




## Forensic age prediction using inter-platform analysis of DNA methylation

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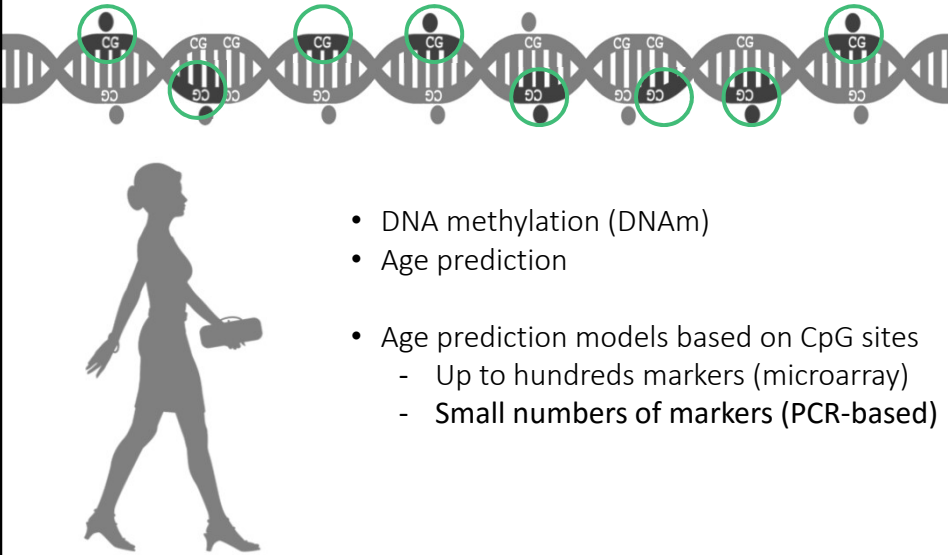
### Introduction

Epigenetic age prediction



### Introduction

Age prediction models



- DNA methylation (DNAm)
- Age prediction
- Age prediction models based on CpG sites
  - Up to hundreds markers (microarray)
  - Small numbers of markers (PCR-based)

### Introduction

Age prediction models in various DNAm analysis platforms

**MPS (Massively parallel sequencing)**

**Vidaki et al. (2017)**  
 Naue et al. (2017, 2018)  
 Aliferi et al. (2018)  
 Heidegger et al. (2020)  
 Woźniak et al. (2021)  
 Heidegger et al. (2021)

**Hong et al. (2019)**

**Freire-Aradas et al. (2020)**

**Mass array**

Xu et al. (2015)  
 Giuliani et al. (2016)  
 Freire-Aradas et al. (2016,2018)

Feng et al. (2018)  
 Montesanto et al. (2020)

**SBE (Single-base extension)**

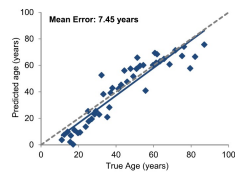
Lee et al. (2015)  
 Hong et al. (2017)  
 Lee et al. (2018)  
 Jung et al. (2019)  
 Dias et al. (2020)

Weidner et al. (2014)  
 Bekaert et al. (2015)  
 Zbiec-Piekarska et al. (2015)  
 Park et al. (2017)  
 Cho et al. (2017)  
 Thong et al. (2021)  
 Koop et al. (2021)

**Pyrosequencing**

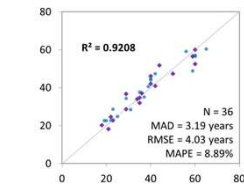
## Introduction

Increased errors in different platforms



Vidaki et al. *FSIG* (2017)

- MPS -> array-based model
- Direct application
- 7.45 years of mean error



Hong et al. *FSIG* (2019)

- MPS & SBE integrated model
- Adding a platform variable

Technology	Group	MSE	RMSE	%CP ± 5	%CP ± 10
EpiTYPER <sup>®</sup>	Training (N = 118)	43.06	4.68	72.8%	76.69%
	Testing (N = 84)	53.09	5.32	71.96%	75.61%
MBOx	Training (N = 84)	43.31	5.77	69.29%	80.23%
	Testing (N = 84)	44.71	7.28	53.57%	44.05%
MBOseq	Training (N = 103)	33.8	6	65%	77%
	Testing (N = 84)	43.22	6.73	69.51%	74.92%
EpiTYPER <sup>®</sup>	Training (N = 84)	42.81	5.93	77.38%	85.71%
	Testing (N = 84)	43.12	7.26	67.86%	75.10%
SNaPshot <sup>™</sup>	Training (N = 103)	42.88	4.43	76.09%	77%
	Testing (N = 84)	44.1	6.43	4.88%	23.71%
SNaPshot <sup>™</sup>	Training (N = 84)	42.88	4.43	76.09%	77%
	Testing (N = 84)	44.1	6.43	4.88%	23.71%
MBOx	Training (N = 84)	41.159	12.49	14.25%	47.62%
	Testing (N = 84)	33.6	8.2	58.22%	82.77%
EpiTYPER <sup>®</sup>	Training (N = 121)	43.89	5.46	64.46%	79.12%
	Testing (N = 84)	42.9	5.27	68.29%	82.93%
MBOx	Training (N = 84)	43.03	5.29	73.31%	79.21%
	Testing (N = 84)	43.8	7.43	65.1%	77.38%

Freire-Aradas et al. *Front Genet* (2020)

- MPS, SBE, mass array, pyrosequencing
- Combined training set
- Z-transformation

## Objective

Initial questions

- Are they identical? How different?
  - MPS vs SBE (semi-quantitative)
- How accurate is it?
  - MPS-based age prediction model
  - Applying SBE data
- Is it simple?
  - Web-based age calculator

## Materials and Methods

Panel using common five age-correlated markers

- Five age-correlated markers for blood
  - ELOVL2, FHL2, KLF14, MIR29B2C (C1orf132), and TRIM59
- Widely studied markers
  - Pyrosequencing
  - SBE
  - Mass array (EpiTYPER)
  - MPS

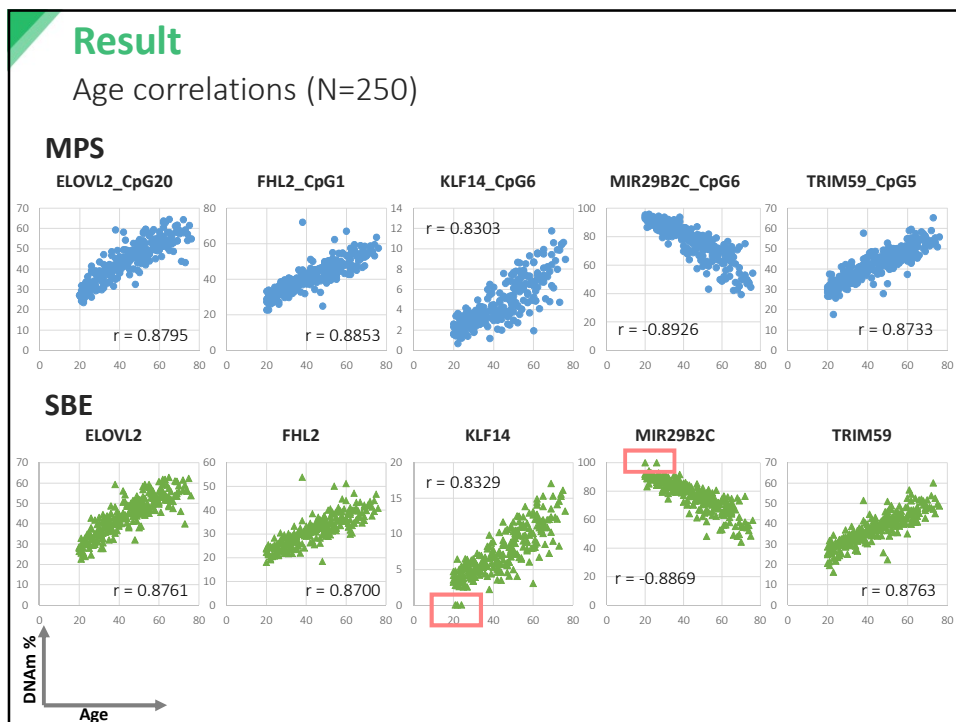
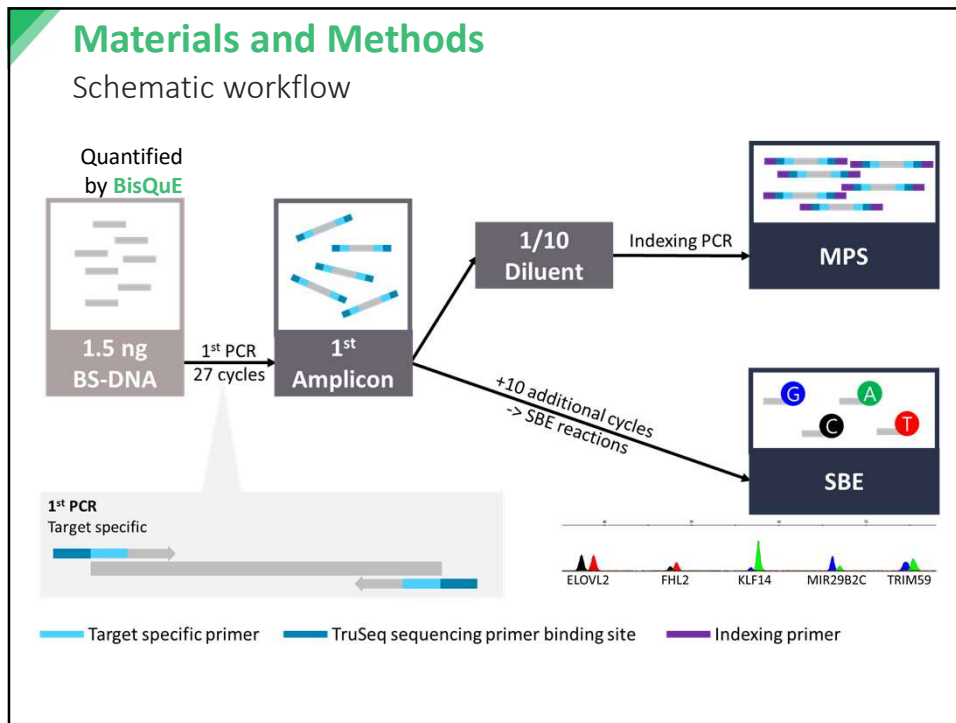


## Materials and Methods

Samples and bisulfite-converted DNA (BS-DNA) preparation

Age	Male	Female
20s	25	25
30s	25	25
40s	25	25
50s	25	25
60s +	25	25
<b>Total</b>	<b>125</b>	<b>125</b>

- Blood samples
  - SNU Asian Sample Network
- EZ DNA Methylation-Lightning (Zymo)
- Quantified via [BisQuE](#)  
Hong and Shin Front Genet (2021)

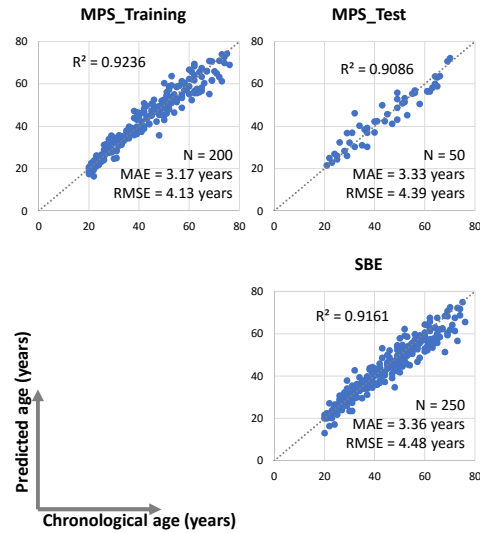


## Result

MPS-based age prediction model

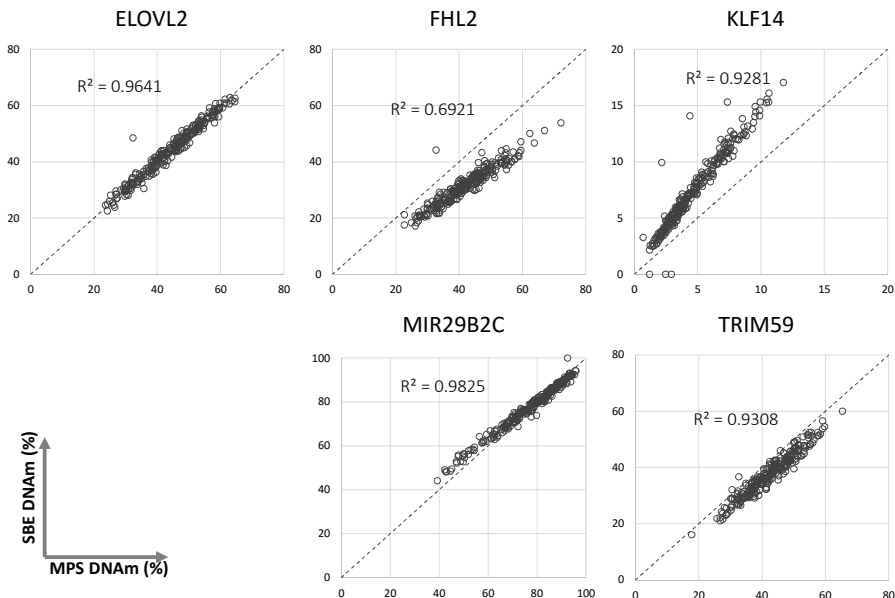
Markers	Coefficient	p-value
(intercept)	-9.0421	0.2966
*P(ELOVL2)	4.6551	< 0.001
FHL2	0.2741	< 0.001
KLF14	1.0935	< 0.001
MIR29B2C	0.4479	0.0734
(MIR29B2C) <sup>2</sup>	-0.0062	< 0.001
*TRIM59	0.2674	< 0.001

\*Power transformation;  $\lambda = 0.24391419$



## Result

Difference between MPS and SBE



## Result

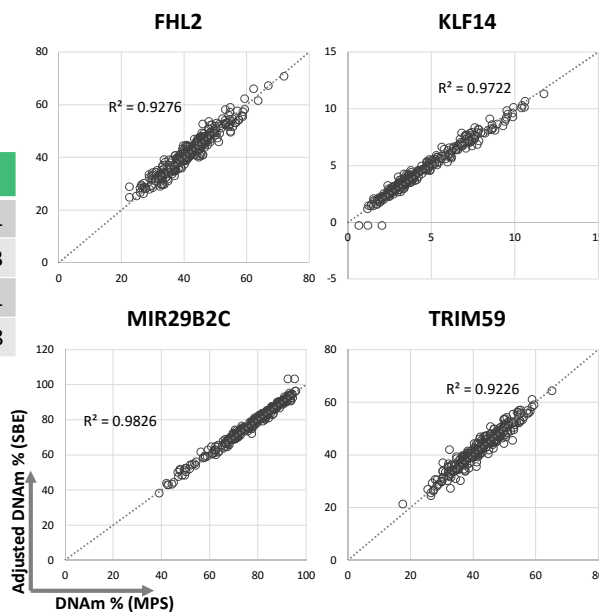
Difference between MPS and SBE

Gene	ICC(2,1)		Passing –Bablok Regression			
	Value	p-value	Slope (95% CI)		Intercept (95% CI)	
			Lower	Upper	Lower	Upper
ELOVL2	0.99	< 0.001	0.9644	1.0026	-0.3855	1.5541
FHL2	0.48	< 0.001	0.7303	0.7891	-1.7677	0.7821
KLF14	0.63	< 0.001	1.4156	1.4787	0.4893	0.7697
MIR29B2C	0.98	< 0.001	0.8375	0.8650	10.9629	13.2226
TRIM59	0.82	< 0.001	0.9485	1.0120	-5.2816	-2.4901

## Result

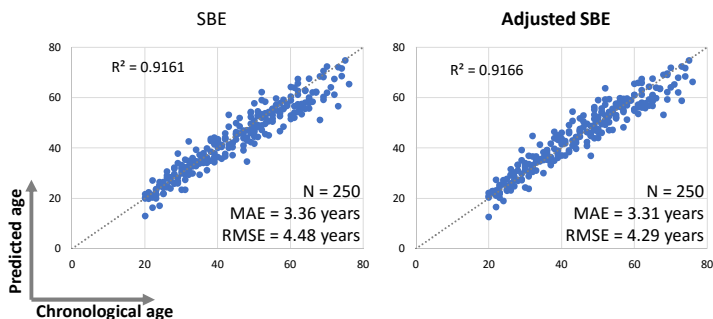
Adjustment of SBE data

Markers	(Intercept)	x
FHL2	1.5992	1.2831
KLF14	-0.3072	0.6793
MIR29B2C	-13.3361	1.1631
TRIM59	5.2748	0.9828



### Result

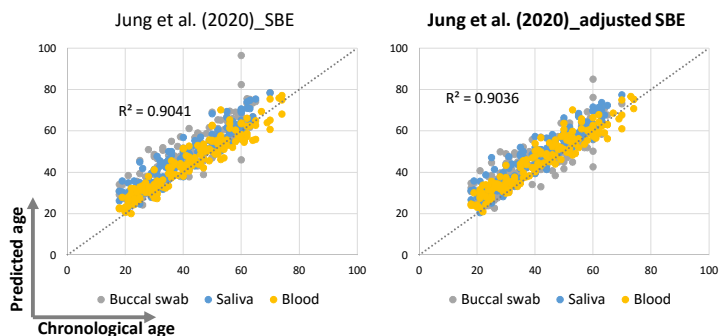
Performance of MPS-based model in SBE data



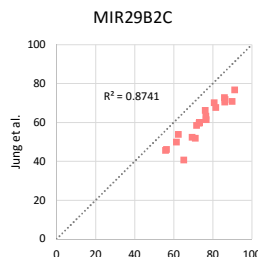
Age	SBE		Adjust_SBE	
	MAE	RMSE	MAE	RMSE
60s +	5.47	6.63	4.66	5.68
<b>Total</b>	<b>3.36</b>	<b>4.48</b>	<b>3.31</b>	<b>4.29</b>

### Result

Performance of MPS-based model in SBE data



Age	Jung		Adjust_Jung	
	MAE	RMSE	MAE	RMSE
Blood	4.28	5.30	4.86	6.03
Saliva	6.61	7.85	6.87	8.71
Buccal	7.69	9.73	7.00	8.27
<b>Total</b>	<b>6.19</b>	<b>7.83</b>	<b>6.24</b>	<b>7.76</b>





## Result

### Web-based age calculator

- Snipper 2.5
- [http://mathgene.usc.es/snipper/age\\_models.php](http://mathgene.usc.es/snipper/age_models.php)
- Age prediction models with DNA methylation
- Age Calculator
- <http://forensic.yonsei.ac.kr/Age5CpG/index.html>

Choose one of our age prediction models with DNA methylation:

- [Blood, 7 CpGs, EpiTYPER](#)
- [Semen, 3 CpGs, SNaPshot](#)
- [Tissue combined \(blood & buccal & saliva\), 5 CpGs, SNaPshot](#)
- [Blood, 5 CpGs, SNaPshot](#)
- [Buccal swab, 5 CpGs, SNaPshot](#)
- [Saliva, 5 CpGs, SNaPshot](#)

Gene	CpG ID	GRCh38 chromosome position
ELOVL2	none	chr6:11044628
FHL2	cg06639320	chr2:105399282
KLF14	cg14361627	chr7:130734355
MIR29B2C	none	chr1:207823681
TRIM59	cg07553761	chr3:160450189

Name	ELOVL2	FHL2_cg06639320	KLF14_cg14361627	MIR29B2C	TRIM59_cg07553761
Values	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

#### Age Calculation by 5 CpGs

Methylation ↻

- ELOVL2  (%)
- FLH2  (%)
- KLF14  (%)
- MIR29B2C  (%)
- TRIM59  (%)

Analysis method

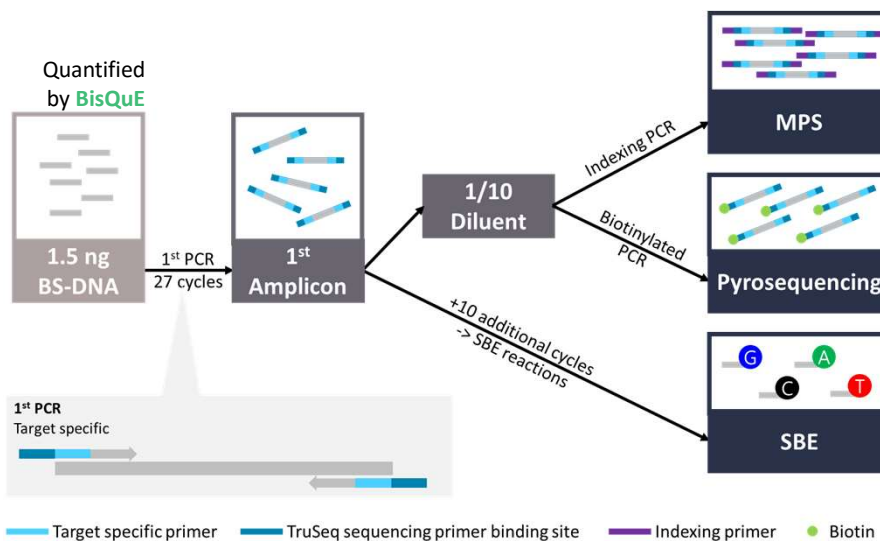
Massively parallel sequencing  
 Methylation SNaPshot (SBE)

Calculated age: 32.25 (yrs)

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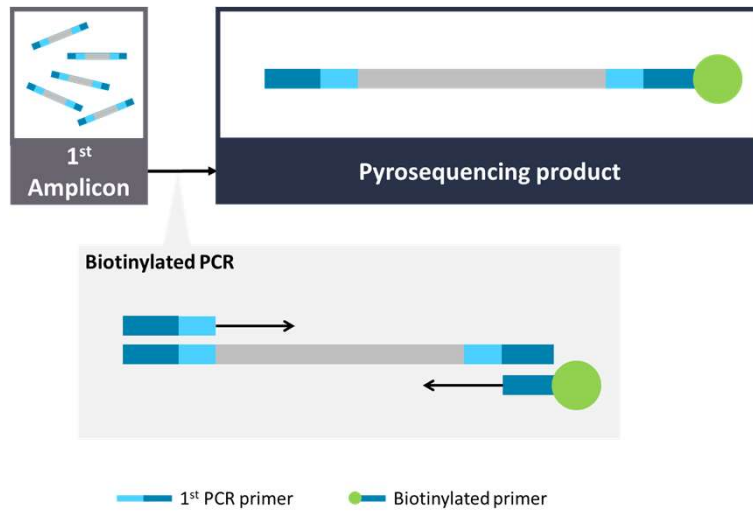
## Further

### Expansion to pyrosequencing



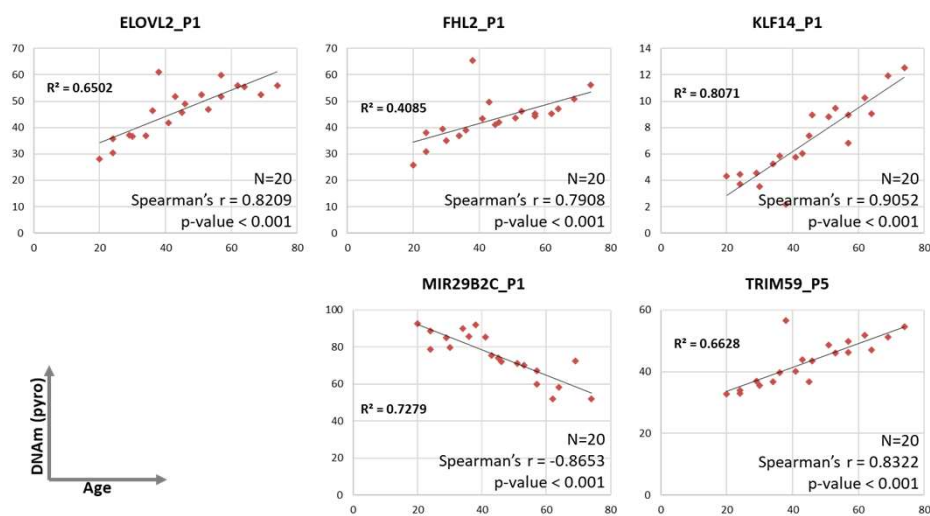
### Further

Expansion to pyrosequencing



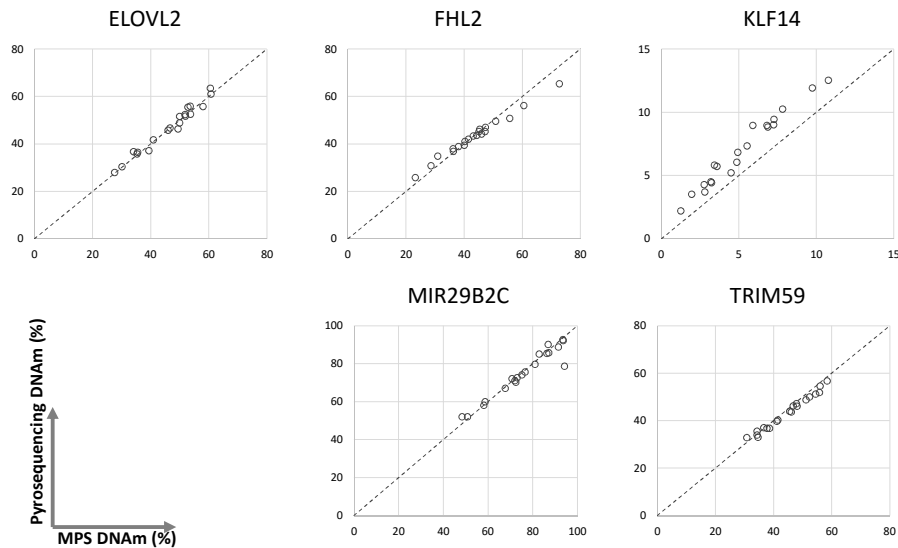
### Further

Expansion to pyrosequencing



## Further

Difference between MPS and pyrosequencing



## Further

Concerns and considerations

- Adjustment of SBE
  - Fluorescence dye
  - primer interactions; CpG island (preference)
  - Instrument dependent (*So et al. Electrophoresis (2021)*)
- Minimum quantity of BS-DNA for age prediction
  - Sensitivity test (quantification)
  - Data-driven approach
  - Reliability / stochastic effects

## Conclusion

Take-home messages

- MPS is highly recommended to build the age prediction model.
- ELOVL2 showed no difference between MPS and SBE.
- The constructed age prediction models based on MPS showed high accuracies in both MPS and SBE.
- Inter-platform analysis can be achieved by using the same amplicons and adjusting DNAm values.

# Thank you

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