Princess Margaret Genomics Centre
101 College Street
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Toronto, ON, M5G 1L7

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

This document contains key information on sequencing coverage assurance, sample quality standards, general and assay-specific sample submission requirements, chemistry information, elution buffers, and shipping information. We strongly encourage you to review this document prior to the submission of your samples to our facility.

Sequencing coverage assurance:

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

Chemistries we use for NGS library preparation require strict 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, never Nanodrop).
- **DNA integrity** check via gel/Tapestation (For HMW/intact samples only).
- 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 comparisons.
- Please submit your samples in **adequately labelled 1.5mL tubes** (0.2mL, 0.5mL tubes or strip tubes are not accepted). Please include **lab name**, **submission date** and **sample ID** on the tube.

General Sample Submission Requirements:

- HMW/intact samples: >50ng/uL in no more than 30 µL volume.
- FFPE samples: >80ng/uL in no more than 20 µL volume.
- Minimum volume: 10 µL.
- Elution buffer: low (0.1mM) to no EDTA.
- There is no need to normalize samples. In fact, we discourage clients from normalizing samples prior to submission, especially based on Nanodrop values.

Note: For FFPE samples, we offer enzyme cocktails for DNA repair before NGS preparation. Let us know if you need this service.

Tanja Durbic
For Bulk DNA inquiries,
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Tanja.Durbic@uhn.ca

Dr. Troy Ketela, Head of Operations
For new project inquires,
(416) 634-8816
Geneservice@pmgenomics.ca

Low input amount considerations:

- Avoid minimum input amounts to prevent compromised ligation, reduced yield, and inconsistency in library quality and low library complexity.
- Minimal sample input amounts 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).

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We currently offer the following bulk genomic and bulk epigenomic assays:

Assay Type, Starting Material and Quality	Kit / *Assay Sample Input Range*	Submission Volume/elution buffer:
Whole Genome Sequencing (WGS) **Starting Material:** pure genomic DNA (gDNA), plasmids, cDNA, amplicons, FFPE	NEBNext Ultra II FS DNA Library Prep 100 *pg* - 0.5 *ug*	No more than 30 *uL* in EB, H2O, Low TE, 0.1X TE.
Whole Genome Sequencing for FFPE samples (WGS-FFPE) **Starting Material:** pure genomic DNA (gDNA), plasmids, cDNA, amplicons, FFPE **Quality:** FFPE DIN > 2	NEBNext FFPE DNA Repair Module v2 + NEBNext Ultra II DNA Library Prep 25 *ng* - 1 *ug*	No more than 30 *uL* in EB, H2O, Low TE, 0.1X TE.
Whole Exome Sequencing (WES) **Starting Material:** pure genomic DNA (gDNA), FFPE	SureSelect XT HS2 DNA Kit + Human/Mouse All Exon v8 10 *ng* - 200 *ng*	No more than 12 *uL* in EB or H2O.
Enzymatic Methyl-Seq (EM-Seq) **Starting Material:** pure genomic DNA (gDNA), cfDNA, FFPE	NEBNext Enzymatic Methyl-Seq 10 *ng* - 200 *ng*	No more than 30 *uL* in EB, H2O, Low TE, 0.1X TE.
5 Letter Methyl-Seq (5L-Seq) 6 Letter Methyl-Seq (6L-Seq) **Starting Material:** pure genomic DNA (gDNA), cfDNA (not validated for FFPE)	duet multiomics solution +modC/evoC 10 *ng* - 80 *ng* gDNA, 10 *ng* – 50 *ng* cfDNA	No more than 30 *uL* in EB, H2O, Low TE.
Bulk ATAC-Seq **Starting Material:** fresh / frozen cells, fresh / cryopreserved tissue **Quality:** > 90% viable cells	NEBNext High-Fidelity 2X PCR Master Mix. Illumina Tagment DNA Enzyme and Buffer Small Kit. 150,000 of viable cells 30 - 50 mg of tissue	Input Volume: NA
ChIP-seq (library construction and sequencing) **Starting Material:** ChIP DNA product + control input DNA **Quality:** DNA fragment size range: 100-500 bp	ThruPLEX DNA-Seq Kit (Takara Bio) > 250 pg/uL	Input Volume: NA

* Assay sample input range is the amount of material that the referenced kit can handle.
For sample submission requirements see 'General Sample Submission Requirements' on page 1. Sample submission input is the amount of material we require to process a sample through our intake QC steps and library preparation stage: for intact samples, sample concentration should be >50ng/uL in 10-30uL; for FFPE samples, sample concentration should be >80ng/uL in 10uL-20uL volume.

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Sample elution buffers:

Elution buffer is a crucial component of any NGS library prep. Elution buffers can significantly impact the enzymology of NGS reactions, especially if they contain chelating agents like EDTA. EDTA binds divalent metal ions such as Mg2+ and Ca2+, which are essential cofactors for many enzymes used in NGS protocols, including polymerases, ligases, and nucleases.

Here are some common elution buffers and their composition for your reference:

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

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:
University Health Network (UHN)
Attn Tanja Durbic PMCRT Room 9-601A
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Ontario, Canada

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