TECHNICAL NOTE

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Development and Population Study of an Eight-Locus Short Tandem Repeat (STR) Multiplex System*

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ABSTRACT: Amplification of short tandem repeat (STR) loci has become a useful tool for human identification applications. To improve throughput and efficiency for such uses, the polymorphic STR loci CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818, F13A01, FESFPS, F13B, and LPL have been evaluated, developed, and configured into fluorescently labeled multiplex systems. Eight of these STR loci were combined to generate the PowerPlex™ System, a two-color multiplex system that supports rapid, accurate, reliable analysis and designation of alleles. The remaining four loci comprise the FFFL System, a one-color multiplex system. The PowerPlex™ System may be evaluated alternatively as two one-color, four-locus multiplex systems, CTTv Multiplex and GammaSTR™ Multiplex. The products of multiplex amplification may be analyzed with a variety of fluorescence detection instruments. Determination of genotypes of over 200 individuals from each of three different population/ethnic groups revealed independence of inheritance of the loci and allowed calculation of matching probability, typical paternity index, and power of exclusion for each multiplex.

KEYWORDS: forensic science, DNA typing, short tandem repeats, multiplex, polymerase chain reaction, CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818, F13A01, FESFPS, F13B, LPL, PowerPlex

DNA profiling using short tandem repeat (STR) loci (1–3) has become widely used for human identification in forensic sciences and other related fields. The polymorphic repeat region of STR loci is small, allowing amplification via the polymerase chain reaction (PCR) to generate fragments 100 to 400 base pairs long. This small, defined size range of each locus has allowed development of multiplex sets of STR loci (4,5). Refining these sets with multicolor fluorescent detection provides an effective method to meet rapid, accurate, high-throughput needs of population database applications (6,7).

In this work we describe the development of the PowerPlex™ System (an eight-locus, two-color multiplex system containing the loci CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818) and the component GammaSTR™ System (a four-locus, one-color multiplex system containing the loci D16S539, D7S820, D13S317, D5S818). These systems are highly discriminating and compatible with several fluorescence detection instruments. We have also evaluated at least 200 individuals in each of three distinct populations to determine the allele and genotype frequencies of the eight loci contained within the PowerPlex™ System along with four additional loci contained in the previously described four-locus, one-color FFFL Multiplex (4) containing the loci F13A01, FESFPS, F13B, and LPL. Independence of these loci and determination of several commonly used statistics in forensic and paternity determinations were defined.

Materials and Methods

DNA Purification, Amplification and Detection

DNA isolation from human samples was performed essentially as previously described (8,9). Approximately 1 to 2 ng template DNA was included in each 25 μL amplification reaction when using the *GenePrint*™ PowerPlex™ 1.1 System (Promega, Madison, WI), *GenePrint*™ PowerPlex™ 1.2 System (Promega, Madison, WI) or the *GenePrint*™ FFFL Fluorescent System (Promega, Madison, WI) as recommended in the *GenePrint*™ PowerPlex™ System or Fluorescent STR System technical manuals (10,11). These amplifications were performed using a Perkin-Elmer GeneAmp™ PCR System 9600 Thermal Cycler (Foster City, CA). The cycling profile for the PowerPlex™ System is as follows: 96°C for 1 min; then 10 cycles of 94°C for 30 s, 68 s ramp to 60°C and hold for 30 s, 50 s ramp to 70°C and hold for 45 s; then 20 cycles of 90°C for 30 s, 60 s ramp to 60°C and hold for 30 s, 50 s ramp to 70°C and hold for 45 s; then 60°C for 30 min. For the FFFL Multiplex System, mineral oil is added to the reactions, the thermal

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cycler lid is not used, and the cycling profile is as follows: 96°C for 1 min; then 10 cycles of 50 s ramp to 94°C and hold for 1 min, 34 s ramp to 60°C and hold for 1 min, 25 s ramp to 70°C and hold for 1.5 min; then 20 cycles of 45 s ramp to 90°C and hold for 1 min, 30 s ramp to 60°C and hold for 1 min, 25 s ramp to 70°C and hold for 1.5 min; then 60°C for 30 min.

Amplification products of the *GenePrint*™ PowerPlex™ 1.1 System, or the FFFL Multiplex System were combined with Fluorescent Ladder (CXR), 60-400 Bases (Promega, Madison, WI), separated in a 4% polyacrylamide denaturing gel (43 cm long, 0.4 mm thick) containing 0.5X TBE and 7M urea for 1 h at 60 watts (W) using a SA43 gel electrophoresis unit (BRL, Bethesda, MD), detected with the FMBIO® II Fluorescent Scanner (Hitachi Software Engineering America, Ltd., San Bruno, CA) and analyzed using the FMBIO® Analysis software. The Fluorescent Ladder (CXR), 60-400 Bases is used as an internal lane standard (ILS) and contains 16 regularly spaced DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400 bases in length.

Amplification products of the *GenePrint*™ PowerPlex™ 1.2 System were combined with the ILS, separated in a 5% Long Ranger™ (FMC BioProducts, Rockland, ME) gel (36 cm long, 0.2 mm thick) containing 1X TBE and 7M urea, detected with the ABI Prism™ 377 DNA Sequencer (Perkin-Elmer Applied Biosystems Division, Foster City, CA) and analyzed using the GeneScan™ Analysis software.

Before the development of the fluorescent multiplexes, the population genotyping was performed using the *GenePrint*™ CTT or FFv Multiplex STR Systems (Promega, Madison, WI), or a combination of *GenePrint*™ F13A01 and LPL STR Systems (Promega, Madison, WI) as recommended in the *GenePrint*™ STR Systems technical manual (8). Approximately 10 to 15 ng template DNA was added to the reactions to ensure detection following post-amplification staining. Amplified samples were separated in 4% polyacrylamide denaturing gels (32 cm long, 0.4 mm thick) containing 0.5X TBE and 7M urea and detected by staining the gel with a 1:10000 dilution of SYBR™ Green II (Molecular Probes, Eugene, OR) for 15 min. The resulting fluorescent signals were detected using the FMBIO® II Fluorescent Scanner (Hitachi Software Engineering America, Ltd., San Bruno, CA).

Population Analyses

Over 200 individuals in each of three population/ethnic groups were analyzed at the CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818, F13A01, FESFPS, F13B, and LPL loci. The African-American and Caucasian-American paternity samples were collected in 50 states and the Hispanic-American paternity samples were collected in the Southwest United States, primarily the states of Texas and Arizona. Allele and genotype frequencies were determined for each locus. Heterozygosity (12) was calculated from the observed numbers of heterozygotes and homozygotes within each sample set. Possible departure from Hardy-Weinberg equilibrium (HWE) was determined by the exact test (13). Matching probability (12), typical paternity index (14), and power of exclusion (14) were calculated as previously defined.

Results and Discussion

Multiplex Development

Candidate loci were originally selected for development based upon their high degree of polymorphism, robust amplification, and minimal artifact bands (15). Fluorescent STR multiplex development has been previously described for the CSF1PO, TPOX, TH01, and vWA loci of the CTTv Multiplex System, and the F13A01, FESFPS, F13B, and LPL loci of the FFFL Multiplex System (4).

With the multiple color detection formats now becoming more broadly available, multiplex systems can be constructed to differentiate loci by both size and color. The PowerPlex™ System is an eight-locus, two-color multiplex which utilizes this approach. It combines primers for the loci of the CTTv Multiplex (CSF1PO, TPOX, TH01, and vWA) with those of the GammaSTR™ Multiplex (D16S539, D7S820, D13S317, and D5S818). The eight STR loci are combined in a single reaction tube for the amplification of genomic DNA. Originally, both multiplexes were developed with one primer for each locus labeled with fluorescein. To achieve separation of all eight loci within the 100 to 400 bp range, the CTTv Multiplex was modified to contain one primer for each locus labeled with carboxy-tetramethylrhodamine (TMR) while the GammaSTR™ Multiplex remained unchanged (i.e., one primer for each locus labeled with fluorescein). All 16 primers for the eight STR loci are combined in one mixture. Allelic ladders (9) have been developed for all eight loci to simplify and accurately determine allele designations.

During the development of the PowerPlex™ System, the various primer concentrations were adjusted to obtain similar product yields for the alleles representing all eight loci. It was apparent from the beginning that unique primer mixes were required for each fluorescent instrument due to the differences in the excitation efficiency of the fluorescent dyes detected by the different instrument lasers. Thus, the PowerPlex™ 1.1 System is customized for the FMBIO® Fluorescent Scanners, while the PowerPlex™ 1.2 System is customized for the Prism™ 377 DNA Sequencer, but will also work with the 373 DNA Sequencer and 310 Genetic Analyzer, a capillary electrophoresis system. Each PowerPlex™ primer mixture was developed independently for each instrument by decreasing and increasing primer concentrations for each locus to obtain a ''balanced'' system (similar amplification yield and sensitivity for all eight loci). This involved examining the allele intensities within a group and between the fluorescent dyes such that the sensitivity with fluorescein- and TMR-labeled loci would be comparable for the eight loci in the multiplex. Both the PowerPlex™ 1.1 System and the PowerPlex™ 1.2 System were balanced to achieve successful amplification when using 1 ng template DNA. In addition to balancing, the amplifications were examined for artifact bands due to primer interaction with other primers in the mixture or with irrelevant genomic DNA sites. For one particular locus, locus D7S820, the primer sequences were modified to eliminate artifact bands generated by incomplete terminal addition of adenine to amplified DNA fragments by Taq DNA Polymerase. Lastly, two distinct PowerPlex™ allelic ladder mixes were prepared and balanced to accommodate the two different instruments.

The PowerPlex™ System has been tested under a range of conditions to validate the system using TWGDAM guidelines. The thermal cycling annealing temperature has been evaluated, as well as the number of amplification cycles, 10X primer concentration, selection and concentration of Taq DNA Polymerase, mixed samples, blood and semen samples, etc. This work has been completed and is in preparation for publication.

A series of DNA templates amplified using the PowerPlex™ 1.1 System and detected using the Hitachi FMBIO® Fluorescent Scanner is shown in Fig. 1. The four loci of the CTTv Multiplex are displayed in red and the GammaSTR™ Multiplex loci are dis-

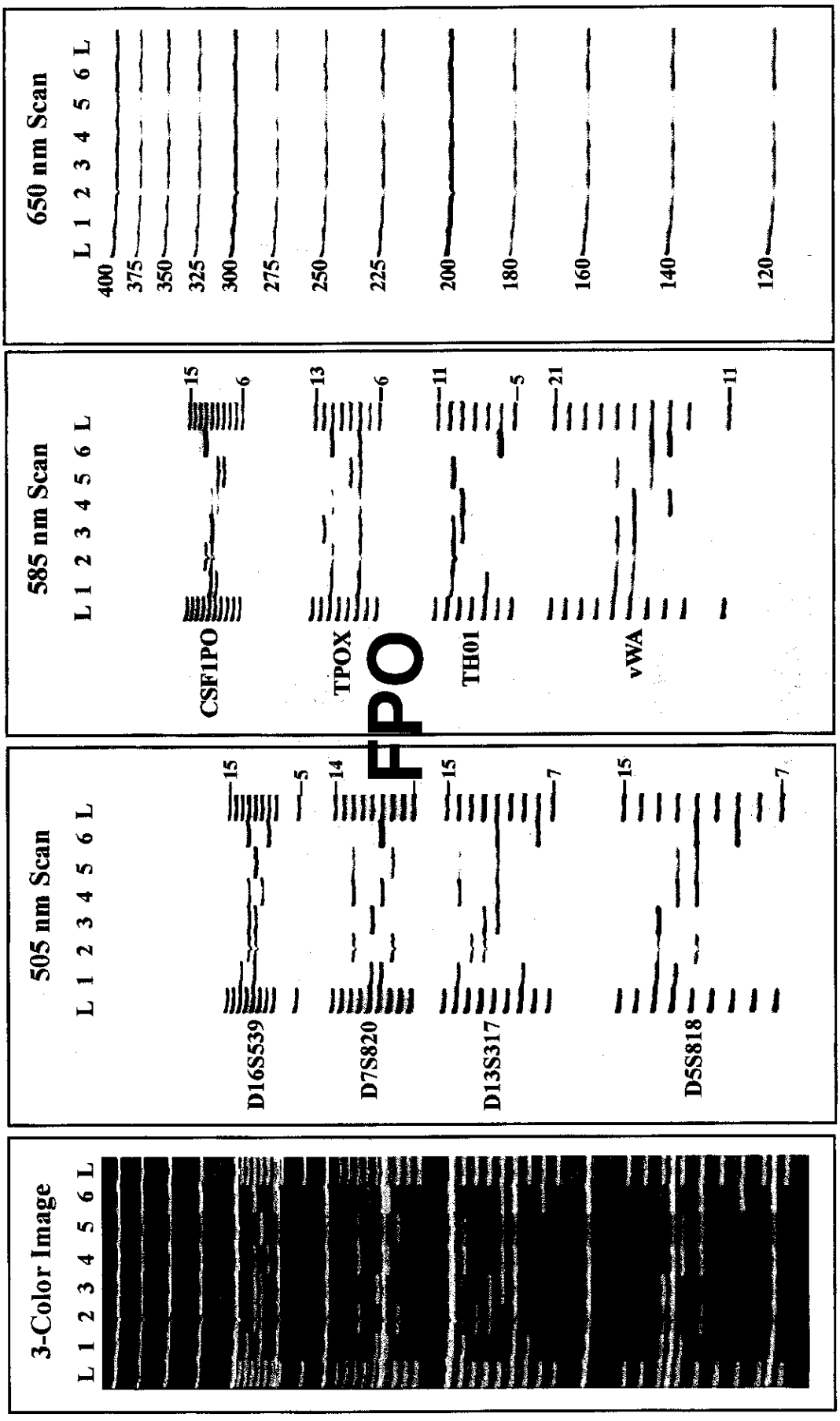

FIG. 1—*The* GenePrint™ *PowerPlex™ 1.1 System detected using the Hitachi FMBIO® Fluorescent Scanner. Six genomic DNA samples (lanes 1–6) were amplified at eight polymorphic loci using the PowerPlex™ 1.1 System, separated in a denaturing 4% polyacrylamide gel, and analyzed in a single gel lane. Panel A displays the three-color image. The CSF1PO, TPOX, TH01 and vWA loci (CTTv Multiplex component) are displayed in red and the D16S539, D7S820, D13S317, and D5S818 loci (GammaSTR™ Multiplex component) are displayed in green. Lanes (L) contain the allelic ladders for each of the corresponding eight loci. The ILS is shown in blue. The color image can be separated into the three individual black-and-white images. Panel B represents the 505 nm scan to detect the fluorescein-labeled fragments (green), Panel C represents the 585 nm scan to detect TMR-labeled fragments (red), and Panel D represents the 650 nm scan to detect CXR-labeled fragments (blue). In Panels B and C, the locus names are labeled to the left and the number of repeats contained within the largest and smallest alleles for each locus are positioned on the right. In Panel D, the sizes of the fragments contained in the ILS are labeled on the left.*

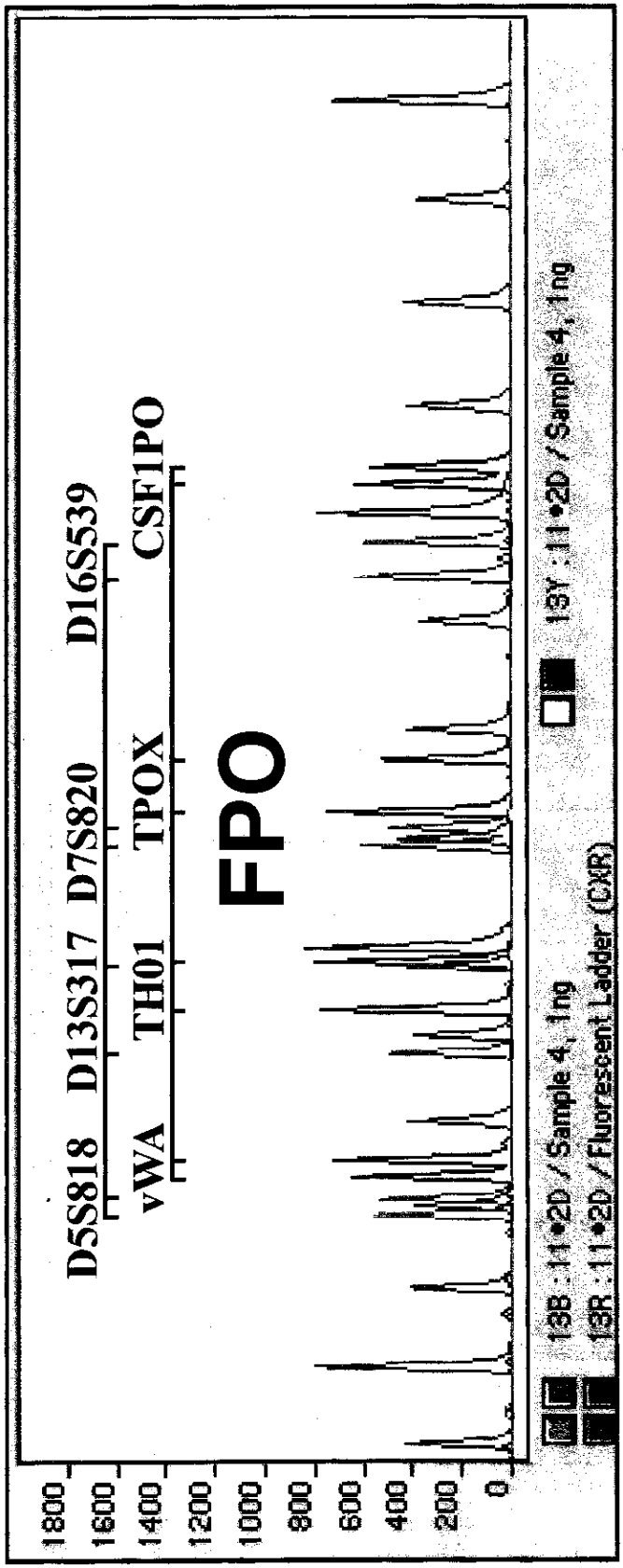

FIG. 2—*The* GenePrint™ *PowerPlex™ 1.2 System. A single genomic DNA sample was amplified using the PowerPlex™ 1.2 System and detected using the ABI Prism™ 377 DNA Sequencer. The CTTv Multiplex loci (CSF1PO, TPOX, TH01, vWA) are displayed in black, the GammaSTR™ loci (D16S539, D7S820, D13S317, D5S818) are displayed in blue, and the ILS is displayed in red.*

played in green. The ILS is shown in blue. Figure 2 is an electropherogram of a single DNA sample amplified using the PowerPlex™ 1.2 System and detected using the ABI Prism™ 377 DNA Sequencer. The locus names are aligned above the corresponding alleles. In this image, the ILS is displayed in red. The amplification of all eight loci is reproducible, with minimal stutter bands (i.e., minor bands one repeat unit smaller than the authentic allele and generated by the amplification of some tandem repeat loci) (16,17). All the loci, except vWA and D5S818, have an average stutter of less than 5% (manuscript in preparation). The loci vWA, D5S818, and several loci not employed in these systems have an average stutter greater than 5%. The low amount of stutter bands is an important feature of these loci, especially in forensic cases in which mixed DNA samples are of concern.

The PowerPlex™ System may be combined with the Amelogenin locus (18,19) for gender identification to provide amplification and detection of nine loci in one reaction tube. Figure 3 shows all nine amplified loci detected using the FMBIO® Fluorescent Scanner. Amelogenin is labeled with carboxy-tetramethylrhodamine (TMR) and displays a 212 base X chromosome-specific band and a 218 base Y chromosome-specific band. The sensitivity of the PowerPlex™ 1.1 System is shown in Fig. 3. As little as 0.2 ng template DNA is successfully amplified with the PowerPlex™ 1.1 System and Amelogenin combination. This combination allows for gender identification and evaluation of eight polymorphic loci for a DNA sample in a single reaction.

Multiplex Characteristics

The chromosome locations, known alleles, and allele size ranges for 12 STR loci are listed in Table 1. All the loci are located on different chromosomes except for the CSF1PO and D5S818 loci, which are more than 50cM apart on chromosome 5 and therefore segregate independently (20).

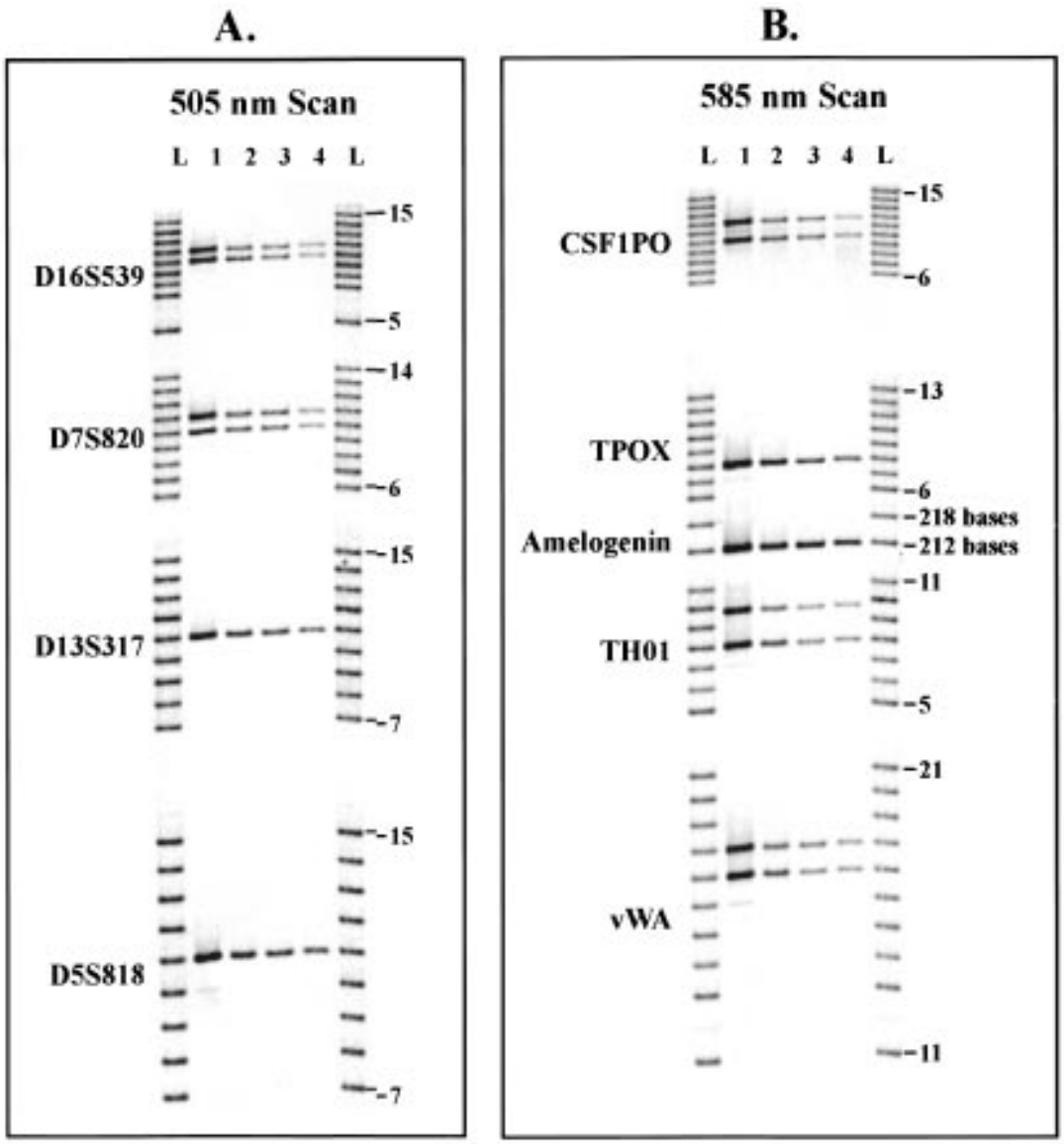

FIG. 3—*Sensitivity of the* GenePrint™ *PowerPlex™ 1.1 System and the* GenePrint™ *Fluorescent Sex Identification System-Amelogenin (TMR). Various amounts of template DNA were amplified using the PowerPlex 1.1 System and Amelogenin, separated in a denaturing 4% polyacrylamide gel, and detected using the Hitachi FMBIO® Fluorescent Scanner. Lanes 1–4 contain 2 ng, 1 ng, 0.5 ng, and 0.2 ng template, respectively. The GammaSTR™ Multiplex loci (D16S539, D7S820, D13S317, D5S818) are displayed in Panel A. The CTTv Multiplex loci (CSF1PO, TPOX, TH01, vWA) and Amelogenin are displayed in Panel B. All nine loci were amplified in one reaction tube and analyzed in a single gel lane.*

TABLE 1—*STR locus characteristics.*

Multiplex system	Locus	Fluorescent dye*	Repeat sequence Edwards	Repeat sequence ISFH	Chromosome location	Known alleles (frequency > .001)	Allele size range (bases)
PowerPlex™ System	CSF1PO	TMR	AGAT	TAGA	5q33.3-34	6-15	291-327
	TPOX	TMR	AATG	TGAA	2p25.1-pter	6-13	224-252
	TH01	TMR	AATG	TCAT	11p15.5	5-9, 9.3, 10-11	179-203
	vWA	TMR	AGAT	TCTA	12p12-pter	11, 13-21	127-167
	D16S539	FL	AGAT	GATA	16q24-qter	5, 8-15	264-304
	D7S820	FL	AGAT	GATA	7q11.21-22	6-14	215-247
	D13S317	FL	AGAT	TATC	13q22-q31	7-15	165-197
	D5S818	FL	AGAT	AGAT	5q23.3-32	7-15	119-151
FFFL Multiplex	F13A01	FL	AAAG	GAAA	6p24.3-25.1	3.2, 4-16	281-331
	FESFPS	FL	AAAT	ATTT	15q25-qter	7-14	222-250
	F13B	FL	AAAT	TTTA	1q31-q32.1	6-12	169-193
	LPL	FL	AAAT	TTTA	8p22	7-14	105-133

*TMR=carboxy-tetramethylrhodamine; FL=fluorescein

TABLE 2—*STR allele sequence variations.*

Locus	Allele(s)	Sequence structure (5'-3')	Sequence variation (5'-3')
CSF1PO	6-15	$[AGAT]_{6-15}$	
TPOX	6-13	$[AATG]_{6-13}$	
TH01	5-11	$[AATG]_{5-11}$	
TH01	9.3	$[AATG]_6[-ATG][AATG]_3$	
vWA	11, 13	$[TCTA][TCTG]_3[TCTA]_{7,9}$	
vWA	14	$[TCTA][TCTG]_3[TCTA]_{10}$	$[TCTA][TCTG]_5[TCTA]_3[TCCA][TCTA]_3$
vWA	15	$[TCTA][TCTG]_4[TCTA]_{10}$	
vWA	16	$[TCTA][TCTG]_4[TCTA]_{11}$	$[TCTA][TCTG]_3[TCTA]_{12}$
vWA	17-20	$[TCTA][TCTG]_4[TCTA]_{12-15}$	
vWA	21	$[TCTA][TCTG]_6[TCTA]_{14}$	
D16S539	5, 8-15	$[AGAT]_{5,8-15}$	
D7S820	6-14	$[AGAT]_{6-14}$	
D13S317	7-15	$[TATC]_{7-15}$	
D13S317	10	$[TATC]_{10}[AATC]$	$[TATC]_{10}[TATC]$
D5S818	7-15	$[AGAT]_{7-15}$	
F13A01	3.2	$[AAAG]_4[A--]$	
F13A01	4-16	$[AAAG]_{4-16}[AGT]$	
FESFPS	7-14	$[AAAT]_{7-14}$	
F13B	6-12	$[AAAT]_{6-12}$	
LPL	7-14	$[AAAT]_{7-14}$	

All 12 loci comprise true tetranucleotide repeats (i.e., each allele within a locus differs by four bases). Within the group, only two microvariants with an allele frequency greater than 0.001 have been observed: TH01 allele 9.3 (21) and F13A01 allele 3.2 (22). The repeat sequences for each locus as defined by two different methods are listed in Table 1. The method described in 1991 by Edwards et al. (1) uses the first alphabetical representation of the STR motif to define the repeat sequence regardless of the strand. Alternatively, in August 1997, the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) published recommendations for STR nomenclature (23). The report states, ''1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5′ nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used.'' Regardless of which method is employed, the allele designations for each of the loci listed are identical except for the locus F13B. In this case, the alleles are one repeat unit larger when using the method described by the DNA Commission of the ISFH. Standardization of the F13B nomenclature will have to be defined.

Allelic ladders (15) were developed for all 12 loci and are analyzed along with unknown samples to allow quick and reliable designation of alleles. All the alleles included in the allelic ladders have been sequenced to confirm the DNA sequence and number of repeat units contained within each allele. The repeat sequence for each allele is listed in Table 2. Within the group of sequenced alleles, we identified one allele for locus D13S317 and two alleles for locus vWA which have two forms that migrate the same distance but differ slightly in sequence. These sequence exceptions are listed in Table 2. Sequence variations in the locus vWA have been previously reported (24).

Statistical Analysis

The allele frequencies, calculated as appropriate sums of genotypic frequencies, for each of the 12 loci and for African-Americans, Caucasian-Americans, and Hispanic-Americans are listed in Tables 3, 4, and 5, respectively. The sample size, observed number of homozygotes and observed number of heterozygotes are shown for each locus. These populations were analyzed to determine if the allele frequencies for each locus are consistent with Hardy-Weinberg equilibrium. The allele frequency data have been compared and correlate closely with previously reported allele frequency data for CSF1PO, F13A01, FESFPS, and LPL (3); TH01 (21); CSF1PO, TPOX, and TH01 (25); TH01, vWA, F13A01, and FESFPS (26).

TABLE 3—*Allele frequencies for African-Americans.*

CTTv Multiplex Components

CSF1PO			TPOX			TH01			vWA		
homozygotes		40	homozygotes		55	homozygotes		60	homozygotes		38
heterozygotes		180	heterozygotes		166	heterozygotes		161	heterozygotes		182
total samples		220	total samples		221	total samples		221	total samples		220
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N
5	0.002	1	6	0.050	22	5	0.005	2	11	0.009	4
6	0.002	1	7	0.034	15	6	0.152	67	12	0.000	0
7	0.066	29	8	0.353	156	7	0.376	166	13	0.011	5
8	0.073	32	9	0.192	85	8	0.233	103	14	0.064	28
9	0.041	18	10	0.113	50	9	0.127	56	15	0.211	93
10	0.273	120	11	0.210	93	9.3	0.090	40	16	0.264	116
11	0.232	102	12	0.048	21	10	0.018	8	17	0.207	91
12	0.261	115	13	0.000	0	11	0.000	0	18	0.143	63
13	0.045	20	All	1.000	442	All	1.000	442	19	0.073	32
14	0.002	1							20	0.016	7
15	0.002	1							21	0.002	1
All	1.000	440							All	1.000	440

GammaSTR™ Multiplex Components

D16S539			D7S820			D13S317			D5S818		
homozygotes		48	homozygotes		52	homozygotes		66	homozygotes		48
heterozygotes		167	heterozygotes		163	heterozygotes		149	heterozygotes		167
total samples		215	total samples		215	total samples		215	total samples		215
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N
5	0.002	1	6	0.002	1	7	0.002	1	7	0.000	0
6	0.000	0	7	0.012	5	8	0.033	14	8	0.065	28
7	0.000	0	8	0.179	77	9	0.019	8	9	0.019	8
8	0.023	10	9	0.084	36	10	0.026	11	10	0.060	26
9	0.205	88	10	0.351	151	11	0.309	133	11	0.258	111
10	0.093	40	11	0.235	101	12	0.414	178	12	0.342	147
11	0.316	136	12	0.112	48	13	0.149	64	13	0.226	97
12	0.202	87	13	0.019	8	14	0.049	21	14	0.028	12
13	0.133	57	14	0.007	3	15	0.000	0	15	0.002	1
14	0.026	11	All	1.000	430	All	1.000	430	All	1.000	430
15	0.000	0									
All	1.000	430									

FFFL Multiplex Components

F13A01			FESFPS			F13B			LPL		
homozygotes		49	homozygotes		44	homozygotes		59	homozygotes		67
heterozygotes		169	heterozygotes		176	heterozygotes		161	heterozygotes		152
total samples		218	total samples		220	total samples		220	total samples		219
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N
3.2	0.087	38	7	0.009	4	6	0.384	169	7	0.000	0
4	0.076	33	8	0.109	48	7	0.157	69	8	0.002	1
5	0.342	149	9	0.057	25	8	0.100	44	9	0.146	64
6	0.131	57	10	0.241	106	9	0.243	107	10	0.370	162
7	0.195	85	11	0.355	156	10	0.114	50	11	0.151	66
8	0.067	29	12	0.182	80	11	0.002	1	12	0.272	119
9	0.009	4	13	0.045	20	12	0.000	0	13	0.059	26
10	0.005	2	14	0.002	1	All	1.000	440	14	0.000	0
11	0.009	4	All	1.000	440				All	1.000	438
12	0.011	5									
13	0.032	14									
14	0.021	9									
15	0.014	6									
16	0.002	1									
All	1.000	436									

TABLE 4—*Allele frequencies for Caucasian-Americans.*

CTTv Multiplex Components

CSF1PO			TPOX			TH01			vWA		
homozygotes		47	homozygotes		76	homozygotes		50	homozygotes		38
heterozygotes		168	heterozygotes		139	heterozygotes		163	heterozygotes		175
total samples		215	total samples		215	total samples		213	total samples		213
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N
6	0.000	0	6	0.002	1	5	0.007	3	11	0.000	0
7	0.000	0	7	0.000	0	6	0.237	101	12	0.000	0
8	0.002	1	8	0.528	227	7	0.148	63	13	0.000	0
9	0.033	14	9	0.093	40	8	0.117	50	14	0.131	56
10	0.251	108	10	0.056	24	9	0.155	66	15	0.082	35
11	0.309	133	11	0.284	122	9.3	0.331	141	16	0.211	90
12	0.330	142	12	0.037	16	10	0.005	2	17	0.265	113
13	0.060	26	13	0.000	0	11	0.000	0	18	0.202	86
14	0.014	6	All	1.000	430	All	1.000	426	19	0.087	37
15	0.000	0							20	0.021	9
All	1.000	430							21	0.000	0
									All	1.000	426

GammaSTR™ Multiplex Components

D16S539			D7S820			D13S317			D5S818		
homozygotes		57	homozygotes		43	homozygotes		61	homozygotes		60
heterozygotes		153	heterozygotes		167	heterozygotes		149	heterozygotes		150
total samples		210	total samples		210	total samples		210	total samples		210
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N
5	0.000	0	6	0.002	1	7	0.000	0	7	0.005	2
6	0.000	0	7	0.010	4	8	0.143	60	8	0.002	1
7	0.000	0	8	0.155	65	9	0.052	22	9	0.010	4
8	0.026	11	9	0.152	64	10	0.052	22	10	0.057	24
9	0.107	45	10	0.295	124	11	0.305	128	11	0.369	155
10	0.079	33	11	0.195	82	12	0.307	129	12	0.350	147
11	0.319	134	12	0.121	51	13	0.083	35	13	0.190	80
12	0.269	113	13	0.057	24	14	0.057	24	14	0.012	5
13	0.167	70	14	0.012	5	15	0.000	0	15	0.005	2
14	0.031	13	All	1.000	420	All	1.000	420	All	1.000	420
15	0.002	1									
All	1.000	420									

FFFL Multiplex Components

F13A01			FESFPS			F13B			LPL		
homozygotes		49	homozygotes		64	homozygotes		51	homozygotes		47
heterozygotes		158	heterozygotes		149	heterozygotes		154	heterozygotes		157
total samples		207	total samples		213	total samples		205	total samples		204
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N
3.2	0.085	35	7	0.000	0	6	0.100	41	7	0.000	0
4	0.041	17	8	0.014	6	7	0.020	8	8	0.002	1
5	0.208	86	9	0.007	3	8	0.259	106	9	0.047	19
6	0.287	119	10	0.284	121	9	0.215	88	10	0.412	168
7	0.329	136	11	0.439	187	10	0.402	165	11	0.287	117
8	0.017	7	12	0.225	96	11	0.002	1	12	0.203	83
9	0.000	0	13	0.028	12	12	0.002	1	13	0.049	20
10	0.000	0	14	0.002	1	All	1.000	410	14	0.000	0
11	0.000	0	All	1.000	426				All	1.000	408
12	0.002	1									
13	0.005	2									
14	0.017	7									
15	0.010	4									
16	0.000	0									
All	1.000	414									

TABLE 5—*Allele frequencies for Hispanic-Americans.*

CTTv Multiplex Components

CSF1PO			TPOX			TH01			vWA		
homozygotes		66	homozygotes		72	homozygotes		53	homozygotes		52
heterozygotes		152	heterozygotes		148	heterozygotes		163	heterozygotes		160
total samples		218	total samples		220	total samples		220	total samples		212
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N
6	0.000	0	6	0.005	2	5	0.000	0	11	0.000	0
7	0.002	1	7	0.002	1	6	0.239	105	12	0.000	0
8	0.005	2	8	0.502	221	7	0.309	136	13	0.005	2
9	0.025	11	9	0.089	39	8	0.086	38	14	0.066	28
10	0.241	105	10	0.052	23	9	0.139	61	15	0.101	43
11	0.296	129	11	0.248	109	9.3	0.218	96	16	0.295	125
12	0.358	156	12	0.102	45	10	0.009	4	17	0.271	115
13	0.060	26	13	0.000	0	11	0.000	0	18	0.165	70
14	0.007	3	All	1.000	440	All	1.000	440	19	0.080	34
15	0.007	3							20	0.017	7
All	1.000	436							21	0.000	0
									All	1.000	424

GammaSTR™ Multiplex Components

D16S539			D7S820			D13S317			D5S818		
homozygotes		45	homozygotes		38	homozygotes		46	homozygotes		48
heterozygotes		162	heterozygotes		169	heterozygotes		161	heterozygotes		159
total samples		207	total samples		207	total samples		207	total samples		207
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N
5	0.000	0	6	0.000	0	7	0.000	0	7	0.063	26
6	0.000	0	7	0.019	8	8	0.087	36	8	0.005	2
7	0.000	0	8	0.099	41	9	0.184	76	9	0.058	24
8	0.012	5	9	0.075	31	10	0.077	32	10	0.058	24
9	0.101	42	10	0.283	117	11	0.229	95	11	0.384	159
10	0.181	75	11	0.266	110	12	0.244	101	12	0.312	129
11	0.300	124	12	0.220	91	13	0.121	50	13	0.114	47
12	0.268	111	13	0.031	13	14	0.053	22	14	0.007	3
13	0.118	49	14	0.007	3	15	0.005	2	15	0.000	0
14	0.019	8	All	1.000	414	All	1.000	414	All	1.000	414
15	0.000	0									
All	1.000	414									

FFFL Multiplex Components

F13A01			FESFPS			F13B			LPL		
homozygotes		45	homozygotes		68	homozygotes		80	homozygotes		66
heterozygotes		177	heterozygotes		142	heterozygotes		137	heterozygotes		144
total samples		222	total samples		210	total samples		217	total samples		210
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N
3.2	0.225	100	7	0.002	1	6	0.051	22	7	0.000	0
4	0.113	50	8	0.012	5	7	0.018	8	8	0.002	1
5	0.227	101	9	0.010	4	8	0.129	56	9	0.029	12
6	0.164	73	10	0.176	74	9	0.362	157	10	0.502	211
7	0.227	101	11	0.452	190	10	0.435	189	11	0.224	94
8	0.014	6	12	0.233	98	11	0.005	2	12	0.207	87
9	0.000	0	13	0.110	46	12	0.000	0	13	0.033	14
10	0.000	0	14	0.005	2	All	1.000	434	14	0.002	1
11	0.007	3	All	1.000	420				All	1.000	420
12	0.000	0									
13	0.005	2									
14	0.005	2									
15	0.007	3									
16	0.007	3									
All	1.000	444									

An exact test for independence of the alleles within individuals was performed for each locus, with the significance levels ($p < 0.050$) found by permutation (2000 permutations of alleles). These p values are shown in Table 6. Only one value (D13S317 in the Caucasian-American database) was below 0.050, and that value was above 0.010. From statistical variation in data analysis alone, we would expect, on average, to observe 1.8 values below 0.050 in the data set of 36 tests described here if the data are in Hardy-Weinberg equilibrium. Thus, the observed uniform distribution of the 36 p-values over the range 0 to 1 is the result expected if there are no departures from HWE at these 12 loci in these three databases.

Associations between frequencies at two loci have been investigated by comparing two-locus genotype frequencies to the products of the corresponding allele frequencies, assuming independence of alleles both within and between loci. The p-values are displayed in Table 7. Overall, there are 15 of 198 values less than 0.050. The rates for African-Americans (3 of 66 values) and Caucasian-Americans (3 of 66 values) are close to the nominal 5% level, and the rate for Hispanic-Americans (9 of 66 values) is higher than the expected normal.

It is noteworthy that 5 of the 9 significant values for Hispanic-Americans involve CSF1PO for which the one-locus p-value was clearly lower than for the other 11 loci in that sample. This observation prompted a retesting for two-locus independence with the genotypes at one locus (e.g., CSF1PO for Hispanic-American database and D13S317 for the Caucasian-American database) held intact and only the alleles at the second locus permuted. This procedure was described by Zaykin et al. (27). For each two-locus test involving CSF1PO or D13S317, the genotypes at that locus were

TABLE 6—*Exact test* p-*values for association at single loci.*

Locus	African-American	Caucasian - American	Hispanic-American
CSF1PO	0.897	0.635	0.072
TPOX	0.128	0.179	0.767
TH01	0.250	0.847	0.822
vWA	0.984	0.982	0.311
D16S539	0.916	0.612	0.377
D7S820	0.462	0.889	0.693
D13S317	0.843	0.021	0.720
D5S818	0.347	0.126	0.346
F13A01	0.213	0.752	0.250
FESFPS	0.570	0.674	0.643
F13B	0.489	0.377	0.679
LPL	0.148	0.079	0.214

TABLE 7—*Exact test* p-*values for association for pairs of loci.*

Loci	African-American	Caucasian-American*	Hispanic-American†
CSF1PO, TPOX	0.080	0.812 (0.833)	0.308 (0.553)
CSF1PO, TH01	0.960	0.548 (0.569)	0.019 (0.054)
CSF1PO, vWA	0.882	0.994 (0.992)	0.387 (0.650)
CSF1PO, D16S539	0.733	0.131 (0.130)	0.355 (0.664)
CSF1PO, D7S820	0.319	0.881 (0.890)	0.413 (0.700)
CSF1PO, D13S317	0.669	0.182 (0.596)	0.003 (0.008)
CSF1PO, D5S818	0.867	0.764 (0.744)	0.043 (0.126)
CSF1PO, F13A01	0.114	0.755 (0.759)	0.004 (0.006)
CSF1PO, FESFPS	0.787	0.854 (0.854)	0.008 (0.038)
CSF1PO, F13B	0.603	0.878 (0.881)	0.497 (0.798)
CSF1PO, LPL	0.290	0.816 (0.826)	0.191 (0.255)
TPOX, TH01	0.263	0.384 (0.391)	0.254 (0.267)
TPOX, vWA	0.239	0.340 (0.340)	0.398 (0.396)
TPOX, D16S539	0.715	0.222 (0.217)	0.770 (0.773)
TPOX, D7S820	0.083	0.126 (0.122)	0.272 (0.263)
TPOX, D13S317	0.035	0.251 (0.698)	0.523 (0.530)
TPOX, D5S818	0.026	0.463 (0.487)	0.096 (0.097)
TPOX, F13A01	0.247	0.532 (0.522)	0.382 (0.356)
TPOX, FESFPS	0.334	0.616 (0.612)	0.510 (0.501)
TPOX, F13B	0.183	0.266 (0.260)	0.330 (0.334)
TPOX, LPL	0.023	0.677 (0.667)	0.603 (0.611)
TH01, vWA	0.453	0.235 (0.244)	0.294 (0.283)
TH01, D16S539	0.337	0.164 (0.161)	0.257 (0.260)
TH01, D7S820	0.155	0.559 (0.559)	0.624 (0.628)
TH01, D13S317	0.101	0.346 (0.776)	0.120 (0.110)
TH01, D5S818	0.193	0.226 (0.232)	0.169 (0.176)
TH01, F13A01	0.371	0.936 (0.944)	0.718 (0.718)
TH01, FESFPS	0.732	0.355 (0.357)	0.390 (0.371)
TH01, F13B	0.189	0.705 (0.695)	0.582 (0.565)
TH01, LPL	0.073	0.793 (0.793)	0.561 (0.574)
vWA, D16S539	0.059	0.308 (0.309)	0.360 (0.341)
vWA, D7S820	0.702	0.891 (0.890)	0.373 (0.355)
vWA, D13S317	0.517	0.103 (0.489)	0.115 (0.120)
vWA, D5S818	0.796	0.420 (0.448)	0.258 (0.273)
vWA, F13A01	0.596	0.345 (0.338)	0.165 (0.172)
vWA, FESFPS	0.903	0.345 (0.363)	0.016 (0.013)
vWA, F13B	0.499	0.323 (0.325)	0.111 (0.110)
vWA, LPL	0.168	0.864 (0.866)	0.606 (0.613)
D16S539, D7S820	0.673	0.345 (0.348)	0.788 (0.796)
D16S539, D13S317	0.086	0.012 (0.073)	0.471 (0.467)
D16S539, D5S818	0.796	0.029 (0.028)	0.383 (0.388)
D16S539, F13A01	0.934	0.326 (0.306)	0.463 (0.474)
D16S539, FESFPS	0.992	0.957 (0.958)	0.261 (0.254)
D16S539, F13B	0.427	0.187 (0.181)	0.471 (0.453)
D16S539, LPL	0.293	0.449 (0.465)	0.663 (0.654)
D7S820, D13S317	0.433	0.358 (0.857)	0.703 (0.698)
D7S820, D5S818	0.710	0.096 (0.090)	0.422 (0.426)
D7S820, F13A01	0.657	0.767 (0.770)	0.162 (0.178)
D7S820, FESFPS	0.342	0.668 (0.657)	0.550 (0.550)
D7S820, F13B	0.437	0.848 (0.855)	0.338 (0.340)
D7S820, LPL	0.663	0.528 (0.548)	0.638 (0.667)
D13S317, D5S818	0.299	0.199 (0.633)	0.395 (0.376)
D13S317, F13A01	0.064	0.065 (0.385)	0.030 (0.028)
D13S317, FESFPS	0.953	0.087 (0.375)	0.015 (0.020)
D13S317, F13B	0.696	0.030 (0.133)	0.084 (0.082)
D13S317, LPL	0.393	0.641 (0.969)	0.357 (0.347)
D5S818, F13A01	0.458	0.118 (0.123)	0.469 (0.475)
D5S818, FESFPS	0.484	0.432 (0.405)	0.179 (0.166)
D5S818, F13B	0.217	0.052 (0.060)	0.568 (0.557)
D5S818, LPL	0.474	0.558 (0.562)	0.971 (0.972)
F13A01, FESFPS	0.720	0.476 (0.467)	0.419 (0.410)
F13A01, F13B	0.216	0.458 (0.472)	0.160 (0.158)
F13A01, LPL	0.145	0.977 (0.974)	0.689 (0.689)
FESFPS, F13B	0.248	0.414 (0.417)	0.031 (0.032)
FESFPS, LPL	0.445	0.813 (0.807)	0.137 (0.147)
F13B, LPL	0.225	0.531 (0.537)	0.626 (0.635)

* Values in parentheses obtained by holding D13S317 genotypes intact.
† Values in parentheses obtained by holding CSF1PO genotypes intact.

held fixed and all the alleles at the second locus were permuted. This leads to a joint test of independence of alleles at the second locus, and independence between alleles at the second locus and genotypes at the first locus. Therefore, there is still a between-locus component to the test. The results are shown in parentheses in Table 7. There is only one significant value for the Caucasian-American database and seven for the Hispanic-American database. Seven is still more than expected by chance, although it can be argued that a more stringent significance level should be used when so many tests (e.g., 66) are being conducted. The analysis of a larger Hispanic-American database will confirm or reject this result.

In 1996, the National Research Council published a report entitled, ''The Evaluation of Forensic DNA Evidence'' (28). This work recommended that attention to formal testing for independence for alleles within and between loci no longer be emphasized. Instead, acknowledgment is to be given to the possibility of departures from Hardy-Weinberg equilibrium at single loci, and recognition is to be given to the fact that any dependencies among loci would be small. Even though our two-locus independence testing showed more significant values than expected, the one-locus testing is consistent with HWE. In addition, the majority of significant values for the two-locus independence testing occurred in only one of three databases. The results of the two-locus independence testing in this report, therefore, have some scientific interest but little bearing on the forensic uses of these data.

We plan to provide our collated genotype data on the Internet so that other scientists may analyze, compare, or combine our population data with their own results. The Internet site will provide a very comprehensive collection of genotype data gathered for various population/ethnic groups. The allele frequency data for all three population groups are currently available in the *GenePrint*™ STR technical manuals and a subset of the frequency data has been published elsewhere (29).

Mutation Rates

At least 300 mother-child genotype pairs were examined for each of the 12 STR loci for direct evidence of mutation. At the locus D13S317, one of 317 mother-child pairs showed evidence of mutation. In this one occurrence, the genotype for the mother is an 11,11 and the child is a 12,12 homozygote. The ethnic background of the mother-child pair is African-American. The mutation rate is estimated as $1/n$ with a standard deviation estimated as $\sqrt{(n-1)/n^3}$ where n is the sample size (opportunities for recombination). Thus, the calculated mutation rate for D13S317 is $3.15 \times 10^{-3} \pm 3.15 \times 10^{-3}$. For the other 11 STR loci, no mutation events were observed. As a combined group, the average mutation rate is 2.6×10^{-4}, approximately tenfold lower than the observed average mutation rate (2.1×10^{-3}) with other described tetranucleotide STR loci (30,31).

Power of Exclusion and Typical Paternity Indices

For parentage testing, the typical paternity index and the power of exclusion were calculated and are shown in Table 8. The typical paternity index is above 2600 and the power of exclusion is above 0.99974 in each population group tested when using all 12 loci described in this work. For each of the three population groups, the dataset consisted of at least 100 paternity cases previously determined to be exclusions by RFLP analysis. In the over 250 exclusionary cases examined at all 12 loci, each alleged father was excluded by at least one locus out of a total of 12 loci. Table 9 lists the number of exclusions observed when 12 loci were employed and when using only 8 loci of the PowerPlex™ System.

TABLE 8—*Multiplex system population characteristics.*

	African-American	Caucasian-American	Hispanic-American
CTTv Multiplex			
Matching probability	1 in 25236	1 in 6796	1 in 7219
Typical paternity index	29.39	19.26	10.51
Power of exclusion	.967	.953	.918
GammaSTR™ Multiplex			
Matching probability	1 in 10872	1 in 16790	1 in 20106
Typical paternity index	16.93	13.51	30.40
Power of exclusion	.946	.934	.967
PowerPlex™ System (CTTv Multiplex and GammaSTR™ Multiplex)			
Matching probability	1 in 2.74×10^8	1 in 1.14×10^8	1 in 1.45×10^8
Typical paternity index	498	260	319
Power of exclusion	.9982	.9969	.9973
FFFL Multiplex			
Matching probability	1 in 16802	1 in 2658	1 in 3276
Typical paternity index	16.83	15.28	8.23
Power of exclusion	.946	.941	.902
All Twelve Loci (PowerPlex™ System and FFFL Multiplex)			
Matching probability	1 in 4.61×10^{12}	1 in 3.03×10^{11}	1 in 4.75×10^{11}
Typical paternity index	8373	3976	2627
Power of exclusion	.99990	.99981	.99974

TABLE 9—*Parentage exclusions using a combination of STR loci.*

Number of excluding loci (out of 12)	Number of non-fathers excluded			Number of excluding loci (out of 8)	Number of non-fathers excluded		
	African-American	Caucasian-American	Hispanic-American		African-American	Caucasian-American	Hispanic-American
0	0	0	0	0	1	0	2
1	2	0	1	1	2	1	4
2	2	1	3	2	8	9	5
3	1	4	1	3	19	10	14
4	4	7	9	4	18	30	18
5	16	15	15	5	23	25	22
6	25	22	17	6	21	14	18
7	12	16	14	7	4	8	4
8	18	17	16	8	1	0	0
9	10	5	5				
10	3	3	2				
11	1	0	0				
12	0	0	0				
Totals	94	90	83	Totals	97	97	87
Average	6.5	6.4	6.2	Average	4.4	4.4	4.3

Using 12 loci, the average number of exclusions per paternity trio is six exclusions. None of the exclusionary cases were falsely included when using the PowerPlex™ System and FFFL Multiplex (12 loci). However, when only eight loci are employed, three out of 281 cases did not show evidence of an exclusion.

Matching Probability

In forensic science, the genotype data from as few as two samples are compared to determine if the samples originated from the same person. If the samples are determined to be a match, the probability of such an occurrence is determined. The matching probabilities for the PowerPlex™ System, its component CTTv Multiplex and GammaSTR™ Multiplex, as well as the FFFL Multiplex are shown in Table 8. When all 12 loci are evaluated in a pair of amplification reactions employing the PowerPlex™ System and FFFL Multiplex System, the matching probabilities are at least 1 in 303 billion for each population group studied.

Summary

The 12 STR loci described in this work display low stutter bands, contain few microvariants, and exhibit low mutation rates, making them ideal for human identification applications. The PowerPlex™ System, FFFL Multiplex, CTTv Multiplex, and GammaSTR™ Multiplex developed for the fluorescence detection and analysis of short tandem repeat loci are reliable and highly discriminating. The high-throughput methods allow the fast, accurate genotyping of large numbers of samples as required for generating databases. In addition, the allelic ladders developed with each multiplex system provide accurate, reliable designation of alleles. The use of allelic ladders and independent in-lane size standards allows laboratories to compare and share data confidently, even if using dissimilar gel electrophoresis methods and detection methods.

The genotype data for over 200 individuals in three distinct population/ethnic groups have been used to calculate the allele frequencies for all 12 loci. The exact test was applied to show Hardy-Weinberg equilibrium and independence of alleles both within and between loci. The product rule may be used for statistical determinations as the mapping data for all 12 loci indicate that the loci segregate independently. For forensic science applications, the PowerPlex™ System and FFFL Multiplex may be employed to obtain a matching probability of at least 1 in 303 billion. The low mutation rates for all 12 STR loci and typical paternity indices which exceed 2600 support the usefulness of STR multiplex systems for parentage applications.

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References

1. Edwards A, Civitello A, Hammond HA, Caskey CT. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 1991;49:746–56.
2. Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 1992;12:241–53.
3. Hammond HA, Jin L, Zhong Y, Caskey CT, Chakraborty R. Evaluation of 13 short tandem repeat loci for use in personal identification applications. Am J Hum Genet 1994;55:175–89.
4. Lins AM, Sprecher CJ, Puers C, Schumm JW. Multiplex sets for the amplification of polymorphic short tandem repeat loci—silver stain and fluorescence detection. BioTechniques 1996;20:882–9.
5. Kimpton CP, Gill P, Walton A, Urquhart A, Millican ES, Adams M. Automated DNA profiling employing multiplex amplification of short tandem repeat loci. PCR Methods and Applications 1993; 3:13–22.
6. Schumm JW, Lins AM, Sprecher CJ, Micka KA. High throughput systems for analysis of STR loci. Proceedings from the Sixth International Symposium on Human Identification. 1996;10–9; Scottsdale (AZ).
7. Oldroyd NJ, Urquhart AJ, Kimpton CP, Millican ES, Watson SK, Downes T, et al. A highly discriminating octoplex short tandem repeat polymerase chain reaction system suitable for human individual identification. Electrophoresis 1995;16:334–7.
8. Promega Corporation. GenePrint™ STR Systems Technical Manual, Part #TMD004 (revised 2/96). Madison, WI, 1996.
9. Puers C, Lins AM, Sprecher CJ, Brinkmann B, Schumm JW. Analysis of polymorphic short tandem repeat loci using well-characterized allelic ladders. Proceedings from the Fourth International Symposium on Human Identification. Scottsdale (AZ): Promega Corporation. 1994;161–72.
10. Promega Corporation. GenePrint™ PowerPlex™ 1.1 System Technical Manual, Part #TMD008 (revised 9/97). Madison, WI, 1997.
11. Promega Corporation. GenePrint™ Fluorescent STR Systems Technical Manual, Part #TMD006 (revised 8/97). Madison, WI, 1997.

12. Jones DA. Blood samples: probability of discriminations. J Forensic Sci 1992;12:355–9.
13. Weir, BS. Genetic data analysis II. Sunderland: Sinauer Associates, Inc., 1996.
14. Brenner C, Morris JW. Paternity index calculations in single locus hypervariable DNA probes: validation and other studies. Proceedings for the International Symposium on Human Identification. Promega Corporation. 1989;21–53.
15. Sprecher CJ, Puers C, Lins AM, Schumm JW. A general approach to analysis of polymorphic short tandem repeat loci. BioTechniques 1996;20:266–76.
16. Levinson G, Gutman GA. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol 1987;4:203–21.
17. Schlotterer C, Tautz D. Slippage synthesis of simple sequence DNA. Nucl Acids Res 1992;20:211–5.
18. Sullivan KM, Mannucci A, Kimpton CP, Gill P. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. BioTechniques 1993;15: 636–41.
19. Mannucci A, Sullivan KM, Ivanov PL, Gill P. Forensic application of a rapid and quantitative DNA sex test by amplification of the X-Y homologous gene amelogenin. Int J Leg Med 1994;106:190–3.
20. The Genome Database, An international collaboration in support of the Human Genome Project. *http://gdbwww.gdb.org/gdb/*
21. Puers C, Hammond HA, Jin L, Caskey CT, Schumm JW. Identification of repeat sequence heterogeneity at the polymorphic short tandem repeat locus HUMTH01 $[AATG]_n$ and reassignment of alleles in population analysis by using a locus-specific allelic ladder. Am J Hum Genet 1993;53:953–8.
22. Puers C, Hammond HA, Caskey CT, Lins AM, Sprecher CJ, Brinkmann B, et al. Allelic ladder characterization of the short tandem repeat polymorphism located in the 5′ flanking region to the human coagulation factor XIII A subunit gene. Genomics 1994;23:260–4.
23. Bar W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, et al. DNA recommendations: Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. Int J Legal Med 1997;110:175–6.
24. Moller A, Meyer E, Brinkmann B. Different types of structural variation in STRs: HumFES/FPS, HumVWA and HumD21S11. Int J Leg Med 1994;106:319–23.
25. Comey C, Koons BW, Budowle B. Analysis of four populations at the tetrameric short tandem repeat (STR) loci CSF1PO, TPOX, and TH01. Proceedings from the Fifth International Symposium on Human Identification. Promega Corporation. 1994;165.
26. Gill P, Evett I. Population genetics of short tandem repeat (STR) loci. Genetica 1995;96:69–87.
27. Zaykin D, Zhizotozsky L, Weir BS. Exact test for association between alleles at arbitrary number of loci. Genetica 1995;96: 169–78.
28. National Research Council. The Evaluation of Forensic DNA Evidence. Washington, DC: National Academy Press, 1996.
29. Schumm JW, Lins AM, Micka KA, Sprecher CJ, Rabbach DR, Bacher JW. Automated fluorescent detection of STR multiplexes—Development of the *GenePrint*™ PowerPlex™ and FFFL multiplexes for forensic and paternity applications. Proceedings from the First European Symposium on Human Identification. Toulouse (France). 1997;90–104.
30. Weber JL, Wong C. Mutation of human short tandem repeats. Hum Mol Genetics 1993;2:1123–8.
31. Heyer E, Puymirat J, Dieltjes P, Bakker E, de Knijff P. Estimating Y chromosome specific microsatellite mutation frequencies using deep rooting pedigrees. Hum Mol Genetics 1997;6:799–803.

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