Guidelines for Preparing and Submitting Cells for Single Cell Experiments

Requirements in advance of submitting samples:

In order to proceed with your run, a submission form must be received by your project manager 48 working hours in advance. If the submission form is not received, the run WILL BE CANCELLED.

Your samples are precious, this requirement is to ensure that they are processed correctly and safely. The submission form asks for information about your samples but the only information that you may not have in advance is the sample names, in this case you may add temporary sample names and ask the person doing the run to change it on the day.

Here is more about the steps required in advance of submitting samples.

- When you are considering a 10X experiment, the first step is to decide on an experimental design and request a quote, including the correct kit (Single Cell 3prime, Single Cell VDJ (5’ GEX +/T-Cell+/B-Cell), Single Cell ATAC. This may take some discussion with the single cell team so you should start this process well in advance of requesting a quote.
- Once you have a quote, you must send the PO to your project manager along with a completed 10X submission form. It is critical to fill in the targeted no of cells per channel on the form (100-10,000), without this information we will not be able to generate a project number. Sample name can be amended later if necessary, as can the volume.
• After receipt of the submission form and PO, your project manager will confirm your project number and it will then be possible to confirm run scheduling.
• You will be able to pencil in a run prior to receiving your project number but if a project number has not been supplied 2 days before the run, the run will need to be cancelled.
• You should be aware that due to heavy workloads it can take our project managers a number of days to act on or reply to an email. This must be taken into consideration when you plan your experiment, the sooner you get the quote in place and provide the project manager with the necessary paperwork the better.

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 a paper copy of the sample submission form for easier use.
• Bring extra media for dilutions.
• Deliver your cells to Wellcome Centre by 3pm (on an arranged day).
• Let us know if your samples do not meet the requirements, we may still be able to proceed

On the day of the run, the person who brings over the samples may be asked to provide a rapid decision on whether or not to proceed if the QC is suboptimal. There will be no time to seek advice/opinions from other members of the team so we suggest that you have some discussion within your group before samples are brought over.

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.
For Cell Preparation Guides go to https://support.10xgenomics.com
– 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.

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.