Representative Publications on Sequencing and DNA/RNA Assays

A one-page selection of peer-reviewed publications
(Selected from 2005 to 2011)

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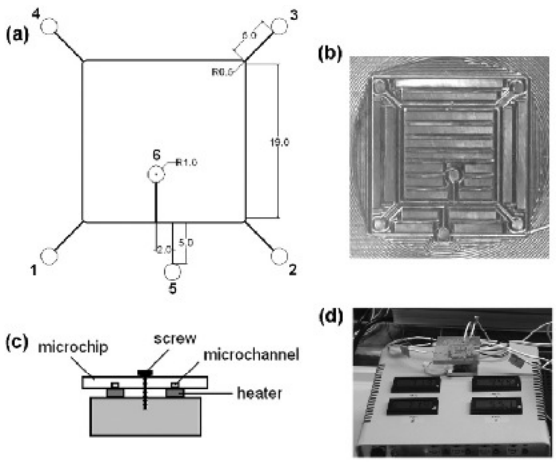
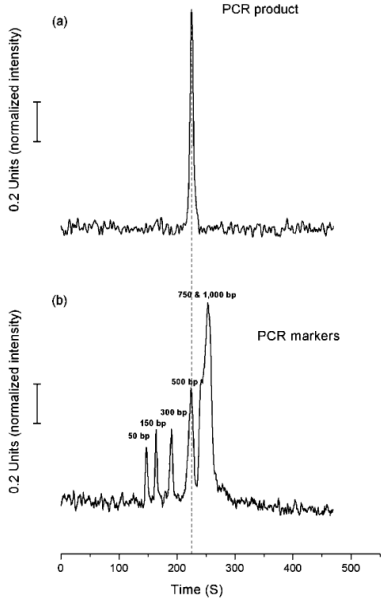
Electrokinetically-synchronized polymerase chain reaction (PCR) microchip fabricated in polycarbonate

Role: Co-Author Status: Published (Analytical Chemistry, 77: 658-666, 2005)

This work presents a novel method for DNA thermal amplification using the polymerase chain reaction (PCR) in an electrokinetically driven synchronized continuous flow PCR (EDS-CF-PCR) configuration carried out in a microfabricated polycarbonate (PC) chip. The synchronized format allowed patterning a shorter length microchannel for the PCR compared to nonsynchronized continuous flow formats, permitting the use of smaller applied voltages when the flow is driven electrically and also allowed flexibility in selecting the cycle number without having to change the microchip architecture. A home-built temperature control system was developed to precisely configure three isothermal zones on the chip for denaturing (95 °C), annealing (55 °C), and extension (72 °C) within a single-loop channel. DNA templates were introduced into the PCR reactor, which was filled with the PCR cocktail, by electrokinetic injection. The PCR cocktail consisted of low salt concentrations (KCl) to reduce the current in the EDS-CF-PCR device during cycling. To control the EOF in the PC microchannel to minimize dilution effects as the DNA "plug" was shuttled through the temperature zones, Polybrene was used as a dynamic coating, which resulted in reversal of the EOF. The products generated from 15, 27, 35, and 40 EDS-CFPCR amplification cycles were collected and analyzed using microchip electrophoresis with LIF detection for fragment sizing. The results showed that the EDS-CF-PCR format produced results similar to that of a conventional block thermal cycler with leveling effects observed for amplicon generation after ~25 cycles. To the best of our knowledge, this is the first report of electrokinetically driven synchronized PCR performed on chip.



(a) Topographical layout of the PC-based microchip (units are in mm). The width of the microchannel was 100 µm, and the depth was 70 µm. The access channels (connecting reactor to solution reservoirs) had the same size as the reactor channel. The reservoirs marked as 1-4 were used to set up the synchronized pattern. Reservoirs 5 and 6 were used for sample injection. (b) Micrograph of the brass molding die used for hot embossing replicates in PC. (c) A simple holding apparatus for microchip. (d) EDS-CF-PCR electronics system.

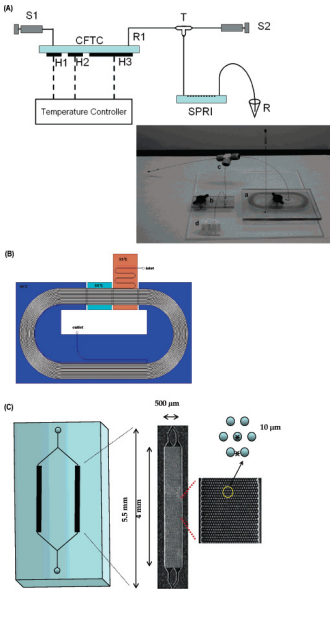


Electropherograms of PCR products and PCR markers. (a) Separation of a 500-bp PCR product that was collected from the EDS-CF-PCR chip. The S/N ratio for PCR product electropherogram is 3.6. (b) Electropherogram of PCR markers using PMMA microchip. The migration time of the 500-bp PCR product was 228 s, and the migration time of a 500-bp DNA marker was 225 s.

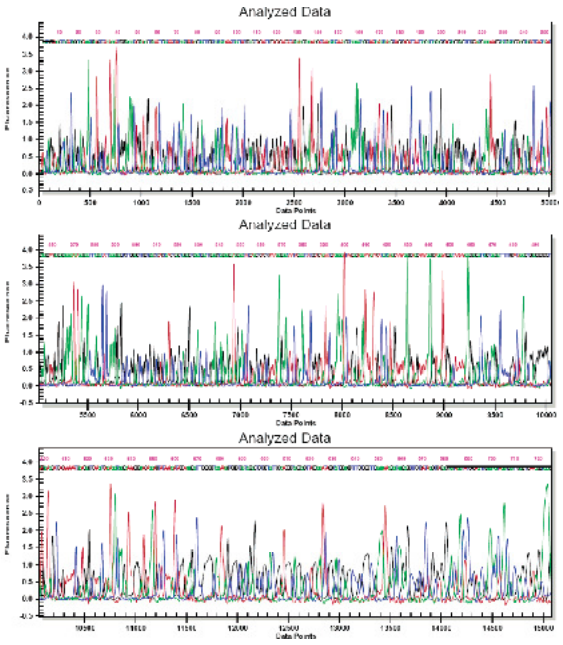
A continues flow thermal cycler microchip for DNA cycle sequencing

Role: Co-Author Status: Published (Analytical Chemistry, 78: 6223-6231, 2006)

We report here on the use of a polymer-based continuous flow thermal cycler (CFTC) microchip for Sanger cycle sequencing using dye terminator chemistry. The CFTC chip consisted of a 20-loop spiral microfluidic channel hot-embossed into polycarbonate (PC) that had three well defined temperature zones poised at 95, 55, and 60 °C for denaturation, renaturation, and DNA extension, respectively. The sequencing cocktail was hydrodynamically pumped through the microreactor channel at different linear velocities ranging from 1 to 12 mm/s. At a linear velocity of 4 mm/s resulting in a 36-s extension time, a read length of >600 bp could be obtained in a total reaction time of 14.6 min. Further increases in the flow rate resulted in a reduction in the total reaction time but also produced a decrease in the sequencing read length. The CFTC chip could be reused for subsequent sequencing runs (>30) with negligible amounts of carryover contamination or degradation in the sequencing read length. The CFTC microchip was subsequently coupled to a solid-phase reversible immobilization (SPRI) microchip made from PC for purification of the DNA sequencing ladders (i.e., removal of excess dye-labeled dideoxynucleotides, DNA template, and salts) prior to gel electrophoresis. Coupling of the CFTC chip to the SPRI microchip showed read lengths similar to that obtained from bench top instruments but did not require manual manipulation of the cycle sequencing reactions following amplification.



(A) Schematic diagram of the integrated CFTC/SPRI microchips for producing cycle sequencing DNA ladders that can be directly processed via capillary gel electrophoresis. CFTC, continuous flow thermal cycler chip; SPRI, solid-phase reversible immobilization chip; S1 and S2, syringe pumps; H1, H2, and H3, heaters; T, micro tee connector; R, sample receiving microfuge tube. Also shown is a picture of the integrated CFTC/SPRI system consisting of the (a) CFTC, (b) the SPRI microchip, (c) micro-tee, and (d) the receiving microfuge tube for the purified cycle sequencing reactions. (B) Layout of the thermal cycler chip and the isothermal zones placed on the chip. (C) Topographical layout and optical micrograph of the SPRI bed, which contain microposts for increasing the DNA load.

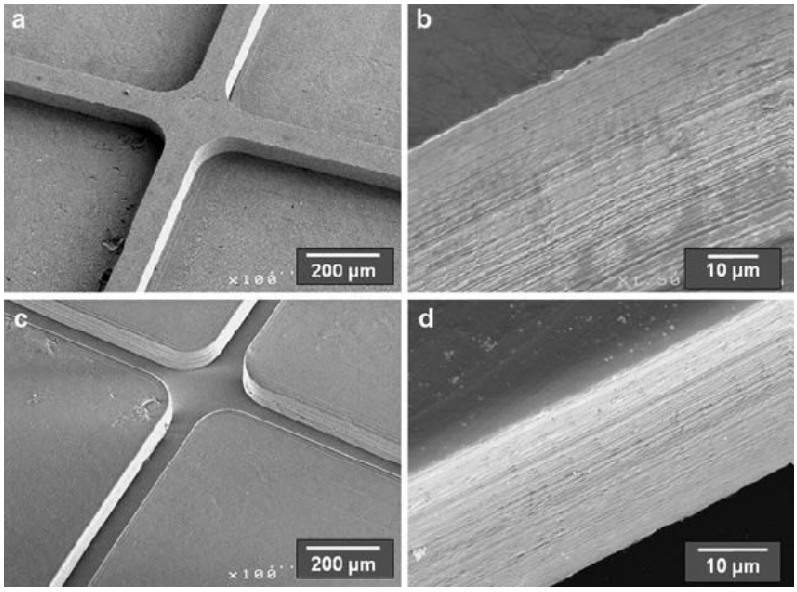


Four-color fluorescence sequencing trace obtained from the thermal cycler microchip coupled to the SPRI microchip. The sequencing trace was analyzed with the default parameters set by the Sequence Analysis Module in the CEQ 8000 system. The sequencing reaction was run through the CFTC chip at a linear flow rate of 1 mm/s, with the same flow rate used to introduce this sample into the SPRI chip. The binding buffer was infused into the SPRI chip at a linear velocity of 3.6 mm/s. Following air-drying of the SPRI chip, the purified sequencing fragments were eluted form the SPRI bed using 20 µL of electrophoresis loading buffer (deionized formamide).

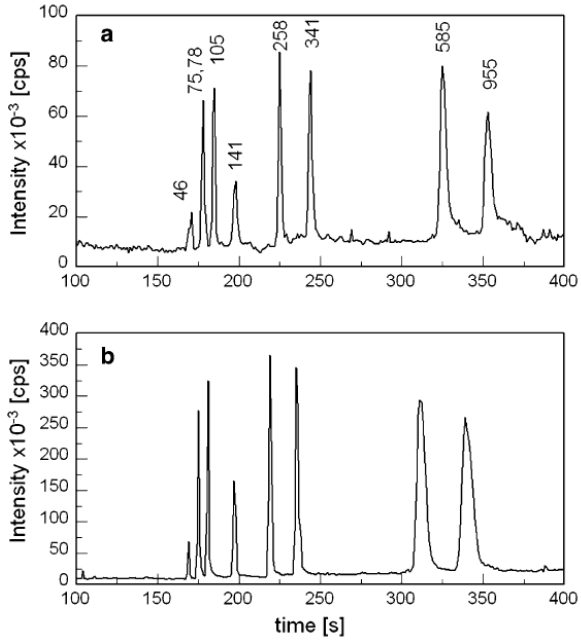
Evaluation of micromilled mold masters for inexpensive replication of plastic microchips for bioanalytical, biomedical and pharmaceutical applications

Role: Co-Author Status: Published (Microfluidics and Nanofluidics, 3: 1-11, 2007)

High-precision micromilling was assessed as a tool for the rapid fabrication of mold masters for replicating microchip devices in thermoplastics. As an example, microchip electrophoresis devices were hot embossed in poly(methylmethacrylate) (PMMA) from brass masters fabricated via micromilling. Specifically, sidewall roughness and milling topology limitations were investigated. Numerical simulations were performed to determine the effects of additional volumes present on injection plugs (i.e., shape, size, concentration profiles) due to curvature of the corners produced by micromilling. Elongation of the plug was not dramatic (< 20%) for injection crosses with radii of curvatures to channel width ratios less than 0.5. Use of stronger pinching potentials, as compared to sharp-corner injectors, were necessary in order to obtain short sample plugs. The sidewalls of the polymer microstructures were characterized by a maximum average roughness of 115 nm and mean peak height of 290 nm. Sidewall roughness had insignificant effects on the bulk EOF as it was statistically the same for PMMA microchannels with different aspect ratios compared to LiGA-prepared devices with a value of ca. 3.7×10^{-4} $cm^2/(Vs)$. PMMA microchip electrophoresis devices were used for the separation of pUC19 Sau3AI double-stranded DNA. The plate numbers achieved in the micromilled-based chips exceeded 1 million/m and were comparable to the plate numbers obtained for the LiGA-prepared devices of similar geometry.



SEM photomicrographs of micro-milled molding master finished with a 100 lm radius milling bit (a, b) and its replicate hot-embossed into PMMA (c, d). The width and depth of the channel, as determined by the dimensions of the mold master, was 100 and 90 µm, respectively

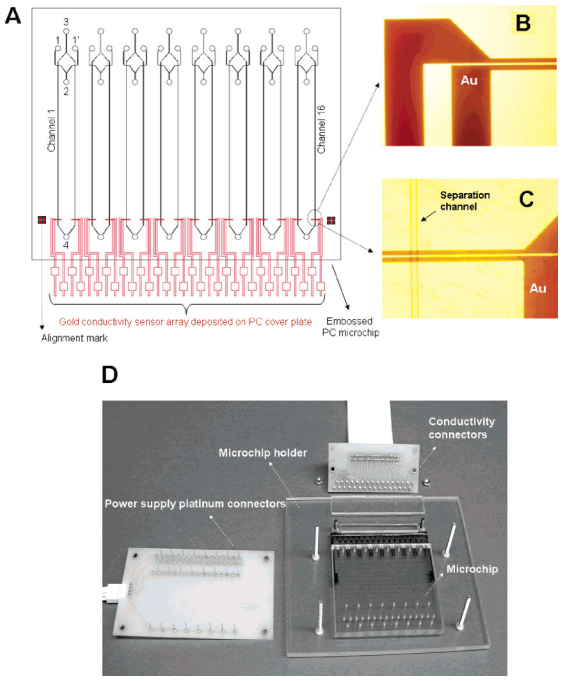


Separation of pUC19 Sau3AI double-stranded DNA stained with 1 µM TO-PRO-3 dye using a PMMA microchip with an offset T injector. E inj = 400 V/cm; t inj = 25 s; E sep = 110 V/cm; separation length 3.5 cm; 4% LPA was used as the sieving matrix. PMMA microchips were hot embossed using either a high precision micro-milled master or b LiGA-made master

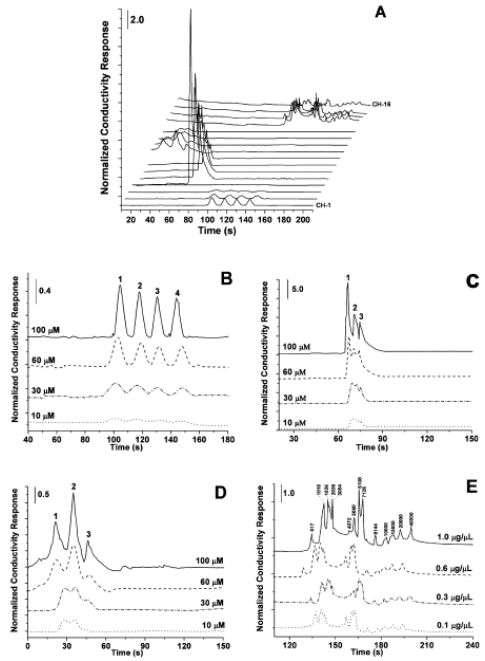
A portable microfluidic system with multichannel plastic microchip and integrated electrochemical biosensor array for parallel separation and detection of biomolecules

Role: First Author Status: Published (Analytical Chemistry, 79: 870-878, 2007), *Most Assessed Article*

The microfluidic network consisted of 16 separation channels that were hot-embossed into polycarbonate (PC) using a high-precision micromilled metal master. A gold (Au) sensor array was lithographically patterned onto a PC cover plate and assembled to the fluidic chip via thermal bonding in such a way that a pair of Au microelectrodes (60 μm wide with a 5 μm spacing) was incorporated into each of the 16 channels and served as independent contact conductivity detectors. The spacing between the corresponding fluidic reservoirs for each separation channel was set to 9 mm, which allowed for loading samples and buffers to all 40 reservoirs situated on the microchip in only five pipetting steps using an 8-channel pipettor. A printed circuit board (PCB) with platinum (Pt) wires was used to distribute the electrophoresis high-voltage to all reservoirs situated on the fluidic chip. Another PCB was used for collecting the conductivity signals from the patterned Au microelectrodes. The device performance was evaluated using microchip capillary zone electrophoresis (μ-CZE) of amino acid, peptide, and protein mixtures as well as oligonucleotides that were separated via microchip capillary electrochromatography (μ-CEC). The separations were performed with an electric field (*E*) of 90 V/cm and were completed in less than 4 min in all cases. The separation efficiency was found to be 6.4×10^4, 2.0×10^3, 4.8×10^3, and 3.4×10^2 plates for the μ-CEC of the oligonucleotides and μ-CZE of the amino acids, peptides, and proteins, respectively, with an average channel-to-channel migration time reproducibility of 2.8%.



(A) Topographical layout of the multichannel microfluidic network. The center-to-center spacing of each fluidic reservoir was fixed at 9 mm. The line trace shown in red provides a topographical layout of the lithographically printed-Au conductivity sensor array. Shown is the detection region of one Au-electrode pair before (B) and after (C) thermal annealing of the cover plate to the microfluidic chip. Each contact conductivity electrode was 60 μm in diameter with an end-to-end spacing of 5 μm. (D) Photograph showing the microchip and the holder setup with connectors for the high-voltage power supply and conductivity detection units.

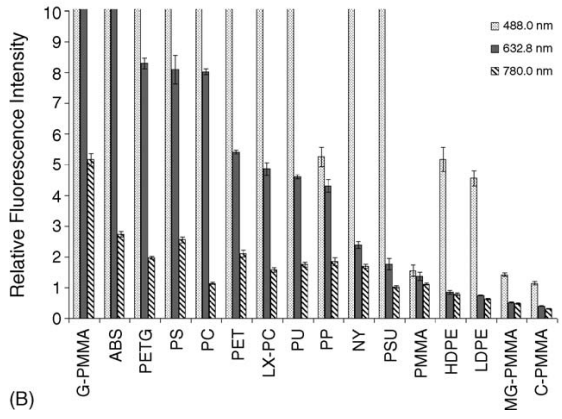


(A) Sixteen-channel microchip electrophoresis of different samples using the PC microchip and setup. Channels 1-4, μ-CZE of amino acids; channels 5-8, μ-CZE of peptides; channels 9-12, μ-CZE of proteins; channels 13-16, μ-CEC of oligonucleotides. (B) An expanded view of the electrophoretic trace for the μ-CZE analysis of amino acids. (C) An expanded view of the μ-CZE analysis of peptides. (D) Expanded view of the electrophoretic trace for the μ-CZE analysis of proteins. (E) Expanded view for the μ-CEC analysis of a 1 kbp oligonucleotide ladder.

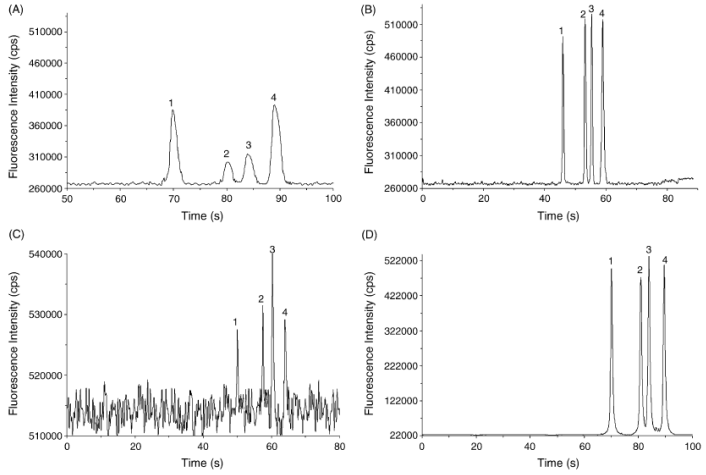
Physiochemical properties of various polymer substrates for use in manufacturing disposable plastic microchips for bioanalytical and pharmaceutical applications

Role: First Author Status: Published (Journal of Chromatography A, 1111: 238-251, 2006)

A suite of polymers were evaluated for their suitability as viable substrate materials for microchip electrophoresis applications, which were fabricated via replication technology. The relevant physiochemical properties investigated included the glass transition temperature (*T*g), UV–vis absorption properties, autofluorescence levels, electroosmotic flow (EOF) and hydrophobicity/hydrophilicity as determined by sessile water contact angle measurements. These physiochemical properties were used as a guide to select the proper substrate material for the intended microchip electrophoretic application. The T_g of these polymers provided a guide for optimizing embossing parameters to minimize replication errors (REs), which were evaluated from surface profilometer traces. RE values ranged from 0.4 to 13.6% for the polymers polycarbonate (PC) and low-density polyethylene (LDPE), respectively. The absorption spectra and autofluorescence levels of the polymers were also measured at several different wavelengths. In terms of optical clarity (low absorption losses and small autofluorescence levels), poly(methyl methacrylate), PMMA (clear acrylic), provided ideal characteristics with autofluorescence levels comparable to glass at excitation wavelengths that ranged from 488–780 nm. Contact angle measurements showed a maximum (i.e., high degree of hydrophobicity) for polypropylene (PP), with an average contact angle of 104° ± 3° and a minimum exhibited by gray acrylic, G-PMMA, with an average contact angle of 27° ± 2°. The EOF was also measured for thermally assembled chips both before and after treatment with bovine serum albumin (BSA). The electrophoretic separation of a mixture of dye-labeled proteins including; carbonic anhydrase, phosphorylase B, β-galactosidase, and myosin, was performed on four different polymer microchips using laser-induced fluorescence (LIF) excitation at 632.8 nm. A maximum average resolution of 5.04 for several peak pairs was found with an efficiency of 6.68×10^4 plates for myosin obtained using a BSA-treated PETG microchip.



LIF background levels measured at three different excitation wavelengths, 488 nm, 632.8 nm, or 780 nm. The LIF system was configured in an epi-illumination format. In all cases, ~2 mW of average laser power was used at the polymer surface. The collected photons were filtered using an interference filter that possessed a center wavelength red-shifted by ~30 nm from the excitation wavelength with a half-band width of ~10 nm. The average fluorescence intensity (cps) of the polymers was normalized with respect to the value obtained for glass at the same excitation wavelength.



Electrophoretic separation of several proteins in polymer microchips: (A) Native PP; (B) BSA-treated PP; (C) BSA-treated PETG; and (D) BSA-treated C-PMMA. All separations were performed with a running buffer consisting of 100 _M Tris–HCl, 1% SDS, pH 9.2; *E* = 300 V/cm; *L*eff = 30 mm; LIF detection (excitation = 632.8 nm; ~2 mW average power). The electrophoretic peaks are identified as: (1) carbonic anhydrase; (2) phosphorylase B; (3) β-galactosidase; and (4) myosin. In all cases, the concentration of the proteins used for the electrophoresis was 300 nM.