Submission of samples for preparation of Chromium libraries

The CGR cannot guarantee good results from samples that do not meet the requirements set out in this document.

Maximising sample quality

10x Genomics Chromium single cell transcriptome libraries require a suspension of viable cells, methanol fixed cells, or freshly isolated nuclei as input. Minimising the presence of cellular aggregates, dead cells, non-cellular nucleic acids and potential inhibitors of reverse transcription is critical to obtaining high quality data.

It can be challenging to generate high quality single cell suspensions, especially when working with fresh tissue, so this should be considered when planning the experiment. We strongly recommend a test run to make sure you can generate single cell suspensions that meet the criteria described in this document.

To maximise sample quality, we recommend that:

- Cells/nuclei are kept on ice at all times.
- Cells/nuclei are treated as gently as possible throughout the extraction process. This includes gentle pipetting (possibly via the use of wide-bore pipette tips) and the use of low centrifugation speeds.
- Sample extraction/preparation times are kept to a minimum. Some cell types are particularly fragile, and their viability can be decreased significantly if they are not processed immediately. As a general rule, samples perform best within 8 hours of extraction. Dead cells contain a lot of contaminating ambient RNA, which will interfere with the subsequent library preparation. If the viability of the cells is not sufficiently high, consider removing dead cells by following the Technical Note: Removal of Dead Cells from Single Cell Suspensions Improves Performance for 10x Genomics Single Cell Applications.
- Cells/nuclei are washed with a PBS and 0.04% bovine serum albumin solution to remove contaminants, such as ambient RNA and unwanted buffer components.

For information about how variation between similar samples can influence the outcome of your experiment, we recommend reading the Technical Note: Biological and Technical Variation in Single Cell Gene Expression Experiments.

Assessing the quality and quantity of samples prior to submission

Please note that cell/nuclei counting, viability assessment and sample clean-up is not included in our standard single cell library preparation protocols, so we request that you perform these steps prior to sample submission. We would strongly recommend that you follow the 10x Genomics cell counting flowchart as part of this process. If any cell-counting or sample clean-up is required after sample submission, this will incur additional charges.

In brief:

- Visually inspect the processed samples under a microscope to ensure that you have isolated single cell/nuclei populations.
- Use a strainer to ensure the cells/nuclei are completely dissociated and free from debris and aggregates. Clumps of cells/nuclei and debris are highly likely to block the microfluidics channels.
- Ensure the cells/nuclei are counted in triplicate. Accuracy of counting is crucial to avoid over- or under-loading, which could have a major detrimental effect of the quality of the resulting library. Remember to count the cells/nuclei after any processing steps, e.g., FACS. Please see the Technical Note: Guidelines for Accurate Target Cell Counts Using 10x Genomics Single Cell Solutions for more information.

Sample submission requirements

- Cells/nuclei must be submitted as a homogeneous solution, strained if necessary to remove any debris or aggregates.
- Cells/nuclei must be counted in triplicate and be in the range specified in the corresponding 10x Genomics protocol, listed below.
- Non-fixed cells should be >90% viable, ideally. The lower limit we would recommend proceeding with is 70%.
- Cell size is a consideration. Large cells can block the microfluidic channels (anything >30 microns is unlikely to perform well).
- Cells/nuclei need to be resuspended in a compatible buffer such as PBS/BSA.

Cell/nuclei suspension concentration requirements for different library preparation methods can be found in the official 10x Genomics protocols:

- 3' Single Cell Gene Expression (see the tables on pages 18 and 27).
- 5' Single Cell Gene Expression (see the table on pages 19 and 28).
- Single Cell V(D)J Profiling for T-Cell and/or B-Cell Receptors (see the tables on pages 20 and 29).
- Single Cell Assay for Transposase-Accessible Chromatin (ATAC) (see the tables on pages 16 and 22).
- Single Cell "Multiome" (combined Gene Expression and ATAC) (see the tables on pages 22 and 29).

If you are unable to meet the stated requirements for your library type, please contact us at CGR_Lab@liverpool.ac.uk and we will be happy to offer further advice.

Samples should be submitted to the CGR no later than 2 pm. If your samples cannot be submitted by this time, please contact the CGR lab team as early as possible on **0151 795 4551** to advise them of the expected submission time.

Complying with the Human Tissue Act

If you are submitting human samples, your project will be governed by the Human Tissue Act. Under this legislation, there is a strictly limited period of time that we are allowed to store human material. We request that you retrieve any remaining samples within ~3 days of submission. If you do not collect remaining material within 10 days, we will be forced to destroy this material and this will incur additional fees.

We will require you to fill out some additional documentation before we can take receipt of any human material. If you have not already received this documentation, please contact us at CGR.Enquiries@liverpool.ac.uk.