

An Introduction to Single-Cell Genomics

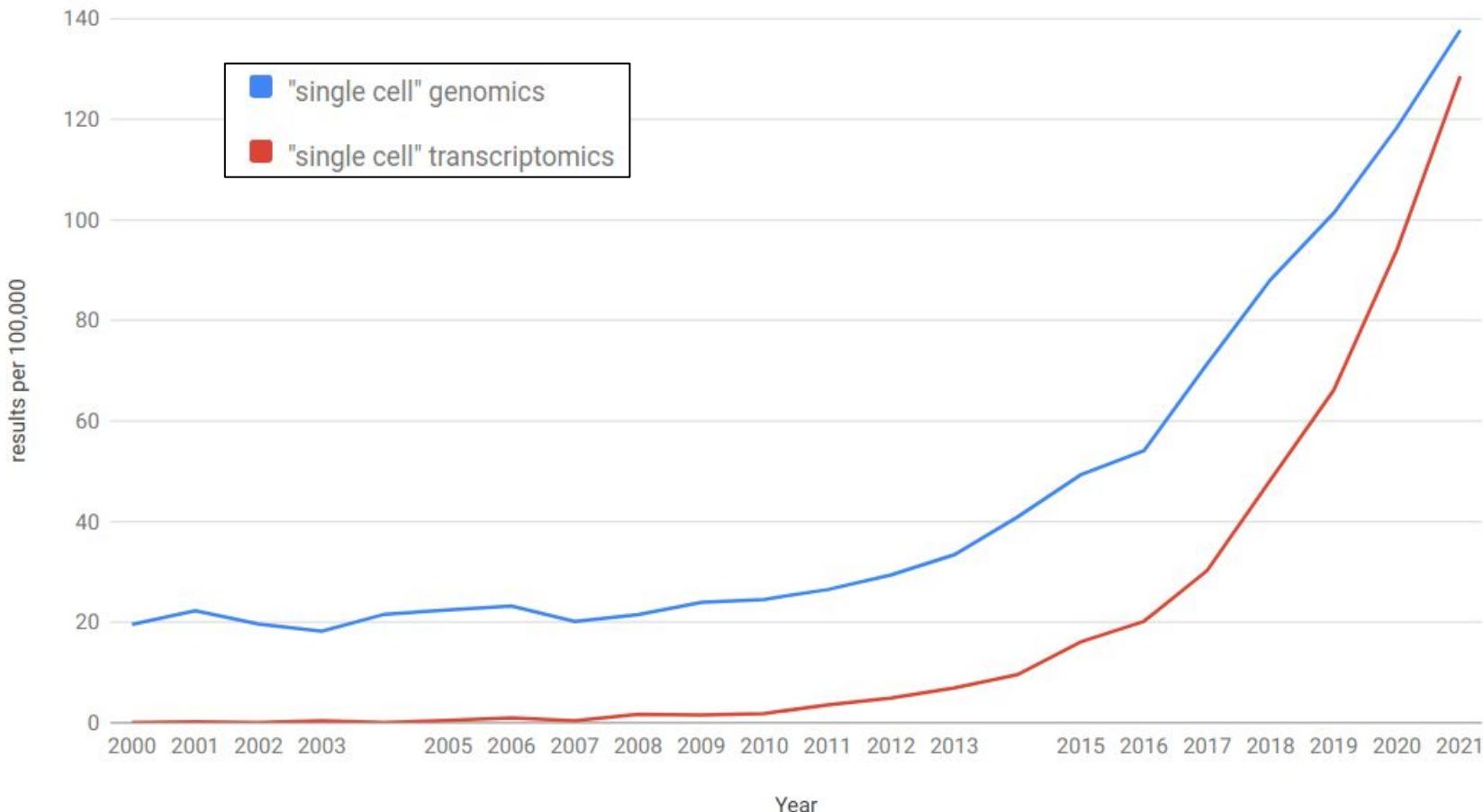
(Actually, mostly transcriptomics)
(Actually, mostly from 10X Genomics)

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INSERM / Gustave Roussy

*So you say you've heard
About
Single cell ?*

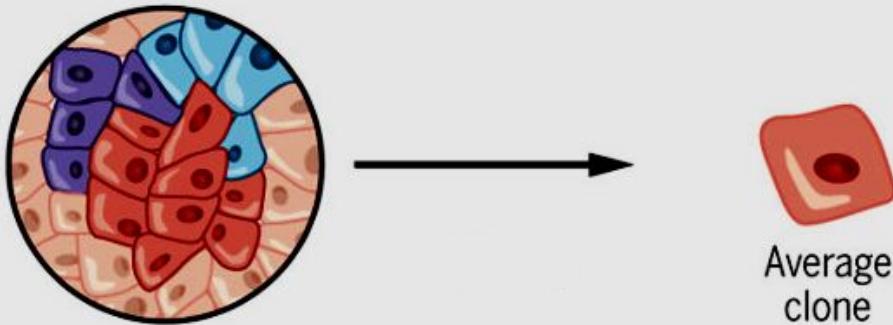


Single cell in peer-reviewed publications (2021)

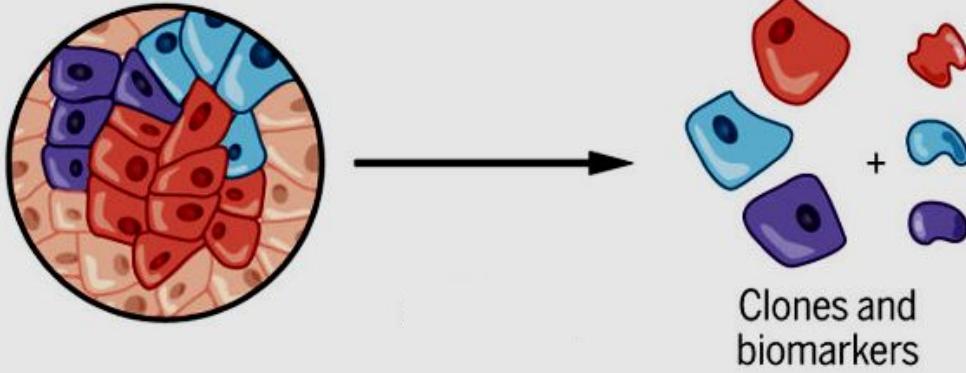


Why so much hype ?

A Bulk analysis



B scRNA analysis

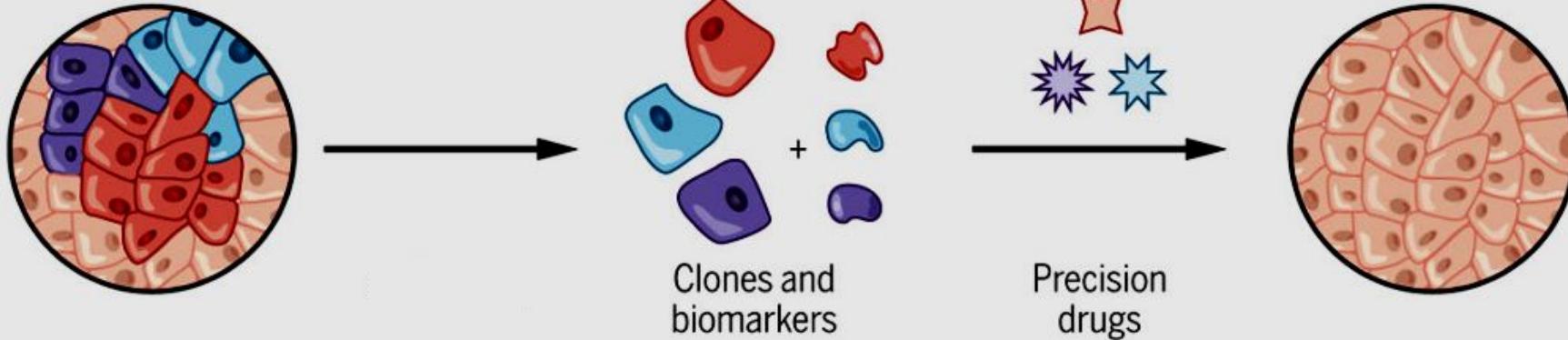


Why so much hype ? (pathology)

A Bulk analysis



B scRNA analysis



Why so much hype ?

Bulk



Single cell



Spatial single cell



Why so much hype ?

Bulk



Single cell



Spatial single cell



Full-length spatial single cell

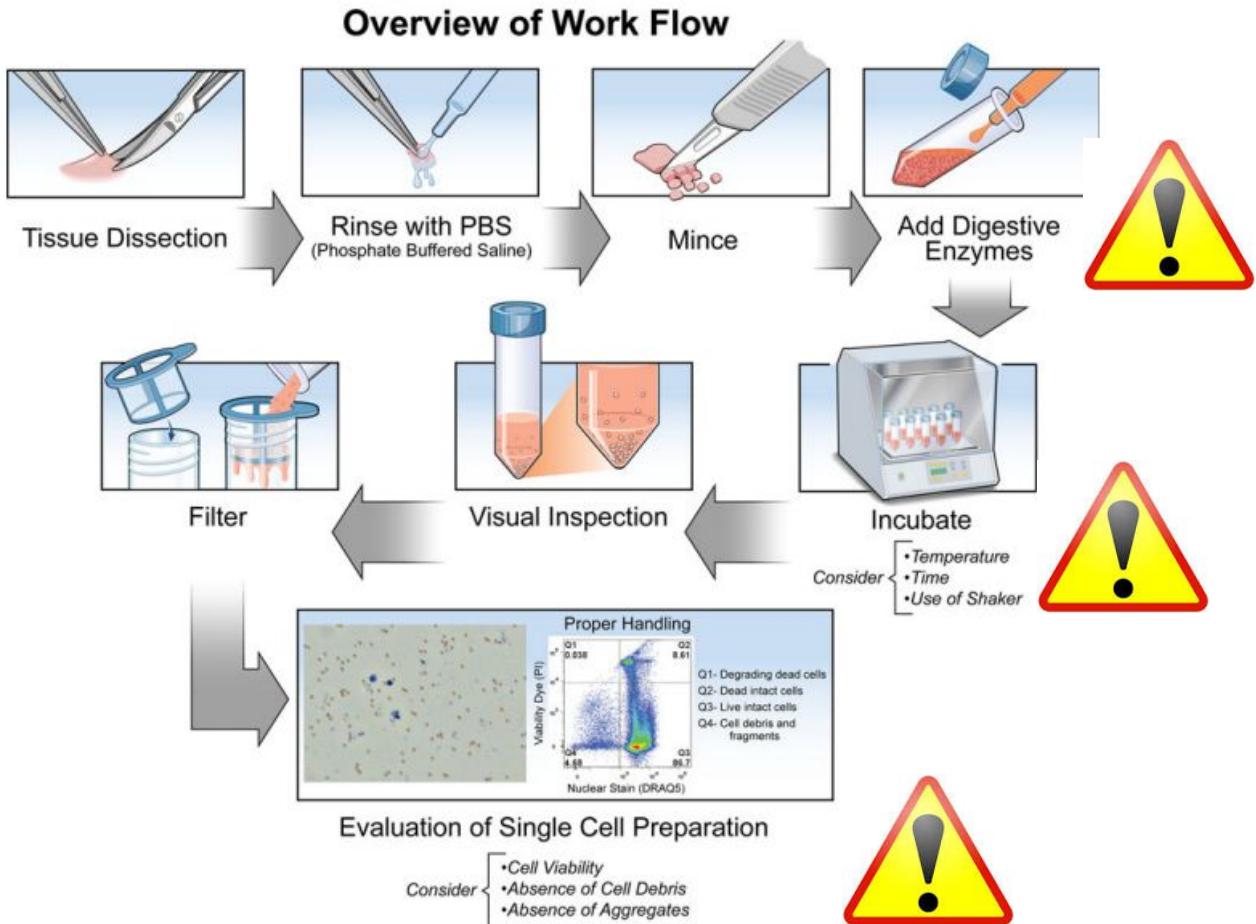




*From
Broad tissue
To
Isolated cells*

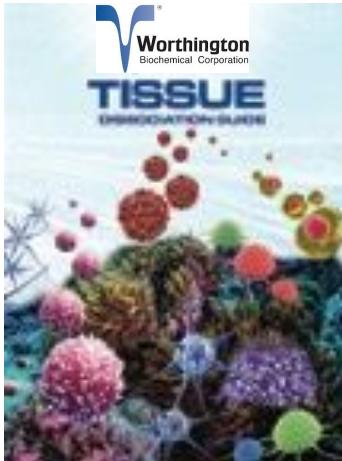


Cells health and dissociation



Cells health and dissociation : Worthington helpdesk

1. Type of tissue
2. Species of origin
3. Age of the animal
4. Genetic modification(s) (knockouts, etc.)
5. Dissociation medium used
6. Enzyme(s) used
7. Impurities in any crude enzyme preparation used
8. Concentration(s) of enzyme(s) used
9. Temperature
10. Incubation times



Tissue Tables (references, grouped by tissue type and species)

Adipose/Fat	Adrenal	Bone	Brain
Cartilage	Colon	Endothelial	Epithelial
Eye	Heart	Intestine	Kidney
Liver	Lung	Lymph nodes	Mammary
Miscellaneous	Muscle	Neural	Pancreas
Parotid	Pituitary	Prostate	Reproductive
Scales	Skin	Spleen	Stem
Thymus	Thyroid/Parathyroid	Tonsil	Tumor

II. Cell Isolation Theory

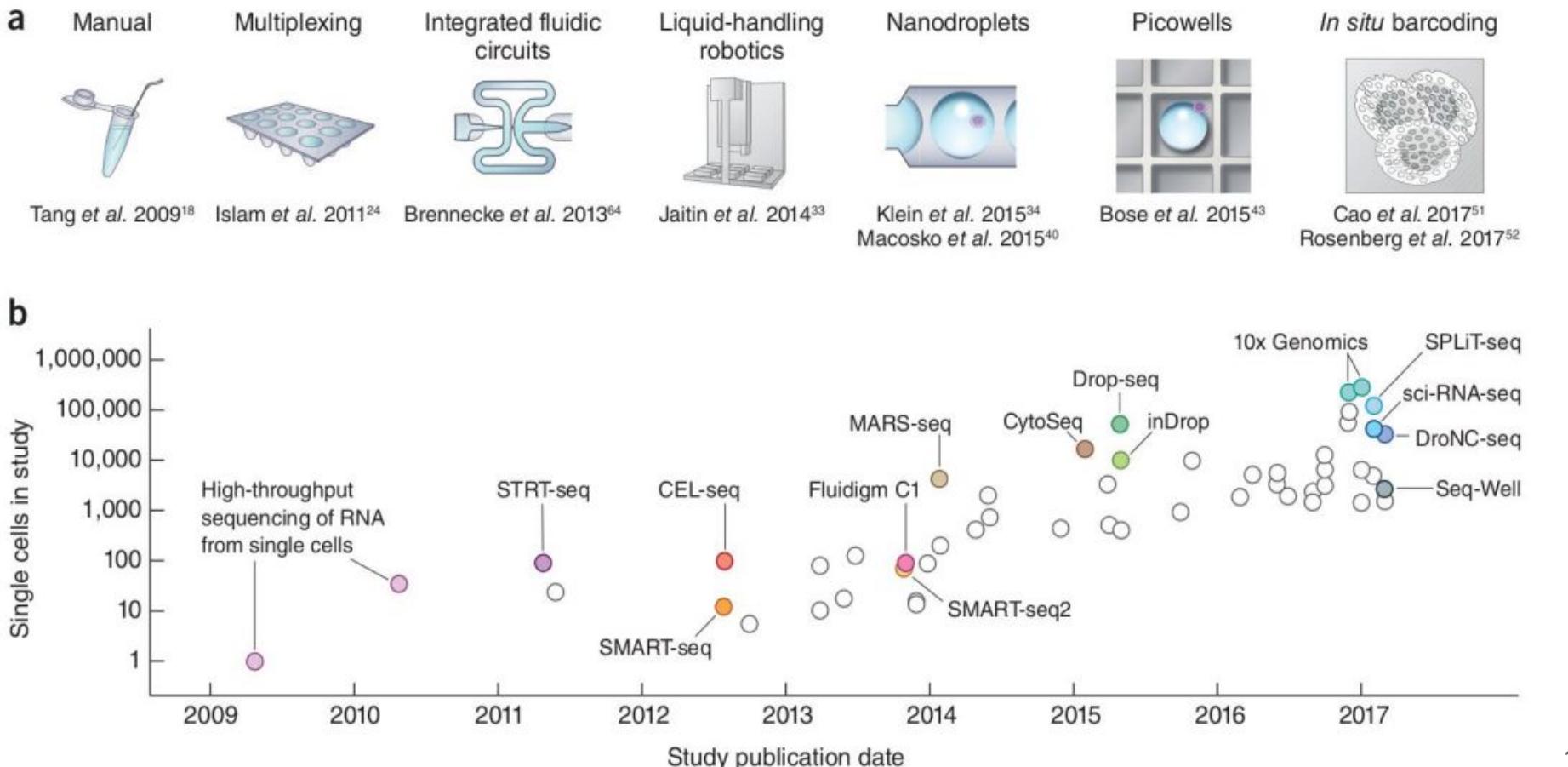
- Tissue Types
 - Epithelial Tissue
 - Connective Tissue
- Dissociating Enzymes
 - Collagenase
 - Trypsin
 - Elastase
 - Hyaluronidase
 - Papain
 - Chymotrypsin
 - Deoxyribonuclease I
 - Neutral Protease (Dispase)
 - Trypsin Inhibitor
 - Animal Origin Free (AOF) Enzymes
 - Celase® GMP

III. Cell Isolation Techniques

- Methods & Materials
 - Working With Enzymes
 - Basic Primary Cell Isolation
 - Equilibration with 95%O₂:5%CO₂
 - Trituration
 - Enzymatic Cell Harvesting
 - Cell Adhesion and Harvesting
 - Trypsin for Cell Harvesting
 - Cell Release Procedure
- Optimization Techniques
 - General Guidelines
 - Optimization Strategy
 - Cell Quantitation
 - Measure of Viability

IV. Use-Tested Cell Isolation Systems

Cells isolation : technologies over the last decade

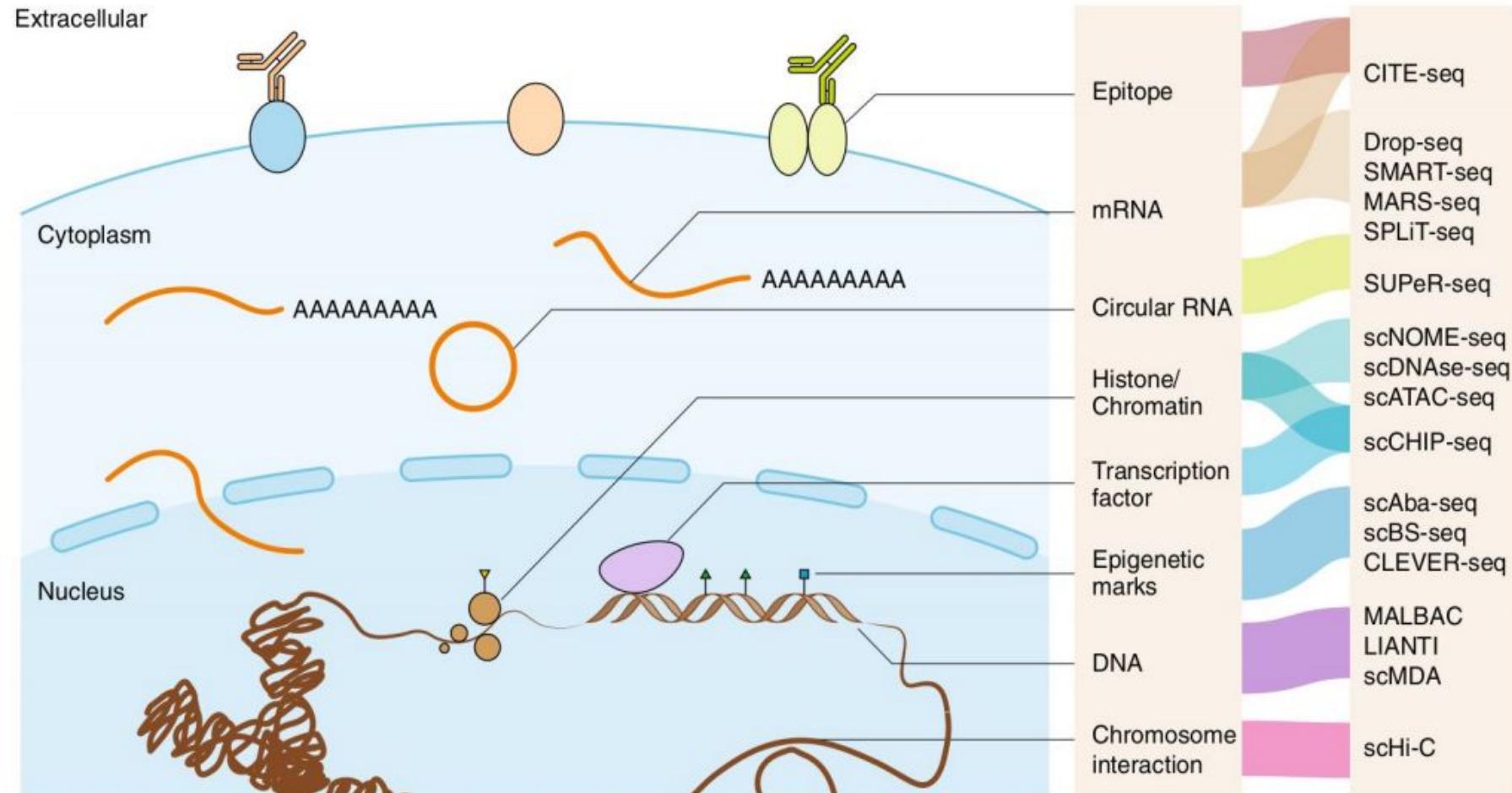




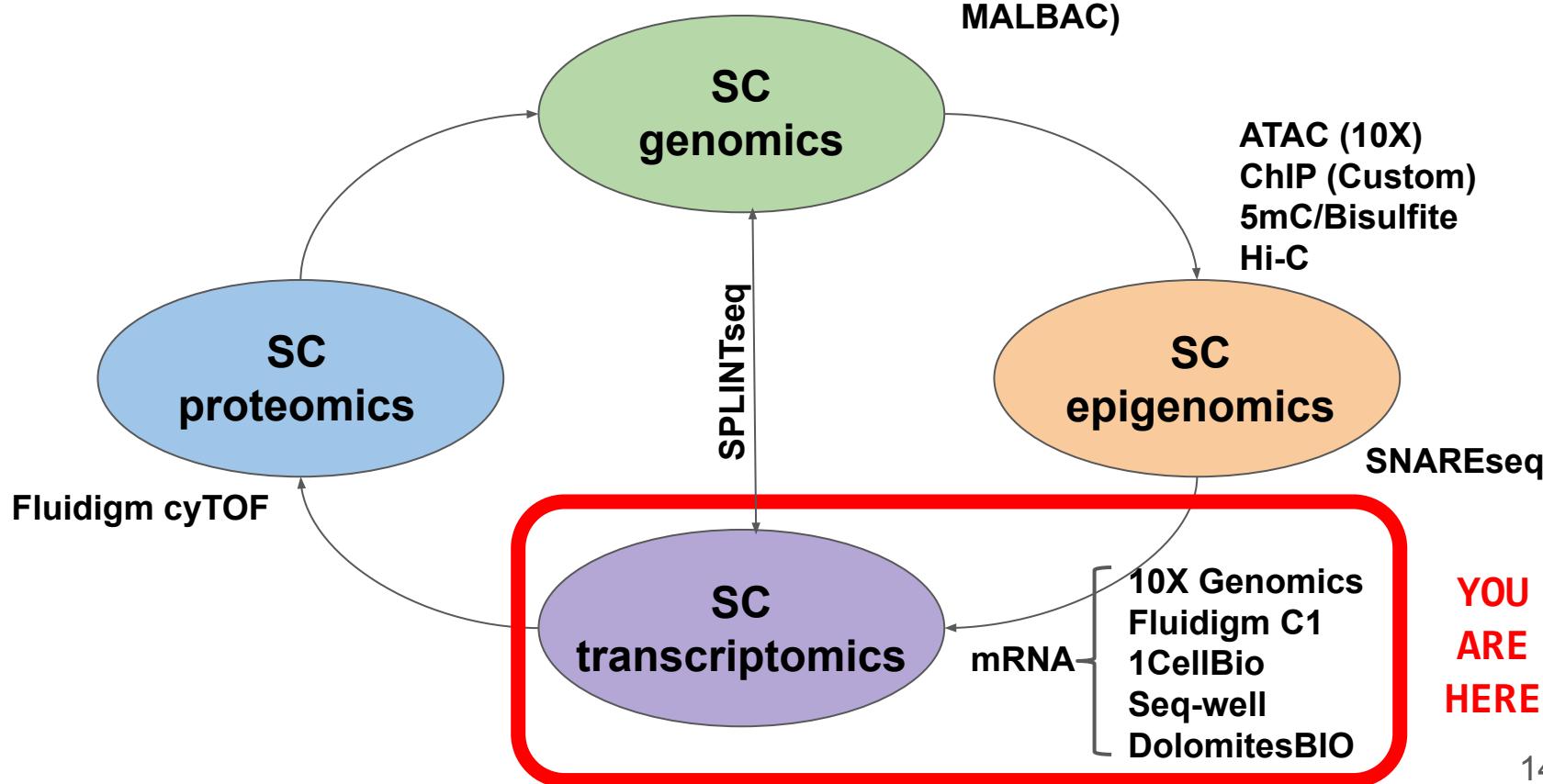
*From
Isolated cells
To
Nucleotide sequences (reads)*



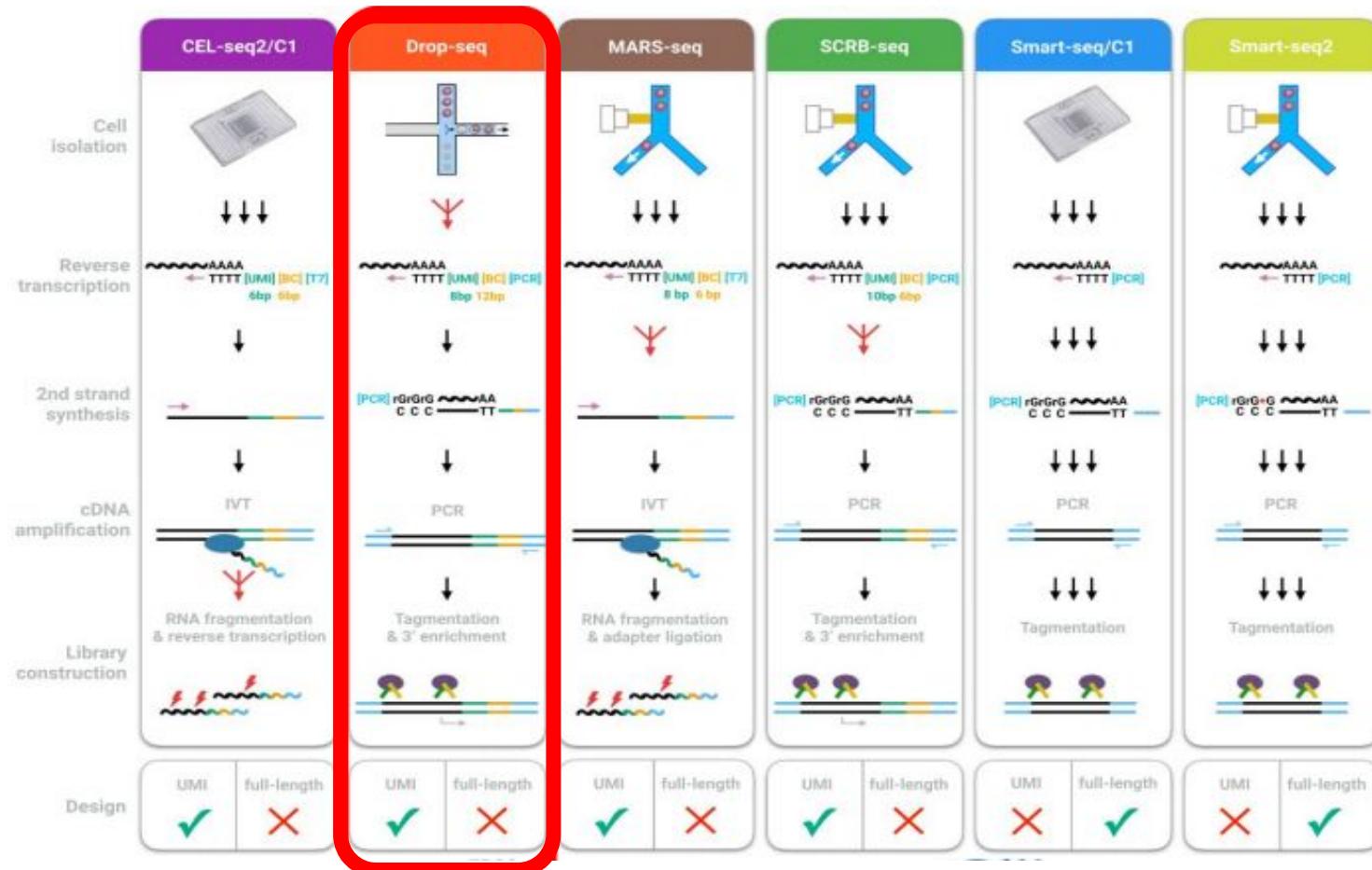
Several protocols for several purposes



Single Cell RNaseq



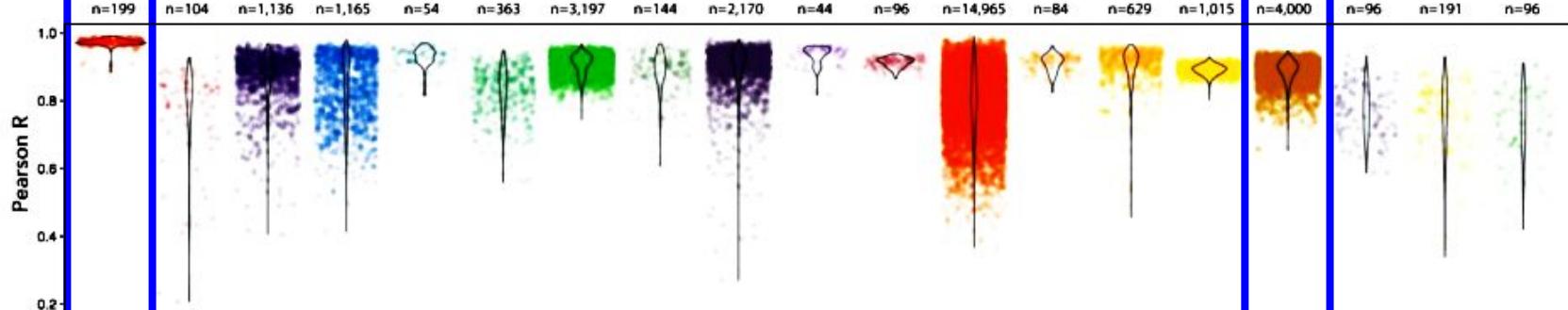
From isolated cells to sequences (Drop-seq / 10X)



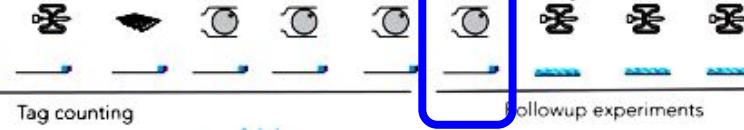
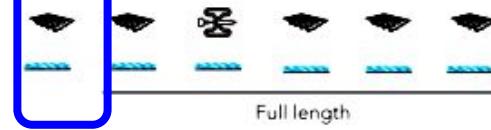
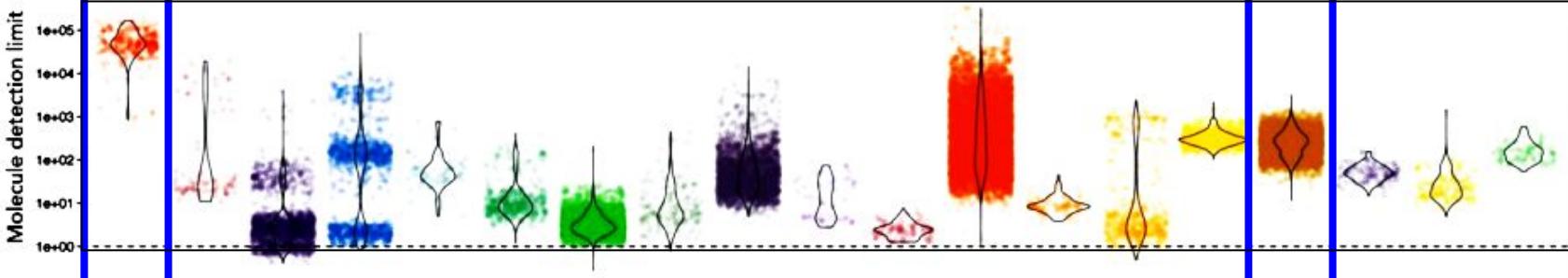
Bulk

10X

Accuracy

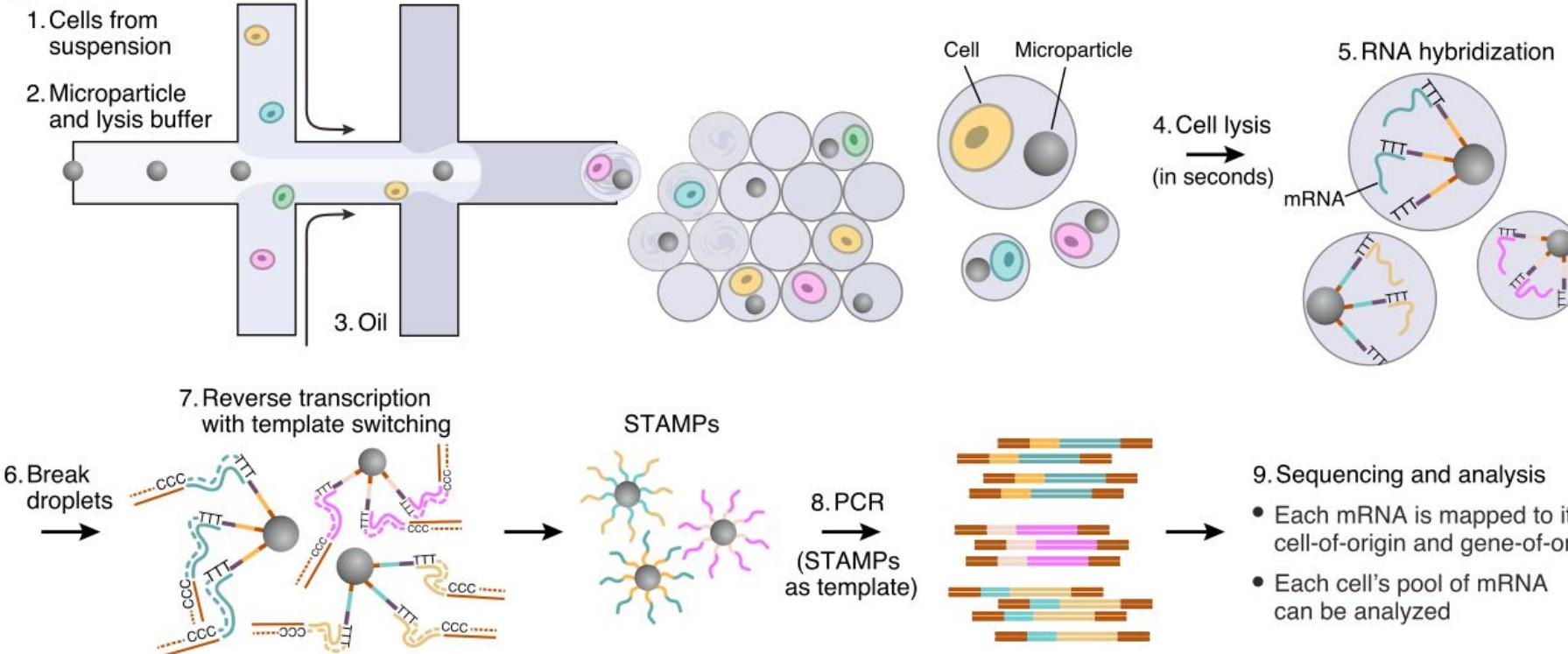


Sensitivity



Drop-seq

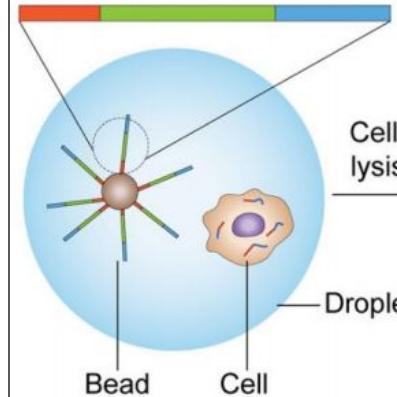
A



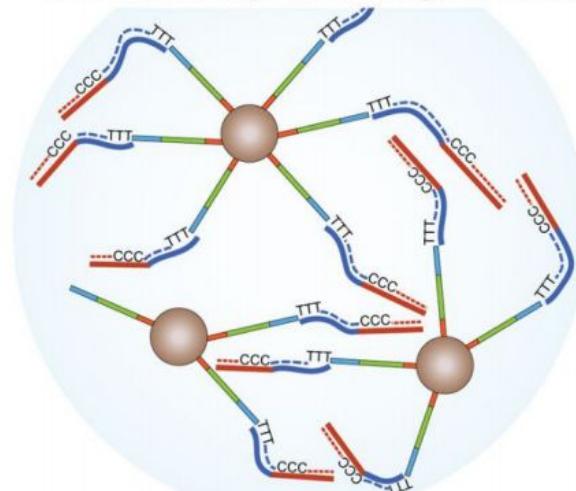
10X Chromium (3')

Structure of the barcode primer bead

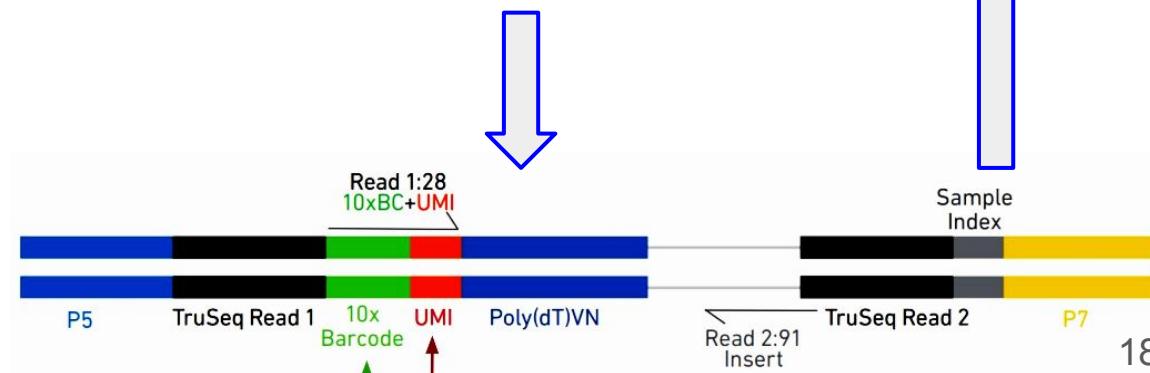
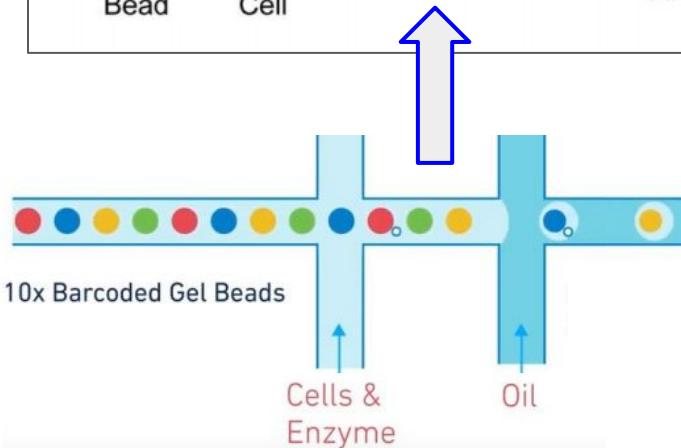
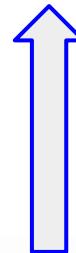
PCR
handle Cell barcode UMI



Reverse transcription with template switching



Sequencing



Sample Index



*From
Nucleotide sequences
To a
Raw count matrix*

Sample	Base	Count
S1	A	100
S1	T	100
S1	G	100
S1	C	100
S2	A	100
S2	T	100
S2	G	100
S2	C	100
S3	A	100
S3	T	100
S3	G	100
S3	C	100
S4	A	100
S4	T	100
S4	G	100
S4	C	100
S5	A	100
S5	T	100
S5	G	100
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S6	A	100
S6	T	100
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S8	A	100
S8	T	100
S8	G	100
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S9	A	100
S9	T	100
S9	G	100
S9	C	100
S10	A	100
S10	T	100
S10	G	100
S10	C	100
S11	A	100
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S11	G	100
S11	C	100
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S12	C	100
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S99	T	100
S99	G	100
S99	C	100
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S100	T	100
S100	G	100
S100	C	100

CERTIFIED



BIOINFORMAGICIAN

Reads QC

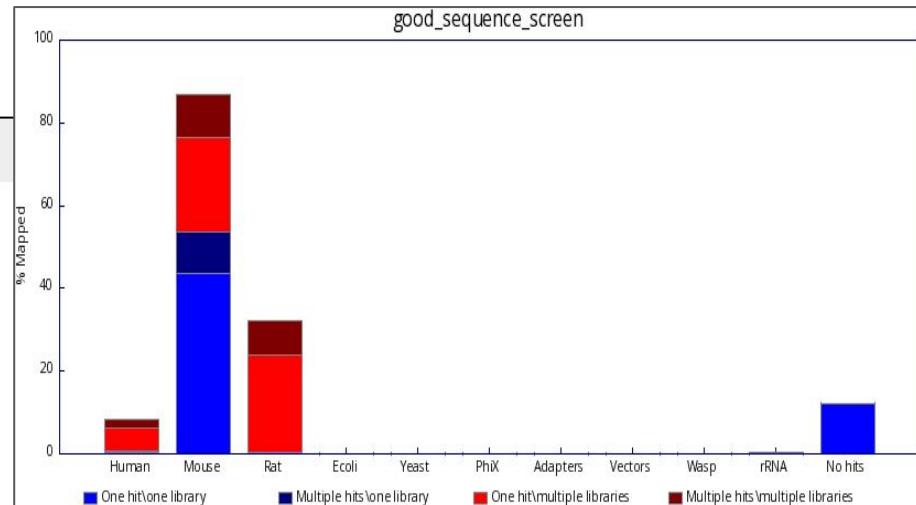
FastQC Report

Summary

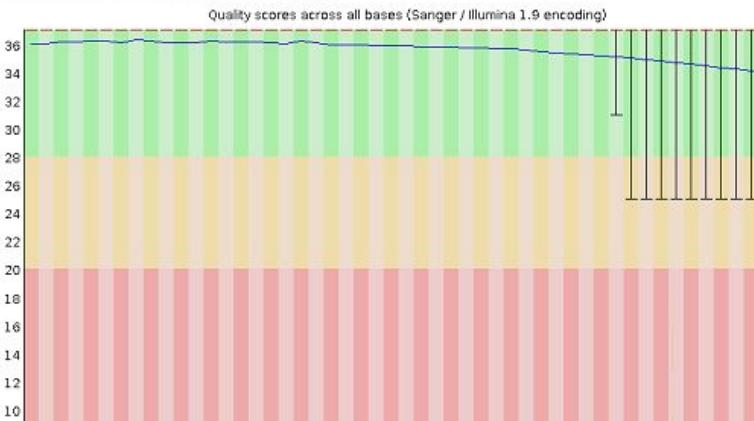
- ✓ Basic Statistics
- ✓ Per base sequence quality
- ✗ Per tile sequence quality
- ✓ Per sequence quality scores
- ✗ Per base sequence content
- ✓ Per sequence GC content
- ✓ Per base N content
- ✓ Sequence Length Distribution
- ✗ Sequence Duplication Levels
- ✗ Overrepresented sequences
- ✓ Adapter Content

Basic Statistics

Measure	Value
Filename	BC_392_1_529_R2_001.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	109443265
Sequences flagged as poor quality	0
Sequence length	91
%GC	43



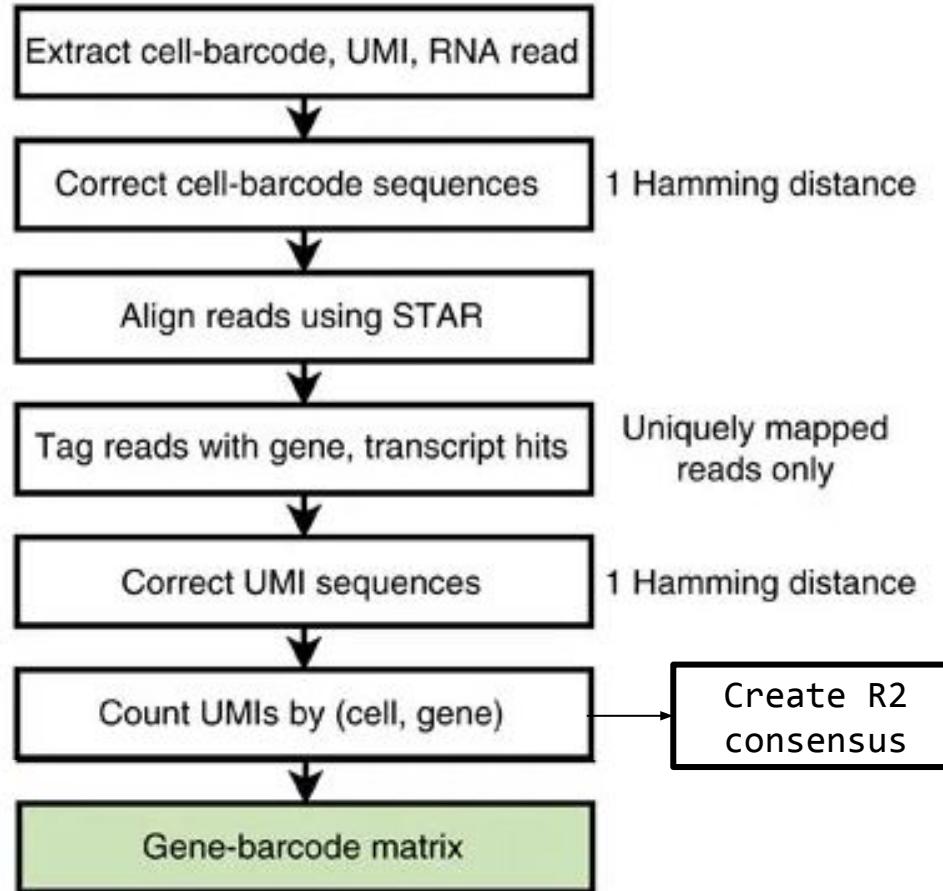
Per base sequence quality



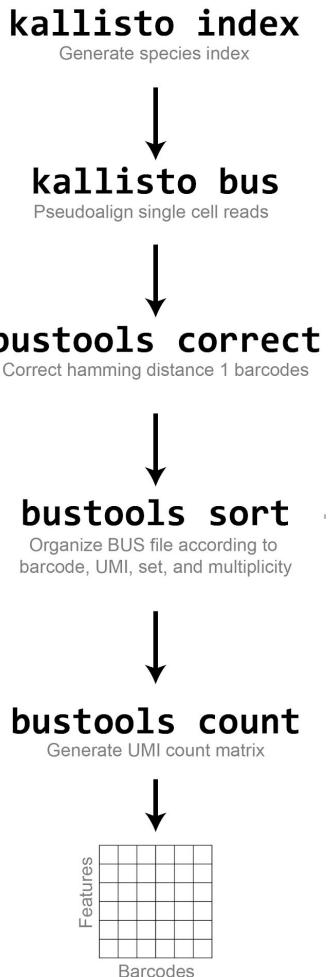
- As usual : FASTQC, FastqScreen, ...
- 10x :
 - Special attention to R1 : cell barcode + UMI (no N)
 - Control of the 4 sample libraries

Reads processing workflows

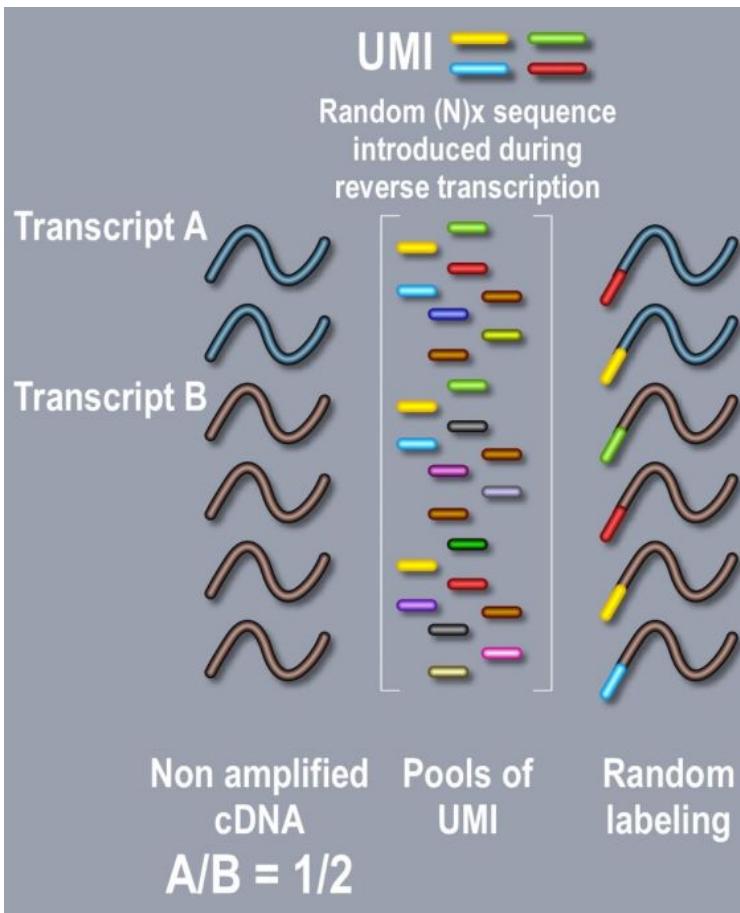
Mapping-based (STAR)



Pseudomapping-based (kallisto bustools)

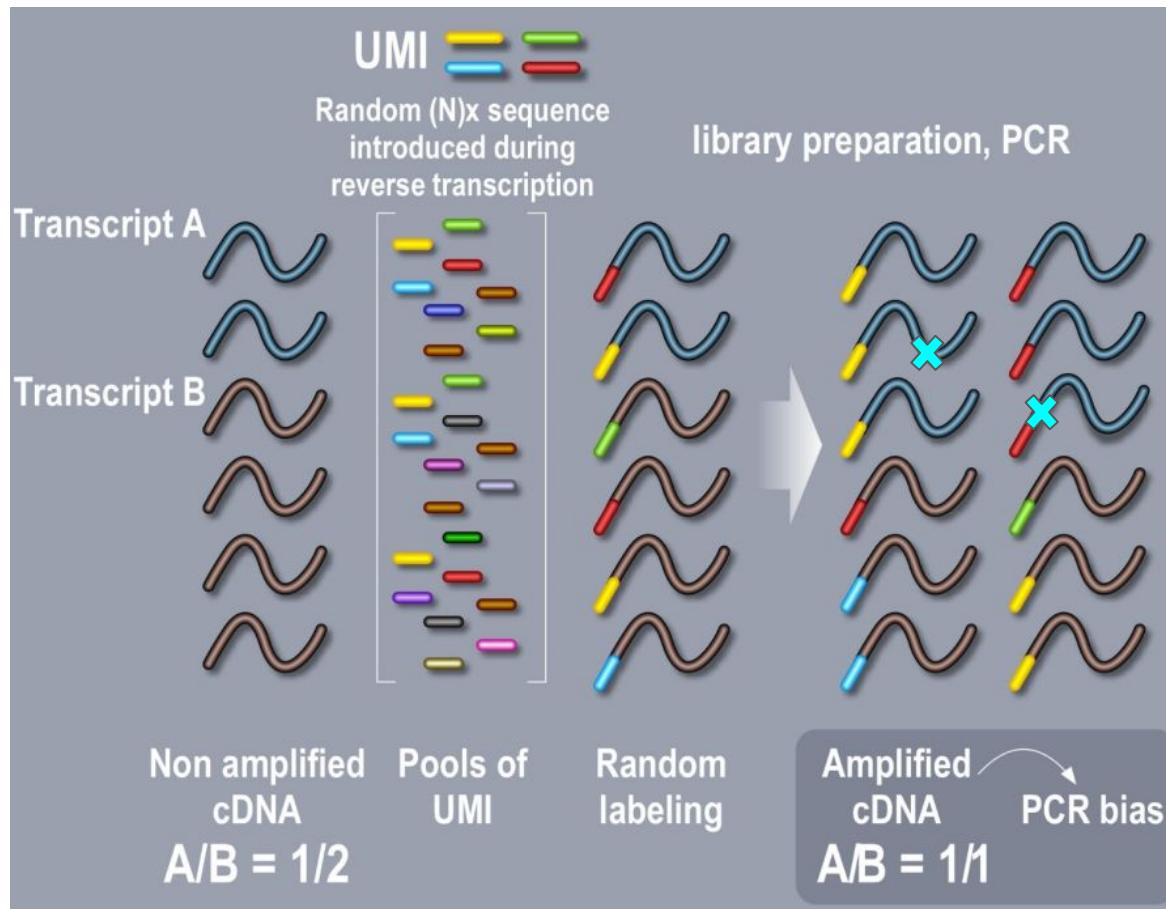


Focus on : Unique Molecule Identifiers



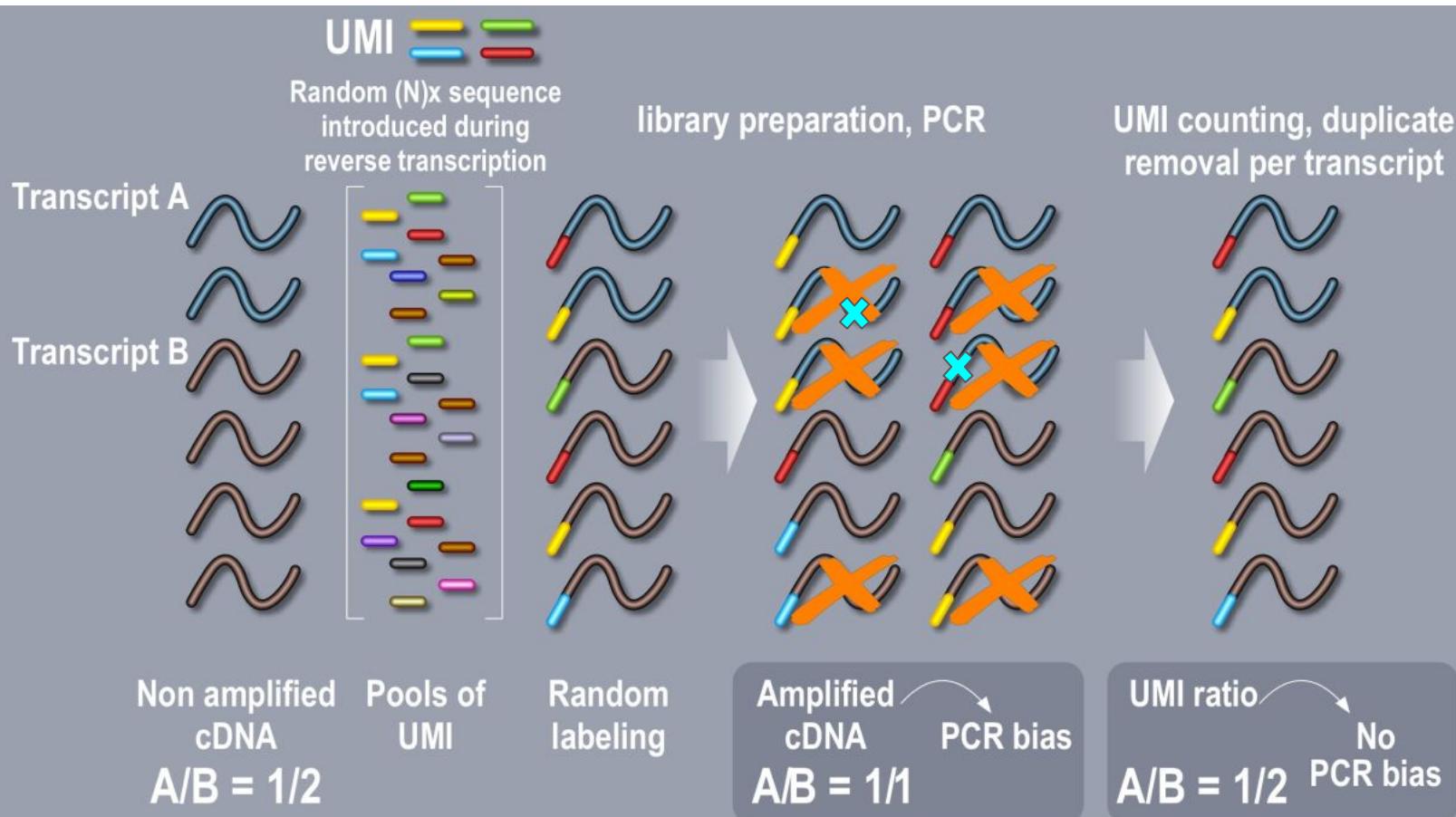
Islam et al., Nature Methods (2014)
Study by Agnès Paquet

Focus on : Unique Molecule Identifiers



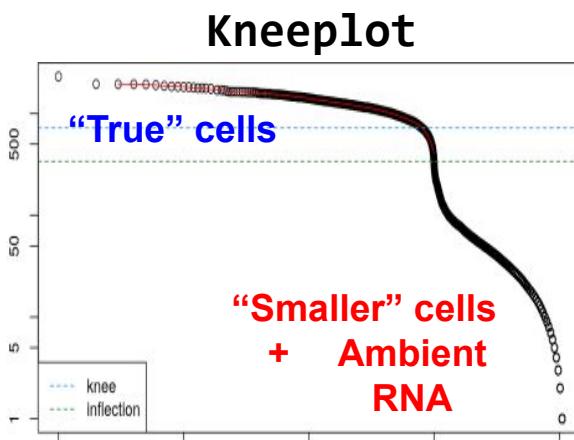
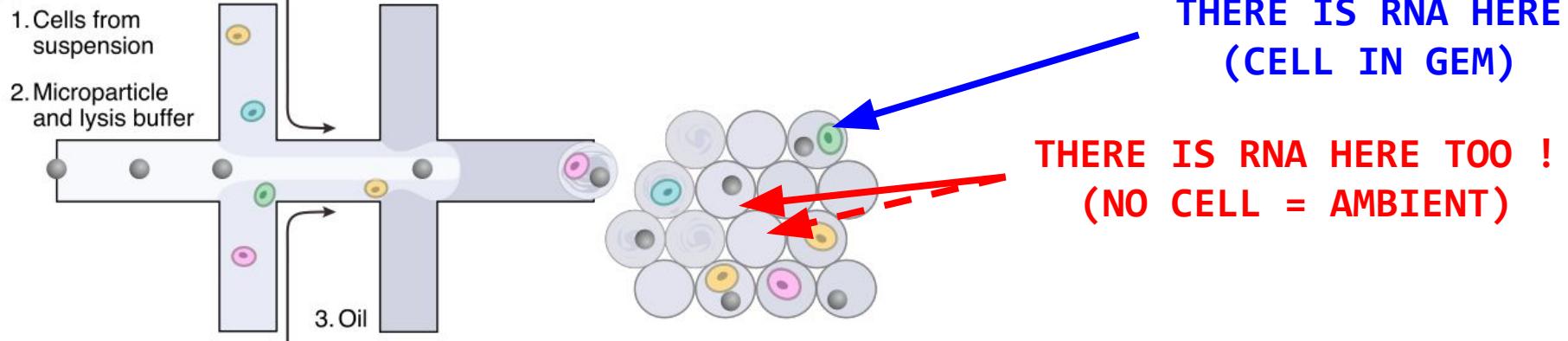
Islam et al., Nature Methods (2014)
Study by Agnès Paquet

Focus on : Unique Molecule Identifiers

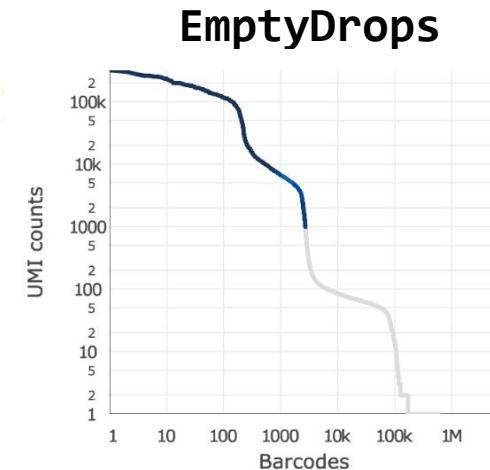
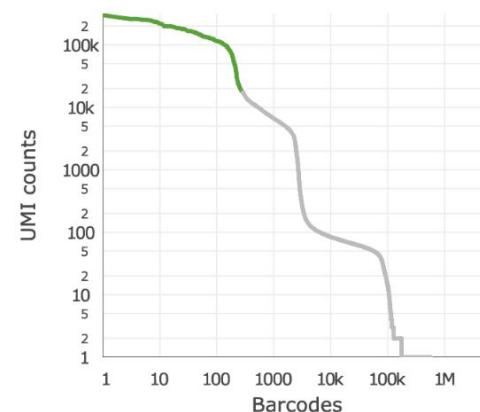


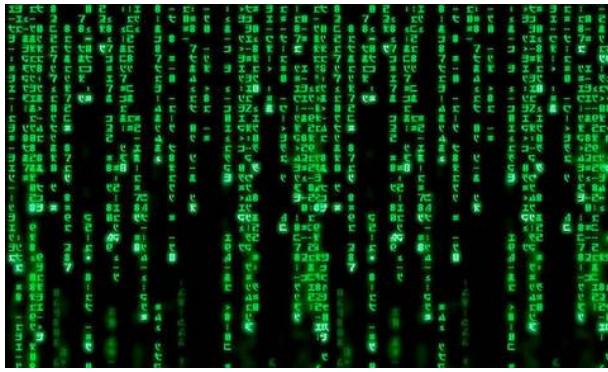
Islam et al., Nature Methods (2014)
Study by Agnès Paquet

Focus on : Empty droplets filtering



<<





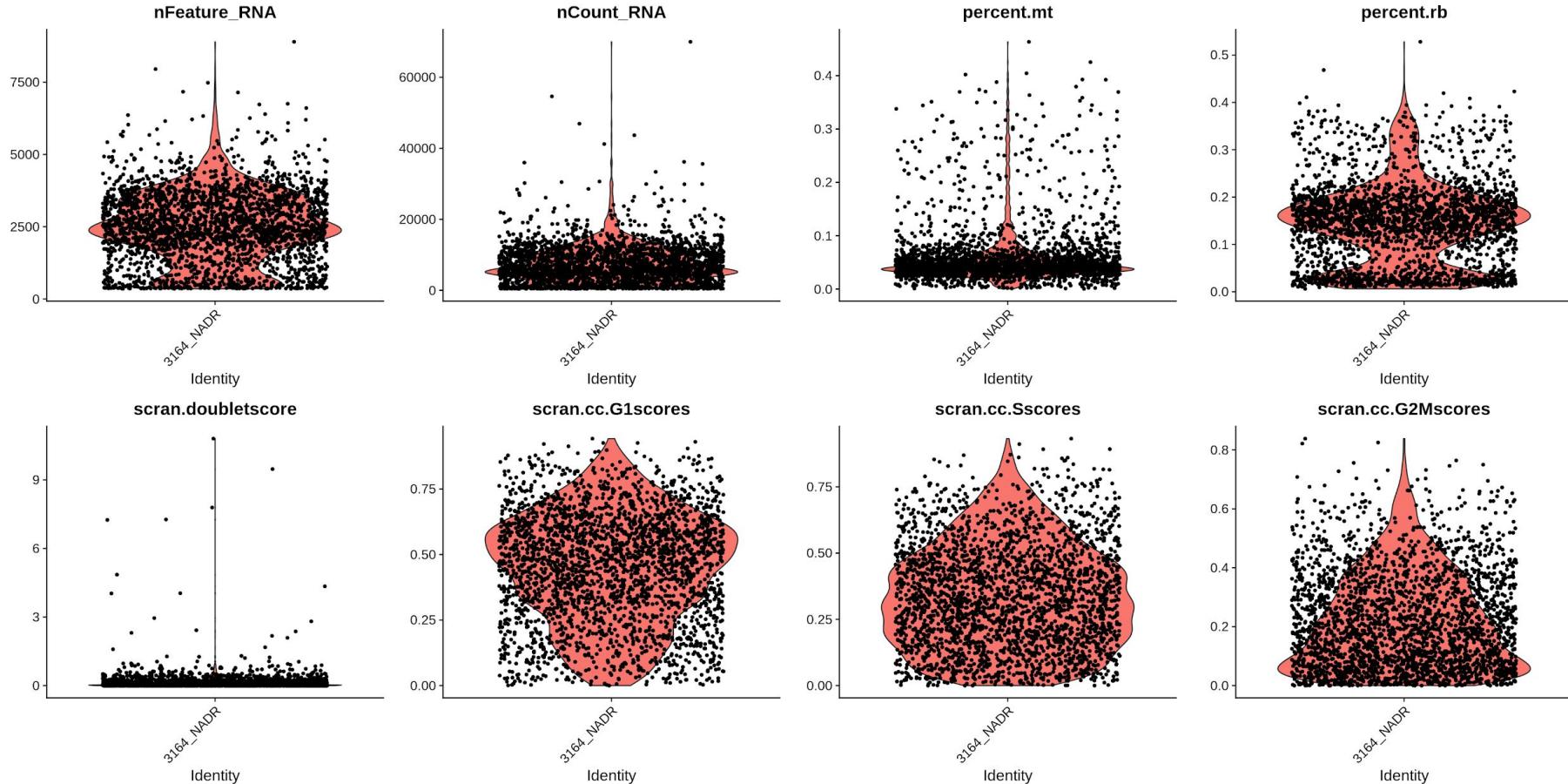
*From a
Raw count matrix
To a
Normalized matrix*



Cell QC considerations

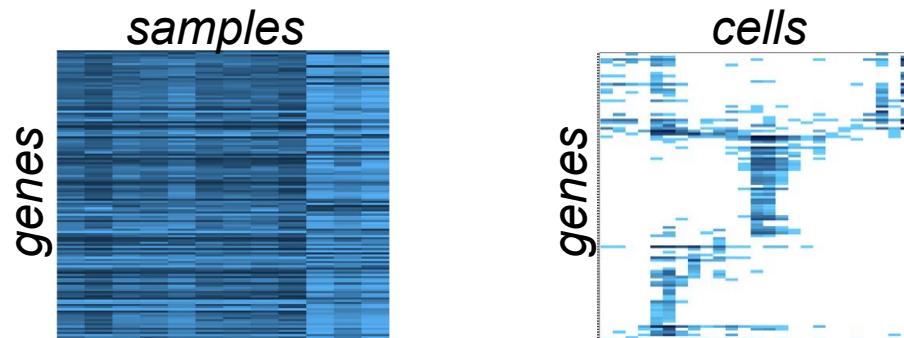
- The number of unique genes detected in each cell :
 - Low-quality cells or empty droplets will often have very few genes
 - Cell doublets or multiplets may exhibit an aberrantly high gene count
- Similarly, the total number of molecules detected within a cell (correlates strongly with unique genes)
- The percentage of reads that map to the mitochondrial genome :
 - Low-quality / dying cells often exhibit extensive mitochondrial contamination
- Other QC criteria to measure :
 - Cell cycle phase / score
 - Nuclear riboprotein-coding genes expression

Cell QC : metrics



Matrix normalization : Houston, we have a problem...

	BULK	SINGLE-CELL
Total RNA	100 ng (~10.000 cells)	10 pg (per cell)
mRNA	~ 5 ng (~10.000 cells)	<< 1 pg (per cell)
Reads	~100 million	~ 50 k (per cell)

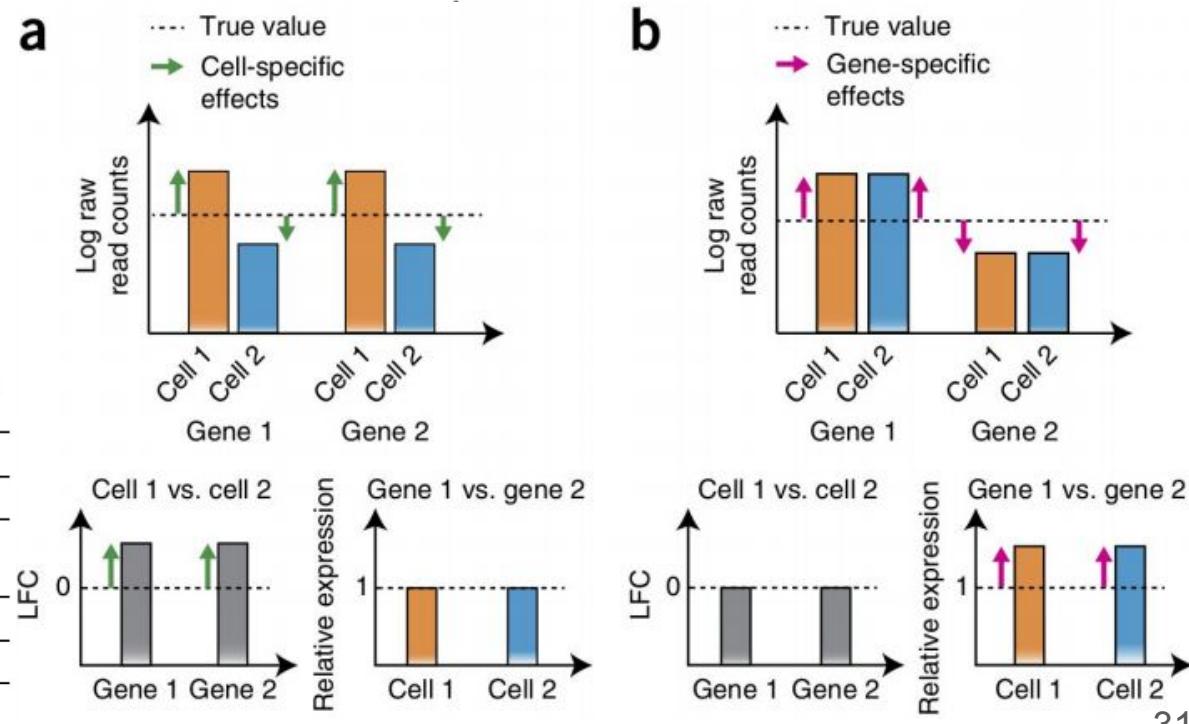


**SC MATRIX IS SPARSE !
(ie, mostly filled with zeros)**

Matrix normalization : different levels

- Process of **identifying** and **removing** systematic variation not due to real differences between RNA treatments i.e. differential gene expression.

- Cell-specific effects
- Gene-specific effects



	Cell-specific effects	Gene-specific effects	Not removed by UMIs
Sequencing depth	✓		✓
Amplification	✓	✓	
Capture and RT efficiency	✓	✓	✓
Gene length		✓	
GC content	✓	✓	✓
mRNA content	✓		✓

Bulk normalization methods are **KO**

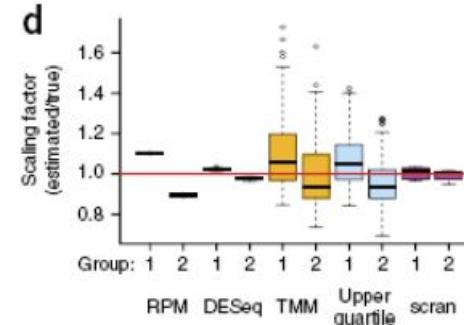
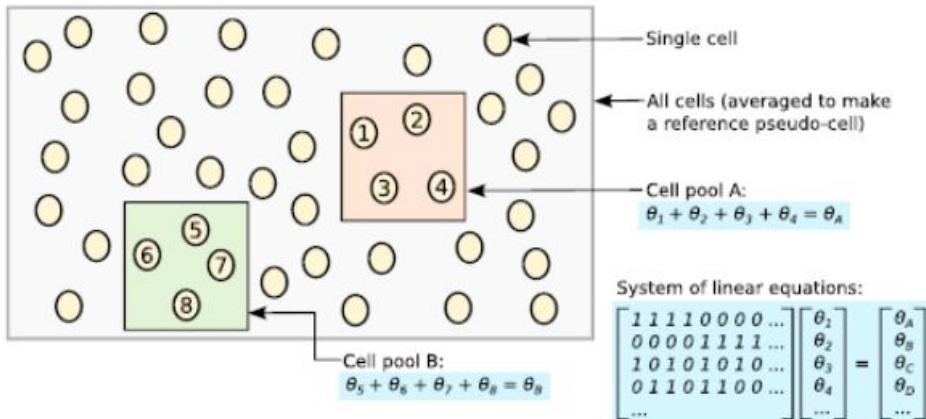
- RPKM/FPKM (Reads/Fragments per kilobase of transcript per million reads of library) : Normalize for sequencing depth and transcript length at the same time => **KO if you DO NOT have full-length data**
- Global scaling (eg: Upper Quartile) : **KO if you have too many zeros**
- Size factors calculation (eg: Estimation of library sampling depth) :
 - DESeq2, edgeR suppose that $\geq 50\%$ of genes are **NOT DE**
 - **KO if you have too many zeros**
- TPM/CPM : **KO** if a small number of genes carry most of the signal

=> Rough solution : global log-normalization / Z-scoring



Matrix normalization : scaling by factors

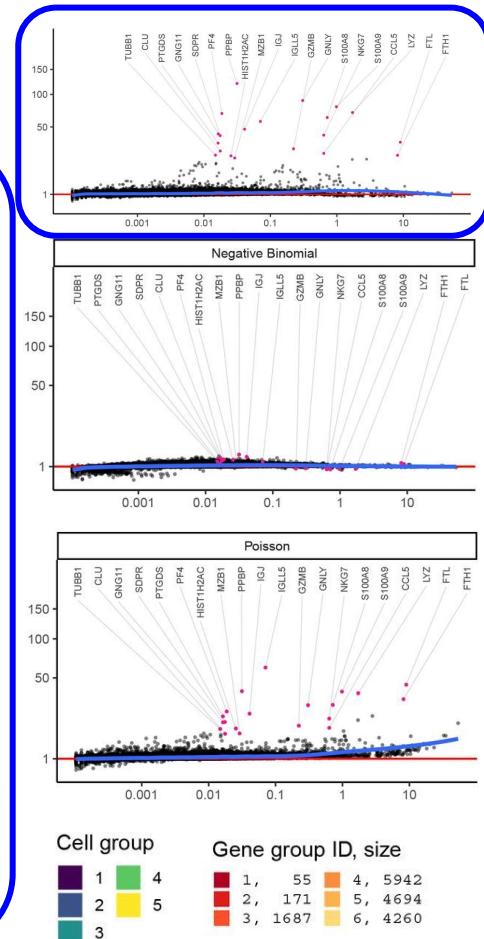
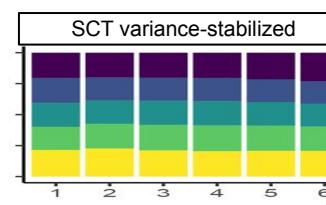
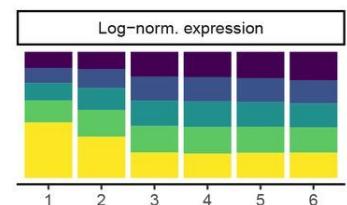
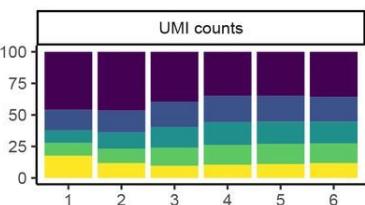
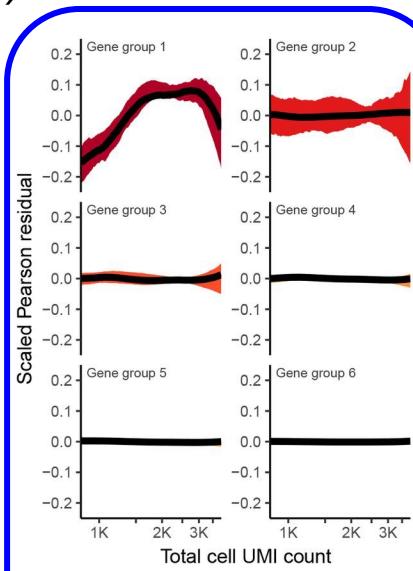
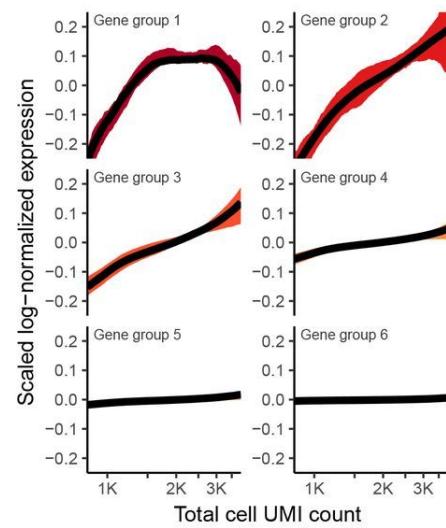
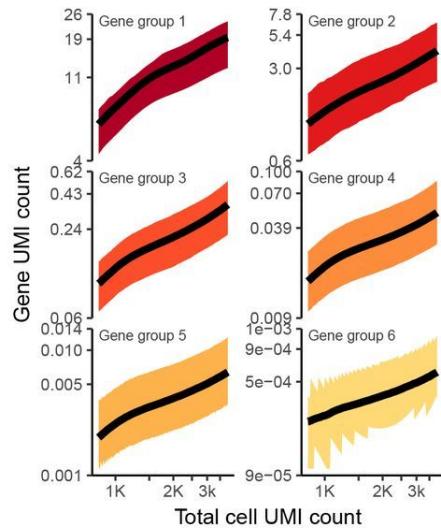
- Alternative method to compute the size factors
- Pool cells to reduce the number of zeros
- Estimate the size factors for the pool
- Repeat many time and use deconvolution to estimate each cell size factor
- Implemented in **scater/scran** packages



Vallejos C, 2017

Matrix normalization : variance stabilization

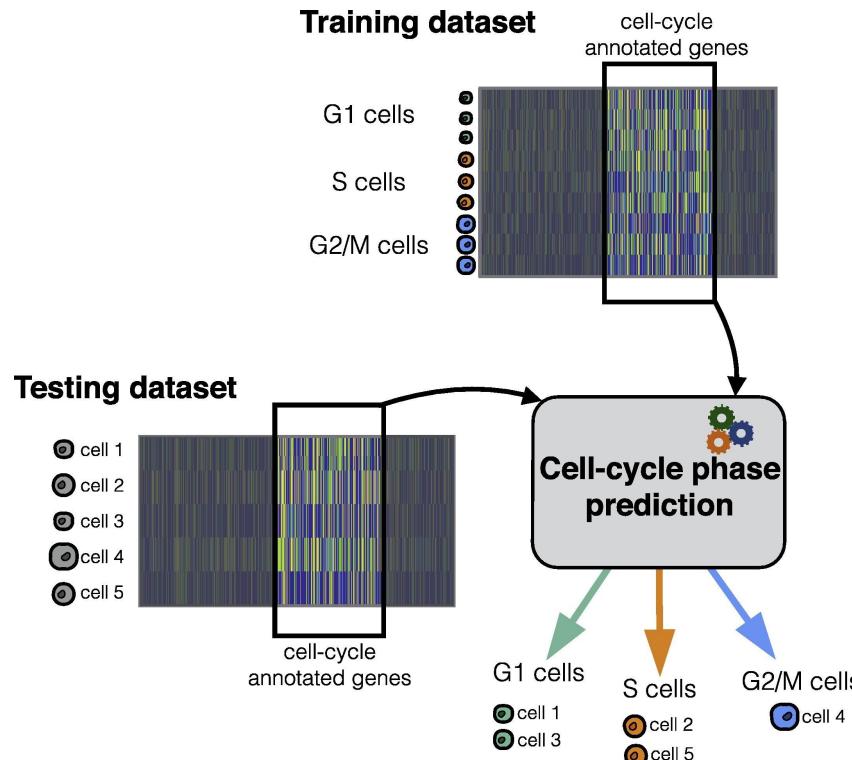
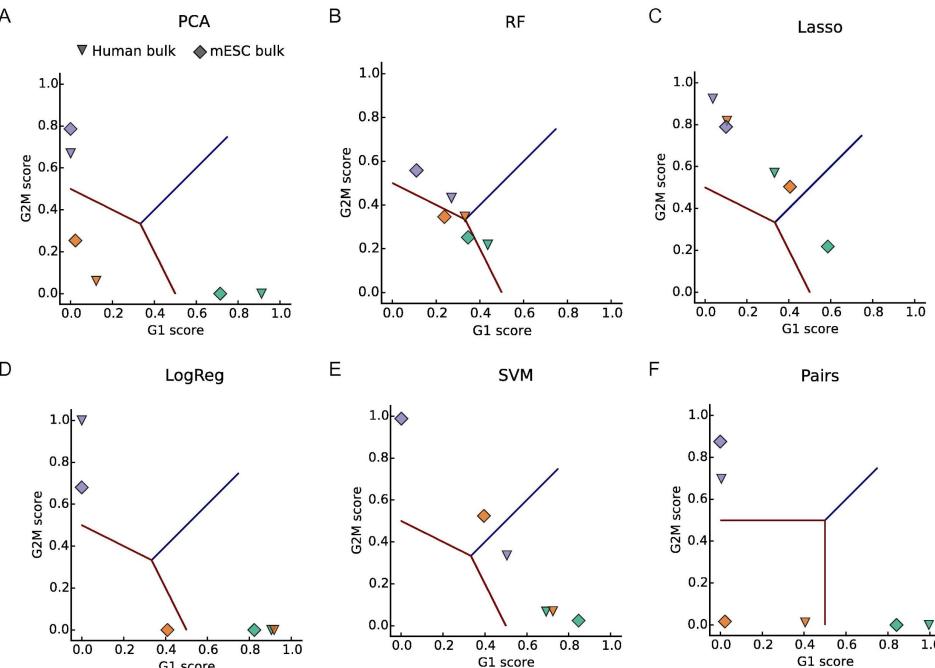
- Regularized negative binomial regression
- Implemented in **sctransform** (*Seurat*)



Hafemeister et al, BioRxiv (2019)

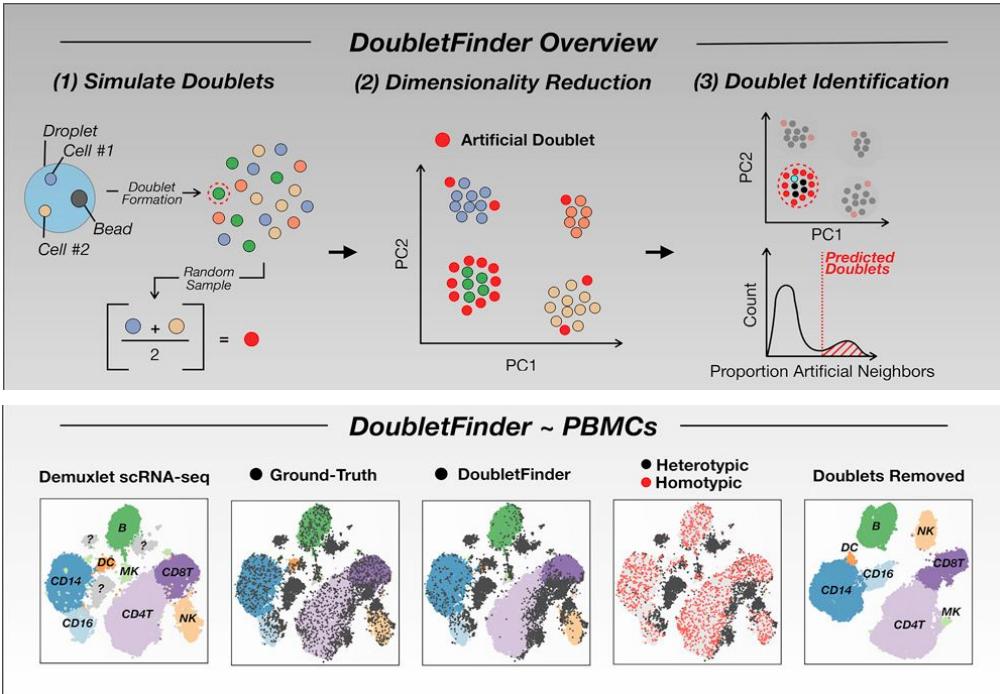
Known biases : Cell cycle phase

- Training on reference set with the 3 phases identified
- Use pairs of differential genes
- Apply model pairs to new dataset and assign phases
- Implemented in **cyclone** (*scran*)



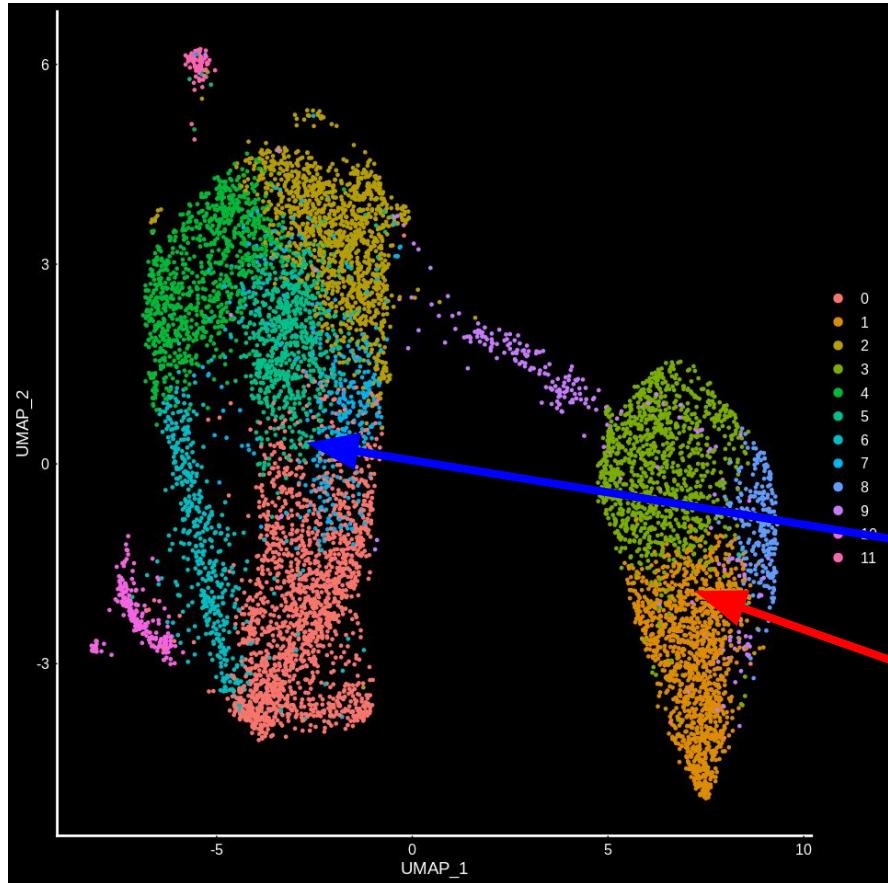
Known biases : Cell doublets

- Two types of doublets :
 - Cells of the same type => higher global expression
 - Cells of the different types => artificial hybrid
- Methods : generate random artificial doublets, capture all



	AUC	pAUC90	pAUC95	pAUC97.5	AUPRC
ch_cell-lines					
• libsize	0.60	0.54	0.53	0.52	0.17
• features	0.60	0.55	0.54	0.53	0.19
• dblCells	0.64	0.62	0.61	0.60	0.37
• cxds	0.65	0.59	0.57	0.55	0.26
• dblDetection	0.66	0.66	0.65	0.65	0.44
• scrublet	0.69	0.65	0.64	0.63	0.41
• dblFinder	0.69	0.66	0.65	0.65	0.45
• hybrid	0.70	0.64	0.63	0.61	0.40
• bcds	0.70	0.66	0.64	0.62	0.43
ch_pbmc					
• dblCells	0.63	0.57	0.56	0.54	0.31
• libsize	0.78	0.63	0.57	0.54	0.44
• scrublet	0.78	0.67	0.63	0.59	0.52
• cxds	0.78	0.69	0.65	0.61	0.54
• features	0.79	0.62	0.57	0.54	0.45
• bcds	0.81	0.71	0.66	0.60	0.58
• hybrid	0.82	0.73	0.67	0.62	0.61
• dblDetection	0.82	0.75	0.69	0.62	0.63
• dblFinder	0.84	0.74	0.68	0.62	0.64
demuxlet					
• dblCells	0.79	0.70	0.65	0.60	0.46
• libsize	0.81	0.58	0.55	0.53	0.30
• features	0.85	0.62	0.57	0.55	0.37
• scrublet	0.87	0.74	0.68	0.62	0.53
• cxds	0.89	0.71	0.63	0.57	0.49
• hybrid	0.91	0.78	0.68	0.58	0.57
• dblDetection	0.91	0.79	0.69	0.58	0.57
• bcds	0.91	0.79	0.71	0.62	0.61
• dblFinder	0.92	0.79	0.70	0.63	0.62
hg-mm					
• libsize	0.87	0.66	0.59	0.54	0.27
• features	0.89	0.68	0.60	0.55	0.30
• dblCells	0.93	0.88	0.84	0.79	0.73
• bcds	0.96	0.87	0.80	0.71	0.64
• hybrid	0.98	0.94	0.90	0.87	0.88
• scrublet	0.99	0.96	0.94	0.91	0.91
• cxds	0.99	0.98	0.98	0.97	0.97
• dblDetection	0.99	0.99	0.98	0.98	0.97
• dblFinder	1.00	0.99	0.99	0.99	0.99

Known biases : an IRL example

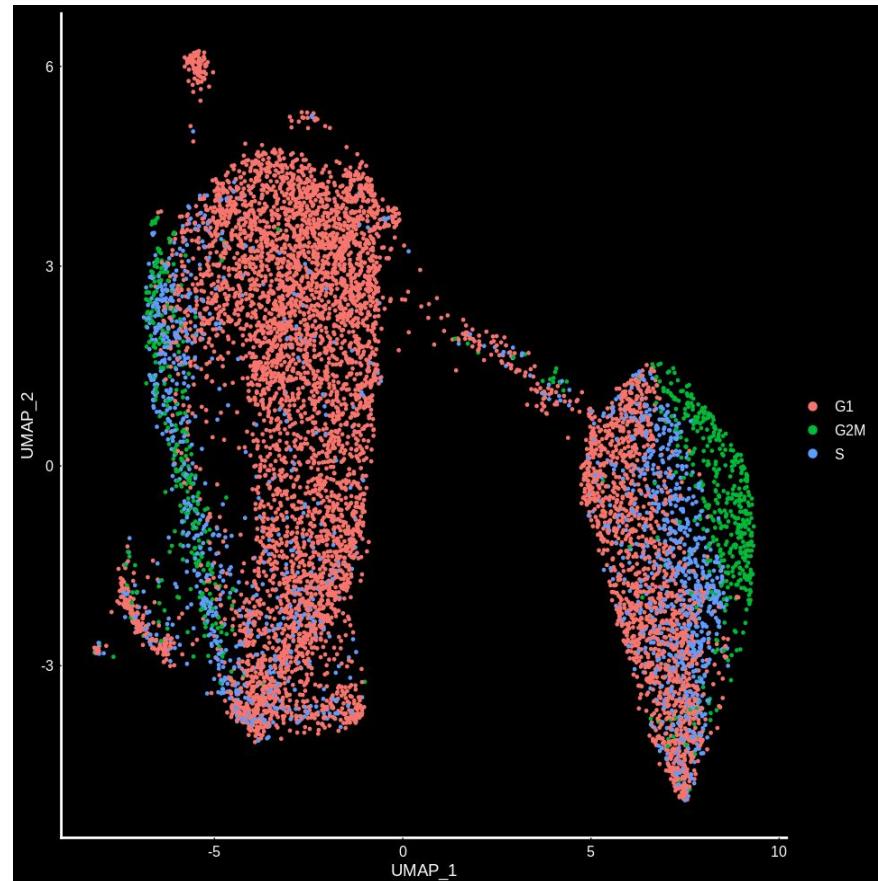
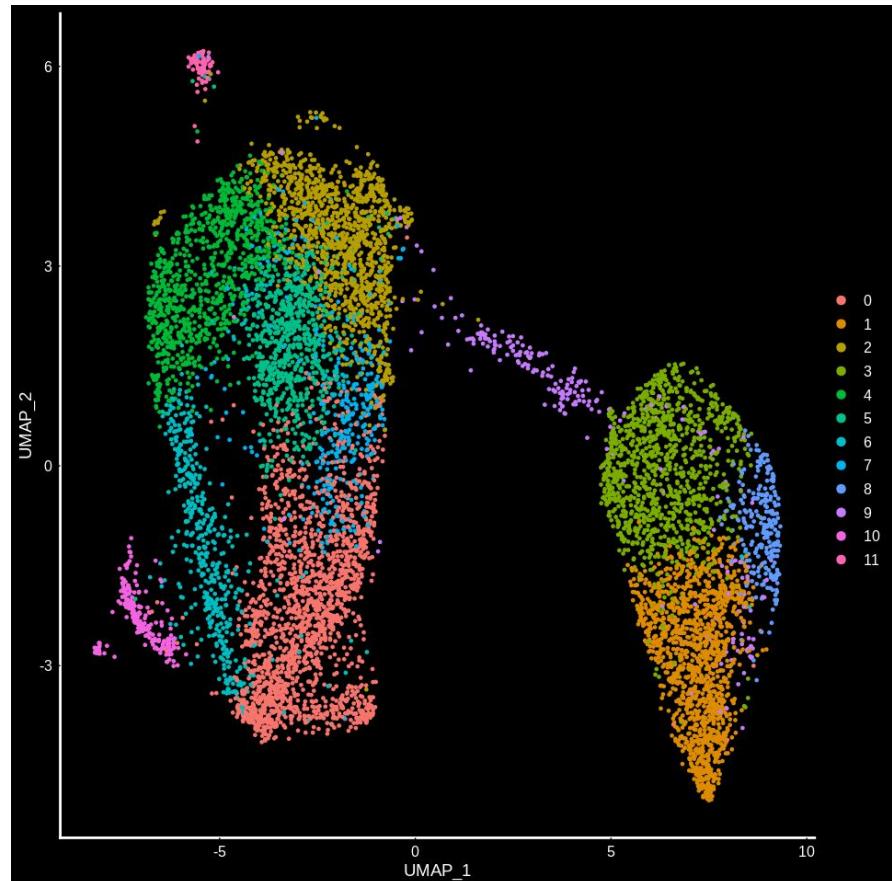


- 10X 3' scRNAseq v2
- Osteosarcoma metastasis
- 8911 cells x 18613 genes
- PCA (109 PCs retained)
- Louvain clustering
 - 12 clusters
- uMAP representation

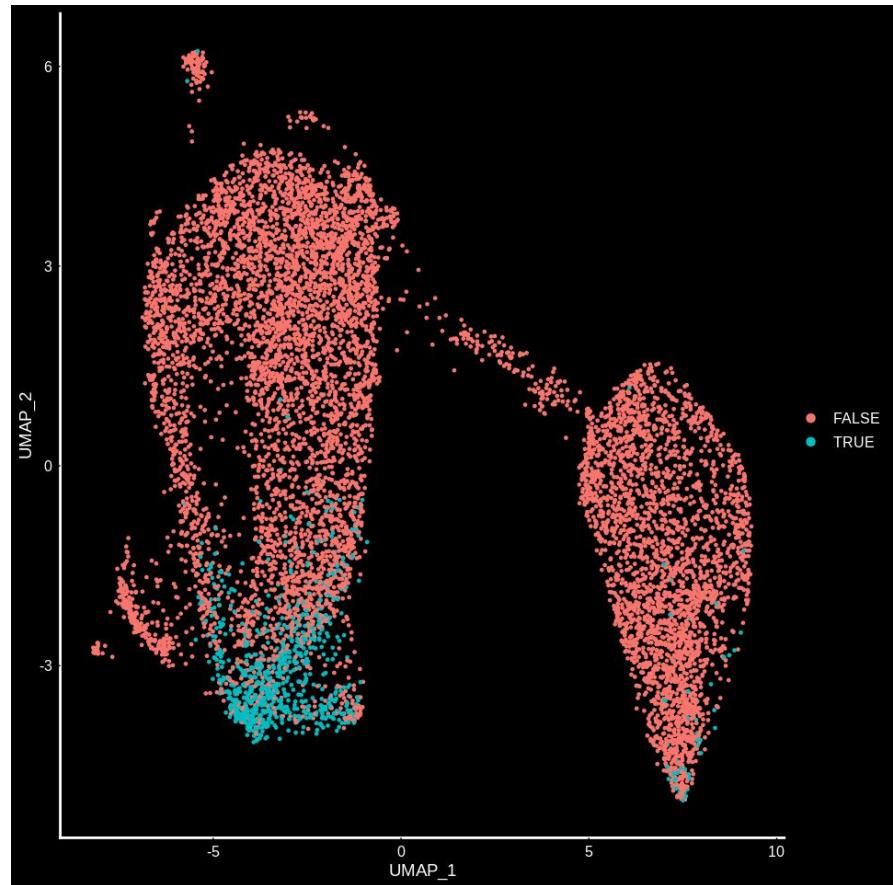
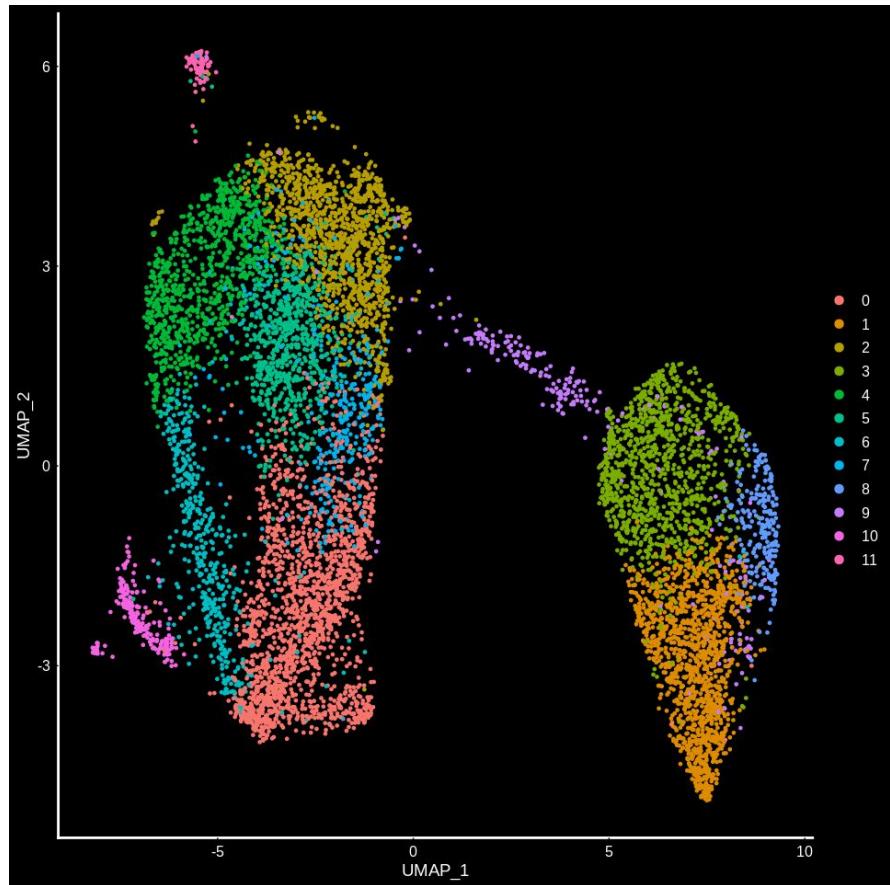
Osteoblasts

Osteoclasts

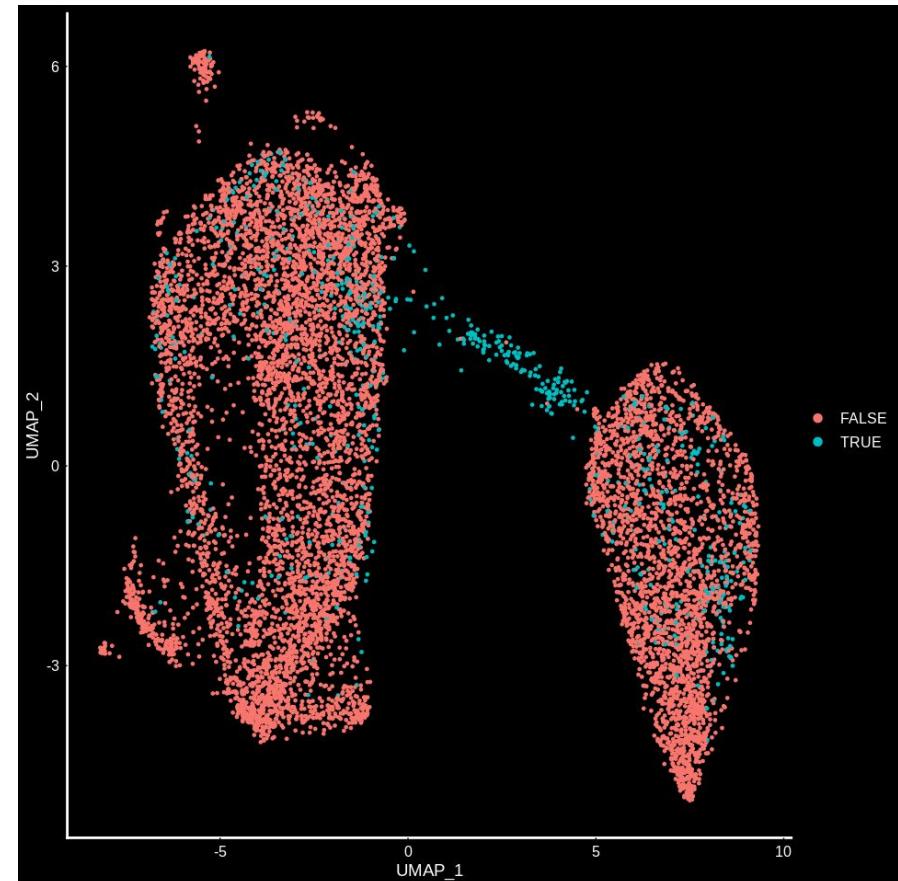
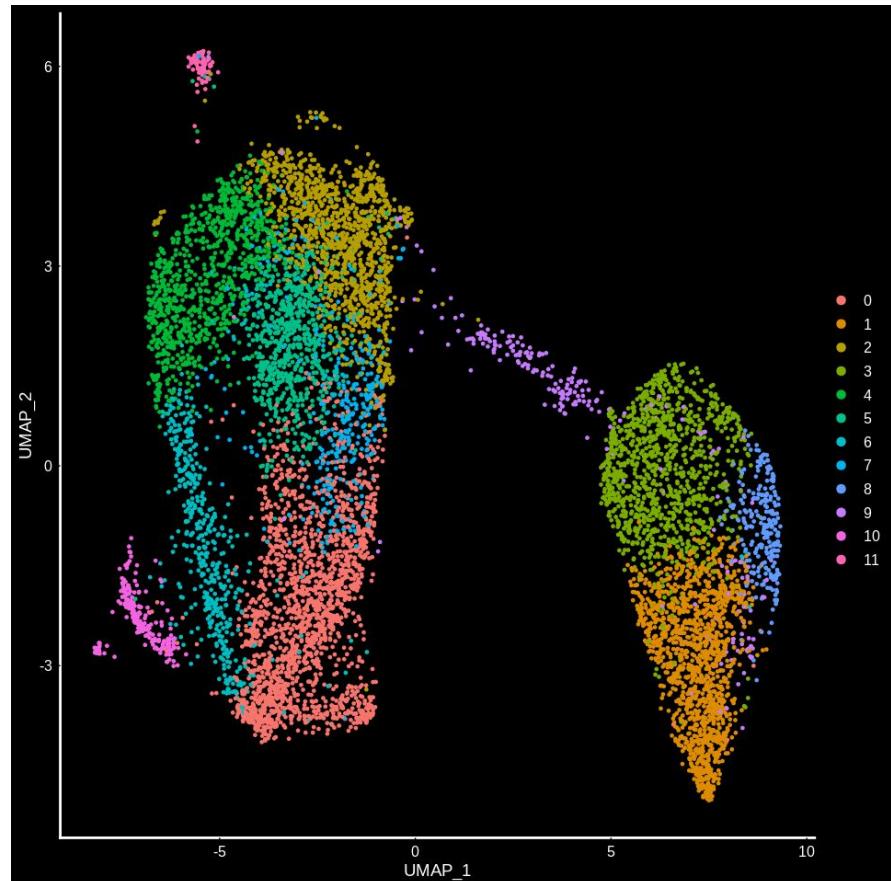
Bias : Cell cycle phases / scores



Bias : Dying cells status / score

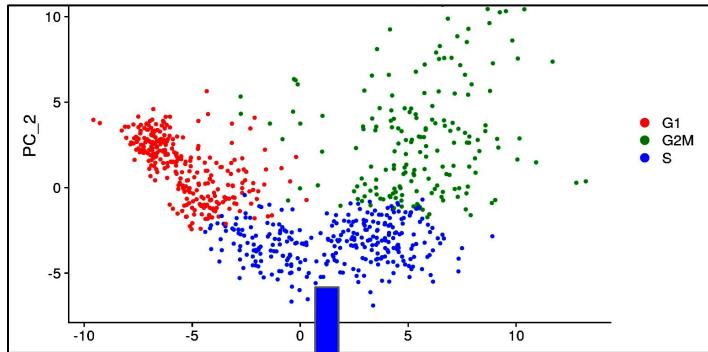


Bias : Cell doublet status / score

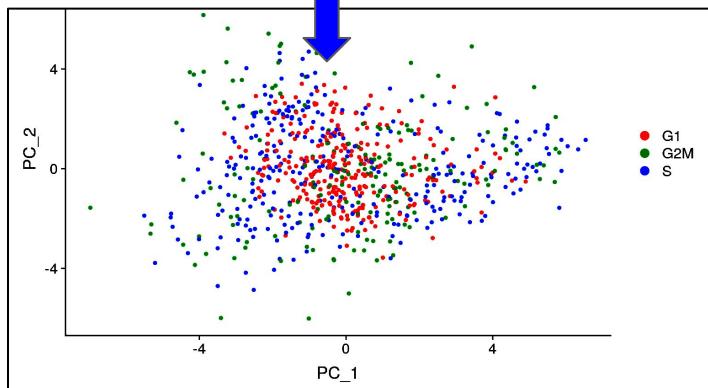


Bias normalization : regression / deblocking

Ex : Cell cycle score

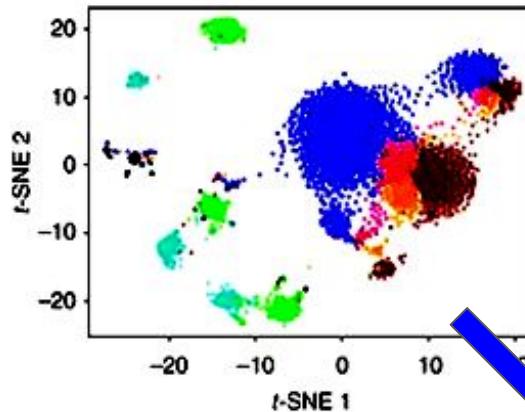


Regression

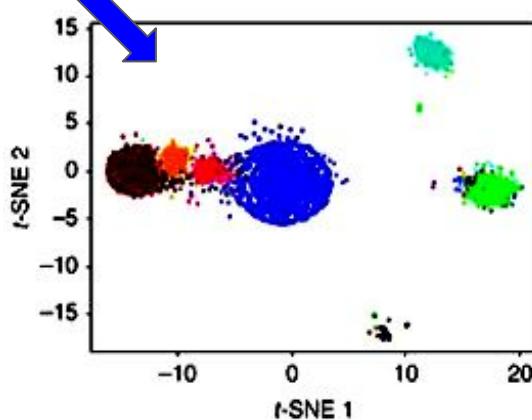


Seurat tutorial

Ex : Batch effect

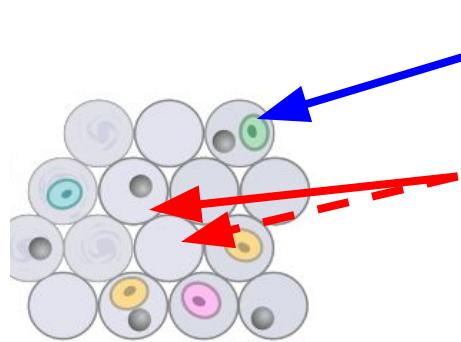


Deblocking



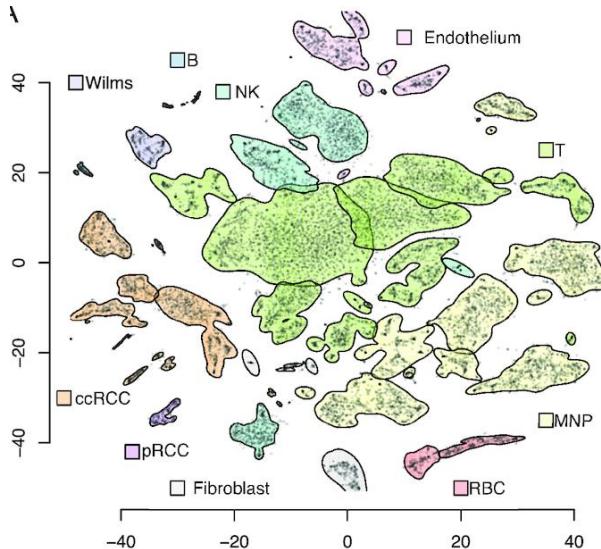
Ambient RNA filtering (soupX)

- emptyDrops : removed empty droplets (contained only ambient RNA)
- **BUT** non-empty droplets **ALSO** have ambient RNA !
- **soupX** determines the amount of ambient RNA in counts, removes it

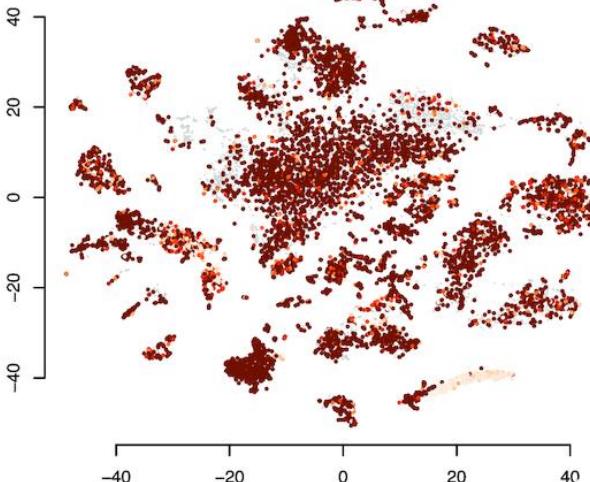


THERE IS RNA HERE
(CELL IN GEM RNA
+ AMBIENT)

THERE IS RNA HERE TOO !
(NO CELL = 100% AMBIENT)

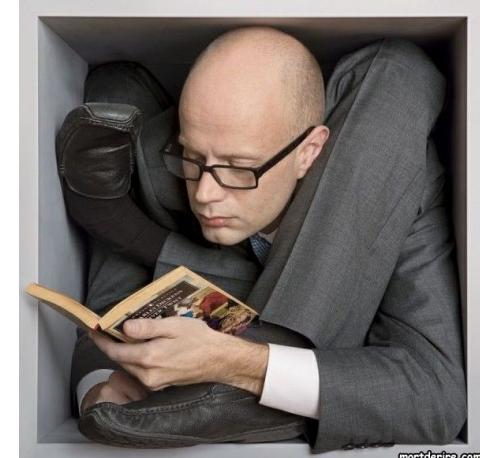


tSNE1
HBB expression removal





*From a
Normalized matrix
To a
Reduced space*



Feature selection : Highly variable genes (HVGs)

Postulate : genes with the highest variability should be the most useful to

1. Assess effect of unwanted sources of variation (cell to cell variation)
2. Quantify true biological differences (population to population variation)

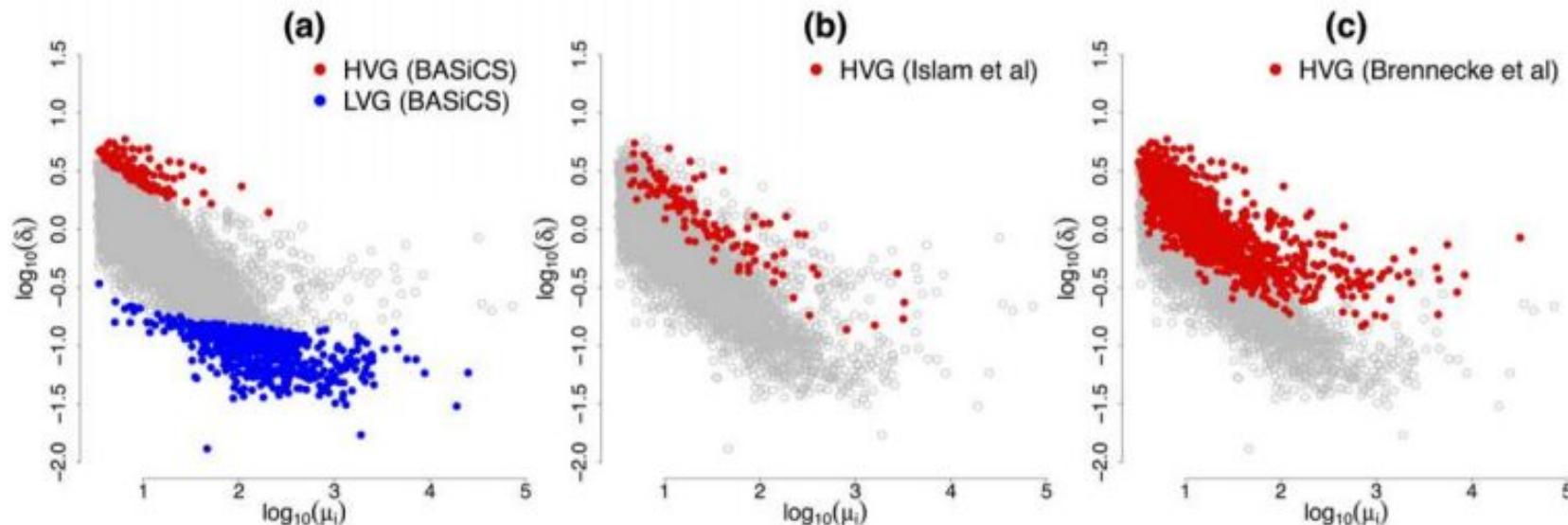


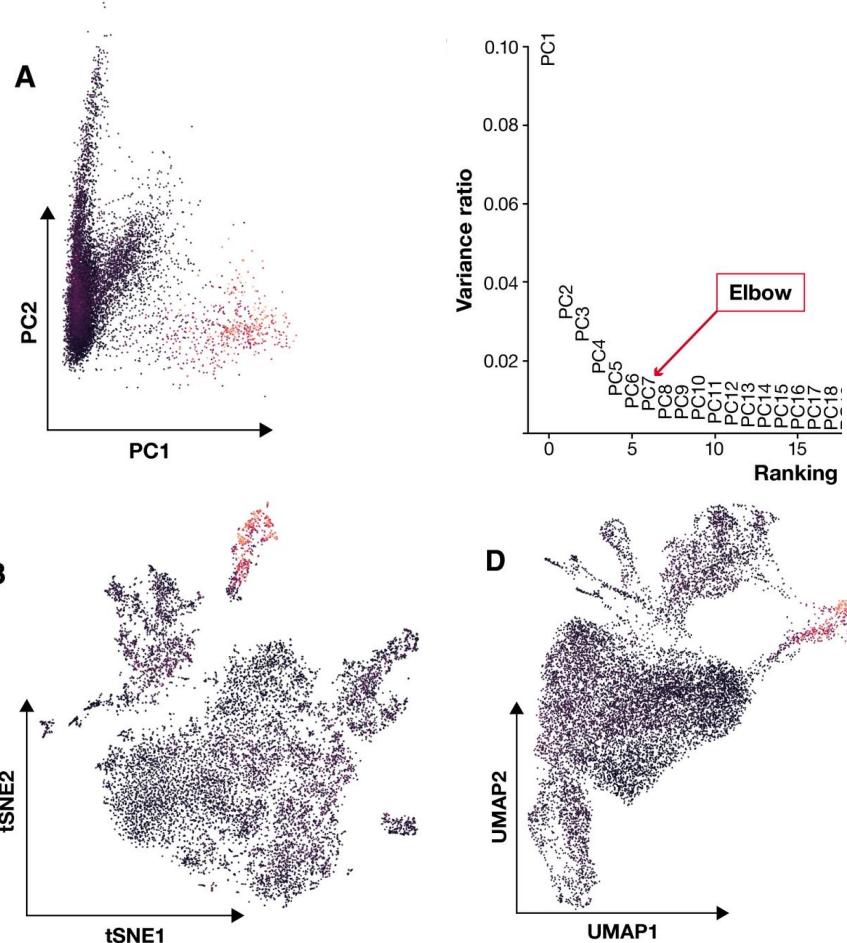
Fig 8. Comparison of HVG detection among different methods. For each of the 7,895 biological genes, posterior medians of biological cell-to-cell heterogeneity term δ_i (log scale) against posterior medians of expression level μ_i (log scale). While the methods described in [16] and [5] only provide a characterisation of HVG, BASiCS is able to detect those genes whose expression rates are stable among cells.

Dimensionality reduction : simplification + selection

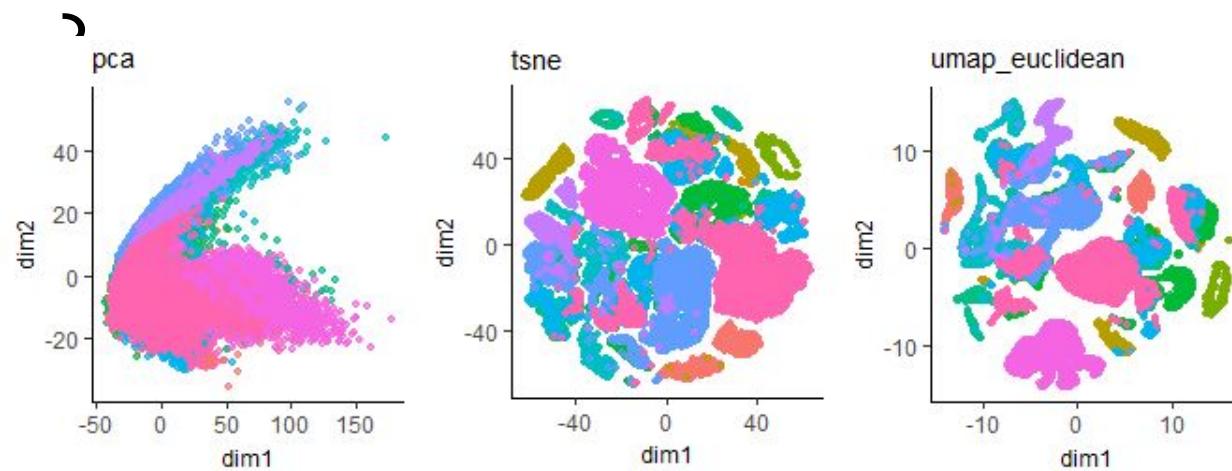
1. Need of an orthogonal space
2. Minimize curse of dimensionality
3. Filter out noise
4. Allow visualization
5. Reduce computational load

Popular methods used for single-cell data analysis:

1. PCA
2. ICA
3. tSNE
4. UMAP
5. Others : Diffusion map, Isomap



Dimensionality reduction : PCA / tSNE / uMAP

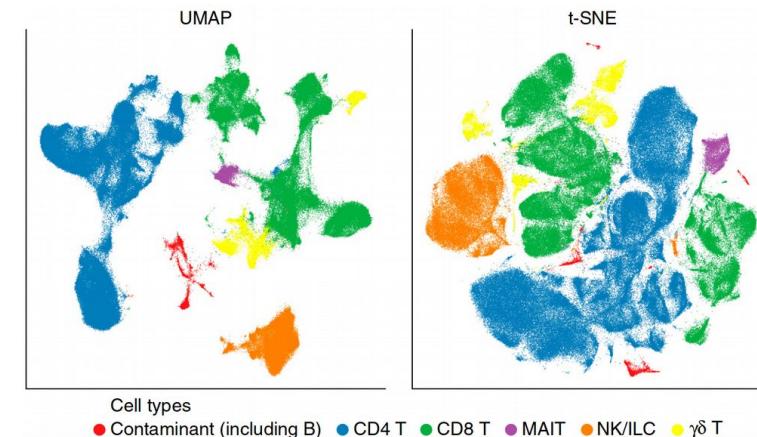


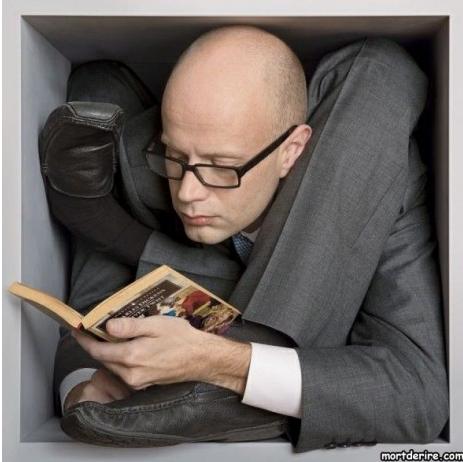
Reduction :

- PCA (on single cell data) is unable to concentrate relationships in 2-3 dimensions only

Visualization : uMAP > tSNE

- Better compaction
- Mostly retains inter-cluster distances
 - Subpopulations
 - Trajectory
- More robust to parameters modification
- (Slightly faster to generate)

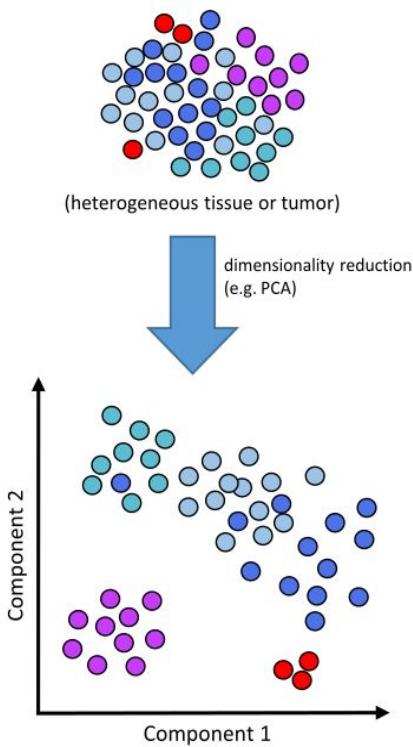




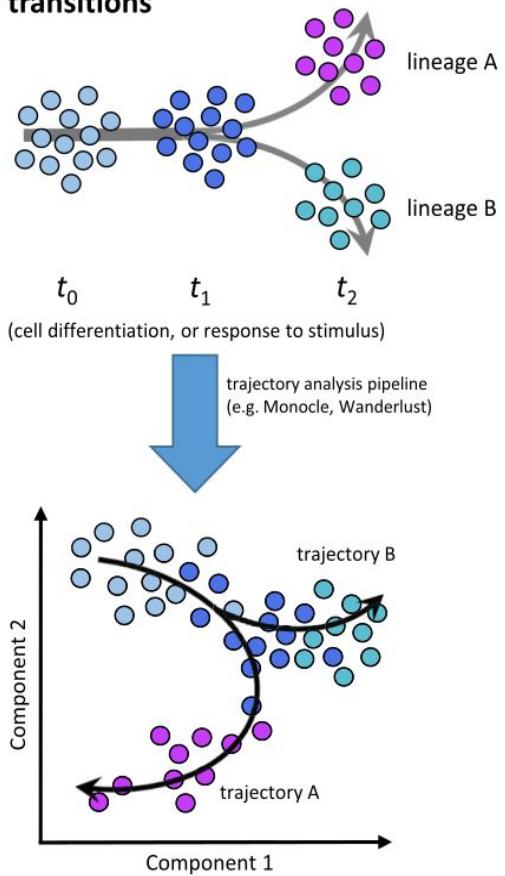
*From a
Reduced space
To ...
... finally what you wanted !*



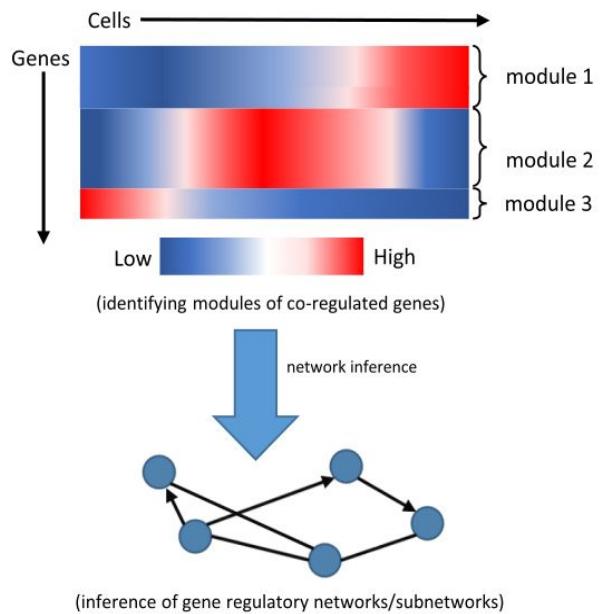
a) Deconvolving heterogeneous cell populations



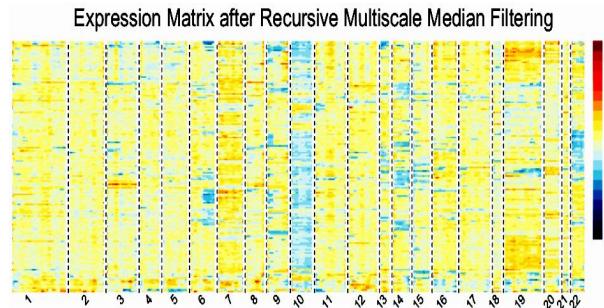
b) Trajectory analysis of cell state transitions



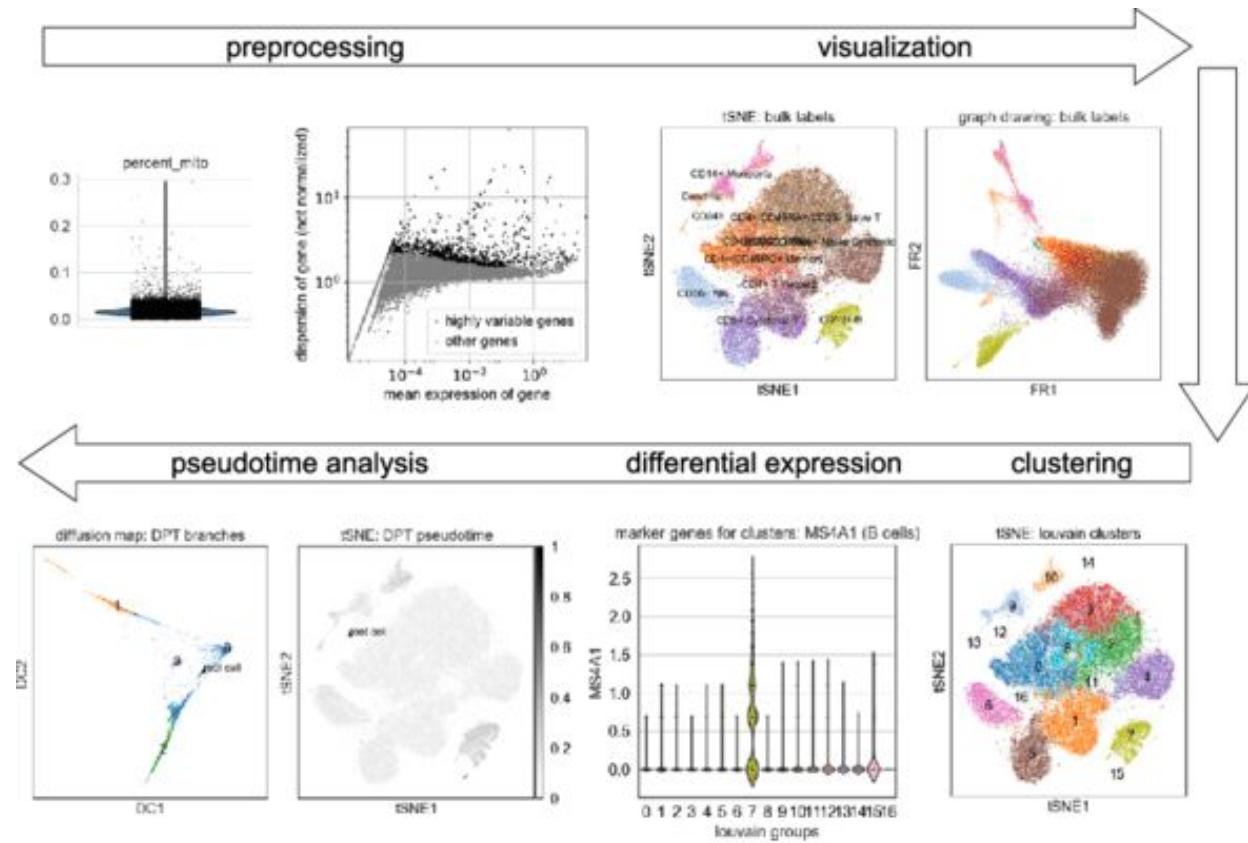
d) Network inference



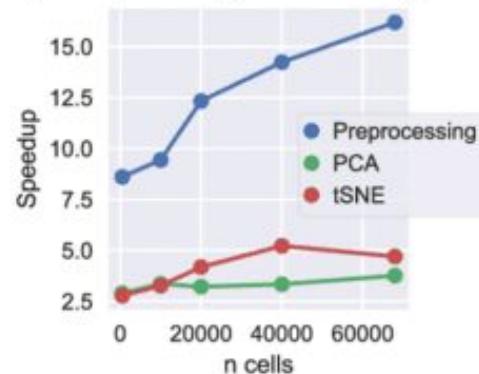
e) Copy number estimation



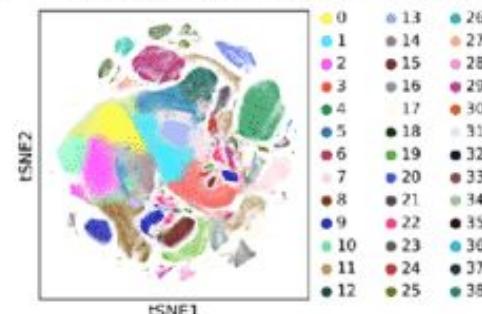
The all-in-one Python toolbox : Scanpy



b Speedup: Scanpy vs. Cell Ranger R



c tSNE of clustered 1.3 million cells



The all-in-one R toolbox : Seurat

Seurat 4.0.4

Install

Get started

Vignettes ▾

Extensions

FAQ

News

Reference

Archive



Official release of Seurat 4.0

We are excited to release Seurat v4.0! This update brings the following new features and functionality:

- **Integrative multimodal analysis.** The ability to make simultaneous measurements of multiple data types from the same cell, known as multimodal analysis, represents a new and exciting frontier for single-cell genomics. In Seurat v4, we introduce weighted nearest neighbor (WNN) analysis, an unsupervised strategy to learn the information content of each modality in each cell, and to define cellular state based on a weighted combination of both modalities. In our new paper, we generate a CITE-seq dataset featuring paired measurements of the transcriptome and 228 surface proteins, and leverage WNN to define a multimodal reference of human PBMC. You can use WNN to analyze multimodal data from a variety of technologies, including CITE-seq, ASAP-seq, 10X Genomics ATAC +

Links

Download from CRAN at
[https://cloud.r-project.org/
package=Seurat](https://cloud.r-project.org/package=Seurat)

Browse source code at
[https://github.com/satijalab/
seurat/](https://github.com/satijalab/seurat/)

Report a bug at
[https://github.com/satijalab/
seurat/issues](https://github.com/satijalab/seurat/issues)

License

[GPL-3](#) | file [LICENSE](#)

Community

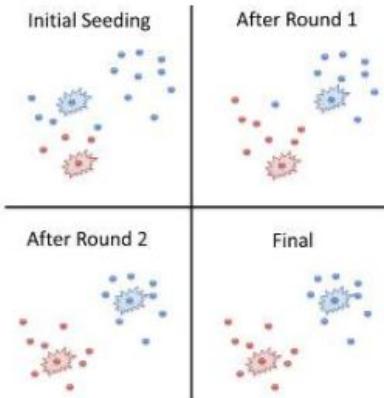
[Code of conduct](#)

Citation

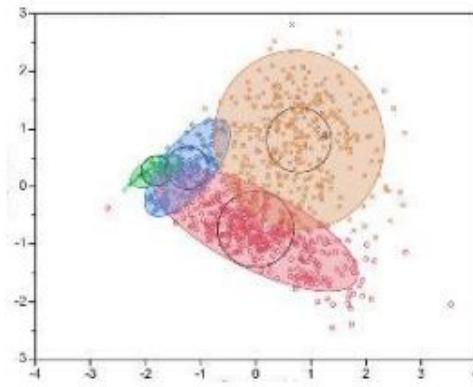
[Citing Seurat](#)

Cell clustering : methods

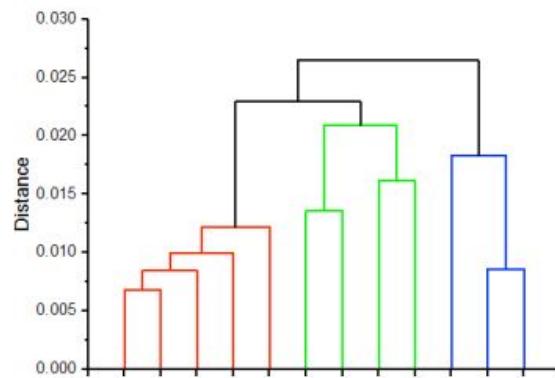
1) K-means based



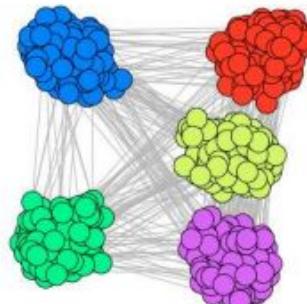
3) Model-based clustering (Mclust)



2) Hierarchical clustering

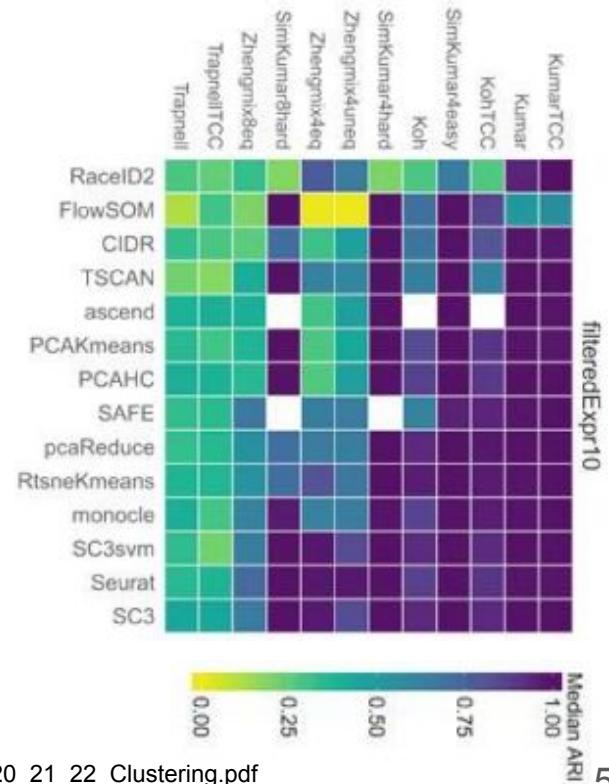


4) Graph-based clustering

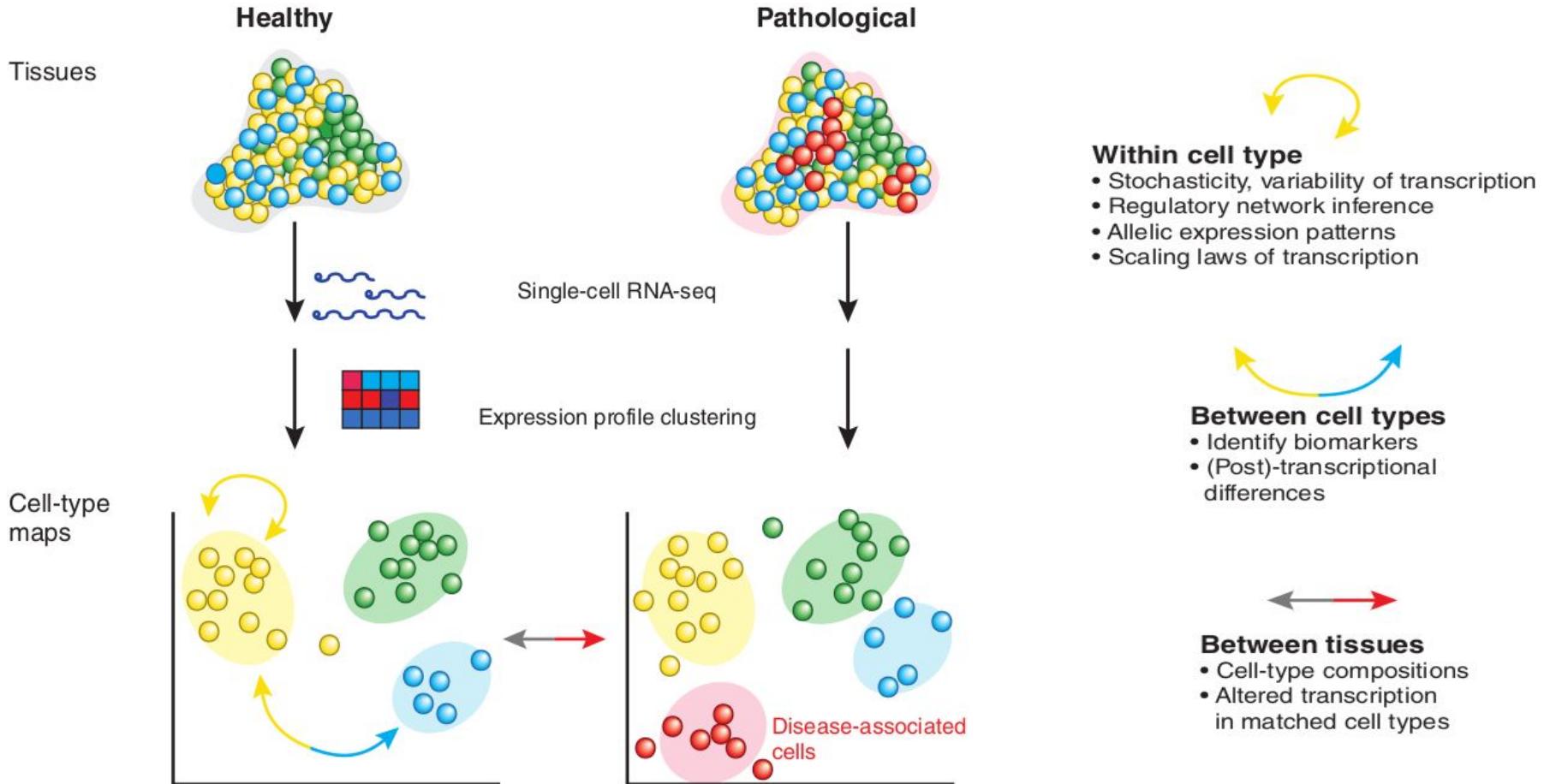


See : https://www.cse.msu.edu/~cse802/S17/slides/Lec_20_21_22_Clustering.pdf

5) Single-cell specific methods

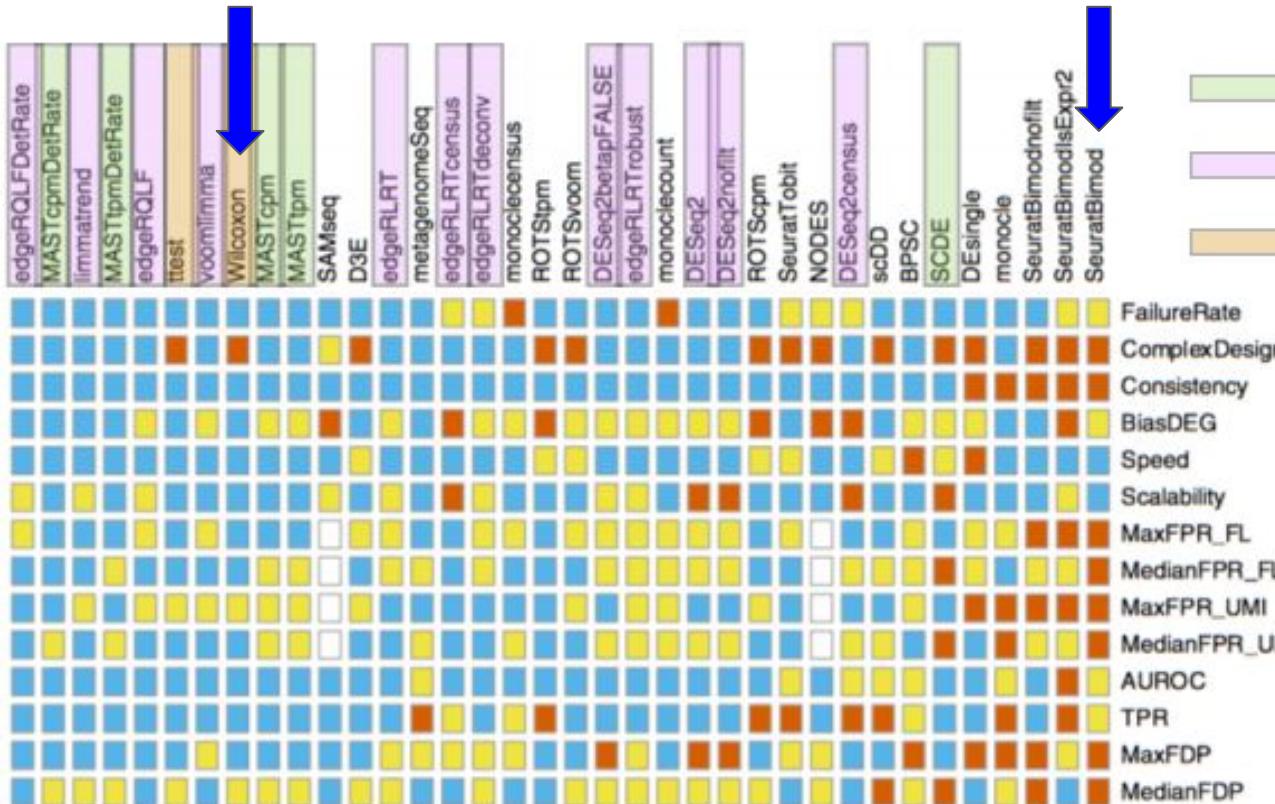


Differential expression analysis



Differential expression analysis : methods

Seurat v3



Seurat v2

Bayesian 3-component model adapted to single-cell data

Methods borrowed from bulk-RNA-seq

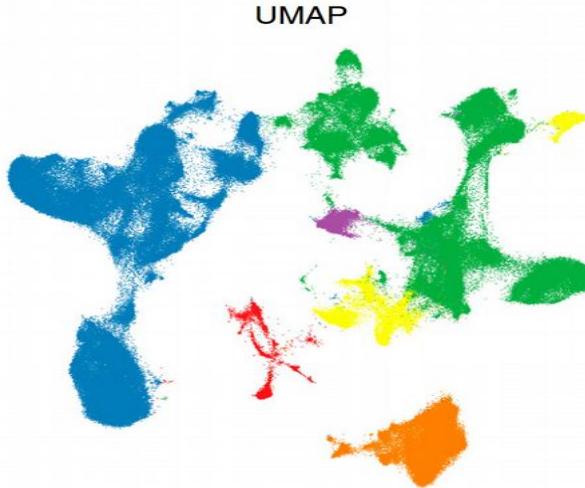
Naïve approaches

Good

Intermediate

Poor

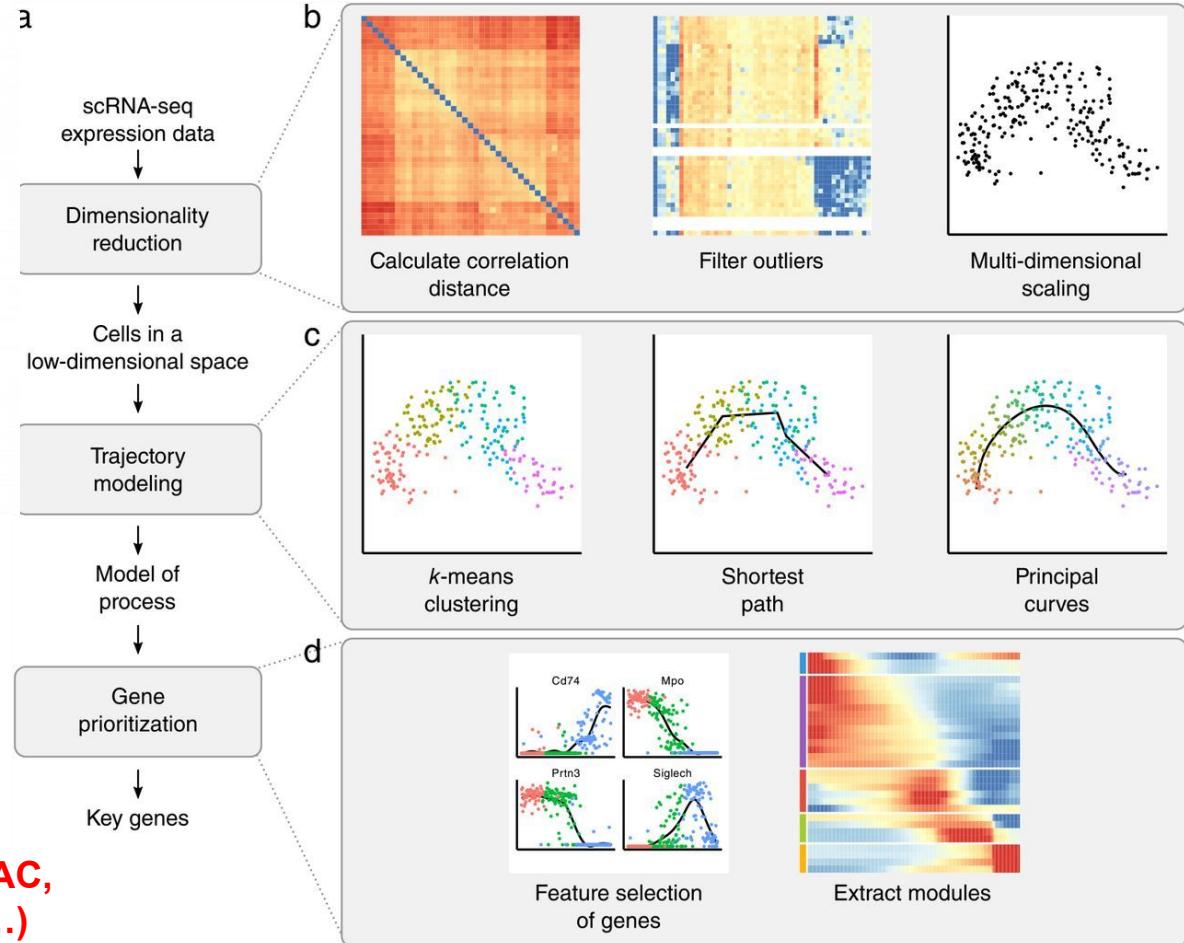
Cell trajectory : methods



Most adopted tools :

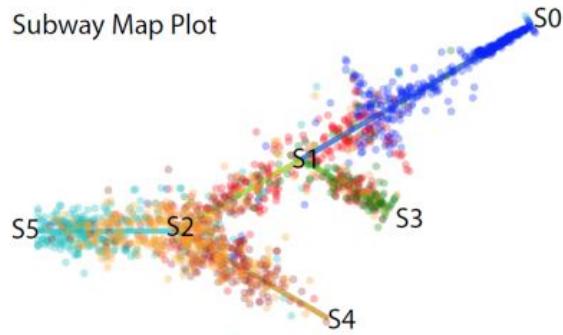
- Monocle 3
- PAGA
- STREAM
- Scorpius
- Slingshot

Not limited to scRNAseq ! (ATAC, CITE, multiomics, imagery-based ...)

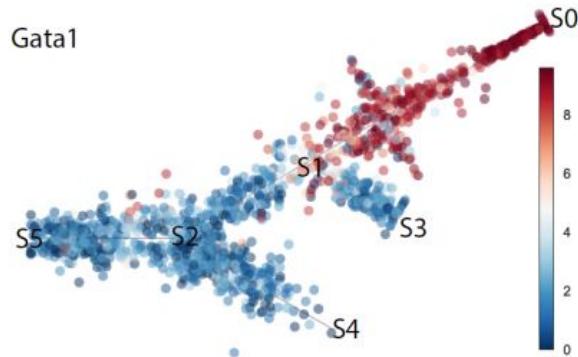


Cell trajectory : visualization

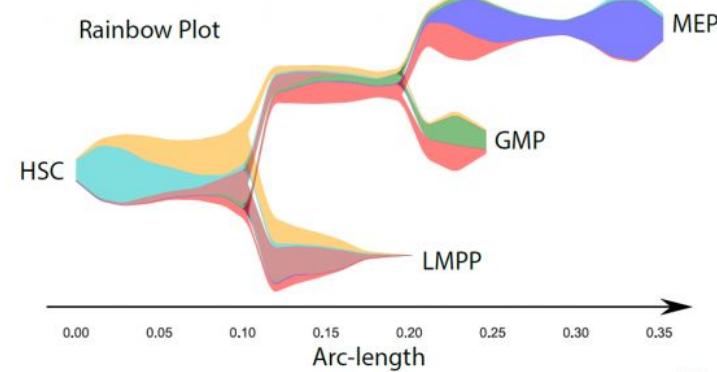
Cell distance to path + cell types



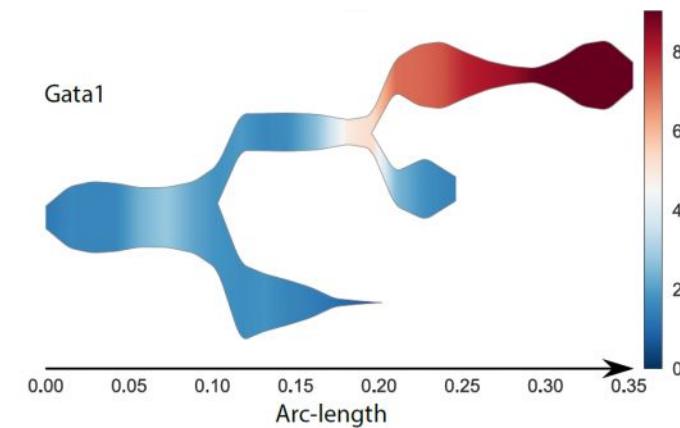
Cell distance to path + gene expression



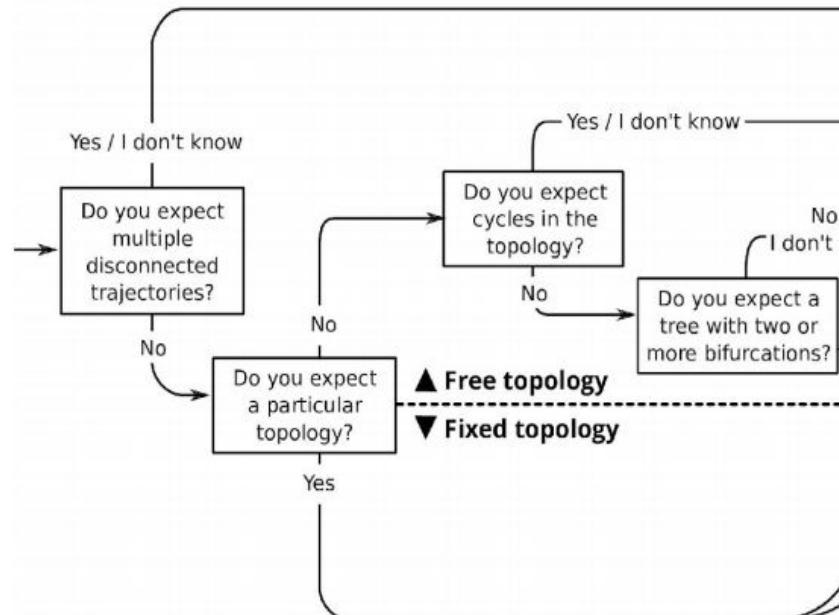
Cell types density



Cells density + gene expression



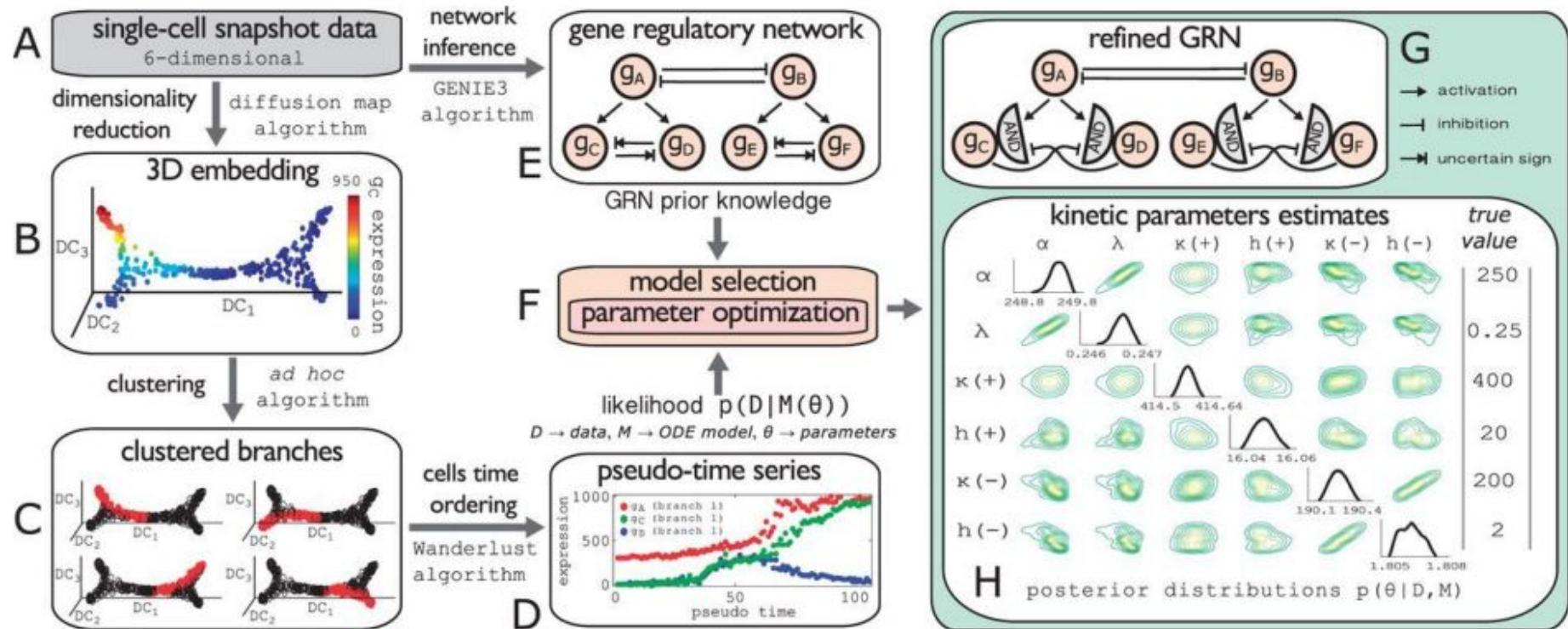
Cell trajectory : Contexts



	Estimated running time (cells × features)					
	Accuracy	Usability	100k×1k	10k×10k	1k×100k	Required priors
PAGA RacelID / StemID	+	±	7m	55s	19s	Start cell(s)
	-	±	1d	1h	1h	
PAGA RacelID / StemID SLICER	+	±	7m	55s	19s	Start cell(s)
	-	±	>7d	2h	31s	Start cell(s)
Slingshot PAGA Monocle ICA MST	+	+	11h	56m	2m	Start cell(s)
	±	±	7m	55s	19s	Number of end & start states
PAGA MST Slingshot RacelID / StemID	+	±	7m	55s	19s	Start cell(s)
	±	+	8m	12m	2m	
STEMNET Slingshot PAGA FateID	+	±	36m	12m	7m	End cell(s), Cell clustering
	+	+	11h	56m	2m	
Slingshot FateID GrandPrix STEMNET	+	±	7m	55s	19s	Start cell(s)
	+	±	6h	1h	26m	Cell clustering, Start & end cells
SCORPIUS Embeddr TSCAN Slingshot	+	±	7m	28m	>7d	# end states
	+	+	36m	12m	7m	End cell(s), Cell clustering
Angle EIPiGraph cycle reCAT RacelID / StemID	+	±	1h	4m	4m	
	±	±	2d	33m	2m	
Angle EIPiGraph cycle reCAT RacelID / StemID	+	+	7m	9m	7m	
	±	+	11h	56m	2m	

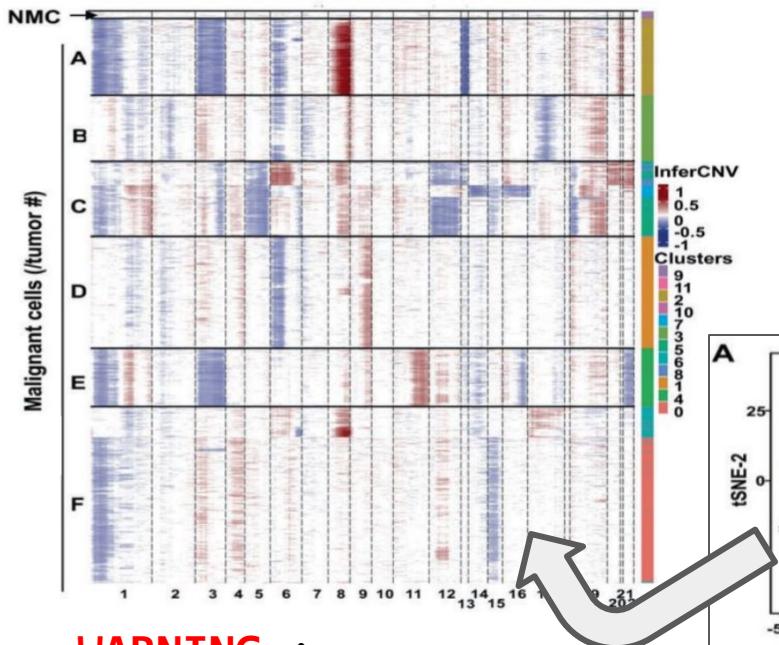
Network inference

Using cell ordering from trajectory analysis + co-occurring / correlated genes



Copy number estimation from scRNASeq

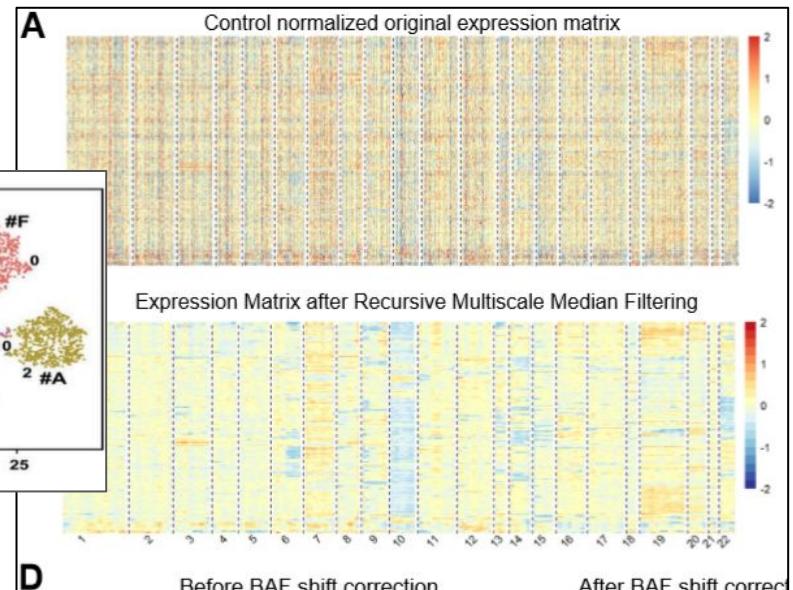
InferCNV (Broad Institute)



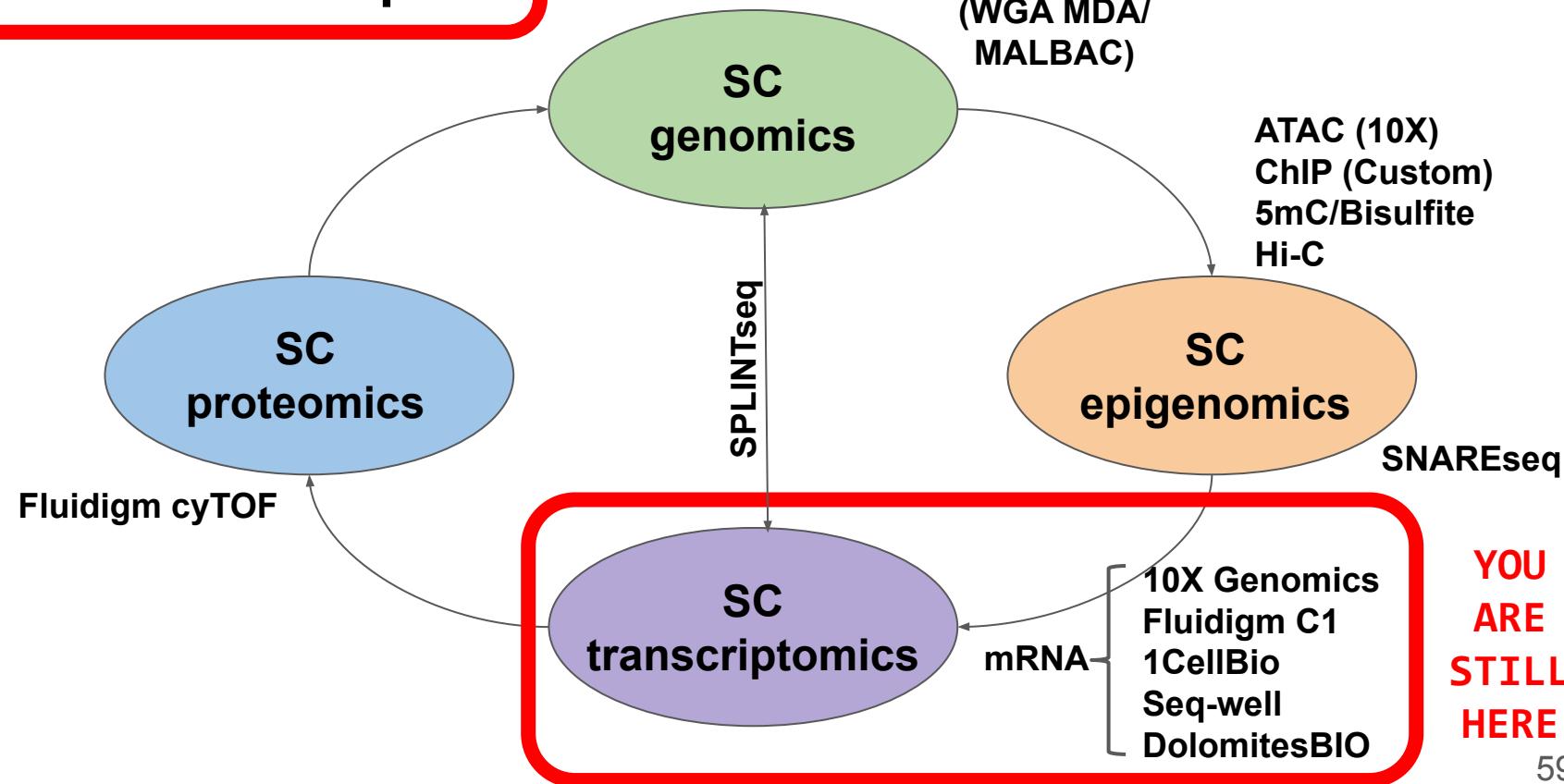
WARNING :

- Coarse grain (> 10 Mb)
- Requires > 75,000 reads / cell

CaSpER (Armanci et al, BioRxiv 2019)

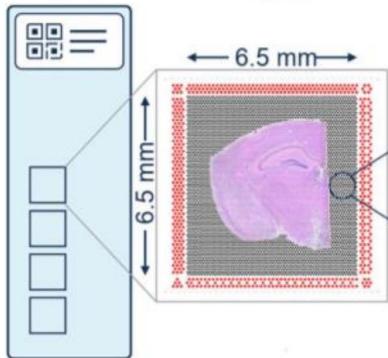


Spatial Single Cell RNAseq



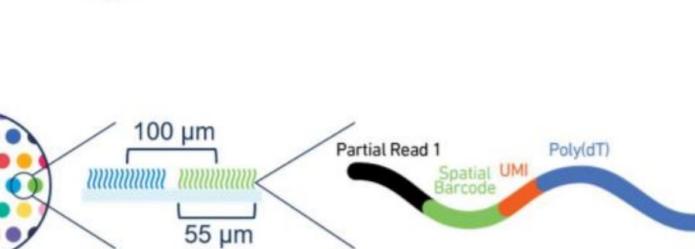
10x Genomics Visium

Visium Spatial
Gene Expression
Slide



Capture Area with
~5000 Barcoded
Spots

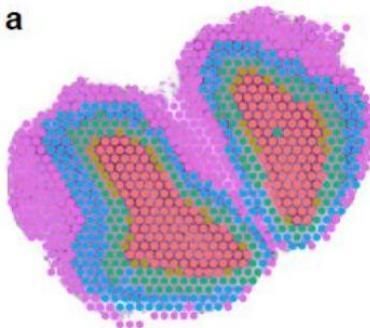
Visium Gene
Expression Barcoded
Spots



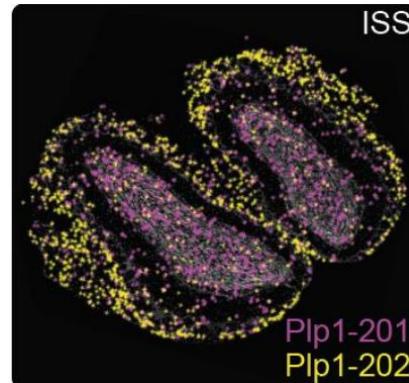
IHC



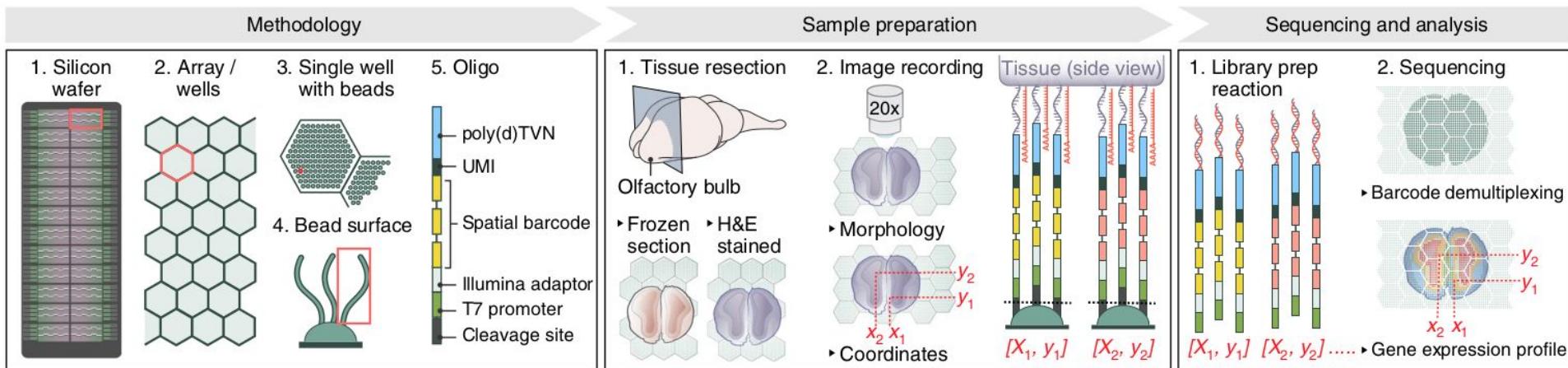
Clusters



RNA FISH

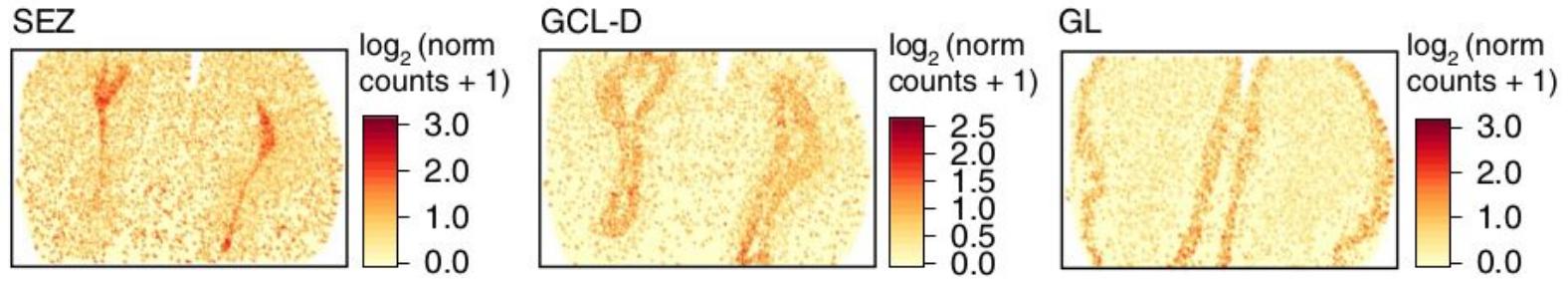


Illumina “HD Spatial Transcriptomics”

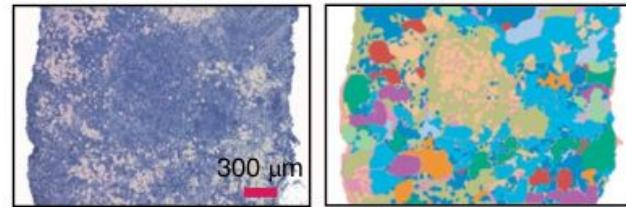


- 2,893,865 individual barcoded beads
- 1,4 M wells
- Well diameter $\sim 2 \mu\text{m}$
 - << median cell diameter (20 μm)
 - $\sim 1,400 \times$ higher resolution than “standard” ST
 - $\sim 25 \times$ compared to SLIDE-seq
- Array reading time $\sim 3 \text{ H}$
- Challenging analysis strategy (very low capture rate) ...
- “Commercially available in 2020” : *never released* ...

Illumina HDST



H&E Annotations



- Fatty tissue, immune/lymphoid
- Fibrous tissue, invasive cancer
- Fibrous tissue, immune/lymphoid
- Invasive cancer, immune/lymphoid
- Immune/lymphoid
- Fatty tissue, fibrous tissue, invasive cancer
- Fibrous tissue
- Fibrous tissue, invasive cancer, immune/lymphoid
- Fatty tissue
- Fatty tissue, fibrous tissue, invasive cancer, immune/lymphoid
- Fatty tissue, invasive cancer, immune/lymphoid
- Invasive cancer

C

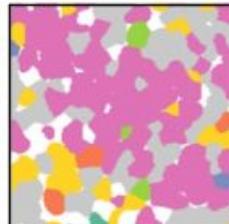
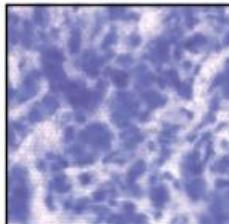
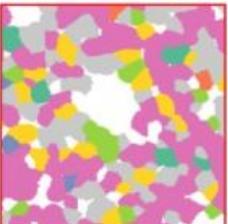
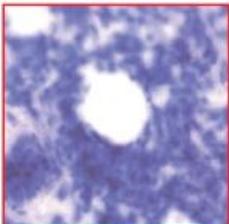
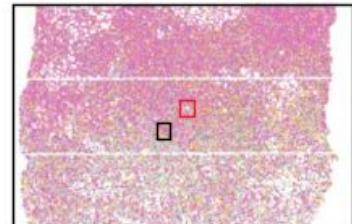
Cell types
in sn-like data

H&E
enlargement

sn-like
enlargement

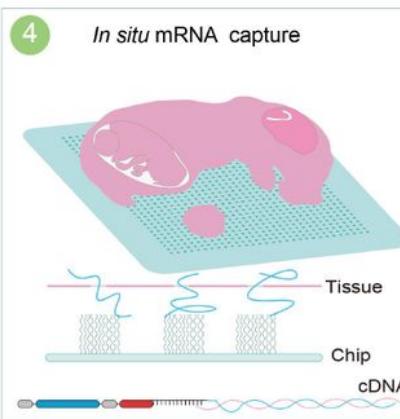
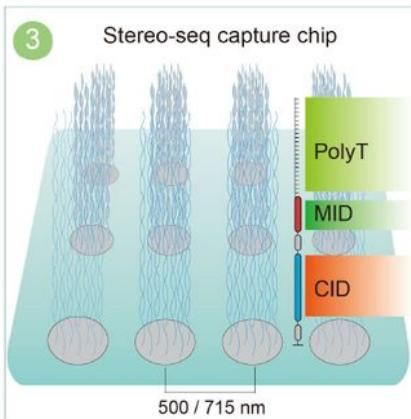
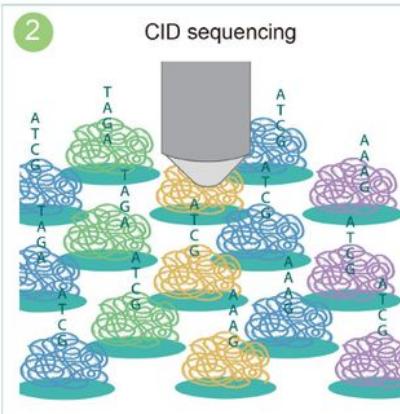
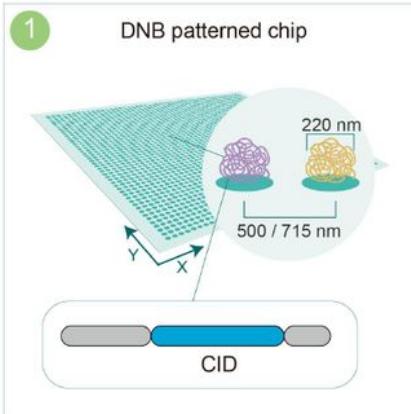
H&E
enlargement

sn-like
enlargement



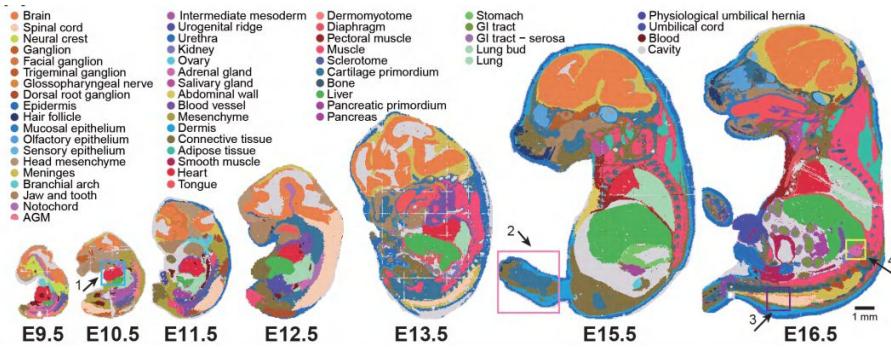
- T cells
- B cells
- Endothelial cells
- Epithelial cells
- Macrophages
- Stroma
- Unassigned nucleus

“StereoSeq” : Ultra HD spatial long reads

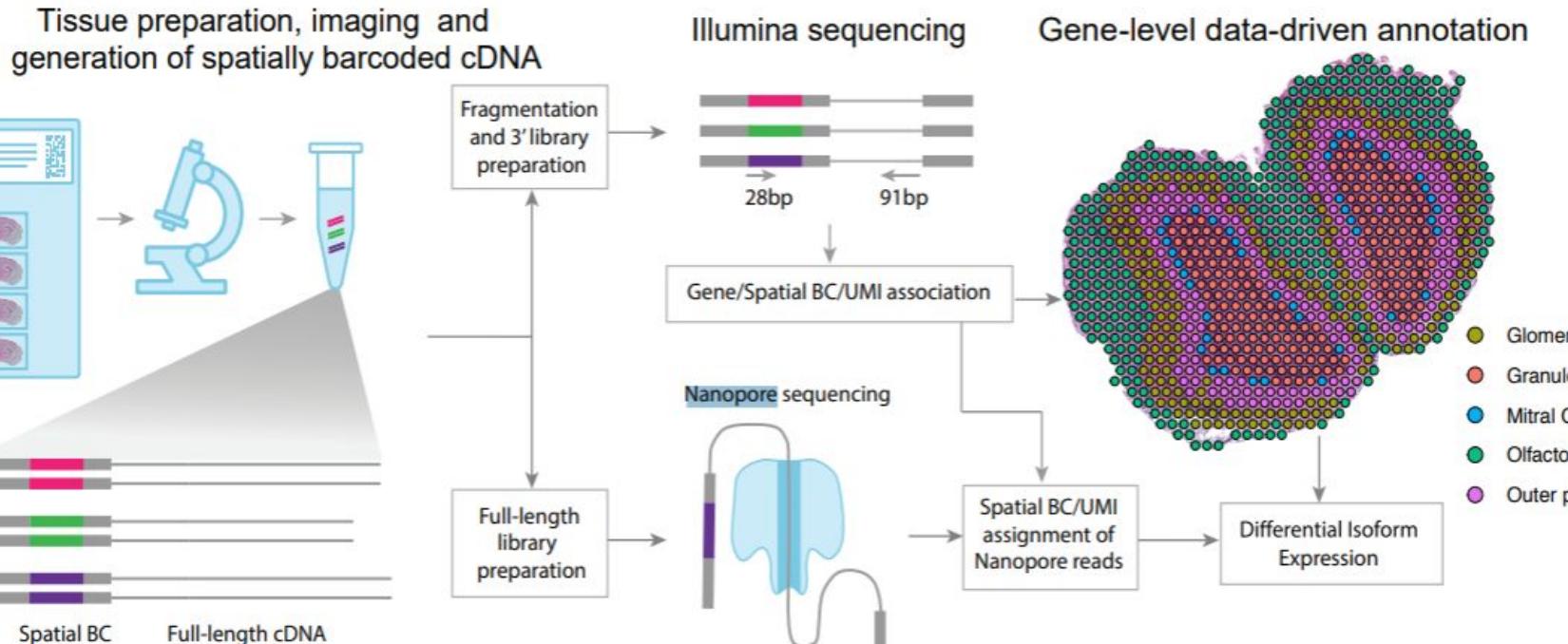


- Based upon “DNB” (DNA Balls) arrays by MGI/BGI
- Claimed resolution of **500-715 nm**
- Active surface of **200 mm²**
- Performed a developmental analysis of **FULL** mice embryos !

Cheng et al, BiorXiv 2021.01



Spatial long reads



Single Cell CNV

CNV

SC
proteomics

Fluidigm cyTOF

SC
genomics

WES
CNV
VDJ/TCR
(WGA MDA/
MALBAC)

NOW, YOU
ARE HERE

SC
transcriptomics

mRNA

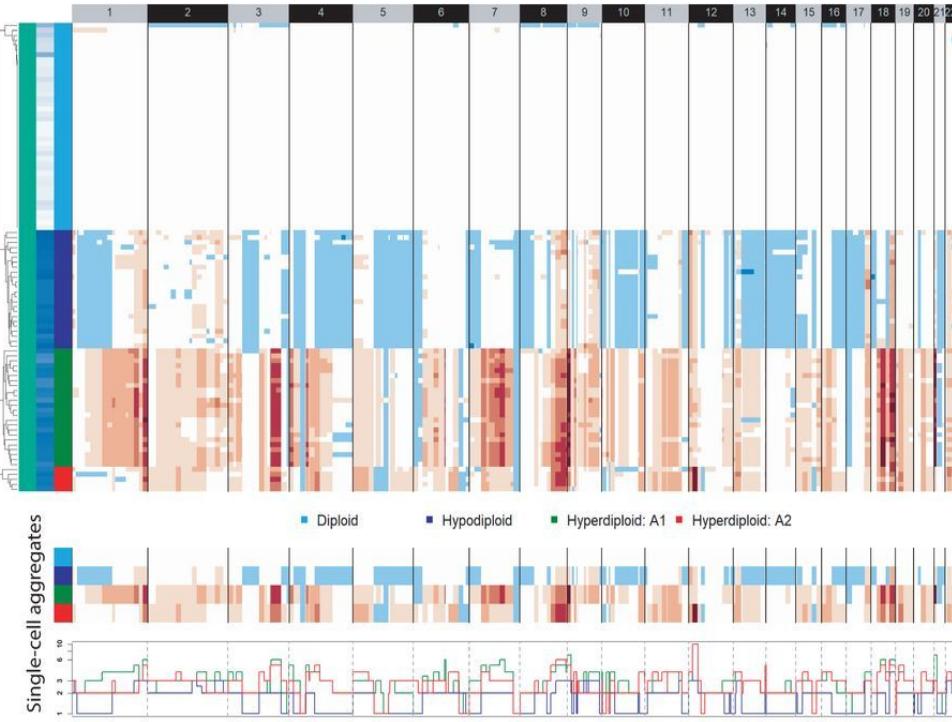
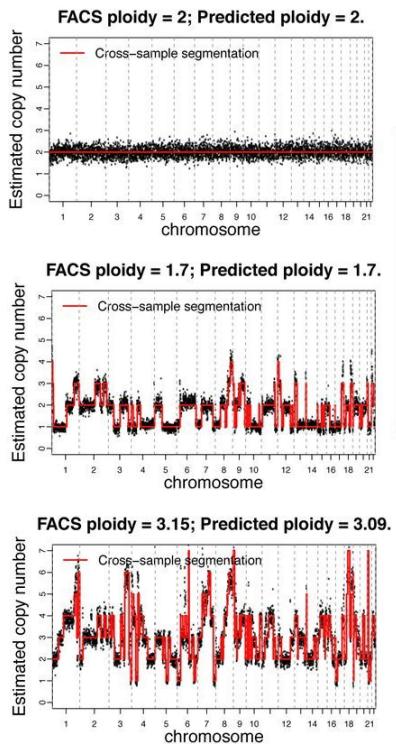
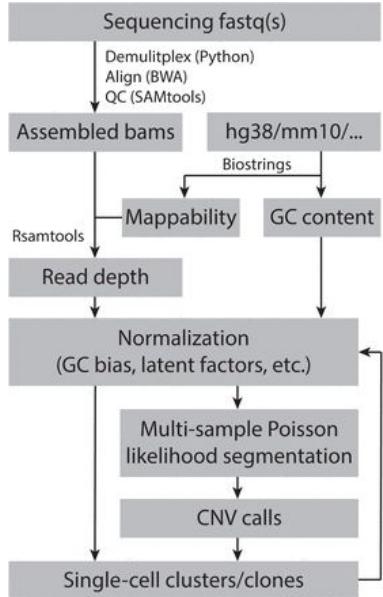
- 10X Genomics
- Fluidigm C1
- 1CellBio
- Seq-well
- DolomitesBIO

SC
epigenomics

SNAREseq

SPLINTseq

scCNV results (SCOPE)

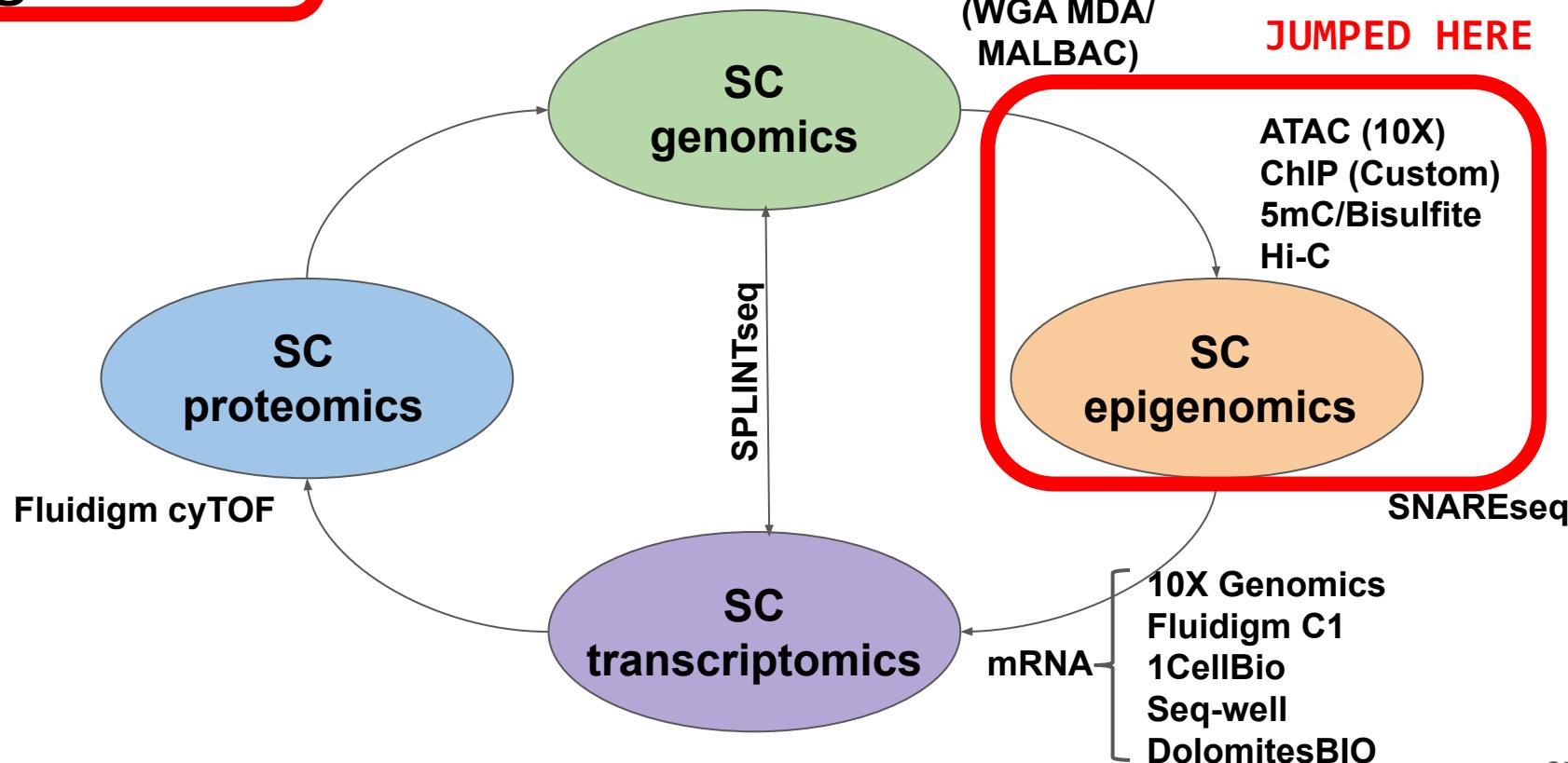


WARNING :

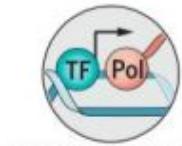
- Limited resolution : > 2 Mb (binning)
- Requires > 750,000 reads / cell

ALSO available : SCYN

Single Cell Epigenomics

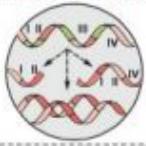


Overview of scEpigenomics techniques

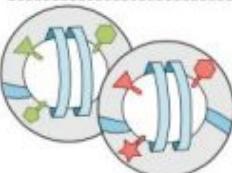


Transcription factor binding

TF binding interacts with DNA methylation and chromatin accessibility



Transcription and RNA maturation



Histone modifications

Modifications can be active marks (e.g., H3K4me3 in green) or repressive marks (e.g., H2K27m3 in red)



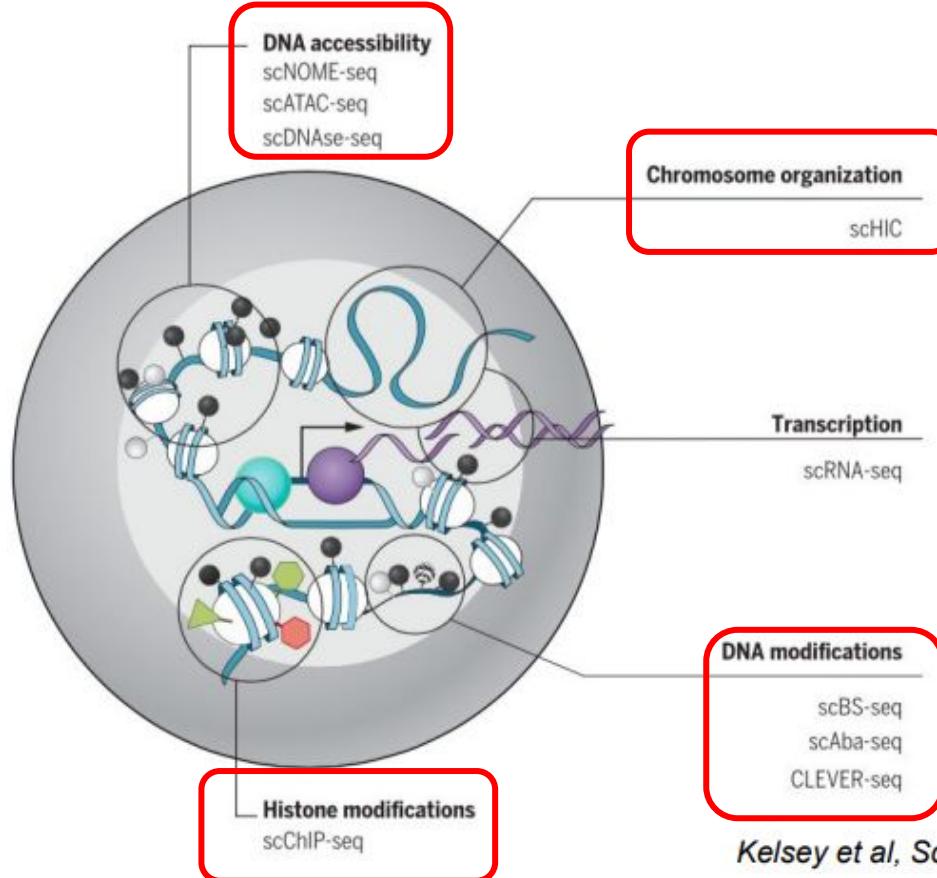
DNA modifications

● C ● 5mC
● 5hmC / 5fC / 5caC



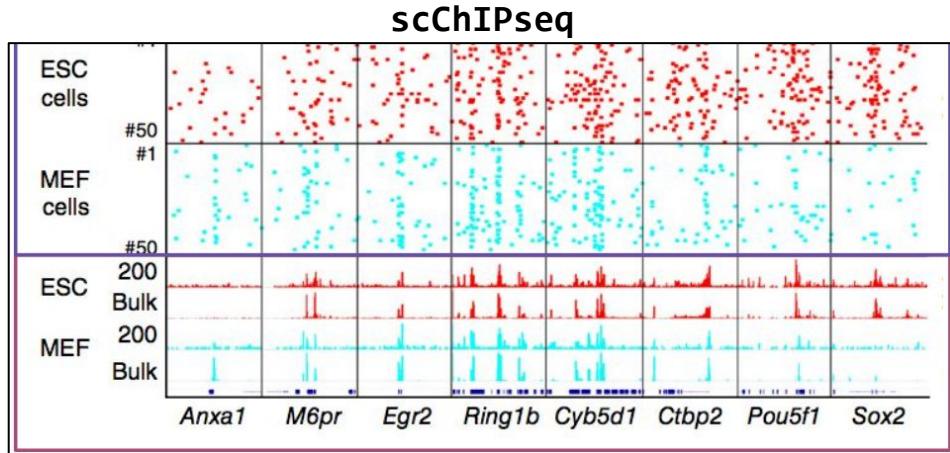
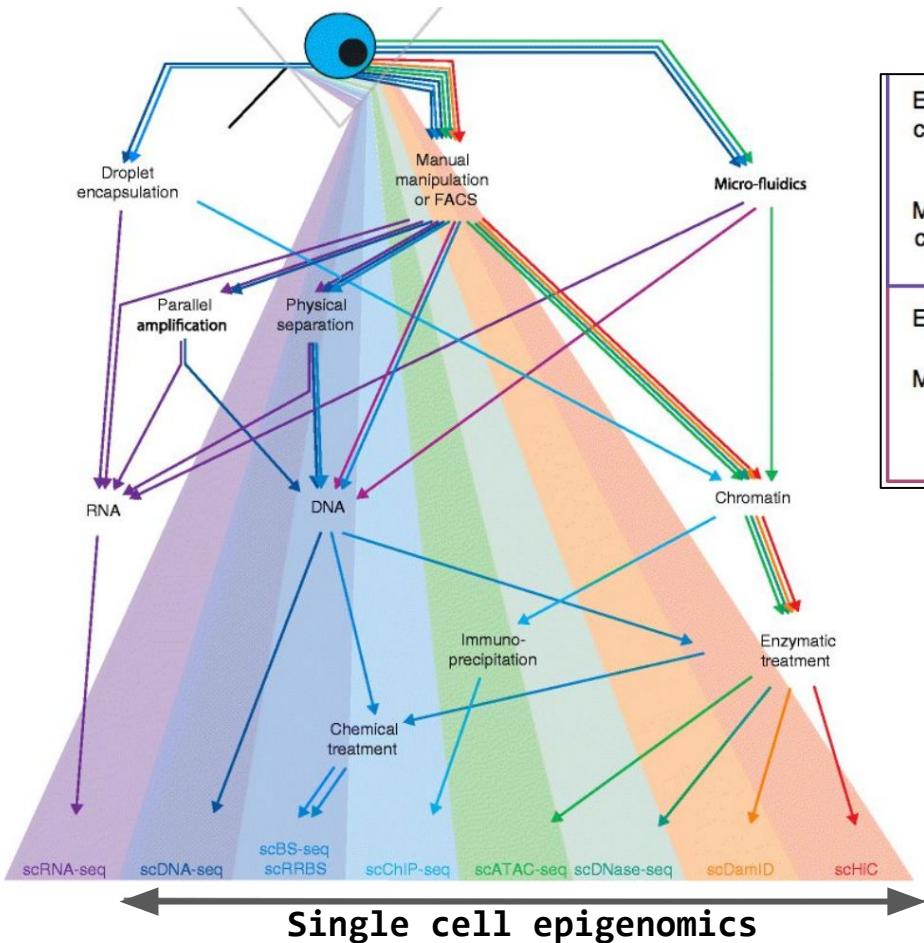
Chromosome organization

Higher-order chromatin organization into LADs and TADs



Kelsey et al, Science, 2017

Overview of scEpigenomics techniques



- scChIP : improvements in 2019
- scMeth : low coverage, low sensitivity (<20% CpG read)
- scHi-C : stable protocol & analysis still needed
- scATAC : most popular technology, numerous tools available

*Single cell (RNAseq) resources
(some)*

Tabula Muris

ARTICLE

<https://doi.org/10.1038/s41586-018-0598-4>

Single-cell transcriptomics of 20 mouse organs creates a *Tabula Muris*

The Tabula Muris Consortium*

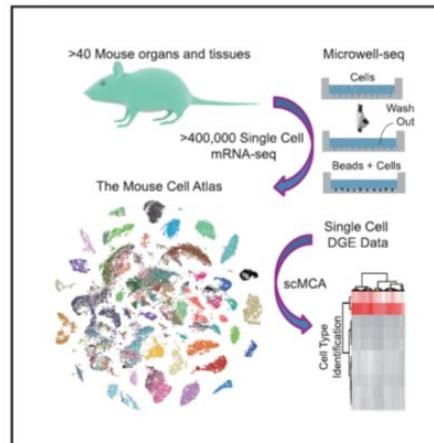


- ~100k cells
- 20 organs
- 2 techniques :
 - Droplet 3', short reads
 - FACS, long reads
- <http://bis.zju.edu.cn/MCA/>

Cell

Mapping the Mouse Cell Atlas by Microwell-Seq

Graphical Abstract



Resource

Authors

Xiaoping Han, Renying Wang, Yincong Zhou, ..., Guo-Cheng Yuan, Ming Chen, Guoji Guo

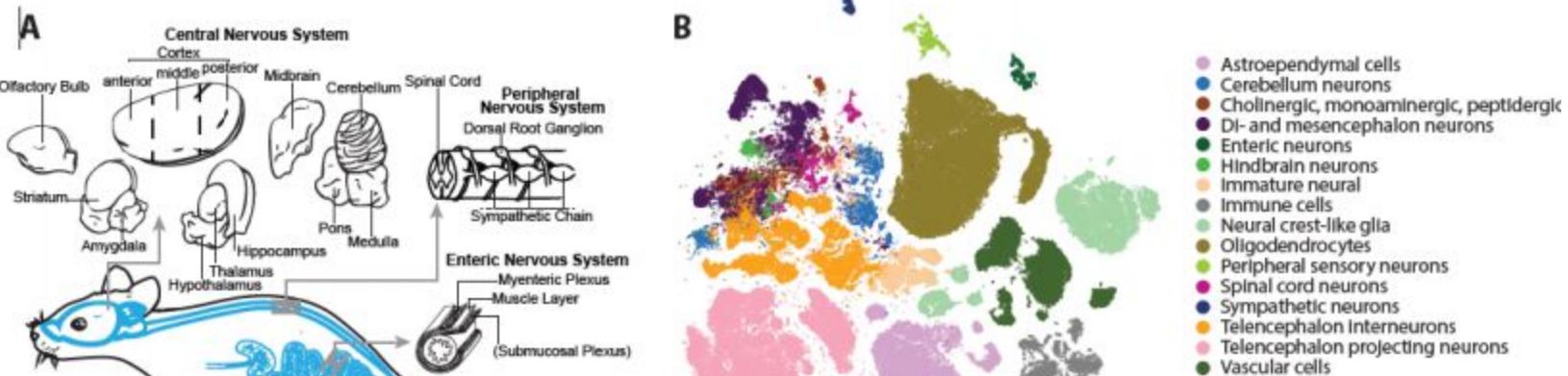
Correspondence

xhan@zju.edu.cn (X.H.), ggj@zju.edu.cn (G.G.)

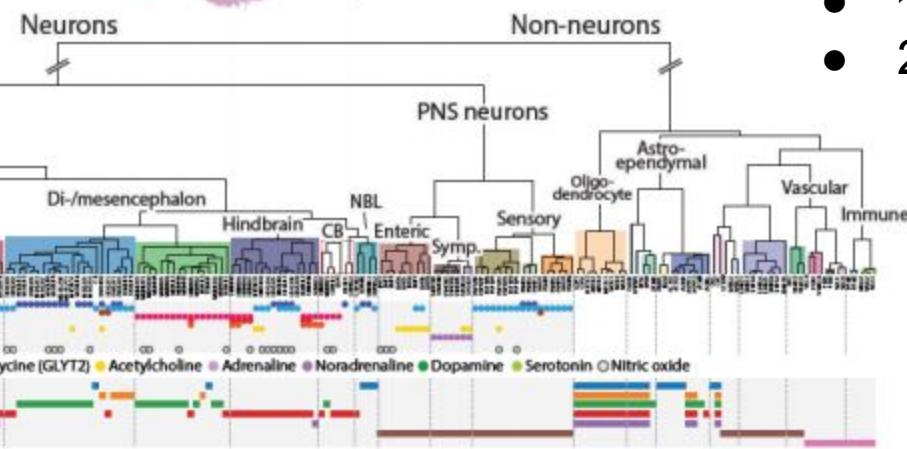
In Brief

Development of Microwell-seq allows construction of a mouse cell atlas at the single-cell level with a high-throughput and low-cost platform.

The Mouse Brain Atlas (mousebrain.org)



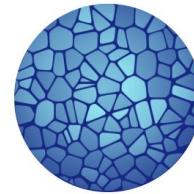
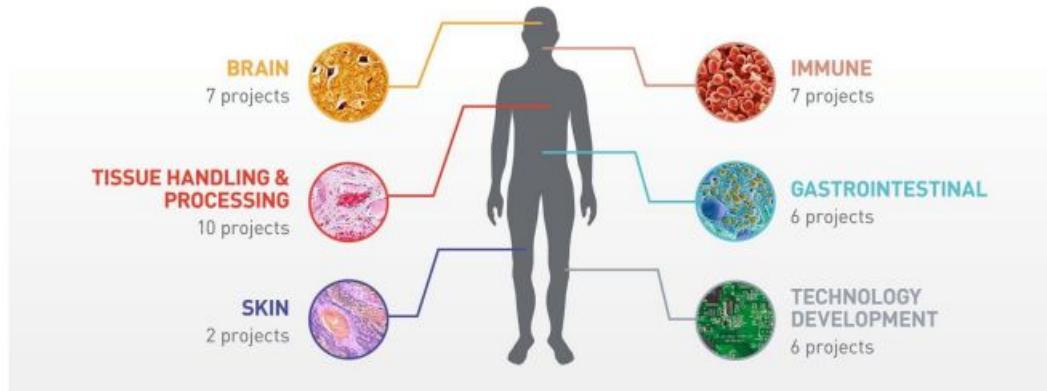
C



The Human Cell Atlas (humancellatlas.org)

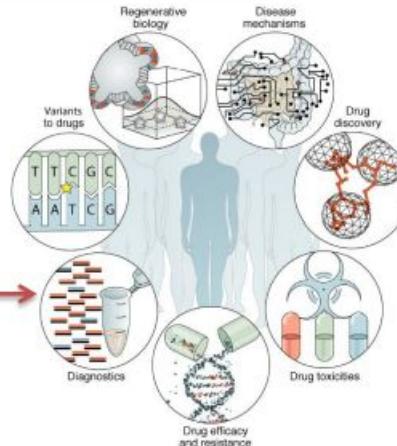
MAPPING THE BASIC UNITS OF LIFE

CZI proudly supports 38 new projects in these six areas for the Human Cell Atlas.

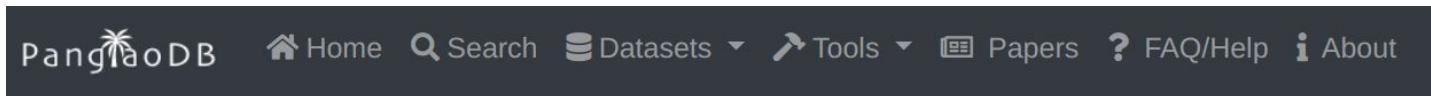


HUMAN
CELL
ATLAS

- Every cell type in the body
- First: define how to proceed
 - Best experimental practice / organ
 - Best bioinformatics methods
- Data will be made available to all



PanglaoDB

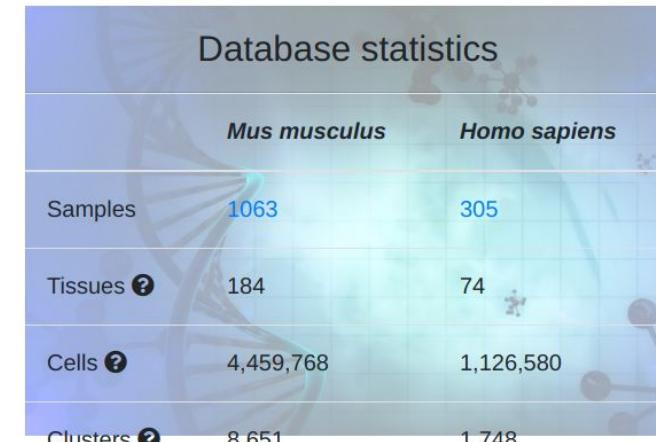


The navigation bar at the top of the page includes the PanglaoDB logo, Home, Search, Datasets, Tools, Papers, FAQ/Help, and About links.

PanglaoDB is a database for the scientific community interested in exploration of single cell RNA sequencing experiments from mouse and human. We collect and integrate data from multiple studies and present them through a unified framework.

Usage examples

- Run a gene search for [SOX2](#), [PECAM1](#) or [ACE2](#)
- Browse the full list of [samples](#)
- Explore the list of cell type markers for [Schwann cells](#)
- Browse cell types of the mouse [retina](#)
- Look at the expression of [CRX](#) in photoreceptor cells



A table comparing database statistics for *Mus musculus* and *Homo sapiens*.

	<i>Mus musculus</i>	<i>Homo sapiens</i>
Samples	1063	305
Tissues	184	74
Cells	4,459,768	1,126,580
Clusters	8,651	1,748

Dataset of the day

Take a closer look at the cellular composition of [Heart](#), using a dataset which consists of 871 cells. Clustering of this dataset resulted in 7 cell clusters, containing among others, [Smooth](#)

WYSIWYG analysis frameworks
(mainly for scRNAseq / scATACseq)

SeuratV3Wizard

The SeuratV3Wizard interface consists of three main panels:

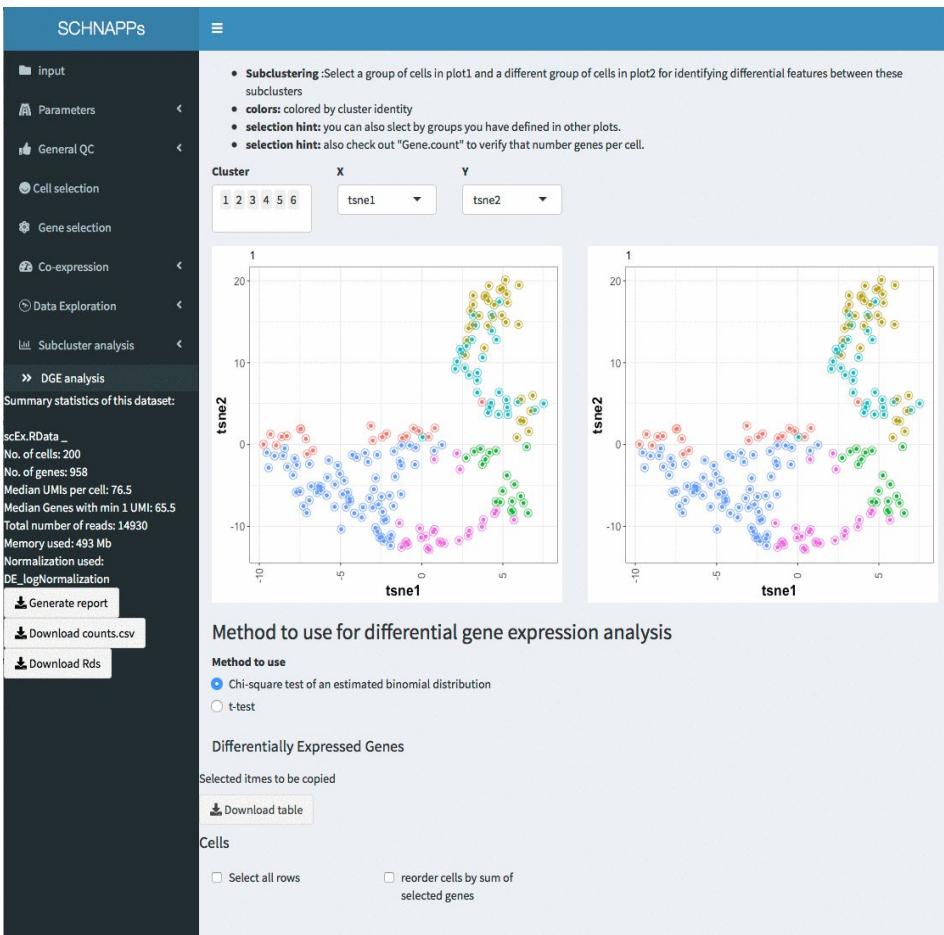
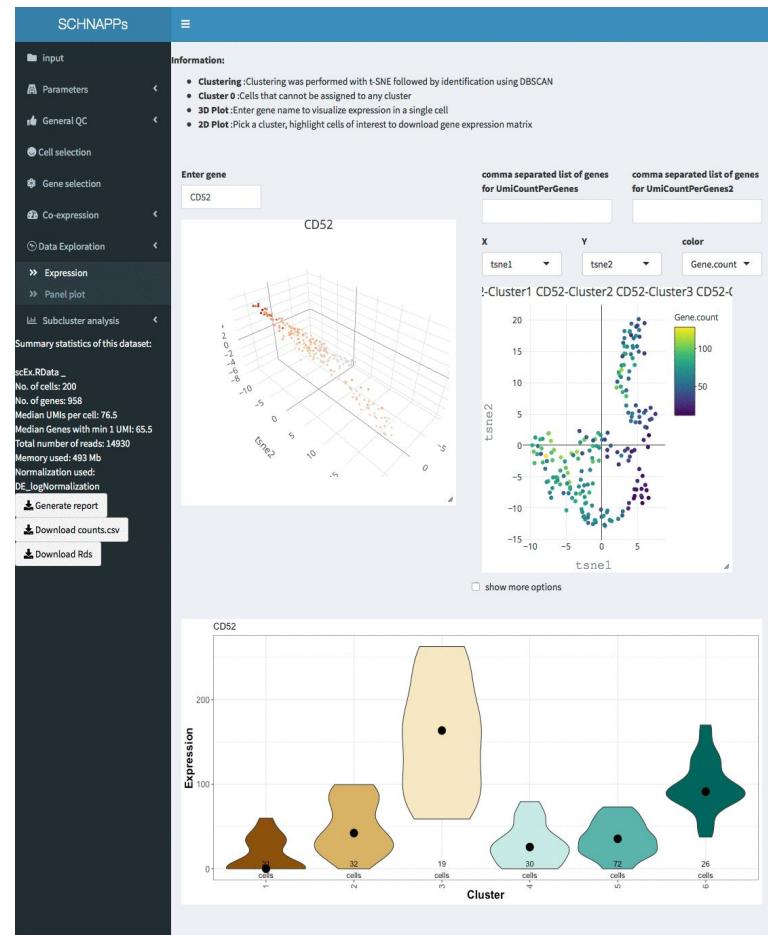
- Vin Plot (Filter Cells) Panel:** Shows two scatter plots: "nFeature_RNA" and "nCount_RNA". The "nFeature_RNA" plot has a red dashed horizontal line labeled "low.threshold" at approximately 100 and a blue dashed horizontal line labeled "high.threshold" at approximately 2000. The "nCount_RNA" plot shows a sharp peak at 1. A subset name "nFeature_RNA" is selected in the dropdown.
- PCHeatmap Output Panel:** A configuration panel for PCA heatmaps. It includes:
 - Input Data: QC & Filter, VinPlot (Filter Cells), Norm/Detect/Scale, PCA Reduction, Viz PCA Plot, PCA Plot, PC heatmap, Elbow/JackStraw, Cluster Cells, Non-linear Reduction, Cluster Markers, Viz Markers, Download Seurat Obj.
 - PCs to use: PC 1 to 6.
 - Number of cells to use: 500.
 - Plot Download Options: Plot height (in cm): 30, Plot width (in cm): 30.
 - Download File Type: PDF (selected).
- Run Non-linear dimensional reduction Panel:** A configuration panel for non-linear dimensionality reduction:
 - Input Data: QC & Filter, VinPlot (Filter Cells), Norm/Detect/Scale, PCA Reduction, Viz PCA Plot, PCA Plot, PC heatmap, Elbow/JackStraw, Cluster Cells, Non-linear Reduction, Cluster Markers, Viz Markers, Download Seurat Obj.
 - Choose reduction method to proceed with:
 - tsne (radio button selected)
 - umap
 - UMAP Plot: A scatter plot showing clusters of cells in UMAP space (UMAP_1 vs UMAP_2). The plot includes a color key from 0 to 9.

A vertical sidebar on the left lists all available steps in the workflow, each with a green checkmark indicating completion.

<http://nasqar.abudhabi.nyu.edu/SeuratV3Wizard/>



SCHNAPPS : A R-shiny app for biologists

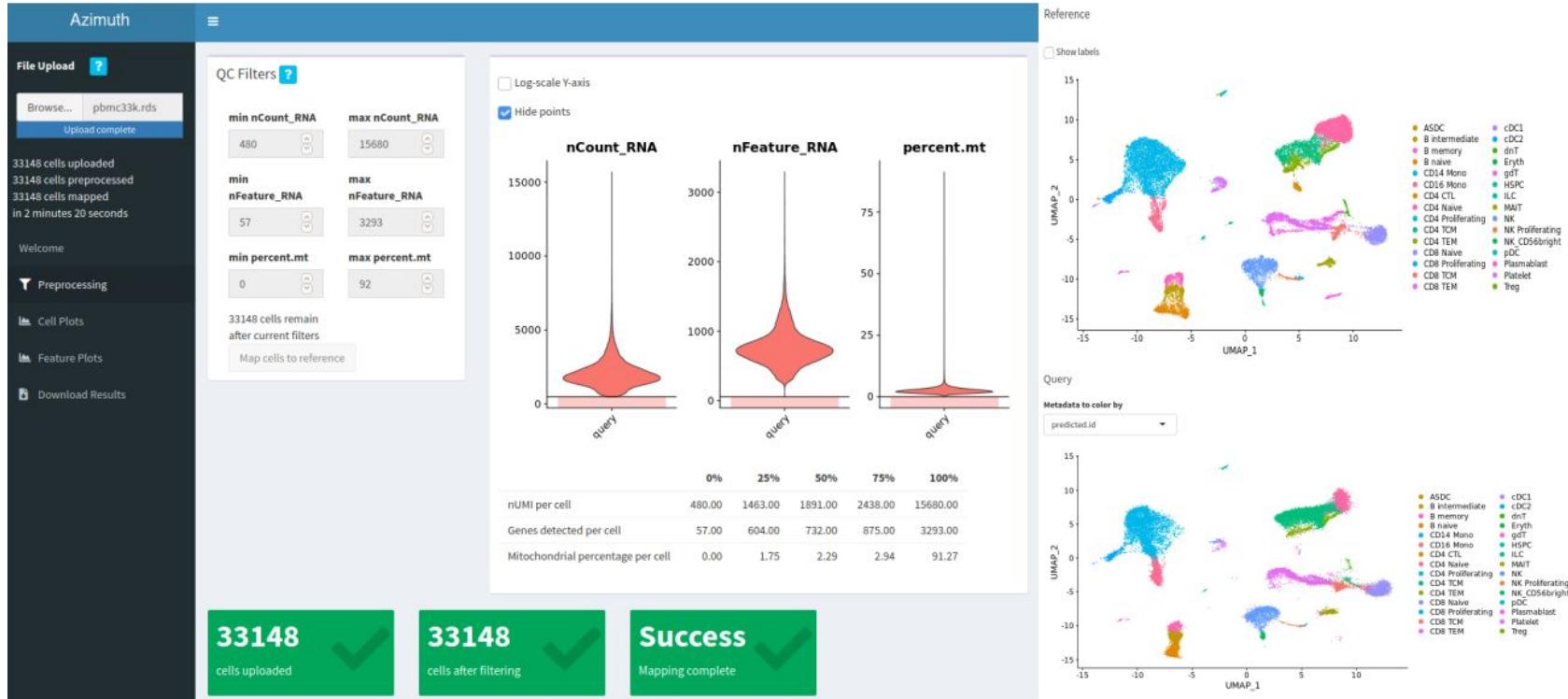


By Bernd Jagla (Pasteur Paris)

<https://c3bi-pasteur-fr.github.io/UTechSCB-SCHNAPPS>

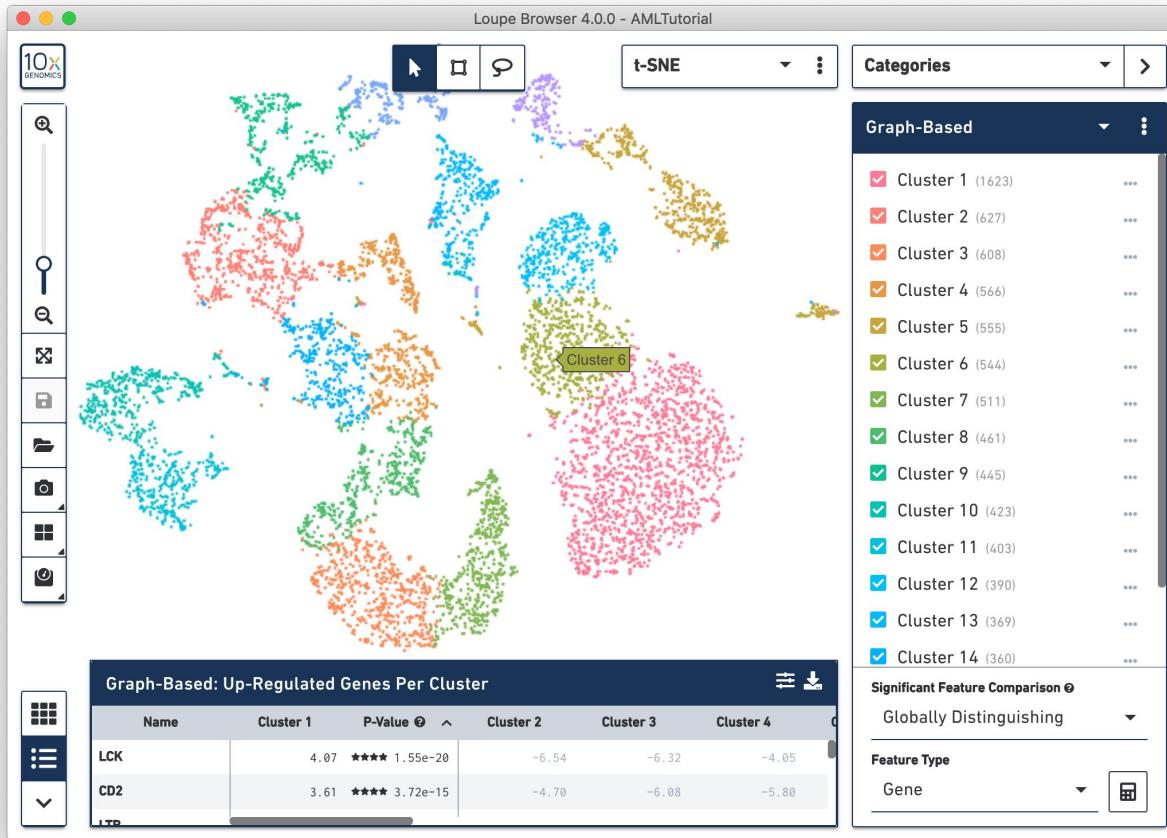
<https://github.com/C3BI-pasteur-fr/UTechSCB-SCHNAPPS>

Azimuth (from Satija Lab)

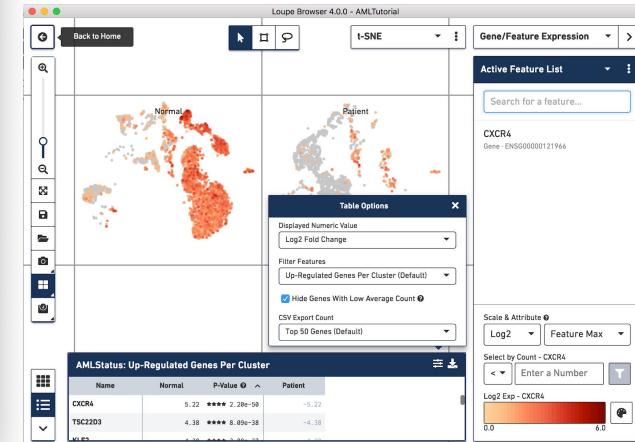


Visualization tools
(mainly for scRNAseq)

10x Genomics Loupe Browser

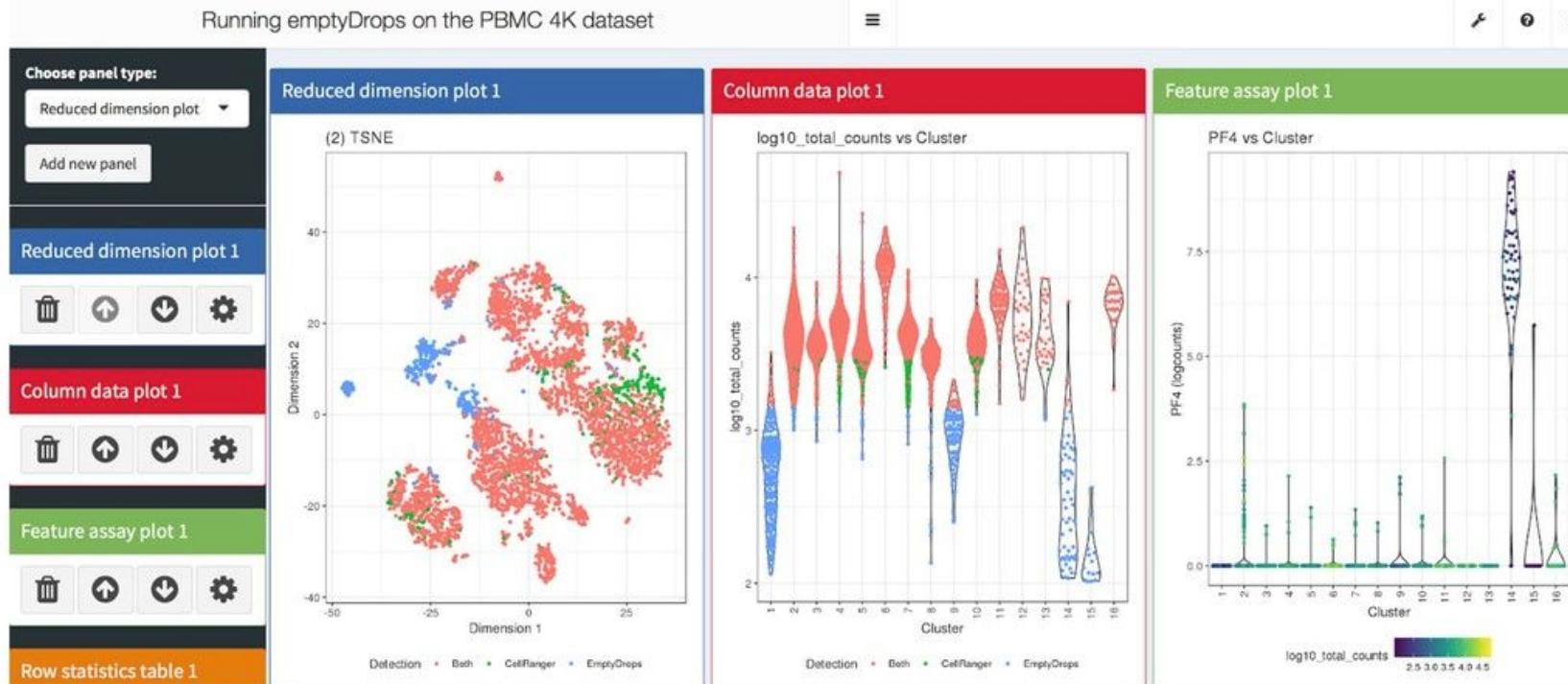


- Compatible with output from 10x Cell Ranger (“cloupe” files)
- Linux / OSX
- Supports Visium (Spatial)



Interactive Data Visualization (iSEE)

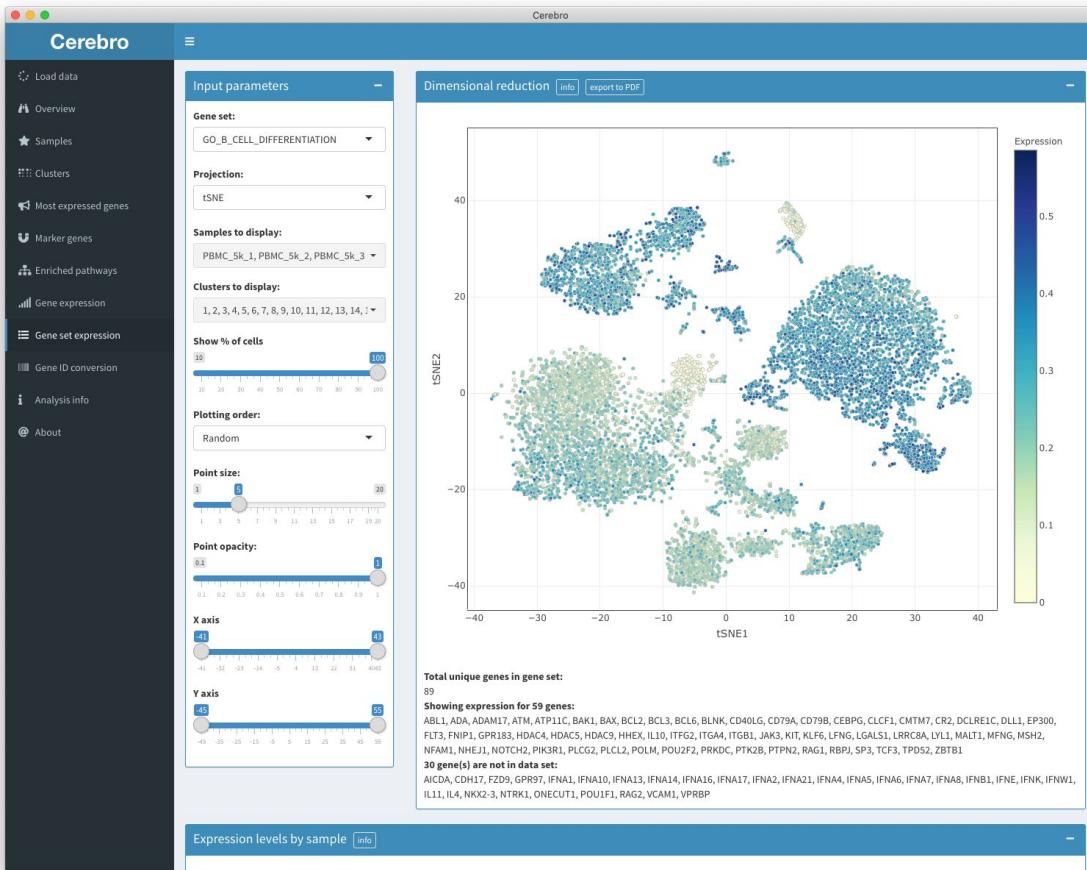
Running emptyDrops on the PBMC 4K dataset



Creators: Federico Marini,
Aaron Lun, Charlotte Soneson,
and Kevin Rue-Albrecht

https://marionilab.cruk.cam.ac.uk/iSEE_pbmc4k/

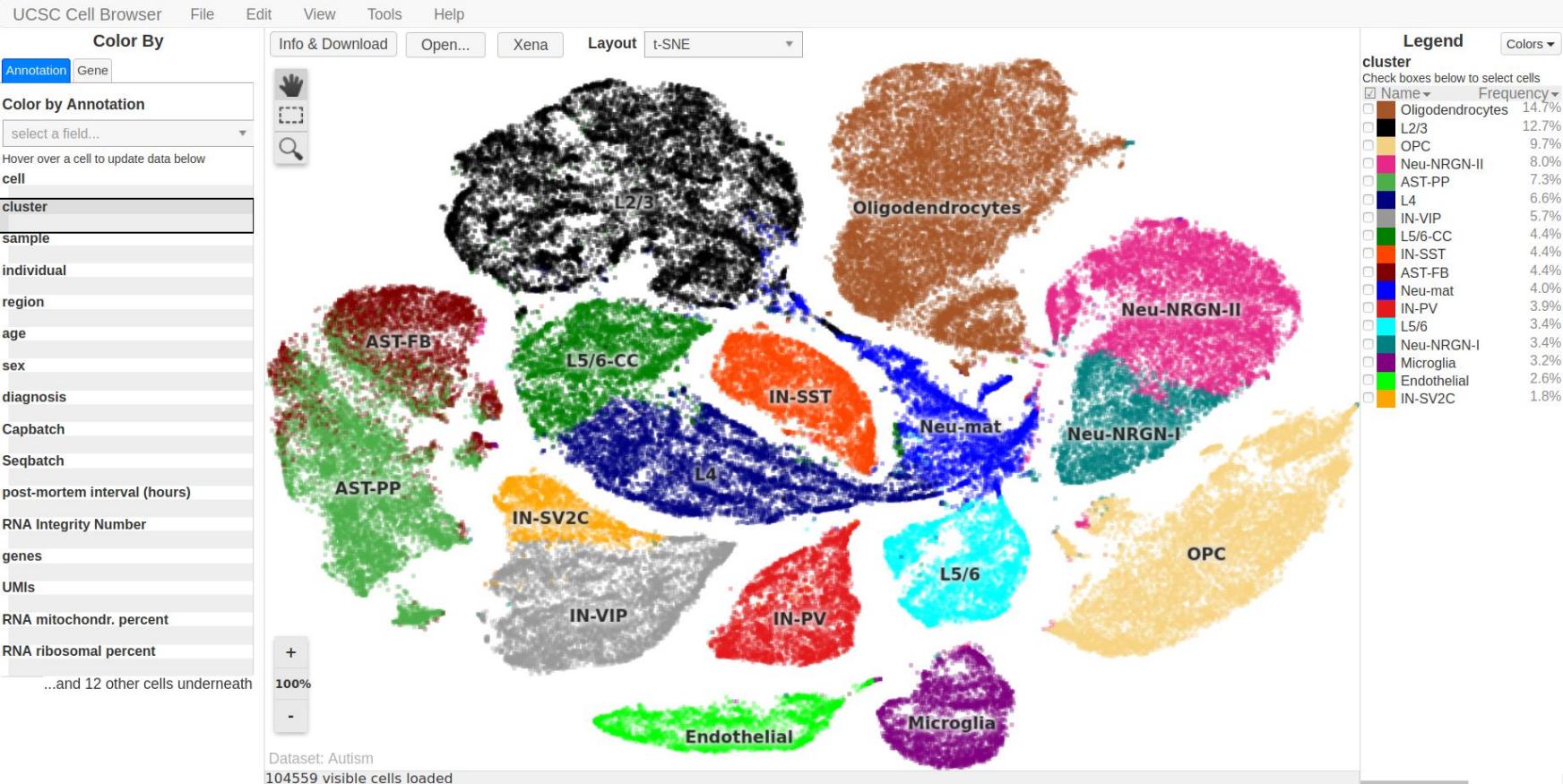
CerebroApp



- ShinyApp (web GUI over some R)
- Binary format (CRB), converted from SeuratObject / SCE
- From QC to trajectory
- Development recently stopped ...

(still my favorite one)

UCSC Cell Browser

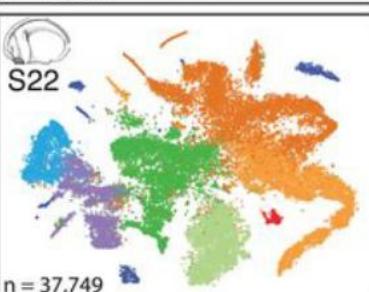
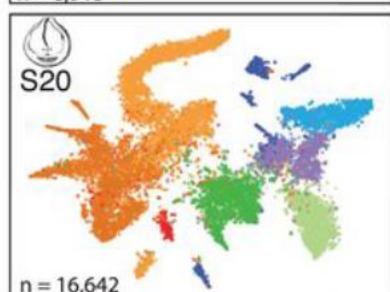
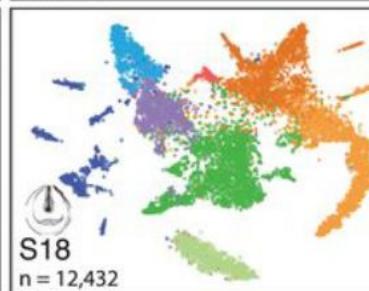
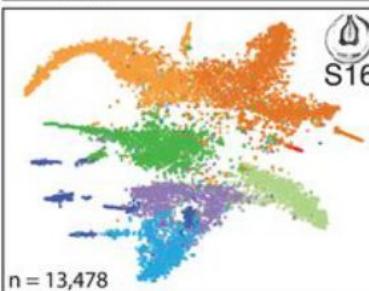
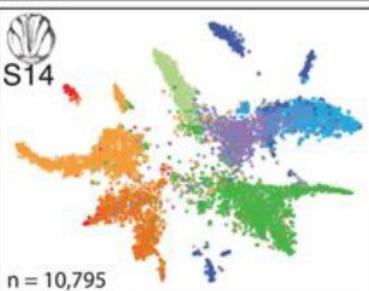
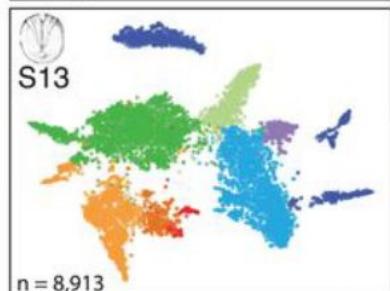
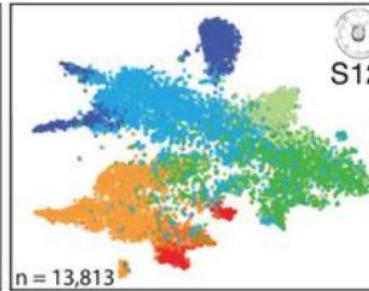
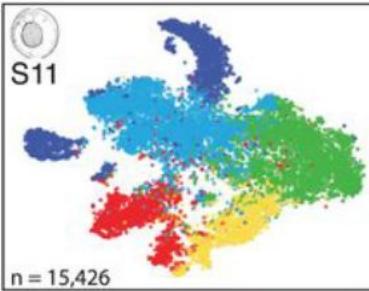
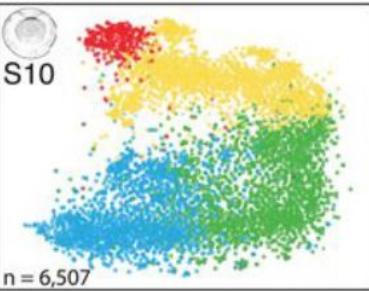
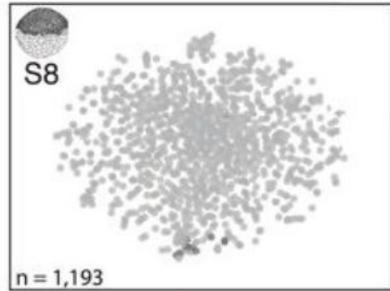


<https://github.com/maximilanh/cellBrowser> (demo : <https://cells.ucsc.edu>)

Some Findings and Applications
(mainly for scRNAseq)

Xenopus embryo development

tSNE dimension 2

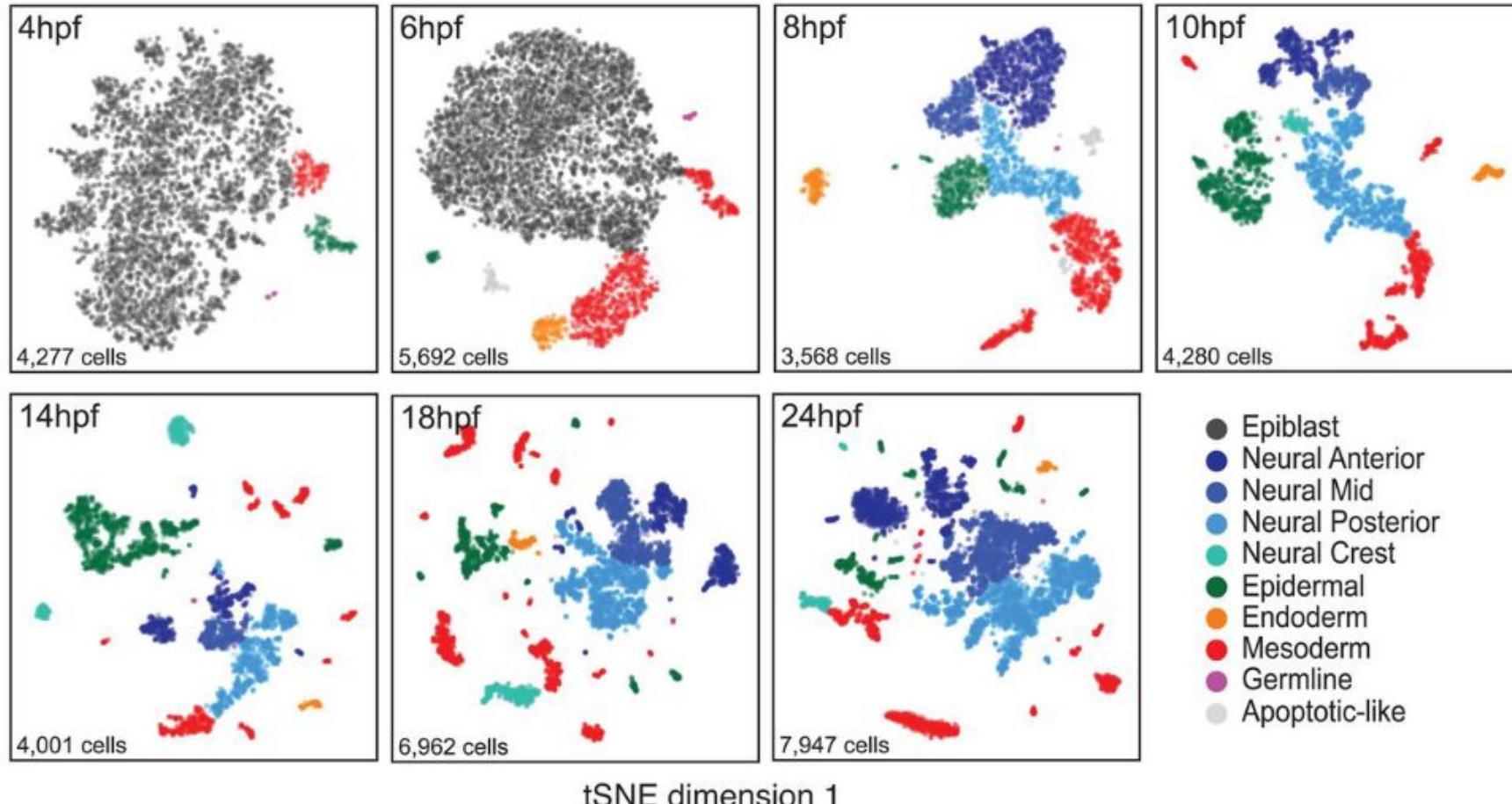


- Pluripotent blastula
- Germline
- Non-neural ectoderm
- Placodal
- Specialized epidermis
- Neural
- Neural crest
- Marginal zone
- Dorsal mesoderm
- Vent/lat/int. mesoderm
- Endoderm

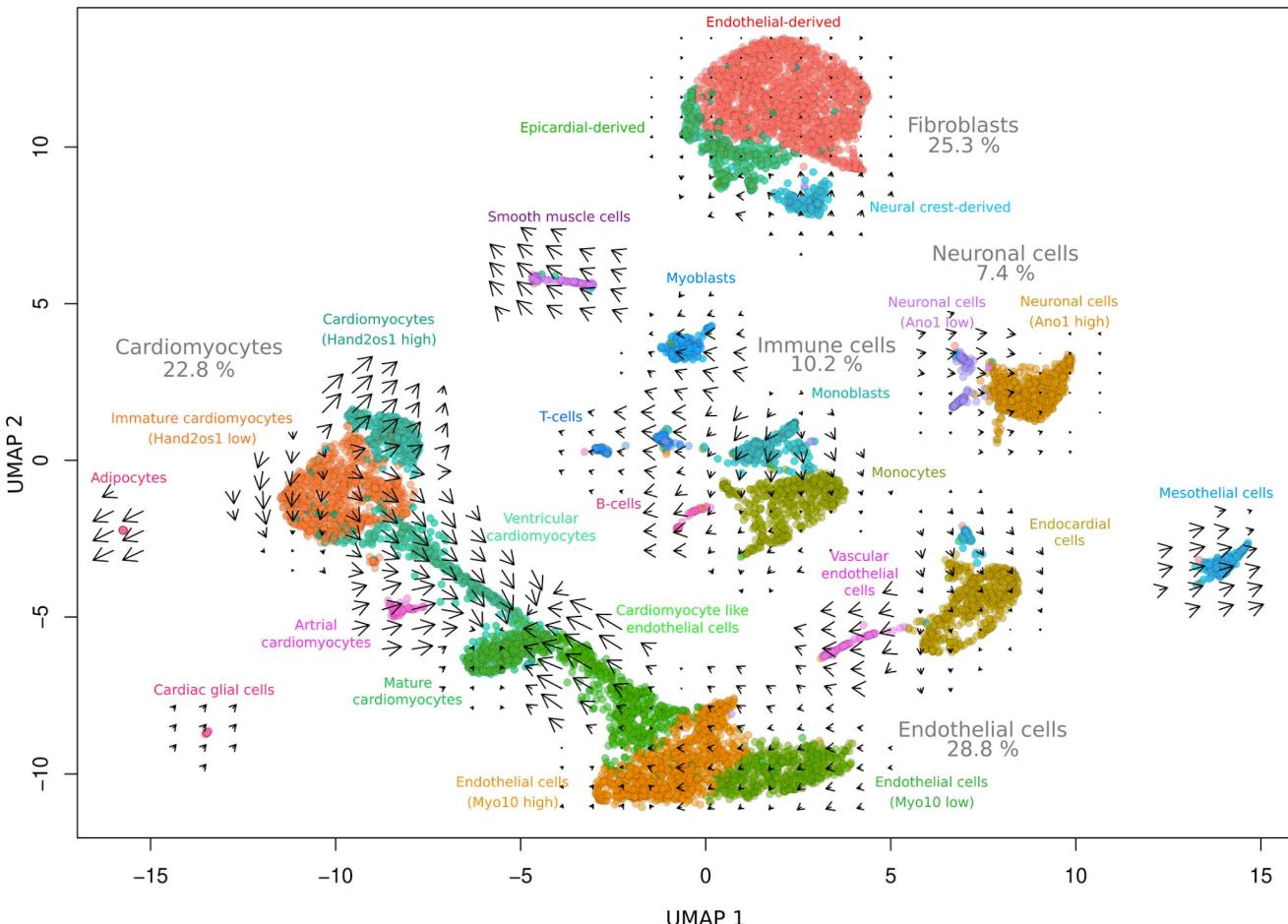
tSNE dimension 1

Zebrafish embryo development

tSNE dimension 2



Entire mouse heart : expression & velocity



<https://doi.org/10.3390/cells9020318>

Some things I muted (*and many, many more*)

- scRNAseq :
 - RNA velocity
 - Protein activity modelization
 - Stemness scoring
 - Variants detection
 - Integration:
 - Multiple samples
 - Multiple omics data
 - All non-droplet methods !
- scEpigenomics : quite everything !
- Other single cell technologies :
 - Genomic :
 - Long reads
 - Non-genomic :
 - Imagery
- Other : ERCCs, PDX, ...

Acknowledgements

Marc Deloger

Morgane Thomas-Chollier

Agnès Paquet

Marine Aglave

Antonio Rausell

Wouter Saelens

Nathalie Gaspar

... and you !



SINGle-cellING in the RAINaseq (1952)
A joke © Jacques van Helden

APPENDIX

Announced by 10x on twitter in January

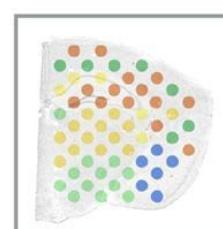
New High Throughput Instrument



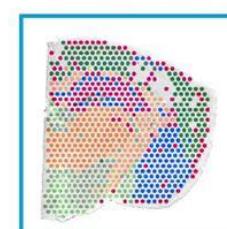
Making 1 million cell experiments routine



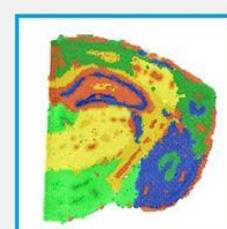
Visium at Single Cell Resolution



First Generation



Visium
Resolution 4x



Visium HD
Resolution 1,500x

Unlocking More Samples for Spatial

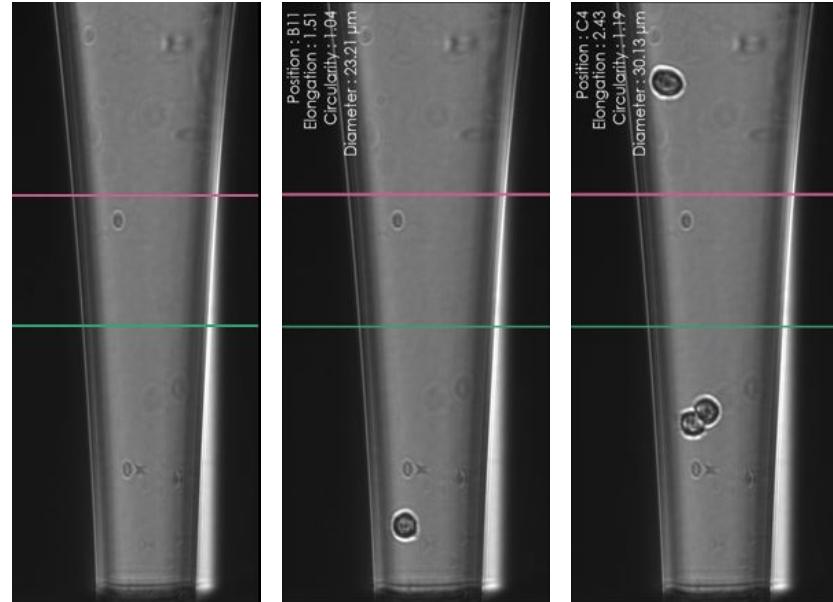


Accessing archived and pre-mounted samples

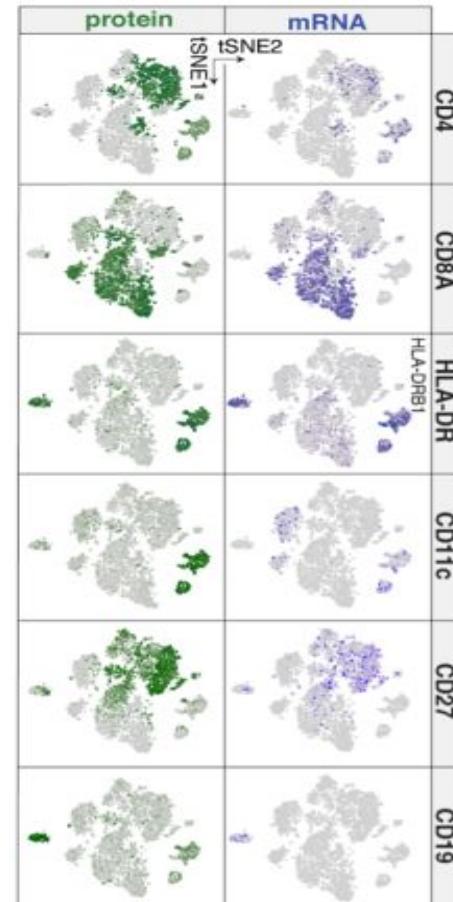
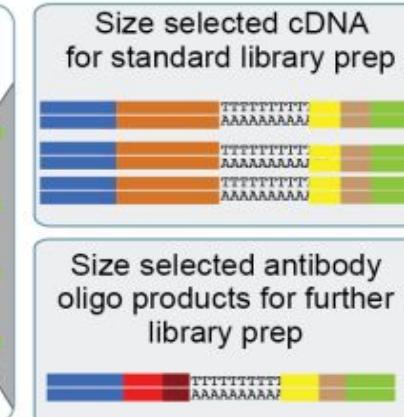
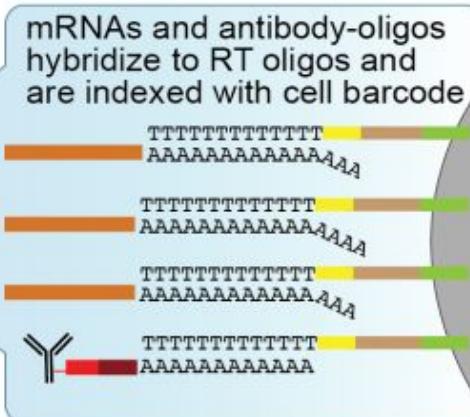
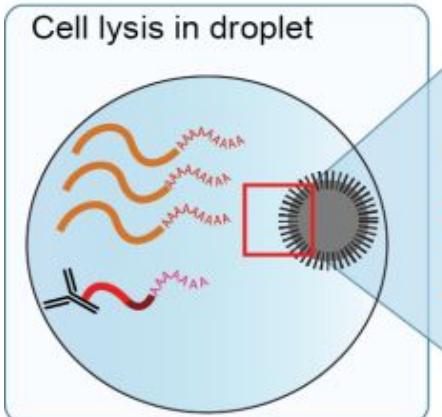
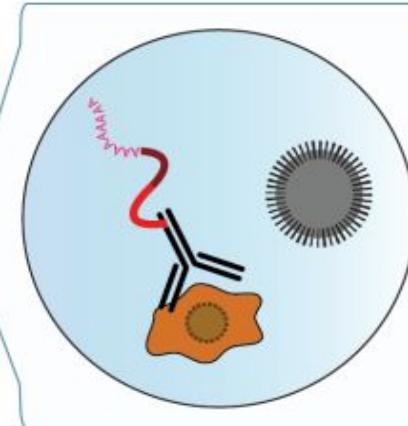
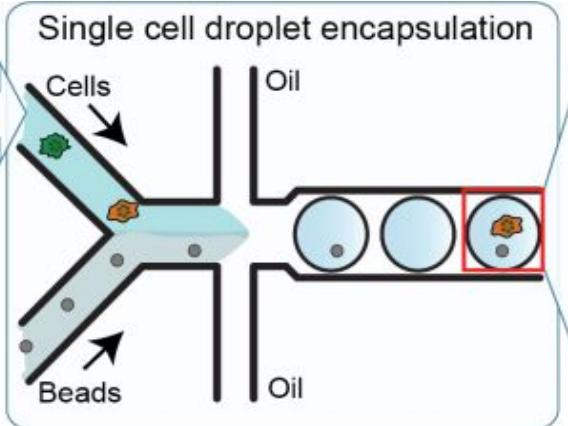
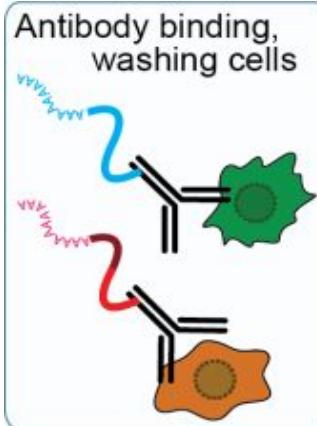
Alternative isolation method :

IBSCI™ (Image Based Single Cell Isolation)

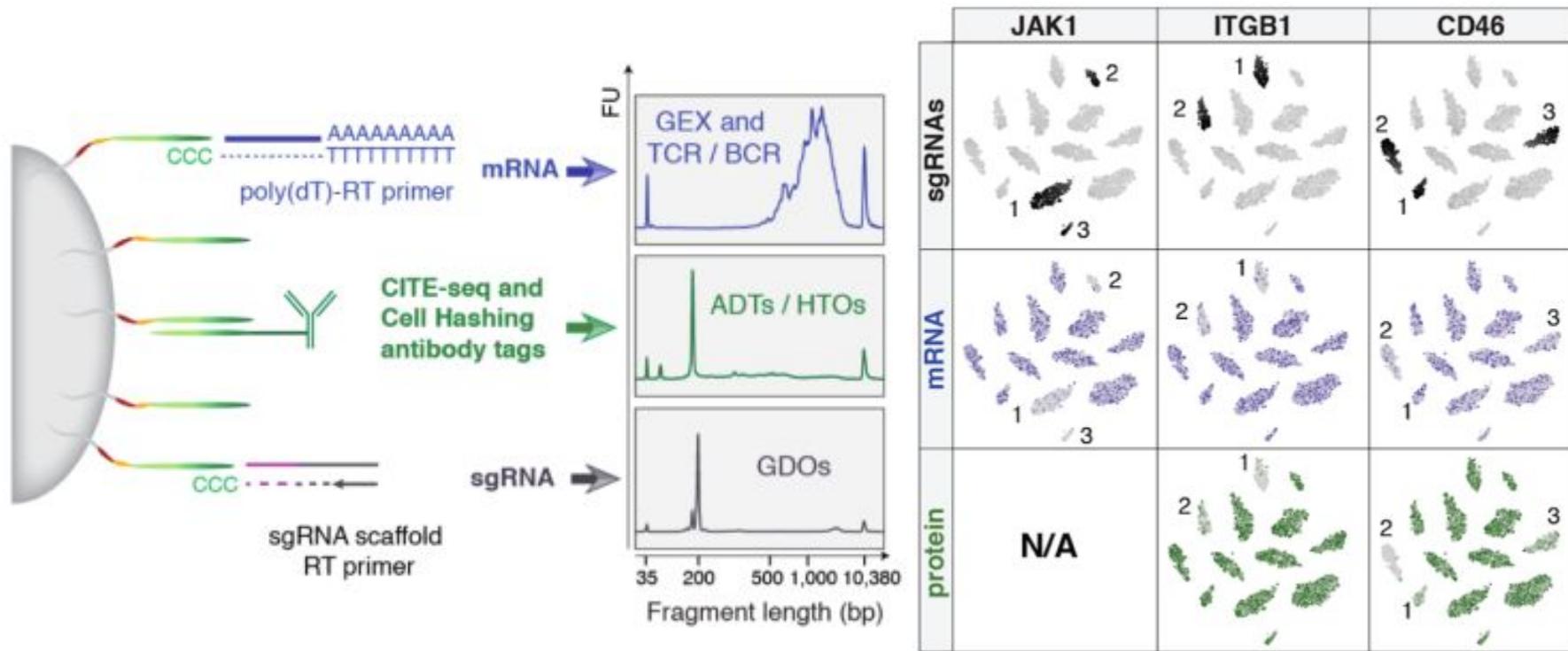
- Capillary real-time video recording :
 - Cell or no cell ?
 - More than 1 cell ?
 - Cell size ?
- Acoustic dispersion (more gentle)
- Middle scale :
 - Plate-based
 - Up to 1536 cells
- Cell recovery rate over 95%
- Open platform
 - Scalable, compatible
 - Custom reaction kits



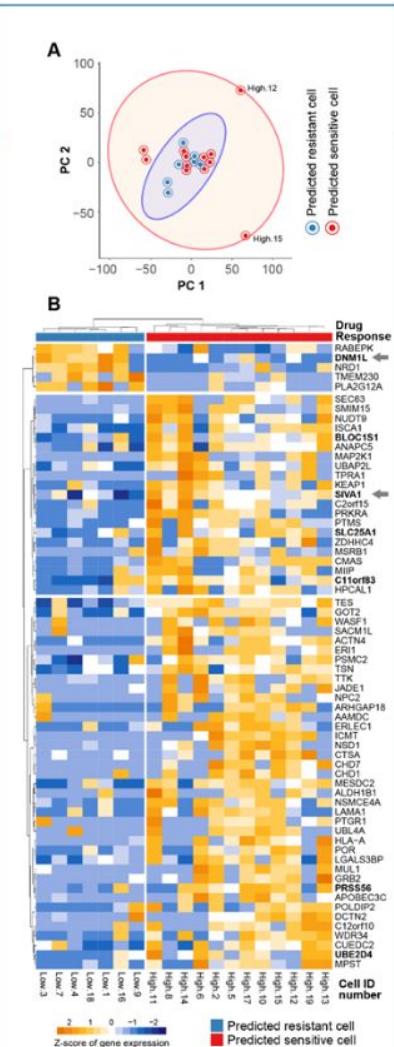
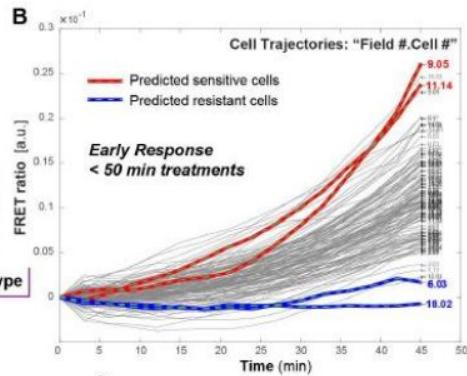
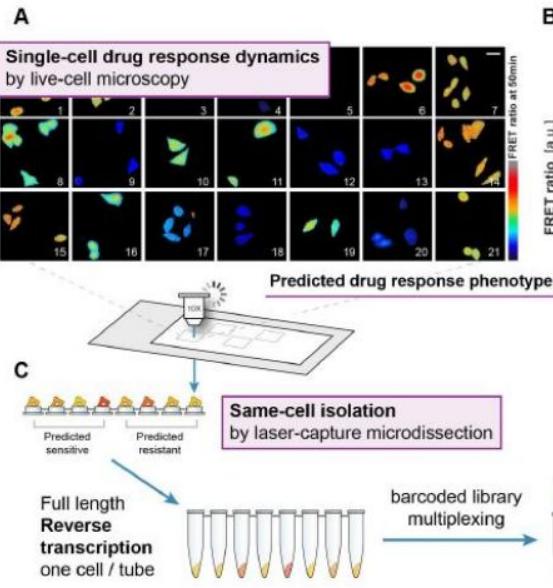
CITEseq : Cellular Indexing of Transcriptomes and Epitopes by Sequencing (scRNAseq + membrane proteins)



ECCITEseq : Extended CRISPR-compatible Cellular Indexing of Transcriptomes and Epitopes by Sequencing (5') (CITEseq + CRISPR/Cas9 gDNA)

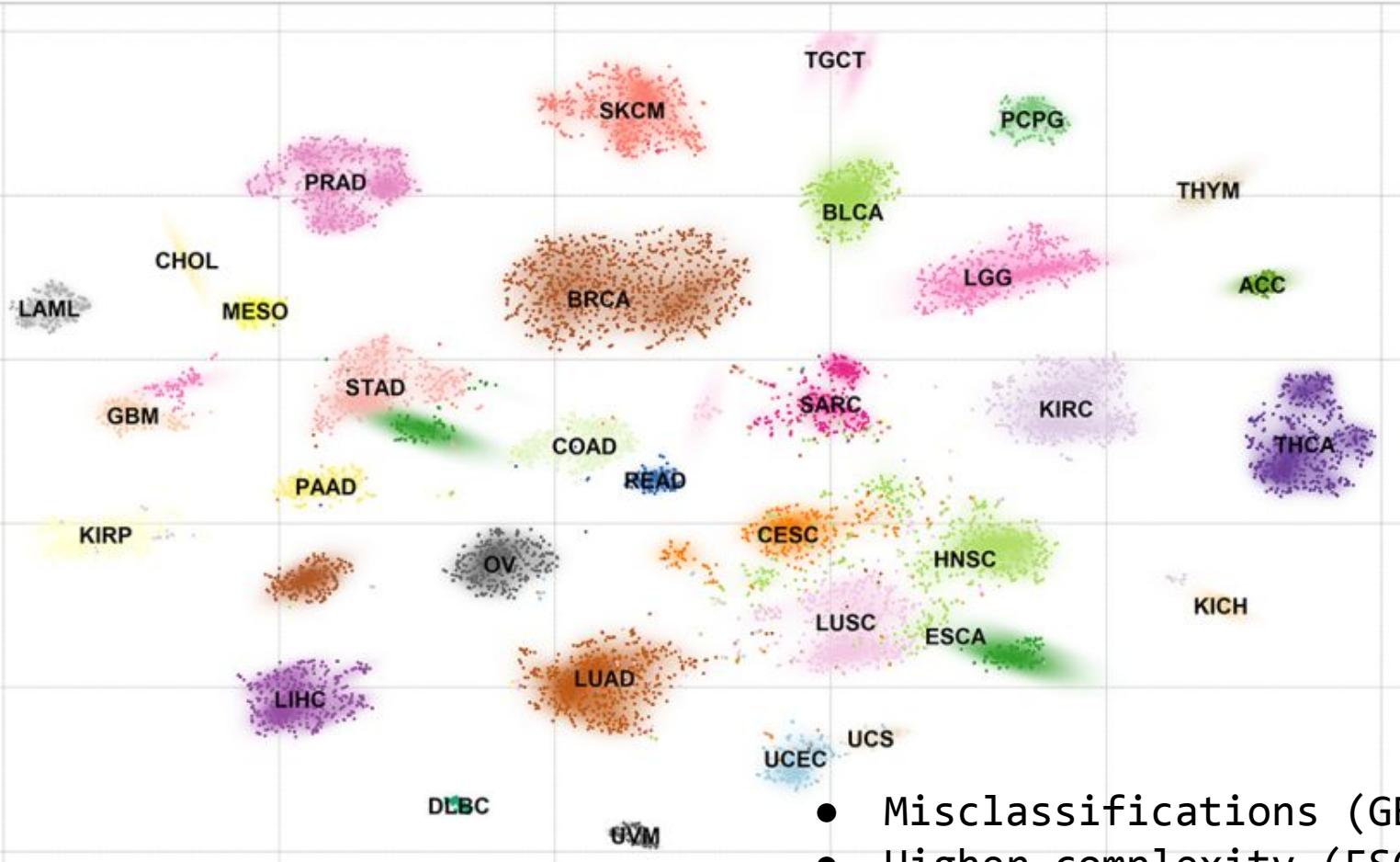


Live cell imaging + scRNA-seq



Musings

t-SNE of the whole TCGA project (*not SC*)



- Misclassifications (GBM-LGG)
- Higher complexity (ESCA-STAD)

Single cell results and the community

TYPES OF SINGLE-CELL SEQUENCING PAPER

