DNA methylation profiling for body fluid identification

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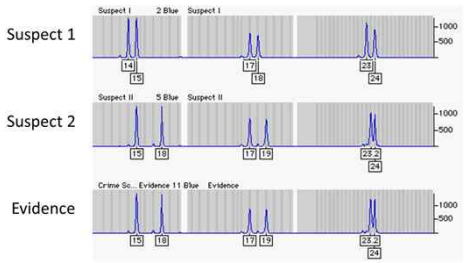
Current Forensic DNA Typing

- Forensic cases -- **matching suspect with evidence**
- Paternity testing -- **identifying father**



Involves generation of DNA profiles usually with the same genetic markers and then ***MATCHING TO REFERENCE SAMPLE***

Picture from www.cstl.nist.gov/strbase/NISTpub.htm

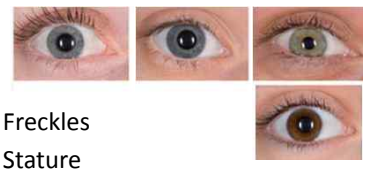
Challenges in Crime Scene Investigation

- Technical challenges in forensic DNA typing include
  - Degraded DNA
  - Low amount of DNA
  - Increasing discrimination power
  - Familiar searching….
- Important challenges remain for **the use of genetic and epigenetic approaches** in forensic investigations

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

Future of Crime Scene Investigation

- The ability to infer **biogeographic ancestry** and **external visible characteristics** from DNA
  - Eye (IrisPlex), hair and skin color



  - Freckles
  - Stature
  - Hair morphology
  - Male baldness, etc.

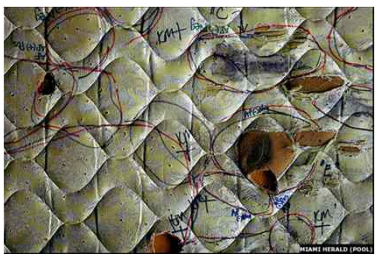
Kayser M and de Kniff P, Nat Rev Genet (2011)

Drawing s from http://meglikestodraw.tumblr.com/

Future of Crime Scene Investigation

- **Body fluid identification** can provide information linking sample donors with actual criminal acts
- **Detailed reconstruction of crime events** is possible by determining the time of sample donation and sample age

Sex offender's trial



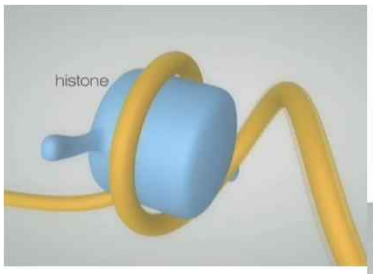
Body fluids founds on the bed

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

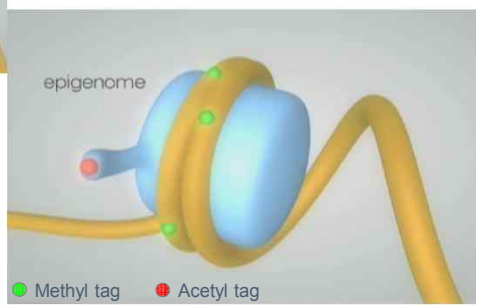
Genetics and Epigenetics

- **Epi** (on top of or in addition to) + Genetics
- Epigenetics is the study of **heritable** changes in gene expression or cellular phenotype caused by mechanisms **other than changes in the underlying DNA sequence**
- Genome – Epigenome, Genetic code – Epigenetic code

What is a Epigenome?



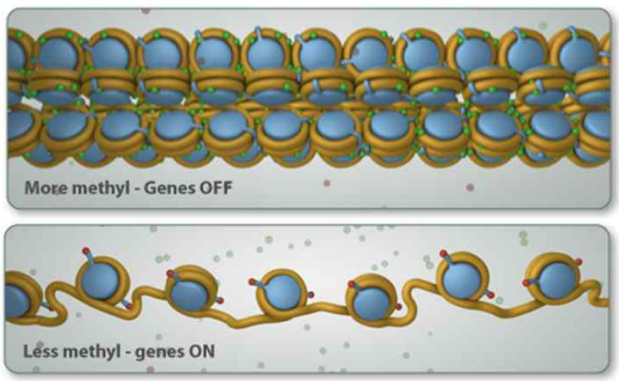
- DNA methylation
- Histone modification
- MicroRNA



http://learn.genetics.utah.edu/content/epigenetics/intro/

Gene Control by the Epigenome

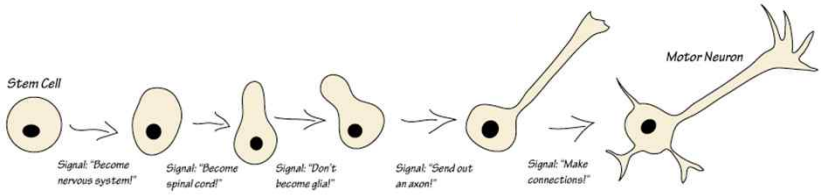
- Epigenome is flexible and epigenetic tags react signals, e.g., diet, stress, etc



http://learn.genetics.utah.edu/content/epigenetics/

Epigenetic Tags and Cellular Memory

- A cell's **epigenetic profile** – a collection of tags that tell genes whether to be on or off – is the sum of the signals it has received during its lifetime

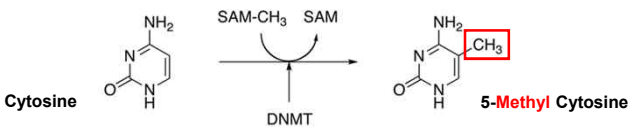


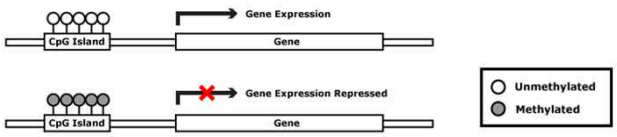
- Epigenetic profiles are specific to **tissue, age and environmental factors**

http://learn.genetics.utah.edu/content/epigenetics/

DNA Methylation

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





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

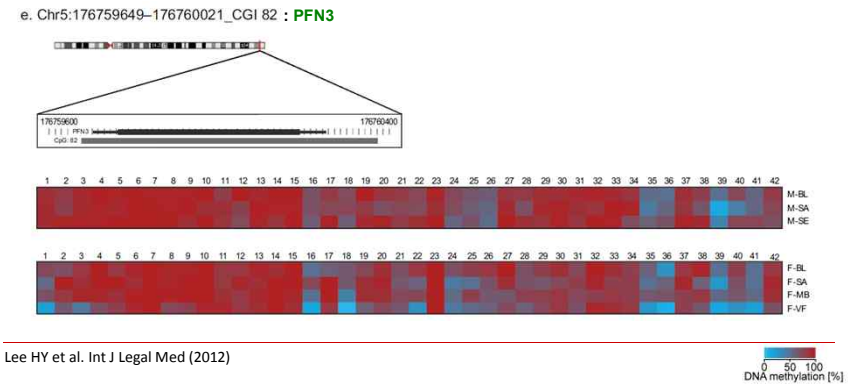
Table. Genomic information for candidate tDMRs for body fluid identification

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	HOXA4	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 HY et al. Int J Legal Med (2012)

Differential DNA Methylation

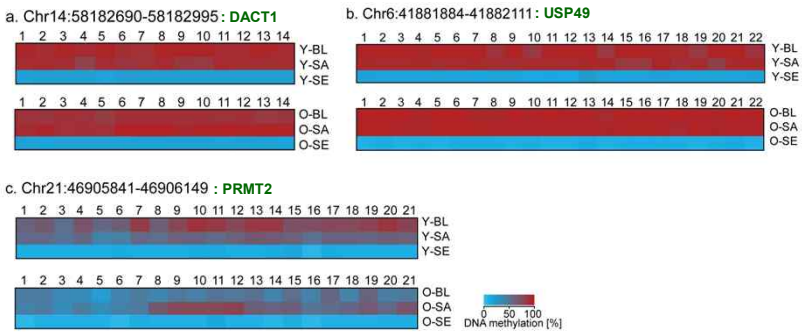
- DNA methylation profiles for the **DACT1, USP49, HOXA4, PRMT2 and PFN3 tDMRs** in pooled DNA samples from blood, saliva, semen, menstrual blood, and vaginal fluid



Lee HY et al. Int J Legal Med (2012)

DNA Methylation and Aging

- The **DACT1, USP49 and PRMT2 tDMRs** in pooled DNA samples from blood, saliva, and semen obtained from 20 young (< 30 y) and 15 elderly (> 50 y) men



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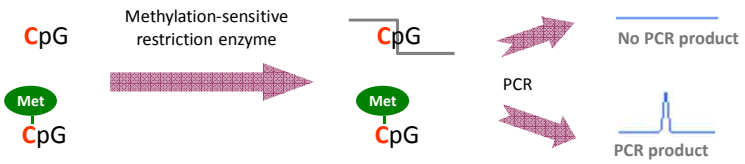
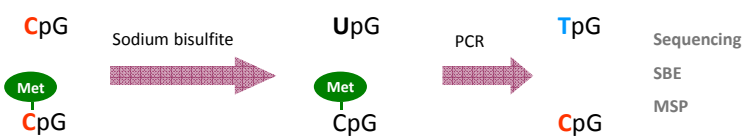
Analysis of DNA Methylation

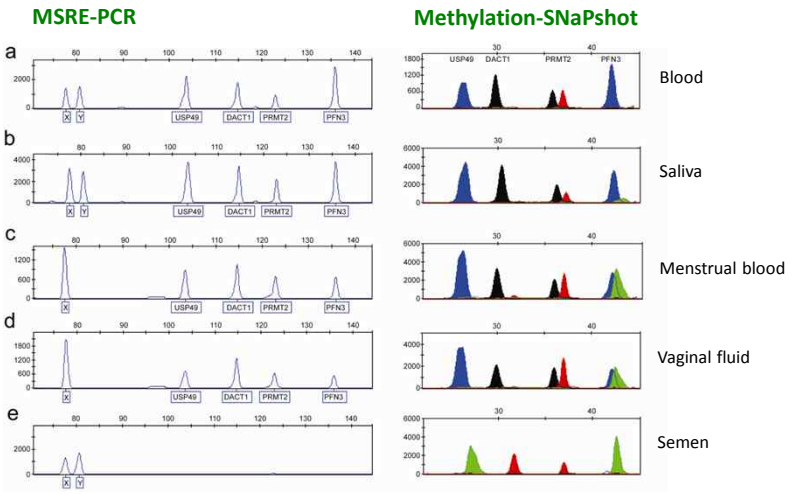
- Methylation Sensitive Restriction Enzyme (MSRE)-PCR



- Sodium bisulfite conversion



Methylation-Specific Multiplex Assays



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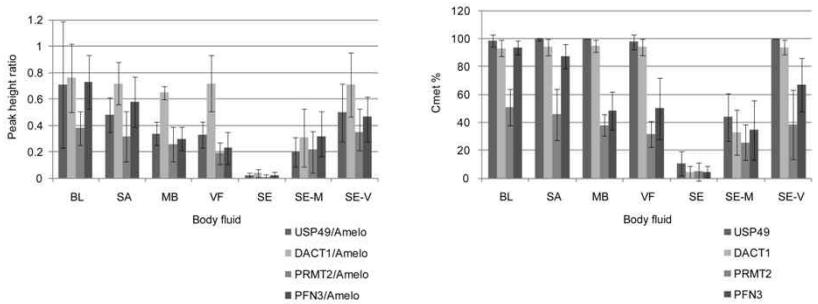
Methylation-Specific Multiplex Assays

- Methylation status of the **USP49, DACT1, PRMT2, and PFN3 tDMRs** in body fluid samples in 40 men and 6 women



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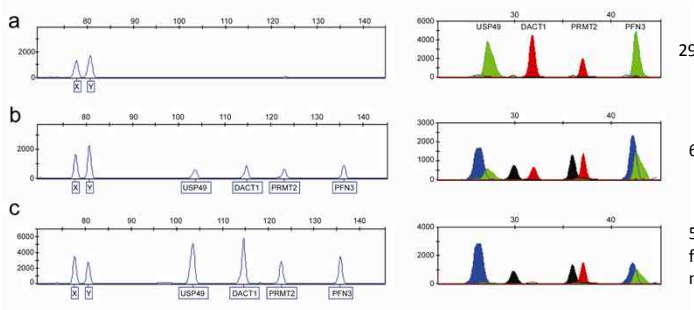
Semen Detection

- **Multiplex methylation-SNaPshot assay** enabled detection of semen samples including spermatozoa



29 semen samples

6 semen samples

5 semen samples from vasectomized males

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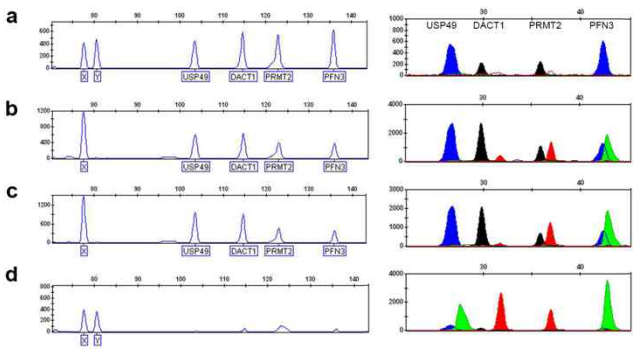
Analyses of Aged Samples

- DNA methylation profiles could be successfully obtained from samples **environmentally exposed for 75 days**



Blood

Menstrual blood

Vaginal fluid

Semen

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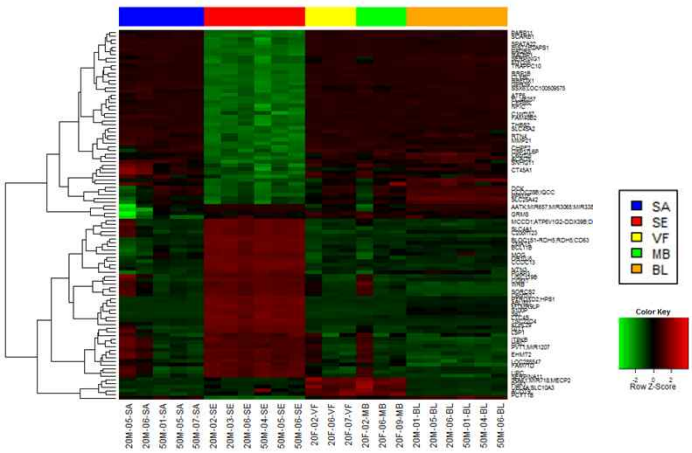
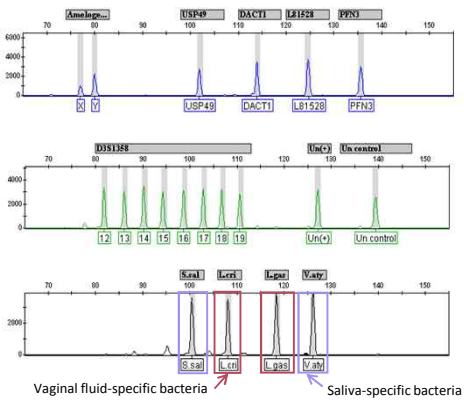
Epigenome-Wide Methylation Analysis

- DNA methylation analysis of various body fluid samples using Illumina Infinium Human Methylation450K



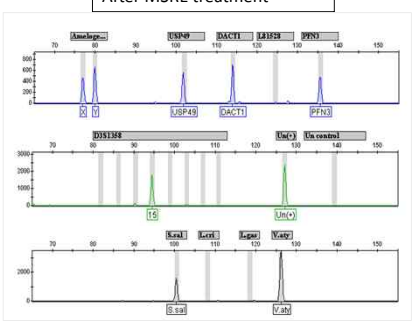
tDMRs and Microbial DNA



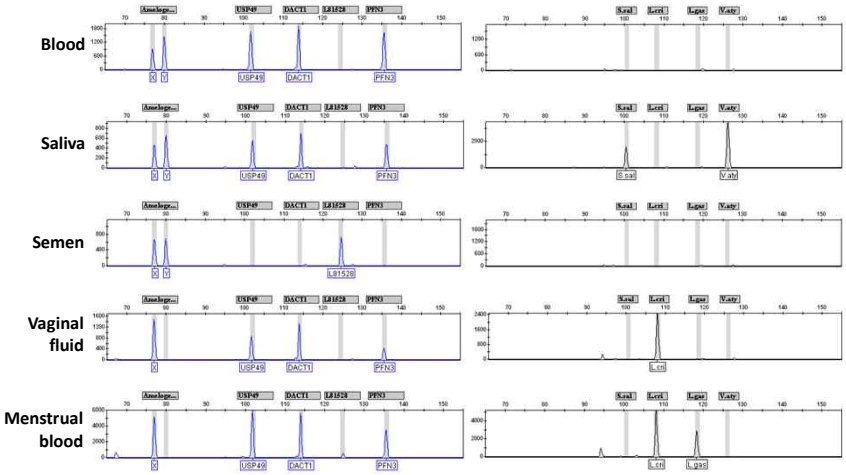
Vaginal fluid-specific bacteria

Saliva-specific bacteria

After MSRE treatment



tDMRs and Microbial DNA

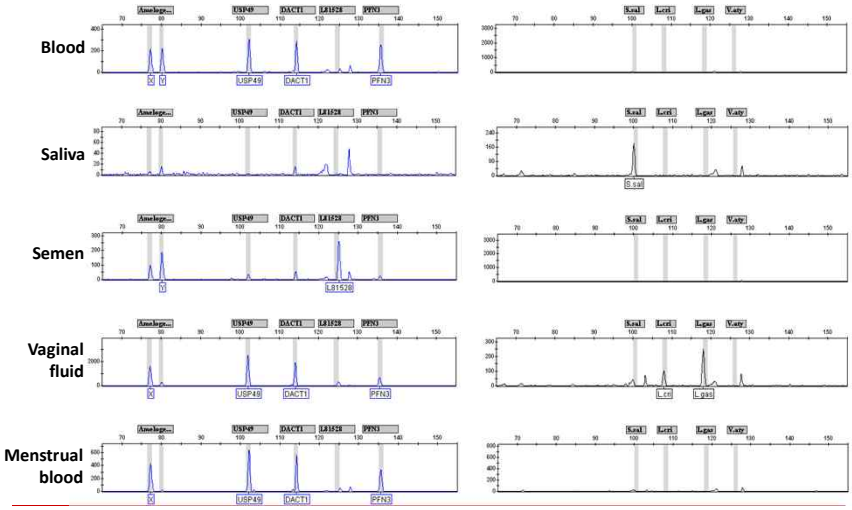


Body Fluid-Specific Microbial DNA

- Combined use of 4 tDMRs and 4 body fluid-specific bacteria markers would be helpful to discriminate blood, saliva, semen, and vaginal fluid-menstrual blood

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	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

Analysis of Aged Samples



Concluding Remarks

- 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 several tDMRs and/or microbial DNA could be used to discriminate blood, saliva, semen and vaginal fluid-menstrual blood
- These results adds to the support that DNA-based body fluid identification could be a valuable tool for forensic analysis of body fluids

The Future of Forensic DNA Typing

Genetics, Genomics, Epigenetics, Epigenomics, Epitranscriptomics…

Thank you for your attention!

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