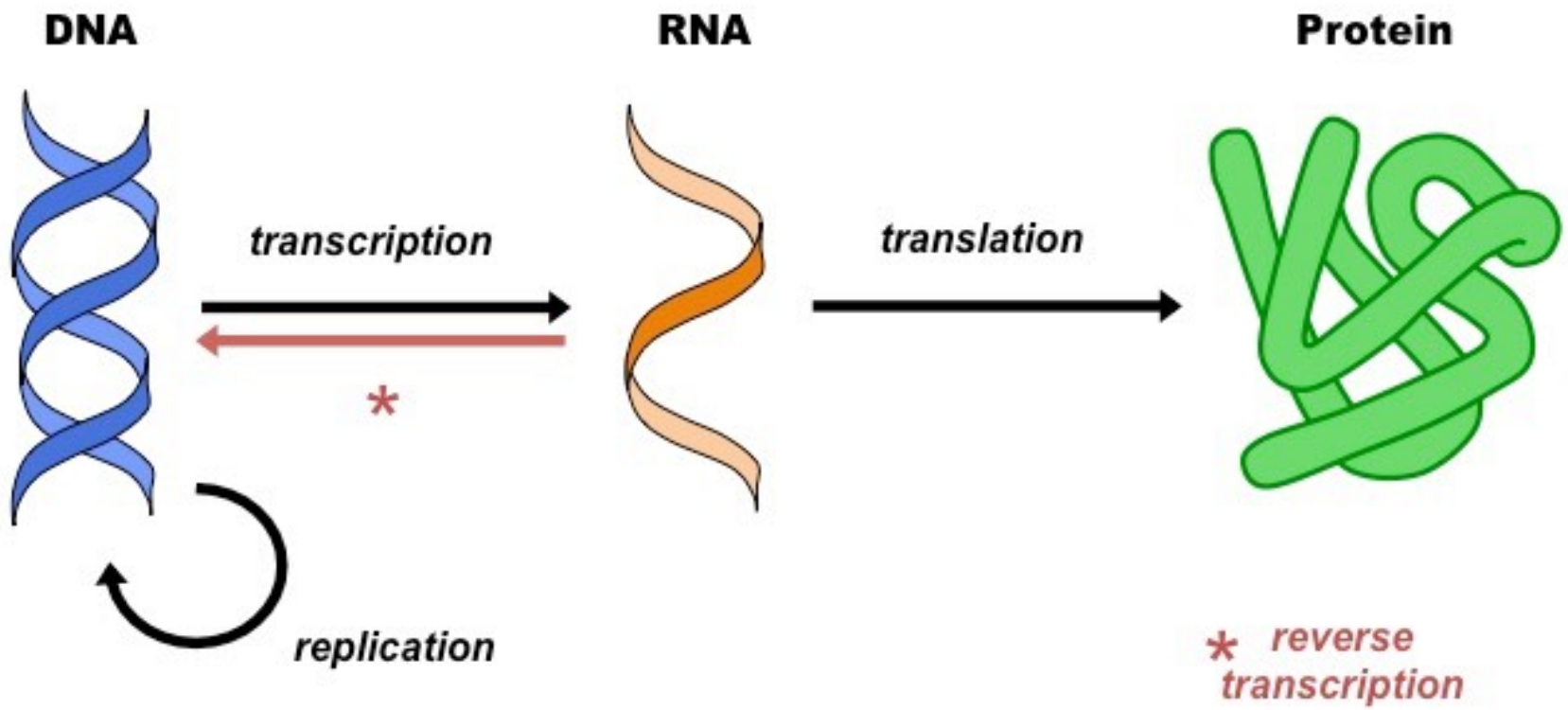
Single-Cell Transcriptomics

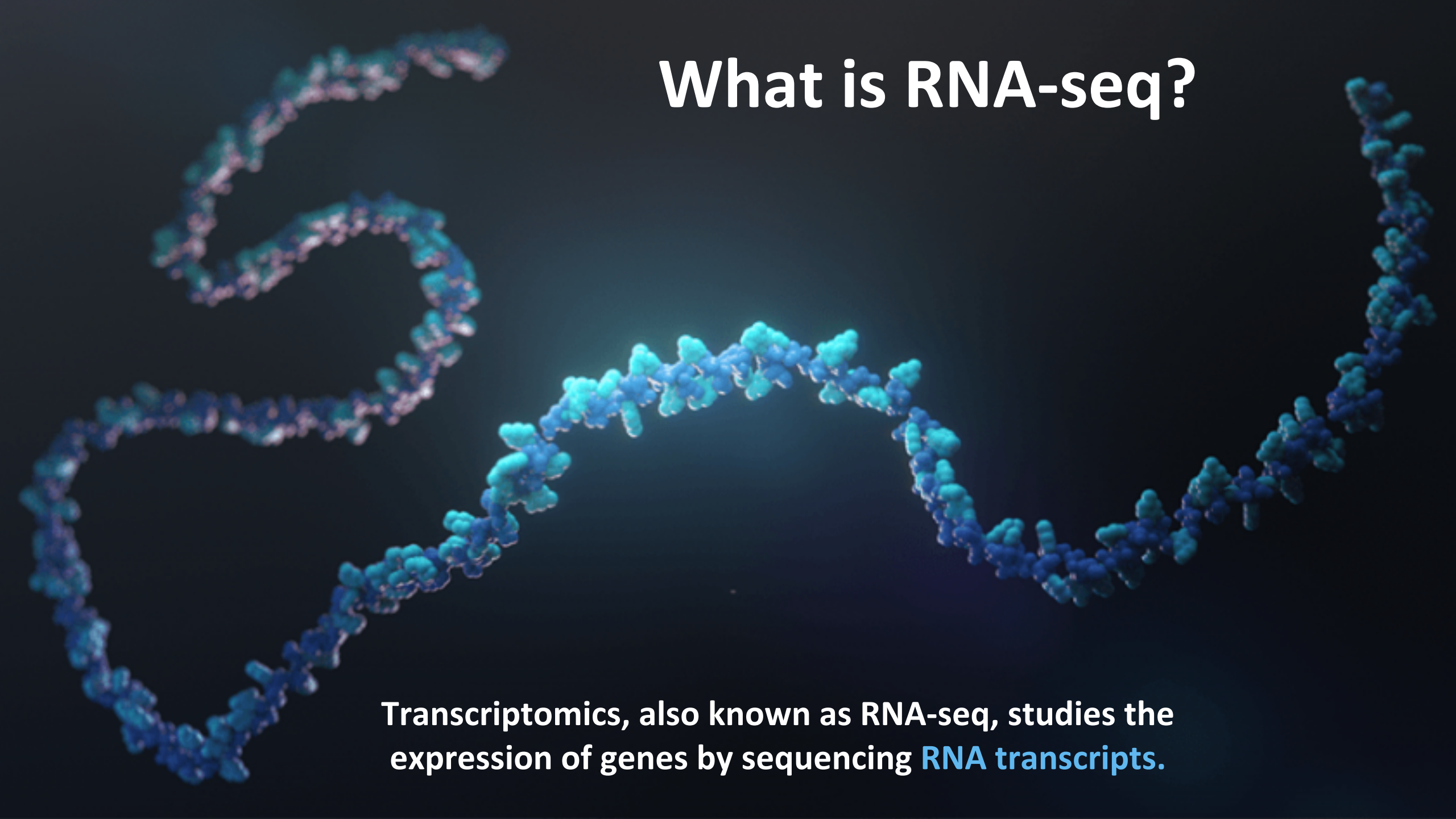
By Will McKinley

What is transcription?



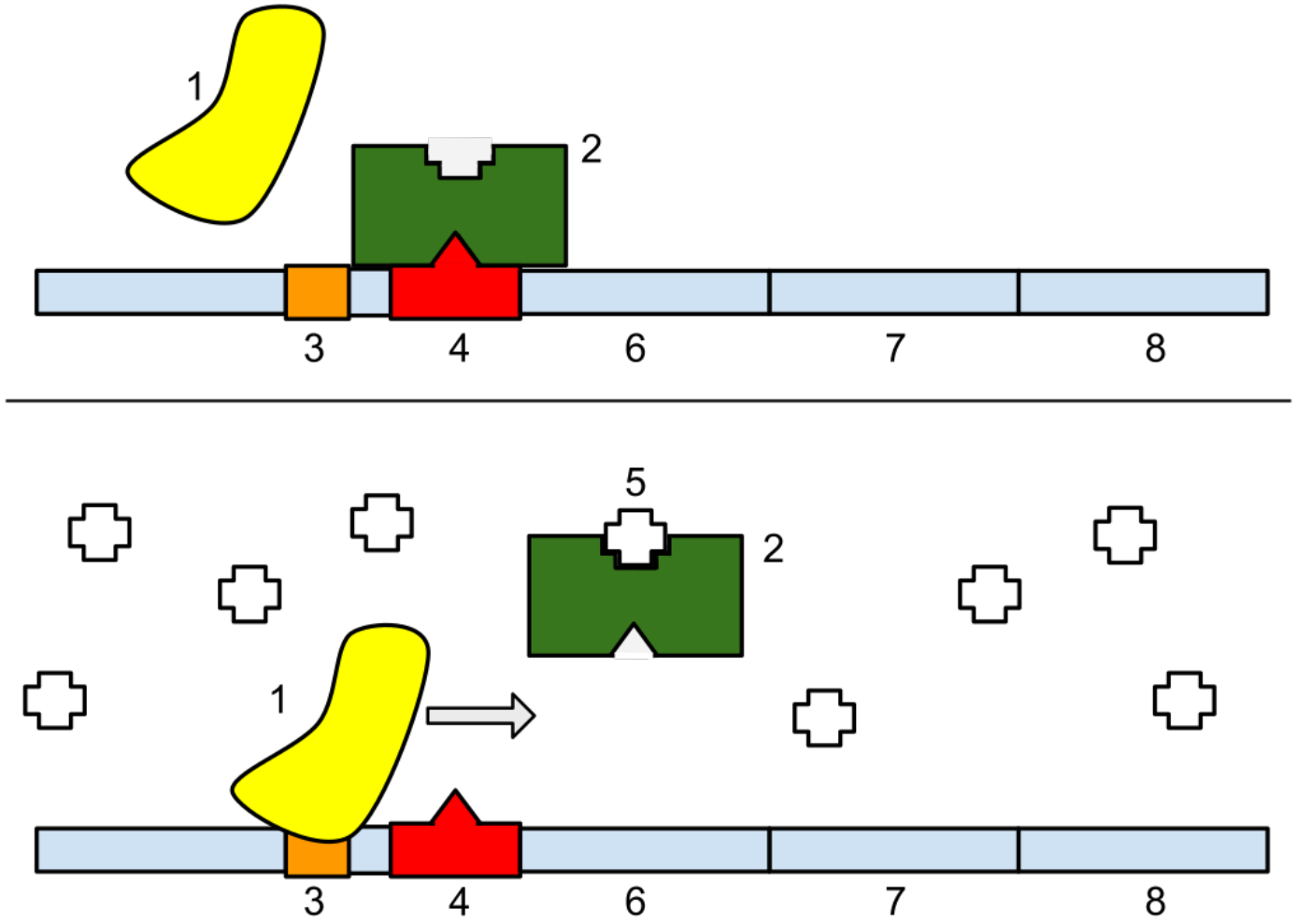
DNA is transcribed into RNA, which is translated into protein.

What is RNA-seq?



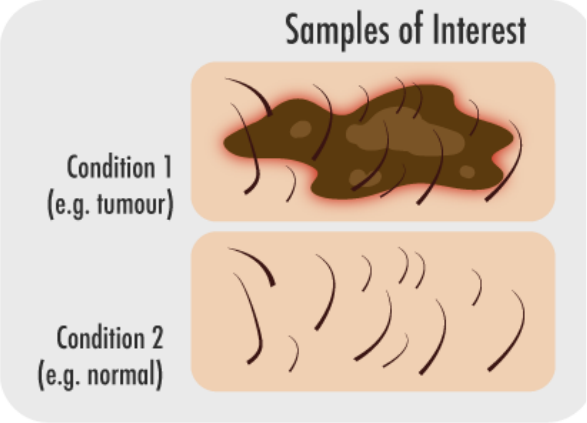
Transcriptomics, also known as RNA-seq, studies the expression of genes by sequencing RNA transcripts.

Why is RNA-seq important?

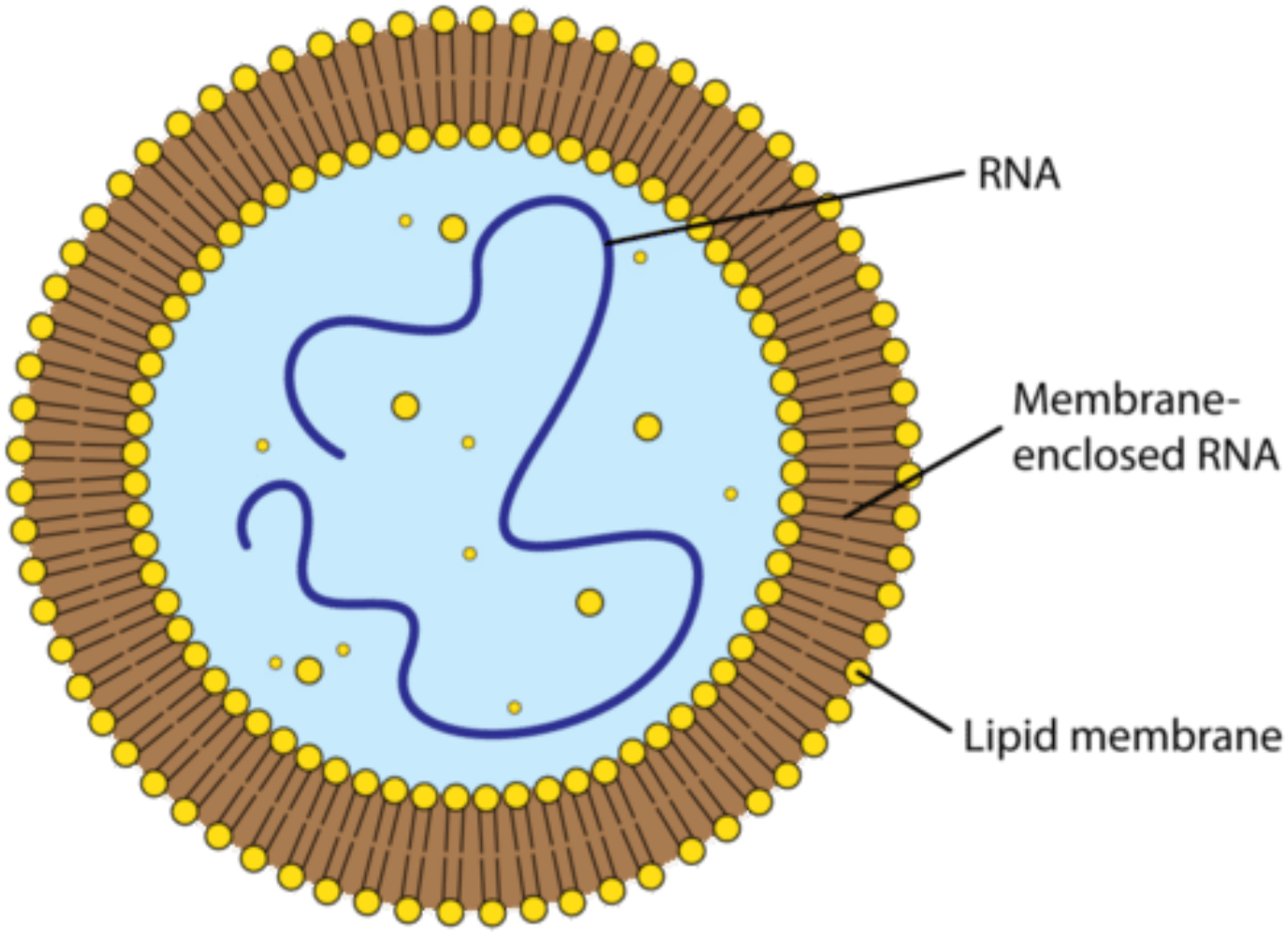


Transcriptomic studies let us look at how genes are expressed differently between cells and circumstances.

How do you do an RNA-seq experiment?



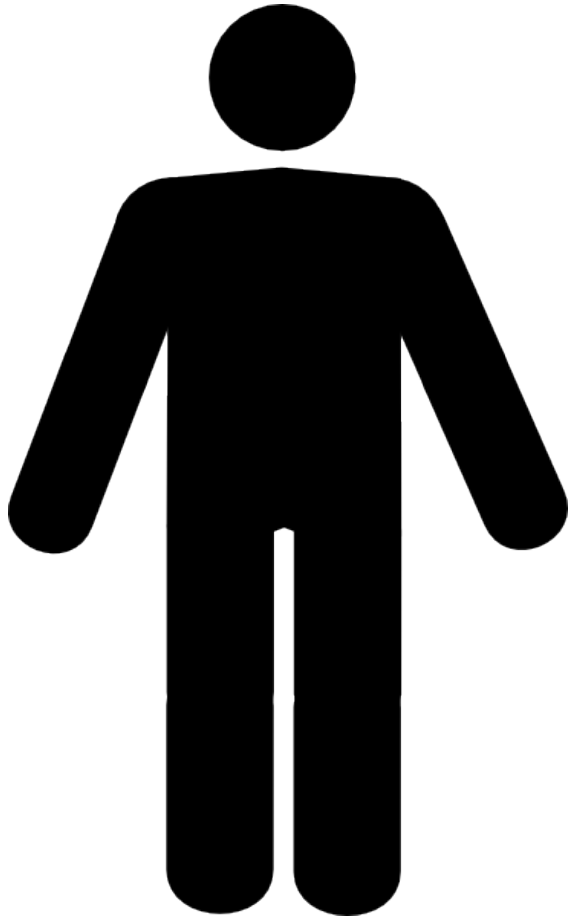
What is single-cell RNA-seq?



Single-cell RNA-seq (scRNA-seq) is when the RNA transcripts from a single cell are sequenced.

Why is single-cell RNA-seq powerful?

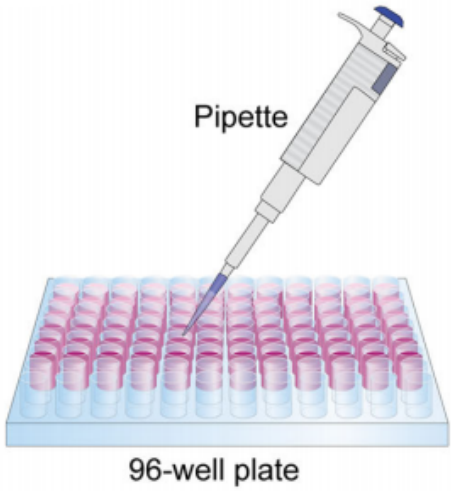
VS



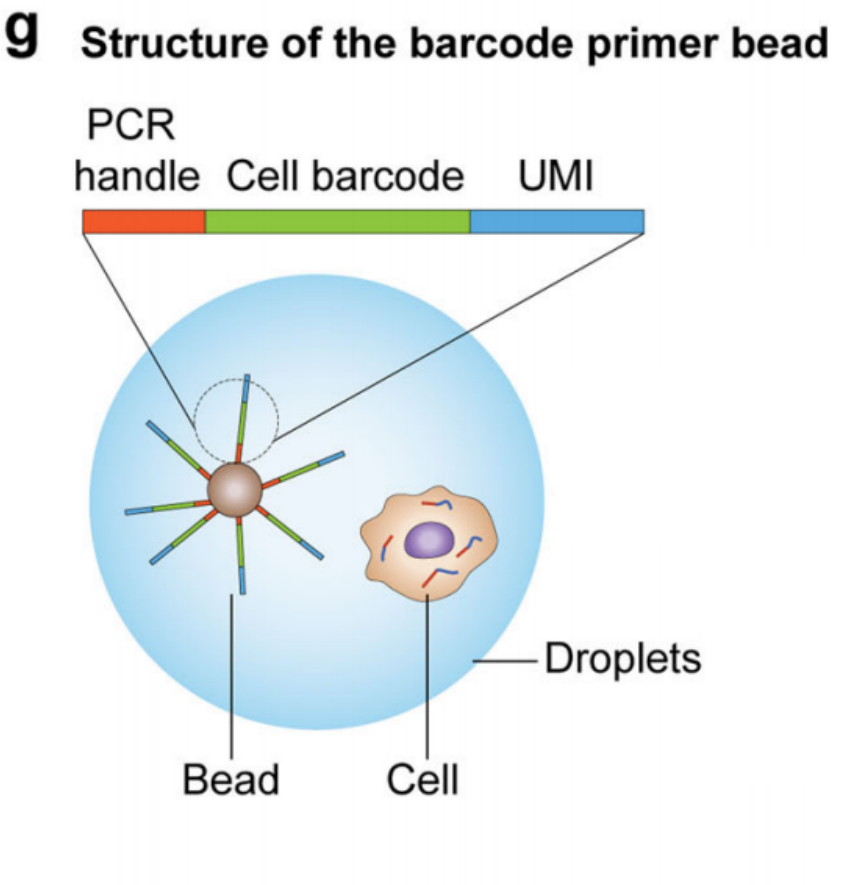
Regular RNA-seq: Tells you what the average cell is doing.

scRNA-seq: Tells you what particular cells are doing.

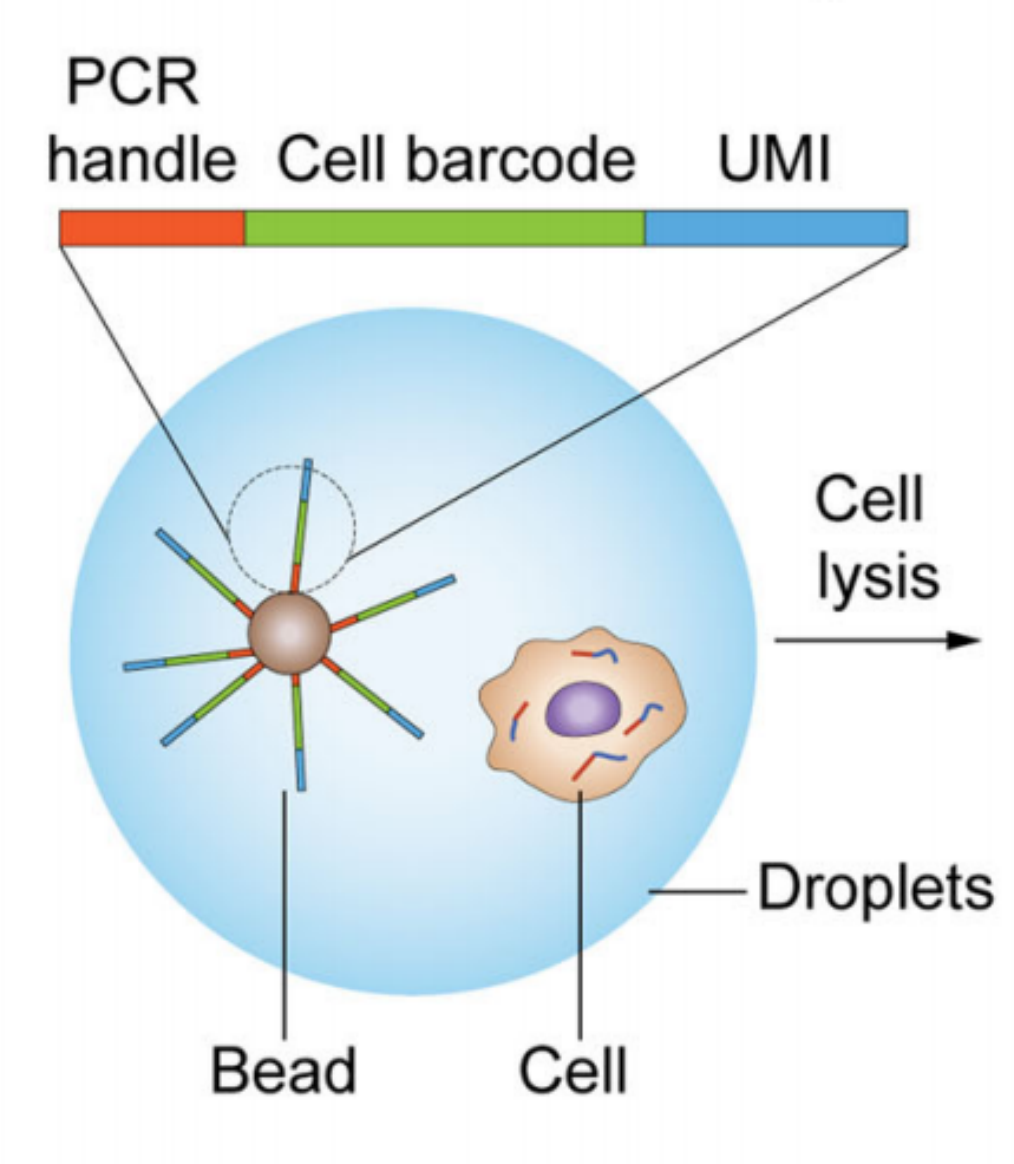
How do you isolate single cells for scRNA-SEQ?



How do you generate sequences from single cells?

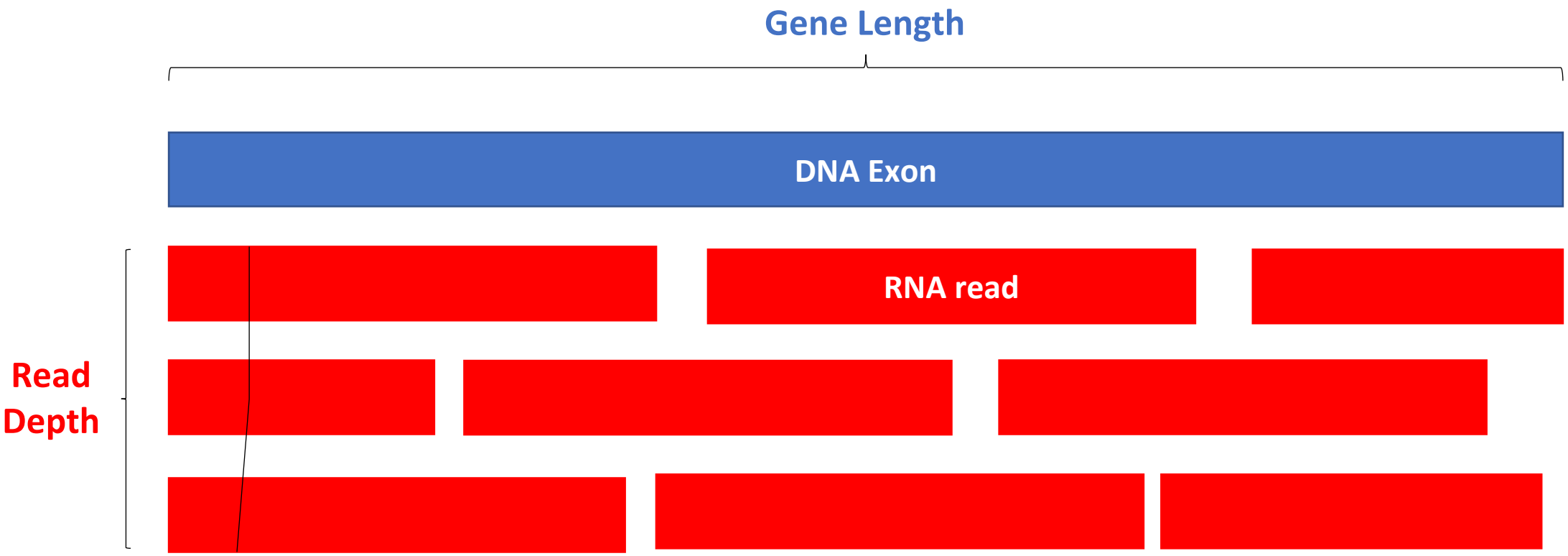


How do you identify which cell each sequence is from?



Unique sequences, such **as cell barcodes** or **UMIs (unique molecular identifiers)** associated with specific cells are reverse-transcribed with the RNA.

How do you normalize RNA-seq data?



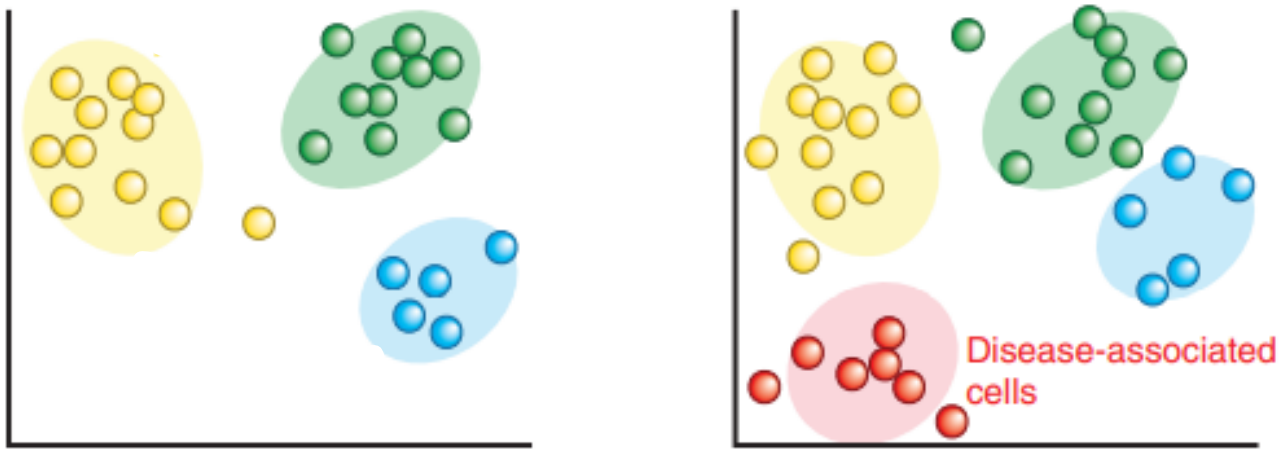
Divide raw transcript numbers by **read depth** (number of times a nucleotide is sequenced) and **gene length**.

What units are used for RNA-seq transcripts?

Gene	Length	Cell 1	Cell 2	Cell 3
A	2kb	20	24	60
B	4kb	40	50	120
C	1kb	10	16	30
D	10kb	5	0	5

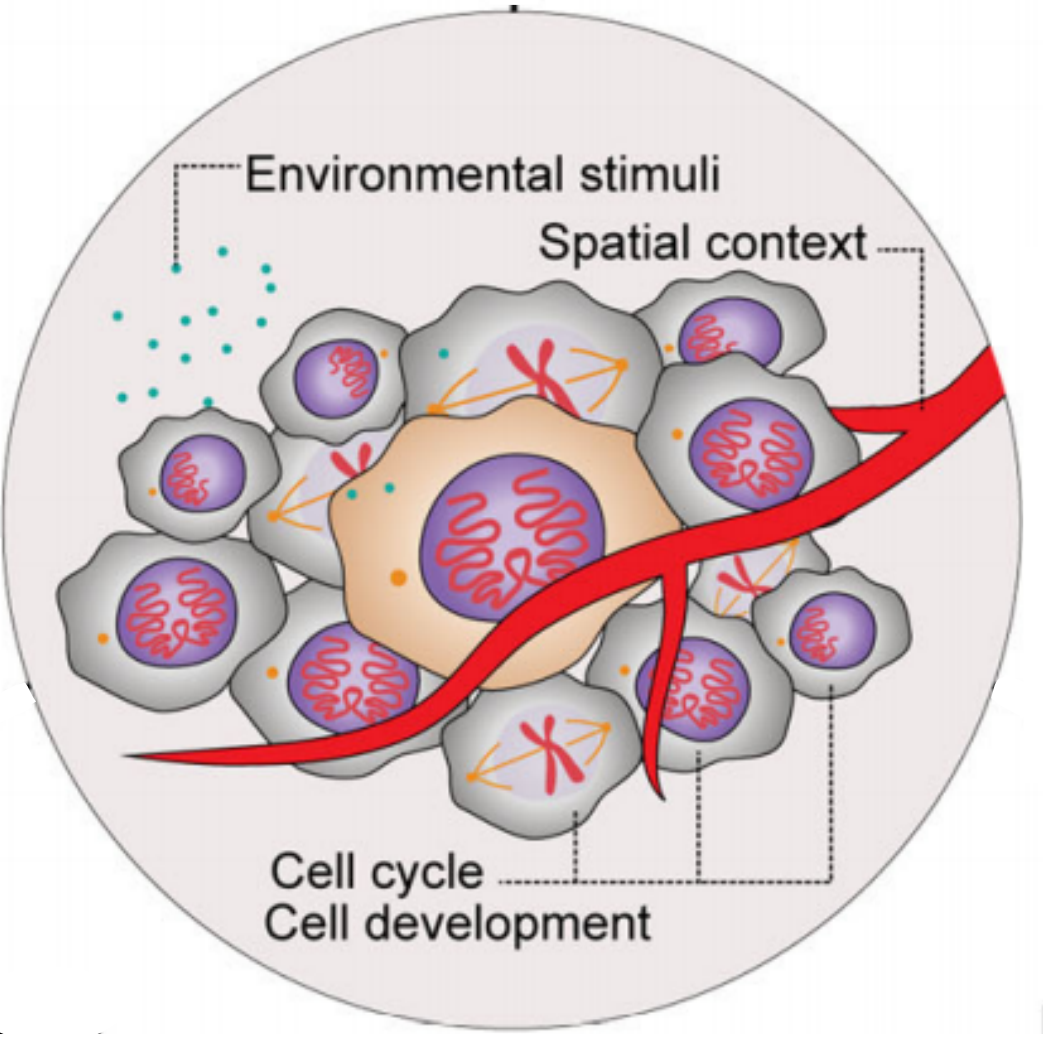
What can scRNA-seq data be used for?

Cell-type maps

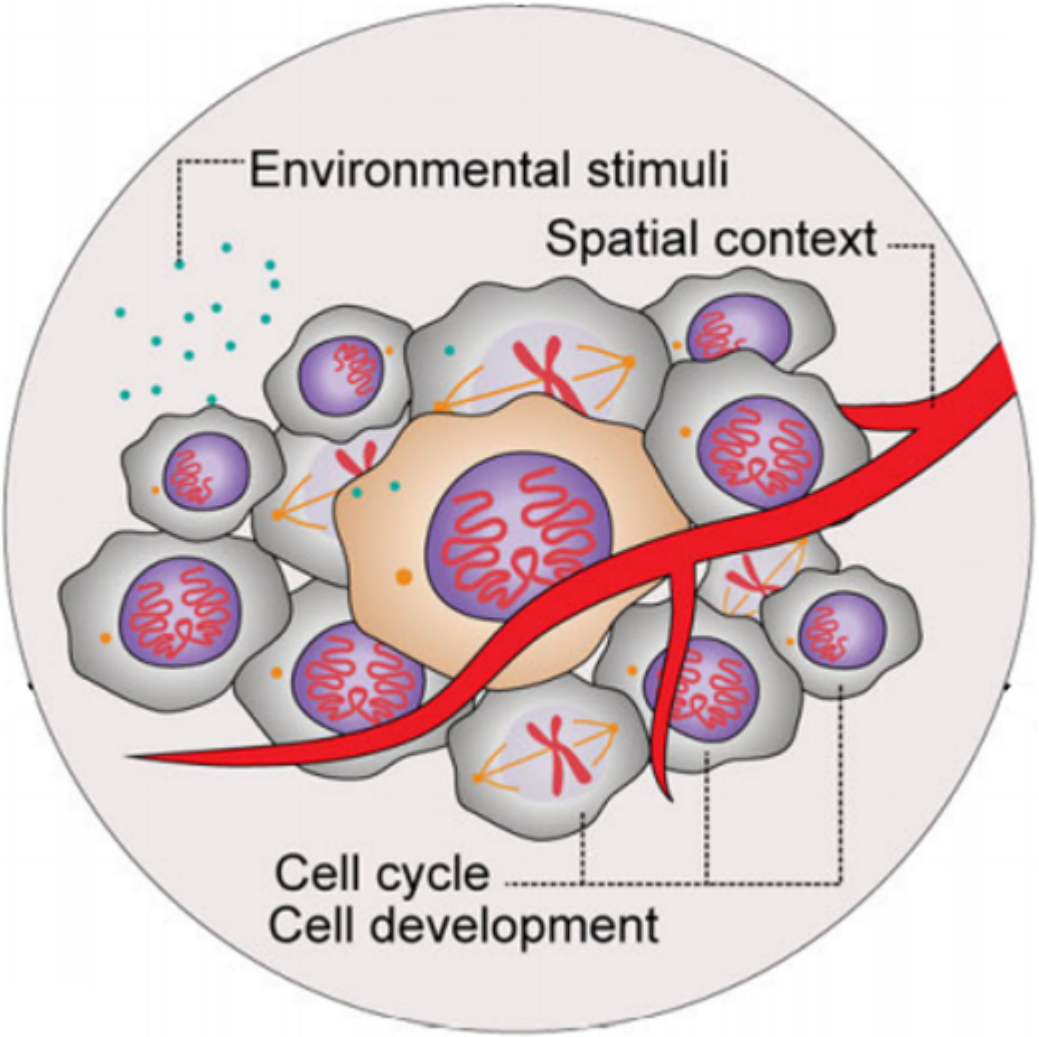


Types of analyses

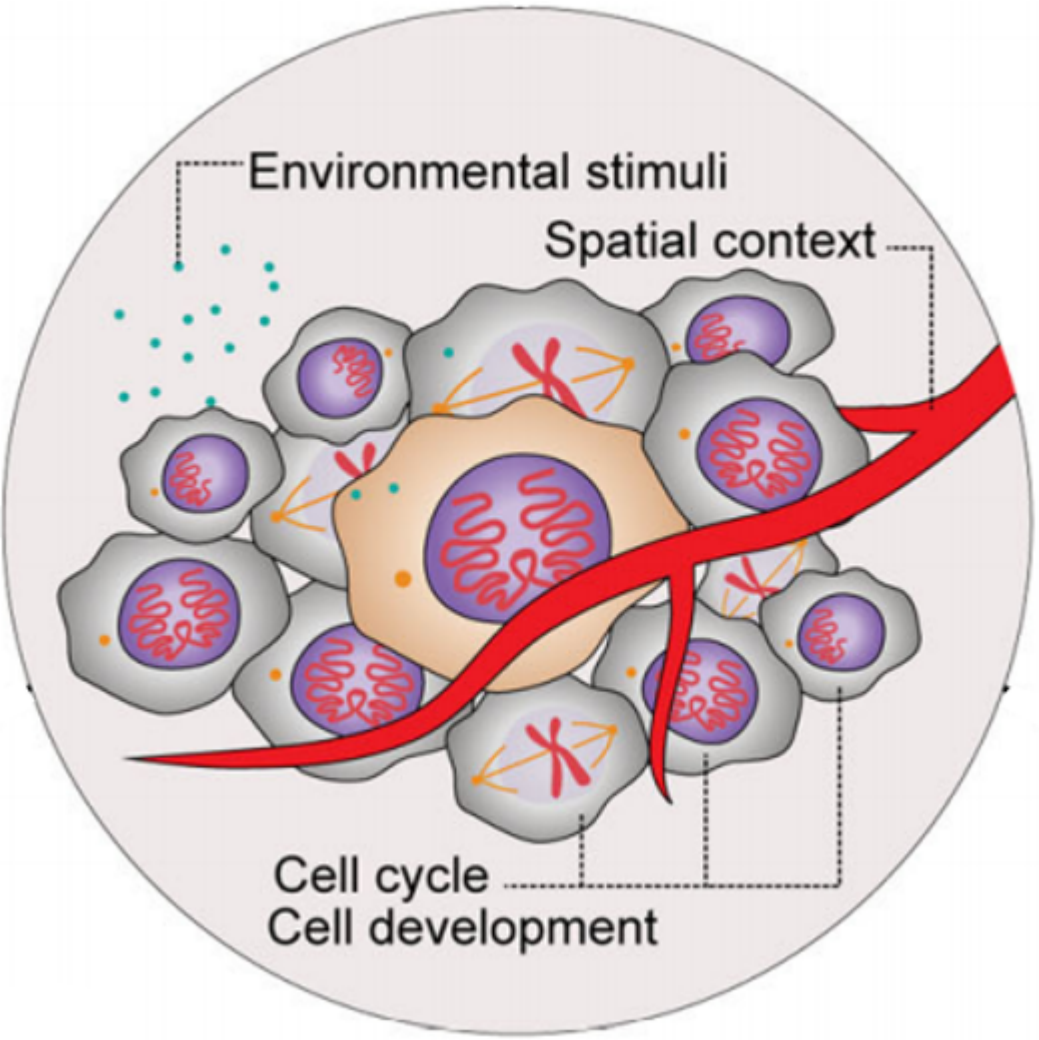
How can scRNA-seq be used to identify cell types?



What can scRNA-seq tell us about regulatory networks?

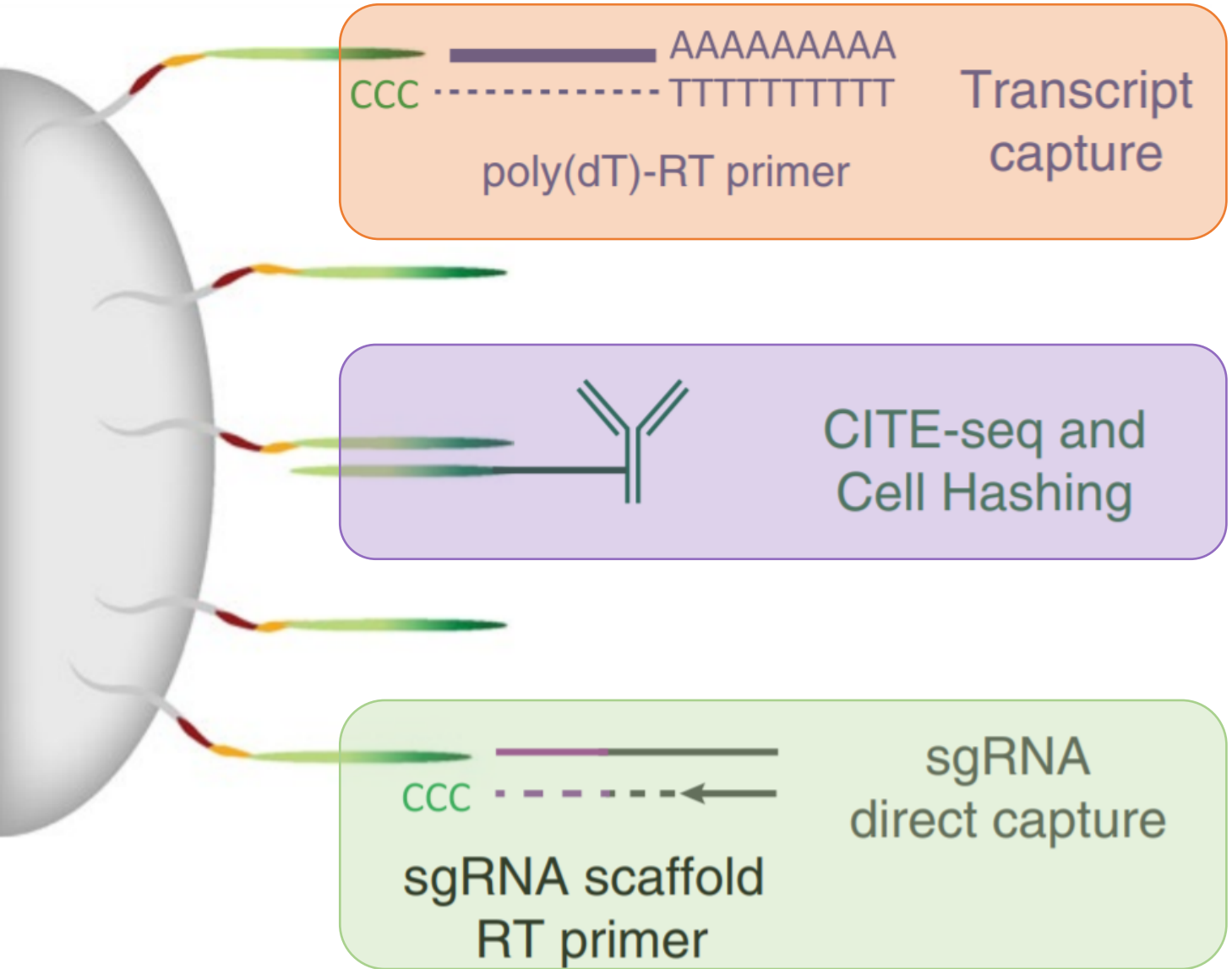


What can scRNA-seq tell us about cell hierarchy?



What is ECCITE-seq?

Expanded **C**RISPR-compatible **C**ellular **I**ndexing of **T**ranscriptomes and **E**pitopes by **SEQ**uencing



Allows cellular RNAs to be captured and sequenced.

Uses oligo-tagged antibodies to identify cells by their surface proteins.

Captures CRISPR guide RNA; shows which gene is knocked out in this cell.

What does Sanjana Lab study?

Led by Dr. Neville Sanjana (center), this NYU/NY Genome Center lab works on cancer genetics, neuroscience, and bioengineering. They have developed many genome editing and CRISPR tools.

Review

RNA-seq allows you to quantify gene expression by observing which genes are transcribed in response to a stimulus.

scRNA-seq allows you to quantify gene expression in particular cells, giving you a more precise idea of what each cell is doing.

The data from scRNA-seq can be used in many ways, including identifying cell types, figuring out regulatory networks, and reconstructing cell hierarchies.


Questions?

Literature References

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