Guidelines for Preparing and Submitting Cells for Single Cell Experiments

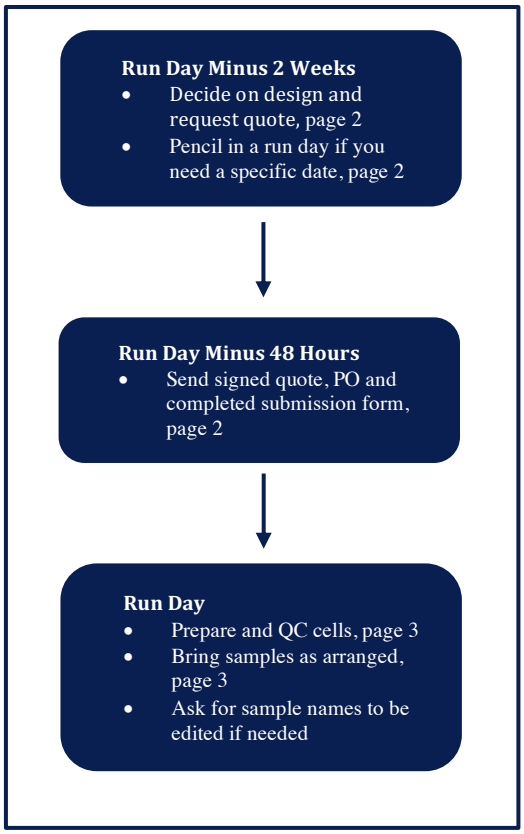
OGC receives thousands of samples every month. Help us to keep your samples safe and to return good quality data in a timely manner by taking the time to read and follow these instructions. Failure to follow these guidelines and incorrect submission of samples could:

1. Delay the initiation of your project
2. Put your samples at risk
3. Lead to poorer quality data
4. Result in additional charges being applied to your project

Obviously, as we've written this document for you, we think it is all important but there are some really key points, which are highlighted with the symbol below. If using a screen reader, these will be marked up as the "key points" style.

If in doubt at any point then please consult your project manager.

The schematic below is designed to give you an overview of the steps that you are required to take prior to the day of your run. Further details are provided below the schematic. We have outlined the steps to take in order, however, we recommend reading the whole document through prior to sample extraction and collection.



If your quote is for anything other than 10X library preparation, then this is not the document for you, please return to the main page or contact your project manager if you are unsure.

Project Planning and Setup

What do I need to do before I prepare my samples?

First and foremost, you need to discuss the project design with a member of the OGC team, to ensure the best experimental and data analysis design. This can take some time, so you should start the process well in advance. Once the design is finalised, you need to request a quote. We recommend that you do this **at least two weeks** before the day of the run. Once you have a run date in mind, you can also check availability and pencil it in with your project manager.

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.

In order to proceed with the project, follow the steps below:

Step 1
Sign your quotation and return via email along with a PO number. If you are based outside the University of Oxford, please provide a PDF copy of the PO.

Step 2
Download the latest 10X submission form from our website. It is important to always use the most recent version, because we make improvements and old versions will not work with our login process.

Complete the sample submission form. When subsequently labelling your sample tubes, ensure that they match exactly the entry in the submission form.

To ensure that there are no delays in initiating your project, please confirm that all the requested information is provided on the submission excel form. There are more details on the form itself, but the minimum required information is:

- sample name
- sample type
- reference genome (from the dropdown or discuss with your project manager if not available)
- pooling (mpx1- for samples to go on 1 sequencing unit, mpx2- for samples than need to go on different sequencing unit)
- targeted no of cells per channel (500 – 10,000 cells)

In order to proceed with your run, a signed quote, completed 10X submission form and PO 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 can either add an identifier or you may add temporary sample names and ask the person doing the run to change it on the day.

Once the samples have been logged in, the project manager will confirm your **project number** and put you in touch with the team member who will be carrying out the run.

Preparing and Bringing Cells

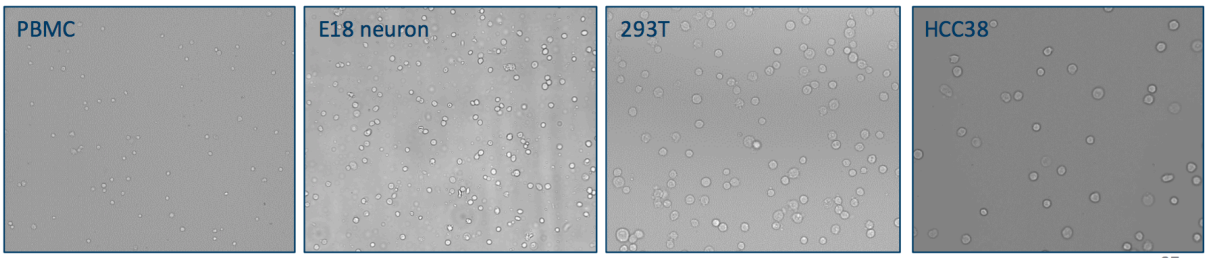
How should I prepare my cells?
10X Genomics have published a range of guides on how to prepare cells for their platform. Go to https://support.10xgenomics.com to find the relevant information for your project. We need cells to be provided as a **suspension of viable single cells**.

- 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.

QC your cells and provide the cell count and viability information (samples with viability below 70% may produce compromised results).

Minimizing the presence of cellular aggregates, dead cells, cell debris, fibres, non-cellular nucleic acids and potential inhibitors of reverse transcription is critical to obtain high quality data. They can interfere with the counting of the cells (which is a critical step during the 10X protocol) causing an inaccurate outcome. Cell debris and large clumps could also potentially clog the chip, resulting in an experiment failure.

Examples of good sample preparations can be seen in the images below, where there are healthy cells spread throughout the sample without the presence of clumps or debris.



How do I wash my cells?
All cells should be washed and resuspended in PBS prior to submission. The 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 loss 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.

How should I provide my cells and in what?
Cell suspensions should be provided in a **1.5ml Eppendorf tube.** The concentration should be in the range of **1000-2000 cells/µl,** in a volume of **more than 100µl** and with a **viability of 90%** or more. If these requirements cannot be achieved, please contact your project manager, as working with lower concentrations and lower volumes is also possible, but needs to be reviewed carefully as it impacts on the cell recovery.

If your samples do not meet our criteria on the day then please get in touch as we may still be able to proceed.

How should I package and ship my libraries?

Cells should be brought on ice along with extra media for dilutions and a paper copy of the sample submission form for easier use. Delivery to the team member running your samples should be carried out by **3pm** (on the arranged day). NB due to COVID-19 restrictions, it will not be possible to come into the lab.

What should I expect on the day of the run?

On the day of the run, we may ask someone to provide a rapid decision on whether or not to proceed if the QC is suboptimal so we will need a contact number or for you to be available on Microsoft Teams. 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.

The essential steps of this process are summarized as a checklist in Appendix 1.

Appendix 1

Checklist for Cells

After reading the full text, this checklist can be used to ensure all the steps are carried out prior sending any samples to OGC. These are only brief summaries of each step and this checklist should not be used as a standalone document.

No.	Processes	Tick
	In advance of run day	
1.	Discuss the experimental design	
2.	Pencil in a run date	
3.	Return signed quote	
4.	Send PO	
5.	Send completed submission form	
	Run day	
6.	Prepare, wash and QC cells	
7.	Ensure correct concentration, volume and viability	
8.	Package on ice	
9.	Include extra media and a paper copy of the submission form	
10.	Deliver to OGC by 3pm and be available on Teams/phone to discuss QC if needed	