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Developing eight SNP-STR markers for DNA mixture detection

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ABSTRACT

SNP-STR marker is a compound marker constituted by a STR marker and a SNP in the flanking sequence. SNP-STR marker has been introduced to improve the discrimination power of STR markers in previous reports. Recently, some researchers have reported that SNP-STR markers performed well in detecting unbalanced DNA mixtures by designing two allele-specific primers. In this study, STR markers were selected from previously reports which had a high polymorphism in Chinese Han population. Then SNP markers with minor allele frequency (MAF) above 0.10 and locate no more than 250 bp from the repeat sequence of STR markers were screened from SNP database. Based on these criteria, eight SNP-STR markers were finally selected and amplification refractory mutation system (ARMS-PCR) method was used to achieve allele-specific amplification. 20 samples were used to estimate the polymorphism of SNPs in Chinese Han population. Six of the eight loci had a high polymorphism in SNP (above 0.20) which indicated the potential of discriminating DNA mixtures. Most of the primers target minor DNA at an excess of 100-fold major DNA. However, discrimination power is not high enough using these six loci. Therefore, further studies are necessary in developing more SNP-STR markers.

1. Introduction

DNA mixtures are commonly obtained during forensic caseworks. The standard way to detect DNA mixtures is autosomal STR kits. These STR primers are locus-specific, which means STR primers will anneal to both the DNA of major contributors and that of minor contributors. However, when a mixture ratio is beyond 20:1, minor DNA is often undetected [1]. Many factors lead to the genotype failure of minor DNA, such as the detection threshold of capillary electrophoresis platforms and amplification bias. In these situations, it can be addressed by applying allele-specific primers which could specifically anneal to minor DNA's sequence.

Recently, DIP-STR marker has been found to perform well in targeting minor DNA in unbalanced DNA mixtures [2,3]. DIP-STR markers take the advantage of allele-specific primers(S-primer and L-primer) to specifically anneal to the two different allele sequences of DIPs. SNP-STR marker share similar features with DIP-STR in discriminating DNA mixtures. In previous studies, SNP-STR worked well in targeting minor DNA at 40-fold background DNA [4]. In this study, we developed eight new SNP-STR markers and studied the performance of these SNP-STRs in interpreting unbalanced DNA mixtures.

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 phenol/chloroform extraction method.

2.2. Candidate loci selection

STR markers were selected from previous reports which showed high polymorphism in Chinese Han population. Then we searched SNP loci in the flanking sequence of these STR markers based on the following criteria: (1) SNP loci in the flanking sequence located no more than 250 bp away from the repeat region of STR markers; (2) Minor allele frequency (MAF) of the SNP loci should above 0.1.Finally, eight SNP-STR loci were screened for this study. (Table 1)

2.3. Primer design

ARMS-PCR method was used to design allele-specific primers for SNP-STR markers. The principle of ARMS-PCR is to design two specific primers which the 3 end of the primers are complementary to the SNP sequence. To enhance the specific amplification of primers, a deliberate

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Table 1
SNP-STR markers list.

Loci	Chr. Location	Distance[a]	SNP allele	SNP MAF
rs258105-D5S2500	5q11.2	85bp	A/C	A = 0.4377
rs11222421-D11S4463	11q25	52bp	A/T	T = 0.4447
rs67295842-D18S535	18q12.3	87bp	C/T	T = 0.2562
rs11668164-D19S253	19p13.12	191bp	C/G	C = 0.3926
rs12673151-D7S1517	7q31.32	38bp	A/G	A = 0.2758
rs1276598-D6S474	6q21	171bp	A/G	G = 0.2965
rs9527324-D13S1492	13q21.1	31bp	A/C	C = 0.1410
rs7897966-D10S2325	10p13	12bp	C/T	T = 0.3309

[a] The distance between SNP and the repeat sequence of STR.

Table 2
Genetic diversity of seven SNP-STR markers.

Loci	Pm	DP	PE	Obs. Het	*I*
rs258105-D5S2500	0.105	0.850	0.355	0.650	0.374
rs11222421-D11S4463	0.065	0.935	0.795	0.900	0.375
rs67295842-D18S535	0.090	0.910	0.695	0.850	0.374
rs11668164-D19S253	0.095	0.905	0.428	0.700	0.342
rs12673151-D7S1517	0.070	0.930	0.795	0.900	0.342
rs1276598-D6S474	0.195	0.805	0.599	0.800	0.288
rs9527324-D13S1492	0.065	0.935	0.898	0.950	0.129

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

mismatch is introduced at the antepenultimate or penultimate base at the 3 end of primers.

2.4. PCR conditions and genotyping

All SNP-STR markers were amplified with a final reaction volume of 10 μl which contained 1 μl DNA (1 ng/μl), 0.5 μl of each forward primer and reverse primer mix(4.0 μM of each), 5 μl of 2 x Taq reaction mix (QIAGEN, Germany) and 3.5 μl ddH_2O. The thermal cycle process was performed at 95 °C for 15 min, followed by 94 °C 30 s, 58 °C 90 s (for rs6032016-D20S481-G primer and rs6041468-D20S482-A primer,the annealing temperature was 56 °C), 72 °C 30 s for 28 cycles and a final extension temperature at 60 °C for 30 min in the GeneAmp 9700 PCR System (Applied Biosystems). Then PCR products was genotyped using the 3130 Genetic Analyzer (Applied Biosystems) and the results were analysed with GeneMapper ® ID-X v1.1 (Applied Biosystems) according to the manufacturers' instructions.

2.5. Simulated mixture analysis

DNA mixtures were simulated by adding two person's DNA together with a total amount of 10 ng into a single reaction. A series of different ratios (i.e., 1:10, 1:20, 1:50, 1:100) constituted by a major DNA and a minor DNA were developed. Major DNA is homozygous in SNP while minor DNA is heterozygous in SNP.

2.6. Statistics and mixture explanation

Allele frequencies, observed heterozygosity and power of discrimination (DP) were calculated using Powerstats V12 (Promega).To evaluate the probability of discriminating unbalanced DNA mixtures using these eight loci, the probability of informative genotypes (*I*) previously published [2] was calculated. In this study, *I* was modified as: $I = 2A^2B^2 + 2A^3B + 2AB^3$. A and B represent the allele frequency of SNP at a SNP-STR marker, respectively.

3. Results

3.1. Polymorphism information of chinese han population

Table 2 lists the allele frequencies of the seven loci in Chinese Han population (Rs7897966-D10S2325 was eliminated because we cannot design suitable allele-specific primers.). DP of the seven loci varied from 0.650 to 0.950. *I* value varied from 0.129 to 0.375.

3.2. Mixture assays

All the primers could target minor DNA at 1:100 ratio mixture.

4. Discussion and conclusions

In this study, we developed seven SNP-STR markers and these loci could target minor DNA at 1:100 ratio. In comparison with 1:20 ratio using common STR primers, SNP-STR demonstrated a distinctive advantage in discriminating unbalanced DNA mixtures. All the loci showed *I* value larger than 0.25 except for rs9527324-D13S1492. The combining *I* value was 0.934419, which means there will be nearly 93% chance to discriminate a mixture using these seven loci.

However, random match probability of minor DNA was calculated based on detected alleles of informative markers. Obviously, the discrimination power was not high enough using these seven loci.

In conclusion, SNP-STR marker will be a promising method to discriminate unbalanced DNA mixtures in the future, but more loci should be developed to get higher discrimination power.

Conflict of interest

None

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