ELSEVIER

Journal of Chromatography A, 676 (1994) 185–189

JOURNAL OF CHROMATOGRAPHY A

Molecular size determinations of DNA restriction fragments and polymerase chain reaction products using capillary gel electrophoresis

Michael A. Marino*, Lisa A. Turni, Susie A. Del Rio, Patrick E. Williams

Developmental Laboratory, Armed Forces DNA Identification Laboratory, Armed Forces Institute of Pathology, Washington, DC 20306-6000, USA

Abstract

Commercially available capillary electrophoresis (CE) systems offer advantages over traditional slab gel methodologies. The capillary format allows the use of higher voltages (225 V/cm), which results in faster migration, higher resolution and greater efficiency without excessive heating. The ability to automate the system increases the unattended sample analysis throughput. For this study, the CE system was configured with a μPAGE 3% T, 3% C polyacrylamide gel capillary with an effective length of 40 cm and μPAGE Tris–borate urea buffer system. The analysis of DNA restriction fragments and polymerase chain reaction products, with an internal standard of Boehringer Mannheim DNA marker XI were completed in less than 70 min. All samples were analyzed at 260 nm. The data establishes automated capillary gel electrophoresis as a high-resolution, reproducible method for analysis of samples under 1000 base pairs.

1. Introduction

DNA is found in virtually every cell of the human body and is unique to the individual. By analyzing the DNA it is possible to identify suspects, victims of crime, and casualties of mass disaster. In some cases, identification is completed by using DNA extracted from samples of tissue, bone, semen, blood or hair.

One general approach to DNA profiling uses markers that are based upon restriction fragment length polymorphism (RFLP) [1]. As originally conceived, variation in the length of target DNA fragments is based upon differences in the presence or absence of endonuclease restriction sites. Two drawbacks often associated with the detection of RFLP markers is the necessity of a relatively large DNA sample (20–100 ng) and detection using radioactive ^{32}P labels.

In humans, RFLP loci with as many as 80 different alleles [2] have been reported. Such loci, referred to as variable number tandem repeat (VNTR) loci [3] consist of sets of tandemly repeated oligonucleotide core sequences and were termed "minisatellites" by Jeffreys *et al.* [4]. The core sequence vary in length from 11 to 60 base pairs and the repetitive region is flanked by conserved endonuclease restriction sites. Thus, the length of the restriction fragment produced by this type of genetic locus is proportional to the number of oligonucleotides core units it contains. Alleles at these loci are visual-

* Corresponding author.

0021-9673/94/$07.00 © 1994 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(94)00119-T

ized via Southern hybridization. Theoretically, hundreds of alleles varying in length from 9 to 30 base pairs can be identified at such a loci. Hybridizing bands on a Southern blot varying in length by only a few core sequences are extremely difficult to differentiate. This difficulty, combined with the possibility of band shifting, have caused concern with the accuracy of human DNA fingerprinting [5].

A new type of genetic marker that allows more exact determination of allelic profiles was suggested in 1989 [6–8]. Rather than repeat units in the range of 11 to 60 base pairs in length, these workers suggested that high levels of length polymorphism exist in dinucleotide tandem repeat sequences. A dinucleotide repeat such as $(C–A)_n$ were reported to occur in human genome as many as 50 000 times with n varying from 10 to 60. These reiterated sequences have been referred to as microsatellites, simple sequence repeats (SSR) [9], simple sequence length polymorphism (SSLP) [10] or short tandem repeats (STR) [7,11,12]. The detection of STRs is based upon variations in the length of polymerase chain reaction (PCR) products [13]. The PCR basis of the system reduces the DNA required for detection a number of orders of magnitude below that of RFLP-based procedures.

The DNA restriction fragments and PCR products are traditionally separated by conventional slab gel electrophoresis, but the fragments have similar charge-to-mass ratios, and thus are separated by length-induced drag. Capillary gel electrophoresis (CGE) is a new technique used in molecular biological analysis which offers advantages over conventional slab gel methodology [14]. CE, using a thin walled capillary of 50–150 μm I.D., provides excellent heat transport, thus allowing higher applied voltages to produce quicker separations. The recent development of polyacrylamide gel capillary and buffer systems have sufficient resolving power to separate these alleles. CGE also uses a smaller sample volume, generally 5 ng sample per injection. The system detector is on-column, which increases sensitivity [14,15]. Consequently, DNA fragments can be detected at 260 nm without radioactive labels.

The purpose of this study was to apply the speed and resolution of CGE to DNA analysis of PCR products and DNA restriction fragments. This study includes examples of PCR products such as soybean STRs and DNA restriction fragments of ΦX174/Hinf1.

2. Materials and methods

2.1. Instrumentation

All analyses used the Dionex CE system CES-1 (Sunnyvale, CA, USA). Separations were performed using μPAGE (3% C, 3% T[a]) polyacrylamide gel electrophoresis column from J & W Scientific (Folsom, CA, USA). The effective column length was 40 cm, and the applied voltage was −225 V/cm. The DNA fragments were detected at 260 nm. The data were collected by the Dionex AI 450 software package (version 3.31) on a ZEOS 486DX (MS-DOS 5.00 and Windows 3.10).

2.2. Reagents

All water used in this study was reagent-grade HPLC water. The buffer used was μPAGE Tris–borate and urea buffer supplied by J & W Scientific with 10 μM ethidium bromide. The standard DNA ladder, DNA molecular mass marker XI (Boehringer Mannheim, Indianapolis, IN, USA) and DNA restriction digest, ΦX174/Hinf1 (BRL 9; Gibco, Gaithersburg, MD, USA) were prepared using a 20-ml aliquot. After dialysis on a MF-Millipore membrane filter (Millipore, Bedford, MA, USA) for 15 min, 20 μl of the ladder were added to 20 μl of dialyzed sample.

2.3. Sample preparation

The PCR products and allelic ladders used in this study were prepared by Ms. Rhonda Roby, Ms. Demeris Lee and Dr. Mitchell Holland from our institute according to standard amplification

[a] C = g N,N′-methylenebisacrylamide (Bis)/%T; T = (g acrylamide + g Bis)/100 ml solution.

procedures as described by Roche Molecular Systems (Alameda, CA, USA). The PCR products of soybeans used were prepared by Dr. Perry Cregan (Soybean & Alfalfa Research Laboratory, US Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA).

3. Results and discussion

The study started with the analysis of DNA molecular mass marker XI. The marker contains double stranded, non-phosphorylated, blunt-ended DNA fragments of 50, 100, 200, 300, 400, 500, 700 and 1000 base pairs (see Fig. 1.). The method used for size determination of a DNA restriction fragment or PCR product was based on the production of a calibration plot. In this method the DNA fragments of known size were separated by CGE and the corresponding retention time were plotted. Fig. 2. is a calibration curve generated from the analysis of the molecular mass ladder.

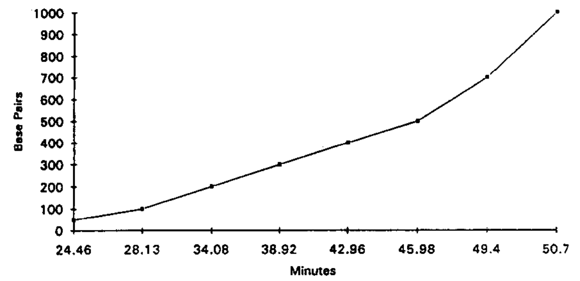

Fig. 2. A calibration curve generated from the analysis in Fig. 1.

The high resolution and reproducibility of CGE gives this technique the potential to perform routine DNA analysis. Fig. 3. shows the analysis of the mitochondrial dinucleotide repeat. The STR is a two-base pair repeat (AC) at location 514 in the D-Loop of the mitochondria [11,16]. CGE has the resolution capable of sizing the PCR products which are a single repeat apart (two base pairs). The size of the mitochondrial repeat was confirmed by direct DNA sequencing of the PCR product.

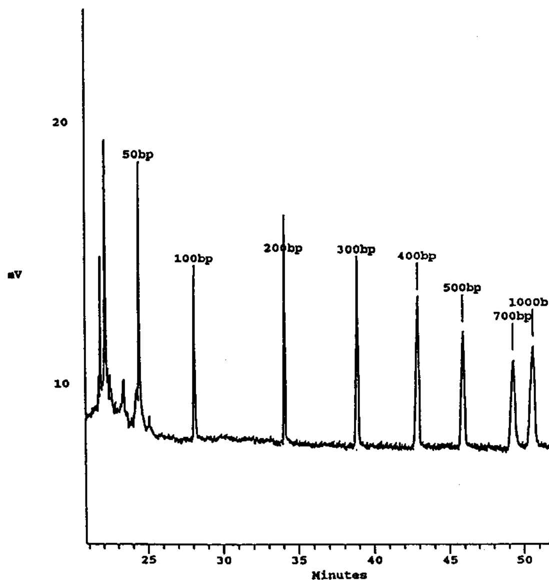

Fig. 1. Analysis of DNA molecular mass marker XI (Boehringer Mannheim) with size as indicated (bp = base pairs). Conditions: μPAGE (3% C, 3% T) polyacrylamide gel electrophoresis column from J & W Scientific. The effective column length was 40 cm. Injection of standard was at −7 kV for 9 s. Run voltage was −225 V/cm. System detector was set at 260 nm.

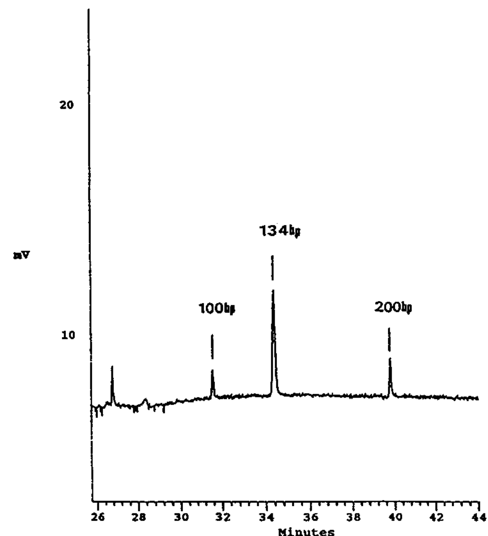

Fig. 3. Analysis of STR at location 514 in the D-loop of the mitochondria. Conditions: μPAGE (3% C, 3% T) polyacrylamide gel electrophoresis column from J & W Scientific. The effective column length was 40 cm. Injection of sample was at −7 kV for 9 s. Run voltage was −225 V/cm. System detector was set at 260 nm.

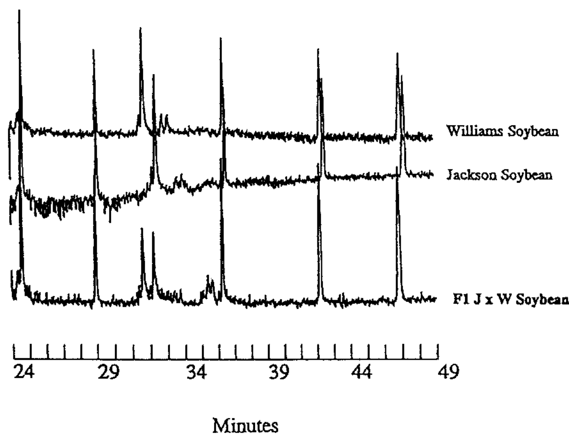

Fig. 4. Analysis of the parental genotypes Jackson and Williams and their using the same conditions as Fig. 1. The molecular mass markers 100 and 200 base pairs are at 29 and 36 min, respectively. The SSR-containing fragments for the genotypes are found between 30 and 33 min. F1 generation is heterozygous.

Analyses of STR-containing PCR products were also performed using soybean genotype [17,18]. Fig. 4. is the electropherograms of parents and the F_1 generation. The allelic STRs are located between 30 and 33 min (flanked by the molecular mass marker). Notice the F_1 generation is a heterozygote, containing the alleles of both parent genotypes.

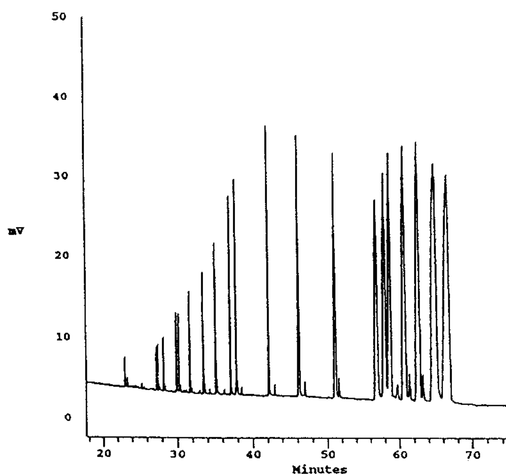

Fig. 5. ΦX174/Hinf1 restriction fragment digest containing 22 fragments from 22 to 726 base pairs in size (conditions as in Fig. 1).

An example of the separation the ΦX174/Hinf1 restriction fragment digest is found in Fig. 5. The resolution of the CGE separates all 22 restriction fragments from 22 to 726 base pairs in size. This system also provides 4-base pair resolution between 413–417 base pair fragments. This separation is complete within 60 min and is superior to the traditional slab gel methods.

4. Conclusions

It is possible to analyze and determine the allelic profile of plants and humans using CGE. The method requires a reference sample and molecular mass markers or internal reference marker. This method is rapid, sensitive and reproducible. Advances in the gel capillary production have greatly increased their life expectancy (over 160 h at 225 V/cm). The automation of the CE allows for continual unattended operation. These advantages make this analysis desirable for many biological studies.

5. Disclaimer

The opinions or assertions herein are those of the author and do not necessarily reflect the views of the Department of Army of the Department of Defense.

6. References

[1] D. Botstein, R.L. White, M. Skolnick and R.W. Davis., *Am. J. Hum. Genet.*, 32 (1980) 314–331.
[2] I. Balazs, M. Baird, M. Clyne and E. Meade, *Am. J. Hum. Genet.*, 44 (1989) 182–190.
[3] Y. Nakamura, M. Leppert, P. O'Connell, R. Wolff, T. Holm, M. Culver, C. Martin, E. Fujimoto, M. Hoff, E. Kumlin and R. White, *Science*, 235 (1987) 1616–1622.
[4] A.J. Jeffreys, V. Wilson and S.L. Thein, *Nature*, 314 (1985) 67–73.
[5] R. Chakraborty, R. and K.K. Kidd, *Science*, 254 (1991) 1735–1739.
[6] M. Litt and J.A. Luty, *Am. J. Hum. Genet.*, 44 (1989) 397–401.
[7] J.L. Weber and P.E. May, *Am. J. Hum. Genet.*, 44 (1989) 388–396.

[8] D. Tautz, *Nucleic Acids Res.*, 17 (1989) 6463–6471.
[9] H.J. Jacob, K. Lindpaintner, S.E. Lincoln, K. Kusumi, R.K. Bunker, Y.-P. Mao, D. Ganten, V.J. Dzau and E.S. Lander, *Cell*, 67 (1991) 213–224.
[10] N.G. Copeland, N.A. Jenkins, D.J. Gilbert, J.T. Eppig, L.T. Maltais, J.C. Miller, W.F. Deitrick, A. Weaver, S.E. Lincoln, R.G. Steen, L.D. Stein, J.H. Nadeau and E.S. Landers, *Science*, 262 (1993) 57–66.
[11] A. Bodenteich, L. Mitchell, M. Polymeropoulos and C. Merril, *Molec. Gen.*, 1 (1992) 40.
[12] A. Edwards, A. Civitella, H. Hammond and C.T. Caskey, *Am. J. Hum. Genet.*, 44 (1991) 746–756.
[13] K. Mullis, F. Faloona, S. Scharft, R. Saiki, G. Horn and H. Erlich, *Quant. Biol.*, 51 (1986) 263–275.
[14] I.S. Krull and J.R. Mazzeo, *Nature*, 357 (1992) 92–94.
[15] L.M. Smith, *Nature*, 349 (1991) 812–813.
[16] P.E. Williams and K. Turner, *Crime Lab. Digest.*, (1994) in press.
[17] M.A. Marino, L.A. Turni, S.A. Del Rio, P.E. Williams and P.B. Cregan, *Appl. Theoret. Electrophoresis*, submitted for publication.
[18] M.S. Akkaya, A.A. Bhagwat and P.B. Cregan, *Genetics*, 132 (1992) 1131–1139.