Sample requirements for single-cell applications on 10x Genomics Chromium System

General remarks:

1) Fresh or cryopreserved cells can be used for single-cell applications.
2) For snap-frozen samples nuclei have to be prepared.
3) The system requires a single cell / single nuclei suspension.
4) Clumps will cause clogging and failure in droplet formation.
5) Please filter cells / nuclei with a regular FACS filter if necessary.

Sample preparation:

1) Cryopreserved samples have to be washed to remove any freezing buffer.
2) Free RNA / DNA will cause severe contamination and the detection of single cells in the data analysis will fail.
3) Please remove any debris / dead cells with appropriate methods (e.g. gradient centrifugation, apoptosis separation kit, washing, etc.).
4) Cells / nuclei should be counted to achieve optimal loading of the chip.
5) If the samples were processed by FACS, please use the total count after sorting divided by 1.5 as a cell number estimate.
6) Typically 50% of the input number of cells are captured. Input cell number may range from 500 to 20.000 cells.
7) Please provide the cell / nuclei suspension in 35 µl with the total cell numbers as stated above (500 – 20.000). Buffer may contain FCS, BSA or sucrose. Do NOT exceed 35 µl as this is the maximal volume that can be loaded on the Chromium machine.
8) If you wish to provide larger volumes / higher cell number / higher cell concentration due to sample stability please count the cells (and provide the cell concentration as number of cells / µl).
9) Processing should be done as quickly as possible – please let us know at least 30 min prior to the start of the run as the chemistry has to be thawed and prepared.
10) Eight samples can be loaded on one chip. Fewer samples are possible however – please state the number of samples in the 10X check list below.

Cost estimates for consumables:

Library preparation/sample: 1,500 €

10x check list

Lab:

Cell type:

Method: ☐ *scRNA-seq* ☐ *sc-V(D)J*

Date:

Single cell suspension:

☐ *Fresh cells* ☐ *Cryopreservation* ☐ *snap-frozen*
☐ *FACS* ☐ *dissociated tissue* ☐ *Nuclei*
☐ *filtered*

Buffer:

☐ *PBS* ☐ *+ BSA*
☐ *+ medium / FCS* ☐ *other, please specify:* _____

Source:

☐ *cell culture* ☐ *primary samples*

Species:

☐ *Human* ☐ *Mouse* ☐ *Other*

Cell number:

sample								
# cells / µl								
# cells loaded *								

* will be filled out in the sc-open lab