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Long-Read Sequencing: A New Frontier for Estimating Biomarkers

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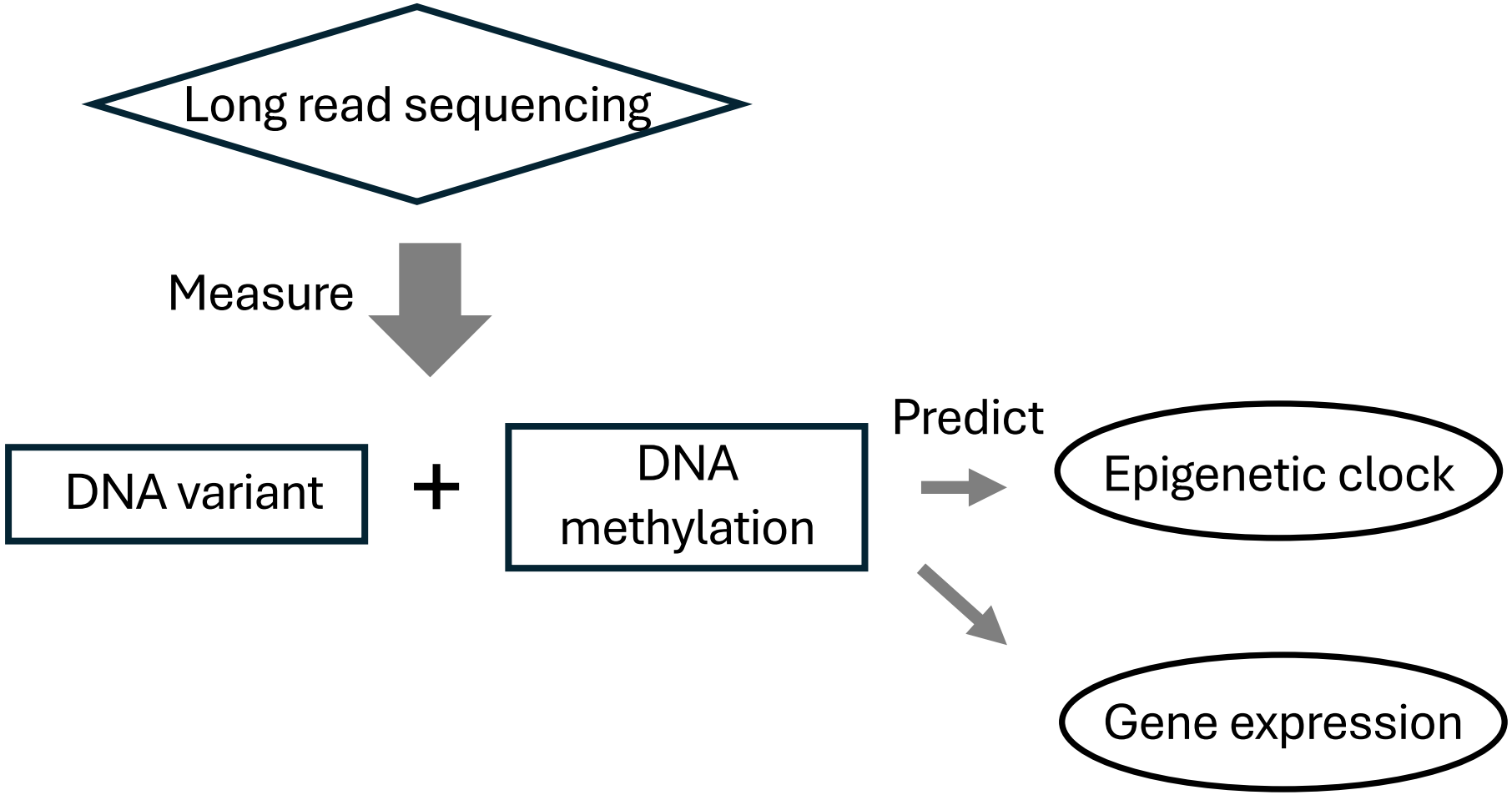
Disclosure Risk Review:

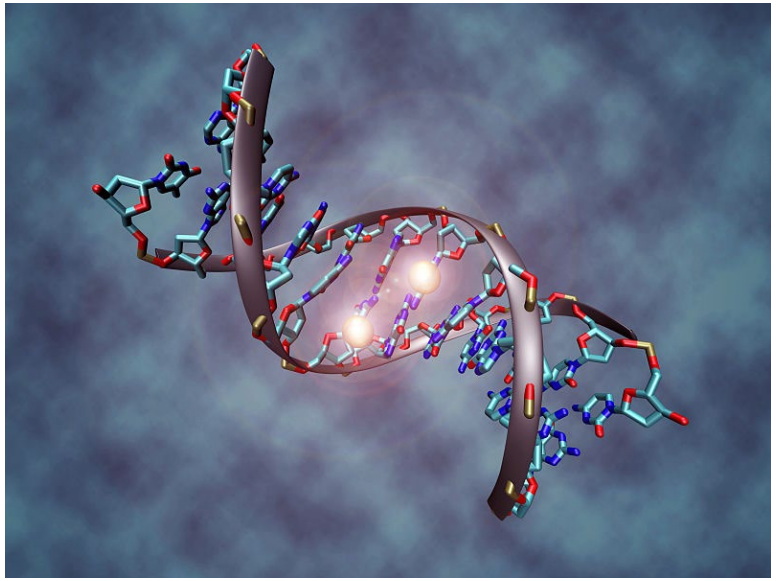
License Number: 21090019

No disclosure risks were identified

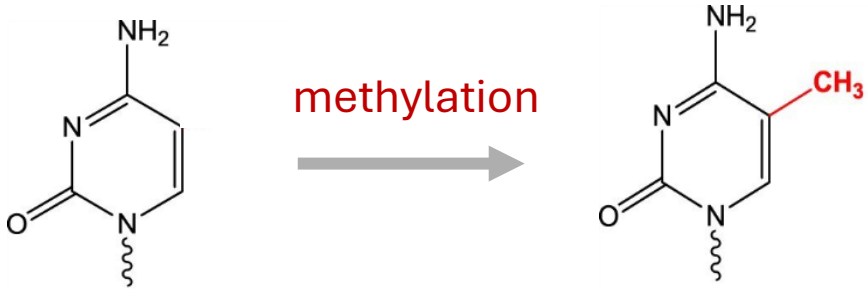
Outlines


Long read sequencing
Measure
DNA variant
+
DNA methylation
Predict
Epigenetic clock
Gene expression

DNA sequences
G, A, T, C



Chemical modifications to DNA



C

5-mC

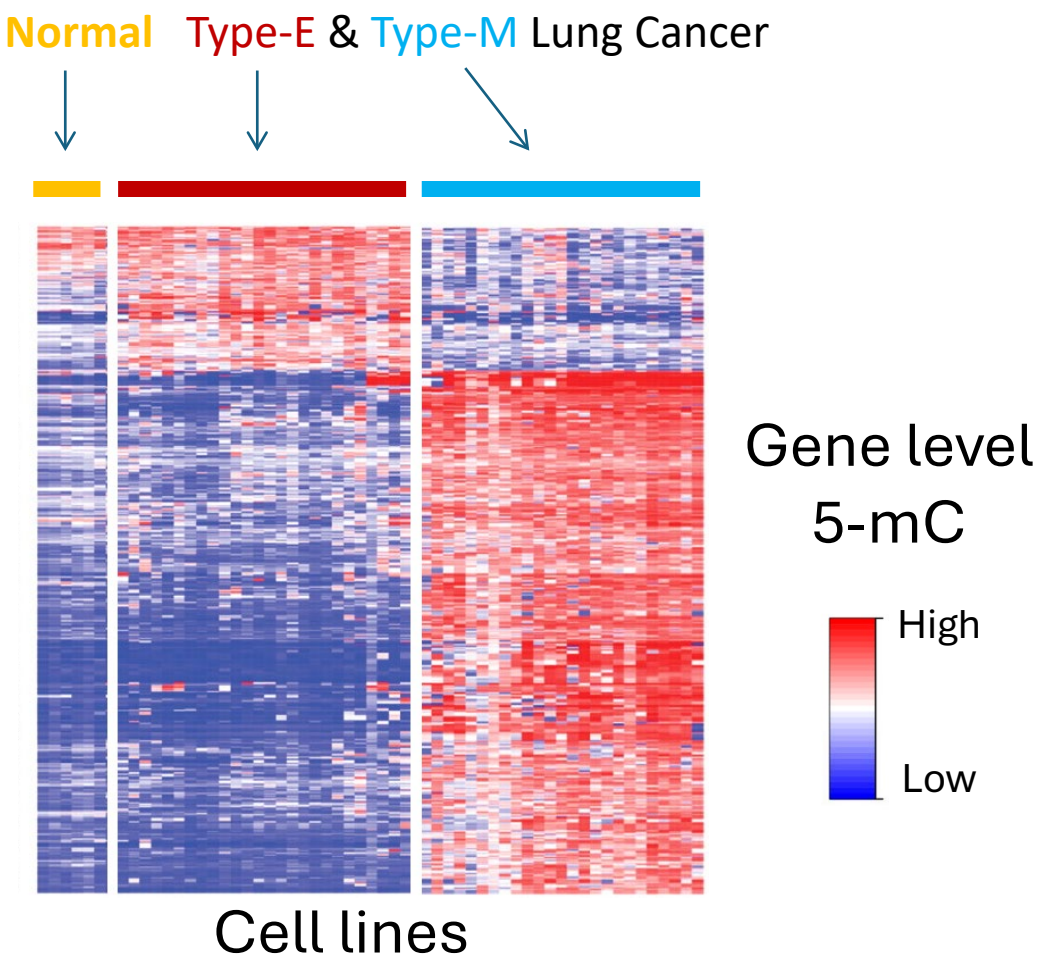
Extensively studied 5-mC

Development

Gene Silencing

© W. P. Armstrong 2000

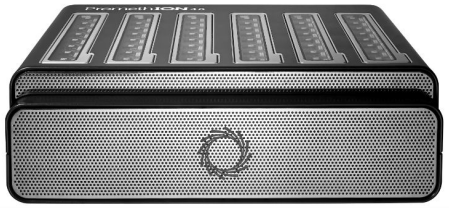
Diseases



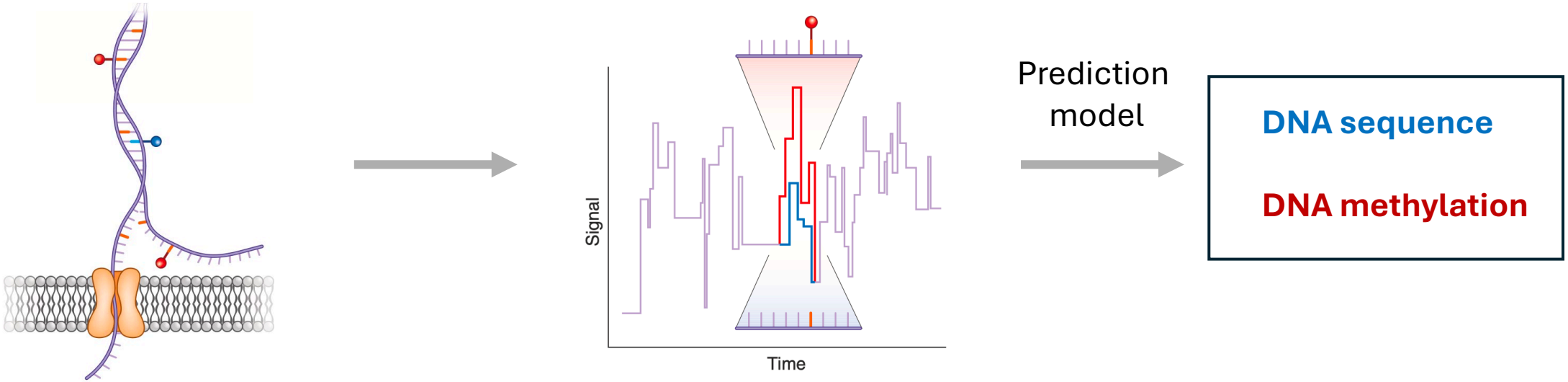
Walter et al. CCR, 2012

3rd Gen Sequencing:
Simultaneous profiling of DNA sequence and methylation

PromethION machine



- **Price:** comparable to Illumina sequencing
- **Throughput:** up to 290 Gb per flow cell
- **Read lengths:** up to >4Mb



(Michael Schatz, Nat Methods, 2017)

Datasets (Long read + EPIC + Gene expression)

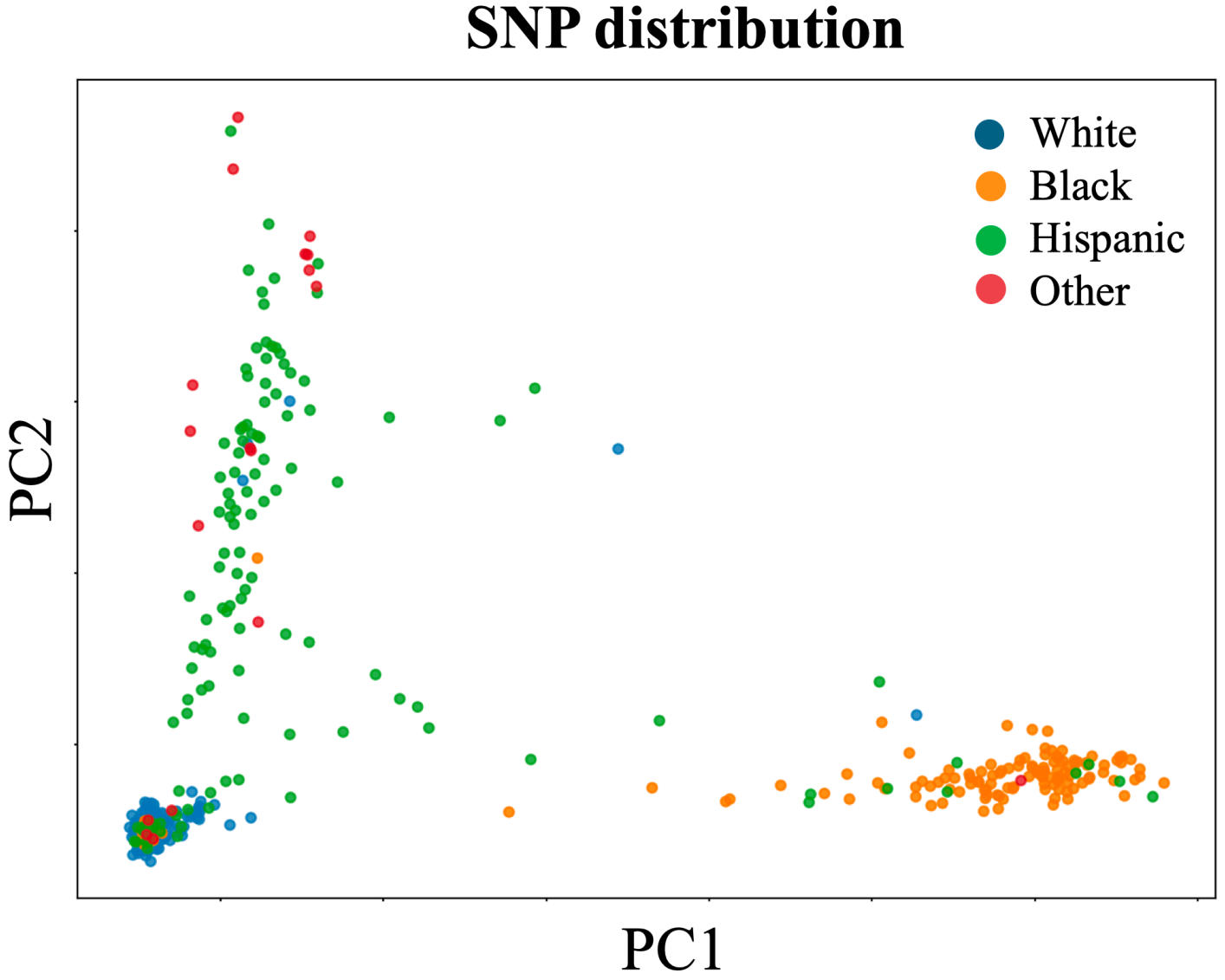
- **Human Retirement Study (HRS):**
  - Nanopore long read data (50 samples)
  - Illumina EPIC v1 data (50 samples + 4,000 samples)
  - Gene expression (50 samples + 4,000 samples)

- **High School and Beyond (HS&B:80):**
  - Nanopore long read data (510 samples)
  - Illumina EPIC v2 data (510 samples)

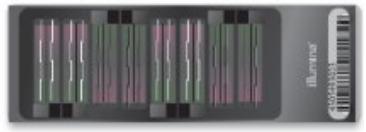
LR variant calling (HS&B:80)

Variant	Subtype	Count
SNP	Indel	2,622,021
	SNV	575,719
SV	Insertion	14,081
	Deletion	9,948
	Duplication	6080

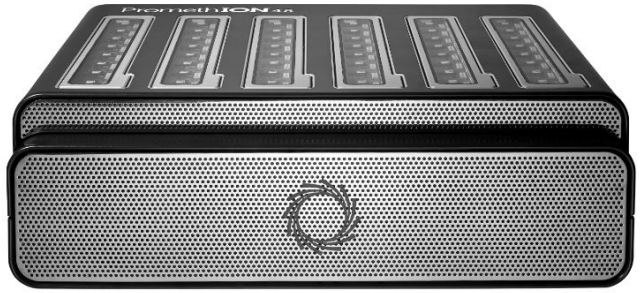
(epi2me/wf-human-variation)



Illumina MethylationEPIC BeadChip



Nanopore PromethION



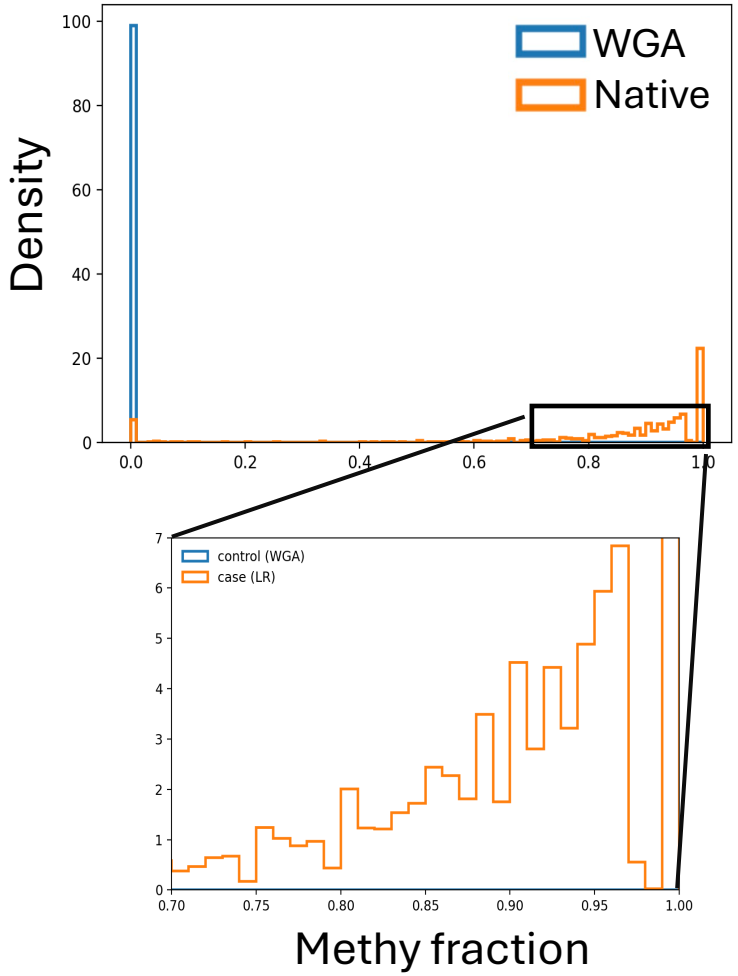
	Illumina methyaltionEPIC v1	Nanopore long read
Detection method	Bisulfite conversion	Ionic current
Information	Only methylation	WGS + methylation
Throughput	>850k (annotated enhancer, promoter, etc)	Whole methylome
Methylation type	5mC	5mC, 5hmC, 6mA

How accurate is the long-read DNA methylation calling?

Accuracy (1): LR prediction model

- **WGA: negative control with no methylation**

5mC



6mA

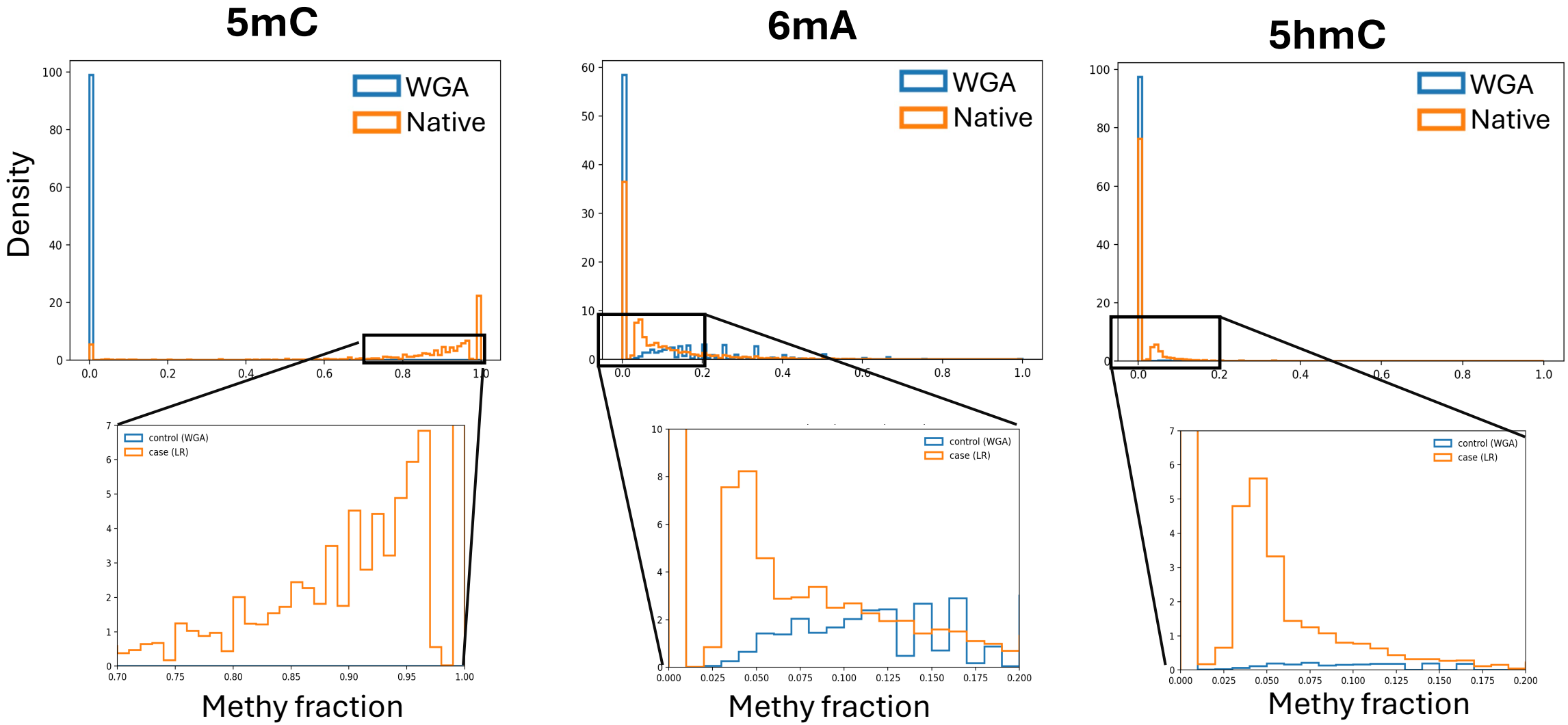
Existence of 6mA in multicellular organisms, including human, is still controversial

5hmC

Low accuracy of 5hmC detection

Accuracy (1): LR prediction model

- **WGA: negative control with no methylation**



Accuracy (2): Consistency between platforms

Datasets (Long read + EPIC + Gene expression)

- **Human Retirement Study (HRS):**
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  - Gene expression (50 samples + 4,000 samples)

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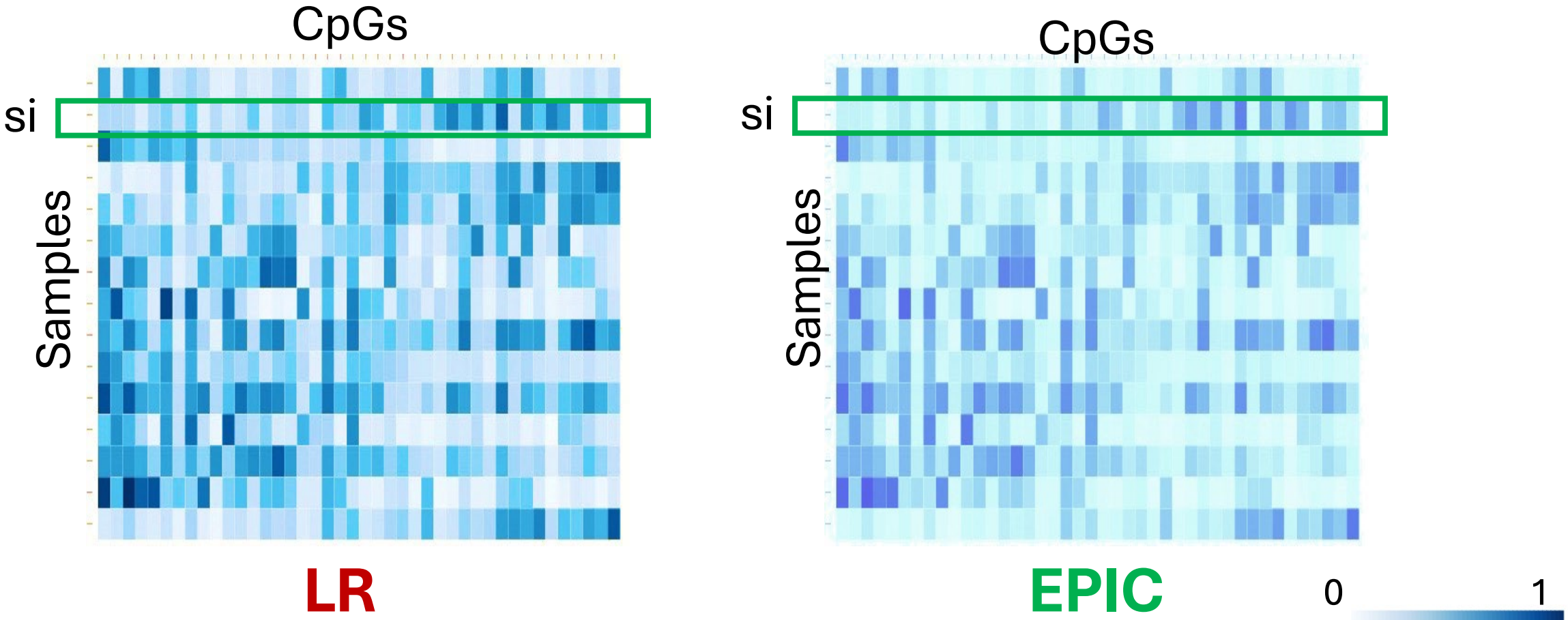
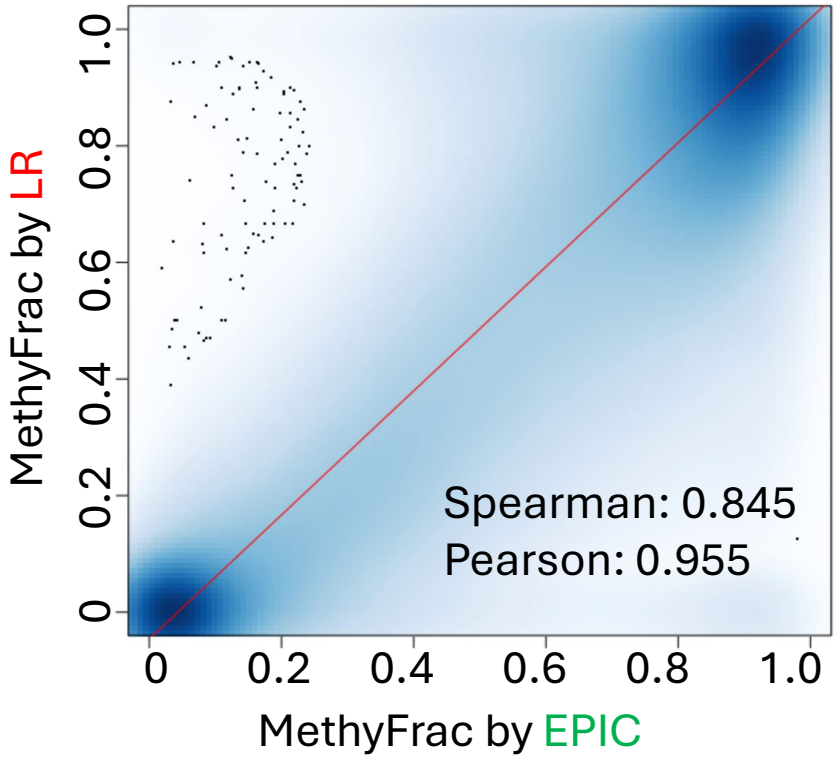
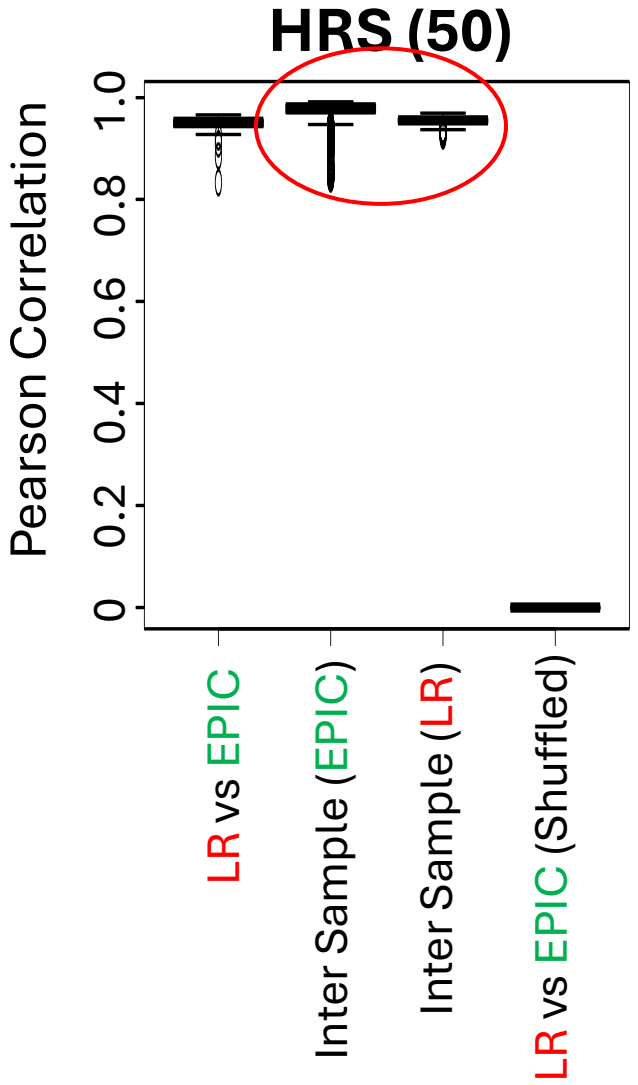
5mC platform comparison (Sample-level)



5mC platform comparison (Sample-level)

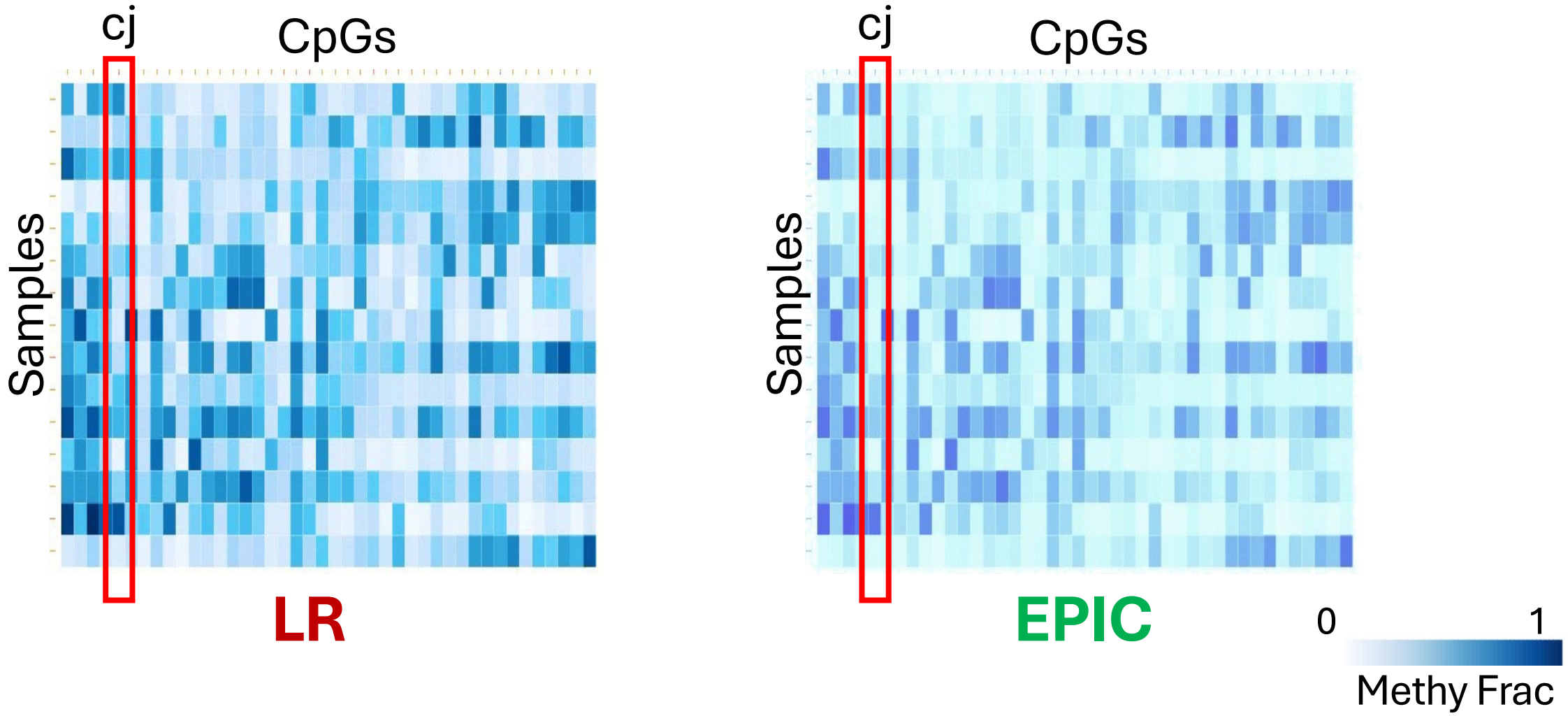


~830k overlap between LR and EPIC



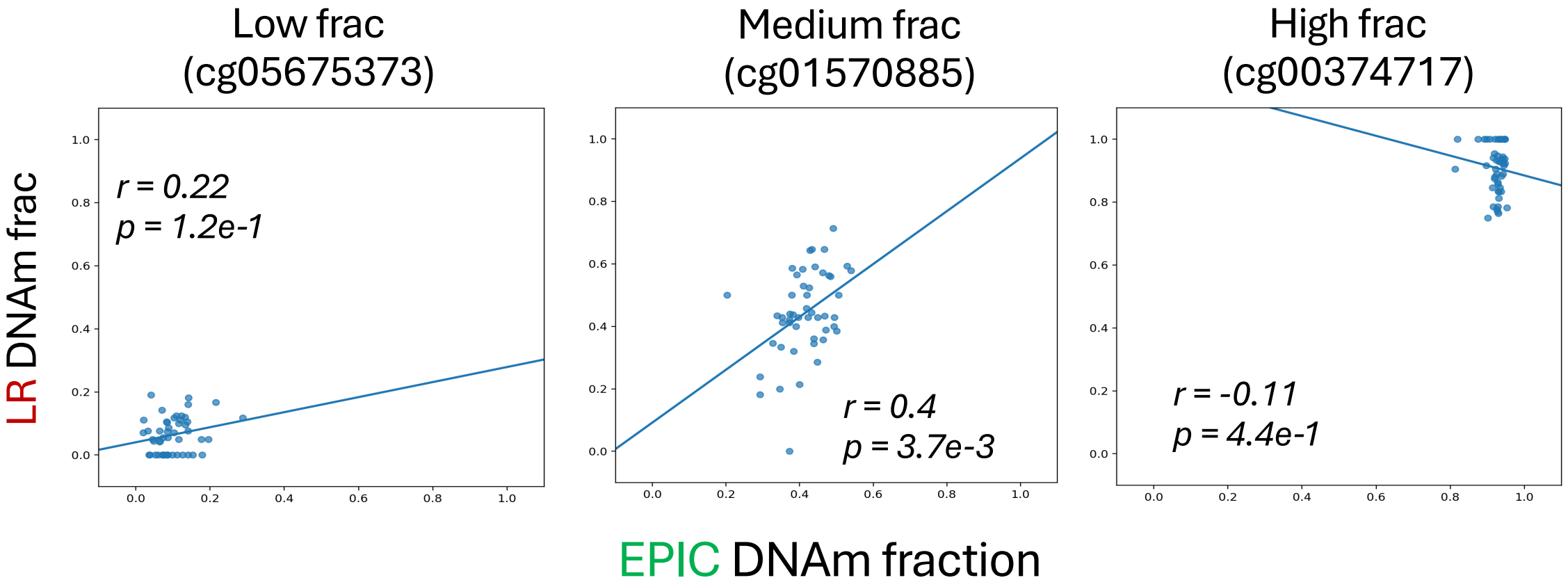
Correlation of each sample Meth fraction between LR and EPIC across all CpG sites

5mC platform comparison (CpG-level)

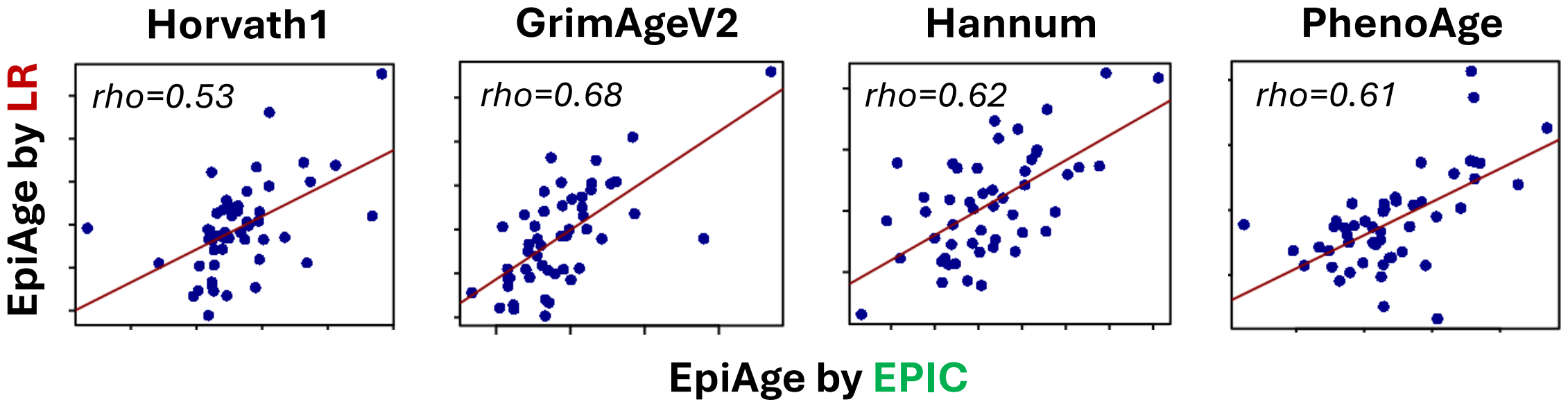


5mC platform comparison (CpG-level)

- Three representative CpGs from Horvath clock

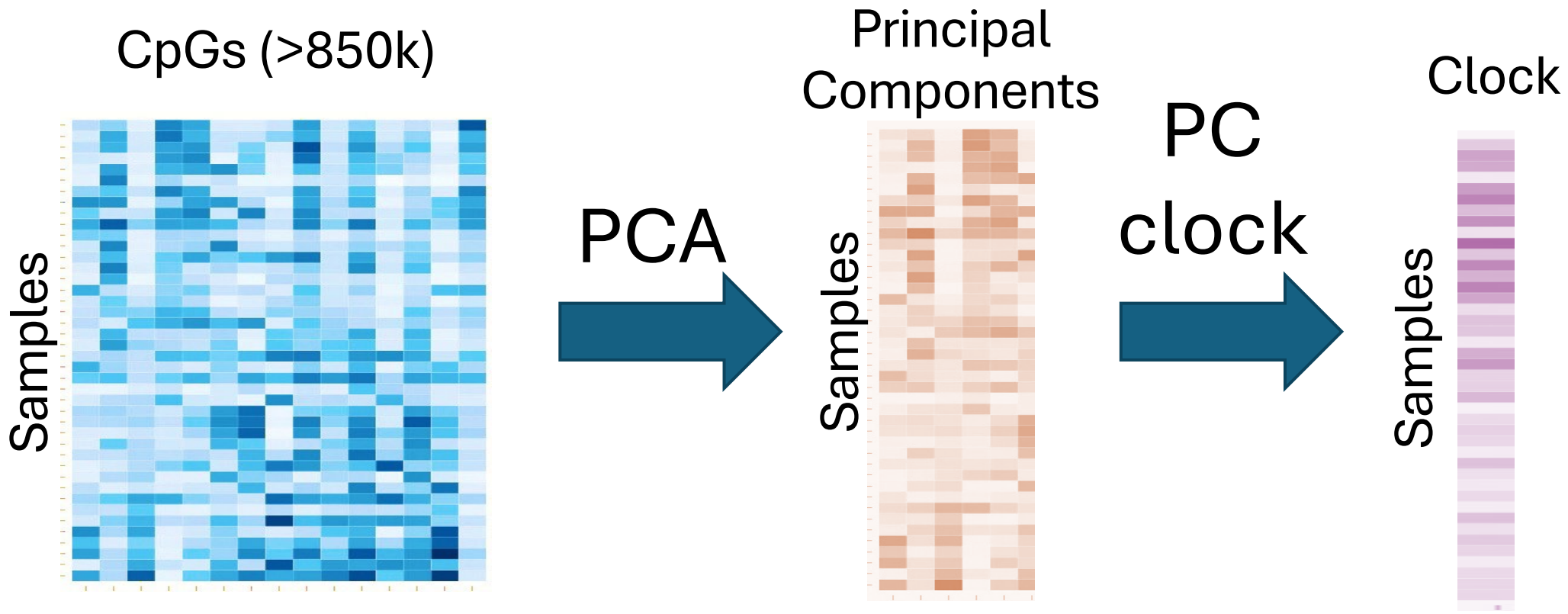


CpG-level inconsistency impacts the downstream analysis



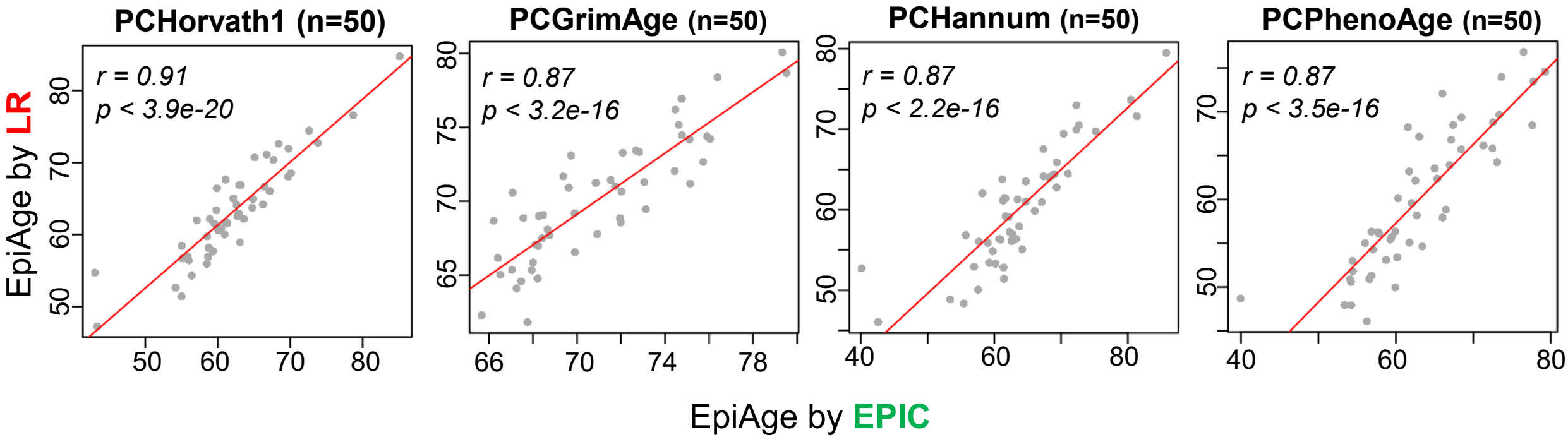
How to improve the consistency?

PC clock improves the consistency

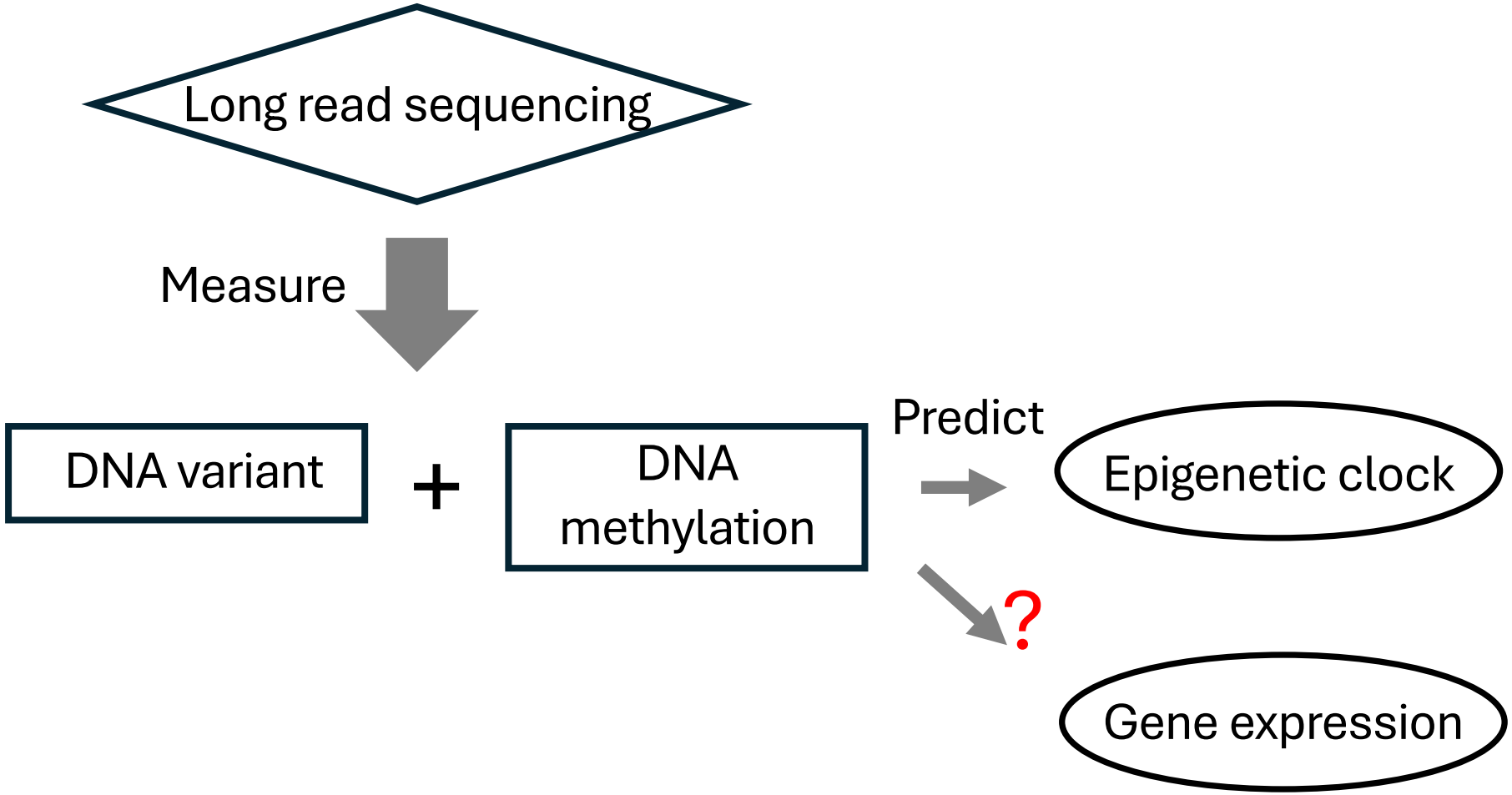


(Higgins-Chen, et al., Nature Aging, 2022)

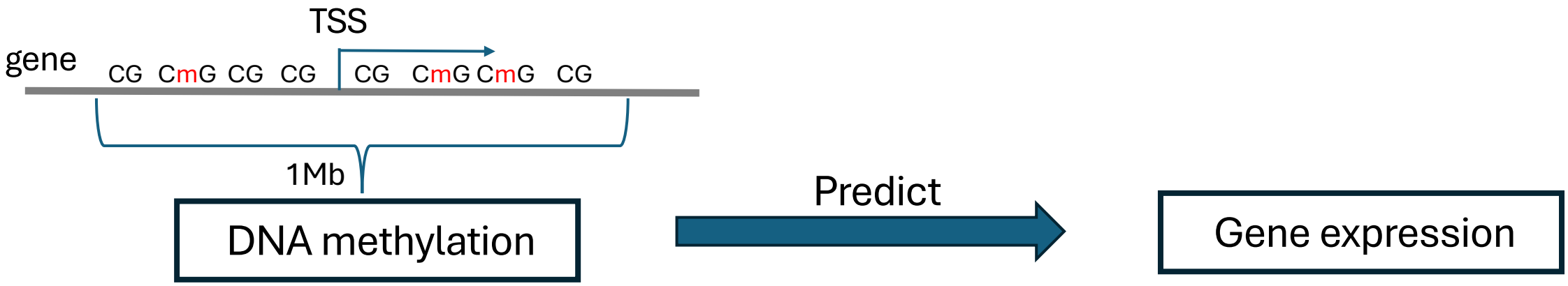
PC clock improves the consistency



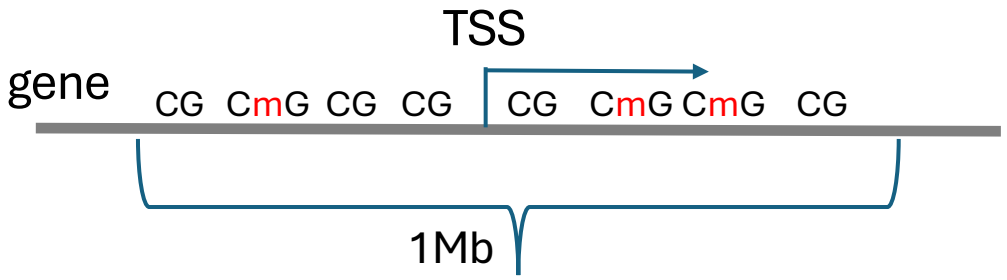
Summary (1)


Long read sequencing
Measure
DNA variant
+
DNA methylation
Predict
Epigenetic clock
?
Gene expression

Transcriptomics prediction



Transcriptomics prediction



DNA methylation

PCA

DNA methylation
Principal components

Predict

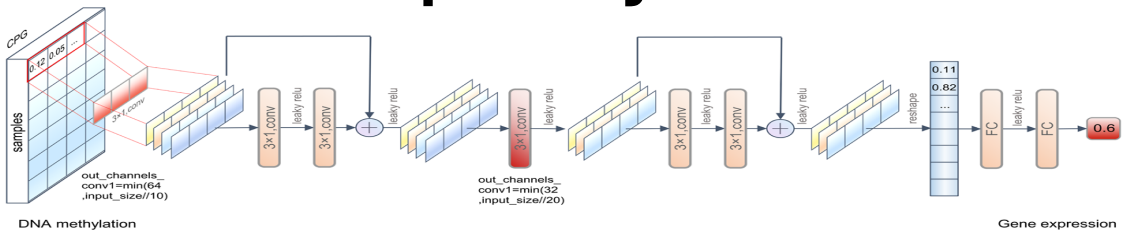
Gene expression

Training dataset

(~4,000 HRS samples)

- **Exposure**: DNAm by EPIC
- **Outcome**: Gene expr by RNAseq

DeepMethyGene

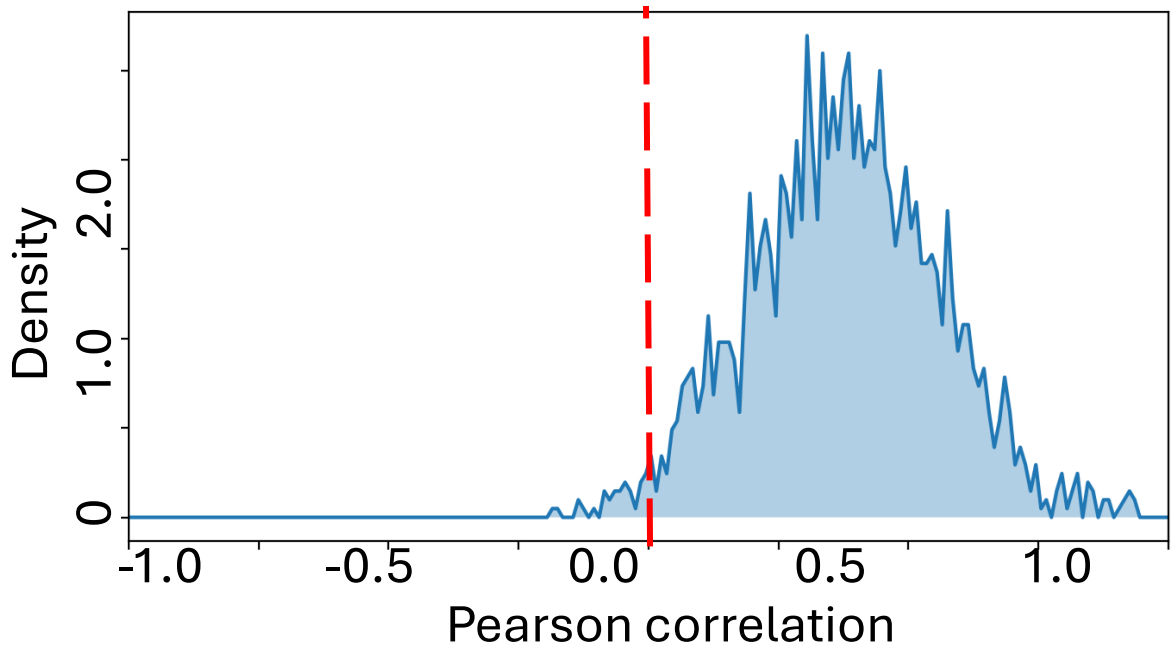


An adaptive recursive convolutional neural network model (Yan, et al., BMC Bioinformatics, 2025)

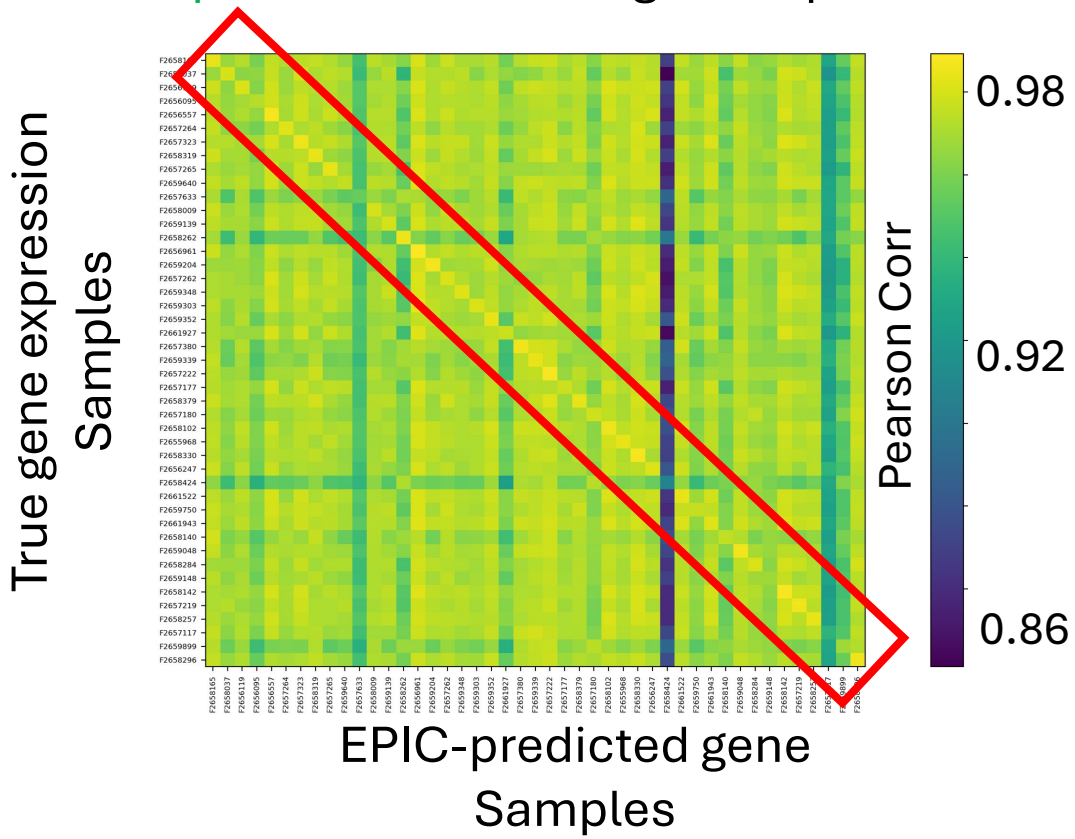
Transcriptomics prediction (same platform testing)

- **Trained on EPIC (~4,000 samples) → tested on EPIC (50 samples)**

Per-gene correlation between EPIC-predicted and **true** gene expression (>16,000 genes)

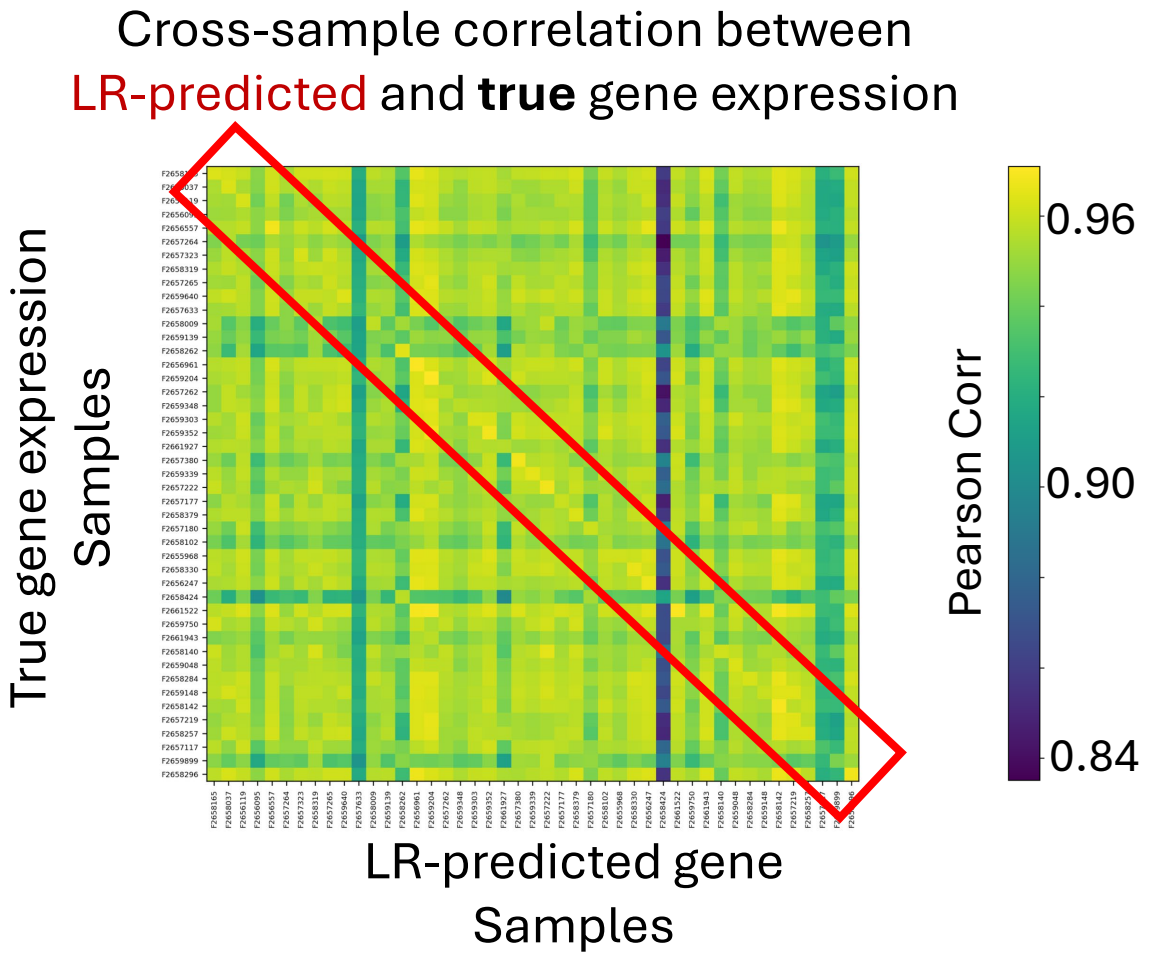


Cross-sample correlation between EPIC-predicted and **true** gene expression

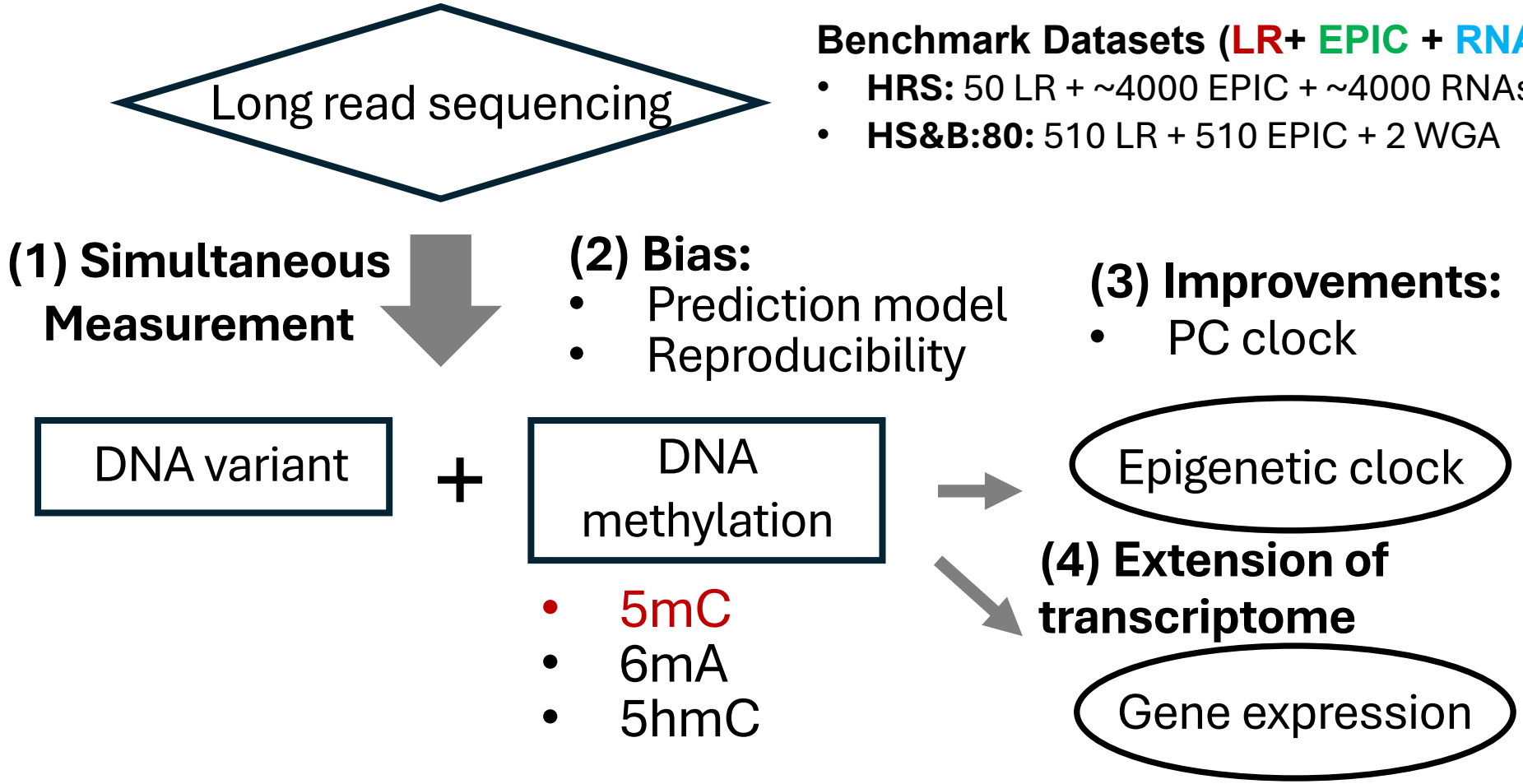


Transcriptomics prediction (platform transferring)

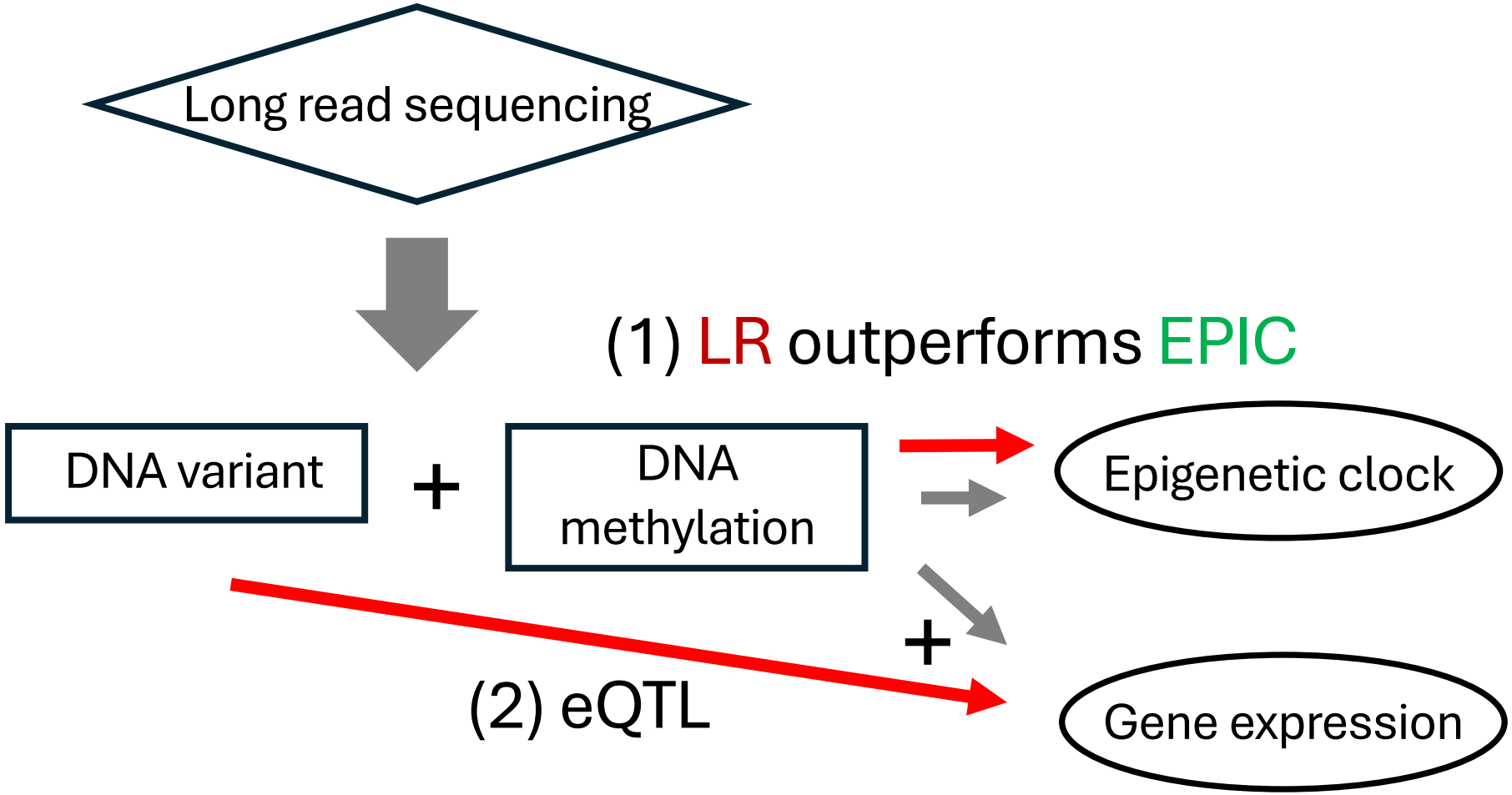
- **Trained on EPIC (~4,000 samples) → tested on LR (50 samples)**



Summary (2)


Long read sequencing
Benchmark Datasets (LR+ EPIC + RNAseq)
• HRS: 50 LR + ~4000 EPIC + ~4000 RNAseq
• HS&B:80: 510 LR + 510 EPIC + 2 WGA
(1) Simultaneous Measurement
(2) Bias:
• Prediction model
• Reproducibility
(3) Improvements:
• PC clock
DNA variant
+
DNA methylation
• 5mC
• 6mA
• 5hmC
Epigenetic clock
(4) Extension of transcriptome
Gene expression

Ongoing works



Acknowledgements

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High School and Beyond Study
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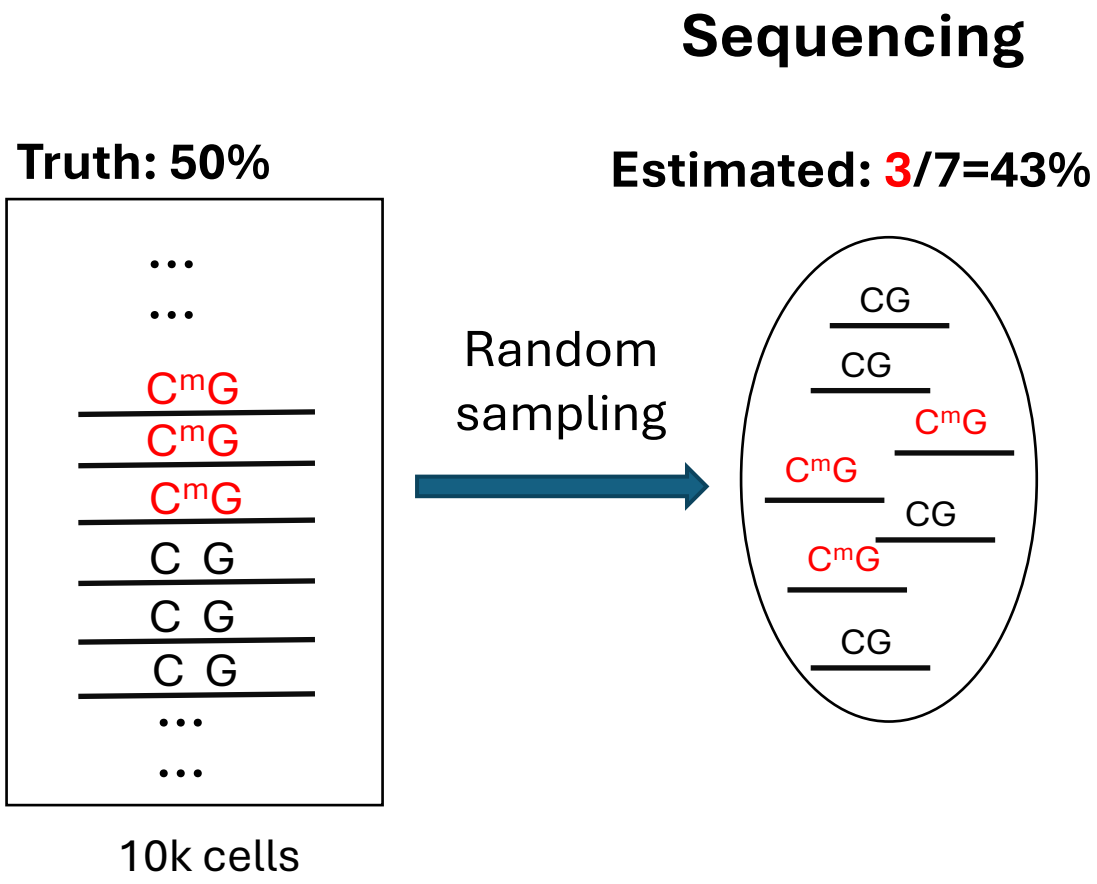
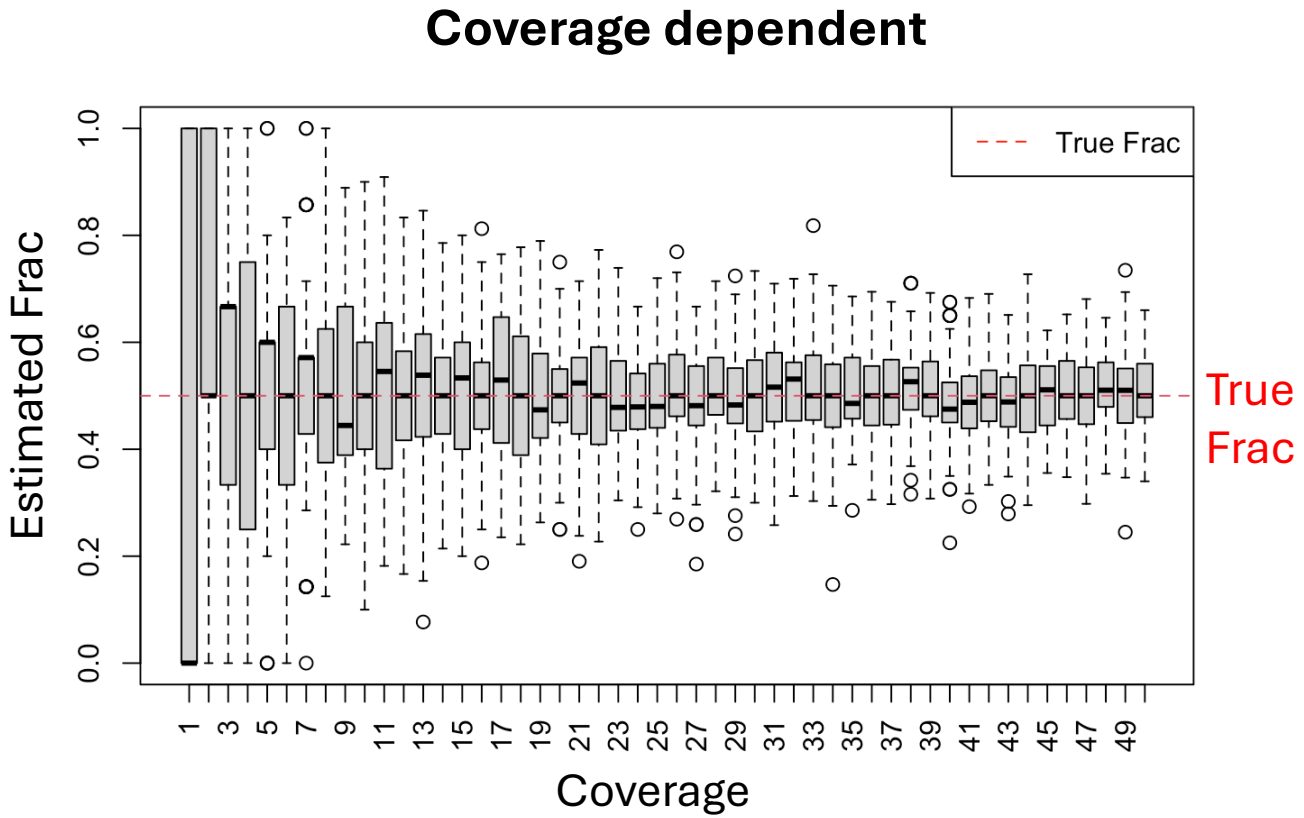
Chandra Muller
Adam Brickman
Jennifer Manly
Mateo Farina

Human Retirement Study
Eilleen Crimmins
Jessica Faul

Bias (2): LR read coverage

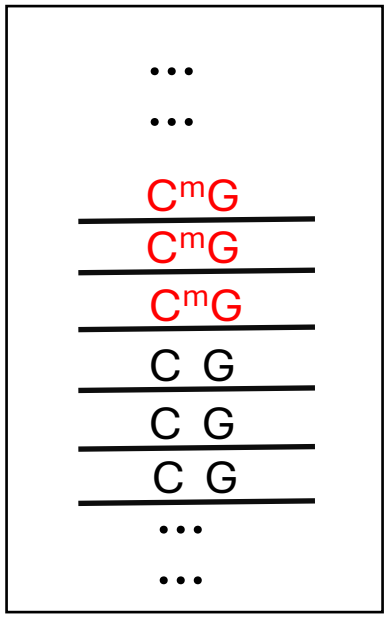




Bias (2): LR read coverage

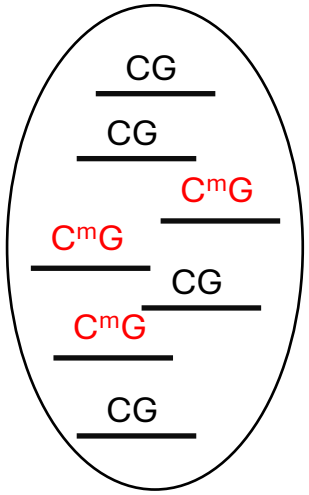
Sequencing

Truth: 50%



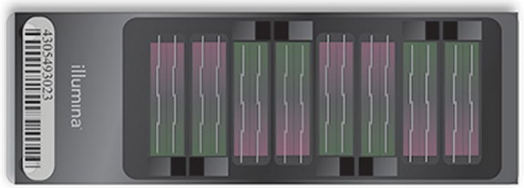
Random sampling

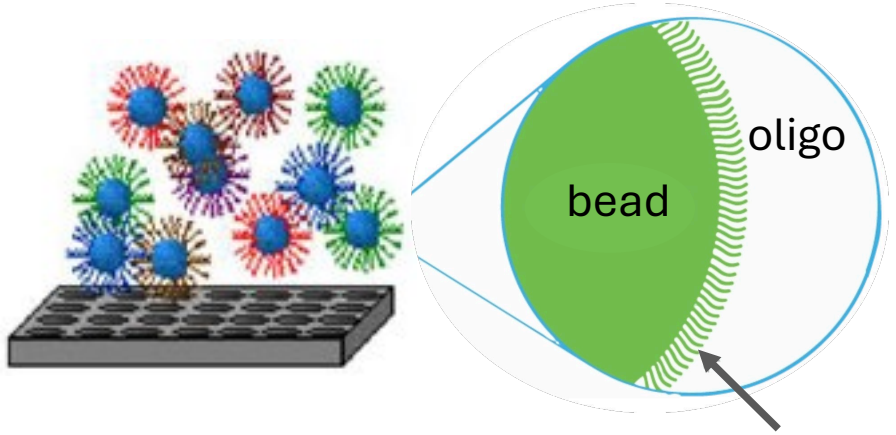
Estimated: 3/7=43%



VS.

BeadChip





Thousands of specific oligonucleotide probe to target a unique CpG site.