Guidelines for First-Time Single Cell RNAseq (scRNAseq) & ATACseq (scATACseq) Customers

The scRNAseq/ scATACseq libraries are prepared using the 10X Genomics Chromium Controller Platform.

**1. Cell/nuclei counting is essential, and performed by the customer on the day of the library prep.**

The customers are responsible for the following:

* Filtering the cell suspension to remove debris/aggregates. Customers should decide whether to proceed in cases where debris/aggregates are present
* Assessing the cell viability rate. A minimum of 90% is required.
* Performing manual or automated cell counting. The cell counting must be performed 3-4 times per sample. The standard deviation between these cell counts should be less than 25%.
* Preparing the final cell suspension, which should be between 700-1200 cells/uL. Target 1000 cells/ul

**2. Time is of the essence.**

The time from beginning cell/nuclei suspension to delivering them after conducting the above-mentioned steps should be as short as possible. Less than one hour is highly recommended.

**3. Cell suspension buffer:**

Cells must be provided in the recommended buffer and at their final intended concentration.

Customers should provide information about the resuspension buffer used (the presence of EDTA or Mg will inhibit the RT reaction), and provide extra buffer, in case the cell suspension needs to be washed or filtered, or the cell stock concentration adjusted.

* The recommended buffer is PBS with 0.04% BSA.

**4. Cell viability:**

Running cell suspension with a lower viability count will likely result in poor or failed library preparation. It is critical to understand that the QC during the library preparation will not reveal how much of an impact viability had, and customers will have to sequence their libraries and only then find that the data may be impacted. If the customers choose to proceed with below recommendation viability, they will be charged for all work being done regardless of the outcomes of the experiment.

* The viability of the cell suspension should be above 90%.

**5. Scheduling and submission times:**

Customers MUST schedule time to deliver their samples at least two weeks in advance. This is to be sure that we are ready to processes the samples as soon as they arrive. In order to complete the first part of the library preparation and reach a safe stopping point, we will need to have the cell suspension dropped off at or by **11am** on the scheduled day. Customers will need to provide all information mentioned above in # 1.

**6. Full consultation before preparing samples for 10x runs:**

Prior to starting a scRNAseq experiment, we highly recommend scheduling a consultation meeting. It is critical that customers understand the sample requirements for the 10X platform, as well as the recommended sequencing depth. Customers will also need to decide how many samples and how many cells are needed to run.

**Tips:**

• The key to the assay is intact, viable cells. Customers may want to review the Cell Preparation Guide: <https://support.10xgenomics.com/single-cell-gene-expression/sample-prep/doc/demonstrated-protocol-single-cell-protocols-cell-preparation-guide>

* For scATACseq, please review the nuclei isolation protocol:

<https://support.10xgenomics.com/single-cell-atac/sample-prep/doc/demonstrated-protocol-nuclei-isolation-for-single-cell-atac-sequencing>

• ***Never*** have the first experiment with a new cell type be with a scarce or precious sample.

• If viability looks poor on the day of their experiment, feel free to cancel and reschedule. We completely understand.

• We recommend dry runs where customers perfect their cell preparation protocol prior to a full prep with sequencing.