10X Genomics Chromium Single Cell Gene Expression best practices

A guide to working with single cells upstream of Next Generation Sequencing

Application Space

- High-throughput, single cell gene expression dynamics detection
- Molecular profiling and clustering of heterogeneous cell populations
- Identify rare cell types in heterogeneous populations
- Immunology, cancer, and stem cell research

Planning your 10X experiment

10x Genomics single cell technology is a powerful tool in any biological field where cell heterogeneity exists. Single cell transcriptomes (scRNA-seq) technology allows for the identification of subpopulations down to ~1% abundance.

A single cell suspension with high quality cellular mRNA is critical for a successful experiment. Minimizing the presence of cellular aggregates, dead cells, non-cellular nucleic acids and potential inhibitors of reverse transcription is critical to obtaining high quality data. The total number of cells required is determined by the cell recovery target defined by the user and ranges from 870 cells to 17,400 cells.

When planning your scRNA-seq experiment, several factors will inform the number of cells you need to load: previous experimental results, the purpose of your experiment (cluster heterogeneous cell population, isolate a small number of rare cells, and/or study expression dynamics), tolerance of multiplet loading (where more than one cell is captured in a bead), and sequencing budget. OICR scientific staff can provide detailed advice on any of these aspects of your study once you have submitted a Project Information Form to us.

10X offers several cell specific protocols as well as their general guide which OICR staff can review with you as we design a project with you.

Determining input and submission requirements

The next step is to decide how cell samples will be transported to OICR. Generally, we recommend submitting live cell suspensions where possible (10X's recommended media is PBS + 0.04% BSA), but cryopreservation, nuclear extraction, or methanol fixation is also an option, especially for labs outside of the core GTA. Cell delivery can usually be coordinated with OICR staff to minimize time between suspension and library creation.

A recommended starting input of 1,700 cells per sample is expected to produce data for approximately 1,000 cells while keeping the incidence of multiplets low. Online tools exist to help you determine the minimal amount of cells to provide for your experiment based on the abundance of cell subpopulations that you need to detect.

Multiple Rate (%)	# of Cells Loaded	# of Cells Recovered
~0.4%	~870	~500
~0.8%	~1,700	~1,000
~3.9%	~8,700	~5,000
~7.6%	~17,400	~10,000

Table 1: Approximate multiplet rates versus cell loading

Cell suspensions should be loaded as soon as possible after preparation, ideally within 30 minutes. Depending on your cells of interest, viability may significantly decrease when cells are kept in suspension for a prolonged period of time before use. Some cells types, such as peripheral blood mononuclear cells (PBMCs), can form persistent clumps when they are kept in PBS for an extended period of time. Clumping will decrease the effective concentration of single cells in the input suspension, reduce the percent of cells recovered and increase the rate of multiplets.

Accurate cell counting and loading is crucial to generation of the expected cell yield. OICR scientific staff can provide hemocytometer services, to ensure accuracy in

the cell counts. In addition, clumps, debris and fibers in the cell suspension can interfere with the accuracy of cell counting, especially when using automated cell counters. Minimization of these components by filtering or other techniques is necessary for both accurate counts and proper loading.

Input cell suspensions should contain more than 70% viable cells. Non-viable and dying cells generally contain less RNA that is often more fragmented and may not be efficiently captured by 10x Genomics Single Cell Solutions. If you think that you cannot maximize cell viability further and still have dead cells, we have successfully used the dead cell removal protocol offered by 10X.

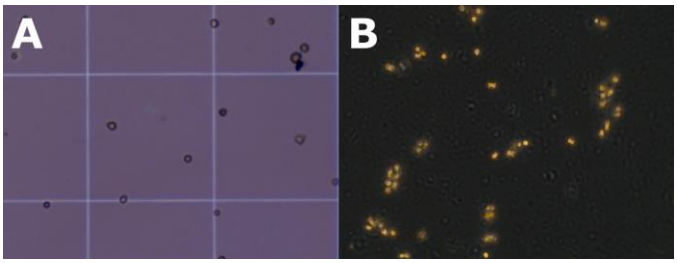


Figure 1: A) Well separated single lymphoblasts under bright field illumination. B) Lymphoblasts exhibiting a high degree of clumping, which would result in a high multiplicity rate for 10X experiments

To prepare your cells for a single cell experiment with us, we recommend you wash your cells in 1x PBS (calcium and magnesium free) containing 0.04% weight/volume BSA (400ug/ml), centrifuging at 150 rcf for 3 min at room temperature for larger cells such as immortalized cell lines, or 300 rcf for 5 minutes at room temperature for smaller cells like PBMCs. Ideal centrifugation conditions will generate a solid, but not too tightly packed, cell pellet with minimal cells remaining in the supernatant. Tight packing increases the risk of cell damage during resuspension. If possible, centrifugation conditions can be optimized by counting cells in the supernatant to ensure that losses are low, especially if your samples contain cells of different sizes.

To minimize any physical damage to the cells during resuspension, we recommend using wide-bore pipette tips and gentle pipetting. If regular bore pipette tips are used, it is especially critical to focus on gentle and slow pipetting during resuspension as the risk of shearing and damaging cells is higher. Resuspension in the PBS and BSA solution described above is recommended but alternative buffers can be used to maximize viability of primary cells, stem cells, and other sensitive cell types.

When washing and resuspending cells, always use sufficient volumes to maintain concentrations at less than 5000 cells/µl. Higher concentrations of cells can cause aggregation and clumping that are not ideal for generation of single cell. OICR scientific staff will work with you to determine the optimal final cell concentration for 10X experiments.

Single cell RNA library preparation

The 10X Single Cell 3' Protocol is a scalable platform for 3' gene expression profiling of 500 – 10,000 individual cells per sample. The technology uses a pool of ~750,000 distinct barcodes to separately index each cell's transcriptome, by partitioning single cells into nanoliter-scale Gel Bead-In-EMulsions (GEMs), where all generated cDNAs in a GEM share a common 10X barcode. Libraries are generated and sequenced from the cDNA and the 10x barcodes are used to associate individual reads back to individual cells.

The OICR genomics core can readily process 8 samples at the same time and has undertaken projects creating 100s of 10X libraries. Please contact us to discuss projects with large throughput.

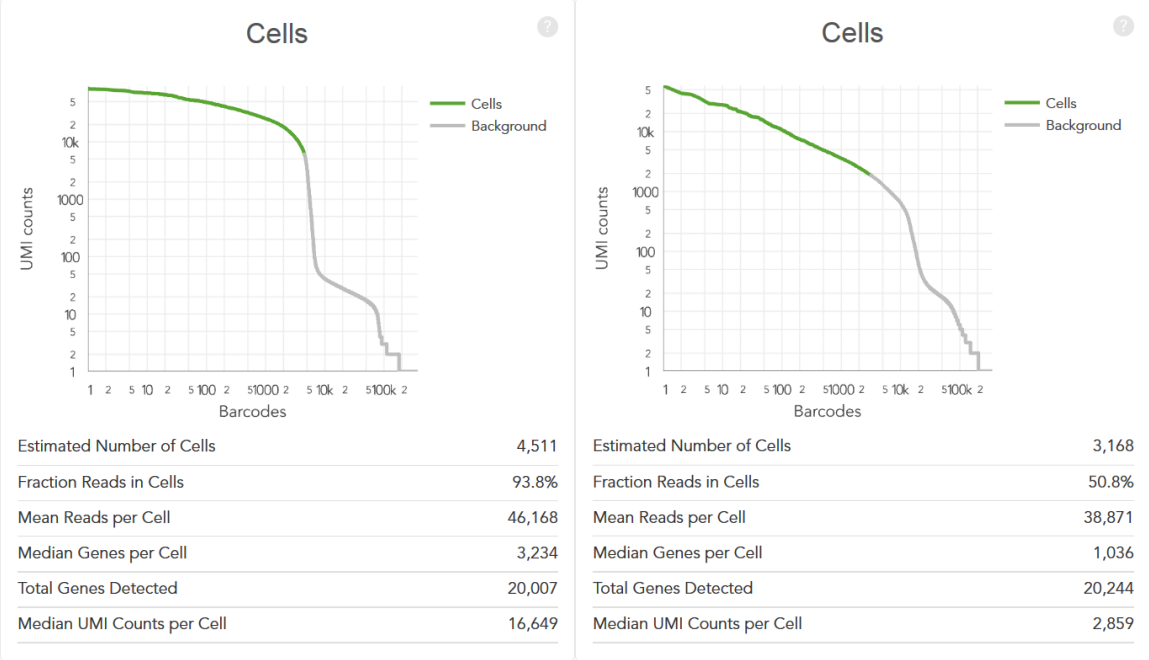
Quality control metrics are an intrinsic part of all OICR genomics services. The submitted sample, amplified single cell transcriptome, and final library will all undergo strict quality control steps to ensure biologically relevant results.

Bioinformatics Analysis

OICR's Genome Sequencing Informatics (GSI) team runs a variety of 10X tools for QC and data analysis. Basic analysis provides properly demultiplexed, paired FASTQ Illumina sequencer reads so that users can run their own analysis. Primary analysis consists of alignment, filtering, and UMI counting , followed by generation of cell specific gene-expression matrices, using 10X's Cell Ranger software. Automated pipelines are available for rapid and consistent analysis of data generated in high volume

projects, to allow rapid turnaround from sample submission to data handoff. Additional analysis is project specific, and can be discussed with our bioinformatics team or specified in our Project Information Form. Please see our website for details on additional analysis approaches and past projects.

Poor 10X scRNA-seq runs can be quickly spotted from Cell Ranger QC information. Ideally, the number of Barcodes (an indicator of cells captured in GEMS) and UMI counts (transcripts per GEM) is initially high, followed by a sharp drop to background levels (Figure 1). Poorly prepared samples (Figure 2) will show a gradual decline in the UMI counts, an indication of random capture of RNAs in GEM beads that arises from broken cells releasing RNA into supernatant.



Estimated Number of Cells	4,511
Fraction Reads in Cells	93.8%
Mean Reads per Cell	46,168
Median Genes per Cell	3,234
Total Genes Detected	20,007
Median UMI Counts per Cell	16,649

Estimated Number of Cells	3,168
Fraction Reads in Cells	50.8%
Mean Reads per Cell	38,871
Median Genes per Cell	1,036
Total Genes Detected	20,244
Median UMI Counts per Cell	2,859

Figure 2: QC data from good samples shows a characteristic drop-off in UMI counts once most cell associated barcodes have been identified (Left panel). Poor samples do not show a clear threshold of UMI counts delineating cell containing GEM beads (Right panel).

Summary

10X scRNA-seq experiments are a powerful addition to your research program. One of the most important determinants of having a successful run is preparing your cell sample upstream of library preparation. By working with our experienced 10X team during the project design phase, we can help to optimize your experiments by minimizing cell input requirements, ensure consistent output, provide rapid turnaround and analysis of the data and reduce the overall cost of your project.

For more information please visit genomics.oicr.on.ca