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A new approach to detect a set of SNP-SNP markers: Combining ARMS-PCR with SNaPshot technology

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ABSTRACT

Microhaplotypes have become a new promising forensic genetic marker in recent years. The microhaplotype composed of two SNPs, SNP-SNP, indicates strong application potential because of the shortest fragment and good polymorphism and without the interference of stutter and high mutation rate as short tandem repeats (STR) and low polymorphism as a single SNP. Currently, the most common method to detect microhaplotypes is massively parallel sequencing (MPS), however its high cost and the need for special instruments limit its use in general forensic laboratories. In this study, we screened out 8 new SNP-SNP loci and established a new detection method by associating multiplex ARMS-PCR and SNaPshot technology. Firstly, we introduced ARMS-based PCR for SNP1. Then, SBE primers for SNaPshot assay were designed as 20–25 bp upstream complementary sequence next to the position of SNP2. Finally, 8 loci were built into one panel based on different SBE primer lengths and fluorescence colors. In brief, by combing ARMS-PCR and SNaPshot technology, it is easy and fast to profile the SNP1 and SNP2 orderly of the SNP-SNP microhaplotype based on CE platform. Our results suggested that the 8 loci have relatively high polymorphism as well as robust performance.

1. Introduction

Microhaplotype is a new genetic marker proposed by Kidd's laboratory firstly. MPS can identify each parental microhaplotype allele at a specific site whether from a single or a mixed source. However, compared with the CE platform, time-consuming, laborious, expensive and complex analysis of MPS restrict the large-scale application of microhaplotype among global laboratories currently [1].

ARMS-based PCR was a classic and mature SNP genotyping method [2]. Nowadays, SNaPshot technology has been used to analyze SNPs of forensic DNA with capillary electrophoresis as its detection system with the advantage of fewer time requirements and greater cost-effectiveness [3]. In this study, we focused on the SNP-SNP marker, the microhaplotype composed of two SNPs with the shortest fragment and good polymorphism. We used ARMS method to design allele-specific primers to target the two alleles of SNP1. After the first step PCR, we performed the amplification products using single base extension (SBE) primers designed to target SNP2. Therefore, we conducted multiplex ARMS-PCR and SNaPshot analysis of 8 SNP-SNP microhaplotypes among 20 individuals of Southwest Chinese Han and explored their application in forensics.

2. Materials and methods

2.1. Sample collection

Blood samples from 20 unrelated Chinese Han individuals were collected after obtaining informed consent. Genomic DNA was extracted using Whole Blood Genomic DNA Rapid Extraction Kit (Bioteke, Wuxi, China).

2.2. SNP-SNP selection

Target SNP-SNPs were filtered from the published 1000 Genomes

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Table 1
Eight SNP-SNP markers lists and genetic diversity.

SNP-SNP	Chr	Location	SNP1 MAF	SNP1 allele	SNP2 allele	Pm	DP	PE	Obs.Het
rs10445426–rs57907290	18	12195773–12195803	G = 0.4519	G/T	A/G	0.905	0.095	0.898	0.950
rs12101725–rs55649144	15	92575365–92575386	A = 0.4559	A/G	A/T	0.300	0.700	0.188	0.500
rs3843625–rs12422436	12	58858690–58858715	T = 0.4409	C/T	C/T	0.285	0.715	0.188	0.500
rs9938522–rs9940690	15	13552242–13552251	A = 0.4469	A/T	A/T	0.230	0.770	0.147	0.450
rs3109851–rs6848611	4	26473621–26473631	T = 0.4545	C/T	A/C	0.380	0.620	0.599	0.800
rs468851–rs468852	22	29956137–29956156	T = 0.4535	C/T	C/T	0.295	0.705	0.147	0.450
rs2527748–rs2527749	8	5396199–5396225	T = 0.2286	G/T	C/T	0.215	0.785	0.291	0.600
rs35443929–rs6462431	7	32935434–32935439	A = 0.2206	A/C	A/G	0.280	0.720	0.428	0.700

Pm: Matching Probability; DP: Power of Discrimination; PE: Power of Exclusion; Obs.Het.: Observed Heterozygosity.

Phase III using VCFtools (https://vcftools.github.io/man_latest.html). Appropriate SNP-SNPs were selected based on the following steps: The distance of the two SNPs was less than 50bp; Minor allele frequency (MAF) of the two SNP loci given in the dbSNP database was above 0.2 (especially among CHB and CHS); Haploview software was used to screen out these haplotypes with a minimum number of 3 and minimum frequency of no less than 0.2. Finally, eight SNP-SNP loci were screened for this study (Table 1).

2.3. Primer design

ARMS-PCR method was used to design two allele-specific primers for the SNP1 of SNP-SNP markers. Then SBE primers for SNaPshot assay were designed as 20–25 bp upstream compensate sequence next to the site of SNP2 with the SBE Primer program.

2.4. ARMS-PCR conditions

All the 8 loci were divided into two panels and two separate multiplex ARMS-PCR amplifications were amplified parallelly with a final reaction volume of 10 µl, which contained 5 µl of 2× Taq reaction mix (QIAGEN, Germany), 2 µl of ddH_2O, 1 µl of forward and reverse primers mixture and 1 µl of DNA (1 ng/µl). The PCR conditions consisted of a pre-incubation for 15 min at 95 °C, followed by 30 cycles of 94 °C for 30 s, 57.8 °C for 90 s, and 72 °C for 60 s and a final extension temperature at 60 °C for 30 min.

2.5. SNaPshot conditions and genotype

After ARMS-PCR, SBE reaction was performed using the SNaPshot™ Multiplex kit (Applied Biosystems) following the manufacturer's instructions. The extension reaction was set up in a total volume of 5 µl, containing 1.5 µl SNaPshot™ Ready Reaction Mix, 1.7 µl RNase-free water, 0.3 µl extension primers mixture and 2.5 µl enzyme-treated PCR products. The mixture was submitted to 28 cycles at 96 °C for 10 s, 53 °C for 5 s, and 60 °C for 30 s. After amplification and purification, the above products were separated by capillary electrophoresis on a 3130 Genetic Analyzer (Applied Biosystems).

2.6. Statistics

Polymorphisms and forensic parameters of the 8 SNP-SNPs were calculated using Powerstats v12 software (Promega, USA).

3. Results

3.1. Polymorphism information

Figure.S1 shows the genotype of a single sample (see supplementary material). Table 1 also lists the allele frequencies of the eight loci in Chinese Han population. Of the eight loci, the observed heterozygosity higher than 0.45 with rs10445426–rs57907290 having the largest value of 0.950; DP varied from 0.620 to 0.785 (except rs10445426–rs57907290 was 0.095) and the combined power of discrimination (CDP) was 0.9998798996 (data not shown).

4. Discussion and conclusions

In this study, 8 SNP-SNP microhaplotypes were screened out and a new detection strategy based on CE platform was successfully constructed, i.e. multiplex ARMS-PCR combined with SNaPshot technology. Results showed that all the loci showed high polymorphisms in the selected Han population. In addition, the characteristics of good polymorphism and short fragment make these loci possible to perform on mixture interpretation and noninvasive prenatal test.

However, the cumulative random match probability was 0.0001201004 (data not shown). Obviously, the discrimination power was not high enough using these eight loci. Therefore, more SNP-SNPs should be selected to enrich the multiplex for the further applications.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.fsigss.2019.09.058.

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