Single-Cell RNA-Seq

Q1. What is meant by Single-Cell RNA-Seq ?

A1. Bulk RNA-Seq provides an expression profile reflecting the average state of the cells contained in the bulk sample. When the bulk sample contains a mixture of cell types this can provide misleading information about the state of the cells. By examining individual cells, one can classify the various cell types in a sample, determine the different cell states that exist in a sample, and even discover previously unknown cell types. NISC has experience sequencing many different types of Single-Cell RNA-Seq libraries. These methods vary in the number of cells that can be processed together from 100s to tens of thousands.

Q2. What material should I send to be analyzed by Single-Cell RNA-Seq ?

A2. There are three options for sending Single-Cell RNA-Seq samples to NISC:

- Pre-made libraries – NISC can sequence pre-made libraries from 10X Genomics, Drop-Seq, FAC-sorted cells in plates, Fluidigm C1, and In-Drop. Since some methods require custom primers and/or custom read lengths, it is important to discuss your approach with NISC before sending the libraries.
- cDNAs – Since the amount of RNA isolated from a single cell is so small, the samples are not as stable as naked RNA. We suggest that you convert the samples to cDNA before submitting them to NISC.
- Suspension of cells for 10X Chromium – In general, cells should be processed as quickly as possible after FAC sorting or harvesting, so it is best to find a device close to your lab. The processing on the Chromium is very quick, but you would then need to follow the protocol through the generation of the cDNA. It is then safe to bring the cDNA and remaining library reagents to NISC to complete the library construction.

Q3. How should the material be qualified ?

A3. Pre-made libraries – The investigator must submit an image of an analytical agarose gel or a trace showing the library size and demonstrating that there are no detectable adapter-dimers remaining.

cDNAs – Our experience is that cDNA from single cells is generally too low in concentration to detect by NanoDrop or High-Sensitivity Bioanalyzer. NISC will qualify the final library before proceeding to sequencing.

Q4. How many reads are required for Single-Cell RNA-Seq ?

A4. Recommendations range from 40,000-100,000 reads per cell. In the case of 10X Genomics libraries, NISC is able to estimate the number of cells represented in the data from a library QC run on a MiSeq using Cellranger Software from 10X Genomics. This allows us to more accurately target the number of reads to obtain.

Q5. What data are returned by NISC ?

A5. Typically, NISC returns to the investigator fastq files containing basecalls and quality scores. The investigator is expected to provide data analyses; this is not offered by NISC.

Data files can become quite large. For efficiency, a sequencing lane typically will contain a pool of barcoded samples, so demultiplexing is part of our data processing. Sequence data for each sample will be in a separate file. Please note that the quality of input nucleic acid will greatly influence the actual amount of quality sequences recovered. Also, poorly annotated genomes can make data analysis significantly more difficult.