UF | ICBR NextGen DNA Sequencing UF | ICBR Gene Expression & Genotyping

10x Genomics Libraries

Linked-reads libraries

Sample requirements:

1. High quality HMW gDNA. DNA < 50 kb can be problematic, and DNA <20 Kb can be highly problematic. Size-selection using SageELF can help to enrich for >50 Kb.
2. At least 3 ng of gDNA in 5 ul, although we prefer to have 10 ng in >10 ul.
3. For recommendations on gDNA extraction methods, please visit: https://kb.10xgenomics.com/hc/en-us/articles/217233726-Recommended-genomic-DNA-extraction-protocols

Sequencing requirements:

1. For de novo assembly, Supernova (10x Genomics software) requires 2x150 reads.
2. Sequence 38X to 56X coverage (https://support.10xgenomics.com/de-novo-assembly/guidance/doc/achieving-success-with-de-novo-assembly)

Best Practices for Handling HMW gDNA (from Chromium™ Genome reagent Kits v2 user guide)
The following tips are designed to maintain the integrity of HMW gDNA through extraction:

- Elute and store HMW gDNA in TE.
- Never vortex tubes containing HMW gDNA.
- If possible, use a wide-bore pipet tip and pipet HMW gDNA slowly to prevent DNA from shearing.
- Avoid repeated freeze/thaw cycles of HMW gDNA.
- Extracted HMW gDNA samples (>10 ng/µl) can be stored at 4°C for up to 2 weeks, or at −20°C for up to 6 months.
- Prepare multiple aliquots at −20°C if a stored HMW gDNA solution will be analyzed multiple times.
- Use nuclease-free reagents and consumables.

Single Cell ATAC

Sample requirements:

Please contact the NextGen Core for a planning of your experiment (ICBR-Nextgen@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. We will need 5 ul of nuclei suspension for immediate processing.

For more information, please visit 10x Genomics website or download the following protocols:
Demonstrated Protocol Nuclei Isolation ATAC

Sequencing: https://assets.ctfassets.net/an68im79xiti/18T8YsKiwKcGUakQmc8U22/086d0daa06444314fb757e35828f3c08/CG000169_DemonstratedProtocol_NucleiIsolation_ATAC_Sequencing_RevA.pdf

Single Cell Protocols Cell Preparation
Guide: https://assets.ctfassets.net/an68im79xiti/56DlUZEsVOWc8sSG42KQis/35cbcf6dcd4b0c0196263ee93815b0ae/CG000053_CellPrepGuide_RevC.pdf

Sequencing requirements:

- 10x Genomics recommends 25,000 reads from R1 and 25,000 from R2 per nucleus.
- PhiX Spike-in: 1 %
- Sequencing configuration: 50/8/16/50
- Supported sequencers: HiSeq 3000, NextSeq, MiSeq
- https://support.10xgenomics.com/single-cell-atac/sequencing/doc/specifications-sequencing-requirements-for-single-cell-atac

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10x Genomics Single Cell libraries: Gene Expression v3, CNV, V(D)J (TCR or Ig) and 5' Gene Expression libraries

Sample requirements:

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
Sequencing Requirements for Single Cell 3' RNAseq: https://support.10xgenomics.com/single-cell-gene-expression/sequencing/doc/specifications-sequencing-requirements-for-single-cell-3Single Cell V3 ***user guider for CRISPR Screening:***
https://assets.ctfassets.net/an68im79xiti/7oWTi4259uwu06kmeCQG4g/caacae48b97b58660c7547cea9c067b6/CG000184_ChromiumSingleCellSingleCell3v3_FeatureBarcodingtechnology_CRISPR_RevA.pdf
Sequencing Requirement for Single Cell DNA: https://support.10xgenomics.com/single-cell-dna/index/doc/specifications-sequencing-requirements-for-single-cell-dna
Impact of sequencing depth on copy number detection: https://support.10xgenomics.com/single-cell-dna/software/pipelines/latest/interpret/read_depth
Single Cell V(D)J: https://support.10xgenomics.com/single-cell-vdj/sequencing/doc/specifications-sequencing-requirements-for-single-cell-vdj
10xGenomics Questions & Answers: https://kb.10xgenomics.com/hc/en-us
Single Cell CITEseq Announcement: https://www.10xgenomics.com/news/10x-genomics-partners-with-biolegend-and-immudex-to-extend-research-applications-of-its-new-single-cell-feature-barcoding-technology/

Sample requirements for sequencing of pre-constructed 10x Genomics single cell libraries:

Please provide us with at least 15 ul, 5 nM of your final library. Quantification must be done by QUBIT and qPCR.

10x Genomics recommends qPCR for library quantification, our lab provides Qubit and TapeStation services for library evaluation and qPCR quantification service is provided by the Gene Expression & Genotyping Core. Please bring your libraries to our lab for library evaluation services.

Please refer to 10x Genomics user guide for loading concentration and PhiX % recommendations. Illumina sequencing platform and format is also recommended in the user guide, feel free to contact our lab for pricing and questions.

Single-cell RNASeq

The sequencing depth recommendation for 10x 3'SC libraries is 50,000 read pairs/cell, so for each sample you will need 50 million read pairs per 1,000 cells or 500 million read pairs per 10,000 cells (i.e ~1.6 lanes on a HiSeq). The sequencing format is 26x8x98 (132 cycles total)

ATAC Seq

For de novo assembly, Supernova (10x Genomics software) requires 2x150 reads. Sequence 38X to 56X coverage (https://support.10xgenomics.com/de-novo-assembly/guidance/doc/achieving-success-with-de-novo-assembly)

Example of number of reads' calculations

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).

For either the HiSeq or the NextSeq we will need to use the reagent kit for 150 cycles. As you can see below, there is a large difference in sequencing cost depending on which instrument you use. So, how you plan experiments and how quickly you need the data will have a big impact on sequencing cost.