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D1S80 Population Data in African Americans, Caucasians, Southeastern Hispanics, Southwestern Hispanics, and Orientals

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ABSTRACT: Allele frequencies for the locus D1S80 were determined in African American, Caucasian, Southeastern Hispanic, Southwestern Hispanic, and Oriental sample populations using the polymerase chain reaction and subsequent electrophoresis and silver staining of the amplified products. Due to the presence of anodal and cathodal electrophoretic variants (in reference to the steps in an allelic ladder), allele frequencies were established using a classification protocol based on the steps in the allelic ladder. All sample populations met Hardy-Weinberg expectations for D1S80. In addition, there was no evidence for association of alleles between the loci D1S80 and D1S7. The product of allele frequencies from the data from the sample populations in this study can be used in forensic analyses and paternity tests to estimate the frequency of a D1S80 DNA genotype.

KEYWORDS: pathology and biology, VNTR, allele frequency, population databases, PCR, AMP-FLP, Hardy-Weinberg Expectations

Amplification of variable number tandem repeat (VNTR) sequences by the polymerase chain reaction (PCR) [*1–9*] has been shown to be a valuable tool for the analysis of forensic biological evidence [*10–18*]. The technique of typing VNTR-PCR products using electrophoresis has been termed amplified fragment length polymorphism (AMP-FLP) analysis. AMP-FLP analysis offers certain advantages over restriction fragment length polymorphism (RFLP) typing of VNTR loci. These advantages include an increased sensitivity of detection, an avoidance of the need for radioisotopic labels, and the ability to type DNA that has been degraded past the point of utility for RFLP analysis. In addition, because of their smaller size, amplified fragment alleles can be resolved into more discrete fragment bands than alleles typically found at VNTR loci by RFLP typing.

One of the most forensically characterized AMP-FLP markers is the polymorphism at the locus D1S80 [*6,7,10–12,15,16,19–22*]. D1S80 is a variable number of tandem repeats (VNTR) locus, generally with a 16 base pair repeat size [*6*]. After the D1S80 alleles are amplified by PCR, they can be resolved and detected by polyacrylamide gel electrophoresis and silver staining [*7*]. Alleles vary in size as a function of the number of repeat sequences contained within them. More than 22 different alleles have been observed at the D1S80 locus [*12*].

For the use of the D1S80 locus in identity testing cases in the United States, it is desirable to collect allele/genotype data from relevant population(s) so that the forensic scientist can calculate an estimate of the frequency of a particular genetic profile. This paper presents allele frequency data for the D1S80 locus in United States Caucasian, African American, southeastern Hispanic, southwestern Hispanic, and Oriental population samples. The data demonstrate that the product of D1S80 allele frequencies can be used to estimate D1S80 genotype frequencies for forensic identity cases. Furthermore, there is no detectable deviation from expectation of independence between the alleles at D1S80 and at the VNTR locus D1S7, a locus used in forensic RFLP typing [*23*].

Materials and Methods

Sample preparation: The sources of the Caucasian, African American, Southeastern Hispanic, and Southwestern Hispanic samples were described previously [*23*]. Oriental samples were obtained from C. T. Caskey (Baylor University, Houston, TX), K. Kidd (Yale University, New Haven, CT) and P. Mills (Department of the Army, San Francisco, CA). The DNA from the Oriental samples was extracted from whole blood or bloodstains according to the organic/microcon method of Comey et al. [*24*]. The quantity of DNA in each sample was estimated using the slot-blot procedure described by Waye et al. [*25*].

D1S80 Typing: The DNA was amplified by using the D1S80 primers described by Kasai et al. [*6*] and by following the PCR protocol described by Baechtel et al. [*26*]. The PCR was carried out in 50 μl reaction volumes containing 5 ng template DNA, 10

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mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 1 nmole of each of the four deoxyribonucleoside triphosphates, 12.5 pmoles of each primer, and 2.5 units of Taq DNA polymerase. The reactions were placed into a Perkin Elmer 9600 thermal cycler and were subjected to 27 cycles of denaturation at 95°C for ten seconds, primer annealing at 67°C for ten seconds, and primer extension at 70°C for 30 seconds. The D1S80 allelic ladder was constructed in-house (see Ref *26*). The amplified products were typed by horizontal polyacrylamide gel (20 cm long) electrophoresis on an ICE Apparatus (EC Corporation, Clearwater, FL) and subsequent silver staining according to previously described procedures [*7,26*]. The quantity of the PCR sample that was applied to the horizontal gels was 4.5 μl. Additionally, some samples were typed in vertical polyacrylamide gels using a variation of the horizontal electrophoretic procedure. In particular, an acrylamide solution (7.5%T, 2.0%C; cross-linker was piperazine diacrylamide) was poured between two glass plates separated by 0.4 mm thick spacers. Gel bond (FMC Corp., Rockland, ME) was affixed to one plate such that the hydrophilic side was in contact with the gel. The gel dimensions were 17 cm by 33.5 cm. The gel buffer was Tris-formate, pH 9.0, which was 60 mM with respect to the formate ion. An 18-tooth comb (catalogue #11092-095, GIBCO BRL, Gaithersburg, MD) was used. The gel was permitted to polymerize for one hour at ambient temperature followed by an incubation period of at least of 30 minutes at 4°C prior to use. The gel was placed in a SA 32 apparatus (GIBCO BRL, Gaithersburg, MD) and 250 mL of 28 mM Tris-borate, pH 9.0, (28 mM with respect to the borate ion) was placed in the bottom reservoir. To the top buffer reservoir 300 mL of 60 mM Tris-formate was added. Four μl of amplified DNA sample were mixed with 2.5 μl of loading solution (25 μg xylene cyanol, 25 μg bromophenol blue, and 4 g sucrose per 10 mL of 120 mM Tris-formate, pH 9.0). The entire sample volume was applied to a gel well at the cathodal end of the gel submersed in the Tris-formate buffer. After all samples were loaded onto the gel, the upper reservoir buffer was removed by draining and replaced with 300 mL of 28 mM Tris-borate. Electrophoresis was performed at ambient temperature with settings of 995 V, 200 mA, and 50 W, and

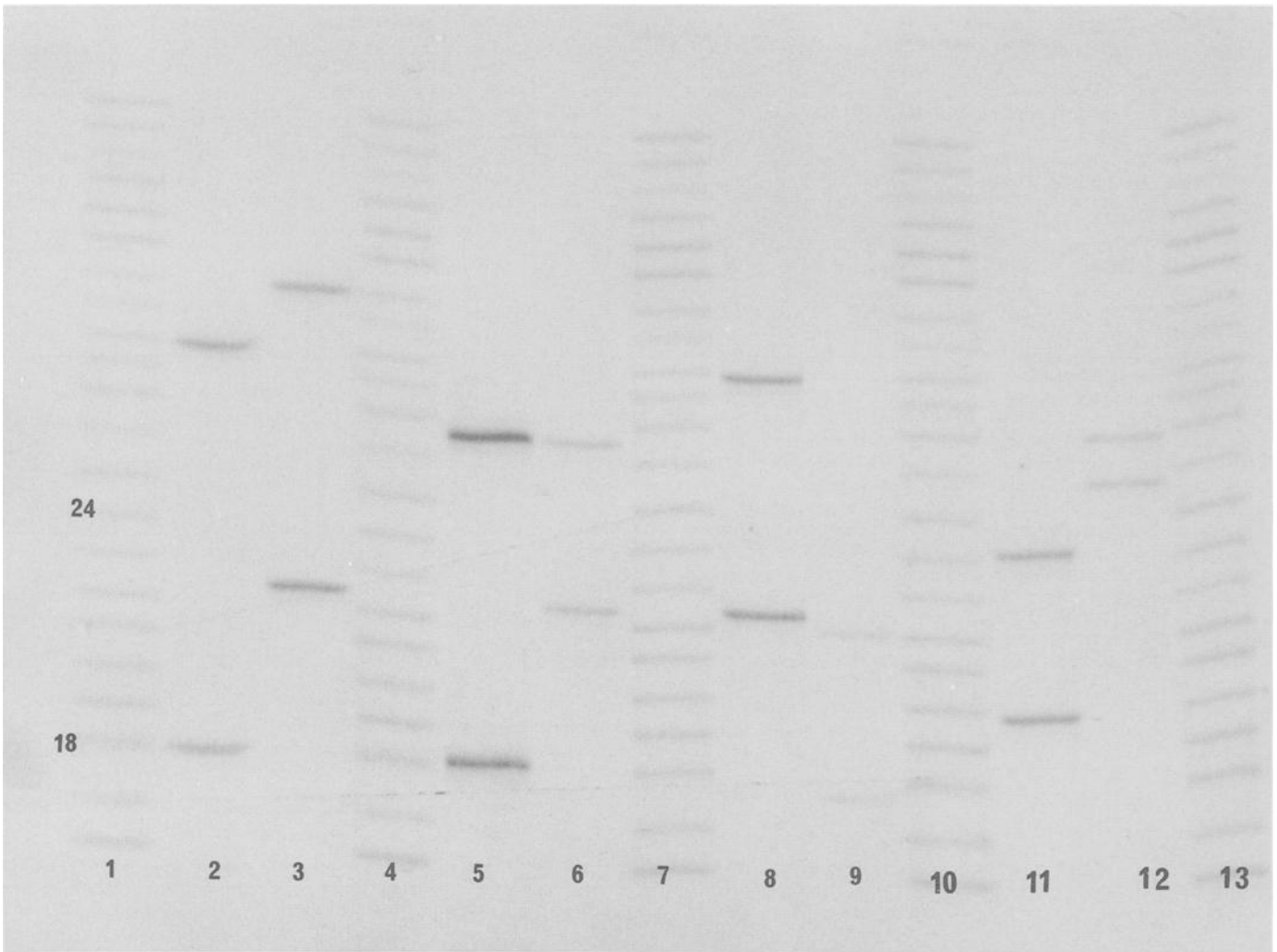

FIG. 1—*A horizontal polyacrylamide gel displaying several D1S80 types. The allelic ladder is in lanes 1, 4, 7, 10, and 13. Lane 2 = 29-18, lane 3 = 31-22M, lane 5 = 26M-18, lane 6 = 26M-22M, lane 8 = 29-22M, lane 9 = 22C-18A, lane 11 = 24C-20A, and lane 12 = 27-25M. Variants designated with an "A" are anodal to the reference ladder allele; variants designated with an "B" are cathodal to the reference ladder allele; and variants designated with an "M" are midway between two reference ladder alleles. The cathode is at the top.*

was allowed to continue until the xylene cyanol tracking dye migrated to the top of the lower reservoir buffer. The gel also was stained with silver as described previously [7]. Allele designations were determined by comparison of the sample's fragments with those of the allelic ladders.

Statistical Analysis

The frequency of each allele for D1S80 was calculated from the numbers of each genotype in the sample set (that is, the gene count method). Unbiased estimates of expected heterozygosity

TABLE 1—*D1S80 allele frequencies in five sample populations (values in parentheses are number of individuals typed).*

Allele[a]	African American[b] (*N* = 606)	Caucasian[c] (*N* = 718)	Southeastern Hispanic[d] (*N* = 247)	Southwestern Hispanic[e] (*N* = 162)	Total Hispanic[f] (*N* = 409)	Oriental[g] (*N* = 204)
15	0.000	0.000	0.000	0.003	0.001	0.000
16	0.002	0.001	0.004	0.019	0.010	0.034
17	0.028	0.002	0.012	0.003	0.009	0.025
18	0.073	0.237	0.225	0.222	0.224	0.152
19	0.003	0.003	0.004	0.006	0.005	0.022
20	0.032	0.018	0.010	0.019	0.013	0.007
21	0.115	0.021	0.030	0.025	0.028	0.034
22	0.081	0.038	0.028	0.019	0.024	0.017
23	0.014	0.012	0.014	0.000	0.009	0.017
24	0.234	0.378	0.316	0.315	0.315	0.230
25	0.045	0.046	0.059	0.093	0.072	0.027
26	0.006	0.020	0.008	0.006	0.007	0.000
27	0.008	0.007	0.012	0.022	0.016	0.047
28	0.130	0.063	0.081	0.074	0.078	0.076
29	0.053	0.052	0.079	0.019	0.055	0.042
30	0.009	0.008	0.018	0.071	0.039	0.123
31	0.054	0.072	0.051	0.056	0.053	0.093
32	0.007	0.006	0.006	0.003	0.005	0.012
33	0.004	0.003	0.004	0.003	0.004	0.005
34	0.086	0.001	0.008	0.003	0.006	0.005
35	0.002	0.003	0.000	0.000	0.000	0.005
36	0.001	0.004	0.014	0.006	0.011	0.005
37	0.000	0.001	0.006	0.000	0.004	0.007
38	0.000	0.000	0.000	0.000	0.000	0.000
39	0.003	0.003	0.002	0.006	0.004	0.005
40	0.000	0.000	0.000	0.000	0.000	0.000
41	0.002	0.000	0.000	0.006	0.002	0.007
>41[h]	0.007	0.001	0.008	0.003	0.006	0.002

[a]Alleles were determined by comparison with an allelic ladder [*26*].

[b]African American population sample—Observed Homozygosity = 0.130; Expected Homozygosity (unbiased) = 0.114; HWE—Homozygosity Test (P = 0.194), Likelihood Ratio Test (P = 0.850), Exact Test (P = 0.891); there were four alleles that were middle electrophoretic variants in this sample determined by horizontal polyacrylamide gel electrophoresis.

[c]Caucasian population sample—Observed Homozygosity = 0.216; Expected Homozygosity (unbiased) = 0.215; HWE—Homozygosity Test (P = 0.973), Likelihood Ratio Test (P = 0.082), Exact Test (P = 0.120); there were 17 alleles that were middle electrophoretic variants in this sample determined by horizontal polyacrylamide gel electrophoresis.

[d]Southeastern Hispanic population sample—Observed Homozygosity = 0.194; Expected Homozygosity (unbiased) = 0.171; HWE—Homozygosity Test (P = 0.319), Likelihood Ratio Test (P = 0.409), Exact Test (P = 0.298); there were five alleles that were middle-electrophoretic variants in this sample determined by horizontal polyacrylamide gel electrophoresis.

[e]Southwestern Hispanic population sample—Observed Homozygosity = 0.204; Expected Homozygosity (unbiased) = 0.171; HWE—Homozygosity Test (P = 0.266), Likelihood Ratio Test (P = 0.786), Exact Test (P = 0.705); there were four alleles that were middle-electrophoretic variants in this sample determined by horizontal polyacrylamide gel electrophoresis.

[f]Total Hispanic population sample (that is, pooled southeastern and southwestern Hispanics)—Observed Homozygosity = 0.198; Expected Homozygosity (unbiased) = 0.170; HWE—Homozygosity Test (P = 0.125), Likelihood Ratio Test (P = 0.628), Exact Test (P = 0.534); there were nine alleles that were middle-electrophoretic variants in this sample determined by horizontal polyacrylamide gel electrophoresis.

[g]Oriental population sample—Observed Homozygosity = 0.093; Expected Homozygosity (unbiased) = 0.113; HWE—Homozygosity Test (P = 0.380), Likelihood Ratio Test (P = 0.482), Exact Test (P = 0.425); there were three alleles that were middle-electrophoretic variants in this sample determined by horizontal polyacrylamide gel electrophoresis.

[h]All alleles migrating slower than the largest allele in the ladder (that is, allele #41) are placed in the >41 allele class.

TABLE 2—*Observed and expected heterozygous and homozygous classes for D1S80 in five sample populations*

	African American (*N* = 606)	Caucasian (*N* = 718)	Southeastern Hispanic (*N* = 247)	Southwestern Hispanic (*N* = 162)	Total Hispanic (*N* = 409)	Oriental (*N* = 204)
Heterozygotes observed	106	81	68	51	86	76
Heterozygotes expected ± SE[a]	106.2 ± 5.4	82.5 ± 5.0	64.3 ± 5.0	48.1 ± 4.5	84.7 ± 5.5	74.0 ± 5.5
Homozygotes observed	9	8	6	6	9	8
Homozygotes expected ± SE[a]	9.4 ± 1.2	7.2 ± 1.2	5.4 ± 1.2	4.7 ± 1.1	6.6 ± 1.3	6.1 ± 1.3

[a]SE = standard error.

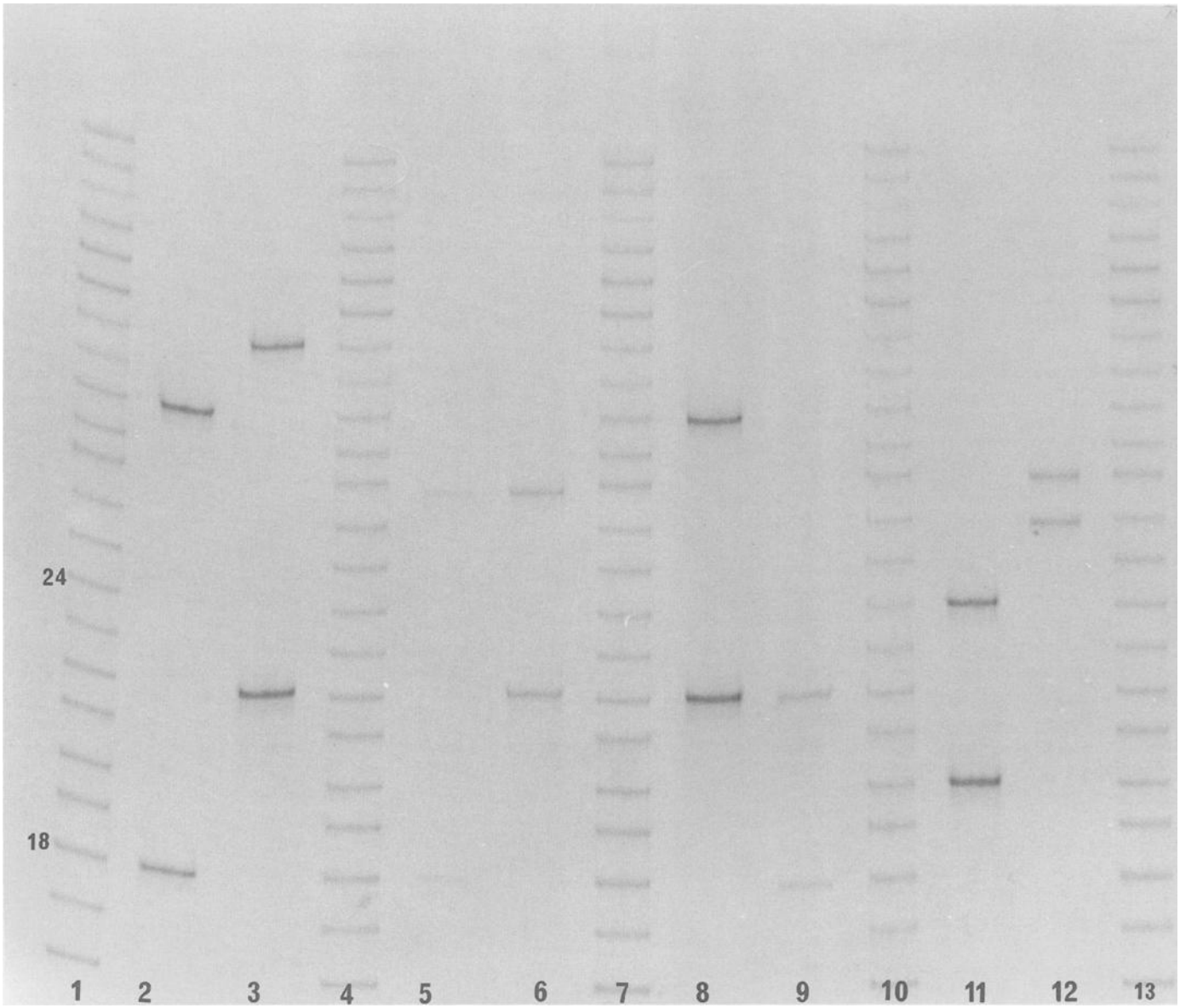

FIG. 2—*A vertical polyacrylamide gel displaying several D1S80 types. These samples are from the same PCR as those in Figure 1. Note that the sample in lane 5 is weaker in Fig. 2 than in Fig. 1; this is probably due to technical or temporal differences between the two approaches, such as the presence of some acrylamide in the vertical well or that the sample may have degraded some between analyses. The allelic ladder is in lanes 1, 4, 7, 10, and 13. Lane 2 = 29-18, lane 3 = 31-22, lane 5 = 27-18, lane 6 = 27-22, lane 8 = 29-22, lane 9 = 22-18, lane 11 = 24-20, and lane 12 = 27-26. The cathode is at the top.*

were computed as described by Edwards et al. [*9*]. The expected numbers of distinct homozygous and heterozygous genotypes and their standard error (SE) were calculated according to the method described by Chakraborty et al. [*27,28*]. Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies [*27,29,30*], and by the log likelihood ratio test criterion [*9,28,31*] and the exact test [*32*]. An interclass correlation criterion [*33*] was used for detecting potential disequilibrium between loci (that is, between D1S80 and D1S7; Ref. *23*). Independence between these two loci also was determined by examining whether or not the observed variance of the number of heterozygous loci in the population sample is outside its confidence interval under the assumption of independence [*34–36*].

Results and Discussion

Figure 1 shows a typical separation of amplified D1S80 alleles by high-resolution discontinuous horizontal polyacrylamide gel electrophoresis. As noted earlier [*37*] some D1S80 alleles migrate anodically or cathodically to the alleles represented in the reference ladder. This is particularly evident with samples separated by horizontal gel electrophoresis (Fig. 1). These variant alleles are due presumably to differences in sizes and/or sequences of the DNA fragments. The anodal and cathodal variants cannot be unequivocally classified as particular off-ladder variants. Thus, it was recognized that to establish allele frequencies and to perform statistical analyses pertinent to identity testing an allele binning protocol would be necessary. The D1S80 variant alleles typed by horizontal

electrophoresis can be categorized readily by their proximity to a distinct fragment in the allelic ladder.

Alternatively, D1S80 PCR products can be separated electrophoretically using vertical polyacrylamide gels (Fig. 2). With the vertical gel format the anodal and cathodal D1S80 variants that have been analyzed tend to migrate to the nearest step in the allelic ladder. Thus, the detection of anodal and cathodal electrophoretic variants is reduced, yet the alleles are proximal to the same fragments in the allelic ladder as determined by horizontal electrophoresis. This observation suggests that most of the anodal and cathodal variants are due to sequence polymorphisms. The AMP-FLPs separated in vertical gels are in a warmer environment (45–50°C) compared with those separated in horizontal gels (15°C). Conformational effects on the DNA fragments due to sequence polymorphisms are less likely to manifest themselves during electrophoresis in a warmer gel (data not shown). Reynolds (Roche Molecular Systems, Alameda, CA, personal communication) has sequenced some D1S80 allelic variants and confirmed that they contain different sequences.

The distribution of allele frequencies for African Americans, Caucasians, southeastern Hispanics, southwestern Hispanics, and Orientals is shown in Table 1. There was a total of 32 alleles (determined using horizontal polyacrylamide gel electrophoresis), out of a total of 3874 chromosomes typed across the five databases, which migrated by horizontal electrophoresis approximately equidistant between two steps of the reference ladder (Table 1). The most common middle electrophoretic variant ($f = 0.012$) was an allele that resided between alleles 22 and 23 in the Caucasian sample population. To place these few middle variants in their appropriate allelic bin class, the samples were run on vertical gels. Thus population data classified in this manner, either generated by vertical or horizontal polyacrylamide gel electrophoresis, are equivalent. There were 24 different nominal alleles observed in each of the population samples, except for the Hispanic samples, each which contained 23 alleles. The observed heterozygosity for the African American, Caucasian, southeastern Hispanic, southwestern Hispanic, and Oriental sample populations is 87.0%, 78.4%, 80.6%, 79.6%, and 90.7%, respectively.

A test for independence for the alleles within a locus, based on the number of distinct heterozygote and homozygote genotypes, was performed (Table 2). There was no deviation from expected values for the five sample populations. Moreover, the distribution of D1S80 genotypes for the five sample populations does not deviate from HWE based on the homozygosity test, log likelihood ratio test criterion, and the exact test (Table 1). The same observations generally hold true even for a pooled Hispanic population sample containing the total southeastern and southwestern data (Tables 1 and 2). Therefore, the data support that D1S80 allele frequencies, derived from the populations in this study, can be multiplied together to estimate genotype frequencies.

Currently, it is not anticipated that PCR-based AMP-FLP markers and RFLP-generated VNTR markers will be analyzed routinely on the same case evidence in the FBI Laboratory. Thus, there was no immediate need in this study to determine whether or not there are any detectable associations between D1S80 alleles and RFLP-based VNTR alleles. These can be done easily on a case-by-case basis. However, while it might be expected that for loci on different chromosomes their alleles generally would be independent [*31,38–41*], this may be less likely for loci residing on the same arm of a chromosome. An examination of population data for the loci D1S80 and D1S7 [*42*] could be useful for determining a potential association between genetically linked loci used in forensic analyses. Both D1S80 and D1S7 reside on chromosome 1p, and the FBI Laboratory has African American, Caucasian, southeastern Hispanic, and southwestern Hispanic population data for both of these loci [*23*]. Analyses of the population data for D1S7 are described elsewhere [*23,31,38*]. Further, analyses were performed to determine whether or not there were any detectable associations between D1S80 and D1S7. An interclass correlation test [*33*] analysis demonstrated that there was no evidence for correlation between the alleles of the two loci (Table 3).

As an additional test for association, independence between D1S80 and D1S7 was evaluated by examining whether or not the observed variance (s_K^2) of the number of heterozygous loci in the population sample is outside its confidence interval under the assumption of independence using the procedure described by Brown et al. [*34*]. Chakraborty [*35*] showed that the use of this test criterion results in some loss of information in summarizing multiple locus genotype data, because each genotype is classified as either a homozygote or a heterozygote only. However, this test criterion is more powerful than the classical goodness-of-fit χ^2 test (which is based on the distribution of the number of heterozygous loci to detect dependence of alleles across loci). This test has sufficient power to detect association of alleles across more than two loci that might have an impact on estimating multiple locus genotype frequencies using the product rule. There was no evidence of association between the alleles at D1S80 and D1S7 for the four sample populations using the s_K^2 criterion (Table 4).

In conclusion, D1S80 population databases have been generated for African Americans, Caucasians, southeastern Hispanics, southwestern Hispanics, and Orientals. The distribution of the genotype frequencies meet HWE, and there is no evidence of association

TABLE 3—*D1S80/D1S7 Interclass Correlation test for four sample populations (two-sided probability).*

	African American	Caucasian	Southeastern Hispanic	Southwestern Hispanic	Total Hispanic
Number of Individuals	209	411	229	122	351
D1S80/D1S7 (*P* value)	0.328	0.153	0.621	0.555	0.447

TABLE 4—*Test for association of alleles—the variance of the number of heterozygous loci—for D1S80 and D1S7 in four sample populations.*

	African American	Caucasian	Southeastern Hispanic	Southwestern Hispanic	Total Hispanic
Number of individuals	209	411	229	122	351
Observed variance	0.182	0.233	0.181	0.190	0.184
95% confidence interval	0.142–0.250	0.203–0.278	0.132–0.205	0.127–0.237	0.143–0.203

of alleles with another VNTR locus used in forensic analyses, D1S7, that resides on the same chromosomal arm as D1S80. The data demonstrate that, when using the sample populations in this study, D1S80 genotype estimates can be estimated by the product of the allele frequencies.

References

[1] Saiki, R. K., Scharf, S., Faloona, T., Mullis, K. B., Horn, G. T., Erlich, H. A., and Arnheim, N., "Enzymatic Amplification of Beta-Globin Genomic Sequences and Restriction Analysis for Diagnosis of Sickle Cell Anemia," *Science*, Vol. 230, 1985, pp. 1350–1354.

[2] Jeffreys, A. J., Wilson, V., Neumann, R., and Keyte, J., "Amplification of Human Minisatellites by the Polymerase Chain Reaction: Towards Fingerprinting of Single Cells," *Nucleic Acids Research*, Vol. 16, 1988, pp. 10953–10971.

[3] Boerwinkle, E., Xiong, W., Fourest, E., and Chan, L., "Rapid Typing of Tandemly Repeated Hypervariable Loci by the Polymerase Chain Reaction: Application to the Apolipoprotein B 3′ Hypervariable Region," *Proceedings of the National Academy of Science USA*, Vol. 86, 1989, pp. 212–216.

[4] Horn, G. T., Richards, B., and Klinger, K. W., "Amplification of a Highly Polymorphic VNTR Segment by the Polymerase Chain Reaction," *Nucleic Acids Research*, Vol. 17, 1989, p. 2140.

[5] Ludwig, E. H., Friedl, W., and McCarthy, B. J., "High-Resolution Analysis of a Hypervariable Region in the Human Apolipoprotein B Gene," *American Journal of Human Genetics*, Vol. 48, 1989, pp. 458–464.

[6] Kasai, K., Nakamura, Y., and White, R., "Amplification of a Variable Number of Tandem Repeat (VNTR) Locus (pMCT118) by the Polymerase Chain Reaction (PCR) and Its Application to Forensic Science," *Journal of Forensic Sciences*, Vol. 35, 1990, pp. 1196–1200.

[7] Budowle, B., Chakraborty, R., Giusti, A. M., Eisenberg, A. J., and Allen, R. C., "Analysis of the VNTR Locus D1S80 by the PCR Followed by High-Resolution PAGE," *American Journal of Human Genetics*, Vol. 48, 1991, pp. 137–144.

[8] Edwards, A., Civitello, A., Hammond, H. A., and Caskey, C. T., "DNA Typing and Genetic Mapping with Trimeric and Tetrameric Tandem Repeats," *American Journal of Human Genetics*, Vol. 49, 1991, pp. 746–756.

[9] Edwards, A., Hammond, H., Jin, L., Caskey, C. T., and Chakraborty, R., "Genetic Variation at Five Trimeric and Tetrameric Repeat Loci in Four Human Population Groups," *Genomics*, Vol. 12, 1992, pp. 241–253.

[10] Hochmeister, M. N., Budowle, B., Borer, U. V., Eggmann, U. T., Comey, C. T., and Dirnhofer, R., "Typing of DNA Extracted from Compact Bone Tissue from Human Remains," *Journal of Forensic Sciences*, Vol. 36, 1991, pp. 1649–1661.

[11] Hochmeister, M. N., Budowle, B., Jung, J., Borer, U. V., Comey, C. T., and Dirnhofer, R., "PCR-Based Typing of DNA Extracted from Cigarette Butts," *International Journal of Legal Medicine*, Vol. 104, 1991, pp. 229–233.

[12] Kloosterman, A. D., Budowle, B., and Daselaar, P., "PCR-Amplification and Detection of the Human D1S80 VNTR Locus: Amplification Conditions, Population Genetics, and Application in Forensic Analysis," *International Journal of Legal Medicine*, Vol. 105, 1993, pp. 257–264.

[13] Potsch, L., Meyer, U., Rothschild, S., Schneider, P. M., and Rittner, C., "Application of DNA Techniques for Identification Using Human Dental Pulp As a Source of DNA," *International Journal of Legal Medicine*, Vol. 105, 1992, pp. 139–143.

[14] Prinz, M., Grellner, W., and Schmitt, C., "DNA Typing of Urine Samples Following Several Years of Storage," *International Journal of Legal Medicine*, Vol. 106, 1993, pp. 75–79.

[15] Rand, S., Puers, C., Skowasch, K., Wiegand, P., Budowle, B., and Brinkmann, B., "Population Genetics and Forensic Efficiency Data of Four AMPFLPs," *International Journal of Legal Medicine*, Vol. 104, 1992, pp. 329–333.

[16] Sajantila, A., Strom, M., Budowle, B., Karhunen, P. J., and Peltonen, L., "The Polymerase Chain Reaction and Post-Mortem Forensic Identity Testing: Application of Amplified D1S80 and HLA-DQ Alpha Loci to the Identification of Fire Victims," *Forensic Science International*, Vol. 51, 1991, pp. 23–34.

[17] Wiegand, P., Budowle, B., Rand, S., and Brinkmann, B., "Forensic Validation of the STR Systems SE 33 and TC 11," *International Journal of Legal Medicine*, Vol. 105, 1993, pp. 315–320.

[18] Wiegand, P., Bajanowski, T., and Brinkmann, B., "DNA Typing of Debris from Fingernails," *International Journal of Legal Medicine*, Vol. 106, 1993, pp. 81–83.

[19] Nakamura, Y., Carlson, M., Krapcho, V., and White, R., "Isolation and Mapping of a Polymorphic DNA Sequence (pMCT118) on Chromosome 1p (D1S80)," *Nucleic Acids Research*, Vol. 16, 1988, p. 9364.

[20] Alonso, A., Martin, P., Albarran, C., and Sancho, M., "Amplified Fragment Length Polymorphism Analysis of the VNTR Locus D1S80 in Central Spain," *International Journal of Legal Medicine*, Vol. 106, 1993, pp. 311–314.

[21] Sajantila, A., Budowle, B., Strom, M., Johnson, V., Lukka, H., Peltonen, L., and Enholm, C., "PCR Amplification of Alleles at the D1S80 Locus: Comparison of a Finnish and a North American Caucasian Population Sample, and Forensic Casework Evaluation," *American Journal of Human Genetics*, Vol. 50, 1992, pp. 816–825.

[22] Skowasch, K., Wiegand, P., and Brinkmann, B., "pMCT118 (D1S80): A New Allelic Ladder and Improved Electrophoretic Separation Lead to the Demonstration of 28 Alleles," *International Journal of Legal Medicine*, Vol. 105, 1992, pp. 165–168.

[23] Budowle, B., Monson, K. L., Anoe, K., Baechtel, F. S., Bergman, D., et al., "A Preliminary Report on Binned General Population Data on Six VNTR Loci in Caucasians, Blacks, and Hispanics from the United States," *Crime Laboratory Digest*, Vol. 18, 1991, pp. 9–26.

[24] Comey, C. T., Koons, B. W., Presley, K. W., Smerick, J. B., Sobieralski, C. A., Stanley, D. M., and Baechtel, F. S., "DNA Extraction Strategies for Amplified Fragment Length Polymorphism Analysis," *Journal of Forensic Sciences* (in press).

[25] Waye, J. S., Presley, L., Budowle, B., Shutler, G. G., and Fourney, R. M., "A Simple Method for Quantifying Human Genomic DNA in Forensic Specimen Extracts," *Biotechniques*, Vol. 7, 1989, pp. 852–855.

[26] Baechtel, F. S., Smerick, J. B., Presley, K. W., and Budowle, B., "Multigenerational Amplification of a Reference Ladder for Alleles at Locus D1S80," *Journal of Forensic Sciences*, Vol. 38, 1993, pp. 1176–1182.

[27] Chakraborty, R., Smouse, P. E., and Neel, J. V., "Population Amalgamation and Genetic Variation: Observations on Artificially Agglomerated Tribal Populations of Central and South America," *American Journal of Human Genetics*, Vol. 43, 1988, pp. 709–725.

[28] Chakraborty, R., Fornage, M., Guegue, R., and Boerwinkle, E., "Population Genetics of Hypervariable Loci: Analysis of PCR Based VNTR Polymorphism Within a Population," in, *DNA Fingerprinting: Approaches and Applications*, T. Burke, G. Dolt, A. J. Jeffreys, and R. Wolff, Eds., Birkhauser Verlag, Berlin, 1991, pp. 127–143.

[29] Nei, M. and Roychoudhury, A. K., "Sampling Variances of Heterozygosity and Genetic Distance," *Genetics*, Vol. 76, 1974, pp. 379–390.

[30] Nei, M., "Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals," *Genetics*, Vol. 89, 1978, pp. 583–590.

[31] Weir, B. S., "Independence of VNTR Alleles Defined by Fixed Bins," *Genetics*, Vol. 130, 1992, pp. 873–887.

[32] Guo, S. W. and Thompson, E. A., "Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles," *Biometrics*, Vol. 48, 1992, pp. 361–372.

[33] Karlin, S., Cameron, E. C., and Williams, P. T., "Sibling and Parent Offspring Correlation Estimation with Variable Family Size," *Proceedings at the National Academic Science U.S.A.*, Vol. 78, 1981, pp. 2664–2668.

[34] Brown, A. H. D., Feldman, M. W., and Nevo, E., "Multilocus Structure of Natural Populations of Hordeum Spontaneum," *Genetics*, Vol. 96, 1980, pp. 523–536.

[35] Chakraborty, R., "Detection of Nonrandom Association of Alleles from the Distribution of the Number of Heterozygous Loci in a Sample," *Genetics*, Vol. 108, 1984, pp. 719–731.

[36] Chakraborty, R., Zhong, Y., Jin, L., and Budowle, B., "Non-Detectability of Restriction Fragments and Independence of DNA-Fragment Sizes Within and Between Loci in RFLP Typing of DNA," *American Journal of Human Genetics*, 1994, pp. 391–401.

[37] Budowle, B., Baechtel, F. S., and Comey, C. T., "Some Considerations for Use of AMP-FLPs for Identity Testing," in, *Advances in Forensic Haemogenetics* 4, C. Rittner and P. M. Schneider, Eds., Springer-Verlag, Berlin, 1992, pp. 11–17.

[38] Chakraborty, R., Srinivasan, M. R., and De Andrade, M., "Intraclass and Interclass Correlations of Allele Sizes Within and Between Loci in DNA Typing Data," *Genetics*, Vol. 133, 1993, pp. 411–419.

[39] Risch, N. and Devlin, B., "On the Probability of Matching DNA Fingerprints," *Science*, Vol. 255, 1992, pp. 717–720.

[40] Hochmeister, M. N., Budowle, B., Borer, U. V., and Dirnhofer, R., "Swiss Population Data on the Loci HLA-DQ∝, LDLR, GYPA, HBGG, D7S8, Gc, and D1S80," *Forensic Science International*, Vol. 67, 1994, pp. 175–184.

[41] Hochmeister, M. N., Jung, M. M., Budowle, B., Borer, U. V., and Dirnhofer, R., "Swiss Population Data on Three Tetrameric Short Tandem Repeat Loci VWA, HUMTHO1, and F13A1—Derived Using Multiplex PCR and Laser Fluorescence Detection," *International Journal of Legal Medicine*, Vol. 157, 1994, p. 34.

[42] Wong, Z., Wilson, V., Patel, I., Povey, S., and Jeffreys, A. J., "Characterization of a Panel of Highly Variable Minisatellites Cloned from Human DNA," *Annals of Human Genetics*, Vol. 51, 1987, pp. 269–288.

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