When submitting samples, please follow these simple guidelines:

1) Bulk samples

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

As part of our service we will do a QC wherever possible, so don't worry if you have not got access to Qubit or tapestation.

1a) RNA samples

RNA can be extracted using any commercially available RNA extraction kit. For any RNA library prep, we require clean, high-quality RNA in RNase free water. 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 We will quantify and run samples on bioanalyzer/tapestation to check quality.

Please make the labelling easy and understandable!

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 protocol:

Samples with total RNA amounts of 50ng to 1000ng can be processed with the standard kit (stranded), and 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).

Low input samples from 250pg to 10ng will be processed with a low input kit (stranded), which has proprietary ribosomal depletion built into the protocol. The required input volume for this is 8ul, the sample you supply will need to be in no more than 12ul volume. This kit will also be used for degraded samples.

We also have a single cell kit available for very low input/single cell samples (below 500pg). This is non-stranded, and requires good quality RNA.

ERCC spike-ins are also an option, if you need them. Due to the requirement of matching the amount of spike-ins to the amount of input RNA, the spike-ins will be increasingly more error-prone, the lower the input amount of RNA.

1b) DNA samples

Genomic DNA should be free of RNA, and you should check the integrity and size before submitting. 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!

2) Single cell 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.

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 are on the ground floor, in the single cell room just behind reception. If you don't 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.

We will receive your cells, which you will have accurately counted and resuspended in the required volume (we will supply submission guidelines specific top the protocol you use). 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.

We will process your samples and keep you updated about their progress.

3) Spatial transcriptomics samples

Tissue sectioning, fixation, staining, and imaging are typically performed at the Histology core facility or by the research groups themselves. If you are planning to perform any of these steps yourself, contact us both in advance since there are strict guidelines for the spatial transcriptomics workflow.

Sample requirements

3a) Visium HD WT panel

Limited to human, mouse, and rat samples

Two capture areas are available: both either 6.5x6.5mm or 11x11mm.

Tissue types: fresh frozen, fixed frozen, FFPE

We receive your sections on standard glass slides. These need to be placed into an allowable area on the slide, to be compatible with the CytAssist.

Some tissue types might be prone to detachment from standard glass slides – please consider special coated slides for samples like that, such as Schott Nexterion H slides.

It is recommended to perform a DV200 analysis / RIN value / RNA per cell of the same tissue block as your sample before submitting your sections (ask if you need us to help you with this).

We require you to check that the morphology of the sections is good enough for cell type annotations.

The workflow will require close collaboration between facilities – and careful planning. Histology will be able to assist you with sectioning, placing sections, deparaffinisation and H&E staining – as well as imaging on the in-house histology scanner. Imaging will be able to help with acquiring alternative good quality images from your sections. After imaging and decoverslipping, the slides can be stored for 2 weeks at 4 degrees.

3b) Visium HD 3’

Species agnostic, as technology is based on capturing PolyA tails.

Size of capture areas: 6.5x6.5mm

Tissue type: fresh frozen

We receive your sections on standard glass slides. These need to be placed into an allowable area on the slide, to be compatible with the CytAssist.

It is recommended to assess the RIN value of the same tissue as your sample before submitting your sections (ask if you need us to help you with this).

The workflow will require close collaboration between facilities – and careful planning. Histology will be able to assist you with sectioning and placing sections. After this point, the slides can be stored in -80 for 4 weeks. We will undertake the steps of fixing, permeabilization, and H&E staining, but we would require you be present for imaging to select the regions and to check that the morphology of the sections is good enough for cell type annotations.

Information about Visium tissue preparation, RNA quality assessment, allowable areas on tissue slides, and other tips and tricks can be found here: https://www.10xgenomics.com/support/cytassist-spatial-gene-expression/documentation/steps/tissue-prep/visium-cyt-assist-spatial-gene-expression-for-ffpe-tissue-preparation-guide

3c) Xenium FFPE

Before fixation, tissues should be handled gently to avoid mechanical stress. Prolonged ischemia and Post Mortem Interval (PMI) can negatively affect tissue quality.

Under- or over-fixation of tissues can negatively impact assay performance. Fixation time may need optimization for specific organisms, tissue types, and disease states. Please consult this document: https://www.10xgenomics.com/support/instruments/xenium-analyzer/xenium-in-situ-spatial-profiling-for-ffpe-%E2%80%93-tissue-preparation-guide

Sectioning: Recommended section thickness is 5 - 10 µm. FFPE tissue blocks can be trimmed or scored to fit multiple sections onto the Sample Area on the Xenium slide. Optimal water bath temperature and section floating time are critical for tissue section expansion.

QC: After section placement on blank slides, one or more serial sections should be DAPI and hematoxylin and eosin (H&E) stained to examine tissue morphology and nuclei integrity. Use the H&E staining to look for signs of over- or under-fixation and sampling artifacts. Use this information to determine if scoring is needed to select the area of interest.

RNA QC: While not mandatory, assessing RNA quality may be beneficial for troubleshooting. DV200 less than 30% is correlated with low transcript density.

Section placement: Xenium slides include an imageable area outlined by a white line measuring 12 mm x 24 mm. The Sample Area is surrounded by fiducials, which must not be obstructed by tissue. Ensure that placed tissue sections do not overlap.

Storage: Slides containing tissue sections that have been incubated at 42ºC for 3h and dried overnight at room temperature in a desiccator can be stored for up to 4 weeks at room temperature in a desiccator.

Pathology: Regions of interest must be accurately annotated if required; consult pathology services if necessary.

Information about tissue preparation, RNA quality assessment, allowable areas on slides, and other tips and tricks can be found here: https://www.10xgenomics.com/support/in-situ-gene-expression/documentation/steps/tissue-prep?tag%5BstepSlugs%5D=tissue-prep&tag%5BproductSlugs%5D=in-situ-gene-expression&tag%5BisAssayDocumentStr%5D=true

4) **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.