

Efficiency of Two Different Nine-Loci Short Tandem Repeat Systems for DNA Typing Purposes

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Background: Genotyping based on short tandem repeat (STR) regions is widely used in human identification and parentage testing, in gene mapping studies, and as an approach to studies on the etiopathogenesis and diagnosis of hereditary diseases. We wished to study a new analytical approach that uses capillary electrophoresis and multicolor fluorescence in place of slab gel electrophoresis.

Methods: We evaluated the efficiency for parentage and forensic purposes of the AmpFLSTR Profiler Plus™ typing kit that is used with the ABI Prism 310 Genetic Analyzer (System-2 STR), and that of a widely used panel of nine STRs analyzed with conventional slab-gel electrophoresis followed by radioactive detection (System-1 STR). System-2 STR, based on automated capillary electrophoresis and automated sizing of the alleles by Genotyper 2.0 software, was used to determine the allele frequency of the nine loci in 157 Caucasian subjects from southern Italy. On the basis of the data obtained, we submitted 40 trios to parentage testing.

Results: A higher median probability of paternity attribution and power of exclusion were obtained with System-2 STR vs System-1 STR: respectively, 99.99% and 99.95% ($P < 0.05$) for attribution; and five and four excluding loci ($P < 0.05$) for exclusion. The most informative and highly discriminating loci were *D18S51*, *D21S11*, and *FGA*. The combined probability of matching-by-chance for all nine STRs was 1.36×10^{-12} for System-2 compared with 1.11×10^{-7} obtained with the

other system. The internal standard and allelic ladder of the System-2 STR facilitated accurate and precise genotyping; furthermore, System-2 STR was faster than the conventional System-1 STR.

Conclusions: The System-2 STR allows rapid testing with higher probabilities of attribution and a higher power of exclusion than with the comparison method with slab-gel electrophoresis.

The application of DNA typing in laboratory medicine is increasing, with uses in paternity and maternity assessment; human identification; bone marrow transplantation follow up; genetic disease diagnosis validation, including testing for chorionic villi contamination by maternal blood during prenatal diagnosis; and genetic mapping (1–5). In the clinical laboratory, most DNA-typing applications frequently have legal and ethical implications. Hence, there is a particular need for tests that have high reliability and diagnostic efficiency.

The most widely used methodology for DNA typing is PCR analysis of various short tandem repeat (STR) loci (6, 7). The STR loci are polymorphic markers consisting of a variable number of tandem repeats, also called “microsatellites”, ranging from two to six nucleotides per locus. Because of their short span, they can be amplified easily, and the corresponding radiolabeled or fluorescent-stained alleles can be separated by acrylamide gel electrophoresis (7–9). Amplified STR fragments are generally separated and detected by slab-gel electrophoresis; however, very recently capillary electrophoresis (10) combined with a genetic analyzer to automate the methodology (11, 12) has been proposed for this procedure.

The aim of this study was to apply a nine-loci STR system based on the latter technology (System-2 STR) to a variety of DNA-typing cases to determine the parameters indicative of the efficiency of the test. We used the AmpFLSTR Profiler Plus™, which includes a panel of nine STRs (*D3S1358*, *FGA*, *vWA*, *D21S11*, *D18S51*, *D5S818*,

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D7S820, *D13S317*, and *D8S1179*), combined with the fully automated ABI Prism 310 Genetic Analyzer. This instrument is based on capillary electrophoresis and multicolor fluorescence. We first estimated the allele frequencies of the STR-2 loci in 157 subjects of a Caucasian population from southern Italy; we then evaluated the performance of this approach for paternity testing on 40 paternity trios. We then compared the efficiency of System-2 STR for the attribution and/or exclusion of paternity and for forensic purposes with the efficiency of the conventional methodology, also based on the nine-loci STR (System-1 STR) currently used in our laboratory (7). The results of this study show that System-2 STR is better than System-1 STR for routine DNA typing.

Materials and Methods

SAMPLE POPULATION

Peripheral blood, obtained at the blood bank of our Medical School (University of Naples "Federico II") from 157 volunteer donors resident in the Campania area, was collected by venipuncture using EDTA as anticoagulant. The study was approved by the Ethics Board on Human Subjects of our University, and informed consent was obtained from all individuals directly (adults) or from the parents (children). The subjects analyzed were unrelated, and their parents and grandparents had been born in the Campania area. The volunteers were screened for all conventional disease markers, and none of them reported a history of genetic diseases. We calculated the allele frequencies of this population, using System-2 STR; the allele frequencies of the same population obtained with System-1 STR are described elsewhere (7), as are the allele frequencies in a representative sample of the general US Caucasian population obtained with System-2 STR (13). We also examined 120 DNA samples from paternity trios (mother, child, and alleged father) with both systems.

GENOMIC DNA

Genomic DNA was isolated from leukocytes of all subjects with a phenol-proteinase K procedure (14). The purified DNA was quantitated by ultraviolet absorbance spectrophotometry at 260 nm.

SYSTEM-1 STR ANALYSIS

Genomic DNA (20 ng) was amplified using TC 480 or TC 600 instruments (Perkin-Elmer Applied Biosystems). We analyzed nine STR loci (see Table 1, System-1 STR), using three triplex PCR reactions (6, 7, 15). The PCR products labeled with ³²P-dCTP were diluted 1:1 in formamide loading buffer, and 2.5 mL were electrophoresed on 0.4-mm thick, 4% acrylamide: bis-acrylamide (39:1, by weight) gels containing 7 mol/L urea. Electrophoresis was performed for 2 h at 1.5 V on a slab-gel electrophoresis apparatus (Model S2 Sequencing Gel; Life Technologies). The alleles were detected by autoradiography after exposure of the slab to film for 18–24 h at –70 °C. Allele sizes were assigned either using a sequence ladder or by

Table 1. Characteristics of the nine STR loci analyzed with System-1 and System-2 STR.

Locus	Chromosome	Product length, bp ^a	Common sequence motif
System-1 STR			
<i>HUMHPRTB</i>	Xq26	259–299	(AGAT) _n
<i>HUMFABP</i>	4q28–q31	199–220	(AAT) _n
<i>HUMCD4</i>	12p12–pter	125–175	(AAAAG) _n
<i>HUMCSF1PO</i>	5q33.3–34	295–327	(AGAT) _n
<i>HUMTHO1</i>	11p15.5	179–203	(AATG) _n
<i>HUMPLA2A1</i>	12q23–pter	118–139	(AAT) _n
<i>HUMF13A01</i>	6p24–25	281–331	(AAAAG) _n
<i>HUMCYAR04</i>	15q21.1	173–201	(AAAT) _n
<i>HUMLIPOL</i>	8p22	125–175	(AAAT) _n
System-2 STR			
<i>D3S1358</i>	3p	114–142	TCTA (TCTG) _{1–3} (TCTA) _n
<i>vWA</i>	12p12–pter	157–197	TCTA (TCTG) _{3–4} (TCTA) _n
<i>FGA^a</i>	4q28	219–267	(TTTC) ₃ TTTT TTCT (CTTT) _n CTCC (TTCC) ₂
<i>D8S1179</i>	8	128–168	(TCTA/G) _n
<i>D21S11^b</i>	21	189–243	(TCTA) _n (TCTG) _n (TCTA) ₃ TA (TCTA) ₃ TCA (TCTA) ₂ TCCA TA (TCTA) _n
<i>D18S51^b</i>	18q21.3	273–341	(AGAA) _n
<i>D5S818</i>	5q21–31	135–171	(AGAT) _n
<i>D13S317</i>	13q22–31	206–234	(GATA) _n
<i>D7S820</i>	7q	258–294	(GATA) _n

^a bp, base pairs.

^b Complex tetramers that has a 2-bp deletion, giving rise to effective dimerism.

comparison with samples with known alleles (RJK 1094 and RJK 1258 cell lines) (6).

SYSTEM-2 STR ANALYSIS

Genomic DNA (2 ng) were amplified in a final volume of 25 μL, using the TC 480 or TC 9600 instruments with the AmpFLSTR Profiler Plus PCR amplification kit (Perkin-Elmer Applied Biosystems). This kit contains an AmpFL-STR PCR reaction mix, AmpliTaq Gold DNA polymerase, and a primer set (one of each pair is labeled with the fluorescent dye 5-FAM, JOE, or NED from Perkin-Elmer Applied Biosystems). We used the kit to amplify nine STR loci (Table 1, System-2 STR). The kit can also be used to amplify in a single reaction tube the *Amelogenin* locus for sex determination (13). The amplification conditions consisted of an initial denaturation of the double-stranded DNA by heating to 95 °C for 11 min, followed by 28 cycles of amplification, with the following conditions: 1 min at 94 °C for denaturation, 1 min of annealing at 54 °C, and 1 min of extension at 72 °C. The last cycle was followed by an additional 45 min at 72 °C to obtain the final extension. For each PCR analysis of a group of DNA patients, we always analyzed both a negative-control sample (no DNA) and a positive-control sample (DNA typed previously).

PCR products were then analyzed by capillary electrophoresis on the ABI Prism 310 (Perkin-Elmer Applied Biosystems) together with an allelic ladder that contained all of the most common alleles for the analyzed loci that were present in US Caucasians (13). Typically, 1.5 μ L of each sample was diluted in 15 μ L of deionized formamide; each sample was supplemented with 0.5 μ L of an internal size standard (ROX 500; Perkin-Elmer Applied Biosystems) labeled with an additional fluorophore. The samples were denatured at 95 °C for 4 min and then placed in the autosampler tray (maximum of 96 samples) on the ABI Prism 310 for automatic injection into the capillary. We assigned allele size at each locus by comparing the sizes obtained for the alleles in the unknown samples to the sizes obtained for the alleles in the allelic ladder. This approach reveals differences down to one to two base pairs at each locus (11, 12).

ELECTROPHORESIS INSTRUMENTATION

The ABI Prism 310 Genetic Analyzer is an argon ion laser-based capillary electrophoresis system. The voltage for electrophoresis is between 100 and 15 000 V, and the temperature is maintained between room temperature and 60 °C. The high denaturing conditions used are essential to obtain reproducible results for fragment sizes (12).

The ABI Prism GeneScan 2.1 and Genotyper 2.0 software give the exact size of each DNA fragment, automatically assign genotypes, and create a table of allele sizes in each sample.

STATISTICAL ANALYSIS

The SE of allele frequencies, the test of Hardy-Weinberg equilibrium (16), mean, median, regression analysis, *t*-test, and Wilcoxon signed-rank test (17) were performed using StatView 4.5 software for Macintosh computers. Mathematical formulas for forensic purposes (mean probability match, typical paternity index, typical probability of paternity, power of exclusion, and discrimination power) were calculated as described previously [see the appendix in Ref. (7) and Refs. (18, 19)], using Excel 5.0 software.

Results

The allele frequencies of the nine STRs obtained with System-2 STR in 157 unrelated Caucasian subjects from southern Italy are reported in Table 2. The allele frequencies of the nine System-1 STR loci are reported elsewhere (7).

Regression analysis did not reveal significant differences in the allele frequencies of the nine System-2 STR loci between the Italian Caucasian population and the US Caucasian population (see Table 2). The regression plot obtained for locus *D18S51* is shown, as an example, in Fig. 1.

The numbers of heterozygotes detected in all loci analyzed were compared with the expected numbers, and no excess homozygosity emerged. Thus, we concluded that our population was in Hardy-Weinberg equilibrium for these loci (Table 3). Seven parameters useful for the

Table 2. Allele frequencies (percentage \pm SE) at nine STR loci^a (System-2 STR).

<i>D3S1358</i>		<i>FGA</i>		<i>VWA</i>	
Allele ^b	Frequency (\pm SE)	Allele	Frequency (\pm SE)	Allele	Frequency (\pm SE)
13	0.6 (\pm 0.4)	17	0.3 (\pm 0.3)	13	0.3 (\pm 0.3)
14	6.7 (\pm 1.4)	18	1.3 (\pm 0.6)	14	7 (\pm 1.4)
15	26.4 (\pm 2.4)	19	6.4 (\pm 1.3)	15	11.5 (\pm 1.7)
16	23.9 (\pm 2.4)	20	9.5 (\pm 1.6)	16	22.3 (\pm 2.3)
17	23.3 (\pm 2.3)	21	16.3 (\pm 2.0)	17	30.3 (\pm 2.5)
18	17.8 (\pm 2.1)	22	17.5 (\pm 2.1)	18	19.4 (\pm 2.2)
19	1.3 (\pm 0.6)	22.2	1.3 (\pm 0.6)	19	7.3 (\pm 1.4)
		23	16.9 (\pm 0.7)	20	1.9 (\pm 0.7)
		24	16.9 (\pm 0.7)		
		25	8.6 (\pm 1.5)		
		26	3.8 (\pm 1.0)		
		27	0.3 (\pm 0.3)		
		28	0.6 (\pm 0.4)		
		30	0.3 (\pm 0.3)		
<i>D21S11</i>		<i>D18S51</i>		<i>D5S818</i>	
Allele	Frequency (\pm SE)	Allele	Frequency (\pm SE)	Allele	Frequency (\pm SE)
26	0.6 (\pm 0.4)	10	0.6 (\pm 0.4)	7	0.3 (\pm 0.3)
27	2.2 (\pm 0.8)	11	3.2 (\pm 0.9)	8	0.9 (\pm 0.5)
28	13.8 (\pm 1.9)	12	14 (\pm 1.9)	9	2.9 (\pm 0.9)
29	24.5 (\pm 2.4)	13	15.7 (\pm 2.0)	10	6.7 (\pm 1.4)
29.2	0.3 (\pm 0.3)	13.2	0.3 (\pm 0.3)	11	31.5 (\pm 2.6)
30	24.9 (\pm 2.4)	14	13.4 (\pm 1.8)	12	35.4 (\pm 2.6)
30.2	4.8 (\pm 1.2)	15	15.7 (\pm 2.0)	13	20.7 (\pm 2.2)
31	3.5 (\pm 1.0)	16	14.3 (\pm 1.9)	14	1.6 (\pm 0.7)
31.2	10.2 (\pm 1.6)	17	11.1 (\pm 1.7)		
32	0.6 (\pm 0.4)	18	5.7 (\pm 1.3)		
32.2	10.5 (\pm 1.6)	19	3.5 (\pm 1.0)		
33.2	3.5 (\pm 1.0)	20	1.6 (\pm 0.7)		
34.2	0.6 (\pm 0.4)	21	0.3 (\pm 0.3)		
		22	0.6 (\pm 0.4)		
<i>D7S820</i>		<i>D13S317</i>		<i>D8S1179</i>	
Allele	Frequency (\pm SE)	Allele	Frequency (\pm SE)	Allele	Frequency (\pm SE)
7	0.9 (\pm 1.6)	8	8.9 (\pm 1.6)	8	2.2 (\pm 0.8)
8	17.2 (\pm 2.1)	9	10.8 (\pm 1.7)	9	1.9 (\pm 0.7)
9	9.5 (\pm 1.6)	10	8 (\pm 1.5)	10	10.2 (\pm 1.7)
10	29.1 (\pm 2.5)	11	25.1 (\pm 2.4)	11	11.8 (\pm 1.8)
11	22.6 (\pm 2.3)	12	33.8 (\pm 2.6)	12	9.5 (\pm 1.6)
12	18.8 (\pm 2.2)	13	12.1 (\pm 2.8)	13	22.6 (\pm 2.3)
13	1.9 (\pm 0.7)	14	1.3 (\pm 0.6)	14	19.2 (\pm 2.2)
				15	17.5 (\pm 2.1)
				16	3.8 (\pm 1.0)
				17	1.3 (\pm 0.6)

^a For all loci, 314 chromosomes were examined.

^b Alleles are identified by the number of repeats.

application of STR to forensic and paternity tests (see *Materials and Methods*) are also reported in Table 3. The most informative loci for the highest power of exclusion (ranging from 0.82 to 0.75) and for the lowest mean probability match (ranging from 0.01 to 0.03) were

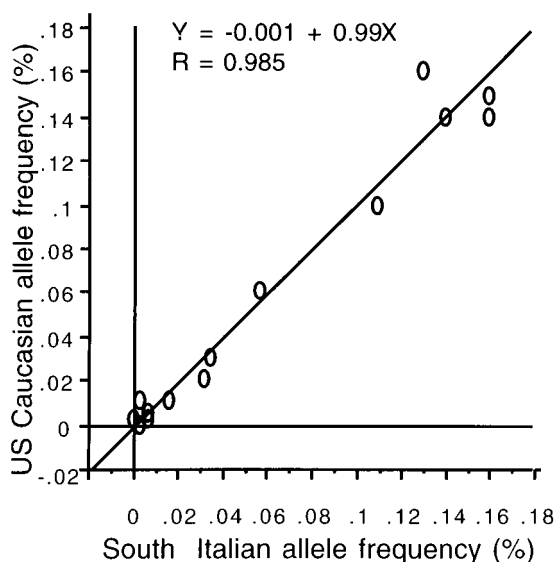


Fig. 1. Example of the regression analysis performed between the allele frequencies detected in the US and southern Italian Caucasian populations at the *D18S51* locus by System-2.

D18S51, *D21S11*, and *FGA*. The same parameters for the System-1 STR loci are reported elsewhere (7).

The combined match probability of the nine System-2 STR loci was lower than that of the nine System-1 STR loci, i.e., 1.36×10^{-12} vs 1.11×10^{-7} , respectively, thus indicating that the System-2 STR has a much stronger interindividual discriminative power.

We tested 40 paternity trios, analyzed previously in our laboratory by System-1 STR, by the System-2 STR to establish the diagnostic efficiency of the latter loci for both the attribution and exclusion of paternity. Fig. 2A shows an example of paternity attribution with System-2 STR; Fig. 2B shows an example of paternity exclusion.

Twenty of the 40 paternity trios were attributions. They were all confirmed when evaluated with the System-2

STR, which yielded a cumulative median paternity probability (%) higher than that obtained with System-1 STR, i.e., 99.99% vs 99.95% ($P < 0.05$). In only two paternity cases did System-1 STR appear slightly better than System-2 STR. The other 20 cases were paternity exclusions. The exclusion results obtained with System-2 STR were consistent with those of System-1 STR; however the mean number of excluding loci with System-2 STR was greater than that obtained with System-1 STR, i.e., five vs four ($P < 0.05$).

The sizing reproducibility of System-2 STR for parentage testing was assessed by analysis of the same paternity trio for 5 days under identical analytical conditions. We invariably obtained identical allele sizing for each allele at each locus and for each of the three samples of the trios [between-day imprecision (CV) = 0%].

To verify the within-day imprecision of the ABI Prism 310 in sizing DNA fragments from injection to injection, each day we analyzed an allelic ladder as a blind sample for the machine (in addition to the sample ladder measured routinely). We obtained the identical size for all the alleles in the ladder.

Discussion

PCR analysis of highly polymorphic STR loci is the method of choice for human identification, in that it can also be used to type old or severely degraded DNA, which is often available only in very low amounts (6). The detection of radioactive- or fluorescent-labeled PCR-amplified DNA fragments is usually performed by manual or semiautomated slab-gel electrophoresis (6–9). Recently, a new instrument, the ABI Prism 310 Genetic Analyzer, which incorporates capillary electrophoresis and is capable of detecting fluorescent DNA fragments at four wavelengths simultaneously, has become available (11, 12).

We tested the panel of nine STR loci included in the

Table 3. Seven parameters calculated for the System-2 STR loci in the southern Italian Caucasian population useful for their application in paternity and forensic tests.^a

	<i>D3S1358</i>	<i>FGA</i>	<i>vWA</i>	<i>D8S1179</i>	<i>D21S11</i>	<i>D18S51</i>	<i>D5S818</i>	<i>D13S317</i>	<i>D7S820</i>
Observed heterozygotes (n = 157) ^b	121	137	126	130	139	143	123	123	127
Expected heterozygotes ± SE	124.1 ± 5.1	145.3 ± 3.2	126.4 ± 4.9	133.2 ± 4.4	131.5 ± 4.6	137.7 ± 4.1	115.8 ± 5.5	122.9 ± 5.1	122.7 ± 5.1
Mean probability of match	0.10	0.03	0.08	0.05	0.02	0.01	0.09	0.09	0.07
Typical paternity index	2.17	3.8	2.5	2.9	4.36	5.5	2.3	2.3	2.63
Typical paternity probability	0.68	0.79	0.71	0.74	0.81	0.84	0.69	0.69	0.72
Power of exclusion	0.57	0.75	0.62	0.67	0.77	0.82	0.59	0.59	0.64
Discrimination power	0.917	0.961	0.924	0.949	0.947	0.964	0.871	0.919	0.921

^a For details see Refs. (7, 16, 18, 19).

^b Number of genotypes examined.

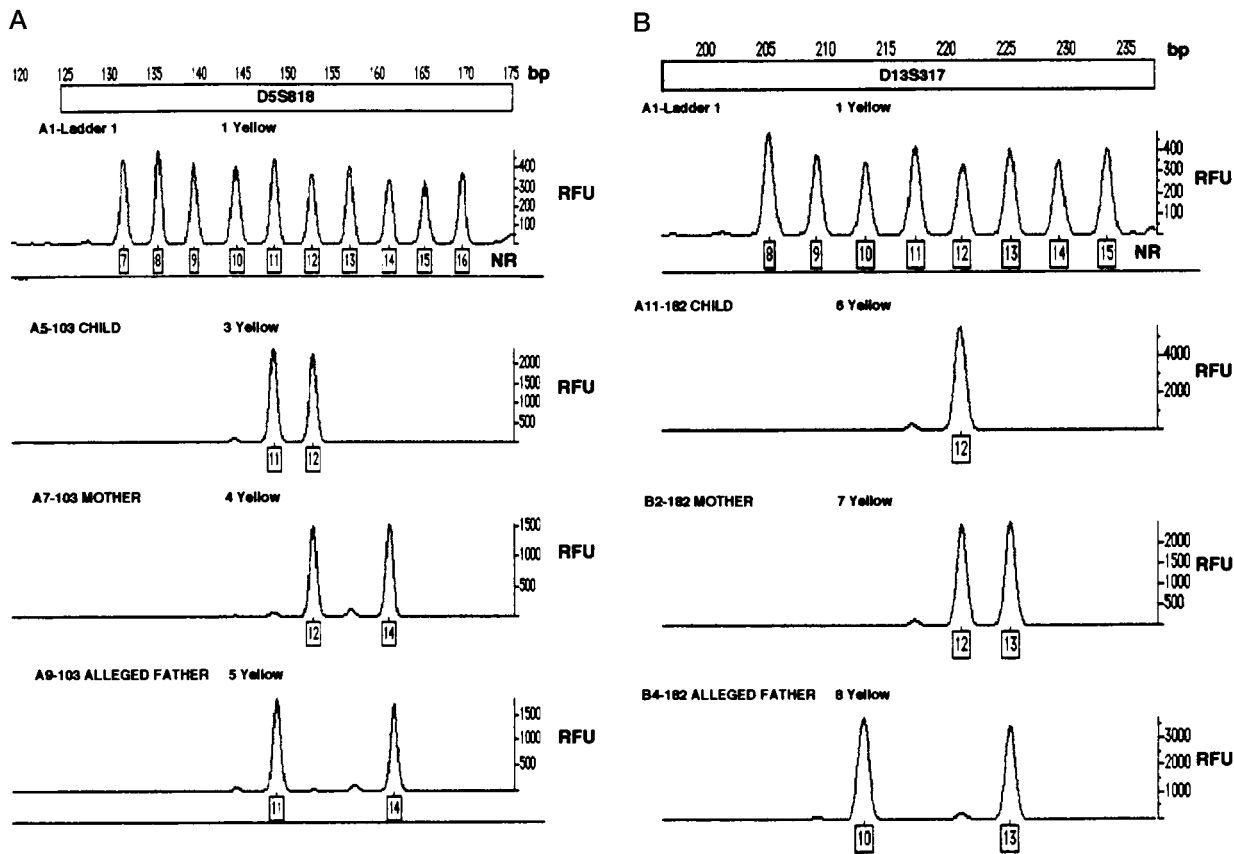


Fig. 2. An example of parentage testing using the ABI Prism 310 and the experimental conditions described under *Materials and Methods*.

The uppermost electropherograms show the alleles in the AmpFLSTR ladder for the STR *D5S818* (panel A) and *D13S317* (panel B) loci. The alleles are indicated as number of repeats (NR), and the peak sizes represent the relative fluorescence units (RFU). (A), the sizes of the *D5S818* alleles indicate an attribution of paternity (DNA typing shows that the child is heterozygous at this locus and that he shares allele 11 with the alleged father and allele 12 with the mother). (B), the sizes of the *D13S317* alleles suggest an exclusion of paternity (DNA typing shows that the child is homozygous; allele 12 is shared with the mother but is absent from the alleged father).

AmpFLSTR Profiler Plus kit with this new methodology (System-2 STR), to evaluate its efficiency for parentage testing and human identification purposes. All nine loci were unlinked, and for each locus an allelic ladder containing all the most common alleles present in the population at that locus was tested in the same series of DNA samples.

We first calculated the allele frequency distributions of the System-2 STR in a Caucasian population from southern Italy and compared them to data obtained from a representative sampling of the general US Caucasian population (13); there was no difference in the allele distribution of any of the nine loci (System-2 STR) between the two Caucasian populations. Our population was in agreement with the expectation of the Hardy-Weinberg equilibrium, which is in agreement with the fact that no significant immigration of individuals from other major ethnic groups has occurred recently.

We next calculated the power of these new polymorphic loci in human identification and paternity testing on the basis of the allele frequencies obtained in our area and compared the results with those of our conventional

System-1 STR panel (7). The combined matching probability, that is the probability of two unrelated individuals matching by chance at all nine loci of the System-2 STR, was much lower (1.36×10^{-12}) with respect to System-1 STR (1.11×10^{-7}) and also lower than those reported by others: 1×10^{-9} with eight loci in one study (20) and 1.5×10^{-10} also with eight loci in another study (21). The most informative loci in System-2 STR were *D18S51*, *D21S11*, and *FGA*: these STRs are complex repeats and have more alleles than STRs formed by simple repeats. We concluded that the System-2 STR is a more powerful tool for individual identification.

In 40 cases of parentage assessment evaluated retrospectively with System-2 STR, we obtained results comparable to those obtained with System-1 STR; however, both the median probability of paternity (20 of 40 cases were attributions) and the number of excluding loci (20 of 40 cases were exclusions) were higher for System-2 STR than for System-1 STR ($P < 0.05$), thus indicating a higher overall efficiency for System-2 STR with respect to System-1 STR.

Some general characteristics of DNA typing by the two

methodologies were also evaluated: (a) the time required to analyze each DNA sample with System-2 STR is approximately one-third of the time required for System-1 STR; (b) with System-2 STR up to 96 DNA samples (maximum tray capacity) can be automatically typed in a single series, with respect to a maximum of 15 DNA samples by System-1 (maximum slab-gel capacity); (c) because of the allelic ladder and the internal size standard in System-2 STR, alleles can be sized automatically and with high precision, thus normalizing differences attributable to electrophoretic mobility or injections, whereas a lower precision is associated with slab-gel manual electrophoretic detection of radiolabeled DNA fragments (absence of an internal standard, slippage of bands, difference in migration between gel lanes); (d) System-2 STR requires less DNA (2 ng) than System-1 STR (20 ng) and thus is more suitable for identification of humans from blood stains or for assessment of DNA chorionic villi contamination with maternal blood, where only minute samples are available; and (e) the automatic electrokinetic injection of samples in the electrophoretic capillary of System-2 STR is much a less labor-intensive sample loading procedure than that of the slab-gel electrophoresis of System-1.

In conclusion, the results of DNA typing by multiplex System-2 STR are comparable with those obtained with System-1 STR; however, the former has a higher power in terms both of paternity attribution and exclusion probabilities and a much lower combined match-by-chance probability for personal identification. In addition, the automated capillary electrophoresis of System-2 STR yields a more precise and accurate sizing of the alleles, allows typing of a greater number of DNA samples simultaneously, and greatly reduces the time for the analysis with respect to the slab-gel electrophoresis of System-1 STR.

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