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Adriano Tagliabracci · Loredana Buscemi
Corrado Sassaroli · Massimo Paoli · Daniele Rodriguez

Allele typing of short tandem repeats by capillary electrophoresis

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Abstract Capillary electrophoresis with laser-induced fluorescence was applied to the analysis of six STRs and the amelogenin sex test with the purpose of verifying accuracy and precision of the sizing method with the GS500 internal standard. Sequenced dye-labeled, PCR-amplified alleles from amelogenin, HumVWA31, HumTH01, HumF13A01, HumFIBRA, D21S11 and HumCSF1PO loci were run several times on the same capillary and on multiple capillaries and the offset of computer-measured fragment sizes from the expected molecular weights was calculated and analysed. All loci except D21S11 showed a poor degree of accuracy. Precision results from run-to-run and day-to-day injections displayed a maximum standard deviation (SD) > 0.15 nt for HumVWA31, HumF13A01, D21S11 and HumFIBRA, although the maximum range of calculated sizes in multiple runs was lower than 1 basepair. No variation in precision was observed according to the quality of the DNA template. Allele typing by comparison with allelic ladders for each locus is recommended.

Key words STRs · Capillary electrophoresis

Introduction

Capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection is a high-resolution and light-sensitive analytical technique recently introduced in DNA analysis [1–4]. Due to the high voltages which may be applied to the thin-walled fused silica capillaries containing the separation medium, CE is more efficient and faster than conventional slab-gel electrophoresis and the labor-intensive and time-consuming procedures intrinsic to slab gel techniques are therefore avoided. Capillary electrophoresis has recently been described as a useful tool in genomic DNA typing of the D1S80 and amelogenin loci [5–7], as well as short tandem repeats (STRs) [8–14]. With respect to early experiments, presently available instruments and software can collect and analyse DNA data and sizing may be achieved by comparing the retention times of forensic samples against molecular weight markers or the allele ladder. The development of multi-wavelength detection technology for CE analysis, as used in automated slab-gel instruments, allows samples and standards to be run and analysed simultaneously, avoiding the lack of precision in sequential injections [15]. The use of different fluorescent dye markers also allows CE to discriminate the PCR products of multiple STRs coamplified in a single multiplex reaction, even from loci with overlapping allele size ranges.

Before introducing this new tool into forensic analysis, it is however necessary to validate the method for the DNA systems used by the scientific community in forensic identification and paternity testing. Several STRs routinely employed in human identification were therefore amplified and submitted to capillary electrophoresis with laser-induced fluorescence to verify the precision, accuracy and efficiency of this technique in DNA sizing analysis.

A. Tagliabracci (✉) · L. Buscemi · C. Sassaroli · M. Paoli
D. Rodriguez
Istituto di Medicina Legale, Università di Ancona,
Policlinico Torrette, I-60020 Ancona, Italy

Materials and methods

DNA was extracted from fresh whole blood according to standard procedures by phenol-chloroform, followed by slot blot quantitation using a specific higher primate probe [16].

Analysis was performed on the HumVWA31 [17], HumTH01 [18], HumF13A01 [19], HumFIBRA [20], D21S11 [21], HumCSF1PO [22] and amelogenin [23] loci, using primers commercially synthesised and labelled from Perkin Elmer/Applied Biosystems (PE/ABD, Foster City, Calif.) (Table 1). Except for HumCSF1PO, these loci have been investigated and extensively applied in forensic casework by the Forensic Science Service (Birmingham, UK) in first and second generation multiplexes [24, 25].

Amplification reactions were carried out on a Perkin Elmer 2400 thermal cycler as either multiplex: HumVWA31 and HumF13A01 (tetraplex HumVWA31, HumTH01, HumFES/FPS, HumF13A01); HumFIBRA, D21S11 and amelogenin (triplex

Table 1 Principal features of STRs and amelogenin sex test employed in this study

Locus	Chromosome location	Repeat sequence	Size range	GenBank access. no.	Dye label
HumVWA31 [17]	12p-12-pter	AGAT	127–167	M25858	JOE
HumTH01 [18]	11p15.5	AATG	169–189	D00269	6-FAM
HumF13A01 [19]	6p24-25	AAAG	181–247	M21986	JOE
HumFIBRA [20]	4q28	AAAG	254–294	M64982	JOE
D21S11 [21]	21q11.2-q21	Complex	211–247	M84567	6-FAM
CSF1PO [22]	5q33.3-34	AGAT	281-317	X14720	JOE
HumAMXA [23]	Xp22.1-p22.3	–	107	M86932	6-FAM
HumAMGY	Yp11.2	–	113	M86933	

HumFIBRA, D21S11 and amelogenin); HumTH01 and HumCSF1P0 (triplex HumTH01, HumTPOX and HumCSF1PO) or singleplex, following the manufacturer's recommendations (Protocols P/N 903223 and 903843 from PE/ABD). PCR amplification was performed using 1–3 ng of genomic DNA in a final volume of 50 µl.

Capillary electrophoresis was carried out on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif.) using 1 µl of the PCR product and 0.5 µl of an internal size standard (GS500) denatured with deionized formamide and samples were run in a 47-cm length capillary (36 cm to detector) with an internal diameter of 50 µm. The capillary was filled with the run buffer Performance Optimized Polymer POP4 (PE/ABD), consisting of linear dimethylacrylamide, 8 M urea, 5% 3-pyrrolydinone and 1 mM EDTA. Samples were electrokinetically injected in 5 s at 15 KV and electrophoresis was carried out for 24 min at 413 Vcm^{-1} and 60 °C.

The length of amplified fragments was established automatically from the internal run standard by the Southern Local method [26] using the manufacturer's software (GeneScan Analysis 2.0.2).

Casework samples

A series of 38 samples involving various kinds of biological evidence collected in our laboratory over a period of 4 months was also considered in this study. Table 2 lists the samples and methods of extraction [27–32]. DNA was tested for quality and quantity by agarose gel and slot blot quantitation. These samples were amplified and submitted to capillary electrophoresis in a daily work session for each singleplex or multiplex and sized as detailed. Allelic ladders were injected at the beginning and end of the work session, to compare forensic samples with allelic ladder allele sizes.

Allelic ladders for the HumVWA31, HumF13A01, D21S11 and HumFIBRA systems made by mixing a series of alleles of known molecular weight, preliminarily sequenced using Taq-Dye-Deoxy-Terminator Cycle Sequencing, or supplied in the Green I triplex (HumTH01, HumTPOX, HumCSF1P0) (PE/ABD) were used for allele designations.

Table 2 Types of biological evidence from casework submitted to analysis

Type	Number	Extraction method
Bloodstains	17	Budowle and Baetchel [27]
Semen stains	7	Budowle and Baetchel [27]
Cigarette butts	4	Hochmeister et al. [28]
Bone	3	Hochmeister et al. [29]
Soft tissues	5	Lassen et al. [30]
Paraffin-embedded tissues	2	Wright and Manos [31] followed by phenolchlorophorm extraction [32]

Taq-cycle-sequencing was performed on both strands using the Taq-Dye-Deoxy-Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, Calif.) in the conditions indicated in the P/N 402078 protocol, with some modifications: 15–20 ng DNA template and half the amount of dye-labeled terminators were used, taken to 20 µl with deionized water. Analysis was carried out by capillary electrophoresis on an ABI Prism 310 (Applied Biosystems). The data were analysed with PE/ABD Sequencing Analysis 3.0 software.

Results

The best sizing method for STRs of forensic interest should be able to identify all alleles of loci submitted to analysis and to give precise sizing results. Since STRs may show alleles differing from each other by up to only 1 bp, the ideal automatic system of sizing should have similar resolution power. The six STRs commonly used in forensic analysis and employed in this study were classified, on the basis of sequencing analysis [33] as simple (HumTH01, HumF13A01, HumCSF1P0), compound (HumVWA31) and complex repeats (D21S11, HumFIBRA). These loci and the amelogenin sex test were chosen because of their current use in forensic science, because they are imperfect tetranucleotide STRs, composed of tetrameric repeat alleles as well as intermediate alleles which differ by 3, 2 or only 1 bp (e.g. TH01 allele 9.3, F13A01 allele 3.2, FIBRA alleles 21.2, 22.2, etc.) and because they cover a broad range of sizing analysis (from 107 bp in the shorter amelogenin allele to 317 bp in the longest HumCSF1PO alleles analysed here).

Like any sizing method, capillary electrophoresis with internal standard GS500 has an inherent measurement error which may cause the sized PCR products not to reflect actual nucleotide sizes. Additionally, the molecular weight values obtained may vary between different instrument platforms or from one run to the next inside the same daily work session, depending on differences in running conditions, e.g. buffer, temperature, electric field, etc. The accuracy of the sizing method may be estimated by calculating how close any given allele is to the actual value, and the measurement reproducibility of the sizing method may be evaluated by running the same allele several times. The latter may be indicated as precision, which is expressed by the standard deviation in the size values obtained for alleles run in several injections.

Table 3 Maximum deviation from true allele size, average precision of sizing, standard deviation (SD) for each locus and maximum range observed for a given allele

Locus	Accuracy bp	Precision[a] (%)	SD (bp)	Maximum range (bp)
Amelogenin	–3.38	99.93	0.089	0.14
HumVWA31	–3.53	99.85	0.202	0.73
HumTH01	–1.75	99.95	0.094	0.30
HumF13A01	–1.72	99.91	0.181	0.83
D21S11	–0.41	99.89	0.237	0.91
HumFIBRA	–4.50	99.95	0.136	0.65
HumCSF1PO	–0.95	99.97	0.076	0.25

[a] Precision calculated formula: % precision = 1– SD/exp × 100, where SD is standard deviation of observed band sizes for a given allele and exp is theoretical allele size

These two parameters were investigated by running the same alleles 3 times consecutively in the same set on a single capillary and 10 times in different sets on multiple capillaries, to verify run-to-run and day-to-day variations in reproducibility results.

Sizing accuracy

CE analysis of the six loci and the amelogenin sex test considered in this study showed poor accuracy in allele sizing using the molecular weight marker GS500. Comparable results were obtained within a set of capillary injections and between sets on multiple capillaries, with a shift between expected allele size and the mean of estimated sizes generally varying by more than 1 bp, with maximum values exceeding 4 bp for HumFIBRA (Table 3). Unlike reports in automated slab-gel analysis with GS500 standard size, where differences between true and observed sizes increased with the allele size of STR loci [34], by CE analysis the results tended to be independent of the molecular weights of alleles.

Sizing precision

Reproducibility in CE analysis with the internal size marker GS500 was very good within the same set of injections. Results were equally good between different sets of injections on multiple capillaries, with a very slight increase in standard deviation values. Performance was independent of the molecular weights of the examined loci, since the best precision was observed for the amelogenin sex test, HumTH01, HumFIBRA and HumCSF1PO loci, which cover a broad size range of alleles submitted to analysis. Instrument precision [35] exceeded 99% on average, ranging from 99.85% for HumVWA31 to 99.97% for HumCSF1PO (Table 3).

Alleles separated from by four repeats showed a standard deviation of less than 0.33 bp (maximum SD observed for allele 17 from HumVWA31 locus). The maximum standard deviation for alleles differing by 2 bp was 0.266 for HumFIBRA 30.2 and 0.066 and 0.113 were the maximum standard deviations for HumTH01 9.3 and HumTH01 10 alleles respectively, separated by only 1 bp. Standard deviation for each locus and maximum range observed for a given allele are reported in Table 3.

Resolution

Alleles 9.3 and 10 from HumTH01 locus, sizing at respectively 188 and 189 bp, displayed computer-generated peaks clearly separated from each other in CE analysis. The resolution power of CE analysis using capillaries filled with polymer POP 4 (ABD) was comparable with the results obtained from slab-gel instruments on standard-length gels [34, 36, 37]. The wear condition of the capillary is a critical parameter for resolution, because the degree of separation decreases as the capillary ages, the resulting peaks from the two alleles being progressively closer to each other, finally fusing in only one peak (Fig. 1).

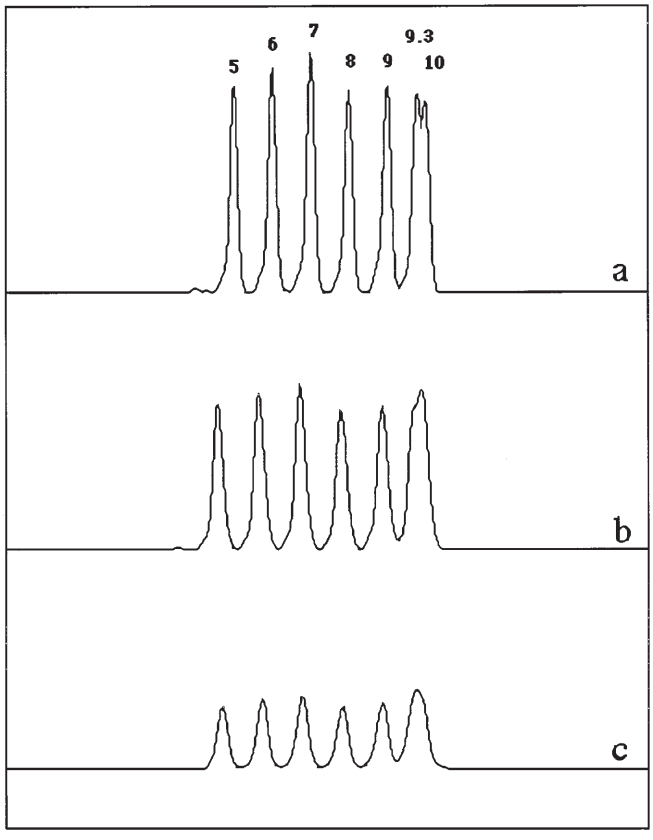

Fig. 1 a–c Resolution power of capillary electrophoresis with ABI Prism 310 and POP4 polymer according to capillary aging. Allele ladder composed of HumTH01 5, 6, 7, 8, 9, 9.3 and 10 alleles was repeatedly run in the same capillary on different days. Ladder sample and buffer were new at each run, without changes in electrical parameters. Electropherogram results on capillary a) just installed, b) after 90 runs, at warranted end of life, c) after 120 runs. Note progressive peak coalescence up to fusion in only one peak in worn capillary between HumTH01 9.3 and 10 alleles (188 and 189 bp respectively)

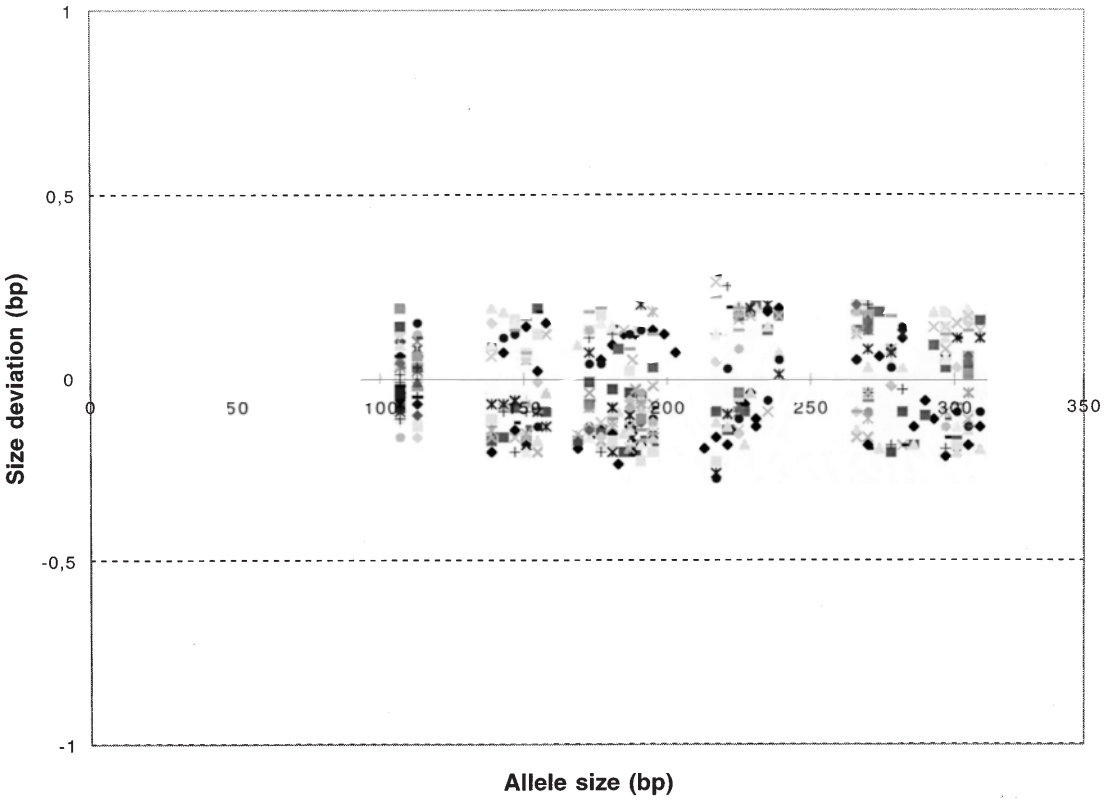

Fig. 2 Sizing precision data of unknown samples of different sources listed in Table 1. y-axis: deviation (in basepairs) of unknown fragment from allelic ladders injected in the same work session. x-axis: each allele in sequenced allelic ladders. Dashed lines: 1 bp range for allele assignment. All unknown fragments, sized by GS500 and represented by different symbols, fall inside the window for nominal allele designation

HumFGA and D21S11 intermediate alleles separated by 2 bp each showed baseline resolution.

Casework analysis

The biological evidence listed in Table 2 was analysed following the same procedures adopted for the experiments. The samples summarized a work period collection of our laboratory. They ranged from bloodstains prepared with fresh blood in paternity cases, to soft and bone tissues from human remains, exposed to the air or buried and aged from a few months to 10 years, in which extracted DNA was heavily degraded. Evidence which was positive for identification analysis routinely performed for loci analysed in this study was considered. Singleplex or multiplex loci were examined in a CE work session for each system. Fragment sizes were calculated from internal standard GS500 and compared with allelic ladder samples injected in the same work session. The difference between each unknown sample allele and the reference allelic ladder (Fig. 2) were inside the bin of only 1 bp discrimination. No variation in allele sizing between fresh blood and aged tissue samples was observed when the DNA templates from various sources were amplified, independently of the degradation status of the sample. Amplification was correctly achieved in the range 0.5–1 ng of DNA template amount. Below this value locus drop-out was observed in multiplex systems. For DNA template amounts below 100 pg, the lack of the largest fragment depending on allelic drop-out was also observed (data not shown).

Discussion

Variations in sizing accuracy mainly depend both on the size standard used [38] and on the STR system studied [35]. Since the Southern Local method [26] of sizing calculates the size of an unknown sample by comparison with the closest standard fragments on either side of it, the molecular weights of the GS500 reference digests are an important factor influencing accuracy. This may explain the overall better and more linear behaviour in sizing observed in our study for loci with alleles in the range very close to the main reference fragments of GS500. However, results obtained in this study for the various loci with alleles sizing in the same range, the lack of correlation in theoretically-measured size differences increasing the molecular weight of alleles and the rough offset in accuracy between adjacent alleles inside the same locus confirmed that the sequence-dependent secondary structures assumed by DNA fragments play an important role in the CE mobility of such molecules [39, 40].

Another factor affecting accuracy and precision, specific for capillary electrophoresis, is the separation matrix used to fill the capillary [11, 41].

Sizing precision of STRs analysed by capillary electrophoresis and the GS500 sizing marker gave similar results to those obtained by the more recent sizing methods suggested for slab-gel techniques [36], the maximum variation in sizing observed for any allele of each locus being within ± 0.5 bp of its observed mean value (Table 3). Lazaruk et al. [11] recently described the results obtained analysing the ten loci belonging to the FISTR Profiler Kit (PE Applied Byosystem) by CE in the same operative conditions applied in this study. Accuracy and precision data reported for HumTH01 and HumCSF1PO loci, amplified using the same primers as in our work, showed almost identical results, with maximum SD < 0.1 bp for both studies. However, these authors obtained better precision results for HumFGA and HumVWA31 loci, but using primers which amplify different regions.

The resolution power of the capillary electrophoresis instrument used here was capable of separating fragments differing by only 1 bp (Fig. 1). The state of wear of the capillary is critical for single basepair resolution because progressive broadening of the peak occurs near the end of column life and the final fusion of the two adjacent peaks does not distinguish alleles differing by 1 bp. Formamide is responsible for the wear condition of capillaries and Buel at al. [42] using formamide deionized with various mixed bed resins, improved the resolution and life of columns significantly.

The resolution power of capillary electrophoresis and the standard deviations from allele mean values measured for the STR loci into this analysis indicate the possibility of discriminating between any allele of each locus. Taking into account that rare variant alleles differing by only 1 bp may occur for any locus, the reliable statistical criterion for unambiguous typing represented by a mean size difference of ± 3 SD defines non-overlapping windows only for the amelogenin, HumTH01 and HumCSF1PO loci. The maximum standard deviation found in this study was > 0.15 nt for the HumVWA31, HumF13A01, D21S11 and HumFIBRA loci, although for the latter three only one allele exceeded this statistical threshold. However these values are higher than the reasonable upper limits for confident discrimination between alleles differing by only 1 bp. Nevertheless, the maximum range of calculated size was lower than 1 bp for any allele in multiple runs performed over several days, with extreme values obtained for the D21S11 locus (0.91 bp) and the amelogenin test (0.14 bp) (Table 3).

Although capillary electrophoresis with the GS500 size marker is a reliable method of sizing STR loci, the considerable shift between expected and observed allele sizes for some loci preventing automatic conversion of the measured DNA fragment to the nominal allele. Interlaboratory differences in the analytical system used and day-to-day laboratory variations due to electrophoresis conditions – polymer and buffer concentration, temperature, field, etc. – further affect fragment sizing and indicate that this approach to allele assignment should not be used. Genotype assignment to measured sample fragments should therefore be reached by comparison with an allelic ladder, injected at the beginning and end of every set of samples run, as suggested by the manufacturer. This procedure, which allows visual genotype designation by overlap of computer-generated peaks of sample and allelic ladder (Fig. 3) is recommended for unambiguous typing.

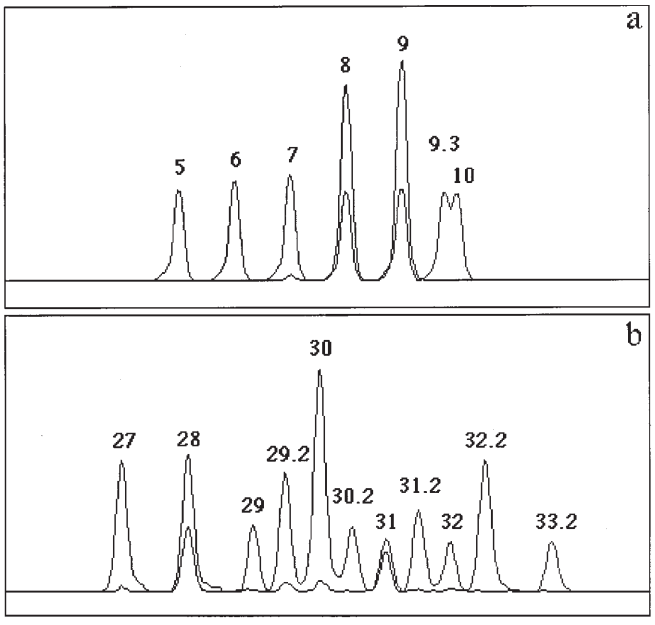

Fig. 3 a, b Visual allele designation of unknown sample by profile overlap with a) HumTH01 ladder composed of 5, 6, 7, 8, 9, 9.3 and 10 alleles, b) D21S11 ladder composed of 27, 28, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2 and 33.2 alleles. Resulting profile of unknown sample was HumTH01 8–9 and D21S11 28–31 heterozygous

CE analysis performed in routine forensic casework showed no variation in precision sizing depending on degradation or amount of DNA template. When the optimal range of 0.5–1 ng of sufficient molecular weight DNA template was used, fresh and highly degraded evidence showed the same performance. The resulting fragment sizes were generally very close to the corresponding alleles of ladder samples used for allele typing, allowing unambiguous designations. According to previous reports [43, 44], a locus or allele drop-out phenomenon was observed when amplifying low copy numbers of DNA template, depending on unequal amplification resulting from a stochastic effect. These observations indicate that amplification is the critical point of analysis because when amplified, DNA samples of different quality and quantity are not able to influence capillary electrophoresis precision in fragment sizing.

In conclusion, capillary electrophoresis with laser-induced fluorescence detection is a robust technique for STR analysis, and the GS500 internal standard method permits reproducible sizing of fragments and identification of alleles differing from each other by up to only 1 bp can be easily achieved by comparison with allelic ladders at each particular locus.

References

1. Karger BL (1989) High-performance capillary electrophoresis. Nature 339:641–642
2. Schwartz HE, Ulfelder K, Sunzeri FJ, Busch MP, Brownlee RG (1991) Analysis of DNA restriction fragments and polymerase chain reaction products towards detection of the AIDS (HIV-1) virus in blood. J Chromatogr 559:267–283
3. Zhang JZ, Chen DY, Wu S, Harke HR, Dovichi NJ (1991) High-sensitivity laser-induced fluorescence detection for capillary electrophoresis. Clin Chem 37:1492–1496
4. Landers JP, Oda RP, Spellsberg TC, Nolan JA, Ulfelder KJ (1993) Capillary electrophoresis: a powerful microanalytical technique for biologically active molecules. Biotechniques 14:98–111
5. Isenberg AR, McCord B, Koons BW, Budowle B, Allen RO (1996) DNA typing of a polymerase chain reaction amplified D1S80/amelogenin multiplex using capillary electrophoresis and a mixed entangled polymer matrix. Electrophoresis 17:1505–1511
6. Mitchell JW, Walsh D (1998) Analysis of the D1S80 locus by capillary electrophoresis. Electrophoresis 19:81–85
7. Pouchkarev VP, Shved EF, Novikov PI (1998) Sex determination of forensic samples by polymerase chain reaction of the amelogenin gene and analysis by capillary electrophoresis with polymer matrix. Electrophoresis 19:76–79
8. Mansfield ES, Robertson JM, Vainer M, Isenberg AR, Frazier RR, Ferguson K, Chow S, Harris DW, Barker DL, Gill PD, Budowle B, McCord BR (1998) Analysis of multiplexed short tandem repeat (STR) systems using capillary array electrophoresis. Electrophoresis 19:101–107
9. Holland MM, Turni LA, DelRio S, Marino M, Lofts RS, Fisher DL, Ross J, Schumm JW, Williams PL (1994) Typing human DNA using capillary electrophoresis: comparison of slab gel and capillary formats. In: Bär W, Fiori A, Rossi U (eds) Advances in forensic haemogenetics 5. Springer, Berlin Heidelberg New York, pp 156–159
10. Kloosterman AD, Schans van der MJ, Janssen HJT, Everaerts FM (1996) Rapid sex-typing by fluorescent based PCR of the X-Y homologous Amelogenin gene and analysis by CGE. In: Carracedo A, Brinkmann B, Bär W (eds) Advances in forensic haemogenetics 6. Springer, Berlin Heidelberg New York, pp 343–345
11. Lazaruk K, Walsh PS, Oaks F, Gilbert D, Rosenblum BB, Menchen S, Schoibler D, Wenz HM, Holt C, Wallin J (1998) Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. Electrophoresis 19:86–93
12. Isenberg AR, Allen RO, Keys KM, Smerick JB, Budowle B, McCord BR (1998) Analysis of two multiplexed short tandem repeat systems using capillary electrophoresis with multiwavelength fluorescence detection. Electrophoresis 19:94–100
13. Dimo-Simonin N, Grange F, Kratzer A, Brandt-Casadevall C, Mangin P (1998) Forensic validation of the short tandem repeat HUMACTBP2 using capillary electrophoresis. Electrophoresis 19:256–261
14. Van Hoofstat DEO, Deforce DLD, Millecamps REM, Brochez VM, Van Geldre EGL, Vergauwe A, Sijs A, Van den Eeckhout EG (1998) Population study of four short tandem repeat loci in the Belgian population, using capillary electrophoresis. Electrophoresis 19:719–722
15. Zhang N, Yeung ES (1996) Genetic typing by capillary electrophoresis with the allelic ladder as an absolute standard. Anal Chem 68:2927–2931
16. Waye JS, Presley L, Budowle B, Shutler GG, Fourney RM (1989) A simple method for quantifying human genomic DNA in forensic specimen extracts. Biotechniques 7:852–855
17. Kimpton C, Walton A, Gill P (1992) A further tetranucleotide repeat polymorphism in the vWF gene. Hum Mol Genet 1:287
18. Polymeropoulos MH, Xiao H, Rath DS, Merril CR (1991) Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (TH). Nucleic Acids Res 19:3753
19. Polymeropoulos MH, Xiao H, Rath DS, Merril CR (1991) Tetranucleotide repeat polymorphism at the human coagulation factor XIII A subunit gene (F13A1). Nucleic Acids Res 19:4036
20. Mills KA, Even D, Murray JC (1992) Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Hum Mol Genet 1:779
21. Sharma V, Litt M (1992) Tetranucleotide repeat polymorphism at the D21S11 locus. Hum Mol Genet 1:67
22. Hammond HA, Jin L, Zhong Y, Caskey CT, Chakraborty R (1994) Evaluation of thirteen STR loci for use in personal identification applications. Am J Hum Genet 55:175–189
23. Mannucci A, Sullivan KM, Ivanov PL, Gill P (1994) Forensic application of a rapid and quantitative DNA sex test by amplification of the X-Y homologous gene amelogenin. Int J Legal Med 106:190–193
24. Kimpton CP, Gill P, Walton A, Urquhart A, Millican FS, Adams M (1993) Automated DNA profiling employing "multiplex" amplification of short tandem repeat loci. PCR Methods Appl 3:13–22
25. Oldroyd NJ, Urquart AJ, Kimpton CP, Millican ES, Watson SK, Downes T, Gill PD (1995) A highly discriminating octoplex short tandem repeat polymerase chain reaction system suitable for human individual identification. Electrophoresis 16:334–337
26. Elder JK, Southern EM (1987) Computer-aided analysis of one-dimensional restriction fragment gels. In: Bishop MG, Rawlings (eds) Nucleic acid and protein sequence analysis. A practical approach. IRL Press, Oxford, pp 165–172
27. Budowle B, Baechtel FS (1990) Modifications to improve the effectiveness of restriction fragment length polymorphism typing. Appl Theor Electrophoresis 1:181–187
28. Hochmeister MN, Budowle B, Jung J, Borer UV, Comey CT, Dirnhofer R (1991) PCR-based typing of DNA extracted from cigarette butts. Int J Legal Med 104:229–233
29. Hochmeister MN, Budowle B, Borer UV, Comey CT, Dirnhofer R (1991) Typing of deoxyribonucleic acid (DNA) extracted from compact bone from human remains. J Forensic Sci 36:1649–1661
30. Lassen C, Hummel S, Herrmann B (1994) Comparison of DNA extraction and amplification from ancient human bone and mummified soft tissue. Int J Legal Med 107:152–155
31. Wright DK, Manos MM (1997) Sample preparation from paraffin-embedded tissues. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols. A guide to methods and applications. Academic Press, San Diego, pp 153–158
32. Romero RL, Juston AC, Ballantyne J, Henry BE (1997) The applicability of formalin-fixed and formalin fixed paraffin embedded tissues in forensic DNA analysis. J Forensic Sci 42:708–714
33. Urquhart A, Kimpton CP, Downes TJ, Gill P (1994) Variation in short tandem repeat sequences – a survey of twelve microsatellite loci for use as forensic identification markers. Int J Legal Med 107:13–20
34. Dupuy BM, Olaisen B (1997) A dedicated internal standard in fragment length analysis of hyperpolymorphic short tandem repeats. Forensic Sci Int 86:207–227
35. Sullivan KM, Pope S, Gill P, Robertson JM (1992) Automated DNA profiling by fluorescent labeling of PCR products. PCR Methods Appl 2:34–40
36. Frazier RR, Millican ES, Watson SK, Oldroyd NJ, Sparke RL, Taylor KM, Panchal S, Bark L, Kimpton CP, Gill PD (1996) Validation of the Applied Biosystem Prism 377 automated sequencer for the forensic short tandem repeat analysis. Electrophoresis 17:1550–1552

37. Moscetti A, Boschi, I, Dobosz M, Destro-Bisol G, Pescarmona M, d'Aloja E, Pascali VL (1995) Fluorescence-based classification of microsatellites using a single-wavenlength semiautomatic sequencer: genotype assignment and identity tests by analysis of comigrating peak profiles. Electrophoresis 16: 1875–1880
38. Kline MC, Duewer DL, Newall P, Redman JW, Reeder DJ, Richard M (1997) Interlaboratory evaluation of short tandem repeat triplex CTT. J Forensic Sci 42: 897–906
39. Konrad KD, Pentoney SL Jr (1993) Contribution of secondary structure to DNA mobility in capillary gels. Electrophoresis 14: 502–508
40. Chastain PD, Eichler EE, Kang S, Nelson DL, Levene SD, Sinden RR (1995) Anomalous rapid electrophoretic mobility of DNA containing triplet repeats associated with human disease genes. Biochemistry 34: 16125–16131
41. Vainer M, Enad S, Dlonik V, Xu D, Bashkin J, Marsh M, Tu O, Harris DW, Barker DL, Mansfield ES (1997) Short tandem repeat typing by capillary electrophoresis: comparison of sizing accuracy and precision using different buffer systems. Genomics 41: 1–9
42. Buel E, Schwartz MB, LaFountain MJ (1998) Capillary electrophoresis STR analysis: comparison to gel-based systems. J Forensic Sci 43(1): 164–170
43. Kimpton C, Fisher D, Watson S, Adams M, Urquhart A (1994) Evaluation of an automated DNA profiling system employing amplification of four tetrameric STR loci. Int J Legal Med 106: 302–311
44. Lygo JE, Johnson PE, Holdaway DJ, Woodroffe S, Whitaker JP, Clayton TM, Kimpton CP, Gill P (1994) The validation of short tandem repeat (STR) loci for use in forensic casework. Int J Legal Med 107: 77–89