Forensic Science International: Genetics Supplement Series 4 (2013) e206–e207

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/FSIGSS

SNP–STR polymorphism: A sensitive compound marker for forensic genetic applications

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ARTICLE INFO

Article history:
Received 10 September 2013
Accepted 2 October 2013

Keywords:
SNP
STR
ARMS

ABSTRACT

Many routine STR and SNP multiplex kits can analyze a large number of markers but cannot type STRs and SNPs in the same reaction. We have explored a method for typing STRs and SNPs in a single reaction named SNP–STR that links SNPs from a flanking region with the STR polymorphism. This allows defining subtypes of STR alleles based on the linked SNP allele observed in the flanking region. The SNP–STRs are widespread in the genome and the genotyping techniques are the same as for routine STR profiling. Pairs of SNPs and STRs linked to each other and at a distance of less than 500 bp were selected from the UCSC genome browser. The method amplifies the target SNP using the amplification refractory mutation system (ARMS). The two forward allele-specific primers are labeled by different fluorescent and the reverse primer is located at the other flanking region of the STR which is linked to the SNP. Through this method, both alleles of the STR and the SNP can be genotyped by the size of the amplicons and the different colors of the amplicons in one reaction. The rs25768 is located 12 bp from the D5S818 which is frequently used in forensic identification. Two allele-specific primers for rs25768 were designed according to the requirement of ARMS-PCR which were labeled by JOE and 6-FAM at the 5′-terminus respectively, and the reverse primer located at the other side of the STR sequence. To increase the specificity, a deliberate mismatch was introduced to the allele-specific primers at −1 and −2 from the 3′-terminus. The DNA samples were extracted from 95 unrelated European individuals through EZ1. 1ul DNA was amplified and profiled by Genetic Analyzer ABI 3130. A set of two-person unbalanced mixture were generated artificially from 1:40 to 40:1, and the lowest concentration is 0.05 ng. We used the both two allele-specific primers and only one of them separately to genotype the mixtures.

1. Introduction

Many routine STR and SNP multiplex kits can analyze a large number of markers but cannot type STRs and SNPs in the same reaction. We have explored a method for typing STRs and SNPs in a single reaction named SNP–STR that links SNPs from a flanking region with the STR polymorphism. This allows defining subtypes of STR alleles based on the linked SNP allele observed in the flanking region. The SNP–STRs are widespread in the genome and the genotyping techniques are the same as for routine STR profiling [1]. Pairs of SNPs and STRs linked to each other and at a distance of less than 500 bp were selected from the UCSC genome browser. The method amplifies the target SNP using the amplification refractory mutation system (ARMS) [2,3]. The two forward allele-specific primers are labeled by different fluorescent and the reverse primer is located at the other flanking region of the STR which is linked to the SNP (Fig. 1). Through this method, both alleles of the STR and the SNP can be profiled by the size of the amplicons and the different colors of the amplicons in one reaction.

2. Materials and methods

The rs25768 is located 12 bp from the D5S818 which is frequently used in forensic identification. Two allele-specific primers for rs25768 were designed according to the requirement of ARMS-PCR which were labeled by JOE for allele A and 6-FAM for allele G at the 5′-terminus respectively, and the reverse primer located at the other side of the STR sequence. To increase the specificity of the rs25768-D5S818 assay, a deliberate mismatch was introduced to the allele-specific primers at −1 and −2 from the 3′-terminus respectively. The DNA samples were extracted from 95 unrelated European individuals through EZ1. 1 ng DNA was amplified through the rs25768-D5S818 assay and D5S818 assay

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http://dx.doi.org/10.1016/j.fsigss.2013.10.106

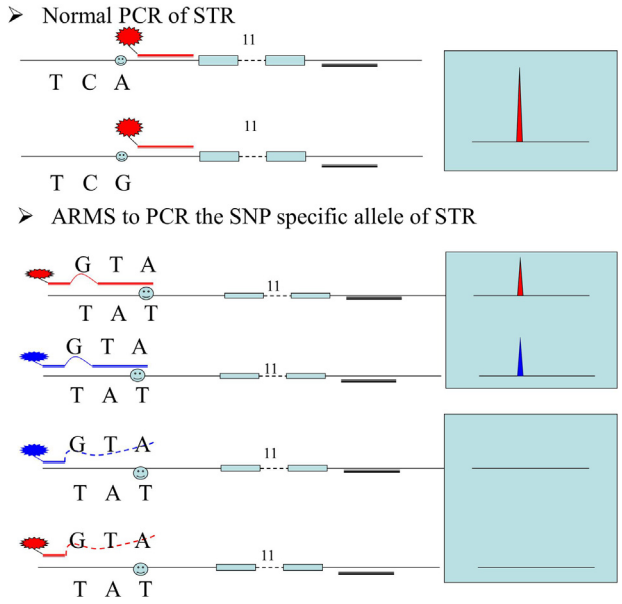

Fig. 1. The SNP–STR is a SNP linked to STR, and the primer designed for the STR and ARMS assay designed for the SNP–STR.

which was used to compare the genotype of the new method. The products were profiled by Genetic Analyzer ABI 3130. A set of two-sample unbalanced mixture were generated artificially from 1:40 to 40:1, and the lowest concentration is 0.025 ng. The genotype of two sample are A-11,A-14 and G-11, G-12. We used the both two allele-specific primers and only one of them separately to genotype the mixtures.

3. Results

10 alleles of rs25768-D5S818 are genotyped and 6 alleles of D5S818 were genotyped in 95 samples. And the D5S818 genotyping result of both assay are matching to each other in every sample. Allele A of rs25768 was not found linked with allele 9 of D5S818. The allele A frequency of rs25768 is 25.26% and the genotype of AA and GA of rs25768 are 6.32% and 37.89%, respectively. Nonspecific amplification was not found in the profiling. In mixture study, all the mixtures in different proportions are genotyped successfully. A drop out of allele G-12 was found in proportion of 40:1when amplified by both allele-specific primers, and the concentration of drop out sample is 0.025 ng [4].

4. Discussions and conclusions

The results showed that the new method is successfully developed. Both the DP and PE of rs25768-D5S818 are higher than D5S818 alone. The advantages of the new SNP–STR markers are: first, the forensic efficiency of SNP–STRs is higher compared to standard autosomal STRs; second, allele-specific primers can be used to detect the minor DNA component with higher sensitivity than normal STRs; third, the application of the SNP–STRs can be improved because they are widespread in the genome; fourth, the difference in mutation rates of SNPs and STRs may have valuable information in evolutionary studies when SNP–STRs are applied.

Role of funding

This study was supported by grants (No. 30801317; No. 81000515 and No. 81202387) from the National Nature Science Foundation, China.

Conflict of interest

None.

Acknowledgements

The authors wish to thanks Iva. Gomez and Daniel. Zaumsegel from Institute of Legal Medicine, Faculty of Medicine, University of Cologne for their support.

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