Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 947–953

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Direct endonuclease digestion and multi-analysis of restriction fragment length polymorphisms by microchip electrophoresis

Rie Akamine[a], Shouki Yatsushiro[a], Shouhei Yamamura[a], Jun-ichi Kido[b], Yasuo Shinohara[c,d], Yoshinobu Baba[a,e,f], Masatoshi Kataoka[a,*]

[a] Health Technology Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Hayashi-cho 2217-14, Takamatsu 761-0395, Japan
[b] Department of Periodontology and Endodontology, Institute of Health Bioscience, University of Tokushima, Kuramoto 3-18-15, Tokushima 770-8504, Japan
[c] Department of Molecular and Pharmaceutical Biotechnology, Graduate School of Pharmaceutical Sciences, University of Tokushima, Shoumachi 1-78, Tokushima 770-8505, Japan
[d] Division of Protein Expression, Institute for Genome Research, University of Tokushima, Kuramoto 3-18-15, Tokushima 770-8503, Japan
[e] Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Furo-cho, Nagoya 464-8603, Japan
[f] MEXT Innovative Research Center for Preventive Medical Engineering, Nagoya University, Furo-cho, Nagoya 464-8603, Japan

ARTICLE INFO

Article history:
Received 21 April 2009
Received in revised form 15 June 2009
Accepted 17 June 2009
Available online 25 June 2009

Keywords:
Microchip electrophoresis
Multi-analysis
Restriction fragment length polymorphisms
Endonuclease
ABO genotype

ABSTRACT

A high-performance multi-analysis system for genotypic mutation by means of restriction fragment length polymorphisms (RFLP) involving endonuclease treatment of PCR-amplified DNA on a microchip and subsequent analysis by microchip electrophoresis for DNA sizing was developed. A Hitachi SV1210 system, with which 12 samples can be analyzed on a plastic chip with good accuracy as to DNA sizing between 25 and 300 bp, was employed for RFLP analysis. We performed RFLP analysis of the ABO genotypes of blood donors for whom the ABO type was known. Six blood samples were analyzed by PCR to amplify two different regions of the genomic DNA, each of the amplified DNAs containing a different nucleotide polymorphism. To analyze the genes at polymorphic sites 261 and 526, restriction endonucleases *Kpn* I and *Ban* I were employed, respectively. When an amplified DNA was digested with each endonuclease on a microchip for 20 min, sequential analysis revealed the presence or absence of the respective restriction site. This analysis was performed within 7 min using a 1/10 volume of a DNA sample in comparison with the conventional method, and the estimated DNA size differed from the predicted size by less than 10 bp. The results indicate the potential of microchip electrophoresis for RFLP with on-chip direct endonuclease digestion and sequential analysis, offering high resolution in a short time.

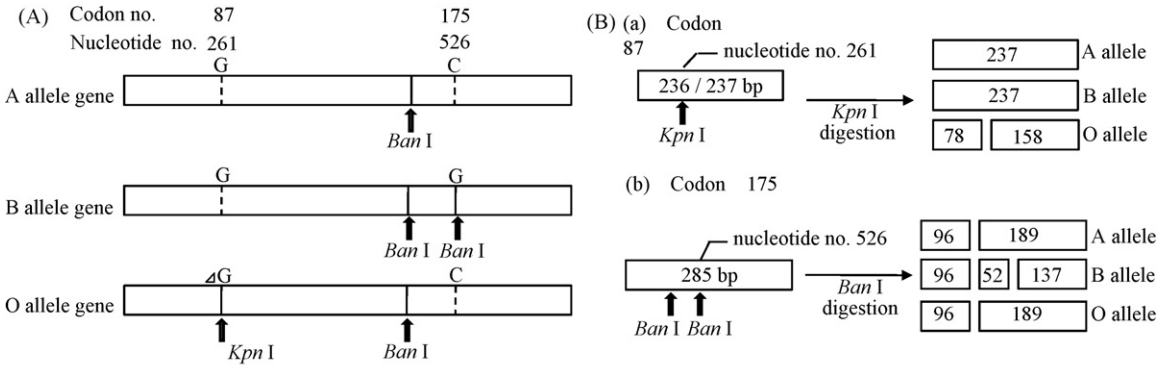
1. Introduction

The molecular basis of histo-blood group ABO antigens has been determined, and the genes encoding the group A and B glycosyltransferases and a non-functional group O transferase have been cloned and sequenced [1–3]. Although the nucleotide sequences of the A and B genes are highly homologous, the presence of single nucleotide polymorphisms (SNPs) is well known, and the A and B transferase genes differ in seven base substitutions (297, 526, 657, 703, 796, 803 and 930). These base substitutions create allele-specific endonuclease digestion sites, especially at position 526 of the *Ban* I digestion site for the B allele [4]. The O and A transferase genes are identical except for a single base deletion at position 261 of the O allele, which shifts the codon frame and results in the synthesis of an entirely different peptide that is enzymatically inactive [1,3]. This single base deletion creates a *Kpn* I digestion site in the O allele. These allele-specific endonuclease restriction sites can be used for restriction fragment length polymorphism (RFLP) analysis for ABO genotyping in transfusion medicine, physical anthropology, cases of disputed parentage, human identification, and forensic analysis [4–9].

In RFLP analysis, digestion of DNA fragments amplified by PCR of a particular region of the genomic DNA with a restriction endonuclease is often performed for the determination of the existence of a particular restriction endonuclease digestion site. A common practice in DNA analysis is to cut a DNA fragment at one site with a restriction endonuclease. For DNA sizing of the resulting DNA fragment, agarose gel electrophoresis is performed with linear DNA sizing markers, followed by ethidium bromide staining. Although manual calibration for DNA sizing with plotting of the migration distances of molecular markers vs. $\log_{10}$ size following agarose gel electrophoresis is possible, it is difficult to distinguish differences in several base pairs. These methods are manual and time-consuming; each endonuclease treatment and run on an agarose gel require about 1 h, and microgram amounts of DNA fragments are needed. Furthermore, after the electrophoresis, a separate step of imaging

* Corresponding author. Tel.: +81 87 869 3576; fax: +81 87 869 4113.
E-mail address: m-kataoka@aist.go.jp (M. Kataoka).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.06.034



(C)

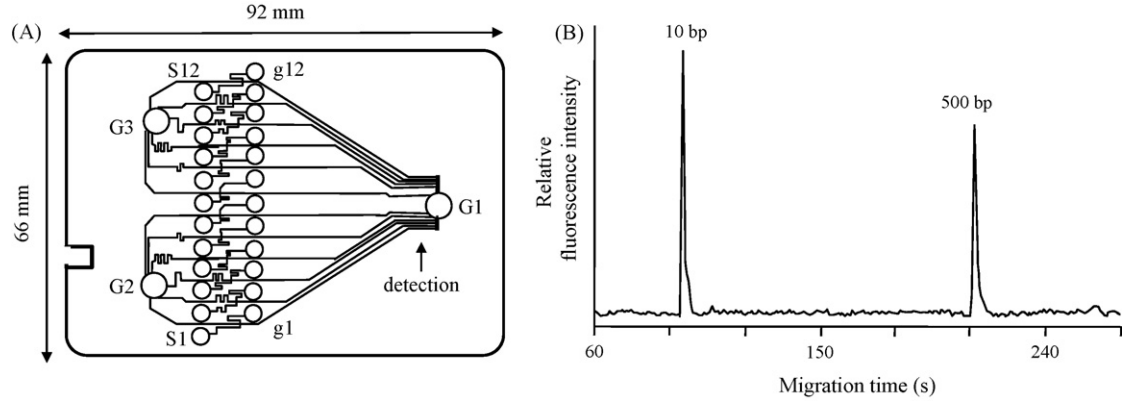
phenotype	genotype	*Kpn* I	*Ban* I
A	AA	–/–	–/–
	AO	+/–	–/–
B	BB	–/–	+/+
	BO	+/–	+/–
O	OO	+/+	–/–
AB	AB	–/–	+/–

–/– PCR product remains undigested.
+/– one allele of the PCR product is digested, the other remaining undigested.
+/+ PCR product is completely digested.

Fig. 1. Scheme for the presence of restriction endonuclease digestion sites on the ABO alleles determined by PCR-RFLP. (A) The presence or absence of a *Kpn* I site at codon 87 and a *Ban* I site at codon 175 of ABO allelic cDNA, respectively. (B) Distinction of the O allele from the A or B one with *Kpn* I at codon 87, and the B allele from the A or B one with *Ban* I at codon 175. (C) The endonuclease digestion results for each genotype are tabulated.

involving densitometric scanning of a photograph or CCD imaging of the stained gel is necessary [10].

Microchip electrophoresis has recently attracted much attention for DNA analysis due to its high efficiency, high throughput, time-saving ability, easy operation, and low consumption of samples and reagents [11]. Some commercial instruments, such as the Agilent 2100 Bioanalyzer, Shimadzu MCE2010, and Hitachi SV1100 and SV1210, have been developed, which have greatly promoted the further application of microchip electrophoresis. In microchip electrophoresis, nucleic acid fragments are separated by capillary electrophoresis on a chip with microfabricated channels, with automated detection as well as on-line data evaluation. Microchip electrophoresis has been employed for analysis of RFLP for rapid genotyping [12–16].



(C)

Peak No.	Migration time (s)	Size (bp)	Area (%)	Concentration (ng/μl)
1	95.4	10	586 (70)	0
2	211.6	500	247 (30)	2.0

Fig. 2. Design of the *i*-chip and the data output of the assay on a microchip. (A) The chip allows capillary electrophoresis in each of 12 different channels, and thus 12 samples can be analyzed on this chip. (B) Analysis of internal controls using the SV1210 software with the Hitachi SV1210 to present the results in the form of electropherograms. (C) The corresponding analytical results for each internal control DNA fragment are tabulated, DNA sizes of 10 and 500 bp, and a 500 bp DNA concentration of 2 ng/μl being estimated automatically, respectively.

Table 1
Reproducibility of migration times of fragments of internal controls in one channel on three successive electrophoreses.

Electrophoresis number	Migration time (s)	
	10 bp	500 bp
1	96.0	214.0
2	97.6	217.2
3	100.2	220.4
Average	97.9	217.2
RSD (%)	2.2	1.5

In the present study, we examined the ability of the Hitachi SV1210, with which 12 samples can be analyzed at the same time, to generate constant results as to the migration times of internal control DNA fragments of 10 and 500 bp on electrophoresis in different channels. The accuracy of DNA sizing with molecular size marker DNA was also investigated. We had already reported the potential of on-microchip endonuclease treatment of DNA with sequential analysis using a Hitachi SV1100 and a commercially available polymethylmethacrylate (PMMA) microchip, which offers high resolution in a short time [17]. Furthermore, we described the feasibility of the microchip electrophoresis method combined with on-chip endonuclease digestion of PCR-amplified DNA for RFLP analysis for ABO genotyping.

2. Materials and methods

2.1. Reagents and sample preparation

Restriction endonucleases *Kpn* I and *Ban* I were purchased from Toyobo (Tokyo, Japan). Genome DNA from hair roots was extracted with SMITEST EX-R&D (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan) according to the instruction manual. PCR was performed using KOD-plus-ver-2 as the DNA polymerase (Toyobo, Tokyo, Japan), and samples were amplified for 30 cycles consisting of denaturation at 94 °C for 15 s, annealing at 65 °C for 30 s, and elongation at 68 °C for 1 min. The PCR products were separated by 4% agarose gel electrophoresis and purified with a QIAquick Gel Extraction Kit (QIAGEN, Tokyo, Japan) for RFLP, and the amplified DNA was adjusted to 10 ng/μl concentration with double distilled water (DDW). PCR primers corresponding to human glycosyl transferase cDNA were synthesized by Sigma–Aldrich (Tokyo, Japan). The sequences of the 5′-primers and 3′-primers were as follows: 5′-primer for analysis of nucleotide position 261: 5′-ATGTGGGTGGCACCCTGCCA-3′ (44–63); 3′-primer for analysis of nucleotide position 261: 5′-ACTCGCCACTGCCTGGGTCTC-3′ (280–261); 5′-primer for analysis of nucleotide position 526: 5′-GTGGCTTTCCTGAAGCTGTTC-3′ (1290–1310); and 3′-primer for analysis of nucleotide position 526: 5′-GCCCACGTGGTCGCGGAACTC-3′ (1574–1554). The concentrations of purified DNA fragments were determined with a NanoDrop ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE). A low molecular weight DNA ladder (NEB, Ipswich, MA) was used for DNA sizing analysis on agarose gel electrophoresis. NoLimit DNA Ladder 10 and 500 bp (Fermentas, Ontario, Canada) were used as internal controls instead of the original internal control on microchip electrophoresis with the Hitachi SV1210. PCR Marker (NEB) was used for examination of the relationship between base pair-sizes and the migration times of the DNA fragments on microchip electrophoresis.

2.2. RFLP analysis by agarose gel electrophoresis

Two separate amplification reactions followed by digestion with diagnostic restriction enzymes, *Kpn* I and *Ban* I, are required for ABO genotyping (Fig. 1). The primers used for analysis of nucleotide position 261 generate a 236/237 bp PCR DNA product (*Kpn* I digest), and those used for analysis of nucleotide position 526 generate a 285 bp PCR DNA product (*Ban* I digest). As shown in Fig. 1, to differentiate the O allele from the A and B ones, 236 or 237 bp PCR-amplified DNA fragments (one nucleotide deletion in the O allele with the appearance of a digestion site for *Kpn* I) including nucleotide position 261 and *Kpn* I were employed for RFLP analysis. Also, 285 bp PCR-amplified DNA fragments including nucleotide position 526 and *Ban* I were employed for RFLP to differentiate the B allele from the A and O ones (appearance of a digestion site for *Ban* I in the B allele). To examine the digestion of an amplified DNA with a restriction enzyme by the conventional method, 5 μl of 10 ng/μl amplified DNA fragment, 2 μl of 70 mM $MgCl_2$, 2 μl of restriction enzyme, and 11 μl of DDW were mixed in an Eppendorf tube. After 1 h incubation at 37 °C, 20 μl/well reaction mixture was subjected to 4% agarose electrophoresis followed by staining with ethidium bromide for visualization of the digestion products. The digested product sizes were calculated from the standard curve obtained with molecular weight markers on the same gel.

2.3. Microchip preparation

Disposable *i*-chips 12 (Hitachi Chemical Co., Ltd., Tokyo, Japan), which are fabricated from PMMA, and comprise an interconnected network of fluid reservoirs and microchannels, were used for all of the separation experiments (Fig. 2A). Twelve samples can be analyzed on one of these chips at one time. For analysis of short length DNA, an *i*-SDNA 12 Kit (Hitachi Chemical) including a gel, and internal controls (10 and 500 bp) were employed. Twenty microliters of loading gel containing a fluorescent dye was infused from well G1 into the microchannels of an *i*-chip 12 by using a syringe, wells G2 and G3 were filled with 20 μl of gel using a pipette, and wells

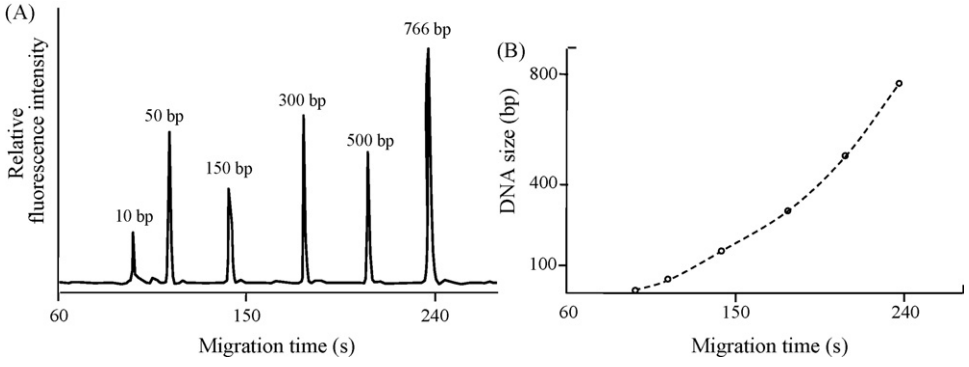


Fig. 3. Relationship between base pair-sizes and migration times of the DNA fragments. (A) Electrophoretic separation of the DNA fragments (10–766 bp) using the SV1210 software with the Hitachi SV1210. (B) Relationship between the base pair-sizes and migration times.

g1–g12 were filled with 10 μl of gel. Wells S1–S12 were the sample wells, and a pipette was used to fill each well with 9.0 μl of the original internal control, comprising 40 ng/μl of 10 bp and 2 ng/μl of 500 bp dsDNA fragments as markers for DNA sizing, and 1.0 μl of sample. Only a 500 bp dsDNA fragment was used as a reference for quantification. The samples could be analyzed in parallel within 6 min.

2.4. Instrumentation

Experiments were performed with a Hitachi SV1210 microchip electrophoresis instrument (Hitachi Electronics Co., Tokyo, Japan) with a semiconductor laser CCD detector (excitation at 635 nm and measurement of fluorescence at 660 nm). The instrument consists of a bench-top device (chip reader) that is connected with a personal computer. The SV1210 software (version 1.6.1) includes data collection, presentation, and interpretation functions. The data are displayed as both simulated gel images and electropherograms. Electropherograms of the internal controls, i.e., 10 and 500 bp DNA fragments, are shown in Fig. 2B. The sizing and quantification of DNA fragments can also be presented in tabular form (Fig. 2C). The chip reader includes programmable high voltage power supplies, each of which is connected to a platinum electrode. These electrodes can be used for multiple injections and other fluid manipulations from specific sample wells.

2.5. Microfluidic separation

All chips, except those used for analysis of RFLP, were used according to the manufacturer's instructions with the supplied materials (gel and internal controls). To examine variations in DNA size after sequential electrophoresis in different channels and in a single channel, 9.0 μl of an internal control comprising 10 and 500 bp DNA fragments, and 1.0 μl of TE buffer instead of a sample were added to the sample well. The sample well was connected through a network of channels to the separation line, which was used to perform the DNA separation. For analysis of a DNA ladder consisting of 10–766 bp fragments, 1.0 μl of 300 ng/μl DNA ladder, 1.0 μl of 100 ng/μl of 10 bp DNA fragment and 8.0 μl of TE buffer were added to the sample well, followed by analysis. The ABO genotyping protocol requires two separate amplification reactions followed by digestion with restriction enzymes *Kpn* I and *Ban* I. For analysis of on-chip RFLP, 2.0 μl of 10 ng/μl amplified DNA fragments, 1.0 μl of 70 mM $MgCl_2$, 4.0 μl of DDW, and 1.0 μl each of 20 ng/μl 10 bp DNA and 10 ng/μl 500 bp DNA instead of the original internal control were added to the sample well, followed by analysis on the microchip. Then, 1.0 μl of restriction enzyme was added to this well, and analysis was performed by just pushing the start button after 20 min incubation of the microchip in the Hitachi SV1210.

3. Results and discussion

The Hitachi SV1210 performs capillary electrophoresis in each of 12 different channels, and thus 12 samples can be analyzed on one chip. To determine the reproducibility of electrophoresis in the different channels, the migration times in individual channels were examined in comparison with those of internal controls, i.e., 10 and 500 bp DNA fragments. The relative standard deviations (RSD) in 60 different channels for the migration times of the 10 and 500 bp fragments were 0.98 and 1.11%, respectively, indicating the reproducibility of the electrophoresis even in different channels (data not shown). Sequential electrophoresis in one channel was performed three times at 7 min intervals. Although there was a tendency of an

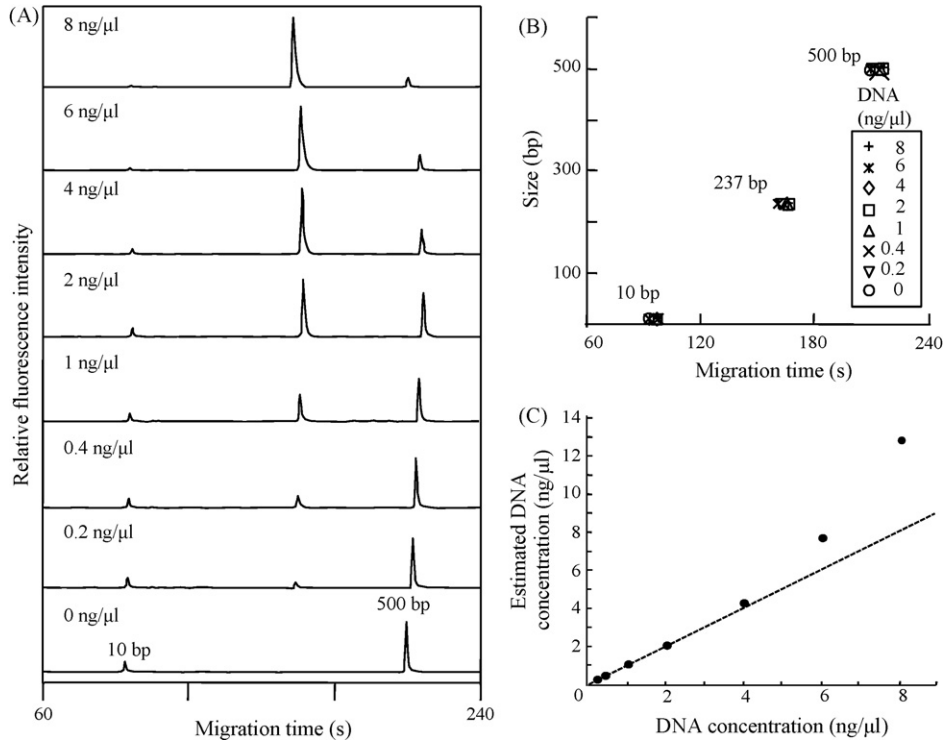


Fig. 4. Effects of the DNA concentration on the accuracy of DNA sizing and quantification with the Hitachi SV1210. (A) Electropherograms obtained on analysis of the 237 bp DNA fragment with different DNA concentrations. In all cases, the peak corresponding to the 237 bp DNA fragment appeared at a similar migration time. (B) The estimated size of the 237 bp DNA fragment is very close to the predicted sizes for all concentrations. (C) Plots of DNA concentrations estimated with the Hitachi SV1210. The filled circles indicate the estimated DNA concentrations of the samples, and the dashed line indicates the precise relationship between the estimated DNA concentrations and the applied sample concentrations. The error in DNA quantification increases in a dose-dependent manner.

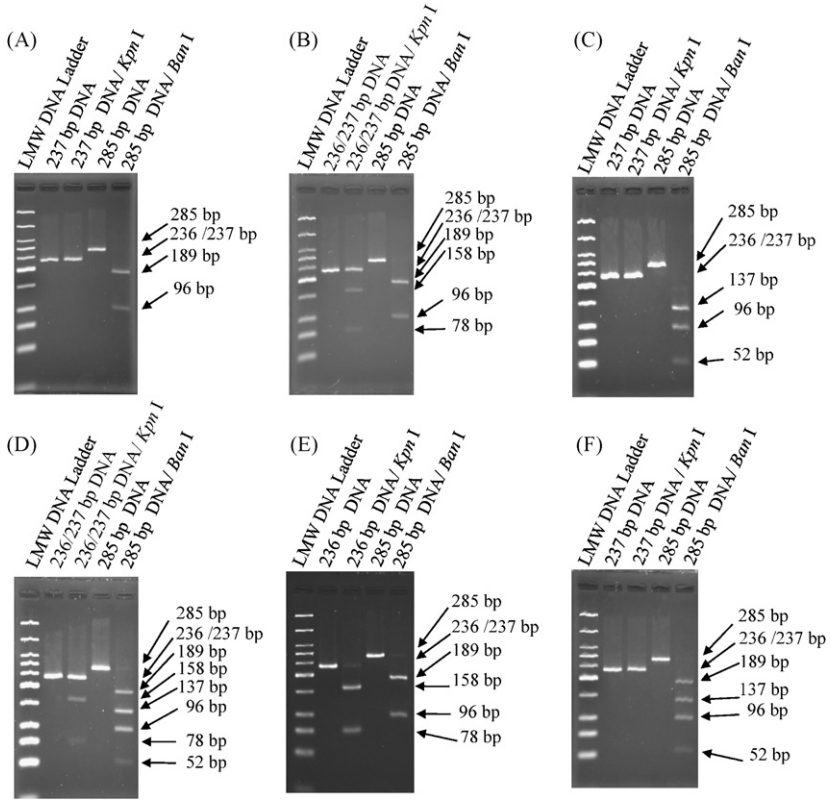


Fig. 5. RFLP analysis of ABO genotypes by conventional 4% agarose gel electrophoresis for genotypes AA (A), AO (B), BB (C), BO (D), OO (E), and AB (F). The 236 or 237 bp DNA fragment was amplified from codon 87 and digested with *Kpn* I, and the 285 bp DNA fragment was amplified from codon 175 and digested with *Ban* I, respectively. The DNA sizes are indicated on the right.

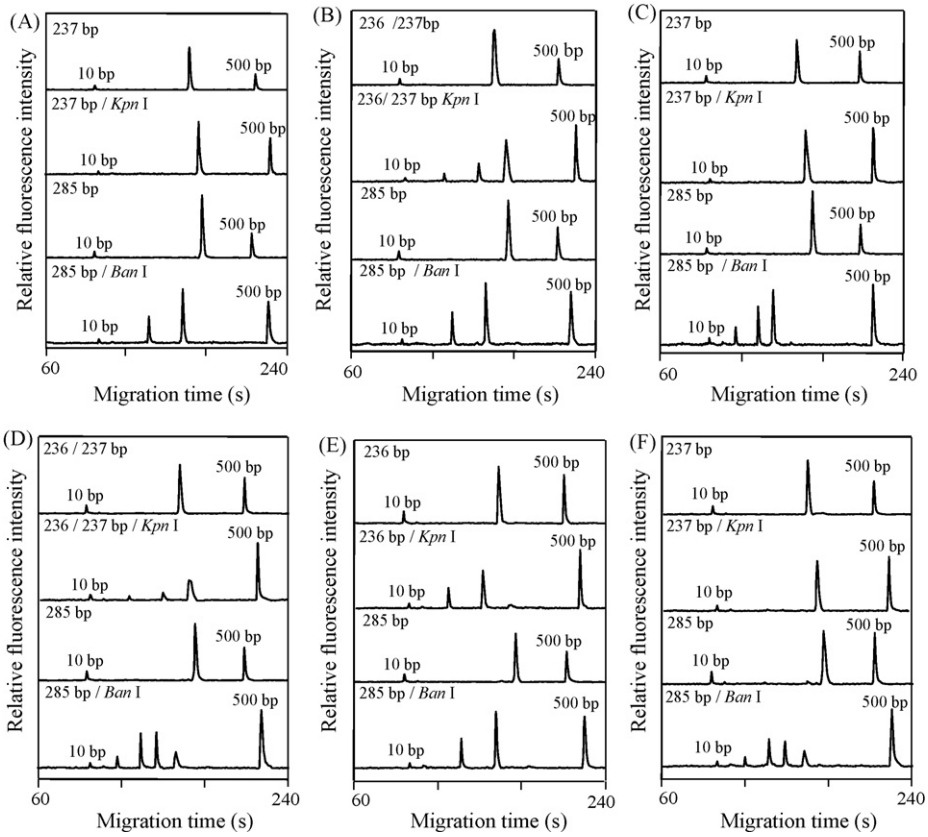


Fig. 6. Electropherograms on RFLP analysis for genotypes AA (A), AO (B), BB (C), BO (D), OO (E), and AB (F). The 236 or 237 bp DNA fragment was amplified from codon 87 and digested with *Kpn* I, and the 285 bp DNA fragment was amplified from codon 175 and digested with *Ban* I, respectively. The two peaks corresponding to 10 and 500 bp DNA in each column are internal controls.

increase in the migration time on sequential electrophoresis, only slight variation of the migration times of the 10 and 500 bp fragments was observed, the RSD for the fragments being 2.2 and 1.5%, respectively (Table 1). These results demonstrated the possibility of performing successive electrophoresis for the sequential analysis of a DNA sample in one channel.

An electropherogram of DNA 10–766 bp fragments is shown in Fig. 3A. Six peaks corresponding to the 10–766 bp fragments were separated clearly. A calibration curve was constructed by plotting each migration time against the DNA size, a linear relationship being obtained for fragment sizes of 50–300 bp (Fig. 3B). The decreased mobility of a large DNA fragment may be due to the loading gel containing a fluorescent dye employed for this microchip electrophoresis [17].

To examine the effects of the DNA concentration on the accuracy of sizing and quantification of amplified DNA fragments, samples containing 0.2, 0.4, 1.0, 2.0, 4.0, 6.0, and 8.0 ng/μl of the 237 bp amplified fragment, which was within the range of DNA sizing with this chip, were analyzed with the Hitachi SV1210 (Fig. 4A). As shown in Fig. 4B, the estimated DNA size was very close to the predicted size of the 237 bp fragment for all concentrations, and the DNA sizing results were found to be independent of the DNA concentration. Similar results have been obtained with other commercial instruments [18]. The accuracy of quantification of the 237 bp fragment was <2.0 ng/μl DNA (Fig. 4C). Similar tendencies as to DNA sizing and quantification were observed using the 285 bp DNA fragment. These results indicate that the accuracy of quantification and sizing was as expected, <2.0 ng/μl (20 ng/sample well), on DNA analysis, so we employed 2.0 ng/μl of each of the 236/237 bp and 285 bp amplified DNA fragments for direct endonuclease digestion on a microchip for RFLP analysis.

Regarding the endonuclease digestion of DNA fragments, a 70 mM $MgCl_2$ solution was used instead of the original 10× buffer because the Mg ion is required for the endonuclease reaction [19]. The signal intensity on microchip electrophoresis decreased with high salt concentrations [18,20]. Each of endonucleases *Kpn* I and *Ban* I was able to act effectively under low salt conditions (0 mM NaCl), so we employed only $MgCl_2$. An endonuclease-treated DNA sample was subjected to 4% agarose gel electrophoresis followed by staining with ethidium bromide for visualization for ABO genotyping (Fig. 5). The AA allele (Fig. 5A), AO allele (Fig. 5B), BB allele (Fig. 5C), BO allele (Fig. 5D), OO allele (Fig. 5E), and AB allele (Fig. 5F) were determined from the endonuclease digested pattern. These conventional methods are manual and time-consuming, each endonuclease treatment and run on an agarose gel requiring 1 h, and consuming microgram amounts of DNA samples. Furthermore, only rough estimation was possible for DNA sizing using the DNA ladder as a reference. The electropherograms obtained on ABO genotyping are shown in Fig. 6. After 20 min incubation of a DNA with an endonuclease on a microchip, the peaks corresponding to the expected DNA fragment and internal control were clearly separated. On sequential microchip electrophoresis analysis, the migration times of the 10 and 500 bp internal controls were increased in all cases. This phenomenon is also shown in Table 1. Although the migration time of each internal control was obviously increased, its size could be estimated exactly (Tables 2 and 3). In all cases, the estimated DNA size with or without endonuclease treatment differed from each predicted size by less than 10 bp (Tables 2 and 3). On 236/237 bp DNA digestion with *Kpn* I, DNA fragments corresponding to 78 and 158 bp originating from the O allele were clearly observed on electropherograms, and these sizes could be estimated more precisely than in the case of the conventional method. Similar precise sizing was also observed for 285 bp DNA digestion with *Ban* I, and the estimated sizes of DNA fragments corresponding to 52 and 137 bp originating from the B allele differing from each predicted size by only several bp (Table 3).

Table 2
Analysis of *Kpn* I digestion of 236/237 bp DNA fragments by microchip electrophoresis.

Sample no.	Before digestion			After digestion		
	Peak no.	Migration time (s)	Size (bp)	Peak no.	Migration time (s)	Size (bp)
1	1	97.0	10	1	100.0	10
	2	167.6	230	2	175.0	227
	3	217.0	500	3	228.6	500
2	1	96.0	10	1	99.4	10
	2	165.4	231	2	128.4	72
	3	213.6	500	3	154.0	152
				4	174.4	231
				5	226.2	500
3	1	94.6	10	1	96.8	10
	2	161.8	230	2	167.4	227
	3	212.2	500	3	217.6	500
4	1	94.0	10	1	96.6	10
	2	162.0	231	2	124.8	73
	3	209.0	500	3	149.4	155
				4	169.8	236
				5	218.4	500
5	1	95.2	10	1	98.4	10
	2	163.4	231	2	127.6	73
	3	211.8	500	3	151.8	152
				4	222.2	500
6	1	96.2	10	1	99.6	10
	2	165.2	229	2	173.0	226
	3	213.6	500	3	225.6	500

Table 3
Analysis of *Ban* I digestion of 285 bp DNA fragments by microchip electrophoresis.

Sample no.	Before digestion			After digestion		
	Peak no.	Migration time (s)	Size (bp)	Peak no.	Migration time (s)	Size (bp)
1	1	96.4	10	1	99.6	10
	2	177.0	282	2	137.2	96
	3	217.0	500	3	162.4	182
				4	228.6	500
2	1	95.4	10	1	99.8	10
	2	175.6	284	2	134.6	96
	3	213.6	500	3	159.2	182
				4	221.8	500
3	1	94.0	10	1	96.2	10
	2	172.4	284	2	115.4	50
	3	208.2	500	3	132.4	96
				4	143.2	133
				5	217.2	500
4	1	94.6	10	1	97.6	10
	2	173.0	283	2	117.0	50
	3	209.0	500	3	133.8	96
				4	145.0	133
				5	158.6	183
				6	220.0	500
5	1	95.8	10	1	98.4	10
	2	175.8	283	2	136.8	96
	3	211.8	500	3	161.8	181
				4	225.8	500
6	1	96.2	10	1	99.4	10
	2	176.4	283	2	119.4	50
	3	213.6	500	3	136.8	96
				4	148.2	133
				5	161.8	183
				6	225.2	500

The RSD in three different experiments for the migration times of endonuclease-digested DNA fragments on the microchip ranged from 0.88 to 1.89% (data not shown). Twenty-seven minutes was required to obtain these results, 20 min for endonuclease treatment on a microchip, and 7 min for electrophoresis and analysis. For analysis after endonuclease treatment of DNA by a conventional method, several hours are required, and microgram levels of sample are needed.

Microchip electrophoresis is being developed predominantly for the sizing and quantification of DNA, RNA, proteins and metabolites. Microchip electrophoresis for the sizing of restriction endonuclease-digested DNA has been applied for RFLP analysis [12–16]. Xie et al. [16] reported the potential of microchip electrophoresis combined with restriction endonuclease digestion of DNA on a microchip for RFLP analysis. They used a hybrid glass–PDMS microchip with combined heaters, resistance temperature detectors, a temperature-controlled reactor, chip electrophoresis, and pneumatic valves/pumps. A dynamic coating procedure for hybrid glass–PDMS microchips was employed for DNA separation. Furthermore, 45 min was required for the enzyme reaction on a microchip. We had already reported the feasibility of endonuclease digestion of plasmid DNA in the sample well on a microchip and subsequent analysis by microchip electrophoresis for DNA sizing [18]. In the present study, we showed the potential of microchip electrophoresis for multi-analysis of RFLP with a simple method involving direct endonuclease digestion of DNA in the sample well on a microchip by 20 min incubation without any heat treatment, and just pushing of the start button for DNA sizing using a commercial instrument. Genetic variations including RFLP are known to contribute to acquired and inherited human diseases, and DNA analysis is becoming a standard method for clinical diagnosis [21–23]. We believe that this method has considerable advantages, such as the accuracy of evaluation of DNA sizes, high efficiency, easy operation, low amounts of samples needed, and suitability for multi-analysis of RFLP.

References

[1] F. Yamamoto, H. Clausen, T. White, J. Marken, S. Hakomori, Nature 345 (1990) 229–233.
[2] F. Yamamoto, J. Marken, T. Tsuji, T. White, H. Clausen, S. Hakomori, J. Biol. Chem. 265 (1990) 1146–1151.
[3] F. Yamamoto, S. Hakomori, J. Biol. Chem. 265 (1990) 19257–19262.
[4] J.C. Lee, J.G. Chang, J. Forensic Sci. 37 (1992) 1269–1275.
[5] E. Hosoi, Jpn. J. Clin. Pathol. 43 (1995) 391–396.
[6] D.F. Stroncek, R. Konz, M.E. Clay, J.P. Houchins, J. McCullough, Transfusion 35 (1995) 231–240.
[7] C. Ladd, M.T. Bourke, C.A. Scherczinger, E.M. Pagliaro, R.E. Gaensslen, H.C. Lee, J. Forensic Sci. 41 (1996) 134–137.
[8] Z. Tun, K. Honda, M. Nakatome, M.N. Islam, H. Bai, Y. Ogura, H. Kuroki, M. Yamazaki, M. Terada, C. Wakasugi, J. Forensic Sci. 41 (1996) 1027–1030.
[9] A. Villa, F. Drago, R. Misto, F. Molelati, F. Poli, G. Sirchia, Haematologica 81 (1996) 492–496.
[10] J. Sambrook, D.W. Russell, Molecular Cloning: A Laboratory Manual, third ed., Cold Spring Harbor Laboratory Press, New York, 2001.
[11] L. Zhang, F. Dang, Y. Baba, J. Pharm. Biomed. Anal. 30 (2003) 1645–1654.
[12] I. Nachamkin, N.J. Panaro, M. Li, H. Ung, P.K. Yuen, L.J. Kricka, P. Wilding, J. Clin. Microbiol. 39 (2001) 754–757.
[13] J. Qin, Z. Liu, D. Wu, N. Zhu, X. Zhou, Y. Fung, B. Lin, Electrophoresis 26 (2005) 219–224.
[14] A. Minucci, E. Delibato, M. Castagnola, P. Concolino, F. Ameglio, C. Zuppi, B. Giardina, E. Capoluongo, J. Sep. Sci. 31 (2008) 2694–2700.
[15] K. Watanabe, J. Pestic. Sci. 33 (2008) 249–260.
[16] H. Xie, B. Li, R. Zhong, J. Qin, Y. Zhu, B. Lin, Electrophoresis 29 (2008) 4956–4963.
[17] J. Sigmon, L.L. Larcom, Electrophoresis 17 (1996) 1524–1527.
[18] M. Kataoka, S. Inoue, K. Kajimoto, Y. Shinohara, Y. Baba, Eur. J. Biochem. 271 (2004) 2241–2247.
[19] K. Yoshikawa, Y. Shinohara, H. Terada, S. Kato, Biophys. Chem. 27 (1987) 251–254.
[20] O. Mueller, K. Hahnenberger, M. Dittmann, H. Yee, R. Dubrow, R. Nagle, D. Ilsley, Electrophoresis 21 (2000) 128–134.
[21] D.N. Cooper, J. Schmidtke, Hum. Genet. 73 (1986) 1–11.
[22] D.N. Cooper, J. Schmidtke, Hum. Genet. 92 (1993) 211–236.
[23] R. Todd, R.B. Donoff, Y. Kim, D.T. Wong, J. Oral Maxillofac. Surg. 59 (2001) 660–667.