



Usefulness of X STR Haplotype markers in Forensic DNA Profiling

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Abstract:

Haplotype is a lineable combination of alleles at multiple loci that are transmitted together. The importance and application of these haplotypes has been gradually increased in forensic sciences. After Y STR's on Chromosome Y and Mitochondrial DNA now the current focus is on haplotype study involving X STR's on Chromosome X. Fathers transmit their X chromosome to daughters as haplotypes. The X-chromosome is 155 million base pairs (Mb) long and carries approximately 1250 known genes. Women carry two copies of X chromosome while men are hemizygous for all X chromosomal markers. Research findings suggest that Mutations on the X chromosome occur less frequently than on autosomes. The analysis of X-chromosome markers can be valuable in particular situations, for example, deficiency kinship cases, where the putative father cannot be typed. X-chromosome short-tandem repeats (X-STRs) are widely used in forensic genetics. X chromosome-linked short tandem repeat (X STR) analysis have been used in paternity testing, more complex deficiency paternity cases, when half-sisters and/or grand mothers are to be examined; paternity testing including blood relatives; and rare cases of maternity testing. Its usefulness is also seen in cases when female DNA traces have to be analyzed against a male background which is more common in the case of sexual assault with children. In present paper the utility of X STR is presented along with a pilot database of a tribal population of Bhil from Madhya Pradesh, to establish frequencies for the single STRs and their haplotypes in Indian population. DNA isolation was done by automated DNA extraction system 12GC from PSS Japan, DNA was quantified using real time PCR ABI 7000 and X STR profiles were generated using Investigator Argus X-12 amplification kit on ABI 3100 genetic analyser. The work was done with the financial support from Madhya Pradesh council of Science and Technology, Bhopal (MP) India.

Keywords: Forensic, DNA Fingerprinting, X STR, DXS10148, DXS10135, DXS8378, DXS7132, DXS10079, DXS10074, DXS10103, HPRTB, DXS10101, DXS10146, DXS10134, DXS7423.

Introduction:

Application of autosomal and Y-chromosomal STR markers forensic investigation is in routine practice now. In some kinship analyses involving maternal relatives, mitochondrial DNA analysis is also being used. But the use of mitochondrial DNA analysis is still not that common and most of the forensic laboratories are not doing mitochondrial DNA analysis because of the fact that protocol of genotyping is comparatively easier and also ready to use pre validated multiplex markers are available. In case of mitochondrial sequencing situation is not the same. Use of X-chromosomal short tandem repeats (X-STR) loci is also started for forensic practice in some complex kinship cases. The commercially available kit of Investigator Argus X-12 (Qiagen, Hilden, Germany) makes it possible to examine the markers belonging to four linkage groups of X-chromosome, linkage group 1: DXS10148-DXS10135-DXS8378, linkage group 2: DXS7132-DXS10079-DXS10074, linkage group 3: XS10103-HPRTB-DXS10101 and linkage group 4: DXS10146-DXS10134-DXS7423 in a single PCR. The availability of Argus X-12 Kit for simultaneous amplification of twelve X-STR loci has created increasing interest among forensic scientists for analyzing X STR markers. Statistical parameters obtained in different studies on X-STR indicate their usefulness in forensic genetics investigations. A large number of short tandem repeat (STR) markers on the entire human X chromosome have been described and established for use in forensic genetic testing [1]. These markers located on X chromosome have a particular inheritance pattern: women are dizygous and men are hemizygous, with the latter receiving the single X from the mother similarly father transmit their X chromosome to daughter as haplotypes. Analysis of X-chromosomal loci might be beneficial in deficiency paternity cases, where half-sisters and/or grandmothers are examined [2]. True daughters carry always the whole paternal X-chromosome (ChrX) and therefore both of the daughters ChrX haplotypes can also be completely recognised when a father and his daughter are submitted to a jointed typing. Likewise, jointed ChrX typing for a mother and her son reveals also both of

the maternal haplotypes. The latter, however, applies with a high certainty only to closely linked loci [3–7]. Due to their particular mode of inheritance, X STRs often allow easy and informative haplotyping in kinship analyses. Moreover, some X-STRs are known to be tightly linked so that, in combination, they constitute even more complex genetic markers than each STR taken individually. As a consequence, X-STRs have proven particularly powerful in solving complex cases of disputed blood relatedness [1]. And can serve as a complement to autosomal STRs and mitochondrial DNA polymorphism [8]. Research findings suggest that Mutations on the X chromosome occur less frequently than on autosomes due to several times lower nucleotide mutation rate in women than in men, which also reduces the X chromosome genetic diversity[9]. Although a considerable amount of information on polymorphism and pairwise genetic distance (Fst) based on the allele frequencies have been carried out in the past few years in different parts of the world on X STR markers [10-18]. But, there exist still a lacuna for this type of study on Indian populations. The present study is an attempt to create a pilot X-STR database with comparative allelic distribution of each locus in population of the Bhil tribes of Madhya Pradesh, India, by using Investigator Argus X-STR 12 kit (Qiagen, Hilden, Germany) which will be helpful not only in criminal investigation such as personal identification and kinship testing but for other anthropological purposes as well. This is first pilot study on the population of Bhil tribes of India. A case of paternity inclusion and exclusion is also presented to show case the utility of X-STR 12 marker multiplex kit for its use as autosomal markers in cases where paternity of female child is in question. It can also be used to confirm the paternity and/maternity of the child where in one or two locus a mismatch is observed with autosomal STR.

Material and Methods:

The study site:

Madhya Pradesh is a medley of ethnic groups and tribes, castes and communities. The contemporary population of Madhya Pradesh is a mixture of indigenous and migrated groups that came along Narmada valley, settled and flourished here since prehistoric times. The central Indian state Madhya Pradesh has always been an important region, in terms of studying evolution and genetic diversity among human populations due to its geographic location. It is located in the heart of India, spread over an area of 23° 10' 12" North to 77° 12' 36" East,

bordering the states Uttar Pradesh, Chhattisgarh, Maharashtra, Gujarat and Rajasthan. Except for the valleys of the Narmada and the Tapti, Madhya Pradesh consists of a plateau, straddled by the Narmada River and interspersed with the mountains of the Vindhya and the Satpuda ranges. The geographical area of the state is 308,144 km² with a total population of 60,575,333 (Census, 2011), giving a density of 196 per square kilometer. The state is endowed with rich and diverse forest resources. The forest area of the state is 95,221 km² (36,765 sq m) constituting 31% of the geographical area of the state and 12.44% of the forest area of the country. The state has a subtropical climate having hot dry summer (April-June) followed by monsoon rains (July-September) and a cool and relatively dry winter. Madhya Pradesh is a medley of ethnic groups and tribes, castes and communities. The contemporary population of Madhya Pradesh is a mixture of indigenous and migrated groups that came along Narmada valley, settled and flourished here since prehistoric times.

The population:

Bhils are primarily an Adivasi people of Central India. Bhils are also settled in the Tharparkar District of Sindh, Pakistan. They speak the Bhil languages, a subgroup of the Western Zone of the Indo-Aryan languages. Bhils are listed as Adivasi residents of the states of Gujarat, Madhya Pradesh, Chhattisgarh, Maharashtra and Rajasthan - all in the western Deccan regions and central India - as well as in Tripurain far-eastern India, on the border with Bangladesh. Bhils are divided into a number of endogamous territorial divisions, which in turn have a number of clans and lineages. Most Bhils now speak the language of the region they reside in, such as Marathi and Gujarati. They mostly speak a dialect of Hindi. Tribals in Madhya Pradesh constitute a sizeable population. The population of Tribals in Madhya Pradesh is 12.233 million constituting 20.27% of the total population of Madhya Pradesh (60.385 million), according to the 2001 census. There were 46 recognized Scheduled Tribes and three of them have been identified as "Special Primitive Tribal Groups" in the State. The main tribal groups in Madhya Pradesh are Gond, Bhil, Baiga, Korku, Bhariya, Halba, Kaul, Mariya, and Sahariya. Dhar, Jhabua and Mandladistricts have more than 50 percent tribal population. In Khargone, Chhindwara, Seoni, Sidhi and Shahdol districts 30 to 50 percent population is of tribes. Maximum population is that of Gond tribes followed by Bhils.

Sample collection:

For population study saliva samples from 50 female bhil tribes were collected after informed consent and were transported to laboratory under ice. Collected saliva samples were stored at - 80°C. Sample Collection was done from Bhil tribes of Shivgarh, Sailana, and Rawti area of Ratlam district of Madhya Pradesh. Referral blood samples of both parents and female child from two real cases was used to see the utility of X STR markers for forensic purpose. A case of paternity inclusion and exclusion were taken to show the applicability of X STR multiplex for identification purpose.

DNA extraction and quantity assessment:

DNA isolation from all the samples was done by Automated DNA extraction system 12 GC (PSS, Japan) by using prefilled cartridges supplied by manufacturer. Quantity assessment was done by ABI 7000 Real Time PCR (Applied Biosystems, Foster City, CA).

Table 1: Autosomal STR DNA profile generated from AmpflSTR identifier kit.

Genetic Markers	FATHER	CHILD	MOTHER
D8S1179	10,13	10,10	10,12
D21S11	30,32.2	27,32.2	27,30
D7S820	8,12	12,12	8,12
CSF1PO	10,10	10,11	9,11
D3S1358	14,19	16,19	15,16
TH01	6,8	8,9	9,10
D13S317	11,12	11,12	11,12
D16S539	11,11	10,11	10,12
D2S1338	22,25	25,25	19,25
D19S433	12,14	13,14	13,13
vWA	14,14	14,16	15,16
TPOX	8,8	8,8	8,11
D18S51	12,16	16,16	16,16
D5S818	12,13	12,13	12,13
FGA	23,24	23,24	21,23
AMELOGENIN	XX	XX	XY

Note- Forensic DNA test is based on the fact that the child shares the allele from his parents. For example on IInd loci tested i.e. on D21S11 the child is 27, 32.2, one of these two allele has to come from father

(32.2 in this case) and the other one from his mother (27 in this case). This has to be tested on all the 16 loci in the kit. One or two mismatch in this test shows exclusion.

PCR and Genotyping:

Multiplexed PCR amplifications of the 12 X STR Loci: DXS10103, DXS8378, DXS7132, DXS10134, DXS10074, DXS10101, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB and DXS10148 and Amelogenin (sex determining marker) was performed using the Quigen Argus X-12 PCR Amplification Kit (Quigen) according to the manufacturers recommended protocol [19]. Multiplexed PCR amplifications of the 15 autosomal STR Loci : D8S1179 , D21S11 , D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and Amelogenin was performed using the AmpflSTR identifier plus Kit (Applied Biosystems, Foster City, CA) according to the manufacturers recommended protocol [20] . The PCR reagents have been standardized in the laboratory for consistency of results. PCR was performed by taking the ½ reaction volume of the manufacturer's recommended protocol and by using Gene Amp 9700 thermal cycler (Applied Biosystems, Foster City, CA) [21]. The amplified PCR products were run on Genetic Analyzer (ABI 3100) following the manufactures protocol. The samples were prepared using 10 µL of Hi-Di formamide, with appropriate 0.1 µL size standard and 0.5 µL of the amplified PCR product. The samples were denatured for 3 min at 95°C and snap cooled on ice before loading onto the instrument. The analyzer was equipped with 36 cm arrays, with separation of fragments using POP-4 polymer (Applied Biosystems, USA) and the data were analysed with Gene Mapper ID Analysis Software. A peak detection threshold of 50 RFUs was used for allele designation.

Statistical analysis:

The allele frequencies of the 12 X STR markers were calculated using power stat v 2.1 software [22]. Several Statistical parameters of forensic importance such as the power of Discrimination (PD), Polymorphism information content (PIC), matching probability (Pm), power of Exclusion (PE), and typical paternity index (TPI), were calculated using the Excel Power stats spread sheet. Hardy Weinberg *p* values, observed heterozygosity (Ho), expected heterozygosity (He) were calculated by the Arlequin software package, version 3.1[23] .

Results and Discussion:

Autosomal STR DNA profile of mother, father and female child trio is presented in Table 1. All the allele in the autosomal STR DNA profile of female child is matching with both the parents on all the 15 locus establishing the identity of the child ie a case of inclusion. Data of DNA profile of same three samples with Argus X12 kit is presented in Table 2. In X STR also all the allele in the autosomal STR DNA profile of female child is matching with both the parents on all the 12 locus. As the female child is having two X, one from father and the other from mother, so this can be shown by generating X STR DNA profile of the trio.

Table 2- X STR DNA profile generated from Argus X 12 kit

Genetic Markers	Father	Child	Mother	+ve Cntrl.	-ve Cntrl
DXS10103	16	16,18	18,19	16,19	–
DXS8378	12	11,12	11,11	10,12	–
DXS7132	14	14,14	14,15	13,14	–
DXS10134	34	34,36	36,37	36,38.3	–
DXS10074	14	14,18	18,18	18,20	–
DXS10101	33	31,33	29.2,31	28.2,28.2	–
DXS10135	20	20,29	27,29	16,30	–
DXS7423	14	14,14	14,14	14,15	–
DXS10146	25	26,25	25,26	26,46.2	–
DXS10079	19	19,21	19,21	18,21	–
HPRTB	13	13,15	11,15	12,13	–
DXS10148	27.1	23,27.1	18,23	18,23.1	–
AMELOGENIN	XY	XX	XX	XX	–

Note- Here also for example on 1st loci tested i.e. on DX10103 the child is 16, 18, one of these two allele has to come from father (16 in this case) and the other one from his mother (18 in this case). As there is match on all 12 loci, this is a case of inclusion.

Allele frequency distribution and statistical evaluation of the 12X STR loci analyzed from the Bhil tribes are presented in Table 4-5. In total, 100 alleles were observed in the Bhil population with the corresponding allele frequencies ranging from 0.010 to 0.480, The HPRTB and DXS7423 loci were found to have a maximum allele frequency range with allele 13 (0.480) and 14(0.480) respectively. The power of discrimination ranged from a minimum of 0.733 for DXS7423 to a maximum of 0.967 for DXS10148 (Table 6). DXS10135 was the most polymorphic X-STR with 17 allele, and DXS7423 was the least polymorphic one with 4 allele. The ideal PIC value is one; meaning that the polymorphism is informative in any random mating and the ideal heterozygosity is 100%. As shown in

table 6, the PIC of the twelve loci proved to be 0.5 or more than 0.5, which indicates that the twelve loci are of high polymorphism in the investigated Bhil population of Madhya Pradesh. The degree of polymorphism of every marker shows that the DXS7423 (0.50) is the least polymorphic marker while DXS10135 (0.91) is the most polymorphic marker in the population of Madhya Pradesh. The polymorphic nature of microsatellites makes them the markers of choice in characterization and genetic diversity studies. The high PIC values of the selected markers confirm their usefulness for genetic polymorphism studies and linkage mapping program in human as well. The parameters of population genetics and forensic interest calculated on the basis of allele frequencies strongly support the use of the set of genetic markers used in the present for personal identification testing. The overall distribution of the allele frequencies in bhil population was found to be in fair accordance with Hardy-Weinberg rule. All the studied loci were consistent with Hardy Weinberg Equilibrium (HWE) except deviation at loci DXS10101 and DXS10135 (p=0.0000). Statistical parameters of forensic importance indicated the usefulness of the loci in forensic individual identification and paternity testing in population of M.P. There is significantly greater inter-individual variation within studied population.

Table 3- X STR DNA profile generated from Argus X 12 kit

Genetic Markers	Father	Child	Mother	+ve Cntrl	-ve Cntrl
DXS10103	19	16,19	17,19	16,19	–
DXS8378	11	11	11,11	10,12	–
DXS7132	13	12,13	13,14	13,14	–
DXS10134	35	33,36	33,39.3	36,38.3	–
DXS10074	7	8,16	16,17	18,20	–
DXS10101	28.2	28.2,32	28.2,31	28.2,28.2	–
DXS10135	30	28,33	28,28	16,30	–
DXS7423	14	14,16	14,14	14,15	–
DXS10146	25	28,31	31,31	26,46.2	–
DXS10079	19	19,20	19,20	18,21	–
HPRTB	11	11,12	11,11	12,13	–
DXS10148	18	24.1,27.1	24.1,24.1	18,23.1	–
AMELOGENIN	XY	XX	XX	XX	–

Note- In this case which is a case of exclusion with father. As shown here on 4th loci tested i.e. on DX10134 the child is 33, 36. At this locus father is 35 but this 35 is not there in daughter while mother is 33,39.3 and the child is having one of the two allele i.e. (33 in this case).

Table 4 Locus wise allele frequency 12 X STR markers

Allele/n	DXS10103	DXS8378	DXS7132	DXS10134	DXS10074	DXS10101	DXS10135	DXS7423	DXS10146	DXS10079	HPRTB	DXS10148
N	50	50	50	50	50	50	50	50	50	50	50	50
7					0.030							
8					0.060							
9		0.020			0.010							
10		0.320									0.020	
11		0.400	0.040								0.040	
12		0.220	0.060								0.280	
13		0.040	0.290								0.480	
14			0.370		0.010			0.480		0.010	0.100	
15			0.190		0.050			0.420			0.070	
16	0.340		0.030		0.130			0.090		0.010	0.010	
17	0.080		0.020		0.370			0.010		0.040		
18	0.170				0.200		0.090			0.210		0.140
19	0.350				0.100		0.030			0.220		0.040
20	0.040				0.030		0.050			0.340		0.030
21	0.020				0.010		0.120			0.090		0.070
21.1							0.010					
22							0.080			0.060		
22.1							0.010					0.030
23							0.140			0.020		0.020
23.1												0.020
24							0.110		0.040			0.030
24.1												0.090
25							0.020		0.020			
25.1												0.160
26						0.010	0.020		0.120			
26.1												0.140
27						0.050	0.090		0.160			
27.1												0.130
27.2						0.040						
28						0.020	0.090		0.220			
28.1												0.090
28.2						0.040						
29						0.060	0.070		0.160			
29.1												0.010
29.2						0.110						
30						0.050	0.030		0.160			
30.2						0.030						
31						0.080	0.030		0.110			
31.2						0.110						
32				0.040		0.170	0.020		0.010			
32.2						0.070						
33				0.090		0.110						
33.2						0.020						
34				0.190		0.010						
34.2												
35				0.230		0.010						
35.3				0.080								
36				0.100								
37				0.080								
37.3				0.060								
38				0.060								
38.3				0.030								
39				0.020								
39.2				0.010								
40.3				0.010								

Table 5 Locus wise allele number in 12 X STR markers

Allele/n	DXS10103	DXS8378	DXS7132	DXS10134	DXS10074	DXS10101	DXS10135	DXS7423	DXS10146	DXS10079	HPRTB	DXS10148
N	50	50	50	50	50	50	50	50	50	50	50	50
7					3							
8					6							
9		2			1							
10		32									2	
11		40	4								4	
12		22	6								28	
13		4	29								48	
14			37		1			48		1	10	
15			19		5			42			7	
16	34		3		13			9		1	1	
17	8		2		37			1		4		
18	17				20		9			21		14
19	35				10		3			22		4
20	4				3		5			34		3
21	2				1		11			9		7
21.1							1					
22							8			6		
22.1							1					3
23							14			2		2
23.1												2
24							11		4			3
24.1												9
25							2		2			
25.1												16
26						1	2		12			
26.1												14
27						5	9		16			
27.1												13
27.2						4						
28						2	9		22			
28.1												9
28.2						4						
29						6	7		16			1
29.1												
29.2						11						
30						5	3		16			
30.2						3						
31						8	3		11			
31.2						11						
32				4		17	2		1			
32.2						7						
33				9		11						
33.2						2						
34				19		1						
34.2						1						
35				23		1						
35.3				8								
36				10								
37				8								
37.3				6								
38				6								
38.3				3								
39				2								
39.2				1								
40.3				1								

Table 6 Locus wise forensic parameters for 12 X STR markers

Statistical Variables	DXS10135	DXS7423	DXS10146	DXS10079	HPR1B	DXS10145	DXS10103	DXS8378	DXS7132	DXS10134	DXS10074	DXS10101
FORENSIC												
Matching Probability	0.034	0.267	0.059	0.089	0.158	0.033	0.138	0.162	0.123	0.040	0.080	0.037
Expressed as 1 in ...	29.80	3.70	16.90	11.30	6.30	30.50	7.30	6.20	8.10	25.00	12.50	27.20
Power of Discrimination (PD)	0.966	0.733	0.941	0.911	0.842	0.967	0.862	0.838	0.877	0.960	0.920	0.963
PIC	0.91	0.50	0.83	0.75	0.63	0.88	0.68	0.63	0.69	0.86	0.76	0.90
PATERNITY												
Power of Exclusion (PE)	0.398	0.316	0.562	0.398	0.369	0.637	0.493	0.398	0.398	0.562	0.316	0.460
Typical Paternity Index (TPI)	1.56	1.32	2.27	1.56	1.47	2.78	1.92	1.56	1.56	2.27	1.32	1.79
ALLELE FREQUENCIES												
Homozygotes	32.0%	38.0%	22.0%	32.0%	34.0%	18.0%	26.0%	32.0%	32.0%	22.0%	38.0%	28.0%
Heterozygotes	68.0%	62.0%	78.0%	68.0%	66.0%	82.0%	74.0%	68.0%	68.0%	78.0%	62.0%	72.0%
Total Alleles	100	100	100	100	100	100	100	100	100	100	100	100
P-value	0.0000	0.9778	0.0875	0.2545	0.0853	0.5063	0.4115	0.5907	0.1572	0.3036	0.0739	0.0000
Observed Hetero.	0.6800	0.6200	0.7800	0.6800	0.6600	0.8200	0.7400	0.6800	0.6800	0.7800	0.6200	0.7200
Expected Hetero.	0.9232	0.5909	0.8548	0.7858	0.6810	0.9010	0.7319	0.6941	0.7438	0.8786	0.7960	0.9188
Most common allele	21	14	28	20	13	25.1	19	11	14	34	17	32
Least common allele	21.1	17	32	14,16	16	29.1	21	9	17	39.2,40.3	9,14,21	34,26,35

Conclusion:

This is the first report of X-STR data on Bhil population of Madhya Pradesh state of India. The created population data base allows the STR markers included in the Argus X-12 Kit to be applied in routine case work. High values of the forensic statistical parameters combined with in dependent inheritance of the studied loci make them a very useful marker set for forensic genetics investigations. Application of X-STR loci may constitute a valuable addition to autosomal markers, particularly in complex kinship and paternity cases, e.g. when instead of the alleged father, his mother (i.e. the putative grandmother of the child) is the source of the reference DNA sample.

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Quality Control:

All the authors have passed proficiency testing of the GITAD, Spain <http://gitad.ugr.es/principal.htm>.

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