Body fluid identification based on tissue-specific differential DNA methylation

Ajin Choi[1], Ja Hyun An[1], Myung Jin Park[1], Kyoung-Jin Shin[1,2], Woo Ick Yang[1], and Hwan Young Lee[1,2]

(1) Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea
(2) Human Identification Research Center, Yonsei University, Seoul, Korea

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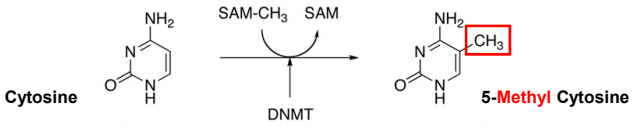
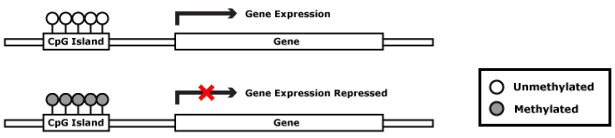
DNA methylation

- **DNA methylation** is the addition of a methyl group to the DNA base cytosine followed by a guanine (**5' CG 3'**).



- **DNA methylation** of a gene's CpG island **represses gene expression**. **Different cell types have different methylation patterns**, which contributes to the differences in gene expression in different cell types.



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tDMRs and body fluid identification

- Chromosome pieces called **tDMRs (tissue-specific differentially methylated regions)** show different DNA methylation profiles according to the type of cell or tissue.
- **The potential of tissue-specific differential DNA methylation for body fluid identification** was examined.

Table 1. Genomic information for candidate tDMRs for body fluid identification

Tissue	UCSC location (Mar. 2006)	CGI	Gene	Function	References
Testis	chr14:58182690–58182995	cpgi50	DACT1	Dapper 1 isoform 2	Genomics. 89:326
Testis	chr6:41881884–41882111	cpgi46	USP49	Ubiquitin carboxyl-terminal hydrolase 49	Genomics. 89:326
Blood	chr7:27135995–27136879	cpgi87	HOX44	Homeobox protein Hox-A4	PLoS Biol. 6:e22
Blood	chr5:176758438–176760564	cpgi82	PFN3	Profilin-3	PLoS Biol. 6:e22
Blood	chr21:46905647–46905874	cpgi55	PRMT2	Protein arginine N-methyltransferase 2	PLoS Biol. 6:e22

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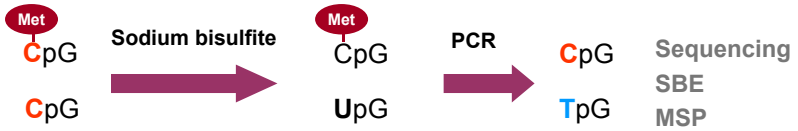
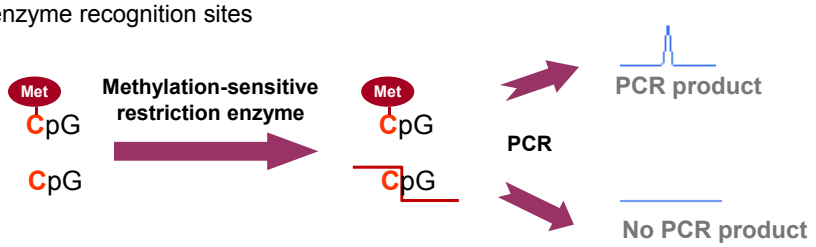
Analysis of DNA methylation

- **Sodium bisulfite treatment** of CpG motifs



- **Methylation-sensitive restriction enzyme treatment** of CpG motifs in the enzyme recognition sites



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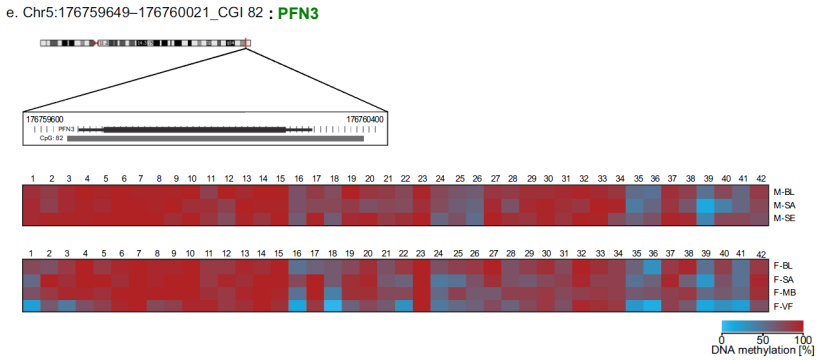
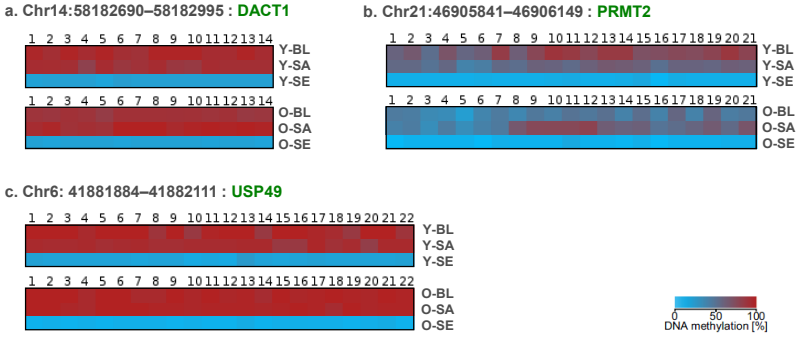
Differential DNA methylation in body fluids

- DNA methylation profiles for the tDMRs for the genes **DACT1, USP49, HOXA4, PRMT2, and PFN3** were produced by sequencing of bisulfite-treated **pooled DNA from blood, saliva, semen, menstrual blood, and vaginal fluid** obtained from **10 males and 6 females**.



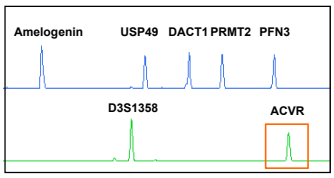
DNA methylation and aging

- DNA methylation profiles of 3 tDMRs for the genes **DACT1, PRMT2, and USP49** were further analyzed by sequencing of bisulfite-treated **pooled DNA from blood, saliva**, and **semen** obtained from **20 young (< 30 y) and 15 old (> 50 y) men.**

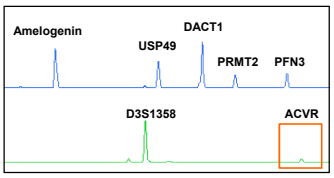


A multiplex PCR using methylation-sensitive restriction enzyme

- A multiplex PCR was developed for determination of DNA methylation status of **4 CpG loci at the USP49, DACT1, PRMT2, and PFN3 tDMRs** using **HhaI**, which recognizes and cuts unmethylated G**CG**C sequence.
- **Complete enzyme digestion** was confirmed by the removal of PCR product for the **ACVR CpG island**. **DMSO** was added at the amplification step due to the **high GC contents of the selected tDMRs** (> 60%).



PCR without HhaI digestion



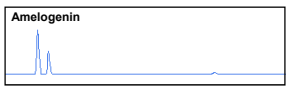
PCR after HhaI digestion

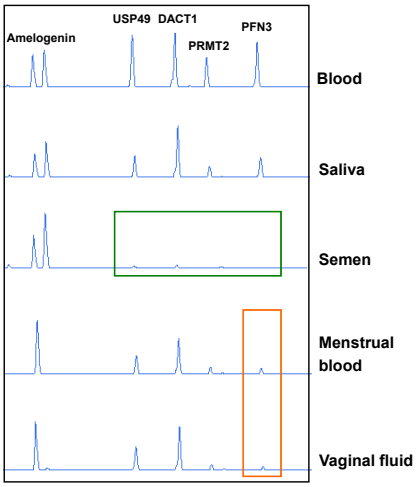
A multiplex PCR using methylation-sensitive restriction enzyme

- The tDMRs for **USP49, DACT1, PRMT2, and PFN3** showed **semen-specific unmethylation**.
- The tDMR for **PFN3** showed **hypomethylation in menstrual blood and vaginal fluid**.

Methylated control DNA

Unmethylated control DNA





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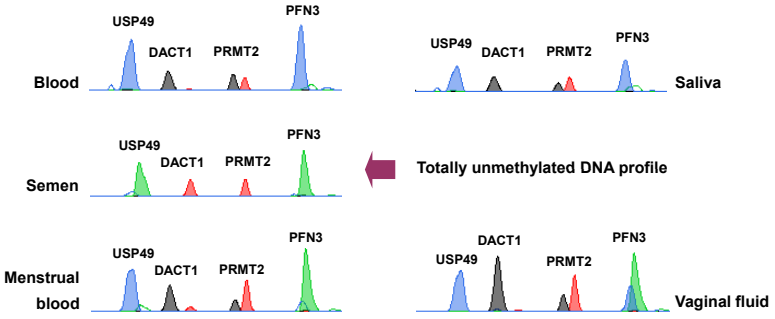
A multiplex SBE design for bisulfite-treated DNA

- **A multiplex SBE** was developed for determination of DNA methylation status of **4 CpG loci at the USP49, DACT1, PRMT2, and PFN3 tDMRs.**
- A multiplex SBE results were consistent with those of the multiplex PCR using methylation sensitive restriction enzyme.



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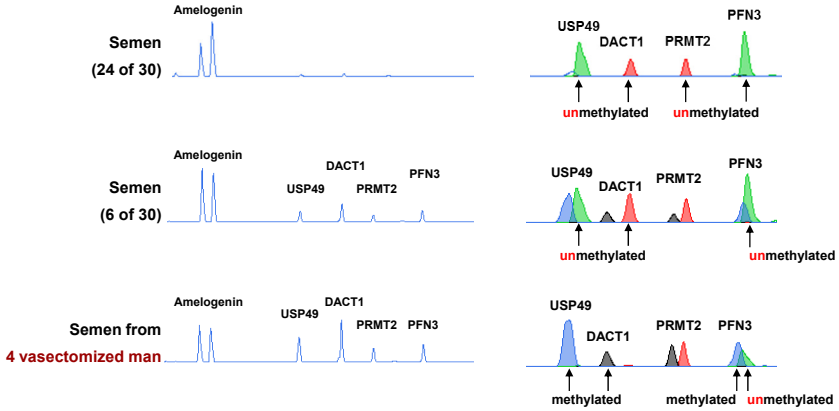
Semen profiles of the two multiplexes

- **Semen samples including sperm cells** displayed **unmethylation signal at all CpG loci in SBE analysis** for bisulfite-treated DNA.



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Concluding remarks

- The analysis of **tissue-specific differential DNA methylation** was proposed as a promising new method for the identification of body fluids.
- The multiplex PCR system, which allows combined use of tDMRs for **USP49, DACT1, PRMT2, and PFN3**, could be used to discriminate blood-saliva, semen and vaginal fluid-menstrual blood.
- Future genome-wide DNA methylation analysis using various body fluid samples will be useful to identify additional body fluid-specific tDMRs and enable the subsequent development of efficient analysis methods for forensic casework.

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