FAQ

How do I get in touch
Contact Maike Paramor (mp600@cam.ac.uk) or the facility account ngs-core@jcbc.cam.ac.uk

What if I have a request that is not on your list
We will always try and accommodate new protocols that we have not previously done. It makes our work exciting and may benefit other groups eventually. Contact us!

How do I submit my samples
Please contact us to arrange a handover, and please fill in the online submission form here.

How much does it cost
For smaller projects, I can provide a price calculator. We will prepare quotations for larger projects and grant applications. Please contact me for details.

What are your turnaround times
It will depend on the type of project, but in general we are aiming to have all incoming projects fulfilled within 2 weeks. This might be more at especially busy times.

Who can access the facility
The NGS facility is prioritising projects from the Stem Cell Institute, however, we are also accepting a wide range of projects from across the University, and from externals.

How will I receive the sequencing data
Once your sequencing run has finished, we will send you information on how to download the data over FTP. The output will be the FASTQ files, and some quality reports and demultiplexing statistics.

Do you provide bioinformatics support
The Stem Cell Institute has a very experienced bioinformatics facility, please contact Irina Mohorianu (iim22@cam.ac.uk) for further details.

How do I fill out the online submission form
How much data do I need

It depends on your individual requirements!
Here are some general guidelines for NGS libraries:
https://emea.illumina.com/science/education/sequencing-coverage.html;
A minimum of 20,000 read-pairs per cell are recommended for libraries constructed with the 10X
Genomics single cell kits.

What is the difference between the different Novaseq flowcells

The Novaseq has different sized flowcells which adjust to different output needs. SP, S1 and S2
flowcells have 2 lanes. The S4 flowcell has 4 lanes.

Here is a table to illustrate the amount of paired end reads (in million) per lane.

<table>
<thead>
<tr>
<th>Machine</th>
<th>Illumina Specified Million reads per lane</th>
<th>Observed Millions of reads per lane (maximum yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novaseq SP</td>
<td>350</td>
<td>500</td>
</tr>
<tr>
<td>Novaseq S1</td>
<td>800</td>
<td>950</td>
</tr>
<tr>
<td>Novaseq S2</td>
<td>2050</td>
<td>2000</td>
</tr>
<tr>
<td>Novaseq S4</td>
<td>2500</td>
<td>2700</td>
</tr>
</tbody>
</table>

What are the main library kits you are using

- NEB small RNA library kit
- Qiagen QIseq FastSelect kit
- NEB PolyA kit
- NEB Ultra II directional RNA kit
- Nextflex rapid DNA kit
- NEB Adapters for multiplexing up to 384 samples
- Thruplex DNA-seq kit
- Nextera XT kit with 384 barcodes
- NuGen Ovation RRBS Methyl-Seq System 1-16 with TrueMethyl oxBS
- SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian
- Takara Smartseq HT kit for single cells / very low RNA amounts
- 10X Chromium Reagent Kits (Chromium Single Cell 3’ v3.1 Reagent Kits with or without feature barcodes, or contact us for your requirements!)

Does the NGS facility have any equipment that groups can access

- You can book the Bioanalyzers and the tapestation on PPMS. Please ask for training/access if necessary.
- We can offer training or access to the Covaris E220 (https://www.covaris.com/e220)
We have recently acquired a miniaturised liquid dispenser, the Mantis (https://formulatrix.com/liquid-handling-systems/mantis-liquid-handler/), which will be available to users who have had training.

What do I do if my samples fail your QC

We would normally ask you to either replace those samples that failed the QC, or continue the project without them. In some cases it might be necessary to work with degraded RNA samples, and in those cases we have the appropriate kits available that deal with degraded RNA.

What recommendations are available for Single Cell preparations?

The 10X Genomics Cell Preparation Guide describes best practices and methods for washing, concentrating, resuspending, and counting cells. Some useful information in this Q&A section.

Which buffer should be used to resuspend cells in preparation for a 10X Genomics Single Cell RNA sequencing experiment?

Cells should be washed a minimum of two times and resuspended in 1X PBS (calcium and magnesium-free) with 0.04% BSA. Solutions should not contain EDTA and surfactants as they will inhibit further reactions. Alternative buffers can be found here.

How do I count my cells before submitting?

It is very important that we receive the correct number of cells in the correct volume. For accurate cell counting, please refer to this guide.

What cell diameter is compatible with the 10X Genomics Single Cell platform?

10X recommends to use cells with a diameter of up to 30µm.

How can I prevent dead cells and debris in my sample?

A 40 µm Flowmi Cell Strainer can be used to reduce the prevalence of aggregates within a cell suspension. To avoid incorporating noisy data from dead cells in your experiment, 10X Genomics provides a demonstrated protocol for dead cell removal.

Can I sort my cells prior to running through the 10x Single Cell assay?

Yes, FACS samples are compatible with the 10x Single Cell workflow. It is a good method to enrich for specific cell types based on cell surface markers, and to get a clean cell suspension as it also removes the dead cells and debris. For some tips about best practises go here.