APPLICATION OF SNP PROBE CAPTURE ENRICHMENT AND MASSIVELY PARALLEL SEQUENCING IN THE ANALYSIS OF DEGRADED AND MIXED SAMPLES

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DNA evidence found at crime scenes and mass disaster events can be highly degraded and mixed in nature, raising issues with the conventional methods of DNA analysis using short tandem repeats (STRs) and capillary electrophoresis (CE). Highly degraded samples typically fail to amplify with PCR-based methods due to the absence of intact primer binding sites and current methods of STR analysis by CE are unable to detect minor contributions less than 10% in mixture evidence. STR analysis of forensic mixtures is further complicated by allele drop-out, allele drop-in and stutter artifacts due to degradation and sporadic contamination. Single nucleotide polymorphisms (SNPs) offer an alternative method in the analysis of highly degraded samples due to their small genetic footprint. The addition of multi-allelic markers, such as microhaplotypes, tri-allelic and tetra-allelic SNPs, and haploid chromosomal SNPs offer additional mixture detection capabilities, improving the resolution of mixtures compared to using bi-allelic SNPs alone. Probe capture enrichment coupled with next-generation sequencing (NGS) technology alleviates the need for intact primer binding sites by using a highly redundant tiling strategy to capture targeted regions of interest, enabling the analysis of challenging samples such as highly degraded mixtures which would typically fail STR analysis due to the short DNA fragment size. DNA mixtures can be enriched and clonally sequenced, allowing for bioinformatic read counting to estimate major and minor contributions. We applied two versions of a SNP probe capture enrichment panel targeting 426 (v1.0) and 436 (v2.0) SNPs to contrived mock-degraded and control mixtures. Varying DNA amounts ranging from 25 ng – 1 ng were assessed with minor contributions ranging from 2.5% - 50%. The detection of a minor component was achieved at a minor contribution of 2.5% in mock-degraded and control mixtures. Major and minor contributions were accurately and precisely estimated in mixtures with 25 ng of input DNA, including mock-degraded mixtures. The application of a one standard deviation criterion helped to improve accuracy in estimating the minor contribution in mixtures with 10 ng of input DNA. We show proof-of-concept of the application of SNP probe capture enrichment in the analysis of highly degraded mixtures where conventional STR analysis may fail.