

BD Rhapsody™ Single-Cell Multi-Omics

Find The Impossible!

High-dimensional biology for single-cell studies

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BD Biosciences
Snr Single Cell Solutions Architect



Outline

- Introduction
- BD Rhapsody technology & multi-omics workflow
- Performance & reproducibility
- Applications
- Use case:
 - *Resolving the elusive and controversial identity of innate lymphoid cells*



BD Cell Sorter

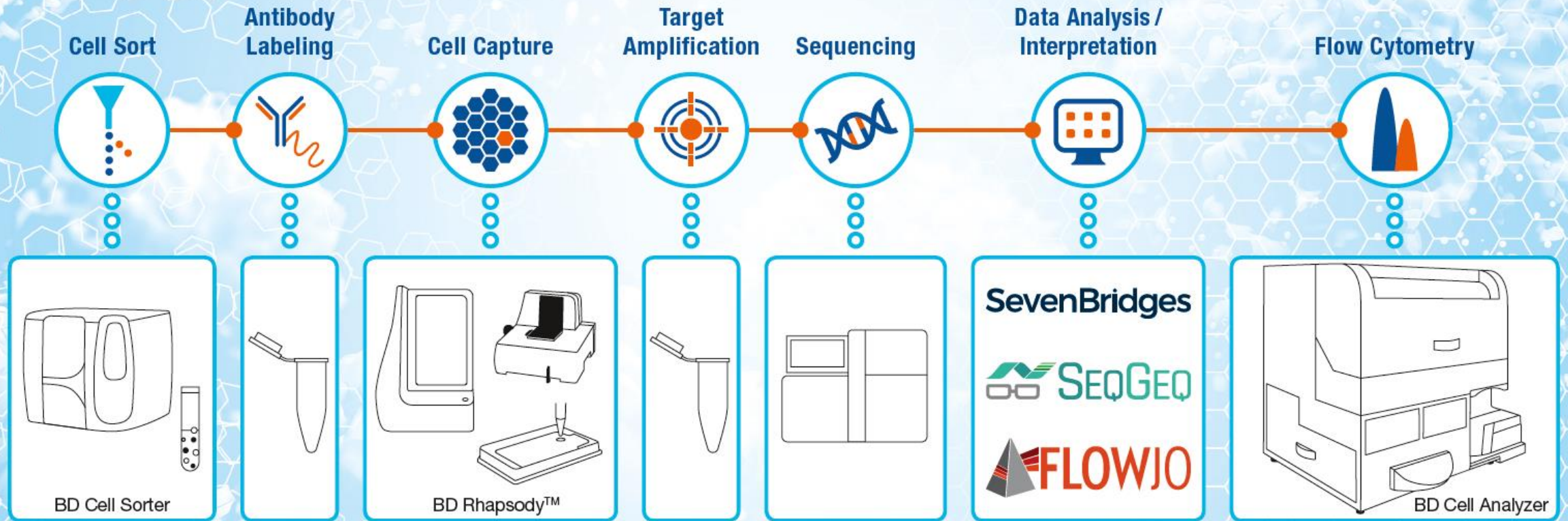
&

BD Rhapsody™

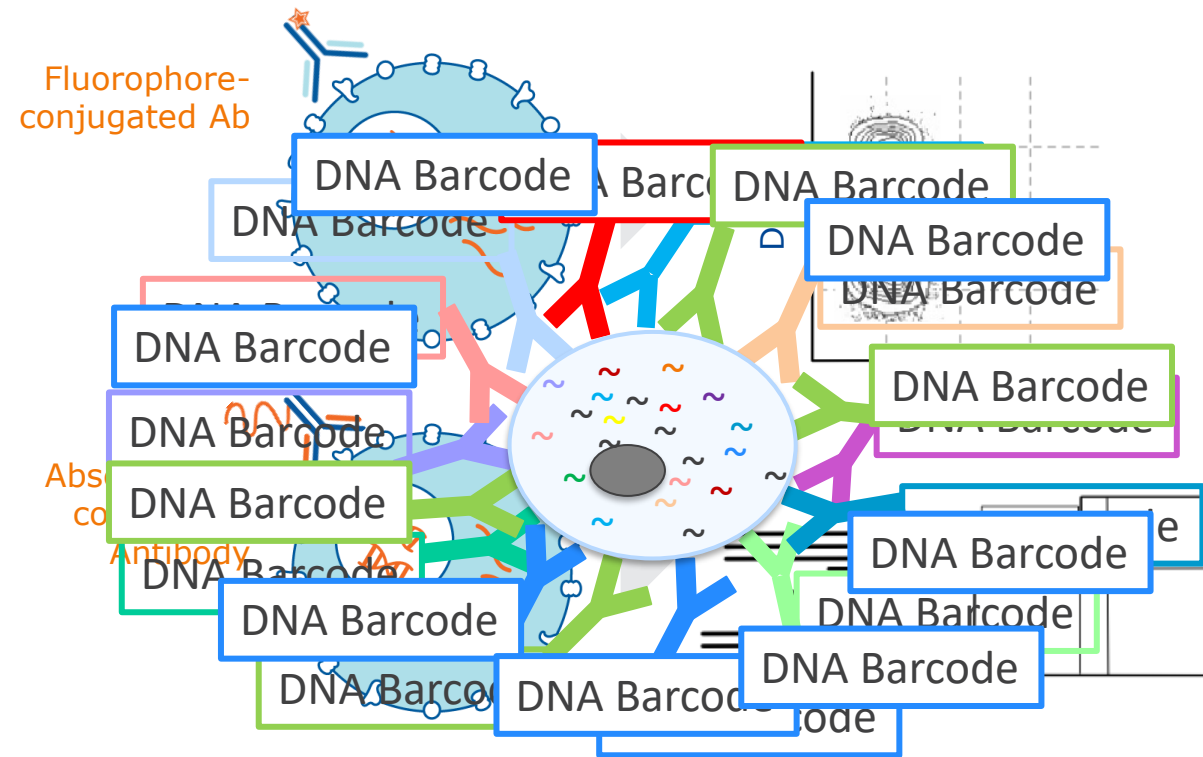
&

BD Cell Analyzer

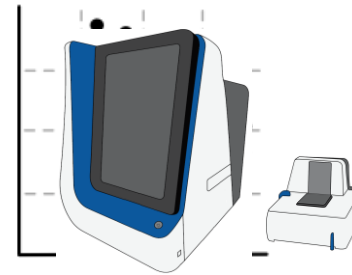
The complete BD Single Cell Workflow



Antibody sequencing readout using conjugated oligonucleotide barcodes



- Expression analysis of
- 100s AbSeq Cell surface markers
 - Upto 20 000+ genes

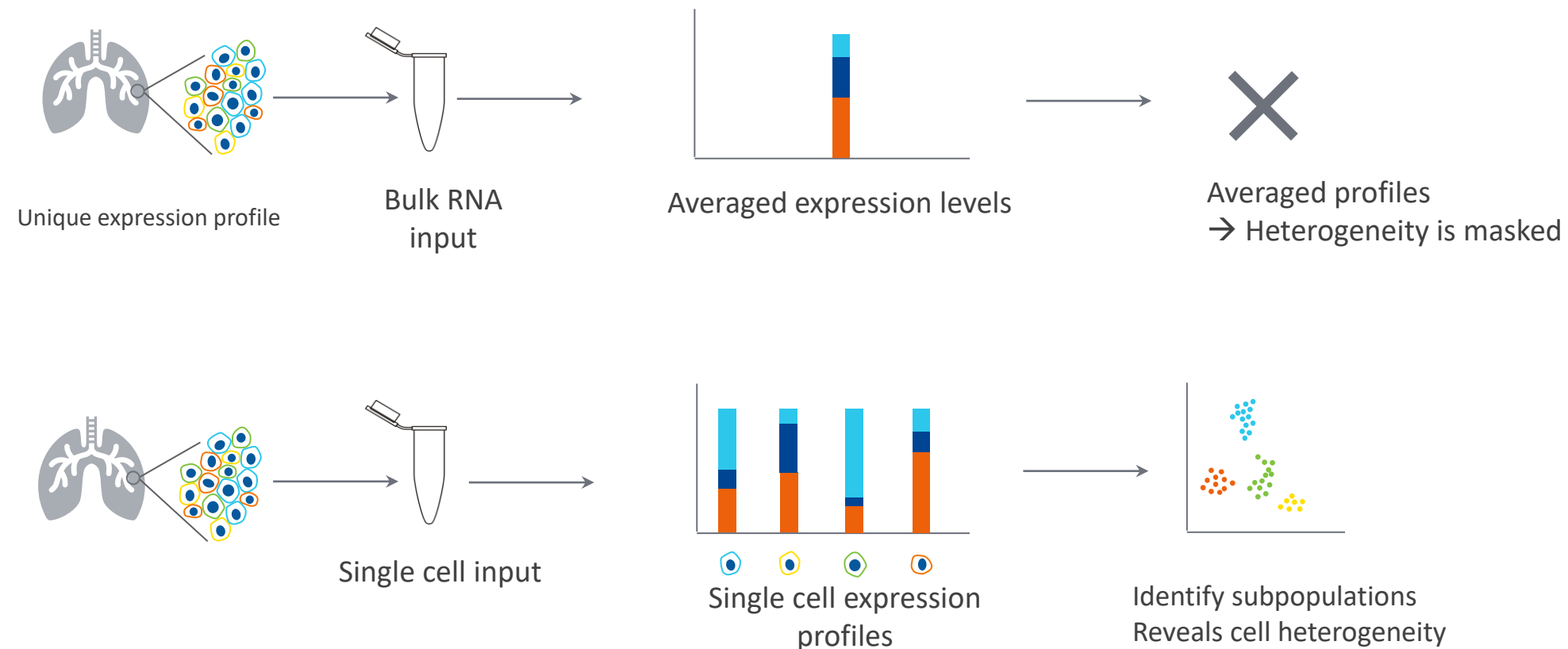


BD Rhapsody™
Single Cell Analysis System

**High parameter
multi-dimensional
data**



Why Single Cell analysis – Reveals differential expression

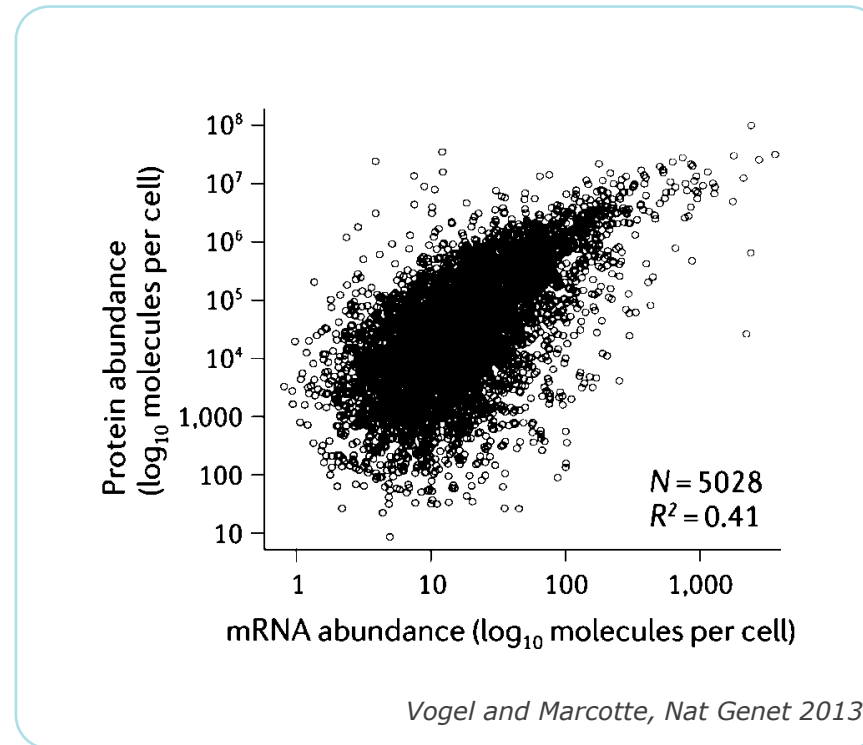


Resolve cellular heterogeneity



RNA & Protein (multi-omics) – Why both

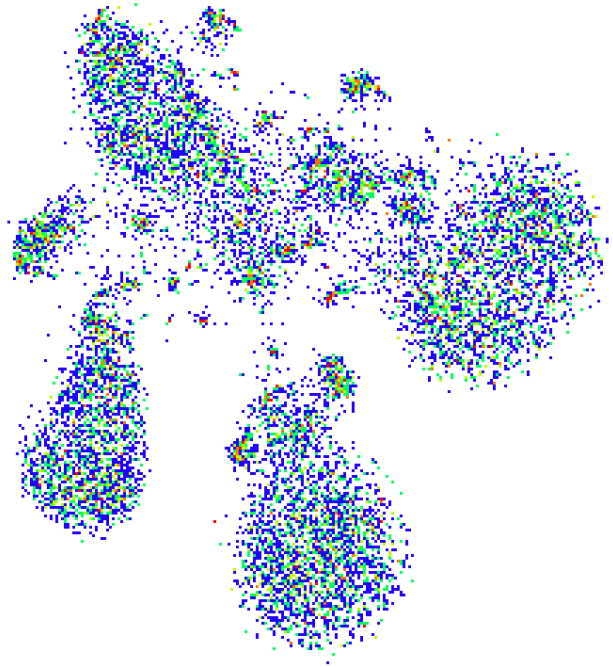
**mRNA/protein expression
not always correlated**



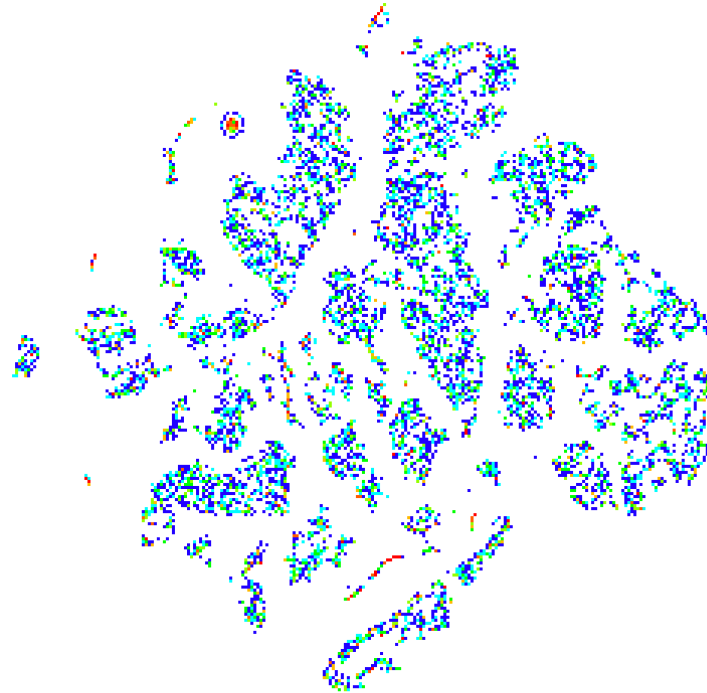
Resolve cellular heterogeneity: See the full picture



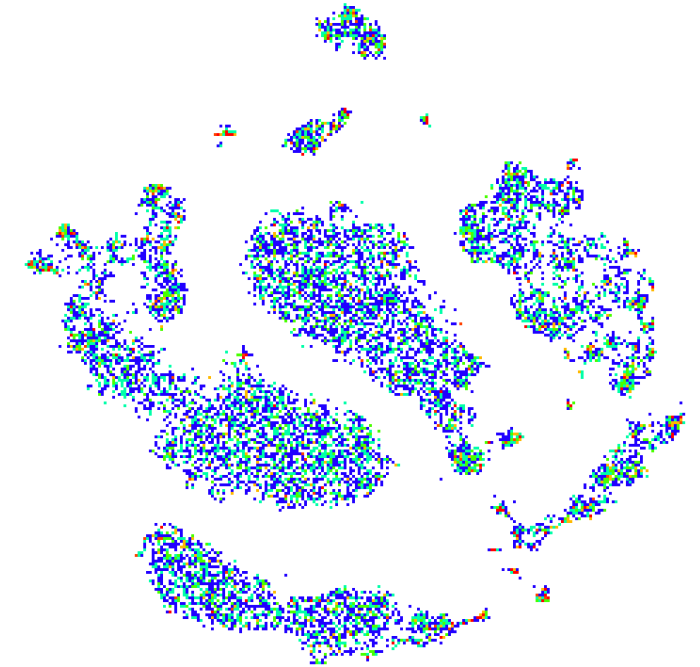
The combination of AbSeq with gene expression analysis improves cell clustering



tSNE: Ab Alone



tSNE: mRNA Alone



tSNE: Abs + mRNA

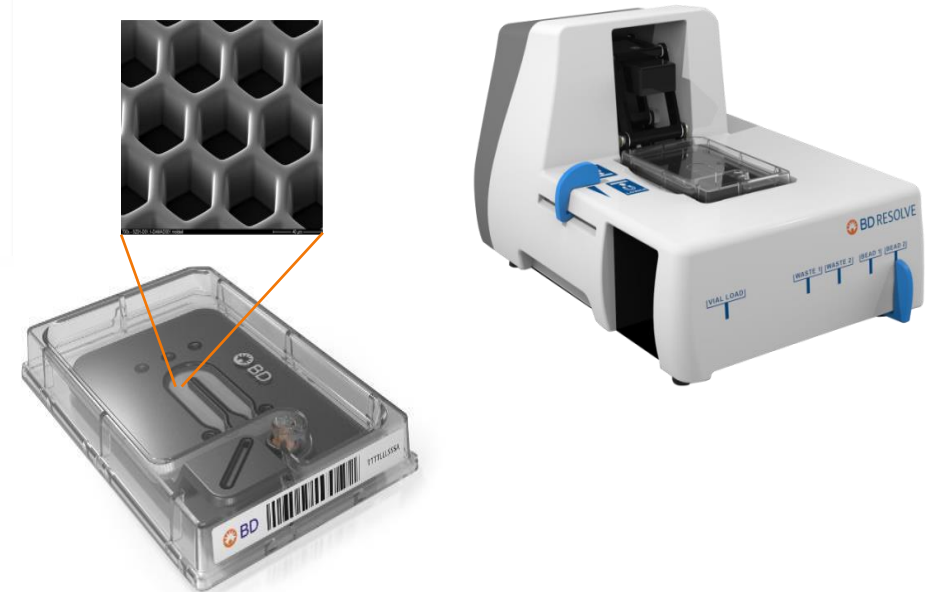
30 AbSeq Cell Surface markers and 399 Genes

Why high parameter multidimensional single cell analysis

- Uncover novel cell populations
- Identify biomarkers
- Discover biological insights



BD Rhapsody Multiomics : The workflow components

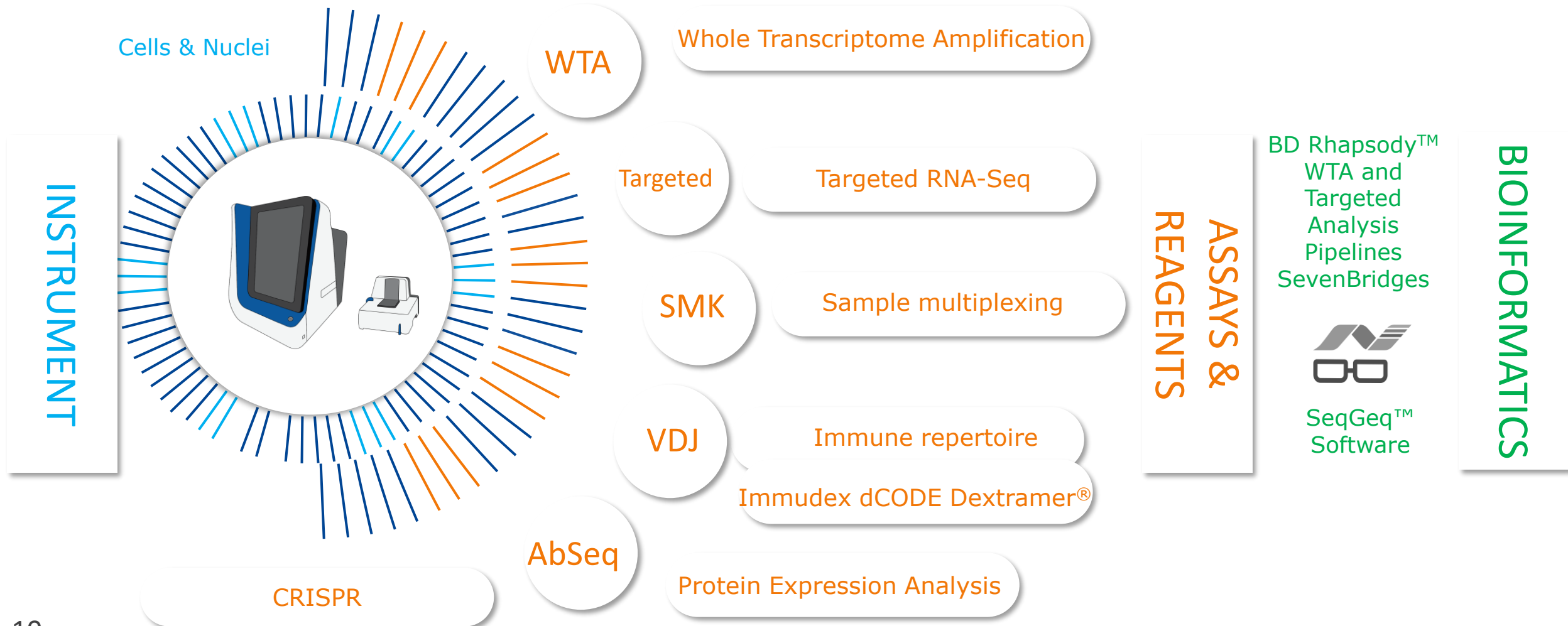


A complete single-cell multiomics offering

BD Rhapsody™ Single-Cell Analysis System

BD Rhapsody™ Assays & Reagents

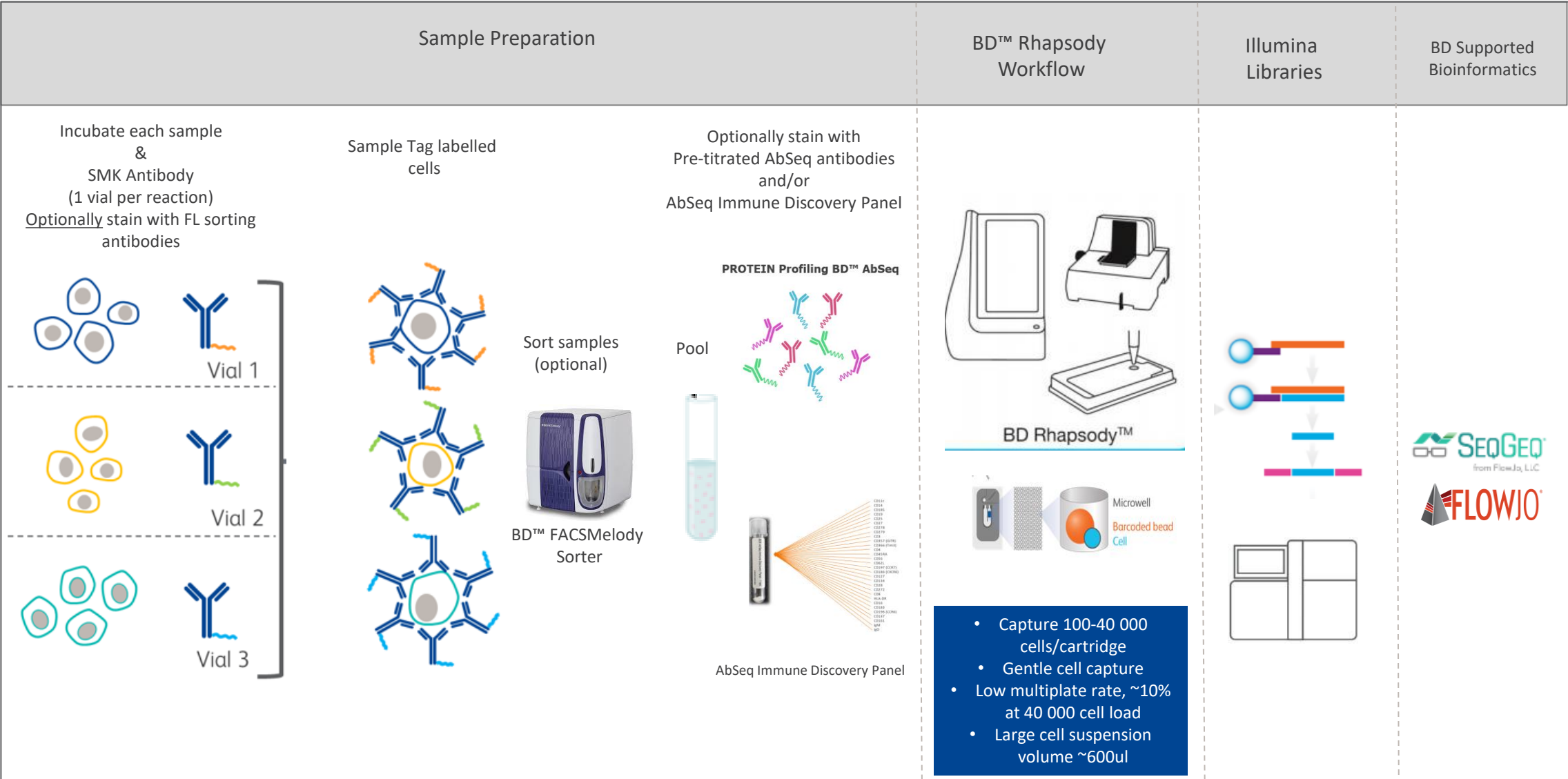
BD Rhapsody™ Analysis Pipelines & SeqGeq™ Software



BD Rhapsody Workflow

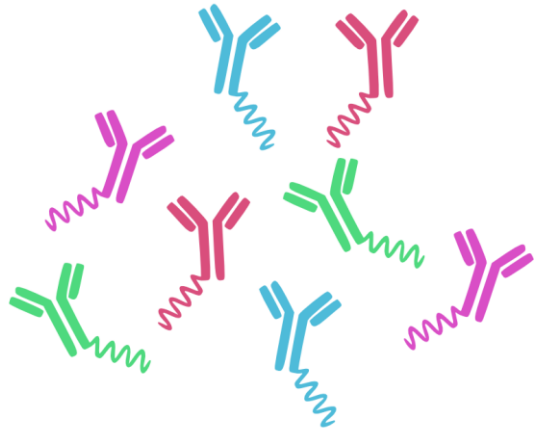


BD Rhapsody workflow - overview

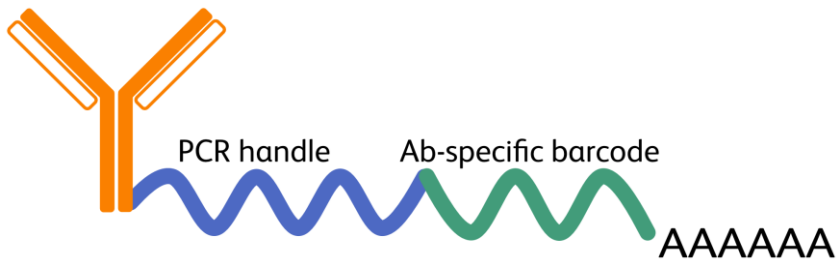


BD's oligo-conjugated antibody technologies

PROTEIN Profiling BD™ AbSeq

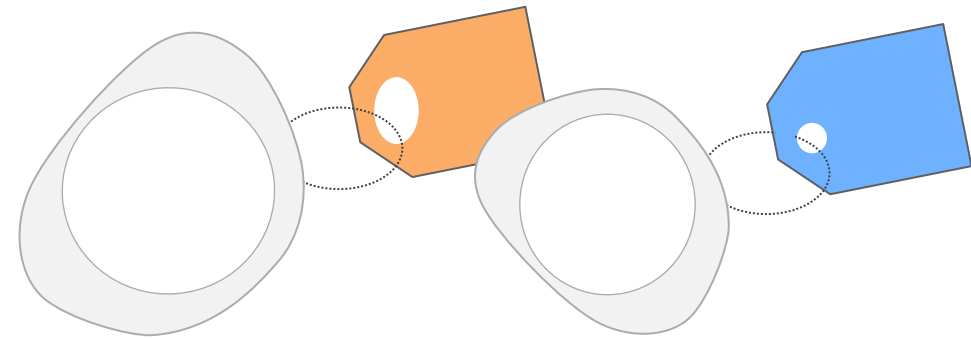


- ✓ Unique barcode conjugated to a variety of antibodies from BD's portfolio
- ✓ Pre-titrated (25 tests/vial)-no optimization
- ✓ Pharmingen Flow Cytometry portfolio

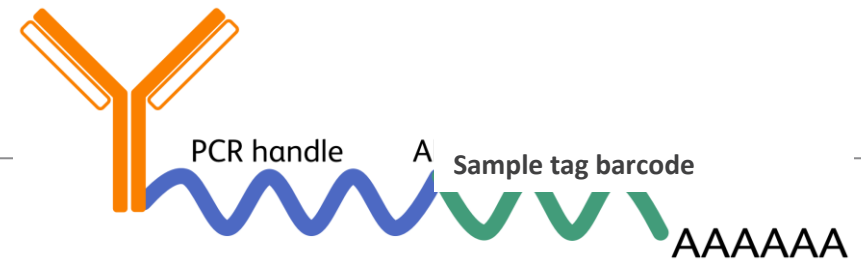


BD™ Single-Cell Multiplexing Kits Hu/Mm

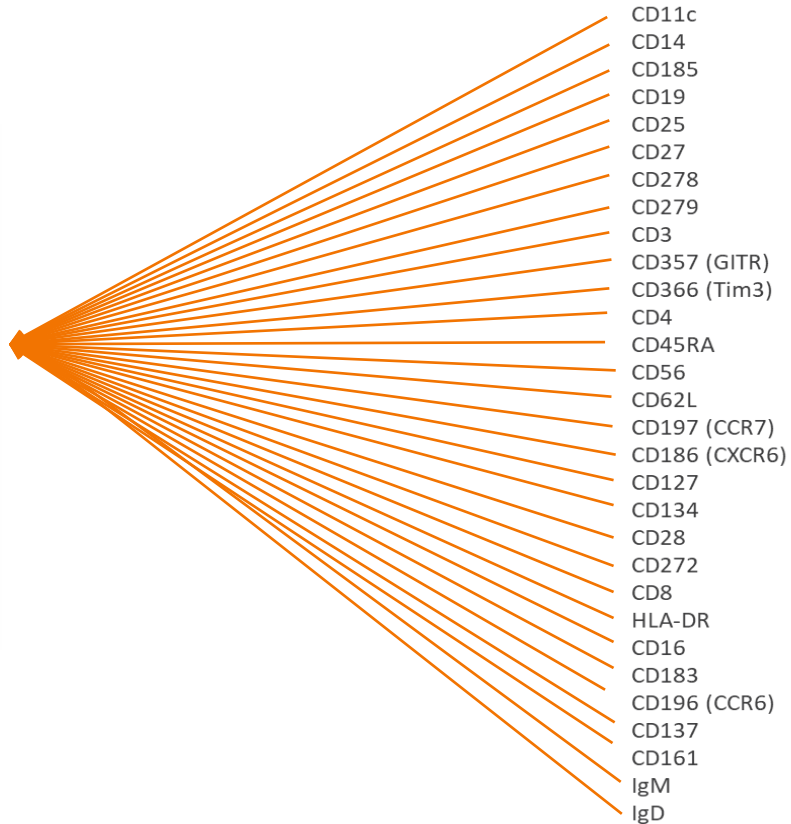
12 sample-specific tags
Process multiple samples in a cartridge



- Human: universal antibody
 - Mouse CD45
 - Mouse MHC-I



BD[®] AbSeq Immune Discovery Panel, Human

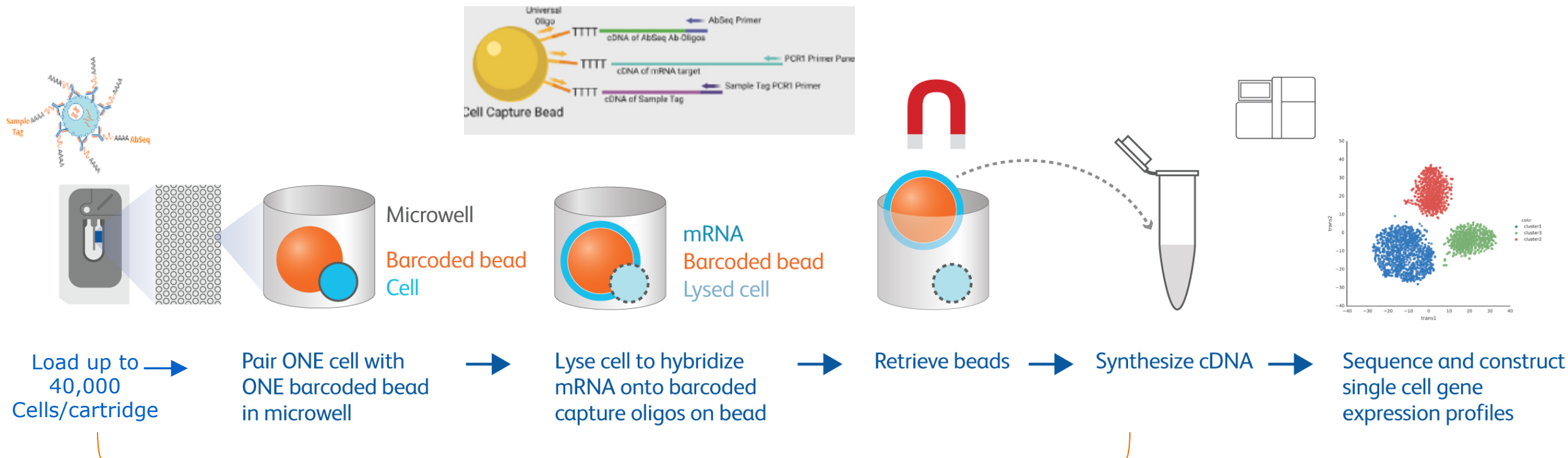


Achieve high parameter profiling

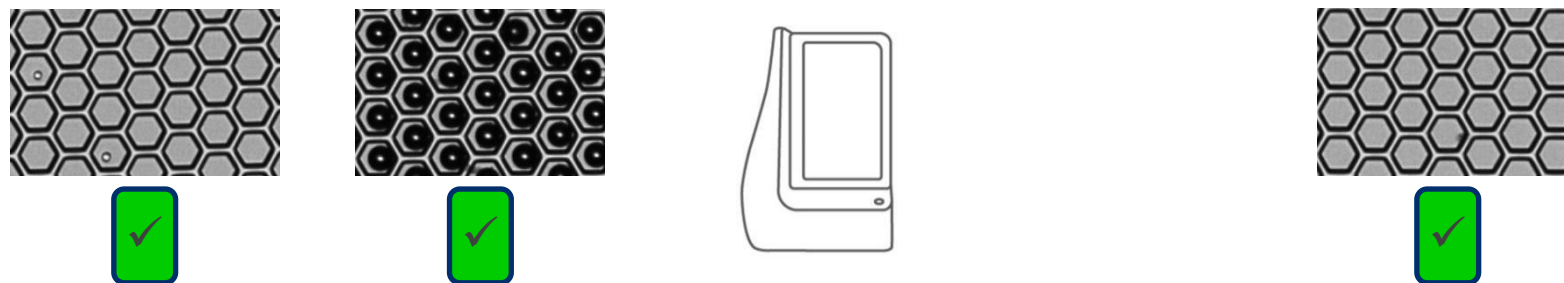
- 30 BD[®] AbSeq Antibodies against major immune markers
- Lyophilized into a single tube (5 tests/vial)
- Validated against flow and individual AbSeq of the same clone
- Tested to work on the BD Rhapsody[™] System
- Add drop-ins



BD Rhapsody Cartridge workflow-



Monitor and QC

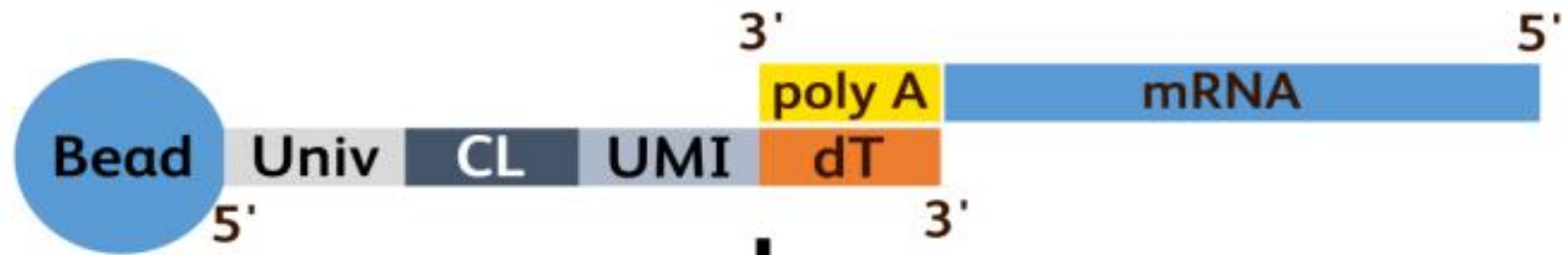


For Research use only.



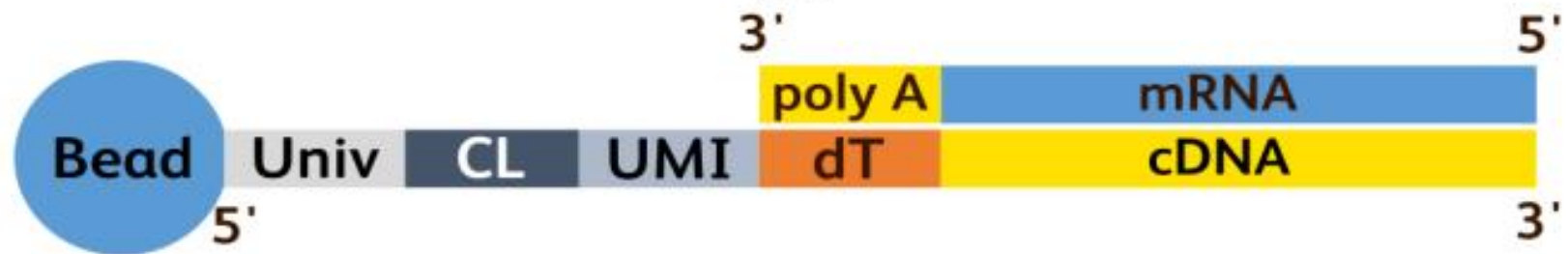
cDNA Synthesis on bead

Beads with hybridized mRNA retrieved from cartridge



Reverse transcription

cDNA archived on bead and tagged with CL and UMI



Superior capture rate for various cell types

Cell type	Tissue	Sample prep	Live cells loaded to cartridge*	Cartridge Capture rate**
CAR-T cells	Blood	Cell in-house manufacturing	25,000	72%
Mesenchymal stem cells (MSC)	Fatty tissue	Frozen, ON grown and trypsinised cells	25,000	73%
Tumor xenograft	Head and neck cancer	Dissociated tumor on Mice (PDX - paw)	25,000	67%
Total CD4+ T cells	Blood	Magnetic isolation (negative selection of total CD4 T cells)	25,000	74%
CD45+ immune cells	Duodenal biopsies	FACS sorted	25,000	68%
FACS sorted NK and T cell subsets	Blood	FACS sorted	15,000	66%
MSC (cryopreserved)	Cell lines	Cryopreserved	11,443	80%
iPSC, Adipocyte (primary fresh), GABA Neurons (cell line), Hepatocytes (primary)	Primary cells/Cell lines	Live/Cryopreserved	22,885	60%
Myeloma cell lines	Cell lines	Flask grown	11,000	73%

*Based on hemocytometer cell counts

**Capture rate = # of wells with viable cells and a bead/live cells loaded to cartridge
of wells with viable cells and a bead is good estimation of the number of cells that could be recovered in sequencing

High correlation between cell types seen with the BD Rhapsody™ System and those seen using flow cytometry

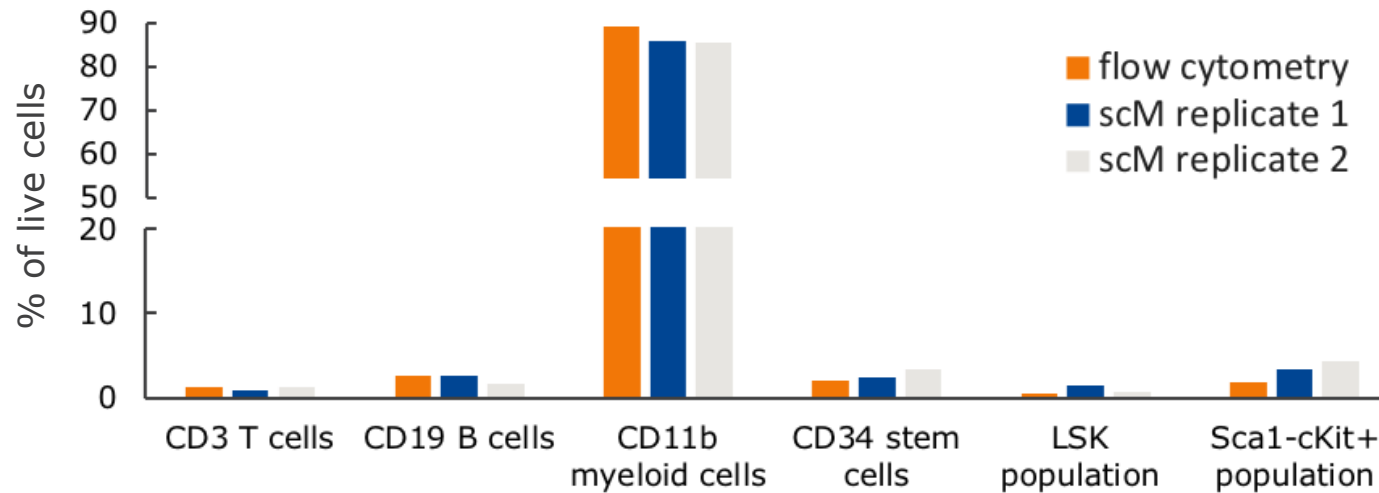
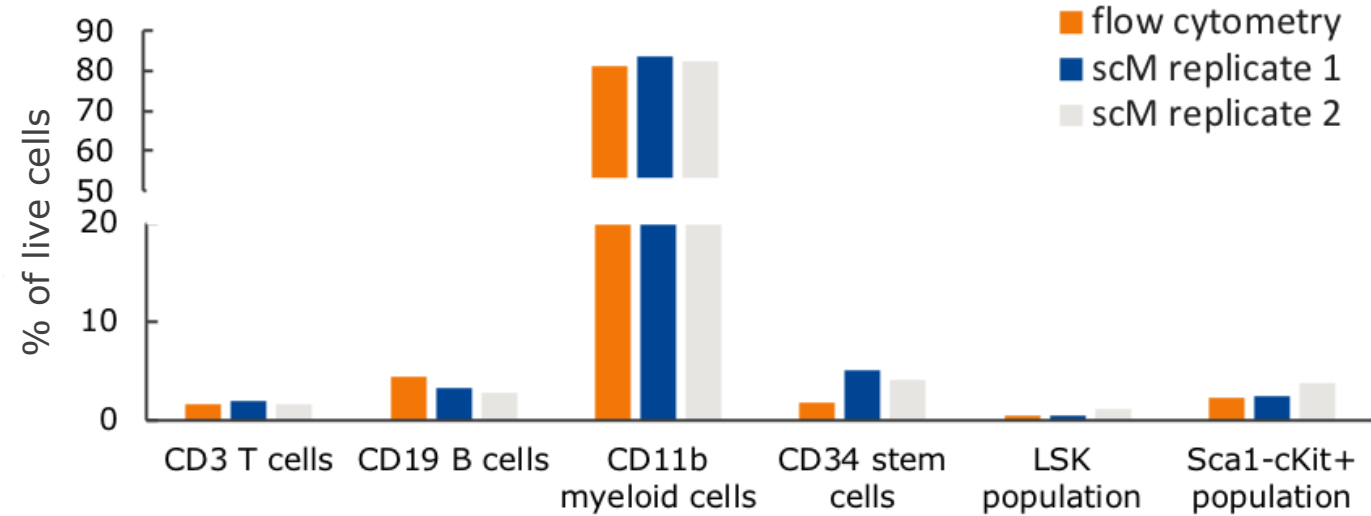


Mouse 1

Bone marrow extracted

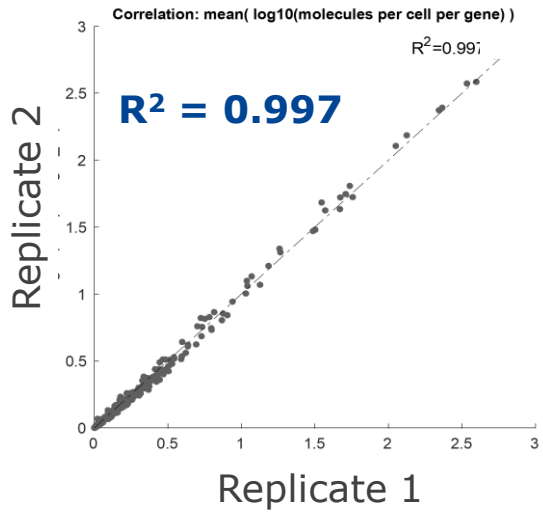


Mouse 2

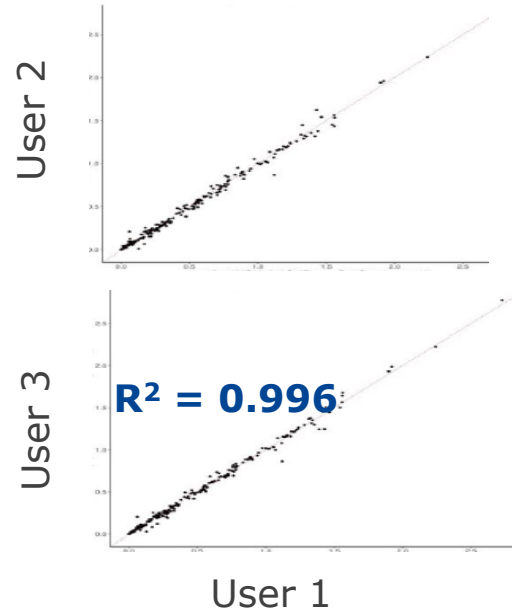


Consistent performance with pre-formed microwell partitions

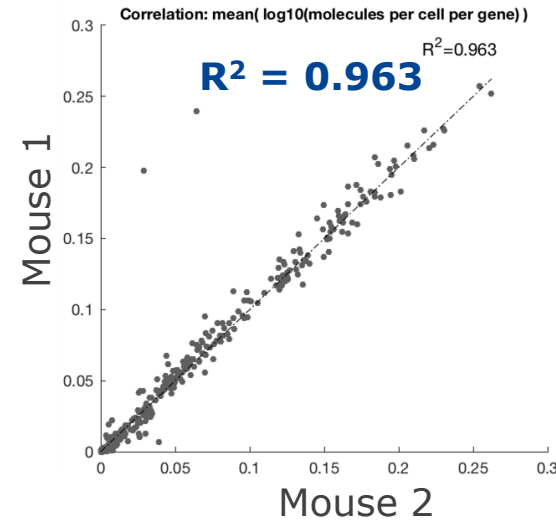
Technical replicates



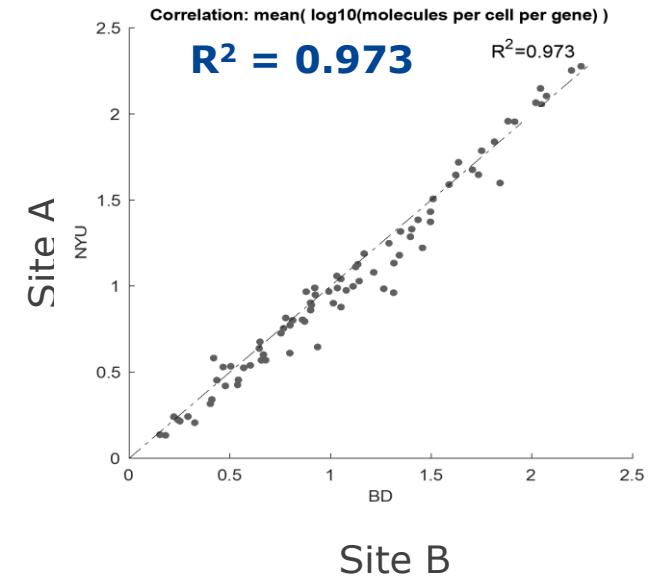
User-to-user



Biological replicates



Site-to-site



Scanner for workflow QC

No need to fly blind, confidence with every experiment



Visual Workflow QC with Every Step



Estimate Cells Expected from Sequencing



See Before You Commit to Sequencing budget



Troubleshooting and Optimization



Metrics before loading cell in cartridge

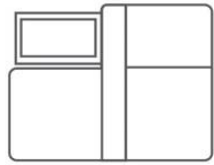
- Cell concentration of sample
- % Viable cells

Metrics during the cartridge workflow

- # of captured single cells with bead
- # of multiplets
- Bead loading
- Bead retrieval

Number of viable cells captured with a bead, 6,549
Cell multiplet rate, 1.58%
Bead loading efficiency, 94.85%
Bead loading acceptance PASS
Excess bead rate, 0.00%
Excess bead acceptance, PASS
Cell retention rate, 94.23%
Cell retention acceptance, PASS
Bead retrieval number of titles process, 26
Bead retrieval efficiency, 95.51%
Bead retrieval acceptance, PASS

BD Rhapsody Bioinformatics



illumina®

FastQ

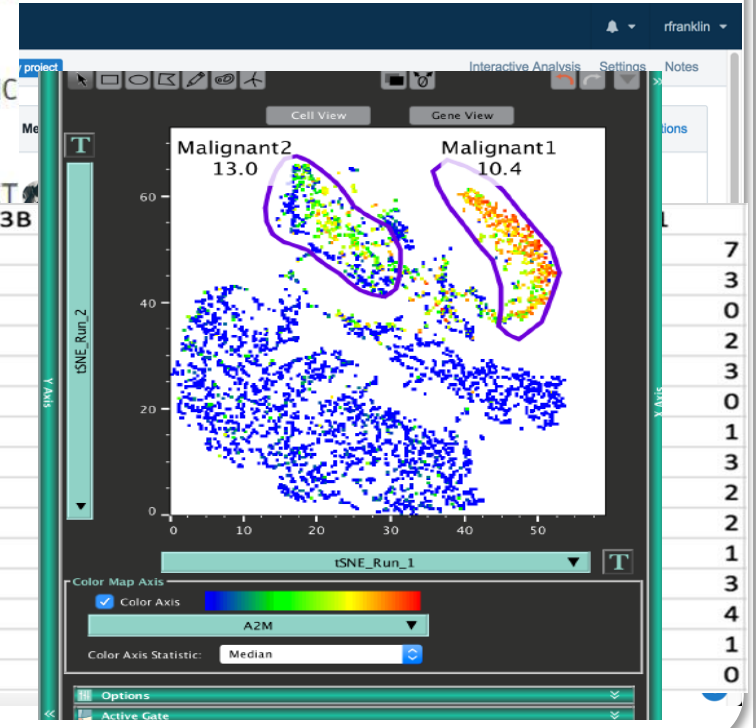
```

--->gzip -cd L21_s1
@M00805:5:000000000
NTTGTATCAGCTGAAGATGAAATAGGATGTAACAGGAAGCAGATTTTGCTAAT
TTGGAAGTACAGCTGAGGATCCTGTGAGCGAAGTTCCGGCAGTGTCACAGCAC
+
#55<<?BBDBDDDDDDFFFFFHFFFFFFHFAFHFFFFFFHFBHHHHHFFHHHHHHHDGDGHC
AFHFFFFFFHFGHDDHFBHDFHFFHFFHFFFA=@BEEEEED)@<B?BE3==?EEEE
@M00805:5:000000000-A0VLL:1:1101:15023:1321 1:N:0:1
NAGAAATCACAGACATACAAAGCAGTCTGTGTCCTTAGGTCCTGAGCAGCCTCCAGCACATTCT
AGCATCTGCCGTCACATT
+
#55???BBDEDDDDDDGG
HHHHHHHHHHHHHHHHHH
@M00805:5:000000000
NTTTCGTGGAAGTGGGTT
ACATGAGTGCCTCTCTTT
    
```



csv File

Cell_Index	ABCB1	ABCG2_ALT1	ACTA2	ACTR3B
52803	0	0	1	
718930	0	0	3	
160403	0	0	59	
720136	0	0	1	
727124	0	0	1	
815380	0	0	70	
257971	0	0	83	
46714	0	0	12	
825447	0	0	0	
443121	0	0	0	
493478	0	0	4	
315769	0	0	1	
543894	0	0	3	
580637	0	0	56	
398887	0	0	42	



Secondary analysis



 v1.3 published July 09, 2019 A clustering algorithm that uses K-Means density estimation.	 v1.0.1 published December 3rd, 2020 Using K-means clustering to identify populations.	 v1.5.0 published January 09, 2021 ClusterExplorer illustrates a profile of relative intensity values across parameters in flow cytometry data.
 v0.4.2 published October 3rd, 2019 Automatically Find the Shortest Gating Strategy for a Population of Interest.	 v2.3 published May 18th, 2020 Automated cleaning of flow data.	 v1.1 published October 13th, 2020 Helps correct for technical variability within flows by normalizing batches of flow data.
 v1.1 published April 14th, 2020 Embed FlowSOM maps into 2D space.	 v0.7 published September 15th, 2020 Automatically gate wells from BD indexed sort data.	 v1.0 published May 30th, 2019 Calibrate samples using a standard curve.
 v1.0.0 published March 4th, 2021 Calculate Stain Index in the click of a button.	 v1.0 published June 4th, 2019 Auto-gate wells from index sorting.	

 v1.3 published March 2nd, 2020 Subset your sample in a specified event count.	 v2.0 published November 20th, 2020 Cluster using Self-Organizing Maps.	 v1.1 published March 24th, 2020 A dimensionality reduction technique similar to t-SNE.	 v1.0 published September 23rd, 2020 Delineate clusters by unsupervised nearest-neighbors grouping of biological data.
 v0.5.1 published December 7th, 2020 Fast Fourier Transform-accelerated interpolation-based t-SNE.	 v1.1 published February 3rd, 2021 Generate violin plots and box and whisker plots.	 v1.1.1 published December 7th, 2020 Create interactive 3D plots, DimReduce, t-SNE, and Clustering.	 v0.6 published December 7th, 2020 This Wizard utility helps install and setup plugins for FlowJo and SeqGeq.
 v0.1 published February 09th, 2020 Dimensionality reduction, analogous to t-SNE or UMAP. This algorithm is used as visualization for high-dimensional data.	 v0.1 published April 30th, 2020 Population hierarchies in an R-based Shiny App.	 v0.1 published September 09th, 2020 Visualize population hierarchies and regression paths across those hierarchies.	 v0.6.1 published December 3rd, 2020 Automatically gate categorical parameters.
 v0.8 published October 16th, 2020 Measure the quality of clustering in n-dimensional space using two statistical methods: ClustCheck and Taylor.			

 v4.1.1 published June 17th, 2019 Pseudotime project and scRNA-Seq analysis.	 v2.0 published March 20th, 2020 Single-Cell Immune Repertoire Profiling.
 v0.9 published June 4th, 2020 Correct for batch effects between combined samples directly within SeqGeq.	 v1.1 published November 16th, 2020 A utility tool for demultiplexing samples and virtual in-tube things.
 v1.0.0 published May 30th, 2019 Impute the dropout values in scRNA-seq data.	 v1.0 published May 20th, 2019 Dropout imputation using a low-rank approximation.
 v3.0.1 published March 1st, 2021 Seurat pipeline developed by the Satija Lab.	 v1.5 published May 27th, 2019 Deep-learning Cell Classification.



BD Rhapsody workflow summary



Microwell technology

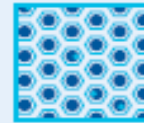
- No sample loss due to clogging of channels
- No electronics, portable
- 575 μ L cells suspension loading volume



Visual workflow QC

Confidence with every experiment

Up to **80%** cartridge capture rate
for certain cell types



Low multiplet rate

2–3% @ 10,000 cell load

8–10% @ 40,000 cell load



Broad range of cell throughput

100–40,000 cells per cartridge

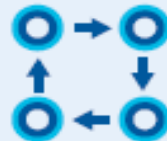


Capture and analyze fragile cells

Granulocytes, neutrophils, CAR-T cells, stem cells, tumor xenograft-derived cells, myeloma, T cells, NK cells and more

Minimal batch effects*

Consistent, reliable results with technical, biological, site-to-site and user-to-user replicates



High correlation with flow data

The same trusted BD antibodies for flow and single-cell multiomics



Subsample beads

Flexibility with experimental design, tool to measure reliability, work with collaborators



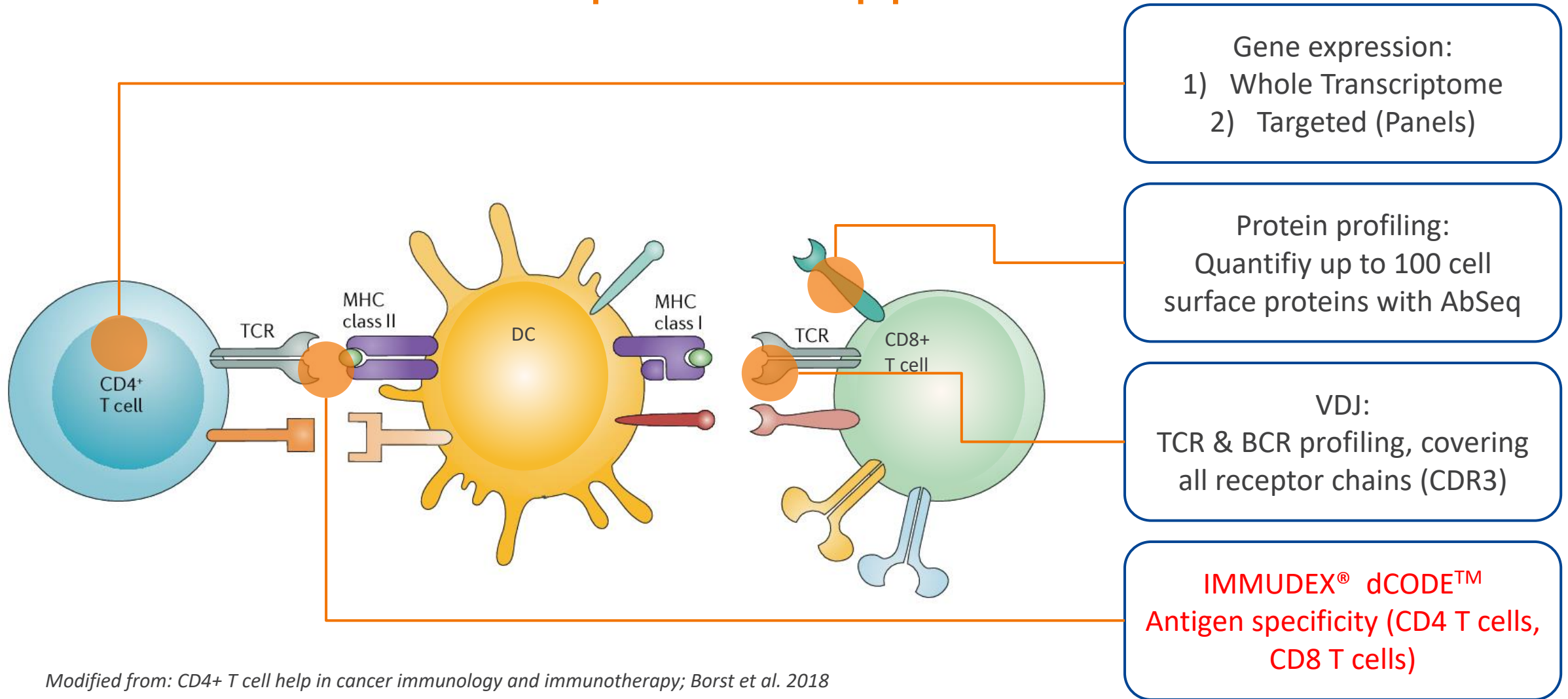
Archive beads

Equivalent data obtained from fresh beads and beads stored for several months

*Results may vary based on sample type and experimental conditions.



Expanded Applications



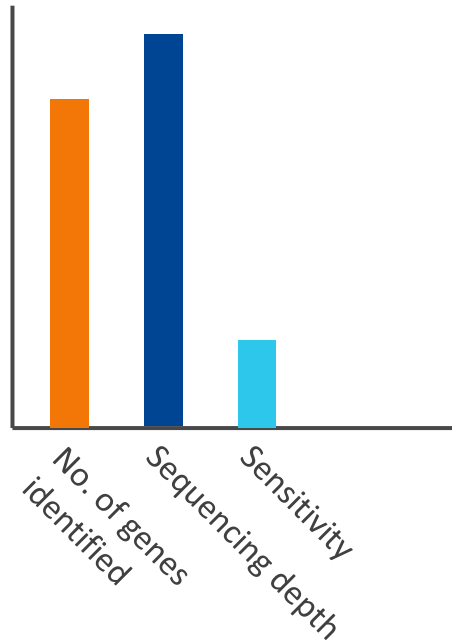
Modified from: CD4+ T cell help in cancer immunology and immunotherapy; Borst et al. 2018



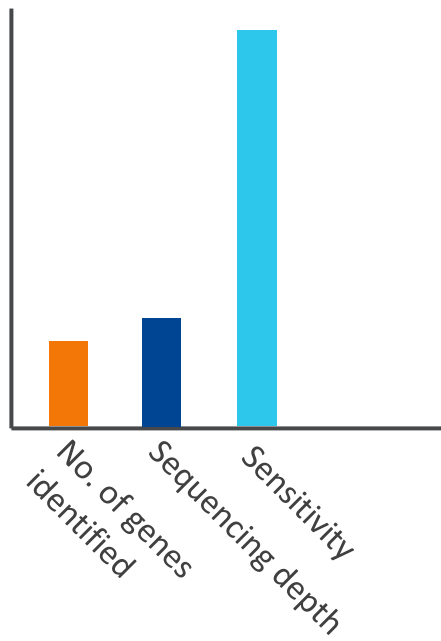
Gene expression profiling - Rhapsody Strategy



Exploratory:
WTA



Focused:
Targeted



Benefit

Increased sensitivity

Improved Resolution

Economy of Sequencing Budget

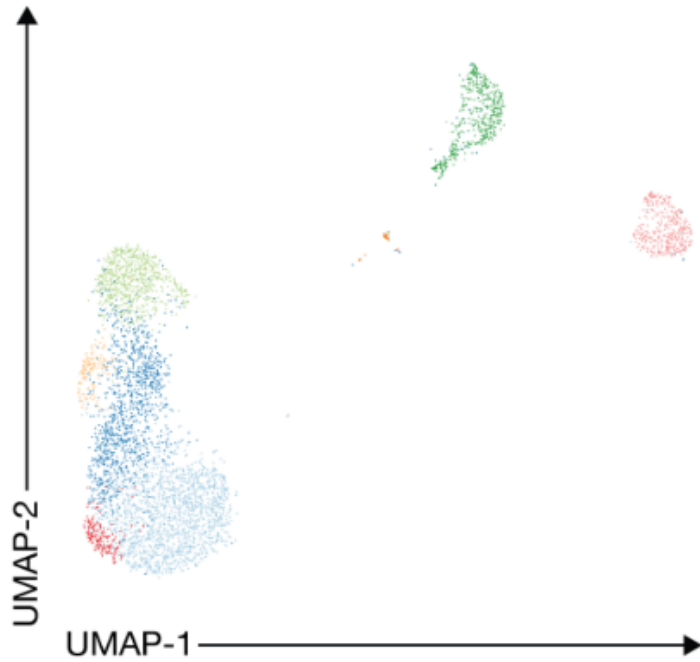
Simplified Analysis



Targeted RNAseq on the BD Rhapsody

WTA @ 50,000 reads/cell

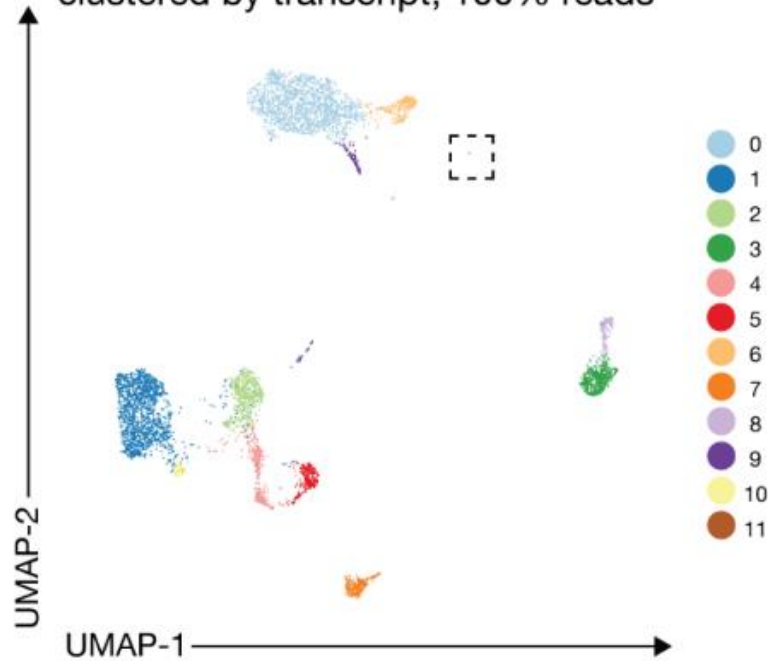
UMAP plot of bulk PBMCs clustered by transcript



- CD4⁺ T cells
- CD8⁺ T cells
- CD56⁺ NK cells
- CD14⁺ monocytes
- CD19⁺ B cells
- RP-high T cells
- gd T cells
- CD11c⁺ DCs
- unknown

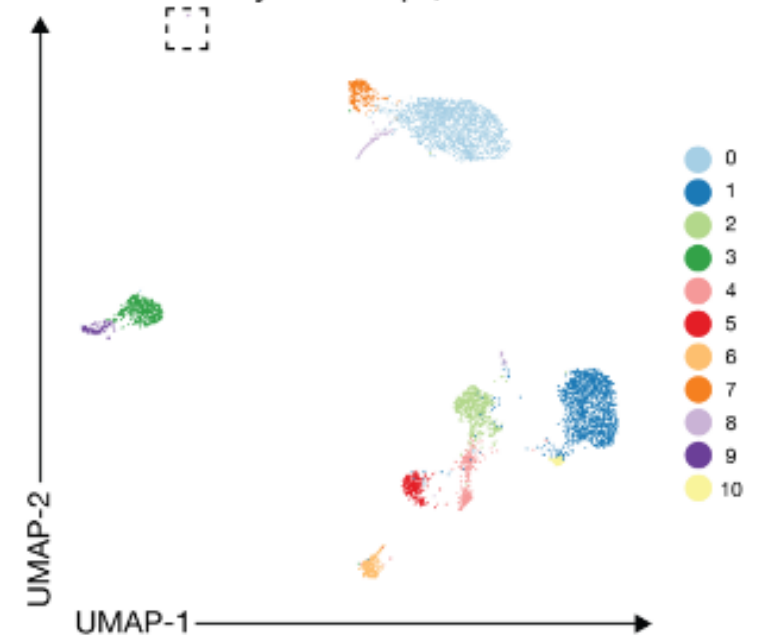
Targeted @ 10,000 reads/cell

UMAP plot of bulk PBMCs clustered by transcript, 100% reads



Targeted @ 2,000 reads/cell

UMAP plot of bulk PBMCs clustered by transcript, 20% reads



From Mair et al., Cell Reports 31; 2020



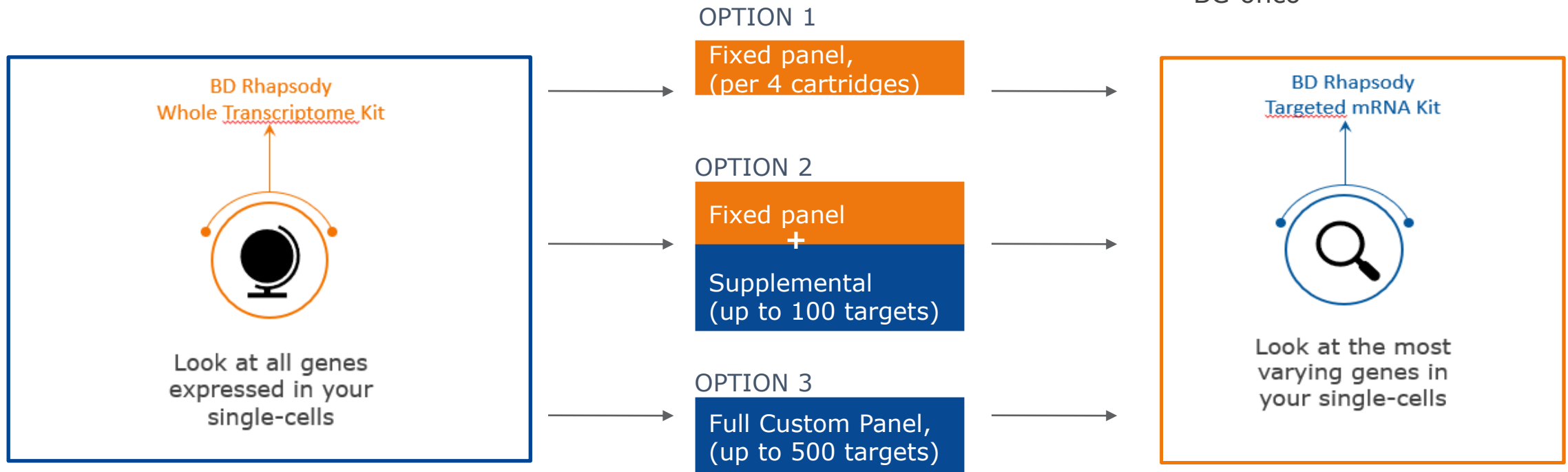
Targeted sequencing strategy

Discovery

- Small sample numbers
- High cost per experiment

Fixed panels

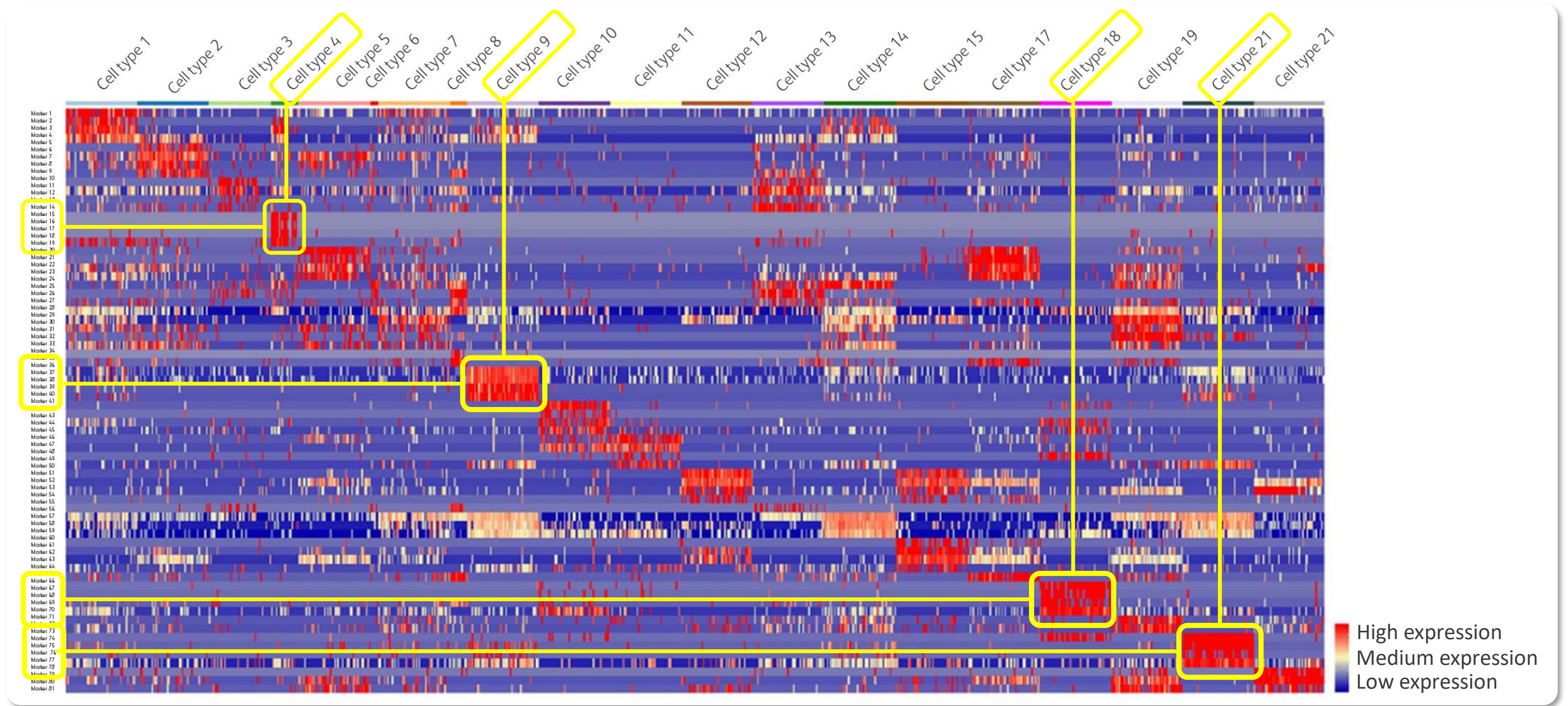
- [Immune Response, Hu](#)
- [Immune Response, Ms](#)
- [T-Cell Gene Expression](#)
- BC-onco



For Research use only.



The BD Rhapsody as a screening tool for Flow Cytometry

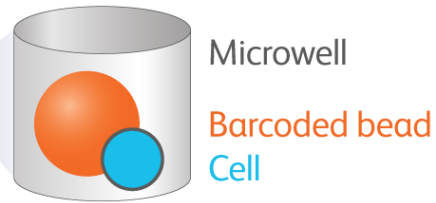
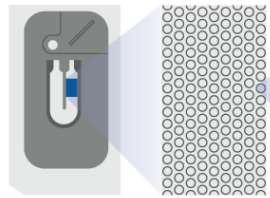
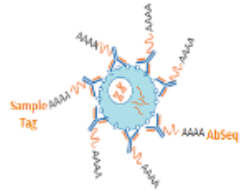


Rhapsody Applications

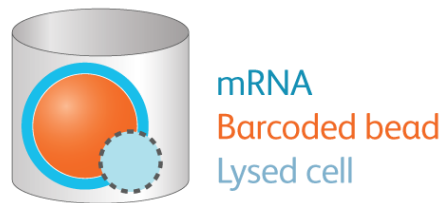
- *VDJ Clonotyping*



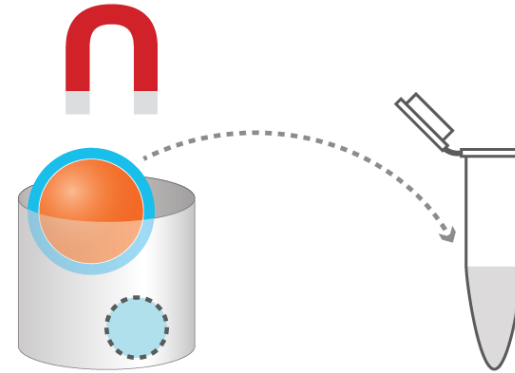
BD Rhapsody Cartridge workflow- VDJ CDR3 Clonotyping



Microwell
Barcoded bead
Cell



mRNA
Barcoded bead
Lysed cell



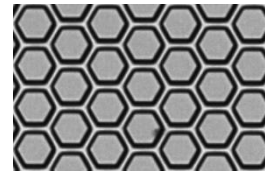
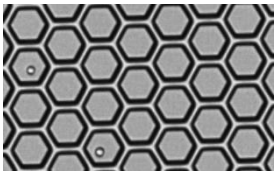
Load up to 20,000 cells

Pair ONE cell with ONE barcoded bead in microwell

Lyse cell to hybridize mRNA onto barcoded capture oligos on bead

Retrieve beads

Monitor and QC



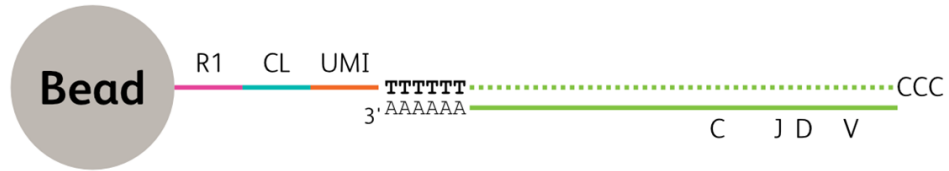
Decision point

For Research use only.



VDJ CDR3 Workflow - Targeted

cDNA synthesis

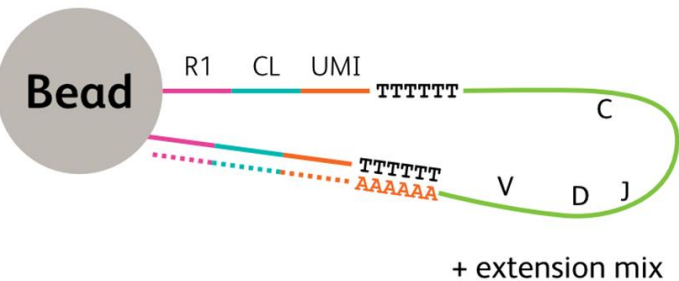


TSO:
GGGTTTTTT

Template switching

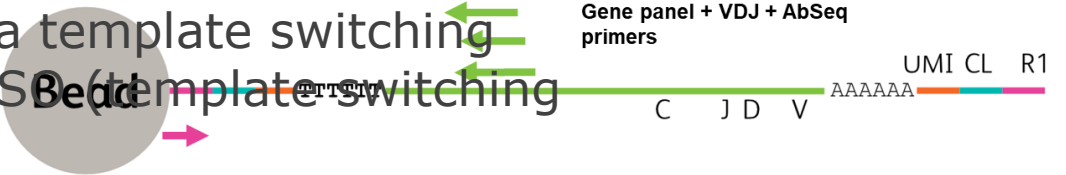


Hybridization and extension



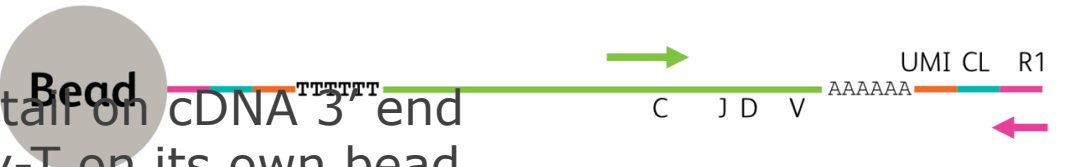
Highly multiplex targeted amplification with 3' targeted Immune Response Panel

New synthetic poly-A tail is added on cDNA 3' end via template switching with a poly-T TSO oligo (template switching oligo)



TCR and BCR enrichment and library prep

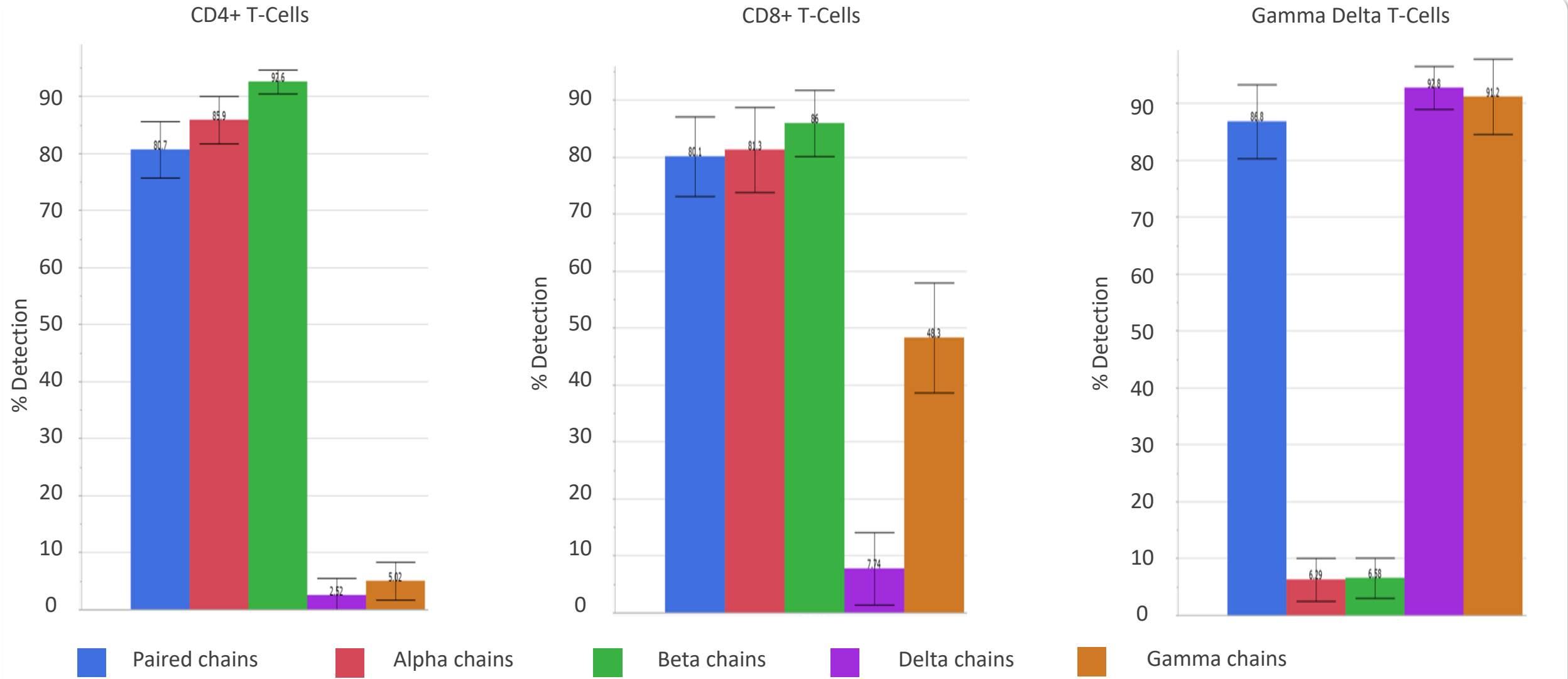
Synthetic poly-A tail on cDNA 3' end hybridizes to poly-T on its own bead.



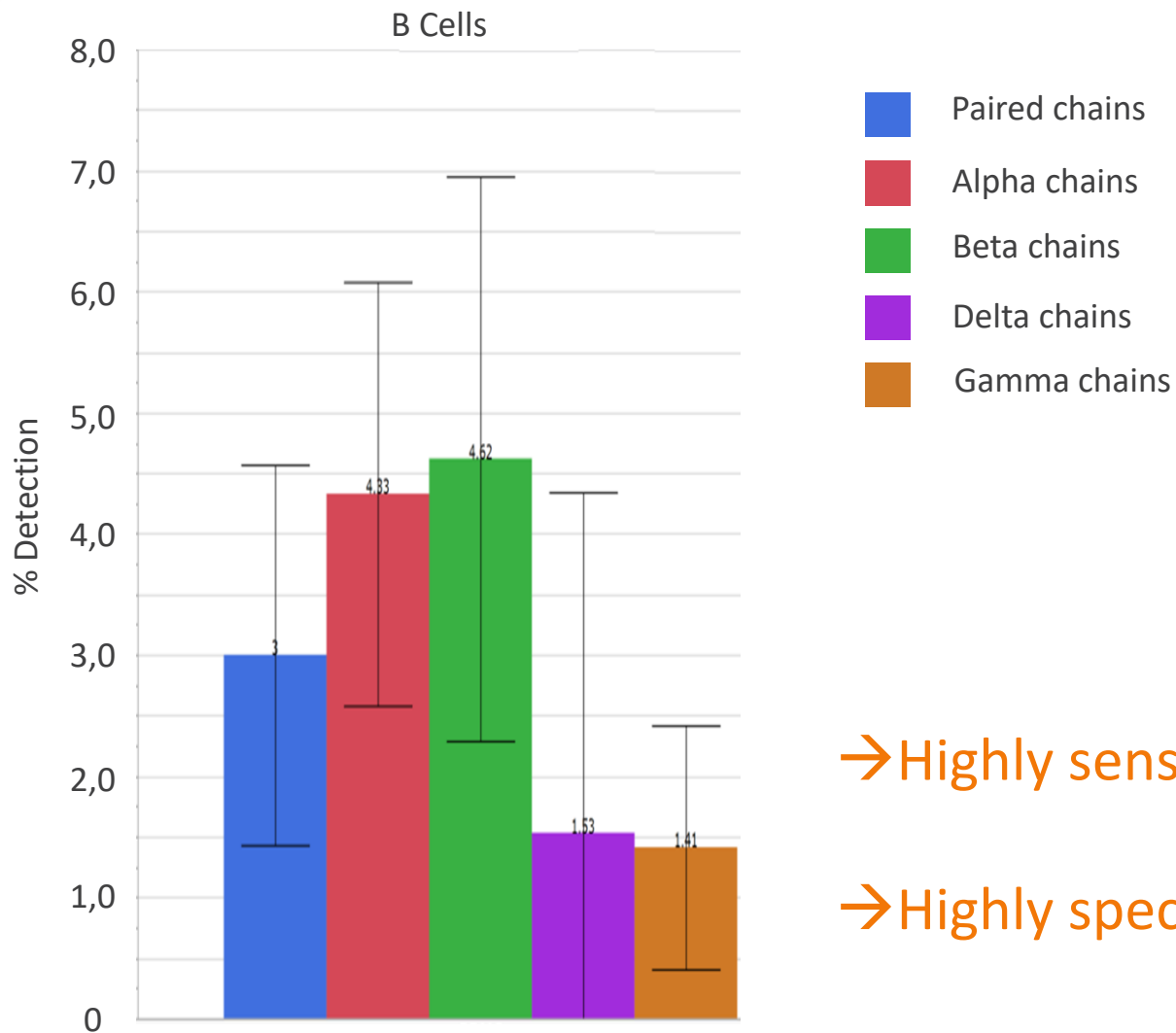
Addition of DNA polymerase and reagents allows copying of new barcodes to cDNA 5' end (equivalent to mRNA 5' end)



TCR assay: Sensitivity



TCR assay: Specificity (% non-T cells with TCR CDR3)

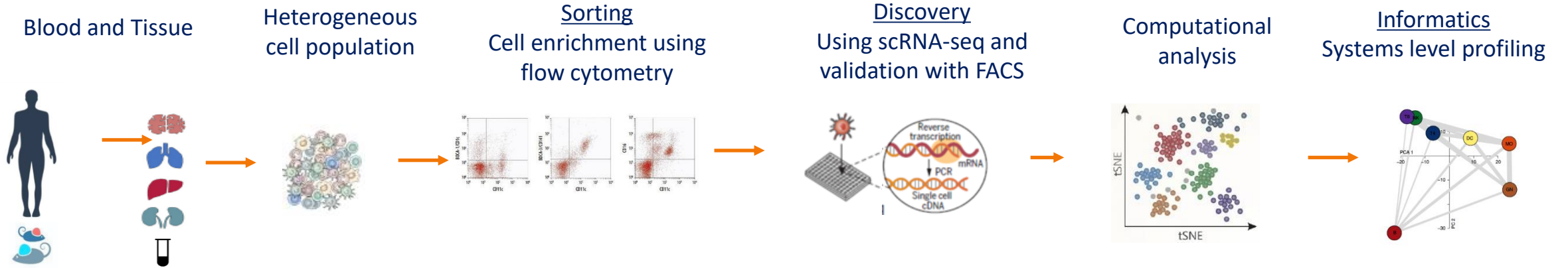


→ Highly sensitive assay, high % paired chains

→ Highly specific assay with very low false positive calls



Single-cell Analysis and Discovery – Enabling Deeper Insights



Adapted from *Systems immunology allows a new view on human dendritic cells*; Schultze, Aschenbrenner; *Semin Cell Dev Biol* (2018)

Blood and Tissue
Collection & Dissociation



Cell Staining
Antibodies, **BD**[®]
AbSeq, **SMK** Panels,
Dyes, & Reagents



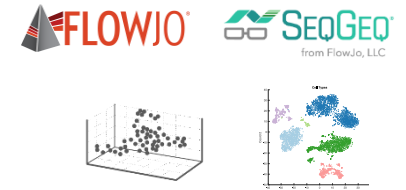
Flow Cytometry
Cell Sorters and Cell Analyzers



Single-Cell Multiomics
WTA, Targeted, VDJ,
Sample Multiplexing



Informatics
Flow and RNAseq
Analysis



Thank you!

Class 1 Laser Product.
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Questions ?

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