Genome-Wide Methylation Profiling and a Multiplex Construction for the Body Fluid Identification Using Epigenetic Markers

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Introduction

The identification of body fluids found at crime scenes can contribute to solving crimes by providing information that can reveal important insights into crime scene reconstruction. Recently, DNA methylation profiling has been in the spotlight as a promising new tool for distinguishing between different types of body fluids because of the high specificity, compatibility with existing STR typing protocols, and fit with current forensic casework application. Different cell types have different methylation patterns, and chromosome segments called tDMRs (tissue-specific differentially methylated regions) are known to show different DNA methylation profiles according to cell or tissue type. Here, we developed a multiplex methylation SNaPshot system for forensic body fluid identification using body fluid-specific epigenetic markers, which were identified from genome-wide epigenetic analysis of 23 samples using the Illumina Infinium HumanMethylation450 BeadChip array.

Materials and Methods

Samples Body fluid samples (venous blood, saliva, semen, menstrual blood and vaginal fluid) were collected from 49 volunteers (34 males and 15 females) using procedures approved by the Institutional Review Board of Severance Hospital, Yonsei University in Seoul, Korea. DNA was extracted from an aliquot of blood, saliva and semen or a single cotton swab of menstrual blood and vaginal fluid using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Extracted DNA was quantified using a Quantifiler® Duo DNA Quantification Kit (Applied Biosystems, Foster City, CA, USA). Bisulfite-converted DNA was obtained by modification of 20-200 ng genomic DNA using the Imprint™ DNA Modification Kit (Sigma-Aldrich Inc., St. Louis, MO, USA) according to the manufacturer's protocol.

Genome-wide DNA methylation profiling using BeadChip array We analyzed DNA methylation profile of 485,000 CpG loci in various body fluids DNA from a group of volunteers using HumanMethylation450 BeadChip array (Illumina, San Diego, CA, USA). We assayed 23 samples, including each 5 or 6 samples of blood, saliva, semen and each 3 samples of menstrual blood and vaginal fluid. Methylation values are reported as betas (fraction between 0 and 1), which represent the methylation level of each CpG site. Based on delta mean value (difference in average beta for each body fluid), we selected body fluid-specific candidate methylation CpG markers. Clustering analysis of selected candidate CpG markers was performed with BDPC program (http://services.ibc.uni-stuttgart.de/BDPC/).

Site-specific analysis using methylation SNaPshot We performed site-specific analysis for 66 candidate markers in a larger group of samples, including each 5 samples from each body fluid, using methylation SNaPshot method. PCR primers and single-base extension (SBE) primers were designed using the MethPrimer program (http://www.urogene.org/ methprimer/index1.html) or the BatchPrimer3 program (http://wheat.pw.usda.gov/demos/BatchPrimer3). One to ten nanogram of bisulfite-converted DNA was amplified in a 20 μL final volume containing 3.0 U of AmpliTaq Gold® DNA Polymerase (Applied Biosystems), 2.0 μL of Gold ST*R 10× Buffer (Promega, Madison, WI, USA), and appropriate concentrations of each primer. Thermal cycling was conducted under the following conditions: 95˚C for 11 min; 34 cycles of 94˚C for 20 sec, 56˚C for 1 min, 72˚C for 30 sec; and a final extension at 72˚C for 7 min. For the following SBE reaction, 5.0 μL of PCR product was purified with 1.0 μL of ExoSAP-IT (USB, Cleveland, OH, USA). SBE reaction was carried out with a SNaPshot™ Multiplex Kit (Applied Biosystems) according to the manufacturer's instructions. Extension products were analyzed using a 3130 Genetic Analyzer (Applied Biosystems) and GeneMapper® ID software v.3.2 (Applied Biosystems).

Multiplex methylation SNaPshot A multiplex methylation SNaPshot system was constructed to determine the methylation status of eight final selected CpG markers simultaneously. Sensitivity and efficacy of the developed multiplex were tested using serially diluted DNA, aged body fluid samples and artificial casework samples.

Results

Fig. 1. Clustering analysis of 66 body fluid-specific CpG marker candidates (A) Heatmap of DNA methylation at 66 CpG sites in 23 body fluid samples. (B) Hierarchical clustering of 23 samples with the 66 CpG markers .

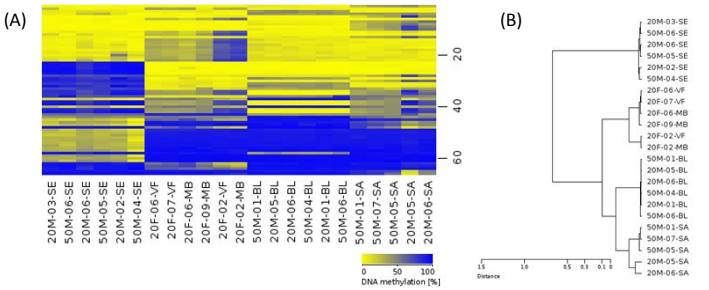


Fig. 2. Representative electropherograms of body fluid identification using multiplex methylation SNaPshot The CpG IDs in the HumanMethylation450 BeadChip array for the markers, SE1, SE2, SE4, BL1, BL2, VF1, VF2 and SA1 are cg17610929, cg26763284, cg17621389, cg06379435, cg01543184, cg09765089, cg26079753 and cg09652652, respectively. Markers with differential DNA methylation which are specific to a certain body fluid were indicated in blue.

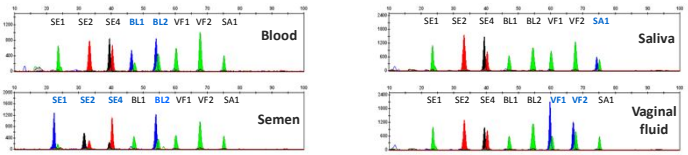


Fig. 3. Validation test for the multiplex methylation SNaPshot (A) Body fluid identification with samples aged while exposed to the ambient environment for 75 days (B) Artificial casework sample test. Sources were post-coital vaginal samples or a penile sample. The semen-specific peaks were indicated by arrows.

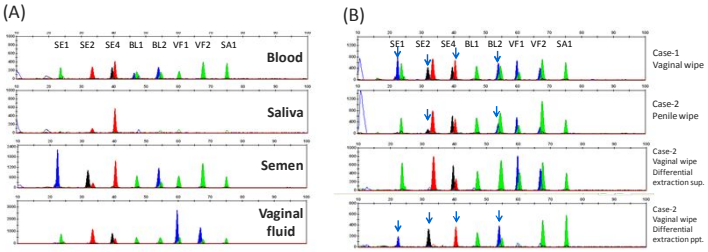
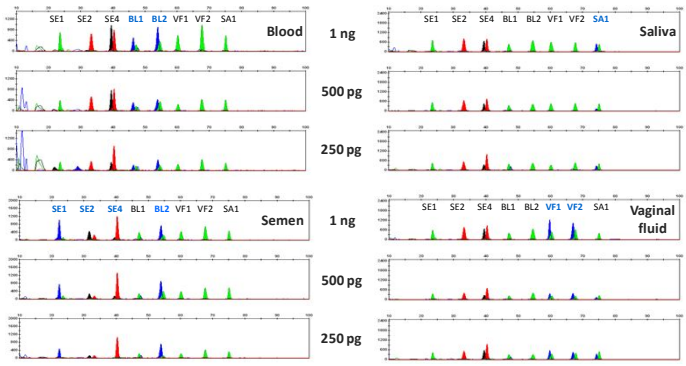


Fig. 4. Sensitivity test for the multiplex methylation SNaPshot



Conclusion

- We analyzed DNA methylation profiles of various body fluids including venous blood, saliva, semen, menstrual blood and vaginal fluid using the Illumina Infinium Human Methylation450 BeadChip array and selected 66 body fluid-specific CpG marker candidates .
- Through the further validation test using methylation SNaPshot reaction, we selected eight CpG markers specific to certain body fluid types and constructed a multiplex methylation SNaPshot system to analyze methylation patterns of the eight CpG markers simultaneously.
- The developed multiplex system was able to discriminate four types of body fluids, i.e. venous blood, saliva, semen and vaginal fluid-menstrual blood, and allowed successful identification even with aged or artificial casework samples.

Acknowledgement

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