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SHORT COMMUNICATION

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Genetic diversity of nine STRs in two northwest Iberian populations: Galicia and northern Portugal

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Abstract The genotyping of two population samples from Galicia and northern Portugal was performed for nine STR loci using a single multiplex reaction with the AmpF*l*STR Profiler Plus PCR amplification kit which co-amplifies the systems D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820 and the X-Y homologous gene amelogenin. Allele frequencies for these nine tetranucleotide repeat markers were calculated and no significant differences were observed when comparing these two populations. Conformity with Hardy-Weinberg equilibrium proportions was good for all systems in both samples. The combined power of exclusion was 99.981% and 99.980% in Galicia and northern Portugal, respectively and the combined power of discrimination was greater than 99.999%. Segregation analysis of all loci detected two incompatibilities, one in D3S1358 (out of 112 meioses) and another in D7S820 (out of 104 meioses). Both could be explained by single-step mutations. In general co-amplification was good except for some relatively degraded samples in which poor amplification was observed for the largest STRs. Nevertheless the system is technically robust even when small amounts of template DNA are used and in addition is highly informative and time-saving. However, caution should be taken in the interpretation of profiles in degraded samples and the apparently high mutation rate of D3S1358 and D7S820 should also be kept in mind.

Key words DNA polymorphisms · STRs · Population studies · Profiler plus

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Introduction

Short tandem repeat polymorphisms are increasingly being used in forensic and population genetic studies. The technical and informative potential of these genetic markers was recently increased by the development of multiplex systems together with the use of automatic sequencers and the introduction of fluorescent technology. The co-amplification of several STR loci in one single reaction has several benefits, such as reduction in the quantity of sample required for analysis, decreased probability of contamination, lower labour costs and less time spent on analysis. In addition, much more information can be obtained from minute or relatively degraded samples, a common situation in forensic routine analyses.

The AmpF*l*STR Profiler Plus PCR amplification kit is a commercial kit that co-amplifies the nine highly polymorphic STRs D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820. Some of these STRs (e.g., vWA, FGA and D21S11) have already been widely studied and validated for forensic proposes. However, previous validation studies were performed using different technical conditions with different sets of primers. There is also a lack of population and genetic data from most of these STRs despite the fact that this commercially available multiplex is increasingly being used in forensic casework.

In this paper, this multiplex was used to study the genetic variability of these STRs in Galicia and northern Portugal. In addition, segregation analyses were carried out and the suitability of the system for forensic applications evaluated.

Materials and methods

Samples

Samples from two populations from the Iberian Peninsula, Galicia (NW Spain) and northern Portugal (region including Portuguese districts to the north of the Douro River) were investigated.

Table 1 Allele frequencies for the nine STRs in populations from Galicia and northern Portugal

STR	Allele frequencies																*N*
D3S1358	13	14	15	16	17	18	19										
Galicia	0.0082	0.0861	0.2787	0.2582	0.2131	0.1393	0.0164										122
Northern Portugal	0.0000	0.0650	0.3250	0.2300	0.2150	0.1600	0.0050										100
VWA	13	14	15	16	17	18	19	20	21	22							
Galicia	0.0000	0.1312	0.1393	0.1926	0.2623	0.1803	0.0820	0.0082	0.0041	0.0000							122
Northern Portugal	0.0047	0.0748	0.1542	0.2523	0.2056	0.1962	0.1028	0.0047	0.0000	0.0047							107
FGA	18	19	20	20.2	21	22	23	24	24.2	25	26	27	28				
Galicia	0.0210	0.0714	0.1218	0.0042	0.1597	0.2521	0.1429	0.1135	0.0000	0.0672	0.0252	0.0168	0.0042				119
Northern Portugal	0.0192	0.0721	0.1346	0.0000	0.1635	0.1683	0.1875	0.1346	0.0096	0.0577	0.0385	0.0096	0.0048				104
D8S1179	8	9	10	11	12	13	14	15	16	17							
Galicia	0.0087	0.0000	0.0826	0.1087	0.1000	0.2957	0.2174	0.1478	0.0261	0.0130							115
Northern Portugal	0.0100	0.0100	0.0650	0.0950	0.1300	0.3250	0.2000	0.1350	0.0250	0.0050							100
D21S11	24.2	25	27	28	29	29.2	29.3	30	30.2	31	31.2	32	32.2	33.2	34.2		
Galicia	0.0000	0.0420	0.0208	0.1333	0.2125	0.0042	0.0042	0.2875	0.0292	0.0375	0.1125	0.0125	0.1083	0.0292	0.0042		120
Northern Portugal	0.0050	0.0000	0.0200	0.1950	0.1700	0.0050	0.0000	0.2150	0.0500	0.0750	0.1300	0.0050	0.0900	0.0300	0.0100		100
D18S51	9	10	11	12	13	14	15	16	17	18	19	20	21	22			
Galicia	0.0044	0.0261	0.0087	0.1696	0.1130	0.1261	0.1696	0.1348	0.1130	0.0565	0.0391	0.0304	0.0087	0.0000			115
Northern Portugal	0.0000	0.0278	0.0046	0.1296	0.1157	0.1759	0.1806	0.1435	0.1065	0.0602	0.0139	0.0278	0.0093	0.0046			108
D5S818	8	9	10	11	12	13	14	15									
Galicia	0.0167	0.0167	0.0458	0.3333	0.3833	0.1708	0.0292	0.0042									120
Northern Portugal	0.0248	0.0049	0.0594	0.3515	0.3812	0.1584	0.0198	0.0000									101
D13S317	8	9	10	11	12	13	14	15									
Galicia	0.1529	0.0744	0.0413	0.3141	0.2562	0.1033	0.0537	0.0041									121
Northern Portugal	0.1400	0.0450	0.0400	0.3200	0.2850	0.1150	0.0550	0.0000									100
D7S820	7	8	9	10	11	12	13										
Galicia	0.0292	0.1708	0.1417	0.3250	0.1917	0.1208	0.0208										120
Northern Portugal	0.0234	0.1402	0.1635	0.2664	0.2056	0.1916	0.0093										107

Blood samples for the population study were collected by venipuncture using EDTA as anticoagulant from unrelated healthy blood donors.

A segregation analysis was performed in more than 100 meiosis from Portuguese families.

Genomic DNA was extracted as described by Valverde et al. (1993) and quantification was performed using fluorescence detection with DyNAQuant 200 (APB, Uppsala, Sweden).

Amplification

The PCR co-amplification of the nine STR loci D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820 was performed using the AmpF*l*STR Profiler Plus amplification kit (PE Applied Biosystems, Foster City, Calif.), according to the manufacturer's instructions, using 5 ng of template DNA in a 10 µl PCR reaction volume.

Amplification was carried out in a DNA thermocycler 480 (PE Applied Biosystems). After a 95 °C pre-incubation step for 11 min, PCR amplification was performed for 28 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min and extension at 72 °C for 1 min, followed by a 45 min final extension at 60 °C.

Detection system

PCR products (0.5 µl) were mixed with 2 µl of deionised formamide, 0.4 µl of loading buffer and 0.4 µl of the internal size standard GS-500 (ROX) (PE Applied Biosystems). After denaturation at 97 °C for 5 min, 2 µl was loaded onto a 4.25% denaturing gel. Electrophoresis was carried out for 4 h at a constant power of 1680 V on an ABI Prism 377 DNA sequencer (PE Applied Biosystems). The results were analysed using the Genescan 2.1 analysis software and allele designations were made by comparison with the allelic ladders provided with the kit.

Statistical analysis

Allele frequencies were estimated by gene counting. Accordance with Hardy-Weinberg equilibrium was checked using an exact test (Guo and Thompson 1992) and the software package GENEPOP (Raymond and Rousset 1995). The discrimination power was calculated according to Fisher (1951). Unbiased heterozygosity was calculated according to Nei (1978). Heterogeneity analysis was performed by χ^2-contingency tables of allele frequencies using GENEPOP (Raymond and Rousset 1995). Bonferroni correction was performed using Daan Uitenbroek's SISA online statistical analysis.

Quality control criteria

The groups taking part in this study have successfully approved the proficiency testing program of the Spanish and Portuguese ISFH Working Group (GEP-ISFH) for all the STRs included in this study.

Results and discussion

Population data

Allele frequencies for both populations are shown in Table 1. The observed genotype distributions (data not shown but available upon request) of the nine STRs showed no deviations from Hardy-Weinberg expectations.

No significant differences were observed between the populations for any of the STRs analysed (Table 2).

A comparison of the frequencies obtained with data from Caucasians and Afro-Americans (available from Perkin-Elmer) showed no significant differences with the Caucasians except for vWA (Galicia vs Caucasians $p = 0.039 \pm 0.003$, northern Portugal vs Caucasians $p = 0.025 \pm 0.003$). However, considering the number of tests, and applying the Bonferroni correction (SISA) the significance level would be lowered to 0.0085 and therefore, at the moment, this finding does not deserve special discussion. On the contrary, significant differences with Afro-Americans were found in all STRs analysed.

Segregation data

The number of meioses analysed for each system is shown in Table 3. Two incompatibilities were detected in D3S1358 with an offspring typed as 15/16 from a 15 (father) × 15/18 (mother) mating and in D7S820 an offspring 12 from a 8/12 × 8/13 mating. In both cases the probability of maternity and paternity calculated using the other systems was higher than 99.9997% (a priori *Po* = 0.5). Both observations could be explained by a single-step mutation event. While in the D7S820 system the mutation was probably of maternal origin, in the D3S1358 system it was not possible to conclude whether the mutation came from the father or mother. The parents in this latter case were 35 (father) and 25 (mother) years old. In the D7S820 the mother was 21 years old.

Table 2 Comparison of allele frequencies between populations from Galicia and northern Portugal (*P* Probability value of the differentiation test, *P* non-differentiation)

	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
P	0.36174 ± 0.0099	0.21381 ± 0.0098	0.62884 ± 0.0100	0.86684 ± 0.0096	0.37105 ± 0.0098	0.86346 ± 0.0090	0.89760 ± 0.0020	0.91447 ± 0.0086	0.21533 ± 0.0100

Table 3 Number of meioses studied for the STRs included in the multiplex and number of mutations found

	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
Number of meioses	122	122	124	118	116	110	118	118	104
Mutations	1 (0.82%)	0	0	0	0	0	0	0	1(0.96%)

Table 4 Forensic efficiency values (*H* Nei's heterozygosity, *PIC* polymorphic information content, *PD* power of discrimination, *PEX* a priori paternity exclusion chance)

STR system	Galicia				Northern Portugal			
	H	PIC	PD	PEX	H	PIC	PD	PEX
D3S1358	0.78632	0.74902	0.91898	0.55185	0.76952	0.72713	0.90672	0.52135
VWA	0.82154	0.79299	0.94180	0.61644	0.81940	0.78958	0.94061	0.61121
FGA	0.85539	0.83537	0.96206	0.68899	0.86714	0.84764	0.96680	0.70713
D8S1179	0.81744	0.78990	0.94153	0.61733	0.80920	0.78066	0.93790	0.60580
D21S11	0.82972	0.80557	0.94895	0.64387	0.85653	0.83523	0.96179	0.68762
D18S51	0.88019	0.86366	0.97304	0.73526	0.87288	0.85468	0.96935	0.71935
D5S818	0.71226	0.65957	0.86640	0.44796	0.70497	0.64940	0.85887	0.43610
D13S317	0.79480	0.76279	0.92834	0.57895	0.78080	0.74478	0.91882	0.55440
D7S820	0.79581	0.76341	0.92807	0.57627	0.80680	0.77350	0.93199	0.58522
Combined			> 0.99999	0.99981			> 0.99999	0.99980

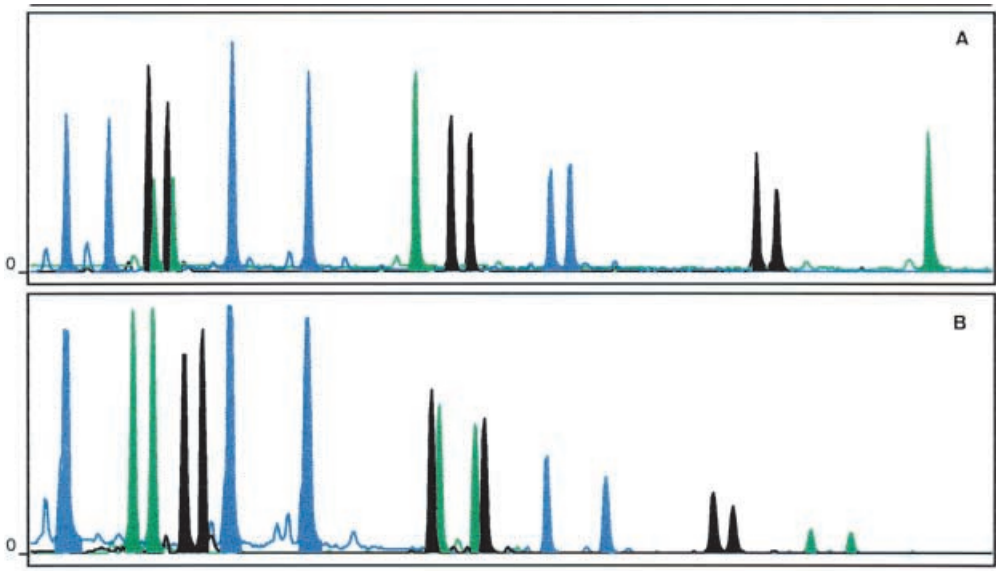

Fig. 1 **A** AmpF*l*STR profile obtained from a fresh blood sample and **B** from a relatively degraded sample

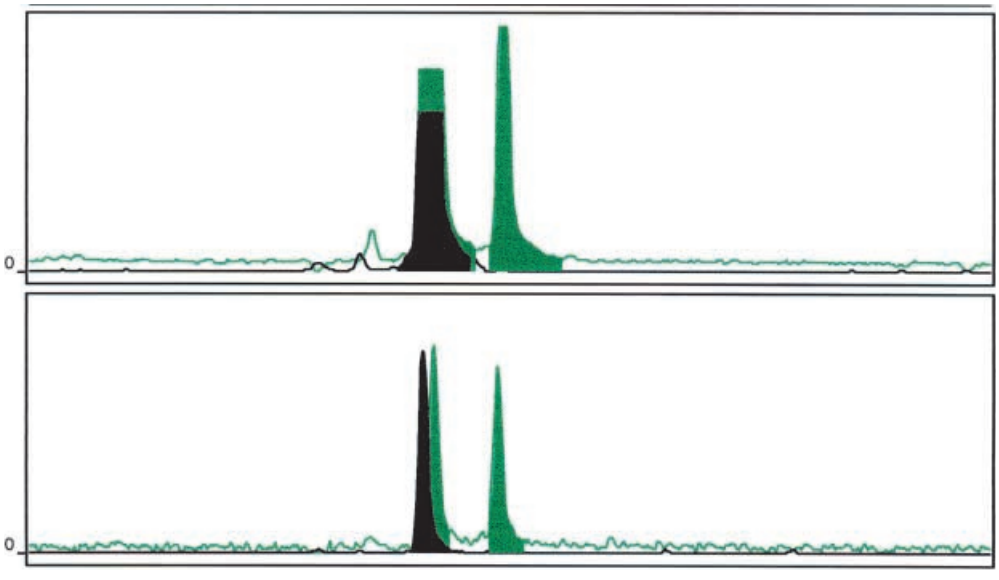

Fig. 2 An over-amplification is observed for D13S317 (*NED*) where yellow (*in black*) pull- ups produce green peaks that overlap in size with the alleles included in the D21S11 allelic ladder (*JOE*)

Other mutations have been reported in Caucasians for D3S1358 (Mohrinweg et al. 1998) and another mutation was found in the 1998 proficiency testing program of the Spanish and Portuguese ISFH Working Group (GEP-ISFH). Although the average size of the amplified D3S1358 products is small, the number of uninterrupted repeats in the variable stretch is high, which is in agreement with the recent observation of Brinkmann et al. (1998). For these reasons, the mutation rate for this system could be relatively high and therefore special attention should be paid when this system is used for paternity testing.

Parameters of forensic genetic interest

The informative potential of this multiplex system, as measured by some standard parameters such as the paternity exclusion probability and the discrimination power, is very high in both populations as shown in Table 4.

Technical data

DNA extracted from well preserved samples was efficiently co-amplified using the manufacturer's protocol (Fig. 1A) for all systems. Nevertheless in badly preserved samples some relatively poor amplification results were observed in the largest STRs. This is in agreement with the earlier observations of Álvarez-García et al. (1996) which correlated the short size of the STRs and positive results in degraded samples. Figure 1B exemplifies this by illustrating the use of this multiplex in an identification case from skeletal remains.

Accurate amplification can be affected in the case of a pull-up effect due to over amplification, e.g. systems labelled with NED, in which a yellow pull-up produces a green peak falling into the read region of the systems labelled with JOE (green) (Fig. 2). In these cases we recommend reanalysing the sample with smaller quantities of DNA.

With respect to forensic applications such as paternity cases, we can conclude that the multiplex is technically robust (even when small amounts of template DNA are used), technically reproducible (all samples were typed twice without any contradictory results), highly informative and time saving.

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