BD Rhapsody Demonstration/Training (Feb 2022)

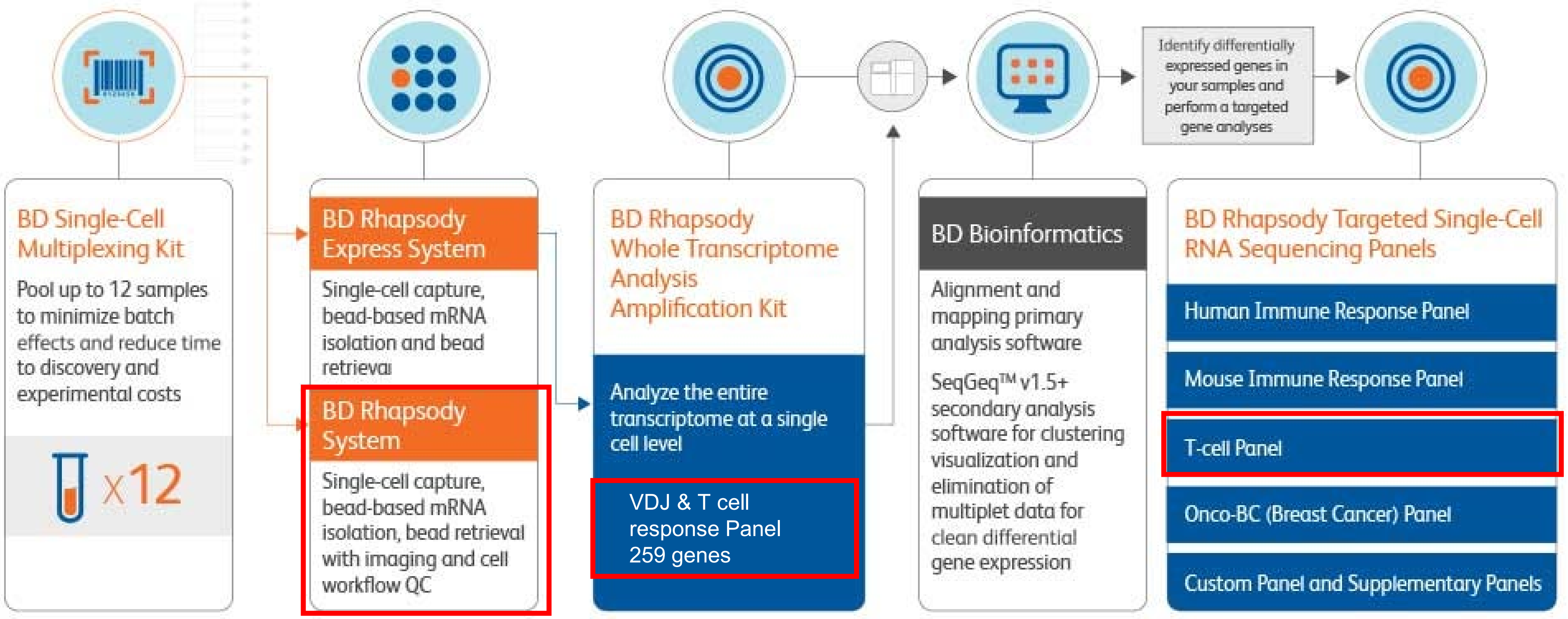
4 Groups

Nicole (Mifsud Group)
Pirooz (La Gruta Lab)
Chris (Degli-Esposti Lab)
Pryanka (Davey Group)

Experimental Design

Nicole: Human targeted VDJ/T cell panel for gene expression
Pirooz: Mouse targeted VDJ/T cell panel for gene expression
Chris: Human Whole Transcriptome Analysis (WTA)
Priyanka: Human targeted VDJ/T cell panel for gene expression

BD Rhapsody Overview



BD Single-Cell Multiplexing Kit

Pool up to 12 samples to minimize batch effects and reduce time to discovery and experimental costs

x12

BD Rhapsody Express System

Single-cell capture, bead-based mRNA isolation and bead retrieval

BD Rhapsody System

Single-cell capture, bead-based mRNA isolation, bead retrieval with imaging and cell workflow QC

BD Rhapsody Whole Transcriptome Analysis Amplification Kit

Analyze the entire transcriptome at a single cell level

VDJ & T cell response Panel 259 genes

BD Bioinformatics

Alignment and mapping primary analysis software

SeqGeq™ v1.5+ secondary analysis software for clustering visualization and elimination of multiplet data for clean differential gene expression

Identify differentially expressed genes in your samples and perform a targeted gene analyses

BD Rhapsody Targeted Single-Cell RNA Sequencing Panels

- Human Immune Response Panel
- Mouse Immune Response Panel
- T-cell Panel
- Onco-BC (Breast Cancer) Panel
- Custom Panel and Supplementary Panels

Nicole: Targeted VDJ and T cell panel for gene expression

3 samples generated

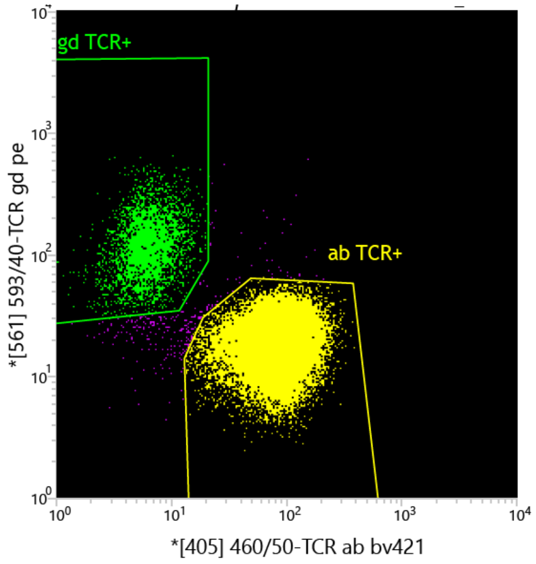
Sample tags – multiplexing (up to 12 samples)

mRNA – gene expression (259 genes)

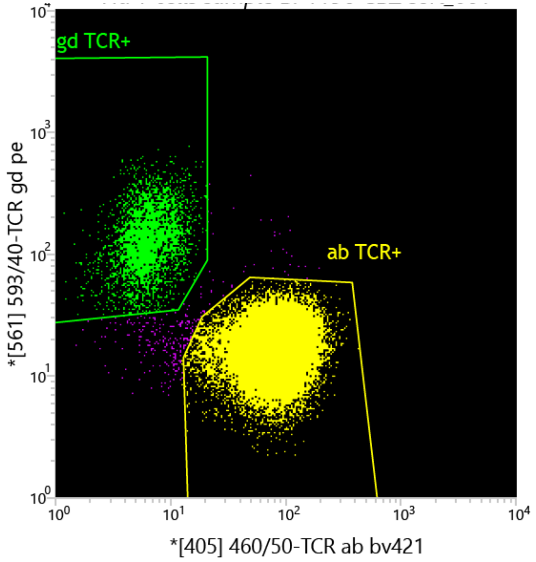
TCR – VDJ analysis

pre-Rhapsody FACSort & Sample Multiplexing

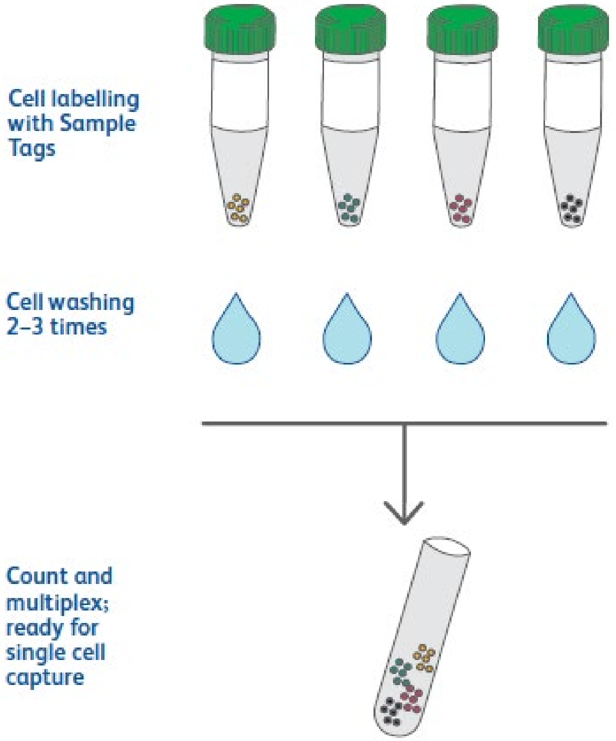
untreated



treated



12 Sample Tags per Multiplex Kit
Input cells = 1 x 10^6



Cell labelling with Sample Tags

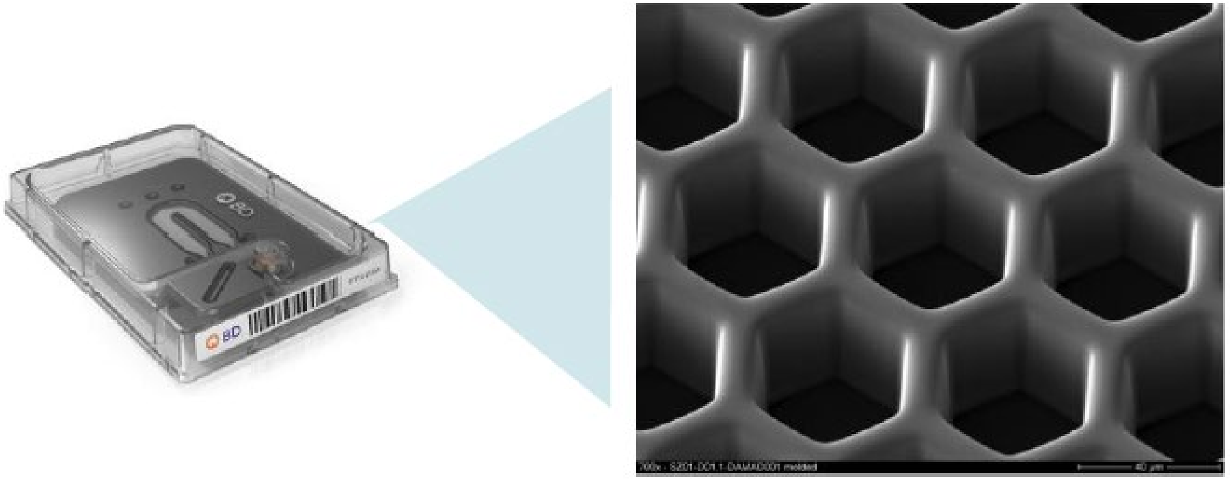
Cell washing 2–3 times

Count and multiplex; ready for single cell capture

Cartridge load range: one sample 10-20,000 cells; multiplex 25,000 cells

BD Rhapsody

Scanner

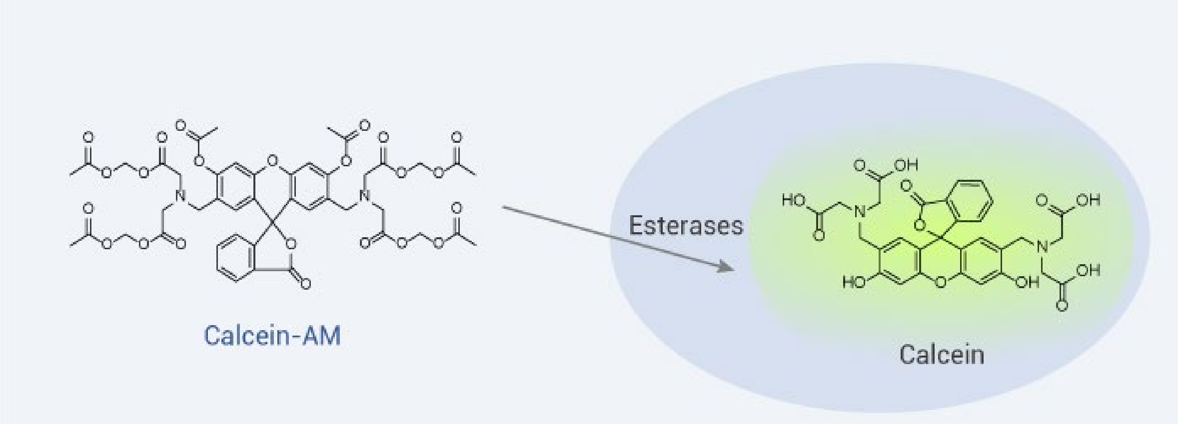


Microwell-based single-cell partitioning

QC Viability Markers

Calcein AM in live cells the nonfluorescent calcein AM is converted to a green-fluorescent calcein after acetoxymethyl ester hydrolysis by intracellular esterases.

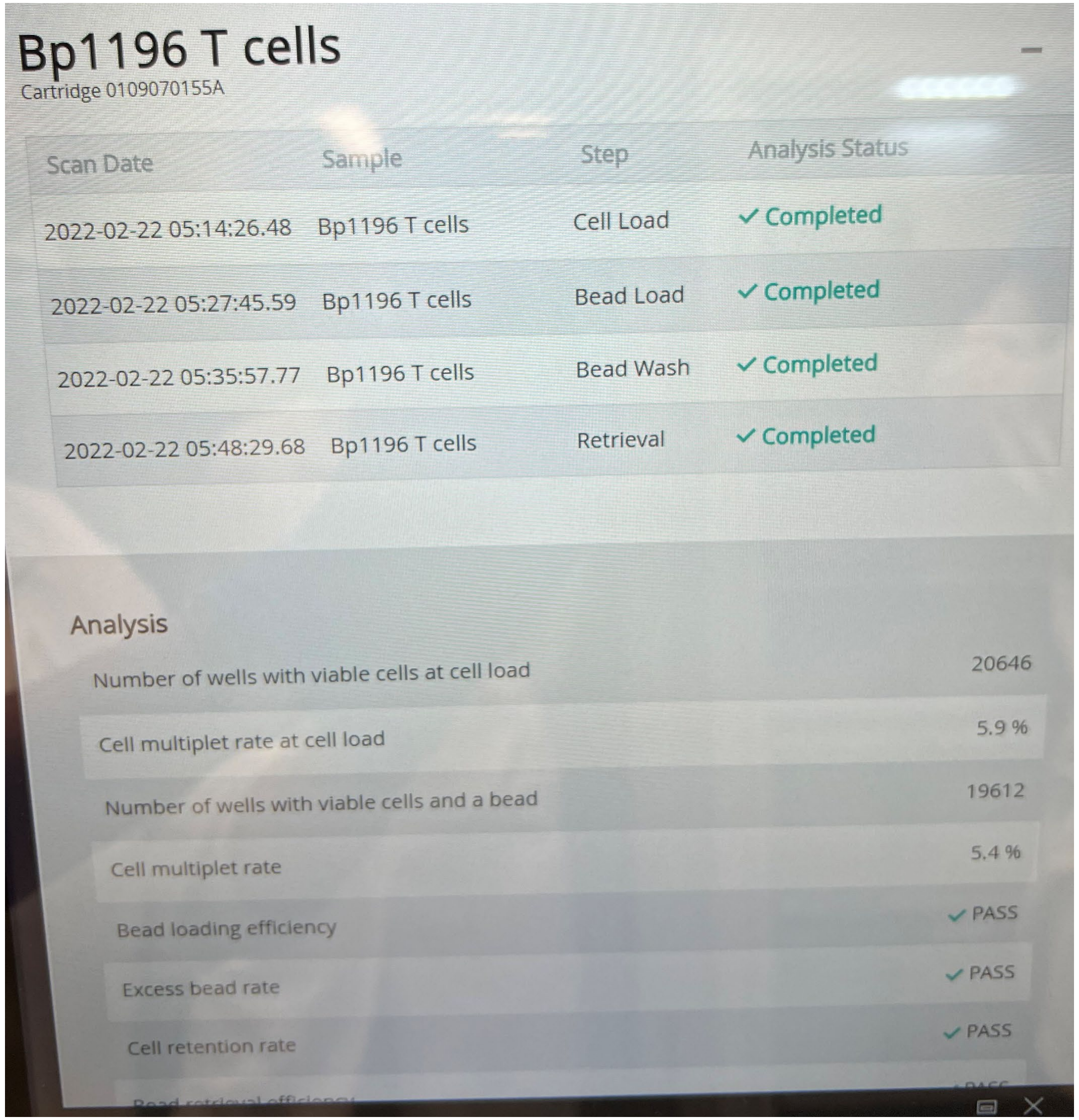
Live cells



DRAQ7™ (Deep Red Anthraquinone 7) is cell impermeable to intact cells

Dead cells

Quality Control Scanner



Bp1196 T cells

Cartridge 0109070155A

Scan Date	Sample	Step	Analysis Status
2022-02-22 05:14:26.48	Bp1196 T cells	Cell Load	✓ Completed
2022-02-22 05:27:45.59	Bp1196 T cells	Bead Load	✓ Completed
2022-02-22 05:35:57.77	Bp1196 T cells	Bead Wash	✓ Completed
2022-02-22 05:48:29.68	Bp1196 T cells	Retrieval	✓ Completed

Analysis

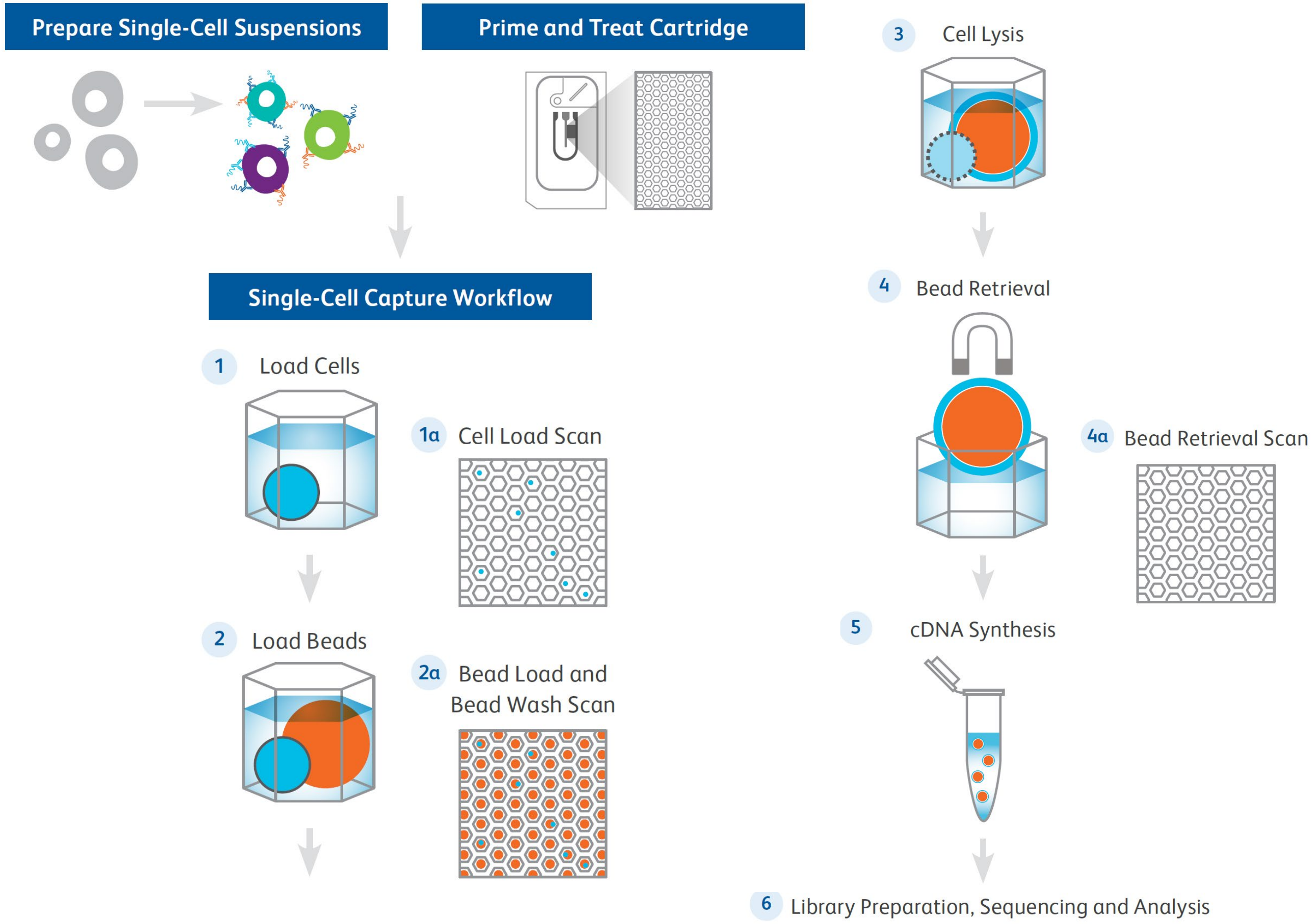
Number of wells with viable cells at cell load	20646
Cell multiplet rate at cell load	5.9 %
Number of wells with viable cells and a bead	19612
Cell multiplet rate	5.4 %
Bead loading efficiency	✓ PASS
Excess bead rate	✓ PASS
Cell retention rate	✓ PASS
Bead retrieval efficiency	✓ PASS

82% viability

95% efficiency

Single Cell Capture - cDNA Synthesis – Template switching

TSO (template switch oligo) is **an oligo that hybridises to untemplated C nucleotides added by the reverse transcriptase during reverse transcription**. The TSO adds a common 5' sequence to full length cDNA that is used for downstream cDNA amplification.



Prepare Single-Cell Suspensions

Prime and Treat Cartridge

Single-Cell Capture Workflow

1 Load Cells

1a Cell Load Scan

2 Load Beads

2a Bead Load and Bead Wash Scan

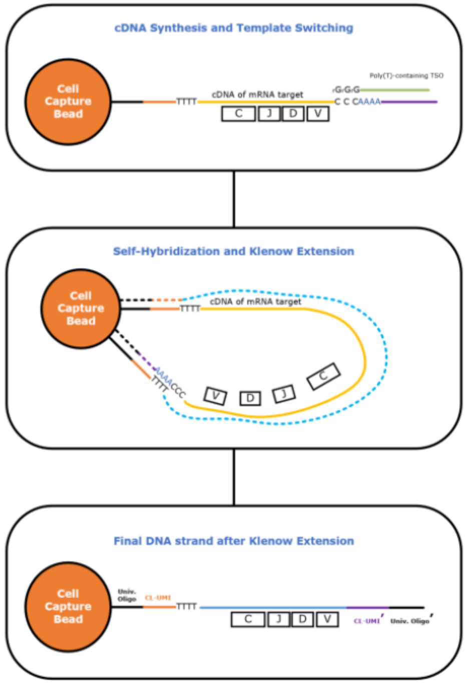
3 Cell Lysis

4 Bead Retrieval

4a Bead Retrieval Scan

5 cDNA Synthesis

6 Library Preparation, Sequencing and Analysis



New synthetic poly-A tail is added on cDNA 3′ end via template switching with a poly-T TSO (template switching oligo)

Synthetic poly-A tail on cDNA 3′ end hybridizes to poly-T on its own bead.

Addition of DNA polymerase and reagents allows copying of new barcodes to cDNA 3′ end (equivalent to mRNA 5′ end)

Ability to amplify from the 5′ end of cDNA

BD Rhapsody

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Optimized workflow for single-cell transcriptomics on infectious diseases including COVID-19

Elena De Domenico,[1,2,4,5] Lorenzo Bonaguro,[2,3,4] Jonas Schulte-Schrepping,[2,3,4] Matthias Becker,[1,2] Kristian Händler,[1,2] and Joachim L. Schultze[1,2,3,6,*]

Volume 1, Issue 3, 18 December 2020, 100233

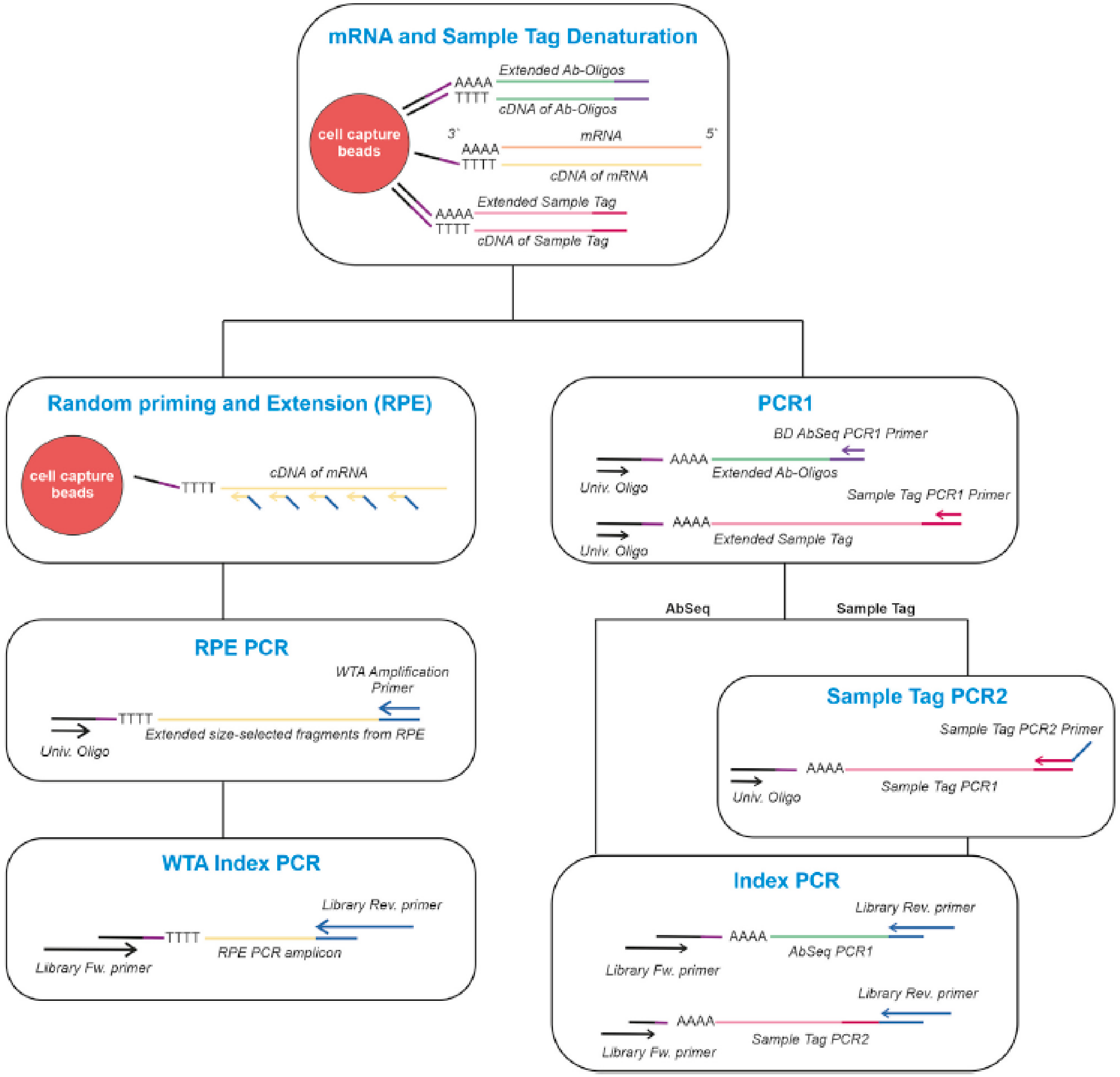


Figure 4. Schematic overview for the combination of WTA, sample tag, and AbSeq

Micromon DNA QC Fragment Analysis

Agilent 2100 bioanalyzer

Sample Name	Volume (uL)	Conc (ng/uL)	Dilution	Volume loaded (uL)
mRNA	3	17.5	1:20	1
ST	3	3.77	1:5	1
TCR	3	4.88	1:5	1

Electrophoresis File Run Summary

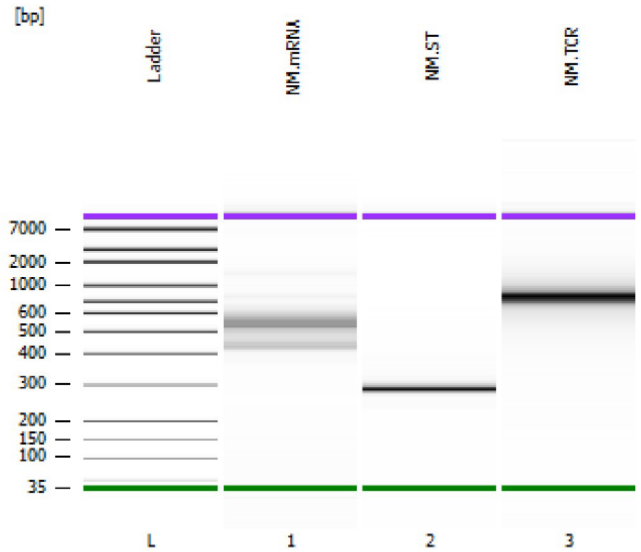
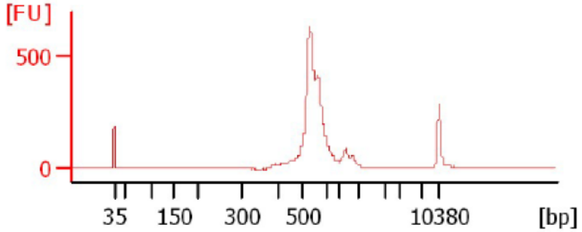


Figure 1 BD Rhapsody Immune Response Panel Hs (human)

Fragment distribution of ~450–700 bp.



NM.mRNA

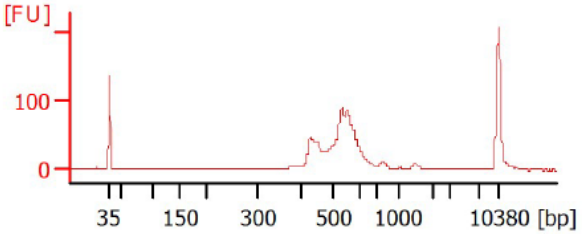
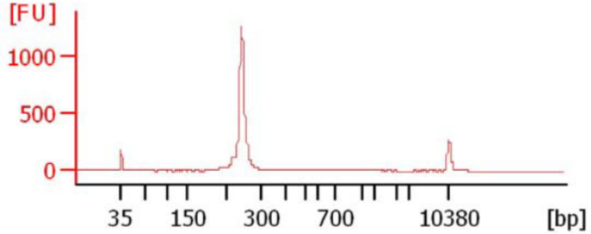


Figure 4 BD Rhapsody Sample Tag Library

Fragment distribution of ~150–300 bp.



NM.ST

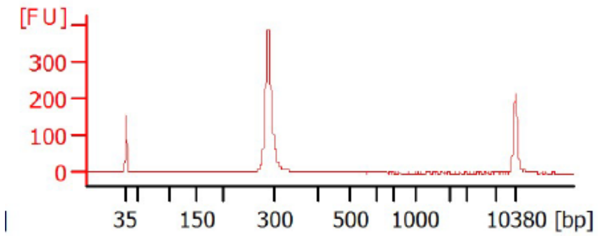
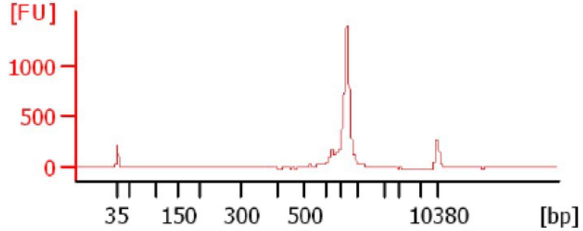
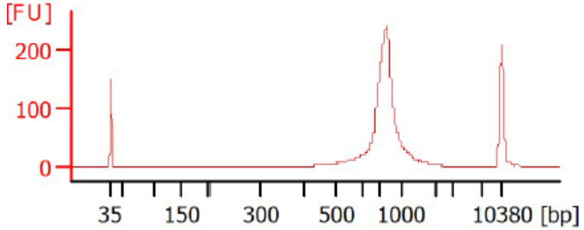


Figure 2 BD Rhapsody TCR Library

Fragment distribution of ~600–1,000 bp.



NM.TCR



MHTP Illumina NextSeq 2000

Dr Trevor Wilson
Scientific Manager, MHTP Medical Genomics Facility
$2500 for 6 samples (multiplexed – share flow cell cost)

MHTP
Monash Health
Translation Precinct
Medical Genomics Facility

Pooled sample – final volume 20 uL

Sample Name	Volume (uL)	Conc (ng/uL)
mRNA	2.31	17.5
ST	1.54	3.77
TCR	16.15	4.88

Sequencing requested: NSQ2k run using P2 300cycle kit – 75/225nt PE reads.
Anticipated read coverage: up to 400M read pairs per run
Library construction: N/A
Cluster generation and sequencing: On-board Denaturing and clustering using 650pM of library pool with 20%Phix.
Illumina Protocol 1000000109376 v3 Nov2020.
Parsing and Base Calling Dragen BCLConvert 3.7.4.

ULN	Sample ID	Library pool mean size (bp)	Library nM (qPCR)	i7 Index	Read Pairs (M)
22-1058	PiroozZareie	700	5.1	AAGAGGCA	161.7
22-1059	NicMifsud	717	2.9	GCTACGCT	277.7
	PhiX (target 20%)				108.4
		Total (includes PhiX and unassigned reads)			551.3

Output Files:
NicMifsud_S2_R1_001.fastq.gz (10 GB)
NicMifsud_S2_R2_001.fastq.gz (35 GB)

Seven Bridges Genomics

https://igor.sbgenomics.com/ SevenBridges

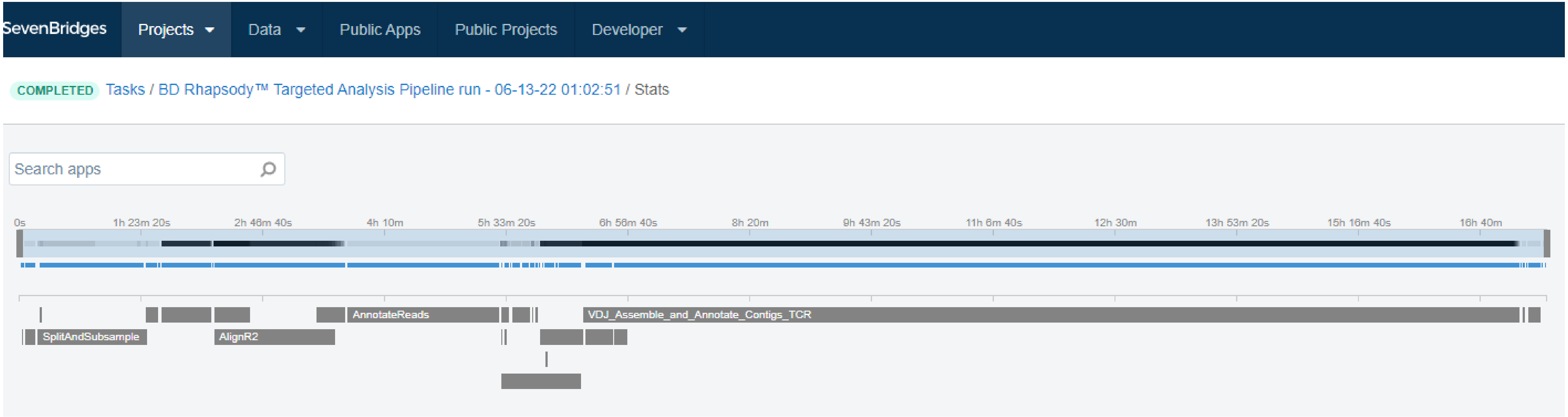
BD Rhapsody™ Targeted Analysis Pipeline run

1. Create Project
2. Upload NextGen Sequencing files (R1 and R2 fastq) and Reference file (BD fasta)

NicMifsud_S2_R1_001.fastq.gz (10 GB)
NicMifsud_S2_R2_001.fastq.gz (35 GB)

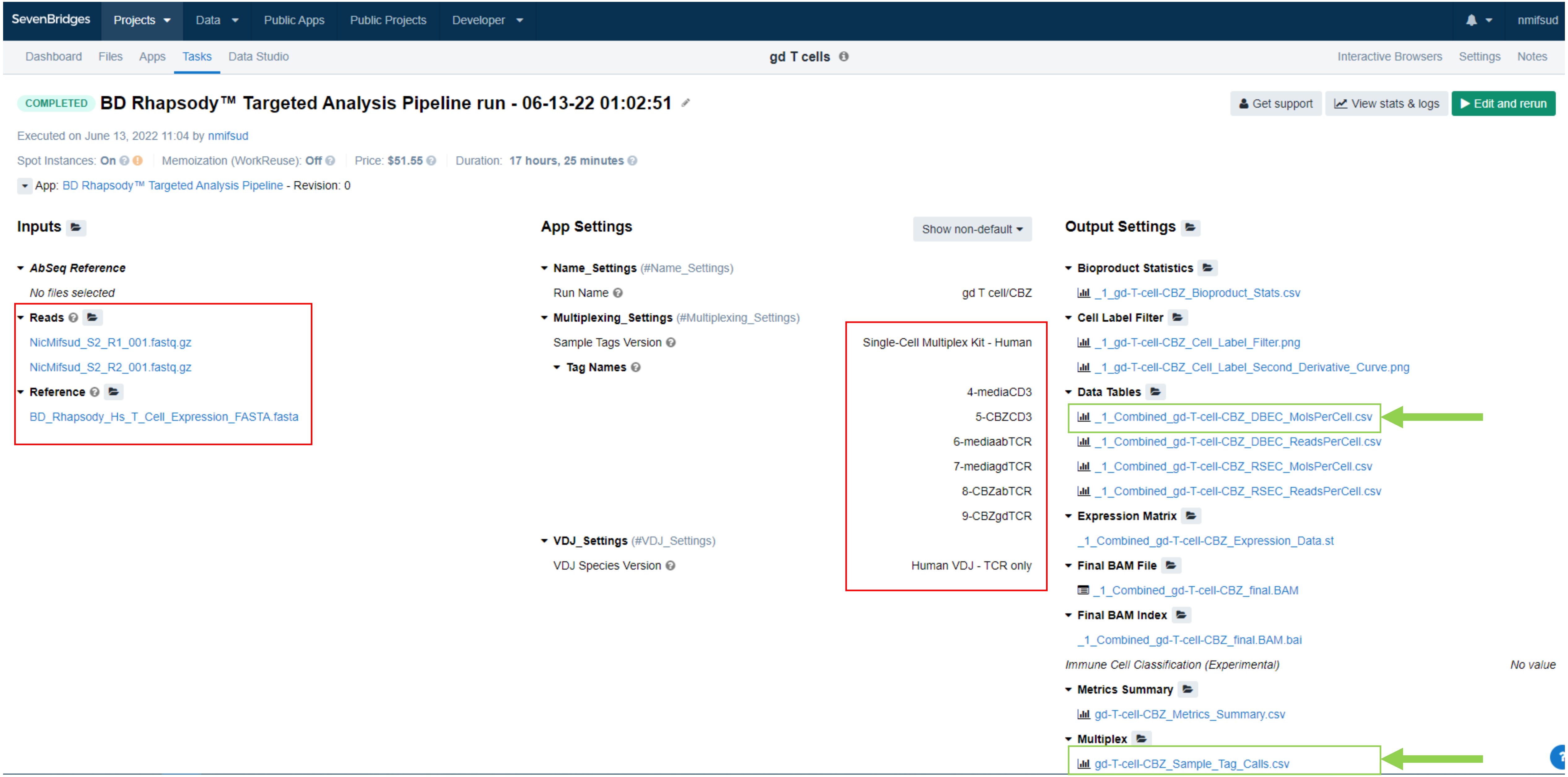
BD_Rhapsody_Hs_T_Cell_Expression_FASTA.fasta

3. Run analysis: 17 hours 25 min – run overnight



COMPLETED Tasks / BD Rhapsody™ Targeted Analysis Pipeline run - 06-13-22 01:02:51 / Stats

Seven Bridges Genomics: File Outputs



COMPLETED **BD Rhapsody™ Targeted Analysis Pipeline run - 06-13-22 01:02:51**

Executed on June 13, 2022 11:04 by nmifsud

Spot Instances: **On** | Memoization (WorkReuse): **Off** | Price: **$51.55** | Duration: **17 hours, 25 minutes**

App: BD Rhapsody™ Targeted Analysis Pipeline - Revision: 0

Inputs

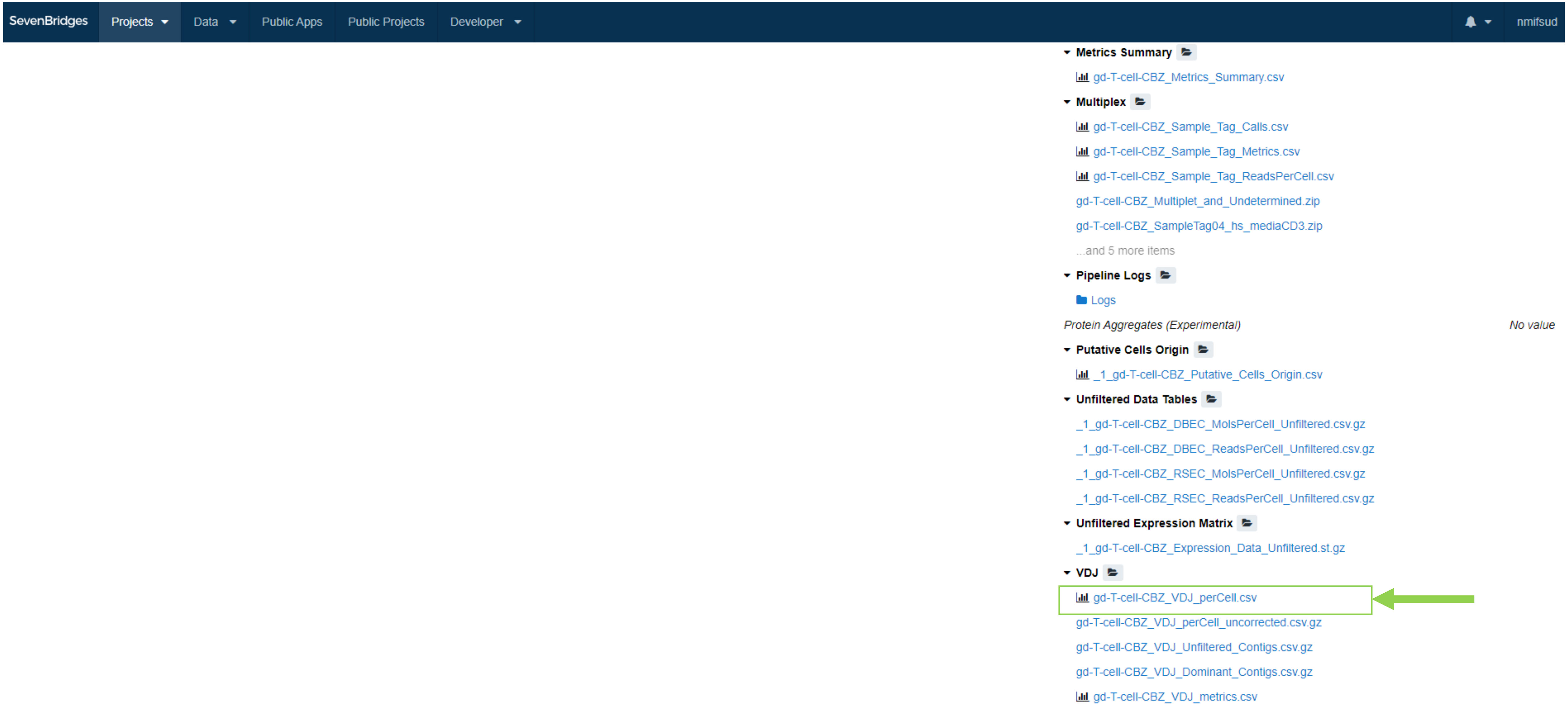
- ***AbSeq Reference***
  - *No files selected*
- **Reads**
  - NicMifsud_S2_R1_001.fastq.gz
  - NicMifsud_S2_R2_001.fastq.gz
- **Reference**
  - BD_Rhapsody_Hs_T_Cell_Expression_FASTA.fasta

App Settings

- **Name_Settings** (#Name_Settings)
  - Run Name: gd T cell/CBZ
- **Multiplexing_Settings** (#Multiplexing_Settings)
  - Sample Tags Version: Single-Cell Multiplex Kit - Human
  - **Tag Names**
    - 4-mediaCD3
    - 5-CBZCD3
    - 6-mediaabTCR
    - 7-mediagdTCR
    - 8-CBZabTCR
    - 9-CBZgdTCR
- **VDJ_Settings** (#VDJ_Settings)
  - VDJ Species Version: Human VDJ - TCR only

Output Settings

- **Bioproduct Statistics**
  - _1_gd-T-cell-CBZ_Bioproduct_Stats.csv
- **Cell Label Filter**
  - _1_gd-T-cell-CBZ_Cell_Label_Filter.png
  - _1_gd-T-cell-CBZ_Cell_Label_Second_Derivative_Curve.png
- **Data Tables**
  - _1_Combined_gd-T-cell-CBZ_DBEC_MolsPerCell.csv
  - _1_Combined_gd-T-cell-CBZ_DBEC_ReadsPerCell.csv
  - _1_Combined_gd-T-cell-CBZ_RSEC_MolsPerCell.csv
  - _1_Combined_gd-T-cell-CBZ_RSEC_ReadsPerCell.csv
- **Expression Matrix**
  - _1_Combined_gd-T-cell-CBZ_Expression_Data.st
- **Final BAM File**
  - _1_Combined_gd-T-cell-CBZ_final.BAM
- **Final BAM Index**
  - _1_Combined_gd-T-cell-CBZ_final.BAM.bai
- *Immune Cell Classification (Experimental)*: *No value*
- **Metrics Summary**
  - gd-T-cell-CBZ_Metrics_Summary.csv
- **Multiplex**
  - gd-T-cell-CBZ_Sample_Tag_Calls.csv



Metrics Summary

- gd-T-cell-CBZ_Metrics_Summary.csv

Multiplex

- gd-T-cell-CBZ_Sample_Tag_Calls.csv
- gd-T-cell-CBZ_Sample_Tag_Metrics.csv
- gd-T-cell-CBZ_Sample_Tag_ReadsPerCell.csv
- gd-T-cell-CBZ_Multiplet_and_Undetermined.zip
- gd-T-cell-CBZ_SampleTag04_hs_mediaCD3.zip
- ...and 5 more items

Pipeline Logs

- Logs

Protein Aggregates (Experimental) *No value*

Putative Cells Origin

- _1_gd-T-cell-CBZ_Putative_Cells_Origin.csv

Unfiltered Data Tables

- _1_gd-T-cell-CBZ_DBEC_MolsPerCell_Unfiltered.csv.gz
- _1_gd-T-cell-CBZ_DBEC_ReadsPerCell_Unfiltered.csv.gz
- _1_gd-T-cell-CBZ_RSEC_MolsPerCell_Unfiltered.csv.gz
- _1_gd-T-cell-CBZ_RSEC_ReadsPerCell_Unfiltered.csv.gz

Unfiltered Expression Matrix

- _1_gd-T-cell-CBZ_Expression_Data_Unfiltered.st.gz

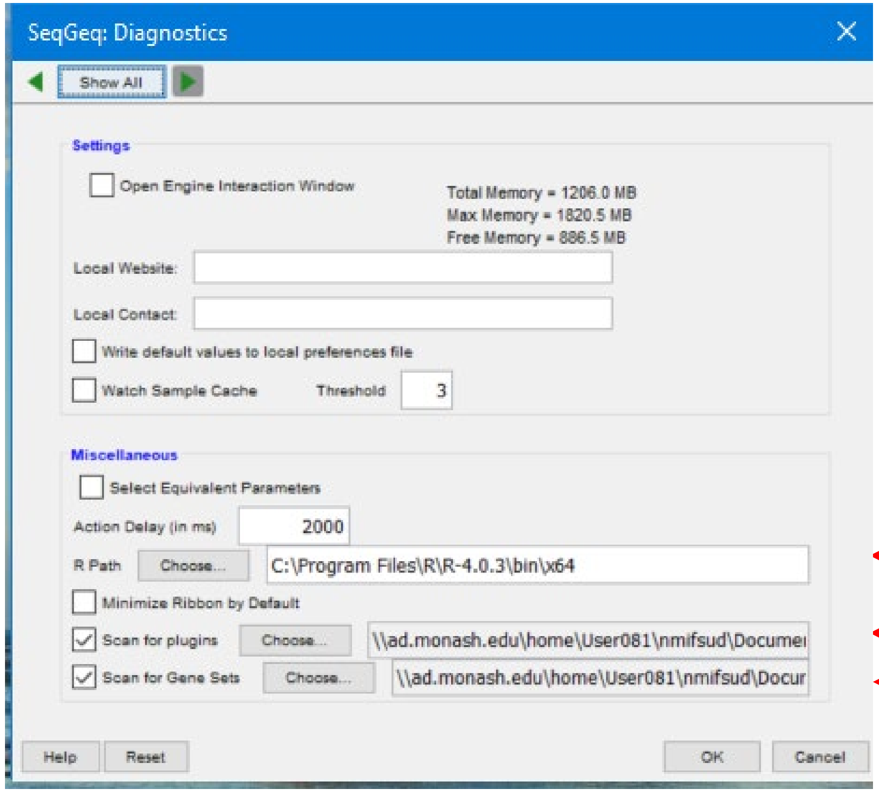
VDJ

- gd-T-cell-CBZ_VDJ_perCell.csv
- gd-T-cell-CBZ_VDJ_perCell_uncorrected.csv.gz
- gd-T-cell-CBZ_VDJ_Unfiltered_Contigs.csv.gz
- gd-T-cell-CBZ_VDJ_Dominant_Contigs.csv.gz
- gd-T-cell-CBZ_VDJ_metrics.csv

SeqGeq

60 day free trial

Preferences: Diagnostics



Settings

☐ Open Engine Interaction Window

Total Memory = 1206.0 MB
Max Memory = 1820.5 MB
Free Memory = 886.5 MB

Local Website:

Local Contact:

☐ Write default values to local preferences file

☐ Watch Sample Cache   Threshold 3

Miscellaneous

☐ Select Equivalent Parameters

Action Delay (in ms) 2000

R Path   Choose...   C:\Program Files\R\R-4.0.3\bin\x64

☐ Minimize Ribbon by Default

☑ Scan for plugins   Choose...   \\ad.monash.edu\home\User081\nmifsud\Documei

☑ Scan for Gene Sets   Choose...   \\ad.monash.edu\home\User081\nmifsud\Docur

R path

Plugins

Gene sets

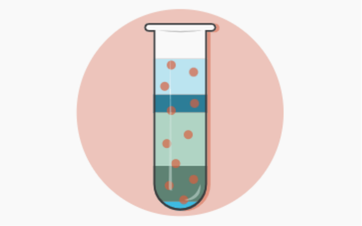
Exchange Plugins

VDJ Explorer

v4.1 published January 17th, 2022

Single-Cell Immune Repertoire Profilling

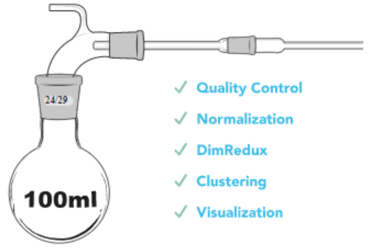
Lex



v1.1 published November 16th, 2020

A utility tool for demultiplexing samples and other fun things.

Seurat



v4.0.4 published December 21st, 2021

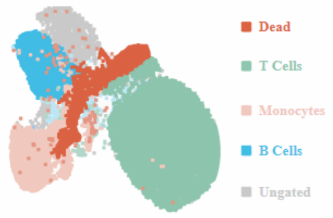
Seurat pipeline developed by the Satija Lab

Phenograph

v3.0 published September 25th, 2020

Delineate clusters by unsupervised nearest-neighbors grouping of biological parameters.

UMAP

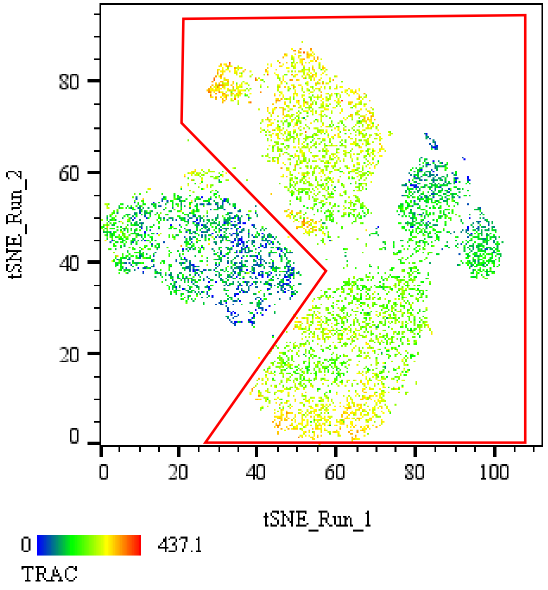


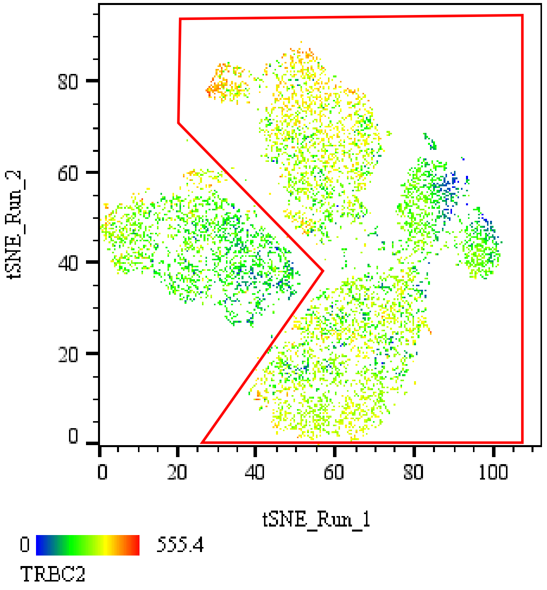
v3.1 published March 24th, 2020

A dimensionality reduction technique similar to t-SNE

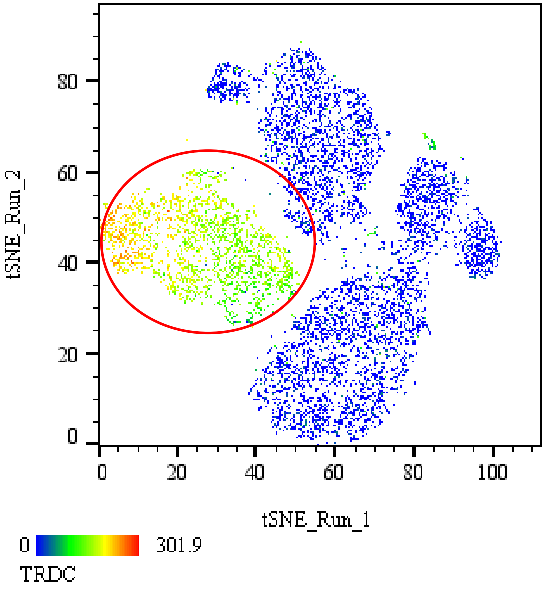
αβ vs γδ T cell subsets

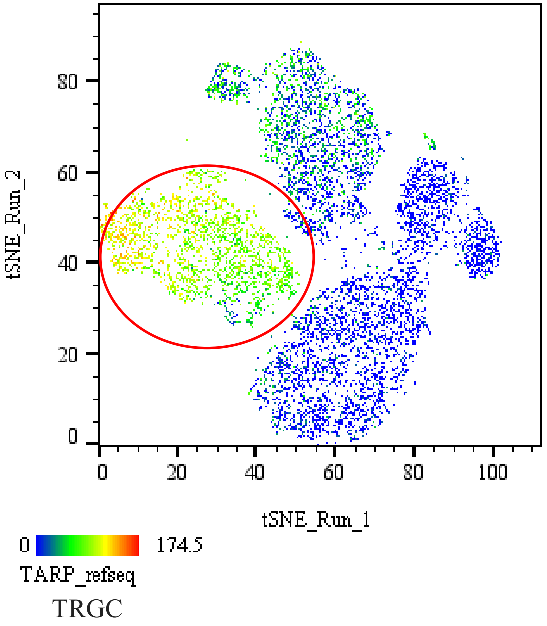
αβ T cells

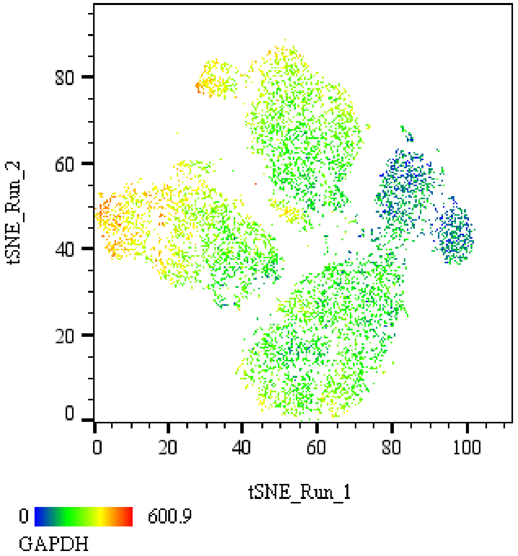




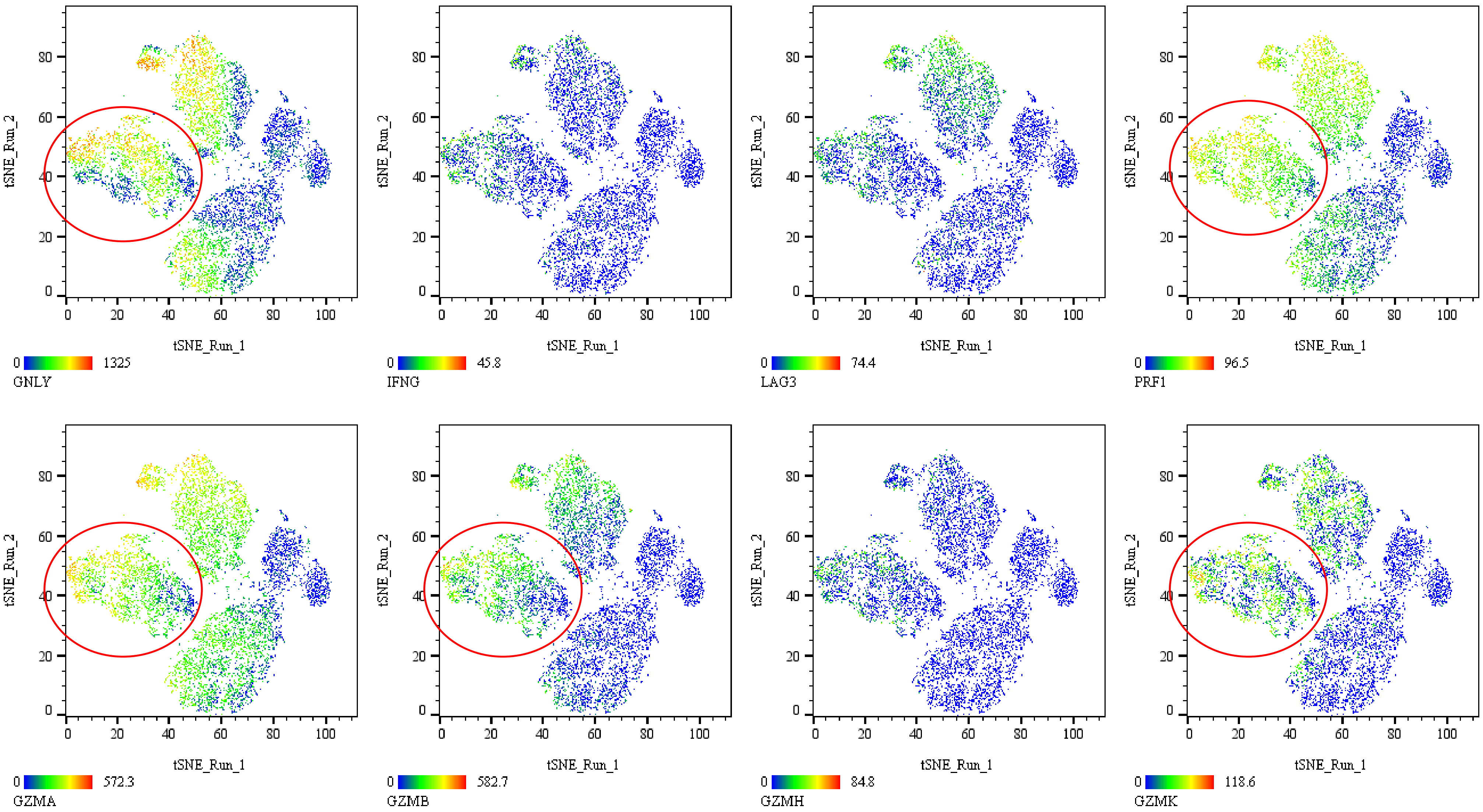
γδ T cells





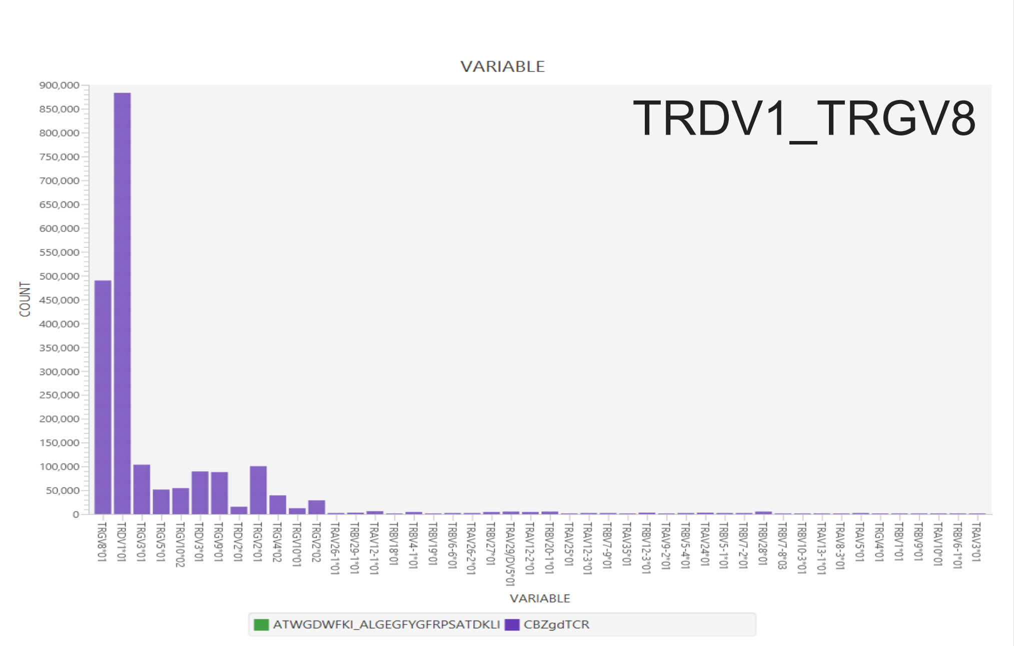


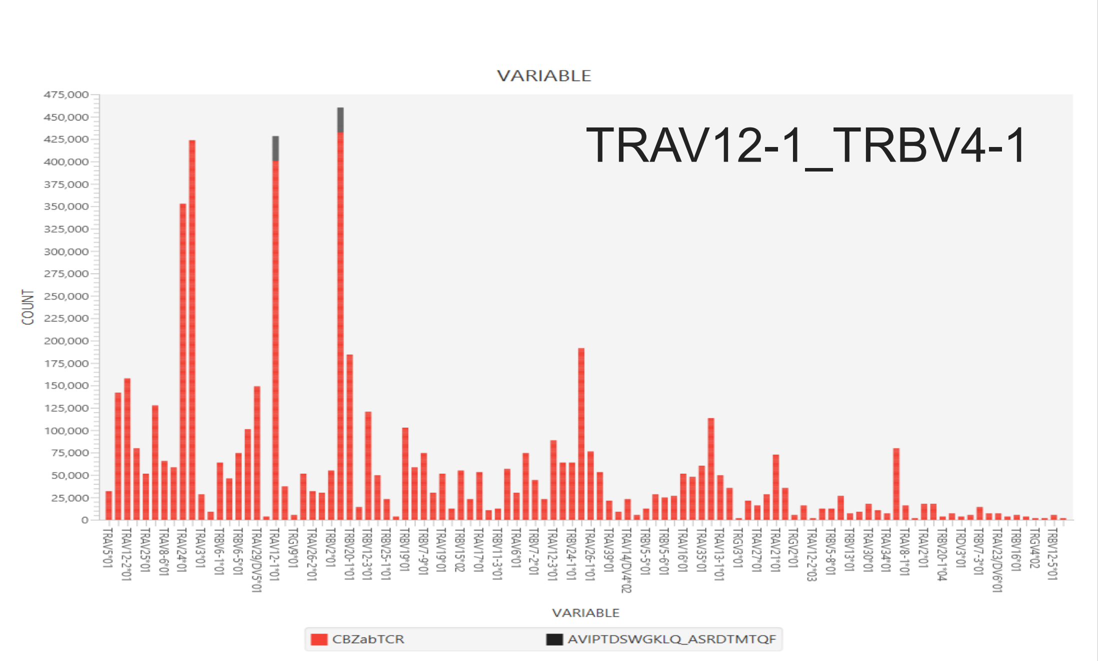
Activation/cytotoxic markers

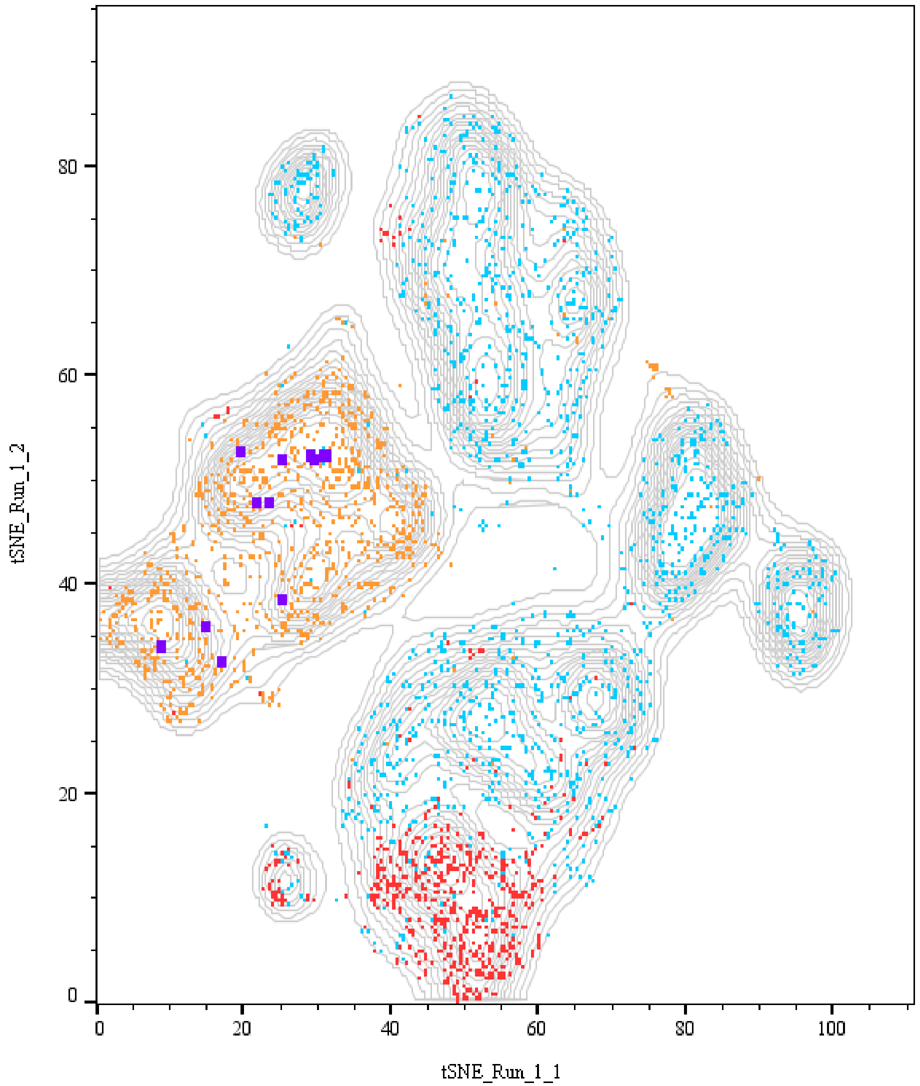


γδ T cells: granulysin, perforin, granzyme A/B/K better biomarkers than IFNγ

Clonotype mapping of prominent TCRs







	Sample Name	Subset Name	Count
🟥	Combined_gd-T-cell-CBZ_DBEC_MolsPerCell.csv	AVIPTDSWGKLQ_ASRDTMTQF	689
🟪	Combined_gd-T-cell-CBZ_DBEC_MolsPerCell.csv	ATWGDWFKI_ALGEGFYGFRPSATDKLI	12.0
🟧	Combined_gd-T-cell-CBZ_DBEC_MolsPerCell.csv	CBZgdTCR	1025
🟦	Combined_gd-T-cell-CBZ_DBEC_MolsPerCell.csv	CBZabTCR	1773
⬜	Combined_gd-T-cell-CBZ_DBEC_MolsPerCell.csv	Ungated	9709

So… what does it cost?

BD Rhapsody: 6 multiplexed samples

BD™ Human Immune Single-Cell Multiplexing Kit (12 tags, used 6)	$1812/2 = $906
TCR/BCR Multiomic Assay for Targeted (4 pack, used 1)	$9525/4 = $2381
Immune Response Panel Hs (4 reactions, used 1)	$320/4 = $80
Total	$3367

Micromon

Fragment analysis	$97.50

MHTP Sequencing

NovaSeq Flowcell (shared with NLG lab)	$5000/2 = $2500

Experiment total	$5964.50

Cost per cell	19612 cells analysed	~$0.30/cell

(maximum number 10-20K/cartridge or up to 25K multiplexed)