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Development of a SNP-STRs multiplex for forensic identification

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ABSTRACT

The short tandem repeat (STR) and single nucleotide polymorphisms (SNP) are widely distributed in human genomes. SNP-STR, a compound genetic marker combining a STR locus with tightly linked SNPs, is more informative than any single polymorphism. The amplification refractory mutation system (ARMS) can allow DNA sample with specific base (e.g., SNP) amplify successfully, which avoids the common PCR bias in the analyses of mixtures. This study aims at enriching the rs25768-D5S818 (D5S818, from U.S. Core Loci) primers obtained in the previous study to develop a forensic SNP-STRs multiplex. Two SNP-STRs (rs2246512-D10S1248 and rs9531308-D13S317) were screened from the UCSC genome browser. Two forward SNP allele-specific primers labelled with different fluorescent dyes for each SNP-STR marker were designed with the ARMS, and a common reverse primer was designed near the 3′ region of STR. The amplicons of the three SNP-STRs were profiled via Genetic Analyzer ABI 3130. The sensitivity and specificity of the multiplex were confirmed by using the standard DNA (9947A). The SNP-STR genotype of minor component (0.05 ng) in the artificial extremely unbalanced two DNA mixture (ratio 1:40) was detected.

1. Introduction

The short tandem repeat (STR) and single nucleotide polymorphisms (SNP) widely exist in human genomes. SNP-STR, a compound genetic marker combining a STR locus with tightly linked SNPs, is more informative than any single polymorphism. The amplification refractory mutation system (ARMS) allows DNA sample with specific base (e.g., SNP) amplify successfully, which avoids the common PCR bias in the analyses of mixtures with different bases [1]. In our prior study, we explored a novel method to amplify STRs and SNPs in a single PCR reaction depending on ARMS, and the STR and SNP alleles of SNP-STR could be determined by the amplicons sizes and the different colors (Fig. 1) [2].

2. Materials and methods

Two SNP-STRs, SNP rs2246512 in the 5′ flanking regions of STR D10S12486 (from Extended European Standard Set) and rs9531308 in the 5′ flanking regions of D13S317 (from U.S. Core Loci), were screened from the UCSC genome browser. Allele-specific primers for rs2246512-D10S1248 and rs9531308-D13S317 were designed according to the requirement of ARMS-PCR which were labelled with JOE for allele rs2246512-A, 6-FAM for allele rs2246512-G, ROX for rs9531308-A and TAM for rs9531308-C at the 5′-terminus, respectively. The reverse primer was located at the other side of the corresponding STR sequence. An artificial mismatch was introduced to each allele-specific primer at 1–2 bp from the 3′-terminus. The two pairs of primers were mixed with the primers of rs25768-D5S818 [2] for the PCR reaction.

Peripheral blood samples were collected from 73 unrelated Chinese individuals based upon written informed consent and DNA was extracted by using the phenol–chloroform protocol. 9947A was used as control. The PCR amplifications were performed with 5 μL Multiplex PCR Mix (Qiagen, Germany), 0.5 μL primer mix, 3.5 μL nuclease-free water and 1 μL DNA (1 ng/μL). The number of thermal cycle was 32. The PCR products were profiled via Genetic Analyzer ABI 3130. Two samples (marked SA and SB) with different genotypes (genotype of SA: G11, G12 at rs25768-D5S818, G13, A13 at rs2246512-D10S1248 and C10, C11 at rs9531308-D13S317; genotype of SB: A11, A13 at rs25768-D5S818, A14, A16 at rs2246512-D10S1248 and A8, A10 at rs9531308-D13S317) were mixed artificially from 1:40 to 40:1 with the lowest concentration of 0.025 ng. The A-allele-specific primers and the G/C primers were separately used to amplify the mixtures.

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1875-1768/

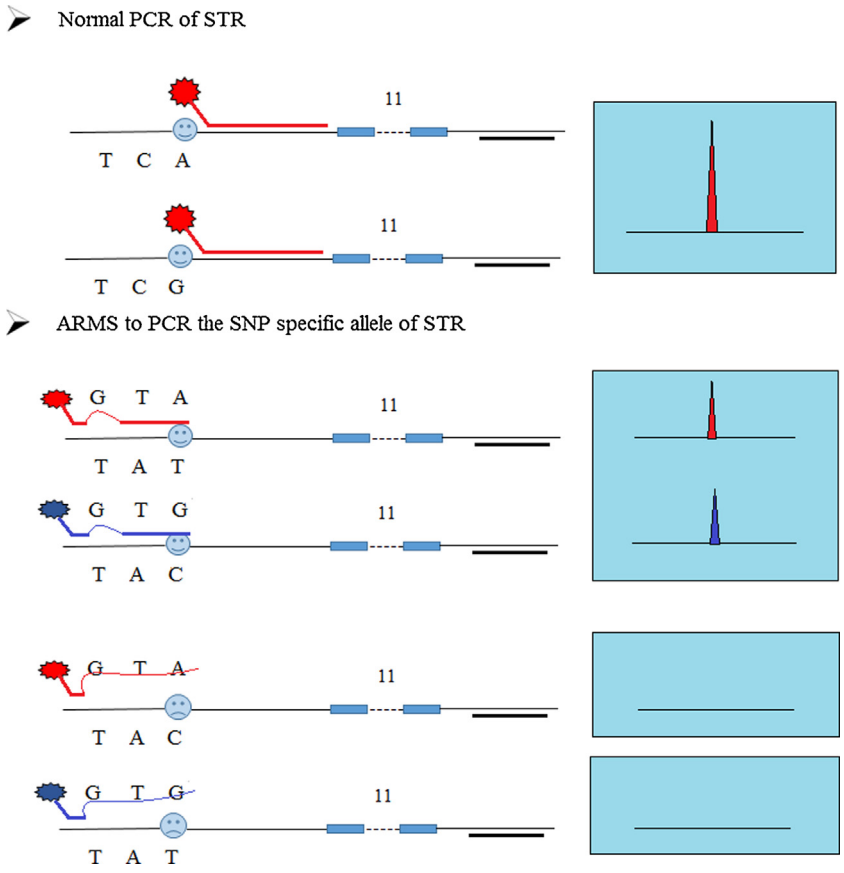

Fig. 1. The primer designed for the STR in a SNP-STR and ARMS assay designed for the SNP-STR.

3. Results

The three SNP-STRs genotypes of 9947A and 73 samples were profiled successfully (Fig. 2). The allelic numbers of rs25768-D5S818, rs2246512-D10S1248 and rs9531308-D13S317 typed in the study were 9, 12 and 12, respectively. The corresponding allelic numbers of STRs: D5S818, D10S1248 and D13S317 were 6, 8 and 7, respectively. The frequencies of AA and GA for rs25768 were 1.37% and 13.70%, and for rs2246512 were 47.95% and 46.58%. The AA and CA frequencies for rs9531308 were 21.92% and 54.80%, respectively. The frequencies of allele A for rs25768, rs2246512 and rs9531308 were 8.22%, 28.77% and 49.32%.

In the mixture study, when sample SB was the major proportion and the G/C allele-specific primers were used, all the genotypes of minor one-SA were profiled correctly. When sample SB was the minor proportion and the A allele-specific primers were used, one drop out of allele A11 (at rs25768-D5S818) and two drop out of A14, A16 (at rs2246512-D10S1248) were found in proportion of 40:1.

4. Discussion and conclusions

This study demonstrates that the genotyping of more than one SNP-STR in a single reaction is feasible and more convenient than the profiling of DIP-STRs [3]. Considering the influence of

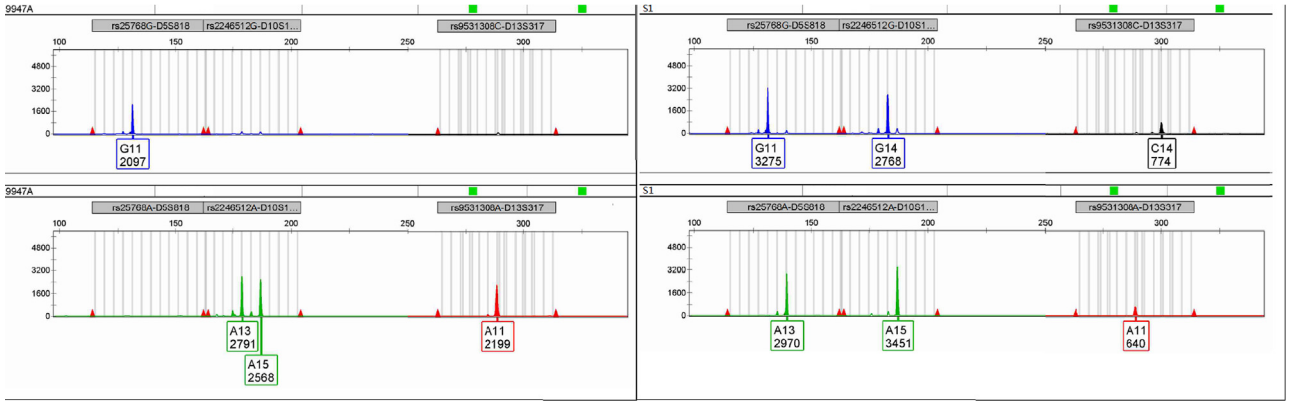

Fig. 2. Sample electropherograms of the SNP-STRs multiplex run with 1 μL PCR product of 9947A and one sample.

amplification for different SNP-STR loci, the allele-specific primers should be separately amplified when involved in the analyses of unbalanced DNA mixtures. For the further applications of forensic genetic practice, more SNP-STRs should be selected to enrich the multiplex in the future studies.

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Conflict of interest

None.

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