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Continuous Flow Thermal Cycler Microchip for DNA Cycle Sequencing

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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 benchtop instruments but did not require manual manipulation of the cycle sequencing reactions following amplification.

Recently, attention has focused on developing microfabricated devices for a variety of biomedical and biological discovery applications with a number of devices directed toward DNA amplifications that require temperature cycling, such as PCR[1−7] and dideoxy cycle sequencing.[8] These efforts have been driven by the importance of thermal cycling reactions in DNA analyses and the fact that microthermal reactors can offer several advantages compared to their benchtop counterparts including lower thermal capacitance, smaller amounts of reagents required for the reaction, and the ability to provide a high degree of automation in the assay by integrating the micro-PCR to subsequent processing steps configured on chips as well. During the past decade a number of groups have designed chamber-type PCR microchips, where a stationary PCR cocktail in a confined space is alternately heated and cooled.[1,3,4,6] DNA amplification can also be achieved in a microchip by shuttling the PCR cocktail repetitively through different isothermal zones using a continuous flow (CF) format.[2,5,6] One of the attractive features associated with the CF-PCR approach is that the thermal cycling process can be conducted at relatively high speeds, since it is not necessary to heat and cool the large thermal masses associated with the amplification chamber. In the CF formats, only small packets of fluid that enter the isothermal zones are required to be heated and cooled, allowing ultrafast thermal cycling due to the improved thermal management of the system. Our group has developed a spiral microchannel with 20 loops hot-embossed into polycarbonate (PC) for ultrarapid PCR. Successful PCRs using a CF-PCR device were determined primarily by enzyme kinetics (AmpliTaq polymerase) using a flow velocity of 15 mm s^{-1} resulting in successful amplification of a 500-bp fragment in 1.7 min at a cycling rate of 5 s $cycle^{-1}$.[2]

While many examples have appeared in the literature using either chamber-type or continuous flow microchips to perform thermal cycling for PCR applications only one report has appeared that described the use of a microthermal cycler for DNA cycle sequencing.[8] This report used a chamber-type format with noncontact heating produced via an infrared source. However, the authors only reported a read length of 400 bp with no information provided on system optimization in order to produce longer effective read lengths.

While new strategies for high-throughput DNA sequencing are evolving that do not require Sanger dideoxynucleotide formats,[9−16] Sanger methods based on cycle sequencing continue to be a work

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horse in most large-scale genome sequencing projects.[17−19] Therefore, micro-based thermal cyclers, especially those using a CF format, could potentially provide another tool for developing fully automated instrument platforms for large-scale, high-throughput DNA sequencing.[20−26]

In this report, we will discuss the use of a polymer-based microchip for cycle sequencing consisting of a CF format. The microchip was fabricated in PC via hot embossing from a metal master and contained a spiral configuration capable of performing 20 thermal cycles. The cycle sequencing cocktail was shuttled between three different isothermal zones required for the reaction. Effects of cycling speed and ratio of residence times within three different temperature zones required for the thermal cycling on DNA sequencing read length will be discussed. In addition, coupling of this thermal cycling chip to a solid-phase cleanup microchip for removing excess dye-labeled terminators prior to gel electrophoretic sorting will be discussed as well. The integrated thermal cycler and solid-phase cleanup chips were used to prepare cycle sequencing reactions that could be directly subjected to capillary electrophoresis for size sorting and subsequent automated base calling.

EXPERIMENTAL SECTION

Continuous Flow Thermal Cycler (CFTC) Microchip Fabrication. The brass mold master used for microreplicating polymer parts was milled with a high-precision micromilling machine (Kern MMP 2522, Kern Micro- und Feinwerktechnik GmbH & Co., KG) using procedures described previously.[27] CFTC chips (see Figure 1B) were obtained by hot-embossing the micropattern into PC (GoodFellow) substrates using a HEX 02 hot embossing system (Jenoptik Mikrotechnik GmbH, Jena, Germany). The chip provided the ability to perform 20 thermal cycles (20 loops) with the channel dimensions being 100 μm × 100 μm (total length of reactor channel 3.5 m, average loop length 17.3 cm). Following embossing, two 1-mm-diameter reservoirs were drilled into the chip to serve as conduits for flowing samples in to and out of the fluidic chip. Finally, the CFTC microchip was sealed with a PC cover plate at 150 °C in a circulating air oven for 20 min.

Three different temperature zones were maintained by Kapton flexible heaters (KHLV-0502/5-P, 0.5 × 2 in; KHLV-103/5-P, 1 × 3 in; Omega Engineering, Inc.) attached to the PC cover plate with a precoated pressure-sensitive adhesive. Temperatures in each zone were controlled by closed-loop PID temperature/process controllers (CN77R340, Omega Engineering, Inc.). Temperature feedback was provided through type K thermocouples

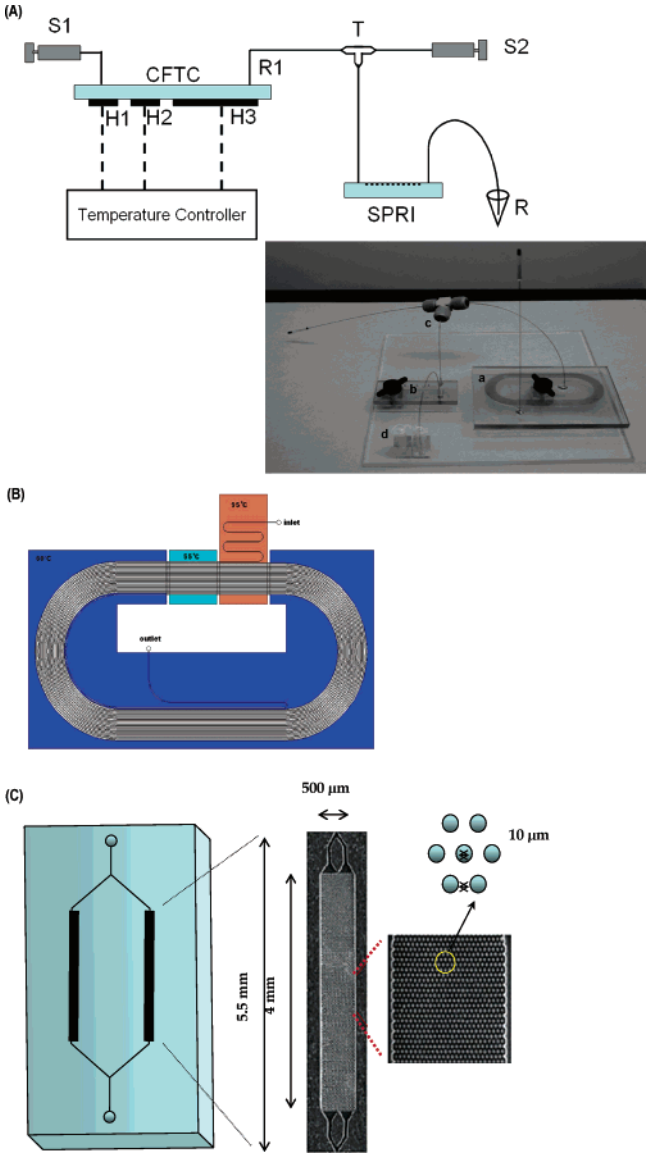

Figure 1. (A) Schematic diagram of the integrated CFTC/SPRI microchips for producing cycle sequencing DNA ladders that can be directly processed via capillary gel electrophoresis. CFTC, continuous flow thermal cycler chip; SPRI, solid-phase reversible immobilization chip; S1 and S2, syringe pumps; H1, H2, and H3, heaters; T, microtee 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) microtee, 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.

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Table 1. Linear and Volumetric Flow Rates, Residence Times, and Total Reaction Times Using the Continuous Flow Thermal Cycler Chip That Was Built To Provide 20 Thermal Cycles[a]

residence times (s)[b]			CFTC microchip			Benchtop thermal cycler		
95 °C	55 °C	60 °C	linear flow rate (mm/s)	volumetric flow rate (μL/min)	total time[c] (min)	set times/ cycle (s)	real time[d]/ cycle (s)	total time[e] (min)
12	12	144	1	0.6	58.3	168	180	62
6	6	72	2	1.2	29.2	84	97	34
3	3	36	4	2.4	14.6	42	56	21
1	1	12	12	7.2	4.9	14	32	13

[a] Also shown are set times at each temperature of a thermal cycle and the total reaction time using a benchtop thermal cycler. [b] Average residence time within each isothermal zone using a ratio of 1:1:12, denature/renature/extend. [c] Total travel time for the reaction mixture to flow through the 3.5-m microchannel. [d] Includes the transition time for heater block to reach the set temperature. [e] Includes a 1-min denaturation time and a 1-min final extension time.

(5TC-TT-K-36-36, Omega Engineering, Inc.) embedded between the PC cover plate and the Kapton heaters.

Solid-Phase Reversible Immobilization (SPRI) Microchip Fabrication. Fabrication of the SPRI microchip has been previously reported by our group.[28] Briefly, the procedure involved fabricating a metal molding die in Ni via LiGA with the micropattern transferred into a PC substrate using hot embossing. The topology of the SPRI chip is depicted in Figure 1C. The immobilization bed was 500 μm in width, 50 μm in depth, and 4 mm in length. This bed was filled with an ordered array of microposts (d = 10 μm; spacing 10 μm) to provide a higher load of DNA compared to an open channel of the same dimensions. Following hot embossing, the SPRI chips were rinsed with copious amounts of water and washed briefly with isopropyl alcohol. The final chips were exposed to UV radiation for 15 min and sealed with a PC cover plate (also exposed to UV radiation) at 140 °C in a circulating air oven for 20 min.

Coupling the CFTC Microchip to the SPRI Microchip. A schematic of the integrated microfluidic system is depicted in Figure 1A. Syringe pump S1 (PHD 2000, Harvard Apparatus, Holliston, MA), fitted with a 100-μL glass syringe (SGE International Pty. Ltd.), was used to pump the sequencing cocktail through the CFTC chip. A second syringe pump (S2, see Figure 1A), fitted with a 250-μL syringe, was used to pump the immobilization, washing, and elution buffers through the SPRI chip. Fused-silica capillaries connected the syringes to the receiving reservoirs of the microchips. The syringes were connected to the capillary with low dead volume leur-to-capillary adapters (P/N 3-2302S, Innova Quartz Inc.). The distal end of the capillaries was glued into a reservoir on the CFTC or SPRI chip with an epoxy adhesive. A MicroTee (P-888, Upchurch Scientific) was used to connect the CFTC chip to the SPRI chip. One arm of this tee accepted the output from the CFTC chip while another arm was used to deliver the binding buffer, wash buffer, or elution buffer into the SPRI chip. The final arm of the microTee was fitted with an additional capillary that carried the eluant from the SPRI chip, containing the purified sequencing ladder, into a well of a microtiter plate that was subsequently placed in the automated capillary gel electrophoresis instrument.

Cycle Sequencing Chemistry. Dye Terminator Cycle Sequencing Quick Start Kit (P/N 608120) from Beckman Coulter Inc. was used in all cases. The cycle sequencing reaction, 20 μL total volume, contained 8 μL of the master mix (dATP, dCTP, dTTP, and dITP; WellRED-labeled ddUTP, ddGTP, ddCTP, and ddATP; Thermo Sequenase DNA polymerase I; Tris-HCl, $MgCl_2$ reaction buffer, pH 8.9, and pyrophosphatase), 2 μL of a 1.6 μM 24-base M13 -47 sequencing primer (5′CGC CAG GGT TTT CCC AGT CAC GAC 3′), 1 μL of 0.2 μg/μL M13mp18 DNA template (P/N 70704, USB Corp.), and 9 μL of PCR grade water (Sigma). The volume of the cycle sequencing reaction (20 μL) was selected to accommodate the input requirements of the capillary electrophoresis machine.

Operation of the CFTC Microchip. Prior to the initial use of the CFTC microchip, 50 μL of 0.1% (w/v) BSA was flowed through the chip at 0.2 mm/s (0.12 μL/min) to coat the interior walls to prevent nonspecific adsorption of the Thermo Sequenase DNA polymerase to the walls of the device, which could result in deactivation of the polymerase. Using this single-coating procedure, we found that >30 cycle sequencing reactions could be run on the same chip before requiring recoating with BSA. Sixty microliters of a 1× sequencing buffer (GenomeLab Methods Development Kit, P/N 608000, Beckman Coulter Inc.) was used to flush the reactor channel between individual cycle sequencing runs.

Three temperature zones were used for cycle sequencing, which were set at 95 °C for template denaturation, 55 °C for primer renaturation, and 60 °C for polymerase extension. The system was brought to thermal equilibrium prior to use. Then, 20 μL of the sequencing cocktail followed by 35−50 μL of mineral oil or air flowed through the channel at a linear velocity ranging from 1 to 12 mm/s (10 to 120 nL/s). The residence time of the sequencing cocktail through each temperature zone was calculated based on the linear velocity and the length of the isothermal zone. To evaluate the efficiency of the CFTC microchip in terms of sequencing read length, the same sequencing cocktail was inserted into a benchtop thermal cycler (Mastercycler Epgradient, Eppendorf North America, Inc.). Denaturation, renaturation, and extension temperatures and set times were the same as those used for the CFTC microchip (see Table 1).

To evaluate carryover contamination, 20 μL of sequencing buffer was allowed to flow through the CFTC microchip at 1 mm/s

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following the 60-μL wash. The 20-μL sample was collected and subjected to SPRI cleanup and then electrokinetically injected into the capillary gel column using conditions similar to that used for the actual sequencing reactions.

Off-Chip SPRI Purification. Crude cycle sequencing products were collected at R1 (see Figure 1A) and purified with a CleanSEQ dye terminator removal kit (Agencourt Bioscience Corp.) according to the protocol suggested by the manufacturer. Briefly, 10 μL of CleanSEQ magnetic bead solution was added to the PCR tube containing 20 μL of the crude reaction products followed by the addition of 62 μL of 85% ethanol and pipet mixing 7 times. Then, the PCR tube was placed onto a magnetic plate for 5 min to separate the beads from the solution. The supernatant was aspirated from the tube and discarded. After washing the beads with 100 μL of 85% ethanol, 20 μL of deionized formamide (CEQ sample loading solution (SLS), P/N 608082, Beckman Coulter Inc.) was added and incubated with the beads for 5 min to elute the isolated DNA from the magnetic beads. Finally, the eluant was transferred to a 96-well microtiter plate, which was loaded into a Beckman Coulter CEQ 8000 genetic analysis system for DNA electrophoretic sorting and automated base calling. The electrophoresis operating parameters were set to the manufacturer's suggested conditions to optimize the read length. These electrophoretic conditions were used throughout the reported studies to ensure that the read length was only influenced by thermocycling parameters.

SPRI Microchip Purification. The binding buffer was prepared by mixing 93 μL of 85% ethanol with 15 μL of the supernatant aspirated from the CleanSEQ kit. A total of 100 μL of binding buffer was continuously infused into the microTee connector by S2 (see Figure 1A) to allow mixing with the crude sequencing reaction with the binding buffer. The flow ratio of S1 and S2 was set to 1:3.6 to provide the correct volume mixing ratio of the crude sequencing reactions with the immobilization buffer. Following immobilization onto the PC capture bed, 100 μL of 85% ethanol was used to wash the bed of the SPRI microchip. Air was then pumped through the SPIR chip to dry the immobilization bed with the purified sequencing products eluted by flowing 20 μL of the SLS solution through the chip. Finally, the eluant was transferred to a 96-well microtiter plate for electrophoretic sorting and automated base calling using the Beckman CEQ 8000.

RESULTS AND DISCUSSION

Cycle Sequencing Using the CFTC Microchip. In our previous work using the CFTC unit for PCR, we incorporated into the device a 1:1:4 ratio of the three isothermal zones (denaturation/renaturation/extension) to produce the desired PCR product consisting of a 500-base pair amplicon in 1.7-min processing time.[2] We therefore set out to test the utility of this CFTC microchip for generating sequencing ladders using dye terminator chemistry and Thermo Sequenase DNA polymerase cycle sequencing. The sequencing ladders were generated in the CFTC chip followed by off-chip SPRI cleanup and subsequently electrophoresed via capillary gel electrophoresis. The bases were called using the electrophoresis system's automated base-calling algorithms with a 98.5% base calling accuracy criterion to set the effective read length. The linear flow velocity initially used was 1 mm/s producing a residence time of approximately 12, 12, and 48 s for denaturation, renaturation, and extension, respectively, within the chip. As a reference, the same cycle sequencing conditions in terms of temperature set times were used with a benchtop thermal cycler. The sequencing read lengths were found to be ~600 and ~550 bp for the microchip and the benchtop thermal cycler, respectively (data not shown). Previous results have shown that the denaturation and renaturation steps are almost instantaneous and the processing time of ultrafast PCR using the CFTC microchip was determined by enzyme kinetics. In dye terminator thermal cycling reactions, the incorporation rate exhibited by the polymerase is slower than PCR because deoxyinosine triphosphate (dITP), which is used in the reaction cocktail to eliminate secondary structures that can produce zone compressions during electrophoresis, and dye-labeled ddNTPs are used as substrates as opposed to deoxynucleotides used for PCR.[2,29] On the basis of these considerations, we changed the ratio of the residence times within the three temperature zones to 1:1:12 and increased the total length of the microchannel from 1.6 to 3.5 m. To improve the thermal homogeneity within each isothermal zone using one-sided heating by reducing channel depth, the microchannel dimensions for the CFTC chip were changed from 50 μm $\times$ 150 μm to 100 mm $\times$ 100 μm. We also investigated, using a benchtop thermal cycler, the necessary number of thermal cycles to provide the optimal read length. When using up to 60 thermal cycles, the fluorescence signal intensities did slightly increase with an increase in the number of cycles, but the read length increased marginally with cycle number (data not shown). Considering the limited patterning space for the microchip, we kept the 20-cycle design for our CFTC in these experiments. Using a linear velocity of 1 mm/s with the aforementioned changes to the chip design, a read length of ~680 bp was found (see Figure 2). Further increases in the relative residence time within the extension zone with respect to the denaturation and renaturation zones did not increase sequencing read length (data not shown). The remaining experiments reported herein used a temperature ratio of 1:1:12 for denaturation/renaturation/extension.

In the next series of experiments, sequencing cocktails were shuttled through the CFTC microchip at different linear velocities ranging from 1 to 12 mm/s to monitor the effects of reaction time on the DNA sequencing read length. The residence times within each temperature zone, volume flow rates, and total reaction times for a 20-μL sequencing mixture are shown in Table 1 using the CFTC microchip. As a comparison, the sequencing cocktails were also run using a conventional benchtop thermal cycler at comparable set times for each step of the cycle sequencing reaction. The reaction time per cycle and the total reaction time for the block thermal cycler are also shown in Table 1. As can be seen from the values depicted in Table 1, the total reaction times for the CFTC microchip are significantly less than that for the benchtop thermal cycler at smaller set times (see last row of Table 1) due to the larger relative contribution of the temperature transition times associated with the block thermal cycler and the absence of such transition times for the CFTC due to the fact that the CFTC system is brought to thermal equilibrium prior to thermal cycling.

The DNA read lengths at different residence times are shown in Figure 2. At a 144-s extension time generated using a 1 mm/s

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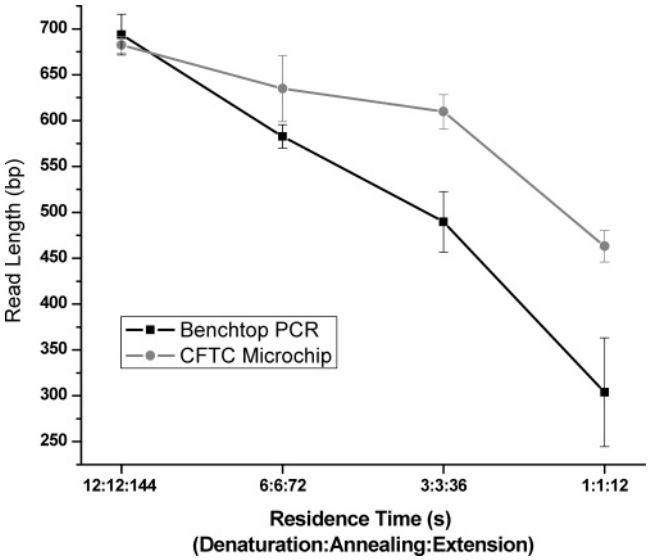

Figure 2. Read length (bp) versus the residence time in each isothermal zone. The residence time used for the benchtop thermal cycler in this plot was equal to the set time for each temperature step of the cycle and did not include the transition time (see Table 1). The sequencing traces were analyzed with the default parameters set by the Sequence Analysis Module of the CEQ 8000 system. The read length was set by the number of bases called at a calling accuracy equal to 98.5%. The error bars represent the read length secured from 3 replicate sequencing runs.

linear velocity, the average read lengths were 682 and 693 bases for the CFTC chip and the benchtop thermal cycler, respectively. When the linear velocity was increased to 4 mm/s equivalent to a 36-s extension time, the average read length for the CFTC chip was 609 bases, while the read length was 490 bases for the benchtop thermal cycler. When the linear velocity for the CFTC was increased to 12 mm/s, producing a 12-s effective extension time, the read length was 463 bp while that for the block thermal cycler using the same extension set time was 304 bp. The observed performance improvement obtained for the CFTC microchip at the shorter dwell times for extension can be explained by the larger surface-to-volume ratio (SVR) associated with this chip compared to the benchtop thermal cycler. The SVR for the microchip was 40 mm^{-1}, and the heat-transfer distance (the depth of the channel) was only 100 μm, while the SVR was 1 mm^{-1} and the heat-transfer distance was ~2 mm for the PCR tube used in the case of the block thermal cycler. Both the large SVR and the smaller heat-transfer distance allow for rapid thermal equilibrium to be obtained within the reaction cocktail when using the CFTC, ensuring a uniform temperature profile throughout the reaction cocktail in a shorter time period. Our previous results using finite element analysis simulations of a similar CFTC microchip for PCR showed that the temperature of a solution traveling through an isothermal zone was at the set value for >90% of its residence time within the extension region at a linear velocity of 12 mm/s[2].

The importance of cleaning up dye terminator sequencing reactions prior to capillary gel loading has been well demonstrated by our group and others as well.[28,30,31] Excess dye terminators can mask electrophoretic bands generated from extension products early in the trace, and excess salts can hamper selective loading of the extension products during electrokinetic injection into the capillary gel column. There are a variety of methods that can be used to purify cycle sequencing reactions, such as precipitation or solid-phase extraction (SPE) techniques. SPE methods are particularly attractive for microfluidic platforms because they do not require centrifugation steps. The solid-phase reversible immobilization strategy adopted here is a solid-phase extraction method that can be implemented using a low-cost PC microchip embossed from a master followed by a UV exposure.[28]

Figure 3A shows raw data obtained from an unpurified cycle sequencing reaction produced using the CFTC microchip, while in Figure 3B, a similar cycle sequencing reaction was subjected to an off-chip CleanSEQ purification, which uses an SPRI protocol with magnetic bead capture. As can be seen from these traces, the electropherogram in (A) is dominated by major bands produced from excess dye terminators (see bands from 20 to 24 min) with faint bands appearing in the trace resulting from low electrokinetic loading of the extension products. However, when the sequencing reaction was subjected to SPRI cleanup, the excess dye terminator bands are absent from the trace and the intensity of the extension products is significantly enhanced due to the removal of excess salts used in the cycle sequencing reaction by SPRI cleanup. The decay in the signal profile (fluorescence intensity) shown in Figure 3B is commonly seen in sequencing traces and is due to electrokinetic injection biases.

We were also interested in investigating whether the CFTC chip could be effectively cleaned up and used for subsequent cycle sequencing reactions without showing carryover contamination or requiring a recoating with BSA to eliminate polymerase nonspecific adsorption to the walls of the microreactor, creating failed reactions. In Figure 3C is shown raw data from a control experiment in which a 20-μL aliquot of sequencing buffer was run through the CFTC chip following a cycle sequencing reaction and a 60-μL washing step. As can be seen from these data, very faint bands appeared in the trace that were significantly lower in intensity compared to those produced from extension products generated from the cycle sequencing reactions. Compared with the sequencing trace depicted in Figure 3B, the carryover contamination was found to be less than 5%. Subsequent cycle sequencing reactions run on the same CFTC chip produced results similar to those shown in Figure 3B. No significant degradation in signal appeared until ~30 reactions were run on the same chip (data not shown).

Continuous Flow Thermal Cycler Microchip Coupled to SPRI Microchip. As shown in Figure 3A, the major interferences using dye terminator chemistry arise from unincorporated dye-labeled ddNTPs and excess salts, which may mask sequencing bands appearing early in the electrophoretic trace or prevent effective loading during electrokinetic injection of the extension products.[30,31] As a result, it is important to purify the crude sequencing cocktail before electrophoretic analysis. While conventional purification methods such as ethanol precipitation, phenol−chloroform extraction, and spin column filtration are used frequently in large-scale DNA sequencing protocols since they

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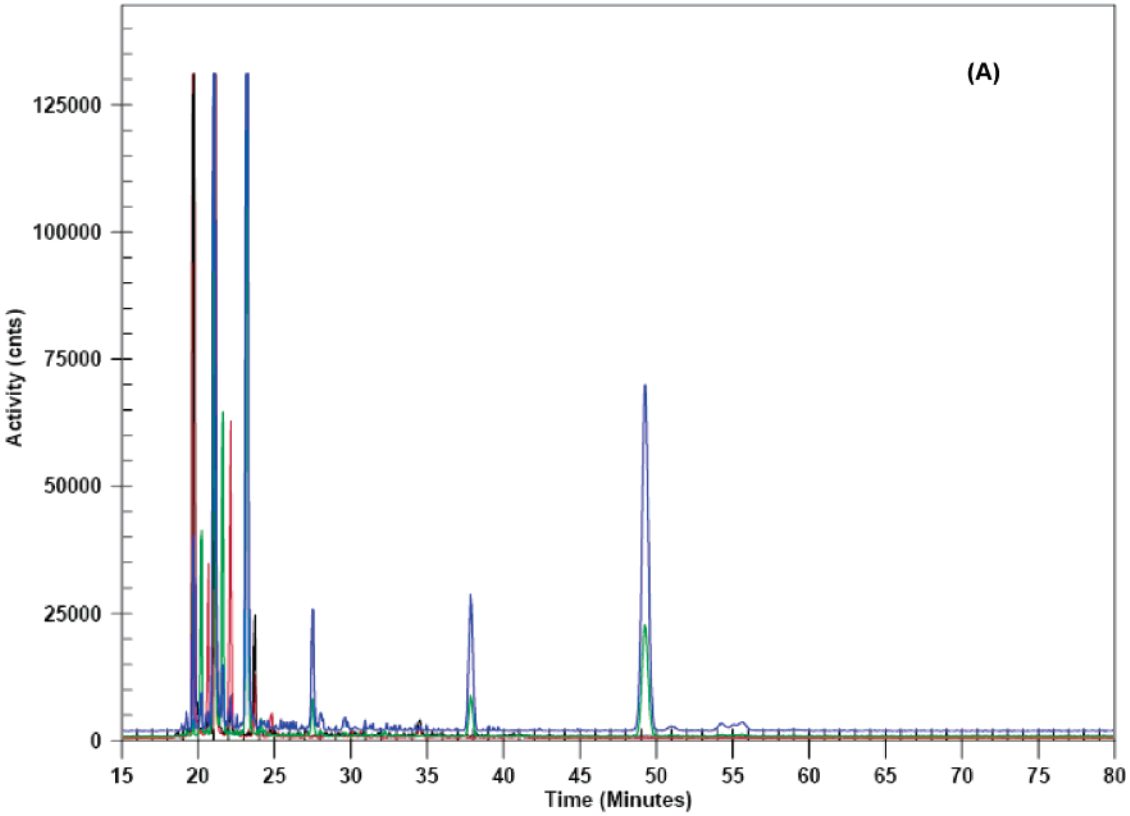
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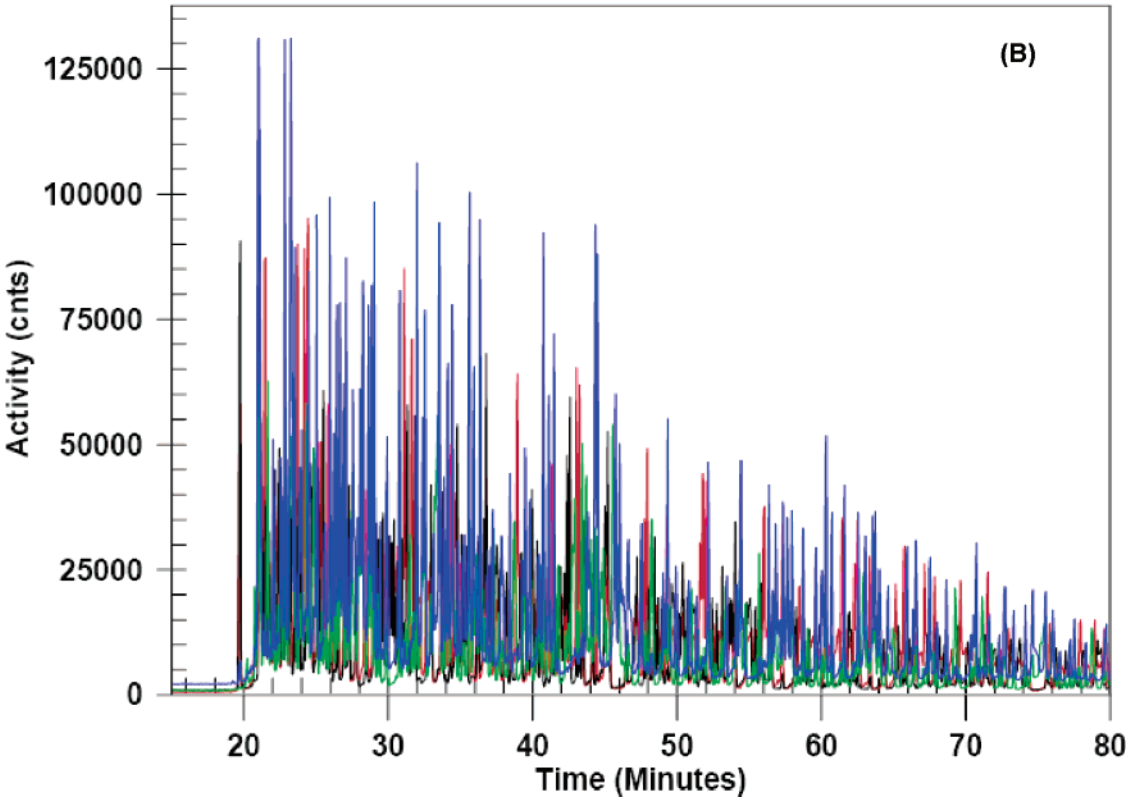
Time (Minutes)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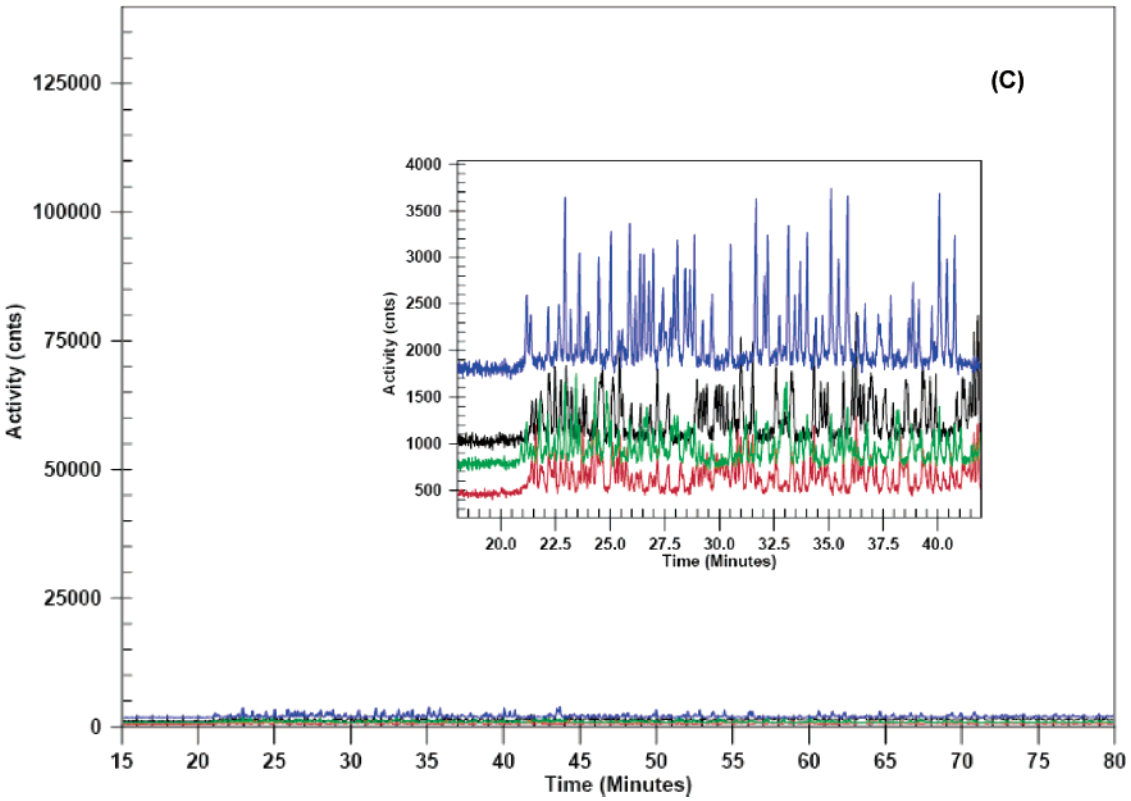

Figure 3. (A) Raw sequencing trace obtained from an unpurified thermal cycle sequencing reaction produced using the CFTC chip with dye terminator chemistry. (B) Raw sequencing trace obtained from a SPRI-purified thermal cycle sequencing reaction produced using the CFTC chip. The thermal cycle reaction was subjected to off-chip SPRI cleanup prior to gel loading on the capillary gel electrophoresis (Beckman CEQ 8000) instrument. (C) Raw sequencing trace obtained from the CFTC chip that was washed with 60 μL of buffer, and then a 20-μL plug of buffer was pumped through the CFTC chip, collected, and subjected to off-chip SPRI cleanup with the eluant analyzed by capillary gel electrophoresis. The data shown in this figure were not subjected to postelectrophoresis signal processing. The reaction cocktails were run through the CFTC chip at a linear velocity of 1 mm/s, producing residence times of 12 (denaturation), 12 (renaturation), and 144 s (extension).

can be implemented in a 96-well (or higher) titer plate format, they require centrifugation, which is difficult to incorporate into a microchip format. Several reports have demonstrated the successful separation and purification of nucleic acids using a solid-phase reverse immobilization,[28,32−35] which uses either a magnetic bead approach or capture on an immobilized support when integrated into a microfluidic platform.

In this set of experiments, we integrated our CFTC microchip to an SPRI microchip to purify the cycle sequencing products prior to electrophoretic sorting. This was accomplished by connecting the output of the CFTC chip to the input of the SPRI chip (see Figure 1A). The purified products from the SPRI chip could be subsequently be collected into a microtiter plate and directly introduced into a conventional capillary gel electrophoresis machine for separation and automated base calling. The system required the operation of two syringe pumps, one to flow the sample through the interconnected microchips and the other to introduce the binding and elution buffers into the system. The reversible binding process could be carried out during the thermocycling reaction, followed by washing and elution with the entire process requiring less than 15 min. Figure 4 shows the bases called from a sequencing run using the CFTC chip coupled to the SPRI chip. From the trace, 681 bases were called with a 99% calling accuracy.

CONCLUSIONS

The results reported herein represent the first example of performing Sanger thermal cycle sequencing reactions in a CFTC microchip. For cases where rapid thermal cycling is required, for example to improve throughput by reducing processing time, the CFTC microchip provides superior performance compared to block thermal cyclers in terms of DNA read length when using rapid cycling times due to its better thermal management capabilities. By coupling the CFTC chip to a SPRI purification chip, we could prepare a sample for electrophoretic sorting in less than 30 min. When the chips are configured into a multichannel format, they can be used to prepare cycle sequencing reactions in an automated, rapid, and high-throughput fashion to deliver electrophoresis-ready products into a capillary array instrument. In addition, if the coupled CFTC/SPRI chips are interfaced to a microchip electrophoresis unit, which would eliminate the need for off-chip sample handling, sample volumes much smaller than 1 μL could be envisioned significantly reducing reagent consumption required for performing DNA cycle sequencing.

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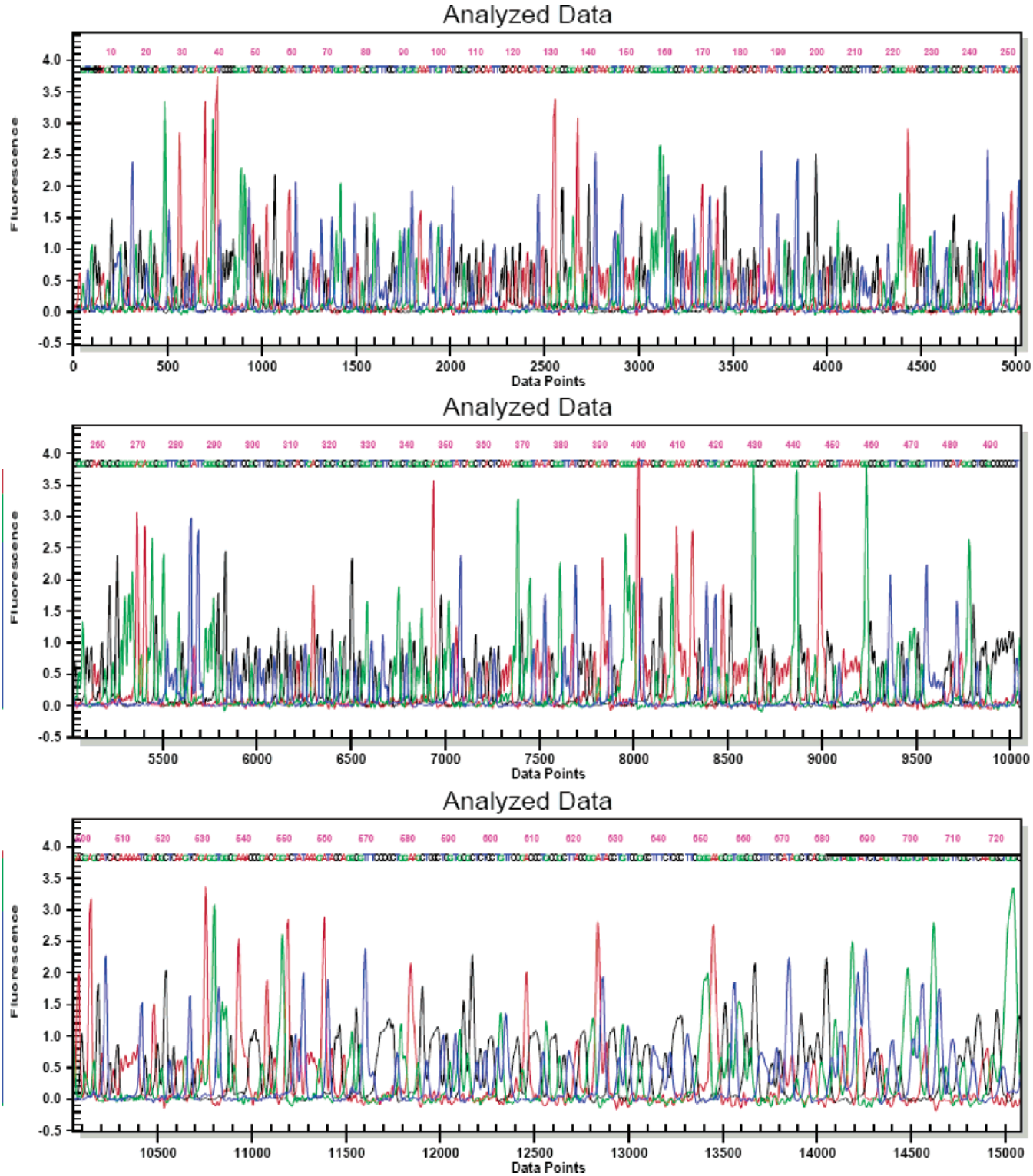

Figure 4. Four-color fluorescence sequencing trace obtained from the thermal cycler microchip coupled to the SPRI microchip. The sequencing trace was analyzed with the default parameters set by the Sequence Analysis Module in the CEQ 8000 system. The sequencing reaction was run through the CFTC chip 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).

In the present system, which incorporated a thermal cycling and solid-phase cleanup chips, each step was poised on different chips. Due to the fact that the material required for each step is similar (PC in this case), one could envision placing both steps onto a single chip to eliminate the need for implementing the interconnection between chips. However, for high-throughput applications where multiple fluidic processors must be operated in parallel to simultaneously process many samples, the limited real estate that can be patterned on a single chip would limit the number of processors the system could incorporate. Therefore,

for high-throughput applications, the approach of placing only a single processing step on a single chip will allow populating the wafer with many more independently operated processors. Work is currently underway in our laboratory to build thermal cycling and solid-phase purification chips composed of 96-channel processors to accommodate high-throughput applications with the appropriate multichannel interconnects.

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