Single-Cell Sequencing

Q1. What is meant by Single-Cell Sequencing ?

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.

10X Genomics also offers single-cell ATAC-Seq, and single-cell VDJ libraries.

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

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

- Pre-made libraries – NISC can sequence pre-made libraries from your method of choice. 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. Additional reagents from the system you are working with may need to be shipped to NISC along with the cDNAs.

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 Sequencing ?

A4. Recommendations range from 20,000-50,000 reads per cell for scRNA-Seq, depending on the cell type. For scATAC-Seq 10,000-25,000 reads per cell are recommended. For scVDJ-Seq ~5,000 reads per cell are recommended.

Q5. What data are returned by NISC ?

A5. Typically, NISC returns to the investigator fastq files containing basecalls and quality scores. NISC also has the capability to process 10X Genomics data using CellRanger if desired. The investigator is expected to provide further 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.