Enhanced Single Cell DNA Methylation Analysis Using Combinatorial Indexing

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Introduction

Single cell-omics has transformed our understanding of cellular heterogeneity and cell function. Technology and informatics advances have accelerated single cell analysis but limitations in sample and cell throughput and high library cost per cell remain, creating a barrier to conduct larger, more complex studies. Additionally, extensibility to new assay modalities like methylation has proven to be challenging.

Single cell combinatorial indexing MET-seq (sciMET) has previously been proposed to scale throughput and reduce cost of single-cell methylation profiling (e.g., Nichols et al. 2022). Nuclei pass through a unique combination of barcoded wells using pooling and splitting, resulting in a unique combination of barcodes being attached to DNA for each cell. The advantages of combinatorial indexing are: 1) cell fixation for sample storage and transport, 2) sample and cell scalability, and 3) sub-linear cost scaling resulting in lower cost per cell.

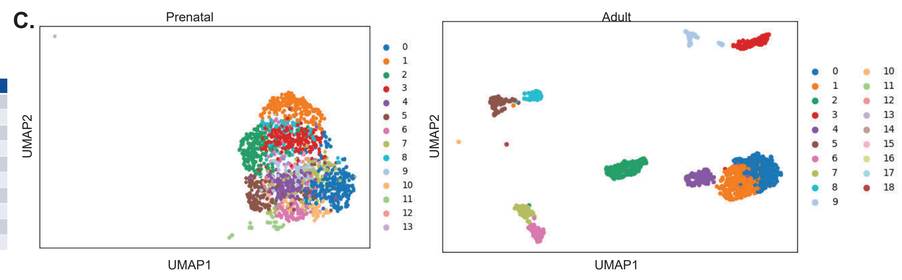
In this study, we use ScaleBio™ Single Cell Methylation Kit to investigate DNA methylation patterns during development and oncogenesis, focusing on methylation changes in prenatal and adult brain tissue, and IDH-mutant glioma tumor tissue. Our analysis of single-cell methylomes showcases high single cell recovery and robust cytosine coverage. We identified cell-type specific clusters during the developmental states using Differentially Methylated Regions (DMR) and hypo-and hyper methylated regions. Comparisons with known bulk methylation profiles revealed unique single-cell methylation profiles, unveiling cellular heterogeneity and trajectories obscured by (pseudo) bulk analysis.

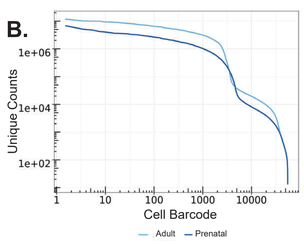
In conclusion, the ScaleBio™ Single Cell Methylation Kit is a first of its kind commercial solution providing a comprehensive genome-wide view of single cell methylomes with scalable throughput, high sensitivity and specificity offering unprecedented insights into cellular heterogeneity and trajectories during developmental and disease-driven methylation.

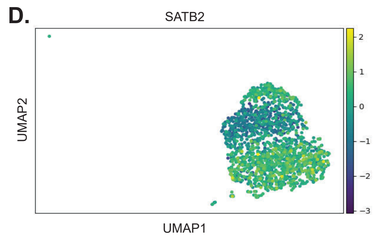
scMET Analysis of Human Adult and Prenatal Brain Tissue Reveals Diverse Cell Methylomes

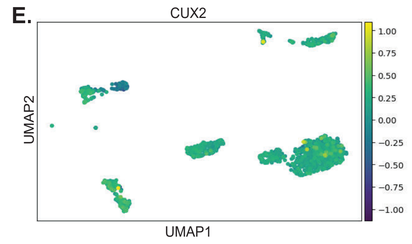
A.

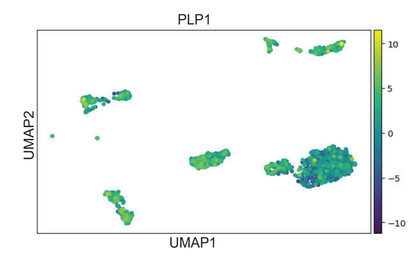
	Metric	Result
Nuclei	Passing Nuclei	4,140
Reads	Total Reads	19,526,823,710
	Mapped Reads	95.2%
	Reads in Nuclei	98.36%
Median per passing cell	Total Reads	2,638,331
	Unique Reads	595,769
	CG sites covered	246,869
	CH sites covered	21,981,574
	CG Methylation	80.01%
	CH Methylation	0.97%











A. Cell metrics from 4140 nuclei isolated from prenatal and adult brain tissue show robust recovery and mapping rates

B. Knee plot shows uniform signal, confident cell calling, and low background across the brain nuclei population

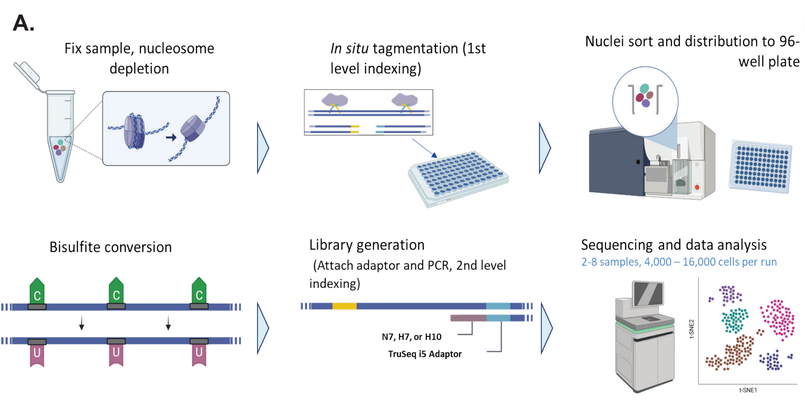
C. UMAP identifies multiple distinct cell types across cells in human prenatal (left) and adult (right) brain tissue

D. SATB2 hypomethylation observed on the %CG methylation UMAP identifies upper layer neurons

E. CUX2 and PLP1 hypomethylation observed on the %CG methylation UMAP identifies upper layer neurons and oligodendrocytes, respectively

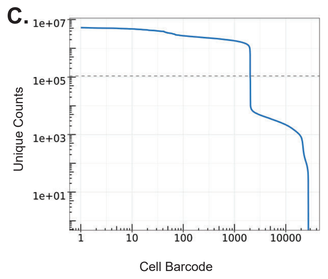
ScaleBio scMET kit: A Modular & Streamlined Workflow Generates Comprehensive & High Quality Data

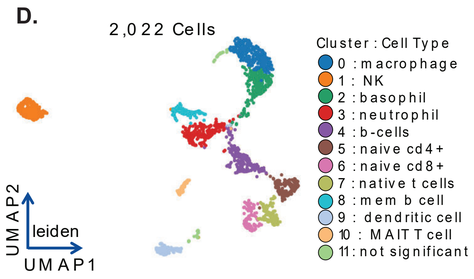
A.

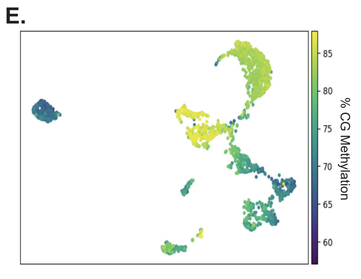


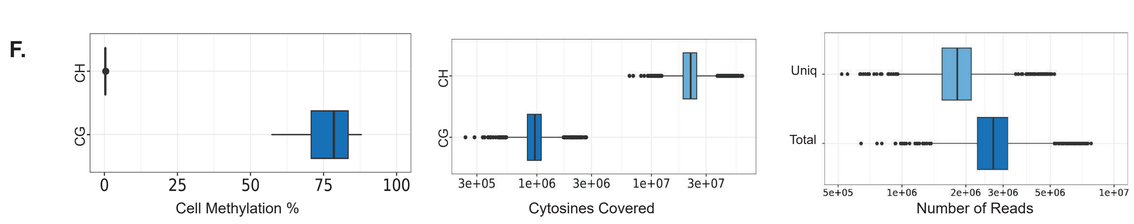
B.

	Metric	Result
Nuclei	Passing Nuclei	2,022
Reads	Total Reads	5,854,657,838
	Passing Reads	91.9%
	Reads in Nuclei	89.4%
Median per passing cell	Total Reads	2,698,010
	Unique Reads	1,821,945
	CG sites covered	961,700
	CH sites covered	21,981,485
	CG Methylation	78.5%
	CH Methylation	0.33%









A. ScaleBio™scMET workflow - nuclei isolation and fixation, nucleosomal disruption and indexed tagmentation, sorting and bisulfite conversion, followed by library generation and sequencing, and bioinformatic analyses

B. Data summary for 2,022 PBMCs with ScaleBio™ scMET kit. In this sample we show ~89% of reads in nuclei and a median of 961,720 CpG sites covered

C. Read count vs. cell barcode ("knee") plot showing unique reads across ranked nuclei shows clear distinction between nuclei and background reads. This plot is used to identify 2,022 passing nuclei, each containing a median of 1.82M unique mapped reads (see B).

D. UMAP of the 2,022 nuclei shows distinct clusters that can be identified as known PBMC cell types from CG methylation profiles

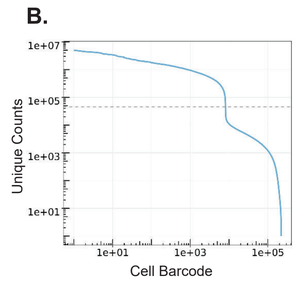
E. UMAP from figure D shaded by %CG methylation shows differential % methylation across different PBMC cell types

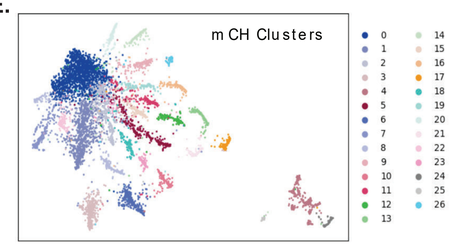
F. Passing cell metrics per nuclei showing CG and CH percent methylation, covered cytosines in the CG and CH contexts, and total mapped reads and uniquely mapped reads

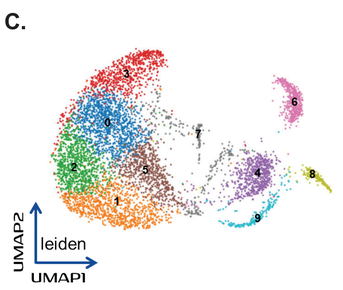
scMET Analysis of Human IDH^{mut} Glioma Tumor Produces Distinct Cell Type Clusters Based on Methylomes

A.

	Metric	Result
Nuclei	Passing Nuclei	8,094
Reads	Total Reads	19,212,954,426
	Passing Reads	97.4%
	Reads in Nuclei	87.8%
Median per passing cell	Total Reads	2,805,108
	Unique Reads	508,676
	CG sites covered	168,974
	CH sites covered	4,290,169
	CG Methylation	84.35
	CH Methylation	0.5%

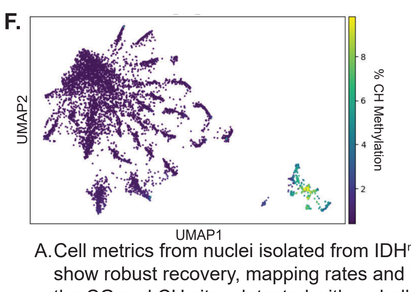


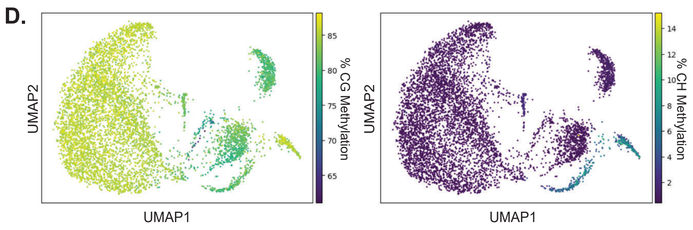




Cluster: Cell Type

- : Cancer stem cells
- : IDH^{mut} marker cells
- : Astrocytes
- : Tumor associated microglia
- : Oligodendrocytes
- : Inhibitory neurons
- : Cortical pyramidal neurons





A. Cell metrics from nuclei isolated from IDH^{mut} glioma tissue show robust recovery, mapping rates and broad coverage in the CG and CH sites detected with a challenging sample.

B. Knee plot shows uniform signal, confident cell calling, and low background across the tumor nuclei population

C. UMAP shows distinct clusters that can be identified as known glioma and associated cell types from CG methylation profiles

D. UMAP from figure C shaded by %CG and %CH methylation shows differential % methylation across different cell types

E. UMAP shows distinct clusters identified from CH methylation profiles

F. UMAP from figure E shaded by %CH methylation shows differential % methylation across different cell types

Conclusions

- The ScaleBio™ Single Cell Methylation Kit (scMET kit) provides the first commercial solution to analyze single cell methylation at scale
- The workflow generates clean data with highly sensitive chemistry and low background. Starting with nuclei isolated from cells or tissues the scMET kit can process up to 18,400 nuclei per experiment
- Demonstrated ability to analyze a wide range of inputs from PBMCs to complex samples like human brain and tumor tissues with robust cytosine coverage and expected CH and CG detection
- In conclusion, the ScaleBio™ scMET kit offers a robust and highly scalable analysis solution for genome-wide single cell methylation profiling