10X GENOMICS SINGLE CELL GENE EXPRESSION

Sample Submission Guidelines

Chromium Single Cell 3′ Gene Expression

Chromium Single Cell VDJ Immune Profiling

The quality of the cell suspension to be used with the single cell methods from 10X Genomics play a crucial role in obtaining high quality sequencing data of captured cells. Please visit 10X Genomics webpage for "Demonstrated Protocols" on how to obtain high quality single cell suspensions based on your starting material (https://support.10xgenomics.com/single-cell-gene-expression/index), and we also recommend you to watch a video from 10X Genomics discussing sample preparation and clean-up steps, including tips if some of the standard steps do not work for your specific sample (https://www.10xgenomics.com/solutions/single-cell/#)

ESCG recommends that you test the protocol to generate your single cell suspension before scheduling an actual run with the 10X methods. It is important that you show ESCG the quality of the suspension by either sending a picture representative of the whole suspension (several cells in the image field) by email, or that you book a time to bring the suspension to the facility for evaluation.

In order to obtain high quality data from your experiment the cell suspension should have:

- *Been prepared in low-binding tubes*
- *A viability of >90% when cells are derived from fresh tissue / cultures*
- *No aggregates (cell clumps)*
- *No observable debris*
- *Less than 5% of red blood cells*
- *A concentration of 700-1,200 cells/µl*
- *No inhibitors of reverse transcription or GEM generation*
- *Enough extra cells for counting them, to perform accurate cell loading*
- *Smallest possible volume for suspensions coming from FACS*

Please see below for further explanations.

Prior to loading your sample onto the 10X Chromium chip, we inspect it under the microscope and count the cells. If possible, we request that you stay with us during these steps in case we see a non-optimal cell suspension and need to discuss it with you before starting the experiment. In the end, ESCG always advice for the best possible outcome based on our experience, but it is the user decision to proceed or not with a non-optimal sample.

Explanations to High Quality Cell Suspension Requirements

Cell viability:

Ideally, the cell suspension should contain **more than 90% viable cells**. To minimize physical damage to cells from shearing forces, it is critical to pipette gently and slowly during cell resuspensions. Depending on cell type, viability may significantly decrease when cells are kept in suspension for a prolonged period of time, and some cells types such as peripheral blood mononuclear cells (PBMCs) can even form clumps when kept in PBS for extended periods of time. Cell suspensions should therefore be loaded on the Chromium chip as soon as possible after preparation, ideally **within 30 min**.

If your cell suspension contains many non-viable cells, please use the **Dead Cell Removal kit** (Miltenyi Biotec) and follow the 10X "Demonstrated Protocol Removal of Dead Cells from Single Cell Suspensions for Single Cell RNA Sequencing". Many of our users have used it with success.

If you are FACS-sorting your sample prior to submitting it to us at ESCG, it is advisable to include a viability marker. There are a variety of markers to use, for example PI, 7-AAD and DAPI.

Cellular aggregates:

Cellular aggregates can cause clogging of the 10X microfluidic chip and will increase the likelihood of having doublets/multiplets of cells.

Washing and resuspension of your cells should be performed in 1X PBS (calcium and magnesium free) + 0.04% BSA (BSA is added primarily to minimize cell losses and aggregation), but if you see cell clumps in your suspension then **higher BSA concentrations** (up to 2% BSA) in the buffer might contribute sterically to prevent the clumping and will not have any adverse effects on workflow or data.

If the clumping is not helped by a higher BSA-concentration, then you can pass your suspension through a **cell strainer** prior to the loading on the 10X chip here in the facility. For low volume suspensions use a **Flowmi™ Cell Strainer** (Merck), for low cell number suspensions use a **MACS SmartStrainer** (Miltenyi Biotec).

Debris:

Tissue/Cell debris in your cell suspension can cause clogging of the 10X microfluidic chip, contamination of your single cells with ambient RNA, and inaccurate cell counting. If you see debris in your cell suspension, please use a **cell strainer**.

For larger debris, the **MACS SmartFilter** (Miltenyi Biotec) is recommended, for low cell suspension volumes, the **Flowmi™ Cell Strainer** (Merck) or the **pluriStrainer® Mini** (pluriSelect) are recommended.

For smaller debris, the **Debris Removal Solution** (Miltenyi Biotec) allows for debris removal through centrifugation.

Red blood cells:

Red blood cells (RBCs) can be seen under the microscope as small cells, very uniform in shape with a yellow coloring and a small black dot in the middle. Removing RBCs may not be necessary before loading your sample, however, if they are not removed, they will contribute to the total number of cells that are loaded and will increase the amount of sequencing you will need to perform to detect the other cell populations of interest.

It is possible to remove the RBC population bioinformatically during Loupe Cell Browser analysis if you do not, or cannot, remove them during sample prep.

If you want to remove RBCs before loading your sample, 10X "Demonstrated Protocol for tumor dissociation" includes a RBC lysis step using a **Red Blood Cell Lysis Solution** (Miltenyi Biotec). You can also use **Ficoll purification** to remove RBCs from PBMC samples prior to loading the cells.

Cell number and concentration:

If possible, we request you to bring at least roughly **three times the number of cells** you aim for as output, meaning if you aim for having 5,000 cells sequenced, we need you to bring 15,000 cells.

The optimal cell concentration is in the range of **700 to 1,200 cells/µl**. If your cell suspension is too diluted when arriving at the facility, you can use our centrifuges for concentrating it, please remember to bring extra buffer in case it is needed.

Inhibitors of reverse transcription and GEM generation:

When submitting your sample to ESCG the recommended buffer for the cell suspension is 1X PBS (calcium and magnesium free) containing 0.04% BSA (400 µg/ml). Sensitive cell types may require suspension in alternative buffers to maximize viability, so if necessary, PBS can be replaced with most common cell culture buffers.

If cell viability cannot be maintained in a buffer, it is also possible to resuspend your cells in most common cell culture media, with up to 10% FBS or up to 2% BSA. Media should not contain excessive amounts of **EDTA** (> 0.1mM) or **magnesium** (> 3mM) as those components will inhibit the reverse transcription reaction. Any **surfactants** (Tween-20, etc) should also be avoided as they may interfere with GEM generation.

Possible failures during the 10X method run

When running samples using the 10X method, occasionally a sample clog may occur. This is generally caused by sub-optimal sample preparation (suspension containing cell clumps or debris), or by loading more than the recommended number of cells. If clogging occurs during the generation of the emulsions, these will not be formed properly, and your cells will not be encapsulated in single droplets and the processing generally cannot proceed. If the sample suspension was sub-optimal and we had discussed the risk of a clog occurring, then the cost of the run is on the user. If the quality of the sample suspension was such that no clogging was to be suspected, then

we can relate to a run failure, and this will be covered by the 10X Genomics company. In either case, if there is enough cell suspension left and if decided by the user, we can run the sample again. However, if it is a very precious sample and you do not have any more cells to load, it may be possible to continue with the protocol and generate cDNA, although at lower levels and coming from a reduced number of cells.

Another type of failure that can occur during a sample run is a wetting failure. This often has no obvious cause and will therefore be covered by the 10X Genomics company. We recommend rerunning the sample in the case of a wetting failure.

At ESCG we have experienced about a 1% run failure rate.