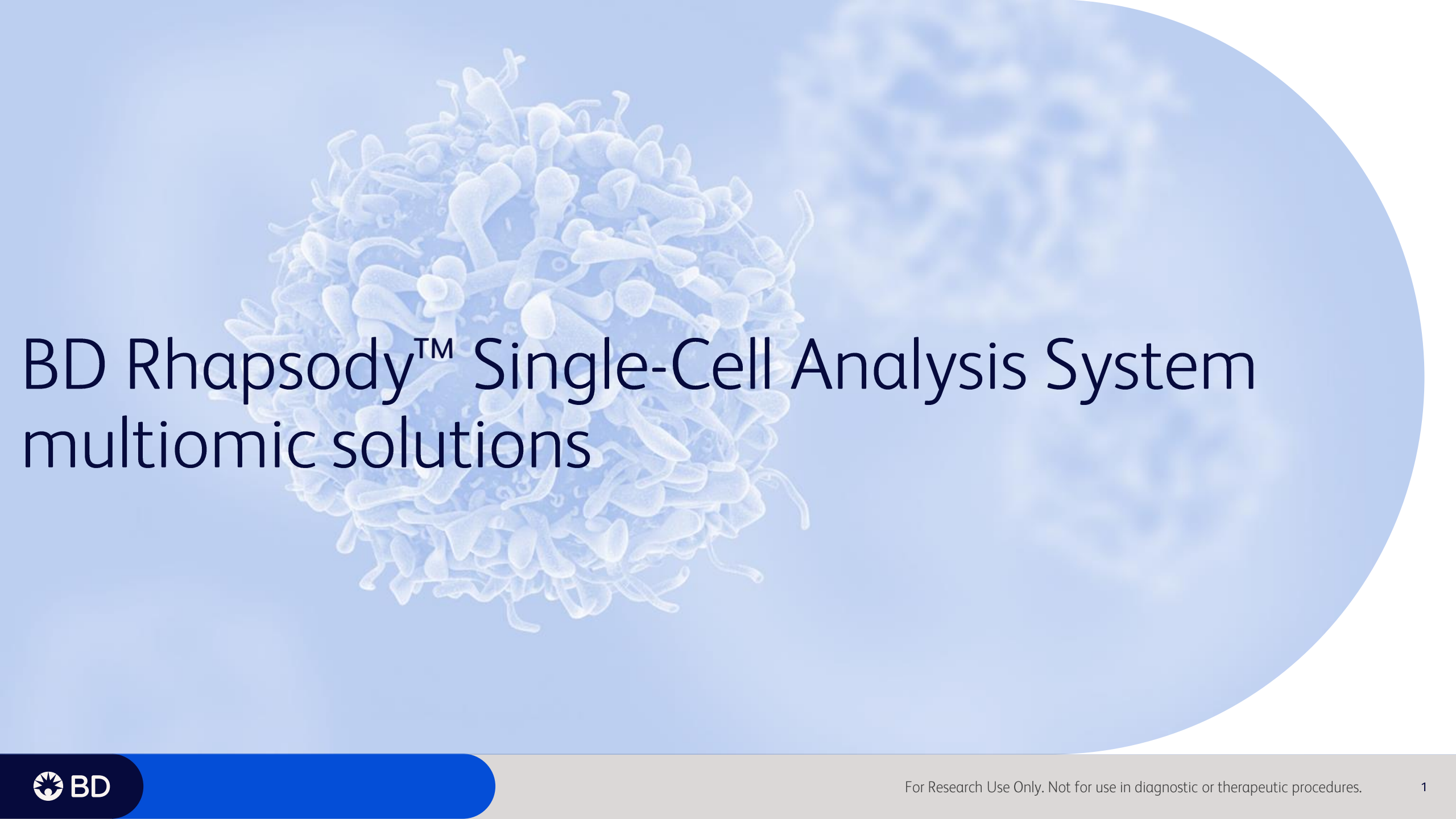
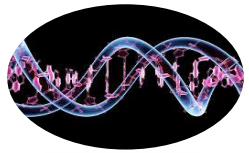


BD Rhapsody™ Single-Cell Analysis System multiomic solutions

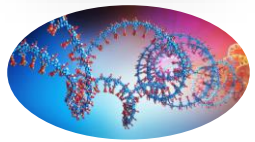
BD single-cell multiomics

Supporting multiomics through providing a complete and comprehensive product solution

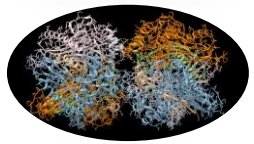
Multiple analytes from a single cell



DNA



RNA

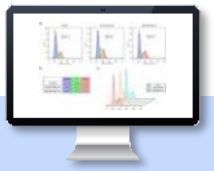


Protein

Our solutions enable comprehensive characterization '*one cell at a time*'

Instruments

Sample prep RNA kits Protein reagents



Informatics solutions

Research areas served

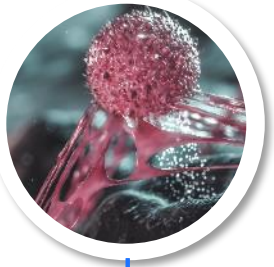
- Immunology
- Oncology
- Neurology
- Cell biology
- Microbiology
- Stem cell

Single-cell multiomics: Example areas of interest

Neurology

- Neurodeg. diseases
- Psychiatric disorders
- Develop. neuroscience

Oncology

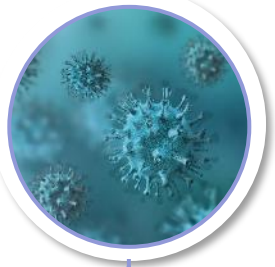


- Tumor heterogeneity
  - Therapy research
    - Tumor microenvironment
- Immuno-oncology

Immunology

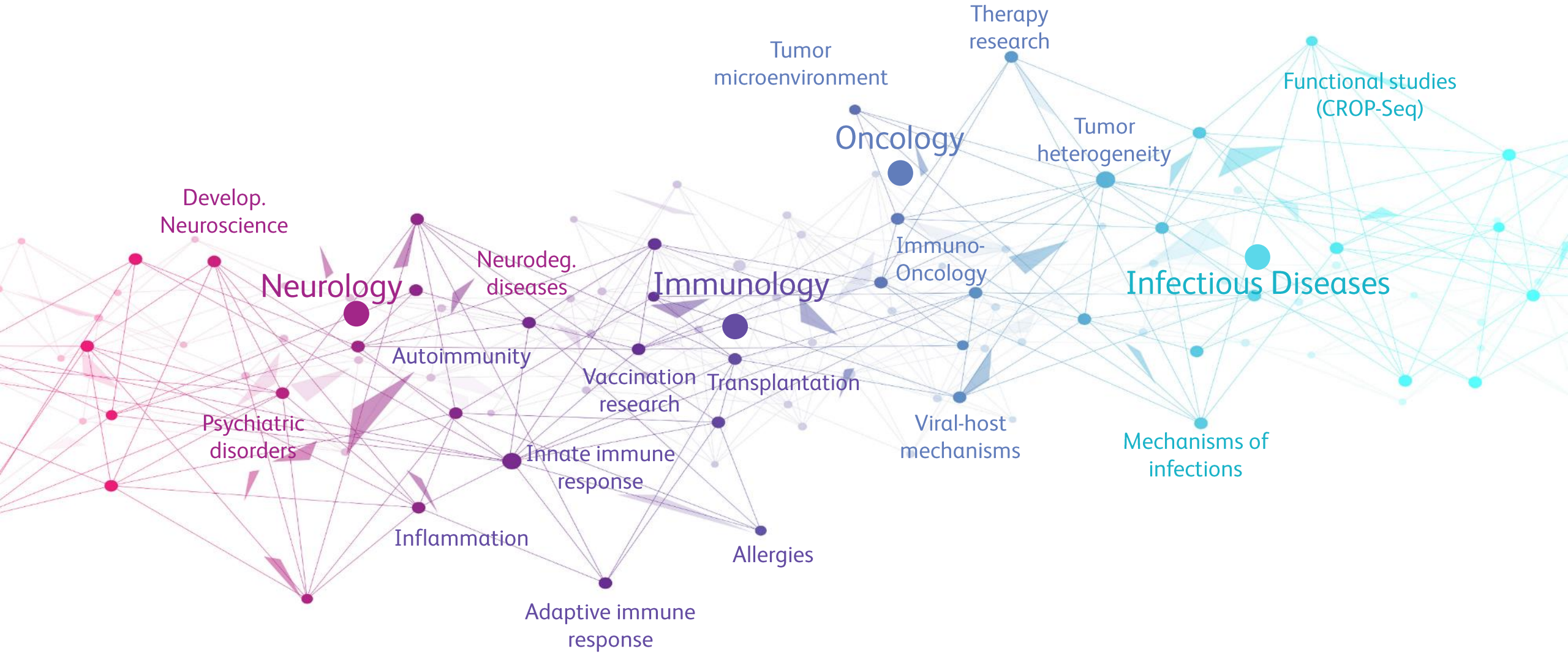
- Autoimmunity
  - Transplantation
    - Allergies
      - Inflammation
- Vaccination research
  - Innate immune response
    - Adaptive immune response

Infectious diseases

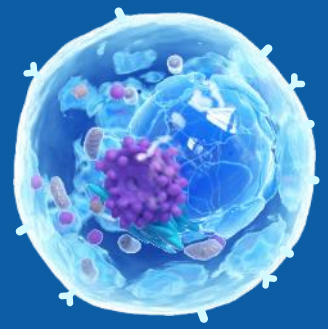


- Mechanisms of infections
  - Viral-host mechanisms
    - Functional studies (CROP-Seq)

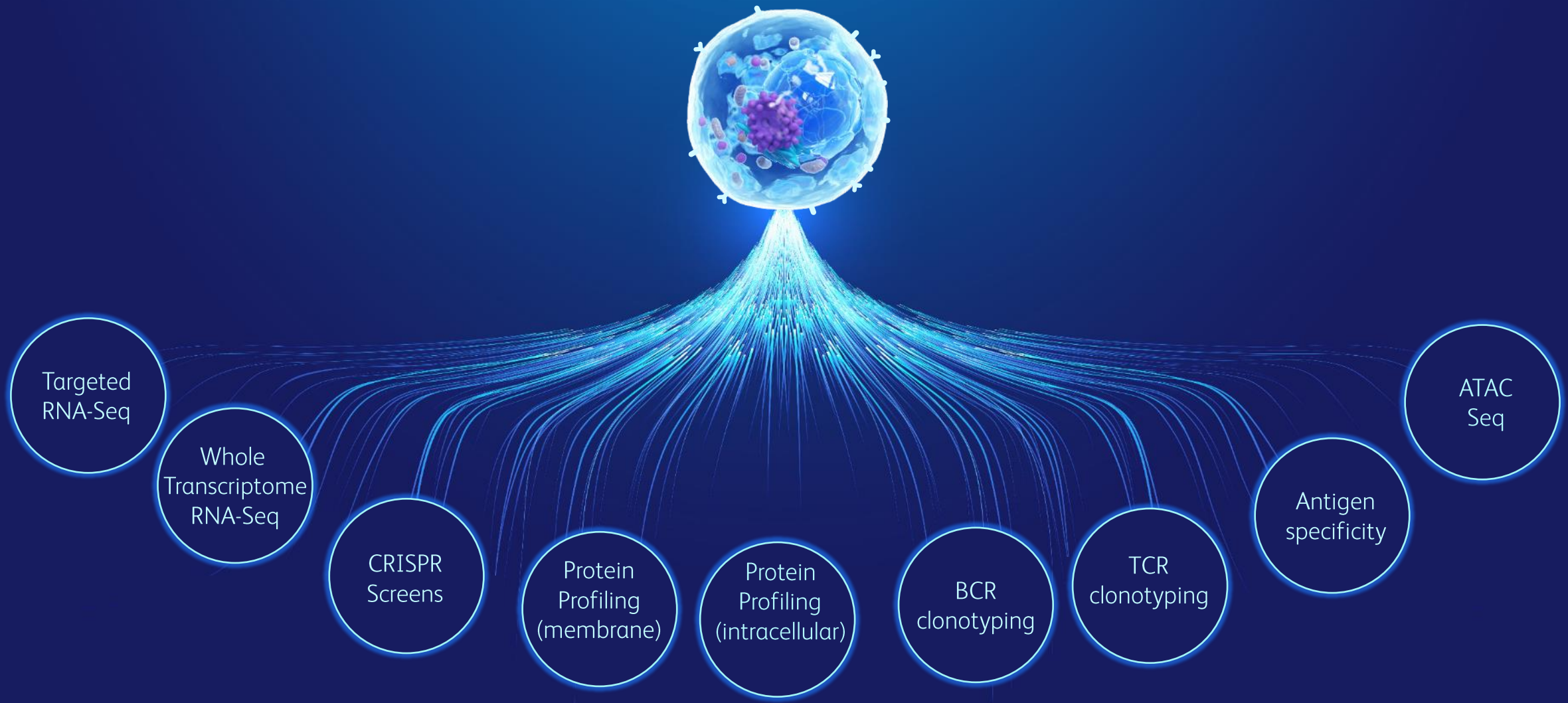
Single-cell multiomics: Example areas of interest



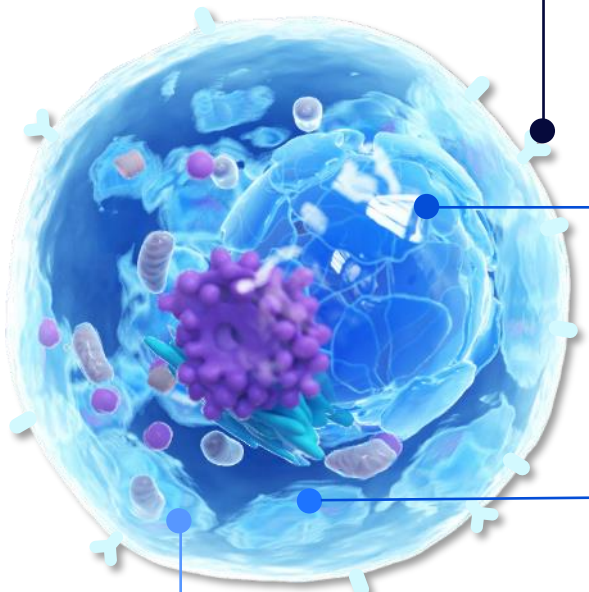
BD Rhapsody™ System: True single-cell multiomics



BD Rhapsody™ System: True single-cell multiomics solutions



BD Rhapsody™ System: True single-cell multiomics



(1) Antigen binding

(2) Gene expression regulation

(3) Gene expression

(4) Protein expression

Antigen specificity:
Immudex™ dCODE (RiO)

TCR clonotypes: Full length V(D)J or CDR3 only

Gene expression regulation:
ATAC-Seq

Gene expression:
WTA RNAseq

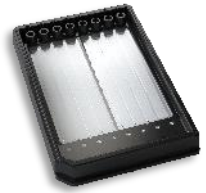
Gene expression:
Targeted RNAseq

Cell surface marker:
BD® AbSeq Ab-Oligo

Intracellular marker:
IC BD® AbSeq Ab-Oligo

BD Rhapsody™ System portfolio

BD Rhapsody™ Scanner



BD Rhapsody™ HT Xpress System

BD Rhapsody™ Express System

BD® Single-Cell Multiplexing Kit (Cell Hashing)

BD® AbSeq Ab-Oligos

BD Rhapsody™ Targeted mRNA Kit

BD Rhapsody™ WTA Amplification Kit

BD Rhapsody™ TCR/BCR Next Multiomic Assays

BD Rhapsody™ ATAC-Seq Assays

BD Rhapsody™ System: Complete single-cell multiomics solution

Instruments

BD Rhapsody™ Express System

- Microwell based; portable
- Process thousands of cells
- Single-lane cell cartridge

BD Rhapsody™ HT Xpress System

- High-throughput system
- Handles ~million cells
- 8-lane flexible cartridge design

BD Rhapsody™ Scanner

- Imaging system for QC
- Helps assess cell quality
- Unique to BD scM

Cell Prep Reagents

BD® OMICS-Guard Sample Preservation Buffer

- Sample preservation reagent
- Protects RNA, protein at 4 °C
- Compatible for RNA-seq, CITE-seq, flow cytometry, qPCR

Genomic Reagents

Whole transcriptome (WTA)

- Unbiased RNA analyses
- Reproducible; repeatable
- Identify genes for Targeted RNA panels via customs

Targeted assays

- Saves sequencing costs
- Immune response panel (Hu; Ms), T cell (Hu), Breast cancer panel (Hu)

Custom panel

- *Supplemental* – Add genes to current panels
- *Custom panel* – Totally new panel

TCR/BCR CDR3/Full Length

- Mouse, Human
- Supports AbSeq, SMK, Targeted and WTA RNA assays

BD Rhapsody™ TCR/BCR Next Multiomic Assays
High pairing TCR/BCR multiomic kit

BD Rhapsody™ ATAC-Seq Assays
Standalone and multiomic (+WTA) kits

Proteomic Reagents

Ab-oligo vials

- BD® AbSeq Ab-Oligos (Hu, Ms)
- Pre-titrated into test size
- Compatible with RNA assays

Custom BD® AbSeq Ab-Oligos

- Ab-oligo from eligible clones
- Antibodies - BD catalog or user provided clones

Ab-oligo panel

- 30-plex panel for CITE-seq
- Flexible backbone panel
- Supports WTA, Targeted, SMK

Intracellular AbSeq

- Intracellular CITE-seq
- Works with cell surface AbSeq and WTA assays

Multiplexing

- Combine multiple samples
- Tag using any primary antibody (BD® Flex SMK)

Bioinformatics

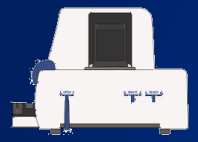
Primary analysis

SevenBridges

- Free-of-cost pipeline
- Available in a cloud format
- Easy-to-use format

BD Rhapsody™ System: Complete single-cell multiomics solution

Instruments

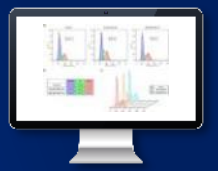


BD Rhapsody™ Express

BD Rhapsody™ HT Xpress

BD Rhapsody™ Scanner

Bioinformatics



Data Analysis

Genomics

Whole transcriptome (WTA)

Targeted Assays

Custom panel

TCR/BCR CDR3/Full Length

Cell Prep

BD® OMICS-Guard Sample Preservation Buffer

Proteomics

Cell surface and intracellular Ab-oligos BD® AbSeq

Ab-oligo panel

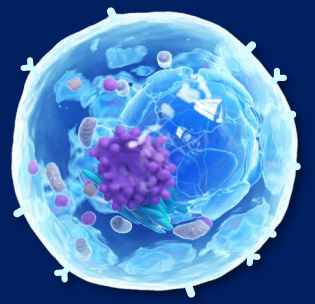
Custom AbSeq

Sample multiplexing

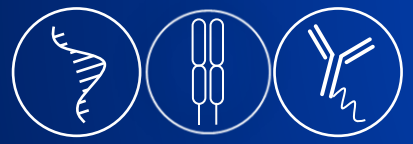
BD

Accelerating single-cell multiomics

New product launches



BD Rhapsody™ TCR/BCR Next Multiomic Assays



Immunology

Delivers in-depth single-cell insights about T and B cell biology

BD Rhapsody™ ATAC-Seq Multiomic Assays

Epigenomics

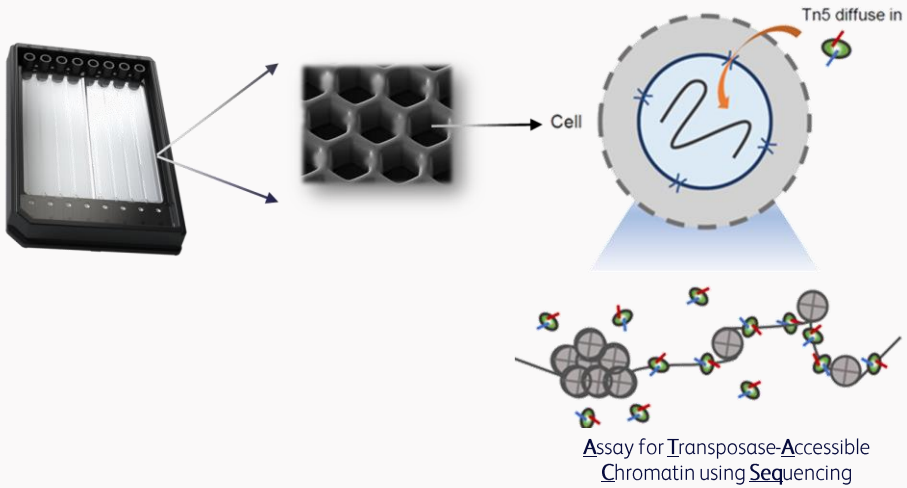
First offering from BD to help researchers understand DNA regulation

Single-cell multiomics—New product launches

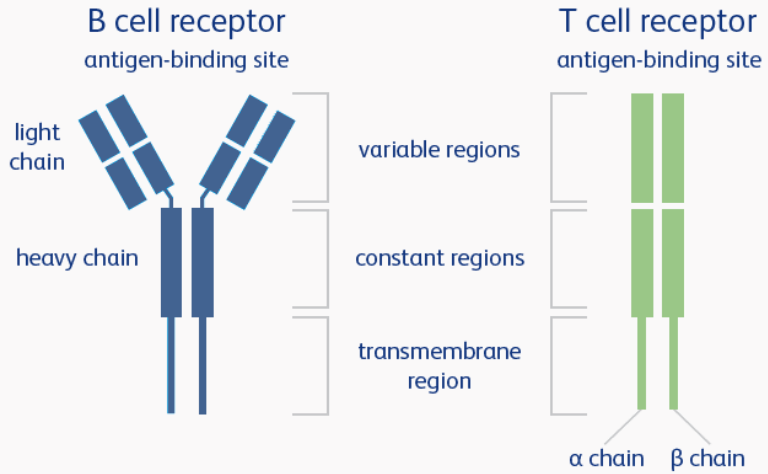
BD Rhapsody™ ATAC-Seq Assays

BD Rhapsody™ TCR/BCR Next Multiomic Assays

Products



Assay for Transposase-Accessible Chromatin using Sequencing



Features

Performance	✓ High specificity and sensitivity for cell lines, primary cells and tissues
Multiomics	✓ Perform integrated epigenomic and transcriptomic characterization
Flexible	✓ Compatibility with custom nuclear antibody tags
Workflow	✓ Compatible over a wide range of nuclei input

- ✓ Superior sensitivity in detecting full-length TCR and BCR pairing efficiency.
- ✓ Simultaneous Targeted, whole transcriptome profiling and CITE-seq.
- ✓ Intuitive data analysis pipeline that visualizes raw data alongside filtered results.
- ✓ Compatibility with BD® OMICS-Guard Sample Preservation Buffer.

BD Rhapsody™ System: Added value for your research

Reliable

No clogging errors

No wetting errors

No sample loss reported to us since 2017

Costs

Low multiplet rate
Spend less money for data waste

Low running costs

Process up to 192 samples per cartridge with multiplexing
→ Lower costs per sample

Flexible

Partial loading enabled
→ load unused lanes on a different day

Combine different assays
→ Run different assays on individual lanes on one cartridge

Capture beads
→ Beads allow storage and shipment at 4 °C and subsampling

Cell number range
→ Process from 100 to 1000s of cells

Insights

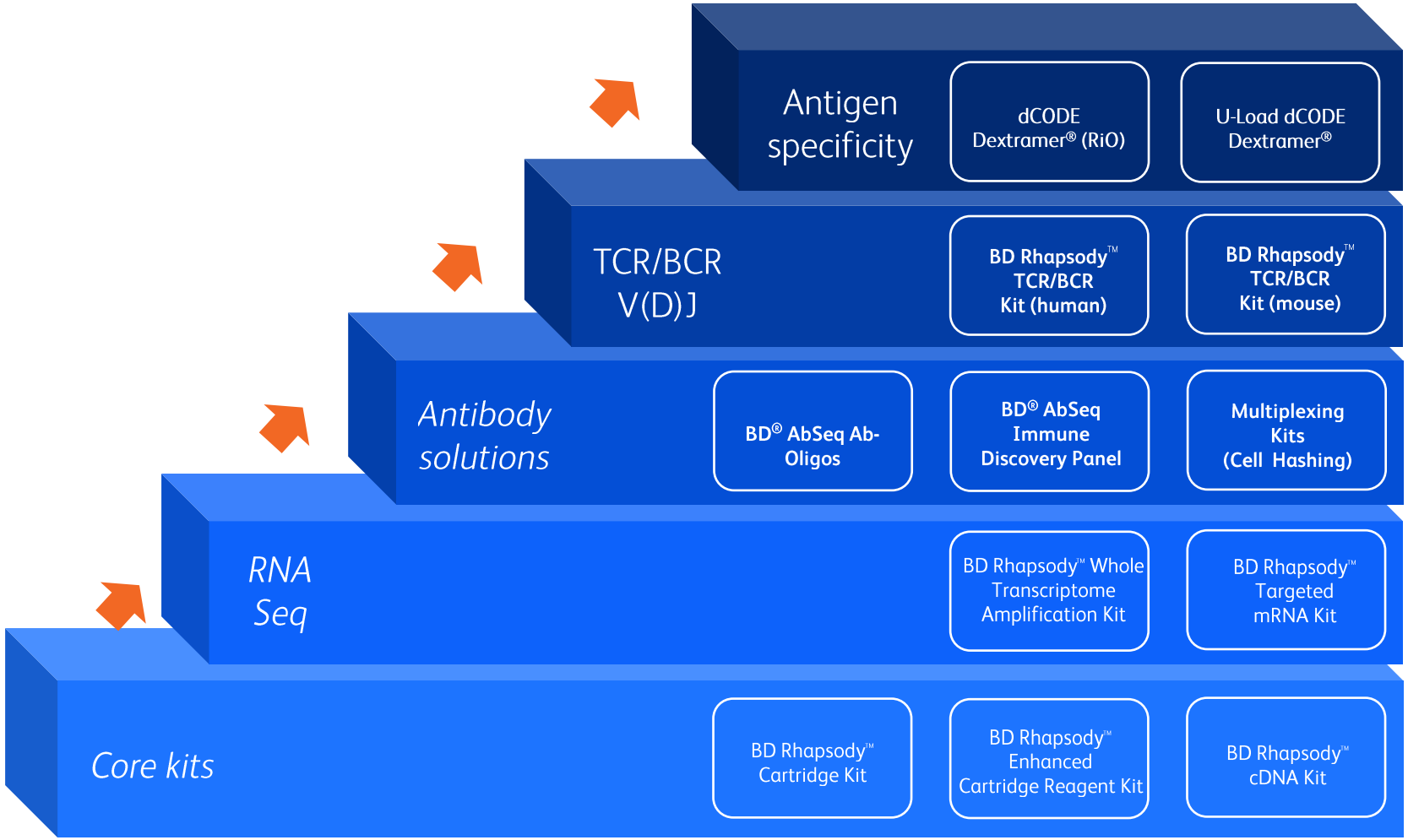
Capture rate:
→ Up to 80%, get more insights from your samples

Capture fragile cells
→ Don‘t miss information from your precious samples

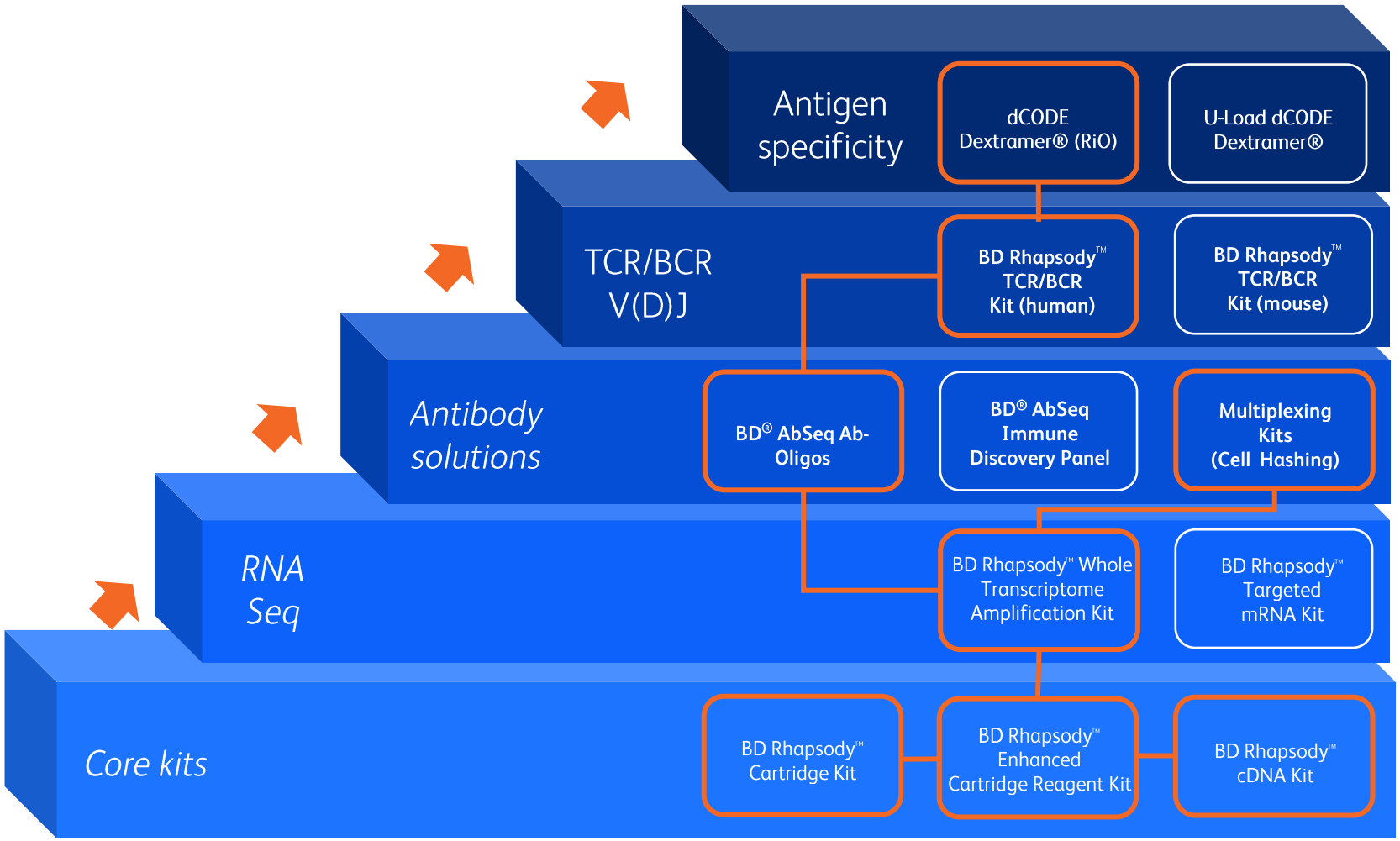
Support

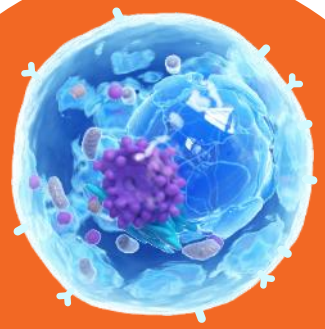
Workflow support from one partner
→ Sample prep is critical step. BD can not only support the SC workflow but also the sample prep

BD Rhapsody™ System assay portfolio: Compatibility of 3' and 5' assays



Multiomics study example: Deep T-cell characterization





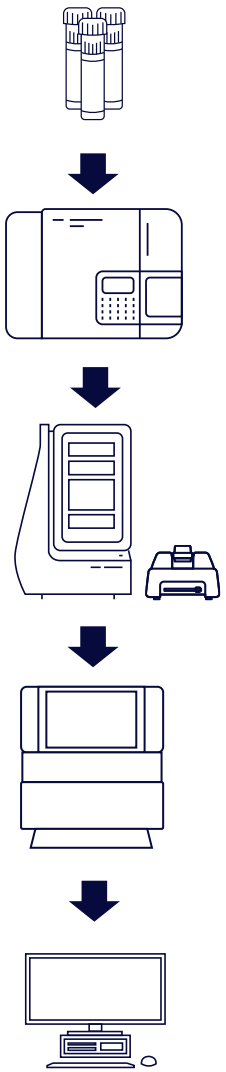
Example study:

Sorted human T-cells:

- WTA RNA-seq
- Protein profiling
- Cell hashing
- TCR clonotyping
- Antigen specificity

Multiomics study example: Deep T-cell characterization

https://www.immudex.com/media/2266/advancements-in-single-cell-multiomic-profiling-of-antigen-specific-t-cells-with-dcode-dextramer.pdf



Sample prep
(BD® SMK, Dextramers, BD® AbSeq Ab-Oligos, Flow abs)

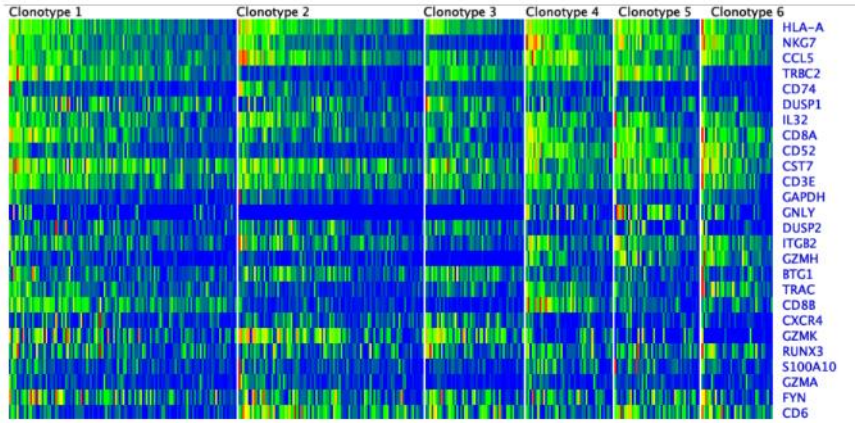
Cell sorting

Cell capture, barcoding and library prep

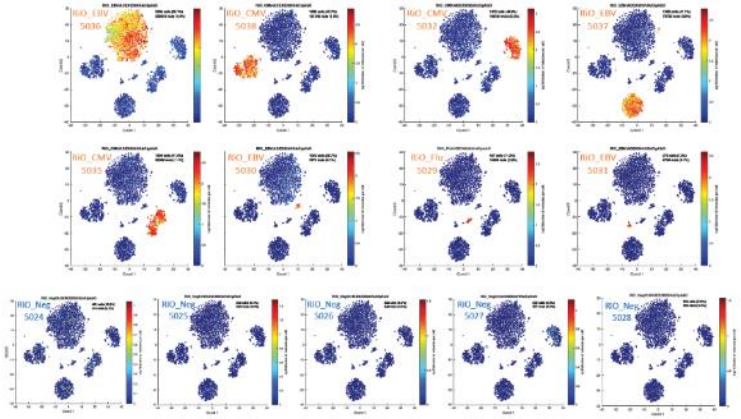
Sequencing

Data analysis and interpretation

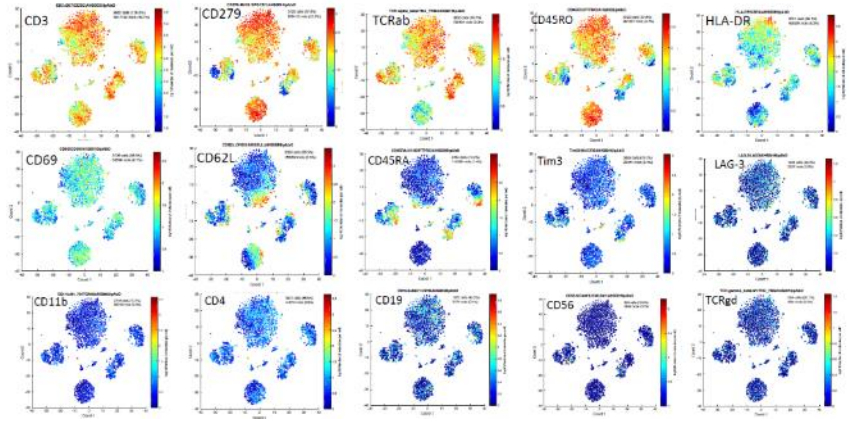
Gene expression (WTA, RNA-seq)



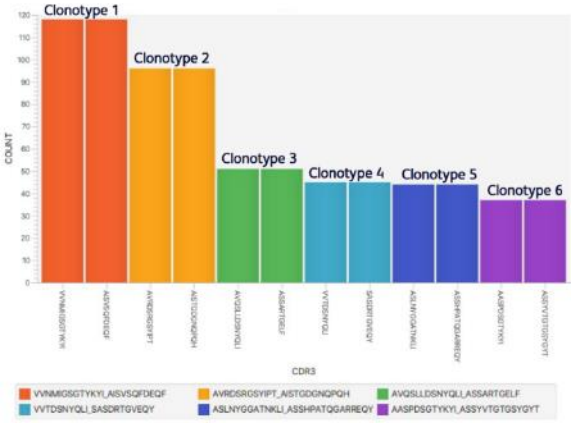
Antigen Specificty (dCODE Dextramer® (RiO))



Protein profiling (BD® AbSeq Ab-Oligos)



TCR clonotyping

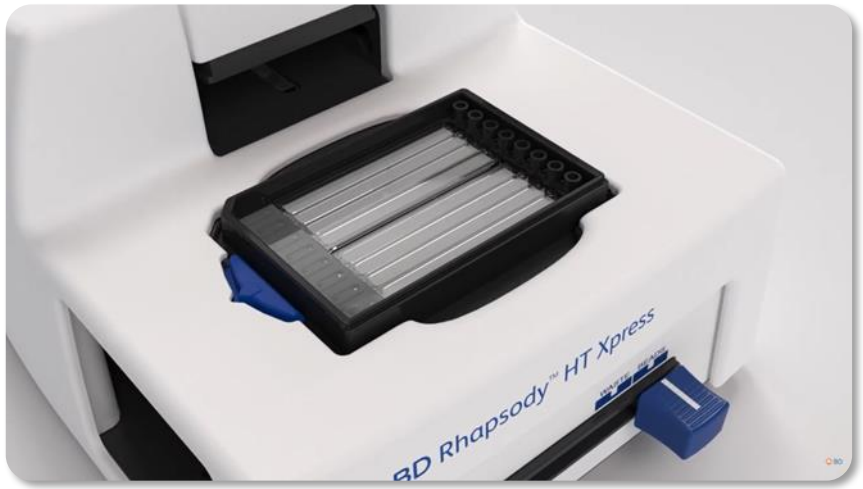


BD Rhapsody™ System

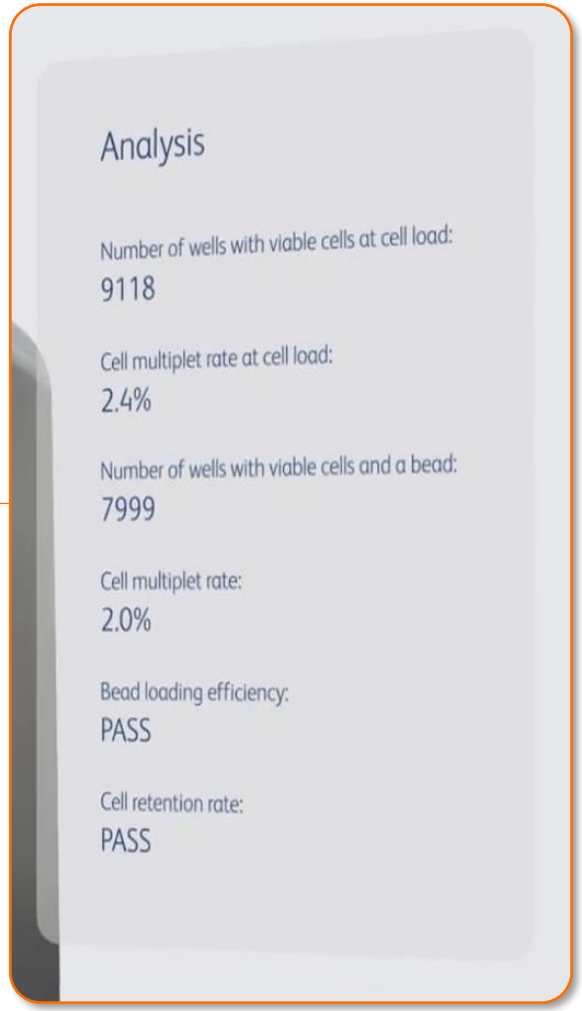
Microwell technology–based high-throughput single-cell capture system

BD Rhapsody™ HT Single-Cell Analysis System

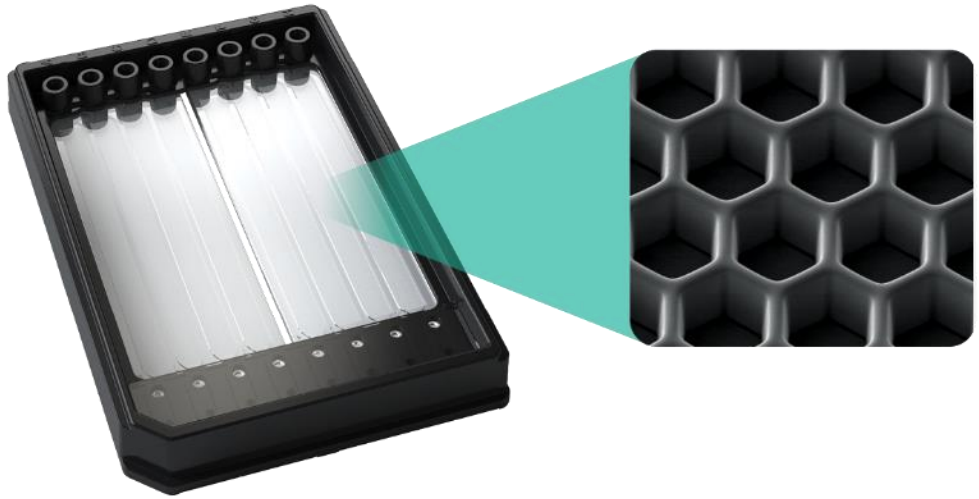
BD Rhapsody™ HT Xpress System



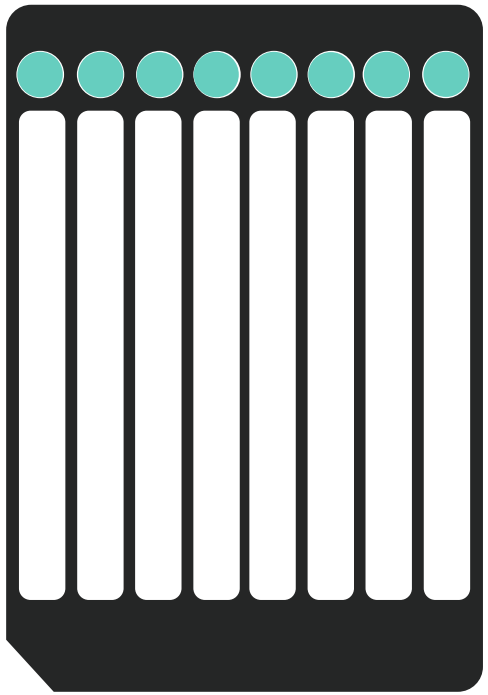
BD Rhapsody™ Scanner



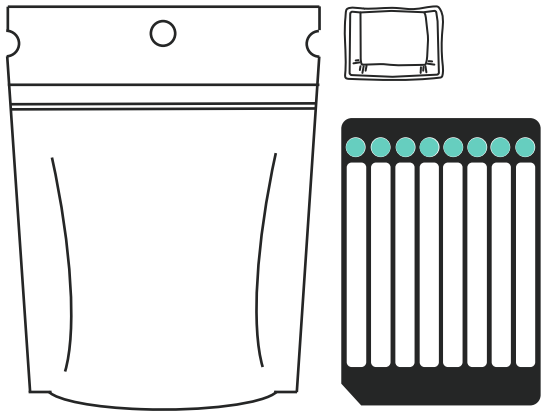
BD Rhapsody™ HT Cartridge



BD Rhapsody™ HT Cartridge



BD Rhapsody™ HT Cartridge, resealable pouch and desiccant bag



Flexible cartridge design

Up to 8 tests per cartridge

>267,000 microwells per lane

320-µL cell suspension loading volume

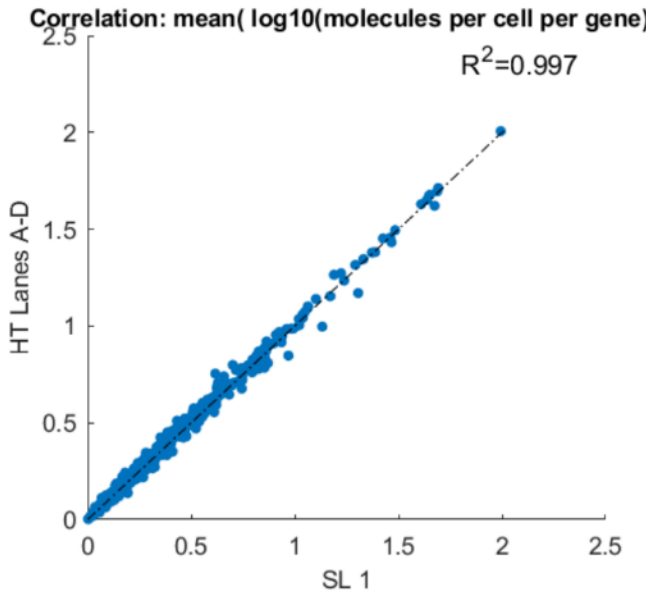
Up to 55,000 cells per lane recommended

Run more or different types of experiments

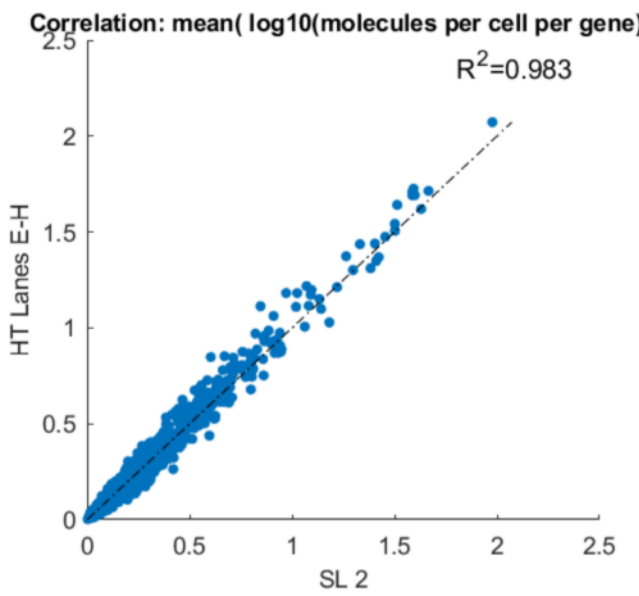
Process samples together or on different days

High gene correlation expression across lanes in an 8-lane cartridge with biological and site-to-site replicates

A.



B.

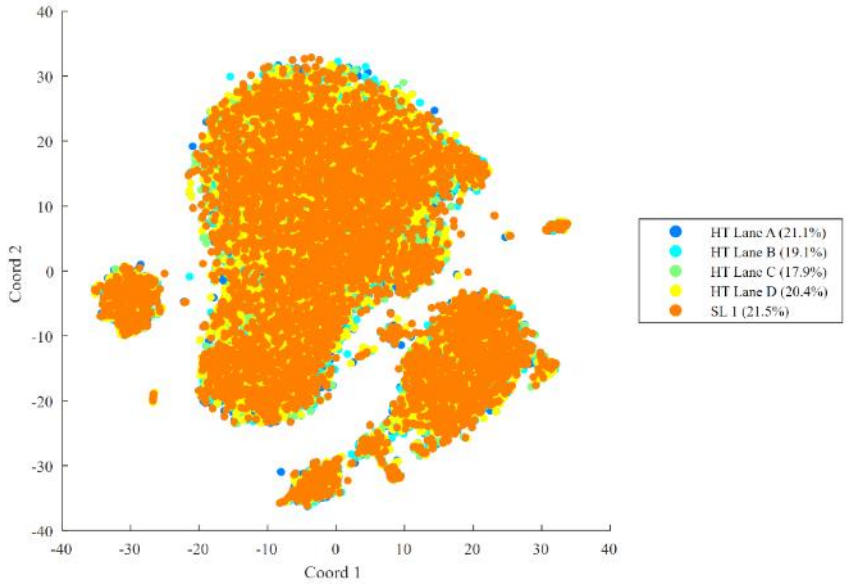


- Gene correlation expression between SL1 and HT (8-lane cartridge) samples A–D is $R^2 = 0.997$
- Gene correlation expression between SL2 and HT (8-lane cartridge) samples E–H is $R^2 = 0.983$

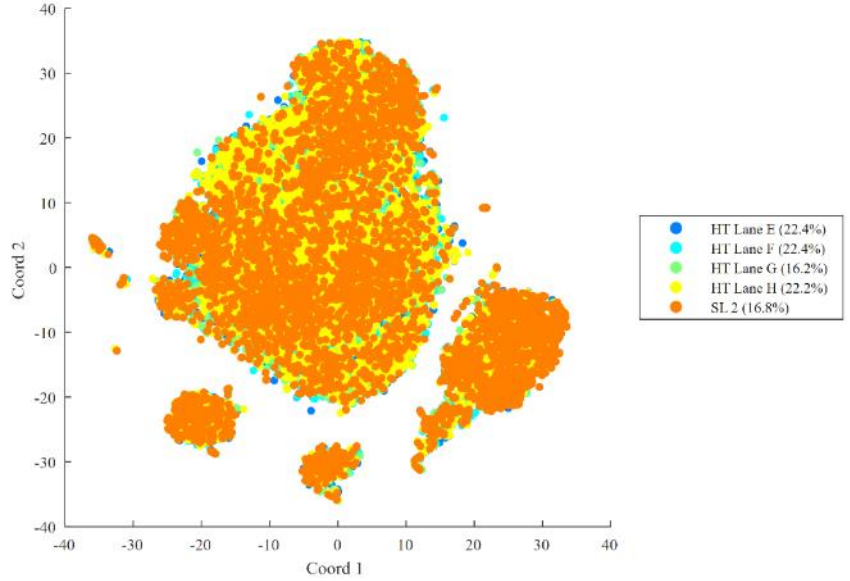
A) Cells (20,000 per lane) from a single PBMC donor were loaded into four lanes (A–D) in a BD Rhapsody™ 8-Lane Cartridge and cell capture was performed on a BD Rhapsody™ HT Xpress System. **B)** A second set of cells from the same PBMC donor were loaded at 20,000 cells per lane into four lanes (E–H) of a different 8-lane cartridge and cell capture was performed on a different BD Rhapsody™ HT Xpress System. WTA libraries were prepared from subsampled beads at 6,000 cells. All BD Rhapsody™ 8-Lane Cartridge results were compared to a BD Rhapsody™ Single-Lane Cartridge control.

Minimal batch effects across lanes in an 8-lane cartridge with biological and site-to-site replicates

A.



B.



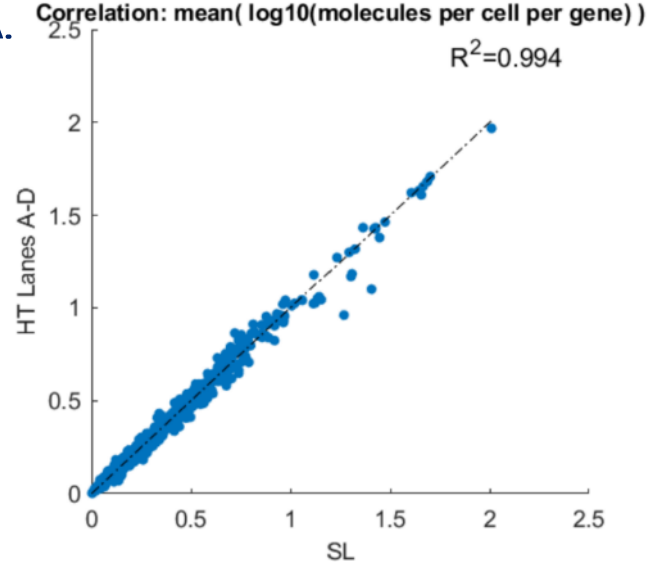
- NO batch effect with HT (8-lane cartridge) samples A–D compared to SL1
- NO batch effect with HT (8-lane cartridge) samples E–H compared to SL2

A) Cells (20,000 per lane) from a single PBMC donor were loaded into four lanes (A–D) in a BD Rhapsody™ 8-Lane Cartridge and cell capture was performed on a BD Rhapsody™ HT Xpress System. **B)** A second set of cells from the same PBMC donor were loaded at 20,000 cells per lane into four lanes (E–H) of a different 8-lane cartridge and cell capture was performed on a different BD Rhapsody™ HT Xpress System. WTA libraries were prepared from subsampled beads at 6,000 cells. All BD Rhapsody™ 8-Lane Cartridge results were compared to a BD Rhapsody™ Single-Lane Cartridge control.

High gene correlation expression across lanes in an 8-lane cartridge with biological, instrument-to-instrument and user-to-user replicates

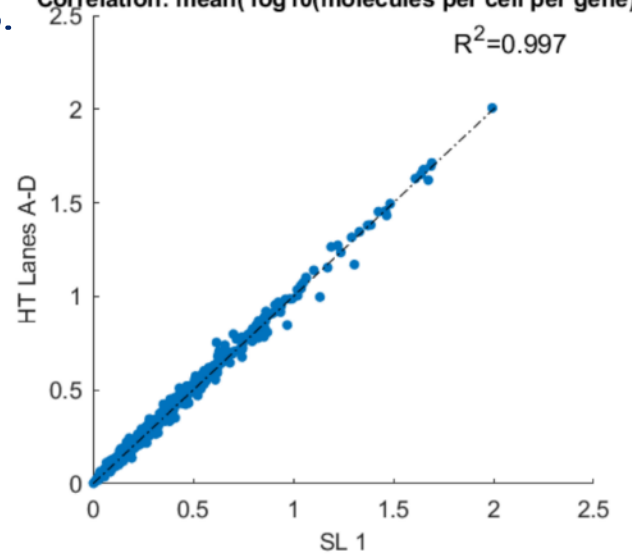
Operator 1 BD Rhapsody™ HT Xpress System 1

A.



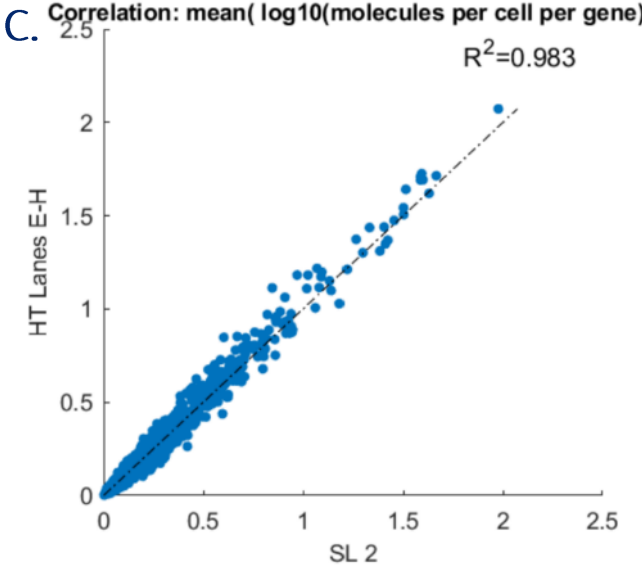
Operator 2 BD Rhapsody™ HT Xpress System 1

B.



Operator 2 BD Rhapsody™ HT Xpress System 2

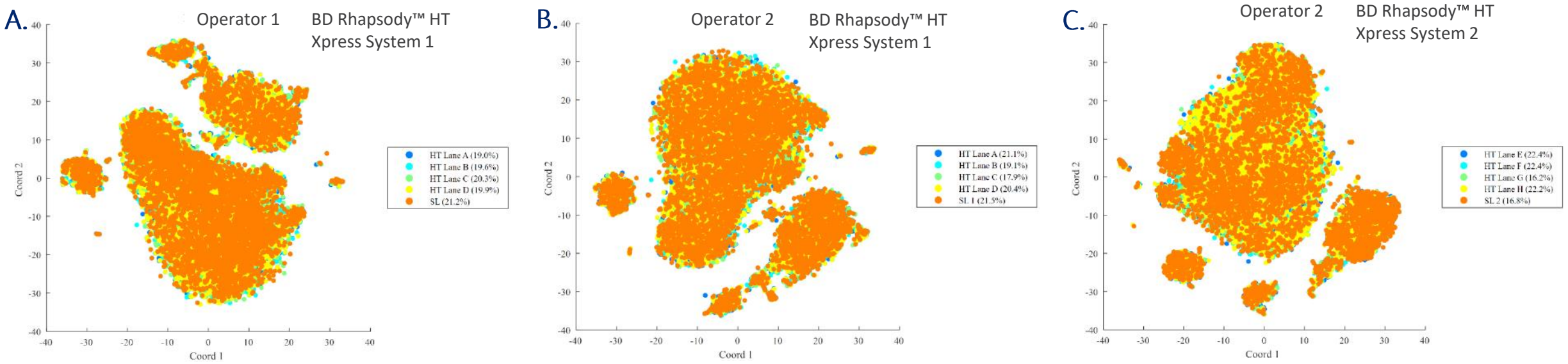
C.



- Operator 1: Gene correlation expression between SL and HT (8-lane cartridge) samples A–D is $R^2 = 0.994$
- Operator 2: Gene correlation expression between SL1 and HT (8-lane cartridge) samples A–D is $R^2 = 0.997$
- Operator 2: Gene correlation expression between SL2 and HT (8-lane cartridge) samples E–H is $R^2 = 0.983$

A) Cells (20,000 per lane) from a single PBMC donor were loaded into four lanes (A–D) of a BD Rhapsody™ 8-Lane Cartridge and cell capture was performed on a BD Rhapsody™ HT Xpress System by operator 1. **B)** A second set of cells from the same PBMC donor were loaded by operator 2 at 20,000 cells per lane into four lanes (A–D) of a different 8-lane cartridge and cell capture was performed on the same BD Rhapsody™ HT Xpress System used by operator 1. **C)** A third set of cells from the same PBMC donor were loaded by operator 2 at 20,000 cells per lane into four lanes (E–H) of a third 8-lane cartridge and cell capture was performed on a different BD Rhapsody™ HT Xpress System than the experiment ran on lanes A–D. WTA libraries were prepared from subsampled beads at 6,000 cells. All BD Rhapsody™ 8-Lane Cartridge results were compared to a BD Rhapsody™ Single-Lane Cartridge control.

Minimal batch effects across lanes in an 8-lane cartridge with biological, instrument-to-instrument and user-to-user replicates

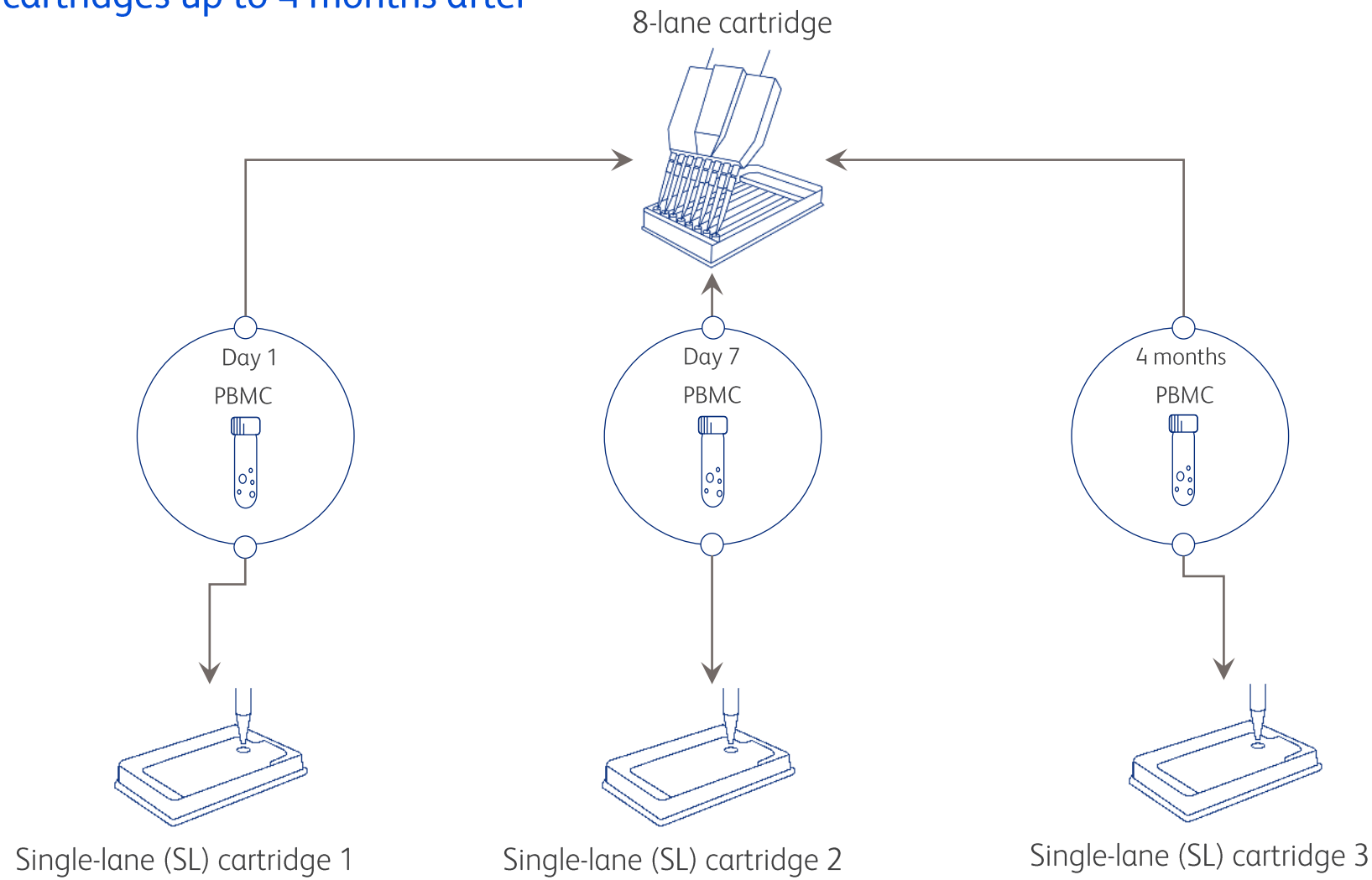


- Operator #1: NO batch effect with HT (8-lane cartridge) samples A–D compared to SL
- Operator #2: NO batch effect with HT (8-lane cartridge) samples A–D compared to SL1
- Operator #2: NO batch effect with HT (8-lane cartridge) samples E–H compared to SL2

A) Cells (20,000 per lane) from a single PBMC donor were loaded into four lanes (A–D) of a BD Rhapsody™ 8-Lane Cartridge and cell capture was performed on a BD Rhapsody™ HT Xpress System by operator 1. **B)** A second set of cells from the same PBMC donor were loaded by operator 2 at 20,000 cells per lane into four lanes (A–D) of a different 8-lane cartridge and cell capture was performed on the same BD Rhapsody™ HT Xpress System used by operator 1. **C)** A third set of cells from the same PBMC donor were loaded by operator 2 at 20,000 cells per lane into four lanes (E–H) of a third 8-lane cartridge and cell capture was performed on a different BD Rhapsody™ HT Xpress System than the experiment ran on lanes A–D. WTA libraries were prepared from subsampled beads at 6,000 cells. All BD Rhapsody™ 8-Lane Cartridge results were compared to a BD Rhapsody™ Single-Lane Cartridge control.

Flexibility with partial use of the BD Rhapsody™ 8-Lane Cartridge

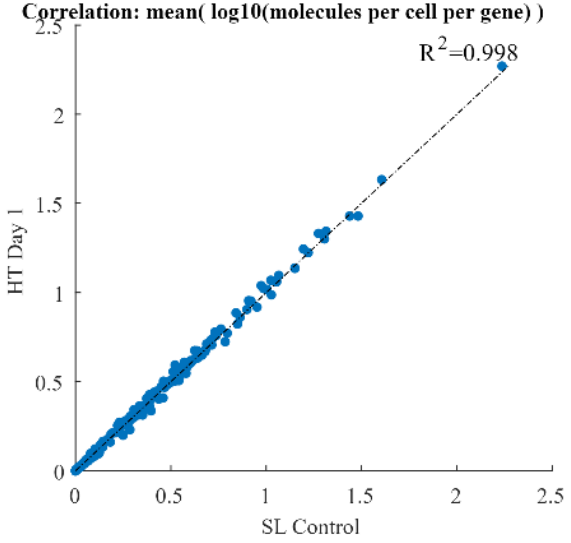
Use partially used cartridges up to 4 months after opening

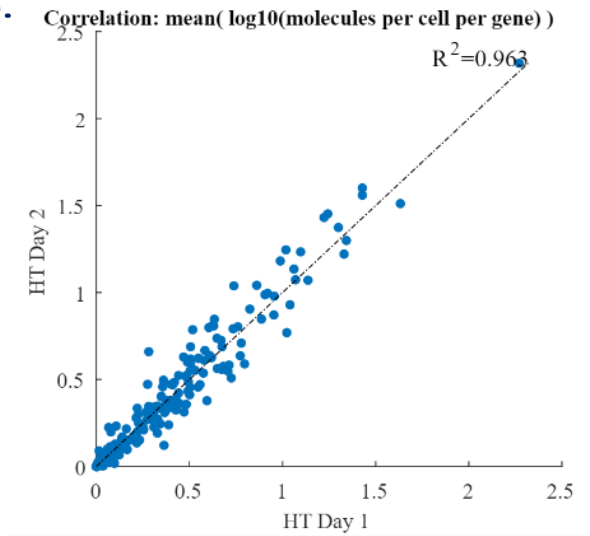


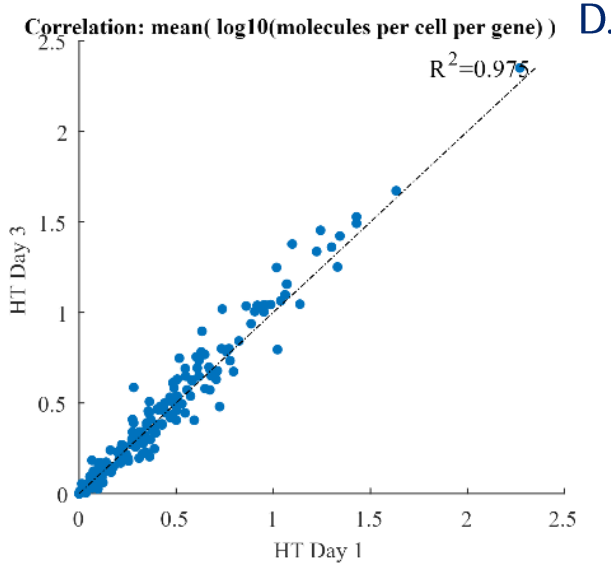
Flexibility with partial use of the BD Rhapsody™ 8-Lane Cartridge

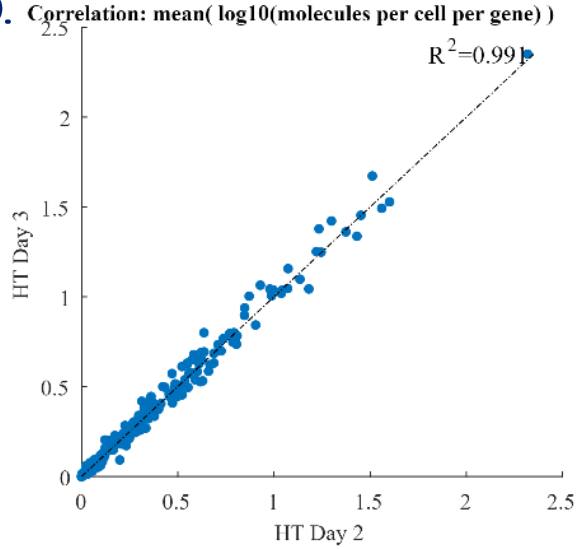
Use 1–8 lanes at a time and re-use the remaining lanes at a different time for the same or different assays.

8-lane cartridge (HT)
Day1- Lanes 1,2
Day2- Lanes 3,4,5
Day3- Lanes 6,7,8

A.


B.


C.


D.


→ **Differential gene expression analysis** $R^2 \geq 0.95$

Two cell types (Jurkat and Ramos) were pooled at 1:1 ratio. Lanes 1 and 2 were loaded with 20,000 cells on Day 1, lanes 3–5 on Day 2 and lanes 6–8 on Day 3. Targeted and SMK libraries were prepared from subsampled beads at 3,500 cells using the BD Rhapsody™ Targeted mRNA Kit with the BD Rhapsody™ Immune Response Panel (Hs) and BD® Single-Cell Multiplexing Kit. **A-B)** Assay performance of the Day 1 samples on the 8-lane cartridge was compared with a single-lane cartridge control or another HT cartridge. **C-D)** The assay performance on the lanes used on different days were also compared. No batch effect was observed, and the correlation of gene expression was high between the single-lane and 8-lane cartridge, and lane-to-lane variability was minimal. Results may vary based on cell type and isolation method.

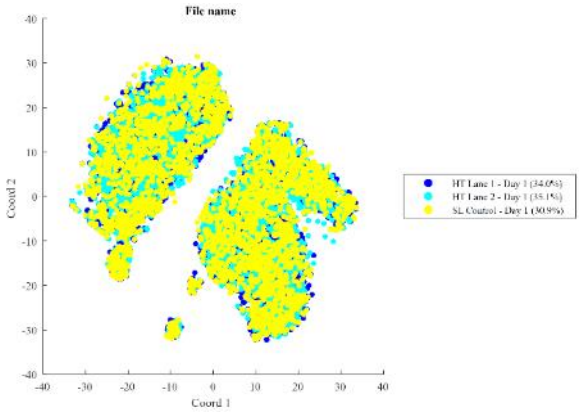
Flexibility with partial use of the BD Rhapsody™ 8-Lane Cartridge

Example: Prolonged stability of partially used cartridges up to 4 months.
Day 1 and 7 on different lanes show tight correlation with single-lane control

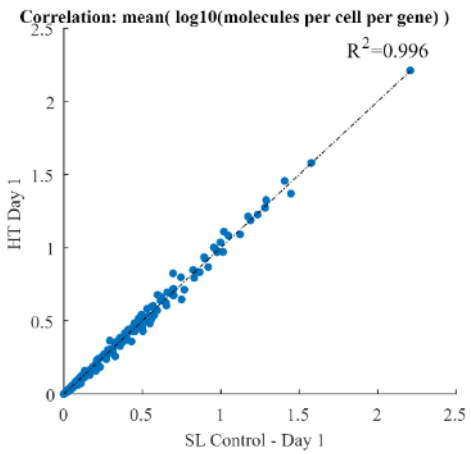
Day 1: Targeted + SMK assay, Lanes 1,2
1:1 Jurkat/Ramos Cells

Day 7: WTA+Abseq assay, Lanes 3,4
PBMCs

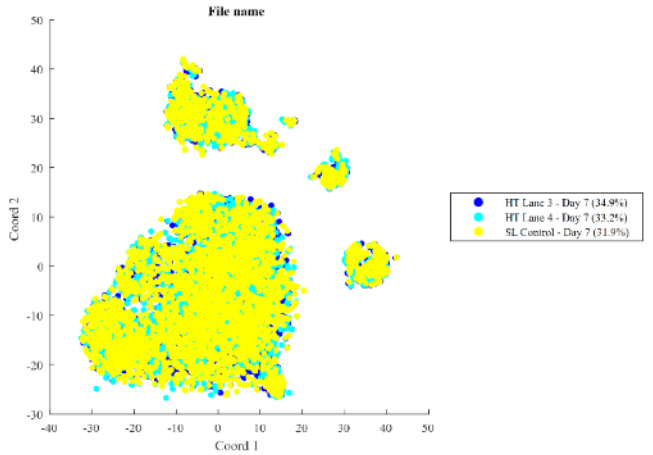
A.



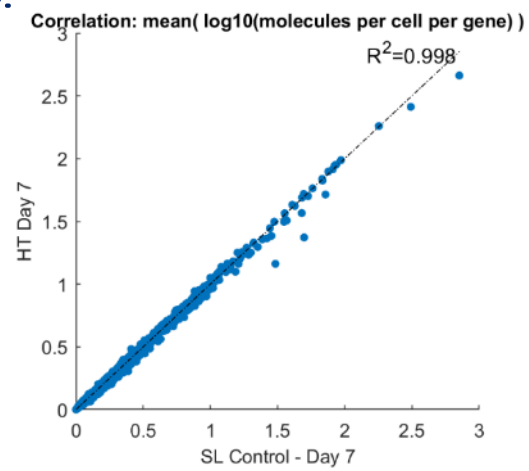
B.



C.



D.



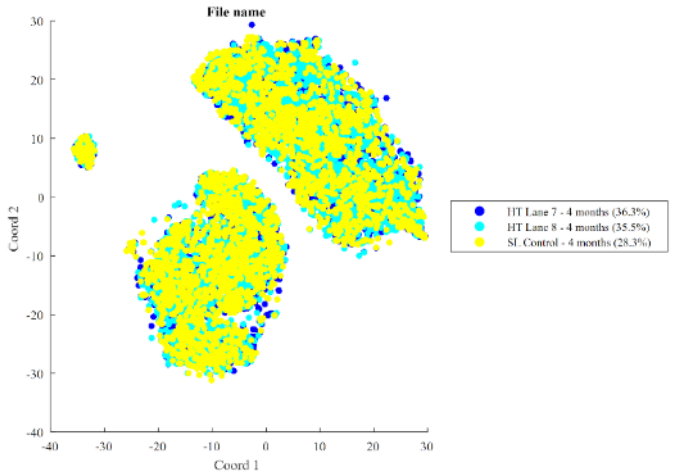
Lanes 1 and 2 were loaded with 20,000 cells consisting of Jurkat and Ramos cells at 1:1 ratio on Day 1, Lanes 3 and 4 were loaded with 20,000 cells from a single PBMC donor on Day 7, and Lanes 7 and 8 were loaded with 20,000 cells consisting of Jurkat and Ramos cells at 1:1 ratio at four months storage of the partially used cartridge. Targeted and SMK libraries were prepared from subsampled beads at 3,500 cells on Day 1 and 4-month storage using the BD Rhapsody™ Targeted mRNA Kit with the BD Rhapsody™ Immune Response Panel (HS) and BD® Single-Cell Multiplexing Kit. **A-B)** Assay performance of the Day 1 samples on the 8-lane cartridge was compared with a single-lane cartridge control. **C-D)** WTA and AbSeq (10-plex) were prepared from subsampled beads at 3,500 cells on Day 7 storage using the BD Rhapsody™ WTA Amplification Kit. No batch effect was observed, and the correlation of gene expression was high between the single-lane and the 8-lane cartridge, and lane-to-lane variability was minimal. Results may vary based on cell type and isolation method.

Flexibility with partial use of the BD Rhapsody™ 8-lane cartridge

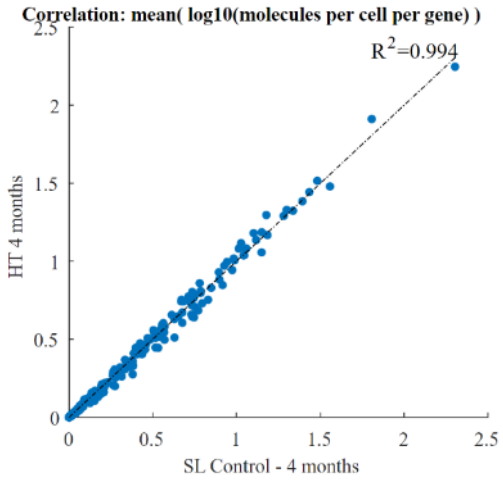
Example: Prolonged stability of partially used cartridges in progress up to 4 months. 4-month storage show tight correlation with single lane control

4 months: Targeted + SMK assay, Lanes 7,8
1:1 Jurkat/Ramos Cells

A.



B.



Lanes 1 and 2 were loaded with 20,000 cells consisting of Jurkat and Ramos cells at 1:1 ratio on Day 1, Lanes 3 and 4 were loaded with 20,000 cells from a single PBMC donor on Day 7, and Lanes 7 and 8 were loaded with 20,000 cells consisting of Jurkat and Ramos cells at 1:1 ratio at four months storage of the partially used cartridge. Targeted and SMK libraries were prepared from subsampled beads at 3,500 cells on Day 1 and 4-month storage using the BD Rhapsody™ Targeted mRNA Kit with the BD Rhapsody™ Immune Response Panel (HS) and BD® Single-Cell Multiplexing kit. **A-B)** Assay performance of the Day 1 samples on the 8-lane cartridge was compared with a single-lane cartridge control. Targeted and SMK (4-plex) were prepared from subsampled beads at 3,500 cells on month four storage using the BD Rhapsody™ WTA Amplification Kit. No batch effect was observed, and the correlation of gene expression was high between the single-lane and the 8-lane cartridge, and lane-to-lane variability was minimal. Results may vary based on cell type and isolation method.

BD Rhapsody™ Single-Lane vs 8-Lane Cartridge

Instruments	BD Rhapsody™ HT Xpress System	BD Rhapsody™ Express System
Description	A high-throughput system with flexible capture and analysis of multiomic information	Standard throughput system for capture and analysis of multiomic information
Technology	Microwell (>267K per lane)	Microwell (220K per lane)
Consumable	BD Rhapsody™ 8-Lane Cartridge (partial use)	BD Rhapsody™ Single-Lane Cartridge (single use)
Cell throughput (input)	100 to 55,000 per lane recommended (100–440,000 per cartridge)	100 to 40,000 recommended
Sample throughput (with multiplexing)	24 samples per lane (192 samples per cartridge)	24 samples
Cell capture rate	Up to 80%	
Barcoding system	BD Rhapsody™ Enhanced Cell Capture Beads v2	
Assay compatibility	• BD Rhapsody™ Single-Cell Multiplexing Kits • BD® AbSeq Assays • BD Rhapsody™ Targeted mRNA or Whole Transcriptome Amplification Kits • BD Rhapsody™ TCR/BCR Multiomic Assays	

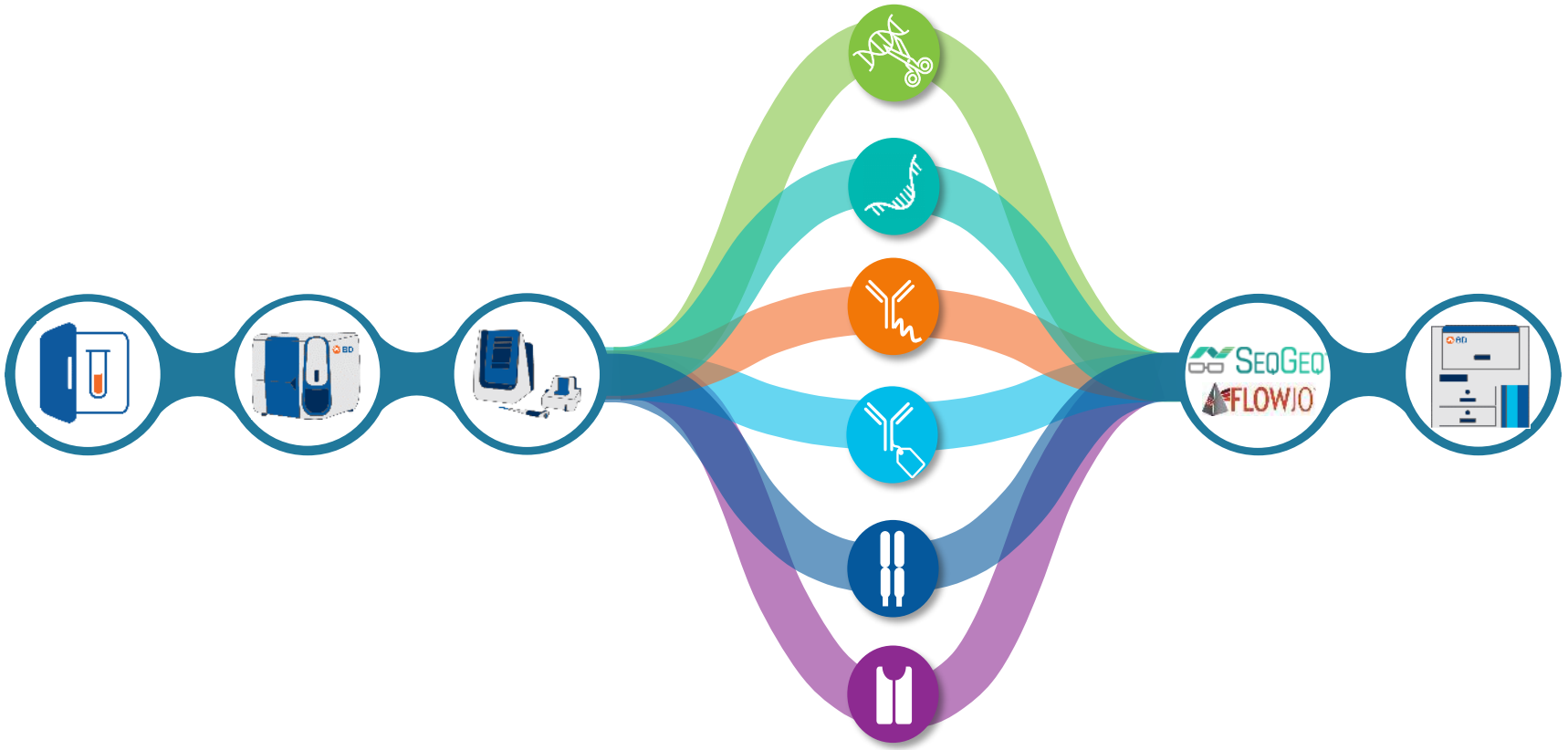
The three steps of single-cell multiomics

1. Capture single cells

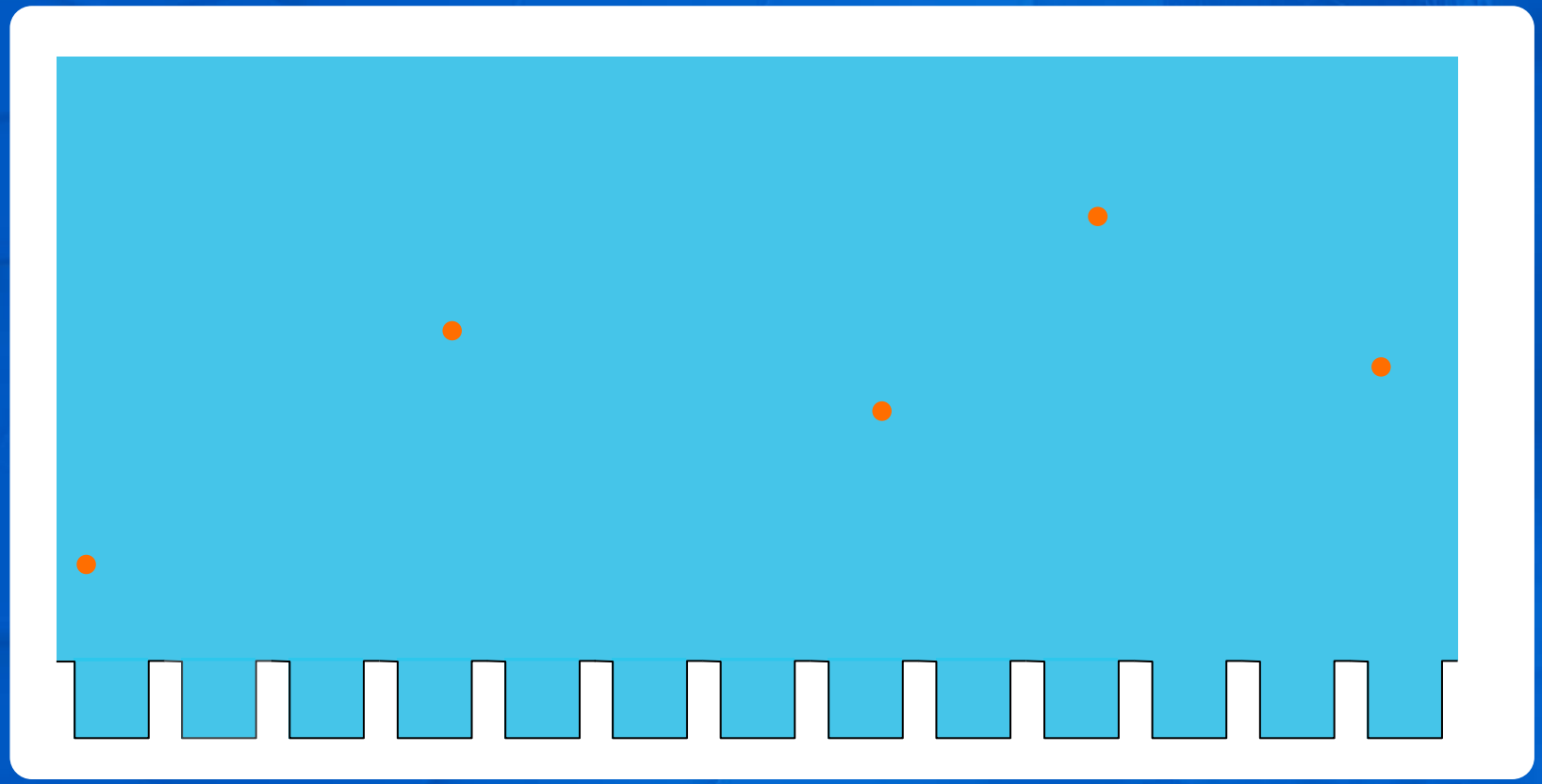
2. Barcode analytes

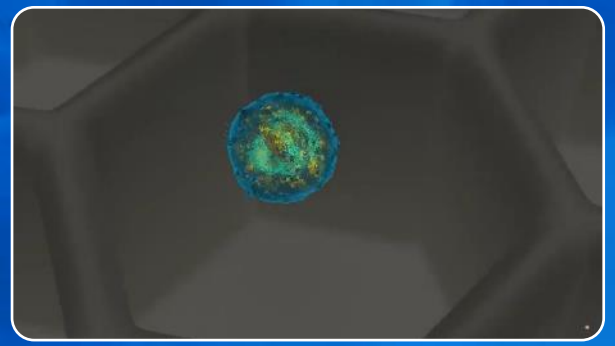
3. Library prep and NGS

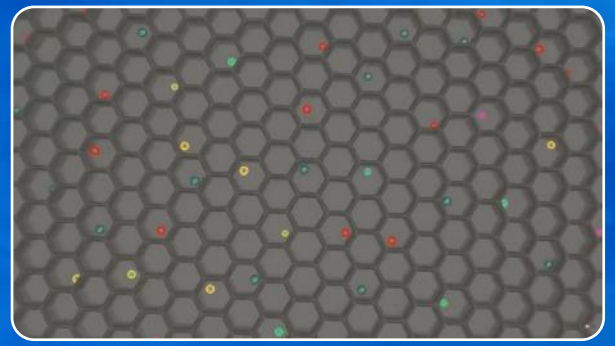
BD Rhapsody™ Cartridge workflow overview



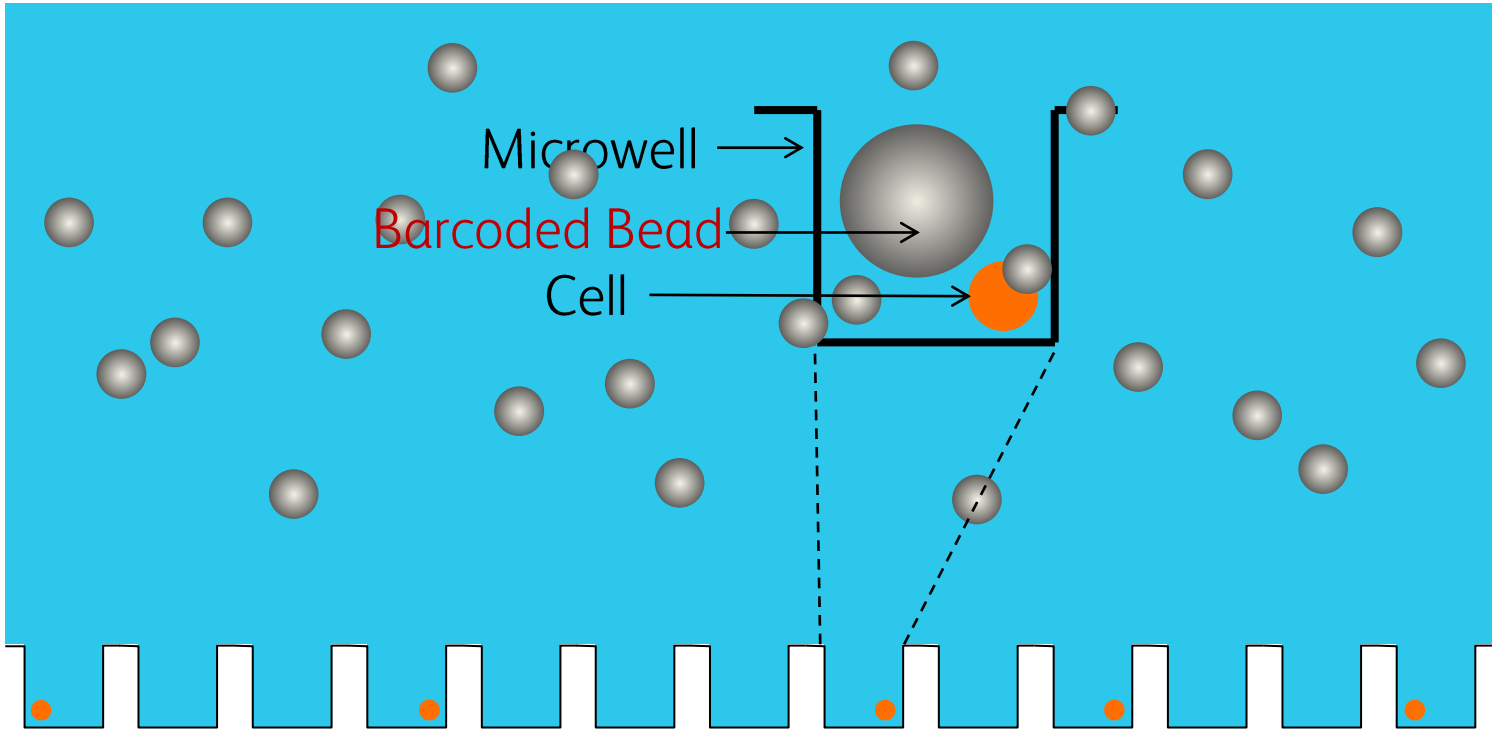
BD Rhapsody™ Cartridge workflow



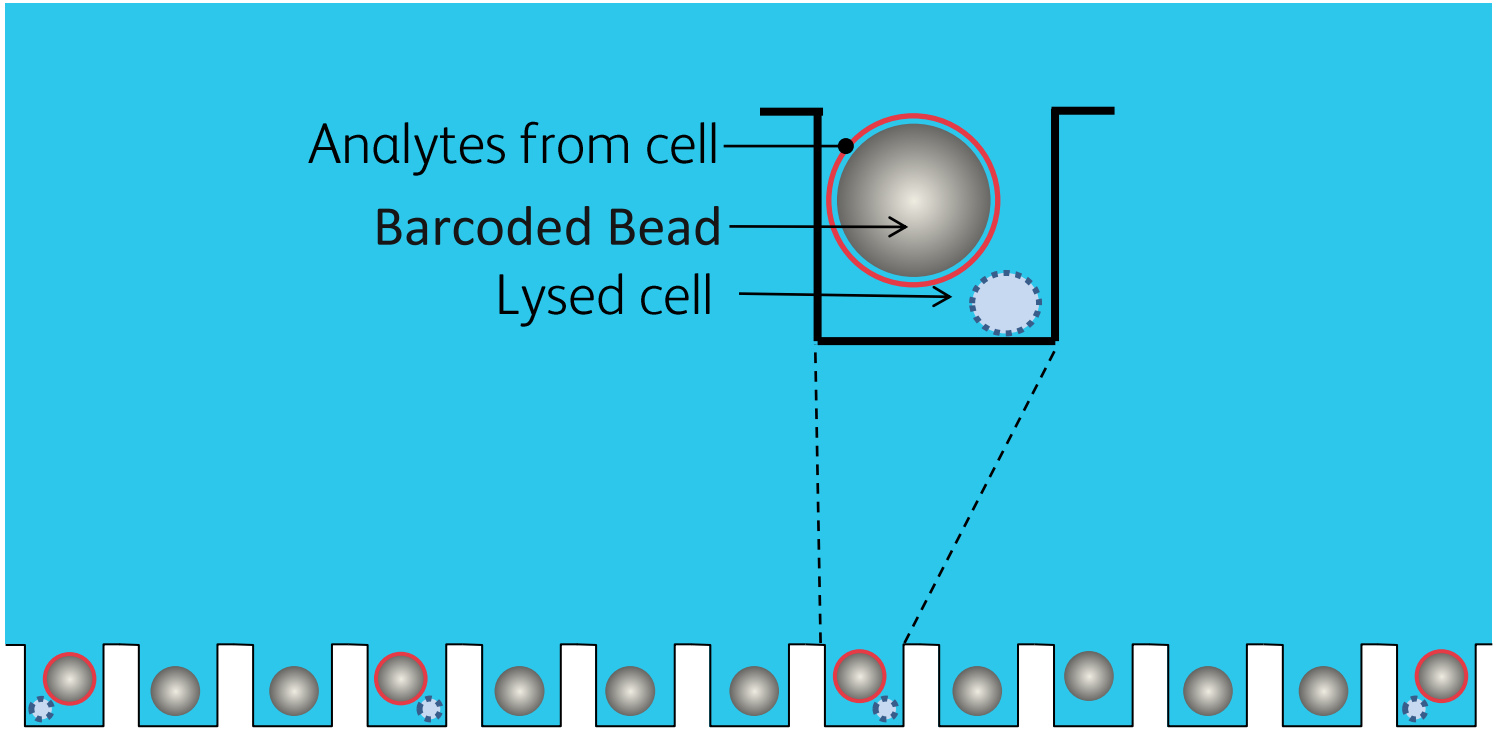


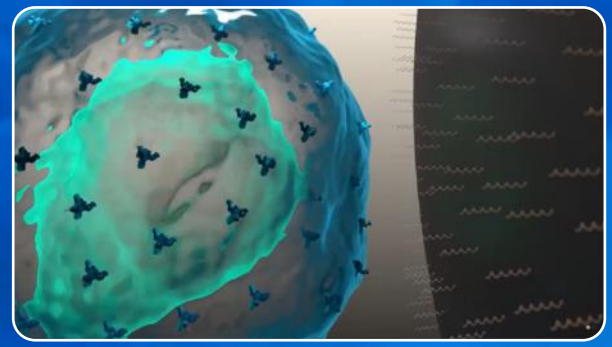


BD Rhapsody™ Cartridge workflow

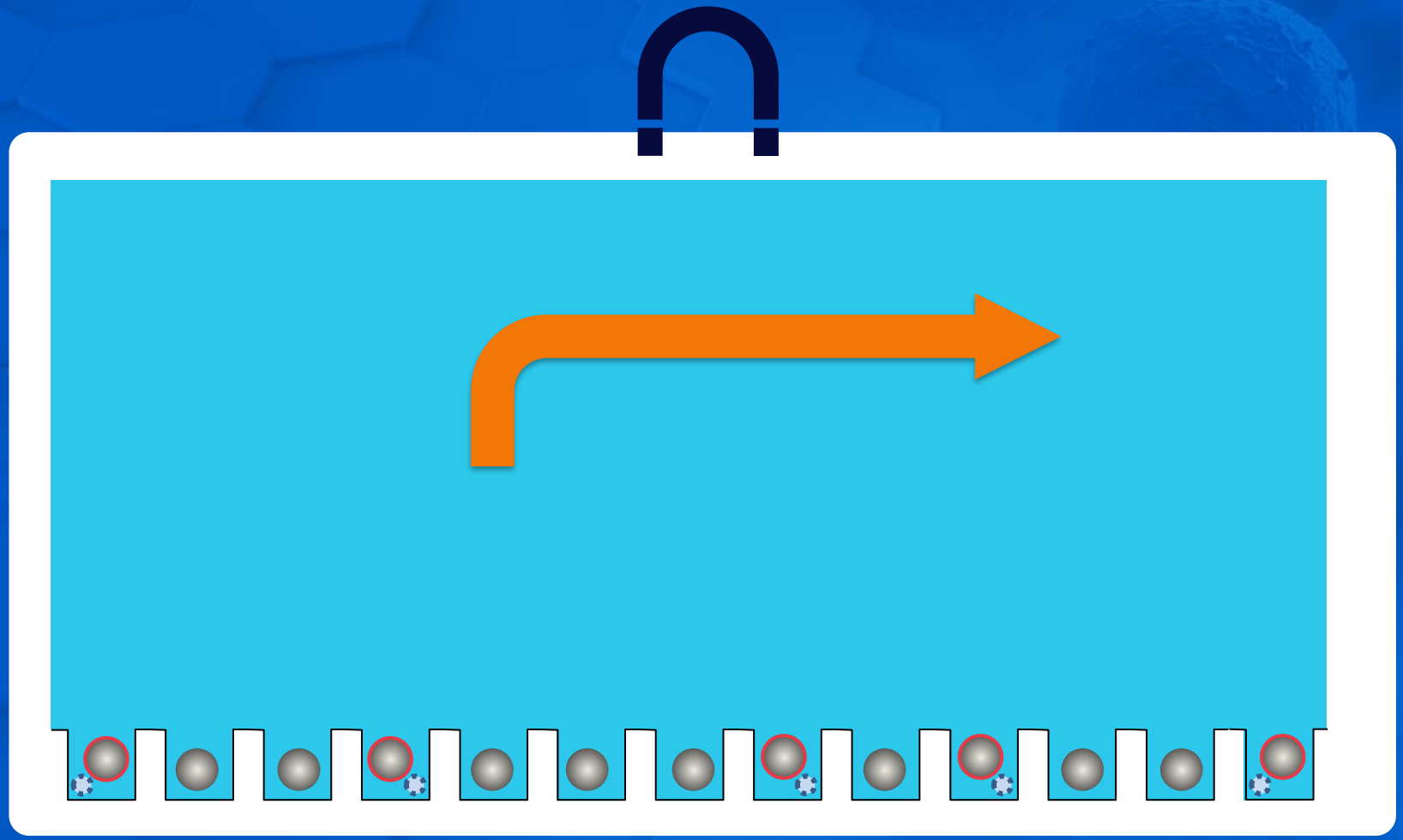


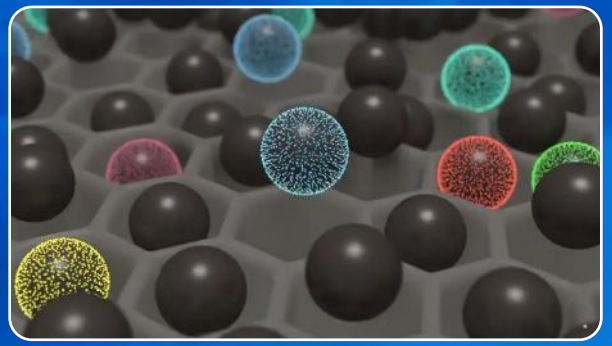
BD Rhapsody™ Cartridge workflow





BD Rhapsody™ Cartridge workflow

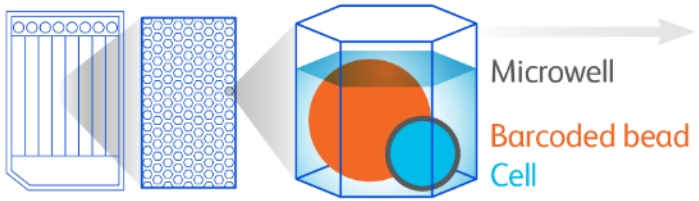




BD Rhapsody™ Cartridge workflow overview

Load cells and beads

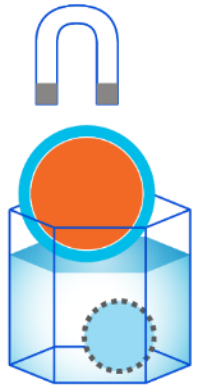
Pair ONE cell with ONE barcoded bead in microwell



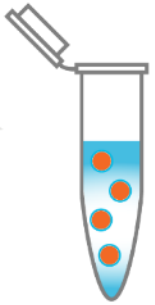
Lyse cells

Lyse cell to hybridize mRNA onto barcoded capture oligos on bead

Retrieve beads

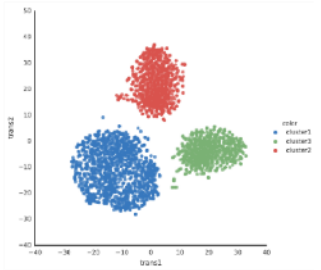


Synthesize cDNA



Analyze data

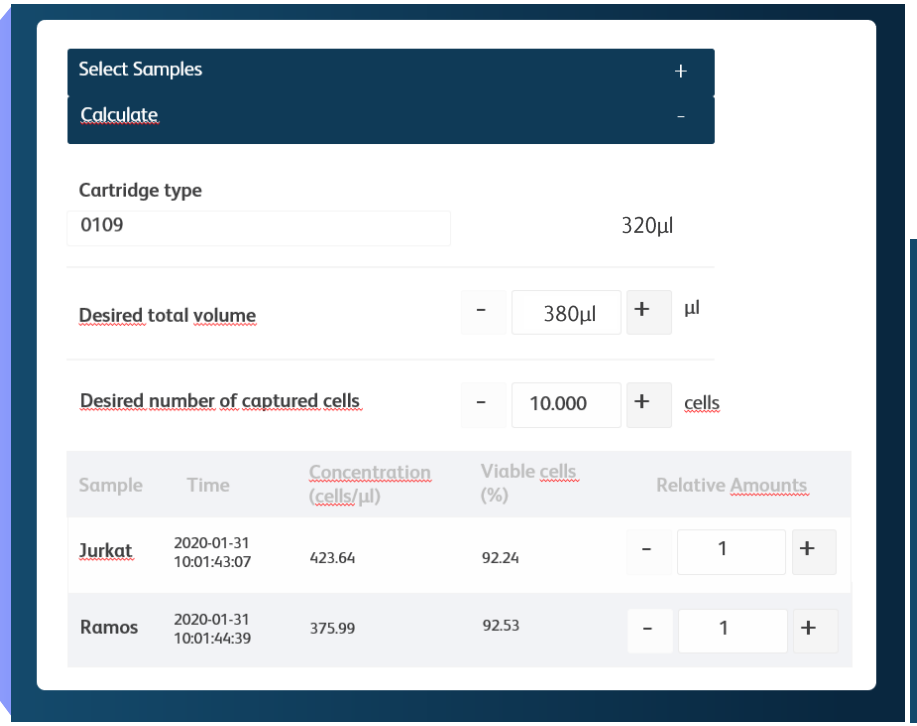
Library preparation, sequencing and data analysis

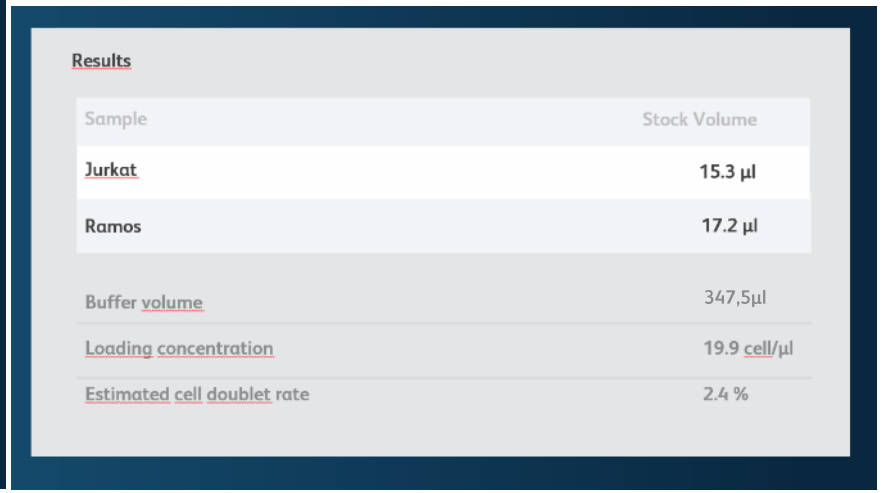


The BD Rhapsody™ Single-Cell Analysis System workflow

Why microwells to capture single cells?

BD Rhapsody™ Scanner: Visual sample QC



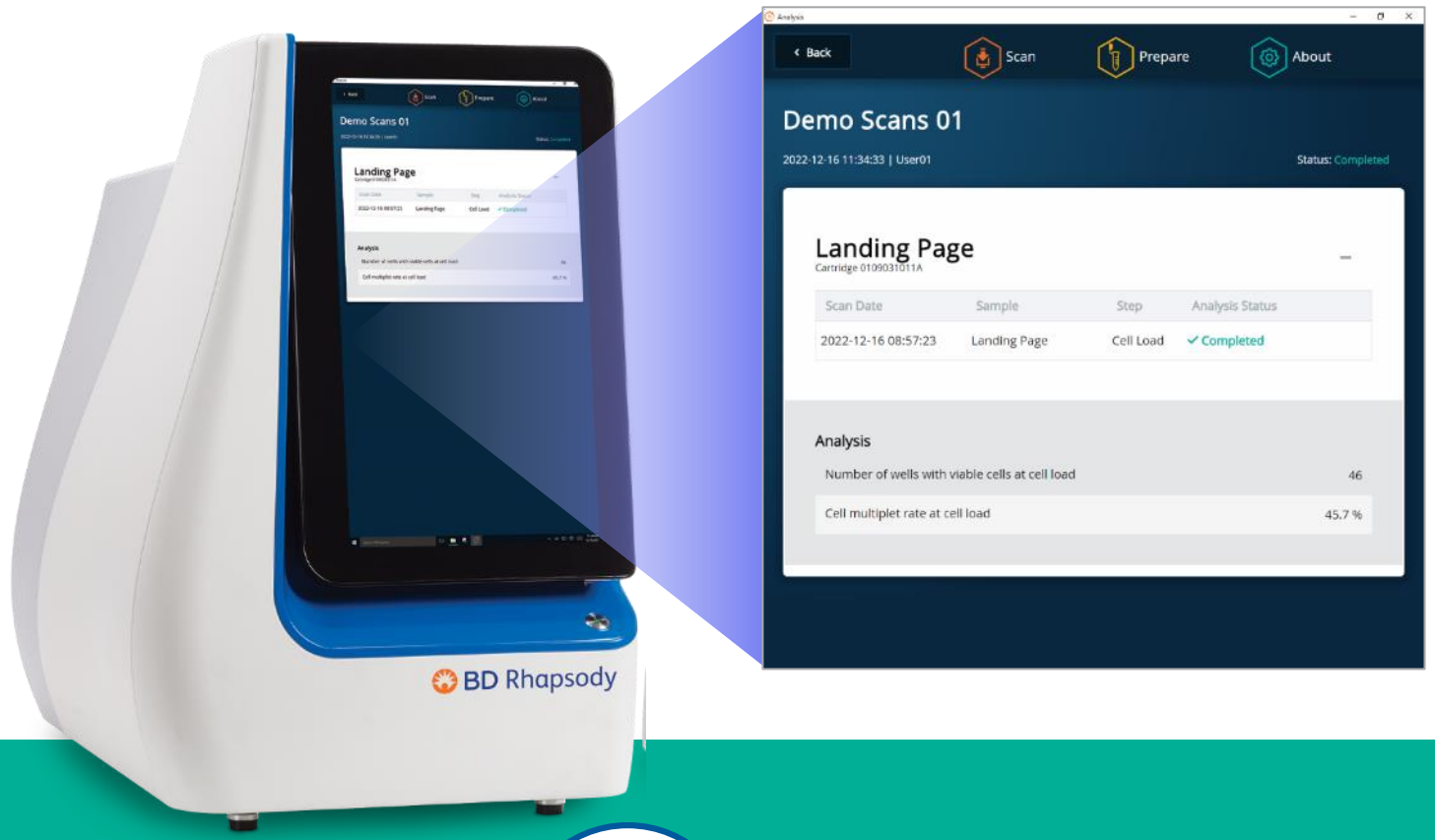


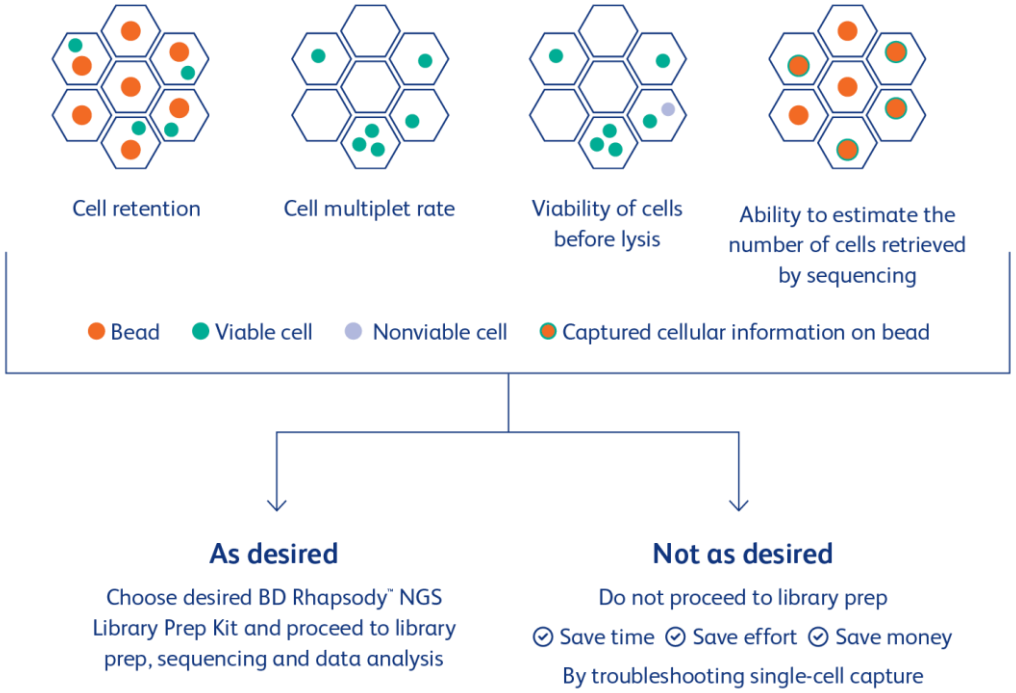
Visual sample QC

Measurement of both, total cell concentration and percentage of viable cells

System automatically calculates loading scheme for desired cell number

BD Rhapsody™ Scanner: Visual workflow QC





Visual workflow QC

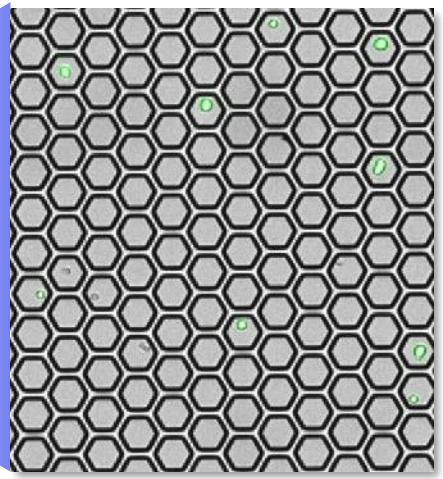
Make real-time decisions before sequencing

Be certain about your cell capture with every single-cell experiment

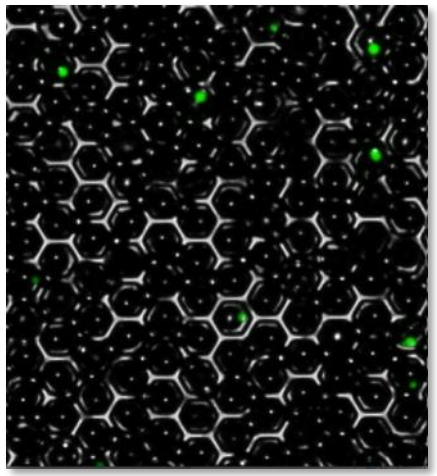
Save time and cost on expensive downstream sequencing

BD Rhapsody™ Scanner: Visual sample QC

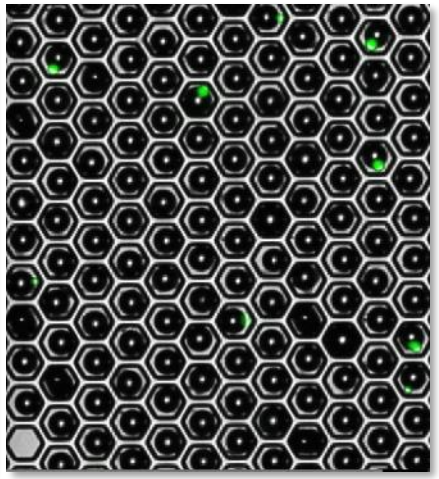
1. Cell loading



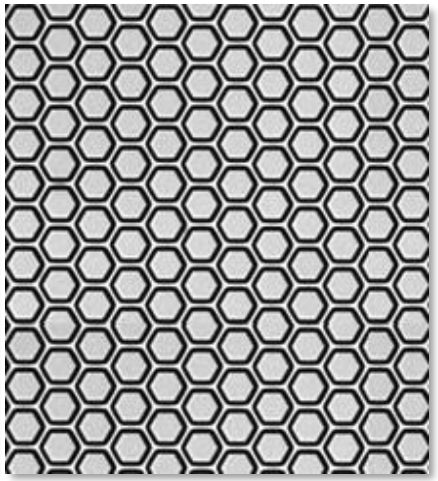
2. Bead loading



3. Remove bead excess



4. Retrieve beads



Visual workflow QC

Make real-time decisions before sequencing

Be certain about your cell capture with every single-cell experiment

Save time and cost on expensive downstream sequencing

BD Rhapsody™ Scanner: Visual sample QC

Analysis	
Number of wells with viable cells at cell load	9.118
Cell multiplet rate at cell load	2.4%
Number of wells with viable cells and a bead	8.399
Cell multiplet rate	2.0%
Bead loading efficacy	✓ PASS
Excess bead rate	✓ PASS
Cell retention rate	✓ PASS
Bead retrival efficiency	✓ PASS

Visual workflow QC

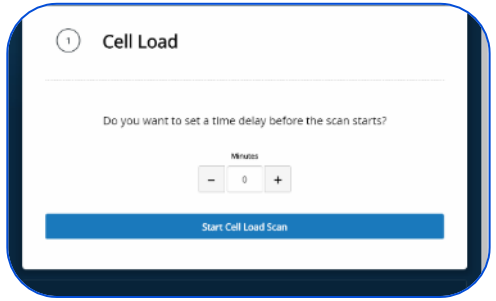
Make real-time decisions before sequencing

Be certain about your cell capture with every single-cell experiment

Save time and cost on expensive downstream sequencing

Intuitive user interface for a multi-sample workflow

The BD Rhapsody™ Scanner can be used to provide quality control measures at different stages of the workflow by direct imaging through an intuitive user interface for a multi-sample workflow.



Experimental setup – Enter sample and experiment information to track samples through single-cell workflow

Scan and status – Watch the status of the lanes being scanned in real time

Multi-sample selection – Allow users to select and name up to eight lanes for processing through the cartridge scan workflow

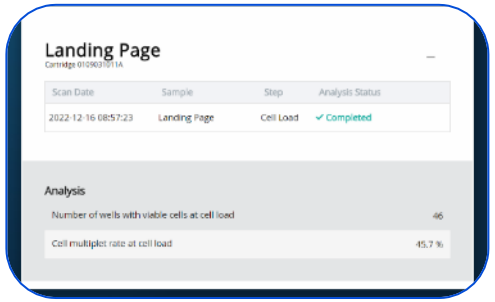
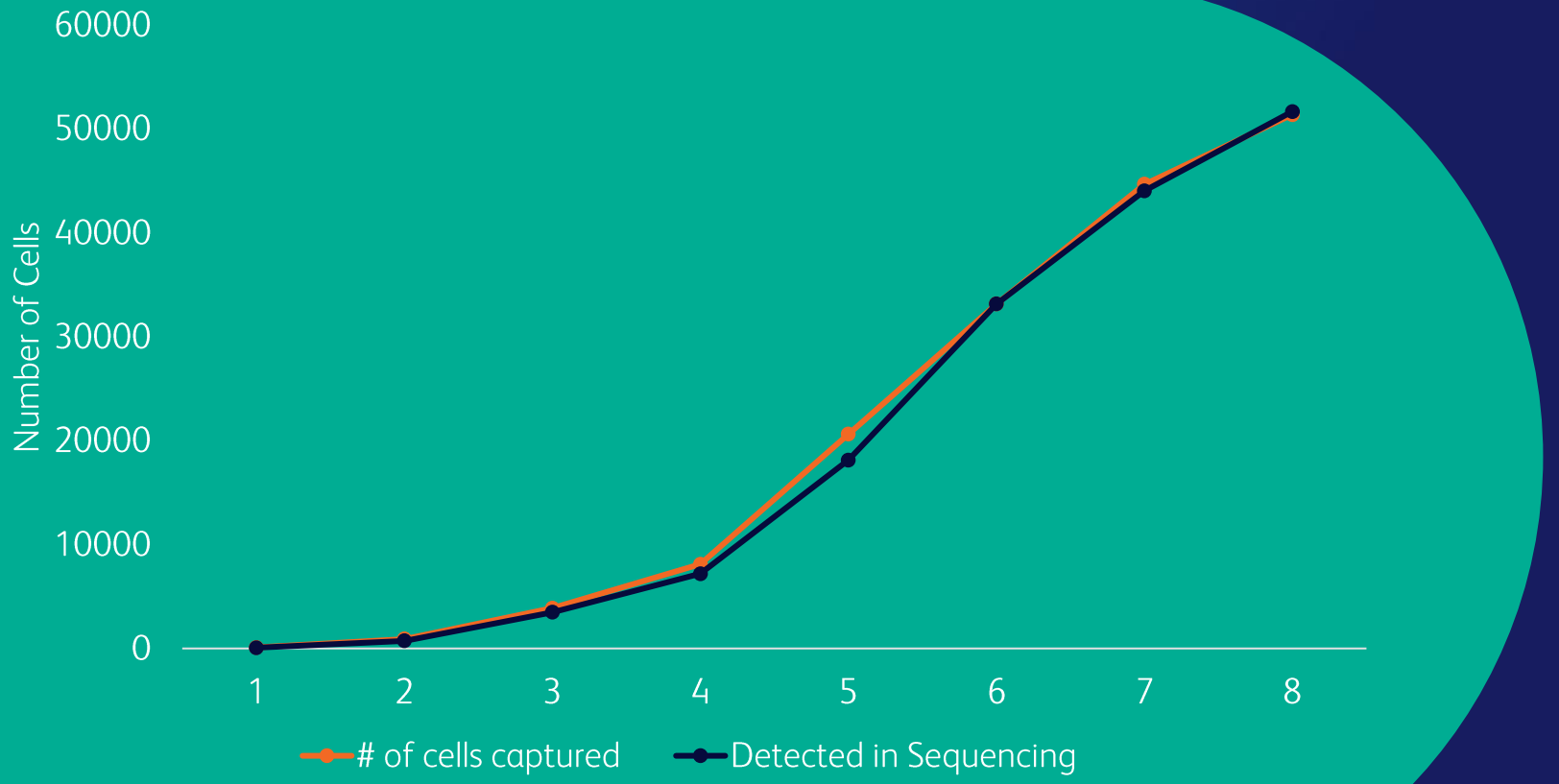


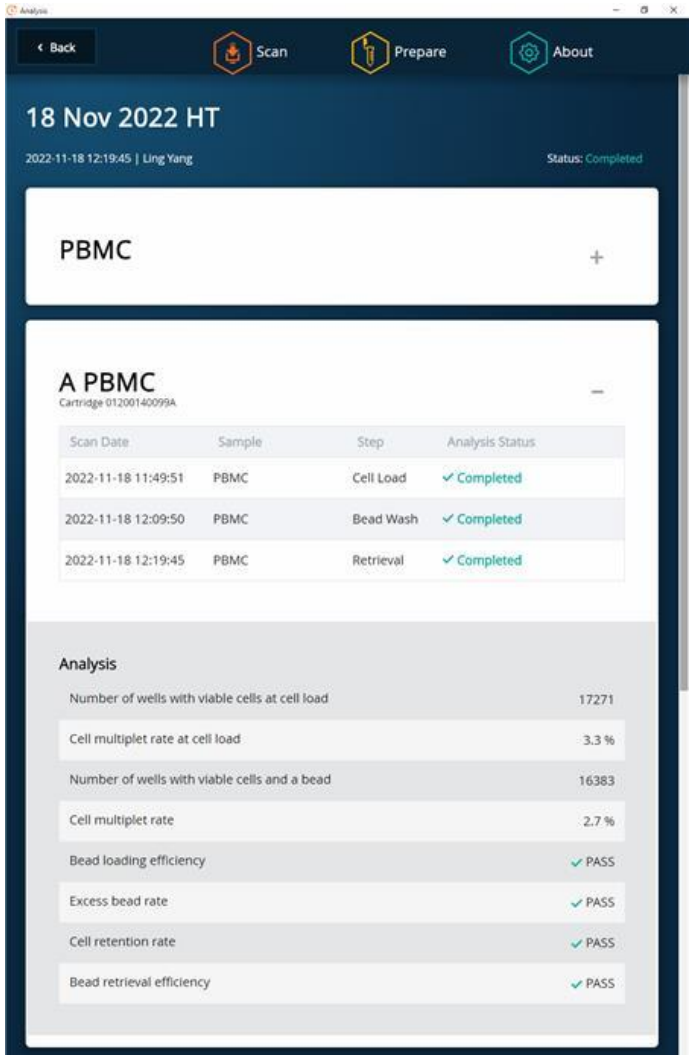
Image analysis – Review the scanner quality metrics and make informed decisions on whether to proceed with library prep

High correlation of scanner metrics with cells recovered by sequencing



Two cell types (Jurkat and Ramos) were pooled at 1:1 ratio. An 8-lane cartridge was loaded with 100, 1,000, 5,000, 10,000, 25,000 and another 8-lane cartridge was loaded with 40,000, 55,000 and 65,000 cells. The number of cells captured reported by the BD Rhapsody™ Scanner correspond to the number of putative cells detected after sequencing libraries using the BD Rhapsody™ Targeted mRNA Kit with the BD Rhapsody™ Immune Response Panel (Hs) at different cell load concentrations. Results may vary based on cell type and isolation method.

Analysis application and metrics at every step

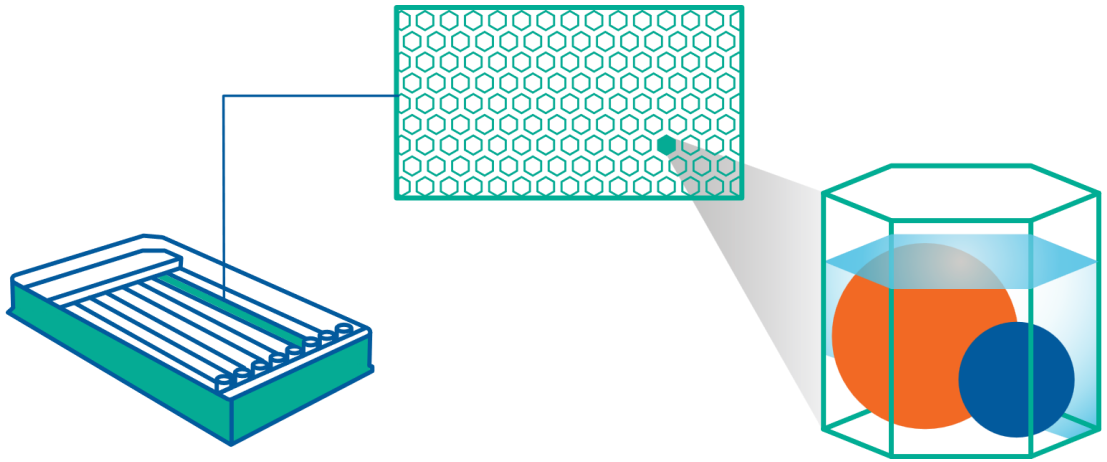


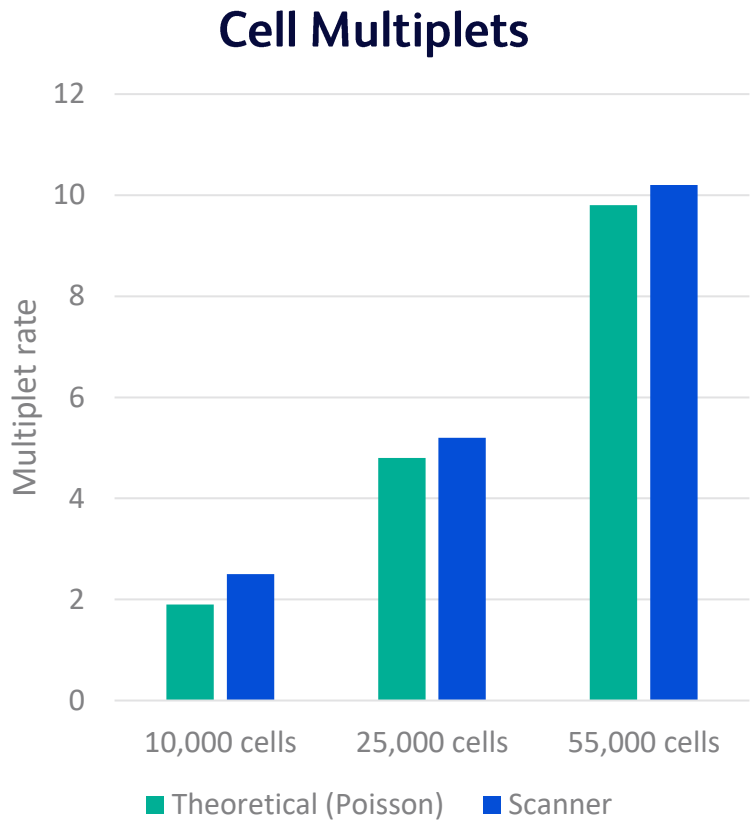
Scanner metric	Use
Number of wells with viable cells at cell load	Provides a preliminary estimate of the number of wells with viable cells captured
Cell multiplet rate at cell load	Provides a measure of cell clumping
Number of wells with viable cells and a bead	Provides an estimate of the number of wells with viable cells captured with beads
Cell multiplet rate	Provides an estimate of the occurrence of multiple cells captured by the same bead in a well
Bead loading efficiency	Indicates if the cartridge is significantly underloaded with beads
Excess bead rate	Indicates the percentage of the beads that are not in wells
Cell retention rate	Indicates if a significant number of cells initially loaded into wells are lost or died during the workflow prior to cell lysis
Bead retrieval efficiency	Indicates if the number of beads retrieved is significantly lower than expected

High cell capture and low multiplet rate across cell inputs

Desired number of cells	Live cells loaded**	Viable cells captured in well with a bead	Capture rate
55,000*	57,749	45,412	0.79
25,000*	26,256	20,977	0.80
10,000*	10,506	8,410	0.80

*Mix of PBMC, Jurkat, Ramos and THP1 cells
**BD Rhapsody™ Scanner hemocytometer count

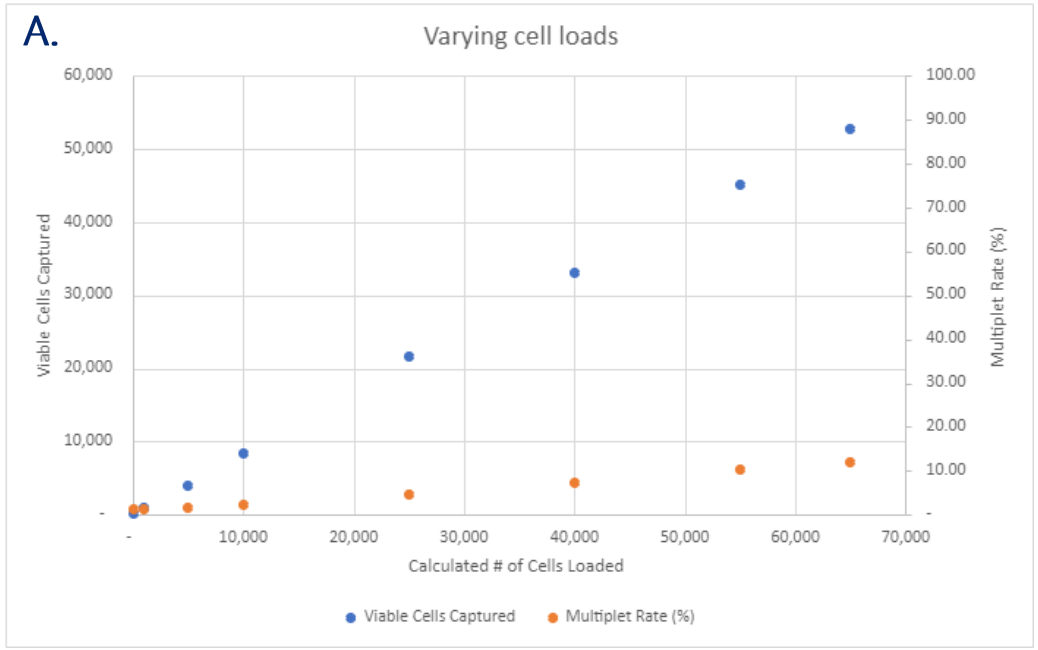


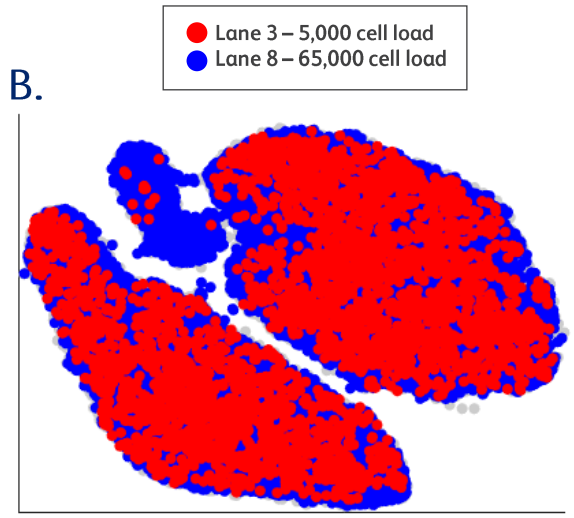


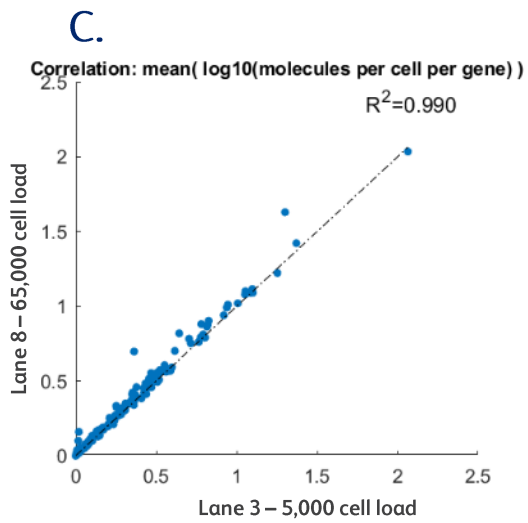
Four cell types (PBMCs, Jurkat, Ramos and THP1) were pooled and loaded in duplicate at 10,000, 25,000 or 55,000 cells per lane on an 8-lane cartridge. Cell capture rates were high and multiplet rates were low at all cell load concentrations. The BD Rhapsody™ Scanner provides a measure of actual multiplet rate for cells loaded onto each lane in the 8-lane cartridge. Capture rates from the scanner were recorded up to 80%. The mutiplet rate for 55,000 cell input was 10.2%. Results may vary based on cell type and isolation method.

Broad range of cell input per lane

Varying cell loads: 100 cells to 65K cells per lane, with up to 80% capture







Multiplet rate follows Poisson rates

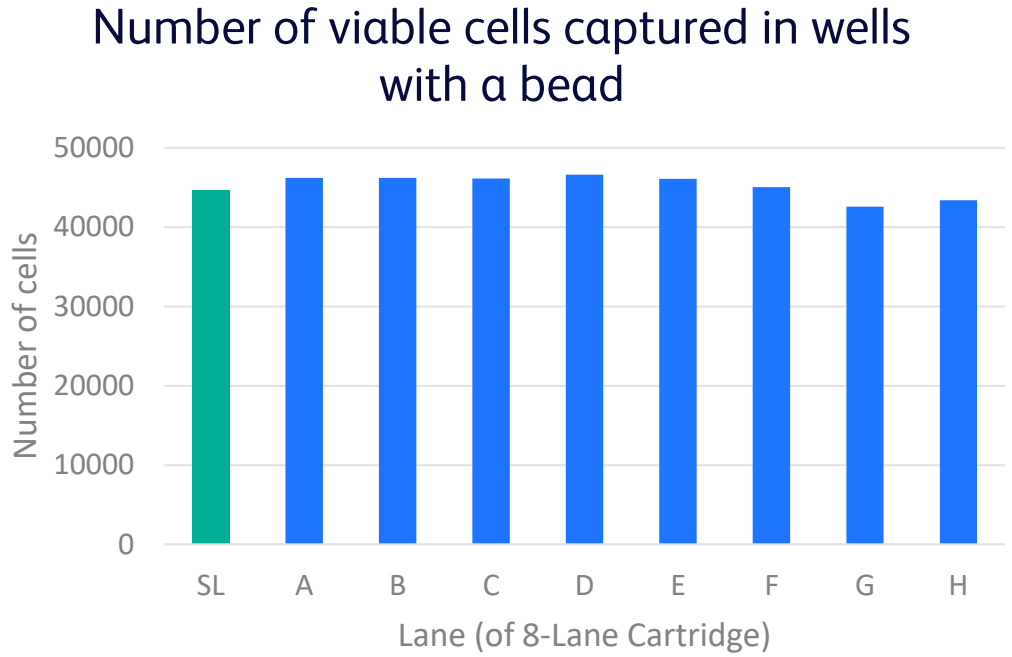
No batch effect between varying cell loads on different lanes

Two cell types (Jurkat and Ramos) were pooled at 1:1 ratio. Each lane on an 8-lane cartridge was loaded with 100, 1,000, 5,000, 10,000, 25,000, 40,000, 55,000 and 65,000 cells. **A)** Viable cells captured correspond to the increasing number of cells loaded and multiplet rates were low at all cell load concentrations. The BD Rhapsody™ Scanner provides a measure of actual multiplet rate for cells loaded onto each lane in the 8-lane cartridge. **B–C)** No batch effect was observed, and the correlation of gene expression was high between the 5,000 and 65,000 cell loads using the BD Rhapsody™ Targeted mRNA Kit with the BD Rhapsody™ Immune Response Panel (Hs). Results may vary based on cell type and isolation method.

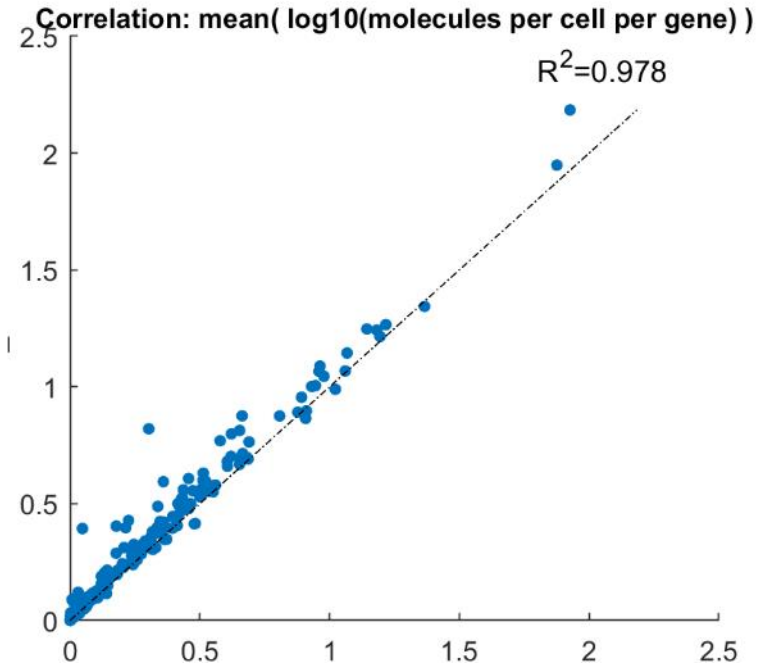
Million cell studies now possible

>40K cells per lane or >320K viable single cells captured across 8 lanes

A.



B.



- Up to 80% capture rate
- Multiplet rate comparable to single-lane BD Rhapsody™ Express System
- Less than 0.5% cell label collision enabled by higher diversity beads.
- NO batch effect: High gene expression correlation between lanes and with single-lane BD Rhapsody™ System

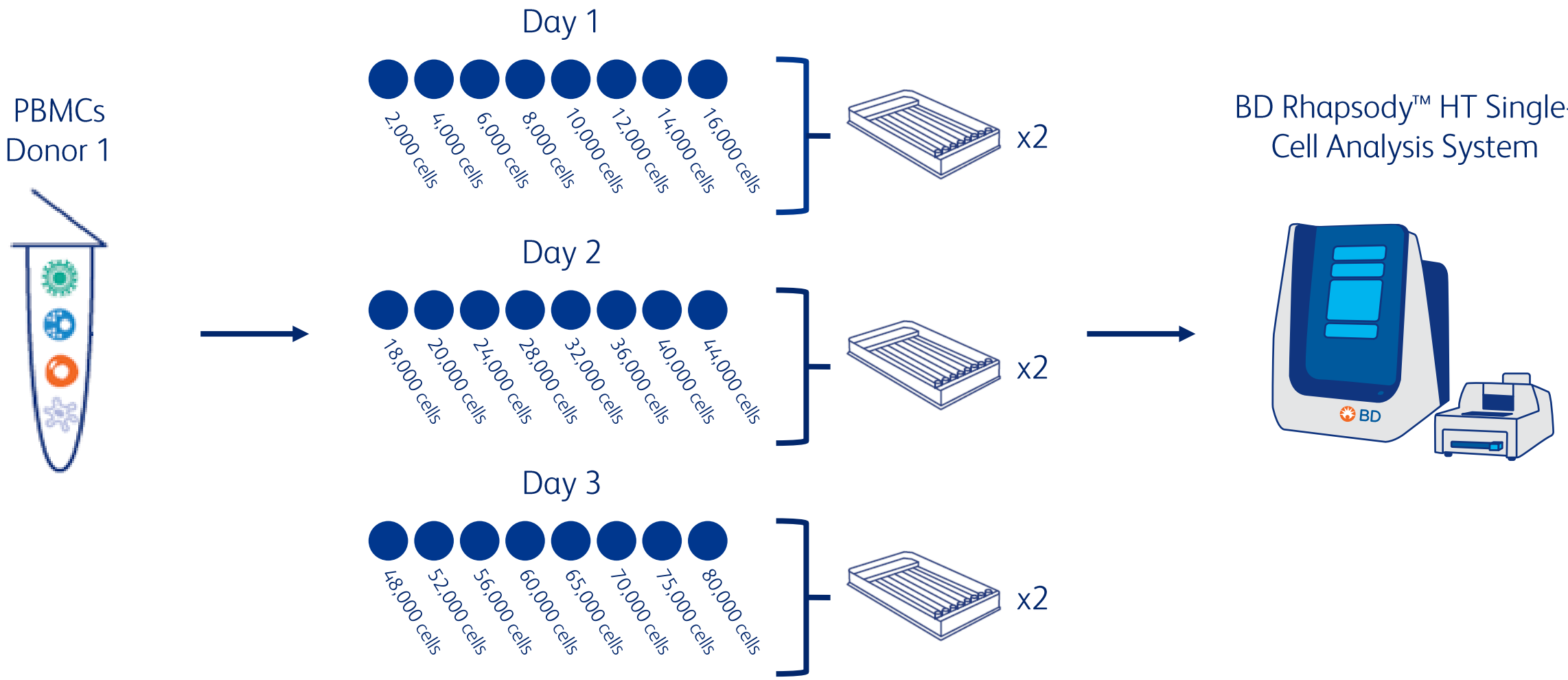
Two cell types (Jurkat and Ramos) were pooled and stained with sample tag and loaded at 55,000 cells per lane on an 8-lane cartridge versus a single-lane cartridge. **A)** The throughput of the 8-lane cartridge is upwards of 320,000 cells or greater than 40,000 cells per lane. The BD Rhapsody™ Scanner provides the number of viable cells captured in wells with a bead in the 8-lane cartridge, showing up to 80% capture rate. **B)** In addition, there is high gene expression correlation across lanes when compared to single-lane cartridge.

Capture over a million cells and store your samples on BD Rhapsody™ Beads for a year

BD Rhapsody™ HT Xpress System

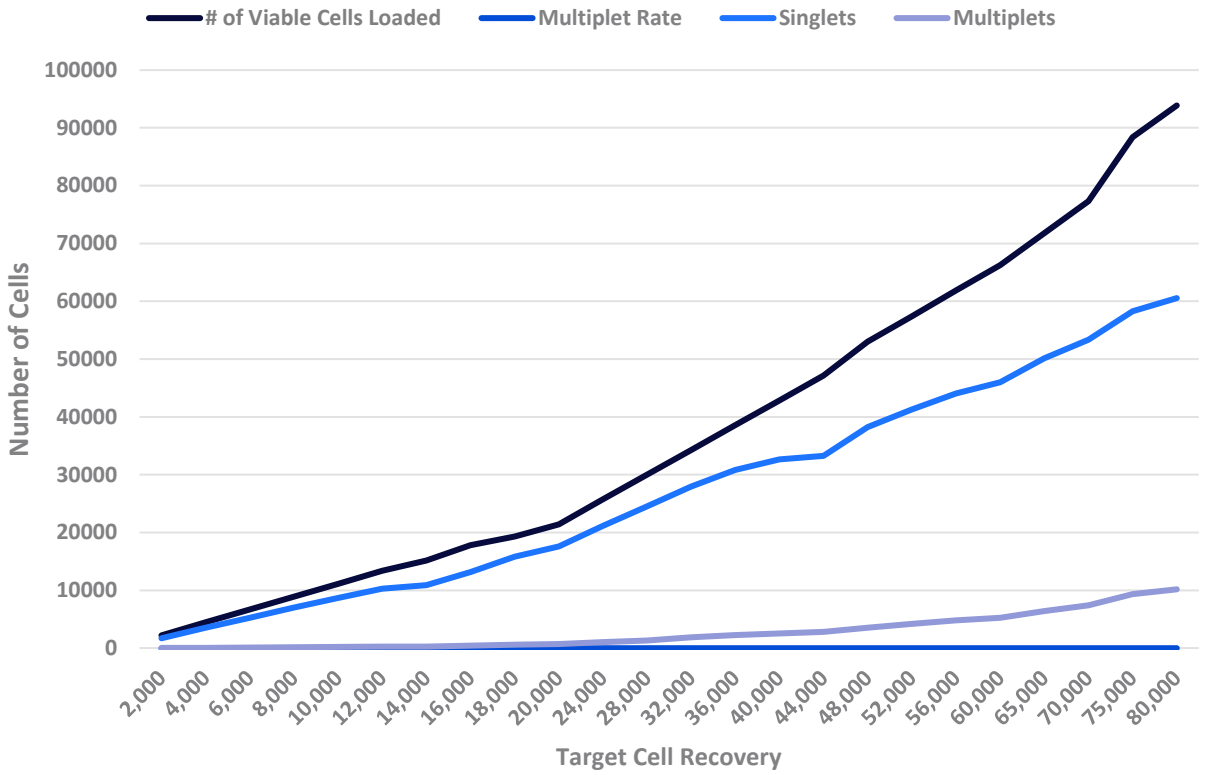
Expanded range of cell input experimental design

Process more than a million cells across 2 cartridges



Expanded range of cell input per lane

Varying cell loads: 2,000 cells up to 80K cells per lane, with up to 80% capture

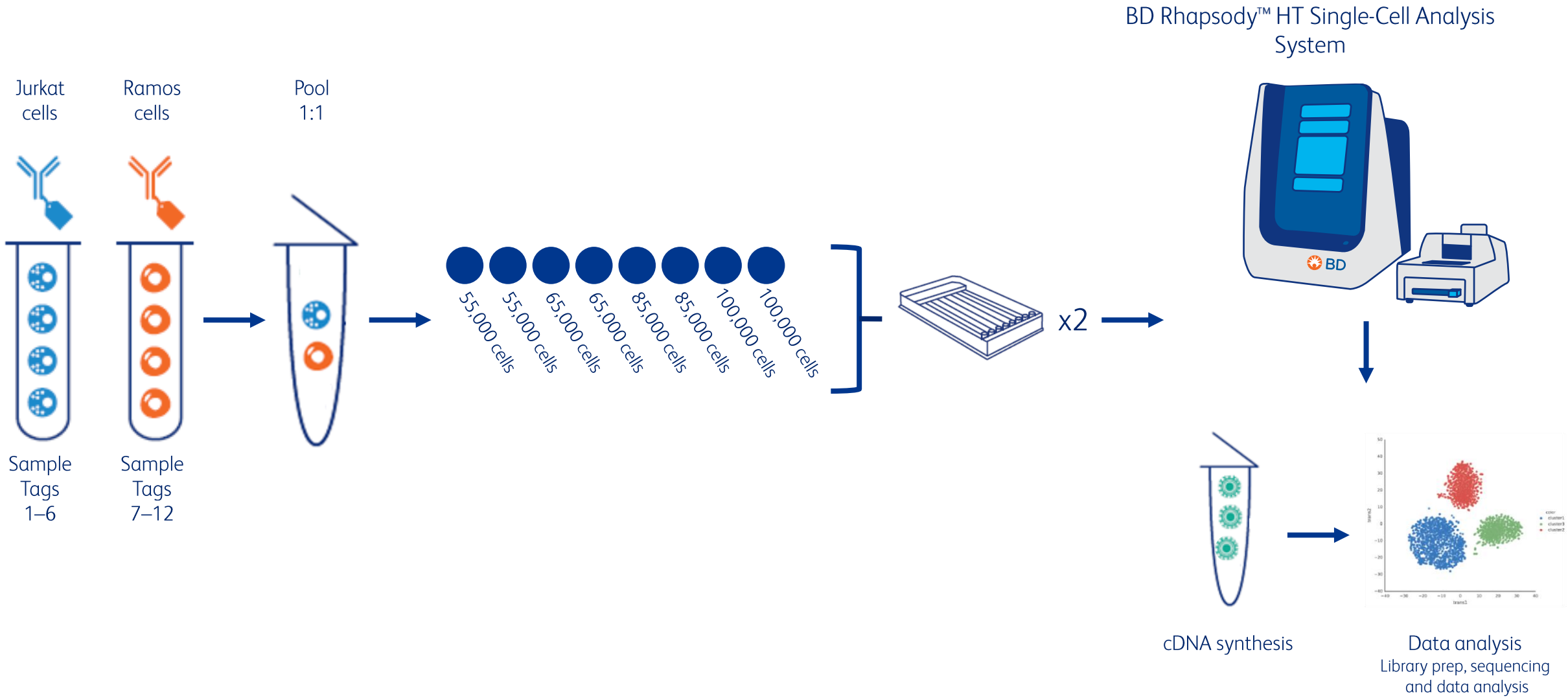


Desired number of cells	Live cells loaded**	Multiplet rate	Singlets	Multiplets	Capture rate
2,000	2,213	0.85%	1,692	14	0.77
8,000	8,902	1.95%	7,027	140	0.81
14,000	15,168	2.50%	10,869	279	0.73
20,000	21,413	3.80%	17,578	694	0.85
32,000	34,271	6.20%	27,927	1,846	0.87
44,000	47,130	7.85%	33,257	2,830	0.77
56,000	61,860	9.80%	44,052	4,787	0.79
70,000	77,317	12.20%	53,356	7,414	0.79
80,000	93,868	14.40%	60,550	10,186	0.75

*PBMCs
**BD Rhapsody™ Scanner hemocytometer count

A graphical and tabular representation of singlets and multiplets recovered across a range of targeted cell loads. Human PBMCs were prepared and captured using the BD Rhapsody™ HT Single Cell Analysis System. Data were collected from two 8-lane cartridges loaded separately by individual users on the same day for each of the different cell input loads, for a total of six 8-lane cartridge for the entire range of cell input. The number of cells sequenced might be less than the number of cells captured due to bead loss during handling, assay choice and sample composition. The number of viable cells captured in the cartridge might be less than the targeted number of captured cells if the viability of the sample is <100%.

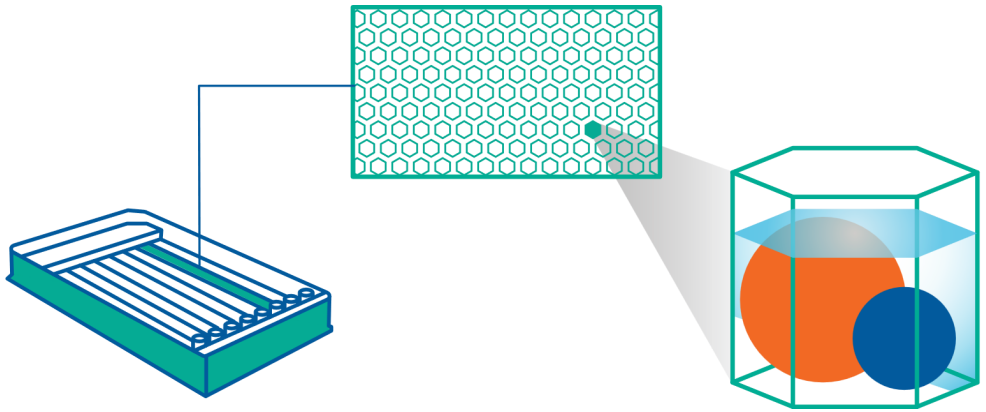
Sample multiplexing to test signal preservation during high cell loading

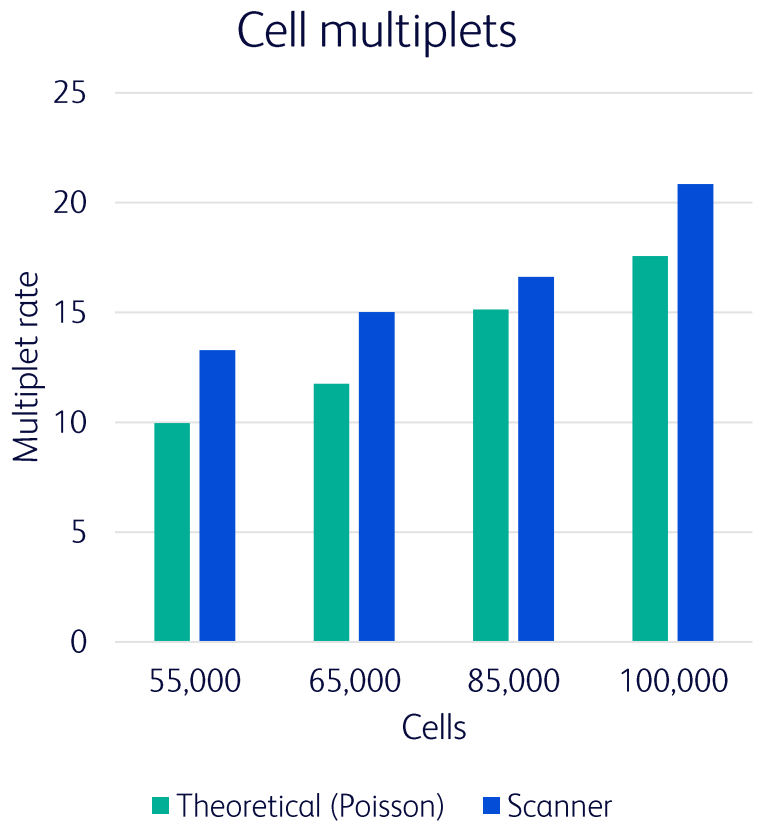


High cell capture and low multiplet rate across high cell inputs

Desired number of cells	Live cells loaded**	Viable cells captured in well with a bead	Capture rate
100,000*	106,156	86,919	0.82
85,000*	90,197	76,621	0.85
65,000*	68,996	58,839	0.85
55,000*	58,385	49,956	0.86

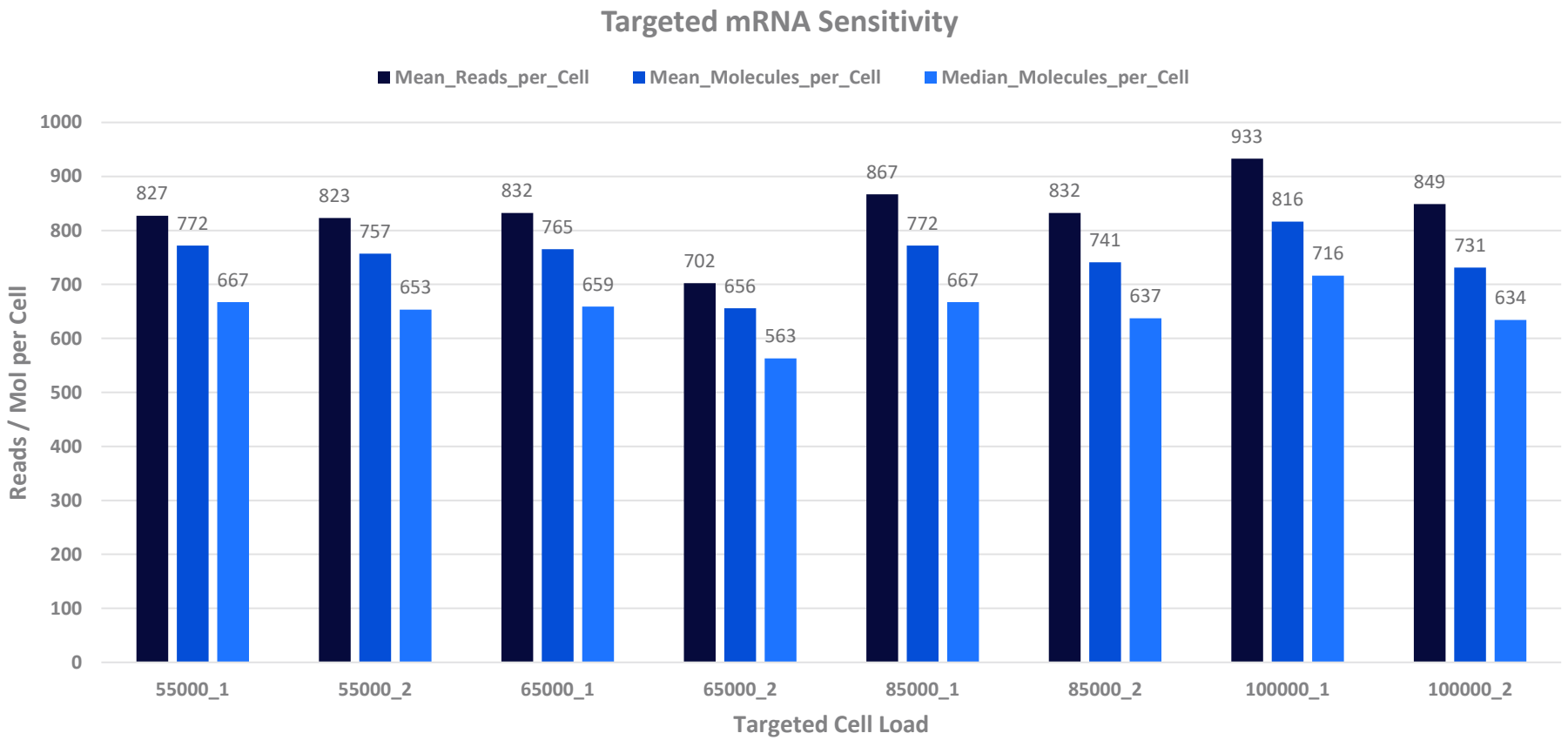
*Mix of Jurkat and Ramos cells
**BD Rhapsody™ Scanner hemocytometer count





Two cell types (Jurkat, sample tag 1–6 and Ramos, sample tag 7–12) were sample tagged, pooled and loaded in duplicate at 55,000, 65,000, 85,000 or 100,000 cells per lane on two 8-lane cartridges. Cell capture rates were high and multiplet rates were low at all cell load concentrations. The BD Rhapsody™ Scanner provides a measure of actual multiplet rate for cells loaded onto each lane in the 8-lane cartridge. Capture rates from the scanner were recorded over 80%. The mutiplet rate for 100,000 cell input was 20.9%. Results may vary based on cell type and isolation method.

Sample multiplexing to test signal preservation during high cell loading



Two cell types (Jurkat, sample tag 1–6 and Ramos, sample tag 7–12) were sample tagged, pooled and loaded in duplicate at 55,000, 65,000, 85,000 or 100,000 cells per lane on an 8-lane cartridge, ran in duplicate. BD Rhapsody™ Targeted mRNA Kit and Sample Tag libraries were prepared with no bead sub-sampling. Similar molecule detection normalized using the same number of sequencing reads per cell on target cell loads up to 100,000 single cells per lane. Multiplets from sample multiplexing were bioinformatically removed. Results may vary based on cell type and assay used.

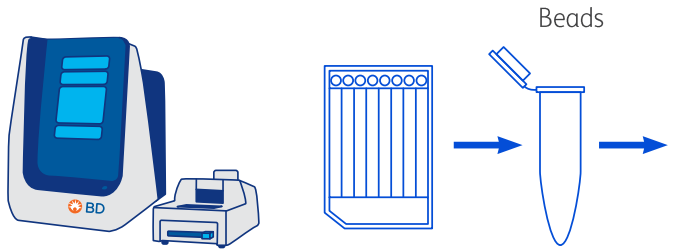
Sample multiplexing to test signal preservation during high cell loading

Desired number of cells	Sample Tag sensitivity
55,000_1	99.81
55,000_2	99.88
65,000_1	99.92
65,000_2	99.94
85,000_1	99.92
85,000_2	99.96
100,000_1	99.92
100,000_2	99.01

Sample Tag specificity for 100,000_1 Cell Input					
Sample Tag	Jurkat	Ramos	Sample Tag	Jurkat	Ramos
1	99.69	0.05	7	0.00	99.90
2	99.75	0.02	8	0.03	99.95
3	99.60	0.05	9	0.05	99.78
4	99.63	0.02	10	0.05	99.87
5	99.73	0.00	11	0.02	99.85
6	99.58	0.07	12	0.00	99.95

Two cell types (Jurkat, sample tag 1–6 and Ramos, sample tag 7–12) were sample tagged, pooled and loaded in duplicate at 55,000, 65,000, 85,000 or 100,000 cells per lane on an 8-lane cartridge in duplicate. Sample Tag sensitivity was >99%. Sample Tag specificity from a 100,000 targeted cell load was >99% showing minimal to no cross-talk between Sample Tags used on different cell types.

Single-cell data obtained from BD Rhapsody™ Beads stored for 1 year

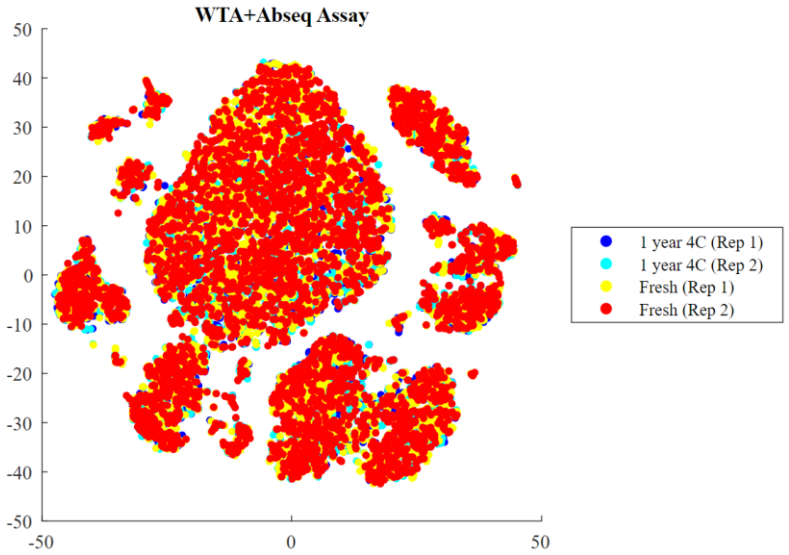


Beads

Split beads:

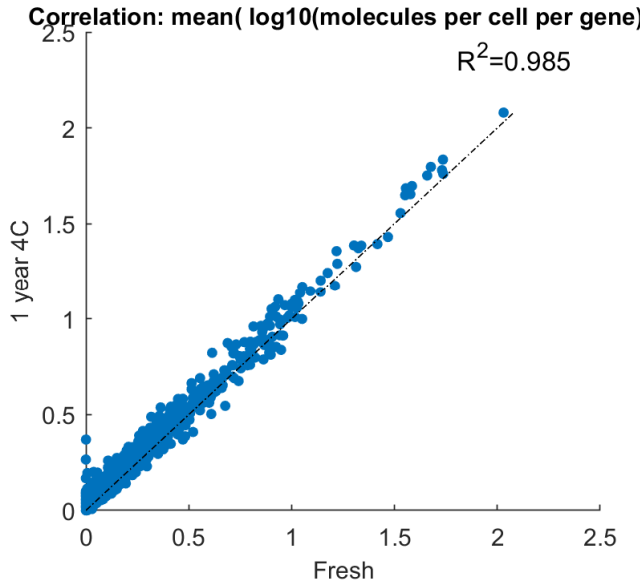
- Fresh
- 1 year (4 °C)

A.

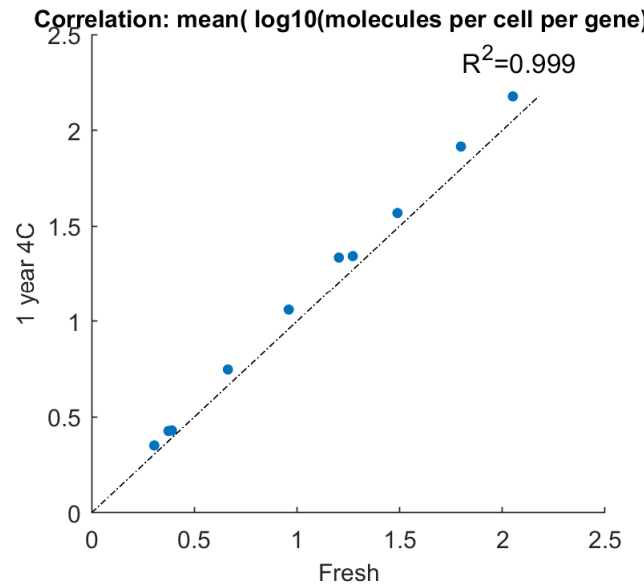


B.

WTA



AbSeq



PBMCs were labeled with AbSeq (10-plex), loaded and captured on duplicate lanes of an 8-lane cartridge. Beads were stored after cDNA synthesis and libraries using the BD Rhapsody™ WTA Kit together with BD® AbSeq Assay were prepared after 1 year compared to control libraries prepared with no storage. **A)** No batch effect was observed and **B)** correlation of gene expression was high between the samples tested.

BD Rhapsody™ System quality, higher throughput

Flexible cartridge design

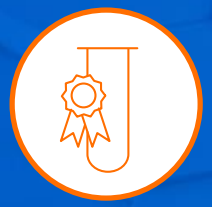
Up to 8 tests per cartridge

Partial use cartridge

Run more or different types of experiments

Process samples together or on different days

Maintain sample integrity



Gentle microwell technology

No sample loss due to clogging of channels

Recover cells with disparate size and morphology, including fragile cell types

Minimal batch effects

Expanded throughput

Easily run million-cell studies

Low multiple rate

Capable of capturing >1M cells

Up to 80% cell capture rate

Visual workflow QC

Save time and sequencing cost

Make real-time decisions before sequencing

Be certain about your cell capture with every single-cell experiment

Subsample and archive beads

Store your processed samples on beads for a year

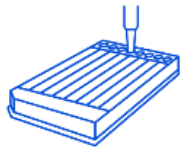
Tool to measure reliability

Supports a collaborative workflow approach

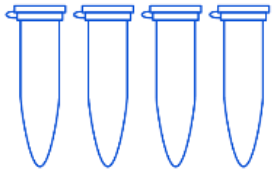
Fail safe for poor or failed library preps

Workflow timing for one test in a BD Rhapsody™ 8-Lane Cartridge

Prepare cartridge
14 min



Cell labeling (optional)
Time varies

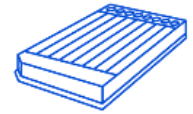


Cell staining and counting
25 min

Stain with Calcein AM and Draq7™

20 min

Count cells

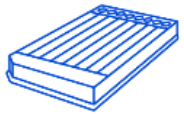


3 min

Review results on scanner

Single-cell capture
50 min

Prep/load cells and prepare cell capture beads



16 min

Image on scanner **Cell load**

4 min

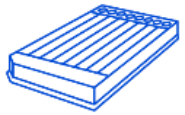
Load beads and image on scanner **Bead Load**

7 min

Perform two bead washes and image on scanner **Bead Wash**

6 min

Lyse and retrieve beads



4 min

Image on scanner **Retrieval**

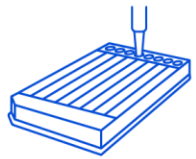
3 min

Wash cell capture beads

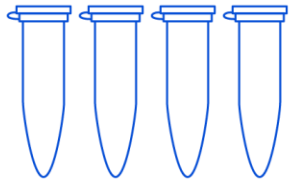
10 min

Workflow timing for eight tests in a BD Rhapsody™ 8-Lane Cartridge

Prepare cartridge
10-15 min



Cell labeling (optional)
Time varies

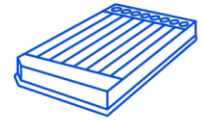


Cell staining and counting
25 min

Stain with Calcein AM and Draq7™

20 min

Count cells

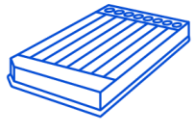


3 min

Review results on scanner

Single-cell capture
52 min

Prep/load cells and prepare enhanced cell capture beads



8 min

Image on scanner **Cell load**

11 min

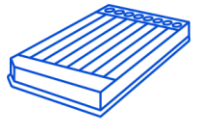
Load beads and perform scanner step **Bead Agitation**

2 min

Perform two bead washes and image on scanner **Bead Wash**

11 min

Lyse and retrieve beads

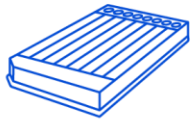


4 min

Image on scanner **Retrieval**

10 min

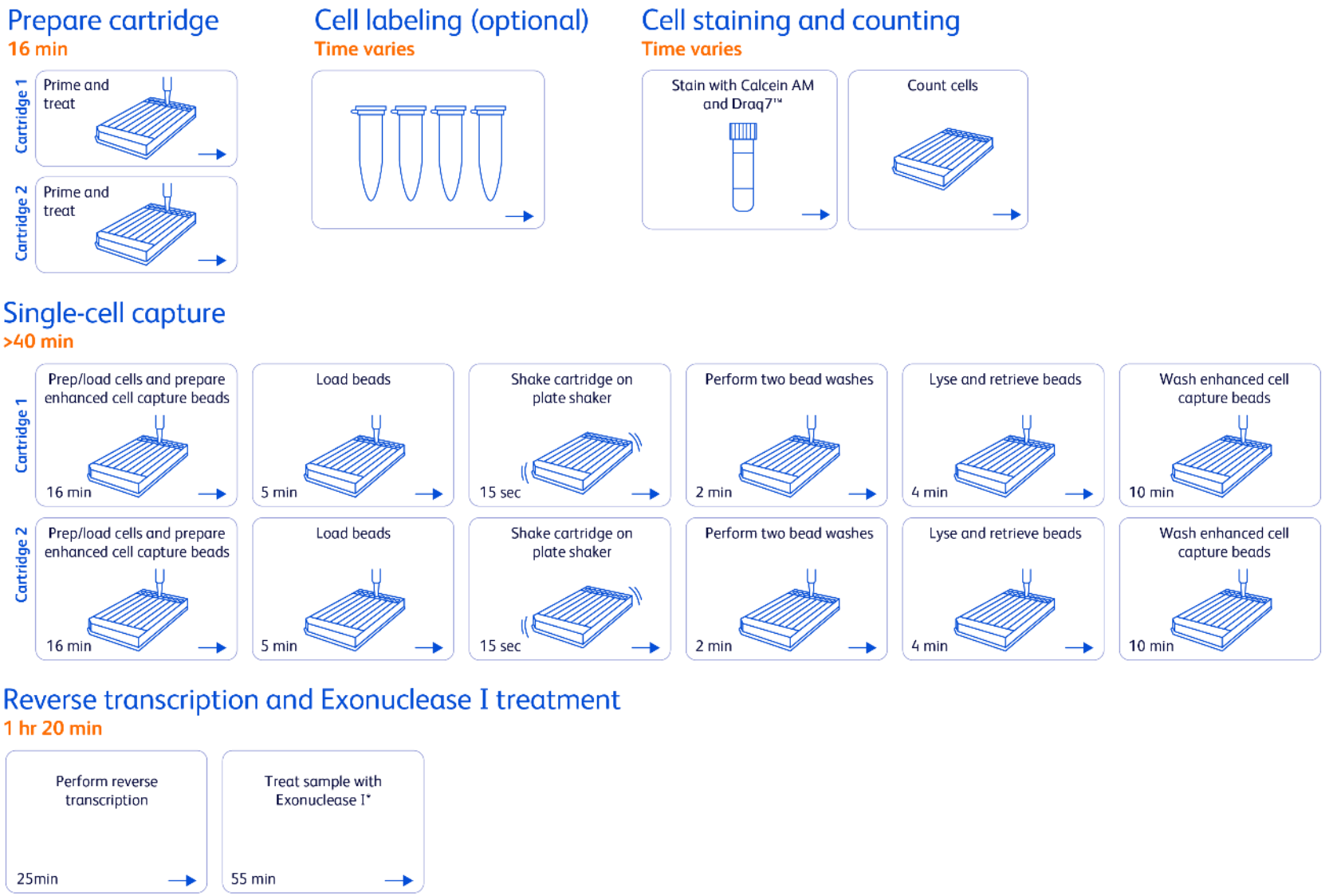
Wash enhanced cell capture beads



variable

Running two BD Rhapsody™ 8-Lane Cartridges in parallel

2x BD Rhapsody™ 8-Lane Cartridges can be processed in parallel in under 2.5 h



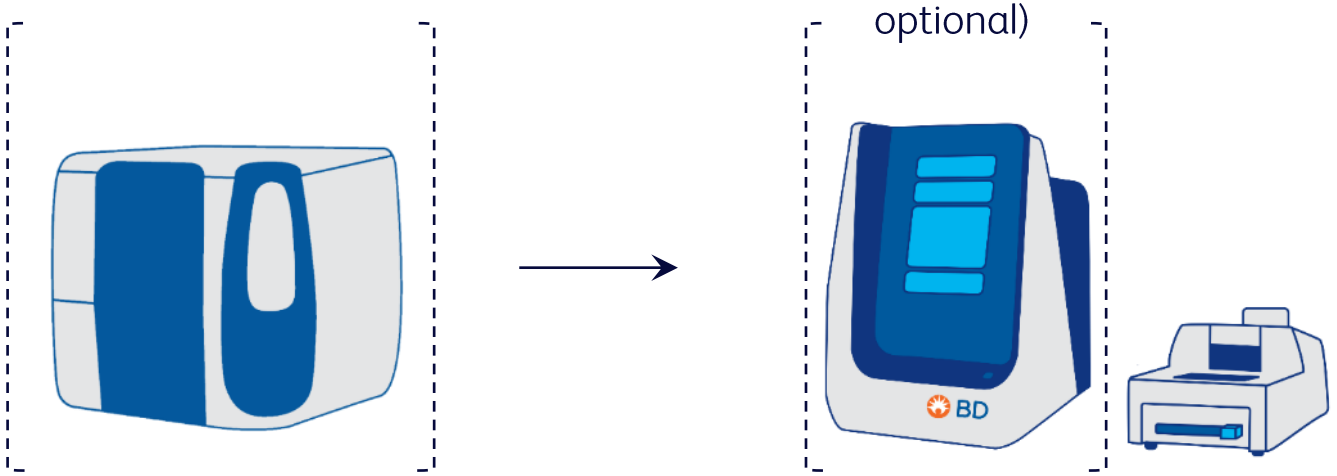
BD Rhapsody™ System: Decentralize single-cell multiomics

BD Rhapsody™ HT Single-Cell Analysis System: Workflow flexibility

Cell sorting (optional)

Cell capture, barcoding (BD Rhapsody™ Scanner optional)

cDNA synthesis



Library preparation

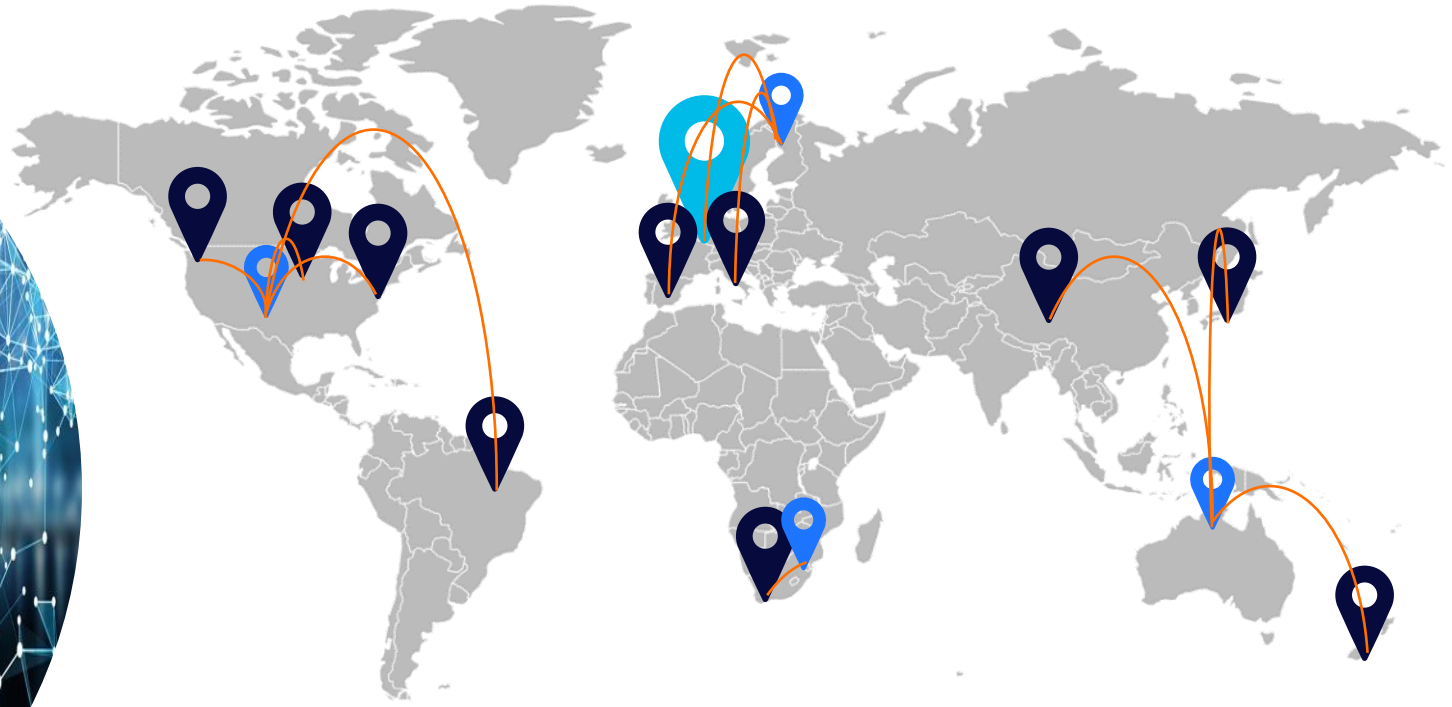
Store beads for up to 12 months at 4 °C

Transport beads to lab

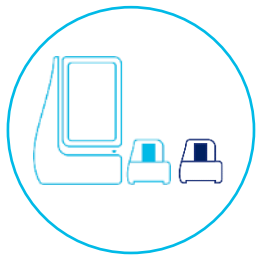
Example 1: Multicenter studies/trials

Establish SOP
Distribute SOP

Example 1: Multicenter studies/trials



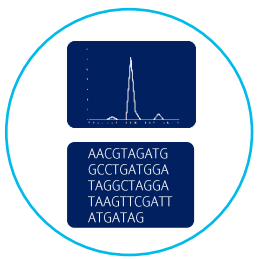
Establish SOP
Distribute SOP



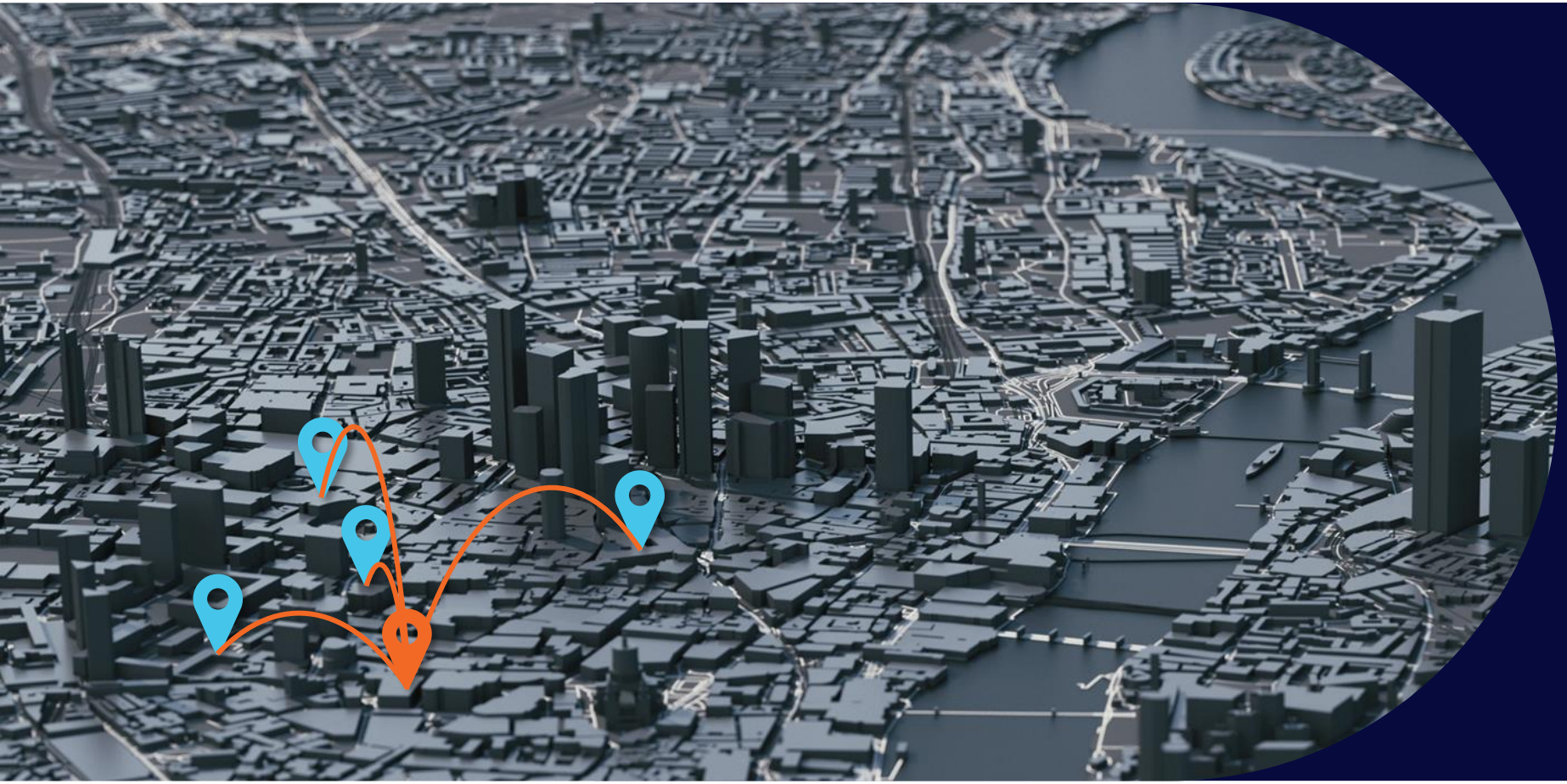
Cell capture, cDNA synth.

Ship beads to CRO
or centralized hub

Library prep & NGS



Example 2: Core facility with decentralized cell capture workflow

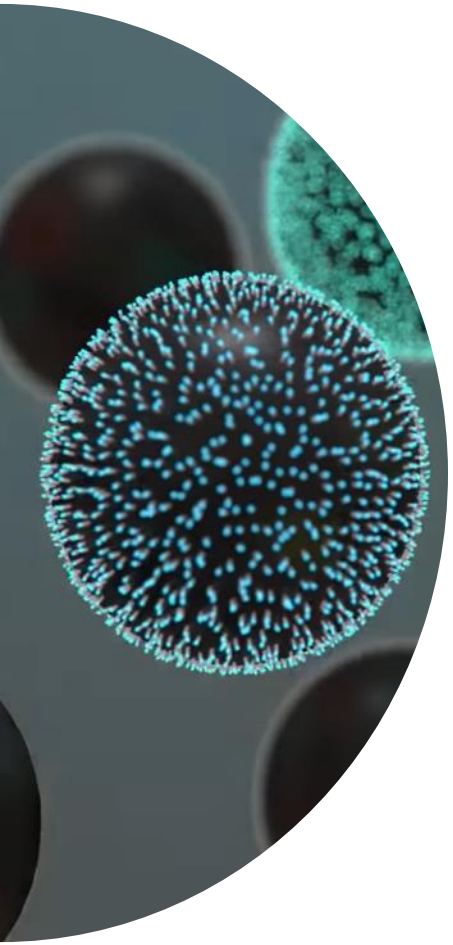


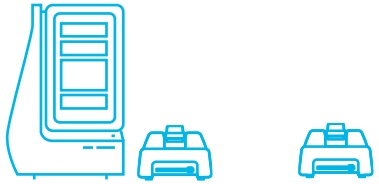
The BD Rhapsody™ HT Xpress System is a benchtop system that can be easily offered for onsite cell capture and barcoding to the customer by the gene core facility

Gene core facility users can easily send back the capture beads with the bound cDNAs to the gene core facility

Decentralized cell capturing: Preserve biology

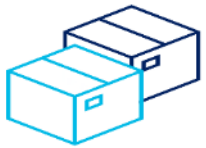
Enables decentralized, straightforward sample processing without fixing the cells!





Flexible configuration

- BD Rhapsody™ Single-Cell Analysis System
  - Includes sample quality control
  - Includes workflow quality control
  - Supports SOP setup
- BD Rhapsody™ HT Xpress System
  - Easy, standardized and robust cartridge workflow
  - Small handheld system
  - Purely mechanical
  - Affordable
- BD® OMICS-Guard Sample Preservation Buffer
  - Preserve samples for up to 72 h and ship
  - No fixation
  - Amenable to RNA-seq, CITE-seq, qPCR, flow cytometry



Subkit concept

- Subkits for cell capture process, cDNA synthesis and library prep enable simplified logistics.

Capture beads

- cDNAs remain covalently bound
- Full assay access
- Shipping at 4 °C enabled
- Stable at 4 °C for up to 12 months

Global pharma company

Global multisite translational research–focused studies already implemented the model.

Multiomics Alliance

Seven KOLs from labs all over Europe implemented BD single-cell multiomics solutions in three distinct translational research projects.

BD flow cytometers are Class 1 Laser Products.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.