International Congress Series 1239 (2003) 283–286

# Genotyping of 9 STR systems in combination with 11 diallelic polymorphisms on the Y-chromosome by fragment analysis and minisequencing

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*Keywords:* Forensic; Y-chromosome; SNPs; STRs

## 1. Introduction

The study of Y chromosomal haplotypes and their relationship to human evolution and variation is increasing rapidly in the fields of anthropology and forensic genetics. Although autosomal STRs are commonly used and very informative for paternity testing and forensic identification, the use of the nonrecombining portion of the Y chromosome is important and provides additional data in cases when the offspring is a male or for mixed male/female crime stains. For this purpose, in the past 2 years, more and more attention has been paid to the examination of diallelic polymorphisms (SNPs) on the Y chromosome [1].

Here, we describe an approach for the typing of 10 Y-chromosomal diallelic polymorphisms (SNPs) using single base extension technology (minisequencing).

The frequencies and haplotype relationships of diallelic polymorphisms (Table 1) in a sample of 95 individuals of Caucasian origin were examined. In addition to the SNPs, we also tested the Y-chromosomal diallelic Alu repeat insertion DYS287 (YAP) [2] as well as

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PII: S0531-5131(02)00323-0

Table 1
Primers used for SNP amplification

| Primer name | Primer sequence (5′ −3′ ) | Primer name | Primer sequence (5′ −3′ ) |
|---|---|---|---|
| SRY 2627-1 | AGGTCTTTTTTGCCTTCTTA | SRY 2627-3 | TTCCTCGGAACCACTACCAG |
| 92R7-2 | GCCTATCTACTTCAGTGATTTCT | 92R7-3 | CTCAGCCTCCCAAAGTTCTG |
| M9-1 | GCAGCATATAAAACTTTCAGG | M9-2 | AAAACCTAACTTTGCTCAAGC |
| SRY 1532-1 | TCCTTAGCAACCATTAATCTGG | SRY 1532-2 | AAATAGCAAAAAATGACACAAGGC |
| M13-1 | TAGTTTATGCCCAGGAATGAAC | M13-2 | ATCCAACCACATTTGCAAAA |
| M17/19-1 | CTGGTCATAACACTGGAAATC | M17/19-2 | TGACCTACAAATGAGAAACTC |
| M20-1 | AGTTGGCCCTTTGTGTCTGT | M20-2 | CATGTTCAGTGCAAATGCAAC |
| SRY 8299-1 | GGTATGACAGGGGATGATGTGA | SRY 8299-2 | CCACGCCCAGCTAATTTTTTGT |
| Tat-1 | GACTCTGAGTGTAGACTTGTGA | Tat-2 | GAAGGTGCCGTAAAAGTGTGAA |

the Y-chromosomal STR systems DYS19, DYS389I+II, DYS390, DYS391, DYS392, DYS393 and DYS385 by fluorescent multiplex fragment analysis.

## 2. Materials and methods

### *2.1. DNA amplification for Y-SNPs and minisequencing*

Our approach is based on the products from two single PCRs for SRY-8299 and Tat, two duplex PCRs (SRY2627 together with 92R7, and SRY1532 together with M9) and one triplex PCR (M13 together with M17/M19 and M20). The amplification was performed in a Perkin Elmer 2400 Thermo Cycler. The 50-μl PCR reaction mixture consisted of 1×Gibco-BRL PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP mix, 250 nM of forward and reverse amplification primer (Table 1), 1 unit of *Taq* DNA polymerase (Gibco-BRL) and 150–300 ng of the DNA. The PCR was carried out for 38 cycles using the following conditions: denaturation at 96 °C for 60 s, annealing at 56–62 °C for 60 s, extension at 72 °C for 60 s, followed by a final extension step at 72 °C for 10 min. The PCR products were controlled by separation in a 1.5% agarose gel.

The PCR products were then pooled, primers and dNTPs were removed by digestion with ExonucleaseI and Shrimp alkaline phosphatase and used for minisequencing with the corresponding primers. The minisequencing reaction is then performed in a single reaction with the commercially available SNaPshot-Kit from Applied Biosystems and needs only one run per sample in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, CA, USA).

### *2.2. DNA amplification for Y-STRs*

The STR analysis was performed as quadruplex and triplex reactions, respectively. For the amplification, a Perkin Elmer 2400 Thermo Cycler was used. The 50-μl PCR reaction mixture consisted of 1×Gibco-BRL PCR buffer, 2 and 3 mM $MgCl_2$ for the quadruplex and triplex reactions, respectively, 300 μM dNTP mix, 100–400 and 100 nM of forward and reverse amplification primer for the quadruplex and triplex reactions, respectively, 2.5 units of *Taq* DNA polymerase (Gibco-BRL) and 100 ng of the DNA. PCR was carried out

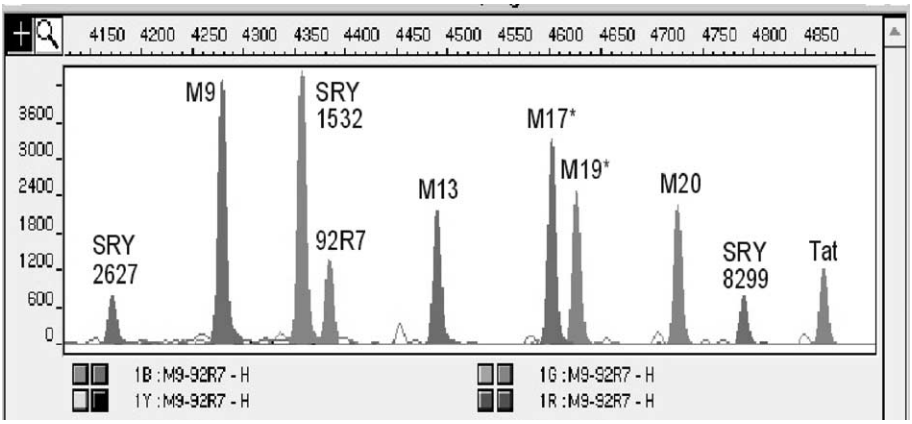


Fig. 1. Minisequencing of 10 Y-chromosomal SNPs.

for 28 cycles using the following conditions: denaturation at 95 °C for 30 s, annealing at 53 °C for 60 s, extension at 72 °C for 20 s, followed by a final extension step at 72 °C for 10 min. The PCR products were separated in an ABI PRISM 310 Genetic Analyzer. The primer sequences used for the Y-chromosomal markers were those described by Kayser et al. [3] and Schneider et al. [4], for the Alu insertion as described previously [2].

## 3. Results and discussion

The typing of SNPs and DYS287 in 95 unrelated Germans results in five different haplogroups. For separation of all 10 SNPs in one CE run, the spacing of SNPs has been

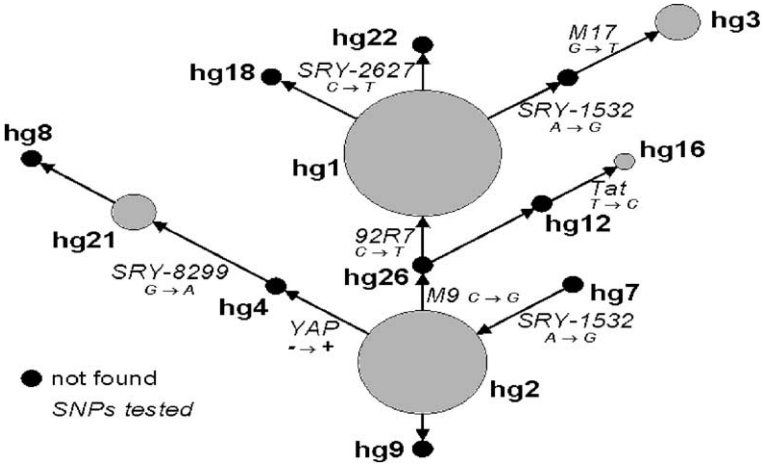


Fig. 2. Unrooted maximum parsimony Y chromosome tree based on 11 diallelicpolymorphisms among 95 Germans [5].

Table 2
Observed haplogroups and haplotypes among 95 Germans

| Haplogroups | *n* | % | STR haplotypes | STR duplicates |
|---|---|---|---|---|
| hg2 | 33 | 34.7 | 32 | 1 |
| hg21 | 11 | 11.6 | 11 | 0 |
| hg16 | 1 | 1.1 | 1 | 0 |
| hg1 | 39 | 41.1 | 35 | 4 |
| hg3 | 11 | 11.6 | 10 | 1 |

adjusted by adding "pig tails" of variable lengths to the 5′ ends of the SNP primers. The individual Y SNPs are analysed by the GeneScan software in one panel. An example is depicted in Fig. 1.

At least six different haplogroup nomenclatures exist at the moment. We have defined them according to the system of Jobling et al. [5] to haplogroups 2, 21, 16, 1, 3 and have arranged our results in a haplotype tree (Fig. 2).

The frequencies of the observed SNP haplogroups and STR haplotypes are shown in Table 2. For the Y STRs, we observed 64 alleles in 89 different haplotypes, 6 pairs of haplotypes were identical and they are always observed in the same haplogroup from apparently unrelated individuals and never between haplogroups.

The amplified DNA fragments generated by the PCR range from 106 to 363 base pairs in length. Therefore, it should be possible to use this method also when the DNA sample contains partially degraded DNA extracted from forensic case work samples.

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