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Y-STR loci multiplex amplification and haplotype analysis in a Chinese Han population

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Abstract. We have developed a multiplex PCR assay dealing with simultaneously amplifying 9 STR loci on Y-chromosome. These Y-STR markers included DYS434, Y-GATA-A10, DYS438, DYS439, DYS531, DYS557, DYS448, DYS456 and DYS444. A total of 101 different haplotypes was found among 120 unrelated males in a Chinese Han population by using the Y-STR-9-plex system, 91 of them being unique. Gene diversity ranged from 0.4394 at DYS434 to 0.7975 at DYS557. The haplotype diversity value calculated from all nine loci combined was 0.9968. The minimum amount of input DNA that could be used to obtain a full 9 Y-STR profile was 0.5 ng. For the male–male mixtures, the minor component in the mixture could be identified to a ratio of 1:9. In male–female DNA mixtures, the Y-STR-9-plex proved to be highly specific for the Y-chromosome in that no significant female DNA products were observed up to 300 ng of female DNA. Our results revealed that the Y-STR-9-plex system was useful for forensic analysis and paternity tests in the Chinese Han population. © 2005 Published by Elsevier B.V.

Keywords: Y-chromosome; Y-STR; Haplotype; Multiplex amplification; Chinese Han population

1. Introduction

Y-chromosome specific STR (Y-STR) loci are valuable tools in forensic casework in special situations. Some of Y-STR loci have proved to be a particularly robust set of genetic markers and have been successfully employed in casework analysis [1–9]. Despite their utility, additional Y-STR loci are also required in order to improve the discriminatory capacity of Y-STR markers. In the present work, we have developed a novel 9-locus Y-

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STR multiplex system that was designed to augment their use by providing significant additional discriminatory power.

2. Material and methods

2.1. DNA samples and extraction

DNA from reference and anonymous donor samples (120 males and 20 females) was obtained from the Han ethnic group in Chengdu of China and was extracted either by phenol–chloroform or Chelex100 procedures.

2.2. Multiplex PCR amplification

The total reaction volume for PCR was 37.5 μL. Amplification reactions contained 9.4 μL of Y-STR 9plex Primer Mix (0.20 to 0.45 μM primers), 2.0 U of home-product MBI Taq polymerase, 0.5–5 ng of DNA template, 200 μM each of dNTP (Pharmacia Biotech, Sweden) and sterile water to raise the volume to 37.5 μL. Amplification reactions were performed in a GeneAmpPCR systems 9600 (Applied Biosystems, Foster City, CA) with conditions as follows: 94 °C, 10 min; 10 cycles of 94 °C, 1 min; 56 °C, 50 s and 72 °C, 40 s; 25 cycles of 94 °C for 30 s, 56 °C for 40 s, and 72 °C for 30 s; 72 °C for 35 min and 4 °C until the samples were removed from the thermal cycler.

2.3. Analysis on a 310 genetic analyzer

PCR amplification products were subjected to capillary electrophoresis on the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). An appropriate matrix was established with matrix standards for the four dyes 6FAM, HEX, TAMRA, and ROX. The samples for analysis were prepared by combining the PCR product and Hi-Di formamide containing 0.2 μL GeneScan®-500[ROX] Size Standard in a 200 μL tube followed by denaturation at 95 °C for 3 min. The denatured products were subjected to electrophoresis on the 310 Genetic Analyzer. The run time was approximately 24 min. The samples were analyzed using Gene-Scan® Analysis and Genotyper® version 3.7 software (Applied Biosystems, Foster City, CA).

3. Results and discussion

3.1. Sensitivity studies

The sensitivity of the system was tested using three different male DNA samples. The optimal quantity of template DNA for Y-STR 9plex ranging from 0.5 to 5 ng proved effective over a wide range of input template DNA. The lower limit of template DNA necessary for a full nine-locus profile was 0.5 ng.

3.2. Male–female mixture studies

The male–female mixture samples were prepared in the proportions 1:0, 1:10, 1:50, 1:100, 1:200, 1:300, 1:400, 1:500, and 1:600 and were analyzed. The amount of male sample was kept constant at 0.5 ng based on previous male–female specificity analysis. A complete 9-locus Y-STR profile was detected in the male–female mixture samples up to the 1:300 ratio, which contained 0.5 ng of male

DNA and 150 ng of female DNA. Thus, it was possible to obtain a male profile in the presence of excess amounts of female DNA using the Y-STR 9plex.

3.3. Male–male mixture studies

DNA from two males was mixed in various ratios (1:1, 1:3, 1:6, 1:9, 1:12, 1:15, 1:20, 1:30). The presence of two individuals was clearly discernible when the minor donor was present up to a ratio of 1:9.

3.4. Population studies

Allelic frequency distributions at loci DYS434, GATA-A10, DYS438, DYS439, DYS531, DYS557, DYS448, DYS456 and DYS444 were investigated in a Chinese Han population sample in Chengdu. Our data showed that differences in allele frequency at loci DYS434, DYS438, DYS439 and DYS456, compared with those of YCC Asian cell lines, can be enormous. Therefore, establishing databases of haplotypes in different populations is a requirement for forensic biostatistic calculation. The combination of the allelic states of the 9 Y-STR loci allowed us to construct highly informative haplotypes. A total of 105 haplotypes were observed in 120 individuals, of which 97 haplotypes were unique and only 8 haplotypes were observed more than once. The cumulative haplotype diversity (HD) was calculated to be 99.68%.

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