CReM
CENTER FOR REGENERATIVE MEDICINE
BOSTON UNIVERSITY BOSTON MEDICAL CLINIC

670 ALBANY STREET, 2ND FLOOR
BOSTON, MA 02118
TEL: 617-414-2969
HTTP://WWW.BUMC.BU.EDU/STEMCELLS/

Summary Sheet:
RNA Seq and scRNA Seq core pricing and sequencing options
Kotton Lab

The Kotton Lab typically performs our bulk (population) RNA sequencing as well as our single cell RNA sequencing (scRNA seq) through our Boston University core facility, with prices listed here: http://www.bumc.bu.edu/singlecell/pricing/

As of 2019 our typical runs are prepared as follows:

For bulk RNA seq:
We prepare bulk organoids or single cell suspensions using either dispase to free cells from 3D Matrigel or dispase/trypsin, respectively. Cell lysates can be prepared immediately or after flow cytometry sorting, centrifugation, and resuspension in Qiazol (Qiagen) following the protocol of the Qiagen miRNeasy kit for RNA extractioin.

For 12-15 samples prepared for bulk RNA Seq:

Library prep for bulk seq:
polyA selection
costs $250/samples. (so $3000 for 12 samples)
using the Illumina mRNA kit

Our sequencing runs are performed on the BU core's Illumina Nextseq as
75 bp paired-end reads in high output mode.
Cost is $3050 for a run (of up to 15 samples combined) and typically results in 400 million reads.

This typically results in the follow read depths:
33 million (M) reads per sample for 12 samples; or
22M reads/sample for 18 samples; or
26M reads/sample for 15 samples

---

scRNA seq using the 10x Genomics system

Costs based on our BU core http://www.bumc.bu.edu/singlecell/pricing/

$6600 for 4 samples (can handle 4 at a time on the 10X machine)
we typically plan to capture 1000 cells per sample with library prep through the BU core.
We request all 4 samples run together in a single Illumina Nextseq run at a cost of $3050; requested as follows through the core:
Nextseq "high output" sequencing mode with "150 cycles" as paired-end reads:
(The paired end reads are at special settings for the 10X Genomics system: 26 bp for one read which covers the barcode and UMI, and the rest for the other paired end).
Prices are here: http://www.bumc.bu.edu/microarray/pricing/

This format typically yields more than 50,000 reads per cell, which is our target read depth).

Once scRNA Seq sequencing files return, we analyze in house using the CReM's bioinformatics pipeline resulting in an "initial look" at the data that includes initial heat maps, basic differentially expressed gene lists for each cluster, and 4 packages for interactive dimensionality reduction visualization: 1) SPRING (from Allon Klein's lab), 2 and 3) tSNE and UMAP, with Louvain clusterings, typically processed with Seurat and/or Cell Ranger with associated spreadsheets of differentially expressed genes with statistics, and 4) Loupe

We prepare tSNE and UMAP gene overlays for both the top differentially expressed genes for each cluster, cell cycle phase overlays, and overlays with standard sets of "genes of interest" developed in the CReM for each group's particular lineage of interest. Follow up statistical analyses and advanced research algorithms (e.g. continuous state hidden Markov modeling for time series, lentiviral bar coding for lineage tracing, etc) is performed ad hoc depending on the needs of each investigator/collaborator, or project.

VERSION HISTORY

2019-03-27	Darrell Kotton	DNK