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

PMGC SAMPLE SUBMISSION GUIDELINES FOR 10X GENOMICS FRESH SAMPLES

Prior to Submission Date

1. Please contact Gurbaksh.Basi@uhn.ca and book an appointment for the date and time when your sample submission is expected.
2. Complete the **PMGC 10X Fresh Single Cell Submission Form** prior to sample submission date and send it to Gurbaksh.Basi@uhn.ca.
3. Ensure an **immediate contact** is available for the submission day and about 2 hours after drop-off. Contact information will only be used for potential issues arising during the on-site PMGC sample quality control check, requiring decisions on further sample processing.
4. Incomplete submission forms or delays in receiving form will result in delays to processing samples under optimal conditions and in a timely manner.

Day of Submission

- **Run sample quality control check with Trypan Blue using hemocytometer and determine total cell yield, viability, and concentration prior to submission and update PMGC.**
- Please update any ETA changes and provide at least 30 minutes lead time. 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 timeslot for efficient processing of your sample on arrival.
- 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 samples Monday – Thursday from 9:00 AM – 4:00 PM and Fridays or last day of the week from 9:00 AM – 3:00 PM.

Sample Drop-off

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.
- If you are a PMCRT internal client, you may place your samples in the icebox located outside Rm 9-601 with pre-approval.
- 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 you have any questions.

Gurbaksh Basi
For 10X Fresh Single Cell inquiries,
(416) 581-7439
Gurbaksh.Basi@uhn.ca

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

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Submission Receiving Conditions (Required)

Preparation of Fresh Single Cell/Nuclei Suspension:

1. Wash samples twice in the loading buffer and final concentration adjusted in the ranges below:

Target Capture Recovery	Required Submission Concentration	Limited Input Submissions *please consult PMGC*
500 - 20,000 cells for Standard GEM-X Chemistries	1300 – 1600 cells/ul or nuclei/ul	50,000 cells or nuclei in 40ul
500 cells - 5,000 cells for OCM GEM-X per sample up to 4 samples	900 -1600 cells/ul or nuclei/ul	10,000 cells or nuclei in 11ul

2. Information on flow sorted samples: What are the best practices for flow sorting cells for 10X Genomics assays?
3. 10X Genomics' standard GEM-X reaction is 68% efficient and 60% for OCM GEM-X chemistries.
4. All samples destined for one OCM reaction need to be similar in origin and in RNA content and prepared with the same sample preparation protocol.
5. If RBC lysis has not been carried out, actual cell ranger cell counts may not reflect the expected cell numbers.
6. Ensure samples are single cell or single nuclei suspensions without clumping.
7. Samples should be devoid of large debris. Samples with large debris >40 µM risk clogging during the initial GEM creation step and potential loss of precious samples.
8. There is small 5% failure rate of all chips loaded into the chromium and droplets fail to generate, we will re-attempt with left over sample and claim the failed reagents from 10X. Unfortunately, limited input submissions (one-shot/load-all) precious samples are lost if this happens.
9. **For cryo-preserved or methanol-fixed cells:** Cells must be at >=80% viability prior to preservation (See 10X Genomics protocols for Fresh Frozen or Methanol-Fixed cells)
10. **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.
11. If samples don't meet the above ideal requirements, please consult us**.**

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.

Supportive Media:

- Please provide your cell samples in a supportive media
- Buffers must be compatible with 10X Genomics. Buffers composed predominantly of PBS can cause cell stress and lead to changes to the transcriptome after extended periods.
- Alternative Supportive Media (up to 10% FBS or up to 2%BSA):
    - EMEM + 10% FBS
    - DMEM + 10% FBS

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  - RPMI + 10% FBS
  - Hams F12 + 10% FBS
  - IMDM + 10% FBS
  - DMEM:F12 + 10% FBS
- Media should not contain excessive amounts of **EDTA (> 0.1mM), or magnesium (> 3mM), or surfactants (Tween-20, etc.)** as those components will inhibit the reverse transcription reaction or interfere with GEM generation.
- Please contact PMGC if there are any concerns with assay compatibility.

WARNING: Plasticware use

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

Sample Shipping

If shipping Cryo-Preserved or Methanol Fixed 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 cells. Within Canada, we recommend FedEX Next Day Priority services.

Shipping address:

Attn: (insert PMGC contact person)
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Canada

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