Forensic Science International: Genetics 4 (2010) 194–199

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Evaluation of seven X-chromosomal short tandem repeat loci located within the Xq26 region

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ARTICLE INFO

Article history:
Received 20 January 2009
Received in revised form 17 July 2009
Accepted 20 August 2009

Keywords:
STR
X-chromosome
Xq26
DXS10101
Mentype Argus X-8 PCR amplification kit

ABSTRACT

In this study a set of 29 X-chromosomal short tandem repeats (STRs) located within the Xq26 region was evaluated. These STRs were found within the 133.14–133.45 Mb region around the HPRTB locus. Evaluation of the microsatellites was performed with regard to polymorphism, reliable amplification, and low stutter artefacts. DXS10101, DXS10102, and DXS10103 were identified as those X-STRs with highest diversity; i.e. PIC values of 0.7174–0.8933. The locus DXS10101 was the optimal candidate for the integration in the commercial available test system Mentype Argus X-8 PCR amplification kit.

1. Introduction

X-chromosomal short tandem repeats (STRs) are very useful for solving complex deficiency cases in the fields of forensics, kinship, and anthropology. The fact that males have only one X-chromosome and that they transmit their X-chromosomal haplotype directly to the child is a great advantage in comparison to autosomal kinship testing. Using a set of X-STR loci with close physical localization could greatly facilitate complex kinship testing [1–6]. So far, only a very small set of polymorphic markers including HPRTB is known within linkage group 3 [7–9]. Therefore, the search for further X-STR loci around HPRTB is necessary.

With accomplishing the "Human Genome Project" in 2004 the sequences of all human chromosomes became available; the findings of the X-chromosome were published by Ross et al. [10]. This data are the basis for a wide screening of STRs on the X-chromosome. The aim of this study was to identify informative X-STRs, closely linked to HPRTB. The distance of the STRs investigated is less than 200 kb.

2. Materials and methods

2.1. Bioinformatics, X-STR database

The contigs of the human reference sequence were downloaded from the GenBank website in September 2004. A search for X-STRs consisting of tri, tetra, penta, and hexanucleotide repeats was performed with the program Tandem Repeats Finder [11,12]. The STR sequences were integrated in an internal custom-designed MySQL database. With this database a search for STR loci consisting of 3–6 nucleotide repeat units was performed.

2.2. DNA samples

The reference DNAs K562, NA3657, NA9948, and NA9947A were purchased from ATCC (Manassas, VA, USA) and Coriell Cell Repositories (Camden, NJ, USA). The reference DNAs XX64, XX74, and XY1 are from Biotype AG (Dresden, Germany). Buccal swab samples from 52 female and 80 male healthy unrelated German volunteers from a Dresden population were collected with written informed consent. DNA was extracted from buccal swabs using the NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany).

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doi:10.1016/j.fsigen.2009.08.010

Table 1
PCR primers for the most variable X-chromosomal microsatellites located in the Xq26 region.

Locus ID	Primer 1 fluorescence label	Primers 1 and 2 concentration [nM]	Primer 1 sequence (5′ → 3′)	Primer 2 sequence (5′ → 3′)
DXS10101	6-FAM	125	TACATGTGGGAGTTCATGATACTATATTT	CACAGCAAATGTCACCTTCTTATTTA
DXS10102	6-FAM	136	TGCCTCAATCTTAATGCCTGT	GTTTCTTTGTAGGTACTCAATAAATGTTTGTCAGA
DXS10103	HEX	616	CCTTCATAATCACATATCACATGAGC	AAACAGAACCAGGGGAATGAA
DXS10104	6-FAM	108	ATAATGCCAGCACTTTGTGAAG	ATTATAGGTGCCAACAACCATGA
DXS10105	6-FAM	96	ATGAACAGGAAGTGAAAGATAAGACA	AATATGCTGTGATTGTGCCACT
DXS10106	HEX	172	TCTGAATCTTGCACCCTTTTT	GCAACAGAGCAAGACTCTGTCTCAC
DXS10107	HEX	192	AAGCATGACATCTTAAACATAAGCA	GGAGGTTGCAGTGAGCTACGAT

The 5′-end of reverse primer of DXS10102 contains a GTTTCTT sequence for increasing the value of template independent adenylation of the 3′-end of the fluorescently labeled strand ("PIG-tailing"; [23]).

2.3. Polymerase chain reaction (PCR)

For a screening of the X-STR loci PCR primers were designed for amplicon sizes between 100 and 150 bp with the program Primer3 ([13]; Primer3 website). For DXS10107, DXS10110, and DXS10116 primers to fit longer amplicons with a maximal length of 280 bp were designed because these sequences were not suitable for the primer design of short amplicons. For the PCR amplification 2 ng DNA, 250 nM primermix, 5 μL reaction mix A (Biotype AG) and 1 U JumpStart *Taq* DNA polymerase (Sigma, Taufkirchen, Germany) were used in a 25-μL reaction volume. The PCR protocol consisted of an initial denaturation step at 94 °C for 4 min, 94 °C for 30 s, 60 °C for 120 s, 72 °C for 75 s over 30 cycles and a terminal step at 68 °C for 60 min. For DXS10101 the annealing temperature was 55 °C. The reaction was carried out on a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA).

For further evaluation of X-STR loci a screening multiplex for DXS10101, DXS10102, DXS10103, DXS10104, DXS10105, DXS10106, and DXS10107 was established. PCR primers were designed for amplicon sizes between 111 and 252 bp and the forward primers were 5′-labeled with 6-carboxyfluorescein (6-FAM) or 4,7,2′,4′,5′,7′-hexachloro-6-carboxy-fluorescein (HEX) (Table 1). PCR was carried out as described with the exceptions of using 5 μL reaction mix B (Biotype AG) and primer pairs in concentrations of 96–616 nM as depicted in Table 1. The HPRTB locus was amplified using the Mentype Argus X-UL PCR amplification kit (Biotype AG).

2.4. Electrophoresis

PCR products amplified with non-labeled primers were separated and visualized via electrophoresis using CleanGel HyRes Plus 36S horizontal gels (ETC Elektrophorese-Technik, Kirchentellinsfurt, Germany) and silver staining according to the manufacturers instructions. The PCR products amplified with 6-FAM or HEX 5′-labeled forward primers were analyzed by capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer with POP-4 polymer (Applied Biosystems).

2.5. Sequence analysis

For the three most diverse loci sequence analysis was performed by cloning and sequencing the reference DNAs NA3657, NA9948, and NA9947A (Coriell Cell Repositories; Camden, NJ, USA) and selected DNAs which were used in the population study. For the less diverse loci one to three alleles of the reference DNAs were sequenced. Cloning and sequencing was performed as published [14]. Selected alleles were submitted to GenBank, accession numbers are BV680544–BV680561.

2.6. Statistical analysis

Allele frequencies and the HWE calculations were performed using the software GenoProof (Qualitype AG, Dresden, Germany). Further calculations on population genetic data were performed by using the Internet database ChrX-STR.org 2.0 ([15]; http://www.ChrX-STR.org website). The amounts of stutter artefacts were calculated for peaks with a height of 1000–4500 RFU, the peak areas were calculated and used for quantitative stutter calculation.

3. Results and discussion

3.1. Evaluation of X-STRs

In this study we identified the 29 unreported X-chromosomal loci DXS10100-DXS10128 in the 133.14–133.45 Mb region of the X-chromosome with a close physical localization to the HPRTB locus. Evaluation of these 29 loci was performed using DNAs of 10 males from a German population. It was shown that 16 of the 29 markers revealed repeat number polymorphism, i.e. more than one allele was observed. Additionally, DNAs of 5 females were utilized for further evaluation of these 16 polymorphic markers. It was found that a number of 7 X-STR loci revealed a minimum of 4

Table 2
Haplotype frequencies of HPRTB and DXS10101.

Haplotype		Frequency
HPRTB	DXS10101	
9	30.2	0.0125
11	26.2	0.0125
11	27.2	0.0125
11	29.2	0.0500
11	30.2	0.0125
12	27	0.0125
12	27.2	0.0125
12	28	0.0375
12	28.2	0.0875
12	29	0.0125
12	30.2	0.0500
12	31	0.0125
12	31.2	0.0625
12	32	0.0125
12	33.2	0.0125
13	28.2	0.0750
13	29.2	0.0500
13	30	0.0250
13	30.2	0.0500
13	31	0.1125
13	31.2	0.0250
13	32	0.0500
13	32.2	0.0375
13	33	0.0250
14	29.2	0.0125
14	30	0.0125
14	30.2	0.0250
14	31.2	0.0125
14	32	0.0250
15	28	0.0125
15	29.2	0.0125
15	33	0.0125
16	32	0.0125