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# Body Fluid Identification by Integrated Analysis of DNA Methylation and Body Fluid-Specific Microbial DNA

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## Body fluid ID based on DNA analysis

- Forensic body fluid Identification

Determining the origin of the biological samples found at a crime scene can provide important clues into **crime scene reconstruction**

- DNA-based body fluid identification method

- DNA methylation analysis

- tDMRs (tissue-specific differentially methylated regions)
    - Semen-specific tDMRs

- Body fluid-specific bacterial identification

- 16S ribosomal RNA (rRNA) gene, 16S-23S rRNA intergenic spacer region
    - Saliva, vaginal fluid specific bacteria



## Limitations

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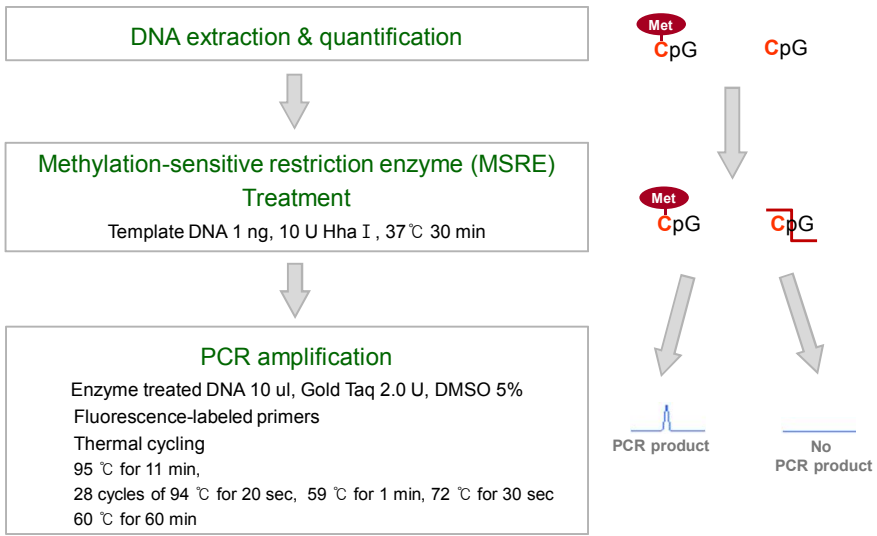
- DNA methylation analysis
    - Can clearly identify semen with sperm cells
    - Imperfect discrimination of detailed body fluids
  - Body fluid-specific bacterial identification
    - Can discriminate only saliva and vaginal fluid
    - Bacteria can reside from body sites that are proximate or can be in contact with the body site
    - Low specificity
- Need to additional markers or integration of other body fluid identification methods for identification of various body fluids in one reaction

## Objects

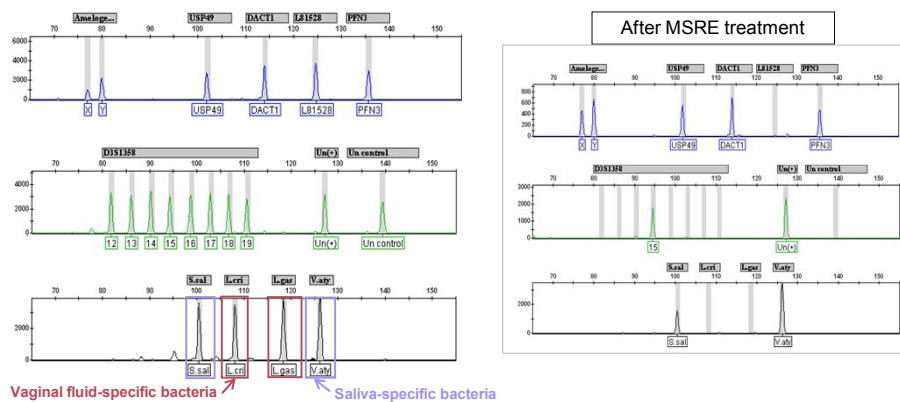
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- Development of multiplex PCR system for efficient body fluid identification by integrated analysis of DNA methylation and body fluid-specific microbial DNA
- Forensic evaluation of developed multiplex PCR system

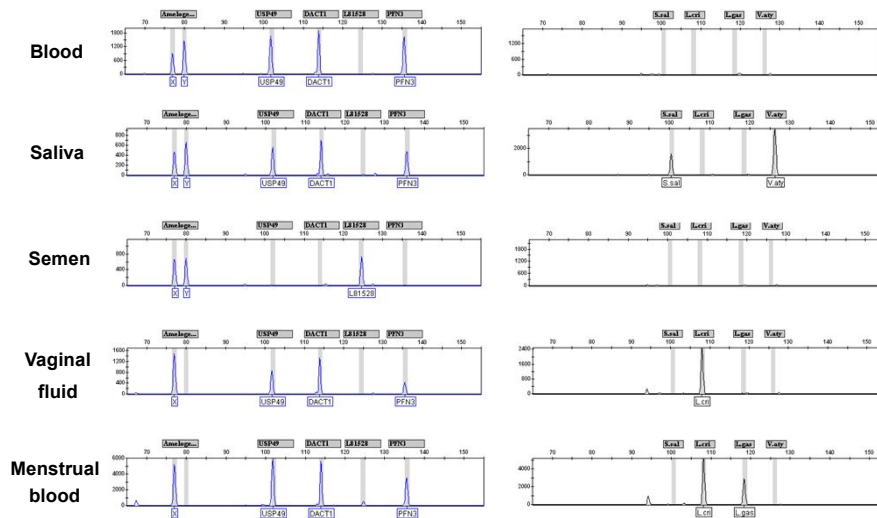
## Experimental Procedures



## Schema of multiplex PCR



## Results of multiplex PCR system

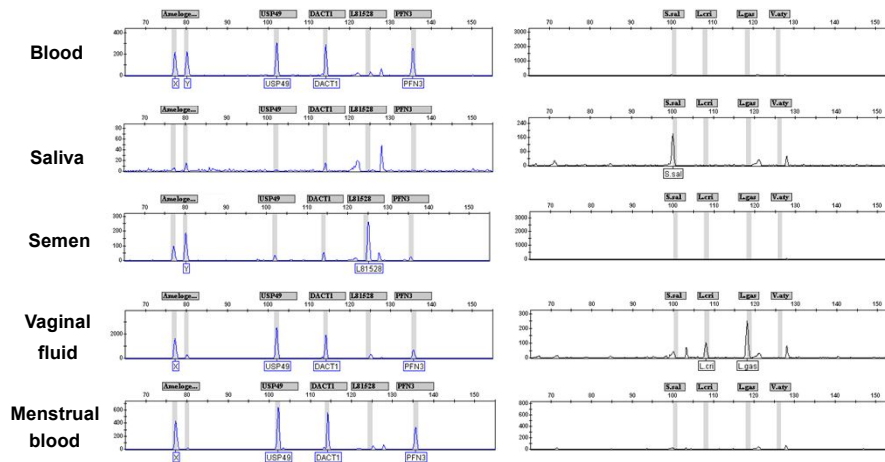


## Bacteria profiles for each body fluid

Body fluid	N	Number of positive samples				Number of negative samples
		<i>L.crispatus</i>	<i>L.gasseri</i>	<i>S.salivarius</i>	<i>V.atypica</i>	
Blood	20	0	0	0	0	-
Saliva	21	1	0	19	13	2
Semen	21	0	0	0	0	-
Vaginal fluid	14	8	9	0	0	1
Menstrual blood	14	8	8	0	0	3

## Stability test

- Aged samples (environment exposure for 75 days) showed almost identical results compared with freshly obtained samples



## Concluding remarks

- The multiplex PCR system, which allows combined use of 4 tDMRs for USP49, DACT1, L81528, PFN3, and 4 body fluid-specific bacteria markers for *L.crispatus*, *L.gasseri*, *S.salivarius*, *V.atypica*, could be used to discriminate blood, saliva, semen, and vaginal fluid-menstrual blood.
- Because developed multiplex system uses the same biological source of DNA for individual identification profiling and simultaneously analyzes various body fluids in one reaction, the method will facilitate more efficient body fluid identification in forensic casework.

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***Thank you for your attention!***



Yonsei DNA Profiling Group  
<http://forensic.yonsei.ac.kr/>

