TECHNICAL NOTE

Carmen Entrala,[1] M.Sc.; Jose A. Lorente,[1] M.D., Ph.D.; Miguel Lorente,[1] M.D., Ph.D.; J. Carlos Alvarez,[1] M.Sc.; Bruce Budowle,[2] Ph.D.; and Enrique Villanueva,[1] M.D., Ph.D.

Spanish Population Data on the Loci D13S317, D7S820, and D16S539 Generated Using Silver Staining (SilverSTR III™ Multiplex)*

REFERENCE: Entrala C, Lorente JA, Lorente M, Alvarez JC, Budowle B, Villanueva E. Spanish population data on the Loci D13S317, D7S820, and D16S539 generated using silver staining (SilverSTR III™ Multiplex) J Forensic Sci 1999;44(5):1032–1034.

ABSTRACT: A set of 212 samples from unrelated Spanish Caucasians living in Andalucia (southern Spain) were analyzed with a new commercially-available kit for multiplex amplification of 3 STR loci (D13S137, D7S820, and D16S539), manual denaturing polyacrylamide gel electrophoresis and silver staining. These three loci are of special interest for the forensic community since they are a part of the 13 CODIS-core STR loci. The results show that the loci D13S317 and D16S539 meet Hardy-Weinberg expectations (HWE), but the locus D7S820 did not meet HWE ($p = 0.003$). However, there was no detectable departures from independence (i.e., linkage disequilibrium) between any pair-wise combination of loci. The D7S820 data were further investigated. The excess homozygosity was due to an excess of D7S820 10, 10 homozygotes. To determine if the allele frequency data are meaningful and can be applied to forensic identity cases, the Spanish D7S820 allele frequency data were compared with four other Caucasian sample populations. The D7S820 allele frequencies were statistically similar; thus, the results support that the allele frequency data can be used reliably for estimating DNA profile frequencies.

KEYWORDS: forensic science, D13S319, D7S820, D16S539, silver staining, DNA typing, short tandem repeats, population genetics, Spain

Typing short tandem repeats (STR) loci is useful for paternity and forensic identity testing. Furthermore, STR loci have been chosen as core markers for CODIS (1). Thus, STR loci typing figure prominently in forensic identity testing for the forseeable future. A new commercially-available kit enables the simultaneous amplification of three of the CODIS core loci: D7S820, D13S317, and D16S539 (DDD) (SilverSTR III™ Multiplex, Promega Corp., Madison, WI). After electrophoretic separation of the STR amplicons, the DNA profiles can be detected by silver staining. Thus, simple, inexpensive procedures can be used to type the DDD triplex. It is well-established in the literature (1–3) that amplification of STR loci by the polymerase chain reaction (PCR), electrophoresis of the amplified products, and detection by silver staining offer useful and reliable approaches for characterizing DNA derived from forensic biological specimens. This study describes results of a population study of unrelated individuals from Andalucia (southern Spain) using the DDD kit.

Material and Methods

Whole blood was obtained in EDTA vacutainer tubes by venipuncture from 212 unrelated Spanish Caucasians from Andalucia (southern Spain). DNA was extracted organically and concentrated and purified by Microcon-100 filtration (4). The quantity of recovered DNA was determined by slot-blot hybridization (5).

The SilverSTR III™ Multiplex kit (Promega Corp., Madison, WI) was used to amplify the loci D7S820, D13S317, and D16S539. The PCR contained 3 to 5 ng of DNA, 2.5 μL of STR 10X buffer, 2.5 μL Multiplex 10× primer pair mix, and 0.75 units of Taq polymerase. Sterile water was used to adjust to a final volume of 25 μL. Amplification was performed in a Perkin-Elmer 9600 for 30 cycles according to the manufacturer's recommendations (Technical Manual. GenePrint™ STR Systems—Silver Stain Detection. Part #TMD004. Promega Corp.).

Electrophoretic separation of the amplified alleles was performed in vertical, denaturing, polyacrylamide gels (4%) using a BRL SA32 apparatus (Life Technologies, Gaithersburg, MD) (2). The gels were pre-run at 40 W for 30 to 45 min in order to reach an approximate temperature of 56°C. Silver staining was performed as described previously (6).

The alleles of the samples were identified by comparison with the allelic ladders in adjacent lanes in the gel and confirmed by a second independent reader. The ladders contain the alleles 7 to 15 for locus D13S317; 6 to 14 for locus D7S820; and 5, 8 to 14 for locus D16S539.

Statistical analysis of the results was performed using the following tests. The frequency of each allele for each locus was calculated from the numbers of each genotype in the sample test. Unbiased estimates of expected heterozygosity were computed as described by Edwards et al. (7). Possible divergence from Hardy-

[1] Laboratory of Genetic Identification. Dept. de Medicina Legal. Facultad de Medicina. Universidad de Granada. E-18012 Granada, Spain.

[2] Forensic Science Research Unit. FSRTC. Laboratory Division. FBI Academy, Quantico, VA.

* This work was supported by a Proyecto de Investigación (PM97-0175) from the DGES, Spanish Ministry of Education and Science (MEC).

Received 28 July 1998; and in revised from 17 Nov. 1998; accepted 5 Jan. 1999.

Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (8–10), the likelihood ratio test (7,11,12), and the exact test by Guo and Thompson (13). An interclass correlation criterion (14) was used for detecting disequilibrium between loci.

Results and Discussion

The results using the SilverSTR™ III kit are easy to obtain and interpret (Fig. 1). For those laboratories familiar with manual gel electrophoresis and silver staining technology, transfer should be relatively easy, and for those laboratories without the more expensive fluorescent detection equipment, the typing of the loci D7S820, D13S317, and D16S539 can still be implemented.

The distribution of observed allelic frequencies, observed and expected homozygosity, tests for independence, probability of discrimination (PD), and probability of exclusion (PE) for the DDD loci are shown in Table 1. The loci D13S317 and D16S539 meet Hardy-Weinberg expectations (HWE), but the locus D7S820 does not meet HWE ($p = 0.003$). The excess homozygosity at the D7S820 locus was due primarily to an excess of observed 10, 10 homozygotes (29 observed versus 21 expected).

This excess of homozygosity could be due to possible population substructure at the locus, selection, typing errors, and/or sampling error. There is no evidence to date to support selection. Typing errors are unlikely because the same samples were typed using the Profiler Plus kit (Perkin-Elmer) and the typing results were the same for the two kits (data not shown). To evaluate if the departure from HWE is due predominately to either substructure or sampling error the Spanish D7S820 allele data were compared with data from three U.S. Caucasian sample populations and one from Italy (all of which meet HWE at the D7S820 locus) using a test for homogeneity, and they were statistically similar (data not shown); thus sampling error at the genotype level appears to be the main

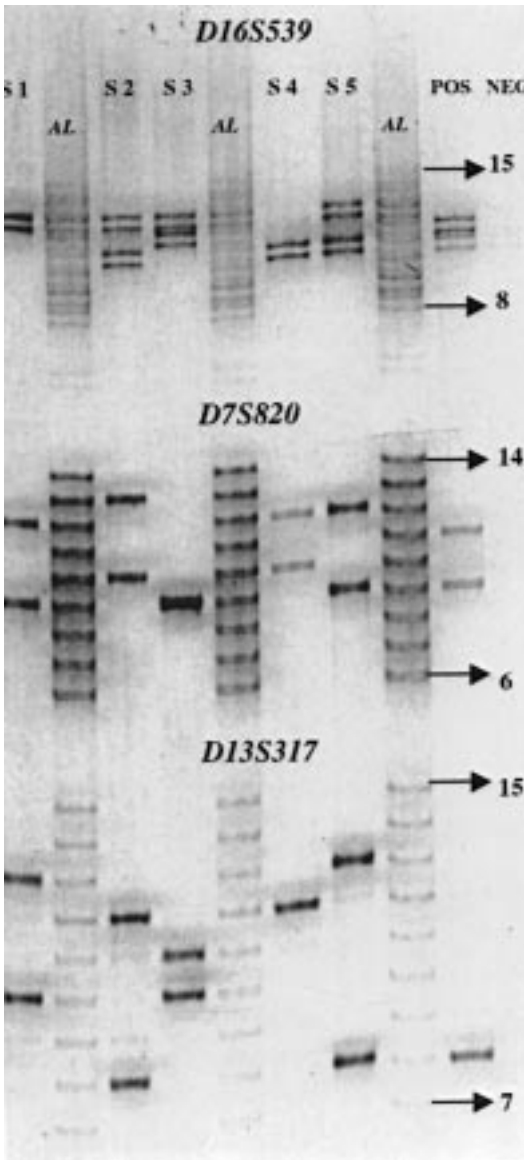

FIG. 1—*SilverSTR™ III genotypes of five samples (S1 to S5), K562 positive control (POS) and negative control (NEG). For each locus, the shortest and largest alleles present in the allelic ladder supplied by the manufacturer are pointed with arrows.*

TABLE 1—*Allele frequencies and parameters of statistical interest to check the Hardy-Weinberg equilibrium of the 3 loci included in the SilverSTR™ III kit. Cummulated PD: 0.9995—Cummulated PE: 0.9333*

Loci	D13S317*		D7S820†		D16S539‡	
Allele	Number	Proportion	Number	Proportion	Number	Proportion
7	—	—	8	0.0226	—	—
8	62	0.1751	54	0.1525	4	0.0113
9	19	0.0536	48	0.1356	42	0.1186
10	24	0.0678	113	0.3192	24	0.0678
11	99	0.2796	68	0.1921	92	0.2599
12	93	0.2627	54	0.1525	110	0.3107
13	39	0.1101	8	0.0226	75	0.2118
14	18	0.0508	1	0.0028	6	0.0169
15	—	—	—	—	1	0.0028

* Observed Homozygosity = 20.8%, Expected Homozygosity = 19.3%, Homozygosity Test ($p = 0.595$); Likelihood Ratio Test ($p = 0.339$); Exact Test ($p = 0.270$); PD = 0.929; PE = 0.618.

† Observed Homozygosity = 25.9%, Expected Homozygosity = 20.0%, Homozygosity Test ($p = 0.030$); Likelihood Ratio Test ($p = 0.006$); Exact Test ($p = 0.003$); PD = 0.927; PE = 0.606.

‡ Observed Homozygosity = 18.4%, Expected Homozygosity = 22.7%, Homozygosity Test ($p = 0.139$); Likelihood Ratio Test ($p = 0.048$); Exact Test ($p = 0.088$); PD = 0.904; PE = 0.556.

cause for the departure of HWE. Furthermore, there was no detectable departures from independence (i.e., linkage disequilibrium) between any pair-wise combination of loci.

In conclusion, the analysis of the DDD loci was facilitated due to the development of a commercial kit. The typing of these STR loci can be performed by most laboratories, since the kit enables manual gel electrophoresis and silver staining to be used to type the markers.

Furthermore, a Spanish population database has been established for the loci D13S317, D7S820, and D16S539. The allelic frequencies of these PCR-based loci can be used to estimate the frequency of a multiple locus PCR-based DNA profile in Spanish population. The data demonstrate that a significant degree of discrimination can be obtained (PD = 0.9995) when all three loci are used to characterize forensic biological evidence; the power of exclussion (PE) reaches 0.9333 for the 3 loci together.

Acknowledgments

We want to thank Randy Nagy and Carol Zabit, (Promega Corporation, Madison, WI), for their continous help and assistance.

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Additional information and reprint requests:
Prof. Jose A. Lorente, M.D., Ph.D.
Dept. Medicina Legal—University of Granada
Av. Madrid 11
E-18012 Granada, Spain
Tel: + 34 958 243546
Fax: + 34 958 246107
E-mail: jlorente@goliat.ugr.es