The BD Rhapsody™ HT Xpress System supports dynamic and high-throughput single-cell capture enabling a seamless workflow for analysis of cellular immune responses

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Poster#
P1029

Abstract

The BD Rhapsody™ Single-Cell Analysis System is an innovative platform that aids high-throughput sequencing of mRNA and proteins in thousands of single cells. Here we present a new eight-lane cartridge design concept, the BD Rhapsody™ HT Xpress System. This novel device architecture enables the users to simultaneously load multiple cell samples while preserving the flexibility to use one lane at a time. To demonstrate the increased throughput capabilities and workflow flexibility of the system, we measured in vitro T cell activation over time. To do this, peripheral blood mononuclear cells from four different subjects were prepared for cell culture in IL-2 or IL-2/anti-CD3/anti-CD28. After two days in culture, cell samples from each culture condition were barcoded using the BD® Single-Cell Multiplexing Kit (SMK). Cells from the same subject were pooled and labeled with the BD® AbSeq Immune Discovery Panel (IDP). Pooled cells from each subject were loaded into the cartridge (one subject per lane, four lanes total). Remaining cells were cultured for another day and similarly barcoded, labeled and loaded into the remaining four lanes of the cartridge. Eight different next generation sequencing libraries were generated and analyzed with the BD Rhapsody™ Analysis Pipeline and DataView Software. This strategy enabled us to assess dynamic changes in both T cell surface protein profiles and transcriptome during cell activation while using a streamlined workflow for concomitant analysis of multiple samples.

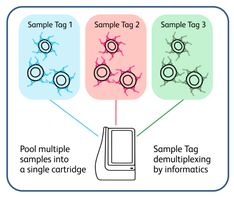
Technology

BD Rhapsody™ HT Xpress System

The BD Rhapsody™ HT Xpress System enables scientists to isolate, barcode and analyze single cells at a high sample throughput — up to eight times more cells than prior versions of BD single-cell analyzers. The system minimizes sample loss and gives scientists the flexibility to analyze multiple samples and different cell sizes and types, such as stem cells or cancer cells, at the same time, to obtain more insights in less time.

BD® Single-Cell Multiplexing Kits

The BD® Single-Cell Multiplexing Kits allows the easy combination and simultaneous processing of up to 12 different samples in one single-cell multiomics experiment on the BD Rhapsody™ Single-Cell Analysis System. The kits are designed to work with all BD Rhapsody™ Assays and include streamlined informatics tools integrated into the BD Rhapsody™ Bioinformatics Analysis Pipeline to automatically demultiplex by Sample Tags and identify individual samples during data analyses.



BD Rhapsody™ T-Cell Targeted Panel (Human)

The BD Rhapsody™ T-Cell Targeted Panel (Human) utilizes multiplex PCR for detecting 259 genes chosen for T-cell profiling. After cells are lysed in the BD Rhapsody™ Cartridge, beads containing captured mRNA are magnetically retrieved for cDNA synthesis. Included primers are used for gene-specific nested PCR for library construction. The final PCR amplification products for sequencing contain sequencing adapters, a cell label, unique molecular index (UMI) and up to 400 bp of the 3' end of the target gene.

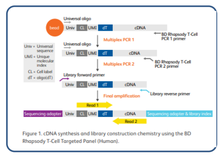


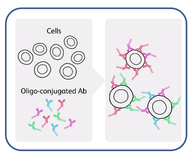
Figure 1: cDNA synthesis and library construction chemistry using the BD Rhapsody T-Cell Targeted Panel (Human).

Example genes by category

Pathway	# of genes in panel
CD marker	25
Cell type marker	21
Chemokine	12
Chemokine receptor	15
Cytokine	11
Cytokine receptor	8
Interleukin	29
Other*	138

BD® AbSeq Antibodies

BD® AbSeq Antibody technology provides a DNA oligonucleotide with a unique molecular identifier conjugated to any one of hundreds of trusted BD antibodies. BD® AbSeq Antibody Conjugates can infer protein expression via Ab binding, where hundreds of variations of protein targets can be evaluated on individual cells in a single assay. This allows the robust detection of protein markers even when their cognate mRNA transcripts are in low abundance. We dropped-in CD69, CD39, LAG-3, CX3CR1 and CD36 on the BD® Abseq Immune Discovery Panel.



BD® Abseq Immune Discovery Panel

Specificity	Clone	Oligo ID	Specificity	Clone	Oligo ID
CD3	UCHT1	AHS0231	**CD137**	4B4-1	AHS0003
CD4	Sk3	AHS0032	**CD161**	HP-3G10	AHS0205
CD8	Sk1	AHS0228	**CD183 (CXCR3)**	1C6/CXCR3	AHS0031
CD11c	B-Ly6	AHS0056	**CD185 (CXCR5)**	RF8B2	AHS0039
CD14	M9HIP9	AHS0037	**CD186 (CXCR6)**	13B 1E5	AHS0148
CD16	3G8	AHS0053	**CD196 (CCR6)**	11A9	AHS0034
CD19	SJ25C1	AHS0030	**CD197 (CCR7)**	2-L1-A	AHS0273
CD25	2A3	AHS0026	**CD272**	J168-540	AHS0052
CD27	M-T271	AHS0025	**CD278**	DX29	AHS0012
CD28	L293	AHS0138	**CD279**	EH12.1	AHS0014
CD45RA	HI100	AHS0009	**CD357 (GITR)**	V27-580	AHS0104
CD56	NCAM16	AHS0019	**CD366 (Tim3)**	7D3	AHS0016
CD62L	DREG-56	AHS0049	**HLA-DR**	G46-6	AHS0035
CD127	HIL-7R-M21	AHS0028	**IgM**	G20-127	AHS0198
CD134	ACT35	AHS0013	**IgD**	IA6-2	AHS0058

Results (1)

1 Experimental Scheme

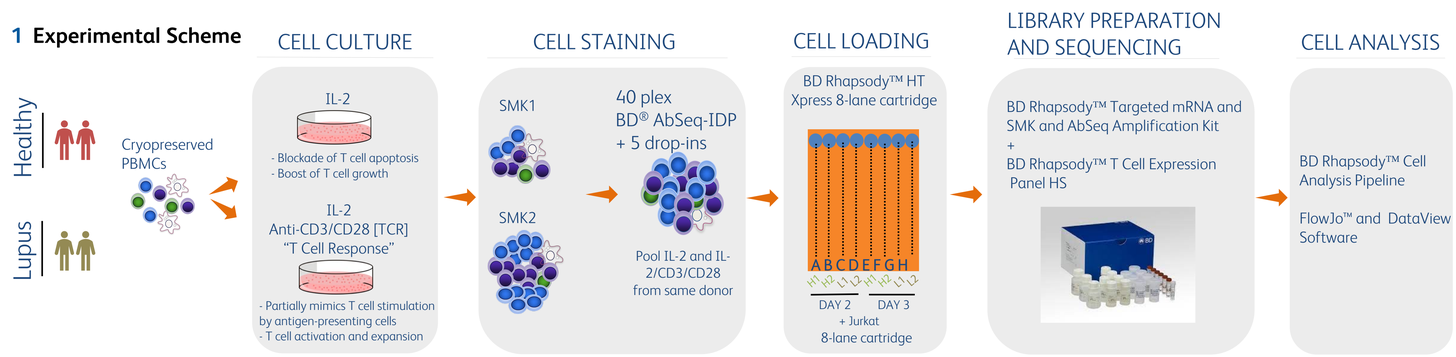


Figure 1: Cryopreserved peripheral blood mononuclear cells (PBMCs) from two healthy donors and two SLE (Systemic lupus erythematosus) patients were cultured with either IL-2 alone or IL-2 and anti-CD3/CD28. The cells were harvested after two days or three days of culture, barcoded with SMKs and stained with BD® AbSeq Immune Discovery Panel prior to loading into the BD Rhapsody™ HT Xpress Cartridge. To incorporate the temporal variable, samples from each of the four donor groups on Day 2 were loaded into half of the cartridge lanes and were stored at 4˚C, the Day 3 samples of respective donors were loaded into the remaining four cartridge lanes following the loading protocol. The cells were profiled using the AbSeq, SMK and the BD Rhapsody™ T-Cell Targeted Panel (Human) containing 259 genes.

2 Experimental QC: stimulation model, index libraries and sequencing quality

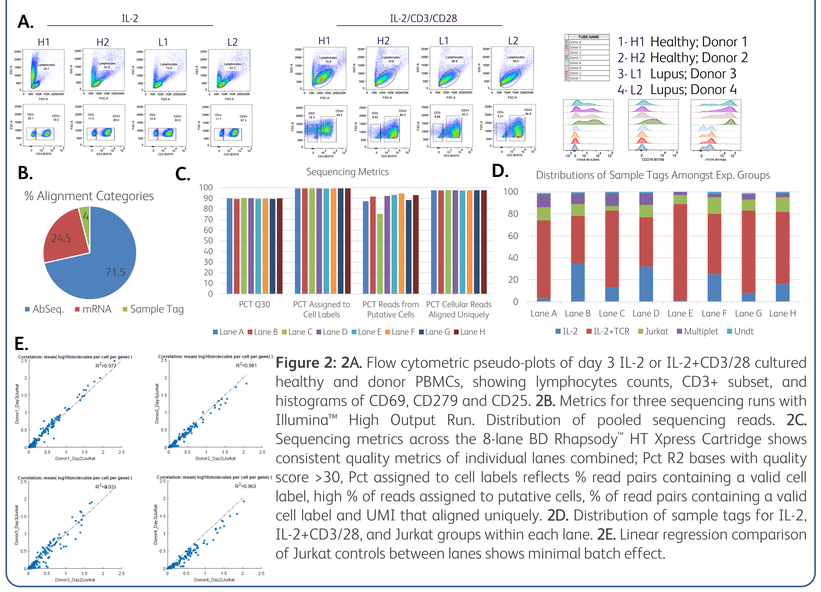


Figure 2: 2A. Flow cytometric pseudo-plots of day 3 IL-2 or IL-2+CD3/28 cultured healthy and donor PBMCs, showing lymphocytes counts, CD3+ subset, and histograms of CD69, CD279 and CD25. **2B.** Metrics for three sequencing runs with Illumina™ High Output Run. Distribution of pooled sequencing reads. **2C.** Sequencing metrics across the 8-lane BD Rhapsody™ HT Xpress Cartridge shows consistent quality metrics of individual lanes combined; Pct R2 bases with quality score >30, Pct assigned to cell labels reflects % read pairs containing a valid cell label, high % of reads assigned to putative cells, % of read pairs containing a valid cell label and UMI that aligned uniquely. **2D.** Distribution of sample tags for IL-2, IL-2+CD3/28, and Jurkat groups within each lane. **2E.** Linear regression comparison of Jurkat controls between lanes shows minimal batch effect.

3 Overall landscape of transcriptome and AbSeq profile

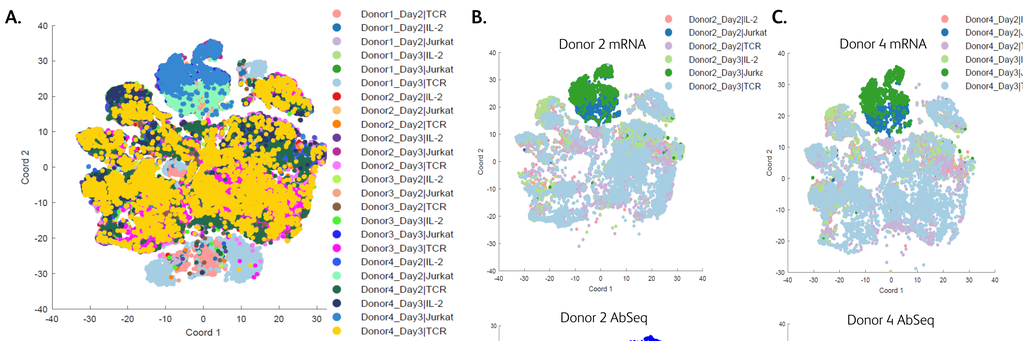


Figure 3: The numerous experimental variables encompassed within can be visualized with the following clusters. **3A.** tSNE plot generated on mRNA and AbSeq gene profile for all experimental groups loaded within the 8-lane BD Rhapsody™ HT Xpress cartridge and sequenced. Clusters are annotated with the sample tags corresponding to the 24 experimental groups of healthy and lupus PBMCs treated cultures with IL-2 or IL-2+CD3/28 stimulated models across 2 time points; all lanes were loaded with SMK-barcoded Jurkat cells, from the same batch, as internal control. **3B. & C.** Samples are deconvoluted by donor. Upper tSNE panel for representative donor 2 (healthy) and donor 4 (lupus) shows distribution of experimental groups at days 2 and 3. Bottom tSNE panels show cluster cell type determined via BD AbSeq cell markers.

Results (2)

4 Differential gene expression of CD4 T cell cluster between IL-2 and IL2+αCD3/CD28 culture conditions

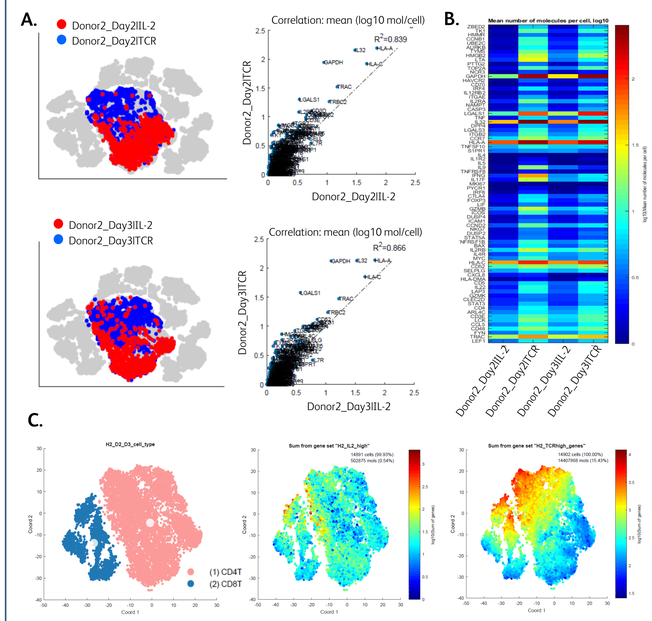


Figure 4: 4A & B. Lymphocyte subset response to different stimulation and time points is demonstrated within the context of Donor 2 (healthy) AbSeq defined CD4 T cell subset. Generation of differentially expressed gene comparison between IL-2 stimulated CD4 subset and IL-2+CD3/28 stimulated CD4 subset at two different time points for the same donor. Comparative populations are denoted by the red and blue clusters. Correlation analysis (left panel) for stimulation comparisons show differentially expressed genes are skewed towards the IL-2/TCR stimulated CD4 cells as opposed to the IL-2 stimulated cluster. **4B.** Heatmap visualizes the generalized patterns of gene expression under different stimulation time points. **4C.** Donor 2 tSNE displays the distribution of CD4 and CD8 clusters with merged timepoints, localized alteration of Donor 2 lymphocyte group under different stimulation models (left to right).

5 Dynamic transcriptomic and proteomic profile of the IL-2+ +αCD3/CD28 stimulated CD8 T cell cluster

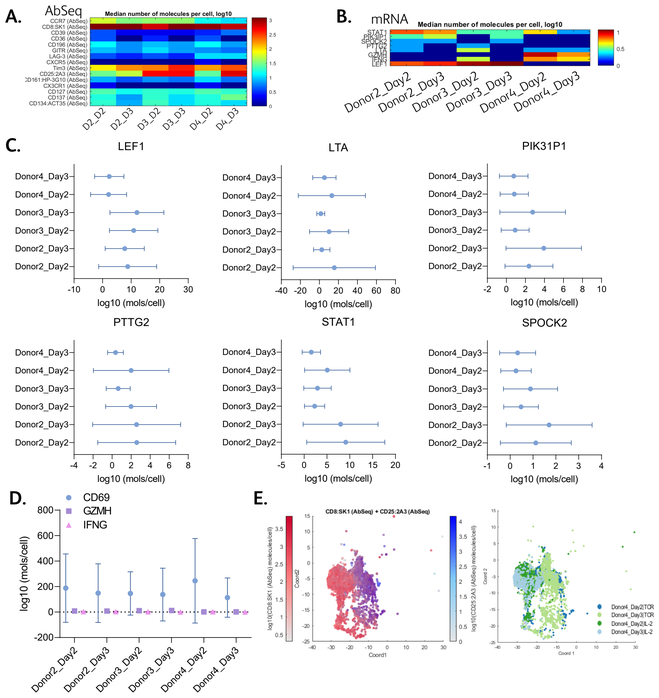


Figure 5: We next investigated the transcriptomic and proteomic changes of AbSeq defined CD8 clusters across donor types (p value < 1E-10). **5A. & B.** Heatmap of median molecule number per cell for differentially express AbSeq and mRNA genes in CD8 cluster across experimental groups. **5C.** Median # of molecules/cell for selected genes exhibit donor and timepoint specific trends in CD8 cluster. **5D.** Median # of molecules/cell of related to T cell activation. **5E.** Representative AbSeq tSNE for Donor 4 CD8 T cell cluster with corresponding CD25 AbSeq signal, right panel shows TCR-cells are localized with increased AbSeq CD25 region.

Conclusions

This study leveraged the BD Rhapsody™ HT Single-Cell Analysis System to characterize the transcriptional lymphocyte response of human PBMCs under in vitro TCR stimulation with IL-2 or anti-CD3/28 stimulation.

Here, out of the total cells captured we sub-sampled ~60,000 cells for sequencing and analysis. Sequenced data encompass 24 different samples from four different donors across two stimulation models and two time points, all in one high-throughput cartridge.

The 8-lane cartridge design allowed for the integration of time points revealing dynamic transcriptional changes with minimal batch effect.

- BD Rhapsody™ Targeted T-Cell Panel (Human) allows the focused investigations of critical genes that orchestrate T-lymphocyte expansion, metabolic adaptations, and function; along with potentially lowered sequencing cost compared to whole transcriptome.
- Incorporation of BD® AbSeq Immune Discovery Panel, in addition with the flexible integration of drop-in targets of interest, allows the concomitant profiling of cell surface profile.
- BD® Single Cell Multiplexing Kit allows for the deconvolution and comparison between the 24 experimental groups, in this application.

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