Rhapsody Single Cell mRNA & Protein "Multi-omics" Analysis Services

Frequently Asked Questions:

1) What kinds of cells can I submit for BD Rhapsody "Multi-omics" Analysis?
   a) Any cell type from 5 µm to 30 µm in diameter can be captured.
   b) Some enzymes used for tissue dissociation may cleave cell surface molecules. Therefore, you should test in advance whether your enzymatic dissociation decreases expression of the surface markers you will stain using BD™ AbSeq or multiplexing antibodies.
   c) Samples must be well dissociated and rid of debris and aggregates prior to single cell capture and isolation. Viability should be at least >70% and >90% for best results.
   d) Please consult us for advice on removing cell debris and dead cells from your sample.

2) What controls should I include?
   a) The sample(s) of interest should always be run in parallel with a control sample when possible.
   b) For example, use cells from a wild-type mouse to compare to those from a mouse mutant, or cells from healthy controls to compare to those from patients.
   c) Choose the Sample Multi-plexing option to combine controls and experimental samples in the same experiment.

3) What is the maximum number of cells and different samples (cell suspensions) that can be loaded into the BD Rhapsody™?
   a) Loading 1,000-20,000 cells is optimal for analysis of a single cell suspension (sample). Above 20,000 cells, multi-plet formation may be unacceptably high (>7%).
   b) You can load up to 45,000 cells if you use the BD™ Single-Cell Multiplexing Kit to load 2-12 samples into the same cartridge, since multi-plets can be identified and removed during data analysis.

4) How many cells should I bring to the facility?
   a) Ideally you should bring 20-50% more cells than the number to be captured, to account for cell losses during washes after staining with multi-plexing or Ab-Seq Abs.
   b) The final recovery rate (cells sequenced/cells loaded) is typically 65-75% when using the BD Rhapsody™ protocol. This means that if you capture 45,000 cells, around 30,000 to 34,000 cells would be identified by sequencing.

5) What buffer should I use for my cells?
   a) If your sample is to be sorted first, please refer to our cell sorting FAQ section: https://lab.research.sickkids.ca/fcf/faq/
   b) Otherwise, your cells can be resuspended in **BD Pharmingen Staining buffer** (Cat# 554656).

6) How long will it take to obtain the results?
   a) We will complete the cell capture, cDNA synthesis and QC assessment within 2-3 business days of starting the experiment.

b) Consult with TCAG to determine their turnaround time for preparing and sequencing your libraries.

7) What Rhapsody library kits are available @TCAG to analyze mRNA in single cells?
   - √ Choose whole transcriptome analysis (WTA) to survey the entire transcriptome at low sensitivity. This assay requires high read-depths which increase sequencing costs
   - √ Choose gene-targeted assays to amplify ~400 mouse or human immune genes at high sensitivity with lower sequencing costs. BD currently offers 4 expression panels here.
   - √ The human and mouse immune panel kits are available at TCAG. Other gene-targeted panels can be directly purchased from BD.
   - √ BD can also design and provide complete custom panels, and up to 100 supplemental targets can be added to a pre-designed panel.
     - To learn more about these options, please contact BD Technical Application Specialist at BDB_Canada_Marketing2@bd.com

8) What are the options for analyzing the raw FASTQ sequencing data files?
   a) Use existing bioinformatics tools and R pipelines to generate .BAM files of aligned R1 and R2 reads as well as a .csv table containing #molecules/cell.
   b) Alternatively, ask a BD bioinformatician to run a Seven Bridges pipeline to generate .BAM files of aligned R1 and R2 reads as well as .csv tables showing transcript count, quality control metrics, filtered or unfiltered molecules/cell and reads/cell.
   c) Use the molecules/cell .csv file to perform downstream clustering and differential expression analysis using:
      (i) standard R bioinformatics pipelines OR
      (ii) SeqGeq™, BD's desktop bioinformatics platform for scRNA-Seq data that uses an intuitive FlowJo-like interface
      (iii) For more information about SeqGeq™ click here

9) What sequencing depth is recommended for WTA assays?
   a) Shallow sequencing (10,000 reads/cell) can be sufficient for cell type identification and clustering.
   b) Moderate (50,000-70,000 reads/cell) or deep (100,000 reads/cell) is needed to detect the highest number of genes/cell and low abundance transcripts. Choose these options to identify more minor subpopulations.
      i) For PBMC, 55,000 reads/cell will detect about 2,000 transcripts/cell, a median of 820 genes/cell and near 100% sequencing saturation.
      ii) Deeper sequencing may be needed to achieve sequencing saturation for other cell types.

10) What sequencing depth is recommended for Targeted assays?
   a) Shallow sequencing (2,000 reads/cell) can be sufficient for cell type identification/clustering.
   b) Deep sequencing (~20,000 reads/cell) is needed to detect the highest number of genes/cell and low abundance transcripts. Choose this option to identify more minor subpopulations.

11) What sequencing depth is recommended for Ab-Seq reads?

a) BD recommends 1,000 reads per antibody per cell for AbSeq reagents.
b) This depth can represent a significant fraction of total reads per library, so it's recommended to consult with us prior to designing your Ab-Seq panel to optimize read depth and cost.

12) What is the cost to sequence libraries prepared using the BD Rhapsody™ Single-Cell Analysis System?
    a) This cost will depend on the number of cells captured, the sequencing depth you require and your choice of Illumina sequencing platforms and flow cell types.
    b) Please contact SickKids' Tara Paton of The Centre for Applied Genomics (TCAG) for formal quotes and to schedule preparation and sequencing of your BD Rhapsody libraries.