Measuring the differences in DNA methylation based on bisulfite-converted DNA quantitation

Sae Rom Hong

Department of Forensic Medicine, Yonsei University College of Medicine

ICGSK2023

Oct 20th, 2023

KRISS

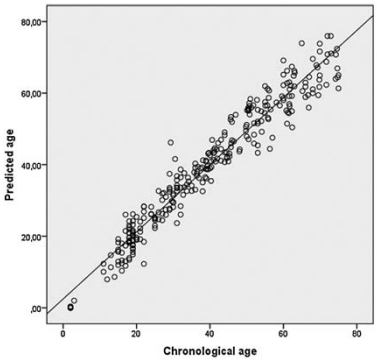
Disclaimer

- Presentations are intended for academic purposes only and do not replace independent professional judgement.
- Statements of fact and opinions expressed are those of the participants individually and, unless expressly stated to the contrary, are not the opinion or position of the Korea Research Institute of Standards and Science.
- Korea Research Institute of Standards and Science does not endorse or approve, and assumes no responsibility for, the content, accuracy or completeness or the information presented.

KRISS

Age prediction based on DNA methylation

- Addition of a methyl group to C in 5'-CG-3'
- Forensic applications



Zbiec-Piekarska et al. (2015) *FSIG*

2

KRISS

Considerations for forensic contexts

Laboratory level

- Low DNA quantity
- Bisulfite-converted DNA (BS-DNA)
  - Enzyme modification
- PCR-based methods
- Small numbers of markers
- Accuracy and errors
- Methodology
- Mixture

Social level

- High scalability
- Cost-effectiveness
- Guidelines for authorities
- Legal and societal issues

3

KRISS

Research questions

To what extent can BS-DNA amount be

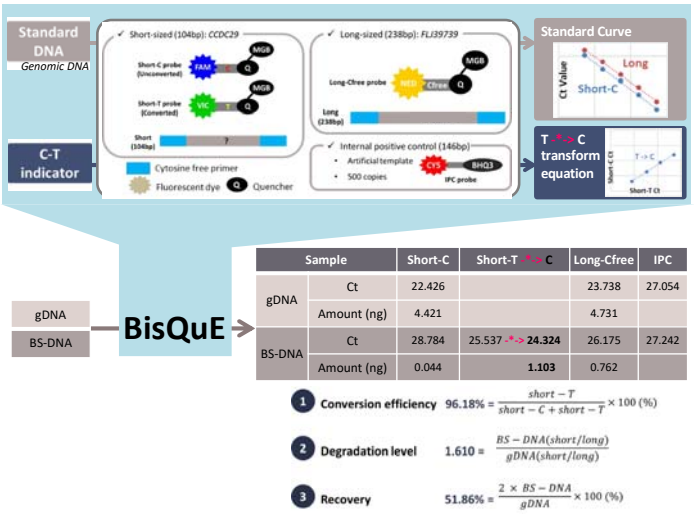
1) reduced without sacrificing accuracy?
2) reduced without sacrificing reliability?
3) considered feasible in forensic cases?

4

KRISS

BisQuE for the quantification of BS-DNA

Bisulfite-converted DNA quantity evaluation



Sample		Short-C	Short-T -*-> C	Long-Cfree	IPC
gDNA	Ct	22.426		23.738	27.054
	Amount (ng)	4.421		4.731	
BS-DNA	Ct	28.784	25.537 -*-> **24.324**	26.175	27.242
	Amount (ng)	0.044	**1.103**	0.762	

1 Conversion efficiency 96.18% = $\frac{short-T}{short-C+short-T} \times 100\ (\%)$

2 Degradation level 1.610 = $\frac{BS-DNA(short/long)}{gDNA(short/long)}$

3 Recovery 51.86% = $\frac{2 \times BS-DNA}{gDNA} \times 100\ (\%)$

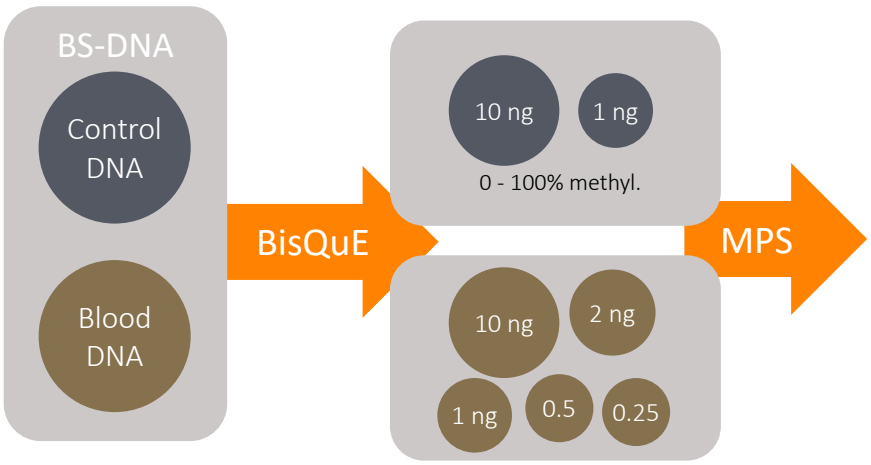
Hong and Shin (2022) *Front Genet*

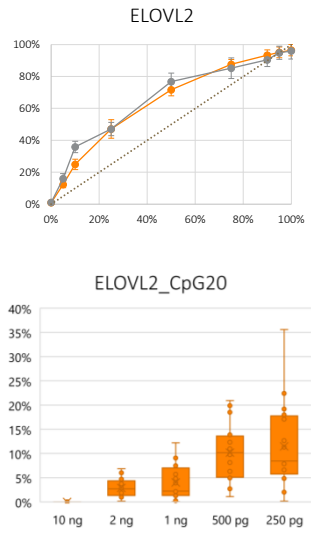
- Real-time PCR
- Multiplex
- Cytosine-free primer sets
- Multi-loci
- Both gDNA and BS-DNA
- Thymine to Cytosine

5

KRISS

Experimental scheme





*MPS: massively parallel sequencing

6

KRISS

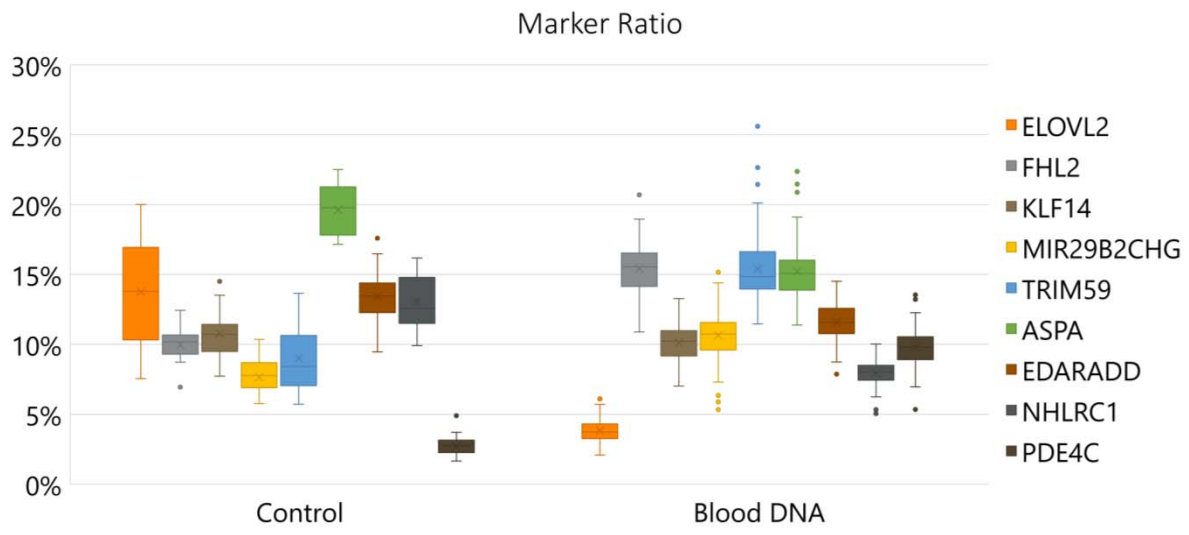
Materials and methods

- BS-DNA Sample
  - DNA methylation control
    - Methylated and non-methylated
    - 0, 5, 10, 25, 50, 75, 90, 95, and 100%
    - 10 ng and 1 ng (BisQuE)
    - Duplicate
  - Blood-derived human DNA samples
    - 20 Korean aged 20-74
    - 10, 2, 1, 0.5, and 0.25 ng (BisQuE)
- Target amplicon-based MPS
  - Age-correlated markers
    - *ELOVL2, FHL2, KLF14, MIR29B2C, TRIM59*
    - *ASPA, EDARADD, NHLRC1, PDE4C*

7

KRISS

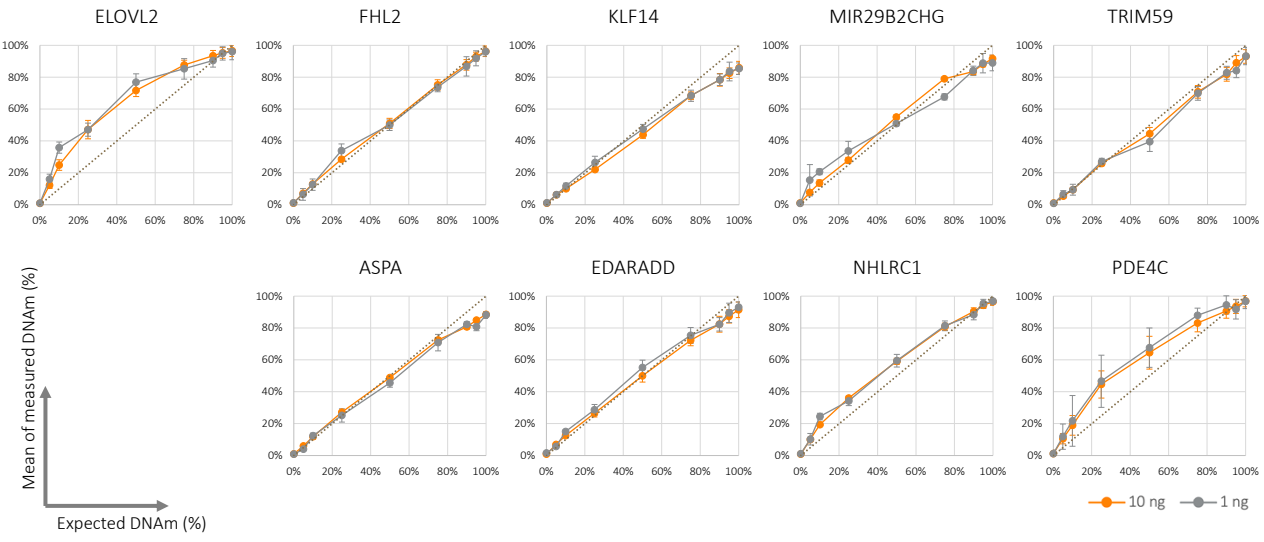
DNAm control DNA does not reflect real samples



8

KRISS

DNA quantity matters: DNAm control DNA



9

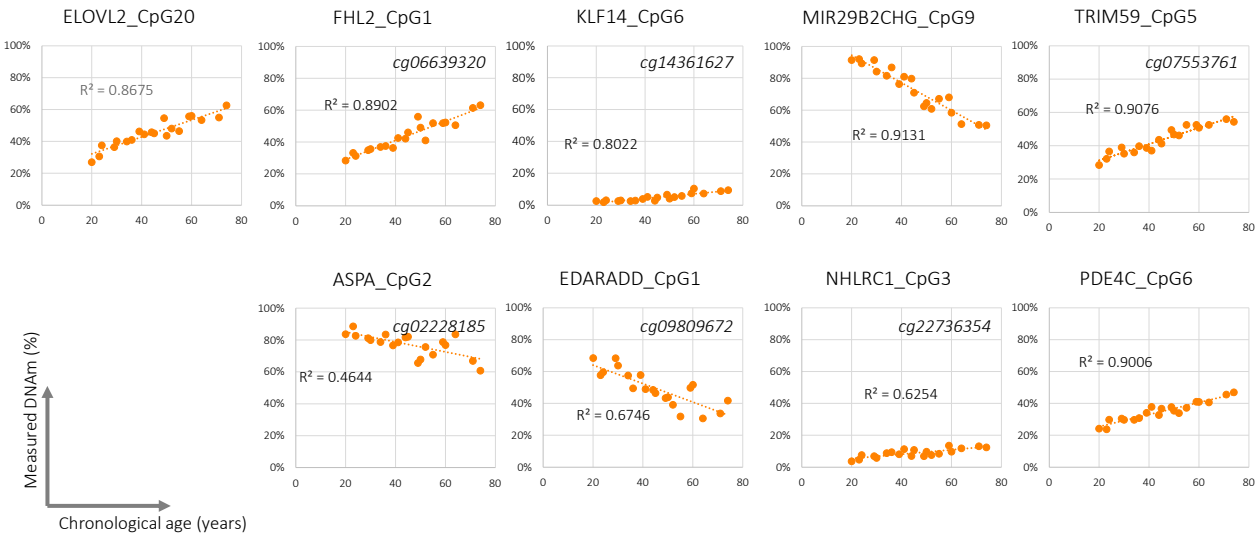
KRISS

DNA quantity matters: DNAm control DNA

- Accuracy
- ELOVL2, NHLRC1, and PDE4C
  - CpGs exist on the primer binding sites.
- Control DNA
  - There were neither 0% nor 100% methylated loci.

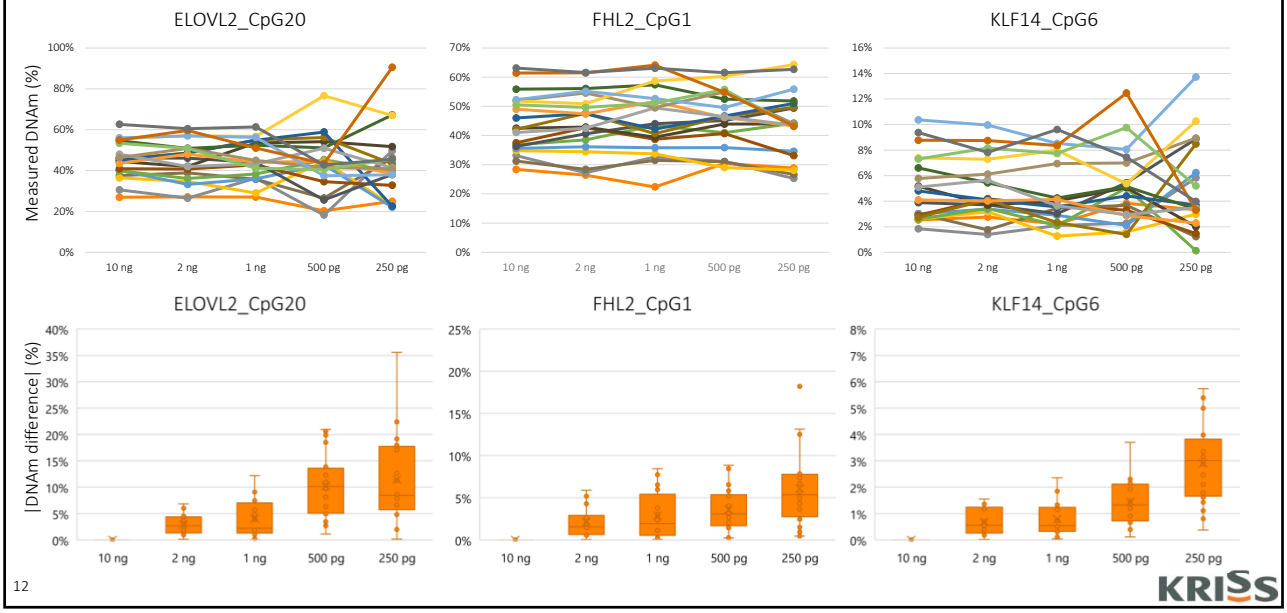
KRISS

Age-correlations in 10 ng of 20 blood DNA samples



KRISS

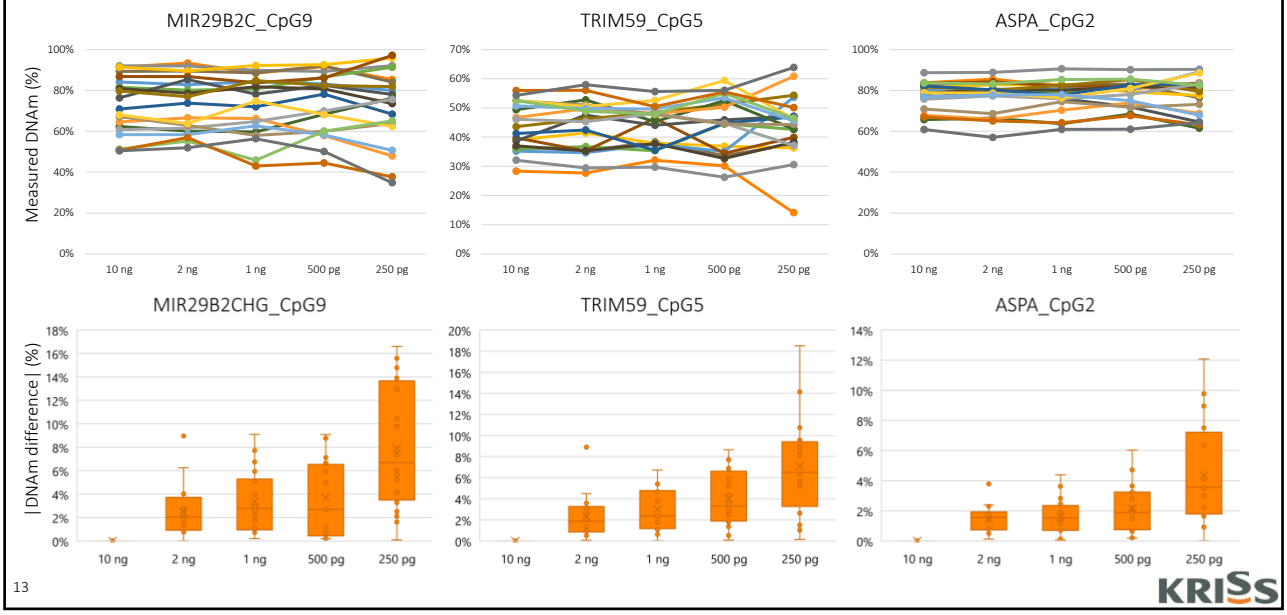
DNA quantity matters: human blood DNA



12

KRISS

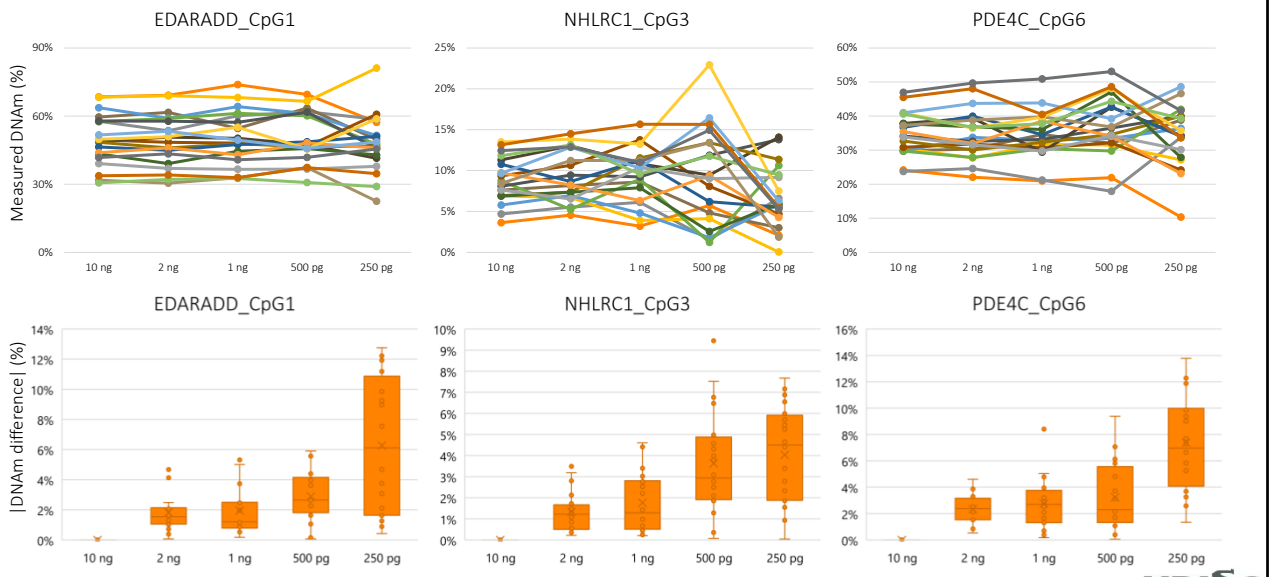
DNA quantity matters: human blood DNA



13

KRISS

DNA quantity matters: human blood DNA



14

KRISS

DNA quantity matters: human blood DNA

Marker	ELOVL2	FHL2	KLF14	MIR29B2C	TRIM59	ASPA	EDARADD	NHLRC1	PDE4C
2 ng	4.62%	5.09%	14.95%	3.58%	4.97%	1.37%	2.43%	10.51%	3.16%
1 ng	8.26%	7.41%	12.41%	4.65%	4.93%	1.58%	2.86%	17.39%	5.40%
0.5 ng	12.12%	6.03%	19.60%	5.48%	6.60%	2.19%	4.13%	27.66%	7.18%
0.25 ng	18.19%	9.63%	56.35%	9.88%	13.98%	4.40%	8.30%	24.13%	13.57%

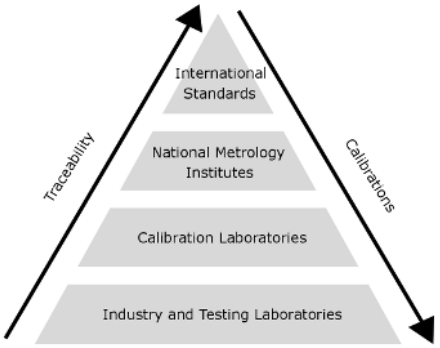
- Relative absolute difference: |DNAm diff.|/ (DNAm_10 ng)
- Accuracy and reliability
- Feasibility

15

KRISS

Reference materials and a consensus are needed

Reference materials for DNAm



A consensus on DNAm age

- DNA quantity
- Primary reference measurement system for DNAm
- Standardized guidelines
- Error reports

16 16

KRISS

Conclusion

- DNAm detection levels are substantially influenced by BS-DNA amounts.
- The thorough quantification of BS-DNA could enhance accuracy and reliability.
- At least, 1 ng of BS-DNA should be used as a template.
- Collaborative works throughout forensic and metrological fields are needed.

17

KRISS

Acknowledgement

- Yonsei University College of Medicine
- Seoul National University College of Medicine
- Korean National Police Agency (0411-20220055)
- KRISS

18

Thank you

Prof. Kyoung-Jin Shin
kjshin@yuhs.ac

Sae Rom Hong
srhong0310@kriss.re.kr