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Alternative Silver Stain Detection protocol for the GenePrint® STR System Applied to Mestizo Population From Jalisco (West of Mexico)

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Abstract

A population sample of 163 unrelated individuals from the Mexican state of Jalisco (West of Mexico) was PCR-typed with the Geneprint® STR System including nine autosomal STRs (CSF1PO, TPOX, TH01, F13A01, FESFPS, vWA, D16S539, D7S820 and D13S317). For this purpose, we implemented an alternative protocol for STR silver stain detection, which provides some economical and technical benefits: 1) make easy the gel handling; 2) simplify the process avoiding the use of some –toxic– reagents; 3) require lesser timer and made-in-home solutions; 4) the original gels can be dried and stored for re-analysis, if necessary. The allele frequencies estimated were similar to a previous report from the same Mexican population, which allowed pooling STR genotype data for upgrade the forensic parameter estimates (n= 472). However, this is the first –and unique– population study in Mexico for F13A01 and FESFPS; thus, the sample size did not increased for these loci (n= 163). In the studied population, genotype distribution by locus and two-loci combination was in agreement with Hardy-Weinberg expectations for all nine STRs. For the STR system, the power of discrimination (PD) and exclusion (PE) were >99.999% and 99.9%, respectively.

Introduction

In Mexico there are two principal populations: the Native American groups, and the Spanish-speaking Mestizo population living in both urban and rural regions throughout the country. Mestizos are the result of admixture occurred after the European contact with the New World, and the process involved Spaniards, Amerindians, and African slaves, principally. Presently, according to linguistic criteria, Mexican-Mestizos represent the majority of the total population (~93%) [1]. Human identification based on DNA is largely based on STR markers; therefore, these loci have been analyzed in worldwide populations for interpretation of the evidence in forensic casework. In Mexico, different studies have been carried out for this purpose [2]. However, to our knowledge, some genetic systems have never been studied in this country, for instance the GenePrint® (Silver Stain Detection) that includes three PCR multiplex systems: FFv, CTT, and Silver STR III. In particular, the population data concerning the loci F13A01 and FESFPS, included into the FFv triplex, is totally absent in Mexico. For this reason, blood samples were obtained of 163 unrelated Mexican individuals living in the state of Jalisco in the West of Mexico. They were classified as Mexican-Mestizos because anyone belonged to a specific Mexican ethnic group. Prior to the inclusion in our study, all volunteers signed an informed consent form, according to the ethical guidelines of the Helsinki Declaration.

Material and Methods

DNA was extracted from dried blood spotted on FTA paper (Whatman, Cliffton, NJ), or from fresh blood by salting-out DNA extraction process. Approximately 20ng of DNA were amplified in a 10μL total volume. The samples were PCR-typed using the GenePrint® STR Systems (Silver Stain Detection) according to the supplier directions (Promega Corp., Madison, USA) [3]. The autosomal STRs included in the kit are: CSF1P0, TP0X, TH01, F13A01, FESFPS, vWA, D16S539, D7S820 and D13S317. Instead of using sequencing electrophoresis systems, products were separated by 6.5% (19:1) denaturing PAGE with urea 7M in vertical gel units of 16.5 x 37.5 x 0.8 cm (DASG250, CBS Scientific) with TBE 0.5X buffer; an aluminum plate of 0.2 cm was adjusted to the electrophoresis system to avoid "smiling" effect. Finally, gels were silver stained by an alternative protocol (Table 1) respect to the recommended by the supplier [3, 4].

Recently STR population data were reported from the state of

	Employed Solution	Time
Fix Solution	10% ethanol, 0.5% acetic acid	10 min
Silver Stainig	0.2% silver nitrate in fix solution	5 min
Washing (twice)	bi-distilled water	20 s and 2 min
Developer Solution	3% NaOH, 0.5% formaldehyde	~5 min Until bands appears
Fix Solution	10% ethanol, 0.5% acetic acid	5 min
Washing	bi-distilled water	5 min

a. Slightly modified from Sanguinetti et al. [4].

Table 1: Rapid silver staining protocol[a] employed for STR detection.

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Jalisco, Mexico [5]. The comparison between our results and those previously reported indicated non-significant differences for the seven loci shared between STR studies. Therefore, we pooled our genotype data with those of the previous study to improve the forensic parameter estimates. For F13A01 and FESFPS, genotype pooling was not possible because, to our knowledge, does not exist previous population data from any Mexican population. Allele frequencies were estimated by gene count method. Statistical parameters of forensic importance like the Power of Exclusion (PE), Probability of Discrimination (PD), Polymorphic Information Content (PIC), and Heterozygosity (Het), Typical Index of Paternity (IP) were computed with the software PowerStats [6]. Hardy-Weinberg expectations (HWE) for each and combined loci were calculated by Fisher exact tests on 3200 shuffling experiments with the software Genetic Data Analysis (GDA version 1.1) [7].

Results and Discussion

Currently, most of the human identification laboratories employ capillary electrophoresis for PCR product detection. However, for economical reasons some laboratories in developing countries still obtain DNA profiles with genetic systems based on silver stain detection [3]. Although the STR kit employed in this work recommends sequencing cameras for electrophoresis of polyacrylamide gels, and a commercial kit for silver stain detection [3], we obtained excellent results with an alternative protocol offering additional benefits (Figure 1) :1) we used vertical gel units, with thicker gels than those run in sequencing cameras (0.8 mm versus 0.4mm), making easy the handling; 2) we avoided the necessity to stick the gel to one glass plate with methacryloxypropyltrimethoxysilane (bind silane), and Gel Slick® to prevent the gel for sticking, which simplified the process enormously and avoid using some –toxic– reagents; 3) we implemented a rapid silver stain protocol that requires solutions easily prepared in the laboratory (Table 1), and about half of the time required by the commercial silver staining protocol (~30 min versus ~1 hour) [4]; 4) finally, the gels can be dried over filter paper, which allows the original results can be stored; eventually the PCR products (bands) can be excised from gel and eluted for re-analysis [4]. In general, this alternative protocol allows saving time and money for human identification purposes, particularly to those forensic geneticists with scarce economical resources that still need using these manual methodologies.

The allele distributions and forensic parameters for the nine STRs loci in the studied population are shown in Table 2. For all nine loci, genotype distribution by locus and two loci combination was in agreement to Hardy-Weinberg expectations. The most informative STRs were D13S317 and F13A01, and the least discriminating were TP0X and FESFPS. The combined power of discrimination (PD) and exclusion (PE) were >0.99999999 and 0.99898, respectively. This STR system had not been reported from this region of Mexico, the second larger city of the country. We did not carry out an extensive population differentiation analysis, because this has been carried out previously [2]. However, we were able to upgrade the forensic parameter estimates in Mestizos from the Mexican population of Jalisco, as observed when comparing the minimum allele frequency (MAF) of F13A01 and FESFPS, which is two-three times greater than those estimated for the STRs whose genotypes were pooled.

Conclusion

We described an alternative –and cheaper– protocol for STR analysis by silver stain detection (GenePrint® STR Systems). This was

Allele	CSF1PO	TPOX	TH01	F13A01	FESFPS	vWA	D16S539	D7S820	D13S317
3.2				0.2239					
4				0.1196					
5			0.0011	0.2178			0.0011		
6		0.0053	0.2627	0.1503				0.0032	
7	0.0032	0.0053	0.3294	0.2607				0.0117	
8	0.0074	0.5456	0.0784	0.0123	0.0061		0.0138	0.1062	0.0837
9	0.0254	0.0477	0.1112	0.0031	0.0092		0.0879	0.0648	0.2140
9.3			0.1706						
10	0.2617	0.0339	0.0466		0.2393		0.1833	0.2611	0.1038
11	0.3093	0.2394			0.4571		0.2712	0.3068	0.2278
12	0.3284	0.1197			0.1994		0.3093	0.1985	0.2140
13	0.0604	0.0021		0.0031	0.0767	0.0038	0.1176	0.0425	0.1123
14	0.0042	0.0011		0.0031	0.0123	0.0416	0.0159	0.0042	0.0445
15				0.0031		0.0869		0.0011	
16				0.0031		0.3627			
17						0.2783			
18						0.1574			
19						0.0642			
20						0.0050			
MAF	0.0061	0.0057	0.0063	0.0180	0.0162	0.0074	0.0062	0.0063	0.0064
Forensic Parameters									
PD	0.8687	0.8154	0.9093	0.9214	0.8596	0.8980	0.9133	0.9158	0.9460
PIC	0.6728	0.5804	0.7386	0.7664	0.6391	0.7156	0.7406	0.7480	0.8005
PE	0.4883	0.3500	0.5657	0.6174	0.3728	0.5460	0.5318	0.5458	0.5928
Typical IP	1.90	1.41	2.29	2.63	1.48	2.18	2.11	2.18	2.46
Het	0.7373	0.6462	0.7818	0.8098	0.6626	0.7708	0.7627	0.7707	0.7966
p-value (HWE)	0.7828	0.7787	0.0540	0.0693	0.9446	0.0262	0.5112	0.0209	0.1859

a. Minimum allele frequency (MAF); Hardy-Weinberg expectations (HWE); heterozygosity (Het); power of discrimination (PD); power of exclusion (PE), Index of Paternity (IP).
b. STR genotype data (*n*= 163) pooled with those of a previous report (*n*= 309) from the state of Jalisco, Mexico [2], excepting for the loci F13A01 and FESFPS.

Table 2: Allele frequencies and forensic parameters[a] for 9 STR loci (GenePrint® STR system) in a Mexican-Mestizo population sample (n= 472)[b] from the state of Jalisco.

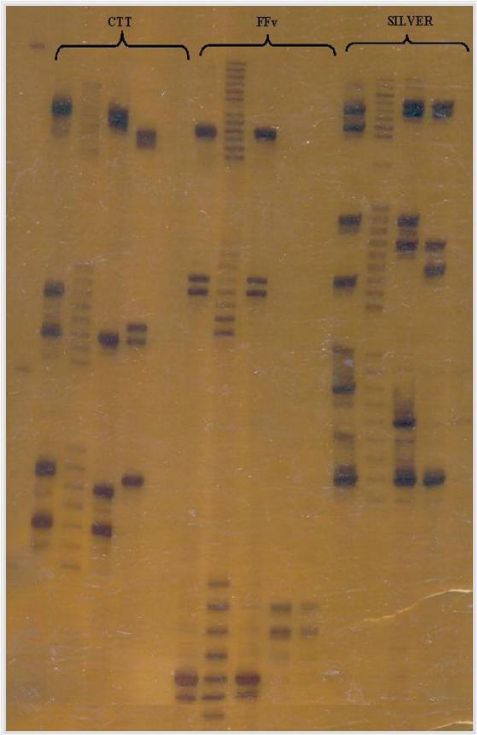

Figure 1: Genotypes for 9 STRs included in the Geneprint STR system (Promega Corp.) obtained with the alternative protocol for electrophoresis and silver stain detection.

applied to upgrade the forensic parameter estimations of Mestizos from the state of Jalisco, describing –for the first time– population data of the loci F13A01 and FESFPS in Mexico.

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