A59 Forensic Tissue Identification Based on DNA Methylation

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After attending this presentation, attendees will become acquainted with a new approach to forensic tissue identification based on methylation analysis.

This presentation will impact the forensic science community by demonstrating the potential of DNA methylation-based forensic tissue identification and the advantages of this approach over existing methods.

Identifying the source tissue of biological material found at crime scenes can be very informative in a number of cases. Current visual, catalytic, enzymatic, and immunologic tests for presumptive and confirmatory tissue identification are applicable only to a subset of samples, suffer limitations such as low specificity, can lack sensitivity of detection, and are substantially impacted by environmental insults. Moreover, these assays are incompatible and thus cannot be multiplexed and are less amenable to automation. In addition their results are operator-dependent. A better alternative approach is tissue identification based on messenger RNA (mRNA) and microRNA (miRNA); however, RNA is not as stable as DNA and requires the use of non-standard procedures by forensic laboratories.

A DNA-methylation based assay for forensic tissue identification can serve as an alternative and potentially overcome the limitations associated with extant methods. In mammalian DNA, methylation occurs at the C5 position of cytosine in some CpG dinucleotides. In the human genome 70 - 80% of all CpGs are methylated, while unmethylated CpGs are mainly grouped in “CpG islands” positioned at the 5’ ends of many human genes. The exact biological function of DNA methylation remains

poorly understood, however differential methylation patterns between different tissues have been demonstrated. Previous research found genomic loci that are consistently methylated and other loci that are consistently unmethylated in natural human DNA samples extracted from forensically relevant tissues, and showed that they could be used to differentiate between natural and artificially synthesized DNA. Herein genomic loci were utilized that were found to be differentially methylated between forensically relevant tissues forming the foundation of a DNA- based forensic tissue identification assay.

The presentation will demonstrate a DNA-based assay that performs tissue identification based on detection of tissue-specific methylation patterns. DNA samples are subject to digestion by a methylation-sensitive restriction endonuclease followed by multiplex amplification of specific genomic targets with fluorescent-labeled primers, capillary electrophoresis of amplification products, and automatic signal analysis by dedicated software, yielding the source tissue of the sample. The single tube assay was designed for easy integration by forensic laboratories (as the assay utilizes the same platforms as current forensic STR profiling). Moreover, the system is fully automatable, provides operator-independent results, and allows combining tissue identification with profiling in a single procedure.

Results will be presented of the tissue identification assay performed in two modes: as a standalone assay and combined with DNA profiling. The assay was tested on 50 DNA samples from blood, saliva, semen, and skin epidermis, and source tissue was successfully identified in all cases. Detection of semen and DNA profiling were combined into one assay and the ability to detect mixtures of semen and saliva in various ratios was demonstrated. The assay correctly detected semen in all samples where it was present, and the calculated percentage of semen was comparable to the fraction of semen in the samples. **Tissue Identification, Differential Methylation, Forensic Science**

 ** Presenting Author*