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Y-SNP typing with the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Abstract. The purpose of our work was to establish a method for analysis of Y-SNP based on the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The results of our study implied that the analysis of Y-SNP was proved to be suitable for forensic application and provided new genetic markers for the forensic purpose. © 2005 Published by Elsevier B.V.

Keywords: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; Single nucleotide polymorphisms; Y chromosome

1. Introduction

The single nucleotide polymorphisms on Y chromosome (Y-SNP) were potential markers for forensic purpose. The matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has become one of the most promising techniques for the analysis of Y-SNP [1–4]. The most widely used MALDI-TOF SNP typing strategy is based on single nucleotide primer extension (SNuPE). However, there is a lack of population data from different ethnic groups. Here, we present the simultaneous genotyping of 3 Y-SNP loci in a Chinese Han male population sample.

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2. Material and methods

2.1. DNA samples and extraction

The blood samples were obtained from 105 male individuals from Chinese Han ethnic group in Chengdu and DNA was extracted by Chelex100 procedures.

2.2. Multiplex PCR amplification

The total reaction volume for PCR was 37.5 μl. Amplification reactions contained 2–10 ng of genome DNA, 0.4 ìM of Primer Mix (Table 1), Mg_2^+ 2.25 mM, 2.0 U Taq polymerase (Femantas, China) and 6.0 μl dNTP (0.2 mM). Amplification reactions were performed in a GeneAmpPCR systems 9600 (Applied Biosystems, Foster City, CA). Cycling conditions were 3 min at 94 °C followed by 32 cycles of 30 s at 94 °C, 30 s at 61 °C, 30 s at 72 °C and a final extension time of 7 min at 72 °C.

2.3. Analysis and purification of PCR products

The PCR products of multiplex PCR amplification for M9, M35 and M98 loci were analyzed by the polyacrylamide gel electrophoresis and visualized by sliver staining. In order to remove excess primers, dNTPs and salts, the genopure dsDNA purification kit for double-stranded DNA (Bruker Saxonia Analytik, Germany) was used. Purified PCR products were recovered in 5 μl elution buffer.

2.4. Multiplex primer extension reaction

The multiplex SNuPE primers were designed by us (Table 2). The purified PCR product was mixed with the extension mix containing 2.0 μl 10× Thermol Reaction Buffer (NEB, England), 3.0 U VentR®(exo-) DNA polymerase (NEB, England), 1 μM mix primers for M9, M35 and M98 polymorphism detection, 200 μM ddGTP or ddCTP (Pharmacia). Cycling conditions were 5 min at 95 °C followed by 45 cycles of 30 s at 95 °C, 40 s at 50 °C, 150 s at 65 °C and a final extension time of 2 min at 72 °C. Excess salts and nucleotides were removed using the genopure oligo purification system (Bruker Saxonia Analytik, Germany).

2.5. Mass spectrometry

1 μl of matrix solution (3-hydroxypicolinic acid, 5 mg/ml and dibasic ammonium citrate, 2 mg/ml in water) was pipetted on an AnchorChipk sample target (Bruker Daltonik) and allowed to dry at room temperature. 1 μl of purified products of extension

Table 1
The primers of multiplex PCR amplification for 3 Y-SNP loci

Locus	The sequence of primers	Amplicon size (bp)
M9	L : cgctgcagcatataaaactttc	233
	R : tgaagctcgtgaaacagattaga	
M35	L : gaaactgagagggcatggtc	130
	R : ggagctgtggtgaatgaaca	
M98	L : agattcacccacccactttg	396
	R : gagcctcaggattcaaagga	

Table 2
The primers of simultaneous primer extension reaction for 3 Y-SNP loci

Locus	Primer size	The sequence of primers (5′–3′)
M98-SNuPE	21 nt	tcccacgggtaattaacactg
M35-SNuPE	24 nt	tttcaattttcctttgggacactg
M9-SNuPE	27 nt	ttttttaacggcctaagatggttgaat

reaction was added to the dried matrix spots and dried at room temperature. The target was introduced into the source region of a BIFLEX III mass spectrometer with SCOUT MTP ion source (Bruker Daltonik), operated in linear mode with 16.3 and 19 kV on the conversion dynode and the sample target, respectively.

2.6. Statistical analysis

Both allele diversity of each locus and haplotype diversity were calculated using the formula $D=(n/n-1)(1-\sum \text{pi2})$ [5].

3. Results and discussion

Our results showed that Y-SNP typing with MALDI-TOF-MS yielded reliable results. In a single triplex reaction, all of alleles at 3 Y-SNP loci were identified with high accuracy by MALDI-TOF-MS. Differences in allele frequency of Chinese population at M9 locus from those of Caucasian and African American population samples described by Vallone and Butler [6] can be enormous. Therefore, establishing databases in different populations is a requirement. The combination of the allelic states of 3 Y-SNP loci allowed us to construct informative haplotypes. A total of 4 haplotypes were observed in 105 male individuals. The haplotype diversity was calculated to be 0.5104.

In conclusion, the results of our study implied that the analysis of Y-SNP was proved to be suitable for forensic application. However, significant difference between different ethnic groups was observed. The quantification of substructure for population at Y-SNP loci requires further intense research efforts.

Acknowledgments

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