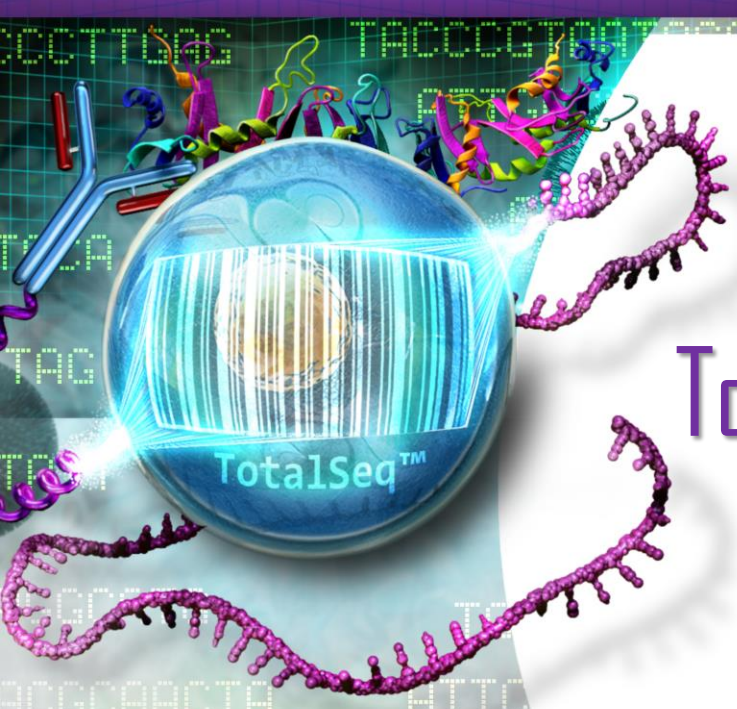


TotalSeq™

Proteomics in the era of high-throughput single-cell sequencing

Jean-Baptiste GUILLERME
Technical Application Scientist
jbguillerme@biolegend.com

www.biolegend.com/totalseq
Email: totalseq@biolegend.com

Our Mission at BioLegend: Enable Legendary Discovery

Gene Lay, D.V.M.
Founder and CEO;
co-founder of PharMingen

BioLegend develops and manufactures world class, cutting-edge **antibodies** and other reagents for immunological and biomedical research.

The principles of our mission:

- Highest quality products
- Outstanding value
- Superior customer service & technical support

TotalSeq™ | Reagents for simultaneous analysis of proteins & RNA expression

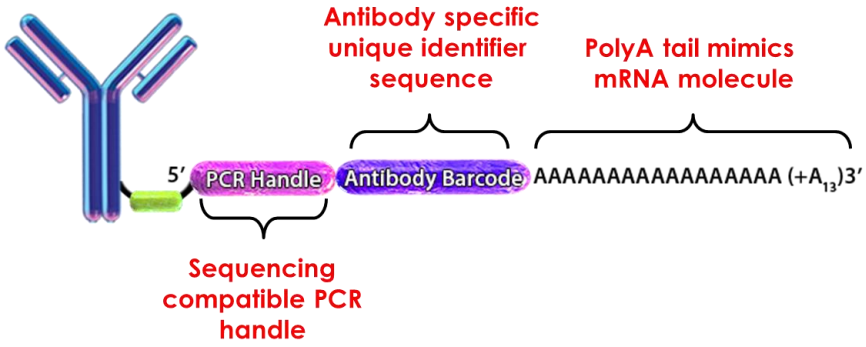
Simultaneous epitope and transcriptome measurement in single cells

Marlon Stoeckius[1], Christoph Hafemeister[1], William Stephenson[1], Brian Houck-Loomis[1], Pratip K Chattopadhyay[2], Harold Swerdlow[1], Rahul Satija[1,3] & Peter Smibert[1]

nature biotechnology

Multiplexed quantification of proteins and transcripts in single cells

Vanessa M Peterson[1,5], Kelvin Xi Zhang[2,5], Namit Kumar[1], Jerelyn Wong[3], Lixia Li[1], Douglas C Wilson[3], Renee Moore[4], Terrill K McClanahan[3], Svetlana Sadekova[3] & Joel A Klappenbach[1]



CITE-seq:

Cellular Indexing of Transcriptomes & Epitopes by Sequencing

REAP-seq:

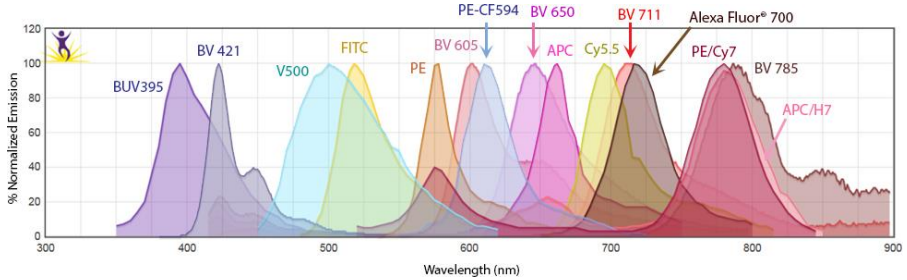
RNA expression and protein sequencing assay

CITE-seq: Tool kit like FACS

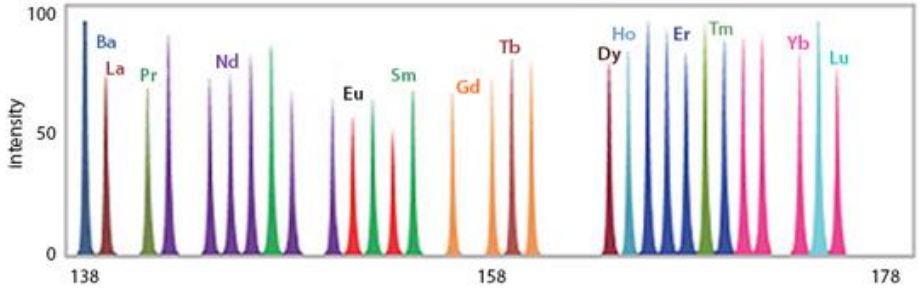
BioLegend®

TotalSeq™ Enables Unparalleled Multiplexed Protein Quantification

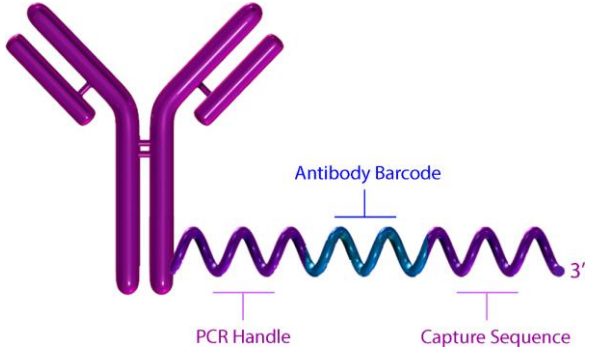
FLOW (Distinct Emissions) 15 to 30 parameters



CYTOF (Distinct Mass Isotopes) 50 parameters



TotalSeq™ (Distinct 15nt Barcodes)



>1 Billion unique barcodes

(4^{15})

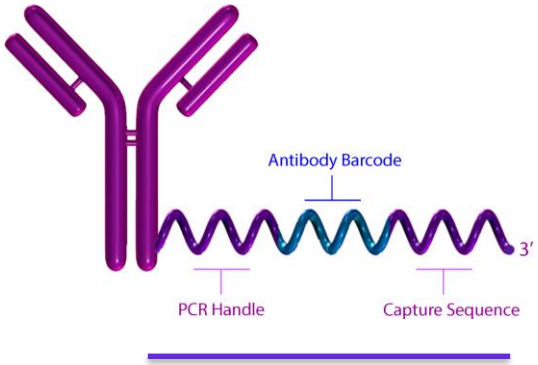
LEGENDScreen

Human: 371 Abs (4 plates)

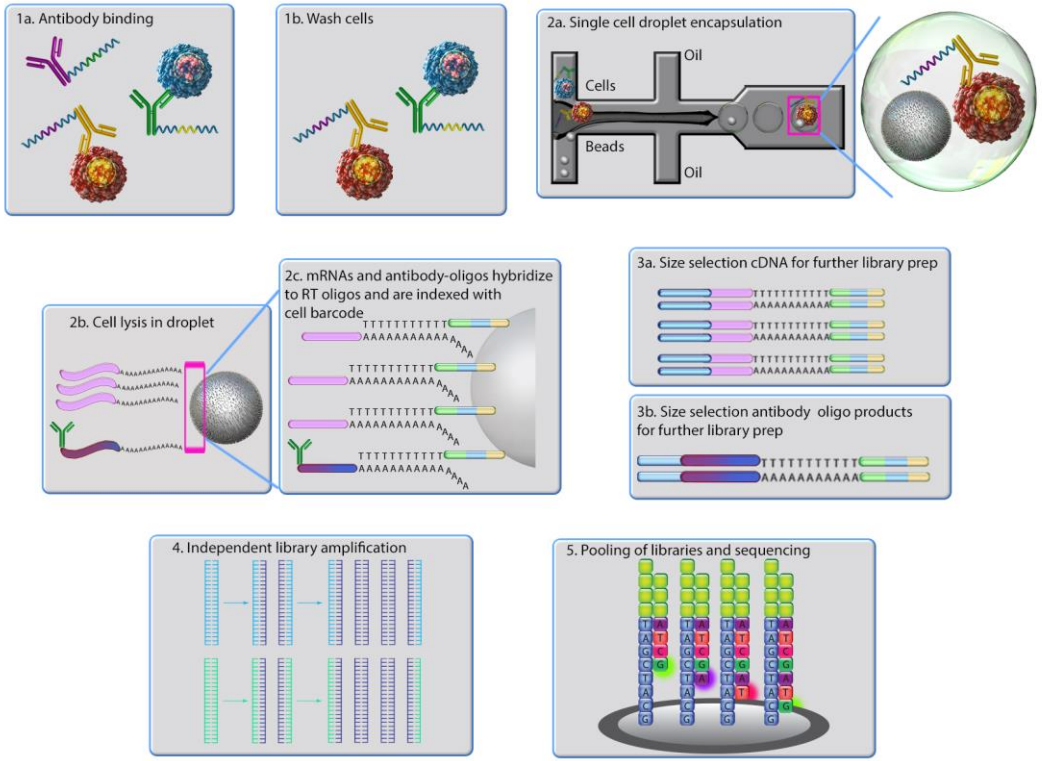
Mouse: 266 Abs (3 plates)

BioLegend®

TotalSeq™ | Overview of the CITE-seq workflow



Antibody Derived Tag
[ADT]



Two TotalSeq™ Reagent Applications:

1) **CITE-seq**
2) **Cell Hashing**

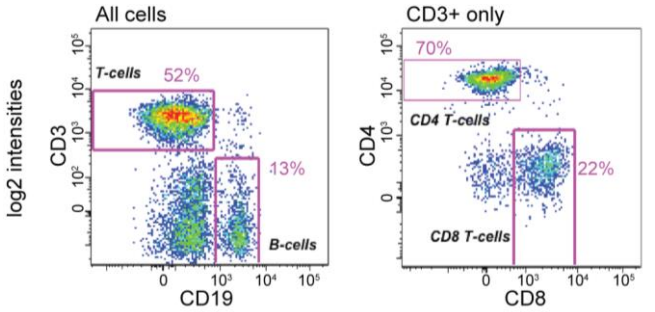
BioLegend®

Complete protocol online @ https://www.biolegend.com/totalseq/

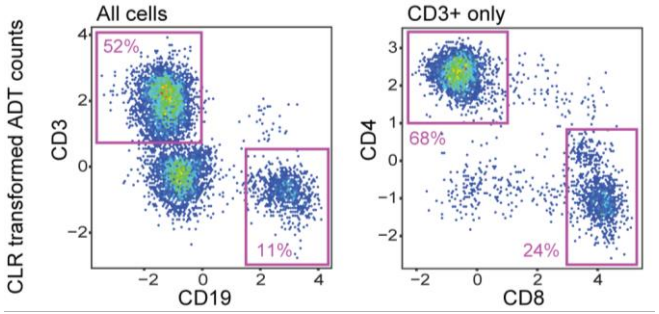
TotalSeq™ | Immunophenotyping: Flow Cytometry Staining Patterns are Reproduced via TotalSeq™

Comparable proportions

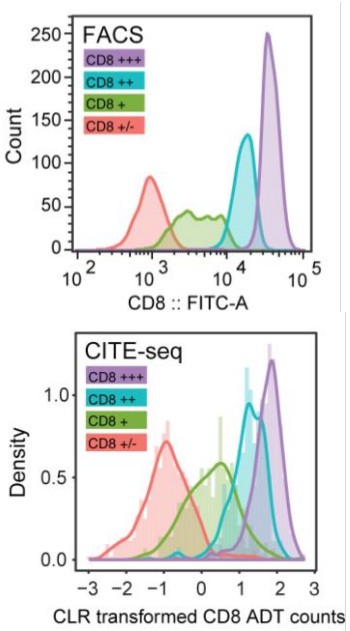
Flow



CITE-seq



Comparable dynamic range

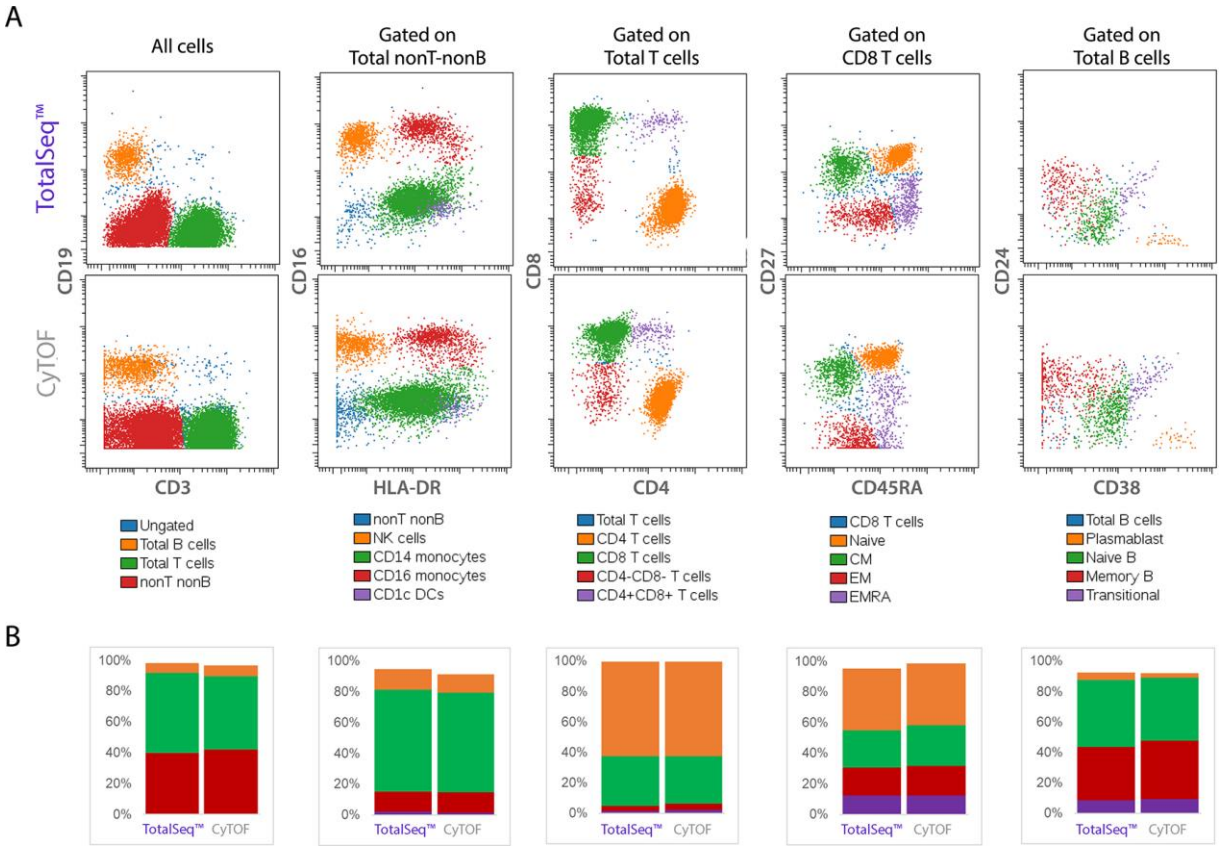


Marlon Stoeckius et al., 2017| **NATURE METHODS**

TotalSeq™ | Immunophenotyping: CyTOF Staining Patterns are Reproduced via TotalSeq™

-Single sample, split in 2

-Stained w/ same clones
- -TotalSeq™-A
- -CyTOF



Data Provided by:
Adeeb H Rahman, PhD

Mount Sinai

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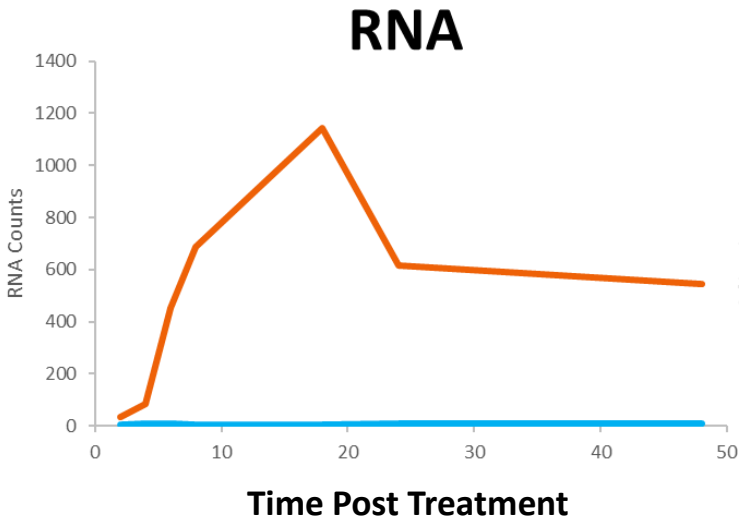
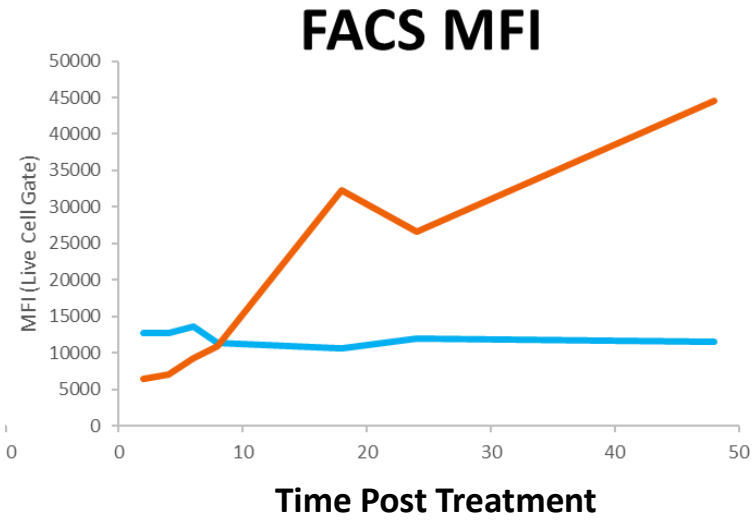
RNA and Protein content do not always correlate

PD-L1

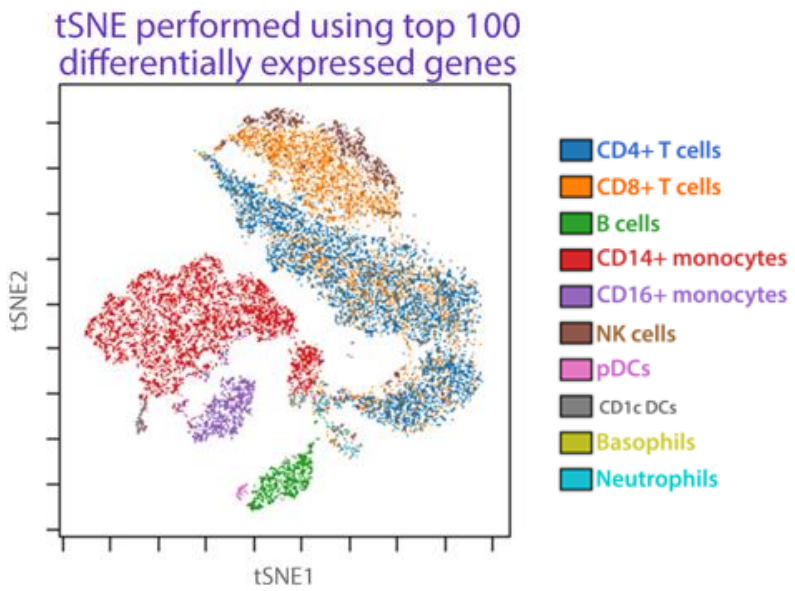


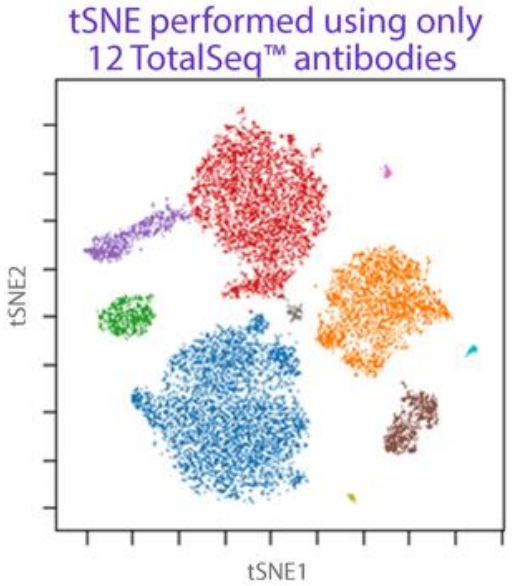


PMA treated cells

Untreated cells

TotalSeq™ | Enhanced Immunophenotyping via Single Cell Sequencing





Enhanced cell type identification compared to RNA

BioLegend®

See similar examples with TotalSeq™-B and TotalSeq™-C data @ https://www.10xgenomics.com/cellebrate-webinars/

10x GENOMICS

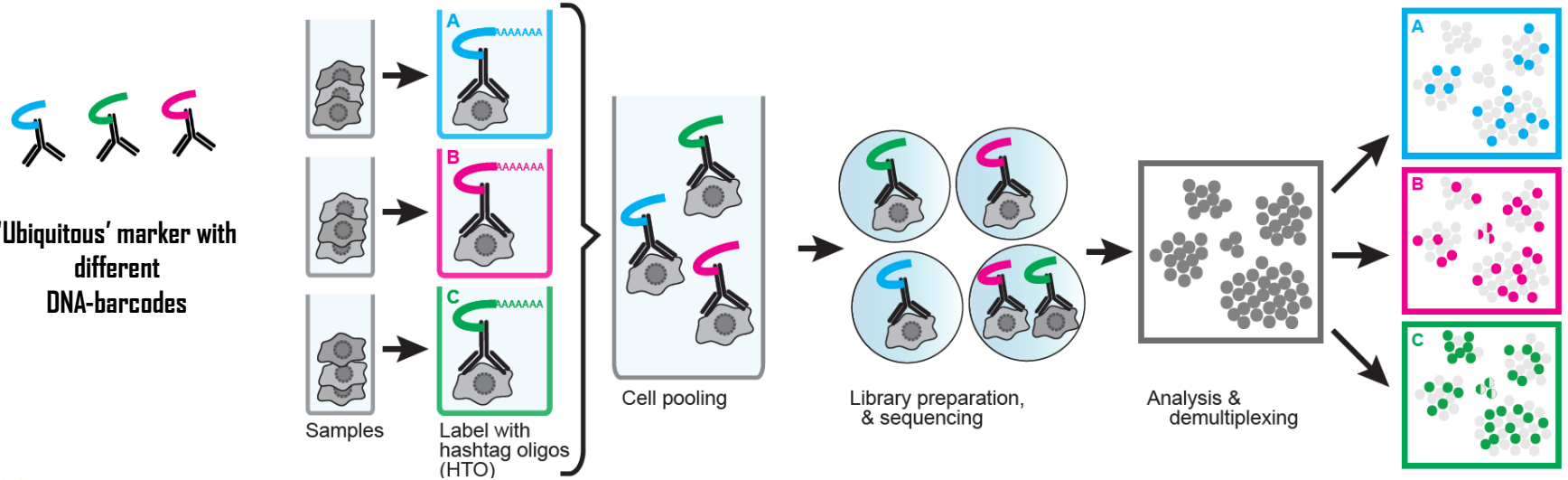
Data Provided by:
Adeeb H Rahman, PhD

Mount Sinai

TotalSeq™ | Cell hashing for multiplexing and doublets exclusion

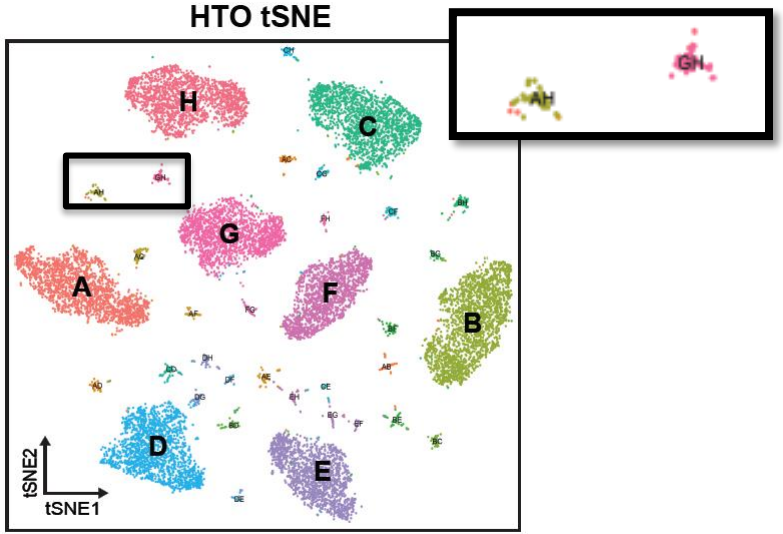
Challenges in scRNA-Seq

- Identification of doublets
- Data normalization when samples are analysed from different runs
- Costs per cell



Marlon Stoeckius et al., 2017| **BioRxiv**

TotalSeq™ | Cell hashing for multiplexing and doublet exclusion

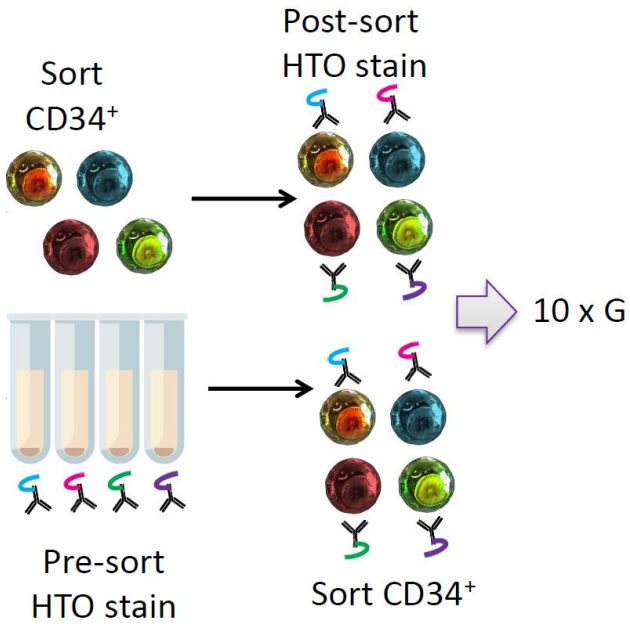


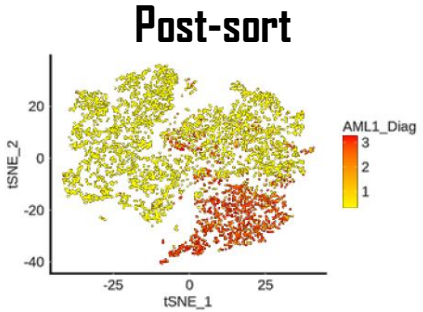
BioLegend provides hashtags (HTO)

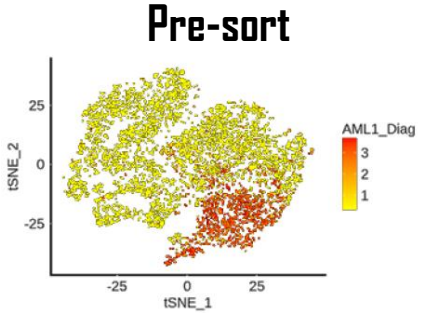
- ✓ **Human:** CD298 and β2 microglobulin
- ✓ **Mouse:** CD45 and H-2/MHC I

Marlon Stoeckius et al., 2017| **BioRxiv**

TotalSeq™ | Cell sorting and TotalSeq™ staining







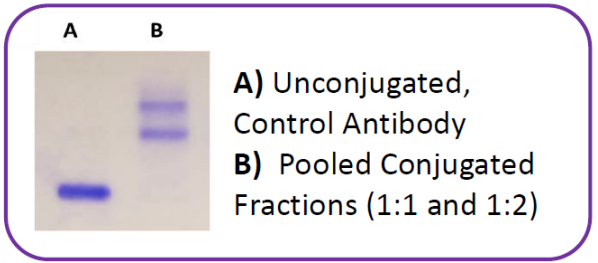
FACS sorting does not affect TotalSeq™ antibody staining

John M. Ashton PhD., MBA. James P. Wilmot
Cancer Institute, University of Rochester

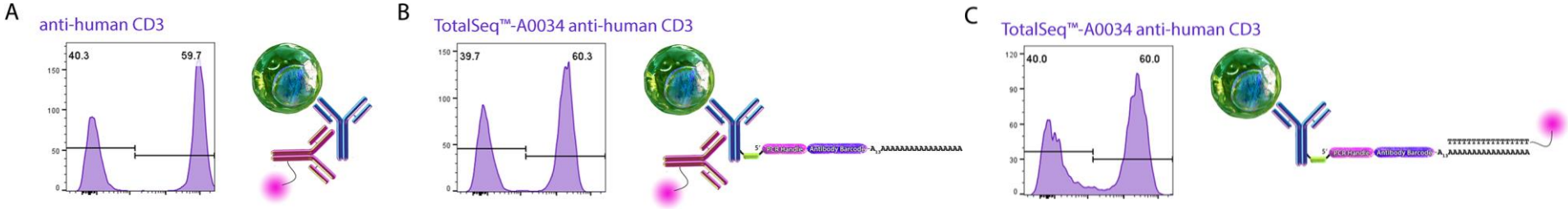
TotalSeq™ | In-house validation of TotalSeq™ Antibodies

✓ Conjugation & Purification

1.5 oligo/Ab
no unconjugated Ab or unbound Oligo
Sequencing of Oligomer



✓ Functional testing

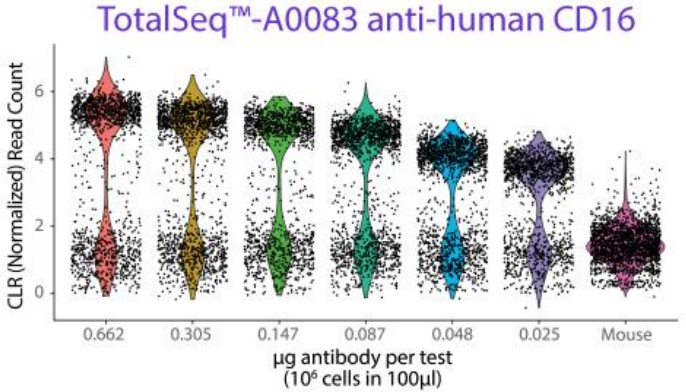


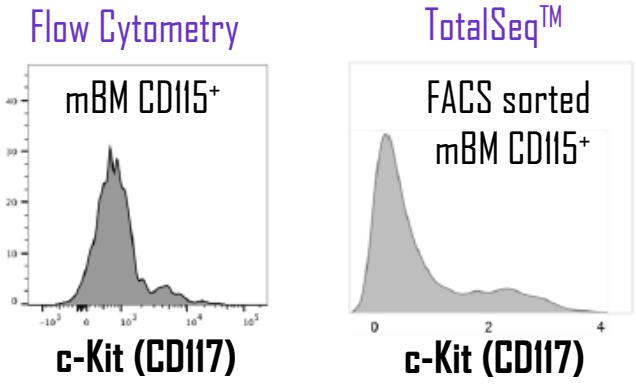
✓ **Titration data** in the future available for each antibody

BioLegend®

TotalSeq™ | Antibody Titration and panel optimization

- ✓ Use your flow cytometry expertise or contact BioLegend for support
- ✓ Titration data for each antibody coming soon
- ✓ 0.5 µg per $1x10^6$ cells is a good starting point
- ✓ Panel design and optimization
- ✓ Pre-defined panels coming soon





Anja Wolf, Cedars-Sinai Medical Center, USA

TotalSeq™ | Multiples Platforms and Applications Compatibility

		Now Available		Coming soon / custom
		TotalSeq™ -A	**TotalSeq™ -B**	**TotalSeq™ -C**
Applications	**Gene Expression**	Yes (3‘)	Yes (3‘)	Yes (5‘)
	Compatibility	Any platform employing the **poly-A tail capture**, apply **CITE-seq protocol**	**10X** Feature barcoding technology	**10X** Feature barcoding technology
		10X 3'**V2** and 3'**V3** kits	10X 3'**V3** kit	10X 5' kit
	VDJ Immune profiling	No*	No*	**Yes**
BioLegend 15nt Barcode	**Barcode#**	A0072	B0072	C0072
	Specificity	**Human CD4**		
	Clone	**RPA-T4**		
	Barcode Sequence	**TGTTCCCGCTCAACT**		
Sample Multiplexing		Yes		
Capture sequence		PolyA Capture	Specific Capture sequence	Specific Capture sequence
NGS Compatibility		**Illumina instruments**		

BioLegend®

10x GENOMICS®

TotalSeq™ | Summary

TotalSeq™ Reagents for **CITE-Seq** (**A**) and **10X** FB Technology (**B&C**):

- ✓ Simultaneous analysis of proteins & RNA
- ✓ Multiplexed protein quantification
- ✓ Enhanced cell type identification compared to scRNA-Seq alone

TotalSeq™ Reagents for **Cell hashing**:

- ✓ Sample multiplexing
- ✓ Doublet exclusion
- ✓ Data normalization
- ✓ Costs per cell

SUPPORT:

- ✓ Antibody usage protocol (titration and flow expertise)
- ✓ Panel design
- ✓ Pre-mixed panels
- ✓ **Data analysis solutions**

BioLegend®

What will you see with TotalSeq™??

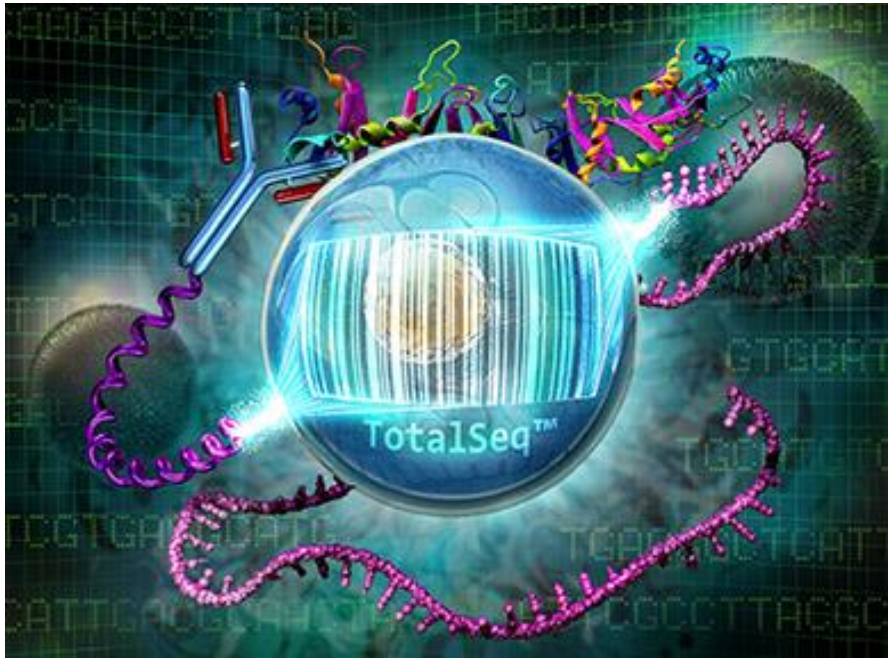
Thanks for your attention!

Questions?

Contact us!

www.biolegend.com/totalseq

jbguillerme@biolegend.com



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