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Review

X-Chromosome short tandem repeat, advantages and typing technology review

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Microsatellites of the X-chromosome have been increasingly studied in recent years as a useful tool in forensic analysis. This review describes some details of X-chromosomal short tandem repeat (STR) analysis. Among them are: microsatellites, amplification using polymerase chain reaction (PCR) of STRs, PCR product evaluation, PCR product purification, separation and detection, data analysis of STR by Identity Software, locus information and allele frequencies for X-chromosomal STR in different populations (DXS101, DXS7423, DXS8377, DXS6789, DXS6807 genetic loci) and advantages of X-chromosomal micro-satellites. In forensic casework and DNA databases, polymorphism in STR is important as a forensic genetic marker.

Key words: Short tandem repeat (STR) loci, forensic, interpretation.

INTRODUCTION

The human X chromosome (ChrX) has so far played a minor role in forensic and in population studies, but the application of ChrX markers in these fields is now accomplishing detailed attention and new doors are opening in population and forensic research (Szibor et al., 2003).

Microsatellites are DNA regions with repeat units that are 2 to 7 bp in length or most generally short tandem repeats (STRs) or simple sequence repeats (SSRs) (Ellegren et al., 2003). The classification of the DNA sequences is determined by the length of the core repeat unit and the number of adjacent repeat units. It may contain several hundred to thousands (Butler, 2012) of these. Tandem repeats occur in the form of iterations of repeat units of almost anything from a single base pair to thousands of base pairs. Mono-, di-, tri- and tetranucleotide repeats are the main types of microsatellite, but repeats of five (penta-) or six (hexa-) nucleotides are usually classified as microsatellites as well.

DNA can be used to study human evolution. Besides, information from DNA typing is important for medico-legal matters with polymorphisms leading to more biological studies (Walkinshaw et al., 1996). Since the STR markers are important for human identification purposes (Rui et al., 2009) the number of repeats can be highly variable among individuals and can be used for identification purposes. There are three types of repeat patterns for STRs. Two or more adjacent simple repeats are considered as compound repeat. Units of similar length are called

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simple repeats (Budowle, 1995; Butler et al., 2009).

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 (Drabek et al., 2004; Shin et al., 2005; Gomes et al., 2007; Hill et al., 2008; Diegoli and Coble, 2011).

AMPLIFICATION USING POLYMERASE CHAIN REACTION OF SHORT TANDEM REPEATS

A billion copies of a given target sequence can be provided by Polymerase Chain Reaction (PCR) in a fast *in vitro* DNA synthesis process. A DNA polymerase may duplicate to result in specific DNA markers to be surfaced. dNTPs, Mg++ and a thermal stable DNA polymerase, (usually Taq polymerase) are five main chemical components. During the cycling of temperatures, the primers are designed to hybridize to the specific markers (for example, STR loci) along the length of the template. A special DNA polymerase that is heat stable is used to copy and amplify the genetic markers using the remaining components after the DNA strands are separated and the primers bind to the template. This happens for a given thermal cycle (Del et al., 2009; Nadine et al., 2010). To analyze the DNA, the process of 28 to 32 heating and cooling cycles is increased. The amplification of multiple samples can be done at one time. In fact in 3 h, 96 samples can be amplified in this manner. The thermal sample contains many sample wells that allows this to happen when several different loci are simultaneously amplified in a single tube when multiple PCR occurs. This is when the typing from a single aliquot of the extracted genomic DNA reduces the sample consumption. It has been found lately that even 15 autosomal short tandem repeats(STRs) have been done at one time using DNA from a very small amount of contaminated sample.

Polymerase chain reaction product evaluation

For DNA amplification product evaluation in order to confirm whether the mtDNA PCR product is amplified, we will need to examine the products of the amplification. To do this, a process called gel electrophoresis is applied. Electric current forces the migration of DNA fragments through a special gel material in this process. Since it is negatively charged, the DNA will move to the positive electrode in the electric field. The electric forces cause the longer portions of the DNA to move slower than the shorter ones (John et al., 2006; Imad et al., 2014c, d).

Polymerase chain reaction product purification

Using specialized binding buffers, EZ-10 spin column purification kits uses a silica gel membrane that selectively absorbs up to 10 µg of DNA fragments. Washed away nucleotides, oligos (<40-mer), enzymes, mineral oil and other impurities do not bind to the membrane. The DNA fragments can then be extracted from the column in small volumes. In downstream applications it can be re-used without further processing.

Separation and detection

After PCR there must be a process of separation and detection of the amplified products. A number of ways can be used to carry out the typing. Among them are:

1. Polyacrylamide gel electrophoresis (PAGE) followed by silver staining or if the primers are fluorescently tagged, detection by fluorescent gel scanners and,
2. Capillary Electrophoresis (CE) with laser induced fluorescence. Because it is automated, this method has become popular. No gel is used and samples can be inserted mechanically.

The resolution of the higher molecular weight loci is usually better than in the PAGE methods since the DNA traverses the entire length of the capillary, the resolution. There are several other components that impact DNA separations within CE systems other than the width of the sample injection zone. Among these are the polymer used for enabling the separation, the capillary, the electrophoresis buffer, and the field strength (John et al., 2004). The object of the exercise is to introduce a different dye onto the 5'-(nonreactive) end of each primer or set of primers (Giusti and Adriano, 1993). The properties of these dyes are quite unusual. Although fluoresce in different regions of the spectra they are all excited by a single argon-ion laser tuned to 488 nm. To determine which dye is present, based on the emission of each fragment as it passes the detector window, a multi wavelength analyzer, such as a charged coupled device (CCD) camera, can then be used. The advantage of this method is it allows the analysis of fragments of DNA that overlap in size as long as they are labeled with different colors, which fluoresce at different wavelengths.

The STR fragments in the sample are amplified using primers with fluorescent tags in the most commonly used

analytical method for detecting STRs. There is fluorescent dye in every new STR fragment made in a PCR cycle. When light is shown over it, each dye will emit a different color. Using electrophoresis in automated “genetic analyzer” machinery the fragments are separated accor-ding to their length. This technology is developed as a by-product of the technology developed for the Human Genome Project that is first carried out to sequence most of the entire genome. In these machines an electric field is used to extract DNA fragments placed at one end of the tube through the entangled polymer or comparable sieving medium. This is done using a long, narrow tube (a “capillary”). The bigger or bulkier fragments move slowly in the medium as compared to the smaller ones. Sending a laser beam through the small glass window in the tube causes it to fluoresce at specific wavelengths as the tagged fragments pass under the light. A kind of electronic camera records the intensity of light emitted by the dye. This can be translated into a graph (an electropherogram), which shows a peak as an STR flashes by. Firstly a short allele will pass by the window and fluoresce first. Later a longer fragment will come by, and this will show another peak on the graph.

DATA ANALYSIS OF STR BY IDENTITY SOFTWARE

GeneScan software

Migration of ssDNA fragments ranging from 20 to 1200 base pairs is sigmoidal and not linear over the entire fragment size range, and carries out three main roles, which are: specifies peaks based on threshold values determined by the user; it differentiates the peaks according to dye color based on a matrix file and it determines the sizes the STR allele peaks based on an internal size standard. It is labeled with a different colored dye that is run for different samples. Because of the different conformations of small and large DNA fragments during migration it is difficult to acquire accurate size calls for data over 800 bps. There is a limited linear range when separating fragments ranging in size from 20-80, 80-900 and over 1000 bps because of the differences in size and shape. It is a normal practice to use different sizing algorithms for size calling. The local Southern are for large fragments and cubic spline and third order least squares are for middle fragments. These methods are all local and do not affect the results beyond the last fragment. Current sizing algorithms limit the analysis of large fragment data.

Genotyper software program

Genotyper software program converts the sized peaks into genotype calls that is (ABI PRISM Genotyper 2.5 Software User’s Manual, P/N 904648, Foster City, CA 2001) takes GeneScan data by comparison of allele sizes in an allelic ladder to the sample alleles to designate its number. In order to make the allele calls from the allelic ladders the manufacturer of a particular STR kit normally provides Genotyper macros. These macros can be made to eliminate stutter peaks (Walsh et al., 1996) that may affect sample interpretation.

GeneMapper

The GeneMapper Software includes an Autoanalysis feature that can eliminate most of the tasks leading up to the analysis of a microsatellite project. A collection of user-defined settings (including an analysis method, size standard, and panel) that determine the sizing and genotyping algorithms is used by the GeneMapper® Software to analyze all sample files in a project.

GeneMarker (®) HID software program

A software tool for the analysis of forensic STR data and as a resource for analysis of custom STR multiplexes is GeneMarker(®) HID. It is easy to be studied and applied. It has features that may curtail user fatigue. STR data from both single-source and mixture profiles were analyzed and compared to profiles interpreted with another software package, to prove its superiority. It shows a 100% concordance for a total of 1898 STR profiles representing 28,470 loci and more than 42,000 alleles. Data obtained from a custom STR multiplex, with simplified and rapid implementation was successfully analyzed using GeneMarker HID. Finally, a time scale study is used to study the impact of the user-friendly design features of the software. It is found that using these software laboratories can save the time required for data analysis by at least 25% by using this software.

LOCUS INFORMATION AND ALLELE FREQUENCIES FOR X-CHROMOSOMAL STR IN DIFFERENT POPULATION

Allele frequencies of the population samples are shown in Tables 1 to 5, respectively. Detailed data are available by the authors.

DXS101 genetic loci

DXS101 genetic loci are highly polymorphic nucleotide. It possesses over 20 different alleles ranging from 12 to 32 repeats in length with repeat sequence - (CTT)x–(ATT)y– A number of same-size, different-sequence alleles have been identified through sequence analysis. Allele frequencies for the DXS101 genetic loci of ten population samples were found in this review (Jeanett et al., 2001; Edelmanna et al., 2004; Coletti et al., 2006; Zarrabeitia et al., 2006; Martins et al., 2008; Yasutaka and Kiyoshi., 2010;

Table 1. Allele frequencies for the DXS101 genetic loci of ten population samples.

Allele	Ireland[1]	Italia[2]	German[1]	German[3]	Spain[4]	Peru[1]	Brazil[5]	Ethiopia[1]	Pakistan[6]	Japan[7]
12	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.008	0.000	0.000
15	0.052	0.053	0.044	0.044	0.000	0.017	0.016	0.008	0.000	0.000
16	0.000	0.000	0.005	0.005	0.000	0.000	0.008	0.024	0.000	0.000
17	0.000	0.019	0.002	0.002	0.011	0.000	0.008	0.008	0.000	0.000
18	0.073	0.178	0.084	0.085	0.046	0.008	0.055	0.098	0.000	0.000
19	0.052	0.046	0.047	0.047	0.032	0.017	0.055	0.024	0.000	0.000
20	0.021	0.033	0.012	0.012	0.000	0.025	0.031	0.024	0.000	0.000
21	0.062	0.026	0.032	0.032	0.032	0.000	0.055	0.049	0.022	0.008
22	0.010	0.033	0.022	0.022	0.096	0.008	0.023	0.073	0.033	0.041
23	0.083	0.046	0.066	0.066	0.222	0.068	0.070	0.065	0.280	0.131
24	0.115	0.177	0.213	0.212	0.170	0.305	0.289	0.122	0.264	0.305
25	0.208	0.132	0.156	0.156	0.149	0.203	0.133	0.114	0.203	0.198
26	0.146	0.092	0.114	0.114	0.117	0.220	0.156	0.171	0.071	0.179
27	0.062	9.098	0.079	0.079	0.053	0.085	0.047	0.089	0.066	0.087
28	0.062	0.033	0.070	0.070	0.021	0.025	0.039	0.081	0.044	0.033
29	0.042	0.026	0.027	0.027	0.000	0.008	0.016	0.032	0.016	0.014
30	0.010	0.007	0.024	0.024	0.000	0.008	0.000	0.008	0.000	0.003
31	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
32	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000

[1] (Edelmanna et al., 2004); [2] (Coletti et al., 2006); [3] (Jeanett et al., 2001); [4] (Zarrabeitia et al., 2006); [5] (Martins et al., 2008); [6] (Muhammad et al., 2012); [7] (Yasutaka and Kiyoshi., 2010).

Table 2. Allele frequencies for the DXS7424genetic loci of seven population samples.

Allele	Ireland[1]	German[2]	German[1]	Peru[1]	Brazil[3]	Ethiopia[1]	Japan[4]
8	0.000	0.001	0.000	0.000	0.000	0.000	0.000
10	0.000	0.004	0.003	0.017	0.008	0.024	0.000
11	0.010	0.006	0.004	0.000	0.000	0.032	0.000
12	0.051	0.039	0.035	0.008	0.023	0.041	0.025
13	0.031	0.072	0.069	0.042	0.094	0.024	0.029
14	0.143	0.219	0.220	0.229	0.164	0.268	0.168
15	0.388	0.303	0.291	0.297	0.281	0.252	0.288
16	0.255	0.224	0.240	0.297	0.313	0.293	0.445
17	0.082	0.098	0.104	0.085	0.102	0.049	0.037
18	0.041	0.020	0.021	0.017	0.008	0.016	0.011
19	0.000	0.013	0.011	0.008	0.000	0.000	0.000
20	0.000	0.001	0.000	0.000	0.008	0.000	0.000

[1] (Edelmanna et al., 2004); [2] (Jeanett et al., 2001); [3] (Martins et al., 2008); [4] (Yasutaka and Kiyoshi, 2010).

Muhammad et al., 2012) (Table 1).

DXS7423 genetic loci

DXS7423 possesses over 12 different alleles ranging from 8 to 20 repeats in length with repeat sequence –(TCCA) x–. Allele frequencies for the DXS7423 genetic loci of seven population samples were found in this review (Jeanett et al., 2001; Edelmanna et al., 2004; Martins et al., 2008; Yasutaka and Kiyoshi, 2010) (Table 2).

DXS8377 genetic loci

A DXS8377 genetic locus is a highly polymorphic nucleotide. It possesses over 24 different alleles ranging from 37 to 60 repeats in length with repeat sequence

Table 3. Allele frequencies for the DXS8377 genetic loci of eight population samples.

Allele	Ireland[1]	Italia[2]	German[3]	German[1]	Spain[4]	Peru[1]	Ethiopia[1]	Japan[5]
37	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000
38	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000
39	0.000	0.000	**0.007**	0.024	0.000	0.000	0.000	0.000
40	0.000	0.007	0.010	0.031	0.011	0.000	0.000	0.000
41	0.033	0.019	0.024	0.044	0.021	0.000	0.008	0.001
42	0.022	0.019	0.031	0.043	0.043	0.000	0.016	0.009
43	0.088	0.053	0.044	0.050	0.053	0.097	0.032	0.031
44	0.055	0.086	0.043	0.069	0.064	0.193	0.081	0.043
45	0.022	0.039	0.050	0.092	0.000	0.150	0.057	0.058
46	0.055	0.079	0.069	0.110	0.096	0.000	0.081	0.105
47	0.176	0.106	0.092	0.113	0.074	0.161	0.073	0.135
48	0.143	0.118	0.110	0.127	0.096	0.086	0.106	0.108
49	0.132	0.118	0.110	0.082	0.096	0.140	0.163	0.158
50	0.077	0.099	0.127	0.063	0.096	0.032	0.073	0.103
51	0.044	0.099	0.082	0.060	0.085	0.054	0.073	0.085
52	0.033	0.046	0.063	0.040	0.064	0.032	0.106	0.55
53	0.022	0.053	0.060	0.014	0.096	0.021	0.073	0.043
54	0.055	0.013	0.040	0.014	0.032	0.021	0.032	0.024
55	0.022	0.026	0.014	0.005	0.021	0.000	0.008	0.240
56	0.022	0.019	0.014	0.001	0.021	0.000	0.000	0.010
57	0.000	0.000	0.005	0.001	0.032	0.011	0.008	0.004
58	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.003
59	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
60	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000

[1] (Edelmanna et al., 2004); [2] (Coletti et al., 2006); [3] (Jeanett et al., 2001); [4] (Zarrabeitia et al., 2006); [5] (Yasutaka and Kiyoshi., 2010).

(GAA)x–(GAG–GAA)y–(GAA)2–GAG–(GAA)6–GAC. A number of same-size, different-sequence alleles have been identified through sequence analysis. Allele frequencies for the DXS8377 genetic loci of eight population samples were found in this review (Jeanett et al., 2001; Edelmanna et al., 2004; Coletti et al., 2006; Zarrabeitia et al., 2006; Yasutaka and Kiyoshi, 2010) (Table 3).

DXS6789 genetic loci

DXS6789 possesses over 13 different alleles ranging from 13 to 25 repeats in length with repeat sequence –TATC–(TATG)x–(TATC)y. Allele frequencies for the DXS6789 genetic loci of nine population samples were found in this review (Jeanett et al., 2001; Edelmanna et al., 2004; Lv,et al., 2004; Coletti et al., 2006; Yasutaka and Kiyoshi, 2010; Muhammad et al., 2012) (Table 4).

DXS6807 genetic loci

DXS6807 possesses over 8 different alleles ranging from 10 to 17 repeats in length with repeat sequence -(GATA)–N7–(GATA)2–GAC–(GATA)x–TGT–.Allele frequencies for the DXS6789 genetic loci of seven population samples were found in this review (Jeanett et al., 2001; Edelmanna et al., 2004; Coletti et al., 2006; Yasutaka and Kiyoshi, 2010) (Table 5).

ADVANTGES OF X-CHROMOSOMAL MICROSETTELITE

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). 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 disequi-librium between the ChrX markers used have to be taken into consideration (Ellegren, 2000).

Table 4. Allele frequencies for the DXS6789 genetic loci of nine population samples.

Allele	Ireland[1]	Italia[2]	German[3]	German[1]	Peru[1]	Ethiopia[1]	Pakistan[4]	China[5]	Japan[6]
13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
14	0.000	0.000	0.002	0.000	0.000	0.000	0.022	0.006	0.008
15	0.070	0.059	0.036	0.036	0.025	0.163	0.192	0.190	0.159
16	0.010	0.013	0.014	0.014	0.161	0.041	0.077	0.308	0.313
17	0.010	0.000	0.001	0.001	0.000	0.008	0.027	0.045	0.017
18	0.000	0.000	0.002	0.002	0.017	0.016	0.000	0.002	0.002
19	0.000	0.046	0.030	0.029	0.051	0.016	0.022	0.033	0.024
20	0.310	0.336	0.374	0.379	0.483	0.317	0.385	0.198	0.174
21	0.340	0.289	0.286	0.286	0.136	0.211	0.170	0.165	0.179
22	0.190	0.145	0.174	0.173	0.085	0.146	0.055	0.045	0.085
23	0.070	0.086	0.064	0.063	0.025	0.065	0.044	0.004	0.036
24	0.000	0.026	0.016	0.060	0.017	0.016	0.005	0.000	0.003
25	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000

[1] (Edelmanna et al., 2004); [2] (Coletti et al., 2006); [3] (Jeanett et al., 2001); [4] (Muhammad et al., 2012); [5] (Lv,et al., 2004); [6] (Yasutaka and Kiyoshi., 2010).

Table 5. Allele frequencies for the DXS6807 genetic loci of seven population samples.

Allele	Ireland[1]	Italia[2]	German[3]	German[1]	Peru[1]	Ethiopia[1]	Japan[4]
10	0.000	0.000	0.000	0.000	0.000	0.016	0.000
11	0.571	0.553	0.492	0.492	0.364	0.658	0.370
12	0.010	0.033	0.021	0.021	0.034	0.041	0.008
13	0.010	0.006	0.011	0.110	0.220	0.049	0.032
14	0.265	0.237	0.235	0.235	0.254	0.146	0.367
15	0.133	0.145	0.202	0.202	0.127	0.041	0.193
16	0.010	0.019	0.029	0.029	0.000	0.032	0.029
17	0.000	0.007	0.010	0.010	0.000	0.016	0.002

[1] (Edelmanna et al., 2004); [2] (Coletti et al., 2006); [3] (Jeanett et al., 2001); [4] (Yasutaka and Kiyoshi., 2010).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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