

Lab on a Chip

Supplementary Information

Integrated sample-in-answer-out microfluidic chip for Rapid Human Identification by STR analysis

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1. Microfluidic chip fabrication

The microfluidic chips were fabricated by MiniFAB Pty Ltd (Scoresby, Australia). The chip was injection molded out of Zeonor 1420R (Zeon Chemicals, Louisville, KY, USA) with microfluidic features on both molding inserts (Fig. S.1a and S.1b). ZEONOR® is a Cyclic Olefin Polymer (COP) that have become widely used in the microfluidic device designs due to biocompatibility and optical properties.¹

The microfeatures on both molding inserts create a single injection molded piece with microfluidic features on two layers that can complete all aspects of the assay. A 100 µm film of the same material was solvent bonded to the bottom side of the microchips (**Fig. S.1c**). The bottom side of the chip contains the separation channel, PCR chamber and several fluidic transport channels (**Fig.S.1d**). Next, gold is sputtered from the top side to create the electrodes for electrophoretic separation. Electrodes were sputtered to a thickness of approximately 100 nm. The pneumatic interface region is layered with a double-sided adhesive, AR90445 (Adhesives Research, Glen Rock, PA, USA), with laser cut holes that aligned to the ports in the molded microfluidic chip. Hydrophobic membrane (PTFE, 0.2 micron, SterliTech, Kent, WA, USA), was then placed over the adhesive to form the pneumatic interface region (**Fig. S.1e**).

A top laminate was an aluminum-acrylic laminate composite and was laser cut to the dimensions of the chip and with several critical features. Two perforated lines were created that align both sides of the pneumatic interface region (**Fig. 1c**). This allowed the top film to be laminated as a single piece yet allowing an easy peel action just prior to reveal the protected hydrophobic membrane (from debris, scratch etc). Cutouts in this layer were created for the detection zone and around the sample waste electrode. A track outlining the sample reservoir was also created to further isolate that electrode as the acrylate layer may not provide a perfect insulating boundary and in some cases allow resistance on the order of the separation channel (1-10 MΩ) causing current leakage. These cutouts and tracks around the electrodes served to reduce this possible failure mode. Polyimide tape (McMaster Carr, Elmhurst, IL, USA) was placed over these cutouts and tracks to ensure

that liquid was contained within the microchip. A cap was placed over the luer connectors until the chip was used to prevent contamination or debris from entering this feature. This assembled chip was then ready for reagent loading (**Fig. S.1f**).

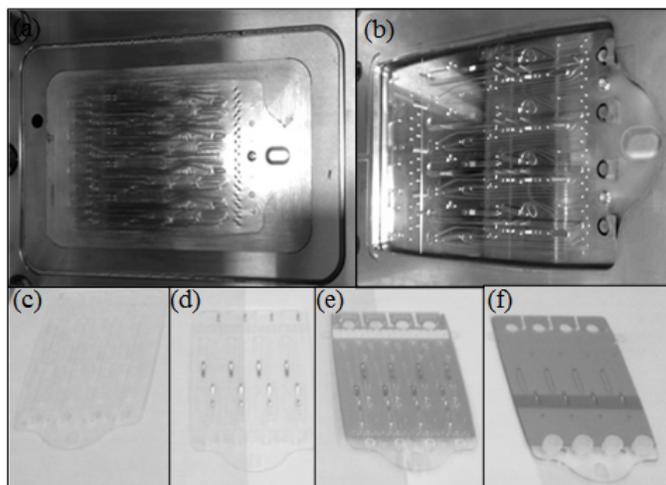


Fig. S.1. Microfluidic chip manufacture process. (a) Picture of the bottom molding part of the insert. (b) Picture of the top molding bottom part. (c) Chip after injection mold and bottom layer bond. (d) Chip after gold sputtering. (e) Chip after different aluminum-acrylic composite and hydrophobic membrane layers bonding. (f) Chip after polyimide tape and luers cap addition.

2. Sample collection

Buccal cells were collected after a 30 seconds mouth rinsing in both cases, by turning the buccal brush swabs against the cheek for 30 seconds and using a DNA collector for FTA™ (sterile foam-tipped applicator, Puritan Medical Products Company LLC, Guilford, ME, USA) after putting it against the tongue for 30 seconds to moisturize it and scrub each of the cheeks with each of the side of the collector. The buccal swab was directly put into the swab acceptor attached to the chip. The DNA collector was applied onto one circle of the FTA mini card to get the maximum of cells.

3. Analytical processes inside the microfluidic chip

Microchip PCR is accomplished by IR-heating and non-contact temperature sensing as described in Le Roux *et al.*². Based on these results, the thermocycling conditions were an initial denature of 94 °C for one minute, 29 cycles of 94 °C for 10 seconds and 59 °C for 40 seconds, and a final extension of 59 °C for 2 minutes. Compared with the previous report², these conditions increase the number of cycles from 27 to 29 and slightly increase the length of the anneal and denature times to increase peak heights. The increased in peak heights with longer anneal and denature times may be a function of differences in thermal mass between the two systems causing a difference in heating and cooling ramp rates.

Microchip electrophoresis for STR analysis was previously demonstrated in a 7 cm effective separation length following a standard cross-T injection method. The microchip used the same effective separation length but with different channel lengths from the cross-T node to the electrodes. The voltage drop along each arm was kept between the two designs requiring different applied voltages. The sample was electrokinetically injected by applying 300 V at the sample waste with the sample electrode grounded for 360 sec. A 6 second gated injection was used with 1500 V at the outlet and the sample electrode again grounded. The separation was performed with 1500 V on the outlet electrode, 250 V and 300 V on the sample and sample waste electrode, respectively, and the buffer electrode grounded. The stage heater temperature that controlled the separation polymer temperature within the microchip channel was set to 43°C.

To test automated alignment of the system, the separation channel was loaded in the same manner as for an end-to-end sample analysis. The sample reservoir was loaded with a fluorescein solution. The channel was aligned as normal by maximizing the signal of the alignment dye. To determine the change in signal with alignment position, the fluorescence intensity is measured when no fluorescein is in the channel and when fluorescein is electrophoresed to the detection zone. The signal is taken as the difference in intensity and the noise is taken as the standard deviation of the fluorescence intensity when no fluorescein is present in the channel. The alignment

actuators are then moved in the Y- or Z-direction away from the optimal alignment position and the measurement is taken again.

4. Conventional analysis

Liquid Extraction/PCR and Capillary Electrophoresis are performed to compare the results of some sub-assays on the microfluidic chip following the manufacturer's instructions and using a GeneAmp® PCR System 9700 thermal cycler (Life Technologies, Carlsbad, CA, USA), a ABI PRISM® 310 Genetic Analyzer (Life Technologies) and a ABI PRISM® 3130-*Avant* Genetic Analyzer (Life Technologies).

5. Instrument modules

The heating modules for liquid extraction and electrophoretic separation used Kapton polyimide heaters (HK5594R9.3L12A and HK5325R3.6L12A Minco Products Inc., Minneapolis, MN, USA) to heat aluminum blocks designed to the shape of the syringes (LE) and chip (ME). Laser induced fluorescence detection utilized a 200 mW laser (1110061, Coherent Inc., Santa Clara, CA, USA) coupled to a multi-mode fiber optic (HPUCO-25-488-M-11AS, OZ Optics Ltd, Ottawa, Ontario, Canada) which then split the light to each of the four channels. The multi-color fluorescence was detected using a transmission grating (1003-2, Wasatch Photonics, Logan, UT, USA) and full frame CCD (D02F-MG43D-UA1001-MN2F, Apogee Imaging Systems Inc., Belfast, UK). IR laser mediated PCR was performed using a custom design laser (AKELA Laser Corp., Monmouth Junction, NJ, USA) with non-contact temperature sensing with a pyrometer temperature sensor (custom, Advanced Circuits, Aurora, CO, USA). High voltage to perform the electrophoretic separation was generated using miniature regulated high voltage DC to DC converters from EMCO (C05 and C30, EMCO High Voltage Corp., Sulfur Creek, CA, USA).

6. Data analysis

Data analysis from both microfluidic and conventional processes was performed with SoftGenetics GeneMarker® HID v2.4.0 software (SoftGenetics, LLC, State College, PA, USA). The microfluidic platform performed the data processing of the assay using proprietary software (Lockheed Martin Corp., Bethesda, MD, USA) that interfaced with GeneMarker®.

7. Aliquot line volume

In preliminary studies, the impact of DNA mass in the PCR chamber on PCR peak heights was investigated. It was found that when DNA mass falls below 1.2 ng in the PCR chamber, peak heights begin to decrease below the target threshold of 1000 RFU on a conventional CE instrument. With an average DNA yield of 9.0ng/μl, and a lower standard deviation bound of 6 ng/μl, the aliquot line microchannel volume was designed to hold 4 μl. This volume provides an average DNA mass of 2.4 ng with a lower standard deviation of 1.6 ng.

8. Supplementary figures

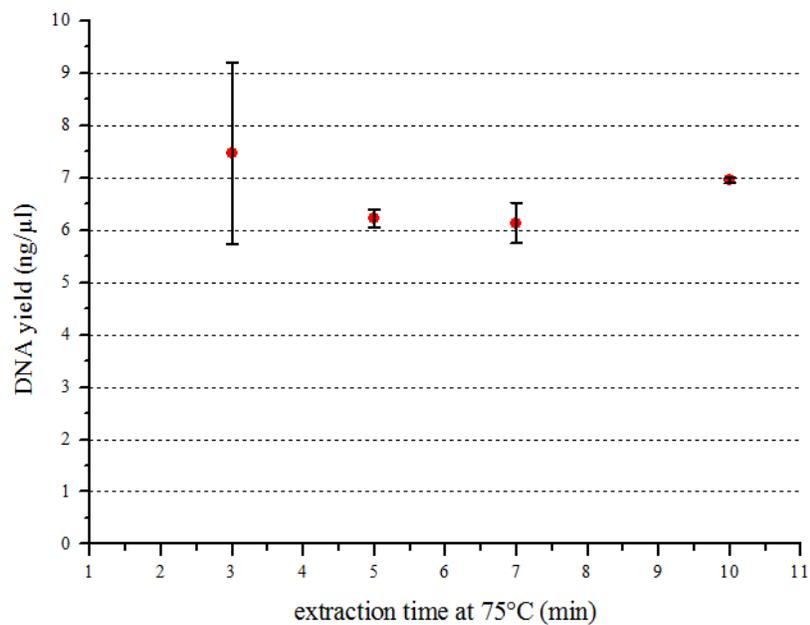


Fig. S.2. DNA yield from liquid extractions on chip with increasing amounts of extraction time (*i.e.* time at 75 °C). n=3.

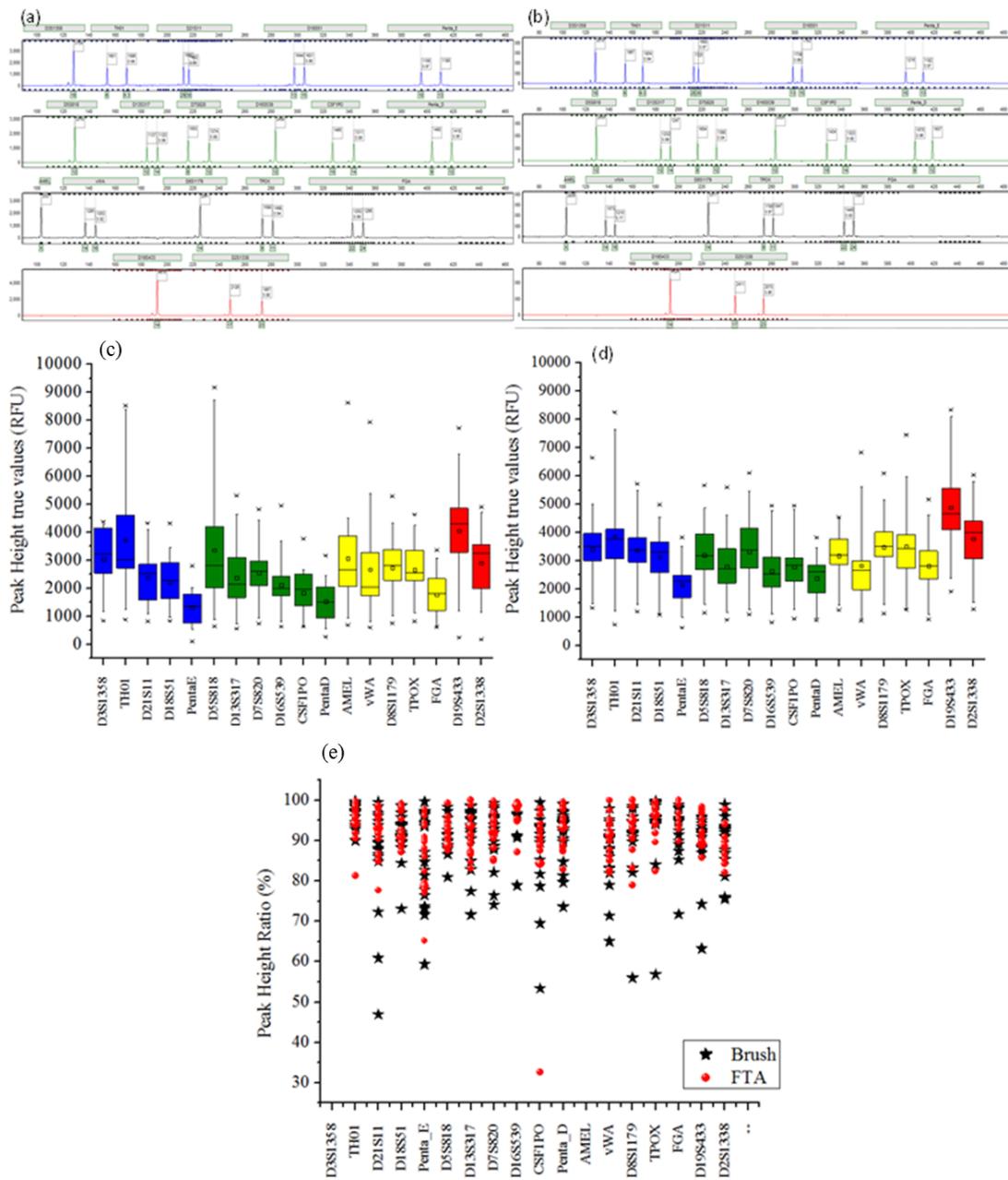


Fig. S.3. Results from LE of buccal brush swab and FTA paper followed by conventional buccal swab extracted inside the instrument (LE on chip), (b) Electropherogram from a 150 mm² FTA paper piece from the same donor extracted inside the instrument. Conventional results from the buccal swabs and FTA paper (20 samples for each DNA source) (c to e). (c) Box plot of the peak height true values from the buccal brush swabs (sum of heterozygote peak heights divided by two or homozygous peak heights divided by two). (d) Box plot of the peak height true values from the FTA paper. (e) Peak height ratio characterizing PCR from buccal swabs and FTA (Higher peak height divided by smaller peak height for heterozygous alleles).

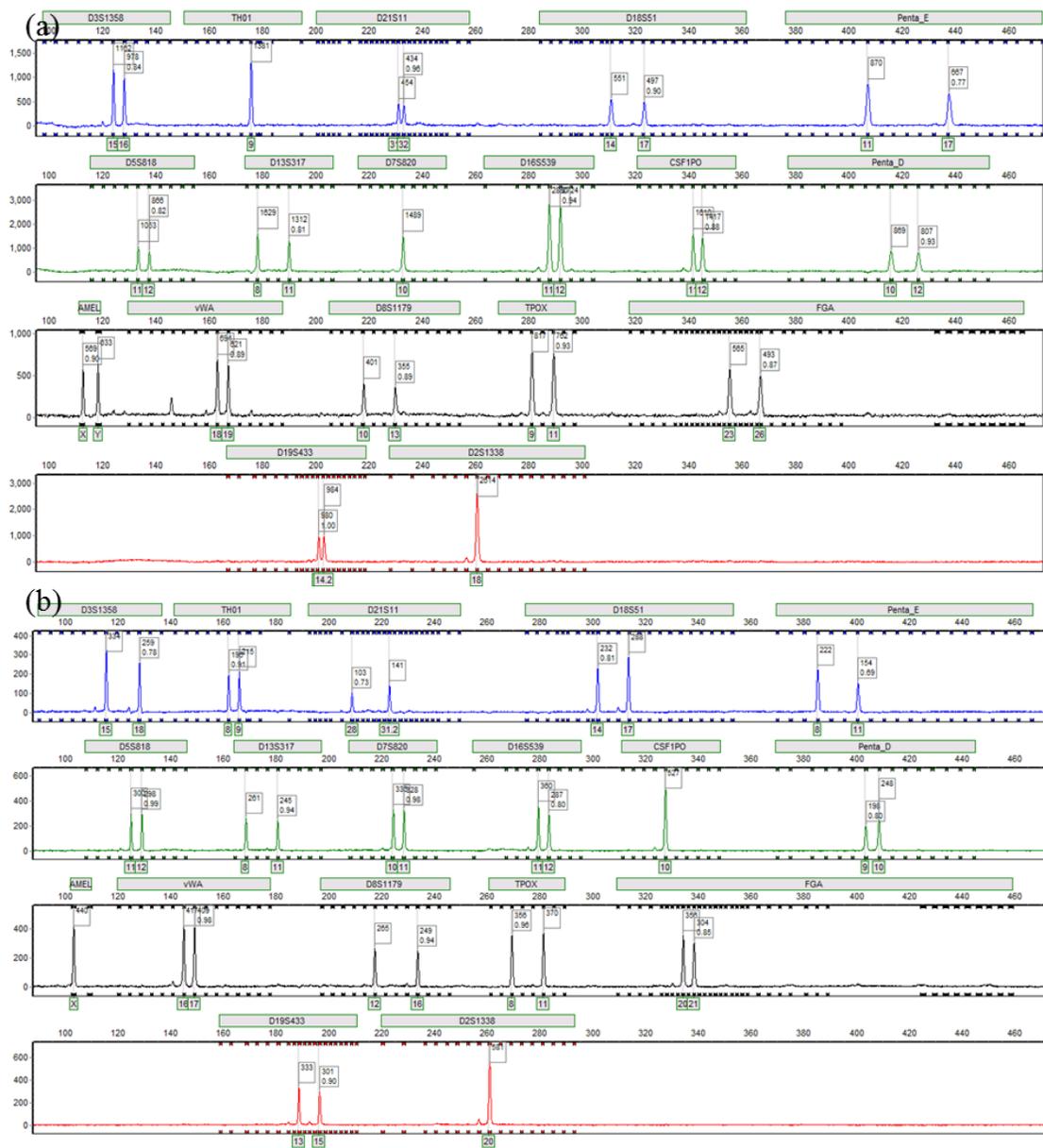


Fig. S.4. Electropherograms examples of LE-PCR on chip from conventional ABI 310 CE instrument of (a) LE-PCR on chip from a buccal swab and (b) a LE-PCR on chip from a 150 mm² FTA paper piece.

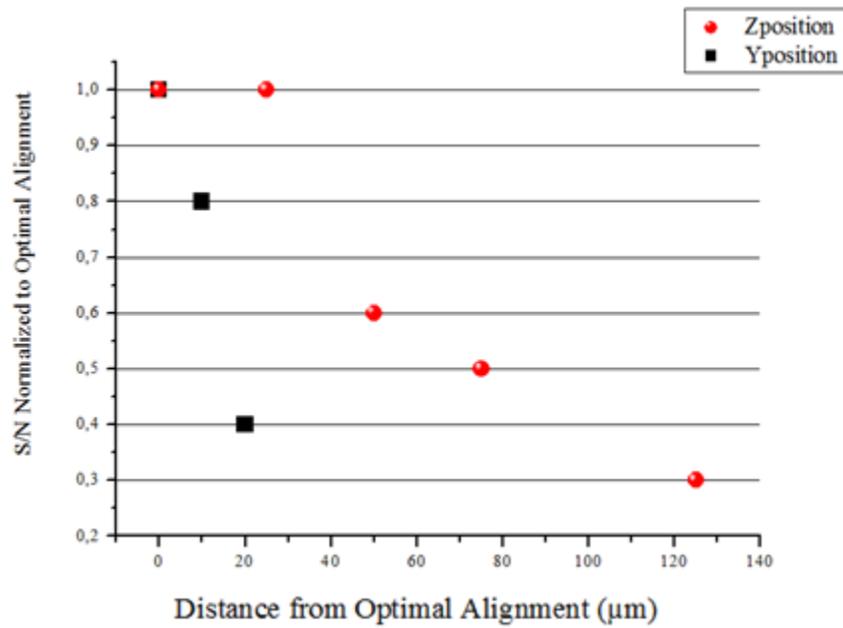


Fig. S.5. Tolerance of sensitivity to misalignment in the Y- and Z- directions. The optics were positioned to maximize the signal from fluorescein the buffer that was loaded in to the channel. The actuators were then moved set distances in the Y- and Z-directions. The signal was calculated from the difference in pixel intensity of when the fluorescein was present in the channel versus a channel with only buffer. The noise was calculated as the standard deviation in signal intensity when no fluorescein is present.

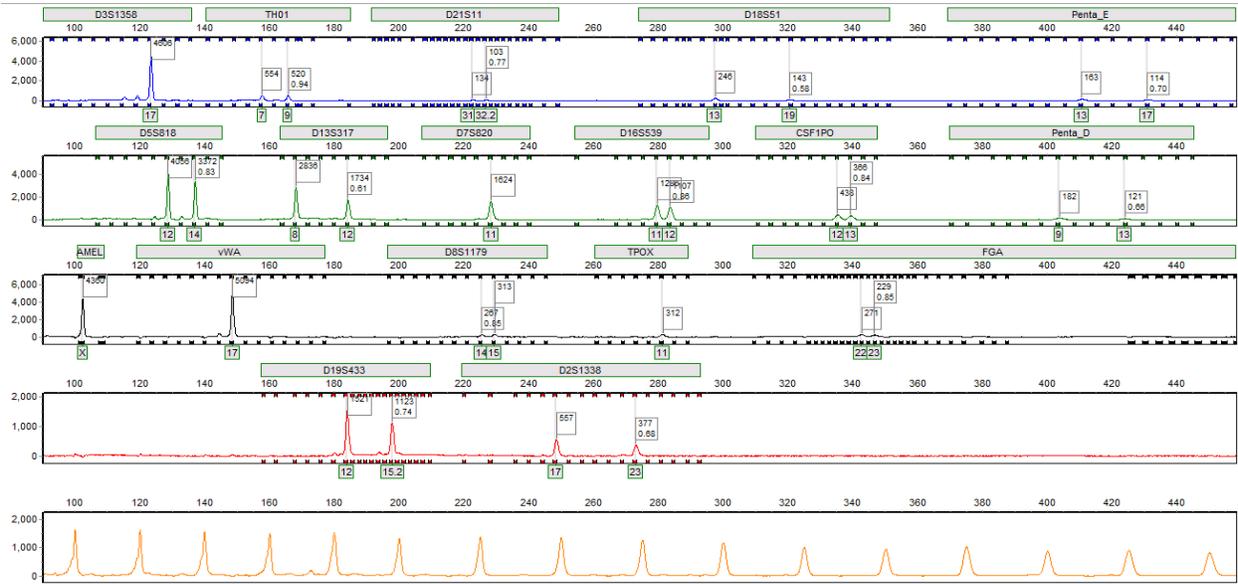


Fig S.6. A PCR-ME performed inside the cartridge. The Internal Lane Standard is well separated as well as the DNA peaks.

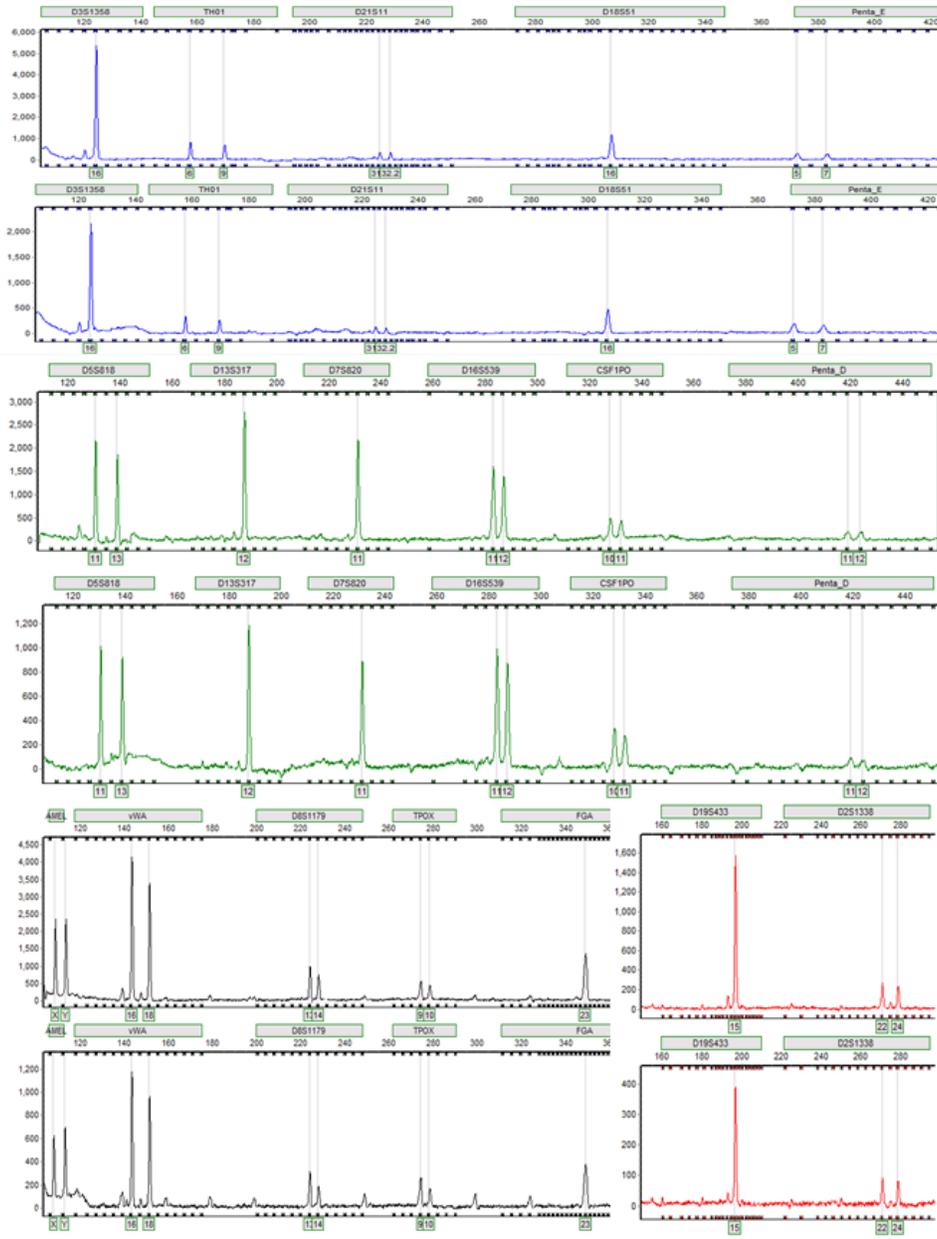


Fig. S.7. Representation of two sample-in-answer-out integrated LE-PCR-ME on microchip from the same donor. The dye color markers are shown together to highlight the similarity between both genetic profiles.

9. References.

- (1) Liu, K.; Fan, Z. H. *Analyst* **2011**, *136*, 1288–1297.
- (2) Le Roux, D.; Root, B. E.; Reedy, C. R.; Hickey, J. A.; Scott, O. N.; Bienvenue, J. M.; Landers, J. P.; Chassagne, L.; de Mazancourt, P. *Anal. Chem.* **2014**.