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Cellular Multiverse in a Nutshell: Advanced Single Cell Multi-Omics Solutions

Julie Laliberte, PhD

Solutions For Single Cell Sequencing

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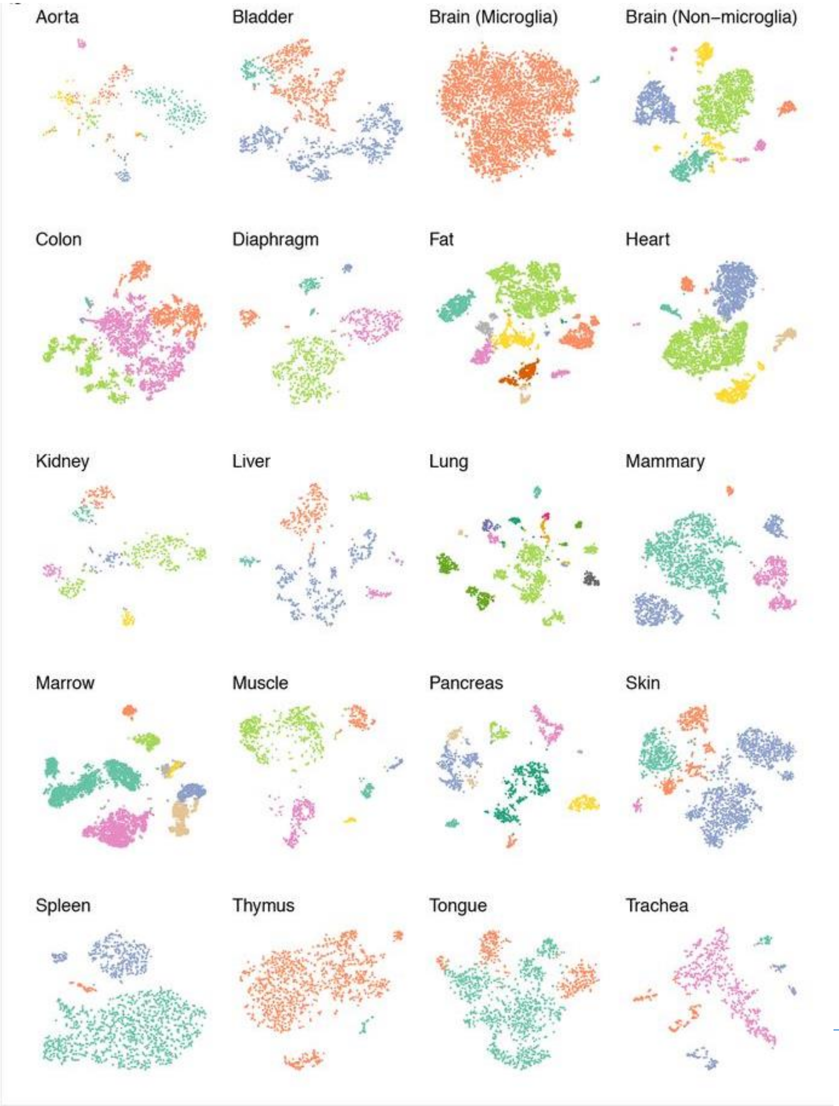
From single cell multi-omics to precision medicine

Why the need of info at single cell level?

Organs and tissues contain a high diversity of cell types.



- To identify the different cell types present in each tissue and gain insights in their role in the context of the tissue.
- Understand the changes in expression level in response to disease at single cell level.
- Understand differences in patient heterogeneity to drug responses.
- Study how cells respond to each other and to their microenvironment.

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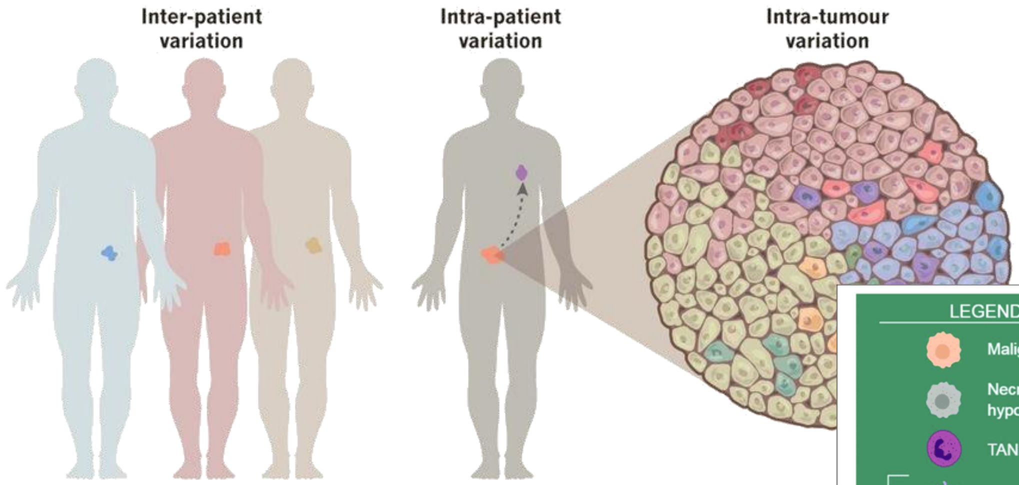
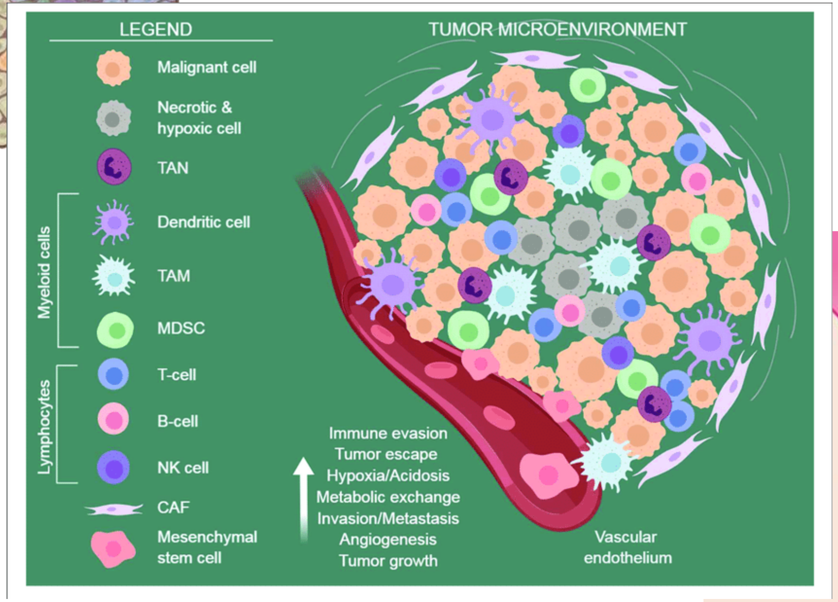
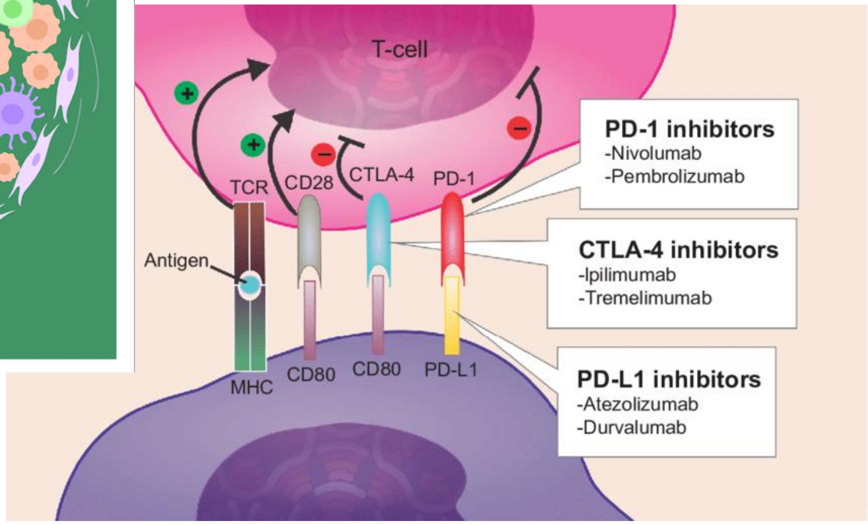
Single-cell Sequencing in Cancer Research



Heterogeneity of cancer cells

Tumor immune microenvironment



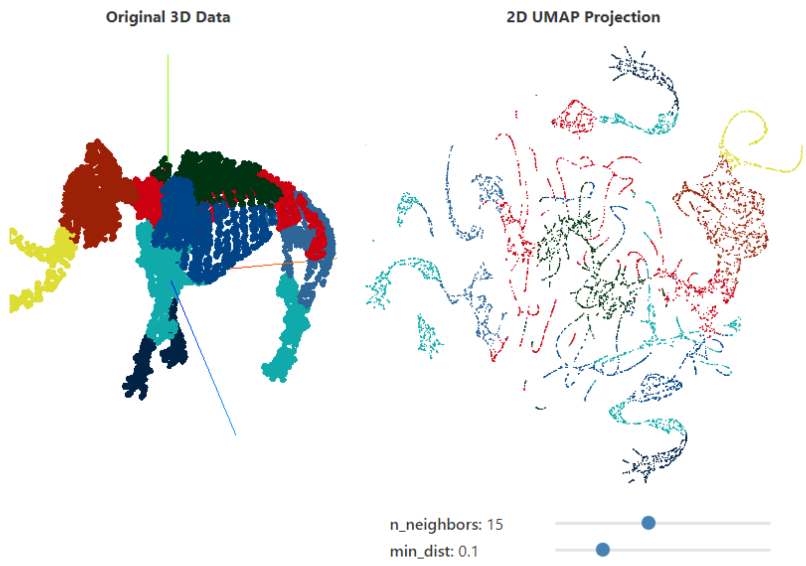


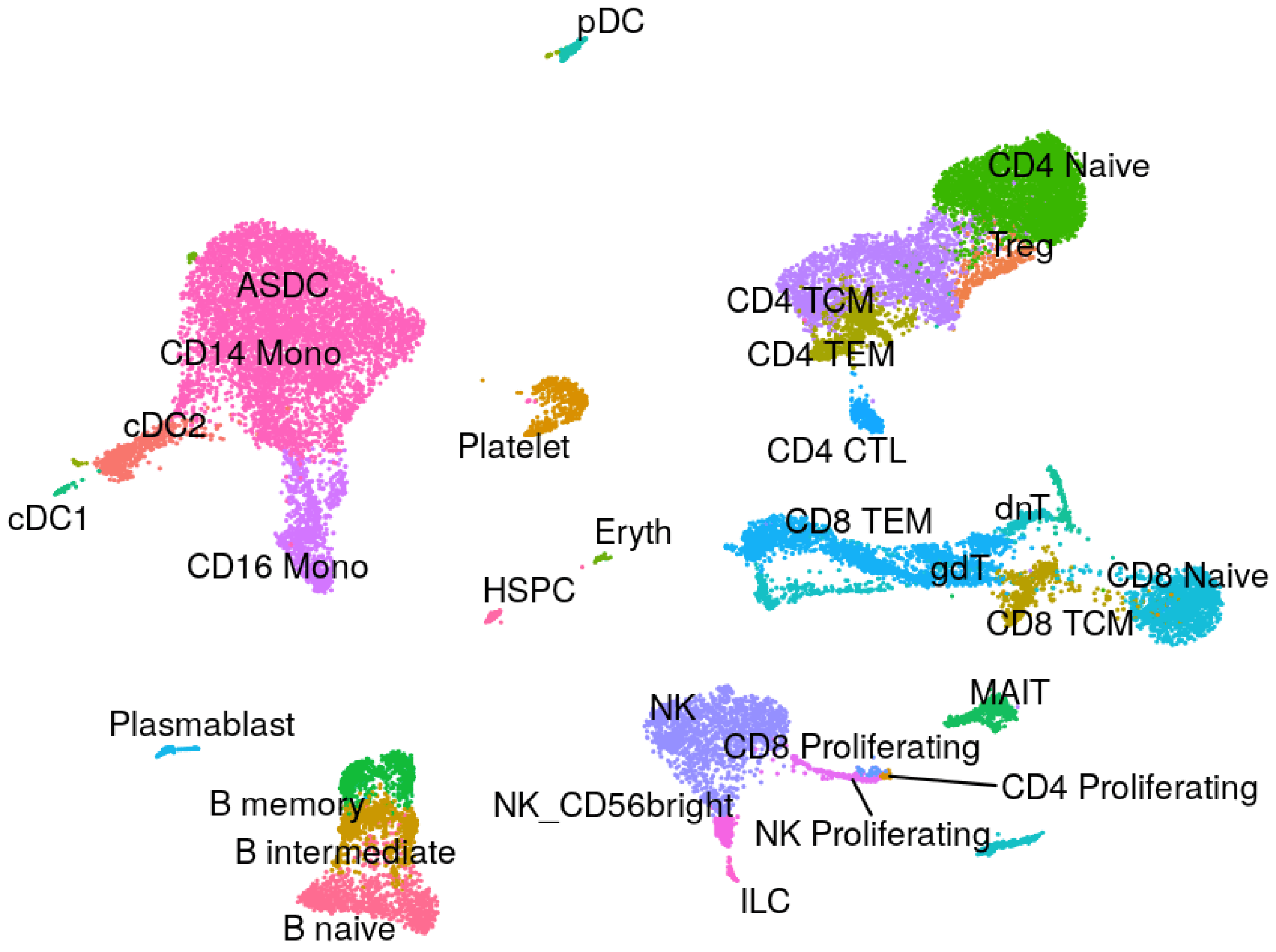
Immune checkpoint inhibitors

The UMAP !

What is a UMAP?

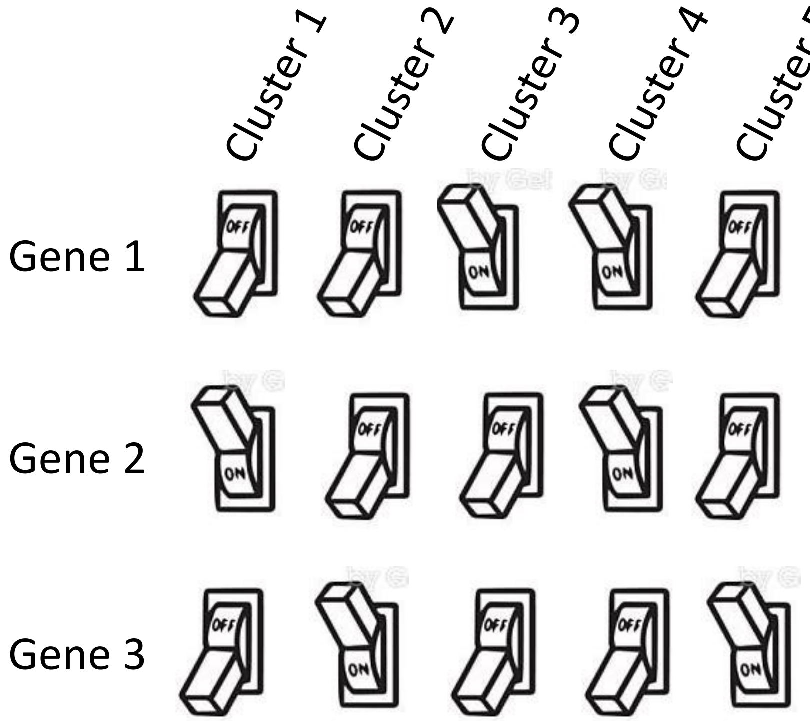
Is a method used to visualize high dimensional datasets in 2 dimensions.

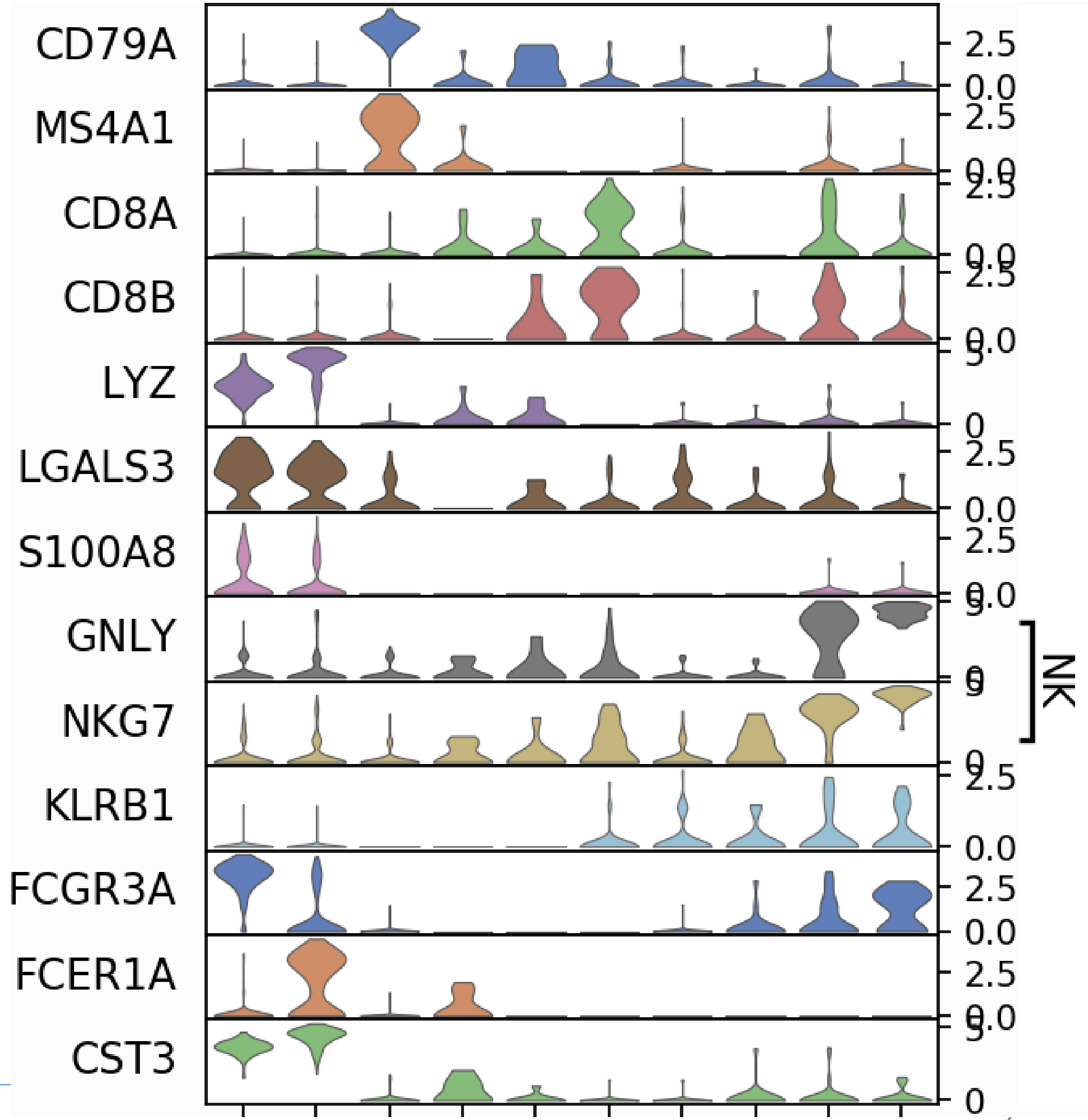




https://pair-code.github.io/understanding-umap/

How to identify your cells clusters?





Singleron's Technologies

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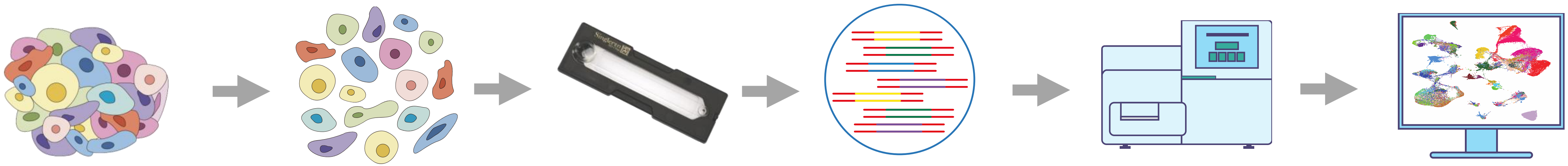
GEXSCOPE scRNAseq Workflow



Tissue → Single-cell suspension → Cell Partitioning, Cell Lysis, mRNA Capture → RT, cDNA Amplification, Library Construction → Sequencing → Bioinformatic Analysis

Tissue

Single-cell
suspension

Cell Partitioning
Cell Lysis
mRNA Capture

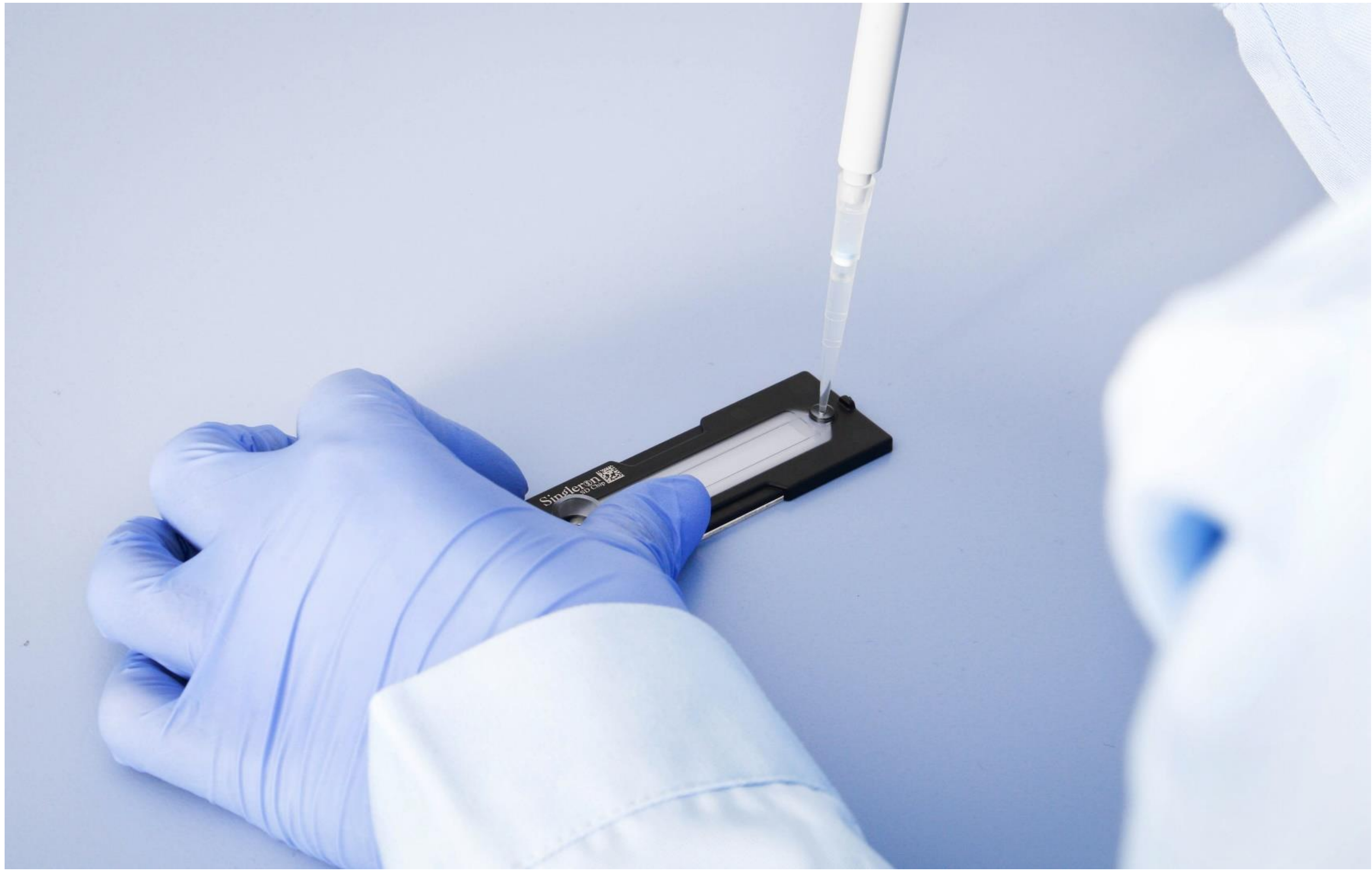
RT
cDNA Amplification
Library Construction

Sequencing

Bioinformatic
Analysis

PythoN **Jr** **Matrix NEO**

Single-Cell Sequencing Solutions: SCOPE-chip®



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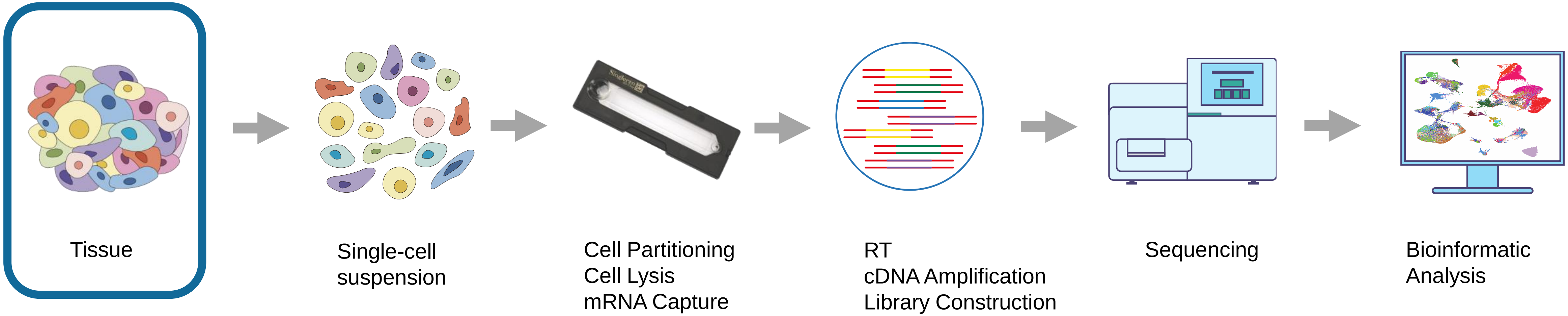
Tissue Preservation

Tissue Preservation Solution

- can effectively preserve the tissue **for 72 hrs at 4 ℃**. (contains no chemical fixatives)



QC:
- Necrosis
- Presence of Blood
- Quantity/Quality

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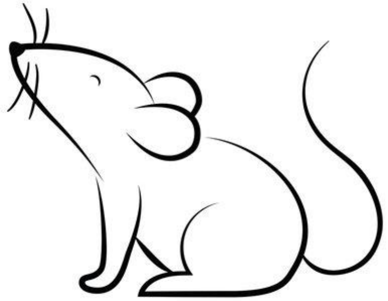
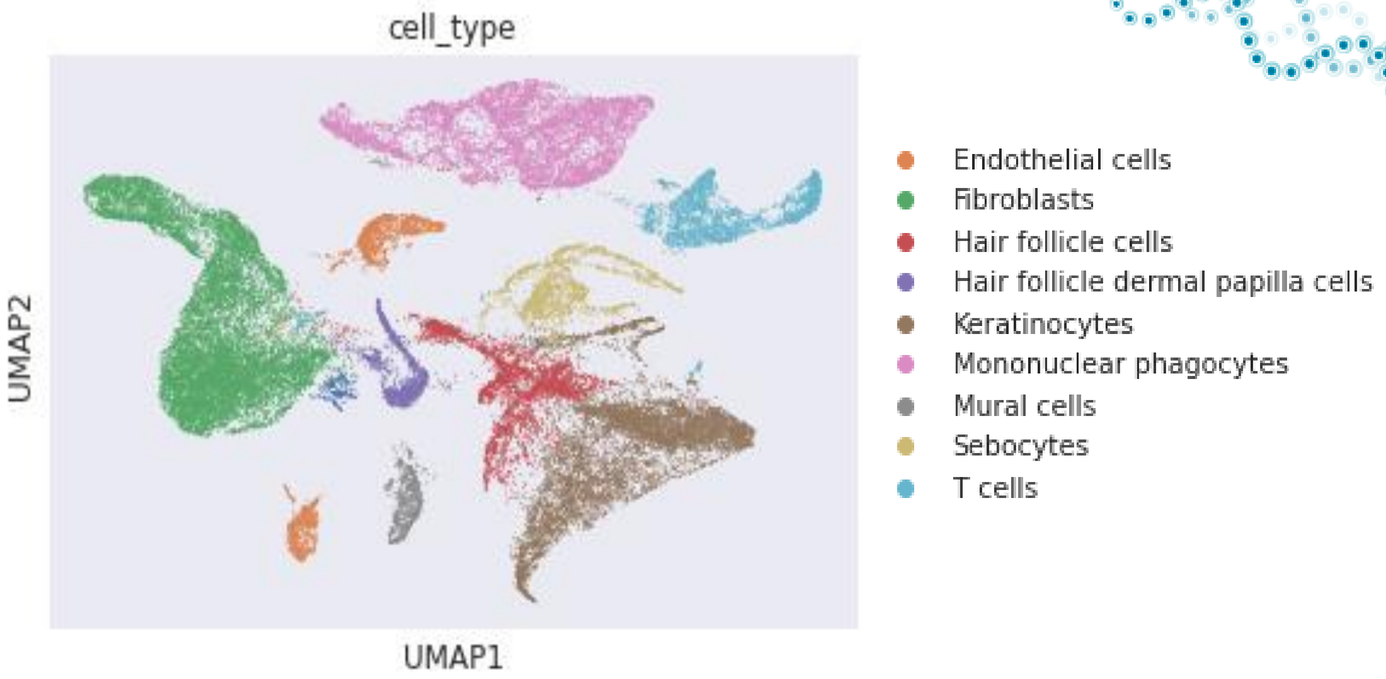
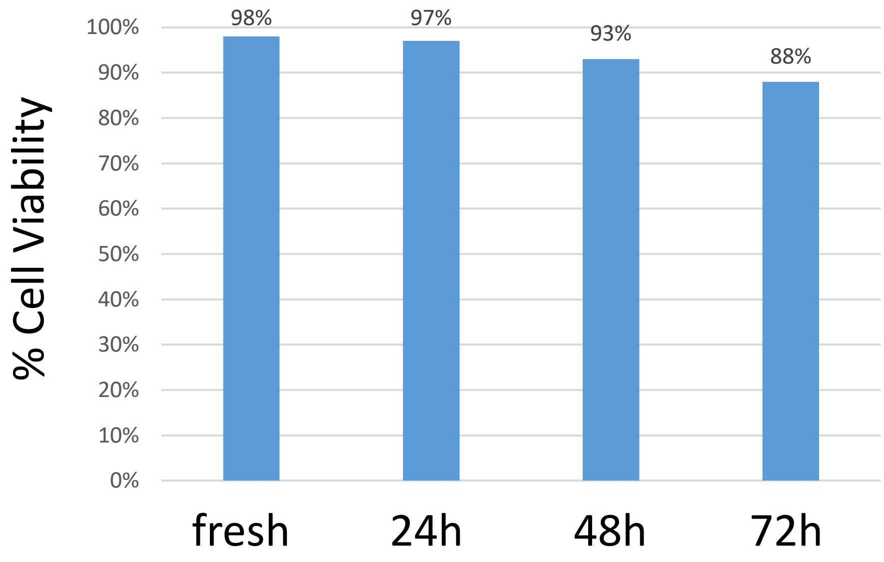
Tissue Preservation

Example of the mouse skin







Pearson correlation of average expression of top 2000 variable genes, comparing with fresh samples

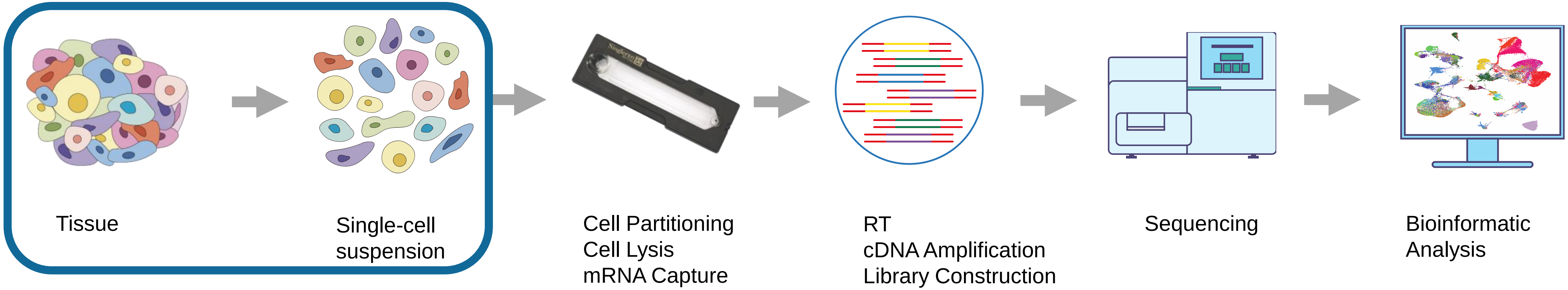
	Fibroblasts	Endothelial cells	Mononuclear phagocytes	Sebocytes	Keratinocytes	T cells	Hair follicle cells	Hair follicle dermal papilla cells	Mural cells
0h	1	1	1	1	1	1	1	1	1
24h	0.965	0.967	0.969	0.982	0.98	0.973	0.967	0.97	0.978
48h	0.961	0.969	0.933	0.961	0.97	0.96	0.959	0.925	0.96
72h	0.957	0.921	0.913	0.826	0.962	0.919	0.933	0.865	0.943

Colour scale: 1.00, 0.98, 0.96, 0.94, 0.92, 0.90, 0.88, 0.86, 0.84

Tissue Dissociation

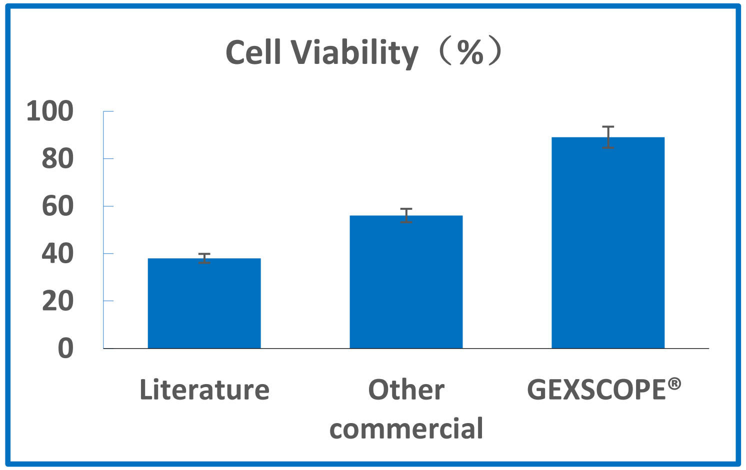
Tissue Dissociation Mix

- can dissociate different types of tissues with high efficiency within **30-60 min**

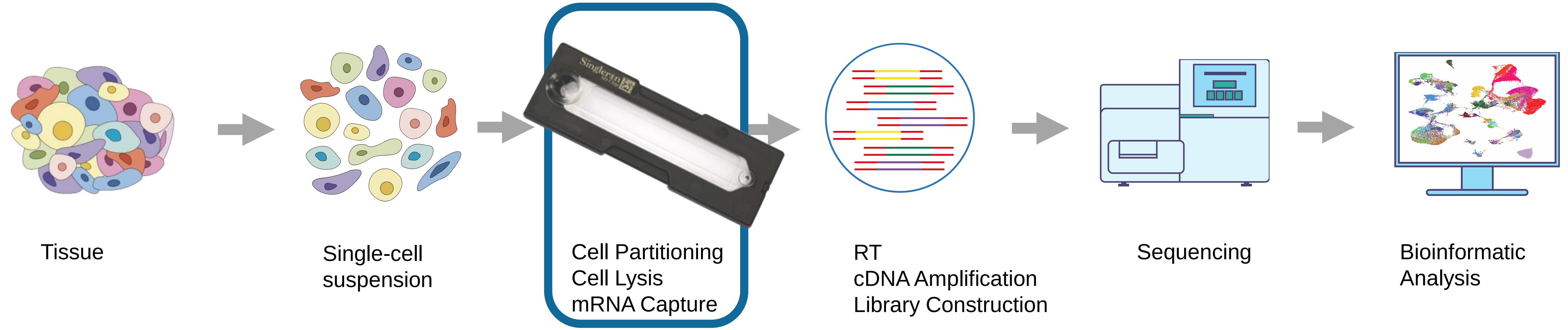


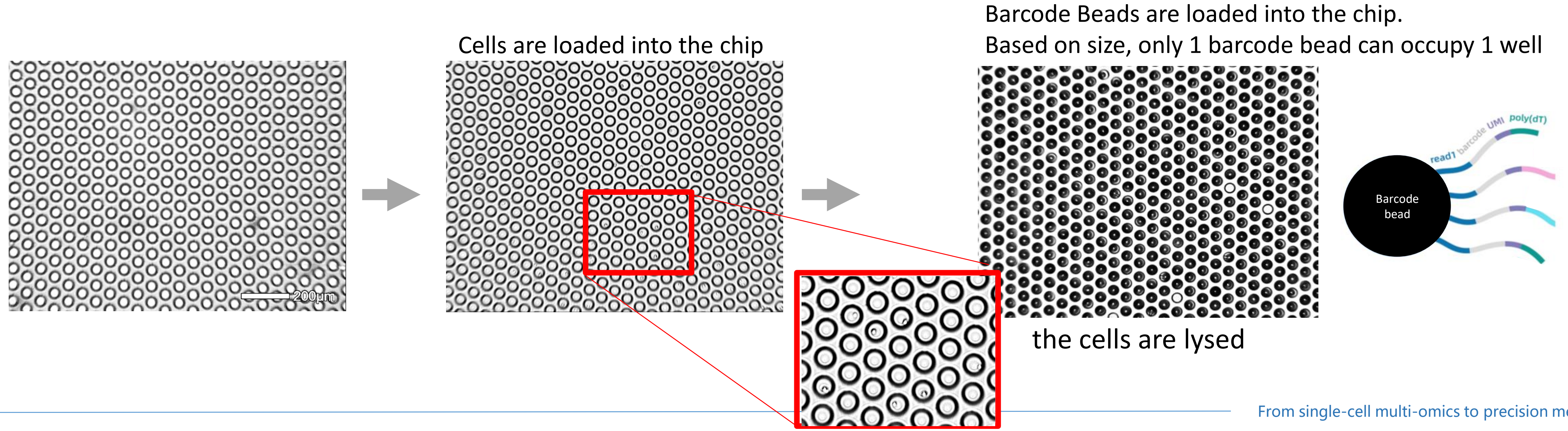
QC:

- Cell aggregation
- Debris
- Red blood cells
- Dead cells
- Accurate cell counting

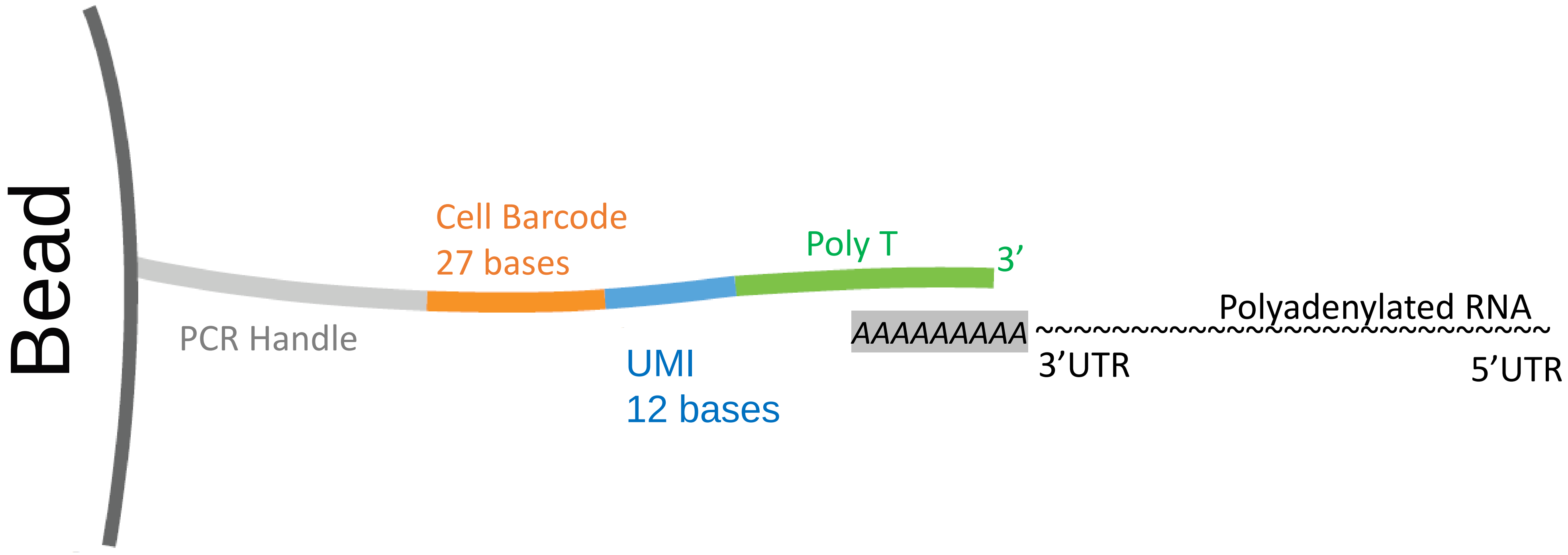


Single Cell Partitioning





Capture of polyadenylated RNA with a unique Cell Barcode



UMI: Unique Molecular Identifier

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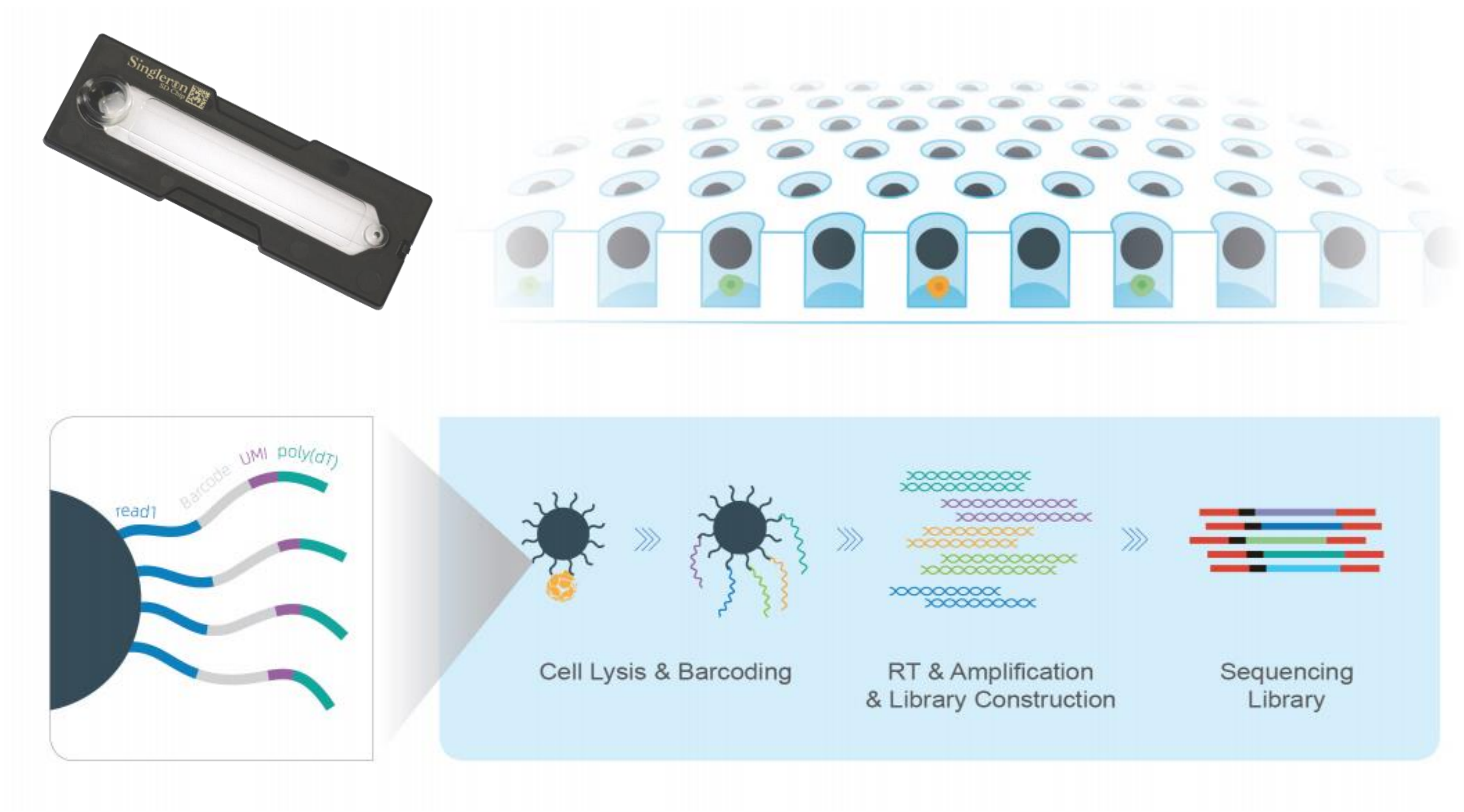
Single-Cell Sequencing Solutions: SCOPE-chip®



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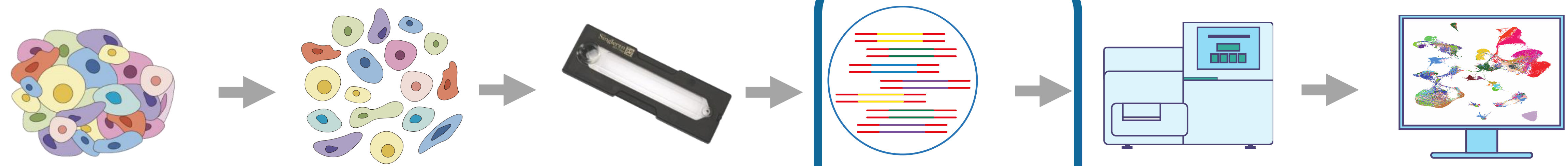
Reverse transcription, cDNA amp and Library Prep



Tissue

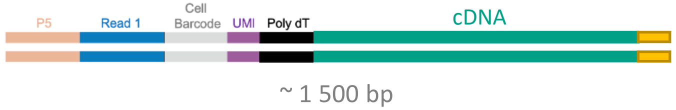
Single-cell suspension

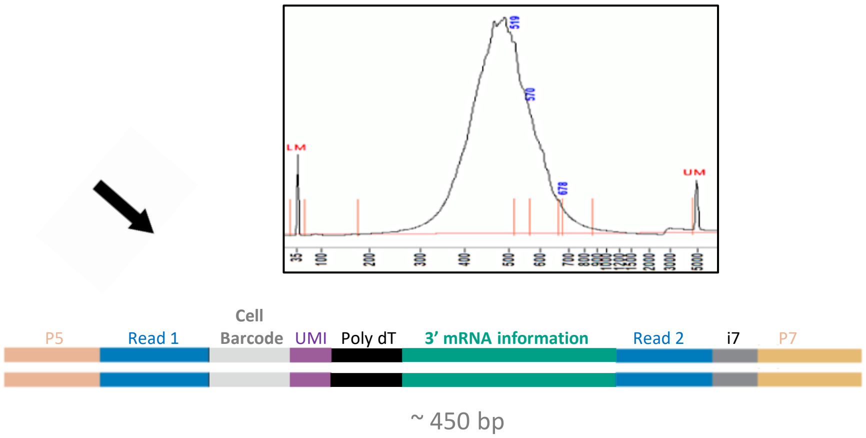
Cell Partitioning
Cell Lysis
mRNA Capture

RT
cDNA Amplification
Library Construction

Sequencing

Bioinformatic Analysis

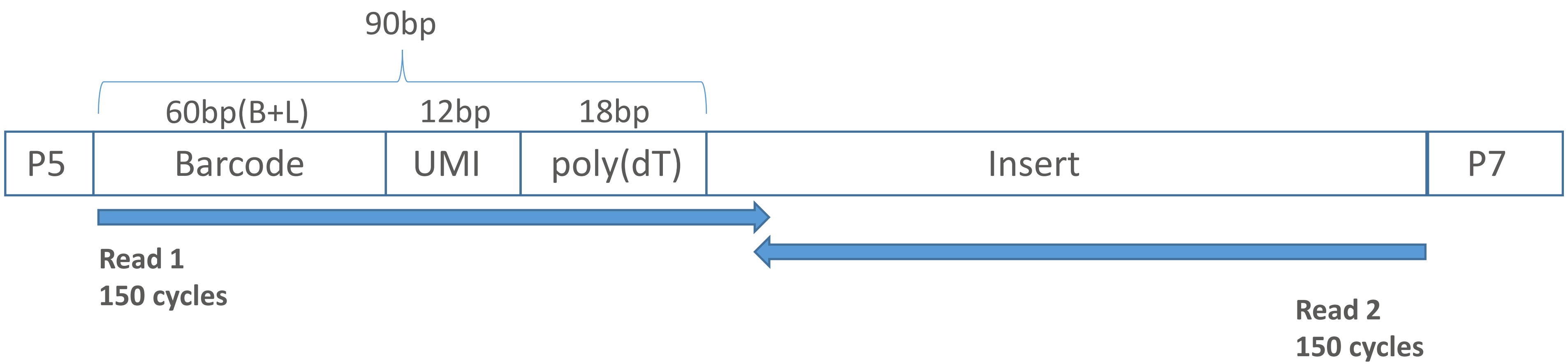


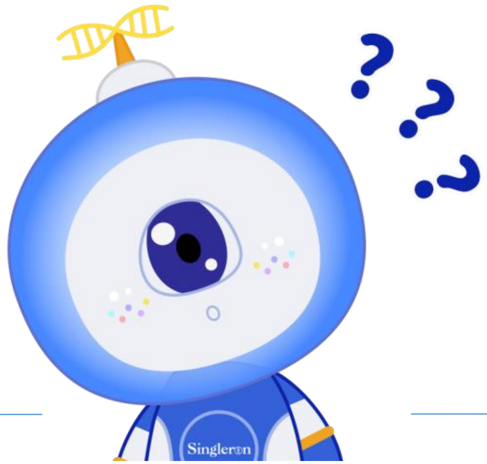


1- The poly-A sequences from the **mRNA** binds the poly T

1- During the RT, the **Cell barcodes** are added to the mRNA
2- cDNA Amplification

Barcode Structure For Pre-Processing





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CeleScope® - Barcode Structure For Pre-Processing

Read 1

5’-Adapter-BBBBBBBBBATCCACGTGCTTGAGABBBBBBBBBTTCAGCATGCGGCTACGBBBBBBBBBLNNNNNNNNNNNNTTTTTTTTTTTTTTT-3’

Cell Barcode
Linker
UMI
polyT

Chemistry	Pattern	Corresponding kit version
scopeV3.0.1	C9L16C9L16C9L1U12T18	Magnetic Bead Kit V2

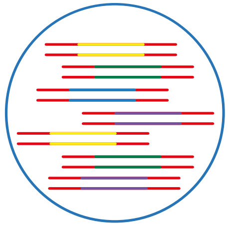
Data Analysis and Interpretation

Fastq files -> trim -> alignement to genome -> feature count and matrix -> QC metrics

CeleScope Software

SynEcoSys Database

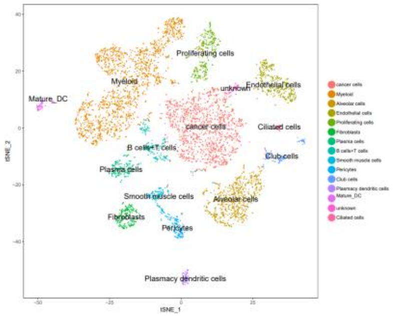
Raw data



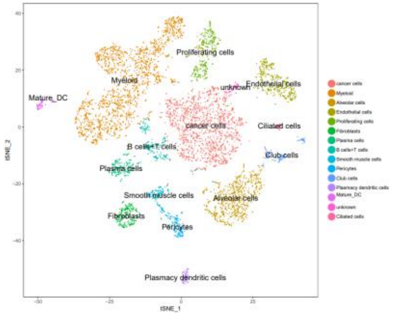
Expression Matrix

Cell:	1	2	···	N
GENE 1	1	2		14
GENE 2	4	27		8
GENE 3	0	0		1
⋮	⋮	⋮		⋮
GENE M	6	2		0

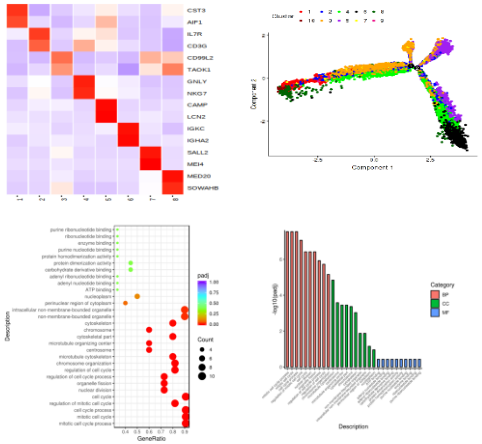
Clustering



Cell Type Annotation



Secondary analysis



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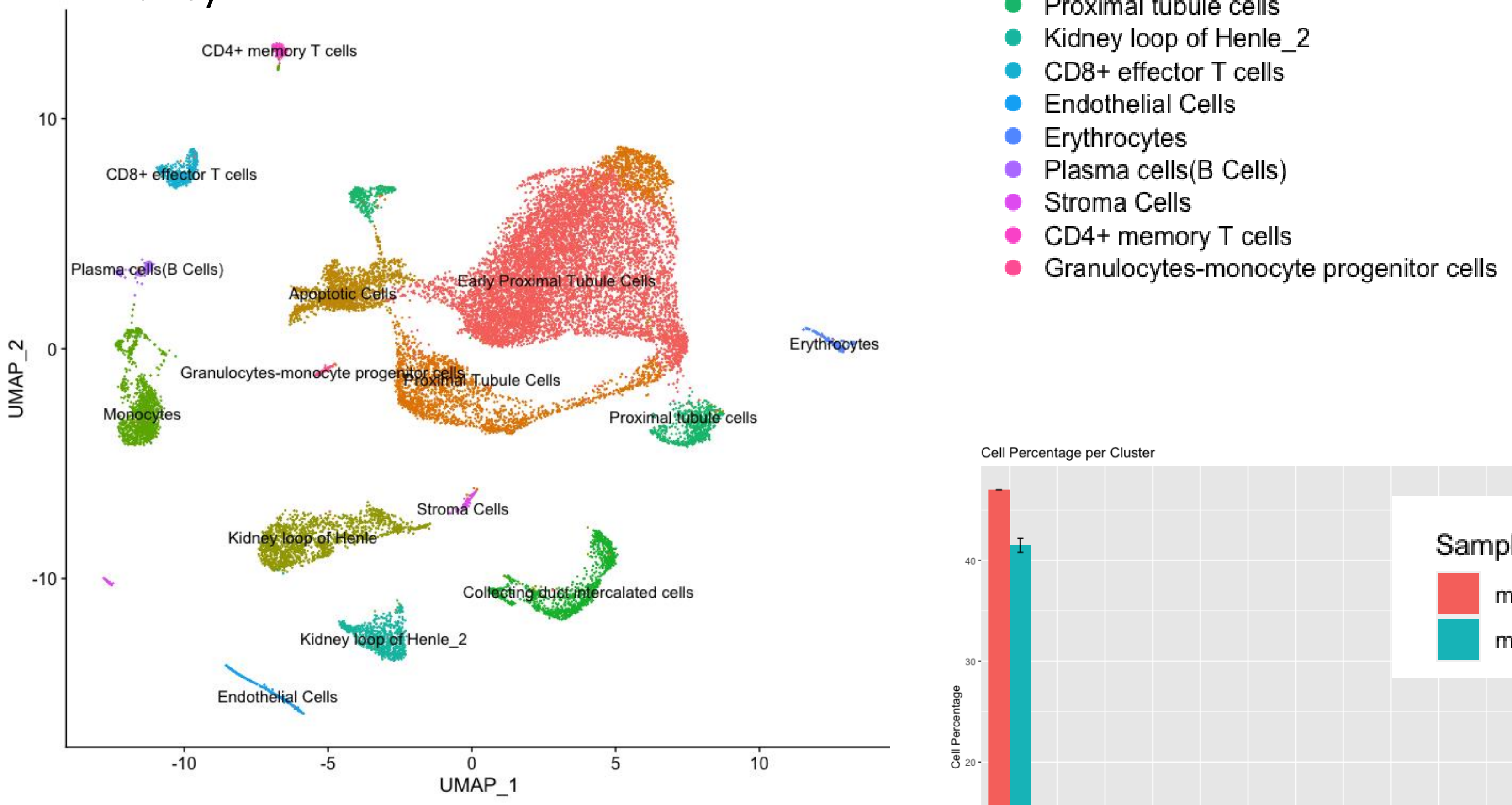
Automation or manual chip loading

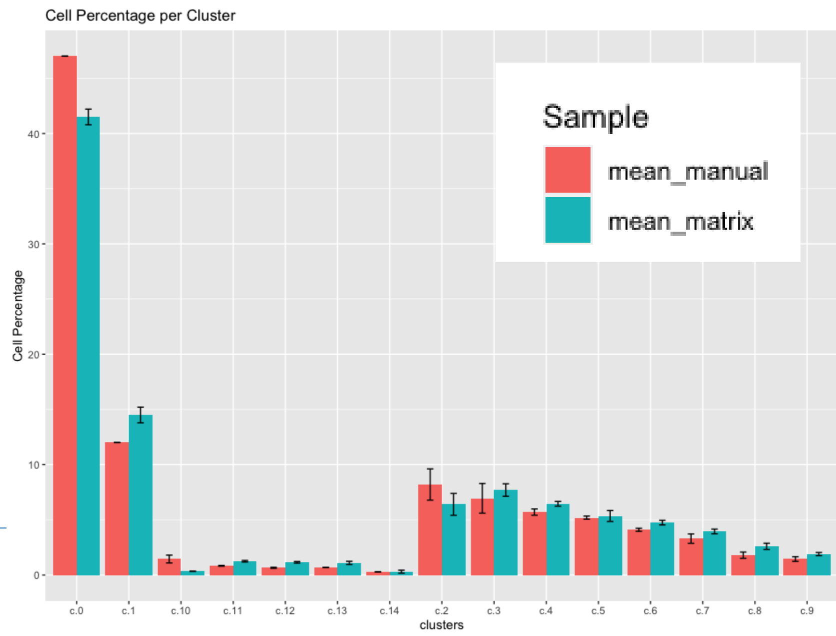
Matrix

VS

Manual

Kidney





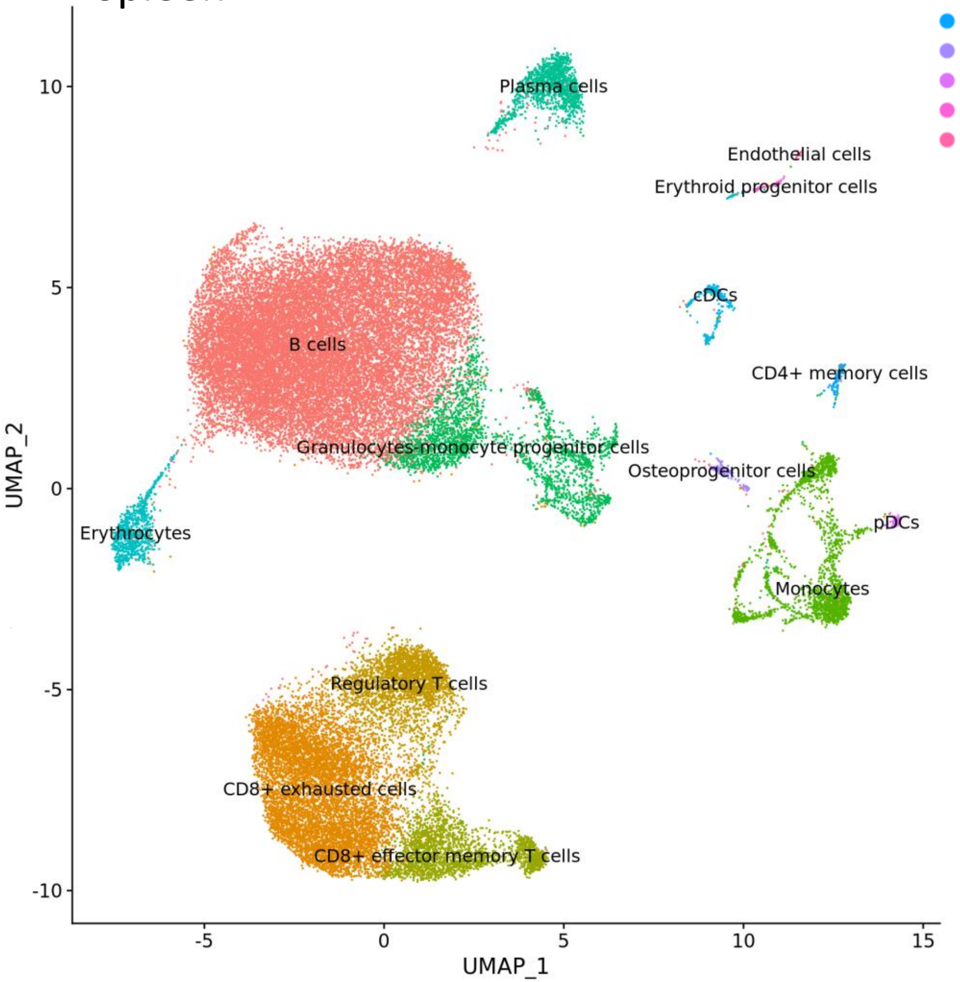
Automation or manual chip loading

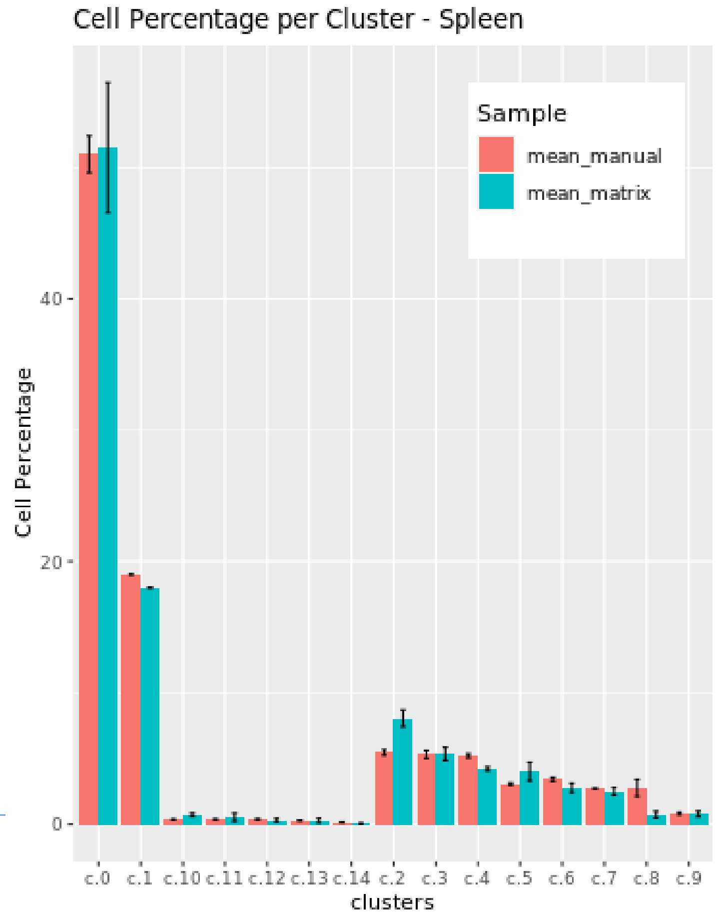
Matrix

VS

Manual

Spleen





Singleron's Service

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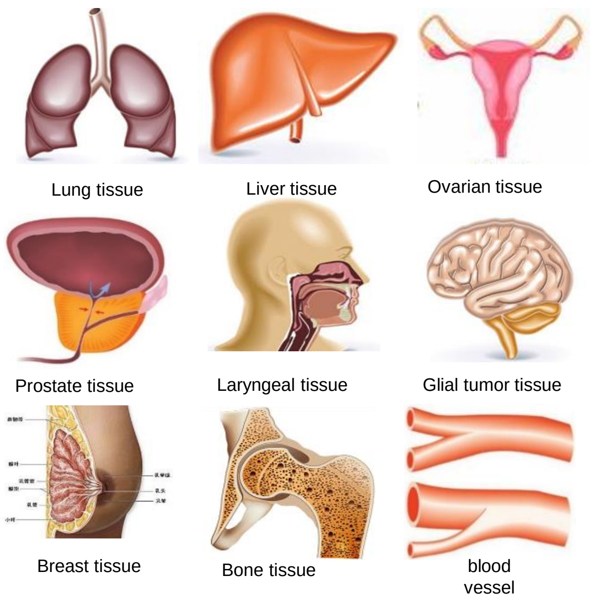
Singleron scRNAseq Service

Bringing groundbreaking single-cell analysis technologies to clinics

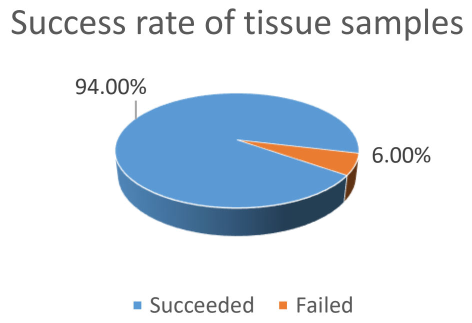
3000+ research projects

- ❑ **500+** customer organizations (~80% are hospitals)
- ❑ 170,000,000+ single cell sequenced
  - ❑ Tissue samples
    - ❑ Surgical samples
    - ❑ Biopsy samples
  - ❑ PBMC
  - ❑ Cell lines/primary cell culture
- ❑ Focus on human & mouse data

400 + different sample types*



High success rate (94%)



Success rate：

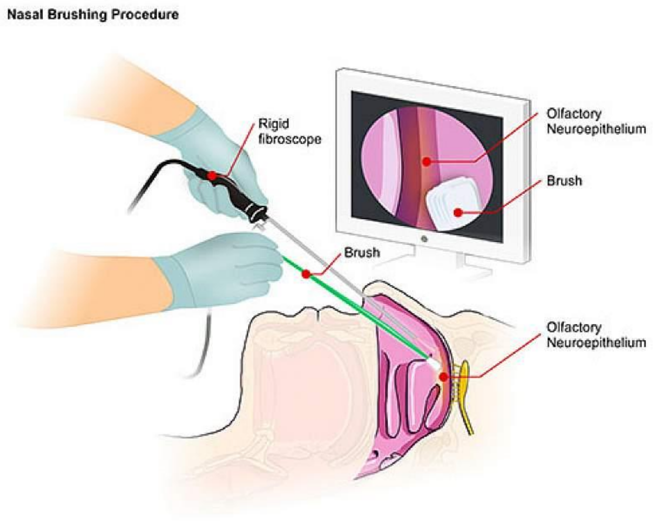
- ❑ Surgical samples：97.3%
- ❑ Biopsy samples：87%
- ❑ PBMC：100%

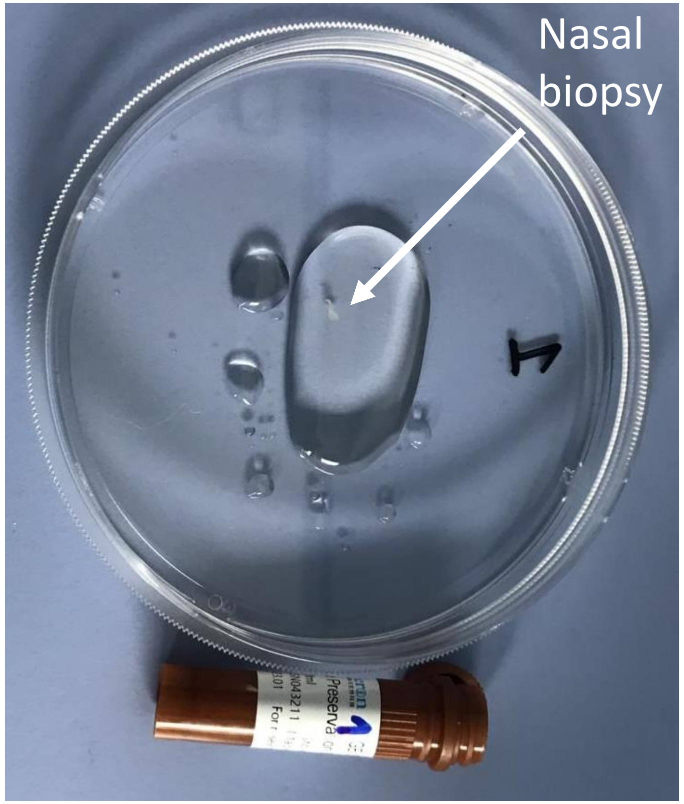
* Updated 03. 2021

Singleron Single cell Service

Example of sample processed by the service lab in Cologne:

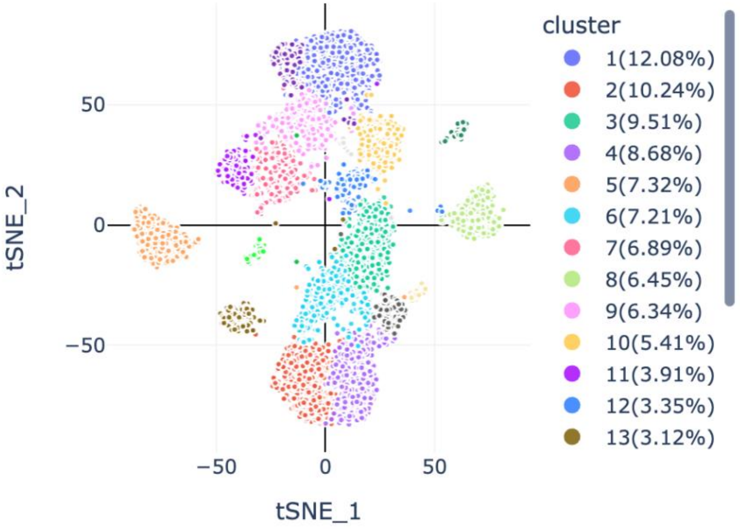
Nasal biopsy
Weight: not measurable
Storage: 24 -36 hours
Cell viability : 95%
Cell number: less than 20,000





Estimated Number of Cells	6,501
Fraction Reads in Cells	63.36%
Median UMI per Cell	4,784
Total Genes	28,169
Median Genes per Cell	1,652
Saturation	78.2%

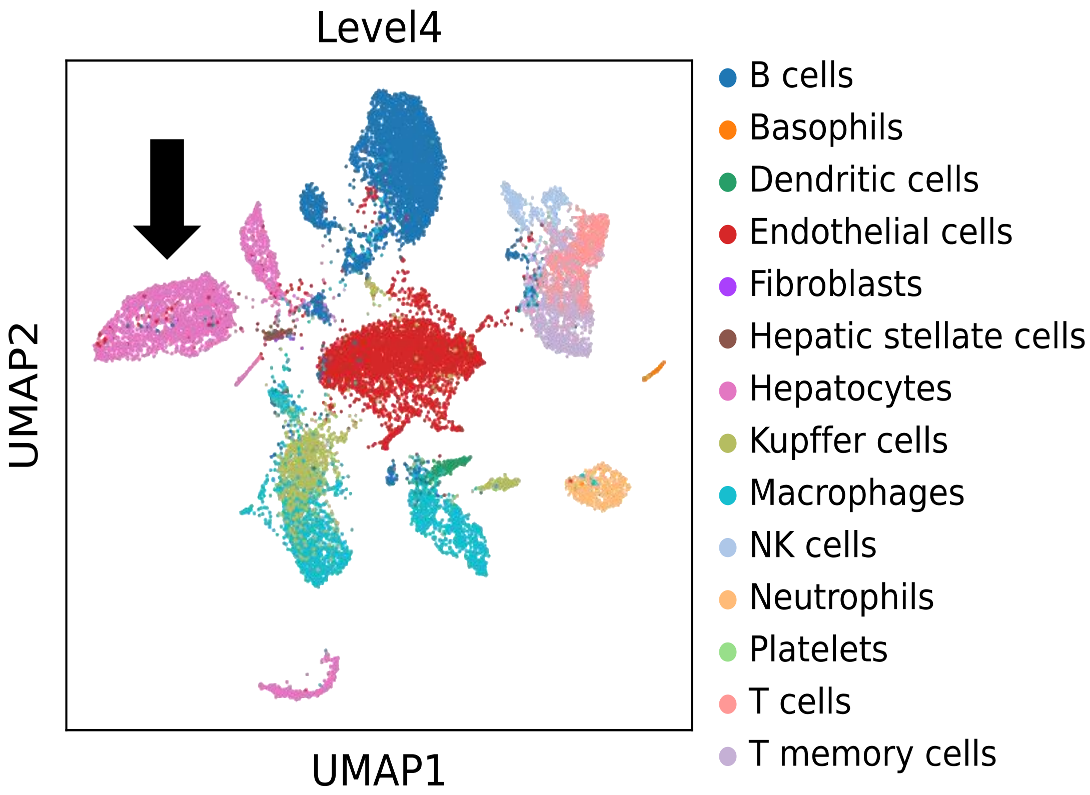
6 501 cells captured



Should I perform Single cells and/or single nuclei ?

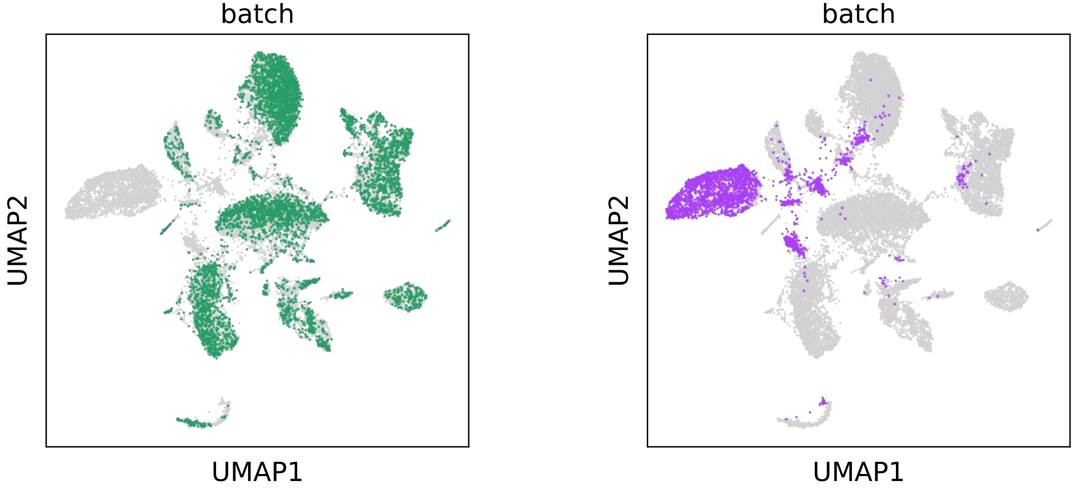
Single cell and/or Single nuclei

Mouse Liver



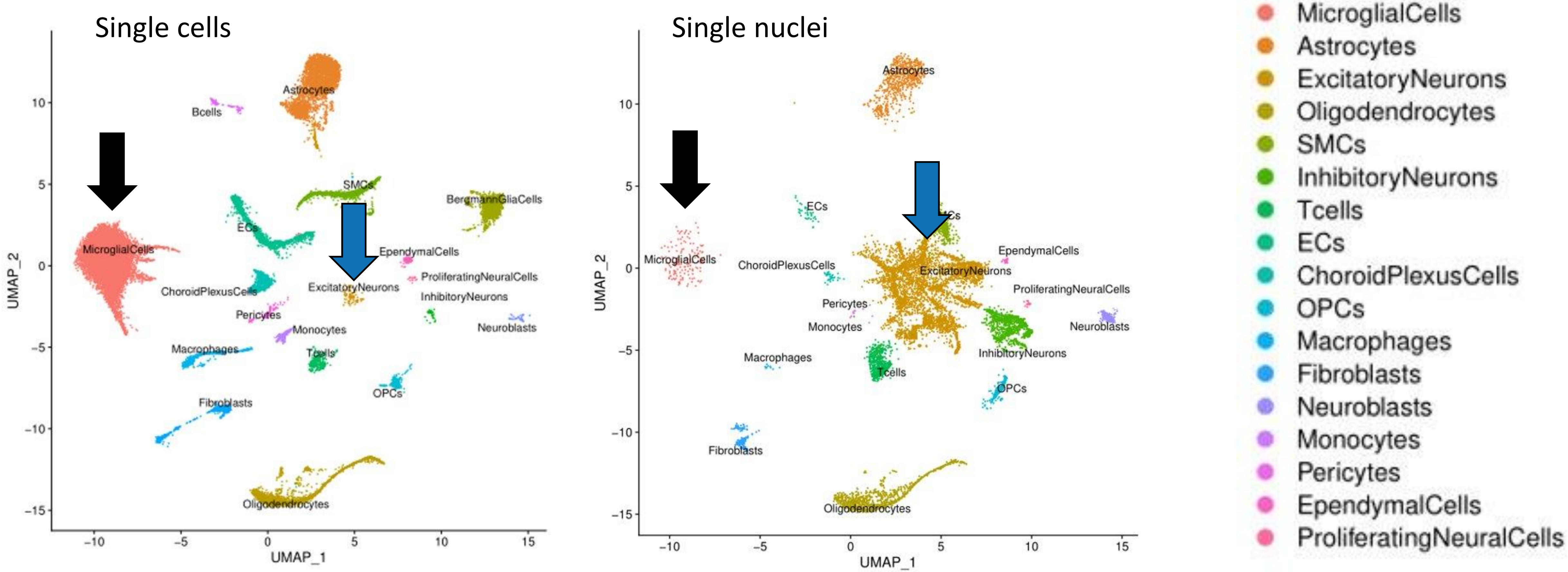
Single cells

Single nuclei

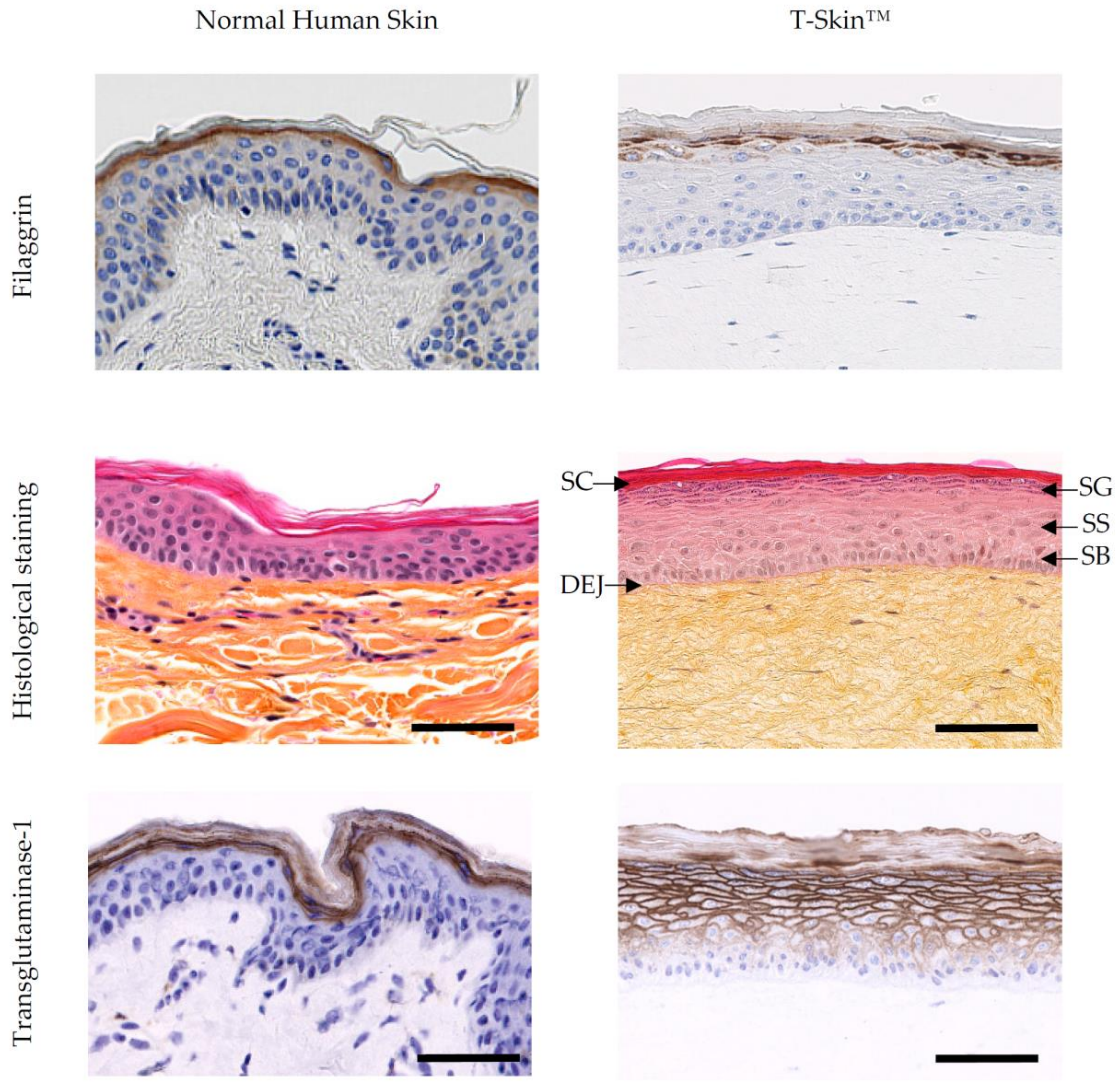


Single cell and/or Single nuclei

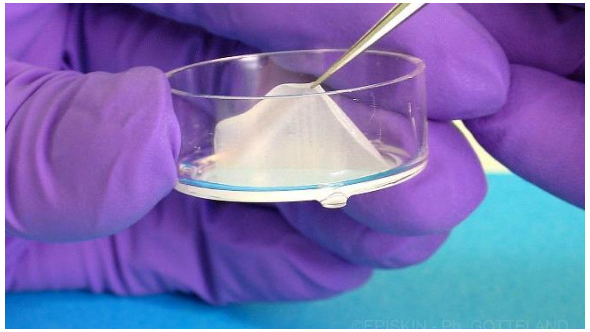
Mouse Brain

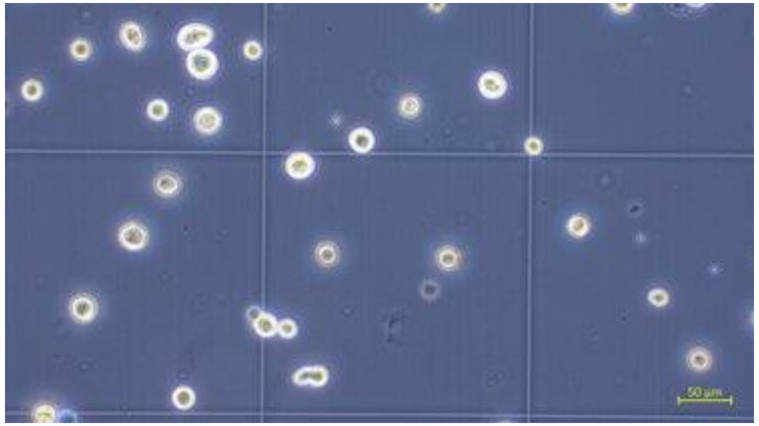


Model system to study human skin

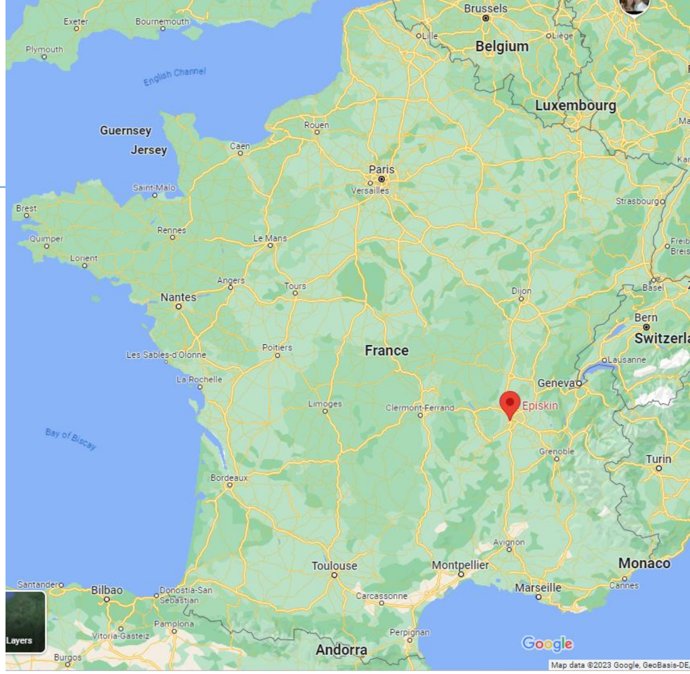


EpiSkin (human skin model)

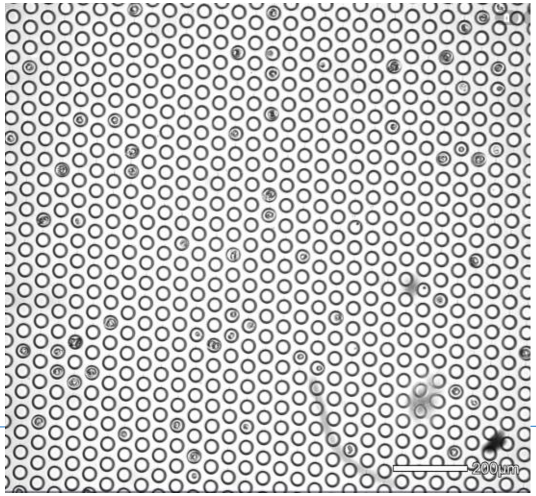


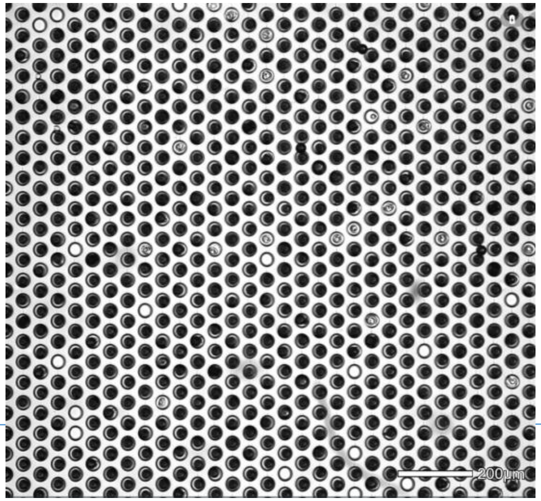


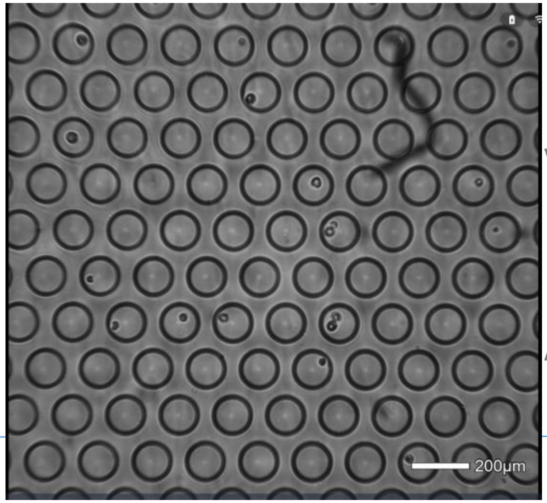
Tissue dissociation into single cell suspension

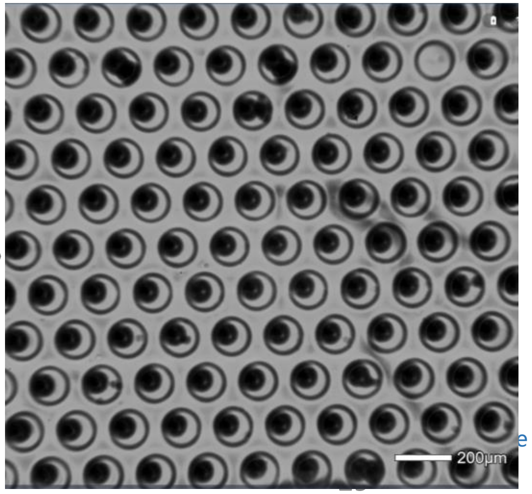


Cells were loaded on the SD chip









Multiomics applications

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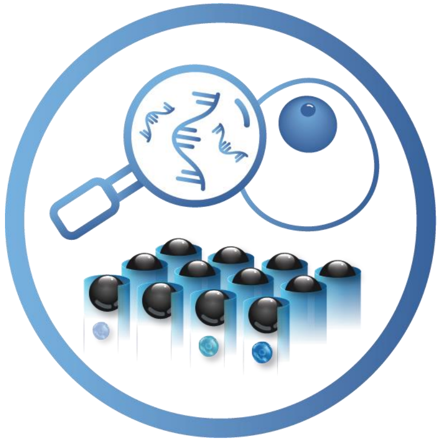
Singleron's Single Cell Multi-Omics Products



Transcriptome profiling

- *Single-Cell*
- *Single-Nucleus*
- *Yeast*

Transcriptome + target sequences

- *Lung/ Blood Cancers*
- *CHIP*
- *EBV Virus*
- *Customizable*

Transcriptome + Glycosylation

- *Detect Surface Proteoglycans*
- *Cell-Level Sensitivity*

Transcriptome + V(D)J profiling

- *CDR3 Profiling*
- *Full-Length TCR/BCR*

Transcriptome With Temporal Resolution

-Cell- & Gene-Level Sensitivity
-Detect Newly Synthesized Transcripts

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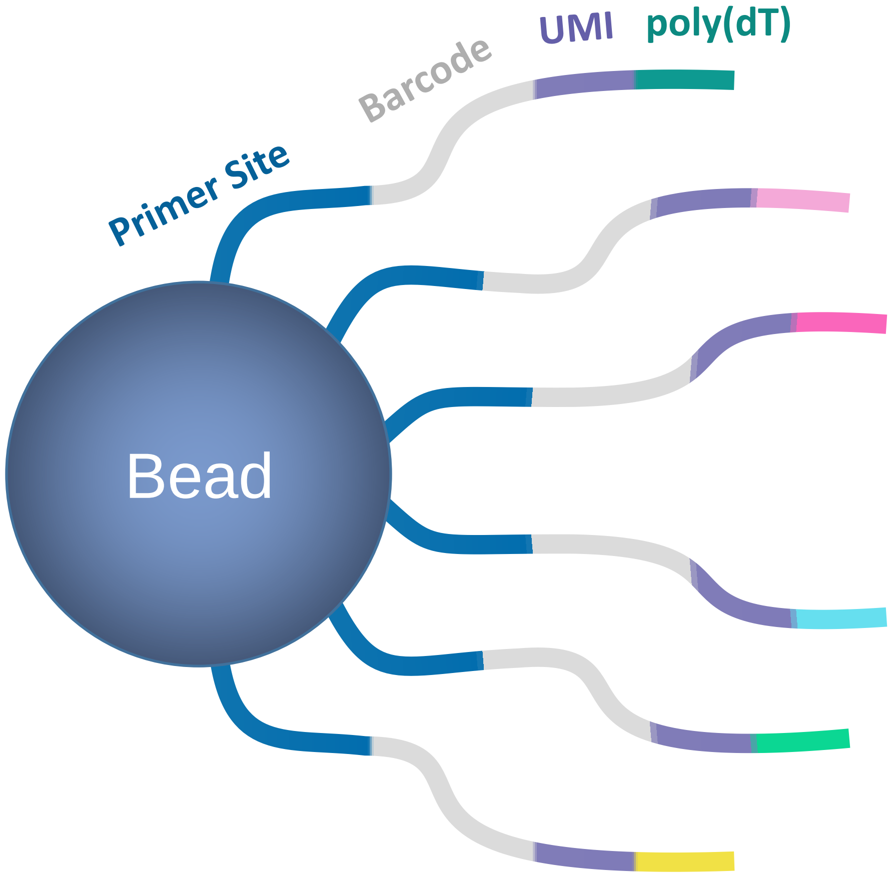
GEXSCOPE® Variations: More Than Just mRNA Profiling

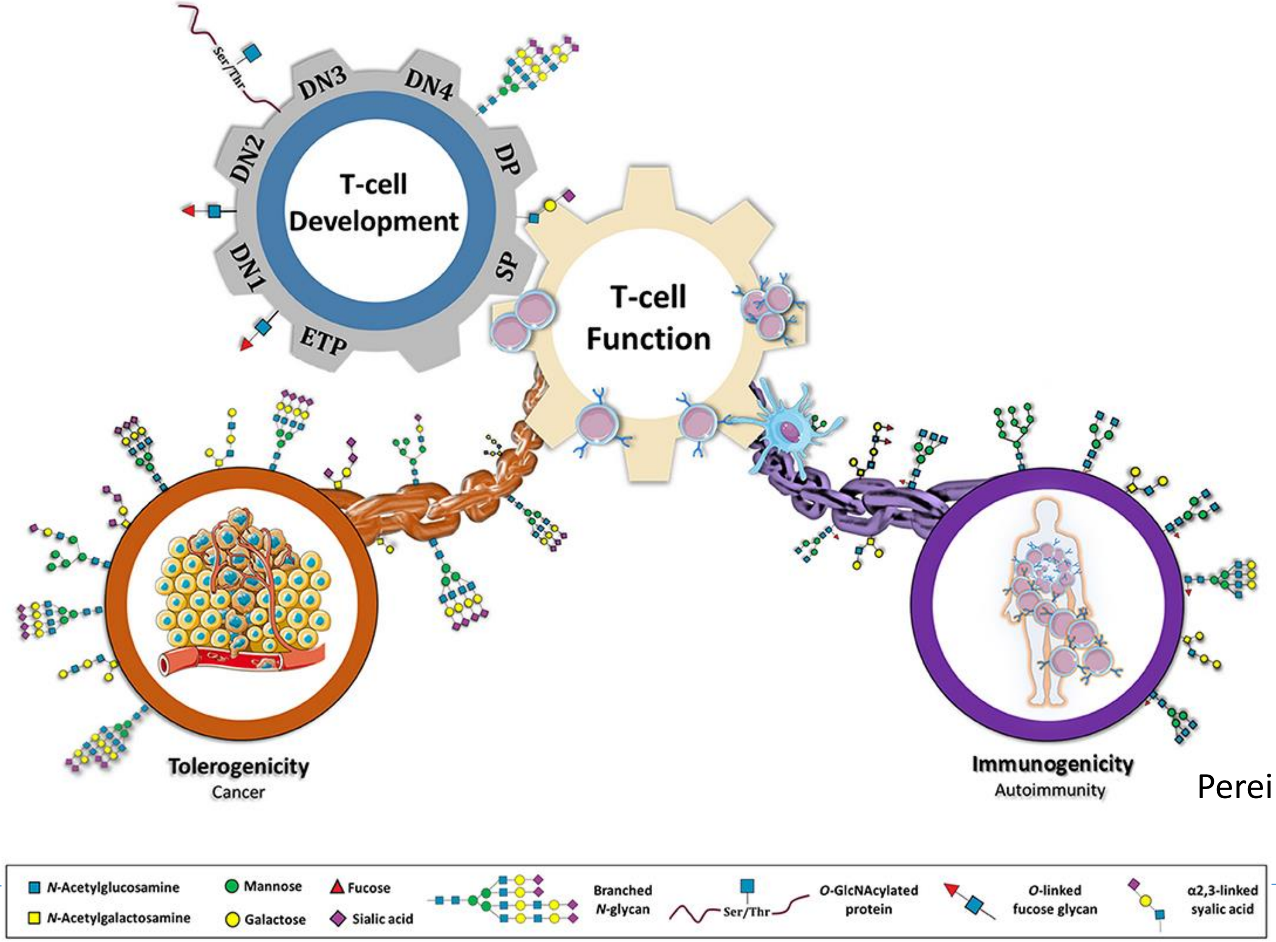


mRNA profiling	GEXSCOPE®
Immune V(D)J Profiling	GEXSCOPE® V(D)J
Full-Length Immunoreceptor Profiling	sCircle™
SNVs, Fusion Genes, Rare Transcripts, And Viral Genes	FocuSCOPE®
Glycosylation Levels	ProMoSCOPE™
Nascent RNA Synthesis	DynaSCOPE®

Cell surface glycosylation

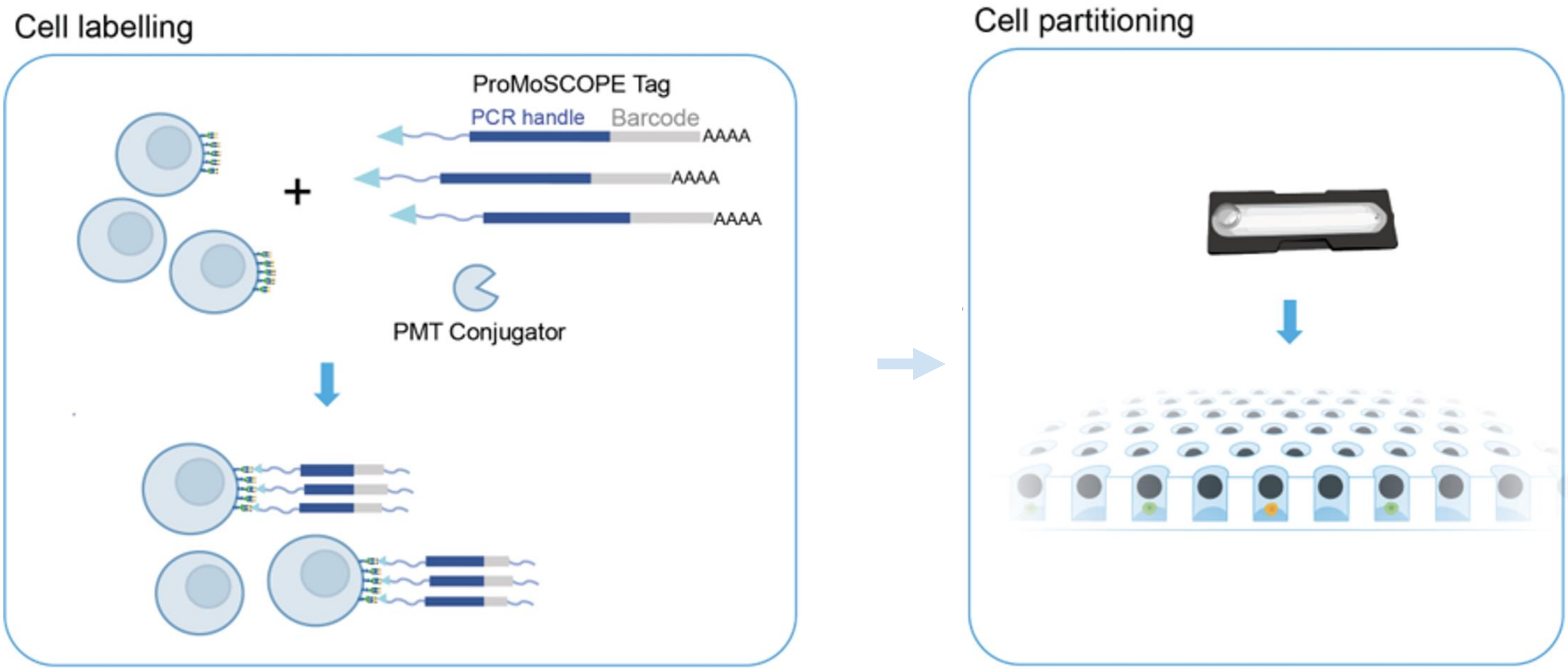
Why monitor the glycan abundance at the surfacce of the cell?

- Cell – cell interactions
- Control T cells response: glycosylation at the heart of immune-unbalanced diseases?



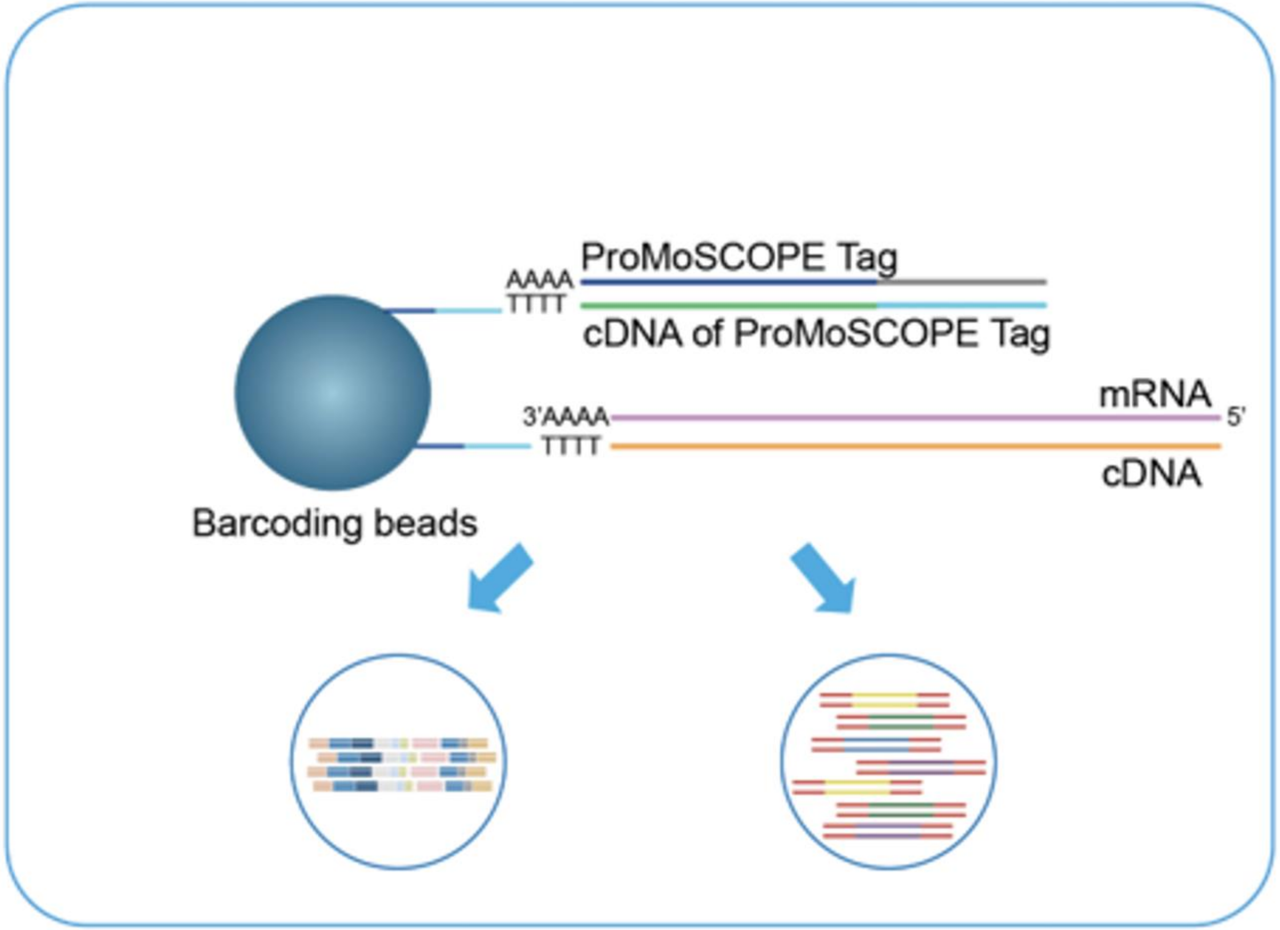
Pereira MS. Frontier in Immunology, 2018

Cell surface glycosylation combined with transcriptome

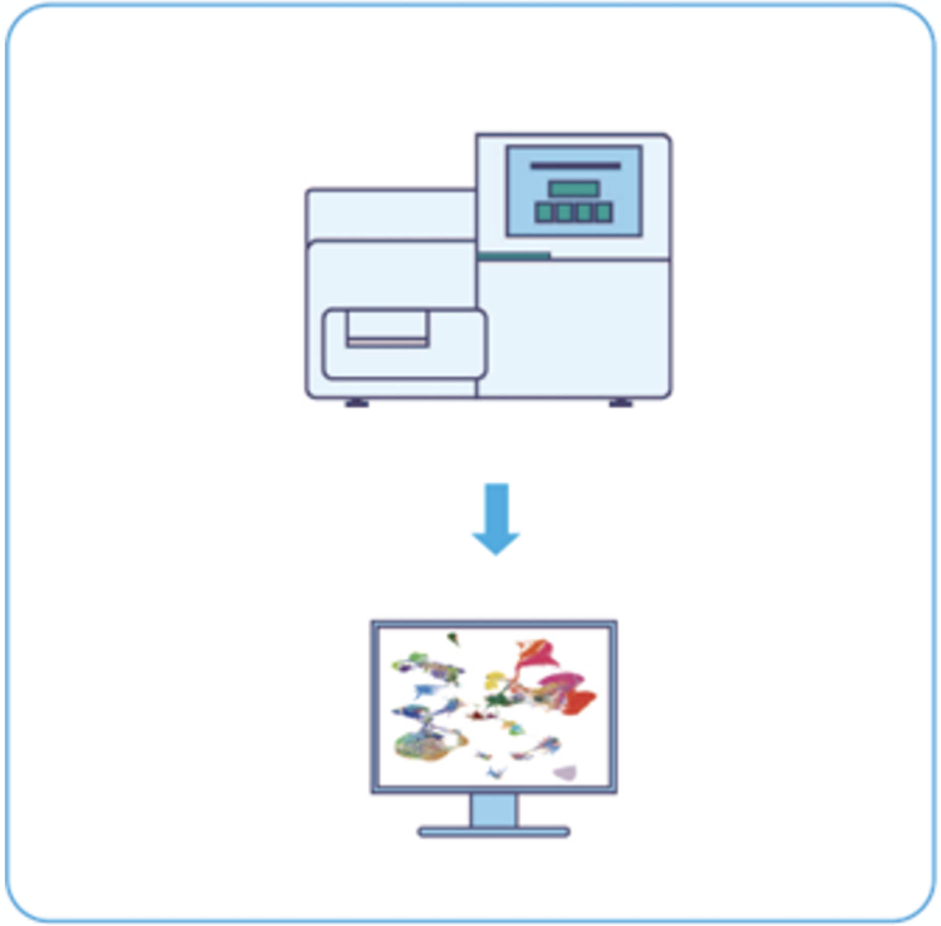


Cell surface glycosylation combined with transcriptome

mRNA capture, RT, library preparation

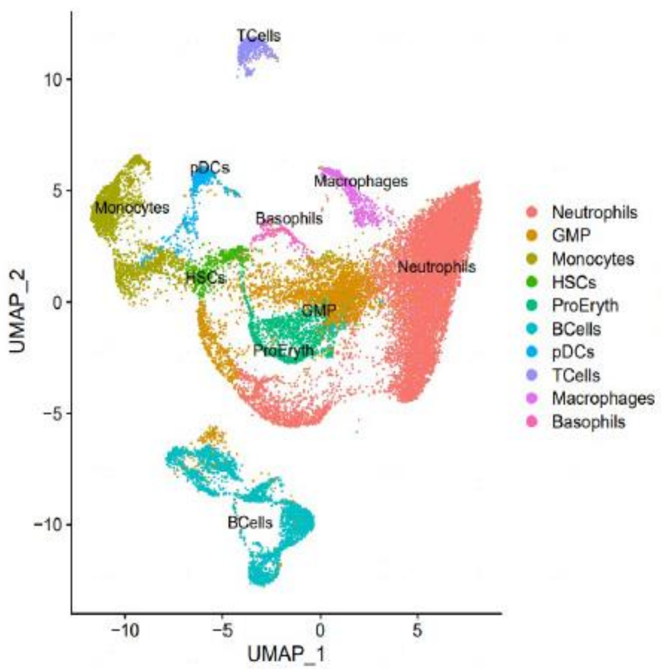


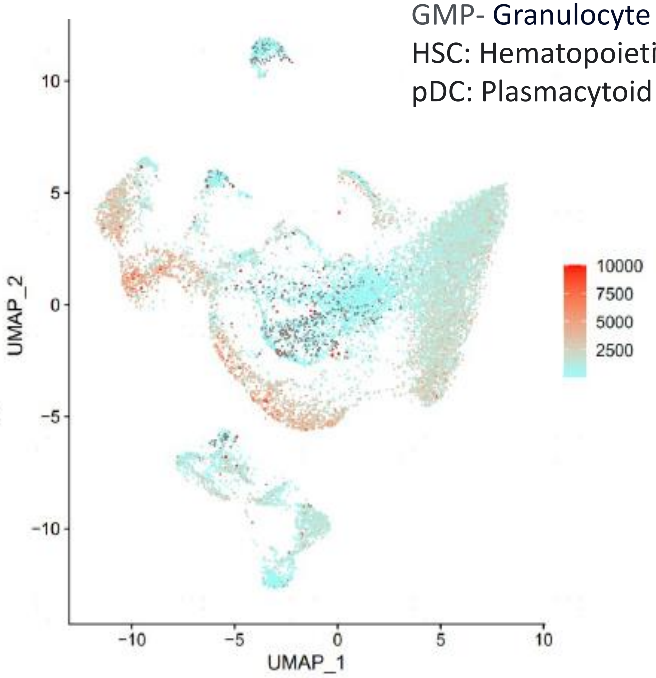
sequencing, data analysis



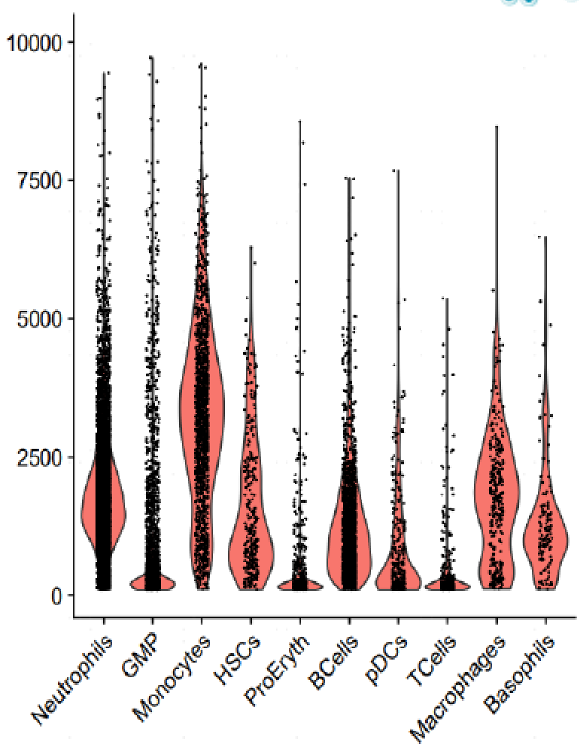
Cell surface glycosylation combined with transcriptome

Cell specific glycosylation abundance in Mouse Bone Marrow

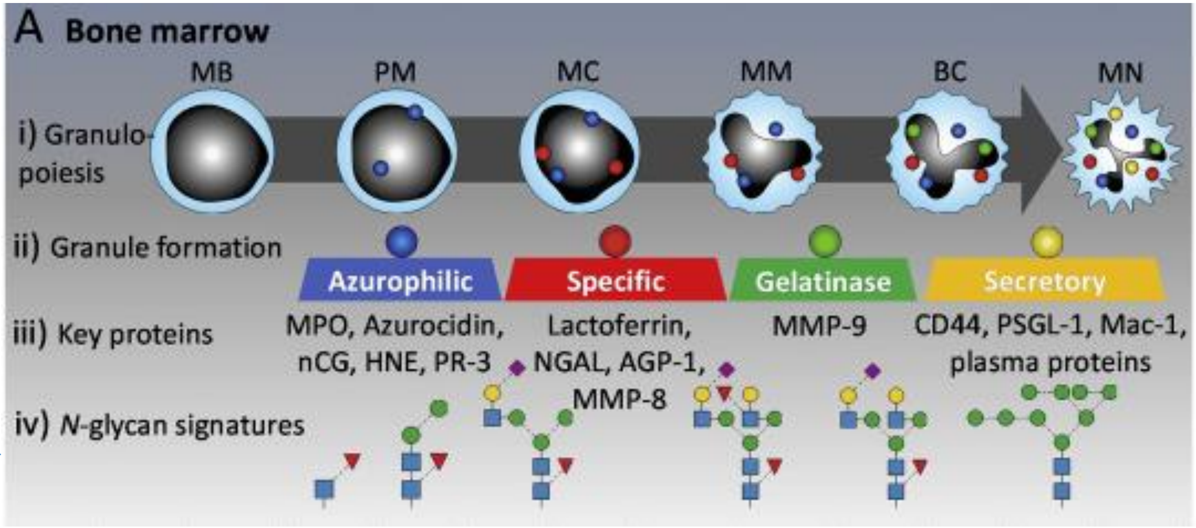




GMP- Granulocyte Monocyte Progenitors
HSC: Hematopoietic stem cells
pDC: Plasmacytoid dendritic cells



During granulopoiesis in the bone marrow, distinct neutrophil granules are successively formed. Distinct receptors and effector proteins, many of which are **glycosylated**, are targeted to each type of granule according to their time of expression, a process called "targeting by timing."



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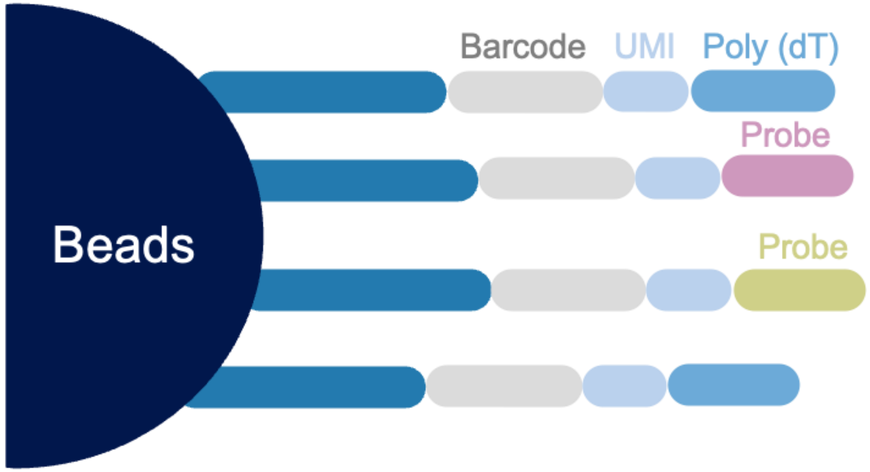
Single Cell Multiomics – Detection of somatic mutations



Lung cancer*	Clonal hematopoiesis*	Blood cancer*	Epstein-Barr Virus*	Custom
EGFR	DNMT3A	WT1	EBNA1	
KRAS	TET2	KRAS	EBNA2	
PIK3CA	ASXL1	IDH1/IDH2	EBER1	
BRAF	JAK2	TP53	EBER2	
TP53	TP53	BCR_ABL1	ZEBRA	
		PML_RARA		

*Specific mutation sites are targeted in the genes showed above.

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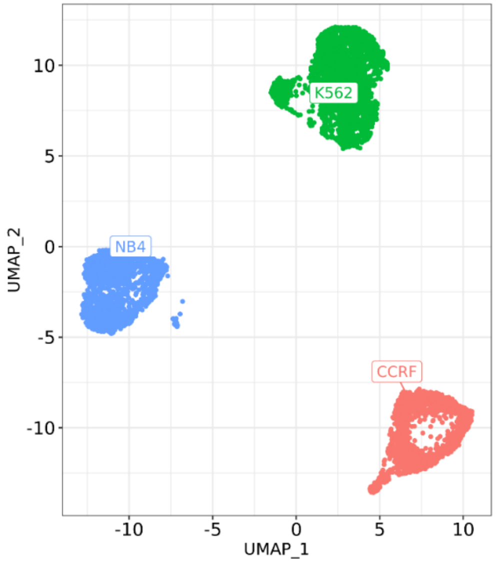
Detection of Different KRAS Mutations

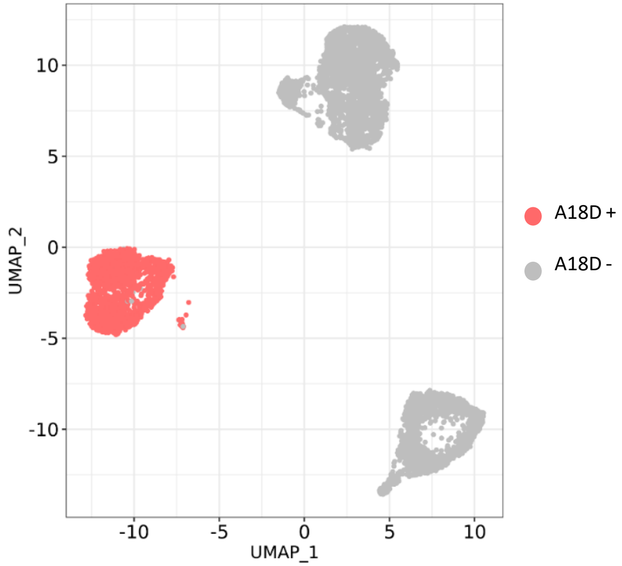
Experimental setup: NB4, CCRF and K562 were mixed in equal proportions.

NB4 cell line contains KRAS (A18D) and TP53 (R248Q) mutations and PML-RARA fusion gene.
CCRF cell line contains KRAS (G12D) and TP53 (R248Q, R175H) mutations.
K562 cell line contains BCR-ABL1 fusion gene.

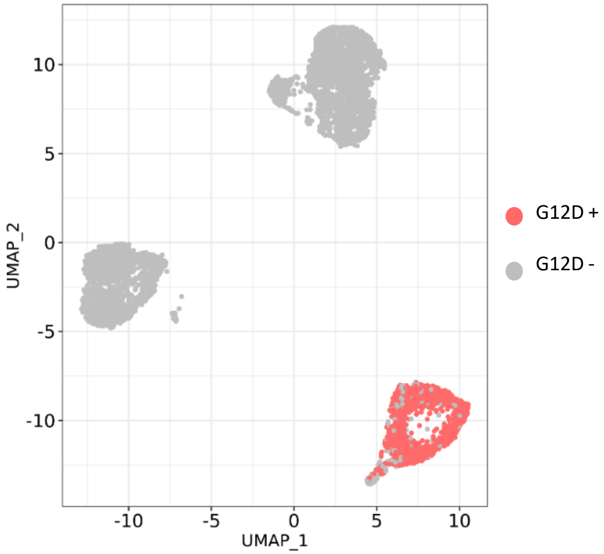
Cell Annotations



A18D-KRAS



G12D-KRAS

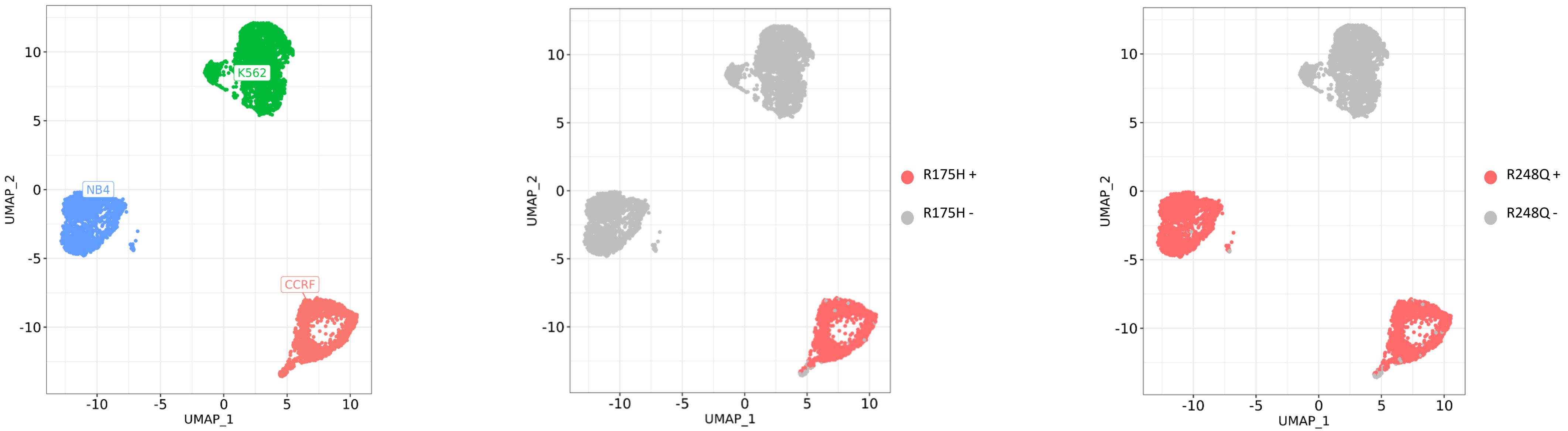


Detection of Different TP53 Mutations

Experimental setup: NB4, CCRF and K562 were mixed in equal proportions.

NB4 cell line contains KRAS (A18D) and TP53 (R248Q) mutations and PML-RARA fusion gene.
CCRF cell line contains KRAS (G12D) and TP53 (R248Q, R175H) mutations.
K562 cell line contains BCR-ABL1 fusion gene.

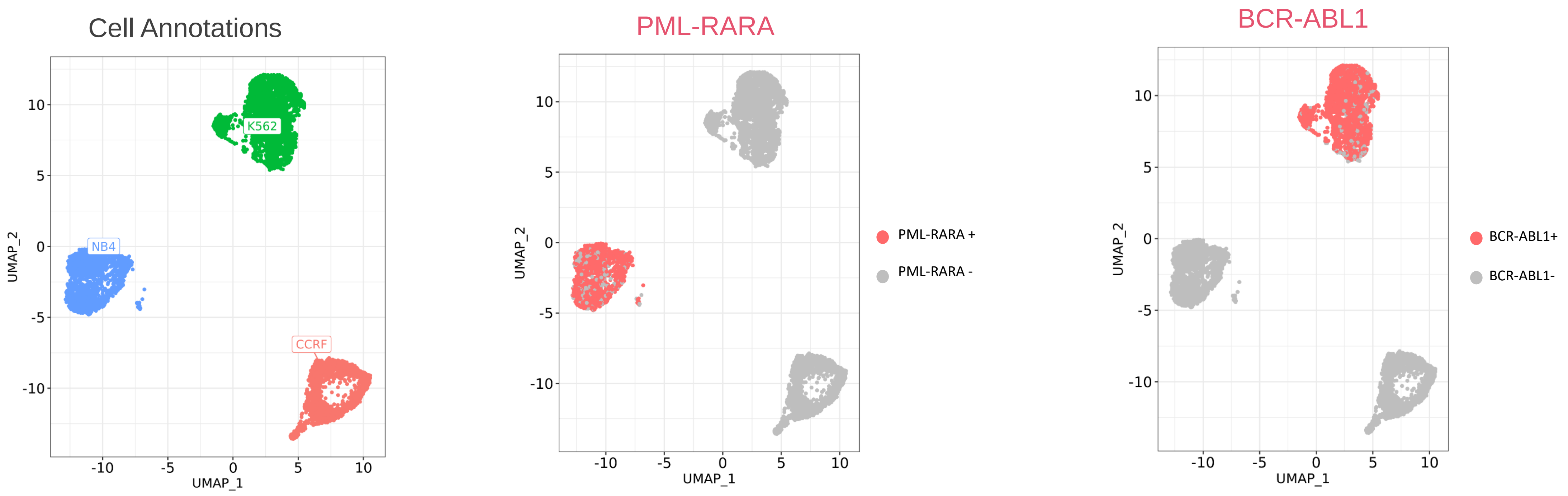
Cell Annotations | R175H TP53 | R248Q TP53



Detection of translocations

Experimental setup: NB4, CCRF and K562 were mixed in equal proportions.

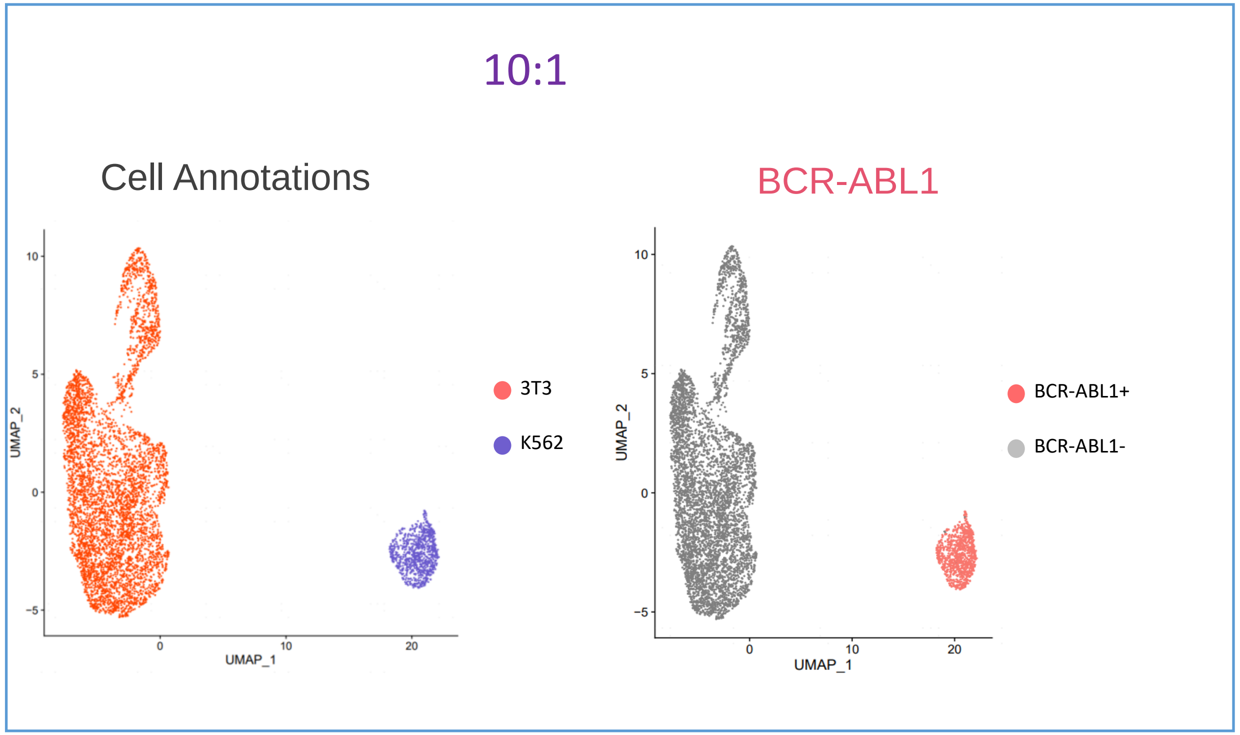
NB4 cell line contains KRAS (A18D) and TP53 (R248Q) mutations and PML-RARA fusion gene.
CCRF cell line contains KRAS (G12D) and TP53 (R248Q, R175H) mutations.
K562 cell line contains BCR-ABL1 fusion gene.

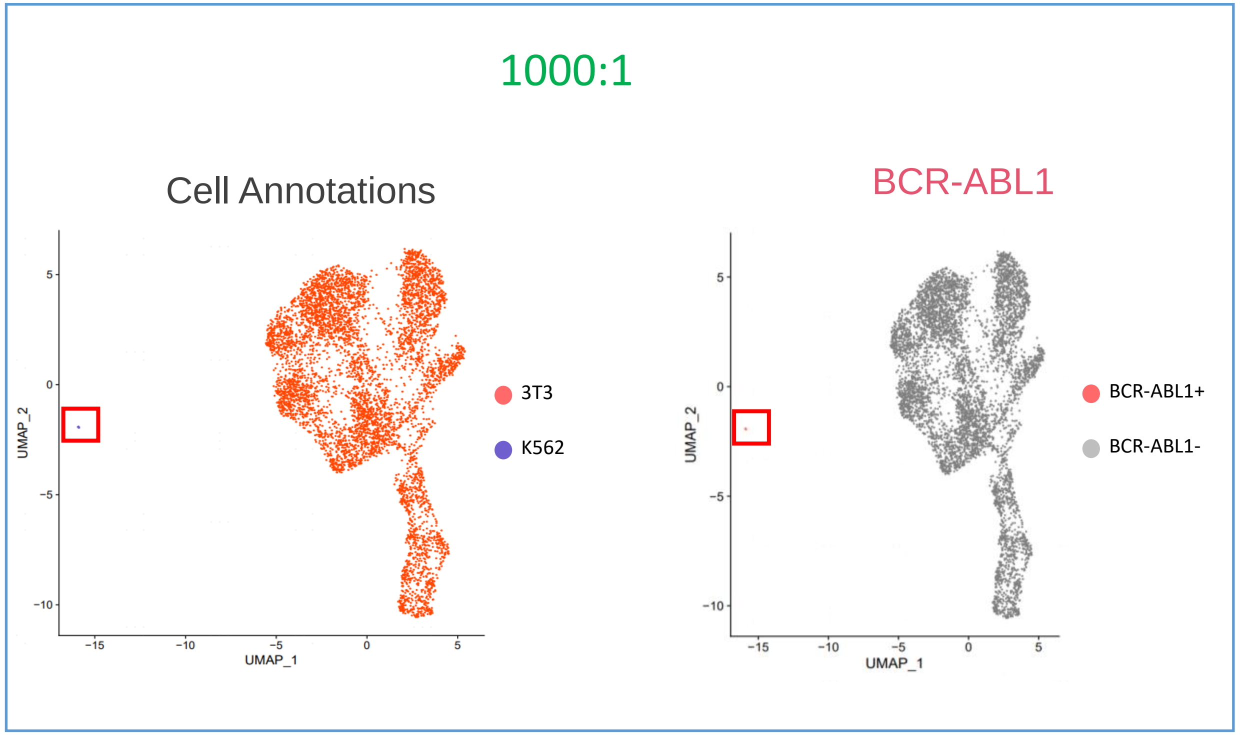


Sensitivity in detecting translocations

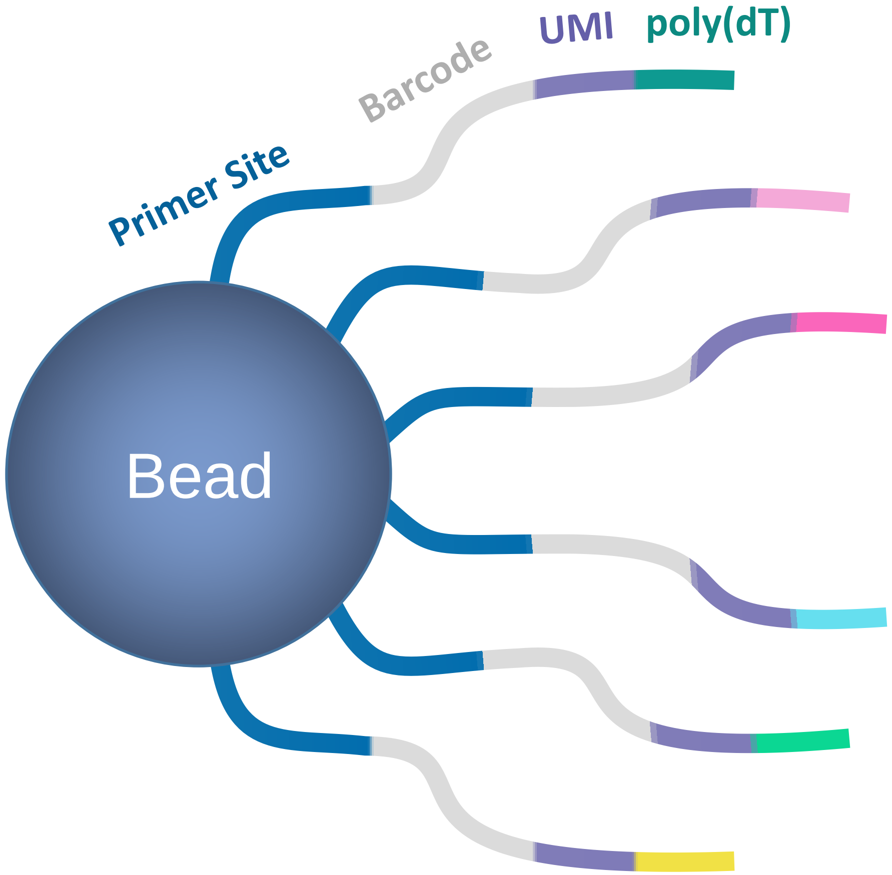
K562 cell line contains BCR-ABL1 fusion gene.
3T3 cell line doesn't contain BCR-ABL1 fusion gene.

Input Ratio	Total Captured Cell Number	3T3 Cells	K562 Cells	Fusion Gene Detected in K562
10:1	6188	5240	948	941 (99%)
100:1	7498	7362	136	136 (100%)
1000:1	5826	5816	10	10 (100%)





GEXSCOPE® Variations: More Than Just mRNA Profiling



mRNA profiling	GEXSCOPE®
Immune V(D)J Profiling	GEXSCOPE® V(D)J
Full-Length Immunoreceptor Profiling	sCircle™
SNVs, Fusion Genes, Rare Transcripts, And Viral Genes	FocuSCOPE®
Glycosylation Levels	ProMoSCOPE™
Nascent RNA Synthesis	DynaSCOPE®

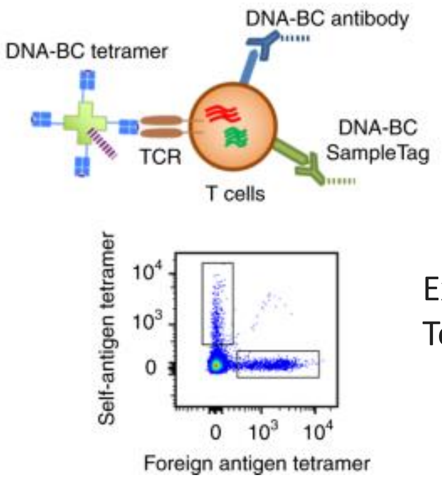
Immune profiling

Learn how the immune system responds and evolves in relation to diseases.

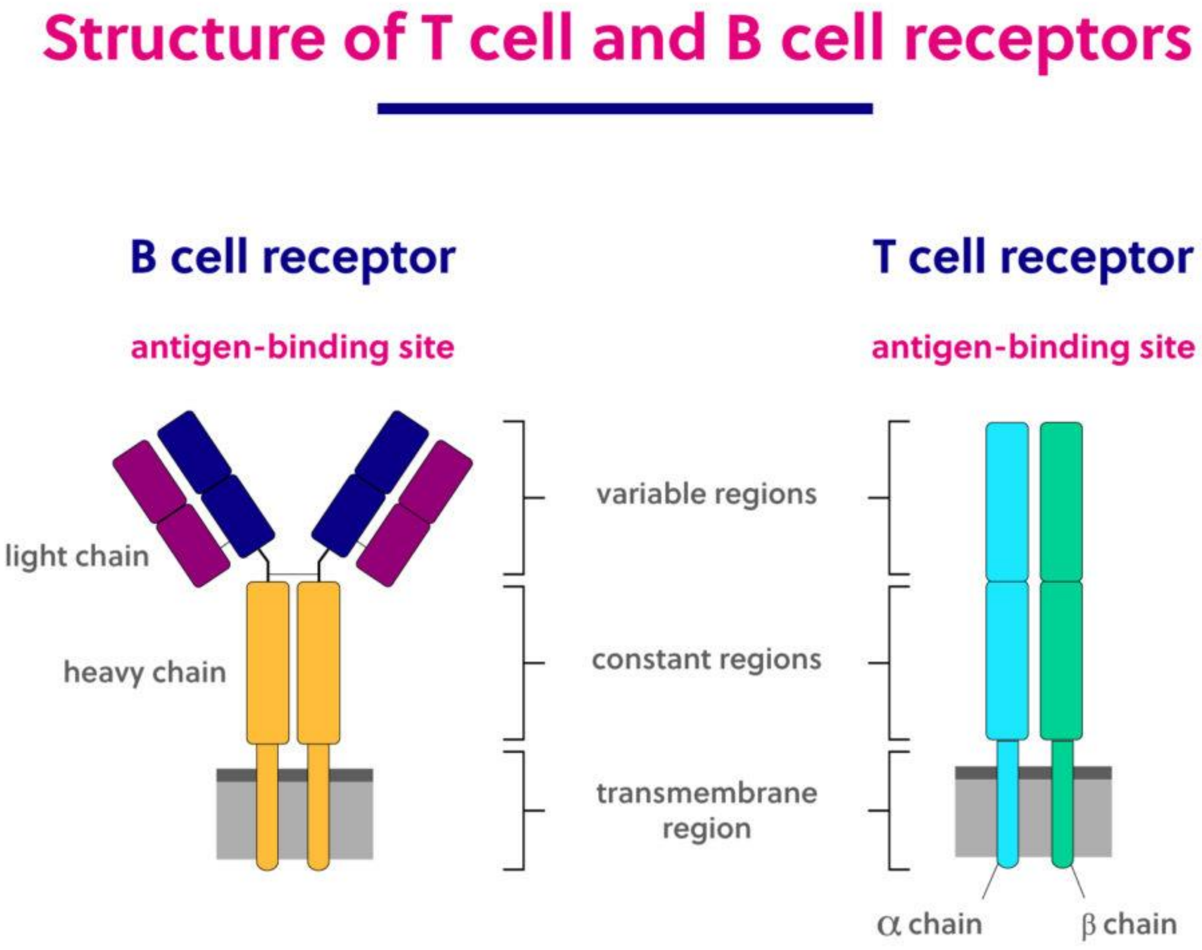
Help decide on treatments and understand disease progression or response to drugs.

Understand the pathway of activation related to specific clonotypes.

Engineer immune cells to destroy cancer cells: T cell receptor (TCR) T cell therapy



Example of TetTCR-Seq



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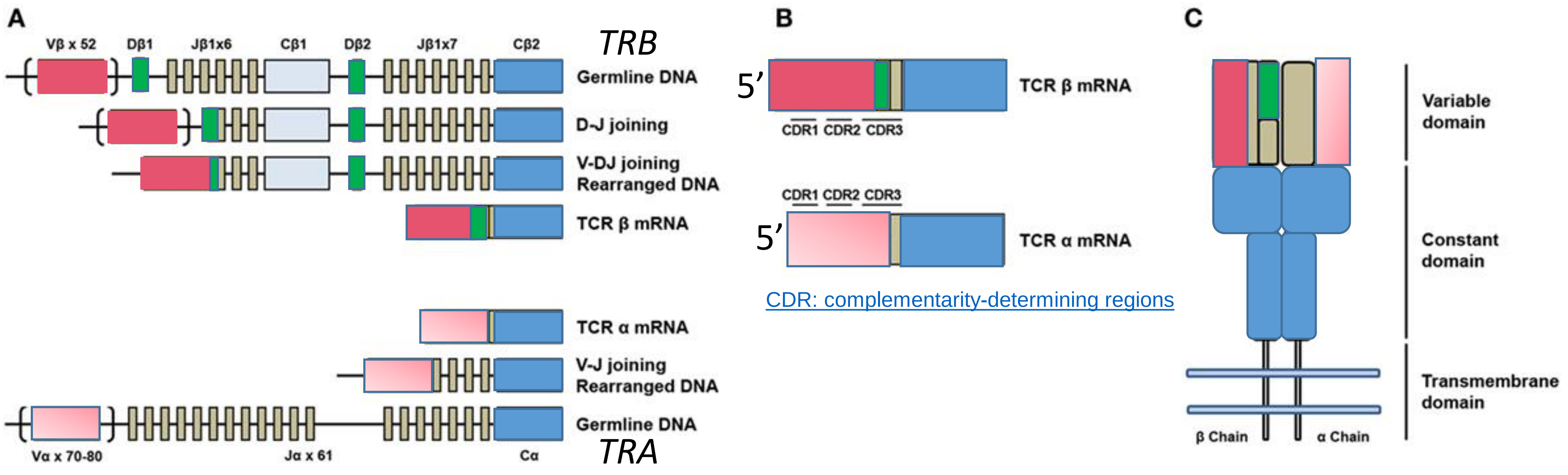
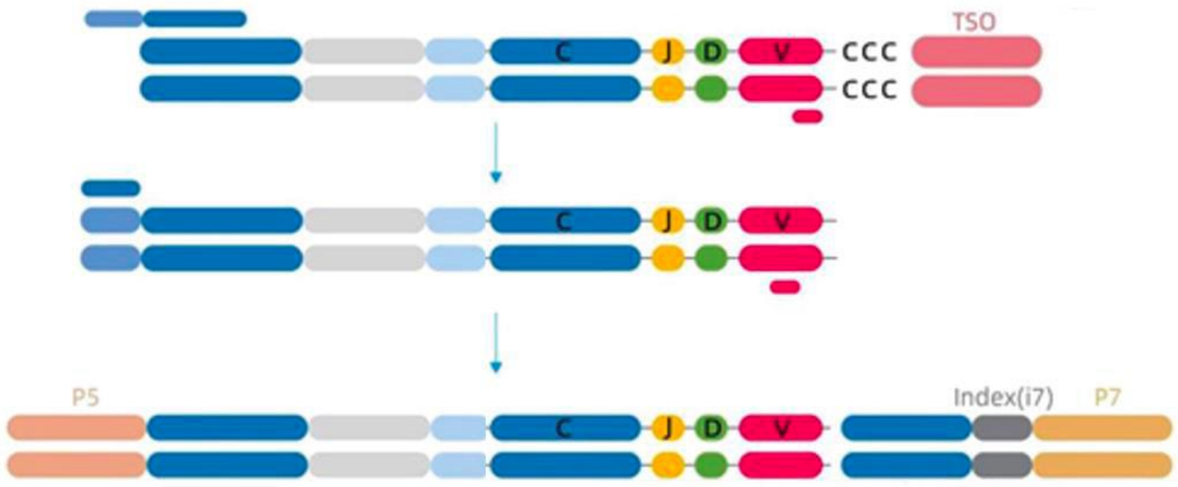
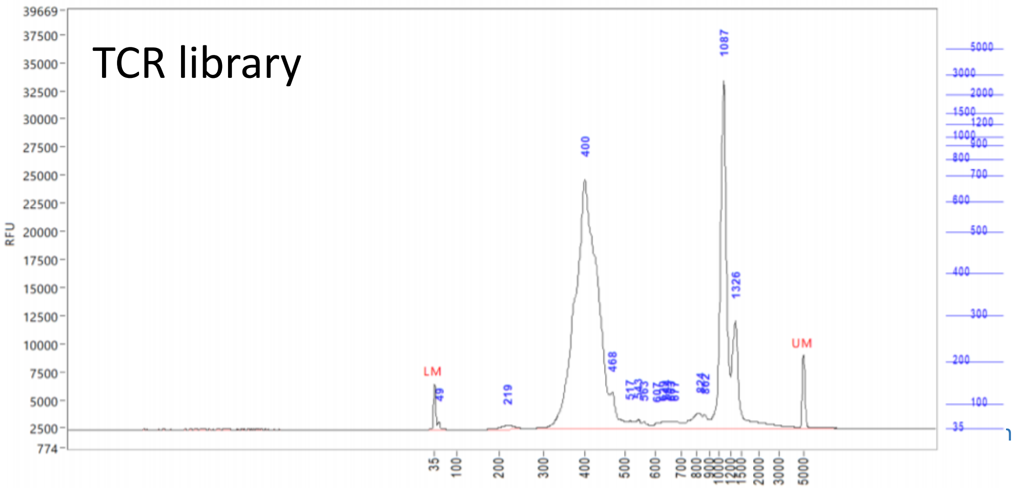
T cell Repertoire







medicine

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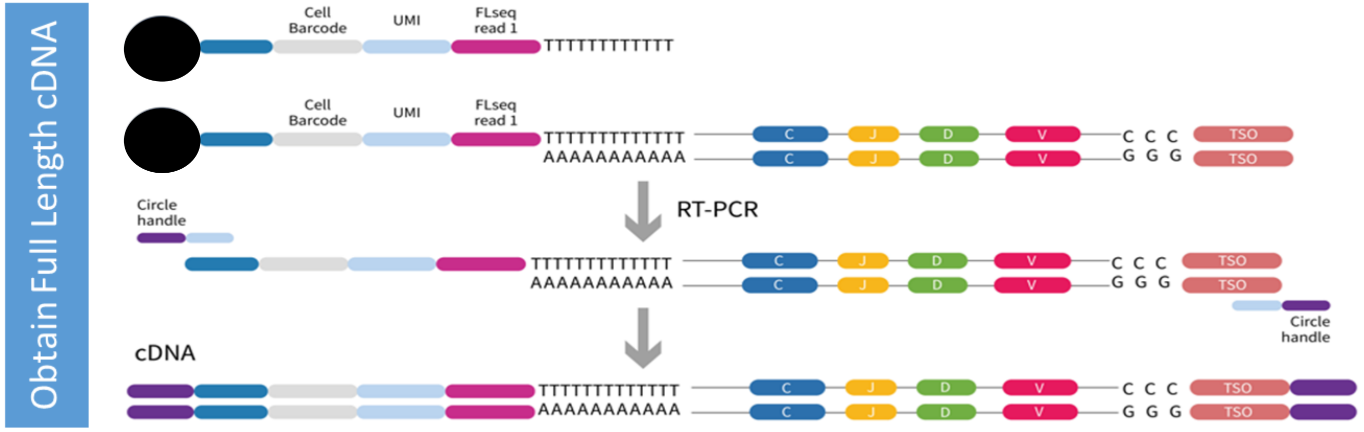
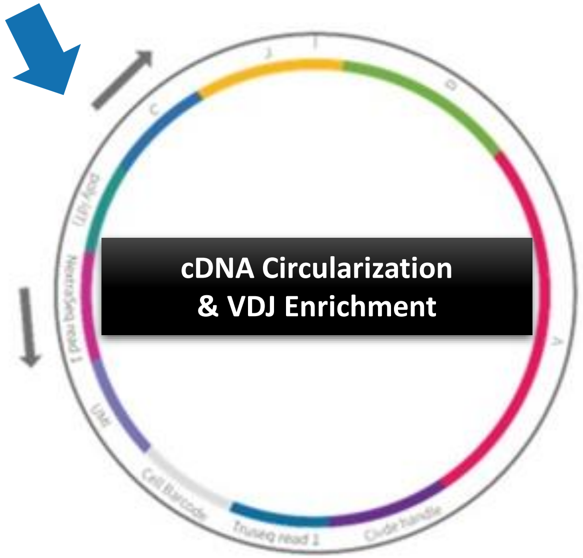
sCircle: Full length immunoreceptor sequencing



Transcriptome library construction



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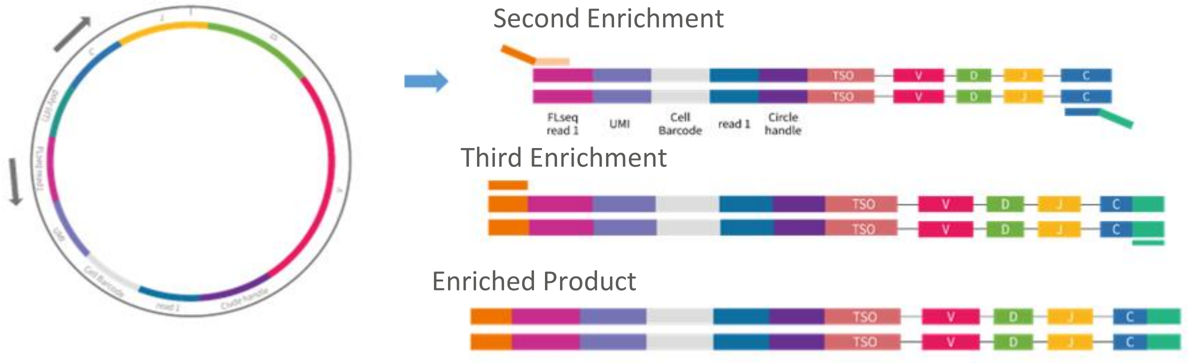
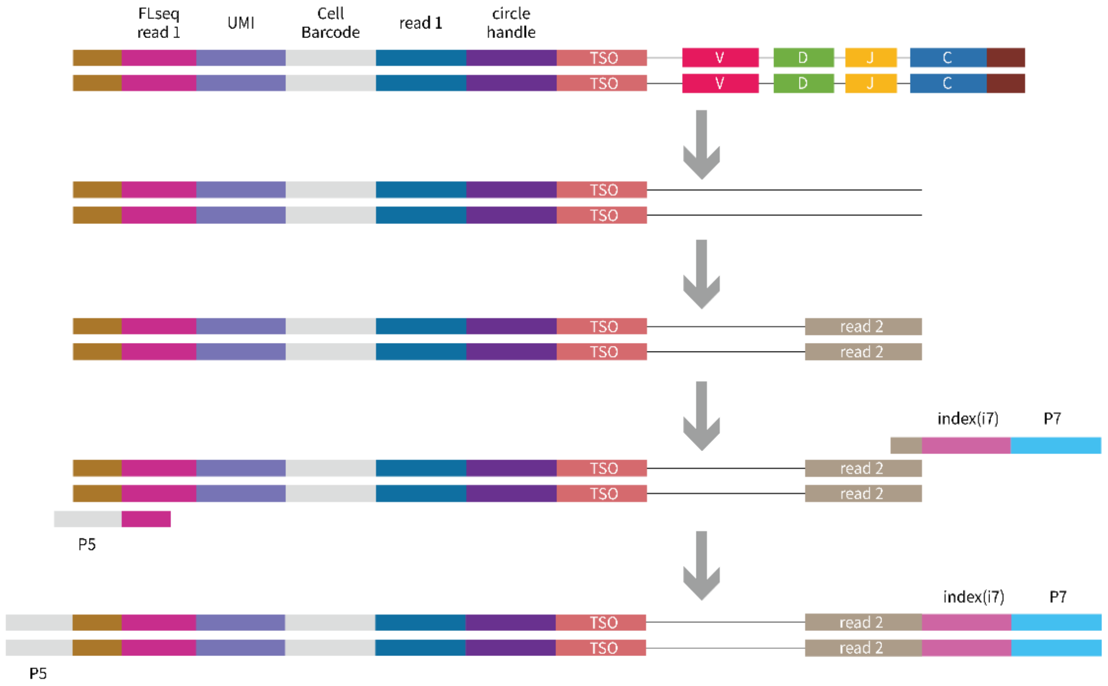
sCircle: Full length immunoreceptor sequencing

Immunoreceptor Enrichment

Circularization And First Enrichment

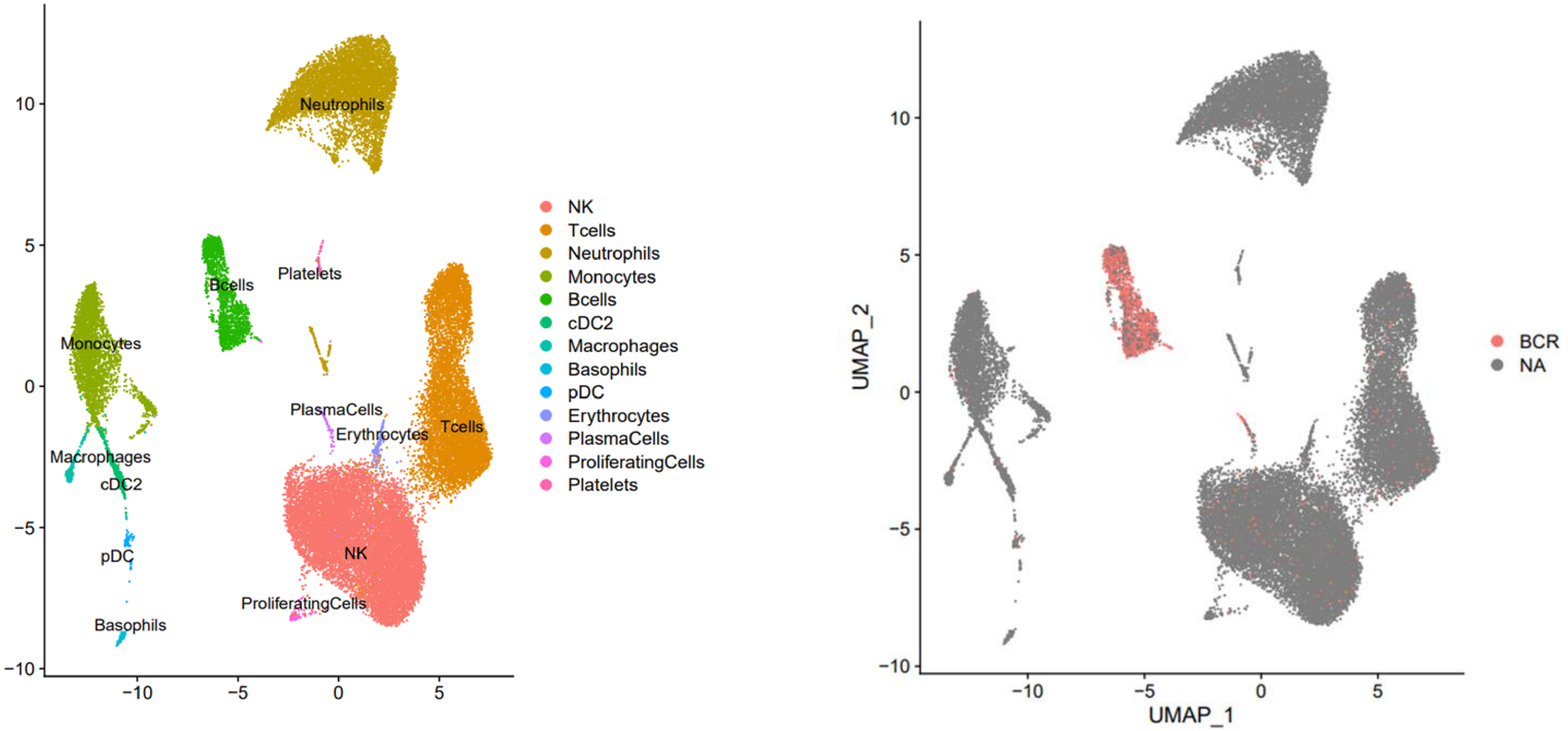


Library Preparation

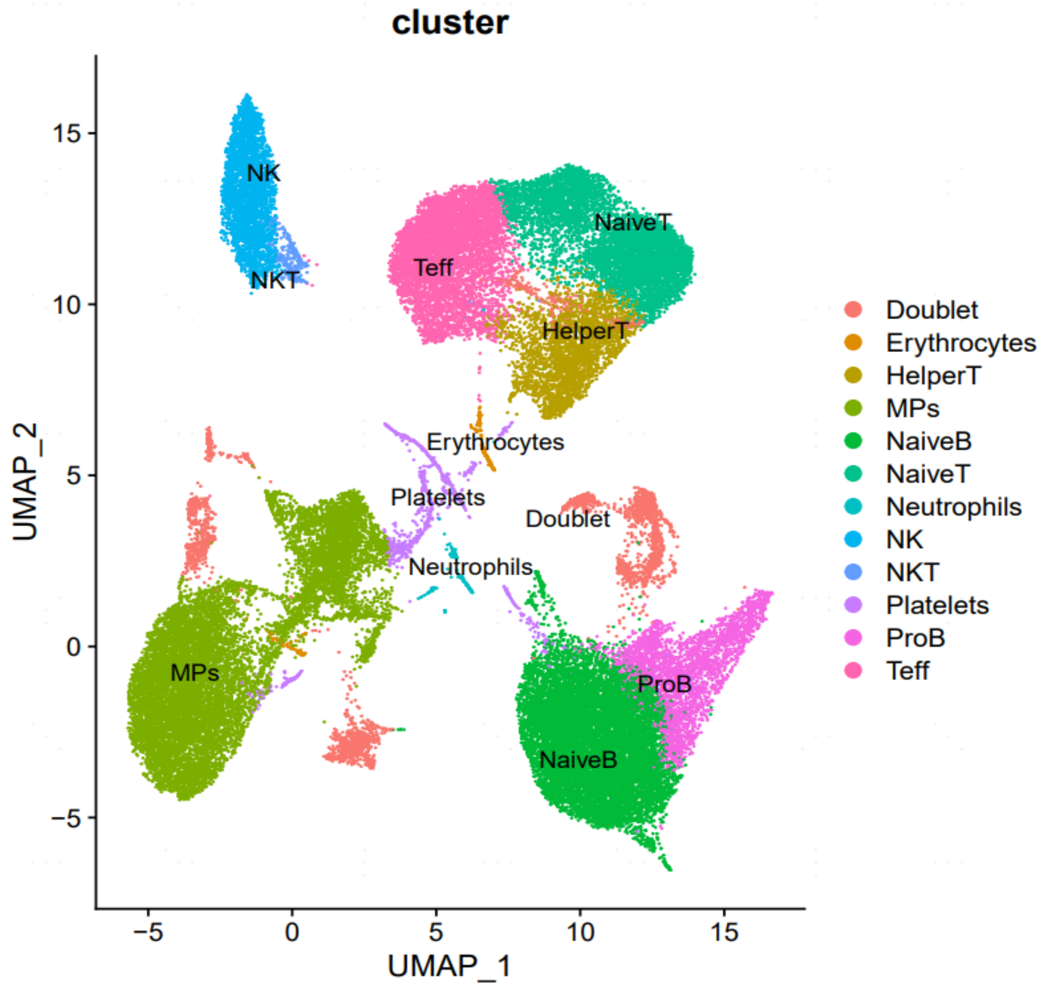


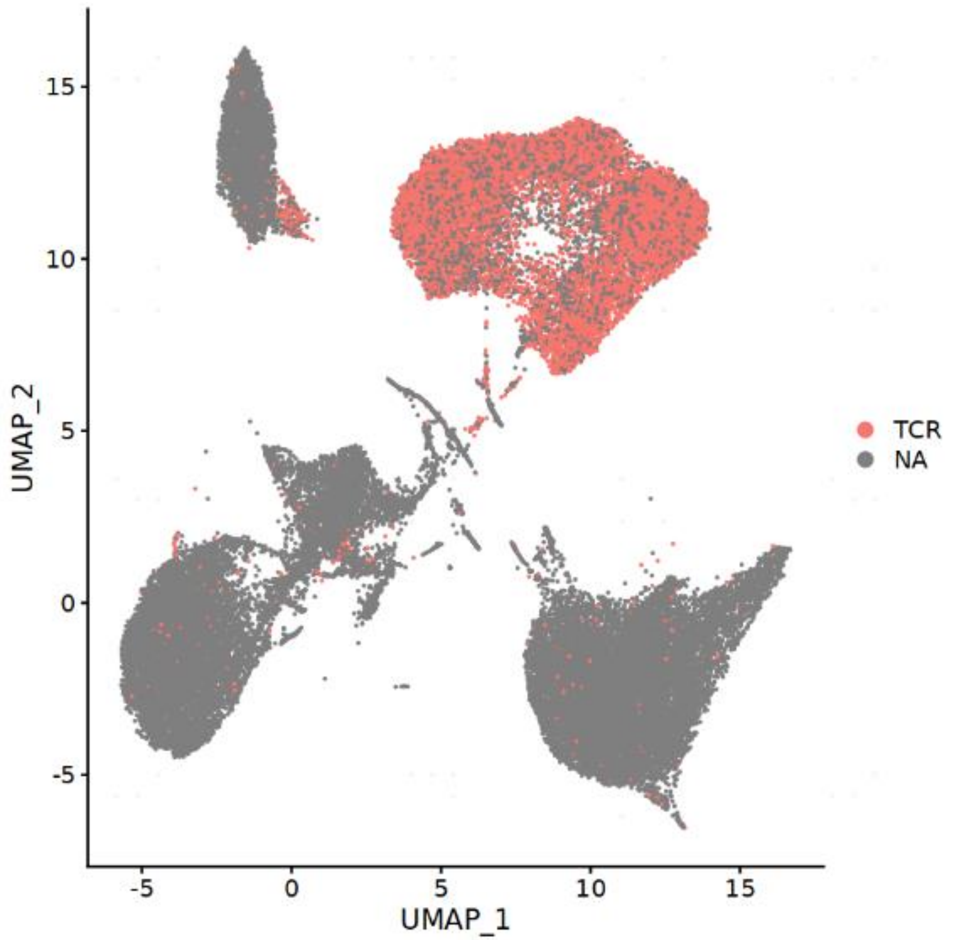
- Premade primers available for human and mouse
    - Primers can be customized for any species

sCircle – human PBMC



sCircle – Mouse PBMC





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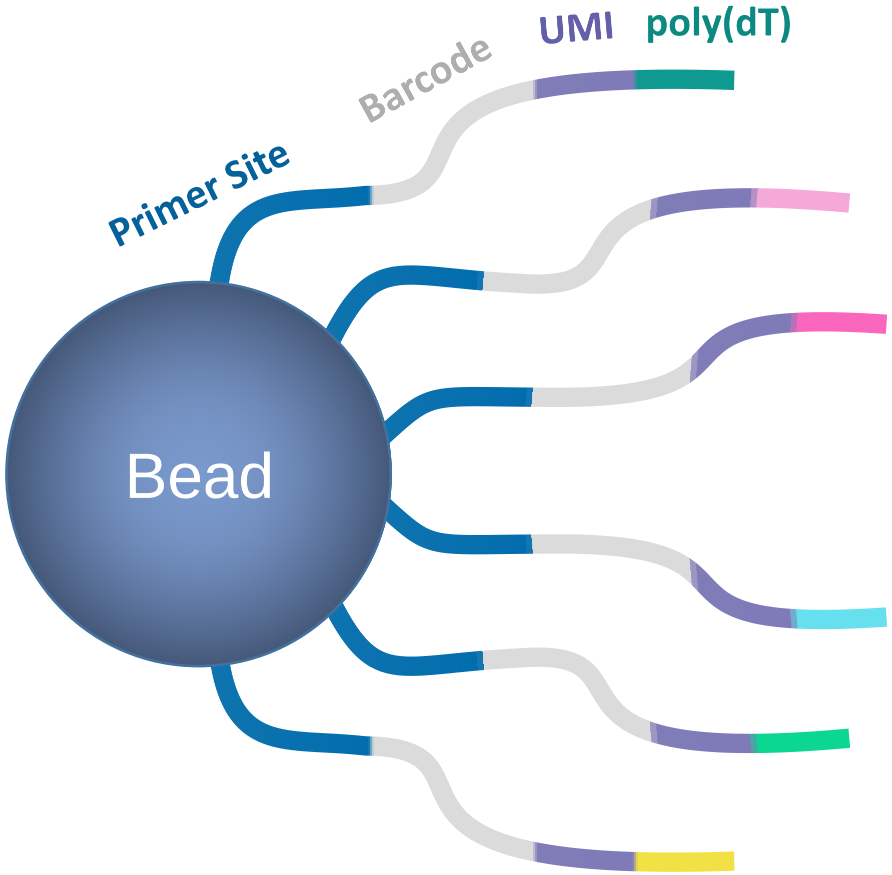
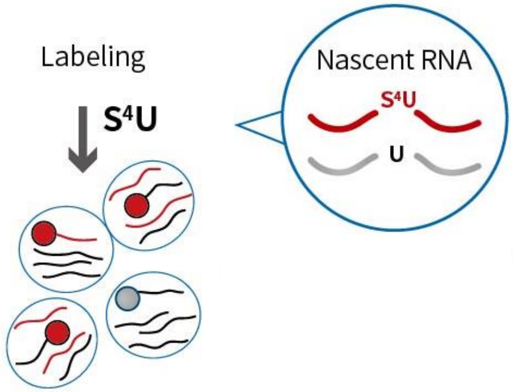
GEXSCOPE® Variations: More Than Just mRNA Profiling



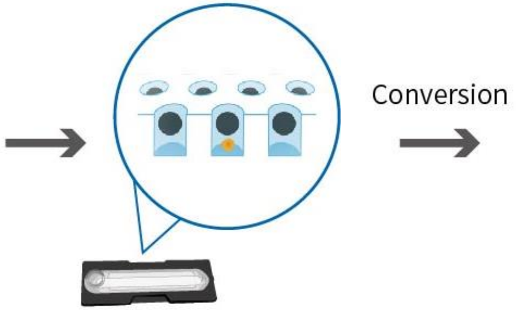
mRNA profiling	GEXSCOPE®
Immune V(D)J Profiling	GEXSCOPE® V(D)J
Full-Length Immunoreceptor Profiling	sCircle™
SNVs, Fusion Genes, Rare Transcripts, And Viral Genes	FocuSCOPE®
Glycosylation Levels	ProMoSCOPE™
Nascent RNA Synthesis	**DynaSCOPE®**

DynaSCOPE ®: Adding time-resolution to single-cell RNA-seq data

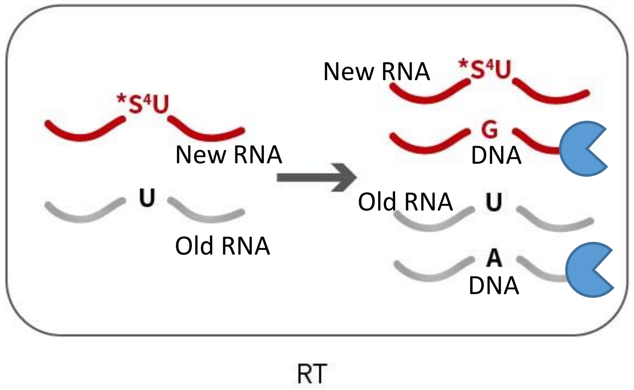
1.) S^4U labeled cells or dissociated tissues

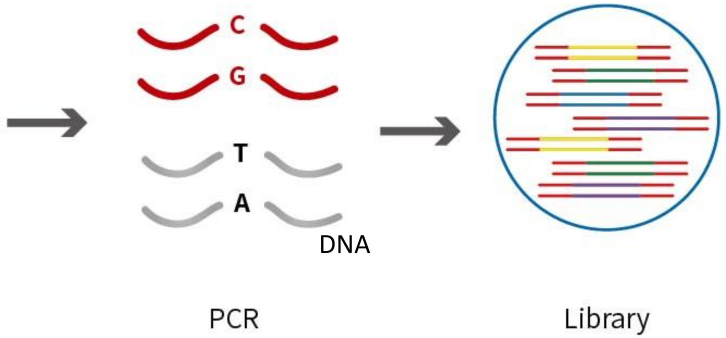


2.) Single cell lysis and barcoding



3.) Conversion of the S^4U into a cytosine analogue



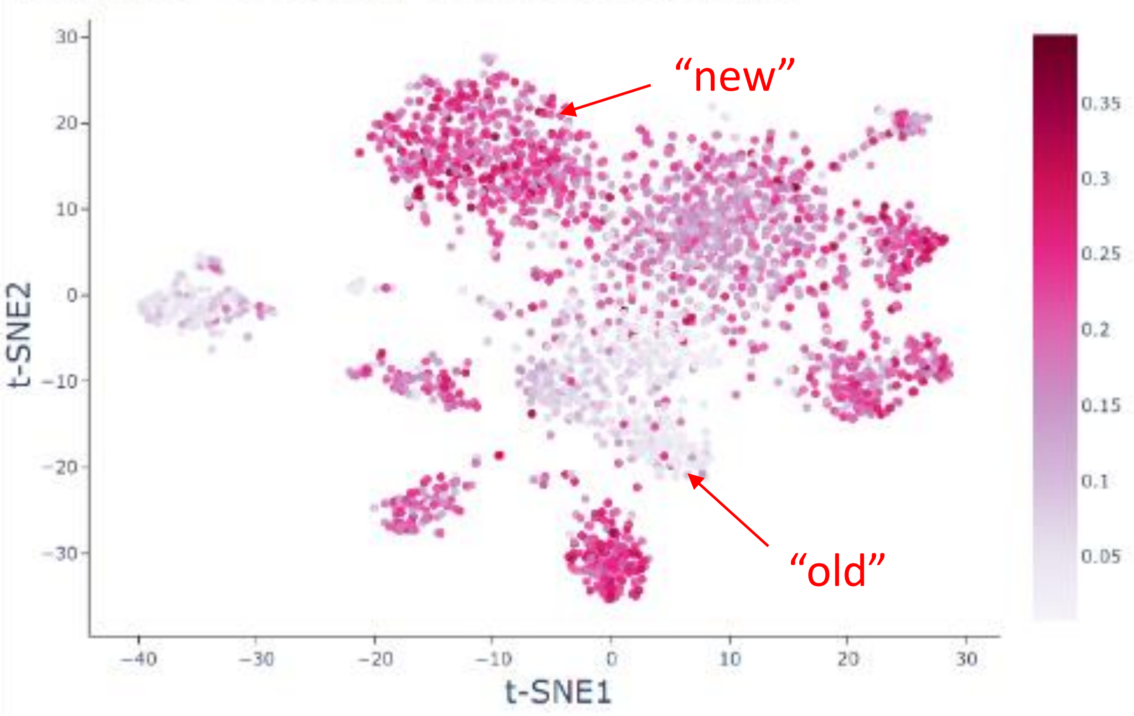


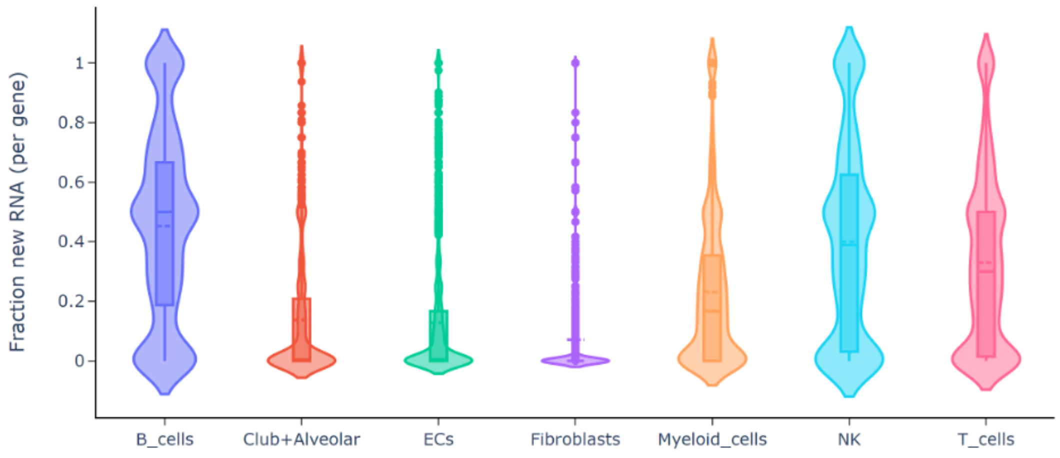
- Mismatched cytosine in nascent RNA
- Uracil in stable transcripts

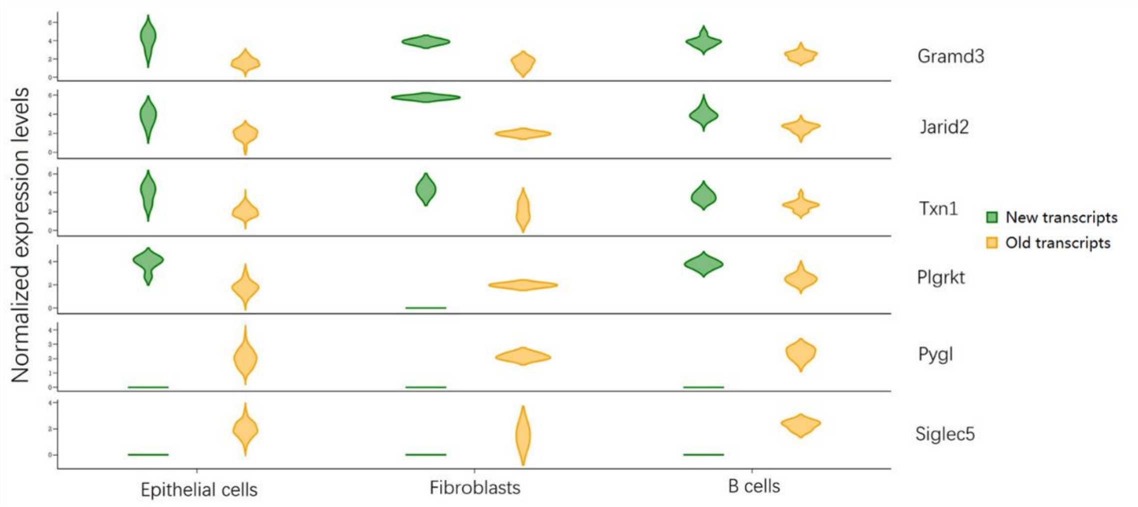
5.) Sequencing: Bioinformatical separation of nascent and long-lived transcripts

DynaSCOPE： Time-resolved transcriptomic dynamics

t-SNE plot colored by substitution rate



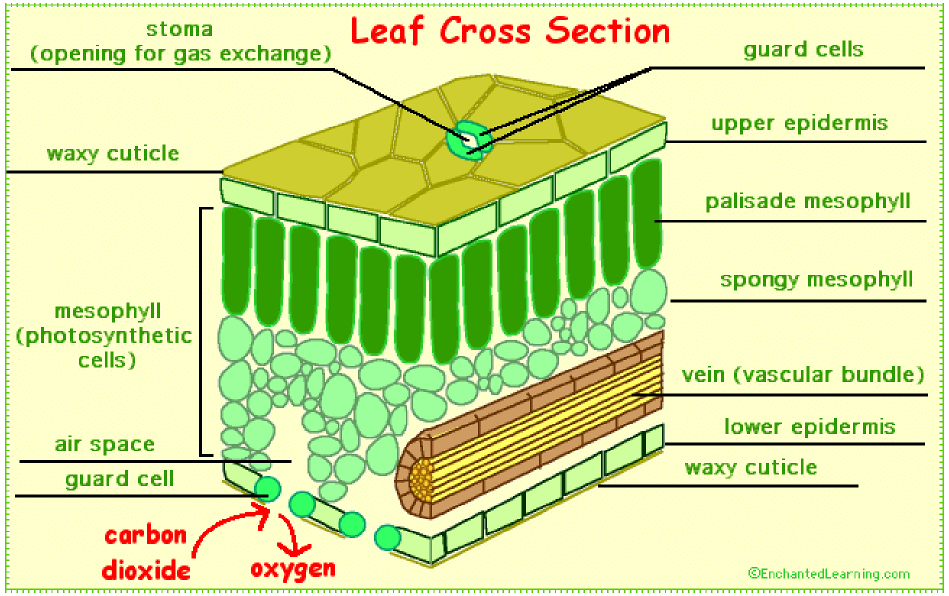


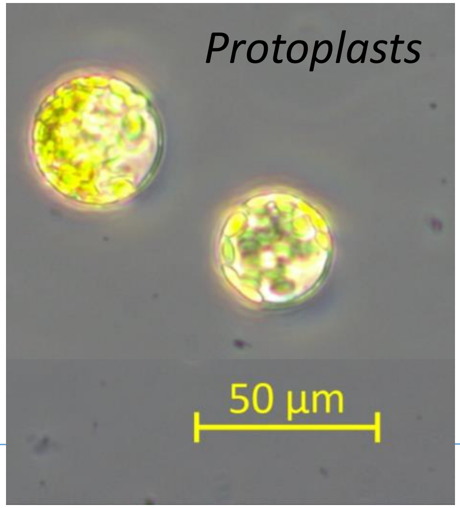


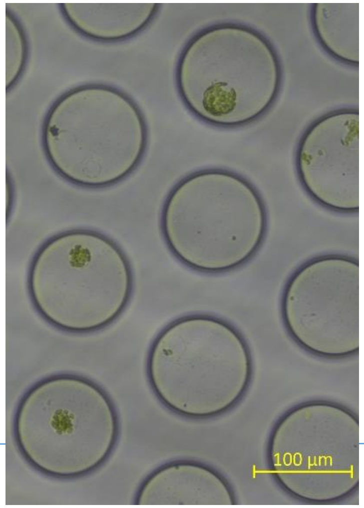
Single cells for non mammalian organisms

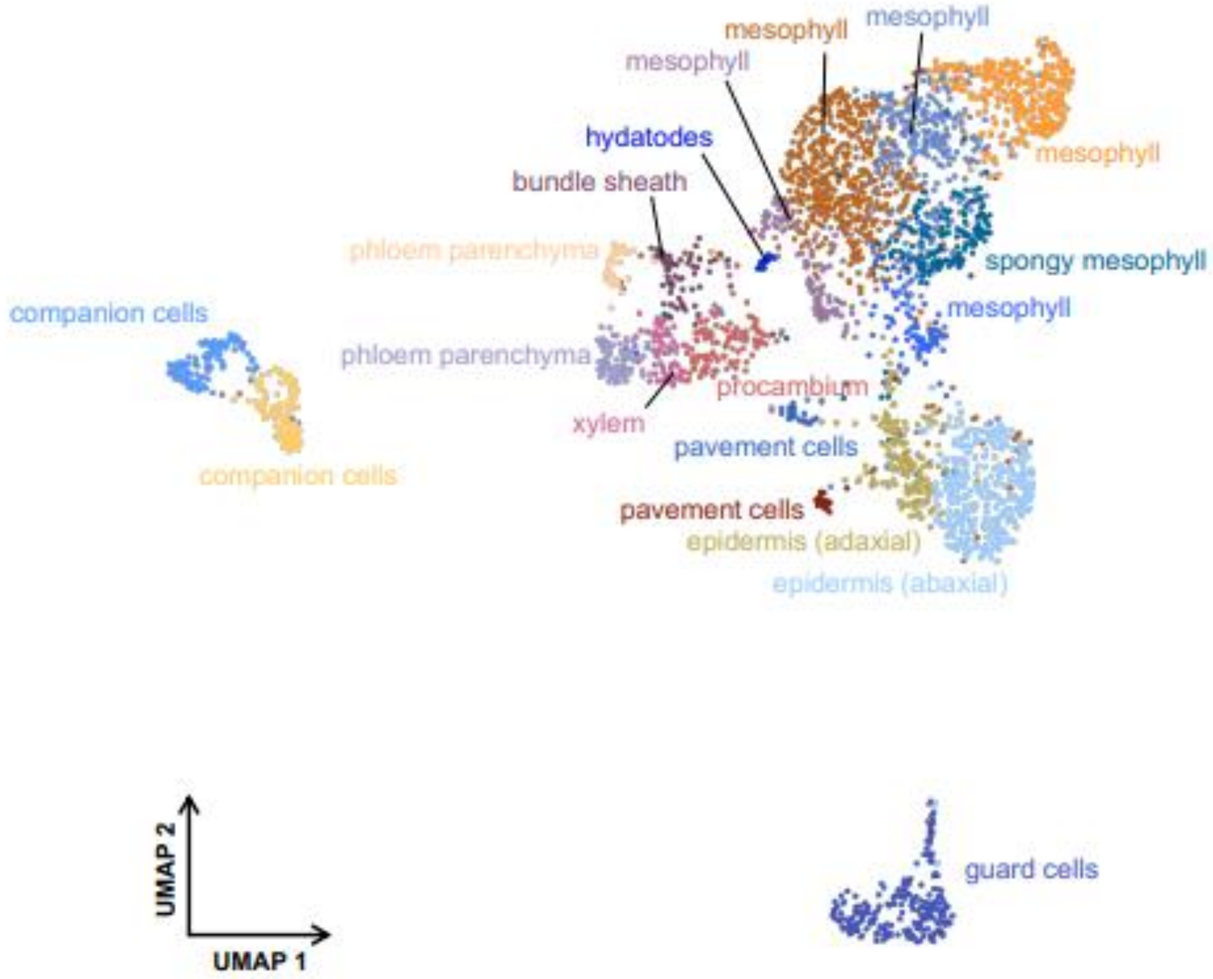
Single cells for plant model – large well chip

Arabidopsis thaliana









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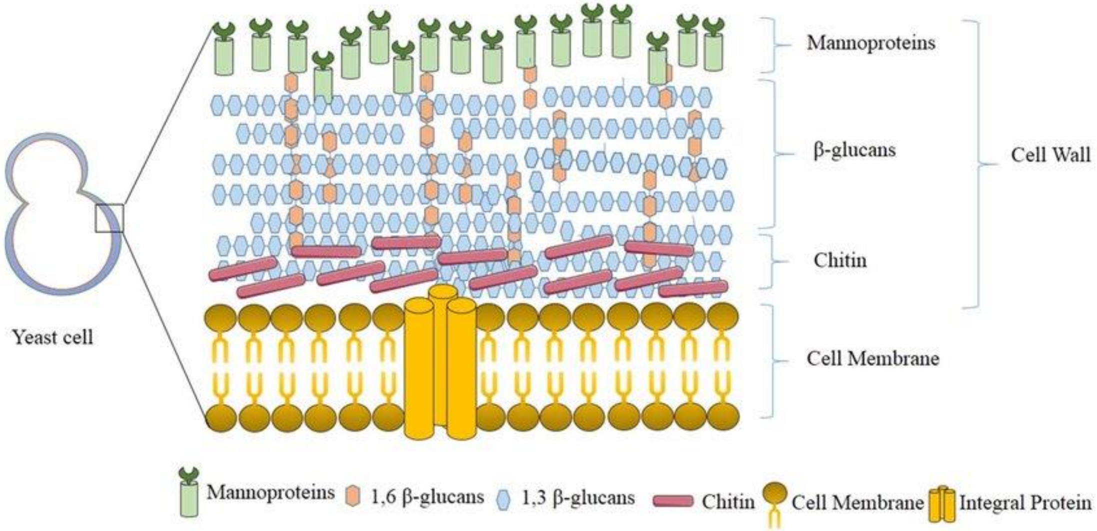
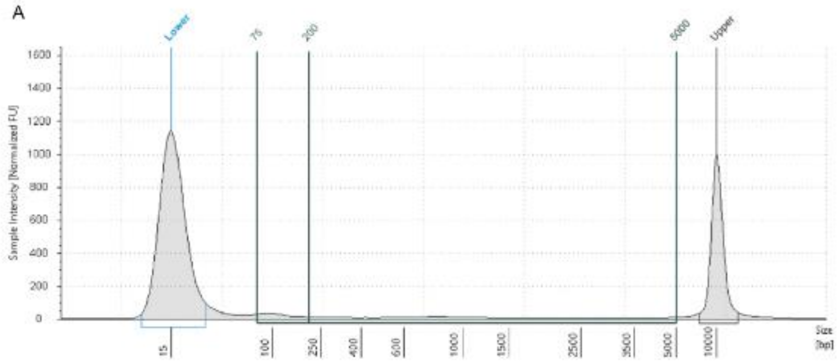
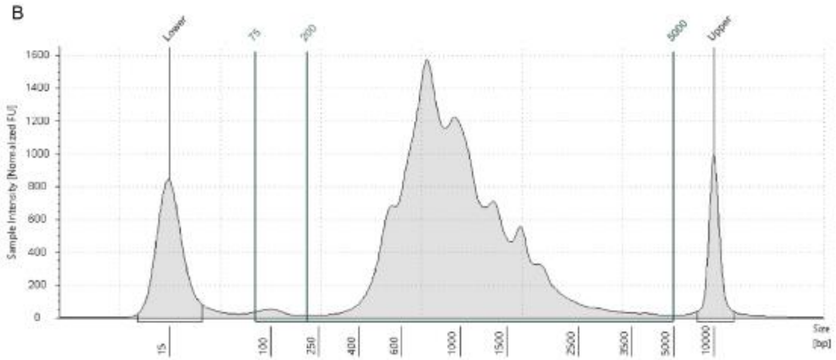
Single cells for yeast model



No cDNA was obtained when cell lysis was unmodified

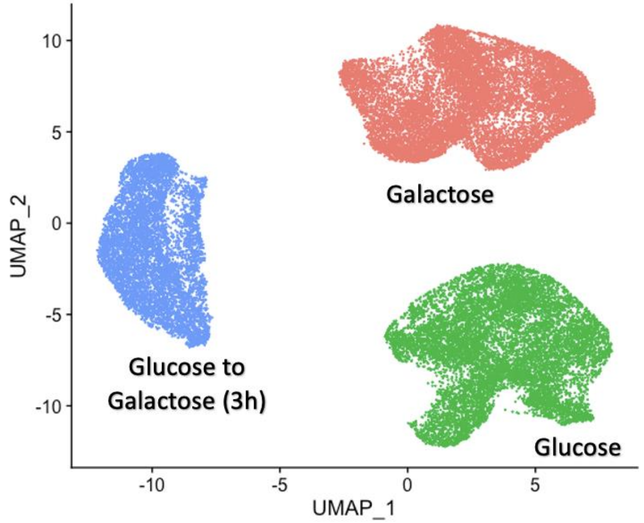


After cell wall digestion, cDNA was obtained from yeasts

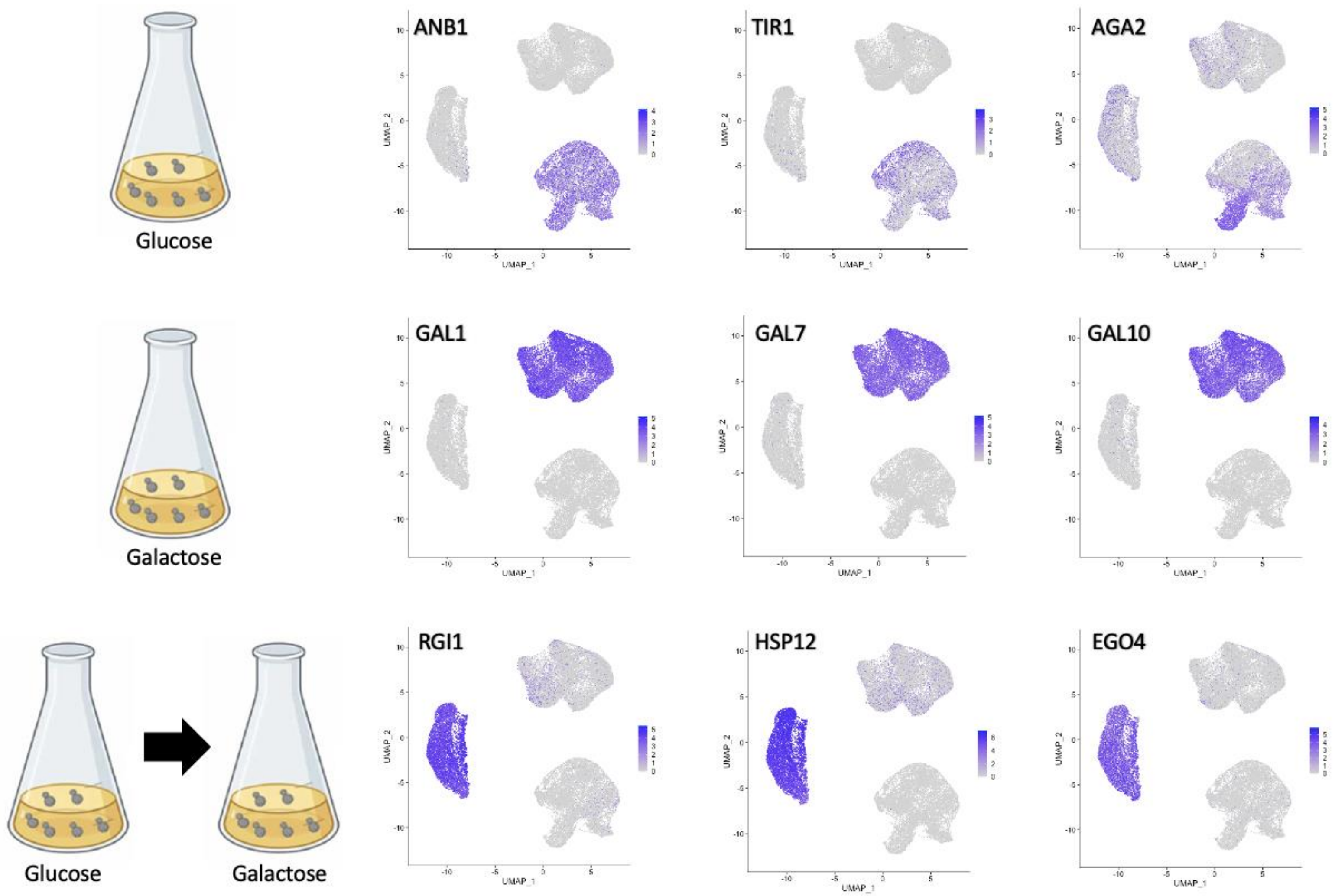


Singleron

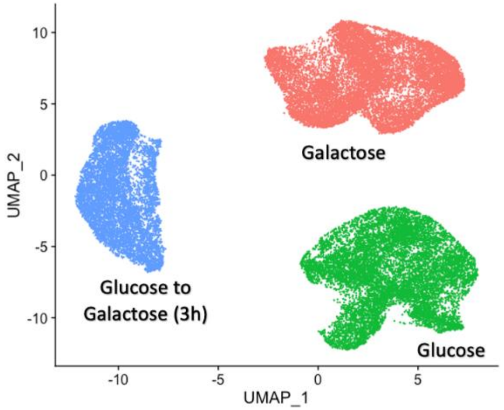
Single cells for yeast model

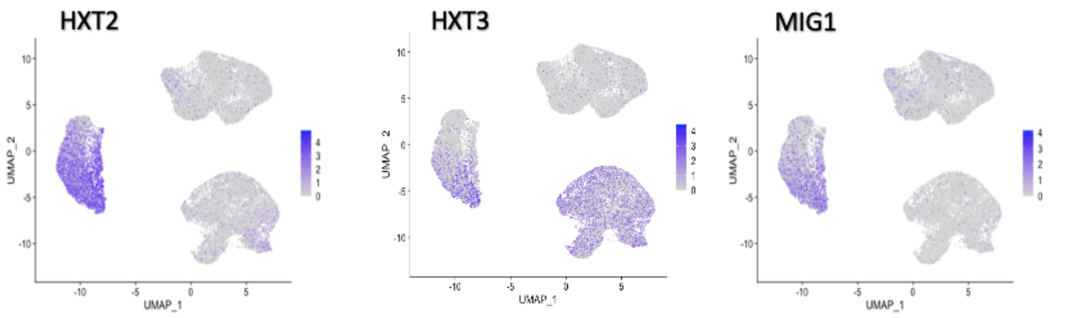


Differentially expressed genes per cluster

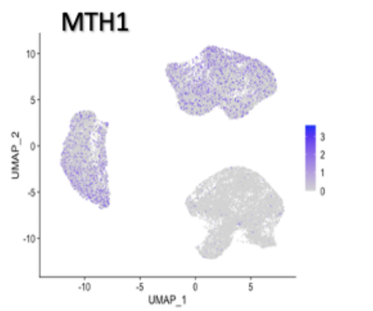


Single cells for yeast model

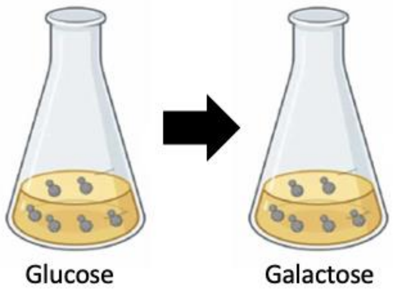


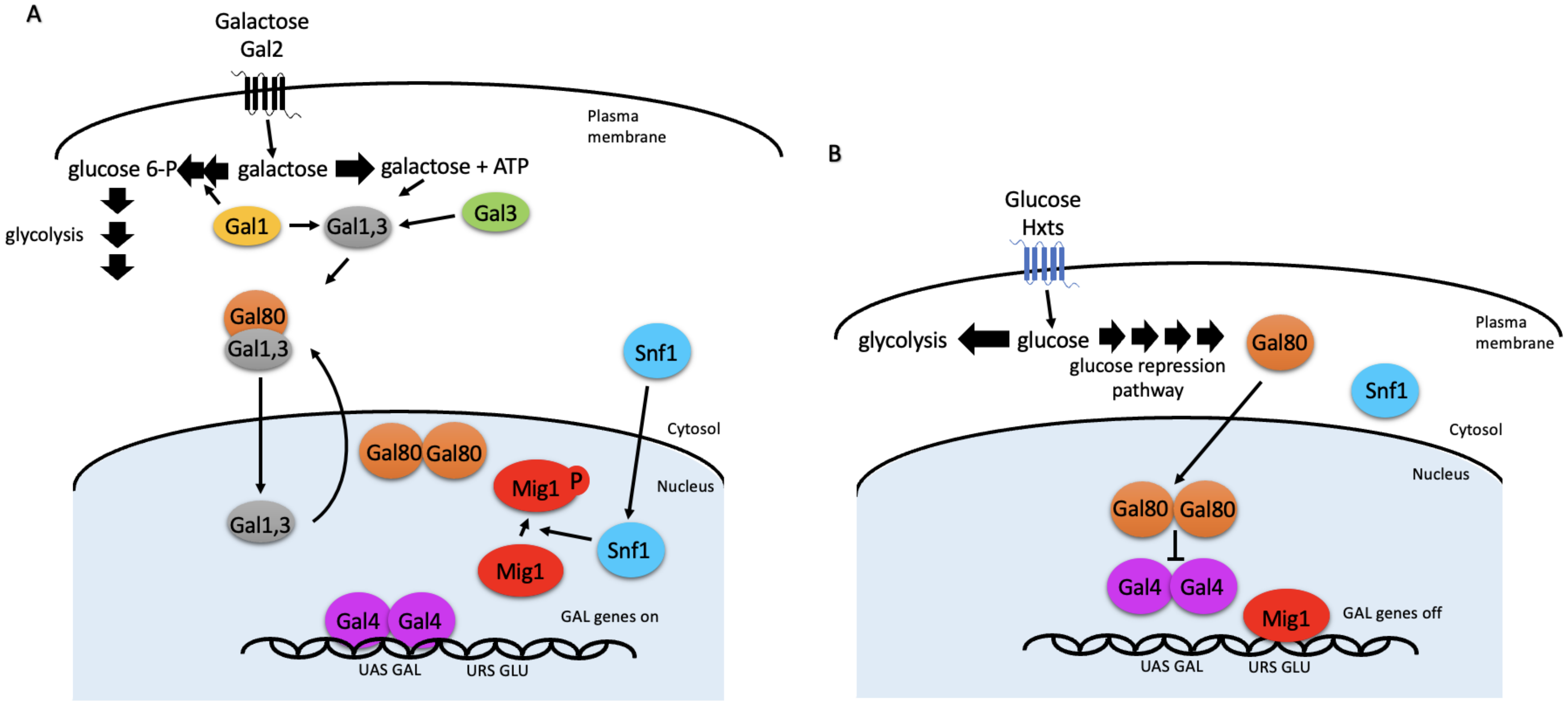


The expression of HXT2/3 and MIG1 was increased in response to glucose reduction



Supression of glucose transporters by MTH1





Surface protein detection

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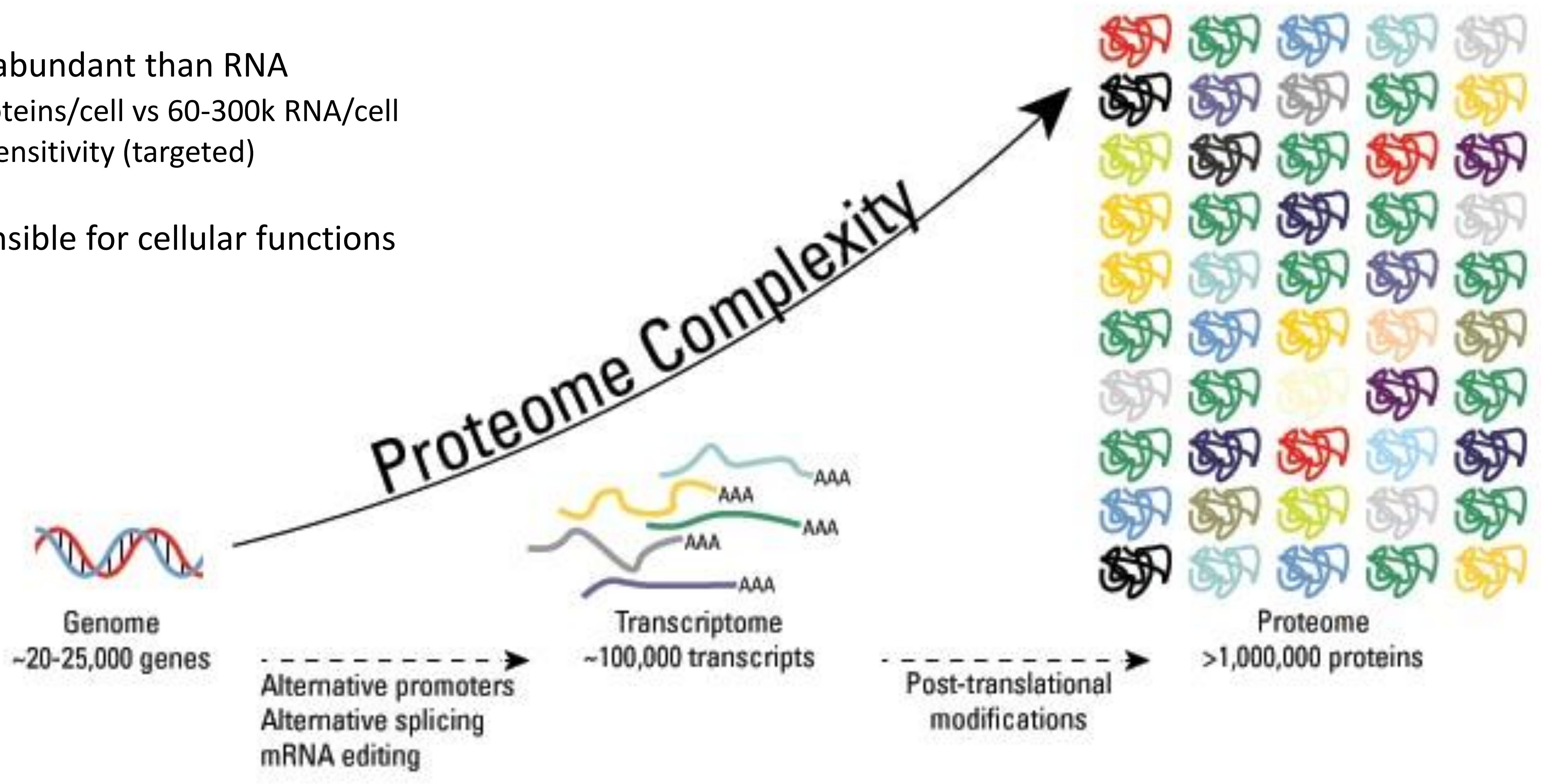
Multiomics: the power of RNA and protein combined

Why Proteins?

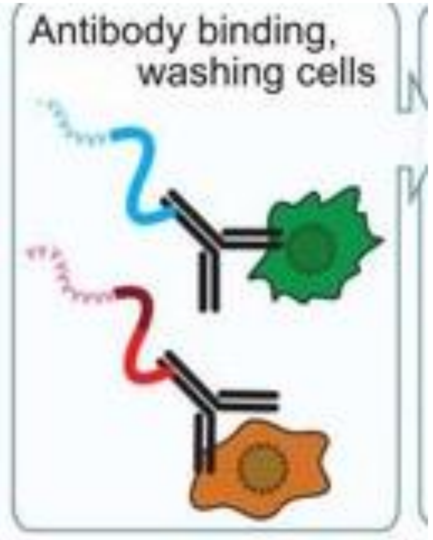
Proteins are more abundant than RNA

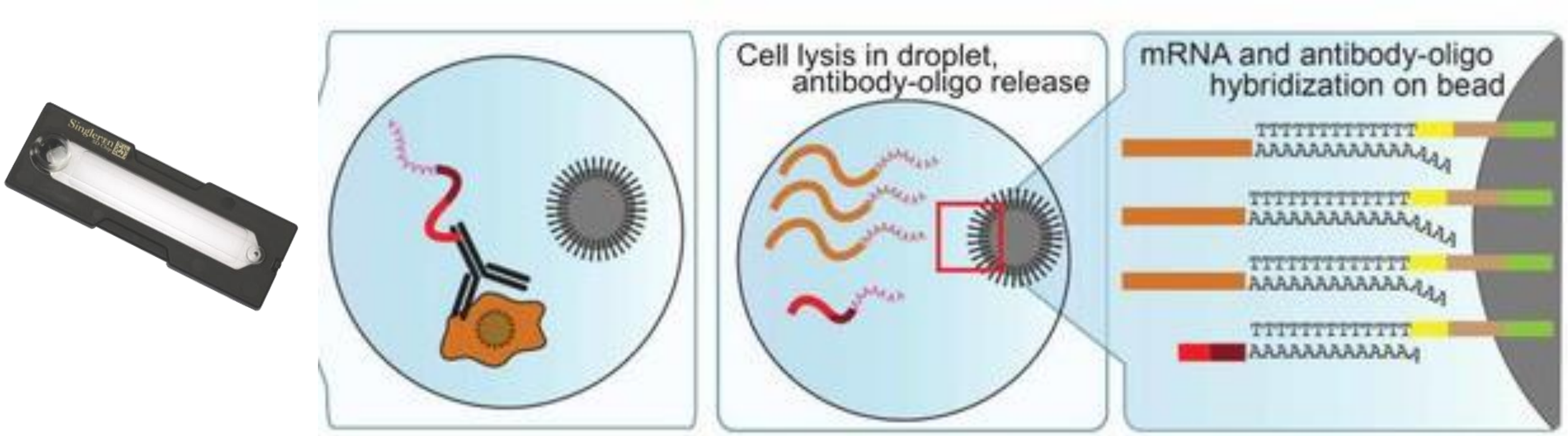
- ~1-3M proteins/cell vs 60-300k RNA/cell
- Increase sensitivity (targeted)

Proteins are responsible for cellular functions



Multiomics: the power of RNA and protein combined





Each antibody is linked to an DNA oligo containing a unique tag and a poly A at the 3’

mRNA and antibody tags are both captured by the poly T on the beads

Stoeckius, M., Hafemeister, C., Stephenson, W. *et al.* Simultaneous epitope and transcriptome measurement in single cells. *Nat Methods* **14**, 865–868 (2017).

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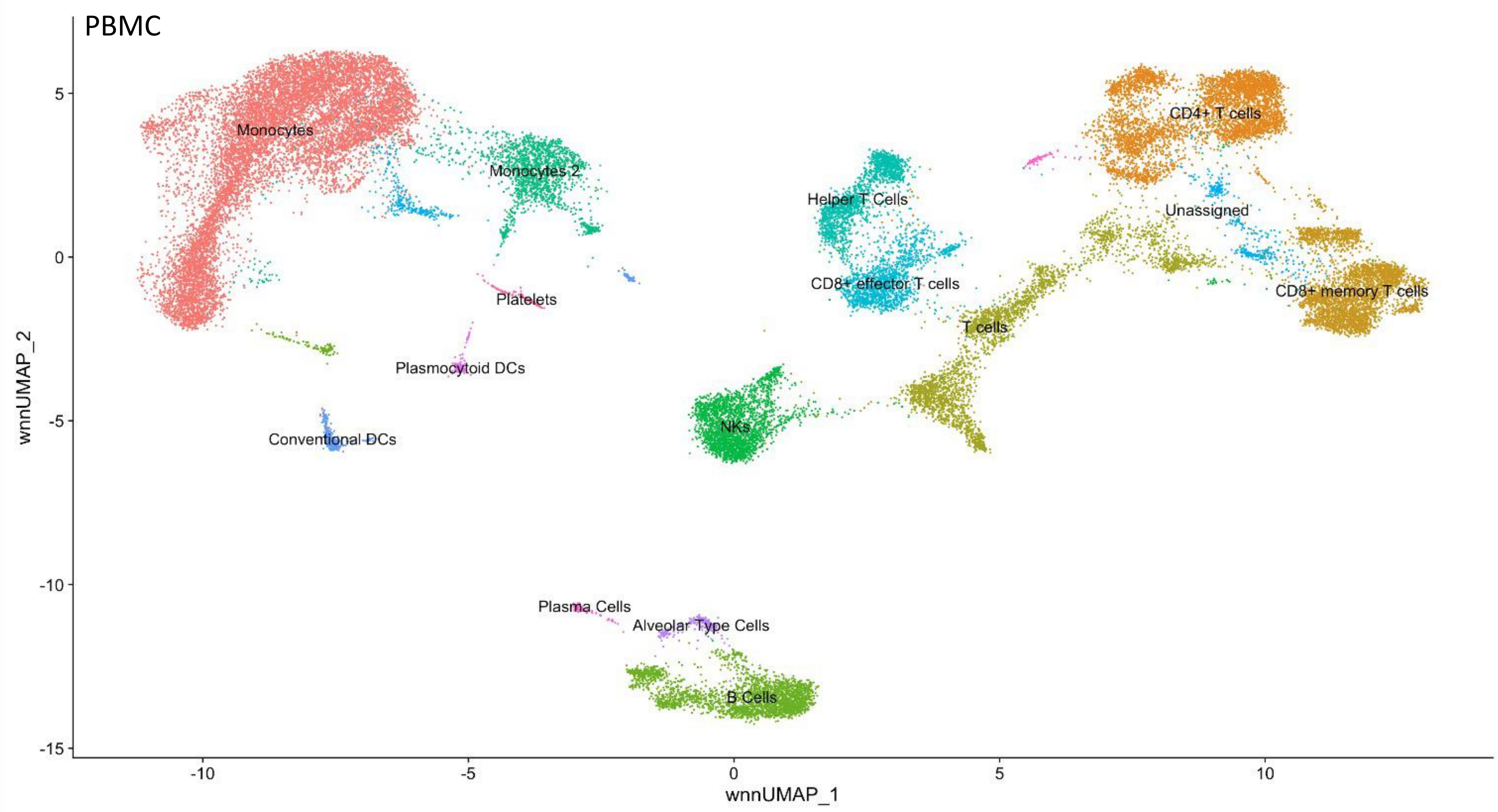
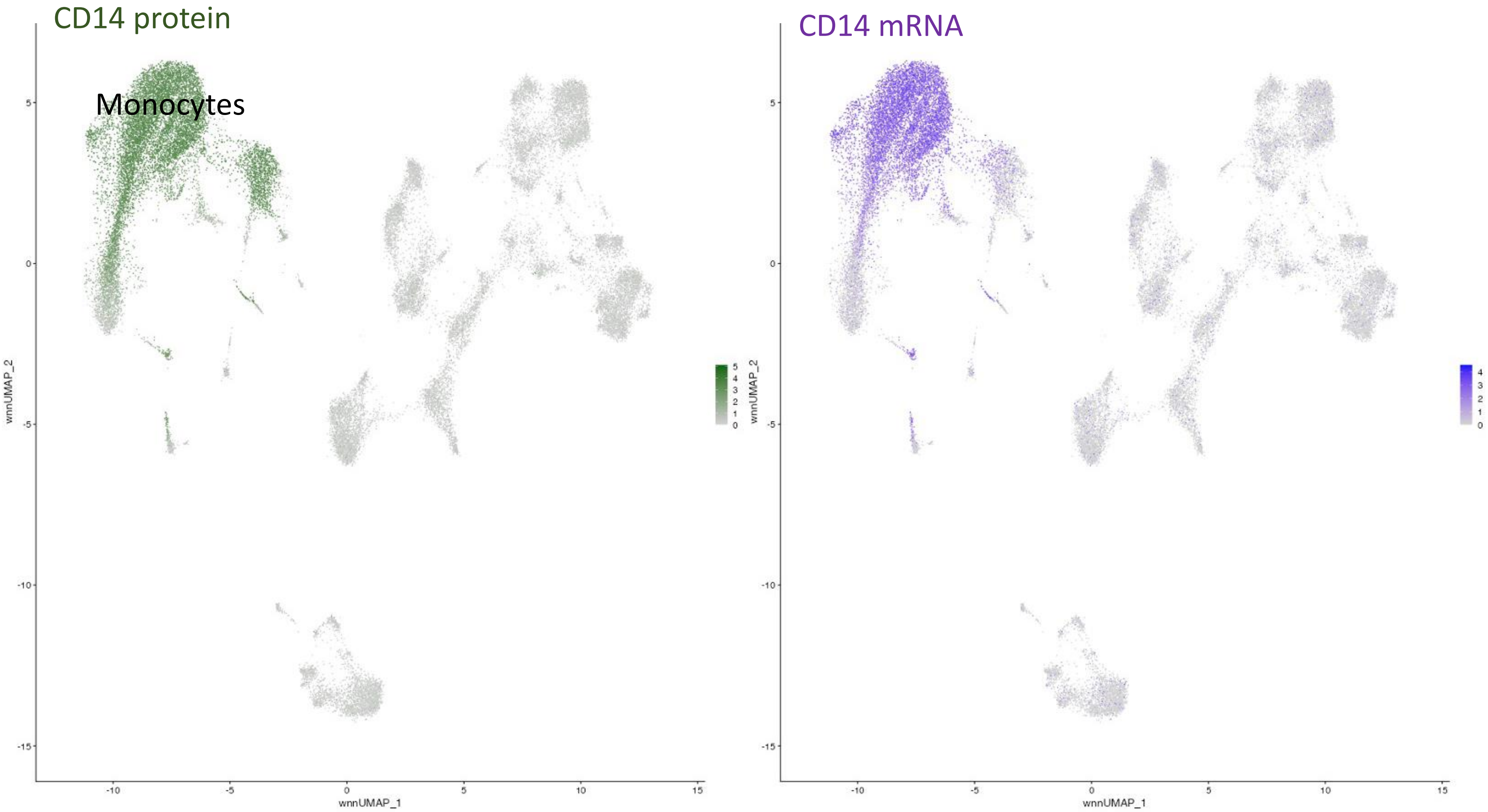
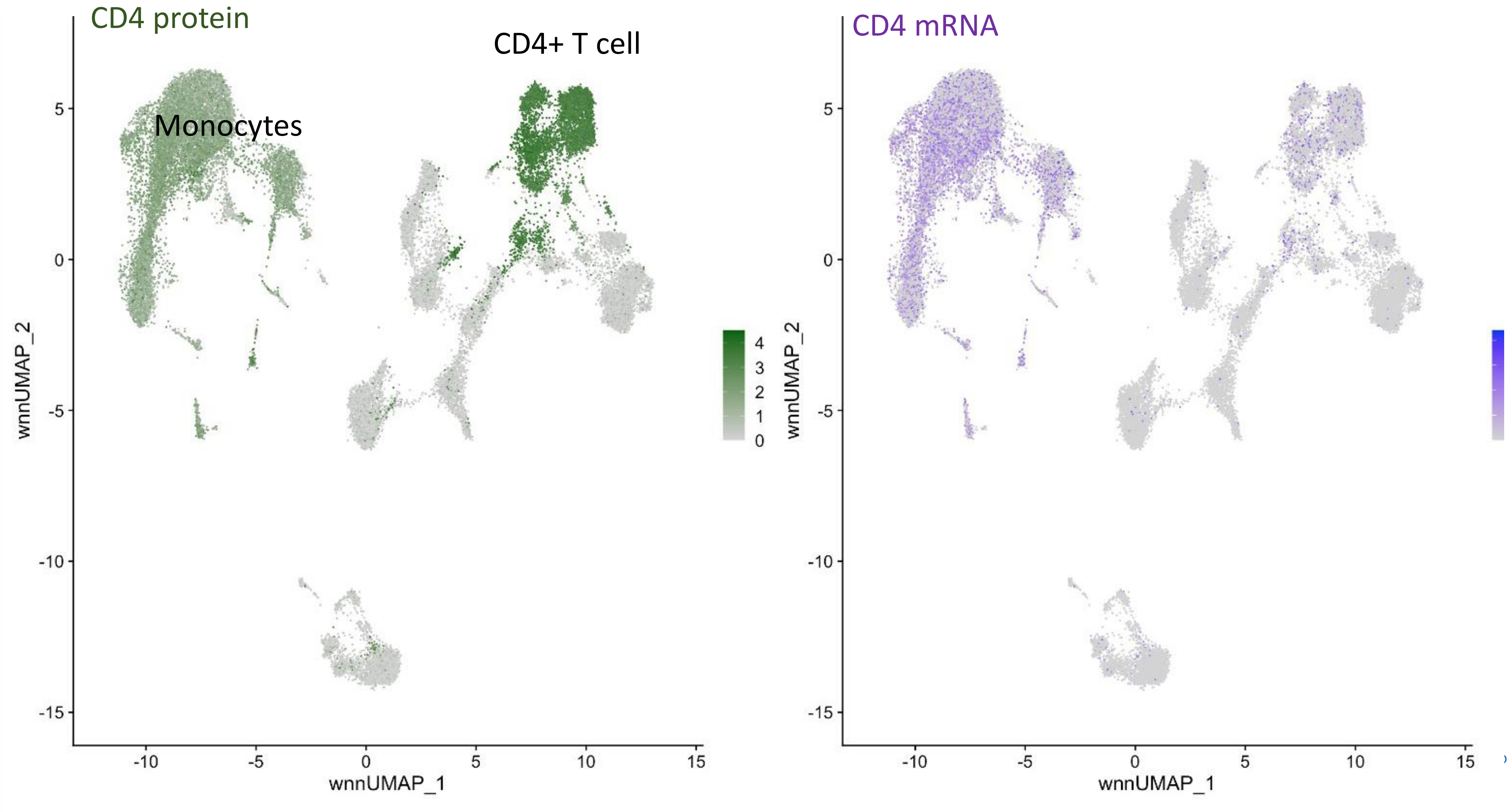
Multiomics, for a better annotation



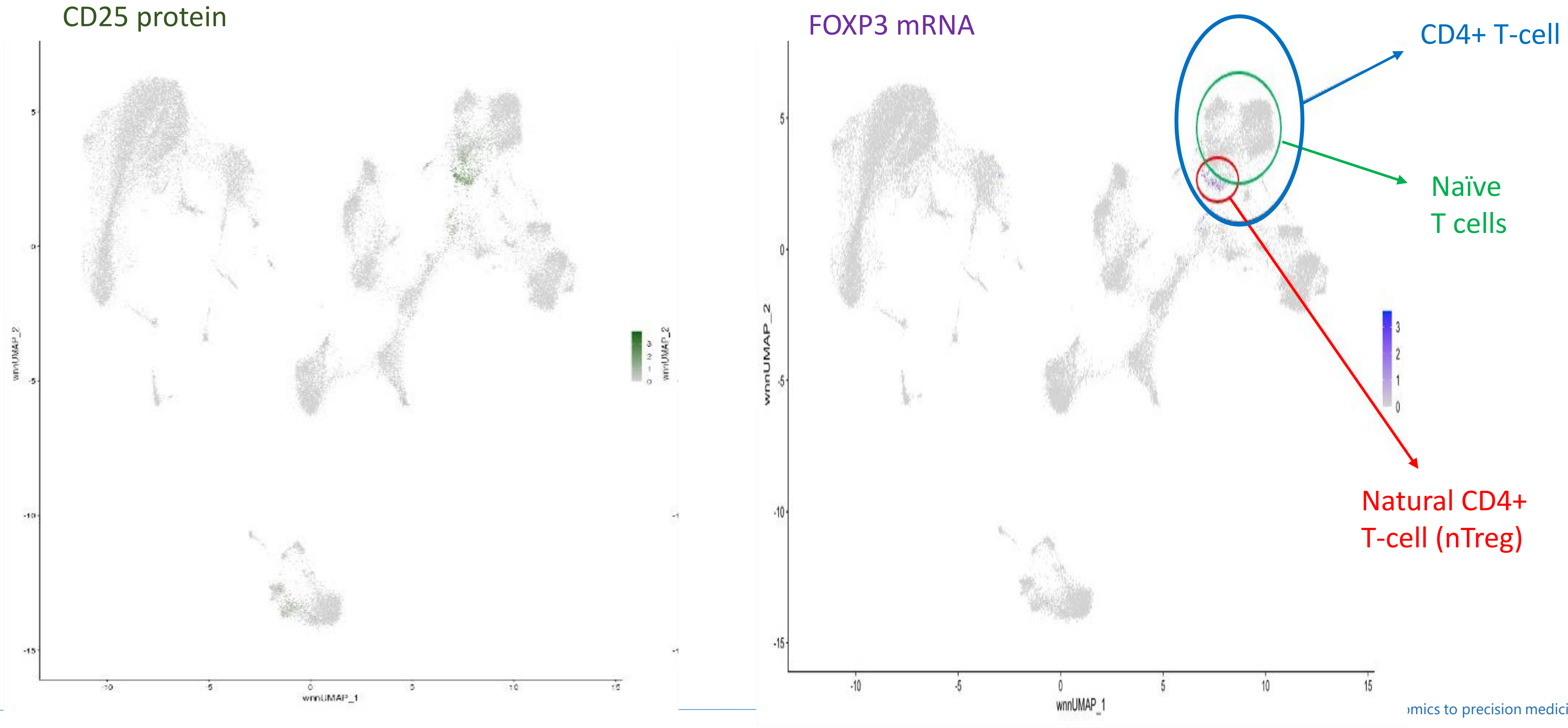
Multiomics: CITE-Seq adaptation



Multiomics, for a better annotation



Multiomics, for a better annotation

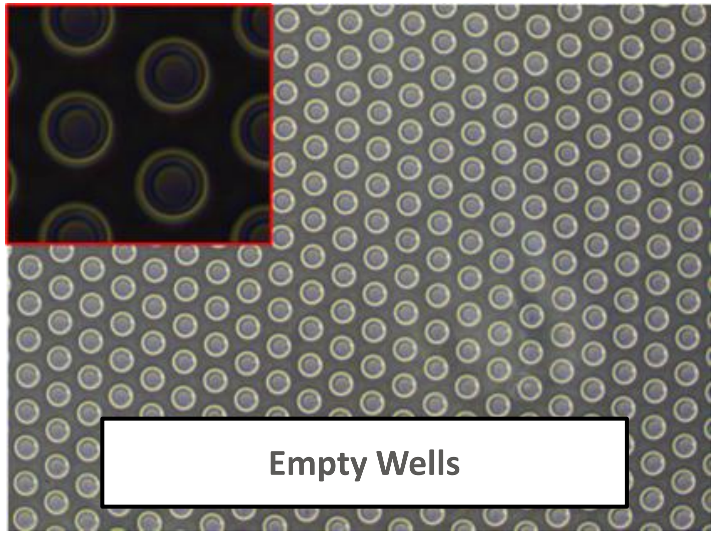


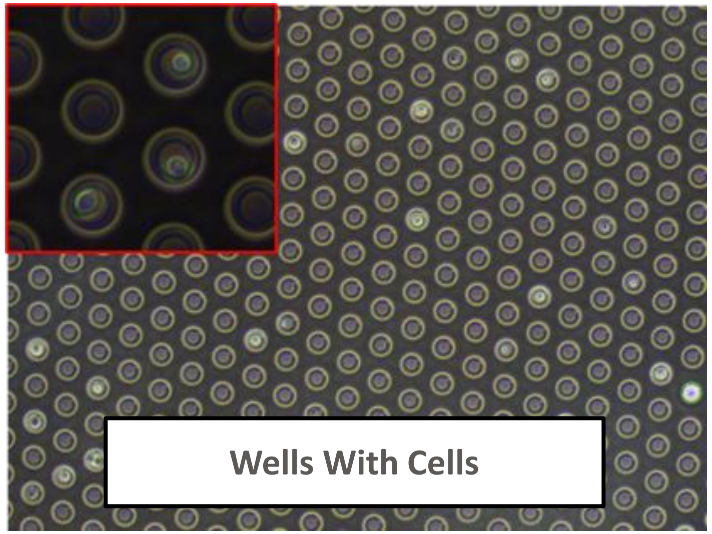
Thanks !

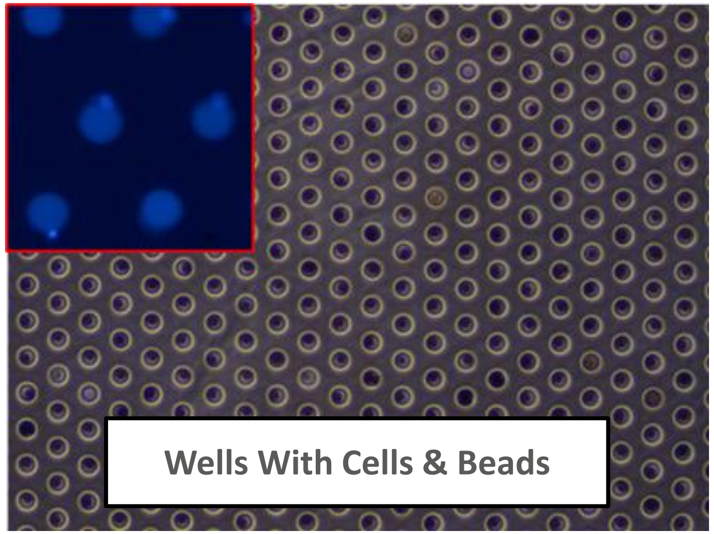
Singleron

Single-Cell Sequencing
SCOPE-chip®

- No Need For Specialized Equipment – Use A P200
- View Cell Loading With Any Standard Microscope
- Similar Size To A Hemocytometer

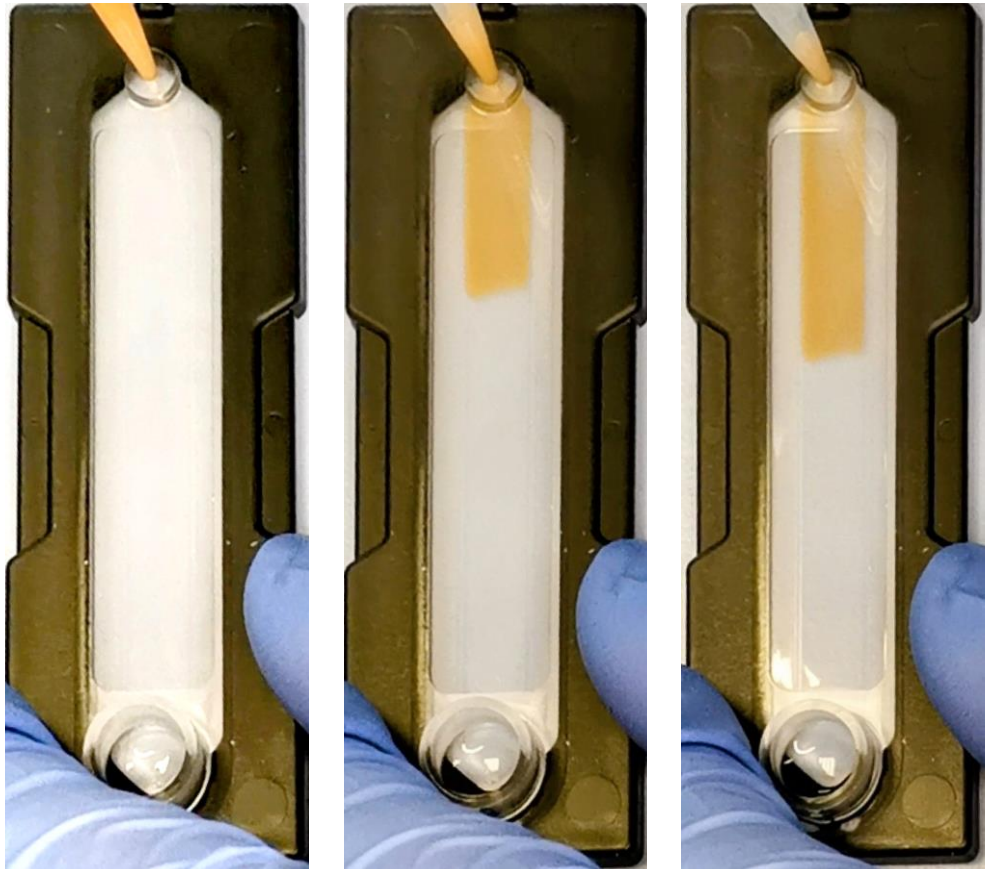




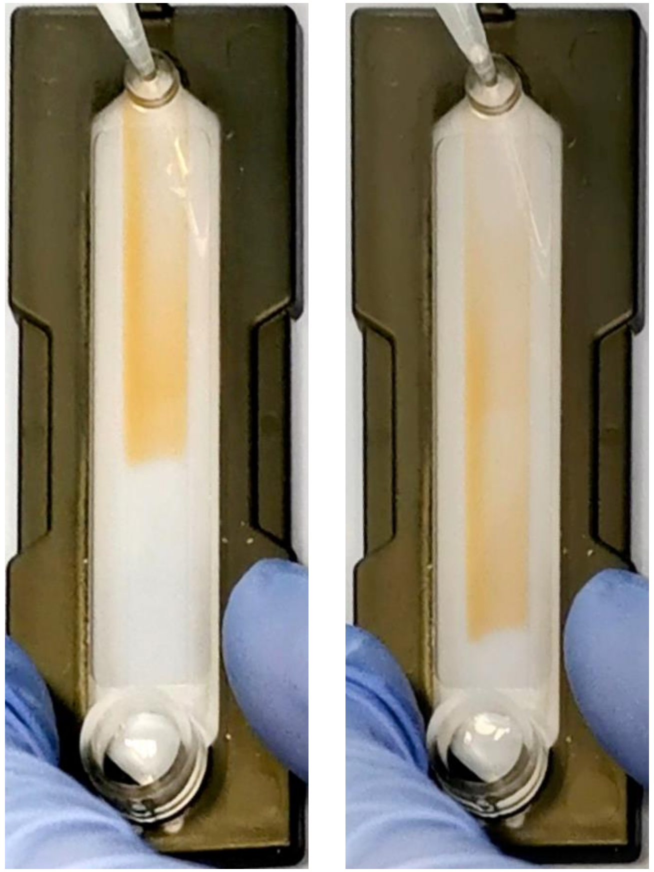


Single-Cell Sequencing Solutions: SCOPE-chip®

Initial Bead Loading



PBS Push #1

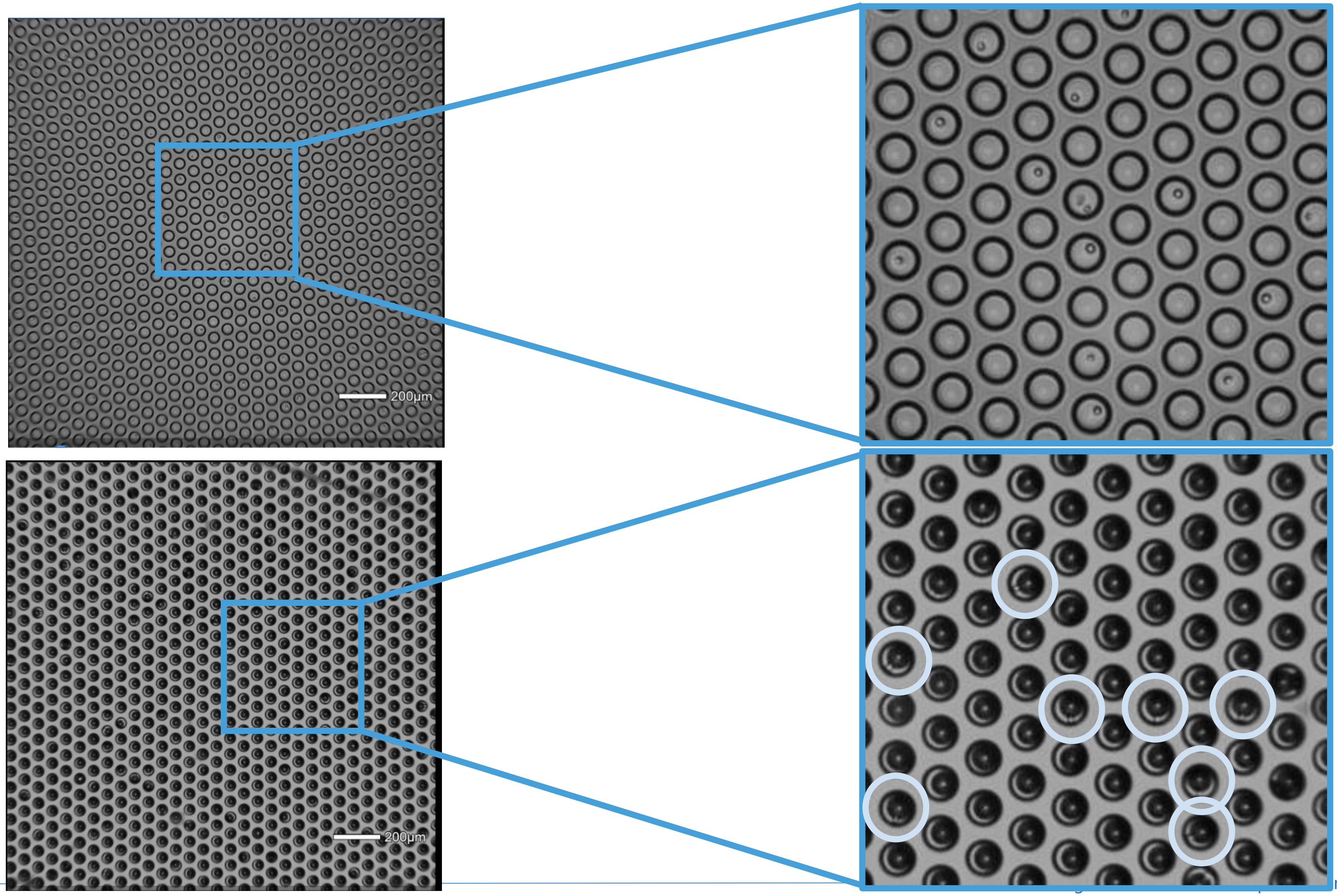


Beginning Of PBS Push #2

Single-Cell Sequencing Solutions: SCOPE-chip®

Cells Only

Cells + Beads



Single-Cell Sequencing Solutions: SCOPE-chip®

Outlet Reservoir Cleaning

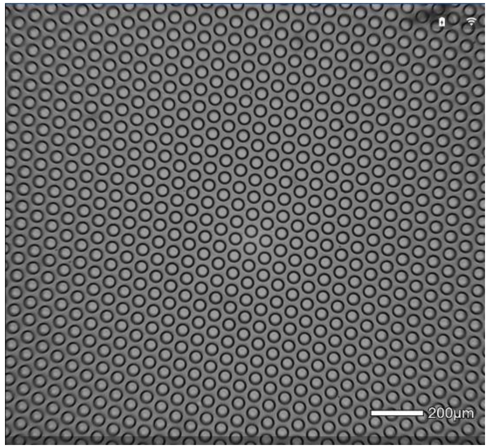
Bead Recovery

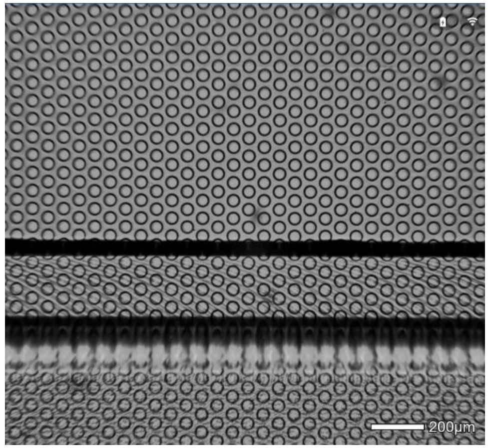
Singleron

Single-Cell Sequencing Solutions: SCOPE-chip®

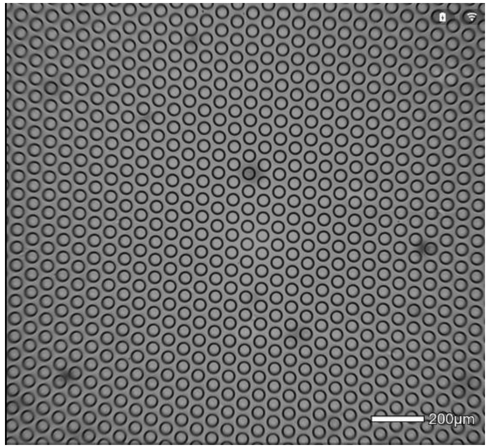
Chip Overview | **Interior Wells** | **Peripheral Wells**

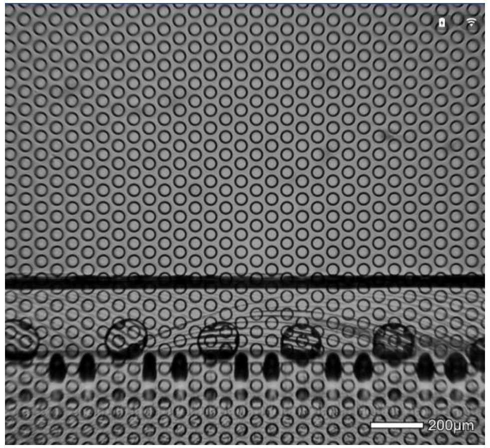
Before Priming





After Priming





QC of cDNA and NGS library