DEVELOPMENT OF A SINGLE-TUBE ASSAY FOR THE SIMULTANEOUS DETECTION OF BLOOD, SEMEN, SALIVA, AND VAGINAL FLUID UTILIZING DNA METHYLATION AND SCREENCLUST HIGH-RESOLUTION MELT SOFTWARE

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Tissue-specific differentially methylated regions (tDMRs)can be used to make determinations about the different body fluids found on a sample. Body fluids at a crime scene are useful evidence items when trying to piece together the events and the people involved.

Different body fluids that are present at the scene can set the tone of what the investigators think might have happened. For example, semen can mean that there was sexual misconduct, while blood could mean that there was some physical struggle. There are tests within the forensic science field that can hypothesize the presence of body fluids, but no test can completely confirm, without the possibilities of false positives or negatives. Since DNA remains the same throughout an individual's body, methylation markers allow different parts of the body to express different traits. When a methyl group is attached to a CpG on the 5' end of a CpG island (CGIs), there will be an inhibition of the transcription start site for the specific region which will ultimately silence the gene expression. After treating DNA with sodium bisulfite, all the cytosine residues that are not methylated will be converted into uracil so that, during PCR, unmethylated cytosines can be distinguished from methylated ones. Once amplified by methylation-specific PCR, the unmethylated cytosines will be transcribed as thymine and methylated cytosines will remain as cytosine residues. This will allow analysts to identify which regions in the DNA have undergone DNA methylation which can be helpful in body fluid detection. Previous research determined that primers such as BCAS4-M, DDX4-U, and DPPA5 are used to distinguish body fluids from high-resolution melt (PCR-HRM). By utilizing the ScreenClust software, the different melt profiles can be statistically separated and analyzed. The research presented here demonstrates the ability to identify different body fluids individually and within mixtures of blood, semen, saliva, and vaginal fluid.