Forensic Science International: Genetics 11 (2014) 229–240

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

An investigation of the potential of DIP-STR markers for DNA mixture analyses

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ARTICLE INFO

Article history:
Received 8 October 2013
Received in revised form 4 March 2014
Accepted 4 April 2014

Keywords:
Unbalanced DNA mixture
Graphical models
Deletion/Insertion Polymorphism
Likelihood ratio

ABSTRACT

The genetic characterization of unbalanced mixed stains remains an important area where improvement is imperative. In fact, using the standard tools of forensic DNA profiling (i.e., STR markers), the profile of the minor contributor in mixed DNA stains cannot be successfully detected if its quantitative share of DNA is less than 10% of the mixed trace. This is due to the fact that the major contributor's profile "masks" that of the minor contributor. Besides known remedies to this problem, such as Y-STR analysis, a new compound genetic marker that consists of a Deletion/Insertion Polymorphism (DIP) linked to a Short Tandem Repeat (STR) polymorphism, has recently been developed and proposed [1]. These novel markers are called DIP-STR markers. This paper compares, from a statistical and forensic perspective, the potential usefulness of these novel DIP-STR markers (i) with traditional STR markers in cases of moderately unbalanced mixtures, and (ii) with Y-STR markers in cases of female–male mixtures. This is done through a comparison of the distribution of 100,000 likelihood ratio values obtained using each method on simulated mixtures. This procedure is performed assuming, in turn, the prosecution's and the defence's point of view.

1. Introduction

The common way to analyse DNA mixtures for forensic purposes is to use the Polymerase Chain Reaction (PCR) and STR markers [2]. One of the limitations of this method is that it does not work successfully if the proportion of the DNA quantities of the two contributors is more extreme than 1:10 [3]. Here, the threshold of 10% is retained as the limit of detection of the minor DNA for blood:blood mixtures. This value varies depending on the types of biological fluids which constitute the mixture and the specific combination of genotypes present in the mixture (as reported in [4]) and should be assessed in the validation procedure [2]. Mixtures with such extreme proportions are referred to in this paper as 'extremely unbalanced mixtures', opposed to 'moderately unbalanced mixtures', that are mixtures for which the proportion of DNA of each contributor is less extreme than 1:10. Situations involving extremely unbalanced mixtures are quite common, such as in cases of sexual assaults when the victim's DNA is largely predominant or cases of microchimerism during pregnancy (where minute quantities of fetal DNA are present in maternal blood). To address constraints implied by these kind of mixtures, Y-STR markers are widely adopted [5], with the limitation that they provide information on the minor contributor only if that individual is male and the major contributor female. To address both the constraints of mixture imbalance and contributors' gender mismatch, an alternative analytical method has recently been developed and proposed [1]. It is based on the use of new compound markers, each formed by an STR marker coupled to a marker in which a Deletion/Insertion Polymorphism (DIP) [6] is known to be present. So far a panel of 9 markers has been provided, called DIP-STR markers.

An object-oriented Bayesian network for the assessment of profiling results obtained with this novel typing technique has been developed [7]. This network approach allows one to calculate a likelihood ratio for mixtures of two contributors, when the major contributor's genotype is known and the two competing hypotheses are 'the minor contributor is the suspect' (H_p) and 'the minor contributor is an unknown person, unrelated to the suspect' (H_d).

This paper aims to compare, from a statistical and forensic perspective, the potential usefulness of these novel DIP-STR markers (i) with traditional STR markers in cases of moderately unbalanced mixtures, and (ii) with Y-STR markers in cases of female–male mixtures. Section 2 starts with a brief introduction to

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http://dx.doi.org/10.1016/j.fsigen.2014.04.001
1872-4973/

the characteristics of the DIP-STR method along with the specification of the chosen STR and Y-STR marker systems. Next, Section 3 will present the interpretative model and the probabilistic tools (among which are graphical models) used to produce (through simulation techniques) likelihood ratio (LR) results for the three methods. Section 4 compares the distributions of the likelihood ratio results for DIP-STR and classical STR, and for DIP-STR and Y-STR. Section 5 focuses on the study of potential usability of the methods, that is the percentage of cases in which they are useful for the purpose of the investigation. The last Section 6 presents a discussion and conclusions, while Appendix provides additional tables and figures.

2. Genetic background

This section briefly introduces the reader to the genetical background of DIP-STR markers. It also specifies the chosen STR and Y-STR marker systems. Particular features of the three methods, which are relevant for the understanding of the forthcoming sections, are also mentioned.

2.1. DIP-STR markers

DIP-STR markers were recently proposed as novel type of genetic markers [1]. The novelty consists in pairing a Deletion/Insertion Polymorphism (DIP) [6] with a standard STR, to form a superlocus where the two composing loci are not independent because they are so close on the chromosomes (less than 500 bp apart) that they cannot recombine, but independence can be assumed between the different DIP-STR markers. Two alternative allele-specific primers overlapping the DIP locus are designed, denoted L-DIP primer and S-DIP primer (L for *long* or S for *short*). Each of these is to be used together with a primer downstream the STR region. They are useful for mixtures of any unbalance proportion (DIP-STR genotypes of minor contributors were successfully typed at a ratio up to 1:1000 [1]) and where one contributor can be assumed as known, but they present a particular interest for extremely unbalanced mixtures, when the use of STR primers leads to masking of the minor contributor's genotype by the major contributor's genotype. This is due to the fact that the STR primers are loci specific. Two contributors necessarily have alleles from the same locus, although of possibly different lengths (i.e., repeat numbers), but STR markers do not differentiate between different alleles of the same locus in case of extremely unbalanced mixtures. In practice it is observed that annealing occurs mainly with those alleles that are present in predominant quantity, so that DNA of a minor contributor will not be successfully replicated. Due to the allele specificity of DIP-STR markers, DIP-STR genotyping allows the selected amplification of the unknown contributor's DNA, as long as it has alleles that are absent in the known contributor's genotype. For the purposes of this article, the known contributor is considered as the major one.

A first important feature of this set of markers concerns the exhaustiveness of the information that can be retrieved about the minor contributor, which depends on the combination of DIP alleles of the two contributors. This is why an initial step in the analysis consists in genotyping the major contributor's DNA, in order to know which DIP-primer to use for each locus of the mixture: if, at a particular locus, the major contributor is homozygous for the DIP alleles (i.e., S–S or L–L), the DIP-primer corresponding to the other DIP allele (L if the major contributor is S–S, S if the major contributor is L–L) will be used. Note that in case the major contributor is heterozygous for the DIP alleles (i.e., S-L), none of the DIP-primers is worth to be used at that particular locus. The best scenario is when the DNA of the major and the minor contributor are homozygous for different DIP alleles (i.e., one S–S and the other L–L, or viceversa). In this case, the possible results can show either two different minor DNA haplotypes or one, depending on the STR-homozygosity or heterozygosity of the minor contributor. On the other hand, when the major contributor is DIP-homozygous and the minor contributor is DIP-heterozygous, only one haplotype of the minor DNA can be retrieved (i.e., the one with the DIP allele opposite to the DIP allele of the major contributor's DNA). A limitation of this method is that, when the predominant DNA is DIP-heterozygous or both contributors are DIP-homozygous of the same type, it is not possible to obtain any result from the analysis of the mixture, since both DIP primers (S and L), if used, will anneal to the major contributor's DNA. Table 1 summarises the possible outcomes. However, it is important to point out that even in those situations for which no alleles of the minor contributor are obtained, if the major contributor is DIP homozygous, some information about the minor contributor are nevertheless obtained, because it indicates that the minor contributor has the same DIP-homozygosity as the major contributor (both S–S or L–L).

A first panel of DIP-STR markers (MID1013-D5S490, MID1950-D20S473, MID1107-D5S1980, rs11277790-D10S530, rs60194384-D15S1514, rs67842608-D5S468, rs66679498-D2S342, rs10564579-D3S1282, rs35708668-D5S2045) was introduced in [1]: they are referred to in this paper as Marker 1, Marker 2, ..., Marker 9, respectively. Data from 103 unrelated Swiss individuals are used here for a Bayesian estimation of the allelic proportions at each of these markers. For further information on this method, see also [7].

2.2. STR markers

STR markers are routinely used to genotype DNA traces [2]. For the purpose of the current discussion, it is important to note that in case of extremely unbalanced mixtures, the use of STR markers generally does not allow one to be aware of the presence of a

Table 1
Informativeness of the different genotypic DIP-STR configurations. This table represents a single locus configuration, and the results in the last column are obtained using the DIP primer opposite to the DIP primer of the major contributor.

DIP genotype of major contributor	DIP genotype of minor contributor	DIP-STR results
S–S	S–S	No results
	L–L	Complete genotype of the minor contributor
	S-L	Only the L DIP-STR allele
L–L	S–S	Complete genotype of the minor contributor
	L–L	No results
	S-L	Only the S DIP-STR allele
S-L	S–S	
	L–L	No results
	S-L	