Customer guidelines for Next-Generation Sequencing

NCCT
NGS Competence Center Tübingen
Core Facility Genomics

Sample requirements RNA Single Cell

General Information

By utilising our core facility services, you confirm that you have carefully read and understood the information provided in the document "NCCT General-Guidelines. This section covers important aspects of the quality and handling of isolated nucleic acid for sequencing at the NCCT.

Short overview

Typically, ~1000 to ~20,000 cells are loaded on the Chromium resulting in 500 to 10,000 sequenced cells with an estimated doublet rate of ~0.4% to ~ 8.0%. For quality control, please provide at least 50% more cells in a maximal total volume of 55 µl. Single cells should be delivered in PBS supplemented with BSA and have a cell viability > 85%, no presence of debris or cell clumps. Transport condition depends on the properties of the cells.

Experiment planning

500 to 10,000 individual cells can be sequenced per sample. To achieve this number, ~1,000 to ~20,000 cells are loaded on the Chromium. Maximally 8 samples can be processed in parallel (1 microfluidic chip). The doublet rate (two cells receiving the same barcode) depends on the cell number, for instance, for 500 cells the estimated doublet rate would be ~0.4%, for 10,000 cells ~8.0%.
The 10x Genomics visualization tools Loupe Cell Browser and Loupe V(D)J Browser are currently only available for human and mouse data.
The recommended minimal targeted sequencing depth for the single cell sequencing protocols 3'GEX & 5'GEX min. are 20,000 read pairs/cell and for the 5'VDJ protocol 5,000 read pairs/cell.

All tubes should be labelled clearly with a sample ID, a date and the name of the sender. We recommend the use of nuclease-free, DNA-free, PCR-clean low-bind 1.5 ml tubes (no autoclaving).

We do not handle S2 (or higher) organisms nor genetically modified organisms (GMO). For these samples using extracted nuclei in suspension might be an option. Please, do not hesitate to contact us to discuss this possibility.

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Preparation of single cell suspensions for the 10x Chromium platform

A cell suspension of fully dissociated, viable single cells (> 85% viable cells recommended) is required. Be aware that non-cellular free-floating RNA from dead cells severely contaminates the sample causing data analysis failure (background noise, single cells can't be detected anymore). Thus, minimize physical damage by pipetting gently during cell resuspension using wide-bore pipette tips whenever possible and centrifuge just fast enough to generate solid, but not too tightly packed cell pellets (150 rcf to 300 rcf depending on the cell type).

Additionally, make sure to minimize cells in the supernatant to be able to represent the entire heterogeneous sample. Be aware that the width of the microfluidic channels is ~50-60 µm. Cells with a size of > 40 µm may not successfully pass but clog the microfluidic channels instead. Additionally, cell debris, clumps or aggregates can cause clogging and failure in droplet formation. To avoid this, filter cell suspensions with a cell strainer (take in account to lose > 30% of cells) and/or remove any debris/dead cells with appropriate methods (e.g. gradient centrifugation, apoptosis separation kit, washing, etc.). Solid tissues and other large cell aggregates must be dissociated using mechanical or enzymatic dissociation or other cell isolation techniques. For cell washes use concentrations at less than 5000 cells/µl to avoid cell aggregation/clumping. Ideally, wash and resuspend cells in 1xPBS (calcium and magnesium free) containing 0.04% w/v BSA (400 ug/ml). For alternative buffers and media tested by the company, please, have a look at the document CG000053_CellPrepGuide_RevC.pdf in the documentation section of the 10xgenomics site.

In general, final buffer or medium must NOT contain Mg^{2+}, EDTA or surfactants. EDTA (> 0.1 mM) or magnesium (> 3 mM) will inhibit the reverse transcription reaction**.** Avoid using RNases and DNases. If they are not washed away and retain residual activity, they will degrade your sample.

The cell concentration in the final cell suspension should always be determined to achieve optimal loading of the chip. Be aware that FACS counts are often overestimated. Additionally, always check for cell debris. To maximize the likelihood of achieving the desired recovery target, the optimal input cell concentration should be 700-1200 cells/µl. The maximal cell suspension volume that can be loaded on the Chromium is 43.2 µl (3'GEX) or 37.8 µl (5'GEX). For quality control of the cells using CellDrop, please provide at least additional ~50% cells than what we will load for you on the Chromium e.g. for ~20,000 cells on the Chromium resulting in ~10,000 recovered cells, please provide ≥ 30.000 cells in a volume of maximally 55 µl. It is crucial that the sample processing is done as quickly as possible. Ideally, the cell suspensions are loaded on the Chromium within 30 min. As the chemistry has to be thawed and prepared, please, let us know at least 30 min prior to the start of the run that you will deliver the samples. Keep the final cell suspension on ice (unless cells prefer other conditions to stay viable).

Be aware that repeated freezing and thawing of the chemistry due to cancelled Chromium runs may result in reduced numbers of sequencing clusters (read pairs/cell). We do not accept samples delivered later than 14:00 for GE

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Pilot experiment and consumable ordering

Due to the short shelf life of the 10x chemistry, the core facility does not stock consumables. Ordering of reagents can take up to 6 weeks to be delivered, with a typical delivery time within 2 weeks.

We advise users to establish the sample preparation and to test the samples on our cell quality QC pipeline prior to the sequencing experiment. In case the sequencing experiment is planned, the core facility needs to order consumables and thaw them on the day of the experiment. If the experiment is cancelled, consumable will be refrozen, reducing the quality of the reagents. These reagents will be accounted to the user and reused in later experiments. If the experiment is completely cancelled, thaw consumable costs will be invoiced, except if another experiment is planned using these consumables. It is not the facility's responsibility to find an alternative usage of the consumables.

Sample processing and sequencing

As default, we propose to process 4 samples in parallel with 8,000 cells per sample (due to 4 rxns per kit) and sequence these 4 samples on one SP flow cell (3'GEX, and 5'GEX). If the targeted cell number and the real cell number match, the recommended minimal targeted sequencing depth will likely be achieved for each sample (3'GEX, and 5'GEX). Depending on the planned sequencing depth, a second SP or S1 flow cell will be required. Using a second flow cell will also help to adjust the sequencing depth to the real number of cells per sample.

For standard projects (4 samples in parallel, 1 flow cell) we may need up to 6 weeks until the samples have been sequenced, for non-standard projects (2nd flow cell e.g. to adjust sequencing depth to real cell number) up to 10 weeks.

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Processed libraries

If you send us already processed libraries, please check our sample requirements:

10x single libraries

Fragment size

- 3'GEX or 5'GEX: (+ feature barcode) 90% < 600 bp
- TCR or BCR: 90% < 1000 bp
- ATAC: Library traces vary depending on the kit used (standalone vs multiome ATAC), sample preservation or biological differences

Concentration > 10 nM; volume > 15 µl in EB. Concentration measured using a Qubit device. Average library size in base pairs (bp), and index used will have to be provided on our sample sheet portal.

10x library pools

Fragment size

- 3'GEX or 5'GEX: (+ feature barcode) 90% < 600 bp
- TCR or BCR: 90% < 1000 bp
- ATAC: Library traces vary depending on the kit used (standalone vs multiome ATAC), sample preservation or biological differences

Concentration > 10 nM; volume > 100 µl in EB. Concentration measured using a Qubit device. Average library size in base pairs (bp), and index used will have to be provided on our sample sheet portal.

If you are doubting to have sufficient material or your samples do not match our criteria, please contact us prior pooling your samples.