Genetic Polymorphism and Forensic Efficiency of Five X-STR Loci in Korean

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Why X-STRs?

- In deficiency cases, the *mean exclusion chance* of X-STRs tends to be higher then that of autosomal STR loci.
  - Males are hemizygous for X-STRs.
- In some *special cases of paternity testing*, the established methods can be effectively supplemented by X chromosome marker investigation.

When are X-STRs applicable?

- If in a kinship case the question to be solved is whether *two women who were separated as children could be sisters*, exclusion can be detected using X-markers in contrast to autosomal STRs.
- X-STR may be valuable to conform a *grandmother-grandchild relationship*.
- X chromosome marks are ***only applicable when the disputed child is female***.

Aim of this study

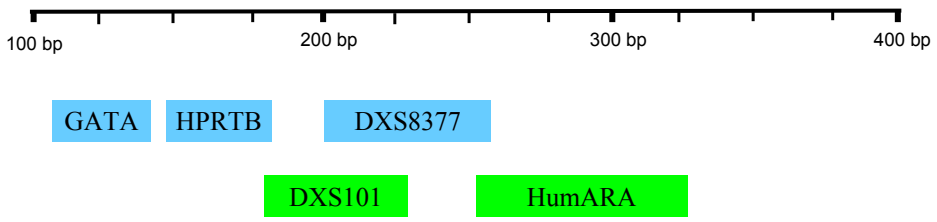
- To explore 5 X-STR's potential utility for forensic application.
  - GATA172D05 (Yuan *et al*. 1997)
  - HPRTB (*Edwards et al.* 1991)
  - DXS8377 (Hu *et al.* 1996)
  - DXS101 (Allen and Belmont 1993)
  - HumARA (*Edwards et al.* 1991)
  - Edelmann *et al*. *Forensic Sci. Int.* 2001
  - Zarrabeitia *et al*. *Forensic Sci. Int. & Int. J. Legal Med.* 2002

Material and Methods

DNA Extraction

- Buccal swab samples were obtained from 150 unrelated Korean men and women.
- Genomic DNA was extracted using QIAamp DNA Mini Kit (QIAGEN) according to the manufactures instructions.

Multiplex PCR Design



Primer of 5 X-STR loci

Locus	Sequence	μM	Dye
GATA172D05			
Primer 1	5'-TAG TGG TGA TGG TTG CAC AG-3'	0.05	FAM
Primer 2	5'-ATA ATT GAA AGC CCG GAT TC-3'		-
HPRTB			
Primer 1	5'-TCT CTA TTT CCA TCT CTG TCT CC-3'	0.06	FAM
Primer 2	5'-TCA CCC CTG TCT ATG GTC TCG-3'		-
DXS8377			
Primer 1	5'-CAC TTC ATG GCT TAC CAC AG-3'	0.2	FAM
Primer 2	5'-GAC CTT TGG AAA GCT AGT GT-3'		-
DXS101			
Primer 1	5'-ACT CTA AAT CAG TCC AAA TAT CT-3'	0.4	HEX
Primer 2	5'-AAA TCA CTC CAT GGC ACA TGT AT-3'		-
HumARA			
Primer 1	5'-TCC AGA ATC TGT TCC AGA GCG TGC-3'	0.8	HEX
Primer 2	5'-GCT GTG AAG GTT GCT GTT CCT CAT-3'		-

PCR Condition

10× PCR Buffer	1.7 ㎕
dNTPs	1.0 ㎕
Primer	2.0 ㎕
Gold Taq Enzyme	0.3 ㎕
Template DNA	0.5 ㎕
dH_2O	7.0 ㎕
Final Volume	12.5㎕

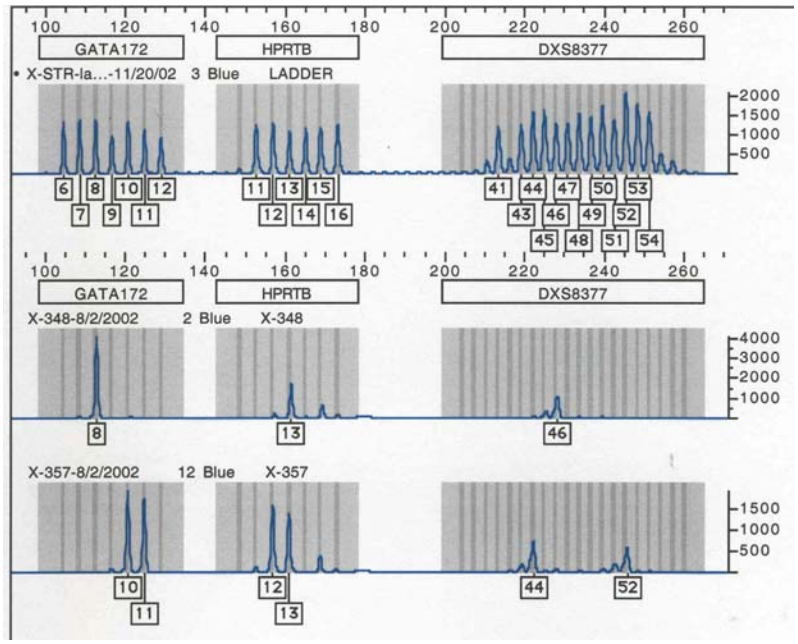
95℃	11 min
× 31 ~ 32 cycles	
94℃	1 min
55℃	1 min
72℃	1 min
60℃	30 min

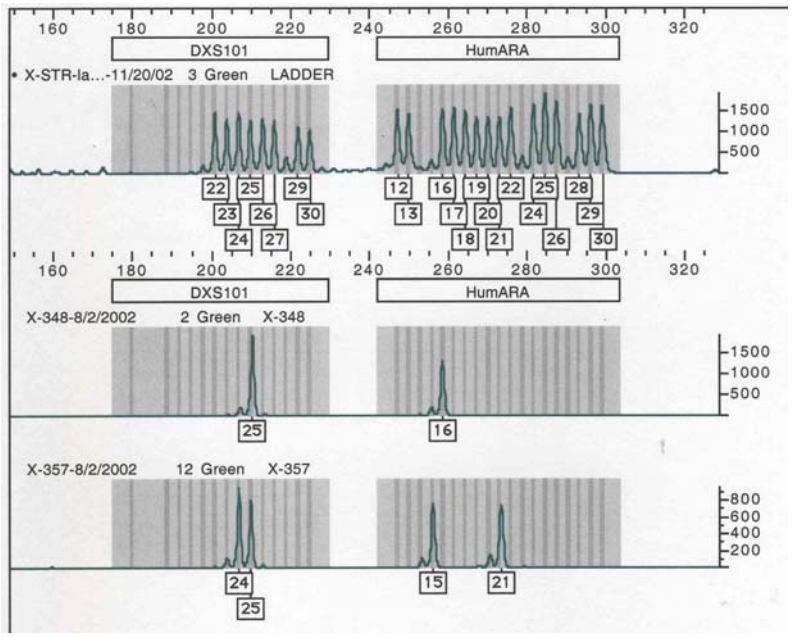
Allelic Ladder Construction

- Fragment Analysis
  - ABI 310 Genetic Analyzer
  - GeneScan 3.1
- Allele Sequencing
  - BigDye Terminator Sequencing Kit 2.0
  - ABI 310 Genetic Analyzer
  - Sequencing Analysis 3.3
  - Sequence Navigator 1.0

Allele Designation

- Allele Nomenclature
  - Generally followed the recommendation by the ISFG Commision.
  - HPRTB and HumARA used the allele nomenclature that appeared in previous studies.
- Create GenoTyper Macro
  - GenoTyper 2.5





Statistical Analysis

- PowerStat (Promega Co.)
  - Heterozygosity
  - Polymorphic Information Content
- Desmarais *et al.* (1998)
  - MEC (trio case)
  - PE (motherless case)
  - PD (male, female)
- Genetic Data Analysis (Lewis)
  - Hardy-Weinberg Equilibrium
  - Linkage Disequilibrium

Results and Discussion

GATA172D05

Allele	Female	Male	Cumulated
6	0.083	0.073	0.080
7	0.003	0.007	0.004
8	0.150	0.167	0.156
9	0.087	0.087	0.087
10	**0.413**	**0.380**	**0.402**
11	0.223	0.220	0.222
12	0.040	0.067	0.049
Het	0.733	–	
PIC	0.706	–	
MEC	0.706	–	
PE	0.596	–	
PD	0.898	0.762	

HPRTB

Allele	Female	Male	Cumulated
11	0.030	0.047	0.036
12	0.323	0.293	0.313
13	**0.420**	**0.407**	**0.416**
14	0.193	0.180	0.189
15	0.027	0.053	0.036
16	0.007	0.020	0.011
Het	0.633	–	
PIC	0.621	–	
MEC	0.621	–	
PE	0.520	–	
PD	0.839	0.711	

DXS8377

Allele	Female	Male	Cumulated
40	0.003	–	0.002
41	0.007	0.013	0.009
42	0.043	0.013	0.033
43	0.030	0.027	0.029
44	0.083	0.100	0.089
45	0.110	0.113	0.111
46	**0.150**	0.087	0.129
47	0.087	0.133	0.102
48	0.120	**0.167**	**0.136**
49	0.130	0.073	0.111
50	0.077	0.080	0.078
51	0.057	0.080	0.064
52	0.047	0.027	0.040
53	0.023	0.033	0.027
54	0.007	0.040	0.018
55	0.013	0.007	0.011
56	0.007	–	0.004
57	0.003	0.007	0.004
58	0.003	–	0.002
Het	0.933	–	
PIC	0.897	–	
MEC	0.897	–	
PE	0.814	–	
PD	0.983	0.901	

DXS101

Allele	Female	Male	Cumulated
21	0.017	–	0.011
22	0.037	0.040	0.038
23	0.113	0.100	0.109
24	**0.310**	0.213	**0.278**
25	0.183	**0.273**	0.213
26	0.167	0.207	0.180
27	0.110	0.120	0.113
28	0.047	0.027	0.040
29	0.007	0.007	0.007
30	0.010	0.013	0.011
Het	0.827	–	
PIC	0.790	–	
MEC	0.790	–	
PE	0.662	–	
PD	0.942	0.810	

HumARA

Allele	Female	Male	Cumulated
12	0.010	0.007	0.009
13	–	0.007	0.002
14	–	–	–
15	0.007	0.013	0.009
16	0.013	0.013	0.013
17	0.017	0.027	0.020
18	0.027	0.033	0.029
19	0.033	0.067	0.044
20	0.067	0.093	0.076
21	0.153	0.107	0.138
22	0.133	**0.207**	**0.158**
23	0.153	0.113	0.140
24	**0.157**	0.093	0.136
25	0.090	0.073	0.084
26	0.037	0.047	0.040
27	0.053	0.027	0.044
28	0.037	0.033	0.036
29	0.007	0.020	0.011
30	0.007	0.020	0.011
Het	0.847	–	
PIC	0.880	–	
MEC	0.880	–	
PE	0.812	–	
PD	0.978	0.899	

HWE & Linkage Test

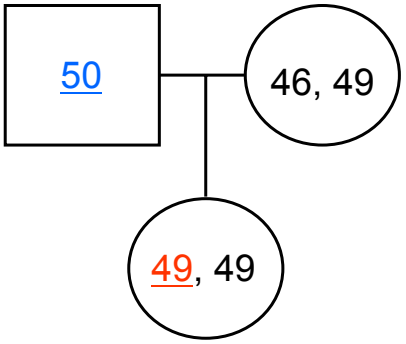
- No deviation from Hardy-Weinberg equilibrium
- No evidence of statistically significant linkage disequilibrium

Mutation Rate of 5 X-STRs

- HPRTB
  - 2 mutations among 580 meioses (Szibor *et al*. 2000)
- DXS8377
  - 1 mutation among 107 meioses (Zarrabeitia *et al*. 2002)
- GATA172D05, DXS101, HumARA
  - No report on mutation rate

Mutation of DXS8377

- 1 mutation among 48 meioses



Conclusion

- DXS8377 and HumARA which have many alleles are polymorphic STRs that can be very useful in forensic cases.
- We believe 5 X-STRs work with reasonable amounts of DNA and may be particularly suitable for difficult deficiency paternity cases.