Single-cell (SC) RNA-Seq on the 10X Genomics system

SC-RNA-Seq projects are conducted in GeneExpression/Genotyping core. Please contact ICBR-Genotyping@ad.ufl.edu (or Yanping Zhang: yanp@ufl.edu) for more details and for planning your experiment.

The sequencing depth recommendation for 10x 3'SC libraries is 50,000 read pairs/cell, so for each sample you will need 50 million read pairs per 1,000 cells or 500 million read pairs per 10,000 cells (i.e ~1.6 lanes on a HiSeq). The sequencing format is 26x8x98 (132 cycles total)

For instance, let's suppose that in your experiment you plan to have 12 samples (# Chromium channels), with an expected # cells capture of 10,000 per sample and a desired reads per cell of 50,000. You will need 12x500 million read pairs total (6000 million read pairs). Since the HiSeq3000 generates ~300 million read pairs per lane, the 12 samples can be sequenced in 20 lanes or 2.5 full flow cells.

Because of the asymmetric sequencing configuration, we may have logistical challenges with filling HiSeq flow cells, unless you request 8 lanes worth of sequencing. For this reason, for smaller experiments, people use the NextSeq500. The NextSeq flow cell generates ~400 million read pairs per run. So, for all practical purposes you can roughly think of one NextSeq500 high output run per sample if you follow the standard recommendations by 10X for scRNA-Seq. Some of our users are sequencing as many as 4 samples (3k-6k cells per sample) on a single NextSeq500 high output run.

If you want to limit your sequencing cost, you can:
1- lower the sequencing depth. For example, you can target 20,000 read pairs/cell (12 samples, 10,000 cells/samples).
2- lower the # of cells captured. For example, you can target 4,000 cells/sample (12 samples, 50K sequencing depth).

For either the HiSeq or the NextSeq we will need to use the reagent kit for 150 cycles. As you can see below, there is a large difference in sequencing cost depending on which instrument you use. So, how you plan experiments and how quickly you need the data will have a big impact on sequencing cost.