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ORIGINAL ARTICLE

The STR cluster DXS10148–DXS8378–DXS10135 provides a powerful tool for X-chromosomal haplotyping at Xp22

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Abstract The evaluation of four pairs of tightly linked chromosome X (ChrX) short tandem repeat (STR)s at Xp22, Xq12, Xq26 and Xq28 led to the creation of the Argus X 8 multiplex amplification kit. These eight STRs are distributed as four closely linked pairs over the entire X-chromosome, and for practical reasons, they are assigned to four linkage groups 1–4. To achieve a further considerable enhancement in discrimination power, we suggest to include additional markers. A recent paper referred to the earlier evaluation of STR clusters at Xq12, Xq26 and Xq28, and here we present the pending data of linkage group 1 at Xp22. The newly established STR updates the Xp22 STR cluster which now presents three polymorphic markers: DXS10148 (PIC = 0.8556), DXS10135 (PIC = 0.9093) and DXS 8378 (PIC = 0.6454). Typing of 398 X-chromosomes provided 278 different and 200 unique haplotypes. All the other haplotypes observed appeared with frequencies in the range between 0.005 and 0.015. Considering this STR triple in the context with the three further triple clusters Xq12, Xq26 and Xq28 published earlier, we announced the development of a next generation of a ChrX STR cluster typing kit.

Keywords X-chromosome · STR · Kinship testing · Haplotyping · Recombination

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Introduction

Recently, four pairs of tightly linked chromosome X (ChrX) short tandem repeats (STRs) i.e. DXS10135–DXS8378, DXS7132–DXS10074, HPRTB–DXS10101 and DXS7423–DXS10134 were assembled to construct the Argus X 8 multiplex amplification kit [2]. To achieve a further considerable enhancement in discrimination power, we suggest the inclusion of additional STRs, i.e. DXS10148 at Xp22, DXS10079 at Xq12[6], DXS10103 at Xq26 (Rodig et al., submitted) and DXS10146 or DXS10147 at Xq28 [4]. These 12 STRs are distributed as four closely linked triplets over the entire X-chromosome at Xp22, Xq12, Xq26 and Xq28, and for practical reasons, they are assigned to four linkage groups 1–4. The test system proposed here can be used for haplotyping of four ChrX STR clusters which show very low haplotype

frequencies at each locus. Characterisation and forensic validation have already been done for the STR clusters at Xq12 [6], Xq26 (Rodig et al., submitted) and Xq28 [4].

Here we report the pending data of an STR triplet at Xp22 consisting of DXS10148, DXS10135 and DXS8378. All markers were found considering the contig AC003684 (Genome Systems Human BAC Library). DXS8378 has already been published and investigated by many groups [3, 5, 8–10, 12, 15] and is included in the Argus X 8 kit. DXS10135 is also included in this kit; however, only few data have been published so far [14]. DXS10148 was chosen as it was evaluated to be the most feasible marker very closely linked to DXS8378 and DXS10135. Haplotype data of DXS10148, DXS10135 and DXS8378 were established by evaluation of 398 chromosomes of a German sample. A recombination study was carried out observing by allele tracking through father–daughter–grandson families.

Materials and methods

The DNA specimens investigated were from a population in middle Germany. They were taken from 80 routine kinship trio cases with daughters and from 79 father–daughter–grandson constellations. The latter group consisted of 59 families with one grandson, 15 families with two grandsons and five families with three grandsons. The first group contributed 240 and the second group 158 ChrX to our investigation, resulting in a total of 398 ChrX. Furthermore, the sample offered the chance to check 159 male and 184 female meioses for mutations. Unfortunately, the father–daughter–grandson samples were anonymous, and therefore, a mutation–age relationship could not be assessed. To study the crossing-over rate for the three markers within the 131.3kb region, we observed the haplotype transmission through the 79 father–daughter–grandson constellations which have the potential for observing 104 meioses. Unfortunately, homozygosity in women provides an uninformative situation, so that the effective number of checked meioses at each locus was smaller. Recombination events can be detected between two markers, when both are heterozygous. A survey of the number of informative and uninformative situations in the 104 mother–son constellations is shown in Table 4.

Primer sequences were established by considering the contig AC003684 sequence (Genome Systems Human BAC Library), and the exact ChrX position was found by University of California, Santa Cruz in silico polymerase chain reaction (PCR; http://www.genome.ucsc.edu/).

Table 1 Xp22 STRs: allele numbers, allele length and allele design and the pattern of control DNAs which can be used for ladder calibration

	DXS10148	DXS10135	DXS8378
Number of alleles	16	16	8
Length (bp)	215–262	159–231	110–142
Nomenclature	18–31.1	14.1–36.2	8–18
Cell line 3657	23.1	25	12
Cell line 9947	22.1/23.1	21.1/27	10/11
Cell line 9948	23.1	22	11

Table 2 Xp22 STRs: allele distribution and parameters of forensic interest for a German sample

	Frequency		
	DXS10148	DXS10135	DXS8378
Allele			
n	380	380	380
8			0
9			0.02
10			0.34
11			0.35
12			0.25
13			0.04
14			0
15			0
18	0.156	0.033	
19	0.025	0.055	
20	0.009	0.063	
21	0.014	0.122	
21.1	0.011	0.011	
22	0.005	0.107	
22.1	0.011		
23	0.005	0.115	
23.1	0.137		
24		0.088	
24.1	0.126		
25		0.107	
25.1	0.191		
26		0.071	
26.1	0.159		
27		0.079	
27.1	0.078		
28		0.060	
28.1	0.030		
29		0.022	
29.1	0.040		
30		0.041	
30.1	0.005		
31		0.019	
32		0.002	
Parameters			
PIC	0.8556	0.9093	0.6454
HET	0.8717	0.9180	0.7042
PD woman	0.9691	0.9866	0.8544
PD man	0.8695	0.9156	0.7023
MEC trio	0.8556	0.9093	0.6454
MEC duo	0.7589	0.8393	0.5007

Table 3 Distribution of the STR haplotypes DXS10148–DXS10135–DXS8378 of 398 X-chromosomes

	German sample (*n*)	Proportion of the sample (%)
Number of investigated chromosomes	398	–
Number of different haplotypes observed	278	–
Most frequent haplotype, 25.1–22–10 (*f*~1.5%)	6	1.51
Haplotypes with frequencies, *f*~1.3%	5	1.26
Haplotypes with frequencies, *f*~1.0%	52	13.06
Haplotypes with frequencies, *f*~0.75%	45	11.31
Haplotypes with frequencies, *f*~0.5%	90	22.61
Number of unique haplotypes (*f*~0.25% each)	200	50.25

Amplifications were carried out as triplexes in 25µl PCR reaction volumes containing approximately 0.2–2ng DNA, 200µM each deoxyribonucleotide triphosphate, 1.5mM $MgCl_2$, 1U Hot-Goldstar-Polymerase (Eurogentec, Seraing, Belgium), 1× PCR buffer and the appropriate primer pairs. The primers were applied at concentrations of 0.4µM for DXS10148, 0.2µM for DXS10135 and 0.4µM for DXS8378. The cycle conditions in PTC-200 cyclers (MJ Research, Watertown, MA, USA) were as follows: 94°C for 10min, (94°C 45s, 52°C 45s, 72°C 45s) × 32 and 72°C for 10min. The primer sequences, their position on the ChrX and the distances between the loci are shown in the table available as ESM 1. The resulting PCR products were resolved and detected by capillary electrophoresis with the denaturing polymers POP4 (Applied Biosystems, Foster City, CA, USA) in the ABI 310 sequencer (Applied Biosystems) as recommend by the manufacturer. Amplicon sizing was supported using the DNA size standard 550 (Biotype AG, Dresden, Germany). Calibration of the allelic ladder was done using sequenced samples. Cycle sequencing was carried out as described earlier [6].

Haplotypes of the three markers at the Xp22 region were obtained as typing results from men. In women, the phase of the three STRs were established indirectly by family analysis.

Table 4 Results of a recombination study in 104 meioses

	DXS10148	DXS10135	DXS8378
NOIM	89		
NOR	1		
NOIM		79	
NOR		1 (or one step mutation)	
Details of case 1			
Grandfather	19	22	11
Daughter	23.1/19	27/22	11/11
Grandson	19	27	11
Details of case 2			
Grandfather	23.1	26	10
Daughter	18/23.1	26/32.2	11/10
Grandson	18	32.2	10

NOIM Number of informative meioses, *NOR* number of recombinations

Results

The three STRs described here span a distance of 131.3kb, and the exact localisation on the ChrX is shown in the table in ESM 1. The three STRs can be proposed for usage as a cluster of the X-chromosomal linkage group 1. The figure, presented as ESM 2, shows the common formulae of the repeat structure and the details of the sequences. A summary of the features of the STRs and of cell line DNA typing patterns are shown in Table 1. These results of the latter can be used as intralaboratory and interlaboratory standards. Table 2 presents allele frequencies and parameters of forensic interest and confirms that DXS10148 and DXS10135 are highly polymorphic markers and could also be used with a considerable impact as single forensic STRs. As known, DXS8378 shows only moderate polymorphic information content but, combined with the three STRs, constitutes a powerful tool for kinship testing. Table 3 presents a review of the distribution and the frequencies of haplotypes from our sample of 398 ChrX in which 50.25% shows haplotype frequencies of only 0.0025 and only about 15% exhibited frequencies of 0.01 or higher. All haplotypes are given in detail as ESM 3.

In a recombination study, we investigated 104 female meioses, but the number of informative meioses was smaller (Table 4). The number of recombinations located between DXS10135 and DXS10148 was one out of 89 informative meioses (case 1 in Table 4). Furthermore, we found one event which can be interpreted as a recombination or as a mutation (case 2 in Table 4), so the stability of this area cannot be evaluated with certainty. Checking the

Table 5 Xp22 STRs: Mutations found in family studies

Meioses	Number of meioses	DXS10148	DXS10135	DXS8378
Female	184	1 (1 repeat loss)	–	–
Male	159	–	1 (2 repeats loss)	1 (1 repeat loss)

STRs with regard to their mutability, we found three events which are displayed in Table 5 and do not consider the uncertain event in DXS8378 which has been mentioned before as being a questionable crossing over.

Discussion and conclusions

Several multiplex systems which utilise X-chromosomal STRs have been reported [1, 5, 10, 11, 13, 16]. However, none of them utilises low recombining STR clusters for haplotyping purposes. The Argus X 8 amplification kit covers eight markers which occur as four duos located at Xp22, Xq12, Xq26 and Xq28. After establishing the STR combination of the Argus X 8 amplification kit, further attempts were aimed at the evaluation of further closely linked STRs for the purpose of haplotyping. For upgrading the multiplex kit, we have chosen STR candidates from each linkage group that showed the closest linkage, the highest degree of polymorphism and amplification and analysing conditions which are robust and easy to handle. Whereas the candidates for upgrading linkage groups 2–4 have already been published [4, 6] (Rodig et al., submitted), the selection of a candidate STR for supplementing the linkage group 1 cluster was still pending. Due to its degree of polymorphism and a reasonably low distance from DXS10135, the DXS10148 STR seems best suited to fulfil these requirements. DXS10148 showed the peculiarity of an upstream association with a poly-A stretch which may be instable. Therefore, there is limited latitude for designing the 5′ primer, and consequently, this primer covers 2.5 repeats of the STR. However, this unusual solution does not cause any problems for an accurate amplification process.

The results of earlier investigations [4, 6] (Rodig et al., submitted) and of this study suggest that an upgrade of each cluster by a further STR is possible. Attempts to include the nonutilised STRs into an extended kit could provide improved potentials to solve complex kinship cases. Initially, the genetic distance within each STR cluster was assumed to span less than 1cM, whereas the pair-to-pair distance is about 50cM or more. These forecasts were deduced from the fundamental ChrX recombination study published by Nagaraja et al. [7]. The first assumption has to be revised. The Xp22 cluster investigated here and the Xq28 cluster [4] show recombination rates in the range of 1–5%. Nevertheless, our preliminary evaluation demonstrates that trios of closely linked STRs investigated during our project provide low-frequency haplotypes. Not only stable haplotypes but also low recombining marker clusters can be used in kinship testing when the crossing over rates are known. To enable reliable kinship calculation, recombination rates have to be established more precisely. However, checking several hundred meioses can be accomplished only by the international community of ChrX marker users.

References

1. Asamura H, Sakai H, Kobayashi K et al (2006) MiniX-STR multiplex system population study in Japan and application to degraded DNA analysis. Int J Leg Med 120:174–181
2. Becker D, Rodig H, Augustin C et al (2008) Population genetic evaluation of eight X-chromosomal short tandem repeat loci using Mentype Argus X-8 PCR amplification kit. Forensic Sci Int Genet 2:69–74
3. Edelmann J, Hering S, Michael M et al (2001) 16 X-chromosome STR loci frequency data from a German population. Forensic Sci Int 124:215–218
4. Edelmann J, Hering S, Augustin C, Szibor R (2008) Characterisation of the STR markers DXS10146, DXS10134 and DXS10147 located within a 79.1kb region at Xq28. Forensic Sci Int Genet 2:41–46
5. Gomes I, Prinz M, Pereira R et al (2007) Genetic analysis of three US population groups using an X-chromosomal STR decaplex. Int J Leg Med 121:198–203
6. Hering S, Augustin C, Edelmann J et al (2006) DXS10079, DXS10074 and DXS10075 are STRs located within a 280-kb region of Xq12 and provide stable haplotypes useful for complex kinship cases. Int J Leg Med 120:337–345
7. Nagaraja R, MacMillan S, Jones C et al (1998) Integrated YAC/STS physical and genetic map of 22.5Mb of human Xq24-q26 at 56-kb inter-STS resolution. Genomics 52:247–266
8. Pepinski W, Skawronska M, Niemcunowicz-Janica A et al (2005) Polymorphism of four X-chromosomal STRs in a Polish population sample. Forensic Sci Int 151:93–95
9. Pereira R, Gomes I, Amorim A, Gusmao L (2007) Genetic diversity of 10 X chromosome STRs in northern Portugal. Int J Leg Med 121:192–197
10. Poetsch M, Petersmann H, Repenning A, Lignitz E (2005) Development of two pentaplex systems with X-chromosomal STR loci and their allele frequencies in a northeast German population. Forensic Sci Int 155:71–76
11. Robino C, Giolitti A, Gino S, Torre C (2006) Development of two multiplex PCR systems for the analysis of 12 X-chromosomal STR loci in a northwestern Italian population sample. Int J Leg Med 120:315–318
12. Shin SH, Yu JS, Park SW et al (2005) Genetic analysis of 18 X-linked short tandem repeat markers in Korean population. Forensic Sci Int 147:35–41
13. Wiegand P, Berger B, Edelmann J, Parson W (2003) Population genetic comparisons of three X-chromosomal STRs. Int J Leg Med 117:62–65
14. Zalan A, Volgyi A, Brabetz W et al (2008) Hungarian population data of eight X-linked markers in four linkage groups. Forensic Sci Int 175(1):73–78
15. Zalan A, Volgyi A, Jung M et al (2007) Hungarian population data of four X-linked markers: DXS8378, DXS7132, HPRTB, and DXS7423. Int J Leg Med 121:74–77
16. Zarrabeitia MT, Amigo T, Sanudo C et al (2002) A new pentaplex system to study short tandem repeat markers of forensic interest on X chromosome. Forensic Sci Int 129:85–89