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Single-step quantitation of DNA in microchip electrophoresis with linear imaging UV detection and fluorescence detection through comigration with a digest

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Abstract

We demonstrate a convenient single-step quantitation technique for double-stranded DNA (dsDNA) fragments in polymerase chain reaction (PCR) products based on microchip capillary electrophoresis (μ-CE)/UV or fluorescence detection. PCR products of polymorphisms on the human Y-chromosome related to spermatogenic failure did not need purification. They were premixed and comigrated with a DNA digest whose concentration was known. Hydroxyethyl cellulose (HEC) dissolved in 5× Tris–borate–EDTA (5× TBE, pH 8.3) was used as a separation matrix in a linear polyacrylamide-coated quartz microchip, while mixed poly(ethyl oxides) (PEOs) of different molar-masses dissolved in 1× TBE (pH 8.3) containing 1 ng/μl ethidium bromide was used as a separation matrix in an uncoated poly(methyl methacrylate) (PMMA) microchip. Elution profiles were monitored under either real-time linear imaging UV detection in the snapshot mode where the total separation time is fixed, or light-emitting diode (LED) confocal fluorescence detection in the finishline mode where solutes migrate over the same separation length. It is found that, in both modes, a linear relation exists between the peak areas (A) and the multiplication of the digest concentrations (C) and the fragment sizes (L) in a DNA restrictive digest. Using the comigration electropherogram of a single-step experiment, the concentrations of PCR products were directly determined using the A versus LC linear relationship. The sole condition to obey is that the chosen digest has different fragment sizes with the PCR products of interest. This condition is easy to obey, because μ-CE owns high separation ability, and many digests are commercially available. The recovery of the technique was between 98 and 105%. The R.S.D. for chip-to-chip concentration measurements was less than 6.0% ($n = 6$). Hence, the technique was accurate and reliable for DNA assays.

Keywords: Chip technology; Fluorescence detection; Light-emitting diodes; Detection, electrophoresis; DNA; Poly(methyl methacrylate)

1. Introduction

The polymerase chain reaction (PCR) is one of the most useful technologies developed for the amplification of genomic materials in medical, forensic, and ecological fields. Analysis of PCR products has been achieved by slab gel electrophoresis (SGE), capillary electrophoresis (CE), and microchip capillary electrophoresis (μ-CE). The SGE and CE are two electrophoretic technologies widely used in the past. The limitations of SGE are the low analytical speed, high labor intensity, and the difficulty to automate. CE in polymer matrices could overcome some of the limitations, because CE allows DNA separations under much higher electric field strengths than SGE on an automating instrument. However, the cost of a parallel capillary array is high and the separation speed needs further elevating [1]. μ-CE is presently the subject of an intense research activity. It offers more advantages than SGE and common CE, such as high speed, miniaturized consumption of samples and reagents, easy integration with reaction units, and feasibility to constitute high throughput and automated platforms [2–4]. In spite of considerable efforts, μ-CE still suffers from difficulties in accurate quantitation of DNA amounts, owing

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to the manipulation of very small sample injection volumes, and multiple variables which affect the quantitation accuracy. Conventional quantitation methods need to generate a standard working curve demonstrating a linear relation between the peak area and the amount of a DNA standard. Subsequently, the peak area of a PCR product obtained in a succeeding electrophoretic run was employed to derive the amount of the product through the previous working curve [5]. The accuracy of peak area determinations by CE will be influenced by many factors, such as batch-to-batch changes in solute properties, buffer composition, separation matrix inhomogeneity, channel surface nonuniformity, injection, temperature, and voltage, etc. [6]. Small differences of these factors will result in very large quantitation errors. Real-world DNA samples usually exist in a dilute, unpurified form dissolved in a high-ionic-strength buffer. Some small ions, i.e., chloride, can interfere with DNA loading on a microchannel [7]. Sample injection is very sensitive to the difference of solvents [8]. Because the PCR products usually contain high amounts of salts and remaining dNTPs, the low-ionic-strength buffer for dissolving DNA standards may not adequately represent the performance expected with the analysis of real-world PCR samples [9]. To overcome the differences, some pretreatment techniques, such as desalting, have been proposed for unpurified PCR products to decrease the interference of DNA buffer on the polyacrylamide-coated capillaries [10]. However, they will complicate the manipulation of automatic lab-on-a-chip. The injection and separation by electrophoretic effects strongly depend on chemical characteristics of the microchannel surface-solution interface. For multiple-step separations of DNA standards and samples, care should be taken to minimize the differences of the microfabricated channels (e.g., geometrical imperfections, wall modification by the transported solutions or analytes, etc.) and of the separation media (e.g., buffer evaporation, electrolysis product existence, etc.) [11,12]. For most implementations of μ-CE separations, one microchannel network reservoir is filled with the sample, and injection is performed by electrokinetic migration of the sample through a cross channel. This approach requires washing and refilling of the polymer solutions and the sample between each run. Thus, how to assure an identical injection and separation condition is particularly challenging. It is reasonable to expect that quantitating in a single-step experiment will be an ultimate solution to the problem.

Our previous work has shown the feasibility of using bare poly(methyl methacrylate) (PMMA) microchips for dsDNA separations without the need to modify the microchannels if some polymer solutions, such as celluloses and poly(ethylene oxide) (PEO), are used as separation matrices [13,14]. The aim of this study was to establish a fast quantitation technique that takes into account of coated and uncoated microchips, and two commonly-used detectors in μ-CE (one is linear imaging UV detector in snapshot mode and the other is LED confocal fluorescence detector in finishline mode). In the snapshot mode, the total separation duration is fixed, as similar as in the slab gel electrophoresis, and molecules of different sizes visit different sections of a matrix-filled channel [15,16]. In the finishline mode, the total separation distance is fixed with a single-point detection, which is nowadays the dominant detection mode in μ-CE. The quantitation technique was validated by the separation reproducibility using PCR products generated from human Y-chromosomes related to spermatogenic failure.

2. Experimental

2.1. Materials and chemicals

Hydroxyethyl cellulose (HEC, M_r 250 000), poly(ethylene oxide) of four molar masses (M_r 8×10^6, 1×10^6, 4×10^5, 1×10^5), and 5× TBE (445 mM Tris–borate, 10 mM EDTA, pH 8.3) were from Aldrich (Milwaukee, WI, USA). HPMC (viscosity of 2% solution at 20 °C, 4000 cP) was from Sigma (St. Louis, MO, USA). ΦX174-*Hae*III and λ-*Hin*dIII digests were from TaKaRa (Shiga, Japan). ΦX174-*Hin*fI digest was from Stratagene (La Jolla, CA, USA). pBR322-*Bst*NI digest was from New England Biolabs (Beverly, MA, USA). pGEM-T vector [3000 base pairs (bp)] was from Promega (Madison, WI, USA). DNA 100 bp ladder was from GibcoBRL (Rockville, MD, USA). Ethidium bromide (EtBr) was from Nippon Gene (Tokyo, Japan). The 18.2 MΩ cm water (Millipore, Bedford, MA, USA) was used for all solution preparation.

2.2. Polymerase chain reaction

Human genomic DNA of Y-chromosome related to spermatogenic failure and the primer sets to yield PCR products [17] of 189, 256, 380, 470, and 600 bp were gifts from Professors Y. Nakahori and T. Shinka of The University of Tokushima. PCR amplifications were performed in a 20-μl reaction mixture containing 200 mM each dNTP, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 4 mM $MgCl_2$, 0.5 unit Z-*Taq* polymerase (all from TaKaRa), 100 ng of genomic DNA, and 0.2 μM of each primer. The mixture was first denatured at 94 °C for 5 s, followed by 30 cycles of denaturation at 94 °C for 2 s, annealing at 60 °C for 15 s, and extension at 72 °C for 15 s. The thermal cycler was LightCycler (Roche Diagnostics, Mannheim, Germany). An aliquot of PCR-amplified product was premixed with an aliquot of DNA digest standard, and then used for the μ-CE quantitation analysis.

2.3. DNA analysis in a coated quartz microchip with snapshot mode UV detection

The quartz microchips were produced at Shimadzu Corp. using traditional photolithographic microfabricated techniques as described previously [18]. The schematic illustration of the microchip is shown in Fig. 1. It had an

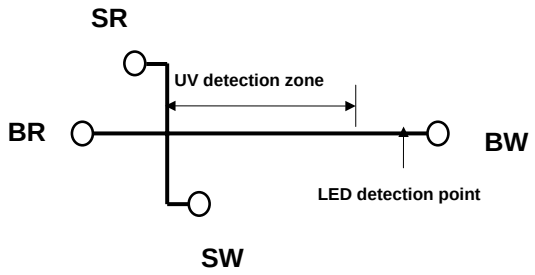

Fig. 1. Diagram of the microchips used in the study. For the quartz chip, the length from the cross point to the sample reservoir (SR), sample waste (SW), buffer reservoir (BR), and buffer waste (BW) was 7.5, 7.5, 7.5, and 33 mm, respectively. The linear polyacrylamide-coated microchannel was 110 μm (width) × 50 μm (depth). There is a 25 mm optical slit in the separation channel for linear imaging UV detection in snapshot mode. For the unmodified PMMA chip, the distance from the cross point to SR, SW, BR, BW was 5.25, 5.25, 5.75, and 37.5 mm, respectively. The microchannels were 100 μm (width) × 30 μm (depth) with 30 mm (effective separation length). In finishline mode, the LED fluorescence detection point was 30 μm from the cross point.

optical slit at the bonding interface to cut off stray light. The simple cross-channel design had a channel dimension of 110 μm in width and 50 μm in depth. The microchip was coated with a thin layer of linear polyacrylamide using the Hjertén protocol [19] to passivate surface charges and annihilate electroosmotic flow (EOF) and DNA adsorption. DNA separations were performed on a Shimadzu MCE-2010 μ-CE instrument with a linear imaging UV detector. For this detector, a 1024 photodiode array repetitively scanned along the cross point to a 25 mm separation channel at a wavelength of 260 nm to improve the sensitivity. The mixtures of DNA digests and PCR samples were put in a 96-well microtiter plate. Each separation was sequentially carried out in fresh polymer matrices. The computer-controlled μ-CE instrumentation automatically performed channel cleaning with water, matrix infusing, sample loading, electrophoretic separation and UV detection. The 2.0% (w/v) HEC matrix was prepared by adding the polymer to 5× TBE and stirring slowly until the solution appeared homogeneous and transparent. For a 50-s sample loading at the applied field strength of 316 V/cm, potentials of 420, 170, 100 V were applied to sample waste (SW), buffer reservoir (BR), and buffer waste (BW), respectively, while the sample reservoir (SR) was grounded. For separations, potentials of 130, 130, 430 V were applied to SR, SW, and BW, respectively, and BR was grounded. The applied field strength for separation was 100 V/cm. The temperature was kept at 25 °C in all experiments. The instrument control and data acquisition were processed using the Shimadzu MCE-2010 software.

2.4. DNA analysis in bare PMMA microchips with finishline mode fluorescence detection

Uncoated PMMA microchips with microchannels of 30 μm in depth and 100 μm in width were purchased from Hitachi (Tokyo, Japan). Its diagram illustration is similar as in Fig. 1, except that the fluorescence signal was recorded at a location in the channel 30 mm downstream from the injection cross. The cross channels were filled with either 1.5% HPMC (w/v) or mixed PEOs, both dissolved in 1× TBE containing 1 μg/ml ethidium bromide (EtBr) as the intercalating dye. Separation was performed on a Hitachi SV 1100 μ-CE instrument. The device detected the fluorescence intensity at 580 nm with an LED laser as the excitation source at 490 nm. Samples, premixed with a concentration-known DNA restriction digest, were loaded into the microchip electrokinetically by applying 300 V (286 V/cm) at SW for 60 s, with other three reservoirs at ground. The separation was performed at a field strength of 168 V/cm (if without other specified) by applying 130 V to SR and SW, 750 V to BW and grounding BR. Each separation was performed in a new microchannel. The data were collected using an SV 1100 software (Hitachi).

3. Results and discussion

In the present study, two detection modes, snapshot and finishline, were adopted. The snapshot mode of linear imaging UV detection enabled a real-time observance of migration pattern of all DNA fragments. Once the separation was satisfactory, the experiment stopped to achieve short separation time. The finishline mode of LED fluorescence detection was performed at a given position near the end of the separation channel, thus all analytes would travel the identical distance.

3.1. Difficulty in keeping consistency of solvent ionic strengths in conventional CE quantitation methods

Except for difficulties in keeping consistency of separation conditions in multiple microchannels and separation matrices, as aforementioned, herein we will discuss another difficulty associated with the influence of solvent ionic strengths in a sample on DNA separations. The same amount of a DNA 100 bp ladder was dissolved in three solvents with different ionic strengths: deionized water, 0.2× TBE, and 1× TBE (all TBE buffers have the same pH value of 8.3), and was separated in PMMA microchips on the Hitachi μ-CE device. The peak area under each fragment peak was determined. It is found from Fig. 2 that the DNA dissolved in deionized water gave larger peak area than dissolved in 0.2× TBE, and the DNA dissolved in 0.2× TBE gave larger peak area than dissolved in 1× TBE. Compared to the peak area of the 500 bp fragment dissolved in 1× TBE, 12-fold increase in peak area was found for the same solute amount dissolved in deionized water and five-fold enhancement in peak area for dissolved in 0.2× TBE. The enhancement in peak area is ascribed to the solute focusing or stacking effect, due to the change of the ionic migration velocity when crossing different ion strengths of solvents with an associated step-like change of the applied field

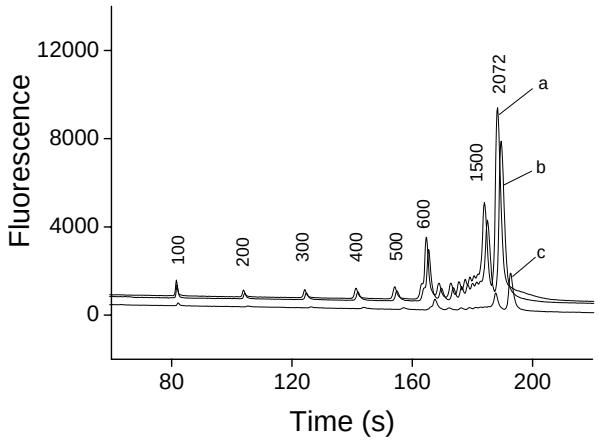

Fig. 2. Comparison of the separations of a 100 bp DNA ladder dissolved in solvents with different ion-strengths. Conditions: SV 1100 μ-CE device and the uncoated PMMA microchip as in Fig. 1; the separation matrix was 1.5% HPMC dissolved in 1× TBE buffer (pH 8.3) with 1 ng/μl EtBr as an intercalating dye; the ladder (30 ng/μl), dissolved in (a) deionized water, (b) 0.2× TBE, or (c) 1× TBE, was injected into the microchip by applying 300 V (286 V/cm) at SW for 60 s, with other three reservoirs at ground; the separation was carried out at 168 V/cm by applying 130, 130, and 750 V to SR, SW, and BW, while BR was grounded; the microchip was maintained at 25 °C; and LED fluorescence detection was set at ex 470 nm/em 580 nm. The numbers in the figure refer to the fragment sizes in base pairs.

strength. Because the buffer of the separation matrix is 1× TBE, deionized water has stronger focusing effect than 0.2× TBE. These results reflect the importance in keeping the consistency of the solvent ionic strengths in the measurement of peak area. In practice, it is not easy to guarantee identical ionic strengths for the peak area determinations between DNA standards and real-life samples, because PCR products commonly situate in a high-ionic-strength solvent and also contain other components, while DNA standards often exist in a low-ionic-strength buffer (even in water). So, it is risky to obtain sample amounts through comparing with the amounts of standard fragments run consecutively in one microchannel. Even worse is to run samples and standards in adjacent two microchannels.

It is also difficult to obtain consistent peak areas of identical fragments from channel to channel. The R.S.D. for the 500-bp peak was as high as 41.8% ($n = 6$). This channel-to-channel peak area variance most likely arises from the nonuniformity of sample amounts injected as a result of inhomogeneities of the polymer matrix, and the surface property, as well as geometrical imperfections of microchannels. Therefore, we attempt to employ a comigration method instead to avoid the problem.

3.2. Microchip separations with snapshot mode UV detection

UV absorption detection is widely used in conventional CE because of its simplicity and versatility. However, sensitivity has been a problem in μ-CE due to the shallow microchannel depth. Using an optical slit at the bonding interface of quartz microchips and repetitively scanning the linear photodiode array located along the separation channel, Shimadzu Corp. successfully developed a novel linear imaging UV detection system [18]. The incident light is cut selectively by the optical slit to eliminate the stray light, and the signal light passing through the microchannel is detected by the photo detector below the microchip. Thus, high sensitivity is achieved.

For an electropherogram, the area under a fragment peak is used to quantitate the fragment concentration. At the same time, longer DNA has more deoxynucleotides (adenine, cytosine, guanine, and thymidine) than shorter DNA, and induces higher absorbance. Hence, the area is also proportional to the length of the fragment, that is:

$$A = a + bLC \quad (1)$$

where A is the peak area, a and b are coefficients, L is the fragment size in base pair, and C is the fragment concentration. When a DNA sample is completely digested, all restriction fragments in the digest will own the same concentration as the digest, i.e., C equals to the concentration of the digest. Therefore, a linear correlation will exist between the peak area of the fragment and the multiplication of the fragment size and the digest concentration. For the fragments in a digest, Eq. (1) is simplified by:

$$\mathrm{A} = a + b'L \quad (2)$$

with b' ($=bC$) to be a constant in a digest. On the MCE-2010 device with a linear imagine UV detector, a short separation time could be obtained, because the detector enables observing a real-time migration pattern of the components in the sample. Fig. 3 shows the separation profile of a ΦX174-*Hae*III digest, using a quartz microchip at an applied field strength of 100 V/cm for 110 s. The X-coordinate of the recorded linear imaging plot was the scanning zoom scale along the channel (the total length of 25 mm), rather than time scale in a conventional electropherogram. When the peak areas of the fragments in the digest were plotted as a function of their sizes, a linear relation was observed. From the inset in Fig. 3, we can see that Eq. (2) could well describe the quantitative relationship between A and L in the migration pattern. If two fragments are not well separated, we can simply use the overlapped peak areas to correlate the sum of both sizes in base pair. For example, 271 and 281 bp in the digest were combined for the correlation in the inset, a high correlation coefficient (r) of 0.996 still existed.

3.3. Microchip separations with finishline mode fluorescence detection

PMMA is one of the most commonly used plastic microchip substrates, and is disposable in μTAS application due to its ease of fabrication and low cost. Simplifying the quantitation experiment into single-step run facilitates the decrease of the run cost and of the demand for high

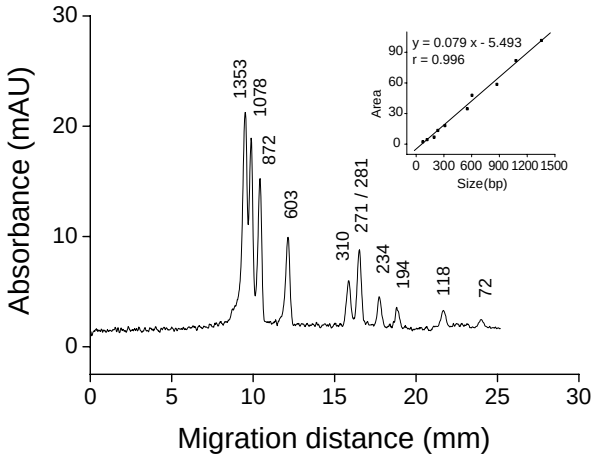

Fig. 3. Analysis of ΦX174-*Hae*III digest (50 ng/μl) for 110 s on the MCE-2010 μ-CE device. Conditions: a linear imaging UV detection at 260 nm was used for scanning a 25 mm separation channel. The separation matrix was 2.0% M_r 250 000 HEC in 5× TBE (pH 8.3). The sample loading was carried out at 316 V/cm for 50 s when the potentials of 420, 170, 100 V were applied to SW, BR and BW reservoirs, respectively, and the SR reservoir was grounded. The separation was carried out at 100 V/cm while the potentials of 130, 130 and 430 V were applied to SR, SW, and BW, while BR was grounded. The chip was maintained at 25 °C. The numbers in the figures indicate the fragment sizes in base pairs. The inset shows the relationship between the peak areas obtained and the fragment length in the digest.

consistency from microfabricated channels, separation matrices and solvents for dissolving analytes. If HPMC and PEO solutions of certain concentrations are used as dsDNA separation matrices, tedious chemical modification of the PMMA channel walls was found unnecessary [20,21]. In the present study, we used the uncoated PMMA microchips on the Hitachi μ-CE device with a finishline mode LED fluorescence detector. As EtBr was used as an intercalating dye, the peak area will be proportional to both the concentration and the length of the DNA fragment. So, Eqs. (1) and (2) will be abided by, too. Previous work has shown that a mixture of several polymers with different molar-masses has better separations of wide size range DNA fragments than a single molar-mass polymer, and at the same time, maintains a relatively low viscosity [14].

The PCR products were amplified in LightCycler with a duration of only 16 min, much quicker than traditional PCR due to minimized reaction volume (only 20 μl) and high heat-transferring ability (20 °C/s) of the device. In practical application, the size of a PCR product is commonly known prior to the concentration quantitation. Because the PCR product is flanked by digest fragments, its size could be easily identified in our electropherograms from the relationship between relative mobility and fragment size obtained in the digest. As the main aim of the present work focuses solely on the quantitation of concentration, we will not discuss more on the other identification topic.

The one-step experiment avoids the chip-to-chip and buffer-to-buffer mismatches between samples and stan-

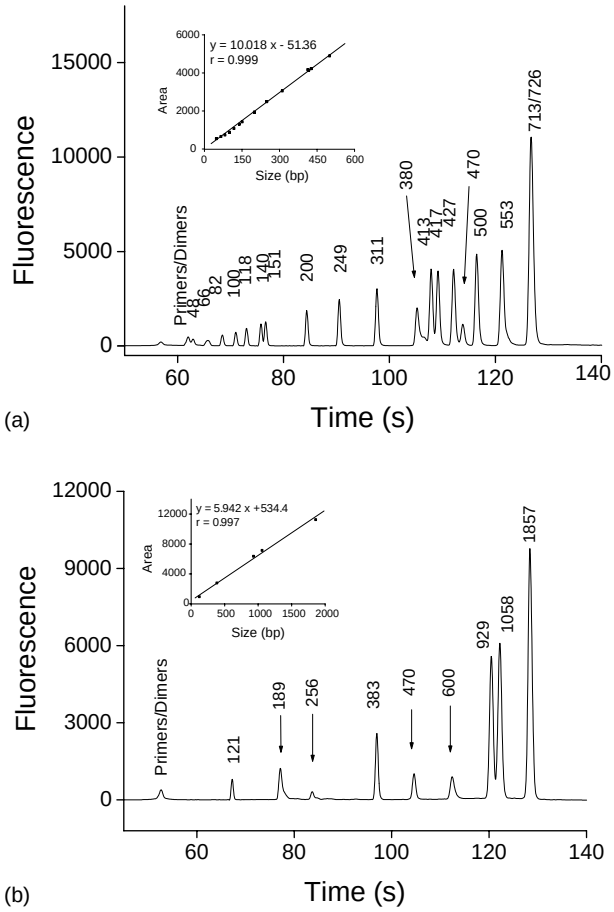

Fig. 4. Comigration of mixtures of PCR products together with different DNA digests on the SV 1100 microchip electrophoresis device to quickly quantitate the PCR products of polymorphisms on the human Y-chromosome related to spermatogenic failure. Arrows show the PCR products to be quantitated. The PCR products were amplified as described in section 2.2. (a) ΦX174-*Hin*fI digest for determination of PCR products of 380 bp and 470 bp; (b) pBR322-*Bst*NI digest for determination of PCR products of 189, 256, 470, and 600 bp. Conditions: the uncoated PMMA microchip as in Fig. 1; mixed polymer matrix containing 0.30% of 8×10^6, 0.60% of 1×10^6, 0.15% of 4×10^5, 0.20% of 1×10^5 M_r PEO and 1 ng/μl EtBr dissolved in 1× TBE; other conditions were the same as in Fig. 2. The numbers in the figure indicate the fragment sizes in base pairs. The insets reflect the obeying of the linear relationship between the peak areas and the lengths of the fragments in the digests.

dards. As a test of the resolving and quantitative abilities of the technique in μ-CE systems, several mixtures of PCR products, which were not desalted, were premixed with different sorts of DNA digests. Some representative electropherograms are shown in Fig. 4 with mixed PEOs of different molar-masses as the separation matrix. All the regression coefficients of the area versus size plots are not less than 0.99. As to overlapping fragments, their areas and sizes were combined and calculated in the A versus L correlation as stated above. The good linearities show the correctness and robustness of Eqs. (1) and (2) in DNA digests. Furthermore, good separations of digested fragments with PCR samples suggest that single or multiple components from PCR products could be simultaneously analyzed

by a single injection and comigration with the digests, e.g., spermatogenic failure-specific PCR-amplified products of 189, 256, 470 and 600 bp could be well separated through using pBR322-*Bst*NI digest (Fig. 4b) and be quantitated using the electropherogram.

The separation of large DNA molecules (i.e., >2 kbp) has numerous applications including large scale gene mapping of microorganisms, and the study of chromosome rearrangements in genetic diseases or cancer. The conventional method for such separations, pulsed field gel electrophoresis, is labor intensive and slow (one separation typically requires 12–24 h [22]). In our study, the 3 kbp product of pGEM-T vector could be separated and quantitated through comigration with λ-*Hin*dII digest in less than 4 min, because a good *A* versus *C* linear relation still exists when the fragment size reaches up to 23 kbp (Fig. 5). Accordingly, the quantitation technique is able to measure DNA products, at least, up to such a large size, only if the *A* versus *C* linear relationship exists.

3.4. Quantitation of PCR products in microchip electrophoresis

To evaluate the accuracy of the proposed quantitation technique, a non-purified PCR mixture of 380 and 470 bp was premixed with ΦX174-*Hae*III digest and comigrated in an unmodified PMMA microchip. The *A* and *L* relationship was obtained from the fragments in the electropherogram of ΦX174-*Hae*III digest, and had a correlation coefficient of 0.999 (Eq. (3) in Table 1). From the known concentration of the digest (65 ng/μl, i.e., 18 nM [23]), the coefficient *b* (=4.132 × 10^8, see Eq. (4)) was obtained. In terms of the peak areas and fragment sizes of the PCR samples separated together with the digest, the concentrations of the PCR products (380 and 470 bp) could be simultaneously and quickly

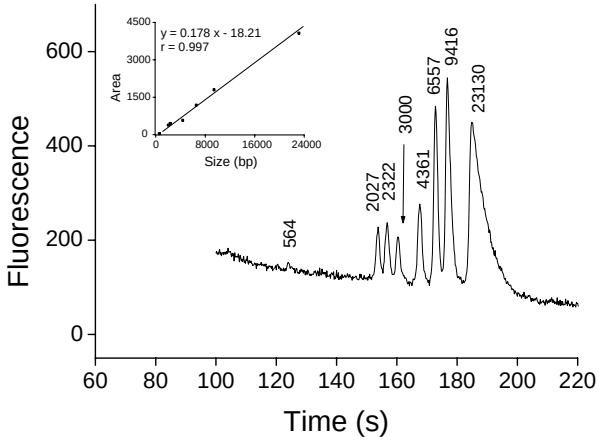

Fig. 5. Microchip electropherogram of a pGEM-T vector (3 kbp) comigrated with the λ-*Hin*dIII digest on the SV 1100 microchip electrophoresis device. Conditions: mixed matrix containing 0.01% of 8 × 10^6, 0.02% of 1 × 10^6, 0.15% of 4 × 10^5, 0.20% of 1 × 10^5 MW PEO and 1 ng/μl EtBr in 1× TBE; the sample was injected into the uncoated PMMA microchip by applying 300 V (286 V/cm) at the SW for 120 s, with other three reservoirs at ground; the separation was carried out at 90 V/cm by applying 70, 70, and 400 V to SR, SW, and BW, while BR was grounded; other conditions were the same as in Fig. 2. The numbers in the figure refer to the fragment sizes in base pairs. The inset reflects the obeying of the linear relationship between the peak areas and lengths of the fragments in the digest. The peak with an arrow is the pGEM-T vector of 3 kbp.

obtained by using Eq. (4), and were shown in Table 1. Independent assays in six different microchannels gave consistent quantitation results, as summarized in Table 2. The precision of the quantitation assay was very high with the run-to-run R.S.D. ($n = 6$) less than 6% for both 380 and 470 bp, Their relative recoveries at two different concentration levels were assessed by the ratio of the calculated concentration divided by spiked concentration. High recoveries between 98 and 105% were obtained, which is very

Table 1
Concentration quantitation of the mixture of PCR products (380 and 470 bp) through comigrating with the ΦX174-*Hae*III digest[a]

Fragment	Area	Equation	Concentration calculated
Digest			
72	598	$A = -12.59 + 7.439\ L$ (3)	
118	828	$r = 0.999$	
194	1390	$A = -12.59 + 4.132 \times 10^8\ LC$ (4)	
234	1596	$r = 0.999$	
271	1960		
281	2144		
310	2559		
603	4327		
872	6418		
1078	7942		
1353	10170		
PCR sample			
380			$C = 3.13 \times 10^{-9}$ M
470			$C = 1.13 \times 10^{-9}$ M

[a] PCR products were premixed with the ΦX174-*Hae*III digest (at a final concentration of 1.8 × 10^{-8} M). The separation conditions were the same as in Fig. 4.

Table 2
Chip-to-chip repeatability and relative recovery for the concentration determination of the PCR mixture of 380 and 470 bp at six independent PMMA chips[a]

Fragment (bp)	Concentration found, C (nM)	Average C (R.S.D.) (nM)	Recovery		Relative recovery (%)
			Concentration spiked (nM)	Concentration found (nM)	
380	3.13 3.07	3.16 (2.3%)	1.00	0.98 ± 0.03	98.0 ± 3.0
	3.10 3.26		5.00	5.07 ± 0.07	101.4 ± 1.4
	3.22 3.18				
470	1.13 1.06	1.14 (5.9%)	1.00	1.05 ± 0.03	105.0 ± 3.0
	1.25 1.09		5.00	4.94 ± 0.10	98.8 ± 2.0
	1.12 1.18				

[a] Conditions were the same as in Table 1.

satisfactory for the quantitation assay of DNA molecules in μ-CE.

4. Conclusions

An easy to operation, one-step quantitation technique for dsDNA analysis by μ-CE in coated and uncoated microchips was demonstrated. The technique needs only a concentration-known digest which could be separated with the sample, and the sample amount is determined through its peak area and the A versus LC linearity obtained in the coexisting digest in the same electropherogram. Artifacts resulting from channel-to-channel, polymer-to-polymer variations could be avoided, and the slight run-to-run differences in the practical experimental conditions hardly affect the quantitation results. The results also showed that the technique could be performed with a minimum sample preparation steps, as sample pretreatments such as desalting were not necessary. The reproducible results over many trials with PCR products suggested the ease, quickness, and accuracy of the quantitation method. The precondition that the sample is separated from the standard digests is easy to meet in μ-CE. Simultaneous identification and determination of multiple unpurified PCR products are available for the sample size up to 23 kbp. The technique will be a powerful alternative for the routine application of dsDNA fragments, when integrated into a fully-unattended μ-TAS system. Further adaptation to a multiple channel array system will allow high-throughput and high-speed operations.

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