

DNA methylation-based age prediction from saliva

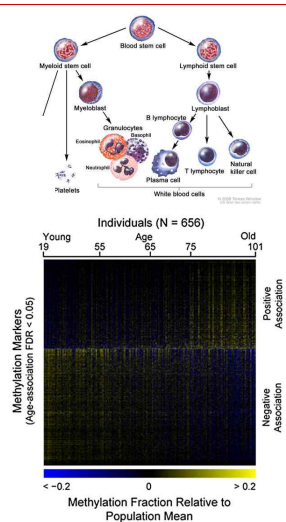
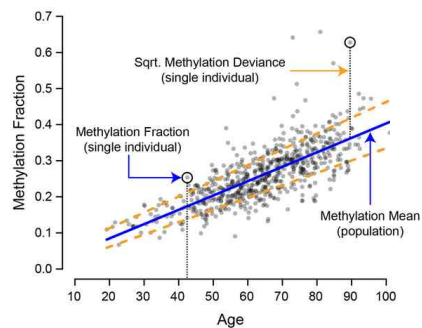
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Age-associated methylation changes

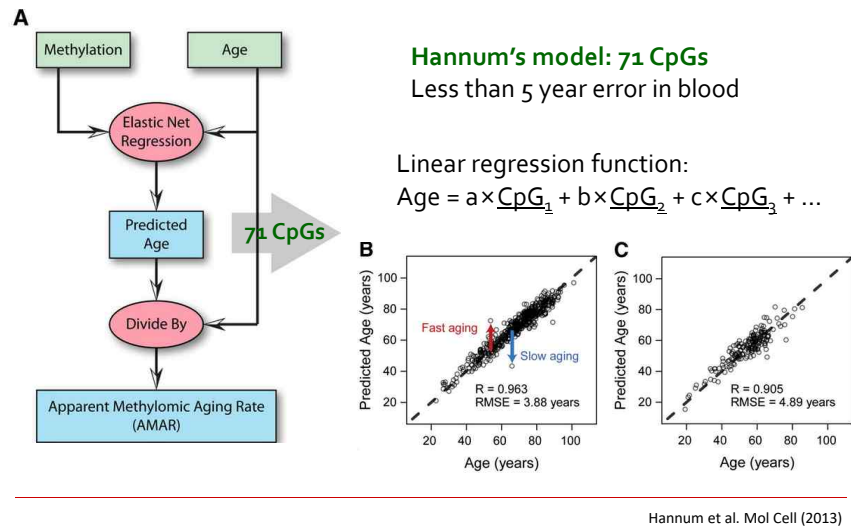
A CpG (cg24724428) in the *ELOVL2* gene

GGCTCAAC**CGT**CCACGGAGCCCCAGGAA

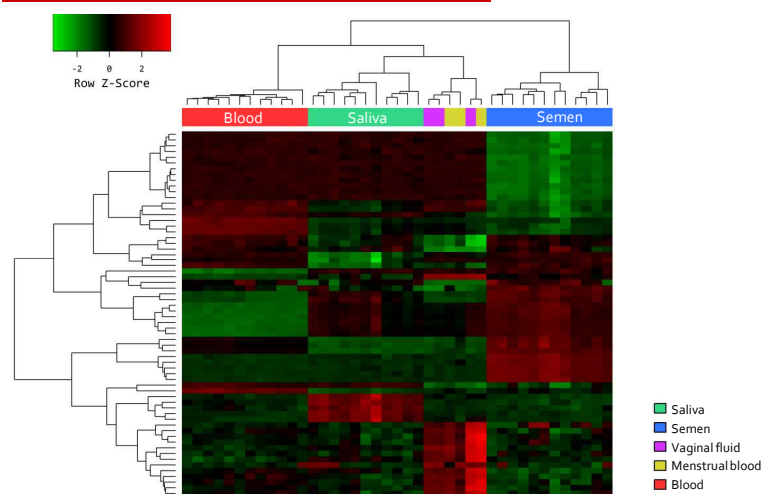


Hannum et al. Mol Cell (2013)

Age prediction with DNA methylation



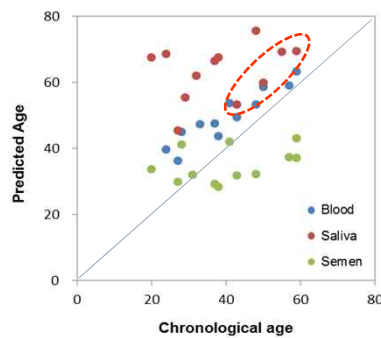
DNA methylation of body fluids



Lee et al. Forensic Sci Int Genet (2015)

Age prediction in different body fluids

- Age predictive values for 36 body fluid samples (GSE59505) were obtained using a model suggested by Hannum *et al.* (2013)



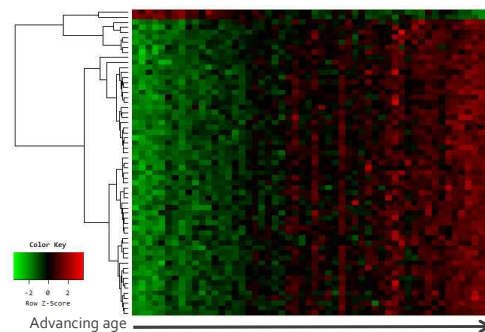
	cg18384097*
20M-03-SA	0.90
20M-05-SA	0.87
20M-06-SA	0.78
20M-02-SA	0.71
30M-08-SA	0.79
30M-05-SA	0.82
30M-04-SA	0.88
40M-07-SA	0.53
40M-10-SA	0.85
50M-01-SA	0.26
50M-07-SA	0.31
50M-05-SA	0.37

*Blood=0.05, Buccal epithelial cell=0.82

Identification of saliva CpG markers

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

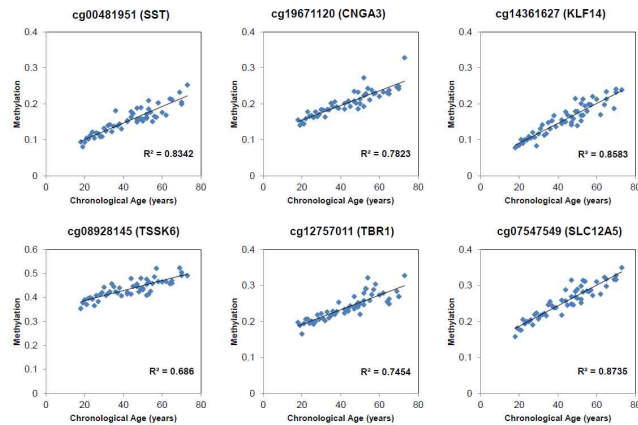
	Analysis	No. CpGs	Cut-off	No. CpGs
Methylated level (avg.beta) [~] Age	Linear regression	445,791	FDR_P<0.05 & R-squared>0.65 & diff>=0.1	62



- Among 62 CpGs, 11 CpGs in 9 genes (*SLC12A5*, *KLF14*, *SST*, *GREM1*, *C10rf132*, *SCGN*, *OTUD7A*, *NHLRC1*, and *TRIM59*) were common to our study and that of Hannum *et al.*
- The *ELOVL2*, *FAM123C*, and *GPR62* genes were common between our study and that of Hannum *et al.*, but the target CpG site differed

Age-associated markers from 450K array

CNGA3, KLF14, TSSK6, SLC12A5 ($R^2 = 0.97$, $RMSE = 3.8$) + SST, TBR1 + cg18384097



Cell type-specific marker cg18384097

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Research Paper

Epigenetic age predictions based on buccal swabs are more precise in combination with cell type-specific DNA methylation signatures

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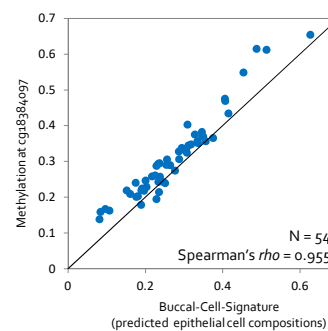
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Key words: aging, predictor, epigenetic, methylation, swab, cell composition, epithelial cells

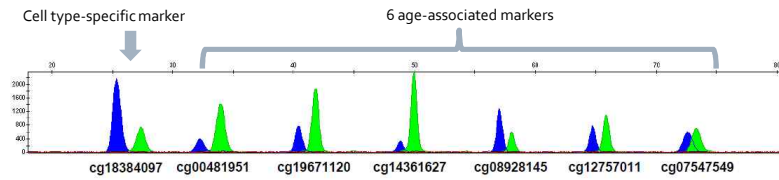
Received: 02/18/16; **Accepted:** 05/18/16; **Published:** 05/31/16

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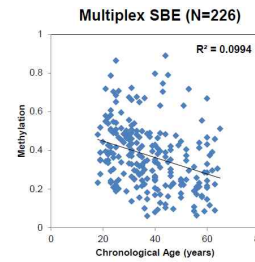
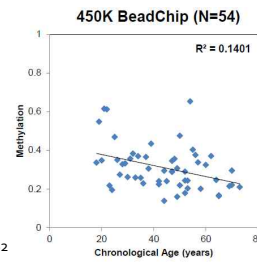
"Buccal-Cell-Signature" was calculated with the function; percentage of buccal epithelial cells (β) = $(99.8 (\beta\text{-value of cg07380416}) + 1.92) / 2 + (-98.12 (\beta\text{-value of cg20837735}) + 88.54) / 2$. The CpG sites, cg07380416 and cg20837735 are located on the *CD6* gene and the *SERPIN5* gene, respectively.

Multiplex methylation SNaPshot

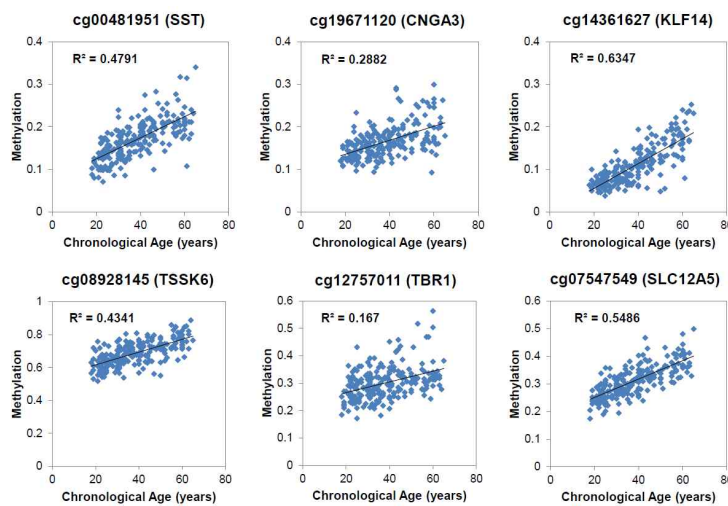


Methylation of
cg18384097

*Blood=0.05
Buccal epithelial cell=0.82

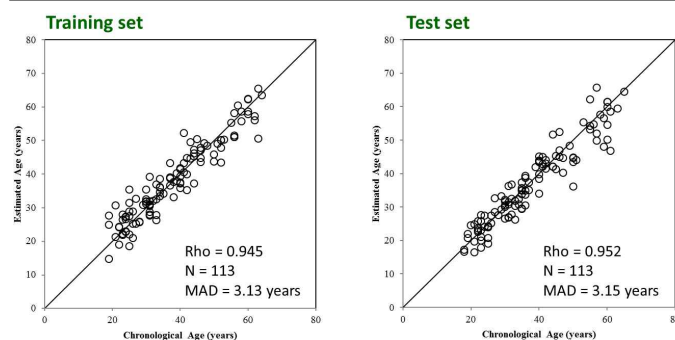


Age-associated markers in 226 samples



Age-predictive model for saliva

Target ID	Gene symbol	Genomic location ^a	Coefficient	P-value	R-squared	RMSE
(intercept)			-27.511	<0.001	0.895	4.16
cg18384097	PTPN7	chr1:202129566	-29.088	<0.001		
cg90481951	SST	chr3:187387650	9.285	0.477		
cg19671120	CNGA3	chr2:98962974	46.992	0.001		
cg14361627	KLF14	chr7:130419116	86.268	<0.001		
cg98928145	TSSK6	chr19:19625364	32.211	<0.001		
cg12757011	TBR1	chr2:162281111	58.699	<0.001		
cg97547549	SLC12A5	chr20:44658225	56.384	<0.001		



Hong et al. Forensic Sci Int Genet (2017)

Prediction performance of the model

Training set (n = 113)					
	No.	MAD (years)	RMSE (years)	±RMSEs No. (%)	±2RMSEs No. (%)
20s or less	31	3.62	5.10	21 (67.74)	28 (90.32)
30s	32	2.39	3.69	25 (78.13)	32 (100.00)
40s	25	2.98	4.72	19 (76.00)	24 (96.00)
50s or more	25	3.63	5.48	15 (60.00)	23 (92.00)
Total	113	3.13	4.16	80 (70.80)	107 (94.69)
Testing set (n = 113)					
	No.	MAD (years)	RMSE (years)	±RMSEs No. (%)	±2RMSEs No. (%)
31	2.43	3.48	24 (77.42)	31 (100.00)	
33	2.50	3.59	24 (72.73)	32 (96.97)	
25	3.26	5.46	20 (80.00)	25 (100.00)	
24	4.69	7.31	12 (50.00)	20 (83.33)	
113	3.15	4.34	80 (70.80)	108 (95.58)	

*95% errors within the range of $\pm 2 \times \text{RMSE}$

Inclusion or exclusion of cell type marker

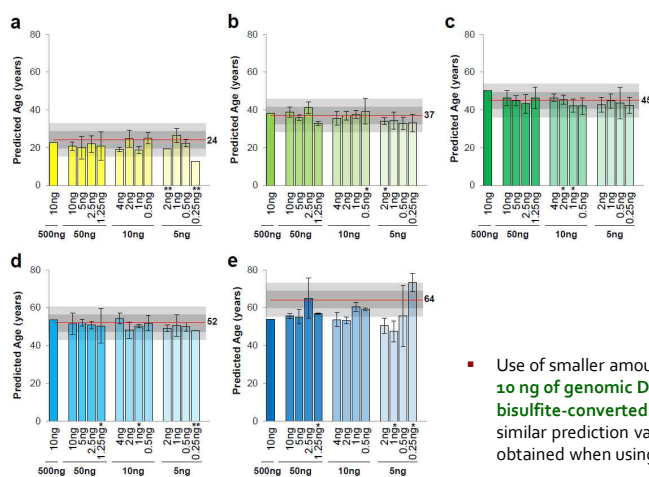
□ 6 age associated markers + 1 cell type-specific marker

	Training set					Testing set				
	No.	MAD (year)	RMSE (year)	±RMSE No. (%)	±2RMSE No. (%)	No.	MAD (year)	Error (year)	±RMSE No. (%)	±2RMSE No. (%)
20's or less	31	3.62	5.10	21 (67.74)	28 (90.32)	31	2.43	3.48	24 (77.42)	31 (100.00)
30's	32	2.39	3.69	25 (78.13)	32 (100.00)	33	2.50	3.59	24 (72.73)	32 (96.97)
40's	25	2.98	4.72	19 (76.00)	24 (96.00)	25	3.26	5.46	20 (80.00)	25 (100.00)
50's or more	25	3.63	5.48	15 (60.00)	23 (92.00)	24	4.69	7.31	12 (50.00)	20 (83.33)
Total	113	3.13	4.16	80 (70.80)	107 (94.69)	113	3.15	4.34	80 (70.80)	108 (95.58)

□ 6 age associated markers

	Training set					Testing set				
	No.	MAD (year)	RMSE (year)	±RMSE No. (%)	±2RMSE No. (%)	No.	MAD (year)	Error (year)	±RMSE No. (%)	±2RMSE No. (%)
20's or less	31	4.13	6.07	20 (64.52)	29 (93.55)	31	3.23	4.48	24 (77.42)	31 (100.00)
30's	32	2.58	4.14	27 (84.38)	31 (96.88)	33	2.87	3.91	24 (72.73)	32 (96.97)
40's	25	3.28	4.87	19 (76.00)	24 (96.00)	25	4.65	8.31	18 (72.00)	24 (96.00)
50's or more	25	4.63	6.57	12 (48.00)	23 (92.00)	24	6.11	8.62	10 (41.67)	20 (83.33)
Total	113	3.61	4.87	78 (69.03)	107 (94.69)	113	4.06	5.66	76 (67.26)	107 (94.69)

Sensitivity of the multiplex assay



- Use of smaller amounts of DNA such as 10 ng of genomic DNA or 4 ng of bisulfite-converted DNA resulted in similar prediction values to those obtained when using more DNA

Summary

- We generated epigenome-wide DNA methylation profiles of 54 saliva samples using Illumina's HumanMethylation450 BeadChip array
 - DNA methylation at the *SST*, *CNGA3*, *KLF14*, *TSSK6*, *TBR1*, and *SLC12A5* genes showed high association with age
 - DNA methylation at 6 age-associated CpGs and a cell type-specific CpG were investigated in 226 saliva samples using methylation SNaPshot
 - A model composed of 7 CpGs exhibited high correlation between predicted and chronological age with a MAD from chronological age of 3.1 years
 - The developed multiplex methylation SNaPshot assay produced reproducible results with a small amount of DNA (4 ng of bisulfite-converted DNA)
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