Guidelines for Preparing and Submitting Cells for Single Cell Experiments

Sample requirements:

- suspension of viable single cells in 1.5ml Eppendorf
- cells washed and re-suspended in PBS (media acceptable in some cases)
- aim for concentration in range **1000-2000 cells/ul**, volume >100ul
- QC your samples, provide: cell count and viability information (samples with viability below 70% may produce compromised results)
- Deliver samples on ice with completed Sample Submission Form and 10x Submission Form (samples can be delivered after successful arrangement of project with OGC project manager)
- Bring extra media for dilutions.
- Let us know your desired **target cell recovery** (100- 10 000 cells/sample) and 10x Kit (Single Cell 3prime, Single Cell VDJ (5’ GEX +/T-Cell+/B-Cell))
- Deliver your cells to Wellcome Centre by 3pm (on a arranged day).
- Let us know if your samples do not meet the requirements, we may still be able to proceed

We expect that your samples will be supplied in the correct containers and at the required concentration and quality (requirements are stated below). Timely completion of your project depends on you following these guidelines.

For further details on packing and submitting your samples, please see the latest version of the following document ‘Guidelines for Preparing and Submitting Samples’.

Sample QC

**10x Genomics® Single Cell Protocols require a suspension of viable single cells as input. Minimizing the presence of cellular aggregates, dead cells, non-cellular nucleic acids and potential inhibitors of reverse transcription is critical to obtaining high quality data.**

- Suspension cell lines, bead-enriched and flow-sorted cells can be used directly after washing
- Adherent cell lines require previous trypsin treatment.
- Single cell suspension from tissues requires optimization of dissociation.
- Cell debris and fibres can interfere during the counting of the cells causing an inaccurate outcome (critical step during the 10X protocol). Cell debris and large clumps could potentially also clog the chip, resulting on an experiment failure.
Examples of good sample preparation:

**Recommended cell washing and resuspension solution** is 1X PBS (calcium and magnesium free) containing 0.04% weight/volume BSA (400 μg/ml). BSA is added to minimize cell losses and aggregation. Primary cells, stem cells and other sensitive cell types may require washing and suspension in alternative buffers to maximize viability. If necessary, PBS can be replaced with **most common cell culture buffers**.

- Required cell concentration depends on targeted cells recovery (check working range with Cell Suspension Volume Calculator Table).
- Optimal sample concentration is 1000 cells/ul, viability > 90% and a volume of around 100ul (including sample needed for the QC).

If these requirements cannot be achieved, please contact the single-cell team, as working with lower concentrations and lower volumes is also possible, but needs to be reviewed carefully. (Table1 for Reference).

**EXPERIMENTAL DESIGN**

We highly recommend a discussion with the single-cell team at the Oxford Genomics Centre in order to plan the best experimental design possible from the lab point of view, also to ensure a sensible data analysis as a result of a carefully planned experiment.

Attached Cell Suspension Volume Calculator Table is submitted for guidelines only; actual volumes of samples loaded on 10X may vary.