Allele Frequencies of 13 Chromosome X STR in Arab Iraqi Population

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Abstract

Short tandem repeat (STR) markers are highly being used for identification of human being in addition to paternity and other forensic cases.The X-chromosome STR (X-STR) markers are a powerful supplementary system particularly in deficiency paternity analysis. Several X-linked microsatellites have been assessed but more studies are necessary to verify the population statistics. In our study, we report allele frequencies of 13 X-linked microsatellites (DXS8378, DXS9898, DXS8377, HPRTB, GATA172DO5, DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS6807, and DXS7424) in the Iraqi arab population. fifty Blood samples were collected from healthy unrelated males. A total number of alleles were detected for for all 13 X-STR loci were (83) and the related frequencies ranging from 0.0200 to 0.5000. Heterozygosity ranged from 0.652 to 0.858. Large values were observed, at least 0.999999994, in combined powers of discrimination. The results powerfully suggest that all the X-linked microsatellites defined here can possibly support the autosomal systems that used in parentage analysis and different forensic case work.

Keywords: *X-chromosome STR, Allele frequency, Forensic parameters, X-STR database, Haplotype.*

Introduction

Armed war and other cases of aggression in Iraq since the 1980s have culminated in the killings of thousands of civilians. Series of events from the Iran -Iraq conflect to newer massacres committed by the Islamic State of Iraq and the Levant (ISIL). Large numbers of people are missing, and their bodies were never identified and returned to their families [1][2]. To try and tackle this problem, Mass Graves Depart-ment in the Iraqi Medico-Legal directorate that was established in 2010 to deal with the missing individuals and it has increased its capability in forensic anthropology and forensic genetics to retrieve and identify the large number of missing people. Recently, The Mass Graves Department has adopted the X-chromosomal STR (XSTR) markers as a one of the important tools that used in human identification purposes [3]. Because of its pattern of inheritance the X- chromosomal short tandem repeat (XSTR) has potential applications for forensic casework and kinship analysis as compared to autosomal STR (A-STR), Y-chromosomal STR (Y-STR), and mitochondrial DNA (mtDNA), [4]. Males usually have one X-chromosome transmitted as haplotypes from their mothers to their daughters, whereas females have two Xchromosomes transmitted from both parents to their offspring in the same manner as autosomes [5]. XSTR analysis can be helpful in trace identification with female minor component and male major component, and in complicated cases of kinship, such as cases of deficiency (father-daughter or mother-son), grandparent-grandchild links, half sister analysis, incest paternity testing, and so on [6] [7].

hile X-Plex PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that used to amplifies 13 X-STR loci and an amelogenin locus located on human chromosome X in a single PCR reaction that is DXS8378, DXS9898, DXS8377, HPRTB, GATA172DO5. DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS6807 and DXS7424 [8] (Table 1).

Table 1: Allelic Ladder of GenePhile X-Plex Kit with its loci, the relating dyes used, alleles contained in the allelic ladder and the genotype of the Control DNA 9947A and K01 are registered in the table [8].

Locus	Designation Alleles Included in X-Plex	Dye Label	9947A Genotype	Control DNA K01 Genotype
DXS8378	8-14	6-FAM™	10,11	11
DXS9898	8.3-16		12,15	12
DSX8377	33-60		45,47	51
HPRTB	6-17		14,14	15
GATA172D05	5-12	VIC®	10,10	11
DXS7423	12-18		14,15	14
DXS6809	27-40		31,34	34
DXS7132	8-19	NED™	12,12	16
DXS101	14-32		24,26	25
DXS6789	13-25		21,22	20
AMEL	X, Y	PET	X,X	X/Y
DXS9902	6-13		11,11	11
DXS6807	11-17		12,14	15
DXS7424	9-18		14,16	16

Autosomal markers are useful for the resolution of most forensic DNA analysis. Some of them, however, require implementing STRs on the sex chromosomes (gonosomes) [9]. Gonosomal STR markers are useful when investigating relationships between individuals of different generations, mainly when key pedigree persons are missing. In addition, the use of gonosomal STRs in forensic analysis of DNA traces is increasing sharply [10]. The development of population studies would improve our understanding of the associated polymorphism and allellic distribution of these loci. As there is a connection between the level of polymorphism at a given locus and the rate of mutation [11].

In this study, we started to create an X-STR database on the population of Iraqi arabs and investigate the polymorphisms and haplotypes of the investigator GenePhile X-Plex loci kit and evaluated their effectiveness to be helpful not only in criminal investigation such as personal identification and kinship testing but for other anthropological purposes as well [12].

Materials and methods

Blood samples were collected from 50 healthy, unrelated and consenting males who were identified themselves as Iraqi Arabs. The consent form were signed by the donor people during the collection of the samples in medico-legal directorate / Baghdad. Finger lancets were used to collecte the samples of blood and then stored on FTA ® Classic Card (GE Healthcare, Pittsburgh, USA). For each sample a 1.2 mm punch was directly amplified following the manufacturer's recommended instruction. PCR using the GenePhile X-Plex Amplification Kit System (GenePhile Bioscience Co., Ltd TAIWAN). with an Applied Biosystems 9700 thermocycler was carried out using the standard manufacturer's recommended instruction: this co-amplified the amelogenin sex marker and 13 STR loci (DXS8378, DXS9898, DXS8377, HPRTB, GATA172DO5. DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS6807 and DXS7424). Capillary electrophoresis was accomplished using a 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA) in accordance with the manufacturer's instructions; ILS-500 (Promega) was used as an internal size standard. Genotyping was carried out using GeneMapper ID-X v1.4 software (Applied

Biosystems). Alleles were designated depending on the published nomenclature; off ladders were termed if they fell into a virtual bin as long as the profile as a whole was judged to be of high quality, before designation, re-run at least once if alleles that were not in a bin or virtual bin.

Statistical Analysis

Once the frequencies achieved, important statistical tests are performed on the data to assess whether the database will be helpful when used in human identification. The allele frequencies were calculated manually for each locus. Match probability (PM), heterozygosities (HET), homozygosities(h), polymorphism information content (PIC), power of discrimination (PD) and power of exclusion (PE) were calculated with ChrX-STR.org 2.0 software web site.

Results and Discussion

Allele frequencies for 13 X-linked microsatellites

(DXS8378, DXS9898, DXS8377, HPRTB, GATA172DO5. DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS6807 and DXS7424) and amelogenin were reported in the Iraqi Arab population. GenePhile X-Plex PCR amplification kit showed that all X-linked markers were highly polymorphic with a high discrimination power.50 (males) samples were collected. Such X-STR markers cover the entire X-chromosome and represent all 4-linkage group previously classified by Szibor

[10]. Supplementary (Table 2) shows allelic frequencies for 13 STR markers. The highest allele frequencies (0.5000) were found in the locus DXS7132, (allele 14).

The minimum number was (3) alleles and the maximum number was (11) alleles were observed per locus, and a total of 83 alleles were observed for all 13 X-STR loci. Heterozygosity varied between 0652.–0.858. Such findings found that DXS8377 is the most informative marker with heterozygosity at 0.844, where it is the least informative as DXS7423 and DXS7132, with heterozygosity at 0.652. DXS8377 was the most polymorphic locus (with 11 alleles, PIC = 0.845), while DXS9902 had the lowest values (with 3 alleles, PIC 0.583). (Figure 1). Power of exclusion (PE) varied from 0.359 (DXS7423,DXS7132) to 0.711 (DXS8377) (Figure 2). Power of discrimination (PD) varied from 0.652 (DXS7423,DXS7132) to 0.858 (DXS8377) (Figure 3). X-STR genotyping can complement the analysis of autosomal, mitochondrial, and Y-chromosomal markers in a complex kinship test. Combined Power of Discrimination (CDP)=0.999999994, and Probability of Combined Exclusion (CEP)=0.9998578. These values mean that the loci can be used safely to establish a database based on DNA for Iraqi populations.

Table 2. Allele frequencies at thirteen X-STR loci of 50 Iraqi unrelated males.

Allele	DXS8378	DXS9898	DXS8377	HPRTB	GATA172D05	DXS7423	DXS6809	DXS7132	DXS101	DXS6789	DXS9902	DXS6807	DXS7424
6					0.12								
8					0.12								
8.3		0.1											
8.5		0.02											
9					0.02								
10	0.28				0.38						0.26		
11	0.46	0.3		0.08	0.26						0.36	0.32	
12	0.2	0.3		0.12	0.1			0.02			0.38		0.02
13	0.06	0.18		0.48				0.16				0.02	0.06
14		0.08		0.26		0.4		0.5				0.18	0.08
15		0.02		0.04		0.4		0.26		0.06		0.42	0.2
16				0.02		0.16		0.06				0.02	0.42
17						0.04						0.04	0.16
18									0.06	0.02			0.06
19										0.04			
20									0.02	0.48			
21									0.06	0.2			
22									0.04	0.18			

Cont... **Table 2. Allele frequencies at thirteen X-STR loci of 50 Iraqi unrelated males.**

24								0.24	0.02				
25								0.14					
26								0.22					
27								0.14					
28								0.06					
29								0.02					
31						0.08							
32						0.12							
33						0.32							
34						0.2							
35						0.16							
36						0.1							
38						0.02							
42		0.06											
45		0.04											
46		0.06											
47		0.12											
48		0.06											
49		0.12											
50		0.28											
51		0.1											
52		0.1											
53		0.02											
54		0.04											

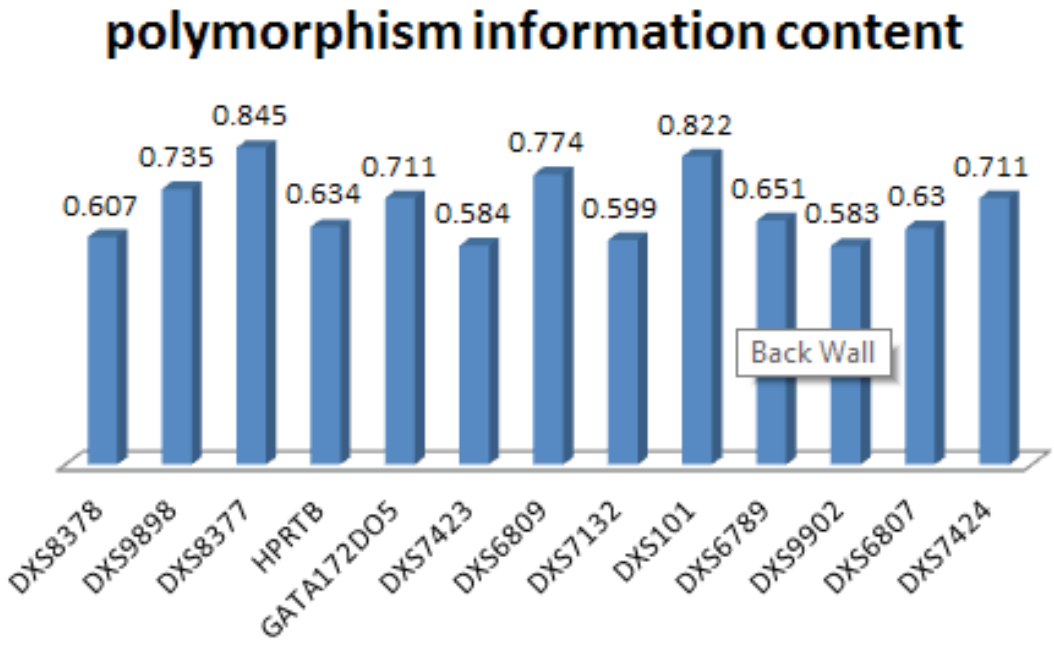

Figure 1. Forensic efficiency parameters: Polymorphism information content (PIC)

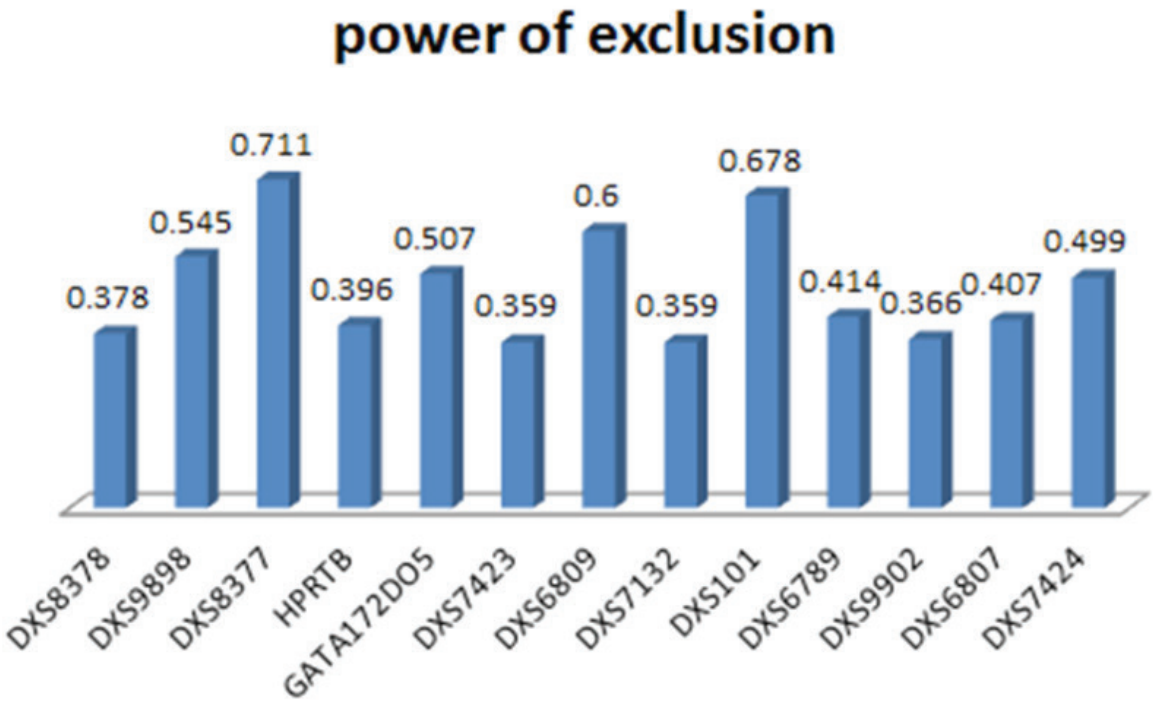

Figure 2. Forensic efficiency parameters: Power of exclusion (PE).

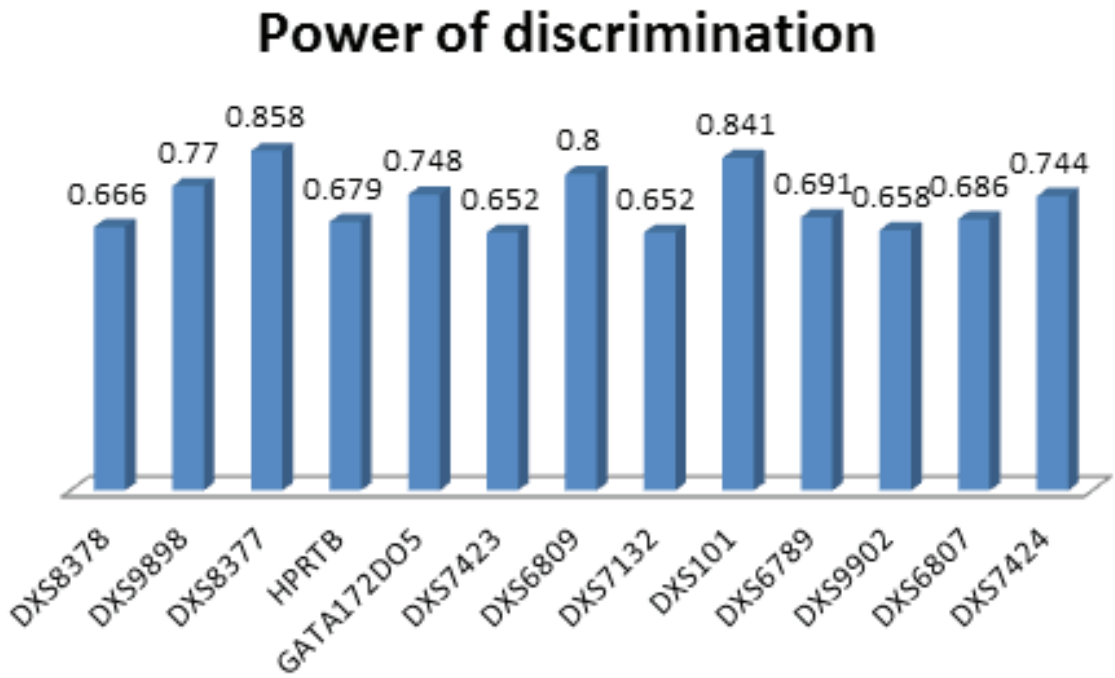

Figure 3. Forensic efficiency parameters: Power of discrimination (PD).

More studies are expected to get a general idea of the X-STR variability in all Iraqi people, and there are plans for inclusion of kurd and turkman in the database.

More studies are expected to provide an understanding of the X-STR variability among all Iraqi populations, and the future plans to inclusion Kurd and Turkman in this database.

Conclusion

The population database created permits for the application of the STR markers included in the GenePhile X-Plex 13 PCR amplification kit to be used in routine case work. High forensic statistical parameter values combined with the studied loci 's in dependent inheritance make them a very useful marker collection for the investigation of forensic genetics. Application of X-STR loci can be a useful addition to autosomal markers, particularly in complex cases of kinship and paternity, e.g. when his mother (i.e. the child's putative grandmother) is the source of the reference DNA sample, instead of the alleged parent.

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Conflict of Interest : Nil.

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