Single-cell full-length TCR/BCR profiling assay with high sensitivity and CITE-seq compatibility enables deeper immune cell characterization

Poster#
B901

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Abstract

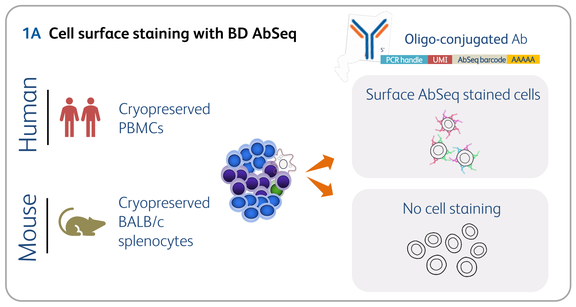
Single-cell full-length TCR/BCR assays provide the ability to profile the highly diverse T cell receptor (TCR) and B cell receptor (BCR) repertoires of the adaptive immune response. The identification of T cell clonotype can be challenging due to insufficient sensitivity to detect low abundance TCR transcripts. Furthermore, cell type identification can be limited using only transcriptomic profiles. Here, we present data with high resolution of TCR clonotype using a new highly sensitive TCR/BCR assay combined with CITE-seq using the BD® AbSeq Assay.

Much deeper insights and resolution of the immune repertoire are achieved when receptor clonotyping is combined with simultaneous transcriptome and surface protein profiling compared to a study with transcriptome profiling only. Adding surface protein profiling enhances detection of cell subsets within a complex population and couples surface protein expression with transcriptomic and clonal information of each cell.

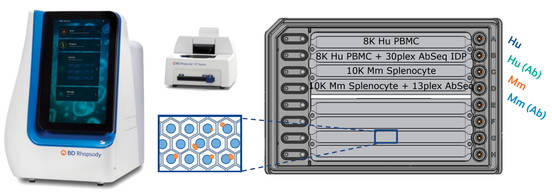
To establish the discovery potential of this improved TCR/BCR assay, we profiled human and mouse T cells alongside whole transcriptome analyses with and without CITE-seq. The enhanced assay showed high clonotype detection with transcriptome-only profiling and with CITE-seq. Results also showed high gene expression correlation, consistent library quality and mapping, and better resolution of cell subtype clustering with CITE-seq.

Methods

1A Cell surface staining with BD AbSeq



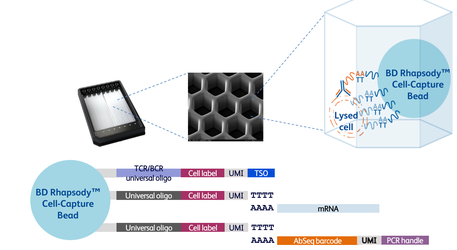
1B Cell and bead loading in microwells



BD Rhapsody™ Scanner and BD Rhapsody™ HT Xpress System

BD Rhapsody™Xpress 8-lane cartridge

1C Cell lysis and capture of mRNA and ab-oligo



1D Library preparation and sequencing

BD Rhapsody™ TCR/BCR Next Amplification Kit
+
BD Rhapsody™ WTA Amplification kit

BD Rhapsody™ Sequence Analysis pipeline

Downsample each library to same raw reads per cell:

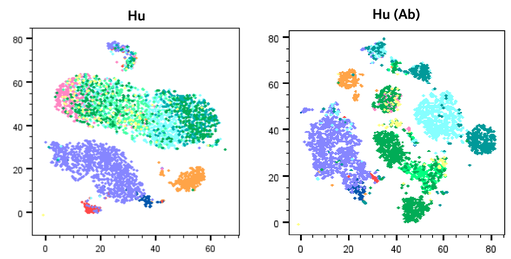
WTA ~10,000
TCR/BCR ~5,000
AbSeq ~1000/ab

Figure 1. Workflow of BD Rhapsody™ TCR/BCR Next Multiomic Assay with mRNA whole transcriptome analysis (WTA) and AbSeq. **(1A)** Cells are stained with BD AbSeq oligonucleotide-antibody markers to tag surface proteins of interest. **(1B)** Single cells are partitioned into microwells and paired with paramagnetic BD Rhapsody™ cell-capture beads. **(1C)** Following cell lysis, bead binds to polyadenylated mRNA transcripts and AbSeq oligo barcodes from lysed cells. **(1D)** On-bead cDNA synthesis followed by 5' capture via the TSO region enables extension of TCR/BCR Universal oligo and cell label information at the 5' end of cDNA. WTA, TCR, BCR, and AbSeq libraries are amplified separately through PCR. Final libraries are sequenced and analyzed via BD Rhapsody™ Sequence Analysis pipeline. All libraries downsampled to minimum recommended raw reads per cell.

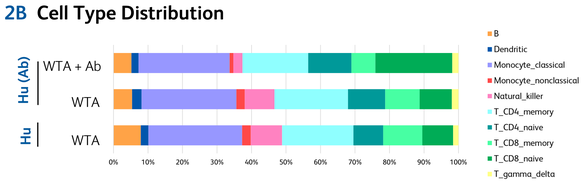
Multiomics data improve cell classification and offer deeper insights in clonotype characterization

2 Improved clustering and more distinct cell subtypes with multiomic tSNE

2A CITE-seq reveal additional cell subpopulations



2B Cell Type Distribution



2C Top ten clonotype clustering

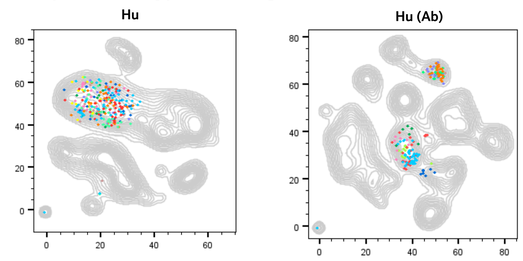
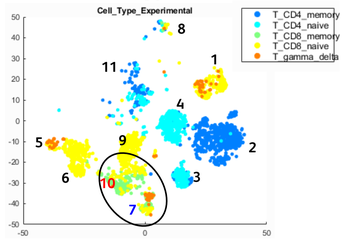


Figure 2. **(2A)** Cell types are annotated using the cell classification algorithm in the BD Rhapsody™ Sequence Analysis pipeline. Better separation and more distinct subpopulations of T cells are observed in the multiomic-derived tSNE. Subpopulations can be further characterized and annotated through differential gene and protein analysis. **(2B)** Hu and Hu(Ab) results in consistent cell type distribution when only WTA data is used by the cell classification algorithm. When AbSeq data is used to supplement WTA data, there is significant number of natural killer cells reclassified as T cells. Additionally, more naïve cells as opposed to memory cells are classified. **(2C)** Top ten clonotypes with the largest group size are plotted on corresponding tSNE contour plot. Clonotypes in multiomic assay are better separated into clusters as opposed to the complete overlap observed in the transcriptomic-only assay.

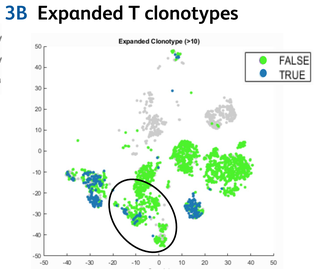
Figure 3. **(3A)** T cells from multiomic Hu (Ab) are graphed in a tSNE with labeled subtypes. 11 different clusters are observed, with two (Cluster 7 and 10) containing T cells classified as "CD8+ memory". **(3B)** Complete T cell clonotypes with TCRα/β pairing are categorized into "Expanded clonotype" TRUE/FALSE status. Cell is labeled TRUE if clonotype group size > 10. In the two clusters of CD8+ memory T cells, only one has an expanded clonotype population. **(3C)** Differential gene expression analysis between "CD8+ memory" cells in Cluster 7 vs 10 is performed to better understand parent population differences between that with vs without clonotype expansion. Gene and protein expression of the CD8+ cells in Cluster 7 are highly attributed to MAIT and NKT cells. Differences in CD56, a common marker for natural killer cells, can only be detected in the protein level and not transcript level. **(3D)** The same cell index from cluster 7 and 10 are labeled in a mRNA derived tSNE from the same Hu (Ab) sample. The unconventional T cells would be difficult to identify through annotation of transcriptomic-only tSNE clusters.

3 Unconventional T cell identification through phenotypic analysis

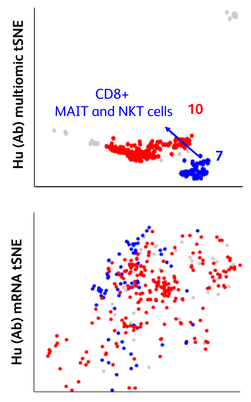
3A T cell subtype



3B Expanded T clonotypes



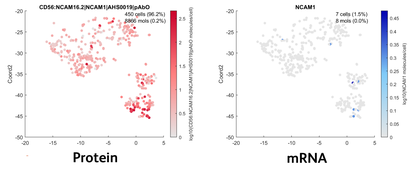
3D CD8 memory T cell clusters



3C Differentially expressed bioproducts

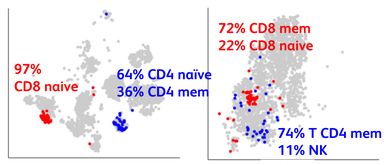
Cluster 7 vs 10

	Gene	Fold Change	P-value
mRNA	SLC4A10	73.9	9.36e-17
	KLRB1	33.3	3.26e-25
	CEBPD	20.7	7.47e-12
Surface protein	CD161	37.1	4.27e-52
	CD196	14.5	6.35e-38
	CD56	4.8	4.74e-14
	CD3	1.1	5.11e-12
	CD8	0.227	1.88e-11



4 Improved cell type classification of expanded clonotypes using surface protein data

4A Clone 1 and Clone 2 clustering

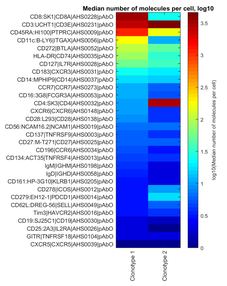


- Clonotype 1 (AVRSGGDTGNQFY_ASSLREYNEQF)
- Clonotype 2 (ATDAPGNTGKLI_ASSFSTGELF)

4B Differentially expressed bioproducts

	Clonotype 1		Clonotype 2	
	Gene	Fold Change	Gene	Fold Change
mRNA	CD8A	12	FGFBP2	6
	TRD-AS1	120		
Surface protein	CD8	33	CD4	24
	CD11c	19	CD62L	13
	CD45RA	6	CD279	11
			CD278	3
			CD3	1.8

4C 30-plex AbSeq IDP



4D Intraclonal phenotypic diversity

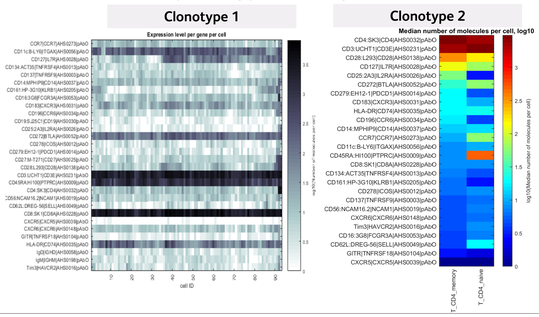


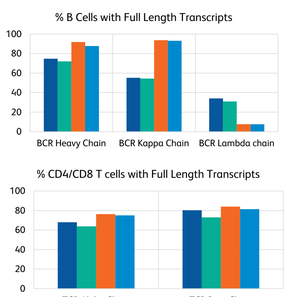
Figure 4. **(4A)** High expansion of clonotype 1 and 2 were observed in both Hu and Hu(Ab) samples. Multiomic assay shows clear separation of the two clonotype population while transcriptomic assay demonstrates some overlap. **(4B)** Differential gene expression analysis with threshold p-value <0.005. Both assays showed consistent upregulated mRNA genes. Except for CD8A, multiomic assay provides further differences amongst surface protein expression that does not correlate with the mRNA differential gene expression. AbSeq data is more aligned with the cell classification results of the multiomics than the mRNA-only cell classification model. **(4C)** From the proteomic data, we can confirm that Clonotype 1 is a CD8+ naïve population, which is consistent with the classification from algorithm. **(4D)** AbSeq can further our analysis by characterizing phenotypic diversity within a clonotype population.

BD Rhapsody™ TCR/BCR Next assay is highly compatible with BD AbSeq

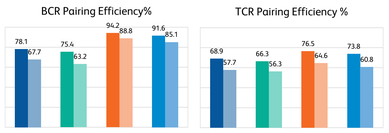
5 Consistent VDJ performance

■ Human PBMC ■ Human PBMC (Ab) ■ Mouse Spleen ■ Mouse Spleen (Ab)

5A Full-length TCR/BCR Transcripts



5B Pairing efficiency



	Hu PBMC -Ab	Hu PBMC +Ab	Mm Splenocyte -Ab	Mm Splenocyte +Ab
No. of Unique Clonotypes	1571	1720	4762	4500
% Paired B Cells with Full Length VDJ	87%	84%	94%	93%
% Paired T Cells with Full Length VDJ	84%	85%	85%	82%

5C Clonotype expansion

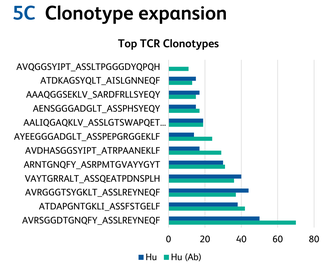
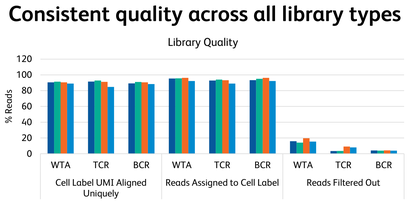


Figure 5. **(5A)** Percent cells with at least one full length TCR/BCR chain recovered remains consistent with CITEseq. **(5B)** Consistent TCR and BCR pairing for both Human and Mouse cell samples between transcriptomic vs CITEseq assay. No drop in clonotype detection observed with AbSeq. Approximately >85% of paired cells have full length VDJ sequence. **(5C)** Clonotype expansion of the same TCR pairing from the same PBMC sample observed in both assays.

6 Consistent quality across all library types



7 No WTA sensitivity loss

7A WTA molecule detection

	Hu PBMC +Ab/-Ab	Mm Splenocyte +Ab/-Ab
Median RSEC Molecules per cell	101.2%	104.8%
Median RSEC genes per cell	100.7%	102.9%

7B Gene expression correlation

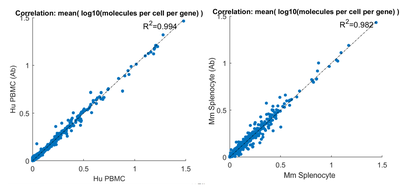


Figure 7. **(7A)** No significant difference in median molecules and genes per cell between transcriptomic only vs multiomic assay. **(7B)** High WTA gene expression correlation between transcriptomic vs multiomic assay for both cell samples (R^2 > 0.98)

Conclusions

- BD Rhapsody™ TCR/BCR Next Amplification kit supports robust multiomic assay capable of simultaneous profiling of the TCR/BCR repertoire, whole transcriptome, and cell surface proteome as demonstrated here with human PBMC and mouse splenocyte models.
- Use of surface protein AbSeq information to complement transcriptomic data results in improved cell clustering and cell subtype classification. Phenotypic information is especially useful to elucidate population differences in cases where the transcriptome expression are similar and protein expression does not correlate with transcriptomic expression.
- BD Rhapsody™ TCR/BCR Next Multiomic Assay allows for full length VDJ profiling of the immune repertoire and provide sensitive chain pairing for clonotype identification and further analysis.