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

PMGC SAMPLE SUBMISSION GUIDELINES FOR 10X GENOMICS FLEX CLIENTS

Prior to Submission Date

- Please complete the **Sample Submission Form** and send to the PMGC contacts below.
- Please check the 10x Flex probe sets to confirm any project critical genes are present in the set. If your samples require custom probes, please make a note in the **Submission Form** and provide the gene sequences to the PMGC contacts.
- If submitting several Flex assay project samples, please separate your samples into their desired processing groups and arrange them in the order of the sample names listed in the **Submission Form.**
- Please ensure sample tube names match the names listed on the **Submission Form** to avoid any processing delays.
- If submitting fixed single cell/nuclei suspensions dissociated from tissue, please confirm the dissociation method compatibility with 10X Genomics' workflow. Ensure that your dissociation method has been optimized and provides adequate single cell suspensions (sufficient cell counts, ≥95% of the sample is single/unclumped cells with minimal debris) prior to submission.
- If submitting FFPE tissue sections or frozen tissue, please provide an additional sample for dissociation optimization/testing if possible.
  ***NOTE:** If you require additional optimization/testing, please contact Troy Ketela (Troy.Ketela@uhn.ca) for recommendations and associated fees.

Day of Submission

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 Allan Slaight Medical Innovation Labs (ASMIL) at your pre-arranged time. ASMIL 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: Paula Chadic
Princess Margaret Genomics Centre
101 College St.
ASMIL, Rm 9-601A
Toronto, Ontario M5G 1L7
Canada

Paula Chadic
For 10x Fixed Single Cell inquiries,
(416) 581-7439
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Aniqa Khan
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Dr. Troy Ketela, Head of Operations
For new project inquires,
(416) 634-8816
Troy.Ketela@uhn.ca

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Submission Receiving Conditions (Required)

Fixed/Frozen Single Cell or Nuclei Suspensions

Storage:

- Store the fixed single cell/nuclei suspension(s) using the 10X Genomics -80°C storage guidelines here. Samples can be stored up to 12 months at -80°C.
- Use 1.5mL Lo-bind tubes or equivalent (*e.g.* Eppendorf DNA LoBind Tube 1.5mL Cat#022431021) to minimize sample loss.

Quality:

- **DO NOT** exceed 10 million cells/nuclei per fixation reaction.
- Count your cells/nuclei after fixation, before freezing down your samples for submission:
  - Ensure a minimum of 400,000 cells/nuclei (targeting 20,000 cells/nuclei)

Our minimum cell/nuclei number requirements differ from those recommended by 10x Genomics. This adjustment accounts for expected cell/nuclei loss during post-storage handling and downstream processing. If your sample falls below our minimum threshold but you're still interested in proceeding with the assay, please reach out to us directly.

- We recommend fixing cells/nuclei suspensions at 4°C for 16-24h (except for granulocytes, leukocytes and bone marrow mononuclear cells). **DO NOT** mix samples with different fixation times into one experiment.
- It is recommended that samples are cleaned up if viability is <80%. Low viability may have more variable cell calling and lower sensitivity, potentially leading to poor data quality downstream.
- The Flex assay works best if the sample is fully dissociated before fixation. Please ensure ≤5% cell aggregates in the fixed single cell/nuclei suspensions.
- Debris should be kept to a minimum for best results. Debris can have associated RNA and negatively affect the data quality. Please reach out to confirm if your clean up methods are compatible with 10X Genomics guidelines.

Flash Frozen Tissue

Storage:

- Use liquid nitrogen or isopentane for snap freezing of fresh tissue; avoid freezing tissues on dry ice or at -80°C, as slower methods can stress and damage cells/nuclei.
- Keep flash-frozen tissue on dry ice in cryovial screw-top tubes (*e.g.* 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.
- Store fixed tissue pieces using the 10X Genomics -80°C storage guidelines here. Submit on dry ice. Samples can be stored for up to 12 months at -80°C.

Quality:

- Ideally a minimum of 30-50mg. May require more based on tissue cellularity. Please schedule a consultation with us if the full tissue piece must be used (i.e. a random sampling cannot be taken) as this may affect assay costs and technical workflow
  - Brain, breast, kidney, liver: 30-50mg
  - Lung, heart, skin, muscle, fat tissue, cartilage: >50mg
- Samples over 5 years from freezing date have been shown to have RNA degradation and this can negatively impact data quality.

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- Samples must not have been any of the following: Freeze-thawed, direct contact with liquid nitrogen, or pre-grounded tissues (if so, please contact us directly).

FFPE Tissue Scrolls

- Obtain and store the FFPE scrolls for processing based on the guidelines here. Scrolls can be stored at **4°C** for up to 1 year.
- On the **Sample Submission Form**, please indicate the date the tissue block was sectioned under "Sample Quality/Collection Date." Please indicate the number of sections x thickness of the scrolls under "Sequencing Requirements and Additional Notes."
- Deliver your scrolls to PMGC at ambient temperature, securing the tubes well to minimize agitation during transport. If scrolls are being shipped to PMGC, a cold pack may be included to mitigate possible temperature fluctuations during transport.
    - For human tissue, it is recommended to use two or more 25 µm sections.
    - For mouse tissue, it is recommended to use two or more 50 µm sections.
- We can process up to six 25 µm scrolls per reaction. If this number is exceeded, additional charges will apply to accommodate the extra reagents needed for processing.

Key Considerations

- Perform optimization experiments to validate preparation methods for specific tissues before performing large scale studies. Check here for Flex tested sample types with special considerations (e.g. spleen tissue, pancreas tissue, bone marrow mononuclear cells).
- Only use reagents recommended by 10X Genomics for the Flex protocol. Please see approved list of Recommended Pipette Tips.
- Each sample may yield different amounts of material and data quality, depending on different factors (*e.g.* age, pre-storage handling, tissue type, pre-fixation quality, tissue density, size/area of tissue)
- There is a mandatory 30 µm filtration step in the Flex assay protocol. Please be aware of the cell sizes submitted in your sample population.
- For samples known to have red blood cells (RBCs), we recommend performing RBC lysis before fixation of samples. RBC contamination can negatively impact data for single cell sequencing as it can take up sequencing reads.
- For multiplex experiments: we do not recommend pooling samples with different RNA content (*e.g.* PBMC samples with tumor samples, control samples with treated samples if treatment impacts RNA levels). More sequencing reads will be distributed to the samples with a higher proportion of RNA content even though all samples have the same sequencing saturation. Thus, samples with more RNA will have more reads per cell compared with those with lower RNA content. It is not possible to add reads to a particular sample.

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Lower input cell/nuclei numbers increase the risk of:

- Reduced target cell/nuclei capture and data output
- Unequal pooling of samples, where samples with low counts may be underrepresented
- Limited material, potentially allowing only a single attempt at Chromium loading
    - While rare, there is a known failure rate during droplet generation on the Chromium platform. If backup material is available, we will attempt a reload. However, for single-use samples, this could result in complete sample loss.

Cell/nuclei loss in wash steps is variable depending on sample type.

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