Abstract We have developed four multiplex genotyping systems (GeneKin Y-STR multiplexes) using silver staining with allelic ladders for ten Y-chromosome STR markers (DYS19, DYS385, DYS388, DYS389I/II, DYS390, DYS391, DYS392, DYS393 and DXYS156Y), with a view towards the application of rapid and simple genotyping assay methods for DNA profiling. The GeneKin Y-STR multiplexes developed have followed the published nomenclature and ISFG guidelines for STR analysis. Allele and haplotype frequencies at these Y-STRs loci were analysed by PCR amplification using the GeneKin Y-STR multiplexes, followed by denaturing polyacrylamide gel electrophoresis in 316 unrelated males in the Korean population. A total of 295 different haplotypes were found, 279 of them being unique. Gene diversity ranged from 0.4026 at DYS391 to 0.9606 at DYS385. The haplotype diversity value (which is the same as the discrimination index) calculated from all ten loci combined was 0.9995, which is informative. Our results revealed that a set of ten Y-STRs can discriminate between most of the male individuals in the Korean population (discrimination capacity: 93.35%). The Y-STR multiplexes thus provide useful information for forensic analysis and paternity tests and can also be of great benefit for providing information not normally available from autosomal DNA systems.

Keywords Allelic ladders · DNA profiling · Koreans · STRs · Y-chromosome haplotypes

Introduction

The usefulness of Y-chromosome markers for studies of human population genetics and forensic analysis has recently been recognised. The non-recombining portions of the human Y-chromosome have the special features of a haploid transmission pattern and father-to-son transmission. The DNA sequence of the non-recombining portions contains a genetic record only of the mutational events that occurred in the past. As a consequence, haplotypes constructed from Y-chromosomal alleles at multiple polymorphic sites can be used to study paternal lineages [1] and to differentiate human population groups [2].

Studies of ancient divergences in human evolution require polymorphisms with low probabilities of back and parallel mutation and for which ancestral states can be determined [3]. Unique polymorphisms, single-base substitutions and small insertions or deletions, are best suited for this purpose. In contrast, most microsatellite, or short tandem repeat (STR) loci have a high level of variability due to high mutation rates [4]. Therefore, Y-STR haplotypes are more useful for investigating and reconstructing the phylogeny of the more recently diversified Y-chromosome [5], as well as for forensic and paternity testing [6].

In this study, we examined whether a set of ten Y-STR loci detected by four multiplex genotyping systems are capable of DNA profiling in the Korean population and here we report Y-haplotype frequency and statistical parameters of forensic interest.

Material and methods

DNA samples

We studied 316 unrelated healthy males selected at random from the South Korean population. All individuals had birthplaces in Korea and Korean surnames. DNA was extracted from whole blood samples by the standard method of phenol-chloroform-isoamyl alcohol extraction [7], or from buccal cells according to Richards et al. [8] and quantified by a DyNA Quant 200 DNA fluorometer (Hoefer Pharmacia Biotech, San Francisco, Calif.).
PCR amplification conditions

The allelic variations at the ten Y-STR loci (DYS19, DYS385, DYS388, DYS389I/II, DYS390, DYS391, DYS392, DYS393, and DXYS156Y) were analysed by PCR amplification with four multiplex systems (System I: DYS19, DYS388, DYS392; System II: DYS390, DYS391, DYS393; System III: DYS389I/II, DXYS156Y; System IV: DYS385, DXYS156Y). Multiplex PCR amplifications (Systems I–III) were performed in a volume of 25 µl containing 10 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, 160 µg BSA, 200 µM dNTPs, 5–10 pM each primer, 2.0 U AmpliTaq DNA polymerase (PE Biosystems). System IV for the amplification of DYS385 was carried out in a 25 µl volume containing 20 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, 160 µg BSA, 2.5 mM MgCl₂, 200 µM dNTPs, 10 pM each primer, 2.0 U AmpliTaq DNA polymerase. The primer sequences for DYS19, DYS388, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DXYS156Y are described in Kayser et al. [4] and for DYS385 in Schneider et al. [9].

Cycling conditions

Amplification reactions were carried out in a Perkin Elmer 9600 thermal cycler, and cycling conditions were as follows:

**Multiplex system I.** Initial denaturation 94 °C for 5 min followed by 35 cycles at 94 °C for 1 min, 57 °C for 1 min, 72 °C for 1.5 min and final extension at 72 °C for 3 min.

**Multiplex system II.** Initial denaturation 94 °C for 5 min followed by 5 cycles at 94 °C for 30 s, 58 °C for 30 s, 72 °C for 45 s, then 30 cycles at 94 °C for 30 s, 54 °C for 30 s, 72 °C for 45 s and final extension at 72 °C for 3 min.

**Multiplex system III.** Initial denaturation 94 °C for 3 min followed by 5 cycles at 94 °C for 20 s, 58 °C for 20 s, 72 °C for 30 s, then 30 cycles at 94 °C for 20 s, 54 °C for 30 s, 72 °C for 30 s and final extension at 72 °C for 3 min.

**Multiplex system IV.** Initial denaturation 94 °C for 3 min followed by 5 cycles at 94 °C for 15 s, 58 °C for 30 s, 72 °C for 1 min, then 30 cycles at 94 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min and final extension at 72 °C for 7 min.

Electrophoresis conditions

Detection of the amplified products was carried out by electrophoresis on a 6% denaturing polyacrylamide gel containing 8 M urea, in 0.5 × TBE buffer, for 2.5 h at a constant 50 W, with a separation distance of 40 cm. Bands were visualised by silver staining [10].

Allele designation and nomenclature

Y-STR alleles were identified using the GeneKin Y-STR multiplexes (Genekotech, Seoul, Korea) based on the number of variable repeats. Initially, our DNA samples were compared to reference samples, kindly provided by M. F. Hammer (University of Arizona, USA: DYS19, DYS390, DYS391, DYS393, and DXYS156Y), C. Tyler-Smith (Oxford University, UK: DYS389I/II) and L. Roewer (Humboldt University, Berlin: DYS385, DYS388, DYS392). Finally, the allelic ladders have been standardised for each locus with the reference DNA samples and sequenced new allelic variants detected in this study. Allele nomenclature follows Kayser et al. [4] and de Knijff et al. [6], for DYS385 and DXYS156Y which is described in Schneider et al. [9] and Karafet et al. [11], respectively.

Table 1 Allele frequencies and gene diversity value at nine Y-chromosome STR loci in the Korean population (n = 316)

<table>
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\[ h^a \] = 0.6832 0.5093 0.6699 0.7349 0.6255 0.4026 0.6842 0.6380 0.6939

\[^a\] h: gene diversity value