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COMMUNICATION

Microchip for continuous DNA analysis based on gel electrophoresis coupled with co-injection of size markers and in-channel staining

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Abstract

A continuous-flow microchip enabling high-accuracy DNA analysis was developed. Serial consecutive analysis for multiple amplified DNA samples was demonstrated. The sample segments were continuously introduced to the microchip from the PCR device which was interfaced to the microchip through capillary tubing. Electrokinetic co-injection of the DNA samples with size marker enabled reproducible and reliable injection of the DNAs into the gel-filled separation channel providing accurate size determination of the DNA samples. Cross-contamination between serially introduced DNA samples was minimized by plugging a washing solution segment following the previous sample segment between two sample plugs. Using this microchip, continuous separation of multiple samples was performed without any inconvenient and labor-intensive sample preparation steps such as sample mixing, staining, and gel loading which are necessary for conventional gel electrophoresis. It has taken about 4 min to separate single DNA sample and taken 37 min for three serially injected samples which implies that this microchip can be a platform device for fast as well as highly accurate DNA analysis.

Keywords Continuous-flow microchip · DNA analysis · PCR product analysis · Electrophoresis

Introduction

Since the development of polymerase chain reaction (PCR) technique by Muller et al., it was used as the most conventional and powerful tool to amplify the amount of DNA molecules and encouraged the development of various research fields such as molecular biology [1, 2], medical diagnosis [3, 4], and forensic science [5, 6]. With the recent development of microfluidics, many microfluidic devices were designed and employed for performing the PCR process taking the beneficial properties such as faster process, lower power, and less reagent consumption [7, 8]. To improve the amplification throughput of many different DNA samples and the device integration efficiency of DNA analysis microfluidic devices, the methods of carrying out the DNA amplification process on a continuous flow was attractive and widely studied [9–12].

In addition to the DNA amplification in PCR process, it is required to confirm if the target sequences were correctly amplified. Mostly, size comparison by performing slab gel electrophoresis was used to ensure the amplified target DNAs following the PCR process. In microfluidic PCR devices, however, this slab gel electrophoresis method has not been well-established because the method is labor-intensive and difficult to automate as well as to integrate which is inappropriate for microdevices. So far, several studies reported microfluidic PCR platforms which were interfaced with capillary (gel) electrophoresis (CE, CGE) [13–16] or fluorescence detection methods [17, 18] for analyzing the amplified DNA fragments. The microfluidic CE or CGE method was mostly employed for evaluating a single DNA sample in both static chamber PCR or continuous flow–type PCR microfluidic devices. However, those single-sample electrophoretic analyses are not sufficient to treat many DNA samples. For the efficient

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analysis of multiple PCR products, static chamber type of PCR increased the throughput by using parallel structures where several PCR chambers were fabricated in a device and each of them has its own separation channel [15, 16, 19]. The parallel operating multi-chamber device has limitations in further increasing the analysis throughput due to the integration capacity and accordingly increased device sizes. On the other hand, continuous flow–type of PCR devices were well-applied for multi-sample analysis with high-throughput capability using segmented sample loading and amplification in a single microfluidic channel without device size limitation [20–23].

Although the continuous flow–type device has shown attractive properties in device integration and sample analysis throughput, it is still challenging to confirm the accurate size of the amplified DNA products which is mostly achieved by gel electrophoresis. The in-channel electrophoresis for the sequentially loaded and amplified segmented multi-samples has not been realized yet but fluorescence intensity was monitored to check the amplified amount of DNAs in each segment. The alternative way of measuring fluorescence, however, has not provided sufficient information to tell the exact size of DNAs but the increased amount of the products. Therefore, to improve the accuracy of sample analysis with high-throughput DNA amplification in microfluidic devices, it is required to develop methods to perform consecutive gel electrophoresis in a channel which is co-integrated or interfaced with PCR devices. In this paper, we developed a continuous-flow DNA analysis microchip and successfully demonstrated precise determination of sizes of the sequentially loaded multi-DNA samples by performing consecutive co-electrophoresis of DNA samples and size markers which were injected in segmented type in a single channel.

Materials and methods

Materials The PCR reagents including reaction buffer, $MgCl_2$, dNTP mixture, and Taq polymerase were purchased from Promega (Madison, WI) as a part of the PCR Core System II kit. Template DNA and their primers for amplifying the 323-bp fragment were obtained from Promega (template; plasmid DNA from bacterial kanamycin resistance gene, primers; upstream, 5′GCC ATT CTC ACC GGA TTC AGT CGTC3′; downstream, 5′AGC CGC CGT CCC GTC AAG TCAG3′), and those for the rest PCR from Takara (template; bacteriophage φX174 DNA) and Bioneer (primers for amplifying the 640-bp fragment; upstream, 5′TCC GCT TCC TCC TGA GAC3′; downstream, 5′GGA AAC ACT GGT CAT AATC3′ and the 850-bp fragment; upstream, 5′AGG CTC TAA TGT TCC TAA CCC TGA3′; downstream, 5′TGA CGG TTA TTT CCT AGAC3′). DNA size marker (50, 150, 300, 500, 750, and 1000 bp) was supplied by Promega. A 6× loading buffer (36% glycerol, 30 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) was obtained from Takara (Shiga, Japan). PDMS was a 10:1 mixture of PDMS oligomer and cross-linking agent (Sylgard 184; Dow Corning, Midland, MI, USA). A stock solution of 5× TBE buffer (pH 8.3) was prepared by mixing 445 mM Tris base, 445 mM boric acid, and 10 mM EDTA. Acrylamide; N,N′-methylenebisacrylamide; isobornyl acrylate (IBA); poly(ethylene glycol) dimethacrylate; and 2,2-dimethoxy-1,2-diphenylethan-1-one (Irgacure 651) were purchased from Sigma (St. Louis, MO). YOYO-1 (Molecular Probes, Eugene, OR) was used as the DNA intercalating fluorescent dye. Perfluorodecalin was purchased from Acros Organics (Morris Plains, NJ). All aqueous solutions were prepared with purified water from a Milli-Q purifying system (Millipore, Milford, MA).

Microchip design Figure 1 shows a schematic design and photographic images of a DNA analysis microchip. Except for V-shaped diagonal microchannels connected to R4, R5, R8, and R9, other microchannels were filled with polyacrylamide gel (Fig. 1A). The microchannels from R4 (R stands for a reservoir) to R8 and from R5 to R9 were used for hydrodynamic introduction of DNA size marker and DNA sample, respectively. Length and width of the diagonal microchannel are 20 mm × 200 μm. The longest channel at the center of the microchip were used as main separation channel (R1 to R10, 32 mm × 300 μm, length and width) and the length from the cross-point to R10 was 20 mm. Laser-induced fluorescence signal from DNAs was collected at the main channel, 5 mm above from R10. Microchannels from R6 and R7 to intersection of each V-shaped microchannel were used to apply injection voltage (10 mm × 150 μm, length and width). Auxiliary separation channels were located between the main separation channel and each V-shaped microchannel (2 mm × 100 μm, length and width). Microchannels from R2 and R3 to each auxiliary separation channel (7 mm length and 200 μm width) were designed to apply voltage to inject DNAs to the short auxiliary channels. All microchannel depths were 50 μm.

Microchip fabrication The microchip was made by bonding PDMS with microchannels and a flat glass substrate (Fig. 1B). Microchannels in PDMS were fabricated by photolithography replica molding method [24, 25]. After assembly of the microchip, surfaces of PDMS microchannels were modified with IBA to fix polyacrylamide gels [26] (see Supplementary Information (ESM) Fig. S1 and S2). An enlarged picture in Fig. 1C (left and right images) shows a selectively modified PDMS region using an IBA mixture with a UV irradiation. The IBA mixture was composed of IBA (92 wt%), poly(ethylene glycol) dimethacrylate (5 wt%), and Irgacure 651 (3 wt%). The mixture was injected into whole microchannels through R1 by a syringe and left for 24 h to be absorbed on the

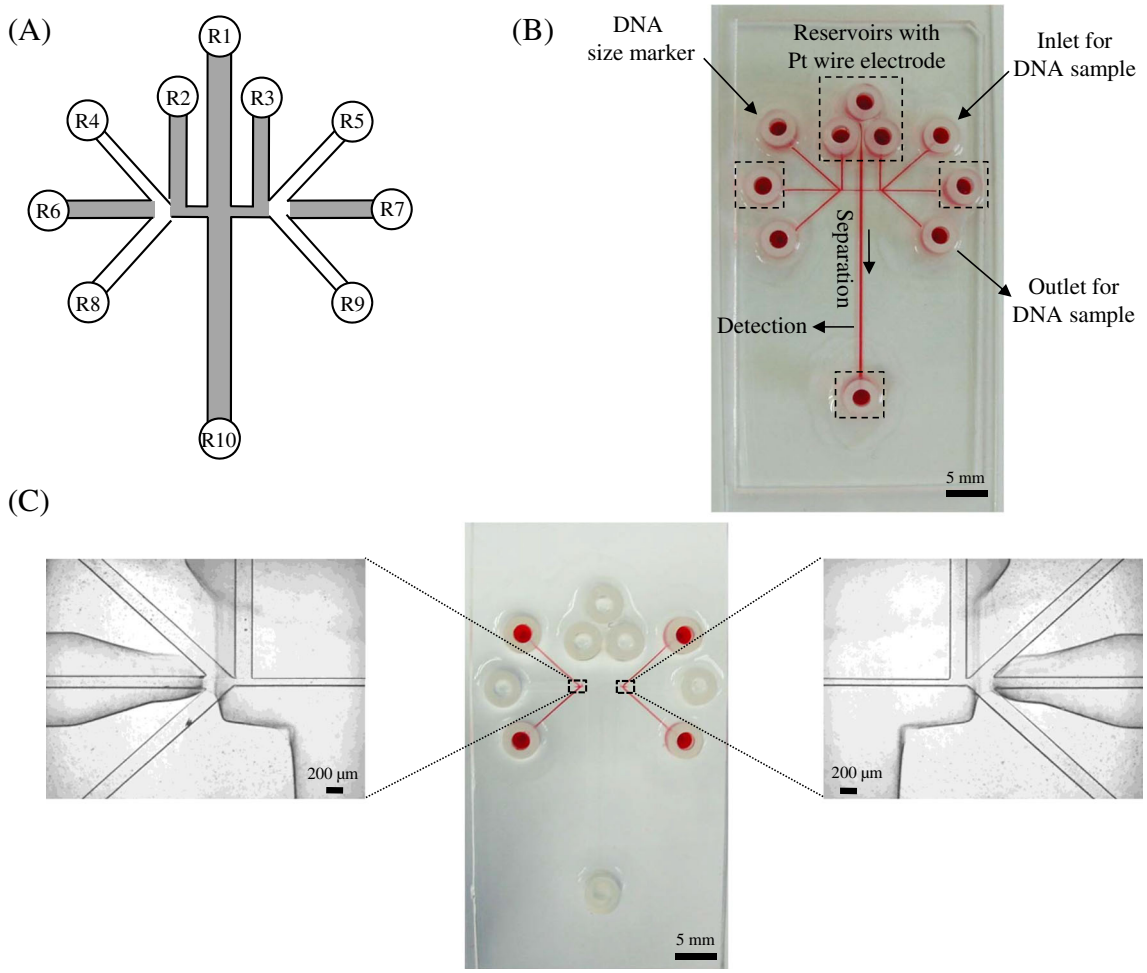


Fig. 1 Microchip for segmented continuous-flow size determination of DNA. **A** Symmetrical microchannel design for simultaneous injection and separation of sample DNA with size marker. Microchannels filled with polyacrylamide gel are shown with gray color. **B** Pt wire electrodes are placed in the reservoirs (box with dotted line) except for DNA size marker and DNA sample channel reservoirs. All microchannel are filled with red ink for clarity (polyacrylamide gel is not formed in this microchip). **C** Pictures of the microchannels selectively filled with polyacrylamide gel. Overview of microchip indicating gel-filled channels and vacant channels filled with red dye solution (middle). Expanded image of vertex region of V-shaped microchannel and a shade of gray indicates selectively modified PDMS by IBA (left and right)

PDMS microchannel surfaces followed by removing the remaining mixture form microchannel by flushing the channel with deionized water and dried. After the IBA mixture absorption on PDMS surfaces, a degassed polyacrylamide gel solution was filled in all the channels from R1 by a syringe. The polyacrylamide gel solution was composed of 5%T (where T is the total monomer concentration) polyacrylamide, 3.3%C (where C is the cross-linker concentration) bis-acrylamide, and 2% (v/v) Irgacure 651 dissolved in 1× TBE buffer. To selectively polymerize the polyacrylamide gels in the microchannels, the microchip was covered by a photomask and exposed to UV light for 10 min (350-W mercury lamp, Osram, Germany). Unpolymerized polyacrylamide gel solution in the V-shaped microchannels where UV light was blocked by the photomask was removed by air and washed with deionized water. V-shaped microchannels and all reservoirs were filled with 1× TBE buffer for preventing dryness of the gels. Platinum wire electrodes for applying high voltage were inserted in each reservoir except for V-shaped microchannel reservoirs (R4, R5, R8, and R9, Fig. 1B).

Instrumentation Sample injection and separation were controlled by computer-controlled high-voltage supplying system equipped with a high-voltage power supply (MP10N24F; Spellman High Voltage electronics Plainview, NY), a high-voltage relay (K45C332; Kilovac, Santa Barbara, CA), and homemade voltage dividing system. A LabVIEW (National Instruments, Austin, TX) program and a multifunction I/O board (CB-68LP; National Instruments) were used for instrumental control and data acquisition. For laser-induced fluorescence (LIF) detection, an argon ion laser (Lexel 95; Lexel laser, Fremont, CA) was employed as an excitation source and the laser beam was incident on the chip at an angle of 45° with a plano-convex cylindrical lens (CLCX-25.4-25.4-

UV; CVI Melles Griot, Rochester, NY). A fluorescence signal was detected by using a microscope (TE300; Nikon, Japan) equipped with a 10× microscope objective lens (numerical aperture, 0.3; Nikon, Japan), fluorescence filter (505-nm dichroic mirror and 515–555-nm barrier filter; FITC filter cube; Nikon, Japan), and photomultiplier tube (H5784-01; Hamamatsu, Bridgewater, NJ).

Continuous-flow PCR A homemade continuous-flow PCR device previously developed was used to amplify multiple samples of DNA in a continuous-flow stream [9, 27, 28]. Briefly, the continuous-flow PCR device was composed of three heating blocks assembled together and PTFE capillary tube (100 μm I.D. and 400 μm O.D.; Ej-06417-72, Cole-Parmer, Vernon Hills, IL) which wound on the assembled blocks. The heating blocks were set at 92 °C, 55 °C, and 72 °C for denaturation, annealing, and extension, respectively. Three PCR mixtures for amplifying 323 bp, 640 bp, and 850 bp were sequentially injected with 5 μL of each sample at regular intervals using an HPLC injection valve (7725i; Rheodyne, Rohnert Park, CA) onto a flowing stream of perfluorodecalin, a hydrophobic carrier fluid. Between each segment of PCR mixtures, 5 μL of 6× loading buffer was injected as a washing solution to prevent sample carryover and also as an indicator to show the position of PCR mixture segments. Two-microliter perfluorodecalin segments were intervening each segment of PCR mixtures and washing solutions. Segments of three PCR mixtures and two washing solutions onto the perfluorodecalin were passing through the device at a rate of 2.0 μL/min by a syringe pump (PHD2000; Harvard Apparatus, Holliston, MA). The outlet of the continuous-flow PCR device was connected to a FEP tubing (254 μm I.D. and 1/16 in. O.D.; 1526, IDEX Health and Science, Oak Harbor, WA), and the sample segments from the PCR device were transferred to the inlet (i.e., R5) of analysis microchip through the FEP tubing (Fig. 3). The sequence of the segments flew into the diagonal microchannel from R5 was carrier fluid—323 bp amplified DNA—carrier fluid—washing solution—carrier fluid—640 bp amplified DNA—carrier fluid—washing solution—carrier fluid—850 bp amplified DNA—carrier fluid. Introduced segments to R5 went through the diagonal V-shaped DNA sample channel and exited through R9 of the DNA analysis microchip.

Continuous DNA analysis procedure Figure 2 shows operating procedure for analysis of DNA in the microchip. Detailed procedure will be explained by following sentences. Except hydrodynamic sample loading of size marker and DNA sample from PCR device to both side of diagonal V-shaped channels, all fluids were operated electrokinetically by applying high voltage thorough Pt wire in microchip reservoirs.

Step 1. Preconditioning step (Fig. 2A). A preconditioning step was for filling and evenly distributing fluorescence dye (YOYO-1) in the main separation channel. A 25-μM YOYO-1 in 1× TBE buffer was placed at R10, and other reservoirs were filled with 1× TBE buffer. Pt wire electrodes were inserted in all reservoirs except for the reservoirs connecting V-shaped microchannels (R4, R5, R8, and R9). The R10 was grounded during all voltage applying procedure. Applying − 200 V (− 62.5 V/cm) to R1 drove positively charged YOYO-1 molecules from R10 to R1, and the voltage application was kept for 30 min for thorough spreading the dyes through the separation channel.

Step 2. Sample loading step (Fig. 2B). A 5 μL of DNA size marker and 323 bp amplified DNA were introduced from R4 and R5, respectively, and they filled V-shaped microchannel of each side.

Step 3. Injection step (Fig. 2C). The DNA size marker and 323 bp amplified DNA were simultaneously injected into auxiliary separation channels by applying − 500 V (− 156.2 V/cm) at the same time to R6 and R7 for 1 s.

Step 4. Positioning step (Fig. 2D). Injected samples were transferred to the cross-region of main separation channel and − 300 V (− 103.4 V/cm) was simultaneously applied for 1 min to R2 and R3.

Step 5. Separation step (Fig. 2E). The separation of the sample and size marker was initiated and maintained by applying − 300 V (− 93.7 V/cm) for 4 min to R1. Injected samples were separated by their size during gel electrophoretic migration toward the R10. They were stained with the fluorescence dye while passing through the main separation channel, and fluorescence signals from the separated samples were detected at 15 mm down from the starting point, cross-region, of the main separation channel.

Step 6. Washing step. A washing solution was following the previous sample to clean up the left trace of DNA for next sample analysis. When the washing solution was passing through the V-shaped DNA sample channel, it was injected solely into the auxiliary separation channel by applying − 500 V (− 156.2 V/cm) to R7 for 2 s. After injecting the washing solution into the main channel, the separation procedure for sample analysis was performed with the same voltage application. Completion of the channel cleaning was followed by next DNA sample segment analysis by repeating steps 2 to 5. Fluorescence signals were continuously obtained during all analysis steps except for the washing processes.

For the multiple DNA samples analysis procedure in the microchip, the operating procedure of multiple DNA samples were basically repetitive cycling of single-sample analysis procedure. The procedure from step 1 (preconditioning) to step 5 (separation) was performed sequentially for the first

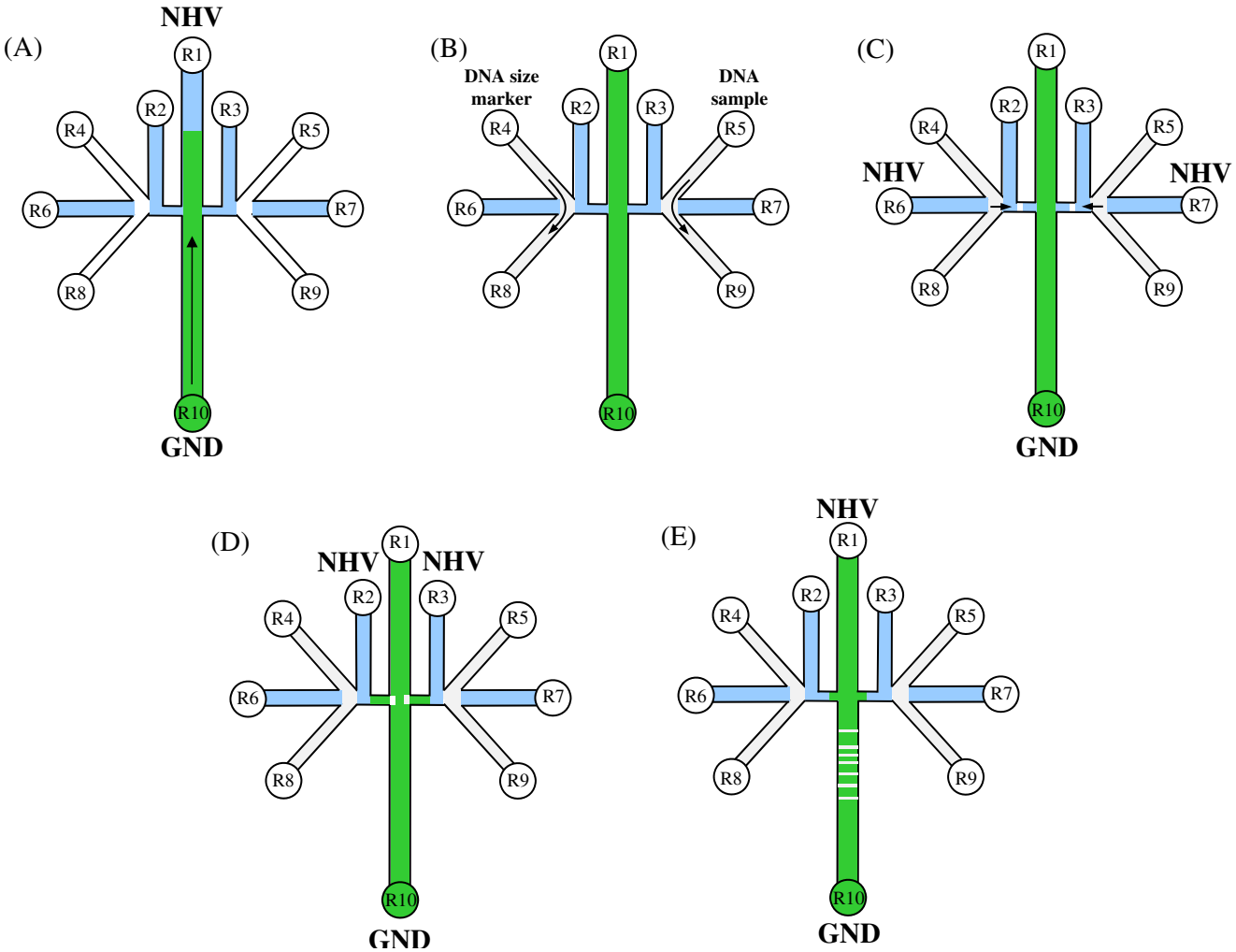


Fig. 2 Operation procedure for size separation of DNA in the microchip. NHV stands for negatively high voltage and GND is ground. **A** Preconditioning step for filling the fluorescence dye into a separation channel. Applying − 200 V to R1 for 30 min drove positively charged YOYO-1 molecules from R10 to R1. **B** Loading step for introducing size marker and DNA sample into each diagonal V-shaped channel. **C** Injection step for co-injecting size marker and DNA sample into auxiliary separation channels. − 500 V was applied to R6 and R7 at the same time for 1 s. **D** Positioning step for transferring size marker and DNA sample to the cross-region of a main separation channel. − 300 V was applied to R2 and R3 for 1 min. **E** Separation step for separation and detection of the injected DNAs. − 300 V was applied to R1 for 4 min

sample and then the procedure from step 2 (loading) to step 5 was performed for the following samples. Because step 1 was essentially the same procedure as step 5, repetitive cycling procedure could be carried out skipping step 1.

Results and discussion

DNA analysis microchip design and fabrication The microchip for performing continuous electrophoresis of segmentally injected samples was designed to have branch channels for simultaneous injection of DNA size markers and DNA samples in a continuous-flow way. The channels were composed of main separation channel (R1 to R10), a V-shaped symmetrical channel for introducing segments of DNA markers (R4 to R8) and samples (R5 to R9), and other branch channels for manipulating the DNA segments. The main separation and manipulation channels were selectively filled with polyacrylamide gel (Fig. 1). To fill the polyacrylamide gel only in selective regions of the microchannels, photopolymerization was employed. To introduce continuous-flowing DNA samples and markers, the V-shaped cannels remained open without filling with gel. Through those selectively open microchannels, the flow of continuous-flowing segmented DNA samples could be introduced from channel R5 to R9 and the size markers from R4 to R8, respectively. As shown in Fig. 1C, only open channels were filled with red dye solutions which were introduced from R4 and R5 implying that the gelation was well carried out selectively in other channel regions and blocked the dye solution.

The polyacrylamide gel in the channel was used as a separation matrix and also played a role for a valve as well as a

passage of electric potential. The gel-filled microchannels which were connected with R2, R3, R6, and R7 were a passage of DNA marker and sample injection potential, respectively. When negatively high potential relative to R10 ground was applied at R6 and R7, DNA size marker and sample were co-injected into the main separation channel region at around the junction of the V-shaped channels. If there was no potential applied, markers and segmented DNA sample flew out through the channels. Thus, the polyacrylamide gel in the region near the open junction of the V-shaped microchannel was supposed to act as a valve for the active injection of DNA sample and DNA size marker.

The channel branches connected to R2 and R3 were designed as passages for applying voltage to move the injected samples to the main separation channel through auxiliary separation channels, a short gel-filled region between the main separation channel and open junction. Based on the previous experience employing DNA analysis microchip which was composed of directly connected injection and main separation channels [29], the auxiliary separation channels were added to prevent the countercurrent flow of fluorescent dyes from the separation channel to V-shaped microchannels during the injection process. Fluorescence dyes, YOYO-1, in the gel have positive charges which were supposed to move in the opposite direction of DNA. When the injection potential was applied, DNAs moved into auxiliary separation channels from V-shaped microchannels, and YOYO-1 moved from separation channel to auxiliary separation channel. If the auxiliary separation channel was not existing, then the separation channel was directly connected to V-shaped microchannels and the dye would move to the sample during the injection process inducing nonspecific fluorescence which interfered the accurate detection of the separated DNA bands in the main channel. Thus, auxiliary separation channels played as buffer region to prohibit florescence dye reacting with DNA samples outside of the main separation channel, which made it possible to use the DNA sample for further process such as cloning.

Operation process for continuous DNA analysis The whole operating procedure of analysis for a DNA sample segment was composed of five steps: preconditioning, loading, injection, lining up, and separation followed by detection (from Fig. 2A to E). To perform the analysis of continuously introduced DNA sample segments in the microchip, the operating procedure had to be carried out seamlessly and repetitively.

The preconditioning step (Fig. 2A) replenished the separation channel with YOYO-1 to facilitate the labeling of DNA samples. For continuous analysis of multiple segmented DNA samples in the same separation channel, fluorescence dye should be replenished into the gel matrix in the channel continuously and repetitively to stain each of the sample segment. YOYO-1 solution was placed in reservoir R10, and this positively charged fluorescence dye moved toward R1 filling the gel matrix in the main separation channel.

The next step was sample loading (Fig. 2B). Segmented DNA samples were continuously introduced through the sample channel by hydrodynamic force using a syringe pump from R5 to R9. DNA size markers were also continuously loaded into the opposite V-shaped side channel from R4 to R8.

The DNA sample and size marker loaded in the V-shaped side channels were injected into auxiliary separation channels from each side as pointed with arrows in Fig. 2C. The simultaneous electrokinetic injections of DNA sample and size marker were performed by applying electric potential at the reservoirs R6, R7, and R10 when the sample segment passes intersection of the right side V-shaped microchannel. This co-injection of a size marker and a DNA sample from both sides of V-shaped channels to the auxiliary channels helped to place the sample and marker at the same starting position, the cross-intersection of the main separation channel before the separation process for accurate analysis. The auxiliary channels also played as barriers to reduce sample contamination by prohibiting the direct contact of DNAs and staining dyes.

After the injection of DNA sample and size maker into each side of the auxiliary channel, the DNAs were transferred to and lined up at the cross-junction of the main separation channel for accurate size determination by applying appropriate potential on reservoirs R2, R3, and R10 (Fig. 2D). During the DNA transferring, the fluorescence dye in the main channel was moving to R2 and R3, staining the DNAs of sample and size marker without contaminating the DNAs in sample channel. This in-channel sample staining method based on counter-current intercalation made it eligible to label the injected segmented DNA samples without additional loading of the dye solution during the intervals of each sample segment which was introduced continuously and sequentially to the analysis microchip. This one-time loading and multiple sample analysis excluded many inconvenience induced by operational complexity.

Finally, simple switching the potential applied in each reservoir, negative high voltage on R1 and ground on R10, started gel electrophoretic separation of sample DNA and size marker mixture (Fig. 2E). The separated DNAs were detected using laser-induced fluorescence at the end of the main channel near R10.

Our microchip was designed to perform continuous analysis for sequentially introduced DNA sample segments as well as a single sample as shown in a schematic drawing (Fig. 3). For the multiple sample analysis, the steps were repeated except for the preconditioning step. Because the preconditioning step to introduce fluorescence dye to the main channel was operated with the same potential application of the final separation step, the preconditioning step could be skipped from

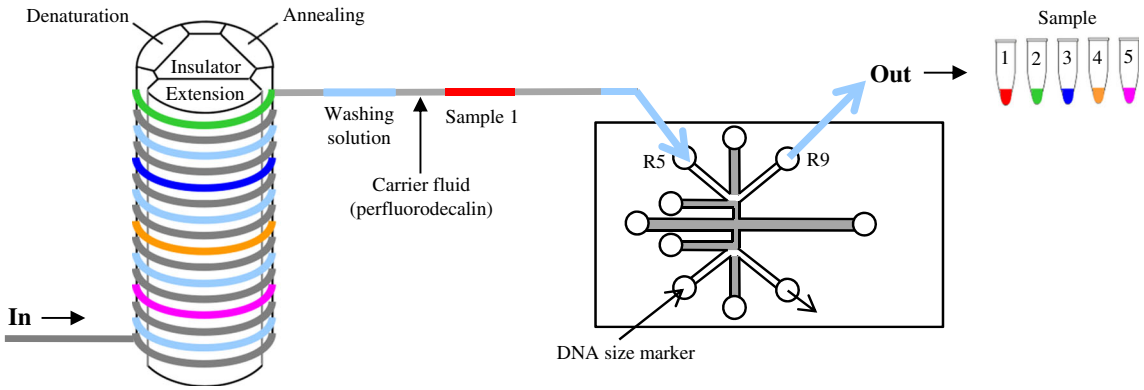


Fig. 3 Schematic diagram of interconnection of DNA analysis microchip with a continuous-flow PCR device

the second segment analysis of the continuous segmented samples.

Single DNA sample analysis Following the described operation process, single PCR amplified DNA sample was analyzed in the microchip. Figure 4 A shows the analysis result of 323-bp PCR product with DNA size marker from the microchip. The PCR product was amplified from the lab-made continuous PCR device and introduced into the right side of the V-shaped microchannel and a DNA size marker was introduced to the left one. For a stricter evaluation of the microchip analysis, three independent separations were conducted, solely injected 323-bp PCR product sample, DNA size marker, and co-injected 323-bp PCR product with size marker. The electropherograms resulted from those three different separations were presented, from top to bottom, 323-bp sample, size marker, and sample with size marker, respectively. The sample peak positions appeared consistently at around 2.6 min right after the 300-bp markers were well matched with that of conventional gel electrophoresis image of the marker and sample (Fig. 4B). Furthermore, separation of the sample and size marker took less than 4 min which is much shorter than conventional gel separation (about 30 min for separation). Comparison of the results from microchip and conventional gel electrophoresis proved that the separation ability of microchip is as good as widely used gel electrophoresis method, and samples can be analyzed using much lesser amounts within a short time.

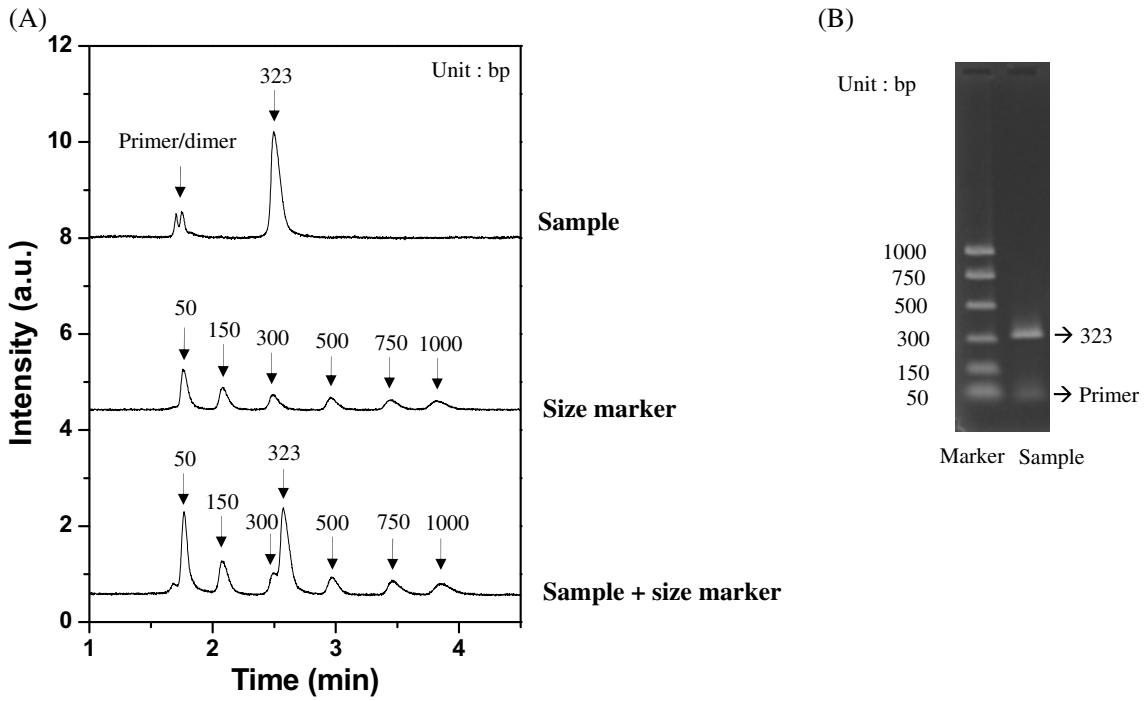


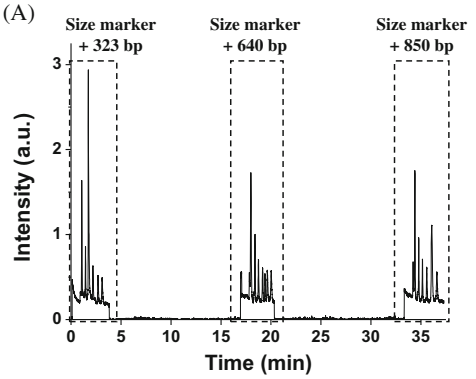
Fig. 4 Single DNA sample analysis results from the microchip and a conventional gel electrophoresis. **A** Microchip analysis result of amplified 323 bp DNA (top), DNA size marker (middle), and 323 bp DNA co-injected with DNA size marker (bottom). **B** Conventional gel electrophoresis result of DNA size marker and amplified 323 bp DNA. The numbers indicate the size of the DNA fragments in base pairs

Continuous analysis of multiple DNA samples Inspired by the successful demonstration of DNA analysis for a single sample with the microchip, continuous analysis was performed for sequentially injected multiple DNA sample segments by repetitive operation of the same process as described in "Microchip fabrication." As samples for analysis, three DNA fragments with different sizes, 323, 640, and 850 bp, were employed. These three samples were injected to the microchip one after another with interval buffer solution between each sample segment for washing the previous sample left in the gel matrix as well as prohibiting cross-contamination between samples. Each sample plug and interval solution was injected with 5 μL volume. Electropherograms for three consecutively injected samples were obtained within 40 min (Fig. 5A). Each sample separation took less than 3 min and the rest of time for intervals. As shown in the details of each electropherogram, the peak for each amplified DNA fragment sample appeared in expected position implying that the sample and size marker were co-injected and separated appropriately (Fig. 5B). Also, the similar peak height of each size marker meant that the electrokinetic injection was consistently reproducible. Even in between the samples as well as in second, and third sample electropherograms, no significant trace of previous sample was found which meant that the buffer plug between samples washed out left over of the DNAs and effectively regenerated the gel in the channel for the next sample separation. Based on the peak position, the sizes of sample DNAs were calculated and discrepancy from their real sizes is listed in Fig. 5C. As shown, the size discrepancy was small for short DNA fragment and large for long one which is also similar with the conventional gel electrophoresis results.

Conclusions

In this work, a continuous-flow DNA analysis microchip was developed. By interfacing with a previously developed continuous-flow PCR device, it was successfully demonstrated to analyze successively introduced multiple DNA sample segments. Most continuous-flow-based devices developed so far have focused on real-time PCR analysis providing the amount of DNA strands in the sample. To evaluate the accurate size of amplified DNA fragment, it was necessary to perform further inconvenient electrophoresis. In our microchip presented in this paper, the amplified DNA samples from thermocycler flew into the analysis chip without any inconvenient sample preparation steps such as taking out the sample, mixing with loading dye, injecting, and staining the DNA. The samples were injected electrokinetically without perturbing the hydrodynamic flow. The direct interfacing the

(A)



(C)

DNA size (bp)	Calculated size (bp)	Discrepancy (%)
323	325	0.9
640	604	−1.7
850	714	−13.2

(B)

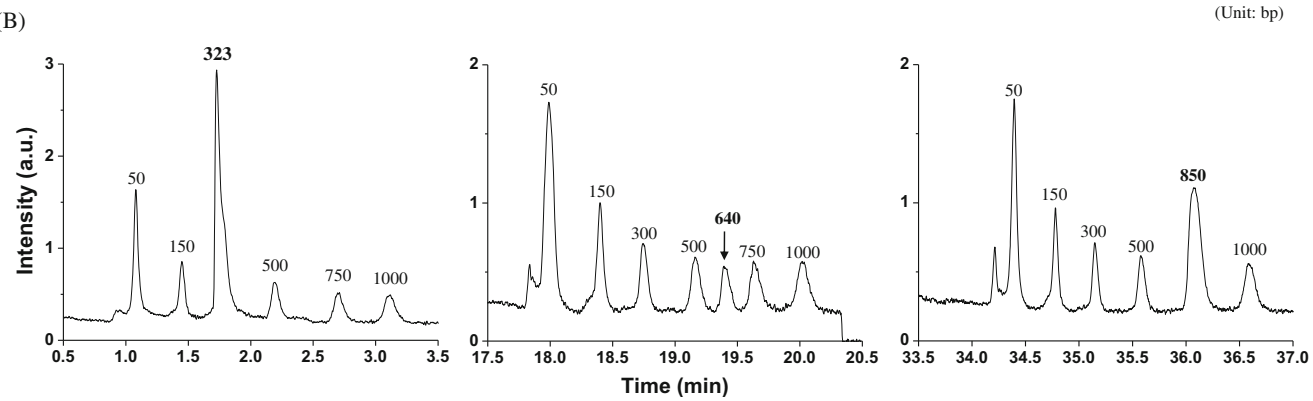


Fig. 5 Multiple DNA sample analysis result. **A** Continuous-flow analysis result of amplified 323 bp, 640 bp, and 850 bp DNA with DNA size marker from the microchip. **B** Enlarged view of dotted box in (**A**) for each sample electropherogram. **C** DNA size and discrepancy calculated by calibration with mobility of size markers and sample DNA

DNA analysis microchip with PCR device helped to protect the sample from contamination risk which could be possible during the manual sample handling process as well as to save the labor. Size marker for referencing the sample fragment size was co-injected with the sample into the separation channel and this co-injection helped to provide exact size estimation of the amplified DNA fragments.

In addition to the convenience of the analysis, the experimental process was also time-saving with a quick process by taking the advantage of microchip. For a single injected sample, it took about 4 min for separation and a total of 37 min took for three consecutively injected samples with 5 μL buffer interval following every sample segment. The washing solution plugged between each sample proved to be effective for rinsing out the previous sample in the separation channel and preventing cross-contamination between samples. Considering the beneficial properties of our DNA analysis microchip described in this work, we expect that this device can be further developed for a convenient, fast, and highly accurate DNA analysis device which has huge potential applicability in the fields of biochemical, medical, and environmental analysis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00216-021-03560-9.

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Declarations

Conflict of interest The authors declare no competing interests.

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