Author's personal copy

Int J Legal Med
DOI 10.1007/s00414-014-1009-x

SHORT COMMUNICATION

Development and characterization of a new 12-plex ChrX miniSTR system

Muhammad Israr · Ahmad Ali Shahid · Ziaur Rahman · Mian Sahib Zar · Muhammad Saqib Shahzad · Tayyab Husnain · Celine Pfeifer · Peter Wiegand

Received: 19 December 2013 / Accepted: 6 March 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Short tandem repeat (STR) markers are extensively used for human identification as well as paternity and forensic casework. X-chromosome STR (X-STR) markers are a powerful complementary system especially in deficiency paternity testing. This study presents the development and characterization of a new X-chromosomal short tandem repeat (STR) multiplex using short amplicon (<200 bp). A total of 366 samples from Punjabi population and 346 samples from Sindhi population were typed for 11 X-chromosomal STR markers: DXS101, DXS6789, DXS6793, DXS7132, DXS7423, DXS7424, DXS8378, DXS9902, GATA31E08, GATA172D05, and HPRTB along with sex-typing locus, amelogenin. Each marker showed a high degree of polymorphism, and the multiplex was sensitive down to 250 pg of human DNA. A total of 78 alleles were found with 5–11 alleles for each marker. The population data can be used as reference database for Sindhi and Punjabi populations.

Electronic supplementary material The online version of this article (doi:10.1007/s00414-014-1009-x) contains supplementary material, which is available to authorized users.

M. Israr (✉) · A. A. Shahid (✉) · Z. Rahman · M. S. Zar · T. Husnain
National Centre of Excellence in Molecular Biology, University of the Punjab, 87-West Canal Bank Road, Lahore -53700, Pakistan
e-mail: israr@cemb.edu.pk
e-mail: ahmadali.shahid@gmail.com

M. Israr
Department of Forensic Sciences, University of Health Sciences, Lahore, Pakistan
e-mail: israr@uhs.edu.pk

M. S. Shahzad
cInstitute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan

C. Pfeifer · P. Wiegand
Abteilung für Rechtsmedizin, Universitätsklinikum Ulm, Prittwitzstraße 6, 9075 Ulm, Germany

Keywords X-Chromosome short tandem repeats (X-STRs) · MiniSTRs · Punjabi population · Sindhi population · Multiplex PCR

Introduction

To alleviate the problems associated with analyzing DNA from degraded samples, a new set of short tandem repeat (STR) primers known as Miniplexes was designed by moving the primers closer to the repeat region leaving the extra sequences out [1, 2]. Using shorter amplicons in polymerase chain reaction (PCR), an improvement has been reported in obtaining results from a forensic evidence or a mass disaster site having degraded specimens [2]. Slightly degraded samples can be typed by traditional STRs, but may yield negative results as the fragmentation increases [3–5].

STRs of the X-chromosome (X-STRs) are routinely used in parentage analysis and kinship investigations such as avuncular and first cousin relationships nowadays. In addition to X-STRs, stable haplotypes of closely associated X-chromosome markers have proven to be a powerful tool in kinship analysis [6] especially for cases when father/daughter relationships are to be tested. It has been reported that a set of 12 X-STRs provides more information than a set of 38 highly informative biallelic autosomal markers for cases involving father–daughter duos [7]. X-STRs have also advantages over autosomal STRs for paternity cases involving close blood relatives as alternative putative fathers and in deficiency paternity cases, i.e., when the DNA sample from putative father is not available and DNA from paternal relative has to be analyzed instead [8]. Further, X-linked STRs can be used to solve siblingship status, without using father's DNA, of two females having the same biological father [9, 10]. X-STRs can determine

Published online: 01 May 2014

Author's personal copy

the relationship of paternal grandmother/granddaughter as the granddaughter theoretically has to carry at least one allele in common with the grandmother [11]. This study reports the successful optimization and testing of a multiplex system, a miniplex, which is capable of parallel amplification of 11 ChrX miniSTRs (DXS101, DXS6789, DXS6793, DXS7132, DXS7423, DXS7424, DXS8378, DXS9902, GATA172D05, GATA31E08, and HPRTB) along with the sex-typing locus, amelogenin.

Materials and methods

Population information

Punjab (*Persian*; five waters) is the largest province of Pakistan by population while Sindh (*Urdu*; River Indus) is the second largest province. According to Population Census Organization of Pakistan (www.census.gov.pk), the Punjab has the largest population with 56 % while Sindh province has 23 % of the country's total population. Both provinces were part of the Indus Valley Civilization, more than 4,000 years old. Throughout history, this region has served as a gateway to the Indian sub-continent, and it came under the rule of Persians, Indians, Arabs, Afghans, Mughals, and British. At the time of Pakistan's independence, Sindh was made a province while the Punjab was divided into Eastern and Western Punjab which joined India and Pakistan, respectively.

DNA extraction, amplification, and typing

Published primer sequences for these STRs [12–17] were analyzed using BLAT and in silico PCR by UCSC Genome Browser [18]. The selected primers were then screened by AutoDimer for complementarities between different primers in a multiplex [19]. As shown in Supplementary Table 1, forward primers of all pairs were labeled at the 5′ end with any of the four fluorescent dyes i.e., 6FAM, VIC, NED, or PET (Applied Biosystems (ABI), Foster City, CA). Schematic diagram of amplicon sizes and dye labeling is shown in Supplementary Fig. 1.

We collected 366 samples (116 females and 250 males) from Punjab and 346 samples (146 females and 200 males) from Sindh after their informed consent was obtained (Supplementary Fig. 2). Phenol–chloroform DNA extraction was performed on each of the blood sample as reported in the literature [20]. DNA concentration was estimated through spectrophotometry using NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE).

PCR was carried out in 10-μL reaction consisting of one unit of AmpliTaq Gold polymerase (ABI), 200 μM of each dNTP, 2 mM $MgCl_2$, 1X (NH_4SO_2) PCR buffer (ABI), and 1 ng of genomic DNA, distilled water, and appropriate amount of labeled primers. The concentrations of primers were adjusted empirically for peak balancing.

The microsatellite markers (STRs) were amplified by polymerase chain reaction (PCR) on ABI GeneAmp® PCR System 2700. The PCR cycling conditions for 12-plex were the following: initial incubation at 95 °C for 11 min, amplification with 33 cycles of 94 °C for 1 min, 59 °C for 1 min, and 72 °C for 1 min, extension at 66 °C for 60 min, and final soak at 4 °C. The capillary electrophoresis was performed using 1 μL of PCR product, 13.5 μL Hi-Di™ Formamide (ABI), and 0.5 μL GeneScan® 500 LIZ™ Size Standard (ABI). The amplified products were separated on an ABI 3130 Genetic Analyzer (ABI). Results were analyzed using GeneMapper Software v3.7 (ABI).

Degraded DNA analysis

Studies were performed on artificially degraded DNA. As previously reported [13], miniSTRs are effective profiling degraded samples which are otherwise difficult to genotype correctly through conventional STR kits. DNA was degraded enzymatically as well as mechanically [21] (Supplementary Fig. 3).

Allele frequencies for each locus were calculated for both males and females collectively using a spreadsheet program. For Hardy–Weinberg exact test and linkage disequilibrium, Arlequin v3.5 was used [22]. PowerMarker v3.1 [23] was used for exact test of population differentiation, gene diversity, polymorphic information content (PIC), and heterozygosity. The ChrX STR website was used for the calculation of paternity index, power of exclusion, power of discrimination in males (PDm) and females (PDf), and mean exclusion chance (MEC) in trios involving a daughter and in father/daughter duos [24].

Quality control

Alleles were assigned as recommended by ISFG (International Society of Forensic Genetics) through comparison with standard DNA 9947A. Allele nomenclature and allele ranges were according to already reported literature [24]. This paper strictly follows the guidelines for publication of population data requested by the journal.

Results and discussion

The markers were selected on the basis of factors such as spacing between allele ranges of different markers in the same dye channel, primer compatibility, and successful amplification in the multiplex environment, small amplicon size, and distribution among different linkage groups. Supplementary Fig. 4 shows electropherogram results for this multiplex using a male human DNA.

Author's personal copy

DNA was extracted from gel in the range of 200–300 bp and also fragments under 200 bp. This DNA was then amplified with the multiplex. Full profile was observed in the fragments of 300 bp while there were partial profiles in the less than 200 bp DNA. The smaller size amplicons were observed but the larger amplicons (>150 bp) dropped out with this highly degraded DNA [21] (Supplementary Fig. 5).

Studies were preformed to evaluate minimum quantity required to obtain the full DNA profile. The GeneScan Analysis threshold for these studies was set at 100 RFU (relative fluorescence units) [25]. The optimal quantity of the template DNA for 12-plex PCR system ranged from 1 to 2 ng. Each amplification at 1–2 ng DNA produced full 11-locus profile with a >100 RFU threshold. Full profile was obtained with DNA concentration as low as 250 pg (Supplementary Fig. 6).

Population genetics and forensic efficiency parameters

Overall, 78 different alleles were found for all the 11 loci in the two populations studied. Comparison of allele frequencies between the two populations at all loci for all markers is given in Supplementary Fig. 7. Maximum of 11 alleles were found for locus DXS6789 while minimum of 5 alleles were found for locus DXS9902 (Supplementary Fig. 8). Alleles found for each locus along with the range of amplicon length observed in the populations studied were used to construct an allelic ladder (Supplementary Table 2). The exact allele sizes were determined by comparing PCR products with standard DNA 9947A.

The allele distribution across the populations was evaluated to determine the presence of population specific pattern to aid the forensic casework as recommended in previous literature [26]. Supplementary Tables 3 and 4 show the allelic frequencies and ranges in Punjabi and Sindhi populations for all the STR loci studied.

Forensic efficiency parameters were calculated for both the Punjabi and Sindhi populations (Supplementary Tables 5 and 6). Polymorphic information content (PIC) ranged from 0.4869 at locus DXS6793 to 0.8116 at locus DXS101 in Punjabi population while it ranged from 0.5482 at locus DXS6793 to 0.7956 at locus DXS7424 in Sindhi population. The combined power of discrimination in females is 4.69×10^{-10} while in male it is 2.27×10^{-6}. In Punjabi population, it is 1.28×10^{-11} in females and 2.64×10^{-7} males. After Bonferroni's correction, the Sindhi population deviates from Hardy–Weinberg equilibrium only at DXS7424. This deviation observed could potentially be due to population sampling effects.

There is only one other study available for five ChrX STR markers in Punjabi population [27], but none of our markers are the same as those reported in the study, and hence, it could not be used for comparison. However, there is another study which reported 13 ChrX STR loci in Pakistani population [17] of which 9 markers are the same as used here.

The exact test for LD was performed in Punjabi and Sindhi populations for all pairs of markers in this study. No evidence of LD was detected in all pair of markers in this study; hence, these are to be considered as independent and are recommended for forensic practice in Punjabi and Sindhi populations. The statistical parameters showed that these markers are appropriate for individual identification, paternity testing involving a female child. In the Punjabi population, DXS6793 showed association with DXS8378 and DXS7423 while DXS7423 showed association with DXS8378. Also, DXS9902 showed association with HPRTB, but after Bonferroni's correction, the LD values were not significant.

Association between pair of markers DXS7424 and DXS101 in either Punjabi or Sindhi population that we studied could not be observed which is consistent with Korean population [28], but significant LD was reported in Germans for same pair of markers [29]. However, a previous study on generic Pakistani population did not report any such association [30]. The association between DXS8378 and DXS7423 has been reported in Waorani population of Ecuador which is an extremely small and highly isolated population of about 3,000 individuals [31].

Conclusion

The specified X marker miniplex concept which was optimized based on primer selection and amplicon length reduction is highly recommended to be used along with the current battery of forensic markers especially on compromised samples as this system shows a high specificity and needs low DNA to give high-quality DNA profiles. Owing to reasonable combined power of discrimination of markers (female=1.28×10^{-11}, male=2.64×10^{-7}) and number of markers in the multiplex (11 plus amelogenin), this multiplex may prove to be a worthwhile tool for deficiency paternity testing. Using X-STRs may give a benefit to obtain more information in contrast to autosomal STRs. In the future, concordance between these miniSTRs and conventional STRs present in commercial kits, like Argus X12, may be studied.

Acknowledgments The authors would like to acknowledge all individuals who participated in this study. The authors would like to thank Dr. Shaheen N. Khan, Obaid Ullah, and M. Akram Tariq for their support in this study. This work was partially supported by Higher Education Commission (HEC), Islamabad, Pakistan.

References

1. Butler JM, Shen Y, McCord BR (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. J Forensic Sci 48:1054–1064

Author's personal copy

2. Coble MD, Butler JM (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. J Forensic Sci 50:43–53
3. Grubwieser P, Muhlmann R, Berger B, Niederstatter H, Pavlic M, Parson W (2006) A new "miniSTR-multiplex" displaying reduced amplicon lengths for the analysis of degraded DNA. Int J Legal Med 120:115–120
4. Holland MM, Cave CA, Holland CA, Bille TW (2003) Development of a quality, high throughput DNA analysis procedure for skeletal samples to assist with the identification of victims from the World Trade Center attacks. Croat Med J 44:264–272
5. Parsons TJ, Huel R, Davoren J, Katzmarzyk C, Milos A, Selmanovic A, Smajlovic L, Coble MD, Rizvic A (2007) Application of novel "mini-amplicon" STR multiplexes to high volume casework on degraded skeletal remains. Forensic Sci Int Genet 1:175–179
6. Szibor R, Hering S, Kuhlisch E, Plate I, Demberger S, Krawczak M, Edelmann J (2005) Haplotyping of STR cluster DXS6801–DXS6809–DXS6789 on Xq21 provides a powerful tool for kinship testing. Int J Legal Med 119:363–369
7. Gomes C, Magalhaes M, Alves C, Amorim A, Pinto N, Gusmao L (2012) Comparative evaluation of alternative batteries of genetic markers to complement autosomal STRs in kinship investigations: autosomal indels vs. X-chromosome STRs. Int J Legal Med 1–5
8. Barbaro A, Cormaci P (2006) X-STR typing for an identification casework. Int Cong Ser 1288:513–515
9. Toni C, Presciuttini S, Spinetti I, Domenici R (2003) Population data of four X-chromosome markers in Tuscany, and their use in a deficiency paternity case. Forensic Sci Int 137:215
10. Toni C, Presciuttini S, Spinetti I, Rocchi A, Domenici R (2006) Usefulness of X-chromosome markers in resolving relationships: report of a court case involving presumed half sisters. Int Cong Ser 1288:301–303
11. Edelmann J, Lessig R, Klintschar M, Szibor R (2004) Advantages of X-chromosomal microsatellites in deficiency paternity testing: presentation of cases. Int Cong Ser 1261:257–259
12. Haas-Rochholz H, Weiler G (1997) Additional primer sets for an amelogenin gene PCR-based DNA-sex test. Int J Legal Med 110: 312–315
13. Asamura H, Sakai H, Kobayashi K, Ota M, Fukushima H (2006) MiniX-STR multiplex system population study in Japan and application to degraded DNA analysis. Int J Legal Med 120:174–181
14. Israr M, Shahid AA, Rahman Z, Shahzad MS, Ullah O, Husnain T (2012) Punjabi population data for seven X-chromosome short tandem repeat (X-STR) loci using a new miniplex system. Afr J Biotechnol 11:10513–10516
15. Edelmann J, Hering S, Michael M, Lessig R, Deischel D, Meier-Sundhausen G, Roewer L, Plate I, Szibor R (2001) 16 X-chromosome STR loci frequency data from a German population. Forensic Sci Int 124:215–218
16. Edelmann J, Deichsel D, Hering S, Plate I, Szibor R (2002) Sequence variation and allele nomenclature for the X-linked STRs DXS9895, DXS8378, DXS7132, DXS6800, DXS7133, GATA172D05, DXS7423 and DXS8377. Forensic Sci Int 129:99–103
17. Tariq M, Ullah O, Riazuddin SA, Riazuddin S (2008) Allele frequency distribution of 13 X-chromosomal STR loci in Pakistani population. Int J Legal Med 122:525–528
18. Kent WJ (2002) BLAT—the BLAST-like alignment tool. Genome Res 12:656–664
19. Vallone PM, Butler JM (2004) AutoDimer: a screening tool for primer-dimer and hairpin structures. Biotechniques 37:226–231
20. Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning : a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
21. Bender K, Farfán MJ, Schneider PM (2004) Preparation of degraded human DNA under controlled conditions. Forensic Sci Int 139:135–140
22. Poetsch M, Sabule A, Petersmann H, Volksone V, Lignitz E (2006) Population data of 10 X-chromosomal loci in Latvia. Forensic Sci Int 157:206–209
23. Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21: 2128–2129
24. Szibor R, Hering S, Edelmann J (2006) A new Web site compiling forensic chromosome X research is now online. Int J Legal Med 120: 252–254
25. Hanson EK, Ballantyne J (2004) A highly discriminating 21 locus Y-STR "megaplex" system designed to augment the minimal haplotype loci for forensic casework. J Forensic Sci 49:40
26. Kashyap V, Guha S, Sitalaximi T, Bindu GH, Hasnain S, Trivedi R (2006) Genetic structure of Indian populations based on fifteen autosomal microsatellite loci. BMC Genet 7:28
27. Nadeem A, Babar ME, Hussain M, Tahir MA (2009) Development of pentaplex PCR and genetic analysis of X chromosomal STRs in Punjabi population of Pakistan. Mol Bio Rep 36:1671–1675
28. Lee HY, Park MJ, Jeong CK, Lee SY, Yoo JE, Chung U, Choi JH, Kim CY, Shin KJ (2004) Genetic characteristics and population study of 4 X-chromosomal STRs in Koreans: evidence for a null allele at DXS9898. Int J Legal Med 118:355–360
29. Edelmann J, Hering S, Kuhlisch E, Szibor R (2002) Validation of the STR DXS7424 and the linkage situation on the X-chromosome. Forensic Sci Int 125:217–222
30. Tariq MA, Sabir MF, Riazuddin SA, Riazuddin S (2009) Haplotype analysis of two X-chromosome STR clusters in the Pakistani population. Int J Legal Med 123:85–87
31. Martins JA, Costa JC, Paneto GG, Gusmão L, Sánchez-Diz P, Carracedo A, Cicarelli RMB (2009) Genetic data of 10 X-chromosomal loci in Vitória population (Espírito Santo State, Brazil). Forensic Sci Int Genet Supp 2:394–395