Swiss Institute of Bioinformatics

SINGLE-CELL TRANSCRIPTOMICS WITH R

Overview of Single-Cell Transcriptomics Workflow Strategies

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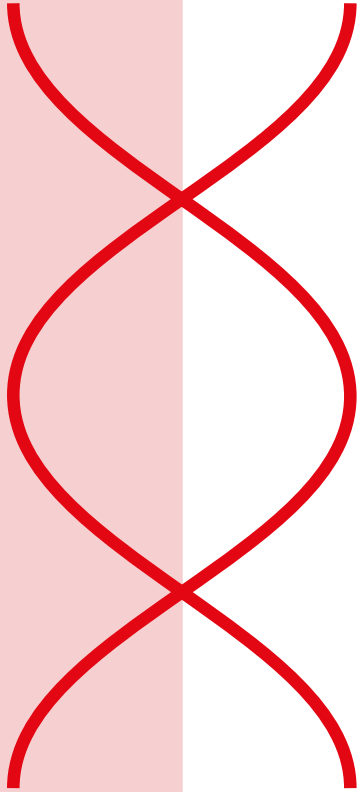
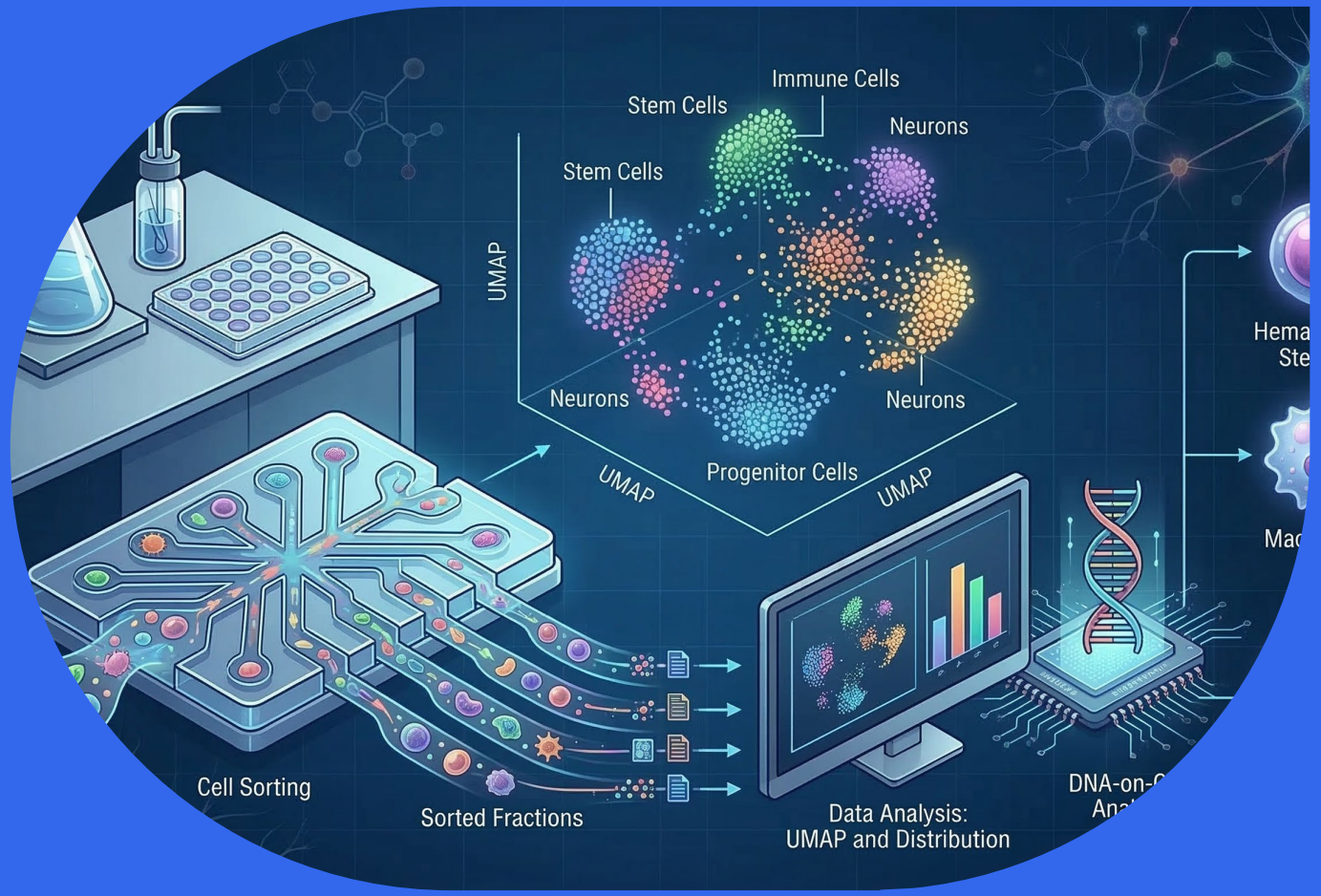
Introduction
The single-cell revolution

General overview of sequencing
NGS technology
Long-read

Single-Cell Transcriptomics
Technology overview
Major commercial technologies
Experimental design





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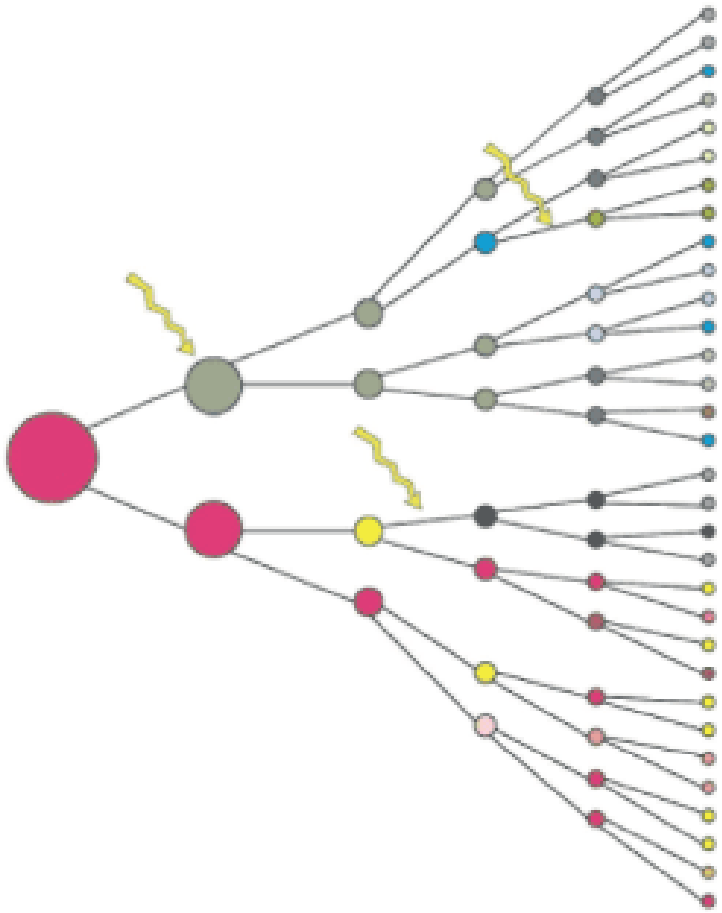
Introduction

The Single-cell Revolution

A human is a mosaic of different cells



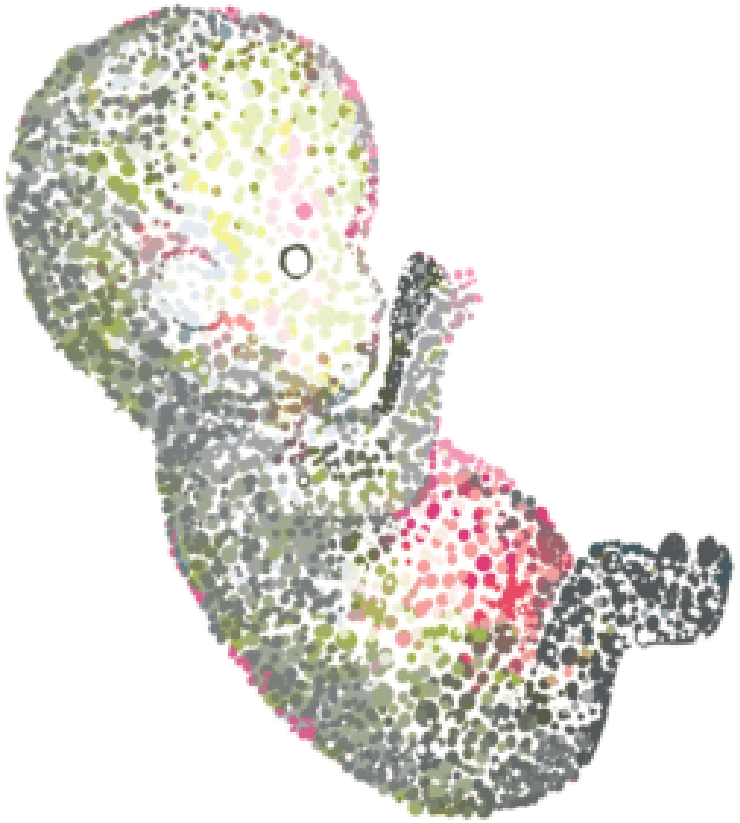
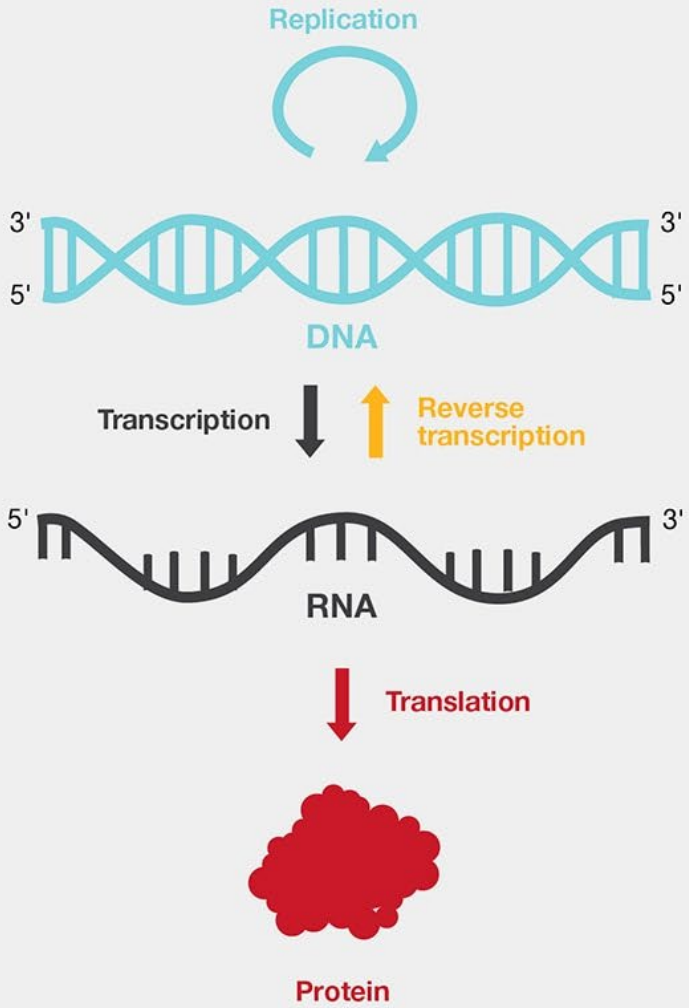


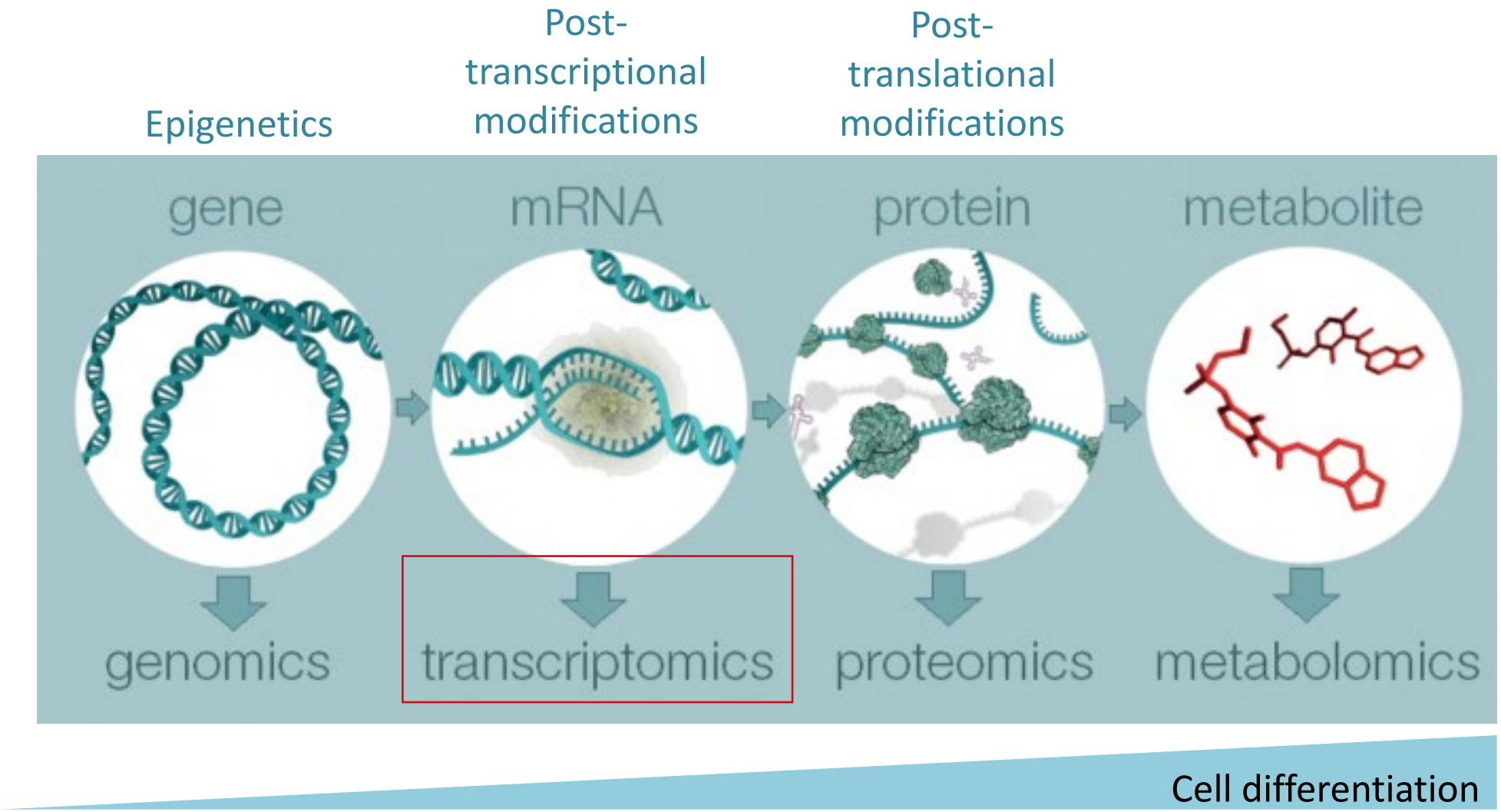
Illustration by Tarryn Porter

The updated dogma of molecular biology



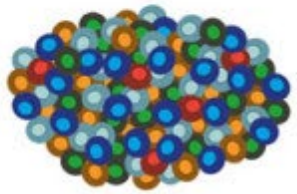
Thermo Fisher, 2026

How can we characterize cell populations?



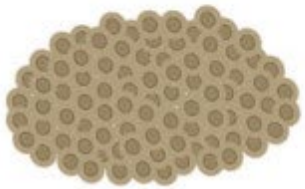
Advantages of single-cell RNA-seq

Bulk tumor

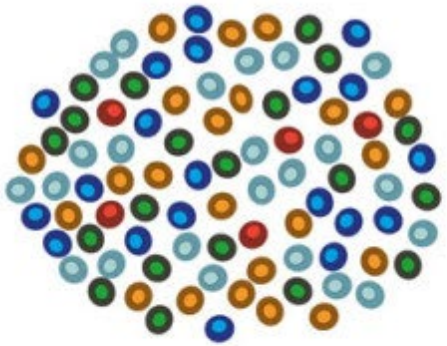


Undetailed and incomplete picture

Inability to resolve cell populations

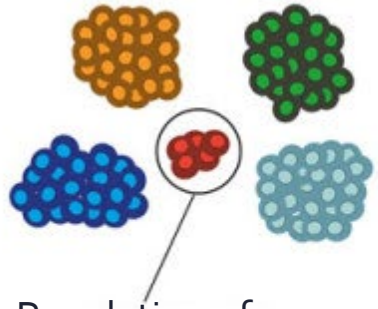


Dissociated tumor



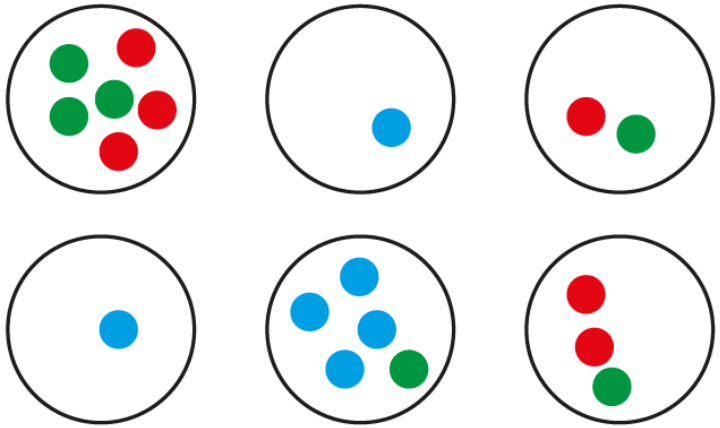
Detailed and complete picture

Identification of different cell populations

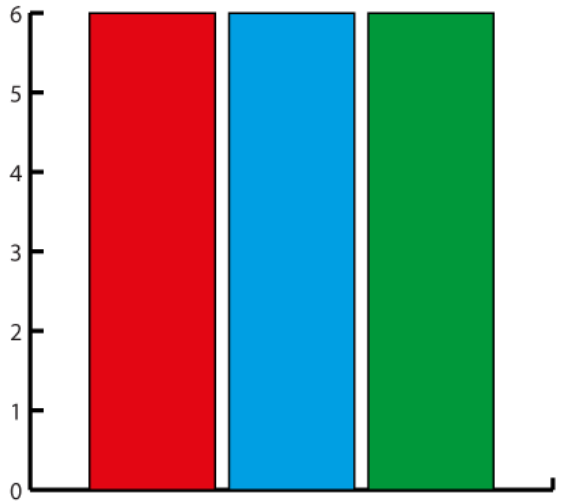


Advantages of single-cell RNA-seq

scRNA-seq



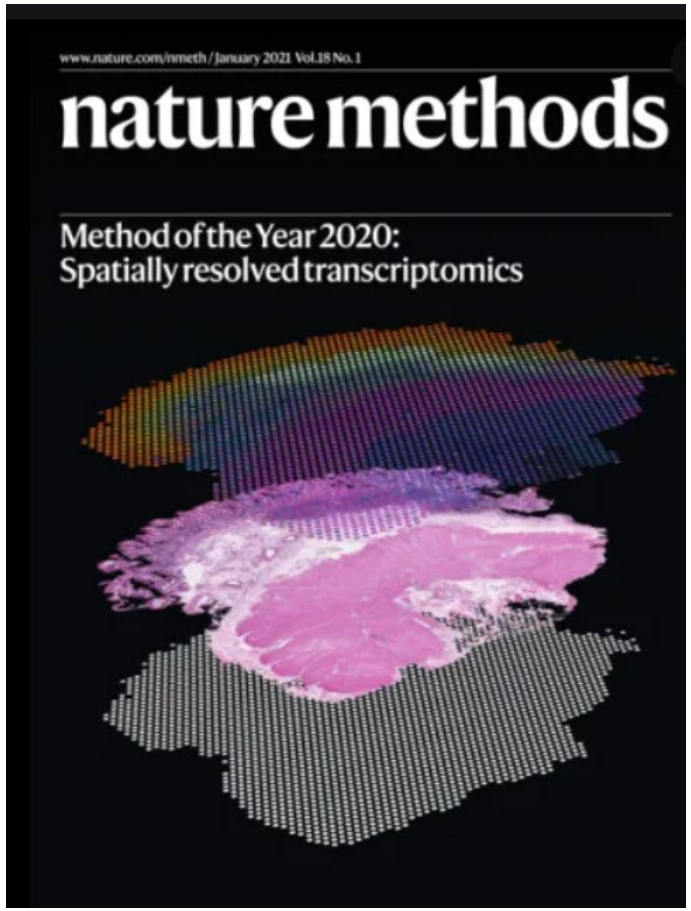
Bulk RNA-seq



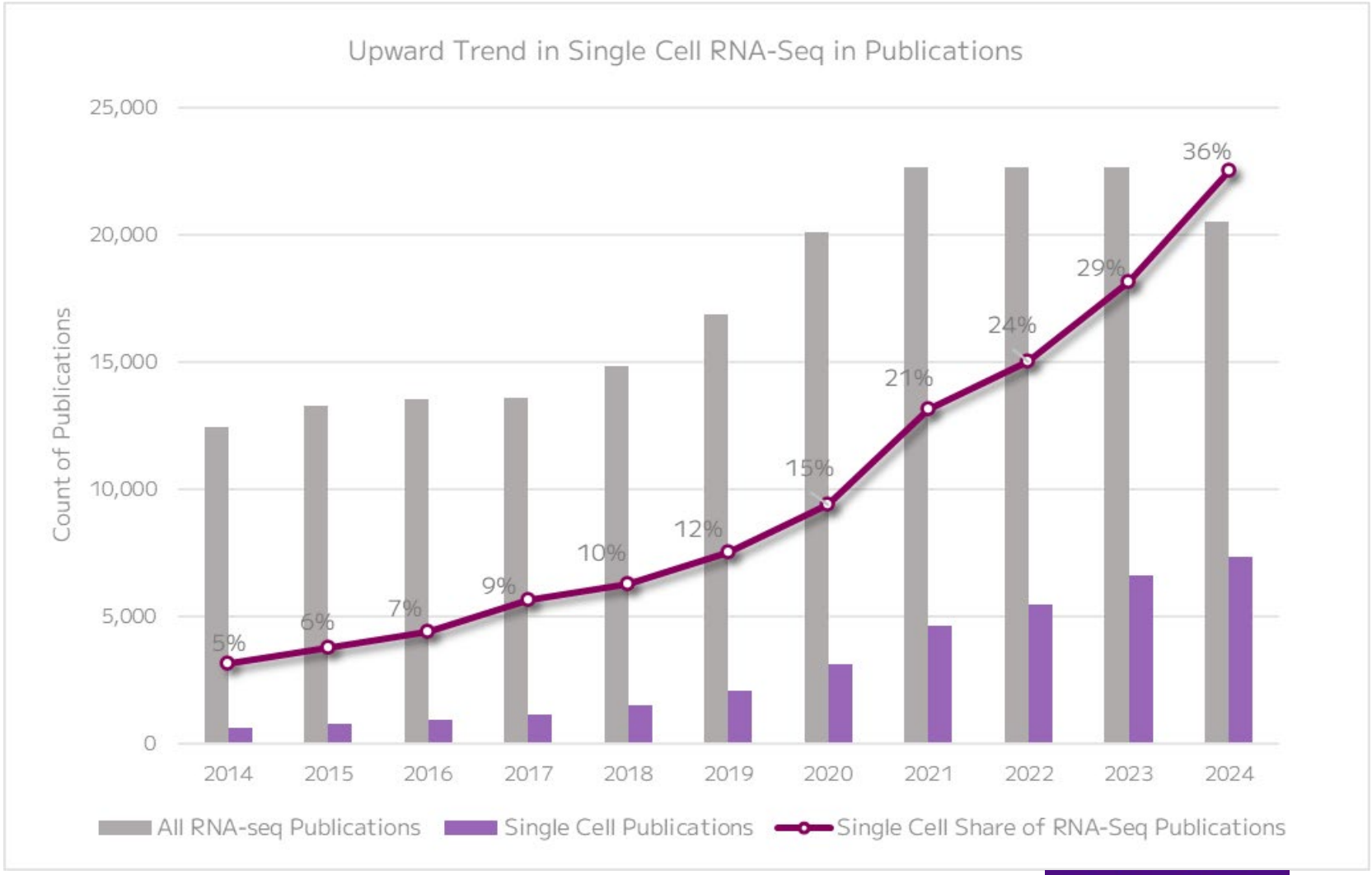
Adapted from Macaulay and Voet, Plos Genetics, 2014.

Power of single-cell transcriptomics technologies

"The single cell revolution is just starting"



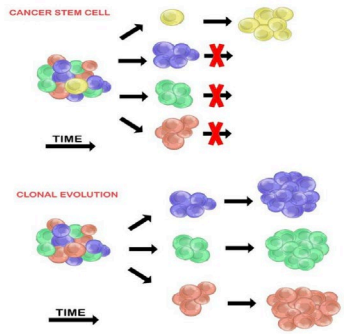
Single-cell RNA-seq studies over time

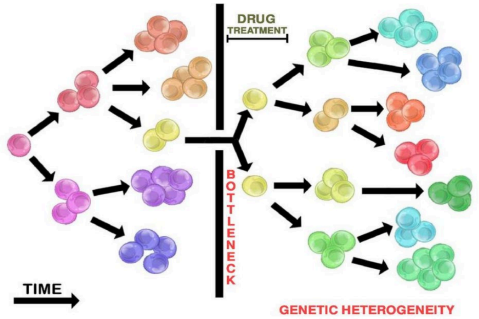


Parse, 2025

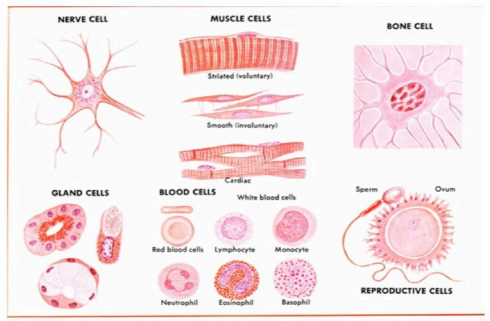
Main application of single-cell transcriptomics

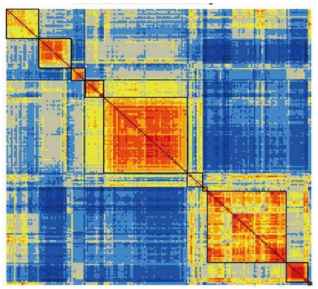
- Cell genetic and response heterogeneity



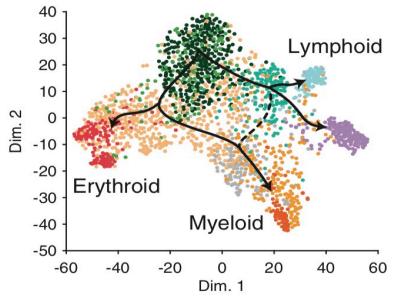


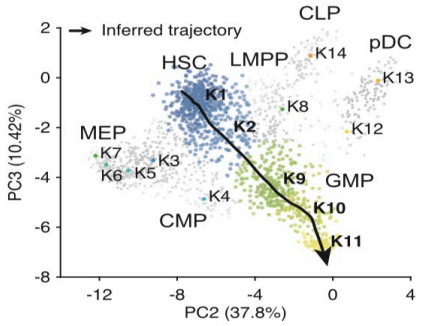
- Classification & identification of cells

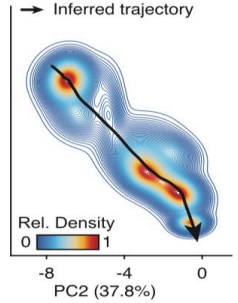




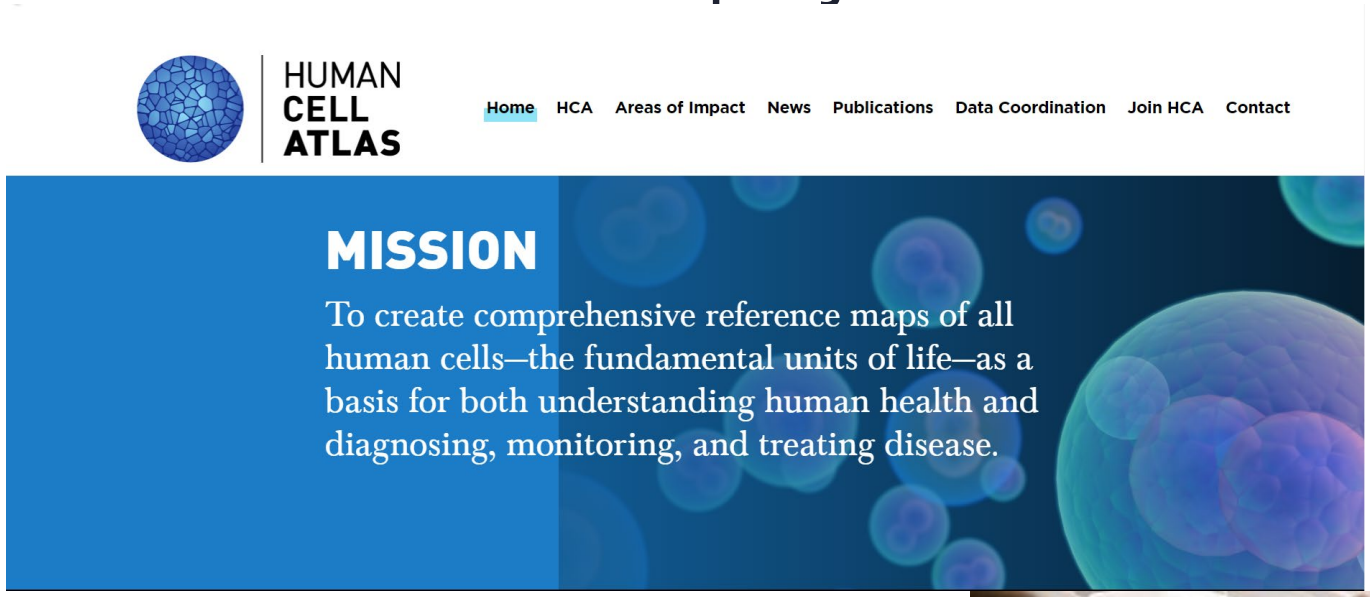
- Stem cell differentiation





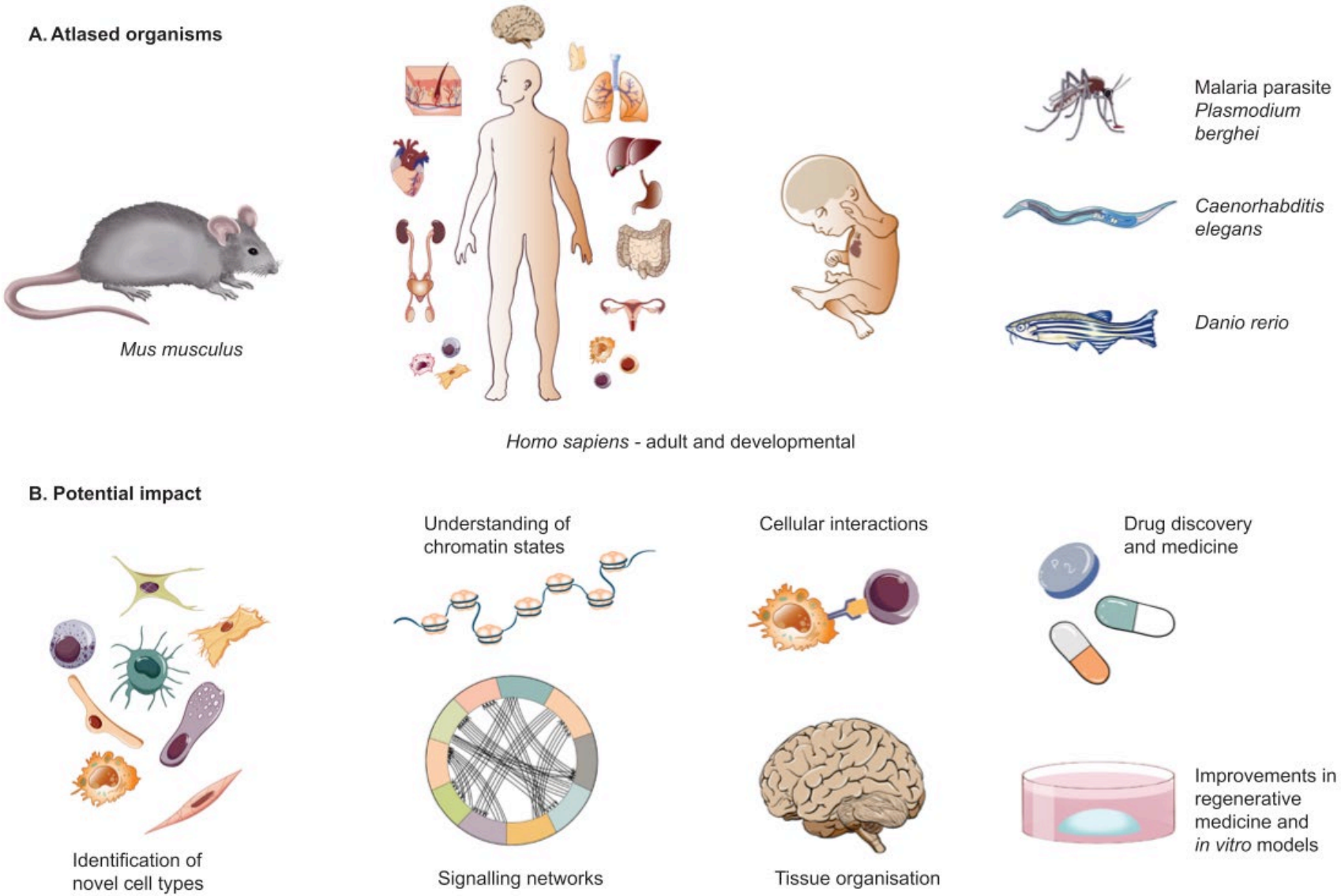


The human cell atlas project



- Sequencing **all** the (37.2 trillion!) cells in the human body
- Sanger Institute, MIT and Harvard
- Mark Zuckerberg and Priscilla Chan donated $3 billion for the project
- Started in 2016, first draft for release in **2026**

Potential impact of atlasing initiatives



Anna W.C. et al., Biochemical Journal, 2020.

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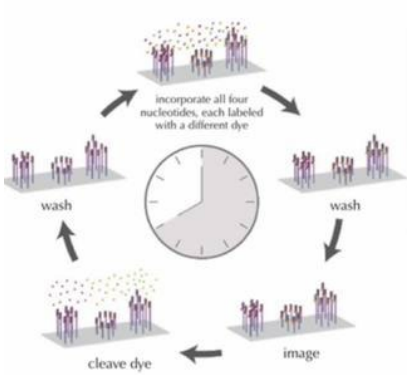
Sequencing Technology Overview

Next Generation and Long-Read Sequencing

Short read vs. long read technology

2nd Generation

- Monoclonal amplification on a solid surface
- Sequencing in cycles
- **Short** reads
- **High number** of reads



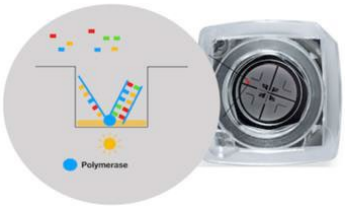
illumina®

Element Biosciences

ULTIMA GENOMICS

3rd Generation

- No amplification
- Sequencing on single molecules
- **Long** reads
- **Low number** of reads



PacBio

Oxford NANOPORE Technologies

SIB

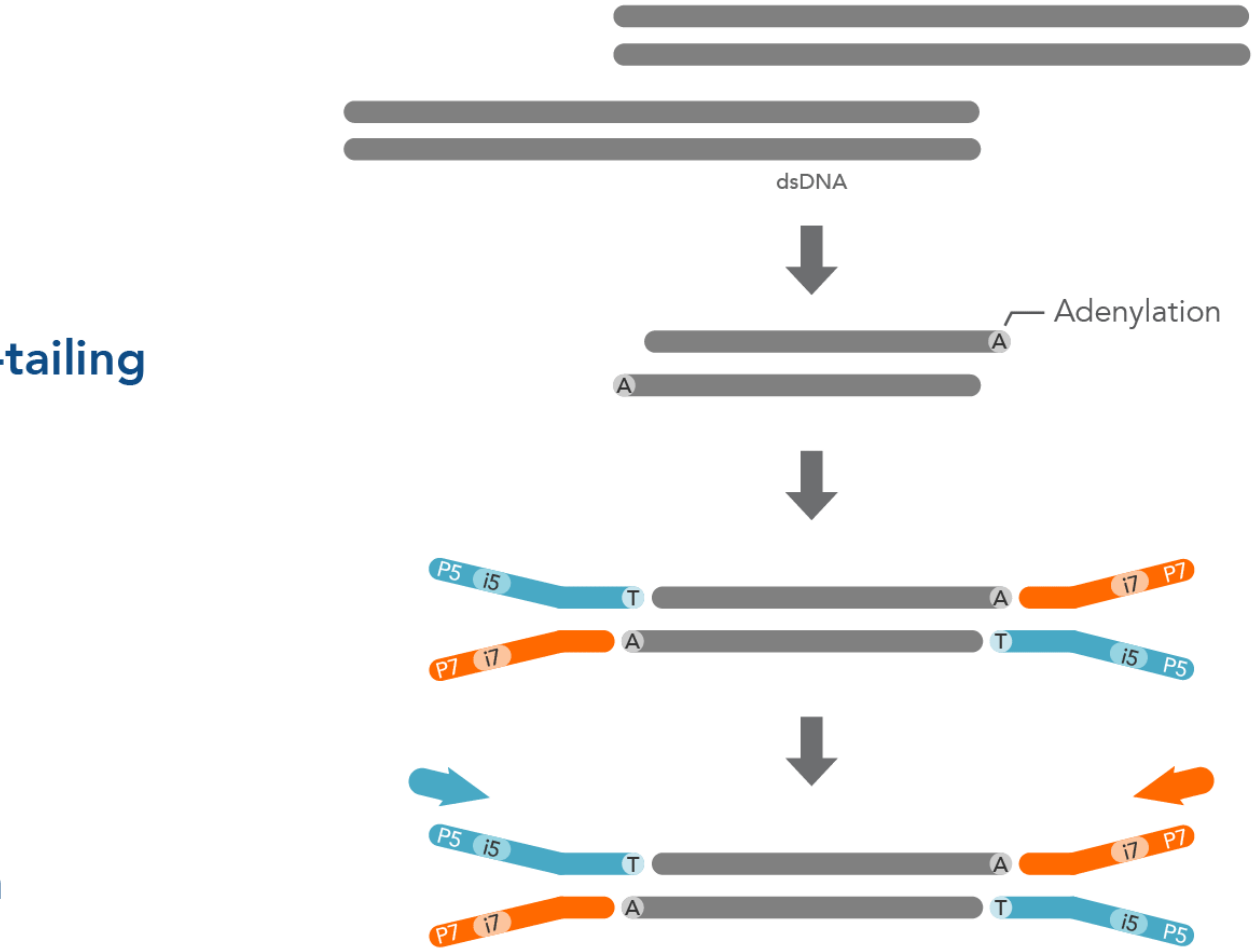
Standard short-read DNA library preparation

Fragmentation

End repair and A-tailing

Ligation

PCR amplification



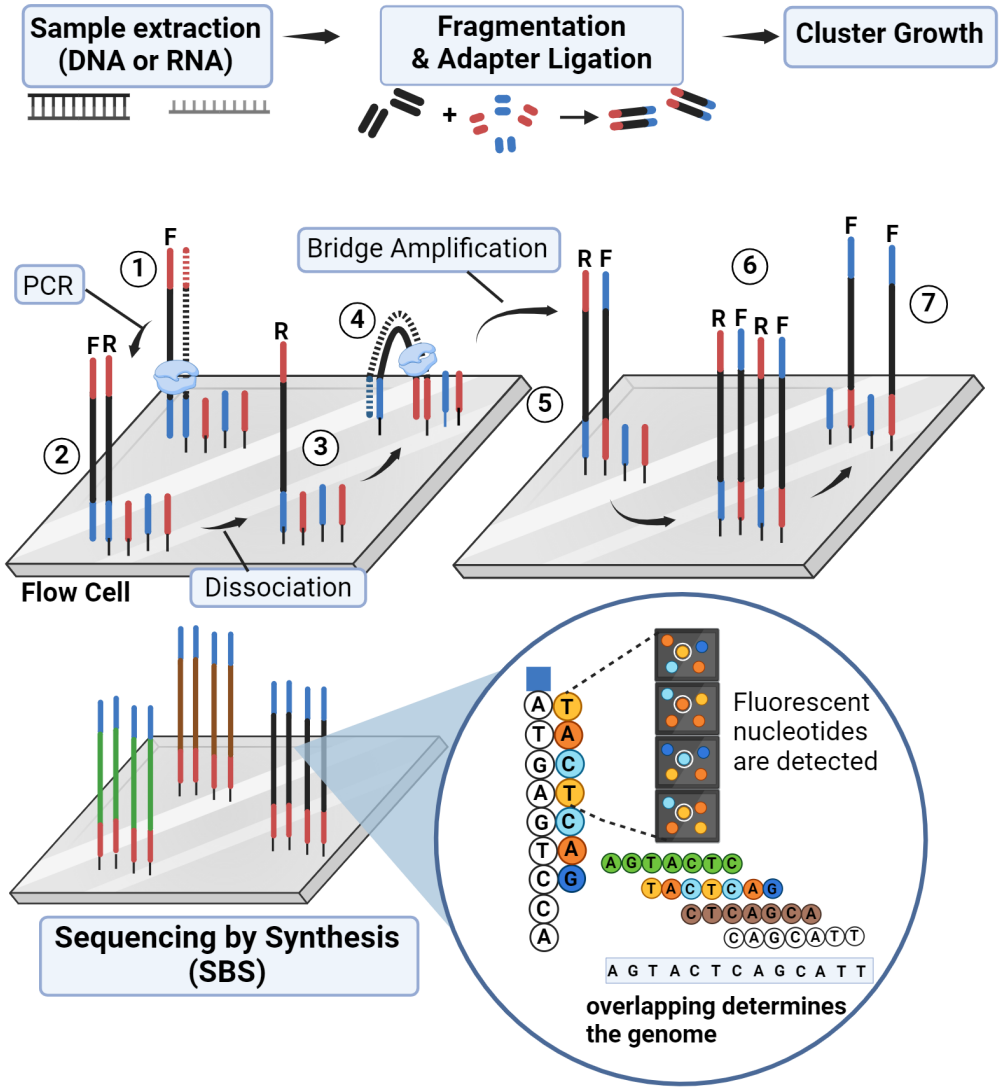
Integrated DNA Technologies, 2026

Illumina sequencers

NovaSeq X

Illumina 2026

Illumina sequencing method



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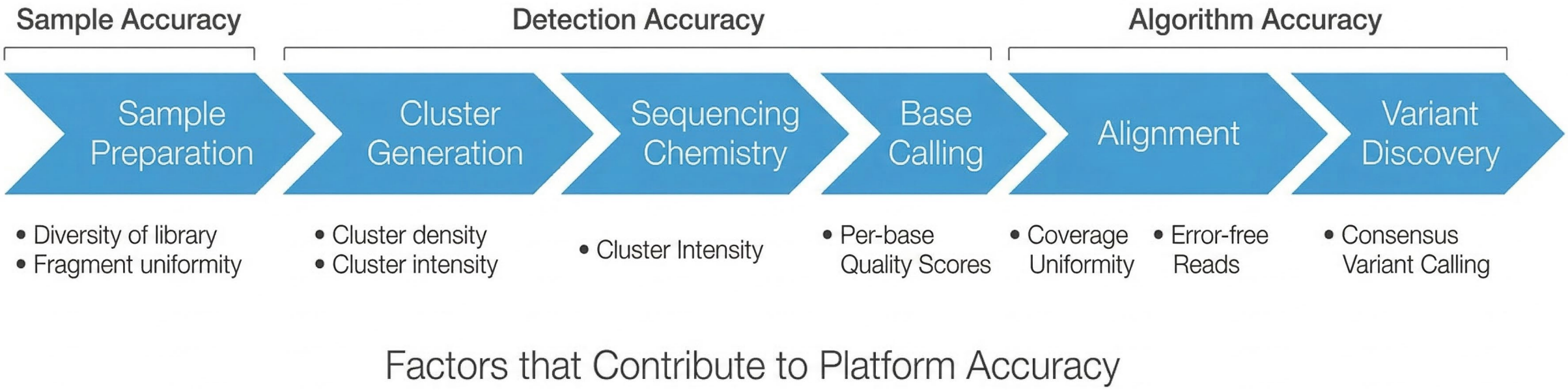
High density cluster formation

1) Fragments annealing to flow cell
2) Reverse strand synthesis
3) Dissociation
4) Bride formation
5) Bridge amplification
6) -Repeat-
7) Dissociation

Sequencing by synthesis

1) Denaturation
2) Incorporation
3) Imaging
4) Cleavage
5) -Repeat-

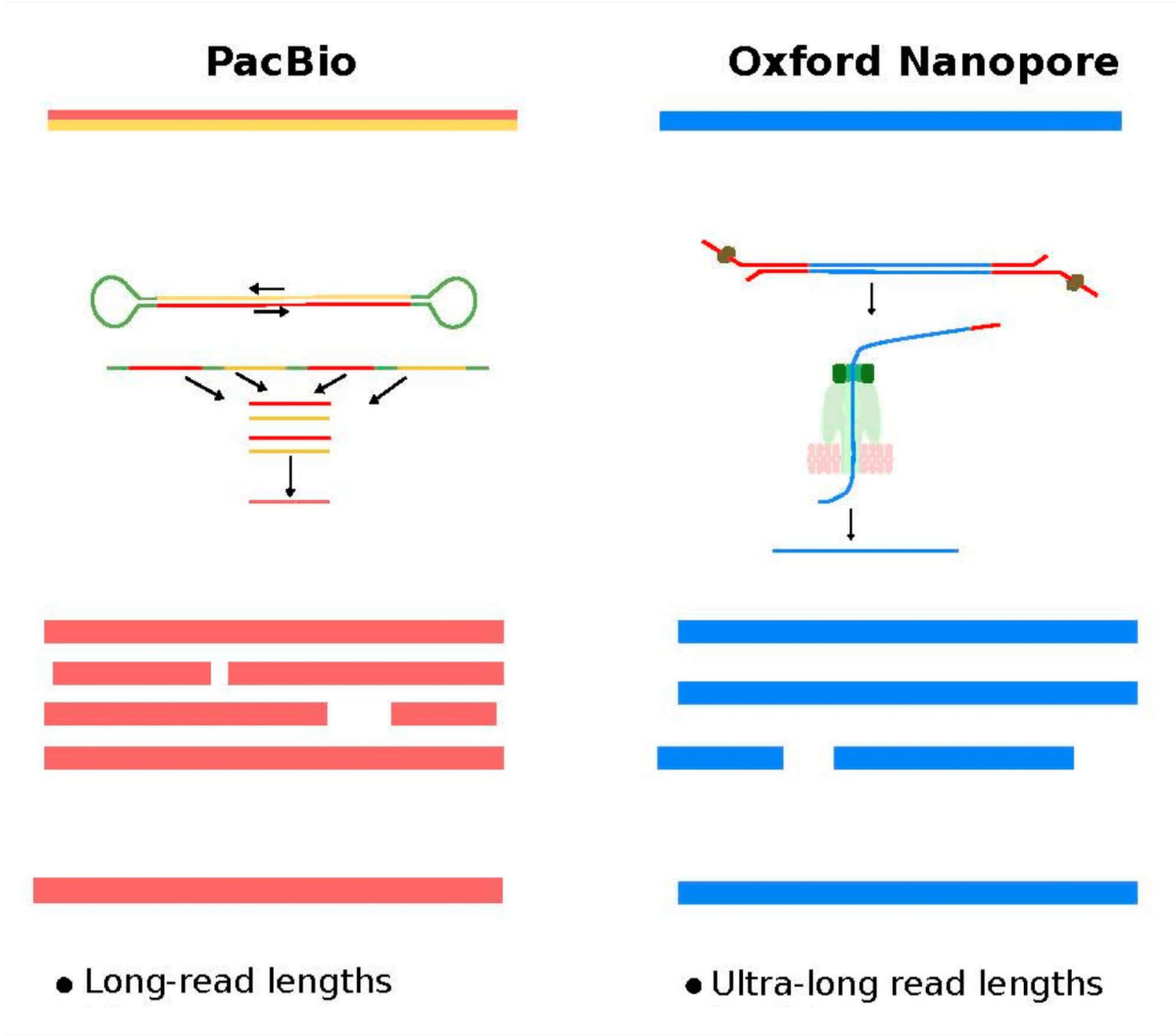
Short-read workflow

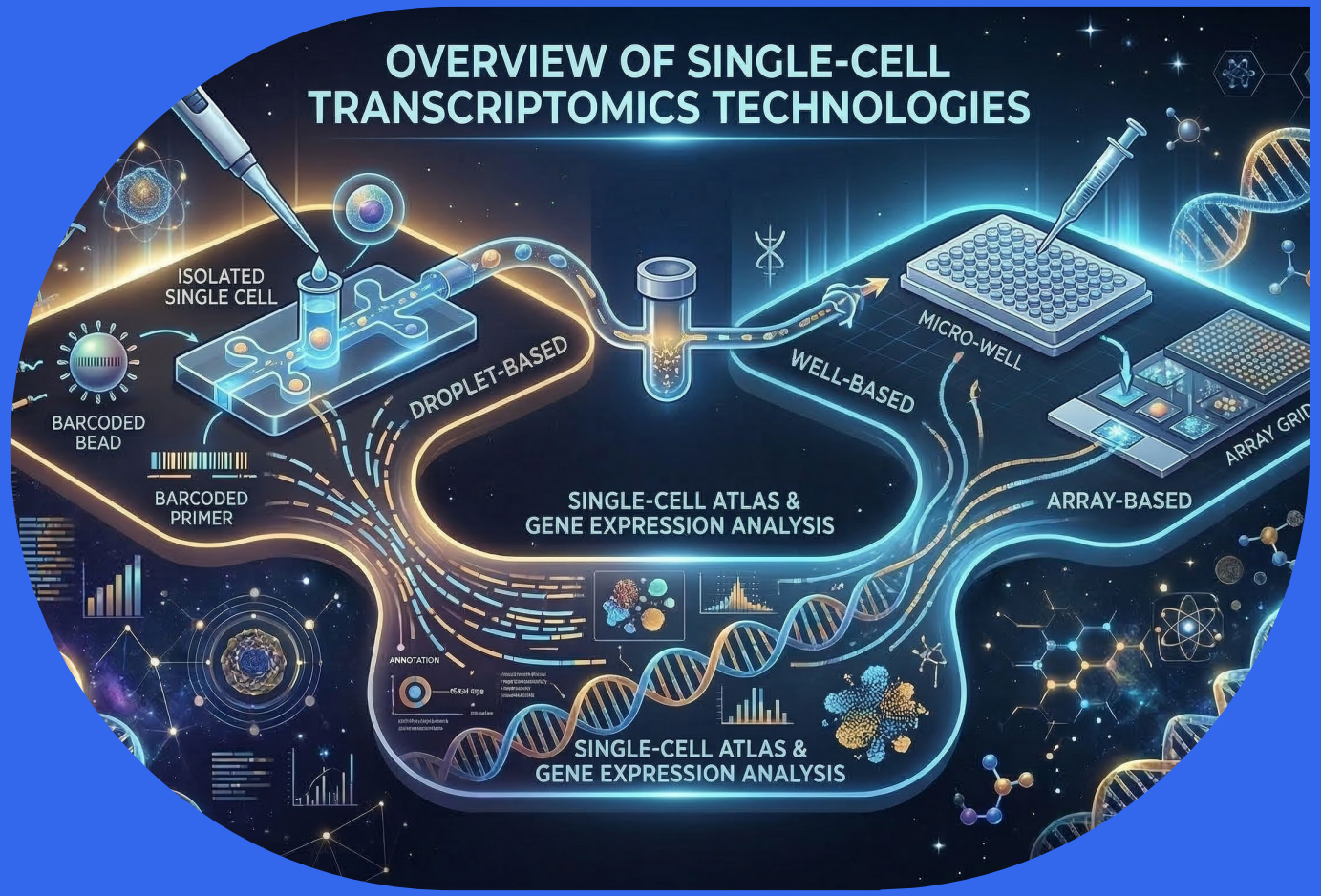


Factors that Contribute to Platform Accuracy

Illumina 2026

Long-read sequencing





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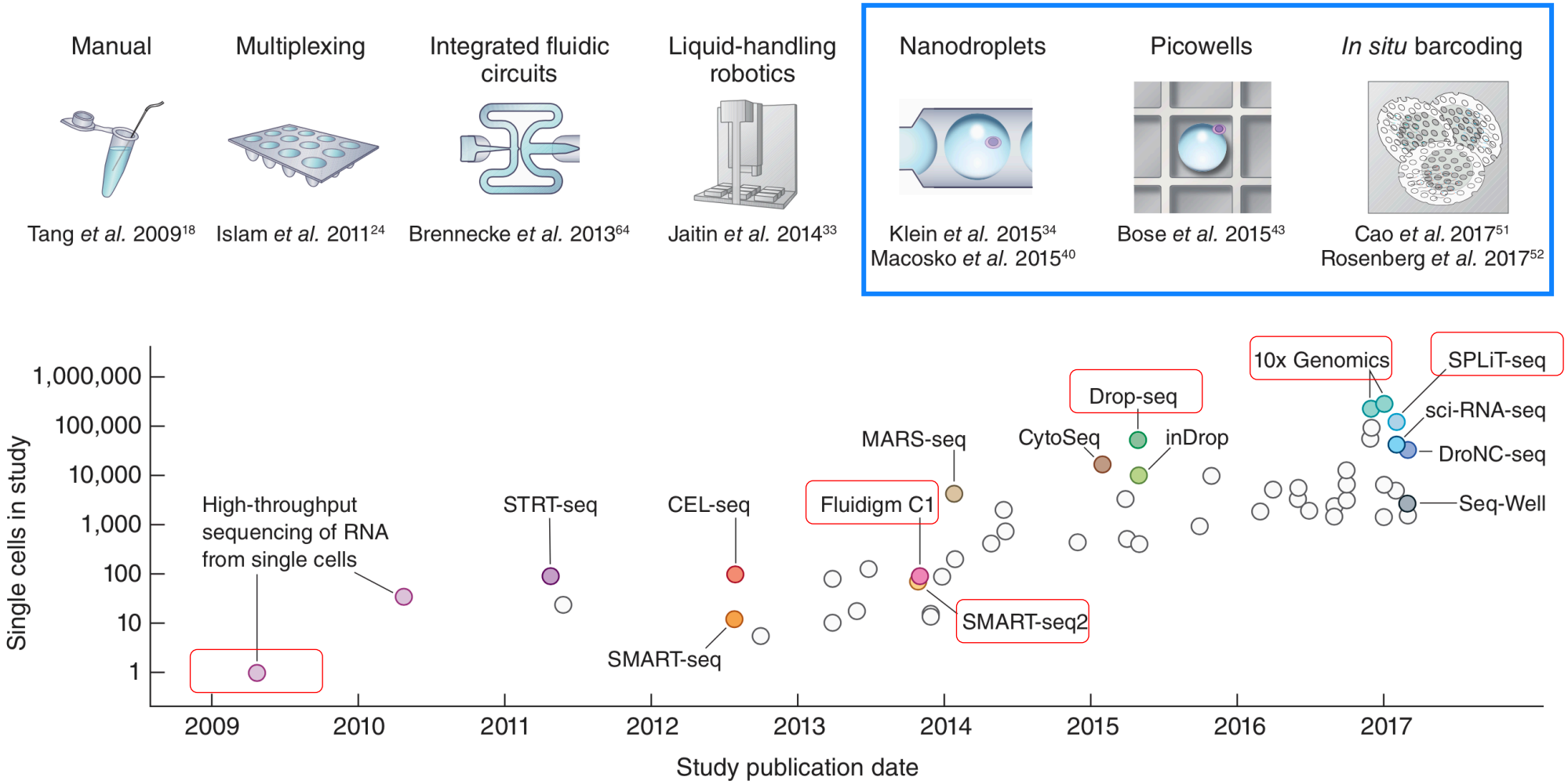
Single-Cell Transcriptomics

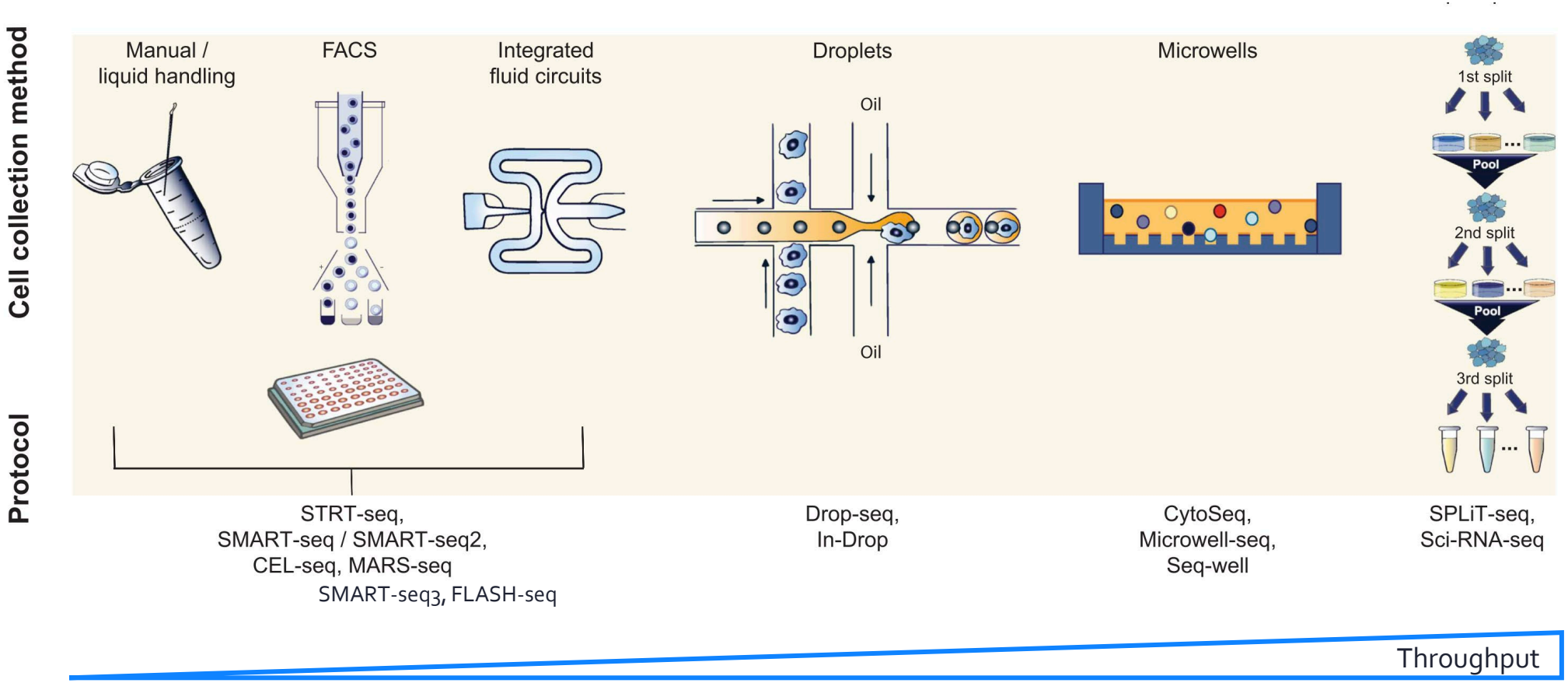
Single-Cell Technology Overview

Increasing throughput of single-cell RNA-seq



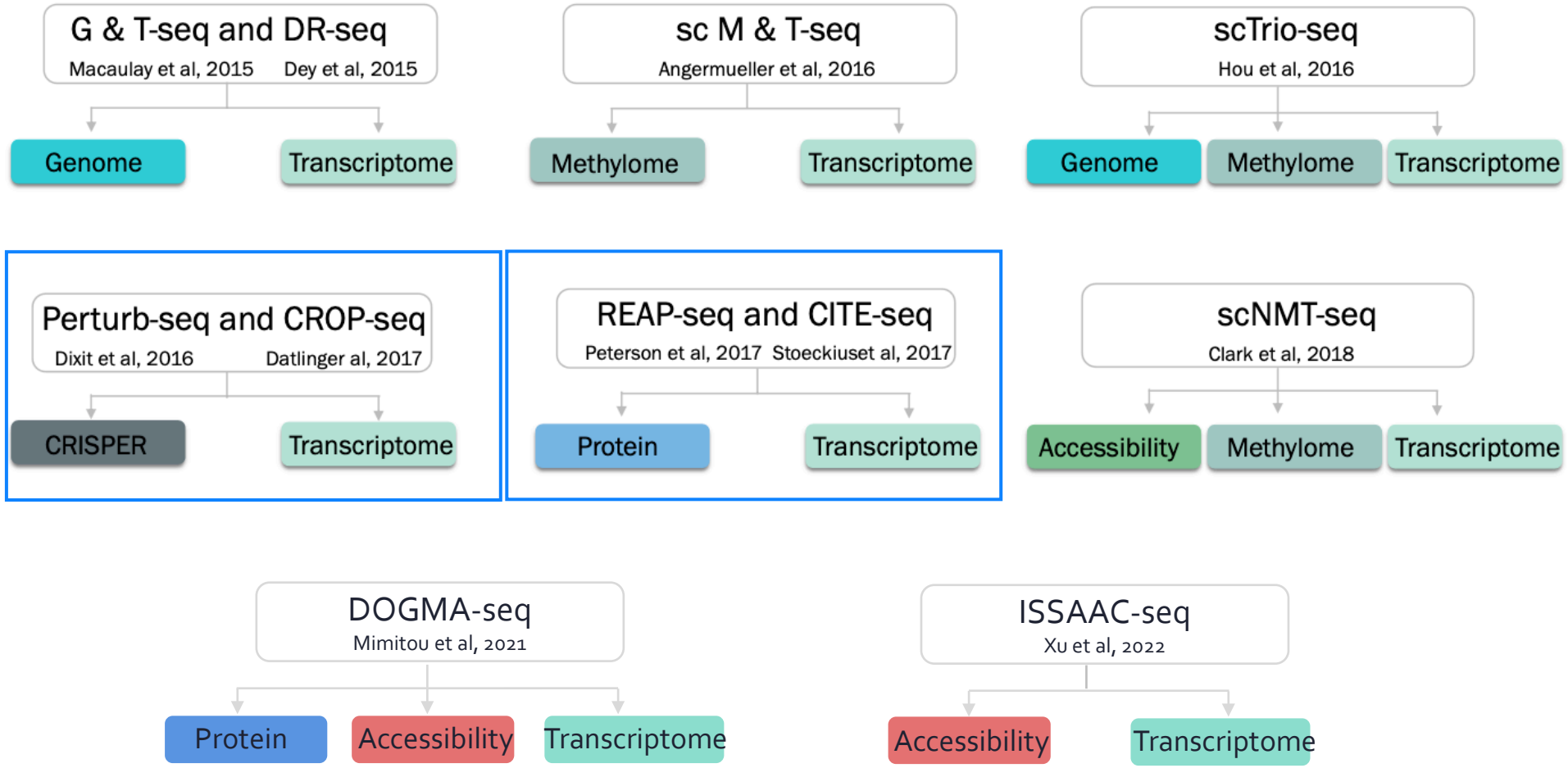
Valentine S. et al., Nature protocol, 2018.

Current single-cell RNA-seq methods



Anna W.C. et al., Biochemical Journal, 2020.

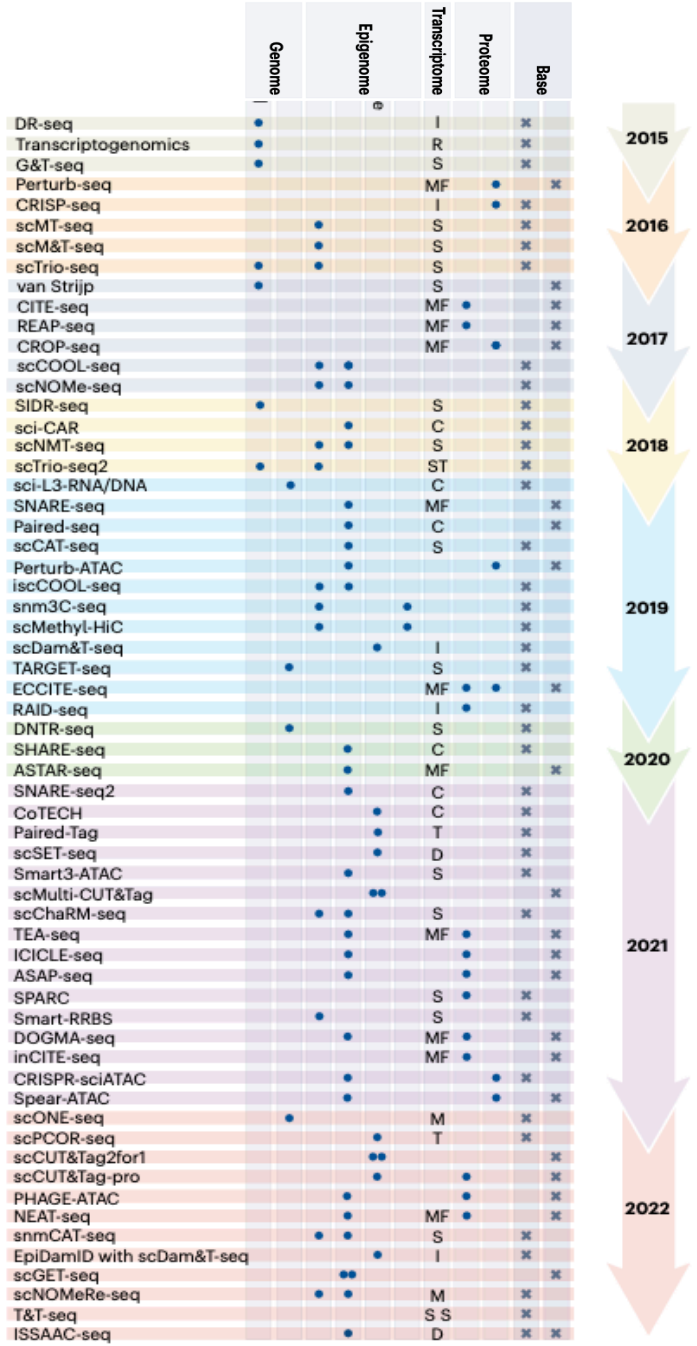
Single-cell multiome methods



Single-cell and Spatial multi-omics, Vandereyken et al., 2023.

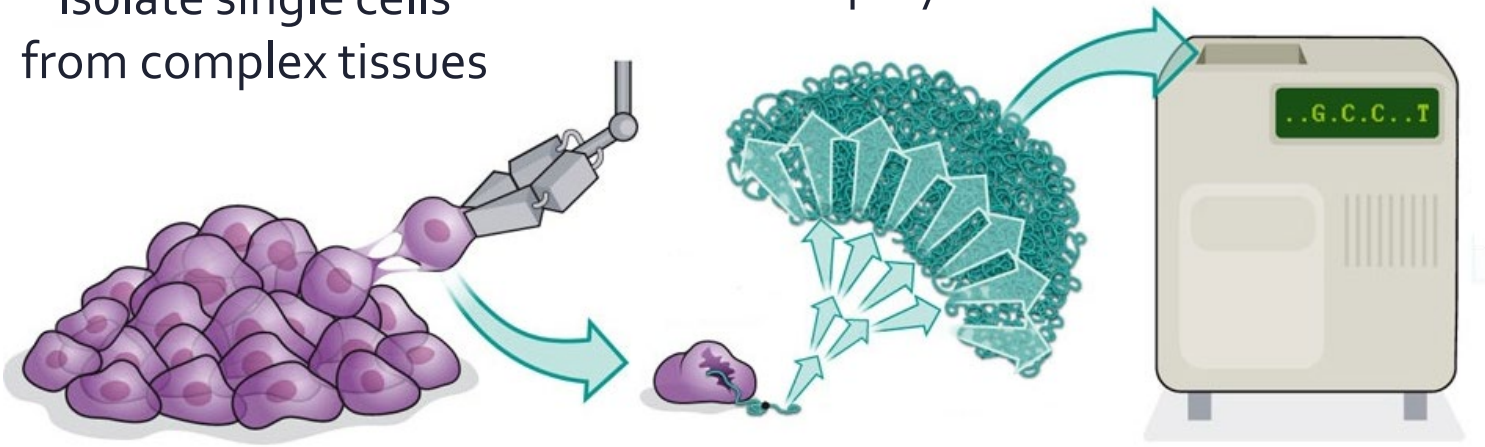
Single-cell multiome methods

- Huge variety of methods available
- Continuously increasing
- Different targets
- Many not commercialized



Single-cell and Spatial multi-omics, Vandereyken et al., 2023.

How does single-cell RNA-seq work?



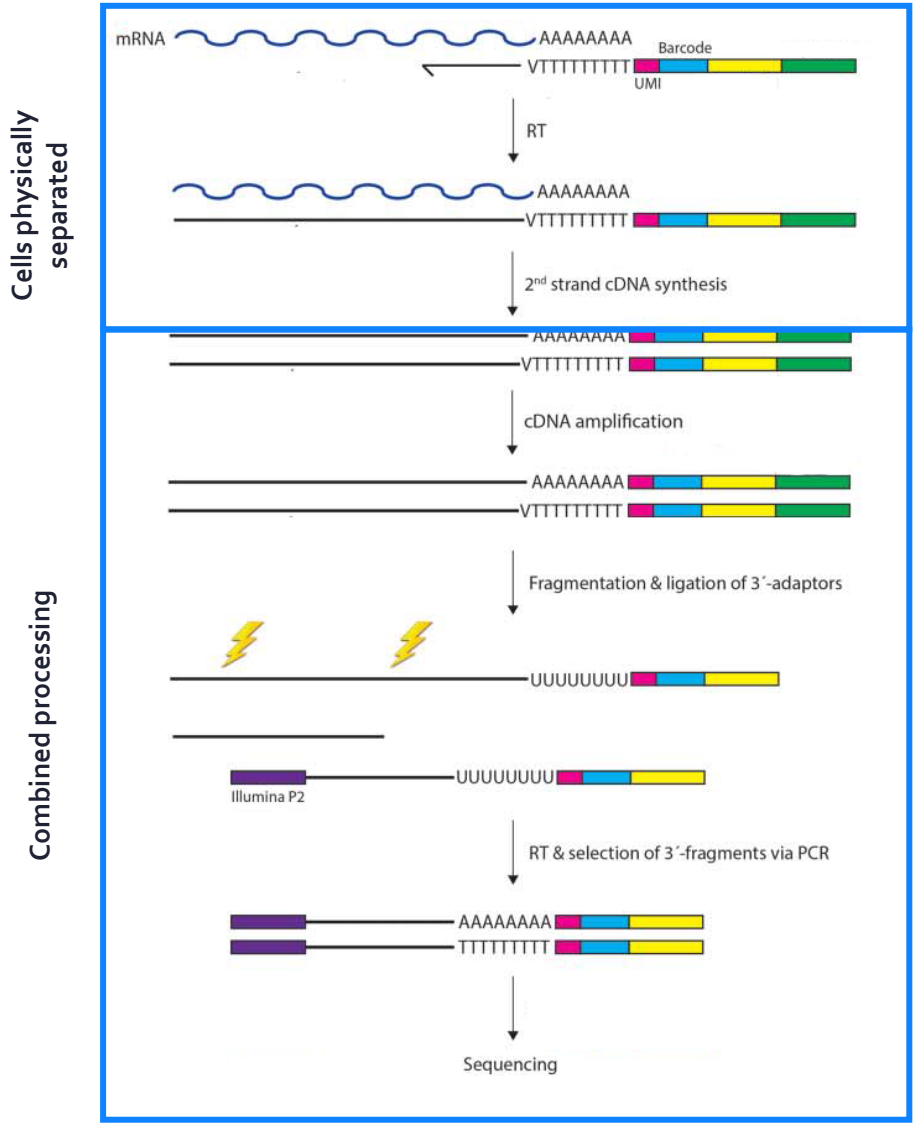
RT-based approach

✓ Captures actual RNA

✓ High-throughput

▪ 3′ or 5′ coverage only

▪ Less sensitive compared to probes



Adapted from Picelli, RNA Biology, 2017.

Probe-based approach

✓ Works with fixed sample

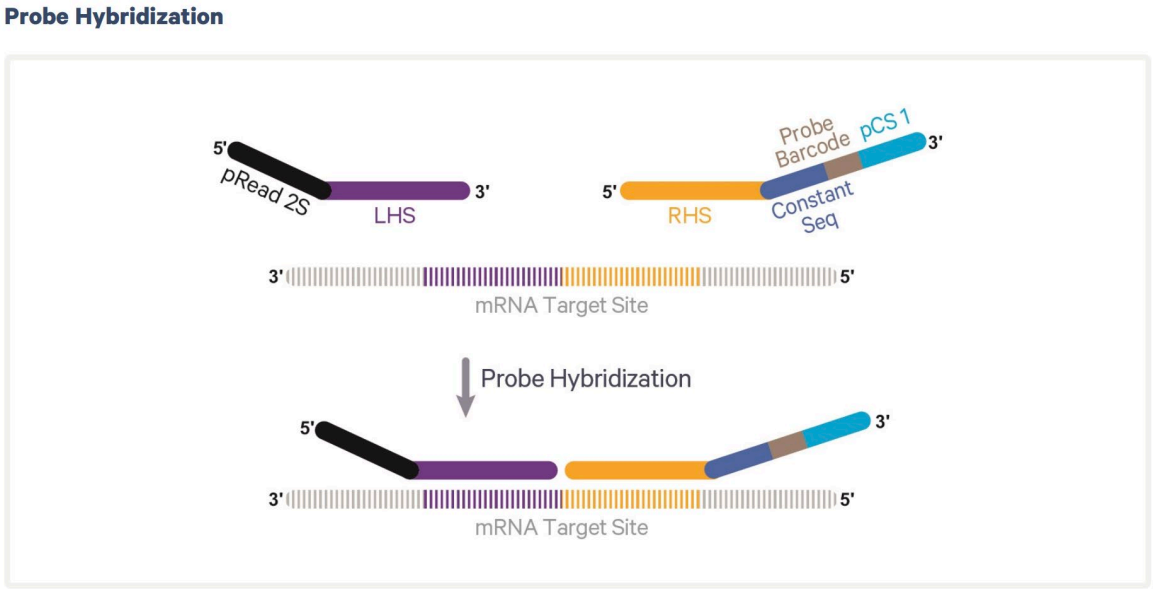
✓ Lower price compared to RT

✓ Very high throughput and sensitivity

▪ Sequencing probes instead of RNA

▪ Organism probeset required

▪ High cell number required



Probe summary: Human WTA Probeset

- **>18,000 coding genes** specifically detected
- >55,000 probe pairs
- Design strategy: maximize **specificity, sensitivity and sequencing efficiency**

10x Genomics, 2022.

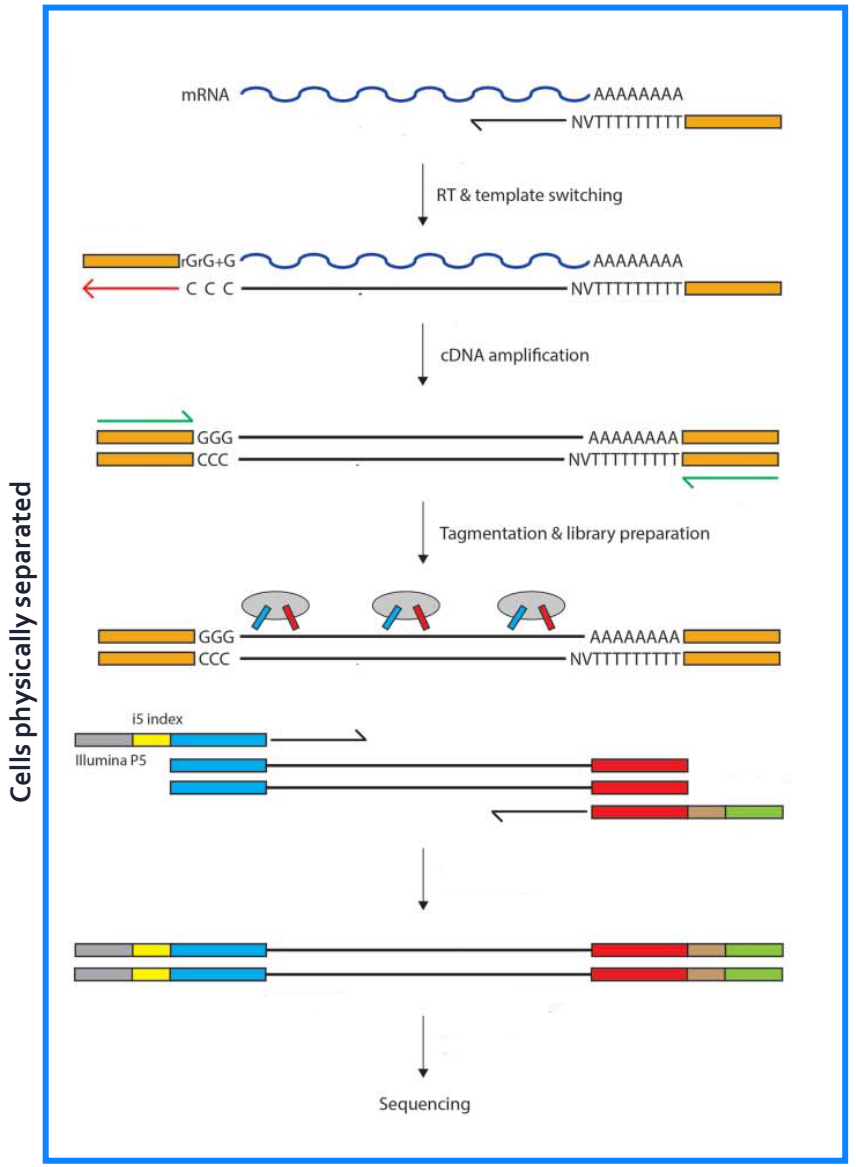
Single-cell RNA-seq methods: full-length methods

✓ Provides full length coverage of the transcripts

✓ High sensitivity

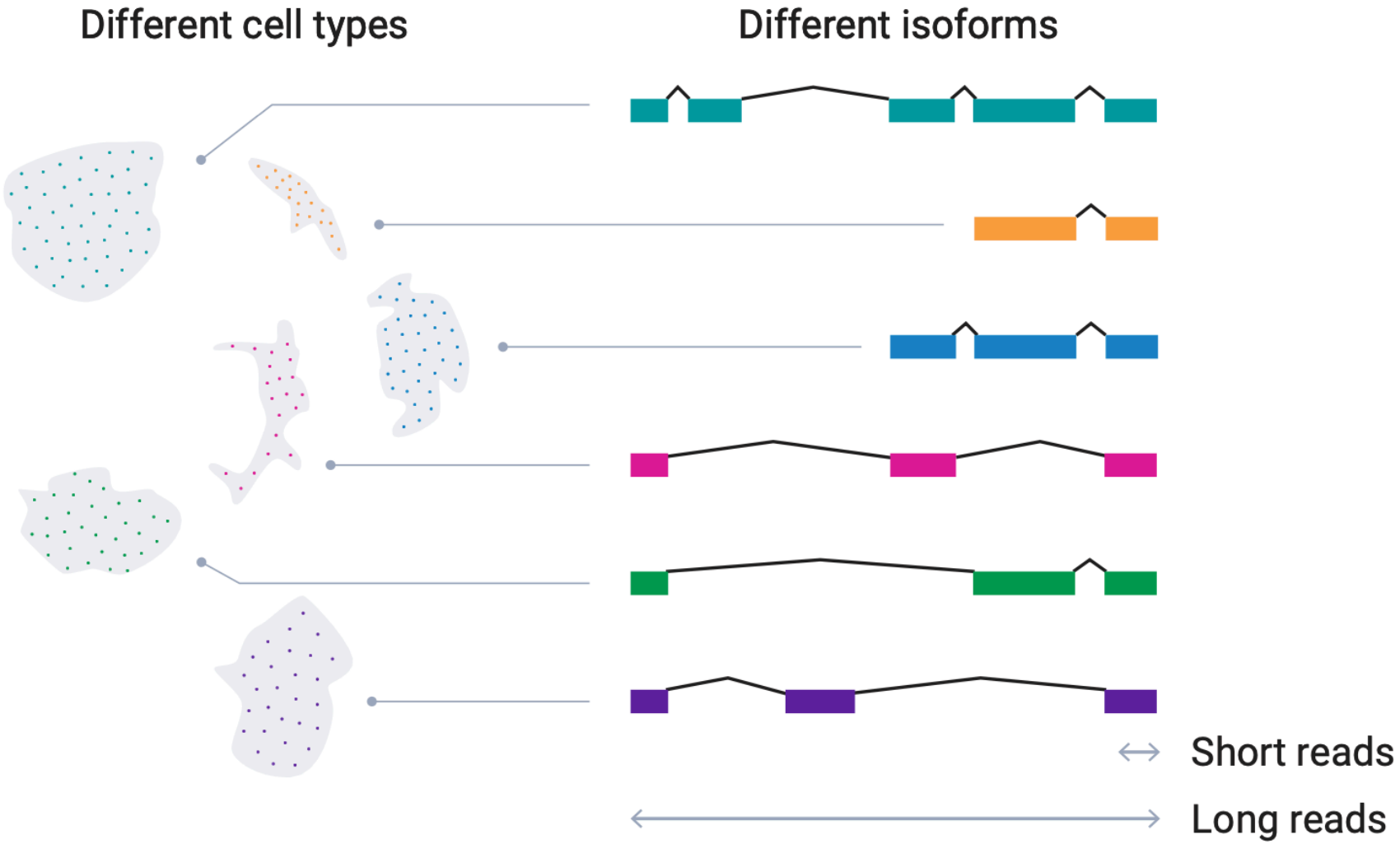
▪Low throughput

▪ Expensive



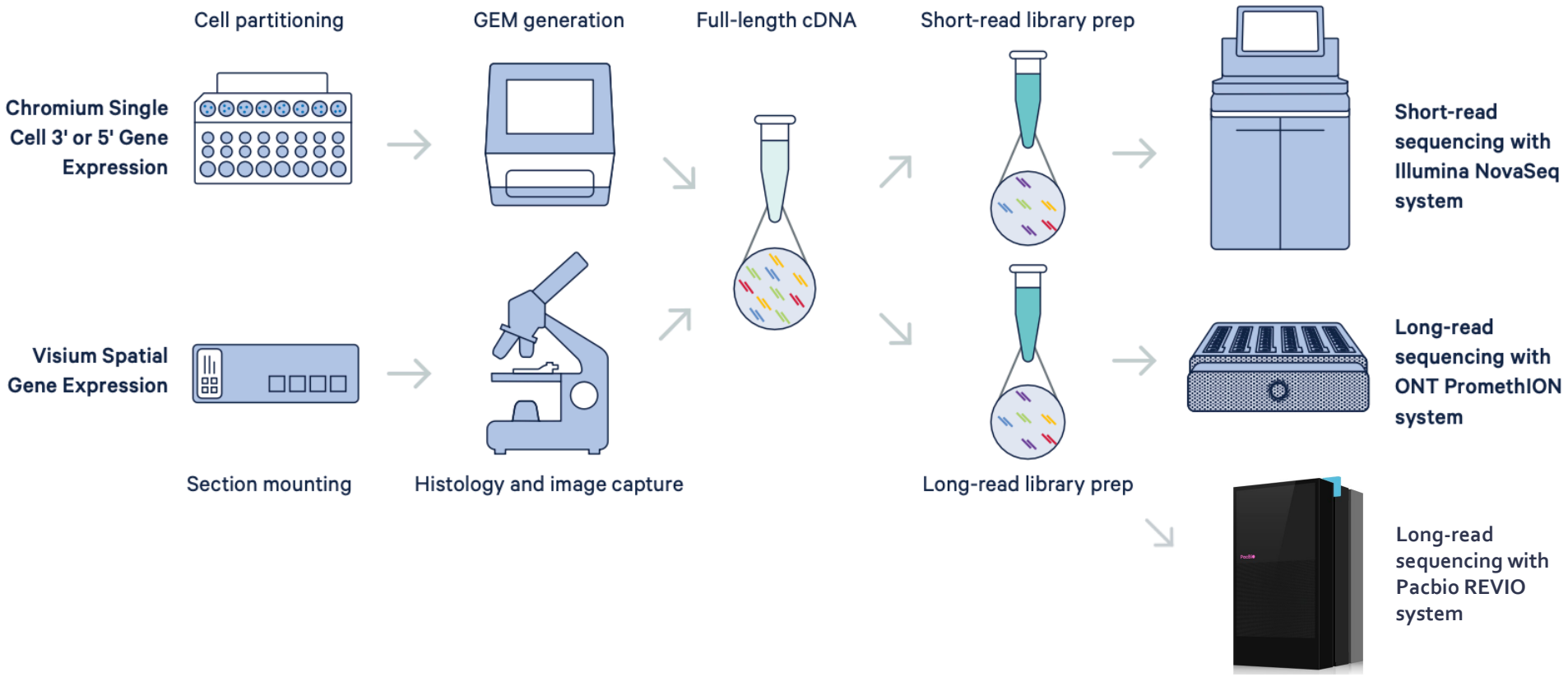
Adapted from Picelli, RNA Biology, 2017.

Single-cell long-read RNA-seq methods

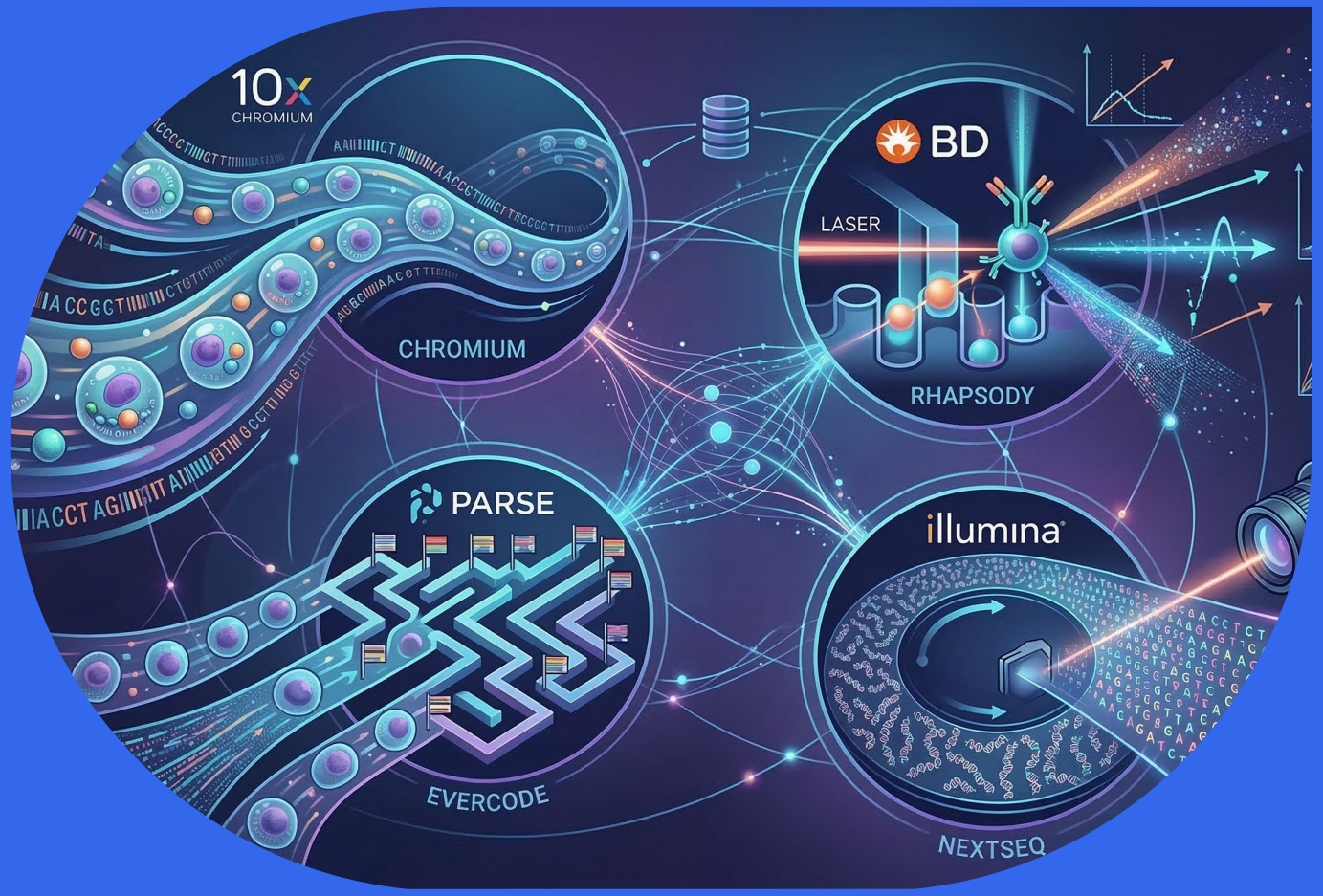


PacBio, 2022

Combining single-cell with long-read sequencing



10x Genomics, 2022



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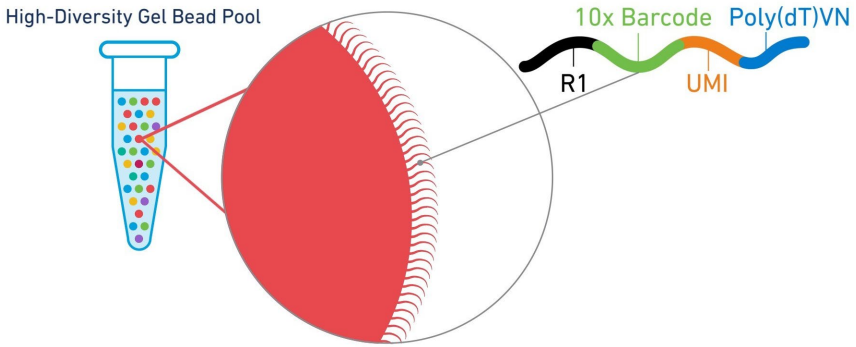
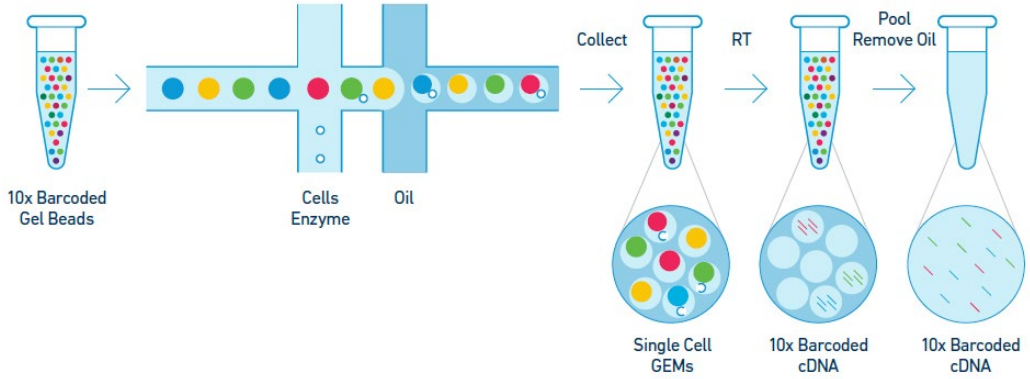
Single-Cell Transcriptomics

Major Commercial Technologies

Droplet based Platform: 10x Genomics

- ✓ Current standard in the field
- ✓ Simple workflow
- ✓ Multiomic applications
- Cell size <50 µm
- Clean samples crucial





10x Genomics, 2022

10x scRNA-seq assay overview

Assay types

Universal 3' Gene Expression

Universal 5' Gene Expression

Epi Multiome ATAC + Gene Expression

Flex Gene Expression

→Gene expression

→Immune repertoire analysis

→scATAC-seq

→Fixed cells

Protocol extensions

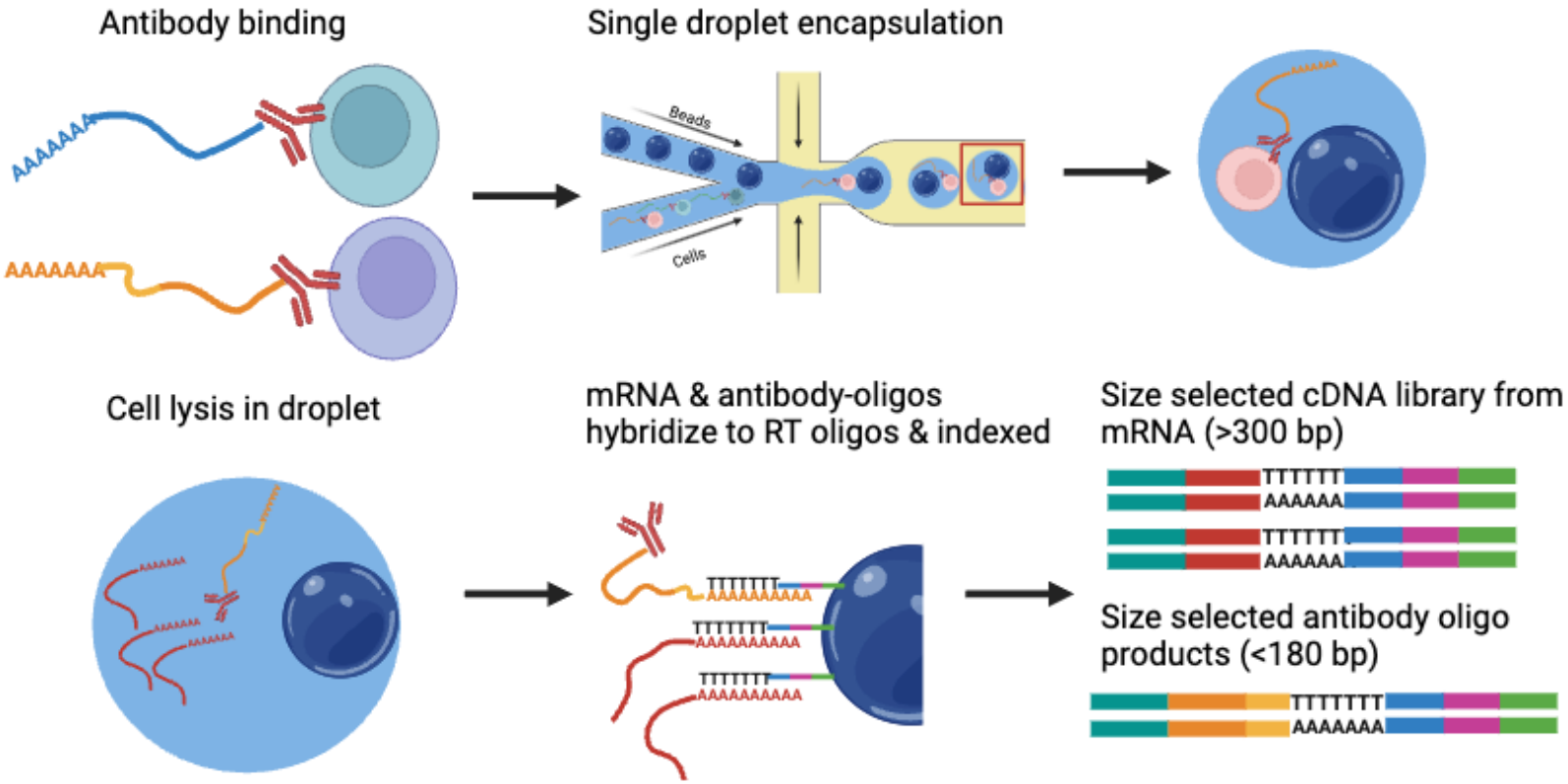
- On Chip Multiplexing
- Cell-surface proteins
- Intracellular proteins

} **CITE-Seq**

- BCR/TCR
- CRISPR Screen
- Long-read

10x Genomics, 2022

CITE-Seq with 10x Genomics



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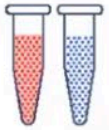
10x Gene Expression Flex (Apex)

Broad sample compatibility

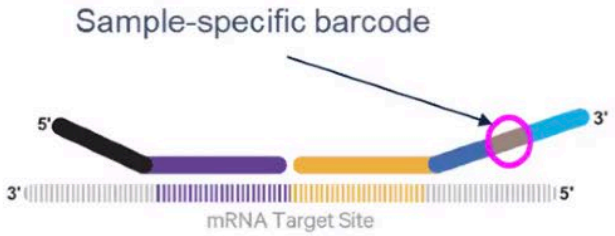
Fresh tissue
Frozen tissue

FFPE tissue



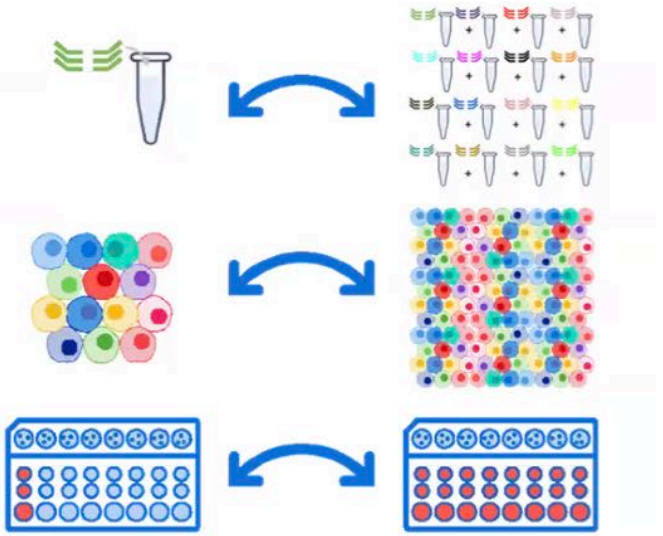
Cell suspensions
Nuclei suspensions

In-line multiplexing



5-fold increase in scale over other multiplexing techniques

Flexible sample and cell input number



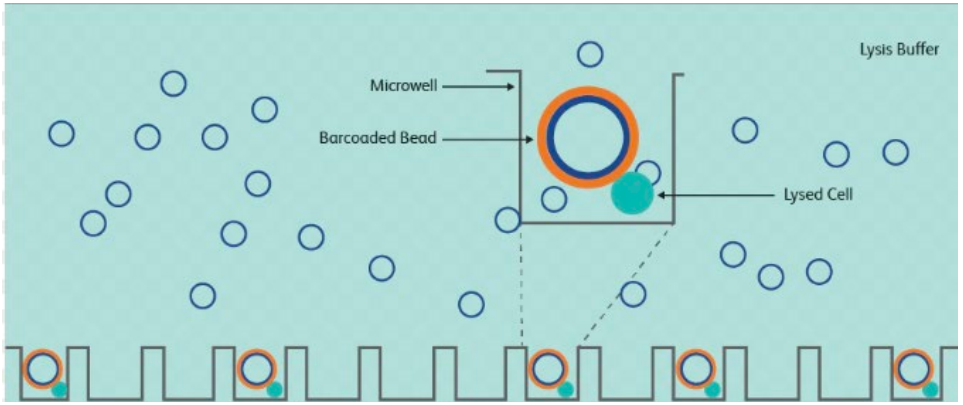
- ✓ Sample are fixed
- ✓ Handling various types of samples (F,FF,FFPE)
- ✓ More flexible for sample multiplexing
- Only avaible for Human and Mouse
- High number of cells required

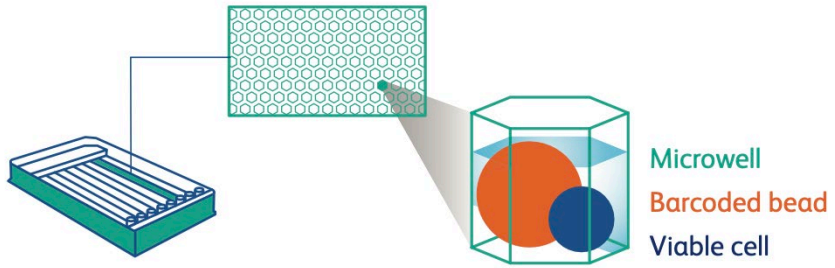
- Up to 3,000 samples per chip
- Up to 8 million cells per chip

10x Genomics, 2023

Microwell-based platform: BD Rhapsody HT

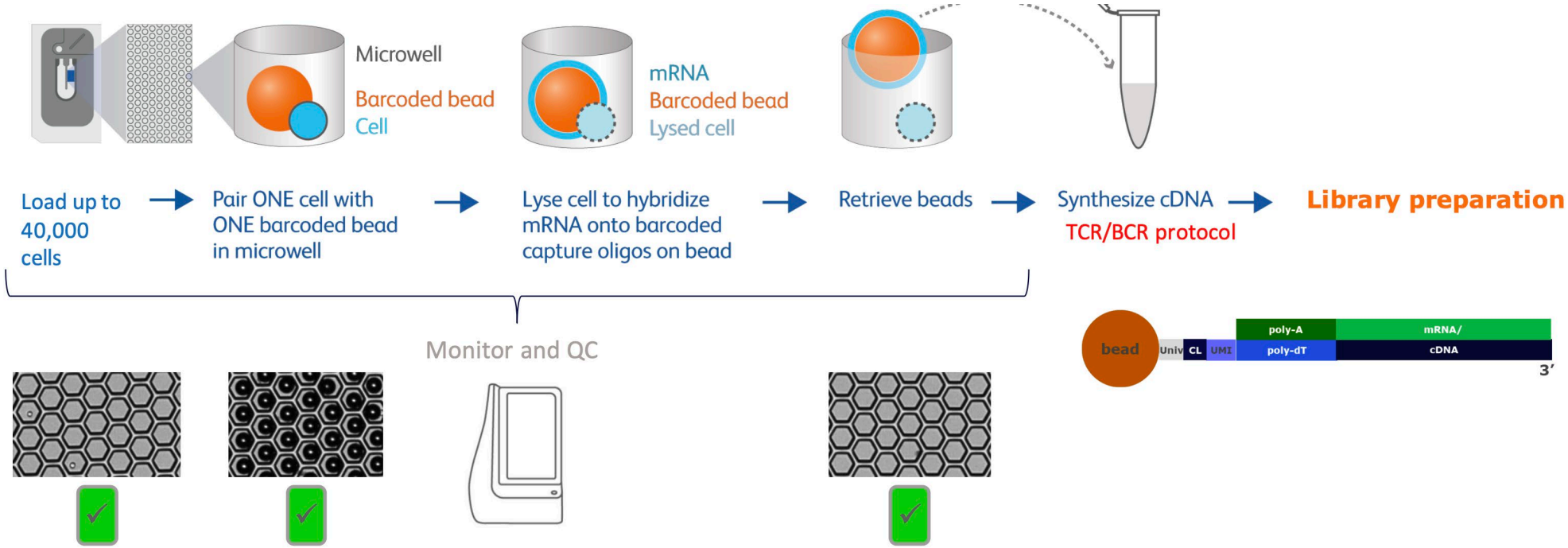
- ✓ Visulization of cell capture
- ✓ Less sensitve for unclean samples
- ✓ Lower multiplet rate than 10x
- ✓ Multiomic analysis
- ▪ Lengthy library preperation protocols
- ▪ Cell recovery lower than 10x





BD, 2025

Microwell-based platform: BD Rapsody HT



BD, 2023

BD scRNA-seq assay overview

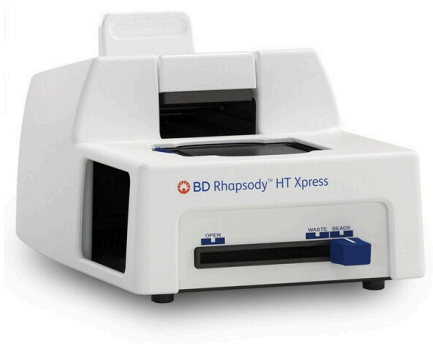
Assay types

WTA Gene Expression → Global gene expression

Targeted Gene Expression → Selected gene expression

VDJ-Seq → Immune repertoire analysis

ATAC-Seq + Gene Expression → scATAC-seq



Protocol extensions

- (Nuclei) Multiplexing
- Omics Guard
- Cell-surface proteins
- Intracellular proteins
- BCR/TCR

} **CITE-Seq**

- CRISPR Screen
- Long-read

BD, 2026

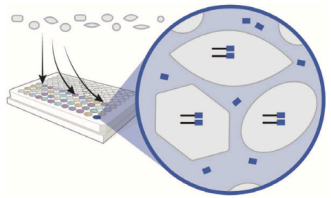
Instrument-free scRNA-seq: Parse bio

- ✓ Fixed samples
- ✓ Sample multiplexing
- ✓ Super high-throughput
- ▪ Complicated and lengthy workflow
- ▪ Low cell recovery rate

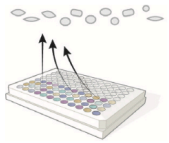
Semi-automation possible

1 Reverse Transcription

Split | Fixed cells/nuclei are distributed into wells, and the first sample-specific barcodes are added by in-cell reverse transcription.

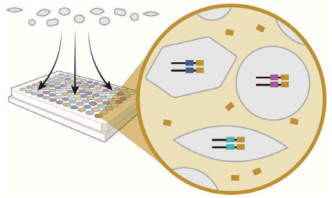


2 Pool | All the cells are pooled together.

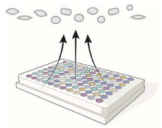


3 Ligation

Split | The pooled cells are distributed across a plate, and an in-cell ligation adds the second barcode.

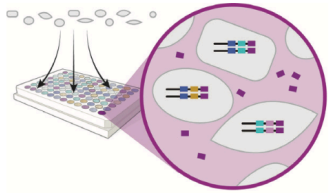


4 Pool | All the cells are pooled together.

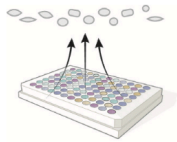


5 Ligation

Split | The pooled cells are again distributed across a plate, and a third barcode is added via in-cell ligation reaction.

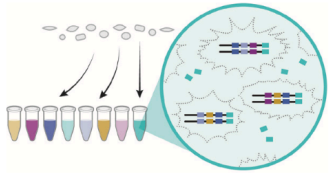


6 Pool | All the cells are pooled together.



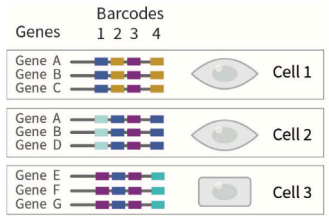
7 Lysis and Library Prep

Split | The pooled cells are distributed across several sublibraries then lysed. The fourth barcode is added via PCR.



8 Sequencing and Analysis

Each transcript is assigned to a single cell based on a unique combination of barcodes.

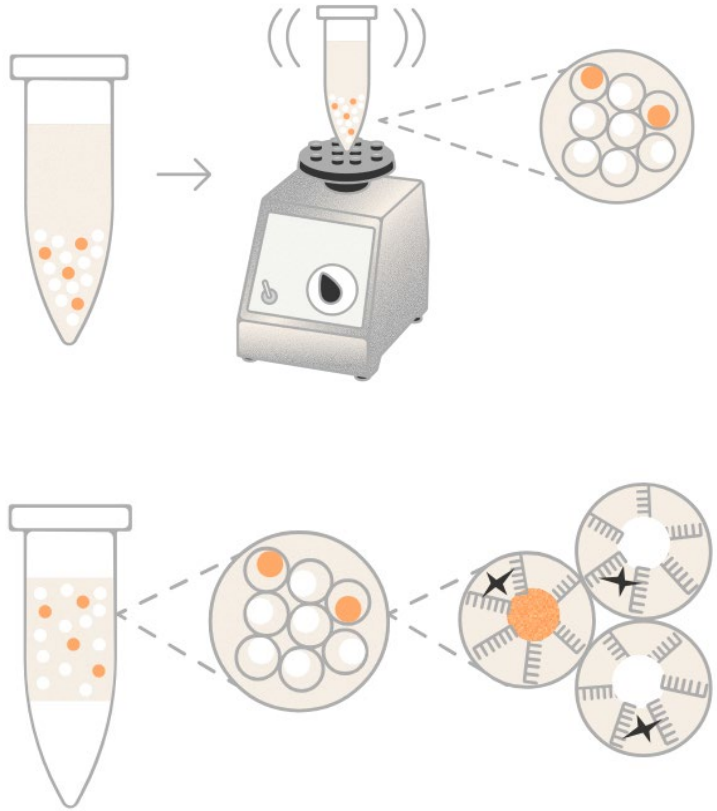


Parse Bio, 2022.

Instrument-free scRNA-seq: Illumina PIP-Seq

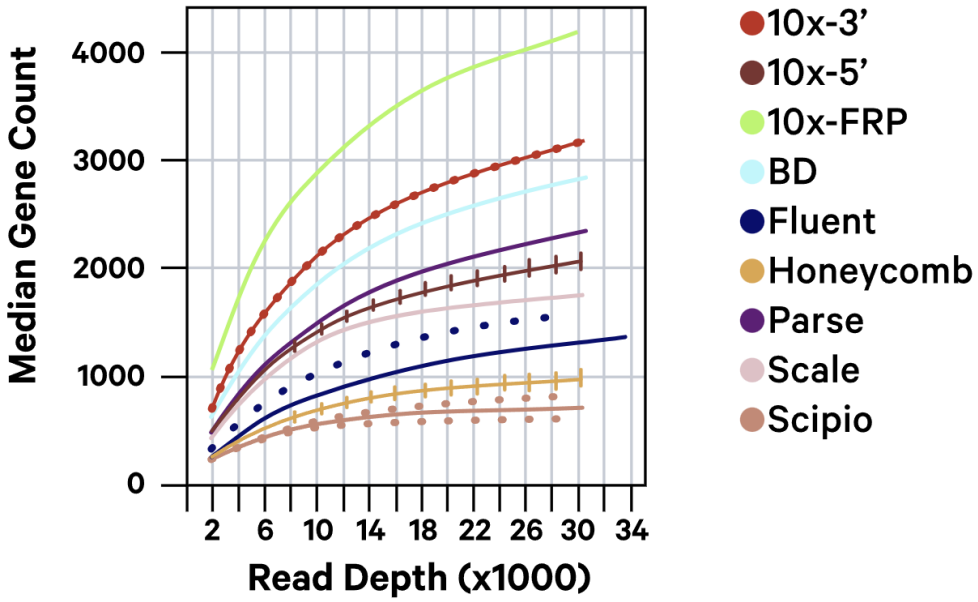
- ✓ Fresh or MeOH-fixed samples
- ✓ Low cost and hands-on time
- ✓ Any (poly-A) species

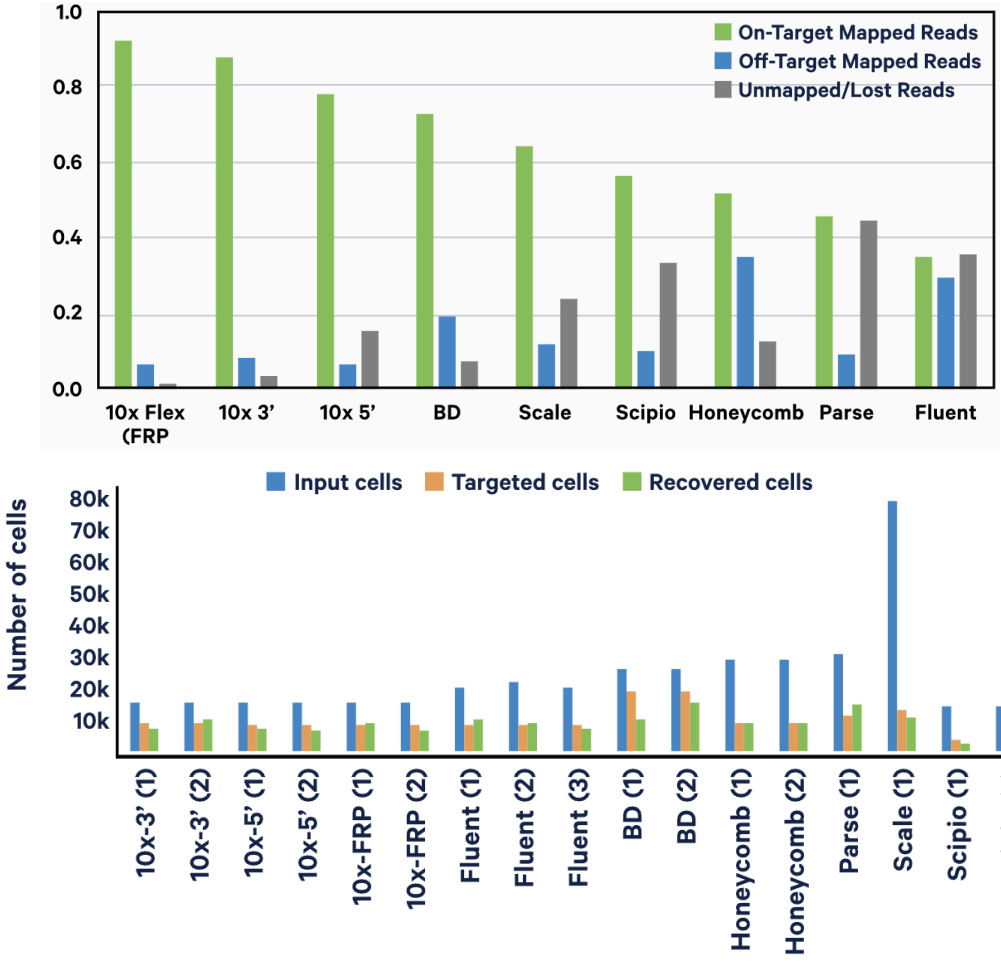
- Lower sensitivity compared to 10x/BD
- Max. 8 samples / 100k cells
- Low cell recovery rate



Illumina, 2025

scRNA-seq methods comparison

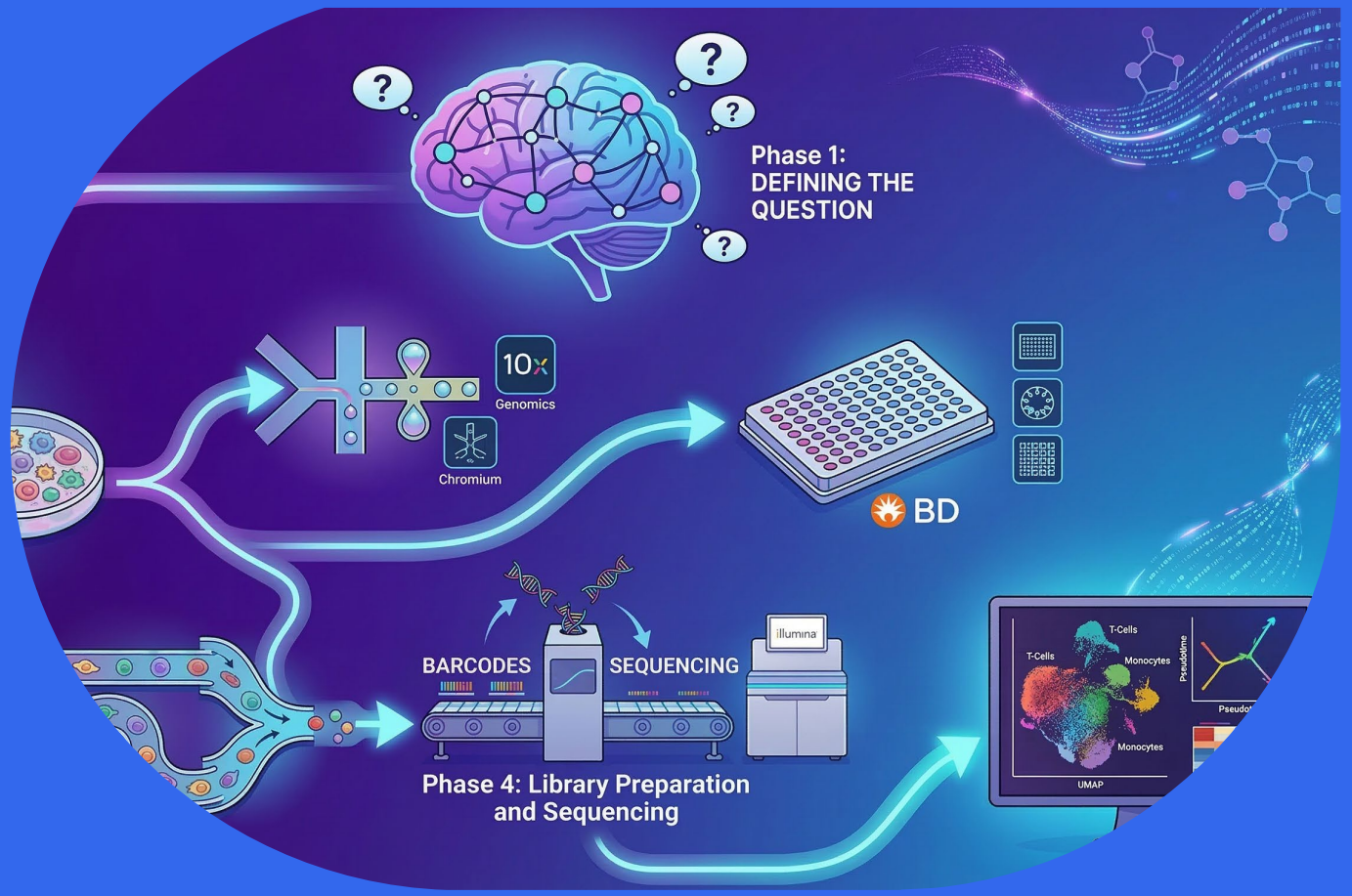




De Simone et al., 2025

Single-Cell Transcriptomics

Experimental Design



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WHAT IS YOUR RESEARCH QUESTION?
Methods
Theory
Hypothesis ?
Scope ?
Literature Review
Data Collection
Methods ?
Curiosity
Impact ?
Methods
Theory
Discovery
Curiosity
AI-Generated by Google Gemini

Some common questions

Which technology?

→Sample type, budget, logistics, throughput

How many replicates?

→More

How many cells?

→What populations are you looking for?

How many reads?

→What genes are you looking for?

Finding the equilibrium

Costs — Data quality

Cell count — Cell viability

Sample number — Sample quality

Most common pitfalls

1) **Complexity too high**

→Increasing quantity usually decreases quality

2) **Technology does not fit sample type**

→Choose wisely, know the parameters

3) **scRNA-seq is not necessary**

→Do you expect different populations?

4) **Pilot experiment skipped**

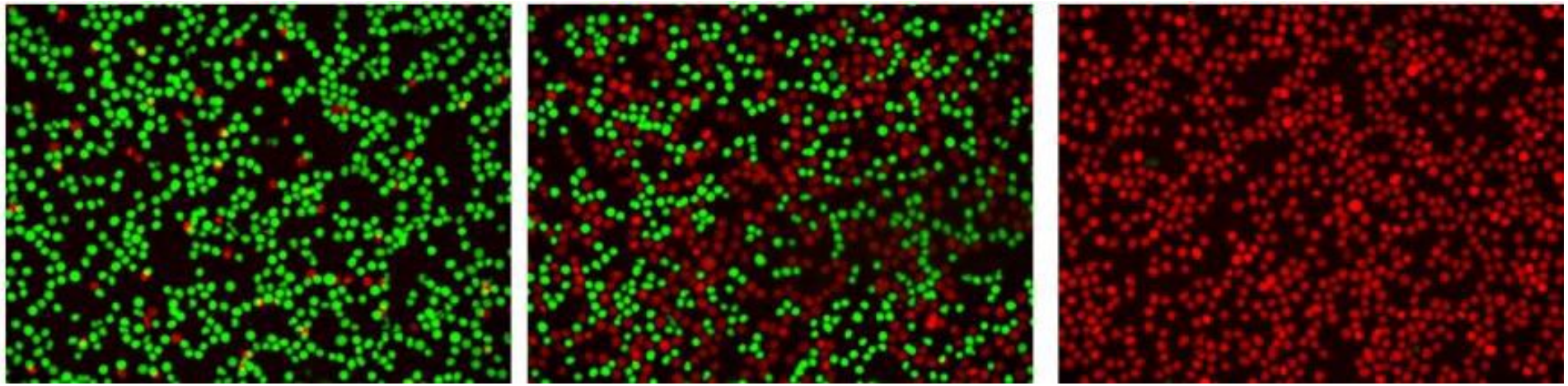
→Don`t!

scRNA-seq requirements

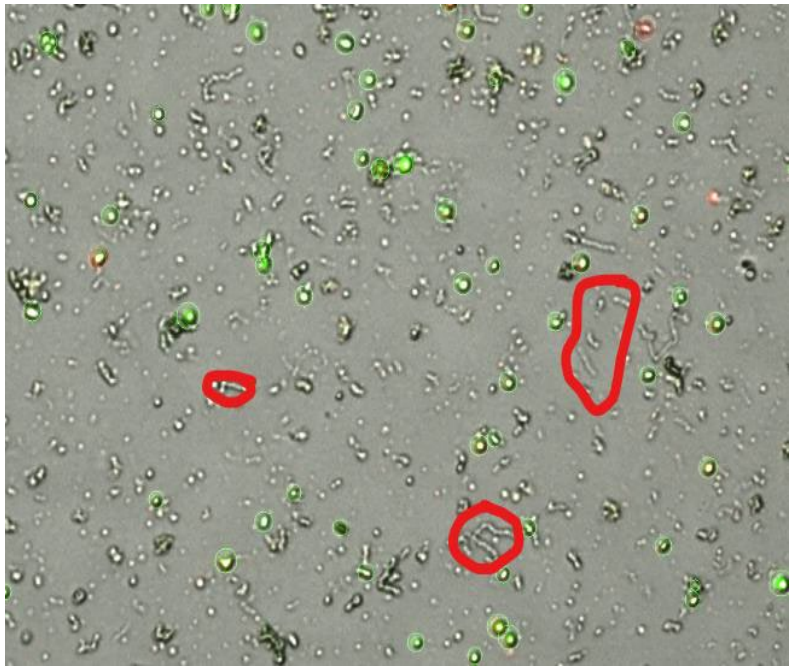
High quality single-cell suspension

- No clumps
- Debris-free
- Sufficient cell number
- High viability

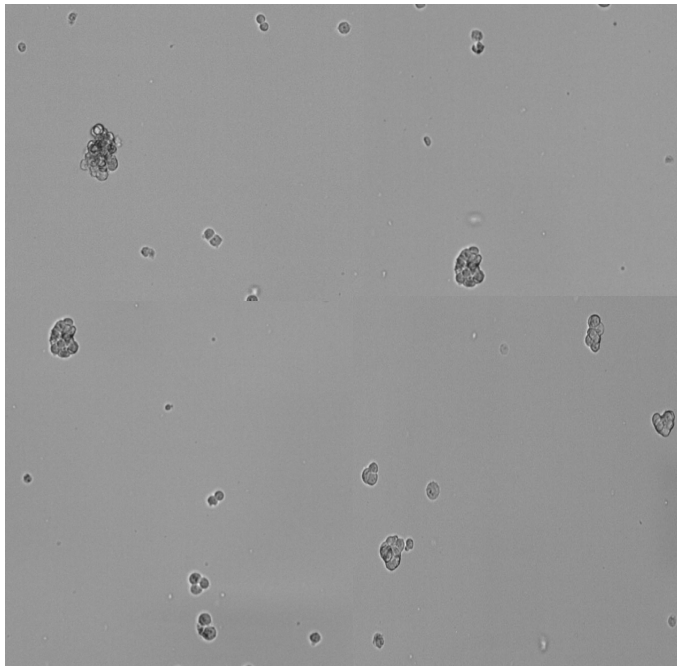
Viability: 90% 50% 0%



scRNA-seq requirements



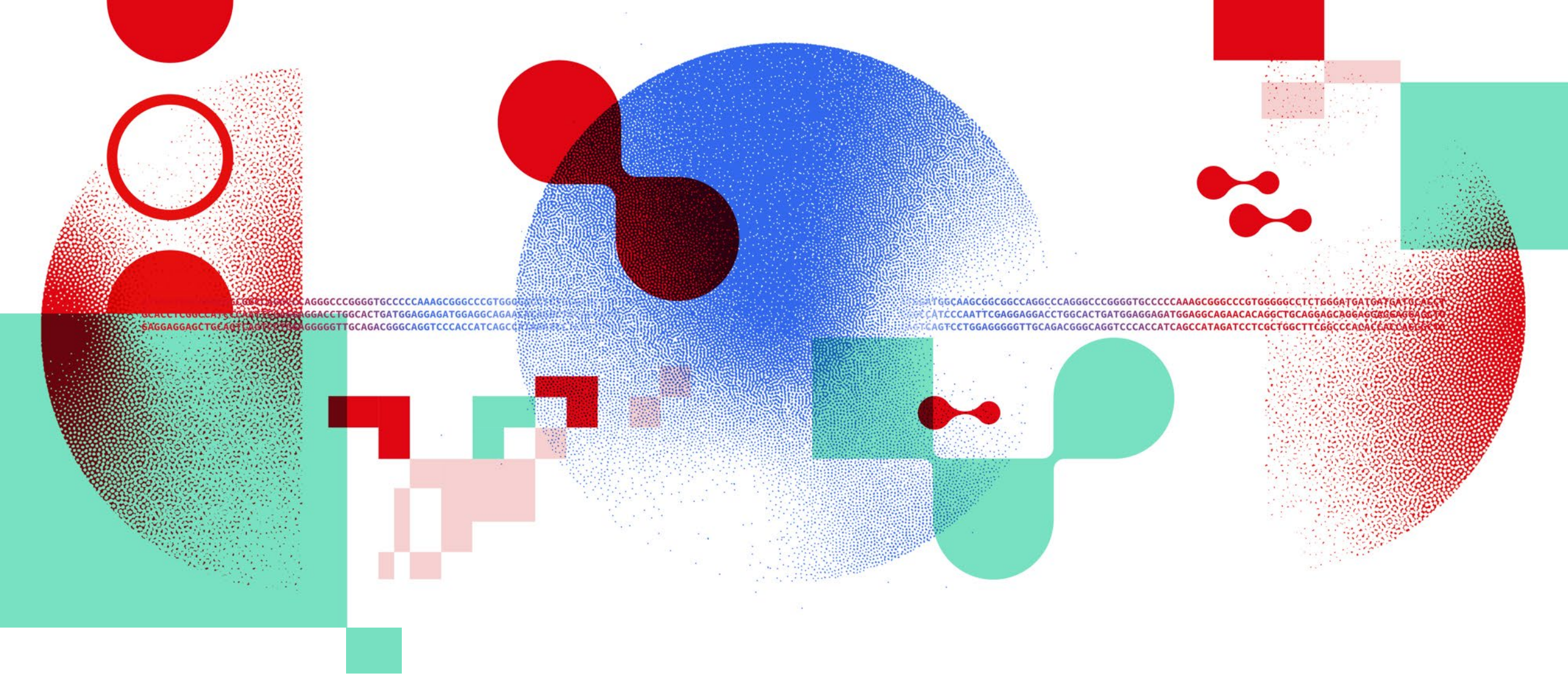
Debris



Clumps

Conclusions

- Sequencing: short and long reads serve **different purposes**
- Most technologies rely on **living cells**
- Many **new technologies** emerging: choose your technology wisely
- Major technology providers: **10x Genomics, BD, Parse**
- Define your **research question** well
- Most crucial parameters: **viability, count, low-debris**



Thank you

DATA SCIENTISTS FOR LIFE

sib.swiss