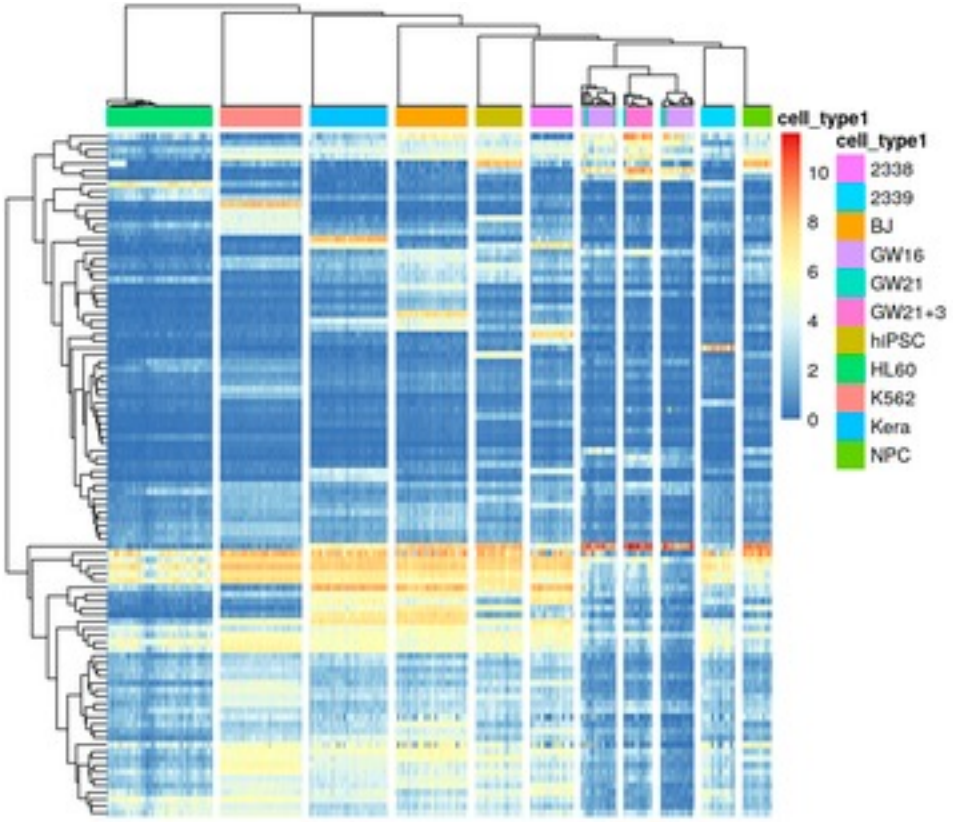
Analysis of single-cell ATAC-seq data

Orr Ashenberg, Jacques Serizay

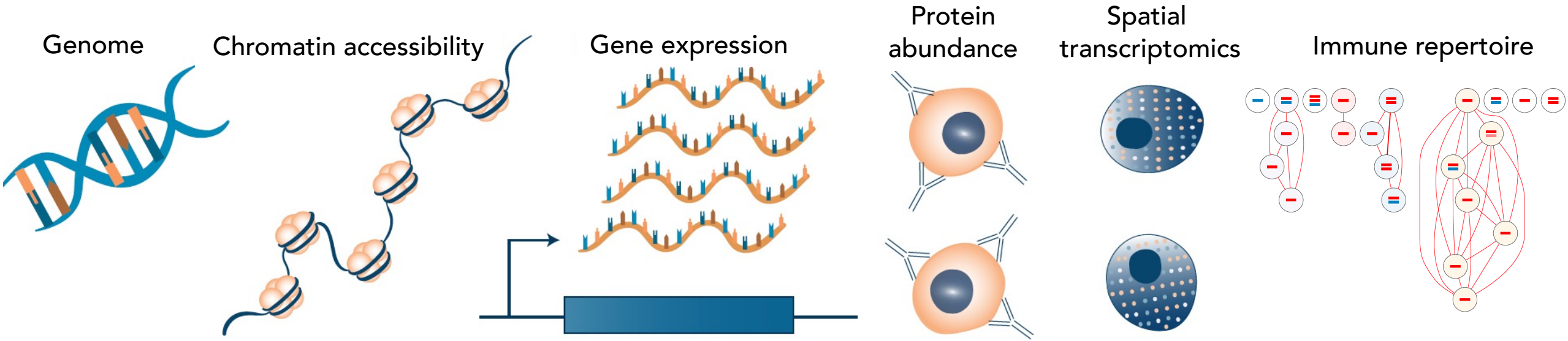
June, 2023

Physalia

Courses



Multimodal measurements

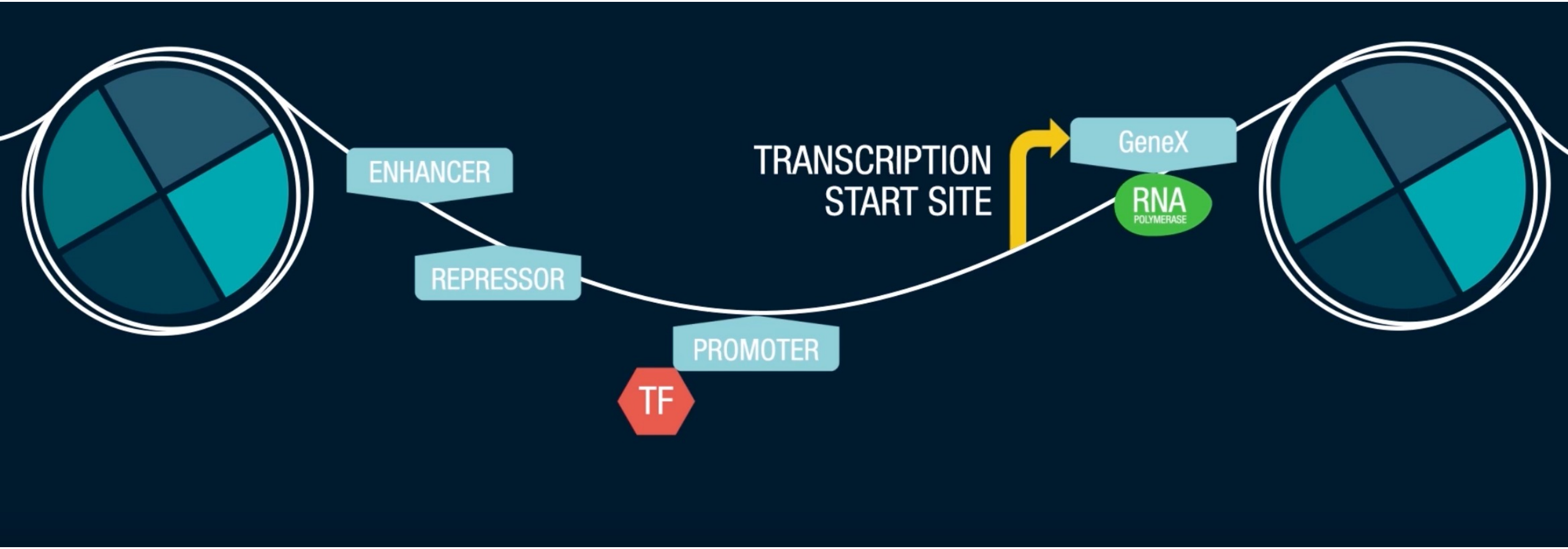


Combining single-cell transcriptomic measurements with other data modalities can reveal gene function and gene regulation.

Efremova M. et al. (2020) *Nature Methods.* 17:11-20.

ATAC-Seq detects accessible chromatin regions

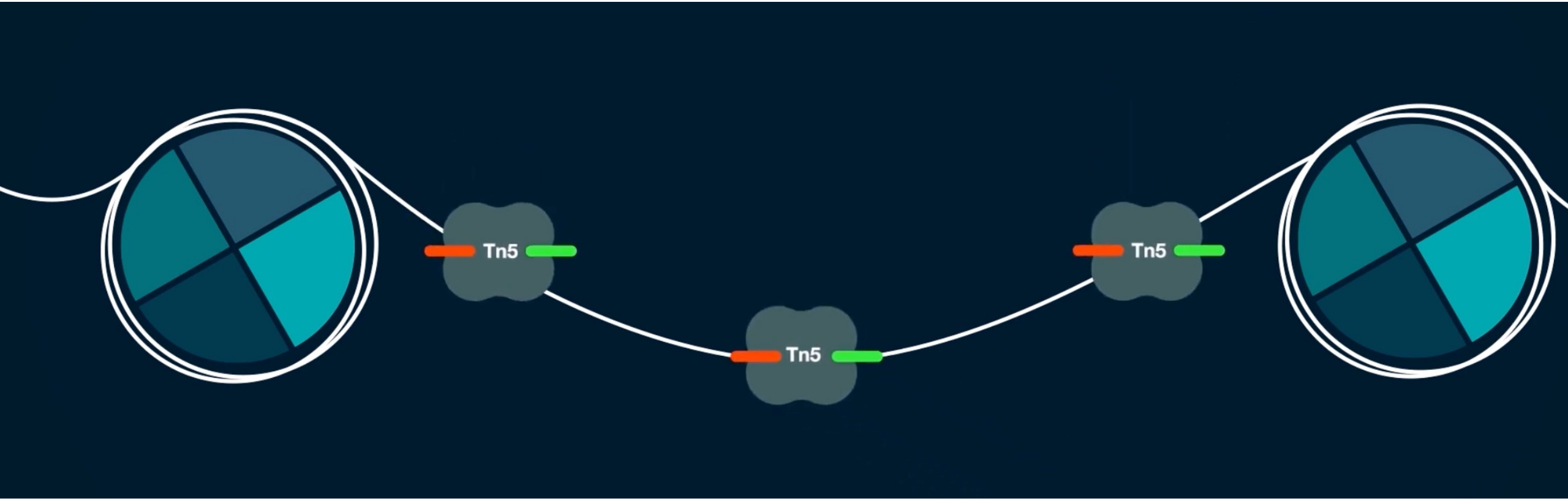
In the cell nucleus, the chromosomes contain tightly packed chromatin material. Part of the chromatin is open and accessible to many regulatory factors who control the expression and suppression of a variety of genes.



"How Single-Cell ATAC-Seq Works", Bio-Rad Laboratories

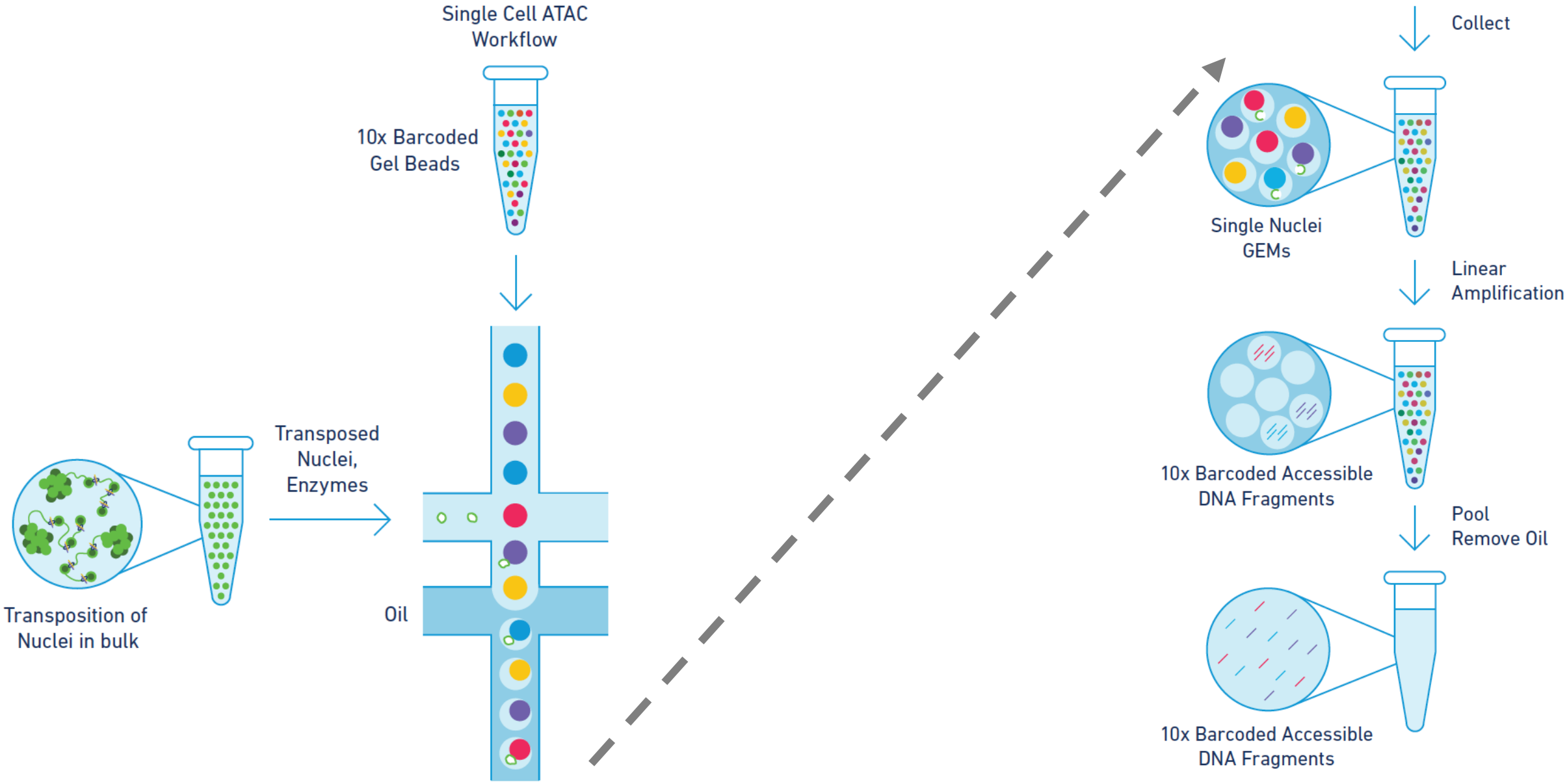
ATAC-Seq detects accessible chromatin regions

ATACseq (as well as scATACseq) measures how open this piece of DNA is. This openness is a proxy of how easily a transcription factor can bind these parts of the genome. ATACseq measures by using an enzyme called Tn5 transposase which binds open chromatin and inserts DNA sequencing adapters.



"How Single-Cell ATAC-Seq Works", Bio-Rad Laboratories

Chromium Single Cell ATAC-Seq (10x)



Single cell resolution reveals cell-type specific regulatory elements

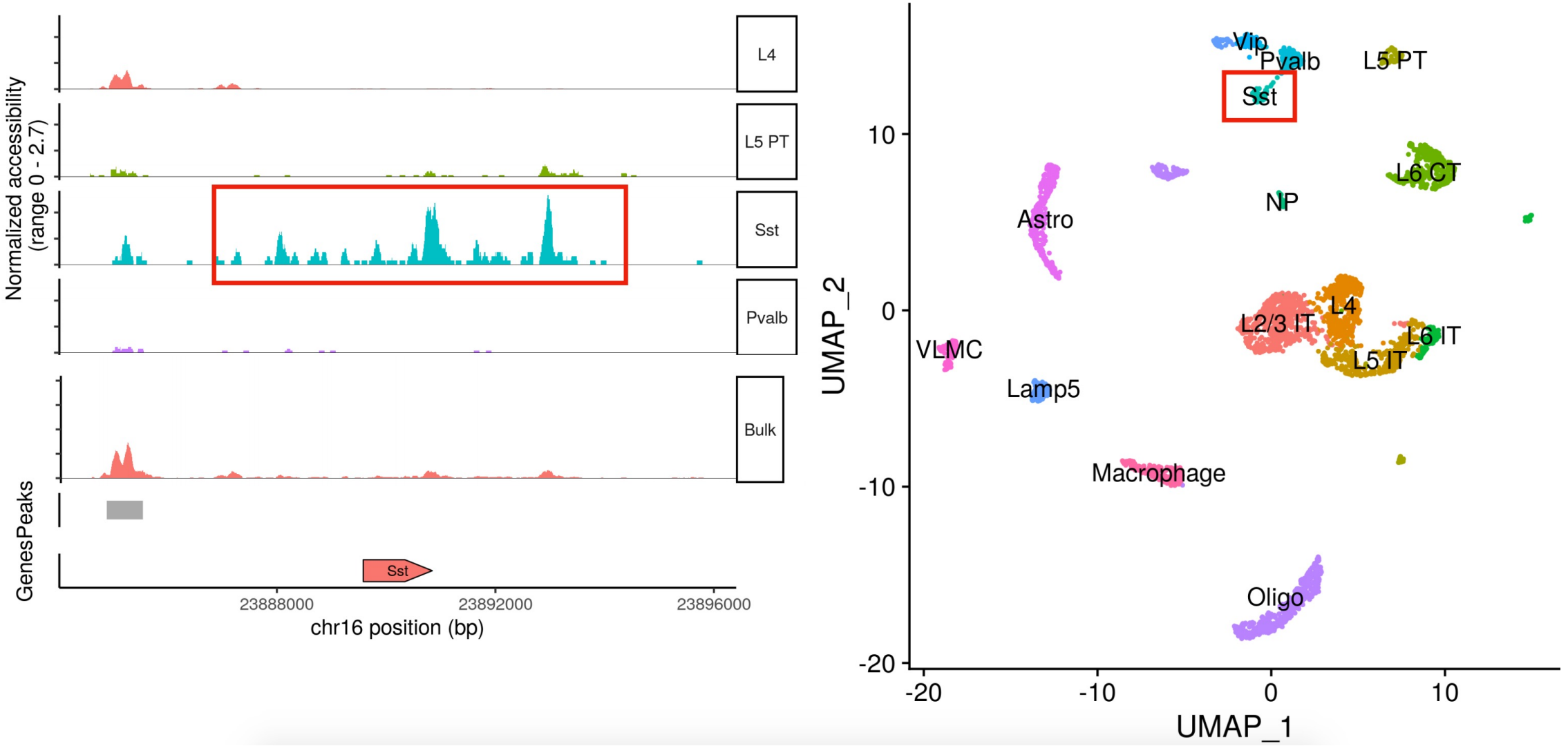


Figure adopted from "Analyzing single-cell ATAC-seq datasets" lecture by Tim Stuart

Pre-processing generates a fragment file and a peak/cell matrix

A full list of **all** unique fragments across all single cells, as opposed to only reads that map to peaks.

1. Indexed fragment file

chrom	start	stop	barcode	reads
chr1	3000141	3000517	GGTTGCGAGCCGCAAA-1	3
chr1	3000159	3000373	CTCAGCTAGTGTCACT-1	1
chr1	3000431	3000621	GAAGTCTGTAACACTC-1	1

2. Large sparse matrix

	AAACGAAAGAGTTTGA-1	AAACGAAAGCGAGCTA-1
chr1:565107-565550	.	.
chr1:569174-569639	.	.
chr1:713460-714823	.	2
chr1:752422-753038	.	.
chr1:762106-763359	.	4

Each value in the matrix represents the number of Tn5 cut sites for each single barcode (i.e. cell) that map within each peak

Figure adopted from "Analyzing single-cell ATAC-seq datasets" lecture by Tim Stuart

scATAC-Seq data is highly sparse

1. Indexed fragment file

chrom	start	stop	barcode	reads
chr1	3000141	3000517	GGTTGCGAGCCGCAAA-1	3
chr1	3000159	3000373	CTCAGCTAGTGTCACT-1	1
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2. Large sparse matrix

	AAACGAAAGAGTTTGA-1	AAACGAAAGCGAGCTA-1
chr1:565107-565550	.	.
chr1:569174-569639	.	.
chr1:713460-714823	.	2
chr1:752422-753038	.	.
chr1:762106-763359	.	4

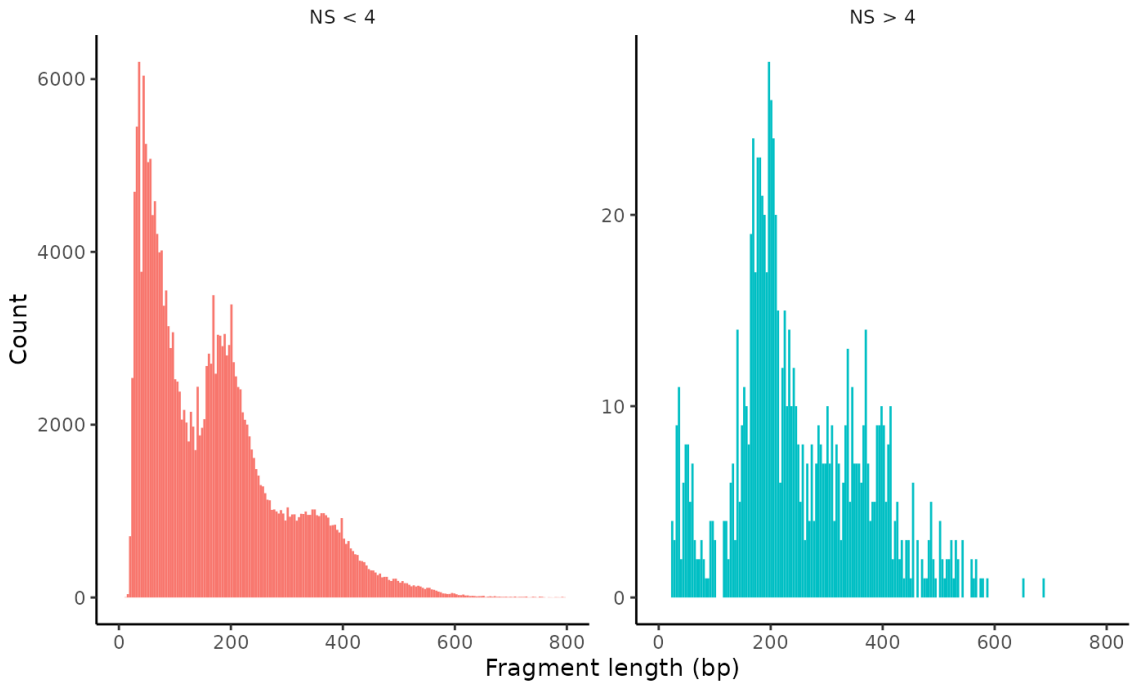
Challenges in comparison to scRNA:

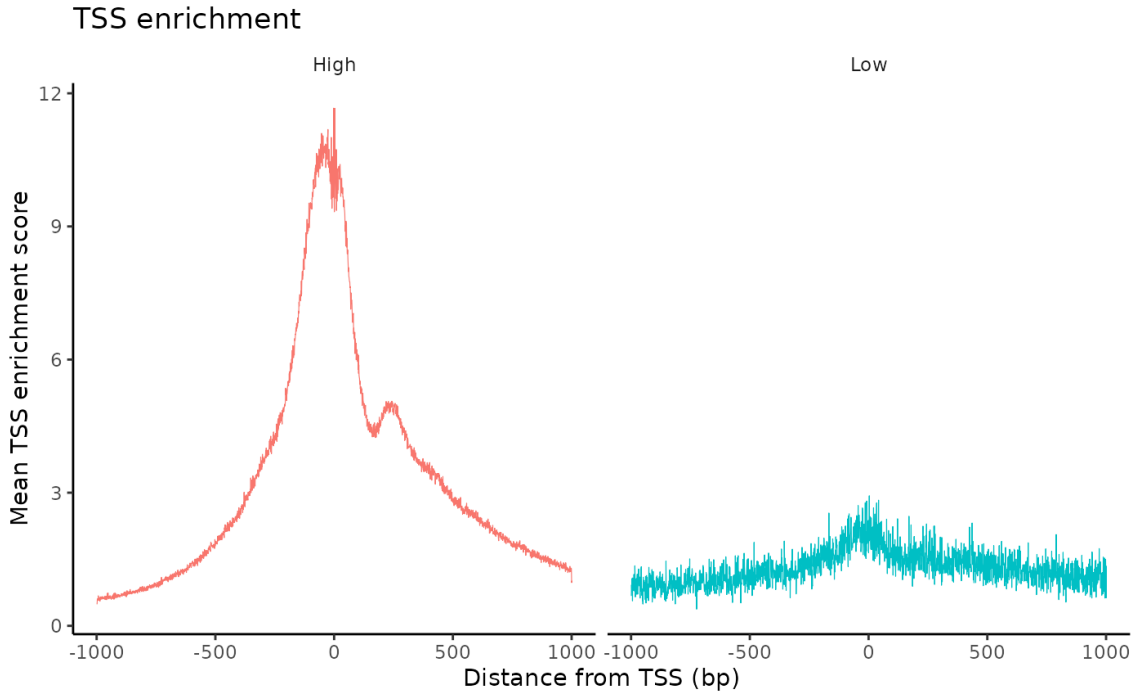
1. More sparse
2. Near-binary data
3. Non-fixed feature set
4. Order of magnitude more features

Figure adopted from "Analyzing single-cell ATAC-seq datasets" lecture by Tim Stuart

Quality control metrics for scATAC-seq data

1. Nucleosome banding pattern
2. Transcriptional start site (TSS) enrichment
3. Total number of fragments in peaks
4. Fraction of fragments in peaks





Overview of scATAC-Seq analysis

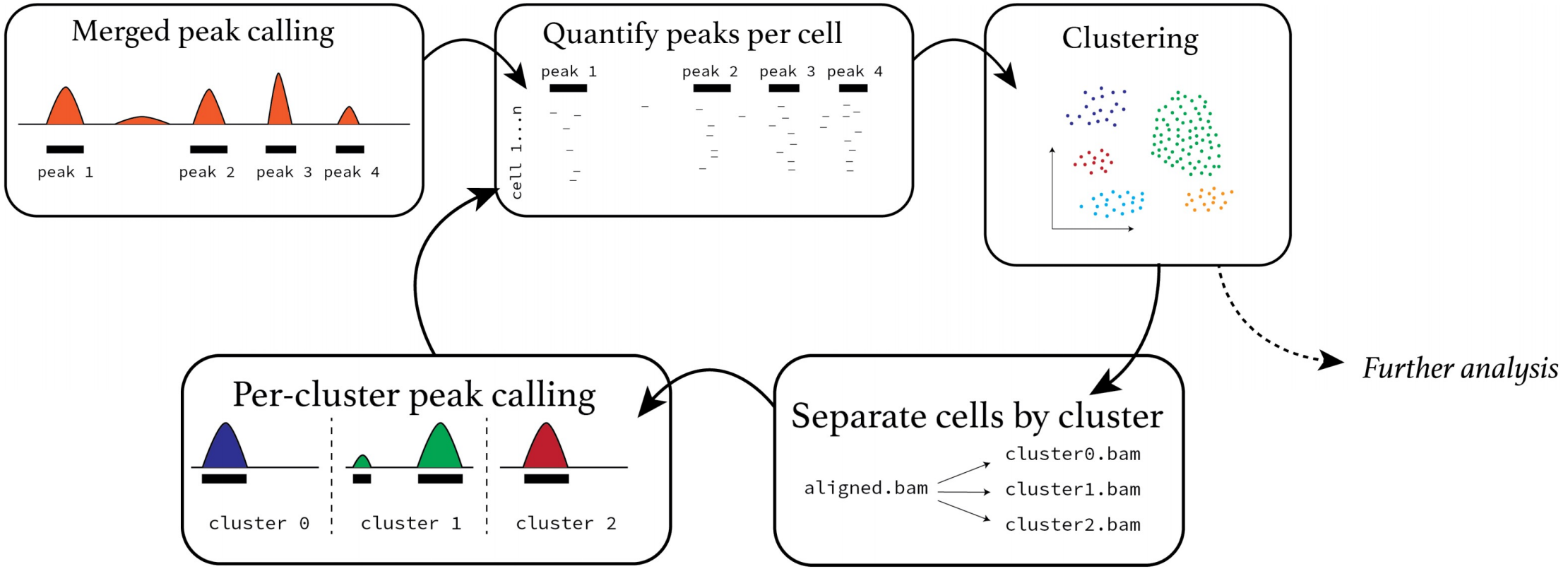


Figure adopted from "Analyzing single-cell ATAC-seq datasets" lecture by Tim Stuart

scATAC-Seq often uses latent semantic indexing (LSI) for dimensionality reduction

- Originally developed for topic modeling / natural language processing (Deerwester et al. 1990) and first applied to scATAC-Seq in 2015 (Cusanovich et al. *Science*)

- term frequency-inverse document frequency (TF-IDF) normalization followed by singular value decomposition (SVD)

- Cell = document and peak = term

```
pbmc <- RunTFIDF(pbmc)
pbmc <- FindTopFeatures(pbmc, min.cutoff = 'q0')
pbmc <- RunSVD(pbmc)
```

- Term frequency: normalize across cells to correct for differences in sequencing depth

- Inverse document frequency: give higher values to more rare peaks

Figure adopted from "Analyzing single-cell ATAC-seq datasets" lecture by Tim Stuart

Visualize clusters in scATAC-Seq

```
pbmc <- RunUMAP(object = pbmc, reduction = 'lsi', dims = 2:30)
pbmc <- FindNeighbors(object = pbmc, reduction = 'lsi', dims = 2:30)
pbmc <- FindClusters(object = pbmc, verbose = FALSE, algorithm = 3)
DimPlot(object = pbmc, label = TRUE) + NoLegend()
```

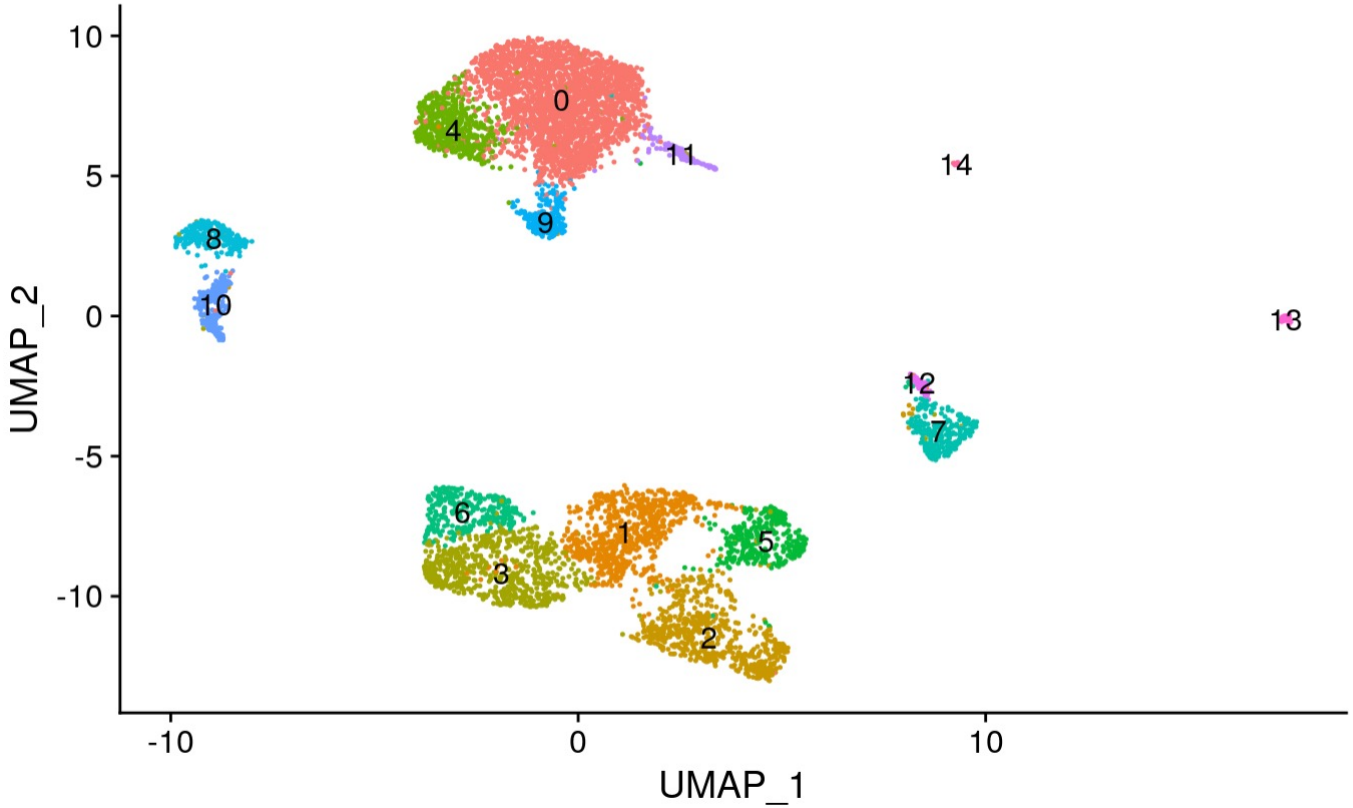


Figure adopted from signac tutorial, https://satijalab.org/signac/articles/pbmc_vignette.html

Fragment file helps infer gene "activity" and annotate clusters

Quantify the activity of each gene in the genome by assessing the chromatin accessibility associated with each gene: count the number of fragments for each cell that map to the promoter + gene body

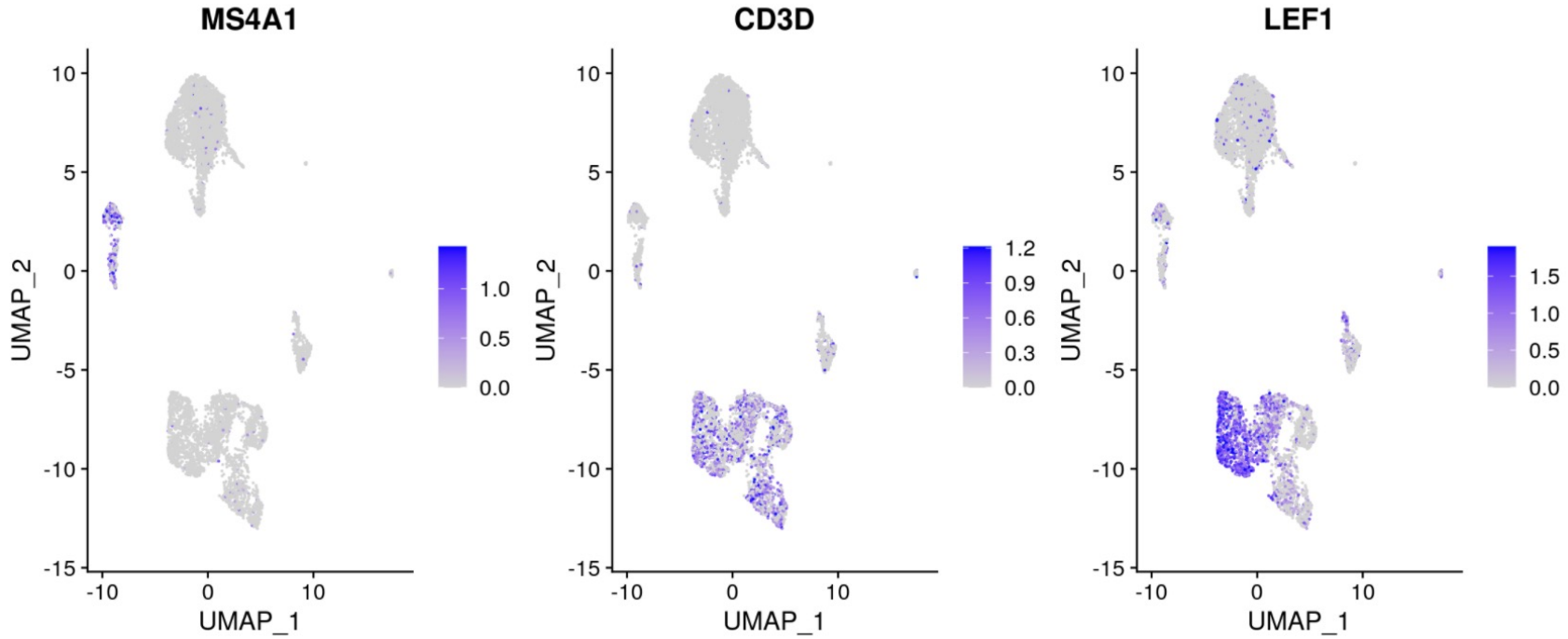
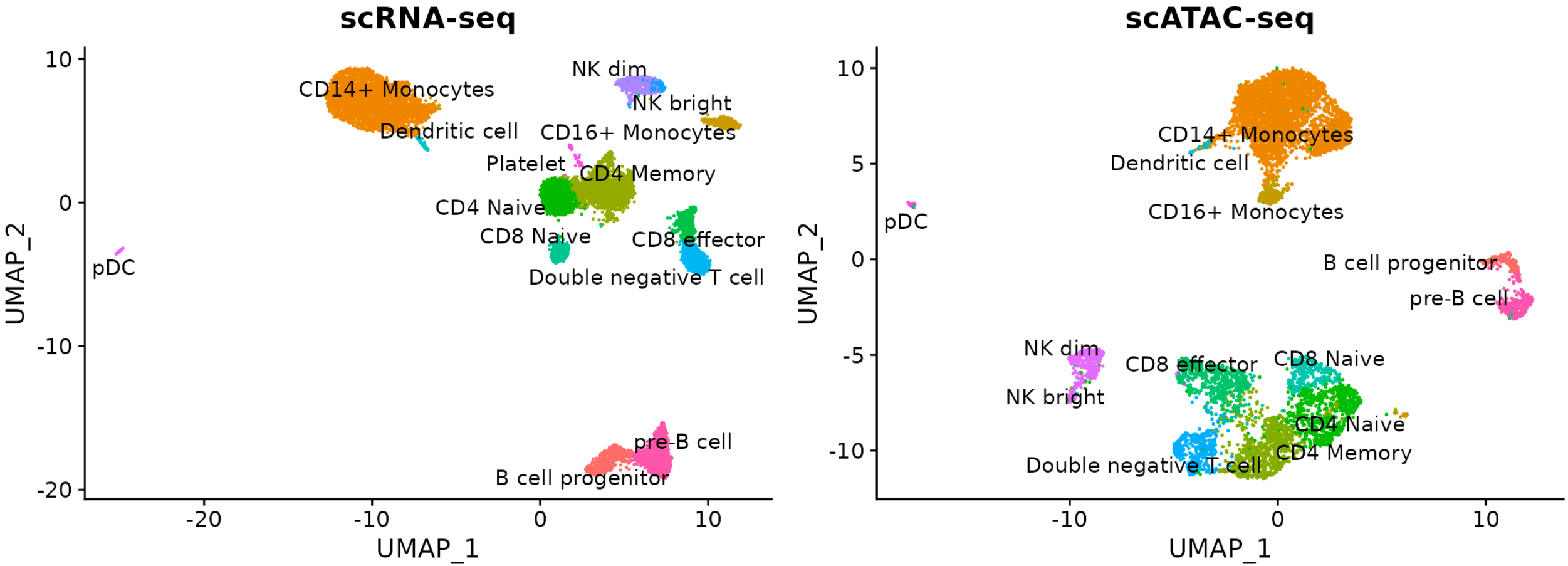


Figure adopted from signac tutorial, https://satijalab.org/signac/articles/pbmc_vignette.html

Integrating with scRNA-seq data using CCA + MNN (Seurat v4)



Critical assumption: there is generally a positive correlation between chromatin accessibility and gene expression!!!!

Figure adopted from signac tutorial, https://satijalab.org/signac/articles/pbmc_vignette.html

Finding overrepresented motifs

To identify potentially important cell-type-specific regulatory sequences, signac searches for DNA motifs that are overrepresented in a set of peaks that are differentially accessible between cell types.

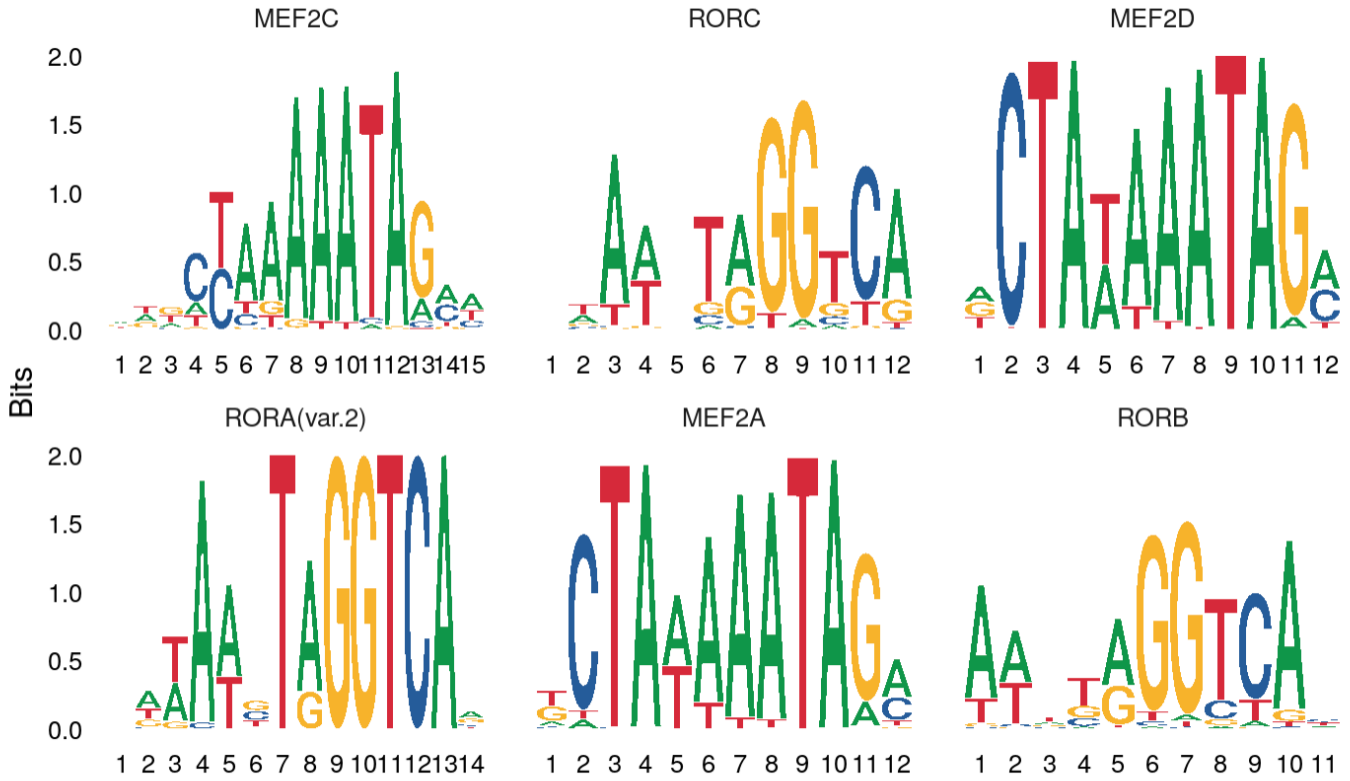


Figure adopted from signac tutorial, https://satijalab.org/signac/articles/pbmc_vignette.html

Computing motif activities

ChromVAR identifies motifs associated with variability in chromatin accessibility between cells.

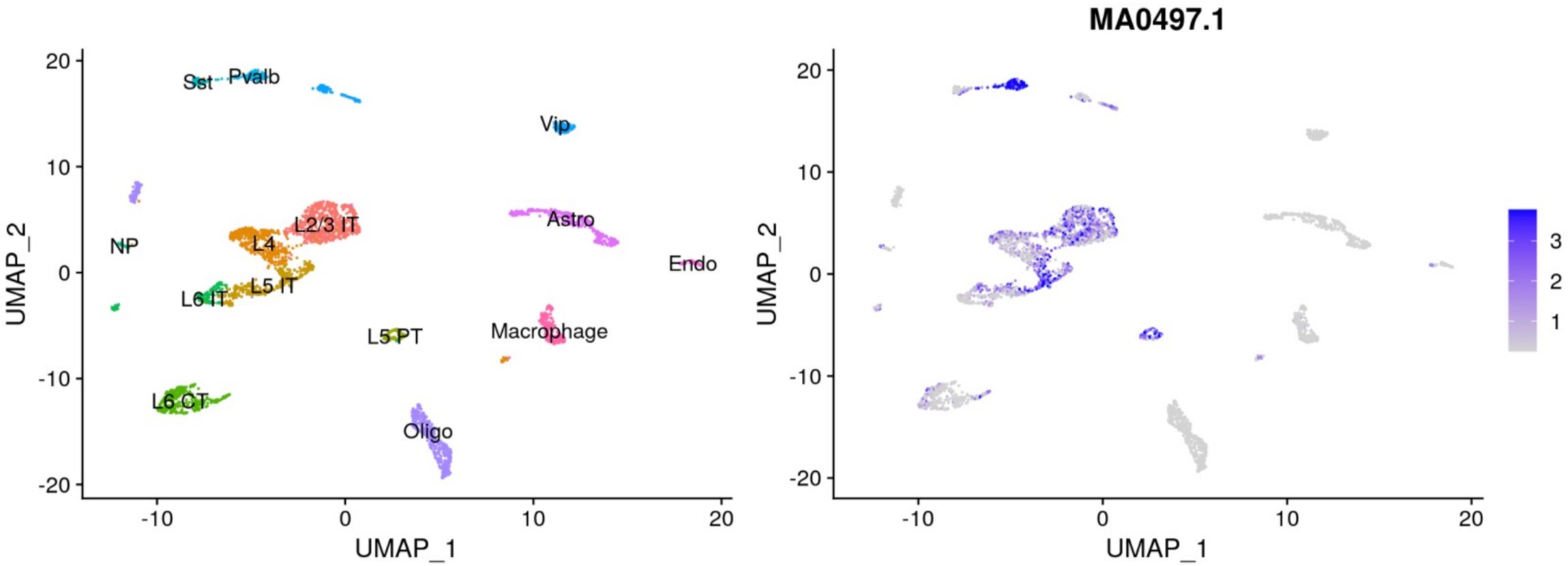


Figure adopted from signac tutorial, https://satijalab.org/signac/articles/pbmc_vignette.html

Software for scATAC-Seq analysis

Signac	*https://satijalab.org/signac/index.html*
SnapATAC	*https://github.com/r3fang/SnapATAC*
ArchR	*https://www.archrproject.com/*
cisTopic	*https://github.com/aertslab/cisTopic*
chromVAR	*https://github.com/GreenleafLab/chromVAR*
CICERO	*https://cole-trapnell-lab.github.io/cicero-release/*

Software for scATAC-Seq analysis

	ArchR	Signac	SnapATAC	
Pre-processing	NR	NA	✓	Data Import
Data import / base file type creation	✓	NA	✓	
QC filter cells	✓	✓	✓	
Matrix creation	✓ (Tile)	✓ (Peak)	✓ (Tile)	
Doublet removal	✓	NP	NP	Doublet Removal
Data imputation with MAGIC	✓	NP	✓	Gene Scores
Genome-wide gene score matrix	✓	✓	✓	
Dimensionality reduction and clustering	✓	✓	✓	Clustering
UMAP and tSNE plotting	✓	✓	✓	
Cluster peak calling	✓	NP	✓	Standard ATAC-seq Analyses
Cluster-based peak matrix creation	✓	NP	✓	
Motif enrichment	✓	✓	✓	
chromVAR motif deviations	✓	✓	✓	
Footprinting	✓	NP	NP	
Feature set annotation	✓	NP	NP	
Track plotting	✓	✓	NP	Data Visualization
Co-accessibility	✓	NP	NP	
Interactive genome browser	✓	NP	NP	
Cellular trajectory analysis	✓	NP	NP	Advanced ATAC-seq Analyses
Project bulk data into scATAC embedding	✓	NP	NP	
Integration of RNA-seq and ATAC-seq	✓	✓	✓	Integration of RNA-seq and ATAC-seq
Genome-wide peak-to-gene links	✓	NP	NP	

NR = Not Required NA = Not Applicable NP = Not Possible

Some approaches to multiome data (scRNA-seq and scATAC-seq)

ArchR: https://greenleaflab.github.io/ArchR_2020/Ex-Analyze-Multiome.html

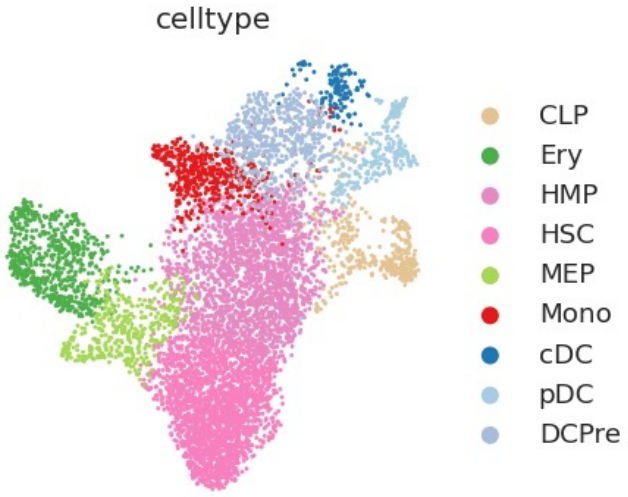
Signac (same people who built Seurat): https://satijalab.org/signac/articles/pbmc_multiomic.html

Methods to overcome sparsity in ATAC-seq data

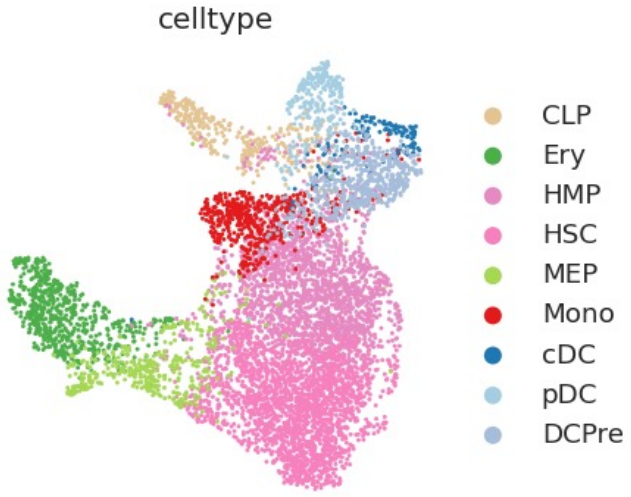
Computing meta-cells is on methodology used to overcome sparsity in scATAC-seq data

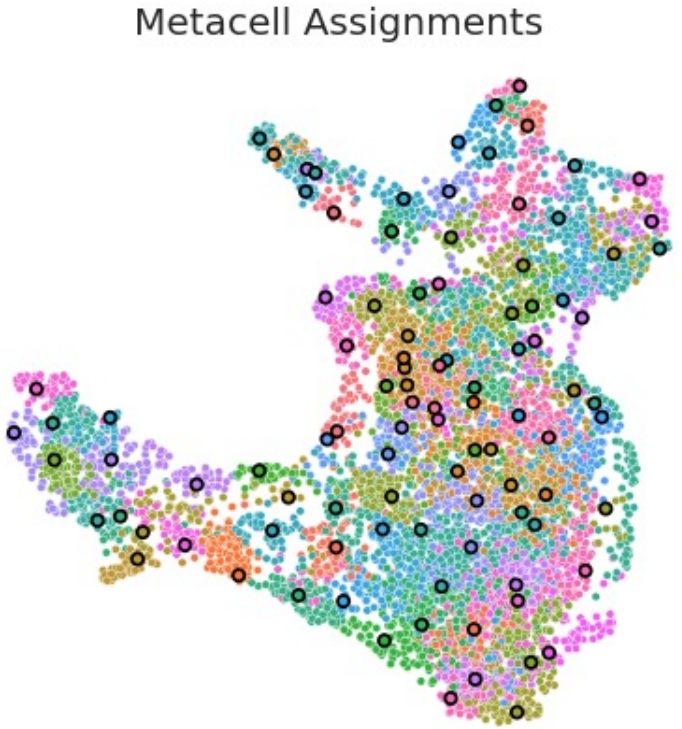
Computing meta-cells (e.g. SEACells algorithm) can improve computation of peak-gene associations

scRNA-seq



scATAC-seq





Methods to overcome sparsity in ATAC-seq data

Generate summarized ATAC and RNA metacells

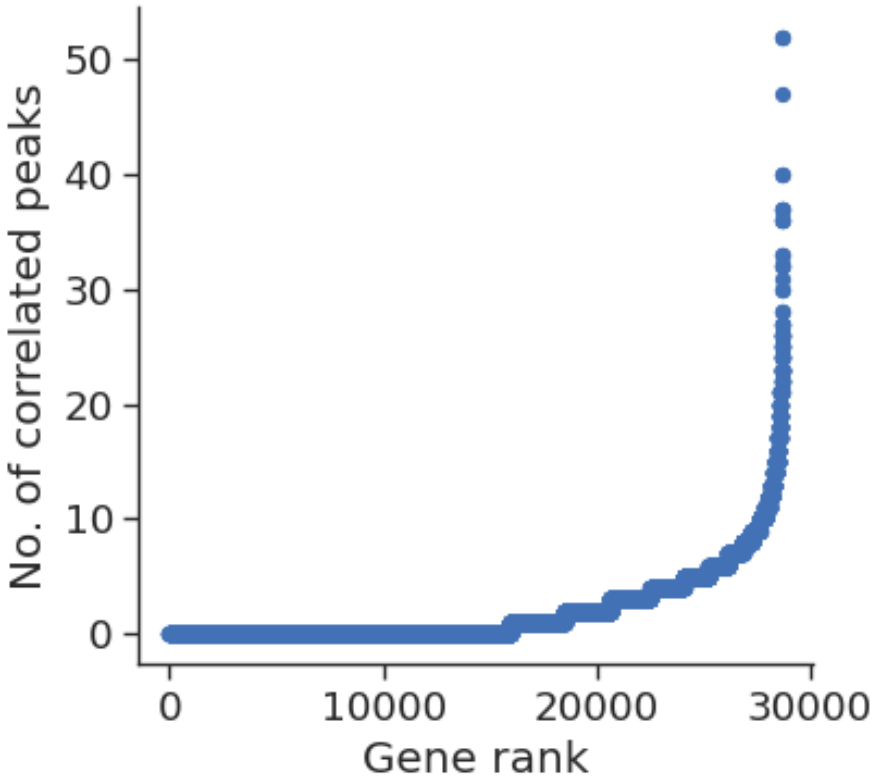
Used the paired multiome metacells to compute the correlation of gene expression and accessibility of peaks within the vicinity of the gene

Pick out highly regulated genes that are correlated with multiple peaks

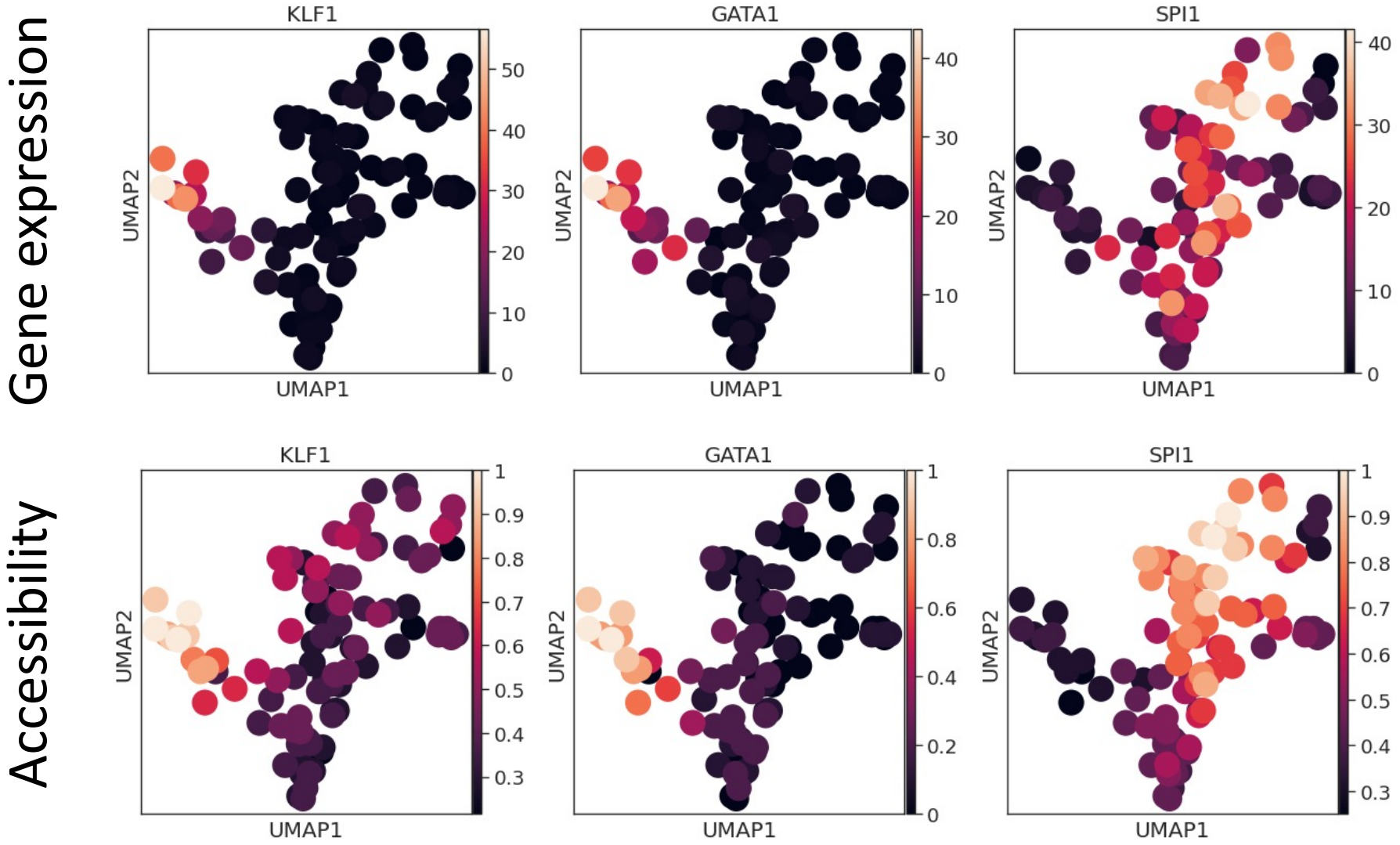
Calculate gene scores as the weighted sum of the accessibility of correlated peaks

To calculate gene accessibility metrics identify the subset of peaks that are open in each metacell

Open peaks are used to compute a gene accessibility metric which represents the fraction of correlated open peaks

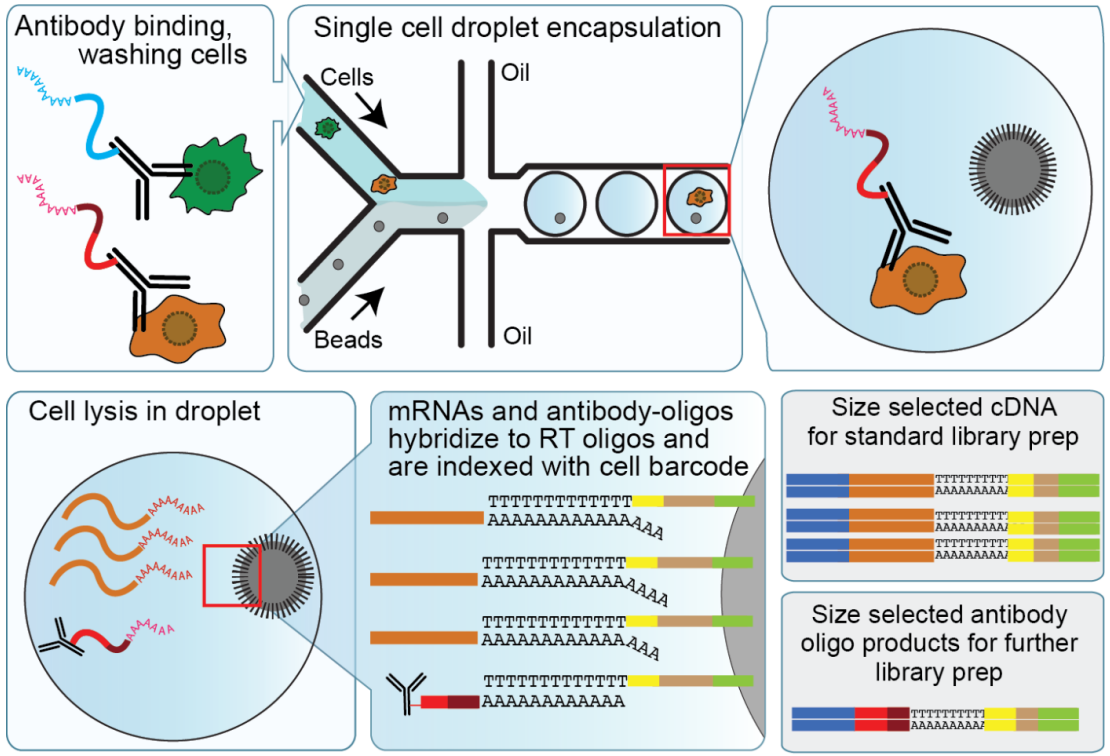


Methods to overcome sparsity in ATAC-seq data



APPENDIX: OTHER MULTIMODAL METHODOLOGIES

CITE-Seq allows simultaneous measurement of RNA expression and surface protein expression in same cell

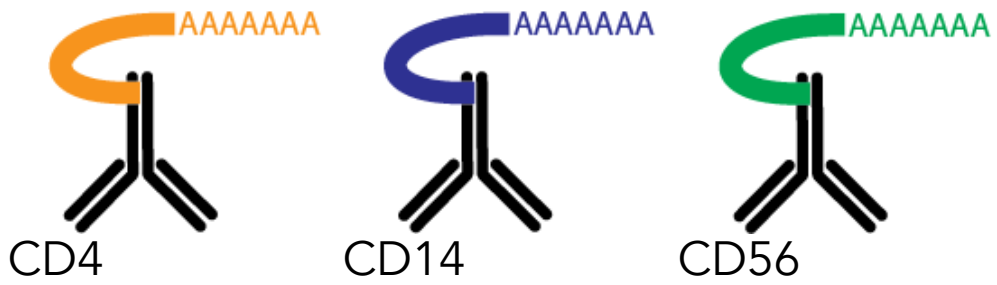


Count matrix1: scRNAseq

Count matrix2: CITEseq

Antibodies are conjugated with DNA-oligos

- DNA-oligo indicates antibody identity and contains poly-A tail



Pre-processing generates RNA count and protein count matrices

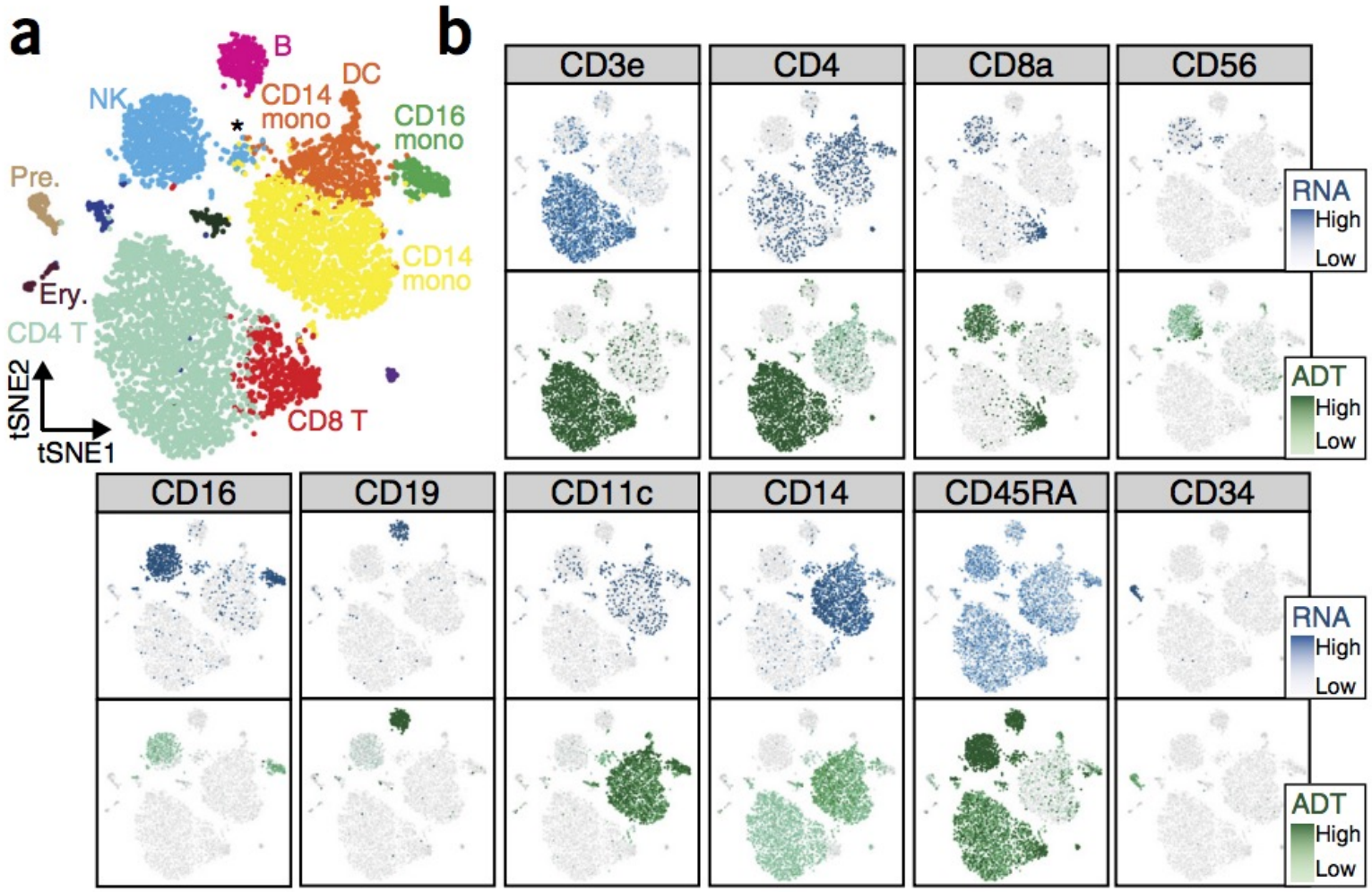
scRNA-Seq

	cell1	cell2	cell3	cell4
Gene1	0	0	0	4
Gene2	0	2	0	0
Gene3	0	0	0	0

CITE-Seq

	cell1	cell2	cell3	cell4
Antibody for CD4	100	0	0	38
Antibody for CD14	0	0	45	0
Antibody for CD56	2	0	0	0

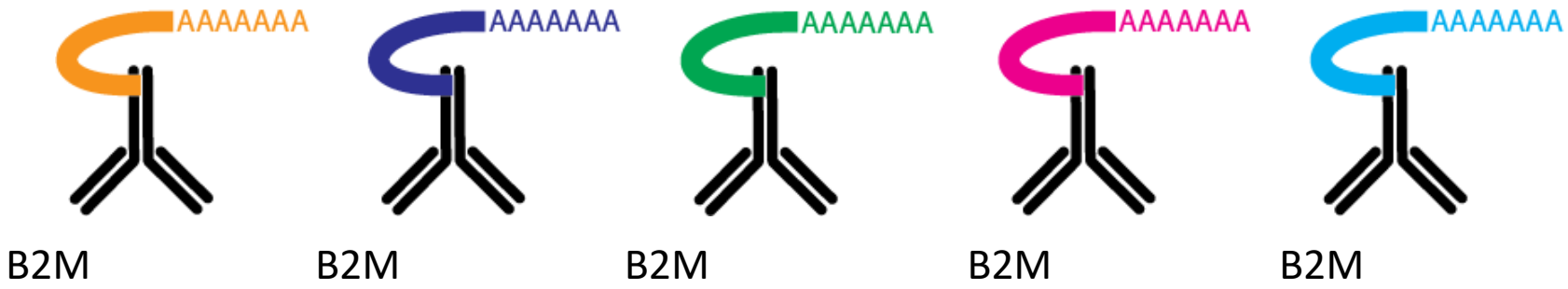
Simultaneous protein and RNA measurement in single cells



Cell hashing using CITE-Seq

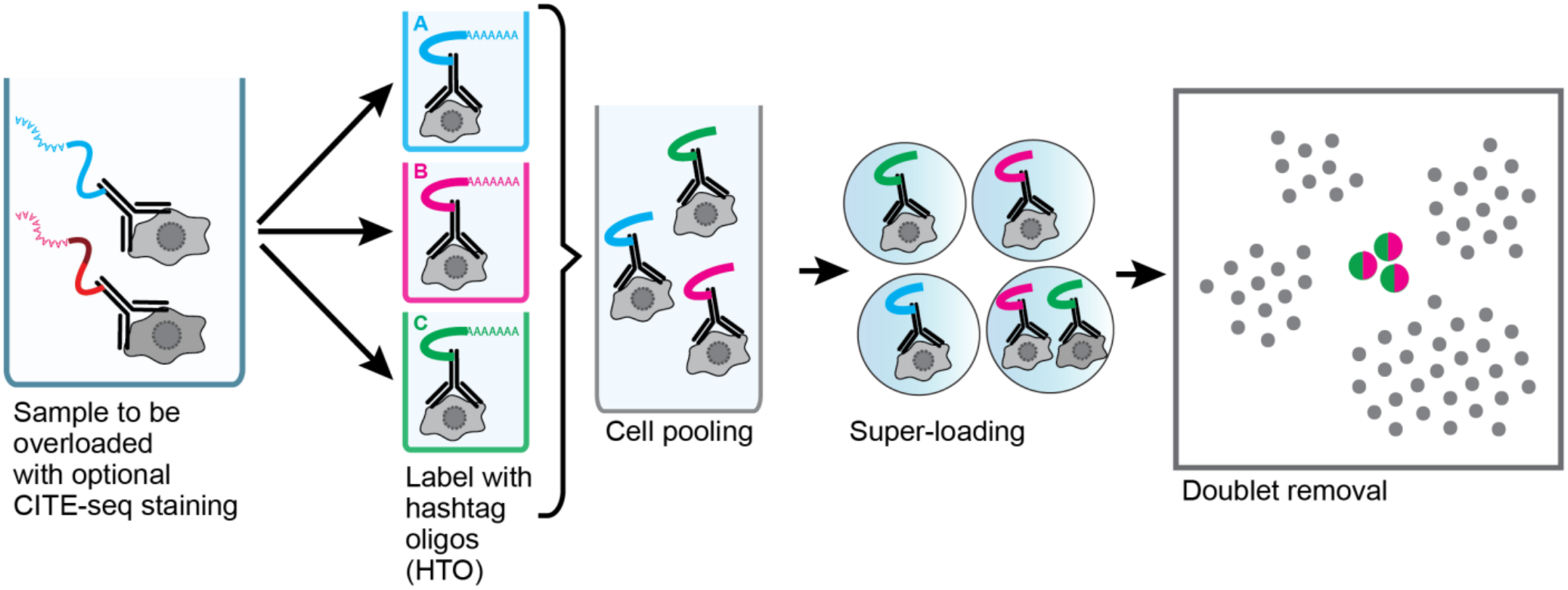
- Cell Hashing enables sample multiplexing and super-loading on single cell RNA-sequencing platforms.

- Cell Hashing uses a series of oligo-tagged antibodies against ubiquitously expressed surface proteins with different barcodes to uniquely label cells from distinct samples, which can be subsequently pooled in one scRNA-seq run.



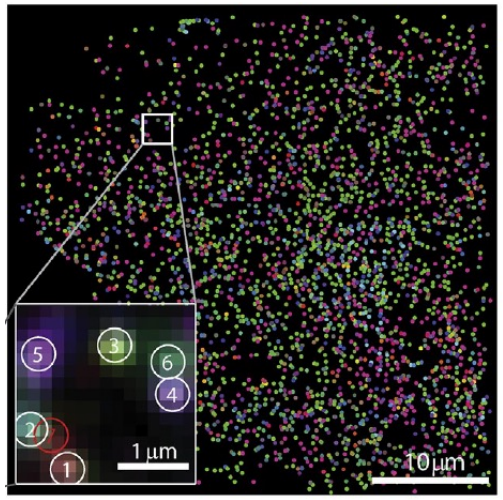
With cell hashing, we can "overload" the 10x channel

By sequencing these tags alongside the cellular transcriptome, we can assign each cell to its sample of origin, and robustly identify doublets originating from multiple samples.



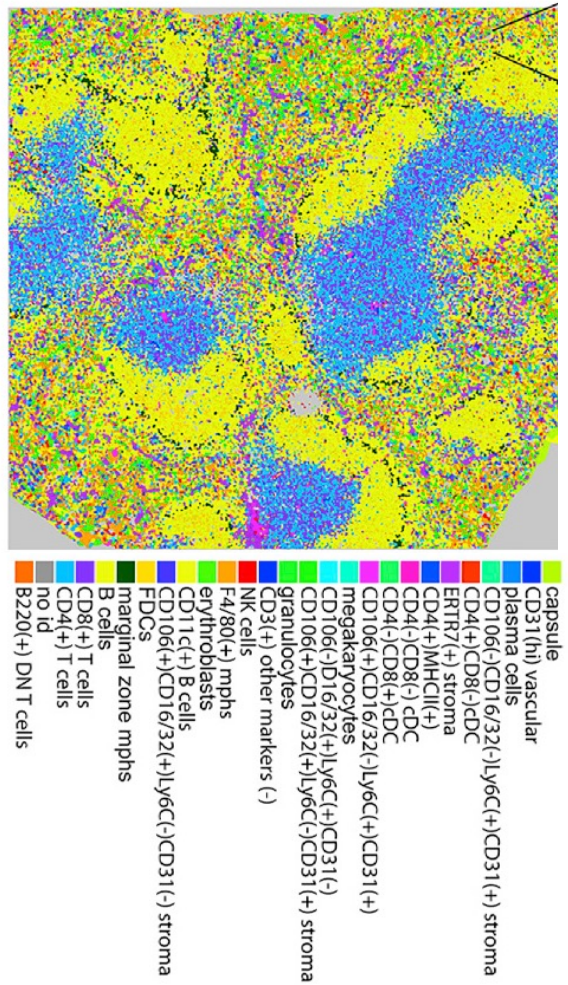
Ongoing developments in single-cell genomics: Growing toolbox for spatial genomics

MERFISH (RNA)



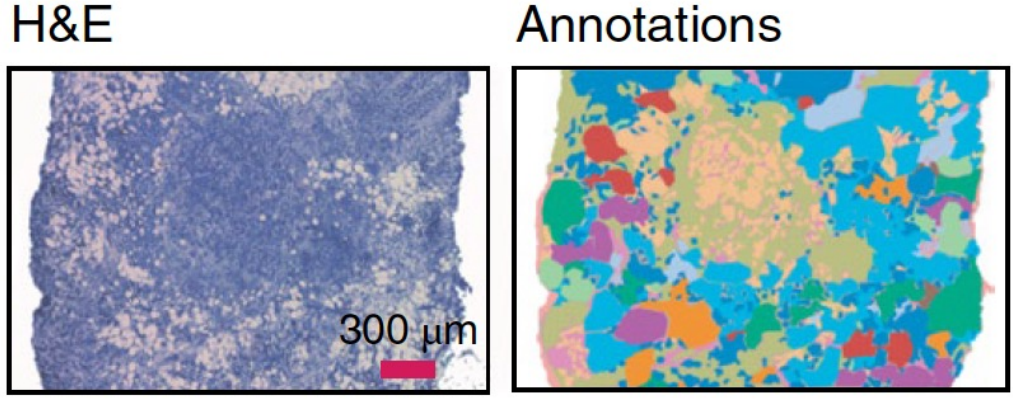
CODEX (protein)

mouse spleen



High Density Spatial Transcriptomics (RNA)

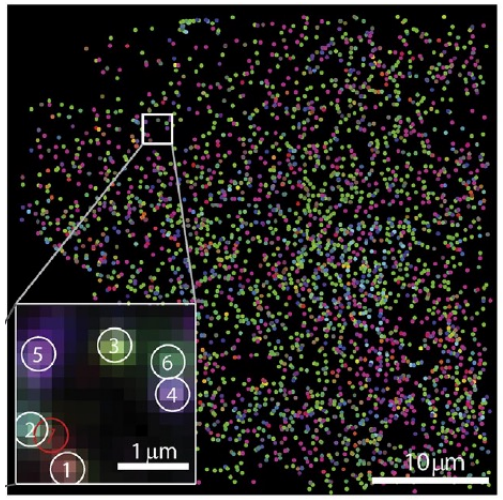
breast cancer



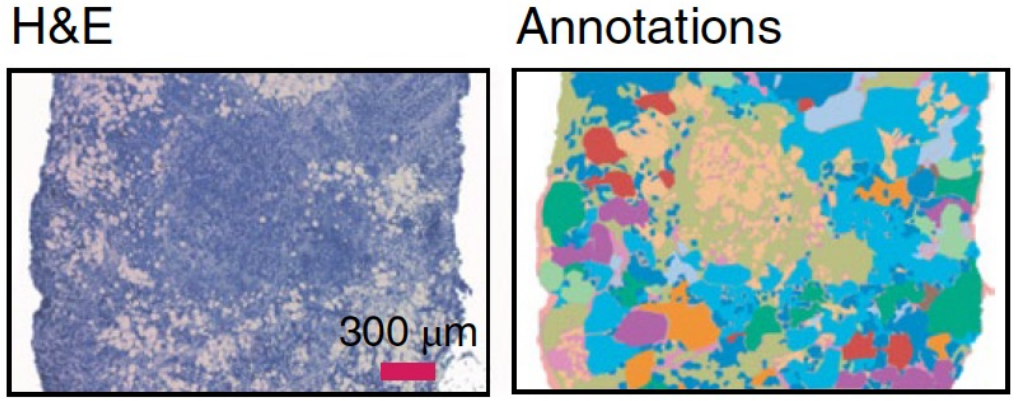
Moffitt J.R. et al. (2016) *PNAS* 113: 11046-11051.
Goltsev Y. et al. (2018) *Cell.* 174: 968-981.
Vickovic S. et al. (2019) *Nature Methods.* 16: 987–990.

Ongoing developments in single-cell genomics: Growing toolbox for spatial genomics

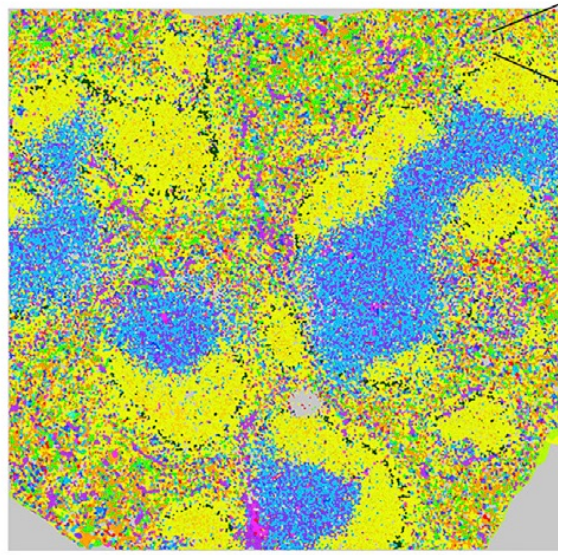
MERFISH (RNA)



High Density Spatial Transcriptomics (RNA)



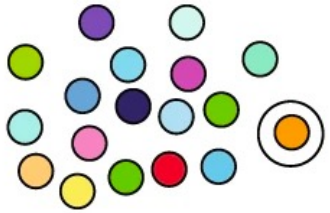
CODEX (protein)

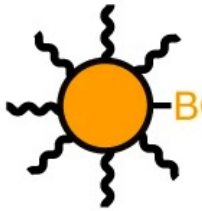


Major method differences

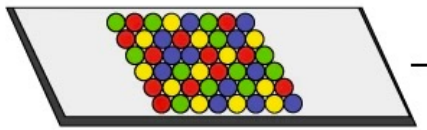
- detecting RNA or protein
- choosing target genes/proteins or full transcriptome
- number of cells profiled
- single-cell or almost single-cell resolution
- *in situ vs* traditional sequencing
- …

Slide-Seq overview (full transcriptome)



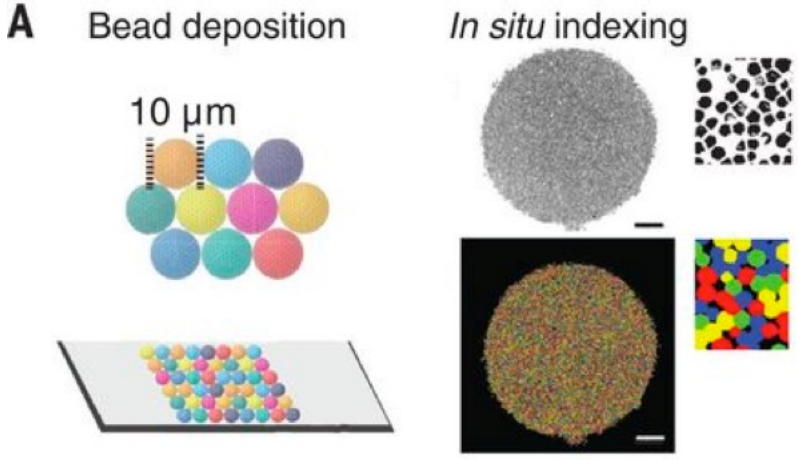


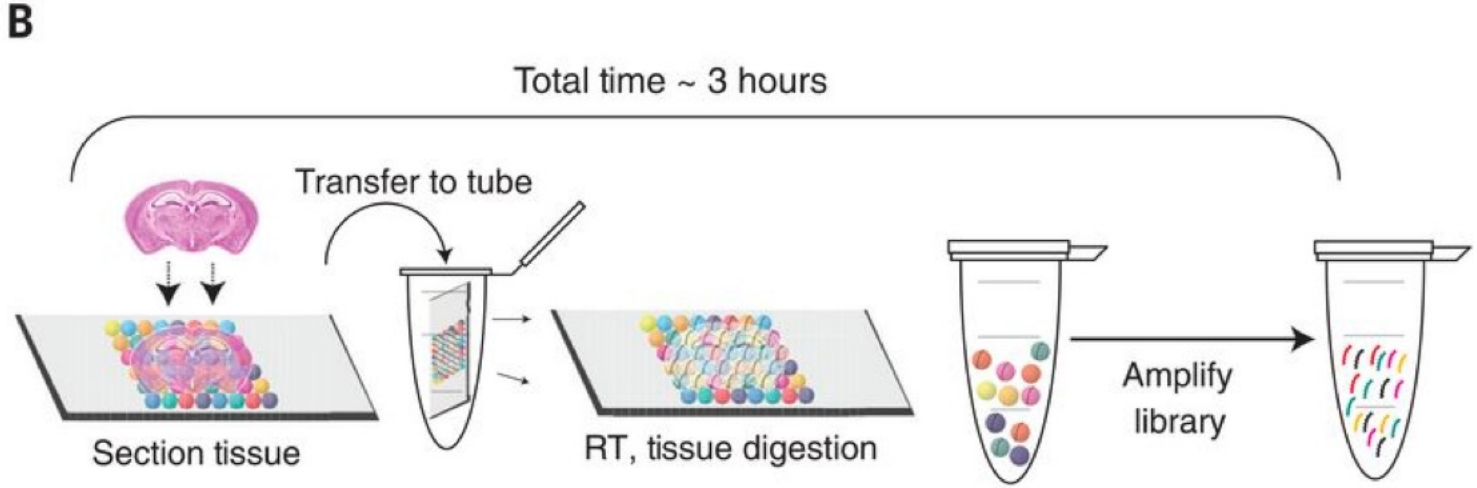
Barcoded bead synthesis



	x	y
BC1	22	34
BC2	68	55
BC3	46	88

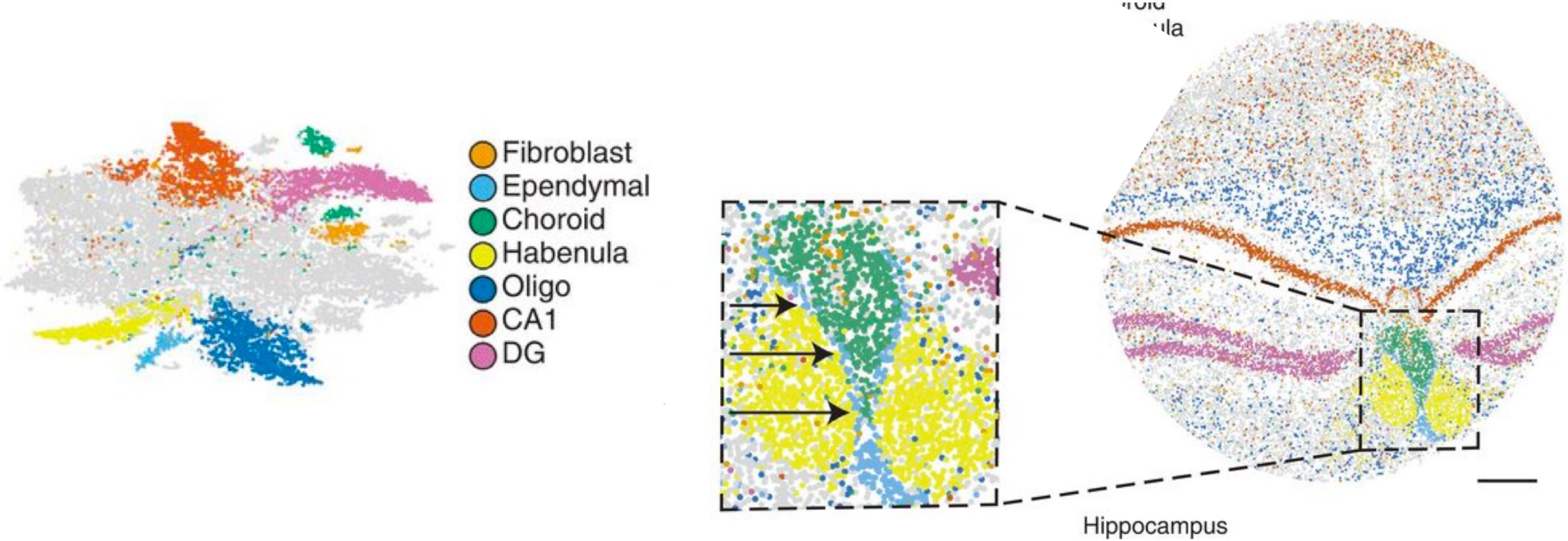
Array generation, indexing





Rodrique, S. et al., Science (2019).
Stickels, R. et al., Nature Biotechnology (2020).

Slide-Seq overview

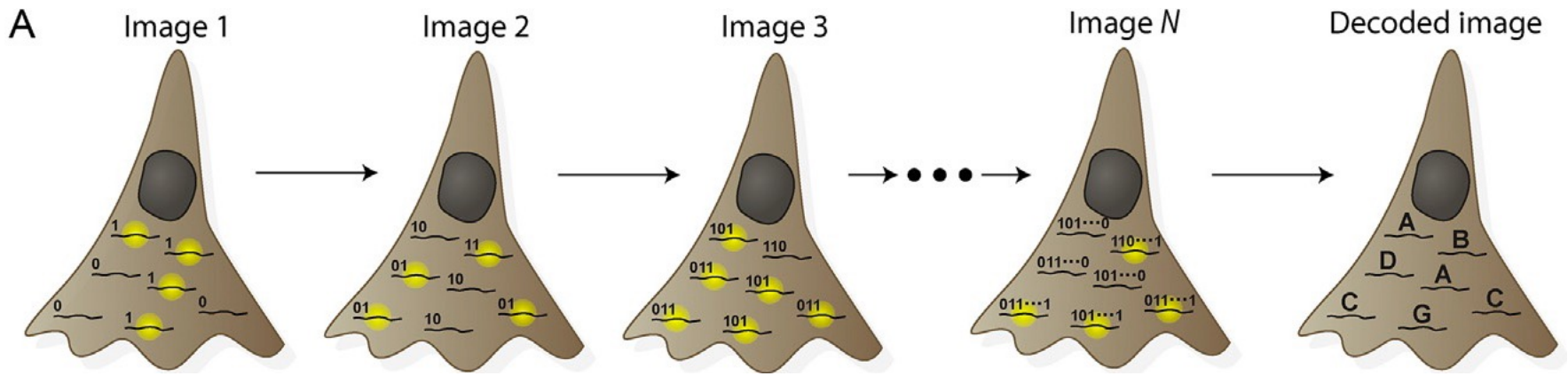


- *Slide-Seq is close to single-cell resolution (10 μm bead) but usually requires deconvolution of the beads to identify the contributing single cells.*

- *Visium (10x) is a related spatial transcriptomic technology.*

Rodrique, S. et al., Science (2019).
Stickels, R. et al., Nature Biotechnology (2020).

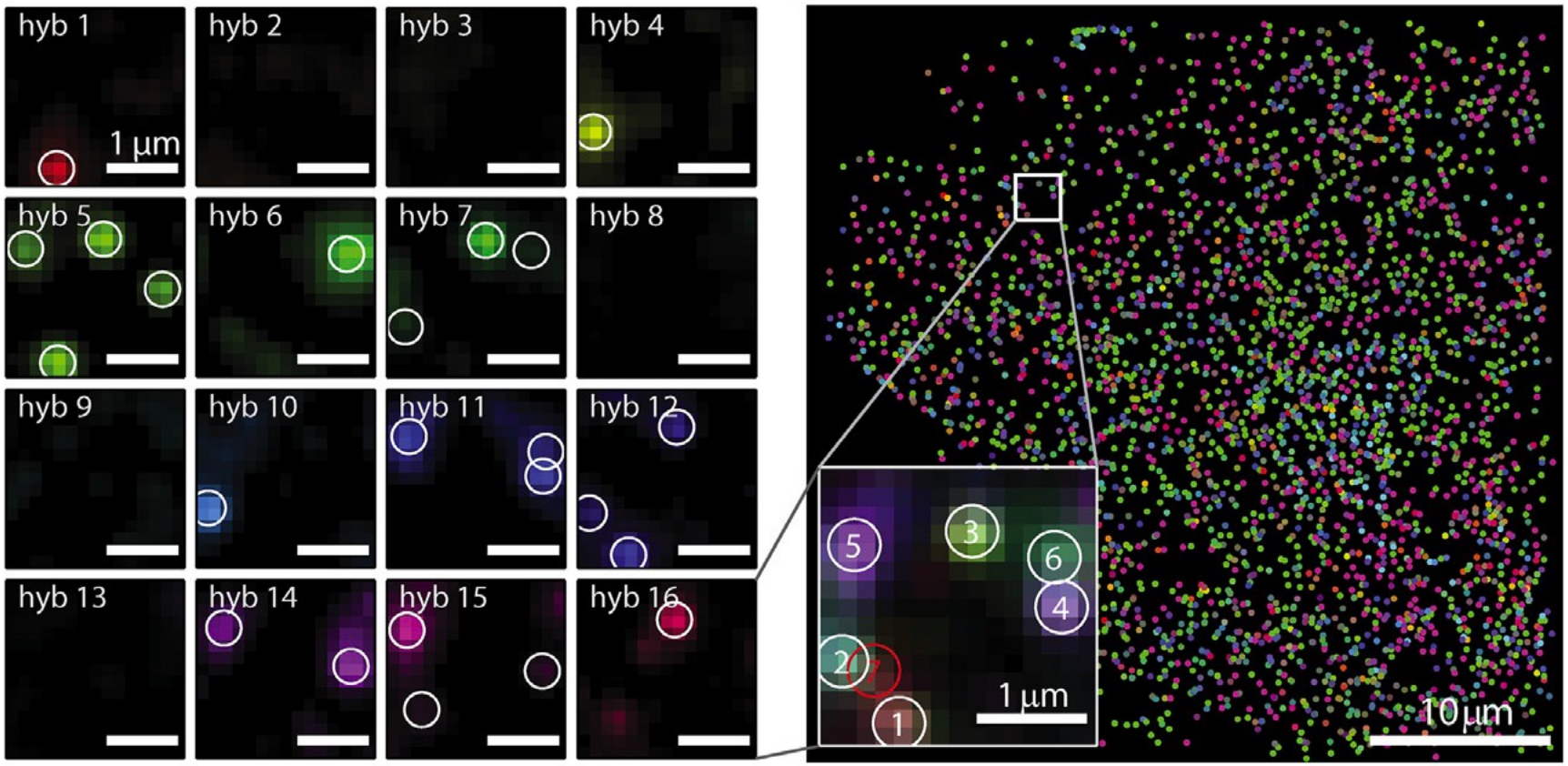
MERFISH overview (choose target genes)



"multiplexed error-robust fluorescence in situ hybridization (MERFISH), a massively parallelized form of smFISH that can image and identify hundreds to thousands of different RNA species simultaneously with high accuracy in individual cells in their native spatial context."

Moffitt, J. R. and Zhuang, X. Methods in Enzymology (2016).

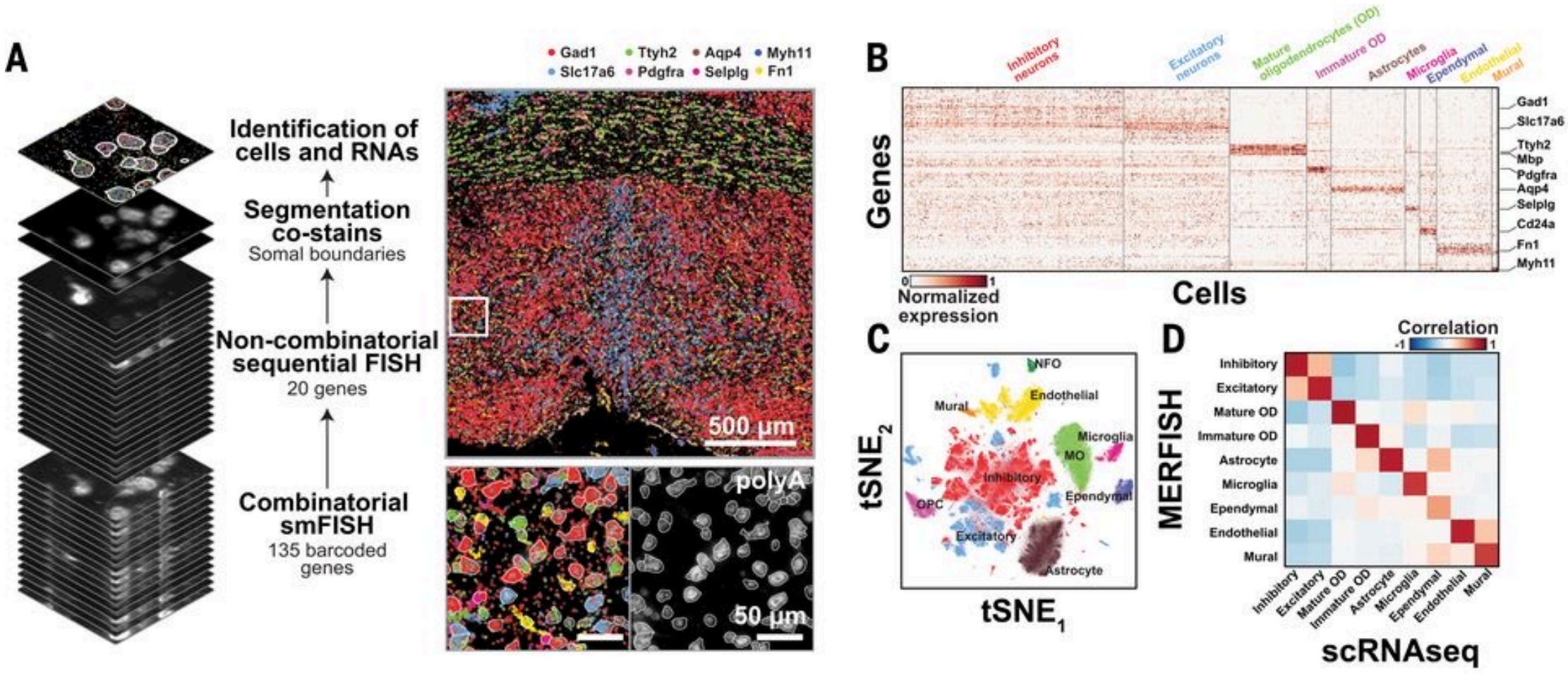
MERFISH overview



Imaging 100s-1000s RNA species in their native cellular and tissue environment

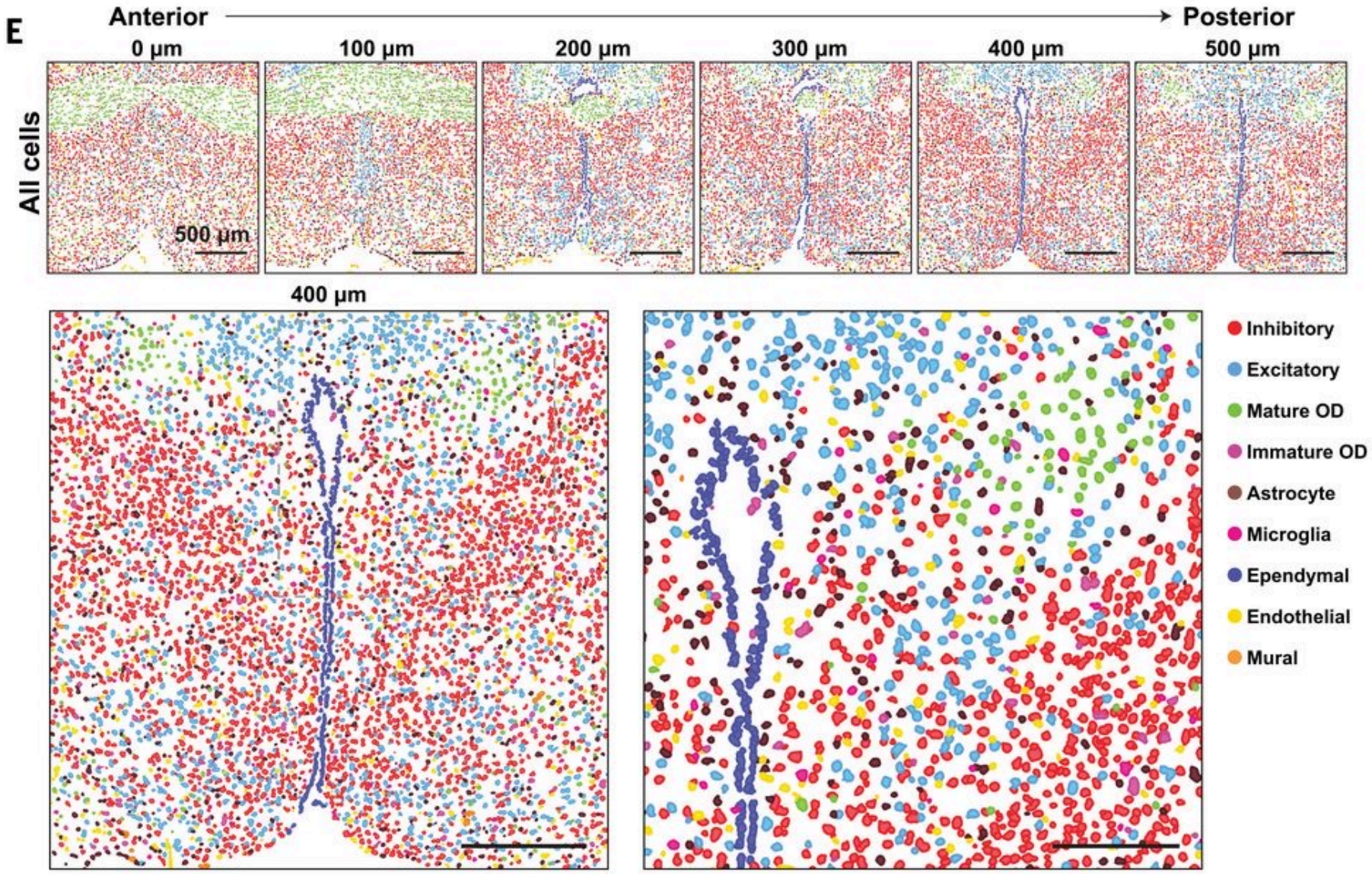
Moffitt, J. R. and Zhuang, X. Methods in Enzymology (2016).

MERFISH overview



Moffitt, J. R. et al., Science (2018).

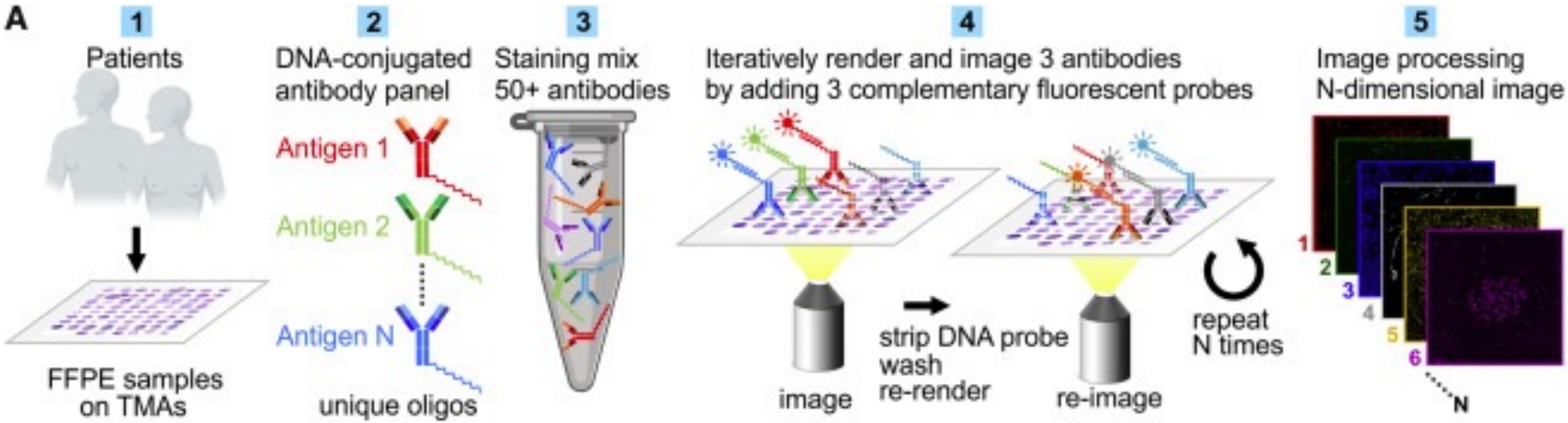
MERFISH overview



Moffitt, J. R. et al., Science (2018).

CODEX overview (choose target proteins)

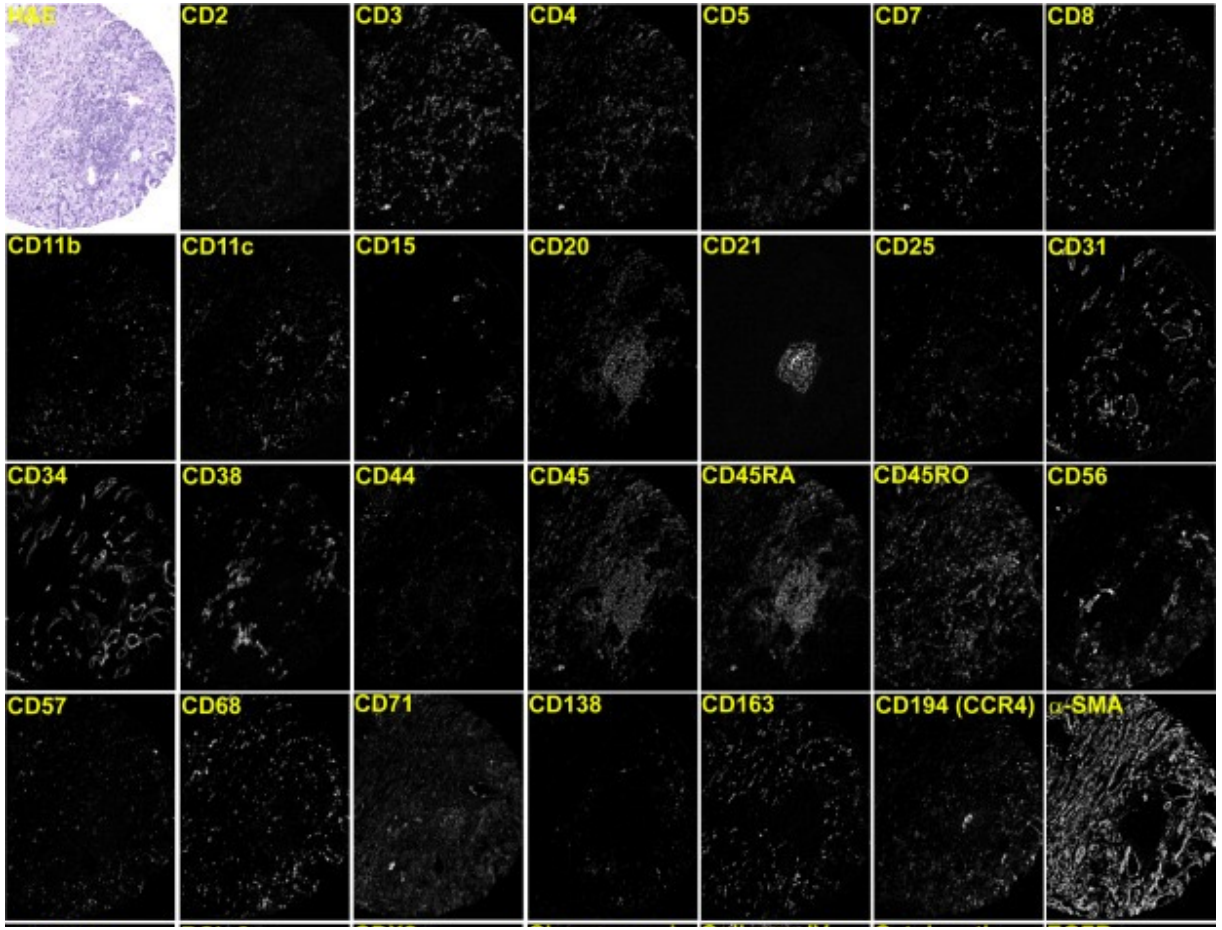
Stain and iteratively image antibodies applied to a tissue sample

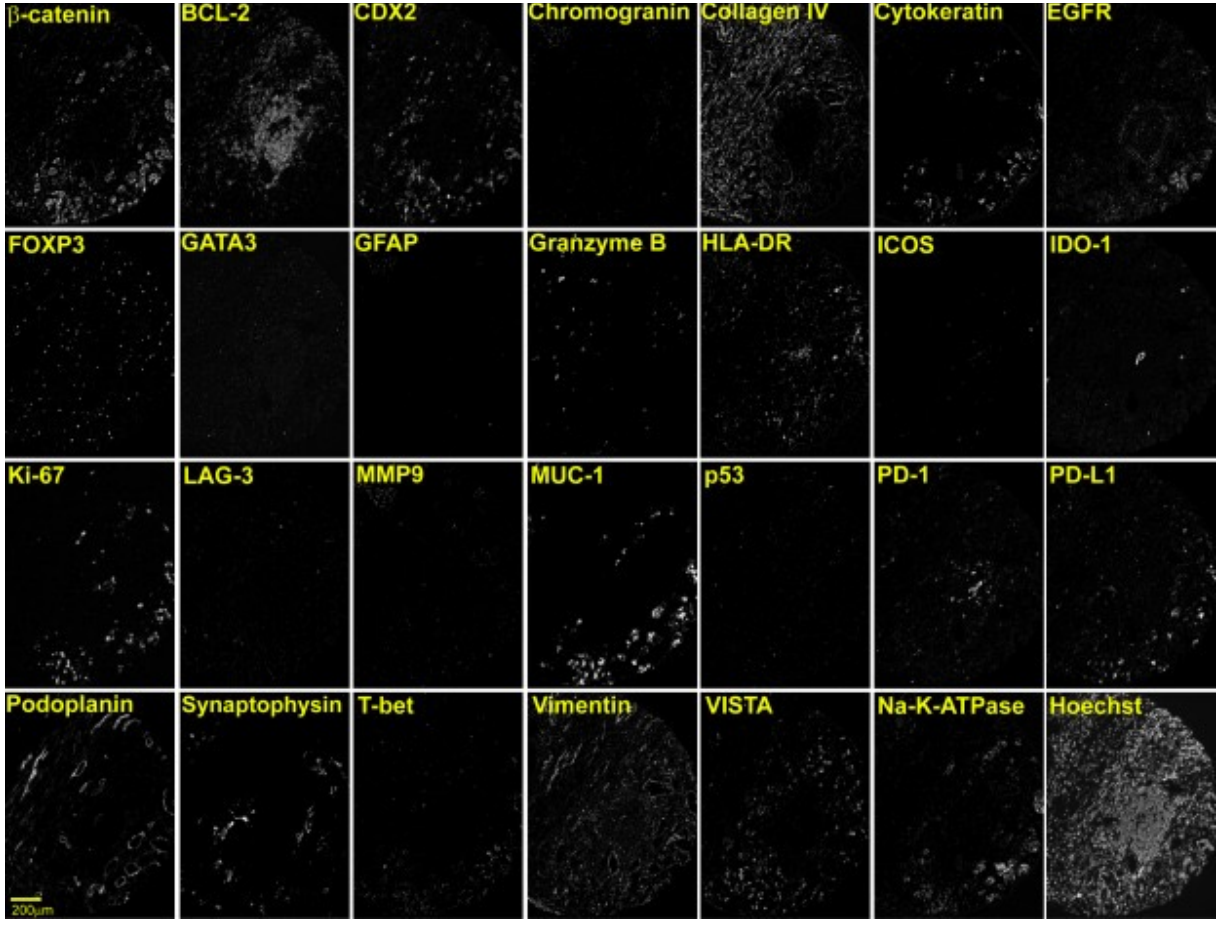


Schürch, C. M. et al., Cell (2020).

CODEX overview: 56 antibody marker panel

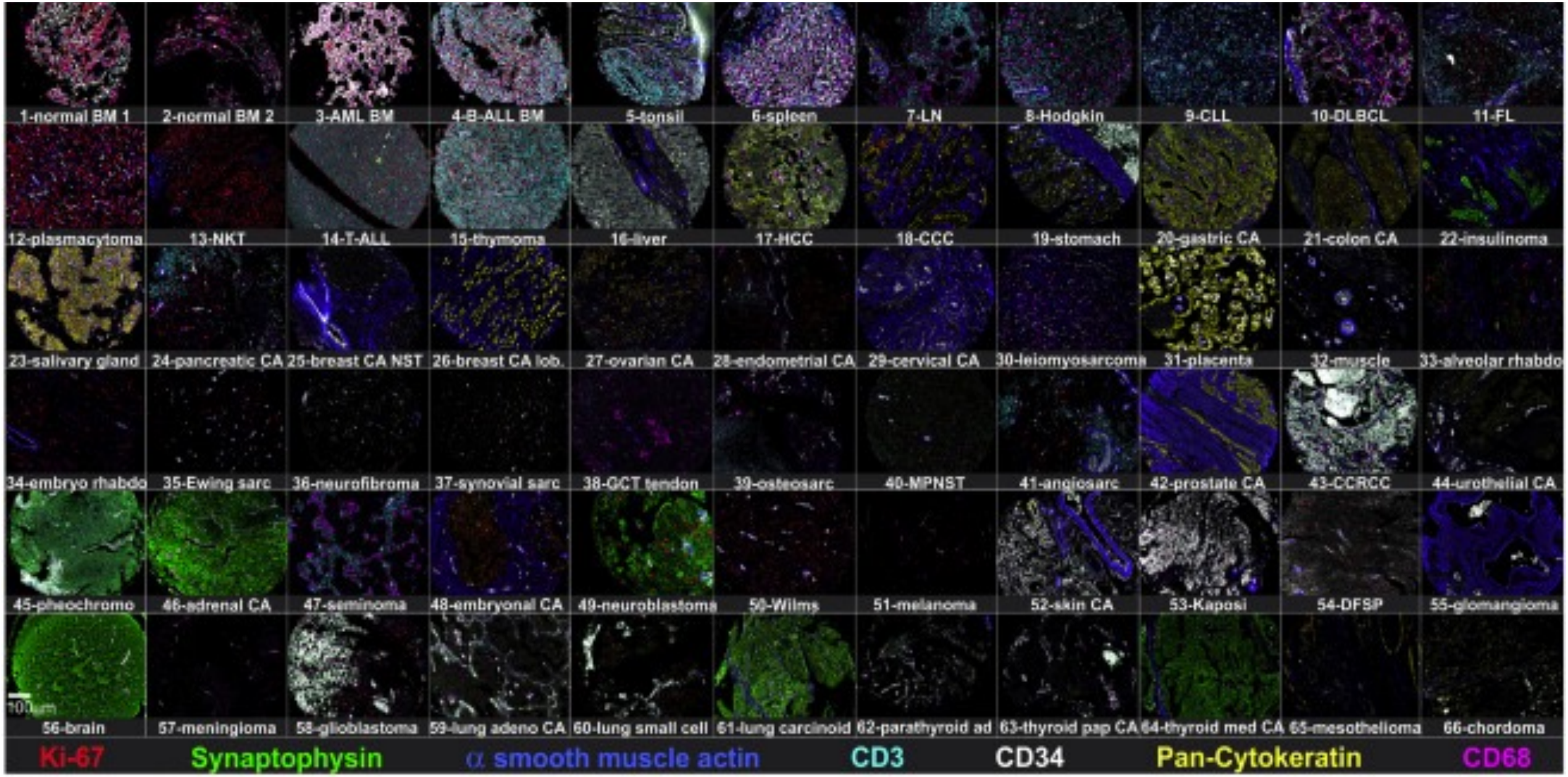
The antibody panel must be validated, and the panel typically includes many immune targets





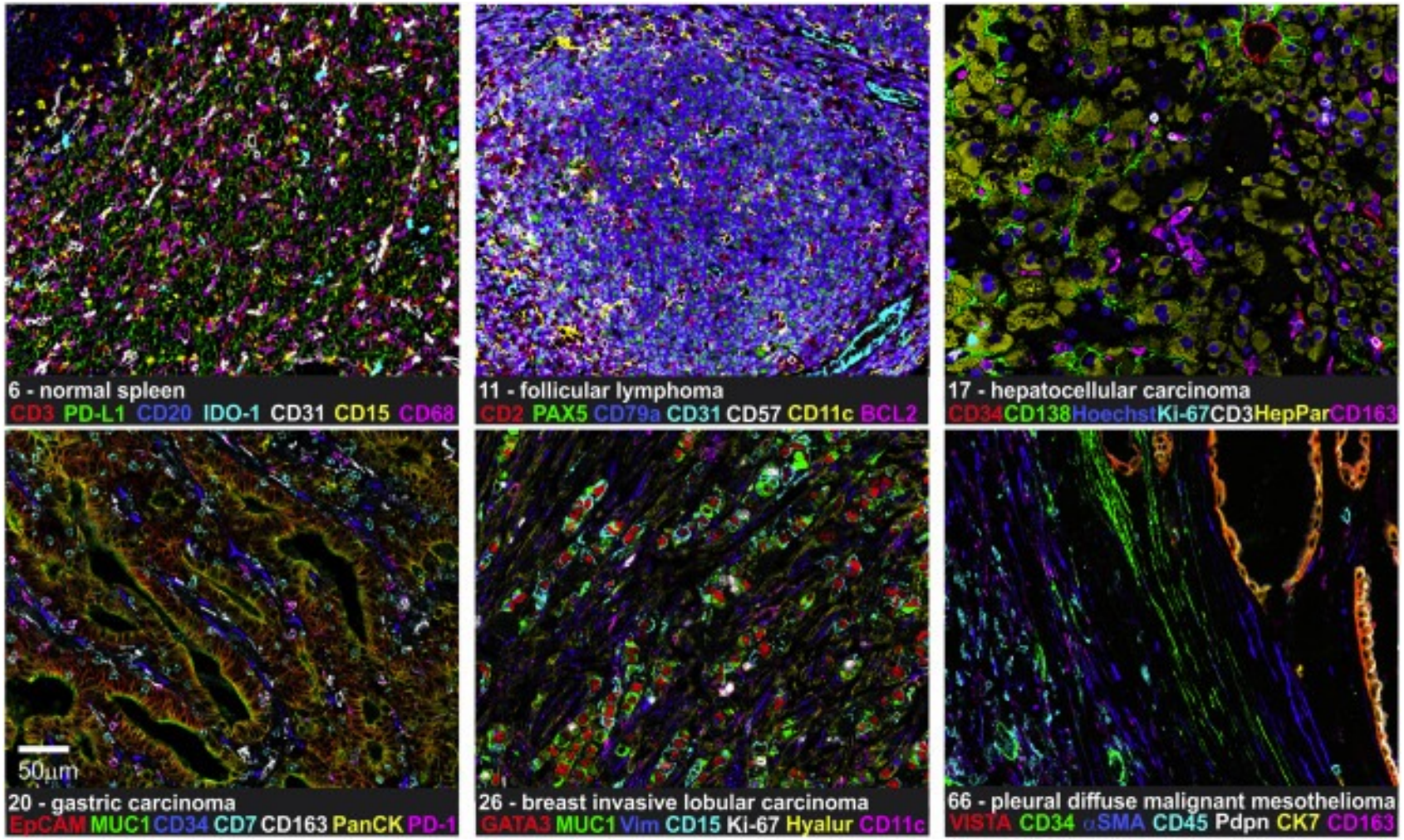
Schürch, C. M. et al., Cell (2020).

CODEX overview: multi-tumor tissue microarray



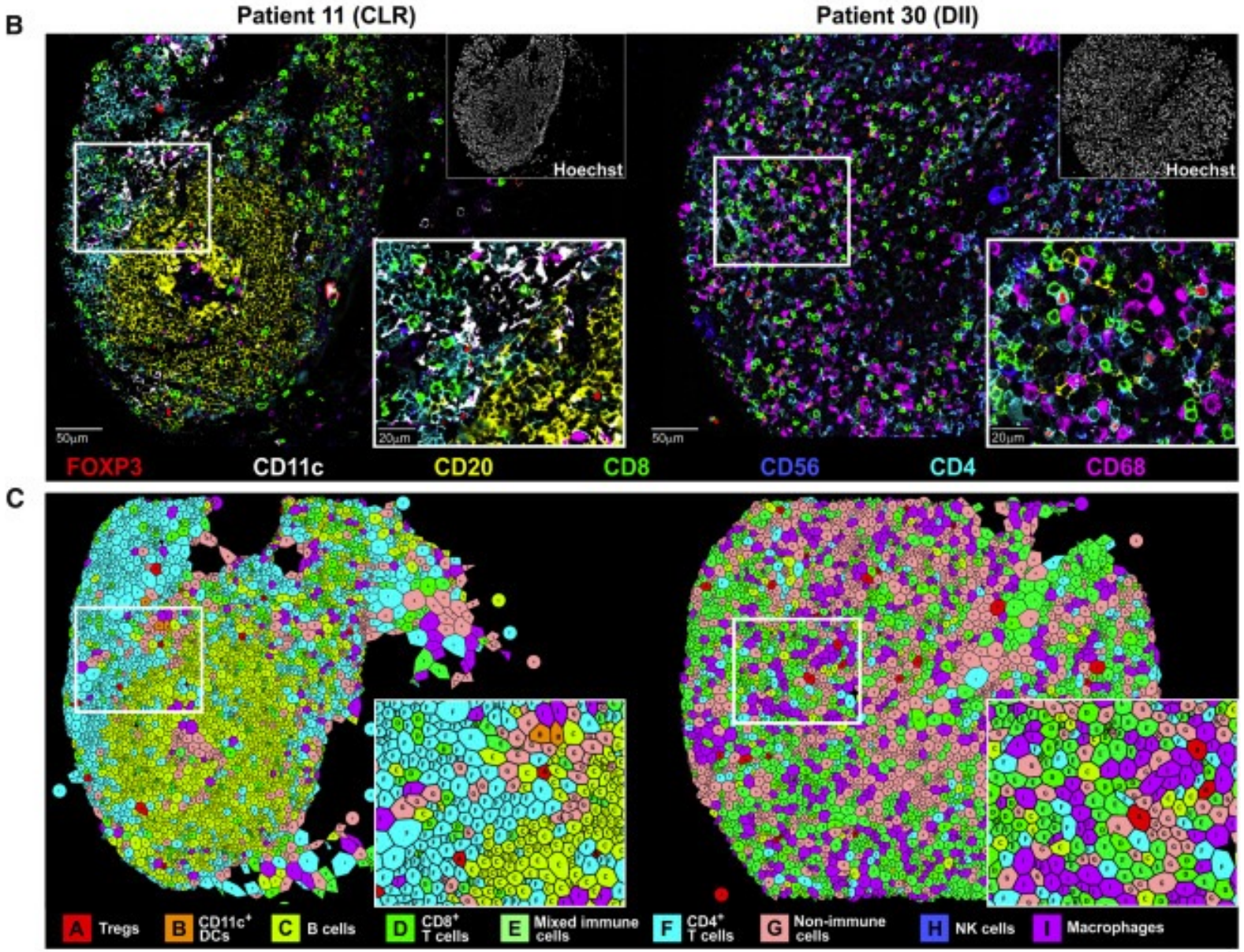
Schürch, C. M. et al., Cell (2020).

CODEX overview: multi-tumor tissue microarray



Schürch, C. M. et al., Cell (2020).

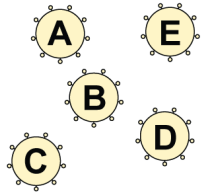
CODEX overview: 56 antibody marker panel



Big challenges

- Imaging
- Segmentation
- Analysis

Perturb-Seq overview



I. Pooled sgRNA library, polydenylated guide barcode

II. Transduce cells (low or high MOI)

III. Capture cells, barcode mRNA

IV. Match guide barcode to transcriptome

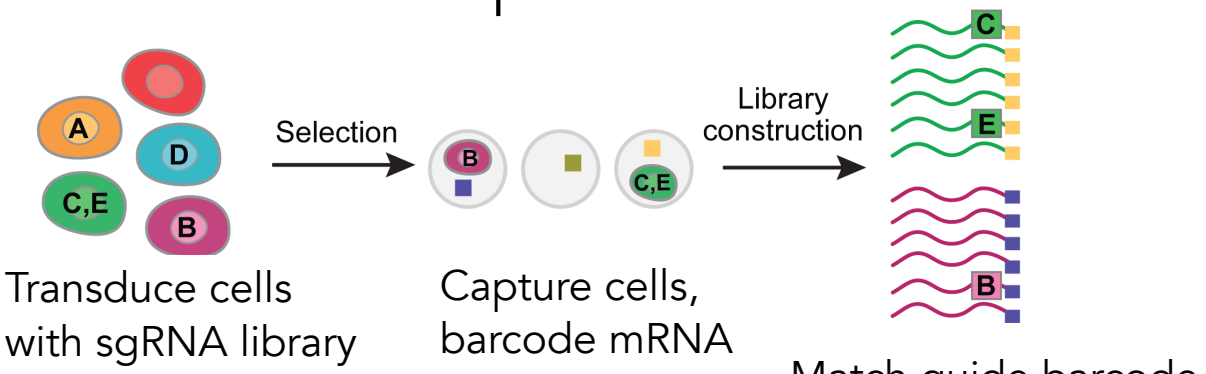
Perturb-Seq advantages

- no need to define a phenotype beforehand
- single cell readout
- pooled format
- higher order interactions

Dixit*, Parnas* et al., *Cell* 2016; Adamson*, Norman* et al., *Cell* 2016

Perturb-Seq overview

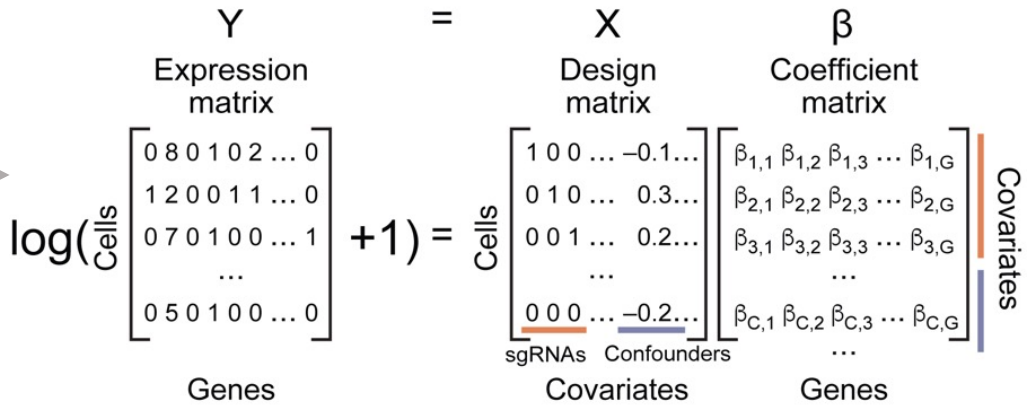
Perturb-Seq links perturbations to their effects on the transcriptome



Transduce cells with sgRNA library

Capture cells, barcode mRNA

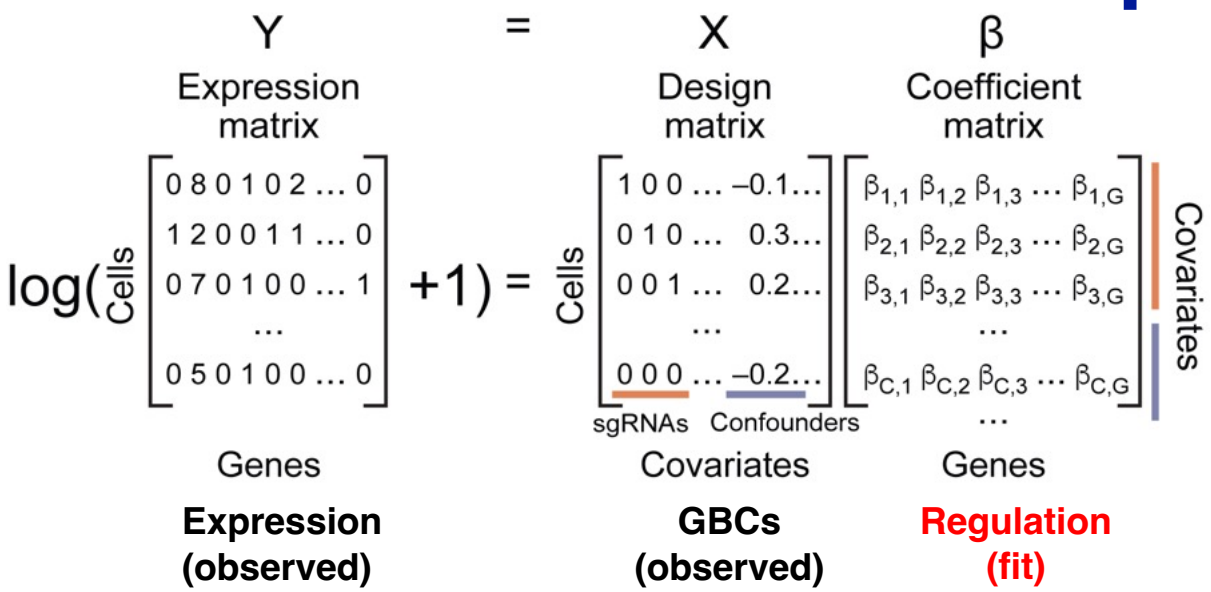
Match guide barcode to transcriptome



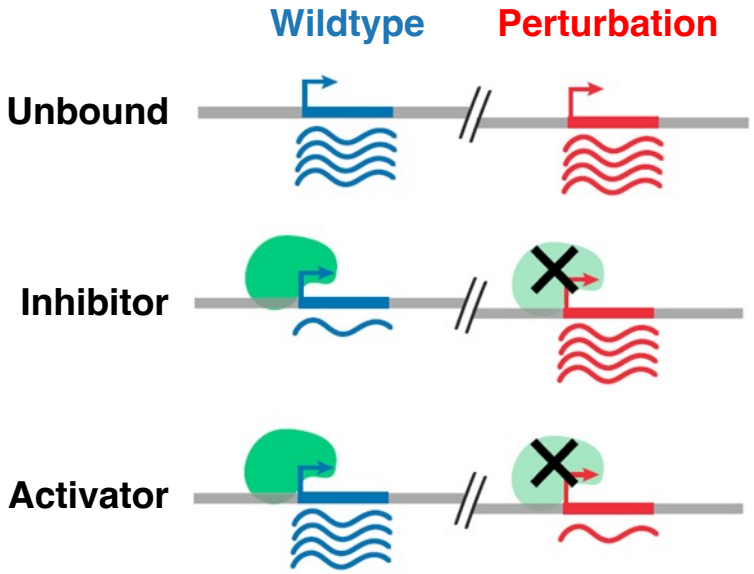
Infer perturbation regulatory effects

Dixit, et al. *Cell* (2016).

Ongoing developments in single-cell genomics: Perturb-Seq



β is the regulatory effect a given guide has on a given gene.



β = 0, no change

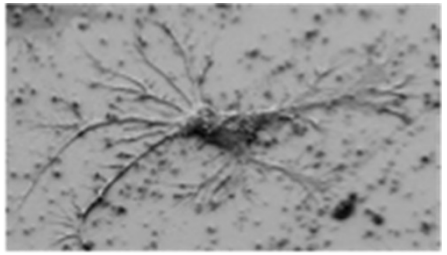
β > 0, increased expression

β < 0, decreased expression

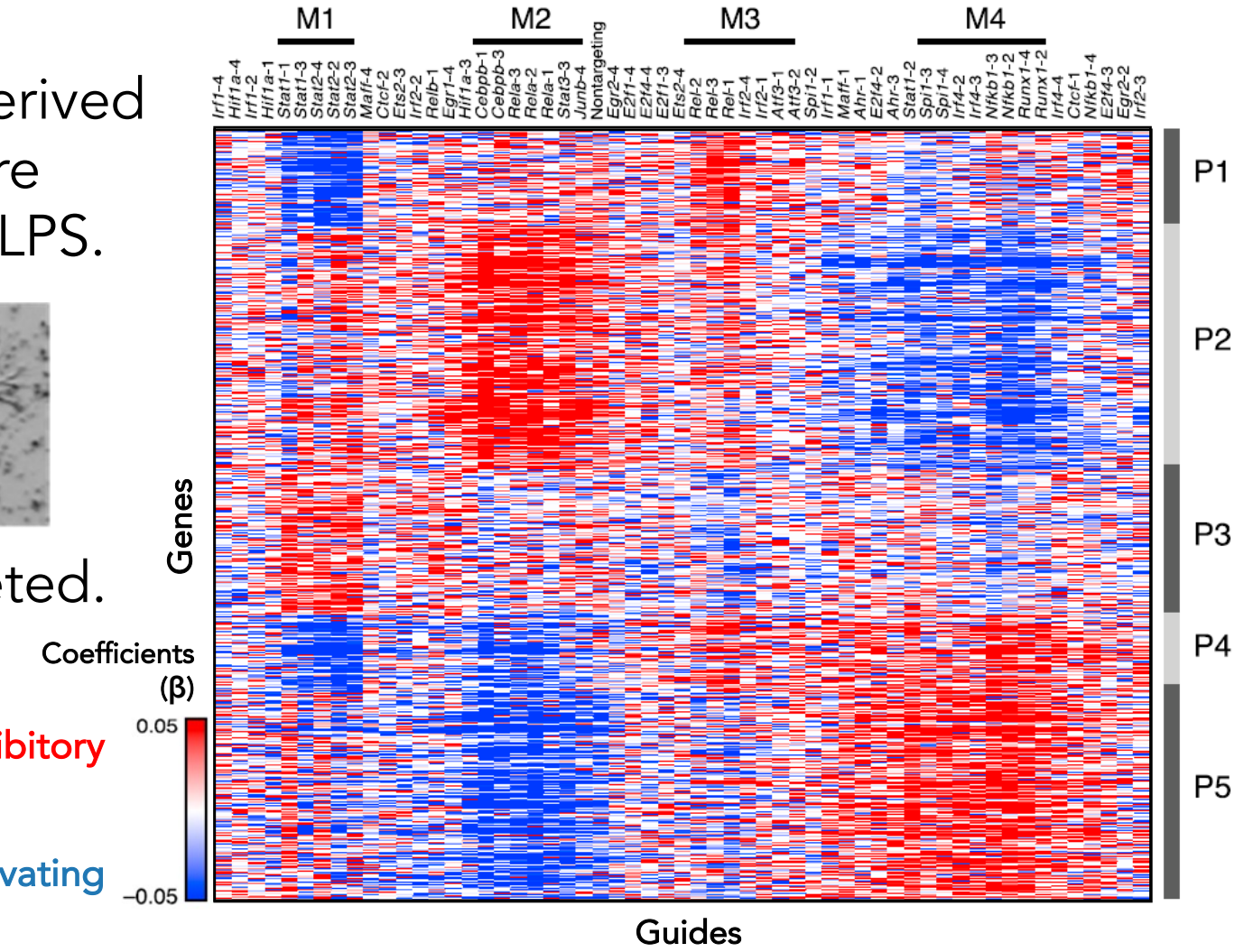
Perturbations reveal gene regulation logic

Guides and genes cluster into transcription factor modules (M) and regulated expression programs (P) respectively.

- Bone-marrow derived dendritic cells are stimulated with LPS.



- 24 TFs are targeted.



Perturbations reveal gene regulation logic