# FORENSIC MIXTURE ANALYSIS USING MIRRORS AND SWAP™ SNPS

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The advantages and disadvantages of interrogating SNPs are well documented: increased degradation resistance is countered by potentially confusing results from mixed DNA template. Surely however, with sufficient commitment and experimental effort, the challenge presented by mixed template DNA can be overcome?

We are developing homogeneous primer extension technology that may contribute to the adoption of SNP profiling even where there is the possibility of a mixed template. We refer to the enabling technology as 'mirror SNP' generation. We are hopeful that a subset of mirror SNPs which we term SWaP™ SNPs, will provide a semi-quantitative analysis strategy that will be of particular utility in examining and interpreting mixtures.

A mirror SNP is an artificially generated copy of the targeted SNP created in the terminal end of a PCR amplicon. This is achieved through use of two almost completely identical primers during PCR. These primers differ at a single base position, and compete evenly for the template binding site. Use of matched/equivalent concentrations of these primers therefore produces an amplicon pool in which there is an artificial heterozygote genotype. This exogenous heterozygote SNP can be used as a reference control to judge the zygosity of the real targeted SNP.

Critically, by controlled manipulation of the concentrations of the two chimeric amplification primers, known asymmetry can be generated at the mirror SNP position. However in order to judge if the real SNP is asymmetrically represented, which may be indicative of a mixture, it requires that a standard curve be produced, necessitating multiple known asymmetries to be generated and measured. SWaP™ SNPs enable the generation of these multiple points in a unimolecular system. They have the unique characteristics of reversing any deliberate asymmetry from one strand to the other, without changing the type of SNP, or the flanking sequence context around the SNP. Each SWaP™ SNP therefore introduces two measurable asymmetries simultaneously, and by introducing asymmetric SWaP™ SNP positions at both ends of the amplicon, sufficient asymmetries can be generated to enable the construction of a standard curve.

This system benefits from being unimolecular, such that all experimental variables are automatically normalized between the mirror SNPs and the real targeted SNP. This system, with sufficient refinement, may enable the identification and subsequent interpretation of mixed DNA samples.