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

PMGC SAMPLE SUBMISSION GUIDELINES FOR 10X GENOMICS NUCLEI SEQUENCING

Prior to Submission Date

1. Please complete the **PMGC 10X NucSeq and Multiome Submission Form** and return a **signed copy of the quote** prior to sample submission. These two documents are required for sample processing.
2. Please ensure an **immediate contact** is available for the day of submission and approximately 2 hours after submission for fresh samples. We will schedule a date for processing frozen samples and the **immediate contact** will be notified of the processing date. Contact information will only be used for potential issues arising during PMGC preparation, which require decisions for sample processing.
3. Incomplete submission forms and quotes will cause delays to processing samples under optimal conditions and in a timely manner.
4. When filling out the submission form, please note that the requested # of reads per cell will be used to determine the total sequencing reads per sample. For example, for a 10k capture with 50,000 reads/nucleus, the total reads delivered for the sample will be 500,000,000(+/- 20%). In case of an over-capture that is related to sample health, we will focus on the total reads delivered vs the reads per cell. Any deeper sequencing will be at an additional cost.
5. If this is a new project, please **arrange a lysis test** for fresh cell suspensions **or nuclei isolation optimization** for frozen tissues **before submitting experimental samples**. This will ensure we are using the correct conditions for your sample type prior to starting the experimental samples.

Day of Submission

Fresh Samples:

- Please email or call/text your PMGC contact person if you are arriving earlier or later than your assigned time slot. Instruments and reagents are prepared as per your submission form to be at their freshest prior to your times lot for efficient processing of your sample on arrival.
- **Count cells on haemocytometer to confirm total cell yield, viability, and concentration prior to submission.**
- Failure to cancel a sample submission without at least 45 minutes notice prior to your scheduled submission date and time will result in a surcharge of $300.
- PMGC can accept fresh samples for nuclei sequencing **Monday – Friday from 9:00AM – 1:00PM**.

Samantha Lee
For 10X Single Nucleus inquiries,
(416) 581-7439
samantha.lee2@uhn.ca

Harshpreet Kaur Dhall
For 10X Single Nucleus inquiries,
(416) 581-7439
Harshpreet.dhall@uhn.ca

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

Frozen Samples:

- Please email or call/text your PMGC contact person if you are arriving earlier or later than your assigned time slot.
- **Frozen samples should be submitted on dry ice.**
- Please weigh the frozen sample or provide a weight estimate (in mg) for each sample.
- PMGC can accept frozen samples for nuclei sequencing **Monday-Friday from 9:00AM – 4:00PM.**

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 for tissues/cells. 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

Preparation of Fresh Single Cell Suspension:

1. Fresh cell suspension can be submitted in the media/buffer that keeps the cells happiest. Please provide the cell suspensions in 1mL volume and preferably in a 1.5ml Lo-Bind tube.
2. Information on flow sorted samples: https://kb.10xgenomics.com/hc/en-us/articles/360048826911-What-are-the-best-practices-for-flow-sorting-cells-for-10x-Genomics-assays-

Samantha Lee
For 10X Single Nucleus inquiries,
(416) 581-7439
samantha.lee2@uhn.ca

Harshpreet Kaur Dhall
For 10X Single Nucleus inquiries,
(416) 581-7439
Harshpreet.dhall@uhn.ca

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

3. A minimum of 250,000 cells are needed, and more if available is preferred. Note: if 250,000 cells is not achievable, we may suggest following a low input protocol with modified lysis and wash steps. In the case of low-input we will need a minimum of 50,000 cells.
4. Ensure samples are single cell suspensions without clumping.
5. Samples should be devoid of large debris. Samples with large debris >40uM risk clogging during the initial GEM creation step and potential loss of precious samples.
6. **For cryo-preserved cells:** Cells must be at >=80% viability prior to preservation (See 10X Genomics protocols for Fresh Frozen cells).
7. **Required viability is >80%.** If samples are precious and lower viability is acceptable, let us know the threshold. NOTE: Data will be messy and require filtering at the data analysis stage.

Storage:

- 1.5mL Lo-bind tubes or equivalent (Ex: Eppendorf DNA LoBind Tube 1.5mL Cat#022431021)
- For Larger Volumes: 5mL FACS Collection tubes or 15mL conical tubes.
- Keep fresh samples on wet ice. Keep viably cryopreserved samples on dry ice.

WARNING: Plasticware use

- Emulsion-safe plastics are required for 10X Genomics sample handling. Non-approved plastic ware raises risk of chip wetting failures due to presence of plasticizers. Please see approved list of Recommended Pipette Tips. **Avoid:** FroggaBio, Axygen and Starstedt tubes and tips.

Preparation of Frozen Samples:

1. We can accept frozen samples in a variety of forms: snap frozen tissue, cyro-preserved cells, and tissue curls are among the most common. We recommend the use of liquid nitrogen or Isopentane for snap freezing tissues and not dry ice to maintain sample integrity. Tissues should also not be frozen by direct transfer from room temperature to a –80 °C freezer. Please contact the PMGC team to discuss preparation for unique sample types.
2. Samples must not have been any of the following: Freeze-thawed, direct contact with liquid nitrogen or pre-grounded tissue (if so, please consult the contacts below).
3. Please provide a minimum of 30 – 50mg of frozen tissue. We may require more based on cellularity of the tissue:
    - Brain, breast, kidney, liver: 30-50mg
    - Lung, heart, skin, muscle, fat tissue, cartilage: >50mg

    Submitting <20mg of low-yield tissues may adversely impact assay performance. Please contact PMGC for further consultation when planning a project with samples expected to have a low yield.
4. Ideally, tissue age should be within 2-3 years from date of freezing.

Storage:

- Keep flash-frozen tissue on dry Ice in cryovial screw-top tubes (Ex: Corning Cryotube with Orange Lid Cat#430488).
- Ideally, place tissue loosely in the tube to prevent stacking. Stacking the tissue can potentially jam at the bottom of tube and be inaccessible to remove with tweezers without partial thawing.

Samantha Lee
For 10X Single Nucleus inquiries,
(416) 581-7439
samantha.lee2@uhn.ca

Harshpreet Kaur Dhall
For 10X Single Nucleus inquiries,
(416) 581-7439
Harshpreet.dhall@uhn.ca

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