Duke Molecular Genomics Core

10x Genomics Single Cell/Nuclei Project Workflow

Consult and/or Cost Estimate

- Email karen.abramson@duke.edu to request a cost estimate and/or consult meeting.
- Information required for estimate: assay type, sample species, how many cells/nuclei you want to target (capture with the assay), input material (fresh cells, cryopreserved cells, or frozen tissue for nuclei), how many samples, how many batches, and do you want data analysis.

Scheduling & Ordering

- **A test run is highly recommended!** Performing a test run provides an opportunity for sample prep optimization. Prep your sample as you would for the real run and bring it to us for a count/viability assessment. **We use AOPI staining which is more accurate than trypan blue.**
- **Submit a service request in CoreResearch@Duke.** We cannot order kits until this is received.
- **Please note, any kits that we order for your project are non-refundable.**
- Email karen.abramson@duke.edu to schedule your test run and your full 10x run.

Preparing for you run

- For your planned 10x run, fill out and email us the "**New Project Summary and Sample Submission**" forms. This is not necessary for the test run.
- **Carefully review sample preparation guidelines! This is critical for the success of your project.**
- Our team will reach out to arrange for sample drop off.

Run day

- Let us know approximately what time you will arrive. We prefer to receive samples by 2pm, but exceptations can be made if you discuss it with us in advance.
- Text or call the lab analyst assigned to your project when you arrive.
- **Remain available by phone for one hour after drop off.** **If the cell viability or nuclei intactness is <80% and/or we don't have enough cells to meet your target, we will not proceed without your approval.**

Post-library generation

- We will contact you to verify the sequencing plan.
- Libraries will be either transferred to the Duke Sequencing Core or back to you.

Post-sequencing at Duke

- If we are providing data analysis, we will download the data and begin processing. If you require a **custom reference** (e.g. you have a transgene), you must inform us before we start.
- The data analysis team will contact you for more details regarding your analysis plan.
- **It is important that you download the raw data**, regardless of whether we are providing the analysis or you are doing it on your own. **We will only store raw data for 3 months.**

Updated March 2023

Duke Molecular Genomics Core

10x Genomic Single Cell and Single Nuclei Sample Preparation Guidelines

Failure to follow the sample preparation guidelines listed below could result in reduced data quality and/or potential issues in GEM generation on the 10x Genomics® Chromium instrument.

It is essential that you follow the sample preparation protocol provided by 10x Genomics® and the MGC.

The 10x Genomics® Chromium instrument has an expected cell capture rate of approximately 60%, meaning if we want to target 10,000 cells we have to load ~16,000. However, the capture rate is dependent on an accurate cell count and concentration, high viability, and a sample free of debris. Due to all these variables **we cannot guarantee a 60% capture efficiency**, despite adhering to best practices and 10x Genomics® recommendations. In addition, there is a **+/- 20% expected difference between cells targeted and cells captured, meaning that if 10,000 cells are targeted, a successful run would yield between 8,000 and 12,000 cells.**

The best way to ensure good quality data is to strictly follow the provided sample preparation guidelines.

- **Cell counting is critical**. If you do not submit enough cells for us to count them twice, or if the concentration of the cells is below 500 cells/ul ($5x10^5$ cells/ml) we cannot guarantee the accuracy of the cell count. An inaccurate cell count could adversely affect the number of cells loaded, and therefore the number of cells targeted and captured. Cell counts are less accurate at low dilutions and/or viability <80%.
- **Despite all best practices and precautions taken, there is always a risk of clogging or a wetting failure on the 10x Genomics® Chromium instrument.** If we have enough cells to re-run a sample that has experienced a clog or wetting failure, we will do so free of charge. Low viabilities, cell clumping, and/or debris all increase the risk of clogging or wetting failure on the instrument.
- **Use Eppendorf DNA LoBind Tubes, 2.0 ml (cat# 022431048). *No substitutions are allowed. Items have been validated by 10x Genomics® and are required for Single Cell workflow, training and system operations.** Use of alternative plastic products increases the risk of clogs and wetting failures.

The full 10x Genomics' Cell Preparation Guide can be found here https://cdn.10xgenomics.com/image/upload/v1660261285/support-documents/CG000053_CellPrepGuide_RevC.pdf.

Key points have been outlined below:

How many cells do we need?

- The minimum is 3-4x the number of cells you want to capture with the assay. We load ~2x the amount you want to capture, and we also need some for the count and viability assessment.

Washing & Resuspension

- The recommended cell washing and resuspension buffer is 1X PBS (calcium and magnesium free) containing 0.04% weight/volume BSA (400 µg/ml).
    - BSA is added to minimize cell losses and aggregation.
        - **Do NOT exceed 2% BSA**
- Primary cells, stem cells, and other sensitive cell types may require washing and suspension in alternative buffers to maximize viability.
    - The following alternative buffers have been verified to not influence performance of 10x Genomics® Single Cell Protocols when profiling mixtures of human 293T/17 and mouse NIH/3T3 cell lines:
        - Dulbecco's Phosphate-Buffered Saline (DPBS)
        - Hank's Balanced Salt Solution (HBSS)

- If cell viability cannot be maintained in one of the above buffers, it is also possible to wash and resuspend in most common cell culture media, with or without serum. The following media have been verified to be compatible with 10x Genomics Single Cell Protocols with little or no loss of performance when profiling mixtures of human 293T/17 and mouse NIH/3T3 cell lines:

    - Eagle's Minimum Essential Medium (EMEM) + 10% FBS
    - Dulbecco's Modified Eagle Medium (DMEM) + 10% FBS
    - Iscove's Modified Eagle Medium (IMEM) + 10% FBS
    - Roswell Park Memorial Institute (RPMI) + 10% FBS
    - Ham's F12 + 10% FBS
    - 1:1 DMEM/F12 +10% FBS
    - M199

- **Do NOT exceed 10% FBS**
- **Do NOT exceed 0.1nM EDTA**
- **Do NOT exceed 3mM magnesium**
- **Do NOT include any surfactants, such as Tween-20**

- When washing and resuspending cells, always use sufficient volumes to maintain concentrations at less than 5000 cells/µl (e.g. less than 5 million cells per tube when using 1 ml washing solution in 2 ml LoBind tubes).
    - Maintaining cells at higher concentrations can cause aggregation and clumping that will interfere with generation of ideal single cell suspensions.
    - **Final concentration for submission should be 1.0-1.5 x 10^6 cells/ml. Lower concentrations affect the accuracy of the cell counting.**

RBC Lysis

- Red blood cells (RBCs) will be encapsulated into GEMs and will count towards your total captured cell number.
    - If your samples contain RBCs and they are not a population of interest you should perform a red blood cell lysis.
    - We do not offer this as a service. If RBC lysis is necessary, it must be performed prior to sample drop-off.

Straining Cell Suspensions

- GEM generation occurs in microfluidic channels that are narrower 50µm. Debris, fibers, or clumped cells can affect optimal system performance and result in GEM generation failure.
    - Filtering cell suspensions with an appropriate cell strainer is helpful for removing large clumps and debris. The strainer should have a pore size that is larger than the maximum cell diameter in the sample, but small enough to catch larger clumps.
    - Flowmi™ Tip Strainer (VWR catalog# 10032-802) is recommended to minimize volume losses. However, the cell concentration can decrease by 30% or more. It is therefore important to measure the cell concentration after straining for an accurate cell count.

Sorted cells

- Sorted cells are often low in concentration, requiring us to spin them down to concentrate them. Between cells lost during sorting and this extra spin, we often end up seeing ~50% of what the sorter says you have. Keep this in mind when you're determining how many cells to sort.

Tissue Handling & Storage for Single Nuclei RNAseq

- Work quickly and minimize handling during all tissue processing steps.
- Wash tissues in a clean **glass** petri dish with cold PBS upon harvest and absorb any excess blood using a laboratory wipe.
- Cut tissues into small pieces (e.g. the size of a rice grain) in a **glass** petri dish for ease of freezing and place in a cryovial. If you use a plastic dish, microscopic pieces of plastic can get into the sample and affect downstream GEM generation on the Chromium instrument.
- To flash freeze, either submerge the cryovial in liquid nitrogen or a liquid-nitrogen cooled bath (e.g. isopentane) or place the tube deep in a bucket of dry ice. Wait at least 2–3 minutes for the tissue to freeze all the way through, and transfer the tube containing the tissue to vapor phase liquid nitrogen for long-term storage.
- Tissues should be stored long-term in a cryovial in liquid nitrogen for best results. Tissues can be stored short-term (1–2 days) at -80°C if needed.
- Once removed from liquid nitrogen, tissues should be maintained at -80°C or on dry ice until use.
- Nuclei isolation may require optimization.

Please don't hesitate to ask questions! We're here to help! Please email DMPI-MGC@dm.duke.edu or karen.abramson@duke.edu.

Updated March 2023