

Abstract

BACKGROUND
 CITE-Seq (Cellular Indexing of Transcriptomes and Epitopes by sequencing) is a powerful approach in single-cell assays used to concurrently characterize both the proteome and transcriptome of individual cells.

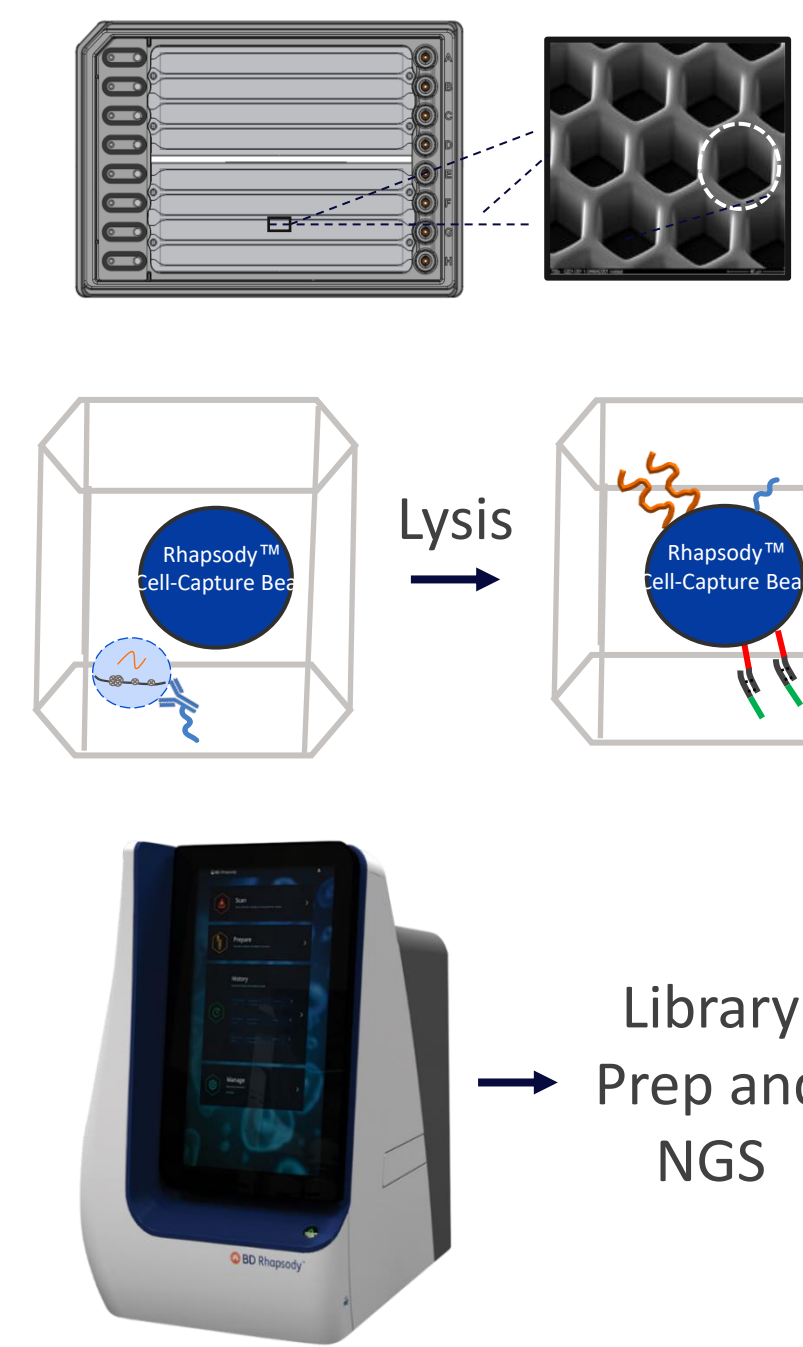
CHALLENGES
 One of the biggest challenges for CITE-Seq workflows is high levels of background signal from unbound oligo-antibodies that often prevents the ability to separate signal from noise, resulting in inaccurate or undetermined measurements of protein expression and increased sequencing costs without the benefit of adding sequencing depth.

SOLUTION
 CITE-Seq is typically done using either a droplet- or microwell-based single-cell system, therefore we questioned whether the system itself could contribute to noise. In a droplet-based system, cells are encapsulated whereby any unbound antibodies are trapped and carried into the assay with no option of removal. Here, we show that by using the open-well based BD[®] Rhapsody Single-Cell System unbound antibodies can be washed away, dramatically enhancing the data.

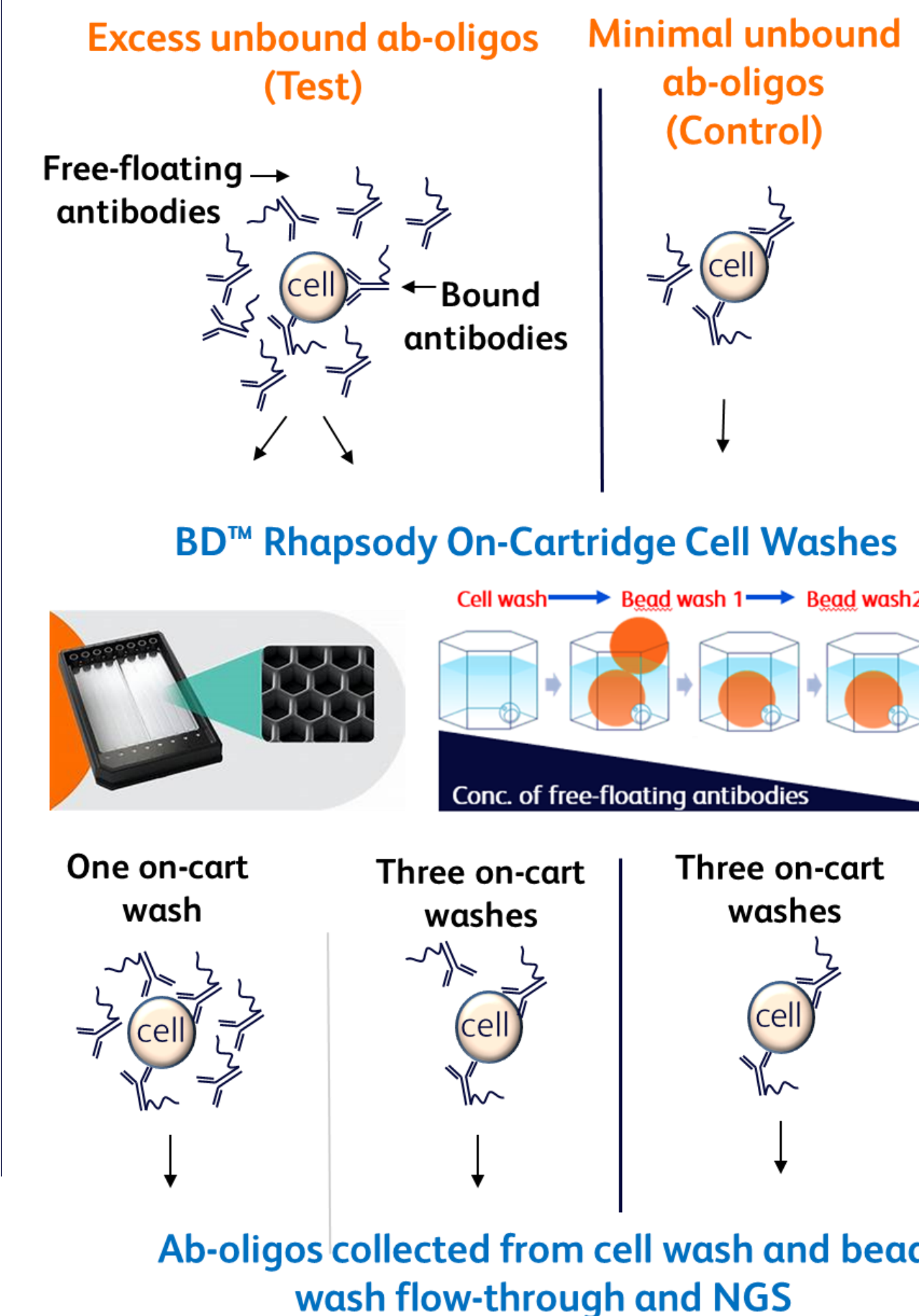
RESULTS and CONCLUSIONS
 We found the open-well based system had inherently significant benefits for removing unbound oligo-antibodies and that additional washing further enhanced signal-to-noise resolution and overall data quality. The specificity and sensitivity of protein data from CITE-Seq assays was improved.

Methods

2A. BD Rhapsody™ Single Cell System



2B. Testing the power of an open well based system



2C. Different cell types and Ab-oligo plexy were tested

Exp	BD [®] AbSeq Plexy	Cell Type	Amount of unbound oligo	On-cartridge cell washes
1	33-plex	Jurkat	Excess	1X
			Excess	3X
			Minimal (Control)	3X
			Excess	1X
2	89-plex	PBMCs	Excess	3X
			Excess	3X
			Minimal (Control)	3X
			Excess	3X

33-plex				
CCR7	CD19	CD56	CD183	LAG-3
CD11c	CD25	CD62L	CD136	CD34
CD127	CD272	CD8	CD256	IgM
CD134	CD278	CXCR5	CD45RA	Tim3
CD137	CD279	CXCR6	CD4	CD28
CD14	CD27	GITR	IgD	CD161
CD16	CD3			

89-plex					
CCR7	CD134	CD27	CD4	EPOR2	CD161
CD103	CD136	CD28	CD54	GITR	CD183
CD109	CD19	CD29	CD58	HLA-ABC	CD184
CD117	CD10	CD30	CD5	HLA-DR	CD275
CD122	CD40	CD31	CD62L	ITGAE	CD278
CD126	CD21	CD35	CD66	IgD	CD279
CD127	CD22	CD36	CD69	IgG	CD45RO
CD133	CD23	CD34	CD73	IgM	CD45
CD134	CD24	CD38	CD79b	Invariant_NK_TCell	CD47
CD137	CD25	CD39	CD80	LAG-3	CD9
CD138	CD267	CD3	CD86	MUC1	CXCR5
CD146	CD268	CD40	CD8	Nectin1	FGFR
CD152	CD26	CD43	CD90	PDPR	Tim3
CD154	CD272	CD44	CD93	TCR_gamma_delta	VISTA
CD155	CD274	CD45RA	CD95	TIGIT	

Unbound ab-oligos are efficiently removed on the cartridge

3A. Cell capture efficiency is not impacted by additional cartridge washes

Experiment	BD [®] AbSeq Plexy	Cell Type	On-cartridge cell washes	Number of wells with viable cells and a bead	Putative cells from pipeline after sequencing
1	33-plex	Jurkat	1X	14,748	14,748
			3X	14,846	15,108
			3X	15,018	14,803
2	89-plex	PBMCs	1X	15,355	14,956
			3X		
			3X		

3B. Open microwell-based system can remove billions of unbound ab-oligos

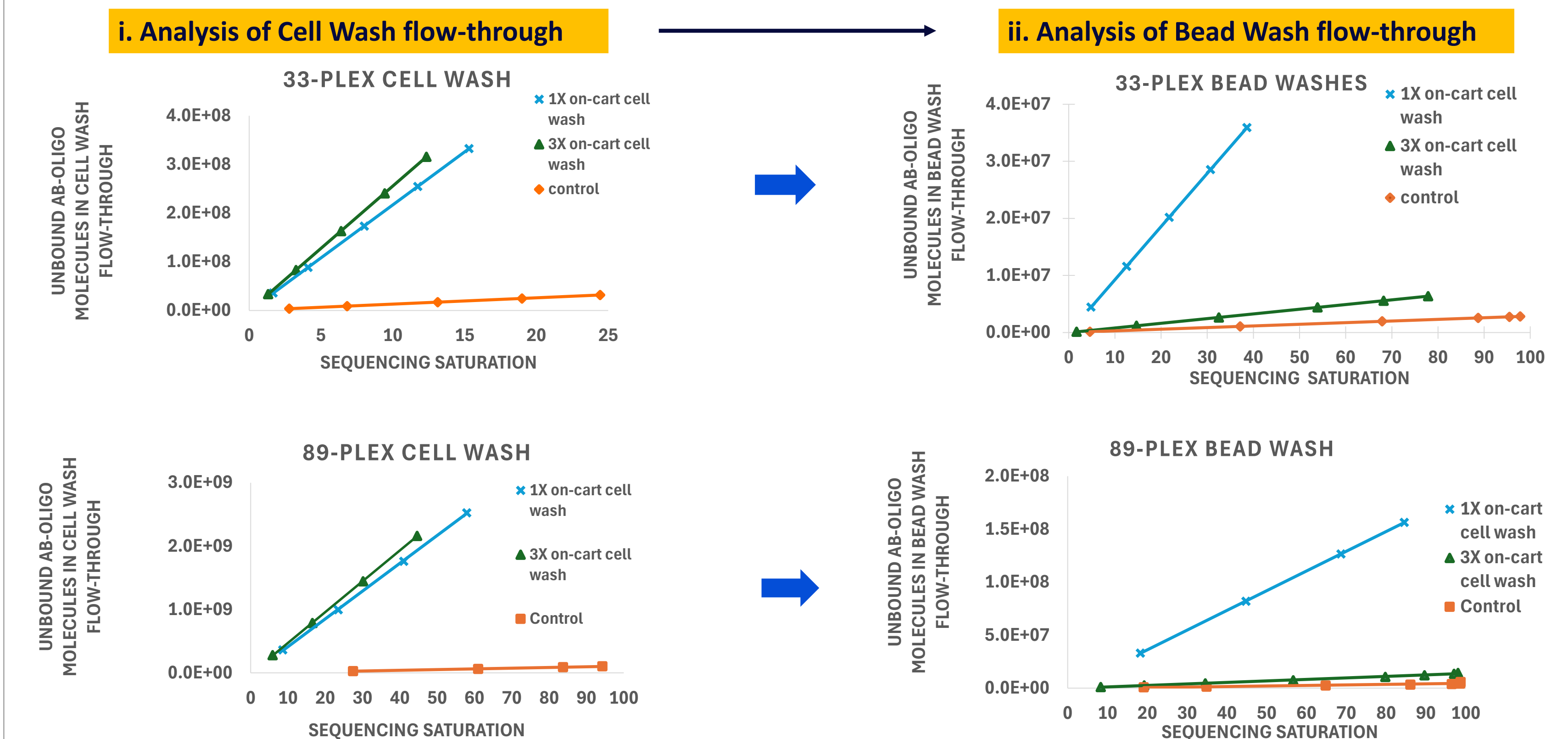
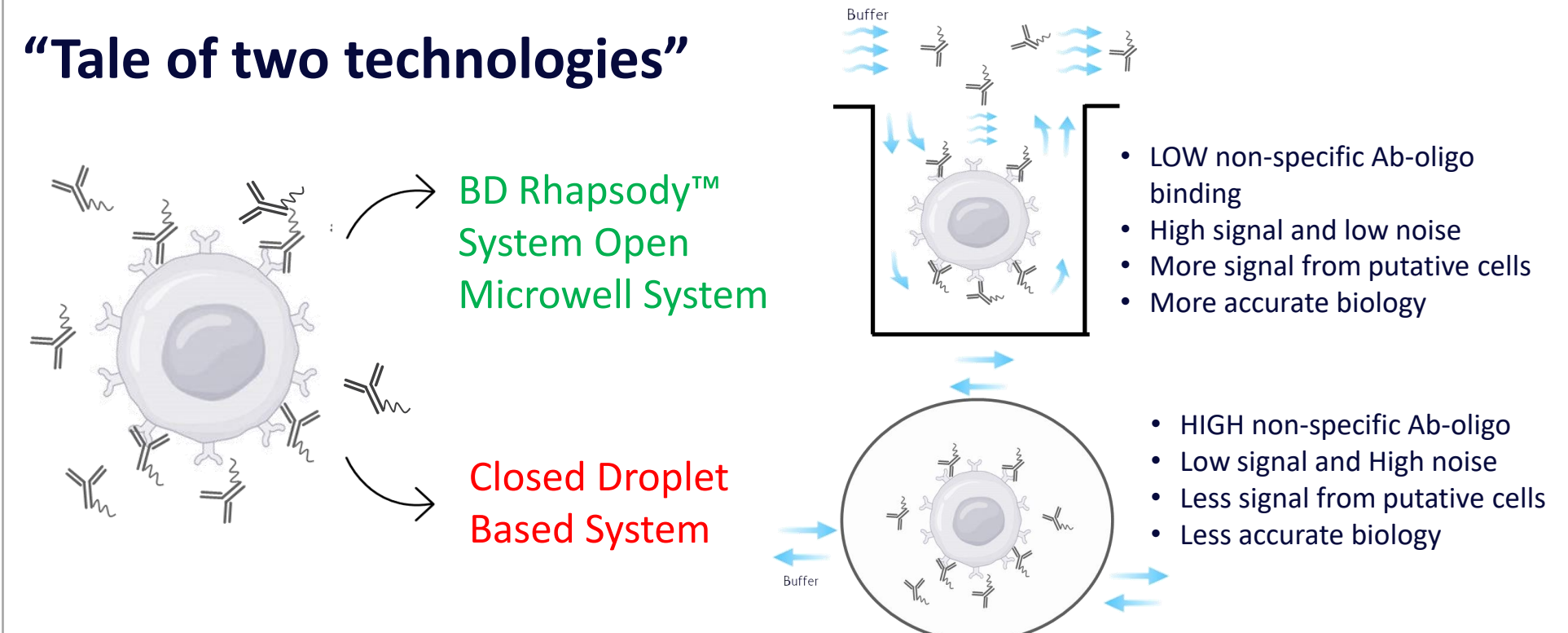


Figure 3. (A). Cell capture was not impacted by additional on-cartridge washes. For each experimental condition, 20,000 cells were loaded onto the cartridge. (B) Graphs showing the number of ab-oligo molecules that are washed away during the (i) cell wash steps or the (ii) bead wash steps. On-cartridge workflow can wash away billions of unbound ab-oligos.

Introduction

1A. CITE-Seq on an open microwell vs. droplet-based system



1B. Signal-to-noise comparison on an open microwell vs. droplet-based system

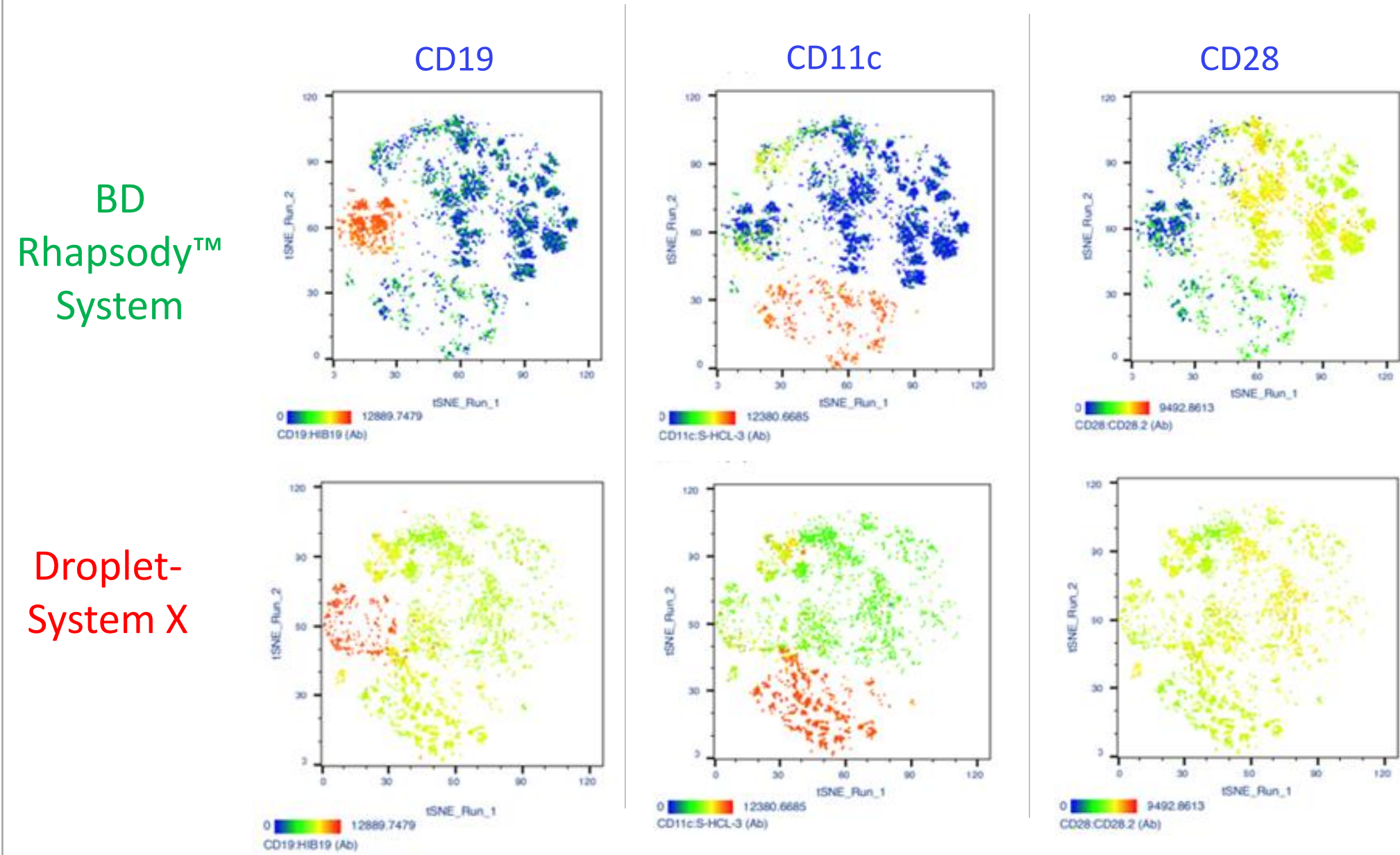
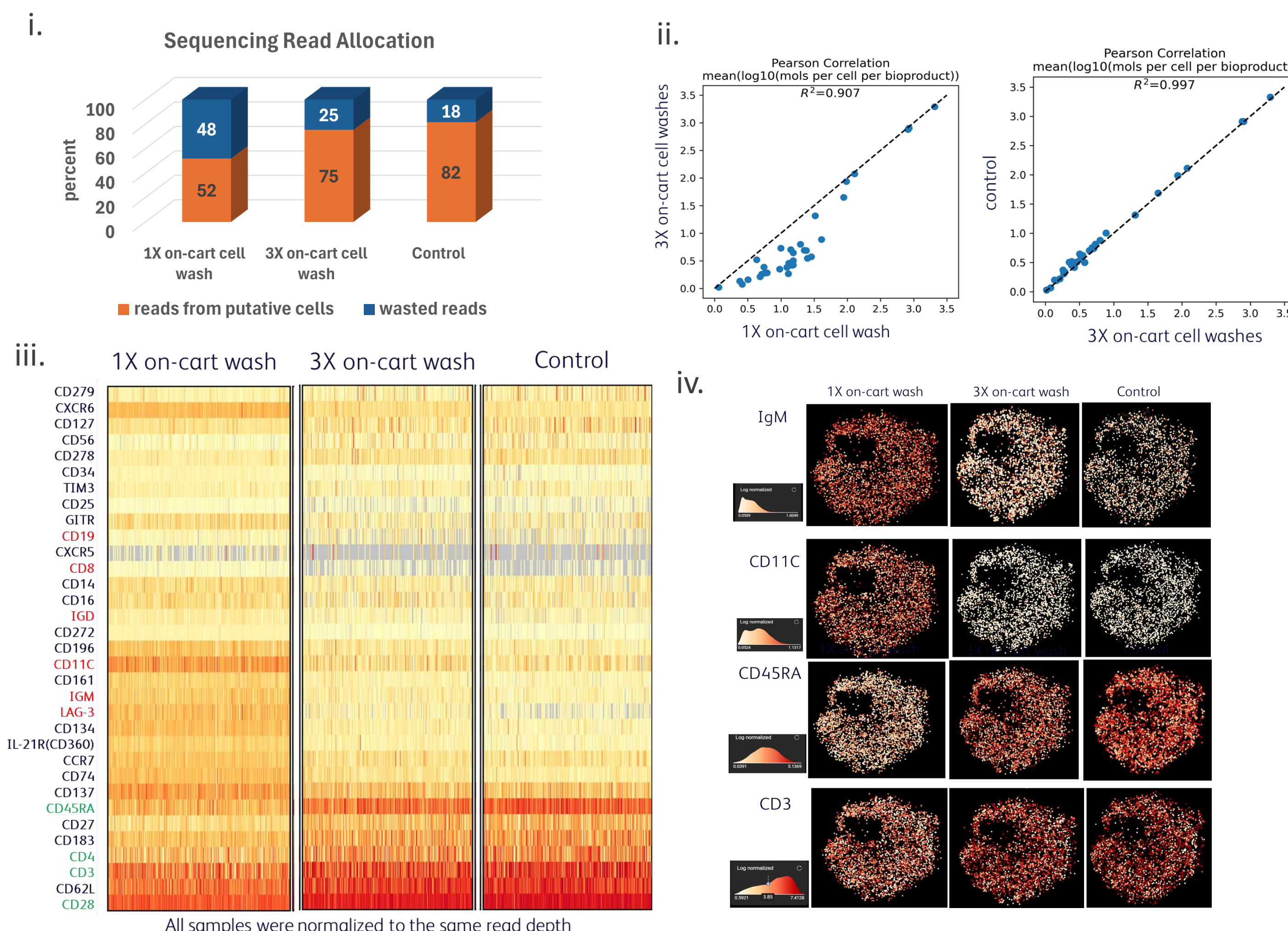


Figure 1. (A) CITE-Seq is typically done on either an open well or droplet-based system. In a droplet based system, any unbound or ambient oligo-antibodies can be trapped and carried into the system generating a lot of background noise and wasted sequencing reads. Alternatively, the open-well based system, like the BD Rhapsody™ System allows for buffer exchange that can remove unbound oligo-antibodies and enhance data quality and provide cost savings on sequencing. (B) BD Rhapsody™ System shows higher specificity (signal-to-noise) for oligo-antibody expression.

Figure 2. (A). Experiments to test open-well based system were completed using the BD Rhapsody™ Single Cell System which partitions cells in a microwell cartridge and allows cells to gently settle via gravity. (B) Cells were stained with BD[®] AbSeq oligo-antibodies and either washed once by centrifugation to obtain “excess antibody sample” or three times to evaluate the abundance of free-floating antibodies that would otherwise be captured in a droplet-based system. Cite-Seq performance was also evaluated using the cells captured in the cartridge. (C) Experimental design using different cells types stained with different BD[®] AbSeq panels

Cartridge washes can restore resolution for samples with high unbound ab-oligos

4A. Experiment 1 (33-plex) Ab-oligo performance in CITE-Seq Assay



4B. Experiment 2 (89-plex) Ab-oligo performance in CITE-Seq Assay

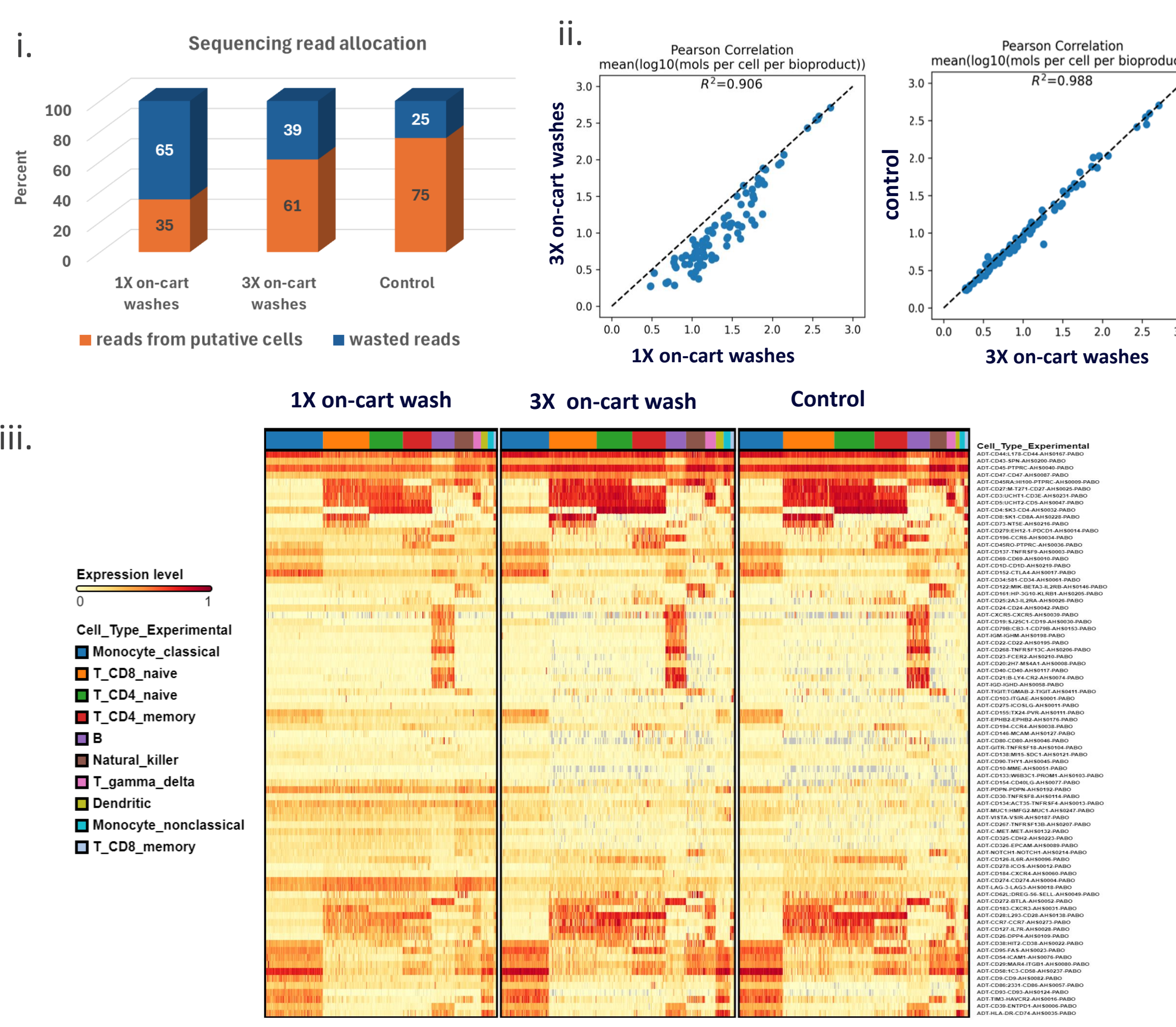


Figure 4. Additional on-cartridge washes can restore samples with high unbound ab-oligos rescuing protein performance. (A) and (B) Data from cells captured with the BD™ Rhapsody Single Cell System. (i) Bar graphs showing the percent useful reads (reads from putative cells) versus wasted reads. Additional cartridge washes can increase the percent of useful reads. (ii) Correlation plots showing ab-oligo performance after either 1 or 3 on-cartridge cell washes. (iii) Heat maps showing high specificity and sensitivity of protein expression after additional on-cartridge cell washes. In (A), ab-oligo markers in red text are those that should not be expressed in Jurkat cells and therefore denote background noise. Green text shows markers expected to be expressed in Jurkat cells. (iv) In (A), tSNE plots showing expression of select markers. Top 2 markers show non-specific binding and bottom 2 markers show sensitivity.

Conclusions

- CITE-seq on BD Rhapsody™ System offers high signal to noise ratio and low wasted reads for ab-oligo data
- On-cartridge washes, a critical feature of “open microwell system”, offers a solution for generating high quality CITE-seq data with higher signal resolution that can also lower sequencing costs
- Removing the need for multiple handling steps after antibody staining offers a path to faster workflows and may minimize stress to cells and cell loss

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