When submitting samples, please follow these simple guidelines:

Always make sure you have got the best possible quality of your RNA or DNA - to get good libraries, you need good starting material!

If you quantify your nucleic acid, use a fluorescent dye (Qubit, picogreen), and avoid the nanodrop! As part of our service we will do a QC wherever possible, so don't worry if you have not got access to this.

RNA samples

RNA can be extracted using any commercially available RNA extraction kit.

It is necessary to include a DNAse treatment to eliminate any DNA background signal in your data.

If you are performing bioanalyzer/tapestation QC yourself, aim for RIN values for >8. We routinely do this quality check for you, so you do not have to have access to a bioanalyzer/ tapestation.

Your samples should be submitted in RNAse free water, and on dry ice if at all possible. Please make the labelling easy and understandable! We like numbers!

We have different RNA protocols, which are aimed at different input amounts. In general, we would like to have as much RNA as you can isolate in the lowest possible volume. The type of protocol we choose for your samples will depend on the outcome of your sample's QC. However, we are constrained by the input amounts of the different protocols:

Low input samples from 250pg to 10ng will be processed with Takara's SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian. The required input volume for this is 8ul, the sample you supply will need to be in no more than 13ul volume. This kit will also be used for degraded samples.

Samples with higher input (100ng to 1000ng) can be either depleted of ribosomal RNA, or selected for PolyA. (PolyA is often chosen when transcript counting and cost limitation are what is required. Ribosomal depletion allows for non-coding RNA to be present and has less 3' bias, but is more costly.) For these two methods we require the samples to be in volumes of 10ul (rRNA depletion) or up to 50ul (PolyA).

DNA samples

Genomic DNA should be free of RNA, and you should check the integrity and size on a 1% gel before submitting.

Clean ChIP DNA should be submitted after you have done the chromatin immunoprecipitation in a volume not larger than 15ul. Do not attempt to measure ChIP DNA in a nanodrop, it will not be accurate enough. Please check the fragment size of your input DNA, and please let us know if your fragments are longer than 500bp.

Your samples should be in water and submitted on ice. If samples have been exposed to phenol or other organic solvents, they should be run through a cleanup column prior to

submission to avoid contaminants that may inhibit the activities of enzymes used in subsequent steps. Please make the labelling easy and understandable! We like numbers!

Single Cell 10X samples

- Please arrange a booking with us in advance. We take samples from 9 to 4 every day, and we need 1.5h per booking.
- We are on the ground floor, in the single cell room just behind reception. If you have access, there is a doorbell that you can ring.
- Please email us 30 min before you are handing over your samples, so that we can defrost the reagents in time. If you become aware that your timings may be off, please let us know as soon as possible so we can fit you around other bookings, if necessary.
- If your experiment is not already submitted in our online submission form, please can you do this, so we have all necessary information there. (https://genomics-sci.atlassian.net/servicedesk/customer/portal/1) It makes sense to submit here for all the samples that will be sequenced together. I understand that this is hard to predict, but we can communicate via this portal to organise things in more detail.
- We will receive your cells, which you will have counted and resuspended, in approx. 45ul. Please look at these documents in detail: (https://assets.ctfassets.net/an68im79xiti/56DlUZEsVOWc8sSG42KQis/35cbcf6dcd4b0c0196263ee93815b0ae/CG000053_CellPrepGuide_RevC.pdf) (https://assets.ctfassets.net/an68im79xiti/57MmukfLzqI4WMeyKQMqom/02f98fb8a503937e38e18e2c9ebc5667/CG000091_10x_Technical_Note_Guidelines_on_Accurate_Target_Cell_Count_RevB.pdf)
- We will be using **43ul** of your cell suspension, this is the volume that you need to have your target number of cells in. We ask you to submit a tiny bit more volume than needed (45-47ul) to avoid bubbles when resuspending and any loss that may occur. (If you can, give us enough volume for two attempts, because wetting failures, although uncommon, do occur from time to time. With added volume, we have another chance to get your cells into GEMs.) **We do not perform cell counting or a viability check in our lab, so we rely on you to be confident about the quality and quantity of the cells you give us.** As speed is critical in 10x Genomics experiments we load cells into the chip immediately after receiving them from you.
- The recommended number of cells loaded per sample is 800-16,000, but there is no optimal cell concentration when submitting for 10x Genomics experiments. It varies due to cell type, experimental design etc. Please expect a **capture rate of 50-60%,** this means that at least 40% of cells loaded into the chip will be lost - but this percentage might be higher in some cell types.
- We can load up to 8 samples into one Chip.
- We will process your samples and keep you updated about their progress.

Logging your project into the system

We require each project to be submitted into the online submission system:

https://genomics-sci.atlassian.net/servicedesk/customer/portal/1

Please create your own account for this system, none of your university affiliations are registered.

We will require information about you, your group, assurance that you have the correct GM and biosafety assessment, your grant code, and of course details about your project. Please see our submission help file for specific questions about these points!

Once submitted, we can arrange a handover for your samples.