A Robust and Integrated Framework for Cross-platform Adaptation of Epigenetic Clocks in Cell-free DNA Sequencing

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Abstract

Epigenetic clocks optimized for array-based profiling exhibit significant cross-platform incompatibility when applied to high-throughput sequencing (HTS) -based cell-free DNA (cfDNA) methylation data. Existing adaptation strategies are frequently non-agnostic or introduce technical artifacts, while a scarcity of paired benchmarks hinders methodological consensus for cfDNA. Through systematic benchmarking using sophisticated paired array and HTS technical replicates, we recovered the methodological requirements to bridge this “platform

gap". The framework establishes optimal parameters, including 10× mean target depth, L2-heavy elastic net regularization, and targeted beta-value imputation. We also demonstrate that transfer learning effectively mitigates platform-specific biases, significantly enhancing clock performance across independent aging and disease cohorts. Our validated framework enables the robust adaptation of legacy clocks for HTS-based cfDNA analysis, providing a standardized pipeline for adapting legacy biomarkers to the HTS domain without sacrificing their established biological interpretations or requiring access to proprietary model architectures.

Keywords: Epigenetic Aging Clocks; cfDNA Methylation; High-throughput Sequencing; Machine Learning; Model Adaptation Framework.

Main

Recent studies increasingly advocate the use of biological age (BA), rather than chronological age (CA; time elapsed since birth), as a more informative indicator of an individual's physiological aging state. BA is estimated from biomarkers of aging (BoAs) through mathematical models known as aging clocks [1–3]. Among diverse BoAs, epigenetic clocks—based on the methylome (DNA methylation profiles)—have distinguished themselves by their outstanding predictive power [4–8]. achieving high accuracy [7–12]. Due to their superior performance, epigenetic clocks are now widely used in health monitoring, age-related disease assessment, and longevity interventions studies [2,6,13–15].

The circulating cell-free DNA (cfDNA or ccfDNA), primarily found in body fluids, enabled minimally invasive testing for early cancer detection and disease monitoring [16–19]. Its utility in epigenetic aging clocks is now being actively explored [20–22]. Different from genomic DNA (gDNA) methylation profiling which adopts array-based platforms such as Illumina HumanMethylation450 BeadChip and Illumina MethylationEPIC BeadChip, cfDNA methylation profiling often rely on high-throughput sequencing (HTS) technologies to overcome technical difficulties faced by these array-based platforms. These difficulties includes sensitivity to low-abundance methylation changes, narrow dynamic range, inability to capture intra-sample methylation heterogeneity [23] and high tissue-of-origin heterogeneity [19], low fragment sizes [24] and low yield (typically 0.1–10 ng/sample, compared to the recommended 200 ng input for arrays [25]),

considering that input amount reduction methods (such as preamplification) may have drawbacks of beta-value distortion [26]. HTS, particularly targeted sequencing with hybridization probe capture, is candidate to overcome these limitations [27–30], hence is in strong coupling with cfDNA methylation profiling [31,32].

Despite their predictive utility, most extant epigenetic clocks are optimized for array-based gDNA profiling, resulting in a significant lack of platform readiness when ported to HTS [4,23,28]. This incompatibility is primarily rooted in divergent data architectures: microarrays generate continuous beta-values based on fluorescent signal intensities, whereas HTS yields discrete, count-based methylation ratios. Consequently, while array-derived variances typically follow predictable distributions, HTS data are inherently heteroscedastic, with variance structures driven stochastically by local sequencing depth. Secondly, array-trained models are restricted to fixed, curated CpG subsets and cannot leverage the expansive genomic landscapes accessible via HTS platforms [23]. These limitations leave a significant technological gap between HTS-based cfDNA epigenetic applications and legacy epigenetic clocks.

To address the platform gap, two primary methodological paradigms have emerged. First, *de novo* HTS models, such as BS-clock and timeseqage [23,33], are trained exclusively on sequencing datasets to bypass platform-specific nuances. However, these models typically identify novel, HTS-specific feature sets rather than preserving the biologically validated biomarker interpretations associated with legacy array-trained clocks. The second paradigm employs adaptation strategies to harmonize array-trained models with HTS data. Data-centric approaches, including batch correction [34–36] and domain adaptation [37,38], can introduce technical artifacts [39] or rely on distributional assumptions that fail to capture the high stochasticity and depth-dependent variance inherent in cfDNA sequencing. Alternatively, model-transferring frameworks like Transfer Elastic Net [40] are fundamentally non-agnostic, requiring architectural transparency, explicit coefficient access, or even original training data, with no guaranteed availability. While recent robust feature selection methods attempt to identify consensus features across platforms [41], their reliance on cell-type-specific tuning is largely incompatible with the intrinsic cell type heterogeneity of cfDNA. Furthermore, the scarcity of systematic benchmarks featuring paired array/HTS technical replicates has hindered the establishment of standardized experimental and bioinformatic protocols. This methodological abscence is particularly pronounced for cfDNA,

where platform-specific biases can easily confound biological aging signals. Consequently, there remains an urgent need for a systematic, model-agnostic framework capable of benchmarking and adapting legacy array-trained clocks for high-precision, HTS-based cfDNA analysis.

To systematically address the platform gap, we tested a controlled benchmarking framework using a matched dataset of cfDNA and gDNA profiled by both HTS and array-based methylation technologies. This design enabled quantitative assessment of clock accuracy, precision, and reproducibility across DNA types and platforms, and allowed us to decompose and correct sources of variance, including coverage depth, CpG missingness and platform biases. Through structured evaluation of these experimental and computational factors, we identified recurrent bias patterns and failure modes arising from cross-platform transferring of epigenetic clocks. Based on these insights, we developed and validated an end-to-end adaptation pipeline integrating experimental design guidance, data harmonization, feature recalibration, and machine-learning-based correction. Together, this work provides a systematic benchmarking resource and a validated methodological framework for robust application of epigenetic clocks to HTS-derived cfDNA data.

Results

Systematic benchmarking of epigenetic profiling across array and HTS platforms

We benchmarked four profiling technologies, Illumina MSA and EPICv2 (arrays) alongside iGeneTech Galaxy and Twist (HTS), using the SRRSH-24 cohort (24 participants; 6 datasets across gDNA and cfDNA with technical replicates; Fig. 1a). The remaining 141 participants (SRRSH-141) were reserved for independent validation.

Platform feature coverage and coverage drift

While HTS capture panels provide significantly larger total marker sets (Galaxy: ~2.0M; Twist: ~3.98M) than arrays (MSA: ~270K; EPICv2: ~935K), this genomic breadth did not translate to higher clock-specific coverage (Fig. S1a-b). Additionally, empirical assessment revealed a “coverage drift” between observed and reference (as calculated from manufacturer’s specifications) CpG coverage of clocks: HTS platforms exceeded design specifications

potentially due to off-target reads (Galaxy: +4.8%; Twist: +1.06%), whereas arrays underperformed potentially due to stringent QC filtering (MSA: -2.5%; EPICv2: -3.0%; Fig. S1b). Despite these discrepancies, HTS datasets maintained ≥ 70% feature coverage, suggesting that while HTS is over-engineered for legacy clocks, it ensures headroom for future *de novo* model development.

CpG-level reproducibility and technical noise

Technical precision was evaluated using 136,437 "Method-shared CpGs" which are observed in all datasets. PCA revealed a systematic platform bias separating HTS-based and array-based methods (Fig. 1c-d). Notably, HTS platforms exhibited significantly lower Intraclass Correlation Coefficients (ICCs) [42] compared to arrays ($P < 1\times10^{-5}$; Fig. 1e). Analysis of the joint distribution showed that low-stability sites (ICC ≤ 0.3) were enriched at beta-value extremes (0 or 1), a burden significantly higher in HTS datasets (30.8-35.7% of total CpGs) than in array-based datasets (60.5-79.9%) (Fig. S2). Aligning with previous studies [12,28], the beta-values close to 0 or 1 exhibit unreliability for their associations with low ICCs. This increased stochastic noise in HTS translated to reduced age-prediction reproducibility, with median ICCs of 0.721 compared to 0.950 for arrays (Fig. 1g). Inter-dataset beta-value correlations further confirmed superior technical consistency within array-based methodologies (Fig. 1f).

Clock categorization and cross-platform robustness

We categorized 53 array-based clocks based on feature coverage and predictive stability. Six models, including five PCA-preprocessed clocks and zhangblup, were designated "Hi-Repro" for superior reproducibility (ICC ≥ 0.9; median RD 0.17-3.81; Fig. S3, S4). Additionally, 20 models were classified as "Hi-Cov-All" (≥ 80% coverage across all platforms) and 12 as "Hi-Cov-nonMSA" (same criteria but except for MSA). Notably, Hi-Repro clocks demonstrated the highest cross-platform correlation in gDNA but showed performance declines in cfDNA (Fig. 1h, S5), likely due to high fragmentation and multi-organ origins reducing the signal-to-noise ratio. Notably, only 17 clocks, including all Hi-Repro clocks, achieved Pearson's R ≥ 0.7 across all platforms, with pcgrimage demonstrating the highest consistency.

Impact of feature selection and platform divergence on predictive stability

To determine if biological feature selection confers cross-platform technical resilience, we benchmarked 2,727 "Clock-shared CpGs" common to the Hi-Cov-All and Hi-Repro subsets. While this curated subset achieved higher global consistency (mean Pearson's R = 0.97; Fig. S1h) than the broader Method-shared CpGs (mean Pearson's R = 0.95; Fig. 1f), it also carried over platform-specific noise (Fig. S1e-f). Array-based platforms maintained superior consistency (Fig. S1c) and reproducibility (Fig. S1g) over HTS platforms. This suggests that while feature selection effectively filters out biological noise, it cannot mitigate the fundamental technical stochasticity inherent in HTS profiling, necessitating further computational adaptation.

Benchmarking the Hi-Repro, Hi-Cov-All, and Hi-Cov-nonMSA subsets across SRRSH-24 datasets revealed a significant accuracy penalty for sequencing-based cfDNA. The gDNA_EPICv2 array served as the technical reference, yielding the highest accuracy (MAE = 2.03-23.08, median 7.39; Fig. 2a&d, S6). Conversely, cfDNA profiles exhibited higher MAEs (5.26-18.75, median 10.83). and lower correlations with CA (0.40-0.92, median 0.76) than gDNA (MAE = 5.02-22.92, median 7.80; R = 0.44-0.96, median 0.80) (Fig. 2c-d). We identified a strong correlation between prediction ICCs and accuracy metrics (Fig. 2g-h), confirming that reproducibility share confounding factors with clocks accuracy in HTS environments. Among the evaluated models, zhangblup demonstrated the greatest cross-platform resilience (MAE ≤ 5.2 years), though its performance collapsed in the gDNA_MSA dataset due to critical (25%) feature coverage deficits (Fig. 2a, S1b, S7). Furthermore, a reciprocal accuracy and reproducibility decline was observed with HTS-trained clocks even applied to HTS-based datasets (Fig. 2b). More intriguingly, using original models, array-trained clocks (hannum, etc.) outperformed HTS-trained models (timeseqage) on an array dataset (GSE245628), while the pattern reversed on an HTS-based dataset (GSE232346) (Fig. 2e-f) . These results indicate that clocks are inherently tuned to dataset or platform-specific patterns [20,23,43], reinforcing the need for targeted adaptation strategies even for HTS-based clocks. In this study, further benchmarking and adaptation focus on array-trained clocks for their better standardized feature set and more versatile clinical applications beyond CA predictions.

Optimizing sequencing depth for HTS-based epigenetic clock applications

We investigated the impact of sequencing depth on beta-value reproducibility, focusing on the stochasticity of observed counts and ratio approximation errors. Benchmarking and randomized downsampling of the SRRSH-141 cohort revealed that MAE and RD stabilize at a minimum mean target depth of 10× [44], and ideally 20× for stability (Fig. 3a-d, S8), which lands in the same range with the guidelines for differential methylation region calling in whole genome bisulfite sequencing [45]. This 10× standard was met by all HTS datasets generated in this study (Fig. S9).

Feature density and L2 regularization enhance reproducibility

Analysis of 20 HCRL clocks confirmed a robust negative correlation between CpG feature count and RD across 11 datasets (Table 1, Fig. S10), with high feature density and PCA-preprocessed models exhibiting superior resilience, reconfirming previous study findings [28,46]. To isolate the drivers of this stability, we systematically tuned elastic net hyperparameters: regularization strength (α) and the L1/L2 mixing parameter (λ). A critical α threshold (~0.01) was identified, beyond which accuracy sharply degraded (Fig. 3e). Within the stable range ($\alpha \leq 0.01$), the correlation between feature count and RD was reaffirmed (Fig. 3f), and sensitivity analysis showed that λ is a significantly more potent determinant of reproducibility (R^2 = 0.67-0.78, $P < 10^{-32}$) than α (R^2 = 0.05-0.21; Fig. 3g-h). The strong positive correlation between λ and RD indicates that skewing models toward an L2 (Ridge) penalty, which retains a broad distribution of small-effect coefficients to minimize the impact of stochastic effects in HTS data. Consequently, L2-heavy architectures are essential for achieving reproducible predictions in sequencing-based applications.

Clock accuracy improvement with the DF-IM-TL adaptation pipeline

To mitigate CpG-level stochasticity, we developed a three-stage HTS data processing pipeline: depth filtering (DF), imputation (IM), and transfer learning (TL).

Preprocessing of stochastic noise mitigation (DF and IM). The DF stage screens out beta-values below specified depth thresholds, which are subsequently handled by IM. Benchmarking eight HTS datasets revealed that technical artifacts at distribution extremes (0 or 1) comprise 18.4%-61.3% of total CpGs, overlapping with 2.0%-34.7% of features in 20 HCRL clocks (Fig. 4a, S11-S14). The IM step significantly enhanced model reproducibility. K-nearest neighbor

(KNN) imputation [47] consistently reduced RD across all platforms, yielding improvements of 0.7-2.1 years in cfDNA and 0.7-1.1 years in gDNA (Fig. 4b). While mean and median imputation achieved comparable RD gains, they introduced significant MAE deterioration (1-5 years) in cfDNA samples, a trade-off absent in gDNA (Fig. 4c). Meanwhile, other common imputation methods such as mean, median had RD improvements and MAE trade-offs similar to KNN, with 0-filling as an exception (Fig. S15), potentially because heuristic imputation methods may amplify the stochastic effects related to cell type heterogeneity such in cfDNA samples. Notably, zhangblup emerged as the most resilient architecture, showing consistent improvements across all datasets (Fig. S16).

TL for cross-platform alignment. To align array-derived clocks with HTS data, we utilized model distillation (Teacher-Student TL). This model-agnostic approach uses an array-trained "teacher model" to guide an HTS-specific "student". To reduce overfitting, we also incorporated PCA-based dimensionality reduction. Five-fold cross-validation confirmed that distillation significantly reduces prediction error across six 1st and 2nd generation representative clocks and stabilizes reproducibility (Fig. 4d-e). Distillation effectively normalized RD across platforms, stabilizing high-variance models like dnamphenoage. Crucially, this recalibration selectively mitigated platform-specific variance while remaining sensitive to DNA source; performance gains were maximized when adaptation and testing datasets shared the same DNA type (Fig. 4f).

Systematic validation and functional benchmarking of the DF-IM-TL pipeline

We evaluated eight serial combinations of the DF-IM-TL pipeline. In the Buccal cohort (N=100), TL-inclusive strategies universally outperformed others, with TL-only as the best providing a median MAE reduction of 10.3 years and a median CA correlation improvement of 0.15 (Fig. 5a-b, S17). Further validation in the SRRSH-141 cohort using PhenoAge as a clinical reference confirmed the pipeline's biological sensitivity. The IM+TL strategy achieved the highest alignment with clinical aging metrics, reducing MAE by a median of 6.6 years and improving Pearson's R by 0.17 (Fig. 5c, S18-S20). Notably, the DF-IM-TL pipeline outperformed the existing MAPLE framework in MAE while maintaining comparable reproducibility (Fig. 5a, S18) both using its pre-trained model or trained from scratch, while the latter one is designed to handle cross-platform biases [48]. The DF-IM-TL pipeline also outperformed other three commonly used adaptation algorithms (quantile mapping, ComBat, and correlation alignment

(CORAL)) in MAE each attempt to align the HTS datasets to gDNA_EPICv2 before prediction with original HCRL clocks. The results in Fig. 5d suggest DF-IM-TL's highly specialized efficacy in correcting HTS platform biases in epigenetic age prediction.

As proof-of-concept for disease sensitivity, we also benchmarked the IM+TL framework's utility for disease detection using an amyotrophic lateral sclerosis (ALS) cfDNA dataset (N=24; GSE164600), where epigenetic delta-age (difference between BA and CA) was a proven discriminative biomarker in recent studies [49,50]. Adaptation significantly increased the Jensen-Shannon Divergence (JSD) between ALS and control samples (Fig. 6a-b). This enhanced distributional separation consequently improved diagnostic performance. An SVM classifier utilizing delta-age from the top four adapted clocks achieved a maximum AUC improvement of 0.125 over non-adapted inputs (Fig. 6c-d). These results demonstrate that the pipeline successfully recovers biological signals from HTS technical noise, enabling high-performance disease-centric liquid biopsy applications.

Discussion

Successful translation of array-trained epigenetic clocks to HTS platforms requires rigorous quantifications of the platform biases between traditional arrays-based and HTS-based profiles. In this study, we generated the SRRSH-24 dataset to address the current scarcity of paired cross-platform datasets, expanding upon previous work that focused primarily on buccal genomic DNA (gDNA) [28]. This dataset with technical replicates across multiple DNA types and profiling technologies enables systematic assessments of measurement reproducibility.

Our benchmarking reveals that HTS technology used as methylation profiling method may introduce significant technical stochasticity, manifested as a significant loss of ICC at CpG level in comparison with array platforms. Crucially, our findings suggest that this limited technical reproducibility is a prerequisite for epigenetic clock reproducibility; however, it is not an ensurance of biological reproducibility as suggested by a previous research [46]. Hence, we suggest that future developed of HTS-ready epigenetic clocks must take this stochasticity into consideration and prioritize RD alongside traditional accuracy metrics such as MAE. Using both

metrics enables disentangling the assessment of model robustness against technological and biological variances.

A generic epigenetic clock is ideally robust against platform-specific noises, which lean towards model architectures that are optimized for stability. Our analysis demonstrates that models with high-density feature sets outperform those with sparse feature sets. This is likely due to the retaining of small effects broadly distributed across numerous CpGs, effectively averaging out the stochastic variances at individual CpG sites. For linear models, this translates to the prioritizing of L2 (Ridge) regularization over L1 (LASSO) in HTS applications. The preference of L2 regularization in elastic net clocks [51] is verified in this study, aligning with empirical approaches with L2-like shrinkage estimator such as PCA-preprocessed clocks [12] and zhangblup [10] (see Supplementary Discussion). As a results, these clocks achieved mean RD as low as 1.6 years across HTS-based datasets (Fig. S3, Fig. S4a) in this study. Overall, we recommend L2 regularization as a practical and promising strategy for improving reproducibility of linear-architecture clocks

To mitigate the technical limitations of HTS-based methylation profiling methods, we propose a three-stage adaptation pipeline using DF, IM and TL to standardize HTS-based epigenetic analysis. Firstly, based on both statistical and downsampling benchmarks, we formalize a recommendation for a minimum 10× mean target depth threshold (with 20× being more ideal) for predictive stability. These guidelines align HTS-based epigenetic clock applications with established standards for whole genome bisulfite sequencing [44,45]. Secondly, while imputing unreliable beta-values consistently improves reproducibility, it can introduce accuracy bias, particularly in cfDNA samples. This trade-off in cfDNA samples likely stems from its higher cellular heterogeneity compared to gDNA [30], leading to low pan-tissue generalizability [23]. We hypothesize that incorporating cell-type deconvolution as a pre-processing step prior to imputation could mitigate these trade-offs by providing more biologically informed priors for missing values [30,52]. Thirdly, our testing identifies distillation as a model-agnostic recalibration tool, correcting systematic distributional shifts between platforms without requiring access to original training data. Our benchmarking results illustrate TL to be the most critical component for adapting legacy array-trained clocks to HTS methylation data in comparison to DF and IM. Unlike de novo training approaches such as timeseqage and BS-clock [23,33], using TL can retain

the biological interpretability of the original epigenetic clocks. Notably, as tested in this study, clocks designed to address cross-platform robustness (e.g. MAPLE [48]) cannot guarantee cross-platform performance. Hence, model-agnostic adaptation methods (such as distillation) are invaluable in epigenetic clock adaptation without the requirement of retraining the clock.

Another key finding of this benchmark is the interchangeability of capture panels. Once a student model is trained via distillation, it can be reliably applied across different HTS platforms (e.g., Twist, Galaxy) as long as the DNA type (gDNA vs. cfDNA) remains consistent. This suggests that the adaptation pipeline corrects for generalized sequencing-related variation rather than panel-specific artifacts.

Furthermore, the validation against clinical PhenoAge and ALS datasets confirms that the DF-IM-TL pipeline preserves biological sensitivity. The adaptation process does not only align predictions with chronological age; it enhances the biological signal required to differentiate diseased states from healthy controls, as evidenced by the increased JSD and improved AUC in ALS classification.

While this study establishes a robust methodological foundation, certain limitations remain. The dataset size and cross-sectional design preclude assessments of sex- or ethnicity-related effects and temporal methylation dynamics. Future work should expand into longitudinal cohorts to evaluate how adaptation influences sensitivity to age-related interventions. While linear models were prioritized for their interpretability and high performance in high-dimensional spaces, the true biological relationship between methylation and aging is not linear-guaranteed. Therefore, though linear epigenetic clocks can already achieve high accuracy [10,12,53], future research into advanced clocks and domain adaptation methods may further close the gap [36,54–56]. Additionally, the challenge of tissue-of-origin heterogeneity in cfDNA remains a critical variable that warrants further investigation to optimize imputation and recalibration strategies. Finally, and recently developed correction method for array platforms (e.g. ComBat-met [35]) still calls for test and verification in HTS environment.

This study provides a comprehensive methodological framework for the cross-platform application of epigenetic clocks. By contributing the SRRSH-24 benchmarking resource, formalizing depth requirements, and validating the DF-IM-TL pipeline, we offer a clear path towards HTS-based aging research. The proposed pipeline framework bridges the extensive

gallery of array-derived epigenetic clocks to be reliably deployed in the emerging HTS-based epigenetic aging and liquid biopsy diagnostics.

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Data availability

The de-identified data generated in this study, including processed methylation matrices, sequencing depth matrices and non-sensitive subject metadata are available at zendoro.org under accession 17453844 (DOI: 10.5281/zenodo.17453844). Raw data available at GSA for Human (https://ngdc.cncb.ac.cn/gsa-human/) under accession HRA014554.

Code availability

The code related to this project are publicly accessible in at https://github.com/regen-bio/cfDNA-HTS-Clock-Analysis.git.

Author contributions

C.O. and G.Z. conceived and supervised the study. G.L., C.O., and G.Z. managed the overall project. Cohort management: L.C., X.C., Z.Y., D.T., X.F., X.H., and V.T. organized the cohort, collected human blood samples, and coordinated resources. X.H. and V.T. also provided essential medical and technical support. Data generation: C.O., Y.G., S.J., B.H., and C.T. performed sample collection, DNA extraction, and library preparation. Bioinformatics analysis: J.W. and W.H. developed the bioinformatics pipeline. J.W. preprocessed HTS and methylation array data and performed downsampling analysis. W.H. conducted reproducibility, consistency, and

accuracy analyses. G.L. investigated clock complexity, unreliable beta filtering, transfer learning, and combination strategies. X.Z. contributed to statistical and machine learning analyses, with advisory support from Y.W. Manuscript preparation: G.L., W.H., X.Z., J.W., and C.O. wrote the manuscript. All authors reviewed and approved the final manuscript.

Competing interests

W.H., X.Z., J.W., Y.G., Z.Y., S.J., B.H., D.T., V.T., C.T., X.F., C.O. and G.Z. are affiliated with Regenerative Bio, a global biotech company powered by AI, providing organ-specific aging assessment models and longevity-focused health solutions. Regenerative Bio was the study sponsor. The other authors declare no competing interests.

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Online Methods

The SRRSH cohorts and ethics declaration

The ethics committee of the Sir Run Run Shaw Hospital (SRRSH, affiliated with Zhejiang University School of Medicine) approved this study (approval number 2024-0547). Written informed consent was obtained from all participants.

The recruitment and data collection for all volunteers in the cohort were conducted under strict protocols. The following criteria were applied for recruiting eligible volunteers: (1) healthy adults aged 20 - 84 years; (2) Within the past 5 years the subject has completed at least two routine health evaluations that included complete blood count, urinalysis, fasting plasma glucose, lipid profile, liver-function tests, renal-function tests, and at least one set of imaging studies: abdominal ultrasound (liver, gall-bladder, pancreas, spleen, both kidneys), chest radiograph, thyroid ultrasound, prostate ultrasound (men), uterus and adnexa ultrasound (women). All reported results must be within normal limits and free of any diagnosed disease; (3) no smoking history within 5 years before screening and agreement to remain abstinent from tobacco for 1 year after consent; (4) body-mass index 18.5-28.0 kg/m^2; (5) without acute infection or active inflammation within 14 days before screening; (6) without cancer-related signs or symptoms (e.g., hemoptysis, rectal bleeding, altered bowel habit, hematuria, dysphagia, palpable mass) within 30 days before screening; (7) without previous organ transplantation or any non-autologous bone-marrow or stem-cell transplantation; (8) without blood transfusion within 7 days before screening; (9) without use of therapeutic drugs for any illness (nutritional supplements excluded) within 30 days before screening; (10) without any surgical procedure within 5 years before screening; (11) pregnant or breastfeeding women were excluded.

Methylation array and capture panel selection

Four methylation profiling methods were included in this study, including two array-based methods and two HTS-based methods with different hybridization probe capture panels: Illumina Human Methylation Screening Array (referred to as MSA), Illumina Methylation EPIC v2.0 BeadArray (referred to as EPICv2), targeted sequencing with iGeneTech BisCap CpG Galaxy Panel for capturing (referred to as Galaxy) and targeted sequencing with Twist Human Methylome Panel for capturing (referred to as Twist). EPICv2 was included because it

encompasses the largest CpG set among currently available methylation arrays, which serve as the basis for training most existing epigenetic clocks. MSA is a newer methylation array with a smaller CpG set than EPICv2, yet is claimed by the manufacturer to be suitable for epigenetic clock applications. Galaxy was included because it is designed as an alternative to EPICv2, covering over 95% of its CpG sites. Lastly, Twist was included as a mature solution for HTS-based methylation profiling with appropriate CpG coverage in the current marketplace. Overall, these methods were selected to cover mature, widely used, and emerging methods in both methylation array and HTS categories.

Experimental design

In total, 165 participants (100 % East Asian / Pacific Islander; aged 20 - 77 years old; 41.8 % female) were recruited as the SRRSH cohort. Among which, 24 subjects were randomly selected (22 - 77 years old; 54.2 % female) for investigating and evaluating the adaptation of epigenetic clocks for cfDNA-based methylation data, referred to as the SRRSH-24 cohort. Though no sample size calculations were conducted *a priori*, a previous study suggested a similar sample size is sufficient to identify 80% of reliable probes [57]. Their cfDNA was extracted for targeted DNA methylation sequencing, and buffy coat gDNA was extracted for both methylation profiling. This resulted in six datasets: gDNA_MSA, gDNA_EPICv2, gDNA_Galaxy, gDNA_Twist, cfDNA_Galaxy, and cfDNA_Twist. Two technical replicates per sample were generated via independent library preparations from the same DNA aliquot. At last, the SRRSH-141 dataset contained Twist-profiled plasma cfDNA methylation data for the remaining 141 participants. In addition, nine phenoage-related [8] plasma biomarkers were collected from the SRRSH-141 cohort, namely albumin, creatinine, glucose, CRP, lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase and white blood cell count.

Epigenetic clock selection

A total of 57 epigenetic clocks were included in this study, including 53 array-trained and 4 HTS-trained clocks (Table S1). The 53 array-trained clocks include 47 integrated in the pyaging package (version 0.1.27) and 6 GP-age glocks (https://github.com/mirivar/GP-age, version 1.0). The 4 HTS-trained clocks include emseqage [20], timeseqage (https://github.com/patricktgriffin/TIME-Seq, Commit 140622f), BS-clock (https://github.com/hucongcong97/BS-clock, Commit 097b800) and komakiage [58]. Two clocks,

BS-clock and komakiage were not included in anlyses beyond feature coverage assessment due to their overall low feature coverage in all six SRRSH-24 datassets.

Biological sample pretreatment and storage

An 8-10 mL whole-blood sample was collected from each volunteer via intravenous collection, after an overnight fast. One anticoagulant tube (Streck, Cell-Free DNA BCT 10ml) was immediately transported to the laboratory for subsequent treatment. The blood was centrifuged at 4 ˚C (1900× g, 10 min) to obtain plasma and buffy coat. All biological samples were stored at -80 ˚C for long-term preservation.

DNA extraction

Cell-free DNA was extracted from 1.0 - 1.5 mL plasma using the QIAamp MinElute ccfDNA Mini Kit (Qiagen, Cat# 55204) following the manufacturer's instructions. Genomic DNA was isolated from approximately 500 μL of thawed buffy coat using TIANamp Genomic DNA Kit (TIANGEN, Cat# DP304) following the manufacture's protocol. The concentration of purified DNA was quantified using a Thermo Fisher Qubit 4.0 fluorometer (Qubit™ dsDNA HS Assay Kit, Cat# Q32854).

Library preparation and sequencing

For genomic DNA, 0.5% (by mass) of unmethylated lambda DNA and 0.05% (by mass) of CpG-methylated pUC19 DNA were spiked into 400-800 nanograms of buffy coat genomic DNA, followed by dilution to 130 μL of 10 mM Tris 0.1 mM EDTA, pH 8.0, following the manufacturer's recommendations. Internal control DNA underwent the same fragmentation procedure as gDNA samples. The DNA mixture was then transferred to a Covaris microTUBE and sheared to an average size of 100-200 bp (peak = 150bp) using a Covaris™ S220 focused ultrasonicator (Covaris, MA, USA).

For cfDNA, unmethylated lambda DNA and CpG methylated pUC19 DNA were respectively sheared to an average size of 100-200 bp (peak = 150bp) in advance. Next, 0.5% (by mass) of fragmented unmethylated lambda DNA and 0.05% (by mass) of fragmented CpG methylated pUC19 DNA were added into 1-10 ng cfDNA samples.

Both cfDNA and sonicated gDNA samples, along with spike-in internal control DNA, were both used to construct EM-Seq libraries with the NEBNext Enzymatic Methyl-seq Kit (New England

Biolabs, Cat# E7120) following the manufacturer's instructions. Libraries were quantified using a Thermo Fisher Qubit 4.0 fluorometer. Then capture-based human methylome enrichment was performed according to Twist's (South San Francisco, USA) protocols for their Twist Human Methylome Panel (Cat# 105521), or according to iGeneTech's (Beijing, China) protocols for their BisCap CpG Galaxy Panel (Cat# PE4000175). The final libraries were analyzed and quantified using TapeStation (Agilent Technologies). The whole-genome libraries were 150 bp paired-end sequenced using the Illumina NovaSeq X Plus sequencer on a 10B or 25B flow cell (Illumina) with 12.5 % PhiX spiked-in, to an average yield of 36.3Gbp raw base per library (Fig S15).

EPICv2 and MSA

Buffy coat genomic DNA samples were sent to Novogene (Tianjin, China) to perform Illumina Infinium MethylationEPIC v2.0 BeadChip array (EPICv2; 937,691 probes), or sent to WeGene (Shenzhen, China) to perform Infinium Methylation Screening array (MSA; 284,318 probes). For each technical replicate sample, 500 nanograms (for EPICv2) or 200 nanograms (for MSA) of genomic DNA were bisulfite treated using the EZ DNA Methylation Kit (Zymo Research, Cat# D5001) following the manufacturer's recommendations for Infinium assays.

As the EPICv2 and MSA arrays have a capacity of 8 and 48 samples per array, respectively, we included two replicates (2 replicates × 24 gDNA samples = 48 positions).

Methylation array data processing

Raw IDAT files from both Infinium MethylationEPIC v2.0 array and Infinium Methylation Screening Array (MSA) were processed using the `minfi` R package (version 1.54.1) according to established protocols. A rigorous quality control and filtering pipeline was applied: probes located on sex chromosomes were excluded, as well as those with a detection p-value greater than 0.01 regarding as unreliable signals. Furthermore, probes known to harbor single nucleotide polymorphisms (SNPs) at or near the CpG site, or those that did not uniquely map to the reference genome, were also removed. Following these filtering steps, the raw intensity data underwent intra-array normalization using the `Noob` method. Finally, beta-values were calculated as the primary methylation metric and prepared for downstream quantitative analyses.

High-throughput sequence (HTS) methylation data processing

Prior to downstream processing, raw paired-end FASTQ files for all samples were downsampled to standardize sequencing depth at approximately 30 Gbp (1 Gbp = 10^9 base pairs). This step was performed to mitigate potential biases stemming from varying sequencing depths and to ensure fair comparisons among samples. Subsequently, these trimmed files underwent adapter removal using `fastp` (version 0.23.4) and were aligned to a comprehensive reference genome, comprising GRCh38, pUC19, and phage lambda sequences, using `bwa-meth` (version 0.2.7). Alignment files were then sorted and indexed using `Samtools` (version 1.21), followed by the identification and marking of duplicate reads using `Samblaster` (version 0.1.26). Methylation levels across CpG contexts were determined and extracted using the mbias and extract modules of `MethylDackel` (version 0.6.1).

Genomic coordinate alignment and probe annotation

Manifest files for the MSA, HM450K, EPICv1, and EPICv2 arrays were obtained from the manufacturers' official websites. Since our analysis was based on the hg38 reference genome, genomic coordinates for the HM450K probes were converted from hg19 to hg38 using the UCSC `LiftOver` tool. The four manifest files were then merged to create a comprehensive probe-to-coordinate reference file.

For sequencing data (Twist and Galaxy), the per-sample output files from `MethylDackel` (https://github.com/dpryan79/MethylDackel, version 0.6.1) were processed. Genomic coordinates and beta-values were extracted for each CpG sites and then merged using `mergeContext` function in `MethyDackel`. These files were subsequently merged into a matrix using `bedtools unionbedg` (version 2.31.1). Finally, the `bedtools intersect` function was employed to annotate the sequencing-derived CpG sites with corresponding probe IDs from the reference file, based on overlapping genomic coordinates.

Dataset benchmarking calculations

Principal component analysis (PCA) was performed using `prcomp()` from R version 4.5.1 (save below). Pearson correlation analysis was performed using `cor()`. Intraclass correlation coefficient (ICC) was calculated using `icc()` from the `irrR` package (version 0.84.1) based on a single-rater, absolute agreement, two-way random-effects model. Missing values (`NaN`) were

removed before these analyses. Benchmarking results were visualized using R packages `ggplot2` (version 4.0.0), `ComplexHeatmap` (version 2.22.0), and `linkET` (version 0.0.7.4) respectively.

CpG coverage analyses were conducted in two levels: (1) across platform; and (2) clock CpGs covered by each platform. Reference CpG sites for each platform were extracted from manufacturer-provided datasheets. In addition, for MSA and EPICv2 arrays, only CpGs with defined genomic coordinates were retained. Input CpGs for each of the 53 clocks were extracted from clock models respectively. Results were visualized using R functions `UpSet()` (`ComplexHeatmap` version 2.22.0) and `pheatmap()` (`pheatmap` version 1.0.13).

Statistical analysis

The comparisons between means and medians of two groups of data were conducted using permutation test (implemented by custom script) by randomly permuting observations between the two groups and comparing the mean/median after permutation with the observed value. The p-values are calculated as the fraction of permutation rounds with its mean/median after permutation being greater than the observed value. Permutation tests make no assumptions of distributions and can be applied to both small and large number of observations.

To account for multiple comparisons, the p-values were adjusted for false discovery rate (FDR) control using the Benjamini-Hochberg (BH) method (`statsmodels.stats.multitest.multipletests(method="fdr_bh")` in the `statsmodels` package, version 0.14.5).

Public datasets

To ensure reproducibility and external validation, we included 11 publicly available datasets. GSE55763 (N = 2711, Illumina HumanMethylation 450K) [60] was downloaded from the Gene Expression Omnibus (GEO) database for model and PCA training for its large size, and was also used to assess age prediction RD using the 72 replicated samples. GSE83944 (N = 48, Illumina HumanMethylation 450K) [61], GSE247193 (N = 23 including one subject without replicate, Illumina Infinium EPIC Human Methylation Beadchip) [62], GSE247195 (N = 23 including one subject without replicate, Illumina Infinium EPIC Human Methylation Beadchip) [62] and GSE247197 (N = 46, Illumina HumanMethylation 450K) [62] were downloaded from the GEO

database to assess age prediction RD as they all contain technical replicates. HTS-based datasets GSE144691 (N = 29) [63] and GSE86832 (N = 4) [64] were downloaded from the GEO database for assessment of unreliable CpGs for not containing replicates. Two additional datasets from the GEO database were downloaded for assessment of age prediction MAE and RD, namely GSE232346 (N = 24, TimeSeq) [33] and GSE245628 (N = 24, Illumina Infinium EPIC Human Methylation Beadchip) [33]. Finally, we also downloaded the Buccal cohort (Shokhirev & Johnson, 2025, N = 100, Twist and Illumina Infinium EPIC Human Methylation Beadchip v2) [28] and GSE164600 (N = 24, cfDNA WGBS) [52] datasets for various validation steps. The ethnicity and sex information can be found in the source dataset but was not considered in this study.

Correlation between clock number of CpGs and RD

20 HCRL clocks were selected to predict biological age with `pyaging` (version 0.1.27). No additional data preprocessing was performed except for filling missing values (`NaN`) with KNN imputation. RD were calculated as the mean absolute difference between the predicted ages between replicates. The linear regression was performed using `scipy.stats.linregress` in the `scipy` package (version 1.15.3). Result p-values were corrected for multiple tests via the Benjamini-Hochberg procedure using custom scripts.

Elastic net model training for independent verification of the correlation between number of CpGs and and RD

A series of elastic net models were trained on the dataset GSE55763 (N = 2711) with `sklearn.linear_model.ElasticNet` implemented in the `scikit-learn` package (version 1.6.1). The 90,394 CpGs shared between GSE55763 and the 6 datasets (gDNA_EPICv2, gDNA_MSA, gDNA_Galaxy, gDNA_Twist, cfDNA_Galaxy and cfDNA_Twist) were extracted, and missing values (`NaN`) were filled with KNN imputation implemented in `pyaging`. No additional data cleaning was performed. The elastic net models were trained with 30 logarithmically spaced α values ranging from 10^{-5} to 10^{2}, and 15 linearly spaced `l1_ratio` (λ) values ranging from 0.0-1.0, resulting in a total of 450 trained models. All other parameters were kept default. Linear regression was performed using `scipy.stats.linregress`.

Minimal sequencing investigation based on binomial stochasticity.

Randomized samples were generated via multi-dimensional binomial model, where the observed methylation counts at each CpG follows independent binomial distributions. For each binomial distribution, the probability parameter was set to the observed beta-value in the original dataset, and the count parameter set to the sequencing depth, given by:

$$beta \sim \frac{1}{depth}\, Binomial(depth, beta_{obs})$$

Where:

depth denotes the simulated sequencing depth under investigation.

$beta_{obs}$ is the empirically estimated true beta-value, calculated as the mean in a replicate pair. Value of $beta_{obs}$ differs across subjects and datasets.

To reduce randomness, 1,000 random datasets were generated at each simulated sequencing depth. Finally, clock prediction metrics (MAE and RD) were calculated with respect to each simulated sequencing depth.

Minimal sequencing investigation based on beta resolution.

This investigation evaluates the impact of uncertainty related to the finite sequencing depths, when beta-values cannot be represented at arbitrary precision. For example, a sequencing depth of 4 can only represent beta-values among 0, 0.25, 0.5, 0.75 and 1.0. To simulate this mechanism, the beta-values were rounded under a given sequencing depth using the following formula before age prediction:

$$beta = \frac{\left\lfloor beta_{obs} \times depth + \frac{1}{2} \right\rfloor}{depth}$$

Where:

depth denotes the simulated sequencing depth under investigation.

$beta_{obs}$ is the empirically estimated true beta-value, calculated as the mean in a replicate pair.

$\lfloor \cdot \rfloor$ is the floor function that maps a real number to the greatest integer less than or equal to that number.

Clock prediction metrics (MAE and RD) were then calculated with respect to each simulated sequencing depth. No repeated tests were necessary since this process is deterministic.

Minimal sequencing investigation based on downsampling.

A downsampling analysis was conducted on five samples, each having an initial sequencing result size ≥50Gb, to assess the effect of varying data volumes on biological age estimation. Raw sequencing data were downsampled at proportions ranging from 0.025 to 0.9 (namely 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, ..., 0.9). At each downsampling point, biological age was estimated using various established models.

Unreliable beta-value imputation at different cut-off depths

For each combination of selected cut-off depth and imputation method, the beta matrix in steps in the following order:

(1) Missing value imputation (first pass): Missing values (`NaN`) were imputed using the chosen method implemented in `pyaging.preprocess`. Briefly, the KNN imputation method replaces missing values with the mean of k nearest neighbors, where distances between samples are computed based on features with non-missing beta-values. The mean and median methods replace missing entries with the mean or median value of the respective feature, calculated from non-missing observations. The 0-filling method assigns 0 to all missing entries. Notably, among these methods, KNN is the only one that considers information across all features rather than only the imputed one.

(2) Unreliable beta-value removal: Selected beta-values were set to `NaN` when both of the following conditions were met: (a) the observation was 0 or 1, and (b) the sequencing depth at the corresponding CpG site was below the defined cut-off.

(3) Missing value imputation (second pass): The selected imputation method was re-applied to impute these newly removed unreliable CpG sites.

This process was repeated for cut-off depths ranging from 1 to 20 across all four HTS-based datasets generated in this study (gDNA_Galaxy, gDNA_Twist, cfDNA_Galaxy, and cfDNA_Twist).

Transfer learning of classic epigenetic models

We used distillation to adopt classic epigenetic models for HTS methylation data. The classic epigenetic models are teacher models. Each HTS dataset were first PCA-transformed using PCA models trained from public dataset GSE55763 (N = 2711) with an output dimensionality of 2500. The transformed dataset was then combined with the output of a teacher model to train and test a student elastic net model using a 5-fold cross-validation (5-fold CV) implemented in the `scikit-learn` package (version 1.6.1). The final evaluation of age prediction MAE and RD were summarized from all testing splits. The hyperparameters (`alpha` and `l1_ratio`) was optimized using an independent 5-fold cross-validation (`sklearn.linear_models.ElasticNetCV(cv=5)`).

Adaptation baseline methods comparison.

Three adaptation methods were evaluated as baselines for comparison with the DF-IM-TL pipeline: quantile mapping, ComBat, and correlation alignment (CORAL). In all cases, target datasets (gDNA_Galaxy, gDNA_Twist, cfDNA_Galaxy, cfDNA_Twist, and Buccal) were adapted to the gDNA_EPICv2 reference prior to age prediction using the original array-trained clocks.

Quantile mapping was applied feature-wise by transforming values in the target dataset to match the empirical distribution of the reference dataset via quantile-to-quantile alignment.

Batch correction using ComBat was performed under an empirical Bayes framework to remove additive and multiplicative batch effects while preserving biological variation.

CORAL was used to align second-order statistics between datasets by matching covariance structures. To accommodate the high dimensionality of methylation data, dimensionality reduction was first performed using truncated singular value decomposition (SVD). CORAL was then applied in the reduced space, followed by projection back to the original feature space.

Quantile mapping and CORAL were implemented using custom scripts using scipy and numpy, while ComBat was applied using the neuroCombat (version 0.2.12) Python package.

PhenoAge calculation.

The PhenoAge of the samples from the SRRSH-141 cohort was calculated from collected clinical biomarkers using the phenoage clock [8] integrated in `pyaging`.

JSD calculation.

Jenson-Shannon Divergence (JSD) were calculated on delta-age predicted by each clock individually. The distribution of delta-age probability density functions (p.d.f.) were estimated using Gaussian kernel density estimation (KDE) (`scipy.stats.gaussian_kde`) independently for normal and diseased samples. JSD was then calculated between the normal and disease sample p.d.f.'s using `scipy.spatial.distance.jensonshannon`. The bandwidth parameter in KDE is automatically determined using the "scott" method.

ALS classification.

The ALS classification was performed via support vector machine (SVM) classifier with a linear kernel (`sklearn.svm.SVC(kernel="linear")`) using the delta-ages calculated before and after model adaptation respectively. The classification predicts if a sample is ALS (1) or normal (0). A stratified 12-fold cross-validation was used to evaluate the classification performance while reducing potential overfitting. Briefly, 1 normal and 1 ALS sample were randomly chosen (without replacement) as testing samples, and the rest were for training. Classification performance was evaluated after 12 iterations, i.e. all samples have been predicted as testing cases using the area under receiver operating characteristic curve (AUC) metric (`sklearn.metrics.auc_score`).

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Figure Legend

Fig. 1| Study design and evaluation of methylation data reproducibility and consistency across six SRRSH-24 datasets. a, The overall design of the study. **b**, ICCs of 55 epigenetic clocks benchmarked in the study. Data are presented as ICC estimates with 95 % confidence intervals and colored by grouped ICC values as follows: Excellent: 0.9 ≤ ICC, Good: 0.75 ≤ ICC < 0.9, Moderate: 0.5 ≤ ICC < 0.75, Poor: ICC < 0.5. Clocks were classified in two different rationales: (1) By generations: First, Chronological age prediction; Second, Phenotypic age or mortality prediction; Third, Pace of aging prediction; Fourth, Causal clocks; Gestational, Gestational age prediction; Other: Telomere length or mitotic age prediction. (2) By CpG coverage across the methylation profiling methods. **c-d**, PCA of all samples colored by datasets (**c**) or replicates (**d**), using beta-values of Method-shared CpGs (N = 136,437). **e**, Intraclass Correlation Coefficients (ICCs) of Method-shared CpGs across six datasets. **f**, Pearson correlations among datasets computed using the mean beta-values of Method-shared CpGs. **g**, Comparisons of per clock ICCs of all array-trained clocks (N = 53). **h**, Pearson's correlations across six datasets calculated using mean of clock predictions for three clock performance categories. P-values in (**e**) and (**g**) were calculated by two-tailed block permutation test with 10,000 permutations, then adjusted for multiple comparisons using the Benjamini-Hochberg procedure. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; N.S., not significant. The center line of each boxplot marks the median, the box body marks the 1st and 3rd quartile, the caps and/or the span of whisker mark the min and max values respectively.

Fig. 2| Decline of epigenetic clock performance in 6 SRRSH-24 HTS-based datasets. a-b, Lollipop plot showing Pearson's R and MAE for (**a**) 6 Hi-Repro clocks and (**b**) 2 sequencing clocks. Each dot represents a replicate. **c-d**, Boxplots showing comparisons and statistical significance of (**c**) R and (**d**) MAE for Hi-Cov-All clocks. The center line of each boxplot marks the median, the box body marks the 1st and 3rd quartile, and the caps mark the min and max of RD values respectively. **e-f**, Performance evaluation of 4 array-trained clocks (hannum, horvath2013, skinandblood and dnamphenoage) and a HTS data-trained clock (timeseqage) cross-applied to array-based dataset (GSE245628, labelled as EPICv1) and HTS-based dataset (GSE232346, labelled as TimeSeq). Only the 24 shared samples in both GSE245628 and GSE232346 were incorporated. **g**, Scatter plot showing the correlation between clock ICC and MAE in the four SRRSH-24 HTS datasets. **h**, Scatter plot showing the correlation between clock ICC and R in the four SRRSH-24 HTS datasets. Lines represent linear regression fits for each replicate group respectively. The p-values in (**c**) and (**d**) were calculated by two-tailed block permutation test with 10,000 permutations, then adjusted for multiple comparisons using the Benjamini-Hochberg procedure. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; N.S., not significant; The R and p-values in (**g**) and (**h**) were calculated by Pearson correlation at replicate level.

Fig. 3| Investigation of optimizing epigenetic clock applications for HTS-based methylation data via sequencing depth and model selection. a, Investigation sequencing depth threshold via beta-value stochasticity effect at individual CpG level on HCRL clocks. **b**, Investigation of sequencing depth threshold via beta-value approximation precision on HCRL clocks. **c-d**, Investigation of the sequencing depth requirements with downsampling simulations with 5 qualified samples from the SRRSH cohort with sequencing result size ≥ 50Gb. **c**, Age prediction MAE at different downsampling depths. **d**, The increasing of the fraction of CpGs with ≥ 20× slowed down above ≥ 20× downsampling depth. **e**, A series of elastic nets were trained on dataset GSE55763 (N = 2711) and tested with the 6 datasets from this study. **f**, The negative correlation observed between number of CpGs and age prediction RD. **g-h**, Comparison of correlation between hyperparameter α, λ and RD. R^2 and p-values were calculated by Pearson correlation.

Fig. 4| Investigating depth filtering (DF), imputation (IM) and transfer learning (TL) to improve epigenetic clock performance across array and HTS platforms. a, HCRL clocks are prone to unreliable beta-values originating from HTS-based methylation profiling technologies (Details shown in Table S3). **b-c**, The K-nearest-neighbor (KNN) imputation technique was tested at varying depth cut-offs; only CpG sites below the cut-off were imputed. **d-e**, Comparison of age prediction MAE (**d**) and RD (**e**) between teacher and student (distillation) clocks, assessed with 5-cold cross validation. **f**, Generalizability benchmarking of transfer learning across HTS capture panels and DNA types. The center line of each boxplot marks the median, the box body marks the 1st and 3rd quartile, and the caps mark the min and max values respectively.

Fig. 5| Testing and validating the DF-IM-TL epigenetic clock adaptation pipeline for biological age prediction on HTS platforms. a, Evaluation of all DF-IM-TL combinations for 20 HCRL clocks on the Buccal cohort (N = 100). **b**, Performance improvements via the best identified strategy (TL-only) with respect to chronological age (CA) on the Buccal cohort. **c**, Performance improvements via the best identified strategy (IM-TL) with respect to biomarker derived PhenoAge on the SRRSH-141 cohort (N = 141). **d**, Adaptation efficacy comparison between DF-IM-TL and other three commonly used adaptation strategies.

Fig. 6| Testing and validating the DF-IM-TL epigenetic clock adaptations strategies for disease classification applications. Validation performed on a public dataset GSE164600 (N = 24) with healthy and amyotrophic lateral sclerosis (ALS) labels. **a**, The distributional change of delta-age predicted by 20 HCRL clocks before and after clock adaptation (IM+TL as the best strategy). The center line in each violin plot marks the median. **b**, The Jenson-Shannon Divergence (JSD) calculated to evaluate the distributional distances between ALS and normal sample. The models in (**a**) were sorted in descending order per change in (ΔJSD) with respect to clock adaptation. Each box marks the 1st -3rd quartile range, cap marks minimum and maximum values, and the center line marks the median. **c**, ALS classification performances collectively using delta-ages predicted by top-ΔJSD models. Classification was conducted using support machine classifier with a linear kernel and was evaluated using area under the receiver operating characteristic curve (AUC). **d**, Maximum AUC achieved using as few as 4 top ΔJSD clocks, leading the non-adapted prediction by 0.125 AUC.

Table 1| Linear regression results between the age prediction replicate difference (RD) and the number of CpGs in 20 HCRL clocks. HCRL denotes 20 linear-architecture clocks classified as Hi-Cov-All or High-Repro. Negative correlations indicate larger number of CpGs is associated with lower age prediction discrepancies between technical replicates. P-values were adjusted for multiple testing using the Benjamini-Hochberg procedure. Full regression outputs for each dataset are provided in Fig. S10.

Dataset	**Slope**	**Intercept**	**Pearson's R**	**p**
gDNA_MSA	-0.36	2.34	-0.94	**3.65E-10**
gDNA_EPICv2	-0.24	1.30	-0.87	**4.67E-07**
gDNA_Twist	-0.28	2.87	-0.90	**4.91E-08**
gDNA_Galaxy	-0.31	3.01	-0.93	**3.49E-09**
cfDNA_Twist	-0.29	3.04	-0.91	**2.69E-08**
cfDNA_Galaxy	-0.28	3.01	-0.90	**5.86E-08**
GSE83944	-0.18	0.75	-0.83	**7.78E-05**
GSE247193	-0.19	1.60	-0.84	**5.65E-05**
GSE247195	-0.22	1.14	-0.85	**2.99E-05**
GSE247197	-0.14	0.55	-0.67	**4.62E-03**
GSE55763	-0.18	0.93	-0.81	**1.65E-04**

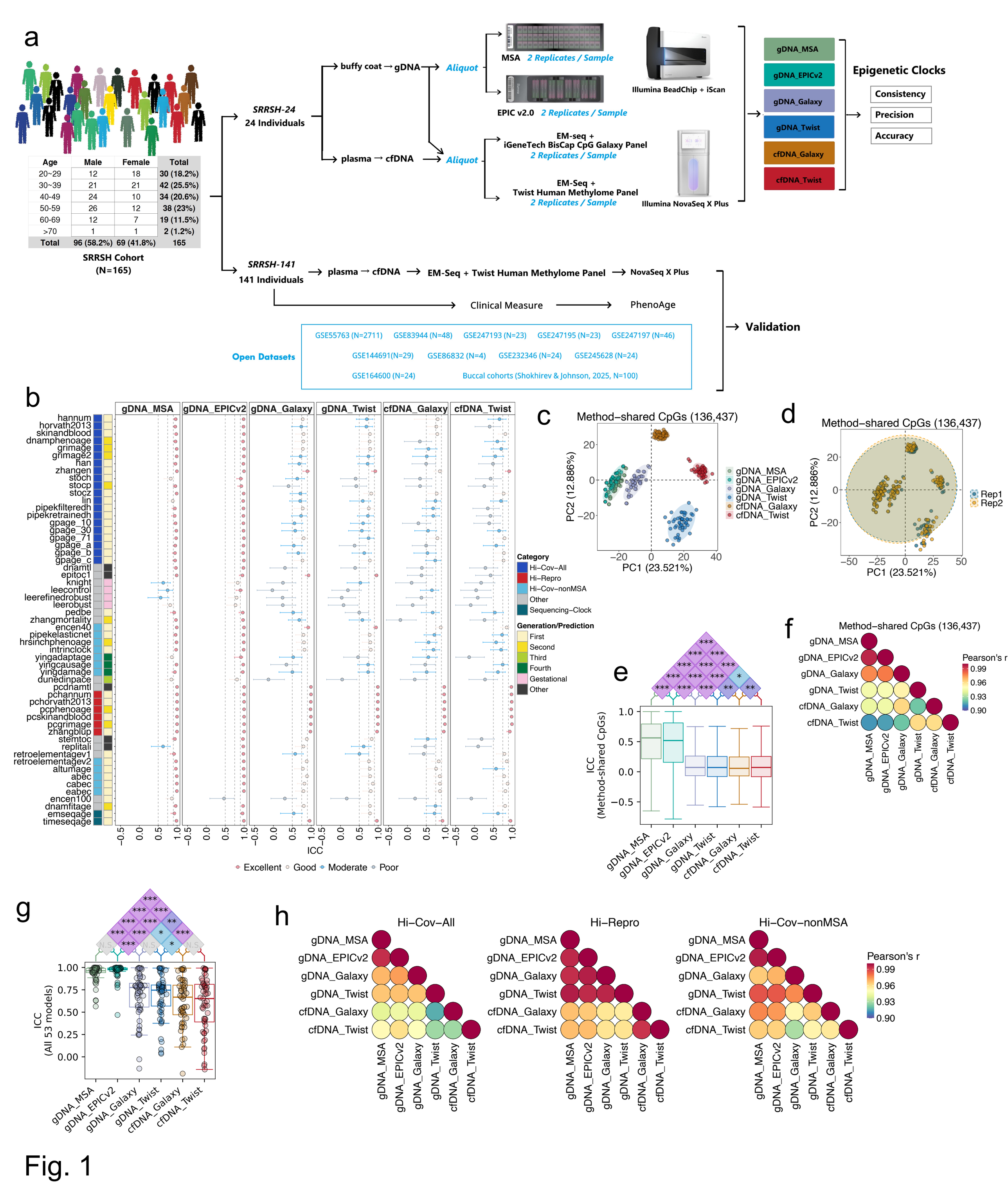
a
SRRSH-24
24 Individuals
buffy coat → gDNA
plasma → cfDNA
Aliquot
MSA 2 Replicates / Sample
EPIC v2.0 2 Replicates / Sample
Illumina BeadChip + iScan
EM-seq + iGeneTech BisCap CpG Galaxy Panel
2 Replicates / Sample
EM-Seq + Twist Human Methylome Panel
2 Replicates / Sample
Illumina NovaSeq X Plus
gDNA_MSA
gDNA_EPICv2
gDNA_Galaxy
gDNA_Twist
cfDNA_Galaxy
cfDNA_Twist
Epigenetic Clocks
Consistency
Precision
Accuracy
SRRSH-141
141 Individuals
plasma → cfDNA → EM-Seq + Twist Human Methylome Panel → NovaSeq X Plus
Clinical Measure → PhenoAge
Open Datasets
GSE55763 (N=2711) GSE83944 (N=48) GSE247193 (N=23) GSE247195 (N=23) GSE247197 (N=46)
GSE144691(N=29) GSE86832 (N=4) GSE232346 (N=24) GSE245628 (N=24)
GSE164600 (N=24) Buccal cohorts (Shokhirev & Johnson, 2025, N=100)
Validation
SRRSH Cohort (N=165)
Age | Male | Female | Total
20~29 | 12 | 18 | 30 (18.2%)
30~39 | 21 | 21 | 42 (25.5%)
40-49 | 24 | 10 | 34 (20.6%)
50-59 | 26 | 12 | 38 (23%)
60-69 | 12 | 7 | 19 (11.5%)
>70 | 1 | 1 | 2 (1.2%)
Total | 96 (58.2%) | 69 (41.8%) | 165
b
gDNA_MSA
gDNA_EPICv2
gDNA_Galaxy
gDNA_Twist
cfDNA_Galaxy
cfDNA_Twist
ICC
Excellent Good Moderate Poor
Category
Hi-Cov-All
Hi-Repro
Hi-Cov-nonMSA
Other
Sequencing-Clock
Generation/Prediction
First
Second
Third
Fourth
Gestational
Other
c
Method-shared CpGs (136,437)
PC1 (23.521%)
PC2 (12.886%)
d
Method-shared CpGs (136,437)
Rep1
Rep2
e
ICC (Method-shared CpGs)
f
Method-shared CpGs (136,437)
Pearson's r
g
ICC (All 53 models)
N.S.
h
Hi-Cov-All
Hi-Repro
Hi-Cov-nonMSA
Pearson's r

Fig. 1

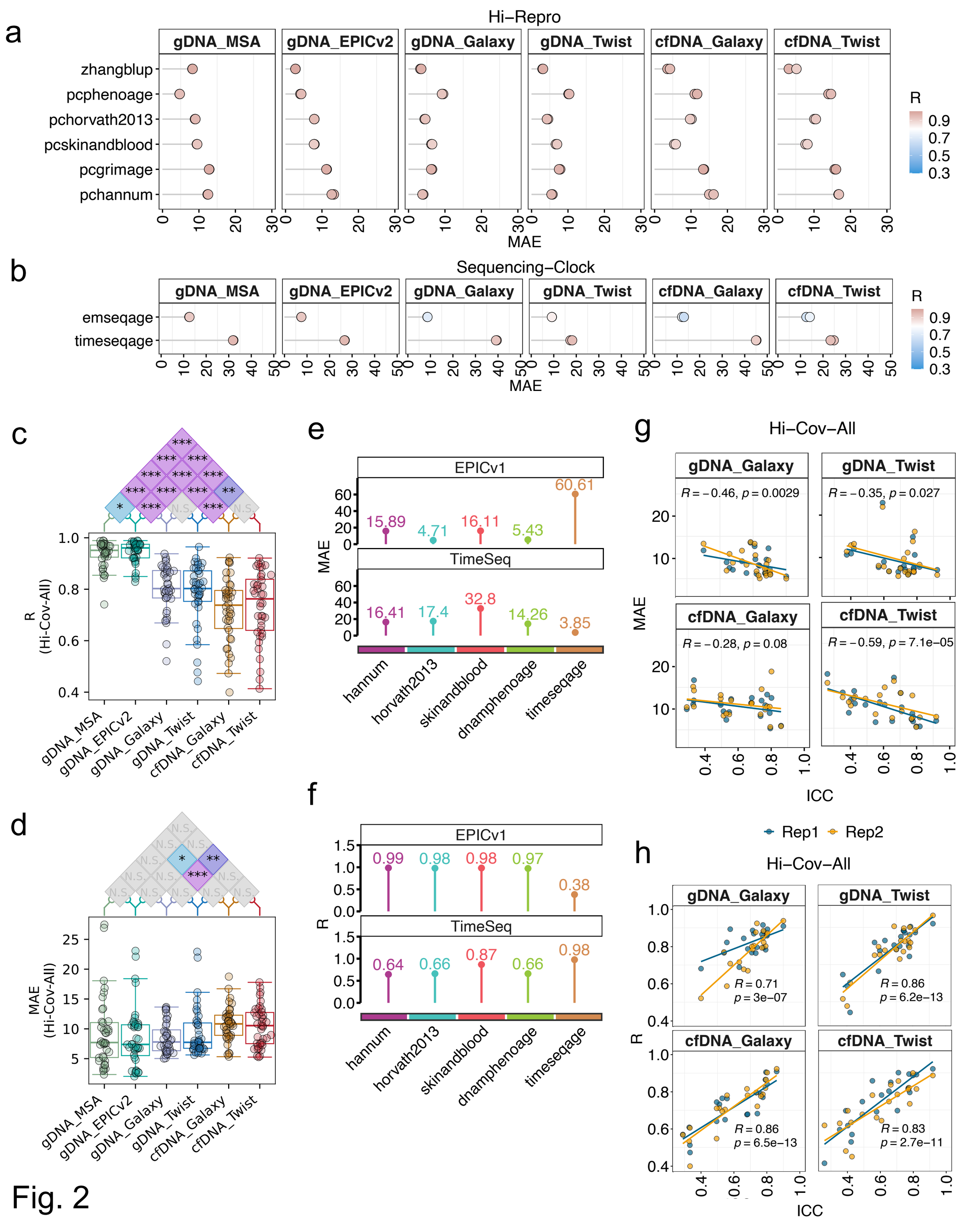
a
Hi−Repro
gDNA_MSA
gDNA_EPICv2
gDNA_Galaxy
gDNA_Twist
cfDNA_Galaxy
cfDNA_Twist
zhangblup
pcphenoage
pchorvath2013
pcskinandblood
pcgrimage
pchannum
MAE
R
0.9
0.7
0.5
0.3
b
Sequencing−Clock
emseqage
timeseqage
c
R (Hi-Cov-All)
d
MAE (Hi-Cov-All)
e
EPICv1
TimeSeq
15.89
4.71
16.11
5.43
60.61
16.41
17.4
32.8
14.26
3.85
hannum
horvath2013
skinandblood
dnamphenoage
timeseqage
f
0.99
0.98
0.98
0.97
0.38
0.64
0.66
0.87
0.66
0.98
g
Hi−Cov−All
R = −0.46, p = 0.0029
R = −0.35, p = 0.027
R = −0.28, p = 0.08
R = −0.59, p = 7.1e−05
ICC
h
Rep1
Rep2
R = 0.71
p = 3e−07
R = 0.86
p = 6.2e−13
R = 0.86
p = 6.5e−13
R = 0.83
p = 2.7e−11

Fig. 2

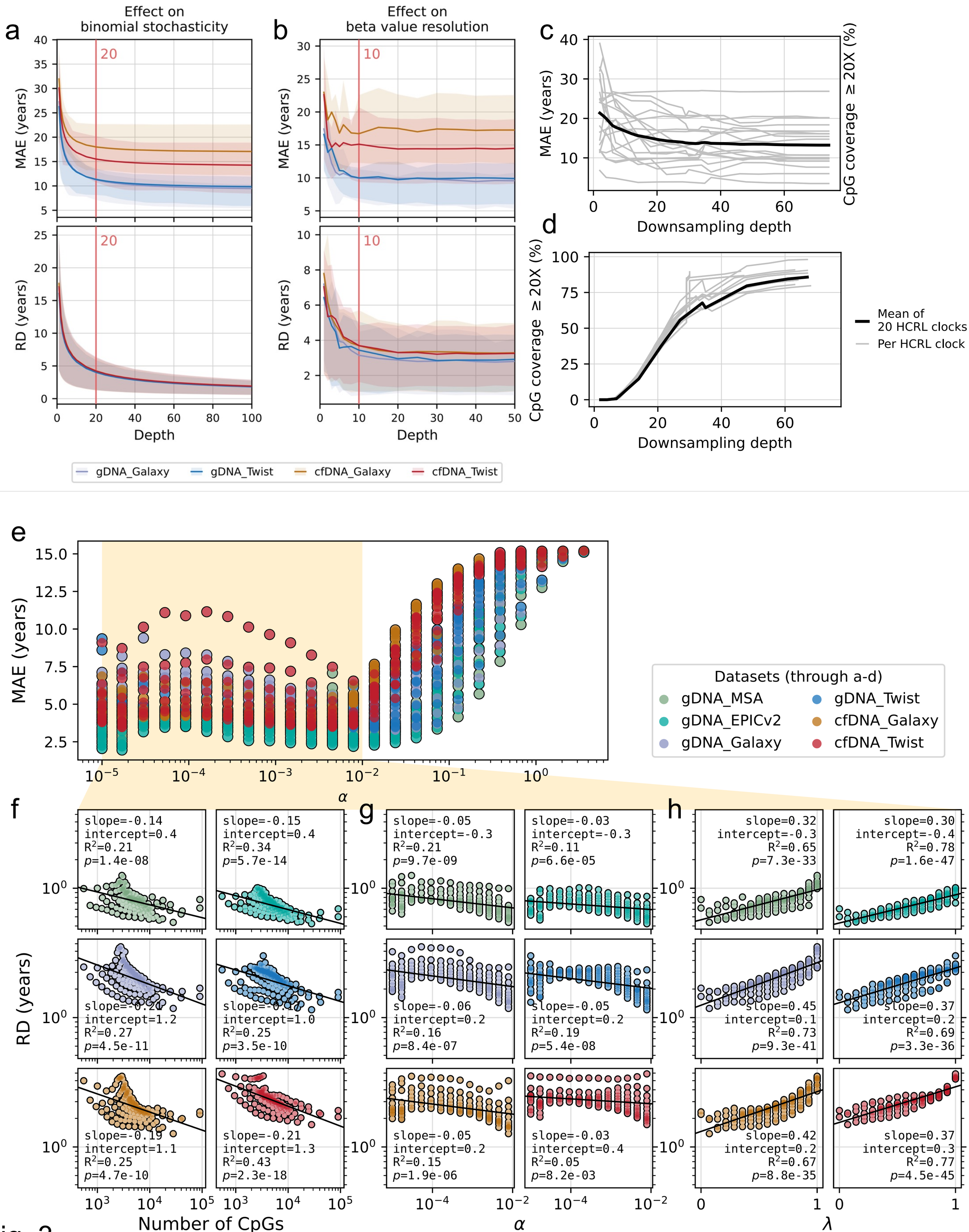
a
Effect on binomial stochasticity
MAE (years)
RD (years)
Depth
b
Effect on beta value resolution
c
MAE (years)
CpG coverage ≥ 20X (%)
Downsampling depth
d
Mean of 20 HCRL clocks
Per HCRL clock
gDNA_Galaxy
gDNA_Twist
cfDNA_Galaxy
cfDNA_Twist
e
MAE (years)
α
Datasets (through a-d)
gDNA_MSA
gDNA_EPICv2
gDNA_Galaxy
gDNA_Twist
cfDNA_Galaxy
cfDNA_Twist
f
g
h
RD (years)
Number of CpGs
α
λ

Fig. 3

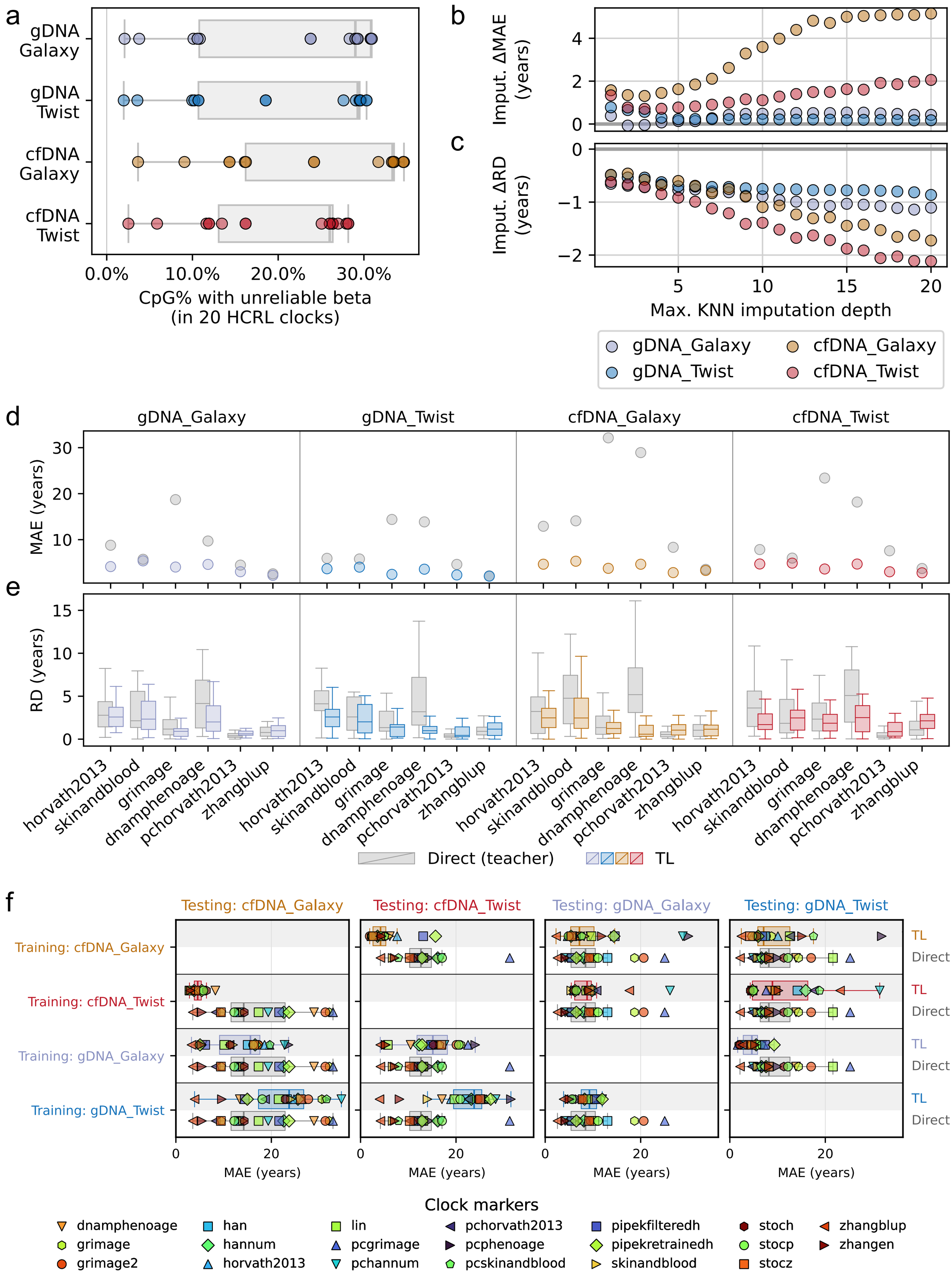
a
gDNA Galaxy
gDNA Twist
cfDNA Galaxy
cfDNA Twist
0.0%
10.0%
20.0%
30.0%
CpG% with unreliable beta (in 20 HCRL clocks)
b
Imput. ΔMAE (years)
c
Imput. ΔRD (years)
Max. KNN imputation depth
gDNA_Galaxy
gDNA_Twist
cfDNA_Galaxy
cfDNA_Twist
d
MAE (years)
e
RD (years)
horvath2013
skinandblood
grimage
dnamphenoage
pchorvath2013
zhangblup
Direct (teacher)
TL
f
Testing: cfDNA_Galaxy
Testing: cfDNA_Twist
Testing: gDNA_Galaxy
Testing: gDNA_Twist
Training: cfDNA_Galaxy
Training: cfDNA_Twist
Training: gDNA_Galaxy
Training: gDNA_Twist
TL
Direct
MAE (years)
Clock markers
dnamphenoage
grimage
grimage2
han
hannum
horvath2013
lin
pcgrimage
pchannum
pchorvath2013
pcphenoage
pcskinandblood
pipekfilteredh
pipekretrainedh
skinandblood
stoch
stocp
stocz
zhangblup
zhangen

Fig. 4

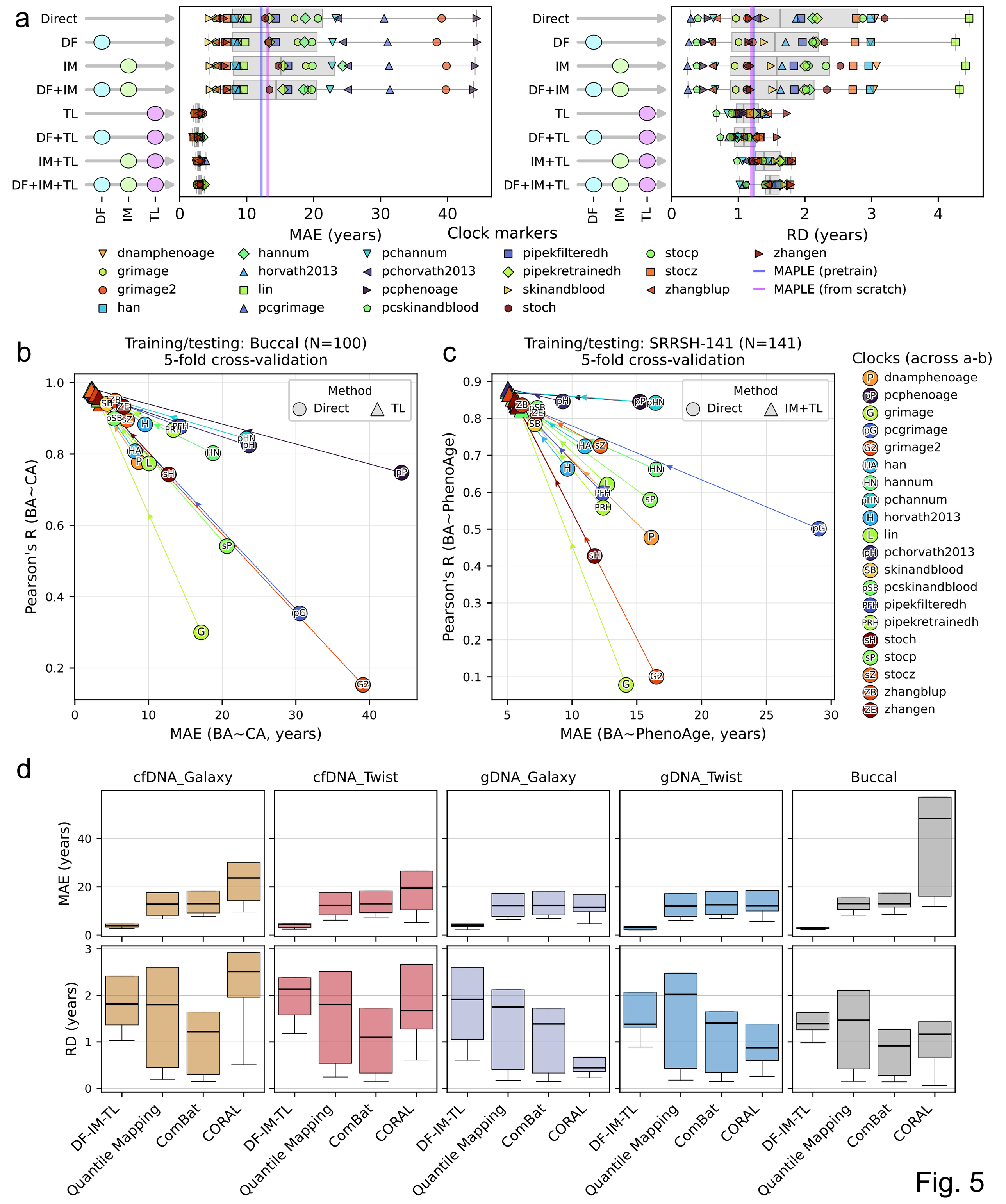
a
Direct
DF
IM
DF+IM
TL
DF+TL
IM+TL
DF+IM+TL
MAE (years)
RD (years)
Clock markers
dnamphenoage
grimage
grimage2
han
hannum
horvath2013
lin
pcgrimage
pchannum
pchorvath2013
pcphenoage
pcskinandblood
pipekfilteredh
pipekretrainedh
skinandblood
stoch
stocp
stocz
zhangblup
zhangen
MAPLE (pretrain)
MAPLE (from scratch)
b
Training/testing: Buccal (N=100)
5-fold cross-validation
Method
Direct
TL
Pearson's R (BA~CA)
MAE (BA~CA, years)
c
Training/testing: SRRSH-141 (N=141)
5-fold cross-validation
Method
Direct
IM+TL
Pearson's R (BA~PhenoAge)
MAE (BA~PhenoAge, years)
Clocks (across a-b)
dnamphenoage
pcphenoage
grimage
pcgrimage
grimage2
han
hannum
pchannum
horvath2013
lin
pchorvath2013
skinandblood
pcskinandblood
pipekfilteredh
pipekretrainedh
stoch
stocp
stocz
zhangblup
zhangen
d
cfDNA_Galaxy
cfDNA_Twist
gDNA_Galaxy
gDNA_Twist
Buccal
MAE (years)
RD (years)
DF-IM-TL
Quantile Mapping
ComBat
CORAL

Fig. 5

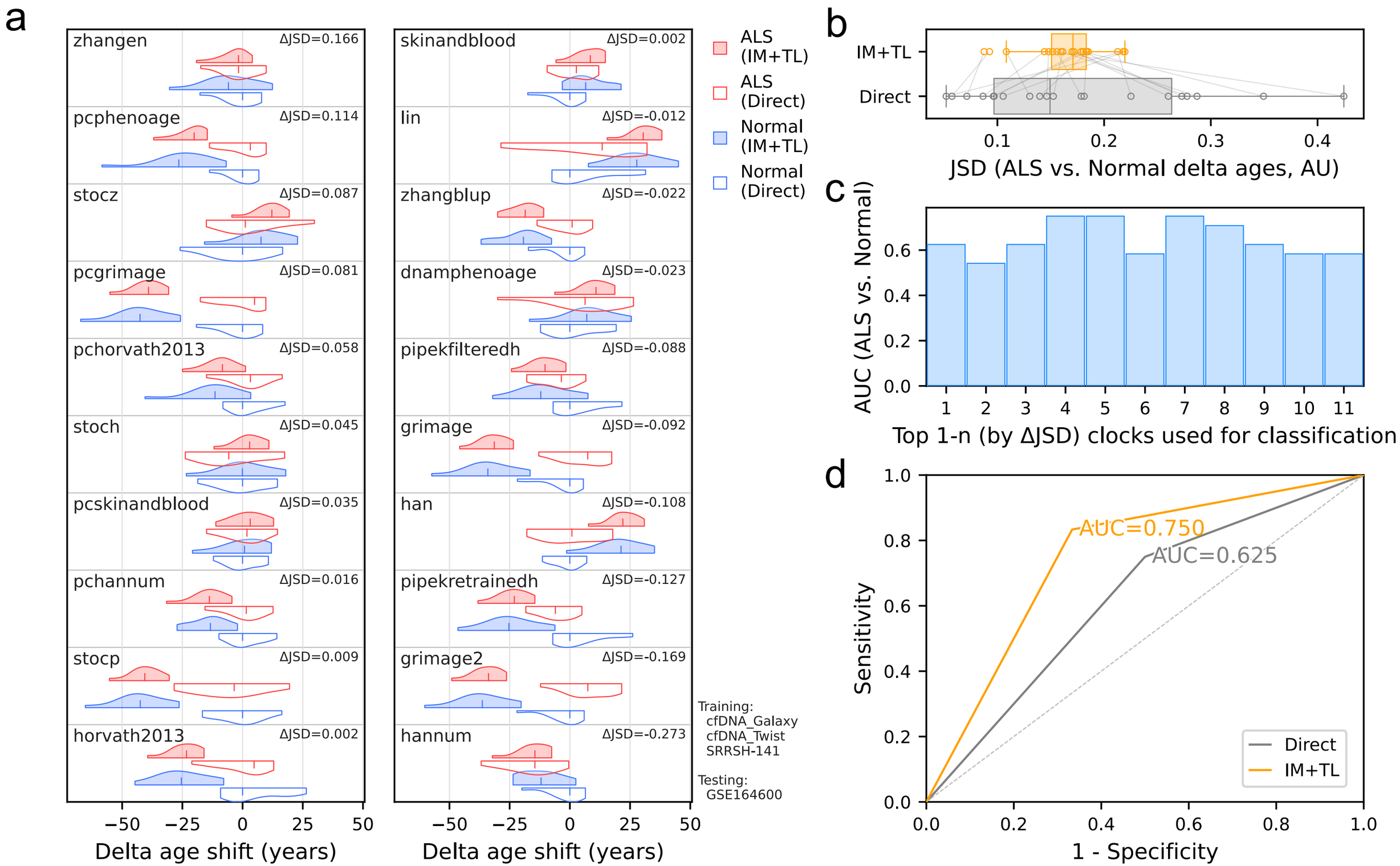
a
zhangen ΔJSD=0.166
pcphenoage ΔJSD=0.114
stocz ΔJSD=0.087
pcgrimage ΔJSD=0.081
pchorvath2013 ΔJSD=0.058
stoch ΔJSD=0.045
pcskinandblood ΔJSD=0.035
pchannum ΔJSD=0.016
stocp ΔJSD=0.009
horvath2013 ΔJSD=0.002
skinandblood ΔJSD=0.002
lin ΔJSD=-0.012
zhangblup ΔJSD=-0.022
dnamphenoage ΔJSD=-0.023
pipekfilteredh ΔJSD=-0.088
grimage ΔJSD=-0.092
han ΔJSD=-0.108
pipekretrainedh ΔJSD=-0.127
grimage2 ΔJSD=-0.169
hannum ΔJSD=-0.273
−50 −25 0 25 50
Delta age shift (years)
ALS (IM+TL)
ALS (Direct)
Normal (IM+TL)
Normal (Direct)
Training:
cfDNA_Galaxy
cfDNA_Twist
SRRSH-141
Testing:
GSE164600
b
IM+TL
Direct
0.1 0.2 0.3 0.4
JSD (ALS vs. Normal delta ages, AU)
c
AUC (ALS vs. Normal)
0.0 0.2 0.4 0.6
1 2 3 4 5 6 7 8 9 10 11
Top 1-n (by ΔJSD) clocks used for classification
d
Sensitivity
1 - Specificity
0.0 0.2 0.4 0.6 0.8 1.0
AUC=0.750
AUC=0.625
Direct
IM+TL

Fig. 6