DNA Separation Using Cellulose Derivatives and PEO by PDMS Microchip

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Poly(dimethyl siloxane) (PDMS) has been employed as a microchip material for DNA separation in microfluidic condition. Different sieving molecules such as cellulose derivatives having glucose building block (methyl cellulose (MC), hydroxyethyl cellulose (HEC), and hydroxypropyl methyl cellulose (HPMC)) and polyethylene oxide (PEO) having linear (ring-opened ethylene oxide) unit were used and their performance was compared in terms of separation efficiency and resolution. In general, PEO showed better separation performance than cellulose derivatives probably due to the nature of linear shape polymer conformation. It was possible to perform at least 15 consecutive running with 1.2% PEO at the electric field strength around 200 V/cm. Fast analysis of the standard ΦX 174 RF DNA/Hae III (less than 130s) was obtained with the number of the theoretical plate around 250,000/m. Our PMDS microchip was applied to the measurement of CAG repeat number, which is related to male infertile disease.

Key Words : PDMS, Microchip, DNA, PEO, Infertile

Introduction

DNA analysis is important in many areas including diagnosis for genetic diseases, DNA profiling with short tandem repeats, forensic analysis, and restriction fragment length polymorphism. In general, slab gel electrophoresis (SGE) has been employed for separation and detection of DNA. Although SGE is still used in many research laboratories, it is labor intensive, hard for automation, and shows long analysis time (> 30 min).[1] Capillary gel electrophoresis (CGE) has shown great potential for DNA analysis with high resolution, and rapid analysis time (< 30 min). It also shows high sensitivity (pg detection limit) when incorporated with laser induced fluorescence (LIF).[2,3] However, it is hard to miniaturize CGE system for point-of-care device and to detect multiple samples simultaneously.[4]

Lab-on-a-chip (LOC) was currently introduced as a method of choice for DNA analysis since it has capabilities for the consumption of extremely small sample volume (several nanoliter), fast analysis time (< 10 min), multiplexing, and miniaturization.[5-9] Polymer materials such as poly(dimethylsiloxane) (PDMS), poly(methyl methacrylate) (PMMA), polycarbonate (PC), polystyrene, cellulose acetate, poly(ethylene terephthalate) (PETP) have been used for the production of LOC.[10-12] Of many materials, PDMS has been widely applied for biomolecule analysis since fabrication of PDMS is easy for nano- and microstructure, optical transparency is excellent for UV/VIS and fluorescence detection at 240-1100 nm, production is cost-effective, and chemical analysis is feasible under high electric field.[13,14] Compared to glass or quartz microchip, PDMS chip shows uneven distribution of surface charge density and adsorption of sample to the channel surface via hydrophobic interaction.[15] Therefore, suppression or control of surface charge distribution is important for DNA analysis in PDMS LOC.

In this paper, water-soluble polymers such as methyl cellulose (MC), hyroxyethyl cellulose (HEC), and poly(ethylene oxide) (PEO) were employed for dynamic coating of PDMS channel surface as well as sieving matrices for DNA separation. It was found that PEO had better dynamic coating effect and showed the number of theoretical plates of 250,000/m at 1.2% with total analysis time less than 130 s. Also, good separation efficiency was obtained for at least 15 consecutive runs with PEO. PDMS microchip was investigated for the potential tool of the diagnosis of genetic disease such as male infertile disease related to trinucleotide tandem repeats.

Experimental Section

Chemicals and reagents. For the production of the master for Lab-on-a-chip, silicon wafer (Siltron Inc., Korea), SU-8 (negative photoresist, Microchem Corp., Newton, MA, USA), poly(dimethyl siloxane, PDMS, Optrontec Inc., Korea) were used. Tris(hydroxymethyl)aminomethane, boric acid, ethylenediaminetetraacetiate (EDTA), ethidium bromide (EB), and ΦX 174 RF DNA/Hae III as a DNA standard marker were from Sigma-Aldrich, Co., MO, USA. Polyethylene oxide (PEO, Mr = 8,000,000), methyl cellulose (MC, 2 wt.% solution in H_2O, 4000 cps), 2-hydroxyethyl cellulose (HEC, Mv *ca.* 1,300,000), hydroxypropyl methyl cellulose (HPMC, 2% aqueous solution, 4000 cps) were also from Sigma-Aldrich. PCR reagents (50 mM KCl, 4 mM $MgCl_2$, 0.5 mM dNTPs, 5 mM Tris-HCl, and 1U Taq polymerase) were from Takara Bio Inc., Japan.

PCR product preparation. Two primers (5'-TCC AGA ATC TGT TCC AGA GCG TGC-3' (left primer) and 5'-GCT GTG AAG GTT GCT GTT CCT CAT-3' (right primer)) were

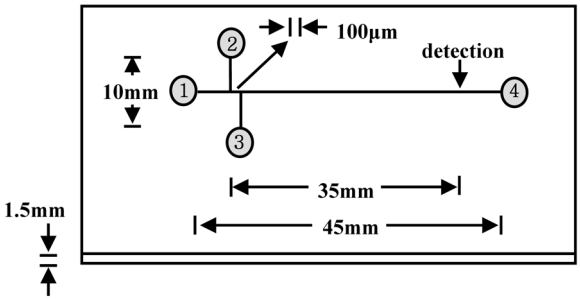

Figure 1. Channel pattern for DNA separation. Separation length; 35 mm, channel width; 80 μm, channel depth; 50 μm.

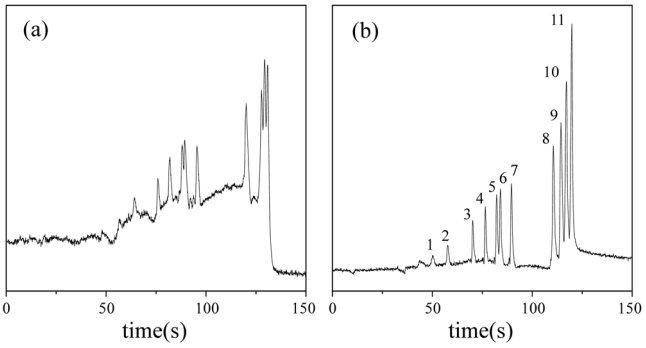

Figure 2. Electropherograms of ΦX 174 RF DNA/Hae III fragments with hydrophobic PDMS chip surface (a), and hydrophilic chip surface (b). PEO was used as both dynamic coating material and sieving matrix in (b). Condition: 1X TBE, sieving matrix: 1.2% PEO, DNA sample: 25 μg/mL ΦX 174 RF DNA/Hae III, field strength: 200 V/cm, EB, 3 μg/mL. Peak assignment; 1 = 72 bp, 2 = 118 bp, 3 = 194 bp, 4 = 234 bp, 5 = 271 bp, 6 = 281 bp, 7 = 310 bp, 8 = 603bp, 9 = 872 bp, 10 = 1073 bp, 11 = 1358 bp.

employed for amplification of CAG repeat region of Xq11-12 exon1. MJ thermal cycle PCR (Waltham, USA) was used with the sequence shown below; 35 cycles of denaturation at 95 °C for 40 s, annealing at 67 °C for 40 s, extension at 72 °C for 2 min, then terminal extension at 72 °C for 10 min.

Fabrication of microchip. A negative photoresist, SU-8 was spin coated at 5000 rpm for 40 s on top of silicon wafer. After it was dried for 10 min, chrome mask with design shown in Figure 1 was laid on silicon wafer and exposed at 365 nm. PDMS oligomer and curing agent (10 : 1) were mixed and the resulting bubbles were eliminated in desiccator. Then, PDMS was poured on the master and dried at 75 °C for 2 hrs. For the formation of the microchannel, another PDMS layer was oxidized by for 10 min by Tesla coil (BD-10A, Electrotechnique Production, Inc., Chicago, IL, USA) and bound with PDMS with engraved channel pattern on it.

Dynamic coating of PDMS channel. In order to reduce the electroosmotic flow (EOF), PDMS channel was dynamically coated by the following procedure. The channel was cleaned with DI H_2O, 0.10 M HCl, 0.10 M NaOH, 1X TBE for 5 min, respectively. A 20 μL of polymer solutions such as MC, HEC, HPMC, and PEO were placed on the reservoir 4 and pushed with constant pressure. The microchip was stood for 10 min before DNA sample injection.

PDMS chip operation. DBMA-100 (Digital Bio Technology, Korea) equipped with DPSS laser (532 nm, 10 mW) and DBHV-100 high voltage supplier was used. A 6 μL of 50 ng/nL ΦX 174 RF DNA/Hae III was placed in reservoir 2. Then, DNA sample was injected by the pinched injection mode;[16] step 1, the reservoir 2 at 0 V, the reservoir 3 at 600 V, the reservoir 1 and 4 at floating for 40s, step 2, reservoir 1 at 0 V, the reservoir 4 at 700 V, the reservoir 2 and 3 at 200 V for DNA separation.

Results and Discussion

Dynamic coating by polymer solution. Although a corona discharge from Tesla coil put energy on the PDMS surface high enough to produce the hydrophilic group (Si-OH), it is known that the surface became hydrophobic in several hours due to the increase of the number of Si-CH_3 group.[17,18] This hydrophobic surface caused low resolution for DNA separation as shown in Figure 2(a). Since PEO is hydrophilic, without dynamic coating process, PEO slowly expelled from the channel to reservoir, resulting in deficiency of sieving matrix inside the channel. In this case, the

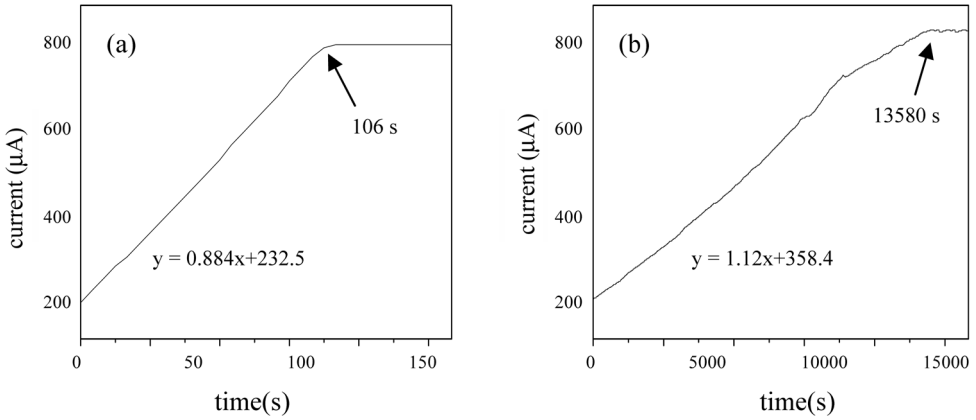

Figure 3. Current monitoring in (a) no dynamic coating, (b) dynamic coating by PEO. Condition: 1x TBE (89 mM Tris, 89 mM borate, 2 mM EDTA) and 0.5x TBE, 1.2% PEO, electric field strength 110 V/cm.

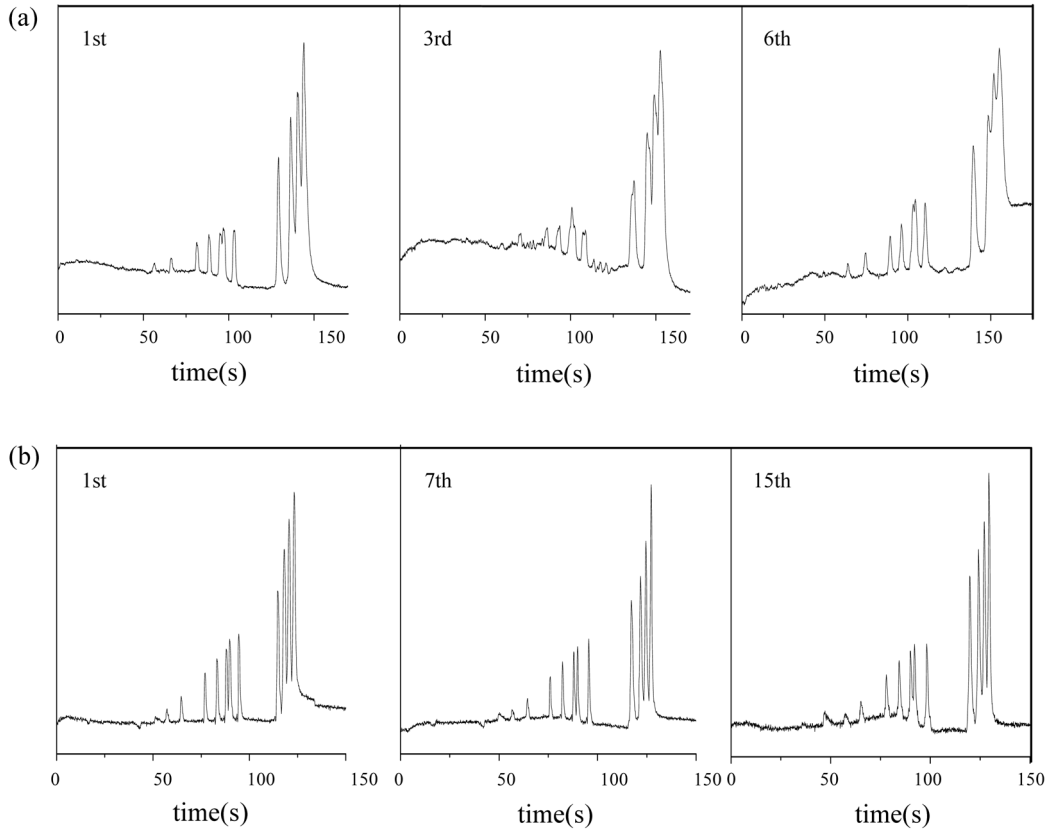

Figure 4. Consecutive running of ΦX 174 RF DNA/Hae III fragments (a) 1.0% MC, (b) 1.2% PEO. Other conditions were the same as in Figure 2(b).

channel was not treated with DI H_2O, 0.10 M HCl, 0.10 M NaOH, 1X TBE for 5 min, respectively. When the channel was treated with the procedure described above, which induced the channel wall hydrophilic, PEO provided good sieving power as shown in Figure 2(b). Note that PEO was used as both dynamic coating material and sieving matrix in Figure 2. It was found that the hydrophilicity of PDMS microchannel could be restored with treatment of channel surface by the reoxdization of channel wall. DNA sample was successfully separated with 7-day-old PDMS microchip after 5 min of the corona discharge treatment.

Dynamic coating effect by PEO was confirmed by current monitoring method.[19]

Since the negatively-charged DNA moves against EOF, the magnitude of EOF should be suppressed to identify DNA at the detection point.[20,21] As shown in Figure 3, EOF inside the channel was reduced from 3.75×10^{-4} $cm^2/V.s$ (Figure 3(a)) to 2.79×10^{-6} $cm^2/V.s$ (Figure 3(b)) by the dynamic coating with PEO. This 100-fold decrease of EOF made it possible of DNA fragments to migrate to the detector and be separated in PDMS microchip.

Separation of DNA with various polymer solutions. Cellulose derivatives (MC, HEC, and HPMC) and PEO were employed as both the dynamic coating material and the sieving matrices, and their performance was compared. Figure 4 shows the consecutive running of the same ΦX 174 RF DNA/Hae III fragments sample with MC and PEO. It was observed that the separation efficiency became worse with MC (Figure 4(a)), especially for last three peaks (872 bp, 1078 bp, and 1358 bp). However, more than 15 runs were feasible with PEO as shown in Figure 4(b). This is probably caused by the unit structure of PEO and capability of hydrogen bonding to the channel wall. PEO is liner so that it can form hydrogen bonding with the channel surface more effectively, compared to MC having relatively bulky glucose unit. Also, the number of monomer unit is higher in PEO (more than 180,000) than in MC (less than 7,500), which provides more chance to form hydrogen bonding per a polymer chain, resulting in stronger dynamic coating effect with PEO. HEC and HPMC showed the same phenomena as in Figure 4(a). The reason that resolution became decreased in Figure 4(a) could be attributed to slow leaking of MC during running due to push caused by EOF, resulting in decrease of separation power.

Separation efficiency and resolution depending on polymer solution was further compared as shown in Table 1. A 234 bp fragment was arbitrarily chosen for the calculation of separation efficiency. Two fragments (271 bp and 281 bp) in ΦX 174 RF DNA/Hae III fragments were used for the measurement of resolution since the size difference is the smallest for those two fragments. Generally, PEO showed better performance compared to HPMC, HEC, and MC. A 1.2% PEO had highest separation efficiency (2.5×10^5), while 1.2% HPMC and MC had 1.5×10^5, 1.0×10^5,

Table 1. Comparison of separation efficiency (234 bp) and resolution (271 bp-281 bp) with various polymer concentrations

Concentration (w/v).	Separation efficiency (N, $\times 10^5$)				Resolution (R)			
	PEO	HPMC	HEC	MC	PEO	HPMC	HEC	MC
1.5%	1.3	0.48	0.61	–	2.2	–	1.4	–
1.2%	2.5	1.5	0.63	1.0	2.8	1.94	1.4	1.8
1.0%	1.1	1.2	0.69	0.88	1.6	0.97	1.7	1.8
0.8%	0.59	–	–	0.68	1.3	–	1.1	1.1
0.6%	0.57	–	–	–	0.27	–	–	–

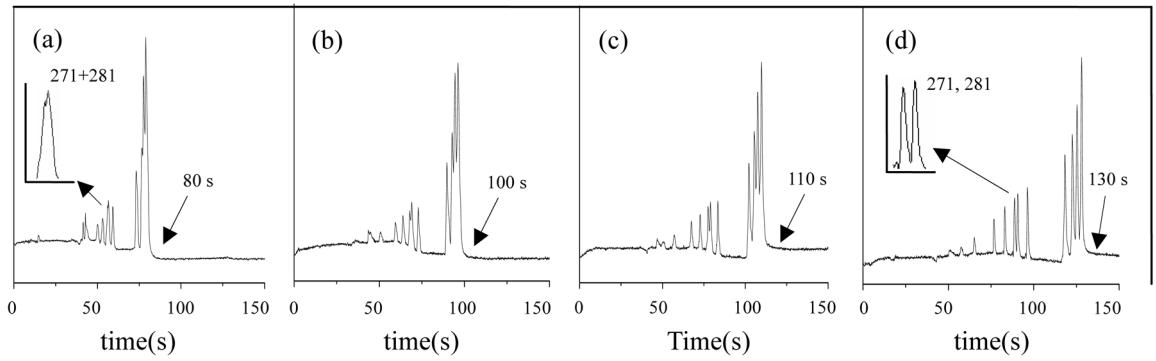

Figure 5. Electropherograms of ΦX 174 RF DNA/Hae III fragments at various PEO concentrations; (a) 0.6%, (b) 0.8%, (c) 1.0%, and (d) 1.2%. Electric field strength; 220 V/cm. Other conditions were the same as in Figure 2(b).

respectively. Resolution was at least 1.5 times higher with PEO than with other cellulose derivatives.

Characteristics of DNA separation with PEO solution. As shown in Figure 5, resolution of ΦX 174 RF DNA/Hae III fragments was the function of PEO concentration. At 0.6% PEO, resolution for two fragments (271 bp and 281 bp) was 0.27 (Figure 5(a)), however, it was 2.8 at 1.2% PEO (Figure 5(d). Separation efficiency was at least four times increased from 6.0×10^4 to 2.5×10^5 for 0.6% and 1.2% PEO, respectively. However, total migration time elongated from 80 s to 130 s for 0.6% and 1.2% PEO, respectively, due to increased viscosity. PEO concentrations higher than 1.2% exhibited lower separation efficiency and resolution than those at 1.2%. For example, the number of the theoretical plate was 1.3×10^5 at 1.5% (Table 1), which is smaller than that at 1.2%.

The electric field strength also affected the separation efficiency and resolution as shown in Table 2. It was observed that separation efficiency was the highest at 1.2% PEO (for 234 bp, 2.5×10^5). As the PEO concentration decreased, so was the separation efficiency. No appreciable separation was obtained at electric field strength higher than 200 V/cm. The opposite tendency was observed for resolution of two DNA fragments (271 bp and 281 bp). As the PEO concentration decreased, the resolution for those fragments improved up to 3.0 (Table 2). However, total migration time for ΦX 174 RF DNA/Hae III fragments was 800 s at 90 V/cm, which was more than six times longer than that at 200 V/cm.

PCR product sizing of Korea infertile male by using PDMS chip. Abnormal trinucleotide repeat expansion of CAG in human genome Xq 11-12 exon 1 causes a genetic disease known as spinal bulbar muscular atrophy (SBMA), resulting in infertile male.[22] The measurement of CAG repeat number and the distribution of repeat number for patients are important in order to find the potential patients and diagnosis.[23] Conventionally, the repeat number was measured by slab gel electrophoresis (SGE). However, the measured value by SGE had error more than 20% due to inherent low resolution of SGE.[24] Our PDMS microchip showed error lower than 5%, therefore, it was applied to the calculation of CAG repeat number for a control and an infertile patient (Figure 6). In this experiment, ΦX 174 RF DNA/Hae III fragment was used as the internal standard for the calibration curve. As shown in Figure 6 and in Table III, the CAG repeat number was easily measured at higher accuracy with nonlinear curve fitting by using PDMS microchip.

Table 2. Comparison of separation efficiency (234 bp) and resolution (271 bp-281 bp) at various electric field strengths

Field strength (V/cm)	1.2% PEO	
	Efficiency	Resolution
200	**2.5×10^5**	2.8
170	1.7×10^5	2.8
140	1.0×10^5	2.1
110	1.7×10^5	2.5
100	1.9×10^5	2.7
90	1.4×10^5	**3.0**

Conclusion

DNA analysis provides the basis for the investigation of

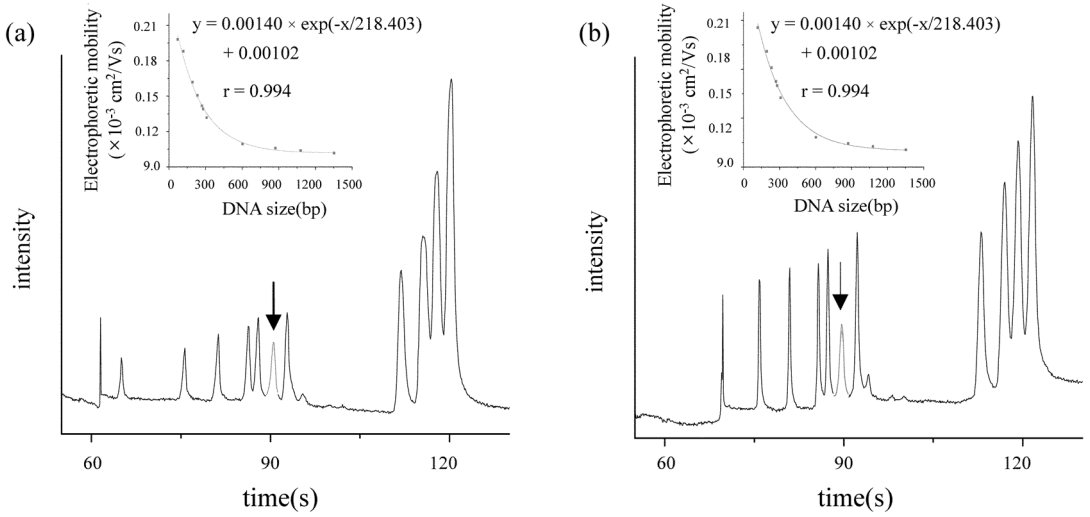

Figure 6. Electropherograms and nonlinear curve fitting for the calculation of the CAG repeat number for (a) a control and (b) an infertile patient. Both 3 μL of 50 ng/mL ΦX 174 RF DNA/Hae III and 3 μL of PCR products were placed in reservoir 2. Other conditions were the same as in Figure 2(b).

Table 3. Calculation of the CAG repeat number of PCR products from a control and an infertile patient

PCR products	Size Calculated (bp)	CAG Repeat Number
Control	289	22
Patient	298	25

genetic diseases, DNA profiling with short tandem repeats, forensic analysis, and restriction fragment length polymorphism. In this paper, the characteristics of DNA separation has been studied using poly(dimethyl siloxane) (PDMS) microchip. Many sieving matrices such as cellulose derivatives (MC, HEC, and HPMC) and polyethylene oxide (PEO) were used and compared in terms of separation efficiency and resolution. At least 15 consecutive running with 1.2% PEO at the electric field strength around 200 V/cm was obtained, which was superior to the results with cellulose derivatives probably due to the nature of linear shape conformation of PEO. High speed separation of the standard ΦX 174 RF DNA/Hae III (less than 130s) was obtained with the number of the theoretical plate around 250,000/m. Our PMDS microchip showed the great potential to investigate the distribution pattern of Korean infertile males by the measurement of CAG repeat number.

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