B92 A New Approach Combining *In Silico* and High-Resolution Melt (HRM) Analysis to Quickly Identify New Loci for Body Fluid Identification Using DNA Methylation Melt Analysis

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After attending this presentation, attendees will be informed of a new combined approach using bioinformatic tools and HRM analysis to quickly and inexpensively identify genome locations from an array dataset for DNA methylation analysis.

This presentation will impact the forensic science community by demonstrating that HRM is an excellent tool to identify epigenetic loci for body fluid identification at a research level and to be used as a body fluid discrimination method easily incorporated into the daily routine of forensic laboratories.

DNA methylation is a natural process in the mammalian genome involving the addition of a methyl group to the 5'carbon of cytosines in a dinucleotide Cytosine-Guanine (CpG) pair.[1] Tissue-specific Differentially Methylated Regions (tDMRs) present different methylation patterns depending on the cell type analyzed, making them interesting targets for body fluid discrimination.[2-7] To fully take advantage of this approach, it is necessary to identify new tDMRs that consistently show methylation differences in body fluids. Whole genome analysis of CpGs using a commercially available array followed by a confirmatory Polymerase Chain Reaction (PCR) -based assay can be costly and time consuming. Alternatively, a computer-based, *in silico* approach can save much effort through the use of bioinformatics analysis.

Bisulfite-modified PCR is a useful method to determine relative levels of DNA methylation. In this procedure, unmethylated cytosines are chemically converted to uracils, which then are converted to adenines during real-time PCR. The methylated cytosines are protected from bisulfite conversion; therefore, methylated DNA will have higher melt temperature (T_M) than unmethylated DNA.[8-10] Since HRM is cost- and time-effective, it constitutes an excellent experimental tool to perform screening for multiple tDMRs quickly. Due to its fast turnaround, HRM also has considerable potential to be used as a standard method to discriminate body fluids in forensic laboratories.

Blood, buccal swabs, vaginal, and semen samples were collected from volunteers according to the approved Institutional Review Board (IRB) 13-0555 from Florida International University. DNA was extracted using the EZ1® DNA Investigator kit and the BioRobot® EZ1 automated purification workstation according to the manufacturer's specifications. Fifty nanograms of DNA were bisulfite modified using the EpiTect® Fast DNA Bisulfite Kit according to manufacturer's instructions. Primers specific for the CpG of interest were designed using either the Methprimer or the BiSearch online tools.[11,12] Bioinformatic analysis was performed using R software to determine relevant CpGs to discriminate blood, saliva, and vaginal epithelia. Array data from the report by Park and colleagues was analyzed and the M-value calculated to perform statistical analysis.[13] CpGs identified as statistically significant

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were further filtered for relevance using the ratio of methylated versus unmethylated probes and comparing such ratio between body fluids. Real-time PCR reactions were performed using the Epitect® HRM kit on a Rotor Gene 6000 real-time machine.

Results illustrate that using bioinformatics tools to perform array data analysis reduces the cost to identify new genome locations for body fluid identification using DNA methylation. The most successful approach was to use the calculated M-values to statistically identify significant differences between blood, saliva, and vaginal epithelia from the array study.[13] The CpGs were further sorted by analyzing the relative light emission from the probes pertaining to one body fluid in comparison to the other two. To date, this approach provided 71% success in identifying new CpGs *in silico.* For example, from seven CpGs identified as potential blood markers, five proved to show a difference in their T_M for blood when compared to saliva, semen, and vaginal epithelia, using HRM.

Using the information gained from these studies, a protocol for a multiplex qPCR with melt analysis that includes semen-, blood-, and vaginal epithelia-specific primers capable of providing results in approximately two hours was successfully tested. In conclusion, the combination of new bioinformatics and HRM analysis provides a cost- and time-effective approach to identify epigenetic loci for body fluid discrimination using DNA methylation. Moreover, due to a high throughput of HRM, this method will allow forensic laboratories to determine differences in DNA methylation for body fluids without the need to invest in expensive DNA sequencers, reagents, or personnel training and time.[2,6,9]

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High-Resolution Melt, DNA Methylation, Body Fluid Identification

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