UF | ICBR Gene Expression & Genotyping

Single Cell Analysis Frequently Asked Questions

Q: What is the sample requirement for single cell transcriptome analysis?

A: Please contact Gene Expression & Genotyping Core for planning of your experiment (ICBR-GeneExpression@ad.ufl.edu). This meeting is important because there needs to be close coordination between your lab and the ICBR cores to make sure that cells are processed without any delay between steps.

For more information, please visit 10x Genomics website
Single Cell 3' RNAseq:

https://cdn.10xgenomics.com/image/upload/v1722285481/support-documents/CG000315_ChromiumNextGEMSingleCell3__GeneExpression_v3.1_DualIndex__RevF.pdf

Single Cell V3 ***user guider for CRISPR Screening:***
https://assets.ctfassets.net/an68im79xiti/7oWTi4259uwu06kmeCQG4g/caacae48b97b58660c7547cea9c067b6/CG000184_ChromiumSingleCellSingleCell3v3_FeatureBarcodingtechnology_CRISPR_RevA.pdf

Impact of sequencing depth on copy number detection:
https://kb.10xgenomics.com/s/article/115002022743-What-is-the-recommended-sequencing-depth-for-Single-Cell-3-and-5-Gene-Expression-libraries

10xGenomics Questions & Answers: https://kb.10xgenomics.com/hc/en-us

GEM-X Flex v2: https://assets.10xgenomics.com/m/643558064873c24c/original/CG000834_GEM-X-Flex-v2_Gene-Expression_UserGuide.pdf

Q: What the sample requirement for single cell ATAC?

A: Please contact the Gene Expression and Genotype Core for a planning of your experiment (ICBR-GeneExpression@ad.ufl.edu). This meeting is important because there needs to be close coordination between your lab and the ICBR cores to make sure that cells are processed without any delay.

For more information, please visit 10x Genomics website or download the following protocols:
Demonstrated Protocol Nuclei Isolation ATAC.
https://cdn.10xgenomics.com/image/upload/v1775599228/user-guides-pdfs/CG000505_Chromium_Nuclei_Isolation_Kit_UG_RevB.pdf

Single Cell Protocols Cell Preparation Guide:
https://cdn.10xgenomics.com/image/upload/v1728078402/support-documents/CG000496_Chromium_NextGEM_SingleCell_ATAC_ReagentKits_v2_UserGuide_RevC.pdf.pdf

Q: How to calculate the number of reads needed?

A For instance, let's suppose that in your experiment you plan to have 12 samples (# Chromium channels), with an expected # cells capture of 10,000 per sample and a desired reads per cell of 50,000. You will need 12x500 million read pairs total (6000 million read pairs). Since the HiSeq3000

generates ~300 million read pairs per lane, the 12 samples can be sequenced in 20 lanes or 2.5 full flow cells.

Because of the asymmetric sequencing configuration, we may have logistical challenges with filling HiSeq flow cells, unless you request 8 lanes worth of sequencing. For this reason, for smaller experiments, people use the NextSeq500. The NextSeq flow cell generates ~400 million read pairs per run. So, for all practical purposes you can roughly think of one NextSeq500 high output run per sample if you follow the standard recommendations by 10X for scRNA-Seq. Some of our users are sequencing as many as 4 samples (3k-6k cells per sample) on a single NextSeq500 high output run.

If you want to limit your sequencing cost, you can:

1- lower the sequencing depth. For example, you can target 20,000 read pairs/cell (12 samples, 10,000 cells/samples).

2- lower the # of cells captured. For example, you can target 4,000 cells/sample (12 samples, 50K sequencing depth).

Q: What is the good volume and concentration of the cells to submit?

A:

- If targeting up to 10,000 cells, the ideal cell suspension conc. is from 700-1,200cells/uL
- If targeting up to 20,000 cells, the ideal cell suspension conc. is from 1,200 – 1,600cells/uL
- The ideal viability is ≥90%
- Ensure that there is no clumping or excessive debris prior to arrival. These issues could lead to clogging and poor cell recovery or total sample loss.
- Please bring a minimum of 50-75uL sample to allow for both counting and loading.

Q: What if my cells are of Poor Quality?

A: If **viability** is poor (60-80%) or very bad (<50%), 10X Genomics do not recommend loading as the libraries and data produced will not be guaranteed.

- If you have a dead cell removal method available to them, you can return to your lab and attempt to improve the sample quality.
- If the customer has only 1 or 2 samples, we could potentially use LeviCell to remove dead cells. Speak to core director about this strategy.

If there are noticeable **clumping or debris,** 10X Genomics do not recommend loading as the sample will likely clog the microfluidics in the loading chip.

Q: What if the cell concentration is too low?

A: If the volume of the cell suspension is high, you can spin down their cells and resuspend at a lower volume to increase concentration.

Q: Do you have other single cell platforms available?

A: Yes, we have Evercode WT from Parse, PIPseq from Illumina and single channel of BD Rhapsody. Parse Evercode WT has strong nuclei performance and is excellent for multi-sample projects. You can process very large studies at a lower cost per cell. Parse has three scales of the kit, Evercode™ WT Mini v3 for up to 10k cells, Evercode™ WT v3 for up to 100k cells and Evercode™ WT Mega v3 for up to 1000k cells. Illumina PIPseq is relatively newer and provides a high-resolution view into gene expression within complex tissues with relatively lower cost. Their gentle isolation technique helps detect fragile and rare cells accurately and can be easily adapted for custom applications.

Please contact the Gene Expression and Genotype Core if you have more questions about the platforms for your experiment (ICBR-GeneExpression@ad.ufl.edu).