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Comparison of slab gel electrophoresis and capillary electrophoresis for the detection of the fluorescently labeled polymerase chain reaction products of short tandem repeat fragments

Dieter L.D. Deforce, Rebecca E.M. Millecamps, David Van Hoofstat, Elfriede G. Van den Eeckhout*

Laboratory for Pharmaceutical Biotechnology, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium

Abstract

The sizing capability of slab gel electrophoresis for short tandem repeat (STR) fragments was compared to the sizing capability of capillary electrophoresis (CE). Both systems used automated laser fluorescence detection to detect four fluorescent dyes, enabling the use of an internal lane standard within each sample. The STR fragments were amplified using a multiplex polymerase chain reaction (PCR) in which the STR fragments Hum CD-4, Hum TH01, Hum D21S11 and Hum SE33 were amplified simultaneously. The reproducibility of the size calling was determined for both systems. The average standard deviation obtained for the slab gel system was 0.2, which was comparable to the standard deviation of 0.12 obtained for the CE system. The CE system produced results comparable to those obtained on the slab gel system, with a level of precision of ±1.0 bp (between instruments). © 1998 Published by Elsevier Science B.V.

Keywords: Polymerase chain reaction; Short tandem repeat fragments; Nucleotides

1. Introduction

Short tandem repeat (STR) sequences are today of major importance in the field of identification of individuals in forensic cases, genomic mapping and genetic linkage analysis [1–5]. It has been estimated that there are approximately 400 million STR loci in the genome [6] and most of these STR sequences show polymorphic alleles that differ in length [7]. The difference in length is caused by a variation in the number of times that a monomer sequence is repeated (for the STR sequences, the monomer length is normally 2–5 bp). Since these STRs can be readily amplified using the polymerase chain reaction (PCR) [8], this technique is very useful in the field of DNA profiling. This technique is more sensitive than conventional techniques, such as restriction fragment length polymorphism (RFLP) [9,10]. This advantage makes them the perfect markers for use in forensic work, where the samples are very often degraded.

For this investigation, four STR systems were evaluated (the Hum CD-4, Hum TH01, Hum D21S11 and Hum SE33 loci) [11]. These STR systems were amplified in a single multiplex PCR [12–15] reaction, this multiplex system was optimized and validated in our laboratory for use in forensic case work. For the validation of the use of this system in forensic case work, a database was produced of the Belgian Caucasian population (these results will be published elsewhere). The method was validated in our laboratory for use on slab gel electrophoresis using automated fluorescence detec-

*Corresponding author.

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tion [11,16]. The system used incorporates automatic sizing of PCR products and eliminates differences in electrophoretic mobility among gel lanes by the inclusion of an internal sizing standard with every sample. One analysis on the slab gel takes a run time of about 14 h, which is independent of the number of samples loaded. Since in forensic cases often only a few samples have to be analyzed, and results are often needed urgently, we were attracted to a capillary electrophoresis system. Recently, a capillary electrophoresis system using laser fluorescence detection became commercially available, offering the same detection capabilities as on the slab gel system. Using the capillary electrophoresis system, the analysis of one sample takes only 32 min, resulting in a much shorter analysis time.

This paper examines the potential to size PCR products on a capillary electrophoresis system using automated fluorescence detection. It also investigates the reproducibility of the results obtained both on the slab gel system and on the CE system. This reproducibility is a measure for the correct attribution of the alleles to the allelic ladder. The precision of the results obtained on both systems was determined by comparing the sizing results, and of the alleles called in comparison to the allelic ladder. This comparison is very important in order to enable a comparison of results obtained by different laboratories [17] and to standardize available procedures.

2. Experimental

2.1. Chemicals

Chelex 100 Resin 100–200 mesh sodium form, biotechnology grade and 40% acrylamide–bisacrylamide solution 19:1 (5% C) were obtained from BioRad (Gent, Belgium). Ethylenediaminetetraacetic acid (EDTA), urea and mineral oil, all of molecular biology grade, were obtained from Sigma (St. Louis, MO, USA). Deionized formamide, of molecular biology grade, was obtained from Eastman Kodak (New Haven, CT, USA). All primers (see Table 1) were synthesized and fluorescently labeled by Perkin Elmer (Netherlands). GeneAmp dNTPs containing the four deoxyribonucleoside triphosphates (dATP, dCTP, dGTP and dTTP) were obtained from Perkin Elmer (Foster City, CA, USA). Gene Amp 10×PCR Buffer II (100 m*M* Tris–HCl, pH 8.3, 500 m*M* KCl) and $MgCl_2$ Solution (25 m*M* $MgCl_2$), AmpliTaq DNA polymerase (DNA deoxynucleotidyltransferase EC 2.7.7.7), all being PCR grade products, were obtained from Perkin Elmer. Genescan 2500 TAMRA (4 n*M* Pst I digestion of phage lambda DNA, ligated with TAMRA-labeled 22-mer oligodeoxynucleotides) and Genescan 500 TAMRA (4 n*M* Pst I digestion of plasmid DNA, ligated with TAMRA-labeled 22-mer oligodeoxynucleotides, and digested with BstU I) internal lane

Table 1
Characteristics of the STR systems studied and the primer sequences employed

System	Repeat unit	Product size (bp)	Primers 5′→3′
CD-4	AAAAG	142–177	TTACGCGTTTGGAGTCGCAAGCTGAACTAGCG (forward) labeled with FAM amidite CCAGGAAGTTGAGGCTGCAGTGAA (backward)
D21S11	TCTA/TCTG	210–242	GTGAGTCAATTCCCCAAG (forward) labeled with FAM amidite GTTGTATTAGTCAATGTTCTCC (backward)
TH01	AATG	179–207	GTGGGCTGAAAAGCTCCCGATTAT (forward) labeled with HEX amidite ATTCAAAGGGTATCTGGGCTCTGG (backward)
SE33	AAAG	231–339	ACATCTCCCCTACCGCTATA (forward) labeled with HEX amidite AATCTGGGCGACAAGAGTGA (backward)

standards were obtained from Perkin Elmer. Performance optimized polymer 4 denatured/8 (POP4) and 10×genetic analyzer buffer containing 1 m*M* EDTA were obtained from Perkin Elmer. HPLC-grade water was obtained from an Elga Maxima Ultrapure Water treatment device. HPLC-grade water, all materials and recipients were autoclaved before use.

2.2. Materials

PCR was performed on a DNA thermal cycler 480 from Perkin Elmer. Slab gel electrophoresis was performed on an ABI 373A DNA sequencer from Applied Biosystems (Perkin Elmer). Collection was performed using the 672 GeneScan Collection software version 1.1 and the data were analyzed using the GeneScan PCR analysis software version 1.2.2-1 (Applied Biosystems). Capillary electrophoresis was performed on an ABI prism 310 genetic analyzer from Applied Biosystems. Collection was performed using the 310 genetic analyzer data collection software version 1.0.2 and the data were analyzed using GeneScan analysis software version 2.0.2 (Applied Biosystems).

2.3. DNA extraction

DNA was extracted from 10 μl of saliva using a slight modification of the Chelex extraction method [18,19]. The saliva samples were obtained from unrelated Caucasians.

2.4. Allelic ladder

An allelic ladder was prepared by pooling DNA extracts with the common alleles for each STR system. These pooled DNA extracts were then subjected to a single locus PCR reaction.

2.5. Multiplex PCR

The DNA extracts were subjected to a multiplex PCR reaction using four primer pairs (see Table 1). Each of the forward primers was labeled with a fluorescent dye marker. To label the primers, two different fluorescent dyes were used, so that neighboring STR systems, some having overlapping alleles (Hum D21S11 and Hum SE 33), were differently labeled. The PCR reaction was performed on 30 μl of the DNA extract using a final concentration of 0.05 μ*M* of each Hum CD-4 primer, 0.27 μ*M* of each Hum D21S11 primer, 0.1 μ*M* of each Hum TH01 primer, 0.11 μ*M* of each Hum SE 33 primer, 22.5 μ*M* of each dNTP, 1.015×Gene Amp PCR buffer II and 2.03 m*M* $MgCl_2$ in a final reaction volume of 44.3 μl. A hot start PCR reaction was performed (adding 1.5 U of AmpliTaq DNA polymerase) followed by 33 cycles at 94°C for 60 s, at 62°C for 60 s and at 72°C for 80 s, followed by a final elongation step at 72°C for 5 min.

2.6. Detection on slab gel electrophoresis

The PCR products were separated and detected on a 42-cm 6% polyacrylamide denaturing (containing 8 *M* urea) slab gel (19:1 acrylamide–bisacrylamide) on the ABI 373 A gene scanner. A 3-μl volume of the PCR reaction was mixed with 0.7 μl of the internal lane standard and 2.5 μl of deionized formamide. Before the samples were loaded on the gel, they were heat denatured (2 min at 90°C). Then 5 μl of the sample was loaded on the gel and electrophoresis was started. Electrophoresis was performed at 1.6 kV for 14 h. The effective separation length for this gel system is 24 cm (from well to detector region). Besides the 14 h required for electrophoresis, another 3 h are required for gel preparation and for loading the samples. When the maximum amount of 24 samples was applied to the gel, this results in a total time of 42.5 min per sample.

2.7. Detection on capillary electrophoresis

Separation on the ABI 310 capillary electrophoresis system was performed using the POP-4 polymer. The composition of the polymer is unknown to us but it is a non-cross-linked polymer, containing urea as a denaturant. It is a viscous fluid, which is removed from the capillary after each sample run.

A 1-μl volume of the PCR reaction was mixed with 0.5 μl of the internal lane standard (GS 500 TAMRA) and 12 μl of deionized formamide. Before the samples were loaded on the autosampler, they were heat denatured (2 min at 90°C) and chilled on ice.

A capillary with a total length of 47 cm was used and the length to the detector was 36 cm. The internal diameter was 50 μm (this capillary was supplied by Perkin Elmer). The same buffer (1× genetic analyzer buffer containing 1 m*M* EDTA) was mounted at both the anode side and at the cathode side. The polymer was injected into the capillary at the anode side using pressure applied by a syringe. The samples were injected electrokinetically at the cathode side, by applying a voltage of 15 kV for 5 s. Electrophoresis was performed at a voltage of 15 kV for 24 min. During electrophoresis, the capillary was kept at a constant temperature of 60°C. The total cycle time for one sample was 32 min.

3. Results and discussion

3.1. Detection on slab gel electrophoresis

In order to determine the reproducibility of the size calling on the slab gel electrophoresis system, a sample was run on ten different gels. These gels were run over a period of one month. The results were obtained using the second order analysis method to calculate the sizes of the PCR products. The following sizes (in base pairs) with standard deviations were observed, for the CD-4 locus, 147.49±0.17; 167.21±0.20; for the TH01 locus, 190.76±0.15; 194.87±0.15; for the D21S11 locus, 209.49±0.20; 225.88±0.22 and for the SE 33 locus, 263.46±0.25; 303.05±0.33. For all alleles, the number of observations was ten. The largest observed difference between the maximum and the minimum length obtained for an allele was 0.94 bp. Since we observed that the alleles differ by at least two base pairs in length (by typing 295 persons), the alleles could always be correctly called in comparison to the allelic ladder. However, when the Local Southern method was used to calculate the sizes, we observed a deviation of up to 10 bp for the results of the larger fragments of the Hum SE33 locus (303–319 bp). When we assume that one fragment (the 361 bp fragment) in the GS-2500 standard shows an abnormality in migration, the second order method will

Table 2
Reproducibility of the size calling using capillary electrophoresis

	Injection number	CD-4	CD-4	TH01	D21S11	SE33	SE33	Age of polymer on system
Capillary 1	59	147.22	167.42	190.46	222.24	268.17	286.63	1 day
	103	147.39	167.62	190.60	222.41	268.10	286.88	1 day
	104	147.32	167.53	190.53	222.23	268.19	286.57	1 day
	105	147.30	167.38	190.48	222.11	268.06	286.53	1 day
	106	147.23	167.40	190.38	222.14	267.80	286.47	1 day
	107	147.13	167.32	190.23	222.03	267.78	286.37	1 day
	108	147.15	167.34	190.36	222.06	267.68	286.39	1 day
Capillary 2	26	147.37	167.50	190.47	222.30	268.06	286.72	1 week
	28	147.15	167.27	190.39	222.07	268.06	286.48	1 week
	30	147.21	167.31	190.31	222.02	267.64	286.41	1 week
	32	147.20	167.30	190.42	222.03	267.66	286.42	1 week
	34	147.17	167.39	190.42	222.05	268.09	286.51	1 day
	36	147.13	167.37	190.29	222.06	268.02	286.45	1 day
	Average	147.23	167.40	190.42	222.13	267.95	286.53	
	Std. dev.	0.09	0.10	0.09	0.12	0.20	0.14	
	Maximum	147.39	167.62	190.60	222.41	268.19	286.88	
	Minimum	147.13	167.27	190.29	222.02	267.64	286.37	
	Difference	0.26	0.35	0.31	0.39	0.55	0.51	

All numbers are expressed as base pairs.
The conditions used are described in the text.

compensate for this error over the whole size calling range, thus spreading the error over the whole range. By spreading this error, the deviation becomes insignificant. The Local Southern method, however, calculates the lengths of the fragments based on the two standard fragments neighboring the unknown fragment. If one of these standard fragments is incorrect, then the calculated length of the fragments in this region will be incorrect due to this local error.

3.2. Detection on capillary electrophoresis

In order to determine the reproducibility of the size calling on the capillary electrophoresis system, a sample was run thirteen times, on two different capillaries. Results were obtained using fresh polymer and using the same polymer after one week. The results were obtained using the second order analysis method to calculate the sizes of the PCR products

Table 3
Accuracy of the results obtained on both systems

	n	ABI 310					ABI 373					373−310
		Average	Std. dev.	Maximum	Minimum	Difference	Average	Std. dev.	Maximum	Minimum	Difference	
CD 4												
142	26	142.25	0.11	142.69	142.14	0.55	141.99	0.25	142.60	141.56	1.04	−0.26
147	16	147.38	0.23	147.96	147.20	0.76	147.01	0.41	148.23	146.52	1.71	−0.37
167	24	167.47	0.05	167.62	167.37	0.25	166.71	0.40	167.63	166.11	1.52	−0.76
TH01												
183	11	182.26	0.19	182.61	182.06	0.55	182.69	0.18	182.97	182.40	0.57	0.44
187	12	186.21	0.15	186.45	186.06	0.39	186.82	0.09	186.98	186.70	0.28	0.61
191	9	190.15	0.05	190.23	190.08	0.15	190.93	0.19	191.35	190.75	0.59	0.78
195	12	194.20	0.11	194.37	194.04	0.33	195.07	0.13	195.38	194.89	0.49	0.86
198	22	197.24	0.06	197.34	197.10	0.24	198.14	0.18	198.71	197.97	0.74	0.90
D21S11												
214	3	213.60	0.09	213.71	213.53	0.18	213.75	0.09	213.85	213.68	0.16	0.15
218	7	217.70	0.06	217.77	217.60	0.17	218.00	0.19	218.40	217.83	0.57	0.30
222	17	221.74	0.10	222.01	221.54	0.47	222.08	0.23	222.61	221.77	0.84	0.33
226	15	225.82	0.11	226.04	225.60	0.44	226.12	0.25	226.64	225.51	1.13	0.29
230	4	230.29	0.10	230.44	230.22	0.22	229.79	0.10	229.94	229.72	0.22	−0.50
232	4	231.83	0.03	231.87	231.80	0.07	232.34	0.18	232.54	232.10	0.44	0.51
234	2	233.98	0.04	234.01	233.95	0.06	234.27	0.01	234.28	234.26	0.02	0.29
236	7	235.99	0.05	236.10	235.93	0.17	236.55	0.29	237.17	236.31	0.86	0.55
SE33												
243	2	243.35	0.08	243.40	243.29	0.11	242.98	0.22	243.13	242.82	0.31	−0.37
247	4	247.56	0.10	247.67	247.45	0.22	247.03	0.34	247.48	246.69	0.79	−0.53
251	4	251.75	0.18	251.90	251.53	0.37	250.82	0.16	251.16	250.82	0.34	−0.93
255	8	255.65	0.09	255.77	255.55	0.22	255.33	0.25	255.72	254.93	0.79	−0.31
259	6	259.94	0.25	260.24	259.61	0.63	259.55	0.22	259.78	259.32	0.46	−0.39
263	5	263.79	0.03	263.84	263.76	0.08	263.67	0.28	264.09	263.39	0.70	−0.12
279	2	278.07	0.14	278.17	277.97	0.20	278.24	0.01	278.24	278.23	0.01	0.16
295	6	294.55	0.26	294.87	294.31	0.56	294.74	0.14	294.87	294.57	0.30	0.19
299	5	298.95	0.26	299.16	298.55	0.61	298.88	0.13	299.01	298.73	0.28	−0.08
303	7	302.68	0.17	303.02	302.53	0.49	302.98	0.24	303.29	302.61	0.68	0.29
307	4	306.72	0.10	306.86	306.62	0.24	307.18	0.31	307.45	306.73	0.71	0.46
311	2	310.90	0.03	310.92	310.88	0.04	311.75	0.20	311.89	311.61	0.28	0.85

ABI 310 is the capillary electrophoresis system and ABI 373 is the slab-gel system.
The first column gives an average length in base pairs obtained on both systems and rounded to an integer, *n* is the number of times this allele was observed.
All values displayed are base pairs.
The last column shows the difference between the average lengths obtained on both systems.

(See Table 2). The largest observed difference between the maximum and the minimum length obtained for an allele was 0.55 bp, which is better than that obtained with the slab gel electrophoresis system. Again, all alleles could confidently be called to the corresponding allele in the allelic ladder. The standard deviation is on average 0.1 better than for the slab gel system described here using the ABI 373, the difference between maximum and minimum length is, on average, 0.3 bp better. The size calling is maintained between capillaries and is not affected by the age of the polymer, at least not when it is changed weekly.

3.3. Precision

In order to determine the precision of both instruments, the fragment lengths obtained on both instruments for 66 samples were compared. Those samples were run on three slab gels, and on two capillaries, with gel age on the system between one day and one week. For both systems, the data were obtained using the second order analysis method. In Table 3, the performance of both systems is compared. The averages were taken of all the alleles with corresponding length, i.e., not by injecting the same sample a number of times but by averaging identical alleles obtained for different samples. Again, the capillary electrophoresis system exhibits a greater reproducibility in size calling than the slab gel system. In order to determine if the data acquired on both systems could be compared, the difference was obtained between the average values obtained on both systems for all alleles. Since this difference was smaller than ± 1 bp, and given that the alleles differ by at least two base pairs, we could conclude that correct sizes were obtained on either system, even without the use of a ladder.

4. Conclusions

When using the POP-4 polymer under the conditions described above, CE is of equal merit as a sizing method for STR fragments as slab-gel electrophoresis. CE, however, offers several advantages over the slab-gel system. First of all, the reproducibility of the data obtained on the CE system is somewhat higher than for the slab-gel system. Furthermore, the equipment to perform CE is less expensive than the slab-gel system and the analysis time is reduced. For a small number of samples, the CE system gives a high gain in time, when five samples have to be analyzed, the run time for the CE would be approximately 2 h 40 min, in contrast to the constant 17 h on the slab-gel system. The CE system also allows a sample to be injected repeatedly, improving the precision of the results. Both of these advantages are very important in forensic case work. It should be stressed that wherever results obtained with two different systems are compared, serious validation for both methods has to be performed. This validation should include the reproducibility, the precision and the reliability of the methods proposed.

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