

Single Cell Sequencing



CSHL Course: Advanced Sequencing Technologies & Bioinformatics

Jon Preall
Research Associate Professor
Cold Spring Harbor Laboratory



Single Cell Biology



Jon Preall

Director



Claire Regan

Comp. Sci Analyst



Evan Zhang

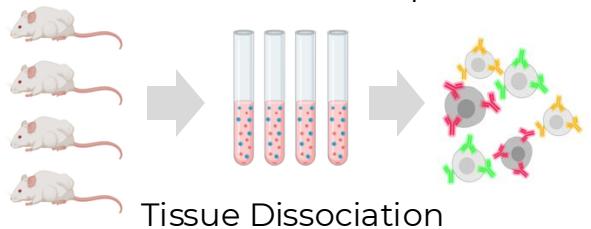
Technician



Lauren Ward

Technician

Study Design



Sony SH800
FACSorter



QC



10X Genomics
Chromium

Library prep & Sequencing

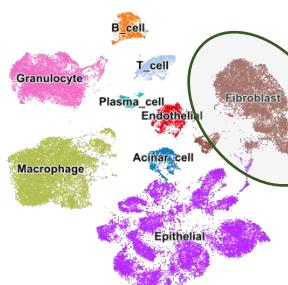


Bench

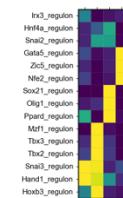
Batch Correction
Data Integration



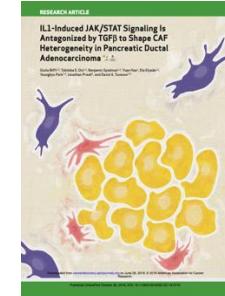
Clustering
Cell Type Identification



RNA Velocity
Trajectory inference
Transcription Factor Modules

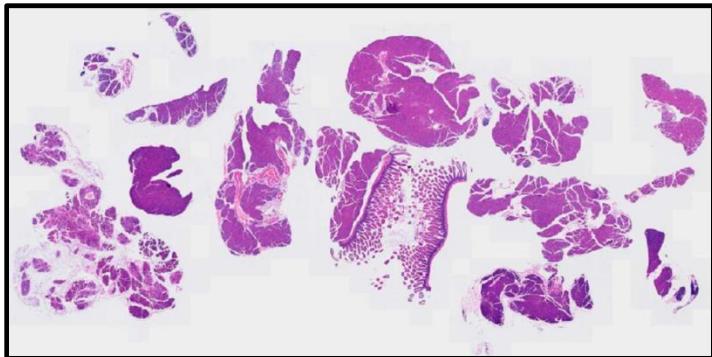


Publication



Lineage Analysis

Spatial Biology Initiative



Histology Core Facility



Kristin Milicich
Manager



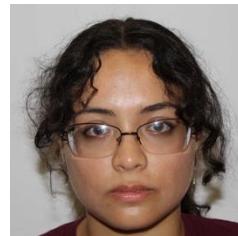
Regina Borello
Histotech III



Denise Cahn
Histotech III



Bella Sanchez
Histotech I



Ashmita Pandey
Histotech I



Lunaphore COMET



Jon Preall

Director

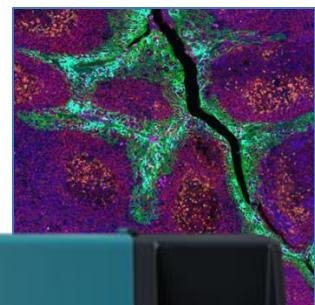
Claire Regan

Comp. Sci Analyst

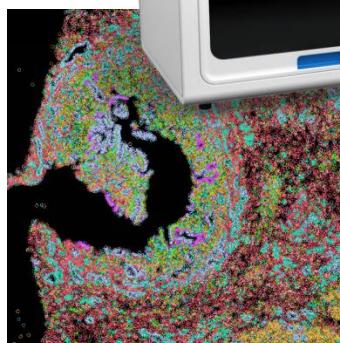
Evan Zhang

Technician

Lauren Ward



Xenium



Lunaphore COMET



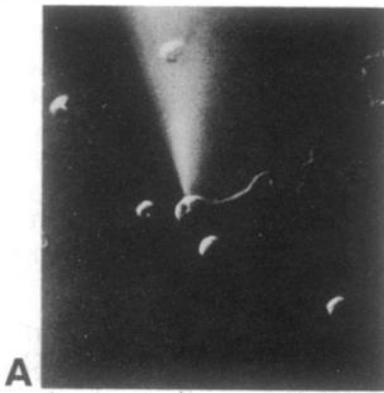
Visium CytAssist

Analysis of gene expression in single live neurons

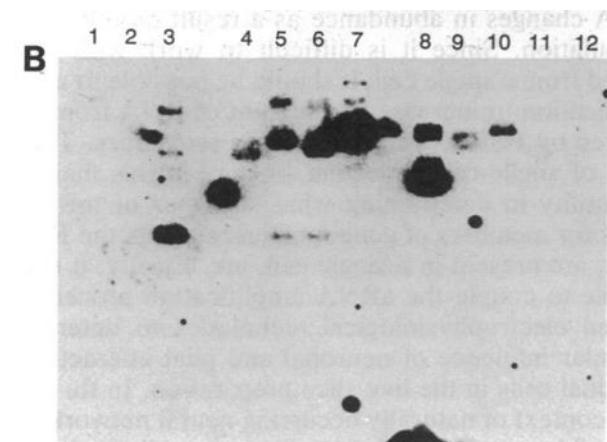
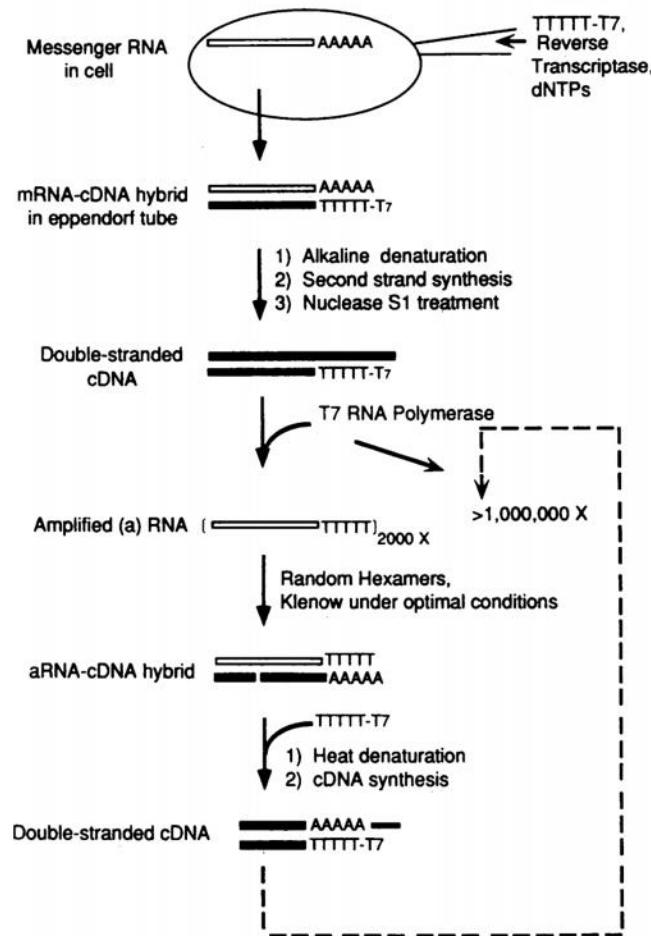
(amplified, antisense RNA/expression profile/mRNA complexity/pyramidal cell)

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

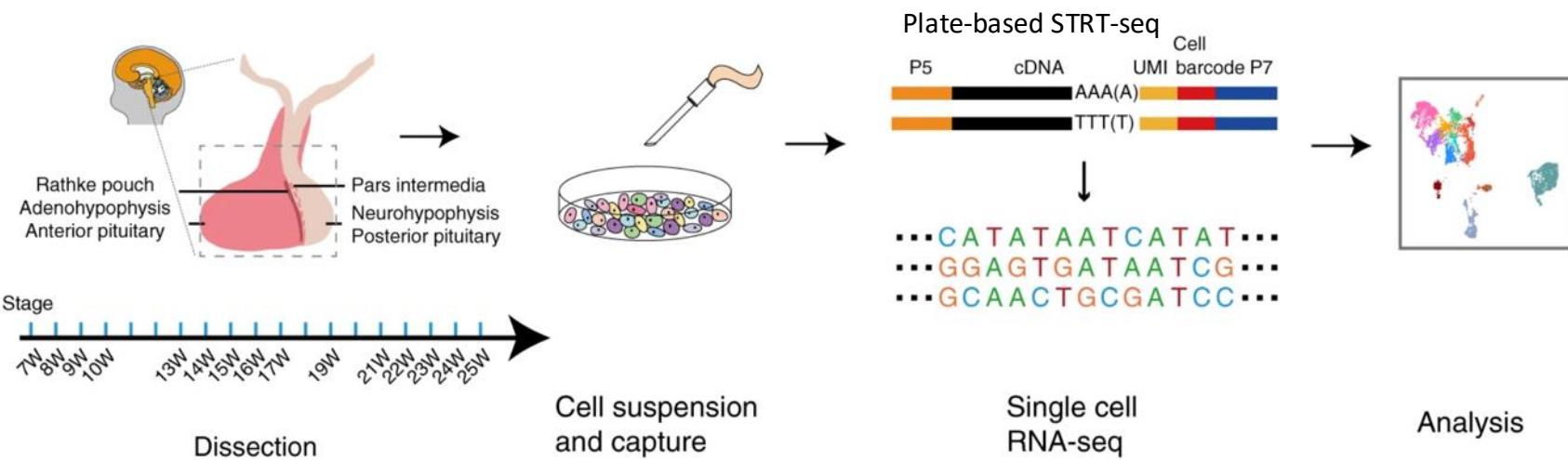
1992

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✉

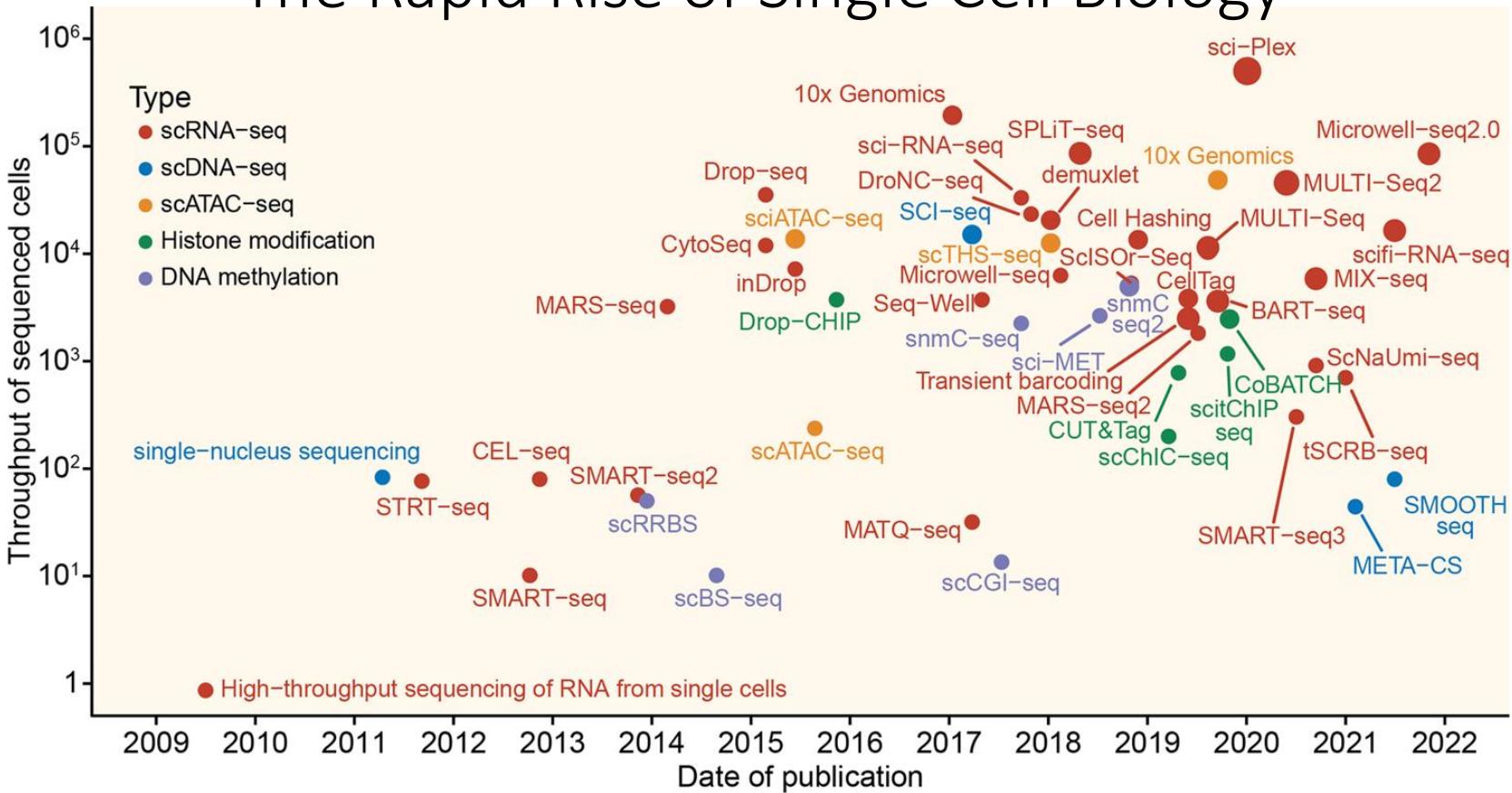
Nature Communications 11, Article number: 5275 (2020) | Cite this article

a



4,113 mouth-pipetted cells!

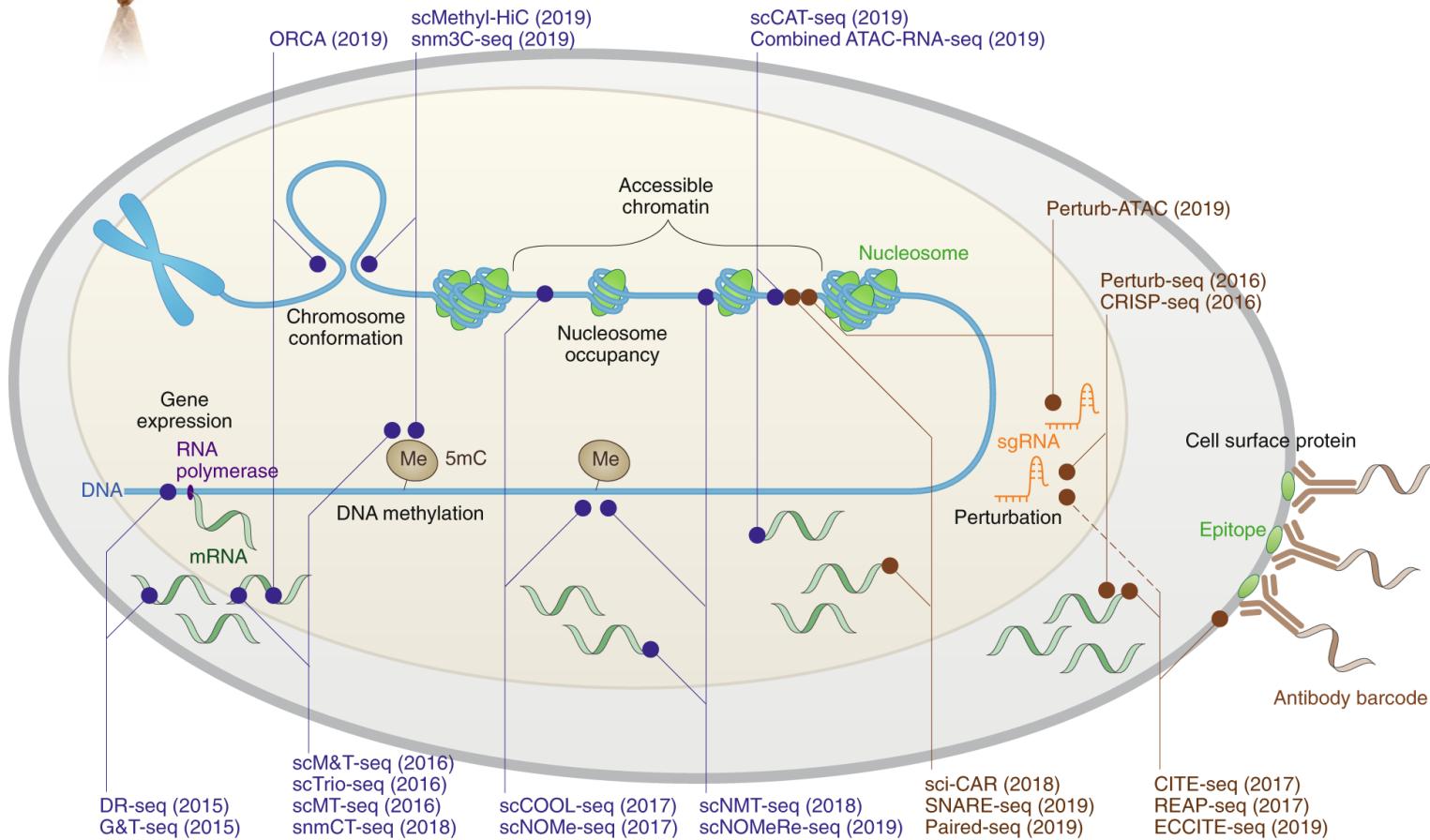
The Rapid Rise of Single Cell Biology



Jia, Q., Chu, H., Jin, Z. et al. High-throughput single-cell sequencing in cancer research. *Sig Transduct Target Ther* 7, 145 (2022)



Many Flavors of Multi- 'Omics



M versus N

Modalities

Replicates

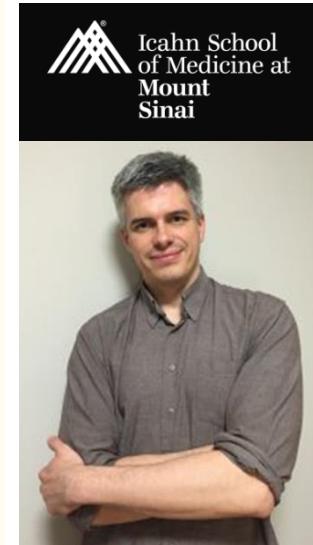
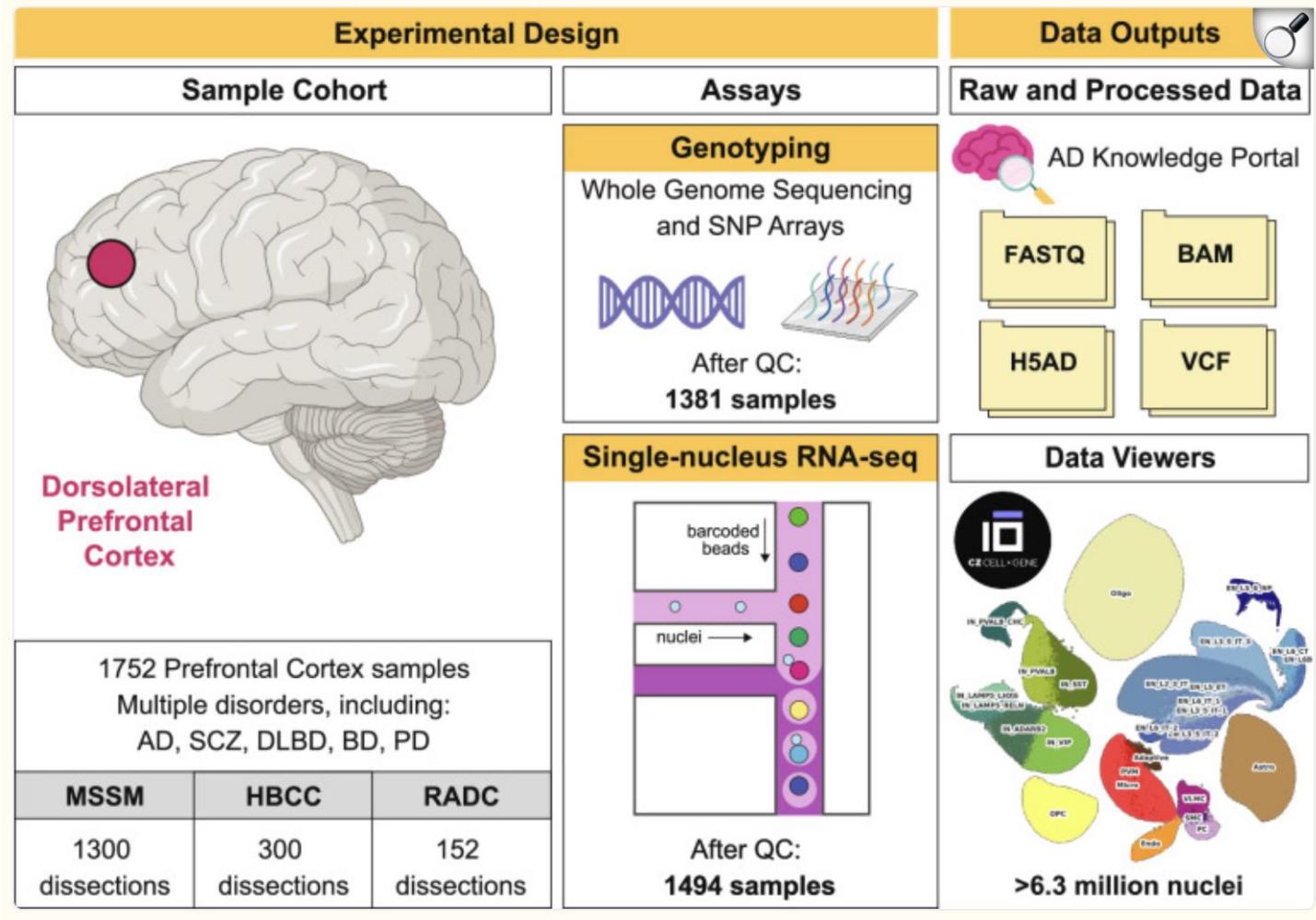
Resources are limited.

Prioritize M: exploratory research, hypothesis generation

Prioritize N: statistical rigor, variable isolation, hypothesis testing

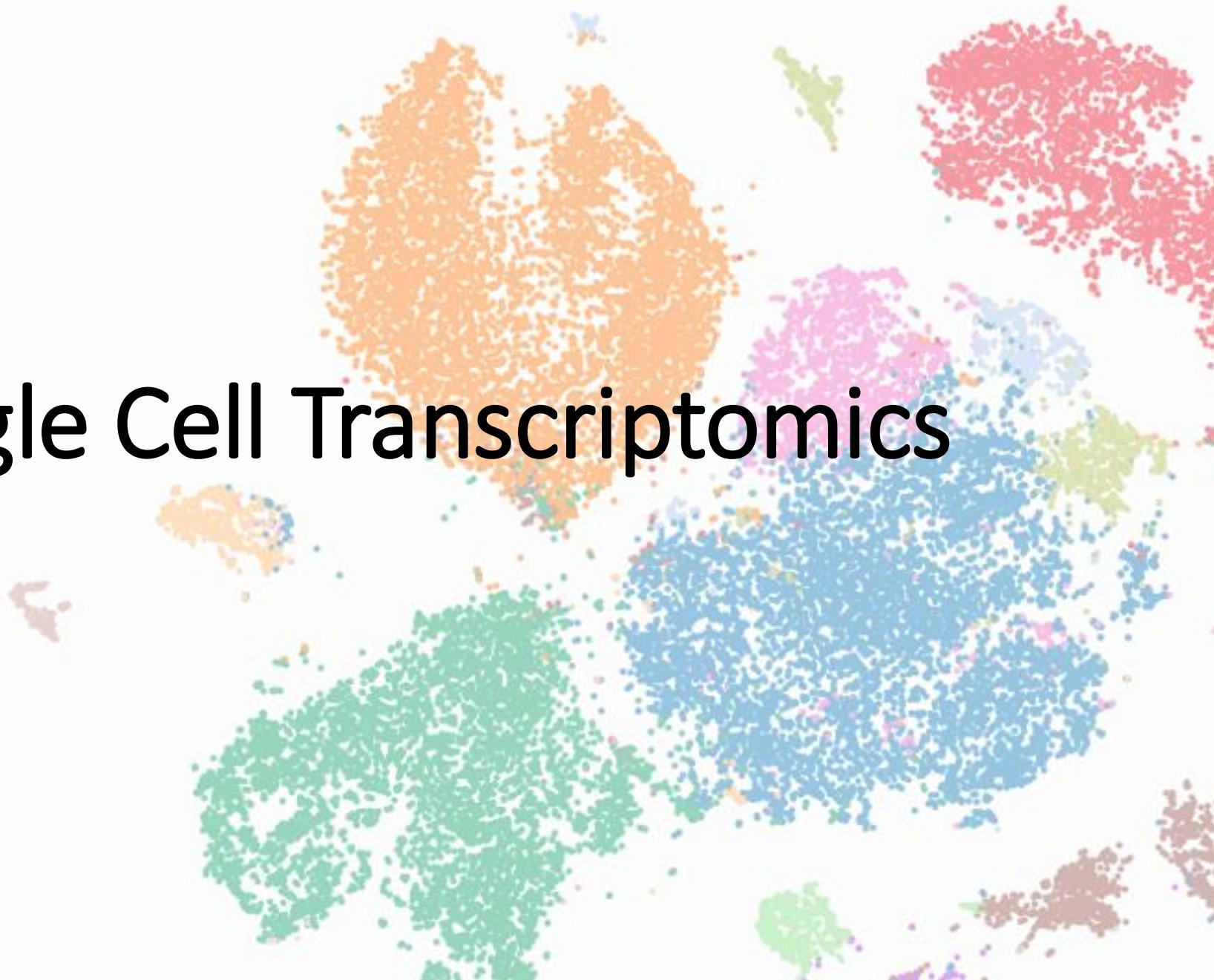


Population-scale cross-disorder atlas of the human prefrontal cortex at single-cell resolution

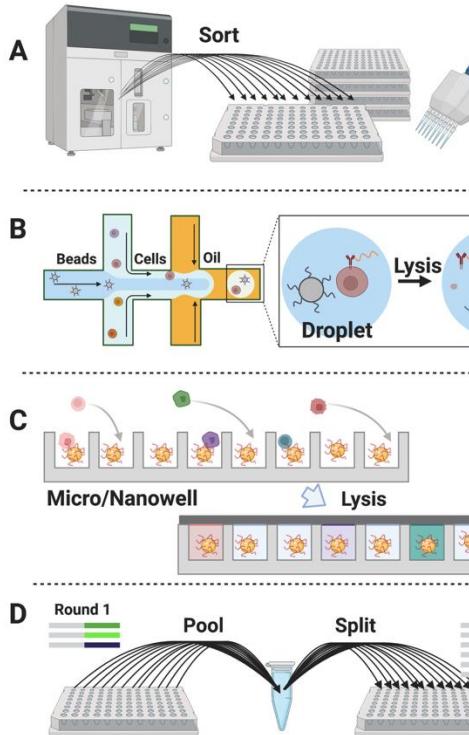


Panos Roussos

Single Cell Transcriptomics



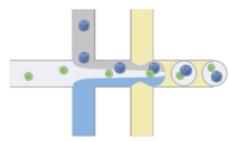
scRNAseq Platforms



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

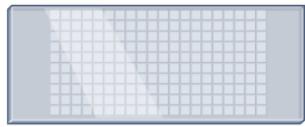
Comparative Analysis of Commercial Single-Cell RNA Sequencing Technologies

Single Cell RNA Sequencing



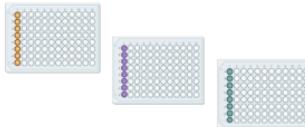
Emulsion:

- Chromium Single Cell 3' Reagent Kit, v3.1 Chemistry (10X Genomics)
- Chromium Single Cell 5' Reagent Kit, v2 Chemistry (10X Genomics)
- Chromium Fixed RNA Profiling (FRP) Reagent Kit (10X Genomics)
- PIPseq T20 3' Single Cell RNA Kit v4.0 (Fluent Biosciences)



Microwell:

- BD Rhapsody WTA Reagent Kit (Becton Dickinson)
- HIVE CLX scRNA Seq Kit (Honeycomb Biotechnologies)
- GEXSCOPE® Single Cell RNA Library Kit (Singleron Biotechnologies)



In plate combinatorial indexing:

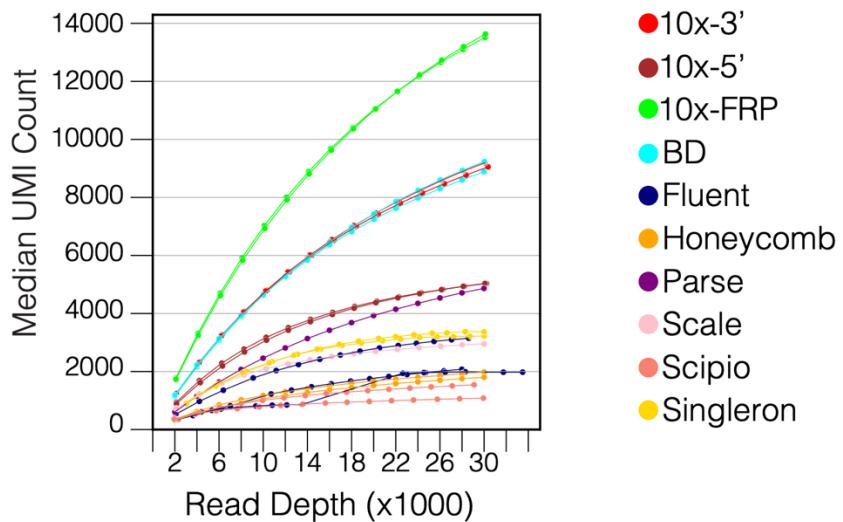
- Evercode WT v2 (Parse Biosciences)
- Single Cell RNA Kit (Scale Biosciences)



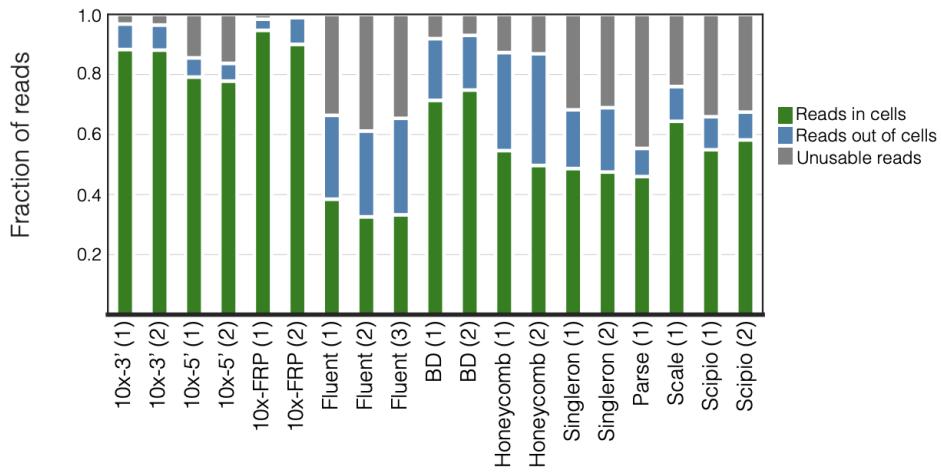
Matrikel:

- ASTERIA Single-cell RNASeq Kit (Scipio Bioscience)

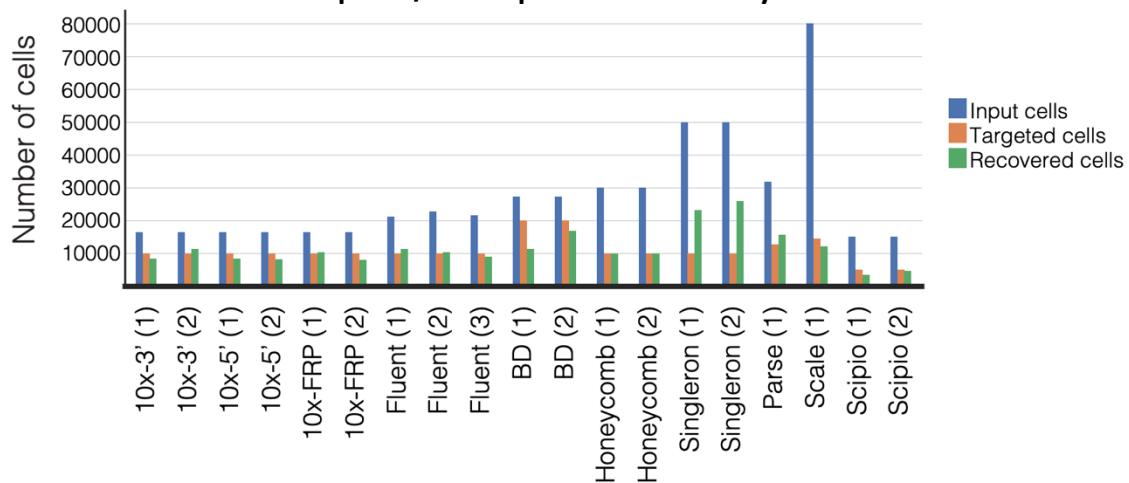
RNA sensitivity



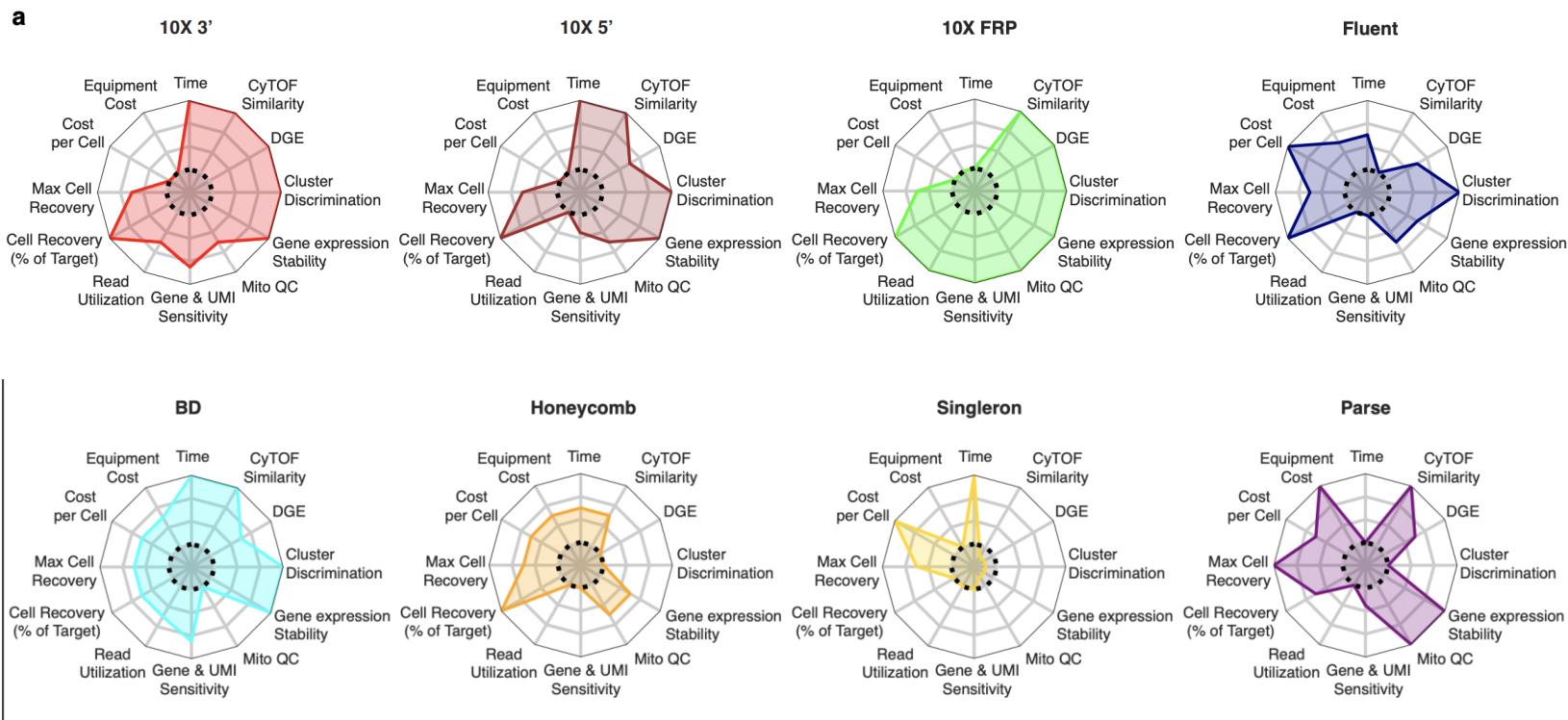
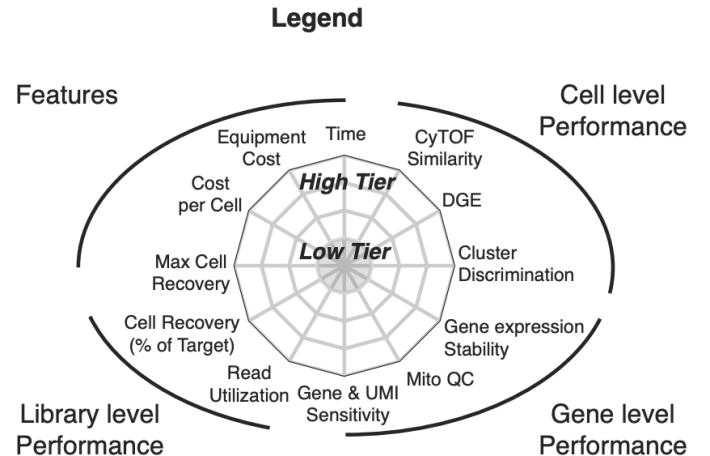
Sequencing efficiency



Cell Input / Output Efficiency



Multi-Dimensional Performance Summary



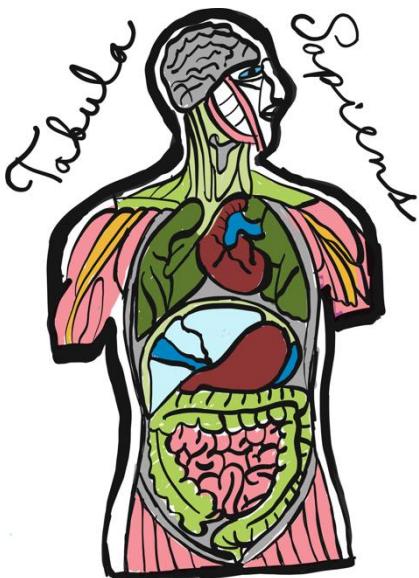
10X Genomics: the *lingua franca* of the single-cell age



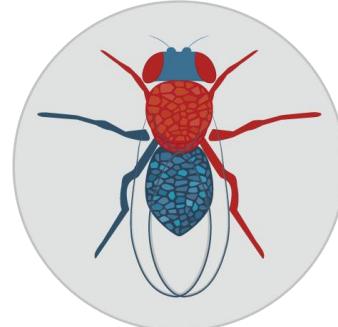
- Easy
- Robust
- Expensive.



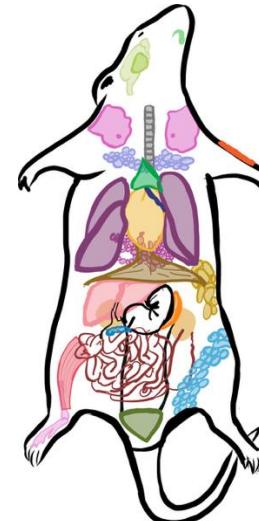
Allen Brain Map



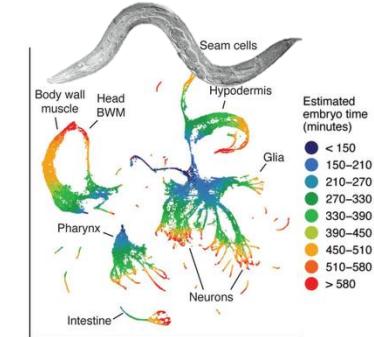
Fly Cell Atlas



Tabula Muris

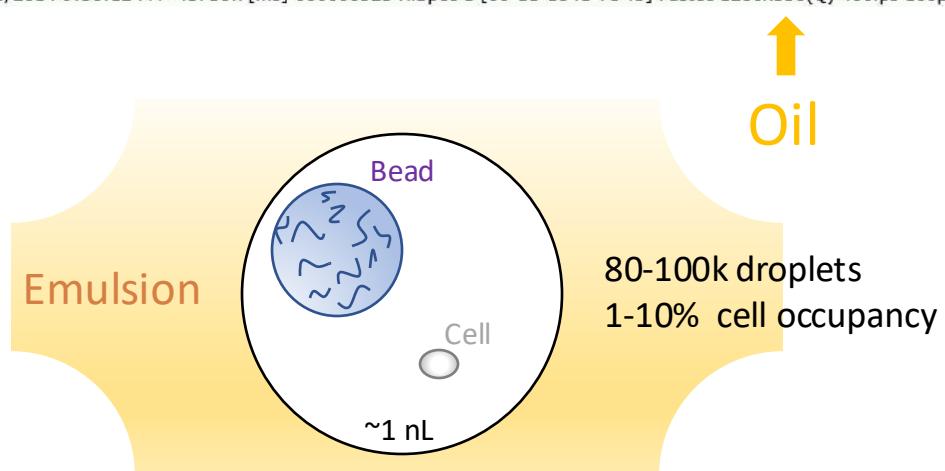
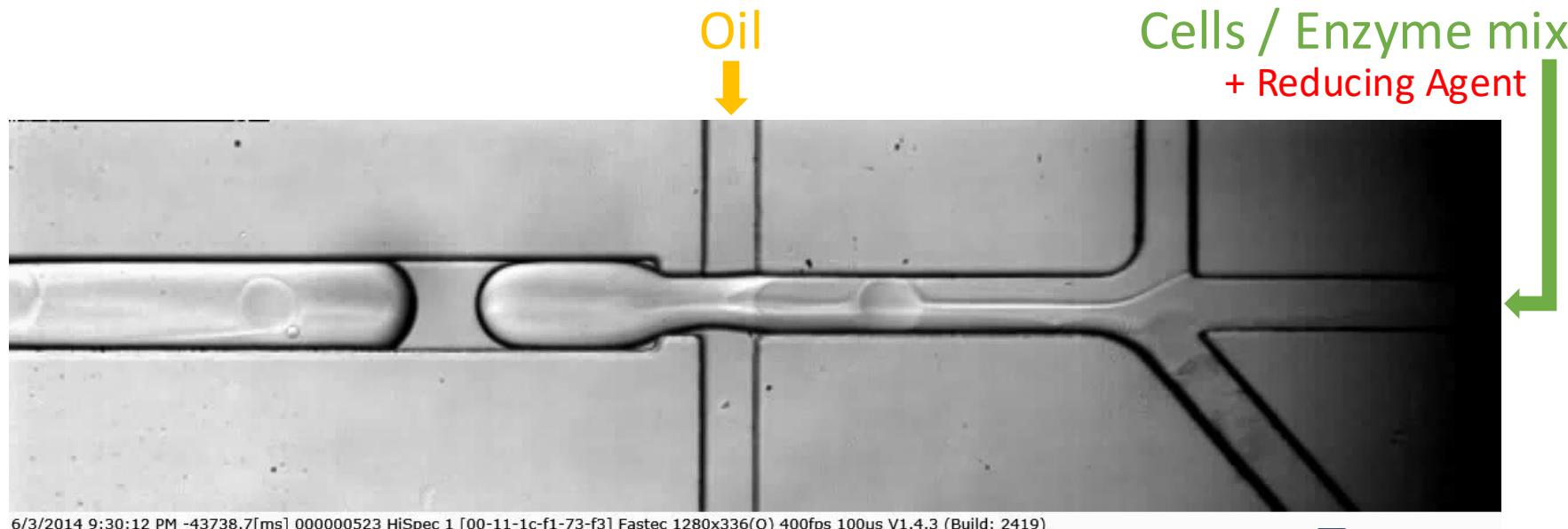


C elegans



Packer et al (2019) Science

Under the Hood

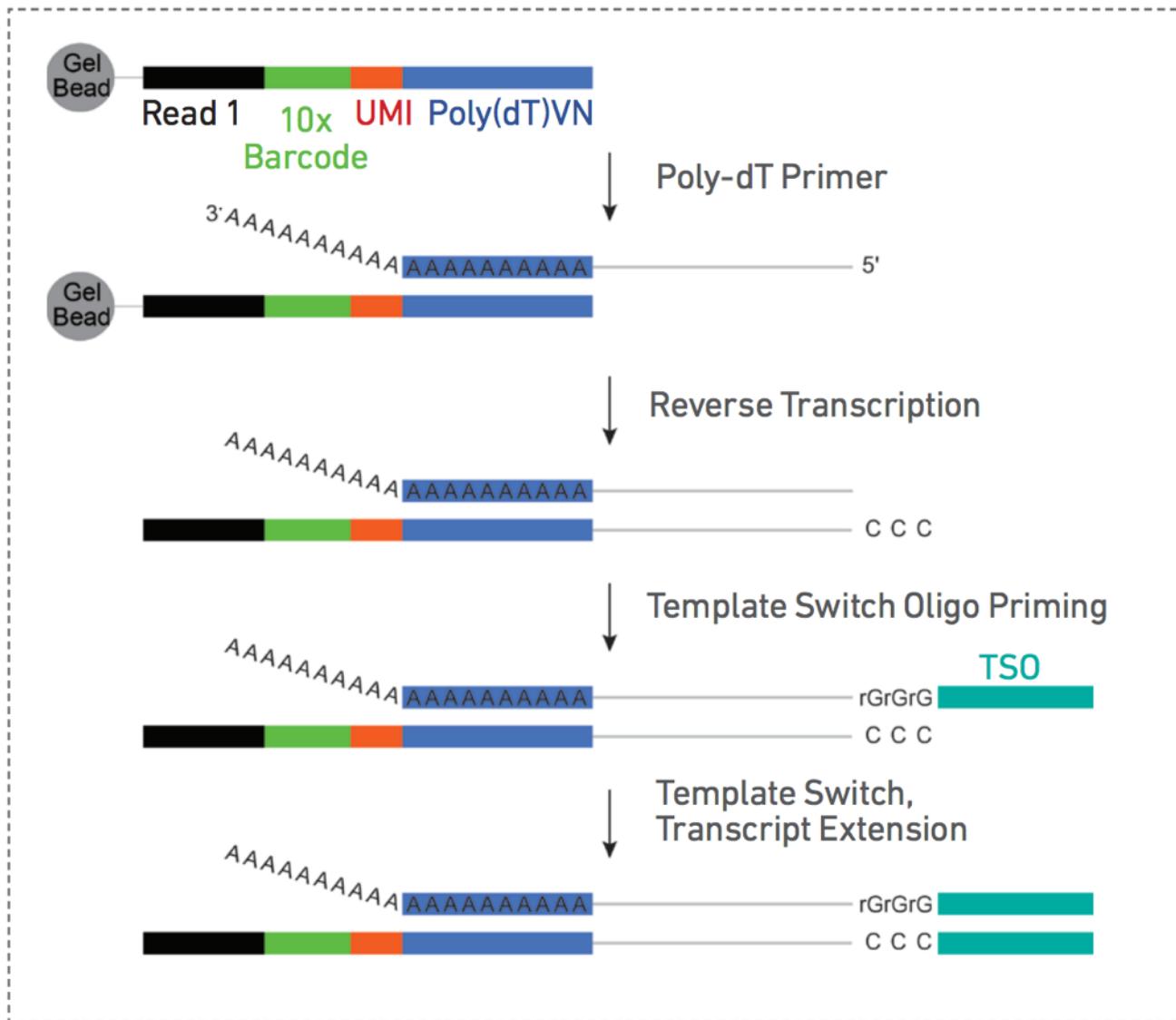


- -S-S- crosslinked hydrogel
- 10^9 primer molecules per bead
- 1 barcode sequence per bead
- Pool of 3M total possible barcodes

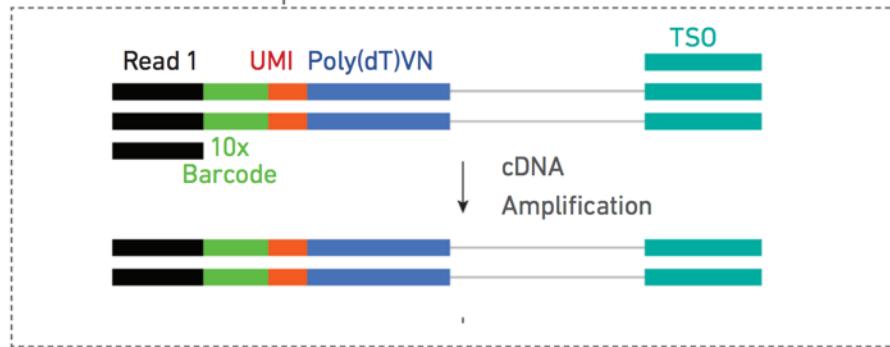


Debris Filters

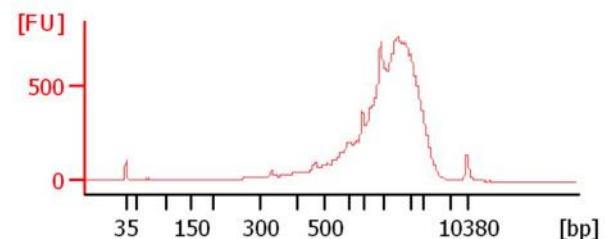
Single Cell 3' Chemistry Overview



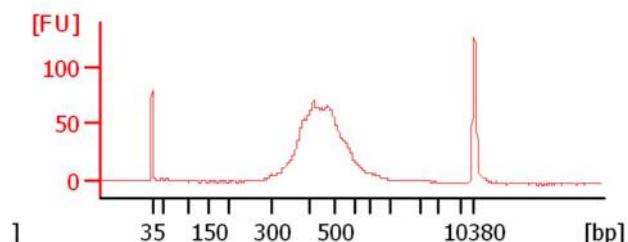
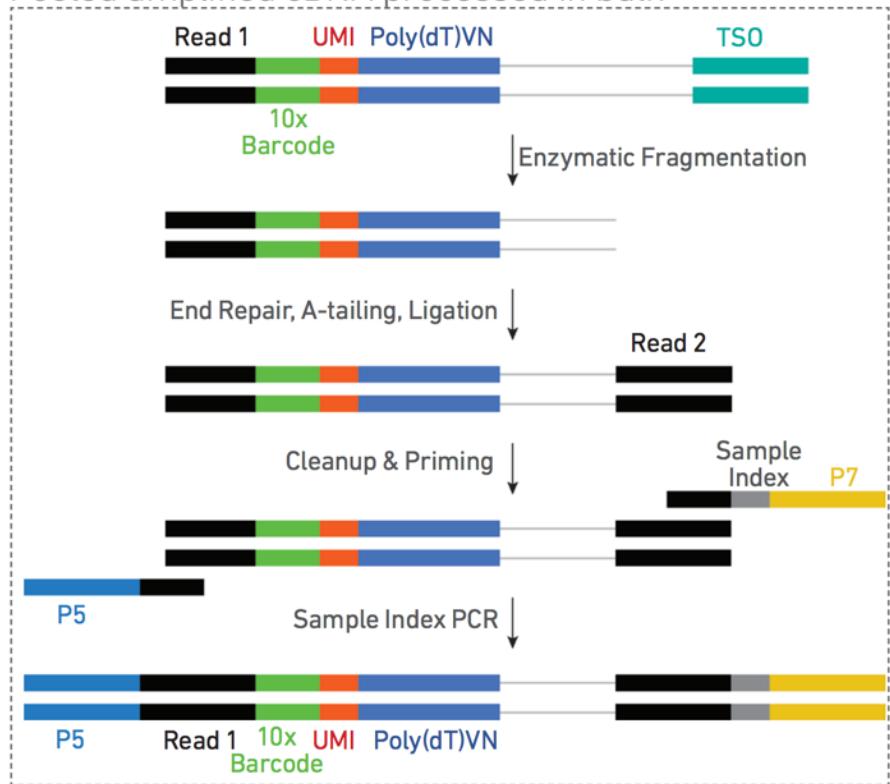
Pooled cDNA amplification



Bioanalyzer



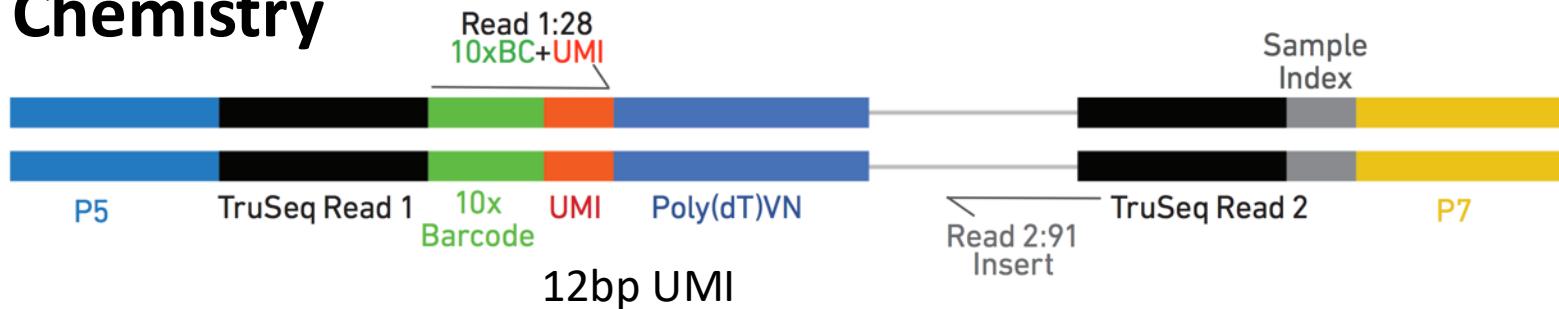
Pooled amplified cDNA processed in bulk



Final Library

Anatomy of a 10X 3'-Single Cell Amplicon

V3 Chemistry



The Best Site On the Internet. Probably.

- https://github.com/Teichlab/scg/lib_structs

Detailed visual guides to dozens of single-cell genomics methods

Adapter and primer sequences:

Barcoded Tn5 sequence s5: 5'- TCGTCGGCAGCTCTTCACG [8-bp Tn5 index] GCGATCGAGGACGGCAGATGTATAAGACAG -

Barcoded Tn5 sequence s7: 5'- GTCTCGTGGGCTCGGCTGTCCCTGTCC [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'- TCGTCGGCAGCGTCTCCACGC -3'

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

P5 index primer: 5'- AATGATAACGGCGACCACCGAGATCTACAC [i5] TCGTCGGCAGCGTCTCCACGC -3'

P7 index primer: 5'- CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGCTCGGCTGTC

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

Index 1 sequencing primer (i7): 5'- CTGTCTCTTATACACATCTGAGGC GGAGACGGT

Product 1 (≤ 5 at both ends) not amplifiable due to semi-suppressive PCR.

5' - TCGTCGGAGCGTCTCCACGC[8-bp Tn5 index] GCGATCGAGGACGGCAGATGTGATAAGAGACXXXXXX...XXX CTGCTCTTATACATCT
TCTACACATATTCTCTGTC XXX...XXXXXXXXXXXXGACAGAGAAATGTGTAGACGGCAGGAGCTAGCG[8-bp Tn5 index] CGCACCTCTGCAACGGCTGCT - 5'

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

5' - **GTCCTGGGCTGGCTGCCCTGTCC**[8-bp Tn5 index]CACCCTCGGCCTCAAGATGTATAAGACAGXXXXXXXXXXXX...XXX CTGTCTCTTACACATCT
TCTACACATATTCTGTCT XXX...XXXXXXXXXXGACAGAGAAATGTGTAGA**CTCCGCCCTGCCAC**[8-bp Tn5 index]**CCTGTCCCTGTGGCTGGTGTCTG** - 5'

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

Unique Molecular Identifier (UMI)

Random ~8-10bp sequence incorporated during oligo synthesis

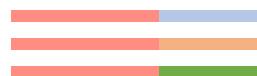
Cell barcode UMI

CCCCCCCCXXXXXXTTTTTTTVN
AAAAAAAAAAABN--- IFNgamma ---

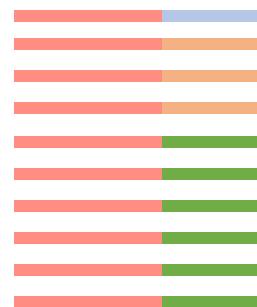
CCCCCCCCXXXXXXTTTTTTTVN
AAAAAAAAAAABN--- IFNgamma ---

CCCCCCCCXXXXXXTTTTTTTVN
AAAAAAAAAAABN--- IFNgamma ---

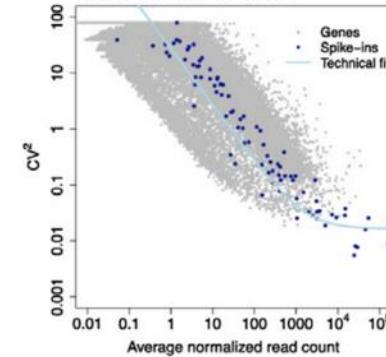
Before PCR



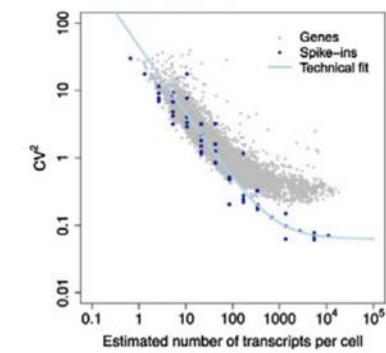
After PCR



without UMIs



with UMIs



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%

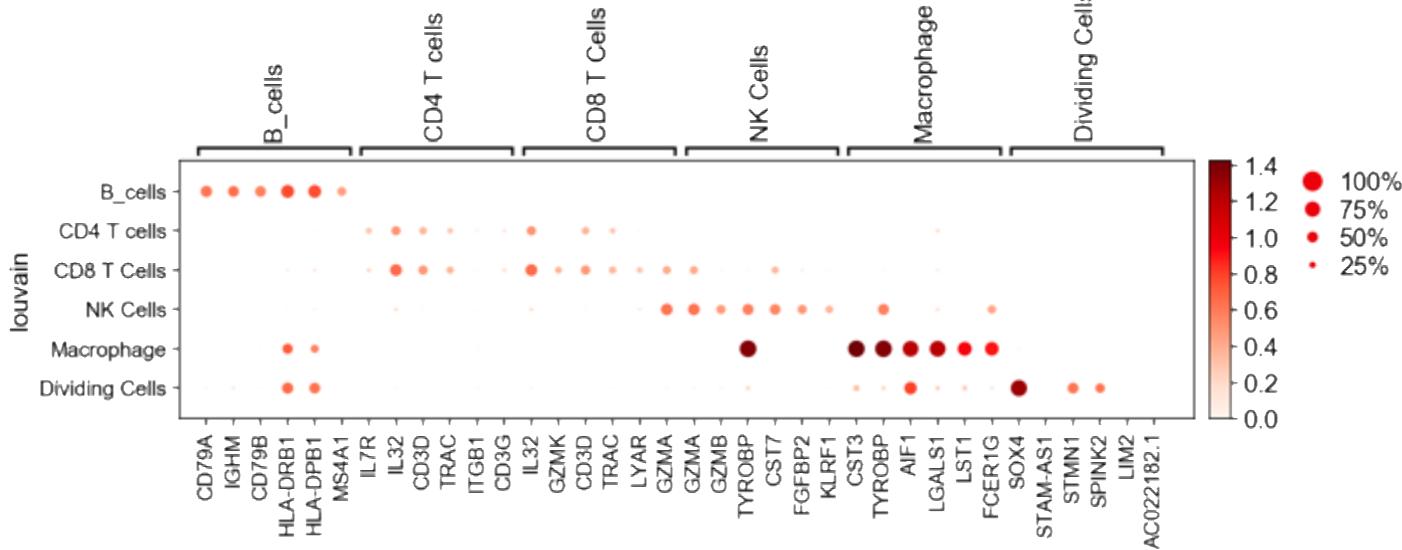
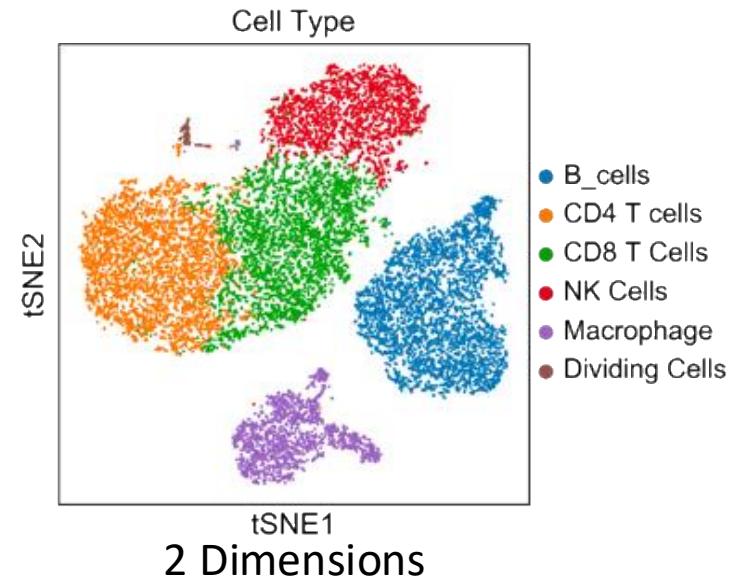
Basic scRNAseq pipeline

	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 →

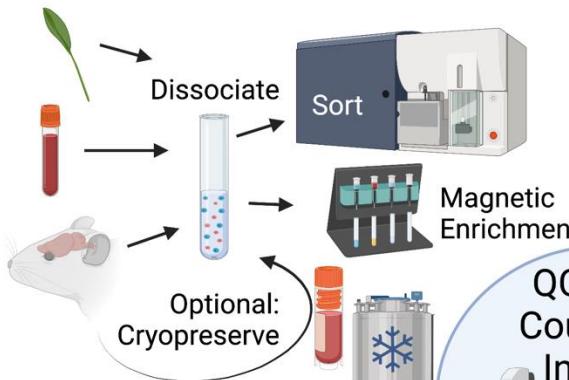
Dimensionality Reduction

Feature Selection
Normalization
PCA / NMF / ICA
tSNE / UMAP



Single Cell Workflow Overview

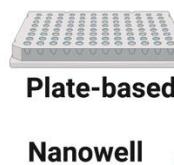
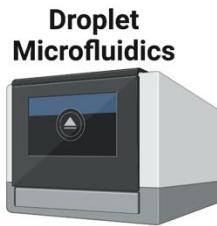
A. Sample Prep



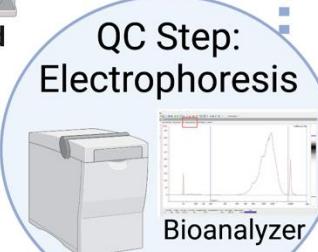
- Collect sample
- Digest / Dissociate
- Enrich for cell types
- Deplete dead cells
- Remove debris

Resources Committed \$ (⌚ ?) ————— \$\$\$ ————— (\$)
Opportunities to Abort and Retry

B. Barcoding / Library Prep



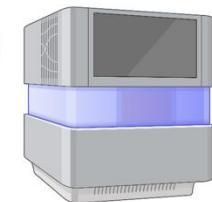
- Capturing and barcoding cells
- Amplifying barcoded cDNA or other library
- Adding sequencing adapters



————— \$\$\$ ————— (\$)
optional

C. Sequencing

Short Reads:
(Gene Exp., ATAC, CNV, Immune Profiling)



and / or

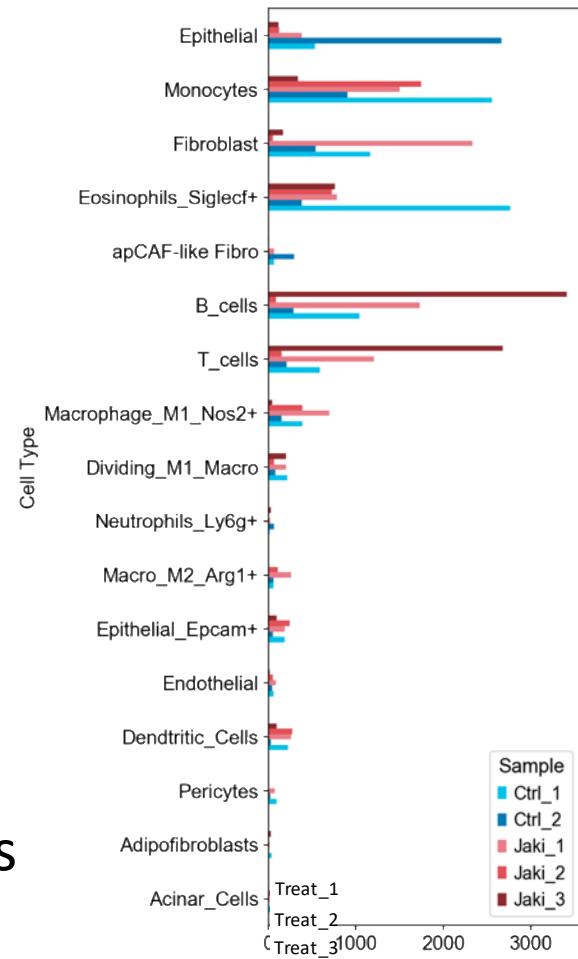
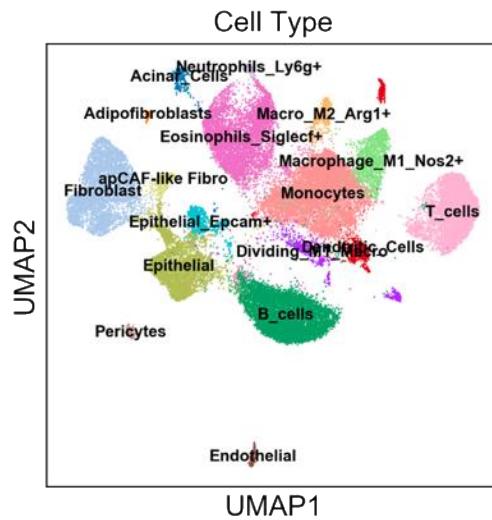
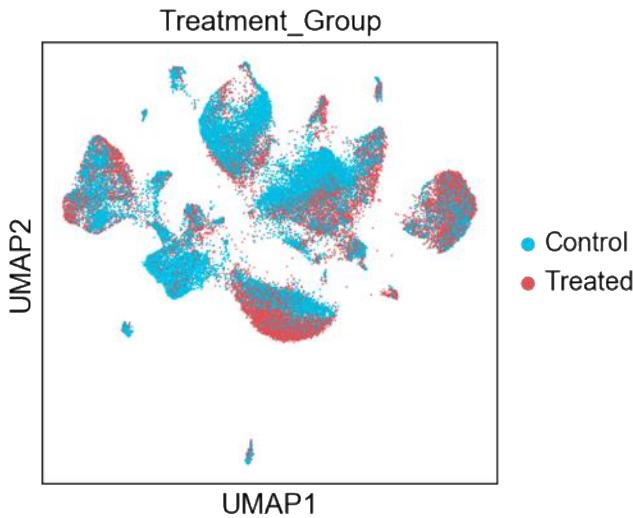
Long Reads:
(immune profiling, isoform seq.)



Oxford Nanopore

PacBio

scRNAseq is a poor cytometry tool



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

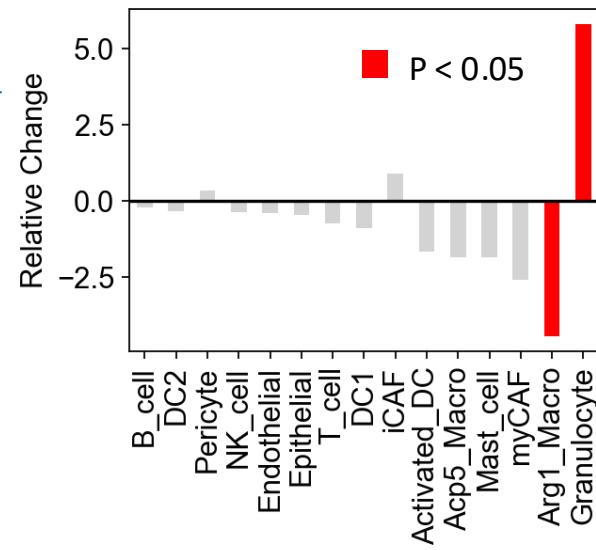
... But if you must, at least use some statistics:

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>

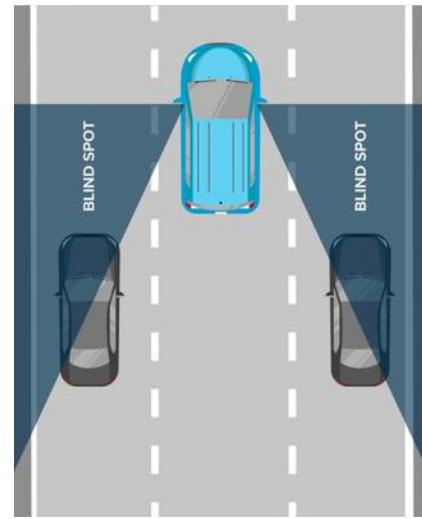
CTDS: Cell Type Diversity Statistic
<https://www.frontiersin.org/articles/10.3389/fgene.2022.855076/full>

DA-seq: Detecting differentially abundant (DA) subpopulations
<https://www.pnas.org/doi/10.1073/pnas.2100293118>

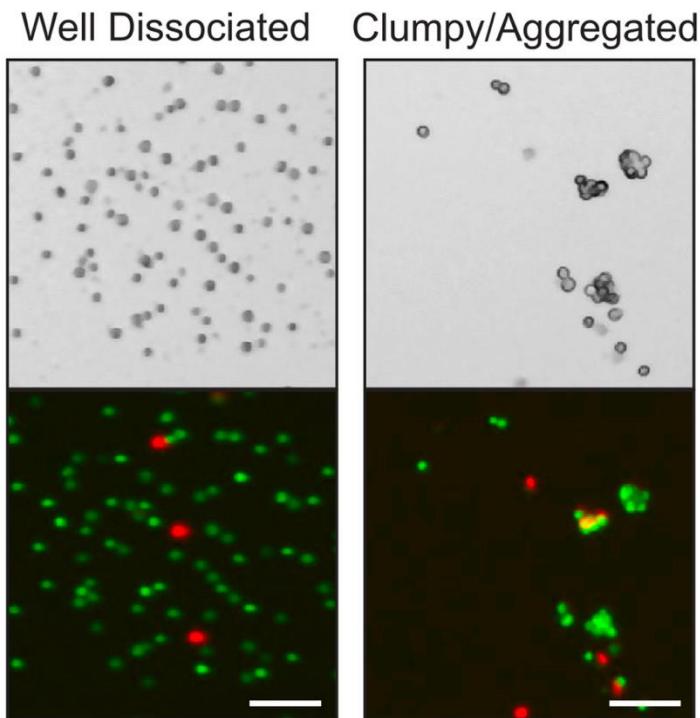
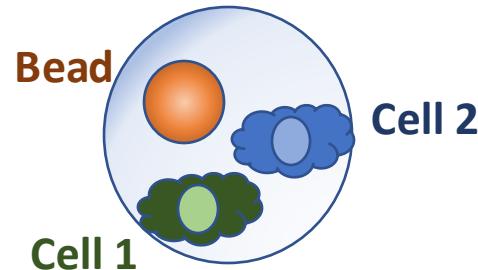
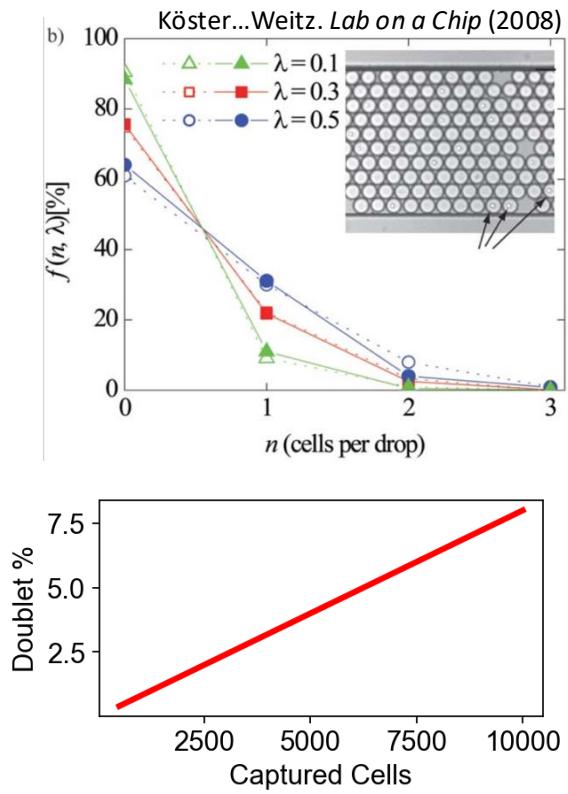


Blind Spots

- 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

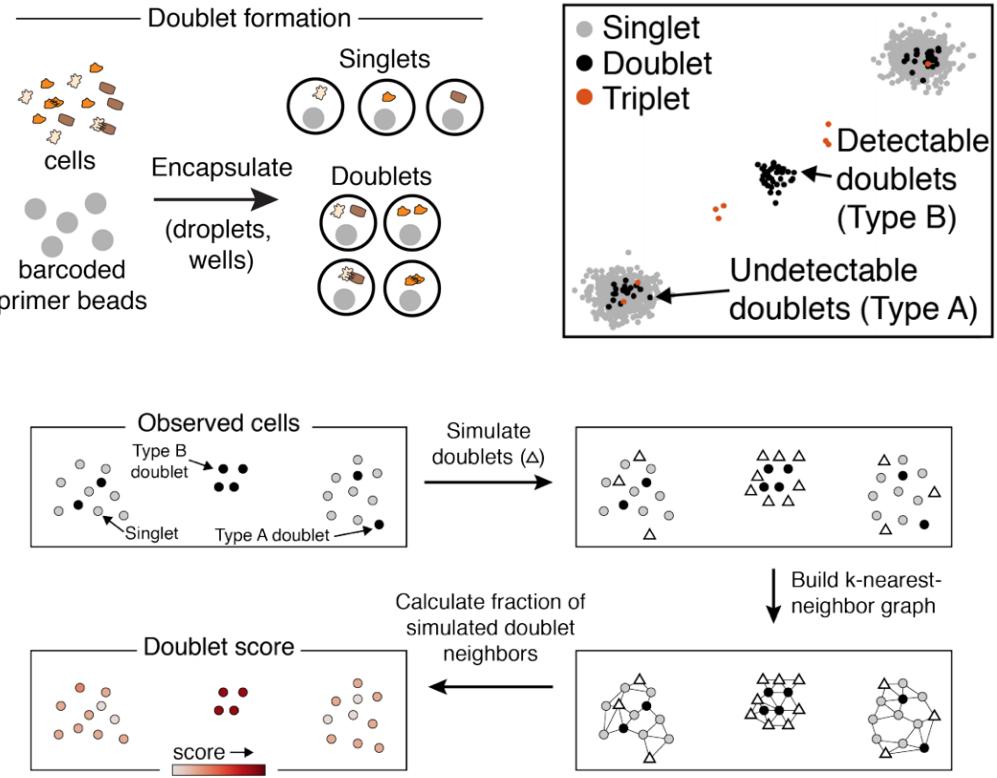
Scrublet

• [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)

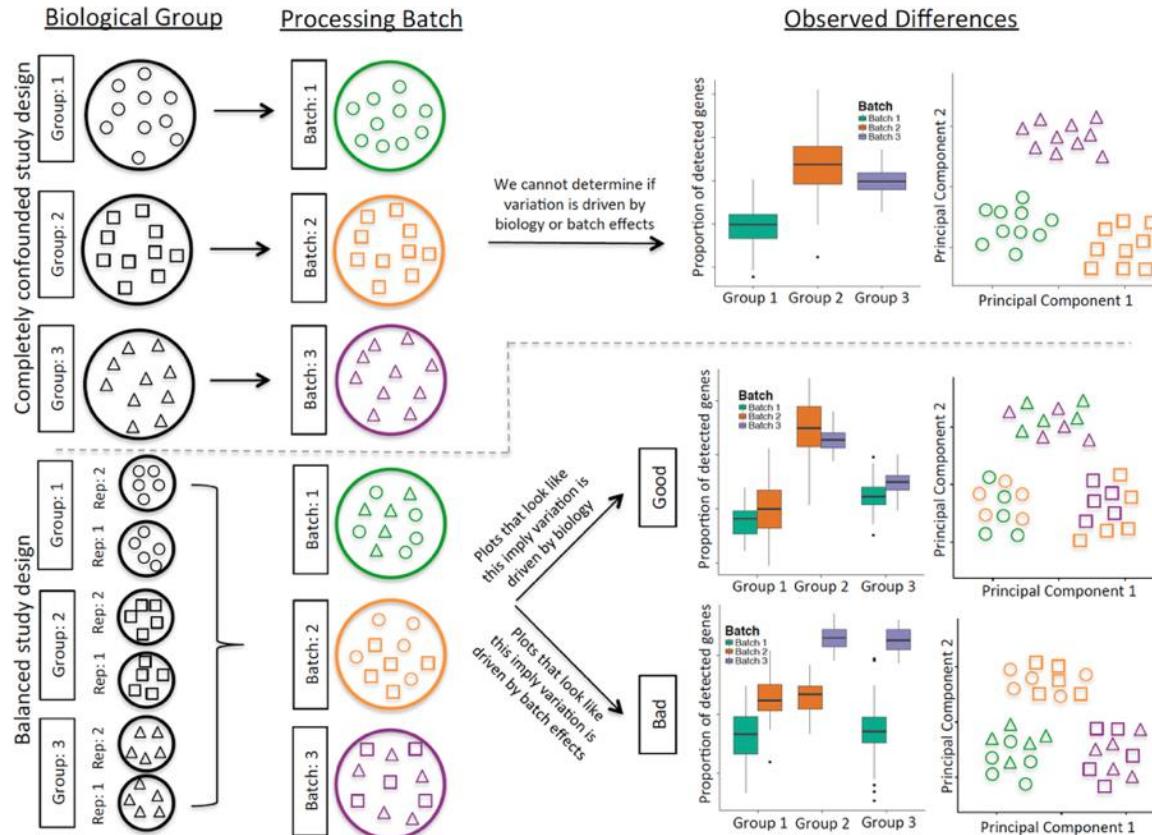
• [DoubletDetection](#) - [R, Python] - A Python3 package to detect doublets (technical errors) in single-cell RNA-seq count matrices. An [R implementation](#) is in development.

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

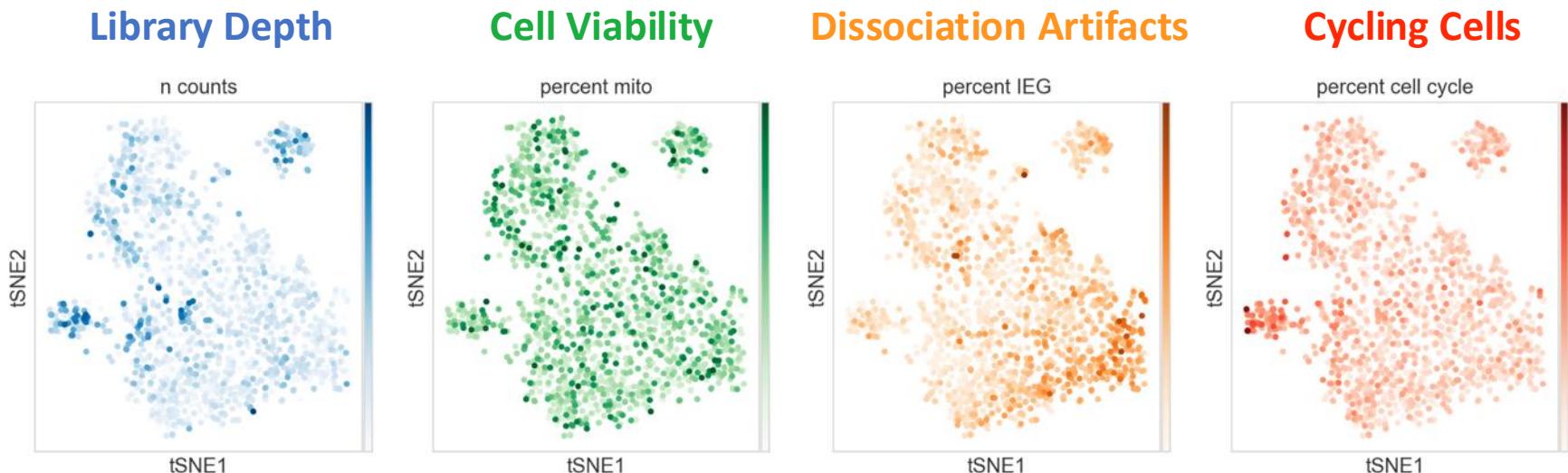


Wolock et al. (2018) bioRxiv

Batch effects and study design



Example Sources of Unwanted Variation & “Batch Effects”



Sex – matched studies are helpful!

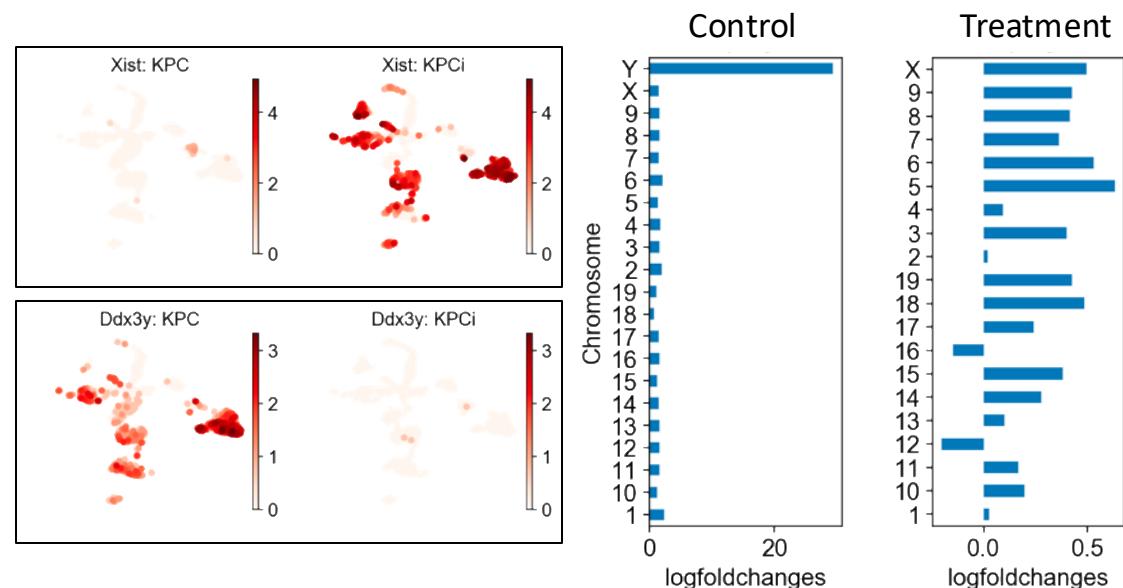
Major confounder: Male / Female

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

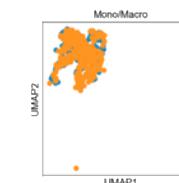
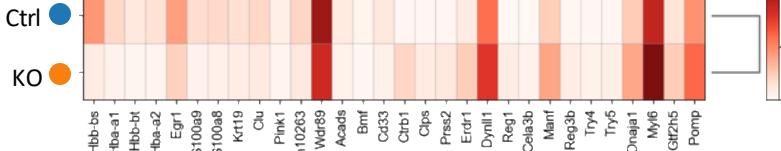
Same colony

Same set of hands

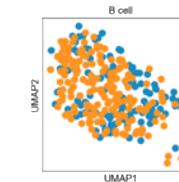
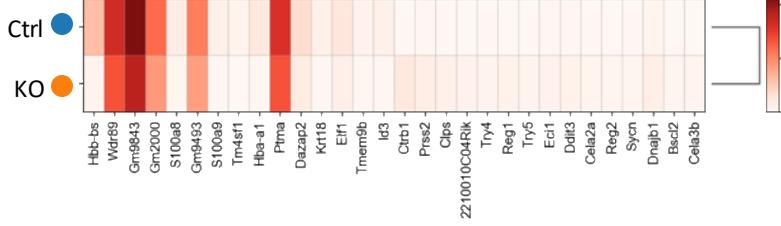
Difex dominated by same genes within every cluster

! major batch effect issues

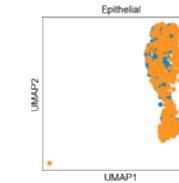
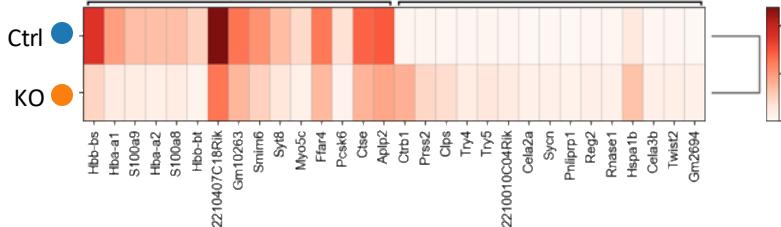
Myeloid



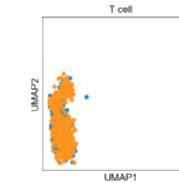
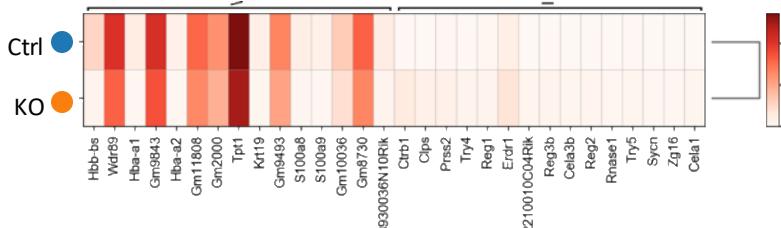
B cells



Epithelial



T cell



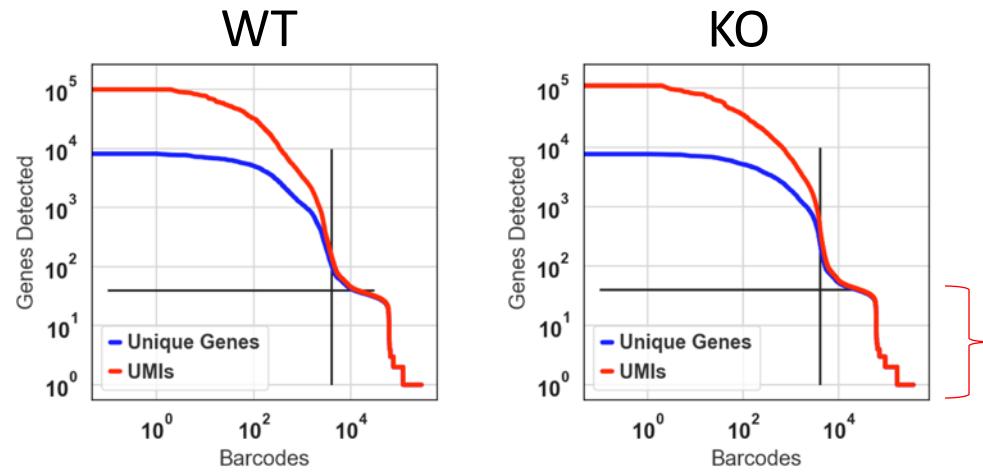
Ctrl
KO

Ctrl
KO

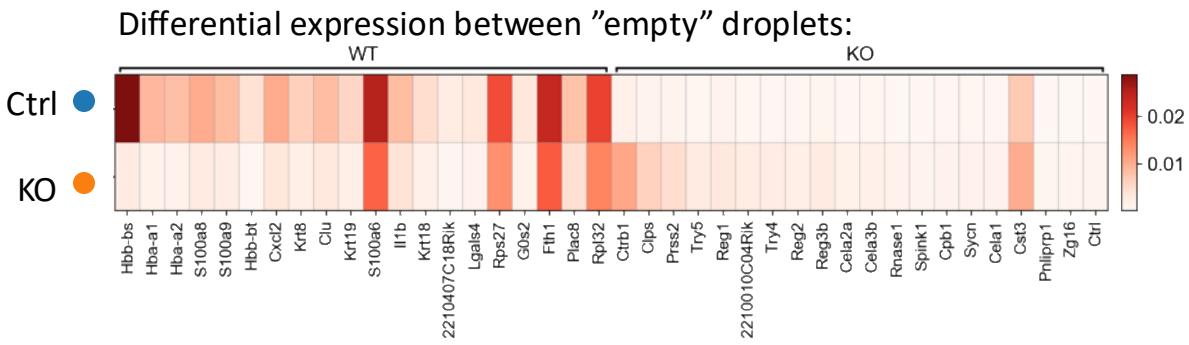
Ctrl
KO

Ctrl
KO

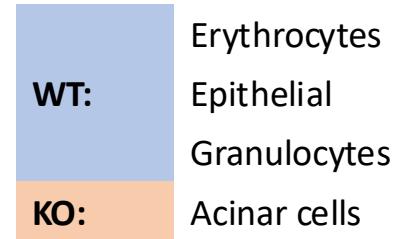
Controlling for batch effects



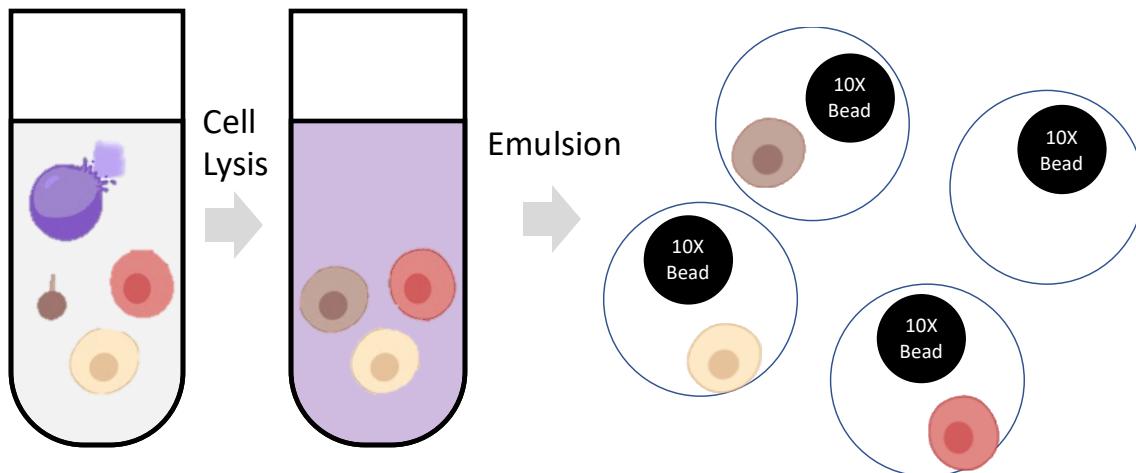
Ambient RNA
in droplets



Significant sources of
contaminating mRNA:



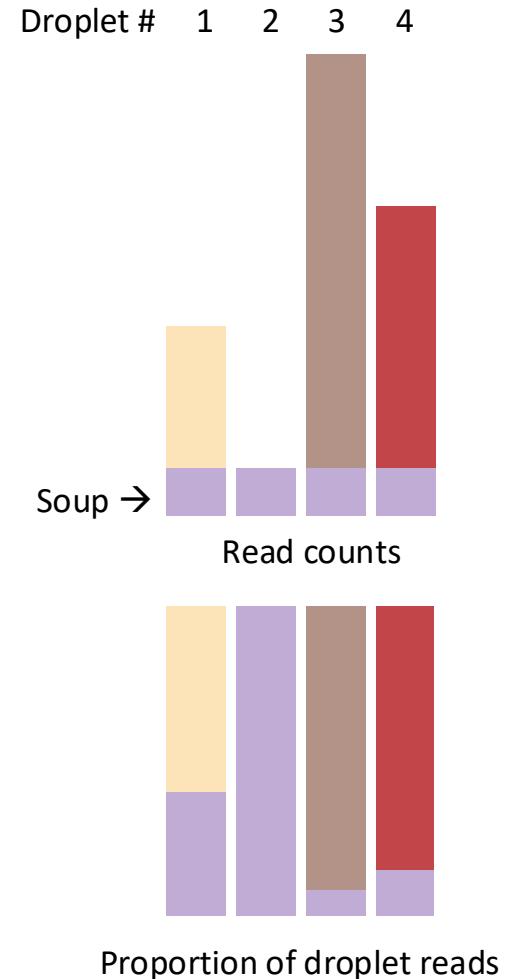
Ambient RNA: "SOUP"



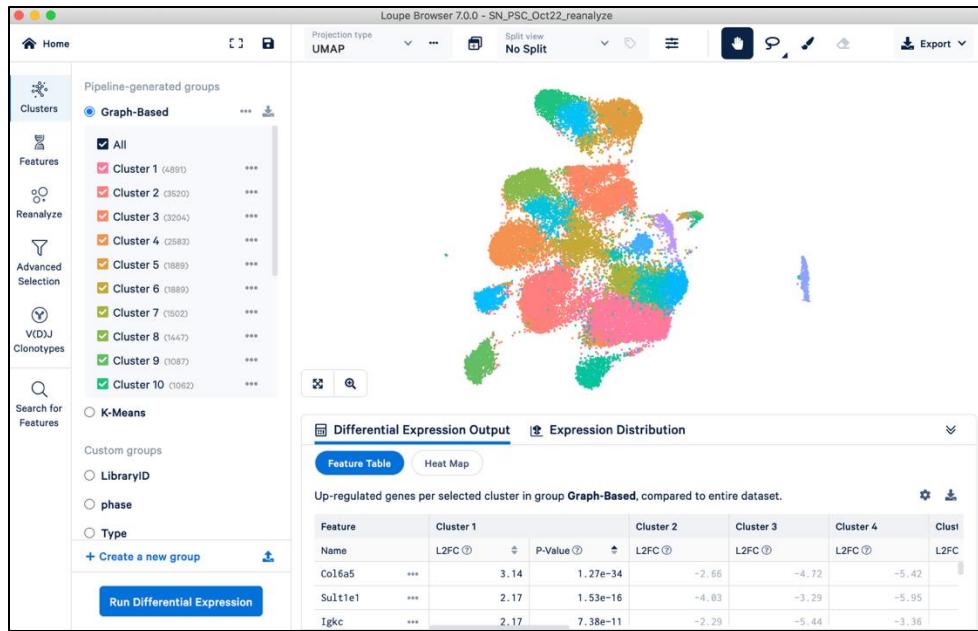
Computational methods to mitigate (but not totally fix) Soup:

- *SoupX*
- *DecontX*
- *Cellbender*

The best time to fix Ambient RNA problems is at the bench!



Loupe Cell Browser



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

Can:

- Quickly visualize genes
- Do guided clustering via marker genes / hand-drawn selections
- Calculate Differential Expression
- Subset cells and re-run PCA / UMAP / Clustering

Can't

- Pseudotime
- Transcription factor analysis
- Sequence-level analysis
- other fancy things

Getting started with your own analyses

Rahul Satija -

R

<https://satijalab.org/seurat>

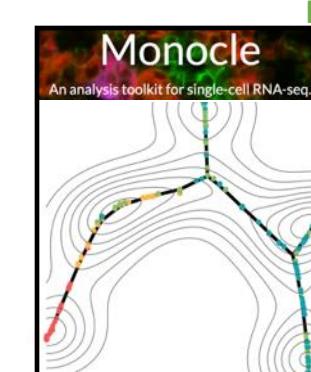


Fabian Theis - München



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

Python



Cole Trapnell – WashU

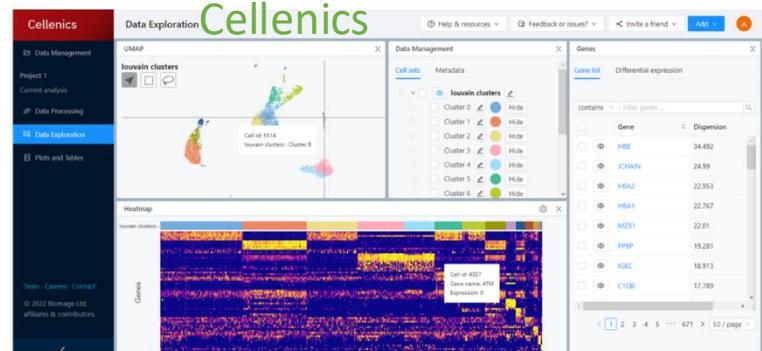
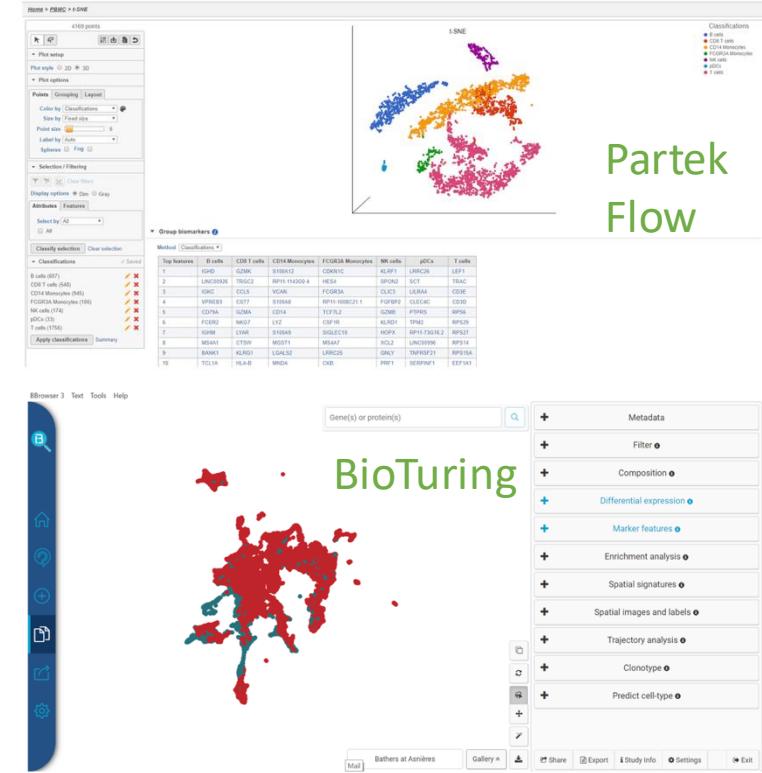
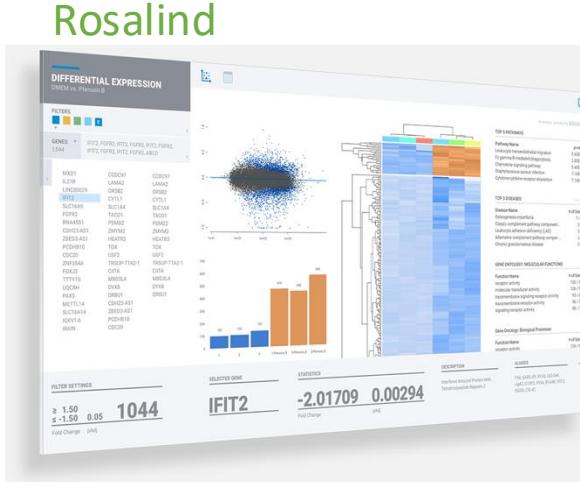


AWESOME SINGLE CELL RESOURCE

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

Or if you fear the command line and have \$\$\$...

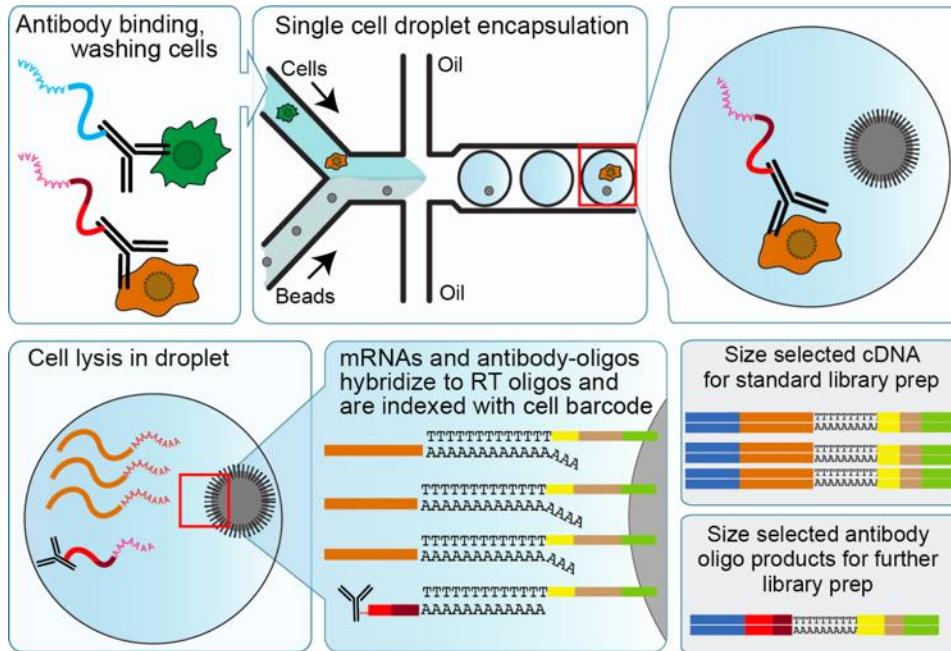
Feature	Cellenics®	BioTuring Browser	10X Loupe Browser	Partek® Flow®	Cellxgene	Rosalind
Type of application	Web	Desktop	Desktop	Desktop/ AWS	Desktop/Web	Web
Open-source?	Yes	No	No	No	Yes	No
Free for academia	Yes	No	Yes	No	Yes	No
Intuitive	Yes	Yes	Yes	No	Partly	Yes
Data import: count matrices	Yes	Yes	No	Yes	No	Yes
Data import: Fastq and h5ad files	No	Yes	No	Yes	Only h5ad	Yes
Data import: Seurat or Scanpy object	No	Yes	No	Yes	No	No
Multiple single-cell technologies supported	Yes	Yes	10X only	Yes	10x only	10x only
Multi-omics technologies supported	No	Yes	Yes	Yes	No	Yes
Species supported	All	All	Limited/All	All	Limited	Limited
Data sharing between researchers	Limited	Yes	No	Yes	Limited	Yes
Data processing: QC and filtering	Yes	Yes	No	Yes	No	Limited
Data integration: support for several algorithms	Yes	Yes	No	Yes	No	Limited
Data normalization: support for several methods	No	No	No	Yes	No	Limited
In-depth data exploration	Yes	Yes	Yes	Yes	Limited	Yes
Cell set prediction	Yes	Yes	No	No	No	Yes
Range of plots: trajectory	Yes	Yes	No	Yes	No	No
A variety of plots available	Yes	Yes	Limited	Yes	Limited	Yes
Plots are fully customizable to publishable quality	Yes	Limited	No	Limited	Limited	Yes



Hacking Droplets



CITE-Seq / REAP-Seq

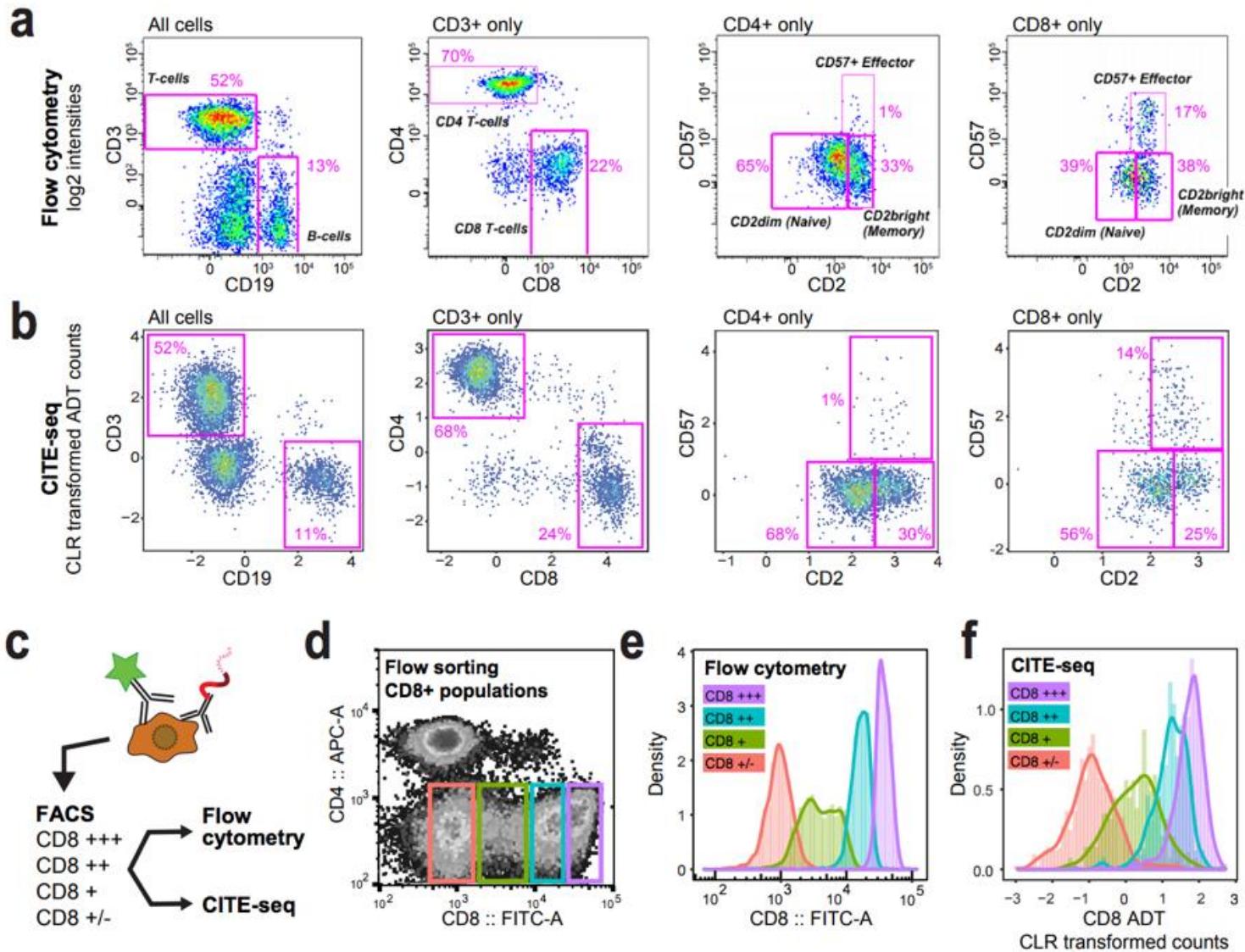


Antibody Derived Tag (ADT)
sequenced as part of normal 10X run

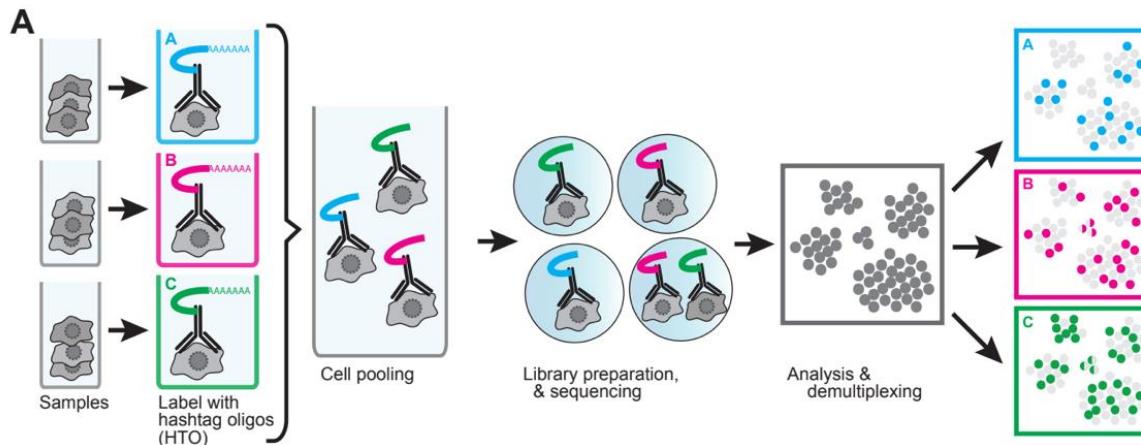
Enables:

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

CITE-Seq / REAP-Seq



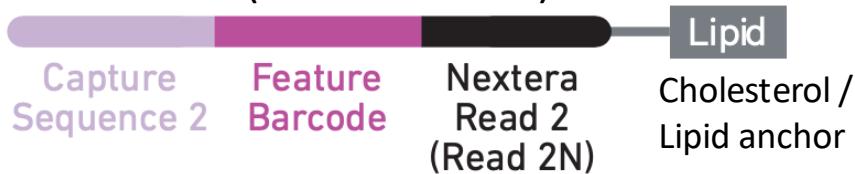
Multiplexing Strategies



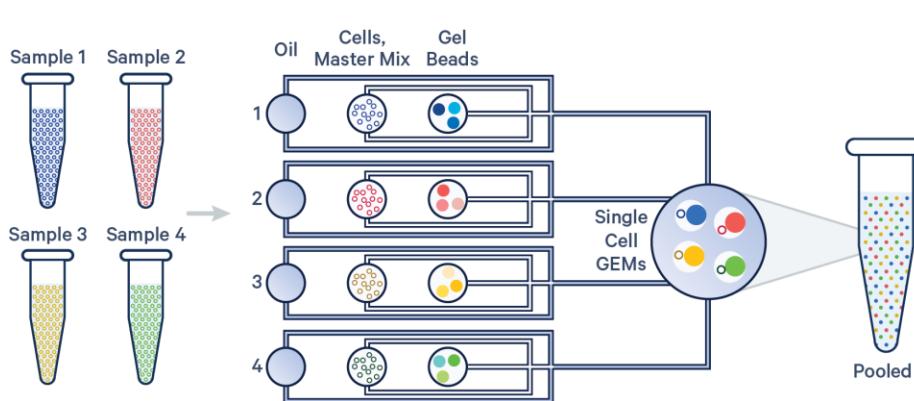
Antibody-based
"Cell Hashing"

Stoeckius, NYGC

CellPlex (10X Genomics)



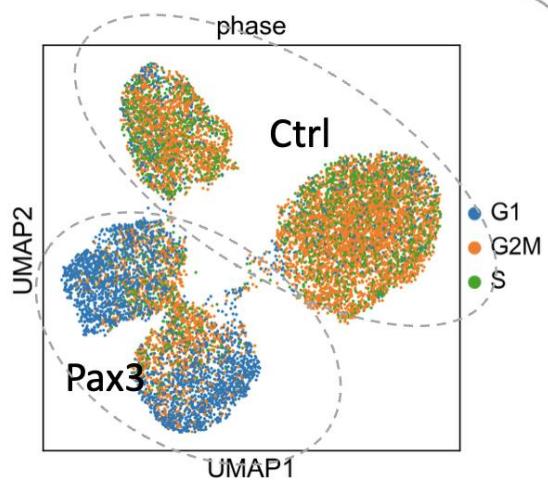
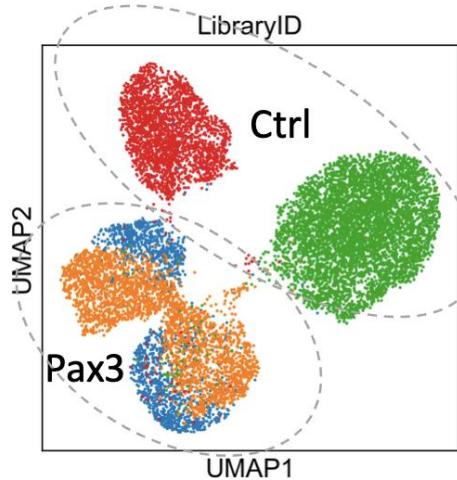
Lipid-Based
"CellPlex" (discontinued?)



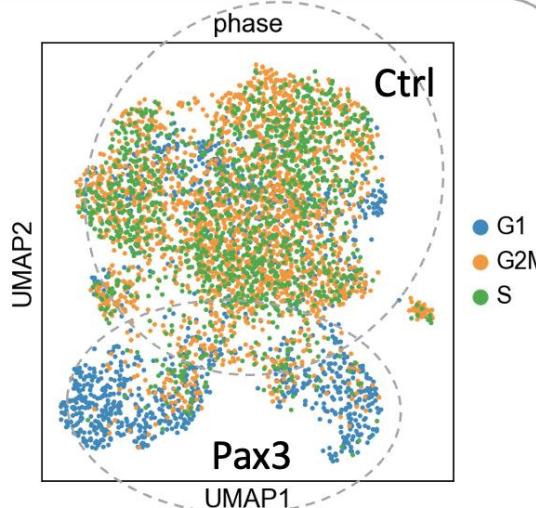
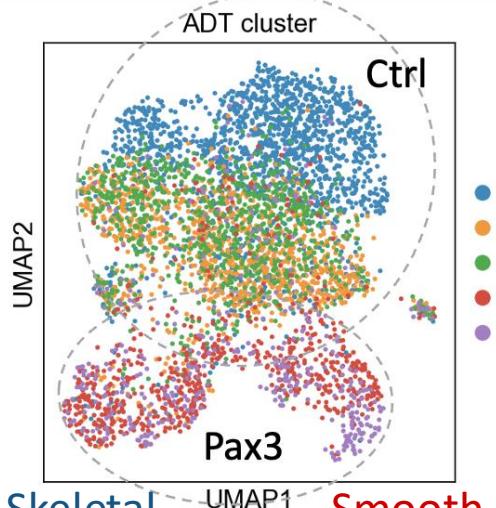
Combining barcode pools
"On-Chip Multiplexing"

Why Multiplex?

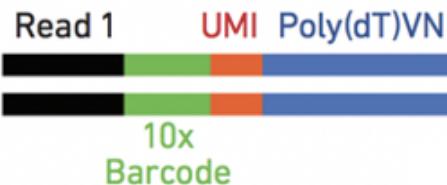
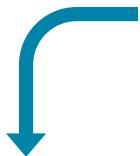
4 Lanes



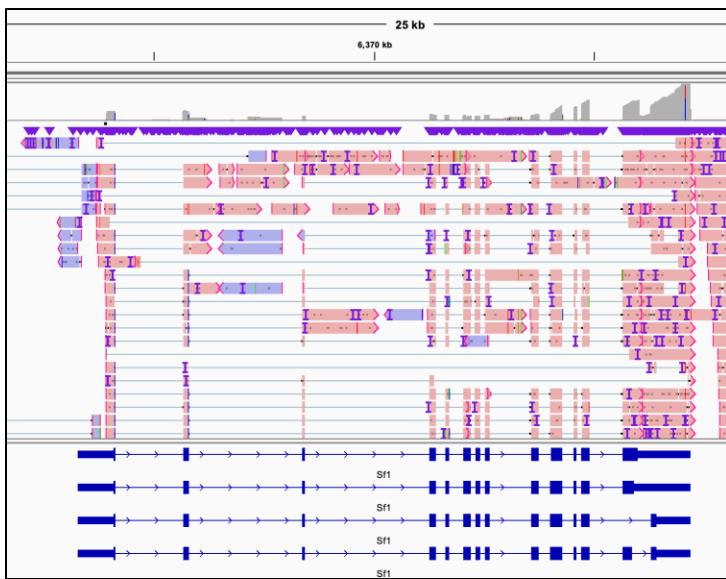
Hashed 1 Lane



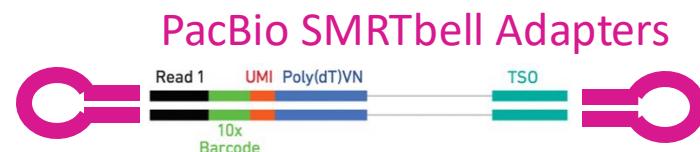
Sequencing full length cDNAs



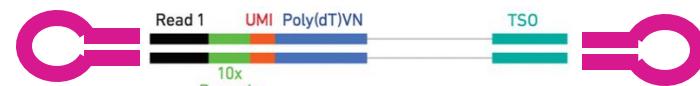
Oxford Nanopore Adapters



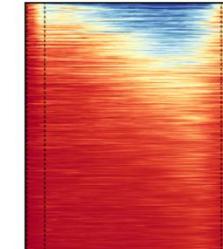
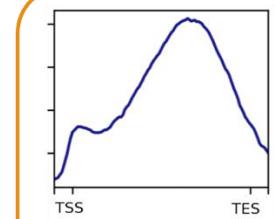
TSO



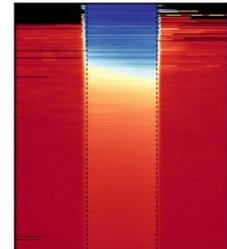
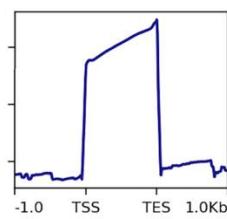
PacBio SMRTbell Adapters



Illumina



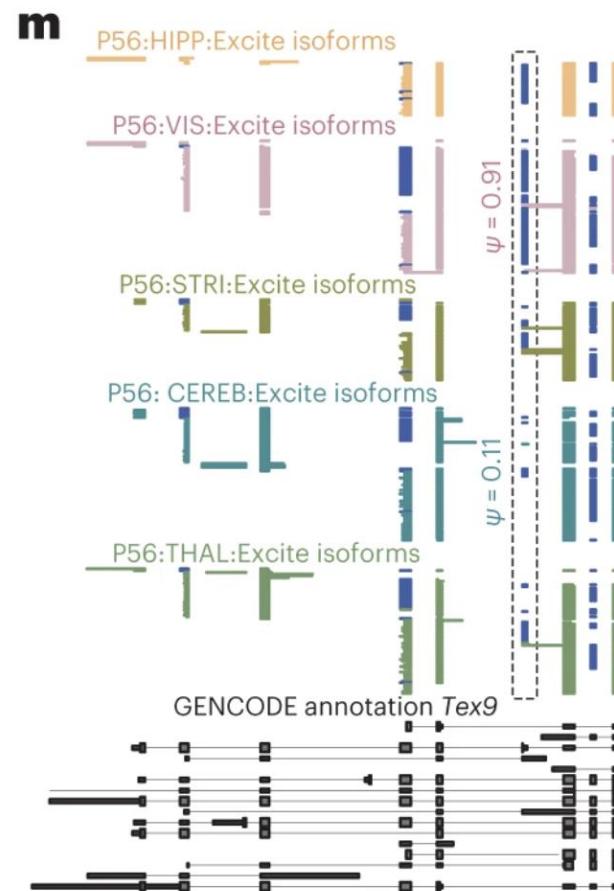
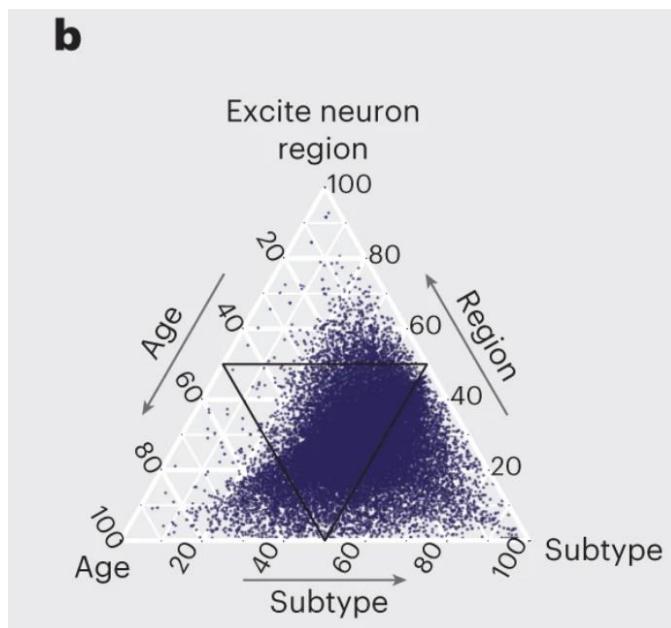
PacBio



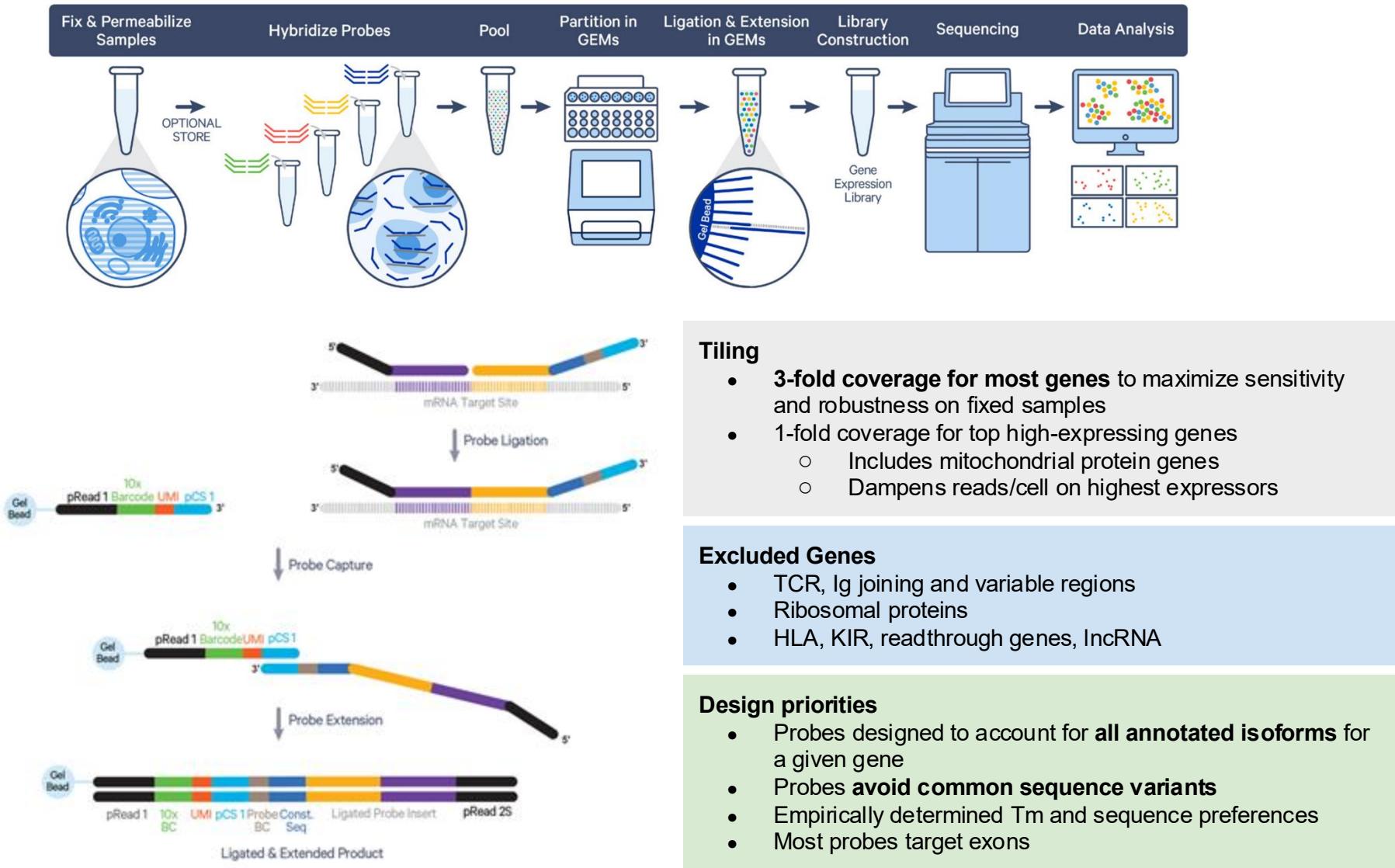
Single-cell long-read sequencing-based mapping reveals specialized splicing patterns in developing and adult mouse and human brain

SciSOr-Seq2

- Nuclei isolation
- 10X cDNA Synthesis
- Deplete non-barcoded cDNA with LAM-PCR
- Enrich for Exons w/capture oligos

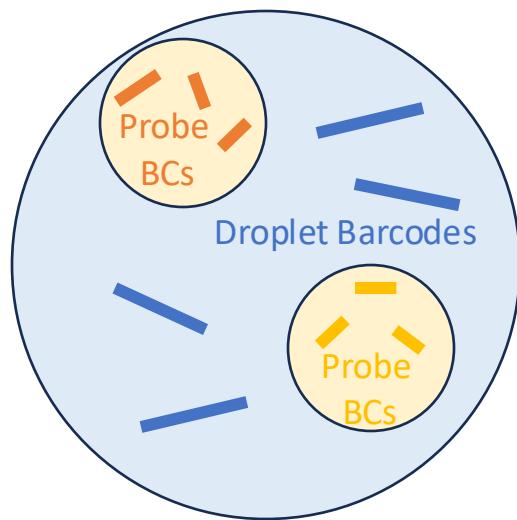
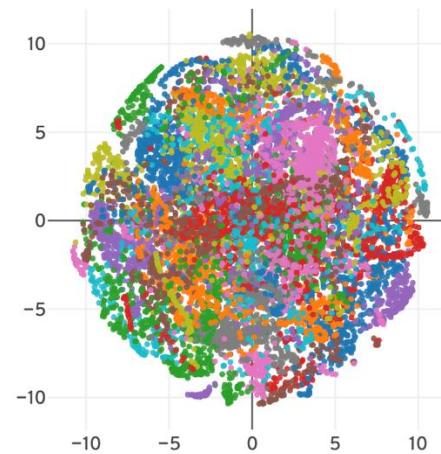
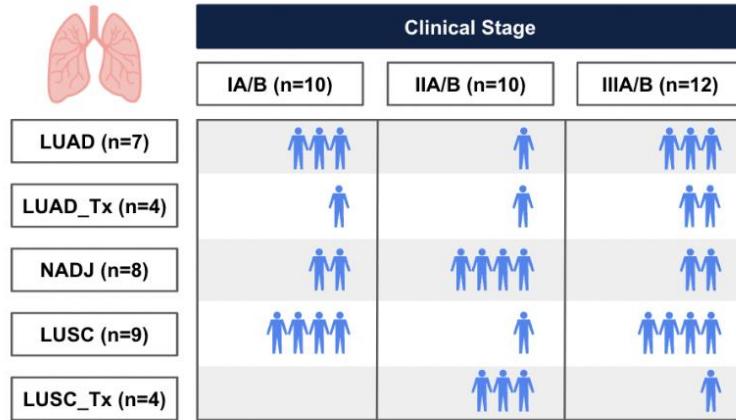


Fixed RNA Profiling



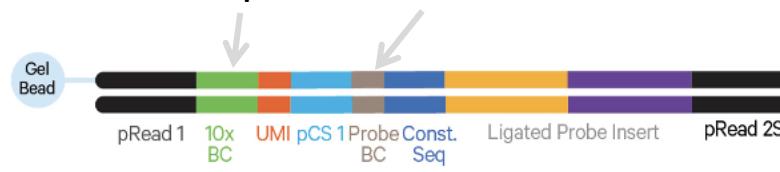
Batching & Multiplexing with Fixation

900K Cell Lung Cancer Cohort – 32 Samples



Higher throughput through hybridization chemistry

- Cells from different batches can share a droplet
- NOT a “doublet” – both transcriptomes are uniquely barcoded
 - DropletBC + ProbeBC



snPATHO-seq – isolation of nuclei from FFPE cells

 CSHL
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, Taiping 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?).



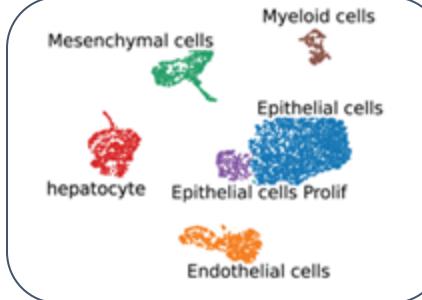
[Abstract](#) [Info/History](#) [Metrics](#) [Preview PDF](#)

Abstract

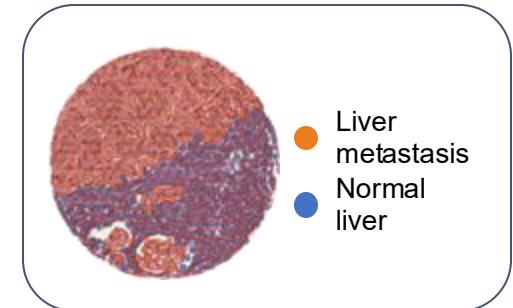
FFPE (formalin-fixed, paraffin-embedded) tissue archives are the largest repository of clinically annotated human specimens. Despite numerous advances in technology, current methods for sequencing of FFPE-fixed single-cells are slow, labour intensive, insufficiently sensitive and have a low resolution,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



Chromium Single Cell FFPE



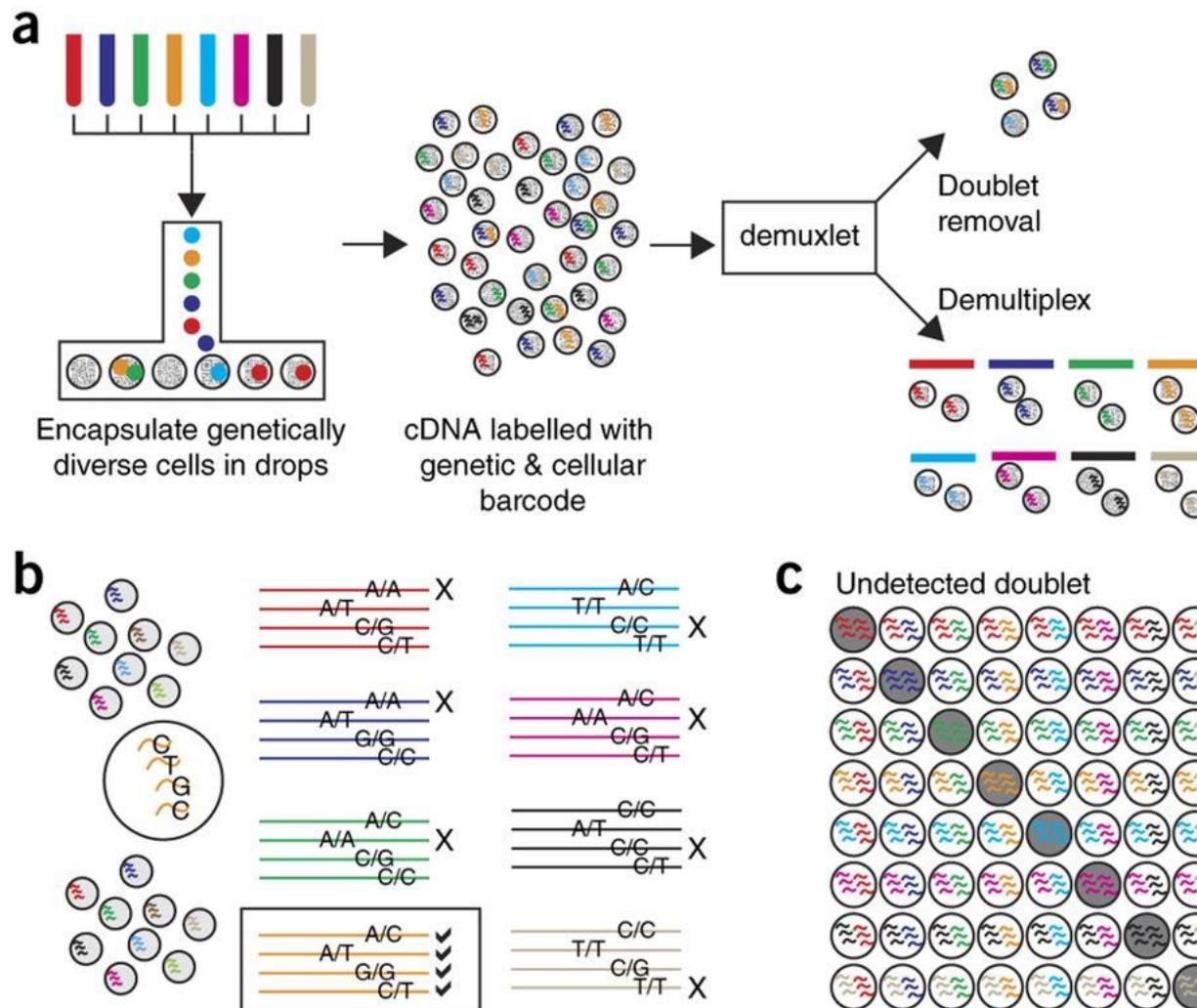
Visium Spatial FFPE



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

Multiplexing Using Natural Genetic Variation

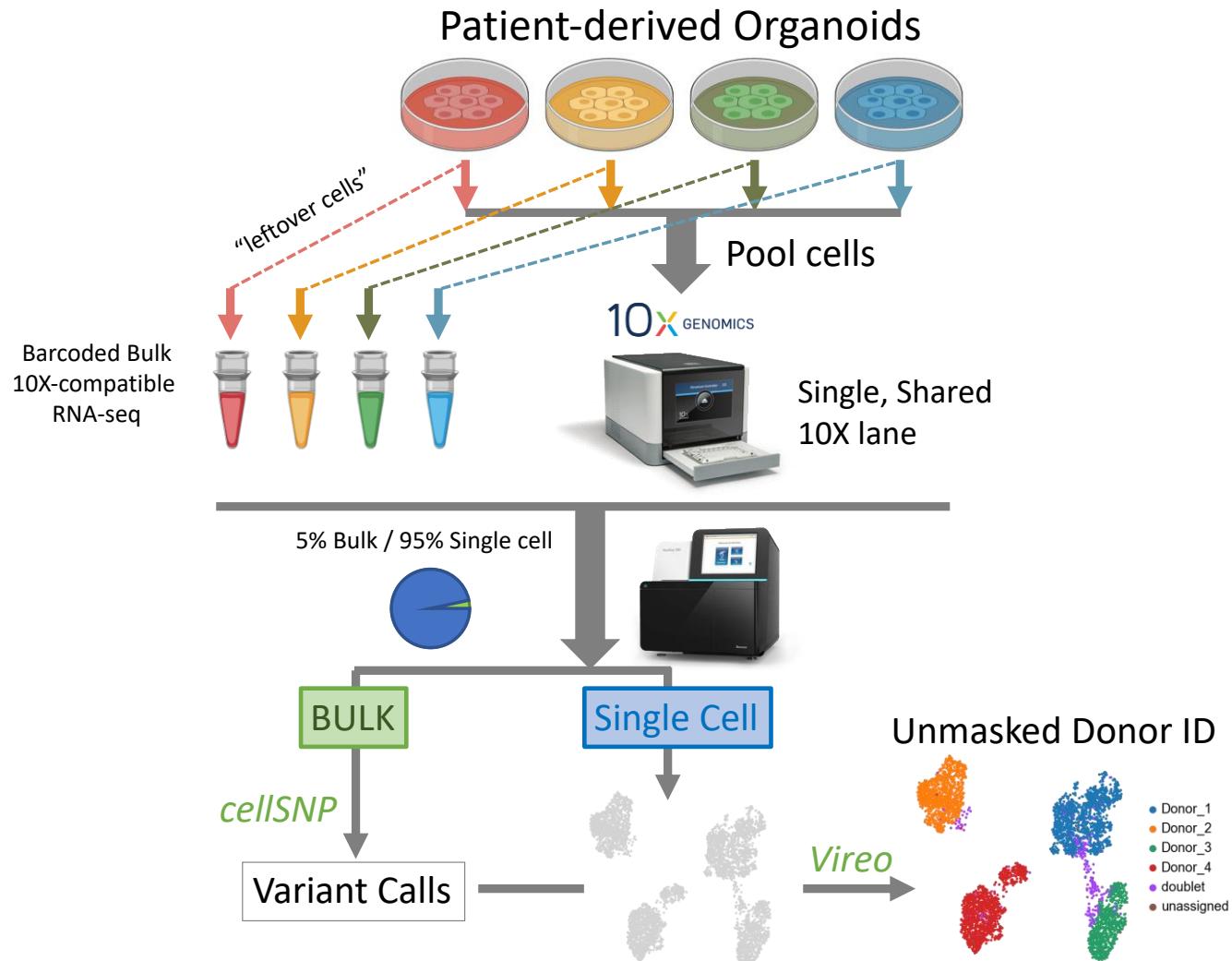
Demuxlet

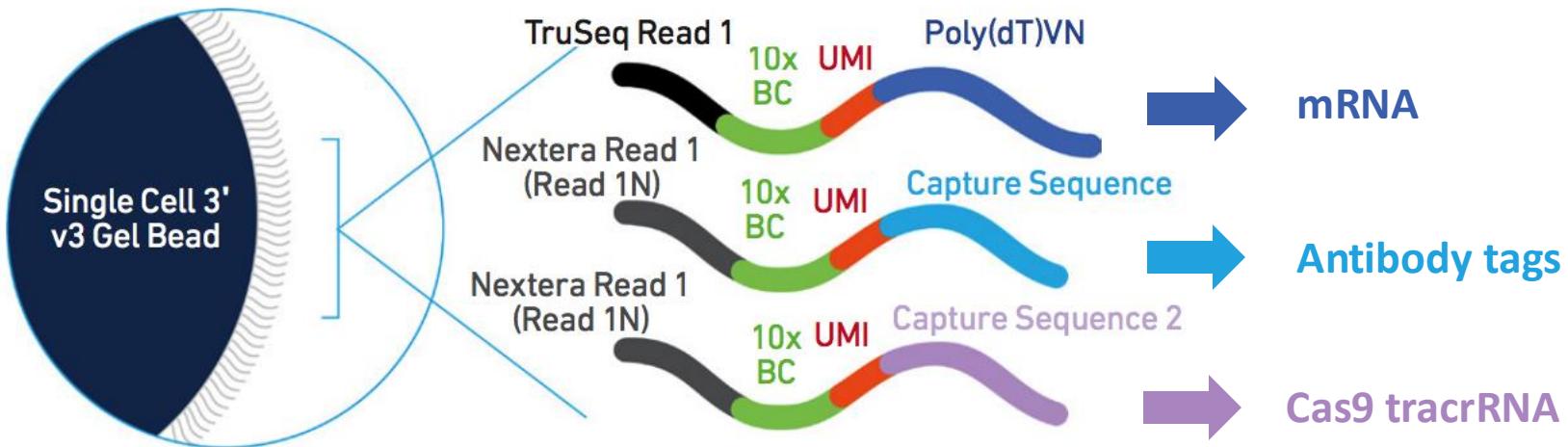


Jimmie Ye lab



Internal WIP nextflow pipeline for Genetic Demultiplexing of 10x Genomics libraries

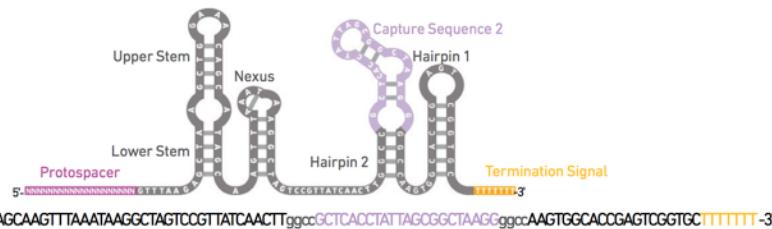




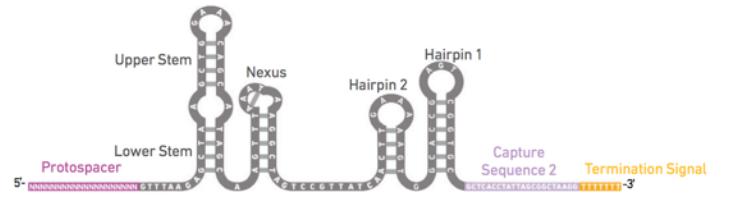
- Multiple RT primer sequences per bead
 - High efficiency capture of antibody tags, CRISPR guides

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

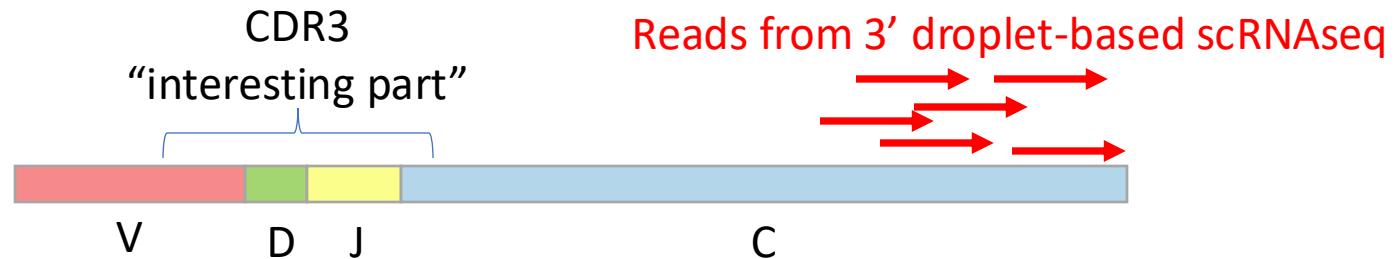
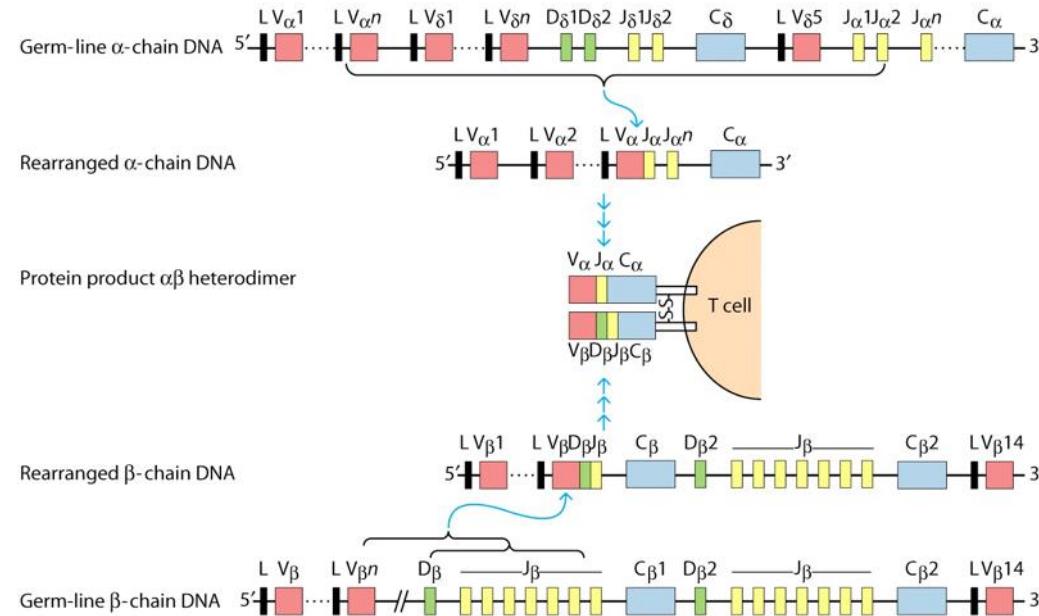
Capture Sequence 2 integrated in sgRNA hairpin



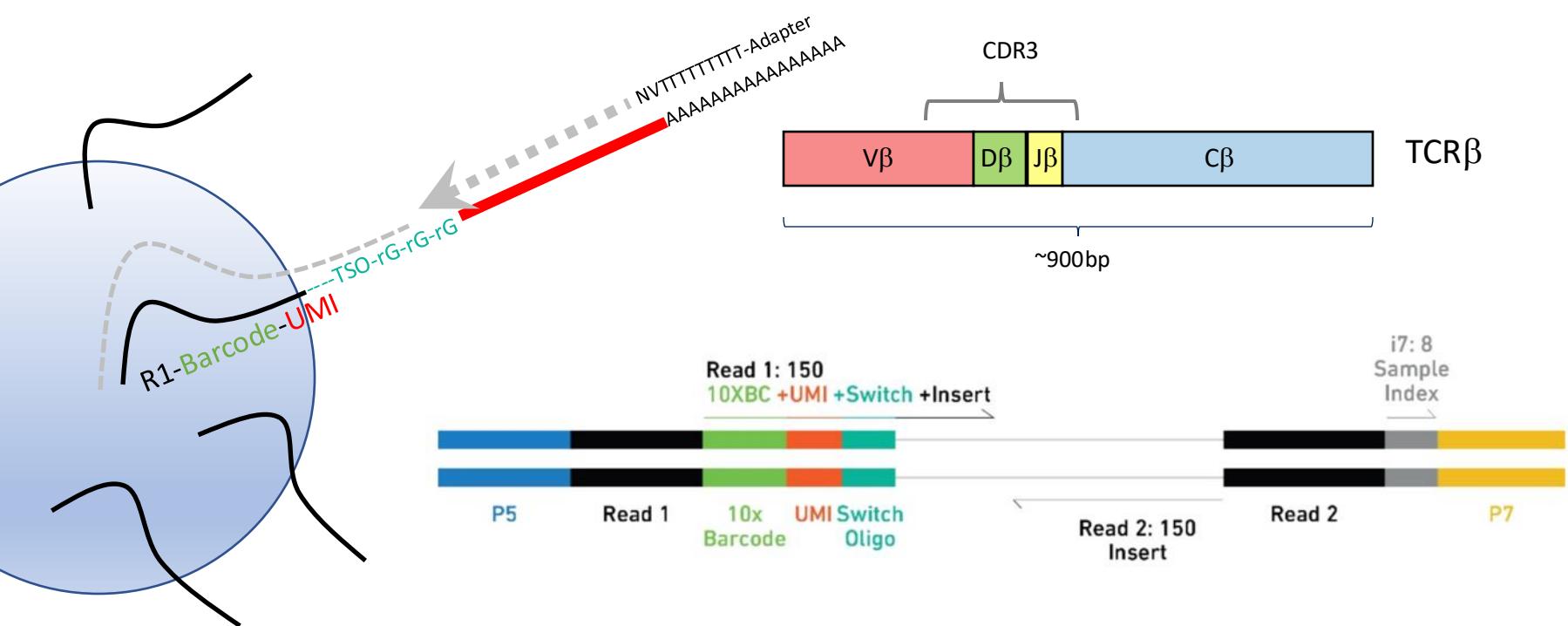
Capture Sequence 2 integrated in sgRNA 3'-end



TCR/BCR Profiling



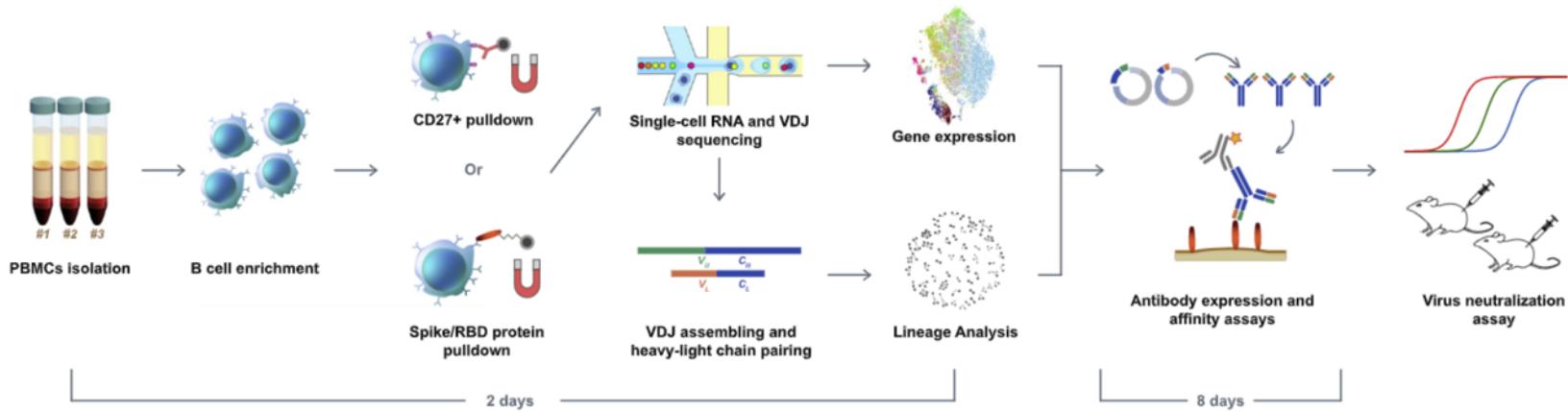
5'-Barcoded Libraries

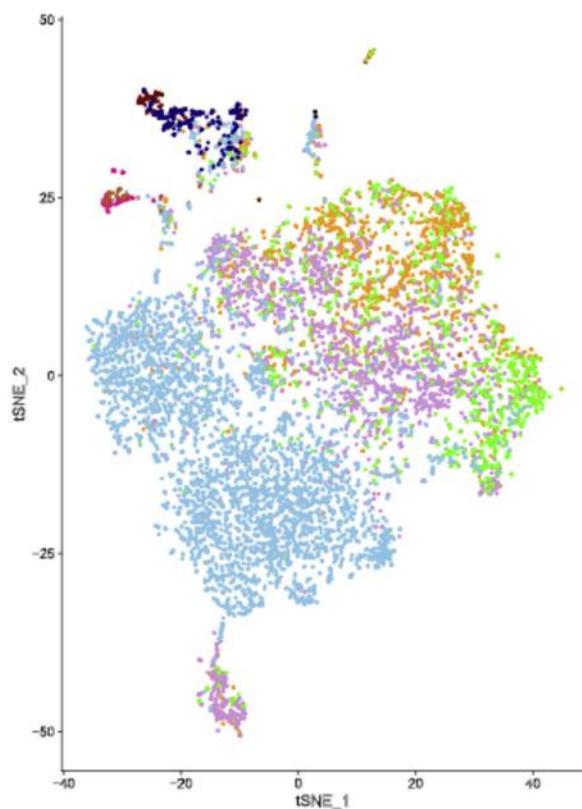
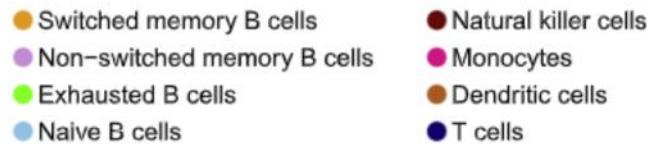


- Problem: standard transcriptome libraries have strong 3' -bias
- CDR3 mapping requires 5'-Barcoded library
- Random fragmentation to sample different 3'-ends of reads
- Require much longer reads (300bp) at a depth of 5,000X / cell

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

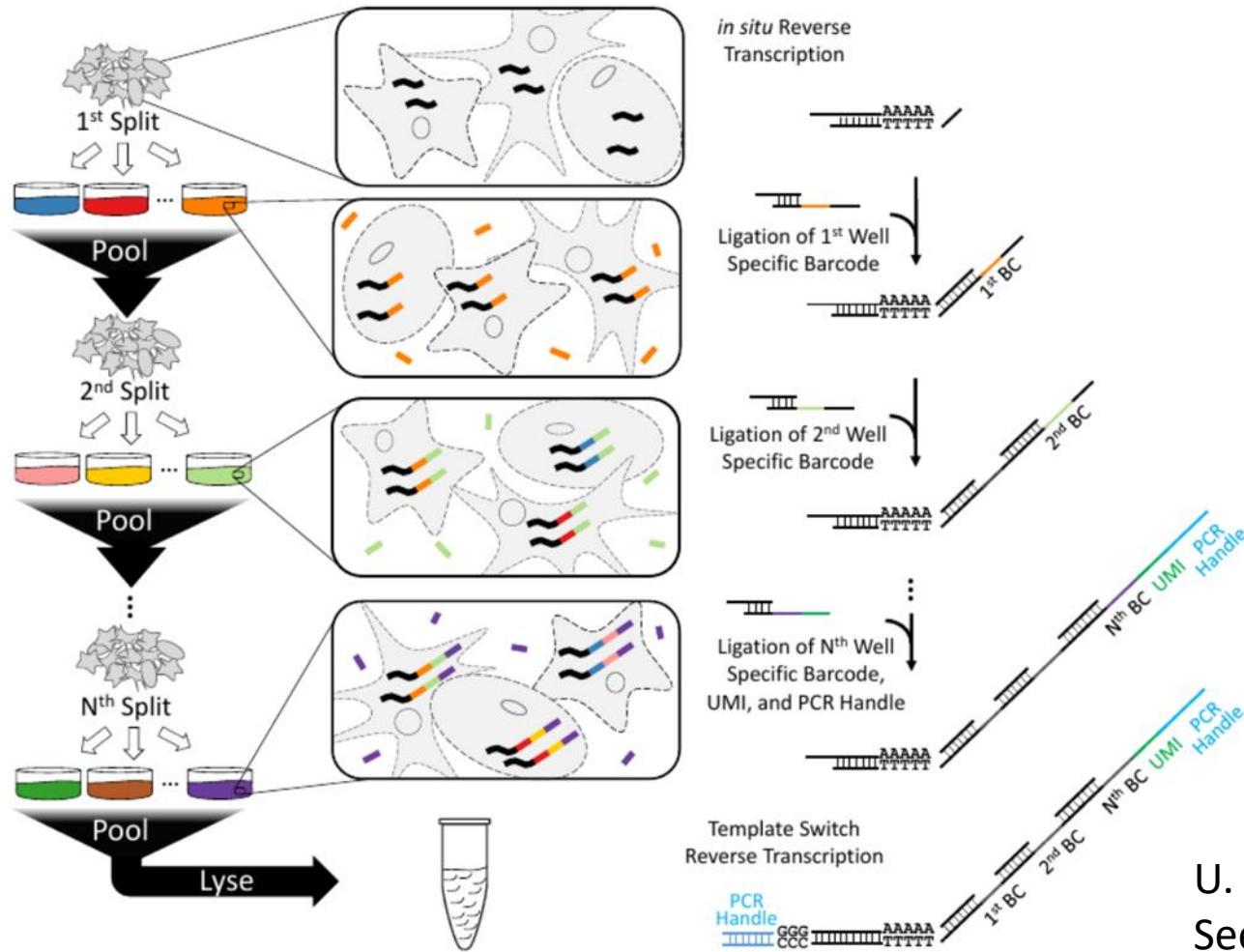
Gene expression analysis

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™ WT Mini

Up to 10K cells, 1-12 samples

Evercode™ WT

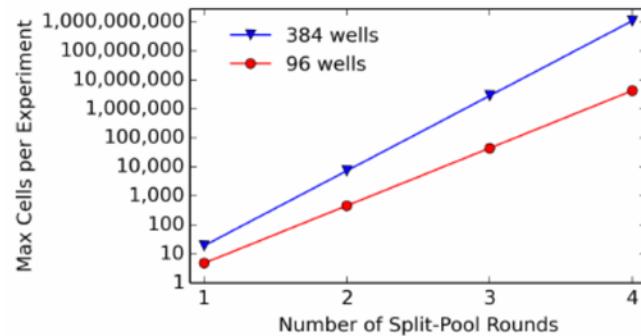
10K-100K cells, 1-48 samples

Evercode™ WT Mega

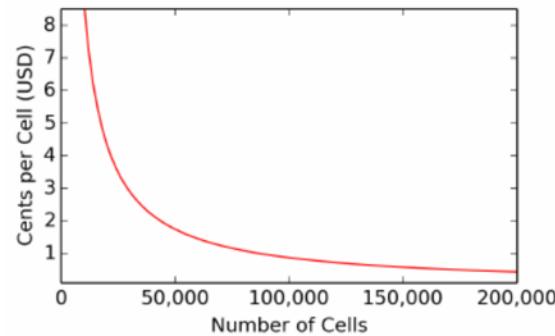
100K-1M cells, 1-384 samples

Evercode™ WT Penta

1M-5M cells, 1-384 samples

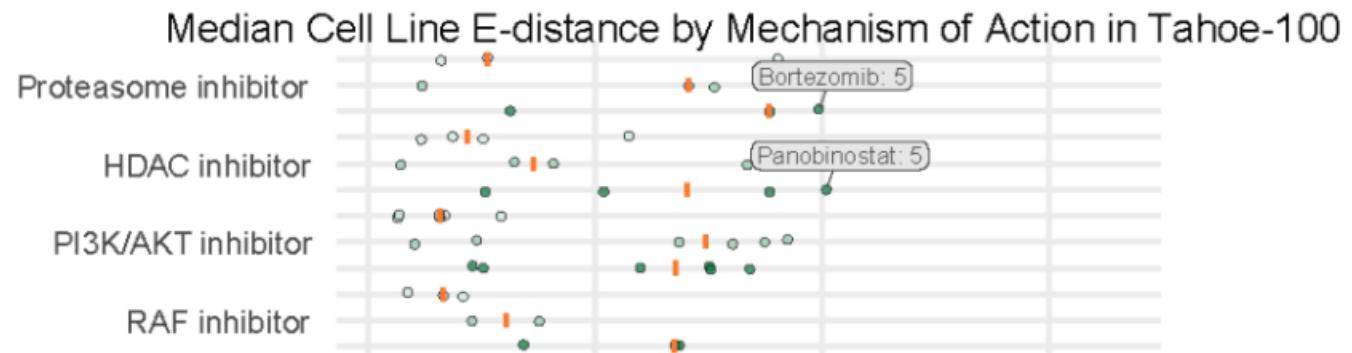
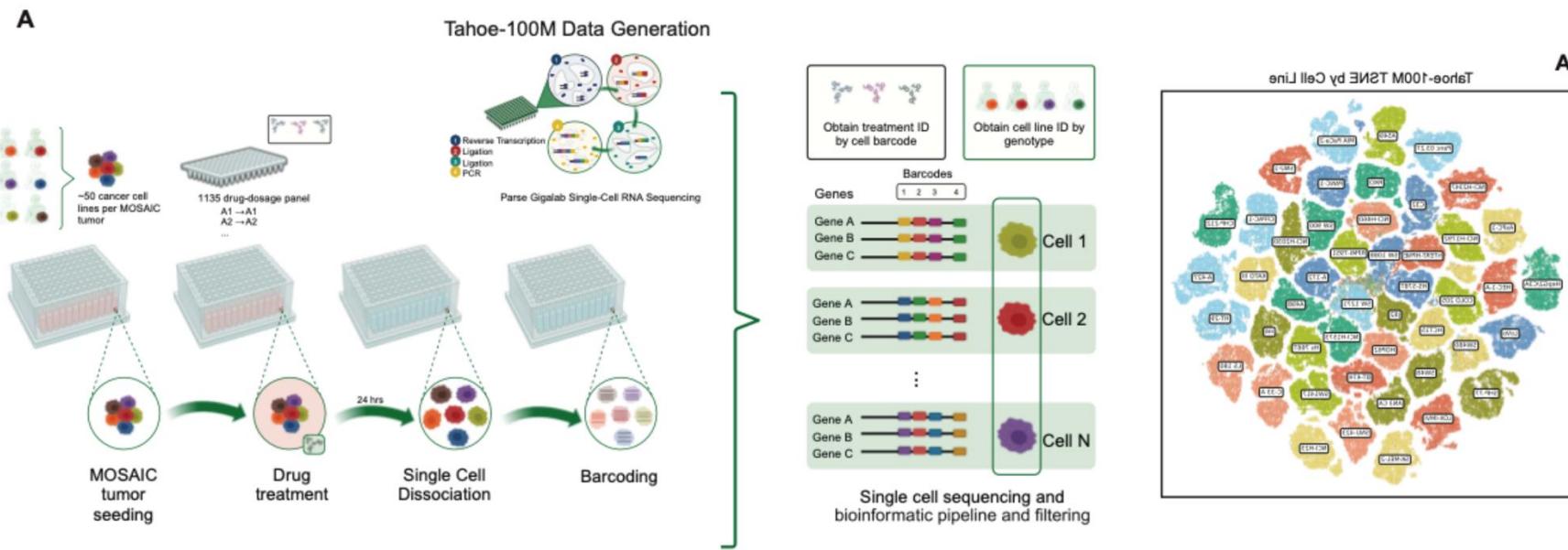


Enormously scalable
Can achieve <\$0.01 per cell

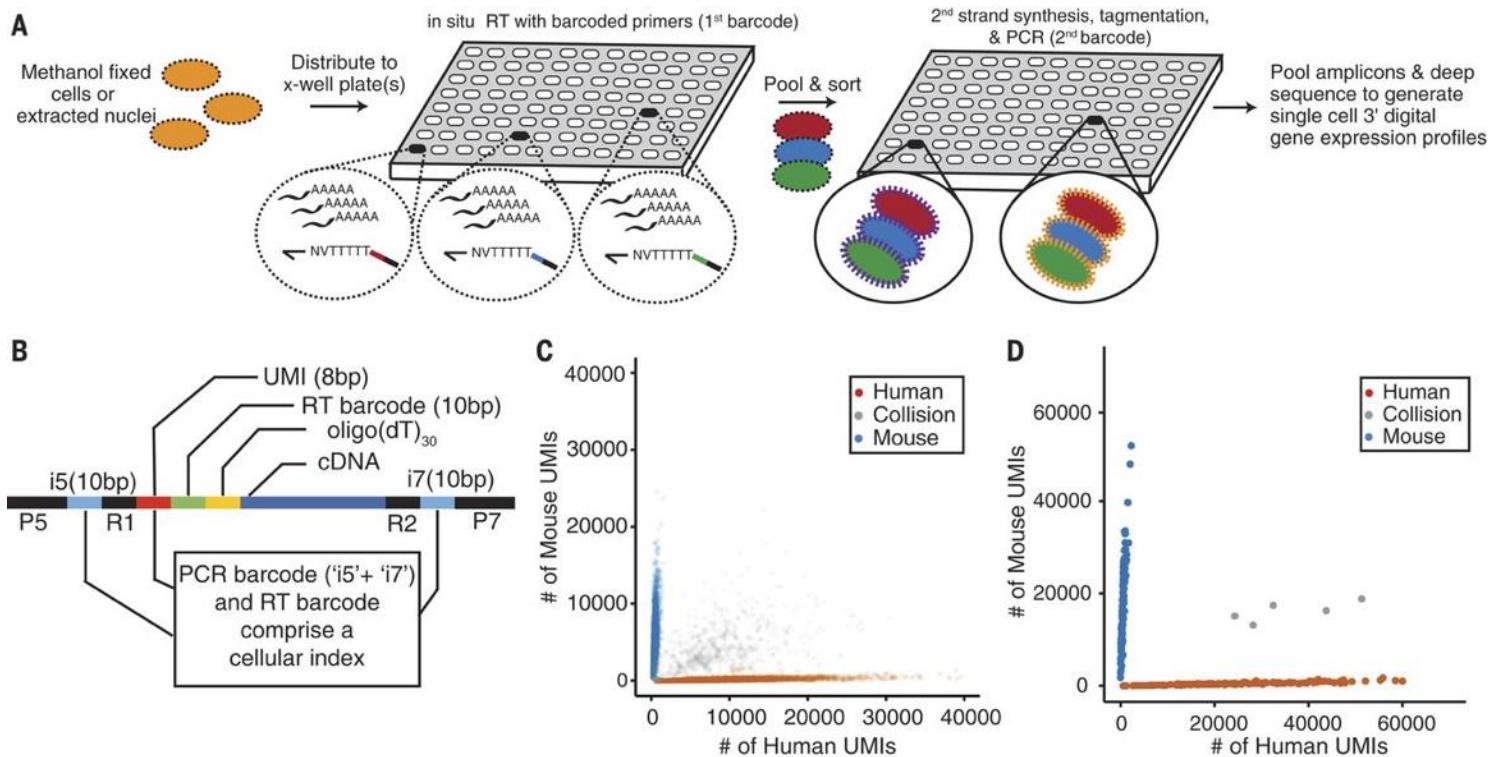


Labor intensive
Significant 'boot-up' cost
Significant validation cost
Who can afford that much sequencing, anyway?

Tahoe-100M: A Giga-Scale Single-Cell Perturbation Atlas for Context-Dependent Gene Function and Cellular Modeling



Combinatorial Indexing sci-Seq



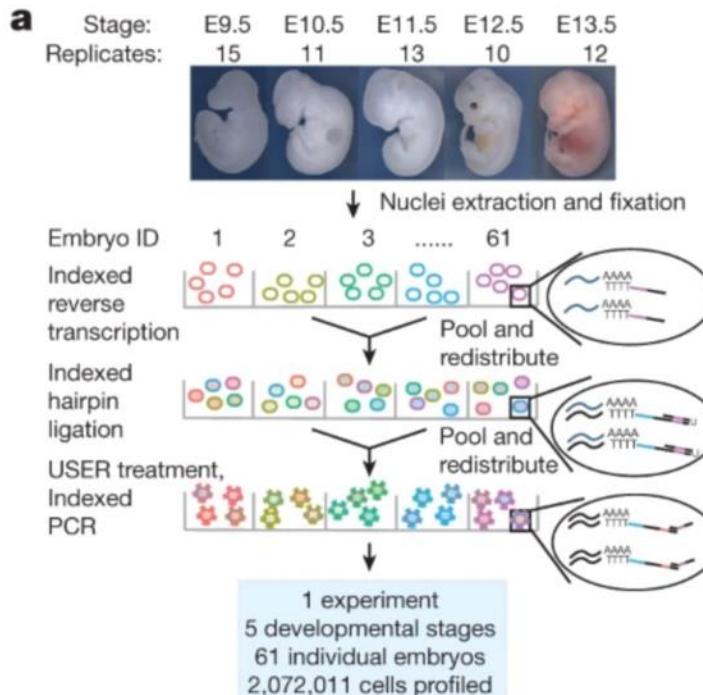
U. Washington
Shendure Lab

The single-cell transcriptional landscape of mammalian organogenesis

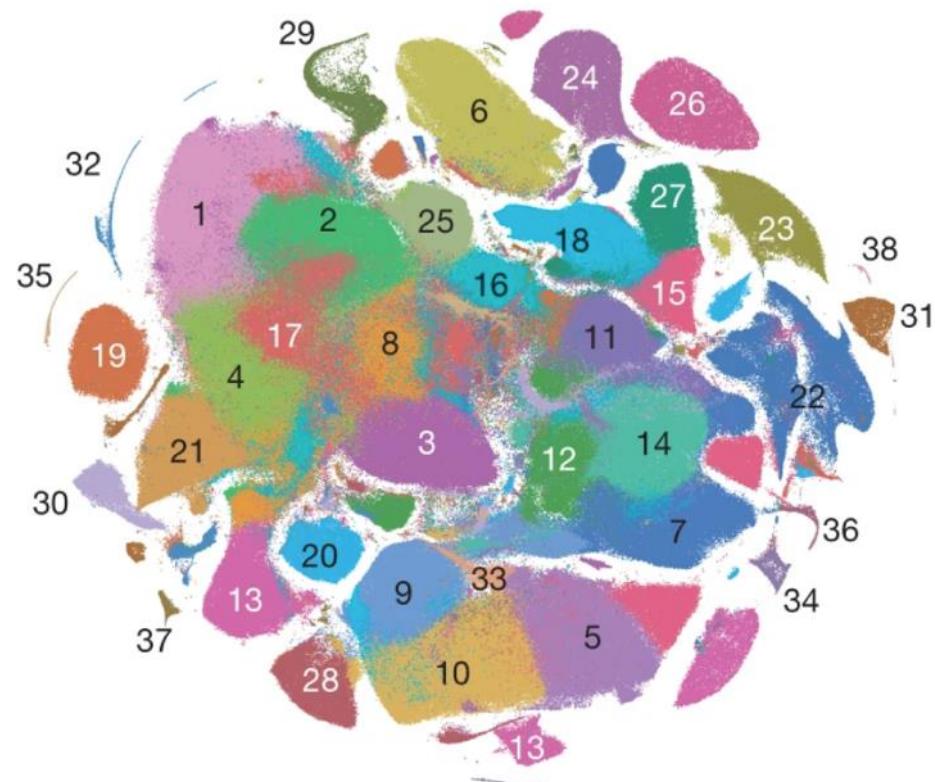
Junyue Cao, Malte Spielmann, Xiaojie Qiu, Xingfan Huang, Daniel M. Ibrahim, Andrew J. Hill, Fan Zhang,
Stefan Mundlos, Lena Christiansen, Frank J. Steemers, Cole Trapnell & Jay Shendure

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

sci-RNA-seq3



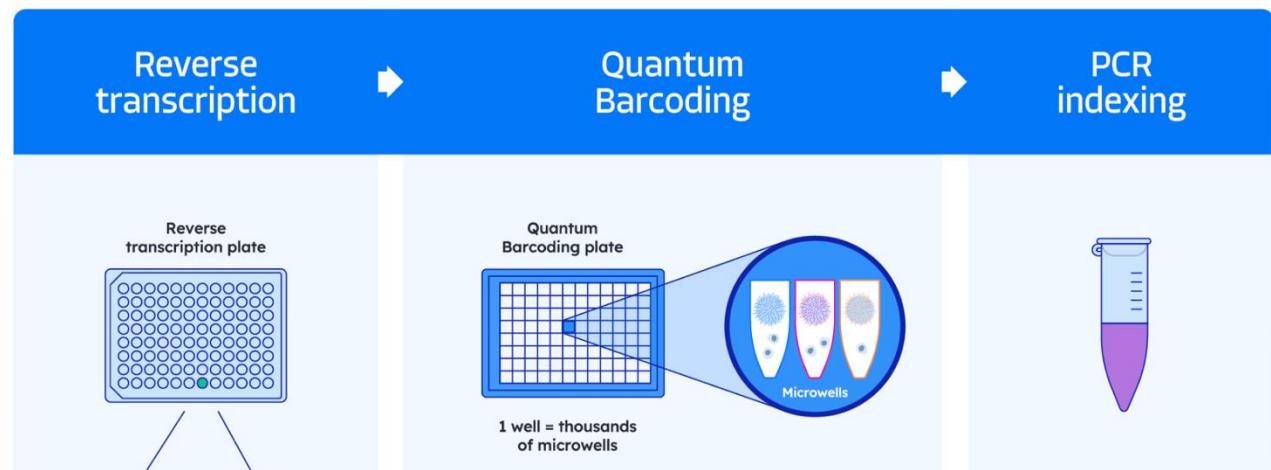
Mouse embryonic development



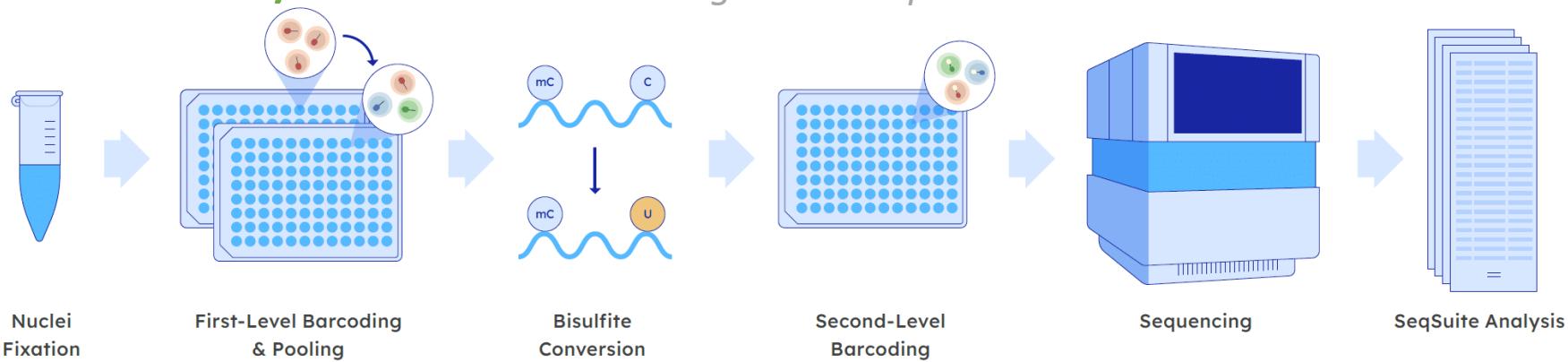
2,058,652 single-cell transcriptomes



scRNA-seq



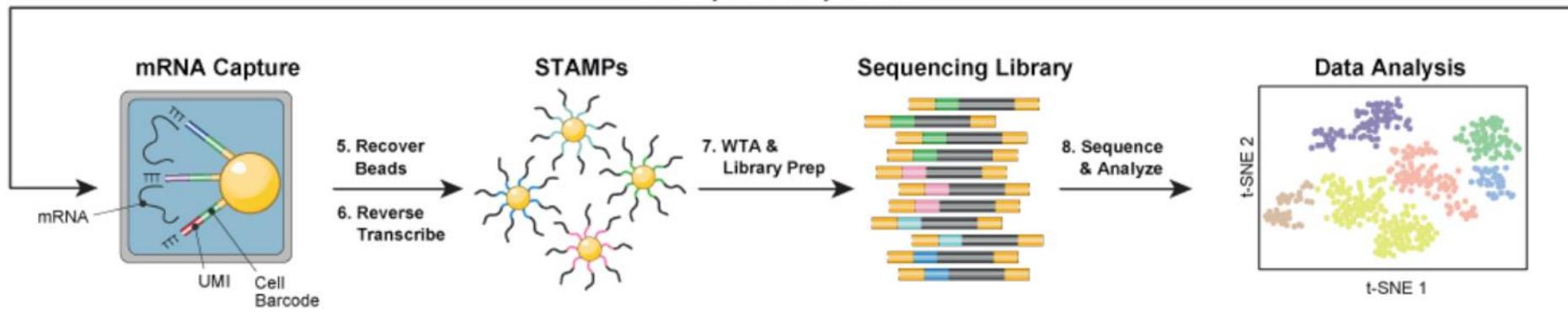
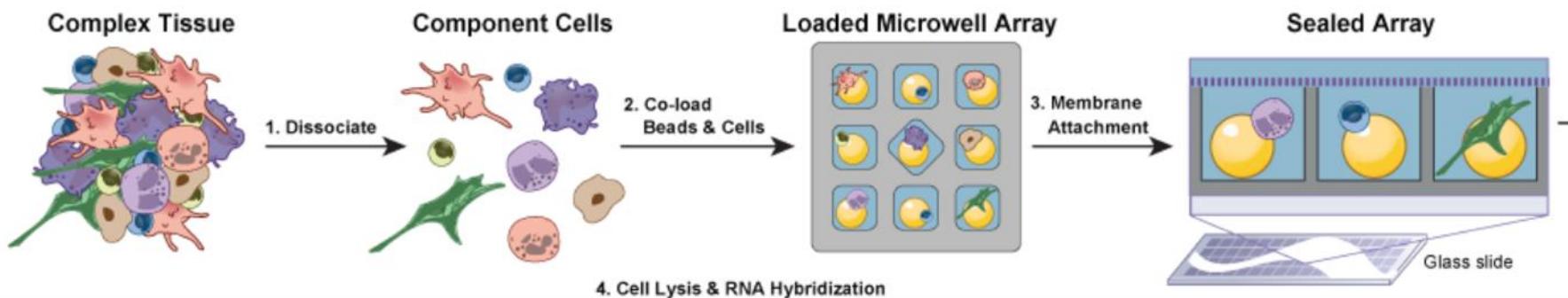
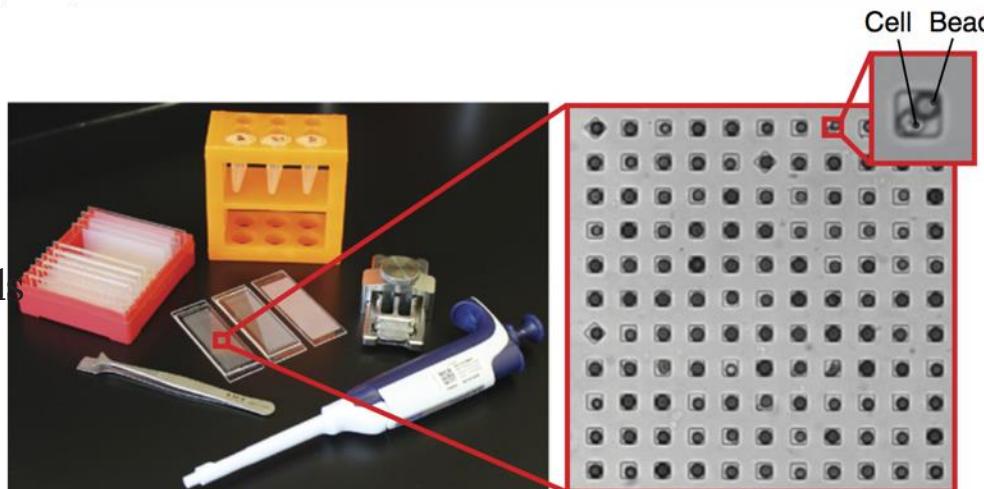
scDNA-methylation



Whole-genome or panel enrichment

Seq-Well

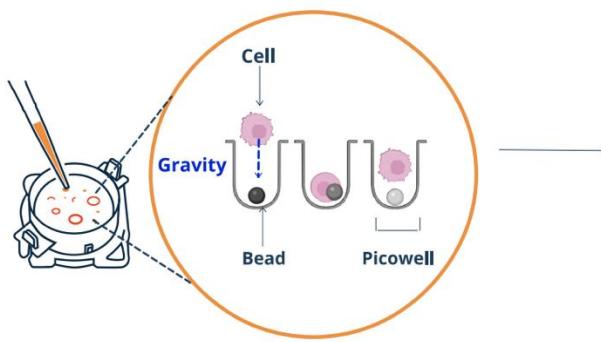
PDMS array of ~86,000 subnanoliter wells
Sized to fit 1 bead per well
Drop-Seq style barcoded beads
Sealed chamber for each cell





THE HIVE

HONEYCOMB

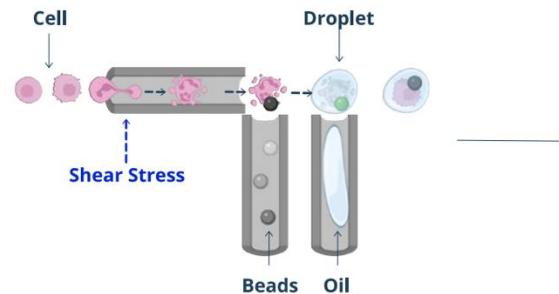


HIVE CELL CAPTURE: Gentle, by gravity or 30g spin.

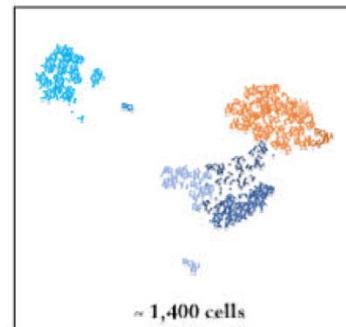
HIVE scRNAseq Method



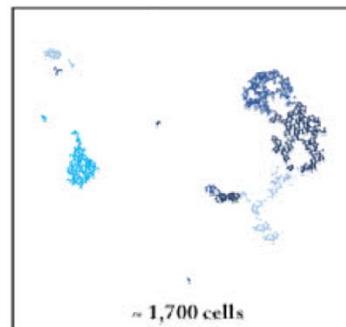
Droplet Based Method



DROPLET CELL CAPTURE: Cells subjected to shear stress.



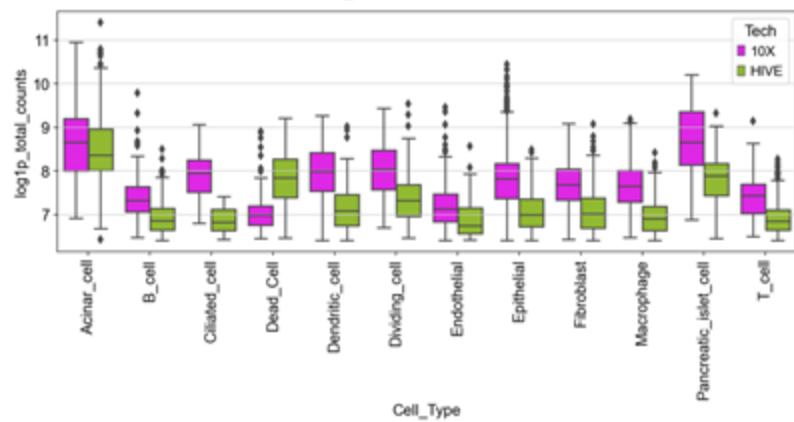
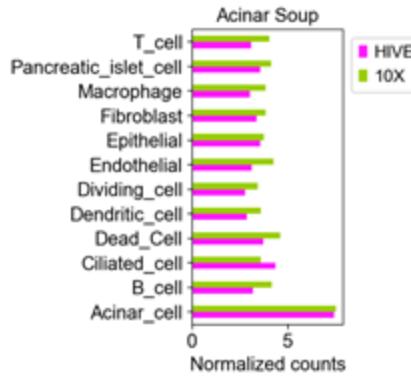
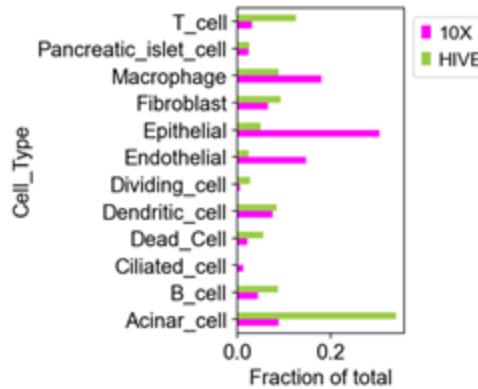
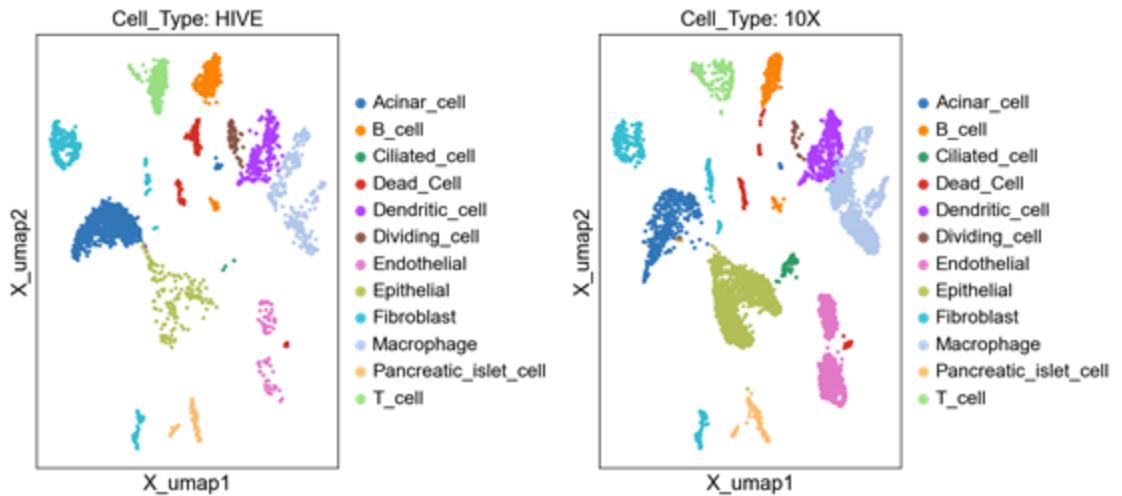
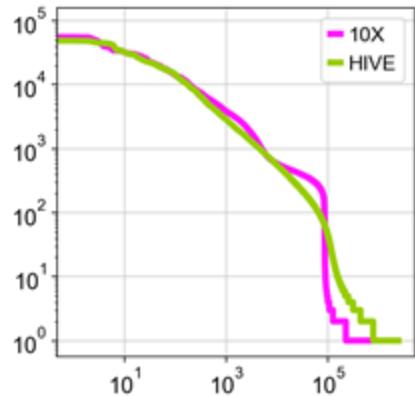
FULL BIOLOGY RECOVERED, including fragile cells like **granulocytes** (neutrophils, eosinophils, and basophils).



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

Cold-dissociated Normal Mouse Pancreas

10X = 132,845,676 reads
 HIVE = 171,905,206 reads

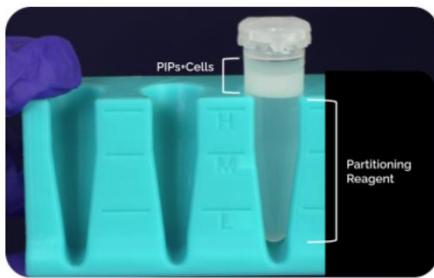
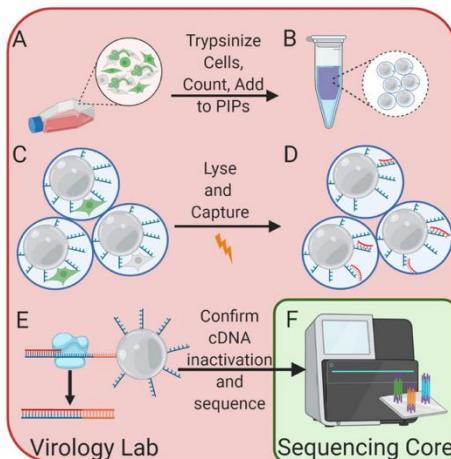
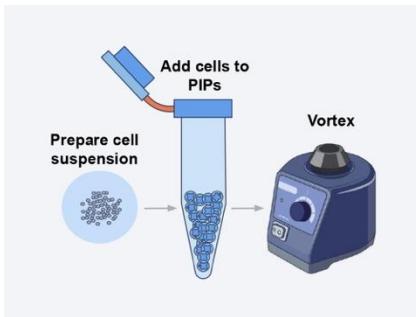




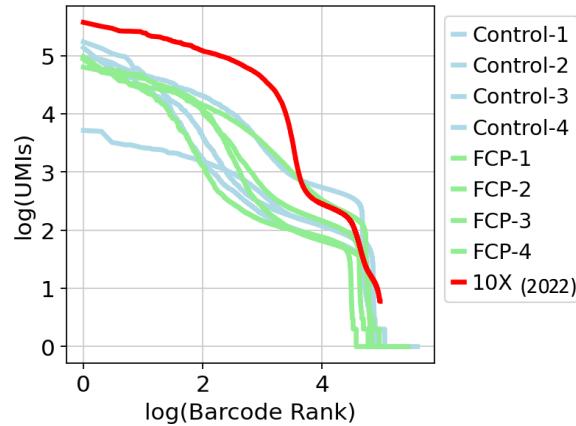
illumina®



PIPseq

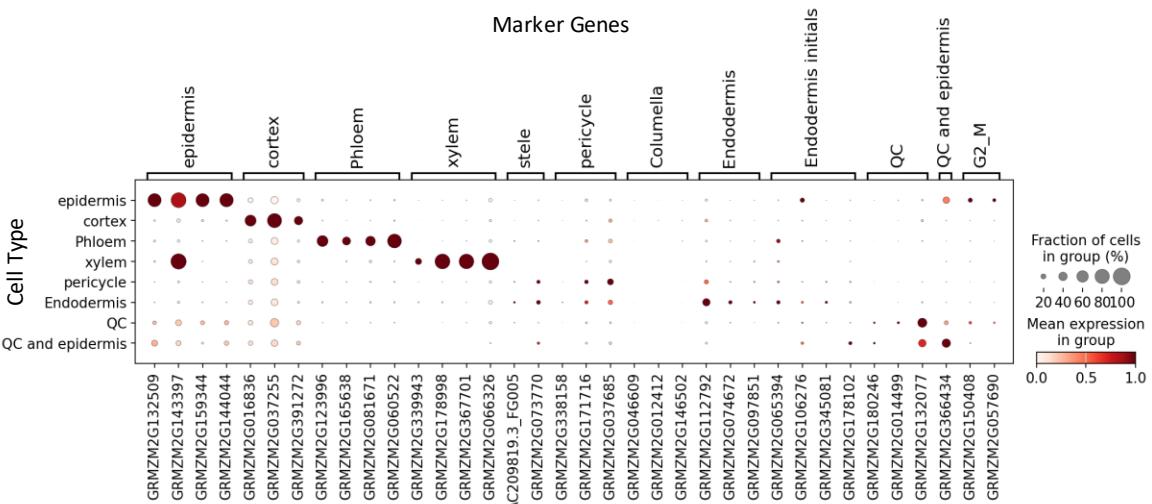
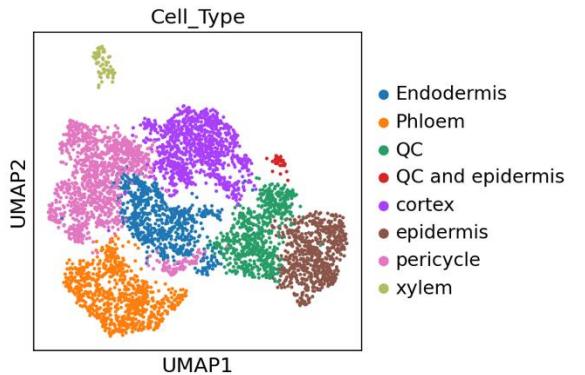
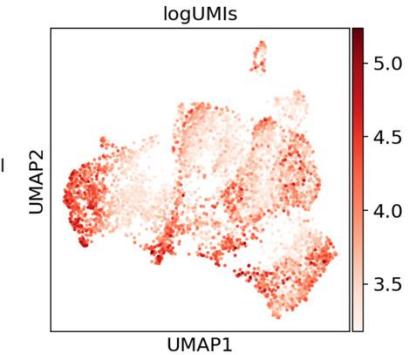
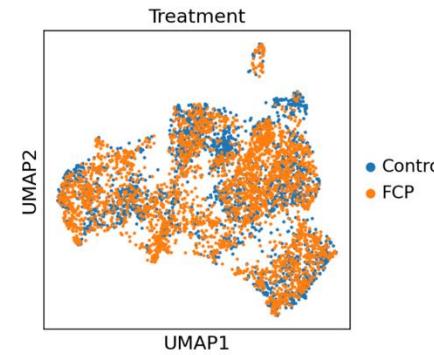


- PIPseq T2:
~2,000 cells
\$300
- PIPseq T20:
~20,000 cells
\$900
- PIPseq T100:
~100,000 cells
\$???



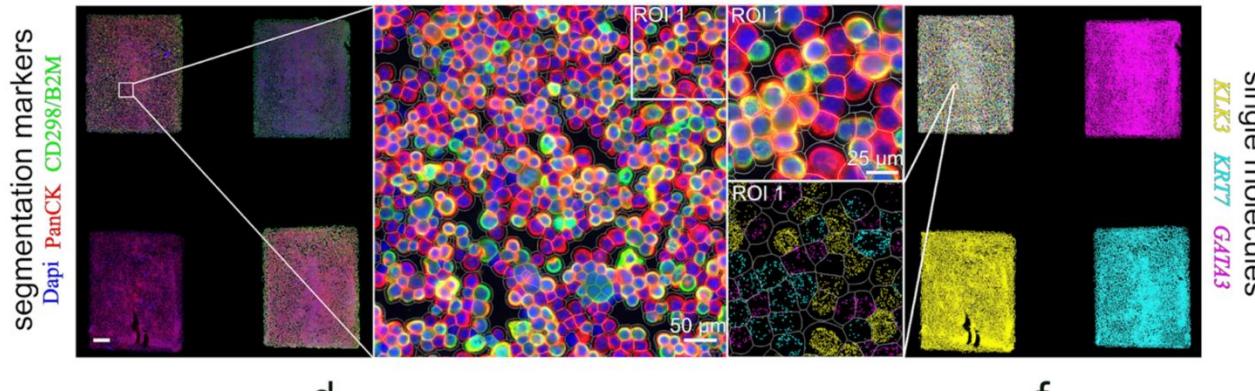
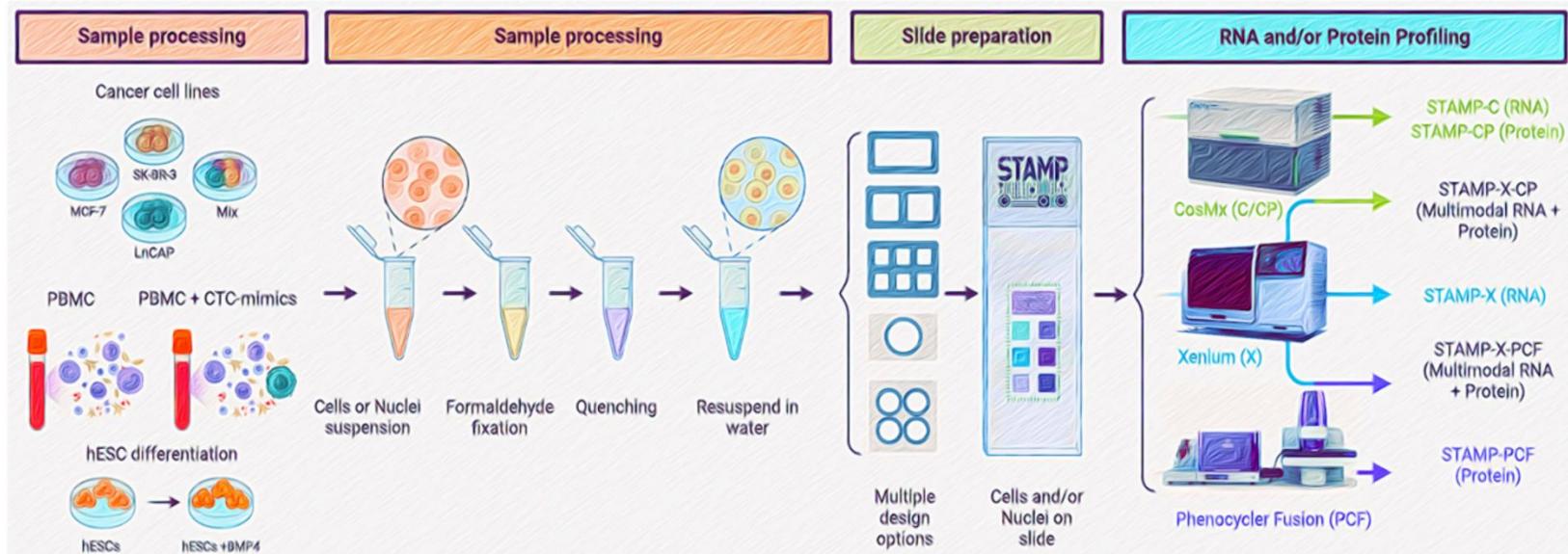
UMIs > 1,500

Sample	Filtered Cells
FCP-3	2134
Control-4	1678
FCP-1	471
FCP-4	341
Control-3	193
Control-2	107
Control-1	103
FCP-2	87



STAMP: Single-Cell Transcriptomics Analysis and Multimodal Profiling through Imaging

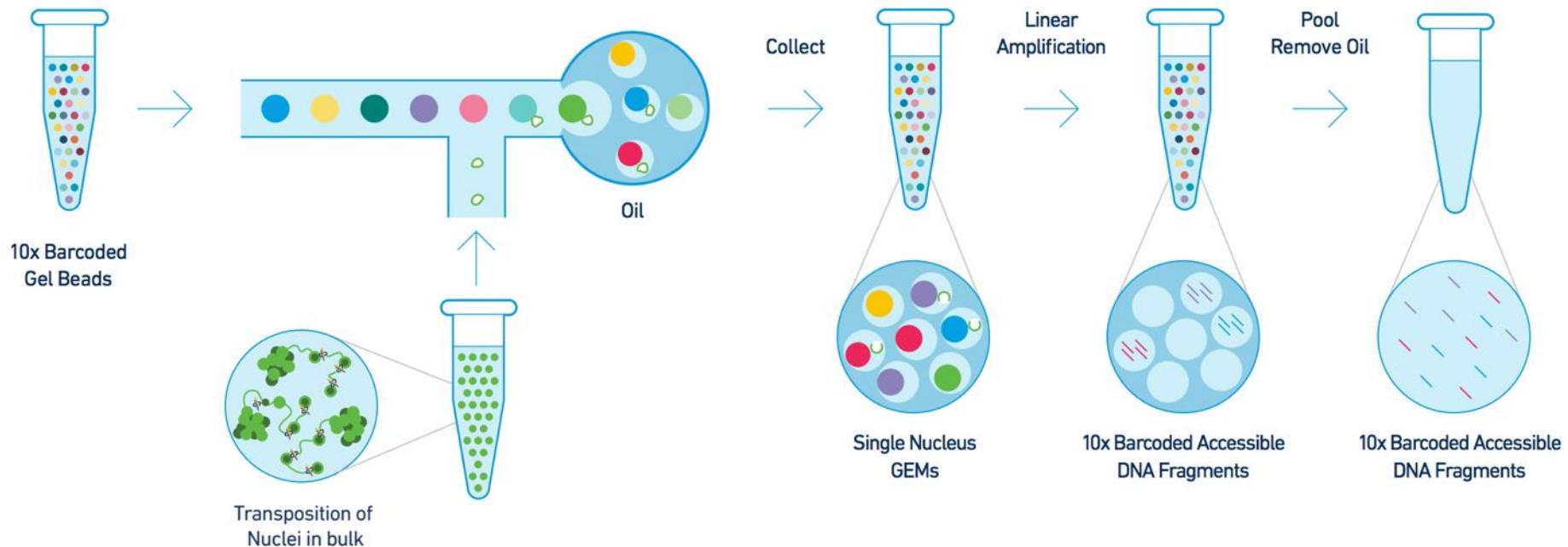
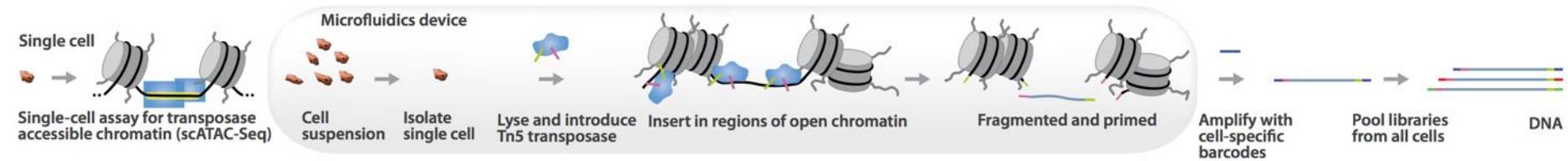
Emanuele Pitino, ... Luciano Martelotto. *bioRxiv*





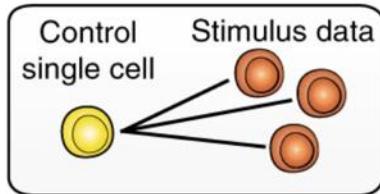
The Other
Nucleic Acid

10X Genomics Single Cell ATAC



a

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



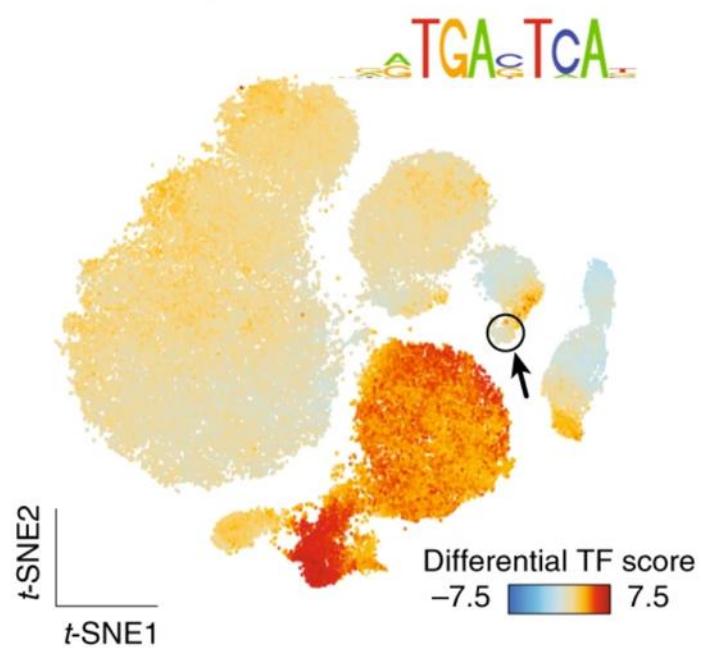
Compute differential TFs

Stimulation – control

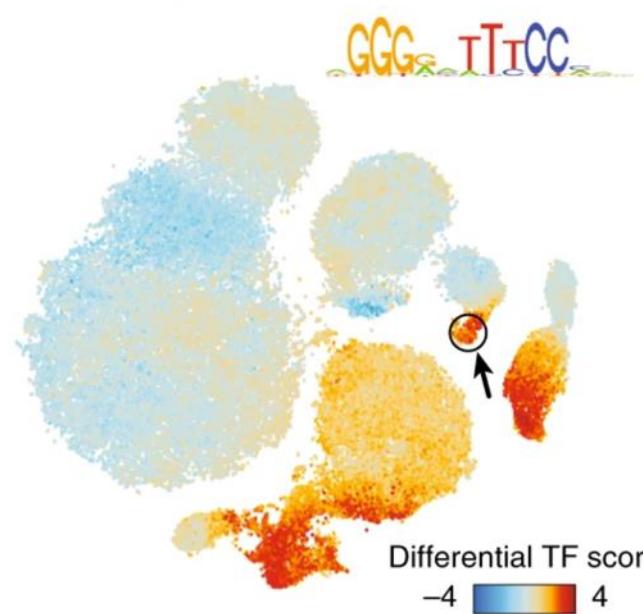
Smooth and display TFs on t-SNE

**b**

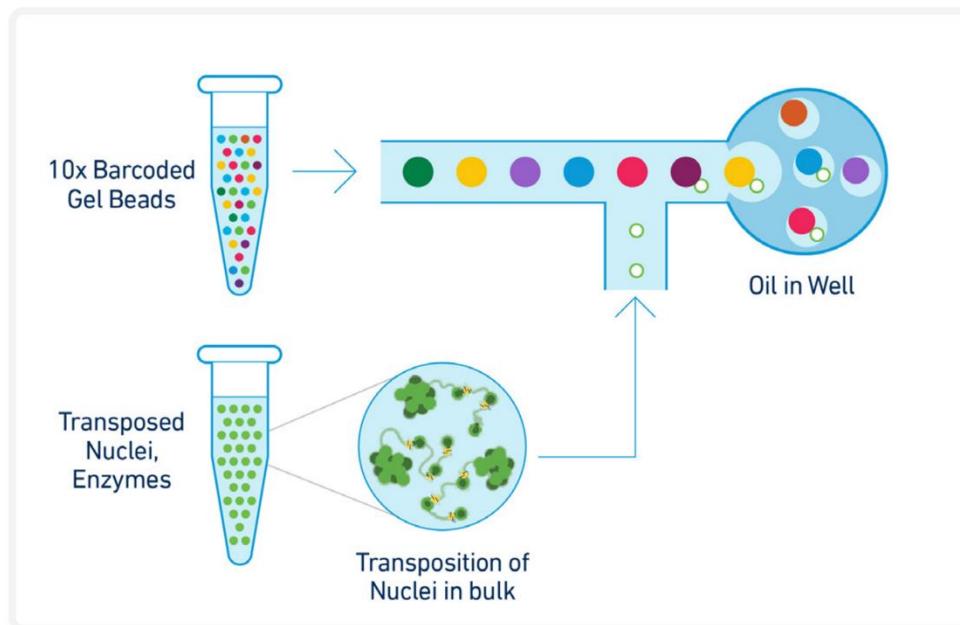
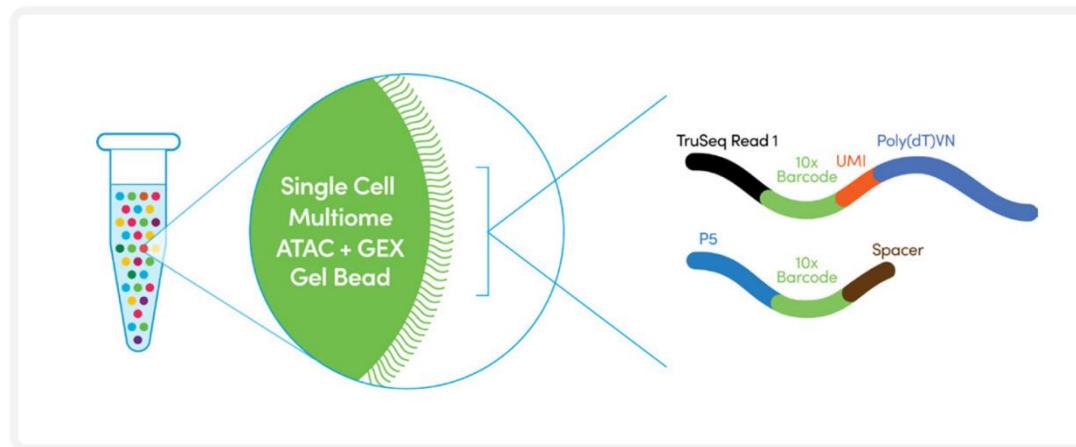
Jun TF motif
Stimulation – control

**c**

NF- κ B TF motif
Stimulation – control

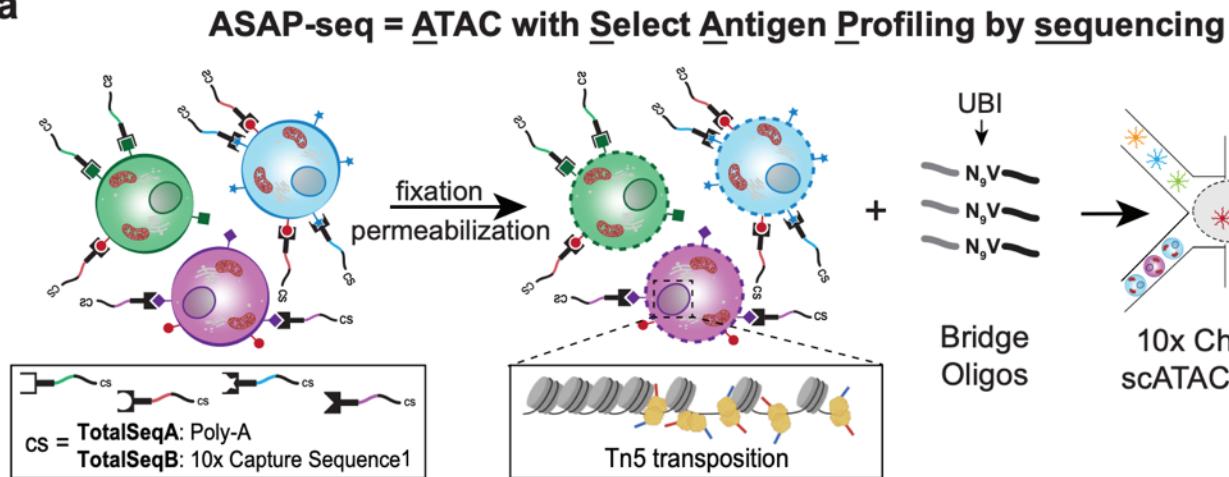


10X Genomics Multiome

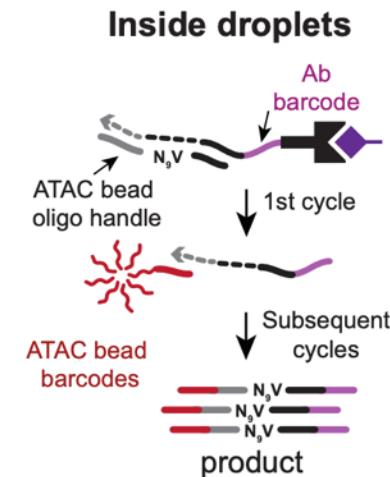


ASAP-seq / DOGMA-seq

a

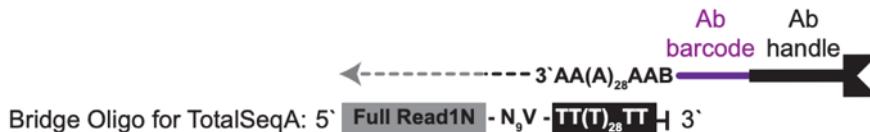


b



b

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



II. Annealing of extended antibody tag with barcoded oligo

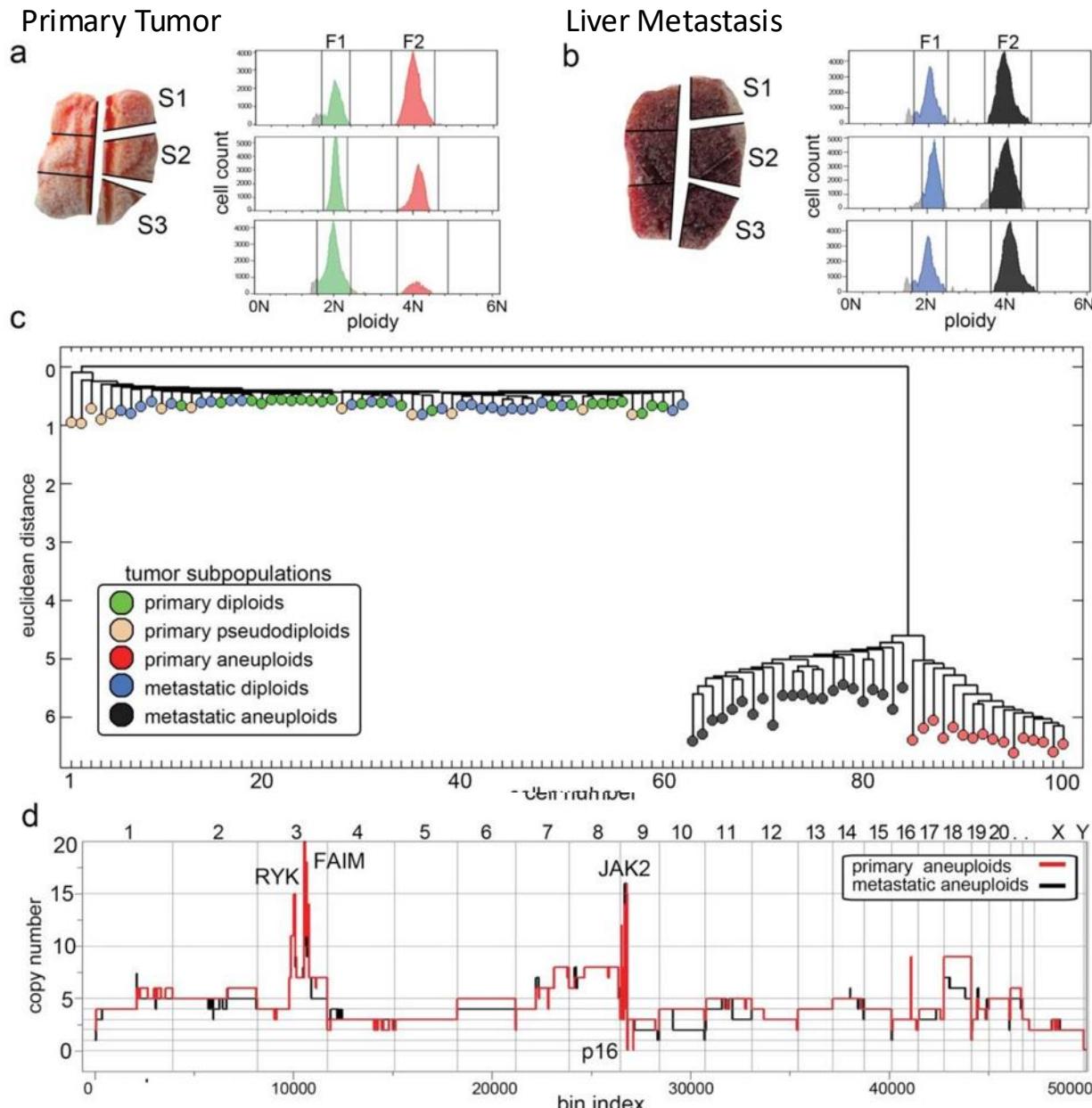
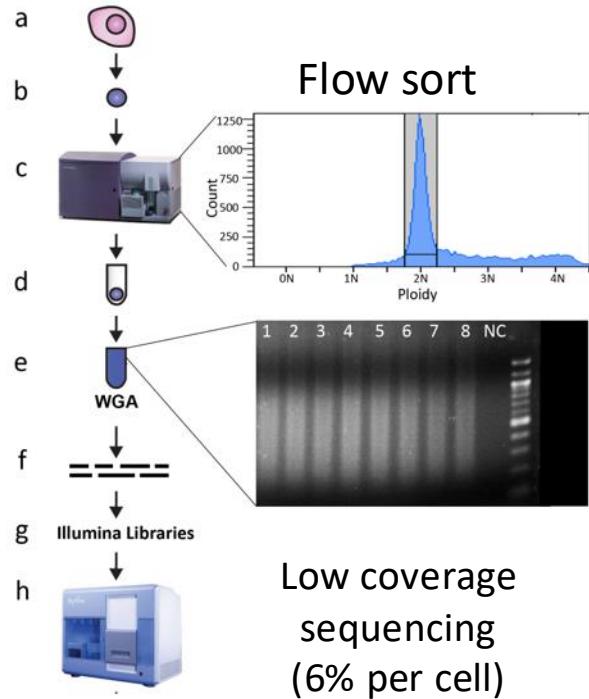


III. Extension of barcoded oligo and amplification for ≤11 cycles

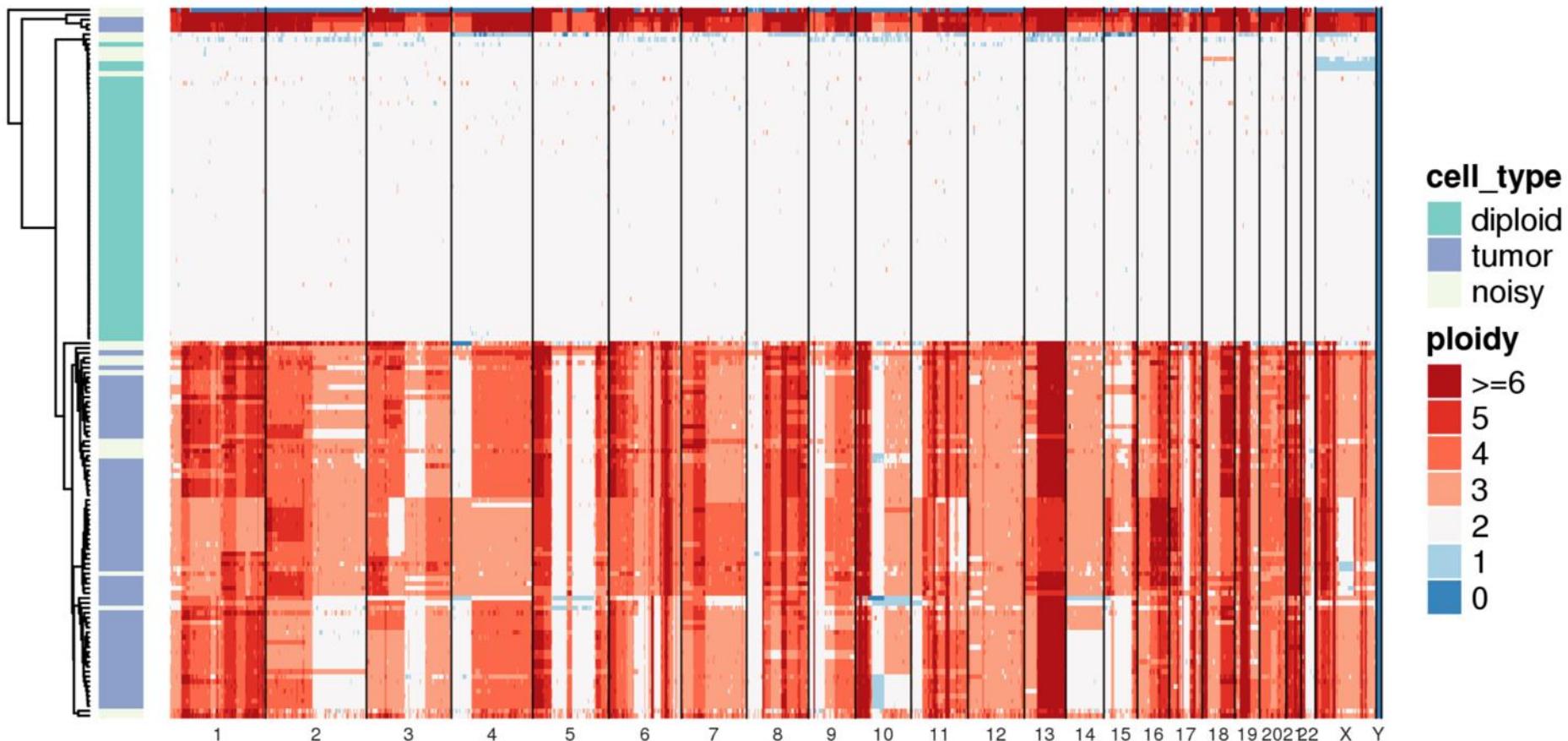


Single cell CNV

Nick Navin, Mike Wigler
CSHL



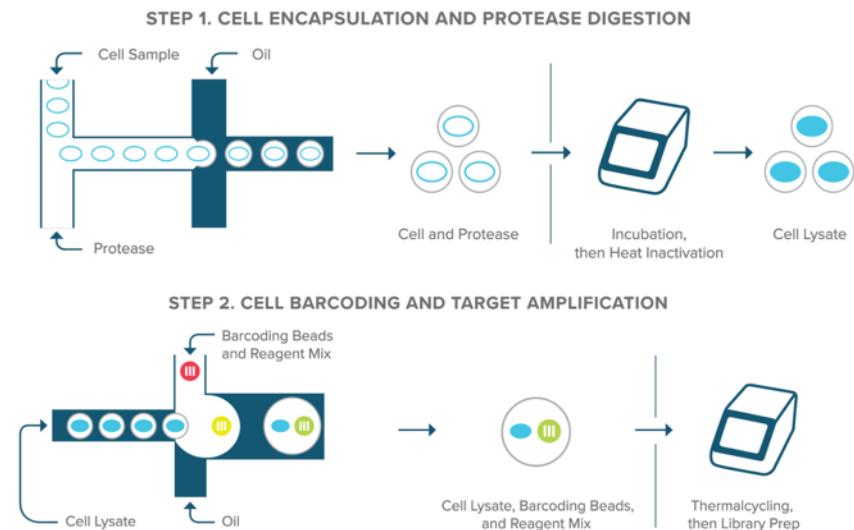
Droplet-based Single Cell CNV



Mission Bio Tapestri

DNA-focused microfluidic platform

For SNV & CNV



Mission Bio Tapestri

DNA-focused microfluidic platform

For SNV & CNV

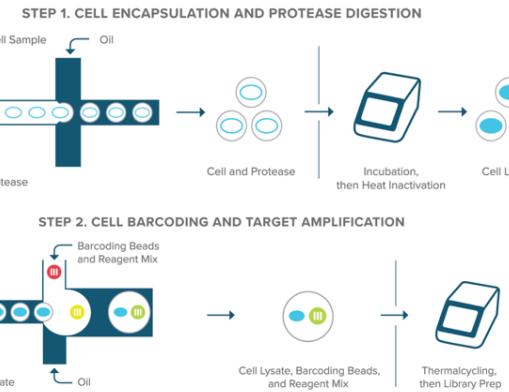


59 GENES - TUMOR HOTSPOT PANEL

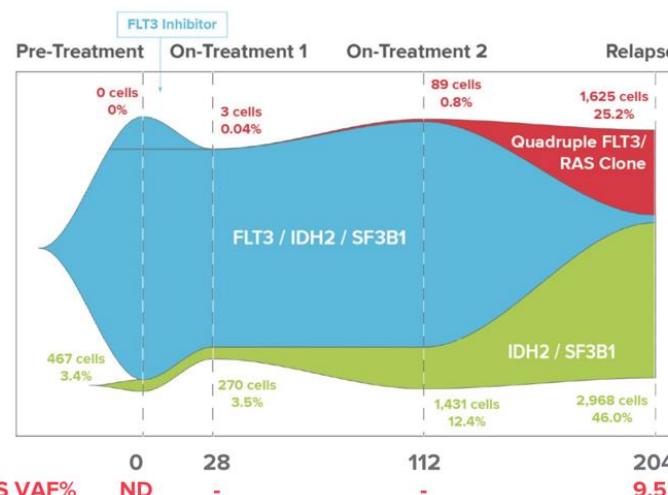
ABL1	CSF1R	FGFR1	IDH2	MLH1	RB1
AKT1	CTNNB1	FGFR2	JAK1	MPL	RET
ALK	DDR2	FGFR3	JAK2	MTOR	SMAD4
APC	EGFR	FLT3	JAK3	NOTCH1	SMARCB1
AR	ERBB2	GNA11	KDR	NRAS	SMO
ATM	ERBB3	GNAQ	KIT	PDGFRA	SRC
BRAF	ERBB4	GNAS	KRAS	PIK3CA	STK11
CDH1	ESR1	HNF1A	MAP2K1	PTEN	TP53
CDK4	EZH2	HRAS	MAP2K2	PTPN11	VHL
CDKN2A	FBXW7	IDH1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

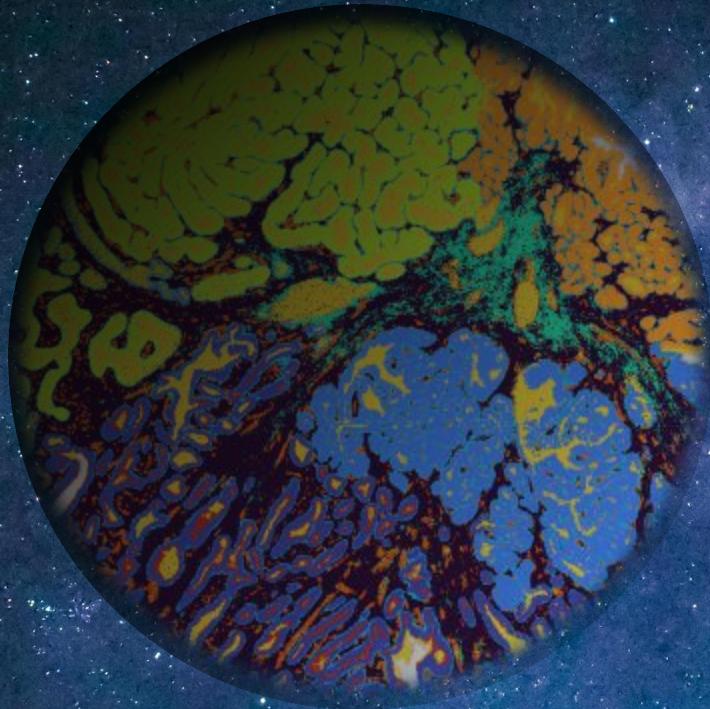
45-GENE MYELOID PANEL

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





LOST
IN
Space