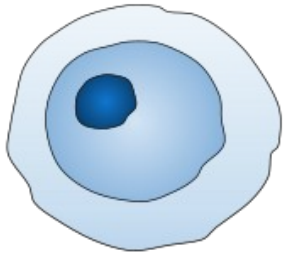
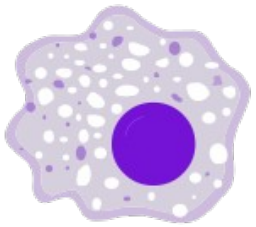
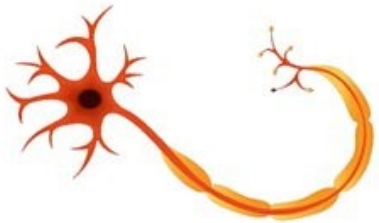




SINGLE-CELL RNA SEQUENCING TECHNIQUE

10x GENOMICS

------What is SINGLE CELL and why does it matter??



Biology is Immensely Complex

40 trillion cells in the human body

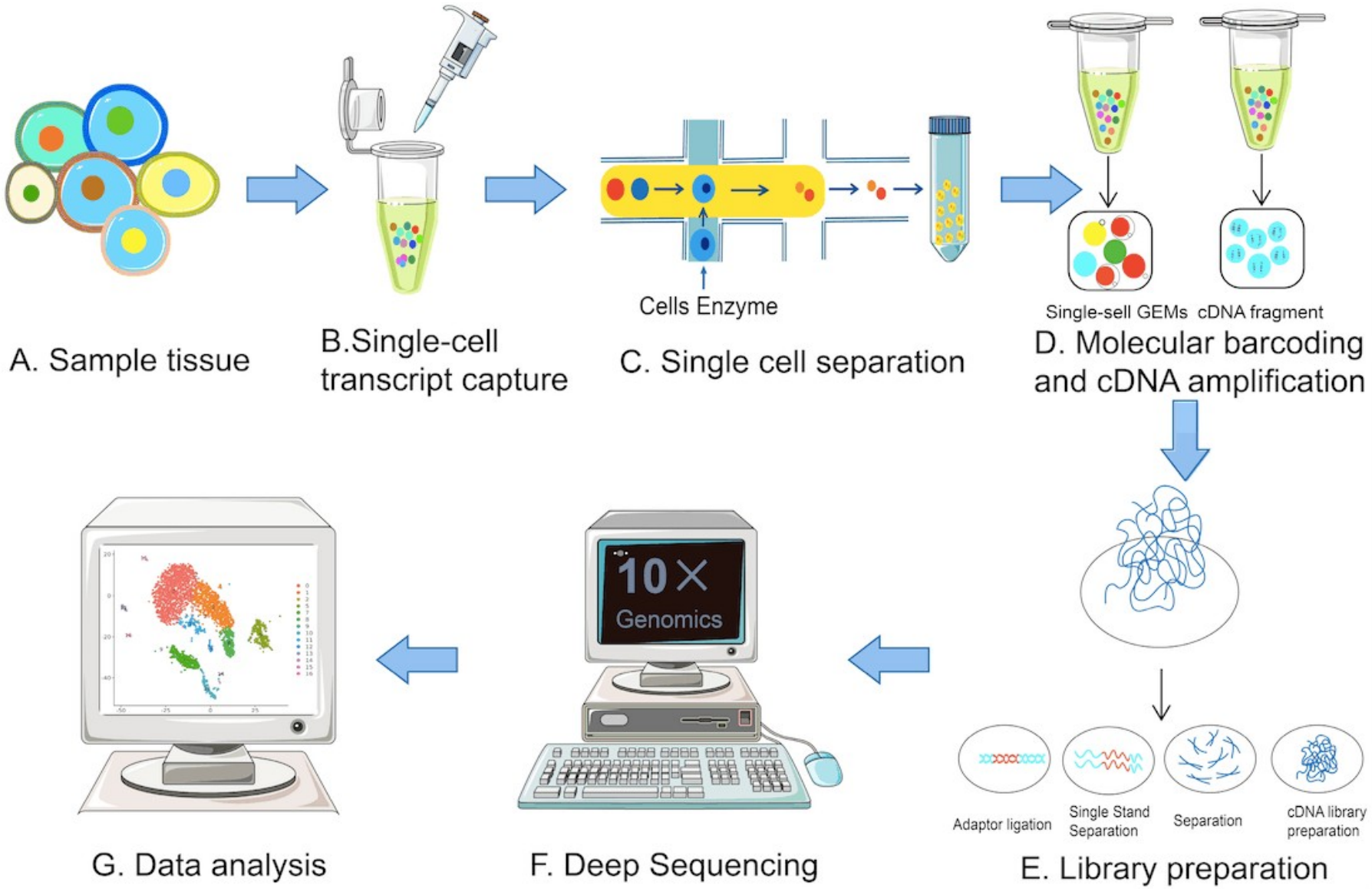
Each with its own set of expressed genes

Enormous complexity within each cell

Interactions of millions of different molecules and molecular machines

Cells form tissues, which form organ

Each tissue with a large diversity of cells and cell types



A. Sample tissue

B.Single-cell transcript capture

C. Single cell separation

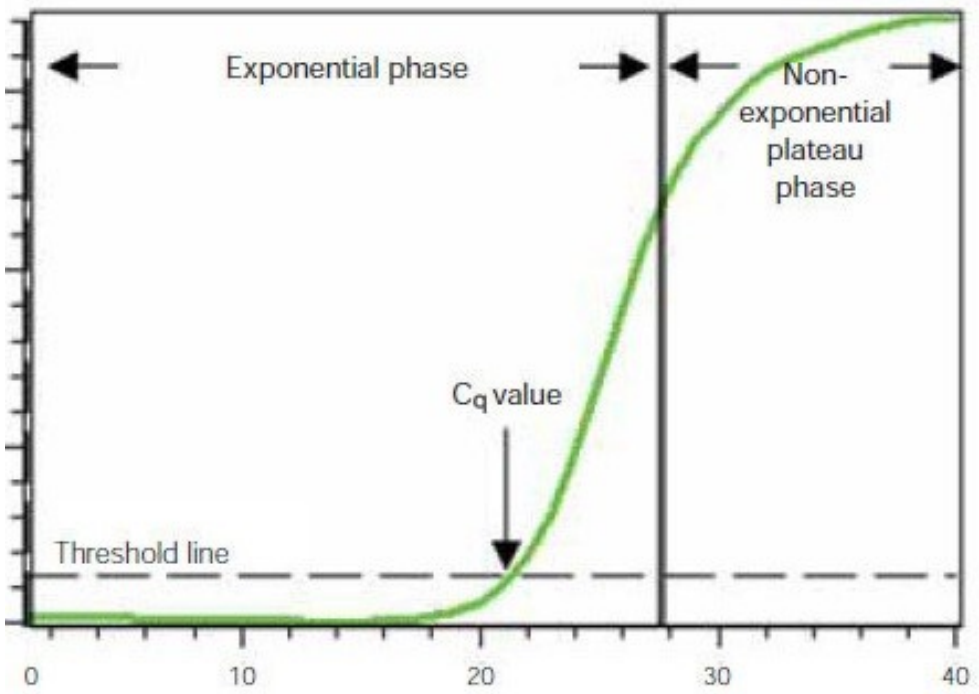
D. Molecular barcoding and cDNA amplification

E. Library preparation

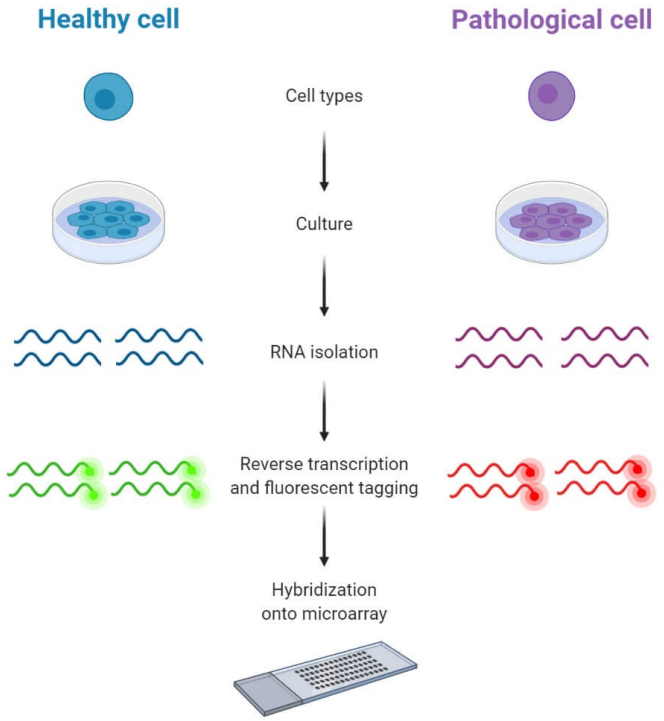
F. Deep Sequencing

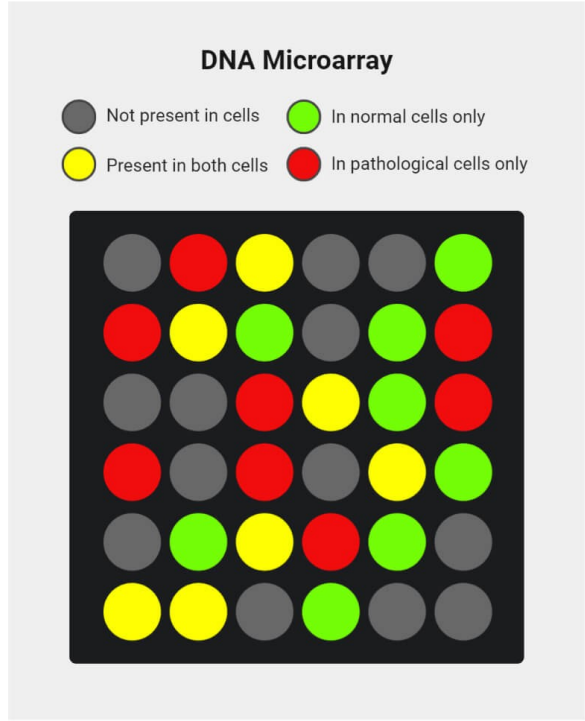
G. Data analysis

A quick glance at more traditional Technologies

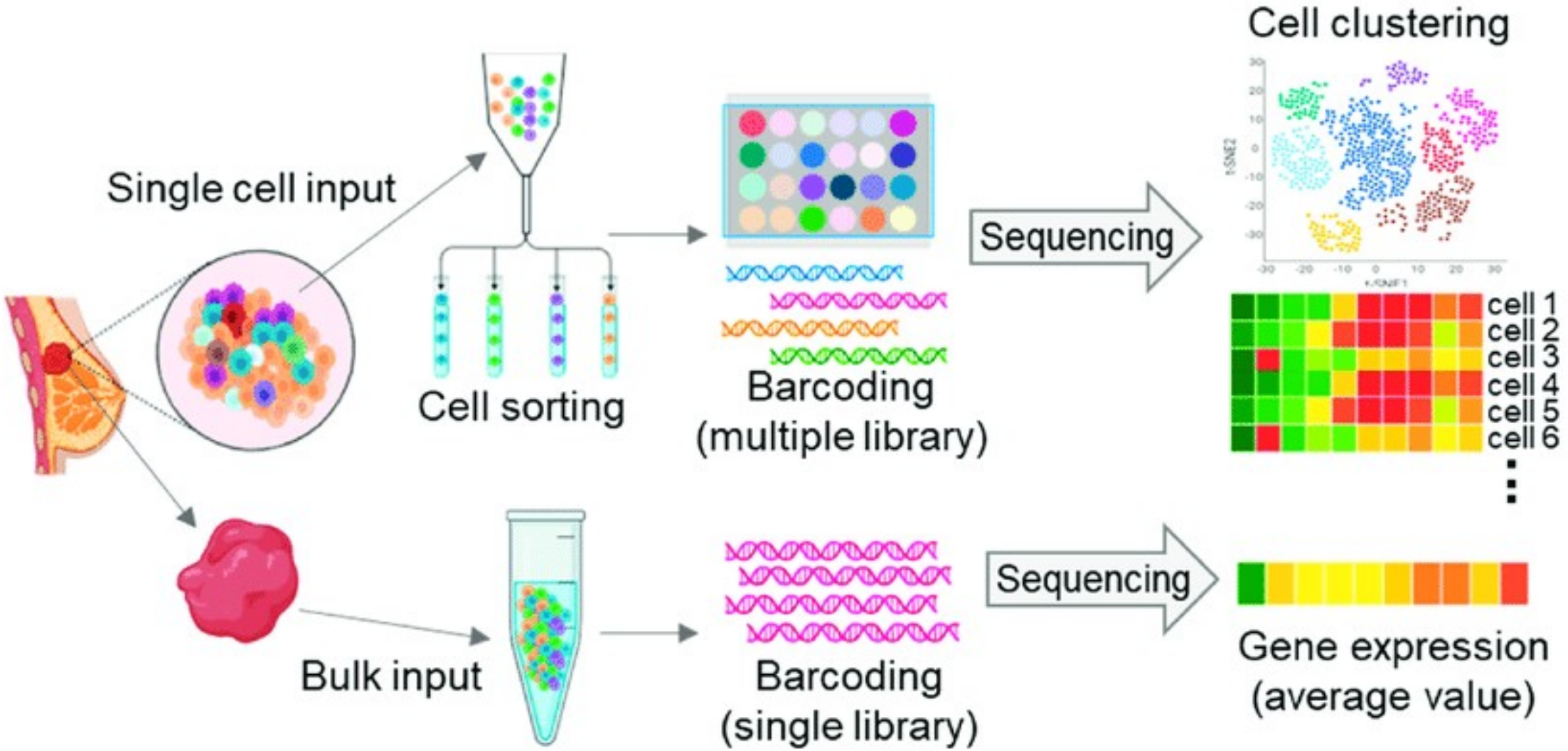


qPCR

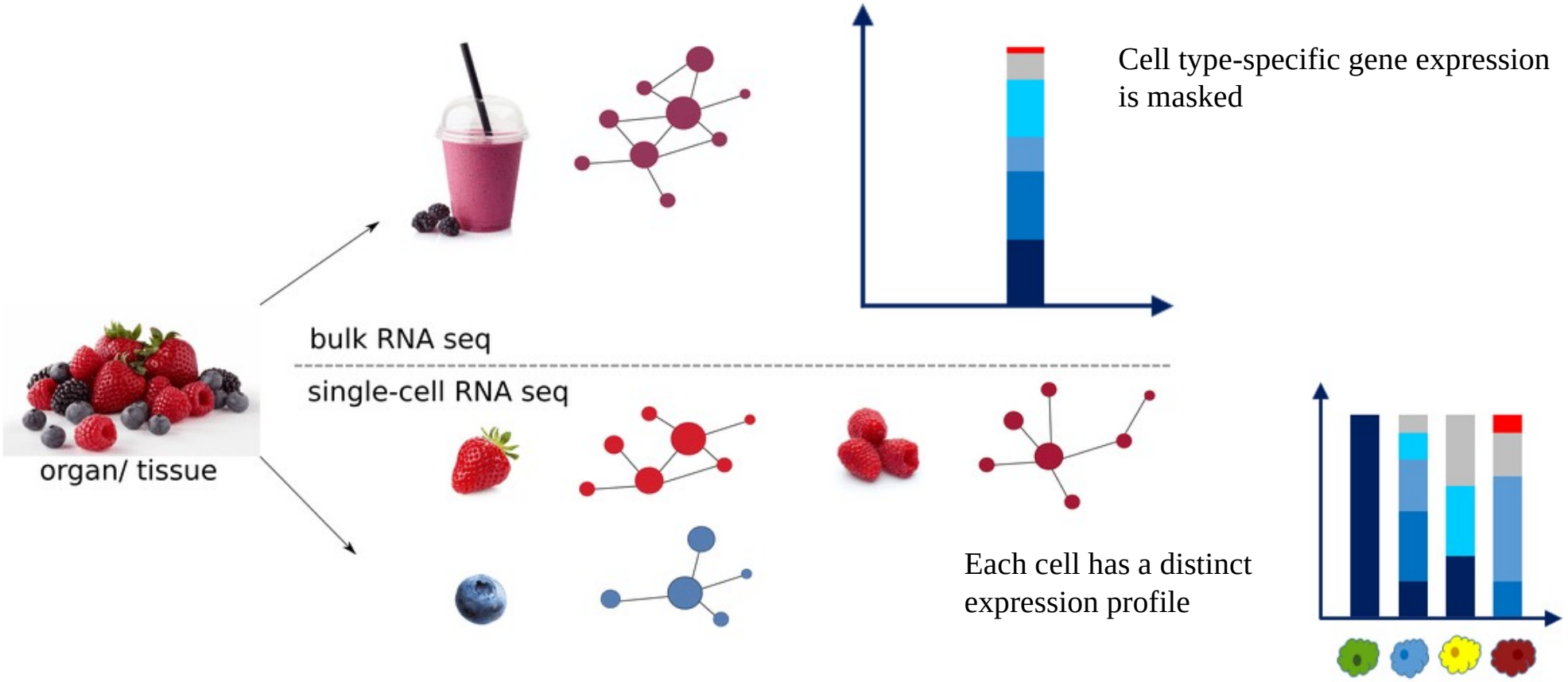




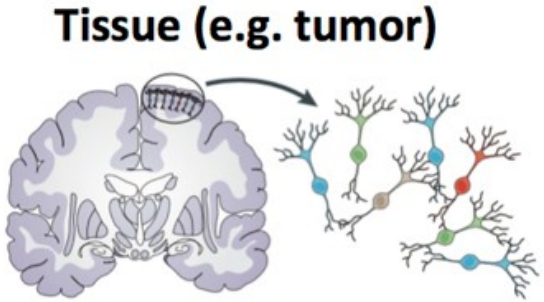
Microarray


Cell clustering
Single cell input
Cell sorting
Barcoding
(multiple library)
Sequencing
cell 1
cell 2
cell 3
cell 4
cell 5
cell 6
Bulk input
Barcoding
(single library)
Sequencing
Gene expression
(average value)

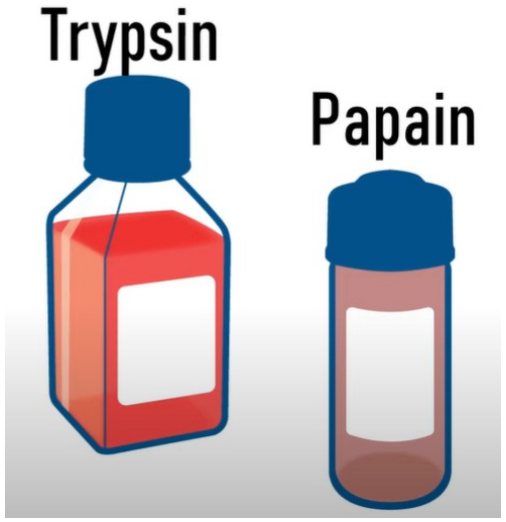
Bulk vs Single Cell RNA Sequence



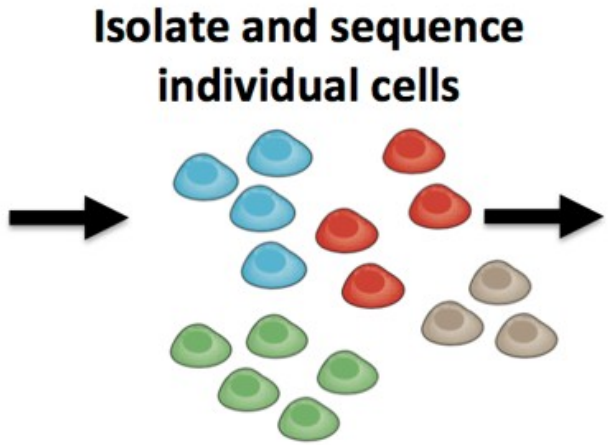
Step 1: Formation of Single Cell Suspension



Tissue with Heterogeneous Cell types



Dissociated with Trypsin or Papain



Formation of Single Cell Suspension

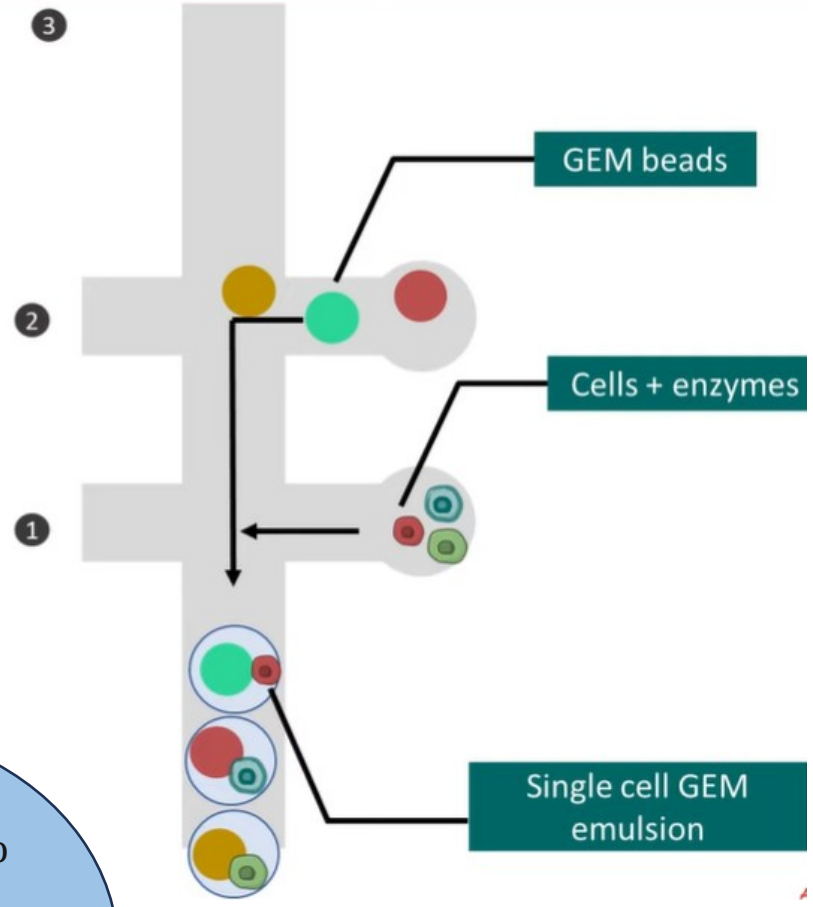
Step 2: Making Emulsions with GEM Bead

Partitioning oil

Gel Beads

Mastermix + sample

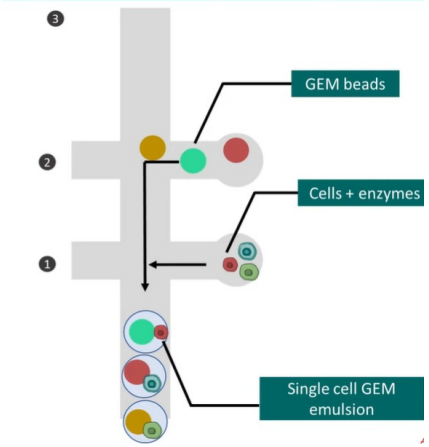
Chromium microfluidics chip

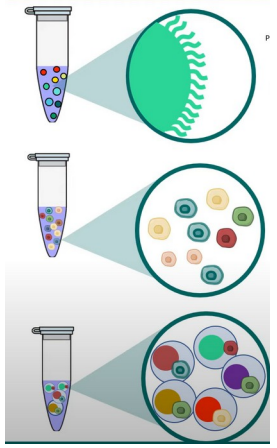


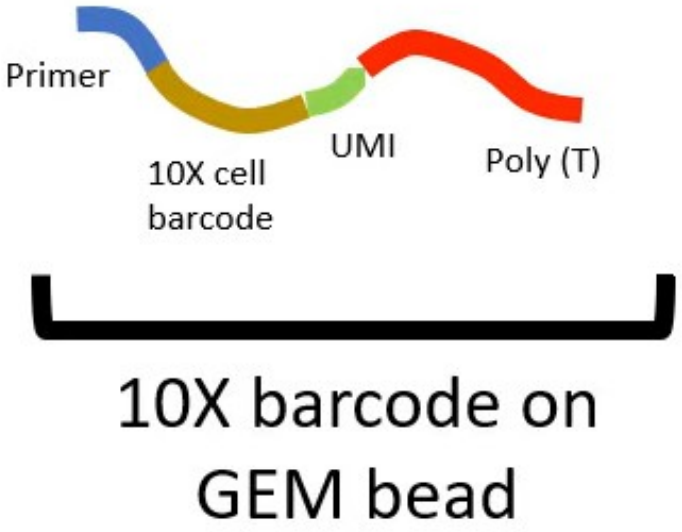
1. Single Cells
2. Reverse Transcription Reagents
3. Gel beads Containing barcoded oligonucleotides
4. Oil

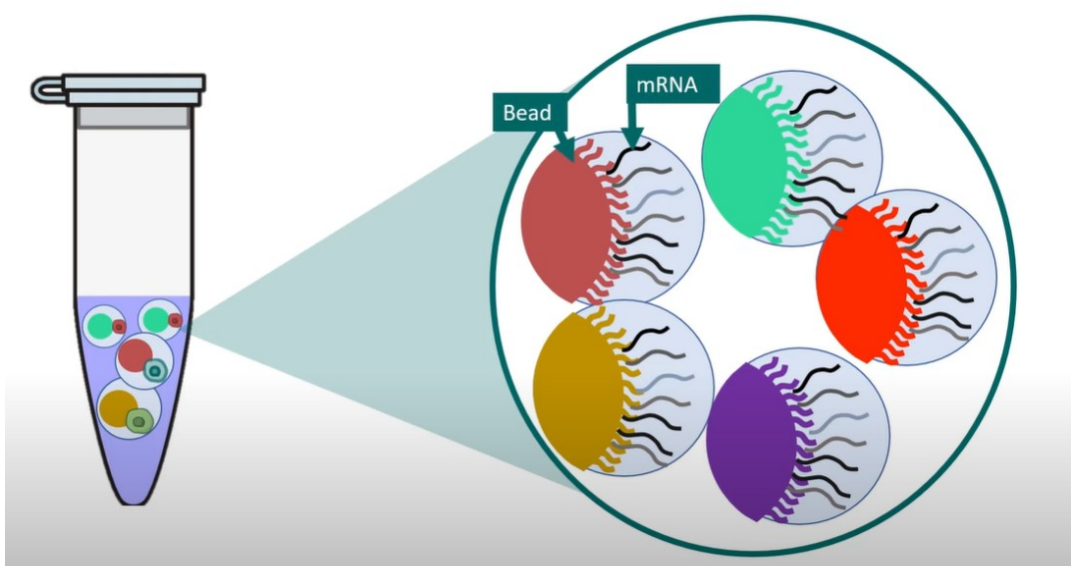
Combined on a microfluidic chip to form a reaction vesicle called Single-cell GEM emulsion

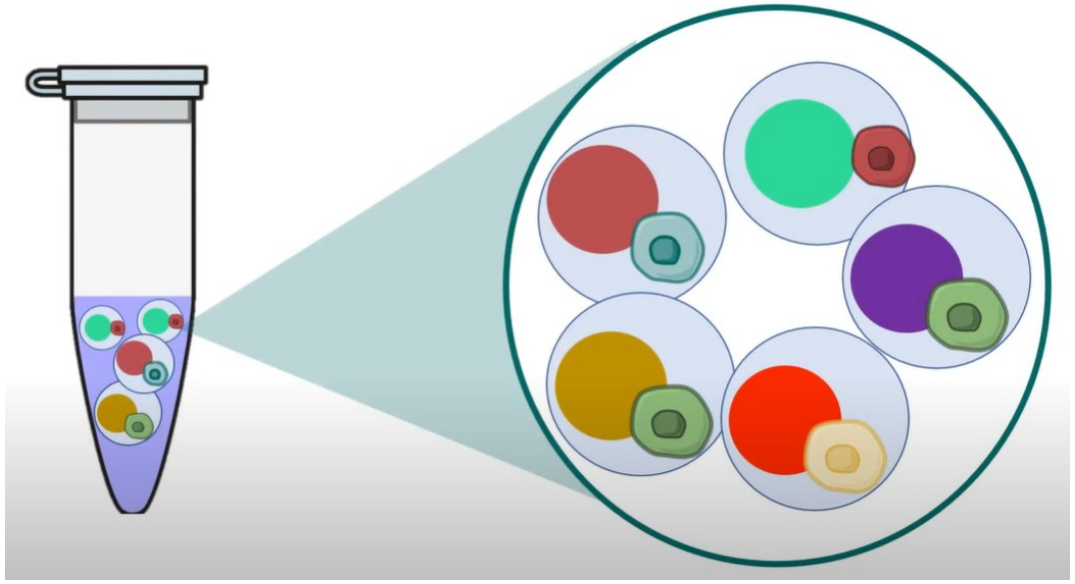
- ***Captures mRNA that comes from the cell***





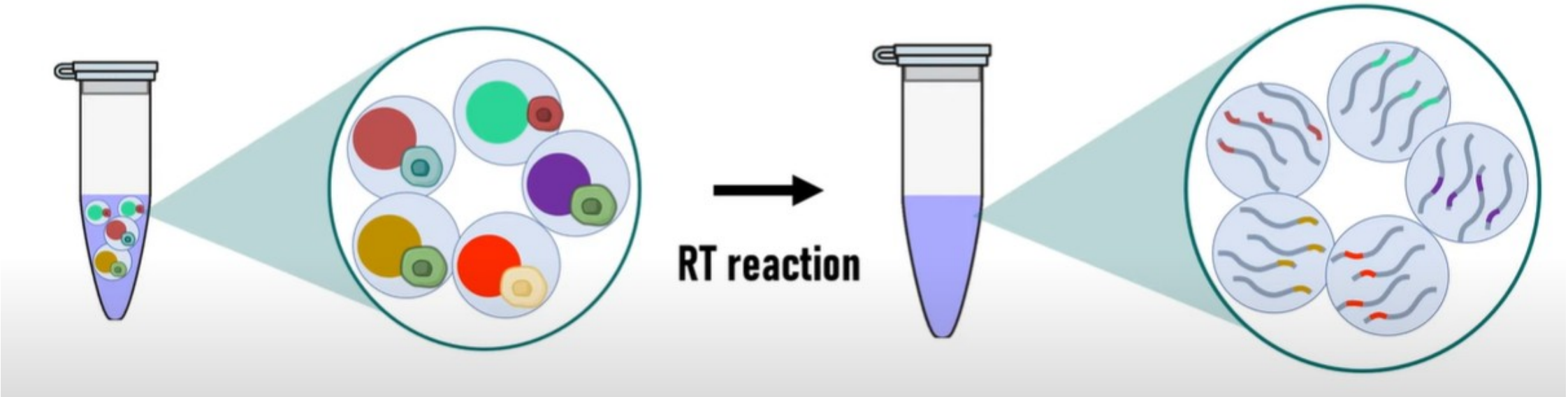


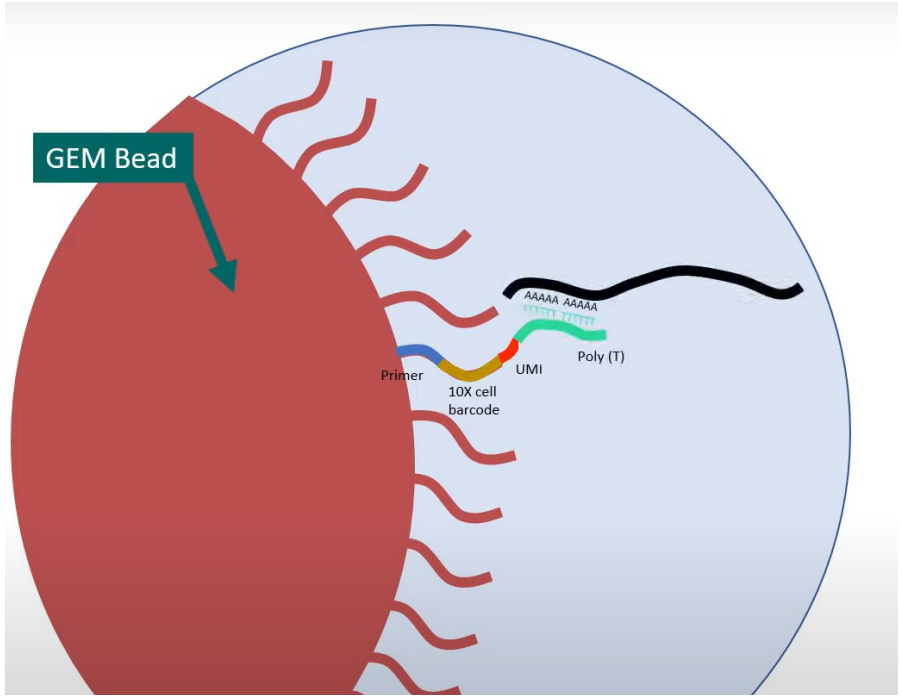




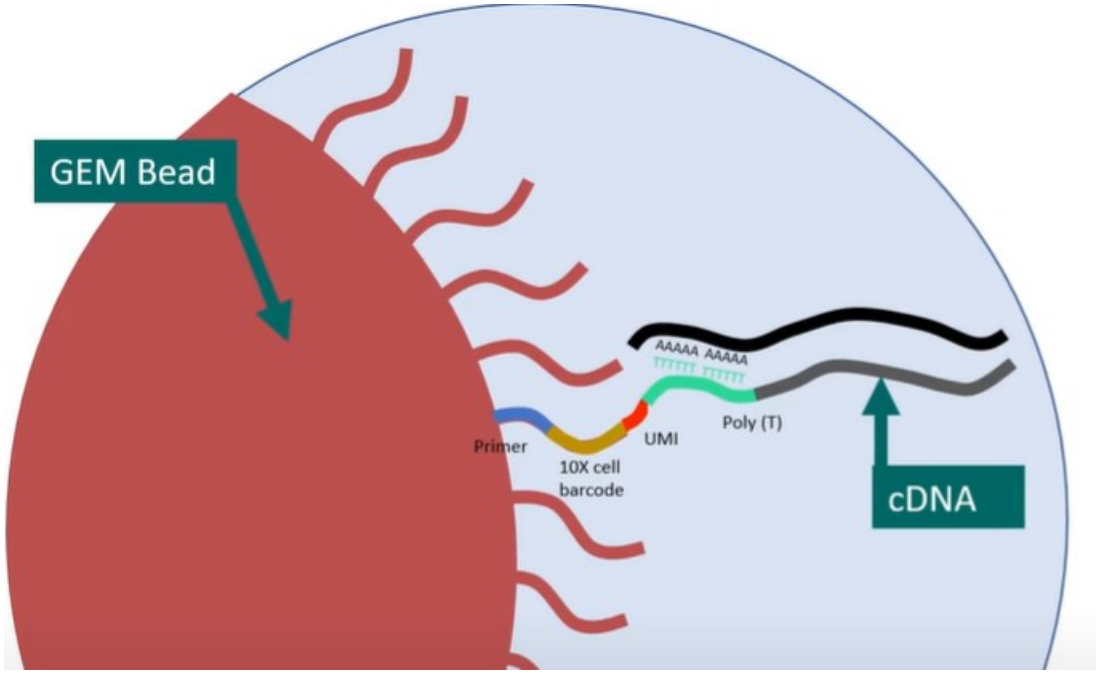
Step 3: Breaking the emulsion GEM Bead

•Sequences with the same barcode can be grouped together as they are coming from same cell source

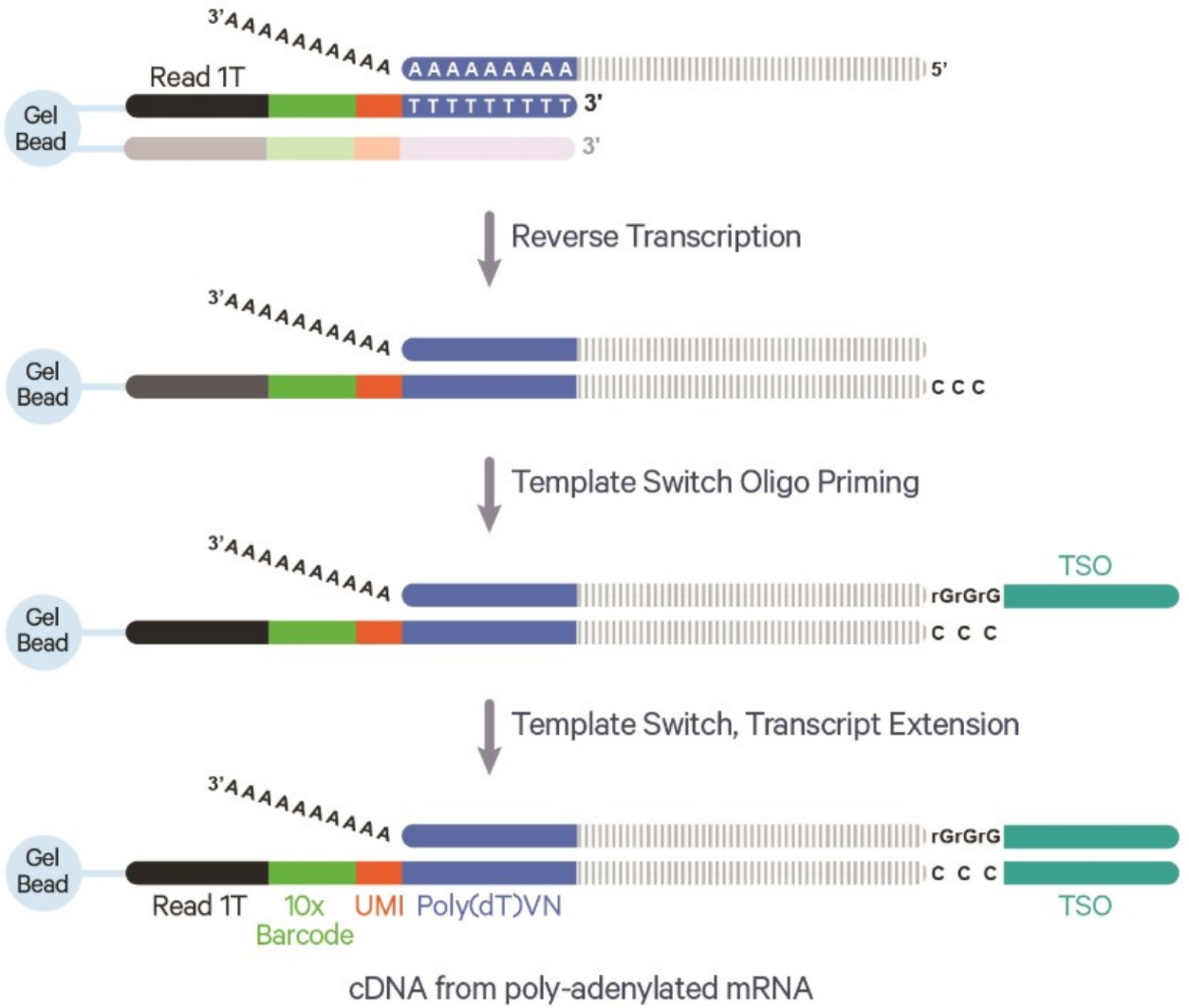




Reverse Transcription happening inside the Emulsion

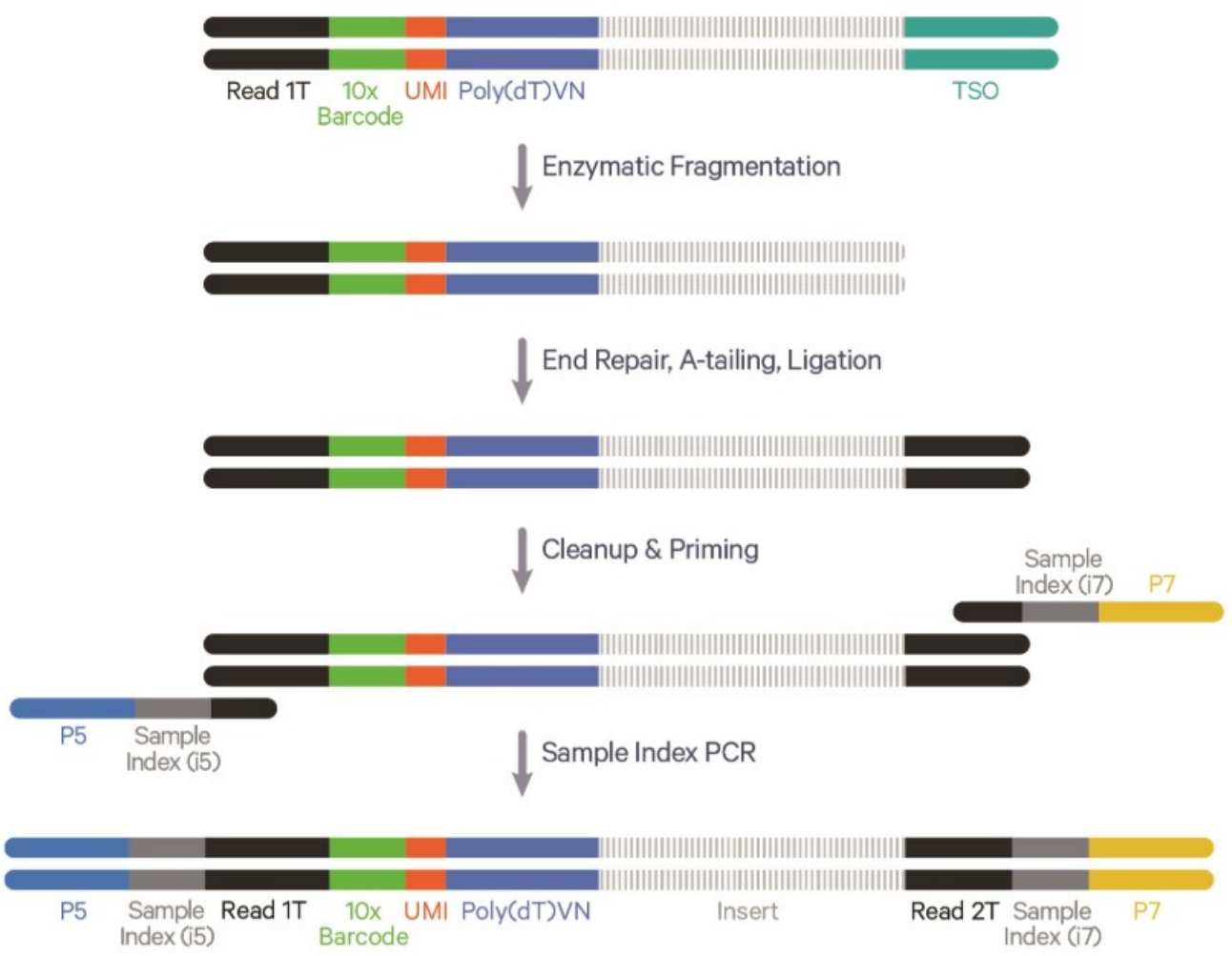


Inside Individual GEMs - A. Gene Expression Primer



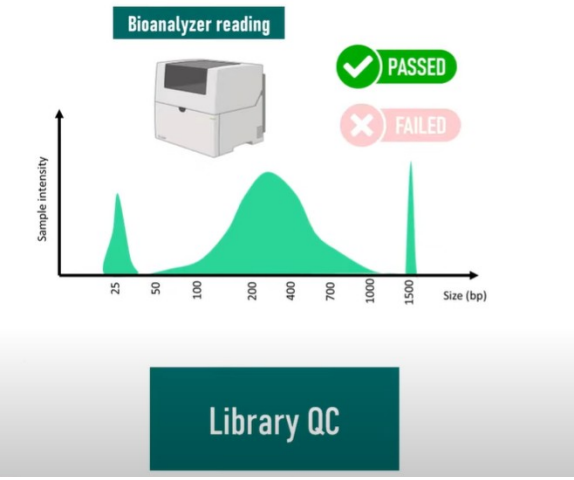
cDNA from poly-adenylated mRNA

Step 4 Library Preparation



Read By a Sequencer

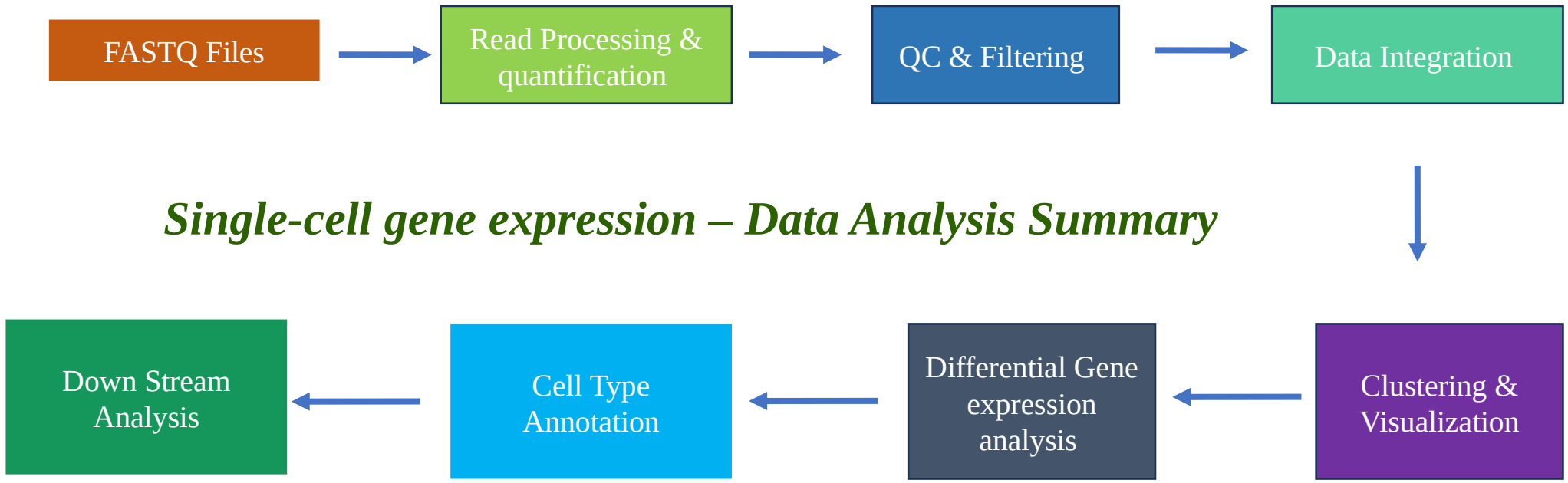
Step 5 Library QC and Sequencing



Passed Library QC

Loaded onto a Sequencer

READS



Single-cell gene expression – Data Analysis Summary

1. Read processing and Quantification

Analysis tool – **CELL RANGER**

Process – Extracting cell barcodes, alignment, UMI counting

Output --- Feature barcode matrix

	barcode1	barcode2	barcode3	barcode4
Gene1	3000	0	1241	1863
Gene2	2	2	2531	3
GeneN	1143	2	2	2522

2. QC & Filtering

Analysis tools – **CELL RANGER & LOUPE**

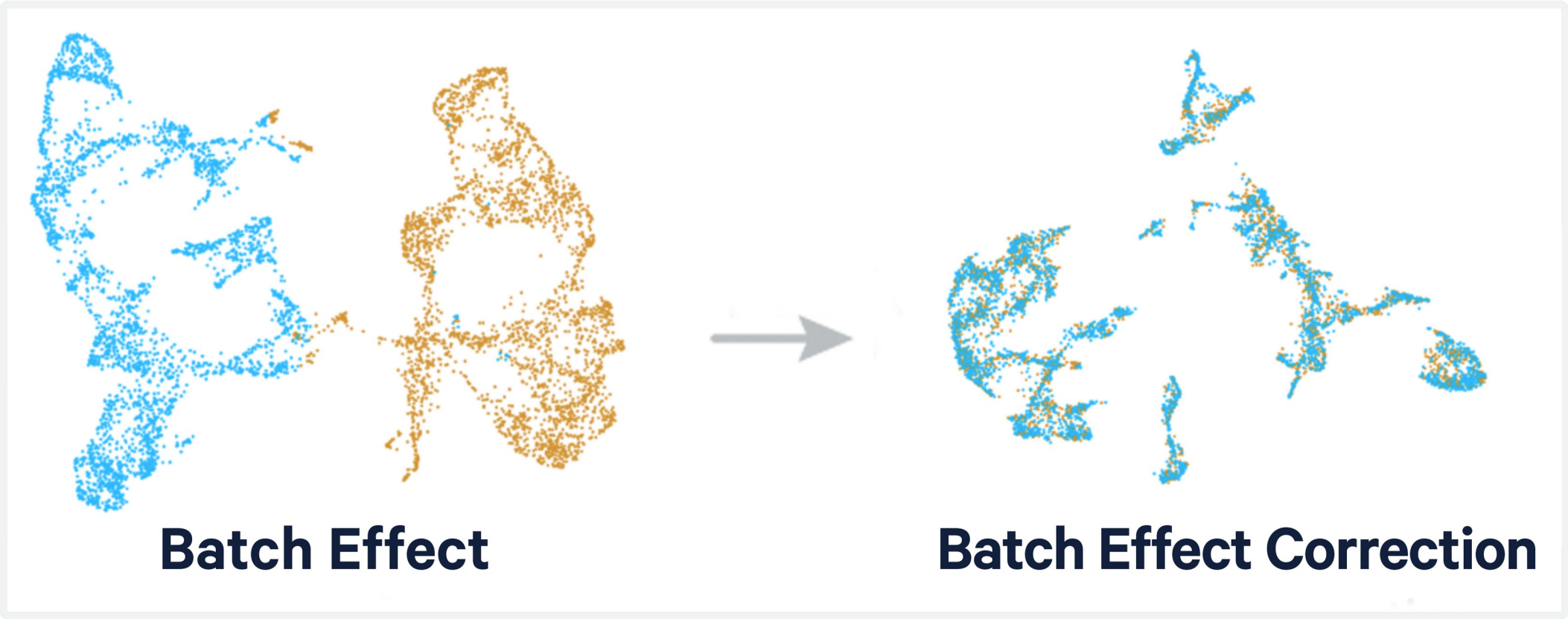
Process – Detecting cell-associated barcodes
Checking mapping and sequence qualities
Filtering cell barcodes based on a number of features, reads, etc..

Output --- Filtered barcodes

	barcode1	barcode3	barcode4
Gene1	3000	1241	1863
Gene2	2	2531	3
GeneN	1143	2	2522

3. Data Integration

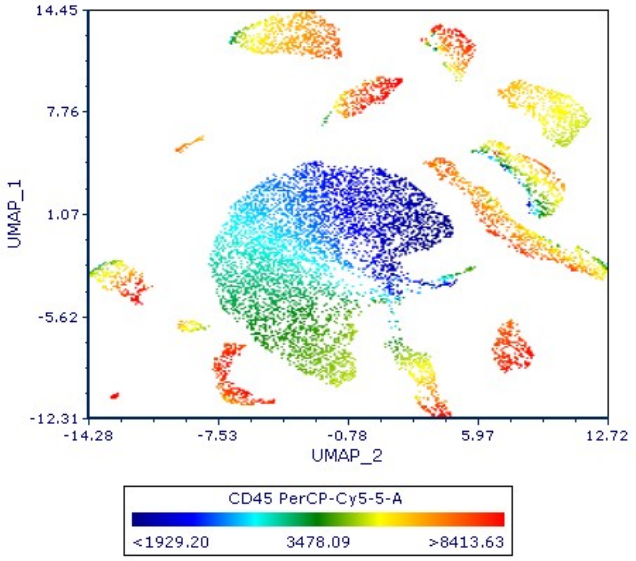
- Analysis tools – **CELL RANGER & Community Developed tools**
- Process – Integrate Multiple samples and correct potential batch effects
- Output --- Corrected, Integrated Data

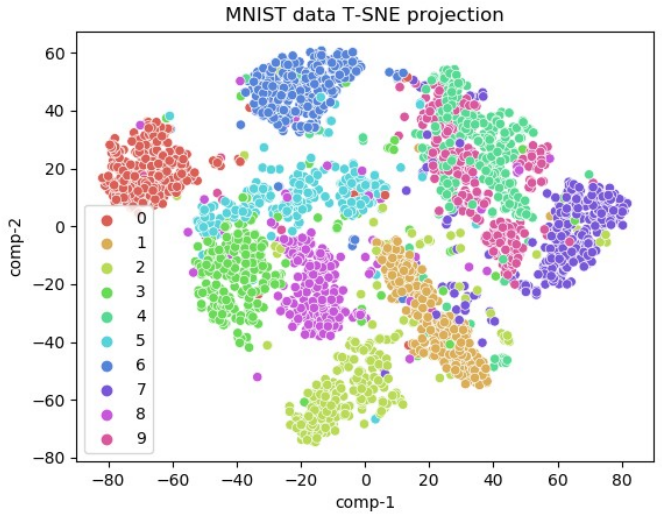


4. Clustering and Visualization

Analysis tools – **CELL RANGER**
LOUPE
Community Developed tools

Process – Group similar cells into clusters and Visualize using tSNE or UMAP
Output --- Cells in clusters and two-dimensional tSNE or UMAP plot





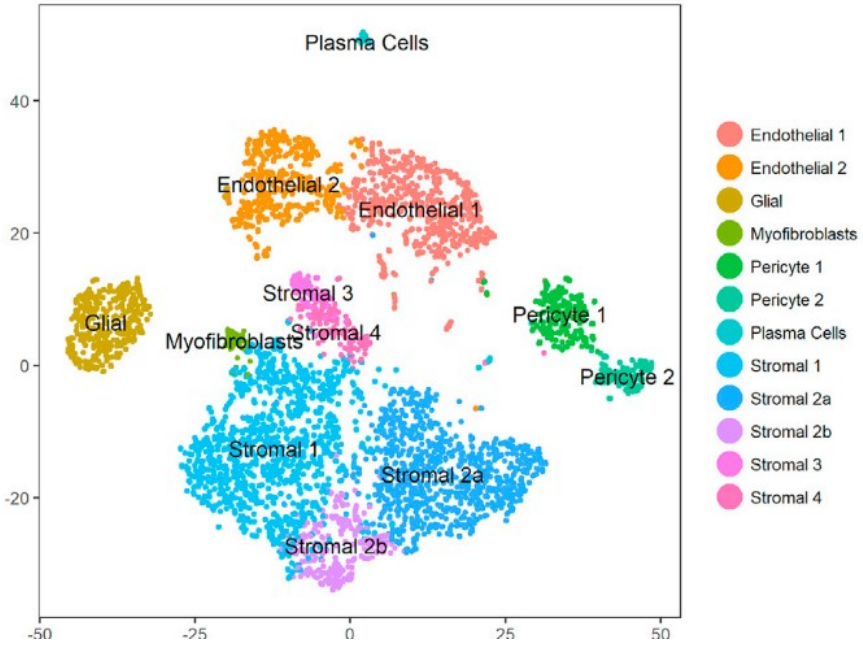
5. Differential Gene Expression Analysis

Analysis tools – **CELL RANGER**
LOUPE
Community Developed tools

Process – Perform differential gene expression analyses for each cluster vs the rest of the cells
Output --- List of enriched genes (markers) in each cluster, with logFC and P values

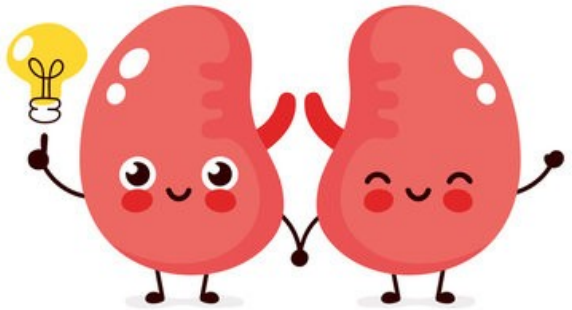
6. Cell type annotation

Process – Annotate cell clusters based on enriched markers or a reference dataset
Output --- Annotated cell populations



Single-cell RNA sequencing of human kidney

Jinling Liao[1,2,3,12], Zhenyuan Yu[1,2,3,4,5,12], Yang Chen[1,2,3,4,5,12], Mengying Bao[1,2,3], Chunlin Zou[6,7], Haiying Zhang[1,2,3], Deyun Liu[4,5], Tianyu Li[4,5], Qingyun Zhang[1,2,3,4,8], Jiaping Li[9,10,11], Jiwen Cheng[1,2,3,4,5*] & Zengnan Mo[1,2,3,4,5*]



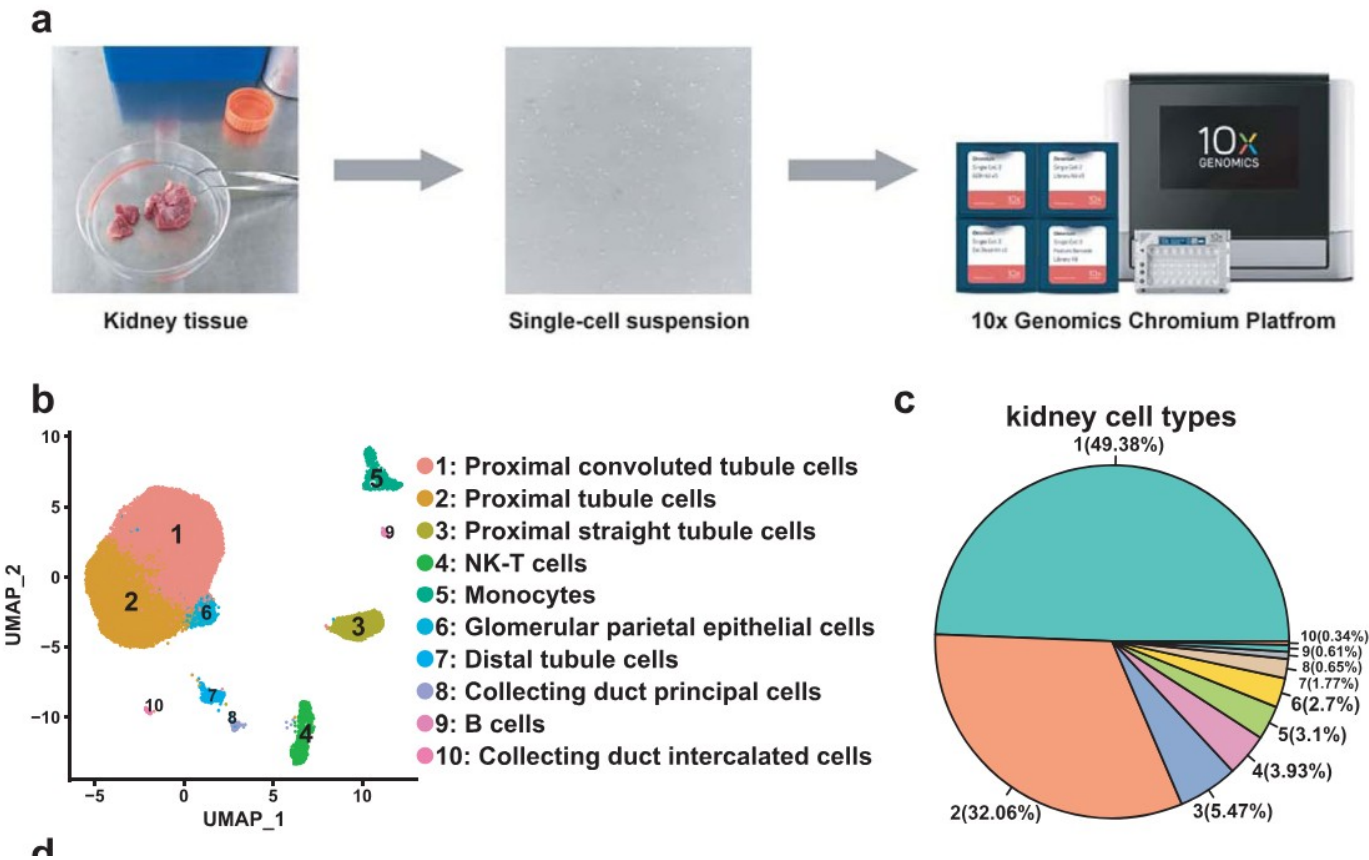
- **Objective**: The study aimed to understand the cellular composition and transcriptomic profiles of the human kidney to provide insights into renal diseases and cancer.

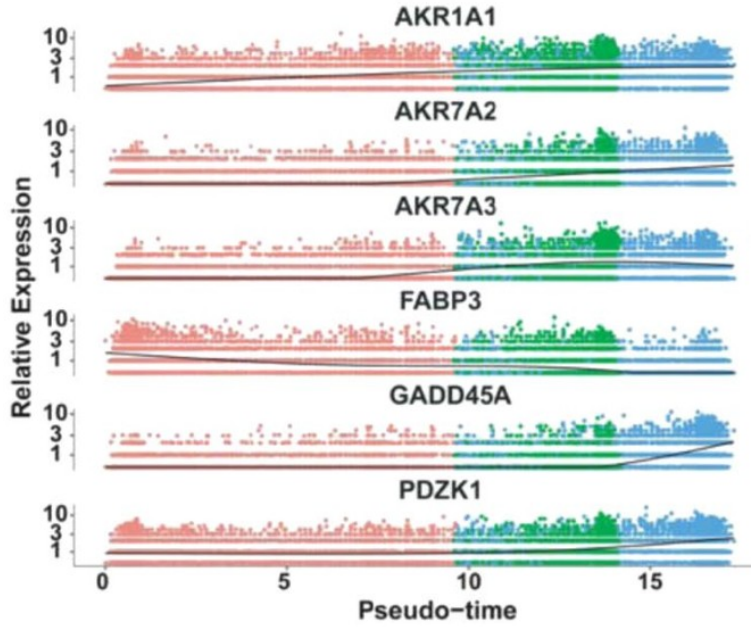
- ✓ Fresh human kidney samples were obtained from patients undergoing radical nephrectomy and radical nephroureterectomy. Normal kidney tissues were collected at least 2 cm away from tumor tissue.
- ✓ Used 10x Genomics Chromium platform for single-cell RNA sequencing
- ✓ scRNA-seq data was generated for **23,366 high-quality cells** from three human kidney donors.
- ✓ **Identified 20,308 PT cells**, highlighting their abundance in the dataset. Classified PT cells into three distinct clusters based on their molecular markers: proximal convoluted tubule, proximal straight tubule, and a group with no accurate classification

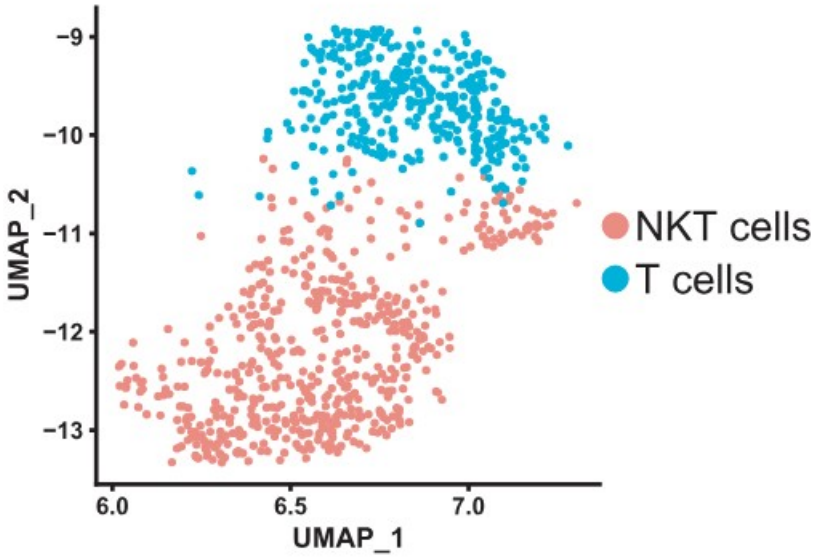
- The study identified 10 clusters of normal human renal cells. Subtypes within proximal tubule (PT) cells (three subtypes) and collecting duct cells (two subtypes) were characterized.
- The data provided detailed transcriptomic information at the single-cell level, allowing for the identification of specific gene expression patterns within different cell types.
- Clustering analysis identified 10 distinct cell types and differentially expressed genes were identified for each cell type.

Findings:

- ✓ **Characterization of Proximal Tubule (PT) Cells**
- ✓ **Characterization of Collecting Duct Cells**
- ✓ **Confirmation of Data Validity**
- ✓ **Detailed Classification of Cell Subsets**







- The top six genes influencing fate decisions are shown as line plots displayed as the expression level over pseudo time by Monocle2
- Classified collecting duct cells into principal cells and intercalated cells according to marker expression
- Cluster 4 highly expressed marker genes of both NK cells and T cells, designated as NK-T cells
- By modifying the parameters cluster 4 can be further classified into two subtypes NKT cells (characterized by specific genes like CD3D, CD3E, GNLY, and NKG7) and T cells (characterized by genes like CD3D, CD3E, and IL7R).

REFERENCES

- https://www.10xgenomics.com/blog/single-cell-rna-seq-an-introductory-overview-and-tools-for-getting-started
- https://youtu.be/eVEDw1YykME?si=ElAcmdANkj4_R5mE
- https://www.10xgenomics.com/support/single-cell-gene-expression/documentation
- file:///C:/Users/admin/Downloads/CG000732_ChromiumGEM-X_SingleCell3_ReagentKitsv4_CellSurfaceProtein_UserGuide_RevA.pdf
- https://www.nature.com/articles/s41597-019-0351-8