BD® AbSeq Immune Discovery Panel

High-dimensional protein discovery tool for single-cell studies

BD

Uncover up to 30 immune markers in a single experiment

Performing protein discovery experiments can be challenging. From reagent handling and marker selection to ensuring accurate execution of all the necessary steps, probing samples for tens of protein markers in a single experiment is no trivial task. Introducing the BD® AbSeq Immune Discovery Panel (IDP), a simple tool that will enable you to uncover up to 30 different immune markers in a single experiment.

The IDP consists of 30 different specificities against major human immune markers combined in a single tube. We developed the IDP by ensuring its performance was comparable to data generated by freshly prepared mixture of the same 30 specificities of our single vial BD® AbSeq Reagents. Designed and optimized to work on the BD Rhapsody™ Single-Cell Analysis System, the IDP is tested to work seamlessly with BD Rhapsody Single-Cell RNA Assays and Multiplexing Kits.

Figure 1A

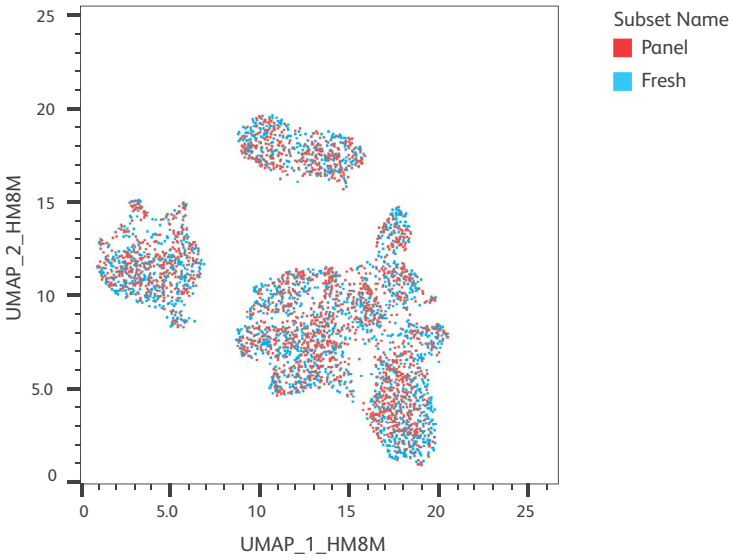


Figure 1B

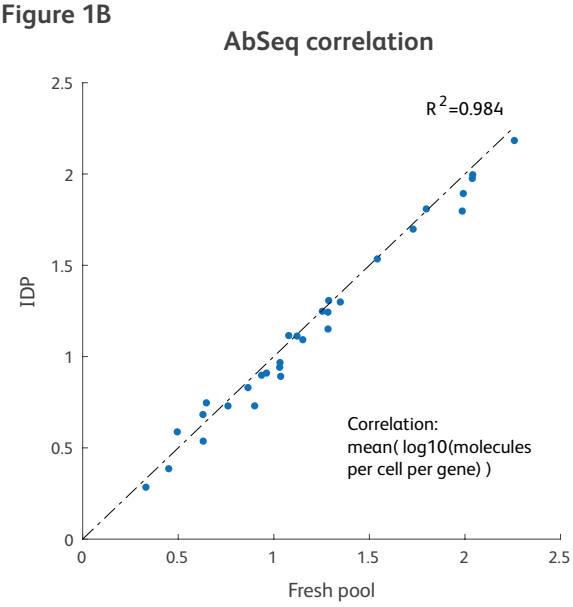
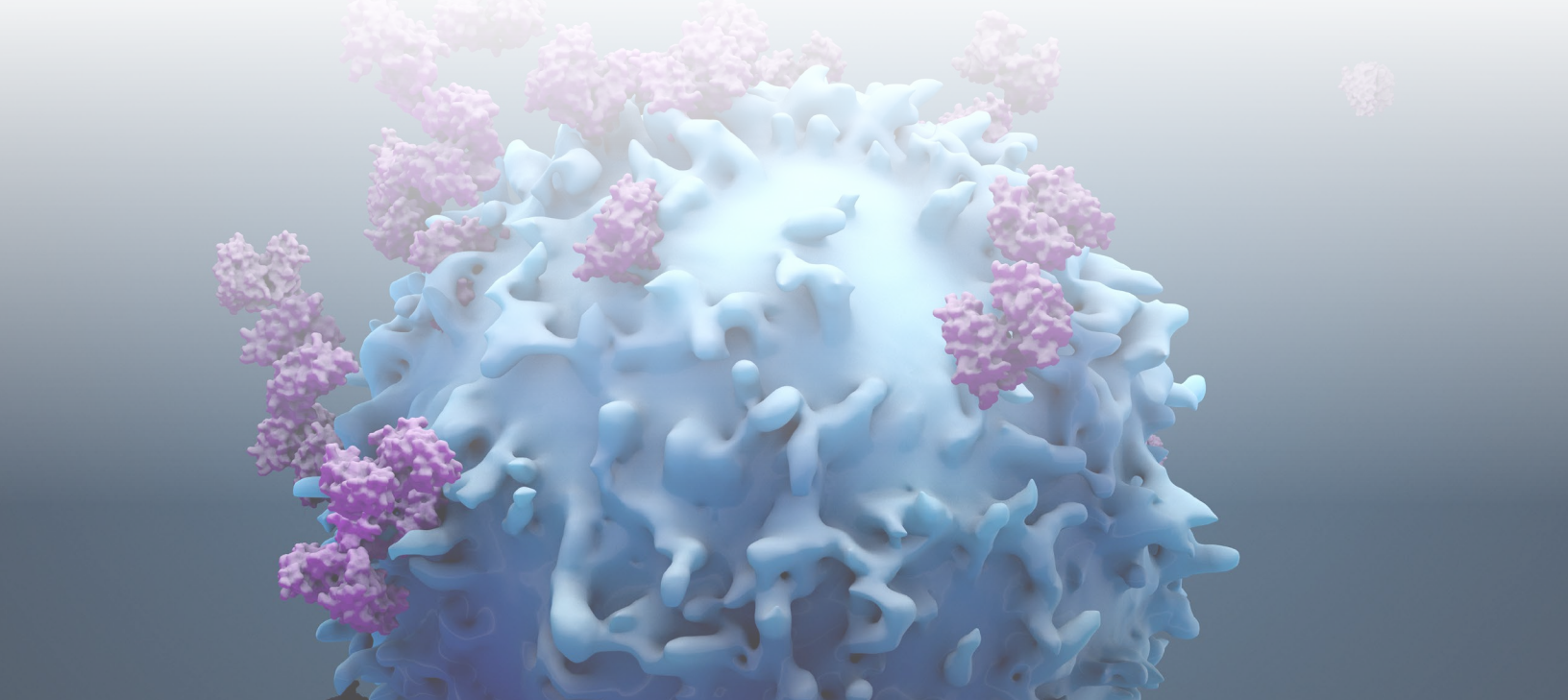


Figure 1. Similar performance between the IDP versus freshly mixed BD AbSeq Antibodies
Following isolation from whole blood, Peripheral blood mononuclear cells (PBMC) were split into resting (untreated) and activated (treated with CD3/CD28 for 24 hours) groups. A 1:1 mixture of the resting and activated cells were stained with either the IDP or a freshly prepared mixture of the same AbSeq specificities. Equal number of cells were then loaded onto BD Rhapsody™ Cartridges and AbSeq and WTA libraries were generated and sequenced (n = 2 individual experiments for this study). Data were analyzed using SeqGeq™ Software and Dataview Software. **A.** UMAP demonstrated strong overlap in the cell groups identified between the IDP and fresh BD® AbSeq Antibody-stained samples. **B.** The total number of AbSeq molecules detected by the IDP and fresh BD AbSeq Antibody mixture showed a high correlation with R^2 greater than 0.98.



Features

ONE-TUBE CONVENIENCE

30 pre-titrated antibodies against major human immune markers in a single tube

EASE OF USE

Lyophilized format; simply reconstitute to stain samples

RELIABLE

Accompanied by comprehensive performance test data

FLEXIBLE

A great backbone panel that allows easy addition of more antibodies of interest

MULTIOMICS ENABLED

Works along with RNA and multiplexing assays

GREAT VALUE

Manage your sequencing costs

30 pre-titrated antibodies								
Specificity	Clone	Oligo ID	Specificity	Clone	Oligo ID	Specificity	Clone	Oligo ID
CD3	UCHT1	AHS0231	CD45RA	HI100	AHS0009	CD196 (CCR6)	11A9	AHS0034
CD4	SK3	AHS0032	CD56	NCAM16	AHS0019	CD197 (CCR7)	2-L1-A	AHS0273
CD8	SK1	AHS0228	CD62L	DREG-56	AHS0049	CD272	J168-540	AHS0052
CD11c	B-Ly6	AHS0056	CD127	HIL-7R-M21	AHS0028	CD278	DX29	AHS0012
CD14	MPHIP9	AHS0037	CD134	ACT35	AHS0013	CD279	EH12.1	AHS0014
CD16	3G8	AHS0053	CD137	4B4-1	AHS0003	CD357 (GITR)	V27-580	AHS0104
CD19	SJ25C1	AHS0030	CD161	HP-3G10	AHS0205	CD366 (TIM-3)	7D3	AHS0016
CD25	2A3	AHS0026	CD183 (CXCR3)	1C6/CXCR3	AHS0031	HLA-DR	G46-6	AHS0035
CD27	M-T271	AHS0025	CD185 (CXCR5)	RF8B2	AHS0039	IgD	IA6-2	AHS0058
CD28	L293	AHS0138	CD186 (CXCR6)	13B 1E5	AHS0148	IgM	G20-127	AHS0198

Steps for reconstituting the lyophilized IDP and staining cells

1. Remove the IDP tube from the foil bag and bring up to room temperature for 5 minutes.
2. Make sure the pellet is located at the bottom of the tube.
3. Add 35 µL of nuclease-free water to the bottom of the tube and allow antibodies to reconstitute for five minutes at room temperature.
4. Store the reconstituted antibodies on ice until the cells are ready for staining.
   Note: Reconstituted antibodies should be used within 24 hours.
5. To make 2X AbSeq labeling master mix, add 65 µL of BD Pharmingen™ Stain Buffer to the solution to bring it to a total of 100 µL.
6. Mix with 100 µL cell suspension prepared following **Single Cell Labeling with BD AbSeq Ab-Oligos** (Doc ID 214394 Rev. 1.0) and stain the cells on ice for 30 minutes.
7. Add 3 mL BD Pharmingen Stain Buffer (FBS) to labeled cells and pipet-mix.
8. Centrifuge each tube at 400 x *g* for 5 minutes.
9. Uncap each tube and invert to decant supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wipe to remove residual supernatant from tube rim.
10. Repeat steps 7–9 twice more for a total of 3 washes.
11. Resuspend pellet in 620 µL cold BD Pharmingen Stain Buffer (FBS) from the BD Rhapsody Cartridge Reagent Kit and proceed to single-cell capture. See the **Single Cell Analysis Workflow with BD Rhapsody Systems** (Doc ID: 220524).

Reliable performance

Ensuring optimal performance of antibodies when multiple antibodies are brought together in a panel is critical for immune discovery experiments. Keeping this in mind, we tested the performance of the individual 30 specificities included in the IDP against PBMCs from multiple donors. Our results demonstrate that all 30 specificities in the panel reliably detect their respective, individual targets.

Figure 2A

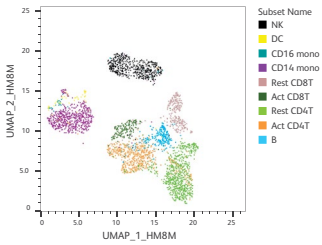


Figure 2B

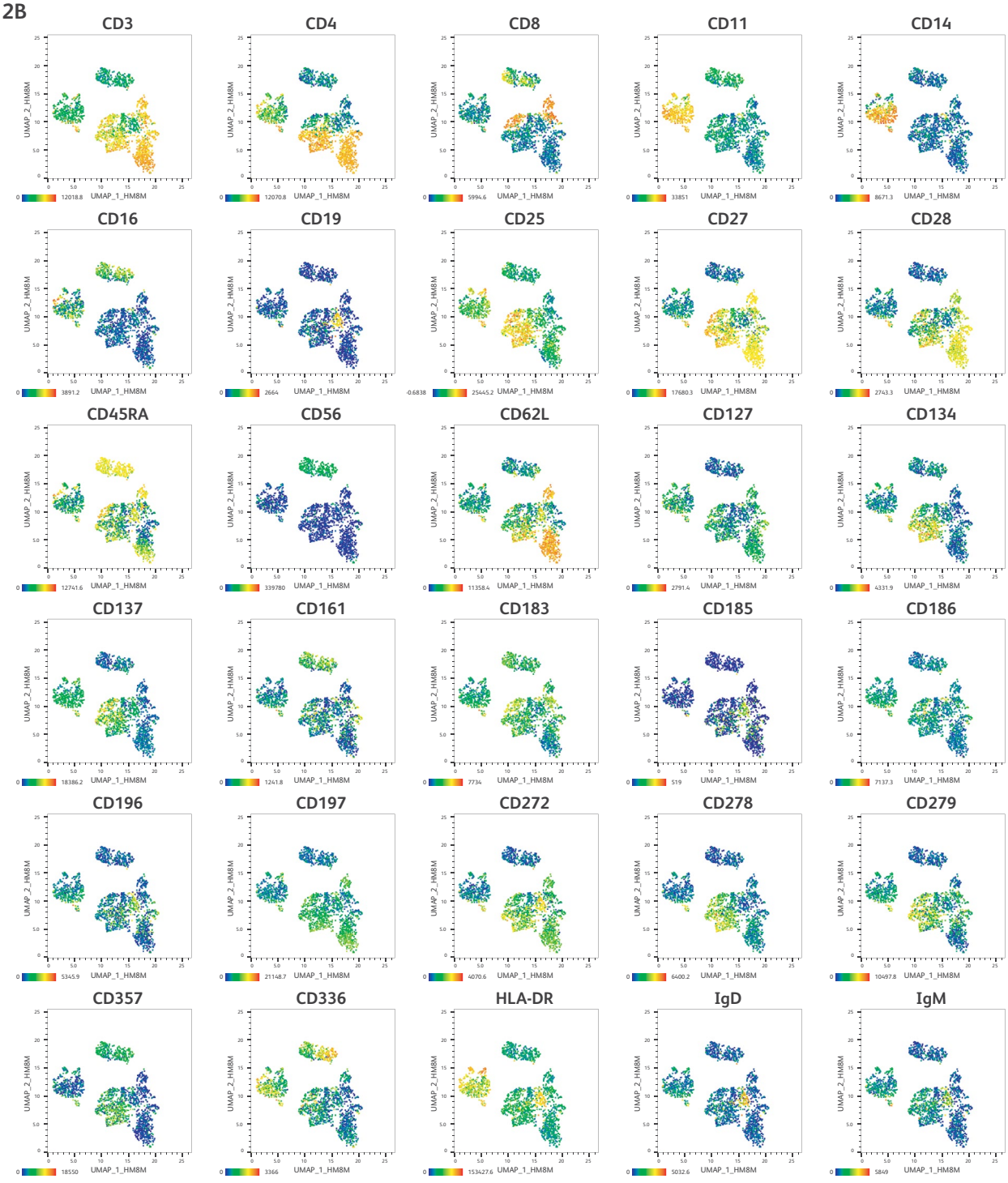


Figure 2. Performance of all 30 AbSeq specificities included in the IDP

PBMC were activated and prepared as described in Figure 1. After staining, cells were captured on the BD Rhapsody System and AbSeq and WTA libraries were generated and sequenced. To obtain over 80% sequencing saturation, the libraries were sequenced at 20,000 reads/cell for WTA and 30,000 reads/cell for AbSeq using the Illumina™ NextSeq™ High-Output Kit. Data were analyzed using SeqGeq Software and Dataview Software. We repeated the above experiments with at least two different donors. The representative figures from one donor are shown here. **A.** Cell annotation of UMAP of resting + activated PBMCs resolved by the IDP antibodies and the WTA mRNA profile. **B.** Heat maps of each AbSeq clone from IDP on UMAP from Figure 2A showing the specificity of AbSeq detection for individual cell type.

Flexibility to add additional specificities of interest

While the IDP provides the ideal backbone panel to quickly probe major immune markers of interest, we recognize there will be additional markers that you would like to include alongside IDP. The IDP is designed to accommodate additional AbSeq specificities and our results demonstrate that adding more BD AbSeq Antibodies does not impact the performance of the IDP or the added antibodies. We specifically chose three distinct specificities to demonstrate the above capability: CD38—a commonly expressed antigen; CD45RO—to test if this specificity complements the CD45RA already included in the panel; and RPA-T8 clone of CD8—to test if alternate clones of the same specificity can be added to the panel as the IDP already includes the SK1 clone of CD8.

Figure 3A

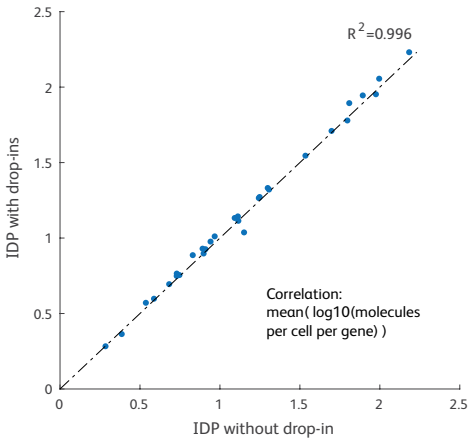


Figure 3B

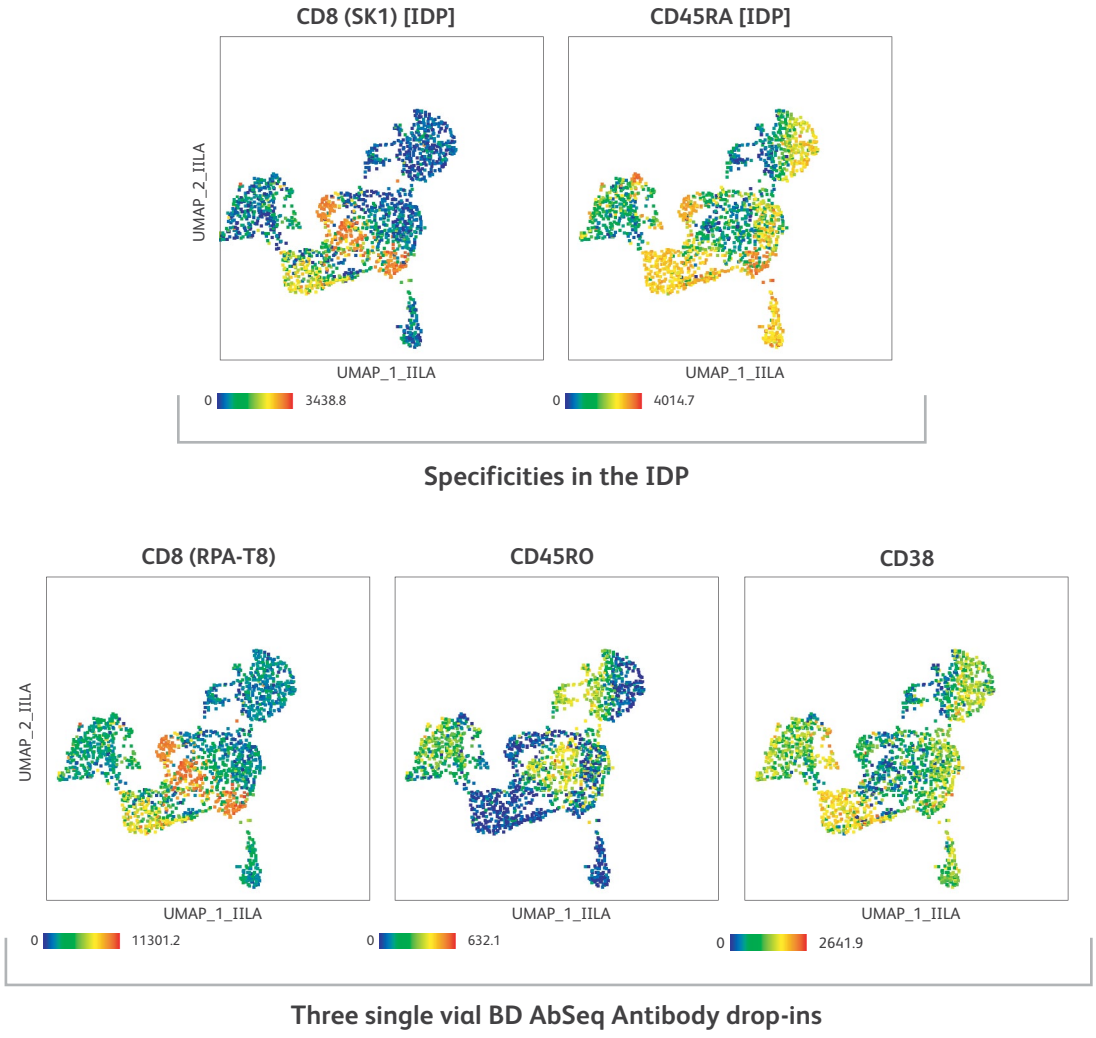


Figure 3. The IDP is a flexible backbone panel and accommodates additional AbSeq specificities

Three BD AbSeq Antibodies were added and mixed with the reconstituted IDP pellet (n = 2). **A.** The IDP performance was not impacted by drop-ins as shown by high correlation (R^2 over 0.99 with or without drop-ins). **B.** The IDP specificities of CD8[SK1] and CD45RA (top row) were assessed against the specificity of drop-ins CD8 [RPA-T8], CD45RO and CD38 (bottom row) and are shown in UMAP. Drop-in for CD38 detected cell types that are expected to be positive for CD38. Drop-in clone for CD8 (RPA-T8) showed a staining pattern very similar to the IDP clone (SK1) suggesting the high specificity of drop-in antibody as well as the compatibility of two clones for the same antigen. The contrasting expression pattern of CD45RO (drop-in) compared to CD45RA (IDP) further confirmed that adding the AbSeq specificities to the IDP had no adverse impact on experimental outcomes.

Multiomics and multiplexing enabled

Perform RNA analyses and combine multiple samples with the IDP

Performing multiomic analyses (RNA + protein) at the single-cell level can reveal deeper insights about your samples. In addition, performing such multiomic analyses on multiple samples together in a single experiment can reduce costs and most importantly help avoid inter-experimental batch effects. The IDP was developed to work seamlessly with BD Rhapsody RNA Assays and Multiplexing Kits.

Figure 4A

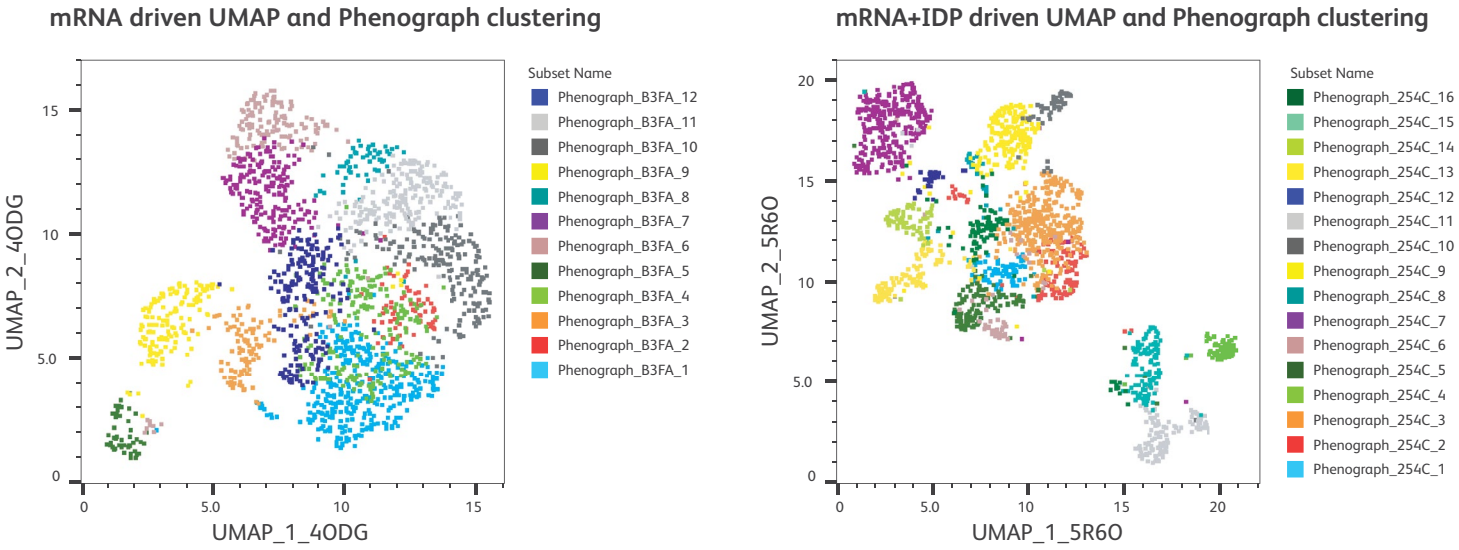


Figure 4B

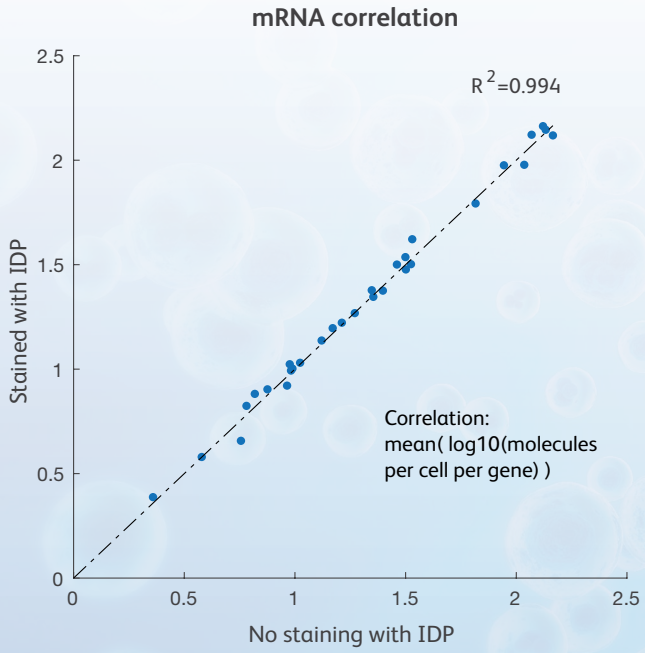


Figure 4. The IDP is designed to work with RNA and multiplexing assays
A. WTA and AbSeq libraries from IDP-stained cells (1:1 mixture of resting and activated PBMCs) were generated and sequenced. To illustrate the power of multiomic analysis, we analyzed the WTA data only (mRNA analysis) and compared with WTA + AbSeq data (mRNA and protein analysis). UMAP coordinates and unbiased clustering (Phenograph) using only WTA (mRNA) data are shown on the left, while coordinates and annotations using WTA + AbSeq (mRNA and protein) data are shown on the right. With a multiomics approach, additional cell types were revealed offering deeper biological insights. **B.** To test the compatibility of the BD® Single-Cell Multiplexing Kit (SMK) and the IDP, we performed cell staining with the SMK and the IDP together and generated WTA, AbSeq and SMK libraries for sequencing. The expression of markers in the IDP was then compared to data generated in the absence of the SMK. These data showed that addition of the SMK does not impact the IDP as demonstrated by high correlation (R^2>0.99) between the IDP + WTA versus IDP + WTA + SMK.

The BD AbSeq Immune Discovery Panel is part of the broad BD portfolio of reagents, instruments and software designed to support your single-cell research.

BD Single-Cell Multiplexing Kit

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

x12

BD Rhapsody Express System

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

BD Rhapsody System

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

- BD Rhapsody™ Whole Transcriptome Analysis Kit
- BD Rhapsody™ Targeted Single-Cell RNAseq Panels
- BD AbSeq Single-Vial Reagents
  BD AbSeq Immune Discovery Panel
- BD Rhapsody™ Custom RNA Panels
  Custom BD AbSeq Antibodies
- BD Rhapsody™ TCR/BCR Profiling Assays

BD Bioinformatics

Sequencing read alignment and mapping primary analysis software

SeqGeq™ v1.6 Secondary Analysis Software for clustering visualization and elimination of multiplet data for clean differential expression

Utilize our expertise and insights to manage the sequencing costs of your single cell experiments. Reach out to your local BD sales representative or contact our help desk **scomix@bdscomix.bd.com** to learn more about managing sequencing costs while using the IDP.

Ordering Information

Panel	
Description	Cat. No.
BD® AbSeq Immune Discovery Panel	625970

To request a quote or place an order, email bdb_custom_orders@bd.com or contact your local BD sales representative.

Suggested Companion Products	
Description	Cat. No.
BD Rhapsody™ Single-Cell Analysis System	633701
BD Rhapsody™ Express Single-Cell Analysis System	633707
BD Rhapsody™ Whole Transcriptome Analysis Amplification Kit	633802
BD Rhapsody™ Targeted mRNA Kit	633771
BD® Human Single-Cell Multiplexing Kit	633781
BD Pharmingen™ Human BD Fc Block™ Buffer Solution	564220
BD Rhapsody™ Immune Response Panel	633750
BD Pharmingen™ Stain Buffer (FBS)	554656

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