

Forensic validation of the X-chromosomal STR-markers GATA165B12, GATA164A09, DXS9908 and DXS7127 in German population

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Abstract. Four X-chromosomal STR markers (GATA165B12, GATA164A09, DXS9908 and DXS7127) were validated for forensic use. We report primer sequences, PCR protocols, allele structures and population data for a German population sample. We investigated up to 767 chromosomes of unrelated individuals and up to 343 meioses. The markers described here revealed a moderate degree of variability (Het=0.69, 0.67, 0.83, 0.76 and PIC=0.62, 0.73, 0.72, 0.72, respectively) low mutation rates and no problems in handling using the automated fragment analysis. All markers are suitable for forensic purpose. © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

The Chromosome X-STRs (ChrX STRs) were recently recognized as useful tools in forensic kinship testing, mainly in solving of complex cases [1]. The highly effective strategy of ChrX microsatellite haplotyping requires the description of numerous STR markers. The aim of this presentation is to add four STRs to the known panel of ChrX markers and to describe their forensic suitability. GATA165B12 and GATA164A09 were characterized recently and population data were published for Korean population samples [2,3]. DXS9908 (also known as GATA182E04) and DXS7127 are not forensically evaluated yet to our knowledge.

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2. Material and methods

We investigated 429 up to 574 unrelated individuals from German population regarding their allele distribution in the above-mentioned STRs. Primers were designed according to GenBank information (www.gdb.org). PCR amplifications were carried out in a 25 μ l reaction volume containing 1–2 ng DNA, 200 μ M each dNTP, 1.5 mM MgCl₂, 0.5 μ M each primer and 1 U *Taq* polymerase using self-established PCR conditions.

Primers and cycle conditions:

GATA165B12:	5′-TATGTATCATCAATCATCTATCCG-3′ 5′-GCATACACAGTGAAAATGATTTAA-3′ PCR: 95°-2 min; 94°-60 s, 56°-40 s, 72°-40 s; 72°-10 min; 30×
GATA164A09:	5′-TTACAGTCCTTTACAGTAGCTCT-3′ 5′-CTCAGTTCAAGGAAATGGGA-3′ PCR: 94°-2 min; 94°-45 s, 53°-60 s, 72°-60 s; 72°-6 min; 29×
DXS9908:	5′-TAGGGTGCAGCAAATCACCA-3′ 5′-TTGCAGAGGGATAGAAATGC-3′ PCR: 94°-2 min; 94°-60 s, 58°-60 s, 72°-90 s; 72°-10 min; 30×
DXS7127:	5′-TGCACCTTAATATCTGGTATGG-3′ 5′-ATTTCTTTCCCTCTGCAACC-3′ PCR: 94°-50 s; 94°-45 s, 57°-45 s, 72°-90 s; 72°-10 min; 29×

PCR products were resolved and detected by capillary electrophoresis on the ABI Prism® 310 Genetic Analyzer (Perkin-Elmer, Foster City, CA, USA).

For the direct Taq-cycle sequencing method we employed the Big Dye Terminator Kit (Perkin-Elmer, Foster City, CA, USA). HWE-analysis was performed using the exact test and different parameters of the STRs regarding their information content were calculated.

3. Results and discussion

In Table 1 we present the allele frequency data for the investigated STRs, further statistical parameters characterizing the analytical usefulness and the localisation on the ChrX.

Sequence structures of the repeat and flanking regions resulted in following allele nomenclature:

- GATA165B12: 10–14, (*Pr*₂₄-*N*₆-[AGAT]₉₋₁₃-*N*₄-[AGAT]-*N*₃₀-*Pr*₂₅);
- GATA164A09: 10–13, (*Pr*₂₂-*N*₈₆-[TATC]₁₀₋₁₃-*N*₁₆-*Pr*₂₀) and 13.3–17.3, (*Pr*₂₂-*N*₈₆-[TATC]₅-ATC-[TATC]₈₋₁₂-*N*₁₆-*Pr*₂₀);
- DXS9908: 18–23, (*Pr*₂₀-*N*₃₆-[TATC]-*N*₁₃-[TATC-TA]₃-[TATC]-TG-[TATC]-CA-[TATC]₁₀₋₁₅-ATC-[TATC]-TCTC-[TATC]-[TA]₅₋₆-*N*₂₈-*Pr*₂₀);
- DXS7127: 23–27.2, (*Pr*₂₂-*N*₇₄-[TA-TATC]₄₋₅-TG-[TATC]₁-CA-[TATC]₁₀₋₁₄-[ATAC]₂-C-[TCTA]₂-[TA]₄₋₅-*N*₂₆-*Pr*₂₀).

Control DNA 9947A (Promega, Madison, WI) can be used for allelic ladder calibration and display the alleles as follows: GATA165B12: 10/12, GATA164A09: 15.3/15.3, DXS9908: 19.2/20, DXS7127: 24.2/25.

The markers described here revealed a moderate degree of variability, low mutation rates and they are easy to handle when the automated fragment analysis was performed on the ABI PRISM™ 310 Analyzers. Information regarding location on the ChrX was drawn from NCBI and additionally established by an own recombination study. Using the exact test for genotype distribution of the STRs we found no significant deviation from Hardy–Weinberg equilibrium.

Table 1
Allele frequency data in a German population

Allele	GATA165B12	GATA164A09	DXS9908	DXS7127
	766 chromosomes	656 chromosomes	767 chromosomes	655 chromosomes
10	0.2846 ± 0.0163	0.0061 ± 0.0030		
11	0.3185 ± 0.0168	0.0381 ± 0.0075		
12	0.3603 ± 0.0173	0.0854 ± 0.0109		
13	0.0352 ± 0.0067	0.0396 ± 0.0076		
13.3		0.0610 ± 0.0093		
14	0.0013 ± 0.0013			
14.3		0.1921 ± 0.0154		
15.3		0.4070 ± 0.0192		
16.3		0.1540 ± 0.0141		
17.3		0.0168 ± 0.0050		
18			0.0078 ± 0.0032	
19			0.2503 ± 0.0156	
19.2			0.0443 ± 0.0074	
20			0.3755 ± 0.0175	
20.2			0.0717 ± 0.0093	
21			0.1669 ± 0.0135	
21.2			0.0169 ± 0.0047	
22			0.0626 ± 0.0087	
22.2			0.0026 ± 0.0018	
23			0.0013 ± 0.0018	0.0122 ± 0.0043
24				0.2366 ± 0.0166
24.2				0.0458 ± 0.0082
25				0.3878 ± 0.0190
25.2				0.0718 ± 0.0101
26				0.1664 ± 0.0146
26.2				0.0153 ± 0.0048
27				0.0626 ± 0.0095
27.2				0.0015 ± 0.0015
Alleles	10–14	10–17.3	18–23	23–27.2
Length (bp)	129–145	184–215	216–236	239–257
Heterozygosity	0.69	0.67	0.83	0.76
Mutation rate	0/343	2/255	0/340	0/264
MEC	0.622	0.731	0.723	0.720
PIC	0.622	0.731	0.723	0.720
PD (females)	0.837	0.914	0.907	0.906
PD (males)	0.687	0.756	0.757	0.754
Position	120 Mb, 77.1 cM	110–130 Mb	142 Mb, 92.8 cM	143–144 Mb

All markers are suitable for forensic purpose and the presented data qualify them as a useful supplementation of the known forensic ChrX marker panel contributing to the development of a ChrX haplotyping procedure as an efficient tool in human kinship testing.

References

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