A NEXT-GENERATION SEQUENCING APPROACH TO EPIGENETIC-BASED TISSUE SOURCE ATTRIBUTION

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The ability to determine the tissue source of biological materials from evidence samples can be highly informative for interpreting forensic data. In this study, a previously published capillary electrophoresis (CE)-based method to probe locus-specific DNA methylation was modified to accommodate detection using next-generation sequencing (NGS) to perform tissue source attribution. DNA samples (1 ng) from each of four different tissue types were digested with the methylation sensitive restriction endonuclease *Hha*1 and polymerase chain reaction (PCR) was used to amplify an optimized subset of ten methylated loci, including positive and negative control loci. The products were prepared as NGS libraries, pooled in a multiplex assay with sample specific barcodes, sequenced with an Illumina MiSeq, and analyzed using a k-Nearest Neighbor algorithm. With this initial effort a concordance rate of 15/16 was demonstrated from samples of varying types: semen, saliva, skin epidermis, and blood. This method also was designed to be compatible with the workflows published to date for NGS of short tandem repeats (STRs). The methylation approach described is highly accurate and, upon further validation and testing, may be potentially used in practice as a confirmatory test in conjunction with other NGS protocols used in forensic laboratories.