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SHORT COMMUNICATION

Development of two multiplex PCR systems for the analysis of 12 X-chromosomal STR loci in a northwestern Italian population sample

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Abstract Two multiplex polymerase chain reaction systems for the automated profiling of 12 X-chromosomal short tandem repeat (STR) markers were developed. Multiplex A consisted of DXS6789, DXS6809, GATA172D05, DXS101, DXS8378, and DXS8377. Multiplex B consisted of DXS7132, DXS6800, DXS6801, DXS7424, HPRTB, and DXS10011. The set of amplified X-STRs was designed to include groups of closely linked markers (DXS101–DXS7424 and DXS6789–DXS6801–DXS6809) to generate highly informative haplotypes for kinship testing. A population genetics study of the 12 X-STRs was conducted in a northwestern Italian population sample (n=160, 80 women and 80 men). A diallelic pattern at locus DXS6789 was observed in one man.

Keywords X chromosome · Short tandem repeats · Multiplex PCR · Italy · DXS6789

Introduction

Typing of short tandem repeat (STR) loci located on the X chromosome can effectively complement autosomal STR data in paternity testing of female children (deficiency cases and cases involving close blood relatives as alternative alleged fathers) and maternity testing of male children [1]. For practical use in forensic casework, multiplex polymerase chain reaction (PCR) systems for the co-amplification and subsequent automated profiling of X-STRs are required to speed up laboratory processes. The development of multiplex PCR assays is also desirable to facilitate the analysis of samples that contain only small amounts of DNA, as occasionally found in disputed kinship cases (e.g., archival histologic material, skeletal remains). Several pentaplex and one heptaplex reaction for rapid X-STR profiling were described [2–4]. In this study, two multiplex PCR assays for the simultaneous analysis of 12 STRs spanning the whole X chromosome were developed. Chromosomal localization of markers and composition of linkage groups as described by Szibor et al. [1] are given in Table S1. The set of amplified X-STRs was designed to include groups of closely linked markers (DXS101–DXS7424 and DXS6789–DXS6801–DXS6809) to generate highly informative haplotypes for kinship testing [5, 6].

As with autosomal STRs, the use of X-STRs in forensic practice requires a precise knowledge of the genetic characteristics of the pertinent population, i.e., degree of polymorphism, allelic distribution, and testing of Hardy–Weinberg equilibrium (HWE) must be determined. Due to the peculiarities of X-linked inheritance, the possibility of linkage disequilibrium (LD) between markers must also be carefully investigated. Since X chromosomes recombine exclusively in females, only two-thirds of X chromosomes recombine in each generation. The measured recombination rate for the X chromosome is, in fact, almost exactly two-thirds of the genome average [7]. As a result, the LD is expected to be greater on the X chromosome and the size of regions with a single genetic history to be larger. In recent years, the amount of available X-STR data for forensic purposes has been slowly but steadily growing, including a newly established Web site surveying current research on X

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chromosome markers (http://www.chrx-str.org) [8]. With this in mind, a local population sample of northwestern Italians (n=240 chromosomes) was investigated by multiplex PCR.

Materials and methods

Buccal swabs were collected from 160 healthy, unrelated Italians (80 women and 80 men) residing in Piedmont (northwest Italy). Sampling was performed anonymously to prevent linkage to the original donor. Genomic DNA was isolated with NucleoSpin Tissue spin columns (Macherey-Nagel, Düren, Germany). X-STRs included in the multiplex PCR assays, primer sequences, concentrations, and dye labeling are listed in Table S2. Polymerase chain reactions were performed in a 25-μl volume containing 1 μl (0.25–10 ng) of template DNA, 10 mM Tris–HCl, 50 mM KCl, 1.5 (multiplex A) or 2 (multiplex B) mM $MgCl_2$, 100 μM each dNTP, and 2.5 U of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). For both multiplex systems, the PCR protocol consisted of a 10-min pre-PCR heat step at 95°C, followed by 32 cycles of denaturation at 95°C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 1 min, with a final 60 min extension step at 72°C. Typing was done on the ABI PRISM 310 Genetic Analyzer in comparison to sequenced allelic ladders and control DNA from K562 and 9947A cell lines (Promega, Madison, WI) [9–11] using the GeneScan software version 3.7. Allelic designation was in accordance with International Society for Forensic Haemogenetics recommendations [12] and followed that suggested by Edelmann et al. [10, 13, 14] and Hering et al. [11], with the exception of locus GATA172D05, where one repeat was added to the nomenclature [15]. Arlequin software version 2.000 [16] was used to perform exact tests (100.000 Markov steps) of population differentiation between male and female allele frequencies, HWE in the female subsample, LD between all pairs of markers in the male subsample, population differentiation between northwestern Italians and other Italian population data from the literature. The following statistical parameters of forensic interest were calculated: expected heterozygosity (h) [17], polymorphism information content (PIC) [18], power of discrimination in females (PD^F) and males (PD^M) [19], mean exclusion chance for autosomal markers in trios (MECI) [20], X-STRs in trios with daughters (MECII) [21], and X-STRs in father/daughter duos (MECIII) [19].

Results and discussion

The multiplex PCR conditions described allowed robust amplification of as little as 0.25 ng of template DNA. Electropherograms showing the observed genotypes of the 9947A cell line and the composition of allelic ladders are supplied as electronic supplementary material (Figure S1). Capillary electrophoresis results of the population genetics study revealed a high mean percentage of stutter (33%) for locus DXS8377, attributable to the long uninterrupted microsatellite array, which favors slippage of DNA polymerase [22]. In female heterozygous genotypes, locus DXS10011 displayed a mean allelic imbalance ratio of 63% (calculated as peak height of the weaker intensity allele peak over that of the stronger intensity allele peak). Preferential amplification of the shorter allele peak was constantly observed and the extent of imbalance was positively correlated (r=0.60, p<0.001) with interallelic size difference.

No significant deviation in the allelic distribution of X-STRs between the female and male subsamples was seen by the exact test, and combined frequencies are thus given

Table 1 Statistical parameters of forensic interest

STR	h	PD^F	PD^M	PIC	MECI	MECII	MECIII
DXS6789	0.709±0.029	0.868	0.706	0.661	0.466	0.655	0.518
DXS6809	0.805±0.026	0.937	0.802	0.778	0.617	0.770	0.656
GATA172D05	0.793±0.026	0.925	0.790	0.760	0.587	0.756	0.631
DXS101	0.895±0.020	0.979	0.891	0.882	0.776	0.875	0.797
DXS8378	0.677±0.030	0.827	0.674	0.607	0.391	0.605	0.460
DXS8377	0.917±0.018	0.986	0.913	0.906	0.816	0.900	0.834
DXS7132	0.742±0.028	0.890	0.739	0.697	0.505	0.692	0.558
DXS6800	0.751±0.028	0.899	0.748	0.710	0.528	0.707	0.574
DXS6801	0.609±0.032	0.791	0.607	0.552	0.358	0.548	0.405
DXS7424	0.755±0.028	0.901	0.752	0.714	0.531	0.711	0.578
HPRTB	0.778±0.027	0.914	0.775	0.740	0.560	0.736	0.607
DXS10011	0.941±0.015	0.992	0.937	0.933	0.874	0.935	0.878

h Expected heterozygosity, *PD^F^* power of discrimination in females, *PD^M^* power of discrimination in males, *PIC* polymorphism information content, *MECI* mean exclusion chance for autosomal markers in trios, *MECII* mean exclusion chance for X-STRs in trios with daughters, *MECIII* mean exclusion chance for X-STRs in father/daughter duos

Table 2 Commonest DXS7424–DXS101 and DXS6801–DXS6809–DXS67`89 haplotypes observed in the northwestern Italian male subsample (n=80) with P values (+0.001 SE) of exact test of pairwise LD

n	Haplotype		n	Haplotype		
	DXS7424	DXS101		DXS6801	DXS6809	DXS6789
5	15	25	6	11	33	20
4	14	24	5	11	33	21
	15	18	4	11	31	22
	15	24	3	11	34	20
	15	26		11	34	21
	16	24		12	33	20
3	15	23		12	34	21
	16	19	2	10	31	20
	16	21		10	34	20
	16	28		11	30	20
2	13	24		11	31	20
	13	25		11	32	16
	14	23		11	32	20
	14	25		11	32	21
	14	26		11	35	20
	15	28		12	28	21
	16	18		12	31	20
	16	20		12	33	23
	16	22		12	34	20
	16	23				
	16	25	P	DXS6801–DXS6809	0.288	
	16	29	P	DXS6801–DXS6789	0.456	
P	DXS7424–DXS101	0.936	P	DXS6809–DXS6789	0.577	

in Table S3 together with observed genotypes of the K562 and 9947A cell lines. All loci were found to be in HWE in the female subsample. Statistical parameters of forensic interest calculated for the 12 X-STR loci are shown in Table 1. The exact test of pairwise linkage between markers in the male subsample did not detect any evidence of LD. In previous studies, LD was described for the Xq22 cluster DXS7424–DXS101 and the Xq21 cluster DXS6801–DXS6809–DXS6789 in a German population [5, 6]. Conversely, Lee et al. [23] failed to demonstrate LD for DXS7424–DXS101 in Koreans. For markers included in Xq22 and Xq21 linkage groups, haplotypes found in northwestern Italian men with frequency n>1 are given in Table 2.

Northwestern Italian allelic frequencies were compared with previously published studies of X-STRs in the Italian population. Data on DXS6789, DXS10011, HPRTB, DXS101 [2], DXS7132, GATA172D05 [24], and DXS8377 [25] were currently available. No statistically significant difference was found by the exact test.

A diallelic pattern (with peaks 22/23 of even intensity) was observed at locus DXS6789 in one man. Due to the anonymity of the samples, cytogenetic and family studies could not be performed. A 47,XXY karyotype was excluded by amplification of the amelogenin locus and a gross chromosomal rearrangement was unlikely in an apparently healthy donor. The abnormal genotype most probably indicated the presence of a microduplication, followed by mutation in one of the duplicated copies of DXS6789. Recent studies demonstrated that the extent to which large duplications/deletions contribute to human genetic diversity was previously unappreciated [26]. Moreover, reciprocal duplications of common X-chromosomal microdeletions are presumably under-ascertained due to the mild degree or lack of noticeable phenotypes [27]. To further investigate this unexpected finding, quantitative PCR analysis and microarray-based comparative genomic hybridization are under way.

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