Forensic DNA methylation profiling for investigative leads
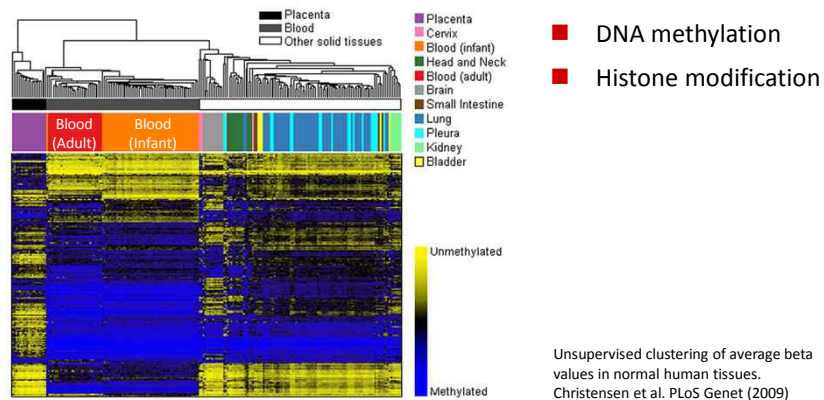
Hwan Young Lee

Department of Forensic Medicine

Yonsei University College of Medicine, Seoul, Korea
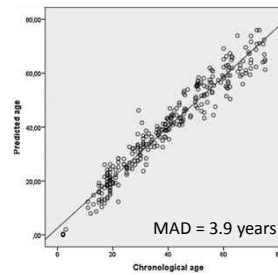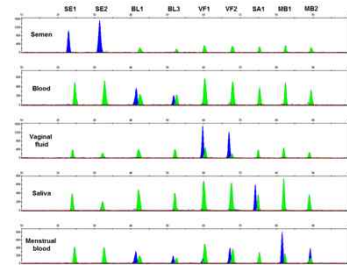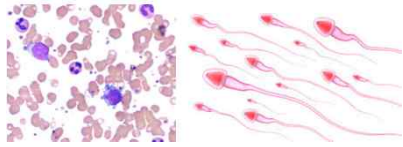

Epigenetic Variation

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

■ DNA methylation

■ Histone modification

Unsupervised clustering of average beta values in normal human tissues.
Christensen et al. PLoS Genet (2009)

1

DNA Methylation in Forensics

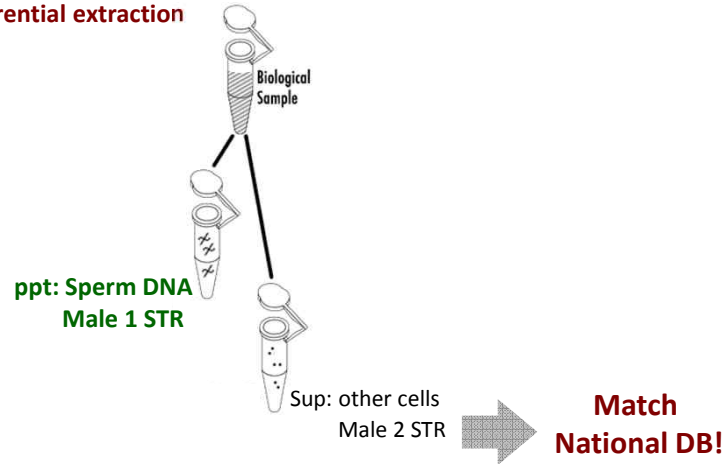☐ Body fluid identification, prediction of age and smoking habits, etc.

MAD = 3.9 years

Analysis of a Casework Sample

☐ A stain preliminary positive for semen and saliva
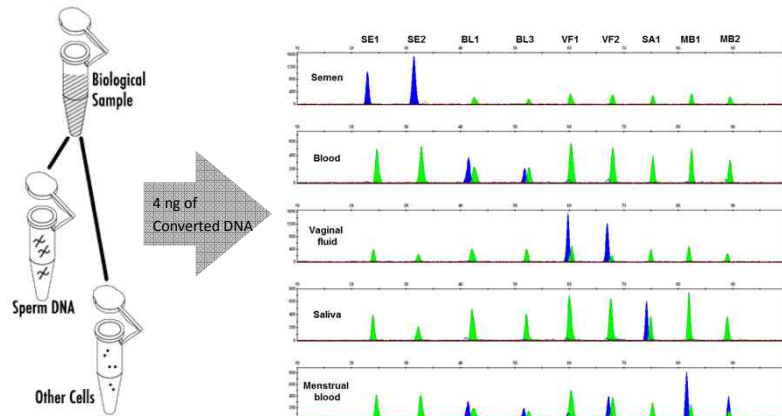☐ Two men's mixed STR profile

A Casework Sample: Mixture Analysis

Differential extraction

**ppt: Sperm DNA
Male 1 STR**

Sup: other cells
Male 2 STR

**Match
National DB!**

A Casework Sample: Mixture Analysis

Differential extraction   **DNA methylation-based body fluid typing**

4 ng of
Converted DNA

Lee HY et al., Forensic Sci Int Genet (2016)

# DNA Methylation-Based Body Fluid ID
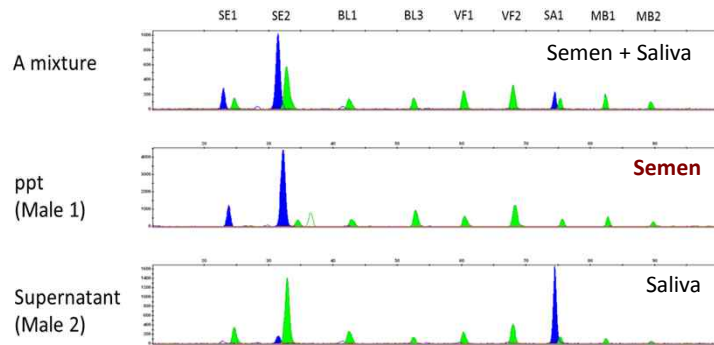
Forensic DNA Phenotyping

- ☐ Forensic DNA phenotyping is expected to be criminally useful in helping **to reduce the number of potential suspects**.

Picture from snapshot.parabon-nanolabs.com

Aging and Forensic DNA Phenotyping

☐ **Age as EVC (externally visible characteristics)** is expected to provide investigative lead to track unknown suspect or to identify missing persons regardless of ethnicity
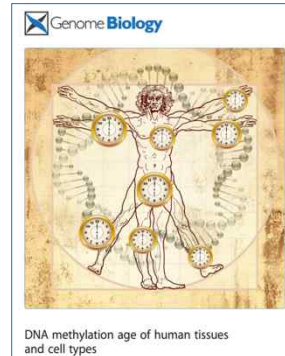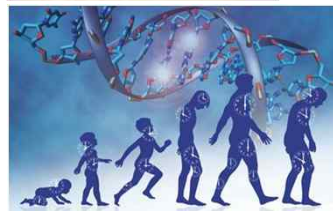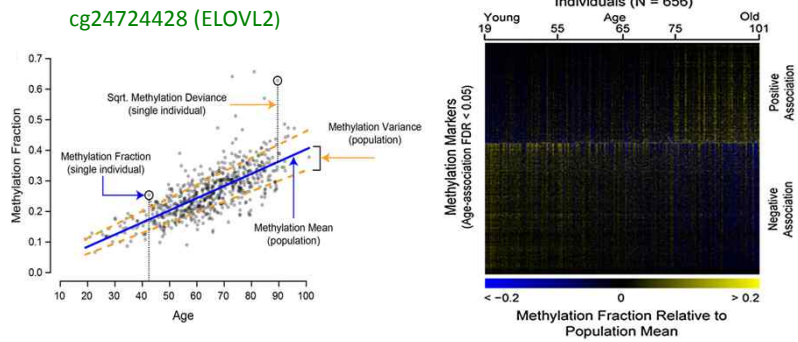

Horvath. Genome Biol (2013)

DNA methylation-based age prediction

☐ Age-predictive models based on the use of blood or even across a broad spectrum of tissues have been reported

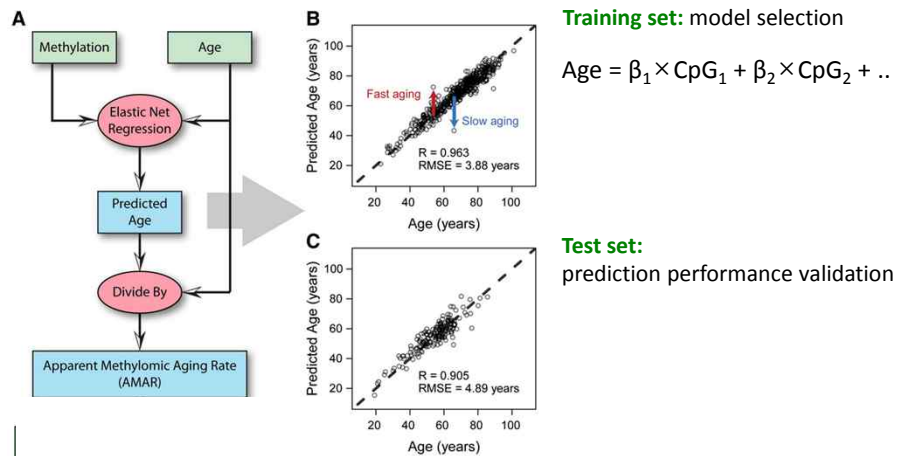|	Age signatures	Tissue	Error (years)
Bocklandt et al. (2011)	EDARADD, TOM1L1, NPTX2	Saliva	5.2
Garagnani et al. (2012)	ELOVL2, FHL2, PENK	Blood	-
Weidner et al. (2014)	ITGA2B, ASPA, PDE4C	Blood	4.3
Zbiec-Piekarska et al. (2015)	ELOVL2, C1orf132, TRIM59, KLF14, FHL2	Blood	3.9
Huang et al. (2015)	ASPA, ITGA2B, NPTX2	Blood	7.9
Lee et al. (2015)	TTC7b, NOX4, cg12837463	Semen	5.2
Hannum et al. (2013)	**71 CpGs** from HumanMethylation450 array	Blood	**3.9**
Horvath (2013)	**353 CpG**s from HumanMethylation27 array	Somatic tissues	**3.6**

Age-related DNA Methylation Changes

- ☐ There are markers which have significant association between methylation fraction and age

cg24724428 (ELOVL2)

Hannum et al. Mol Cell (2013)

Age Predictive Model Construction

Training set: model selection

$Age = \beta_1 \times CpG_1 + \beta_2 \times CpG_2 + ..$

Test set:
prediction performance validation

Hannum et al. Mol Cell (2013)
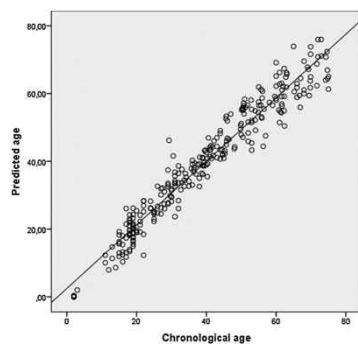
Age Predictive Model by Horvath

☐ An age predictor using 353 CpGs could be applied across a broad spectrum of somatic tissues
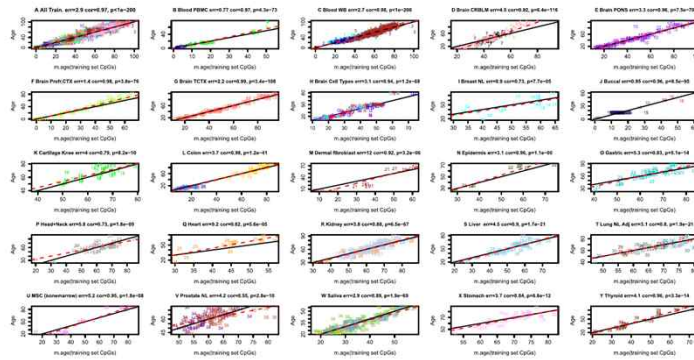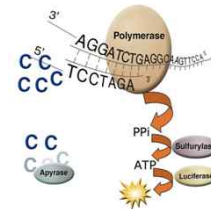

Horvath, Genome Biol (2013)

Age Prediction in Blood

☐ 5 CpGs in the genes **ELOVL2, C1orf132, TRIM59, KLF14 and FHL2**

$$Age = a + b \times \underline{CpG_1} + c \times \underline{CpG_2} + d \times \underline{CpG_3} + \dots$$
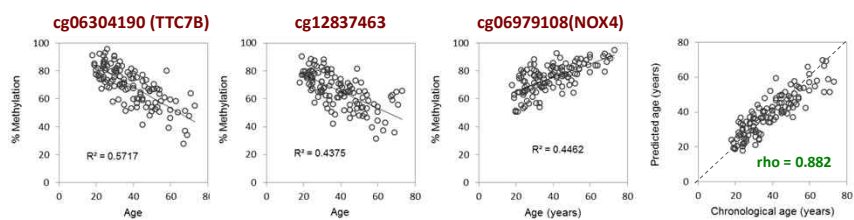
Pyrosequencing

MAD (Mean Absolute Deviation) from chronological age = **3.9 years**

Zbieć-Piekarska et al. Forensic Sci Int Genet (2015)

Age Prediction in Semen

☐ Age correlation of the 3 CpGs and predicted versus chronological ages of 125 semen samples in methylation SNaPshot analysis

cg06304190 (TTC7B)   **cg12837463**   **cg06979108(NOX4)**

Target ID	Estimate (n = 125)	P-value	R-squared	RMSE	MAD	Gene symbol
(Intercept)	46.240	0				
cg06304190	-0.519	0	0.766	6.690	5.2	TTC7B
cg12837463	-0.178	0.007				
cg06979108	0.541	0				NOX4

Lee et al. Forensic Sci Int Genet (2015)

Previously Reported Saliva Markers

☐ Bocklandt et al. (2011), **EDARADD, TOM1L1 and NPTX2** from 27K array
☐ Age vs. methylation from 450K array data of 54 Korean saliva samples
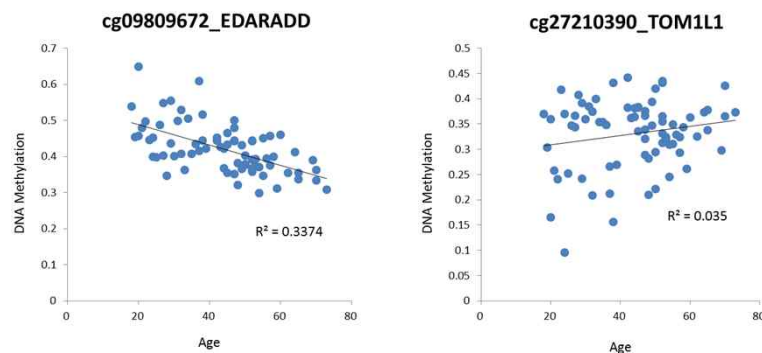
Identification of Saliva Markers

- Saliva samples obtained from 54 males aged 18 to 73 years

Analysis	Statistical method	No. CpGs	Cut-off	No. CpGs
Methylated level (avg.beta)~ Age	**Linear regression**	445,791	FDR_P<0.05	74,807
			FDR_P<0.05 & R-squared>0.65	80
			FDR_P<0.05 & R-squared>0.65 & diff>=0.1	62

(1) 80 CpGs with FDR_P < 0.05 & R-squared > 0.65

Advancing age
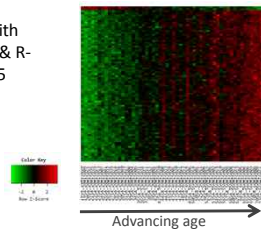
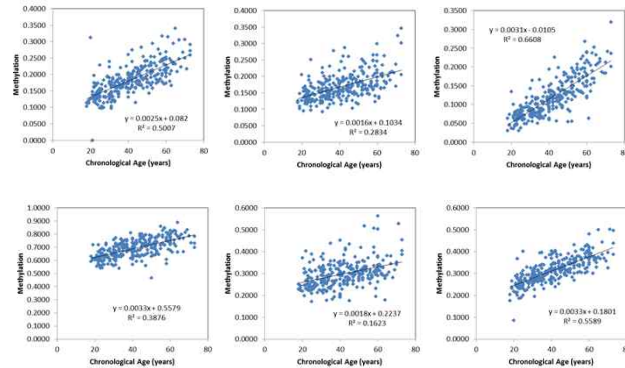(2) 62 CpGs with FDR_P < 0.05 & R-squared > 0.65 & methylation diff >=0.1

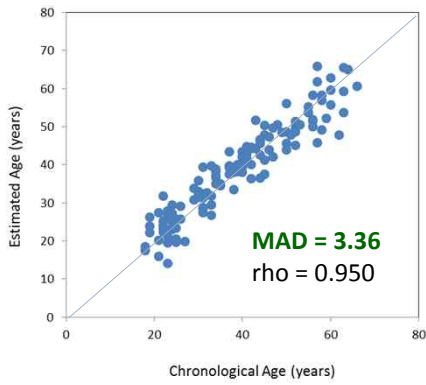Advancing age
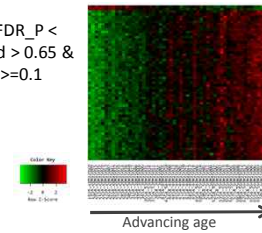
Age-Associated Saliva Markers
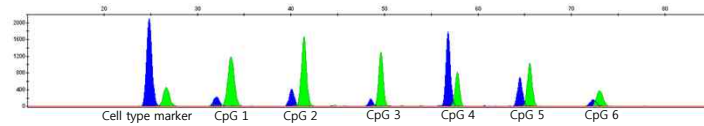
Methylation SNaPshot
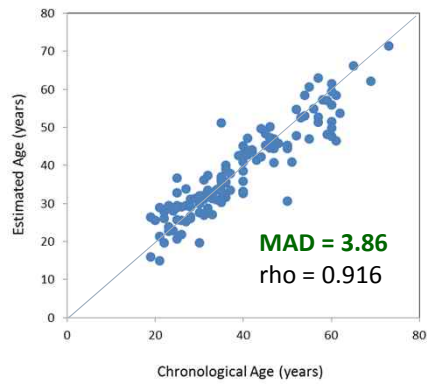
$$\%methyl = \frac{G\ intensity}{(G+A)\ intensity}$$

Age Predictive Model for Saliva

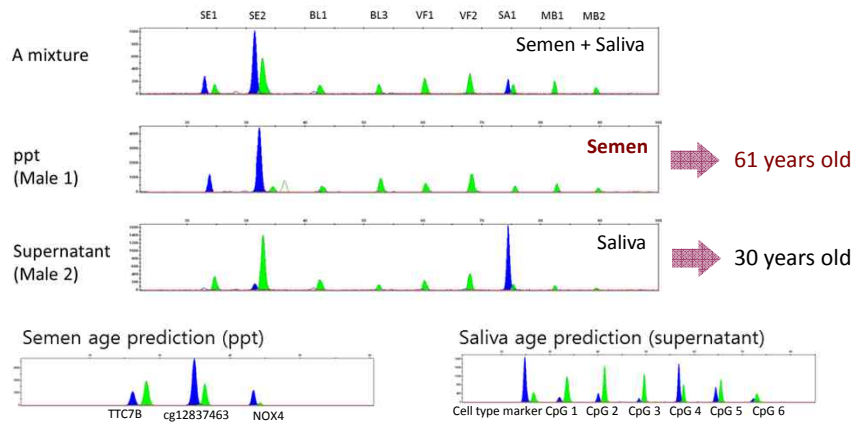**Training set** (n=128):
Model selection

MAD = 3.36
rho = 0.950

Test set (n=126):
Prediction performance validation

MAD = 3.86
rho = 0.916

Casework Samples

| | SE1 | SE2 | BL1 | BL3 | VF1 | VF2 | SA1 | MB1 | MB2 |

A mixture — Semen + Saliva

ppt (Male 1) — **Semen** ➡ 61 years old

Supernatant (Male 2) — Saliva ➡ 30 years old

Semen age prediction (ppt)

TTC7B   cg12837463   NOX4

Saliva age prediction (supernatant)

Cell type marker CpG 1 CpG 2 CpG 3 CpG 4 CpG 5 CpG 6
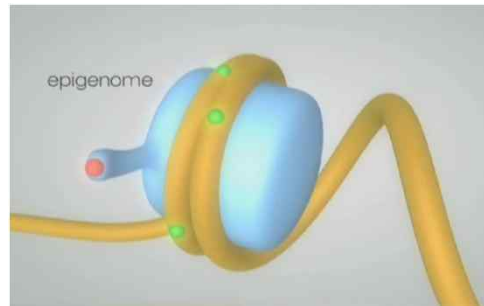
DNA Methylation Profiling in Forensics

- ☐ Just like epigenetics to genetics, DNA methylation analyses are expected to add more informative layers to the forensic genetic analyses of the evidence materials

---

Acknowledgment

- ☐ Thanks to Sang-Eun Jung, Ja Hyun An, Eun Hee Lee, Eun Young Lee, Yu Na Oh, Ajin Choi, Myung Jin Park, Woo Ick Yang and Kyoung-Jin Shin

- ☐ This research was supported by the National Research Foundation of Korea (NRF-2012R1A1A2007031 and NRF-2014M3A9E1069992) and the National Forensic Service (nos. 1315000435 and 1315000544)