# Genetic characteristics of 22 Y-STRs in Koreans: Atypical alleles, deletions in the DYS385 flanking regions, and null alleles associated with *AZFc* microdeletions


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## Introduction

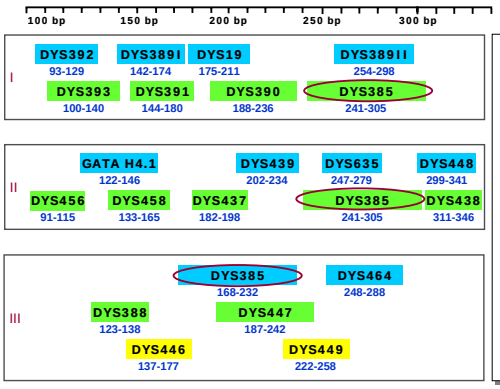
To construct a Korean Y-chromosomal STR database for 22 Y-STRs (DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS446, DYS447, DYS448, DYS449, DYS456, DYS458, DYS464, DYS635 and GATA H4.1), 708 Korean males were analyzed using three in-house PCR sets. During analysis, we could find non-standard alleles at several Y-STR loci. Moreover, discordant allele designations at DYS385 according to primer binding sites and a null type at DYS448 were shown. Here, we present characteristics of atypical alleles, the molecular basis for these allele designation discrepancies and null allele.

## Materials and Methods

### DNA samples

Blood or buccal samples from 708 unrelated Korean males, including 355 males already typed for 22 Y-STRs (Lee et al., FSI 2007;121:128-35) and 301 males typed for 19 Y-STRs (Park et al., FSI 2005;152:133-47), were analyzed. Genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).

### Schematic of 3 multiplex PCRs for 22 Y-STRs




**PCR reaction**: Total 10 μl reaction contained 1 ng of DNA, 1.6 μl of Gold STR 10X buffer (Promega, Madison, WI), 2.0 U of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA), appropriate concentration of each primer.

**Cycling condition**: 95°C 11 min; 94°C 1 min, 55~59°C 1 min, 72°C 1min x 28~30 cycles; 60°C 45 min using the GeneAmp 9600

**Detection system**: ABI prism 310 Genetic Analyzer, GeneScan software 3.1 and Genotyper 3.7 software (Applied Biosystems)

DYS385 is included in all the three multiplexes to detect sample switching, but the multiplex III contains smaller amplicon than the multiplex I/II.

### Multiplex PCR assay to characterize the null allele at DYS448

Five *AZFc*-specific sequence tagged site (STS) markers, sY1161, sY1191, sY1201, sY1206 and sY1291, and a control gene pair ZFX/ZFY were amplified to detect rearrangements at the azoospermia factor c (*AZFc*) region. Primers for the five markers and the ZFX/ZFY gene were the same as used by Lin et al. (Mol Hum Reprod 2006;12;347-51).

## Results

### Atypical alleles in DYS447, DYS449, DYS458 and DYS464

| Locus | Allele | Sequence structure | N |
|---|---|---|---|
| DYS447 | 18 | $(TAATA)_7TAAAA_1(TAATA)_{10}$~~$TAAAA(TAATA)_n$~~ | 2 |
| | 19 | $(TAATA)_7TAAAA_1(TAATA)_{11}$~~$TAAAA(TAATA)_n$~~ | 4 |
| DYS449 | 26 (25)* | $(TTTC)_{12}$-~~tctc~~-N10-N32-cttc-$(TTTC)_{14}$ | 1 |
| | 30 (30.1)* | $(TTTC)_{16}$-tctc-N10-N32-c~~ttt~~c-$(TTTC)_{14}$ | 1 |
| | 33 (42)* | $(TTTC)_{15}$-tctc-N10-[N32-cttc]$_2$-$(TTTC)_{18}$ | 1 |
| | 27.2 | $(TTTC)_3TC(TTTC)_{10}$-tctc-N10-N32-cttc-$(TTTC)_{14}$ | 1 |
| | 28.2 | $(TTTC)_3TT(TTTC)_{11}$-tctc-N10-N32-cttc-$(TTTC)_{14}$ | 1 |
| | 29.2 | $(TTTC)_3TC(TTTC)_{10}$-tctc-N10-N32-cttc-$(TTTC)_{14}$ | 1 |
| | 30.2 | $(TTTC)_{16}$-tctc-N10-N32-cttc- $(TTTC)_{10}TT(TTTC)_4$ | 12 |
| DYS458 | 14.1 | $(GAAA)_{14}G$ | 1 |
| | 17.2 | $(GAAA)_{15}AA(GAAA)_2$ | 1 |
| DYS464 | 12.3 | $(CCTT)_7CTT(CCTT)_5$ | 1 |
| | 14.3 | $(CCTT)_3CTT(CCTT)_{11}$ | 5 |

*** Ostensible alleles at DYS449 are indicated in brackets.**

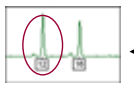
### Duplicated alleles at DYS19, DYS390 and DYS447

| Locus | | Allele | sequence structure |
|---|---|---|---|
| DYS19 |  | 16,17 | $(TAGA)_3tagg(TAGA)_{13,14}$ |
| DYS390 |  | 22,23 | $(TCTG)_8(TCTA)_{9,10}(TCTG)_1(TCTA)_4$ |
| DYS447 |  | 23,24 | $(TAATA)_7TAAAA(TAATA)_{7,8}TAAAA(TAATA)_7$ |

### Characterization of allele designation difference at DYS385

**Difference by 1 repeat in 5 samples**

Multiplex I/II: AmpFISTR® Yfiler™ Powerplex® Y



Multiplex III

Due to 4 bp deletion in this flanking region

$(gagaaaaa)_2$-N38-$(aagg)_{6\to5}$ $(GAAA)_{13}$

**Difference by 2 repeat in 3 samples**

Multiplex I/II: AmpFISTR® Yfiler™ Powerplex® Y

Multiplex III

Due to 8 bp deletion in this flanking region

$(gagaaaaa)_{2\to1}$-N38-$(aagg)_6(GAAA)_{19}$

F primer in the multiplex I/II

```
agcatgggtg acagagctag acaccatgcc aaacaacaac aaagaaaaga aatgaaattc agaaaggaag
gaaggaagga gaaagaaagt aaaaaagaaa gaaagagaaa agaaagaaaa gaaagaaaga gaagaaagag
aaagaggaaa gagaaagaaa ggaaggaagg aaggaaggaa gggaaagaaa gaaagaaaga aagaaagaaa
gaaagaaaga aagagaaaaa gaaaggagga ctatgtaatt ggaatagata gattattttt taaaatattt
ttattacctt tacagttttt ttaaatgccg ccatttcaga aagaaaatctg gtcagcagcc cttaccagct
```

F primer in the multiplex III

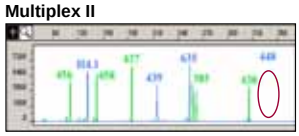
R primer in the multiplex I/II

R primer in the multiplex III

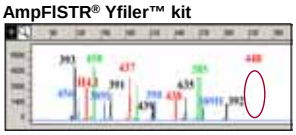
**PCR using the primers in the multiplex III reflects only the variations in DYS385 core repeats.**

### Null alleles associated with *AZFc* microdeletions

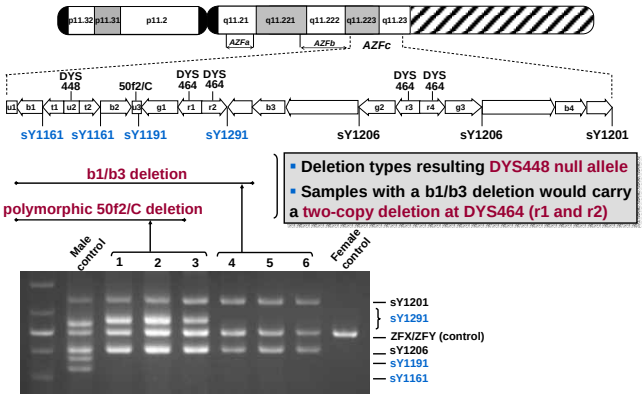
**Multiplex II**



**AmpFISTR® Yfiler™ kit**



**Six samples showed a null allele at DYS448**




- Deletion types resulting DYS448 null allele
- Samples with a b1/b3 deletion would carry a two-copy deletion at DYS464 (r1 and r2)

### Frequencies of null allele at DYS448 in various ethnic groups

| | | | |
|---|---|---|---|
| Korean | 6 in 708 | 0.85 % | Present study |
| Japanese | 10 in 1079 | 0.93 % | Mizuno et al. FSI In press |
| Nepalese | 3 in 769 | 0.39 % | Parkin et al. FSI 2006 |
| Malays, Chinese and Indian in Malaysia | 3 in 980 | 0.31 % | Chang et al. FSI 2006 |
| Kalmyk | 7 in 99 | 7.07 % | Roewer et al. FSI In press |
| Mexican | 1 in 326 | 0.31 % | Gutiérrez-Alarcón et al. Leg Med 2007 |
| Spanish | 1 in 247 | 0.41 % | Sánchez et al. FSI In press |
| Asian | 2 in 330 | 0.61 % | AmpFISTR® Yfiler™ database |
| African American | 2 in 985 | 0.20 % | AmpFISTR® Yfiler™ database |
| Caucasian (USA) | 2 in 1276 | 0.16 % | AmpFISTR® Yfiler™ database |

**The relatively high frequencies of the DYS448 null allele in Asians suggest giving careful consideration to the use of DYS448 for commercial genotyping and further database construction in Asians.**

## Conclusion

- **18 microvariant alleles at DYS449, 6 intermediate alleles at DYS464, 2 intermediate alleles at DYS458, and 1 duplicated allele at DYS19, DYS390 and DYS447 have been discovered and characterized.**
- **At DYS385, two deletion mutations were observed in the upstream flanking region of the core repeat units. Therefore, selection of primer pairs for criminal investigations or for storing DNA profiles in national DNA database should be carried out with more consideration of the purpose of analysis and the possible presence of mutations in flanking sequences.**
- **Deletions of DYS448 and DYS464 were observed in association with *AZFc* rearrangement. A DYS448 null allele was more frequent in Asians than any other population.**
- **These findings will provide useful information in forensic practice for improved interpretation of Y-STR data and database construction.**