HIGH-RESOLUTION MELT ANALYSIS OF DNA METHYLATION PATTERNS CAN DISCRIMINATE BODY FLUID OF ORIGIN IN CRIME SCENE SAMPLES

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Body fluid discrimination can aid the forensic investigation by determining the relevance of the DNA samples collected at a crime scene. We have had success in discriminating body fluids by studying epigenetic DNA methylation (1-4), which is characterized by the presence of a methyl group in the 5' carbon of a cytosine followed by a guanine (CpG) (5).
To expand the number of CpGs for forensics, a recently published methylation study involving 450,000 individual CpGs in the human genome was performed (6). The subsequent results were next examined in detail using pyrosequencing (2, 6) or SNaPshot (7); however, not all of those CpGs showed the expected outcome.
As a result, we have applied a novel, *in silico* analysis of array data in order to define useful loci for discrimination of body fluids.

Our *in silico* analysis gives a higher weight to raw array data and uses a combination of Kruskal Wallis and ANOVA followed by Dunn's and Tukey post-hoc tests, respectively. In our initial study, CpGs with the potential to discriminate vaginal epithelia were prioritized and 8 specific CpGs were identified. The next step involved developing primers for these regions using Methprimer (8) or BiSearch (9) according to the guidelines of Wodjacz (10).

Blood, saliva, vaginal epithelia and semen were collected from volunteers according to the approved IRB protocols. DNA was extracted using the BioRobot® EZ1 automated purification workstation (Qiagen, CA) and 100 ng of DNA were bisulfite-modified using the EpiTect® Fast DNA Bisulfite Kit (Qiagen, CA). Amplification and melt-analysis reactions were performed using the Epitect® HRM kit on a Rotor Gene 6000 real time instrument (Qiagen, CA).

The results obtained show that of the initial list of 8 CpGs identified from the array, 4 produced the expected differences in T_M between vaginal epithelia and other body fluids.
Of the remaining 4 CpGs, 3 were not amplified, a result which probably indicated deviations in annealing temperature between forward and reverse primers. The 4th locus showed a similar melt temperature between semen and vaginal epithelia. The array experiment did not include DNA from semen, which may explain why our *in silico* analysis was unable to predict this similarity.
In conclusion, our combination of *in silico* and HRM analysis results in a fast PCR-based method to identify new CpGs for body fluid discrimination. Application of this process should help to accelerate discovery of additional epigenetic loci for forensic analysis.

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