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Review Article

Application of Y-STR, DIP-STR and SNP-STR Markers in *Interpretation* of Forensic Genetic Profiling: A Narrative Review

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Abstract

Y-STR, DIP-STR, and SNP-STR are useful alternatives for testing the low quantity of DNA in solving the challenges in interpreting forensic genetic profiling. In an unbalanced mixed DNA, partial DNA is often not detected due to the effect of masking by the dominant DNA. Therefore, in such cases interpretation of the results is limited. Furthermore, profiling of these specimens cannot be performed using conventional forensic genetic methods. Biomarkers *including* Y-STR, DIP-STR and SNP-STR perform well in detecting DNA contributes in the mixed sample. In the present research, the performance of each is evaluated separately.

Keywords: Unbalanced mixed DNA; Forensic genetic; Marker; Genetics

Introduction

Eukaryotic genomes have repetitive DNA sequences (1). These DNA sequences are very small in size and are usually determined by the length of the nucleus of each repetitive unit and the number of repeats of the nucleus of each unit. Regions of DNA have repetitive units' 2 to 7 bp microsatellite, or commonly called short tandem repeats (STRs). From repetitive DNA markers, STRs have become popular DNA repeat markers, because the number of repeats of these markers can vary considerably and can be easily amplified by PCR. This exclusivity has made STRs a useful marker for determining human identity (2). In the past two decades, genetic profiling with a number of these STRs has become a common molecular technology to determine the nature and identity of specimens referred to forensic medicine. Consequently this technology is very effective and efficient in solving various cases related to forensic medicine mainly associated with the two purposes of human paternity and investigations related to crime. The use of common autosomal STR markers used in forensics can be challenging in tracking mixed DNA samples found at crime scenes. Mixed biological samples consist of a part of the culprit DNA and a large part of the complainant DNA or the opposite (3).

STRs analyzed through a direct polymerase chain reaction (PCR)-to-capillary electrophoresis (CE) is a common method used in the forensic genetics laboratory. In fact, although autosomal STRs are the main markers used in forensic medicine and they have high discrimination power (3),

However, due to the competition of PCR primers during amplification, these markers have limitations in determining the genetic profile of an unbalanced mixed specimen (3, 4).

In other words, these markers are unable to interpret the genetic profile of a mixture of DNA with two different sources, if one source is 10 or 20 times larger than the other and it is not possible to distinguish and profile both sources. This is one of the most common challenges that makes it difficult to interpret profiling results (5-7).

Although there have been many advances in molecular techniques over the past decade (from differential extraction to massively parallel sequencing (MPS)), none can yet be practically used as a common and inexpensive method in the legal genetics laboratory. The main reasons are the complexity and high cost, low efficiency, high risk of cross-contamination and/or lack of public use of these methods. Therefore, there is still a need to develop simpler and cheaper methods (8-10).

In this research, three simple and feasible methods; Y-STR, DIP-STR and STR-SNP to solve this challenge are reviewed and their limitations and advantages are stated.

Y STRs

The use of Y STRs can be effective in the study of mixed biological specimens (e.g. in cases of rape). In cases where the DNA of a male is mixed with the high level of DNA of a female; although there must be a specific sexual incompatibility for the use of Y-STRs (11,12). However, Y-STR analysis is of great help in identifying DNA specimens with a female DNA background. However, depending on the frequency of haplotype Y in the population, its statistical value can be limited, and the possibility of the presence of DNA of the relatives of a suspect's paternal lineage cannot be ruled out as a contributor in the stain (13-16).

In forensic genetics, Y-STRs are used in order to tracing a relationship between two or more men. Therefore, mutation rate of Y-STRs is key index for this target. Recently, a significant improvement has been reported in tracing a relationship between two or more men by the panel of rapidly mutating Y-STRs which composed of 13 locus with rate high mutation (DYF387S1, DYF399S1, DYF403S1a/b, DYF404S1, DYS449, DYS518, DYS526I/II, DYS547, DYS570, DYS576, DYS612, DYS626, and DYS627). The kit improved discrimination power between Y-STR profiles of related and unrelated males compared to conventional Y-STRs (17,18). Despite the features improvement of Y-SYR, there is a need to develop new methods to allow the complete DNA profile of the unbalanced mixed stains to be determined regardless of the gender of the contributors; two methods have been developed for this purpose: DIP-STR and SNP-STR (18-22).

These markers, marked on autosomal and sex chromosomes, show the expected characteristics of forensic medicine, such as the absence of a peak stutter, lower mutation rates than STRs, and short amplicons. These characteristics are of great importance for the analysis of destroyed samples, determination of human identity, human paternity, etc. (20-27).

Deletion/Insertion polymorphism linked to a STR (indels or DIP-STR)

DIP-STR consists of two parts, including a delete or insert region and a STR polymorphism (18). Therefore, two set of allele-specific PCR primers have been designed, one for insertion) long or "L" allele) and the other for deletion (short or "S" allele). The STR alleles also enhances the discrimination power of this biomarker (18, Fig. 1).

DIP-STRs are broad in genome-wide screening and their type methods are similar to conventional STR (26). In this method, PCR amplified partial DNA to reach a high level of specificity so that a partial DNA can be revealed in the presence of more than 1000 times the dominant DNA (26). The DIP-STR marker detects informative or non-informative genotypes based on the presence of a unique S/L mismatch in a partial donor. As shown in Fig. 2 (28), two haplotypes of DNA partial (red box, informative genotype 1) can be revealed when the partial and

dominant donor's genotypes are opposite each other (SS/LL or LL/SS). Conversely, one partial DNA haplotype detectable when there is a homozygote (SS or LL) in the dominant DNA and a heterozygous (SL) in the partial DNA (blue box, informative genotype 2). Whereas, when the partial DNA *is not unique* to S or L alleles, identified genotypes are non-informative (white box). Therefore, in this condition, the DIP-STR type is similar to conventional STR typing (26, 28). Therefore, the existence of a population database and selection of informative alleles is very important in the use of these markers.

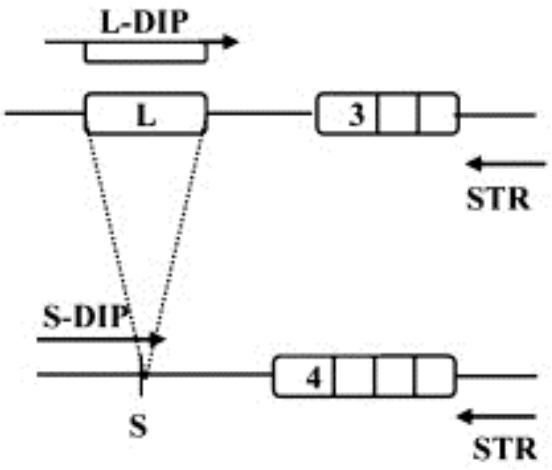

Fig. 1: Schematic of the structure of DIP-STR marker: Insertion polymorphism (long or "L" allele), Deletion polymorphism (short or "S" allele) and STR polymorphism linked to DIP. The right and left arrows indicate the forward and reverse primers

Due to the ability of DIP-STR to detect partial alleles in unbalanced DNA mixtures, this tool also seems to be useful for determining fetal alleles extracted in DNA plasma of pregnant women (27). Besides, DIP-STR with the ability to amplify genomic regions of DNA in the cell-free fetal DNA (cffDNA) can be used as a non-invasive prenatal test to determine paternity (27). Moreover, using DIP-STR can determine an additional DIP-STR allele inherited from the father in the plasma of a pregnant women with a singleton baby, but in a twin pregnancy DZ detects two non-maternal DIP-STR alleles in the plasma of a pregnant woman; of course, the determined alleles are different from the maternal alleles (29). So far, DIP-STR has been performed in a small number of populations, including in the countries Switzerland and China (26,30-33).

In a study, a specific indel-allele-based Real-time PCR method has been reported using two reactions with separate specific primers for unbalanced mixed DNA (30). In this method, by comparing the difference in Ct value for the two reactions, the corresponding indel genotypes are determined for each individual, but electrophoresis is required for final confirmation. With this method, in the Han Chinese population the performance of 14 highly polymorphic loci with a variable level of specificity in different regions was reported. The detection ratio of the mixed DNA for the five indels was from 1:50 to 1:100 and for the remaining sites from 1: 500 to 1:1000 using 1-10 ng of DNA (30). In general, DIP alleles of specific primers allow the verification of partial DNA with higher sensitivity than conventional STRs; moreover, the spatial distribution of DIP-STRs throughout the genome allows for their use in all mixed DNA samples, regardless of the sex of the contributors. Despite the Y-STR, the DIP-STR allows access to individuals rather than the paternal lineage (33).

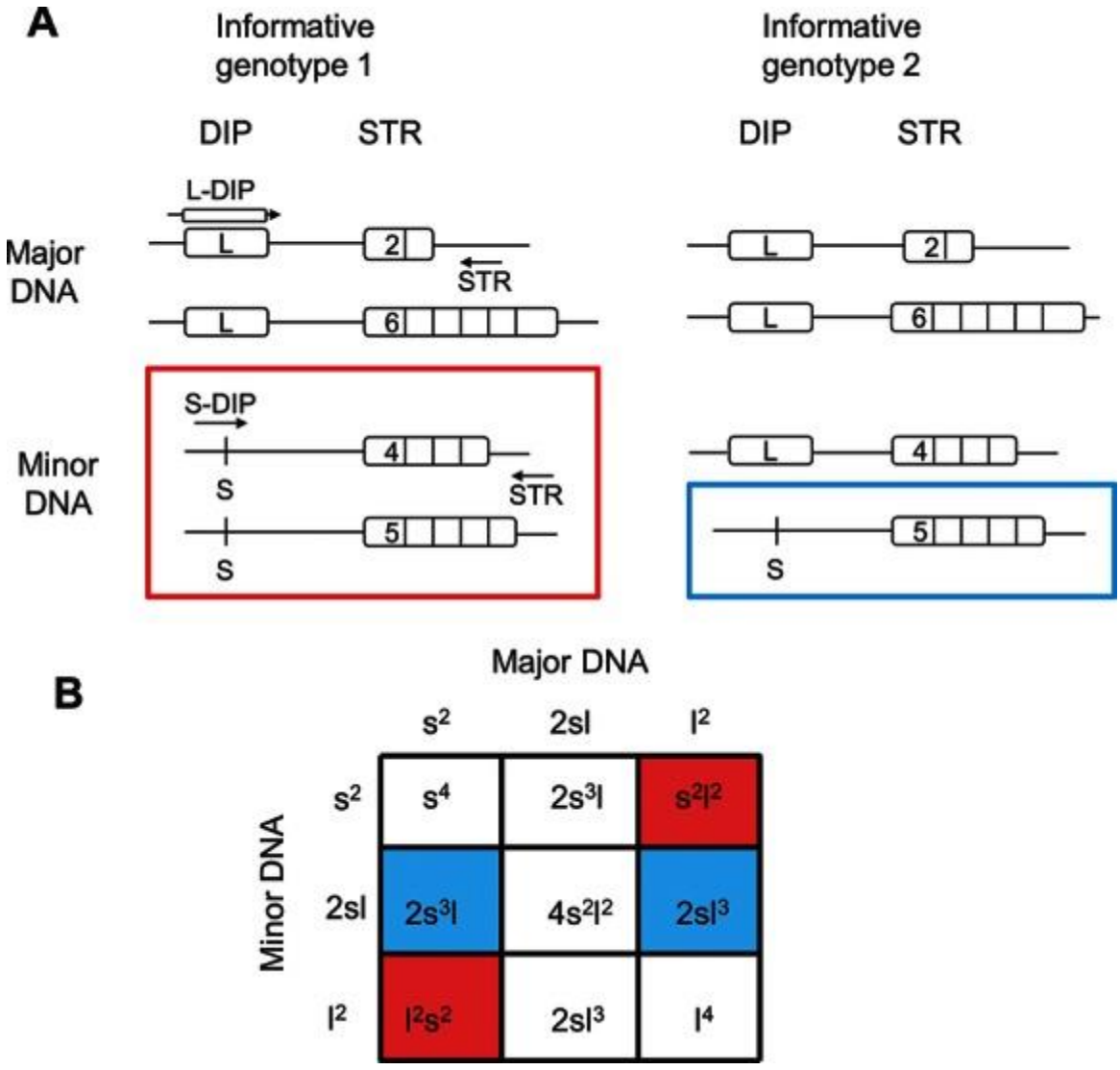

Fig. 2: Description of possible genotypes of DIP–STR in a two-person mixture (A). Table of possible genotype sets according to the formula 2(pq) + (p) 2 + (q) 2 = 1(B). Allelic frequencies are shown by the letters s and l. So, the sum of s2l2+l2s2+2s3l+2sl3 are the possibilities of having informative genotype. Informative genotype 1 (red box), informative genotype 2 (blue box) and non-informative (white box)

Single Nucleotide Polymorphism-STR combinations (SNP –STR)

SNP-STR formed by a bi-allelic SNP that linked to a STR polymorphism (Fig. 3) (34), and targeted a genomic region unique in partial DNA and reduced the negative effect of masking by the dominant DNA (35,36). Using SNP-STR markers in a two-donor mixture, the possible genotypes are identifiable based on the presence of a specific allele in partial donor's genotype and its absence in the dominant donor's genotype. According to the ARMS-PCR method, SNP allele-specific primers are designed and labeled with different fluorescent dyes (Fig. 3). The forward and reverse primers are located in the Upstream and downstream of the STR polymorphism sequence, respectively. PCR results of SNP-STR markers are visible by analysis CE similar to DIP-STR markers method. By adding a deliberate mismatch at the end of the 3 'primers, the proba-

bility of specific PCR amplification is increased in DNA mixture. The bioinformatic analyzes and population database are very effective in selecting SNP-STR markers (35). In an singleplex PCR with SNP–STR markers, there are three observed chances for informative genotype and one chance for uninformative genotype (35). According to the formula 2(pq) + (p) 2 + (q) 2 = 1, the probabilities of effective informative genotypes that specifically targeting partial DNA include $M^2N2 + M^2N^2 + 2M^3N + 2MN^3$ and informative genotypes are $2M^3N + 4M^2N^2 + 2MN^3$. In one study, it was reported that lengths shorter than 550 for targeted amplicons and minor allele frequency (MAF) more than 0.02 were factors influencing the selection of SNP-STR markers in the target population (Fig. 4) (35).

Unlike SNP-STRs, DIP-STRs are more sensitive markers (1: 1000 (28, 37) vs. 1:40 (38, 39) for the analysis of unbalanced DNA mixtures, but they still have disadvantages in forensic purposes. The dispersion of DIP markers in the human genome are significantly lower than SNPs, which severely limits selection of DIP-STRs as biomarker candidates. Furthermore, unlike other STRs used in forensics, including Combined DNA Index System (CODIS), Extended European Standard Set (ESS) and National Institute of Standards and Technology (NIST)-miniSTR, DIP-STRs are almost unavailable. Therefore, it is possible the results of DIP-STR typing are not comparable to the common STR typing (34).

Therefore, SNP-STR may in practice be a more valuable combination of genetic markers than DIP- STRs are for analyzing unbalanced DNAs, and the ability to type SNP-STR in a reaction allows the STR and SNP alleles to be analyzed together in a reaction (32, 33).

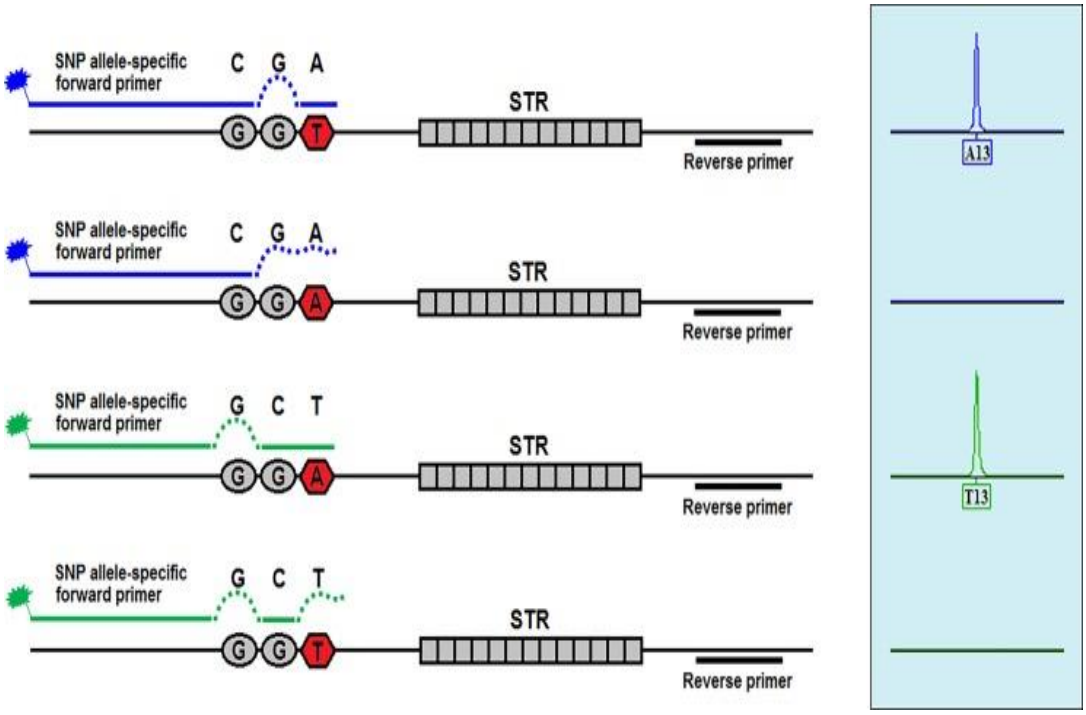

Fig. 3: Schematic of the structure of SNP-STR marker based on ARMS method

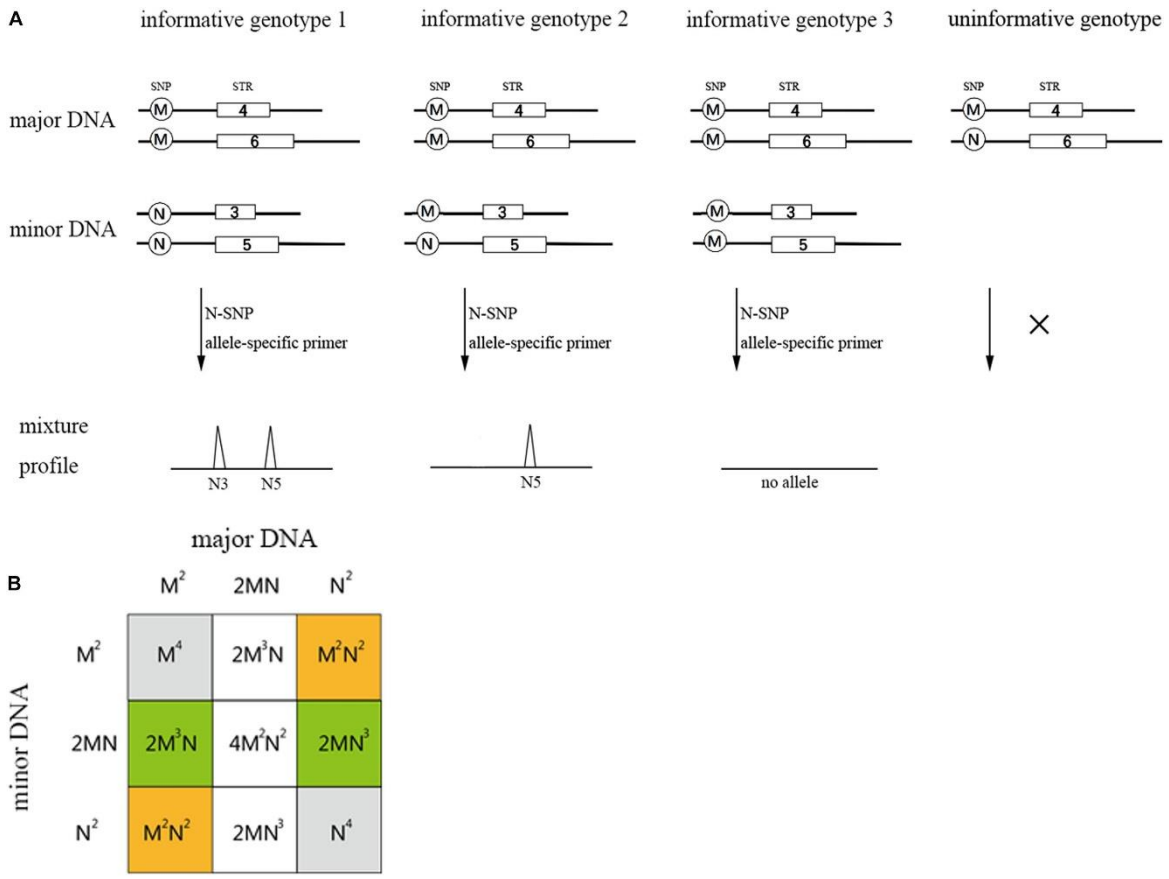

Fig. 4: Description of possible genotypes of DIP – STR in a two-person mixture. A: Informative genotype 1 can be revealed when both donor's DNA are homozygous (MM/NN) for SNP genotypes (orange box). Informative genotype 2 detectable when there is a homozygote (MM) in the dominant DNA and a heterozygous (MN) in the partial DNA (green box). Informative genotype 3 detectable when both donor's DNA are homozygous- MM (Gray box). Non-informative is observed when the SNP genotype is a heterozygous (MN) in dominant DNA and there is no specific SNP allele for partial DNA(white box). **B**: Table of possible genotype sets according to the formula $2(pq) + (p)^2 + (q)^2 = 1$. Allelic frequencies are shown by the letters M and N

Conclusion

The use of Y-STRs in determining two distinct reliable profiles is limited to cases where a man's DNA is mixed with a woman's DNA. If two or more men are in a mixture, this method cannot be used to target partial DNA. In addition, Y-STR markers are haplotypes, so their power of discrimination is very low compared to autosomal STRs, and in some cases it is invalid for differentiating individuals with the same paternal lineage.

Although DIP-STR markers are more sensitive than SNP-STR, to date, according to studies in the literature, DIP-STR markers have no forensic population database as references, while SNP-STR associated STR markers are in CODIS or ESS positions, which can be used as reference database. Statistical ratios and population distributions are essential in trusting the DIP-STR test results and their differential strength, conducting various demographic studies to examine the p-value of these statistical indicators will increase their reliability and accuracy.

The use of these markers in specific casework is practical and the combination of the use of the two markers in the future, as mentioned, will

solve many of the limitations of profiling in forensic medicine.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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