Study biological complexity with BD Rhapsody HT Single-Cell Analysis System

Fast track single-cell research without compromise

UCLL
UNIVERSITY OF APPLIED SCIENCES

BD

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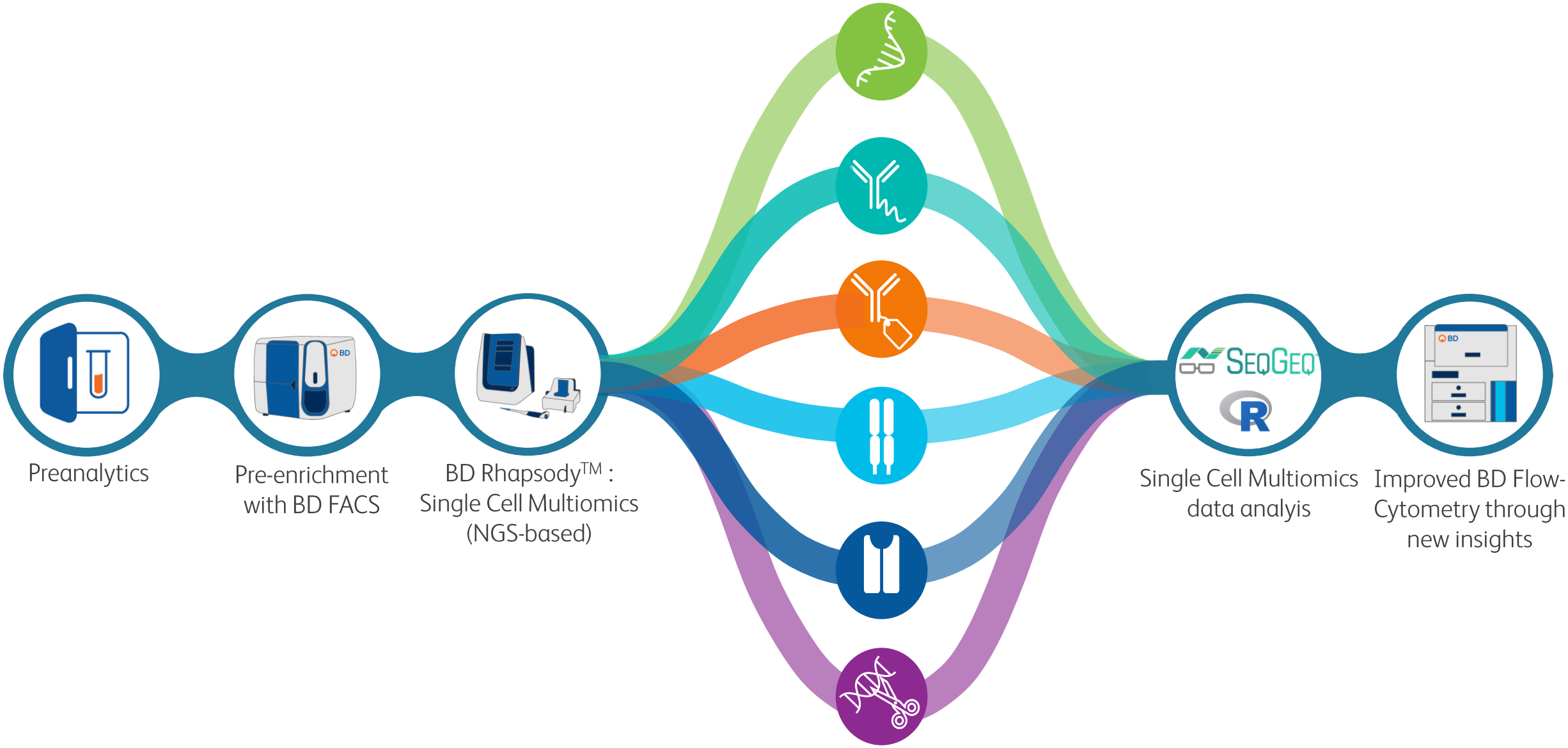
5 February 2024

The BD Rhapsody workflow scRNAseq & multiomics

*A robust **microwell-based** single-cell partitioning system for high-dimensional biology research*

BD Rhapsody™ : Part of BD's Single Cell Environment



BD Rhapsody™ : Part of BD's Single Cell Environment

Preanalytics

Pre-enrichment with BD FACS

BD Rhapsody™ : Single Cell Multiomics (NGS-based)

mRNAseq: Quantify the expression levels of up to thousands mRNA transcripts of a single cell / nuclei (WTA & Targeted)

Abseq/CITE-seq: Quantify up to 200 cell surface & intracellular proteins without antibody panel optimization

Sample Multiplexing: Combine multiple samples on one CBD Rhapsody™ Cartridge for enhanced cost effectiveness

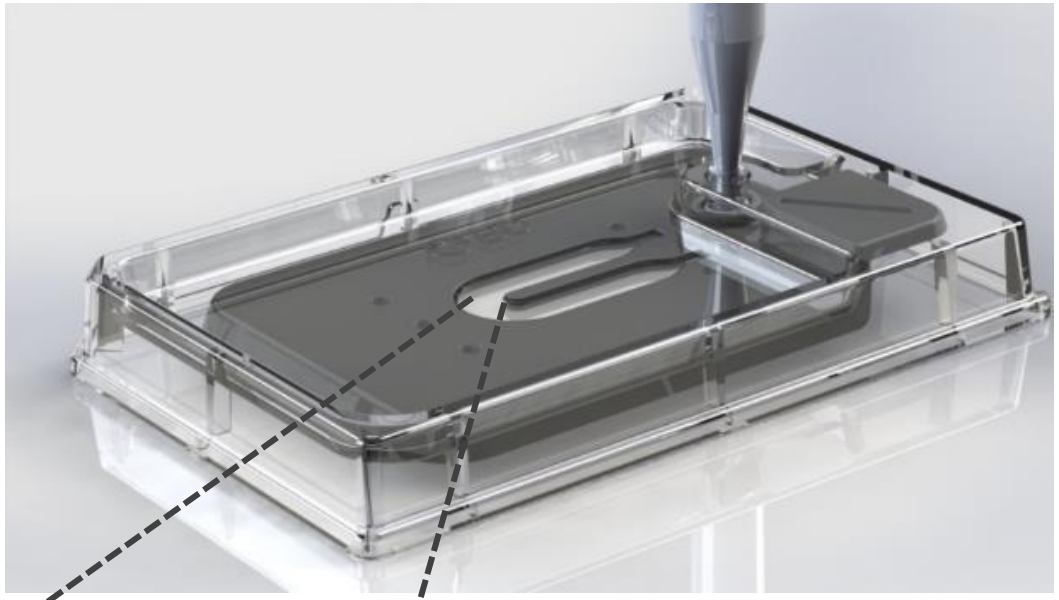
VDJ-Seq: Profile the immune repertoire (TCR & BCR) on a single cell level, cover all receptor chains (TCRs & BCRs)

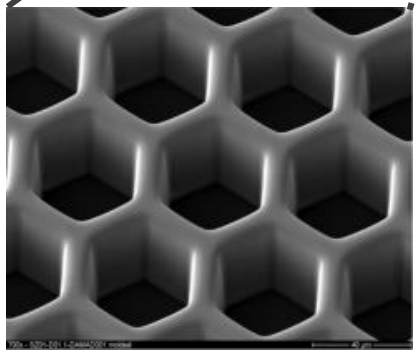
IMMUDEX dCODE®: Antigen Specificity testing with barcoded Dextramers®

CRISPR-Screens (CROP-Seq): Enhanced cost efficacy and dynamic range in high throughput CRISPR screens

BD Rhapsody™ HT Single-Cell Analysis System

Cell capture: Rhapsody HT Xpress





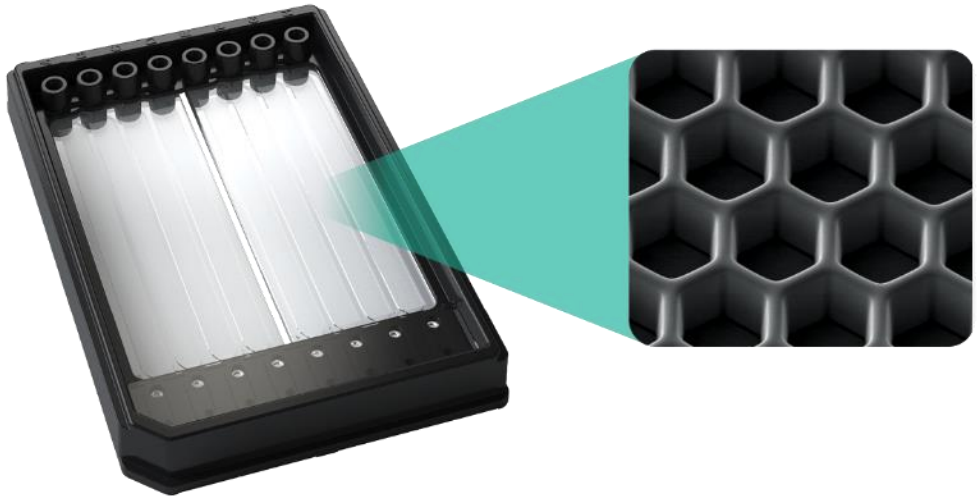
Capture of cells and beads

- Single Use
- Easy to load
- 200k+ microwells

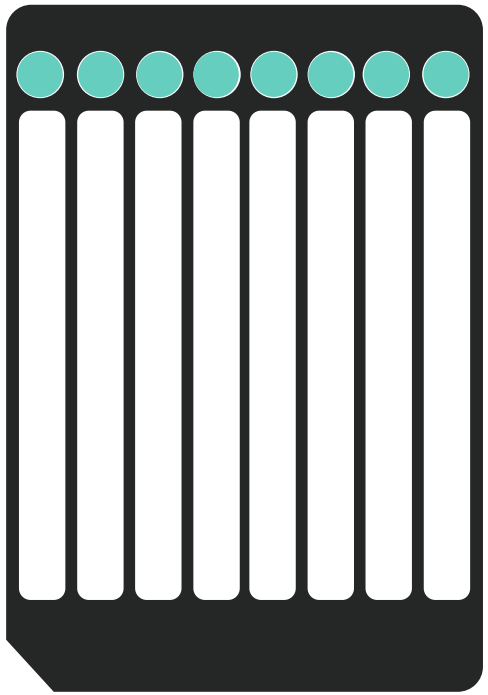
Manipulation of Microwell Cartridge

- Simple and portable
- Fits in a tissue culture hood
- Can be purchased individually

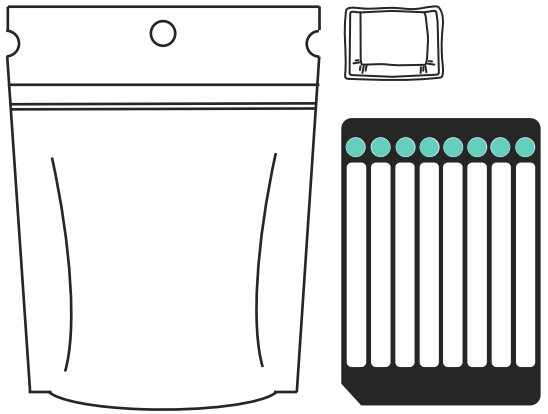
BD Rhapsody™ HT Cartridge



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

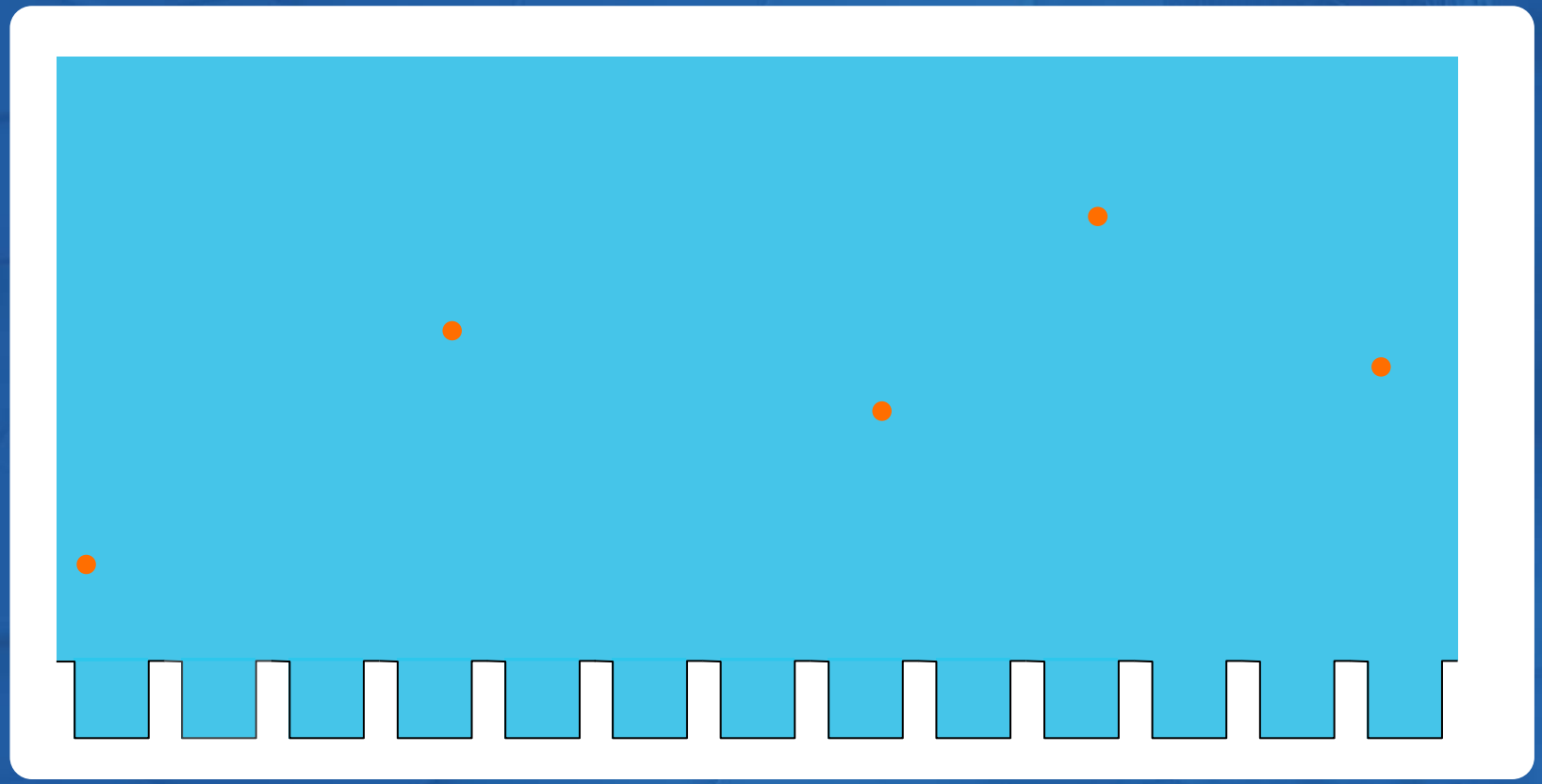
320µl-cell suspension loading volume

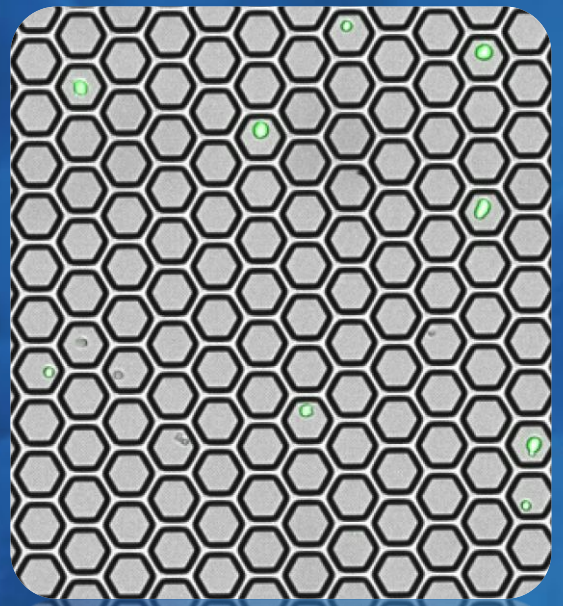
Up to **65,000 cells** per lane recommended

Run more or different types of experiments

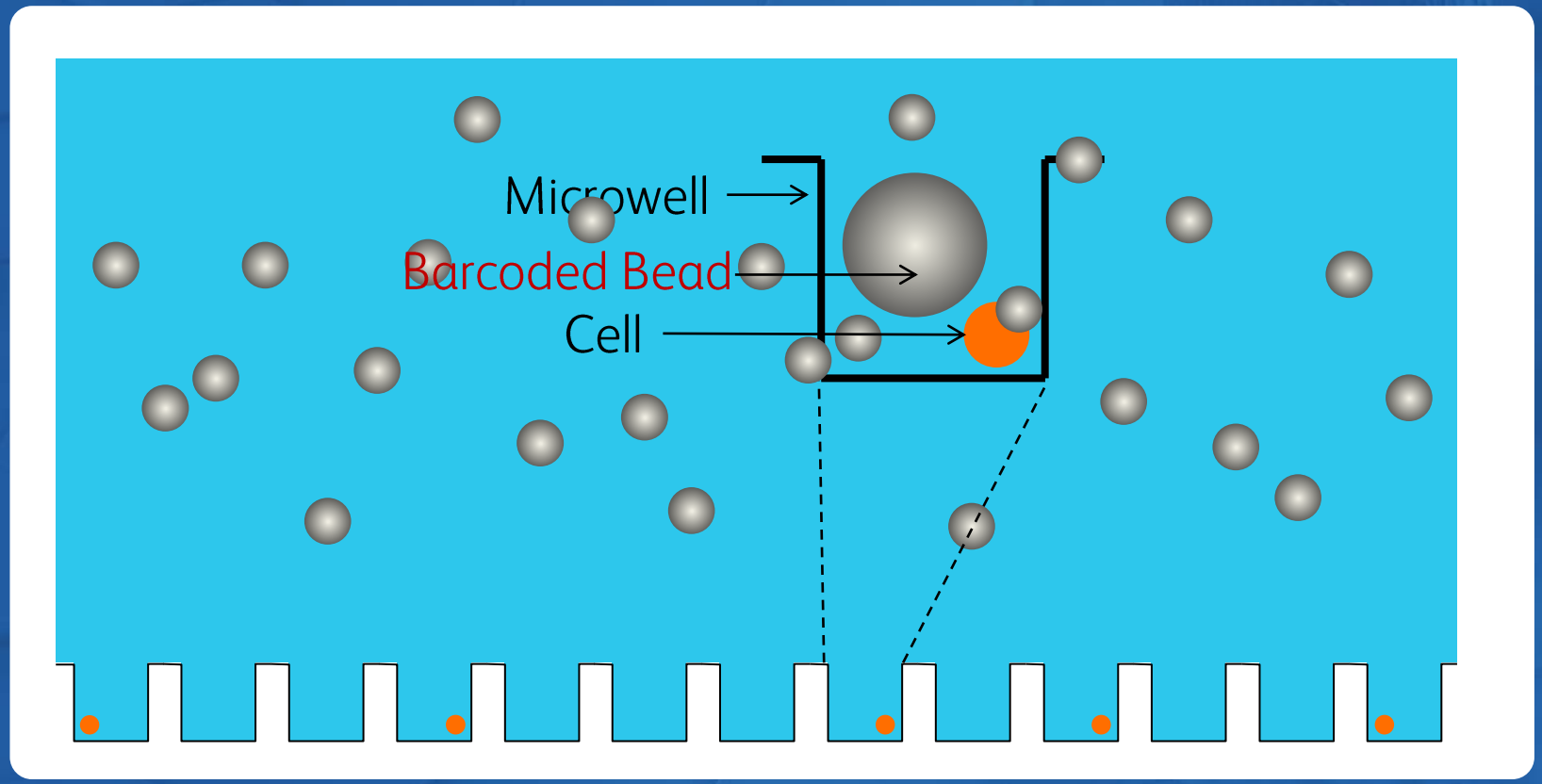
Process samples together or on different days

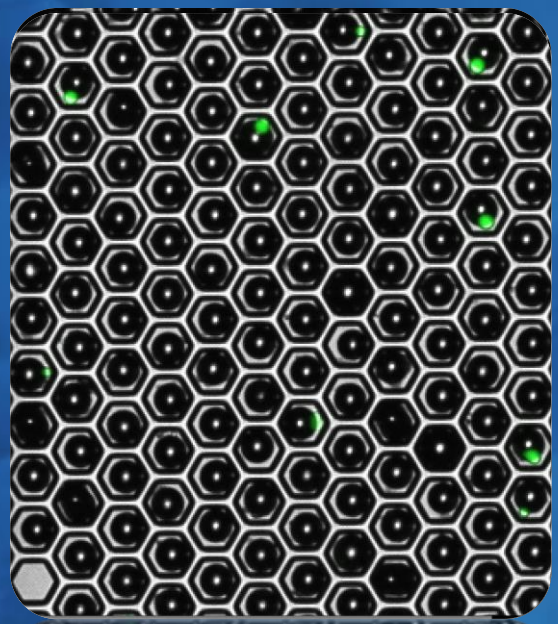
BD Rhapsody™ Cartridge workflow



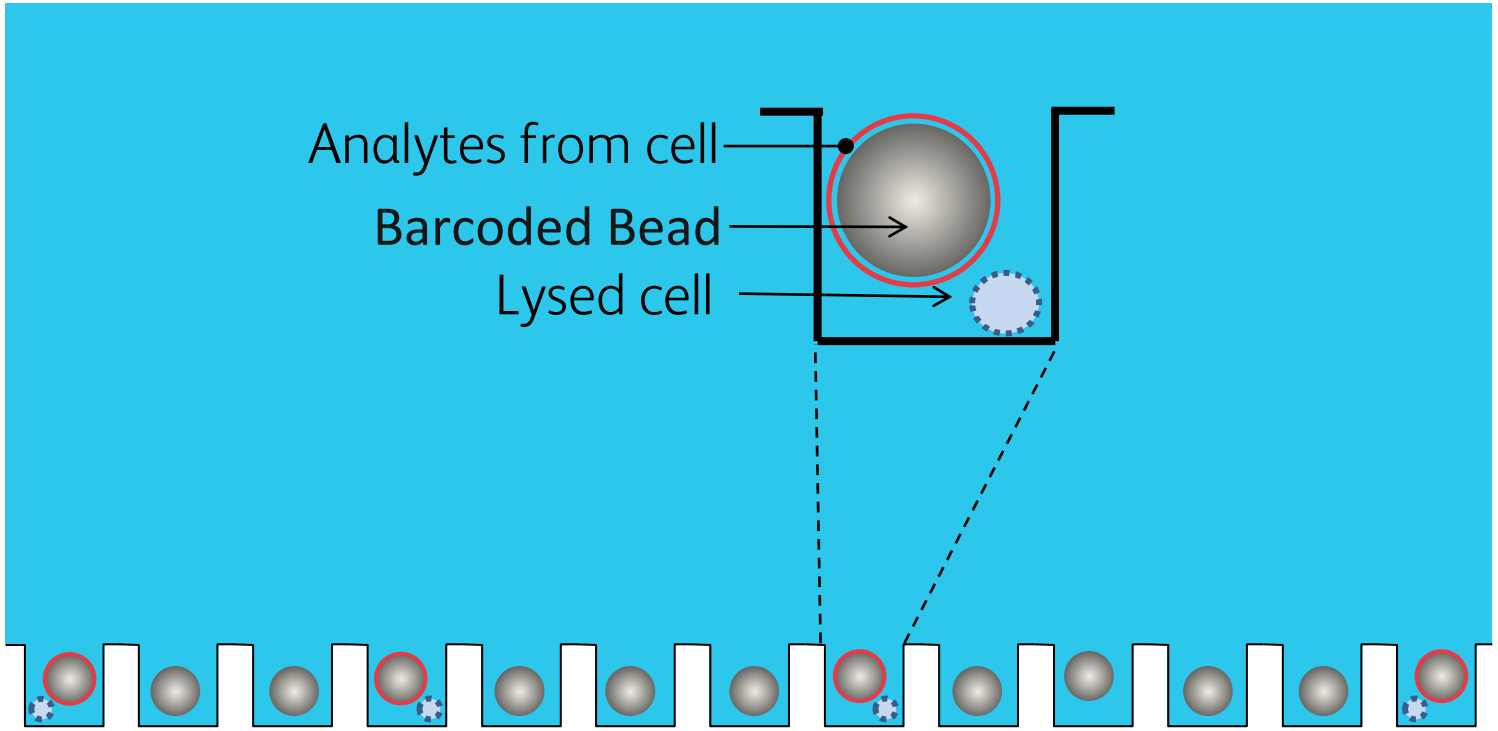


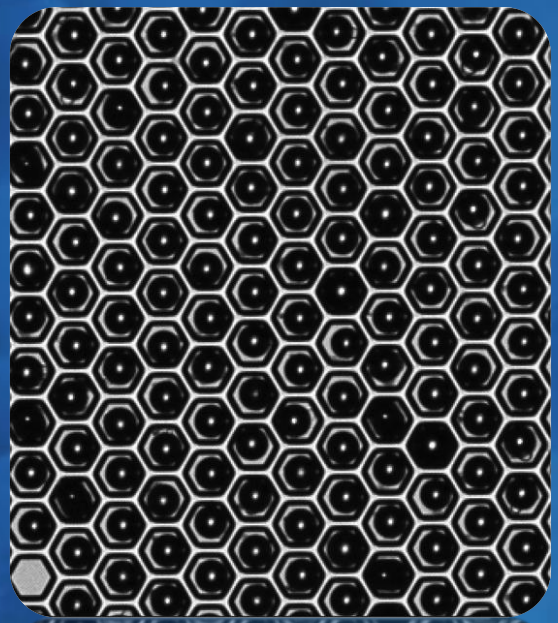
BD Rhapsody™ Cartridge workflow



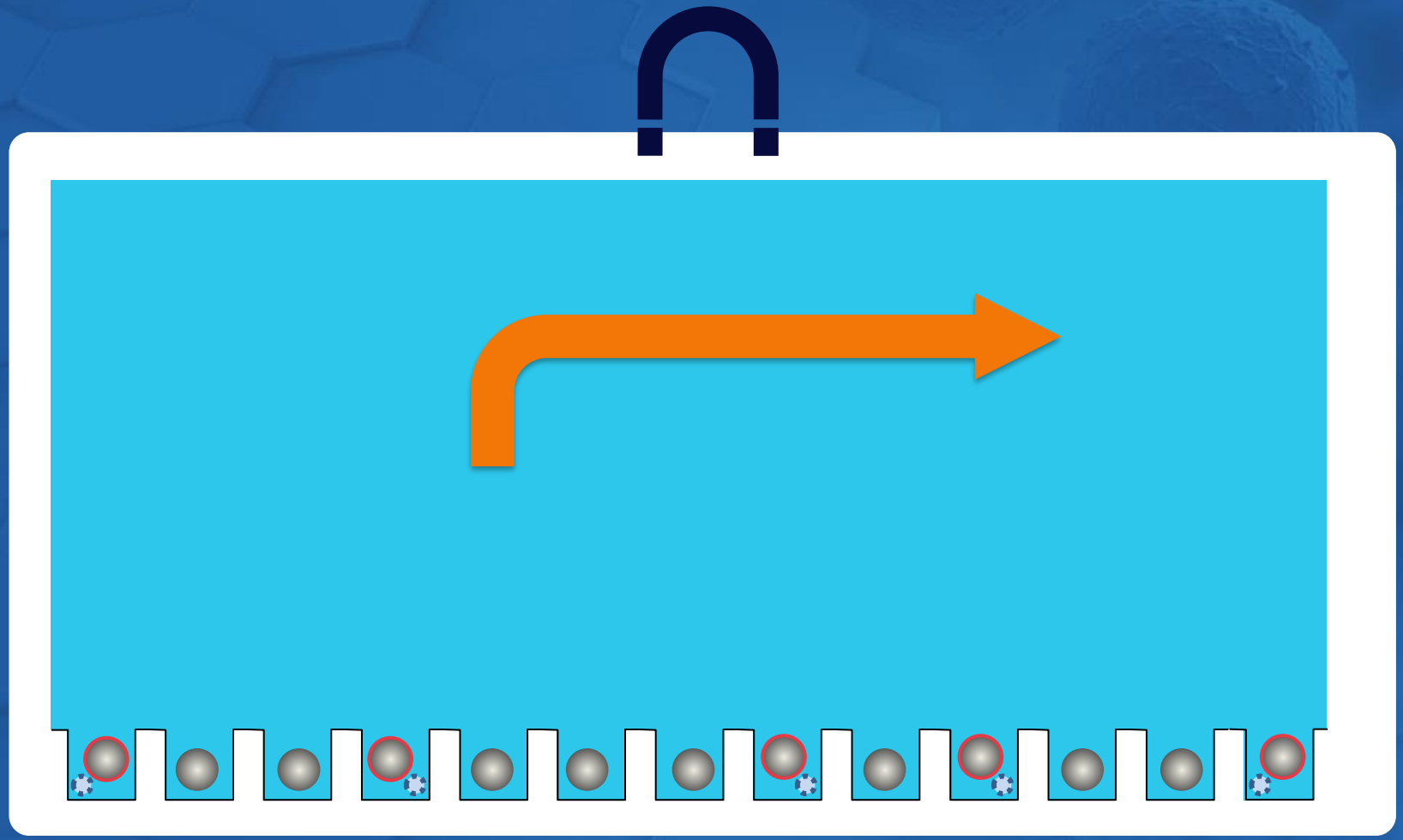


BD Rhapsody™ Cartridge workflow

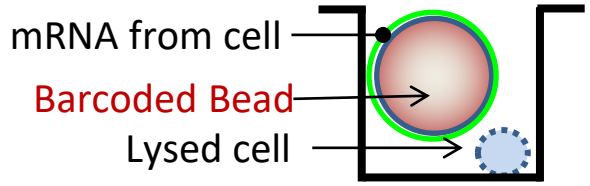




BD Rhapsody Cartridge workflow

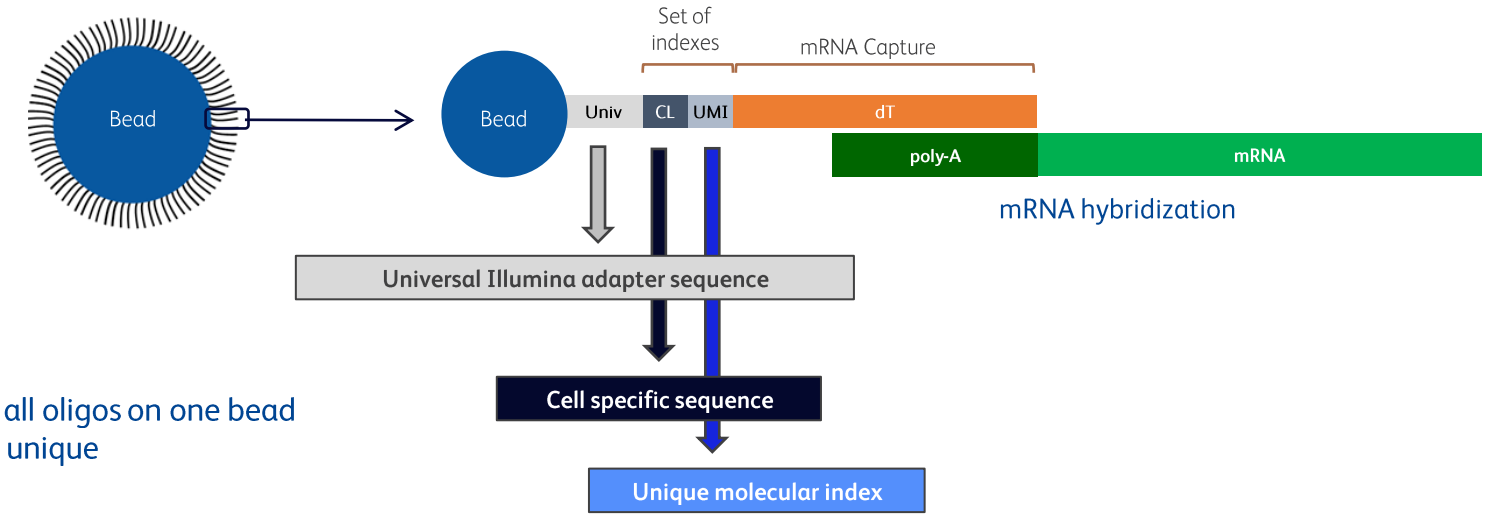


BD Rhapsody workflow- Cell lysis & Barcoding



Cell lysis releases mRNA

MAGNETIC BEAD WITH MILLIONS OF CAPTURE OLIGOS

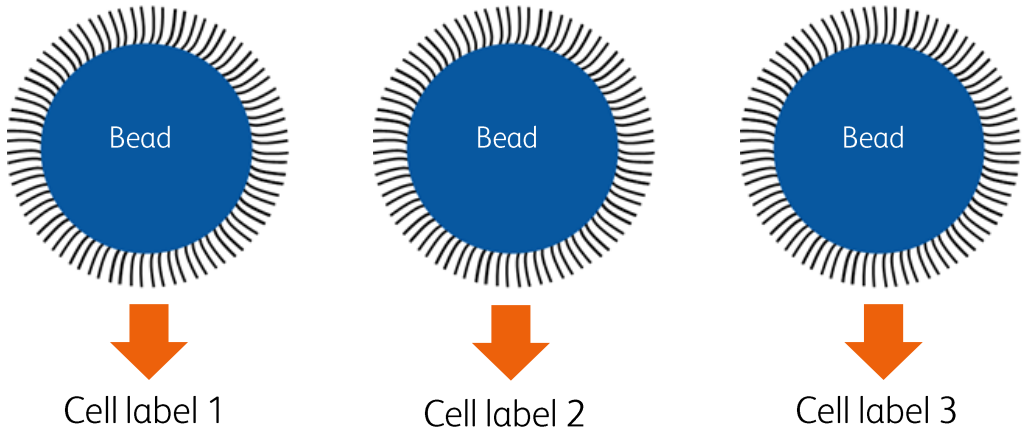


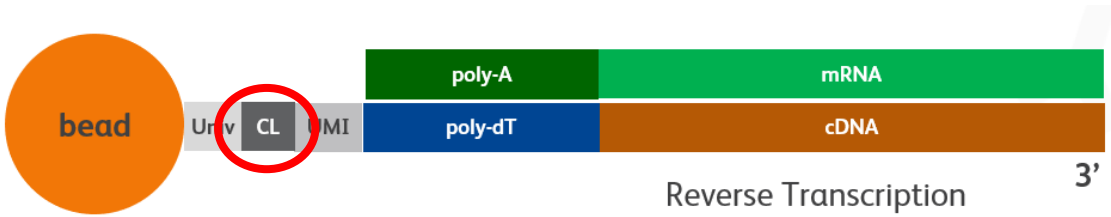
mRNA hybridization

- Identical for all oligos on one bead
- Each bead is unique

BD Rhapsody capture beads:

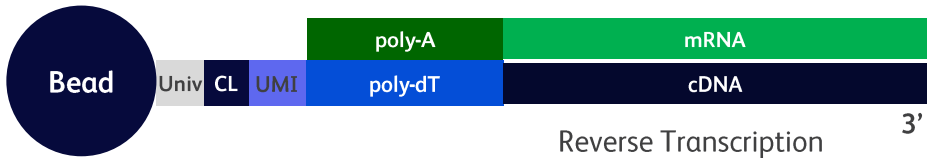
The cell label (CL): Retrieve single cell information

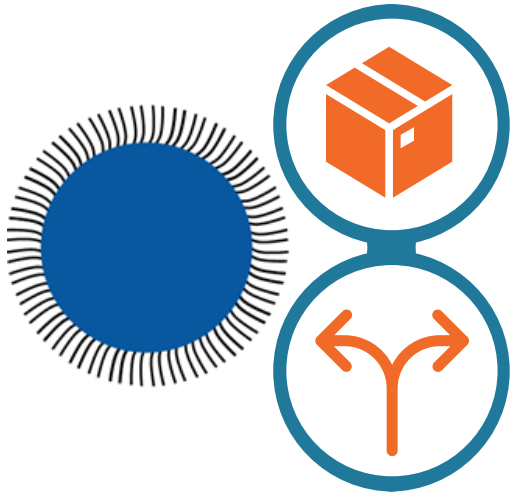




Archivable capture beads

Beads with hybridized mRNA retrieved from cartridge





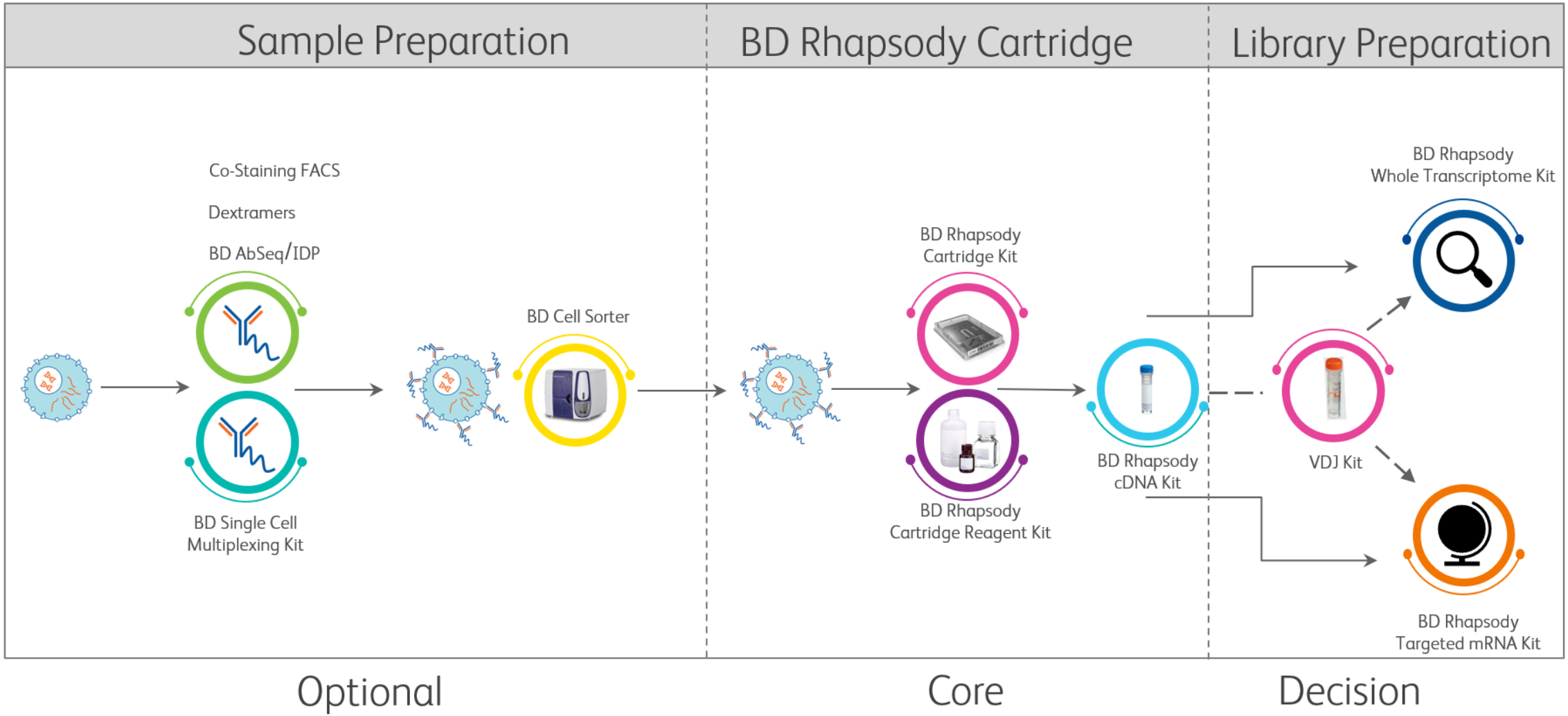
cDNA archived on bead and tagged with cell label and UMI

- All mRNA molecules from a single cell tagged with the same cell label
- Each mRNA molecule within a single cell is tagged with a UMI

cDNA stably (covalently) captured on the beads

- Up to 3 months at 4 degrees Celsius
- Allowing subsampling and amplification with different primers panels

BD Rhapsody workflow options



The BD Rhapsody workflow

Why using microwells to capture single-cells?

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

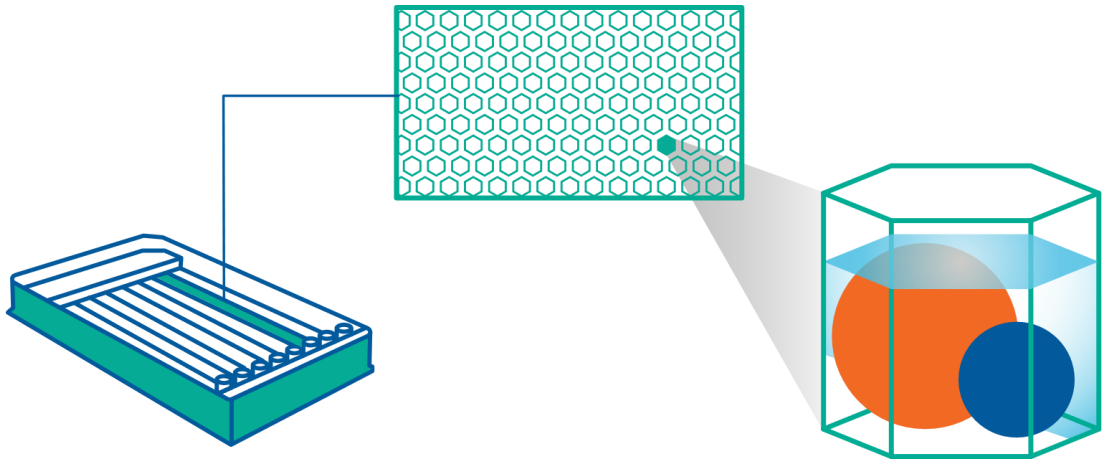
Be certain about your cell capture with every single-cell experiment

Save time and cost on expensive downstream sequencing

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



Cell type	Cartridge Capture rate**
CAR-T cells	72%
Frozen mesenchymal stem cells (MSC)	73%
Tumor xenograft (dissociated)	67%
Total CD4+ T cells	74%
CD45+ immune cells	68%
FACS sorted NK and T cell subsets	66%
MSC (cyropreserved)	80%
iPSC, Adipocyte (primary fresh), GABA Neurons (cell line), Hepatocytes (primary) (Cryopreserved)	60%
Myeloma cell lines	73%

Other highlights:

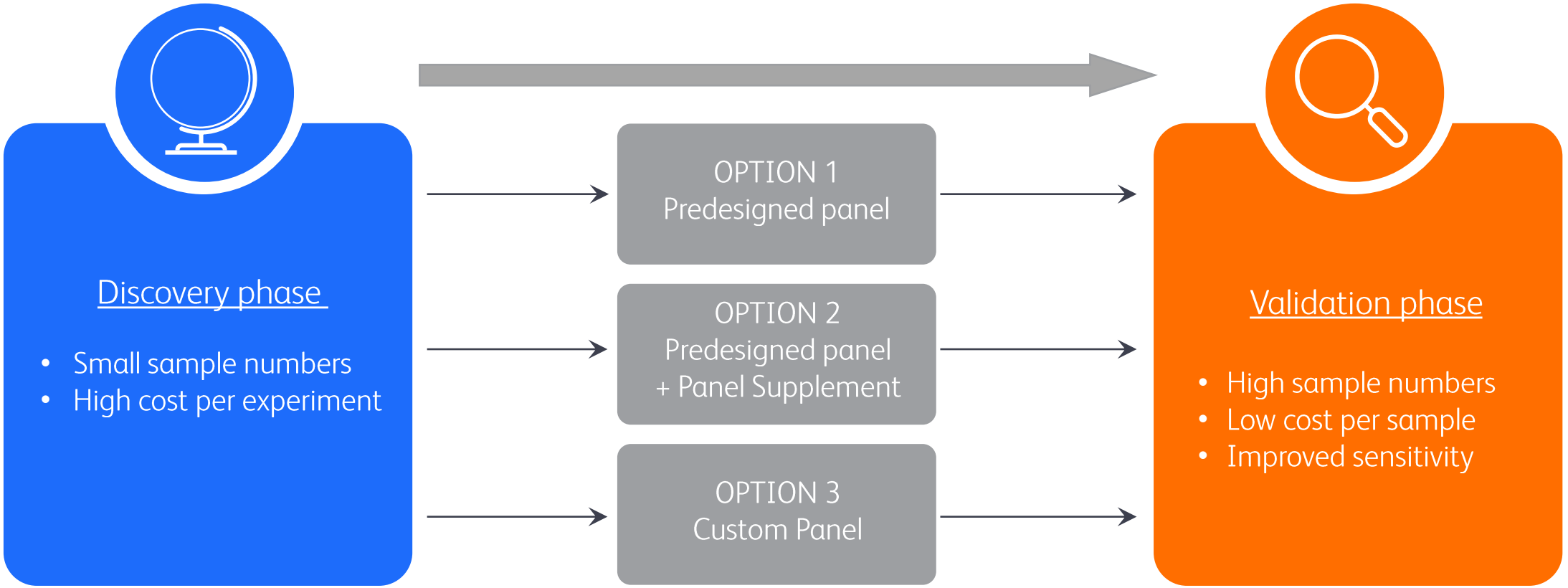
→ Cell input from **100** to **65.000** possible (in 320ul)

→ Low multiplet rate

→ No risk of clogging

Figure Legend: 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.

BD Rhapsody targeted RNA custom options

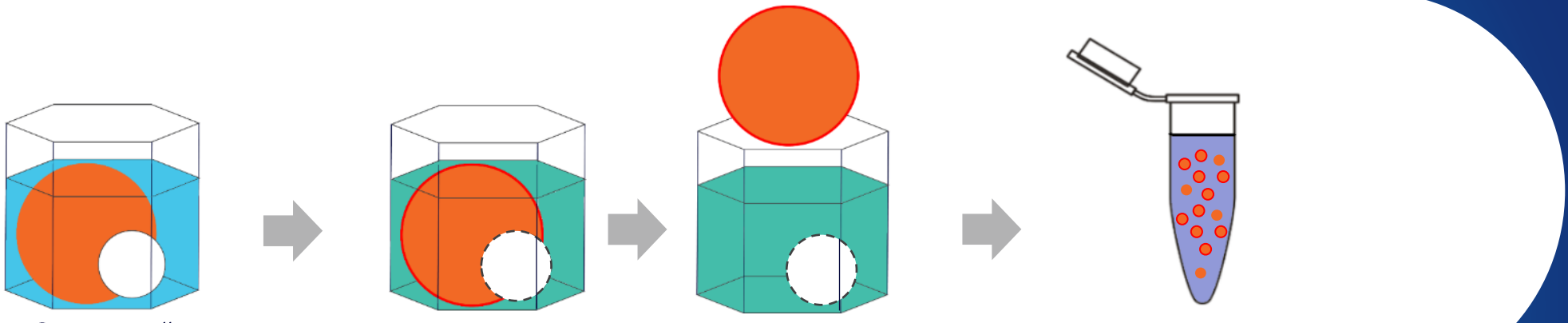


BD Rhapsody™
Whole Transcriptome RNA
Sequencing

- Immune response panel
- T-Cell panel
- Breast cancer panel

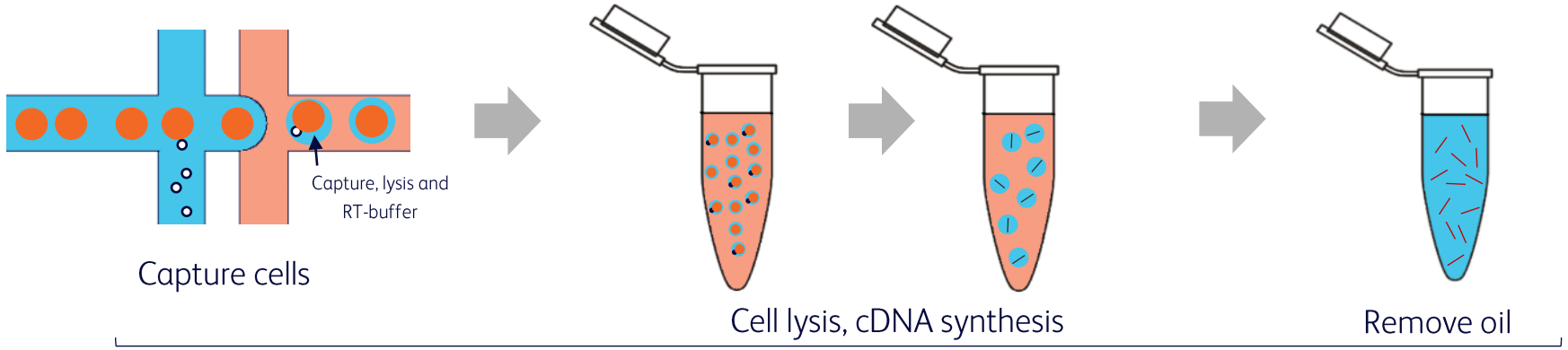
BD Rhapsody™
Targeted
RNA Sequencing

Microwells enable buffer exchanges during the workflow



BD Rhapsody workflow

Droplet-based workflow

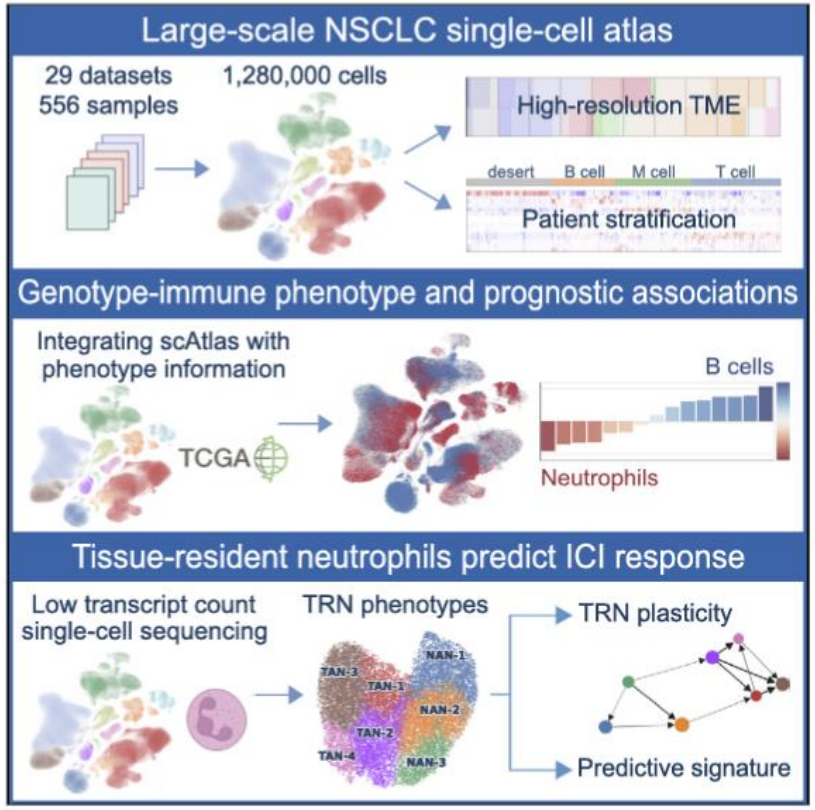


Missing access to the cells → Identical buffer for all workflow steps

Cancer Cell

High-resolution single-cell atlas reveals diversity and plasticity of tissue-resident neutrophils in non-small cell lung cancer

Graphical abstract



Authors

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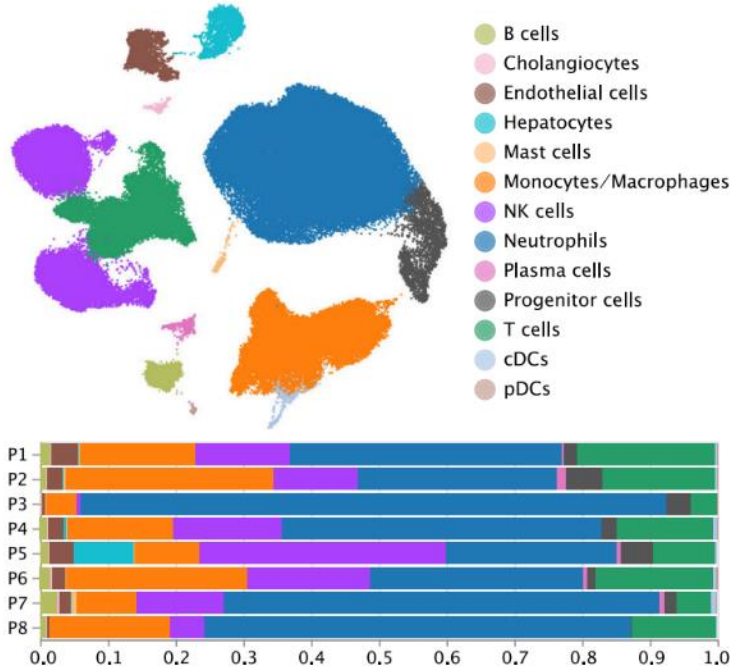
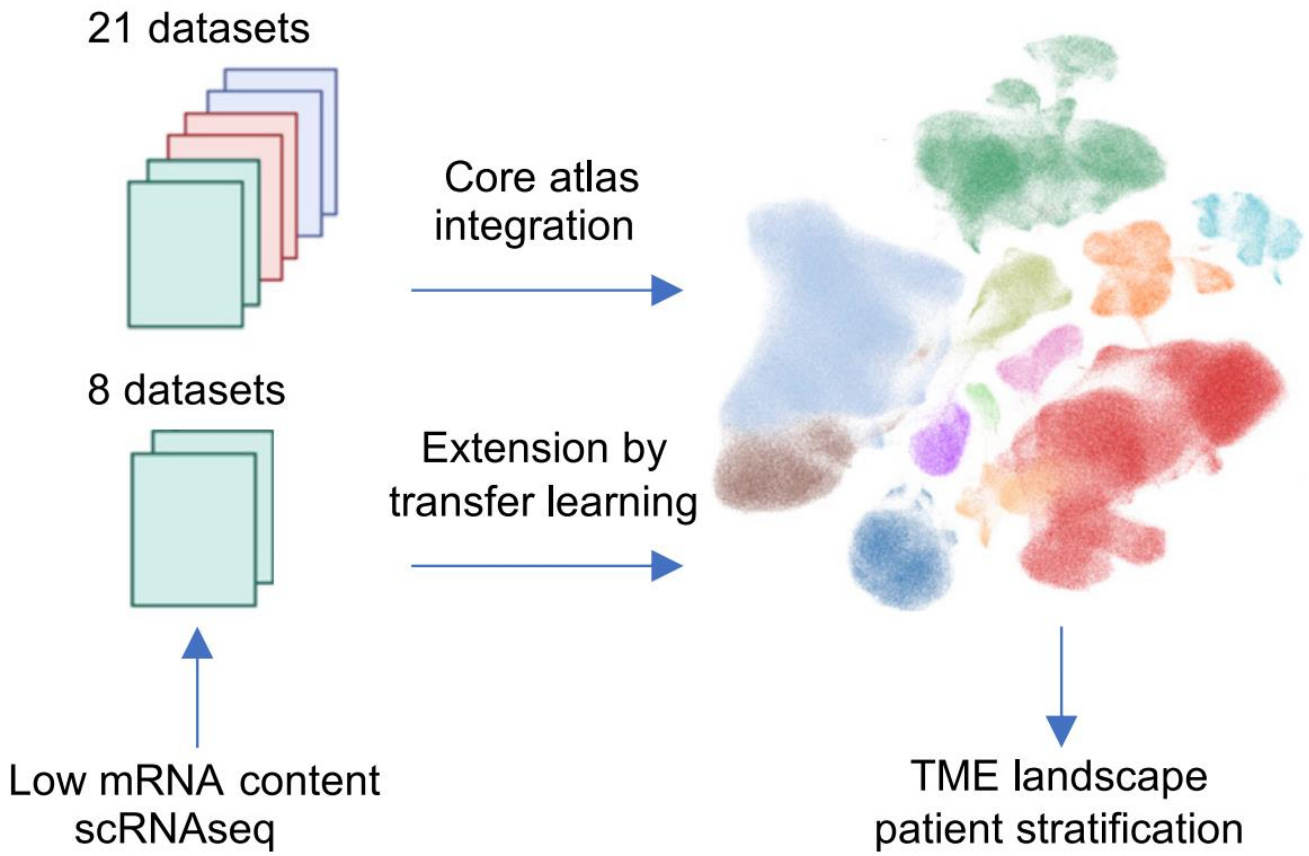


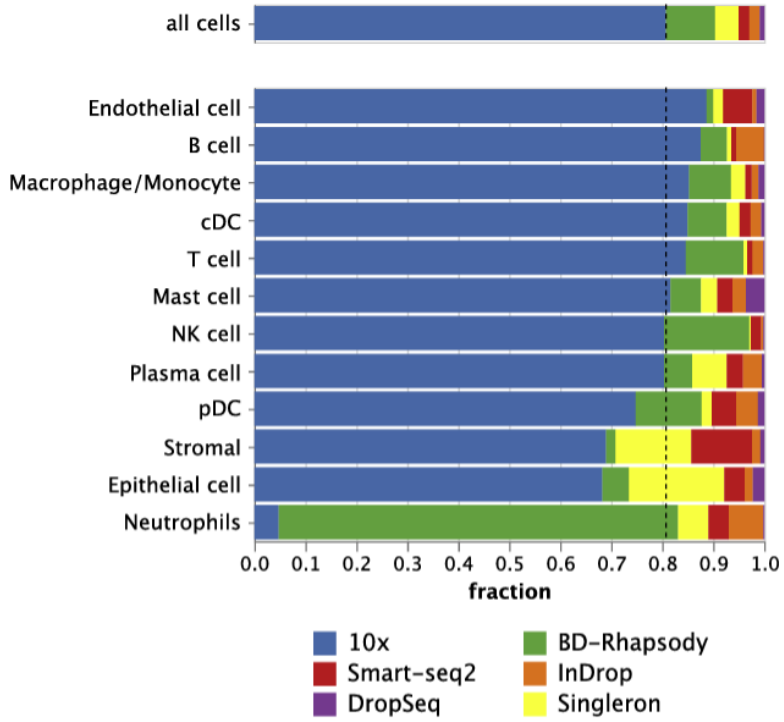
Article | Open Access | Published: 21 April 2023

Immune cell dynamics deconvoluted by single-cell RNA sequencing in normothermic machine perfusion of the liver

T. Hautz, S. Salcher, M. Fodor, G. Sturm, S. Ebner, A. Mair, M. Trebo, G. Untergasser, S. Sopper, B. Cardini, A. Martowicz, J. Hofmann, S. Daum, M. Kalb, T. Resch, F. Krendl, A. Weissenbacher, G. Otarashvili, P. Obrist, B. Zelger, D. Öfner, Z. Trajanoski, J. Troppmair, R. Oberhuber, ... S. Schneeberger

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





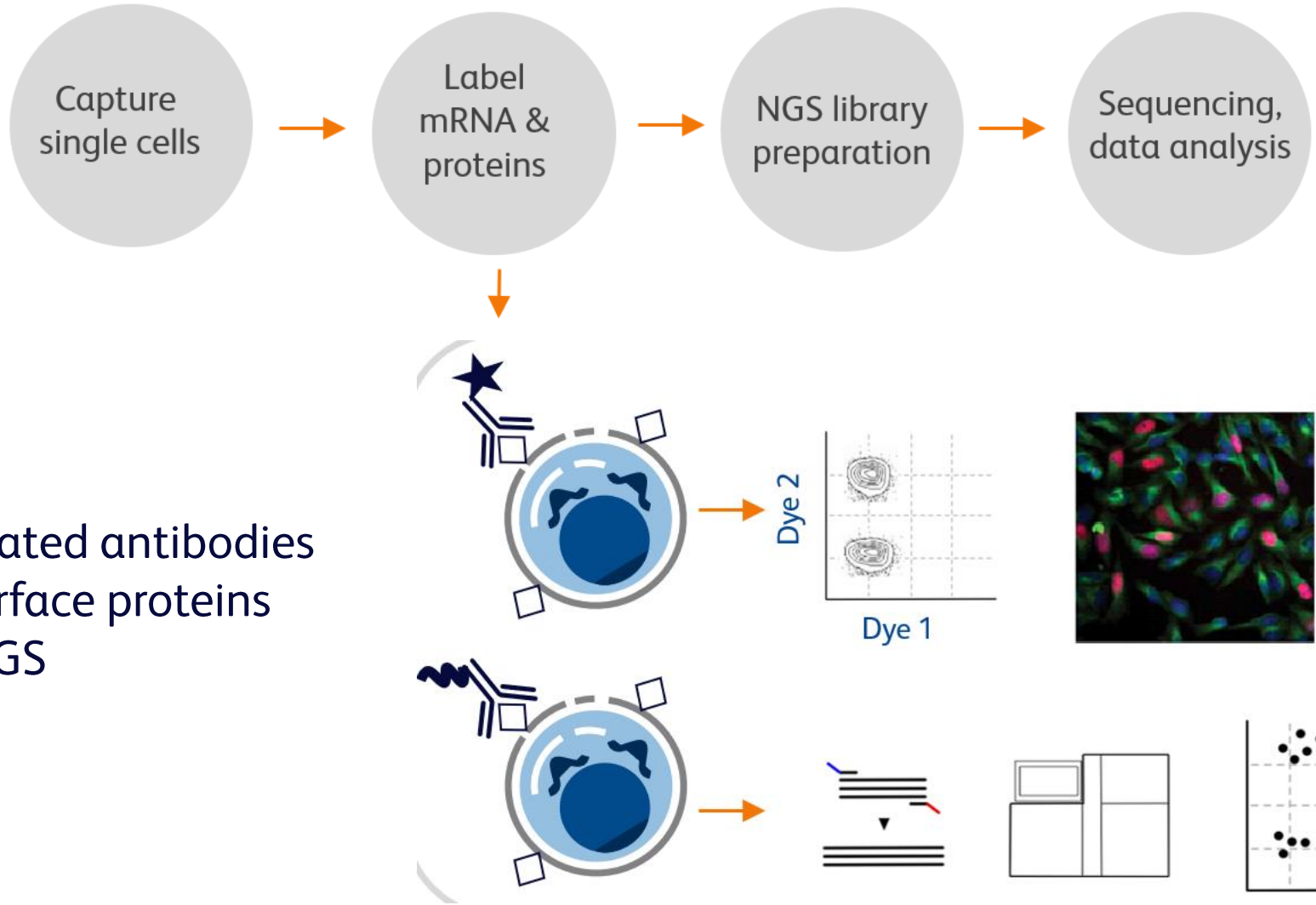
(From: Salcher S. et al., 2023 Cancer Cell)

*The dataset from the **BD Rhapsody** experiment contained the majority of Neutrophils in the extended atlas (data from 6 different single-cell technologies)*

BD Rhapsody Protein Profiling

- *Surface markers using BD® Abseq*
- *Sample multiplexing (cell hashing)*

How to make it multiomic?



DNA-oligo conjugated antibodies (Abseqs) make surface proteins „accessible“ for NGS

BD® AbSeq: Cartridge workflow

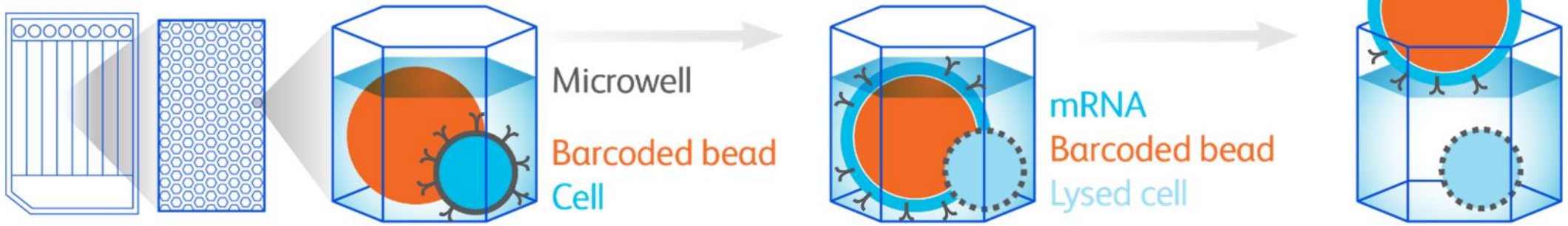
Load cells and beads

Pair ONE cell with ONE barcoded bead in microwell

Lyse cells

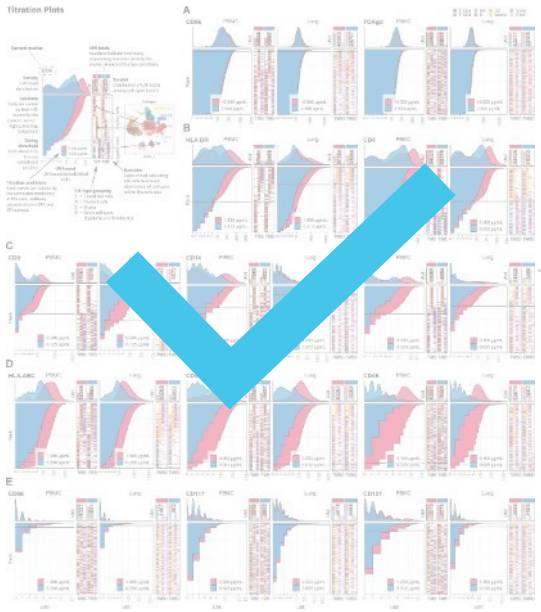
Lyse cell to hybridize mRNA onto barcoded capture oligos on bead

Retrieve beads



BD® AbSeq: Pre-titrated antibodies for the BD Rhapsody

Adjust antibody concentration

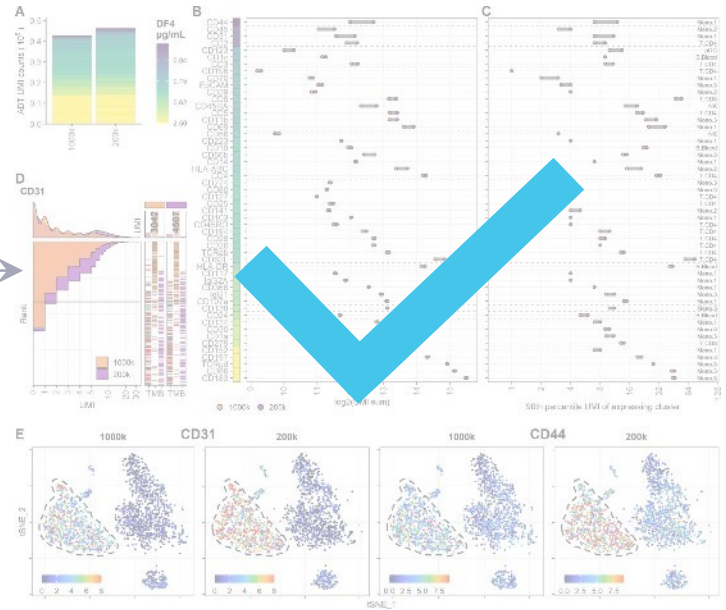


Each BD® AbSeq antibody is pre-titrated for PBMCs

Adjust staining volume

BD Biosciences has developed a standardized staining protocol, 2µl of each antibody is added, the total reaction volume is 100µl/sample

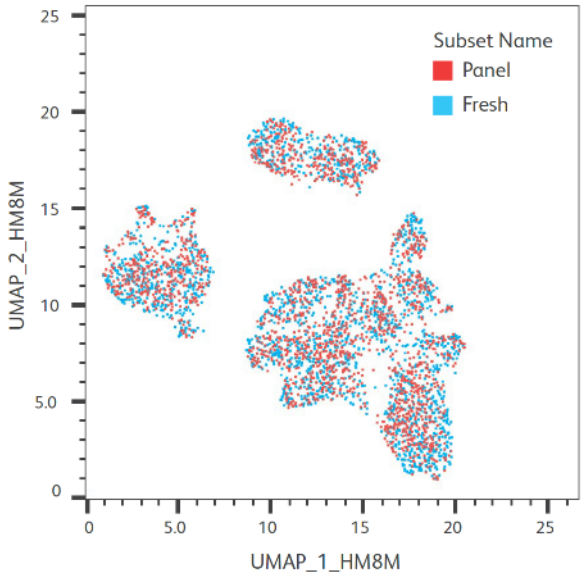
Adjust cell number

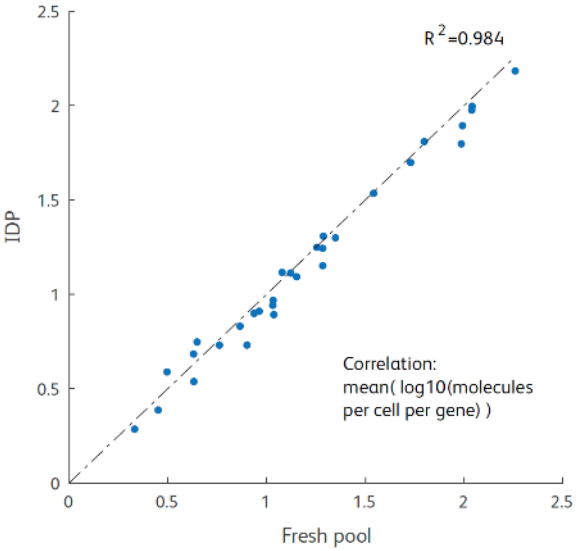


Each BD® AbSeq antibody is tested for 20,000 to 1 million cells

BD® AbSeq Human Immune Discovery Panel

Specificity	Cat.	Clone	Oligo ID
CD3	940307	UCHT1	AHS0231
CD4	940001	SK3	AHS0032
CD8	940305	SK1	AHS0228
CD11c	940024	B-Ly6	AHS0056
CD14	940005	MPHIP9	AHS0037
CD16	940006	3G8	AHS0053
CD19	940004	SJ25C1	AHS0030
CD25	940009	2A3	AHS0026
CD27	940018	M-T271	AHS0025
CD28	940226	L293	AHS0138
CD45RA	940011	HI100	AHS0009
CD56	940007	NCAM16	AHS0019
CD62L	940041	DREG-56	AHS0049
CD127	940012	HIL-7R-M21	AHS0028
CD134	940060	ACT35	AHS0013
CD137	940055	4B4-1	AHS0003
CD161	940283	HP-3G10	AHS0205
CD183 (CXCR3)	940030	1C6/CXCR3	AHS0031
CD185 (CXCR5)	940042	RF8B2	AHS0039
CD186 (CXCR6)	940234	13B 1E5	AHS0148
CD196 (CCR6)	940033	11A9	AHS0034
CD197 (CCR7)	940394	2-L1-A	AHS0273
CD272	940105	J168-540	AHS0052
CD278	940043	DX29	AHS0012
CD279	940015	EH12.1	AHS0014
CD357 (GITR)	940096	V27-580	AHS0104
CD366 (Tim3)	940066	7D3	AHS0016
HLA-DR	940010	G46-6	AHS0035
IgM	940276	G20-127	AHS0198
IgD	940026	IA6-2	AHS0058





BD Rhapsody Protein Profiling

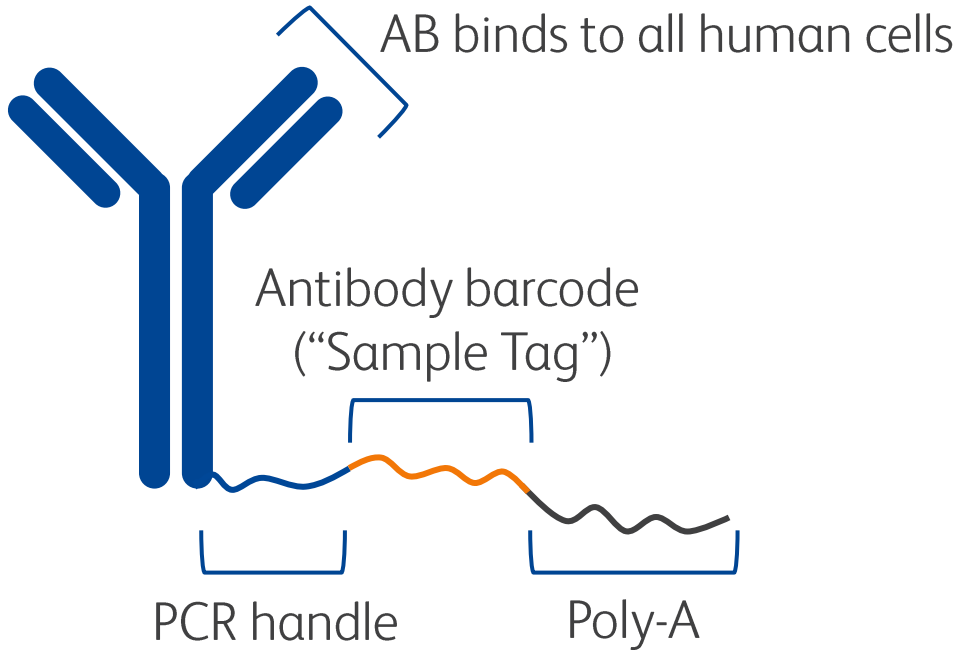
- *Surface markers using BD® Abseq*
- *Sample multiplexing (cell hashing)*

Sample multiplexing kit (SMK) human & mouse

Concept

- Kit enables multiplexing of up to twelve Samples
- Works with all human cells (mouse version available)
- Kit contains twelve AB vials
- Each vial contains identical AB with diff. tags
- DNA barcode acts as RNA mimic to be captured by 3’ based RNA-seq assays

Antibody structure



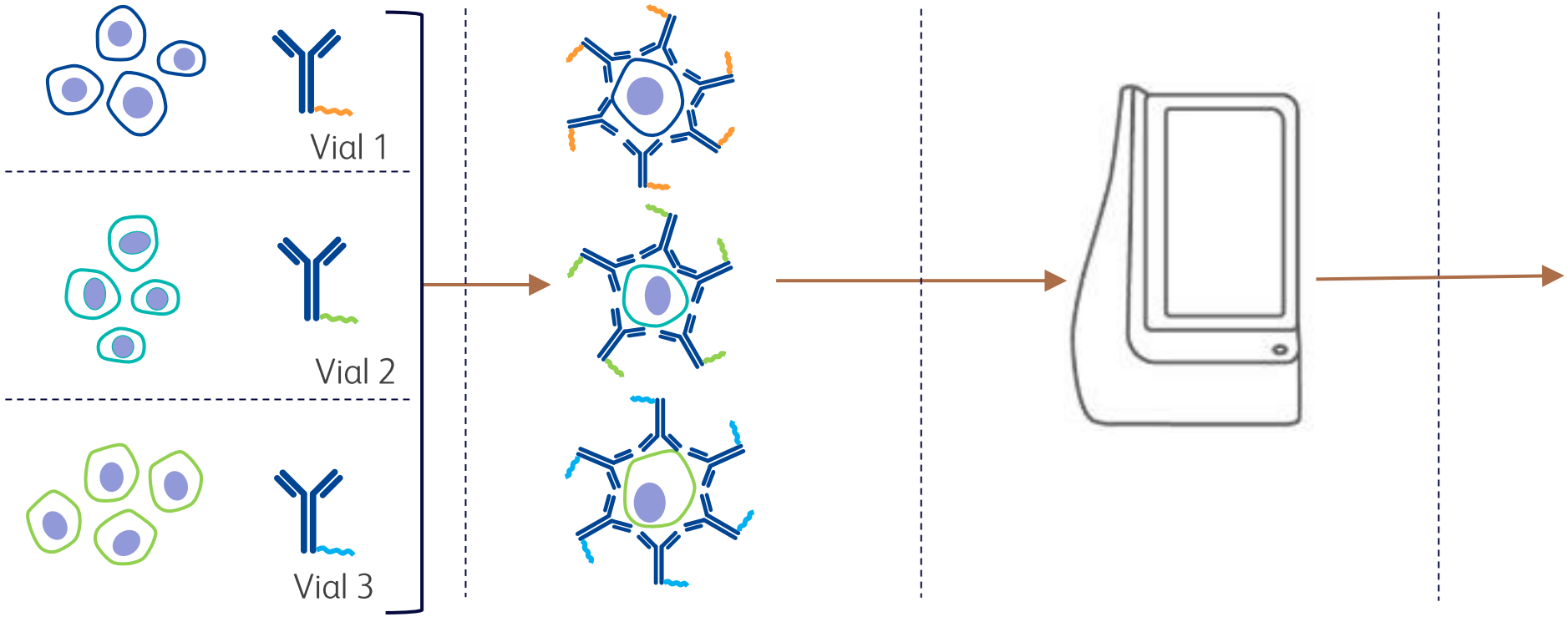
Sample multiplexing kit (SMK), 3-plex example

Incubate each cell sample
&
SMK Antibody
(1 vial per reaction)

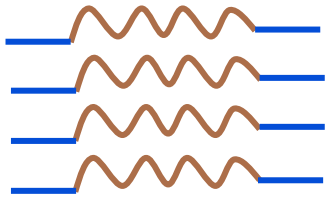
Antibody labeled
cells
(Pool reactions)

Rhapsody SMK WF

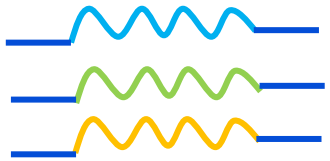
Illumina libraries



cDNA library
(w. UMIs & CLs)



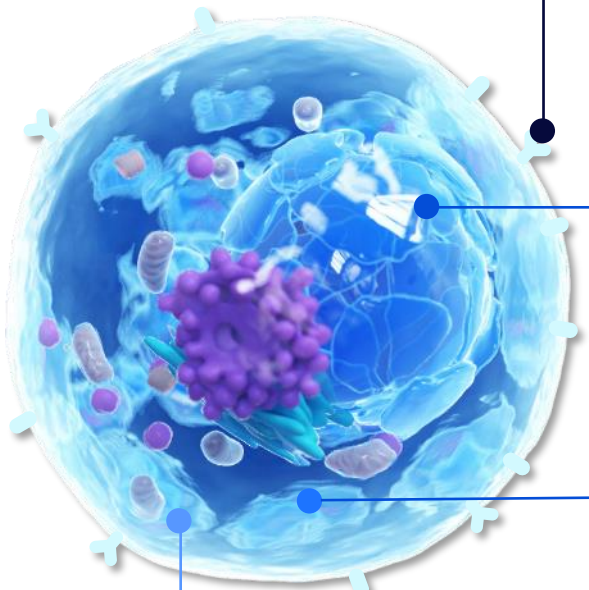
Sample specific tags
(w. *UMIs* & CLs)



BD Rhapsody launches and future developments

*A robust **microwell-based** single-cell partitioning system for high-dimensional biology research*

BD Rhapsody™ menu and future developments



(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

poly-A independent:
RoCK&ROI-Seq*

Cell surface marker:
BD® AbSeq

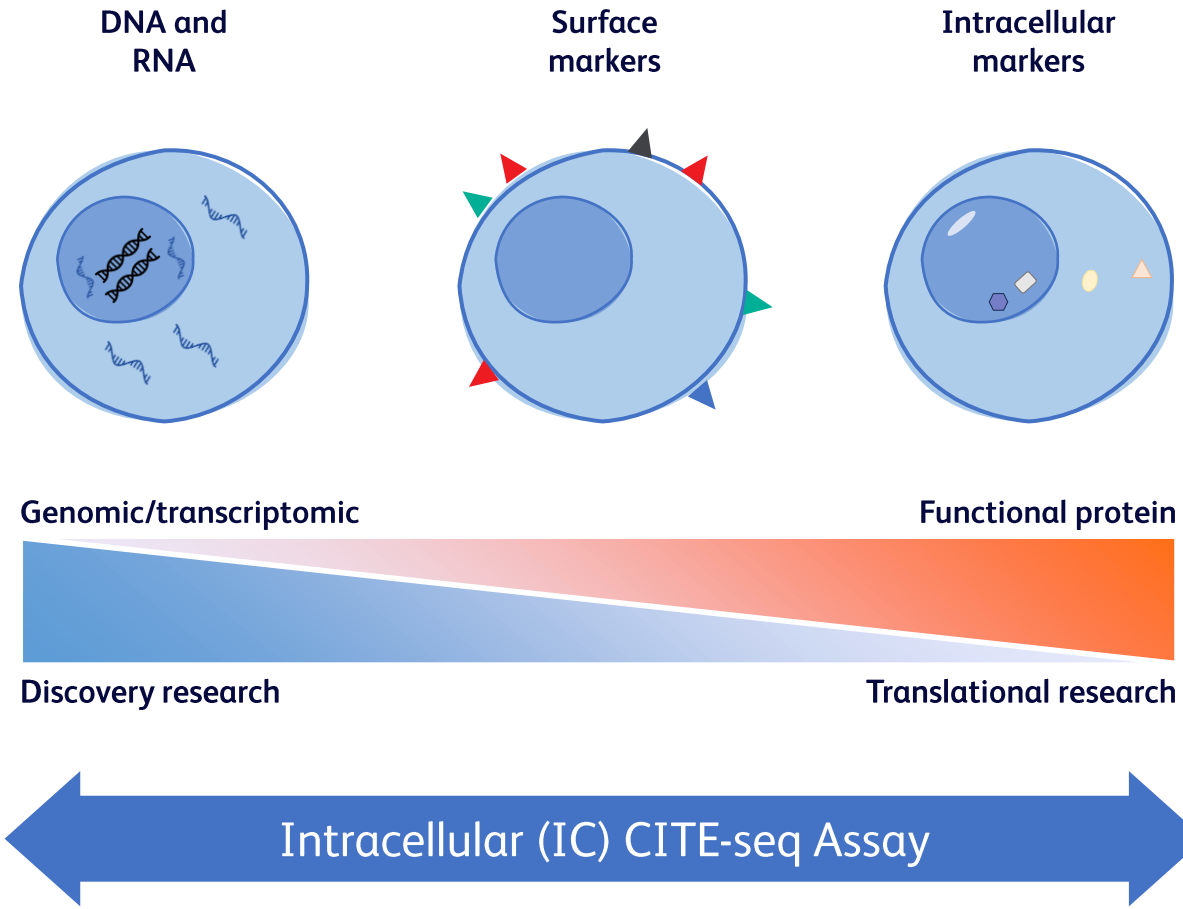
Intracellular & secreted marker:
BD® IC-AbSeq incl. stabilisation reagent*

*In development

New: Intracellular CITE-seq using BD® AbSeq Antibody-Oligos

Intracellular Abseq Multiomics Assay (internally ICAS Assay)

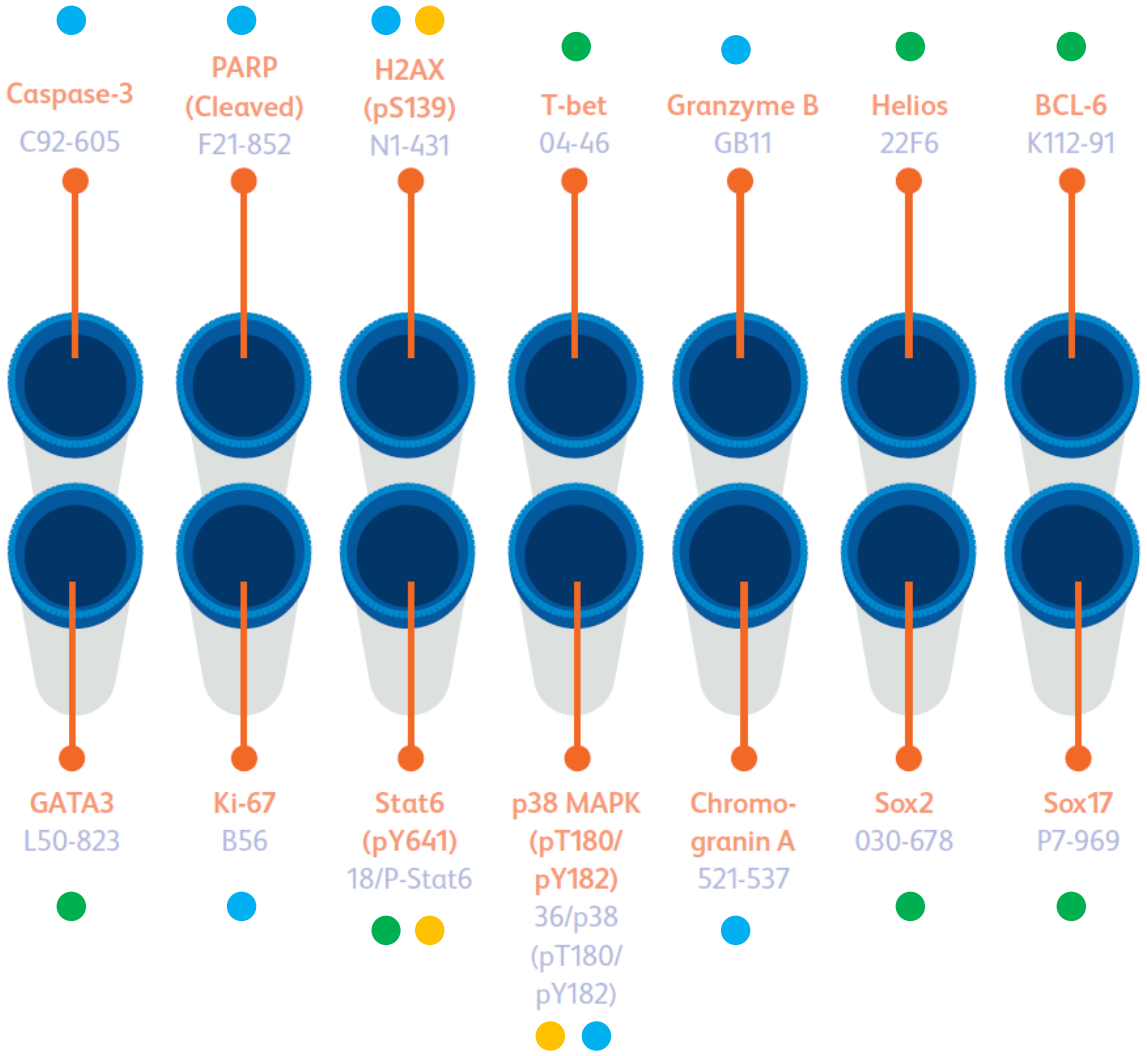
Gain deeper multiomic insights—Intracellular protein + cell surface protein + RNA



- Simultaneous single-cell transcriptomic, surface and intracellular proteomic analysis with NGS readout
- Enables a holistic understanding of cellular dynamics such as transcription regulation and functional states
- Decreases data acquisition time and conserves precious samples

Introducing intracellular BD® AbSeq Ab-Oligos for intracellular protein detection

- *Transcription factor*
- *Plasma protein*
- *Phosphorylation protein*



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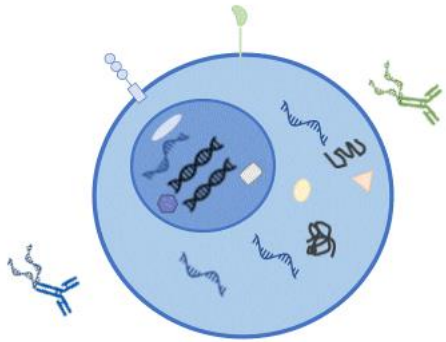
IC CITE-seq Assay chemistry

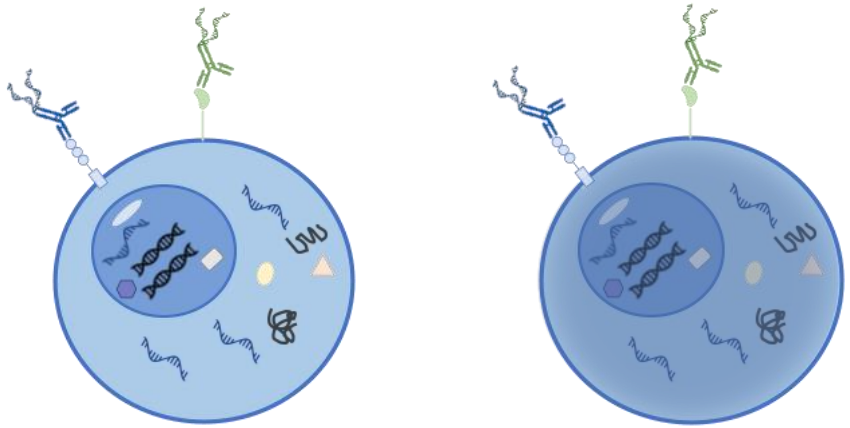


Surface AbSeq Staining

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



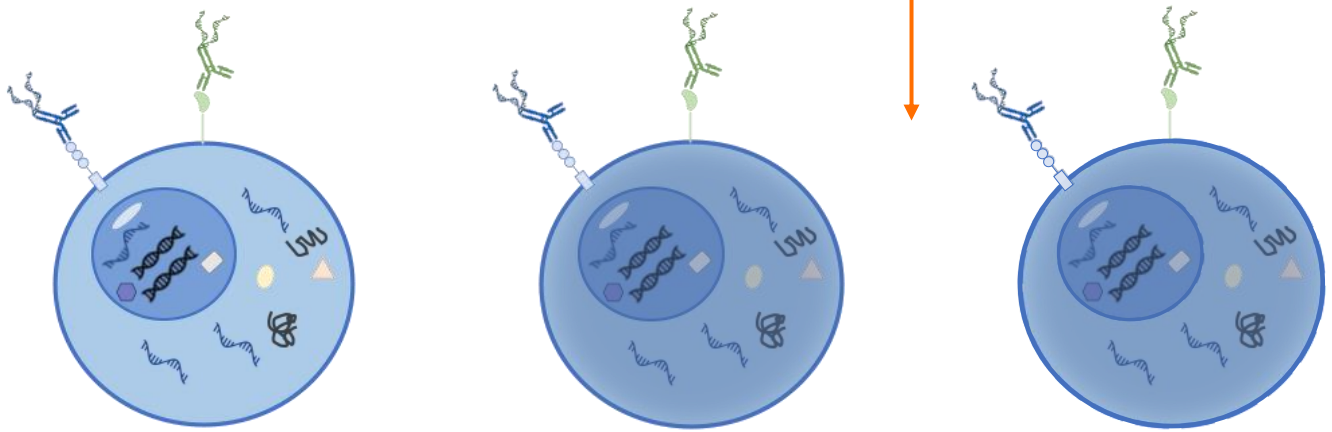
Surface AbSeq Staining

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

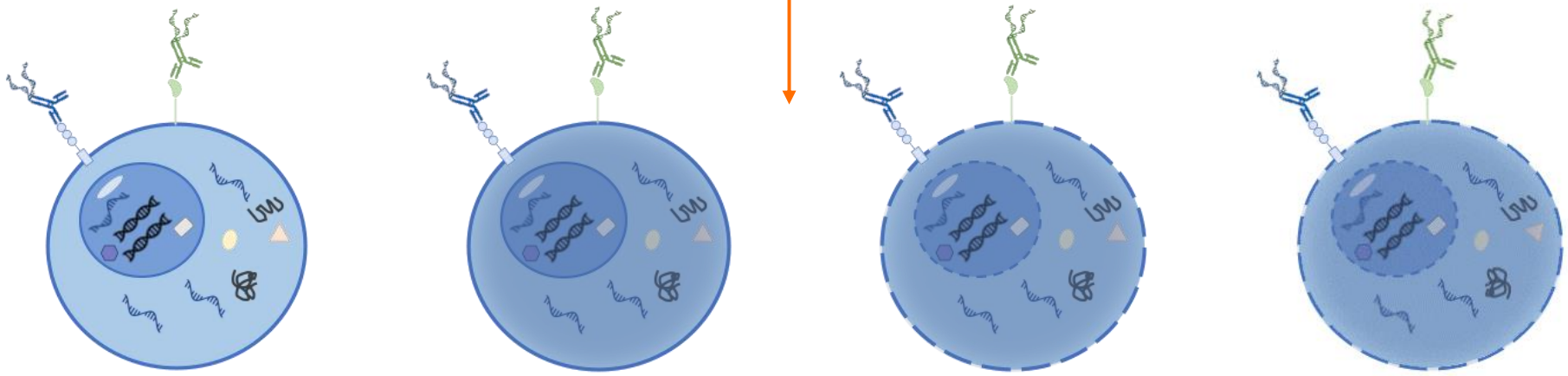


Surface AbSeq Staining | Preservation | Permeabilization

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:
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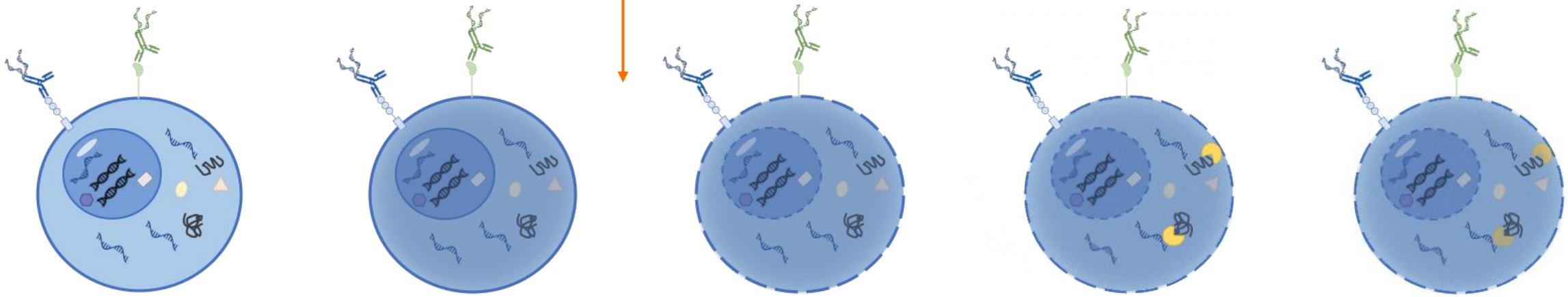


Surface AbSeq Staining | Preservation | Permeabilization | Blocking | IC AbSeq Staining

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

~2 hours

New: BD® OMICS-Guard Sample Preservation Buffer

Protect your samples, guard your science

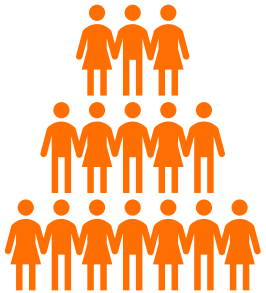
Biological sample preservation is a critical need

Collaborations

When samples need to be processed in a centralized location

Clinical samples for research

When it's hard to predict when samples can be collected and/or processed



Large-scale studies

When there are too many samples to process at the same time

Introducing BD® OMICS-Guard Sample Preservation Buffer

A simple solution for biological sample preservation to provide flexibility when samples cannot be processed at the same time or need to be transported between study sites.

- Stress-free, one-step preservation protocol with minimum hands-on time
- Optimized to preserve cells for a variety of downstream transcriptomic, proteomic and multiomic applications, including RNA-seq, CITE-seq, flow cytometry and qPCR
- Protects cell viability and preserves different cell populations in your samples for up to 72 hours at 4 °C
- Developed and tested across multiple sample types: PBMC and tissue samples
- Available in two, easy-to-use formats: 50-mL bottle or 12 x 1-mL vials
- PFA-free reagent with lower health risks

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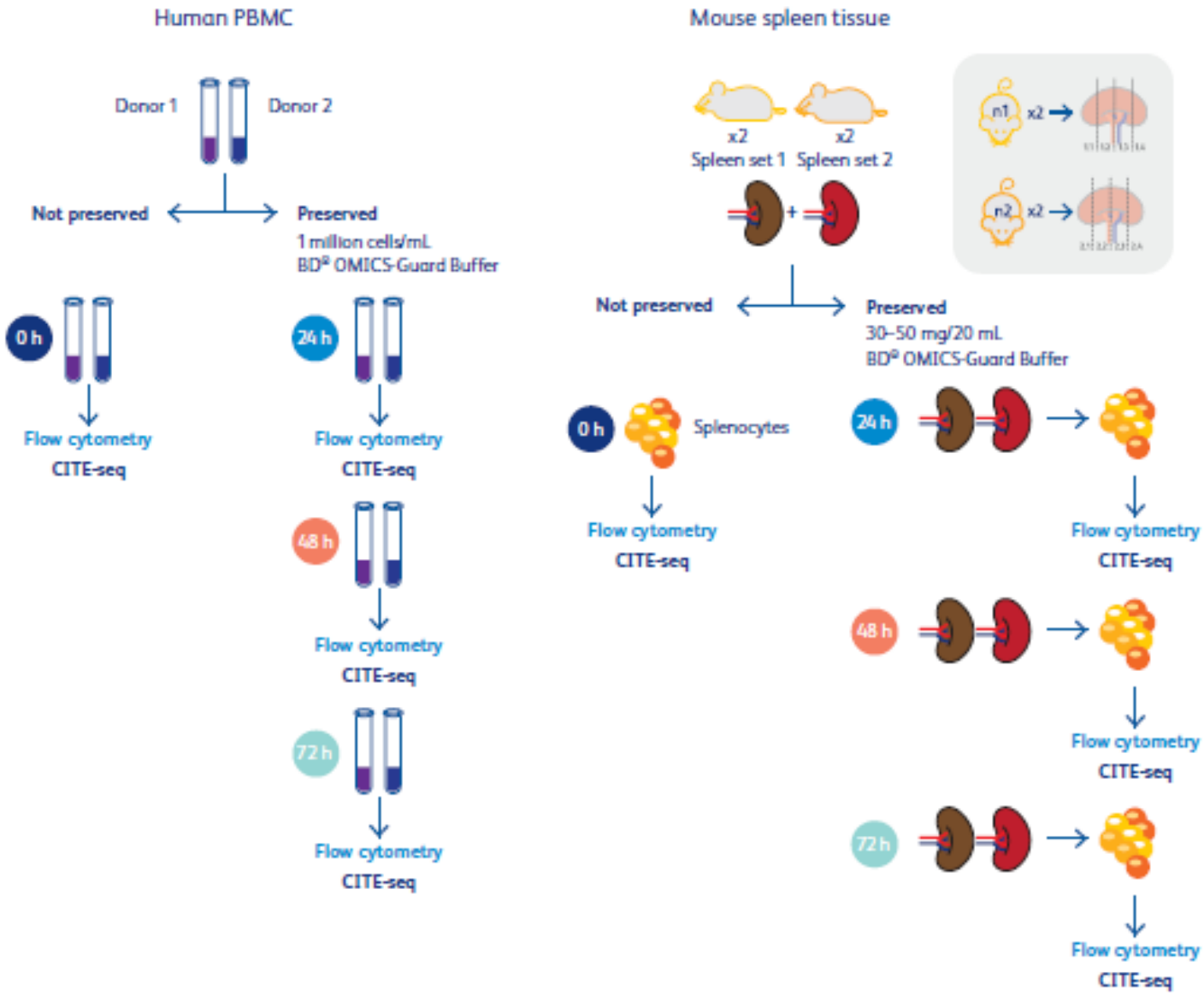
CITE-seq analyses with samples preserved in BD® OMICS-Guard Buffer

Overview of experimental design

CITE-seq analyses were conducted on the BD Rhapsody™ Single-Cell Analysis System with PBMCs and tissues preserved in BD® OMICS-Guard Buffer. Cell viability, 3′ gene expression, surface protein expression and cell populations in both human PBMCs and mouse spleen tissues were analyzed and compared to non-preserved samples (controls) in a time-course study over 72 hours.



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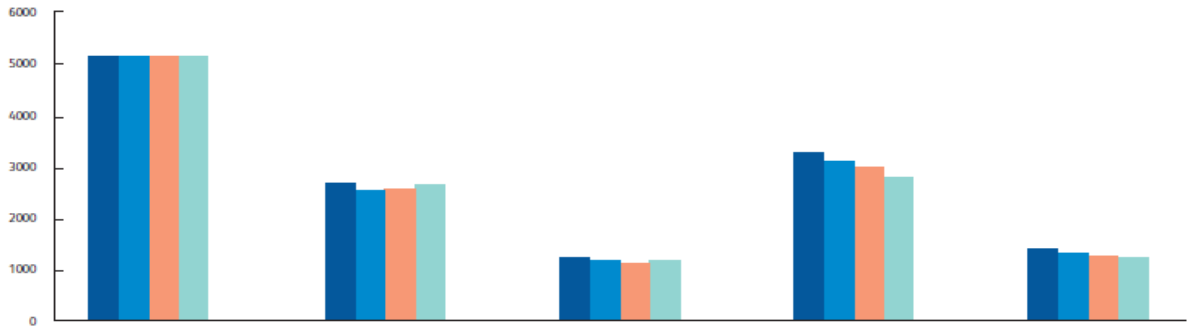
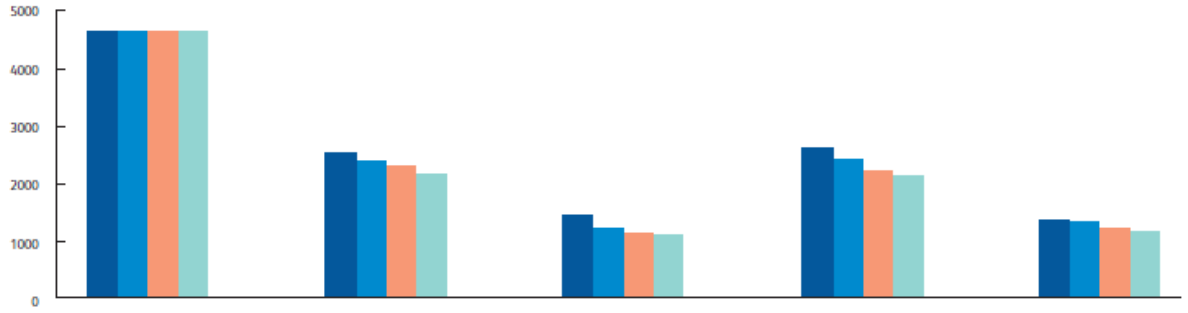
Whole transcriptome analysis (WTA) assay sensitivity metrics

A. Human PBMC



	Donor 1 and Donor 2 Mean reads per cell	Donor 1 Median molecules/cell	Donor 1 Median genes/cell	Donor 2 Median molecules/cell	Donor 2 Median genes/cell
0 h	5211	2712	1222	3285	1402
24 h	5200	2567	1159	3122	1335
48 h	5204	2608	1144	3039	1294
72 h	5206	2663	1148	2841	1203

B. Mouse spleen tissue



	Sample 1 and 2 Mean reads per cell	Sample 1 Median molecules/cell	Sample 1 Median genes/cell	Sample 2 Median molecules/cell	Sample 2 Median genes/cell
0 h	4676.49	2558	1486	2631	1387
24 h	4673.11	2391	1300	2455	1319
48 h	4672.62	2336	1254	2202	1221
72 h	4680.95	2181	1171	2150	1155

WTA assay sensitivity represented by median molecules per cell (median transcripts per cell) and median genes per cell were compared among control samples (0 h) and preserved 24, 48, and 72-h human PBMCs (**A**) and mouse splenocytes (**B**). Sequencing data were normalized to the same read-depth and samples were demultiplexed.

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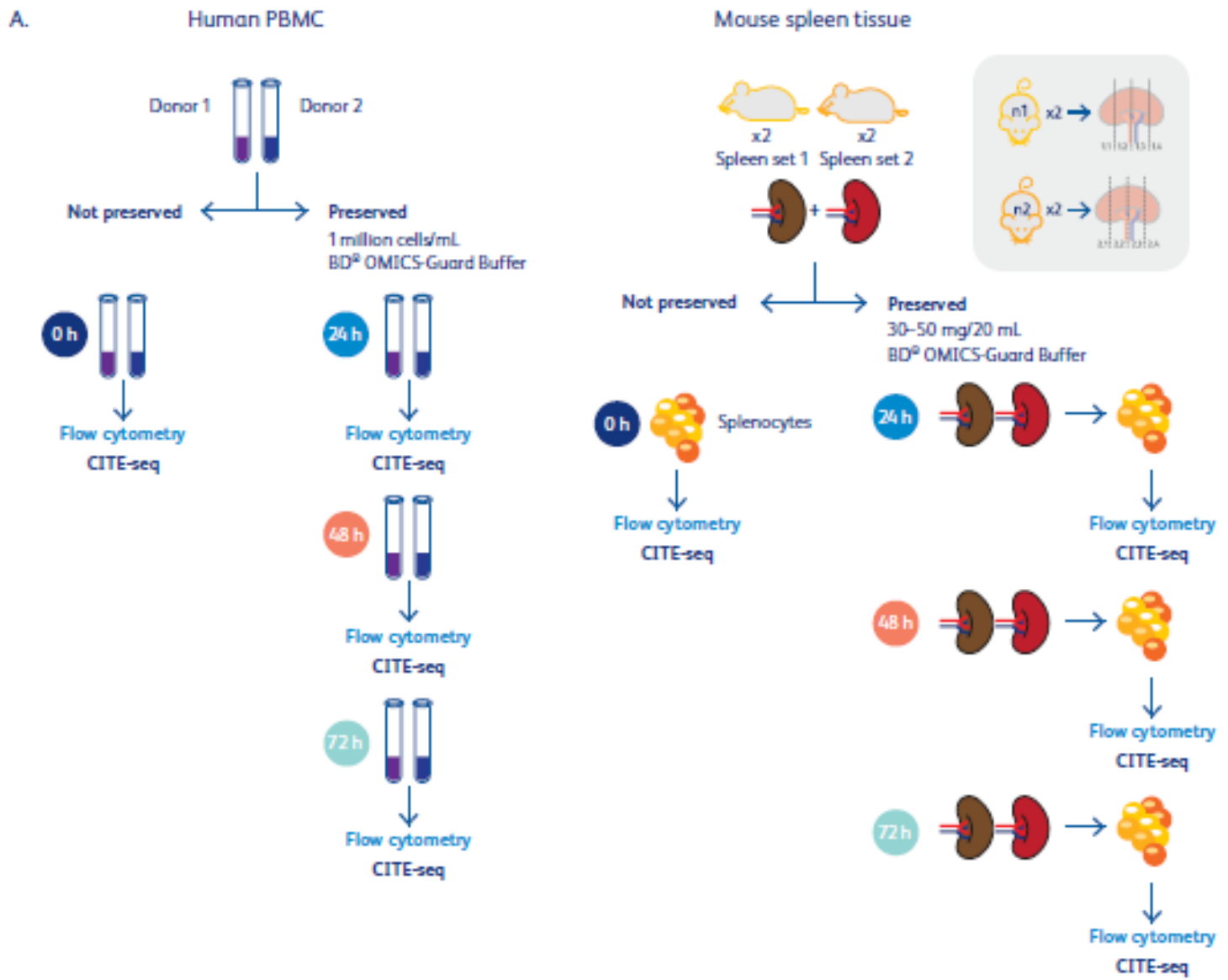
Proteomic profiling of BD® OMICS-Guard Buffer preserved samples using flow cytometry analyses

Overview of experimental design

Flow cytometry analyses were conducted on human PBMCs preserved in BD® OMICS-Guard Buffer and mouse splenocytes from tissue preserved in BD® OMICS-Guard Buffer. Surface protein expression and cell populations in both human PBMCs and mouse splenocytes were analyzed and compared to non-preserved samples (controls) in a time-course study over 72 hours.



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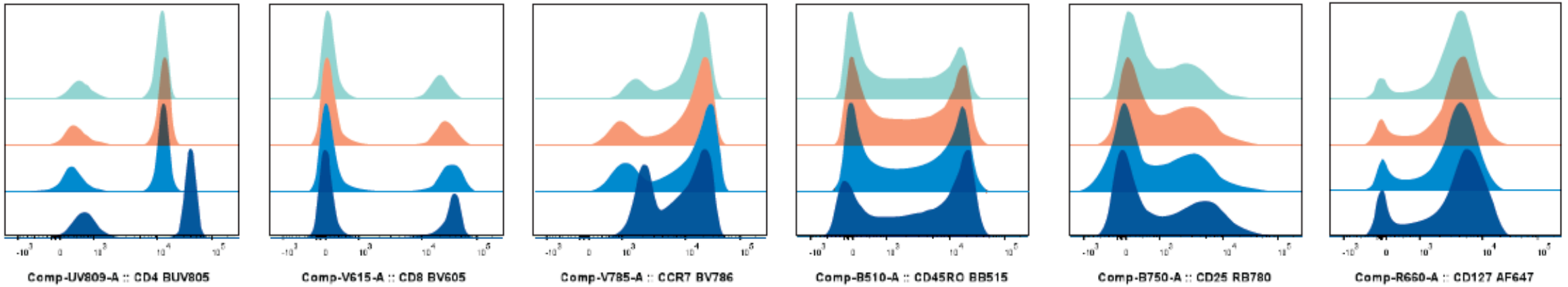
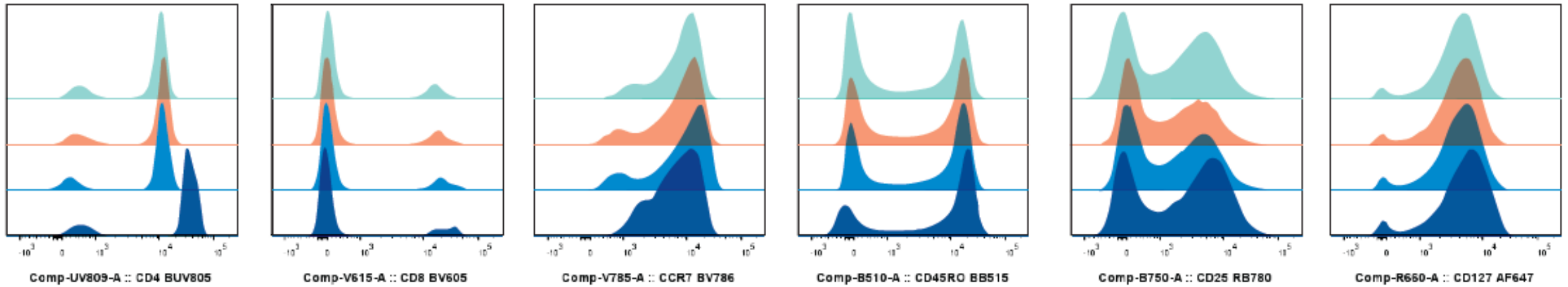
Consistent protein expression over time in human PBMC samples

Donor 1



Donor 2



Flow histogram plots show consistent key protein marker expression represented by fluorescent signal between control (0 h) and preserved samples over time (24, 48, 72 h) for human PBMCs (top: Donor1, bottom: Donor 2). For both samples, CD14 and CD16 positive cells are excluded from live CD45+ leukocytes. NK cells and B cells are identified from CD14-CD16- cells. CD4 and CD8 T cells were gated from CD3 T cells. Effector memory T cells and regulatory T cells are evaluated on CD4 T cells.

0 h
24 h
48 h
72 h

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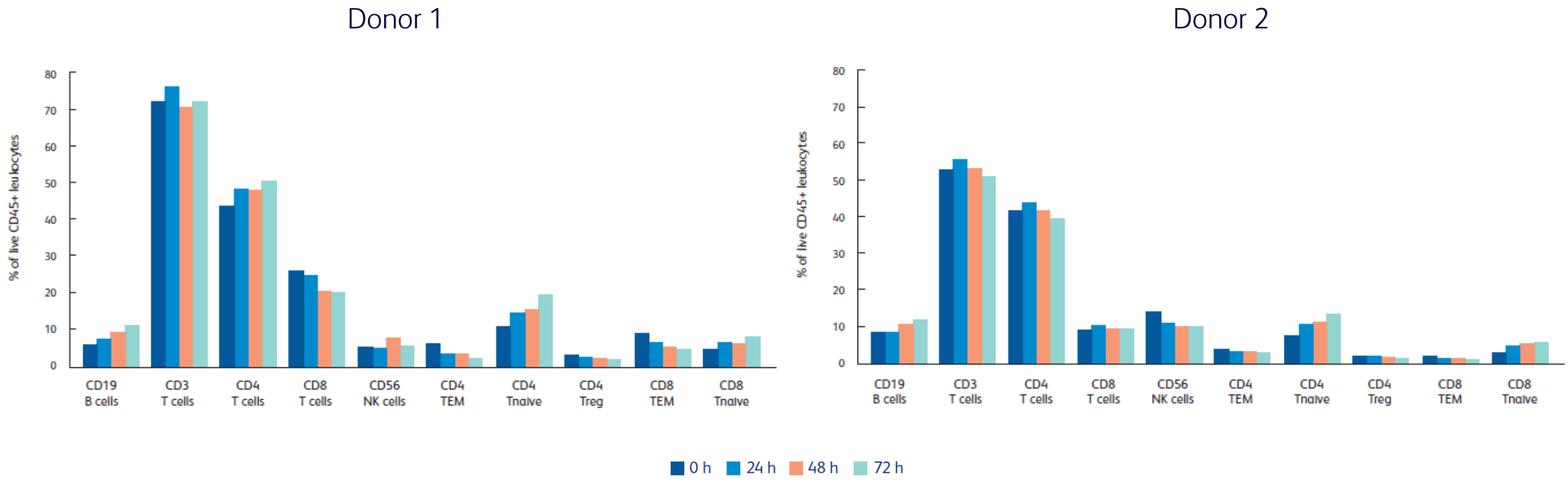
Cell subpopulation frequencies of human PBMCs across preservation time points is consistent



The ratio of different immune populations stays consistent over time for both Donor 1 and Donor 2. Proportions of live CD45+ cell types identified by corresponding cell surface marker(s), as outlined in the gating scheme, across donor and preservation time points.

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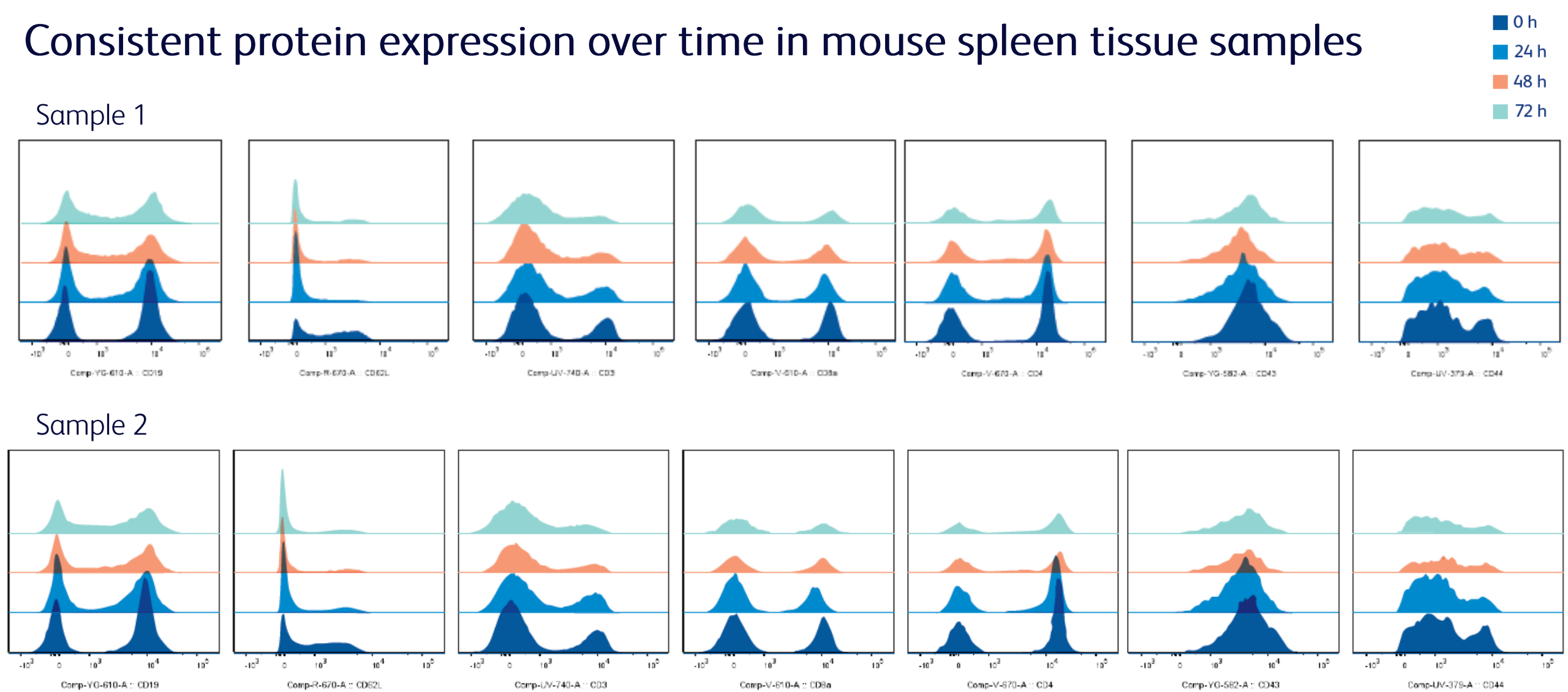
Consistent protein expression over time in mouse spleen tissue samples



Flow histogram plots show consistent key protein marker expression represented by fluorescent signal between control (0 h) and preserved samples over time (24, 48, 72 h) for mouse spleen tissues (top: Sample 1, bottom: Sample 2). For both samples, CD19 and CD3 is gated on CD45+; CD62L is gated on CD19, and CD4 and CD8a histograms are from CD3 gate. Additionally, we show histograms for lymphocyte surface proteins CD43 and CD44 signal in CD4 event clusters. Cell type and surface markers signals are relatively consistent across donor and preservation time.

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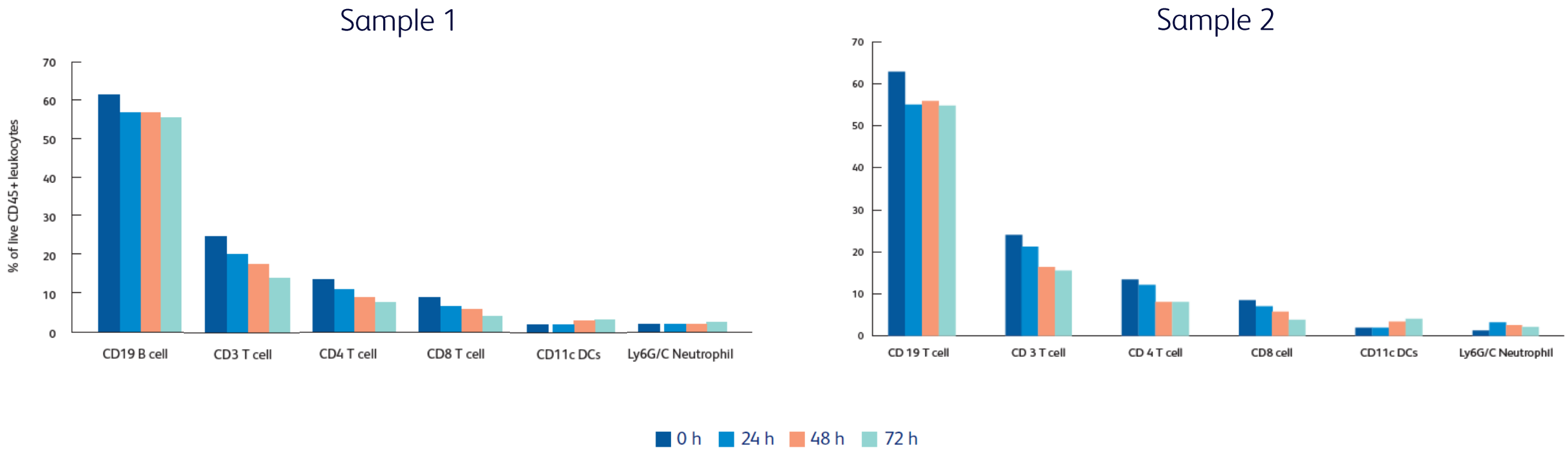
Cell subpopulation frequencies of mouse splenocytes across preservation time points is consistent



The ratio of different immune populations stays consistent across time for both mouse spleen tissue samples. Proportions of major splenic leukocyte CD45+ cell types identified by corresponding cell surface marker, as outlined in the gating scheme, across sample and preservation time points.

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Thank you for your attention