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Population Genetic Analysis of 12 X-STRs in a Bahraini Population Sample

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Abstract

To date, there are very limited genetic studies conducted on the Kingdom of Bahrain and this is one of the first studies directed to evaluate the 12 X-STRs included in the Investigator X-12 QS kit. Bahrain is a small archipelago positioned in the Arabian Gulf. X-STRs are very informative in population genetics studies, human identification and complex kinship analysis. One hundred and fifty-six (156) buccal swabs were collected using cotton swabs from non-relatives' Bahraini males from four different regions of Bahrain. Genomic DNAs were extracted and purified using QIAsymphony SP instrument following quantification with Investigator Quantiplex HYres Kit in the 7500 Real-Time PCR System and detected in ABI 3500xl Genetic Analyzer. Analysis was done using various statistical software to obtain allele and haplotypes' frequencies based upon the available four clusters of Linkage Groups (LGs) as well as different forensic and population parameters. Results indicated the diversity of the Bahraini population in terms of high Power of Discrimination (PD) and Probability of Match (PM) values. The combined values of each forensic parameter such as cPDM, Mean exclusion chance (cMEC) Krüger, CMEC Kishida, and CMEC Desmarais as well as CMEC Desmarais Duo, were 0.9999983, 9999979, 0.9999939, 0.9999996 and 0.9999514 respectively based on the allele and haplotype frequencies. No shared profiles were observed. Number of non-standard alleles and null alleles were obtained, and more profoundly in locus DXS10148. We have constructed phylogenetic tree as well as multidimensional scale to analyze the interpopulation diversity between Bahraini population and other neighboring populations and our findings reflected the geographical and social background of the region. Overall, the results confirmed the importance of X-STRs in discriminating between individuals among Bahraini population and in establishing DNA databases for forensic and kinship studies.

Keywords: Bahraini population; Investigator Argus X-12 QS Kit; Forensic parameters; Population genetics; X-STR

Introduction

The Investigator Argus X-12 QS Kit (Qiagen, Germany) is a multiplex solution that amplifies 12 segments of DNA located in the chromosomal X short tandem repeats (X-STRs). This solution is used for human identification, paternity testing, forensic cases and population genetic studies [1]. X-STR markers are very informative for some cases where autosomal STRs fail to provide crucial answers such as in kinship testing involving female offspring sharing the same father [2]. In this kit, the 12 X-STR markers are clustered into four distinct linkage groups (LG), each

cluster includes three markers: LG1 (DX8378-DXS10135-DXS10148), LG2 (DXS7132-DXS100740-DXS10079), LG3 (DXS10101-DXS10103-HPRTB), LG4 (DXS7423-DXS10134-DXS10146) [3]. The dependency between markers is known as linkage and is the physical proximity of two loci on the same chromosome [2].

To date, there are few population genetic studies conducted on the Kingdom of Bahrain as well as in the Arabian Peninsula region. Knowledge of any such structure is crucial in the interpretation of DNA-based forensic evidence and in the construction of applicable databases. Recent studies were done using autosomal STR and YSTR kits [4, 5].

Kingdom of Bahrain is a small archipelago located in the Arabian Gulf with a total landmass of 760 square kilometers [6] consisting of 33 islands, only the five largest are inhabited. These islands are consisting of Bahrain, Muharraq, Umm and Nasan and Sitra. To the southeast of Bahrain is the State of Qatar, and to its west stands the Kingdom of Saudi Arabia, with which it is connected by a 25-kilometer causeway. To the north and east of Bahrain is the Islamic Republic of Iran [7].

Because of the geographical location of Bahrain, the diversity of the population had been affected which is generally divided into four main ethnic groups: Arabs, Baharna and Persians (Huwala and Ajam) [8-10]. This geographical and social association might be anticipated to have an effect on the patterns of the genetic variations [11]. Currently, the geographical distribution of Bahrain is included into 4 governorates: Northern, Muharraq, Capital and Southern Governorates.

This present study is to genetically characterize the Bahraini population, using Investigator Argus X-12 QS Kit (Qiagen, Germany). We present a database of 12 X-STRs allele and haplotype frequencies for Bahraini population sample including the high combined forensic statistical parameters of these loci, and collectively as haplotypes, illustrate that they are informative and discriminatory. The comparison with worldwide populations demonstrate that they generally reflect biogeography and historical relationships.

Twelve X-STRs were studied to characterize different genetic population and forensic parameters in 156 Bahraini males. These includes the following primers to co-amplify the Amelogenin (AM) used for gender determination, DXS7132, DXS7423, DXS8378, DXS10074, DXS10079, DXS10101, DXS10103, DXS10134, DXS10135, DXS10146, DXS10148 and HPRTB which have been proven to offer consistent DNA typing results and boost the power of discrimination (PD). Furthermore, the autosomal STR marker D21S11 is included as a reliable marker for common database kits and prevents sample mixup and was excluded from the genetic analysis for this study. In addition, the Kit Primer comprises of an internal PCR control (Quality Sensor QS1) to provide support about the efficacy of the PCR. The Quality Sensor is amplified along with the polymorphic X-STRs' markers and was excluded from all the genetic analysis for this study.

Materials and Methods

Sample Collection

One hundred and fifty-six (156) buccal swabs were assembled using cotton swabs (SceneSafe, UK) from healthy unrelatives' Bahraini males. The research study was publicized through different media platforms. Participants who wished to contribute their samples communicated with the corresponding author and presented at the General Directorate of Criminal investigation and Forensic Science – Kingdom of Bahrain to liver their buccal swabs for the research after obtaining informed consent. The age of the participants varied from 19 to 58 years old.

In each case, males with ancestry (to the level of paternal grandfather) from four different geographical subdivisions of the country (Capital Governorate, Muharraq Governorate, Northern Governorate and Southern Governorate) were sampled. Ethical review for conducting tests was obtained and approved by the Research and Research Ethics Committee (RREC) (E007-PI-10/17) in the Arabian Gulf University. All participants provided informed consent prior to contribution their buccal swab samples.

DNA Processing

Genomic DNAs were extracted using QIAsymphony SP instrument (Qiagen, Germany) following magnetic beads principal. Subsequently the extracted DNAs were quantified using Investigator Quantiplex HYres Kit (Qiagen, Germany) in the 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to manufacturer's recommendation. About 0.5 ng of the extracted DNA was amplified using Investigator Argus-X12 QS kit (Qiagen, Germany) with half-volume reactions (12.5µl) following manufacturer's protocol in 27 cycles conditions *via* MicroAmp Optical 96-Well Reaction Plate (Thermo Fisher Scientific, Inc., Waltham, MA, USA) along with the provided positive control and DNA grade water as a negative control.

The PCR products (1µl) were separated by capillary electrophoresis in an ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific Company, Carlsbad, USA) with reference to the BTO size standard (Qiagen, Germany) in total of 12 µl master mix consisting of BTO size standard and Hi-Di formamide (Thermo Fisher Scientific, Inc., Waltham, MA, USA). GeneMapper® ID-X Software v1.4 (Thermo Fisher

Scientific, Inc., Waltham, MA, USA) was used for genotype assignment.

Statistical Analysis

Allele and haplotype frequencies were calculated using StatsX v1.0 software [12]. Also, Linkage groups (LG) were designated as 3 loci in each LG in total of 4 LGs in StatsX v1.0 software [12]. Forensic parameters such as power of discrimination (PD), random matching probability (PM), power of exclusion (PE), polymorphism information content (PIC), number of alleles (Nall) and linkage disequilibrium (LD) between pair of loci were estimated using STRAF [13]. Mean exclusion chance in Duos (MECD), PD for females (PD_f), and PD in males (PD_m) were estimated using ChrX-STR.org 2.0 website [14]. It should be noted that all of the samples were compromised of males, so it was impossible to calculate the Hardy-Weinberg (HW) equilibrium.

Interpopulation pairwise genetic distances based on Fst between the population of Bahrain and the rest of populations extracted from the literature which included Saudi [15], Filipino [16], Emiratis [17], Bengali [18], Egyptian [19], Turkish [20], Indian [21], Algerian [22] and Jewish [23] were calculated using POPTREE2 software [24] and represented by a nonmetric multidimensional scaling (NM-MDS) analysis using IBM SPSS Statistics v21.0 Software to investigate the populations structure between Bahraini population and the abovementioned populations based on Fst's genetic distances. Phylogenetic tree was constructed from allele frequency data by using the neighbor-joining method [25] *via* MEGA X: Molecular Evolutionary Genetics Analysis [26]. The tree is used to compare between different genetic structures of the populations with Bahraini population using the minimum available loci for different populations. The tree was constructed with allele frequency data of twelve STR loci (DXS7132, DXS7423, DXS8378, DXS10074, DXS10079, DXS10101, DXS10103, DXS10134, DXS10135, DXS10146, DXS10148 and HPRTB) for all populations in corrected fixation index (Fst) using neighbor joining for phylogeny in 1000 permutations.

Results

Allele Frequencies, Forensic Parameters and Efficiency: As for the allele frequency score, some alleles show very high frequencies in the Bahrain population such as allele 14 in DXS7423 with highest frequency of 0.462, followed by allele 19 in DXS10103 with frequency of 0.447 (Tables 1-4). The least frequent was 0.0064 for 12 different alleles. The probability that two randomly chosen person have the same unspecified genotype at a locus is the sum squares of the frequencies of all genotypes at that locus. The full set of Bahrain data is available in Table S1. No shared profiles were observed.

In this studied population, number of alleles per locus (Nall) was ranged from 5 for marker DXS8378 to 27 for DXS10135 with an average Nall per locus with 13.66, and a total number of alleles observed was 164. The most polymorphic locus was DXS10135 (Tables 1-4).

The highest gene diversity (GD) was observed for locus DXS10135 with 0.9467 while the smallest observed was for locus DXS7423 with 0.6693 (Tables 1-4).

Based on allele frequencies, we further determined the statistical parameters of forensic interest. Generally, the polymorphism degree of a specific locus can be measured by the Polymorphism Information Content (PIC) which indicates the degree of genetic polymorphism. We have found out that PIC values for all STR loci were highly informative (PIC≥0.6) with an average of 78.9% (Tables 1-4). PM was ranged from 0.0594 for DXS10135 to 0.3403 for DXS7423.

Allele/N*	DXS8378	DXS10135	DXS10148	DXS7132	DXS10074	DXS10079	DXS10101	DXS10103	HPRTB	DXS7423	DXS10134	DXS10146
N	**156**	**154**	**151**	**156**	**156**	**156**	**156**	**150**	**156**	**156**	**155**	**155**
7					0.077							
8					0.160							
9					0.006							
10	0.269								0.006			
11	0.397			0.019	0.006				0.122			
12	0.308			0.192	0.006				0.385	0.013		
13	0.019			0.237	0.013				0.256	0.026		
13.3			0.020									
14	0.006			0.256	0.026				0.179	0.462		
15				0.231	0.096	0.013			0.038	0.327		

16				0.058	0.135	0.013		0.067		0.135		
17		0.019	0.007	0.006	0.186	0.109		0.100	0.013	0.038		
17.1		0.006										
18		0.045	0.152		0.179	0.154		0.220				
18.1		0.013										
19		0.065	0.033		0.083	0.250		0.447				
19.1		0.013										
20		0.065	0.020		0.026	0.212		0.140				
20.1		0.032	0.007									
21		0.117	0.007			0.135		0.020				
21.1		0.019										
22		0.071	0.013			0.109		0.007				
22.1		0.006	0.013									
23		0.052	0.013			0.006						
23.1		0.013	0.013									
24		0.058	0.007									0.013
24.1		0.006	0.152									
24.2							0.006					

Table 1: Allele frequency and forensic efficiency parameters of (156) samples from population of Bahrain.

Allele/N	**DXS8378**	**DXS10135**	**DXS10148**	**DXS7132**	**DXS10074**	**DXS10079**	**DXS10101**	**DXS10103**	**HPRTB**	**DXS7423**	**DXS10134**	**DXS10146**
N	**156**	**154**	**151**	**156**	**156**	**156**	**156**	**150**	**156**	**156**	**155**	**155**
25		0.032	0.007				0.006					0.052
25.1			0.166									
26		0.071					0.006					0.084
26.1		0.006	0.126									
26.2							0.026					
27		0.071	0.013				0.006					0.103
27.1			0.099									
27.2							0.026					
28		0.071	0.007				0.019					0.161
28.1			0.060									
28.2			0.007				0.141					
29		0.032	0.007				0.019					0.187
29.1			0.033									
29.2							0.141					
30		0.071	0.007				0.051				0.006	0.077
30.2							0.167					
31		0.013					0.077				0.006	0.052
31.1			0.007									
31.2							0.096					
32		0.006					0.090				0.032	0.013

32.2							0.045					
33		0.013					0.045				0.084	
33.2							0.006					
34		0.006					0.013				0.148	0.006
34.2												0.013
35							0.013				0.219	
36											0.200	
37											0.168	
37.2											0.006	0.013

Table 2: Allele frequency and forensic efficiency parameters of (156) samples from population of Bahrain.

Allele/N	**DXS8378**	**DXS10135**	**DXS10148**	**DXS7132**	**DXS10074**	**DXS10079**	**DXS10101**	**DXS10103**	**HPRTB**	**DXS7423**	**DXS10134**	**DXS10146**
N	**156**	**154**	**151**	**156**	**156**	**156**	**156**	**150**	**156**	**156**	**155**	**155**
25		0.032	0.007				0.006					0.052
25.1			0.166									
26		0.071					0.006					0.084
26.1		0.006	0.126									
26.2							0.026					
27		0.071	0.013				0.006					0.103
27.1			0.099									
27.2							0.026					
28		0.071	0.007				0.019					0.161
28.1			0.060									
28.2			0.007				0.141					
29		0.032	0.007				0.019					0.187
29.1			0.033									
29.2							0.141					
30		0.071	0.007				0.051				0.006	0.077
30.2							0.167					
31		0.013					0.077				0.006	0.052
31.1			0.007									
31.2							0.096					
32		0.006					0.090				0.032	0.013
32.2							0.045					
33		0.013					0.045				0.084	
33.2							0.006					
34		0.006					0.013				0.148	0.006
34.2												0.013
35							0.013				0.219	
36											0.200	
37											0.168	
37.2											0.006	0.013

Table 3: Allele frequency and forensic efficiency parameters of (156) samples from population of Bahrain.

Allele/N	DXS8378	DXS10135	DXS10148	DXS7132	DXS10074	DXS10079	DXS10101	DXS10103	HPRTB	DXS7423	DXS10134	DXS10146
N	156	154	151	156	156	156	156	150	156	156	155	155
38											0.045	
38.1			0.007									
38.2												0.006
38.3											0.006	
39											0.026	
39.2												0.019
39.3											0.019	
40.2												0.032
41.2												0.013
41.3											0.019	
42.2												0.026
42.3											0.013	
43.2												0.045
44.2												0.032
45.2												0.019
46.2												0.019
47.2												0.013
Forensic Statistics												
PIC	0.607733	0.936221	0.883274	0.74906	0.851058	0.803967	0.683826	0.707199	0.696207	0.601068	0.832856	0.895932
H	0.325484	0.060488	0.107386	0.215992	0.134297	0.173134	0.279558	0.261093	0.262401	0.340264	0.149905	0.09681
HET	0.674516	0.939512	0.892614	0.784008	0.865703	0.826866	0.720442	0.738907	0.737599	0.659736	0.850095	0.90319
PE	0.389949	0.876615	0.780357	0.569705	0.726039	0.649814	0.460588	0.490981	0.488791	0.368758	0.695052	0.801942
Power of Discrimination												
PD female	0.827277	0.99305	0.979128	0.918399	0.967319	0.947126	0.885231	0.900123	0.889754	0.825552	0.960289	0.98337
PD male	0.674516	0.939512	0.892614	0.784008	0.865703	0.826866	0.720442	0.738907	0.737599	0.659736	0.850095	0.90319
Mean paternity exclusion change												
MEC Krüger	0.393295	0.877499	0.784705	0.571867	0.729362	0.654432	0.501305	0.490852	0.508674	0.402189	0.698798	0.80587
MEC Kishida	0.607611	0.936115	0.882727	0.74906	0.850947	0.803853	0.683473	0.6622	0.696207	0.600947	0.829488	0.893765
MEC Desmarais	0.607733	0.936221	0.883274	0.74906	0.851058	0.803967	0.683826	0.707199	0.696207	0.601068	0.832856	0.895932
MEC Desmarais Duo	0.46044	0.883478	0.80012	0.617823	0.752132	0.687488	0.542731	0.570105	0.556593	0.455211	0.727428	0.819724

Table 4: Allele frequency and forensic efficiency parameters of (156) samples from population of Bahrain.

cPD_Male	0.9999983
cMEC_Krüger	0.9999979
cMEC_Kishida	0.9999939
cMEC_Desmarais	0.9999996
cMEC_Desmarais_duo	0.9999514

*N: Number of samples; PIC: Polymorphism information content; h: Homozygotie; HET: Heterozygotie; PE: Power of Exclusion; PD: Power of discrimination; cMEC: Combined Mean paternity exclusion change

The PM was ranged from 0.0594 for DXS10135 to 0.3403 for DXS7423. The power of discrimination (PD) explains level of discriminating between members. As higher the discriminating power of a locus, the more efficient it can be used to discriminate between members. DXS10135 showed the greatest (PD) in Bahraini population with value of 0.9406, whereas DXS7423 gave the lowest value of (PD) with only 0.6597. The average (PD) of the tested loci was 0.815. The combined PD (CPD) and combined MP (CMP) for all the 12 X-STR loci were 99.99999997% and 3.02583E-10 respectively. The combined values of each forensic parameter, cPDM, cMEC Krüger, cMEC Kishida, and cMEC Desmarais as well as cMEC Desmarais Duo, were 0.9999983, 0.9999979, 0.9999939, 0.9999996 and 0.9999514

respectively (Tables 1-4). The high values of the above-mentioned parameters indicated the usefulness of using the 12-XTRs markers included in the Investigator Argus X-12 QS Kit as a discrimination tool in complement with autosomal STRs [4] and can be utilized for genetic characterization of the Bahraini population to differentiate between individuals for forensic and kinship purposes.

Linkage Disequilibrium Analysis: As shown in Table 5, the study showed no significant deviation from linkage disequilibrium (LD) between pairwise STR loci after Bonferroni's correction in Bahraini population except when plotting the following loci; DXS10074 - DXS10135, DXS10148 - DXS10148, DXS10134 - DXS10148, DXS7132 - DXS10146, DXS7132 - DXS7132 and DXS10103 – HPRTB. The highest pairwise LD was 0.999 when plotting DXS10101 with DXS10148 and also when plotting DXS10103 with DXS10146 and the lowest pairwise LD was 3.62E-07 when plotting DXS10148 with DXS10148.

The significance in LD was obtained between different loci in different LGs and within same LGs.

	DXS8378	DXS10135	DXS10148	DXS7132	DXS10074	DXS10079	DXS10101	DXS10103	HPRTB	DXS7423	DXS10134	DXS10146
DXS10146	0.434436	0.834526	0.000114	0.77173	0.623602	0.617766	0.999879	0.762272	0.798729	0.778209	0.460807	
DXS10134	0.856572	0.00014	0.974159	0.890384	0.009892	0.978075	0.91026	0.119297	0.99456	0.198766		
DXS7423	0.12909	0.712395	0.856028	0.58943	0.633612	0.788513	0.874481	0.046661	0.929244			
HPRTB	0.031025	0.360825	0.838351	0.660623	0.719482	0.86349	6.88E-06	0.115648				
DXS10103	0.600294	0.173926	0.236435	0.762466	0.659187	0.750817	0.005649					
DXS10101	0.940213	0.999954	0.803355	0.811644	0.870909	0.472515						
DXS10079	0.245888	0.014217	0.982419	0.491172	0.879475							
DXS10074	0.001805	0.93321	0.963231	0.254482								
DXS7132	0.718462	0.839586	0.000477									
DXS10148	0.517361	2.94E-06										
DXS10135	0.498013											
DXS8378												

Haplotype Allele Frequencies: Haplotype allele frequencies were determined using the four LG clusters as shown in Tables 6-9. The numbers of observed haplotypes in each of the 4 linkage groups-LG1, LG2, LG3, LG4- were 148, 156, 149 and 153, respectively, while the Haplotype Diversity (HD) values were equal to 1.0000. The three most common haplotype for LG1 was 11-21-25.1 displaying 1.99% of haplotype frequency, in LG2 three sets of haplotypes 14-17-19, 14-7-20 and 15-8-19 were observed each with a frequency of 2.56 % LG3 presented haplotypes 29.2-19-12 4.46% and LG4 14-33-29 and 15-37-27 with haplotype frequency 2.63%.

As for LG1, most of haplotype frequencies counted as 1 or 2 which gave very diverse combinations of haplotypes. Unique haplotypes, which were observed once, accounted for 55.9% of all LGs haplotype observed (339/606).

LG1 Haplotype	Count	Frequency	LG2 Haplotype	Count	Frequency	LG3 Haplotype	Count	Frequency	LG4 Haplotype	Count	Frequency
10 17 18	1	0.0068	11 15 21	1	0.0064	24.2 20 12	1	0.0067	12 37 26	1	0.0065
10 17.1 13.3	1	0.0068	11 16 17	1	0.0064	25 19 13	1	0.0067	12 39.3 28	1	0.0065
10 19 25.1	1	0.0068	11 16 22	1	0.0064	26 20 15	1	0.0067	13 33 42.2	1	0.0065
10 19 26.1	1	0.0068	12 15 18	1	0.0064	26.2 16 12	1	0.0067	13 34 29	1	0.0065
10 19.1 26.1	1	0.0068	12 15 19	1	0.0064	26.2 18 12	1	0.0067	13 37 28	1	0.0065
10 20 24.1	1	0.0068	12 15 20	1	0.0064	26.2 18 13	1	0.0067	13 39 30	1	0.0065
10 20 25.1	1	0.0068	12 15 22	1	0.0064	26.2 20 12	1	0.0067	14 31 32	1	0.0065
10 20 26.1	1	0.0068	12 16 19	2	0.0128	27 19 13	1	0.0067	14 32 28	2	0.0131
10 20 27.1	1	0.0068	12 16 20	1	0.0064	27.2 18 12	1	0.0067	14 32 29	1	0.0065
10 20.1 27.1	2	0.0135	12 16 21	1	0.0064	27.2 21 12	1	0.0067	14 32 31	1	0.0065

10 21 18	1	0.0068	12 17 17	2	0.0128	27.2 22 12	1	0.0067	14 33 26	1	0.0065
10 21 24.1	2	0.0135	12 17 18	1	0.0064	28 18 12	1	0.0067	14 33 29	4	0.0261
10 21 26.1	1	0.0068	12 17 19	2	0.0128	28 19 12	1	0.0067	14 34 25	1	0.0065
10 22 27.1	1	0.0068	12 17 21	1	0.0064	28 20 14	1	0.0067	14 34 26	3	0.0196
10 23 25.1	1	0.0068	12 18 19	1	0.0064	28.2 16 10	1	0.0067	14 34 28	1	0.0065
10 24 26.1	1	0.0068	12 18 20	2	0.0128	28.2 16 12	1	0.0067	14 34 29	1	0.0065
10 24 28.1	1	0.0068	12 18 21	2	0.0128	28.2 18 11	1	0.0067	14 34 30	2	0.0131
10 24 29.1	1	0.0068	12 19 18	1	0.0064	28.2 18 12	1	0.0067	14 34 31	1	0.0065
10 24.1 29.1	1	0.0068	12 19 22	1	0.0064	28.2 18 13	3	0.0201	14 34 41.2	2	0.0131
10 25 24.1	1	0.0068	12 7 17	1	0.0064	28.2 19 11	5	0.0336	14 34 44.2	1	0.0065
10 25 27.1	1	0.0068	12 7 18	1	0.0064	28.2 19 12	3	0.0201	14 35 24	1	0.0065
10 26 19	2	0.0135	12 7 19	1	0.0064	28.2 19 13	4	0.0268	14 35 25	3	0.0196
10 26 28.1	1	0.0068	12 7 20	1	0.0064	28.2 20 12	1	0.0067	14 35 26	1	0.0065
10 27 17	1	0.0068	12 8 18	2	0.0128	28.2 20 13	2	0.0134	14 35 27	1	0.0065
10 27 24.1	1	0.0068	12 8 19	1	0.0064	29 16 14	1	0.0067	14 35 28	3	0.0196
10 27 25.1	1	0.0068	12 8 20	1	0.0064	29 19 12	1	0.0067	14 35 29	1	0.0065
10 27 26.1	1	0.0068	12 9 18	1	0.0064	29.2 16 13	1	0.0067	14 35 30	3	0.0196
10 27 31.1	1	0.0068	13 11 22	1	0.0064	29.2 17 12	1	0.0067	14 35 31	2	0.0131
10 28 18	1	0.0068	13 12 20	1	0.0064	29.2 18 11	1	0.0067	14 35 34.2	1	0.0065
10 28 23	1	0.0068	13 14 19	1	0.0064	29.2 18 12	2	0.0134	14 35 37.2	1	0.0065
10 28 28.1	1	0.0068	13 15 17	1	0.0064	29.2 18 13	1	0.0067	14 35 43.2	1	0.0065
10 29 24.1	1	0.0068	13 15 18	1	0.0064	29.2 19 12	7	0.0470	14 35 44.2	1	0.0065
10 29 25.1	2	0.0135	13 15 19	2	0.0128	29.2 19 13	3	0.0201	14 35 47.2	1	0.0065
10 29 27.1	1	0.0068	13 15 20	1	0.0064	29.2 19 14	2	0.0134	14 36 26	1	0.0065

Table 6: Haplotype diversities of four X-Chromosomal linkage groups in Bahraini male individuals.

LG1 Haplotype	Count	Frequency	LG2 Haplotype	Count	Frequency	LG3 Haplotype	Count	Frequency	LG4 Haplotype	Count	Frequency
10 30 19	1	0.0068	13 15 22	1	0.0064	29.2 19 15	1	0.0067	14 36 27	1	0.0065
10 30 19	1	0.0068	13 15 22	1	0.0064	29.2 19 15	1	0.0067	14 36 27	1	0.0065
10 30 25.1	1	0.0068	13 16 15	1	0.0064	29.2 20 12	1	0.0067	14 36 28	2	0.0131
10 30 26.1	1	0.0068	13 16 18	1	0.0064	30 16 14	1	0.0067	14 36 29	2	0.0131
10 31 25.1	1	0.0068	13 16 20	1	0.0064	30 17 12	1	0.0067	14 36 30	1	0.0065
11 17 27	2	0.0135	13 16 21	1	0.0064	30 18 12	1	0.0067	14 36 37.2	1	0.0065
11 18 13.3	1	0.0068	13 17 19	1	0.0064	30 19 12	2	0.0134	14 36 42.2	2	0.0131
11 18 25.1	2	0.0135	13 17 20	1	0.0064	30 19 13	1	0.0067	14 36 43.2	1	0.0065
11 18.1 18	2	0.0135	13 17 21	3	0.0192	30 19 14	1	0.0067	14 36 45.2	1	0.0065
11 19 18	1	0.0068	13 17 22	1	0.0064	30.2 18 11	4	0.0268	14 37 27	1	0.0065
11 19 25.1	2	0.0135	13 17 23	1	0.0064	30.2 18 12	1	0.0067	14 37 28	1	0.0065
11 20 18	1	0.0068	13 18 15	1	0.0064	30.2 18 13	1	0.0067	14 37 29	3	0.0196
11 20 26.1	1	0.0068	13 18 18	1	0.0064	30.2 18 14	1	0.0067	14 37 30	1	0.0065
11 20.1 24.1	1	0.0068	13 18 19	3	0.0192	30.2 18 15	2	0.0134	14 37 42.2	1	0.0065
11 21 18	2	0.0135	13 18 20	2	0.0128	30.2 19 11	3	0.0201	14 37 46.2	1	0.0065

11 21 19	1	0.0068	13 18 21	1	0.0064	30.2 19 12	4	0.0268	14 37.2 29	1	0.0065
11 21 20	1	0.0068	13 18 22	1	0.0064	30.2 19 13	3	0.0201	14 38 28	1	0.0065
11 21 24.1	2	0.0135	13 19 17	1	0.0064	30.2 19 15	1	0.0067	14 38 46.2	1	0.0065
11 21 25.1	3	0.0203	13 19 21	1	0.0064	30.2 20 11	2	0.0134	14 38.3 26	1	0.0065
11 21 26.1	1	0.0068	13 19 22	1	0.0064	30.2 20 12	1	0.0067	14 39 28	2	0.0131
11 21 28.2	1	0.0068	13 8 17	1	0.0064	30.2 20 14	2	0.0134	14 39.3 31	1	0.0065
11 21 29.1	1	0.0068	13 8 18	1	0.0064	30.2 21 12	1	0.0067	14 41.3 27	1	0.0065
11 21.1 27.1	1	0.0068	13 8 20	1	0.0064	31 16 13	1	0.0067	14 42.3 27	1	0.0065
11 22 18	1	0.0068	13 8 21	1	0.0064	31 17 12	3	0.0201	15 30 30	1	0.0065
11 22 22	1	0.0068	13 8 22	1	0.0064	31 17 13	1	0.0067	15 33 28	1	0.0065
11 22 24.1	2	0.0135	14 13 22	1	0.0064	31 18 12	2	0.0134	15 33 29	2	0.0131
11 22 26.1	1	0.0068	14 15 20	1	0.0064	31 18 13	1	0.0067	15 33 31	1	0.0065
11 22 30	1	0.0068	14 16 16	1	0.0064	31 19 13	1	0.0067	15 33 43.2	1	0.0065
11 22.1 19	1	0.0068	14 16 18	1	0.0064	31 19 14	1	0.0067	15 34 28	2	0.0131
11 23 23	1	0.0068	14 16 19	3	0.0192	31 20 12	1	0.0067	15 34 29	1	0.0065
11 23 24.1	1	0.0068	14 16 20	1	0.0064	31 20 13	1	0.0067	15 34 40.2	1	0.0065
11 23 25.1	1	0.0068	14 16 22	2	0.0128	31.2 16 12	1	0.0067	15 35 25	2	0.0131
11 23 26.1	1	0.0068	14 17 18	1	0.0064	31.2 17 14	1	0.0067	15 35 26	1	0.0065

Table 7: Haplotype diversities of four X-Chromosomal linkage groups in Bahraini male individuals.

LG1 Haplotype	Count	Frequency	LG2 Haplotype	Count	Frequency	LG3 Haplotype	Count	Frequency	LG4 Haplotype	Count	Frequency
11 23 28	1	0.0068	14 17 19	4	0.0256	31.2 18 12	1	0.0067	15 35 28	1	0.0065
11 23.1 25.1	1	0.0068	14 17 21	1	0.0064	31.2 19 12	3	0.0201	15 35 31	1	0.0065
11 24 18	1	0.0068	14 18 17	1	0.0064	31.2 19 13	4	0.0268	15 35 34	1	0.0065
11 24 20	1	0.0068	14 18 18	2	0.0128	31.2 19 14	3	0.0201	15 35 39.2	1	0.0065
11 24 23.1	1	0.0068	14 18 19	2	0.0128	31.2 20 14	1	0.0067	15 35 43.2	1	0.0065
11 24 24.1	1	0.0068	14 18 20	2	0.0128	31.2 21 13	1	0.0067	15 35 44.2	1	0.0065
11 24 25.1	1	0.0068	14 18 22	1	0.0064	32 16 14	1	0.0067	15 36 24	1	0.0065
11 25 27.1	1	0.0068	14 19 18	2	0.0128	32 17 12	3	0.0201	15 36 26	1	0.0065
11 26 24.1	1	0.0068	14 19 22	1	0.0064	32 17 14	3	0.0201	15 36 28	3	0.0196
11 26 25.1	1	0.0068	14 20 20	1	0.0064	32 18 13	2	0.0134	15 36 29	2	0.0131
11 26.1 24	1	0.0068	14 20 22	1	0.0064	32 19 14	3	0.0201	15 36 30	1	0.0065
11 27 18	1	0.0068	14 7 17	1	0.0064	32 20 14	2	0.0134	15 36 40.2	3	0.0196
11 27 25.1	1	0.0068	14 7 20	4	0.0256	32.2 18 13	1	0.0067	15 36 46.2	1	0.0065
11 28 24.1	1	0.0068	14 8 18	1	0.0064	32.2 19 12	1	0.0067	15 37 26	2	0.0131
11 28 26.1	1	0.0068	14 8 19	3	0.0192	32.2 19 14	2	0.0134	15 37 27	4	0.0261
11 28 29.1	1	0.0068	14 8 20	1	0.0064	32.2 20 12	2	0.0134	15 37 28	1	0.0065
11 30 24.1	1	0.0068	14 8 21	1	0.0064	32.2 20 13	1	0.0067	15 37 29	2	0.0131
11 30 27.1	1	0.0068	15 13 19	1	0.0064	33 16 13	2	0.0134	15 37 39.2	1	0.0065
11 30 28.1	1	0.0068	15 14 17	1	0.0064	33 17 12	1	0.0067	15 37 47.2	1	0.0065
11 31 28.1	1	0.0068	15 14 20	2	0.0128	33 17 14	1	0.0067	15 38 27	2	0.0131
11 32 22	1	0.0068	15 15 18	1	0.0064	33 19 14	1	0.0067	15 38 29	1	0.0065

11 34 26.1	1	0.0068	15 16 18	1	0.0064	33 19 17	2	0.0134	15 38 34.2	1	0.0065
12 18 18	1	0.0068	15 16 19	1	0.0064	33.2 19 12	1	0.0067	15 38 39.2	1	0.0065
12 18 25	1	0.0068	15 17 16	1	0.0064	34 18 11	1	0.0067	15 39 27	1	0.0065
12 18 26.1	1	0.0068	15 17 17	1	0.0064	34 19 13	1	0.0067	15 39.3 30	1	0.0065
12 19 18	2	0.0135	15 17 19	2	0.0128				15 42.3 28	1	0.0065
12 19 20.1	1	0.0068	15 17 20	3	0.0192				16 33 27	1	0.0065
12 19 38.1	1	0.0068	15 17 21	2	0.0128				16 34 29	1	0.0065
12 19.1 28.1	1	0.0068	15 17 22	1	0.0064				16 34 30	1	0.0065
12 20 18	1	0.0068	15 18 17	1	0.0064				16 34 43.2	1	0.0065
12 20 24.1	2	0.0135	15 18 19	2	0.0128				16 35 29	2	0.0131
12 20 27.1	1	0.0068	15 18 20	1	0.0064				16 35 32	1	0.0065

Table 8: Haplotype diversities of four X-Chromosomal linkage groups in Bahraini male individuals.

LG1 Haplotype	Count	Frequency	LG2 Haplotype	Count	Frequency	LG3 Haplotype	Count	Frequency	LG4 Haplotype	Count	Frequency
12 20.1 18	1	0.0068	15 19 18	1	0.0064				16 35 38.2	1	0.0065
12 20.1 22.1	1	0.0068	15 19 19	1	0.0064				16 35 45.2	1	0.0065
12 21 26.1	1	0.0068	15 19 21	2	0.0128				16 36 26	1	0.0065
12 21 27.1	1	0.0068	15 7 17	1	0.0064				16 36 28	2	0.0131
12 21.1 18	1	0.0068	15 7 19	1	0.0064				16 36 29	2	0.0131
12 22 13.3	1	0.0068	15 7 22	1	0.0064				16 36 31	1	0.0065
12 22 24.1	1	0.0068	15 8 18	2	0.0128				16 37 25	1	0.0065
12 23 27.1	1	0.0068	15 8 19	4	0.0256				16 37 27	2	0.0131
12 24 27.1	1	0.0068	15 8 21	2	0.0128				16 37 29	1	0.0065
12 24 29	1	0.0068	16 15 18	1	0.0064				16 37 44.2	1	0.0065
12 25 26.1	2	0.0135	16 16 20	1	0.0064				17 32 44.2	1	0.0065
12 26 18	2	0.0135	16 18 17	2	0.0128				17 33 25	1	0.0065
12 26 24.1	1	0.0068	16 19 20	1	0.0064				17 34 43.2	2	0.0131
12 26 25.1	1	0.0068	16 20 17	2	0.0128				17 34 45.2	1	0.0065
12 26 27.1	1	0.0068	16 8 20	1	0.0064				17 41.3 40.2	1	0.0065
12 26 28.1	1	0.0068	16 8 21	1	0.0064						
12 27 18	1	0.0068	17 15 20	1	0.0064						
12 27 24.1	2	0.0135									
12 28 25.1	2	0.0135									
12 28 27.1	1	0.0068									
12 28 28.1	2	0.0135									
12 29 25.1	1	0.0068									
12 29 29.1	1	0.0068									
12 30 18	1	0.0068									
12 30 21	1	0.0068									
12 30 25.1	1	0.0068									
12 30 26.1	1	0.0068									
12 33 22.1	1	0.0068									

12 33 26.1	1	0.0068									
13 23 18	1	0.0068									

Table 9: Haplotype diversities of four X-Chromosomal linkage groups in Bahraini male individuals.

Rare Variants, Off-ladder and Null Alleles: Several cases showed off ladder (OL) in various loci; two allelic ladder variants were detected at the DXS10146; Sample#12 indicated OL in 223.05 bp and sample#34 showed OL in 243.87 bp. Four allelic ladder variants were detected at DXS10148; Sample#29 showed OL in 312.62 bp, sample#49 and sample#50 showed OL in 312.57, and sample#123 showed OL in 280.19 bp. One allelic ladder variants were detected with OL in DXS10134; Sample#72 showed OL with 325.61 bp. Null alleles were also observed in two loci; DXS10148 in sample#105 and in DXS10103 showed drop out allele in sample#135 and sample#119 (Figure 1).

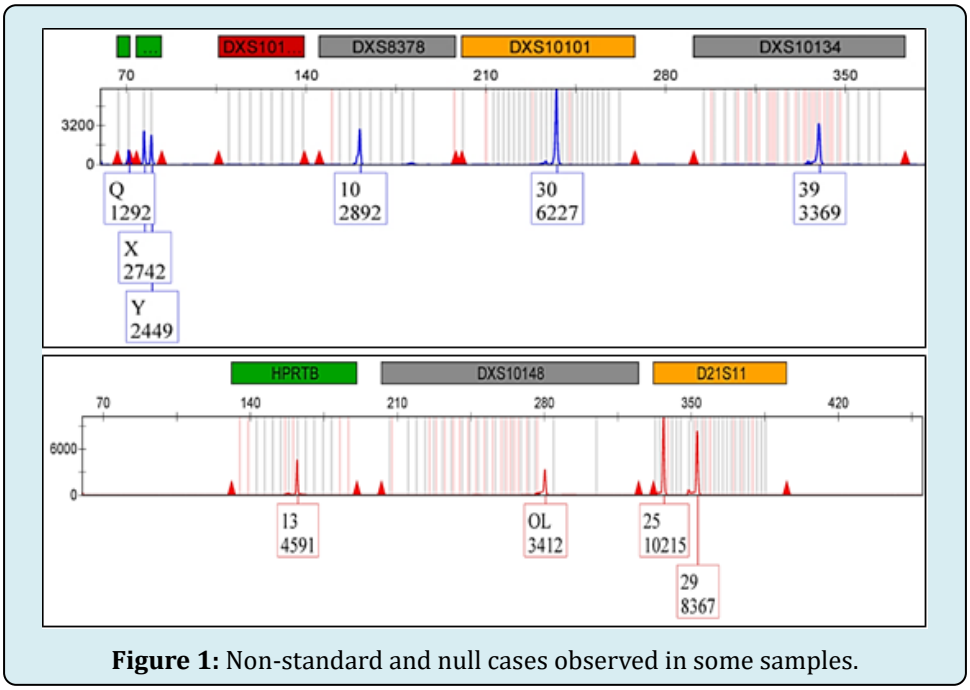

Figure 1: Non-standard and null cases observed in some samples.

High frequent cases of non-standard ladders (OL) and null alleles were at DXS10148, which gave an indication that the Investigator Argus X-12 QS kit provided standard ladder which lacked significant coverage of bins at DXS10148 as it was confirmed in previous literature [17].

More than half of the samples (103/156) showed 0.1 variant in DXS10148 and 18 samples gave the same variant in DXS10135.

More than half of the samples (102/156) also showed 0.2 variant in DXS10101, 39 samples in DXS10146, one sample in DXS10134 and one in DXS10148. As for 0.3 variant; 9 samples were detected in DXS10134 and 3 samples in DXS10148.

Interpopulation Diversity: To measure the diversity between Bahraini population and other populations previously reported, we have constructed the phylogenetic tree from allelic frequencies data by using the neighbor-joining (NJ) method *via* MEGA X: Molecular Evolutionary Genetics Analysis (Figure 2).

We have used 9 populations including: Saudi [15], Filipino [16], Emiratis [17], Bengali [18], Egyptian [19], Turkish [20], Indian [21], Algerian [22] and Jewish [23]. Fst and p-values for allele frequency distribution between Bahraini population and the published groups are shown in (Table 10). It is shown that Bahraini and Emirati populations shared the most genetic relatedness than other populations, as the Saudi population considers being the most geographically close population, however gave more genetic relatedness with Egyptian population than with Bahraini population. The rest of populations stood distant of genetic association with the Bahraini population. We have also constructed the MDS plot using IBM SPSS Statistics v21.0 Software, and it gave correlating results with the phylogenetic tree. As Bahraini, Emirati and Algerian populations gave the same clusters and Saudi, Egyptian and Jewish in another cluster (Figure 3).

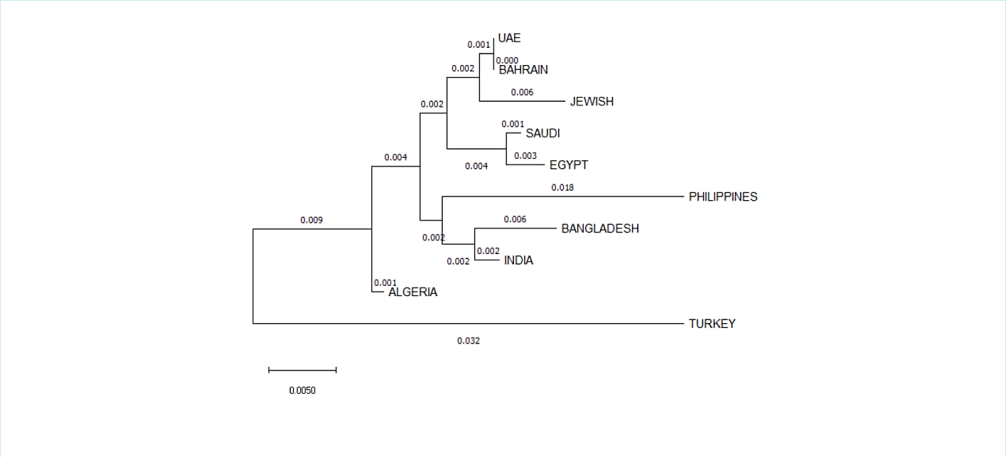

Figure 2: Phylogenetic tree using based on Nei's DA Distances for the 12 X-STR loci estimated among 10 populations.

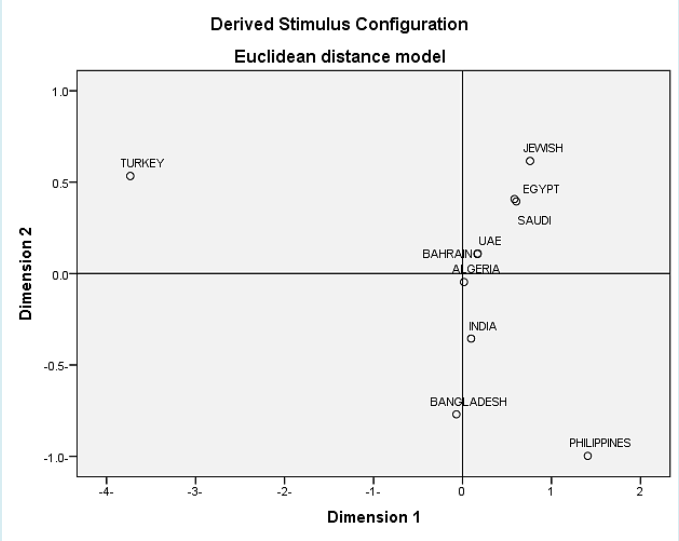

Figure 3: MDS plot constructed between Bahraini population and other populations.

	Saudi	Emirati	Egyptian	Turkish	Algerian	Jewish	Filipino	Bengali	Indian	Bahraini
Saudi	0	0.001	0.003	0.032	0.001	0.007	0.018	0.006	0.002	0.001
Emirati	0.009	0	0.004	0.002	0.002	0.002	0.002	0.002	0.002	0.003
Egyptian	0.004	0.011	0	0.009	0.003	0.004	0.002	0.004	0.004	0.012
Turkish	0.052	0.048	0.055	0	0.009	0.002	0.002	0.003	0.003	0.006
Algerian	0.013	0.01	0.015	0.042	0	0.003	0.018	0.002	0.002	0.006
Jewish	0.012	0.008	0.017	0.059	0.013	0	0.002	0.002	0.002	0.002
Filipino	0.023	0.025	0.027	0.066	0.027	0.03	0	0.002	0.002	0
Bengali	0.022	0.013	0.02	0.051	0.008	0.024	0.029	0	0.002	0.041
Indian	0.017	0.009	0.014	0.05	0.013	0.019	0.02	0.008	0	0.018
Bahraini	0.009	0	0.012	0.051	0.012	0.007	0.027	0.016	0.012	0

Table 10: Nei genetic distance matrix between Bahraini population and other populations. Above the diagonal are p values while below the diagonal are Fst values.

Discussion

The observed deviation from LD (neglecting the Bonferroni's correction) could be a result of the high diversity of the Bahraini population or caused by high polymorphism at the same loci investigated loci. This observation are likely to reflect the high level of inbreeding with consanguinity rates in Bahrain, with intra-familial unions accounting for 20–50% of all marriages compared to other Arab countries [27]. The PD in correlation with PM supports the high degree of polymorphism between Bahraini individuals.

As shown the significance of LD provided of loci in different LGs, suggesting of using individual allele frequency as well as haplotype allele frequencies for population database and likelihood studies.

We have compared Bahraini population data with other populations according to the available data using the accessible loci (Table S2).

It is shown that the Bahraini population shares similar results with the study conducted of Emirati populations using the X-STRs loci. Allele 14 in locus DXS7423 scored the highest frequency for Bahraini, Emirati, Egyptian and Indians populations [17,19,21] whereas allele 15 in same locus were the highest frequency for Filipino and Algerian populations [16,22]. Turkish population gave the highest allele frequency in allele 13 (HPRTB) [20]. Jewish and the Saudi populations shared the highest frequency with allele 19 (DXS10103) [15,23]. Bengali population showed the highest allele frequency in allele 11(DXS8378) [18].

Regarding the Interpopulation diversity, the phylogenetic tree was constructed based upon the data from the nine populations which were consistent with other population data from the region based upon the Fst values obtained. The obtained Fst value of Bahrain is <0.0000 which is less than the recommended value for casework statistics of Fst < 0.01 [28].

As shown, Bahraini and Emirati populations were more genetically related in terms of phylogenetic tree and MDS plot in contrary of Saudi population which was shown in previous papers published being more genetically related to Bahrain [4,5]. This can be explained by studying the origins of the mothers of participants as it wildly affects the X-STRs results.

Once more studies of Arab populations in the region become accessible, it may be more probable to develop a greater understanding of the genetic associations between the different populations for the Arabian Peninsula. Further linkage studies must be conducted to determine if the loci are physically linked.

Conclusion

In conclusion, this is first study to report the allele frequencies and forensic statistical parameters of the X chromosomal STRs included in the Investigator Argus X-12 QS Kit in a sample population of Bahrain. X-STRs panels can be used for some cases for forensics investigations such as human identification and paternity testing.

It is shown that X-STRs included in the Investigator Argus X-12 kit can be utilized for forensic practice in Bahraini population. Our results demonstrate the importance of analyzing diverse populations using X-STRs markers.

Conflict of Interest

The authors declare that they have no conflict of interest

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