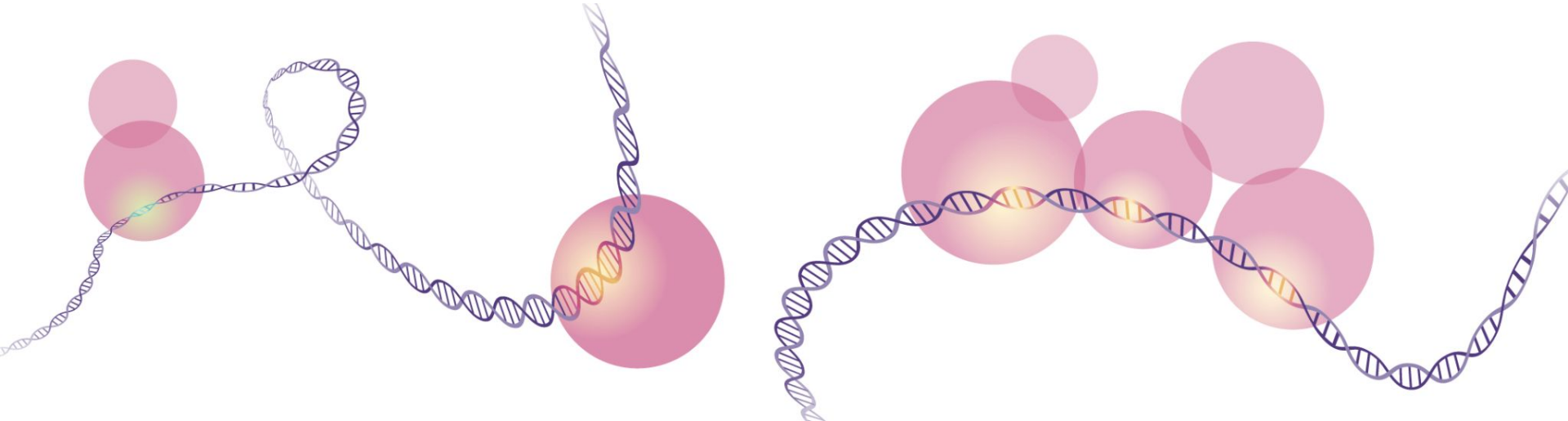
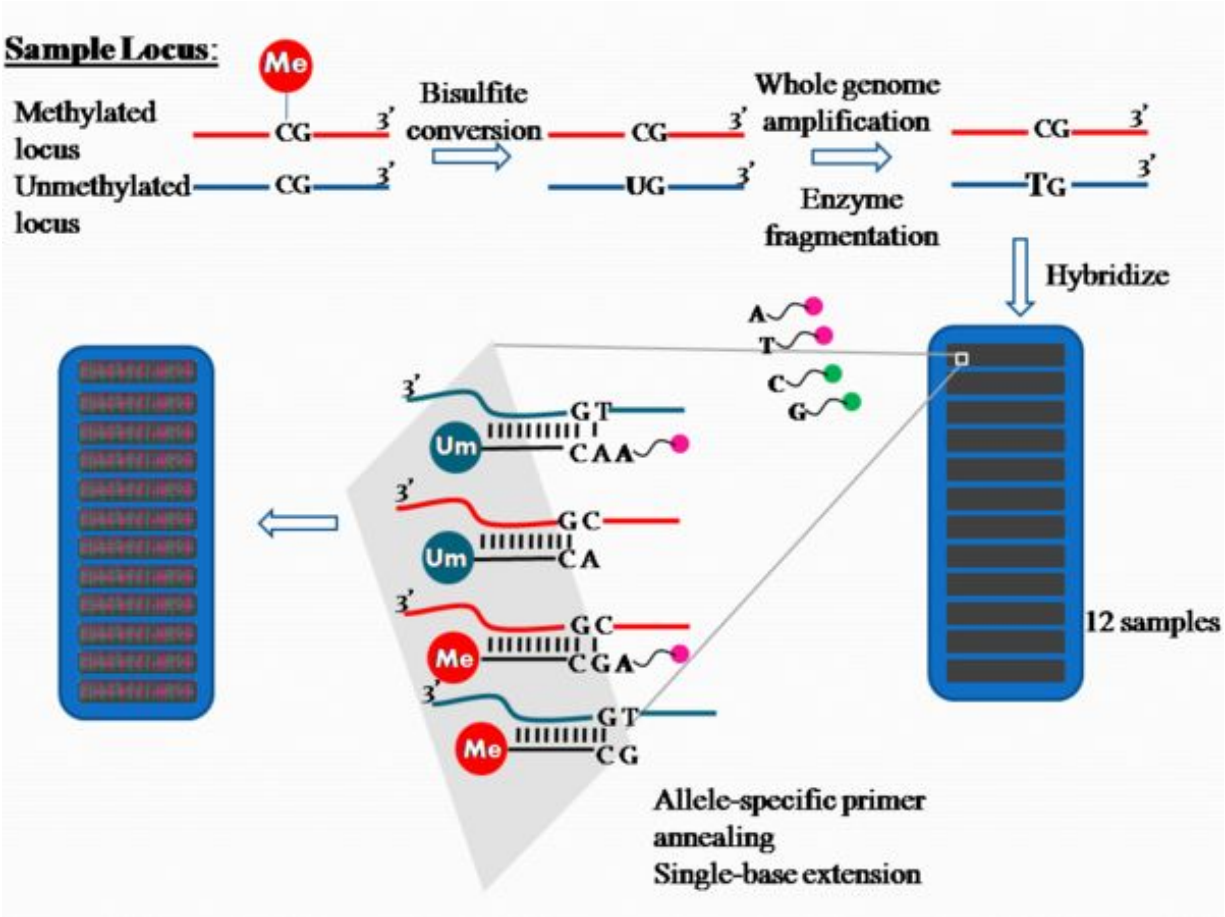
DNA methylation:

Illumina 27K Array

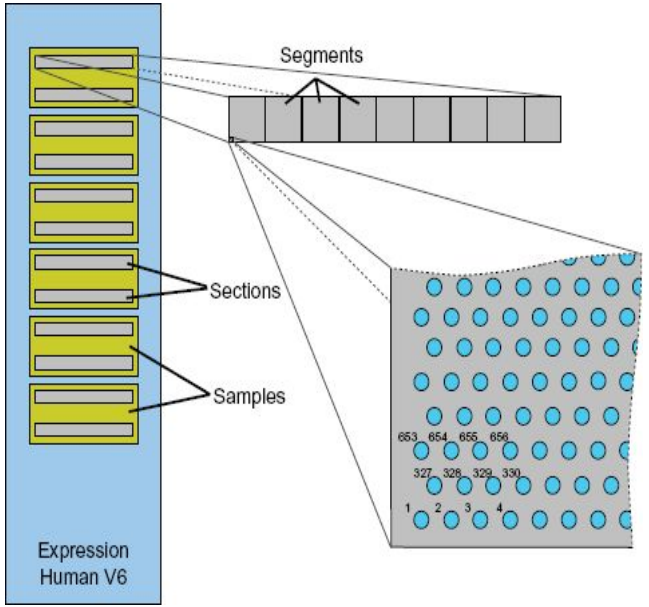
Ali Balubaid, Azari Bantan, Turki Sobahy



Workflow Overview



BeadChip Technology



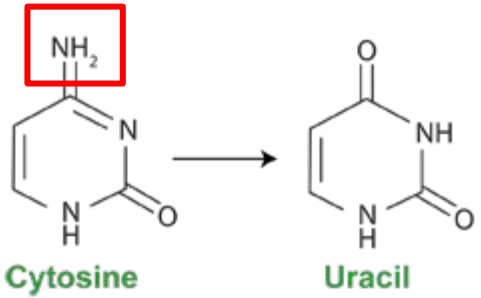
Bisulfite Conversion

CpG island

—--- ATTCTA**GC**

—--- TAAGAT**CG**

Bisulfite cytosine deamination



If methylated

—--- ATTCTA**GC**

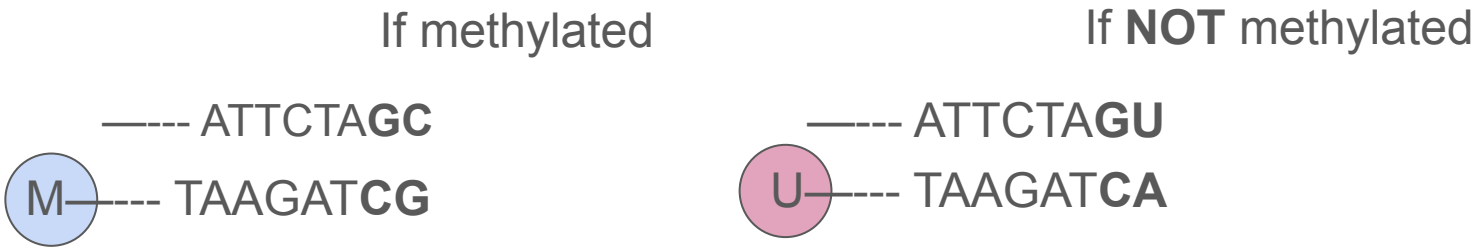
—--- TAAGAT**CG**

If **NOT** methylated

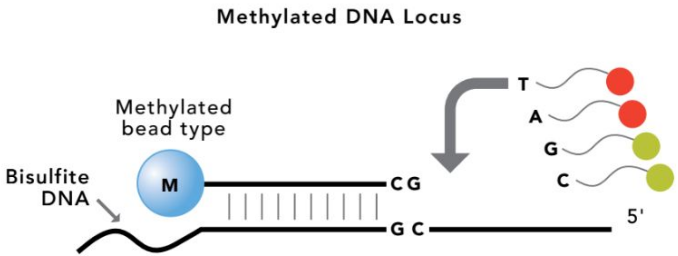
—--- ATTCTA**GU**

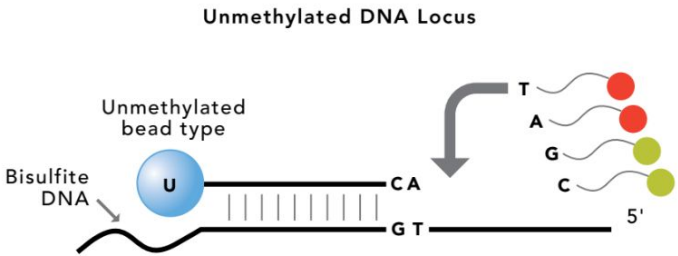
—--- TAAGAT**CG**

Fragment Hybridization



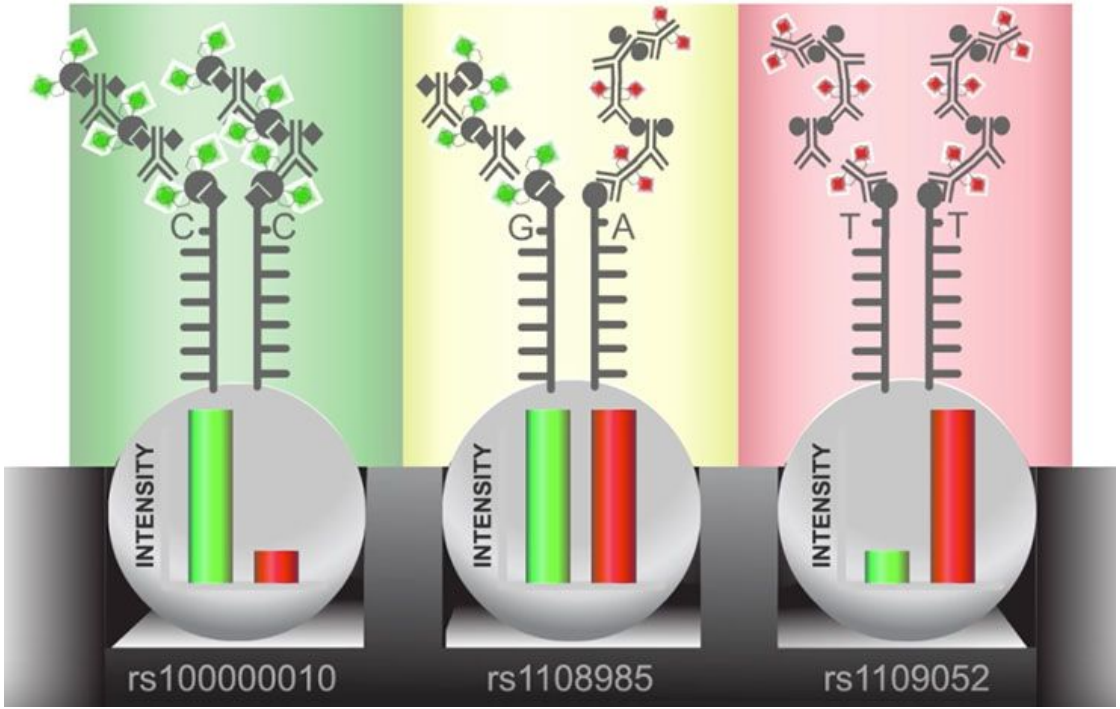
Fragment Extension





https://www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/appnote_dna_methylation_analysis_infinium.pdf

Allele Specificity



Illumina’s Infinium HumanMethylation27 BeadChip

27,578 CpG dinucleatides

14,495 genes

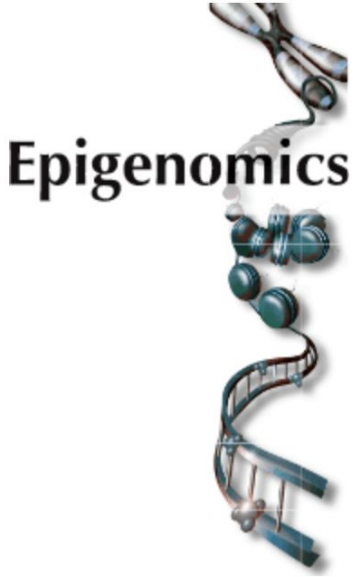
1 µg DNA required

12 sample concurrently

Original Paper

Future Medicine Ltd
Epigenomics
Volume 1, Issue 1, October 2009, Pages 177-200
https://doi.org/10.2217/epi.09.14

Technology Report



Genome-wide DNA methylation profiling using Infinium® assay

Marina Bibikova[1], Jennie Le[1], Bret Barnes[1], Shadi Saedinia-Melnyk[1], Lixin Zhou[2], Richard Shen[1] & Kevin L Gunderson[1,†]

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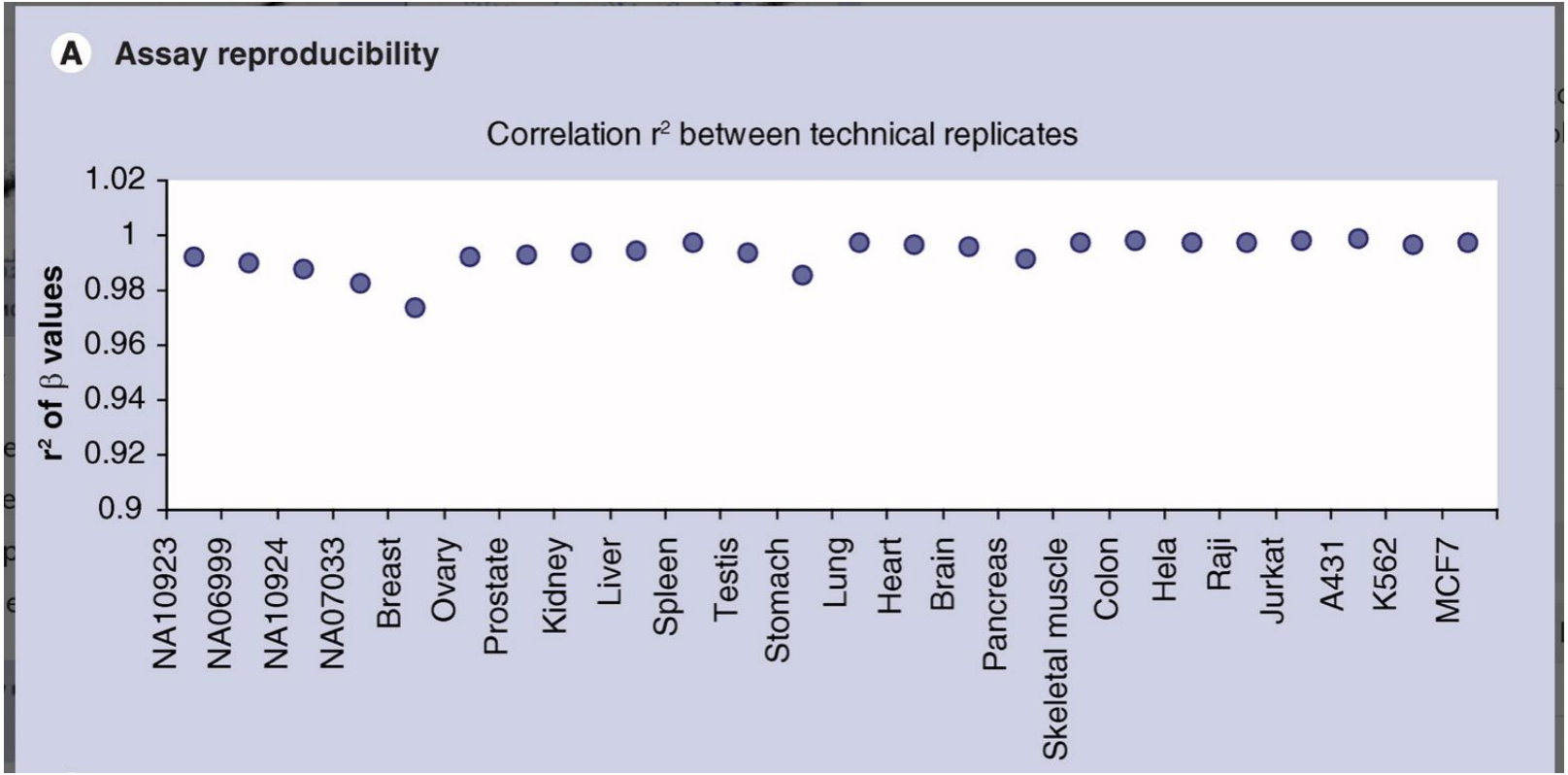
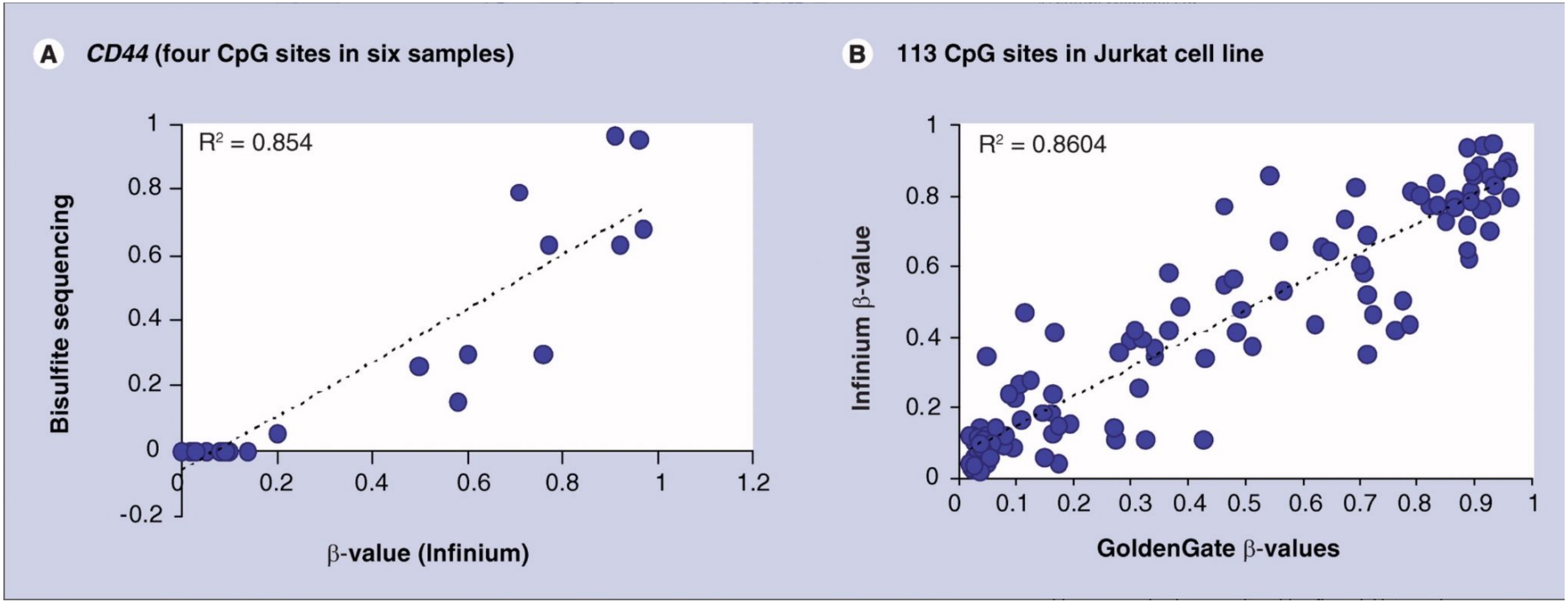
[†]† *Author for correspondence*

Is it reliable ?



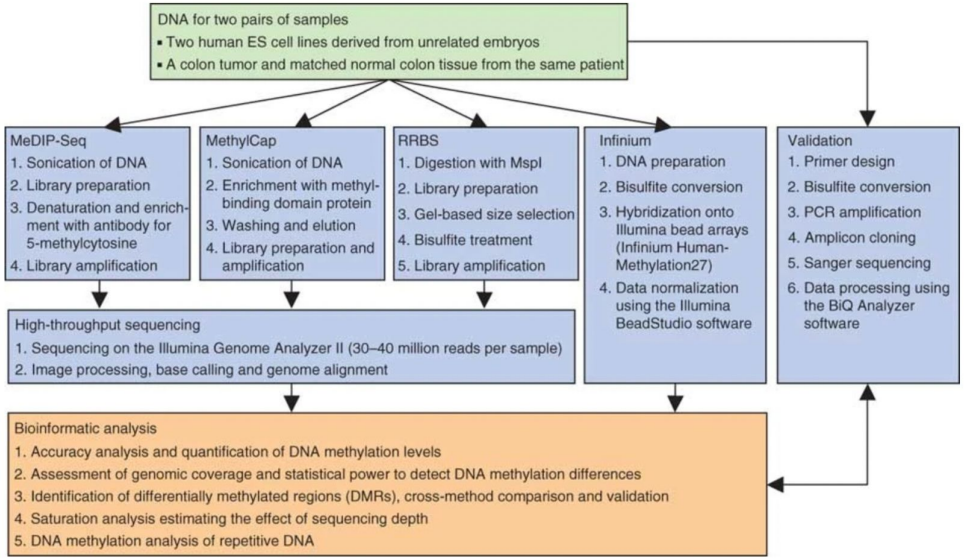
Is it reliable ?



Other comparisons

Figure 1: Outline of the DNA methylation technology comparison.

From: Quantitative comparison of genome-wide DNA methylation mapping technologies



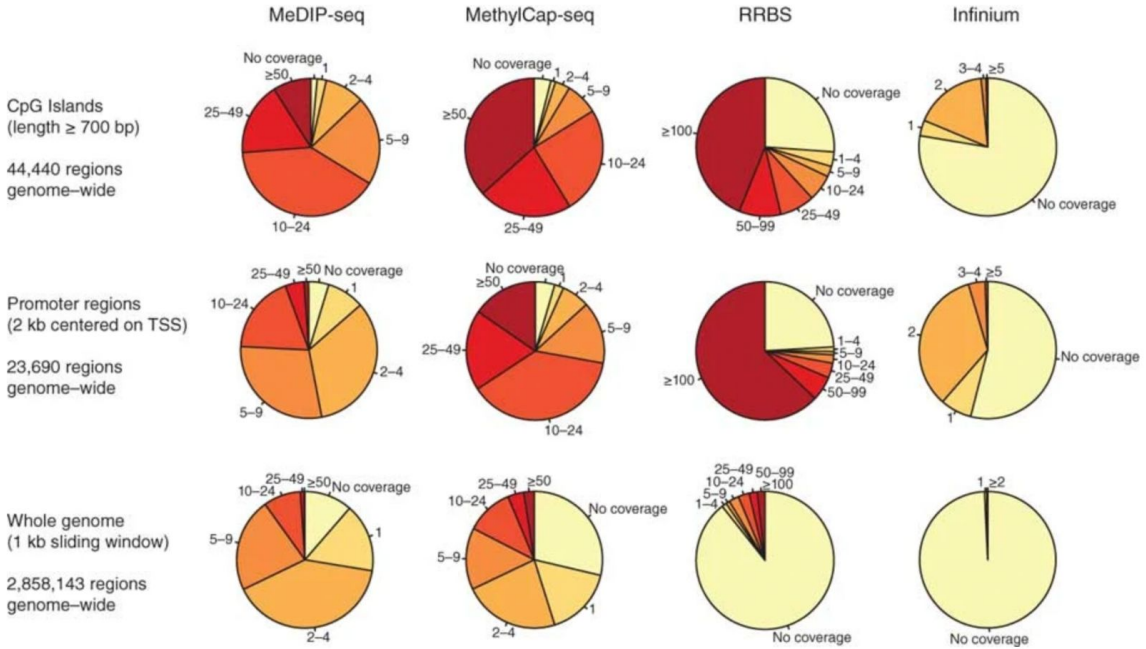
Four methods for DNA methylation mapping were compared on two pairs of samples. The resulting 16 DNA methylation maps were bioinformatically analyzed and benchmarked against each other. In addition, clonal bisulfite sequencing was performed on selected genomic regions to validate DNA methylation differences that were detected exclusively by one method.

https://www.nature.com/articles/nbt.1681

Genome coverage

Figure 4: Genomic coverage of MeDIP-seq, MethylCap-seq, RRBS and Infinium.

From: Quantitative comparison of genome-wide DNA methylation mapping technologies

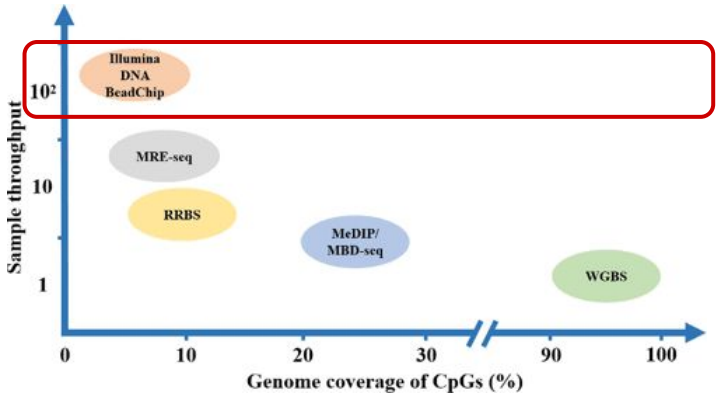


https://www.nature.com/articles/nbt.1681

Illumina 27K Array

Promises

- Reliability
- Sample throughput



Challenges

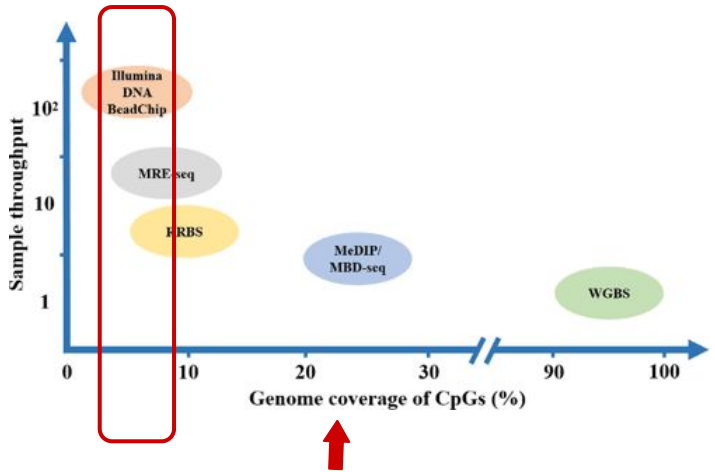
Illumina 27K Array

Promises

- Reliability
- Sample throughput

Challenges

- Coverage (limited to 27k CpG sites; 0.1% of total CpGs)



* 450K (1.7% of total CpGs), EPIC (3% of total CpGs)

Li, S. and Tollefsbol, T.O. *DNA methylation methods: Global DNA methylation and Methylomic analyses*, *Methods.* (2021)

Illumina 27K Array

Promises

- Reliability
- Sample throughput

Challenges

- Coverage (limited to 27k CpG sites; 0.1% of total CpGs)

Table 1: Markers of the HumanMethylation27 BeadChip.[3]

Type of target	CpG sites present	Avg # of CpG sites per target
RefSeq Genes	14,475	1.9 sites
Well-annotated genes described in the NCBI CCDS database (Genome build 36)	12,833	1.9 sites
Methylation hotspots in cancer genes	144	7.6 sites
Cancer-related targets	982	1.9 sites
miRNA promoters	110	2.3 sites

Illumina 27K Array

Promises

- Reliability
- Sample throughput

Challenges

- Coverage (limited to 27k CpG sites; 0.1% of total CpGs)
- Sources of biases

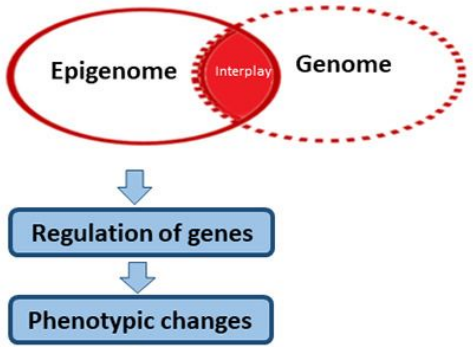
Table 1

Potential sources of bias for different DNA profiling approaches.

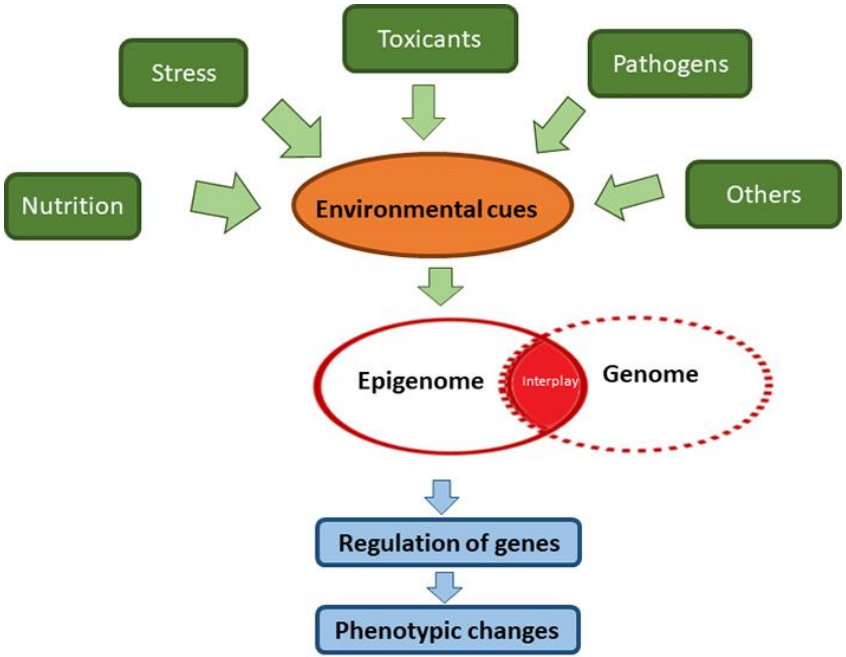
Source of bias	Bisulfite conversion-based		Microarray-based	Endonuclease digestion-based	Affinity enrichment-based	
	WGBS	RRBS	Illumina DNA BeadChip	MRE-seq	MeDIP-seq	MBD-seq
Incomplete bisulfite conversion	√	√	√			
Post-bisulfite conversion PCR	√	√	√			
Cross-hybridization			√			
Fragment size variation				√		
Copy number variation					√	√
CpG density					√	√

Li, S. and Tollefsbol, T.O. *DNA methylation methods: Global DNA methylation and Methylomic analyses*, *Methods.* (2021)

DNA methylation technology …



DNA methylation technology …



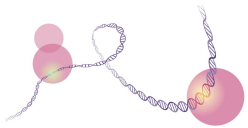
DNA methylation technology …

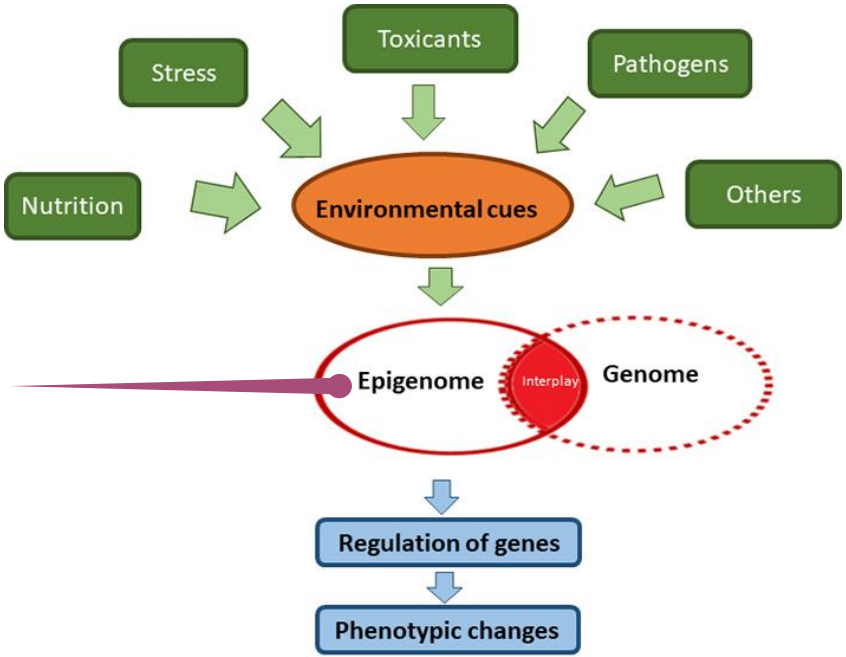
Insights into Epigenetic Regulation

Methylation patterns as biomarkers

Therapeutic targets

DNA methylation studies





DNA methylation technology …

Modifications can be both stable and dynamic

Data Integration is still crucial

DNA methylation studies