Molecular tools for forensic body fluid identification

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Presentation overview

- Introduction: Body fluid identification in forensics
- Recent developments in molecular genetics-based body fluid identification
  - **Messenger RNA profiling**
  - **Bacterial ribosomal RNA analysis**
  - **Micro RNA profiling**
- Potential application of **DNA methylation profiling** for forensic body fluid identification

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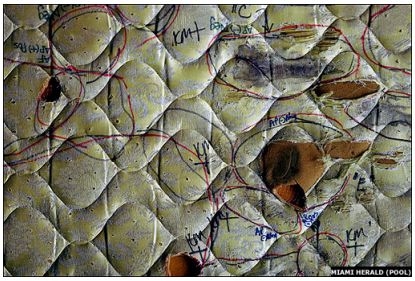
Body fluid identification in forensics

- Information that supports **a link between sample donors who have been identified by DNA profiling and actual criminal acts** is crucial.
- Body fluid identification, **determining the sample's cellular origin** can reveal significant insights into crime scene reconstruction and contribute toward solving crimes.

Sex offender's trial



Body fluids founds on the bed

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Body fluid identification in forensics

- Current methods based on chemo luminescence or immunological tests are mainly presumptive.
- Recent advances in genetics and molecular biology led to the suggestion of **the use of a molecular genetics-based approach to supplant conventional methods**.

Photo of belt with semen illuminated with white light and UV light

Photo of robber mask with saliva illuminated with white light and UV light

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Recent developments in molecular genetics-based body fluid identification

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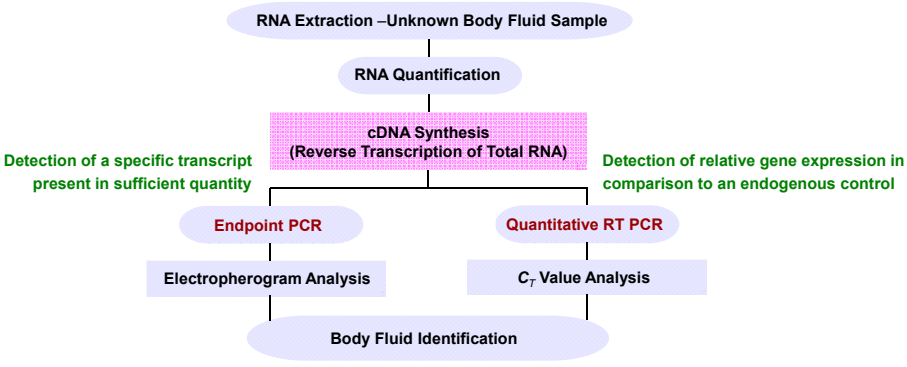
RNA profiling for body fluid identification

- Some **mRNAs and miRNAs are expressed in a tissue-specific manner** and their expression patterns can confirm specific body fluids, even after long periods.



Schema for body fluid identification using RNA profiling

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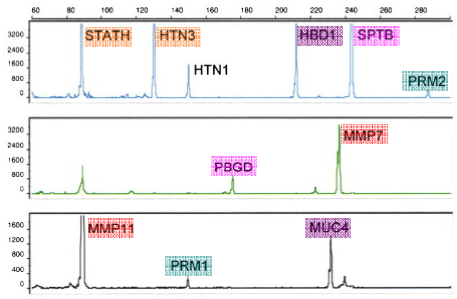
mRNA profiling for body fluid identification

- Several mRNA-based multiplex PCR assays now are available for parallel determination of venous blood, saliva, semen, and menstrual blood.
- Major advantages of mRNA profiling are the **possibility of detecting several body fluids in one multiplex reaction** and the **simultaneous DNA isolation** without loss of material.

	Blood	Saliva	Semen	Vaginal secretions	Menstrual blood	Genomic DNA
SPTB	+	-	-	+	(+)	-
SPTB*	+	-	-	+	+	-
PBGD	(+)	-	-	-	(+)	-
HBB	+	-	-	-	+	-
HBB*	+	-	-	-	+	-
STATH	-	+	-	-	-	-
HTN3	-	+	-	-	-	-
PRM1	-	-	+	-	-	-
PRM2	-	-	+	-	-	-
PRM2*	-	-	+	-	-	-
HBD-1	-	-	-	+	(+)	-
MUC4	-	-	-	+	+	-
MMP-7	-	-	-	(+)	+	-
MMP-7*	-	-	-	+	(+)	-
MMP-11	(+)	-	-	-	+	-
MMP-11*	-	-	-	(+)	+	-
18SrRNA	++	+	+	+++	+++	+
GAPDH	+	-	++	++++	++++	+++
GAPDH*	++	+	+	+++	+++	-

Body fluid specificity of the mRNA markers



Multiplex result of body fluid mixture (Haas et al. FSIG 2009)

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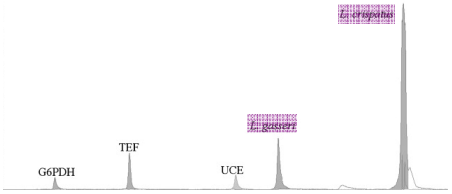
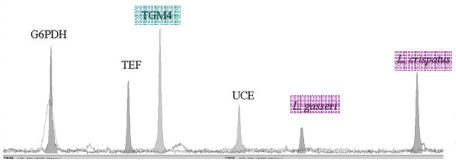
Microbial RNA for vaginal fluid identification

- The 16S-23S rRNA intergenic spacer region of ***Lactobacillus crispatus*** and ***Lactobacillus gasseri*** were found to be a suitable marker for identifying vaginal secretions, and could be incorporated into a mRNA multiplex system.



Multiplex result from a vaginal swab (Fleming *et al.* FSIG 2010)



Multiplex result from a vaginal swab with semen (TGM4: seminal fluid specific mRNA marker)

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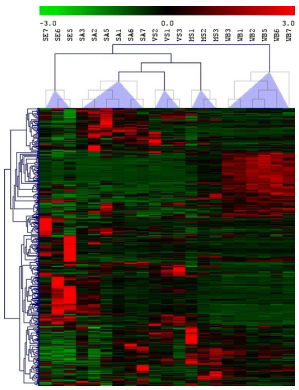
miRNA profiling for body fluid identification

- A clear advantage of microRNAs (miRNAs, 18-25 bases in length) over mRNA is their small size, which makes them likely to have higher ***in vitro*** **stability than mRNAs**.



- Accurate quantification of miRNAs using quantitative RT PCR requires not only a **highly sensitive and specific detection platform** for experiment operation, but also **a reproducible methodology with an adequate model for data analysis**.

Differentiation of forensically relevant body fluids using genome-wide miRNA expression data (Zubakov et al. IJLM 2010)

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Potential application of DNA methylation profiling for body fluid identification

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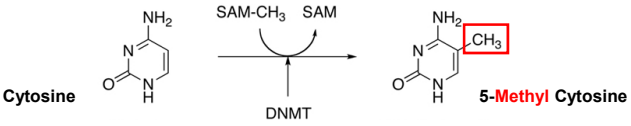
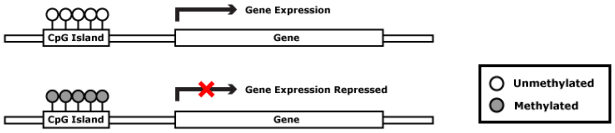
DNA methylation

- **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

Lee et al. IJLM in press

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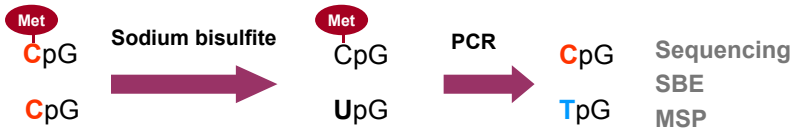
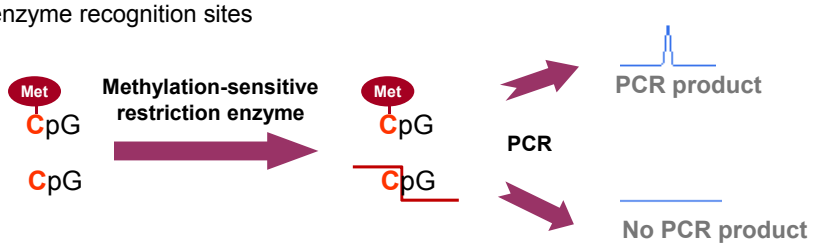
Analysis of DNA methylation

- **Sodium bisulfite treatment** of CpG motifs



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



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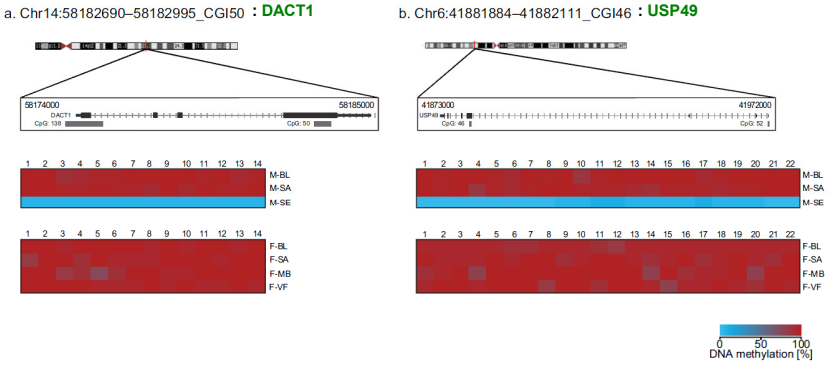
Differential DNA methylation in body fluids

- 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**.



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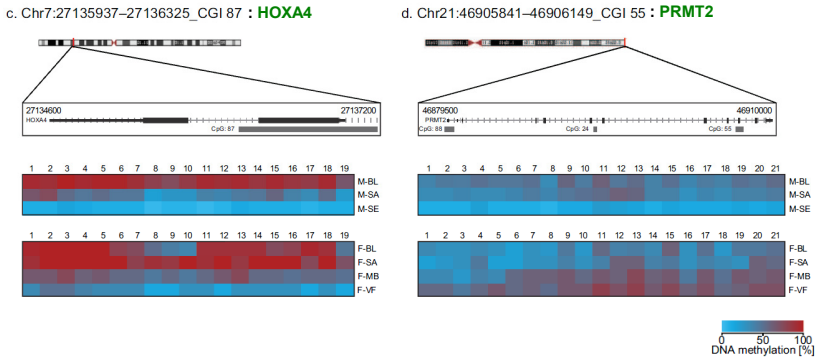

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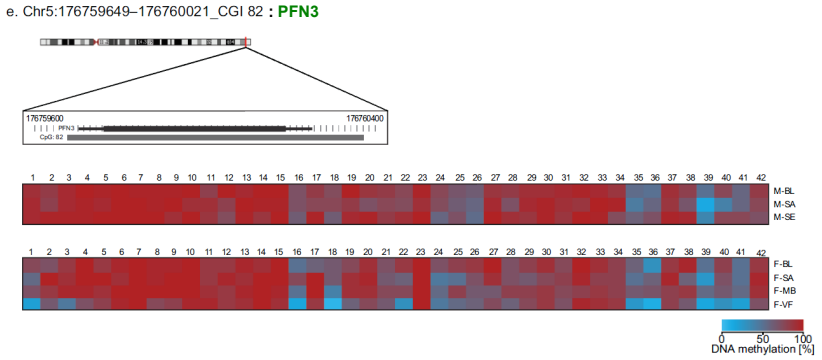

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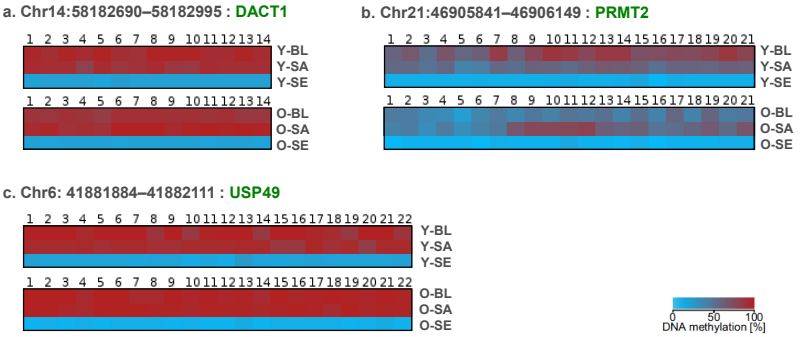
DNA methylation and aging

- 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.**



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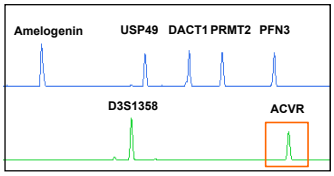
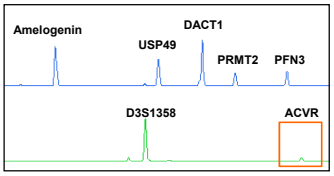
A multiplex PCR using methylation-sensitive restriction enzyme

- 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

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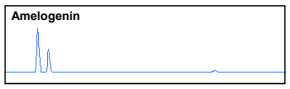
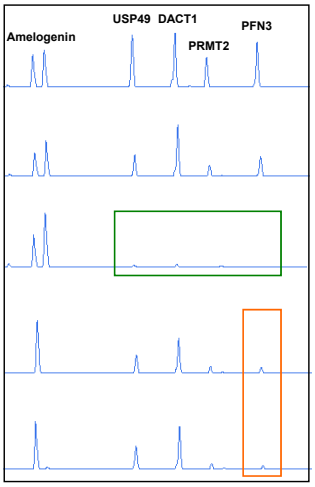
A multiplex PCR using methylation-sensitive restriction enzyme

- 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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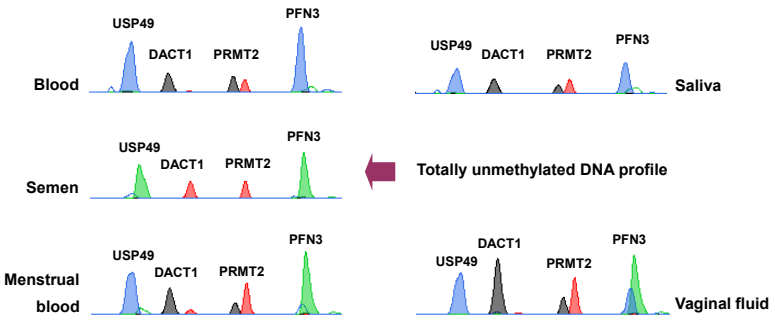
A multiplex SBE for bisulfite-treated DNA

- **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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DNA methylation profiling for body fluid identification

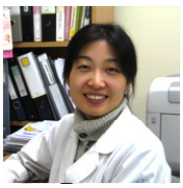
- The analysis of **tissue-specific differential DNA methylation** was proposed as a promising new method **for the identification of forensic body fluids.**
- The multiplex PCR system, which allows combined use of tDMRs for **USP49, DACT1, PRMT2, and PFN3**, could be used **to discriminate blood-saliva, 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.

Concluding remarks

- **Body fluid identification** can reveal significant insights into **crime scene reconstruction** and contribute toward solving crimes.
- Along with recent advances in genetics and molecular biology, lots of studies suggested **RNA markers for differentiating body fluids**, but some of the results for miRNA markers have yet to be confirmed and warrant additional investigation.
- The analysis of **tissue-specific differential DNA methylation** was also proposed as a promising new method for the identification of body fluids, but additional marker development and forensic validation will be necessary for practical use of this new approach in forensic practices.

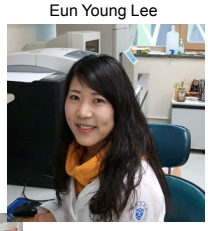
Acknowledgment

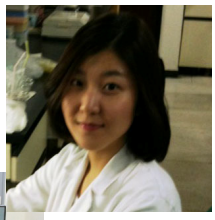
Prof. Kyoung-Jin Shin



Myung Jin Park

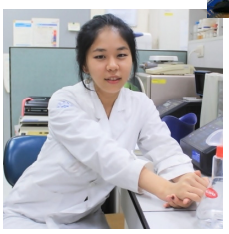
Eun Young Lee





Ja Hyun An

Dr. In Seok Yang



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