Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics 2 (2008) 41–46
FSI GENETICS
www.elsevier.com/locate/fsig

Characterisation of the STR markers DXS10146, DXS10134 and DXS10147 located within a 79.1 kb region at Xq28

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Received 24 January 2007; received in revised form 26 June 2007; accepted 6 August 2007

Abstract

Three polymorphic X-chromosomal STR markers within a 79 kb region at Xq28 were studied and registered in the GDB as DXS10146, DXS10134 and DXS10147. These markers were molecular characterised and evaluated for their forensic usage. As a result DXS10134 was recently integrated in the commercial available test kit Mentype Argus X-8. At locus DXS10146 we found 23 alleles with PIC and HET values of 0.878 and 0.887. Locus DXS10134 showed 17 alleles with PIC and HET values of 0.844 and 0.858. At locus DXS10147 only 5 alleles with some lower PIC and HET values of 0.636 and 0.692 were found. Additionally, the already known and closely linked STR DXS7423 was included into the haplotyping and recombination studies. Testing this cluster a German population of 404 males revealed the presence of 311 haplotypes. Recombination analysis was performed in 109 father–daughter–grandson trios in which two crossing over events were observed located in the 65.8 kb region between DXS10146 and DXS10134. By using this STR complex for haplotyping in kinship testing further genetic analyses are required to establish an exact recombination rate.

Keywords: X-chromosome; Xq28; STR cluster; Haplotyping; Kinship testing

1. Introduction

In males and in certain pedigree situations, typing of closely linked STRs can establish haplotypes which can substitute single STR data providing highly informative tools for kinship testing [1–5]. Initially, we checked internet databases for clusters of linked ChrX microsatellites and established the haplotyping approach using linked markers [2,4]. Thus, it was possible to find clusters consisting from slightly tight linked STRs which span only very few centimorgans. Moreover, our most recent attempts are aimed at establishing haplotypes consisting of very closely linked markers nearly free of recombination. For such purpose we screened GenBank sequences for not described STRs and selected candidates according to their structure, polymorphic degree and localisation. Hence, STRs from different regions on the ChrX were investigated such as Xp22 [Szibor, unpubl.] Xp11 [3], Xq12 [5], Xq26 [6] and Xq28. The aim of the present research was to study the Xq28 STR cluster. We found three polymorphic markers within the human X contig NT_011726, clone AL034384. The markers were characterised and evaluated for their forensic efficiency and registered in the GDB as DXS10146, DXS10134 and DXS10147. Xq28 also hosts the markers DXS7423, DXS8377 [7,8] and DXS10011 [9,10]. Meanwhile DXS10134 and DXS7423 are utilised in the commercially distributed ArgusX-8 amplification kit which also includes closely linked pairs at Xp22, Xq12 [5] and Xq26 [6]. With respect to the haplotype stability our study involves DXS10146, DXS10134, DXS10147 and DXS7423.

2. Materials and methods

2.1. Selection of loci

We screened several Xq28 contig sequences (http://www.ncbi.nlm.nih.gov/) for tri-, tetra- and pentanucleotide

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doi:10.1016/j.fsigen.2007.08.001

repeats using GeneRunner program (http://www.generunner.com). STRs with repeat numbers higher than five were chosen for experimental studies. As a result three markers mentioned above were considered for the forensic evaluation.

2.2. DNA extraction and PCR condition

Blood samples and buccal swabs were obtained from unrelated Germans. DNA was extracted with the QiaAmp Mini Kit (Qiagen). We investigated 169 females for DXS10146 and DXS10147 and 197 females for DXS10134. The numbers of male samples were 499 (DXS10146), 458 (DXS10134) and 498 (DXS10147), respectively. For estimation of the haplotype stability, recombination analysis was performed in 109 female meioses of father–daughter–grandson trios. DXS7423 was also involved in this study.

PCR was carried out using primers according to GenBank sequence information (http://www.gdb.org). Table 1 displays the primer sequences, their X-chromosomal position and the physical distance of the loci. The latter was estimated using the electronic PCR tool in-Silico PCR of http://www.genome.ucsc.edu/ [11].

Amplification was carried out in singleplex PCR 25 μl reaction volume, containing 0.5–2 ng DNA, 200 μM of each dNTP, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 1 U AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, CA) and 1× PCR buffer.

The PCR conditions in a T3 cycler (Biometra, Göttingen, Germany) were as follows:

- DXS10146: 95 °C, 15 min; 94 °C, 40 s, 58 °C, 50 s; 72 °C, 50 s, 28 cycles; 72 °C, 10 min.
- DXS10134: 95 °C, 15 min; 94 °C, 40 s; 60 °C, 50 s; 72 °C, 50 s, 29 cycles; 72 °C, 10 min.
- DXS10147: 95 °C, 15 min; 94 °C, 40 s; 62 °C, 50 s; 72 °C, 50 s, 28 cycles; 72 °C, 10 min.

PCR products were resolved by capillary electrophoresis in the denaturing polymer POP4 and detected with automated fragment analysis using ABI PRISM® 310 sequencer (Perkin-Elmer, Foster City, CA).

2.3. Sequencing

- DXS10146: To facilitate the sequencing procedure we introduced an alternative forward primer 65 bp upstream. The oligonucleotide 5′–AGTAAGATGTCCTGTGAAATG-3′ was used to generate the sequencing templates and as sequencing primer as well. According to their DXS10146 amplicon length 89 male DNA specimens were amplified and sequenced.
- DXS10134: To generate sequencing templates we selected 57 male DNA specimens according to their amplicon length. Amplifications were done using the regular PCR primers, however without any dye labelling. The same oligonucleotides were used as sequencing primers.
- DXS10147: The templates for sequencing ($n = 66$) were generated using the forward primer indicated in Table 1 and an alternative reverse primer beginning 139 bp downstream of the repeat: 5′-GGTGGTGGGCTCTACACATT-3′.

Direct Taq-cycle sequencing was carried out with the BigDyeTerminator Kit (Perkin-Elmer) and a primer concentration of 3 pmol. Sequence data were analysed on the ABI PRISM® 3100 Avant sequencer (Perkin-Elmer, Foster City, CA).

2.4. Data analysis

For each marker the Hardy-Weinberg equilibrium (HWE) was checked by exact test [12] using DNAVIEW software (C. Brenner, Berkeley, CA). Other population genetic parameters of forensic interest such as polymorphism information content (PIC) [13], power of discrimination in females (PDF) and in males (PDM) [14], mean exclusion chance (MEC) [14] and probability of exclusion in motherless cases (PE) [15] were calculated.

3. Results

3.1. Sequencing analysis and nomenclature

All STRs reported here can be described by common formulas in which PrI and PrII and the following numbers indicate the length of the forward and reverse primers. N symbolises the nucleotides in the non-variable blocks. *x* and *y* are variables.

Table 1
Primer sequences and positions

Locus	Primer sequence	Primer position (5′ bp)	Distance (kb)	Distance (kb) (cumulative)
DXS10146	5′-CTGCCTTGCCCTTCCTACC-3′	149,334,927		
	5′-GAAAAAGAAAGAAAGACAGAG-3′	149,335,115		
			65.8	65.8
DXS10134	5′-CCTGGGTGACATAGAGAGAC-3′	149,400,734		
	5′-TGGTTGAGCCCCTGCTTTC-3′	149,400,979		
			13.3	79.1
DXS10147	5′-TGAGCTGAGATTGTGCCACT-3′	149,414,073		
	5′- CTTCATCTACTAATGGCCTGG -3′	149,414,242		
			47.5	126.6
DXS7423	5′-GTCTTCCTGTCATCTCCCAAC-3′	149,461,562		
	5′-AGCTTAGCGCCTGGCACATA-3′	149,461,747		