Analysis of Variable Number of Tandem Repeats of Human Genome D1S80 Locus Using Capillary Electrophoresis with Laser-Induced Fluorescence Detector

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The conventional method of identifying individuals by DNA in the field of forensic medicine is slab gel electrophoresis, which is time-consuming, labor-intensive, and nonquantitative. Accordingly, the use of capillary electrophoresis with a laser-induced fluorescence detector (CE-LIF), human genome D1S80 locus, a DNA marker which has a variable number of tandem repeats (VNTR) on chromosome 1, was examined to improve DNA analysis for identification. Using an internal standard, fragment size of VNTR was accurately and rapidly determined.

Key words capillary electrophoresis; variable number of tandem repeat; D1S80 locus; individual identification; PCR

Variable number of tandem repeat (VNTR) loci are used for individual identification and genetic diagnosis of disease. On repair by various enzymes, DNA is replicated with striking precision, such as approximately 1 base mutation per 1000 million base. But mutation repetition over time causes error (1 in 200—500 bases) and is used as a genetic polymorphism to identify individuals. The DNA polymorphism actually used for identification is of two types, D1S80 locus (type MCT118) on chromosome 1 and type HLADQα on chromosome 6.[1)]

D1S80 is located on chromosome 1 of the human genome and contains a 16 base pair (bp) repeat.[2)] The D1S80 alleles generally contain 14—41 repeats of a basic 16 bp unit. These alleles differ with respect to the number of 16 bp repeat units and range from approximately 369 bp to 801 bp in size.[3)] The D1S80 locus is a powerful marker for individual identification because it has a large number of possible alleles and demonstrates a heterozygosity with many phenotypes and thus is highly polymorphic.[2)]

Recently much attention has been focused on capillary electrophoresis as a tool for DNA analysis.[4—8)] DNA typing has been traditionally performed by slab gel electrophoresis. However, capillary electrophoresis with a laser-induced fluorescence detector (CE-LIF) is more convenient for such typing in terms of automation, high sensitivity, high speed, and small amounts of sample. In addition, CE-LIF can determine the size of PCR-amplified DNA fragments with high accuracy and high resolution. we report here a method for the rapid resolution of D1S80 allele and the accurate determination of its size using CE-LIF.

Two ladders were analyzed using CE-LIF for the optimization of separation conditions and size determination. To obtain the best separation efficiency for the 20 bp DNA ladder by CE-LIF, analytical conditions were investigated in terms of capillary length and electric field.

Figure 1 shows the effect of the applied voltage on the separation of the 20 bp DNA ladder using 50 mM Tris–borate (TB) buffer containing 0.5% methyl cellulose(MC). The migration time of each fragment decreased with increase of the applied voltage. It was difficult to separate DNA fragments larger than 600 bp with an electric field of 150 V/cm (Fig. 1A). Improved resolution was observed at 80 V/cm field compared with 150 V/cm field, however, because at 150 V/cm, the resolving power of MC solution decreased due to band broadening by the stretching DNA. Thus, it was pos-

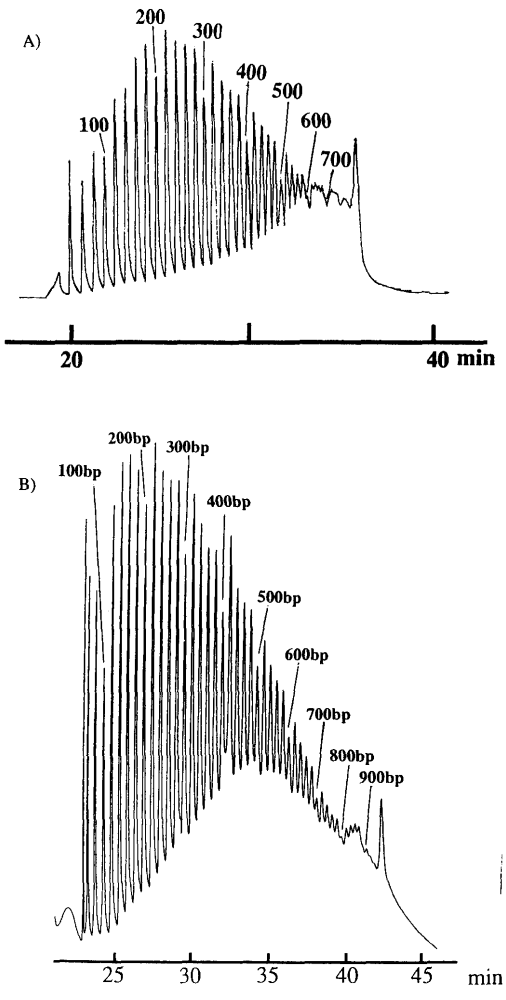

Fig. 1. Effect of Applied Voltage on the CE Separation of the 20 bp DNA Ladder

Capillary: 100 μm i.d., 360 μm o.d., total length 37 cm; effective length 30 cm. Running buffer: 50 mM TB, 0.5% MC, and 0.1 μM YO-PRO-1. Temperature: 25 °C. Voltage: A) 150 V/cm, B) 80 V/cm. Detection: Ex. 488 nm, Em. 560 nm.

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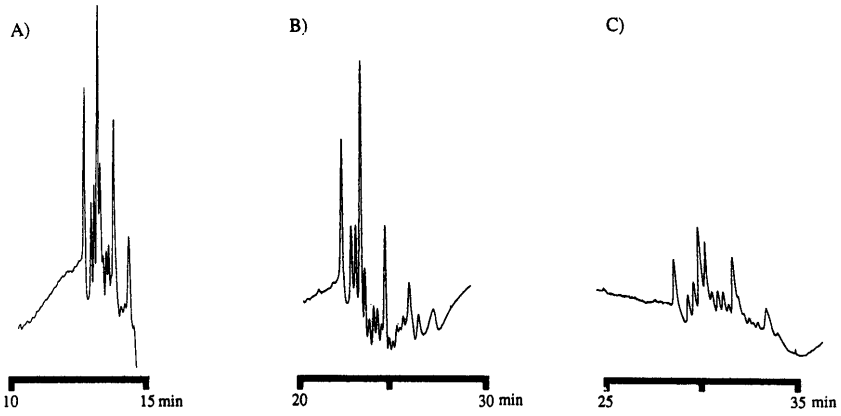

Fig. 2. Effect of Capillary Length on the CE Separation of the D1S80 Ladder

Capillary length: A) 37 cm, B) 47 cm, C) 57 cm. Field: 150 V/cm. Other conditions as in Fig. 1.

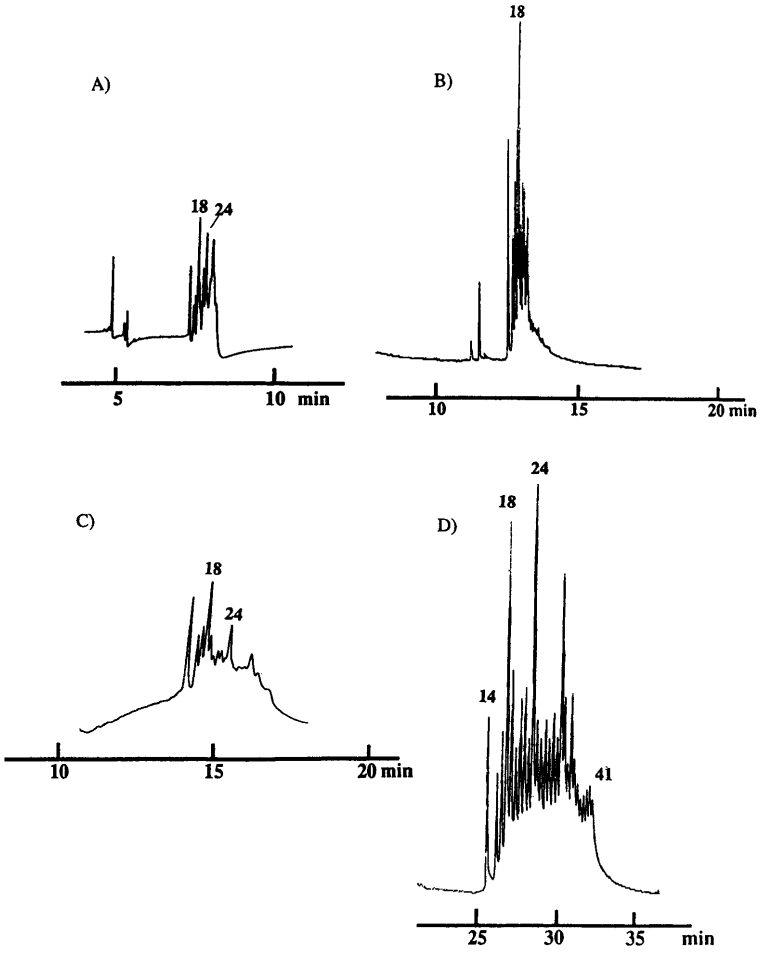

Fig. 3. Effect of Electric Field on the CE Separation of the D1S80 Ladder

Field: A) 300 V/cm, B) 200 V/cm, C) 150 V/cm, D) 80 V/cm. Capillary length: 37 cm. Other conditions as in Fig. 2.

sible to separate DNA fragments larger than 600 bp by low electric field analysis using 50 mM TB buffer–0.5% MC.

Figure 2 shows the effect of the capillary length on the separation of the D1S80 ladder. A D1S80 ladder is a mixture of 27 pooled human alleles, but only two (heterozygote) or one (homozygote) band is observed for the PCR-amplification products from actual human D1S80 loci. From the figure, it is clear that the migration time increased with increasing capillary length, but the resolution changed little in each electropherogram.

Figure 3 shows the effect of electric field on the separation of the D1S80 ladder. A decrease in the electric field markedly improved the resolution of DNA fragments of the ladder sample as shown in Fig. 3D.

DNA fragments of the D1S80 ladder from 300 bp to approximately 800 bp can be perfectly separated with high resolution within 35 min using a 37 cm capillary, an 80 V/cm field, and 50 mM TB buffer containing 0.5% MC.

We next examined the reproducibility in the analysis of the D1S80 ladder. Table 1 shows the reproducibility of the mi-

Table 1. Reproducibility (n=9) of Migration Time, Mobility, and Relative Mobility for DNA Fragments of D1S80 Allelic Ladder

DNA size (repeats)	Migration time (min)	R.S.D. (%)	Mobility ($cm^2V^{-1}s^{-1}$)	R.S.D. (%)	Relative mobility	R.S.D. (%)
PCR150	21.43	0.29	2.92×10^{-4}	0.29	1	—
14	26.04	0.25	2.40×10^{-4}	0.25	0.823	0.06
16	26.67	0.26	2.34×10^{-4}	0.26	0.803	0.08
17	26.95	0.28	2.32×10^{-4}	0.27	0.795	0.08
18	27.24	0.28	2.29×10^{-4}	0.28	0.787	0.10
19	27.53	0.29	2.27×10^{-4}	0.29	0.778	0.12
20	27.82	0.30	2.25×10^{-4}	0.30	0.770	0.13
21	28.10	0.31	2.24×10^{-4}	0.31	0.762	0.14
22	28.36	0.32	2.20×10^{-4}	0.32	0.755	0.17
23	28.63	0.34	2.18×10^{-4}	0.34	0.748	0.18
24	28.87	0.35	2.16×10^{-4}	0.35	0.742	0.21
25	29.14	0.37	2.14×10^{-4}	0.37	0.735	0.23
26	29.39	0.38	2.13×10^{-4}	0.38	0.729	0.25
27	29.66	0.40	2.11×10^{-4}	0.40	0.722	0.27
28	29.89	0.40	2.09×10^{-4}	0.41	0.717	0.29
29	30.13	0.42	2.07×10^{-4}	0.42	0.711	0.31
30	30.38	0.44	2.06×10^{-4}	0.44	0.705	0.32
31	30.61	0.45	2.04×10^{-4}	0.45	0.700	0.35
32	30.81	0.47	2.03×10^{-4}	0.47	0.695	0.36
33	31.04	0.49	2.01×10^{-4}	0.49	0.690	0.38
34	31.25	0.50	2.00×10^{-4}	0.50	0.685	0.40
35	31.46	0.50	1.98×10^{-4}	0.50	0.681	0.41
36	31.68	0.51	1.97×10^{-4}	0.50	0.676	0.41
37	31.88	0.50	1.96×10^{-4}	0.51	0.672	0.42
38	32.08	0.51	1.95×10^{-4}	0.55	0.668	0.41
39	32.28	0.55	1.94×10^{-4}	0.51	0.664	0.42
40	32.45	0.52	1.93×10^{-4}	0.54	0.660	0.43
41	32.64	0.52	1.91×10^{-4}	0.52	0.656	0.42

gration time for the fragments in the ladder, and we used 150 bp PCR product as an internal standard. In practice, the mixture of D1S80 ladder and 150 bp PCR product was analyzed 9 times using the same buffer and same capillary. The capillary was washed first with methanol and then buffer solution in each run. We also investigated the mobility and the relative mobility of the 150 bp PCR product, and found that the migration time and the mobility of each DNA fragment were reproducible to about 0.5% relative standard deviation (R.S.D.) on 9 repeated runs, respectively. The reproducibility of relative mobility was 0.06—0.42% R.S.D., indicating that the CE-LIF technique has satisfactory precision for DNA identification.

Figure 4 shows the analyses of the PCR-amplified DNA fragments from an individual and the D1S80 ladder mixed 150 bp PCR product. The dotted line peaks (B) represent the result for PCR-amplified D1S80 DNA fragments. This individual has two alleles which have different repeat numbers. The full line peaks (A) represent the result for the D1S80 ladder. The peak at around 21 min is a 150 bp DNA fragment of an internal standard in both electropherograms, and from this electropherogram, we can understand that this individual has 18 and 31 repeat units of DNA fragments because the two peaks agree with the repeat number 18 and 31 in the D1S80 ladder. Thus, the result shows that the size of the PCR product can be determined without mixing a sample in the D1S80 ladder when an internal standard is used. Analysis of the D1S80 DNA fragments is accurately achieved within about 32 min. Thus, CE-LIF can been applied to the rapid identification of individuals in forensic medicine in place of slab gel electrophoresis.[9]

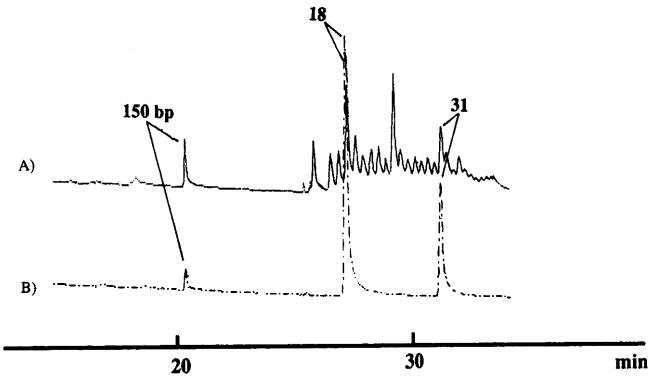

Fig. 4. CE Separation of A) D1S80 Ladder and B) D1S80 PCR Product
Field: 80 V/cm. Other conditions as in Fig. 3.

In conclusion, CE-LIF is a powerful technique to determine the number of repeat units in PCR-amplified VNTR alleles with high accuracy, resolution and speed. This may be a preferred alternative to slab gel electrophoresis and is applicable to the high speed analysis of PCR products and to individual identification with high-precision in the field of forensic medicine.

Experimental

A P/ACE system 2050 capillary electrophoresis apparatus with a laser source (Beckman Instruments , Inc., Fullerton, CA, U.S.A.) was used with negative source polarity. The laser source was operated at a power of 3 mW, excitation was at 488 nm and a 560 nm bandpass filter was used as an emission cut off filter. A DB-17 coated capillary (360 μm o.d., 100 μm i.d.; J&W Scientific, Folsom, CA) with 30—50 cm effective length (37—57 cm total length) was used.

The running buffer solution used in the present study was 50 mM TB. To this buffer solution was added 0.5% MC. Just prior to the CE analysis, 1 μl of fluorescent dye solution 1-(4-[3-methyl-2,3-dihydro-(benzo-1,3-oxazole)-2-methylidene]-quinolinium)-3-trimethyl-ammonium propane diiodide(YO-PRO-1), which was intercalated into the double strand of the DNA, was added to 10 ml of the running buffer solution.

A 20 bp DNA ladder was purchased from Gensura Laboratories Inc.(CA). DNA sample was diluted 10—100 fold with Milli-Q water and stored at −18 °C until use. MC was purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical-reagent or electrophoretic grade and were from Wako (Osaka, Japan).

The 150 bp DNA target of bacteriophage lambda DNA was amplified by PCR as described.[10] PCR was performed on a thermal cycler (Techne, PHC-3, Princeton, NJ, U.S.A.) with 25 cycles of amplification (1 min at 94 °C, 1 min at 37 °C, and 1 min at 72 °C).

Human genomic DNA was amplified by using an *AmpliFLP*™ D1S80 PCR amplification kit (Perkin-Elmer). PCR was performed according to the protocol described by Kasai *et al.* [11] The oligonucleotide primers were 25 nucleotides in length. The sequence of the 5′-PCR primer was 5′-GATGAGTTCGTGTCCGTACAACTGG-3′, and that of the 3′-PCR primer was 5′-GGTTATCGAAATCAGCCACAGCGCC-3′. The PCR was carried out in a final volume of 50 μl, containing 5 ng of genomic DNA, 12.5 pmol of oligonucleotide primers, 1 nmol of the four deoxyribonucleotide triphosphates (dNTPs), 2.5 U of thermostable Taq Polymerase, and 10×buffer (10 mM Tris–HCl; pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01%(w/v) gelatin). The PCR was performed on a GeneAmp PCR System 2400 (Perkin-Elmer, Norwalk CT, U.S.A.) with 29 cycles of amplification (denaturation: 15 s at 95 °C, annealing: 15 s at 66 °C, and extension: 40 s at 72 °C).

Sample solution was electrophoretically introduced into the capillary at a negative polarity of 2.9 kV for 20 s and was run with the running buffer solution described above at a negative polarity of 2.9—11.1 kV (80—300 V/cm) at 25 °C. DNA fragments were detected at 560 nm (Ex.; 488 nm). Prior to the electrophoretic analysis of the samples, the capillary was washed with methanol (50 v/v %) for 5 min and then with the running buffer solution for 5 min.

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