

A MULTIPLEX SNP FRET-BASED ASSAY TO DISTINGUISH INDIVIDUALS

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A fast, inexpensive method to quickly screen crime scene samples would expedite the analysis of probative samples. This new method need not be perfectly definitive nor give a complete profile; it simply must be able to differentiate between most victim and suspect samples. We describe the development of a multiplex SNP, FRET-based real-time PCR assay. In this assay, PCR is performed as usual using two primers flanking the SNP. Two probes are also present: one 5' situated probe with a 3' dye label which covers the SNP, referred to as the reference or sensor probe, and a second, 3' situated probe called the anchor probe, labeled with a 5' quencher and a 3' phosphate (to prevent elongation). The reference/sensor probe is designed as a perfect match to one allele but has one mismatch with the other allele. When the two probes both bind to the PCR product, and the 3' dye is excited by light of the appropriate wavelength, it transfers energy to the 5' quencher which quenches the fluorescence. The quenching will only occur on (and will be proportional to) the amount of PCR product. Determination of which allele(s) is present depends on the melting temperature where the fluorescence is re-gained. When the probe binds to a perfect sequence match, it will melt at a higher temperature than if paired with a 1 base mismatch. Because this system only requires one dye per SNP, up to six SNPs can be multiplexed in one reaction depending on the real-time instrument. We have developed both 6-plex and double 4-plex variations of this assay. This assay can discriminate between approximately 95-99% of samples from different individuals. This assay is fast (~2 hours), much less expensive than STR analysis and uses a real-time PCR instrument which is found in most forensic and molecular biology labs.

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