Enabling high-dimensional biology using BD® AbSeq Antibody Oligonucleotides

Learnings from the use of 100 different AbSeq antibodies together in a BD Rhapsody™ System single-cell experiment

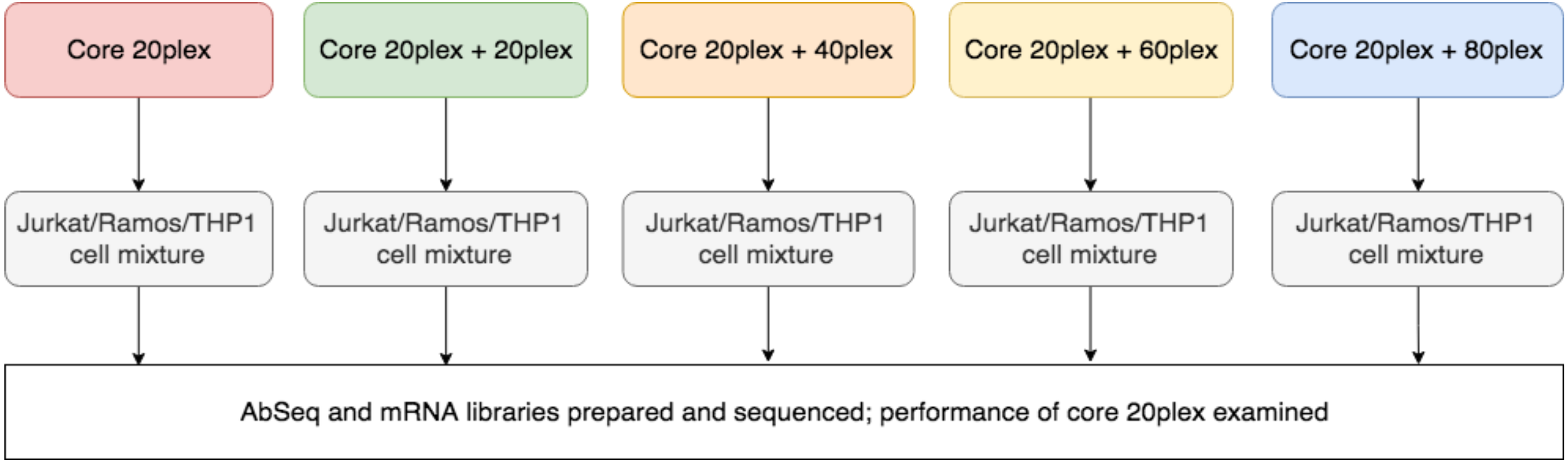
BD

BD® AbSeq Ab-Oligos enable high-plexy protein panels

- Antibody oligos like BD AbSeq Ab-Oligos do not impact each other like other traditional antibodies
- Lack of interference enables use of high-plexy BD AbSeq Antibody-Oligo Protein Panels

Question: Will increasing plexy impact the performance of individual antibodies? (or) Does an antibody behave differently in the presence of 19 other antibodies versus 99 other antibodies?

Setting up a 100-plex AbSeq experiment to study impact of increasing plexy (n=2)



List of AbSeq markers included in this study

Core 20plex	
CD197	HLA-DR
CD20	CD14
CD45RA	CD185
TIM-3	CD45
CD28	CD7
CD8	CD5
CD19	IgG
CD183	TCRab
CD4	CD11a
CD3	CD18

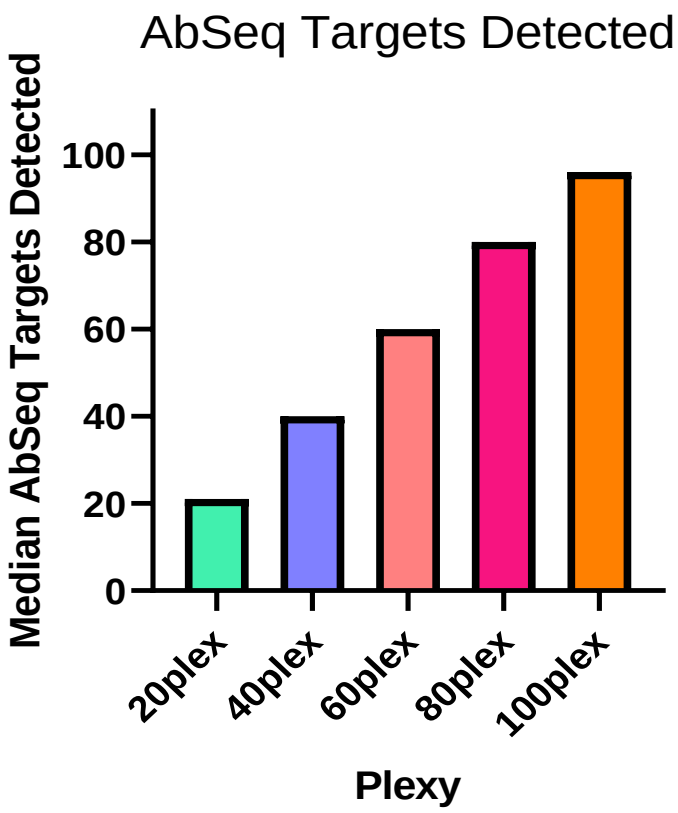
Core 20plex + 20plex	
CD137	CD24
CD11b	CD235a
CD39	CD62L
CD56	CD16
CD38	CD11c
CD27	IgD
CD25	CD54
CD127	CD47
CD196	CD326
CD194	CD133

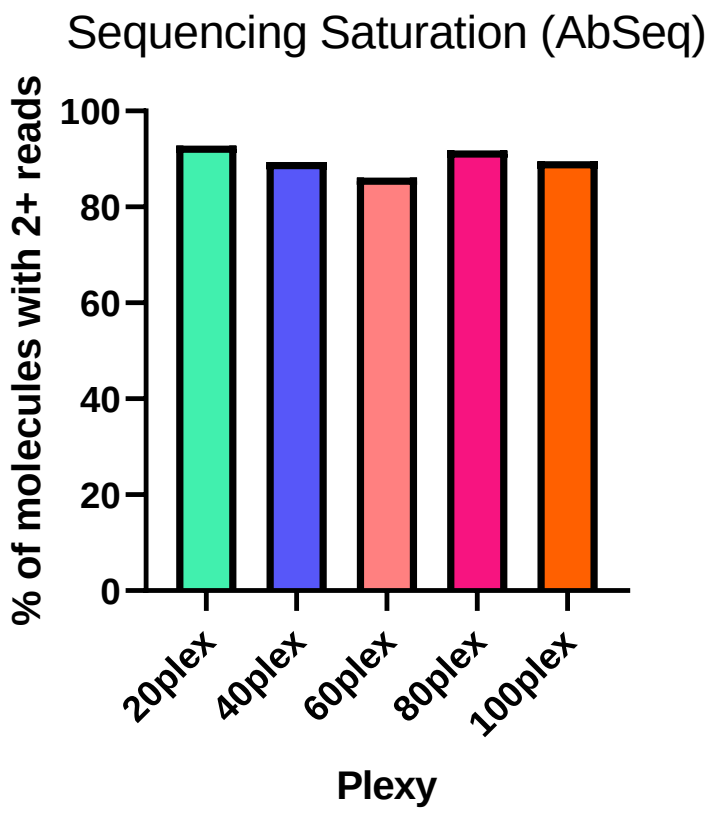
Core 20plex + 40plex	
CD274	HLA-A,B,C
TCRgd	CD154
CD152	CD141
CD95	CD83
CD80	NKp44
CD272	CD178
CD184	CD98
CD163	IL-21R
CD117	B7-H4
CD314	CD26

Core 20plex + 60plex	
CD134	CD21
CD279	CD29
CD2	CD1c
CD45RO	CD66
CD33	CD126
CD10	CD124
CD49d	CD49a
CD1a	GITR
CD335	CD40
CD195	CD49E

Core 20plex + 80plex	
CD69	CD61
CD275	CD206
CD278	CD32
LAG-3	CD273
CD123	CD226
CD81	CD9
CD90	CD49b
CD13	CD270
CD86	CD155
CD34	CD30

Successful detection of 20-80 AbSeq markers on top of core 20-plex (exp 1)



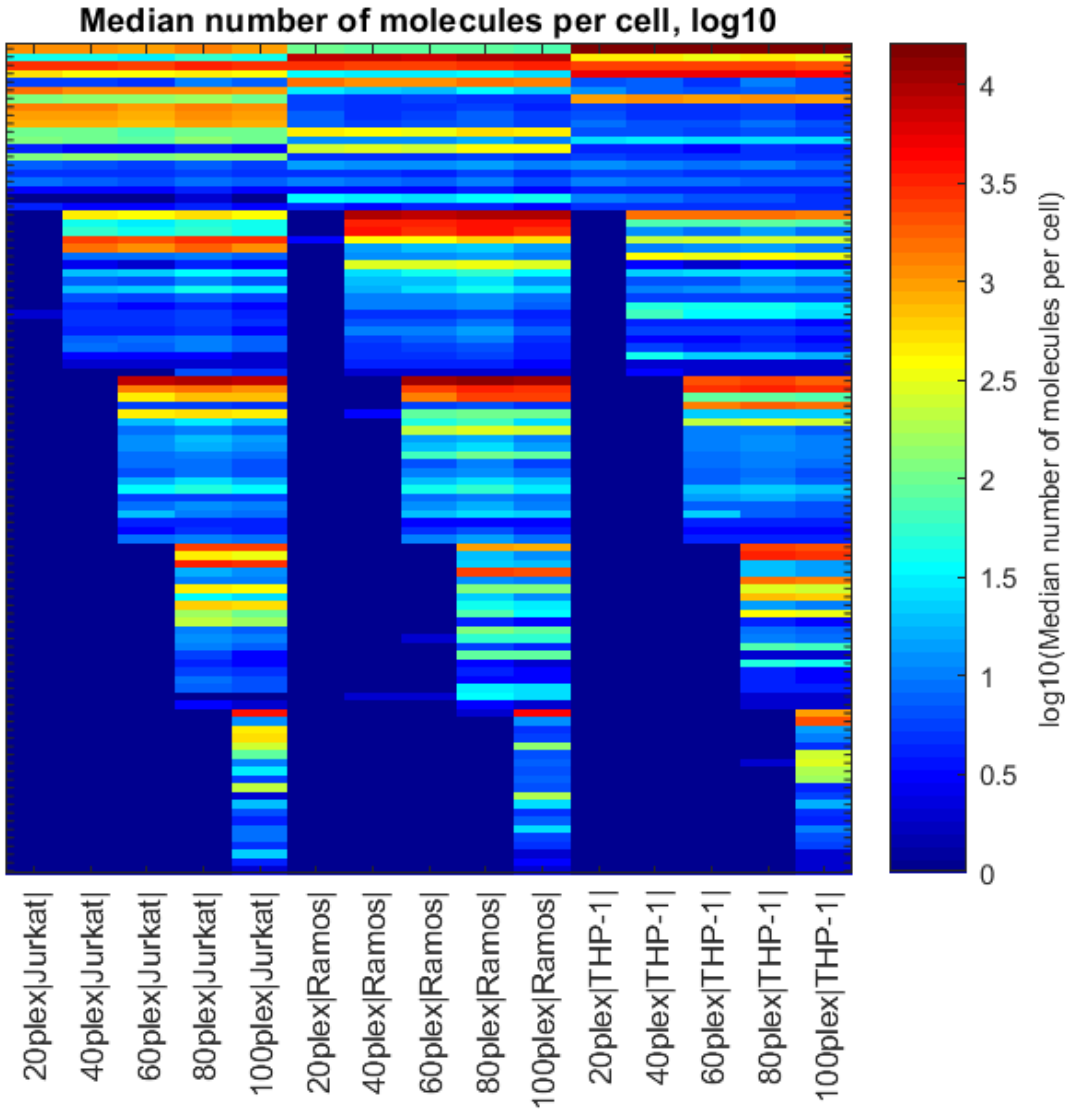


- Each successive 20plex is chosen to include a range of high-, mid- and low-expressed proteins
- Sequencing reads are adjusted to get similar sequencing depth at each plexy

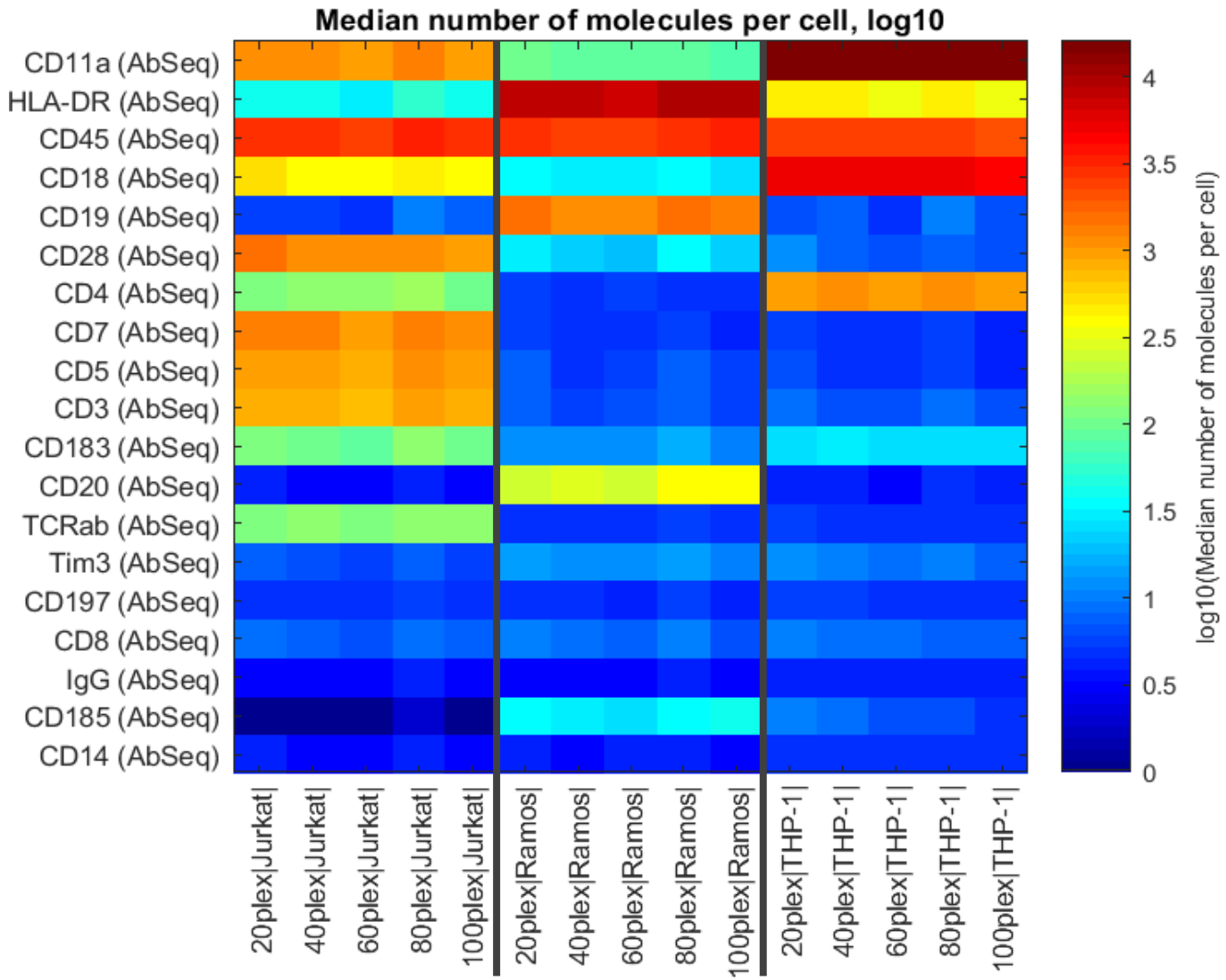
Performance of core 20-plex in each cell type in the presence of 20-80 additional AbSeq markers (exp 1)

Core 20-plex

Additional 20-plex panels added on successively



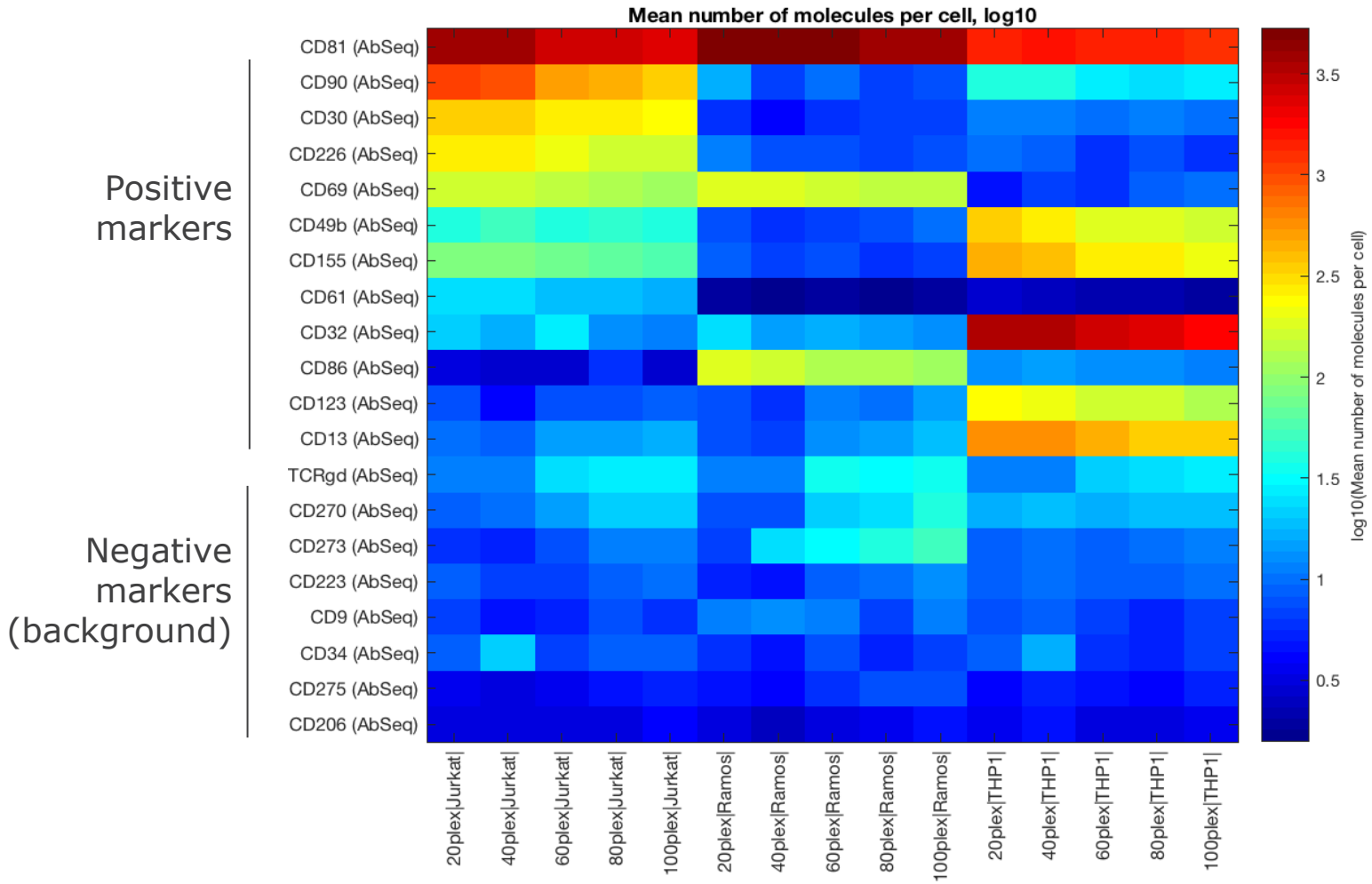
Increasing AbSeq plexy in this experiment did not significantly impact the core 20-plex AbSeq performance (exp 1)



Increasing plexy can lead to changes in signal/noise (exp 2)

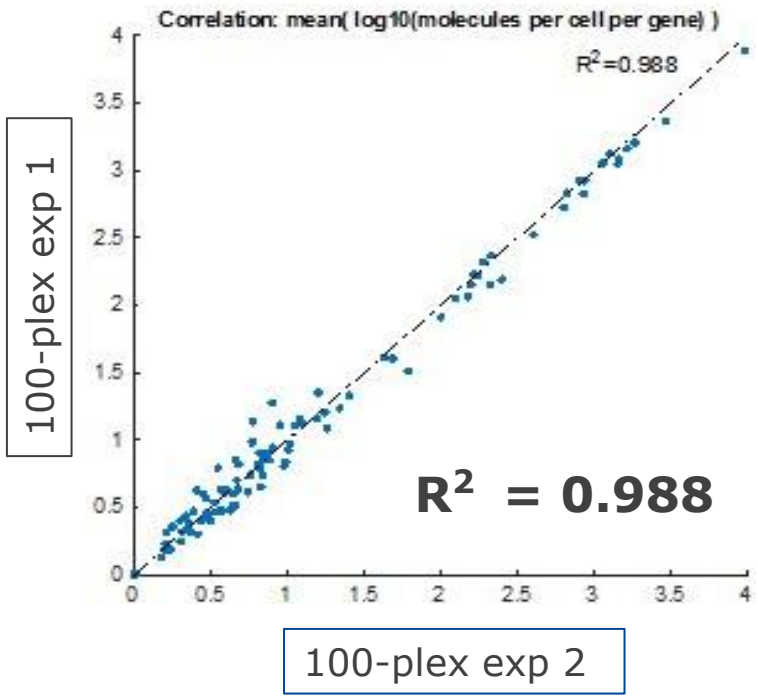
Watch out!

- Possible interactions between antibodies and technical artifacts can impact specificity/ sensitivity in high-plex AbSeq experiments

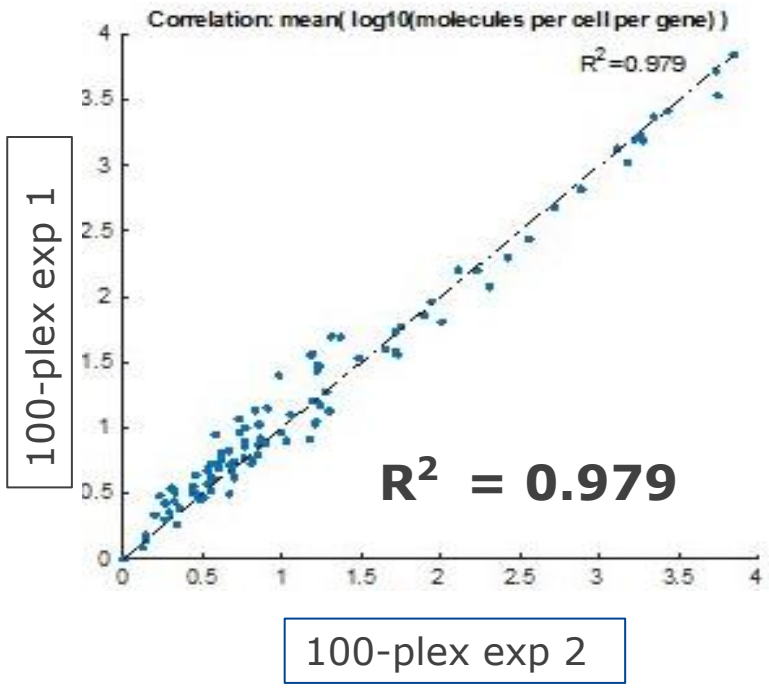


Strong correlation between 100-plex experiments (exp 1 and 2)

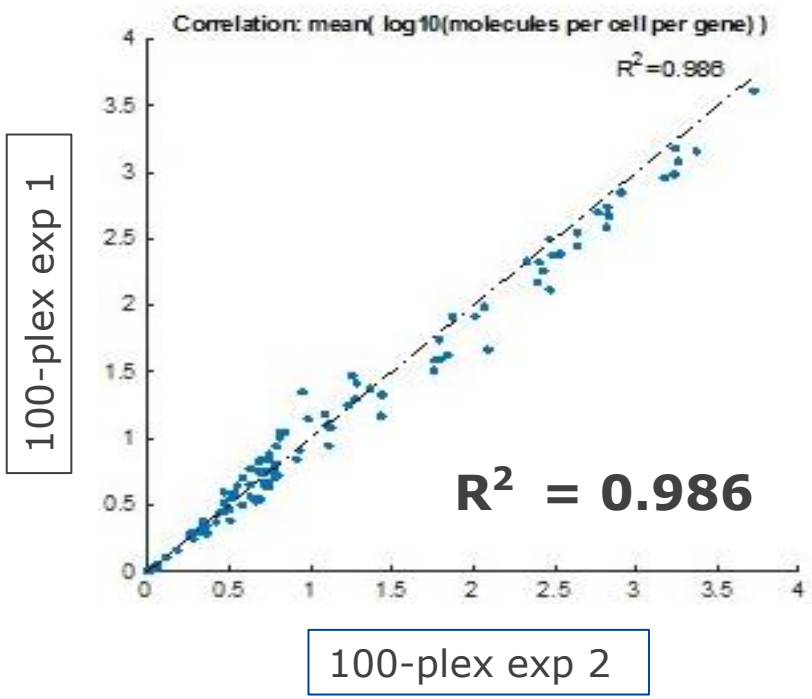
Jurkat



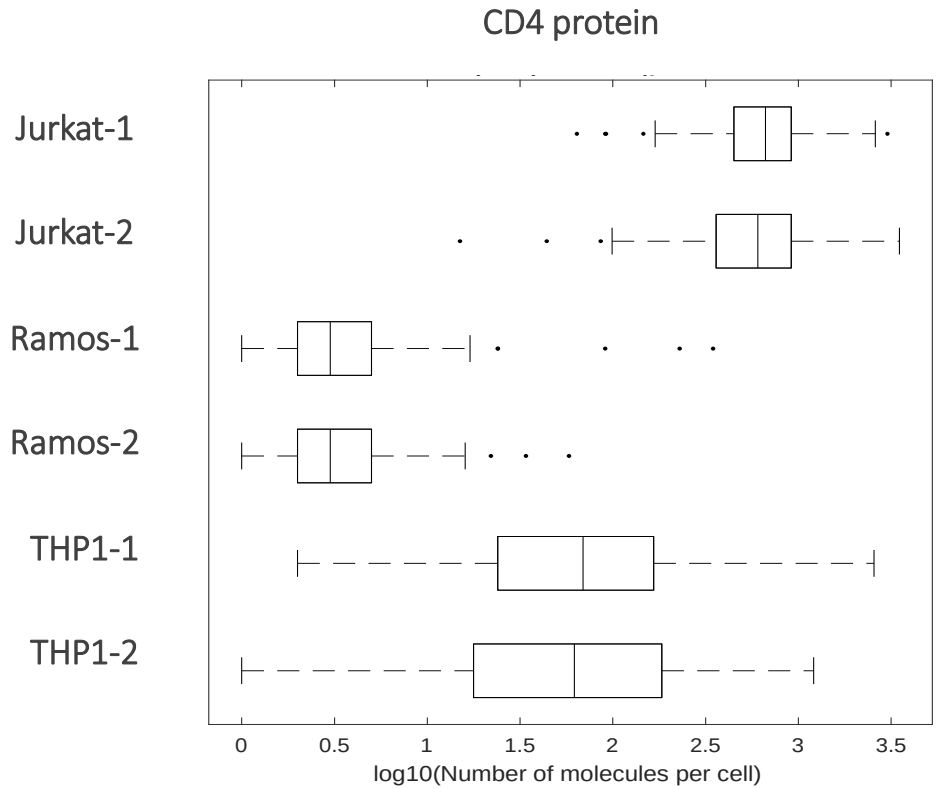
Ramos

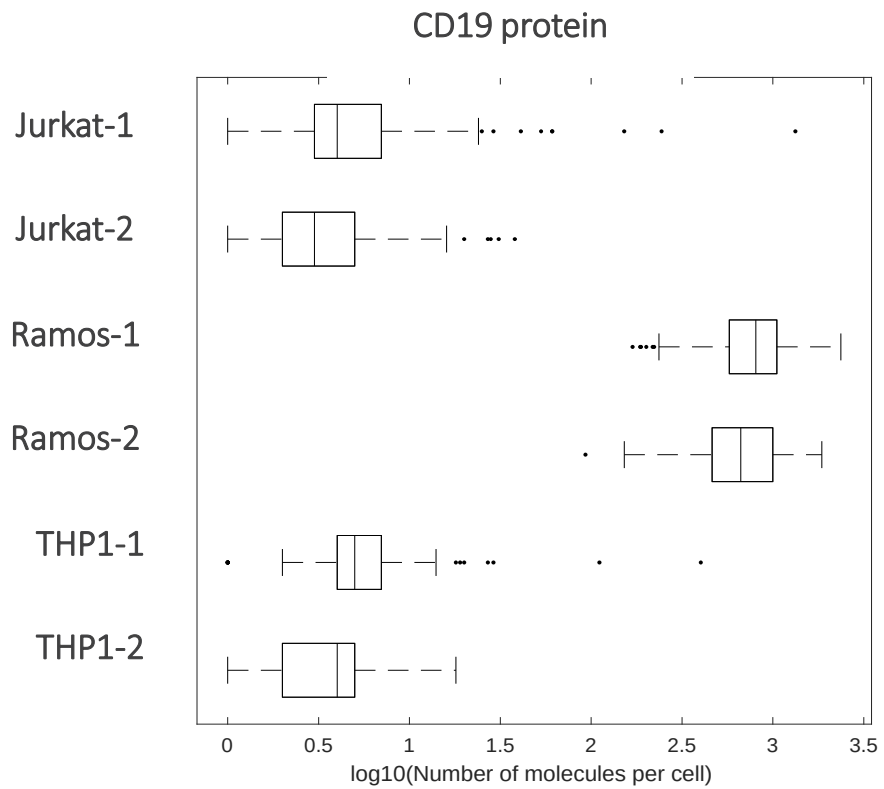


THP-1



Strong correlation between 100-plex experiments (exp 1 and 2)

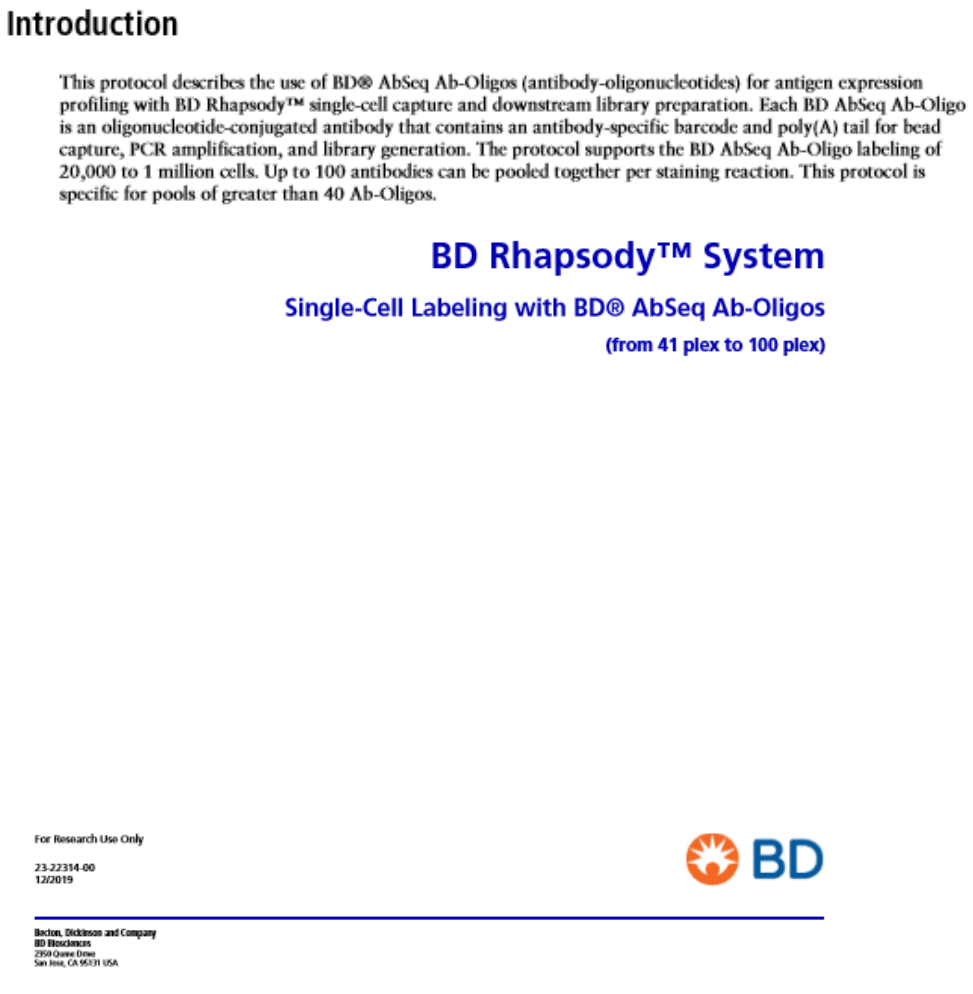




Conclusions

- BD® AbSeq Ab-Oligos enable high-plex protein panel analyses
- AbSeq panels can increase in plexy without significantly impacting AbSeq performance
- Experimental technique (e.g., rigorous cell washing, appropriate sequencing depth) can minimize technical artifacts that arise due to the high number of molecules in a high-plex AbSeq experiment
- Special attention to washing and handling steps is needed for higher plexy experiments

BD® AbSeq Ab-Oligos protocol specific for high-plexy experiments



Introduction

This protocol describes the use of BD® AbSeq Ab-Oligos (antibody-oligonucleotides) for antigen expression profiling with BD Rhapsody™ single-cell capture and downstream library preparation. Each BD AbSeq Ab-Oligo is an oligonucleotide-conjugated antibody that contains an antibody-specific barcode and poly(A) tail for bead capture, PCR amplification, and library generation. The protocol supports the BD AbSeq Ab-Oligo labeling of 20,000 to 1 million cells. Up to 100 antibodies can be pooled together per staining reaction. This protocol is specific for pools of greater than 40 Ab-Oligos.

BD Rhapsody™ System

Single-Cell Labeling with BD® AbSeq Ab-Oligos

(from 41 plex to 100 plex)

For Research Use Only

23-22314-00
12/2019

BD

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

- Supports high-plexy ab-oligo experiments (up to 100-plex)
- Separate protocol for high-plexy experiment (up to 100-plex) + single-cell multiplexing (SMK)

BD

Examining the impact of pre-pooling antibody-oligos on antibody performance

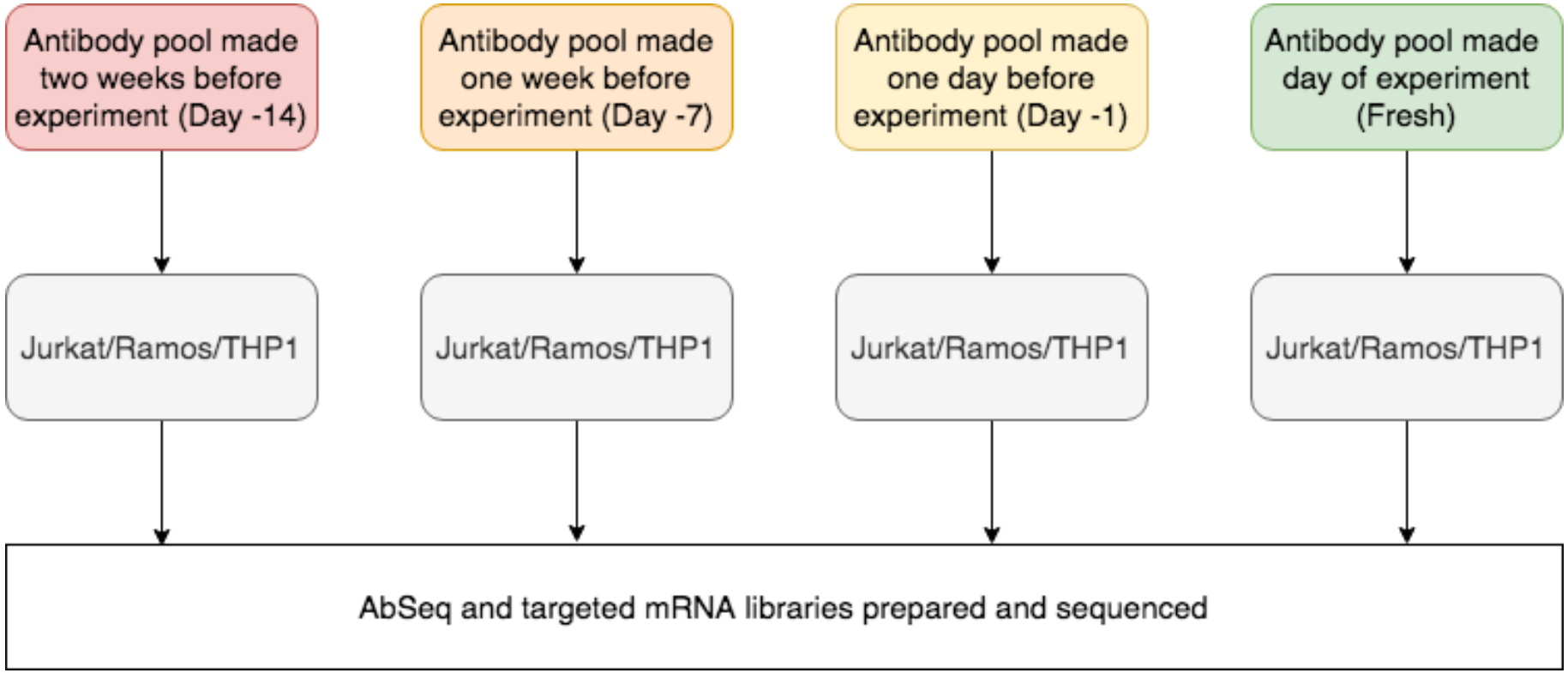
Learnings from performance of pre-pooled 40-plex BD® AbSeq Antibody-Oligos in a time course study

Pre-pooling antibody oligos

- Antibody-oligo technology is revolutionizing protein analyses
- However, rigorous analyses of these tools is still lacking
- We decided to investigate the performance of antibody-oligos (BD® AbSeq Reagents) upon pre-pooling and prolonged storage

Question: What is the performance of 40 ab-oligos that are pooled and stored for different periods before use, versus those that are pooled and used fresh?

Workflow of the pre-pooling experiment



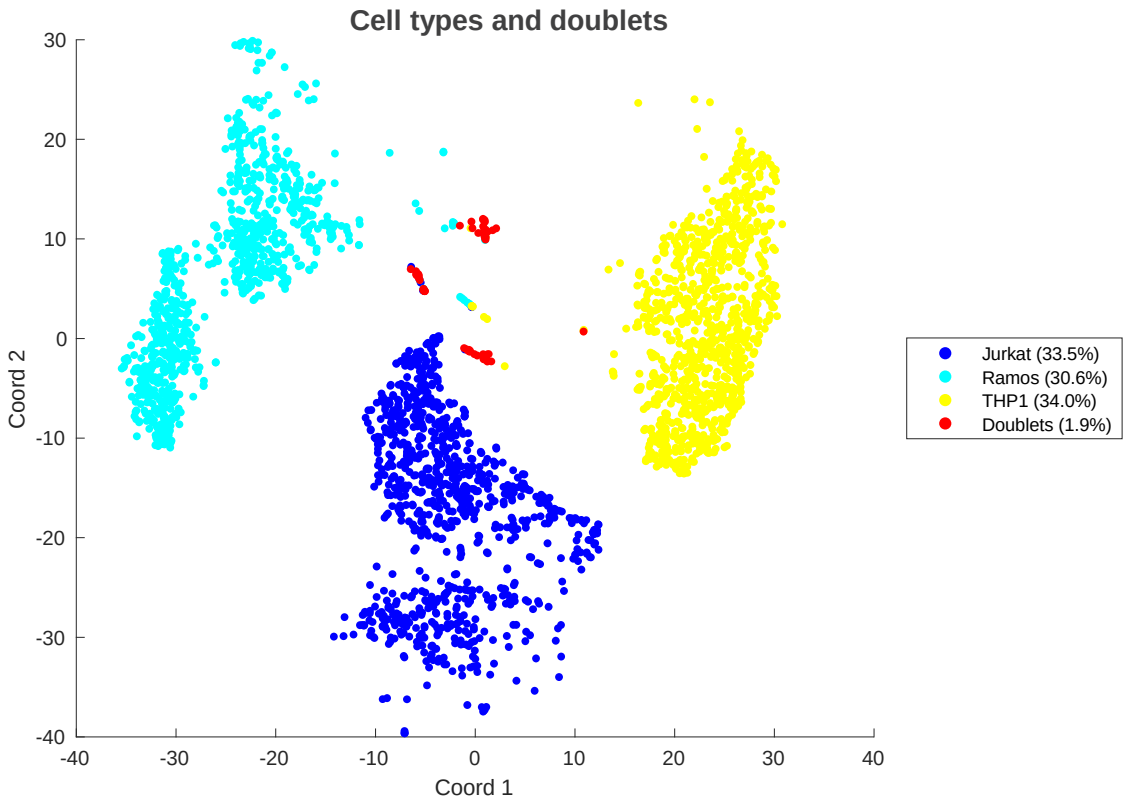
Note: Jurkat (T cells), Ramos (B cells), THP-1 (monocytes) in 1:1:1 mixture

List of BD® AbSeq Reagents used

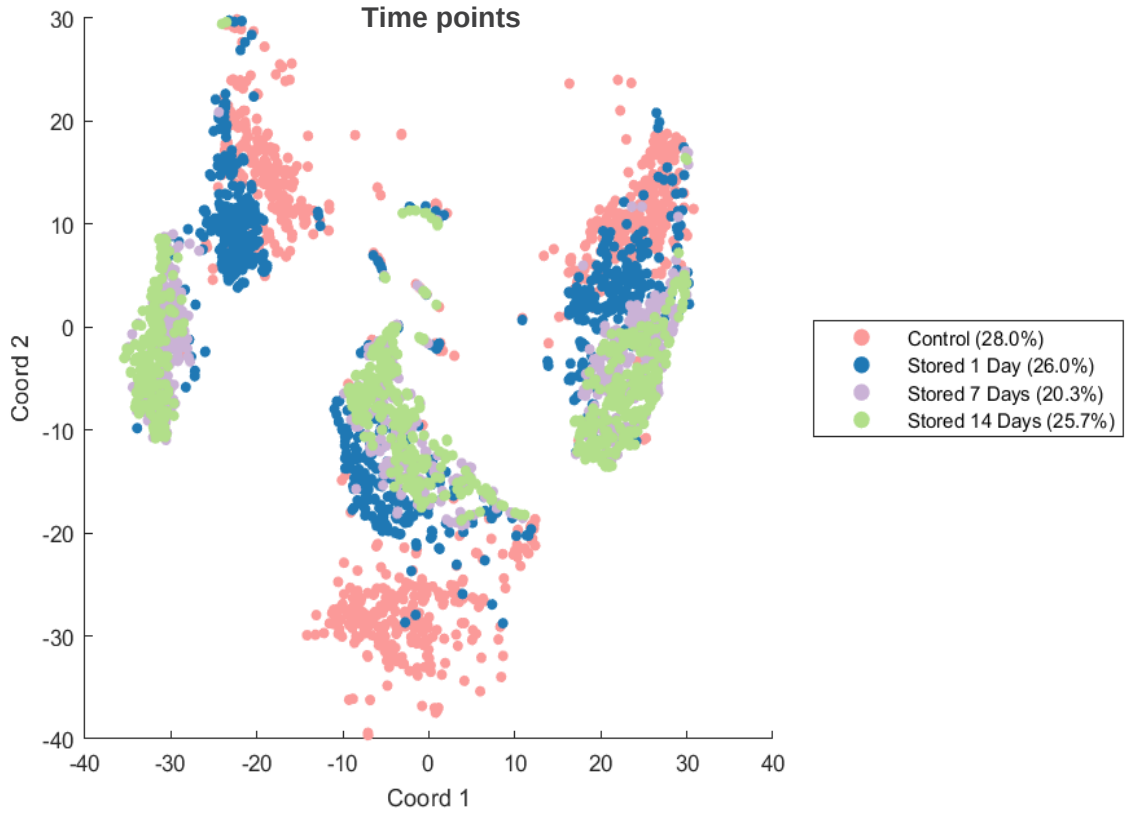
BD® AbSeq Reagents used in this study (40-plex)				
CD20	CD45RA	CD197	CD137	CD194
CD8	CD16	Tim3	CD11b	CD24
CD19	CD7	CD28	CD39	CD235a
CD4	CD5	CD183	CD38	CD62L
CD3	CD11a	CD185	CD27	CD11c
HLA-DR	CD56	IgG	CD25	IgD
CD14	CD54	TCRab	CD127	CD326
CD45	CD47	CD18	CD196	CD133

Impact of pre-pooling across different cell types

Similar proportion observed across different cell types

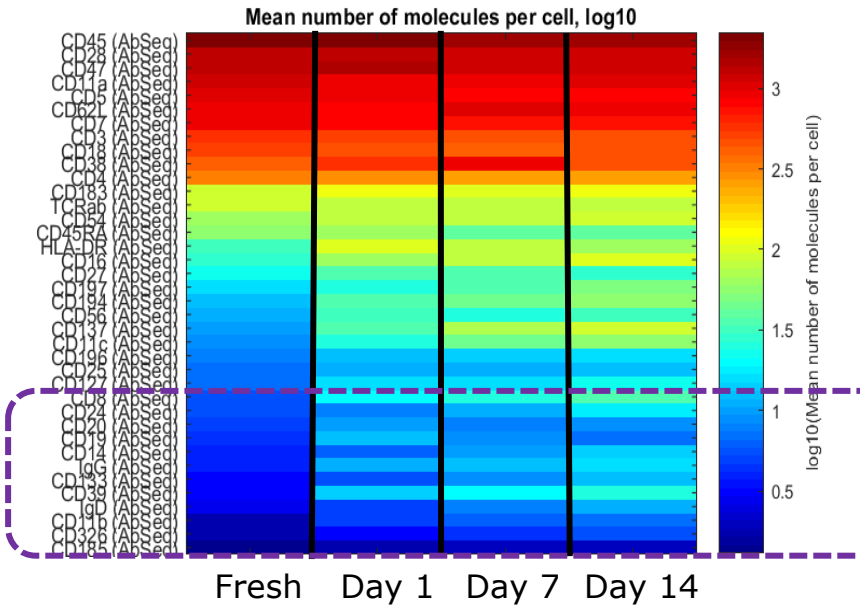


Batch effect observed for all stored samples compared to the fresh sample

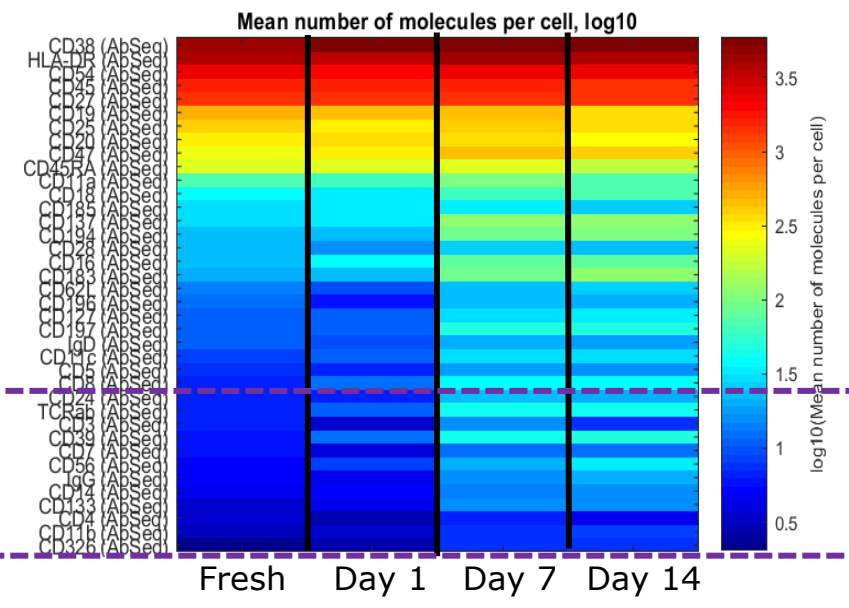


Sensitivity is maintained with increased storage time but background noise increases

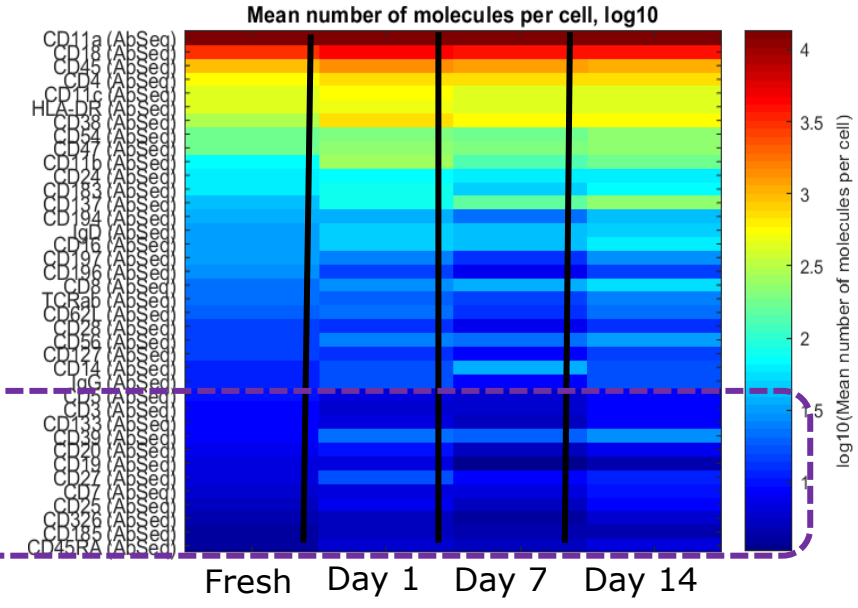
Jurkat



Ramos

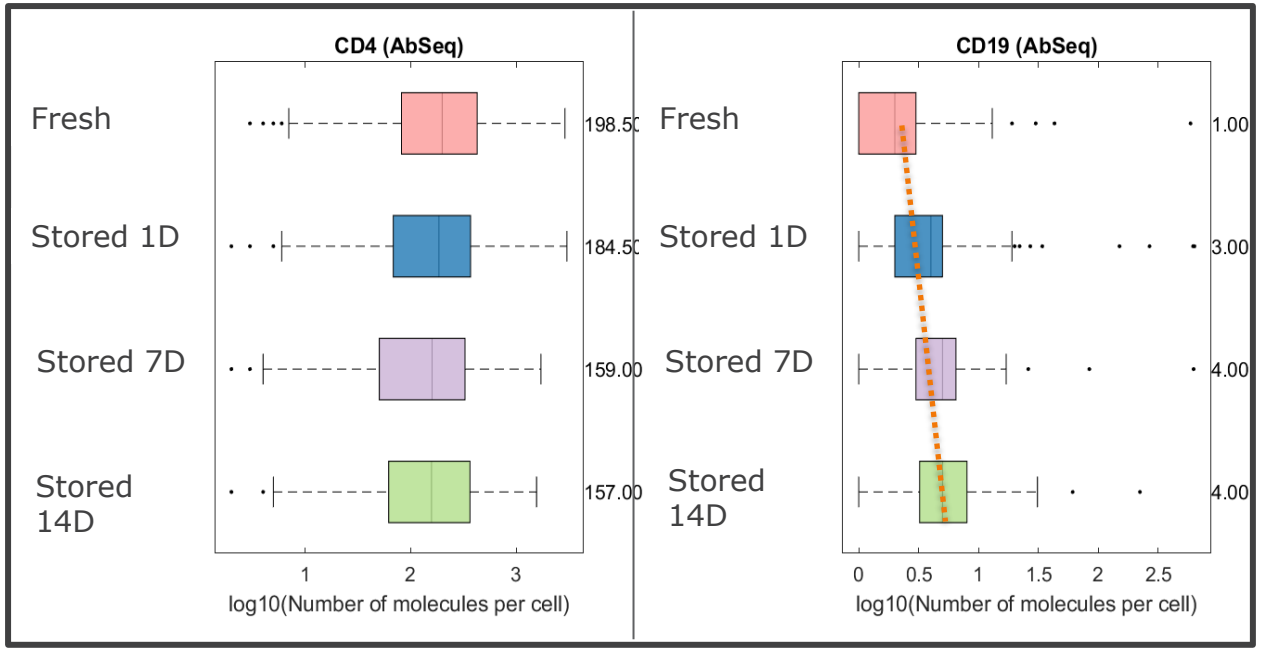


THP-1



Noise increases for pre-pooled ab-oligos without significant impact to sensitivity

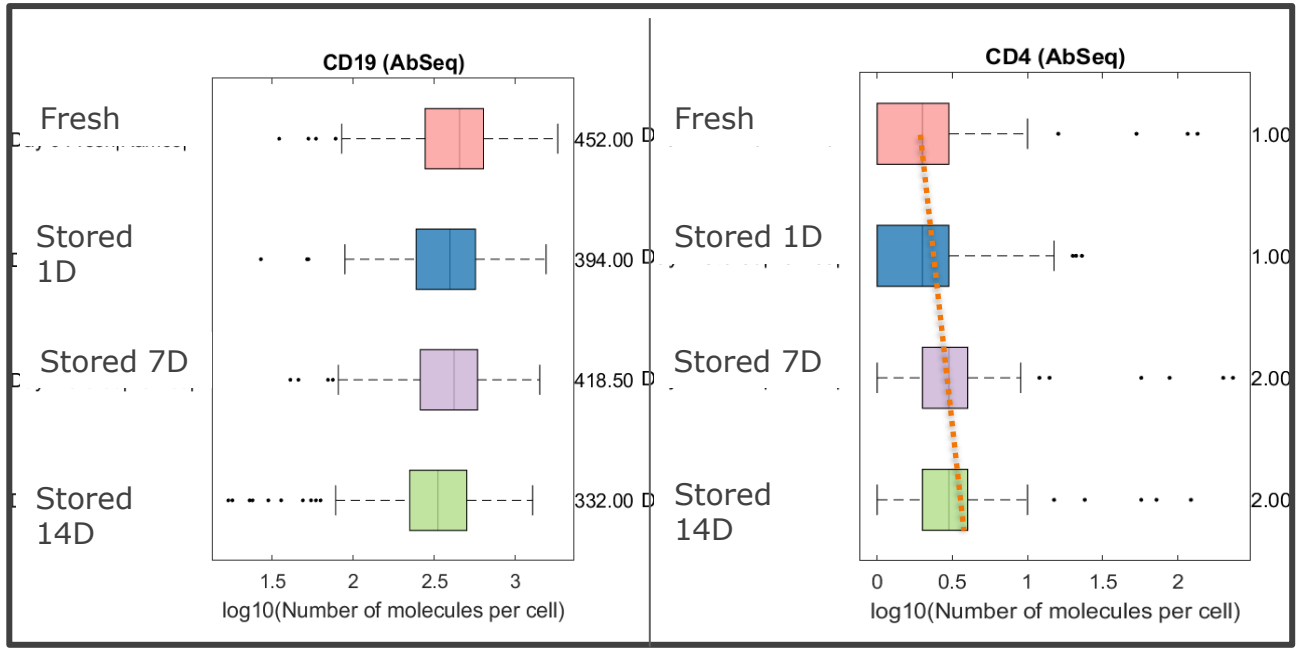
Jurkat (T cell line)



Sensitivity

Noise

Ramos (B cell line)



Sensitivity

Noise

Conclusions

- Some batch effect is observed when the pre-pooled antibody-oligos are stored over time
- Although sensitivity remains mostly unchanged with storage, noise increases with longer storage times

For optimal BD® AbSeq Antibody-Oligo performance, preparing a fresh mixture of pooled antibody-oligos is strongly recommended

Tackling the challenge of sequencing high expressors in a single-cell multiomic experiment

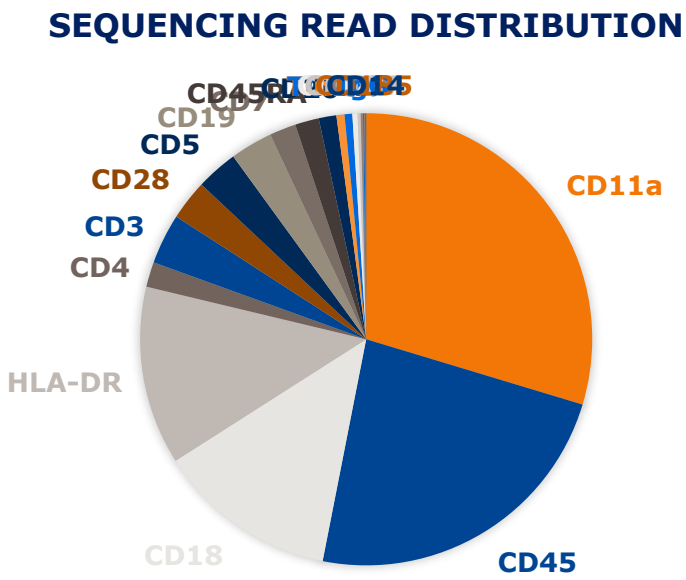
Testing approaches to decrease sequencing costs for multiomic experiments on the BD Rhapsody™ System

BD

Highly expressed antigens increase sequencing costs

20-plex BD® AbSeq Antibodies with high expressers

CD45	CD19	Tim3	CD5
CD11a	CD45RA	CD20	CD3
CD18	CD28	TCRab	CD8
HLA-DR	CD183	CD197	CD14
CD4	CD7	CD185	IgG



83% of sequencing reads devoted to five markers (bold)

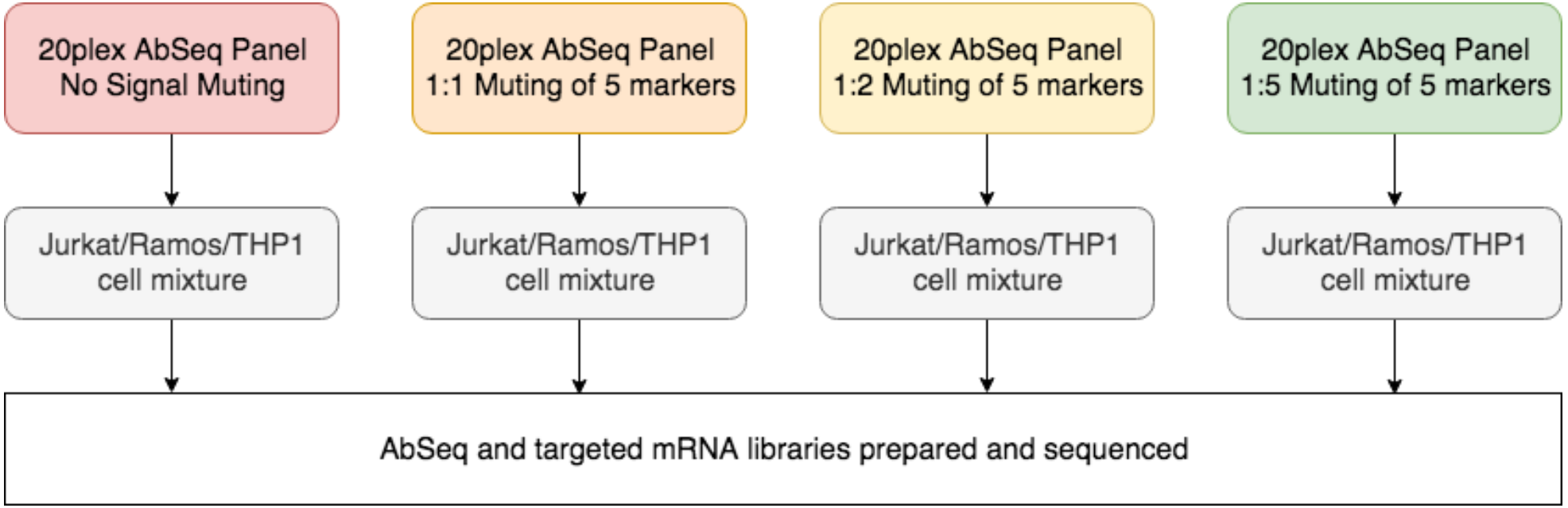
Question: Can muting high expressors decrease sequencing cost by lowering the total number of reads required to resolve low expressed antigens?

Muting the high expressors in the BD® AbSeq Panel

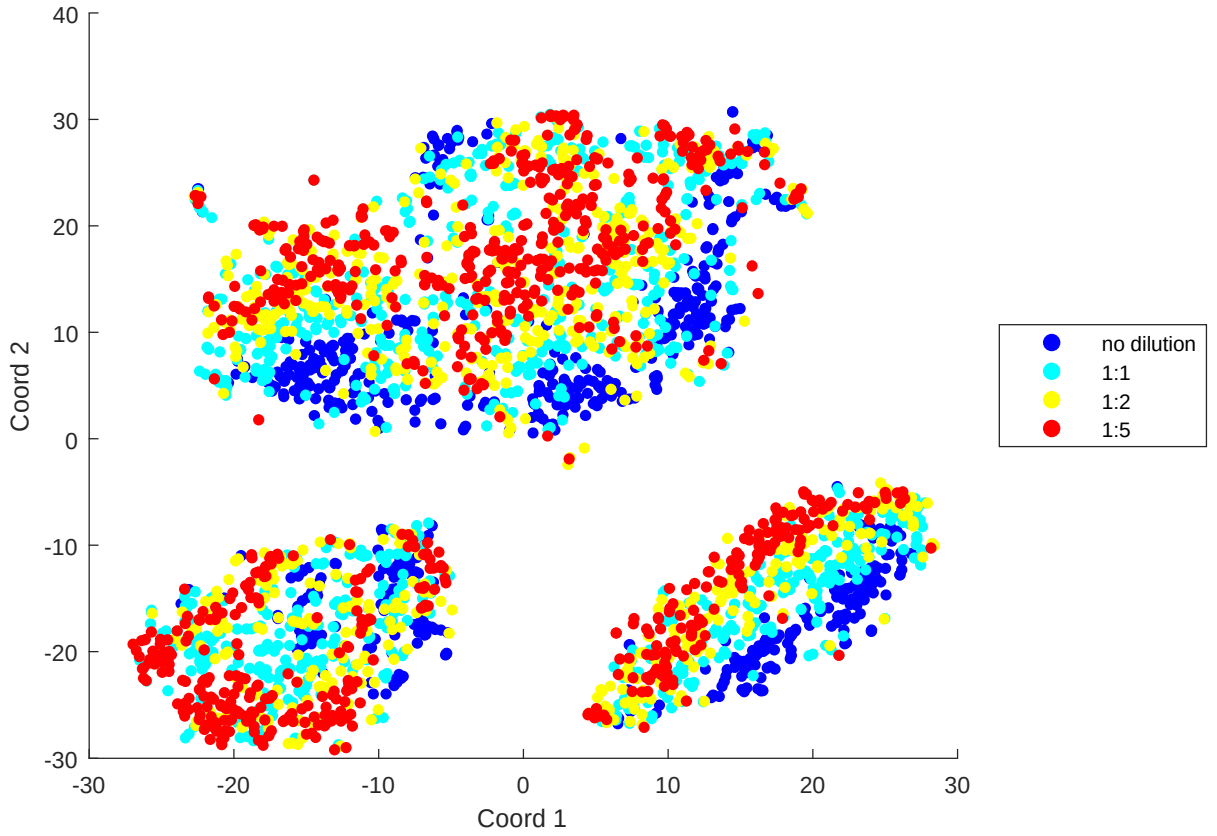
Muted antibodies
CD45
CD11a
CD18
HLA-DR
CD4

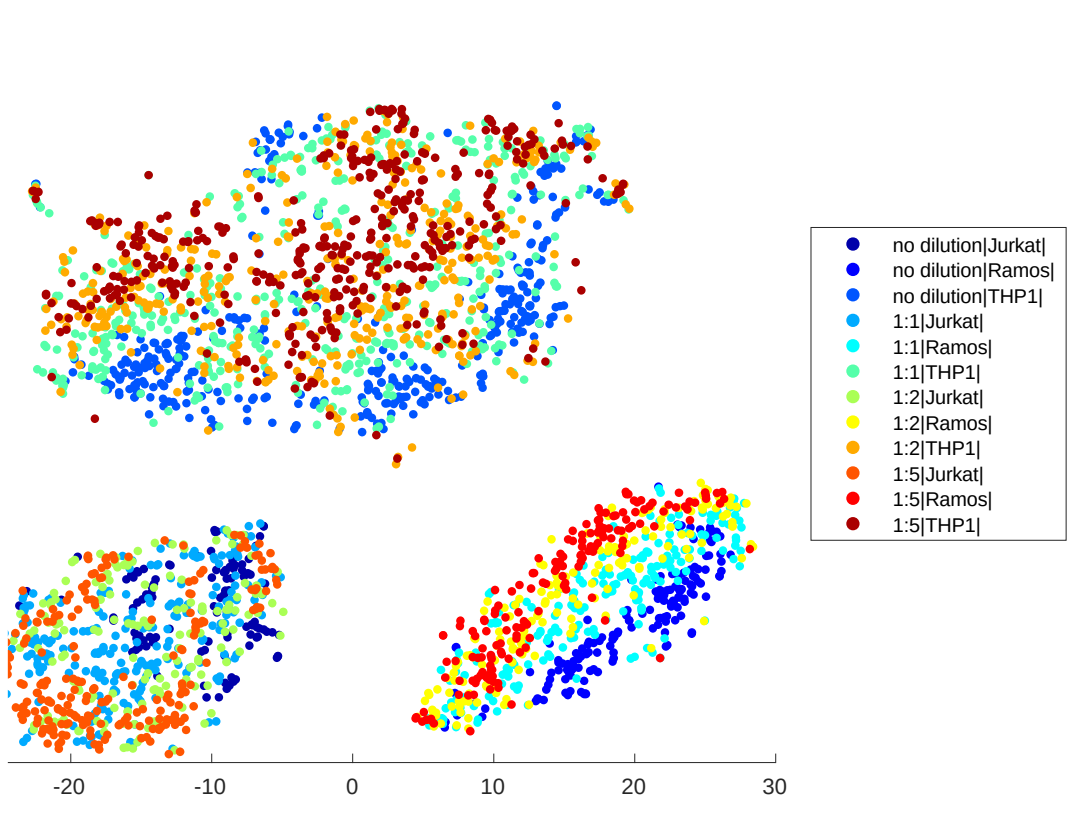
Unmuted antibodies	
CD19	Tim3
CD45RA	CD20
CD28	TCRab
CD183	CD197
CD7	CD185
CD5	CD8
CD3	CD14
IgG	

AbSeq markers can be muted by mixing conjugated and unconjugated antibodies at different ratios

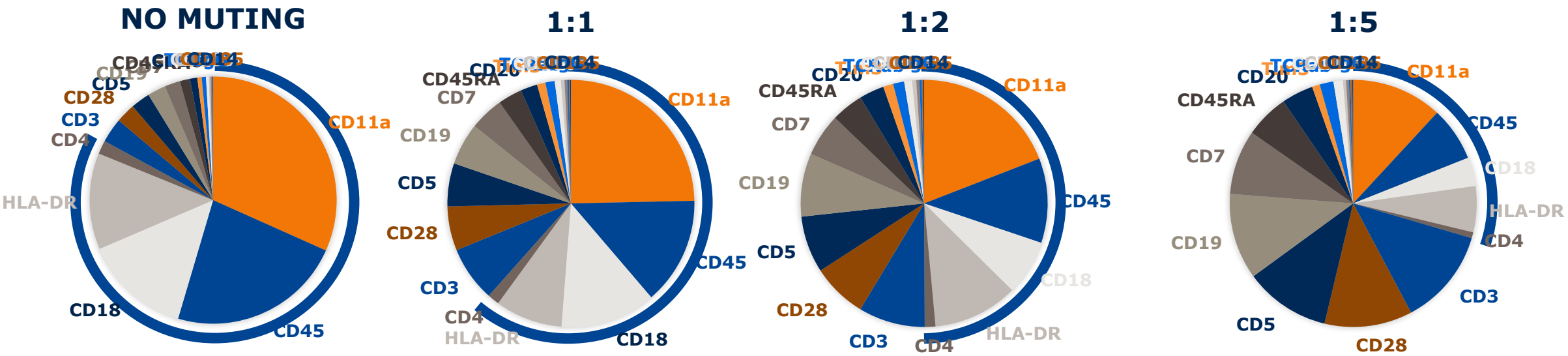


Resolution of cell types is not impacted by signal muting





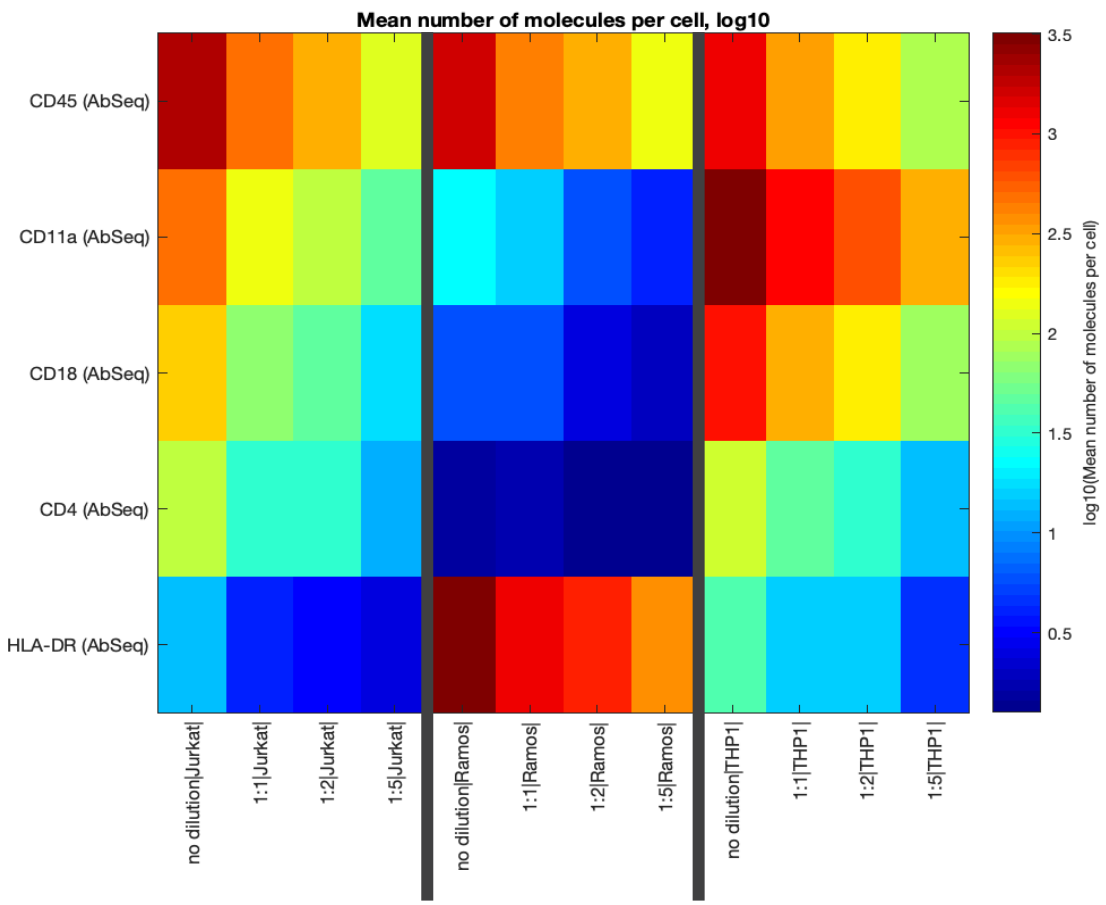
Sequencing reads are re-distributed after signal muting towards non-muted markers

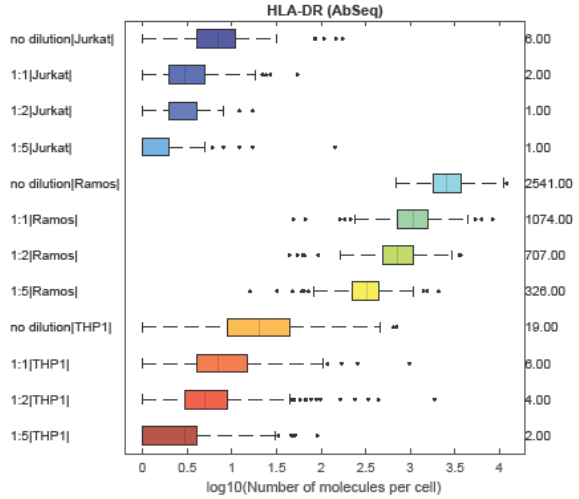


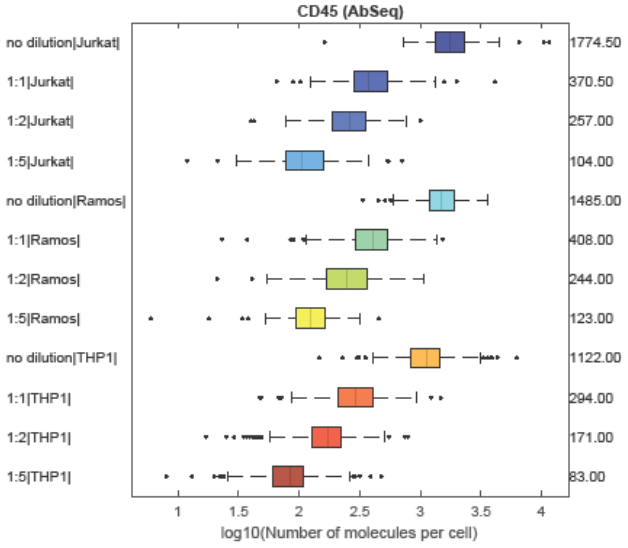
83% 62% 50% 30%

% reads devoted to the five muted antibodies

Fewer molecules/cell observed for muted antibodies

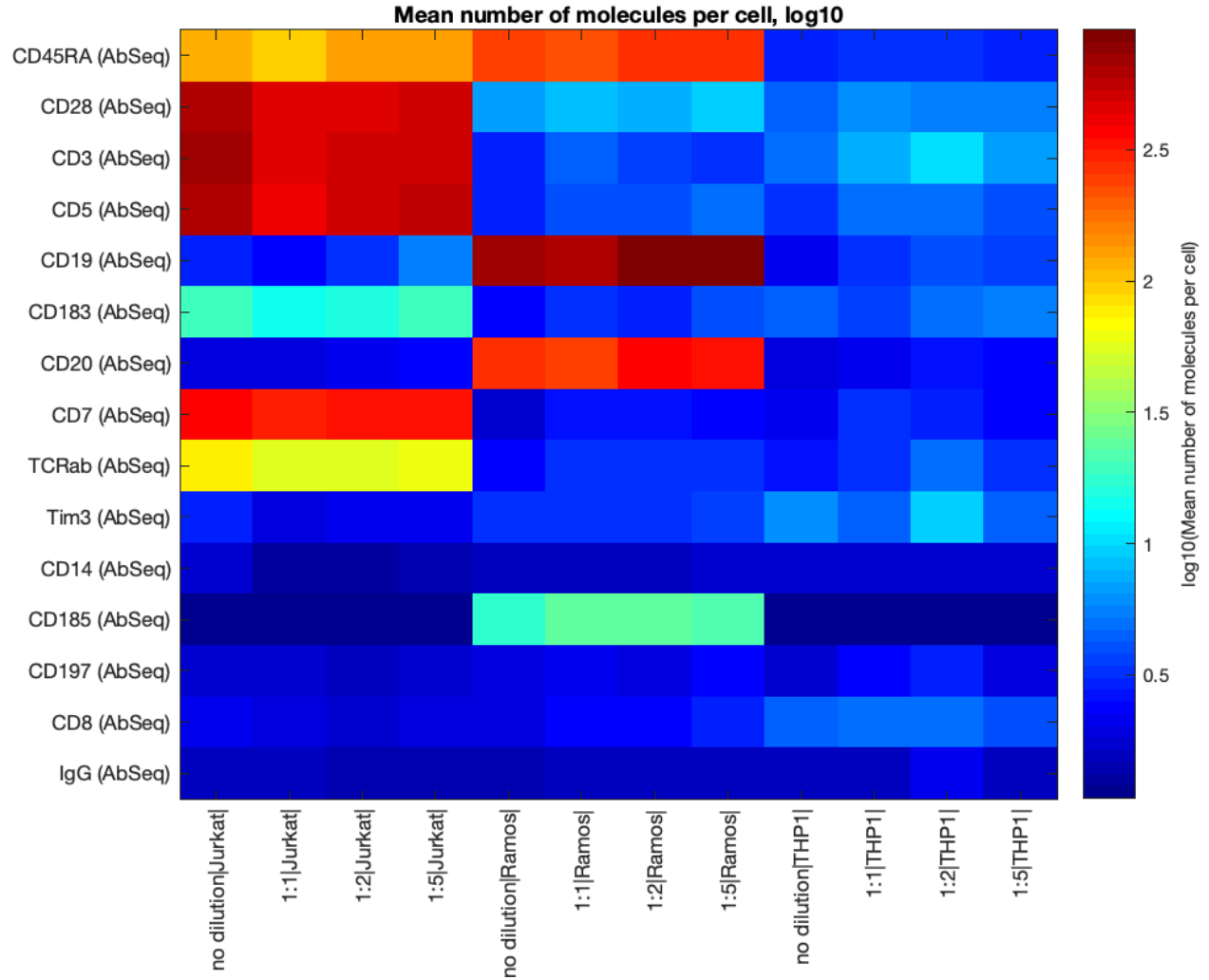






No significant impact in the resolution of unmuted markers at saturated sequencing

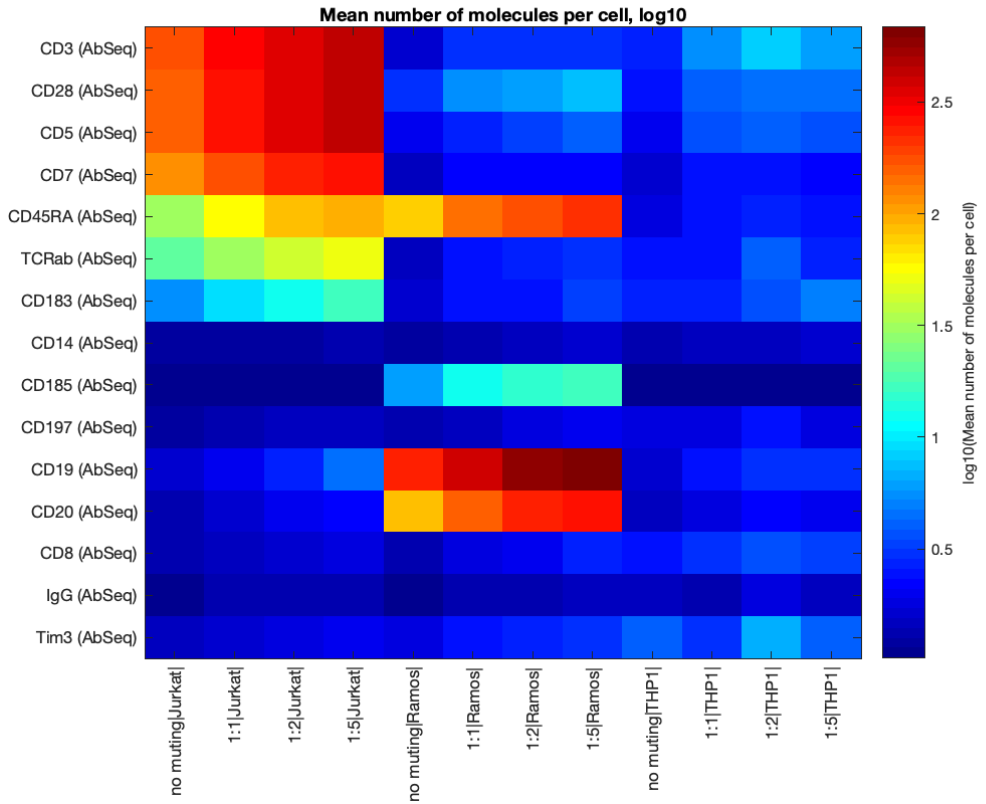
Unmuted antibodies



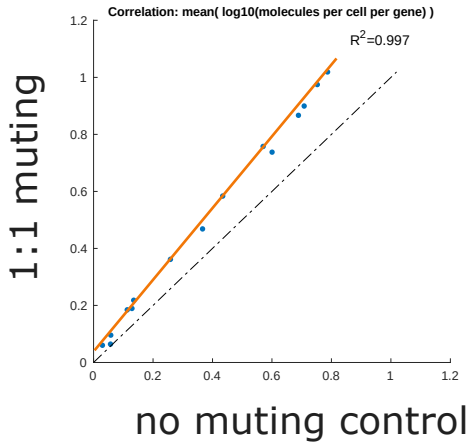
57,000 reads/cell for BD® AbSeq Antibody-Oligos

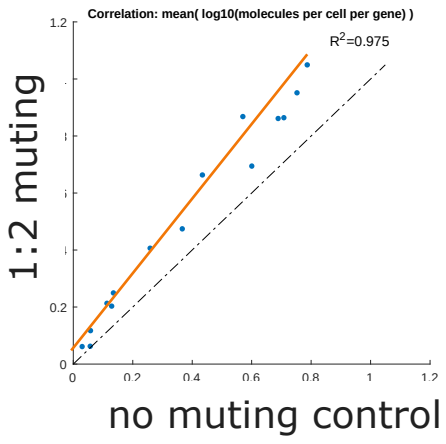
Muting strategy at sub-saturated sequencing increases sensitivity for low expressors

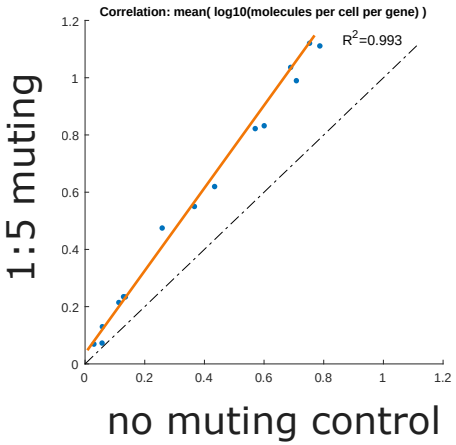
4,000 reads/cell for BD® AbSeq Ab-Oglios



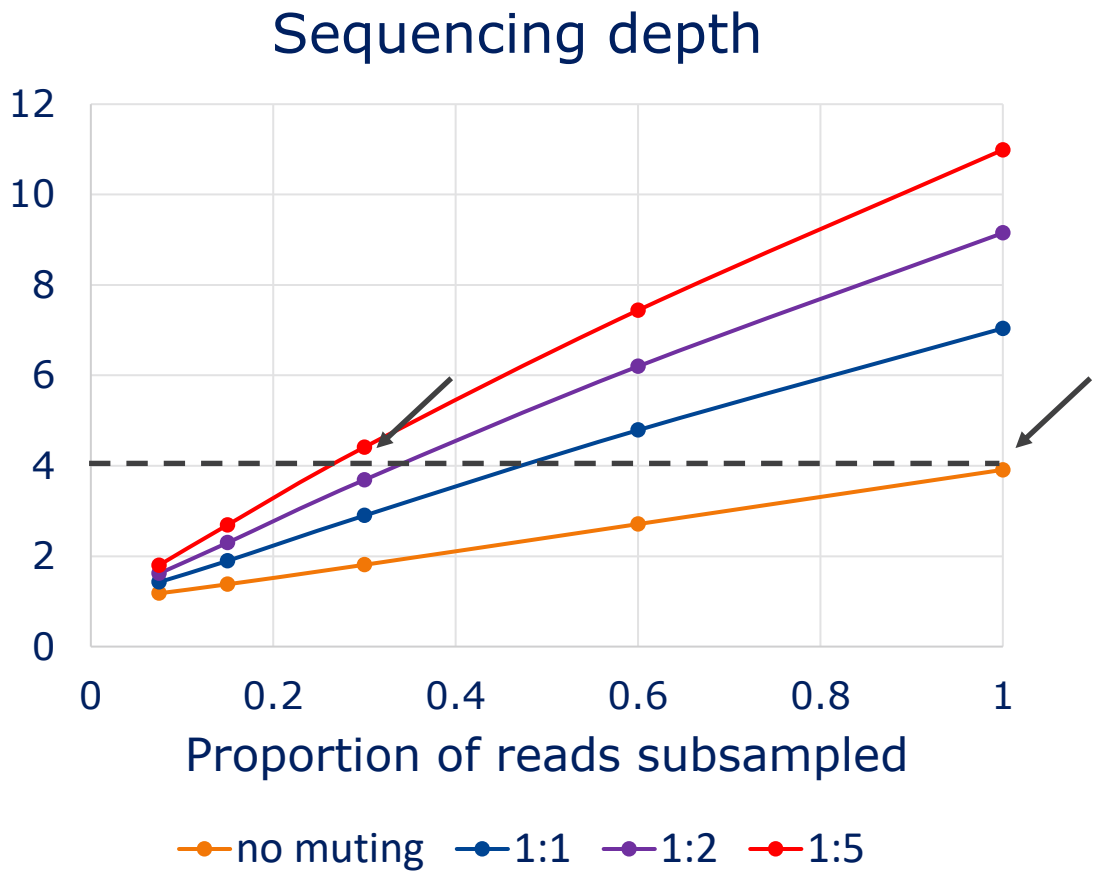
15 unmuted antibodies:mean(log10[molecules per cell per ab-oligos])





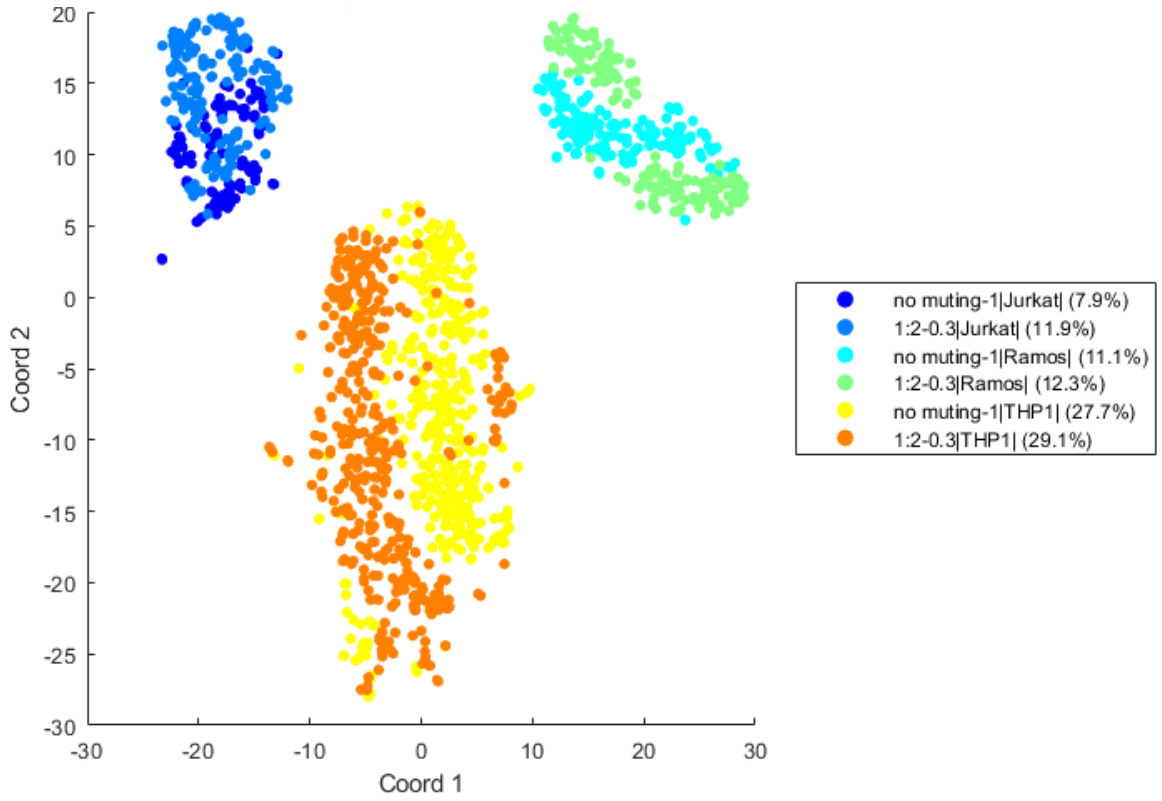


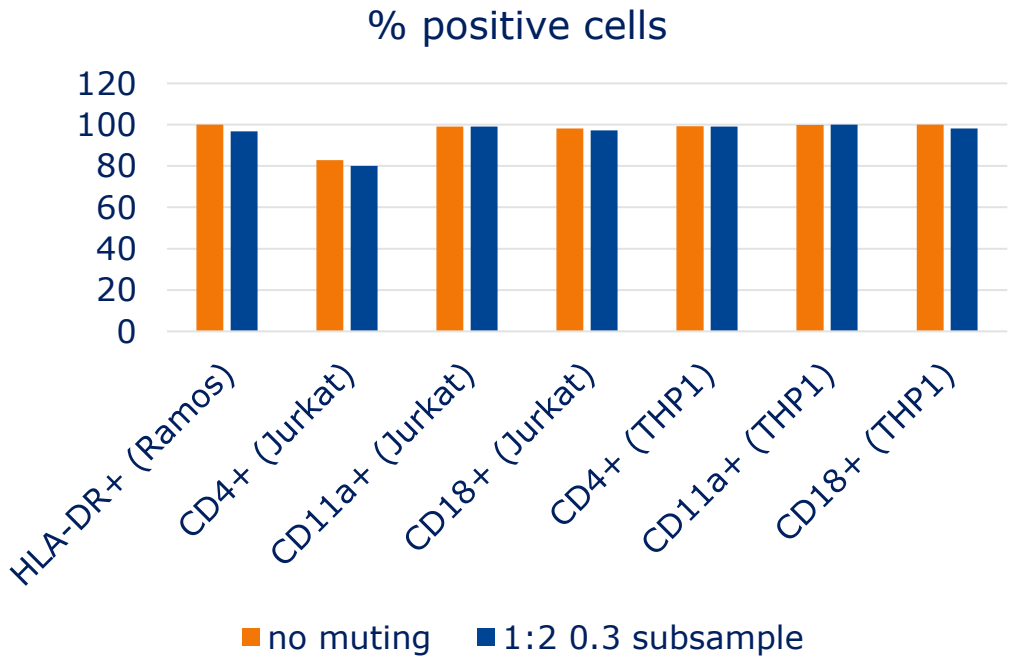
Bioinformatic subsampling of sequencing reads illustrates the impact of muting on sequencing depth



Similar sequencing depth achieved with **30% of sequencing reads** in 1:2 muting sample

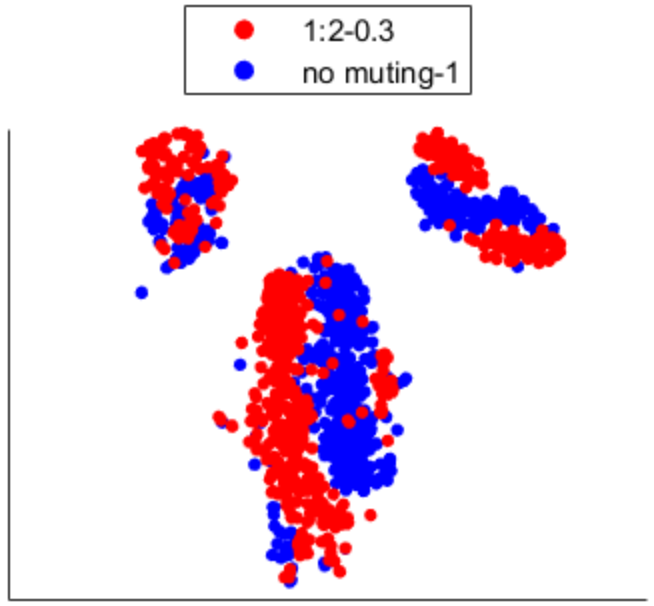
Performance - 1:2 muted at 30% of sequencing vs. unmuted controls

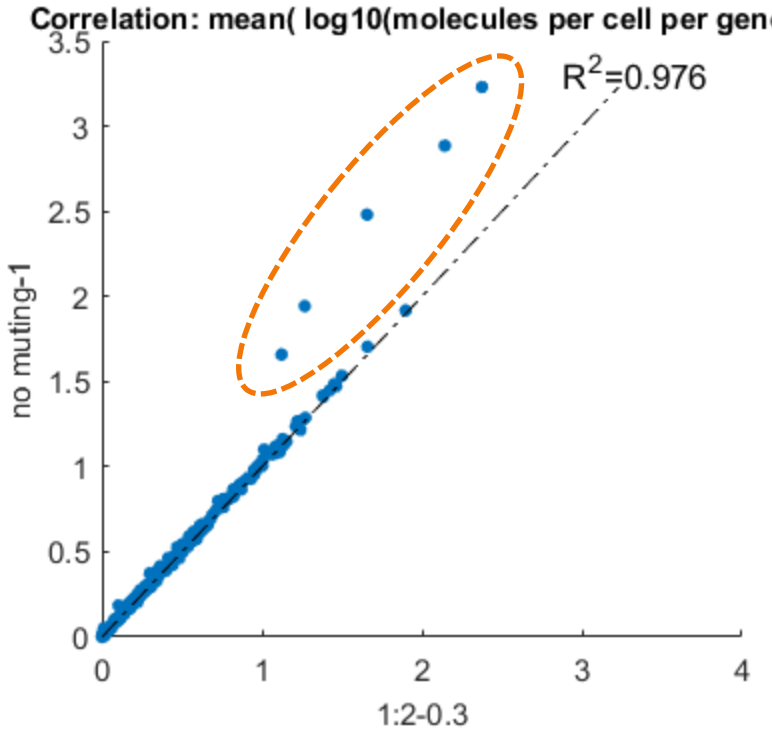


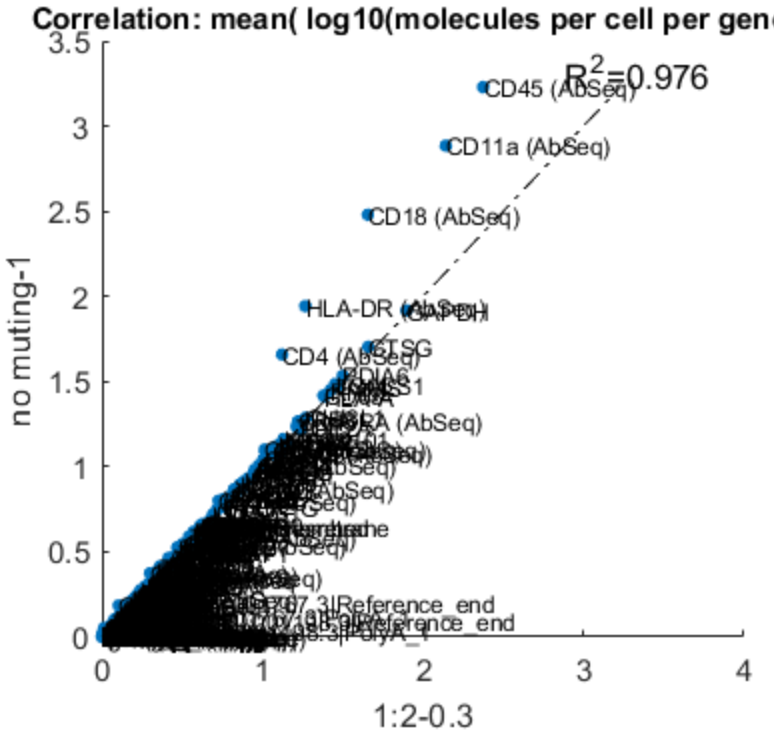


Performance - 1:2 muted at 30% of sequencing vs. unmuted controls

57,000 reads/cell compared to 16,000 reads/cell

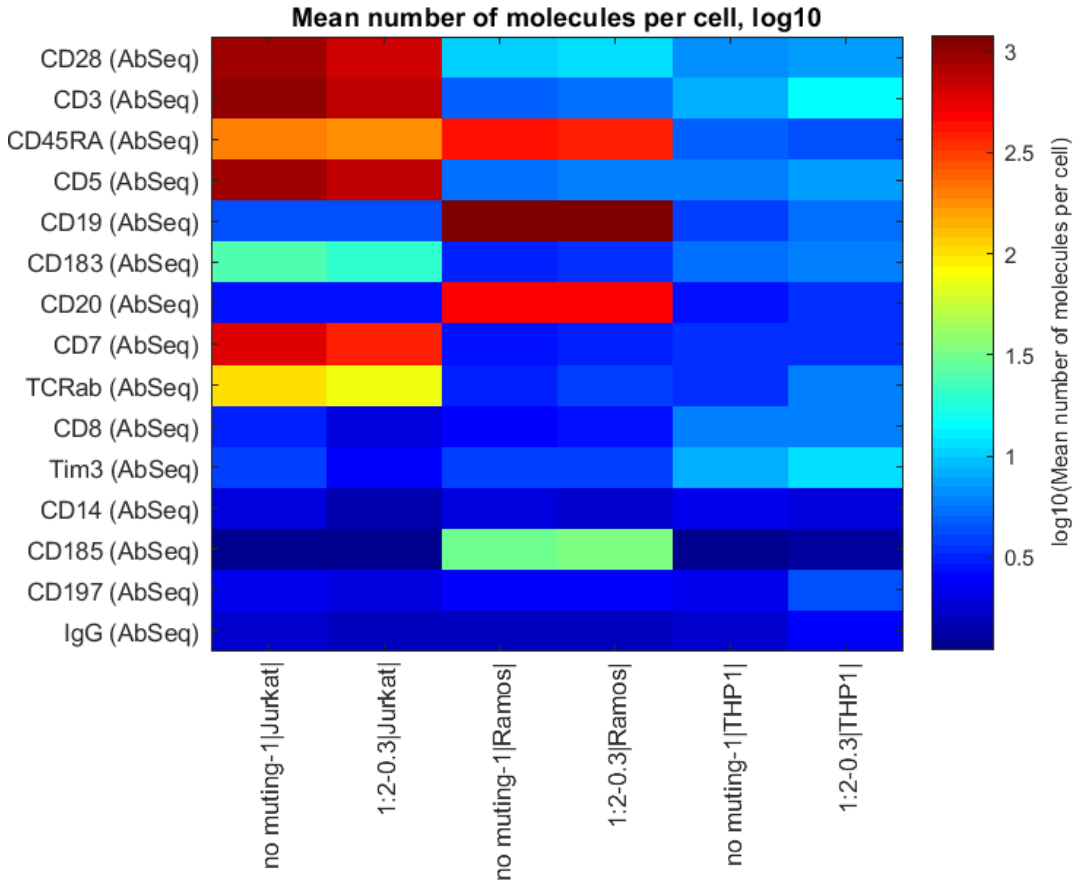


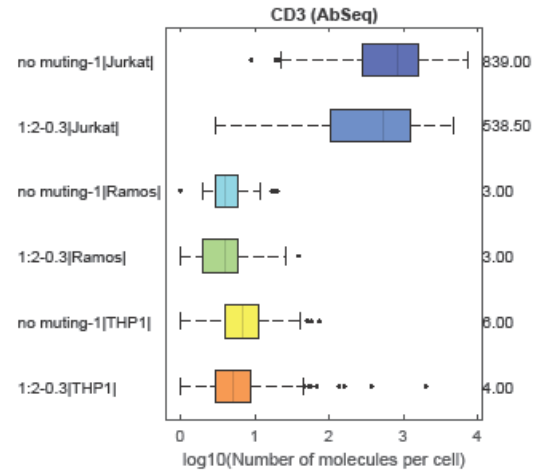


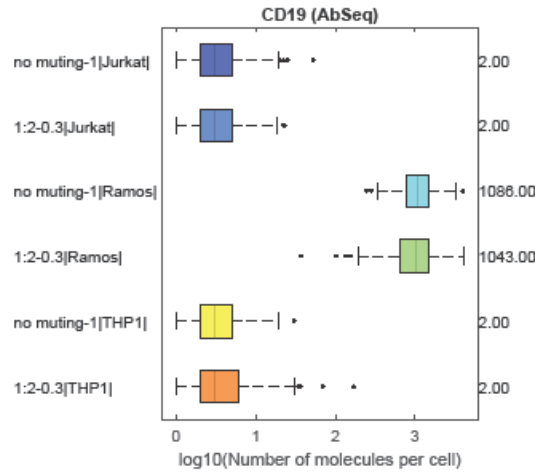


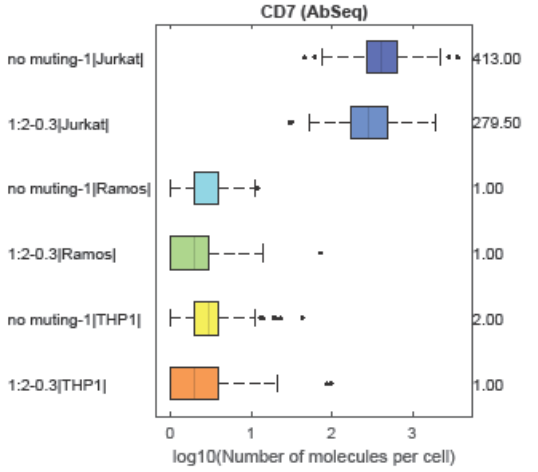
Note: The five targets (circled) that were muted show less molecules detected under muting conditions

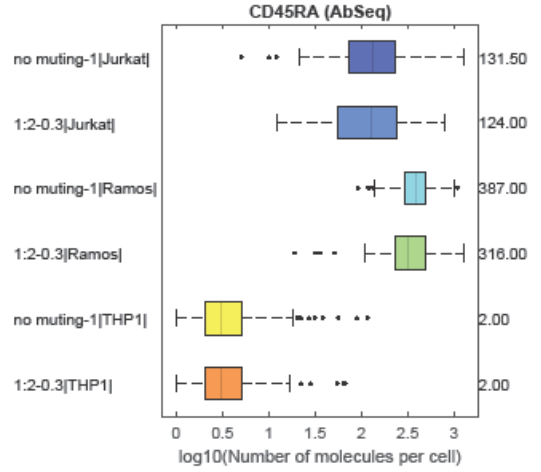
Number of molecules - 1:2 muted at 30% of sequencing reads vs. unmuted controls











Cost comparison: Signal muting enables higher throughput for same experimental costs

	No Muting	With Muting
AbSeq reads needed/cell for this plexy	57,000	20,000
Cells per Illumina NextSeq® System high output	5,614	16,000
Samples that can be run (5k cells/sample)	1	3
Sequencing cost per cell	$0.46	$0.16

Conclusions

- Muting high expressors redistributes reads, increasing resolution of low or weakly expressed markers
- Saturated sequencing shows that there are similar numbers of un-muted molecules in all samples
- Informatic subsampling of reads shows that at lower saturation, muting enables better resolution of low expressed antigens
- A 1:2 signal muted sample showed similar sensitivity for unmuted antibodies with only 30% of reads

Thank you!

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