Body fluid identification by simultaneous analysis of DNA methylation and body fluid-specific bacteria
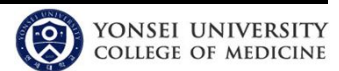
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# Body fluid ID based on DNA analysis

- **DNA methylation analysis**
  - Target : tissue-specific differentially methylated regions (tDMRs)
  - Application : Semen identification

- **Body fluid-specific bacterial detection**
  - Target : 16S rRNA gene, 16S-23S rRNA intergenic spacer region
  - Application : Saliva and vaginal fluid identification

  ➔ Need to add more markers or to integrate existing DNA-based body fluid identification methods for discrimination of more types of body fluids in one multiplex reaction

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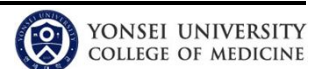
Experimental procedures

DNA extraction & quantification

⬇

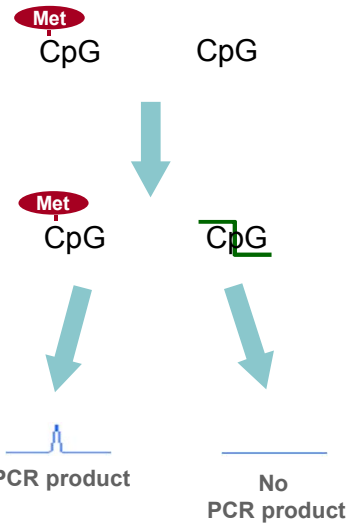Methylation-Sensitive Restriction Enzyme treatment

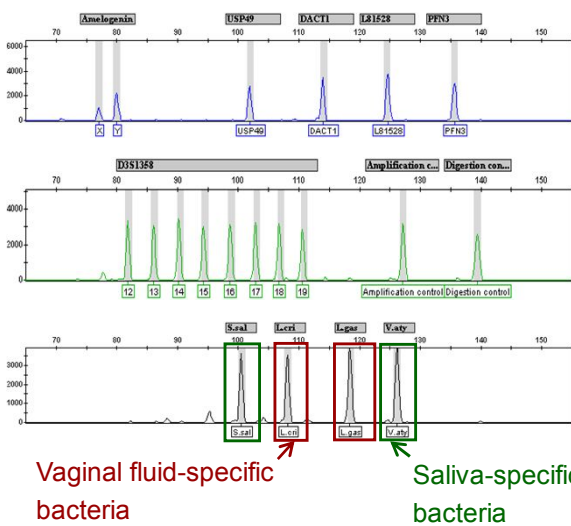1 ng of DNA, 10 U of *Hha* I , 37℃ for 30 min

⬇

PCR amplification

Enzyme treated DNA, 2.0 U Gold *Taq* pol, 5% DMSO, fluorescence-labeled primers
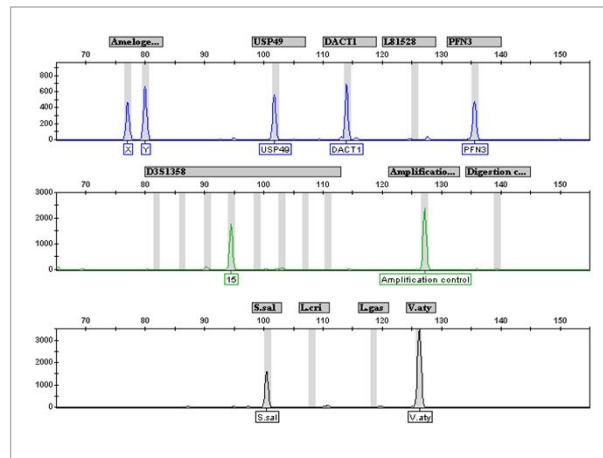
Thermal cycling

95 ℃ for 11 min

94 ℃ for 20 sec
59℃ for 1 min
72 ℃ for 30 sec
X 28 cycles   60℃ for 60 min

Met CpG   CpG

Met CpG   CpG

PCR product   No PCR product

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Schema of multiplex PCR

After MSRE treatment

Vaginal fluid-specific bacteria

Saliva-specific bacteria

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Body fluid ID using multiplex PCR

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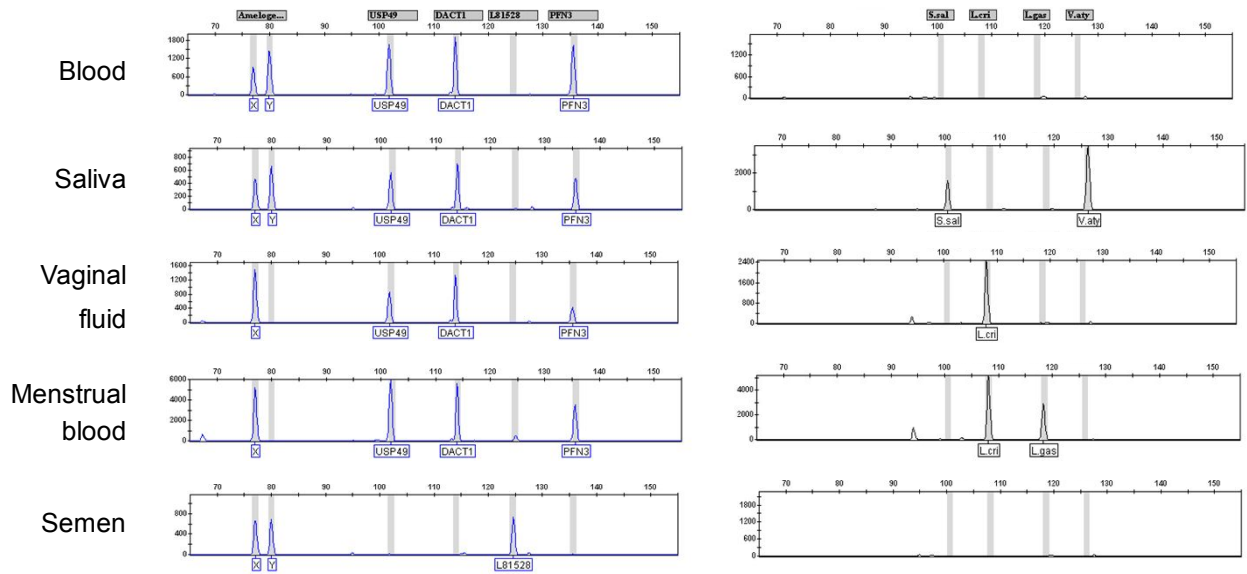
Semen identification using multiplex PCR
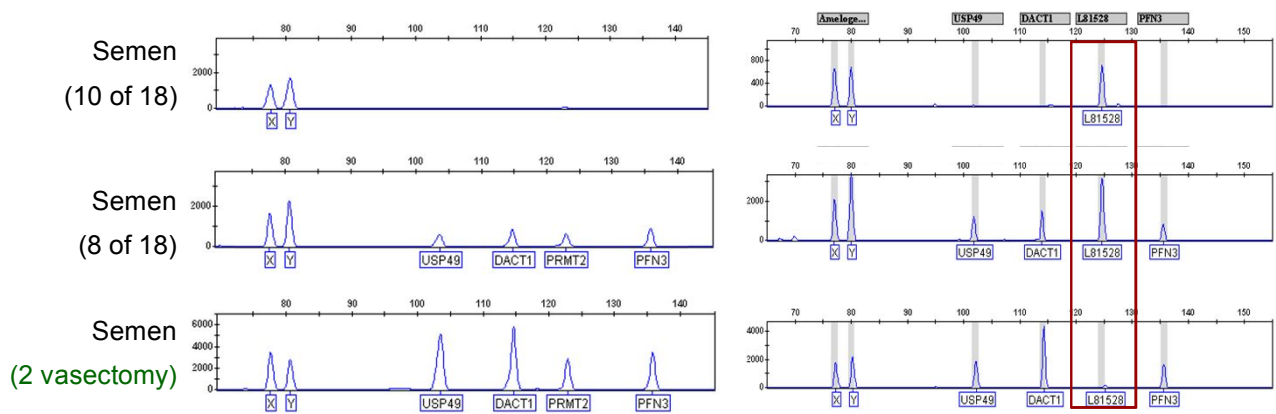
Int J Legal Med (2013) 127:35–43

Multiplex PCR in the present study

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Bacteria profiles for each body fluid

Body fluid	N	Number of positive samples				Number of negative samples
		L.crispatus	*L.gasseri*	*S.salivarius*	*V.atypica*	
Blood	20	0	0	0	0	-
Saliva	20	1	0	18	12	2
Semen	20	0	0	0	0	-
Vaginal fluid	14	8	9	0	0	1
Menstrual blood	14	8	8	0	0	3

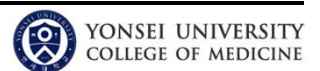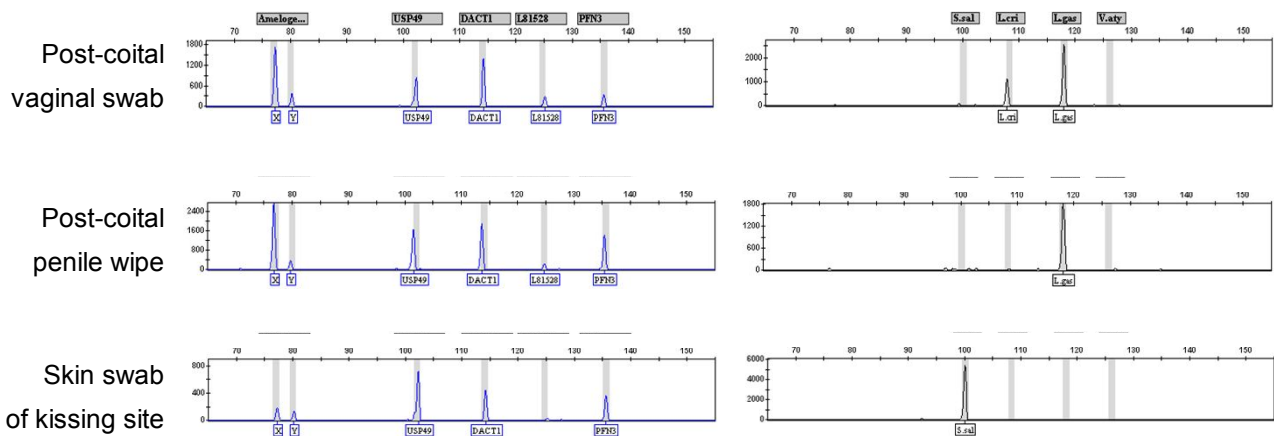# Forensic evaluation - Sensitivity test

500 pg semen

500 pg saliva

125 pg saliva

250 pg vaginal fluid

125 pg vaginal fluid
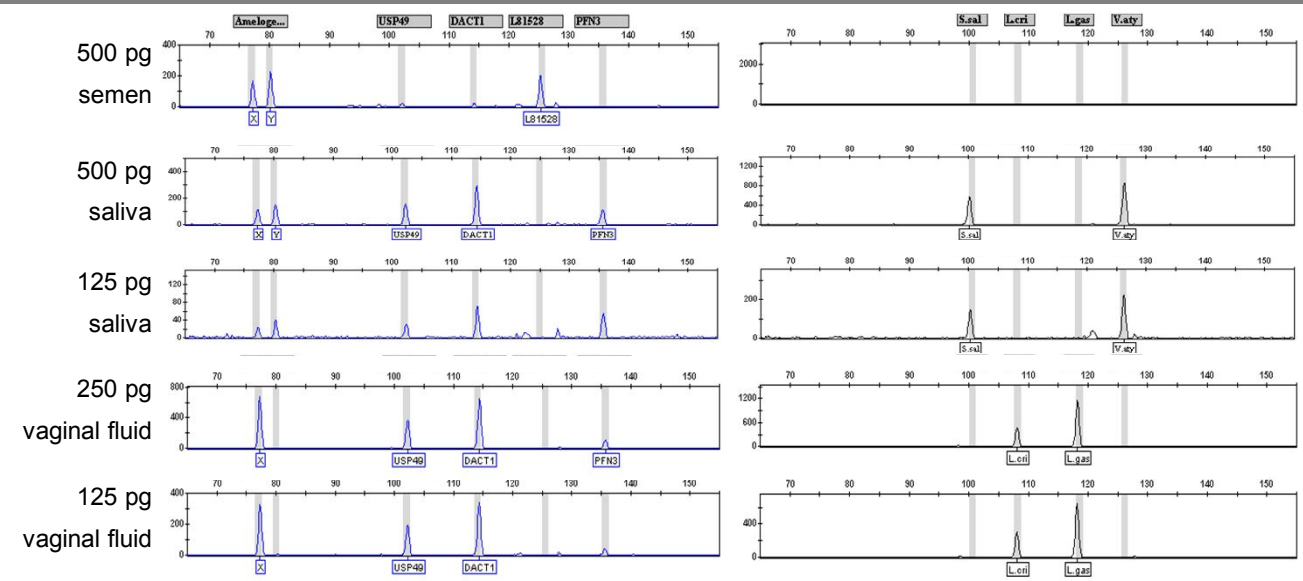
Forensic evaluation - Stability test

- Aged samples showed almost identical results compared with freshly obtained samples

Blood
Saliva
Semen
Vaginal fluid
Menstrual blood
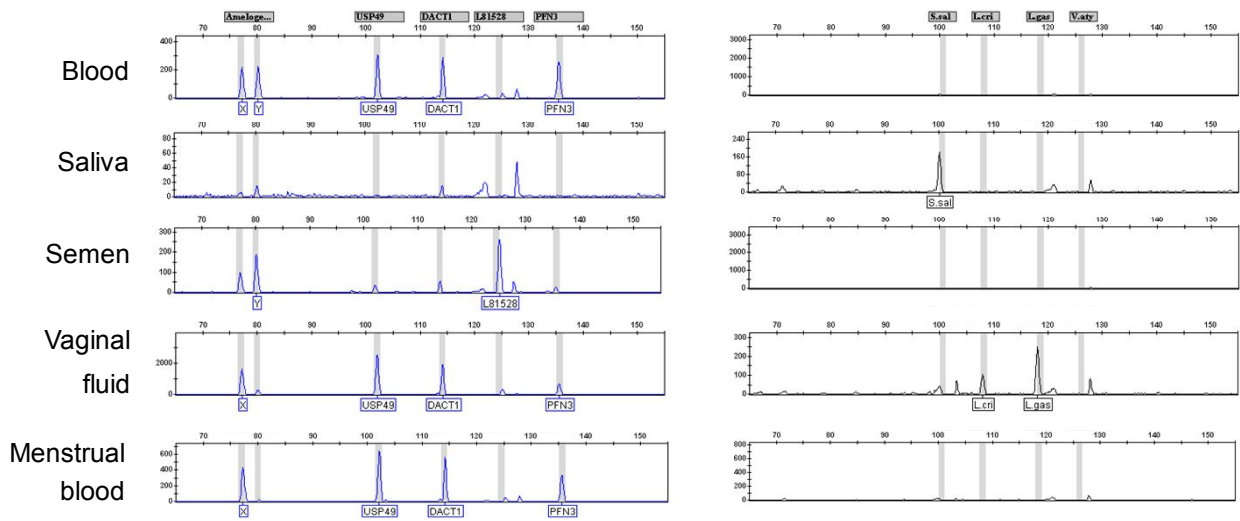
Forensic evaluation – Casework sample test

Post-coital vaginal swab
Post-coital penile wipe
Skin swab of kissing site

Conclusion

- The multiplex PCR system, which allows combined use of 4 tDMRs for USP49, DACT1, L81528 and PFN3, and 4 body fluid-specific bacteria markers for *L.crispatus, L.gasseri, S.salivarius and V.atypica*, could be used to discriminate blood, saliva, semen and vaginal fluid-menstrual blood.
- The newly developed multiplex method ensures forensic applicability as well as high specificity, reliability and sensitivity, thereby facilitating more efficient body fluid identification in forensic casework.

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