Intracellular CITE-seq using BD® AbSeq Antibody-Oligos

Workflow presentation

BD

Redefine CITE-seq: Profile intracellular proteins, surface proteins and RNA in one single-cell experiment

Single-cell multiomics

- Simultaneously detect single-cell **transcriptome, surface and intracellular proteome** profiles
- Yields high WTA sensitivity* for RNA analyses
- Faithfully recover expected cell subsets in your sample

High-parameter proteomics profiling

- Profile up to 100 protein markers including surface and intracellular proteins in a single experiment

Sample multiplexing enabled

- Multiplex up to 12 samples using the BD® Single-Cell Multiplexing Kit (SMK)

Validated assay

- Fully validated on the BD Rhapsody™ System
- Each IC BD® AbSeq Ab-Oligo is validated against flow cytometry controls
- Comparable IC protein performance vs flow cytometry

Simple and free bioinformatics analysis

- Analyze data with the intuitive BD Rhapsody™ Sequence Analysis Pipeline
- Same pipeline for analysis of intracellular and surface proteins

Efficient and flexible workflow

- ~2 hours in addition to the regular BD Rhapsody™ System workflow
- Stopping point for up to 24 hours during the workflow without compromising assay sensitivity

*WTA sensitivity means medium bioproduct/cell and molecule/cell. Based on development data using peripheral blood mononuclear cells (PBMCs)

Required reagents for the IC CITE-seq Assay using BD® AbSeq Ab-Oligos

Required reagents for CITE-seq with BD® AbSeq Ab-Oligos

BD Rhapsody™ Enhanced Cartridge Reagent Kit

Cat. No. 664887, for four cartridge lane runs

BD Rhapsody™ Cartridge Kit

Cat. No. 633733, four cartridge lanes

OR

BD Rhapsody™ 8-Lane Cartridge

Cat. No. 666262, eight cartridge lanes

BD Rhapsody™ cDNA Kit

Cat. No. 633773, for four cartridge lane runs

BD Rhapsody™ WTA Amplification Kit

Cat. No. 633801, for four cartridge lane runs

Order the single-vial surface BD® AbSeq Ab-Oligos separately to suit experimental needs

+

Additional reagents needed for IC CITE-seq Assay

BD Rhapsody™ Intracellular AbSeq Buffer Kit

Cat. No. 570742, for four IC staining tests

- Base buffer, 20 mL
- Perm buffer, 12 mL
- 0.1 M DTT, 20 µL
- Proteinase K, 210 µL
- Nuclease-free water, 20 mL

BD® RNase Inhibitor

Cat. No. 570751, for four IC staining tests

Single-vial reagent, 900 µL

OR

RNase Inhibitor (New England Biolabs, M0314L)

Using RI from other vendors could impact assay performance

BD® AbSeq Enhancer

Cat. No. 570750, for four IC staining tests*

- BD® AbSeq Enhancer 1, 50 µL
- BD® AbSeq Enhancer 2, 50 µL
- BD® AbSeq Enhancer 3, 50 µL

BD® OMICS-Guard Sample Preservation Buffer Kit

Cat. No. 570908, for 12 million cells

12 vials/kit, 1 test/vial (1 mL)

OR

BD® OMICS-Guard Sample Preservation Buffer

Cat. No. 570911

50-mL bottle

Need to aliquot in sterile conditions

Vybrant™ DyeCycle™ Green Stain (Thermo Fisher Scientific, V35004)

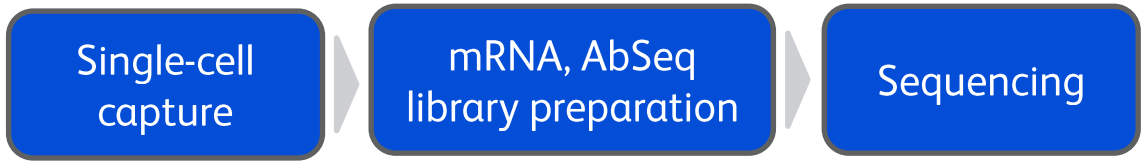
Order the single-vial IC BD® AbSeq Ab-Oligos separately to suit experimental needs

*Optimization of the AbSeq Enhancer may be necessary for untested model systems to optimize signal to noise.

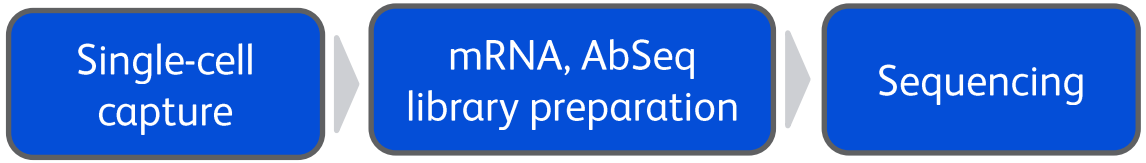
Workflow

IC CITE-seq Assay workflow

Surface CITE-seq Assay workflow



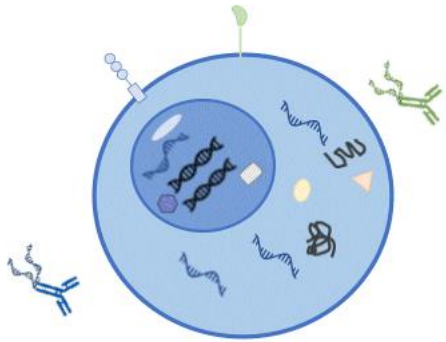
IC CITE-seq Assay workflow



Intracellular BD® AbSeq Ab-Oligos and supporting products

Hands-on time	40 min	40 min	80 min
Total time	120 min	130 min	90 min

IC CITE-seq Assay chemistry

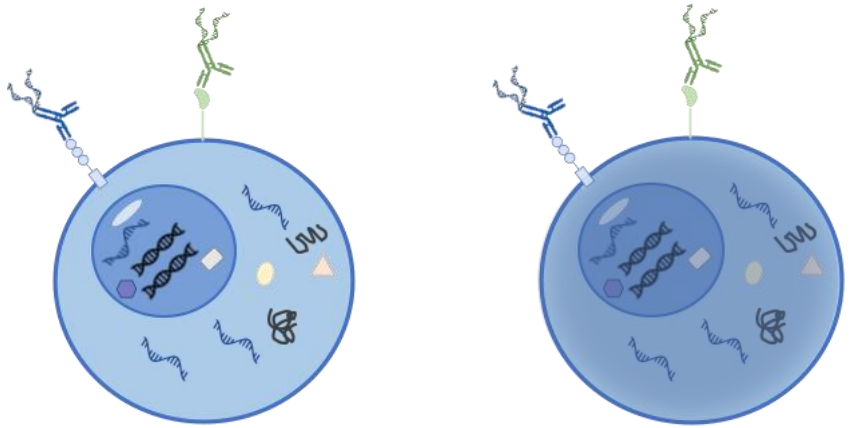


Surface AbSeq Staining

1 **Surface staining:** Surface AbSeq/SMK bind to surface proteins

IC CITE-seq Assay chemistry

SAFE STOPPING POINT:
User will have an option to store the cells for up to 24 h in BD® OMICS-Guard Buffer before moving to permeabilization



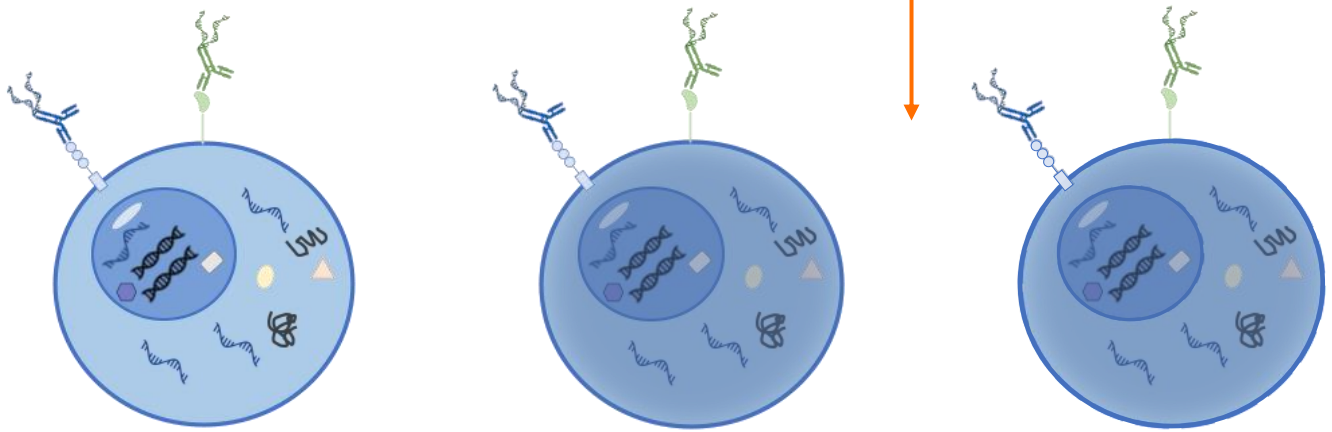
Surface AbSeq Staining

Preservation

1. **Surface staining:** Surface AbSeq/SMK bind to surface proteins
2. **Preservation:** Preserves cell status

IC CITE-seq Assay chemistry

SAFE STOPPING POINT:
User will have an option to store the cells for up to 24 h in BD® OMICS-Guard Buffer before moving to permeabilization

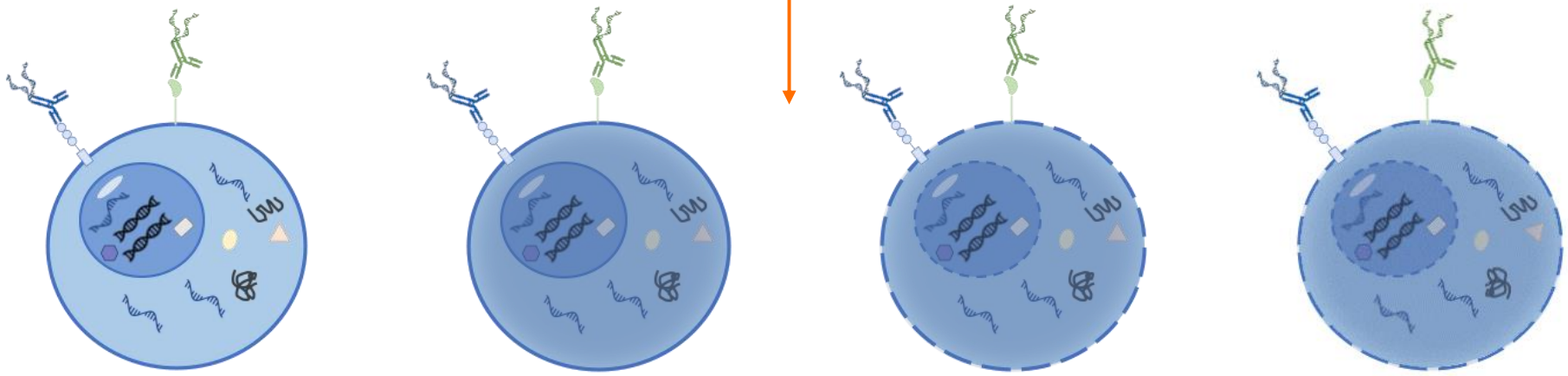


Surface AbSeq Staining | Preservation | Permeabilization

1. **Surface staining:** Surface AbSeq/SMK bind to surface proteins
2. **Preservation:** Preserves cell status
3. **Permeabilization:** Allows entry of AbSeq molecule into cell

IC CITE-seq Assay chemistry

SAFE STOPPING POINT:
User will have an option to store the cells for up to 24 h in BD® OMICS-Guard Buffer before moving to permeabilization

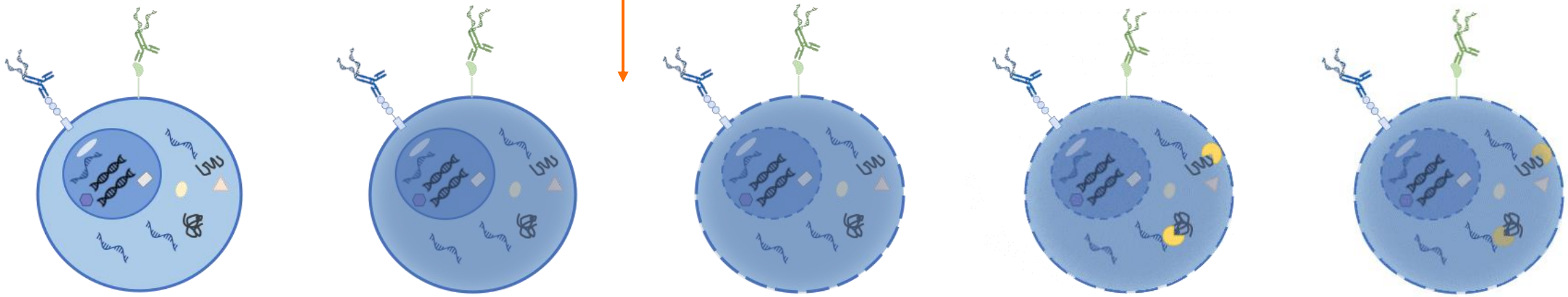


Surface AbSeq Staining | Preservation | Permeabilization | Blocking

1. **Surface staining:** Surface AbSeq/SMK bind to surface proteins
2. **Preservation:** Preserves cell status
3. **Permeabilization:** Allows entry of AbSeq molecule into cell
4. **Blocking:** Limits noise from nonspecific binding of AbSeq Ab-oligo

IC CITE-seq Assay chemistry

SAFE STOPPING POINT:
User will have an option to store the cells for up to 24 h in BD® OMICS-Guard Buffer before moving to permeabilization

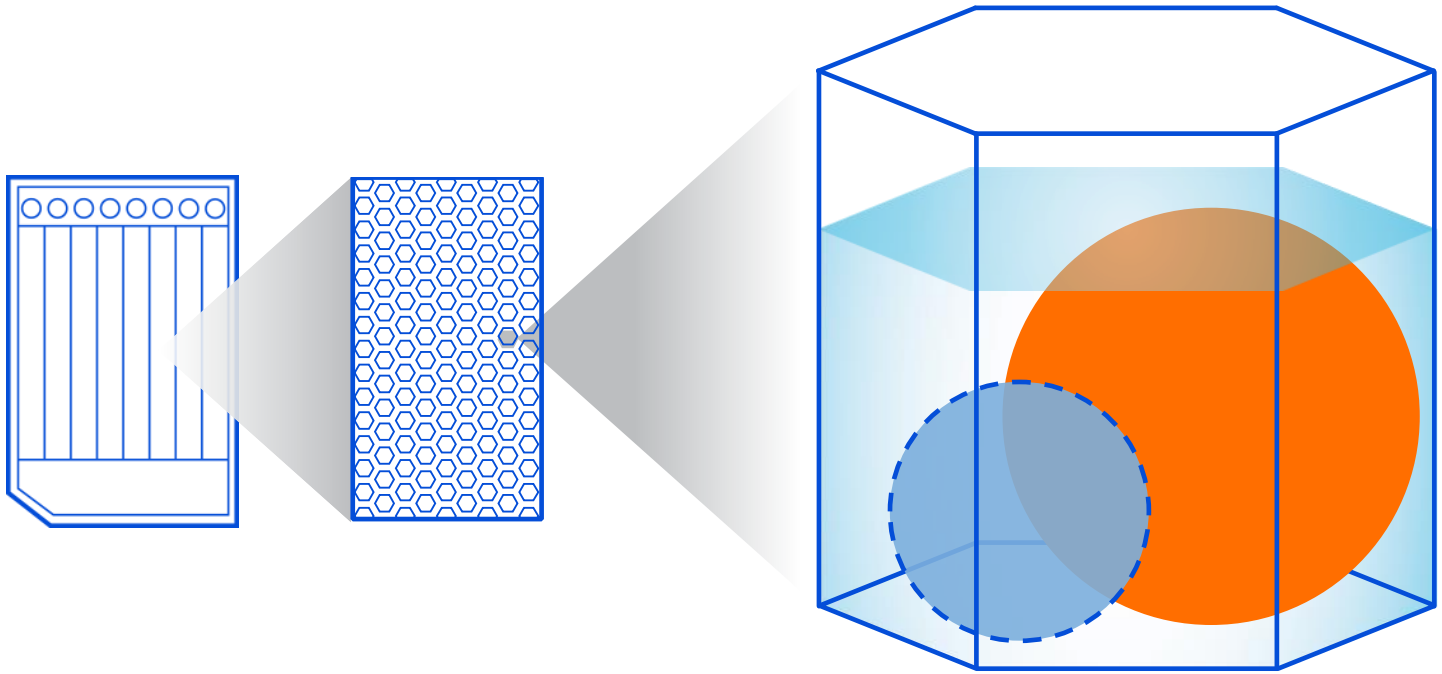


Surface AbSeq Staining | Preservation | Permeabilization | Blocking | IC AbSeq Staining

1. **Surface staining:** Surface AbSeq/SMK bind to surface proteins
2. **Preservation:** Preserves cell status
3. **Permeabilization:** Allows entry of AbSeq molecule into cell
4. **Blocking:** Limits noise from nonspecific binding of AbSeq Ab-oligo
5. **IC staining:** IC AbSeq Ab-oligos bind to intracellular proteins

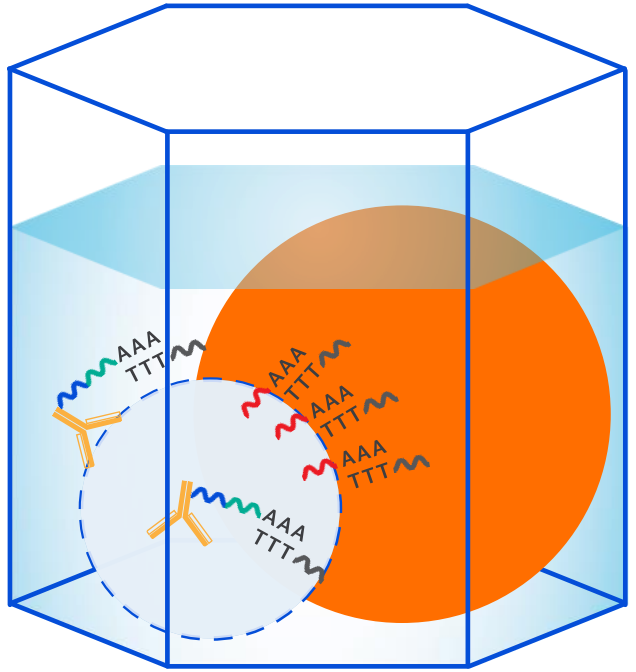
Load cells and beads

Pair ONE cell with ONE barcoded bead in microwell



Lyse cells

Lyse cell to hybridize mRNA onto barcoded capture oligos on bead



Barcoded bead

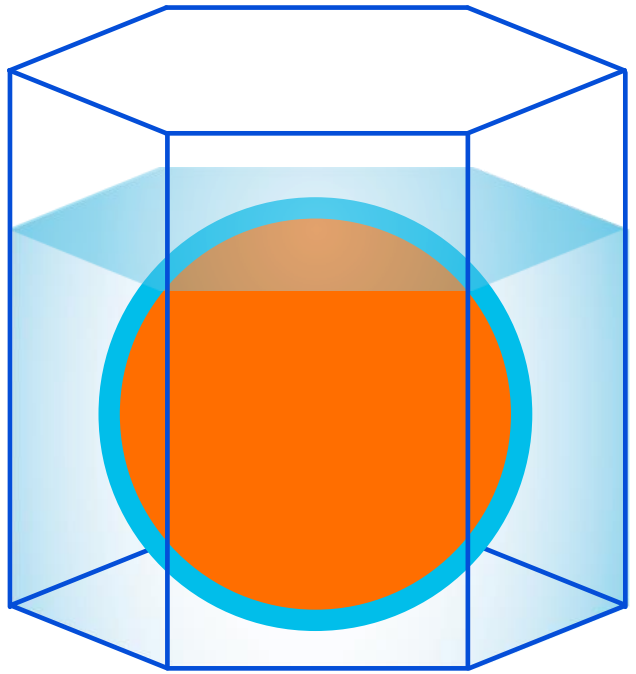
Lysed cell

mRNA AAA

BD® AbSeq Ab-Oligo AAA

Capture oligo TTT

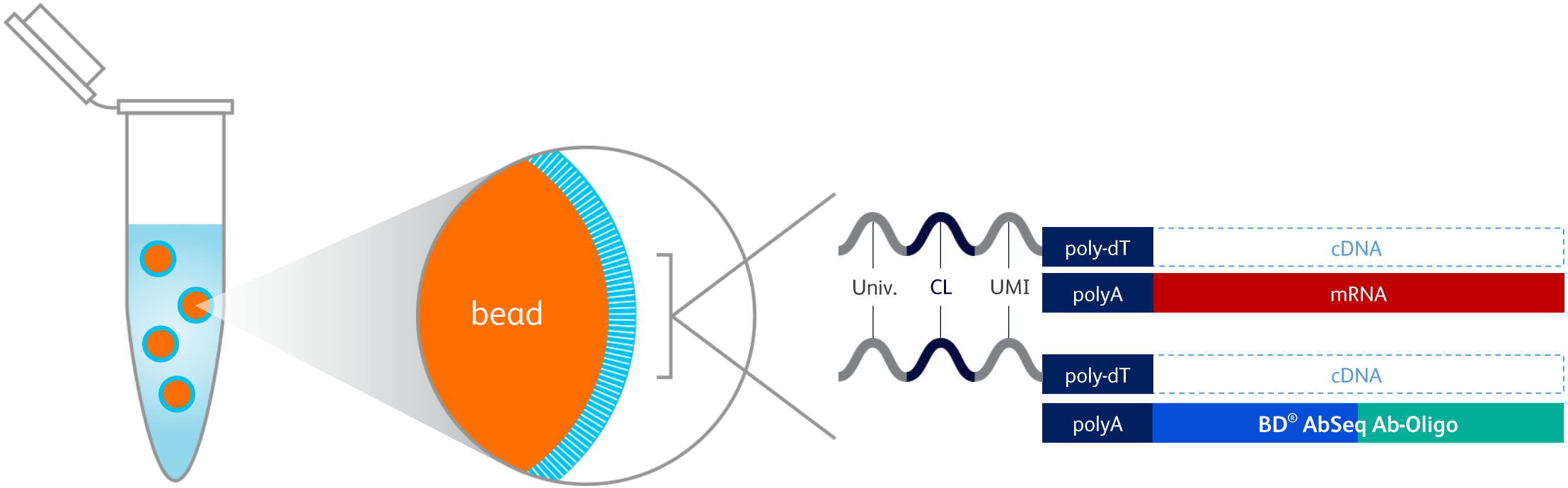
Retrieve beads



Barcoded bead

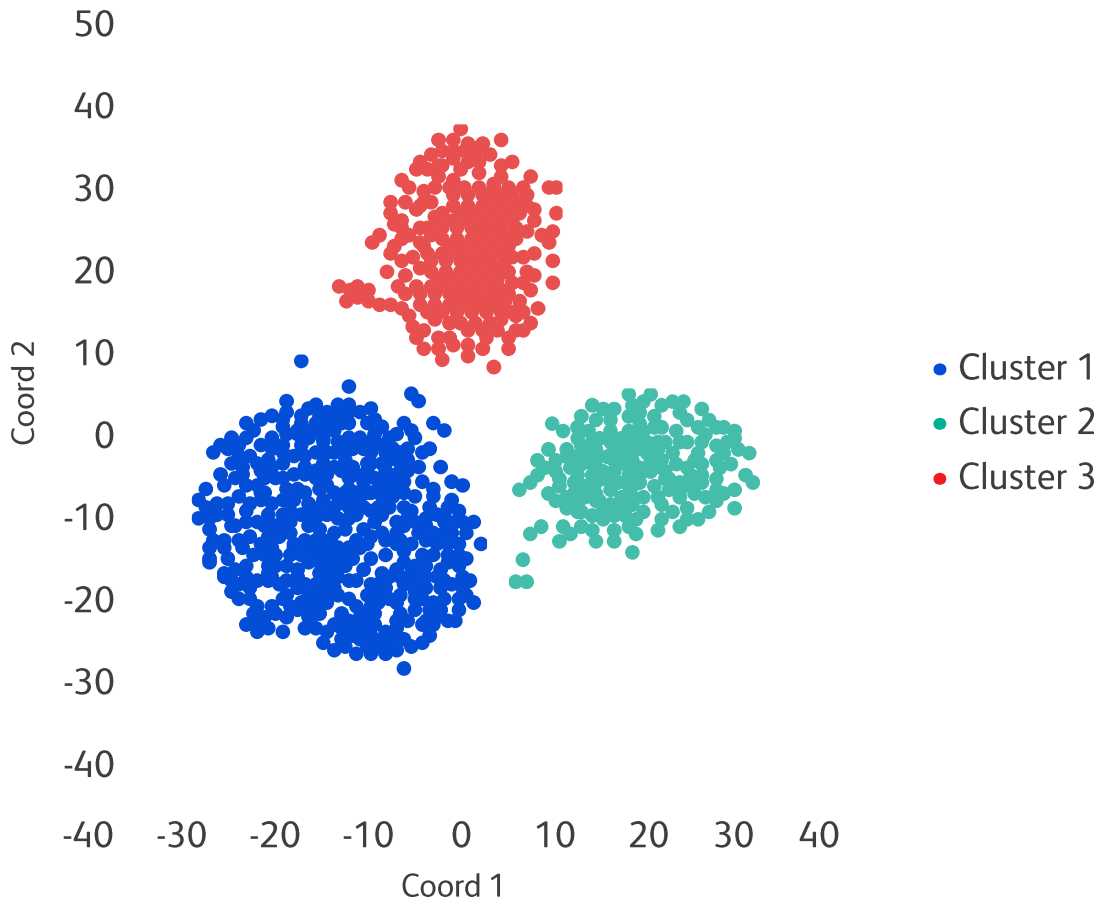
AbSeq oligos and mRNAs

Synthesize cDNA



Analyze data

Library preparation, sequencing and data analysis



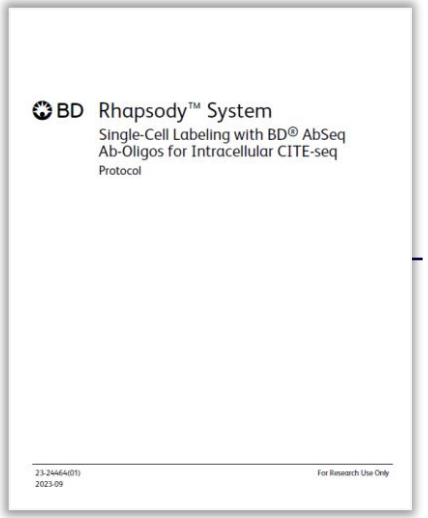
IC CITE-seq Assay protocols

IC CITE-seq Assay

Surface staining → **Preserve** → **Perm** → **Block** → **IC Stain** → **Single-cell capture** → **mRNA, AbSeq library preparation** → **Sequencing and bioinformatics analysis**

Use one of below

- Single-Cell Labeling with BD® AbSeq Ab-Oligos (1 plex to 40 plex)
- Single-Cell Labeling with BD® AbSeq Ab-Oligos (41 plex to 100 plex)
- Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (1 plex to 40 plex)
- Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (41 plex to 100 plex)



Modifications to single-cell capture workflow

Use one of below

- Single-Cell Capture and cDNA Synthesis with BD Rhapsody™ Single-Cell Analysis System 23-22951(02)
- Single-Cell Capture and cDNA Synthesis with BD Rhapsody™ Express Single-Cell Analysis System 23-22952(02)
- BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis 23-24252(01)
- BD Rhapsody™ HT Xpress System Single-Cell Capture and cDNA Synthesis 23-24253(01)

Use one of below

- mRNA Whole Transcriptome Analysis (WTA) and BD® AbSeq Library Preparation Protocol 23-24118(02)
- mRNA Whole Transcriptome Analysis (WTA), BD® AbSeq, and Sample Tag Library Preparation Protocol 23-24120(02)

- BD Rhapsody™ Sequence Analysis Pipeline
- BD Rhapsody™ Single-Cell Multiomics Analysis Setup User Guide 23-21333(06)
- BD Rhapsody™ Single-Cell Multiomics Bioinformatics Handbook 23-21713(05)

IC CITE-seq Assay materials for each step

IC CITE-seq Assay

Surface staining

Materials needed:

- Surface BD® AbSeq Ab-Oligos(MTS AbSeq or custom AbSeq)
- (Optional) BD® AbSeq IDP
- (Optional) BD® Human SMK if sample multiplexing

40 min

120 min

IC CITE-seq Assay materials for each step

IC CITE-seq Assay

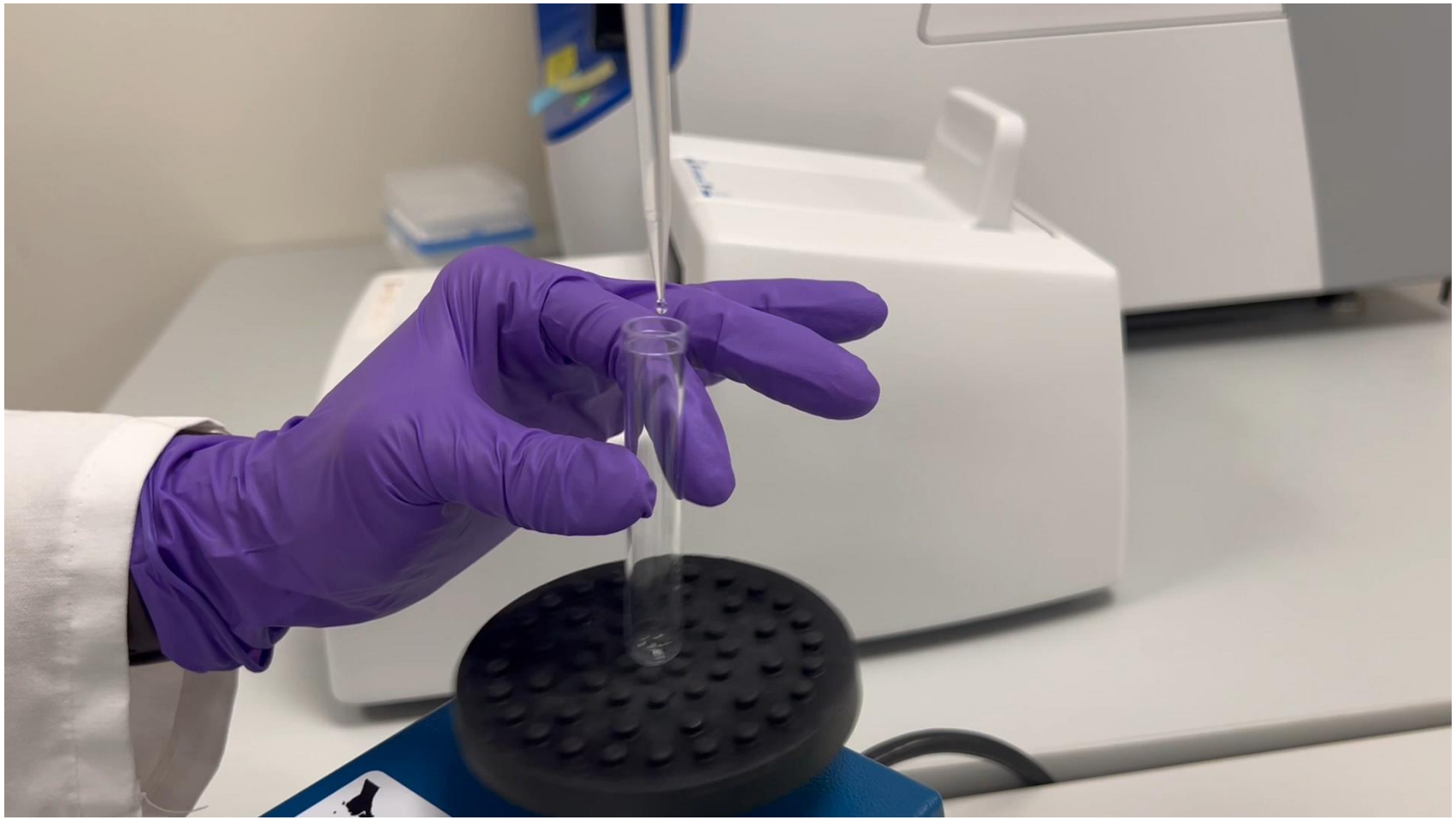
Materials needed:

- BD Rhapsody™ Intracellular AbSeq Buffer Kit
- BD® RNase Inhibitor OR RNase Inhibitor (New England Bio, M0314L)
- BD® OMICS-Guard Sample Preservation Buffer Kit
- BD® AbSeq Enhancer Kit
- Intracellular BD® AbSeq Ab-Oligos
- (Optional) BD® Human SMK if sample multiplexing

40 min

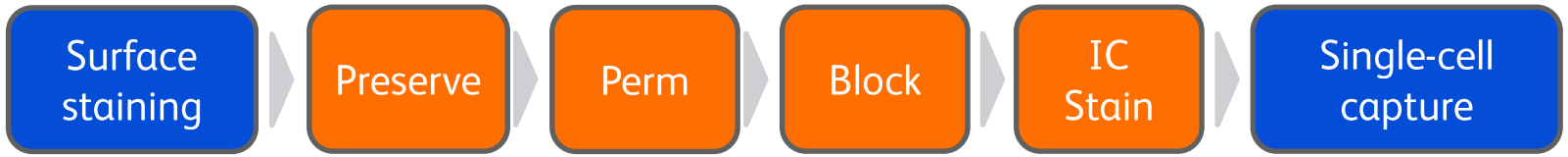
130 min

Critical step:
While slowly vortexing the cells, add drops of ice-cold permeabilization reagent equaling 1 mL



IC CITE-seq Assay materials for each step

IC CITE-seq Assay



Materials needed:

- BD Rhapsody™ HT Xpress System Package or BD Rhapsody™ Express Single-Cell Analysis System Package
- BD Rhapsody™ Enhanced Cartridge Reagent Kit
- BD Rhapsody™ Cartridge Kit or 8-Lane Cartridge
- BD Rhapsody™ cDNA Kit
- Proteinase K from BD Rhapsody™ Intracellular AbSeq Buffer Kit
- (Optional) BD Rhapsody™ Scanner
- (Optional) Vybrant™ DyeCycle™ Green if using scanner

80 min

90 min

IC CITE-seq Assay materials for each step

IC CITE-seq Assay

Materials needed:

- BD Rhapsody™ WTA Amplification Kit

Sequencing recommendation

	Illumina™ Sequencers (RUO)	Element Biosciences AVITI™ Sequencer (RUO)
Read length	Read 1: 51 cycles i7: 8 cycles i5: 8 cycles Read 2: 71 cycles	Read 1: 52 cycles i7: 8 cycles i5: 8 cycles Read 2: 72 cycles
PhiX	1%	1%
Sequencing depth	600–1,000 reads per protein specificity per cell	
Notes	• To determine the ratio of BD Rhapsody™ WTA Assay library to BD® AbSeq Assay library to pool for sequencing, contact your local field application specialist (FAS) or scomix@bd.com to access the sequencing calculator. • Avoid pooling >70% BD® AbSeq Assay library as it may impact mRNA sequence quality.	

IC BD® AbSeq Ab-Oligos data interpretation

Analyze BD® AbSeq Ab-Oligo data using FlowJo™ Software

BD Rhapsody™ Sequence Analysis Pipeline output files

Output files

Output	File	Content
Metrics summary	<sample_name>_Metrics_Summary.csv	Report containing sequencing, molecules, and cell metrics
Pipeline report HTML	<sample_name>_Pipeline_Report.html	Summary report containing the results from the sequencing analysis pipeline run
BAM and BAM Index	<sample_name>.BAM <sample_name>.BAM.bai	Alignment file of R2 and associated R1 annotations
Data tables[a]	<sample_name>_RSEC_MolsPerCell_MEX.zip <sample_name>_DBEC_MolsPerCell_MEX.zip	Molecules per bioproduct per cell, based on RSEC or DBEC
	<sample_name>_RSEC_MolsPerCell_Unfiltered_MEX.zip	Unfiltered tables containing all cell labels with ≥10 reads
Bioproduct Statistics	<sample_name>_Bioproduct_Stats.csv	Metrics from RSEC and DBEC Unique Molecular Identifier adjustment algorithms on a per-bioproduct basis
Single-cell analysis tool inputs	<sample_name>_Seurat.rds <sample_name>.h5ad	Seurat (.rds) input file containing RSEC molecules data table and all cell annotation metadata. Scanpy input file containing RSEC molecules data table and all cell annotation metadata.

a. For a multiplexed samples run, the tables contain counts for putative cells from all samples combined.

Outputs when multiplex option selected

Output	File	Content
Sample Tags metrics	<sample_name>_Sample_Tag_Metrics.csv	Metrics from the sample determination algorithm
Sample Tag calls	<sample_name>_Sample_Tag_Calls.csv	Assigned Sample Tag for each putative cell
Per-sample folder	<sample_name>_Sample_Tag<number>.zip <sample_name>_Multiplet_and_Undetermined.zip	Data tables, metric summary, and expression matrix for a particular sample. **Note:** For putative cells that could not be assigned a specific Sample Tag, a Multiplet_and_ Undetermined.zip file is also output.

Convert MolsPerCell from MEX to CSV using Python script

For multiplexed samples, sample tag specific MolsPerCell files can be found in the per-sample ZIP file

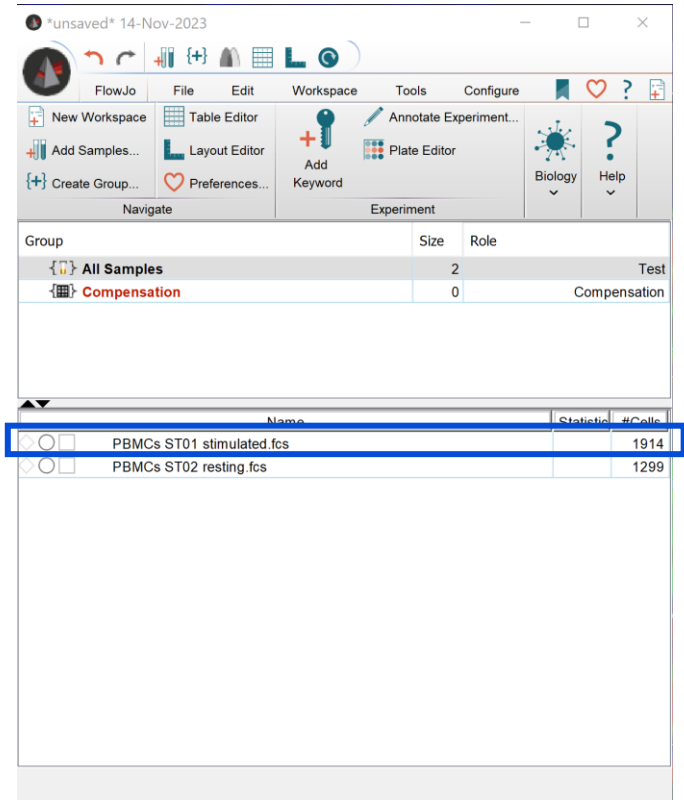
Remove the Cell Index column, find all bioproducts with "*pAbO*" in the header line and transfer all the columns containing "*pAbO*" *to* a new CSV file

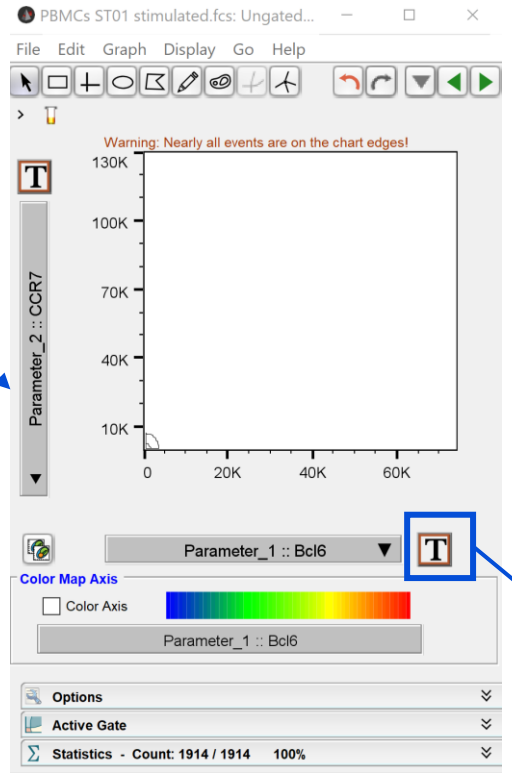
Contact local bioinformatics specialist or scomix@bd.com if you have any questions about file conversion

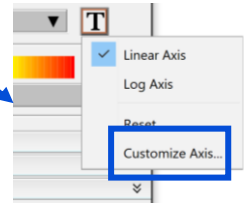
Upload the modified CSV file to FlowJo™ Software

Make sure the treated and untreated samples have the same headers

FlowJo™ Software analysis

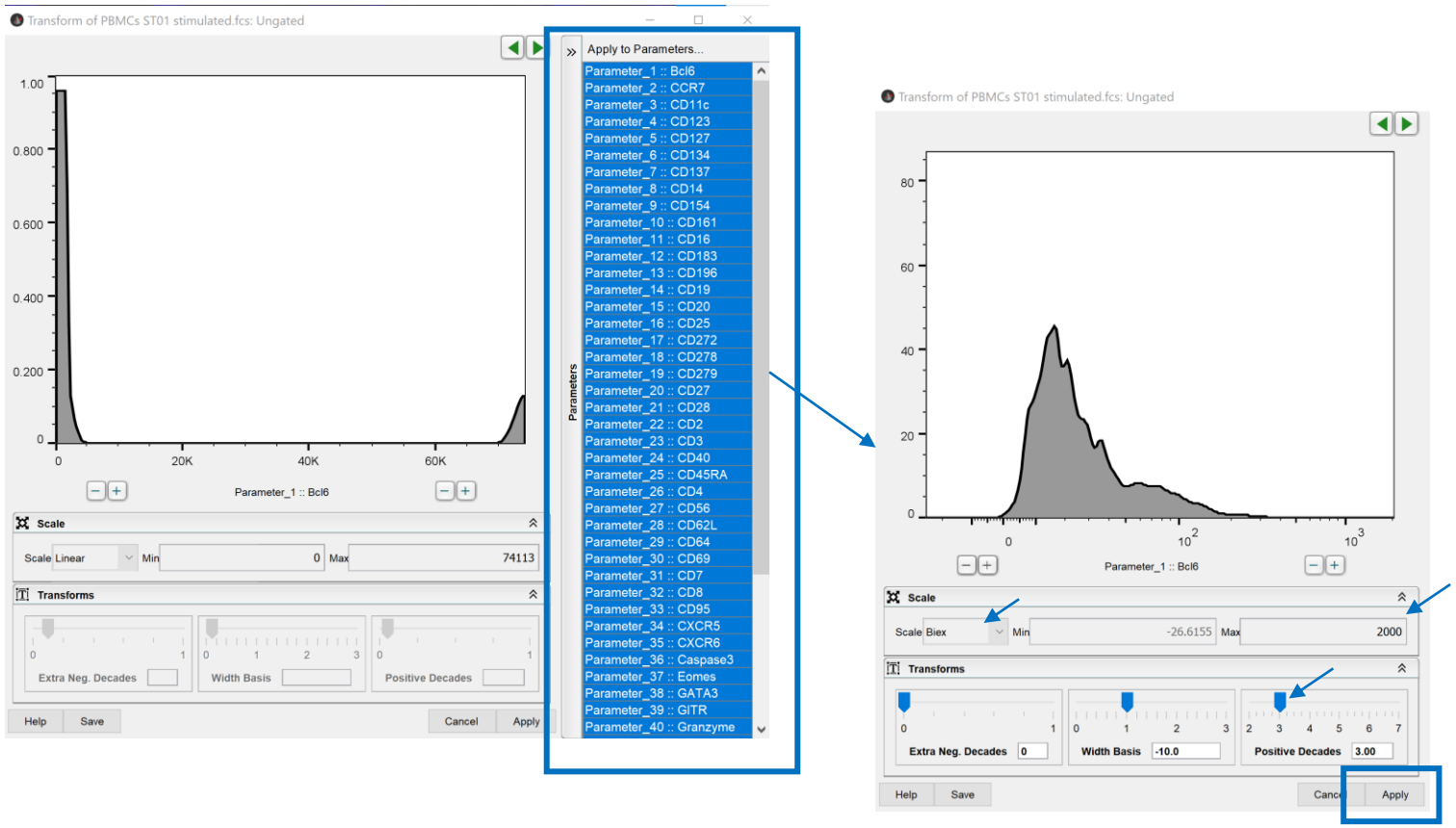






- Open a new FlowJo Workspace and drag in .csv files
- Double-click on sample to open graph window
- Click Transform Data Display drop down
  - Select “Custom Axis …”

FlowJo™ Software analysis—Changing visualization settings (default initial starting point for all parameters)

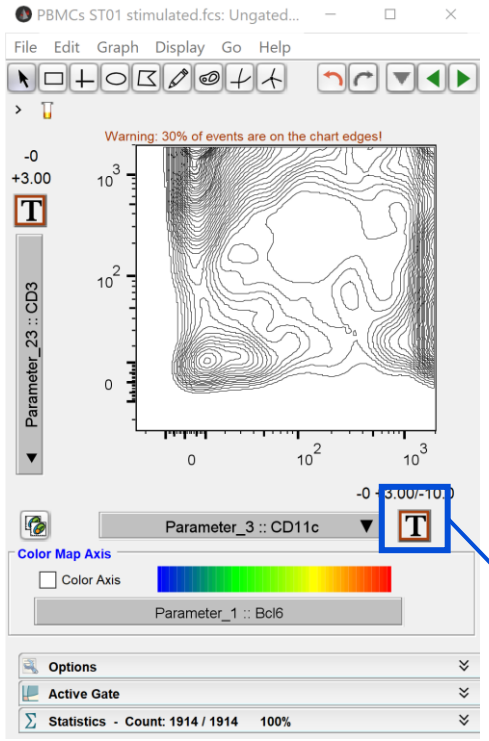


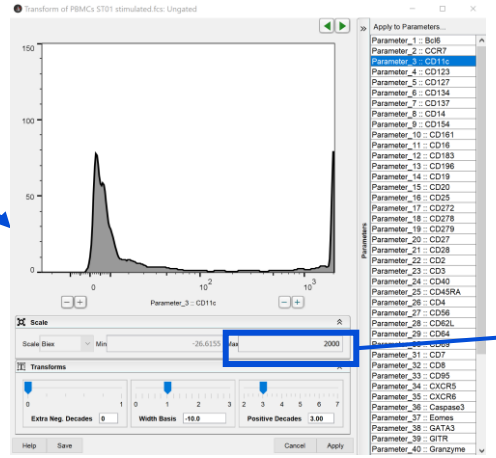
- In Transform Window, select all parameters in “Apply to Parameters …” list

- Change “Scale” to Biex
- Default Initial Settings (adjust as needed for individual data sets)
  - Extra Neg. Decades 0
  - Width Basis -10
  - Positive Decades 3.0
  - Max 2000
- Click Apply

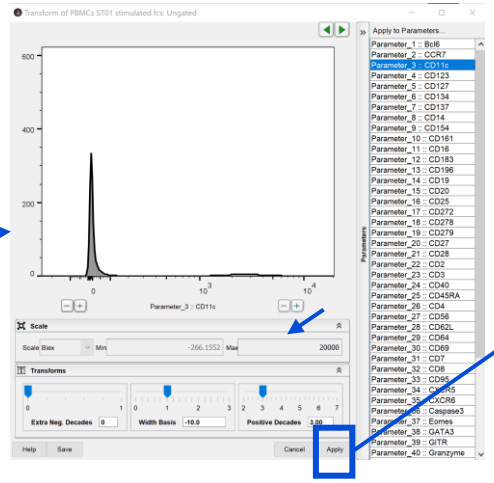
FlowJo™ Software analysis—changing visualization settings (adjusted for individual parameters)

- Look at all parameters of interest in the graph window
- If data is off scale, adjust dynamic range of parameter display
  - Transform Data Display -> Customize Axis …
  - Change Max so data is within the displayed range
  - Click Apply

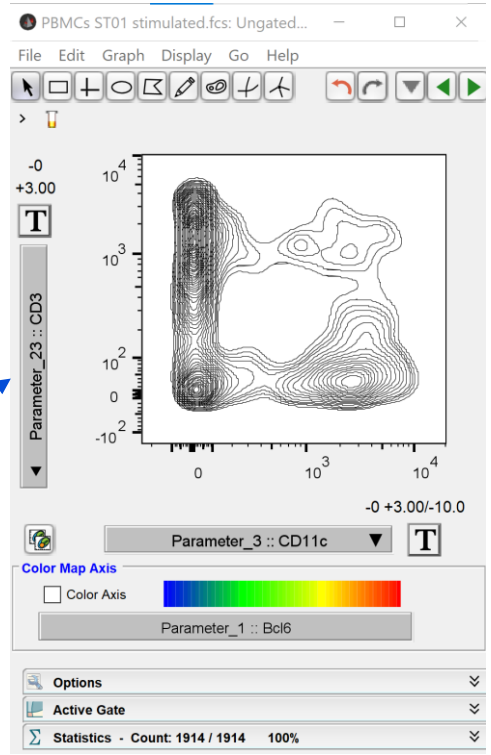




Max changed from 2,000 to 20,000



Max also adjusted for y-axis

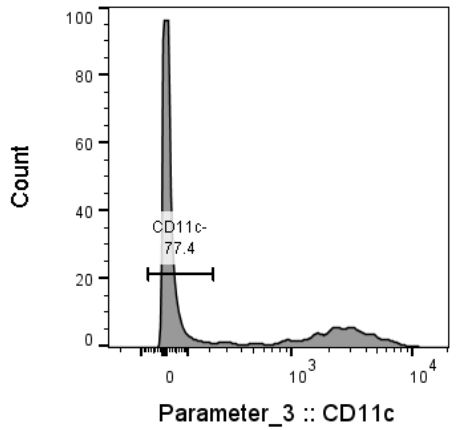


FlowJo™ Software analysis—Phenotype gating

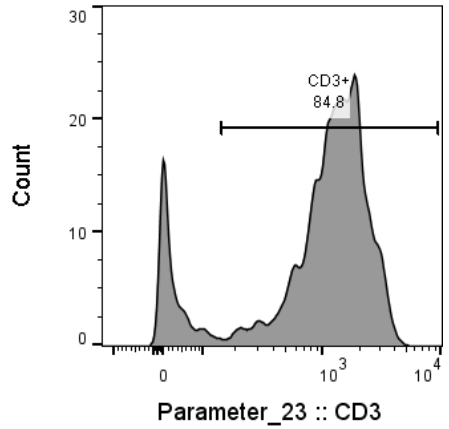
- Gate on cells of interest
  - Example: Lymphocytes, CD3+ subset
  - PBMC sample
    - Gate out CD11c+ Monocytes
      - Gate on CD3+ Lymphocytes

Histogram plots – Interval gates

All cells
gate on CD11c- pop

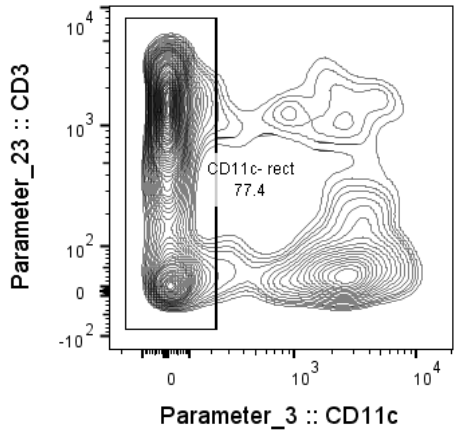


CD11c- Lymphs
gate on CD3+ pop

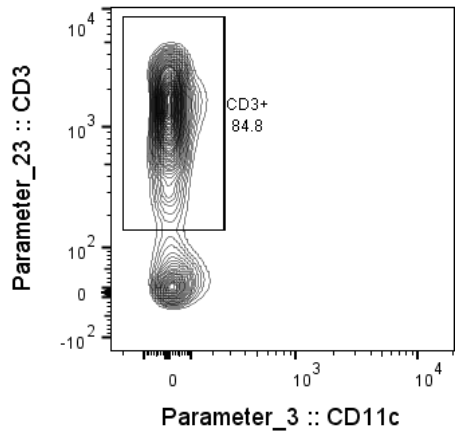


Bivariate contour plots – Rectangle gates

All cells
gate on CD11c- pop



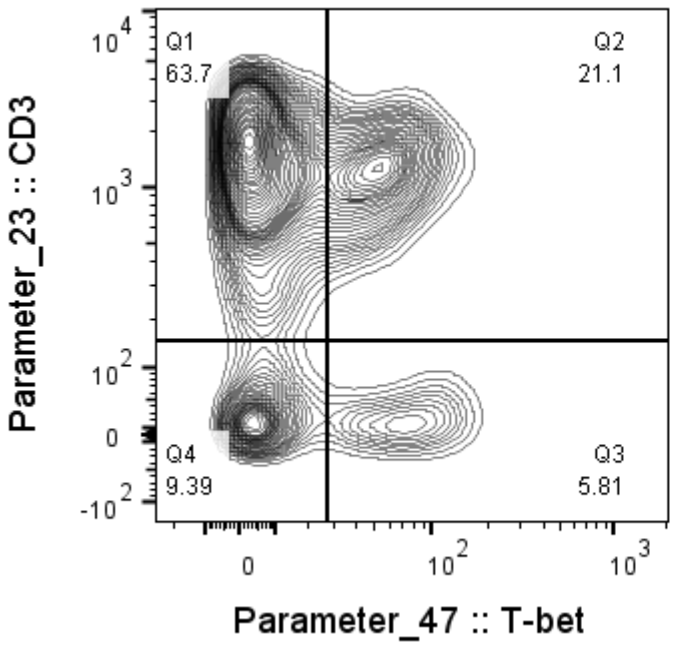
CD11c- Lymphs
gate on CD3+ pop



FlowJo™ Software analysis—IC AbSeq visualization

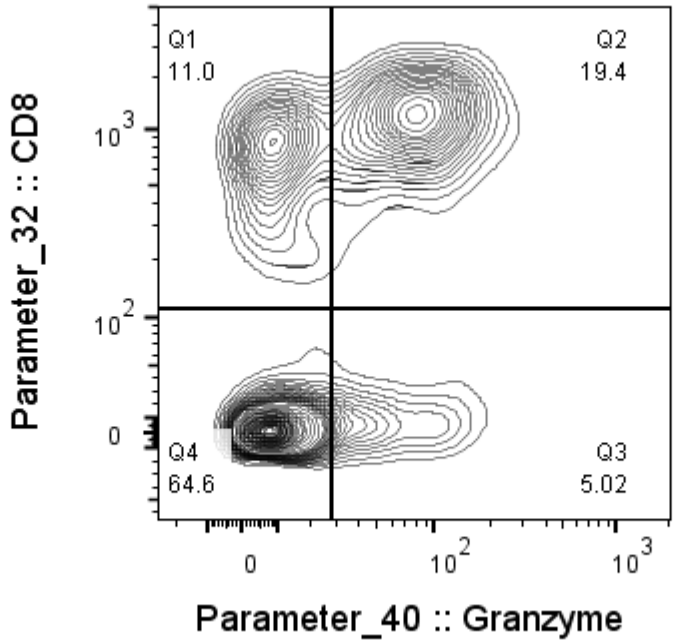
Bivariate plots

T-bet vs CD3
Quadrant gate



PBMC-Lymph population

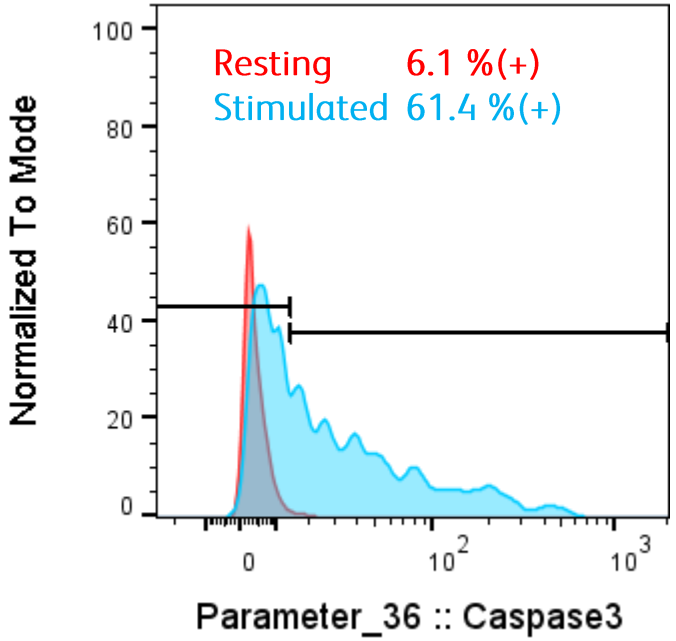
Granzyme B vs CD8
Quadrant gate



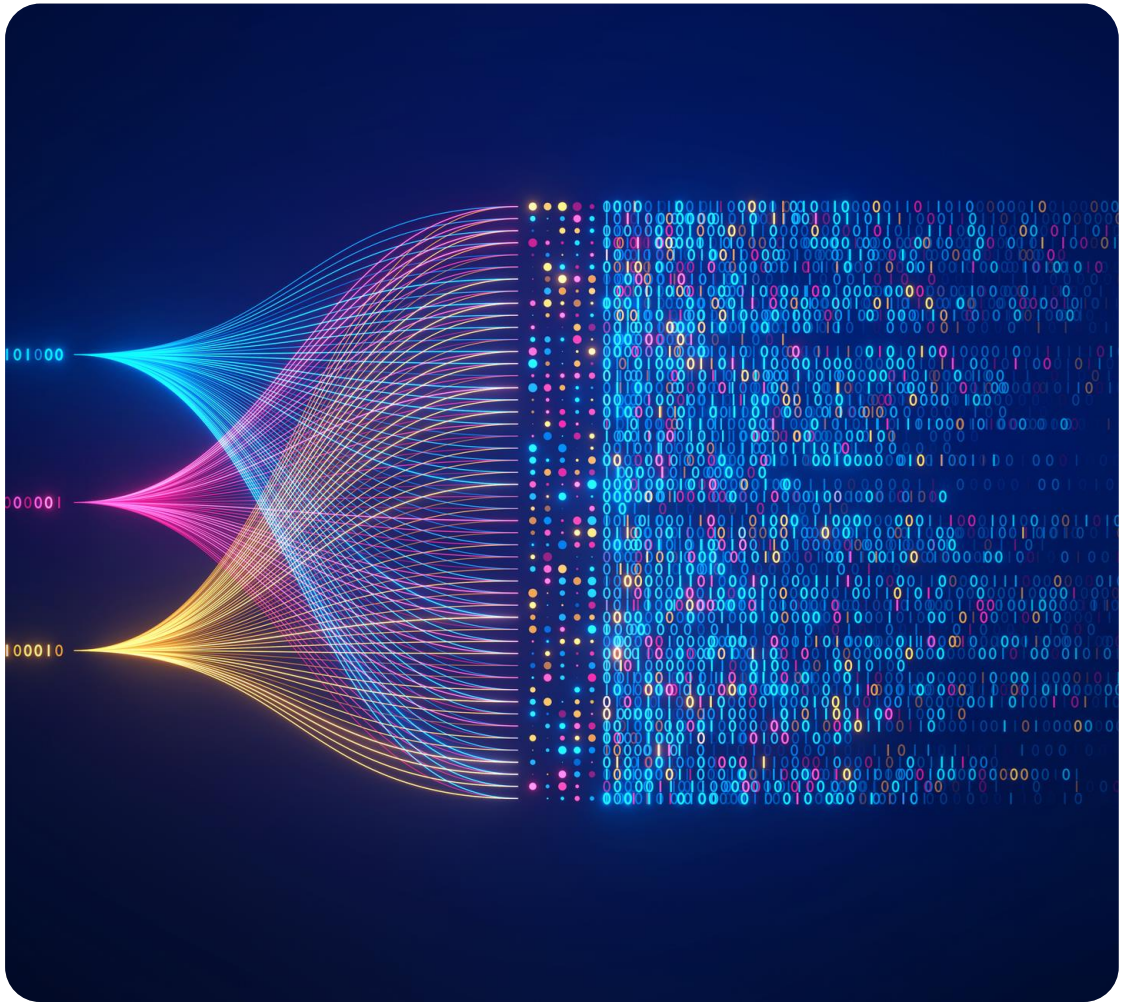
PBMC-L > CD3+ population

Histogram plot

Active Caspase-3
Interval gate



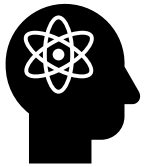
PBMC-L > CD3+ population



Sequencing data analysis using the BD Rhapsody™ Sequence Analysis Pipeline

- Get access to the BD Rhapsody™ Sequence Analysis Pipeline on the Seven Bridges Genomics Platform or on a local installation
- Acquire the AbSeq reference file (.fasta) from the BD AbSeq Panel Generator
- Set up analysis following the *BD® Single-Cell Multiomics Analysis Setup User Guide* (23-21333)

Supporting you with your single-cell experiments



Getting help from single-cell experts

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