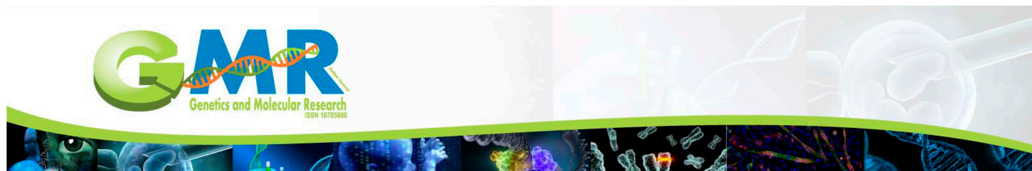

Comparative analysis of short tandem repeat data obtained by automated and gel electrophoresis techniques

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ABSTRACT. Short tandem repeats (STRs) are commonly used as genetic markers. The detection and analysis of STRs can be used to gather information on polymorphisms of interest to forensic geneticists. Denaturing polyacrylamide gel electrophoresis (PAGE) is an affordable method for the detection of minor sequence changes in DNA, while capillary electrophoresis (CE) is the gold standard for genotyping analysis. This appears to be the first study to directly compare data obtained using the two electrophoretic techniques. We analyzed genomic DNA from 209 individuals to compare genotyping results from seven Combined DNA Index System (CODIS) STR markers obtained by

both techniques. The automated electrophoresis was carried out using a MegaBACE™ 1000 DNA analysis system. Full concordance was found in 1297 of 1308 STR allele calls. Kappa and McNemar-Bowker tests indicated that there were no statistically significant differences between the results from the two methods. There was no statistically significant difference in precision between denaturing PAGE followed by silver nitrate staining, despite a longer protocol, compared with CE when applied to population studies. STR allele frequency data from non-automated genotyping techniques seem to be just as reliable as from automated genotyping methods.

Key words: Denaturing polyacrylamide gel electrophoresis; Capillary electrophoresis; STR frequency population data

INTRODUCTION

Since the 1990s, short tandem repeat (STR) markers have been used extensively for forensic and human identification purposes. However, because each population has a different genetic background, studies reporting allelic frequencies are important because they provide statistics that serves as population frequency data for forensic and paternity analysis calculations (Gurkan et al., 2015; Zhu et al., 2015).

Two technologies are commonly used for estimating STR allele frequencies: denaturing polyacrylamide gel electrophoresis (PAGE) followed by silver nitrate staining and capillary electrophoresis (CE). In general, these processes differ in their cost and speed.

Although denaturing PAGE is not used as often today, some researchers have recently adopted the technique for generating population data (Rangel-Villalobos et al., 2010; Gazi et al., 2011; Tenakanai et al., 2011). Denaturing PAGE is a non-automated technique that allows STR marker genotyping at a much lower cost; no expensive instruments are needed and the reagents are cheap because the polymerase chain reaction (PCR) products do not require fluorescent labels.

However, fluorescence detection by CE facilitates multiplex STR genotyping, which can examine more than 15 loci simultaneously. Although the automated process represents a faster and less laborious protocol, expensive equipment and reagents preclude some laboratories from using it routinely.

CE is currently the gold standard for genotyping analysis (Durney et al., 2015). However, since large-scale automated techniques are expensive, the choice of a less costly method might be an interesting alternative (Rangel-Villalobos et al., 2010).

Despite these issues, few studies have compared the concordance and accuracy of results generated by the two techniques.

To analyze the data quality of denaturing PAGE and CE for population studies, we used a comparative analysis to evaluate the results of STR marker genotyping from a sample of 209 individuals using the PAGE procedure and an automated method using the MegaBACE™ 1000 analyzer. This study is important because it provides statistical support for STR data generated by silver-staining detection methods compared with those obtained using a modern genotyping technique.

MATERIAL AND METHODS

Ethics

All participants signed a written informed consent form and the study was approved by the Ethics Committee of the Espírito Santo Federal University (number 015/07).

Blood collection and DNA extraction

Peripheral blood samples from 209 unrelated volunteers living in Espírito Santo State, Brazil, were collected using either FTA Elute Cards® (GE Healthcare, Little Chalfont, Buckinghamshire, UK) or ethylenediaminetetraacetic acid (EDTA) Vacutainer™ tubes (BD, Franklin Lakes, NJ, USA) between 2007 and 2009. Genomic DNA was extracted according to the FTA Elute Card® manufacturer protocol or the salting-out procedure (Miller et al., 1988).

Genotyping

The automated genotyping was carried out using a PowerPlex®16 kit (Promega Corporation, Madison, WI, USA). PCR fragments were analyzed by capillary electrophoresis on a MegaBACE™ 1000 DNA Sequencing System (GE Healthcare) and allele calling was conducted using the Fragment Profiler software. All steps were carried out according to the instructions provided by the manufacturer.

Denaturing PAGE samples were amplified at loci CSF1PO, D13S317, D16S539, D18S51, TH01, TPOX, and vWA. These markers were selected because they are present in commercially available Combined DNA Index System (CODIS) kits and showed effective amplification of degraded DNA in previous experiments (data not shown). The STRBase primers are shown in Table 1 (STRBase, 2016). The PCRs were carried out in a 20-μL reaction volume containing 40 ng genomic DNA, 0.20 mM dNTPs, 1X PCR Buffer, 0.50 mM primers (Invitrogen™, Carlsbad, CA, USA), and 1 U Taq DNA Polymerase (Invitrogen™). The reactions were performed using a GeneAmp® 9700 system (Applied Biosystems™, Foster City, CA, USA) or a Veriti® Thermal Cycler (Applied Biosystems™). The cycling protocol was as follows: initial denaturation at 95°C for 1 min; followed by a specified number of cycles comprising denaturation at 95°C for 30 s, annealing at a specified temperature for 30 s, and extension/elongation at 72°C for 30 s; and a final elongation at 72°C for 30 min. The annealing temperatures and the number of cycles for each primer pair are listed in Table 1. To verify band quality, the PCR products were run on a vertical 7 M urea denaturing gel using an S2 Sequencing Gel Electrophoresis Apparatus (Biometra GmbH, Göttingen, Germany). Allele identification was conducted by silver staining and comparison with previously known alleles used as an allelic ladder (Sprecher et al., 1996).

Statistical analysis

The denaturing gel genotyping results were compared with the corresponding CE results. The Kappa test was used to calculate the proportion of cases with the same outcome. Results were considered to be statistically significant if the P value was less than 0.05. In addition, the McNemar-Bowker P value was used to determine differences in treatment. Data were analyzed using the SPSS 18.0 software.

Table 1. Primers and polymerase chain reaction (PCR) conditions for each short tandem repeat (STR) marker.

STR marker	PCR primer sequences (5' to 3')		Product length (bp)	Annealing temperature (°C)	Number of cycles
	Forward	Reverse			
CSF1PO	AAC CTG AGT CTG CCA AGG ACT AGC	TTC CAC ACA CCA CTG GCC ATC TTC	291-331	68	30
D13S317	ACA GAA GTC TGG GAT GTG GA	GCC CAA AAA GAC AGA CAG AA	175-199	60	30
D16S539	GAT CCC AAG CTC TTC CTC TT	ACG TTT GTG TGT GCA TCT GT	148-172	60	30
D18S51	GAG CCA TGT TCA TGC CAC TG	CAA ACC CGA CTA CCA GCA AC	266-366	68	30
TH01	GTG GGC TGA AAA GCT CCC GAT TAT	ATT CAA AGG GTA TCT GGG CTC TGG	146-190	71	30
TPOX	ACT GGC ACA GAA CAG GCA CTT AGG	GGA GGA ACT GGG AAC CAC ACA GGT	220-256	69	25
vWA	CCC TAG TGG ATG ATA AGA ATA ATC AGT ATG	CCT GTC TAC TAT TTA TGT ATC CTA CCT AC	122-200	49	30

RESULTS

The genotyping data obtained by denaturing PAGE were compared with those obtained by CE for each individual through cross-table analysis. There was full concordance in 1297 of the 1308 STR allele calls.

Differences occurred when allele identification through PAGE did not correspond to the CE allele call. When alleles were equally identified by both methods, results were considered to correspond. Differences in the identification of one or both alleles were considered non-corresponding. There were 11 non-corresponding allele designations in 6 samples. Seven of these differences (I-19, I-163, IP-9, and IP-43) occurred in a single allele of the pair. The other four differences (IP-27 and IP-28) occurred in both alleles of the pair. More details are shown in Table 2. The results from the Kappa and McNemar-Bowker tests are shown in Table 3, and they indicate that there were no statistically significant differences between the methods.

Table 2. Genotypic differences found between techniques.

STR marker	Samples	Alleles found by denaturing PAGE	Alleles found by CE
CSF1PO	I-19	9 and 11	9 and 12
	IP-43 and I-163	10 and 10	10 and 11
D13S317	I-19	8 and 11	11 and 11
D16S539	I-19	11 and 11	11 and 14
D18S51	I-19	13 and 19	19 and 19
TH01	IP-9	7 and 8	7 and 6
TPOX	-	No single difference	No single difference
vWA	IP-27	15 and 18	17 and 17
	IP-28	17 and 17	15 and 18

STR = short tandem repeat; PAGE = polyacrylamide gel electrophoresis; CE = capillary electrophoresis.

Table 3. Results for statistical analysis by each marker.

STR marker	Kappa test	P value for Kappa test	McNemar-Bowker test
CSF1PO	0.984	0.001	0.223
D13S317	0.995	0.001	0.317
D16S539	0.995	0.001	0.317
D18S51	0.317	0.001	0.995
TH01	0.990	0.001	0.317
TPOX	1.000	0.000	-
vWA	0.990	0.001	1

STR = short tandem repeat.

DISCUSSION

Although automatic methods are widely used and have featured in several genetic identification studies, the PAGE technique is still used to analyze STR allelic frequencies in population studies. These studies are important for the forensic community because they provide data for the calculation of forensic and paternity parameters; however, there is a lack of information about the reliability of these results. Our study compared results from the denaturing PAGE and CE techniques. Statistical tests were used to indicate whether the observed differences affected the STR allele frequency results.

Population studies use large sample sizes of non-related individuals. The present study used 209 individuals; however, our choice of cross-table analysis increased the power of

the statistical tests, allowing us to obtain 1308 allele comparisons.

The cross-table analysis shows that the concordance level between the PAGE and CE techniques can reach 99.16% (1297/1308) and the statistical tests confirmed the global concordance of outcomes and the absence of significant differences between results. Moreover, the results were obtained from the analysis of a considerable number of individuals and the same samples were handled in distinct laboratories by different researchers, increasing the reliability of our conclusions. Such findings suggest that denaturing PAGE, involving commercial kits with silver stain detection systems such as the GenePrint® STR System (Promega Corporation), can be an efficient and less costly alternative for population studies. The GenePrint® system can be used to evaluate very small amounts (e.g., 1 ng) of human DNA.

However, one drawback of PAGE is the presence of microvariant alleles, e.g., TH01. In the first analysis, the banding patterns obtained were well defined for STRs, except for the TH01 marker, which has a 9.3-microvariant allele and differs from the 10 alleles by a single nucleotide. Although previous analyses of the TH01 marker did not show a high-resolution pattern for genotyping some individuals, they did provide us with a first screening of individuals carrying the doubtful alleles, i.e., alleles 9.3 or 10. To increase the power of resolution, we changed some electrophoretic conditions: we decreased the voltage applied to the gel, and increased the gel concentration and running time. Using these conditions, we obtained better separation of doubtful PCR products, which allowed the visualization of one-base differences in the size of TH01 alleles, detecting microvariant alleles by PAGE.

Although discrepancies of allele identification between PAGE and CE (Table 2) are not significant for population studies, owing to the large sample size, they can affect paternity, postmortem, and other forensic identifications. In these cases, a single erroneously identified allele can lead to misinterpretation, hinder analysis, and prolong the procedure. The exclusion power increases according to the number of alleles used. Therefore, multiplex kits with 24-27 STR loci, such as the PowerPlex® Fusion 6C System, have higher exclusion power. PAGE allows for the discrimination of single-base pair differences, but the protocols are more time consuming and labor intensive (Schneider, 2007; Butler, 2010).

In spite of the advantages and disadvantages inherent in the PAGE technique, this study suggests that the method is as reliable as CE for STR data analysis in population studies, and supports the reliability of data obtained by previous PAGE studies.

Since STR polymorphisms can be studied in many other species, PAGE may also be an affordable alternative in studies on animals and plants, and is useful for researchers working with a variety of organisms.

Conflicts of interest

The authors declare no conflict of interest.

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