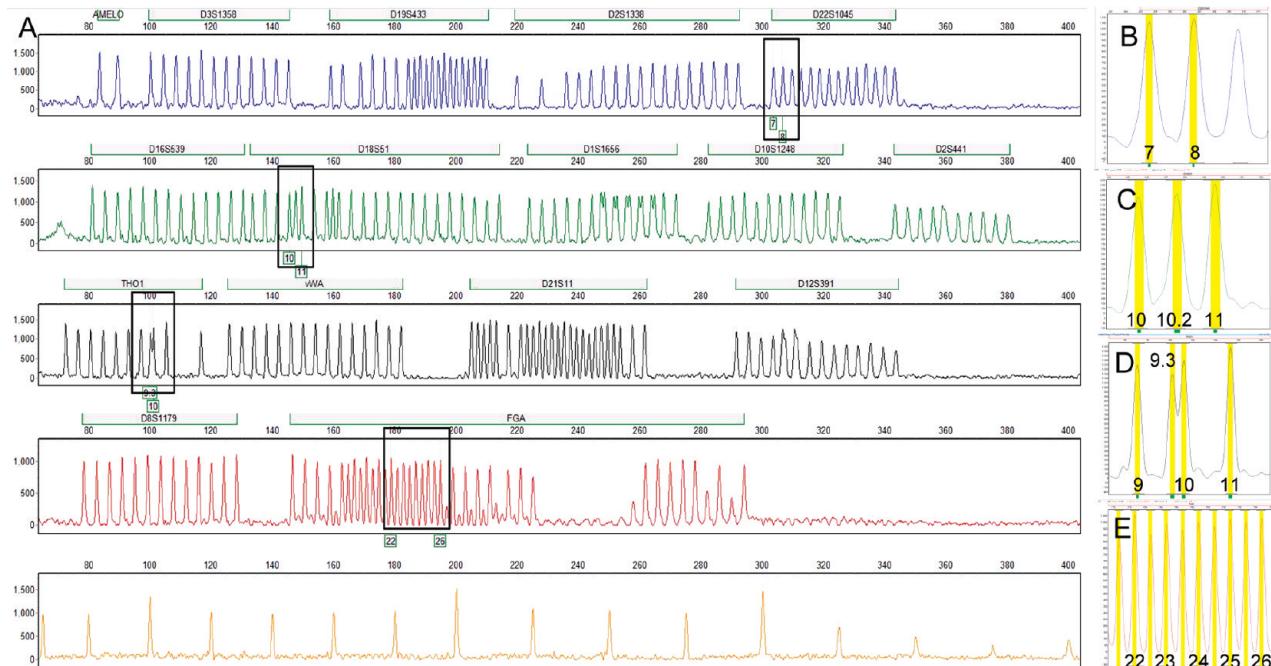


Figure 5. (A) Powerplex ESI16 allelic ladder run using CC5 ILS500 size standard (Promega, Madison, WI) sized using GeneMarker HID 1.76 (SoftGenetics LLC, State College, PA) with appropriate panels and bins provided by Promega. (B) Close-up of the [7–9] allele region (304–310 bp) on the D22S1045 marker. (C) Close-up of the [10–11] region (146–150 bp) on the D18S51 marker. (D) Close-up of the [9–11] region (97–105 bp) on the TH01 marker. (E) Close-up of the [21.2–26] region (177–195 bp) on the FGA marker.

Table 3. Evolution of the Sizing Results for Specific Regions of an Allelic Ladder Run for Three Different Chips and Five Different Runs^a

D22S1045	size of allele no. 7 (bp)	size of allele no. 8 (bp)								
chip 1 - 1st load	304.2	307.3								
chip 2 - 1st load	305.6	308.2								
chip 2 - 2nd load	304.1	307.2								
chip 3 - 1st load	304.3	307.2								
chip 3 - 2nd load	304.0	307.2								
D18S51	size of allele no. 10 (bp)	size of allele no. 10.2 (bp)	size of allele no. 11 (bp)							
chip 1 - 1st load	146.1	148.2	150.2							
chip 2 - 1st load	147.1	149.2	151.1							
chip 2 - 2nd load	146.3	148.3	150.2							
chip 3 - 1st load	145.8	147.7	149.7							
chip 3 - 2nd load	145.9	147.8	150.0							
TH01	size of allele no. 9.3 (bp)	size of allele no. 10 (bp)								
chip 1 - 1st load	100.2	101.2								
chip 2 - 1st load	100.3	101.3								
chip 2 - 2nd load	100.2	101.1								
chip 3 - 1st load	100.1	101.4								
chip 3 - 2nd load	100.2	101.2								
FGA	size of allele no. 21.2 (bp)	size of allele no. 22 (bp)	size of allele no. 22.2 (bp)	size of allele no. 23 (bp)	size of allele no. 23.2 (bp)	size of allele no. 24 (bp)	size of allele no. 24.2 (bp)	size of allele no. 25 (bp)	size of allele no. 25.2 (bp)	size of allele no. 26 (bp)
chip 1 - 1st load	176.8	178.8	180.8	182.8	184.8	186.8	188.9	190.8	192.8	194.8
chip 2 - 1st load	177.2	179.3	181.0	183.0	184.2	186.0	188.0	189.8	191.6	193.3
chip 2 - 2nd load	177.0	178.9	180.7	182.7	184.5	186.6	188.8	190.7	192.6	194.7
chip 3 - 1st load	176.8	178.6	180.7	182.7	184.5	186.6	188.8	190.7	192.6	194.7
chip 3 - 2nd load	176.6	178.5	180.6	182.5	184.7	186.6	188.6	190.6	192.7	194.5

^a The values represent the calculated size of the designated allele in DNA base pairs relative to the ILS in the same run. The markers are chosen to represent 1 marker per color, cover most of the regions of interest [100–300 bp], and correspond to the most challenging peaks to separate.

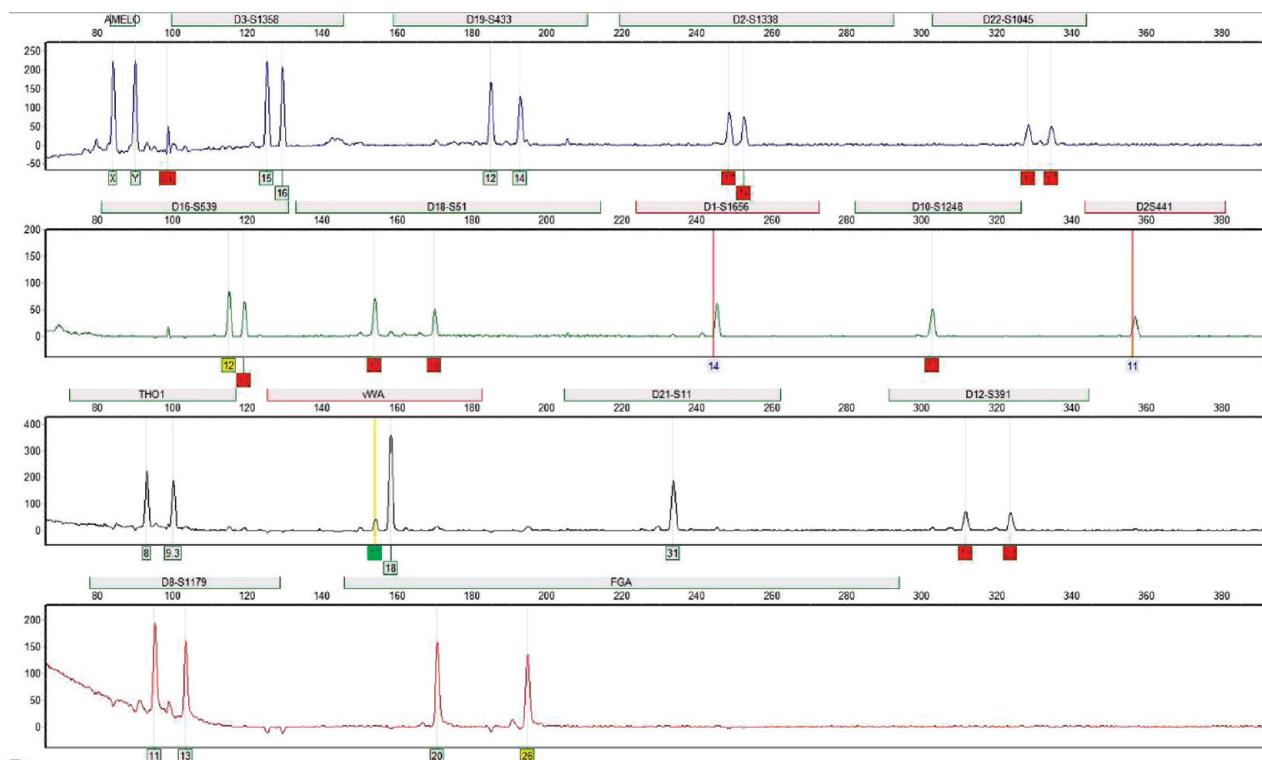


Figure 6. Micro Capillary Electrophoresis profile from a fully integrated run. DNA was purified and amplified on-cartridge from lysed cells from a buccal swab and transferred automatically via Teflon tubing to the glass microchip. Allele designation was performed using GeneMarker HID 1.76 (SoftGenetics LLC).

version of the software is in development which will use the time file provided by the instrument and so align the data correctly on a true time scale.

CONCLUSION

We have demonstrated a successful microTAS approach to the delivery of rapid DNA technology suitable for the forensic

field. DNA profiles were delivered from a crude DNA lysate with no manual input. The automated process is simple to use and provided DNA profiles which were concordant with the expected DNA profile of the donor. Our target is to deliver an evidential quality DNA profile from sample to profile in less than 2 h. While we currently have the capability of processing the sample in under 4 h, we believe that there are a number of areas where we can speed up the process. A major time saving (approximately 1.5 h) can be made simply by reducing the PCR cycling parameters from those published here to thermal profiles similar to those described previously.^{4–6} Optimizing the cartridge operating process to allow parallel processing of valve firings and pump control will further reduce the processing time and we believe we will soon be able to meet the target time of 2 h for delivery of the genotyped DNA profile. The turn around between samples is approximately 10 min to load the solutions into the single-use plastic cartridge, and 75 min for preparation of the CE chip. This can be carried out while the previous sample is running, provided that a number of CE chips are available, reducing the turn around time to little more than 5 min. The resolution obtained on the μ CE module is very close to that of a commercial CE apparatus and the reproducibility of fragment migration was good: With allelic ladders the maximum variation in the positioning of an allele was around 0.2 bp on the TH01 locus, 0.7 bp on the D18S51 locus, 0.4 bp on the FGA locus, and 0.7 bp on the D22S1045 locus. The migration of DNA profiles from human samples does require some improvement if the system is to be used for probative samples as we were unable to successfully type all integrated runs due to a small variation in the apparent migration of fragments. This variation is likely to be minimized by implementation of a new version of the NanoIdentity software which will take the true time file from the instrument, rather than assuming all data points are equidistant in time. The largest variation in the process remains the amount of template DNA input to the PCR reaction, and while this impacts the peak

heights observed in the electropherogram, this does not affect the accuracy of the genotyping of a sample.

Simple substitution of the multiplex chemistry in the cartridge will allow the production of DNA profiles suitable for loading to any established national DNA database. However, the rapid delivery of a DNA profile, whether at a crime scene, or in a custody suite is only a part of the puzzle. If the full impact of these new technologies is to be gained, the rapid delivery of chemistry must be supported by a capability to submit and compare the DNA profile in near real time. Currently neither the CoDIS database, nor the UK National DNA database have the capability to support rapid chemistry protocols.

While the operation of the instrument is straightforward, a good level of training is required before the instrument can be operated but we believe that any individual with a basic scientific education could become competent in the operation of the instrument. Our vision for the future is to have a single fully integrated injection-molded cartridge with reagents preloaded with capability for DNA purification, amplification and μ CE-based separation. Such a cartridge, coupled with automatic channel finding and focusing will minimize set up time and make the system usable by an individual with very basic training.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of Glen McCarty, David Nguyen, and Brett Duane for electronics design and prototyping; Baiju Thomas, Keith Burt, and Matthew Barrett for plastic device fabrication; Dr. Jian Gu and Peter East for microfabrication; and Amol Surve for instrumentation design. We also thank Bruce McCord for the provision of the separation matrix and helpful discussion around microCE and Promega Corporation for the generous gifts of PowerPlex ESI 16 STR kits and ILS 500 CC5.

Received for review May 24, 2010. Accepted July 1, 2010.

AC101355R

RESEARCH PROFILE

Catching suspects in the nick of time

Soon, law enforcement officials in the U.K. may be able to match arrestees to unsolved crimes within hours. In their recent *AC* paper (DOI 10.1021/ac101355r), Andrew Hopwood at the Forensic Science Service (U.K.), Frederic Zenhausern at the University of Arizona, and colleagues describe an instrument that spits out a suspect's DNA profile within a few hours of collecting a cheek swab.

Currently, police face a delay of several weeks as a centralized laboratory analyzes DNA collected from arrested suspects. Suspects are often released from custody while the laboratory processes samples and may commit another offense in that timeframe. To prevent this, law enforcement officials need to be able to do DNA profiling at police stations and booking sites as quickly and efficiently as possible.

So Hopwood, Zenhausern, and colleagues have developed an instrument to do just that. At the heart of the instrument lies a microfluidic platform, which extracts DNA from cheek cells collected on cotton swabs and amplifies the DNA by PCR. Short tandem repeats (STRs) in the DNA that are unique to each individual are identified by capillary electrophoresis laser induced fluorescence (CE-LIF) detection, producing an STR profile. The STR profile could then be compared to those held in the U.K.'s National DNA Database to see if the person under arrest can be connected to an unsolved crime.

"It is the first published device that goes from start to finish to generate a DNA STR profile," states John Butler at the National Institute of Standards and Technology.

The microfluidic platform consists of two cartridges preloaded with the necessary reagents. The two cartridges are connected together with a Teflon tube. The first cartridge, made of polycarbonate, houses the reagents for solid-phase DNA extraction and PCR amplification. The second cartridge for



COURTESY OF FREDERIC ZENHAUSERN

The microfluidic platform is inserted into the instrument for STR profiling of suspects in police custody.

CE-LIF detection of the PCR products is made of either glass or the copolymer COC.

The two-cartridge setup neatly sidesteps problems of making a single cartridge multipurpose. "I thought it was a nice idea because they don't have to do CE on the same [cartridge] as the extraction step and the PCR," says Butler.

Steven Soper at Louisiana State University points out that many of the processing steps the authors describe have already been published in the literature, but the authors get credit for combining the steps. "The microfluidics community needs to realize that integration of processes is not easy at all. Getting steps to be compatible with each other when done in a serial fashion on a chip is not a trivial matter," he says. "The authors are on the right path with their integration efforts."

Zenhausern says that subsequent designs of the microfluidic platform will integrate all the functions onto a single cartridge—they are now working on figuring out which materials work best for fabrication. "You either do every-

thing in the same substrate, such as COC, or you can do a hybrid fabrication where you have different components of the cartridge with different material substrates," he explains. "We are optimizing the fabrication to make the system as cheap as possible."

Zenhausern emphasizes that their instrument isn't a mere laboratory prototype. "It isn't held together with rubber bands!" he quips. "We are already making hundreds of the cartridges with the consumables. We have a process now for manufacturing that can scale up to several thousands of cartridges, and we can go beyond that."

Besides improving the technical aspects, the investigators are focusing on validation studies with users of the instrument. They want to be sure that the overall configuration of the platform meets the users' needs "in a very compelling and competitive manner so they will adopt the technology over others," says Zenhausern.

The legal aspect also has to be considered now that the police themselves may carry out the measurement. "These measurements are not being done in a laboratory, but at the police station," explains Zenhausern. He wonders how these will be accepted in court.

The applications of the instrument aren't limited to just STR profiles of cheek swabs. Zenhausern explains his team originally began working on the platform when the U.S. Federal Bureau of Investigations approached them to analyze semen and vaginal samples from rape cases, so they are currently developing a microfluidic cartridge specifically geared for those cell types. Zenhausern points out that they can also "change the cartridge and the assay chemistry and run the system for infectious disease detection or for other types of measurements based on molecular tests."

—Rajendrani Mukhopadhyay