Giới thiệu phương pháp phân tích dữ liệu phiên mã gene theo cấu trúc không gian và theo từng tế bào đơn

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HCMC, 12/08/2023

Contents

Self-introduction and Acknowledgement

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- NGUYEN PHAN XUAN TRUONG, MD
- TRAN NGUYEN TRONG PHU, MD

All member of VnPathoinformatics group

Pacific Informatics
Your comprehensive bioinformatics solution

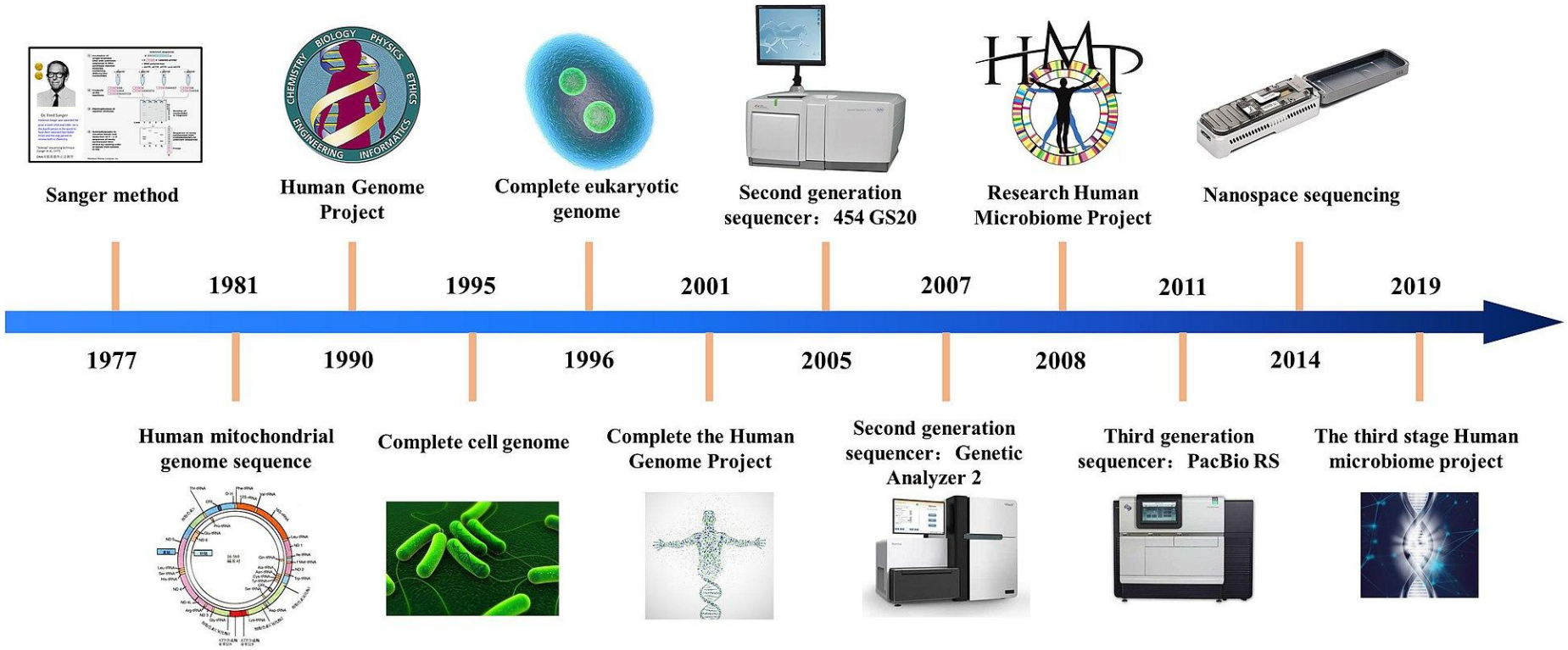
The Bioinformatics Team

ZYMO RESEARCH

Name and Role	Profile Picture	Profile Picture	Name and Role
Luu Phuc-Loi, PhD Team Lead			**Nguyen Viet Tuan**, PhD Consultant
Du Hoang Tien, Msc. Associate			**Phan Vo Thu Nga**, Bsc. Associate
Nguyen Le Duc Minh, Dr. Genetics Consultant			**Tran Thien Tan**, Bsc. Technician
Dao Khuong Duy, Bsc. Technician **Nguyen Minh Hoang**, Bsc. Technician	 		**Le Van Hieu**, Bsc. Technician

Human Genome and Next-Generation Sequencing

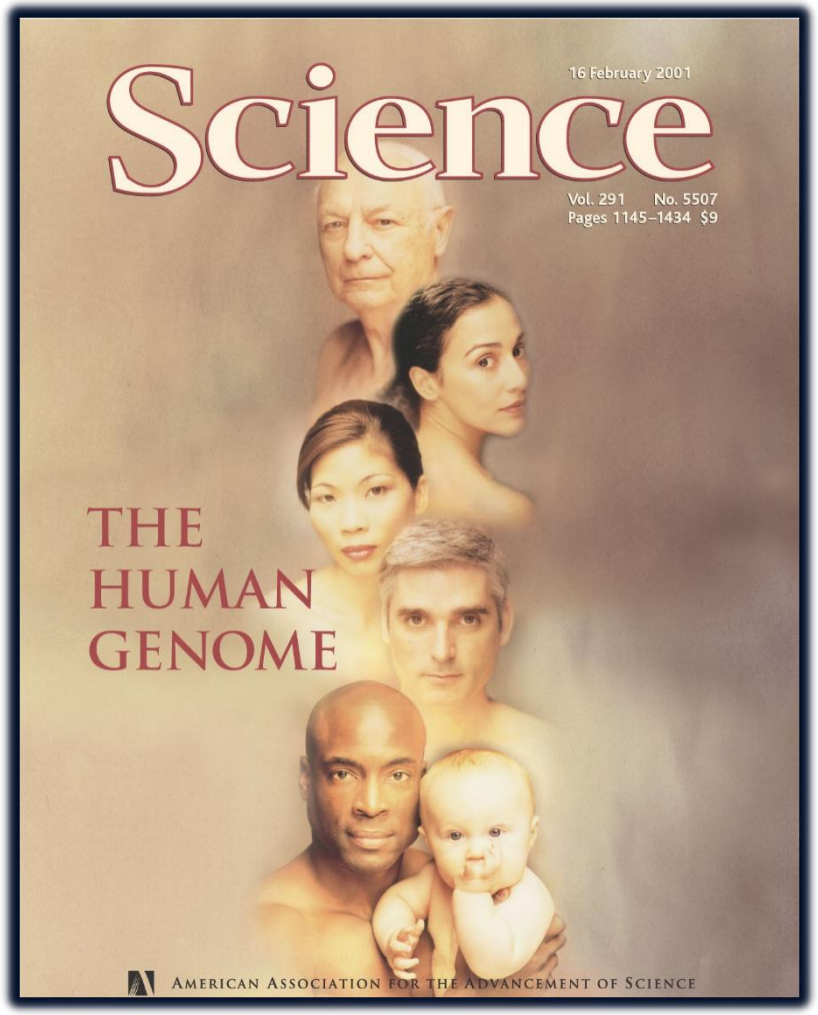
High-throughput sequencing (HTS) methods



https://en.wikipedia.org/wiki/DNA_sequencing

February 2001: Papers Reporting Draft Sequence of Human Genome

HGP Paper



Venter/Celera Paper

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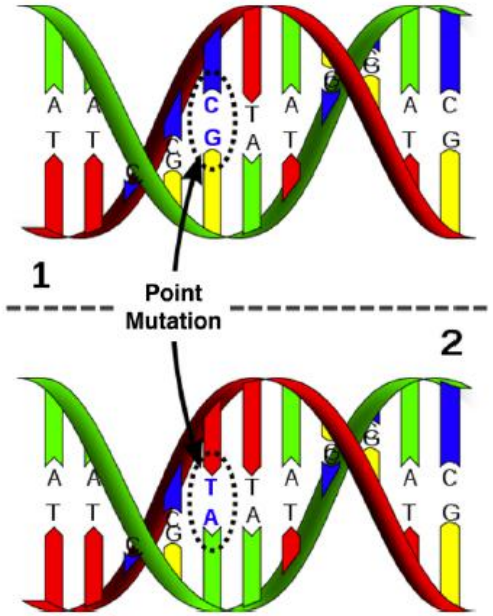
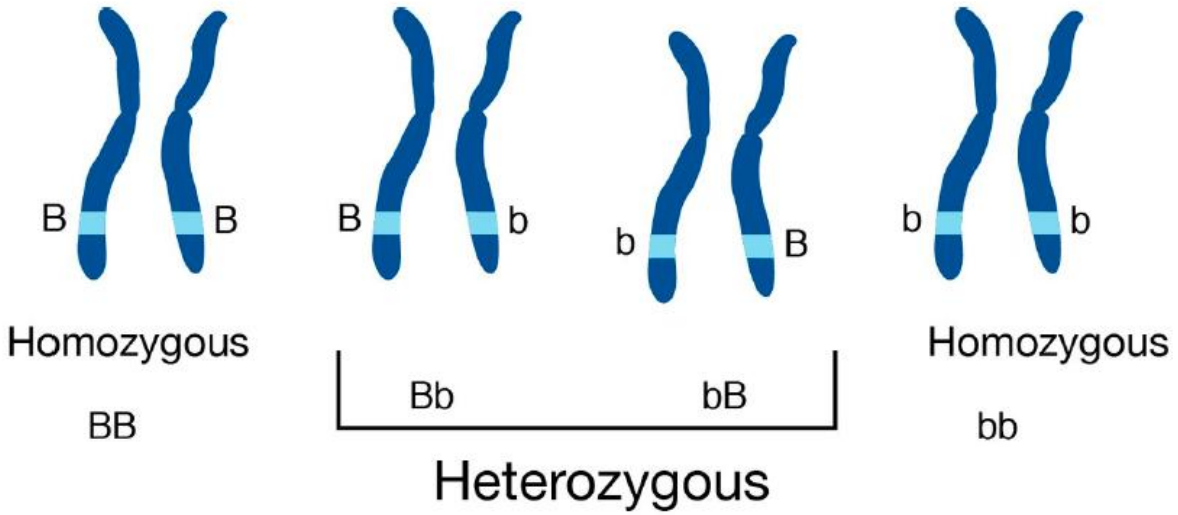
```

https://www.ncbi.nlm.nih.gov/nuccore/806904736

Human Genome Variation

- Humans genomes are >99% similar by sequence
- A typical human genome has ~5 million variants with 3-4 million single-nucleotide variants
- Humans are diploid

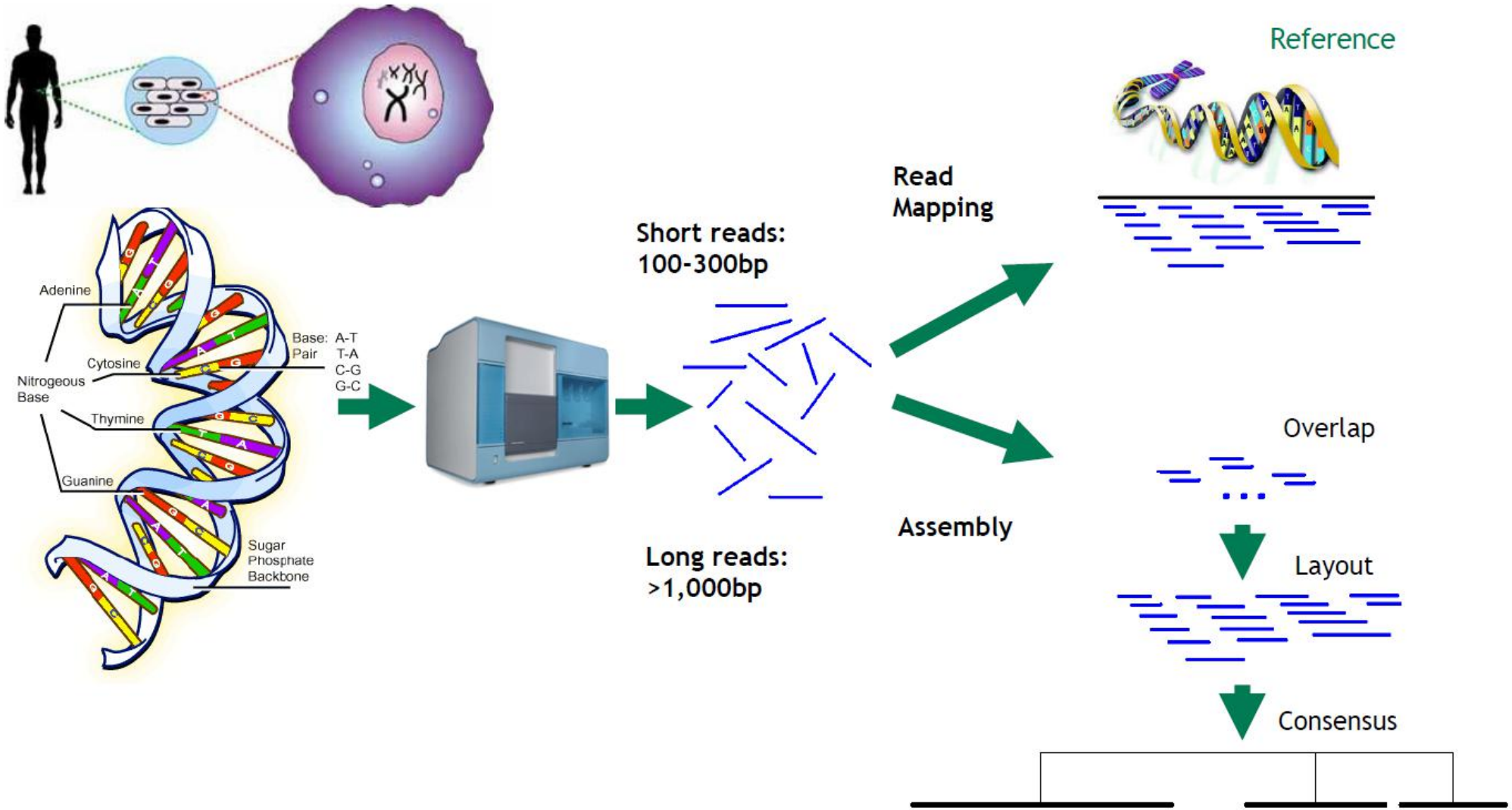




Source: https://rosalind.info/media/point_mutation.png, https://en.wikipedia.org/wiki/Zygosity

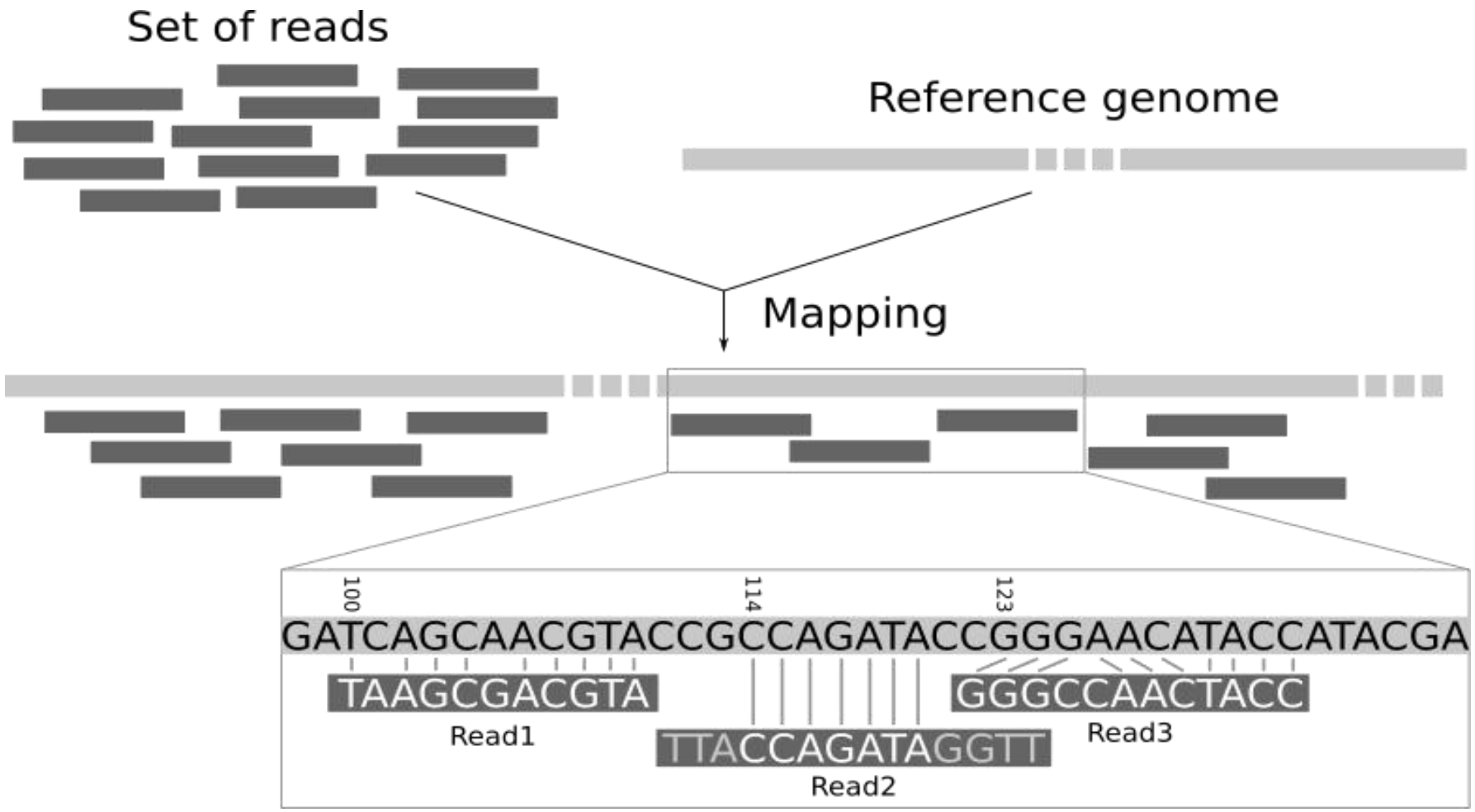
Tobias Rausch

Sequencing DNA



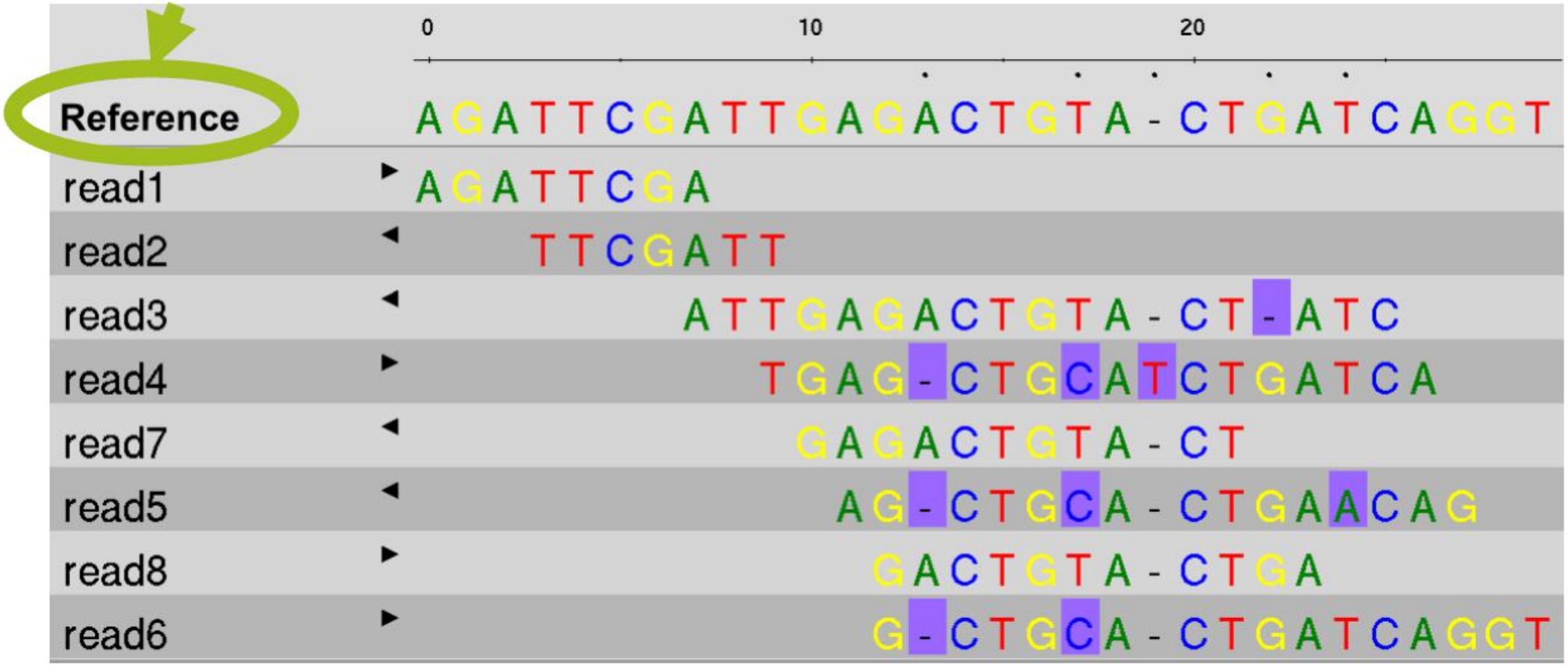
Tobias Rausch

Read Alignment



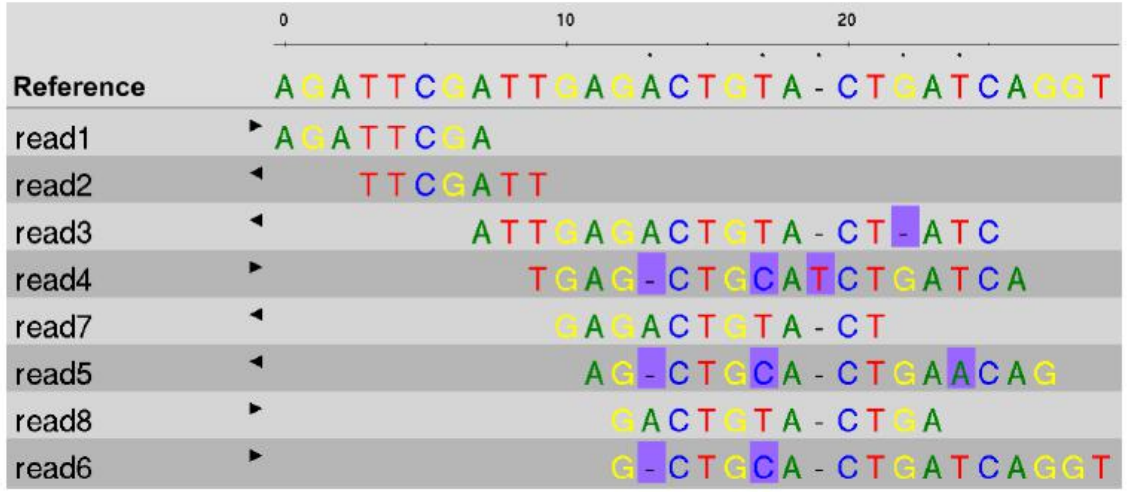
Read Alignment

Reference sequence



Variant Calling - Data Transformation

Alignment



SAM/BAM file

List of Variants

CHR	POS	ID	REF	ALT	GT
chr1	12	.	GA	G	0/1
chr1	17	rs123	T	C	0/1

[No Title]

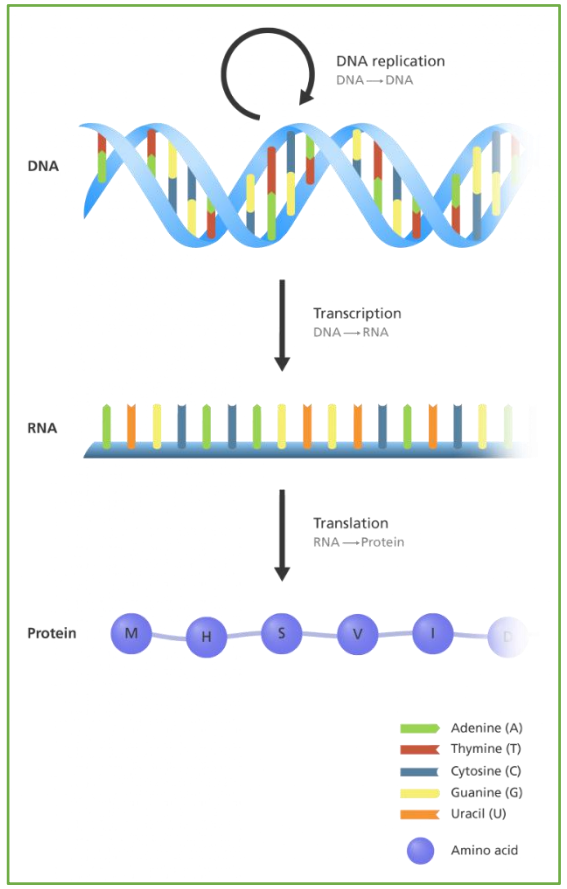
Genotype (GT):

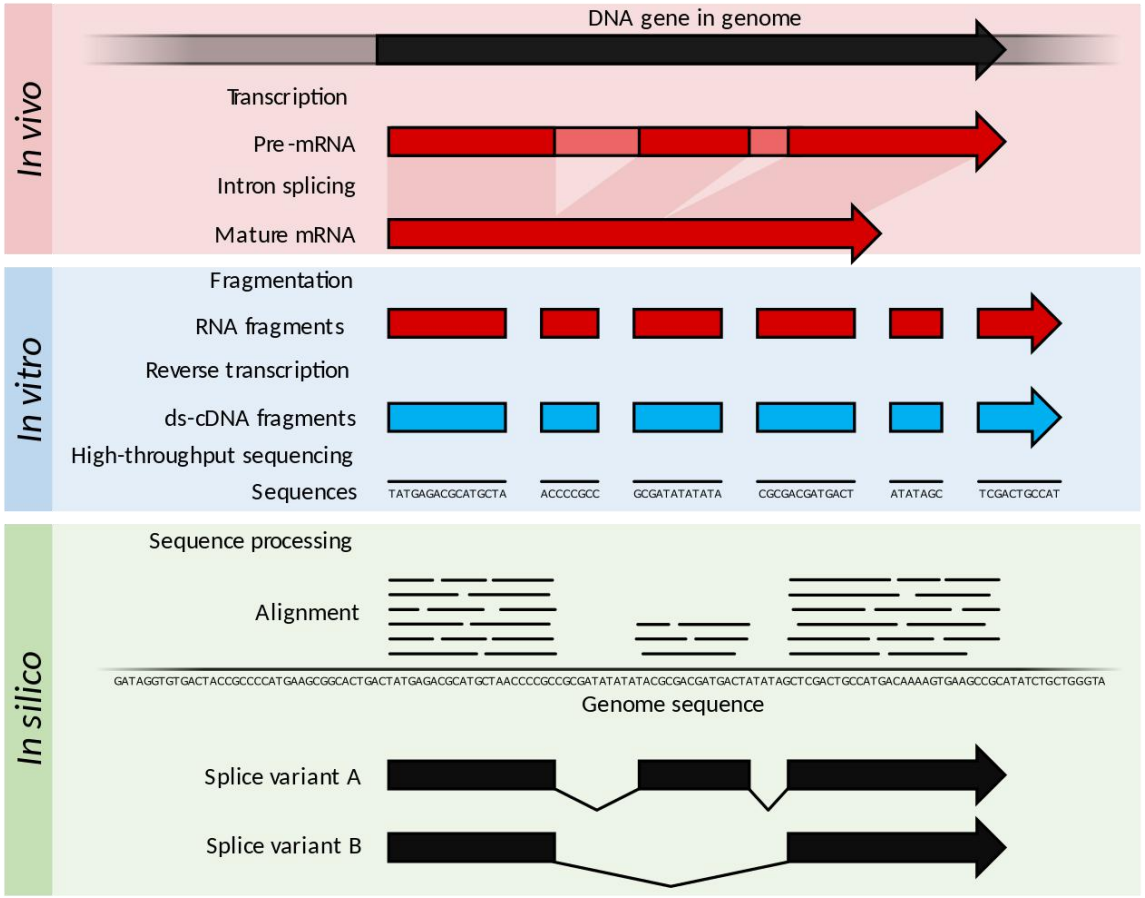
0/0: hom. reference
0/1: heterozygous
1/1: hom. alternative

VCF/BCF file

Bulk RNA Sequencing (RNA-seq)

What is RNA sequencing (RNA-seq)?





https://www.yourgenome.org/facts/what-is-the-central-dogma/

https://en.wikipedia.org/wiki/RNA-Seq

What is RNA sequencing (RNA-seq)?

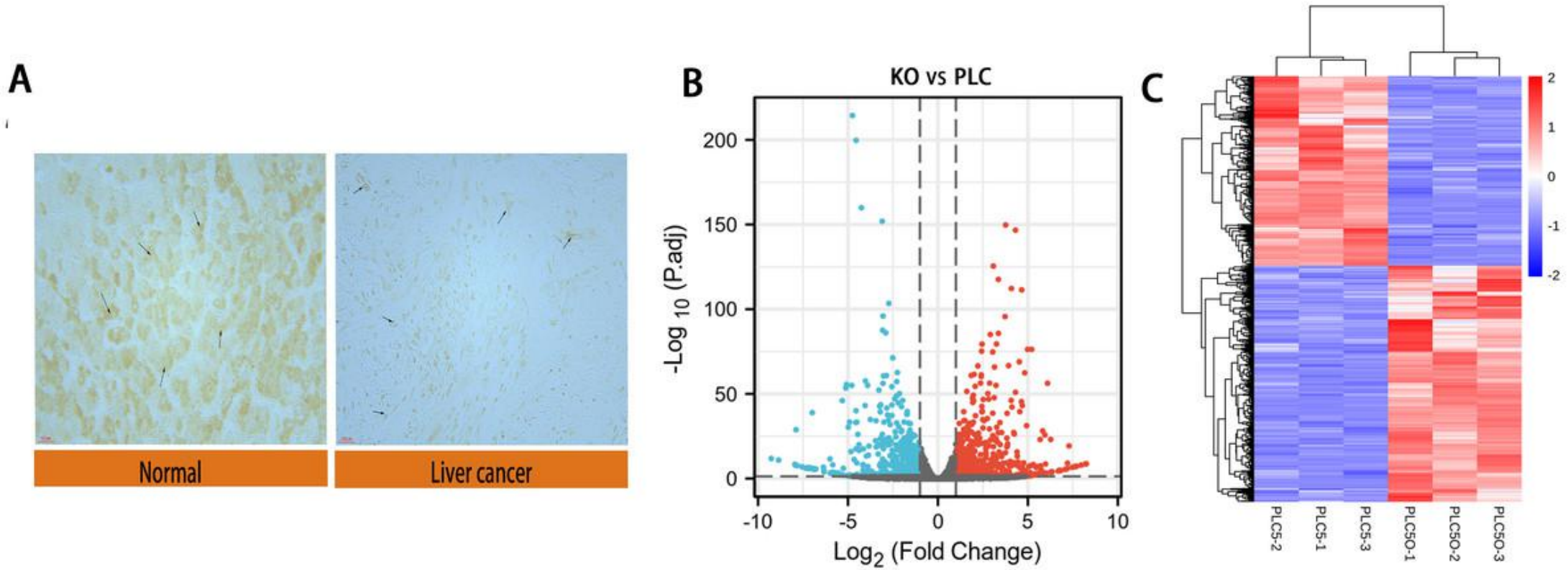


Figure: Differential expression of DAPK1 and volcano plot and heat maps of differential genes.

(A) Relative expression of DAPK1 compared to normal liver tissue samples. (B, C) Volcano plot and heat map showing the 732 differentially expressed genes. The color red indicates upregulated genes, and blue indicates downregulated genes. PLC, PLC/PRF/5 cells; KO, DAPK1–knockout PLC/PRF/5 cells. padj <0.5, logFC 1.

https://www.yourgenome.org/facts/what-is-the-central-dogma/

What is RNA sequencing (RNA-seq)?

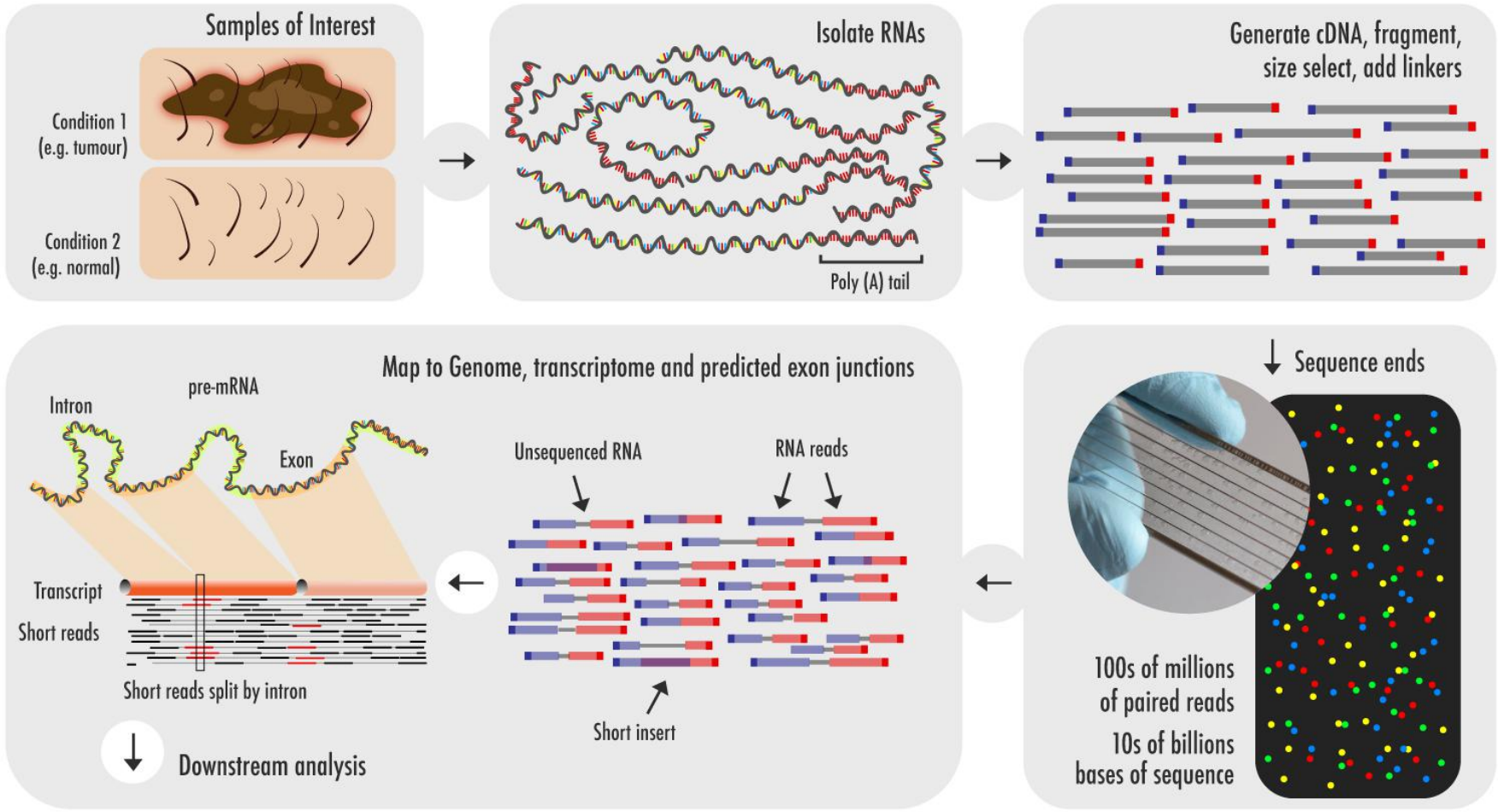
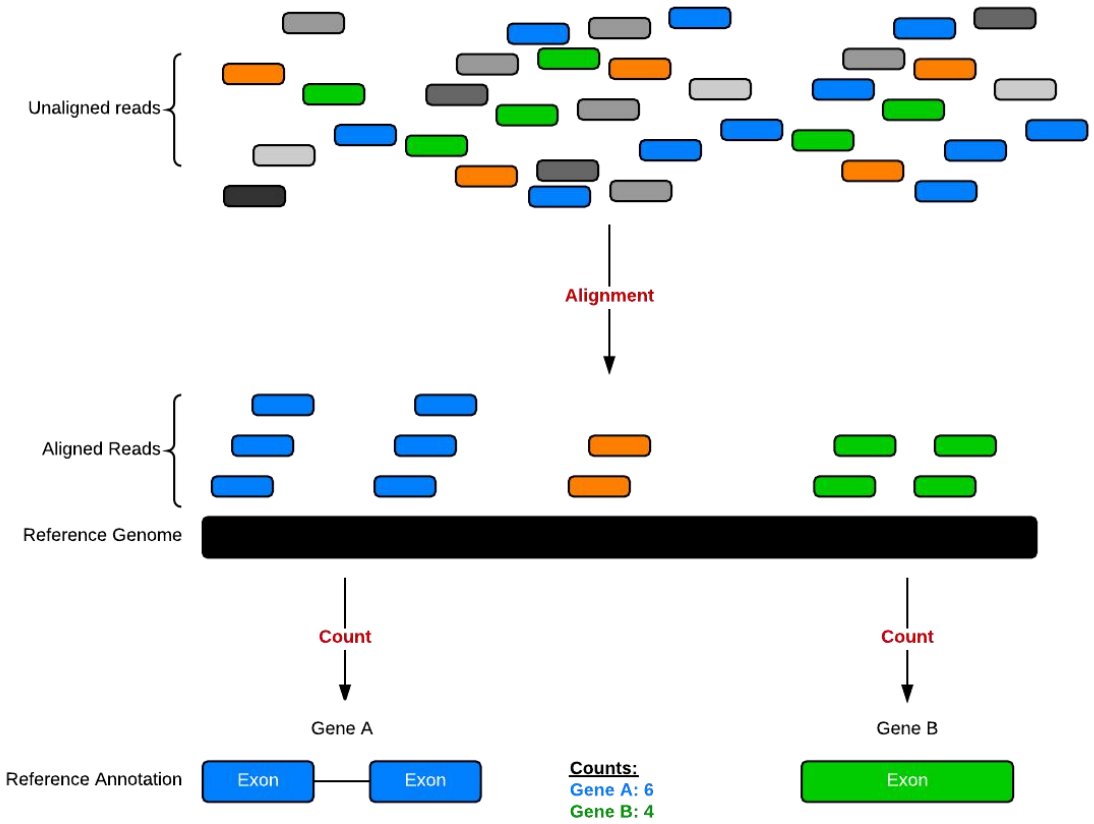


Figure. *A workflow for RNA-seq. Credit: Technology Networks.*

How can RNA sequencing quantify gene expression?



countData

	ctrl_1	ctrl_2	exp_1	exp_1
geneA	10	11	56	45
geneB	0	0	128	54
geneC	42	41	59	41
geneD	103	122	1	23
geneE	10	23	14	56
geneF	0	1	2	0
…	…	…	…	…
…	…	…	…	…
…	…	…	…	…

colData

	treatment	sex
ctrl_1	control	male
ctrl_2	control	female
exp_1	treatment	male
exp_2	treatment	female

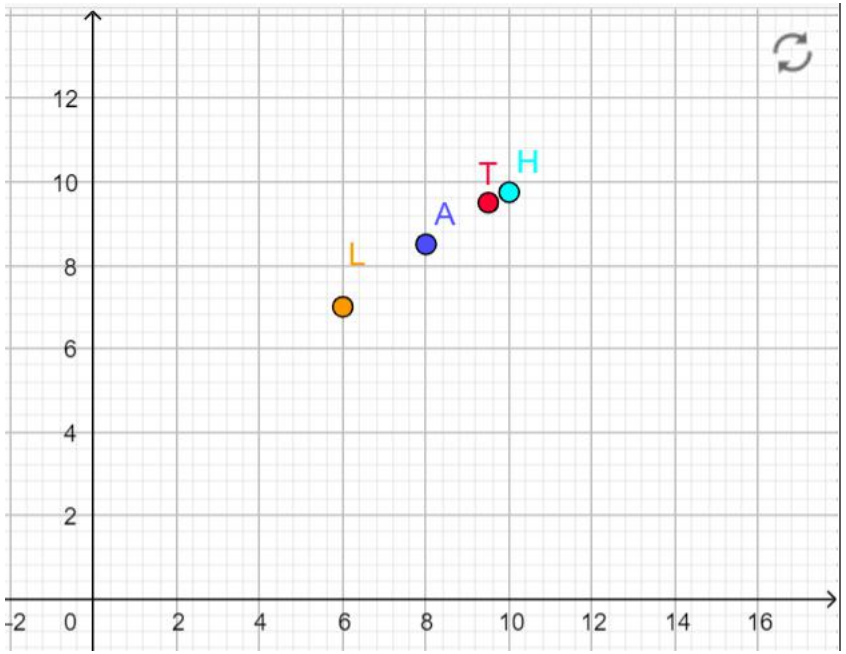
Sample names:
ctrl_1, ctrl_2, exp_1, exp_2

http://bioconnector.github.io/bims8382/r-rnaseq-airway.html

Data of life

	Name	Math	English
1	Kim Anh	8	8.5
2	Hiep	10	9.75
3	Hanh	9.75	10 (French)
4	Loi	6	7
5	Truong	9.5	9.5

English

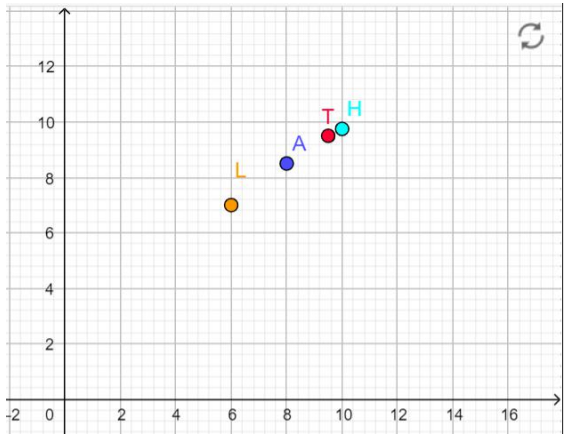


Math

Data of life

	Name	Gender	Age	Math	English	History	isDoc
1	Kim Anh	f	16	8	8.5	10	No
2	Hiep	m	39	10	9.75	6	yes
3	Hanh	f	18	9.75	10 (French)	9.5	yes
4	Loi	m	41	6	7	8	No
5	Truong	m	34	9.5	9.5	9	yes

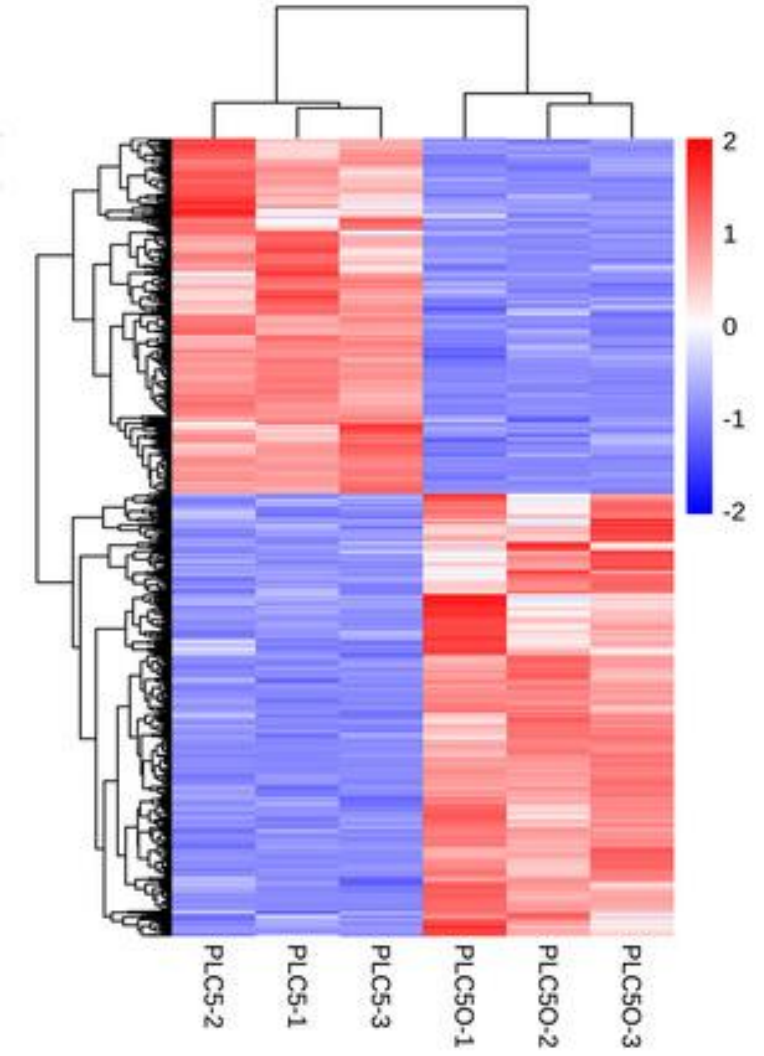
PC2 (14%)



PC1 (62%)

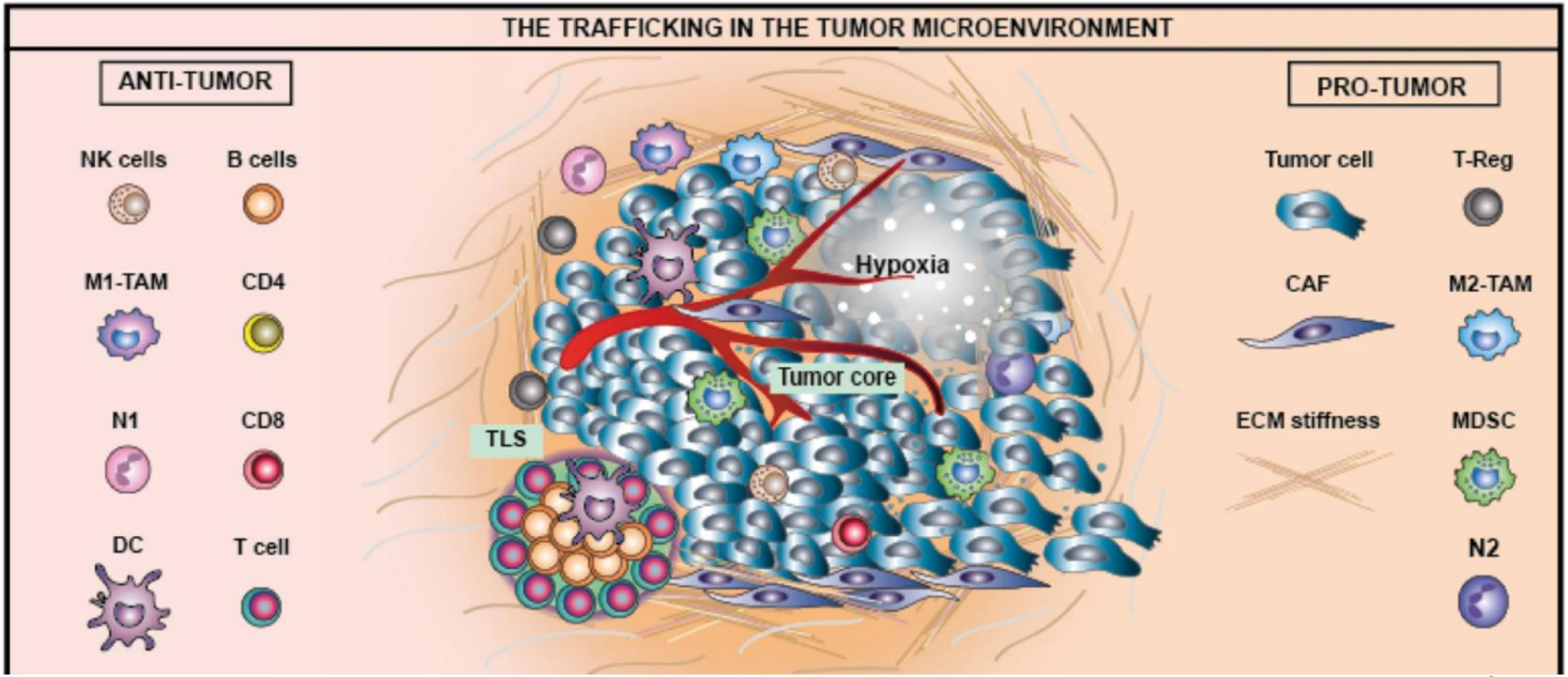
Heatmap

	A	B	C	D
1		2014	2015	2016
2	January	600	708	594
3	February	607	984	749
4	March	901	886	908
5	April	608	615	835
6	May	715	833	734
7	June	520	663	618
8	July	731	521	950
9	August	709	663	987
10	September	633	863	979
11	October	533	651	841
12	November	996	958	749
13	December	792	717	875



Single cell RNA Sequencing (scRNA-seq)

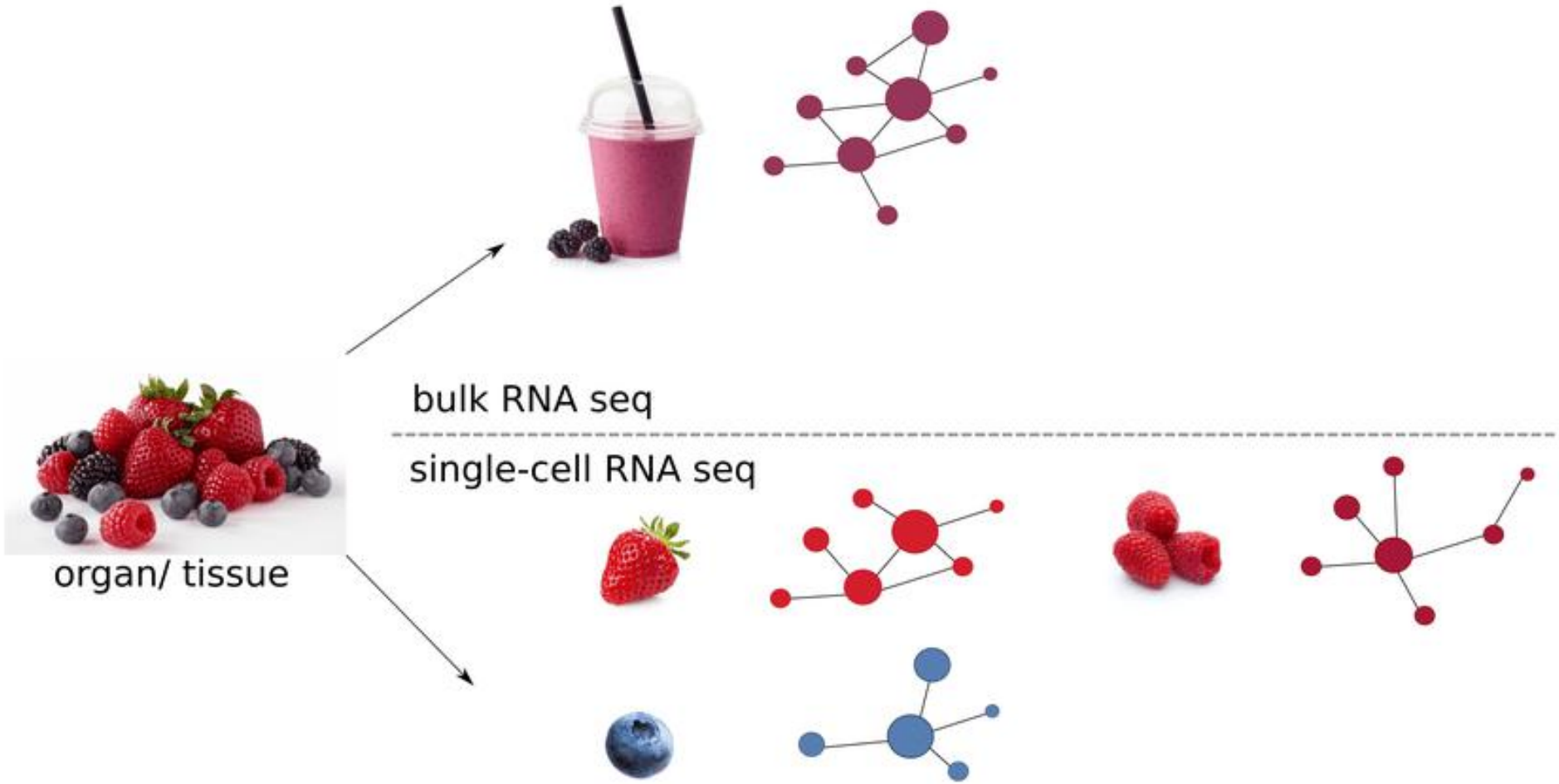
Single-cell applications: Tumor microenvironment



Source: https://doi.org/10.1186/s13046-020-01586-y

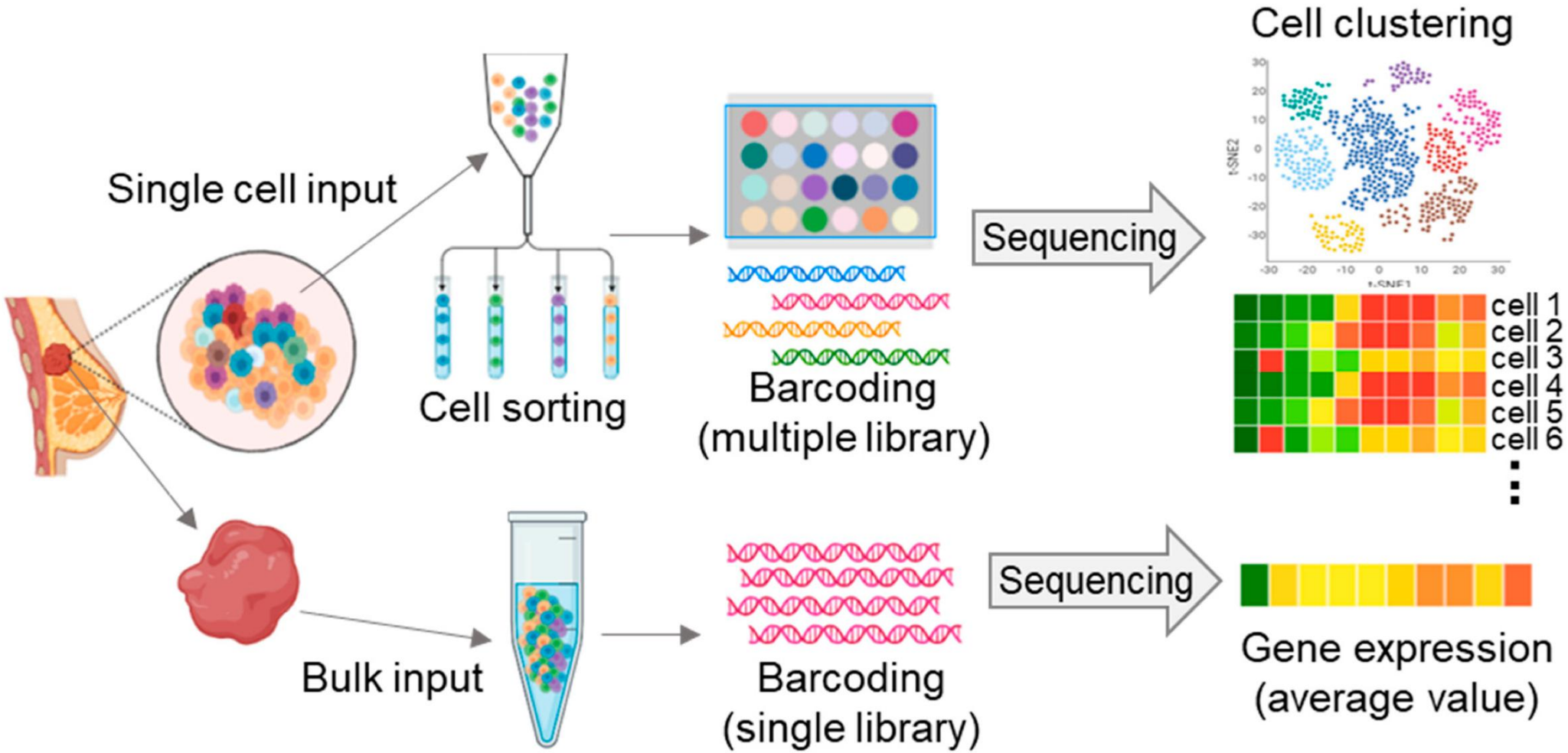
Tobias Rausch

Bulk RNA-seq vs Single-cell RNA-seq



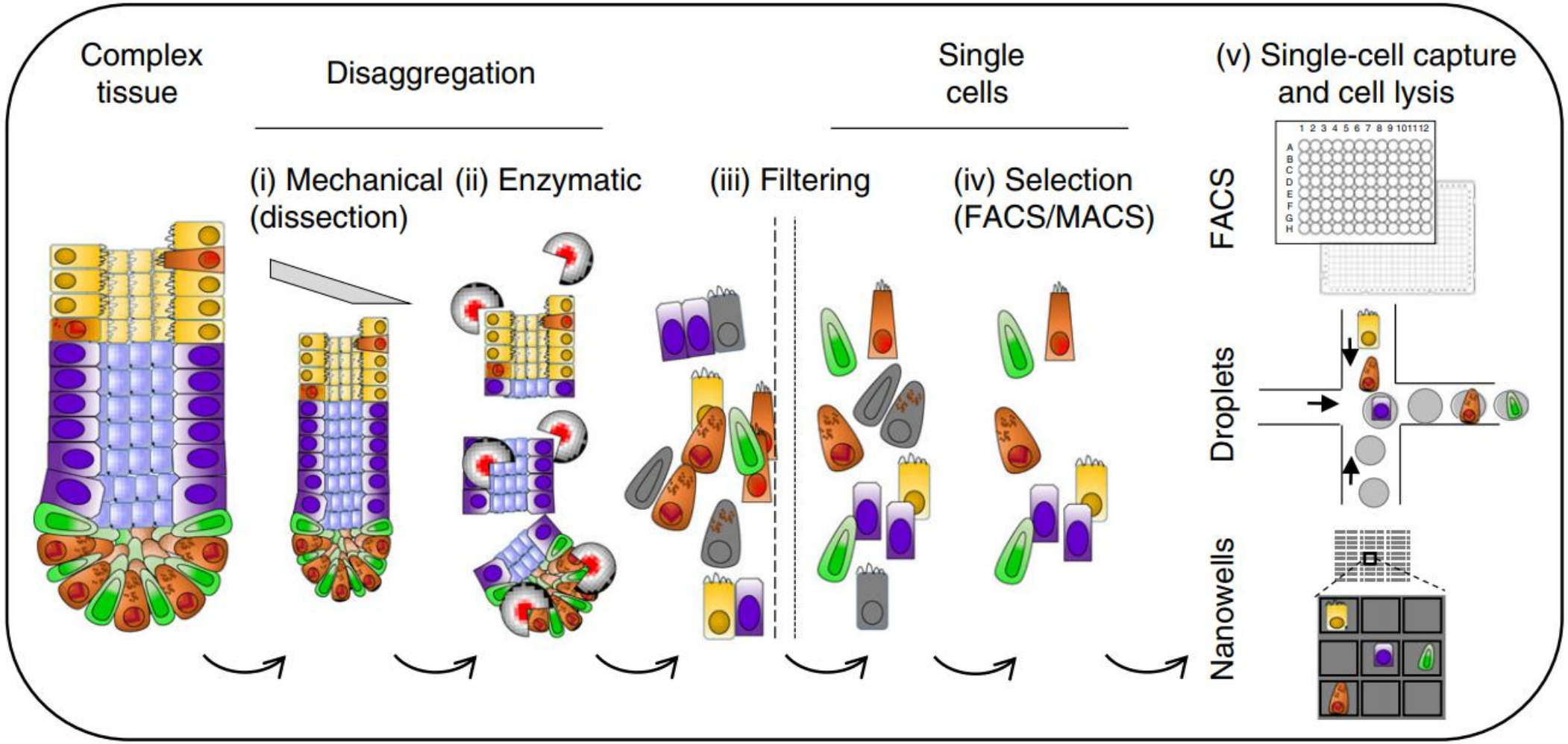
DOI:10.1101/2021.04.02.438193

Bulk RNA-seq vs Single-cell RNA-seq



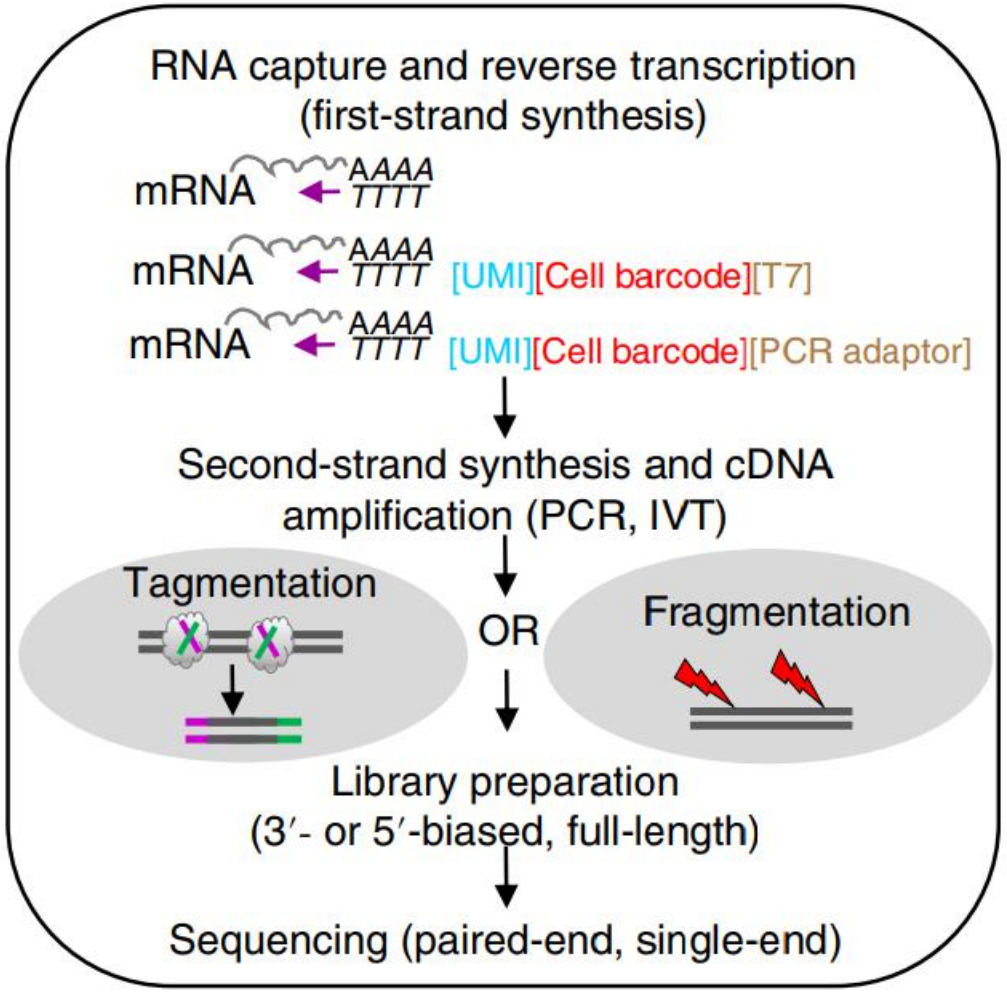
DOI:10.3390/ijms23010216

Single-cell RNA-seq: sample prepraration



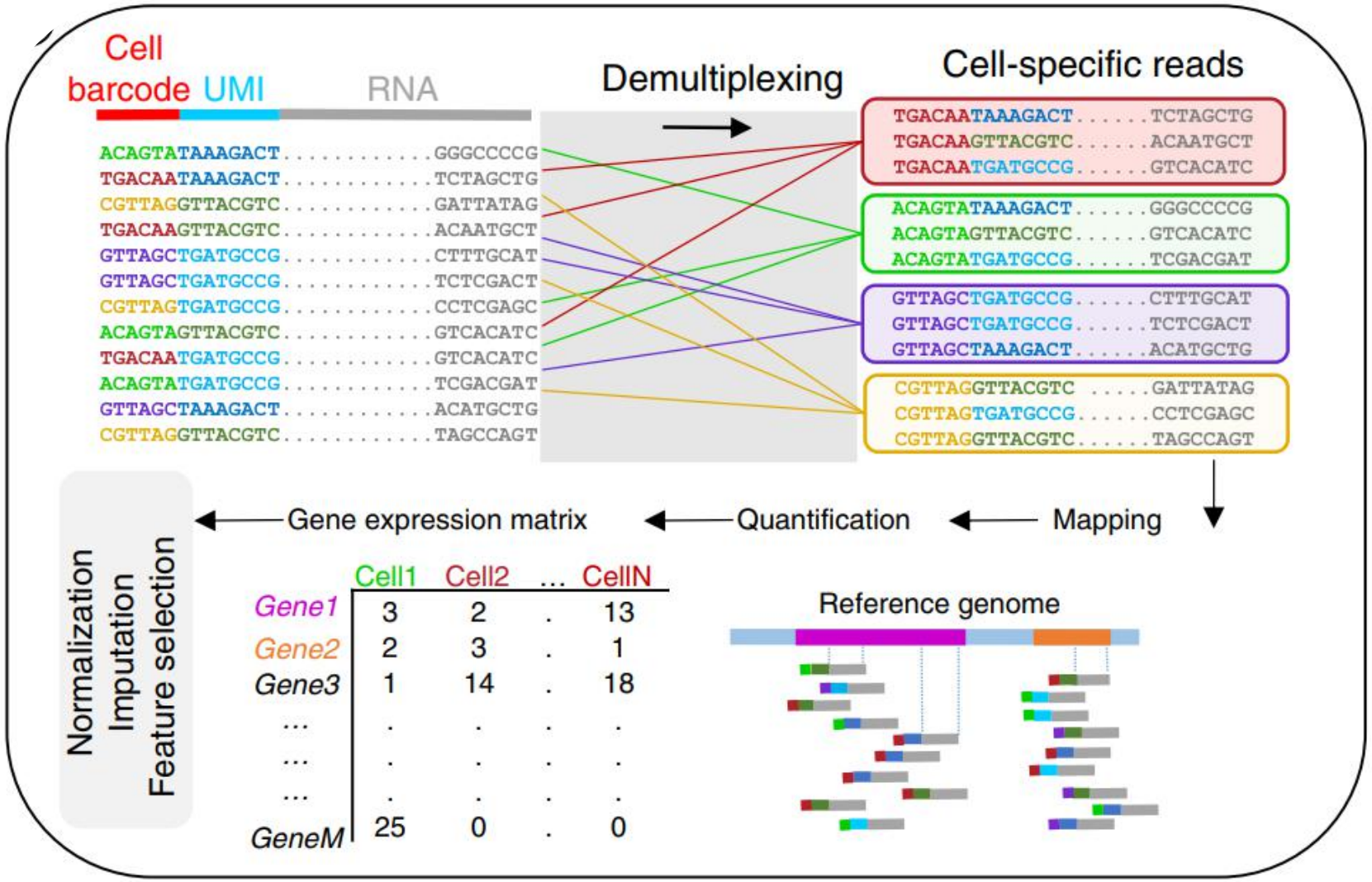
DOI:10.1038/s41596-018-0073-y

Single-cell RNA-seq: sequencing



DOI:10.1038/s41596-018-0073-y

Single-cell RNA-seq: data processing



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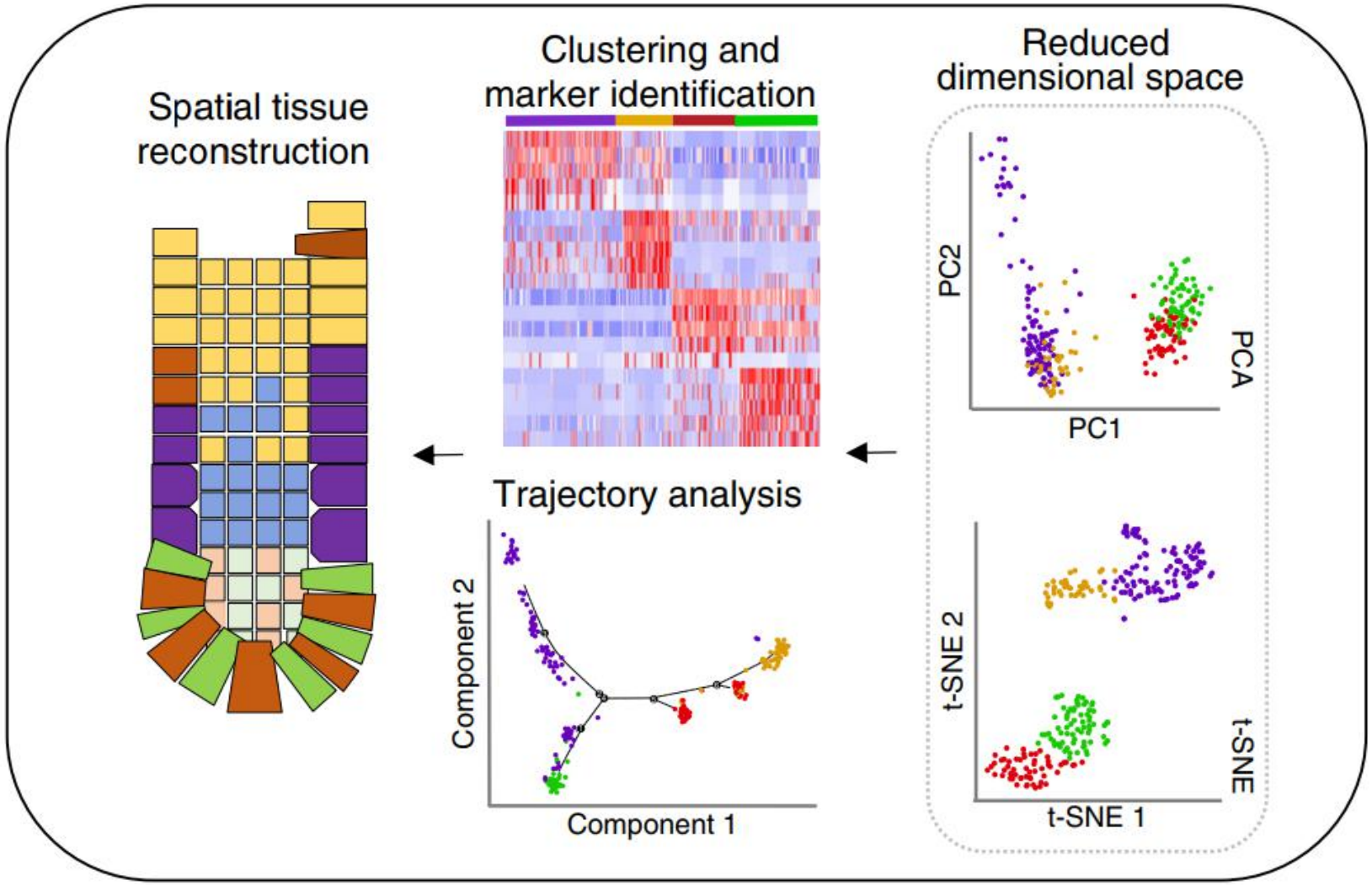
Single-cell RNA-seq: data processing



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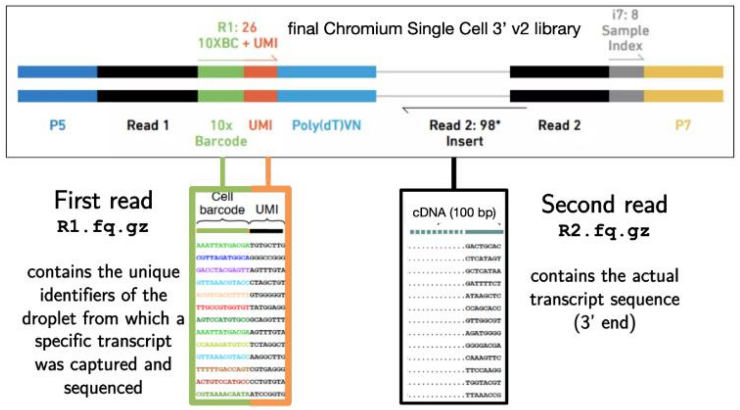
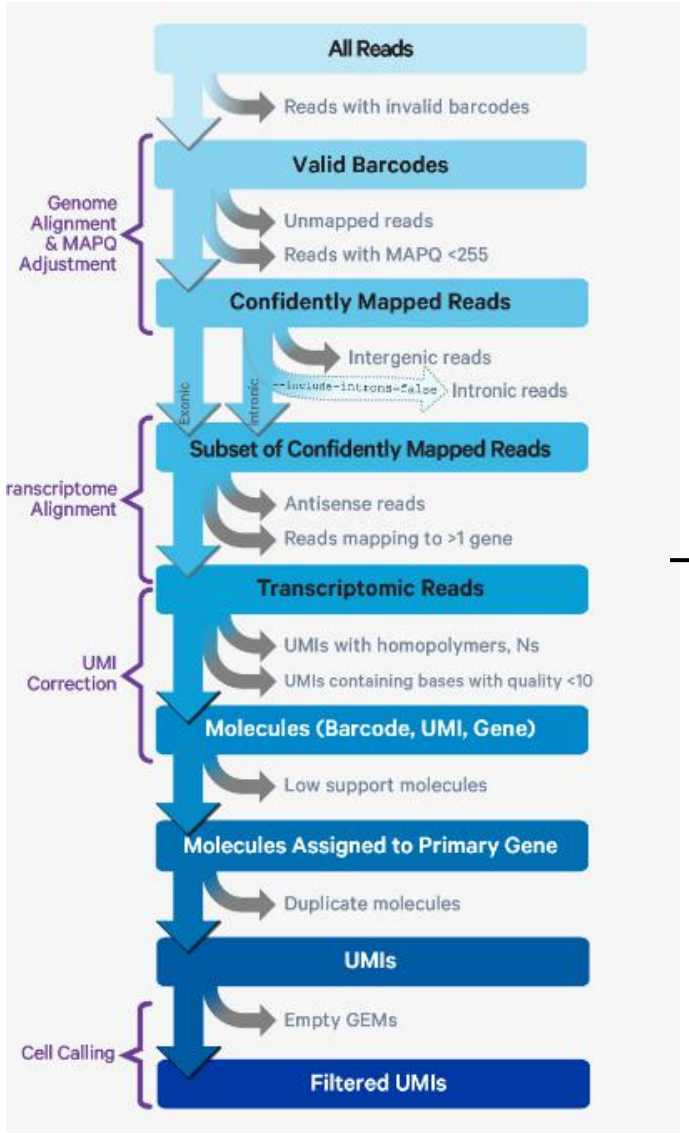
Upstream Analysis Process

Input: Fastq



Sequencing multi-QC



Cellranger Pipeline

Individual sample CellRanger

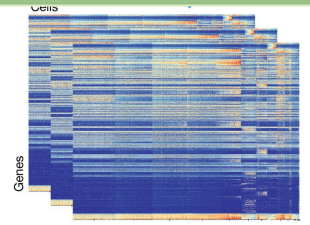
CellRanger Multi-QC report

https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/algorithms/overview#cell_calling

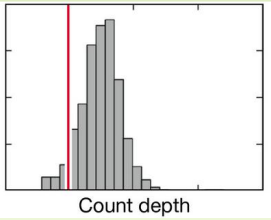
DATA PRE-PROCESSING

Raw data process

Alignment to transcriptome



Quantification into Raw Counts Matrix



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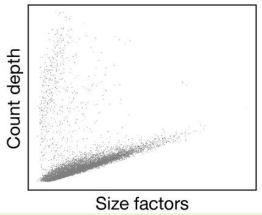
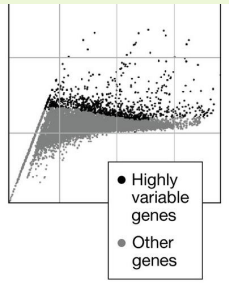


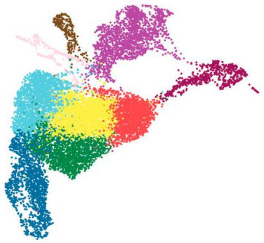
Normalization

Feature selection

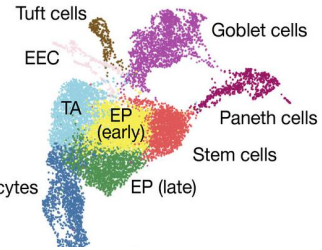


Visualization

Clustering



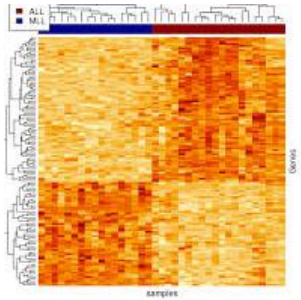
Cluster annotation



SUBSETING

Differentially gene expressed features (cluster biomarkers)

Pseudo-bulk differential gene expression analysis



Gene Ontology analysis

DOWNSTREAM ANALYSIS

Bulk ATAC-seq

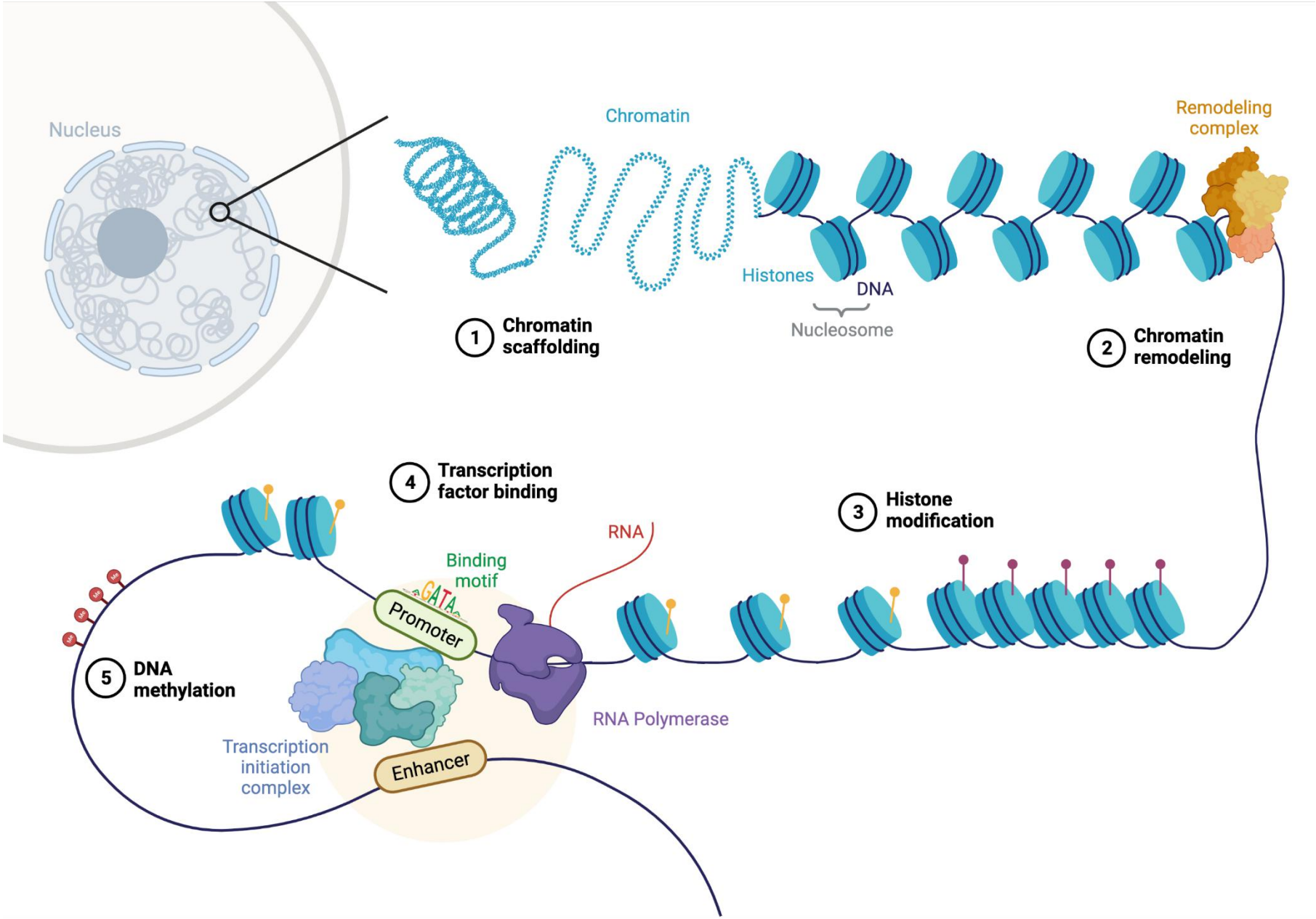


Figure. Overview of mechanisms influencing chromatin accessibility. Created with BioRender.com.

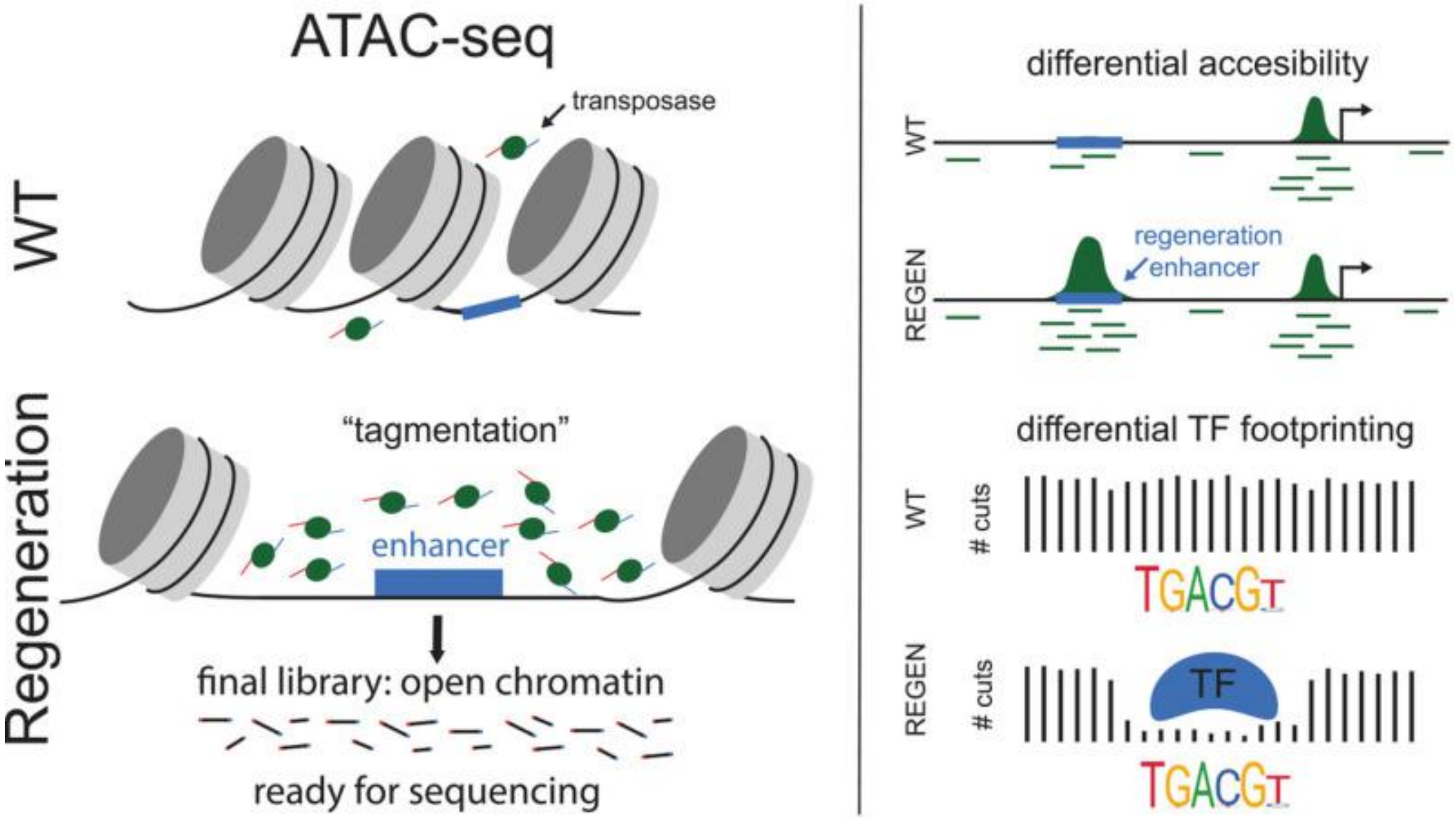


Figure. Overview of an ATAC-seq seq experiment to assay regeneration-responsive chromatin.

https://www.ncbi.nlm.nih.gov/books/NBK586944/figure/ch29.Fig1/?report=objectonly

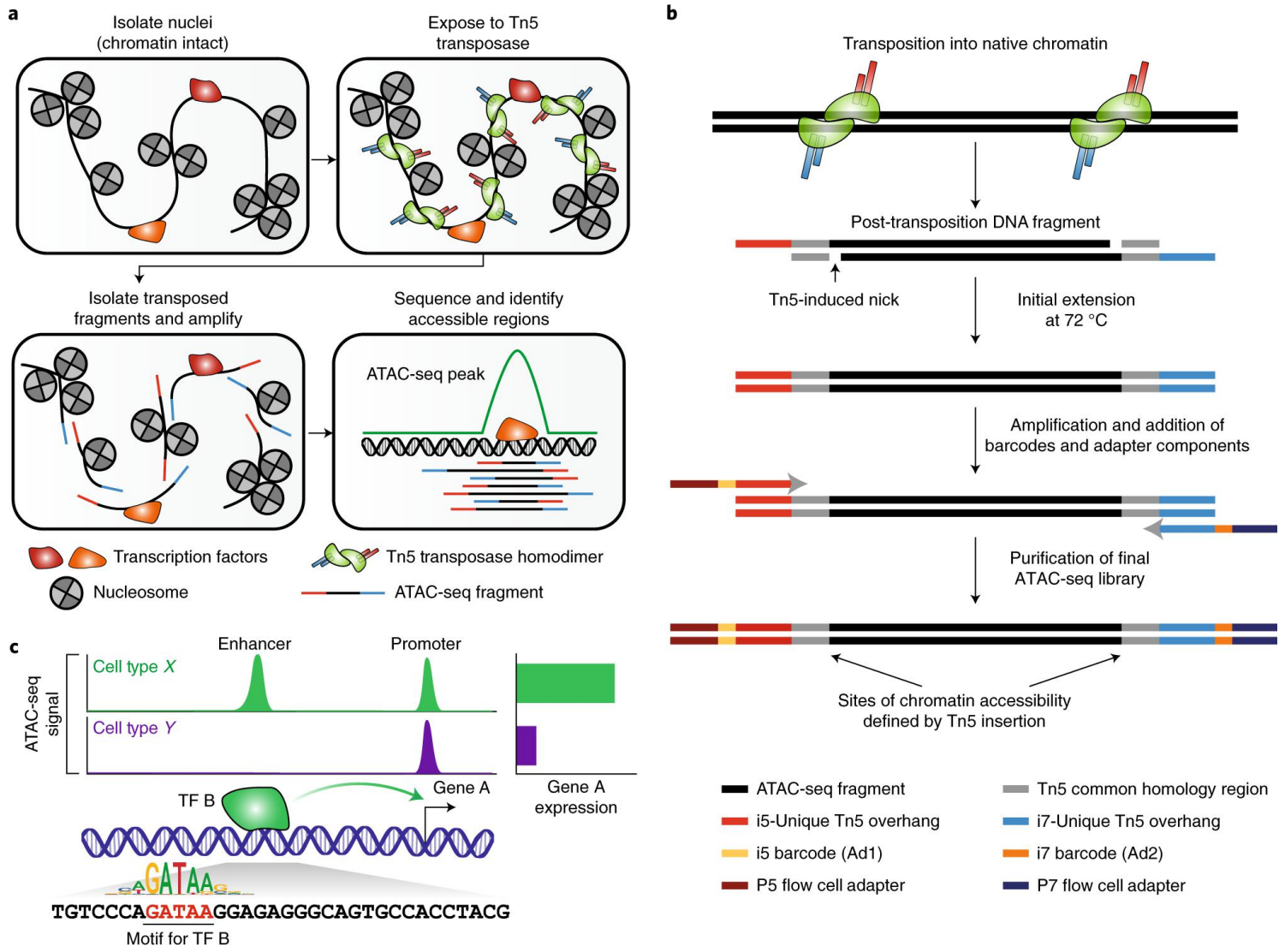
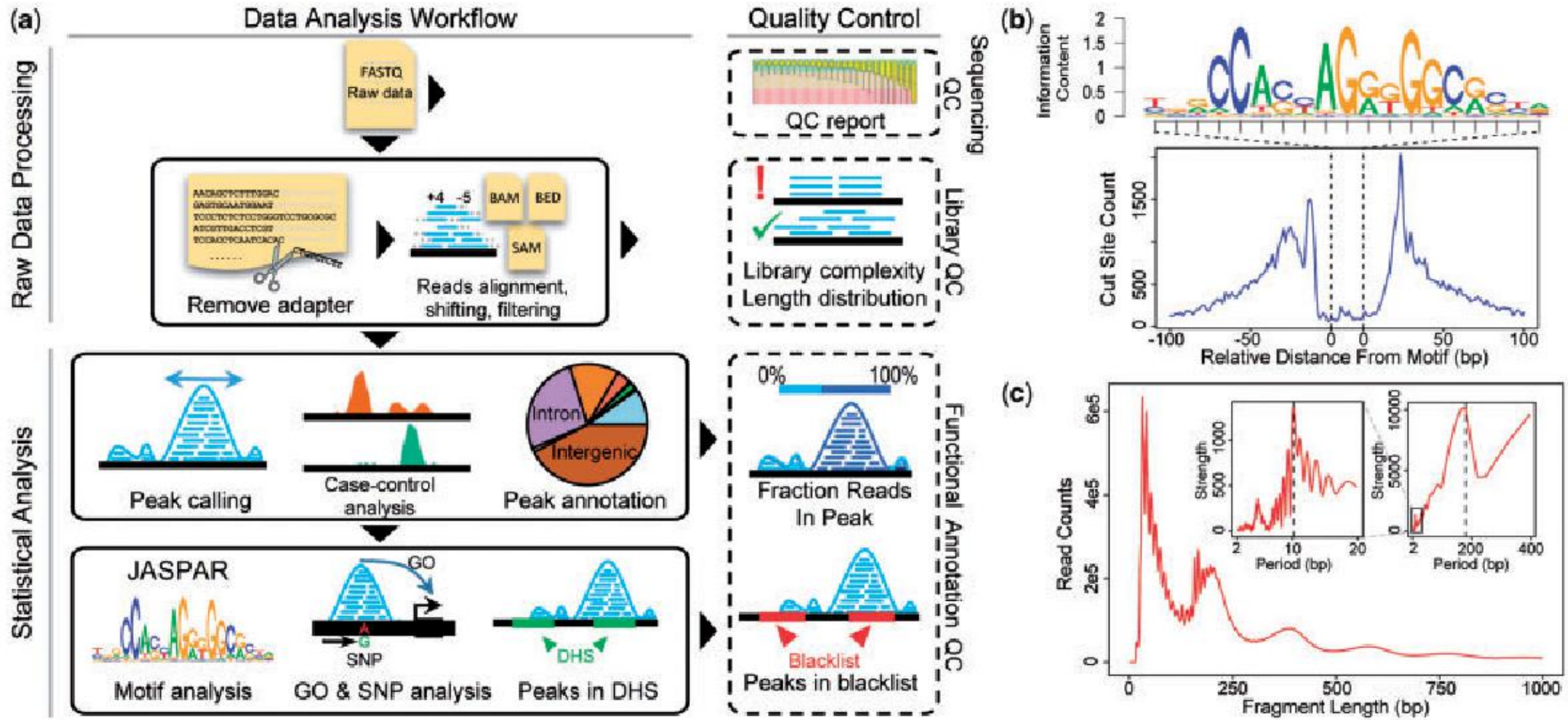


Figure. Schematic of the ATAC-seq transposition reaction and library preparation.

https://www.nature.com/articles/s41596-022-00692-9

Bulk ATAC-seq analysis



DOI:10.1093/bioinformatics/bty141

Single-cell ATAC-seq

scATAC-seq analysis

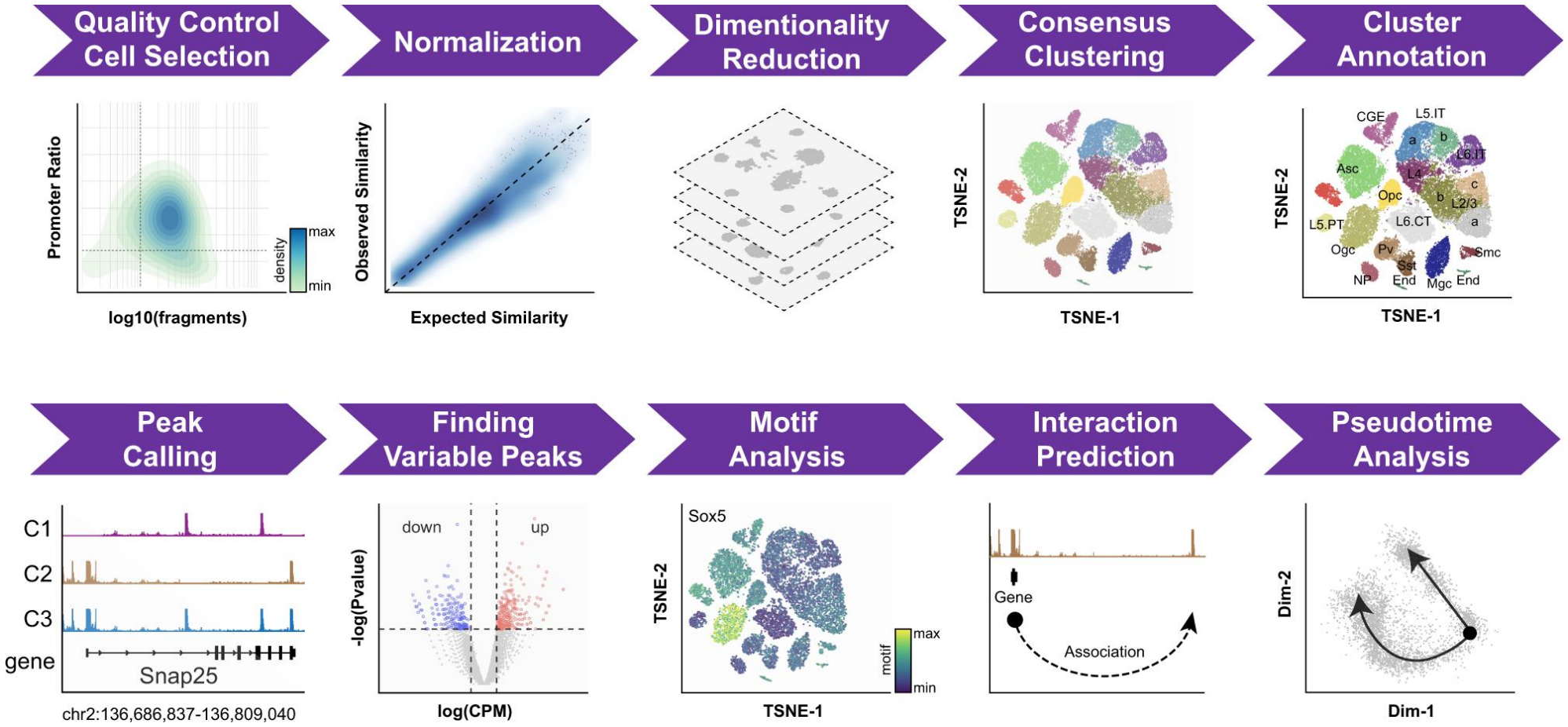
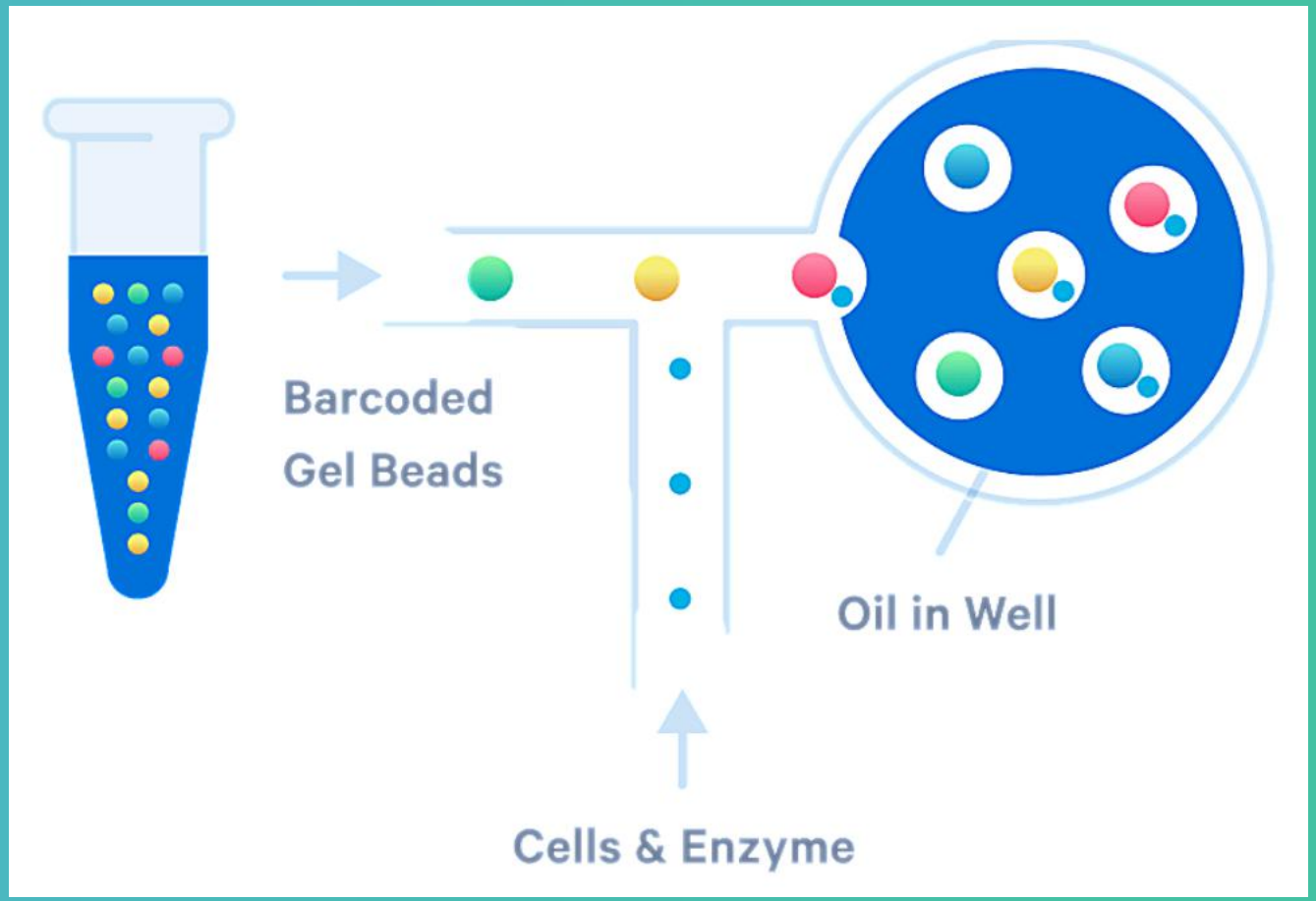
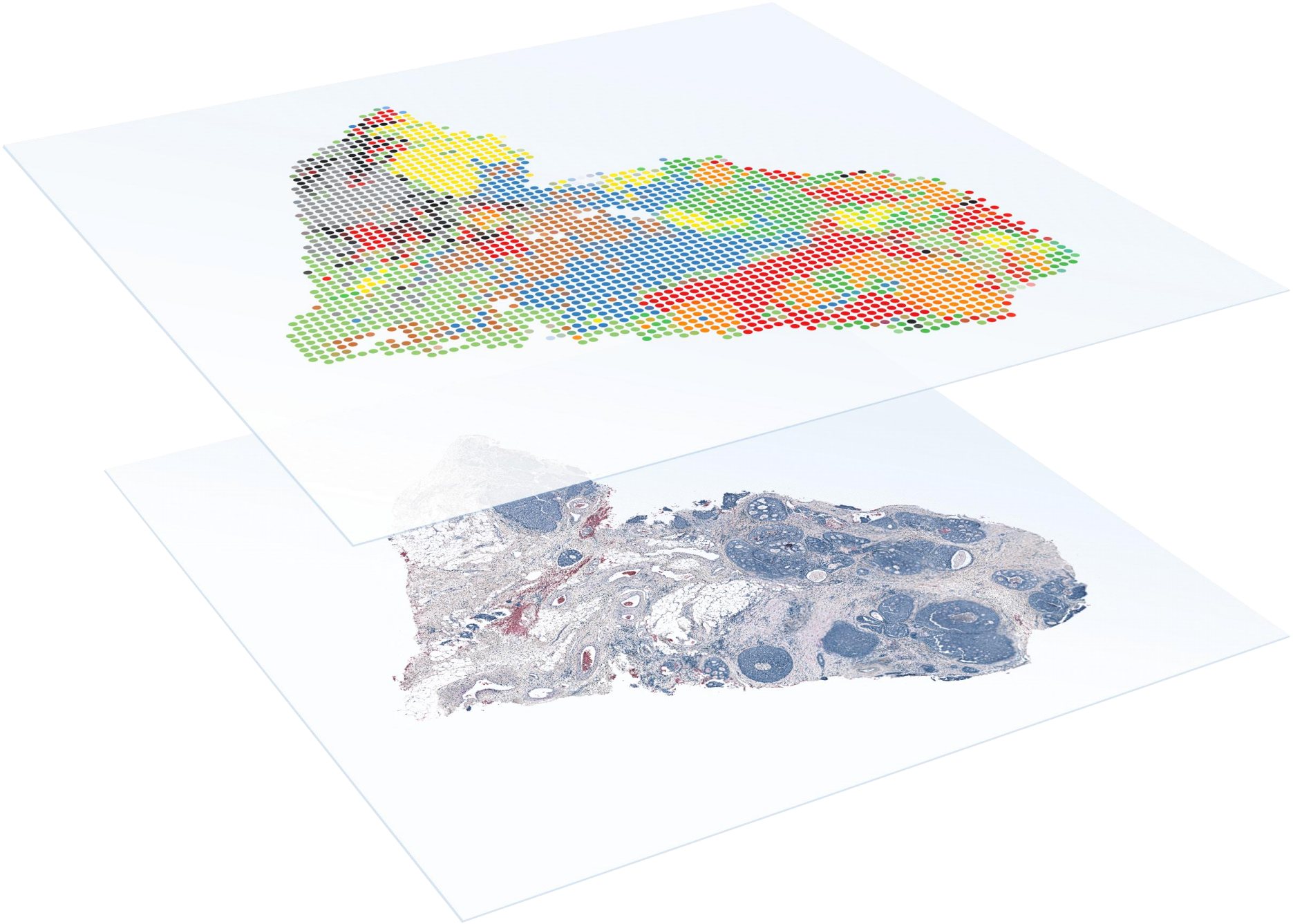


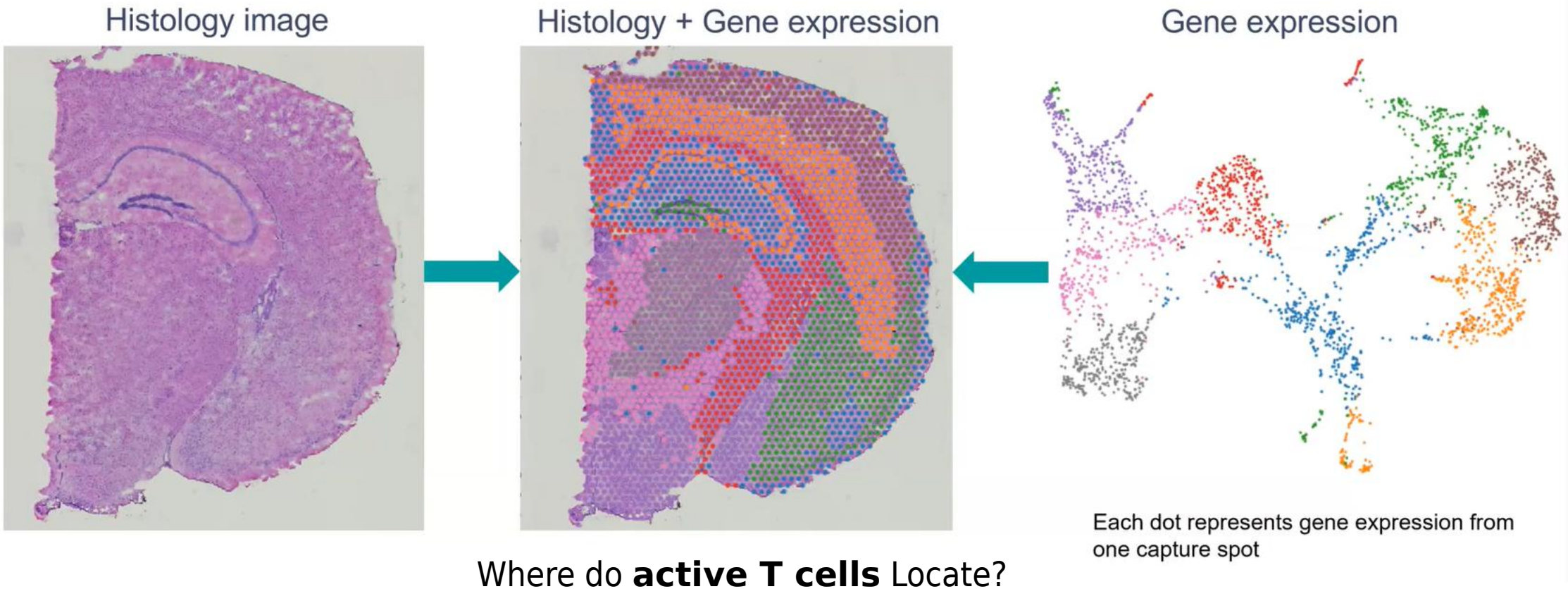
Figure: Schematic overview of SnapATAC analysis workflow.

Chromium Single Cell



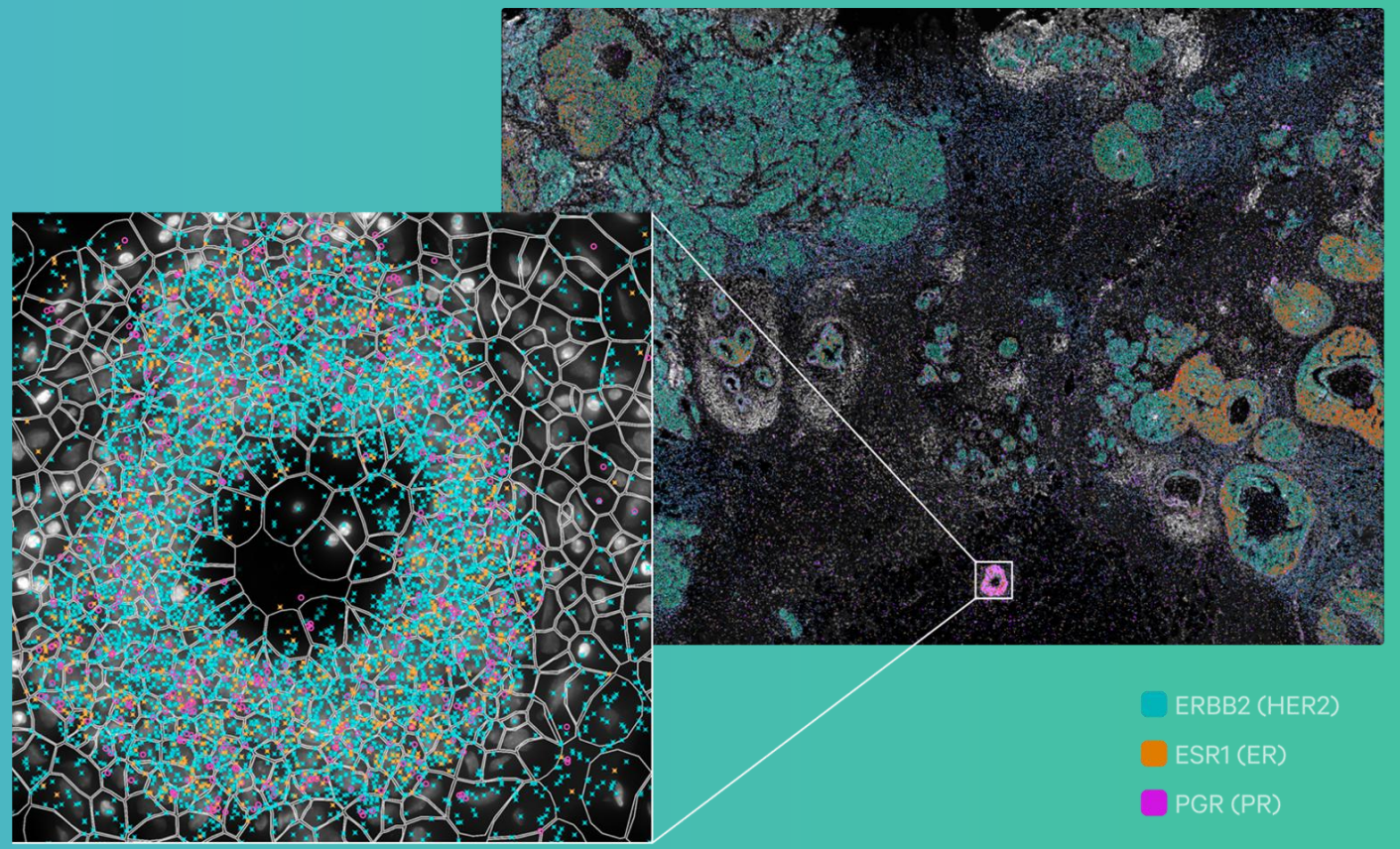


Spatial transcriptomics with Visium



Where do **active T cells** Locate?

Xenium In Situ



In this breast cancer sample
(Stage II-B, ER+/PR−/HER2+), Xenium identifies a
previously unknown triple-positive region.

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THANK YOU FOR YOUR ATTENTION and Q&A!

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For further information!