Princess Margaret Genomics Centre
101 College Street
PMCRT, Rm 9-601A
Toronto, ON, M5G 1L7

PMGC SAMPLE SUBMISSION GUIDELINES FOR SCALEBIO SCMET-SEQ

Scale Biosciences (ScaleBio) offers the first ever commercial solution for single-cell resolution into methylation states (scMET-Seq). The Single Cell Methylation Kits allow you to profile single-cell methylation states across tens of thousands of cells using bisulfite conversion chemistry.
scMET uses proprietary combinatorial barcoding of DNA in nuclei themselves without the need for specialized instrumentation.

Kit Size Options

Two chemistry sizes are available based on desired nuclei barcoding and sequencing:

1. Small kit (up to 6 samples): 4,600 barcoded/processed nuclei for sequencing
2. Large kit (up to 8 samples): 18,400 barcoded/processed nuclei for sequencing

The number of nuclei required for scMET-Seq

The number of nuclei per sample will depend on how many total samples are processed on the same kit. The two tables below list the minimum required and the recommended number of nuclei per samples as well as the expected number of barcoded and processed nuclei for sequencing based on the total number of samples included in the prep.

Recommended Number of Nuclei Per Sample for Fixation

Number of samples	Minimum Required Nuclei	Recommended Nuclei	Small Kit (4,600): Barcoded Nuclei for Sequencing	Large Kit (18,400): Barcoded Nuclei for Sequencing
1	2 Million	2-6 Million	4,600	18,400
2	1 Million	1-3 Million	2,300	9,200
3	1 Million	1-2 Million	1,500	6,100
4	500 000	1 Million	1,100	4,600
5	500 000	1 Million	900	3,600
6	400 000	1 Million	700	3,000
7*	400 000	1 Million	--	2,600
8*	400 000	1 Million	--	2,300

* Additional Methylation Nuclei Preparation Kit required

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Dr. Troy Ketela, Head of Operations
For new project inquires,
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Validated Sample Types

To-date nuclei from primary tissues in fresh dissociated, snap-frozen, and OCT-embedded formats have been demonstrated in addition to PBMCs and cell lines. FFPE samples are not supported.

Computational Resources and Analysis Software

You must have access to computational resources as PMGC does not offer scMET-Seq analysis services. A custom single cell methylation analysis pipeline is provided as a product by ScaleBio to take you from raw sequencing outputs to a cell-methylation state matrix. scMET, like most methylation-profiling pipelines, is computationally intensive. Minimum seq parameters are PE101 (recommended is PE151).

Methylome Enrichment Option

scMET is a whole genome profiling assay and as such it is sequencing and budget intensive (the required minimum read output is 1 million clusters/nucleus). One way to circumvent the high cost of sequencing is by enriching regions of the genome that are biologically relevant methylation markers. The Twist Human Methylome Panel targets 3.98M CpG sites through 123 Mb of genomic content. If you wish to evaluate the Twist Human Methylome Panel for specific target sites check out the Twist Methylome Panel Target BED file.

Sample Submission Options

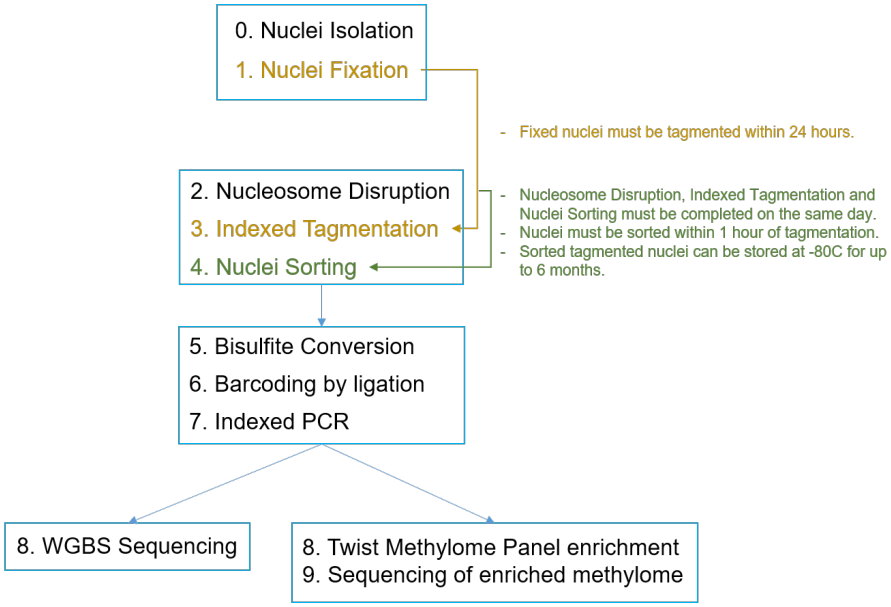
Protocol Constraints: The timing of the first 4 steps of the scMET workflow is critical.

- Freshly fixed samples can be stored for up to 24 hours prior to the Indexed Tagmentation step.
- Indexed Tagmentation and Nuclei sorting must be completed on the same day.
- Sorted tagmented nuclei can be stored in the freezer for up to 6 months.

1. Due to the protocol constraints, we prefer to receive snap-frozen samples.
2. However, if you wish to submit fixed nuclei to PMGC, you need to coordinate the date and time of fixation with the PMGC staff **at least three weeks in advance** as we need be available within a 24-hour period of fixation to further process your samples. We need time to book the FACS facility as well, as they are booked 1-2 weeks in advance.

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Sample Drop-off / Shipping

If dropping off samples: Please **schedule your drop off date and time in advance** with your PMGC contact person.

- Your PMGC contact will meet you at the **9th floor elevator lobby** of the Princess Margaret Cancer Research Tower (PMCRT) at your pre-arranged time. PMCRT is the East Tower of the MaRS building, near the corner of College and Elizabeth Street entrance.
- Email or call/text when you are at the designated meeting area and your PMGC contact will come to collect the samples.
- REMINDER: Transport samples using appropriate means of storage (e.g. on dry ice for frozen samples, wet ice for fresh samples). Please confirm with PMGC if any questions.

If shipping samples: Please ship out on **Monday/Tuesday** to prevent weekend delays. Place a generous supply of dry ice to ensure dry ice will remain for the duration of the delivery time. For international clients, we recommend shipping with World Courier. Within Canada, or if shipping DNA/RNA, we recommend FedEX Next Day Priority services.

Shipping address:

Attn: Christine Pham
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