



Population Genetic Study of Four Closely Linked X-STR Trios in Koreans

Jeong Eun Sim, Hwan Young Lee, Myung Jin Park, Na Young Kim, Woo Ick Yang and Kyoung-Jin Shin

Department of Forensic Medicine and Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea.

Introduction

In case that the X chromosomal short tandem repeat (X-STR) markers are close to each other sufficiently, the X-STR markers represent stable haplotype, thereby providing highly informative tools for kinship testing. In consequence of that, 4 pairs of tightly linked X-STR duos were assembled to the Mentype Arugus X-8 PCR Amplification Kit (Biotype AG, Dresden, Germany). To achieve a further enhancement of discrimination power, there were efforts to add additional 4 X-STRs (i.e. DXS10148 at Xp22, DXS10079 at Xq12, DXS10103 at Xq26, and DXS10146 at Xq28) to the 4 linkage duos. To evaluate these markers for forensic usage in Koreans, we analyzed allele frequencies and forensic parameters of the 12 X-STR markers. And, we presented haplotypes for 4 linkage groups each comprised of 3 X-STRs (i.e. DXS10148-DXS10135-DXS8378, DXS7132-DXS10079-DXS10074, HPRTB-DXS10101-DXS10103, DXS10146-DXS10134-DXS7423) and their frequency data in 300 males. In addition, haplotype diversities and forensic parameters based on halotype frequencies were presented in this study.

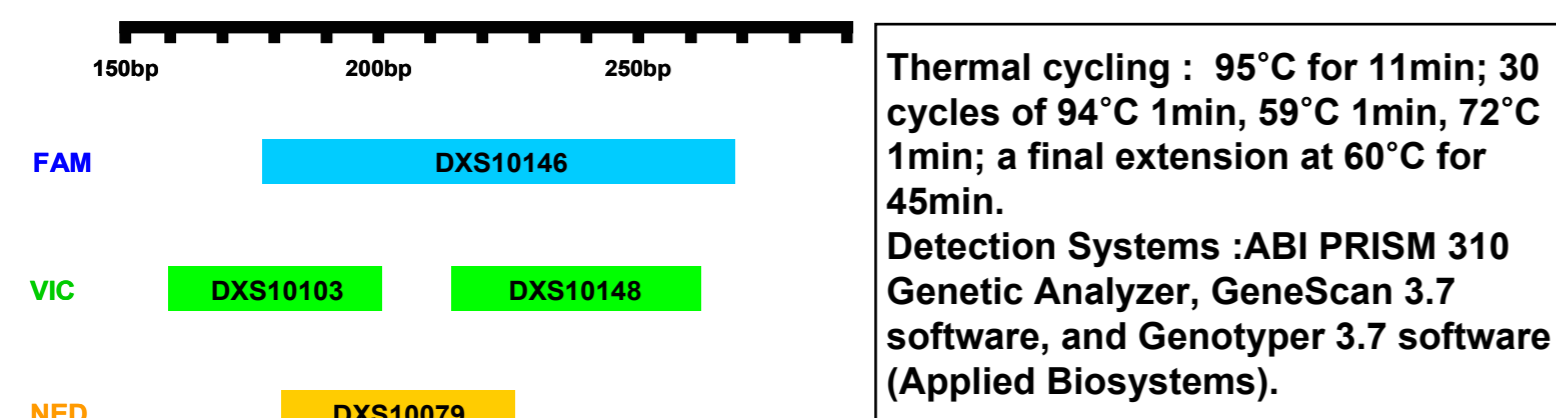
Materials and Methods

DNA Samples

Blood or buccal samples from 450 unrelated Koreans (300 males and 150 females), who had previously been typed for 8 X-STRs using the Mentype Argus X-8 kit were analyzed. Genomic DNA was extracted using a QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

PCR Amplification and Genotyping

An in-house quadruplex was constructed to amplify 4 X-STR markers (i.e. DXS10079, DXS10103, DXS10146, DXS10148). Primer information were provided by Dr. Szibor, but the primer for DXS10079 was redesigned. PCR amplification was carried out in 10 ul reaction mixture: 0.5-2.0 ng DNA, 1.0 ul of 10 x Gold STR buffer (Promega, Madison, WI), 1.5 U of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA) and appropriate concentrations of primers.



Statistical Analysis

Forensic efficiency parameters were calculated at the ChrX research homepage (<http://www.chrx-str.org>). Fisher's exact tests of Hardy-Weinberg equilibrium (HWE) and the population differentiation were carried out using the Alrequin software ver 3.0.

Results

Table 1. Forensic statistical parameters of the Mentype® Argus X-8 STRs in a Korean population

Parameter	DXS7132	DXS7423	DXS8378	HPRTB	DXS10074	DXS10101	DXS10134	DXS10135	Combined
PIC	0.722	0.412	0.494	0.639	0.742	0.875	0.834	0.914	
Het _{obs}	0.767	0.433	0.573	0.653	0.840	0.887	0.887	0.913	
Het _{exp}	0.761	0.485	0.565	0.695	0.777	0.888	0.852	0.921	
MEC _{trio}	0.722	0.412	0.494	0.639	0.742	0.875	0.834	0.914	0.999986
MEC _{duo}	0.586	0.276	0.350	0.494	0.610	0.788	0.729	0.847	0.999662
PD _{female}	0.904	0.662	0.740	0.852	0.916	0.976	0.961	0.988	>0.999999
PD _{male}	0.760	0.484	0.565	0.694	0.776	0.886	0.851	0.920	>0.999995
HWE (p-value)	0.618	0.200	0.199	0.567	0.386	0.264	0.246	0.016	

Table 2. Forensic statistical parameters of additional 4 X-STRs in a Korean population

Parameter	DXS10079	DXS10103	DXS10146	DXS10148	Combined*
PIC	0.787	0.709	0.852	0.913	
Het _{obs}	0.760	0.747	0.907	0.900	
Het _{exp}	0.814	0.751	0.867	0.920	
MEC _{trio}	0.787	0.709	0.852	0.913	>0.999999
MEC _{duo}	0.667	0.572	0.753	0.845	0.999998
PD _{female}	0.940	0.897	0.968	0.988	>0.999999
PD _{male}	0.813	0.749	0.866	0.919	>0.999999
HWE(p-value)	0.659	0.827	0.835	0.419	

*Combined forensic parameters were calculated for 12 X-STRs including Mentype Argus X-8 markers and 4 additional X-STR markers.

Table 3. Numbers of haplotypes and haplotype diversities of 4 closely linked X-STR trios in a Korean population

Linkage group	Haplotype	No. of Haplotype	Haplotype diversity
Linkage group 1	DXS10135-DXS8378	56	0.9640 ± 0.0029
	DXS10135-DXS8378 + DXS10148	197	0.9966 ± 0.0006
Linkage group 2	DXS7132-DXS10074	41	0.9473 ± 0.0038
	DXS7132-DXS10074 + DXS10079	125	0.9894 ± 0.0012
Linkage group 3	HPRTB-DXS10101	59	0.9662 ± 0.0029
	HPRTB-DXS10101 + DXS10103	130	0.9893 ± 0.0014
Linkage group 4	DXS10134-DXS7423	38	0.9157 ± 0.0073
	DXS10134-DXS7423 + DXS10146	137	0.9887 ± 0.0014

Table 4. Forensic statistical parameters of 4 closely linked X-STR trios in a Korean population

Parameter	DXS10148-DXS10135-DXS8378	DXS7132-DXS10079-DXS10074	DXS10103-HPRTB-DXS10101	DXS10146-DXS10134-DXS7423	Combined
MEC _{trio}	0.9933	0.9859	0.9858	0.9852	>0.999999
MEC _{duo}	0.9867	0.9725	0.9723	0.9712	>0.999999
PD _{female}	0.9999	0.9996	0.9996	0.9996	>0.999999
PD _{male}	0.9933	0.9861	0.9860	0.9854	>0.999999

Discussion

- Forensic efficiency parameters for 12 X-STR markers indicated that they are highly informative for forensic application in Koreans.
- We found a discrepancy from a previous report (Hundertmark T *et al.*) on 9948 genotype (23 allele not 23.1 allele) at DXS10148. It has been confirmed by cloning and sequence analysis.
- Population comparison on DXS7132, DXS7423, DXS8378, HPRTB, DXS10074, DXS10101, DXS10134, DXS10135, DXS10079, DXS10146, and DXS10148 showed significant difference between German and Korean populations ($P < 0.001$).
- By addition of 4 X-STR makers to the 4 linkage duos in our previous study (Lim EJ *et al.*), the total number of haplotypes were increased from 38-59 to 125-197 for each linkage group. 77.1% of all haplotype showed frequency less than 0.01. Haplotype diversity values for each X-STR duo were also increased from 91.6-96.6% to 98.9-99.7%.
- Especially, the X-STR duo in linkage group 4 showed the lowest number of haplotype. But, by addition of DXS10146, X-STR trio in linkage group 4 showed the second highest number of haplotype and increased haplotype diversities.
- In order to apply these haplotype data in forensic caseworks, it is necessary to evaluate haplotype stability and independency between linkage groups.

References

- Hundertmark T, Hering S, Edelmann J, Augustin C, Plate I, Szibor R. The STR cluster DXS10148-DXS8378-DXS10135 provides a powerful tool for X-chromosomal haplotyping at Xp22. *Int J Legal Med.* 2008;122:487-92
- Lim EJ, Lee HY, Sim JE, Yang WI, Shin KJ. Genetic polymorphism and haplotype analysis of four tightly linked X-STR duos in Koreans. *Croat Med J.* 2009. In Press.

Acknowledgement

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korean government (MEST) (No. M10640030002-08N4003-00210).