Comparative analysis of CITE-seq on the BD Rhapsody™ and 10X Genomics Chromium Single-Cell Analysis Systems

Introduction

CITE-seq experiments provide researchers with invaluable multiomics data to answer critical cellular biology questions needed to advance immuno-oncology research. When 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.

Previous studies[1,2] have found non-zero median molecules per cell in the negative populations using droplet-based CITE-seq. These results are consistent with publicly available data on the 10X Genomics website[3] and requires deeper sequencing to resolve signal from noise.

Recent work by Buus et al., 2021,[4] showed that signal to noise can be improved by decreasing the concentration of oligo-conjugated antibodies from the manufacturer's recommended concentration; however, finding optimal concentrations for every marker in a panel can be time consuming. It's also unclear if titrating antibodies to an optimal concentration will completely resolve the low signal-to-noise issue that has been observed on droplet systems.

We demonstrate that AbSeq on the BD Rhapsody™ System has superior resolution to TotalSeq™ Antibodies on the 10X Genomics Chromium platform in an experiment comparing CITE-seq performance using two PBMC donors, controlling for antibody concentration and staining volume.

Methods

Antibodies were selected with overlapping clones from both BD and BioLegend (Table 1). PBMCs were thawed, suspended in BD Pharmingen™ Stain Buffer (Cat No. 554656) and treated with BD Fc Block™ Reagent (Cat No. 564219). Cells were split into two tubes and stained with either BD® AbSeq Reagents at optimal stock concentrations or BioLegend TotalSeq™-C Reagents diluted to the same final concentrations. Cells were stained in 200-µL stain buffer for 30 minutes on ice and washed three times with 2-mL stain buffer before they were loaded on their respective platforms. The capture target was 5,000 cells per donor.

After cell capture, each platform's respective protocols[5] were followed to yield whole transcriptome and surface protein libraries. Libraries were sequenced on a NextSeq™ 500 System using each assay's sequencing recommendations. Data from the BD Rhapsody™ System were analyzed on the SevenBridges BD Rhapsody™ System WTA analysis pipeline and data from the 10X Genomics Chromium System were analyzed using the 10X Genomics Cloud. AbSeq reads were downsampled to obtain a similar number of usable reads per cell, defined as the number of reads from cells that were aligned to a reference, to the 10X Genomics samples. Samples were imported into SeqGeq™ Software and normalized before analysis.

Target	Clone
CD19	HIB19
CD3	UCHT1
CD16	3G8
CD4	RPA-T4
CD11c	S-HCL-3
CD14	M5E2
CD8	SK1
CD45RA	HI100
CD28	CD28.2
CD134	ACT35
CD137	4B4-1

Table 1. Antibody clones compared

	Donor 1		Donor 2	
Metric	**10X Genomics Chromium**	**BD Rhapsody™ System**	**10X Genomics Chromium**	**BD Rhapsody™ System**
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%

Table 2. Antibody detection metrics as reported by each respective pipeline.

Results

The reads and molecules per cell detection metrics can be found in Table 2. There was no consistent difference between the platforms in the molecule per cell detection.

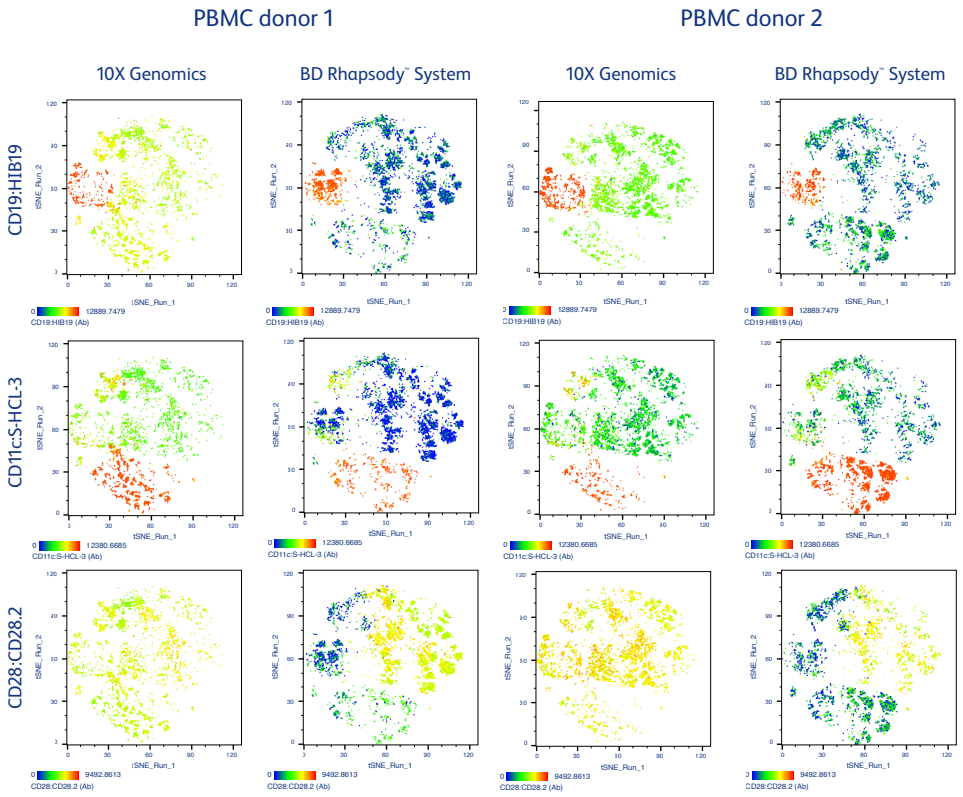

Figure 1 illustrates the signal to noise for a few representative markers. The BD Rhapsody™ System data for both samples show higher specificity (signal to noise) and have more negative cells with near zero molecule detection outside the target population whereas 10X Genomics data show signal outside of the target population.

Figure 1. Multigraph color map of representative markers. tSNE plots were created 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.

The contour plots in Figure 2 illustrate the differences in resolution between the positive and negative populations for each platform. Both platforms show excellent resolution for CD19 even though the 10X Genomics negative population is measuring >10 molecules per cell. BD Rhapsody™ System CD4 negatives are <10 molecules per cell while the 10X Genomics negative CD4 population is >10 molecules per cell, which leads to merging of populations (myeloid intermediate and T cell high expression populations). The BD Rhapsody™ System CD4 data show clean definition between the high, medium and negative expression populations. CD28 expression is known to be lower in expression, and only AbSeq on the BD Rhapsody™ System is able to resolve the CD28 expressing population of cells. BioLegend reagents on the 10X Genomics Chromium System are unable to resolve distinct CD28 negative/positive populations.

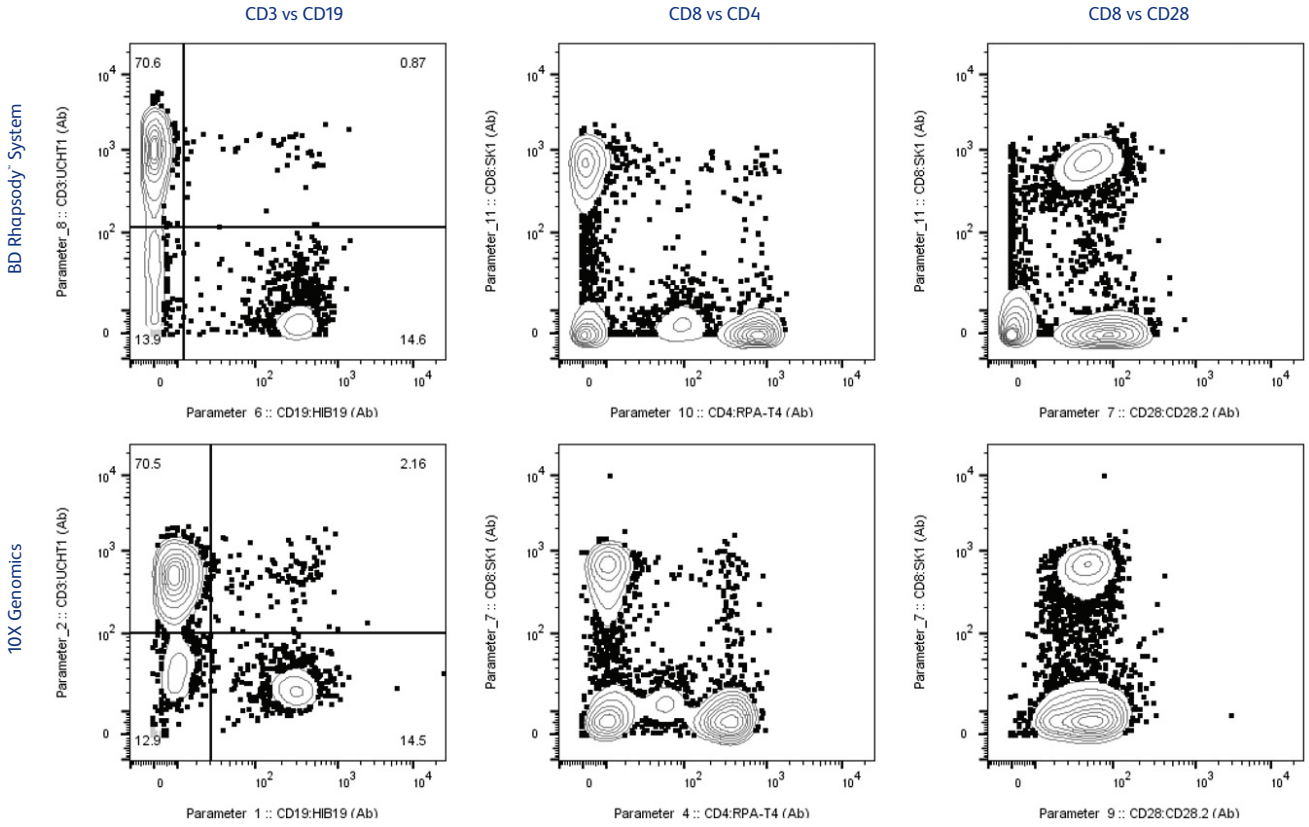

Figure 2. Signal-to-noise contour plots for PBMC Donor 1 (analyzed with FlowJo™ Software). The BD Rhapsody™ System samples (top) show lower median molecules per cell in the negative population for all markers compared to 10X Genomics (bottom).

Figure 3 shows the ratio of BD signal to noise over 10X Genomics 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.

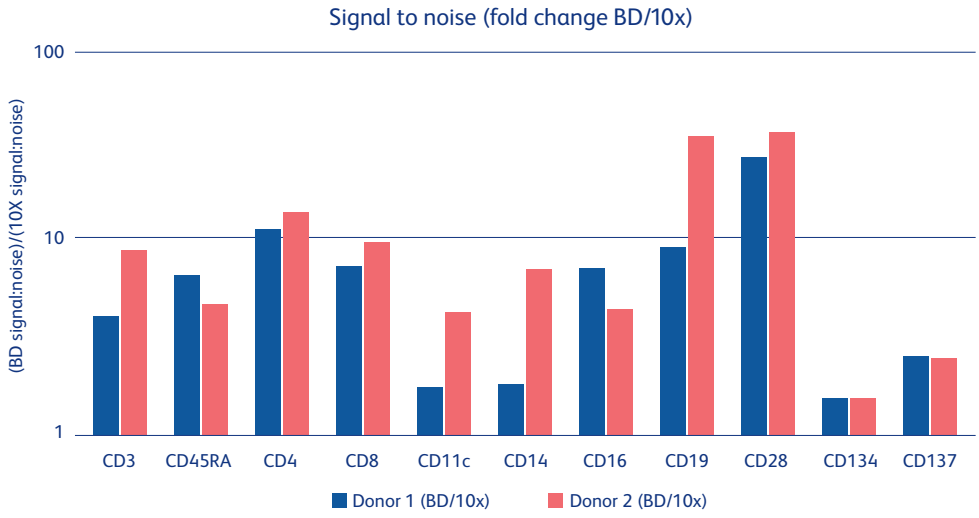

Figure 3. The ratio of BD signal to noise to 10X Genomics signal to noise. Ratio greater than 1 indicates higher resolution in the BD data.

Molecules from the negative population (Figure 4) do not contribute to the data and interfere with downstream analyses if the noise is so great that the positive population can not be resolved. 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 the antibody-derived median molecules per cell metric should be considered in the context of resolution for CITE-seq experimental results.

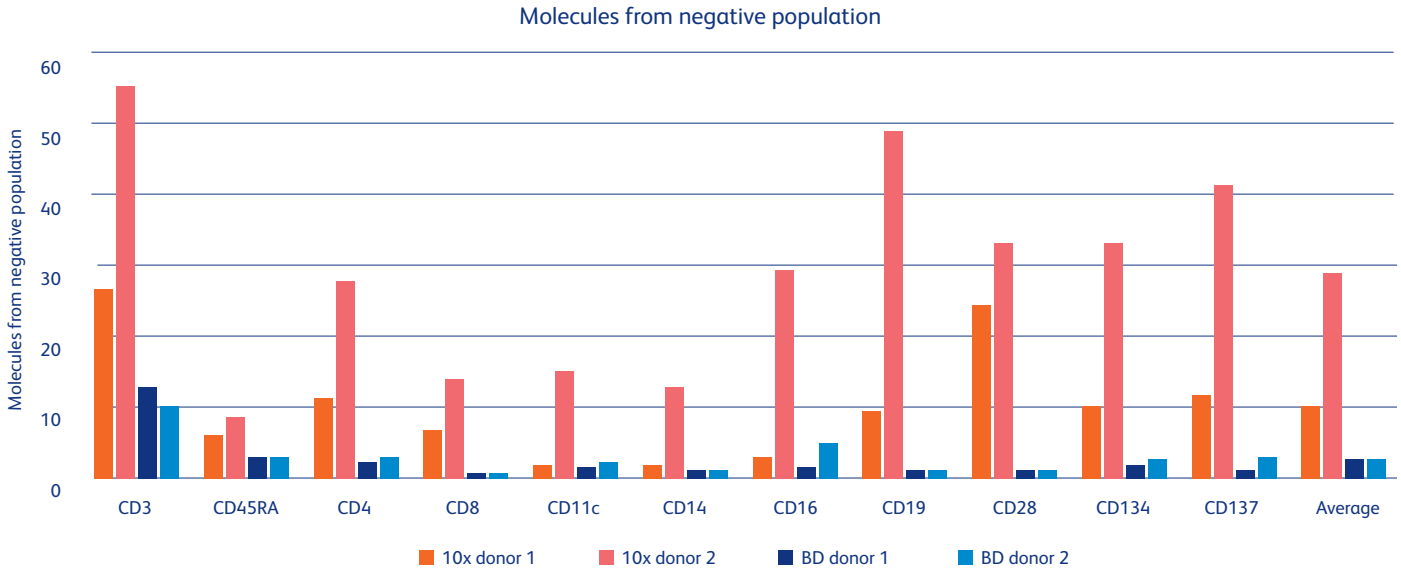

Figure 4. Median molecules per cell detected for each antibody from the negative population. Negative (non-expressing) populations of cells were determined via data analyses in FlowJo™ Software, standard histogram gating and statistical outputs.

Conclusions

CITE-seq is a powerful method used for gaining quantitative and qualitative information on surface proteins with available antibodies on a single-cell level.[6] The method can be performed on several single-cell platforms.[7] This study compared the two most common workflows for CITE-seq: BD Rhapsody™ System with AbSeq and 10X Genomics Chromium with BioLegend TotalSeq™ reagents. For this experiment, AbSeq on the BD Rhapsody™ System was able to provide high-resolution CITE-seq surface feature data with fewer reads from non-target cells than TotalSeq on the 10X Genomics Chromium System.

References

1 Stoeckius M, Hafemeister C, Stephenson W, et al. Large-scale simultaneous measurement of epitopes and transcriptomes in single cells. *Nat Methods*. 2017; 14(9):865-868. doi: 10.1038/nmeth.4380

2 Mulè MP, Martins AJ, Tsang JS. Normalizing and denoising protein expression data from droplet-based single cell profiling. *Nat Commun*. 2022; 13(1):2099. doi: 10.1038/s41467-022-29356-8

3 10X publicly available data:
- https://www.10xgenomics.com/resources/datasets/pbm-cs-of-a-healthy-donor-5-gene-expression-and-cell-surface-protein-1-standard-3-0-0
- https://www.10xgenomics.com/resources/datasets/10-k-pbm-cs-from-a-healthy-donor-gene-expression-and-cell-surface-protein-3-standard-3-0-0

4 Buus TB, Herrera A, Invanova E, et al. Improving oligo-conjugated antibody signal in multimodal single-cell analysis. *eLife*. 2021;10:e61973. doi: 10.7554/eLife.61973

5 Protocols used:
- 10X Genomics Protocol: Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping (CG000330 Rev D)
- Rhapsody Protocol: BD Rhapsody™ System mRNA Whole Transcriptome Analysis (WTA) and AbSeq Library Preparation Protocol (23-24118(01))

6 Mercatelli D, Balboni N, De Giorgio F, Aleo E, Garone C, Giorgi FM. The Transcriptome of SH-SY5Y at Single-Cell Resolution: A CITE-seq Data Analysis Workflow. *Methods Protoc*. 2021. doi: 10.3390/mps4020028.

7 Xie H, Ding X. The Intriguing Landscape of Single-Cell Protein Analysis. *Adv Sci*. 2022;9(12):e2105932. doi: 10.1002/advs.202105932

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