Deciphering Epigenetic Landscapes: A Comparative Analysis of DNA Methylation in 2D versus 3D Cultured Colon Cancer Cells using Scale Bioscience's Single Cell Methylation Kit

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ABSTRACT

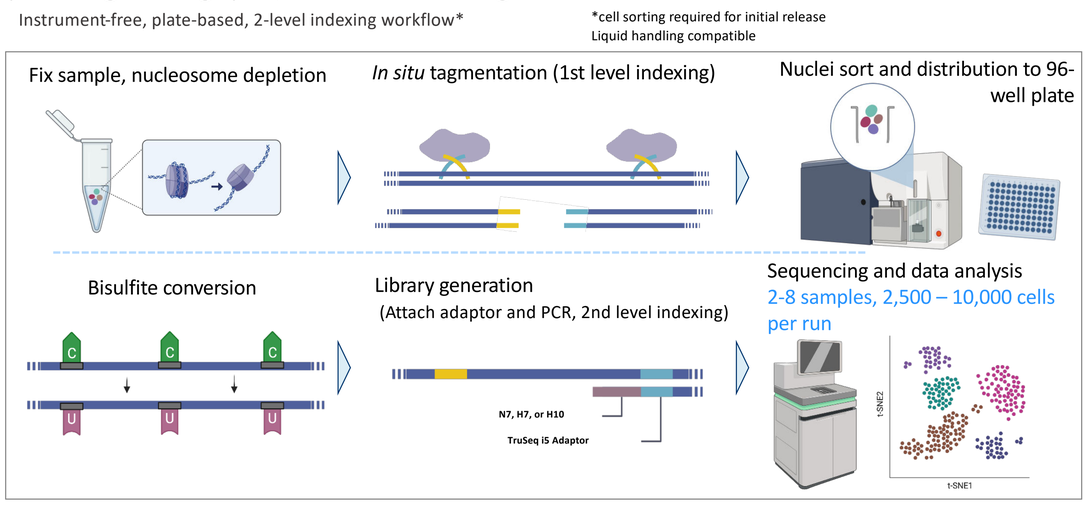
We utilized our Single Cell Methylation Kit to generate DNA libraries from colon cancer cells, comparing the traditional monolayer (2D) setup with the more dynamic spheroid (3D) configuration. The increasing interest in 3D cell culture, especially spheroids, is driven by its potential to better approximate in vivo conditions compared to the more conventional 2D cultures. Our spheroids were cultivated in ultra-low adherence plates, while the 2D cells were maintained in TC treated plates.

Our workflow was highly efficient, yielding significant cytosine coverage and sequencing saturation. Using UMAP analysis, we observed distinct clustering patterns for the 2D and 3D cultured cells. There was some overlap, which we attribute to the cells having been in the 3D environment for only 5 days, suggesting they retained characteristics from their 2D origins.

Additionally, by examining Differentially Methylated Regions (DMR), we could gain insights into how cellular behavior and expression might differ based on culture conditions. Such an approach not only has implications for potential gene discovery but also provides deeper insights into the epigenetic nuances of cells based on their growth environment.

In conclusion, merging our Single Cell Methylation Kit with 3D spheroid culture techniques has provided valuable insights. This approach might prove pivotal in enhancing the precision and understanding of DNA methylation sites and cellular heterogeneity.

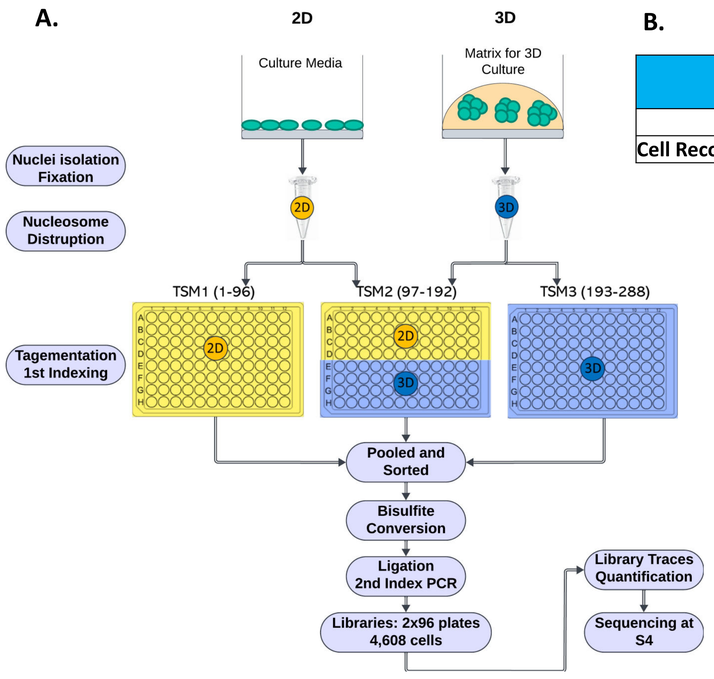
Figure 1: Combinatorial indexing technology can be used to increase single cell sequencing throughput, while decreasing cost



Combinatorial indexing uses repeated rounds of distributing cells across plates and subsequent well-based barcoding. Although multiple cells receive the same barcode in each round, repeated rounds creates a unique combinatorial barcode for the molecules in each individual cell.

Figure 2: Multiplexing of 2D and 3D cultured HCT-116 cells in scMET-seq workflow

A.



B.

	Initial Cell Counts		After Nucleosome Disruption	
	2D	3D	2D	3D
Cell Recovery Rate	3M	2M	55%	30%

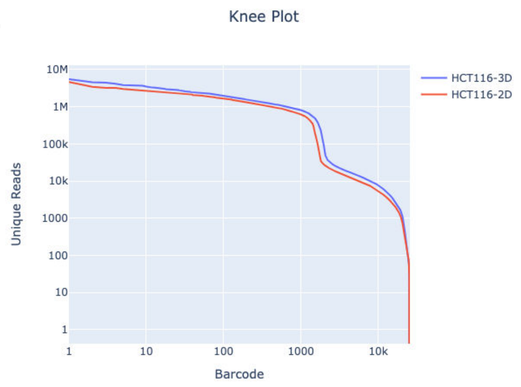
C.

	2D	3D
Theological Initial Cell Counts	2,034	2,034
Passing Cell Counts	1,730	2,025
Passing Cell Rate	75.10%	87.80%

A. HCT-116 cells were cultured in regular cell culture dish for 2D culture vs. low attachment culture dish for 3D culture. Spheroids were cultured for 5 days (they form on day 1). Nuclei from 2D and 3D culture were than purified, fixed, and treated before evenly distributed onto TSM plates for 1st level indexing and multiplexing. **B.** Cell recovery rate from the initial starting materials **C**. Passing cell counts and cell rate after sequencing.

Figure 3: ScaleBio's scMET-seq kit produces high quality data with high mapping rate and low CH methylation percentage

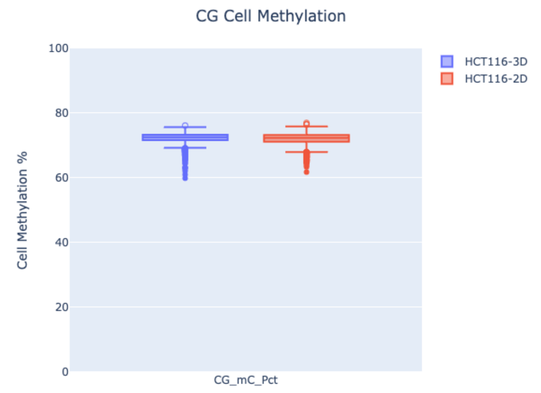
A.



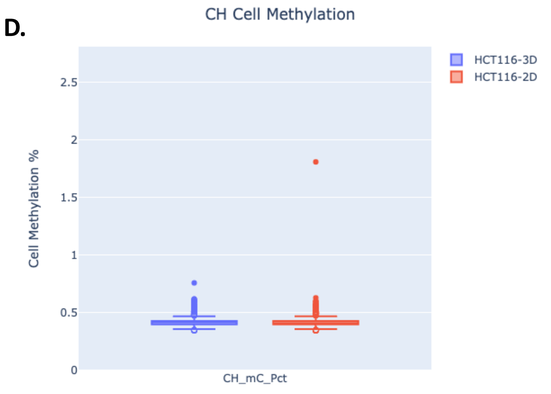
B.

Metric	2D	3D
Total_reads	9,510,131,348	12,655,919,764
Reads_passing_trimming	9,330,675,088	12,427,150,692
Reads_passing_mapping	8,992,574,156	11,938,374,654
Percent pass trimming	98%	98%
Percent mapped	95%	94%

C.



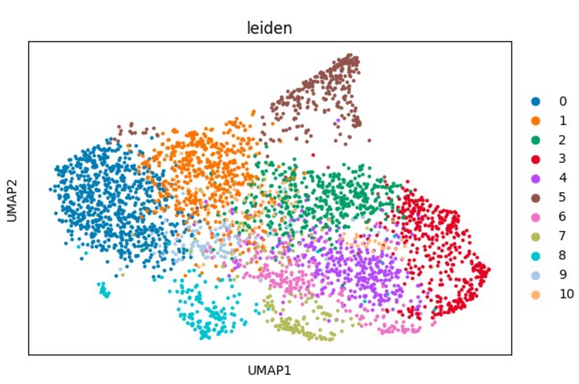
D.



A. Knee plot shows uniform signal, confident cell calling, and low background across 2D and 3D cultured HCT-116 cells. **B.** Metrics show good sequencing and mapping quality. **C.** The median CG methylation percentage is in line with human cells (~75%). **D.** The low median CH methylation percentage (< 0.5%) indicating complete conversion of non-methylated Cytosines in CH context.

Figure 4: UMAP clustering separated 2D and 3D cells based on single cell methylation sequencing results

A.



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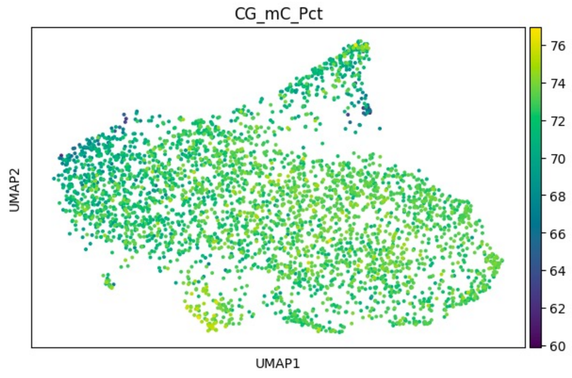
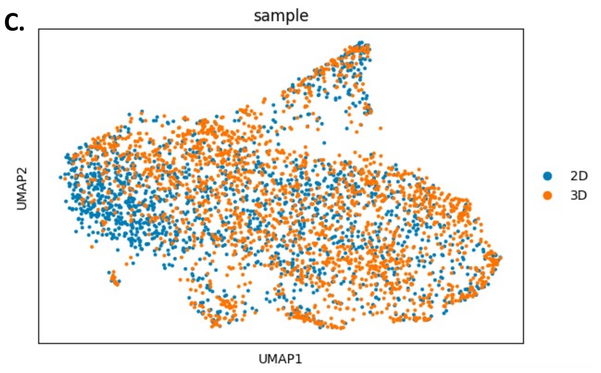
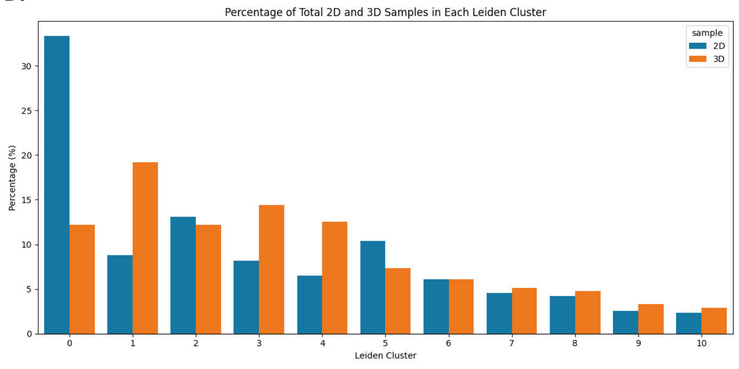


C.



D.



A. UMAP identified 11 clusters based on CG methylation on 50kB genomic regions using Scanpy. **B.** UMAP shows CG methylation percentage across different clusters. **C.** 2D and 3D cells can be separated into different clusters based on single cell methylation information. **D.** The percentage of total 2D or 3D cells presents in individual clusters. More than 31% 2D cells were found in cluster 0.

CONCLUSIONS

- The ScaleBio™ scMET workflow generates clean data with low background
- The ScaleBio™ Single Cell Methylation Sequencing Kits provides high sensitivity and low background single cell methylation information. Data from 2D and 3D cultured HCT-116 cells shows that the kit can separate 2D and 3D cultured cells solely based on methylation information.