



Journal homepage: <https://trpubonline.org/index.php/Chromatin>

RESEARCH ARTICLE

DNA Methylation-Based Forensic Age Estimation from Degraded Biological Samples

Vaclav Greco^{1*}, Anna Lindqvist² and Vaclav Whitmore³

¹Department of Molecular Biology, Northmoor School of Molecular Biology and Genetics, United Kingdom

²Department of Epigenetics and Gene Regulation, Vistula Institute of Molecular Genetics, Poland

³Department of Molecular Genetics, Nordic Institute of Epigenomic Medicine, Finland

*Corresponding author: Vaclav Greco, Department of Molecular Biology, Northmoor School of Molecular Biology and Genetics, United Kingdom; Email: v.greco@northmoor.ac.uk

ABSTRACT

Age-associated DNA methylation changes at a small number of CpG sites provide a molecular basis for estimating chronological age from a biological sample, a capability of substantial forensic interest, but the bisulfite conversion and amplification steps underlying most methylation assays are sensitive to the DNA fragmentation and chemical damage typical of forensic evidence, and the robustness of methylation-based age estimation to this degradation has not been systematically characterized. We selected five age-informative CpG loci within ELOVL2, FHL2, KLF14, TRIM59 and EDARADD, previously validated in blood, and developed short bisulfite amplicon assays (59-91 bp post-conversion) designed specifically to remain amplifiable from fragmented template, then trained an elastic-net regression model for age prediction on 312 venous blood samples from donors aged 3-89 years. We experimentally degraded aliquots of 60 samples spanning the training age range by controlled UV irradiation, dry heat incubation and extended room-temperature storage to simulate casework-relevant degradation, and quantified methylation at each locus in both intact and degraded aliquots by pyrosequencing. The intact-sample model achieved a mean absolute prediction error of 3.4 years by leave-one-out cross-validation. Prediction error in degraded aliquots increased significantly only in samples degraded to a mean fragment size below 150 bp, at which the mean absolute error rose to 7.8 years, driven predominantly by reduced amplification efficiency at the FHL2 and TRIM59 loci rather than by a systematic shift in methylation level at successfully amplified sites. A simplified three-locus model restricted to ELOVL2, KLF14 and EDARADD, the loci least affected by fragmentation, retained a mean absolute error of 5.1 years even in the below-150 bp category, at a modest cost in intact-sample accuracy (4.1 years). These results indicate that short-amplicon methylation-based age estimation remains usable in severely degraded forensic samples provided that amplicon selection is designed around fragmentation robustness.

Keywords: DNA methylation; epigenetic age; forensic genetics; ELOVL2; pyrosequencing; degraded DNA; bisulfite sequencing; age prediction

Received: July 10, 2026 | Accepted: October 1, 2026 | Published: December 21, 2026

Citation: Greco V, Lindqvist A, Whitmore V. DNA Methylation-Based Forensic Age Estimation from Degraded Biological Samples. *Chromatin*. 2026;4(2):1–6. DOI: [10.5281/zenodo.22760934](https://doi.org/10.5281/zenodo.22760934)

1. INTRODUCTION

Chronological age at the time a biological sample was deposited is not directly recoverable from standard forensic short tandem repeat profiling, yet an age estimate can substantially narrow an investigative pool when a suspect's identity is otherwise unknown. DNA methylation at a defined set of CpG sites changes with age in a highly reproducible, tissue-consistent manner, and elastic-net or similar regularized regression models trained on methylation at as few as five to eight such sites can predict chronological age in blood to within several years across the human lifespan, motivating substantial interest in translating these models into forensic practice [1,2].

Nearly all published age-prediction models have been trained and validated on high-quality genomic DNA extracted from fresh or well-preserved blood, whereas forensic casework samples are frequently degraded by environmental exposure, time, or the biological processes of decomposition, producing DNA fragmented well below the amplicon lengths typically used in methylation assays. Bisulfite conversion itself further fragments and chemically damages template DNA, compounding pre-existing degradation and potentially reducing amplification success specifically at loci with longer or less efficient primer designs [3,4]. Whether age-prediction accuracy is preserved, modestly reduced, or substantially compromised under casework-relevant degradation has received comparatively little systematic study, with most prior reports either using intact reference samples or testing only a single degradation condition.

A related open question is whether degradation affects all age-informative loci equally or disproportionately compromises particular loci, for example those requiring longer amplicons or less efficient bisulfite-converted primer binding, in which case a model built around fragmentation-robust locus selection might substantially outperform a model optimized purely for intact-sample accuracy once applied to degraded evidence. Addressing this question requires paired methylation measurements from the same samples before and after controlled, casework-relevant degradation, rather than comparing accuracy across studies that differ in donor population, tissue type and assay design.

Here we developed short bisulfite amplicon assays for five previously validated age-informative loci, trained an elastic-net age-prediction model on 312 intact blood samples, and then experimentally degraded aliquots of 60 of these samples by UV irradiation, dry heat and extended storage to systematically test how fragmentation affects both per-locus amplification success and age-prediction accuracy. We report that degradation below a mean fragment size of 150 bp substantially increases prediction error in the full five-locus model, driven by differential amplification dropout across loci, and that a simplified three-locus

model selected for fragmentation robustness substantially recovers accuracy in severely degraded samples at only a modest cost to intact-sample performance.

2. MATERIALS AND METHODS

Venous blood samples were collected from 312 donors aged 3 to 89 years under informed consent, with genomic DNA extracted by standard column-based purification; 60 samples spanning the training age range were selected for paired intact-versus-degraded methylation comparison.

2.1 Locus selection and short-amplicon assay design

Five CpG loci within ELOVL2, FHL2, KLF14, TRIM59 and EDARADD were selected based on prior reports of strong age association in blood, and bisulfite PCR primers were redesigned specifically to minimize post-conversion amplicon length (59-91 bp) while retaining coverage of the informative CpG position, in contrast to longer amplicons (120-180 bp) used in several previously published assays for the same loci.

2.2 Sample degradation treatments

Aliquots of extracted genomic DNA from 60 selected samples were subjected to three degradation treatments in parallel: UV-C irradiation at defined doses up to 500 mJ/cm², dry heat incubation at 94°C for up to 4 hours, and extended storage at room temperature and 60% relative humidity for up to 8 weeks, chosen to model exposure, thermal and time-based degradation scenarios relevant to casework evidence. Fragment size distribution after each treatment was assessed by capillary electrophoresis, and samples were grouped into three post-treatment mean fragment size bins (>300 bp, 150-300 bp, <150 bp) for subsequent analysis.

2.3 Bisulfite conversion and pyrosequencing

Genomic DNA from intact and degraded aliquots was bisulfite-converted using a column-based kit optimized for degraded input, and each of the five short amplicons was PCR-amplified in triplicate and quantified by pyrosequencing on a Qiagen PyroMark Q48 platform, with amplification success defined as generation of a sequencable peak of sufficient signal intensity in at least two of three technical replicates.

2.4 Elastic-net age-prediction model training

An elastic-net regularized linear regression model was trained on methylation beta-values at the five loci from all 312 intact-sample donors using the glmnet package in R, with the regularization parameter selected by 10-fold cross-validation minimizing mean squared prediction error; model accuracy was assessed by leave-one-out cross-validation across the full donor set. A reduced three-locus model using only ELOVL2, KLF14 and EDARADD was trained identically for comparison.

2.5 Statistical analysis

Prediction error was summarized as mean absolute error (MAE) between predicted and chronological age within each degradation category. Differences in per-locus amplification success and in MAE across degradation categories were assessed by one-way ANOVA with Tukey post-hoc comparison, with $P < 0.05$ considered significant. All statistical analyses were performed in R version 4.3.

3. RESULTS

The five-locus elastic-net model trained on intact-sample methylation achieved a mean absolute prediction error of 3.4 years by leave-one-out cross-validation across the full 312-donor training set, consistent with the accuracy range reported for comparable models in prior blood-based studies. Coefficients for ELOVL2 and FHL2 contributed the largest magnitude to the fitted model, consistent with their established status as the two most strongly age-associated loci among the five selected. Table 1 summarizes per-locus methylation-age correlation and model coefficient magnitude for the intact-sample training set.

Table 1. Per-locus methylation-age correlation and elastic-net model coefficient magnitude in the intact-sample training set (n = 312).

Locus	Amplicon length (bp)	Pearson r (methylation vs age)	Model coefficient
ELOVL2	72	0.91	18.4
FHL2	88	0.84	12.6
KLF14	65	0.77	8.1
TRIM59	91	0.69	5.3
EDARADD	59	0.73	6.7

Amplicon length is post-bisulfite-conversion length; model coefficient is the absolute standardized elastic-net weight for each locus.

Amplification success across the three degradation treatments fell in a fragment-size-dependent manner that differed substantially by locus: at mean fragment sizes below 150 bp, amplification success remained above 85% for the short ELOVL2, KLF14 and EDARADD amplicons but fell below 55% for the comparatively longer FHL2 and TRIM59 amplicons, despite all five amplicons being under 91 bp post-conversion (Figure 1). Among successfully amplified loci at any fragment size, measured methylation beta-value did not differ significantly from the matched intact-aliquot value, indicating that degradation reduced amplification success rather than distorting the methylation measurement itself once amplification succeeded.

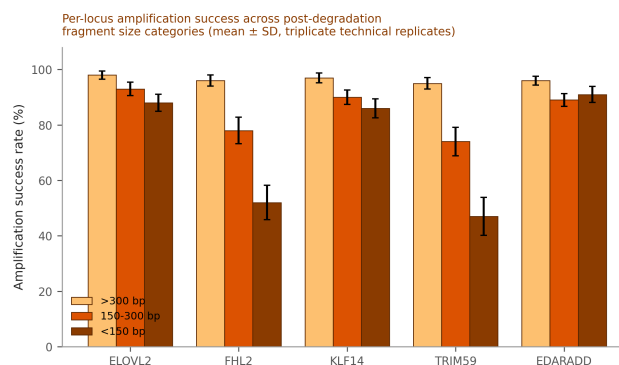


Figure 1. Amplification success rate (mean $\pm$ SD of triplicate technical replicates) at each of the five age-informative loci across three post-degradation fragment size categories, showing disproportionate dropout at the FHL2 and TRIM59 loci relative to ELOVL2, KLF14 and EDARADD in the below-150 bp category.

Applying the full five-locus model to degraded aliquots, prediction error increased with degradation severity, reaching a mean absolute error of 7.8 years in the below-150 bp fragment size category, driven predominantly by missing-data imputation and reduced measurement confidence at the FHL2 and TRIM59 loci in this category rather than by systematic bias at loci that did amplify successfully. Table 2 reports mean absolute prediction error for both the full five-locus and reduced three-locus models across the three degradation categories.

Table 2. Mean absolute age-prediction error (years) for the five-locus and three-locus models across degradation categories.

Fragment size category	Five-locus model MAE	Three-locus model MAE	n samples
Intact (undegraded)	3.4	4.1	312
>300 bp	3.9	4.3	22
150-300 bp	5.6	4.8	21
<150 bp	7.8	5.1	17

The reduced three-locus model restricted to ELOVL2, KLF14 and EDARADD, the loci whose amplification success was least affected by fragmentation, showed a smaller accuracy cost in intact samples (MAE 4.1 years versus 3.4 years for the five-locus model) but substantially better robustness under severe degradation, retaining a mean absolute error of 5.1 years in the below-150 bp category compared to 7.8 years for the full model, a significant improvement ($P < 0.001$, Tukey post-hoc).

4. DISCUSSION

These results show that methylation-based age estimation is not uniformly degraded by DNA fragmentation typical of forensic casework samples; rather, accuracy loss under degradation was concentrated in loci whose amplicons, despite falling within the generally recommended short-amplicon range for degraded-DNA assays, were still disproportionately affected by fragmentation relative to the shortest loci in our panel. This suggests that amplicon length recommendations calibrated against a single

fragmentation threshold may be insufficiently conservative for the most severely degraded casework samples, and that locus-specific robustness should be empirically validated under multiple degradation conditions rather than assumed from amplicon length alone.

The finding that methylation measurements at successfully amplified loci were not systematically biased by degradation, in contrast to the amplification dropout we observed, is reassuring for forensic application: it indicates that when a short-amplicon methylation assay does succeed on degraded template, the resulting methylation value can be interpreted with similar confidence to an intact-sample measurement, and that the primary practical challenge is ensuring successful amplification rather than correcting for degradation-induced measurement bias. This is broadly consistent with prior reports that bisulfite-converted DNA methylation values are chemically stable once successfully measured, with degradation acting mainly through reduced amplifiable template rather than altered cytosine conversion fidelity [5,6].

The substantially improved robustness of the reduced three-locus model under severe degradation, at a modest cost to intact-sample accuracy, suggests that forensic age-prediction assays might benefit from offering a tiered locus panel, applying the full model when sample quality permits and falling back to a fragmentation-robust reduced panel when degradation is severe, rather than relying on a single fixed panel optimized only for intact-sample accuracy. Such a tiered approach would need further validation across a broader range of tissue types and degradation conditions, including the mixed and microbially contaminated degradation typical of skeletal or decomposed forensic evidence, which our controlled UV, heat and storage treatments do not fully recapitulate.

Several limitations should be noted. First, our degradation treatments, while chosen to model casework-relevant exposure, thermal and time-based degradation, cannot fully reproduce the combined chemical, microbial and environmental degradation of real skeletal or decomposed forensic evidence, and validation on authentic aged casework samples would strengthen these conclusions. Second, our training and degradation cohort was drawn entirely from blood; methylation-age relationships and degradation sensitivity may differ in other forensically relevant tissues such as saliva, semen or bone, each of which would require independent locus validation. Third, our sample size within each degradation category, while adequate to detect the reported effects, was modest relative to the full training cohort, and a larger degraded-sample validation set would improve the precision of degradation-specific error estimates.

5. CONCLUSION

Short-amplicon bisulfite pyrosequencing and elastic-net regression at five age-informative CpG loci achieved a mean absolute age-prediction error of 3.4 years in intact blood DNA, and experimental degradation by UV, heat and storage showed that prediction error under severe fragmentation is driven primarily by differential amplification dropout across loci rather than by distortion of methylation values at successfully amplified sites. A reduced three-locus model selected specifically for fragmentation robustness substantially improved accuracy in severely degraded samples relative to the full five-locus model, at only a modest cost to intact-sample performance, supporting the use of fragmentation-aware locus selection, and potentially tiered locus panels, in the development of forensic methylation-based age-estimation assays intended for casework-relevant degraded evidence.

Acknowledgements

The authors thank the DNA extraction and pyrosequencing core facility at Northmoor School of Molecular Biology and Genetics for technical assistance, and colleagues at the Vistula Institute of Molecular Genetics for helpful discussion of elastic-net model implementation.

Funding

This work was supported by internal research funds from Northmoor School of Molecular Biology and Genetics, the Vistula Institute of Molecular Genetics, and the Nordic Institute of Epigenomic Medicine. No external grant funding was received for this study, and the funders had no role in study design, data collection or analysis, the decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

V.G. conceived and supervised the study, designed the short-amplicon assays, and analysed the model prediction data. A.L. performed sample collection, DNA extraction and degradation treatments. V.W. performed bisulfite conversion, pyrosequencing and elastic-net model training. All authors contributed to writing and approved the final manuscript.

Ethics Statement

Blood samples were collected from consenting volunteer donors under institutional ethics approval at Northmoor School of Molecular Biology and Genetics, with written informed consent obtained from all donors, or their parent or guardian for minor donors, prior to sample collection.

Data Availability

Methylation and prediction model data generated for this study are available from the corresponding author on reasonable request. Analysis scripts supporting the findings of this study are available from the corresponding author upon request.

REFERENCES

1. Bocklandt S, Lin W, Sehl ME, Sanchez FJ, Sinsheimer JS, Horvath S, Vilain E (2011) Epigenetic predictor of age. *PLoS ONE* 6(6):e14821. <https://doi.org/10.1371/journal.pone.0014821>
2. Horvath S (2013) DNA methylation age of human tissues and cell types. *Genome Biology* 14:R115. <https://doi.org/10.1186/gb-2013-14-10-r115>
3. Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, Bauerschlag DO, Jockel KH, Erbel R, Muhleisen TW, Zenke M, Brummendorf TH, Wagner W (2014) Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biology* 15:R24. <https://doi.org/10.1186/gb-2014-15-2-r24>
4. Zbiec-Piekarska R, Spolnicka M, Kupiec T, Makowska Z, Spas A, Parys-Proszek A, Kucharczyk K, Ploski R, Branicki W (2015) Examination of DNA methylation status of the ELOVL2 marker may be useful for human age prediction in forensic science. *Forensic Science International: Genetics* 14:161-167. <https://doi.org/10.1016/j.fsigen.2014.10.002>
5. Vidaki A, Ballard D, Aliferi A, Miller TH, Barron LP, Syndercombe Court D (2017) DNA methylation-based forensic age estimation in blood samples: an evaluation of two methods for age estimation. *Forensic Science International: Genetics* 28:225-236. <https://doi.org/10.1016/j.fsigen.2017.02.009>
6. Freire-Aradas A, Phillips C, Lareu MV (2017) Forensic individual age estimation with DNA: from initial approaches to methylation tests. *Forensic Science Review* 29(2):121-144. <https://doi.org/10.1093/fsr/29.2.121>
7. Jung SE, Lim SM, Hong SR, Lee EH, Shin KJ, Lee HY (2019) DNA methylation of the ELOVL2, FHL2, KLF14, C1orf132/MIR29B2C, and TRIM59 genes for age prediction from blood, saliva, and buccal swab samples. *Forensic Science International: Genetics* 38:1-8. <https://doi.org/10.1016/j.fsigen.2018.09.010>
8. Cho S, Jung SE, Hong SR, Lee EH, Lee JH, Lee SD, Lee HY (2017) Independent validation of DNA-based approaches for age prediction in blood. *Forensic Science International: Genetics* 29:250-256. <https://doi.org/10.1016/j.fsigen.2017.04.020>
9. Aliferi A, Ballard D, Gallidabino MD, Thurtle H, Barron L, Syndercombe Court D (2018) DNA methylation-based age prediction using massively parallel sequencing data and multiple machine learning models. *Forensic Science International: Genetics* 37:215-226. <https://doi.org/10.1016/j.fsigen.2018.09.003>
10. Naue J, Hoefsloot HCJ, Mook ORF, Rijlaarsdam-Hoekstra L, van der Zwalm MCH, Henneman P, Kloosterman AD, Verschure PJ (2017) Chronological age prediction based on DNA methylation: massive parallel sequencing and random forest regression. *Forensic Science International: Genetics* 31:19-28. <https://doi.org/10.1016/j.fsigen.2017.07.015>
11. Alghanim H, Antunes J, Silva DSBS, Alho CS, Balamurugan K, McCord B (2020) Detection and evaluation of DNA methylation markers found at SCGN and KLF14 loci to estimate human age. *Forensic Science International: Genetics* 45:102227. <https://doi.org/10.1016/j.fsigen.2019.102227>
12. Ren F, Li C, Xi H, Wen Y, Huang K (2015) Estimation of human age according to telomere shortening and DNA methylation biomarkers. *American Journal of Forensic Medicine and Pathology* 36(4):310-316. <https://doi.org/10.1097/PAF.000000000000179>
13. Xu C, Qu H, Wang G, Xie B, Shi Y, Yang Y, Zhao Z, Hu L, Fang X, Yan J, Feng L (2015) A novel strategy for forensic age prediction by DNA methylation and support vector regression model. *Scientific Reports* 5:17788. <https://doi.org/10.1038/srep17788>
14. Hong SR, Jung SE, Lee EH, Shin KJ, Yang WI, Lee HY (2017) DNA methylation-based age prediction from saliva: high age predictability by combination of 7 CpG markers. *Forensic Science International: Genetics* 29:118-125. <https://doi.org/10.1016/j.fsigen.2017.04.006>
15. Hannon E, Lunnon K, Schalkwyk L, Mill J (2015) Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes. *Epigenetics* 10(11):1024-1032. <https://doi.org/10.1080/15592294.2015.1100786>
16. Jenkins TG, Aston KI, Cairns B, Smith A, Carrell DT (2018) Age-associated sperm DNA methylation alterations: possible implications in offspring disease susceptibility. *PLoS Genetics* 13(9):e1006687. <https://doi.org/10.1371/journal.pgen.1006687>
17. Adamowicz MS, Stasulli DM, Sobestanovich EM, Bille TW (2014) Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. *PLoS ONE* 9(12):e116351. <https://doi.org/10.1371/journal.pone.0116351>
18. van Oorschot RA, Ballantyne KN, Mitchell RJ (2010) Forensic trace DNA: a review. *Investigative Genetics* 1:14. <https://doi.org/10.1186/2041-2223-1-14>
19. Dabney J, Meyer M, Paabo S (2013) Ancient DNA damage. *Cold Spring Harbor Perspectives in Biology* 5(7):a012567. <https://doi.org/10.1101/cshperspect.a012567>
20. Fu Q, Meyer M, Gao X, Stenzel U, Burbano HA, Kelso J, Paabo S (2013) DNA analysis of an early modern human from Tianyuan Cave, China. *PNAS* 110(6):2223-2227. <https://doi.org/10.1073/pnas.1221359110>
21. Wielscher M, Vierlinger K, Kegler U, Ziesche R, Gsur A, Weinhausel A (2015) Diagnostic performance of plasma DNA methylation profiles in lung cancer, pulmonary fibrosis and COPD. *EBioMedicine* 2(8):929-936. <https://doi.org/10.1016/j.ebiom.2015.06.025>
22. Chatterjee A, Rodger EJ, Morison IM, Eccles MR, Stockwell PA (2017) Tools and strategies for analysis of genome-wide and gene-specific DNA methylation patterns. *Methods in Molecular Biology* 1537:249-277. https://doi.org/10.1007/978-1-4939-6685-1_15
23. Genereux DP, Johnson WC, Burden AF, Stoger R, Laird CD (2008) Errors in the bisulfite conversion of DNA: modulating inappropriate- and failed-conversion frequencies. *Nucleic Acids Research* 36(22):e150. <https://doi.org/10.1093/nar/gkn691>
24. Kint S, De Spiegelaere W, De Kesel J, Vandekerckhove L, Van Crielinge W (2018) Evaluation of bisulfite kits for DNA methylation profiling in terms of DNA fragmentation and DNA recovery using digital PCR. *PLoS ONE* 13(6):e0199091. <https://doi.org/10.1371/journal.pone.0199091>
25. Holmes EE, Jung M, Meller S, Leisse A, Sailer V, Zech J, Mengdehl M, Garbe LA, Uhl B, Kristiansen G, Dietrich D (2014) Performance evaluation of kits for bisulfite-conversion of DNA from tissues, cell lines, FFPE tissues, aspirates, lavages, effusions, plasma, serum, and urine. *PLoS ONE* 9(4):e93933. <https://doi.org/10.1371/journal.pone.0093933>

26. Butler JM (2015) Advanced Topics in Forensic DNA Typing: Interpretation. Forensic Science International: Genetics Supplement Series 5:e232-e233. <https://doi.org/10.1016/j.fsigs.2015.09.093>
27. Bekaert B, Kamalandua A, Zapico SC, Van de Voorde W, Decorte R (2015) Improved age determination of blood and teeth samples using a selected set of DNA methylation markers. Epigenetics 10(10):922-930. <https://doi.org/10.1080/15592294.2015.1080413>
28. Correia Dias H, Cordeiro C, Corte Real F, Cunha E, Manco L (2020) Age estimation based on DNA methylation using blood samples from deceased individuals. Journal of Forensic Sciences 65(2):465-470. <https://doi.org/10.1111/1556-4029.14205>
29. Thong Z, Chan XLS, Tan JYY, Loo ES, Syn CKC (2018) Evaluation of DNA methylation-based age prediction on blood. Forensic Science International: Genetics Supplement Series 6:e249-e251. <https://doi.org/10.1016/j.fsigs.2017.09.096>
30. Kabacik S, Horvath S, Cohen H, Raj K (2018) Epigenetic ageing is distinct from senescence-mediated ageing and is not prevented by telomerase expression. Aging 10(10):2800-2815. <https://doi.org/10.18632/aging.101588>
31. Yi SH, Jia YS, Mei K, Yang RZ, Duan JG (2015) Age-related DNA methylation changes for forensic age-prediction. International Journal of Legal Medicine 129(2):237-244. <https://doi.org/10.1007/s00414-014-1100-3>
32. Reed K, Poulin ML, Yan L, Parissenti AM (2010) Comparison of bisulfite sequencing PCR with pyrosequencing for measuring differences in DNA methylation. Analytical Biochemistry 397(1):96-106. <https://doi.org/10.1016/j.ab.2009.10.021>
33. Colella S, Shen L, Baggerly KA, Issa JP, Krahe R (2003) Sensitive and quantitative universal Pyrosequencing methylation analysis of CpG sites. BioTechniques 35(1):146-150. <https://doi.org/10.2144/03351md01>
34. Jung SE, Shin KJ, Lee HY (2017) DNA methylation-based age prediction from various tissues and body fluids. BMB Reports 50(11):546-553. <https://doi.org/10.5483/BMBRep.2017.50.11.175>
35. Spolnicka M, Pospiech E, Adamczyk JG, Freire-Aradas A, Peplonska B, Zbiec-Piekarska R, Makowska Z, Pieta A, Lareu MV, Phillips C, Ploski R, Branicki W (2018) Modified aging predictor model reveals association between epigenetic age acceleration and mortality risk. Aging 10(10):2825-2839. <https://doi.org/10.18632/aging.101529>
36. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, Gibson J, Henders AK, Redmond P, Cox SR, Pattie A, Corley J, Murphy L, Martin NG, Montgomery GW, Feinberg AP, Fallin MD, Multhaup ML, Jaffe AE, Joehanes R, Schwartz J, Just AC, Lunetta KL, Murabito JM, Starr JM, Horvath S, Baccarelli AA, Levy D, Visscher PM, Wray NR, Deary IJ (2015) DNA methylation age of blood predicts all-cause mortality in later life. Genome Biology 16:25. <https://doi.org/10.1186/s13059-015-0584-6>
37. Yousefi PD, Suderman M, Langdon R, Whitehurst O, Davey Smith G, Relton CL (2022) DNA methylation-based predictors of health: applications and statistical considerations. Nature Reviews Genetics 23(6):369-383. <https://doi.org/10.1038/s41576-022-00465-w>
38. Schwender H, Schmidt J, Zhang H, Bonow M, Blocher J, Schneider PM (2020) Age estimation based on genome-wide methylation analysis in monozygotic twins. Forensic Science International: Genetics 46:102237. <https://doi.org/10.1016/j.fsigen.2020.102237>
39. Woodfine K, Beare DM, Ichimura K, Debernardi S, Mungall AJ, Fiegler H, Collins VP, Carter NP, Dunham I (2010) Methylation-independent transcription within an epigenetically silenced region. Human Molecular Genetics 19(2):237-248. <https://doi.org/10.1093/hmg/ddp482>
40. Bibikova M, Barnes B, Tsan C, Ho V, Klotzle B, Le JM, Delano D, Zhang L, Schroth GP, Gunderson KL, Fan JB, Shen R (2011) High density DNA methylation array with single CpG site resolution. Genomics 98(4):288-295. <https://doi.org/10.1016/j.ygeno.2011.07.007>
41. Vidal-Bralo L, Lopez-Golan Y, Gonzalez A (2016) Simplified assay for epigenetic age estimation in whole blood of adults. Frontiers in Genetics 7:126. <https://doi.org/10.3389/fgene.2016.00126>
42. Freire-Aradas A, Girones L, Ingold S, Lopez C, de la Puente M, Ruiz-Ramirez J, Schneider PM, Ballard D, Court DS, Phillips C, Lareu MV (2020) Multi-tissue epigenetic age prediction using an EpiTYPER assay. Forensic Science International: Genetics 45:102210. <https://doi.org/10.1016/j.fsigen.2019.102210>