TECHNICAL NOTE

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Capillary Electrophoresis STR Analysis: Comparison to Gel-Based Systems

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ABSTRACT: Capillary electrophoresis is a relatively new technology for the forensic analysis of STRs, and may be an excellent alternative to traditional gel-based methods. Eighty samples, obtained from six different laboratories which had previously analyzed the samples using a variety of gel-based methods, were reanalyzed by CE. It was determined that CE could obtain the same allelic designation as that reported by the comparison laboratory. Seven different loci employing two separate multiplex amplifications were used for the comparison study. One basepair resolution and average standard deviations within the range of 0.075 to 0.117 basepairs were observed.

KEYWORDS: forensic science, DNA typing, capillary electrophoresis, short tandem repeat, D3S1358, HUMvWFA31(vWA), FGA, CSF1PO, HUMTHO1, HUMTPOX, Amelogenin

The individualization of biological materials using the polymerase chain reaction (PCR) has become an important analytical tool for forensic laboratories. One application of PCR is the amplification and subsequent analysis of short tandem repeats or STRs. Profiling a number of these polymorphic DNA regions scattered throughout the human genome can provide excellent individualization of evidence samples (1,2).

Of the many existing choices for the post-amplification analysis of STRs, the most widely-used methods are all gel-based. Electrophoretic gels are treated in a number of ways to allow interpretation of amplified STR results. Gels can be silver or fluorescently stained for visual evaluation or for scanning with a gel imager. Alternatively, STRs can be amplified using fluorescently tagged primers and evaluated in real time as they migrate within the gel, or analyzed after electrophoresis using an imager as above (3,4). An alternative to gel-based analysis of STRs is capillary electrophoresis (5,6). This separation technology employs the basic principles of conventional electrophoresis within fused silica capillaries. A liquid polymer matrix replaces conventional gel slabs. Semi-automated, this technology allows the rapid separation and characterization of DNA and other molecules. The application of capillary electrophoresis (CE) to forensic DNA analysis is a recent practice and so must be thoroughly investigated before it can be applied to actual forensic casework. In this work we compare the STR results obtained by forensic laboratories performing gel-based analyses to our results on the same samples using CE. These comparisons were made by evaluating seven STR loci: D3S1358, HUMVWFA31(vWA), FGA, CSF1PO, HUMTHO1, HUMTPOX, and amelogenin contained on chromosomes 3, 12, 4, 5, 11, 2 and the X/Y chromosome respectively (7–13). The first three STR loci, D3S1358, vWA and FGA, can be co-amplified using a commercially available kit AmpFISTR Blue (PE Applied Biosystems, Foster City, CA) while the next three STR loci can be co-amplified using a commercially available GenePrint CTT kit (Promega Corp., Madison, WI). The CTT loci can also be co-amplified with the amelogenin locus using the AmpFISTR Green I kit (PE Applied Biosystems).

Materials and Methods

Samples were obtained from six laboratories for the comparison study. Each laboratory provided between 5 to 20 samples that they had previously analyzed. Table 1 lists the participating laboratories and the analytical systems used to generate their STR results. The samples consisted of blood patched on cotton cloth or paper, mouth swabs collected on cotton swabs, or extracted DNA from blood, hair, or abortus tissue. Samples requiring extraction were subjected to proteinase K (Life Technologies, Gaithersburg, MD) digestion with subsequent extraction with phenol/chloroform with the aqueous extract subjected to Microcon-100 (Amicon Inc., Beverly, MA) dialysis (14). Samples were quantitated by mini-gel electrophoresis employing the DNA dye DAPI (15). For low concentration extracts that fell below the 4 to 8 ng detectable level for DAPI, the mini-gel was placed in a gel buffer solution containing a 1:10,000 dilution of SYBR Green I (Molecular Probes Inc, Eugene, Oregon) and gently rocked for twenty minutes. DNA was visualized using UV light and evaluated by comparison of relative fluorescence with a series of human concentration standards ranging from 125 to 0.12 ng per lane. Sensitivity of this SYBR Green I technique was approximately 0.25 ng.

In our laboratory, amplifications were performed using the AmpFISTR Blue and AmpFISTR Green I kits generously provided by

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TABLE 1—*Laboratory analytical systems.*

Laboratory	Systems Assessed	Gel System	Visualization	Detection/Evaluation
Center for Blood Research	Geneprint CTT	4.0% denaturing gel w/ 7.0M urea, 32cm well to bottom of plate	Silver stain	Visual inspection
North Carolina State Bureau of Investigation	Geneprint CTT	6.0% denaturing gel w/ 8.0M urea, 32cm well to bottom of plate	SYBR Green I	Molecular Dynamics gel scanner, Fluorimager
Center of Forensic Sciences, Toronto	AmpFISTR Blue	6.0% denaturing gel w/ 7.0M urea, 36cm well to read distance	Fluorescently tagged primers	ABI PRISM 377 DNA Sequencer
Armed Forces DNA Identification Laboratory (AFDIL)	Geneprint CTTv AmpFISTR Blue	6.5% denaturing gel w/ 7.5M urea, 12cm well to read distance	Fluorescently tagged primers	ABD 373A Gene Sequencer
National Institute of Standards and Technology (NIST)	CTT	*	*	*
Suffolk County Crime Laboratory	AmpFISTR Blue	6% denaturing gel w/ 8.3 M urea, 24cm well to read distance	Fluorescently tagged primers	ABD 373A Gene Sequencer

*NIST data represents consensus data from 33 laboratories using a variety of protocols, none included capillary electrophoresis.
Molecular Dynamics (Sunnyvale, CA).
ABD: Applied Biosystems Division (Perkin-Elmer).

PE Applied Biosystems. The extracts were diluted to approximately 0.2 ng/μL, and 10 μL amplified according to the recommendations of the manufacturer with a single exception—the volume of each component was reduced by half yielding a total reaction volume of 25 μL. The amplifications were performed using a GeneAmp PCR System 9600 thermocycler (PE Applied Biosystems) using the following conditions:

AmpFISTR Blue: 95°C for 11 min then: 28 cycles at: 94°C for 1 min, 59°C for 1 min, 72°C for 1 min, followed by a 30 min hold at 60°C.

These same conditions with one additional cycle for a total of 29, were used for the AmpFISTR Green I amplifications.

The capillary electrophoresis unit employed to evaluate the STR amplicons was an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems). An uncoated 50 μm ID capillary (Perkin-Elmer), 47 cm in length with a 37 cm read length (distance from injection end to laser), was used for the separation. The separation medium was the proprietary polymer product POP4 (PE Applied Biosystems). The run buffer was a 1:10 dilution of 310 Genetic Analyzer Buffer with EDTA (PE Applied Biosystems). Samples were routinely electrokinetically injected at 15 kV for 5 s, followed by a 24 min run at a constant voltage of 15 kV and a constant temperature of 60°C. Prior to each injection the column was back flushed with new polymer by the instrument. Amplified STR products were prepared for CE analysis, as were allelic ladder samples provided with the kits, by mixing 1 μL of the product with 1 μL of GeneScan −350 ROX internal lane standard (PE Applied Biosystems) and 24 μL of deionized formamide. Formamide (Amresco, Solon, OH) was deionized by using a molecular grade mixed bed resin (Sigma, St. Louis, MO). A mixed bed resin from another supplier was inadequate for this process. The sample mixture was then denatured by heating at 95°C for 3 min, then snap cooled for an additional three minutes in an ice water bath. All denatured samples were loaded into the instrument autosampler and the analysis was allowed to proceed. The results were analyzed with ABI PRISM GeneScan Software (Version 2.1) and Genotyper (Version 1.1.1), both from PE Applied Biosystems.

Sizing precision studies were conducted through the analysis of the STR allelic ladders supplied with the AmpFISTR Blue and Green I kits. These studies were conducted after instrument and sample preparation conditions were optimized to yield baseline resolution of the two basepair variant within the FGA locus of the AmpFISTR Blue ladder, and resolved a one basepair variant within the THO1 locus of AmpFISTR Green I ladder. Over 60 separate injections were made of each ladder on at least two different columns with the columns averaging over 200 runs. Standard deviation measurements, average base pair size and range of measurements were calculated for each allele. Each set of CE runs, which averaged between 10 and 15 samples, included allelic ladder run as the first and last sample of each set.

TABLE 2—*Sizing precision results.*

Locus	Average SD Deviation in Base Pairs	Maximum SD Deviation in Base Pairs	Average Range in Base Pairs	Maximum Range in Base Pairs
Amelogenin	0.089	0.089	0.435	0.44
THO1	0.084	0.098	0.413	0.60
TPOX	0.075	0.084	0.20	0.27
CSF1PO	0.089	0.125	0.41	0.63
D3S1358	0.088	0.096	0.536	0.84
vWA	0.113	0.129	0.535	0.63
FGA	0.117	0.131	0.539	0.64

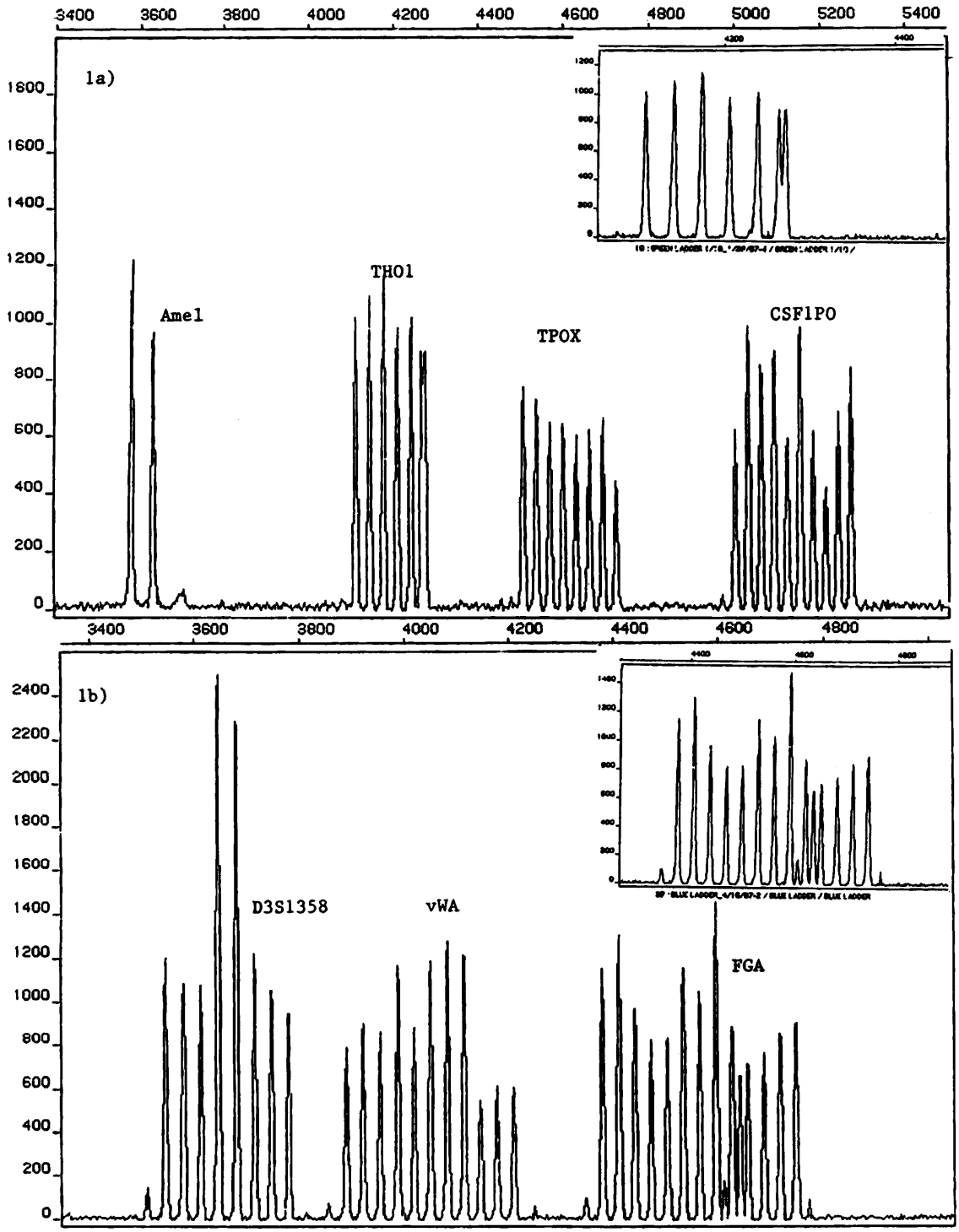

FIG. 1—*Electrophoretograms of AmpFISTR Green I (1a), AmpFISTR Blue (1b) ladders and a representative sample (1c) run using the AmpFISTR Blue system. The horizontal scale represents scan number, the vertical scale indicates relative fluorescence units. The insert in the corner of 1a is an enlargement of the THO1 portion of the ladder demonstrating resolution of the alleles differing by one basepair. The insert in the corner of 1b is an enlargement of the FGA portion demonstrating the two basepair separation. The representative sample contains an off ladder variant that was detectable with CE.*

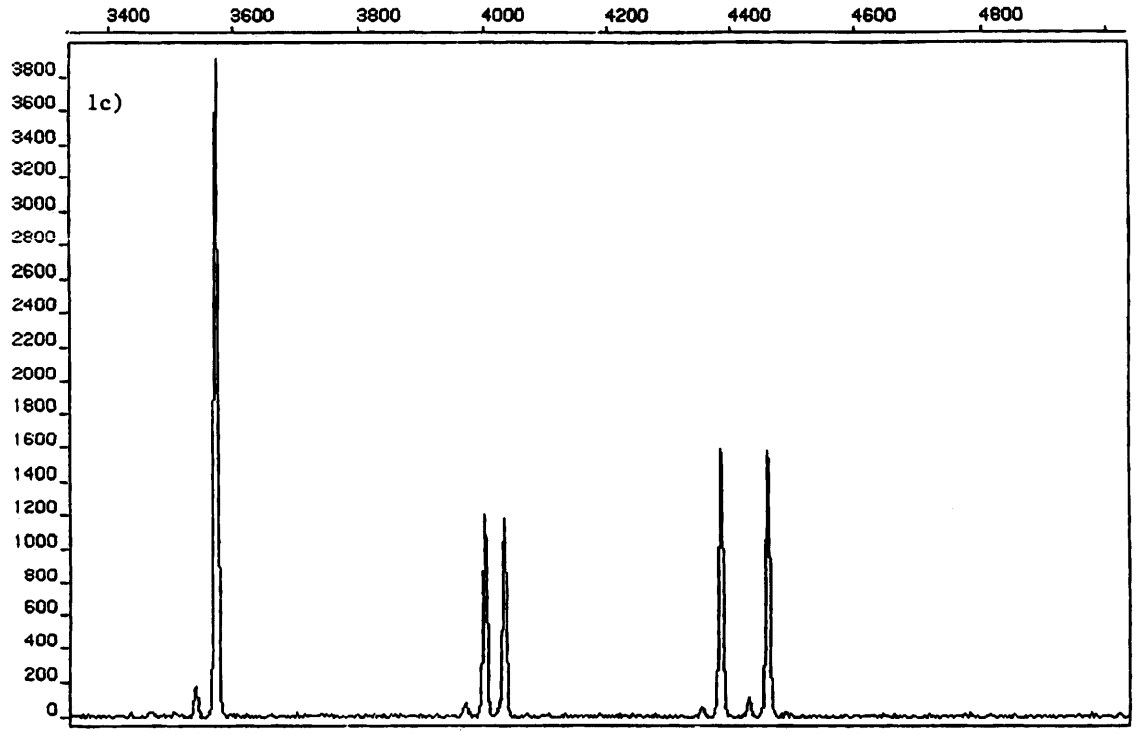

FIG. 1—*Continued.*

Results

Many electrophoretic systems are sensitive to base pair composition or other factors such that base pair size estimates may differ between systems. Since a given fragment will migrate consistently within a system, laboratories can compare their STR analyses to those obtained from other laboratories if standards, such as allelic ladders, are run to assess the migration of the alleles. The use of allelic ladders allow laboratories to make allelic determinations of STR amplicons and compare their allelic calls to those of other laboratories despite the base pair size variations that occur between different analytical systems. In the STR loci examined, differences in alleles are based on differing numbers of 4 base pair repeat units, with some loci displaying variants within this normal 4 base pair repeat. The allelic designation is based upon the number of repeats contained within it and the number assigned to that allele generally represents the number of repeats. The allelic ladders are designed to capture the major alleles and are defined within this nomenclature. Based on our results of the allelic ladder sizing studies conducted using the AmpFISTR Blue and Green I ladders, we obtained average base pair values for the alleles within these ladders (data not shown) which were used to determine precision (Table 2). These values were used to assess the repeatability of the instrument, assign basepair ranges in the typing software and make allelic determinations of the samples supplied by the participating laboratories.

Band size data from the results of the analyses of the comparison samples were compared to the data obtained from the sizing study and allelic determinations made with the Genotyper software. The allele designations obtained using CE were identical to those detailed by the contributing laboratory in all of the comparison samples that yielded sufficient amplified product for analysis. Figure 1 demonstrates resolution of both AmpFISTR Blue and Green I ladders and a representative sample from the comparison samples. All show the typical baseline resolution and balanced amplification that is routinely been observed with CE analysis in our study. Five samples yielded either incomplete or no STR profiles. Reamplification was attempted on these samples, but when that failed, further efforts to achieve an amplification were not pursued. Over all, 80 samples yielded typeable product, providing 532 allelic identifications. The allele designations made using the CE system were the same as those reported by the submitting laboratories and are summarized in Table 3.

Obtaining good resolution of STR fragments differing by one or two base pairs was essential in determining the allelic designations of the comparison samples and for assessing the usefulness of the CE unit. The 9.3 and 10 alleles of THO1, differing by one base pair, were well resolved using the CE. However we found that the quality of the formamide used to denature the samples was critical in achieving good resolution of these closely spaced alleles. Figure 2 focuses on the THO1 9.3 and 10 alleles from two CE runs of the AmpFISTR Green I ladder prepared in formamide deionized with different mixed bed resins. The poorly resolved peaks in Fig. 2a show clear resolution after a different resin was used to treat the formamide (Fig. 2b). These runs were performed on the same column one day apart. This improved resolution of the system allowed for the appropriate designation of the one base pair variant “9.3” in the THO1 system. The change in the resin used to treat the formamide has also increased the number of runs that can be obtained from a capillary column. Prior to switching to the new mixed bed resin we routinely had a column life of only 85 to 90 runs, but now have realized a column life over 200 runs, with one column exceeding 350 runs with no loss in resolution.

Discussion

With any new technology that may be used to replace or supplement an existing one, it is important to establish that the technology

TABLE 3—*Experimental determined genotypes of the comparison samples.*

Laboratory	D3S1358	vWA	FGA	THO1	TPOX	CSF1PO	Amelogenin
Center for Blood Research				9.3, 9.3	9, 11	10, 11	
				8, 9.3	8, 12	10, 13	
				8, 9.3	8, 8	10, 12	
				6, 9.3	10, 12	11, 13	
				7, 9.3	9, 11	10, 11	
				7, 9.3	8, 11	10, 11	
				7, 9.3	8, 11	10, 11	
				6, 9.3	8, 11	10, 13	
				7, 9.3	8, 9	10, 13	
				6, 6	8, 8	10, 12	
				7, 9.3	8, 9	10, 13	
				6, 6	8, 8	10, 12	
				9.3, 9.3	9, 11	10, 11	
				9.3, 9.3	9, 11	ND	
				ND	ND	ND	
				9.3, 9.3	9, 11	ND	
				9.3, 9.3	9, 11	10, 11	
North Carolina State Bureau of Identification				7, 9.3	11, 11	10, 11	
				7, 9.3	8, 8	11, 12	
				9, 9.3	8, 8	13, 13	
				6, 7	8, 8	10, 12	
				9, 9.3	8, 10	10, 12	
				7, 8	8, 10	11, 11	
				8, 9.3	8, 11	10, 12	
				6, 9.3	11, 12	11, 12	
				7, 9.3	8, 10	11, 11	
				7, 9.3	8, 8	10, 12	
				6, 6	8, 11	11, 12	
				8, 9.3	11, 12	11, 13	
				7, 9	8, 8	11, 11	
				6, 9.3	12, 12	10, 12	
				7, 9.3	8, 8	10, 11	
				9, 9.3	8, 11	10, 11	
AFDIL	16, 17	18, 19	19, 23				
	15, 16	14, 17	22, 24	6, 7	11, 11	12, 13	XY
	14, 16	13, 15	23, 23				
	16, 16	15, 19	21, 23				
	15, 18	17, 18	20, 24				
	16, 17	16, 17	22, 22	9.3, 9.3	8, 11	8, 8	XX
	16, 17	15, 17	20, 26	ND	ND	ND	ND
	15, 17	15, 17	20, 25				
	15, 17	17, 17	21, 24	9, 9.3	8, 8	11, 11	XY
	18, 18	17, 18	20, 24				
	16, 16	17, 17	21, 22				
	17, 17	16, 17	20, 20	7, 9.3	8, 11	10, 13	XX
	16, 17	17, 17	21, 21	8, 10	8, 8	9, 11	XY
	16, 16	17, 18	19, 23				
	14, 18	17, 17	22, 22	9, 9.3	8, 8	12, 12	XX
	15, 15	15, 16	22, 24	9.3, 9.3	8, 11	11, 13	XY
	14, 15	17, 18	23, 24	8, 9.3	8, 8	10, 12	XX
Centre of Forensic Sciences, Toronto	15, 16	17, 21	20, 22				
	12, 16	19, 19	19, 25				
	12, 16	16, 17	21, 24				
	14, 16	15, 15	20, 21				
	15, 17	14, 17	23, 25				
	14, 14	16, 17	20, 22.2				
	14, 17	17, 18	22, 22				
	18, 18	16, 20	18, 21				
	16, 17	17, 17	19, 22				
	18, 18	13, 16	21, 26				
NIST				ND	ND	ND	
				6, 9.3	8, 9	10, 11, 12	
				7, 9.3	8, 9	13, 13	
				9, 9.3	8, 8	12, 12	
				6, 9.3	8, 11	12, 14	
Suffolk County Crime Laboratory	15, 16	15, 16	21, 25				
	15, 18	14, 16	19, 20				
	15, 19	16, 17	20, 25				

TABLE 3—*Continued*

Laboratory	D3S1358	vWA	FGA	THO1	TPOX	CSF1PO	Amelogenin
	17, 17	14, 18	22, 23				
	14, 15	16, 17	19, 23				
	16, 16	16, 18	22, 24				
	16, 16	16, 20	22, 24				
	15, 17	16, 16	23, 25				
	15, 18	15, 16	19, 21				
	15, 16	15, 18	20, 21				
	17, 18	16, 19	19, 22				
	14, 18	14, 16	22, 23				
	15, 16	17, 18	23, 24				
	15, 17	14, 17	23, 23				
	16, 17	17, 17	21, 24				

ND = Not detected.

can produce comparable results. The work detailed here examined the ability of CE to separate and type PCR amplified DNA fragments from several STR loci currently in use for forensic individualization. The samples analyzed by CE had previously been run and typed in a variety of gel-based systems in several different laboratories. The results show that capillary electrophoresis yields the same results obtained by those gel-based technologies when allelic ladders are employed to standardize the technique. Results obtained from either technique are comparable so that databases constructed using one technology can be searched or compared directly to another. Proficiency tests and other experimentally derived data can also be easily compared.

Capillary electrophoresis presents a new approach in the analysis of DNA fragments and, in particular, STRs. We have shown that CE is capable of distinguishing alleles with single base pair resolution and that within the CE system, base pair sizing is highly repeatable. As seen in Table 2, the average base pair range determined for any allele within a locus is considerably less than a base pair. Consequently, although base line resolution was not achieved for single base pair variants, the resolution was sufficient to accurately designate alleles on the basis of basepair size which is not overlapping. As demonstrated, CE allows the analyst to correctly identify the STRs examined here and offers some advantages over gel-based technologies. The conventional gel-based technologies require the preparation, loading and subsequent analysis of gels. The space and the time associated with these processes are considerable. The CE unit is relatively small and requires a minimal amount of supporting equipment and space. The capillary columns are

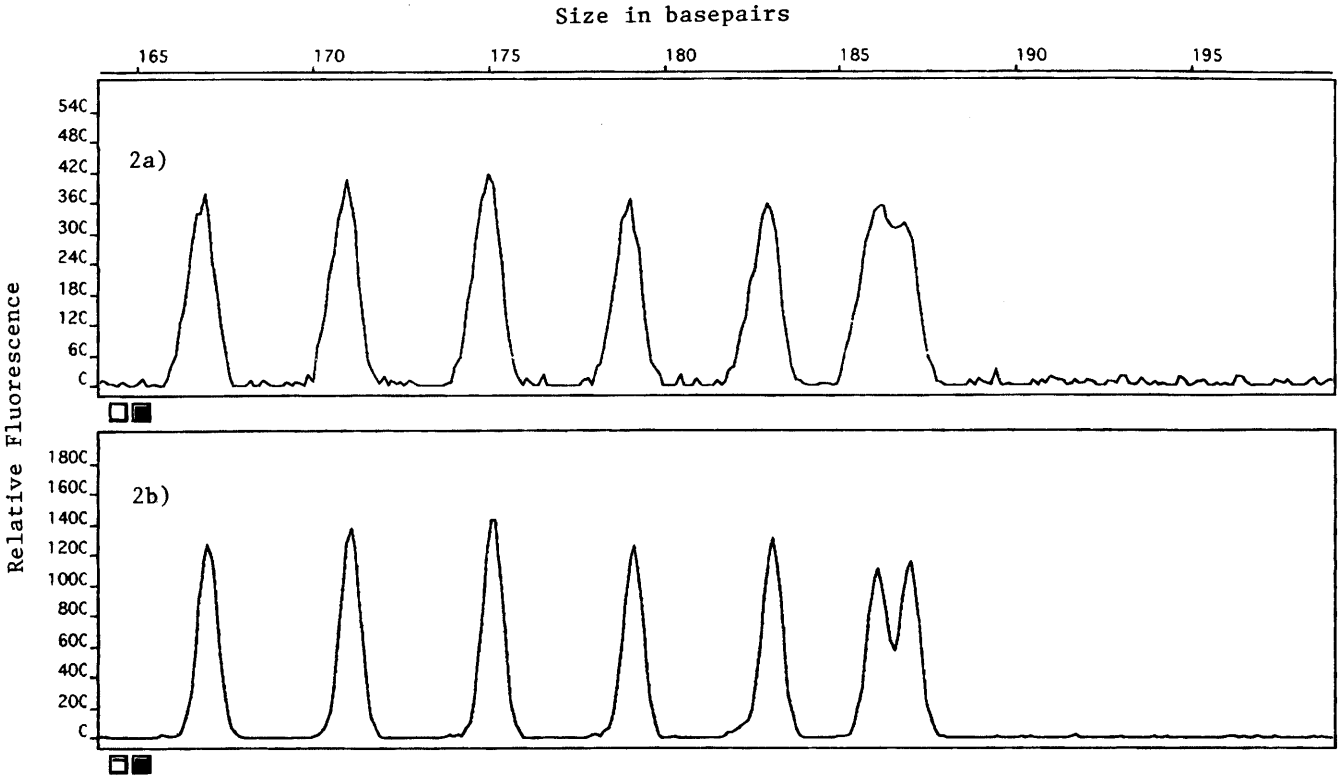

FIG. 2—*Electrophoretograms of the AmpFISTR green I ladder focusing on the 9.3 and 10 alleles in THO1. The only difference between Fig. 2a and 2b is the mixed bed resin used to treat the formamide.*

easy to replace and samples are placed within an autosampler for automatic loading. Samples that require re-running can be automatically reinjected and analyzed by the instrument without the need to remake a gel and reload the samples.

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