

## Genome-wide methylation profiling and a multiplex construction for the identification of body fluids using epigenetic markers

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## Forensic Body Fluid Identification

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- **Body fluid identification** can provide information linking sample donors with actual criminal acts
- **DNA methylation, mRNA and miRNA** are promising new markers for forensic body fluid identification



Sex offender's trial

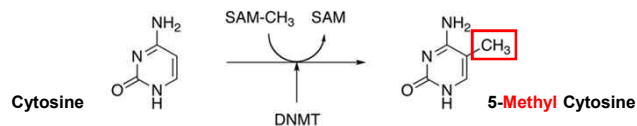


Body fluids found on the bed

Kayser M and de Kniff P, Nat Rev Genet (2011)

## DNA Methylation

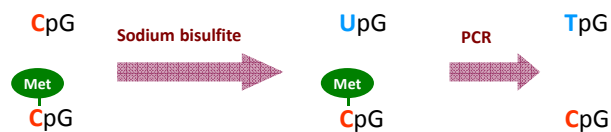
- **DNA methylation** is the addition of a methyl group to the DNA base cytosine followed by a guanine (5' CpG 3')



- Chromosome pieces called **tDMRs** (**tissue-specific differentially methylated regions**) show different DNA methylation profiles according to the type of cell or tissue

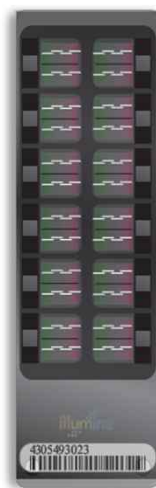
## Analysis of DNA Methylation

- Sodium bisulfite conversion

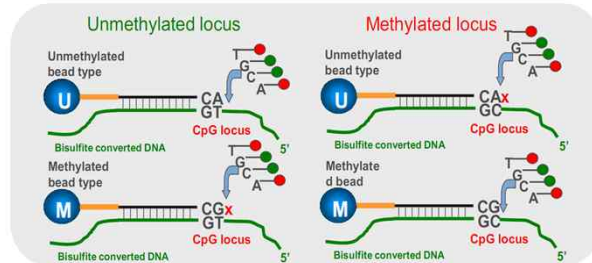


Cloning and sequencing  
Single base extension (SBE)  
Methylation (allele)-specific PCR  
Next Generation Sequencing

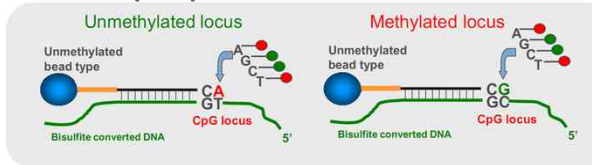
# Human Methylation450 BeadChip



## Infinium I (Allele-specific)



## Infinium II (SBE)



# Methylation Profiling in Body Fluids

- Genome-wide DNA methylation profiles were produced in 42 body fluid samples including **12 blood, 12 saliva, 12 semen, 3 vaginal fluid and 3 menstrual blood** using **Human Methylation450 BeadChip** array (GSE59505)

Sample	Age	Sample	Age	Sample	Age	Samples	Age
BL-01	24	SA-01	20	SE-01	20	VF-01	21
BL-02	27	SA-02	24	SE-02	27	VF-02	21
BL-03	28	SA-03	27	SE-03	28	VF-03	23
BL-04	33	SA-04	29	SE-04	31	MB-01	21
BL-05	37	SA-05	32	SE-05	37	MB-02	23
BL-06	38	SA-06	37	SE-06	38	MB-03	27
BL-07	41	SA-07	38	SE-07	41		
BL-08	43	SA-08	43	SE-08	43		
BL-09	48	SA-09	48	SE-09	48		
BL-10	50	SA-10	50	SE-10	57		
BL-11	57	SA-11	55	SE-11	59		
BL-12	59	SA-12	59	SE-12	59		

Lee HY et al. Forensic Sci Int Genet (2015)

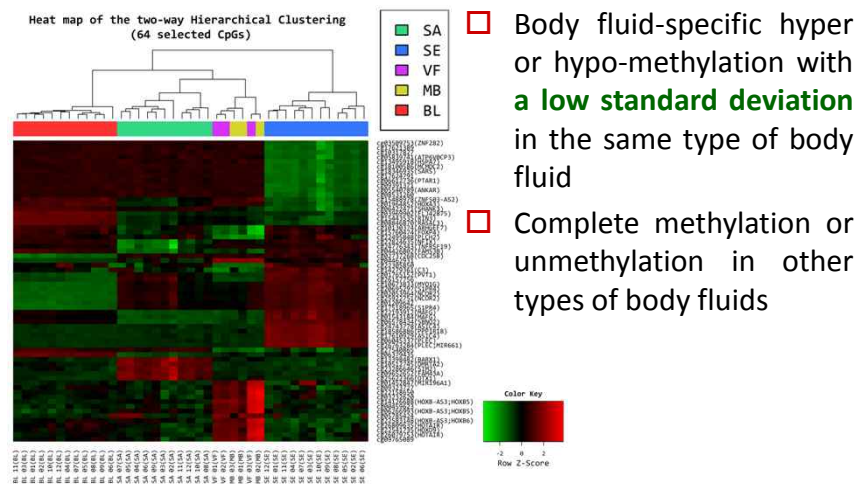
## Significant Probes from 450K Array

	Comparison <sup>a</sup>	Cut-off <sup>b</sup>	No. of CpGs
1	SE vs. BL	Abs (delta_mean) ≥ 0.3, fdr. P < 0.05	64,079
	SE vs. SA	Abs (delta_mean) ≥ 0.3, fdr. P < 0.05	64,305
	SE vs. VF	Abs (delta_mean) ≥ 0.3, fdr. P < 0.05	54,062
	SE vs. MB	Abs (delta_mean) ≥ 0.3, fdr. P < 0.05	45,310
	BL vs. SA	Abs (delta_mean) ≥ 0.3, raw P < 0.05	9,100
	BL vs. VF	Abs (delta_mean) ≥ 0.3, raw P < 0.05	442
	BL vs. MB	Abs (delta_mean) ≥ 0.3, raw P < 0.05	556
	SA vs. VF	Abs (delta_mean) ≥ 0.3, raw P < 0.05	620
	SA vs. MB	Abs (delta_mean) ≥ 0.3, raw P < 0.05	371
	VF vs. MB	Abs (delta_mean) ≥ 0.2, raw P < 0.05	0
2	SE vs. (BL, SA, VF, MB)	Abs (delta_mean) ≥ 0.5, fdr. P < 0.05	20,542
	BL vs. (SA, VF, MB)	Abs (delta_mean) ≥ 0.3, raw P < 0.05	4,252
	SA vs. (BL, VF, MB)	Abs (delta_mean) ≥ 0.3, raw P < 0.05	2,771
	(VF, MB) vs. (BL, SA)	Abs (delta_mean) ≥ 0.2, raw P < 0.05	604

<sup>a</sup>BL, SA, SE, MB and VF represent blood, saliva, semen, menstrual blood and vaginal fluid, respectively

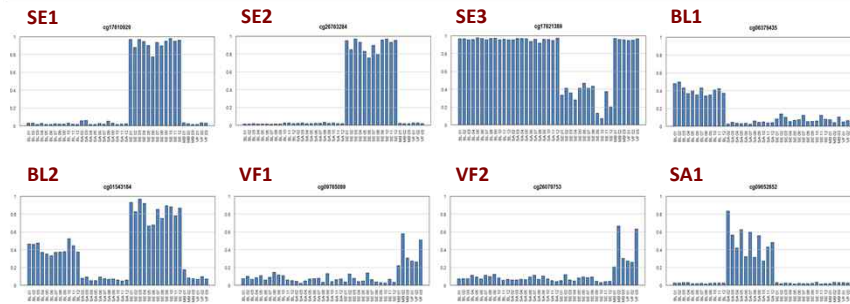
<sup>b</sup>Abs (delta\_mean) represents the discrepancy in the mean values of average  $\beta$ -scores between a certain type of body fluid and the other type of body fluids

## Body Fluid-Specific CpG Candidates



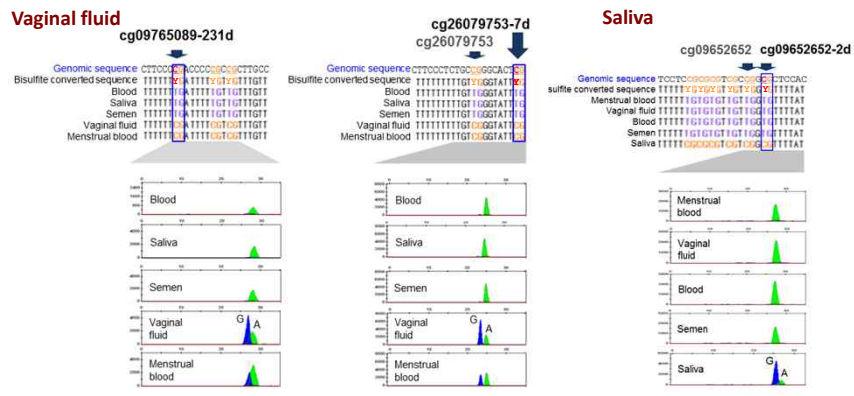
## Selection of Body Fluid-Specific CpGs

Marker	Target ID	Mean beta values $\pm$ SD <sup>a</sup>					Genome build_37		Gene symbol
		SE (n = 12)	BL (n = 12)	VF (n = 3)	MB (n = 3)	SA (n = 11)	Chr	Map info.	
SE1	cg17610929	<b>0.92 <math>\pm</math> 0.06</b>	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.03 $\pm$ 0.02	2	220379044	ACCN4;ASIC4
SE2	cg26763284	<b>0.90 <math>\pm</math> 0.07</b>	0.02 $\pm$ 0.01	0.02 $\pm$ 0.00	0.02 $\pm$ 0.00	0.02 $\pm$ 0.00	8	145018185	PLEC;MIR661
SE3	cg17621389	<b>0.32 <math>\pm</math> 0.13</b>	0.96 $\pm$ 0.01	0.95 $\pm$ 0.01	0.96 $\pm$ 0.01	0.96 $\pm$ 0.02	6	14728290	
BL1	cg06379435	0.08 $\pm$ 0.03	<b>0.40 <math>\pm</math> 0.05</b>	0.05 $\pm$ 0.01	0.07 $\pm$ 0.03	0.04 $\pm$ 0.01	19	3344273	
BL2	cg01543184	<b>0.84 <math>\pm</math> 0.10</b>	<b>0.41 <math>\pm</math> 0.06</b>	0.08 $\pm$ 0.02	0.11 $\pm$ 0.03	0.07 $\pm$ 0.02	17	79881543	MAFG
VF1 <sup>b</sup>	cg09765089	0.06 $\pm$ 0.04	0.09 $\pm$ 0.03	<b>0.35 <math>\pm</math> 0.14</b>	<b>0.37 <math>\pm</math> 0.19</b>	0.06 $\pm$ 0.03	7	27291346	
VF2 <sup>b</sup>	cg26079753	0.07 $\pm$ 0.03	0.09 $\pm$ 0.02	<b>0.39 <math>\pm</math> 0.21</b>	<b>0.39 <math>\pm</math> 0.24</b>	0.07 $\pm$ 0.02	12	54355528	HOTAIR
SA1 <sup>b</sup>	cg09652652	0.02 $\pm$ 0.01	0.02 $\pm$ 0.00	0.03 $\pm$ 0.00	0.03 $\pm$ 0.01	<b>0.49 <math>\pm</math> 0.17</b>	3	194408845	FAM43A



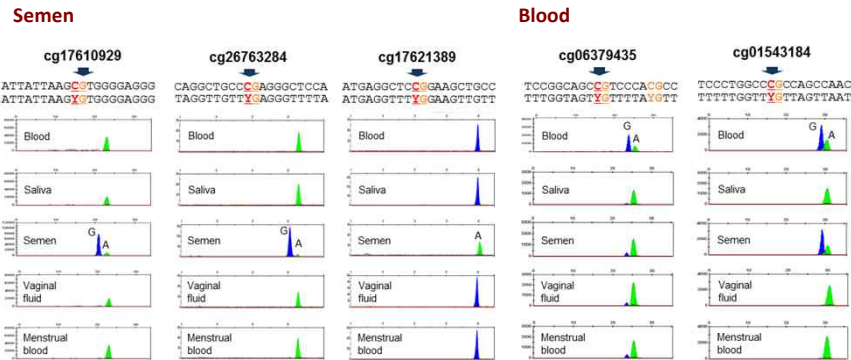
## Validation of Body Fluid-Specific CpGs

- Body fluid-specificity was further investigated using **direct sequencing** and a **SBE reaction** of bisulfite-converted DNA

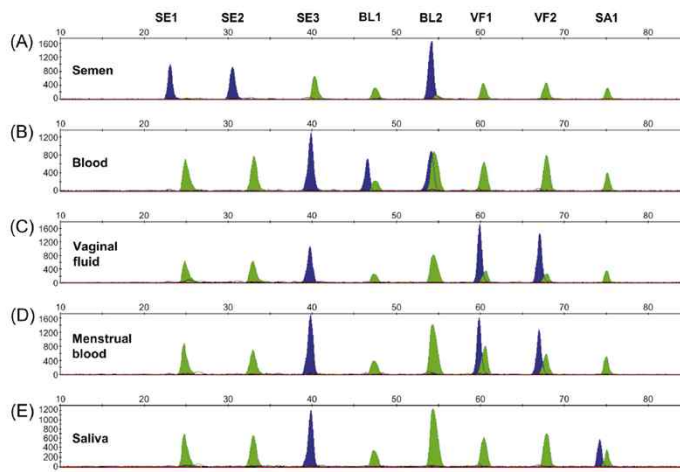


## Validation of Body Fluid-Specific CpGs

- **Body fluid-specificity** was further investigated using **direct sequencing and a SBE reaction** of bisulfite-converted DNA

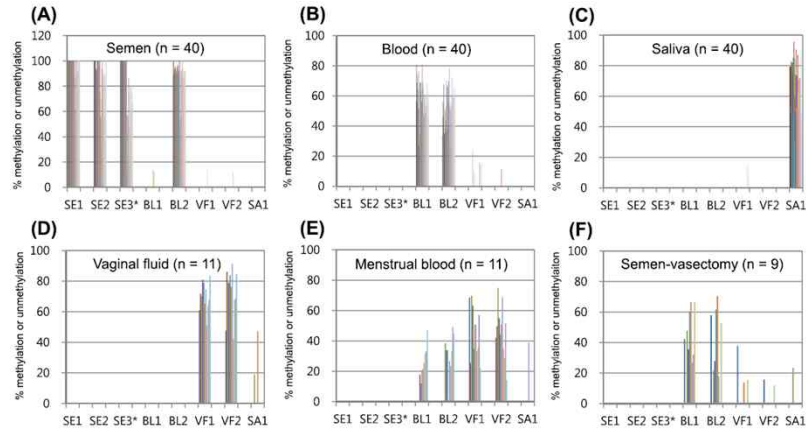


## Multiplex SBE for Body Fluid Identification

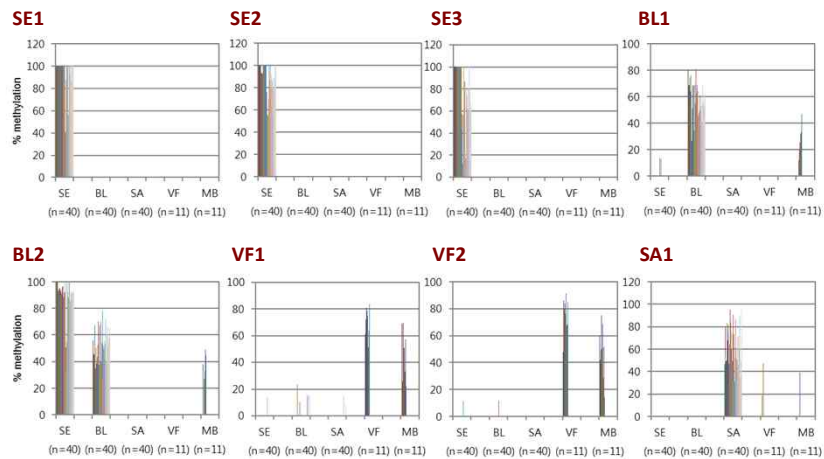


# Multiplex SBE for Body Fluid Identification

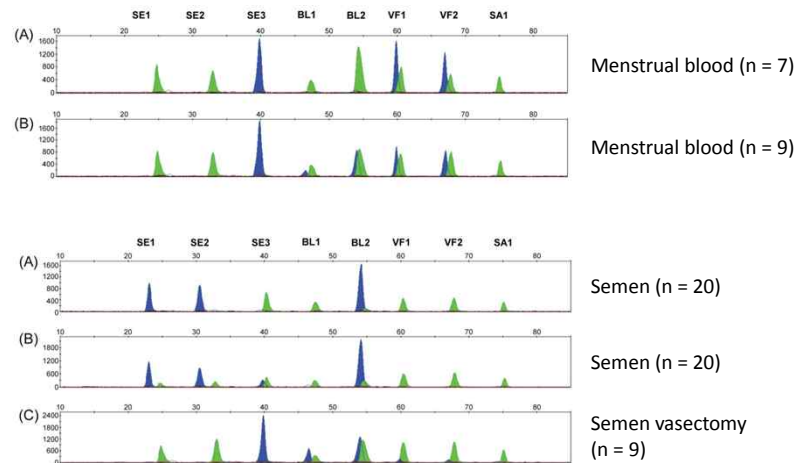
□ A total of 151 samples were analyzed with the multiplex SBE



# Body Fluid Specificity of Selected CpGs

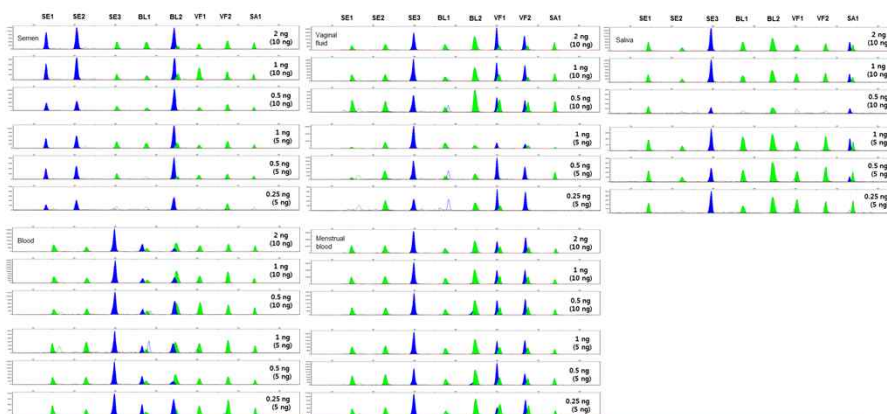


## Menstrual Blood and Semen Profiles



## Sensitivity of the Developed Multiplex

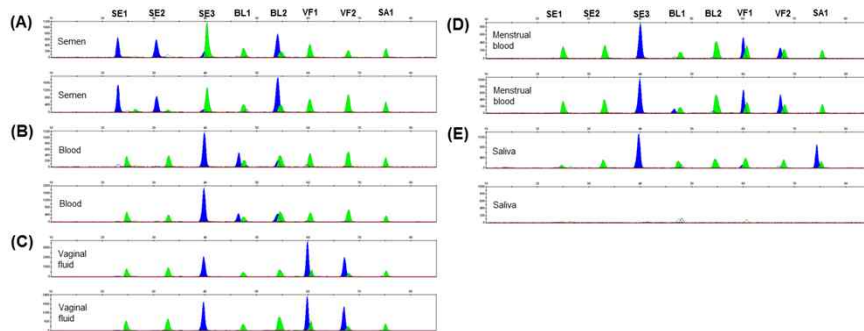
- Successful methylation profiling results could be obtained with approximately **0.5 ng of bisulfite converted DNA**





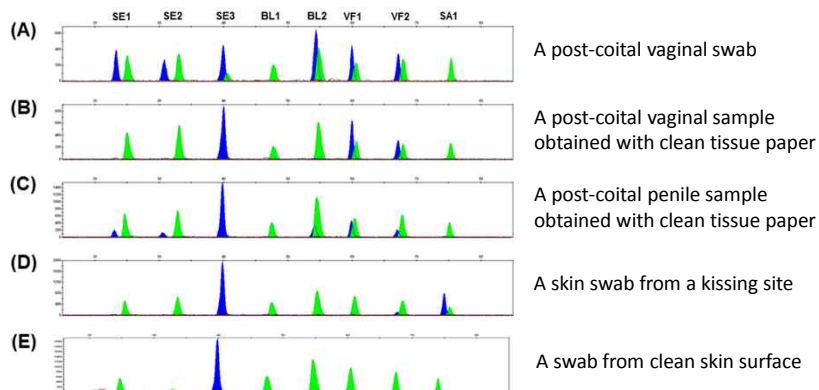
## Aged Sample Test

- **Samples exposed to the environment for 75 days** produced successful DNA methylation profiling results and correct identification results



## Artificial Casework Sample Test

- Successful methylation profiling results could be obtained with **mixed body fluid samples**



Lee HY et al. Forensic Sci Int Genet (2015)

## Summary

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- ❑ Forty two body fluid samples were analyzed by the Illumina BeadChip array.
  - ❑ A total of 64 CpGs were selected as body fluid-specific marker candidates.
  - ❑ A multiplex methylation SNaPshot reaction was constructed using 8 CpG sites.
  - ❑ The multiplex assay differentiates between blood, saliva, semen and vaginal fluid.
  - ❑ The multiplex produced successful methylation profiles in aged or mixed samples
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**Thank you for your attention!**

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