Population Studies and Casework Application with the New *GenePrint*™ SilverSTR™ III Multiplex (D16S539, D7S820, D13S317)

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INTRODUCTION

Typing short tandem repeat (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 will figure prominently in forensic identity testing for the foreseeable 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 that amplification of STR loci by the polymerase chain reaction (PCR), electrophoresis of the amplified products, and detection by silver staining are useful and reliable approaches for characterizing DNA derived from forensic biological specimens [2, 3]. 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 10x 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, USA) [2]. The gels were prerun at 40 Watts for 30 to 45 minutes in order to reach an approximate temperature of 50• C Silver staining was performed as described previously [6].

The alleles of the samples were identified by comparison with the allelic ladders, supplied in the kit, and run in adjacent lanes. The ladders contain the alleles 7 to 15 for locus D13S317; 6 to 14 for locus D7S820; and 5, 8 to14 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-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozgote/heterozygote frequencies [8, 9, 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 vs. 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. The data (not shown) were statistically similar; thus sampling error at the genotype level appears to be the main cause for the departure of HWE. Furthermore, there were no detectable departures from independence (i.e., linkage disequilibrium) between any pair-wise combination of loci.

In a set of 44 paternity trios, no mutations have been found after using these 3 loci included in the kit. Also, results obtained with this triplex using old forensic casework samples are consistent with the results previously obtained with the older Promega's CTT and FFV kits. Further studies are on the way to completely validate this kit for forensic casework.

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. The data demonstrate that a significant degree of discrimination can be obtained in a Caucasian population (PD= 0.9995) when all three loci are used to characterize forensic biological evidence; the power of exclusion (PE) reaches 0.9333 for the 3 loci together.

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	D13S317[a]	D7S820[b]	D16S539[c]
7	-	0.0283	0.0094
8	0.1722	0.1509	0.1203
9	0.0660	0.1391	0.0613
10	0.0660	0.3137	0.2641
11	0.2712	0.1887	0.2995
12	0.2594	0.1557	0.2241
14	0.1156	0.0212	0.0188
15	0.0495	0.0024	0.0024

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. Cumulative PD: 0.9995 - Cumulative PE: 0.9333

a) 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.
b) 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.
c) 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.

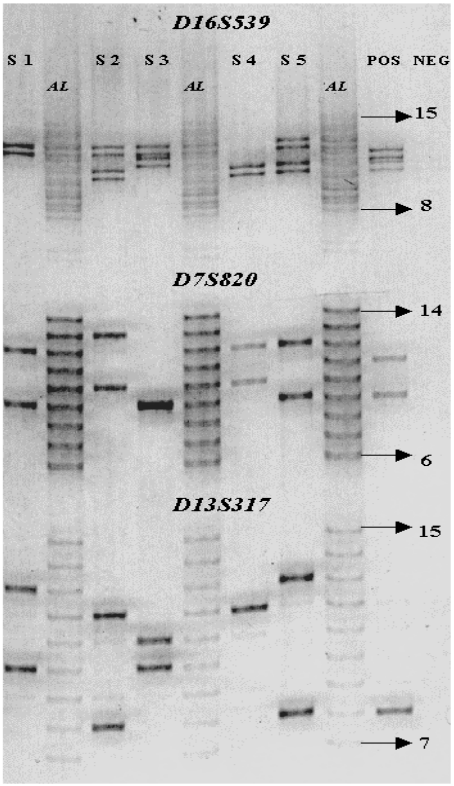

Figure 1. SilverSTR™ *III* genotypes of five samples (S1 to S5), K562 positive control (POS) and negative control (NEG). For each locus, the shortest and largest alleles present in the allelic ladder supplied by the manufacturer are pointed with arrows.