

Genetic Polymorphism and Haplotype Analysis of Four Closely Linked X-STR Trios in Koreans

Jeong Eun Sim¹, Hwan Young Lee^{1,2}, Myung Jin Park¹,
Na Young Kim¹, Jung Ah Yoon¹, Woo Ick Yang¹ and
Kyoung-Jin Shin^{1,2}

¹Department of Forensic Medicine and Brain Korea 21 Project for Medical Science,
Yonsei University College of Medicine, Seoul, Korea

²Human Identification Research Center, Yonsei University, Seoul, Korea

X-STRs in Forensic Genetics

- Gonosomal markers have been efficiently used for solving deficiency case.
- Due to the hemizyosity in males and high mean exclusion chances, X-chromosomal STRs (X-STRs) are particularly helpful in paternity testing and kinship analyses
- Grandmother-granddaughter kinship testing, the kinship testing of putative sisters



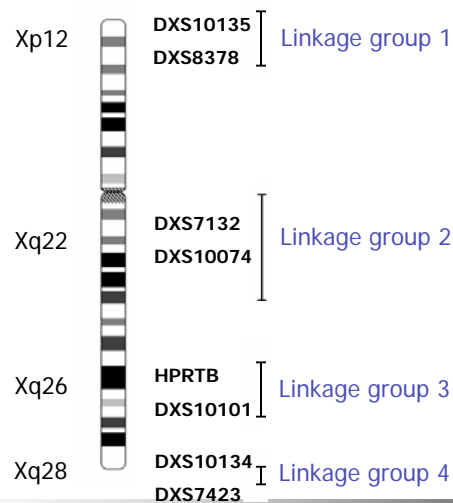
X-STR Clusters

- When second and third degree kinships are investigated, extremely polymorphic STRs might be required. As the degree of polymorphism is limited in most STRs and highly polymorphic STRs like DXS10011 are prone to mutations.
- Haplotype analysis of closely associated markers has proven to be a powerful tool in kinship analysis especially when X-STRs fail to resolve uncertainty in relationship analysis.

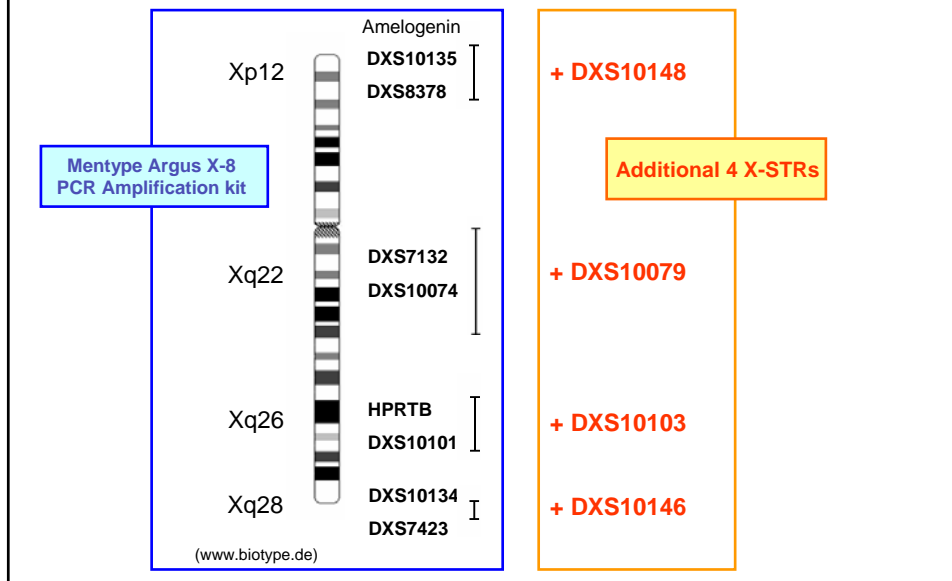
It has recently been suggested that stable haplotypes of closely linked loci are used in kinship testing instead of a single STR.



Mentype Argus X-8 PCR Amplification kit



Four Closely Linked X-STR Trios



Aims of This Study

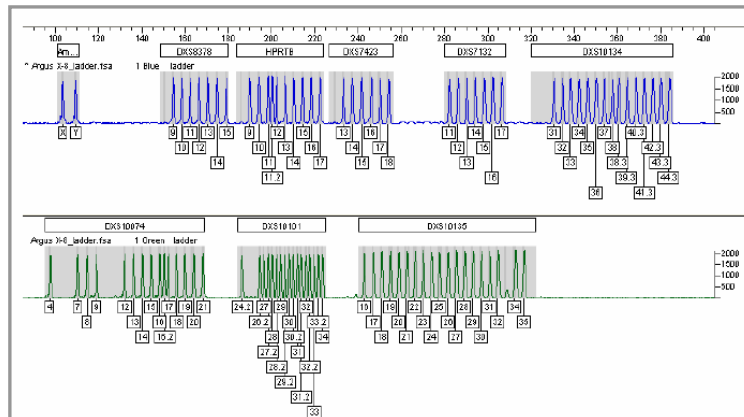
- Study of genetic polymorphism for 8 X-STRs include Argus X-8 multiplex amplification kit and additional 4 X-STRs in Koreans
- Evaluation of usage for adding 4 X-STRs to linkage groups
- Providing basic data to actual use in forensic practice

Materials and Methods

- **Samples:** 450 unrelated Koreans (i.e. 300 men and 150 women)
- **PCR amplification:** Mentype Argus X-8 Amplification kit. In-house multiplex PCR
- **Detection Systems:** ABI PRISM 310 Genetic Analyzer, GeneScan 3.7 software, and Genotyper 3.7 software
- **Statistical analysis :** forensic parameters, Fisher's exact tests of the Hardy-Weinberg equilibrium, the population differentiation, Haplotype frequency and diversity using Arlequin software

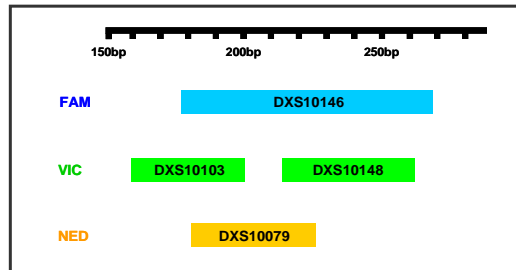


Mentype Argus X-8 PCR Amplification kit



In-house Multiplex PCR Condition

Schematic of Multiplex PCR:



Thermal Cycling:

95°C for 11 minutes, then:
94°C for 1 min
59°C for 1 min
72°C for 1 min
for 30 cycles, then:
60°C for 45 minutes
4°C soak



Analysis of 8 X-STR using Mentype Argus X-8 PCR Amplification kit

Allele Frequencies for 8 X-STRs

| DXS7132 | | DXS7423 | | DXS8378 | | HPRTB | | DXS10074 | | DXS10101 | | DXS10134 | | DXS10135 | |
|---------|--------|---------|--------|---------|--------|--------|--------|----------|--------|----------|--------|----------|--------|----------|--------|
| Allele | Freq. | Allele | Freq. | Allele | Freq. | Allele | Freq. | Allele | Freq. | Allele | Freq. | Allele | Freq. | Allele | Freq. |
| 10 | 0.0017 | 13 | 0.0017 | 7 | 0.0017 | 10 | 0.0033 | 12 | 0.0033 | 26.2 | 0.0017 | 30 | 0.0017 | 16 | 0.0100 |
| 11 | 0.0067 | 14 | 0.2833 | 9 | 0.0050 | 11 | 0.0400 | 13 | 0.0017 | 27 | 0.0117 | 31 | 0.0017 | 17 | 0.0100 |
| 12 | 0.0833 | 15 | 0.6583 | 10 | 0.5767 | 12 | 0.3183 | 14 | 0.0117 | 28 | 0.0167 | 32 | 0.0250 | 18 | 0.0666 |
| 13 | 0.2017 | 16 | 0.0533 | 11 | 0.3000 | 13 | 0.4067 | 15 | 0.0617 | 28.2 | 0.0167 | 33 | 0.0317 | 19 | 0.0882 |
| 14 | 0.3133 | 17 | 0.0033 | 12 | 0.1117 | 14 | 0.1917 | 16 | 0.1833 | 29 | 0.0317 | 34 | 0.0717 | 20 | 0.1032 |
| 15 | 0.2983 | | | 13 | 0.0050 | 15 | 0.0300 | 17 | 0.3067 | 29.2 | 0.0450 | 34.2 | 0.0017 | 21 | 0.1314 |
| 16 | 0.0717 | | | | | 16 | 0.0100 | 17.3 | 0.0033 | 30 | 0.1017 | 35 | 0.1567 | 21.3 | 0.0033 |
| 17 | 0.0183 | | | | | | | 18 | 0.2750 | 30.2 | 0.0917 | 35.3 | 0.0033 | 22 | 0.1265 |
| 18 | 0.0050 | | | | | | | 19 | 0.1267 | 31 | 0.1767 | 36 | 0.2217 | 22.2 | 0.0017 |
| | | | | | | | | 20 | 0.0250 | 31.1 | 0.0017 | 37 | 0.2183 | 23 | 0.0799 |
| | | | | | | | | 21 | 0.0017 | 31.2 | 0.1467 | 37.1 | 0.0017 | 24 | 0.0765 |
| | | | | | | | | | | 32 | 0.1633 | 37.3 | 0.0117 | 25 | 0.0599 |
| | | | | | | | | | | 32.2 | 0.0783 | 38 | 0.1250 | 26 | 0.0333 |
| | | | | | | | | | | 33 | 0.0683 | 38.3 | 0.0267 | 27 | 0.0499 |
| | | | | | | | | | | 33.2 | 0.0300 | 39 | 0.0650 | 28 | 0.0516 |
| | | | | | | | | | | 34 | 0.0150 | 39.3 | 0.0083 | 29 | 0.0349 |
| | | | | | | | | | | 34.2 | 0.0033 | 40 | 0.0150 | 29.2 | 0.0017 |
| | | | | | | | | | | | | 41 | 0.0017 | 30 | 0.0150 |
| | | | | | | | | | | | | 41.3 | 0.0050 | 31 | 0.0166 |
| | | | | | | | | | | | | 42.3 | 0.0017 | 32 | 0.0183 |
| | | | | | | | | | | | | 43.3 | 0.0050 | 33 | 0.0100 |
| | | | | | | | | | | | | | | 34 | 0.0033 |
| | | | | | | | | | | | | | | 35 | 0.0033 |
| | | | | | | | | | | | | | | 36 | 0.0033 |
| | | | | | | | | | | | | | | 37 | 0.0017 |



Forensic Efficiency Parameters for 8 X-STRs

| 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 | 0.920 | 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 | >0.9999 |
| MEC _{duo} | 0.586 | 0.276 | 0.350 | 0.494 | 0.610 | 0.788 | 0.729 | 0.847 | 0.9997 |
| PD _{female} | 0.904 | 0.662 | 0.740 | 0.852 | 0.916 | 0.976 | 0.961 | 0.988 | >0.9999 |
| PD _{male} | 0.760 | 0.484 | 0.565 | 0.694 | 0.776 | 0.886 | 0.851 | 0.920 | >0.9999 |
| HWE (p-value) | 0.618 | 0.200 | 0.199 | 0.567 | 0.386 | 0.264 | 0.246 | 0.016 | |



Haplotype Analysis for Linked X-STR Duo

| Linkage group | Haplotype | No. of Haplotype | Haplotype diversity |
|------------------------|-------------------------|------------------|------------------------|
| Linkage group 1 | DXS10135-DXS8378 | 56 | 0.9640 ± 0.0029 |
| Linkage group 2 | DXS7132-DXS10074 | 41 | 0.9473 ± 0.0038 |
| Linkage group 3 | HPRTB-DXS10101 | 59 | 0.9662 ± 0.0029 |
| Linkage group 4 | DXS10134-DXS7423 | 38 | 0.9157 ± 0.0073 |

68% of all the observed haplotypes showed frequencies < 0.02



Summary of 8 X-STRs Analysis

- Each linkage duo was proved to be highly polymorphic for forensic applications.
- X-STR duo in linkage groups 4 showed low haplotype diversity in Korean, Japanese population but not in European populations.
- There will be a need for discovering a new marker for Asians that can serve as an adequate substitute for DXS7423 or at least complement the existing linkage duo of DXS10134-DXS7423.



Analysis of 4 X-STR using in-house quadruplex

Allele Frequencies for 4 X-STRs

| DXS10079 | | DXS10103 | | DXS10146 | | | | DXS10148 | | | |
|----------|--------|----------|--------|----------|--------|--------|--------|----------|--------|--------|--------|
| Allele | Freq. | Allele | Freq. | Allele | Freq. | Allele | Freq. | Allele | Freq. | Allele | Freq. |
| 14 | 0.0033 | 14 | 0.0017 | 21 | 0.0017 | 34 | 0.0100 | 17 | 0.0033 | 27.1 | 0.1033 |
| 15 | 0.0033 | 15 | 0.0117 | 22 | 0.0033 | 35 | 0.0067 | 18 | 0.1050 | 27.2 | 0.0017 |
| 16 | 0.0167 | 16 | 0.3067 | 23 | 0.0050 | 42.2 | 0.0100 | 18.1 | 0.0017 | 28 | 0.0017 |
| 17 | 0.0550 | 16.2 | 0.0017 | 24 | 0.0100 | 43.2 | 0.0116 | 19 | 0.0767 | 28.1 | 0.0533 |
| 18 | 0.1000 | 17 | 0.0783 | 25 | 0.0765 | 44.2 | 0.0033 | 20 | 0.0367 | 28.2 | 0.0017 |
| 19 | 0.2100 | 18 | 0.1850 | 26 | 0.1082 | 45.2 | 0.0017 | 20.1 | 0.0050 | 29.1 | 0.0500 |
| 20 | 0.2750 | 18.2 | 0.0017 | 27 | 0.1747 | | | 21 | 0.0183 | 29.2 | 0.0033 |
| 21 | 0.2067 | 19 | 0.3317 | 28 | 0.2063 | | | 21.1 | 0.0233 | 30.1 | 0.0300 |
| 22 | 0.1050 | 20 | 0.0750 | 29 | 0.1614 | | | 22.1 | 0.0533 | 30.2 | 0.0017 |
| 23 | 0.0167 | 21 | 0.0067 | 30 | 0.1098 | | | 23.1 | 0.0717 | 31.1 | 0.0183 |
| 24 | 0.0083 | | | 31 | 0.0666 | | | 24.1 | 0.1000 | 32.1 | 0.0017 |
| | | | | 31 | 0.0666 | | | 25.1 | 0.1033 | 32.2 | 0.0017 |
| | | | | 32 | 0.0150 | | | 26.1 | 0.1283 | 33.1 | 0.0017 |
| | | | | 33 | 0.0183 | | | 26.2 | 0.0033 | | |



Forensic Efficiency Parameters for 4 X-STRs

| 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 | >0.9999 |
| MEC_{duo} | 0.667 | 0.572 | 0.753 | 0.845 | >0.9999 |
| PD_{female} | 0.940 | 0.897 | 0.968 | 0.988 | >0.9999 |
| PD_{male} | 0.813 | 0.749 | 0.866 | 0.919 | >0.9999 |
| HWE (<i>p</i>-value) | 0.659 | 0.827 | 0.835 | 0.419 | |



Haplotype Analysis of 4 Linked X-STR trios

| | 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 |

- 94.1% of all haplotype showed frequencies < 0.02
- 77.1% of all haplotype showed frequencies < 0.01

By addition 4 X-STRs to 4 linkage groups, haplotype frequencies showed lower and value of haplotype diversity was increased effectively.



Conclusions

- Four linked X-STR trios are highly polymorphic in Koreans. Therefore, 4 closely linked X-STR trios can provide a powerful tool for solving complex kinship cases in Koreans.
- By addition 4 X-STR markers to 4 linked X-STR duos, it could be overcome the low haplotype diversity values that was showed in X-STR duo of linkage groups 4.
- In order to apply these haplotype data in forensic caseworks, it is necessary to evaluate haplotype stability and independency between linkage groups.



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