

## Epigenetic Age Signatures In Saliva: Age Prediction Using Methylation SNaPshot and Massively Parallel Sequencing

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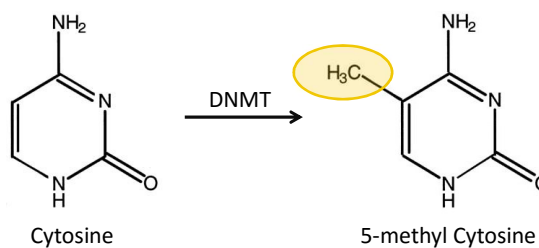
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### DNA Methylation



- Addition of a **methyl group** to **cytosine** followed by guanine
- 5'-CG-3'



## DNA Methylation



### Cell differentiation



### Aging



### Genetic factor



### Environmental factor



## Age Prediction



- Age-related molecular changes
  - Telomere shortening
  - Mitochondrial DNA deletion
  - sjTREC
  - DNA methylation
- DNA methylation-based age predictors
  - Various tissues
    - Koch & Wagner. *Aging (Albany NY)* (2011)
    - Horvath. *Genome Biol.* (2013)
  - Blood
    - Hannum *et al.* *Mol. Cell.* (2013)
    - Zbiec-Piekarska *et al.* *FSI Genet.* (2015)
  - Semen
    - Lee *et al.* *FSI Genet.* (2015)
  - **Saliva & Buccal swab**
    - Bocklandt *et al.* *PLoS One.* (2011)
    - Eipel *et al.* *Aging (Albany NY)* (2016)

## Method

- **Saliva samples**
  - 280 samples (18-73 years)
- **HumanMethylation450 BeadChip Array**
  - 54 males (18-73 years)
- **Targeted Bisulfite Sequencing**

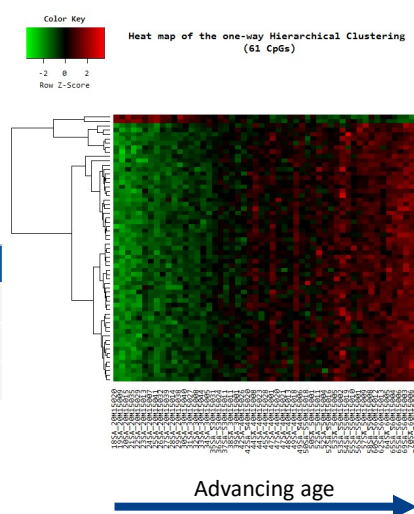
| info   | Training Set | Testing Set | Total |
|--------|--------------|-------------|-------|
| Male   | 47           | 70          | 117   |
| Female | 48           | 61          | 109   |
| Total  | 95           | 131         | 226   |

- Multiplex methylation SNaPshot (226 samples; Both sets)
- Massively parallel sequencing (95 samples; Training set)
- Multivariate linear regression analysis using SPSS

## HumanMethylation450 BeadChip Array

- **Details**
  - 54 males (18-73 years)
  - GSE92767
  - 445,791 CpGs
- **Selection of marker candidates**

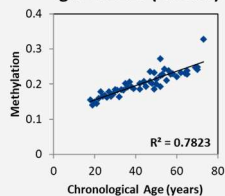
| Criteria   | No. CpGs  |
|--|-----------|
| FDR <sub>p</sub> < 0.05  | 74,807    |
| R <sup>2</sup> value > 0.65  | 80        |
| $ \beta\text{-score}_{\text{MAX}} - \beta\text{-score}_{\text{min}}  \geq 0.1$ | <b>62</b> |



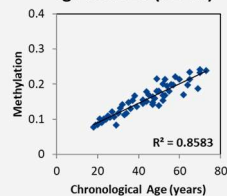
## Age-associated CpG candidates

### Stepwise linear regression analysis

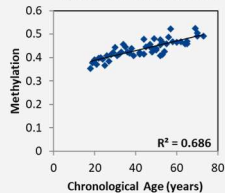
cg19671120 (CNGA3)



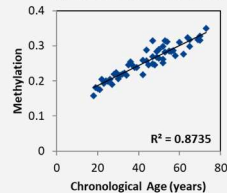
cg14361627 (KLF14)



cg08928145 (TSSK6)

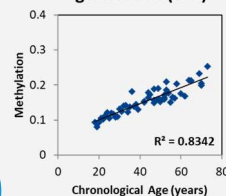


cg07547549 (SLC12A5)

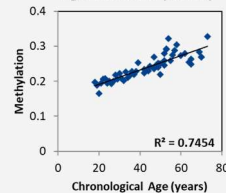


### Additional candidates

cg00481951 (SST)



cg12757011 (TBR1)



+

## Cell Type-specific Marker

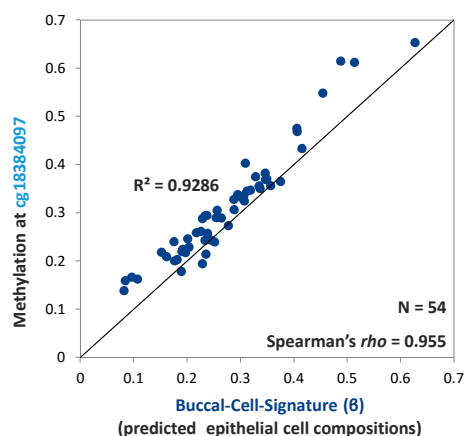
- **Buccal-Cell-Signature ( $\beta$ )**

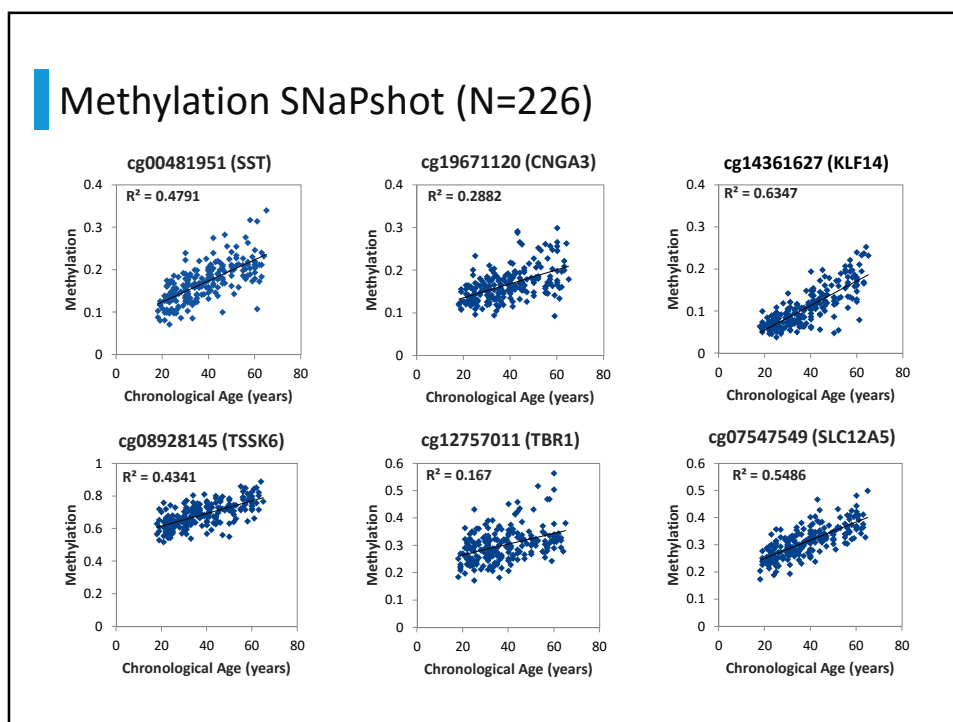
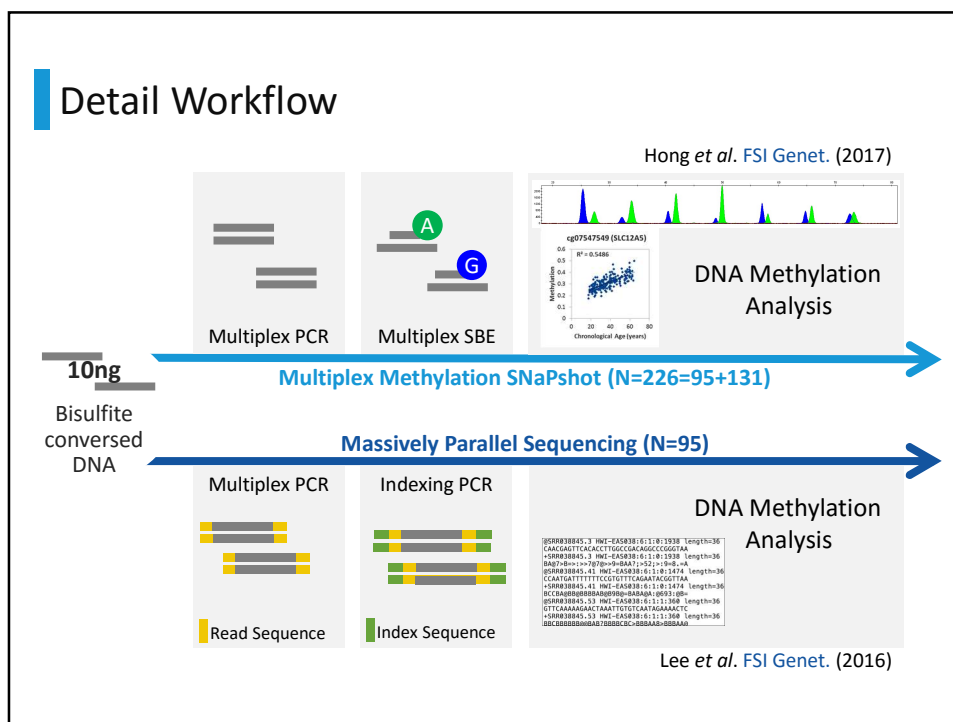
- Eipel *et al.* *Aging (Albany NY)*. (2016)
- cg07380416 (*CD6*)
- cg20837735 (*SERPINB5*)
- Percentage of buccal epithelial cells

$$\beta = \frac{99.8 \times \beta_{cg07380416} + 1.92}{2} + \frac{-98.12 \times \beta_{cg20837735} + 88.54}{2}$$

- **cg18384097 (*PTPN7*)**

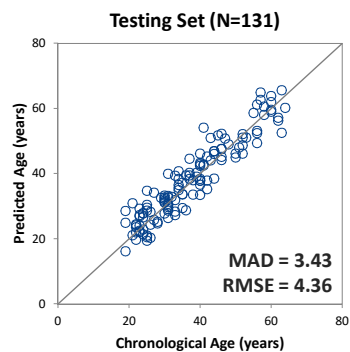
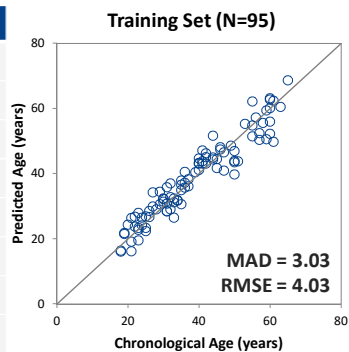
- Souren *et al.* *Genome Biol.* (2013)
- High in buccal epithelial cell
- Low in blood cell
- *PTPN7* gene
  - Protein tyrosine phosphatase (PTP)
  - Preferentially expressed in hematopoietic cells





## Model – Multiplex Methylation SNaPshot

| Target ID   | Coefficient |
|-------------|-------------|
| (intercept) | -24.521     |
| cg18384097  | -31.111     |
| cg00481951  | 6.718       |
| cg19671120  | 23.760      |
| cg14361627  | 81.053      |
| cg08928145  | 24.325      |
| cg12757011  | 53.634      |
| cg07547549  | 89.415      |

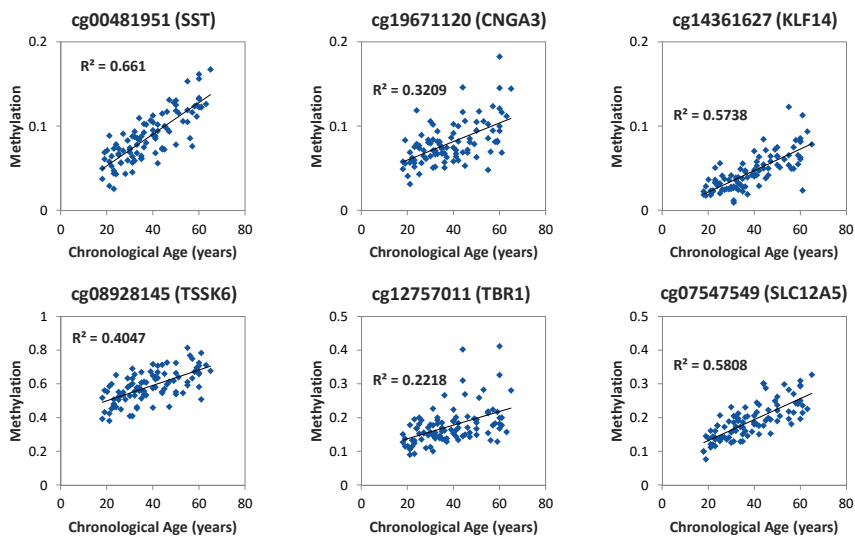


MAD: Mean Absolute Deviation  
RMSE: Root Mean Square Error

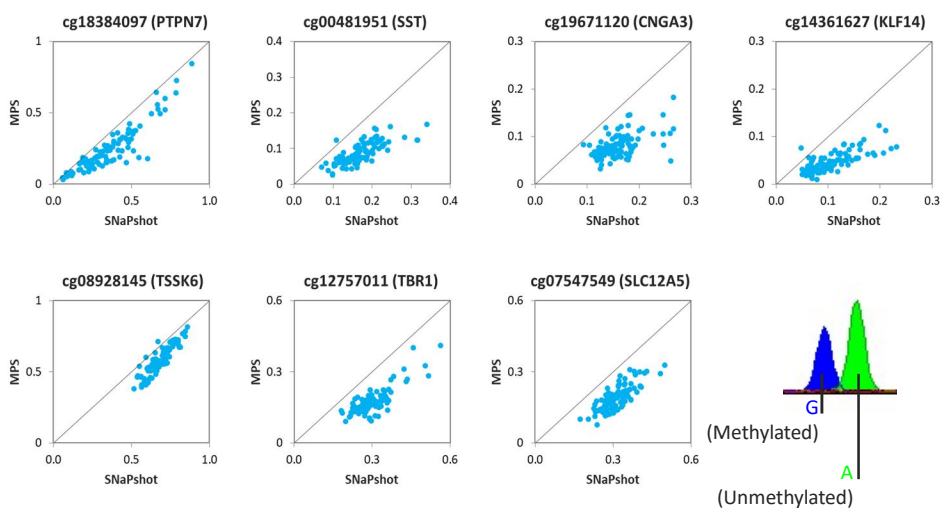
## MPS (N=95) – Read Count



## MPS (N=95) – Methylation value



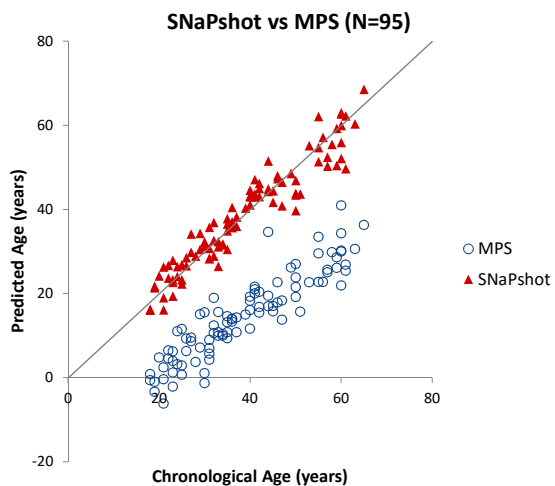
## SNaPshot vs MPS (N=95)



## SNaPshot vs MPS (N=95)

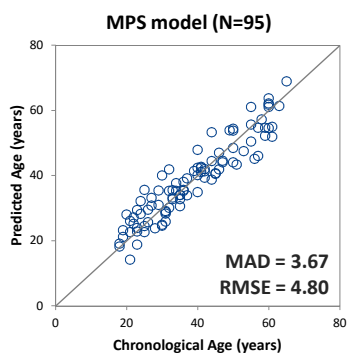
Model - SNaPshot

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| cg14361627  | 81.053      |
| cg08928145  | 24.325      |
| cg12757011  | 53.634      |
| cg07547549  | 89.415      |



## Model – MPS

| Target ID   | Coefficient |
|-------------|-------------|
| (intercept) | -7.282      |
| cg18384097  | -18.170     |
| cg00481951  | 131.995     |
| cg19671120  | 71.822      |
| cg14361627  | 138.619     |
| cg08928145  | 20.377      |
| cg12757011  | -1.307      |
| cg07547549  | 78.467      |





## Conclusion

- A cell type-specific marker (cg18384097) and 6 age-associate markers (cg00481951, cg19671120, cg14361627, cg08928145, cg12757011, and cg07547549) enabled age prediction in saliva with high accuracy.
- Markers can be applied to both Multiplex methylation SNaPshot and MPS.
- The model should be altered as the platform differs.

## Acknowledgement

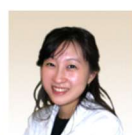


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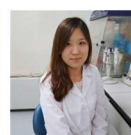
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Mi Hyeon Moon



SeungMin Lim



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