

PMGC SAMPLE SUBMISSIONS GUIDELINES FOR BULK DNA ASSAYS (WGS, WES, EM-Seq, 5L-Seq, 6L-Seq)

This document contains key points on sample quality, sequencing coverage, and low input amounts, along with details on assay type, kitted assays, and chemistry descriptions, as well as specific sample submission requirements for each assay type. We strongly encourage you to review this document prior to the submission of your samples to our facility for downstream processing and sequencing.

Assays listed below require strict and basic sample quality standards:

- **Pure DNA** with 260/280 ratio of 1.8 and 260/230 ratio of 2.0.
- **RNA-free DNA** (DNA treated with RNase for RNA removal prior to submission).
- **Accurately quantified DNA** (use a fluorometric method such as Qubit).

Sequencing coverage assurance:

- Fold coverage may vary due to technical constraints, but read depth is guaranteed.

Input amount considerations:

- Avoid submitting minimum input amounts to prevent compromised ligation, reduced yield, and inconsistency in library quality and low library complexity.
- Minimal inputs increase library preparation failure rate. They also require more PCR cycles, leading to high % duplication rates and decreased mapping rates.
- Low sample concentrations pose challenges in accurate quantification and normalization, especially with RNA contamination, which can skew normalization efforts and introduce noise (crucial point for all samples but especially so for low-input and low-quality samples like FFPE).

Sample submission tube requirements:

- Please submit your samples in 1.5mL tubes (0.2mL, 0.5mL tubes or strip tubes are not accepted).

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For new project inquiries,
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At PMGC, we currently offer the following bulk genomic and epigenomic assays:

Assay Type	Kit	Chemistry description
Whole Genome Sequencing (WGS)	NEBNext Ultra II FS DNA Library Prep Kit	This assay utilizes enzymatic fragmentation.
Whole Genome Sequencing for FFPE samples (WGS-FFPE)	NEBNext FFPE DNA Repair Module v2 + NEBNext Ultra II DNA Library Prep Kit	This assay requires mechanical or acoustic shearing.
Whole Exome Sequencing (WES)	Agilent SureSelect XT HS2 DNA Kit + SureSelect Human/Mouse All Exon Probes	This assay utilizes enzymatic fragmentation.
Enzymatic Methyl-Seq (EM-Seq)	NEBNext Enzymatic Methyl-Seq Kit	Genome-wide methylation profiling assay. Enzyme-based conversion minimizes damage to DNA. This assay enables the detection of 5mC and 5hmC, however it does not distinguish between the two. It does not require custom analysis solutions.
5 Letter Methyl-Seq (5L-Seq)	Biomodal duet multiomics solution +modC	Genome-wide methylation profiling assay. This assay enables phased genetic variant calls and it enables the detection of 5mC and 5hmC, however, it does not distinguish between the two. It requires custom software solution for analysis (provided by Biomodal).
6 Letter Methyl-Seq (6L-Seq)	Biomodal duet multiomics solution evoC	Genome-wide methylation profiling assay. This assay enables phased genetic variant calls and it enables the detection of 5mC and 5hmC. It is able to distinguish between the two modC modes. It requires custom software solution for analysis (provided by Biomodal).

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Specific Sample Submission Requirements for each assay type:

Unless specified otherwise, TE or other buffers that contain EDTA are not compatible with assay chemistries listed below. EDTA is a chelator that adversely impacts enzyme efficiency and function. See page 4 for buffer compositions.

Whole Genome Sequencing (WGS)

Starting Material: genomic DNA (gDNA), plasmids, cDNA, amplicons

Quality: pure DNA

Input Amount: 100 pg - 0.5 ug DNA

Input Volume: No more than 30 uL in EB, H2O, Low TE, 0.1X TE

Whole Genome Sequencing for FFPE samples (WGS-FFPE)

Starting Material: FFPE DNA

Quality: DIN > 2; pure DNA

Input Amount: 25 ng - 1 ug FFPE

Input Volume: No more than 55 uL in in EB, H2O, Low TE, 0.1X TE

FFPE Repair Module is highly recommended for all FFPE samples prior to library generation. FFPE repair chemistry is not compatible with EM-Seq.

Whole Exome Sequencing (WES)

Starting Material: genomic DNA (gDNA)

Quality: pure DNA

Input Amount: 10 ng - 200 ng DNA

Input Volume: No more than 10 uL in EB or H2O

Enzymatic Methyl-Seq (EM-Seq)

Starting Material: genomic DNA (gDNA), cfDNA, FFPE

Quality: pure DNA

Input Amount *: 10 ng - 200 ng gDNA/cfDNA/FFPE

Input Volume: No more than 30 uL in EB, H2O, Low TE, 0.1X TE

5 Letter Methyl-Seq (5L-Seq) and 6 Letter Methyl-Seq (6L-Seq)

Starting Material: genomic DNA (gDNA), cfDNA

Quality: pure DNA

Input Amount: 10 ng - 80 ng gDNA, 10 ng – 50 ng cfDNA

Input Volume: No more than 30 uL in EB, H2O, Low TE

TE BUFFERS

Buffer	Vendor, Cat#	Chemical composition
1X TE	Invitrogen, cat# 12090015	10 mM Tris-HCl, 0.1 mM EDTA (pH 8.0)
1X TE	Promega, cat# V6231	10 mM Tris-HCl, 1 mM EDTA (pH 8.0)
0.1X TE		1 mM Tris-HCl, 0.1 mM EDTA (pH 8.0)
Low TE		10 mM Tris-HCl, 0.1 mM EDTA (pH 8.0)
1X IDTE	IDT, cat# 11-05-01-13 or 11-05-01-15	10 mM Tris-HCl, 0.1 mM EDTA (pH 7.5 or pH 8.0)
EB	Qiagen, cat# 19086	10 mM Tris-HCl, pH 8.5

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: (insert PMGC contact person)
 Princess Margaret Genomics Centre
 101 College St.
 PMCRT, Rm 9-601A
 Toronto, Ontario M5G 1L7
 Canada

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