User Guide: 10X Genomics Chromium Single cell 3’ technology

**TABLE OF CONTENTS ................................................................................................ 1**

**CONTACT INFORMATION .......................................................................................... 1**

**SAMPLE INFORMATION ............................................................................................ 2**

**GUIDELINES FOR SINGLE CELL SAMPLES .................................................................. 3**

IMPORTANT GENERAL CONSIDERATIONS ....................................................................3

SINGLE CELL SEQUENCING USING THE 10X GENOMICS CHROMIUM SINGLE CELL 3’ TECHNOLOGY ……………………………………………………………………………………………………………….......... 4

SAMPLE SPECIFICATIONS ........................................................................................ 5

**SAMPLE SUBMISSION AND SHIPMENT DETAILS........................................................ 6**

SHIPPING INFORMATION ..........................................................................................6

# Contact Information

|  |  |
| --- | --- |
| Name (Contact person)  |  |
| Institution/Company  |  |
| Department  |  |
| Street Address  |  |
| Postal code/City |  |
| Email  |  |
| Telephone  |  |
| Date of Order  |  |
| Shipping Courier  |  |

Sample Information

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No | Sample name | Sample type | Species | Conc. (cells/ul) | Resuspension Buffer | Analysis |
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Guidelines for Single Cell Samples

**Important general consideration**

The guidelines contained herein aim to provide you the best possible sequencing data within the quickest possible turnaround time. Any and all samples that do not conform to the guidelines expressed herein may be refused. The success of a single cell sequencing project is determined by the quality of the cell preparation provided to Genomics and Transcriptome Core. Researcher must coordinate their sample delivery with core personnel prior to submission.

It is recommended to:

* Count cell before submitting them to the Genomics and Transcriptome Core to determine cell concentration and cell viability;
* Submit completely dissociated cell suspension. To ensure a well singulated cell suspension free from cell debris and cell aggregates cell straining can be employed. ensure that pore size is larger than the cell diameter but small enough to catch clumps and debris;
* Submit single cells with viability >90%. Lower cell viability will decrease the apparent efficiency of cell partitioning and recovery since non-viable and dying cells generally contain less RNA which is more fragmented. Cell viabilities <70% will not be processed for single cell library preparation;
* Wash and re-suspend cells in 1xPBS (calcium and magnesium free) containing 0.04% w/v BSA. If necessary, PBS can be replaced with most common cell culture buffers verified by 10X to be compatible with 10X single cell protocols (EMEM+10%FBS, DMEM+10%FBS, IMEM+10%FBS, RPMI+10%FBS, Ham's F12+10%FBS, 1:1 DMEM/F12+10%FBS, M199);
* Submit cell samples with a concentration of 700-1200 cells/ul. Maintaining cells at higher concentrations can cause aggregation and clumping that will interfere with generating ideal single cell suspensions;
* Not over-centrifuge the cells when pelleting. The recommended centrifugation conditions are 150 rcf for 3 min. at RT° for larger cells (ie. immortalized cell lines) and 300 rcf for 5 min. at RT for smaller cells (ie. PBMCs).

Sample identification of the container(s) carrying the sample(s) must correspond exactly to what is specified in the Request Form.

Libraries generated are compatible with Illumina sequencers. In order to read the transcript sequences on one end, and the barcode and UMI on the other end, paired-end sequencing reads are required.

Vender's specification recommends getting at least 50-100K paired-end raw reads per cell for gene expression studies\*.

\* Reads per cell required is very cell-type dependent, it is recommended to obtain more reads per cells at the optimization stage.

**Please acknowledge that you have read and understand the above notes by signing this page and submitting it with your sample submission.**

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**Single Cell sequencing using the 10X Genomics Chromium Single Cell 3’ Technology**

The Chromium Single Cell Gene Expression Solution allows you to analyze transcriptomes on a cell-by-cell basis through the use of microfluidic partitioning to capture single cells and prepare barcoded, next-generation sequencing (NGS) cDNA libraries:

* Partition 100 to 10,000 cells per sample; up to 80,000 cells per chip;
* Recovers up to 65% of cells (typically closer to 50% depending on cell type);
* Low doublet rate: ~0.8% per 1,000 cells;
* 50,000 reads per cell should be sequenced for saturating gene detection.  70,000 reads per cell is appropriate for expression analysis.

**Sample specifications**

**Table 1. Summary of sample requirements for the construction of 10X Chromium single cell 3’ libraries**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Analysis type | Quantity (cells) | Recommended Resuspension Solution | Conc (cells/µL) | Vitality | Cell size |
| 10X 3’ Single cell v3.1 | 10.000 | PBS1X 0.04% BSA (\*1) | 700-1200 | >90% | 30m |

(\*1) If necessary, PBS can be replaced with most common cell culture buffers verified by 10X to be compatible with 10X single cell protocols (EMEM+10%FBS, DMEM+10%FBS, IMEM+10%FBS, RPMI+10%FBS, Ham's F12+10%FBS, 1:1 DMEM/F12+10%FBS, M199).

***For additional information, see 10X Genomics website.***

Sample Submission and Shipment details

Shipment of samples/libraries is always after acceptance of the submission form to the “Centro Piattaforme tecnologiche”.

# Shipping Information

Please send your samples to the following address:

**Centro Piattaforme Tecnologiche (CPT)**

**Università di Verona**

**Pz.Le L.A. Scuro, 10 – Palazzina di Farmacologia**

**37134 Verona - Italy**

Email: monica.castellucci@univr.it

 francesca.griggio@univr.it

Phone: +39 045 802 7220

Fax: