High-quality CITE-Seq assay using the BD Rhapsody™ Single-Cell Analysis System and BD® AbSeq Antibodies

Tracy L. Campbell, [1] Jody Martin, [2] Hye-Won Song, [2] Larry Wang, [1] and Aruna Ayer[1]

[1] BD Biosciences 2350 Qume Dr, San Jose, CA 95131; [2]BD Biosciences 10975 Torreyana Rd, San Diego, CA 92121

Poster#
P1036

Abstract

CITE-Seq experiments can provide researchers with invaluable multiomics data to answer critical cellular biology questions. In selecting the right platform to answer these questions, researchers are confronted with a myriad of options and the benefits of each may not always be clear when comparing summary metrics alone.

Proteomics assays using oligo-conjugated antibodies have metrics beyond sensitivity to consider when choosing the right platform, for example antibody specificity as well as antibody oligo-mediated noise. Noise from negative populations in a CITE-Seq experiment can require additional sequencing to reach the same depth while complicating downstream analysis and obscuring conclusions. Previous studies have revealed signal-to-noise issues with CITE-Seq on the 10X platform. Because BD® AbSeq Antibodies are developed by adding oligos to high-quality BD antibodies and titrated to an optimal concentration for high signal to noise, we performed a side-by-side comparison of the two CITE-seq assays on the 10X vs BD Rhapsody™ Systems.

In this study, we are comparing a CITE-Seq assay using BD® AbSeq Antibodies and the BD Rhapsody™ Whole Transcriptome Analysis (WTA) Kit with the newly launched Enhanced Beads and CITE-Seq assay using BioLegend TotalSeq™ Antibodies and the 10X Genomics 5' Gene Expression Kit.

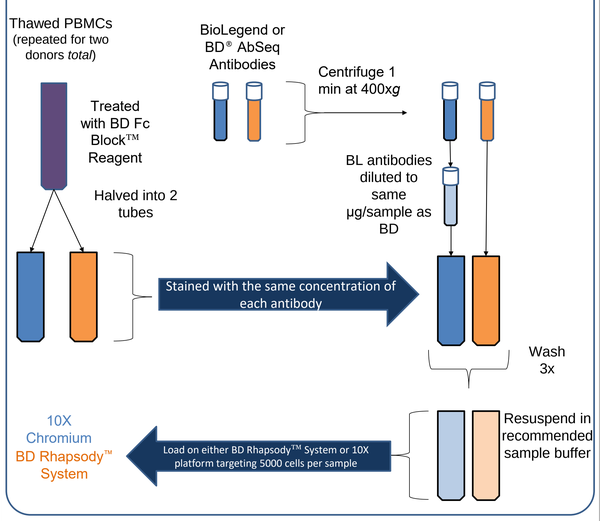
Briefly, two PBMC donors were profiled using the BD Rhapsody™ WTA Kit and 10X Genomics 5' Gene Expression Kit and surface proteins were profiled using BD® AbSeq and BioLegend TotalSeq™ Antibodies, respectively, utilizing antibody clones available on both platforms.

We demonstrate that BD® AbSeq Antibodies on the BD Rhapsody™ System have higher resolution and less noise than BioLegend TotalSeq™ Antibodies on 10X Chromium when comparing CITE-Seq performance across two PBMC donors, controlling for antibody concentration and staining volume.

Methods

Antibodies were selected based on clone overlap from both BD and BioLegend (Table A). PBMCs were thawed, suspended in BD Pharmingen™ Stain Buffer (Cat # 554656) and treated with BD Fc Block™ Reagent (Cat # 564219). Figure A outlines the workflow. Briefly, cells were split into two tubes; one tube of cells was stained with BD Rhapsody™ System reagents at optimal stock concentrations while the other tube was stained with BioLegend TotalSeq™ C Reagents diluted to the same final concentration as the corresponding BD® AbSeq Reagent. Cells were stained in 200 µL stain buffer for 30 minutes on ice and washed three times with 2 mL stain buffer before loading on each respective platform. A quantity of 5,000 cells per donor was targeted for capture.

Figure A Cell staining and washing workflow steps



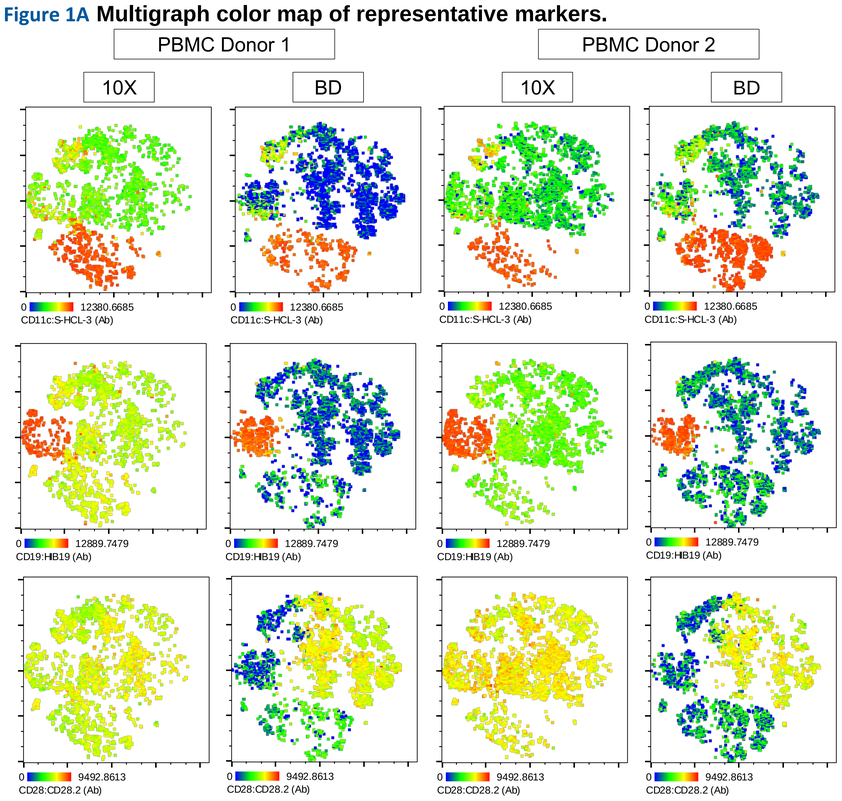
10X libraries were prepared according to the Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology protocol (Rev D). BD libraries were prepared according to the BD Rhapsody™ System mRNA Whole Transcriptome Analysis (WTA) and AbSeq Library preparation protocol (23-24118(01)). Libraries were sequenced on a NextSeq™ 500 Sequencer (Illumina) using a 150-cycle High Output kit v2 according to each assay's sequencing recommendations. BD data were analyzed in SevenBridges BD Rhapsody™ WTA Analysis Pipeline v1.11 and Chromium data were analyzed using the 10X Genomics Cloud. BD® AbSeq Antibody reads were down-sampled to obtain a similar number of usable reads per cell as BioLegend/10X libraries. Molecules per cell files were imported into SeqGeq™ Software and normalized before analysis.

Table A Oligo-conjugated antibodies used with corresponding catalog numbers

Target	Clone	BD Cat.no	BL Cat.no
CD19	HIB19	940247	302265
CD3	UCHT1	940307	300479
CD16	3G8	940006	302065
CD4	RPA-T4	940304	300567
CD11c	S-HCL-3	940265	371521
CD14	M5E2	940257	301859
CD8	SK1	940305	344753
CD45RA	HI100	940011	304163
CD28	CD28.2	940017	302963
CD134	ACT35	940060	350035
CD137	4B4-1	940055	309839

BD® AbSeq Antibodies on the BD Rhapsody™ System show high signal to noise compared to BioLegend on the 10X Genomics' droplet-based system

Figure 1A Multigraph color map of representative markers.



tSNE plots were made with mRNA data normalized to molecule counts per 10,000 and overlaid with the frequency of marker detected per cell, as indicated by the color bar, with blue being zero and red indicating the highest levels of detection. mRNA data were used to produce the tSNE plots so that nonspecific antibody noise wouldn't influence the clustering. The BD Rhapsody™ System data for both PBMC donors show higher signal to noise and have more non-target cells with near zero molecules detected.

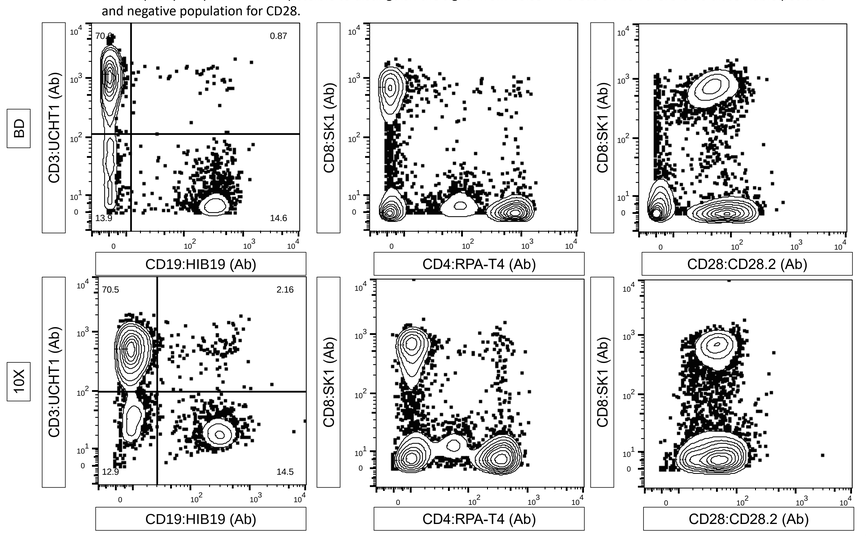
Table 1 Table of antibody-oligo molecule detection metrics

The BD Rhapsody™ System reads were subsampled to have similar usable reads per cell compared to the 10X libraries. Both platforms have similar molecule per cell detection and sensitivity.

	Donor 1		Donor 2	
Metric	10X	BD	10X	BD
Putative Cells	4995	5046	3641	4475
Mean Reads per cell	9292	3108	15230	5140
Usable Reads per cell	1151	2268	2288	2793
Median Molecules per cell	922	1664	1508	1246
Sequencing Saturation	63%	41%	32%	66%

Figure 1B Signal-to-noise contour plots for PBMC donor 1

The BD Rhapsody™ System samples (top) show lower median molecules per cell in the negative population for all markers compared to 10X (bottom). CD19 shows excellent separation between positive and negative for both platforms. The CD4 negative population shows a median at around zero for the BD Rhapsody™ System, whereas for 10X the CD4 negative population median is closer to 10–50 molecules per cell. CD28 has lower signal to noise than the other markers, however, on BD Rhapsody™ System it is still possible to distinguish the signal from noise whereas on 10X there is no discernable positive and negative population for CD28.



BD® AbSeq Antibodies on BD Rhapsody™ System data have fewer molecules from non-target cells

Figure 2A Signal-to-noise fold change (BD signal:noise/10X signal:noise)

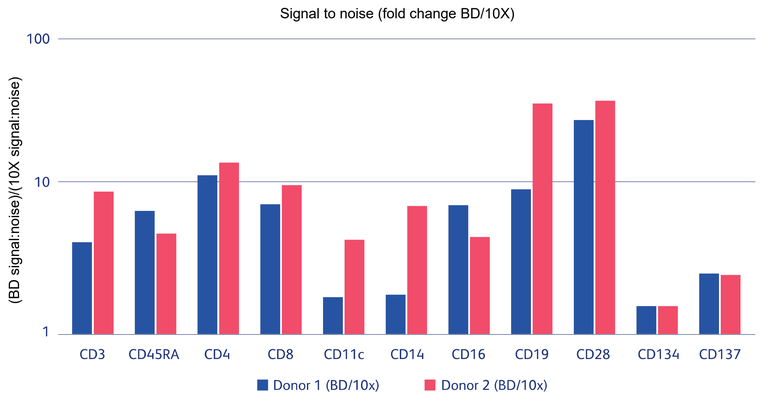


Figure 2A shows the ratio of BD signal to noise over 10X signal to noise for each marker, with a value >1 indicating a BD advantage. Every marker across both donors is positive, indicating that BD has higher resolution for all clones tested.

Figure 2B Median molecules per cell detected for each antibody from the negative population.

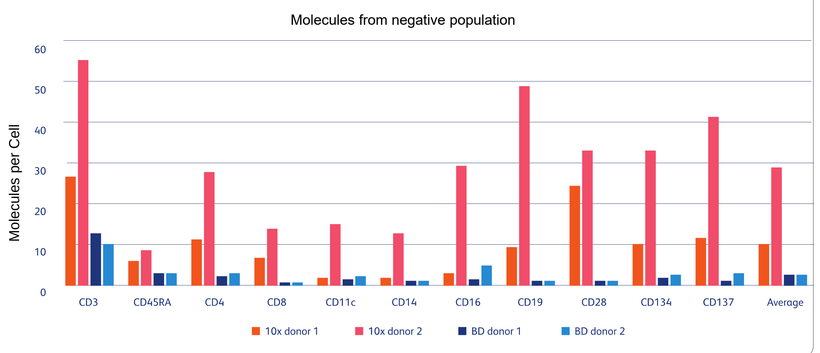


Figure 2B Molecules from the negative population are not only useless but they interfere with downstream analyses if the noise is so great that the positive population can't be resolved (see figure 1B). These molecules represent wasted reads, despite being categorized as 'useful' reads in pipeline metrics. These molecules contribute to the median molecules per cell, an indication that antibody-derived median molecules per cell metric should be considered in the context of signal to noise for CITE-Seq experimental results.

Conclusions

- In this experiment we saw that two PBMC donors analyzed using the BD® AbSeq Antibodies on the BD Rhapsody™ System produced libraries with higher signal to noise than libraries prepared with BioLegend on the 10X Genomics droplet-based system for all antibody-oligo markers used here.
- The impact differed across markers, with CD28, CD19, and CD4 having the largest advantage for AbSeq.
- CD28 positive was unable to be resolved from the negative population in the BioLegend on 10X samples for this experiment.
- Libraries from the BD Rhapsody™ System had fewer unwanted molecules and reads from non-target cells than the BioLegend on 10X droplet-based system.
- The presence of unwanted molecules from noise populations requires more sequencing to get to desired sequencing saturation than if the molecules were not present.
- BD® AbSeq Antibodies with the BD Rhapsody™ System produced high-quality libraries with relatively high signal to noise without requiring numerous antibody dilutions before cell staining.