

Genetic Analysis of Ten Gonosomal STR Loci in an Italian Population Using the Elucigene QST*R-XY Amplification Kit

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Abstract

Genotyping of X-chromosomal short tandem repeats (X-STRs) is an emerging tool in forensic genetics because of its inheritance pattern, and a large number of markers has been characterized. Quantitative fluorescence polymerase chain reaction (QF-PCR) analyses of STR markers on the X-chromosome are performed routinely in medical genetics laboratories for the rapid detection of aneuploidy in chromosome X. In this study, 595 Italian participants were genotyped at 10 gonosomal STRs (DXS680, DXS98, DXS6807, DXS1187, XHPRT, DXS742, DXS6809, DXYS267, DXYS218, and DYS448) using a commercially available QF-PCR kit. Here, we report the allele architecture of DXS1187 and DXYS218, which have not previously been characterized for forensic use. The presence and extent of genetic linkage and linkage disequilibrium between all X-STRs were estimated. Allele and haplotype frequencies in the Italian population were assessed and reported together with statistical parameters.

Keywords: Allele frequencies; Haplotype frequencies; Elucigene QST*R-XY; X-STR markers; DXS1187; DXYS218

Abbreviations:

STR: Short Tandem Repeat; PIC: Polymorphism Information Content; PD: Power of Discrimination; MEC: Mean Exclusion Chance

Introduction

Analyses of X-chromosome markers are useful supplemental tools for genetic investigation, kinship analysis, deficiency paternity cases, and for interpretation of complex profiles in DNA mixtures. Several X-chromosomal markers have been characterized by the forensic DNA community, and assays have been developed to detect of X-chromosomal short tandem repeats (X-STRs) [1]. Commercial kits that analyze STR markers for medical purposes also have emerged. Quantitative fluorescence polymerase chain reaction (QF-PCR) is a routine technique in prenatal genetic diagnosis that allows for rapid, simple, and inexpensive diagnoses of common aneuploidies [2]. Similarly to current forensic DNA kits, highly polymorphic STRs are amplified readily using fluorescent dye-labelled primers, detected with capillary electrophoresis, and analyzed using GeneMapper software [3]. QF-PCR results are interpreted according to the number and the fluorescence intensity of alleles at each locus. The methods and chemistry of QF-PCR diagnostic kits are similar to those currently used in forensic DNA analyses, but these kits have not been evaluated formally by the forensic DNA community. We examined the Elucigene QST*R-XY kit (Gen-Probe Life Sciences Ltd, Abingdon, UK), a DNA-based multiplexed assay for the rapid prenatal determination of sex chromosomal aneuploidies, including Klinefelter

and Turner syndromes. This 12-plex QF-PCR enables the identification of the Amelogenin marker, which amplifies non-polymorphic sequences on the X (104 bp) and Y (110 bp) chromosomes, and of the non-polymorphic Y-specific SRY marker, which permits gender determination. Elucigene QST*R-XY targets (1) the pseudoautosomal STR markers, DXYS267 and DXYS218, located in both the X and Y chromosomes; (2) the X-specific markers, DXS680, DXS98, DXS6807, DXS1187, XHPRT, DXS742, and DXS6809; and (3) the Y-specific marker, DYS448 [4].

In this study, we describe the allele frequencies of these gonosomal STR markers in an Italian population, and we characterize the STR structures of the novel markers, DXS1187 and DXYS218. Statistical and genetic parameters confirm the informativity and usefulness of the Elucigene QST*R-XY for forensic purposes.

Materials and Methods

Samples and data collection

DNA was extracted from buccal swabs of 595 unrelated healthy volunteer donors born in the Central Italy (284 males, 311 females) using a QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany). All individuals provided their written informed consent.

All DNA samples were amplified using Elucigene QST*R-XY kits (Gen-Probe Life Sciences Ltd, Abingdon, UK) by dispensing 2 µl of DNA into a 0.2-ml PCR vial containing 10 µl QST*R-XY reaction mix. Samples were amplified on an Applied Biosystems 9800 Fast Thermal Cycler according to the following program: initial denaturation at 95°C for 15 min, 26 cycles of 95°C for 30 s, 59°C for 90 s, and 72°C for 90 s, final extension at 72°C for 30 min. PCR products were separated

and detected on an *ABI 3130xl* Genetic Analyzer (Applied Biosystems, Foster City, CA). Raw data were analyzed using GeneMapper ID 3.2 software (Applied Biosystems), according to the manufacturer's recommendations.

Analysis of STR structures

To establish a correspondence between electrophoretic lengths (bp) and allele structures, we sequenced the STRs of several homozygote/hemizygote samples. Elucigene QST*R-XY enables the detection of 10 STRs, of which 8 have been described previously for forensic purposes [5-14]. We extensively sequenced and characterized the structures of the novel markers, DXS1187 and DXYS218. Forward and reverse primers (Invitrogen) were designed and used for PCR and sequencing (Table 1). PCR was performed in 25 µl reaction volumes each containing 5-10 ng of genomic DNA, 0.7 µM of each primer, 200 µM of each dNTP, 2 mM MgCl₂, 1 U of AmpliGold Taq DNA polymerase (Applied Biosystems), and 10X PCR buffer (Applied Biosystems) using a GeneAmp® PCR System 9700 (Applied Biosystems). 0 Samples were amplified according to the following program: initial denaturation at 96°C for 10 min, 30 amplification cycles at 96°C for 1 min, 57-60°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 30 min. Following enzymatic purification with 1 U exonuclease I and 2 U alkaline phosphatase (Ambion, Austin, TX), samples were sequenced using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's instructions. Sequencing reaction products were purified from the residual dye terminators using a BigDye XTerminator Purification kit (Applied Biosystems). Repeat structures were determined by direct sequencing of PCR products using an *ABI 3130xl* Genetic Analyzer (Applied Biosystems).

Marker	Primer sequence (5'-3')
DXS6807	F: 5'AAAATACTCCACCCCACT 3'
	R: 5'TGCATGCATGTTACATAACTT 3'
DXS981	F: 5' CGATGAAAGAGTTGGAGATTGTG 3'
	R: 5' CACCATATTGTTCTTGAGTCCT 3'
DXS6803	F: 5'TGTGCTTTGACAGGAAAAACAA 3'
	R: 5' TTCACTTGCTATGTGAAAGAAGC 3'
DXS6809	F: 5' TGCTTTAGGCTGATGTGAGG 3'
	R: 5' CAGGAATACTGAGGCATGA 3'
XHPRT	F: 5' AGAGGGCAGTAGCTTTCAGCTT 3'
	R: 5' TGCCACAGATAATACACATCCCC 3'
DXS7423	F: 5' TGCGAGCCCACTCTTCTAT 3'
	R: 5' TGGCCTTTGTCTCCAGTACC 3'
DXS1187	F: 5' AGGCACCTTTCAGTACTCA 3'
	R: 5' AGAGGGTGATATGGGGGACT 3'
DXYS267	F: 5' GTGGTCTTACTTGTGTCAATAC 3'
	R: 5' GTGTGTGGAAGTGAAGGATAG 3'
DXYS218	F: 5' CTTCTCCAAGCTGGGTCT 3'
	R: 5' CAACTGAGGGGACCTGGAAT 3'

DYS448	F: 5' TGTCAAAGAGCTTCAATGGAGA 3'
	R: 5' TCTTCCTTAACGTGAATTCCTC 3'

Table 1: Forward and reverse primers used in STRs sequencing.

Statistical analyses

ARLEQUIN 3.10 [15] software was used to calculate sex-specific allele frequencies, gene diversity, and heterozygosity. Allele frequency distributions observed for the loci in male and female samples were compared using an exact test of population differentiation. Deviations from Hardy-Weinberg equilibrium at each locus were tested for statistical significance using female data, according to Guo and Thompson [16]. ARLEQUIN 3.10 also was used to estimate the linkage disequilibrium (LD) between all pairs of loci by the exact test. All statistical tests and all calculations of standard deviations (SDs) were based on 10,000 randomizations. To determine linkage values and estimate a genetic map of the markers, we submitted the physical positions of the examined STRs to the Rutgers Map Interpolator application in the Rutgers Combined Linkage-Physical Map of the human genome (v.2) [http://compngen.rutgers.edu/Default.aspx] [17]. The Rutgers Map is a high-resolution genetic map that assembles the largest set of polymorphic markers publicly available. Included in the Rutgers Map are sequence-based positional data, recombination-based data, and genotype data from the CEPH and deCODE pedigrees, the Marshfield map, and the SNP Consortium. Genetic localizations and recombination values were estimated using the formula of Kosambi et al. [18]. The ChrX-STR application [http://www.chrx-str.org] was used to determine the polymorphism information content (PIC) [19], the power of discrimination (PD) for males and females [20], and the mean exclusion chance (MEC) [20-22].

Results and Discussion

Allelic designation and estimation of frequencies

Sequencing data were used to assign alleles in accordance with the ISFG recommendations [23]. Fragment sizes were analyzed by comparison with allelic ladders prepared in house by pooling sequenced alleles. Control DNA from cell line 9947A (Applied Biosystems) was used as reference standard. Repeat structures and alleles observed among the 10 STRs are summarized in Table 2. We examined in detail the structures of two loci that had not previously been characterized for forensic purposes, DXS1187 and DXYS218. Sequencing of 45 chromosomes identified DXS1187 as a tetrameric repeat marker, with 10 alleles and a (GATA)₂-GAT-(GATA)_n sequence structure. According to the recommended nomenclature [23], the proposed designation of DXS1187 alleles ranges from 12 to 20. For DXYS218, sequencing of 42 chromosomes revealed a repeat structure of (AGAT)₂-GAT-AGAT-ACAT-(AGAT)_n in both X and Y chromosomes and alleles ranging from 11 to 17.

Sequence data obtained from the other eight STRs were consistent with published structures [5-14]. As previously described [11] for the XHPRT marker of one sample, we observed an architecture composed by an AG dinucleotide deletion 48-bp downstream of 12-repeat (AGAT) sequence producing an allele fragment that is shorter by two bases. At the DXYS267 locus, corresponding to the DYS393 marker [13], Elucigene QST*R-XY PCR detects the Y-chromosomal del(TTAG) polymorphism located 90-bp downstream of the STR.

Therefore, allele frequencies for the DXYS267 marker were estimated using only female data. Similarly, DXS6807 sequencing indicated that Elucigene QST[®]R-XY PCR amplifies a del(AATAA) polymorphism, 62-bp downstream of the STR, associated to the allele 11.

Locus Name	Repeat Motif	Allele Range	Control 9947A	Reference
DXS6807	PF-N16-(GATA)1-N7-(GATA)2GAC(GATA)8-15-N99-PR	Nov-18	Dec-14	[5]
DXS981	PF-N228-(TATC)11-17-N46-PR	Nov-17	13.3-14.3	[6]
	PF-N145-Δ(TT)-N81-(TATC)10-15(ATC)(TATC)1-N46-PR			
DXS6803	PF-N13-(TCTA)10-14-N88-PR	10-15.3	11.3-12	[7]
	PF-N13-(TCTA)10-14(TCA)(TCTA)1-N88-PR			
DXS6809	PF-N41-(CTAT)7(ATCT)3-N9-(TATC)3(ATCT)3-N10-(ATCT)11-14-N28-PR	27-37	31-34	[8]
	PF-N41-(CTAT)8-10(ATCT)3-N9-(TATC)3(ATCT)5-N10-(ATCT)11-16-N28-PR			
DXS1187	PF-N23-(GATA)2GAT(GATA)5-13(G/AATA)1(GATA)4-N125-PR	Dec-20	18-19	
XHPRT	PF-N77-(AGAT)7-17-N178-PR	Jul-17	14-14	[9,10]
DXS7423	PF-N72-(TCCA)3-N8-(TCCA)10-15-N50-PR	13-18	14-15	[12]
DXYS267	PF-N1-(TATA)1(GATA)7-12(GACA)1(GATA)1-N158-PR (females)	Oct-17	13-14	[13]
DXYS218	PF-N565-(AGAT)2GAT(AGAT)1(ACAT)1(AGAT)7-13-N2-PR	Nov-17	15-16	
DYS448	PF-N48-(AGAGAT)11-13-N10-(AGAGAT)3-N14-(AGAGAT)6-10-N44-PR	17-23	-	[14]

Table 2: Repeat motif and alleles observed in the 10 XY-STRs

The exact test on female data showed no significant deviation from Hardy-Weinberg equilibrium. No significant differences were found for any loci in allele frequencies between the male and female subgroups. For this reason, samples were pooled, and the allele

frequencies of the ten X and Y chromosomal (XY)-STRs in the Italian population were reported (Table 3). At each locus, 6-13 alleles were observed.

Allele	DYS448	DXYS267	DXYS218	DXS6807	DXS981	DXS6803	DXS6809	DXS1187	XHPRT	DXS7423
7									0.0011	
8									0.0033	
9									0.0077	
10		0.0016				0.046			0.0077	
10.3						0.0042				
11		0.0016	0.0048	0.5306*	0.0033	0.3284			0.149	
11.3					0.0055	0.0188				
12		0.1511		0.0143	0.0188	0.2615		0.0011	0.3377§	
12.3					0.1093	0.1068				
13		0.4405	0.0048	0.0022	0.1347	0.1025		0.0044	0.2925	0.0475
13.3					0.287	0.1046				
14		0.2396	0.2636	0.2285	0.1777	0.0188		0.0155	0.1435	0.3653
14.3					0.1015	0.0063				
15		0.1479	0.4871	0.1976	0.0993			0.0265	0.0453	0.3609
15.3					0.0287	0.0021				

16		0.0161	0.2065	0.0254	0.0254			0.1104	0.011	0.1876
16.3					0.0077					
17	0.0106	0.0016	0.0332	0.0022	0.0011			0.1711	0.0011	0.0375
18	0.0565			0.0022				0.2693		0.0011
19	0.4028							0.3411		
20	0.3533							0.0607		
21	0.1519									
22	0.0141									
23	0.0106									
27							0.0042			
28							0.0209			
29							0.0355			
30							0.023			
31							0.2114			
32							0.1653			
33							0.2803			
34							0.1736			
35							0.0628			
36							0.0146			
37							0.0084			
PIC	-	0.658	0.59	0.609	0.815	0.76	0.788	0.73	0.717	0.642
Het	-	0.704	0.612	0.637	0.801	0.798	0.804	0.752	0.74	0.717
PD f	-	0.679	0.817	0.833	0.953	0.927	0.94	0.91	0.902	0.853
PD m	0.689	0.866	0.817	0.659	0.834	0.788	0.813	0.765	0.756	0.697
MEC	-	0.463	0.385	0.609	0.815	0.76	0.788	0.728	0.716	0.642

Table 3: Allele frequencies of the 10 XY-STR markers in the Italian population. *AATAA deletion, 62-bp downstream of the repeat motif, observed with a frequency of 0,0298; § AG dinucleotide deletion, 48-bp downstream of the repeat motif, observed with a frequency of 0,0011. PIC: Polymorphism Information Content; Het: Observed Heterozygosity; PD: Power of Discrimination for females (f) and males (m); MEC: Mean Exclusion Chance.

The allele frequencies of the investigated X-STRs were similar to those reported in other European populations and in Italy [24-26]. No significant differences were detected when an exact test of population differentiations was performed to compare our marker frequencies with those obtained in other studies of Italian populations (100,000 steps in Markov chain).

Linkage and LD

Unlike autosomal STRs, gonosomal markers are syntenic. Both physical dependencies between loci (linkages) and dependencies between alleles at different associated loci (LD) may occur at

gonosomal markers and can affect statistical analyses. In kinship testing, if loci are closely linked and in LD, it is highly indicated to use haplotype frequencies of clustered STRs rather than single STR frequencies [27-29].

Linkage measures the co-segregation of closely positioned loci within families and provides an estimate of the genetic distance between loci. Alleles in X-STR loci recombine in female meioses exclusively and at a frequency dependent upon their genetic distance. Table 4 summarizes the *recombination* fraction theta (θ) values and the genetic distances between the considered X-STR markers obtained from the Rutgers Map.

Locus Name	Cytogenetic Band (NCBI v. 37.1)	Physical Location (bp) (NCBI v.37.1)	Genetic Location (cM) (Rutgers Map v.2)	Distance (cM)	Theta
DXS6807	Xp22.32	4743382	14.76	78.05	0.458
DXS981	Xq11.2	68197359	92.81	6.59	0.065
DXS6803	Xq21.31	86431170	99.4	8.72	0.086
DXS6809	Xq21.33	94938153	108.12	39.82	0.331
DXS1187	Xq26.1	1.31E+08	147.94	1.71	0.017
XHPRT	Xq26	1.34E+08	149.64	34.55	0.299
DXS7423	Xq28	1.5E+08	184.19		

Table 4: Physical and Genetic locations of the X-STR markers. Genetic and physical locations obtained from <http://compgen.rutgers.edu> and <http://www.ncbi.nlm.nih.gov>. Recombination fractions Theta estimated using Kosambi's map function (1944).

Genetic distances confirm the distribution of our X-STRs into the typical four linkage groups on Xp22.32 (DXS6807), Xq11/21 (DXS981-DXS6803-DXS6809), Xq26 (DXS1187-XHPRT), and Xq28 (DXS7423). The linkage groups are located at distances ranging from 35 to 78 cM (θ assessed from 0.30 to 0.46) and were regarded as indicative of independent genotypes. The DXS1187-XHPRT cluster spans approximately 1.7 cM, whereas markers in the DXS981-DXS6803-DXS6809 cluster are associated with distances of 6.59 cM and 8.72 cM, respectively. Similar genetic distances were published recently in a large recombination study [30].

LD measures the non-random associations of alleles at different loci at the population level. For closely linked markers, strong LD may be observed. A considerable LD implies a deviation of population-specific haplotype frequencies from the product of the corresponding allele frequencies. When this occurs, haplotype frequencies cannot be

inferred from single-allele frequencies and instead must be estimated directly from population data.

LD was assessed for all possible pairwise comparisons of loci and between X and Y STRs. Significant LD was only detected for the closest markers, DXS1187-XHPRT ($p=0.043 \pm 0.001$). This result was expected according to the distance between these two X-chromosomal loci (1.7 cM) and given the smaller number of observed haplotypes compared to those expected (37 vs. 99). However, after Bonferroni correction for multiple tests, no significant association was detected in all pairwise comparisons.

Because of the large number of possible haplotypes, a solid evaluation of LD between DXS1187-HPRT would have required an extremely large sample size. Thus, haplotype frequencies were estimated directly from our population (Table 5).

DXS981-DXS6803-DXS6809			
Haplotype	Frequency	Haplotype	Frequency
11 12.3 31	0.0065	14 12.3 34	0.0065
12 11 33	0.0065	14 13 31	0.0065
12 12 33	0.0065	14 13 32	0.0132
12.3 11 31	0.0065	14 13 33	0.0132
12.3 11 33	0.0329	14 13.3 33	0.0132
12.3 11 34	0.0065	14 13.3 29	0.0065
12.3 12 28	0.0065	14 14 35	0.0065
12.3 12.3 34	0.0065	14 14.3 32	0.0065
12.3 13 33	0.0065	14.3 11 31	0.0132
12.3 13 35	0.0065	14.3 11 32	0.0065
12.3 13.3 31	0.0065	14.3 11 33	0.0065
12.3 13.3 32	0.0065	14.3 11 35	0.0065
13 10 32	0.0065	14.3 11.3 32	0.0065

13 10 29	0.0065	14.3 12 32	0.0065
13 11 32	0.0065	14.3 12 33	0.0065
13 11 34	0.0132	14.3 12.3 31	0.0065
13 11 35	0.0065	14.3 12.3 32	0.0065
13 11.3 29	0.0065	14.3 12.3 33	0.0065
13 12 31	0.0065	14.3 12.3 34	0.0065
13 12 33	0.0065	14.3 12.3 36	0.0065
13 12 35	0.0065	14.3 13 31	0.0065
13 13 31	0.0065	14.3 13 34	0.0065
13 13.3 31	0.0065	14.3 13.3 31	0.0065
13 13.3 32	0.0132	14.3 13.3 34	0.0065
13 13.3 34	0.0065	15 11 31	0.0065
13.3 10 33	0.0065	15 11 32	0.0065
13.3 11 31	0.0263	15 11 33	0.0065
13.3 11 32	0.0132	15 11 28	0.0065
13.3 11 33	0.0132	15 11.3 35	0.0065
13.3 11 34	0.0263	15 12 31	0.0065
13.3 11 29	0.0065	15 12 32	0.0132
13.3 12 31	0.0263	15 12 34	0.0065
13.3 12 32	0.0065	15 12 35	0.0132
13.3 12 33	0.0395	15 12 28	0.0065
13.3 12 34	0.0263	15 12.3 31	0.0065
13.3 12 35	0.0065	15 12.3 33	0.0065
13.3 12.3 31	0.0197	15 13 33	0.0065
13.3 12.3 32	0.0132	15 13 34	0.0065
13.3 12.3 33	0.0065	15 13.3 31	0.0065
13.3 12.3 34	0.0065	15.3 10 33	0.0065
13.3 13 32	0.0065	15.3 11 31	0.0065
13.3 13 34	0.0065	15.3 12.3 31	0.0065
13.3 13.3 30	0.0065	15.3 13 31	0.0065
13.3 13.3 31	0.0065	15.3 13 33	0.0065
13.3 13.3 32	0.0132	16 11 32	0.0065
13.3 13.3 34	0.0065	16 12 34	0.0065
14 11 31	0.0197	16 12.3 31	0.0065
14 11 33	0.0197	16 12.3 34	0.0065
14 11 37	0.0065	16 13 31	0.0065

14 11.3 32	0.0065	16 13 32	0.0065
14 12 30	0.0065	16.3 11 31	0.0065
14 12 31	0.0065	PIC	0.9867
14 12 32	0.0132	Het	0.9939
14 12 33	0.0132	PD f	0.9997
14 12 34	0.0132	PD m	0.9869
14 12 35	0.0065	MEC	0.9882
14 12.3 33	0.0197		
DXS1187-XHPRT			
Haplotype	Frequency	Haplotype	Frequency
13 14	0.0035	18 12	0.0669
14 11	0.0035	18 13	0.081
14 12	0.0141	18 14	0.0387
14 13	0.0035	18 15	0.007
14 17	0.0035	19 8	0.0035
15 12	0.0035	19 11	0.0493
15 15	0.0035	19 12	0.0986
15 16	0.0035	19 13	0.1232
16 11	0.0176	19 14	0.0458
16 12	0.0458	19 15	0.0282
16 13	0.0282	20 7	0.0035
16 14	0.0246	20 11	0.0035
16 15	0.0106	20 12	0.0035
17 11	0.0352	20 13	0.0106
17 12	0.0634	20 14	0.0106
17 13	0.0563	20 15	0.007
17 14	0.0317	PIC	0.9376
17 15	0.007	Het	0.9132
17 16	0.0035	PD f	0.9934
18 8	0.0035	PD m	0.9407
18 11	0.0528	MEC	0.9344

Table 5: Haplotype frequencies of the DXS981-DXS6803-DXS6809 and the DXS1187-XHPRT clusters in the Italian population.

Marker informativity

Statistical parameters were calculated for the XY-STRs and for the two considered clusters (Table 5). The heterozygosities and PIC values for most of the markers exceeded 0.7. In particular, DXS981 was associated with 13 alleles and a particularly large PIC of 0.815. Conversely, DXYS218 was the least polymorphic/informative locus

with a PIC of 0.590. The genetic diversity of DYS448, corresponding to the PD, was estimated as 0.689. MEC values ranged from 0.385 to 0.815 in the separated XY-STR markers, whereas increases to 0.988 and 0.934 were observed in cluster I (DXS981-DXS6803-DXS6809) and cluster II (DXS1187-XHPRT), respectively. The low MEC values we obtained for pseudoautosomal DXYS218 and DXYS267 STRs

highlight the reduced efficiency of these markers compared to X-STR systems for kinship testing involving a daughter.

Conclusion

The application of X-STR marker analyses to forensics has been widely recognized, and several X-STR systems have been validated and adapted into multiplex amplification kits for forensic purposes. X-chromosome markers are especially helpful for deficiency cases, such as paternity testing without maternal genotype information or kinship analyses in which only remote relatives are available. X-chromosomal markers are more efficient than autosomal markers in these cases because they are associated with a larger MEC for a comparable PIC. In the present study, an Italian population was genotyped at 10 gonosomal STR markers using the commercially available Elucigene QST*R-XY kit. Most of these markers had already been validated for forensic use. We characterized the following two additional loci to determine their forensic utility: DXYS218, located in the pseudoautosomal region PAR, and DXS1187, located in the region Xq26.1 near the XHPRT locus. Moreover, we like to point out that this kit, widely available in the diagnostic, non-forensic genetic laboratories, can be also used for a fast segregation analysis of X-chromosome during prenatal or postnatal genetic linkage analysis.

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