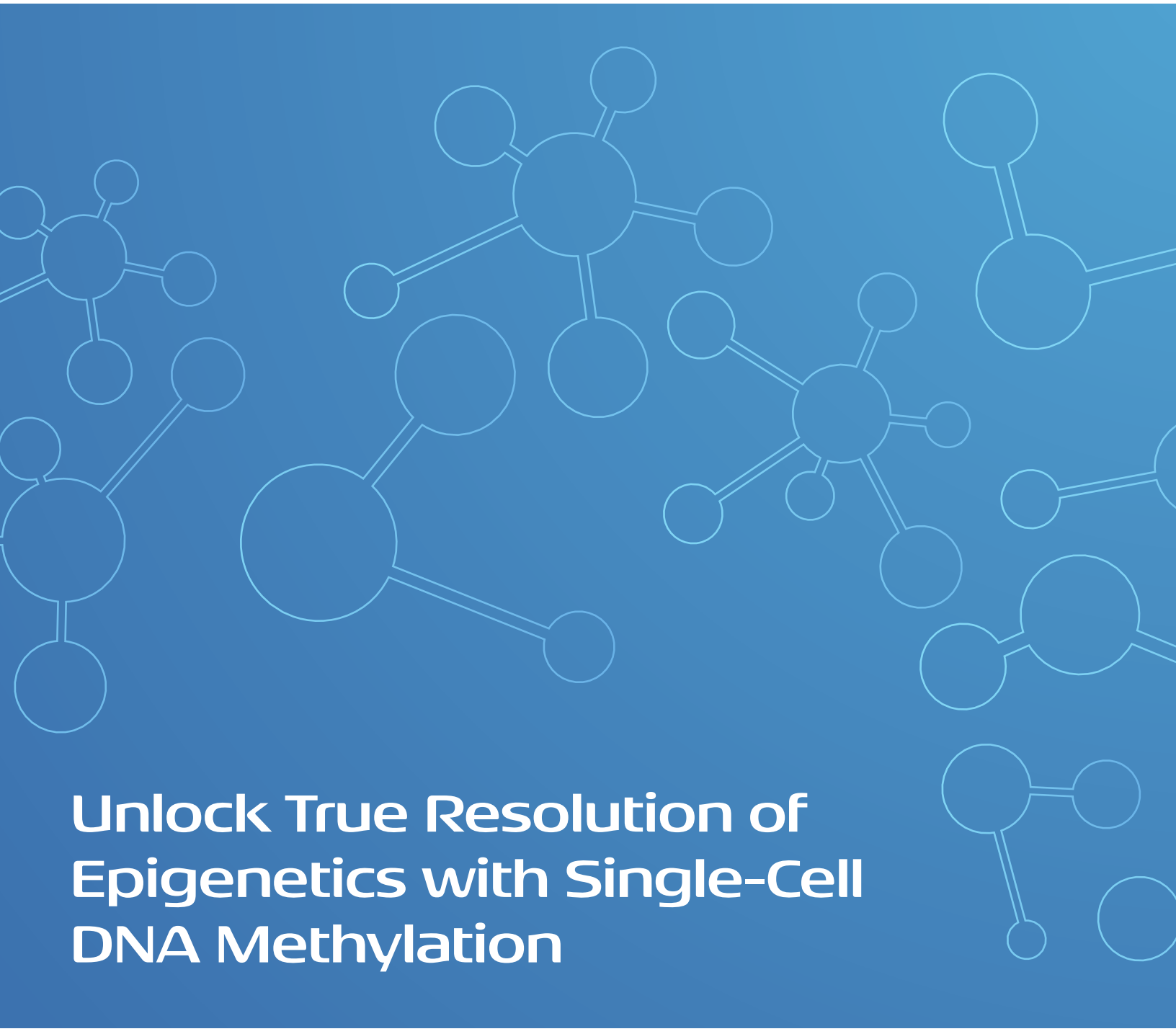
SCALE biosciences



Unlock True Resolution of Epigenetics with Single-Cell DNA Methylation

Scale.bio

Introduction

In recent years, the study of epigenetics and the role it plays in disease has increased dramatically.

DNA methylation is the most widely studied epigenetic modification and is marked by the chemical modification of cytosine by a methyl group, most typically in a CpG dinucleotide context. It influences the binding of proteins to DNA and regulates gene expression (Moore 2013). DNA methylation is essential for cellular differentiation and can be altered by environmental conditions (Suelves 2016). As CpG methylation is heritable during cell division, it serves as a record of cellular history.

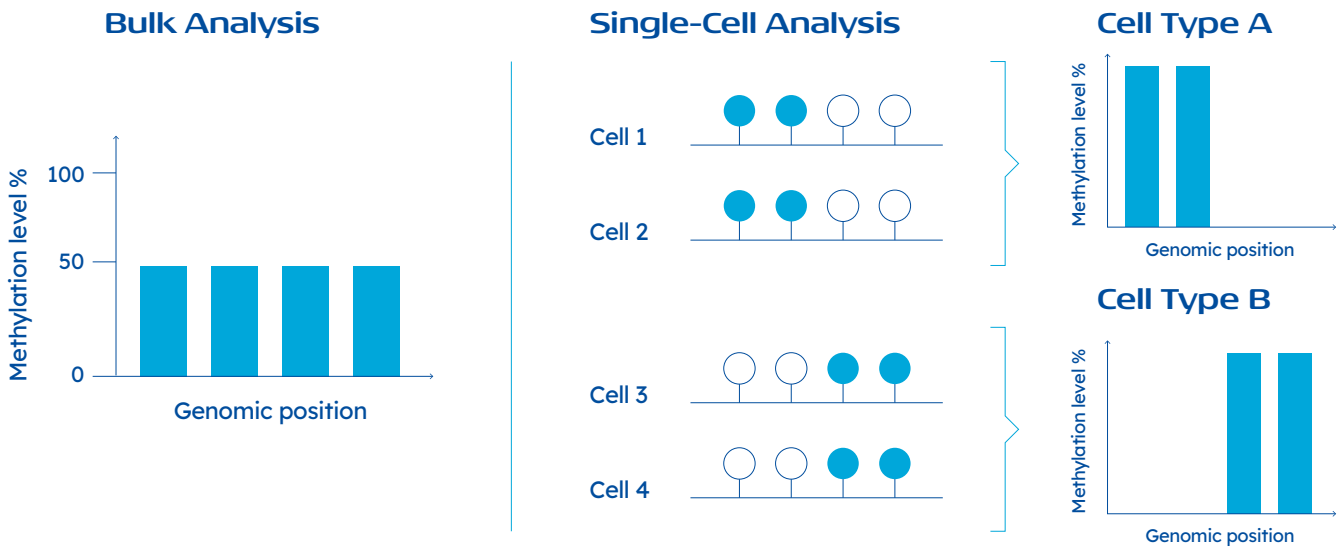
Analysis of DNA methylation is a powerful tool to uncover epigenetic regulation during cellular development and disease. Aberrant DNA methylation patterns are present in diseases such as cancer, and can reveal potential therapeutic targets. It is also a useful biomarker due to its stability and detection in plasma and other bodily fluids (Li 2022).

The most common methods for analyzing methylation, however, profile cells in bulk. Although this approach has revealed important biological insights, bulk analysis has limited ability to detect cell type-specific methylation patterns and can mask critical changes in subpopulations of cells **(Figure 1)**. While computational deconvolution methods can help, they are often limited by the need for reference datasets and may not be able to identify novel or unknown cell types or cellular states. Consequently, single-cell resolution of DNA methylation analysis can provide novel epigenetic insights.

In this primer, we'll review:

- The importance of DNA methylation in cancer and neuroscience research
- How single-cell analysis is critical to gain deeper biological insights in methylation studies

Figure 1. Advantages of single cell methylation analysis. Compared to bulk analysis, which represents an average DNA methylation level across all cells in a sample (left), single cell analysis enables resolution of cell type- and state-specific methylation patterns (right).

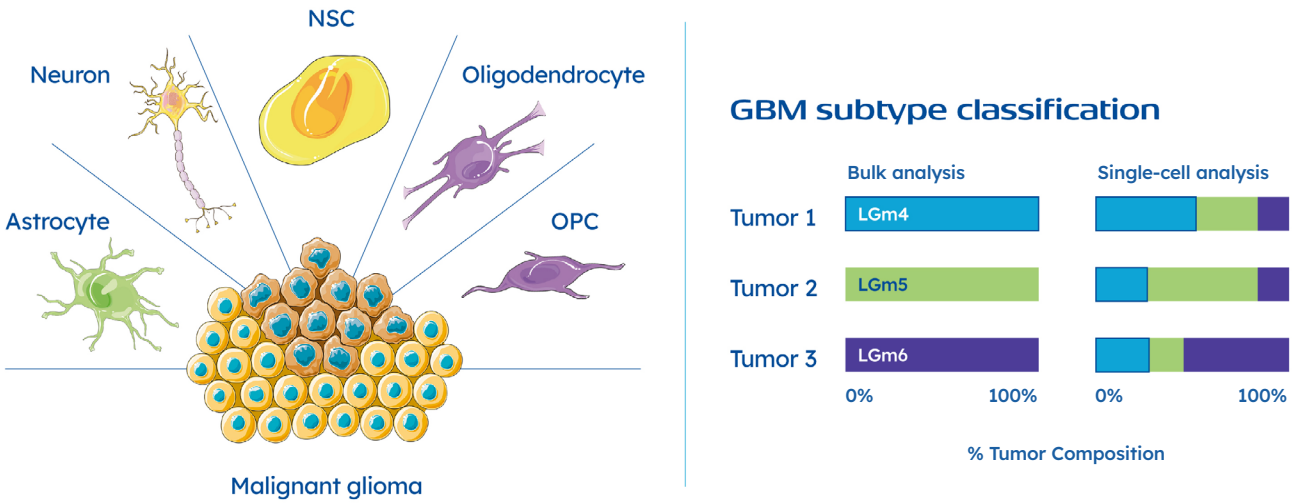


Role of DNA methylation in cancer biology

Altered DNA methylation is a hallmark of many cancers (Jones 2007). Changes in DNA methylation occur early in tumorigenesis and drive tumor progression, metastasis, and resistance to therapy. Tumor genomes are characterized by global hypomethylation, leading to genomic instability, while CpG islands become hypermethylated, leading to silencing of tumor suppressor genes (Ng 2015, Feng 2019, Ozturk 2022). In addition, aberrant methylation patterns have been identified as potential prognostic biomarkers in numerous tumor types (Guo 2019, Ding 2019) and as predictive biomarkers for response to chemotherapy or immunotherapies (Sigin 2020, Xu 2021, Gu 2019, Filipski 2021). Therefore, DNA methylation analysis is highly valuable for both mechanistic and translational inquiries.

A major challenge to deriving biological insights through DNA methylation analysis concerns cellular resolution: while many studies use bulk methylation analysis to characterize tumors, malignant and normal cells are often present together in the same sample **(Figure 2)**. Additionally, malignant cells may be present as different subclones with distinct genetic backgrounds and molecular states. Therefore, bulk analysis may obscure cancer-associated methylation patterns due to averaging of methylation signals across the sample. Reliance upon deconvolution techniques to extrapolate bulk average values is limited by requiring knowledge of cellular populations and proportions, and risks introducing artifacts or losing subclones. As tumor heterogeneity is thought to be a key driver of tumor resistance to therapies (Dagogo-Jack 2018), resolving DNA methylation signals across heterogenous tumor samples is of critical importance.

Figure 2. Tumors reflect a complex mixture of cell types and states. Previous efforts classified gliomas using bulk-level analysis (LGm4-6 subtypes, Ceccarelli et al 2016). With more recent single-cell data, individual tumors show DNA methylation patterns that reflect a mix of tumor subtypes, likely as a result of intratumor cellular heterogeneity (Chaligne 2021).



New approaches to unravel tumor heterogeneity

Higher cellular resolution approaches are needed to help untangle the challenge posed by tumor heterogeneity. In glioma, for instance, single-cell DNA methylation studies identified the epigenetic regulators of distinctive cell states, which recapitulated neurodevelopmental trajectories (Chaligne 2021). Interestingly, the single-cell DNA methylation data could be used to infer copy number alterations (CNAs) with higher resolution than single-cell RNA sequencing. Furthermore, when classifying tumor cells using previously defined glioma tumor subtypes based on bulk DNA methylation (Ceccarelli et al 2016), cells spanned multiple subtypes within individual tumors, suggesting tumor subtyping based on bulk analysis may not be capturing the full complexity of gliomas **(Figure 2)**. As bulk DNA methylation profiling is increasingly used in the clinic, this study demonstrates the critical need to better understand intratumor DNA methylation signatures using single-cell analysis.

Potential applications of single-cell DNA methylation analysis in cancer

- **Tumor Heterogeneity**
  Identify subpopulations of cells within heterogenous tumors, and describe characteristics associated with disease progression.
- **Cancer Subtyping**
  Identify clonal subtypes with unique DNA methylation profiles, enabling more tailored and effective treatment strategies.
- **Epigenetic Drivers**
  Identify epigenetic drivers of cancer by identifying genes or regions with altered methylation patterns that contribute to oncogenesis.
- **Early Detection**
  Identify aberrant DNA methylation patterns in individual cells and ctDNA before a tumor becomes clinically detectable.
- **Monitoring Treatment Response**
  Monitor changes in DNA methylation to identify the emergence of drug-resistant cell populations and provide insights into mechanisms of therapeutic response.
- **Metastatic Potential**
  Identify cells within primary tumors with acquired epigenetic changes associated with metastatic potential, and discover new signatures of metastatic potential.
- **Drug Development**
  Identify new potential therapeutic targets and biomarkers for existing therapies.
- **Biomarker Discovery**
  Identify biomarkers for cancer screening, prognosis, therapy response, and cancer reoccurrence for liquid-based biopsies.

Similarly, improving cell type and state-specific methylation biomarkers by using single-cell DNA methylation could help improve the sensitivity and specificity of liquid biopsy testing. Many tumors release DNA into the bloodstream through various mechanisms (Cheng 2016), and blood-based or liquid biopsies have been developed as a noninvasive way to assay tumors. Circulating tumor DNA (ctDNA) maintains tumor-specific DNA methylation as well as methylation patterns consistent with the cell or tissue of origin, enabling liquid biopsies to be used for early cancer detection, predicting treatment response, and monitoring for minimal residual disease (MRD), among other uses (Luo 2021). Single-cell DNA methylation can be used to develop reference sets for ctDNA-based applications that better reflect tumor cell states, improving their accuracy and prognostic or diagnostic value.

Role of DNA methylation in neuroscience

The human brain is a highly complex tissue with extensive heterogeneity in cell types and states. A recent study using single-cell RNA sequencing identified over 3,000 cell types in the human adult brain (Siletti 2023). Understanding how cellular diversity is encoded is critical for both neurodevelopment and neurological disease research. DNA methylation helps establish cellular identities in development (Bogdanovic 2017), while aberrant DNA methylation patterns have been reported in several neurodegenerative diseases, including Alzheimer's disease (AD) (Kaur 2022).

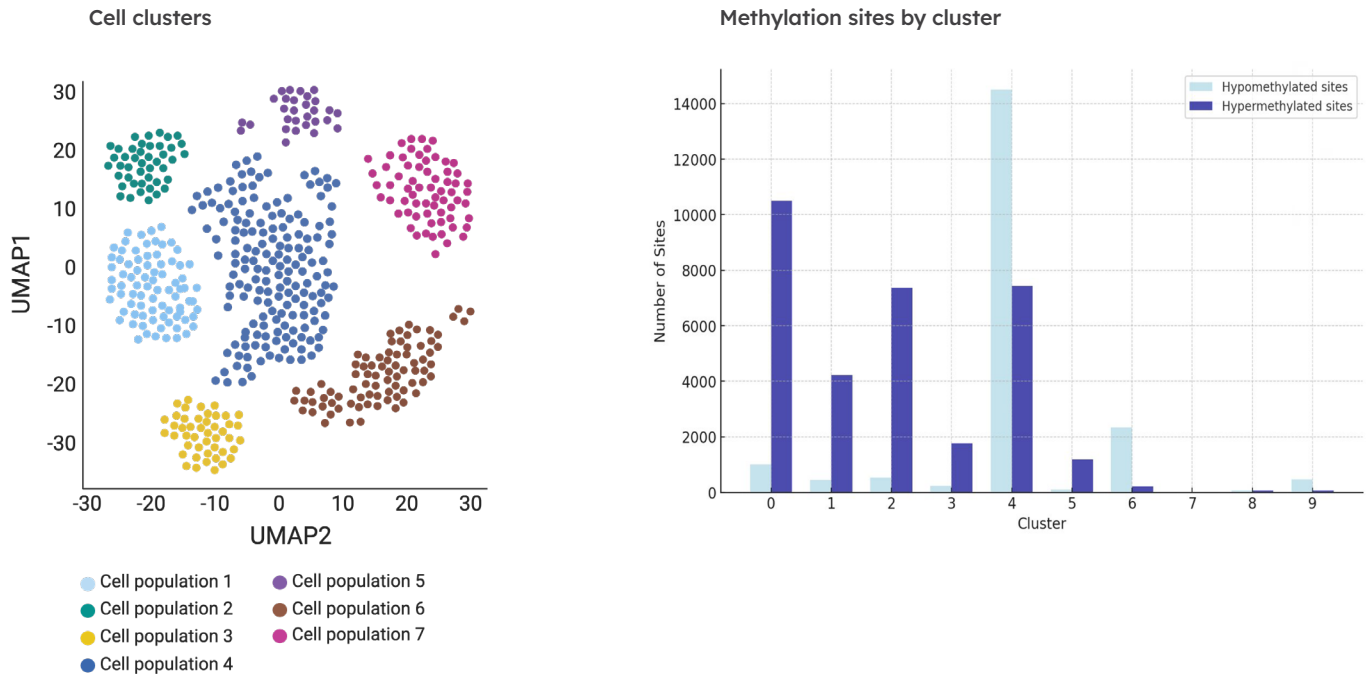
Computational deconvolution of bulk DNA methylation data from healthy and diseased brain tissues has enabled more granular mapping of DNA methylation to different cell types, including recent methods that include more reference cell types (Zhang Z 2023). However, reference data exists for less than a dozen cell types, resulting in cell types not in the reference database to be missing data or potentially confounding the methylation signal for other cell types.

New approaches to unravel neurodevelopment and disease

Higher cellular resolution approaches are helping researchers uncover the molecular diversity of the human brain. Flow cytometry, for instance, is often used to sort cell populations from heterogenous mixtures. For example, in Alzheimer's patients, researchers sorted nuclei from cortical regions prior to DNA methylation analysis (Shireby 2022). They found differentially methylated regions not previously associated with AD pathology. Interestingly, this study revealed that the majority of the differentially methylated regions were associated with non-neuronal cells, such as microglia. However, deconvolution of bulk data was unable to identify disease-associated DNA methylation differences occurring in different cell types, highlighting the importance of using refined cell populations for analysis.

Even with sorting, the cellular complexity in the brain cannot be fully captured. Common methods utilize neuronal nuclei (NeuN) as a marker for neurons and can distinguish between neurons and non-neurons, but individual cell types are not further refined (Zhang Z 2023). However, single-cell technologies are beginning to reveal the molecular diversity of the human brain (Tian 2023), and epigenetic investigations into gene regulation will help us further understand the underpinnings of this diversity **(Figure 3)**. Incorporating single cell methylation analysis into neuroscience research can uncover novel biology of brain function, development, psychiatric disorders, and neurodegenerative disorders by offering a clearer understanding of the epigenetic mechanisms that shape the brain's complexity and disease progression.

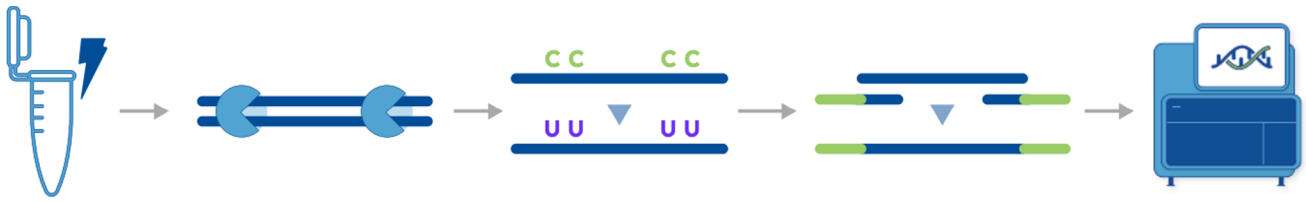
Figure 3. Single-cell DNA methylation analysis eliminates the need for population enrichment and enables analysis of cell types or states without known expression markers. All cell types in a sample can be analyzed simultaneously during DNA methylation analysis.



Potential applications of single-cell DNA methylation analysis in neuroscience

- **Cellular Heterogeneity**
  Allow characterization of the epigenetic differences between neuronal subtypes, glial cells, and other brain cell populations.
- **Neuronal Diversity**
  Uncover the epigenetic modifications that define distinct neuronal subtypes and their functional roles.
- **Neuroinflammation**
  Provide insights into how inflammation-induced changes in DNA methylation affect different cell populations within the nervous system.
- **Drug Efficacy on Epigenetic Targets**
  Provide insights into how therapies can impact epigenetic regulation in different neuronal populations.
- **Long-Term Effects of Environmental Factors**
  Reveal how environmental factors influence DNA methylation patterns in individual neurons or immune cells, helping to elucidate the long-term impact on neuronal function.
- **Synaptic Plasticity**
  Uncover how DNA methylation patterns change in response to learning and memory tasks.
- **Neuronal Development**
  Aid in the identification of key regulators of neural fate determination with a detailed map of DNA methylation changes during neurodevelopment.
- **Epigenetic Regulation of Behavior**
  Link specific DNA methylation patterns to behavioral traits, offering a molecular understanding of the relationship between epigenetics and behavior.

Figure 4. The ScaleBio Single-Cell DNA Methylation workflow. Nuclei samples are fixed and barcoded in situ using tagmentation, enabling sample multiplexing. Bisulfite conversion is then performed, followed by cleanup and the addition of adaptors and a second barcode to complete library construction. Libraries are sequenced and data is analyzed using the ScaleBio Seq Suite.



Enabling the next wave of discoveries: ScaleBio's Single-Cell DNA Methylation Kit

The field of epigenetics has made great strides over the last few decades. Single-cell DNA methylation is driving new discoveries and unmasking biology hidden in bulk methods by enabling researchers to distinguish cell types and states and cellularly resolve methylation patterns. It also enables characterization of novel or uncommon cell types, which are inaccessible to bulk methods even with computational deconvolution, to drive new discoveries in development and disease.

Single-cell DNA methylation protocols can be technically challenging, time consuming, and expensive to optimize without dedicated technical support and validated reagents, and typically offer limited cell throughput. ScaleBio's Single-Cell DNA Methylation Kit enables robust detection of hundreds of thousands of CpGs per cell, across thousands of cells, in a single experiment **(Figure 4)**. The workflow is compatible with target enrichment panels to offer further customization of single-cell DNA methylation experiments to focus on genomic regions or pathways of interest.

To learn more about ScaleBio's Single-Cell DNA Methylation Kit and download example datasets, visit https://scale.bio/scalebio-single-cell-methylation-kit/

SCALE biosciences

References

Bogdanovic, O et al. DNA methylation and the preservation of cell identity. Curr Opin Genet Dev. 2017; 46:9-14.

Ceccarelli M, et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell. 2016 Jan 28;164(3):550-63.

Chaligne R et al. Epigenetic encoding, heritability, and plasticity of glioma transcriptional states. Nat Genet. 2021 Oct;53(10):1469-1479.

Cheng, F et al. Circulating tumor DNA: a promising biomarker in the liquid biopsy of cancer. Oncotarget. 2016 Jul 26; 7(30): 48832–48841.

Dagogo-Jack I., Shaw A.T. Tumour heterogeneity and resistance to cancer therapies. Nat. Rev. Clin. Oncol. 2018; 15: 81-94

Ding W et al. Integrative analysis identifies potential DNA methylation biomarkers for pan-cancer diagnosis and prognosis. Epigenetics. 2019 Jan;14(1):67-80.

Feng LY et al. Hypermethylation of tumor suppressor genes is a risk factor for poor prognosis in ovarian cancer: A meta-analysis. Medicine (Baltimore). 2019 Feb;98(8):e14588.

Filipski K, et al. DNA methylation-based prediction of response to immune checkpoint inhibition in metastatic melanoma. J Immunother Cancer. 2021 Jul;9(7):e002226.

Guo W et al. A four-DNA methylation biomarker is a superior predictor of survival of patients with cutaneous melanoma. Elife. 2019 Jun 6;8:e44310.

He, Y et al. Non-CG Methylation in the human genome. Annu Rev Genomics Human Genet. 2015. 16:55-77.

Jones, PA et al. The epigenomics of cancer. Cell. 2007 128:4, 683-692.

Kaur G et al. DNA Methylation: a promising approach in management of alzheimer's disease and other neurodegenerative disorders. Biology (Basel). 2022 Jan 7;11(1):90.

Li, P et al. Liquid biopsies based on DNA methylation as biomarkers for the detection and prognosis of lung cancer. Clin Epigenet. 2022. 14, 118.

Luo, H et al. Liquid biopsy of methylation markers in cell-free DNA. Trends Mol Med. 2021 27,5:482-500.

Moore, LD et al. DNA methylation and its basic function. Neuropsychopharmacology. 2013. 38:23–38.

Murthy M et al. Neurodegenerative movement disorders: an epigenetics perspective and promise for the future. Neuropathol Appl Neurobiol. 2021;47:897–909.

Ng, JMK, Yu, J. Promoter hypermethylation of tumor suppressor genes as potential biomarkers in colorectal cancer. Int J Mol Sci, 2015 Feb; 16(2): 2472-2496.

Ozturk H et al. ISL2 is a putative tumor suppressor whose epigenetic silencing reprograms the metabolism of pancreatic cancer. Dev Cell. 2022 Jun 6;57(11):1331-1346.e9.

Policicchio, S et al. Fluorescence-activated nuclei sorting (FANS) on human post-mortem cortex tissue enabling the isolation of distinct neural cell populations for multiple omic profiling V.1. Protocols. IO. doi: dx.doi.org/10.17504/protocols.io.bmh2k38e

Shireby G et al. DNA methylation signatures of Alzheimer's disease neuropathology in the cortex are primarily driven by variation in non-neuronal cell-types. Nat Commun. 2022 Sep 24;13(1):5620.

Sigin VO et al. DNA methylation markers panel can improve prediction of response to neoadjuvant chemotherapy in luminal B breast cancer. Sci Rep. 2020. PMID: 32514046

Siletti, K et al. Transcriptomic diversity of cell types across the adult human brain. Science. 2023 13l382(6667).

Suelves, M et al. DNA methylation dynamics in cellular commitment and differentiation. Brief Func Genom. 2016 15; 6: 443–453.

Tian W et al. Single-cell dna methylation and 3d genome architecture in the human brain. Science. 2023. Oct 13; 382(6667): eadf5357.

Wei, A et al. Mammalian DNA methylome dynamics: mechanisms, functions and new frontiers. Development. 2022 Dec 15; 149(24): dev182683.

Xu B, et al. A pan-cancer analysis of predictive methylation signatures of response to cancer immunotherapy. Front Immunol. 2021 Dec 9;12:796647.

Zhang Z et al. Hierarchical deconvolution for extensive cell type resolution in the human brain using DNA methylation. Front Neurosci. 2023 Jun 19;17:1198243.

ScaleBio Single Cell Methylation Sequencing
Support@scale.bio

SCALE biosciences

ScaleBio_scDNA_Methylation_eBook_RevB