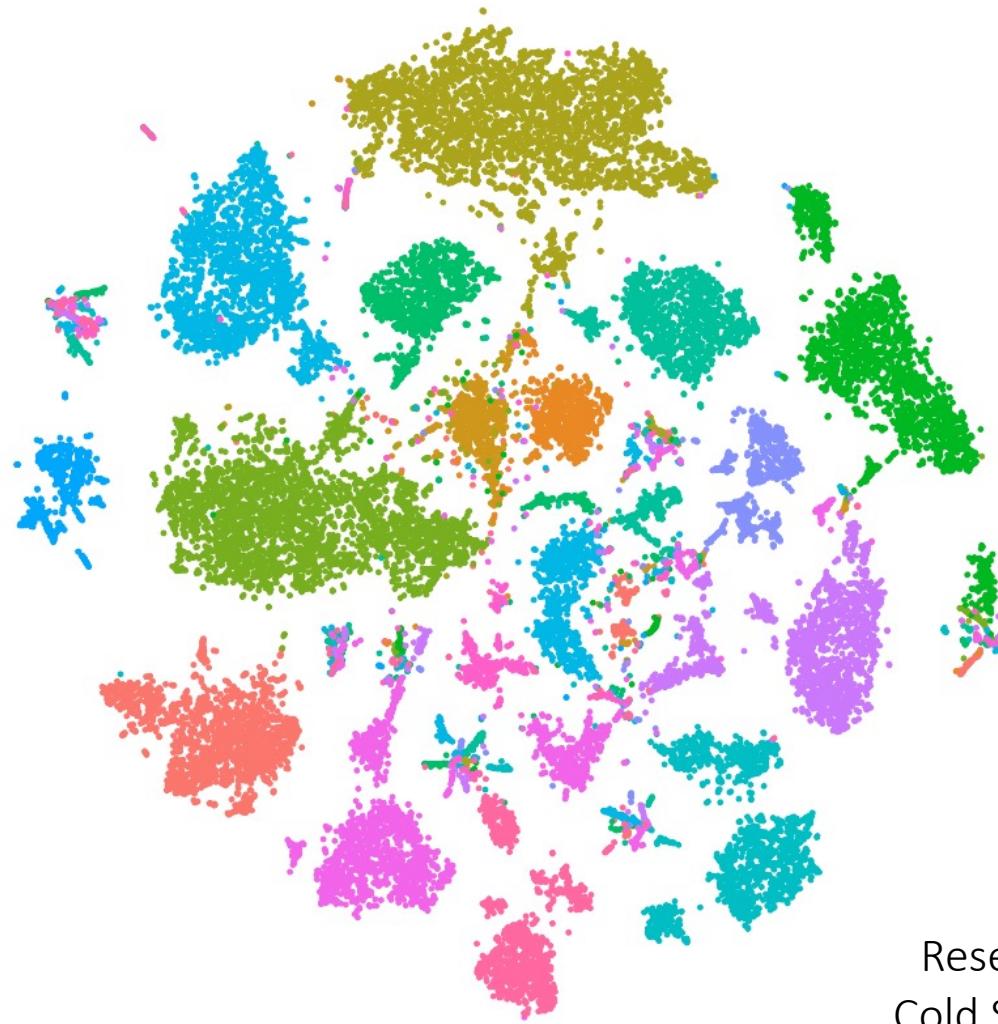


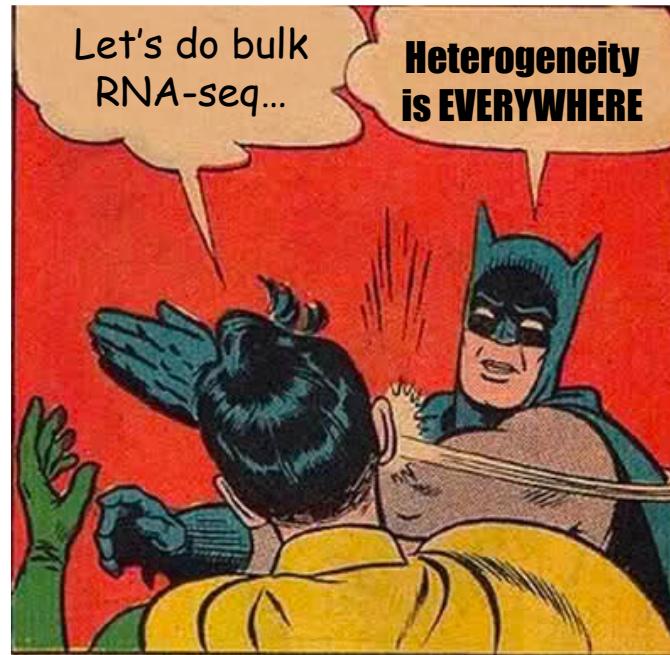
Single Cell Sequencing

CSHL Course: Advanced Sequencing Technologies & Applications

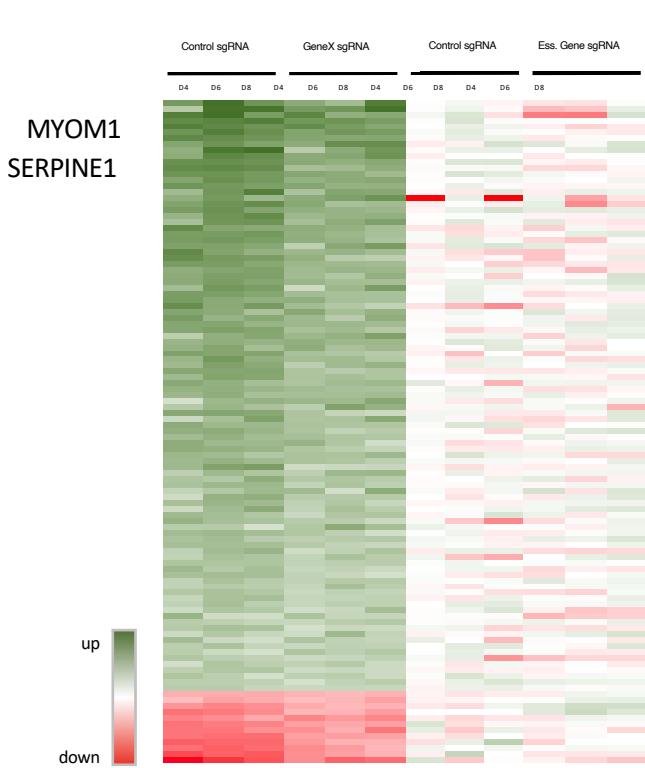


Jon Preall
Research Associate Professor
Cold Spring Harbor Laboratory

Why Sequence Single Cells?



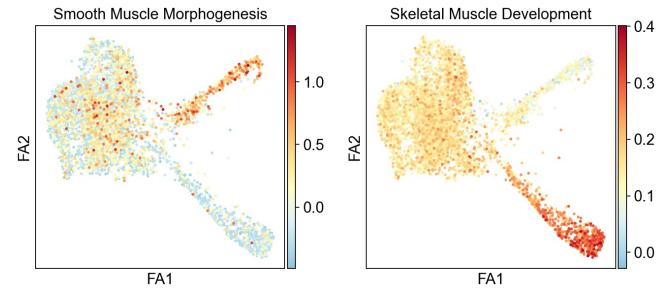
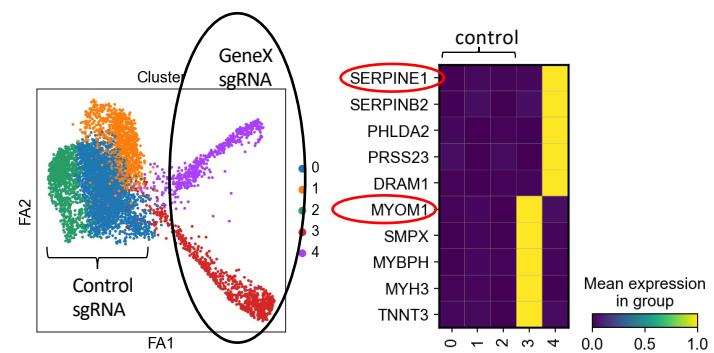
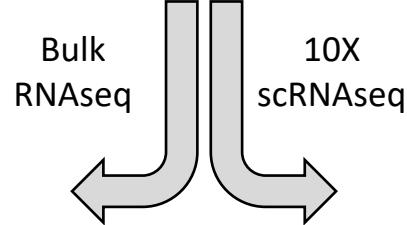
The Importance of Single Cell Resolution



Human
Rhabdomyosarcoma
cell line



CRISPR KO
Driver gene

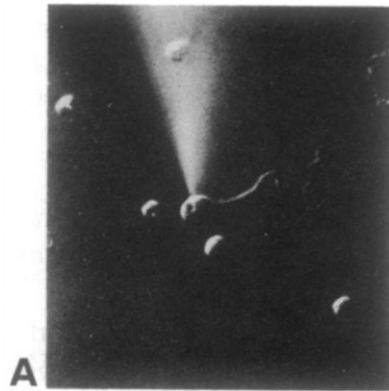


Analysis of gene expression in single live neurons

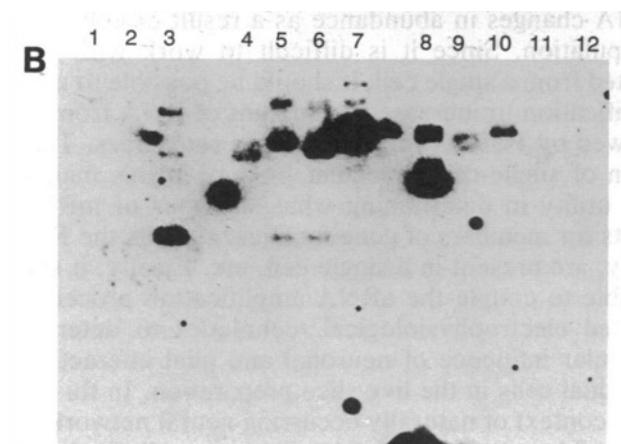
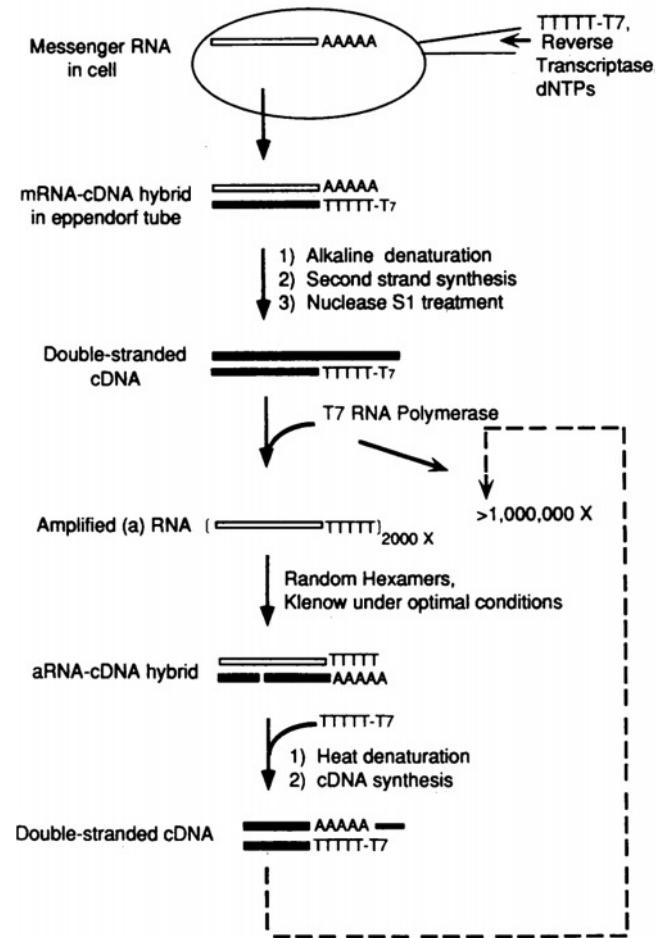
(amplified, antisense RNA/expression profile/mRNA complexity/pyramidal cell)

JAMES EBERWINE*†‡, HERMES YEH§, KEVIN MIYASHIRO*, YANXIANG CAO*, SURESH NAIR*,
RICHARD FINNELL*¶, MARTHA ZETTEL§, AND PAUL COLEMAN§

Departments of *Pharmacology and †Psychiatry, University of Pennsylvania Medical School, Philadelphia, PA 19104; and Department of §Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642



Microinjection of cDNA synthesis reagents directly into single neurons



Southern Blot
Plasmid standards
containing gene of interest
Probed with aRNA

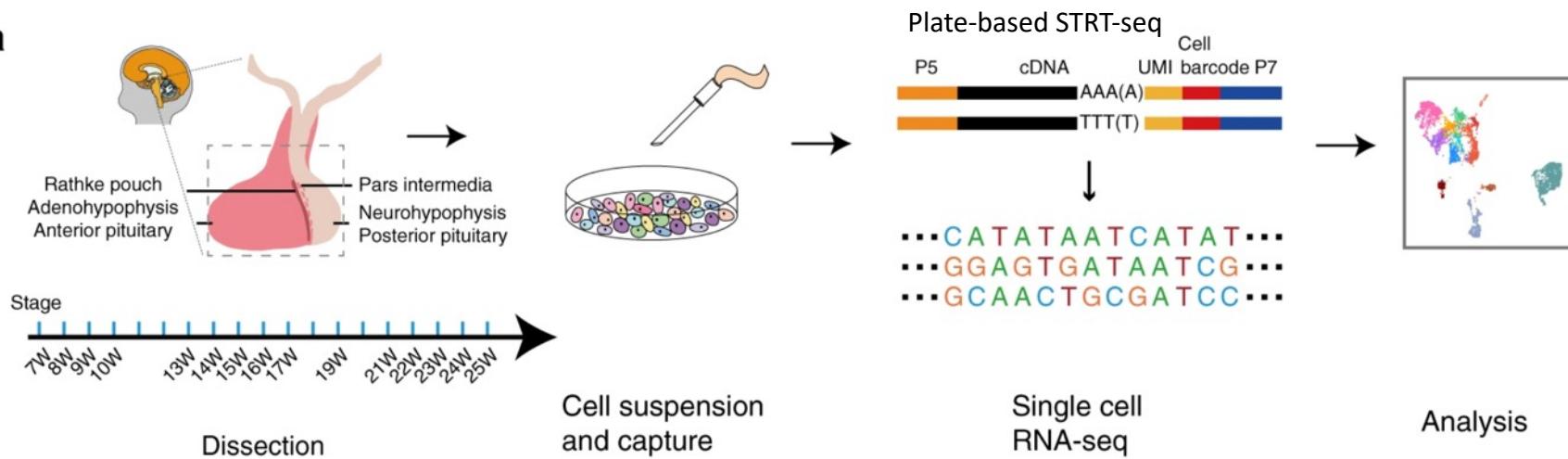
1992

Single-cell transcriptomics identifies divergent developmental lineage trajectories during human pituitary development

Shu Zhang, Yueli Cui, Xinyi Ma, Jun Yong, Liying Yan, Ming Yang, Jie Ren, Fuchou Tang, Lu Wen✉ & Jie Qiao✉

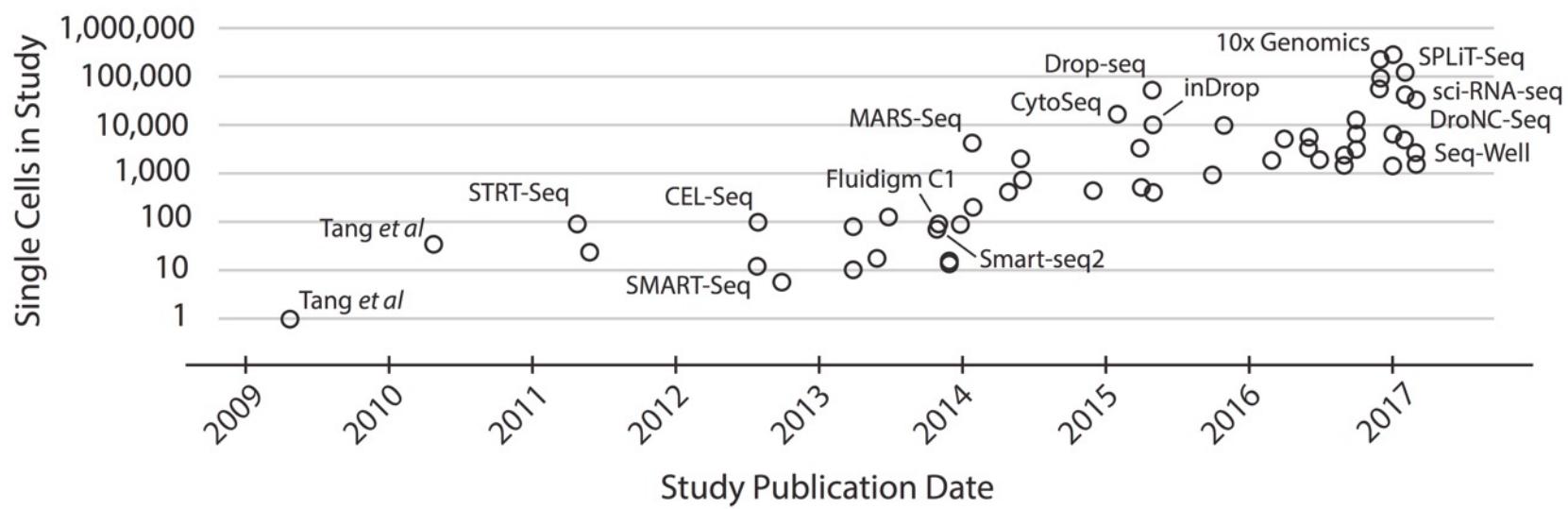
Nature Communications 11, Article number: 5275 (2020) | Cite this article

a



4,113 mouth-pipetted cells!

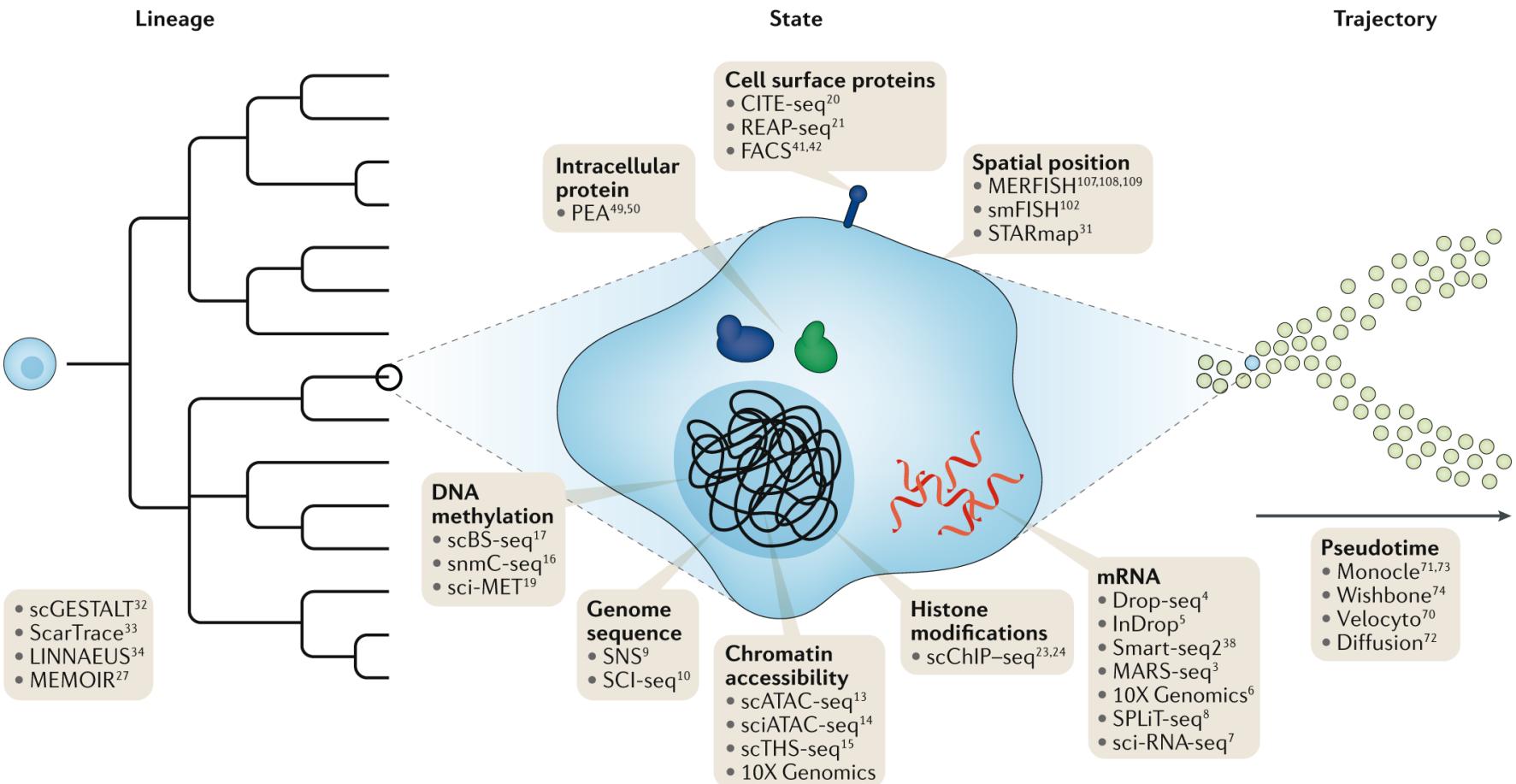
The Rapid Rise of Single Cell Biology



~10-fold increase in # of cells profiled every other year

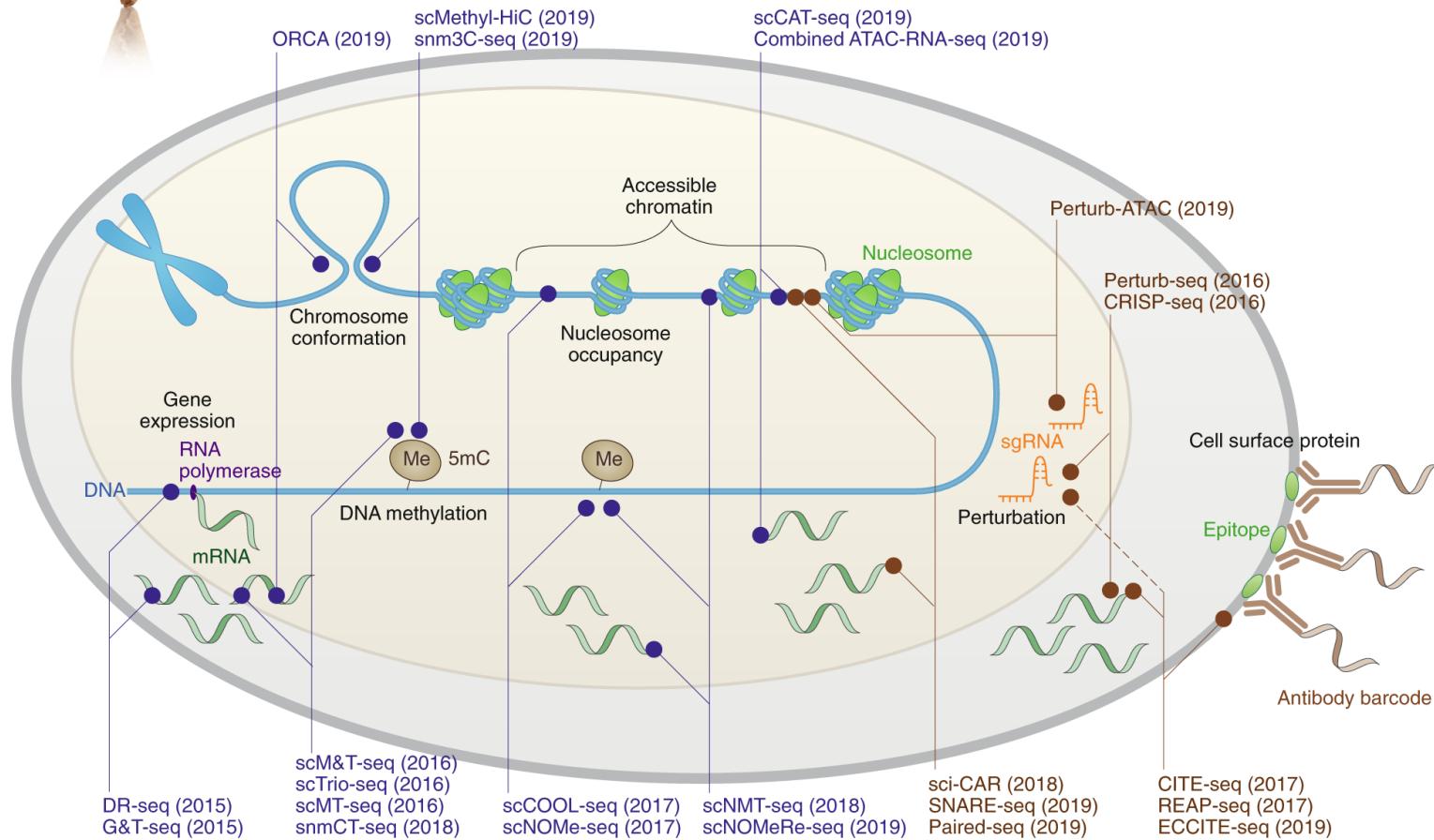


Many Flavors of Single cell 'Omics

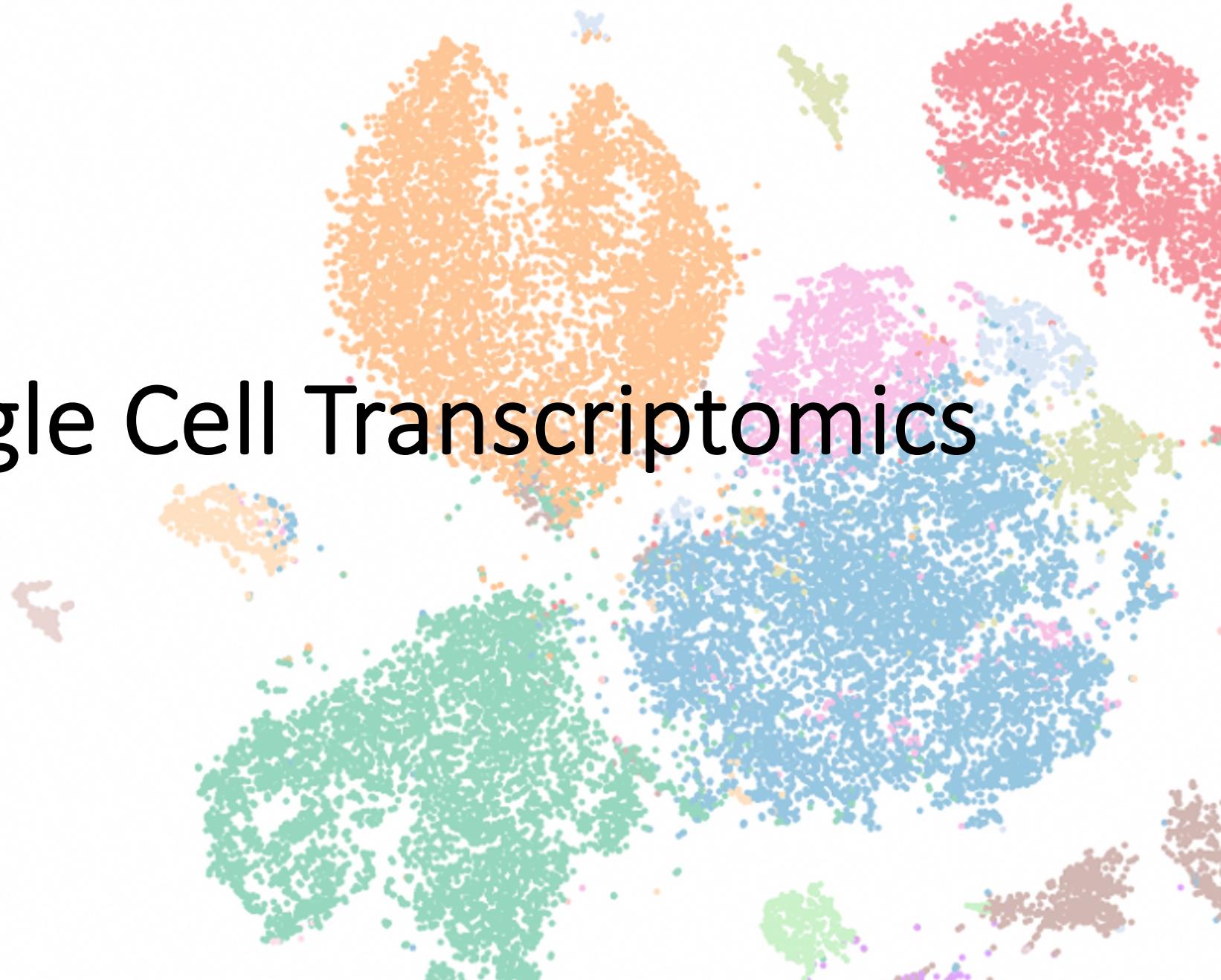




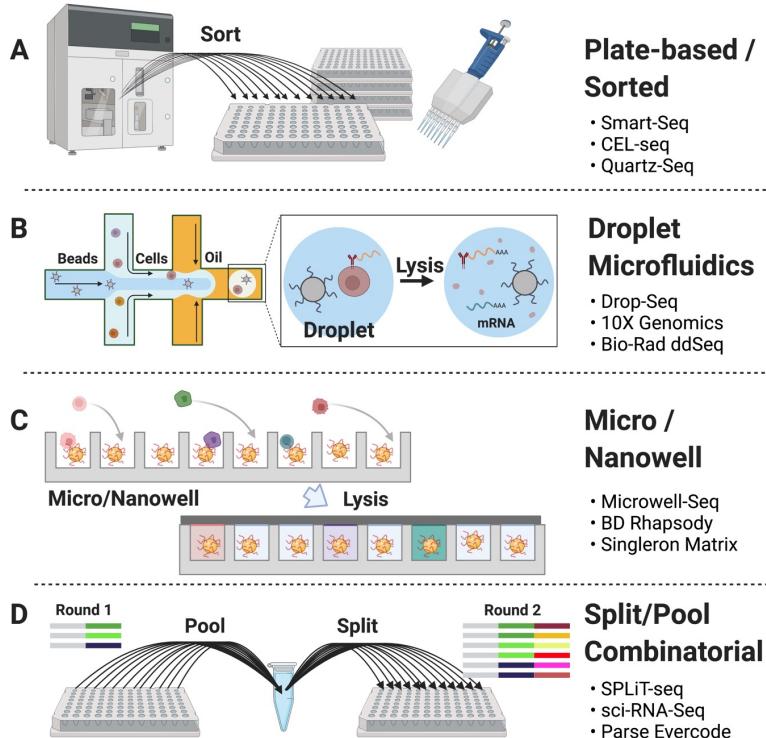
Many Flavors of Multi- 'Omics



Single Cell Transcriptomics

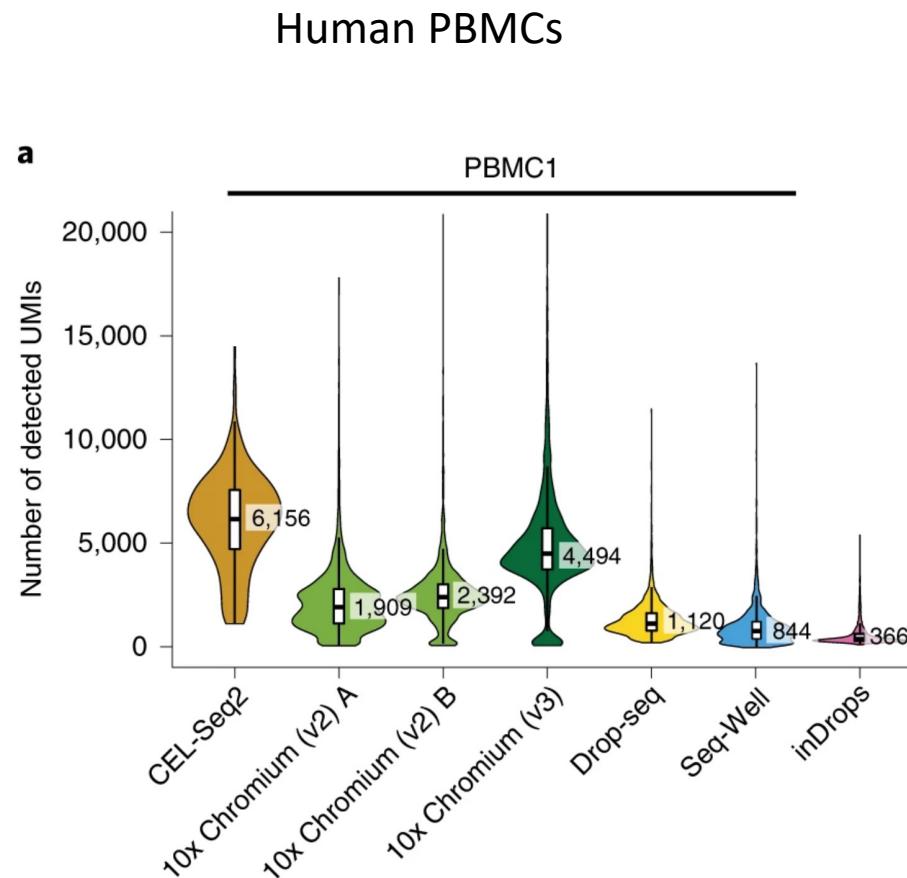


scRNAseq Platforms



Throughput (cost/labor per cell)	Flexibility	Sensitivity / Max Depth	Protocol Simplicity / Accessibility	Adoption / Available public datasets
+	+++	+++	++	++
++	+	++	+++	+++
++	++	++	+	+
+++	++	++	++	++

Systematic comparative analysis of single cell RNA-sequencing methods



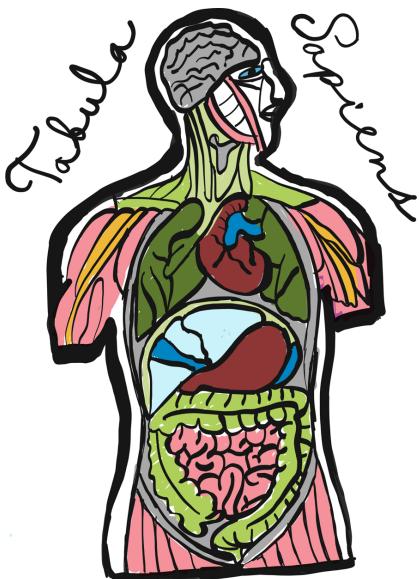
10X Genomics: the *lingua franca* of the single-cell age



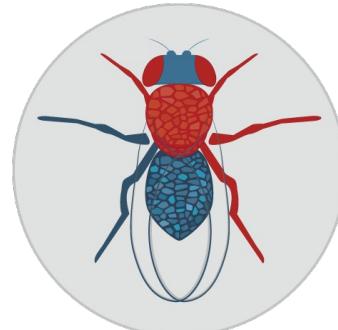
- Easy
- Robust
- Expensive.



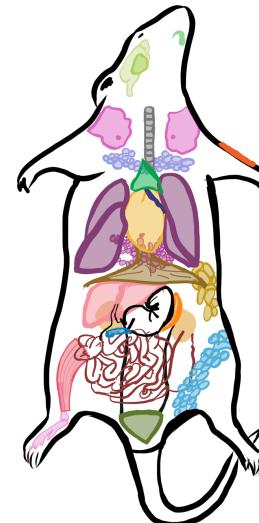
Allen Brain Map



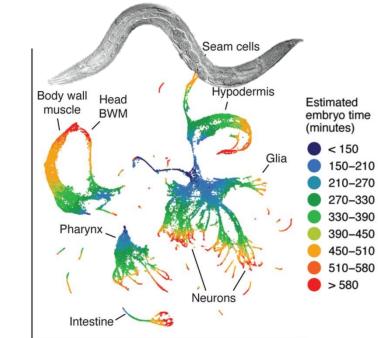
Fly Cell Atlas



Tabula Muris



C elegans



Packer et al (2019) Science

10X Genomics Platform

Chromium Controller Chromium X / iX



Up to ~80,000 cells / run

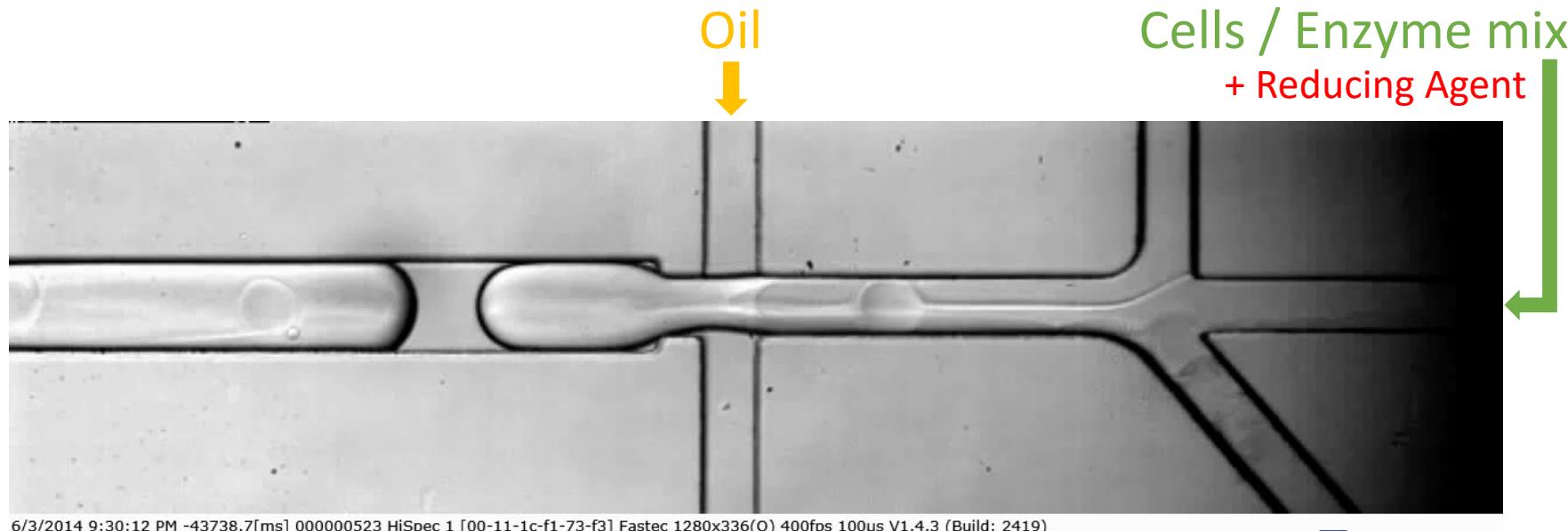


~up to 1M / run*
*using tricks

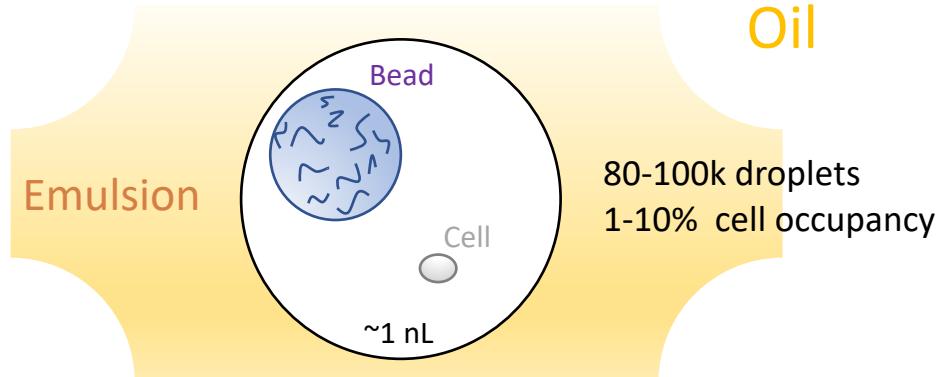
- RNA-seq
 - V(D)J profiling
 - ATAC-seq
 - RNA + ATAC
 - Surface Proteins
 - CRISPR guides
- Fixed RNA profiling

- Most similar to InDrops
 - Hydrogel bead
 - Pseudo-single Poisson Loading
- ~50% of input cells generate usable data
- Partition cells up to 30um in diameter
- ~1% doublet rate, scales linearly w/cell #

Under the Hood

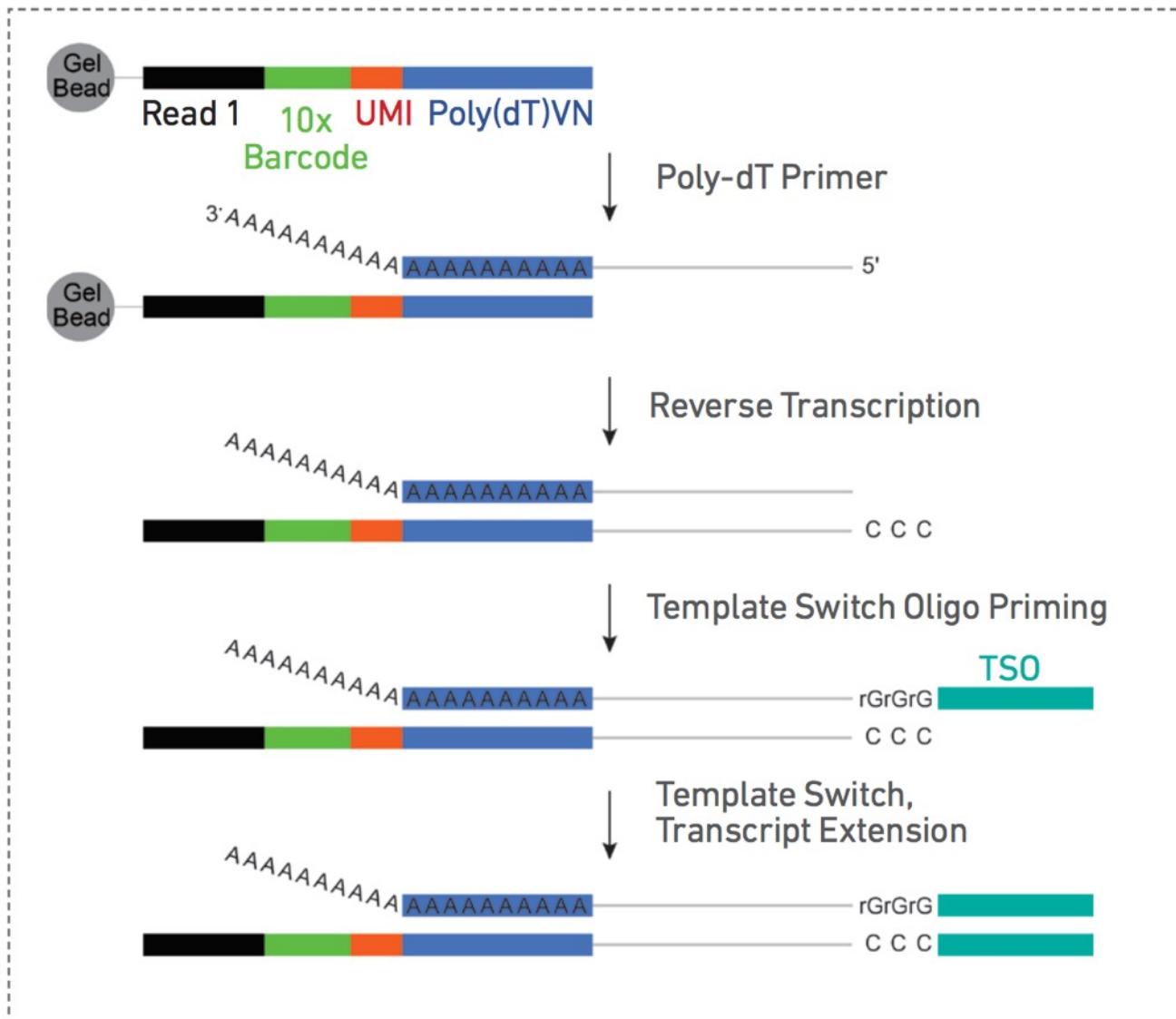


6/3/2014 9:30:12 PM -43738.7[ms] 000000523 HiSpec 1 [00-11-1c-f1-73-f3] Fastec 1280x336(Q) 400fps 100µs V1.4.3 (Build: 2419)

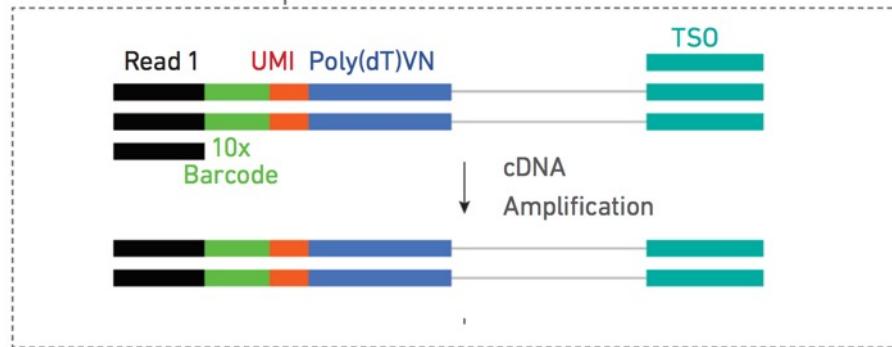


- -S-S- crosslinked hydrogel
- 10^9 primer molecules per bead
- 1 barcode sequence per bead
- Pool of 3M total possible barcodes

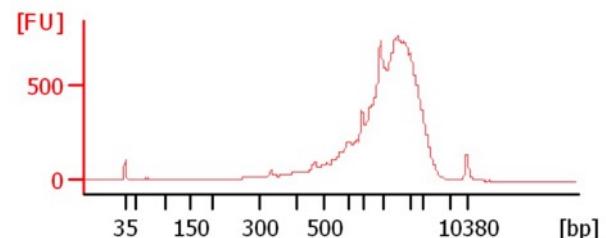
Single Cell 3' Chemistry Overview



Pooled cDNA amplification

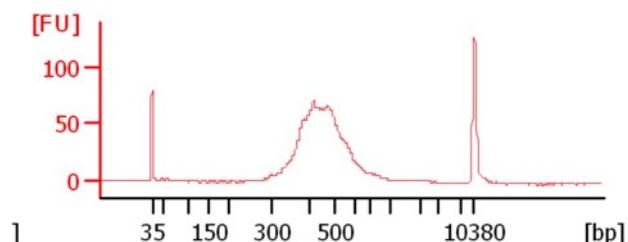
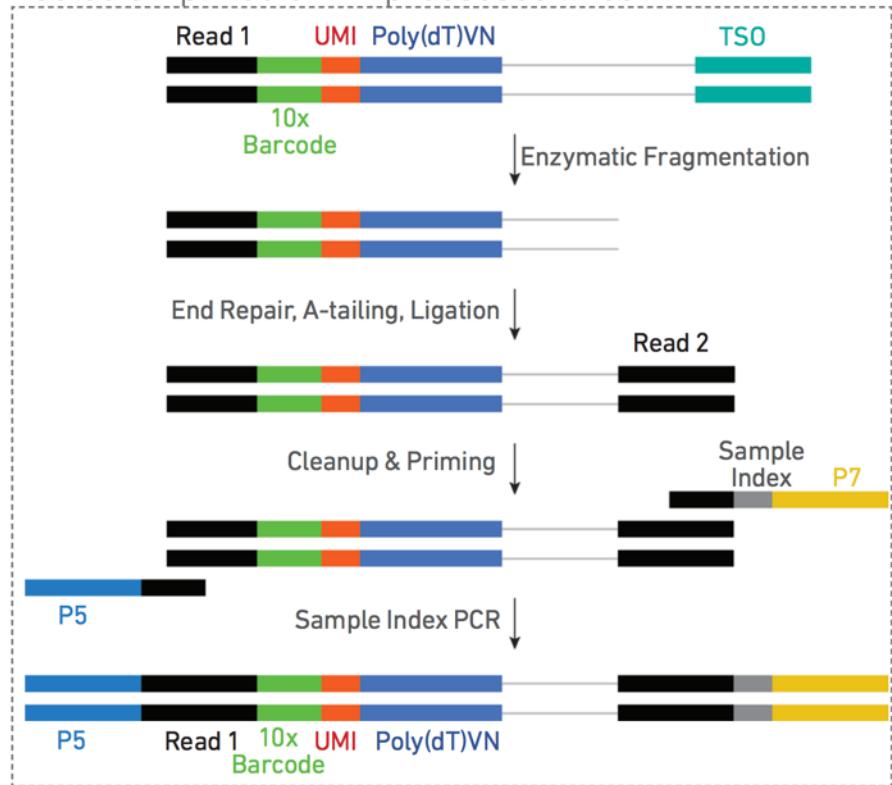


Bioanalyzer



Amplified cDNA

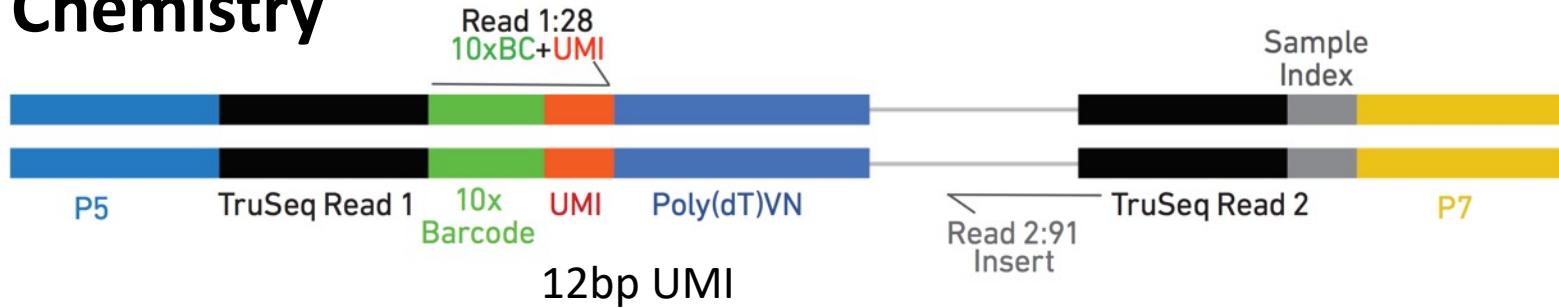
Pooled amplified cDNA processed in bulk



Final Library

Anatomy of a 10X 3'-Single Cell Amplicon

V3 Chemistry



Unique Molecular Identifier (UMI)

Random ~8-10bp sequence incorporated during oligo synthesis

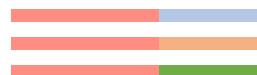
Cell barcode UMI

CCCCCCCCXXXXXXTTTTTTTVN
AAAAAAAAAAABN---IFNgamma---

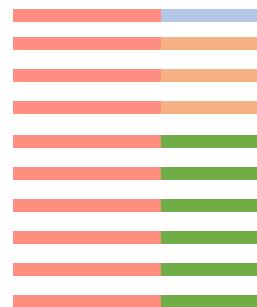
CCCCCCCCXXXXXXTTTTTTTVN
AAAAAAAAAAABN---IFNgamma---

CCCCCCCCXXXXXXTTTTTTTVN
AAAAAAAAAAABN---IFNgamma---

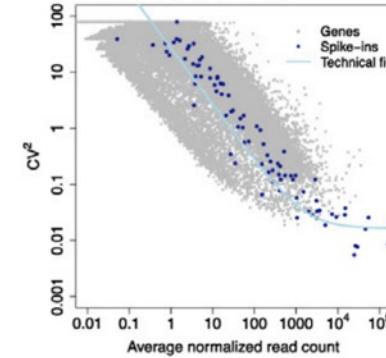
Before PCR



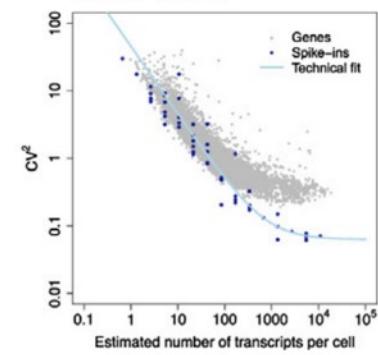
After PCR



without UMIs

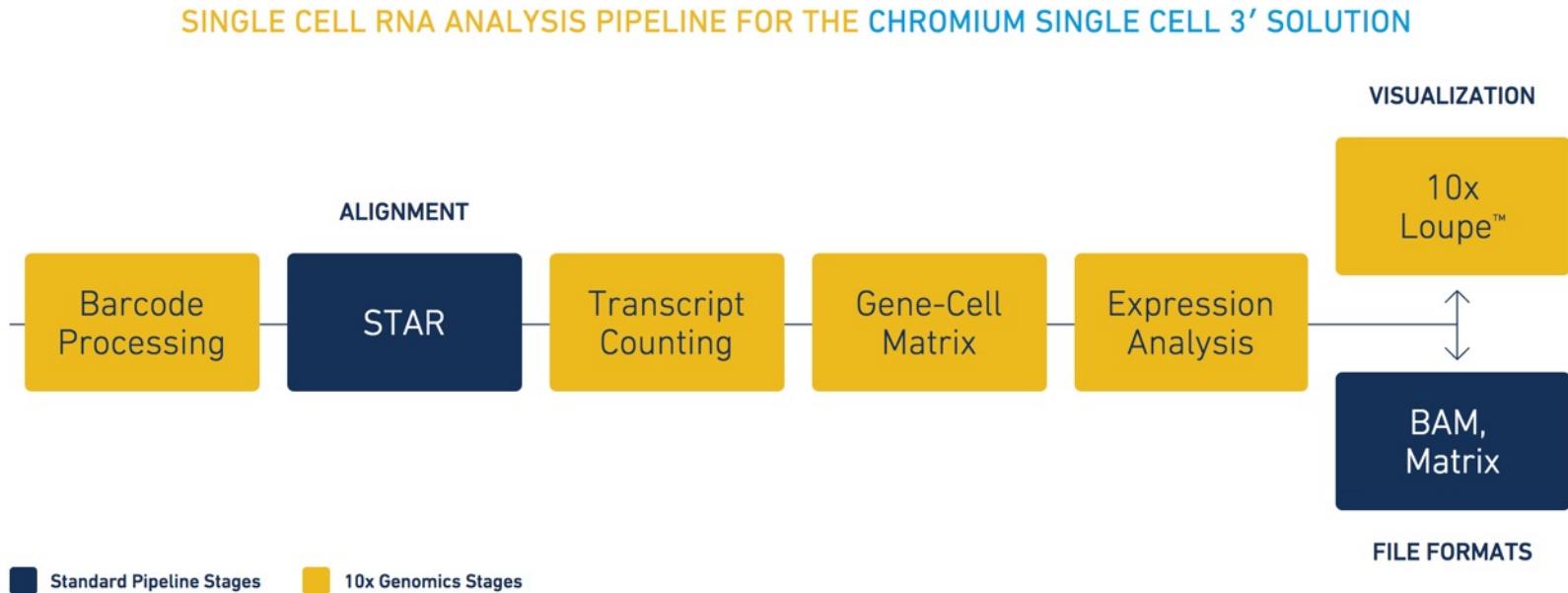


with UMIs



Mapping and Transcript Quantification

Cellranger Count pipeline: [10X Genomics support page](#)



Digital Gene Expression, Not Coverage

“Deep” Single Cell Libraries

Well-based, eg. SmartSeq
Fluidigm C1

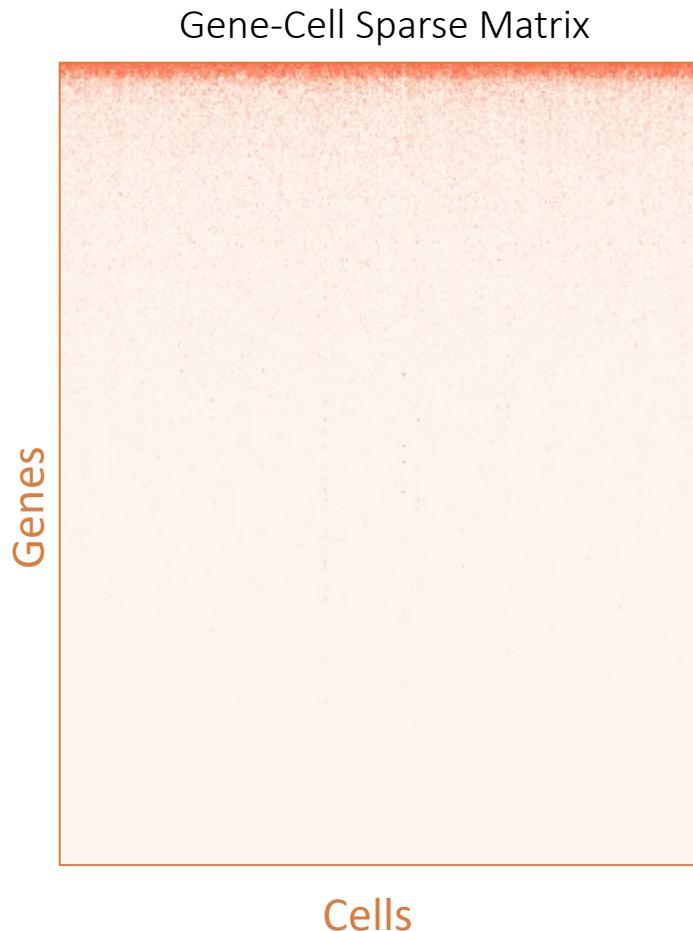


Droplet – Based DGE libraries

Drop-Seq
10X Genomics
Seq-Well



Sparse sampling of gene expression



Top	Gene Expression	US Wealth
1%	15%	35%
10%	55%	73%
20%	73%	86%

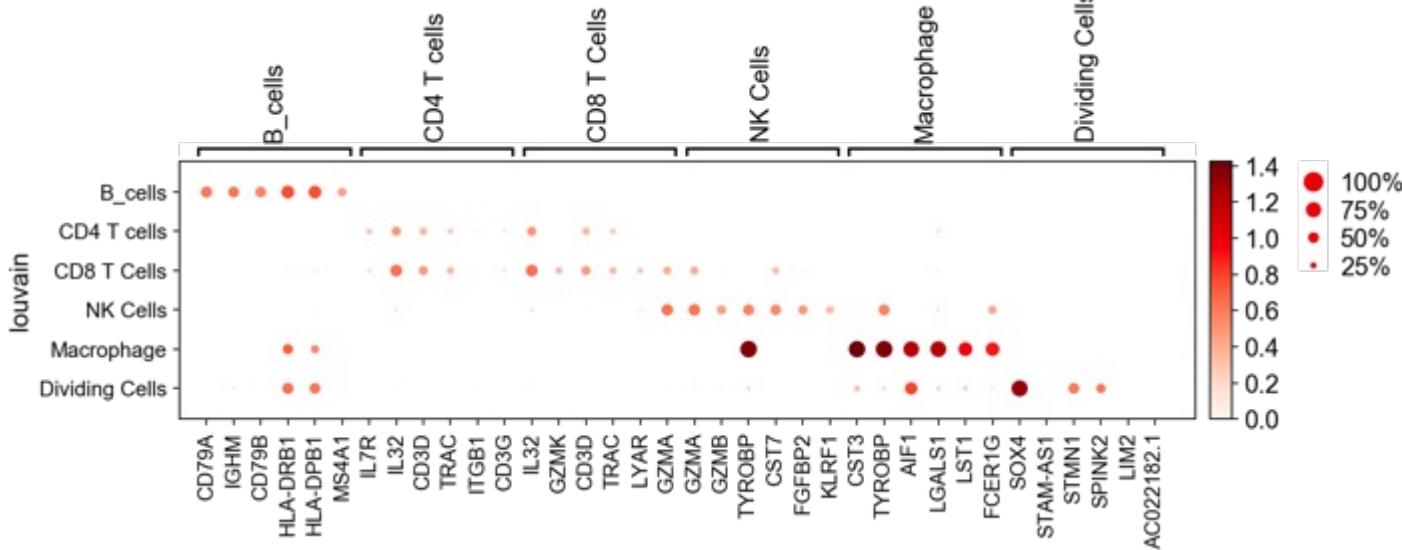
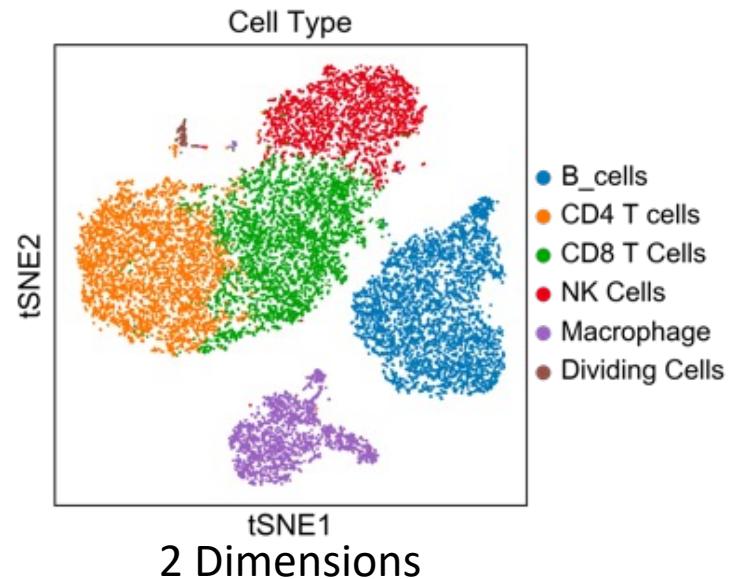
Basic scRNAseq pipeline

	Gene 1	Gene 2	Gene 3	Gene 4
Cell1	0	0	4	1
Cell2	0	1	0	12
Cell3	0	0	0	11
Cell4	5	0	0	2
Cell5	20	1	0	0

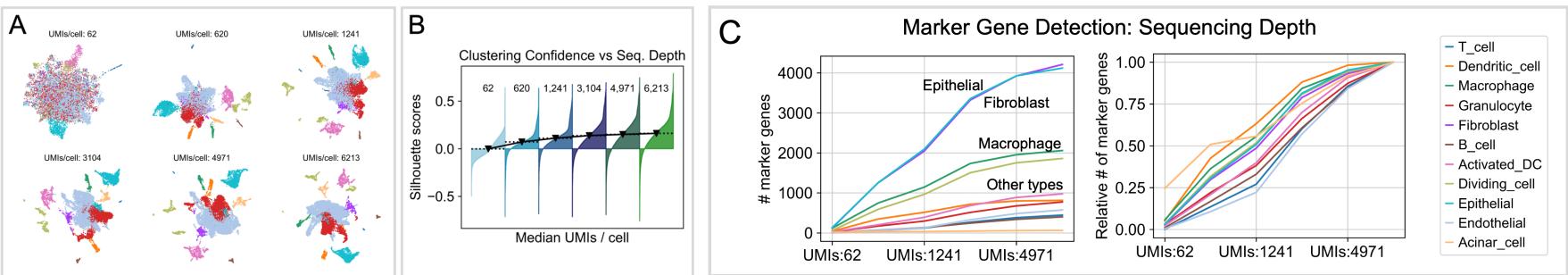
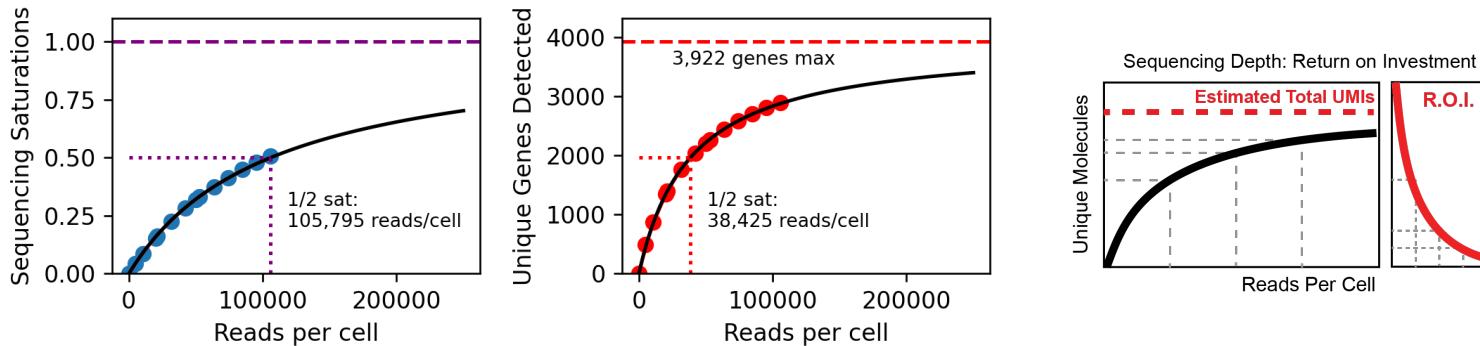
25,000+ Dimensions →

Dimensionality Reduction

Feature Selection
Normalization
PCA / NMF / ICA
tSNE / UMAP

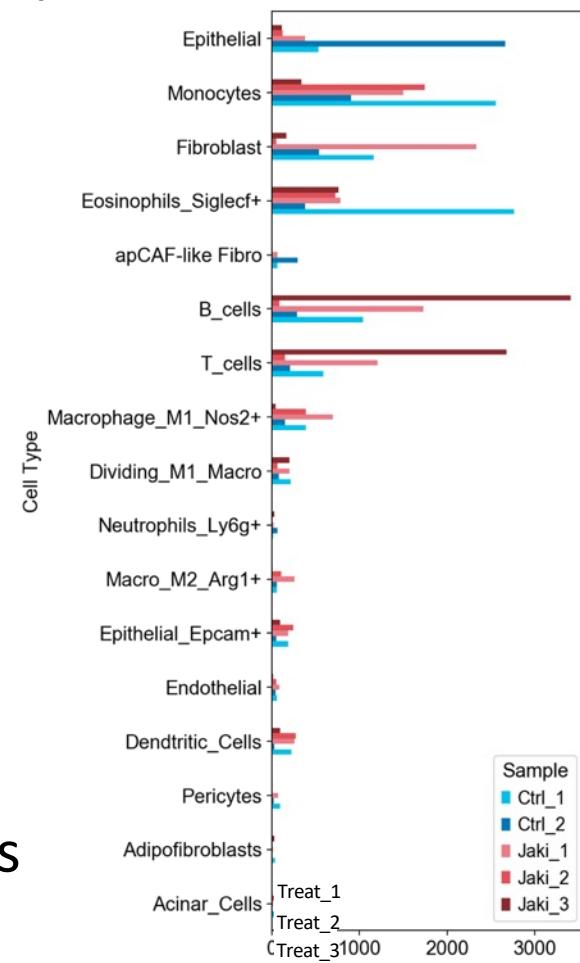
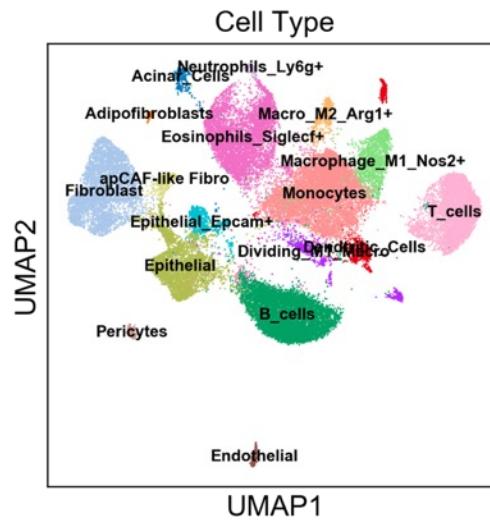
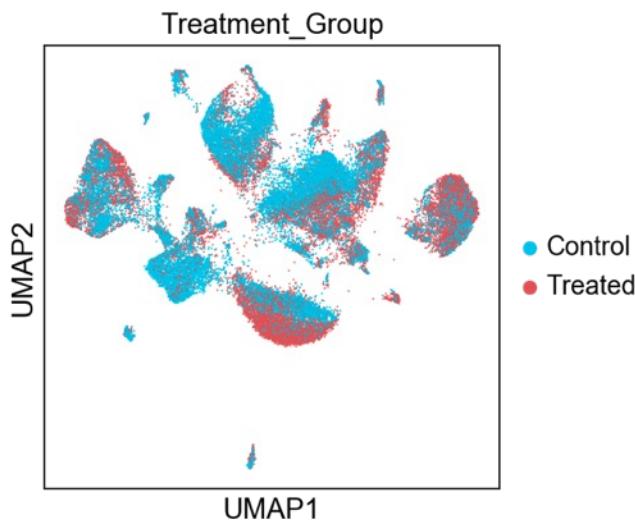


Sequencing Saturation – How Deeply to Sequence?



Regan and Preall, *Current Protocols* (2022)

scRNAseq is a poor cytometry tool



- Unreliable – highly sensitive to conditions
- Expensive
- Low throughput

... But if you must, at least use some statistics:

propeller: testing for differences in cell type proportions in single cell data

<https://www.biorxiv.org/content/10.1101/2021.11.28.470236v1.full>

scDC: single cell differential composition analysis

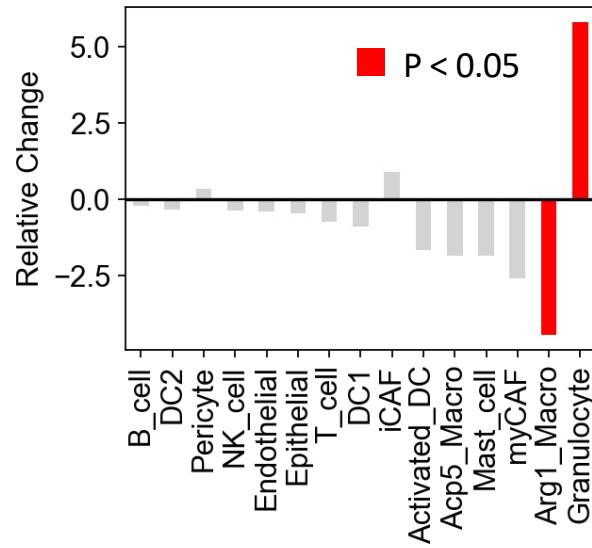
<https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-019-3211-9>

CTDS: Cell Type Diversity Statistic

<https://www.frontiersin.org/articles/10.3389/fgene.2022.855076/full>

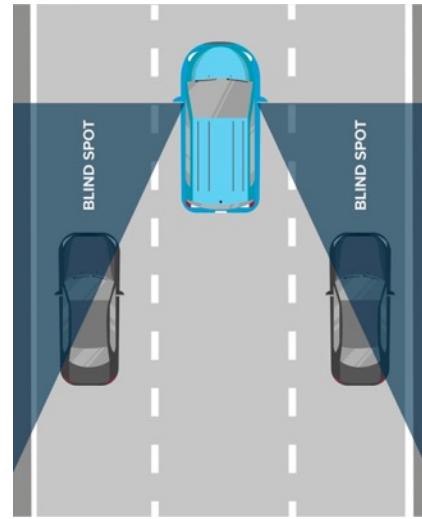
DA-seq: Detecting differentially abundant (DA) subpopulations

<https://www.pnas.org/doi/10.1073/pnas.2100293118>

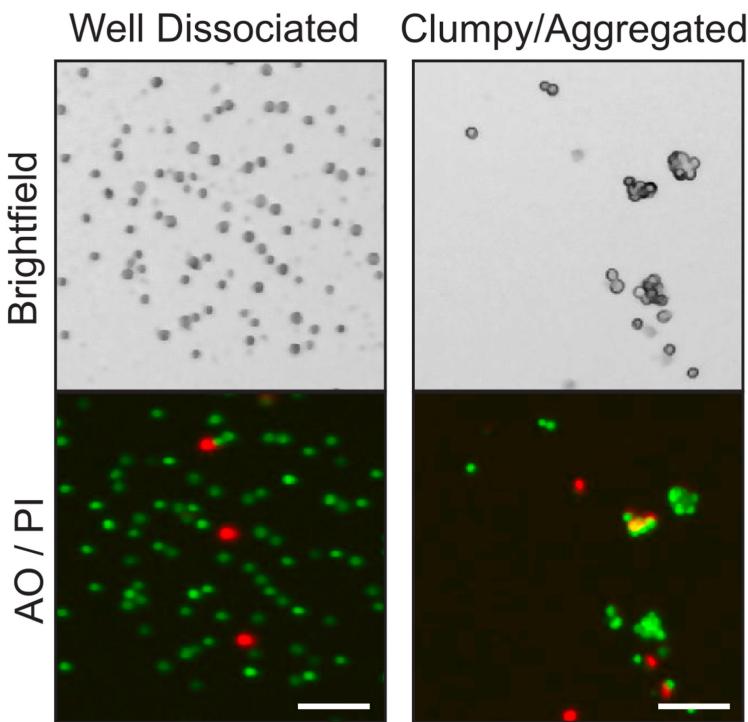
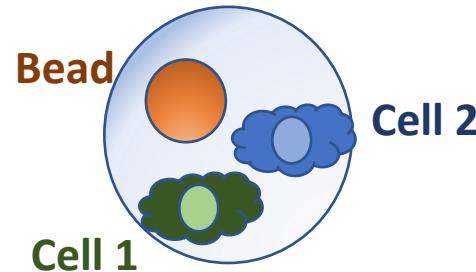
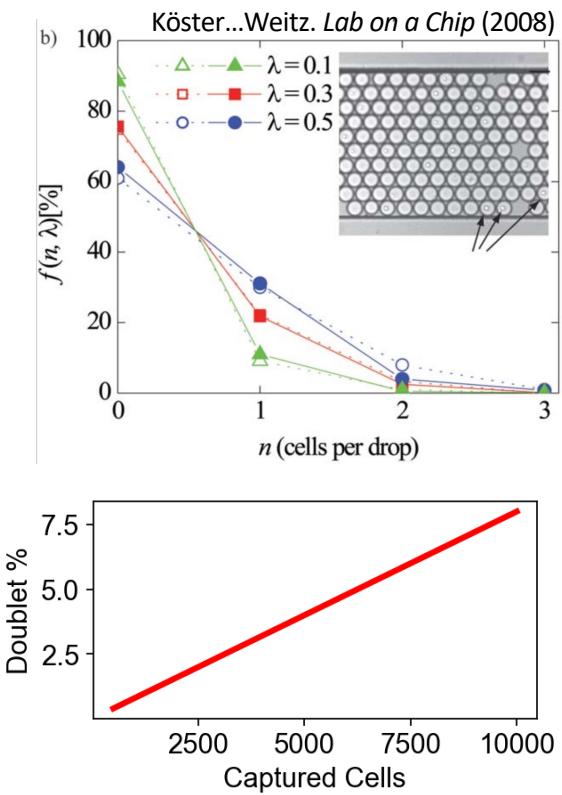


Blind Spots

- Some cell types might be missed
 - Low mRNA count – filtered from matrix
 - Early 10X Genomics Software (v2)
 - Defaulted to exclude lots of lymphocytes
 - Hard to dissociate from tissue
 - Fibroblasts
 - Cells might die quickly during prep
 - Stem cells
 - Fragile: (Acinar cells, Plasma cells)
 - High RNase / protease content (Acinar, Neutrophils)
 - Peripheral blood neutrophils especially!!!
 - Doublets / Multiplets



Doublets / Multiplets



Doublet Filtering

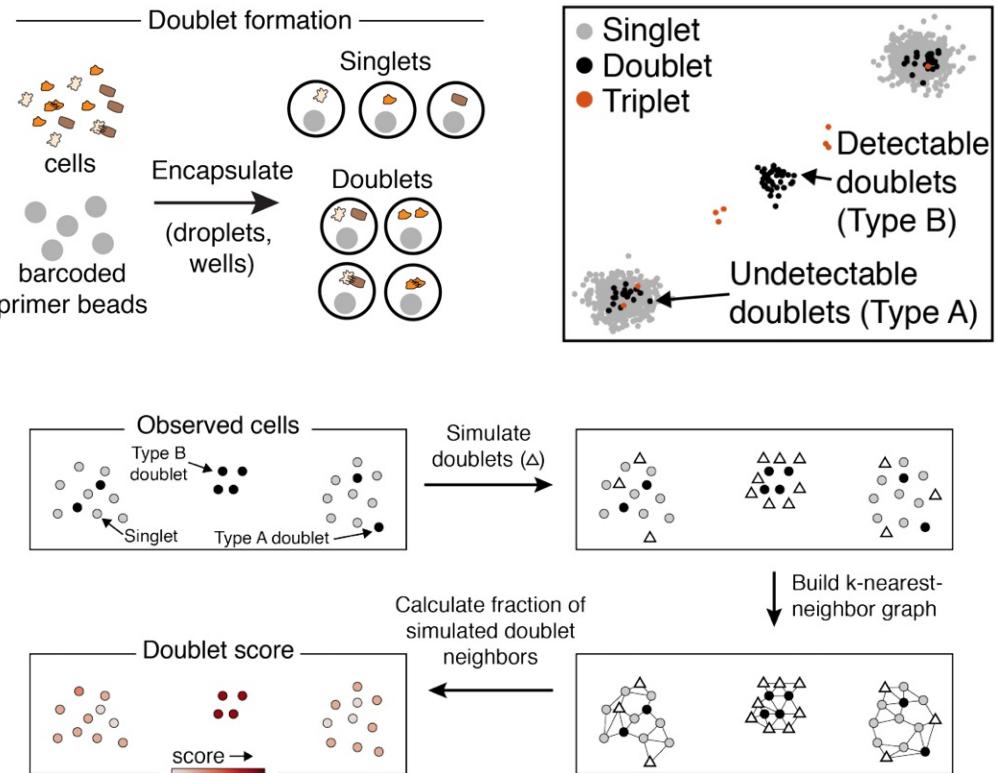
Scrublet

• [DoubletFinder](#) - [R] - Doublet detection in single-cell RNA sequencing data using artificial nearest neighbors. [BioRxiv](#)

• [DoubletDecon](#) - [R] - Cell-State Aware Removal of Single-Cell RNA-Seq Doublets. [\[BioRxiv\]](#) (DoubletDecon: Cell-State Aware Removal of Single-Cell RNA-Seq Doublets)

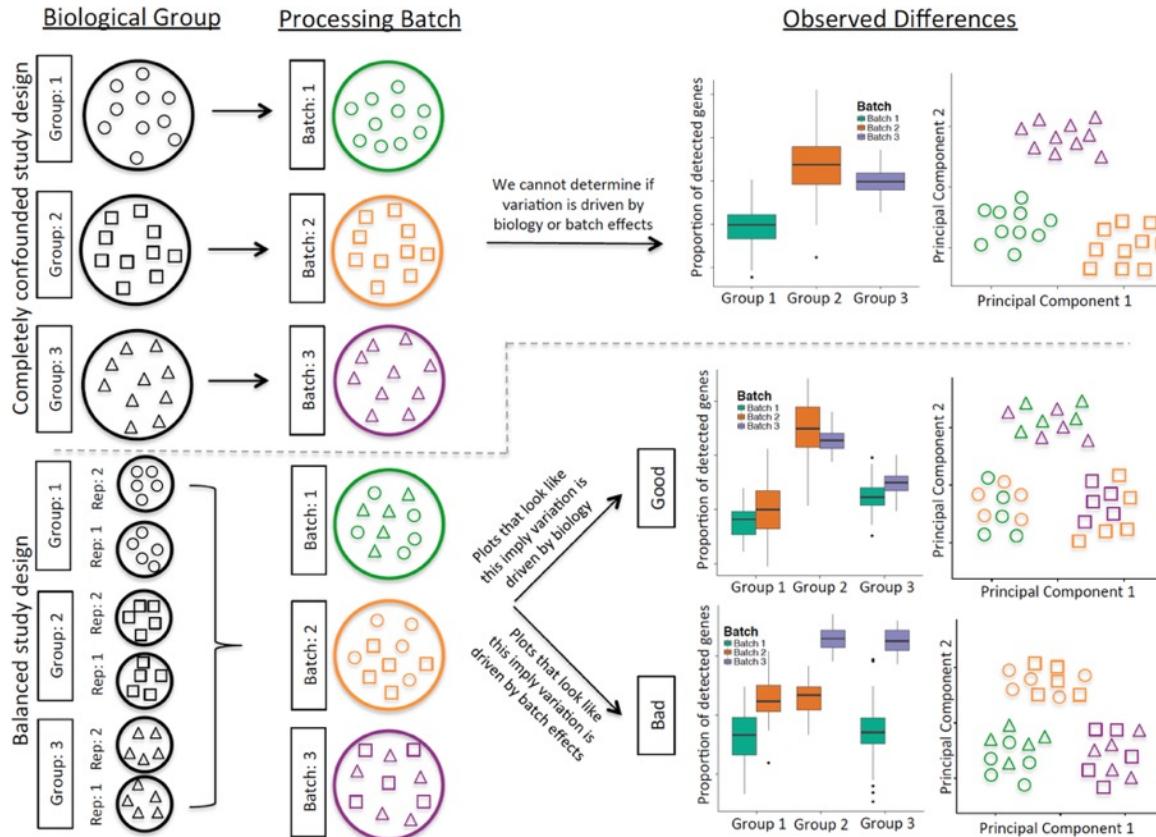
• [DoubletDetection](#) - [R, Python] - A Python3 package to detect doublets (technical errors) in single-cell RNA-seq count matrices. An [R implementation](#) is in development.

• [Scrublet](#) - [Python] - Computational identification of cell doublets in single-cell transcriptomic data. [BioRxiv](#)

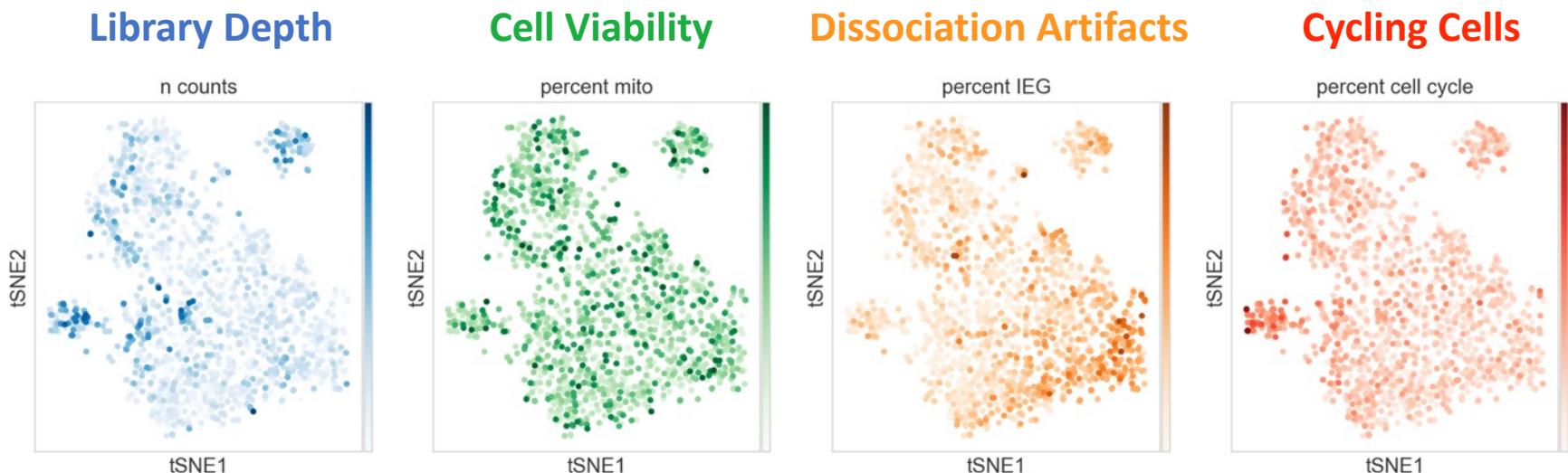


Wolock et al. (2018) bioRxiv

Batch effects and study design



Example Sources of Unwanted Variation & “Batch Effects”



Sex – matched studies are helpful!

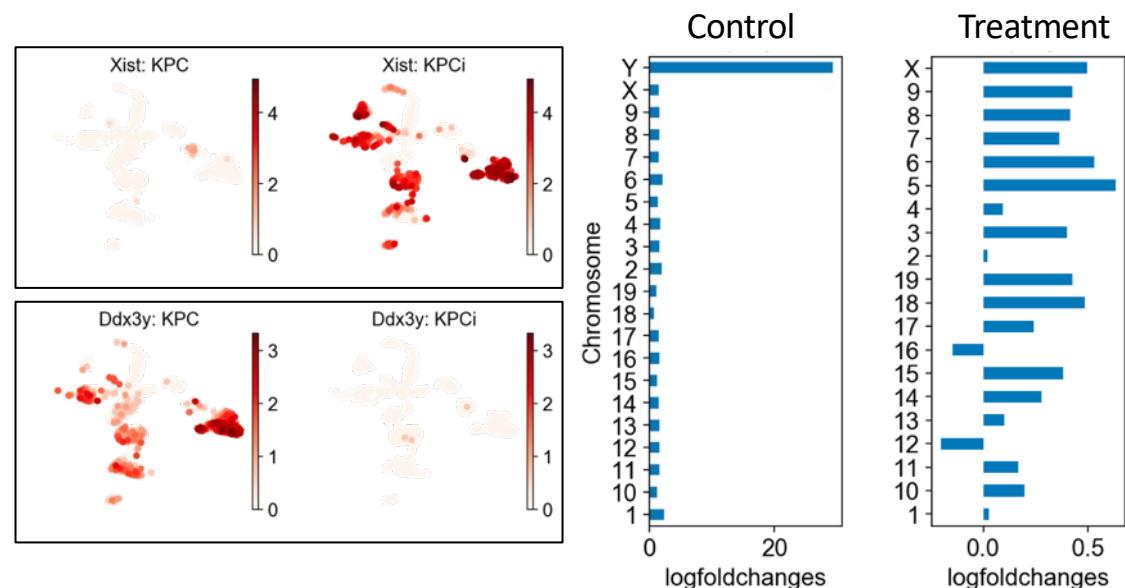
Major confounder: Male / Female

Treatment: Female

Control: Male

Consequence:

Unsupervised differential gene expression calling will be dominated by sex-specific expression. No way of separating this variable from the treatment variable



Batch Correction

Confounded Study Example:

WT and KO mice

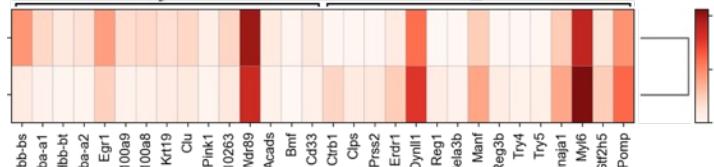
Prepared on same day
Same colony
Same set of hands

DiffeX dominated by same genes within every cluster

! major batch effect issues

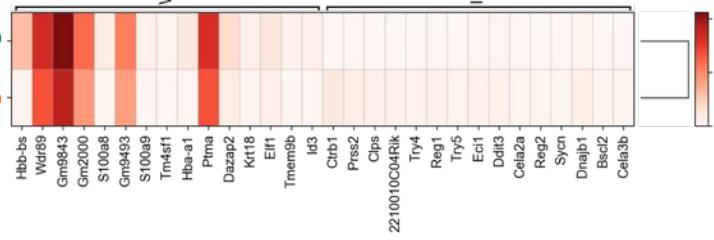
Myeloid

Ctrl
KO



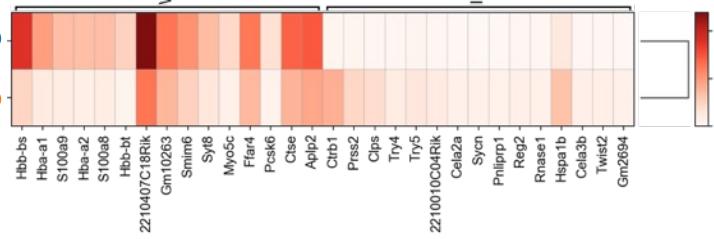
B cells

Ctrl
KO



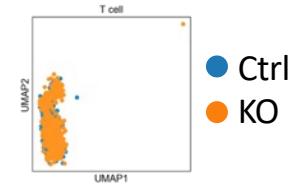
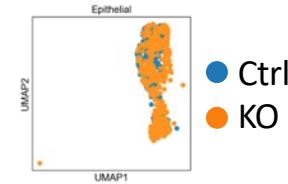
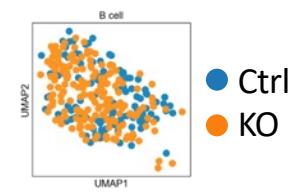
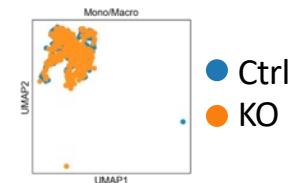
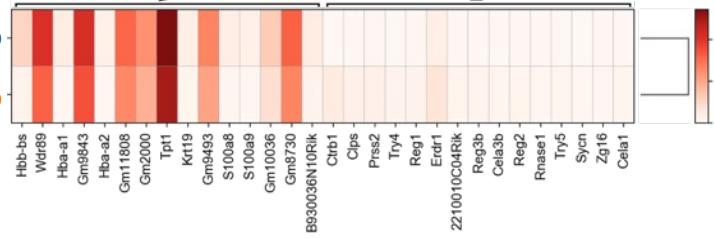
Epithelial

Ctrl
KO

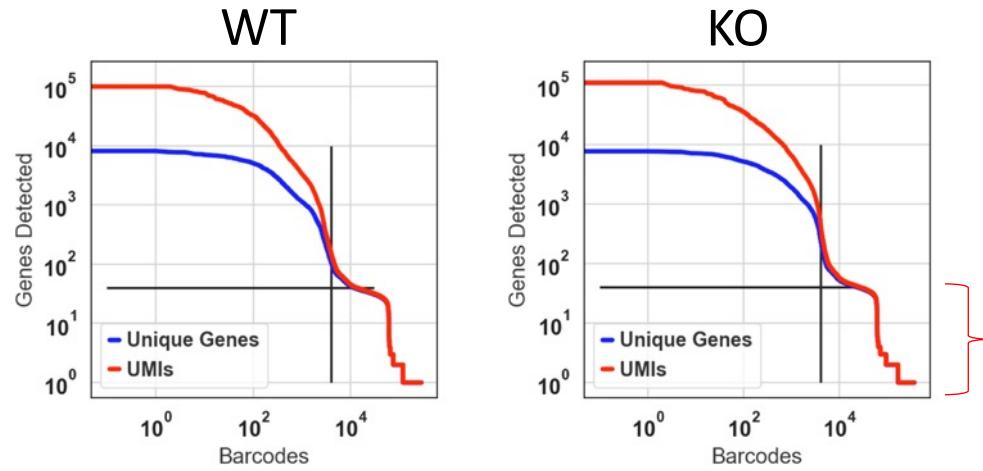


T cell

Ctrl
KO

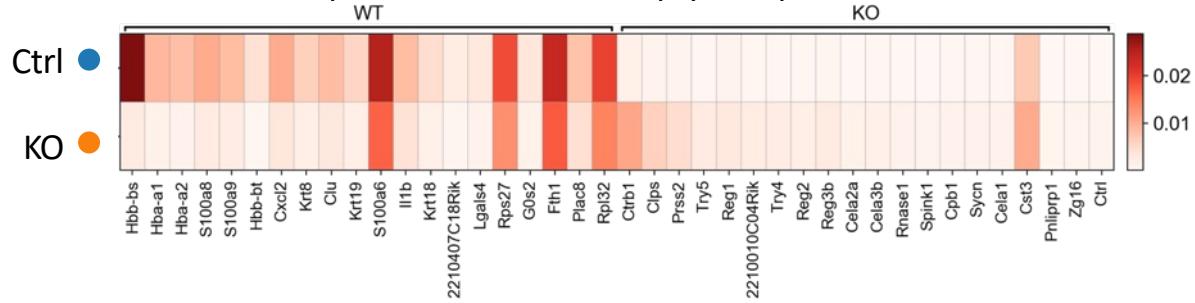


Controlling for batch effects



Ambient RNA
in droplets

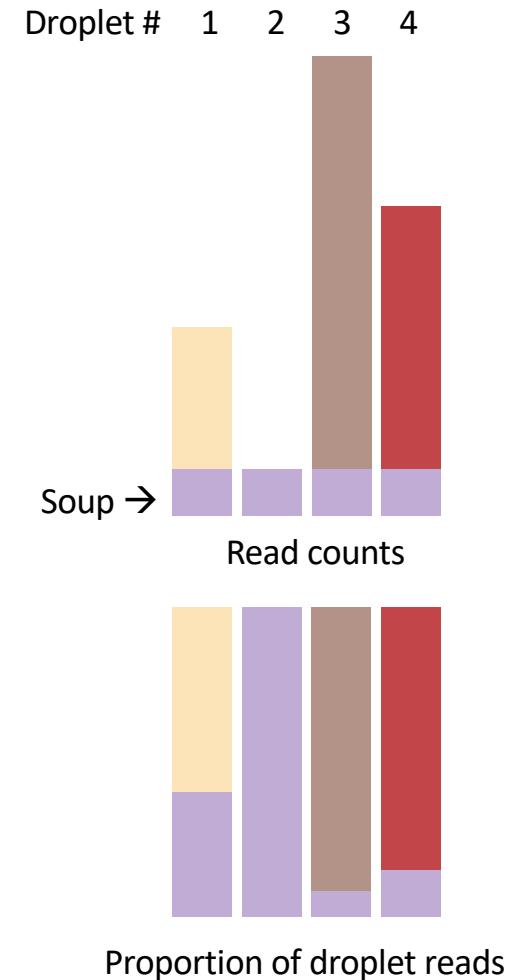
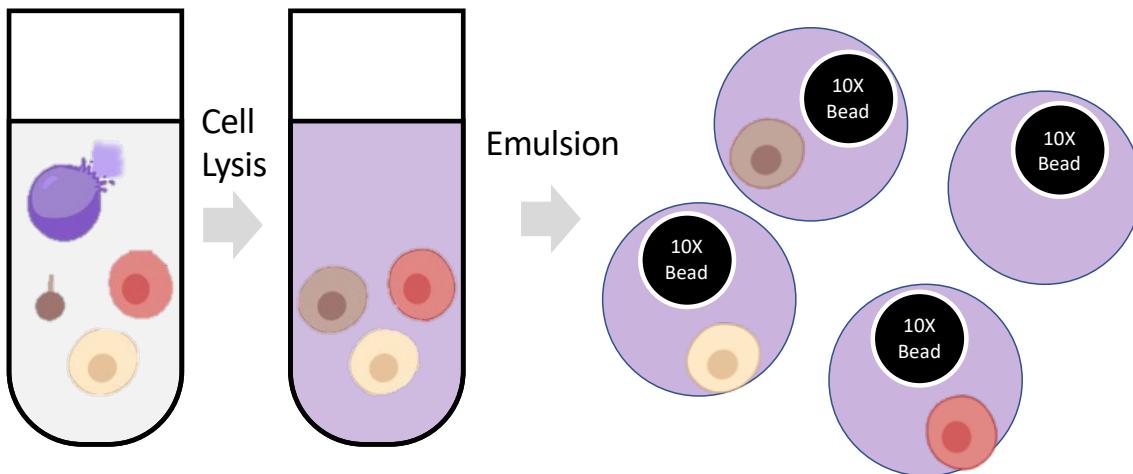
Differential expression between "empty" droplets:



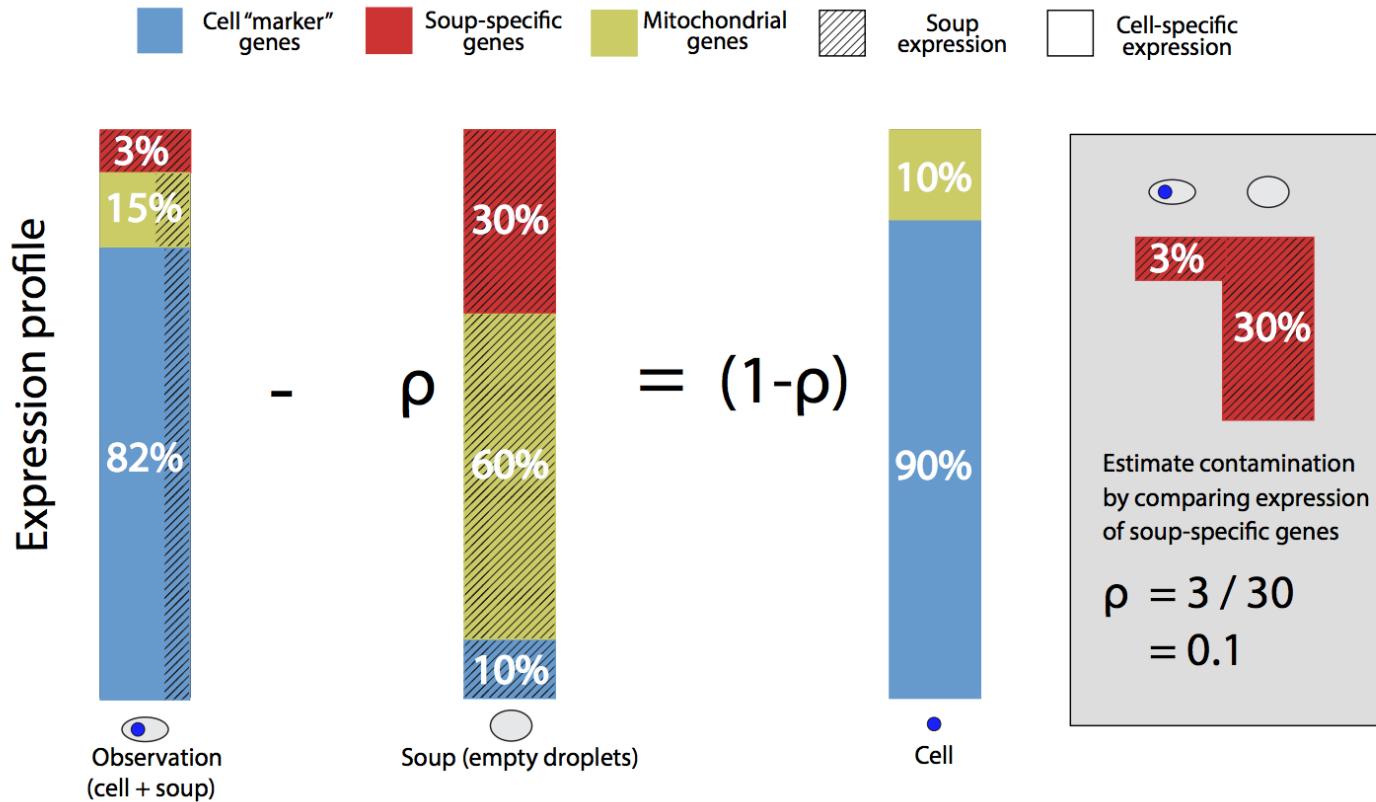
Significant sources of
contaminating mRNA:

WT:	Erythrocytes
	Epithelial
	Granulocytes
KO:	Acinar cells

Ambient RNA: "SOUP"



SoupX



Young and Behati (bioRxiv) 2018.

<https://www.biorxiv.org/content/10.1101/303727v1>

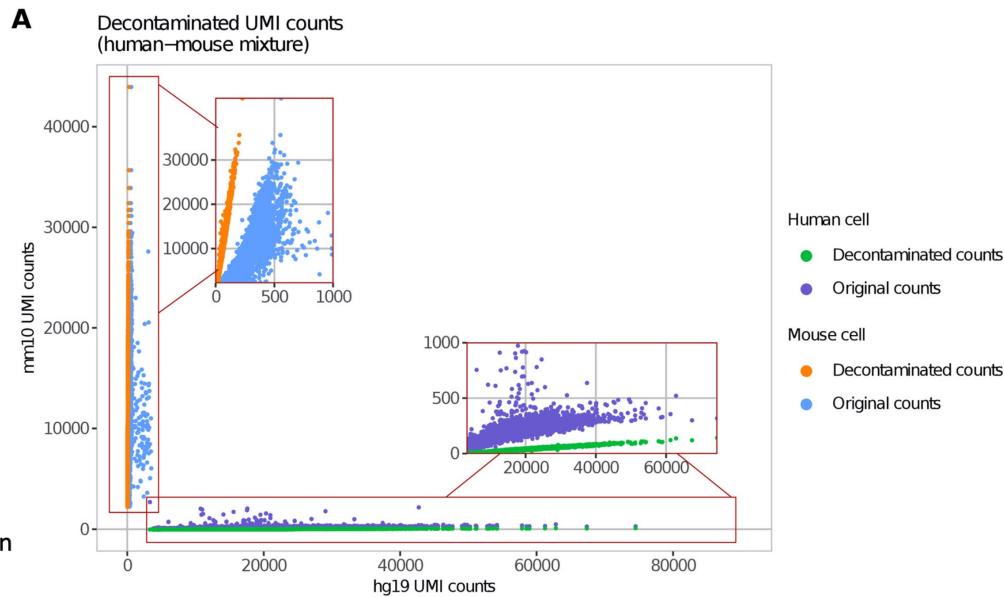
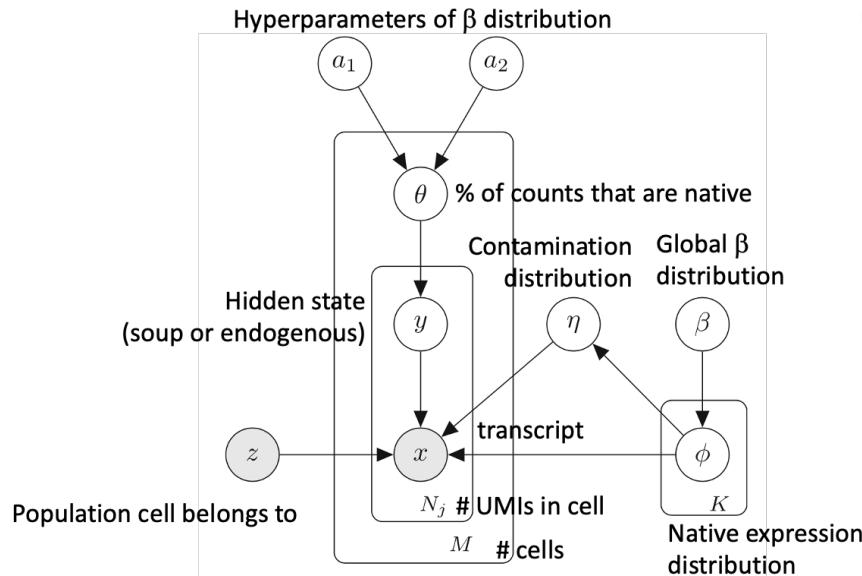


Decontamination of ambient RNA in single-cell RNA-seq with DecontX

Shiyi Yang¹ , Sean E. Corbett¹, Yusuke Koga¹, Zhe Wang¹ , W Evan Johnson¹, Masanao Yajima² and Joshua D. Campbell^{1*}

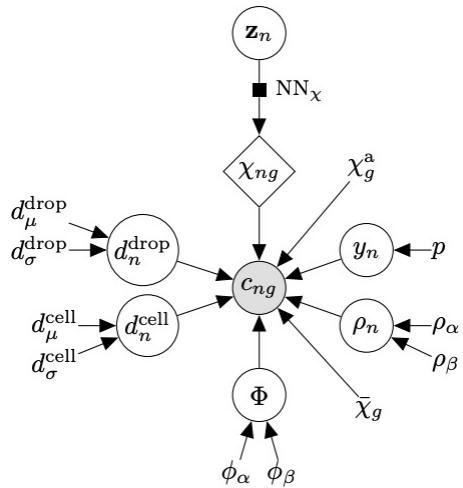
Uses Variational Bayes Inference
Similar to Latent Dirichlet Allocation (LDA)

Models soup as a weighted combination of other cell types in the population



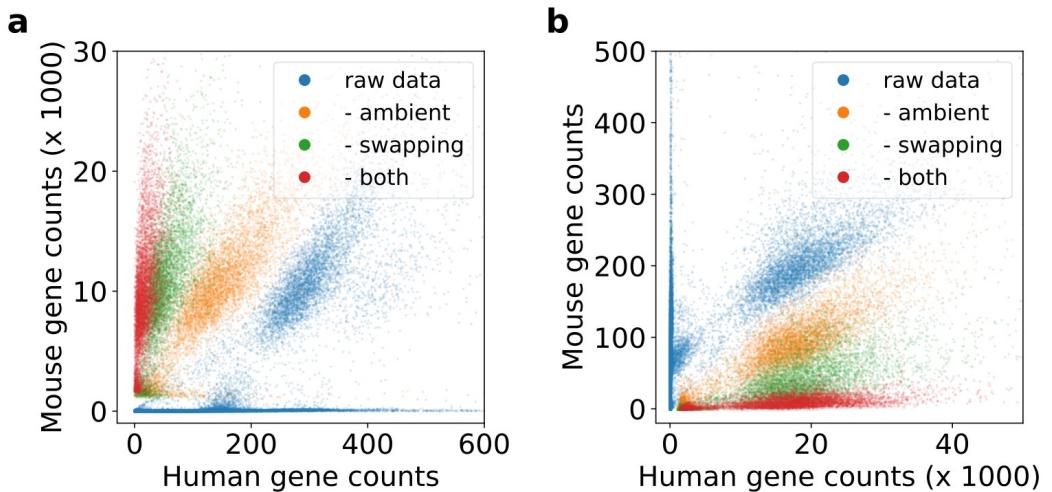
CellBender remove-background: a deep generative model for unsupervised removal of background noise from scRNA-seq datasets

Stephen J. Fleming^{1,2}, John C. Marioni^{3,4}, and Mehrtash Babadi^{1,2}



Variational Inference method
GPU-optimized

Corrects 2 problems:
1. Soup / Ambient RNA
2. Barcode swapping during PCR



Getting started with your own analyses

Rahul Satija -

R

<https://satijalab.org/seurat>

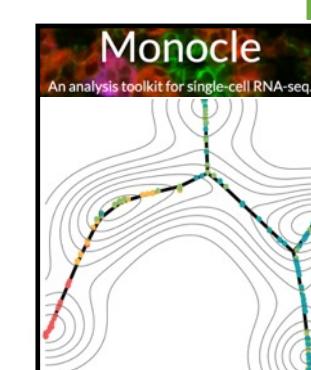


Fabian Theis - München



<https://scanpy.readthedocs.io/en/latest/>

Python



Cole Trapnell – WashU

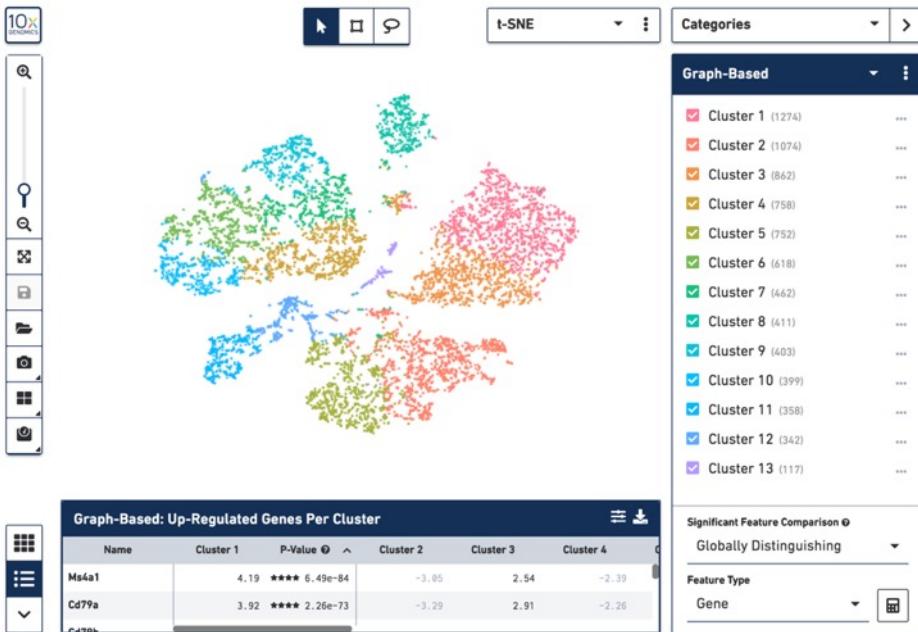


Macosko lab

AWESOME SINGLE CELL RESOURCE

<https://github.com/seandavi/awesome-single-cell>

Loupe Cell Browser



Can:

- Quickly visualize genes
- Do guided clustering via marker genes / tSNE selections
- Calculate Differential Expression
- Export cells and gene sets for reanalysis on Cellranger (cluster)

Can't

- Redo unsupervised clustering / tSNE / UMAP
- Repeat PCA / gene set selection
- Pseudotime, other fancy things

<https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/latest>

The Best Site On the Internet. Probably.

- [https://github.com/Teichlab/scg lib structs](https://github.com/Teichlab/scg_lib_structs)

Detailed visual guides to dozens of single-cell genomics methods

Adapter and primer sequences:

Barcode Tn5 sequence s5: 5' - **TCGTCGGCAGCGTCTCACGC**[8-bp Tn5 index]GCGATCAGGGACGGCAGATGTGTATAAGAGACAG -3'

Barcode Tn5 sequence s7: 5' - **GTCTCGGGCTCGGCTGTCCTGTCC**[8-bp Tn5 index]CACCGTCTCCGCTCAGATGTGTATAAGAGACAG -3'

Tn5 binding site 19-bp Mosaic End (ME) bottom: 5' - /Phos/**AGATGTGTATAAGAGACAG** -3'

P5 index primer entry point (s5): 5' - **TCGTCGGCAGCGTCTCACGC** -3'

P7 index primer entry point (s7): 5' - **GTCTCGGGCTCGGCTGTCCTGTCC** -3'

P5 index primer: 5' - **AATGATAACGGCACCGAGATCTACAC**[i5]**TCGTCGGCAGCGTCTCACGC** -3'

P7 index primer: 5' - **CAAGCAGAACGCGCATACGAGAT**[i7]**GTCTCGGGCTCGGCTGTCCTGTCC** -3'

Read 1 sequencing primer: 5' - GCGATCAGGGACGGCAGATGTGTATAAGAGACAG -3'

Index 1 sequencing primer (i7): 5' - CTGTCCTTACACATCT**GAGGCCGAGACGGTG** -3'

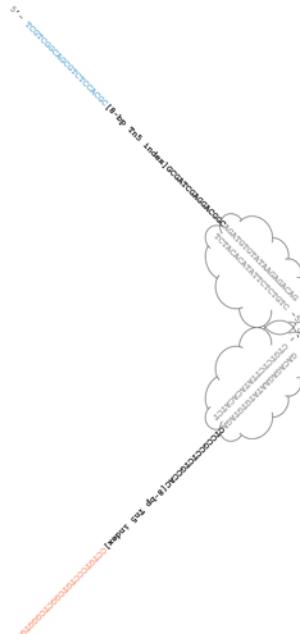
Read 2 sequencing primer: 5' - CACCGTCTCCGCTCAGATGTGTATAAGAGACAG -3'

Product 1 (s5 at both ends, not amplifiable due to semi-suppressive PCR):

5' - **TCGTCGGCAGCGTCTCACGC**[8-bp Tn5 index]GCGATCAGGGACGGCAGATGTGTATAAGAGACAGXXXXXXXXXXXX...XXX
 TCTACACATATTCTCTGCT CTGTCCTTACACATCT
 XXX...XXXXXXXXXXGACAGAGAATATGTGAGACGGCAGGAGCTAGCG[8-bp Tn5 index]CGCACCTCTGGACGGCTCT -5'

Product 2 (s7 at both ends, not amplifiable due to semi-suppressive PCR):

5' - **GTCTCGGGCTCGGCTGTCCTGTCC**[8-bp Tn5 index]CACCGTCTCCGCTCAGATGTGTATAAGAGACAGXXXXXXXXXXXX...XXX
 TCTACACATATTCTCTGCT CTGTCCTTACACATCT
 XXX...XXXXXXXXXXGACAGAGAATATGTGAGACCTCCGCTCTGCCAC[8-bp Tn5 index]CCTGTCCTGTCGGCTGGGTGCTCTG -5'

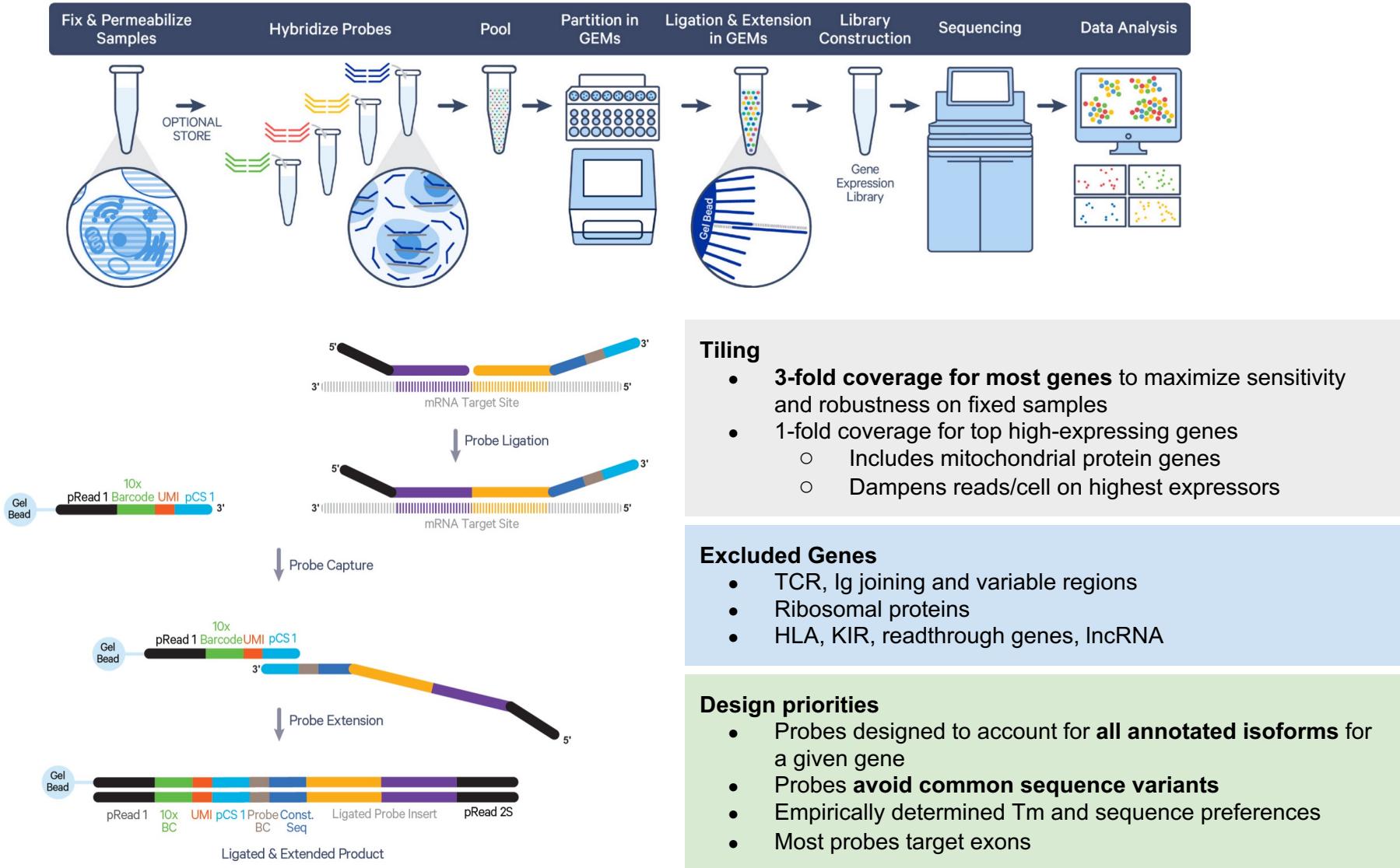


“What I cannot create, I do not understand.” --Feynman

Hacking Droplets

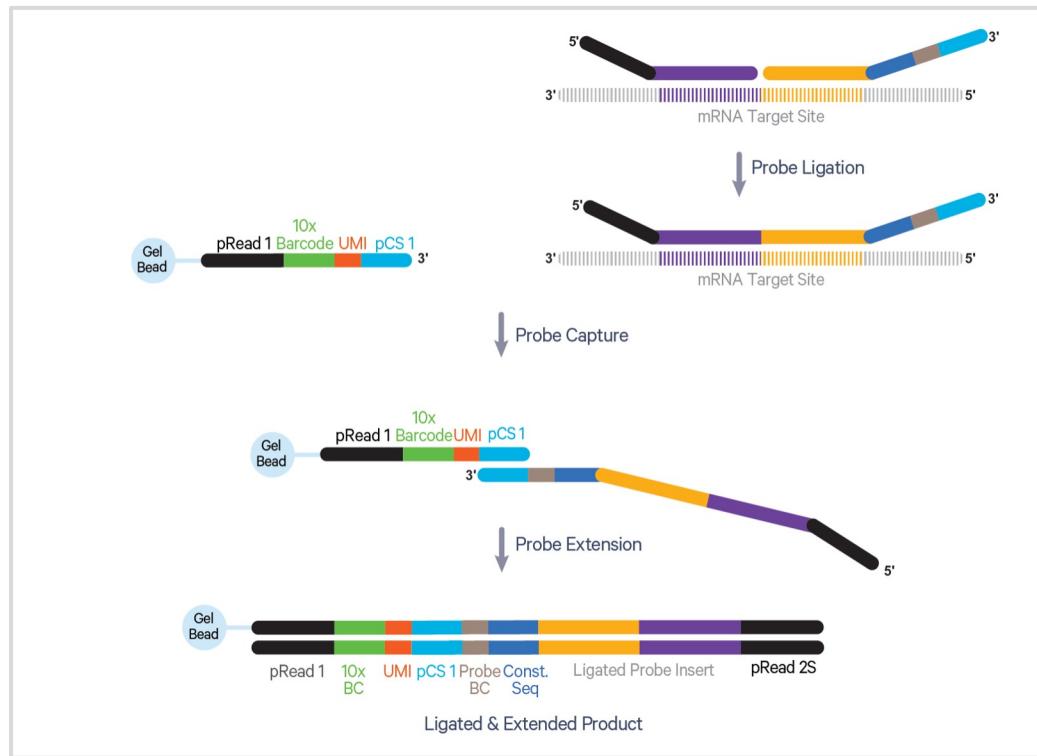


Fixed RNA Profiling



How it Works – Inside the GEMs

Direct capture of ligated probes



Followed by in-bulk amplification of products, and library construction and QC...

snPATHO-seq – isolation of nuclei from FFPE cells

 CSH
Cold Spring Harbor Laboratory

bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

bioRxiv posts many COVID19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive.

New Results [View current version of this article](#) [Follow this preprint](#)

snPATHO-seq: unlocking the FFPE archives for single nucleus RNA profiling

Andres F Vallejo, Kate Harvey, Taopeng Wang, Kellie Wise, Lisa M Butler, Jose Polo, Jasmine T Plummer, Alexander Swarbrick, Luciano G Martelotto

doi: <https://doi.org/10.1101/2022.08.23.505054>

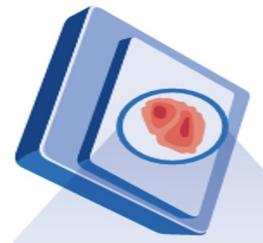
This article is a preprint and has not been certified by peer review [what does this mean?].



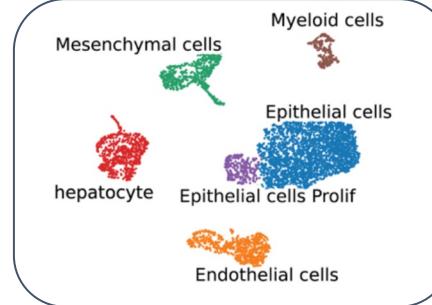
[Abstract](#) [Info/History](#) [Metrics](#) [Preview PDF](#)

Abstract

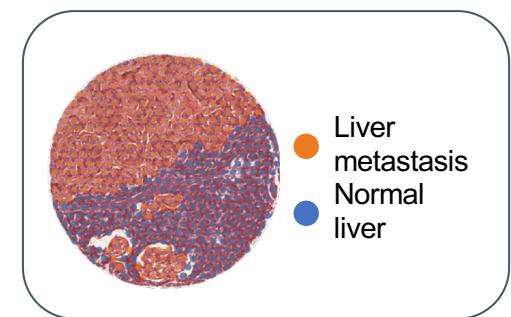
FFPE (formalin-fixed, paraffin-embedded) tissue archives are the largest repository of clinically annotated human specimens. Despite numerous advances in technology, current methods for sequencing of FFPE-fixed single-cells are slow, labour intensive, insufficiently sensitive and have a low resolution, making it difficult to fully exploit their enormous research and clinical



Chromium Single Cell FFPE

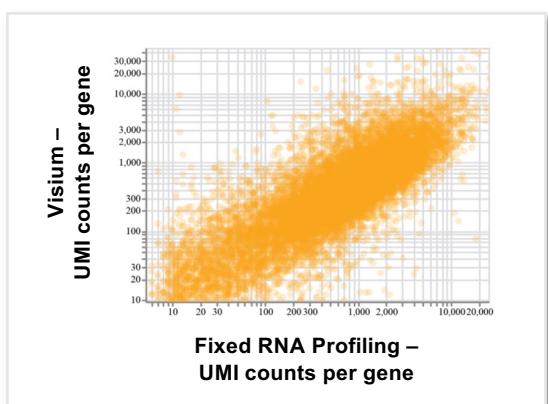
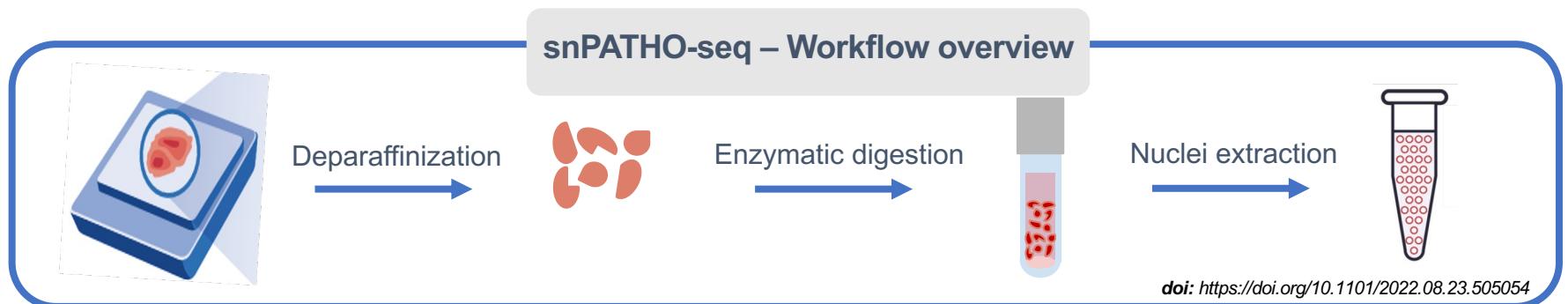


Visium Spatial FFPE



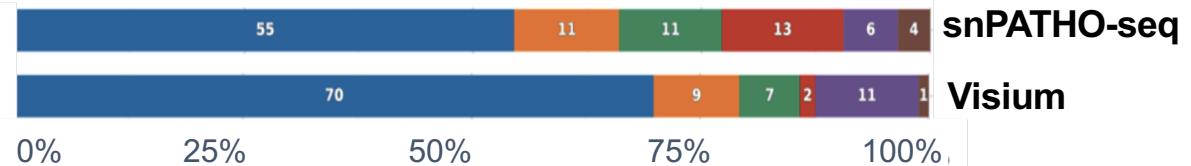
doi: <https://doi.org/10.1101/2022.08.23.505054>

FRP and Visium show strong correlation on FFPE samples

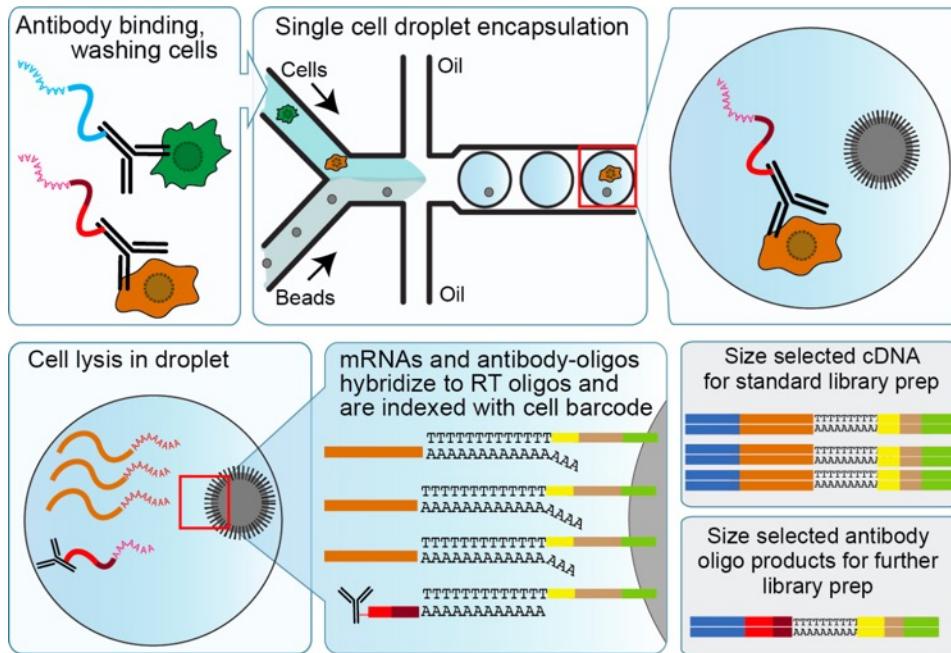


Epithelial cells
Endothelial cells
Mesenchymal cells

Epithelial cells (proliferating)
Hepatocytes
Myeloid cells



CITE-Seq / REAP-Seq



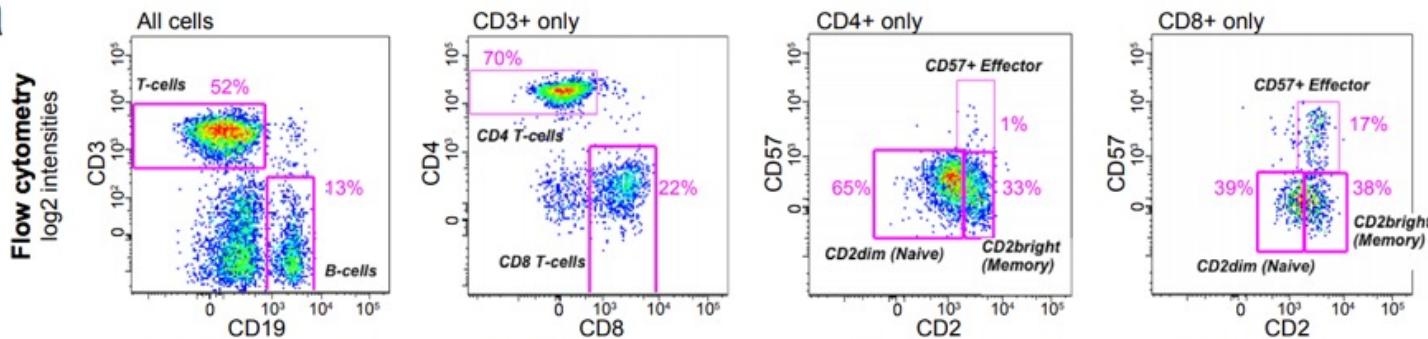
Antibody Derived Tag (ADT)
sequenced as part of normal 10X run

Enables:

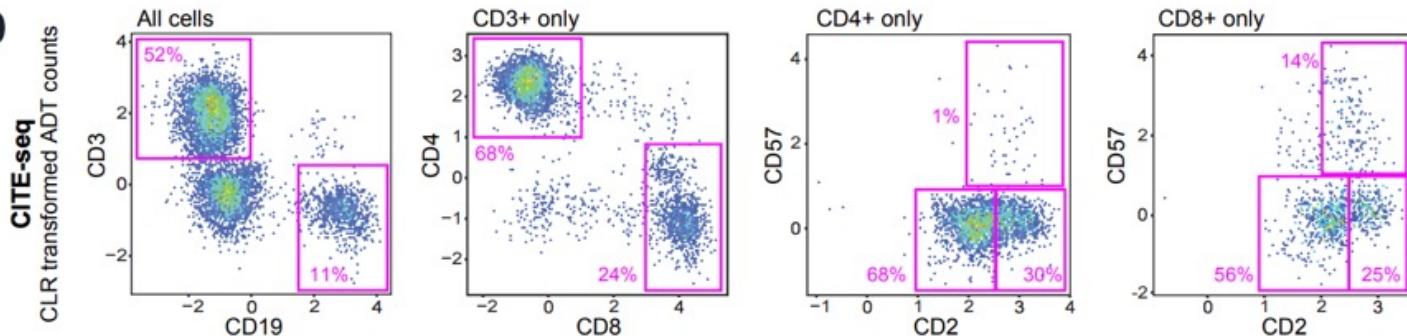
- Simultaneous mRNA + Protein Abundance
- Increased sensitivity to individual targets
- ‘Superloading’

CITE-Seq / REAP-Seq

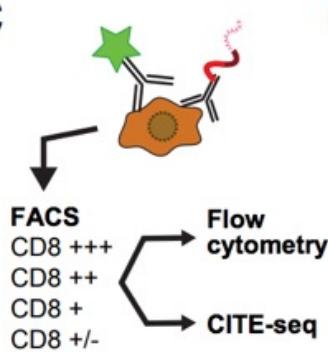
a



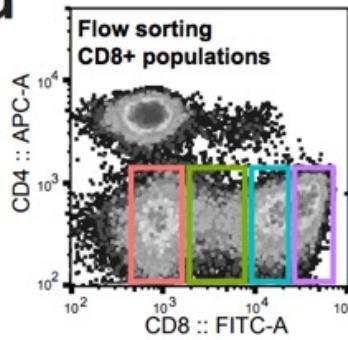
b



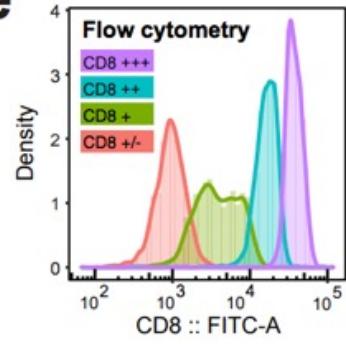
c



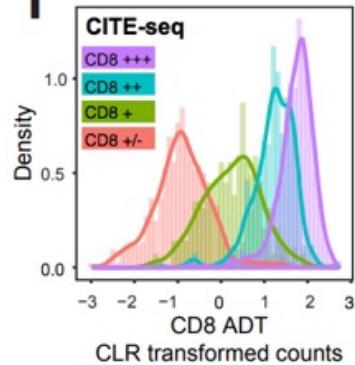
d



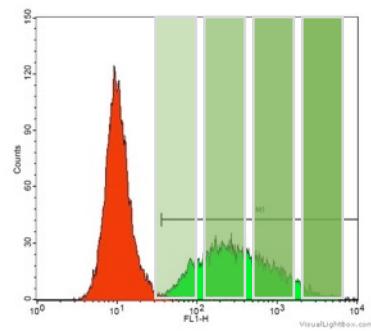
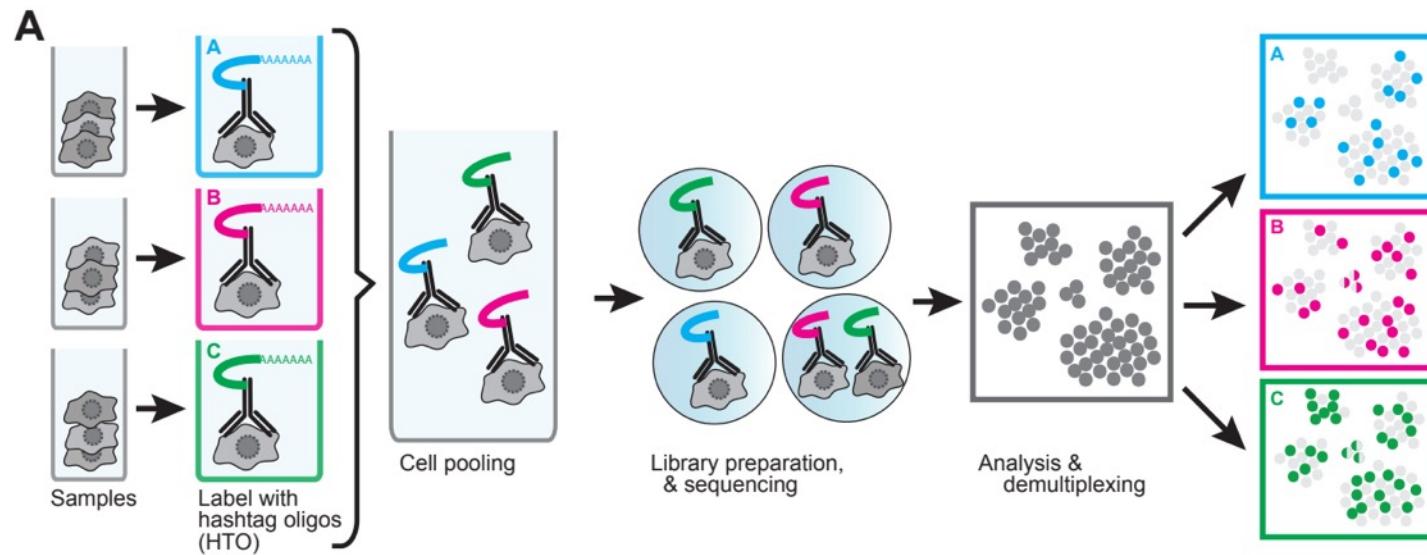
e



f

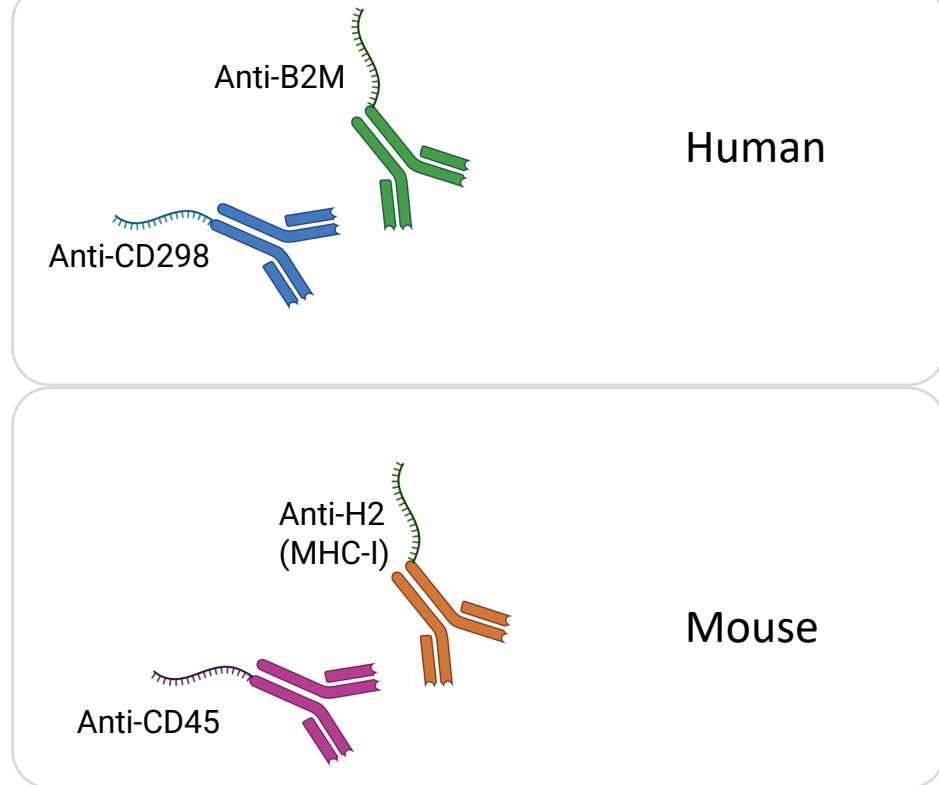


Multiplexing with ADTs: “Cell Hashing”



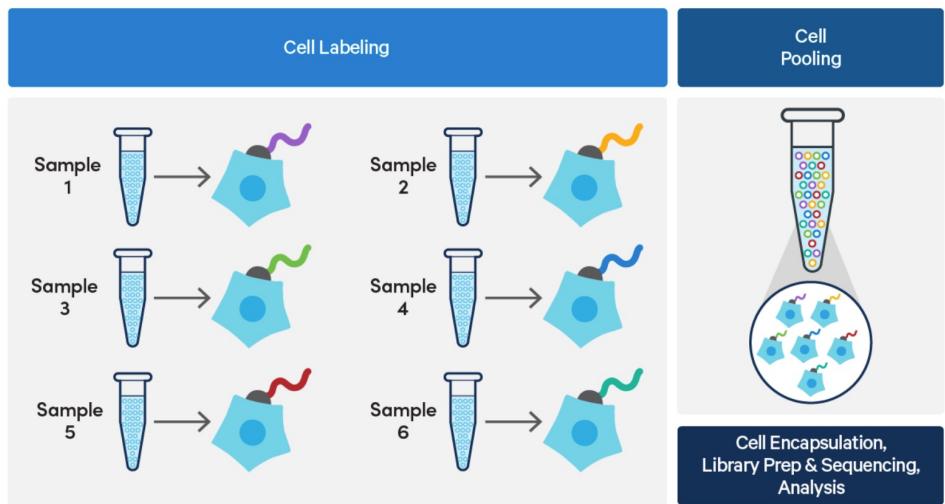
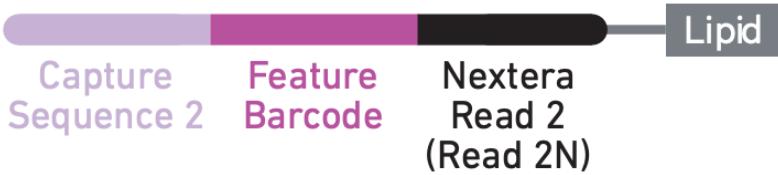
Sort multiple bins → HTO Label → Repool & Capture

Total-Seq (Biolegend)

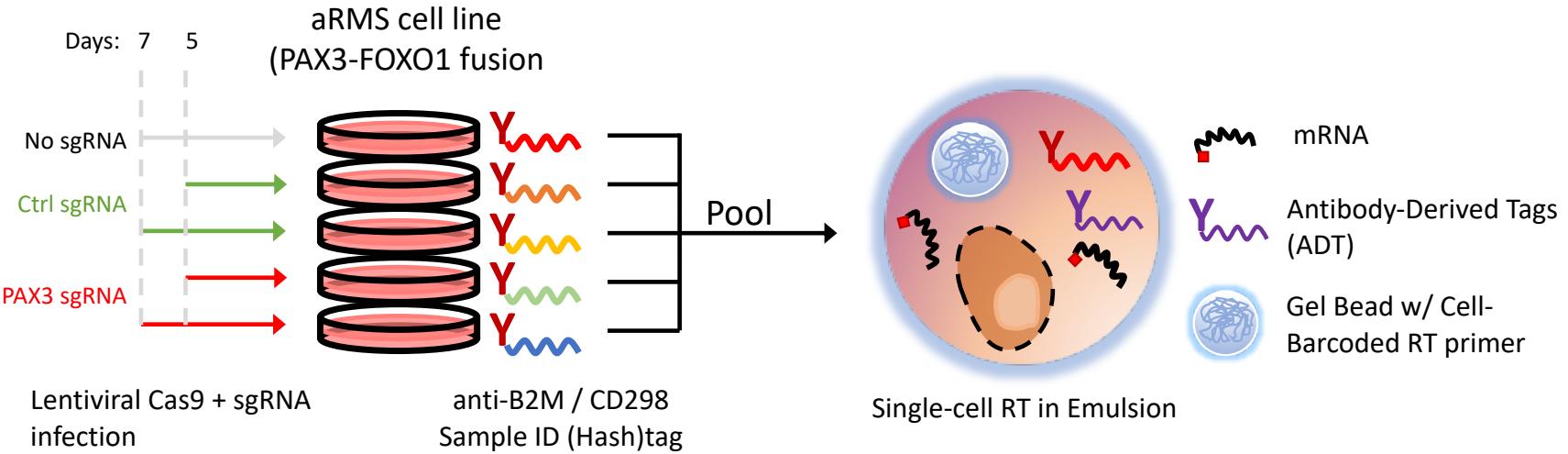


CellPlex (10X Genomics)

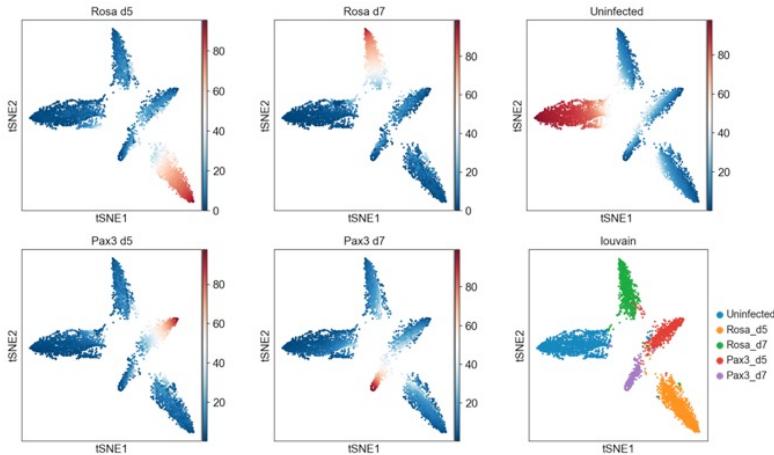
Cholesterol / Lipid anchor



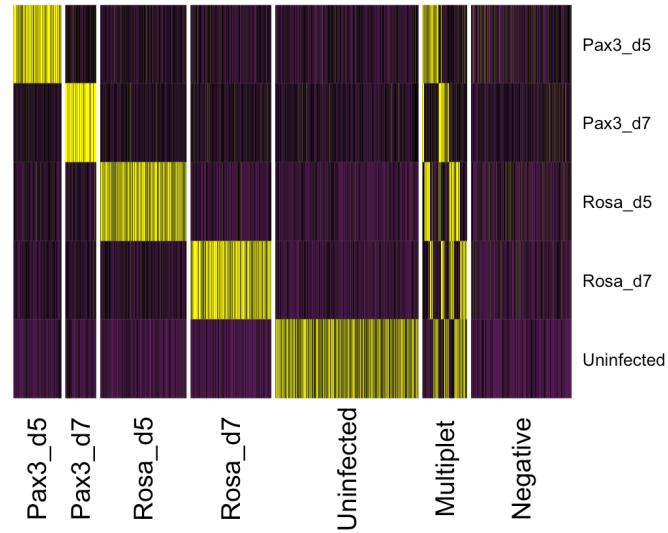
Multiplexing with ADTs: “Cell Hashing”



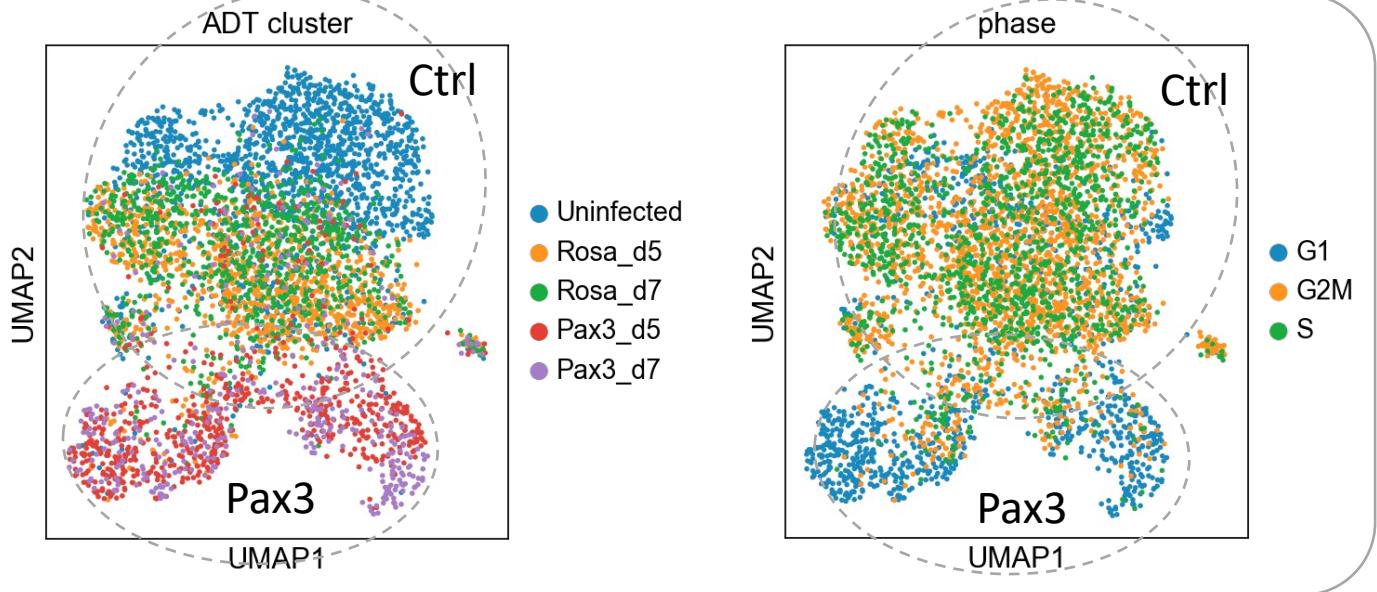
tSNE by Hash Tag



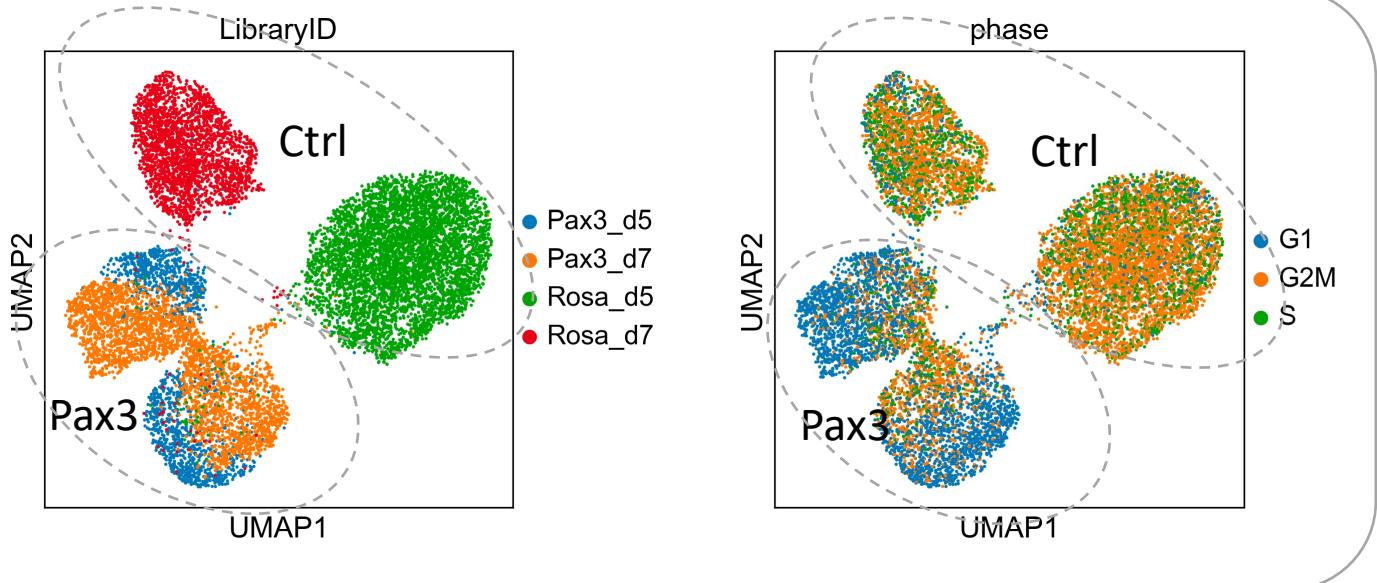
Hash tag Clustering



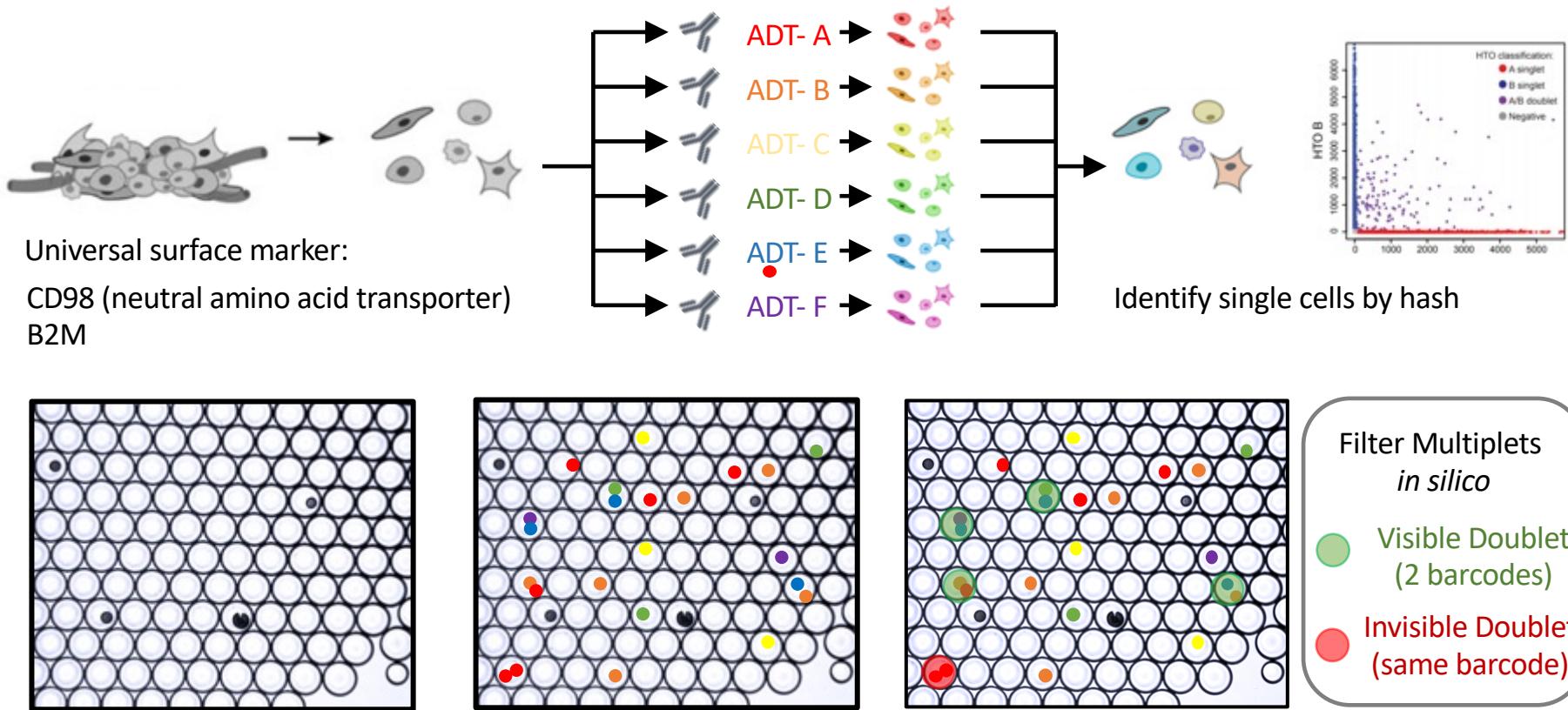
Hashed 1 Lane



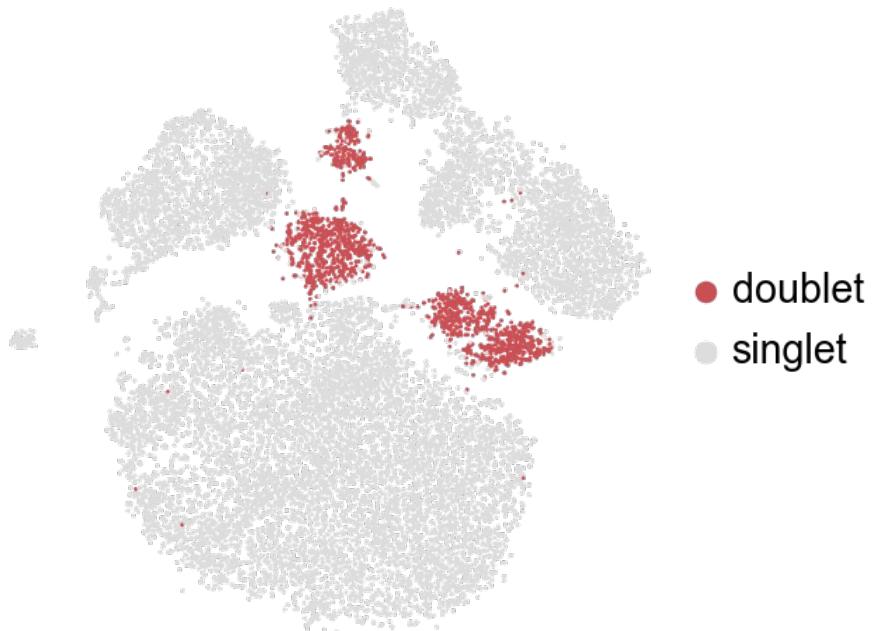
4 Lanes



Superloading with ADTs: “Cell Hashing”

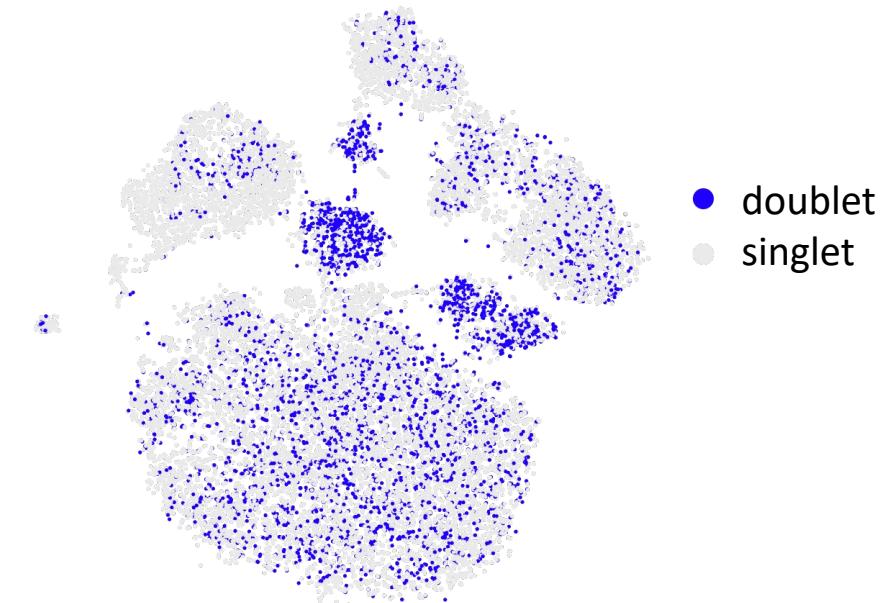


Doublet Detection by Cell Cluster



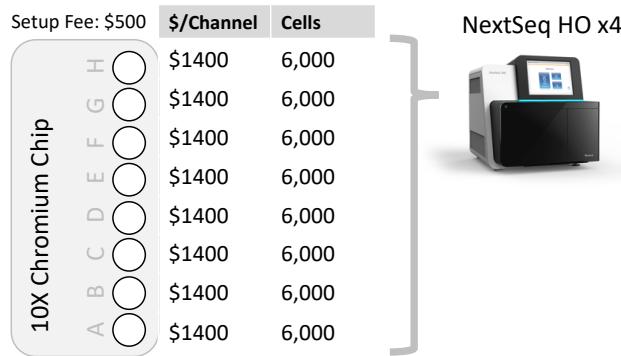
singlet	17,110
doublet	1,668

Doublet Detection by Hash Tag



singlet	15,148
doublet	3,630

Superloading with Cell Hashing Benefits



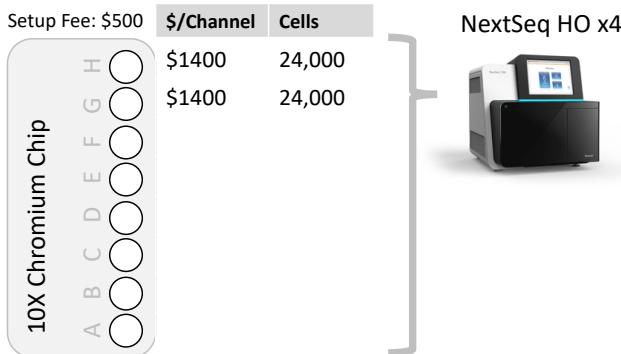
Cost Per Cell:

Capture: \$0.24
Sequencing: \$0.15

Total Experiment Cost:

Total Capture: \$11,700
Total Sequencing: \$7,200

Total: \$18,900



Cost Per Cell:

Capture: \$0.07
Sequencing: \$0.15

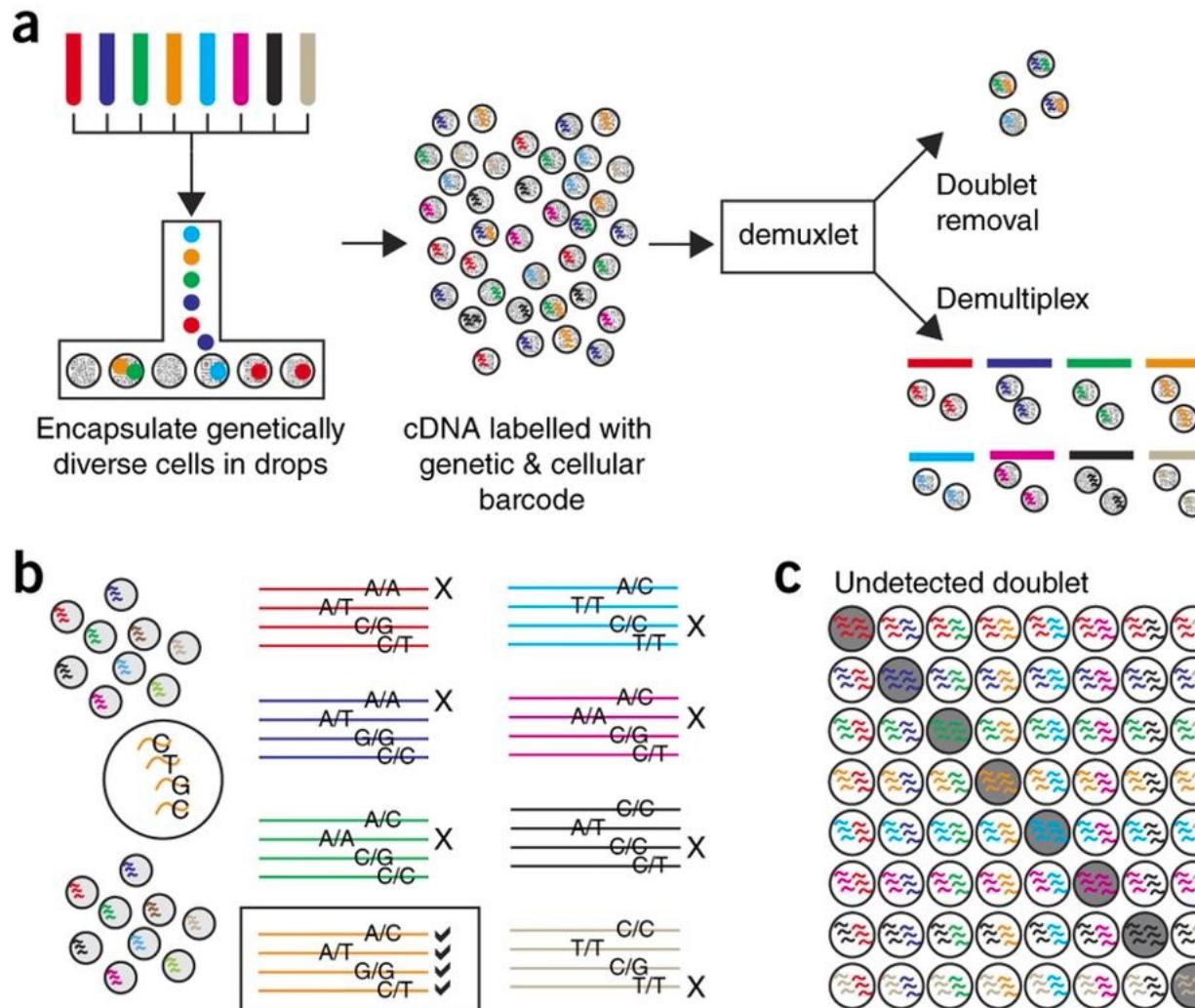
Total Experiment Cost:

Total Capture: \$3,300
Total Sequencing: \$7,200

Total: \$10,500

Multiplexing Using Natural Genetic Variation

Demuxlet



Jimmie Ye lab

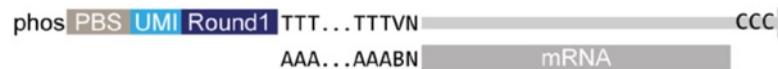
scifi-RNA-seq

Combinatorial fluidic indexing

- Massive improvement in # cells
- Up-front barcoding in plates via RT
- Swaps chemistry of 10X Genomics:
 - Uses 10X Gel beads
 - Ligation instead of RT
- Up to 150,000 cells per channel
 - (15X increase)

scifi-RNA-seq method design

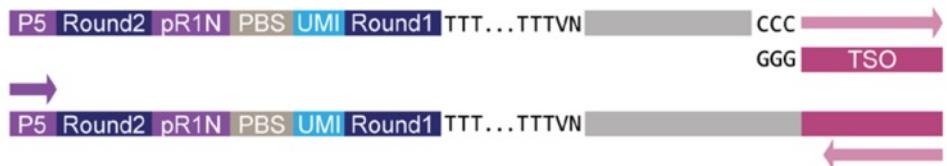
Round 1 indexing by reverse transcription on microwell plate



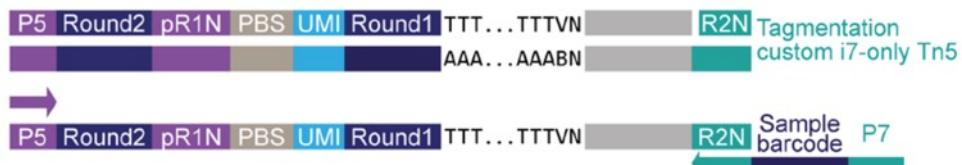
Round 2 indexing by thermoligation in microfluidic droplets



Template switching and cDNA enrichment



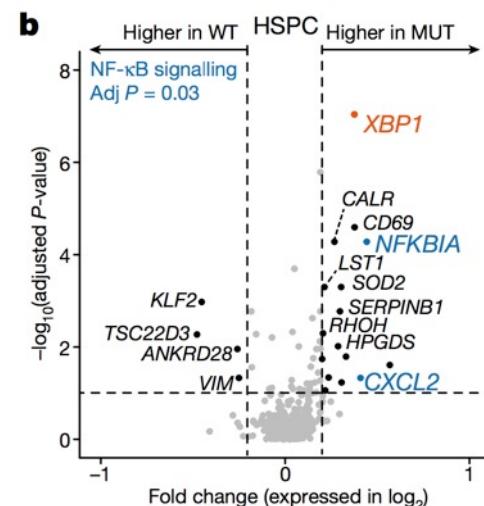
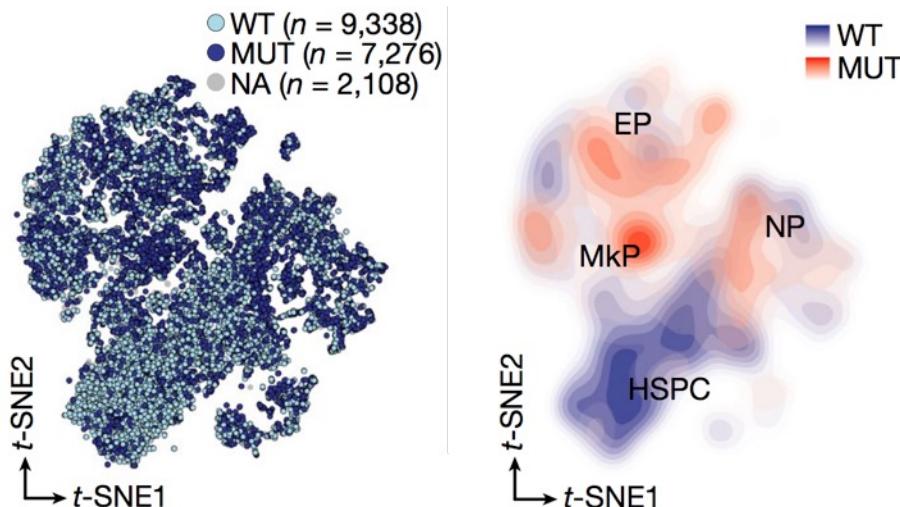
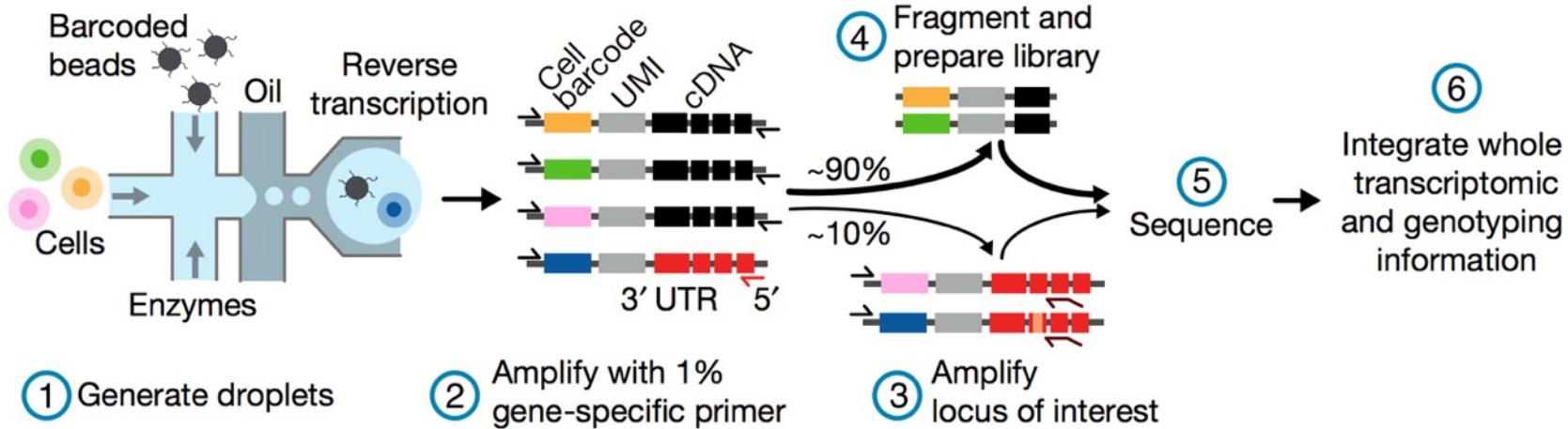
Fragmentation with custom transposome and library enrichment



Next-generation sequencing (Illumina NovaSeq 6000)



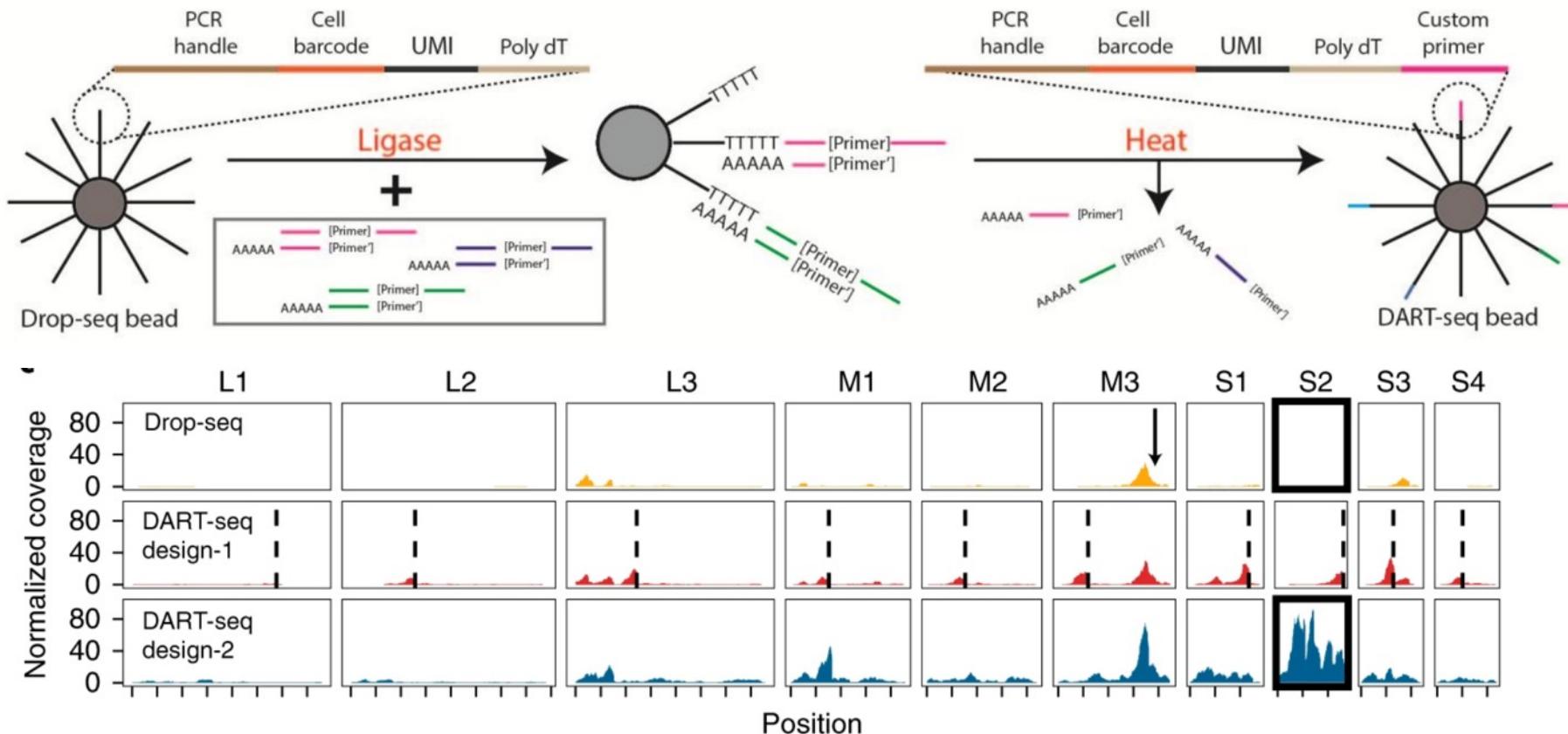
Genotyping of Transcriptomes



DART-seq

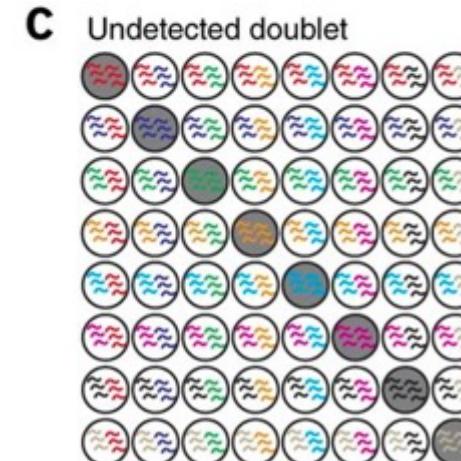
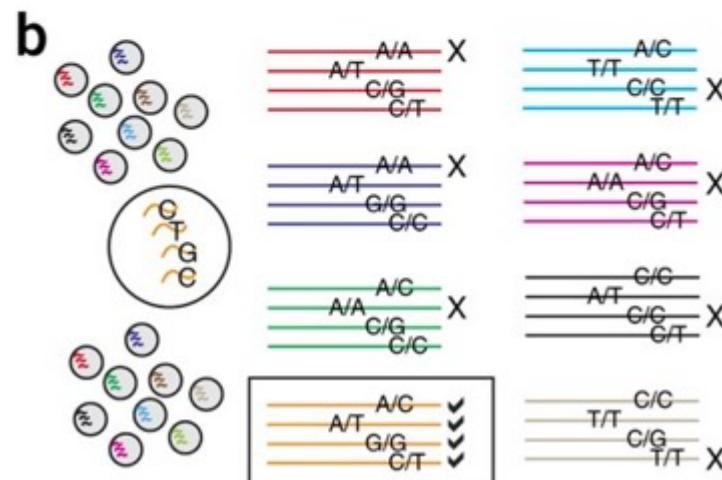
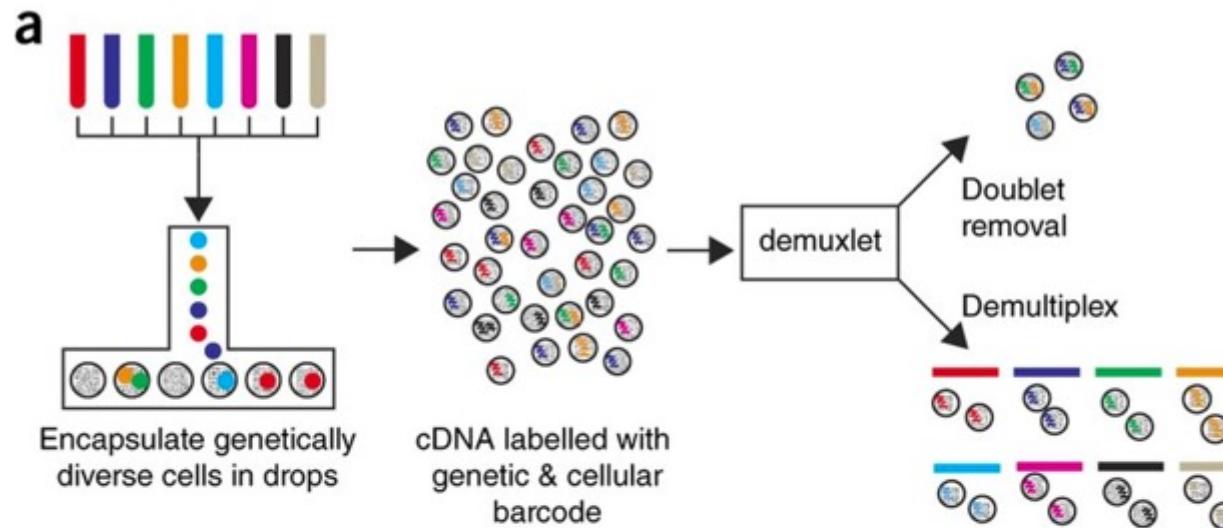
Droplet-Assisted RNA Targeting by single-cell sequencing

- Modification of barcoded bead to prime non-poly(A) transcripts
- Ligate gene-specific primers to subset of oligo-dT sites via bridge oligo
 - Careful titration of primers necessary

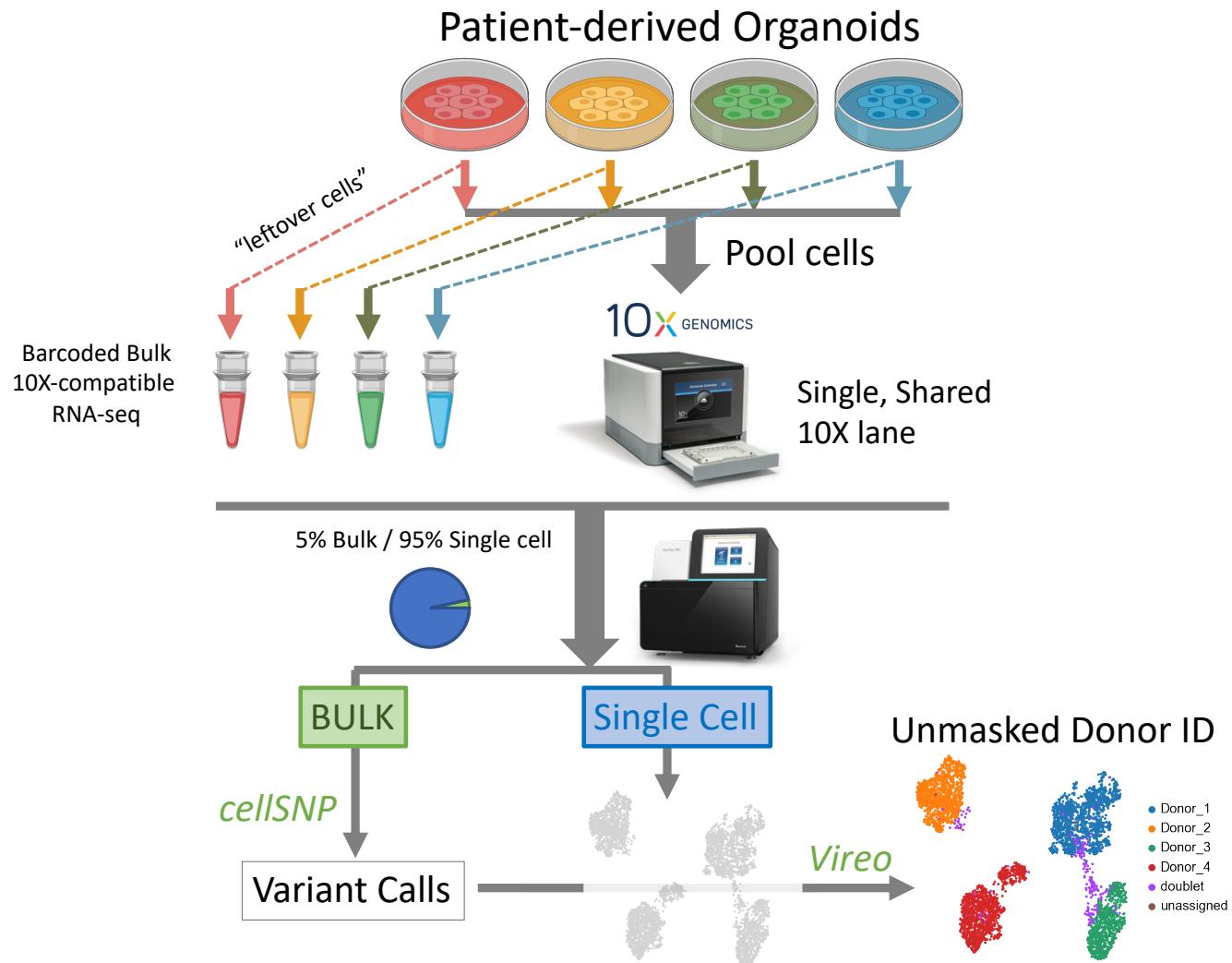


Multiplexing Using Natural Genetic Variation

Demuxlet

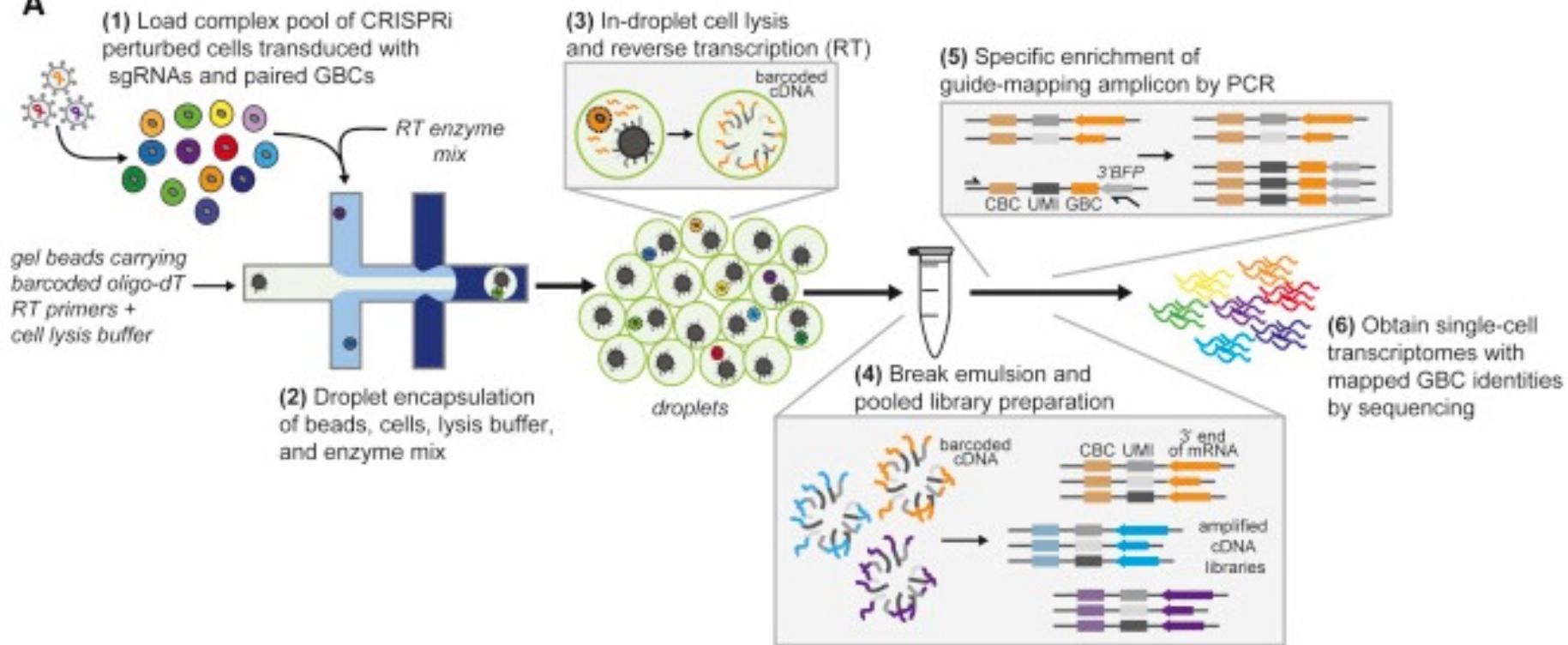


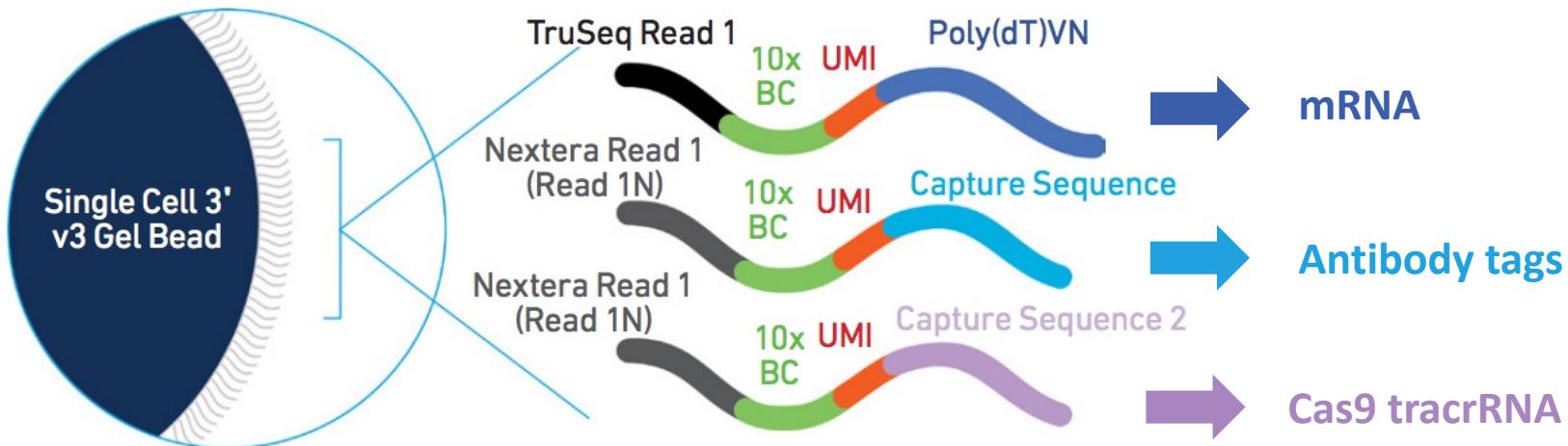
Jimmie Ye lab



Perturb-Seq

A

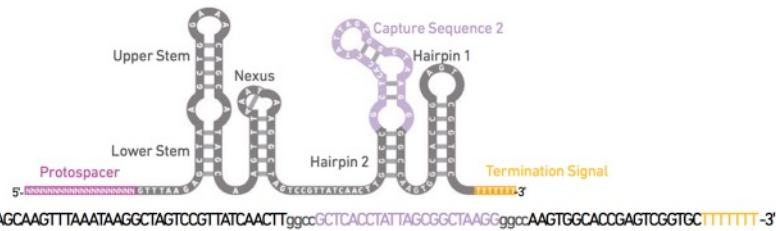




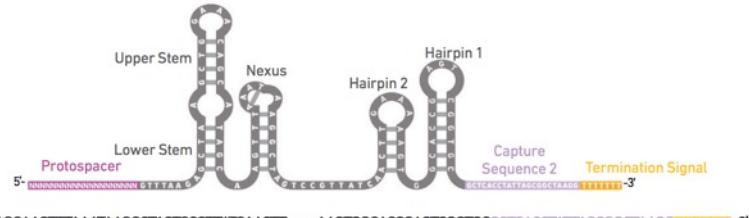
- Multiple RT primer sequences per bead
- High efficiency capture of antibody tags, CRISPR guides

Capture Sequence 2 on Gel Bead: 5'-CCTTAGCCGCTAATAGGTGAGC-3'

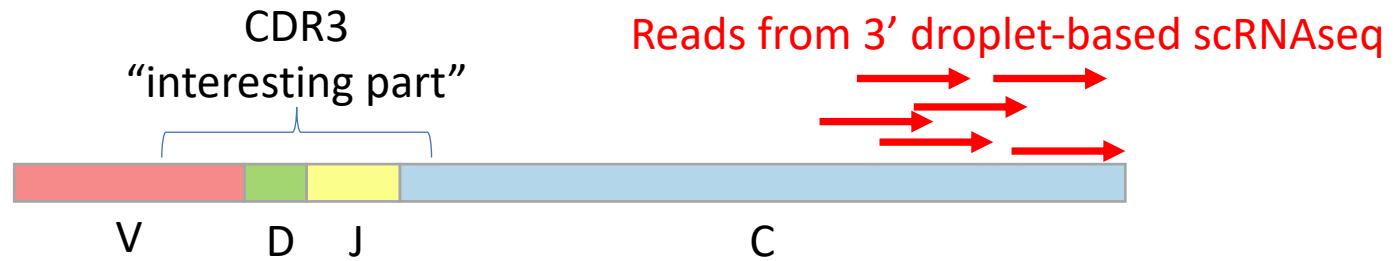
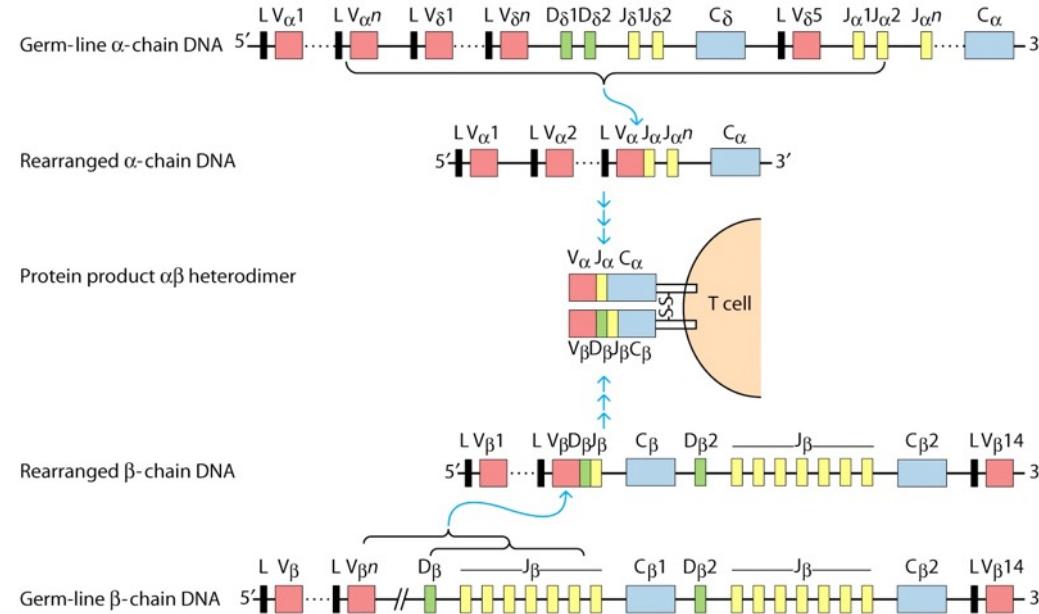
Capture Sequence 2 integrated in sgRNA hairpin



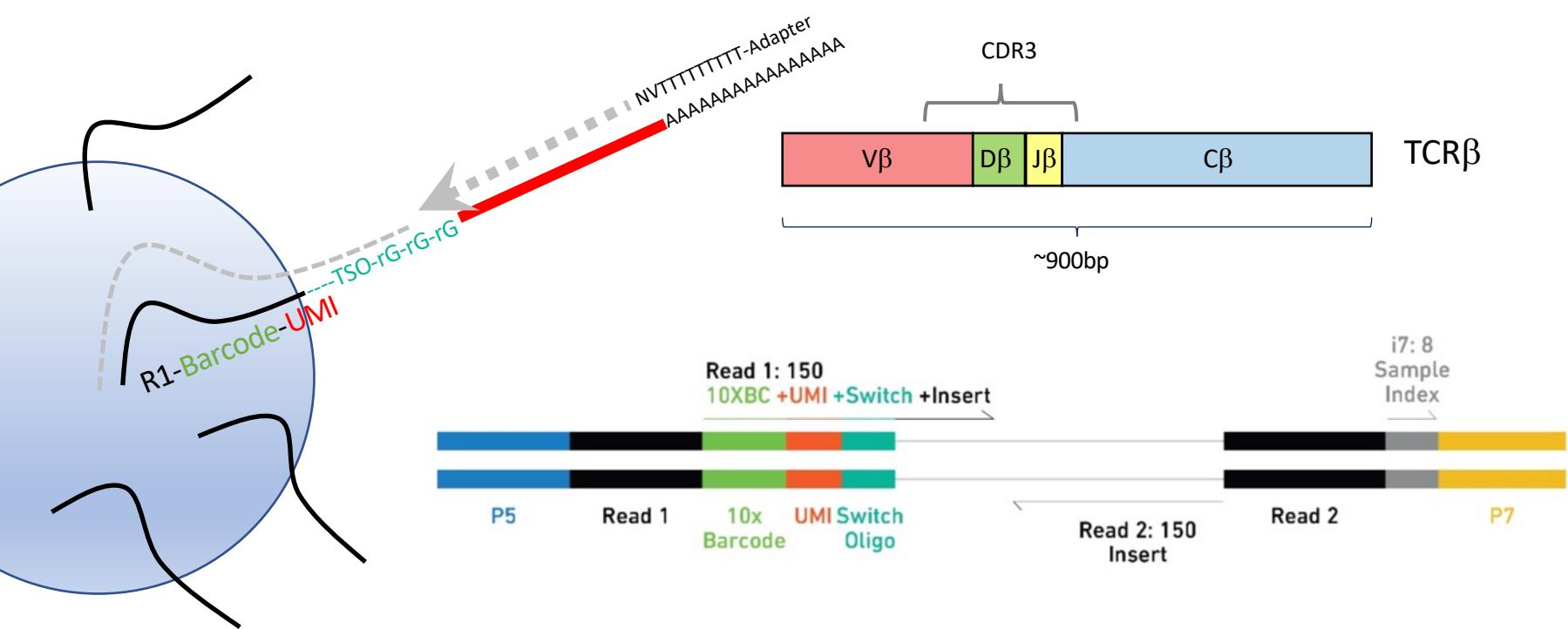
Capture Sequence 2 integrated in sgRNA 3'-end



TCR/BCR Profiling



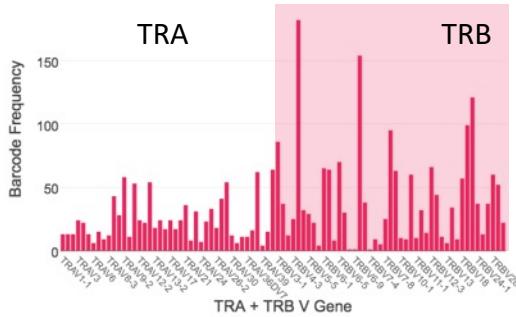
5'-Barcoded Libraries



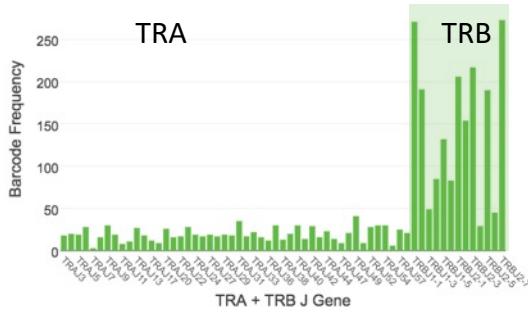
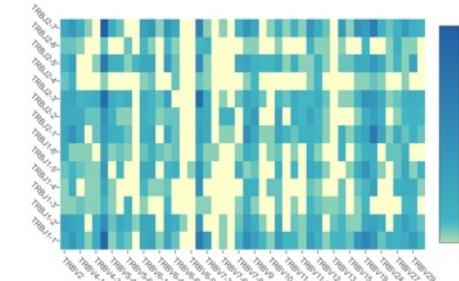
- Problem: standard transcriptome libraries have strong 3'-bias
- CDR3 mapping requires 5'-Barcoded library
- Random fragmentation to sample different 3'-ends of reads
- Require much longer reads (300bp) at a depth of 5,000X / cell

10X VDJ output example

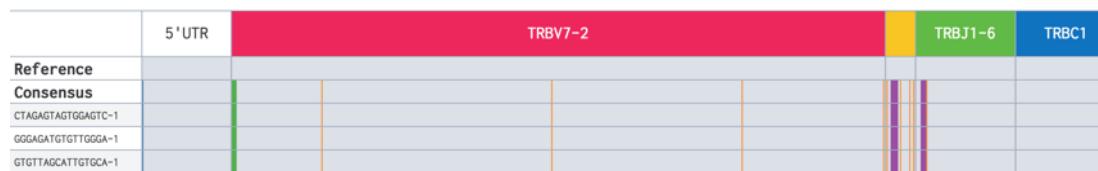
Individual V / J usage



Pairwise V-J usage



Independent
T-cells



Example Rearranged
TCR beta chain

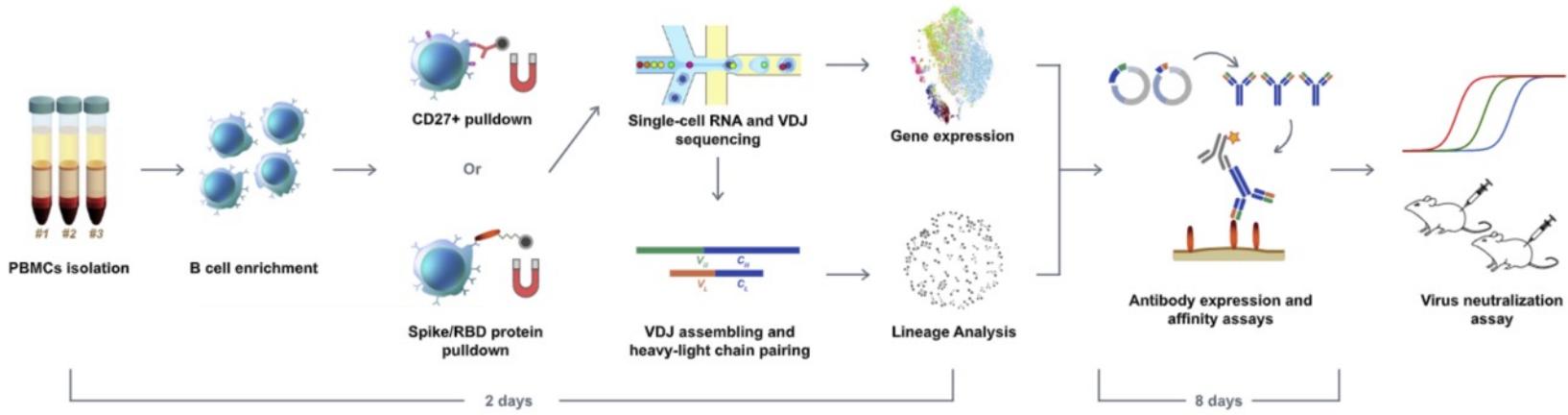
CDR3

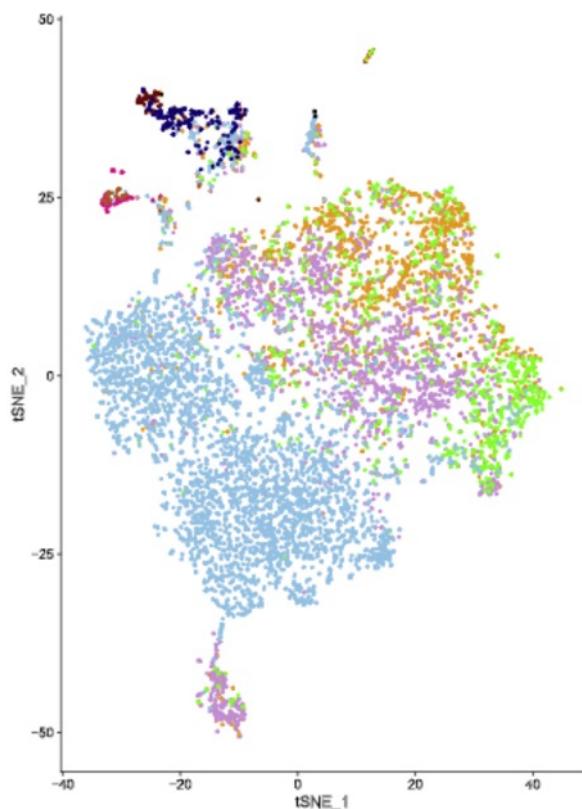
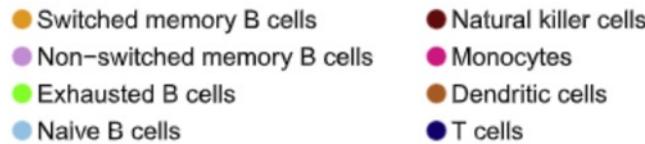
AA: CASRRGGGKTYEQYF

NT: TGTGCCAGCCGCCGGGGCGGGGGAAAACCTACGAGCAGTACTTC

Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients' B Cells

A





Rapid Filter for Neutralizing antibody candidates:

VDJ sequencing:

1. Select only IgG1 isotypes
2. Clones with multiple observed cells
3. Clones with somatic hypermutation

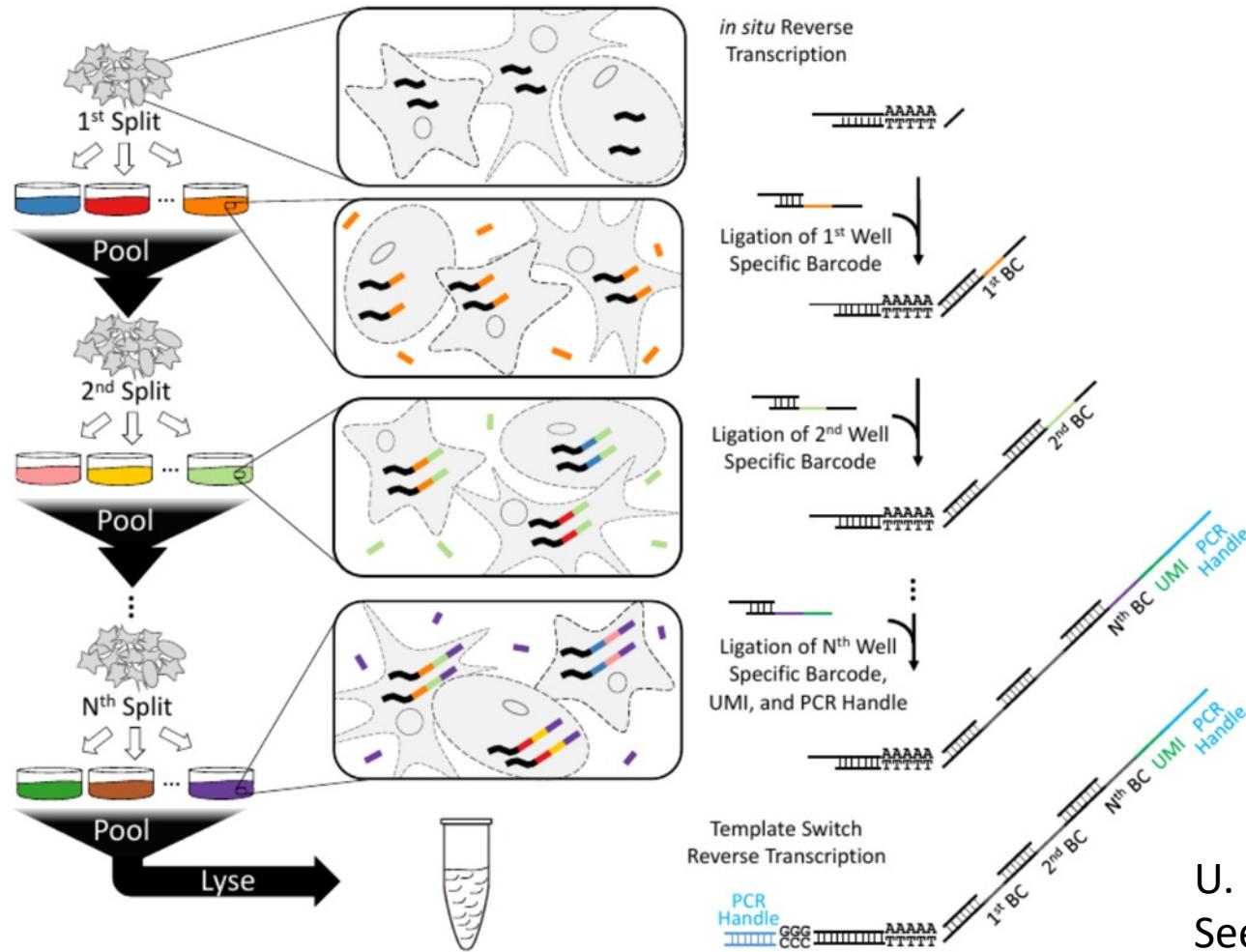
Gene expression analysis

1. Exclude exhausted and naïve phenotypes
2. Favor memory and plasma phenotypes

Other high-throughput platforms

Combinatorial Indexing

Split-Seq



U. Washington
Seelig Lab



Evercode™ Whole Transcriptome

WT Mini

<10k cells

1-12 samples

WT

10k-100k cells

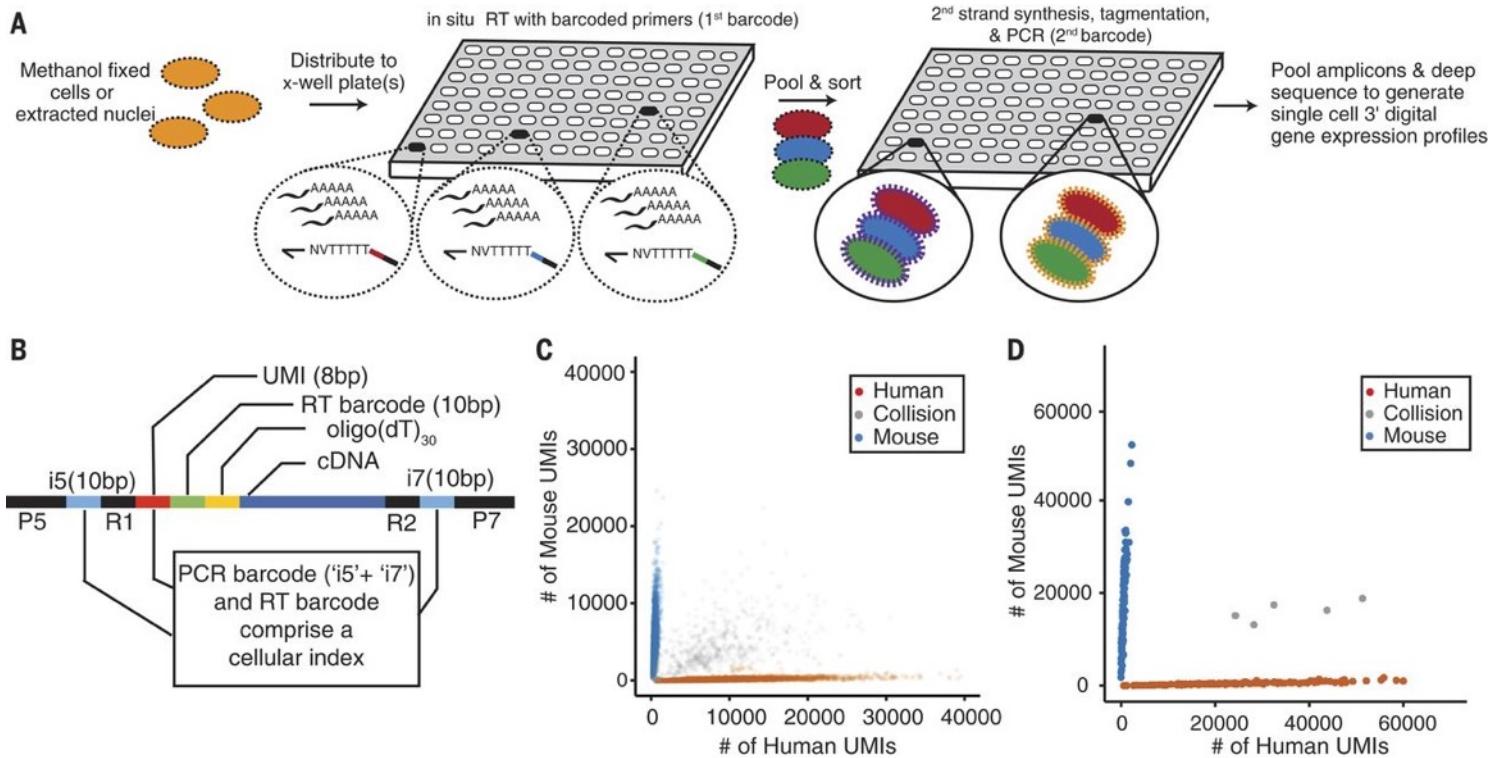
1-48 samples

WT Mega

100k-1M cells

1-96 samples

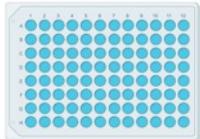
Combinatorial Indexing sci-Seq



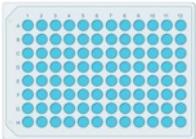
U. Washington
Shendure Lab

Combinatorial Scaling

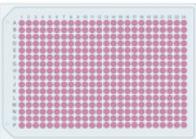
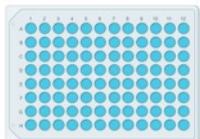
Round 1



Round 2



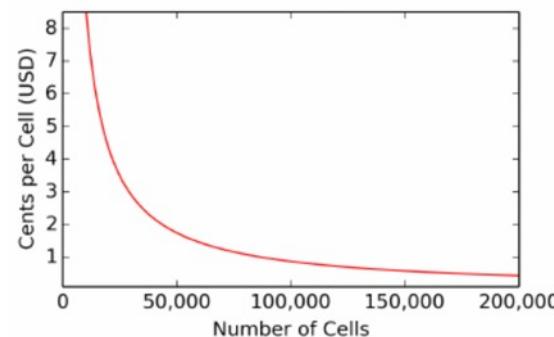
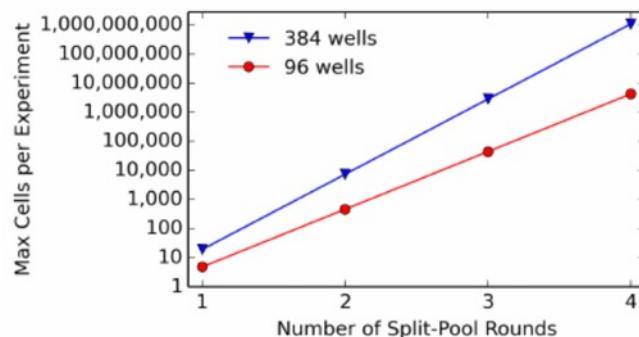
$$96 \times 96 = 9,216 \div 10 = \sim 921 \text{ cells}$$



$$96 \times 384 = 36,864 \div 10 = \sim 3,684 \text{ cells}$$



$$384 \times 384 = 147,456 \div 10 = 14,746 \text{ cells}$$



Enormously scalable
Can achieve <\$0.01 per cell

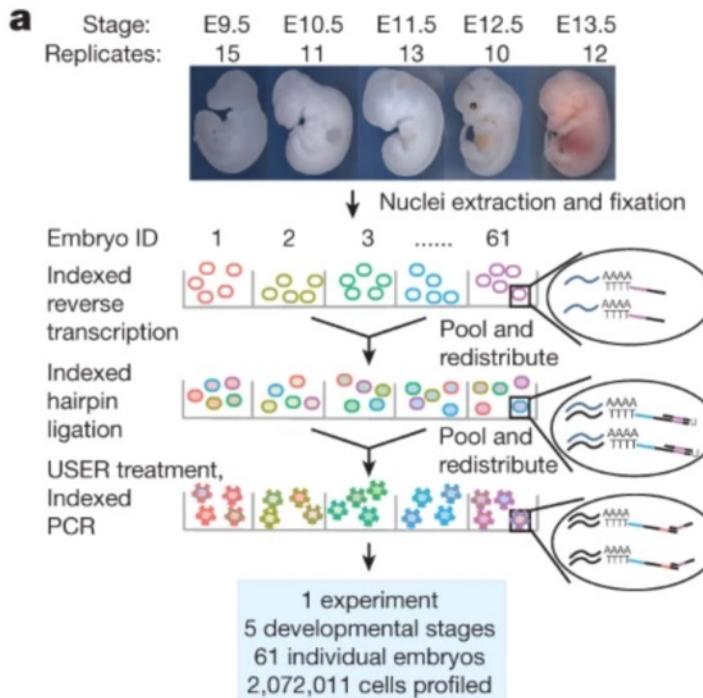
Labor intensive
Significant 'boot-up' cost
Significant validation cost
Who can afford that much sequencing, anyway?

The single-cell transcriptional landscape of mammalian organogenesis

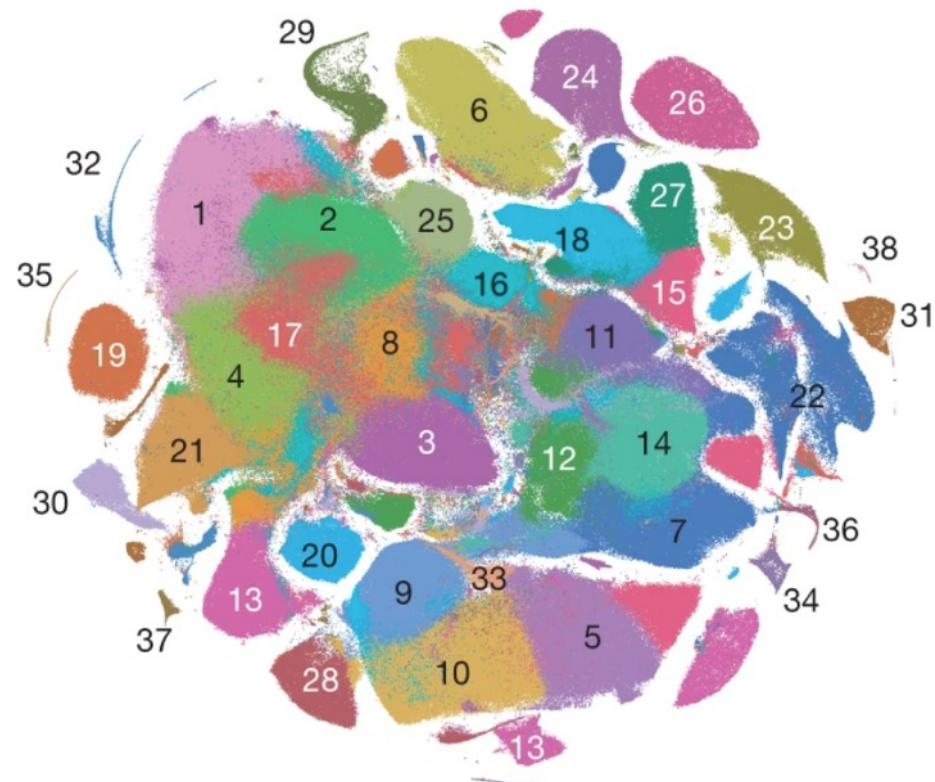
Junyue Cao, Malte Spielmann, Xiaojie Qiu, Xingfan Huang, Daniel M. Ibrahim, Andrew J. Hill, Fan Zhang,
Stefan Mundlos, Lena Christiansen, Frank J. Steemers, Cole Trapnell & Jay Shendure

Nature 566, 496–502(2019) | Cite this article

sci-RNA-seq3



Mouse embryonic development



2,058,652 single-cell transcriptomes

A human cell atlas of fetal gene expression

Junyue Cao^{1,*}, Diana R. O'Day², Hannah A. Pliner³, Paul D. Kingsley⁴, Mei Deng², Riza M. Daza¹, Michael A. Zager^{3,6}, Kimberly A. Aldinger^{2,5}, Ronnie Blecher¹, Fan Zhang⁷, Malte Spielmann^{8,9}, James Palis⁴, Dan Doherty^{2,3,5}, Frank J. Steemers⁷, Ian A. Glass^{2,3,5}, Cole Trapnell^{1,3,10,#}, Jay Shendure^{1,3,10,11,#}

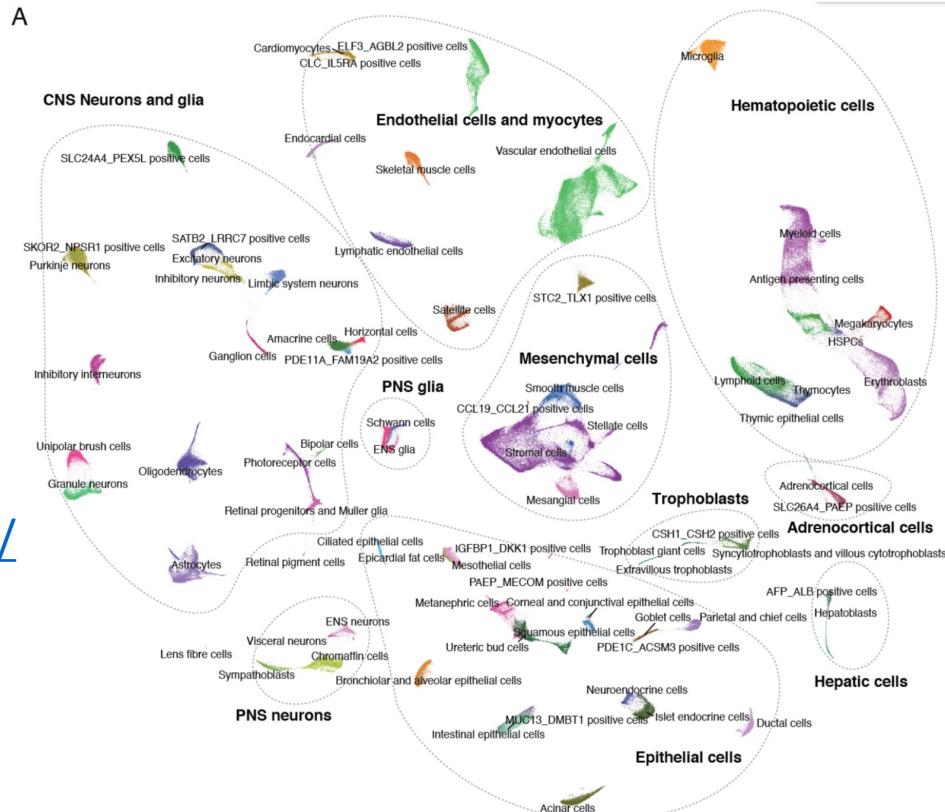
Published in final edited form as:

Science. 2020 November 13; 370(6518): . doi:10.1126/science.aba7721.

sci-RNA-seq3

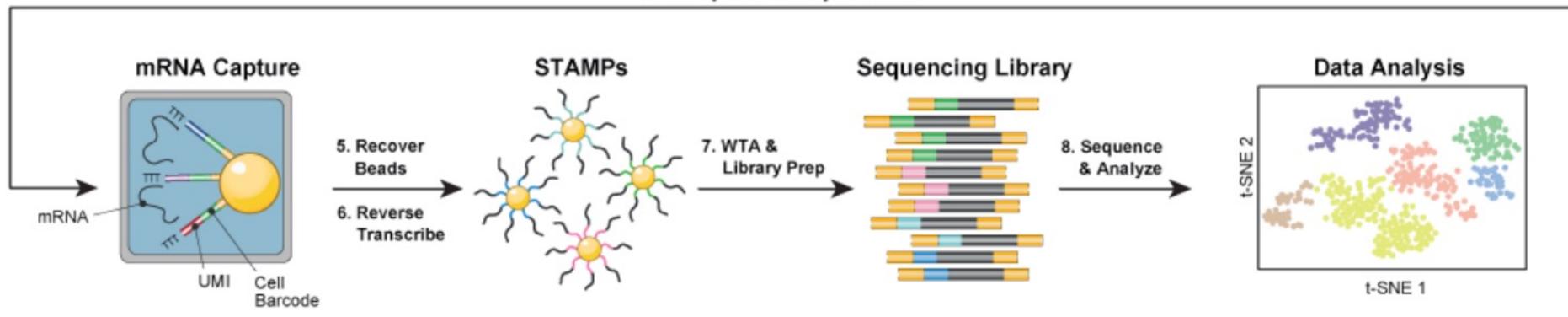
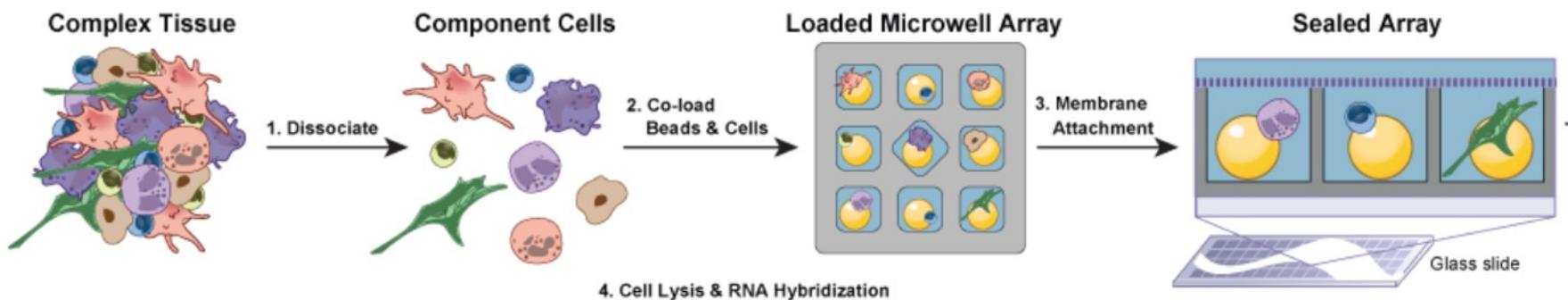
> 4M cells
15 organs
110 samples

<https://descartes.brotmanbaty.org/>



Seq-Well

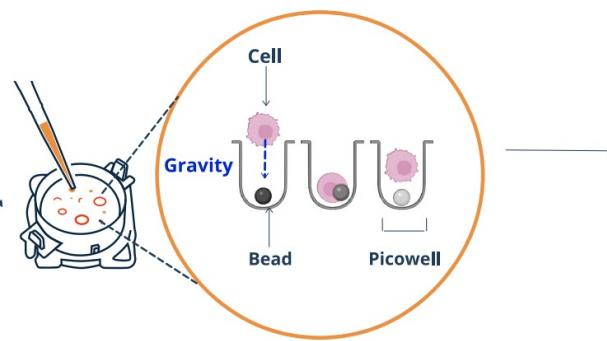
PDMS array of ~86,000 subnanoliter wells
Sized to fit 1 bead per well
Drop-Seq style barcoded beads
Sealed chamber for each cell





THE HIVE

HONEYCOMB

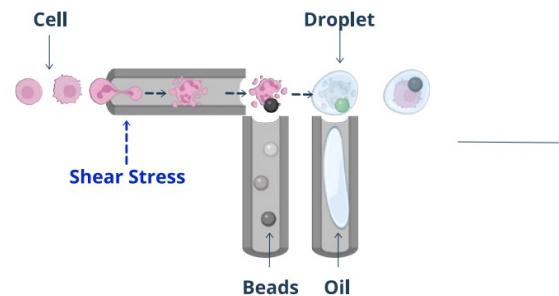


HIVE CELL CAPTURE: Gentle, by gravity or 30g spin.

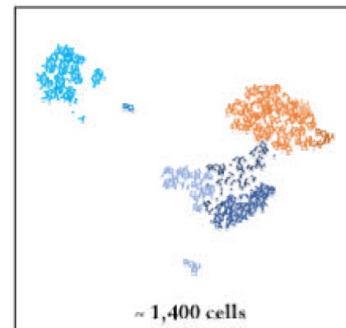
HIVE scRNASeq Method



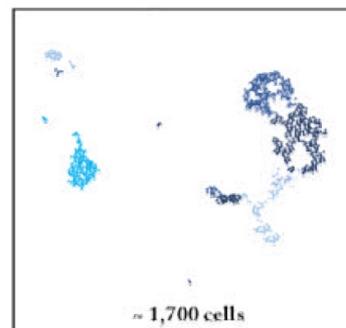
Droplet Based Method



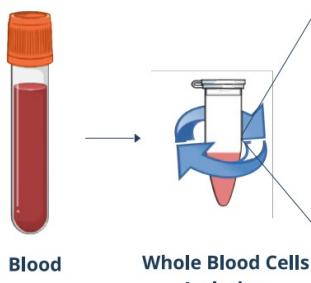
DROPLET CELL CAPTURE: Cells subjected to shear stress.



FULL BIOLOGY RECOVERED, including fragile cells like **granulocytes** (neutrophils, eosinophils, and basophils).



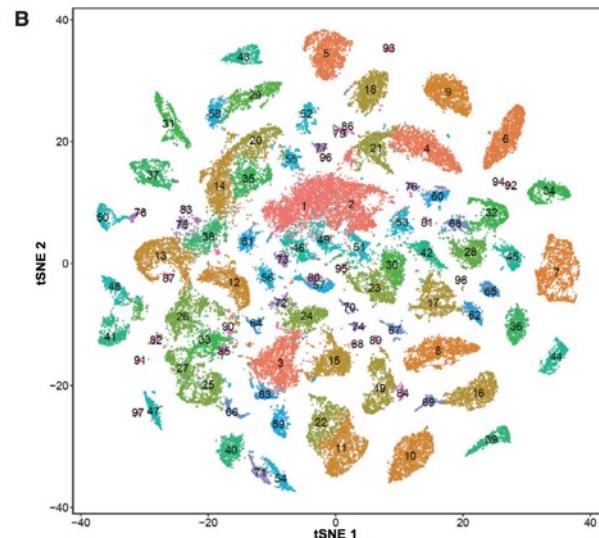
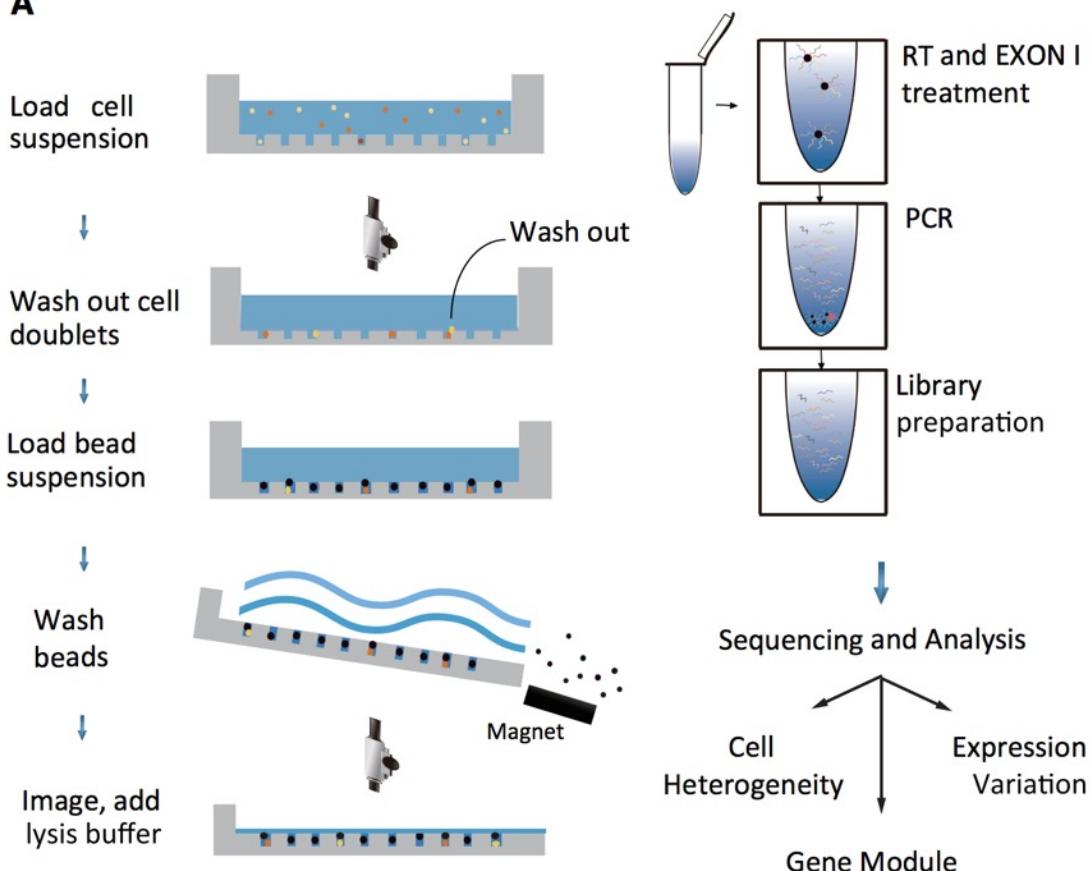
INCOMPLETE BIOLOGY RECOVERED, with fragile **granulocytes** missing.



Mapping the Mouse Cell Atlas by Microwell-Seq

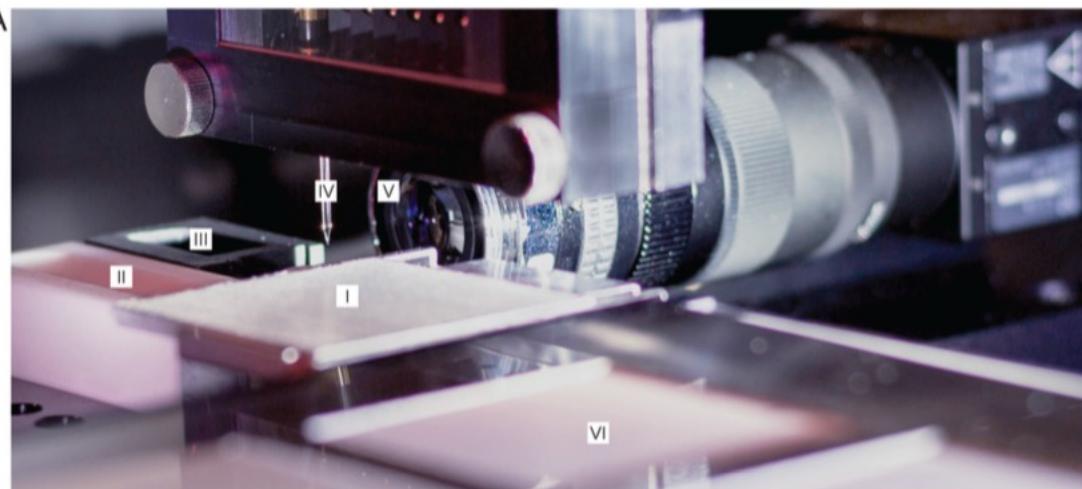
Xiaoping Han,^{1,12,13,*} Renying Wang,^{1,12,13} Yinchong Zhou,^{2,12,13} Lijiang Fei,^{1,12,13} Huiyu Sun,^{1,12,13} Shujing Lai,^{1,12,13} Assieh Saadatpour,¹¹ Ziming Zhou,^{1,12} Haide Chen,^{1,12} Fang Ye,^{1,12} Daosheng Huang,¹ Yang Xu,¹ Wentao Huang,¹ Mengmeng Jiang,^{1,12} Xinyi Jiang,^{1,12} Jie Mao,³ Yao Chen,⁴ Chenyu Lu,⁵ Jin Xie,⁶ Qun Fang,⁷ Yibin Wang,⁸ Rui Yue,⁸ Tiefeng Li,³ He Huang,^{9,12} Stuart H. Orkin,¹⁰ Guo-Cheng Yuan,¹¹ Ming Chen,^{2,12} and Guoji Guo^{1,9,12,14,*}

A



400,000 single cells from
major mouse organs

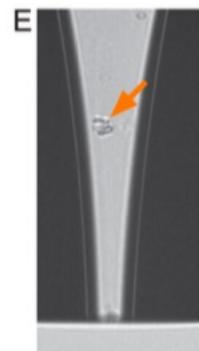
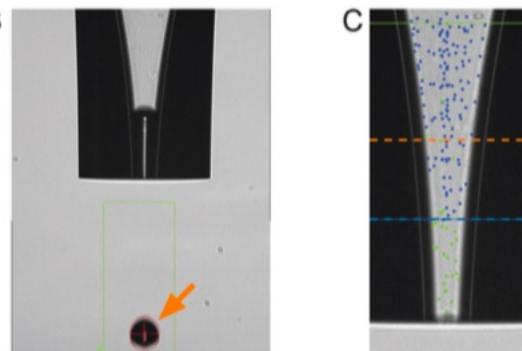
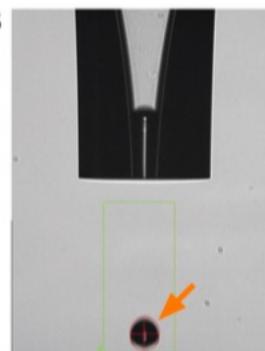
Array-based formats



Cell / reagent arrayers

Eg. Scienion sciFlexarray
Scienion cellenONE

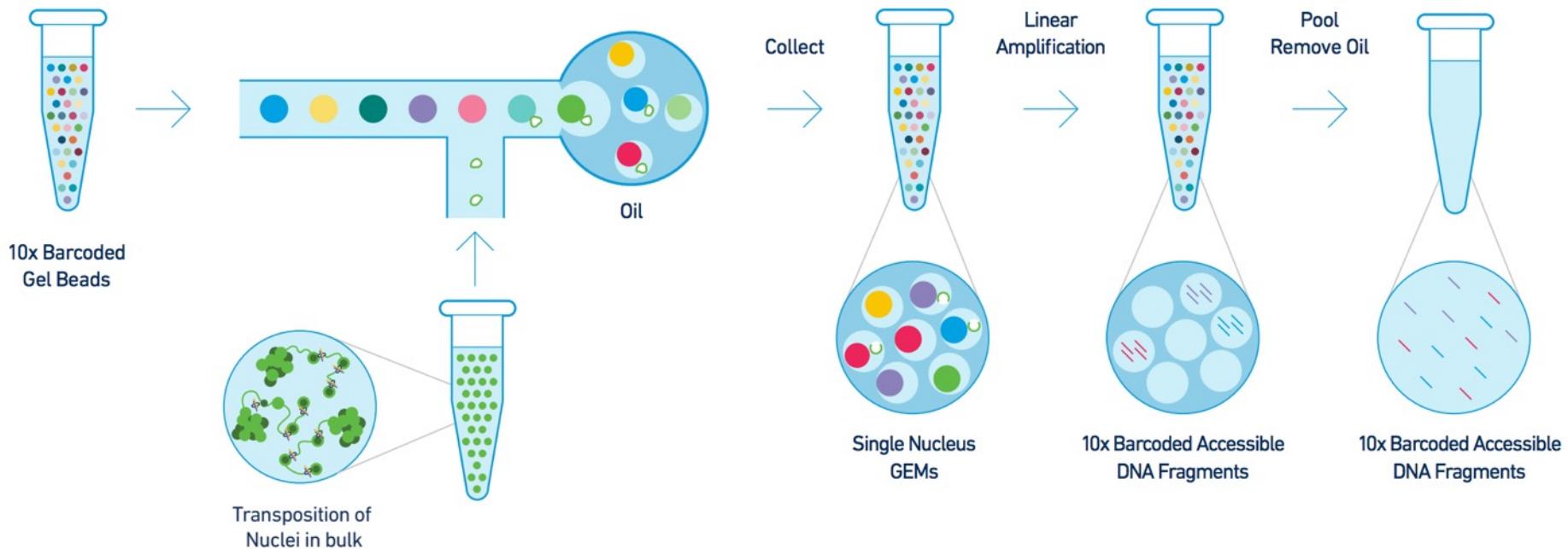
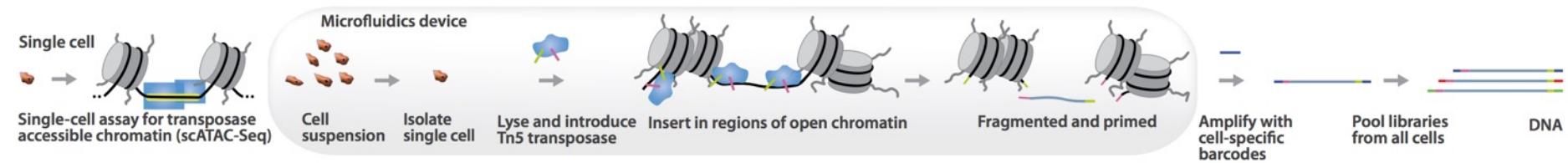
Custom workflows
Imaging-based sorting / rejection





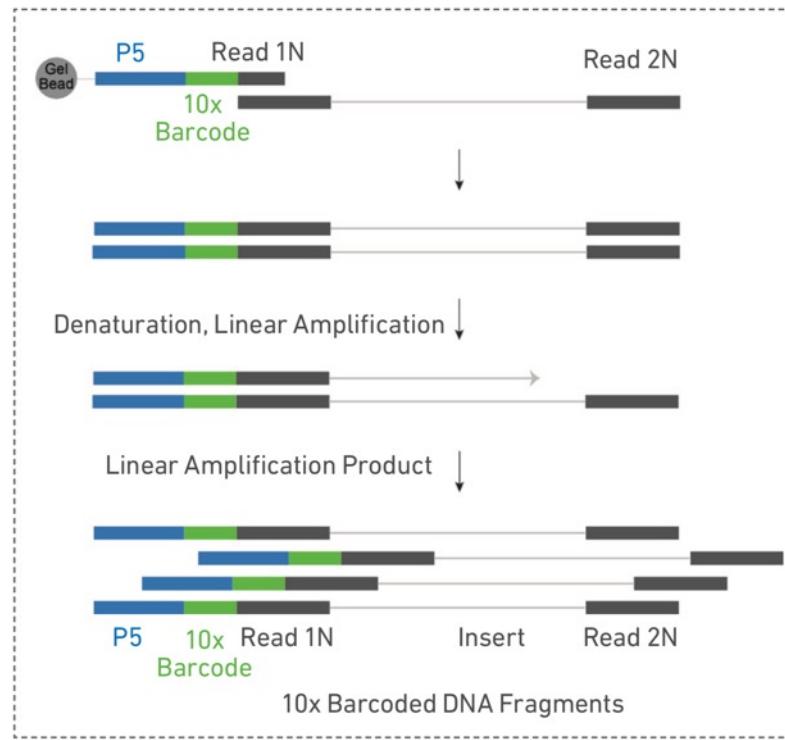
The Other
Nucleic Acid

10X Genomics Single Cell ATAC

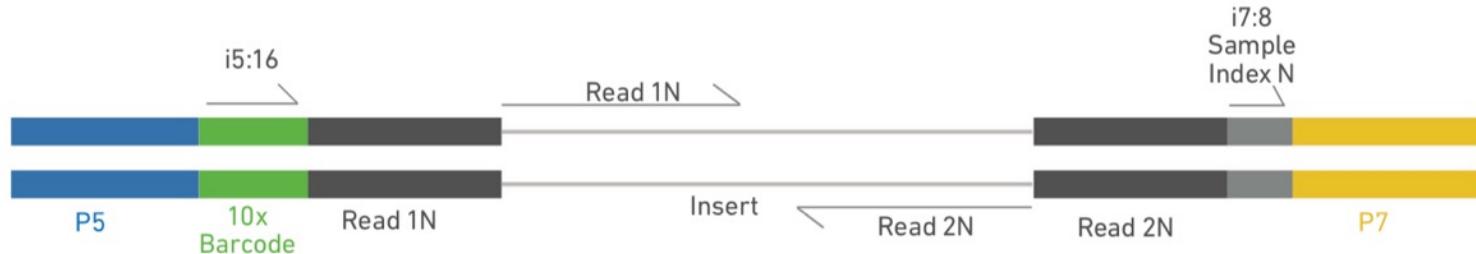


10X Genomics Single Cell ATAC

Inside Individual GEMs

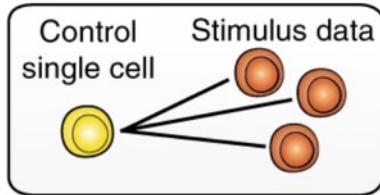


Chromium Single Cell ATAC Library



a

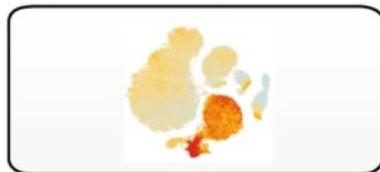
Find k -nearest neighbors ($k = 20$)



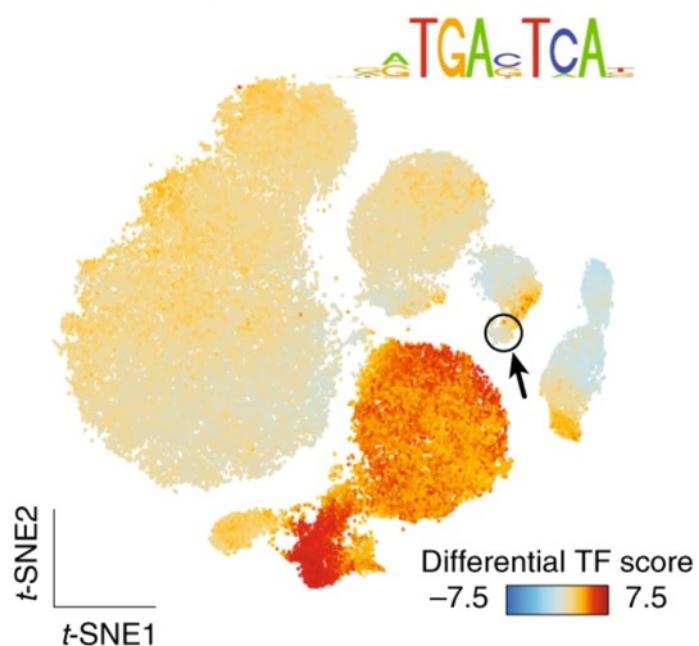
Compute differential TFs

Stimulation – control

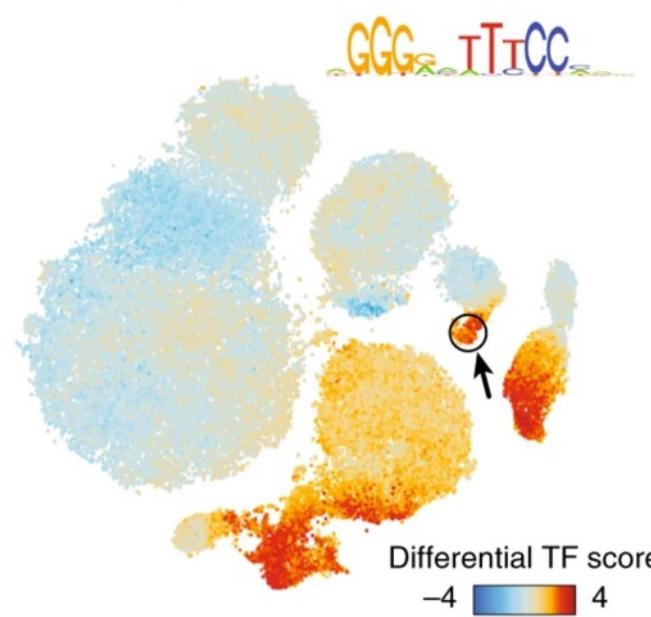
Smooth and display TFs on t-SNE

**b**

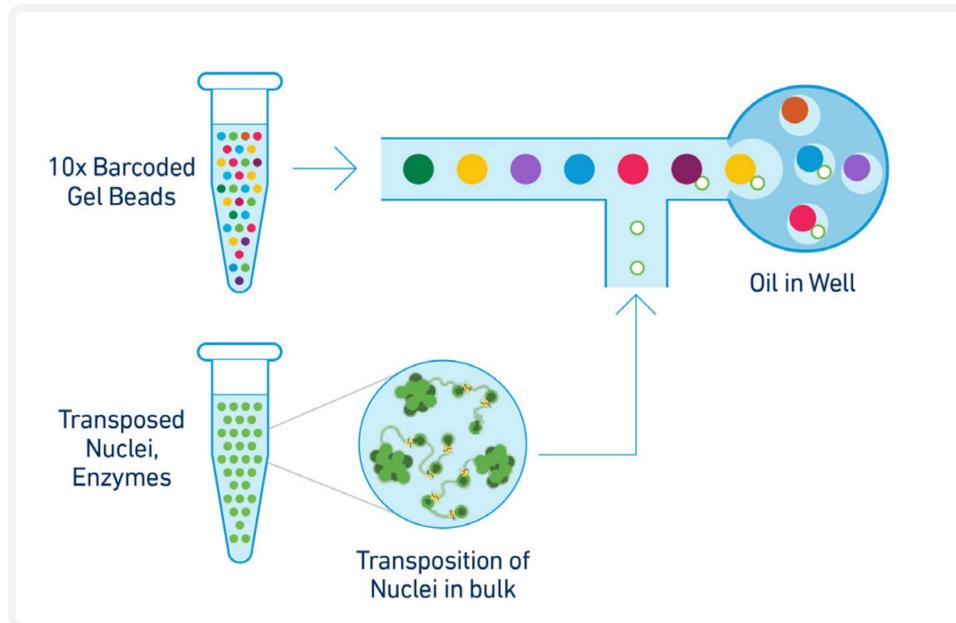
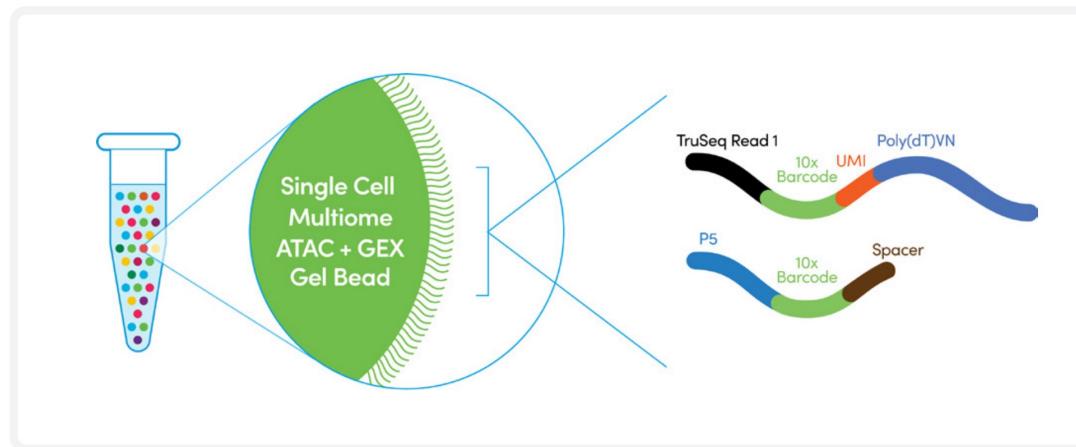
Jun TF motif
Stimulation – control

**c**

NF- κ B TF motif
Stimulation – control



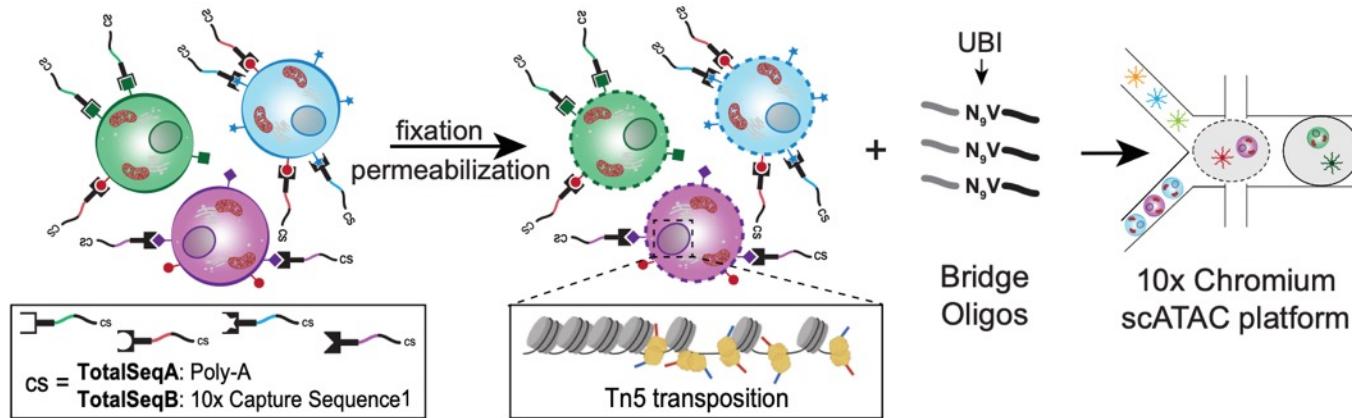
10X Genomics Multiome



ASAP-seq

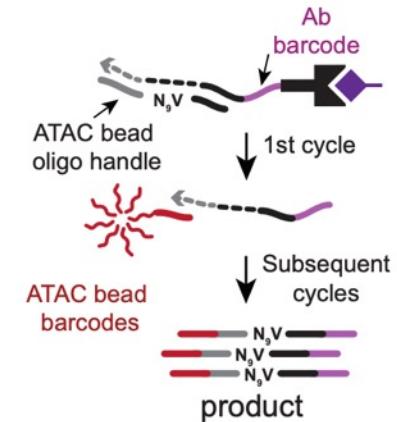
a

ASAP-seq = ATAC with Select Antigen Profiling by sequencing



b

Inside droplets



b

I. Annealing of antibody tag with BOA and extension in droplets



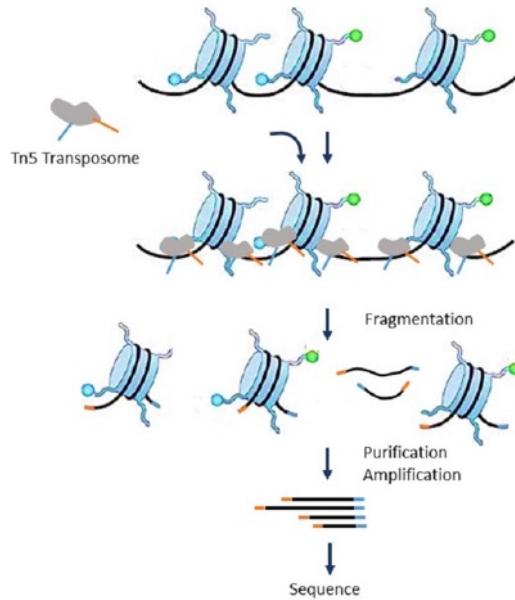
II. Annealing of extended antibody tag with barcoded oligo



III. Extension of barcoded oligo and amplification for ≤11 cycles

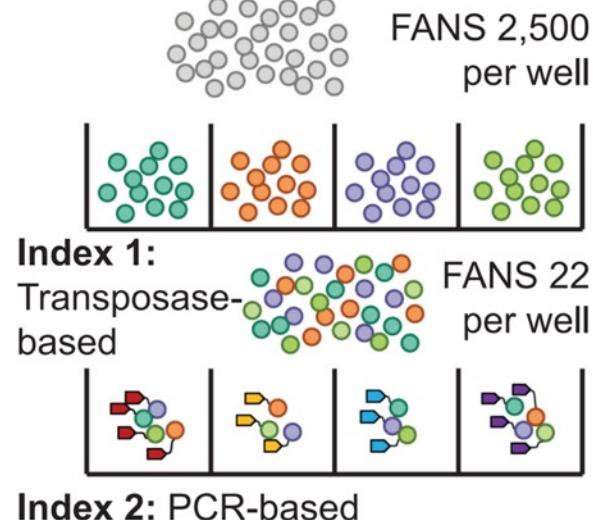


sci-ATAC



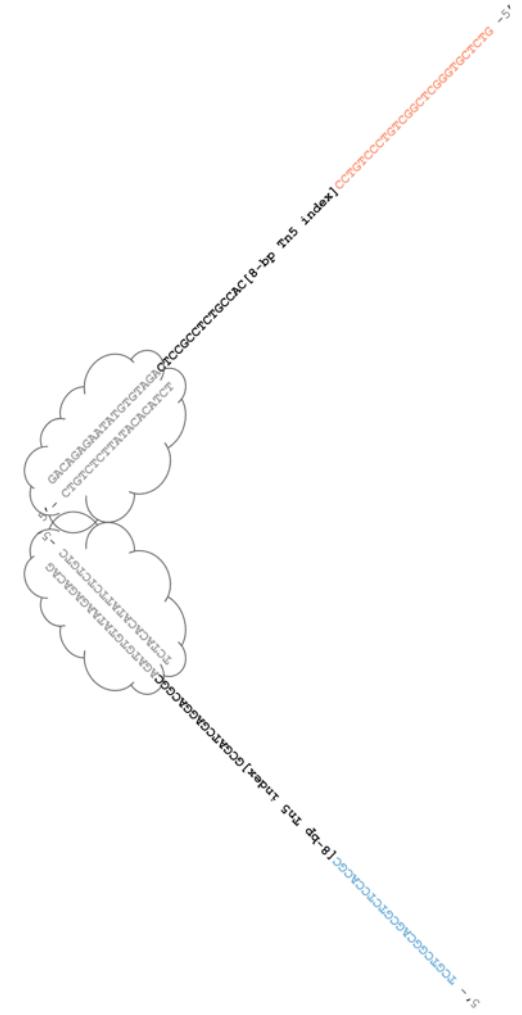
Round 1:
Internally Barcoded
Tn5 transposomes

Round 2:
Barcoded PCR primers



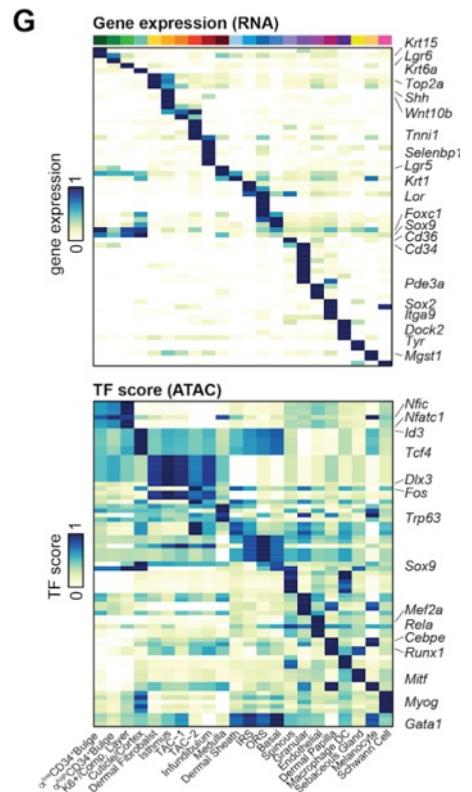
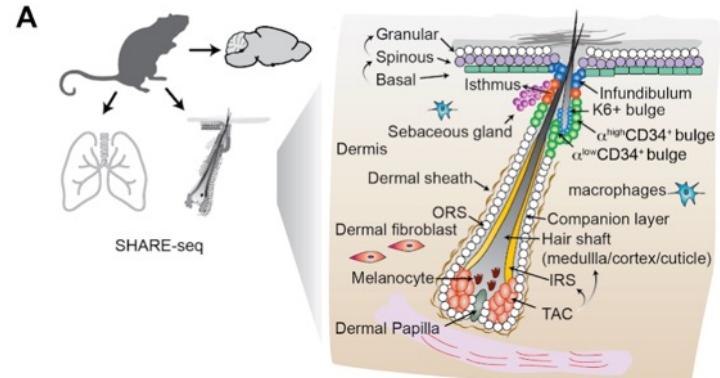
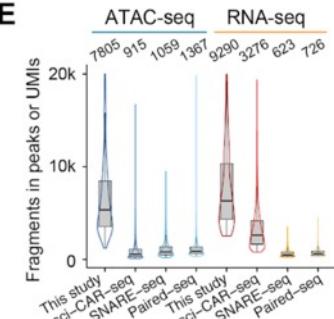
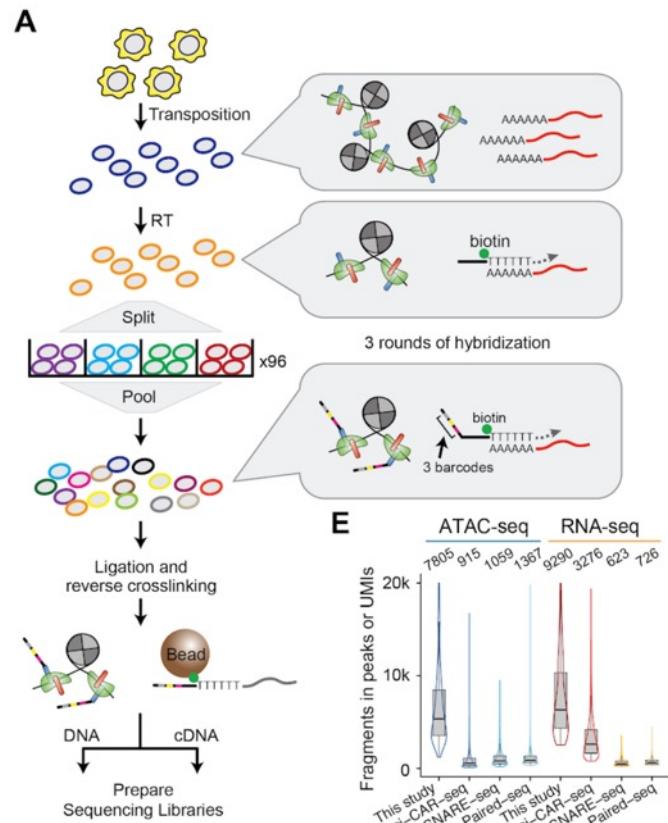
5' - AATGATACTGGCGACCCGAGATCTACACNNNNNNNNTCGTCGGCAGCGTCTCACGNNNNNNNNCGCATCGAGGACGGCAGATGTGATAAGAGACAGXXXXXX...XXXXXXCTGTCCTTATACACATCTGGCGGAGACGGTGNNNNNNNNNGACAGGGACACGGCGAGCCCACGAGACNNNNNNNNATCTCGTATGCCGCTCTCGCTTGG -3'
3' - TTACTATGCCGCTGGTGGCTAGATGTGNNNNNNNNAGCAGCGCTCCAGGGTGCNNNNNNNNCGCTAGCTCTGCCGCTCACATATTCTGTGXXXXXXXXX...XXXXXXGACAGGAATATGTGAGACTCCGCCCTGCCACNNNNNNNNCTGCCCCCTGCCGCTCTGNNNNNNNNTAGAGCATACGGCAGAGACGAAC -5'

Illumina P5 i5 s5 8 bp Tr5 barcode ME gDNA ME 8 bp Tr5 barcode s7 i7 Illumina P7



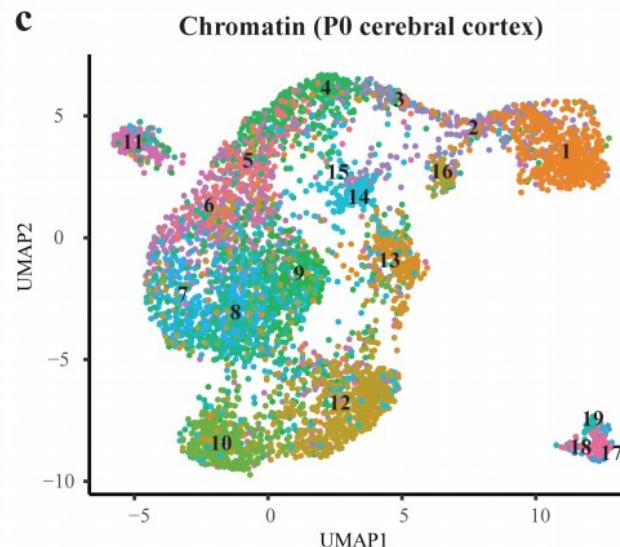
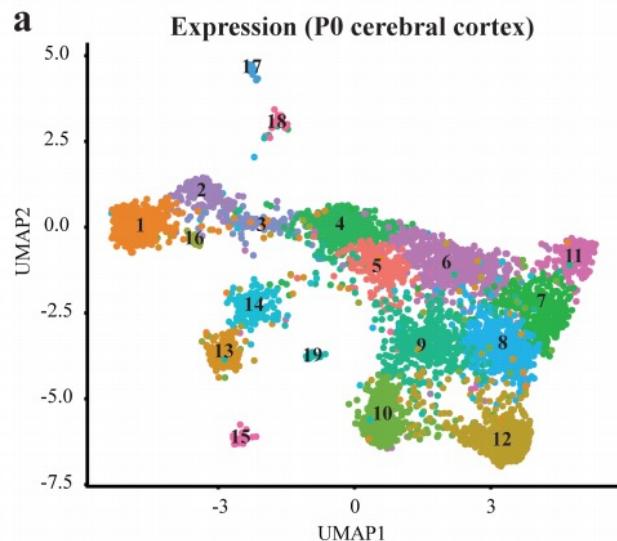
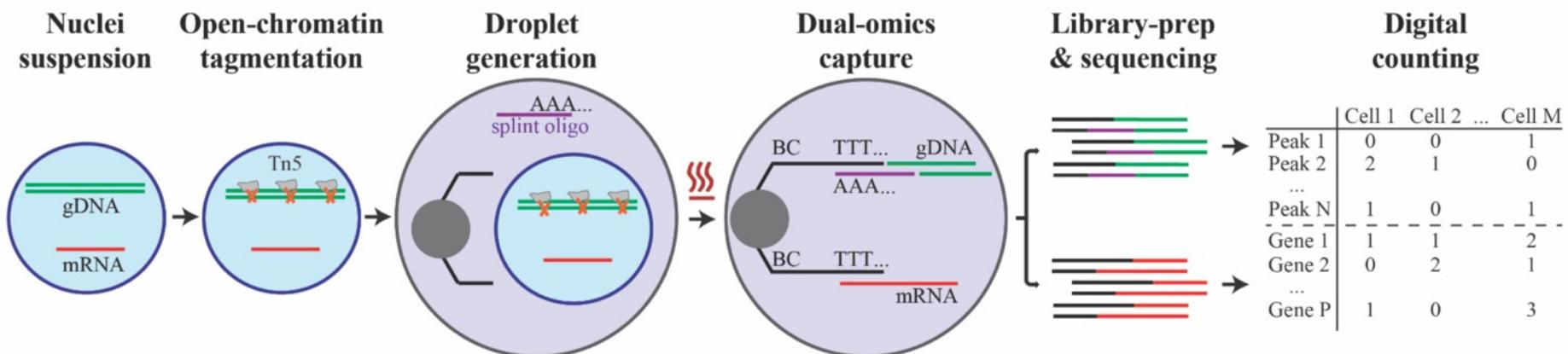
SHARE-Seq

- Same-cell scRNA/ATAC
- Combinatorial split-pool barcoding of adapters



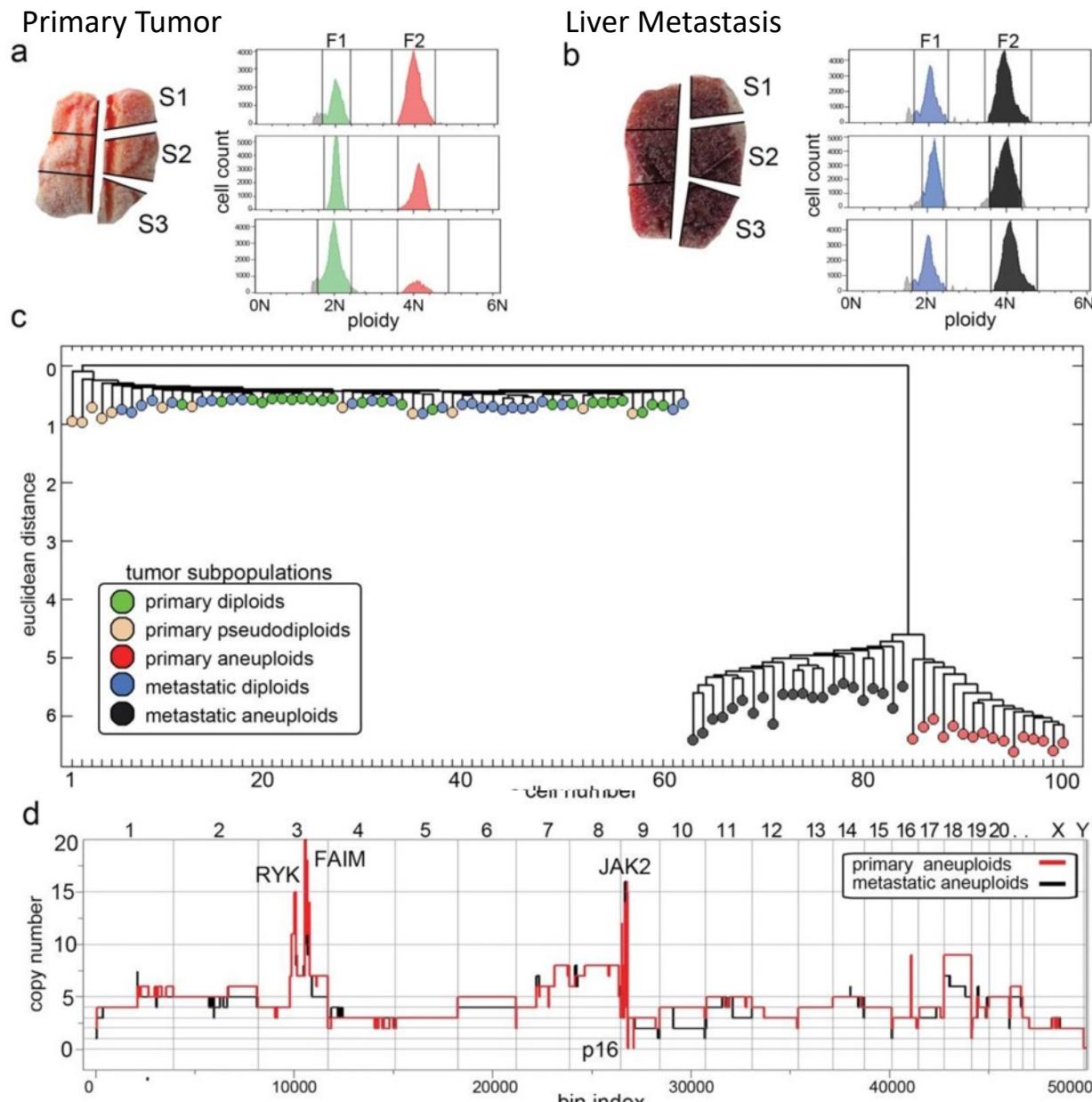
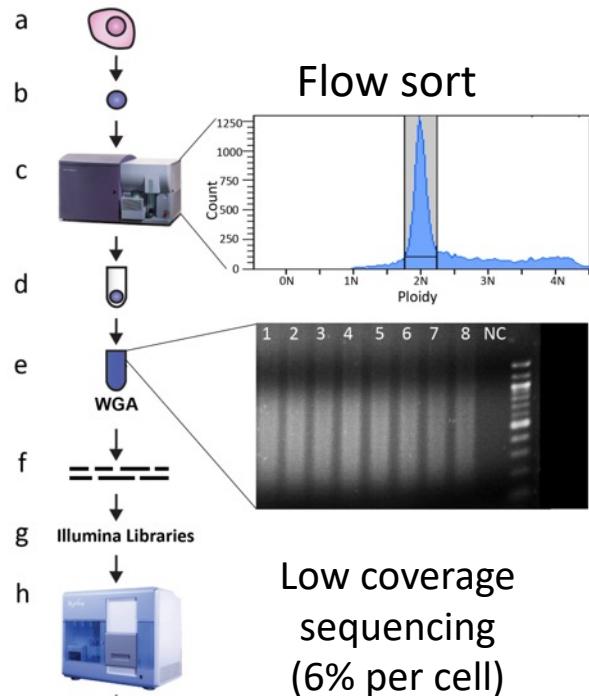
SNARE-seq

a

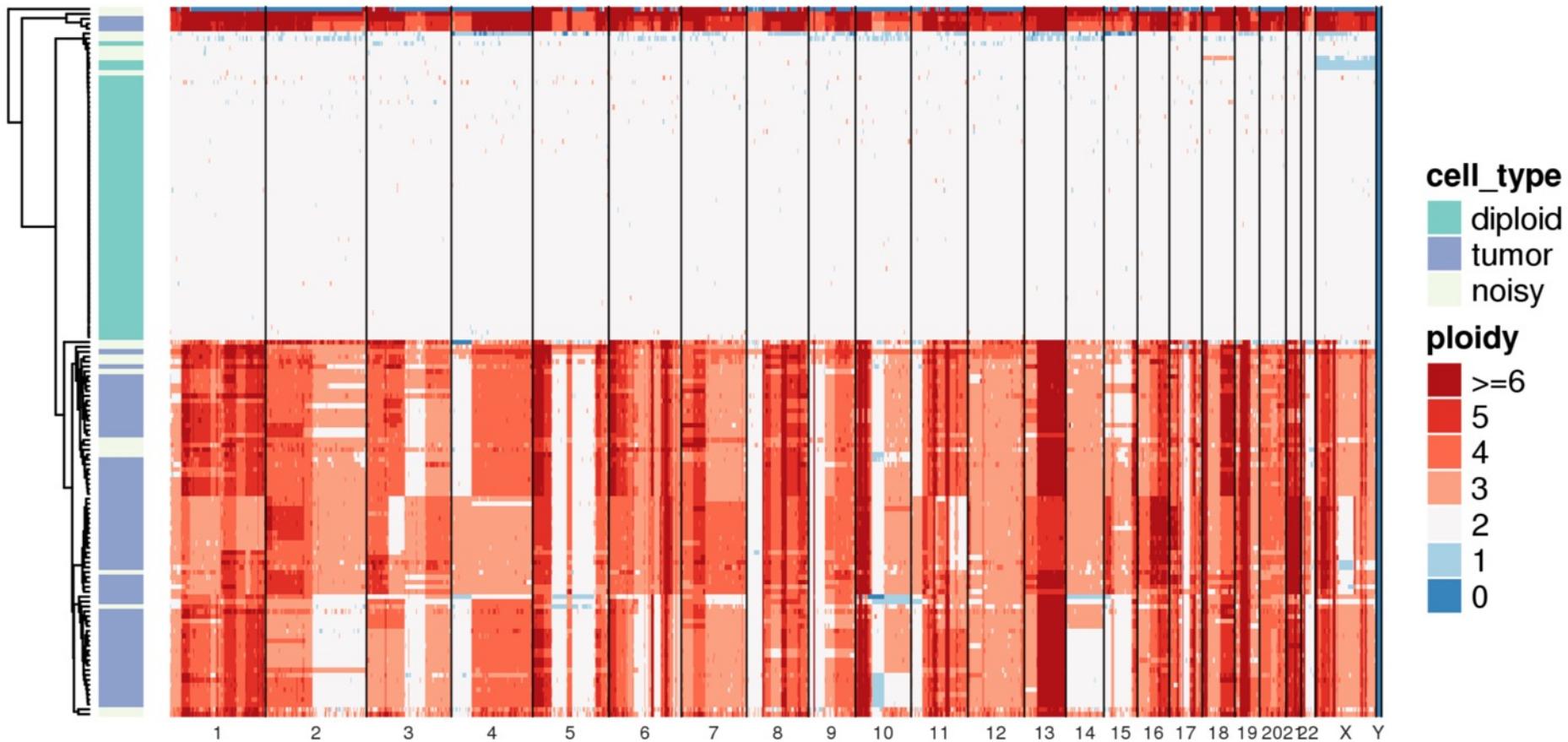


Single cell CNV

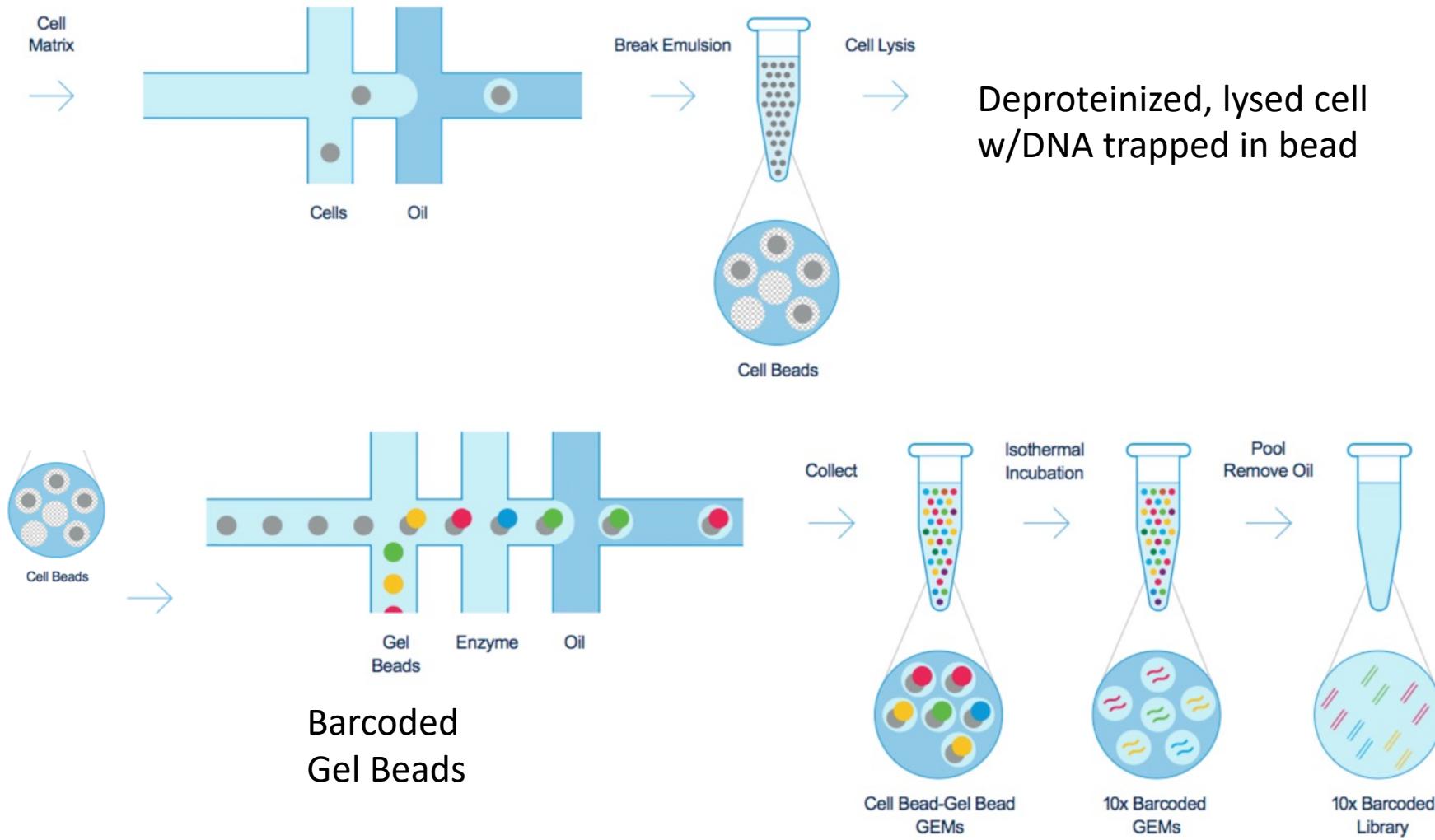
Nick Navin, Mike Wigler
CSHL

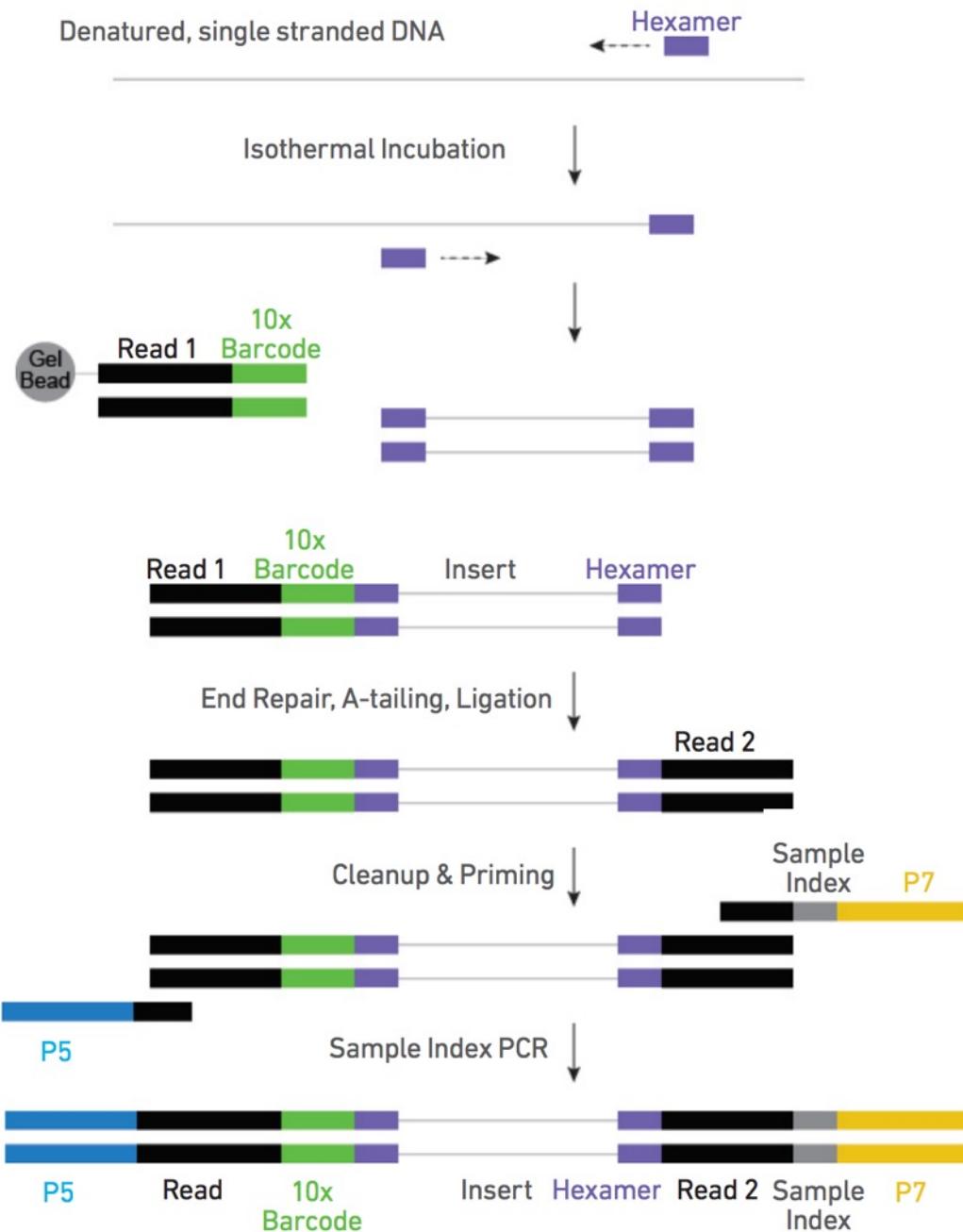


Droplet-based Single Cell CNV



Droplet-based Single Cell CNV

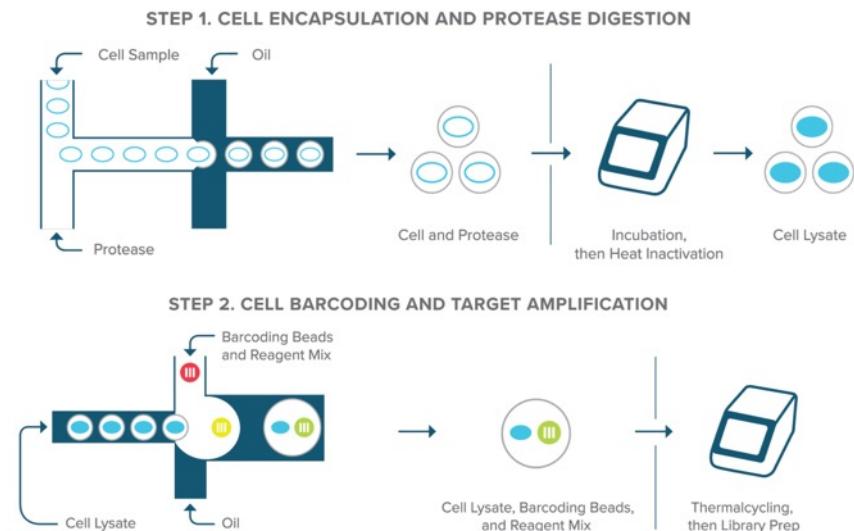




Mission Bio Tapestri

DNA-focused microfluidic platform

For SNV & CNV



Mission Bio Tapestri

59 GENES - TUMOR HOTSPOT PANEL

ABL1	CSF1R	FGFR1	IDH2	MLH1	RB1
AKT1	CTNNB1	FGFR2	JAK1	MPL	RET
ALK	DDR2	FGFR3	JAK2	MTOR	SMAD4
APC	EGFR	FLT3	JAK3	NOTCH1	SMARCB1
AR	ERBB2	GNA11	KDR	NRAS	SMO
ATM	ERBB3	GNAQ	KIT	PDGFRA	SRC
BRAF	ERBB4	GNAS	KRAS	PIK3CA	STK11
CDH1	ESR1	HNF1A	MAP2K1	PTEN	TP53
CDK4	EZH2	HRAS	MAP2K2	PTPN11	VHL
CDKN2A	FBXW7	IDH1	MET	RAF1	

45-GENE MYELOID PANEL

ASXL1	ERG	KDM6A	NRAS	SMC1A
ATM	ETV6	KIT	PHF6	SMC3
BCOR	EZH2	KMT2A	PPM1D	STAG2
BRAF	FLT3	KRAS	PTEN	STAT3
CALR	GATA2	MPL	PTPN11	TET2
CBL	GNAS	MYC	RAD21	TP53
CHEK2	IDH1	MYD88	RUNX1	U2AF1L5
CSF3R	IDH2	NF1	SETBP1	WT1
DNMT3A	JAK2	NPM1	SF3B1	ZRSR2

Clonal Architecture Resolved Over Time

