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Genetic typing and HIV-1 diagnosis by using 96 capillary array electrophoresis and ultraviolet absorption detection

Xiaoyi Gong, Edward S. Yeung*

Ames Laboratory-USDOE and Department of Chemistry, Iowa State University, Ames, IA 50011, USA

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Abstract

Current high-throughput approaches to the analysis of PCR products are based primarily on electrophoretic separation and laser-excited fluorescence detection. We show that capillary array electrophoresis can be applied to HIV-1 diagnosis and D1S80 VNTR genetic typing based simply on UV absorption detection. The additive contribution of each base pair to the total absorption signal provides adequate detection sensitivity for analyzing most PCR products. Not only is the use of specialized and potentially toxic fluorescent labels eliminated, but also the complexity and cost of the instrumentation are greatly reduced.

Keywords: Genetic typing

1. Introduction

Since the invention of the polymerase chain reaction (PCR) in 1985 by Kary Mullis, the ultimate in sensitivity, together with increasing ease in implementation, have placed this technique in a central position in molecular biology research and in clinical diagnosis [1]. In the last ten years, PCR has stimulated numerous investigations in genetic analysis, and is even being used to determine the genetic basis of complex diseases [2]. There is no need to reiterate the development of capillary electrophoresis (CE) as a powerful analytical tool in post-PCR analysis. A large amount of research has been done to explore the advantages of CE over traditional slab gel electrophoresis, including high-speed, high-resolution restriction fragments analysis [3–8], high-speed, high-throughput DNA sequencing, [9–15] rapid and precise DNA typing and sizing [16–22], single-base mutation analysis [23–28] and the analysis of disease causing genes [29–33]. In particular, capillary array electrophoresis along with other micro-fabricated devices [34–36] are promising methods for the purpose of achieving high-throughput DNA analysis.

The conventional protocol for DNA analysis calls for labeling with radionuclides or fluorescent tags before, during or after size-based separation in slab gel electrophoresis or in capillary gel electrophoresis (CGE). This derivatization process involves expensive reagents and raises safety concerns for the operator and for waste disposal because of the toxic nature of these labeling reagents. Single capillaries have been utilized for DNA analysis [3]. Recently, a 96-capillary array electrophoresis system based on a novel absorption detection approach using a single linear photodiode array has been invented in our

*Corresponding author. Tel.: +1-515-294-8062; fax: +1-515-294-0266.

E-mail address: yeung@ameslab.gov (E.S. Yeung)

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laboratory [37]. Based on this system, we are able to design DNA analysis protocols to take advantage of capillary array gel electrophoresis and absorption detection based on the inherent spectral properties of the DNA bases. The fact is that a 100-bp DNA contains 100 absorbing units that can provide excellent net absorptivity for sensitive detection. No special labeling reactions are required. The method was tested on two broadly used PCR protocols using typical concentrations of starting materials. We show that as low as 50 copies of HIV-1 *gag* fragment can provide a positive diagnosis. Also, the human D1S80 VNTR region can be used to provide a DNA (genetic) fingerprint following the same method. Best of all, since the novel absorption detector involves no lasers and is suitable for simultaneously monitoring 96 capillaries (and scalable eventually to 1000 capillaries), high throughput can be achieved in a low-cost rugged instrument.

2. Experimental

2.1. Reagents and materials

1×TBE buffer was prepared by dissolving premixed TBE buffer powder (Amerosco, Solon, OH) in deionized water. Poly(vinylpyrrolidone) (PVP) was obtained from Sigma (St. Louis, MO). The coating matrix was made by dissolving 2% (w/v) of 1 300 000 MW PVP into the buffer, shaking for 2 min and letting it stand for 1 h to get rid of bubbles. Poly(ethylene oxide) (PEO) was obtained from Aldrich Chemical (Milwaukee, WI). The sieving matrix was made by dissolving 2% (w/v) 600 000 MW PEO into the buffer. The solution was stirred vigorously overnight until all the material was dissolved and no bubbles could be observed. Ethidium bromide (EtBr) was obtained from Molecular Probes Inc. (Eugene, OR). 50-bp and 100-bp DNA ladders were purchased from Life Technologies (Gaithersburg, MD).

2.2. Sample preparation

2.2.1. Polymerase chain reaction

2.2.1.1. Multiplexed PCR for variable number of tandem repeats (VNTR) loci. AmpliFLP D1S80 PCR amplification kit was purchased from Perkin–Elmer (Foster City, CA). The kit included D1S80 PCR Reaction Mix (containing two D1S80 primers, AmpliTaq DNA polymerase and dNTPs in buffer), $MgCl_2$ solution and Control DNA 3 (human genomic DNA of D1S80 type 18, 31 in buffer). The PCR mixtures used are described as follows:

Positive control: 20 μl of D1S80 PCR Reaction Mix, 10 μl of $MgCl_2$ solution and 20 μl of Control DNA3.
Negative control: 20 μl of D1S80 PCR Reaction Mix, 10 μl of $MgCl_2$ solution and 20 μl of autoclaved DI H_2O.

The polymerase chain reactions were performed with the following parameters: 30 cycles of denaturation at 95°C for 15 s, annealing at 66°C for 15 s, and extension at 72°C for 40 s. The thermal cycler used was a Perkin–Elmer GeneAmp PCR system 2400.

2.2.1.2. PCR for human immunodeficiency virus (HIV). The HIV testing kit (Perkin–Elmer) included positive control DNA that includes all parts of the HIV-1 genome, negative control DNA, HIV primers, AmpliTaq DNA polymerase, dNTPs, PCR reaction buffer and $MgCl_2$ solution. The PCR mixtures used are listed in Table 1. The protocol for the Perkin–Elmer GeneAmp thermal cycler is 40 cycles of denaturation at 95°C for 30 s, annealing and extension at 62°C for 1 min. The annealing and extension temperatures were the same for this amplification.

2.2.2. DNA purification

All PCR products were purified with Microcon YM-30 centrifugal filter devices (Millipore, Bedford, MA). After the purification, salts, dNTPs and most HIV-1 primers were eliminated from the DNA samples.

2.2.3. Capillary array electrophoresis

The 96 capillary array electrophoresis system with photodiode array absorption detection has been detailed before [37]. A DC-powered mercury lamp (UVP Inc., Upland, CA) was used as the light source, which gave lower noise levels than the AC-powered mercury lamp used in previous work. The absorption wavelength was set at 254 nm by an

Table 1
PCR mixtures for HIV amplification

Component	Addition order	Volume	Final concentration
Autoclaved, deionized water	1	32.8 μl	
10× PCR buffer II	2	5 μl	1×
DNTPs	3	1 μl each	200 μ*M* each dNTP
HIV-1 primer 1 (SK38)	4	1 μl	0.5 μ*M*
HIV-1 primer 2 (SK39)	5	1 μl	0.5 μ*M*
AmpliTaq DNA polymerase	6	0.2 μl	1 unit
25 m*M* $MgCl_2$ solution	7	5 μl	2.5 m*M*
Positive Control DNA *or* Negative Control DNA	8	1 μl	0.5 μg human placental DNA

interference filter (Oriel, Stanford, CT). The total length of the capillaries was 55 cm, with 35-cm effective length. The capillary array was first flushed with deionized water and then with 1 ml of 2% PVP at a pressure of 100 p.s.i. While the injection ends were immersed in the buffer reservoir, 0.5 ml of 2% PEO (600,000 MW) sieving matrix was pushed into the capillary bundle at 100 p.s.i. The procedure roughly took 20 min. After the gel filling, ethidium bromide was added to the buffer reservoirs at the concentration of 1 μg/ml. The system was then pre-run for 10 min with the electric field strength at 150 V/cm. After the pre-run, ethidium bromide should have spread out evenly in the sieving matrix through electrical migration. Ethidium bromide is known to make the DNA fragments more rigid, thereby leading to sharper bands in CGE. It is not expected to significantly alter the absorption strength of the DNA fragments in this study. The samples were injected electrokinetically at 150 V/cm for 15 s. 150 V/cm field strength was employed for the separation. The total current was about 620 μA during the separation process. Twelve different samples were used in the 96-capillary array electrophoresis experiment, which are detailed in Table 2. Each type of sample was injected into and run in eight different capillaries in the 96-capillary array.

3. Results and discussion

Fig. 1 shows the result of the capillary array gel electrophoresis for DNA analysis as a reconstructed ''gel'' image. The vertical direction represents the capillary array arrangement while the horizontal direction represents the migration time. All separations were finished within 25 min. Capillary #84 (Sample type 11, see Table 2) showed bad separation resolution after 350 base pairs. The other 95 capillaries all gave reasonable separation and good signal-

Table 2
Sample description in the capillary array electrophoresis

Sample no.	Description
1	4 μl of HIV-1 primer-1 (SK38)
2	4 μl of purified HIV-1 negative PCR product
3	4 μl of purified HIV-1 positive PCR product
4	4 μl of 100 bp ladder
5	3 μl of purified HIV-1 negative PCR product with 1 μl of 100-bp ladder
6	3 μl of purified HIV-1 positive PCR product with 1 μl of 100-bp ladder
7	4 μl of purified D1S80 negative PCR product
8	4 μl of purified D1S80 positive PCR product
9	4 μl of 50-bp ladder
10	3 μl of purified D1S80 negative PCR product with 1 μl of 50-bp ladder
11	3 μl of purified D1S80 positive PCR product with 1 μl of 50-bp ladder
12	4 μl of DI H_2O

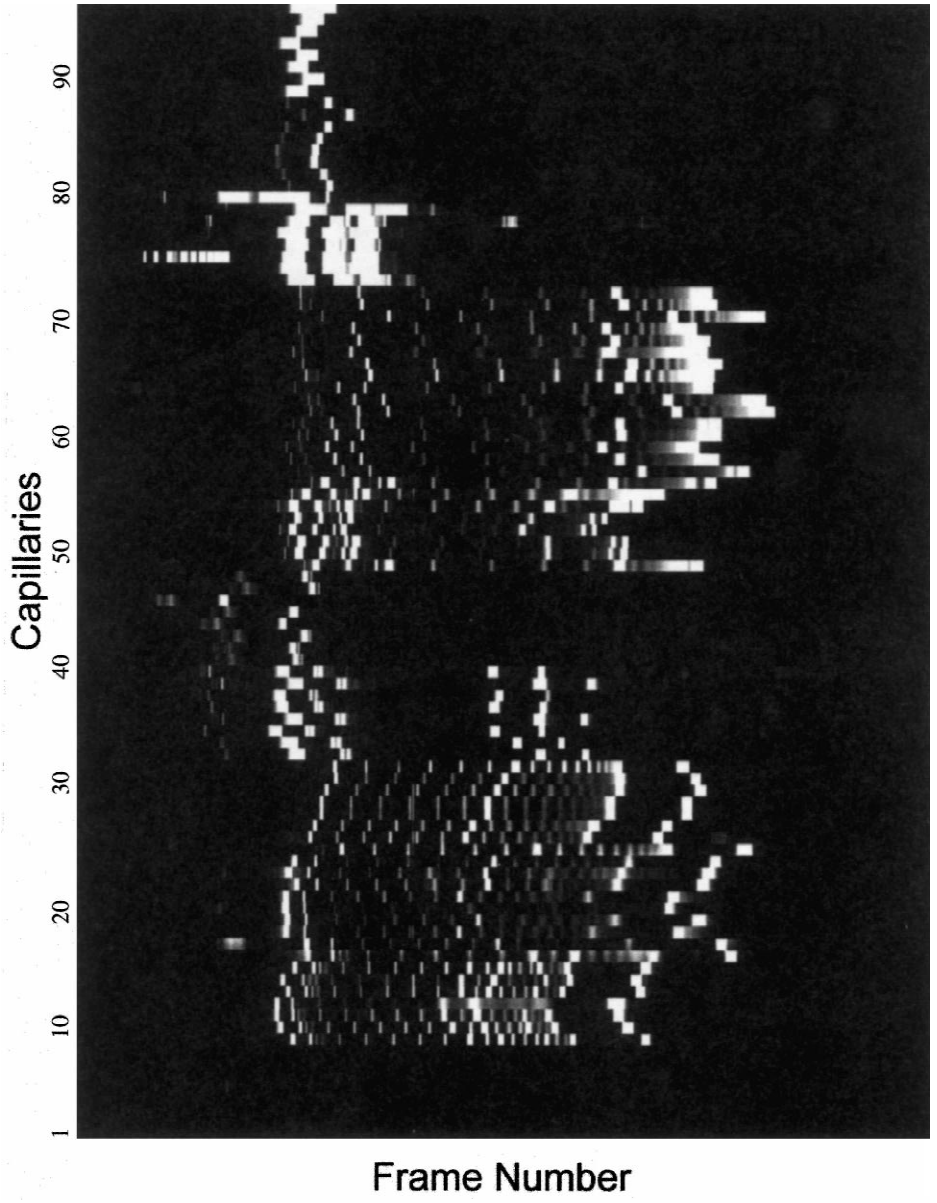

Fig. 1. Reconstructed two-dimensional electropherogram for capillary array electrophoresis. The 96 capillaries are aligned vertically and the migration time direction is from left to right.

to-noise ratios. The migration times and peak intensities were highly non-uniform among the capillaries. This is to be expected from the absence of temperature regulation and variations in the column surfaces. We have demonstrated that an internal standardization scheme can be employed to normalize the results among the capillaries so the migration times and the peak areas are reliable enough for high-throughput applications [38].

Actual electropherograms were extracted from capillaries #3, 9, 17, 25, 33, 41, 49, 57, 65, 73, 85 and 89, to represent each type of sample described in Table 2, and are shown in Fig. 2. In capillaries #9–16 and #17–24, negative and positive HIV-1

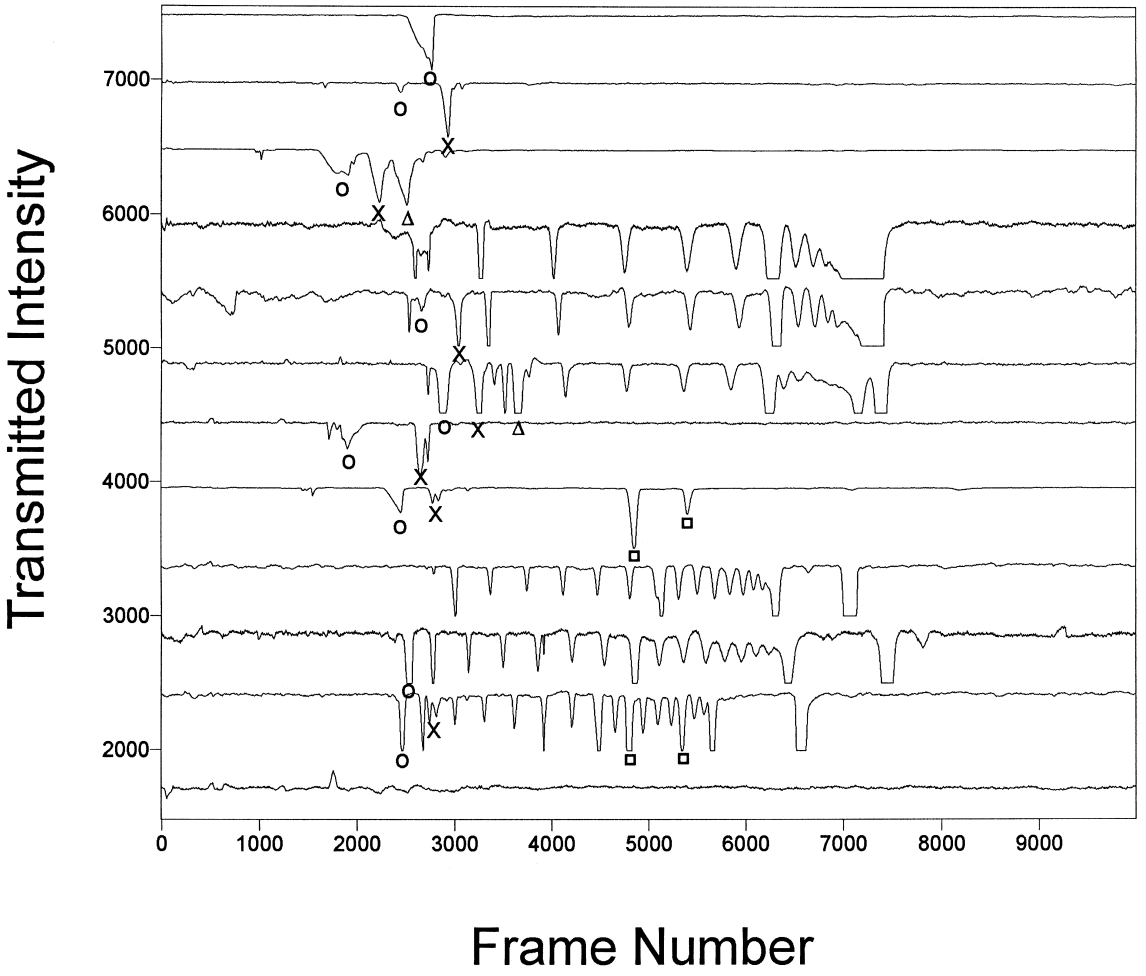

Fig. 2. Selected extracted electropherograms for capillary array electrophoresis. From top to bottom are capillaries #3, 9, 17, 25, 33, 41, 49, 57, 65, 73, 85 and 89. The samples are described in Table 2. ○, primer; ×, primer dimer; △, HIV-1 *gag* PCR product; and □, D1S80 PCR product.

PCR products were injected respectively. The positive and negative results can be easily differentiated through the HIV-1 *gag* fragment peak (triangle) which appeared only in the electropherograms from capillaries #17–24. Both the positive and negative HIV-1 PCR samples also contained the excess primers (circle) and the primer dimers (cross). To provide an even higher level of confidence for identification despite the variation of migration times among capillaries, the HIV-1 *gag* fragments, primers and primer dimers can be sized by mixing the PCR products with 100-bp DNA ladders (capillaries #25–32), and injecting them into capillaries #33–40 and #41–48. The electropherograms from the latter two groups of capillaries showed that the HIV-1 *gag* fragment is about 115 bp and the primer dimer is about 60 bp.

In the electropherograms from capillaries #1–8, the HIV-1 primers gave broad peaks, which we believe are due to sample overloading. Deionized H_2O was injected into capillaries #89–96, which gave blank electropherograms. These electropherograms served as blank references and were subtracted from the signals in the other capillaries to cancel out the flicker noise from the mercury lamp, as reported before [37]. The electropherograms from capillaries

#49–56 showed negative D1S80 PCR results, where only the primer peaks can be observed. The electropherograms from capillaries #57–64 showed positive D1S80 genotyping PCR results. Two component peaks (D1S80 type 18 and 31) can be observed as expected from the heterozygous samples in addition to the very broad primer peaks. Again, to increase the confidence level for identification, the two D1S80 components as well as the primer were roughly sized by mixing each PCR product with a 50-bp ladder (capillaries #65–72) and injecting them into capillaries #73–80 (negative) and #81–88 (positive). The results showed the two D1S80 components to be about 400-bp and 600-bp.

4. Conclusions

We designed DNA analysis protocols to take advantage of high-throughput capillary array gel electrophoresis and simple UV absorption detection based on the inherent spectral properties of the DNA bases. UV absorption detection of DNA products reduces the cost of analysis since it does not require labeling. 96-capillary array electrophoresis analysis of two typical PCR products was demonstrated. Twelve different samples were successfully analyzed simultaneously. The capillary array was flushed with water in between runs and did not show any degradation over tens of runs in a one-month period. Since the sample injection can be fully automated, this work demonstrated that it should be possible to obtain a true DNA analysis throughput that is 100 times (scalable to 1000 times) higher than what commercial single capillary gel electrophoresis systems can achieve, at relatively low cost.

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