METHYLATION MARKERS AND IDENTIFICATION OF BLOOD FOR FORENSIC PURPOSES USING PYROSEQUENCING

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DNA methylation analysis for forensic tissue identification is one of the new and fast developing technologies in the field of forensic science. Herein, we report the usefulness of the differentially methylated regions of the human genome for tissue source identification of a DNA sample. One advantage of this methodology is that it uses DNA, which is already available from case work, without the need of additional use of evidence material. Several differentially methylated regions of the human genome have been explored and here we report the usefulness of four specific CpG sites (cg-xxx630, cg-xxx435, cg-xxx773, cg-xxx495) and 18 other neighboring sites for the differentiation of blood tissue from four other bodily fluids namely, buccal cells, vaginal cells, spermatozoa, and skin cells. In this report, we measure methylation differences using pyrosequencing. Pyrosequencing provides quantitative methylation value for each CpG studied, allowing direct sample to sample comparison. Additionally, this technology allows the possibility of multiplexing, allowing multiple markers to be analyzed in a single run. DNA was extracted from multiple samples of blood, buccal cells, vaginal cells, spermatozoa, and skin cells, bisulfite modified, PCR amplified using site specific primers and pyrosequenced. Mean methylation values of the different tissues were compared using a one-way ANOVA and Tukey's Post Hoc pair-wise comparisons to determine if there were statistically significant differences between the methylation values of the tissues studied. Methylation differences were statistically significant when p-values were less than 0.05. SPSS software package (PASW statistics 18) was used to perform the statistical analysis of the data. All CpG sites studied were found to be hypermethylated in blood presenting differences of 20-40% of methylation when compared to other tissues. Methylation levels in blood were statistically significant ($p<0.05$) compared to other tissues. The methylation markers here identified, together with pyrosequencing technology, can be used in a forensic laboratory to identify the tissue origin of a DNA sample.