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Development of a quadruplex PCR system for the genetic analysis of X-chromosomal STR loci

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1. Introduction

Short tandem repeat systems on the X chromosome are the natural counterpart to the well-established Y-chromosomal STR loci. The X-linked systems are inherited as a single haplotype only in males, whereas in females, the X chromosomes recombine and exhibit the same characteristics as the autosomes. Nevertheless, X-linked systems may provide a useful tool in paternity cases with female offspring, in particular when the alleged father is not available for testing, or in forensic identification cases based on the comparison with first- or second-degree relatives. Only a small number of STR loci have been described on the X chromosome, and a number of these are not highly informative.

Therefore, we have investigated the four X-chromosomal STR loci: DXS101, DXS8377, HPRTB and STRX-1 [1–5], to develop a rapid multiplex PCR typing system suitable for fluorescent detection and to perform population genetic studies among Germans. Whereas HPRTB and STRX-1 contain tetrameric repeats, DXS101 and DXS8377 have trimeric repeats. All four loci are highly polymorphic exhibiting up to 18 common alleles at each locus (Table 1).

2. Methods

The published primer sequences were slightly modified to achieve more uniform PCR conditions required to perform a quadruplex amplification assay. The forward primers of

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Table 1
Characteristics of the selected X-STR loci

System	Localization	Repeat	Fragment sizes (bp)	Ref.
DXS101	Xq22	$(CTT/ATT)_{15-32}$	179–230	[1,2]
DXS8377	Xq28	$(GAA/GAG)_{37-56}$	207–264	[3]
HPRTB	Xq26	$(ATCT)_{12-17}$	274–294	[4,6]
STRX-1	X	$(ATCT)_{11-16}$	298–318	[5,6]

each system were dye-labelled with FAM and HEX, respectively (Table 2). PCR was performed in a 50-μl volume containing 50 ng template DNA, 10 pmol of each primer, 40 nmol dNTPs, 25 nmol $MgCl_2$, 5 μl 10×PCR buffer and 4 U Taq polymerase (Invitrogen). Hot-start PCR was performed by adding the enzyme after the initial denaturation in a Perkin Elmer 9600 thermocycler. A touchdown PCR protocol was carried out as follows: initial denaturation at 94 °C for 4 min, six cycles with 1 min at 94 °C, 61–59 °C for 1 min (decreasing the annealing temperatures by 0.5 °C in each cycle) and 72 °C for 1 min, then 24 cycles with constant annealing at 58 °C, followed by the final extension at 72 °C for 60 min. Separation of the fragments was achieved by capillary electrophoresis using an Applied Biosystems Prism 310 DNA sequencing system.

A population study based on 60 family trios (27 male, 33 female offspring) from routine casework of Western German origin with confirmed paternity was performed to evaluate the forensic efficiency values as well as to investigate the mutation rates of these X-chromosomal STR systems.

3. Results and discussion

After optimization of the PCR conditions, allelic ladders were assembled from selected male DNA samples representing the most common alleles in each system. The allele designation was based on nomenclature proposals from previous publications [2,6]. In the case of DXS8377, a tentative repeat-based nomenclature proposed by J. Edelmann (Institute of Legal Medicine, Leipzig, Germany; personal communication) was used.

The results from population studies were analyzed separately for male and female individuals for each locus, and the resulting allele frequencies compared to each other. No significant differences were observed, hence, the two groups were combined into a single frequency distribution, as shown in Table 3. As the German population sample is composed of 60 male and 60 female individuals, a total number of 180 X chromosomes

Table 2
Primer sequences of the selected X-STR systems

System	Forward primer (5′→3′)	Reverse primer (5′→3′)
DXS101	FAM—ACTCTAAATCAGTCCAAATATCTCCCTT	CAAATCACTCCATGGCACATGTAT
DXS8377	HEX—ACCACTTCATGGCTTACCACAG	TATGGACCTTTGGAAAGCTAG
HPRTB	FAM—ATGCCACAGATAATACACATCCCC	CTCTCCAGAATAGTTAGATGTAGG
STRX-1	HEX—CCTTGTGGCCTTCCTTAAATGG	TCCAGCACCCAAGGAAGTCA

Table 3
X-STR allele frequencies among 120 Germans

DXS101		DXS8377		HPRTB		STRX-1	
Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency
15	0.050	37	0.006	12	0.133	11	0.006
17	0.006	38	0.011	12.2	0.006	11.1	0.006
18	0.061	39	0.022	13	0.317	12	0.011
19	0.050	40	0.011	14	0.294	12.1	0.106
20	0.028	41	0.056	15	0.150	13	0.117
21	0.050	42	0.044	16	0.078	13.1	0.261
22	0.022	43	0.039	17	0.022	14	0.233
23	0.061	44	0.089			14.1	0.106
24	0.206	45	0.139			15	0.072
25	0.150	46	0.128			15.1	0.044
26	0.144	47	0.106			16	0.039
27	0.067	48	0.139				
28	0.072	49	0.061				
29	0.017	50	0.039				
30	0.011	51	0.050				
32	0.006	52	0.022				
		53	0.011				
		54	0.022				
		56	0.006				

has been included. Furthermore, the genotype distributions among the females have been tested for conformity with the Hardy–Weinberg equilibrium applying the exact test provided by the DNA·VIEW™ software. No significant deviations were observed at any of the four loci (Table 4). Furthermore, no significant differences were observed regarding the observed frequency distribution of DXS101 in comparison to another German population study [2]. Also, no mutations were detected at any of the four loci based on 93 meioses. In the STRX-1 system, an unusual group of alleles was found characterized by the presence of a single trimeric repeat as well as a 2-bp deletion in the flanking region. Thus, the corresponding STR fragments could be described as "12.3, 13.3, 14.3, etc. " based on the repeat structure, but as "12.1, 13.1, 14.1 etc." based on the

Table 4
Biostatistical parameters of the quadruplex loci

	DXS101	DXS8377	HPRTB	STRX-1
Hardy–Weinberg exact test (women, *p*)	0.89	0.07	0.39	0.94
Exclusion chance (women)				
Maternity	0.781	0.8	0.549	0.658
Identity	0.978	0.982	0.908	0.948
Exclusion chance (men)	0.865	0.914	0.748	0.822
Combined exclusion chance (women)				
Maternity	0.9932			
Identity	0.9999			
Combined exclusion chance (men)	0.9994			

total fragment length difference in comparison to the allelic ladder. It appears that the group of alleles with the incomplete repeat might have evolved independently from the other alleles with a regular tetrameric repeat structure.

The individual and combined biostatistical parameters are summarized in Table 4. Due to the large number of evenly distributed alleles, DXS8377 is the most informative of the four loci, followed by DXS101, STRX-1 and HPRTB. The combined exclusion chance for identification of the quadruplex exceeds 99.9% both in men and women demonstrating the high forensic efficiency of this combination of X-linked STR loci.

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