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

PMGC SAMPLE SUBMISSION GUIDELINES FOR 10X GENOMICS FLEX CLIENTS

Prior to Submission Date

- Please complete the **PMGC 10X Flex Assay Submission Form** prior to sample submission and email to Mary Shi (mary.shi@uhn.ca). Please be sure to fill out all fields in the form and include N/A when it is not applicable to your project.
- If dissociating **before** submitting, please confirm the dissociation method compatibility with 10X workflow. Ensure that your dissociation method has been optimized and provides adequate single cell suspensions prior to submission.
- If submitting fixed and/or frozen tissue, please provide an additional sample for dissociation optimization/testing if possible.
  ***NOTE:** If you require additional optimization/testing please contact Dr. Troy Ketela (Geneservice@pmgenomics.ca) for recommendations and associated fees.

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

Mary Shi
For 10X Flex Assay inquiries,
(416) 581-7439
Mary.Shi@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)

Fixed/Frozen Single Cell or Nuclei Suspensions

Storage:

- Please store the fixed single cell suspension using the 10X Genomics -80°C storage guidelines here. Samples can be stored up to 6 months at -80°C.
- Please be sure to use 1.5mL Lo-bind tubes or equivalent (Ex: Eppendorf DNA LoBind Tube 1.5mL Cat# 022431021) as this will decrease sample loss.

Quality:

- Minimum of 300,000 total cells OR 500,000 nuclei going into fixation, preferably 1 million cells or nuclei. **DO NOT** exceed 10 million cells/nuclei per fixation reaction.
- **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 down the line.
- Debris should be kept to a minimum for best results. Debris can have associated RNA and affect the data quality. Please reach out to confirm if your clean up methods are compatible with 10X guidelines.

Fixed/Frozen Tissue

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.
- Please store fixed tissue pieces using the 10X Genomics -80°C storage guidelines here. Submit on dry ice. Samples can be stored up to 6 months at -80°C.

Quality:

- Ideal minimum of 30-50mg. May require more based on cellularity of the tissue:
  - 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 impact data quality.
- Samples must not have been subjected to any of the following: Freeze-thawed, direct contact with liquid nitrogen or pre-grounded tissues (if so, please contact us directly).

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FFPE Tissue

- Obtain and store the FFPE scrolls for processing based on the guidelines here.
- Please schedule sample drop off well in advance as scrolls must be processed into single cell suspensions within a week of collection.
- 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, depending on tissue type (check the protocol for cell yields from different FFPE tissue types).

IMPORTANT NOTES:

- We recommend performing optimization experiments to validate preparation methods for specific tissues before performing large scale studies.
- Do not substitute any reagents that are not recommended by 10X Genomics. You may substitute the formaldehyde for any molecular biology grade stock solution of 37% that is ~19% methanol stabilized with little to no precipitant.
- Each cell suspension/tissue/FFPE block may yield different amounts of material and data quality, depending on the age, pre-storage handling, tissue type, pre-fixation quality, tissue density, size/area of tissue, and other factors.
- Due to the nature of this protocol, providing less than the recommended amount of cells/tissue/scrolls will result in significant decrease of target capture number and data output.

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