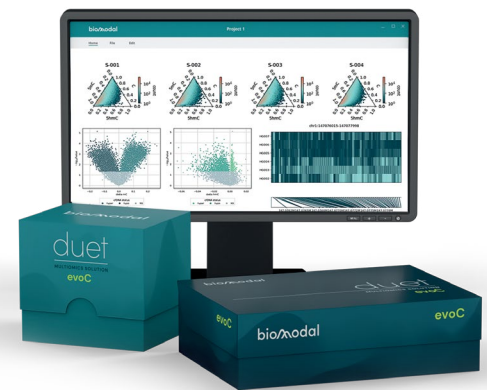


# Understanding the value of DNA Methylation data

From global methylation patterns to differentially methylated regions, high-resolution data can help you get more from your studies

The human genome defines what a cell can be, but it's the epigenome that dictates what a cell becomes. Whether it's in health or disease, the genome's influence only extends as far as the epigenome allows it to. Detailing epigenetic patterns and how they differ among cells can reveal valuable biomarkers, uncover mechanisms of disease, and expose fundamental processes in human genetics.

Among the most studied epigenetic marks are **5-methylcytosine (5mC)** and **5-hydroxymethylcytosine (5hmC)**. Though only a hydroxyl group apart, these two modifications have distinct biological roles and implications. Having the ability to resolve between them opens new possibilities in the study of development, disease, and cellular identity.



**Seeing that methylation patterns have changed is a start. Resolving 5mC and 5hmC allows you to go further, revealing the direction and meaning of that change.**

## What Can You Learn from Global Methylation Data?

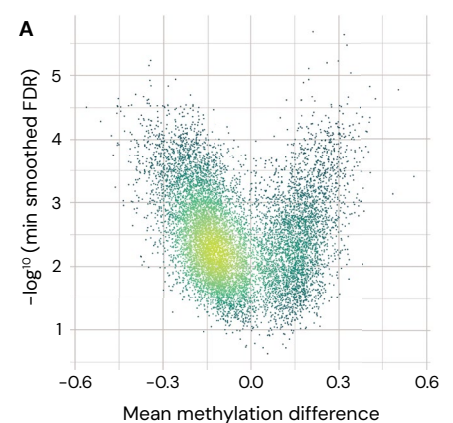
Like a tell-tale fingerprint, changes in DNA methylation patterns can be informative on their own, regardless of their biological impact. Global methylation patterns can serve as valuable biomarkers. They are stable and often cell type-specific.

Studying global methylation patterns and how they change can enable:

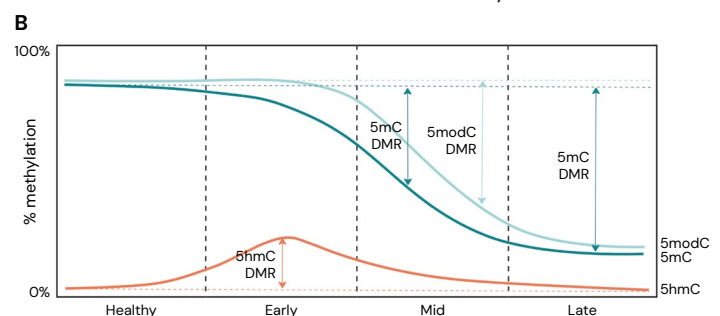
- Defining of cell identities and lineage
- Finer classification of disease states
- Stratification of patient subgroups
- Earlier disease detection

Even without mapping to genes, global shifts can signal that cells are undergoing large-scale reprogramming with biological consequence. Global hypomethylation is commonly detected in stage IV cancers, for example [1].

Importantly, differentiating between changes in 5mC and 5hmC can reveal distinct and complimentary insights.

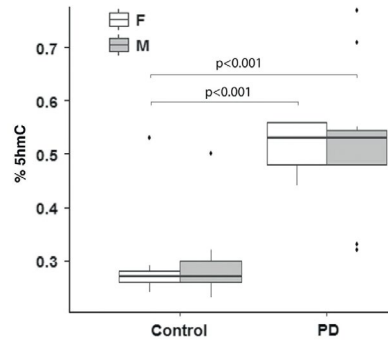


**Figure 1: Difference in global methylation:** (A) Volcano plot showing global changes in DNA methylation (5mC) between stage IV colorectal cancer and adjacent healthy tissue. The X-axis represents change in methylation as measured across different regions between the two samples, and Y-axis represents statistical significance. (B) Schematic showing the expected dynamics of global DNA methylation patterns at different stages of cancer. These dynamics have recently been observed in colorectal cancer, where early stage tumors showed a measurable increase in 5hmC levels with little change in 5mC levels. [2]



## Value of higher resolution global methylation data

In a 2017 study of Parkinson's disease, researchers found no difference in global 5mC levels between patients with or without Parkinson's disease. However, robust changes were detected in 5hmC levels, indicating 5hmC patterns may serve as Parkinson's specific biomarkers. [3]



**Figure 2: 5hmC comparison from [3]:** Quantitation of 5hmC in DNA of the cerebellum from age-matched control (n = 27; 11 females, 16 males) and PD individuals (n = 36; 12 females (F), 24 males (M)).

## Zooming in with DMRs

To move from signal to insight, researchers study **Differentially Methylated Regions (DMRs)**—defined regions with statistically significant methylation shifts.

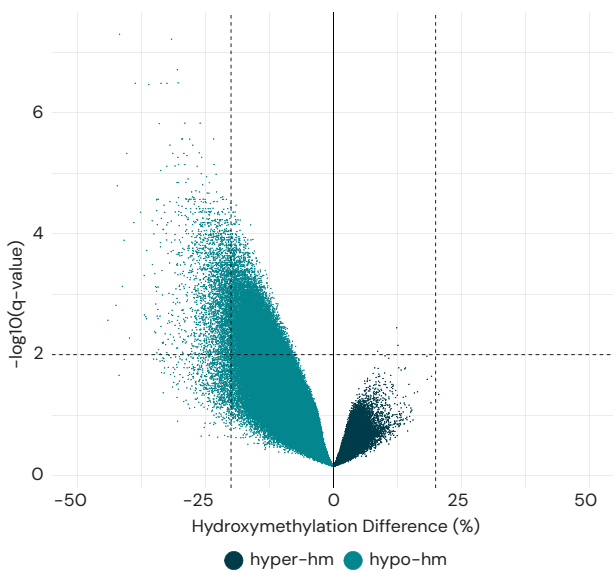
DMRs can be identified using:

- Agnostic approaches, which typically involve scanning tiled windows genome-wide. This is often useful for biomarker discovery, especially when little is known about the biology of the system under study.
- Focused, hypothesis-driven methods, wherein analysis is focused on known enhancers, promoters, or disease-relevant loci.

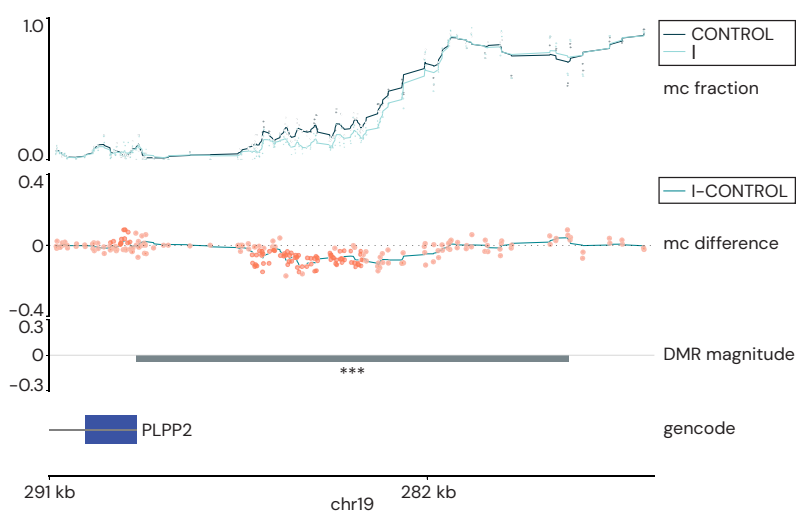
Most researchers iterate between both. Either way, resolving both 5mC and 5hmC reveals deeper biology—exposing genes that are actively changing from a silenced to active state.

**Whether you cast a wide net or start from a hypothesis, DMRs take you deeper.**

### A Enhancer DhMCs CRC Stage IV vs Healthy



### B Healthy Control vs Stage I – 5mc DMR



**Figure 3: DMR analyses in colorectal cancer (CRC)** (A) Volcano plot comparing hydroxymethylation status (5hmC) at gene enhancers between healthy volunteers and patients with stage IV CRC. (B) Track plot showing a promoter and transcription start site (TSS) upstream of the PLPP2 gene. Data compares 5mC methylation in Stage I CRC samples (light teal) and healthy samples (dark teal). On the second track, coral dots represent CpG-level differential methylation and the loci average (teal line). In tracks 3 and 4, the dark teal box represents the promoter's significant DMR and blue boxes represent PLPP2 exon 1.

## 6-Base Sequencing Unlocks More

Bimodal's 6-base sequencing simultaneously detects:

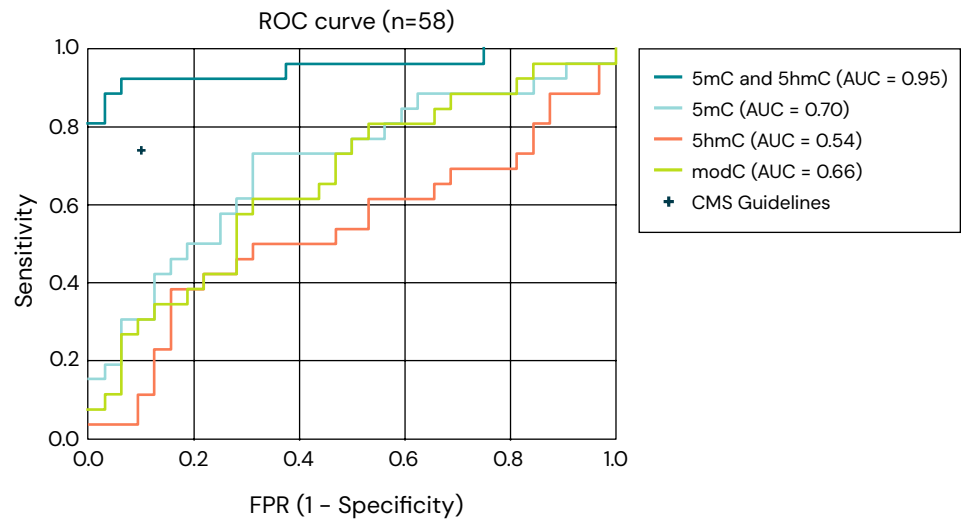
- All four canonical bases (A, C, G, T)
- 5mC (silencing-associated)
- 5hmC (often activation- or enhancer-associated)

This single workflow dataset allows you to:

- Detect regions of active demethylation
- Resolve overlapping or opposing methylation signals
- Identify subtle, disease-relevant changes

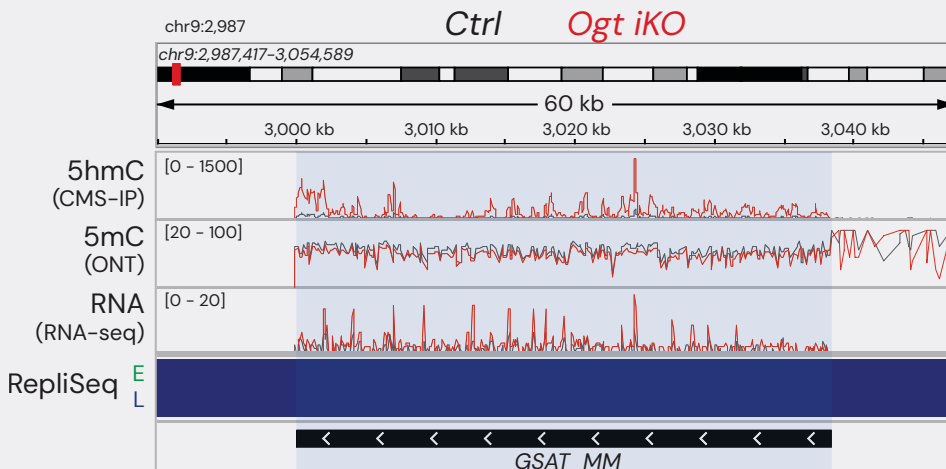
### Early CRC detection

Using TCGA-defined DMRs, researchers applied 6-base sequencing to cfDNA. Early-stage CRC showed 5hmC enrichment – signals missed by 5mC alone. The combined 5mC and 5hmC model achieved 85% sensitivity at 95% specificity. [2]



### Genome instability & TEs

In a study of OGT-deficient cells, global methylation changes were enriched at transposable elements (TE). 6-base sequencing revealed 5hmC DMRs linked to genome destabilization and inflammatory signaling, helping explain a mechanism of TE activation in cancer. [4]



**Figure 4: Changing methylation in transposable elements (TE):** Genome browser view showing increased 5hmC, decreased 5mC and increased expression at TE (GSAT\_MM major satellite) regions showing control (black) and OGT iKO (red). Increased expression was confirmed using total ribo-depleted RNA-seq.

1. Pfeifer, Gerd P., et al. "The Role of 5-Hydroxymethylcytosine in Human Cancer." *Cell and Tissue Research*, vol. 356, no. 3, 1 June 2014, pp. 631–641, <https://doi.org/10.1007/s00441-014-1896-7>
2. Fabio Puddu, et al. "5-Methylcytosine and 5-Hydroxymethylcytosine Are Synergistic Biomarkers for Early Detection of Colorectal Cancer." *BioRxiv (Cold Spring Harbor Laboratory)*, 31 Oct. 2024, <https://doi.org/10.1101/2024.10.30.621123>
3. Stöger, Reinhard, et al. "Elevated 5hmC Levels Characterize DNA of the Cerebellum in Parkinson's Disease." *Npj Parkinson's Disease*, vol. 3, no. 1, 1 Feb. 2017, <https://doi.org/10.1038/s41531-017-0007-3>
4. Sepulveda, Hugo, et al. "OGT Prevents DNA Demethylation and Suppresses the Expression of Transposable Elements in Heterochromatin by Restraining TET Activity Genome-Wide." *Nature Structural & Molecular Biology*, 28 Mar. 2025, <https://doi.org/10.1038/s41594-025-01505-9>

## Let's Talk

Interested in how biomodal's 6-base sequencing can support your research?

+44 (0) 1223 800 700

[info@biomodal.com](mailto:info@biomodal.com)

[biomodal.com](https://www.biomodal.com)



### Footnote

Publications demonstrate the enhancers detailed here regulate the genes MED8, ST3GAL3, CEP152, SHC4, DSG3, FCGR2C and RASSF9.

### Disclaimer

The duet multiomics solution is for research use only.

## biomodal

Chesterford Research Park  
Cambridge, UK  
CB10 1XL

+44 (0) 1223 800 700

[biomodal.com](https://www.biomodal.com)

[info@biomodal.com](mailto:info@biomodal.com)

