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Genetic analysis of eight x-chromosomal short tandem repeat loci in Iraqi population using the Mentype® Argus X-UL PCR amplification kit

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X-Chromosome short tandem repeat (STR) typing can complement existing DNA profiling protocols and can also offer useful information in cases of complex kinship analysis. This is the first population study of 8 X-linked STRs in Iraq. The purpose of this work was to provide a basic data of allele and haplotype frequency for x-linked markers and methods that are commonly used to analyze microsatellites, and the subsequent possibilities of using these specifities especially in forensic genetics. FTA® Technology (FTA™ paper DNA extraction) was utilized to extract DNA. Amplification was performed using the Mentype® Argus X-UL PCR amplification kit. Products were detected using ABI PRISMR 3100 Genetic Analyzer (Applied Biosystems). Forensic efficiency parameters showed that DXS7132, DXS7423, DXS8378, HPRTB, DXS10074, DXS10101, DXS10134 and DXS10135 are suitable for forensic application in Iraq. All the analyzed markers were in Hardy-Weinberg equilibrium (HWE); therefore Hardy-Weinberg laws could be applied for match probability calculation.

Key words: Allele frequency, haplotype frequency, Iraq, short tandem repeat (STR), X-chromosome.

INTRODUCTION

X-Chromosomal short tandem repeats (X-STRs) are particularly helpful in paternity testing and kinship analyses, such as father-daughter, mother-son and grandmother-granddaughter kinship testing, or the kinship testing of putative sisters (Excoffier and Lischer, 2010). The Argus X-8 kit enables simultaneous amplification of eight STR loci located on human chromosome X, that is DXS7132, DXS7423, DXS8378, DXS10074, DXS10101, DXS10134, DXS10135, HPRTB and the locus of Amelogenin (Gomes et al., 2009) (Figure 1). Autosomal markers are helpful in solving most of the forensic tasks in DNA analysis. However, some of them need the implementation of STRs on the sex chromosomes (gonosomes) (Diegoli and Coble, 2011; Mohammed and Imad, 2013). Gonosomal STR markers are helpful in the investigation of relationships among individuals of different generations, especially when key persons of the pedigree are missing. Furthermore, the use of gonosomal STRs in the analysis of DNA traces in forensic purposes is strongly rising (Becker et al., 2008; Muhanned et al., 2015).

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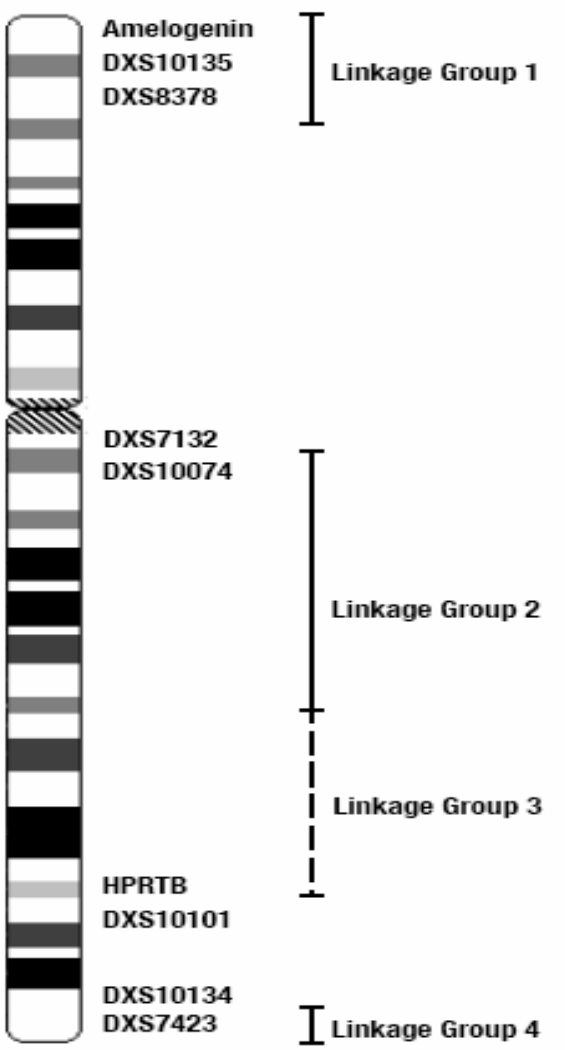

Figure 1. The ideogram of the Mentype® Argus X-8 markers on X-chromosome (www.biotype.de).

Chromosome X short tandem repeats (X-STRs) analysis has recently attracted attention of the forensic community because of its usefulness in complex kinship testing. It is worth-while including X-STRs with autosomal markers for the cases when father/daughter relationships are to be tested. Analysis of ChrX short tandem repeat markers (STRs) can successfully embrace the answer that unravels the challenge presented in particular cases of kinship analysis, when the offspring is female (Szibor et al., 2003). Since fathers transmit the same X chromosome to all their daughters, they are particularly useful in deficiency paternity cases when the child is a female, in maternity testing, and in paternity cases involving blood relatives (Desmarais et al., 1998; Tun et al., 1999; Zarrabeitia et al., 2000; Szibor et al., 2003; Jia et al., 2004; Lee et al., 2004; Imad et al., 2014a,b). Forensic X-STR markers, as per size of amplicons, are just like autosomal and Y chromosomal STRs (Shin et al., 2005; Gomes et al., 2007; Hill et al., 2008; Diegoli and Coble, 2011).

The major advantage of X-chromosomal (ChrX) STRs arises in deficiency paternity cases, that is, when a putative father is not available and DNA from paternal relatives has to be analyzed instead (Szibor, et al., 2000; Imad et al., 2014c). Female individuals fathered by the same man share their paternal ChrX. Males inherit their only ChrX from their mother. Hence, in cases in which the putative grandmother is available for genotyping, the possible ChrX alleles of the putative father can be determined (Ellegren, 2000; Szibor et al., 2003). ChrX marker typing is highly effective in mother-son kinship and in father-daughter testing. However, linkage and possible linkage disequilibrium between the ChrX markers used have to be taken into consideration (Szibor et al., 2006).

In the present study, we investigated the polymorphisms and haplotypes of the Argus X-8 loci in Iraq and evaluated their efficiency in forensic practice.

MATERIALS AND METHODS

Preparation of blood stain samples

Biological samples of blood were collected from 120 healthy unrelated males randomly selected from population living in the middle and south population from Iraq. Total DNA from blood cells was extracted using the Chelex® DNA extraction kit and FTA™ paper DNA extraction. This special paper is useful in storing collected DNA blood samples because it contains a matrix which protects the sample from nuclease degradation and bacterial growth.

DNA amplification for X- chromosomal STR

All samples were amplified using the Mentype® Argus X-8 STRs add 1.2 mm punches from FTA® storage cards containing whole blood into the appropriate wells of the reaction plate. The preferred protocol for use with the GeneAmpR PCR System 9700 thermal cycler is provided below. The estimated total cycle time is 1.5 h, 94°C for 4 min, then: 94°C for 30 s, 58°C for 120 s, 72°C for 75 s, for 30 cycles, then: 68°C for 60 min.

PCR amplicon analysis (capillary electrophoresis)

Capillary electrophoresis is a method in which DNA is separated by size in order to be analyzed. The fragments were separated in POP-4™ polymer using the ABI PRISM® 3130xl Genetic Analyzer instrument for capillary electrophoresis and data collection. The analysis software program used for this study is Applied Biosystems GeneMapper® ID version 3.2, which has precise base sizing capabilities and designates appropriate allele calls. To set up for capillary electrophoresis, a master mix is prepared containing HiDi Formamide and GeneScan™ 500 LIZ (Figure 2). 1 µl of amplified STR product, controls and allelic ladder are added to the appropriate wells. 9 µl of master mix is added to all the reactions, giving a total 10 µl reaction volume. The mixture was denatured at 95°C for 5 min and then immediately snap-cooled on ice for 3 min until the amplicons were loaded on the ABI 3130xl. Analysis of the data was done using GeneScan® 3.7 (Applied Biosystems)

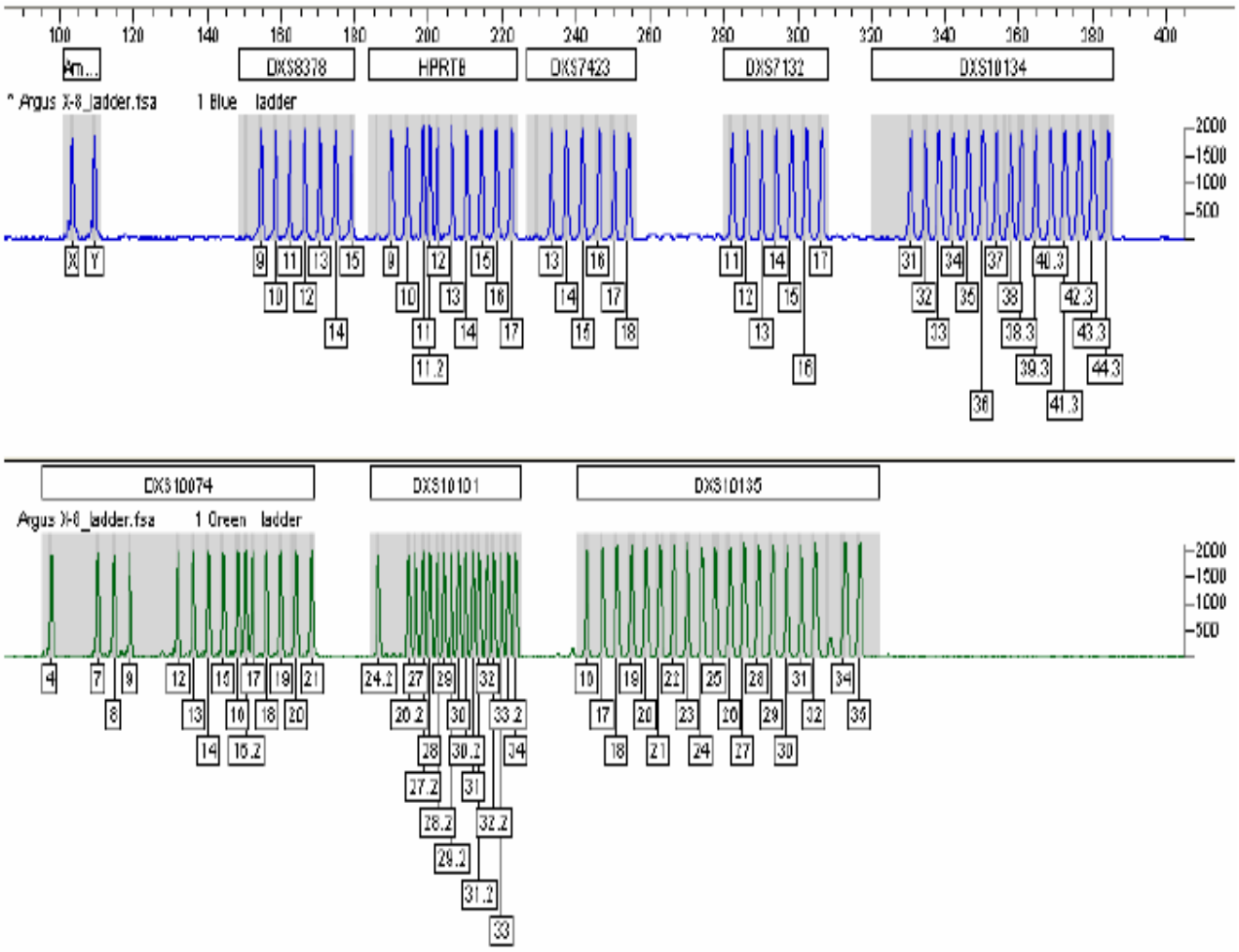

Figure 2. Electropherogram of the allelic ladder Mentype® Argus X-8 analysed on an ABI PRISM® 310 genetic analyzer.

software. Amplicons were then converted to allele numbers using the Genotyper® 3.7 (Applied Biosystems).

Statistical analysis

Once frequencies are obtained, specific statistical tests are conducted on the data to evaluate whether the database will be useful when applied to human identity testing. Allele frequencies for each locus were calculated for males collectively by hand. Observed heterozygosities (HET), polymorphism information content (PIC), power of exclusion (PE) and power of discrimination (PD) were calculated with PowerStats v12 software (http://www.promega.com). Hardy-Weinberg equilibrium (HWE) was calculated by an exact test with Arlequin v3.5 software (Excoffier and Lischer, 2010). Power of discrimination in males (PD) wascalculated with chromosome X web version.

RESULTS AND DISCUSSION

X-Linked markers in Mentype® Argus X-8 PCR amplification kit proved to be highly polymorphic with a high power of discrimination. Allele frequencies for each of the eight short tandem repeat loci in the Iraqi population sample are shown in Table 1. In the locus HPRTB, (allele 13) the highest allele frequencies were found. Haplotype frequencies of four linkage groups were counted in 120 men. The linkage groups 1, 2, 3 and 4 revealed 60, 90, 65 and 88 haplotypes, respectively (Tables 2 and 3). The most frequent haplotypes were 12-12, 12-15 and 17-15 for Linkage Group 1; 11-34 and 13-27 for Linkage Group 2; 31-17 and 38-13 for Linkage Group 3; and 20-13 for Linkage Group 4. DXS10135 was the most polymorphic locus (with 25 alleles, PIC = 0.931), whereas the lowest values were observed for DXS7423 and DXS8378 (both with 5 alleles, PIC 0.506 and 0.589 respectively) (Figure 3). Power of exclusion (PE) ranged from 0.308 to 0.781 in male samples (Figure 4). Power of discrimination (PD) ranged from 0.599 to 0.952 in male samples (Figure 5). In a complex kinship testing, X-STR genotyping can supplement the analysis of autosomal, mitochondrial and Y-chromosomal markers. In the last few years, the need

Table 1. Allele frequencies at eight X-STR loci of 120 unrelated males from Iraq.

Allele	HPRTB	DXS10101	DXS7132	DXS10074	DXS10134	DXS7423	DXS10135	DXS8378
7	0.03032	-	-	0.04007	-	-	-	-
8	-	-	-	0.09916	-	-	-	-
9	-	-	-	0.00810	-	-	-	0.02091
10	0.02083	-	-	0.00275	-	-	-	0.29810
11	0.20090	-	0.00262	0.00300	-	-	-	**0.37100**
12	0.00282	-	0.10970	0.02610	-	-	-	0.19229
13	**0.43620**	-	**0.40010**	0.00991	-	0.08971	-	0.04007
14	0.29410	-	0.28113	0.05190	-	**0.40980**	-	-
15	-	-	0.10002	**0.30160**	-	0.30151	-	-
15.3	-	-	-	-	-	-	0.00459	-
16	0.06910	-	0.03960	0.19500	-	0.21500	0.00265	-
17	-	-	0.00251	0.26600	-	0.02390	0.01184	-
18	-	-	-	0.03957	-	-	0.04251	-
19	-	-	-	0.10061	-	-	0.06256	-
19.1	-	-	-	-	-	-	0.00265	-
20	-	-	-	0.00640	-	-	0.04243	-
20.1	-	-	-	-	-	-	0.03151	-
21	-	-	-	-	-	-	0.05944	-
21.1	-	-	-	-	-	-	0.02073	-
22	-	-	-	-	-	-	0.05697	-
22.1	-	-	-	-	-	-	0.00610	-
23	-	-	-	-	-	-	0.08400	-
23.1	-	-	-	-	-	-	0.01301	-
24	-	-	-	-	-	-	0.08204	-
25	-	-	-	-	-	-	0.08902	-
25.1	-	-	-	-	-	-	0.00825	-
25.2	-	0.02192	-	-	-	-	-	-
26	-	-	-	-	-	-	**0.09320**	-
26.2	-	0.00298	-	-	-	-	-	-
27	-	0.00310	-	-	-	-	0.08989	-
28	-	0.06210	-	-	-	-	0.04100	-
28.2	-	0.01292	-	-	-	-	-	-
29	-	0.10330	-	-	-	-	0.04812	-
29.2	-	0.05972	-	-	-	-	-	-
30	-	0.11922	-	-	0.00401	-	0.02799	-
30.2	-	0.03077	-	-	0.00299	-	-	-
31	-	**0.22011**	-	-	0.01270	-	0.03092	-
32	-	0.07921	-	-	0.00412	-	0.00698	-
32.2	-	0.20930	-	-	-	-	-	-
33	-	0.06033	-	-	0.06009	-	-	-
33.2	-	0.03991	-	-	-	-	-	-
34	-	0.05001	-	-	0.13299	-	0.00419	-
34.2	-	0.00960	-	-	-	-	-	-
35	-	-	-	-	0.20390	-	-	-
35.2	-	-	-	-	**0.36022**	-	-	-
36	-	-	-	-	0.20716	-	-	-
37	-	-	-	-	0.00215	-	-	-
37.1	-	-	-	-	0.00801	-	-	-
37.2	-	-	-	-	0.00719	-	-	-
37.3	-	-	-	-	0.05911	-	-	-
38	-	-	-	-	0.01996	-	-	-

Table 1. Contd

38.3	-	-	-	-	0.02113	-	-	-
39	-	-	-	-	0.04093	-	-	-
39.3	-	-	-	-	0.00416	-	-	-
40	-	-	-	-	0.01968	-	-	-
41	-	-	-	-	0.00296	-	-	-
41.3	-	-	-	-	0.01930	-	-	-
42.3	-	-	-	-	0.02617	-	-	-
43.3	-	-	-	-	0.00805	-	-	-

Table 2. Haplotype frequencies for eight X-STR loci in linkage groups 1 and 2 in Iraq.

Linkage group 1				Linkage group 2			
Haplotype		Number	Frequency	Haplotype		Number	Frequency
DXS7132 - DXS10074				HPRTB - DXS10101			
11	11	1	0.0083	7	29.2	1	0.0083
11	12	2	0.0166	7	30	1	0.0083
11	13	2	0.0166	7	30.2	1	0.0083
11	14	1	0.0083	7	31	2	0.0166
11	15	1	0.0083	7	32	1	0.0083
12	7	1	0.0083	7	32.2	2	0.0166
12	8	4	0.0333	7	33	2	0.0166
12	9	1	0.0083	7	33.2	1	0.0083
12	10	1	0.0083	7	34	1	0.0083
12	11	1	0.0083	7	34.2	1	0.0083
12	12	10	0.0833	10	25.2	1	0.0083
12	13	1	0.0083	10	26.2	1	0.0083
12	14	1	0.0083	10	27	1	0.0083
12	15	6	0.0500	10	28	1	0.0083
12	15.3	1	0.0083	10	28.2	1	0.0083
13	10	1	0.0083	10	29.2	1	0.0083
13	11	1	0.0083	10	30	2	0.0166
13	12	1	0.0083	10	30.2	1	0.0083
13	13	5	0.0416	10	31	1	0.0083
13	14	1	0.0083	10	32	1	0.0083
13	15	1	0.0083	10	32.2	1	0.0083
13	15.3	1	0.0083	10	33	2	0.0166
14	7	1	0.0083	10	33.2	1	0.0083
14	8	2	0.0166	10	34	1	0.0083
14	9	3	0.0250	10	34.2	1	0.0083
14	10	1	0.0083	11	28	1	0.0083
14	11	1	0.0083	11	28.2	1	0.0083
14	12	1	0.0083	11	29.2	1	0.0083
14	13	4	0.0333	11	30	1	0.0083
14	14	1	0.0083	11	30.2	1	0.0083
14	15	1	0.0083	11	31	1	0.0083
14	15.3	2	0.0166	11	32	1	0.0083
15	7	2	0.0166	11	32.2	1	0.0083
15	8	2	0.0166	11	33	1	0.0083
15	9	1	0.0083	11	33.2	1	0.0083
15	10	1	0.0083	11	34	6	0.0500

Table 2. Contd

15	11	1	0.0083	11	34.2	1	0.0083
15	12	5	0.0416	12	25.2	1	0.0083
15	13	1	0.0083	12	26.2	1	0.0083
15	14	2	0.0166	12	27	1	0.0083
15	15	1	0.0083	12	28	1	0.0083
15	15.3	3	0.0250	12	28.2	1	0.0083
16	9	1	0.0083	12	29.2	1	0.0083
16	10	1	0.0083	12	30	1	0.0083
16	11	1	0.0083	12	30.2	1	0.0083
16	12	3	0.0250	12	31	1	0.0083
16	13	1	0.0083	12	32	1	0.0083
16	14	6	0.0500	12	32.2	1	0.0083
16	15	1	0.0083	12	33	1	0.0083
16	15.3	5	0.0416	12	33.2	1	0.0083
17	7	1	0.0083	12	34	4	0.0333
17	8	1	0.0083	12	34.2	1	0.0083
17	9	4	0.0333	13	25.2	3	0.0250
17	10	2	0.0166	13	26.2	1	0.0083
17	11	2	0.0166	13	27	6	0.0500
17	12	1	0.0083	13	28	1	0.0083
17	13	1	0.0083	13	28.2	1	0.0083
17	14	1	0.0083	13	29.2	1	0.0083
17	15	6	0.0500	13	30	1	0.0083
17	15.3	1	0.0083	13	30.2	1	0.0083
-	-	-	-	13	31	4	0.0333
-	-	-	-	13	32	2	0.0166
-	-	-	-	13	32.2	1	0.0083
-	-	-	-	13	33	1	0.0083
-	-	-	-	13	33.2	1	0.0083
-	-	-	-	13	34	1	0.0083
-	-	-	-	13	34.2	1	0.0083
-	-	-	-	14	27	1	0.0083
-	-	-	-	14	28	1	0.0083
-	-	-	-	14	28.2	1	0.0083
-	-	-	-	14	29.2	1	0.0083
-	-	-	-	14	30	1	0.0083
-	-	-	-	14	30.2	1	0.0083
-	-	-	-	14	31	1	0.0083
-	-	-	-	14	32	1	0.0083
-	-	-	-	14	32.2	1	0.0083
-	-	-	-	14	33	1	0.0083
-	-	-	-	14	33.2	1	0.0083
-	-	-	-	16	25.2	1	0.0083
-	-	-	-	16	26.2	1	0.0083
-	-	-	-	16	27	5	0.0416
-	-	-	-	16	28	2	0.0166
-	-	-	-	16	28.2	1	0.0083
-	-	-	-	16	29.2	1	0.0083
-	-	-	-	16	30	1	0.0083
-	-	-	-	16	30.2	1	0.0083
-	-	-	-	16	31	1	0.0083
-	-	-	-	16	32	1	0.0083

Table 2. Contd

-	-	-	-	16	32.2	1	0.0083
-	-	-	-	16	33	1	0.0083

Table 3. Haplotype frequencies for eight X-STR loci in linkage groups 3 and 4 in Iraq.

Linkage group 3				Linkage group 4			
Haplotype		Number	Frequency	Haplotype		Number	Frequency
DXS10134 - DXS7423				DXS10135 - DXS8378			
30	15	1	0.0083	15.3	11	1	0.0083
30	16	6	0.0500	15.3	12	1	0.0083
30	17	1	0.0083	15.3	13	1	0.0083
30.2	13	1	0.0083	16	10	2	0.0166
30.2	16	1	0.0083	16	12	2	0.0166
31	13	1	0.0083	16	13	1	0.0083
31	14	1	0.0083	17	9	1	0.0083
31	15	1	0.0083	17	10	1	0.0083
31	16	1	0.0083	17	11	1	0.0083
31	17	10	0.0833	17	12	2	0.0166
32	14	1	0.0083	17	13	1	0.0083
32	15	1	0.0083	18	11	1	0.0083
32	16	1	0.0083	18	12	1	0.0083
32	17	1	0.0083	18	13	1	0.0083
33	15	1	0.0083	19	9	1	0.0083
33	16	1	0.0083	19	12	1	0.0083
33	17	1	0.0083	19	13	1	0.0083
34	14	2	0.0166	19.1	9	1	0.0083
34	17	2	0.0166	19.1	10	4	0.0333
35	13	2	0.0166	19.1	12	1	0.0083
35	16	2	0.0166	19.1	13	5	0.0416
35	17	1	0.0083	20	9	1	0.0083
35.2	13	1	0.0083	20	10	1	0.0083
35.2	14	1	0.0083	20	11	1	0.0083
35.2	15	1	0.0083	20	12	1	0.0083
35.2	16	2	0.0166	20	13	9	0.0750
35.2	17	2	0.0166	20.1	11	1	0.0083
35.2	13	1	0.0083	20.1	12	1	0.0083
35.2	14	1	0.0083	20.1	13	1	0.0083
36	15	1	0.0083	21	9	1	0.0083
36	16	3	0.0250	21	10	1	0.0083
37	14	1	0.0083	21	11	1	0.0083
37	15	1	0.0083	21	12	1	0.0083
37	16	1	0.0083	21	13	1	0.0083
37.1	13	4	0.0333	21.1	10	1	0.0083
37.1	14	5	0.0416	21.1	11	1	0.0083
37.1	17	1	0.0083	21.1	12	1	0.0083
37.2	13	1	0.0083	22	9	1	0.0083
37.2	14	1	0.0083	22	10	1	0.0083
37.2	15	2	0.0166	22	11	1	0.0083
37.2	17	1	0.0083	22	12	1	0.0083
37.3	16	2	0.0166	22	13	1	0.0083

Table 3. Contd

37.3	17	1	0.0083	23	10	1	0.0083
38	13	10	0.0833	23	11	1	0.0083
38	15	1	0.0083	23	12	1	0.0083
38	16	1	0.0083	23.1	11	1	0.0083
38	17	1	0.0083	23.1	12	1	0.0083
38.3	14	1	0.0083	23.1	13	1	0.0083
38.3	16	1	0.0083	24	10	7	0.0583
39	13	1	0.0083	24	11	3	0.0250
39	17	1	0.0083	24	12	2	0.0166
39.3	15	1	0.0083	24	13	2	0.0166
39.3	16	4	0.0333	25	9	2	0.0166
39.3	17	1	0.0083	25	10	1	0.0083
40	17	1	0.0083	25	11	1	0.0083
41	13	1	0.0083	25	12	1	0.0083
41	16	1	0.0083	25	13	1	0.0083
41.3	15	1	0.0083	25.1	10	1	0.0083
41.3	16	1	0.0083	25.1	11	1	0.0083
41.3	17	1	0.0083	25.1	12	1	0.0083
42.3	13	1	0.0083	26	9	1	0.0083
42.3	14	1	0.0083	26	10	1	0.0083
42.3	17	1	0.0083	26	11	1	0.0083
43.3	14	1	0.0083	27	11	1	0.0083
42.3	16	1	0.0083	27	12	1	0.0083
-	-	-	-	27	13	1	0.0083
-	-	-	-	28	9	1	0.0083
-	-	-	-	28	10	1	0.0083
-	-	-	-	28	11	1	0.0083
-	-	-	-	28	12	1	0.0083
-	-	-	-	28	13	3	0.0250
-	-	-	-	29	10	1	0.0083
-	-	-	-	29	11	1	0.0083
-	-	-	-	29	12	1	0.0083
-	-	-	-	30	9	1	0.0083
-	-	-	-	30	10	1	0.0083
-	-	-	-	30	11	1	0.0083
-	-	-	-	30	12	1	0.0083
-	-	-	-	30	13	1	0.0083
-	-	-	-	31	10	1	0.0083
-	-	-	-	31	11	1	0.0083
-	-	-	-	31	12	1	0.0083
-	-	-	-	32	10	1	0.0083
-	-	-	-	32	11	1	0.0083
-	-	-	-	32	12	1	0.0083
-	-	-	-	32	13	1	0.0083
-	-	-	-	33.2	11	1	0.0083
-	-	-	-	33.2	12	1	0.0083

for commercially available and validated X-STR kits has increased due to a growing number of complex kinship cases. Intensive studies of the X chromosome discover a lot of closely linked X-STR markers, which can be included

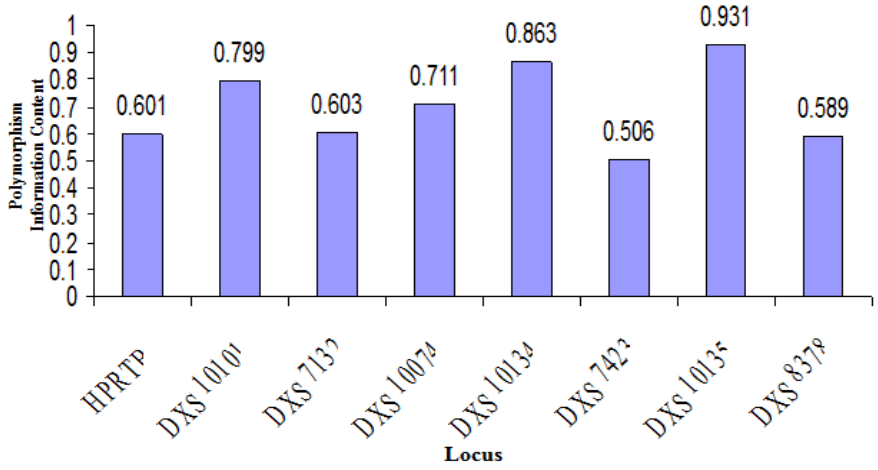

Figure 3. Forensic efficiency parameters: Polymorphism information content (PIC).

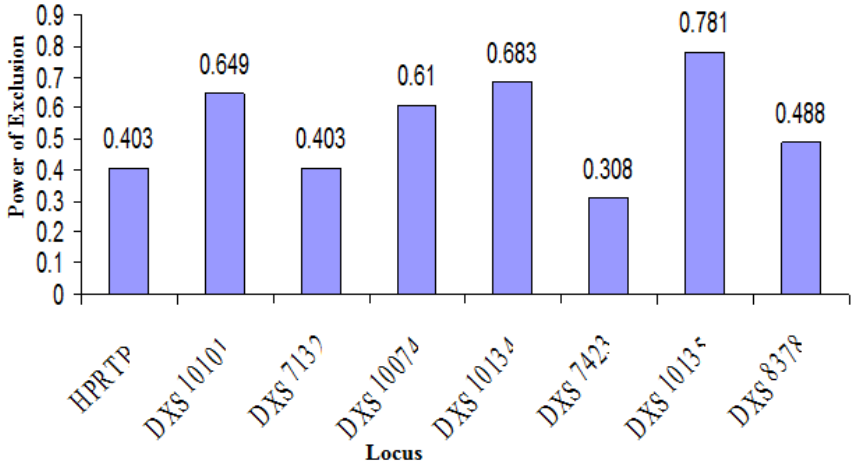

Figure 4. Forensic efficiency parameters: Power of exclusion (PE).

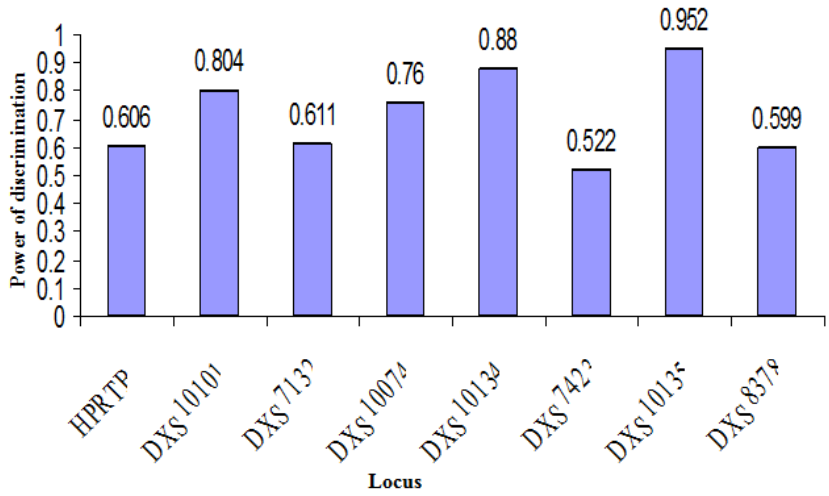

Figure 5. Forensic efficiency parameters: Power of discrimination (PD).

in the commercially available kits (Hering et al., 2006; Edelmann et al., 2008; Edelmann et al., 2009; Hundertmark et al., 2008; Ferreira et al., 2010).

Recent forensic casework, population genetics and anthropological studies have used the relatively new commercially available Investigator Argus X-12 kit. Investigator Argus X-12 presents an improvement as compared to Mentype® Argus X-8 in the sense of increased discriminatory power due to four linkage groups with three markers per group (Amelogenin; DXS10148, DXS10135, DXS8378; DXS7132, DXS10079, DXS10074; DXS10103, HPRTB, DXS10101; DXS10146, DXS10134, DXS7423) (Qiagen. Investigator Argus X-12 PCR handbook. Hilden: Qiagen; 2010). Further studies are planned to get an overview of the X-STR variability in all Croatian regions, and there are plans for inclusion of 12 X-STR loci in the database.

Conclusion

This study is the first of its kind in Iraq, where there are no prior study on the assessment of x-chromosome STRs. X-linked markers in Mentype® Argus X-8 PCR amplification kit proved to be highly polymorphic with a high power of discrimination. Our results suggest that all eight X-STRs described here can efficiently be used in parentage analysis and provide a powerful tool in forensic case work, in particular, to identify the female DNA profile in mixture analysis.

Conflict of interests

The author(s) did not declare any conflict of interest.

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