

Genetic polymorphisms and forensic efficiency of 19 X chromosomal STR loci for Xinjiang Mongolian population

Ling Chen¹, Yuxin Guo^{2,3}, Cheng Xiao¹, Weibin Wu¹, Qiong Lan¹, Yating Fang¹, Jiangang Chen⁴, Bofeng Zhu^{Corresp. 1, 2, 3}

¹ Southern Medical University, Department of Forensic Biology, School of Forensic Medicine, Guangzhou, Guangdong, China

² Xi'an Jiaotong University, Key Laboratory of Shaanxi Province for Craniofacial Precision Medicine Research, College of Stomatology, Xi'an, Shaanxi, China

³ Xi'an Jiaotong University, Clinical Research Center of Shaanxi Province for Dental and Maxillofacial Diseases, College of Stomatology, Xi'an, Shaanxi, China

⁴ Ministry of Public Security, Institute of Forensic Science, Beijing, China

Corresponding Author: Bofeng Zhu

Email address: zhubofeng@mail.xjtu.edu.cn

Aims: X-chromosomal short tandem repeat (X-STR) loci are playing an increasingly important role in some complex kinship cases in recent years. To investigate the forensic efficiency of X chromosomal STRs of Mongolian minority group from Xinjiang Uygur Autonomous Region, China, and further depict the genetic relationship among Xinjiang Mongolians and other populations, 267 blood samples from unrelated healthy Xinjiang Mongolians were amplified by AGCU X-19 STR kit.

Results: No deviations for all 19 X-STR loci were observed from the HWE after Bonferroni correction ($P > 0.0026$) in female samples. The most frequent allele was allele 10 at locus DXS10164 with the frequency 0.5663. The PIC values of the 19 X-STR loci were more than 0.5 with the highest polymorphism at the locus DXS10135. The cumulative power of discrimination were 0.9999999999999999999988761005481 in females and 0.999999999999903 in males, respectively; and the cumulative mean exclusion chances were 0.9999999969738068321121 in duos and 0.99999999998952 in trios, respectively. The 7 linkage groups were extremely informative, with all the haplotype diversities greater than 0.9487. No linkage disequilibrium was observed for a significance level of 0.00029 ($p = 0.05/171$) after Bonferroni correction. The MDS plot, phylogenetic tree based on the 11 overlapping X-STR loci all presented that the Xinjiang Mongolian population was genetically different from other Asian populations, including the Mongolians from Inner Mongolia Autonomous Region, China

Conclusion: This study indicated that the 19 X-STR multiplex PCR system was of high utility value for both forensic practices and population genetic researches in Xinjiang Mongolian group.

Title:

Genetic polymorphisms and forensic efficiency of 19 X chromosomal STR loci for Xinjiang Mongolian

Population

Running Title:

Analysis of 19 X-STR in Mongolian

Ling Chen^{1,#}, Yuxin Guo^{2,3,#}, Cheng Xiao¹, Weibin Wu¹, Qiong Lan¹, Yating Fang¹, Jiangang Chen⁴, Bofeng Zhu^{1,2,3,*}

¹Department of Forensic Biology, School of Forensic Medicine, Southern Medical University, Guangzhou, Guangdong 510515, P. R. China

²Key Laboratory of Shaanxi Province for Craniofacial Precision Medicine Research, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi 710004, P. R. China

³Clinical Research Center of Shaanxi Province for Dental and Maxillofacial Diseases, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi 710004, P. R. China

⁴Institute of Forensic Science, Ministry of Public Security, Beijing, 100038, P. R. China

[#]These authors contributed equally to this work.

16

17 **Abstract**

18 **Aims:** X-chromosomal short tandem repeat (X-STR) loci are playing an increasingly important role in some complex kinship cases in
19 recent years. To investigate the forensic efficiency of X chromosomal STRs of Mongolian minority group from Xinjiang Uygur
20 Autonomous Region, China, and further depict the genetic relationship among Xinjiang Mongolians and other populations, 267 blood
21 samples from unrelated healthy Xinjiang Mongolians were amplified by AGCU X-19 STR kit.

22 **Results:** No deviations for all 19 X-STR loci were observed from the HWE after Bonferroni correction ($P>0.0026$) in female samples.
23 The most frequent allele was allele 10 at locus DXS10164 with the frequency 0.5663. The PIC values of the 19 X-STR loci were more
24 than 0.5 with the highest polymorphism at the locus DXS10135. The cumulative power of discrimination were
25 0.9999999999999999999988761005481 in females and 0.999999999999903 in males, respectively; and the cumulative mean
26 exclusion chances were 0.9999999969738068321121 in duos and 0.99999999998952 in trios, respectively. The 7 linkage groups were
27 extremely informative, with all the haplotype diversities greater than 0.9487. No linkage disequilibrium was observed for a significance
28 level of 0.00029 ($p=0.05/171$) after Bonferroni correction. The MDS plot, phylogenetic tree based on the 11 overlapping X-STR loci all
29 presented that the Xinjiang Mongolian population was genetically different from other Asian populations, including the Mongolians
30 from Inner Mongolia Autonomous Region, China.

Conclusion: This study indicated that the 19 X-STR multiplex PCR system was of high utility value for both forensic practices and population genetic researches in Xinjiang Mongolian group.

Introduction

As one of the most famous nomadic nations in the world, the Mongols mainly live in East-Central Asian. With a population of 5.8 million, Mongolian was the eighth largest ethnic minority on Chinese population data according to the 6th China population census in 2010 (<http://www.stats.gov.cn/tjsj/pcsj/rkpc/6rp/indexch.htm>). Their indigenous dialects are known as the Mongolian language, which belongs to Altaic language family. The traditional Mongolian script was created in the early 13th century based on the script of Huihu (Janhunnen 2011). Based on Chinese historical texts, the ancestry of Mongolians can be traced back to Donghu, a nomadic confederation of tribes with same ethnic origin, different dialects and names. After the Donghu were defeated by Xiongnu, the Xianbei and Wuhuan survived as the main remnants of the confederation. As recorded by the Chinese histories, Xianbei split into three prominent groups: the Rouran, the Khitan and the Shiwei. A subtribe of Shiwei, called "Shiwei Menggu", was held to be the origin of Mogolians. In the thirteenth century, Genghis Khan united a large group of Mongolic-speaking tribes and founded the Mongol Empire. With the expansion of the Mongol Empire, the Mongolians settled over almost all Eurasia and carried on military campaigns from the Adriatic Sea to Indonesian Java island and from Japan to Palestine. In the late 14th century, Mongolia was divided into two parts: Western Mongolia

46 (Oirats) and Eastern Mongolia (Khalkha, Inner Mongols, Barga, Buryats) after the fall of the Mongol Empire. In the modern period, the
47 most prominent Mongol groups are the Inner Mongols concentrated in Inner Mongolia Autonomous Region and Northeast China, the
48 Oirats concentrated in Xinjiang Uyghur Autonomous Region of China, and the Khalkha, known as Outer Mongols, concentrated in State
49 of Mongolia, and approximately sixty percent Mongolians in the world settled in China.

50 In recent years, autosomal short tandem repeats (STRs) have been extensively applied in paternity testing and individual identification.
51 However, autosomal STRs may not be effective in some deficiency paternity cases, such as tests of half-sister without the father's DNA,
52 grandmother-granddaughter without the parent's DNA, paternal aunt-niece or maternal aunt/maternal uncle-nephew without the parent's
53 and grandparent's DNA. X-STRs have special characteristics that make them particularly useful in the forensic cases described above
54 (Chen et al. 2014; Szibor 2007; Szibor et al. 2003b). Therefore, X-STR loci have drawn more and more attention in forensic sciences
55 and been reported its special role for forensic purpose (Liu et al. 2013; Sun et al. 2013). At present, X chromosomal genetic information
56 of Mongolians had been reported by Hou et al. using 9 X-STR Loci, Zhang et al. using 34 X-markers (18-STRs and 16-Indels), and Tao
57 et al. using 12 X-STR loci (Hou et al. 2007; Tao et al. 2018; Zhang et al. 2015). However, these researches only involved in Inner
58 Mongolians, and few studies had aimed at Western Mongols. AGCU-X19 STR kit was a recently developed multiplex amplification
59 system, which can amplify 19 X-chromosomal STR loci simultaneously, including DXS8378, DXS7423, DXS10148, DXS10159,
60 DXS6809, DXS7424, DXS10164, DXS10162, DXS7132, DXS10079, DXS6789, DXS101, DXS10103, DXS10101, HPRTB,

DXS10075, DXS10074, DXS10135 and DXS10134. And Yang et al had indicated it could be used as a supplementary tool in kinship tests in China (Yang et al. 2016). Further, genetic polymorphisms of this X-STR system have been investigated in a number of populations (Liu et al. 2017; Meng et al. 2017; Yang et al. 2017; Zhang et al. 2016). In the present study, we used this 19 X-STR multiplex PCR system to obtain genetic information from Western Mongols, including the allele frequencies and forensic parameters of these 19 X-STR loci, the haplotypic diversities of 7 X-STR linkage groups. And we also estimated the population differentiations between the Western Mongols and other previously reported groups.

Materials and Methods

Population samples

The blood samples were collected from 267 unrelated healthy Xinjiang Mongolians (156 males and 111 females) living in Bortala Mongol Autonomous Prefecture, Xinjiang Uyghur Autonomous Region, China. Genomic DNA was extracted using the Chelex-100 method (Walsh et al. 1991).

Compliance with ethics guidelines

This study was carried according to the approval for research involving human and animals by the ethical committee of Xi'an Jiaotong

76 University Health Science Center, China. Informed consents were signed by all participants prior to sample collection.

77

78 **PCR amplification and STR typing**

79 DNA samples were amplified in a 25µl PCR reaction volume using the AGCU-X19 STR kit (AGCU ScienTech Incorporation, Wuxi,
80 Jiangsu, China), following the manufacturer's instructions. The PCR products were separated through capillary electrophoresis with an
81 ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific, MA, USA) and analyzed with GeneMapper ID-X (Thermo Fisher Scientific,
82 MA, USA).

83

84 **Quality control**

85 This study was carried out strictly following the ISFG recommendations on the analysis of DNA polymorphisms (Schneider 2007) and
86 guidelines for publication of population data (Carracedo et al. 2013; Carracedo et al. 2014). The experiments were conducted under the
87 laboratory internal control standards. Negative control (deionized water for amplified reaction) and positive control (the 9947A DNA
88 sample, Promega, Madison, WI, USA) were genotyped along with each batch of samples.

89

90 **Statistical analyses**

Allelic frequencies of all the 19 X-STR loci were calculated using the PowerStats v1.2 program (Promega, Madison, WI, USA). Hardy-Weinberg equilibrium (HWE) and pair-wise linkage disequilibrium in female samples were conducted by the software of Genepop (<http://genepop.curtin.edu.au/>). Differences of allele frequencies in males and females were assessed by the standard analysis of variance (ANOVA) method using statistical software SPSS Version 19.0 (IBM, USA). And the haplotype frequencies in male samples were also calculated by SPSS Version 19.0. Polymorphism information content (PIC), Heterozygosity (Het), power of exclusion (PE), power of discrimination in females (PDF), power of discrimination in males (PDM), mean exclusion chance (MEC) for deficiency cases, normal trios and duo cases were calculated using the free online-calculation tool of ChrX-STR.org 2.0 database (<http://www.chrx-str.org>). The geographical distributions of the 15 Asian populations were drawn by the R software (<https://www.r-project.org/>). The DA distance and the phylogenetic tree were constructed using Poptree2 Software (Takezaki et al. 2010). Based on the DA distances, the multidimensional scaling (MDS) plot was constructed by SPSS Version 19.0.

101

102 **Results**

103 **Allelic frequencies of 19 X-STR loci**

104 A total of 156 male and 111 female individuals from Xinjiang Mongolian group were analyzed. And the raw genotypes at 19 X-STR
105 loci were displayed in Supplementary Table 1. Exact tests of HWE after Bonferroni correction ($p > 0.05/19 = 0.0026$) demonstrated no

106 significant deviations for all 19 X-STR loci in female samples. There were no significant differences in the allele frequency distributions
 107 between males and females by the results of One-Way ANOVA ($p > 0.05/19 = 0.0026$), which indicated that the allele frequency
 108 distributions of all the 19 X-STR loci had no gender bias. Therefore, the allele frequencies of males and females were pooled together
 109 according to the formula $(2 \times \text{femalefreq} + \text{malefreq})/3$, which were showed in Supplementary Table 2. A total of 223 alleles were
 110 observed at the 19 X-STR loci, and the allele numbers ranged from 5 at DXS7423 to 26 at DXS10148. The most frequent allele observed
 111 was allele 10 at locus DXS10164 with the frequency 0.5663. Since all positive controls were genotyped correctly, we confirmed a series
 112 of off-ladder alleles for the 267 Mongolian individuals, among which allele 18.1 and 19.1 at DXS10079 and DXS10162 were more than
 113 0.1

114

115 Forensic parameters of 19 X-STR loci in Xinjiang Mongolian group

116 Forensic statistical parameters of the 19 X-STR loci were calculated based on the pooled allele frequencies. As displayed on Table 1,
 117 all the 19 X-STR loci were found to be very informative ($\text{PIC} > 0.5$). DXS10135, with the highest PIC (0.9184), Het (0.9235), PE
 118 (0.8437), PDF (0.9890) and PDM (0.9235), had the most polymorphism. The lowest PIC (0.5334), Het (0.5915), PE (0.2808), PDF
 119 (0.7750) and PDM (0.5915) were observed at DXS10164, indicating that DXS10164 was of the lowest polymorphism. The cumulative
 120 PDF and PDM were 0.9999999999999999999988761005481 and 0.9999999999999999999903, respectively. The cumulative PDF and PDM

both showed extremely high values, which confirmed that the 19 X-STR multiplex system would be powerful for individual identification in the Xinjiang Mongolian group. The MEC Krüger, MEC Kishida, and MEC Desmarais Duo ranged from 0.3406 to 0.8448 for the deficiency cases, 0.5334 to 0.9183 for the normal trios and 0.3867 to 0.8539 for the duo cases, respectively. The cumulative MECs were 0.999999983245493 in deficiency cases, 0.99999999998952 in normal trios, and 0.9999999969738068321121 in duo cases, respectively. The high values of these cumulative MECs suggested that the 19 X-STR multiplex system could be useful in paternity tests, especially for some deficiency cases.

Haplotypic structure of 7 linkage groups

According to the study of Zhang et al, the 19 X-STR loci could be divided into 7 linkage groups (Zhang et al. 2016). And DXS8378-DXS10148-DXS10135, DXS7132-DXS10079-DXS10075-DXS10074, DXS10103-DXS10101-HPRTB and DXS10134-DXS7423 (<http://xdb.qualitytype.de/xdb/linkageTable.jsf>) were classified as linkage groups 1, 2, 3 and 7, respectively. DXS6809-DXS6789 (Pasino et al. 2011; Szibor et al. 2003a) was specified as linkage group 4; DXS10159-DXS10162-DXS10164 (Meng et al. 2014; Ye et al. 2014a) as linkage group 5; DXS7424-DXS101 (Edelmann et al. 2002; Szibor et al. 2003a) as linkage group 6. The haplotypic frequencies and haplotypic diversities of the 7 linkage groups were showed in Supplementary Table 3. In this study, 127 haplotypes were observed for linkage group 1, 117 haplotypes for linkage group 2, 84 haplotypes for linkage group 3, 43 haplotypes for linkage group 4, 53 haplotypes

for linkage group 5, 41 haplotypes for linkage group 6, 34 haplotypes for linkage group 7. The 7 linkage groups were proved to be fairly informative in the Xinjiang Mongolians because of the high values of haplotype diversities, which ranged from 0.9487 at linkage group 7 to 0.9969 at linkage group 1. Among these linkage groups, the most common haplotypes were H 33-20 for linkage group DXS6809-DXS6789 and H 16-24 for linkage group DXS7424-DXS101 with a frequency of 0.1090, followed by H 37-15 for linkage group DXS10134-DXS7423 with a frequency of 0.1026. For linkage group DXS8378-DXS10148-DXS10135, which had a large variety of haplotypes with relatively low frequencies, the most common haplotype was H 10-25.1-21, with a frequency of 0.0256. However, H 10-25.1-21 was not found in the Xibe population (Meng et al. 2017) and was observed in the Guanzhong Han population (Zhang et al. 2016) with a relatively lower frequency of 0.0040. For linkage group DXS10103-DXS10101-HPRTB, the most common haplotype in the Xinjiang Mongolians was H 19-30.2-12 with a frequency of 0.0449, followed by H 16-32-13 with 0.0385; while in the Japanese population (Uchigasaki et al. 2013), H 19-30.2-12 and H 16-32-13 were at a frequency of 0.0091, H 16-30-13 with a frequency of 0.0411 was the most common haplotype instead. For linkage group DXS10159-DXS10162-DXS10164, H 24-18-10 had the highest frequency of 0.0833; however, H 26-18-10 was much more common than H 24-18-10 in the Xibe (Meng et al. 2017) and Guangxi Han population (Ye et al. 2014b). For the linkage group DXS7424-DXS101, H 16-24 was of a medium frequency of 0.0403 in the Kazak population (Liu et al. 2017), where H 16-25 with a frequency of 0.0872 was the most common haplotype. These haplotype comparisons indicated that the studied Xinjiang Mongolian population had a different pattern of haplotype distribution, compared to other Asian populations.

151

152 **Linkage disequilibrium analyses**

153 Pair-wise linkage disequilibrium in the female samples were analyzed by Genepop software before the 19 X-STR loci were applied for
154 forensic purposes and population genetics. In this study, 171 pair-wise comparisons were performed (Supplementary Table 4) and the
155 results revealed that 10 pairs of pair-wise loci had p -value < 0.05 . Nevertheless, no linkage disequilibrium were observed for a
156 significance level of 0.00029 ($p = 0.05/171$) after Bonferroni correction. This indicated that there weren't non-random association of
157 alleles at different loci in the Xinjiang Mongolian group.

158

159 **Inter-population comparisons based on the 11 overlapping X-STRs**

160 To investigate the population differentiations between the studied Xinjiang Mongolians and other Asian groups, we collected allele
161 frequencies of 11 overlapping X-STR loci (DXS8378, DXS7423, DXS10148, DXS10134, DXS7132, DXS10079, DXS10103,
162 DXS10101, DXS10074, DXS10135 and HPRTB) from Xibe (Meng et al. 2017), Kazak (Liu et al. 2017), Inner Mongolian (Tao et al.
163 2018), Japanese (Uchigasaki et al. 2013), Shanghai Han (Zhang et al. 2012), Bhil (Shrivastava et al. 2015), Malay (Samejima et al.
164 2012), Taiwanese (Chen et al. 2014), Ili Uygur (Guo et al. 2016), Guanzhong Han (Zhang et al. 2016), Tibetan (Yang et al. 2017),
165 Southern Han (Yang et al. 2017), Hui (Yang et al. 2017) and Korla Uygur (Yang et al. 2017). For a better understanding, the geographical

distributions of these populations mentioned above were showed in Figure 1. The D_A distance (Nei et al. 1983) was often used in forensic science for measuring genetic distance between populations, and the D_A distance was more efficient in obtaining the correct tree topology for microsatellite data than other distance measures including D_{ST} and F_{ST} (Takezaki & Nei 1996; Takezaki & Nei 2008). Therefore, through the comparisons of allelic frequencies of the 11 overlapping X-STR loci, we calculated the D_A distances between these Asian populations. As shown in Supplementary Table 5, the largest D_A distance was observed between Xinjiang Mongolian and Bhil (0.078), and the smallest D_A distance between Guanzhong Han and Taiwanese (0.009). Based on these D_A distances, a multidimensional scaling (MDS) plot was constructed. As shown in Figure 2, the Southern Han, Guanzhong Han, Shanghai Han, Taiwanese, Japanese, Hui, Malay, as well as Inner Mongolian were clustered together at the center part of this plot; however, only the Xinjiang Mongolian was located in the lower right corner, keeping a long distance from other populations. Thus, we can see that the Xinjiang Mongolian population was obviously different from other Asian populations, including Inner Mongolian.

Phylogenetic analyses based on the 11 overlapping X-STRs

To evaluate the evolutionary relationship between the Xinjiang Mongolian and other 14 Asian populations, we constructed a phylogenetic tree using the D_A distance and the unweighted pair-group method with arithmetic means method (UPGMA). As shown in Figure 3, populations with the same ethno-linguistic origin such as Taiwanese, Guanzhong Han, Shanghai Han, Southern Han, Japanese

181 were clustered together; populations with close geographic distance like Korla Uyghur, Kazak were clustered as a terminal clade; only
182 the Xinjiang Mongolian was segregated as a distant outlier, indicating that the Xinjiang Mongolian population was genetically distinct
183 from those compared Asian populations. On the whole, the phylogenetic tree showed a genetic relationship similar to the result of MDS
184 plot.

185

186 **Discussion**

187 Results of this study showed that the 19 X-STR multiplex PCR system was very useful for forensic applications and population genetic
188 researches in Xinjiang Mongolian population. Compared with Investigator Argus X-12 amplification kit (Qiagen, Hilden, Germany),
189 the AGCU 19 X-STR multiplex PCR system enriched eight new X-STR loci (DXS10159, DXS10162, DXS7424, DXS10164,
190 DXS6789, DXS101, DXS6809 and DXS10075). The 19 X-STR multiplex PCR system has been studied in several Chinese populations
191 such as Zhejiang Han (Yang et al. 2016), Guanzhong Han (Zhang et al. 2016), Kazak (Liu et al. 2017), Xibe (Meng et al. 2017), Uyghur
192 (Guo et al. 2016), and these studies also indicated its potential in paternity testing and individual identification. However, there has been
193 no data of the set of 19 X-STR for Western Mongolians so far. There were about 170,000 Mongolians residing in Xinjiang Uyghur
194 Autonomous Region, China according to the 6th China population census in 2010
195 (<http://www.stats.gov.cn/tjsj/pcsj/rkpc/6rp/indexch.htm>). Thus, we performed a study on the polymorphism of 19 X-STRs in Xinjiang

196 Mongolians. In summary, the forensic statistical parameters of the 19 X-STR loci for Xinjiang Mongolian population showed that most
 197 loci owned high PIC, Het and PE, and the new added eight X-STRs were highly polymorphic except DXS10164 (PIC=0.5334). The
 198 combined power of discrimination was more than 0.999999999999 for both the female samples and the male samples. Moreover, the
 199 combined mean exclusion chance was more than 0.999999999999 in normal trio cases and over 0.999999999 in duo cases. These results
 200 suggested that the 19 X-STR multiplex system could provide highly polymorphic information suitable for individual identification and
 201 paternity testing in Xinjiang Mongolian populations. Especially, the combined mean exclusion chance was more than 0.99999999 in
 202 deficiency cases. This meant that the 19 X-STRs could be helpful for deficiency paternity cases.

203 As mentioned above, the 19 X-STR loci were grouped into 7 linkage groups. The results showed the 7 linkage groups could provide
 204 highly haplotype diversity information for the Xinjiang Mongolian population. The obtained X-chromosomal haplotype frequencies of
 205 the 7 linkage groups could be essential for calculate likelihood ratio of kinship. The previous researches showed the haplotyping of the
 206 X-STR loci can be used to analyze some complex kinship testing (Edelmann et al. 2002; Szibor 2007). The MDS plot and the
 207 phylogenetic tree based on the 11 overlapping X-STRs all presented that the Xinjiang Mongolian population was genetically different
 208 from other Asian populations, including the Mongolians from Inner Mongolia Autonomous Region, China.

209 The Xinjiang Mongolians were the subgroup of the Oirats, whose ancestral home was in the Altai region of western Mongolia. The
 210 Oirats share some history, geography, culture and language with the Eastern Mongols. However, they were often at war with the Eastern

Mongols, only few times united as a larger Mongol entity. Gradually, the Oirats and Eastern Mongols had developed separate identities after the collapse of the Mongol Empire (https://en.wikipedia.org/wiki/Main_Page).

Previous studies based on X chromosomal STR loci indicated that Mongolians were closely related with Chinese Han groups (Hou et al. 2007; Tao et al. 2018; Zhang et al. 2015). In fact, the Mongolians involved in these studies were only Inner Mongolians, descendants of the Eastern Mongols. And through the analysis of autosomal SNPs, Xing et al indicated that Deedu Mongolians, living in the Qinghai-Tibetan Plateau, shared genetic ancestry with other Mongolians as well as Tibetan populations (Xing et al. 2013); Nakayama et al demonstrated that the largest fraction of the ancestry of Outer Mongols was mainly shared by ethnic groups in Central and Northeast China (Daur, Hezhen, Oroqen, and Xibe), and by Siberians (Yakut), and this fraction was present to a lesser extent in Southern populations including Japanese, Chinese Han, and other ethnic groups in Southeast Asia (Nakayama et al. 2017). Thus, we can see that many factors like the sample sizes, the origins of the selected samples, the choice of the genetic markers and the coverage of reference populations may lead to the discrepancy in the analysis of genetic relationship. In summary, to achieve a better understanding of genetic relationships between those populations, larger sample sizes, more genetic markers, more population data and further investigations are needed.

Conclusion

In this study, 267 unrelated individuals from Chinese Mongolian minority living in Xinjiang Uyghur Autonomous Region of China were genotyped and analyzed for 19 X-STR loci for the first time. These loci were proved to be highly polymorphic and demonstrated high power of discrimination in Xinjiang Mongolian minority. The present study indicated that the 19 X-STR multiplex PCR system could be of high utility value for forensic practices, especially for the deficient paternity cases. We also found that the Xinjiang Mongolian population was genetically different from other Asian populations, including the Mongolian population from Inner Mongolia Autonomous Region, China.

Acknowledgements

The authors would like to thank Jiangwei Yan for helping us to analysis data.

References

- Carracedo A, Butler JM, Gusmao L, Linacre A, Parson W, Roewer L, and Schneider PM. 2013. New guidelines for the publication of genetic population data. *Forensic Sci Int Genet* 7:217-220. 10.1016/j.fsigen.2013.01.001
- Carracedo A, Butler JM, Gusmao L, Linacre A, Parson W, Roewer L, and Schneider PM. 2014. Update of the guidelines for the publication of genetic population data. *Forensic Sci Int Genet* 10:A1-2. 10.1016/j.fsigen.2014.01.004
- Chen MY, Ho CW, Pu CE, and Wu FC. 2014. Genetic polymorphisms of 12 X-chromosomal STR loci in Taiwanese individuals and likelihood ratio calculations applied to case studies of blood relationships. *Electrophoresis* 35:1912-1920. 10.1002/elps.201300645
- Edelmann J, Hering S, Kuhlisch E, and Szibor R. 2002. Validation of the STR DXS7424 and the linkage situation on the X-chromosome. *Forensic Sci Int* 125:217-222.

- 245 Guo YX, Chen JG, Wang Y, Yan JW, Chen J, Yao TH, Zhang LP, Yang G, Meng HT, Zhang YD, Mei T, Liu YS, Dong Q, and Zhu BF. 2016. Genetic
246 polymorphism analyses of a novel panel of 19 X-STR loci in the Chinese Uygur ethnic minority. *J Zhejiang Univ Sci B* 17:367-374.
247 10.1631/jzus.B1500228
- 248 Hou QF, Yu B, and Li SB. 2007. Genetic polymorphisms of nine X-STR loci in four population groups from Inner Mongolia, China. *Genomics Proteomics
249 Bioinformatics* 5:59-65. 10.1016/S1672-0229(07)60015-1
- 250 Janhunen J. 2011. *The Mongolic Languages*. London: Routledge.
- 251 Liu QL, Li ZD, Li CT, Zhao H, Wu YD, Li Q, and Lu DJ. 2013. X chromosomal recombination--a family study analyzing 26 X-STR Loci in Chinese Han three-
252 generation pedigrees. *Electrophoresis* 34:3016-3022. 10.1002/elps.201300204
- 253 Liu YS, Meng HT, Mei T, Zhang LP, Chen JG, Zhang YD, Chen J, Guo YX, Dong Q, Yan JW, and Zhu BF. 2017. Genetic diversity and haplotypic structure of
254 Chinese Kazak ethnic group revealed by 19 STRs on the X chromosome. *Gene* 600:64-69. 10.1016/j.gene.2016.11.018
- 255 Meng HT, Han JT, Zhang YD, Liu WJ, Wang TJ, Yan JW, Huang JF, Du WA, Guo JX, Wang HD, Zhang YH, Zhou RH, Zhu BF, and Wei X. 2014. Diversity
256 study of 12 X-chromosomal STR loci in Hui ethnic from China. *Electrophoresis* 35:2001-2007. 10.1002/elps.201400045
- 257 Meng HT, Shen CM, Zhang YD, Dong Q, Guo YX, Yang G, Yan JW, Liu YS, Mei T, Shi JF, and Zhu BF. 2017. Chinese Xibe population genetic composition
258 according to linkage groups of X-chromosomal STRs: population genetic variability and interpopulation comparisons. *Ann Hum Biol*:1-8.
259 10.1080/03014460.2017.1318951
- 260 Nakayama K, Ohashi J, Watanabe K, Munkhtulga L, and Iwamoto S. 2017. Evidence for Very Recent Positive Selection in Mongolians. *Mol Biol Evol* 34:1936-
261 1946. 10.1093/molbev/msx138
- 262 Nei M, Tajima F, and Tateno Y. 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *J Mol Evol* 19:153-170.
- 263 Pasino S, Caratti S, Del Pero M, Santovito A, Torre C, and Robino C. 2011. Allele and haplotype diversity of X-chromosomal STRs in Ivory Coast. *Int J Legal
264 Med* 125:749-752. 10.1007/s00414-011-0591-4
- 265 Samejima M, Nakamura Y, Nambiar P, and Minaguchi K. 2012. Genetic study of 12 X-STRs in Malay population living in and around Kuala Lumpur using
266 Investigator Argus X-12 kit. *Int J Legal Med* 126:677-683. 10.1007/s00414-012-0705-7
- 267 Schneider PM. 2007. Scientific standards for studies in forensic genetics. *Forensic Sci Int* 165:238-243. 10.1016/j.forsciint.2006.06.067
- 268 Shrivastava P, Jain T, Gupta U, and Trivedi VB. 2015. Genetic polymorphism study on 12 X STR loci of investigator Argus X STR kit in Bhil tribal population of
269 Madhya Pradesh, India. *Leg Med (Tokyo)* 17:214-217. 10.1016/j.legalmed.2014.11.004
- 270 Sun K, Zhao S, Tian H, Zhang S, and Li C. 2013. Development of the 16 X-STR loci typing system and genetic analysis in a Shanghai Han population from China.
271 *Electrophoresis* 34:3008-3015. 10.1002/elps.201300234
- 272 Szibor R. 2007. X-chromosomal markers: past, present and future. *Forensic Sci Int Genet* 1:93-99. 10.1016/j.fsigen.2007.03.003
- 273 Szibor R, Edelmann J, Hering S, Plate I, Wittig H, Roewer L, Wiegand P, Cali F, Romano V, and Michael M. 2003a. Cell line DNA typing in forensic genetics--
274 the necessity of reliable standards. *Forensic Sci Int* 138:37-43.

275 Szibor R, Krawczak M, Hering S, Edelmann J, Kuhlisch E, and Krause D. 2003b. Use of X-linked markers for forensic purposes. *Int J Legal Med* 117:67-74.
 276 10.1007/s00414-002-0352-5

277 Takezaki N, and Nei M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* 144:389-399.

278 Takezaki N, and Nei M. 2008. Empirical tests of the reliability of phylogenetic trees constructed with microsatellite DNA. *Genetics* 178:385-392.
 279 10.1534/genetics.107.081505

280 Takezaki N, Nei M, and Tamura K. 2010. POPTREE2: Software for constructing population trees from allele frequency data and computing other population
 281 statistics with Windows interface. *Mol Biol Evol* 27:747-752. 10.1093/molbev/msp312

282 Tao R, Zhang J, Bian Y, Dong R, Liu X, Jin C, Zhu R, Zhang S, and Li C. 2018. Investigation of 12 X-STR loci in Mongolian and Eastern Han populations of
 283 China with comparison to other populations. *Sci Rep* 8:4287. 10.1038/s41598-018-22665-3

284 Uchigasaki S, Tie J, and Takahashi D. 2013. Genetic analysis of twelve X-chromosomal STRs in Japanese and Chinese populations. *Mol Biol Rep* 40:3193-3196.
 285 10.1007/s11033-012-2394-1

286 Walsh PS, Metzger DA, and Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*
 287 10:506-513.

288 Xing J, Wuren T, Simonson TS, Watkins WS, Witherspoon DJ, Wu W, Qin G, Huff CD, Jorde LB, and Ge RL. 2013. Genomic analysis of natural selection and
 289 phenotypic variation in high-altitude mongolians. *PLoS Genet* 9:e1003634. 10.1371/journal.pgen.1003634

290 Yang X, Wu W, Chen L, Liu C, Zhang X, Chen L, Feng X, Wang H, and Liu C. 2016. Development of the 19 X-STR loci multiplex system and genetic analysis
 291 of a Zhejiang Han population in China. *Electrophoresis* 37:2260-2272. 10.1002/elps.201500540

292 Yang X, Zhang X, Zhu J, Chen L, Liu C, Feng X, Chen L, Wang H, and Liu C. 2017. Genetic analysis of 19 X chromosome STR loci for forensic purposes in four
 293 Chinese ethnic groups. *Sci Rep* 7:42782. 10.1038/srep42782

294 Ye Q, Tang J, Chen Z, Li F, Yu X, Wang P, Lin H, and Shi M. 2014a. Analysis of linkage disequilibrium and linkage for 12 short tandem repeat loci on chromosome
 295 X. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 31:782-785. 10.3760/cma.j.issn.1003-9406.2014.06.023

296 Ye Q, Tang J, Chen Z, Li F, Yu X, Wang P, Lin H, and Shi M. 2014b. [Analysis of linkage disequilibrium and linkage for 12 short tandem repeat loci on
 297 chromosome X]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 31:782-785. 10.3760/cma.j.issn.1003-9406.2014.06.023

298 Zhang S, Bian Y, Li L, Sun K, Wang Z, Zhao Q, Zha L, Cai J, Gao Y, Ji C, and Li C. 2015. Population genetic study of 34 X-Chromosome markers in 5 main
 299 ethnic groups of China. *Sci Rep* 5:17711. 10.1038/srep17711

300 Zhang S, Zhao S, Zhu R, and Li C. 2012. Genetic polymorphisms of 12 X-STR for forensic purposes in Shanghai Han population from China. *Mol Biol Rep*
 301 39:5705-5707. 10.1007/s11033-011-1379-9

302 Zhang YD, Shen CM, Meng HT, Guo YX, Dong Q, Yang G, Yan JW, Liu YS, Mei T, Huang RZ, and Zhu BF. 2016. Allele and haplotype diversity of new
 303 multiplex of 19 ChrX-STR loci in Han population from Guanzhong region (China). *Electrophoresis* 37:1669-1675. 10.1002/elps.201500425

304

305 **Figure legend**

306 Figure 1. The geographical distribution of the 15 Asian populations.

307 Figure 2. The multidimensional scaling (MDS) plot based on DA distance for the 15 Asian populations.

308 Figure 3. The phylogenetic tree generated by the DA distance and the unweighted pair-group method with arithmetic means method
309 (UPGMA).

310

Figure 1

The geographical distribution of the 15 Asian populations.

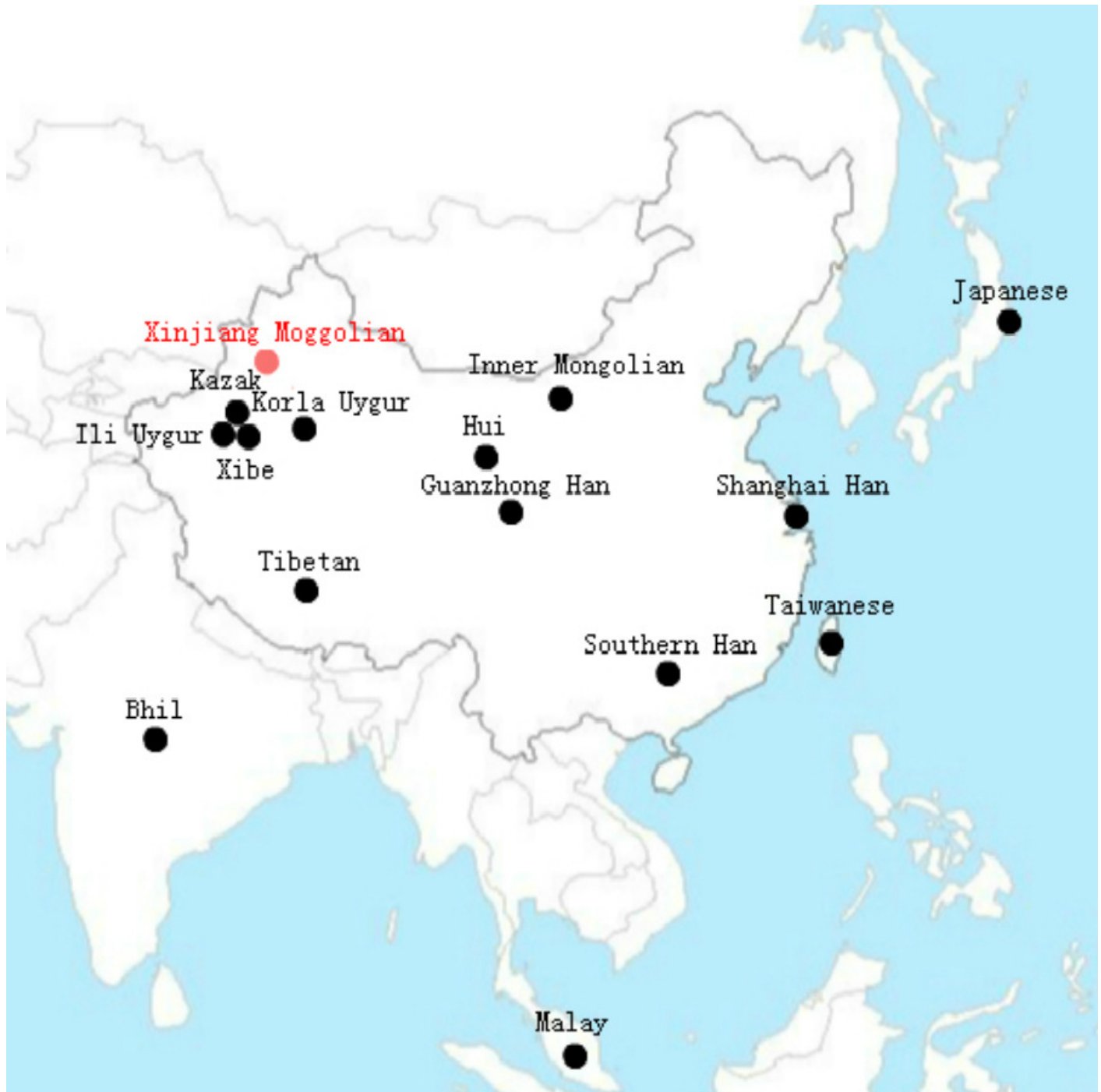
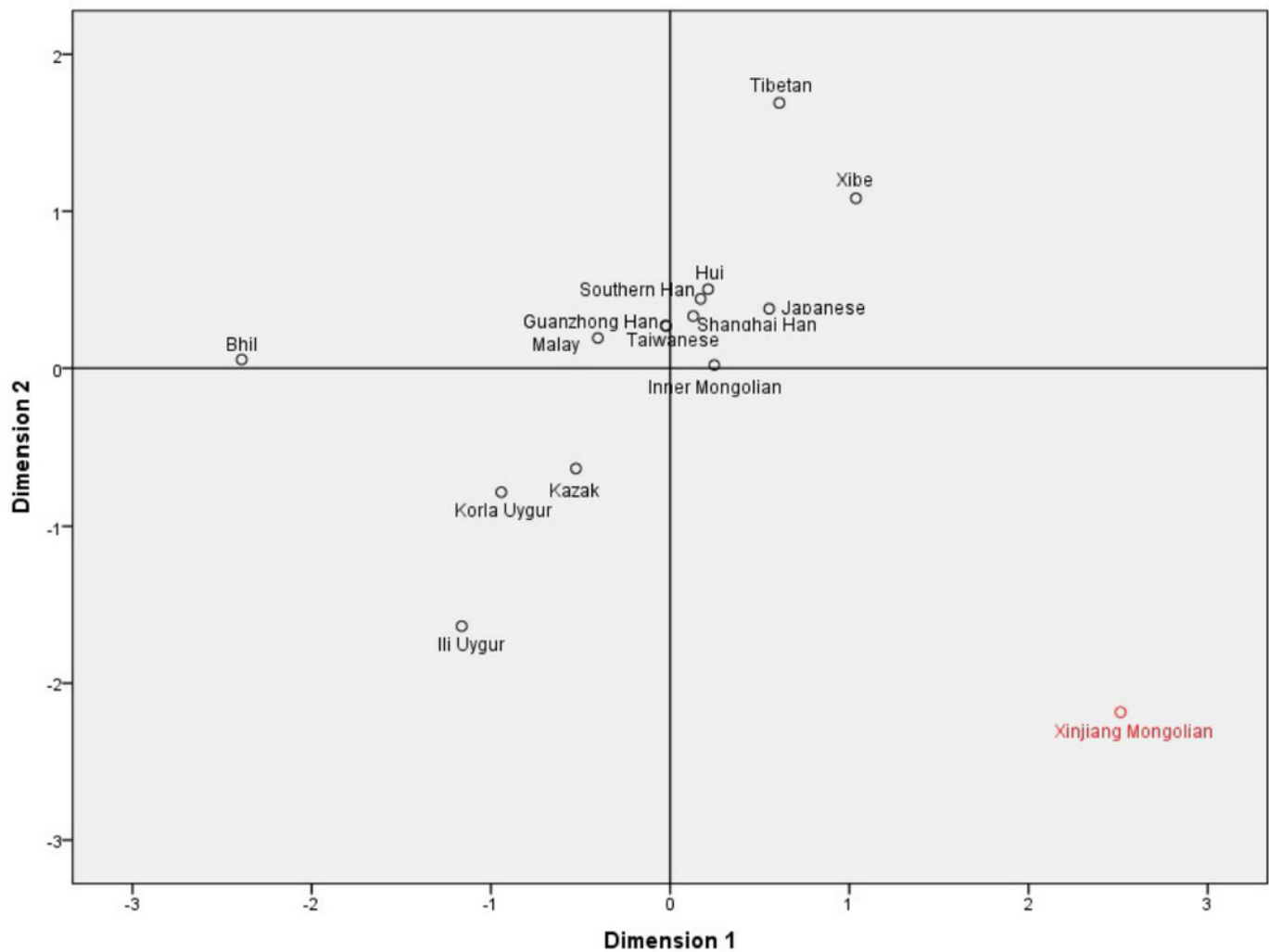


Figure 2

The multidimensional scaling (MDS) plot based on DA distance for the 15 Asian populations.



The phylogenetic tree generated by the DA distance and the unweighted pair-group method with arithmetic means method (UPGMA).

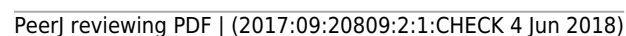


Table 1(on next page)

The forensic efficiency parameters of 19 X-STR loci in Xinjiang Mongolians.

1 **Table 1. The forensic efficiency parameters of 19 X-STR loci in Xinjiang Mongolians.**

Index	PIC	Het	PE	PDF	PDM	MEC Krüger	MEC Kishida	MEC Desmarais Duo
DXS8378	0.6157	0.6687	0.3815	0.8372	0.6687	0.4160	0.6157	0.4687
DXS7423	0.5430	0.6077	0.3003	0.7814	0.6077	0.3442	0.5430	0.3966
DXS10148	0.8980	0.9056	0.8069	0.9835	0.9056	0.8095	0.8980	0.8221
DXS10159	0.7550	0.7882	0.5773	0.9220	0.7882	0.5817	0.7550	0.6254
DXS10134	0.8494	0.8635	0.7217	0.9672	0.8635	0.7296	0.8494	0.7508
DXS7424	0.7185	0.7499	0.5096	0.9061	0.7499	0.5457	0.7185	0.5830
DXS10164	0.5334	0.5915	0.2808	0.7750	0.5915	0.3406	0.5334	0.3867
DXS10162	0.6804	0.7199	0.4598	0.8820	0.7199	0.4958	0.6804	0.5393
DXS7132	0.7047	0.7453	0.5018	0.8945	0.7453	0.5200	0.7047	0.5669
DXS10079	0.7883	0.8130	0.6234	0.9403	0.8130	0.6345	0.7883	0.6681
DXS6789	0.7679	0.7966	0.5927	0.9299	0.7966	0.6050	0.7679	0.6425
DXS101	0.8119	0.8321	0.6600	0.9516	0.8321	0.6707	0.8119	0.6992
DXS10103	0.7559	0.7863	0.5739	0.9240	0.7863	0.5876	0.7558	0.6271
DXS10101	0.8931	0.9015	0.7984	0.9819	0.9015	0.8001	0.8930	0.8141
HPRTB	0.6781	0.7244	0.4669	0.8777	0.7244	0.4844	0.6781	0.5362
DXS6809	0.7799	0.8051	0.6085	0.9368	0.8051	0.6232	0.7799	0.6572
DXS10075	0.6289	0.6845	0.4047	0.8448	0.6845	0.4296	0.6289	0.4839
DXS10074	0.7535	0.7847	0.5710	0.9225	0.7847	0.5845	0.7535	0.6244
DXS10135	0.9184	0.9235	0.8437	0.9890	0.9235	0.8448	0.9183	0.8539

2 PIC: polymorphism information content; Het: Heterozygosity; PE: probability of exclusion; PDF: power of discrimination in females; PDM: power of
3 discrimination in males; MEC Krüger: mean exclusion chance for deficiency cases; MEC Kishida: mean exclusion chance for normal trios; MEC
4 Desmarais Duo: mean exclusion chance in duo cases.