

Mixture analysis using SWaP™ SNPs and non-biallelic SNPs

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Abstract. Improved analysis of degraded samples, increased throughput, and a wider choice of typing platforms are some of the significant advantages offered by single nucleotide polymorphism (SNP) genotyping over established short tandem repeat (STR)-based systems. However, DNA mixtures present a considerable problem to SNP analysis as there is currently no generally accepted technique that allows recognition of the presence of a mixed profile or identification of the individual contributors. We present the first demonstration of SNP mixture analysis with an approach based upon the use of two rare subsets of SNPs: SWaP™ SNPs and non-biallelic SNPs and discuss their value for forensic mixture analysis. © 2006 Elsevier B.V. All rights reserved.

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1. Introduction

Single nucleotide polymorphisms (SNPs) offer many advantages over established short tandem repeat (STR)-based systems, but mixture analysis is still a significant obstacle to their becoming the method of choice for forensic DNA analysis. STRs are highly polymorphic, so a mixture can be recognised with STR analysis simply through the

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presence of multiple peaks at a locus, and two person mixtures can be interpreted relatively easily [1]. However, because the majority of SNPs are biallelic, opportunities for mixture interpretation are limited and are currently restricted to the detection of higher than expected levels of heterozygosity [2].

This paper describes initial work on a new method of mixture interpretation that utilises two rare subsets of SNPs. The first of these are termed SWaP™ SNPs and are complimentary transversions (S or W) located within short palindromic sequences, where SWaP means G/C (=S) or A/T (=W) amid Palindromes. An example of such a SNP might be ${}^5\text{CTG}[\text{G/C}]\text{CAG}{}^3$, as CTG is the reverse compliment of CAG.

Fig. 1 illustrates the main principles of the SWaP SNP assay. Each SNP is amplified using PCR primers with modified tails attached. These tails consist of a random sequence within which is located a replicate of the SNP under examination, termed the ‘real’ SNP, and the two bases of the palindromic sequence on either side. There are therefore two forms of each primer, representing the two alleles of the locus. The primers are added to a PCR in known ratios to create amplicons containing the real SNP with an unknown allele ratio, flanked by two ‘mirror’ copies of the SNP with known allele ratios. Each of the three SNPs is then interrogated in a single-base extension (SBE) reaction.

Because the SNPs are located within palindromic sequences, the 3′ environment for the SBE primers is very similar. The peak ratios from the known mirror SNPs can therefore be used to construct a standard curve from which the allele ratio in the real SNP is estimated. In this way, the assay generates its own internal controls to account for factors that may influence signal ratio, such as 3′ environment or amplicon concentration. In addition, the assay is able to signal the success or failure of the PCR: in negative control reactions, the mirror SNPs still generate signals following SBE, so if only these peaks are detected a problem with the PCR is likely.

The second group of SNPs are termed non-biallelic (NB) SNPs as three alleles occur in the population [3]. Mixtures can therefore be detected by the presence of three alleles at a

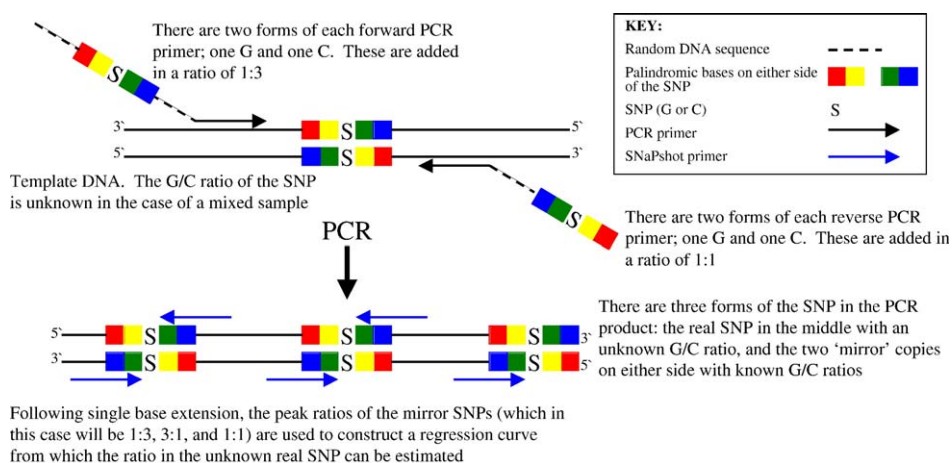


Fig. 1. The principles of the SWaP SNPs technique.

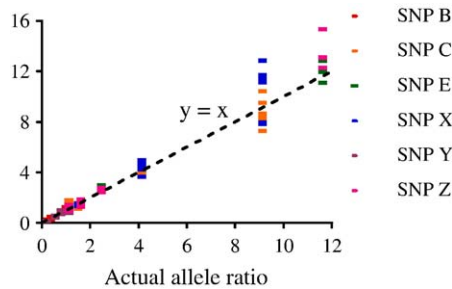


Fig. 2. The relationship between the ratios predicted by SWaP SNPs and Powerplex 16.

locus, and concordant SWaP SNPs analysis could reveal the proportions contributed by each individual.

2. Methods

An assay comprising five G/C (termed SNPs A to E) and three A/T (termed X, Y and Z) SWaP SNPs was developed and tested blind upon artificial mixtures sent to this laboratory as part of a chimerism study. Five such samples were tested, two representing the donor and recipient, and three unknown combinations of these. Signal ratios were estimated as described above and compared with those calculated from Powerplex 16 analysis (the method currently used for chimerism analysis in this laboratory). The same samples were typed for three NB SNPs to investigate their utility in mixture detection.

3. Results and discussion

With the exception of one G/C SWaP SNP, estimates of mixture ratio were very similar to those obtained from Powerplex 16 analysis (Fig. 2), especially for ratios <5. Note that SNP D is not on the figure as the two samples share genotypes. SNP A is not included as it produced a non-linear regression curve so inaccurately predicted mixture ratio. This could be because the SBE primers for SNP A were relatively short and, therefore, may be subject to interference from noise at the start of the electropherogram.

There were slight differences between the predictions obtained from the forward and reverse real primers on the A/T SNPs. Further investigation into this is required, and it may be that the least accurate of the two can be removed and the remaining SBE primers shortened.

NB SNPs were not informative in this case as the two samples tested displayed only two alleles at each locus. However, when other artificial mixtures are typed, three alleles can clearly be detected and the assay has successfully been multiplexed with the eight SWaP SNPs to produce the first SNP-based test for mixture detection and interpretation. This could be particularly useful when STR analysis fails, for example, when samples are degraded.

References

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