

# Introduction and Overview of Commercial Single-Cell Platforms

Susan Kloet

Leiden Genome Technology Center (LGTC)

MGC Course on Single-Cell Analysis

29 October 2024

# Why single-cell?

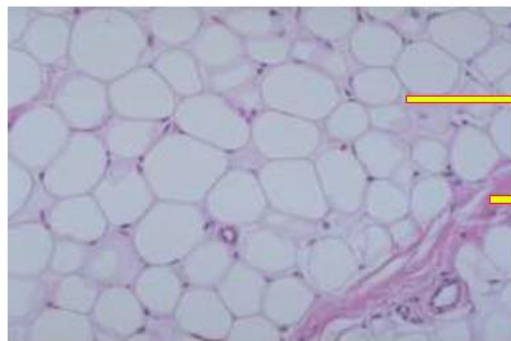
Bulk sample analysis is just like putting a fruit salad into a blender - the taste is an average of all ingredients.



Analyzing single cells is like tasting each individual piece of fruit to gain a much more nuanced understanding of the composition of the fruit salad



# Tissues are heterogeneous



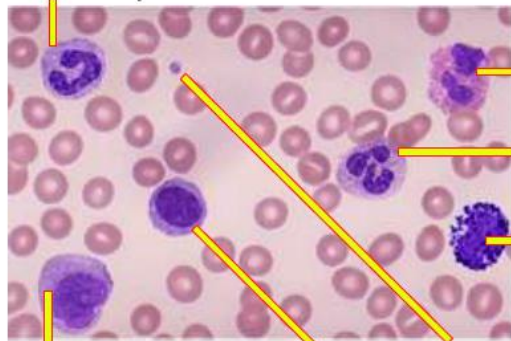
**Adipose**

Fat

Connective  
tissue

Band  
Neutrophil

**Normal Peripheral Blood**



Eosinophil

Segmented  
Neutrophil

Basophil

Monocyte

Platelet  
Lymphocyte

**Small Intestine Mucosa**



Epithelial cells

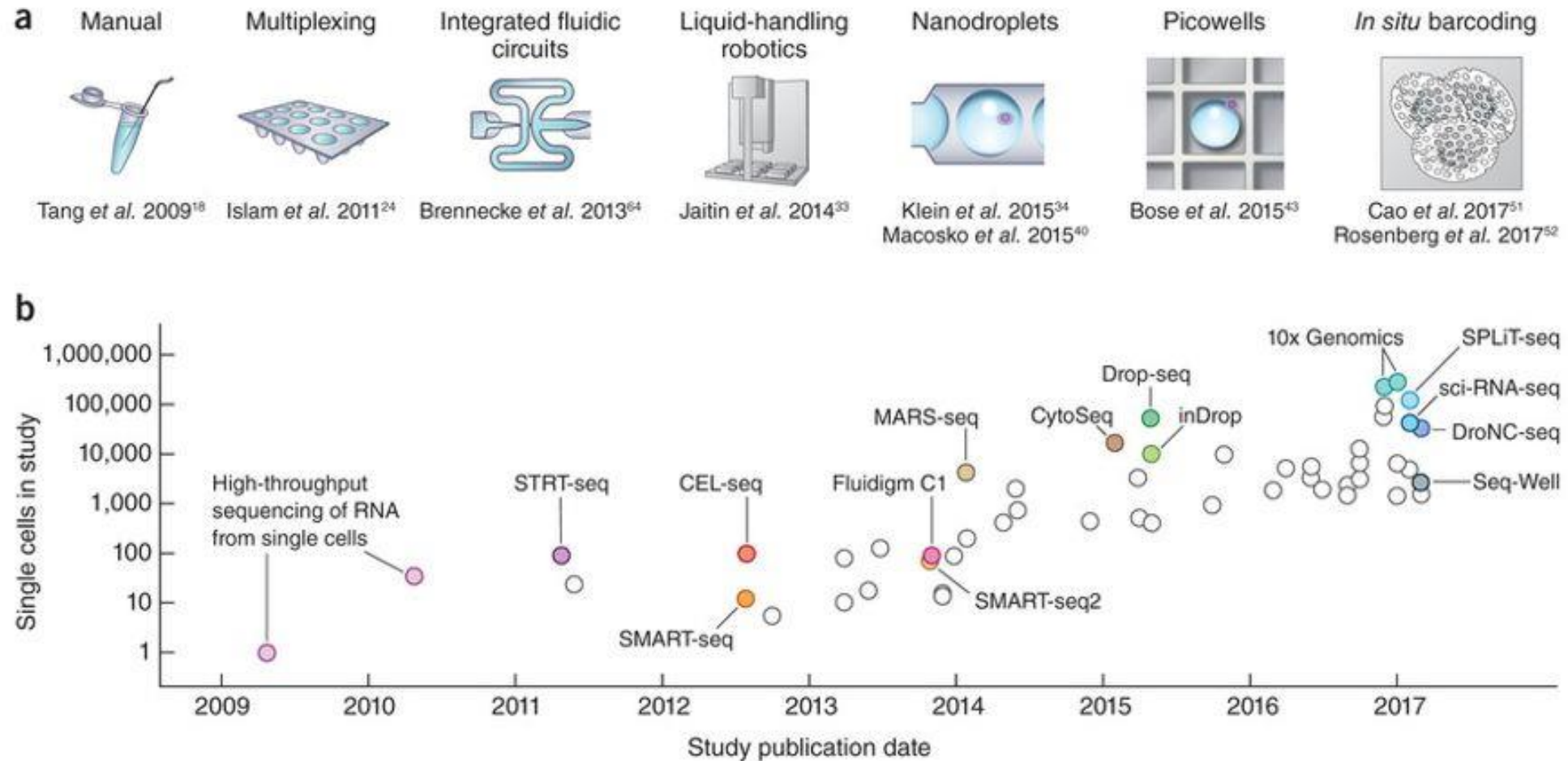
Goblet  
cells

Lamina  
propria

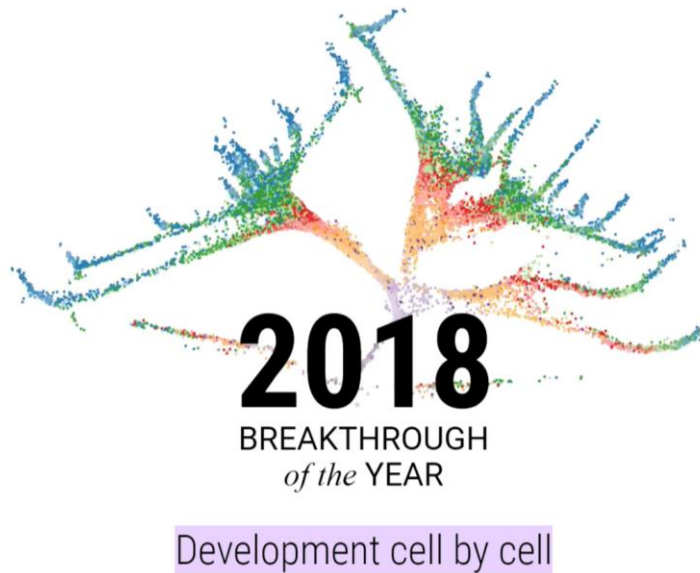
Muscularis mucosa  
(smooth muscle)

Created with figures from [library.med.utah/WebPath/HISTHTML/HISTO.html](http://library.med.utah/WebPath/HISTHTML/HISTO.html)

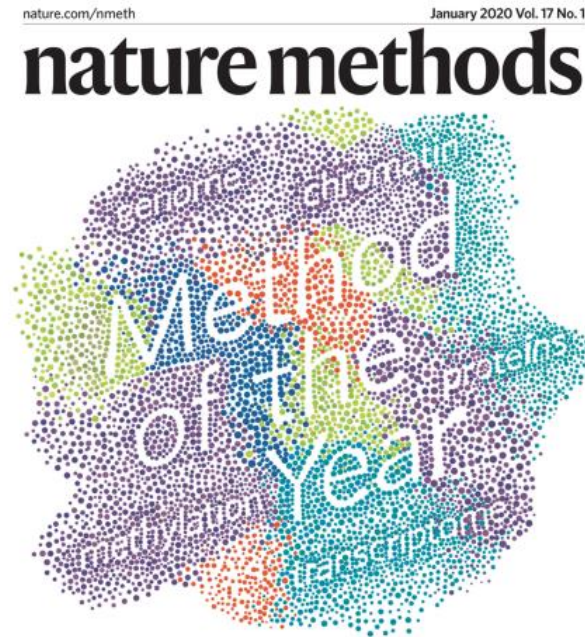
# Exponential scaling of single-cell throughput



# “The single-cell revolution is just starting.”



Science, Vol. 360, p. 367, 27 April 2018



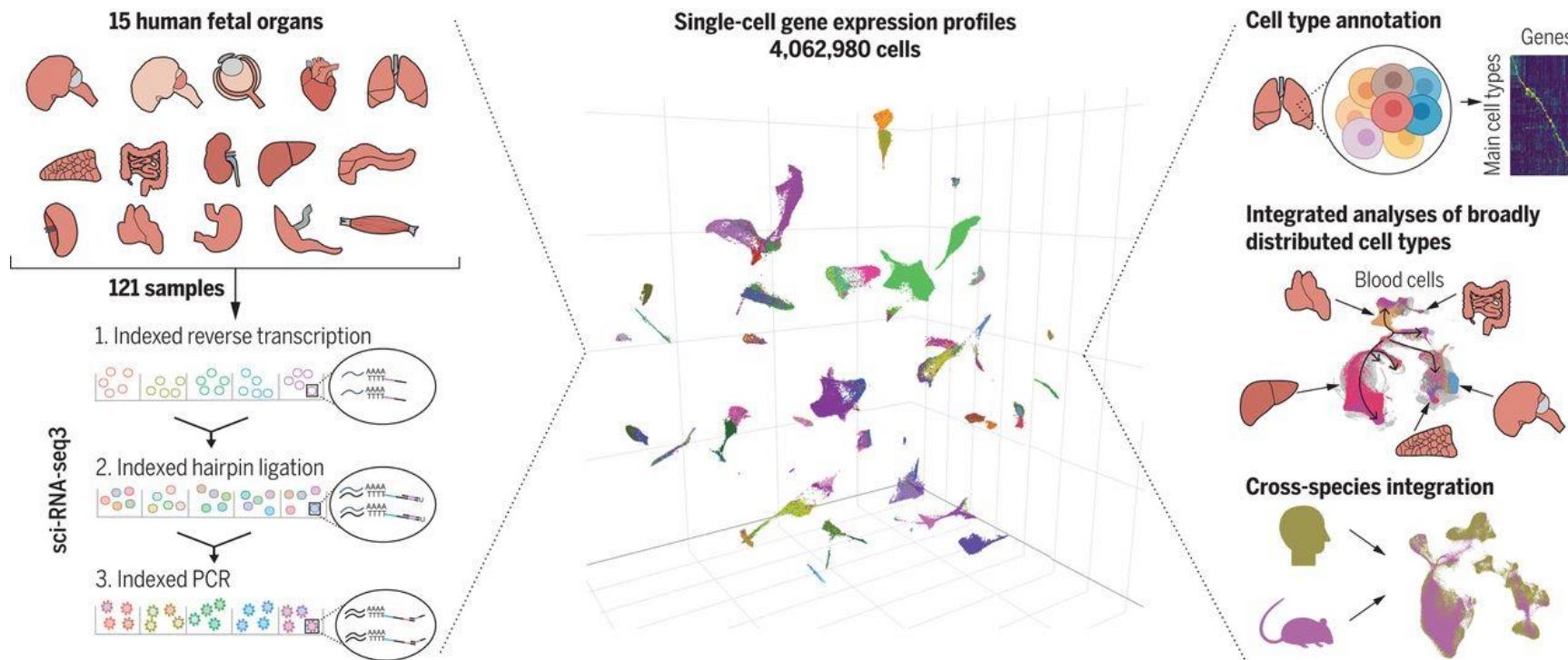
Single-cell multimodal omics  
2019 Method of the Year





# How do we handle all of this data?

Cao et al. A human cell atlas of fetal gene expression. *Science*, 2020 Nov 13;370(6518):eaba7721



4M rows of cells x 20k genes = 80B entries!

# We will cover

- Description of single-cell and spatial assays/platforms/protocols
- Sample prep and experimental design concerns
- Gene and cell filtering
- Normalization
- Dimensionality reduction
- Clustering and cell annotation
- Data integration
- Differential expression
- Trajectory inference

# Participation time!

- Who has (or will soon generate) single-cell RNA data?
- Who has (or will soon generate) single-cell DNA data?
- Who has (or will soon generate) single-cell protein data?
- Who has (or will soon generate) spatial data?
- Anybody working with all of the above?



# In this lecture we will cover:

- Commercial methods for single-cell DNA-seq
- Commercial methods for single-cell RNA-seq
- How 10x Genomics technology works
- What 10x Genomics sequencing libraries look like

# scDNA-seq

WG(B)S (whole genome (bisulfite) sequencing)

DIY methods – Miao's multiomics lecture

Commercial methods – BioScrib, Scale

Chromatin structure and accessibility

ATAC-seq, ChIP-seq, Hi-C, etc.

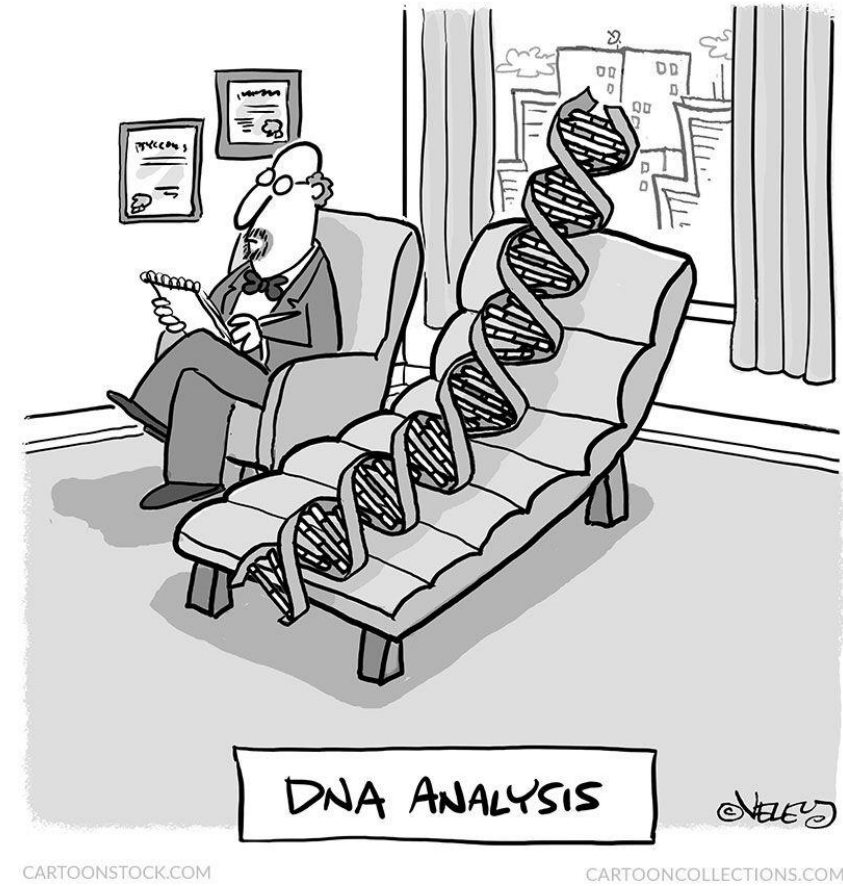
DIY methods – Miao's multiomics lecture

Commercial methods – 10x Genomics (end of this lecture)

Copy number variation (CNV) and single nucleotide variation (SNV)

DIY methods – Miao's multiomics lecture

Commercial methods – Mission Bio



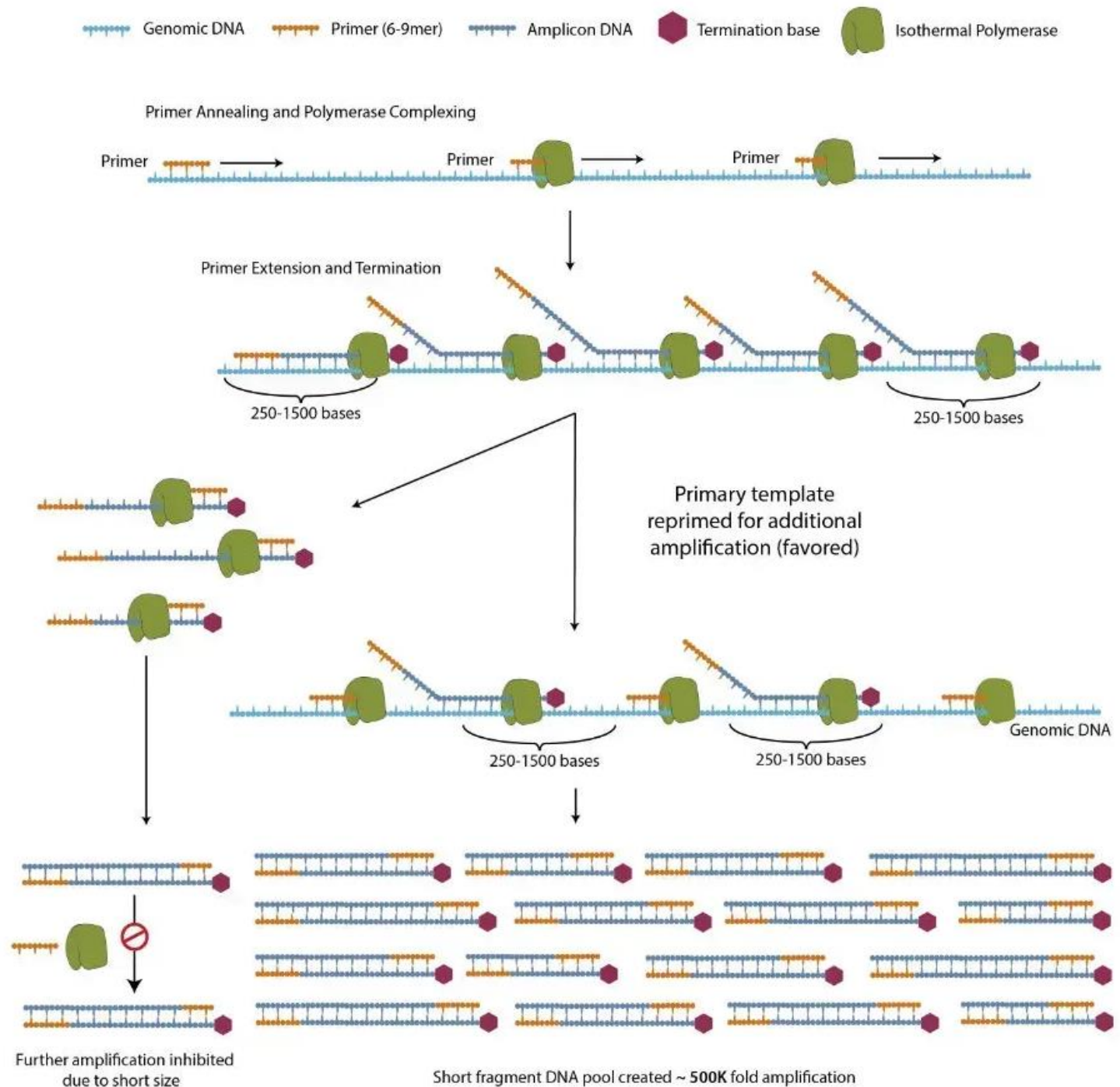
CARTOONSTOCK.COM

CARTOONCOLLECTIONS.COM

# BioSkryb

No instrument required!

WGS with PTA  
(primary template-directed  
amplification)



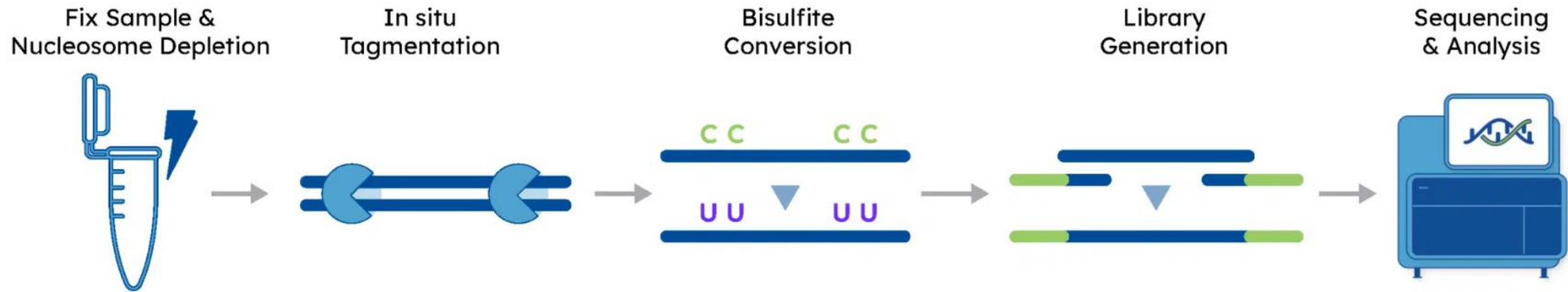
# Scale Biosciences



NEW single-cell methylation kit

Up to 18k cells / run

Capture-compatible



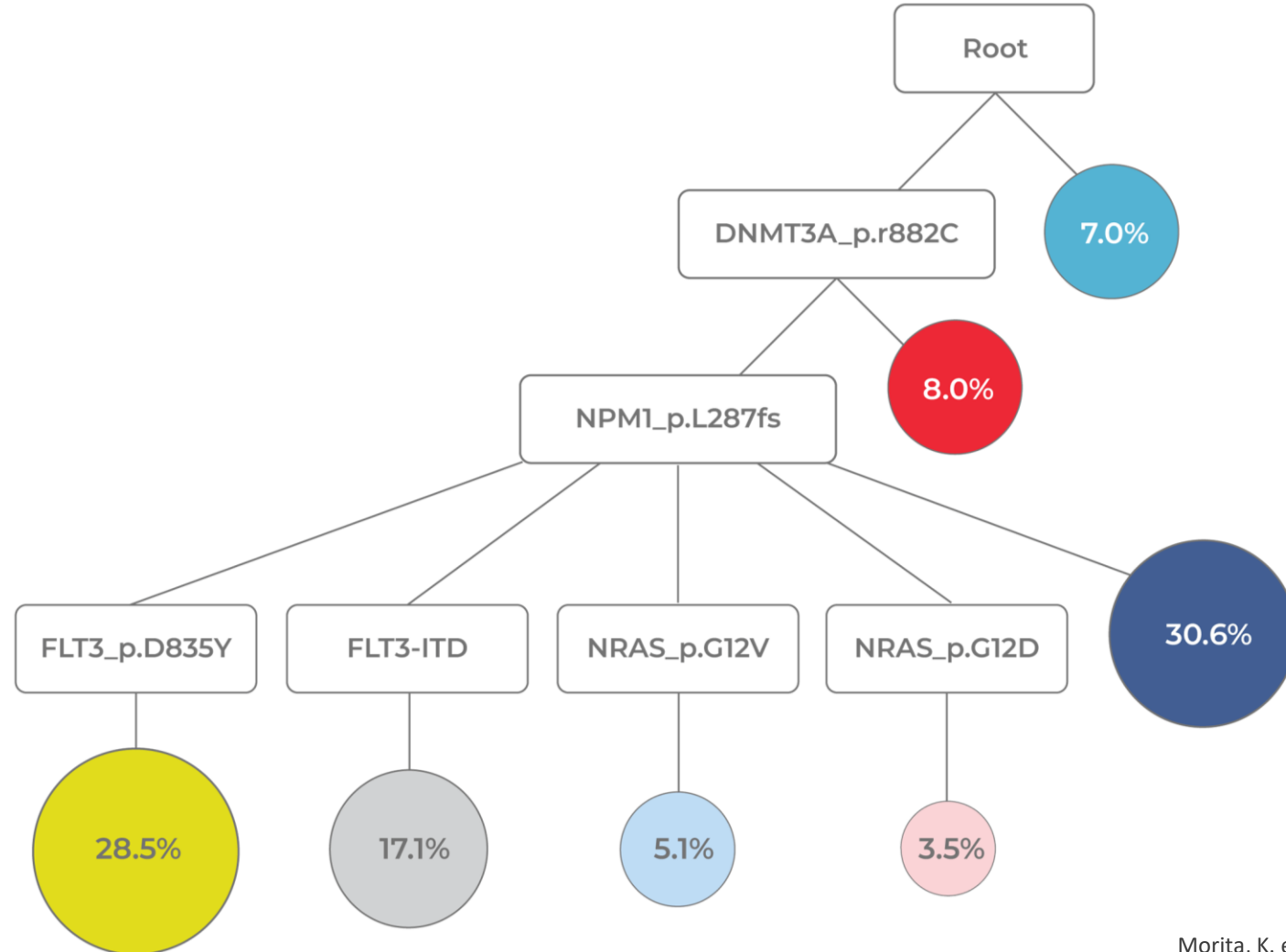
# CNV and SNV detection – Mission Bio

## Tapestri platform

- Droplet-based amplification
- Up to 10k cells
- Panel-based PCR (up to 400 targets)
- Can call both CNVs and SNVs in target regions
- NEW multiomics application: Protein + DNA

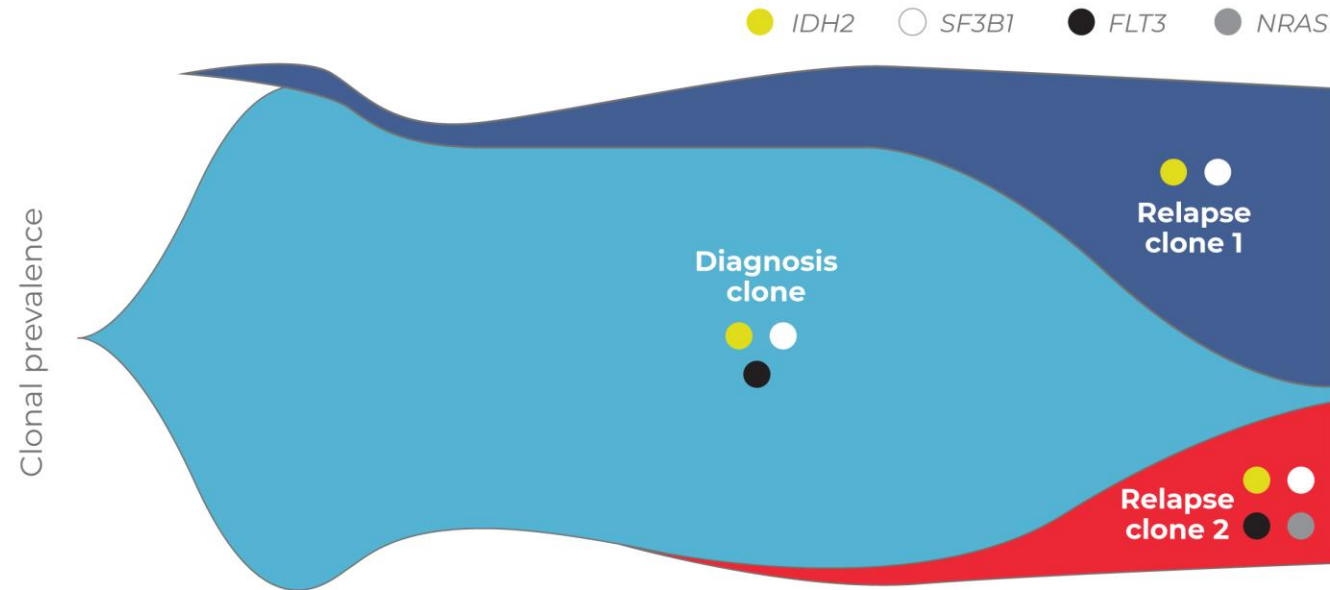


# Track clonal evolution (AML)

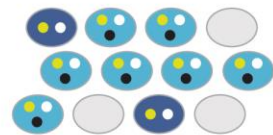




# Reveal therapy resistance

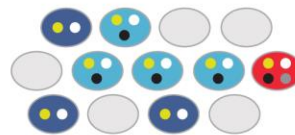


## Diagnosis



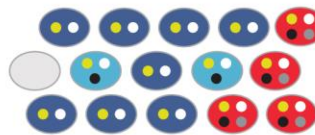
The tumor contains 2 clones. The light blue clone is dominant.

## During Therapy



The therapy targets only the light blue clone. The red clone emerges given a new mutation.

## Relapse

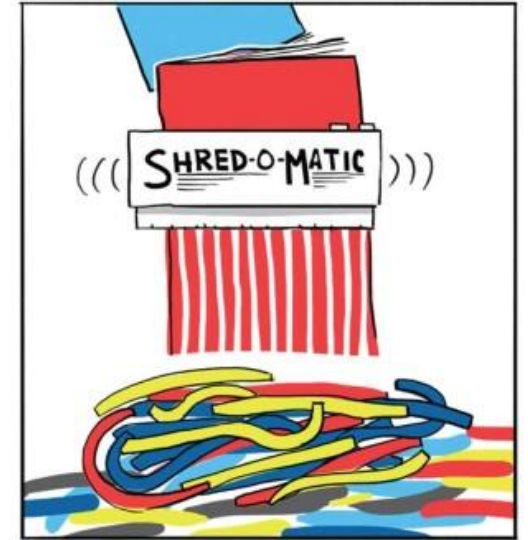


Post-treatment, the red clone & dark blue clone drive relapse.

# scRNA-seq

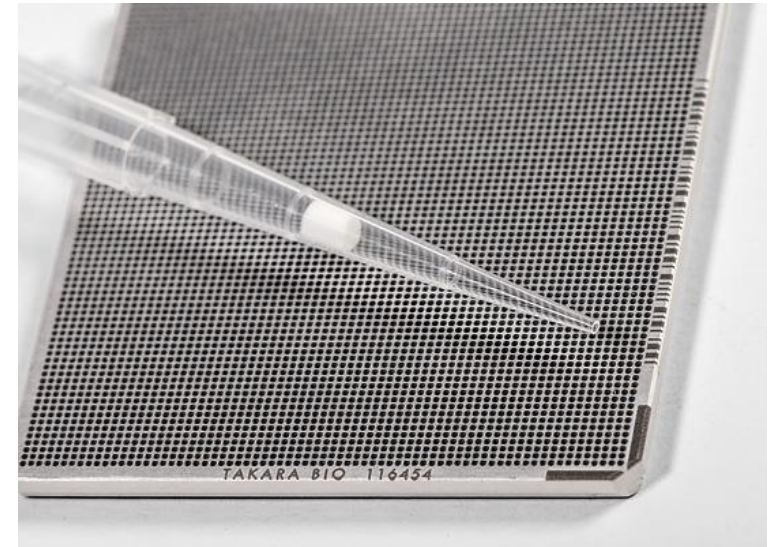
## **MANY** different assays

- Some commercial, some DIY
  - Full transcriptome vs 3' vs 5'
  - Automation varies
  - Throughput varies
  - Cost varies
- 
- Microwell-based
  - Plate-based
  - Droplet-based



# iCELL8 cx

- Available at ErasmusMC
- Uses 5184 nanowell chip, ~1800 cells loaded
- Compatible with immunofluorescence
- Protocols for single-cell
  - SMART-Seq full-length transcriptome analysis
  - Differential expression by 3' end counting
  - TCR profiling and 5' end differential expression
  - ATAC-seq



# BD Rhapsody

- Works with targeted panels to reduce sequencing costs
  - Immune response human/mouse
  - T-cell
  - Oncology – breast cancer
  - Custom panel add-ons
- Up to 400 amplicons / sample
- Includes UMIs to reduce PCR amplification bias
- Increased flexibility
  - Archiving up to 3 months
  - Sub-sampling

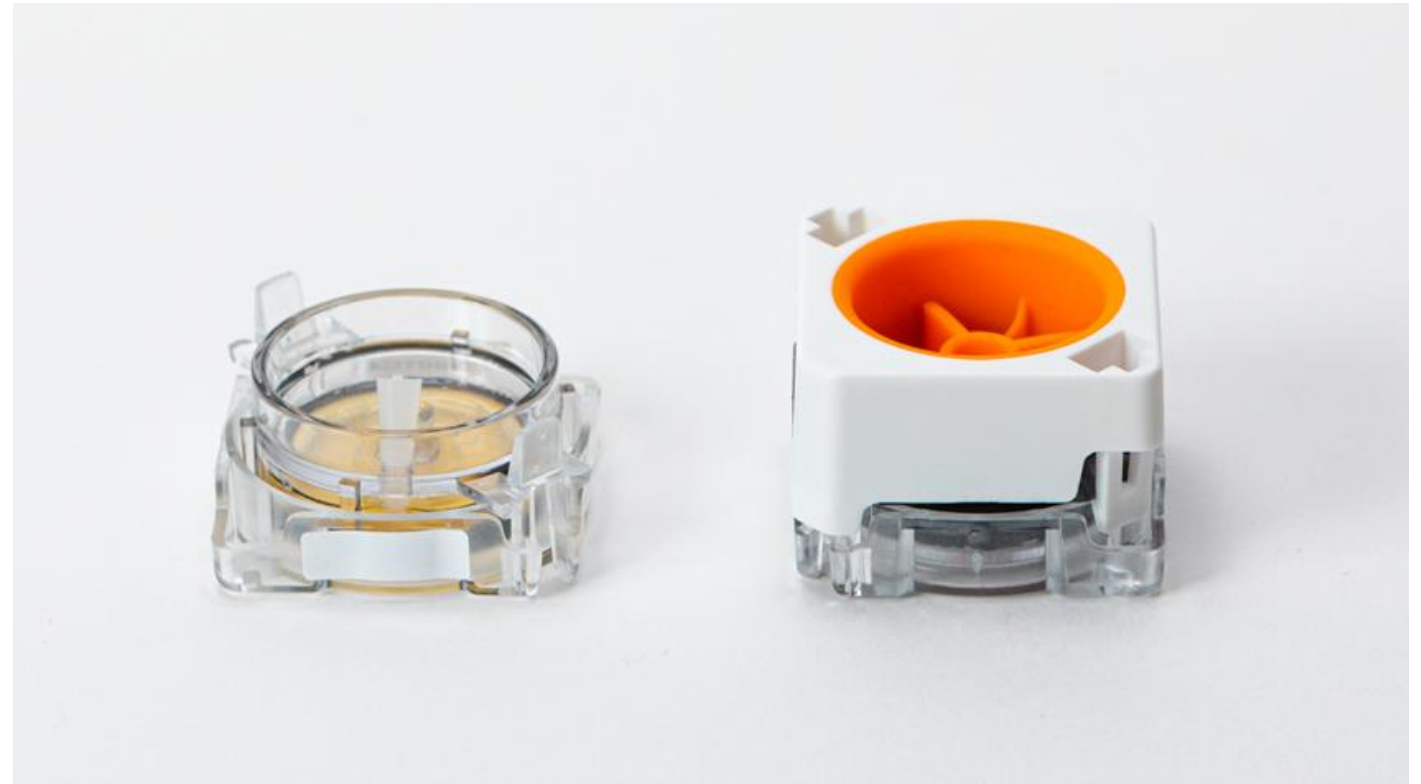
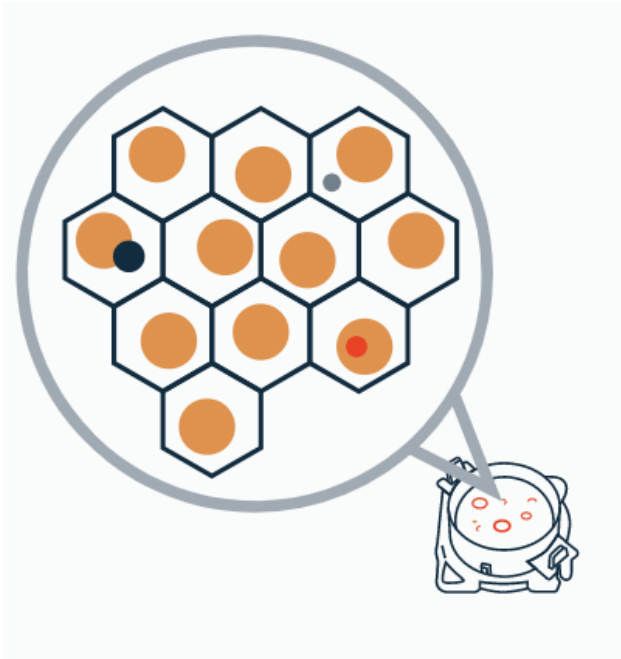


# Honeycomb

The HIVE - portable, single-use microwell chip  
Store samples up to 9 months



**HONEYCOMB**





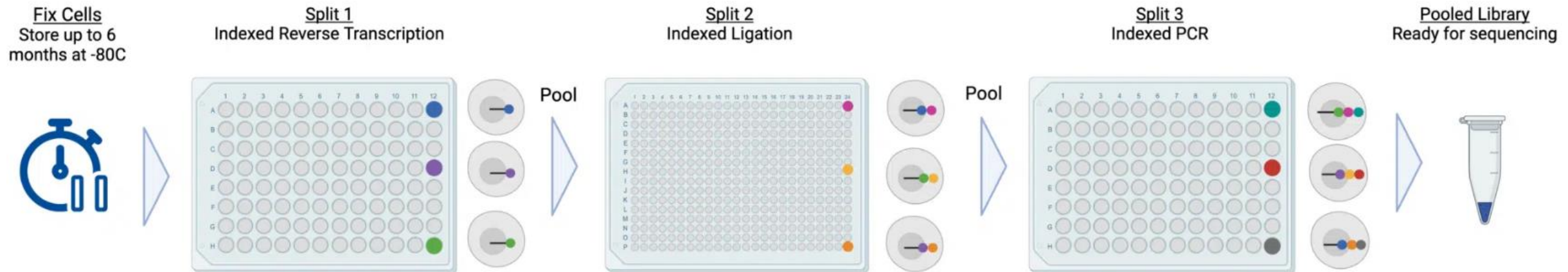
# Scale Biosciences



No instrument required!

Based on sci-RNA-seq

Fix and store up to 6 months





# Parse Biosciences

No instrument required!

Based on SPLiT-seq

Fix and store up to 6 months



## Unlock Single Cell at Scale

Perform scRNA-Seq on up to 1 million cells with a single kit accommodating up to 96 different biological samples or experimental conditions.

### Evercode WT Mega

1 million Cells

### Evercode WT

100,000 Cells

### Evercode WT Mini

10,000 Cells

#### High Throughput scRNA-Seq Achieved

Start pursuing uncompromising science with up to a million cells.

# Dolomite Bio

Nadia Instrument and Nadia Innovate  
Commercialized Drop-seq

scRNA-seq

Plant protoplast RNA-seq

snATAC-seq

Protocol development

Agarose droplet formation

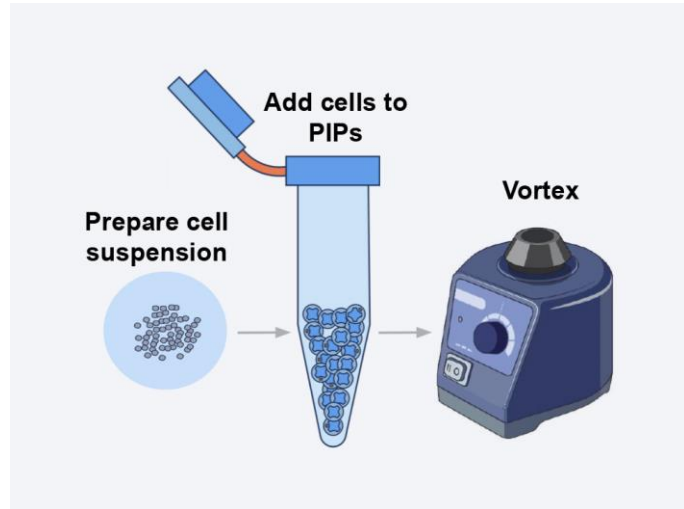


# Fluent Biosciences

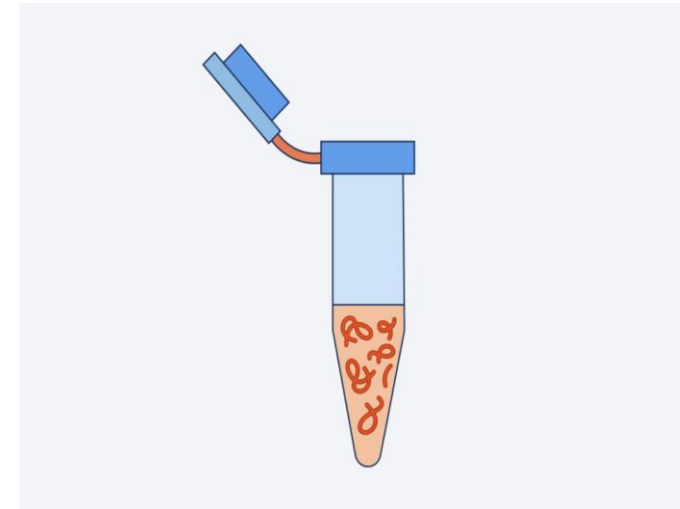


No instrument required!

Based on PIP-seq (particle-templated instant partition)



~5 min for encapsulation  
Thermocycler does the rest

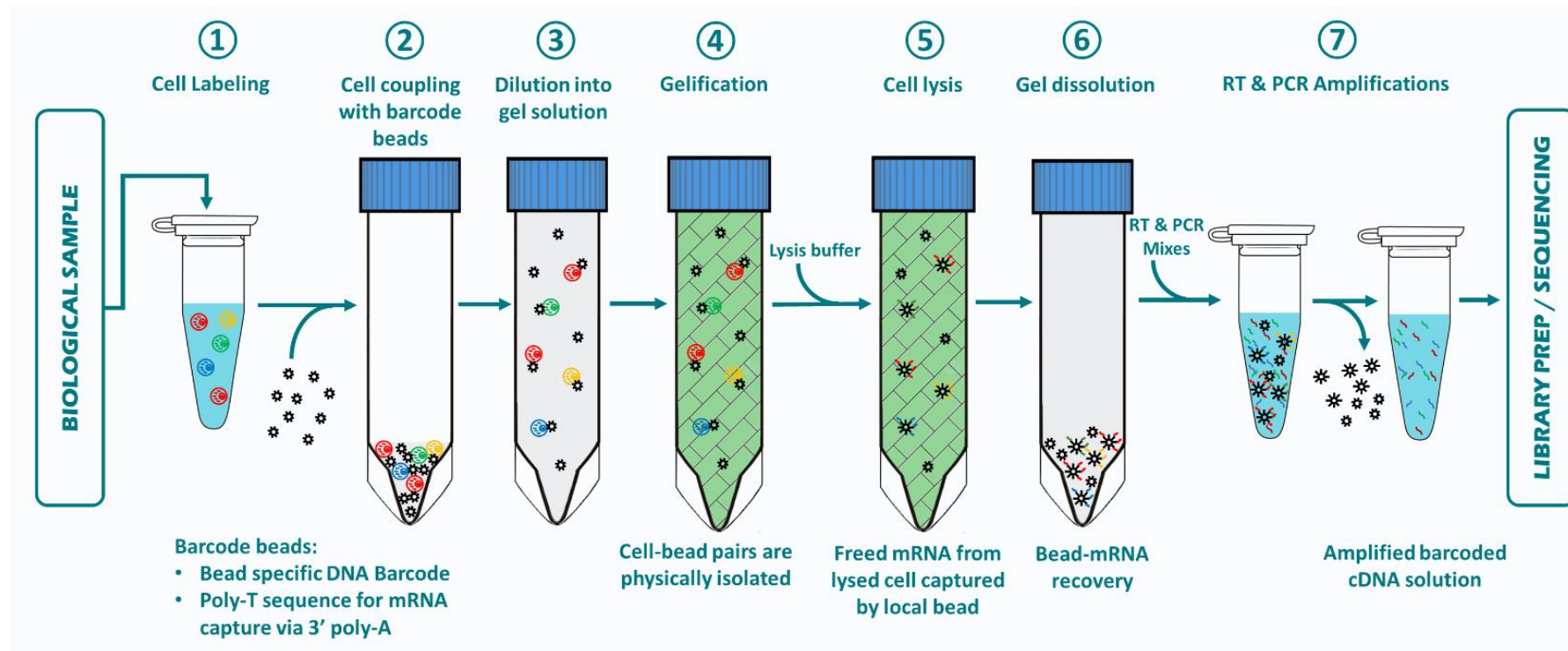
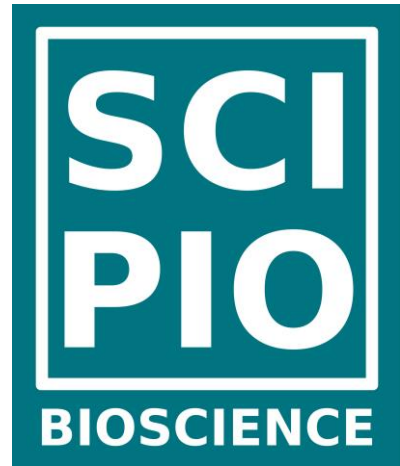


1 tube library prep based on  
standard Illumina workflows

# Scipio Bioscience

No instrument required!

Based on hydrogels for reaction compartmentalization



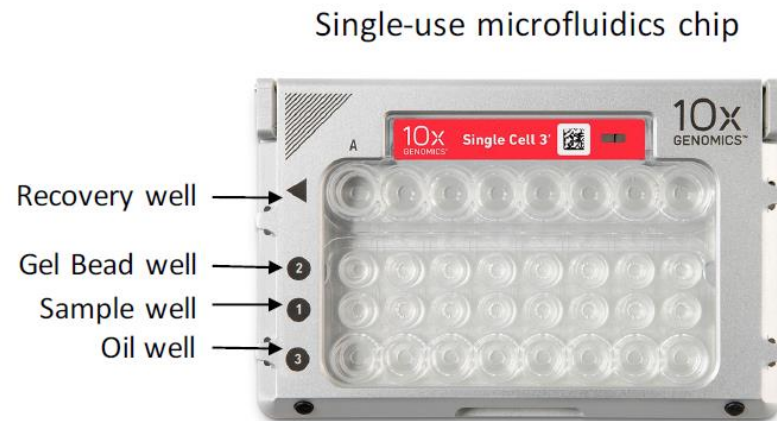
# 10x Genomics Chromium Controller

Commercial launch early 2016

Microfluidics system for reaction compartmentalization

High throughput, up to 80k cells/run

High capture rate, ~50%



# 10x Genomics single-cell products

## Chromium

### Single Cell Gene Expression

3' gene expression profiling at scale with single cell resolution.

### Single Cell Gene Expression Flex

Fixed RNA Profiling assay for comprehensive probe-based gene expression profiling with single cell resolution.

### Single Cell ATAC

Chromatin accessibility profiling at the single cell level.

### Single Cell Immune Profiling

5' gene expression alongside V(D)J repertoire profiling and antigen specificity of T and B cells.

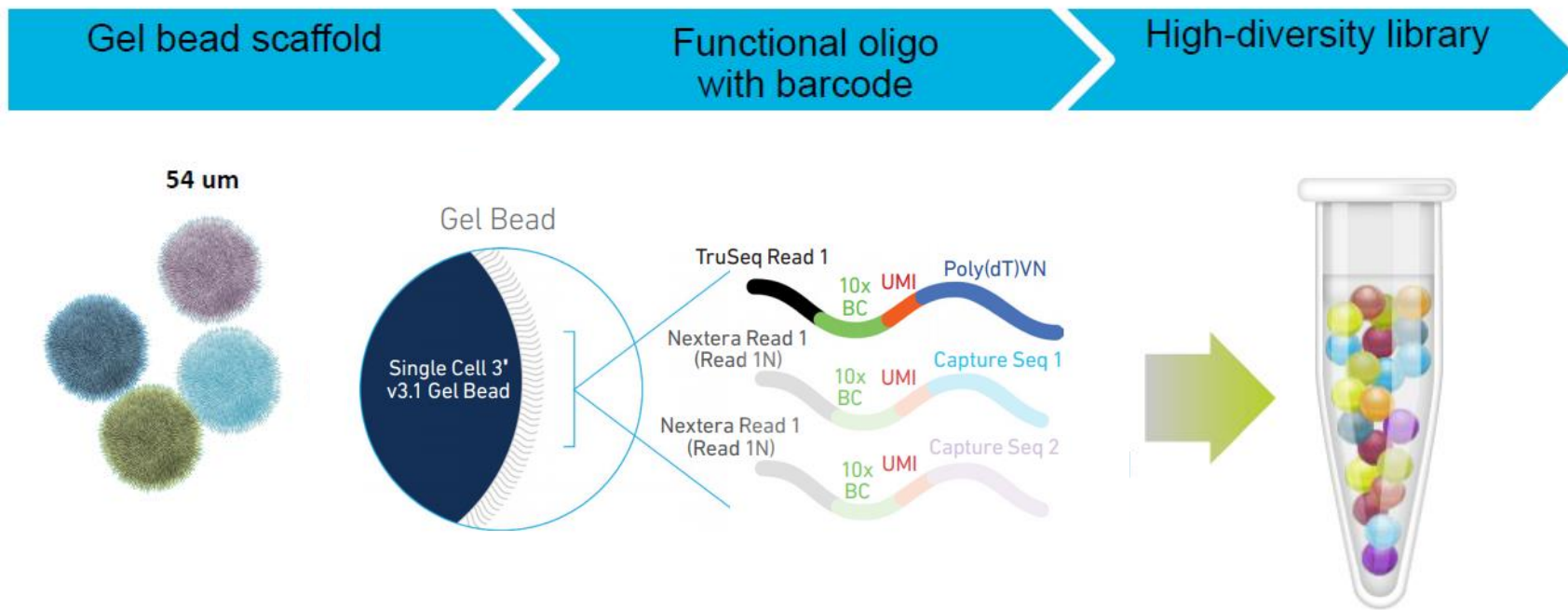
### Single Cell Multiome ATAC + Gene Expression

Combined profiling of 3' gene expression and chromatin accessibility from the same cell.



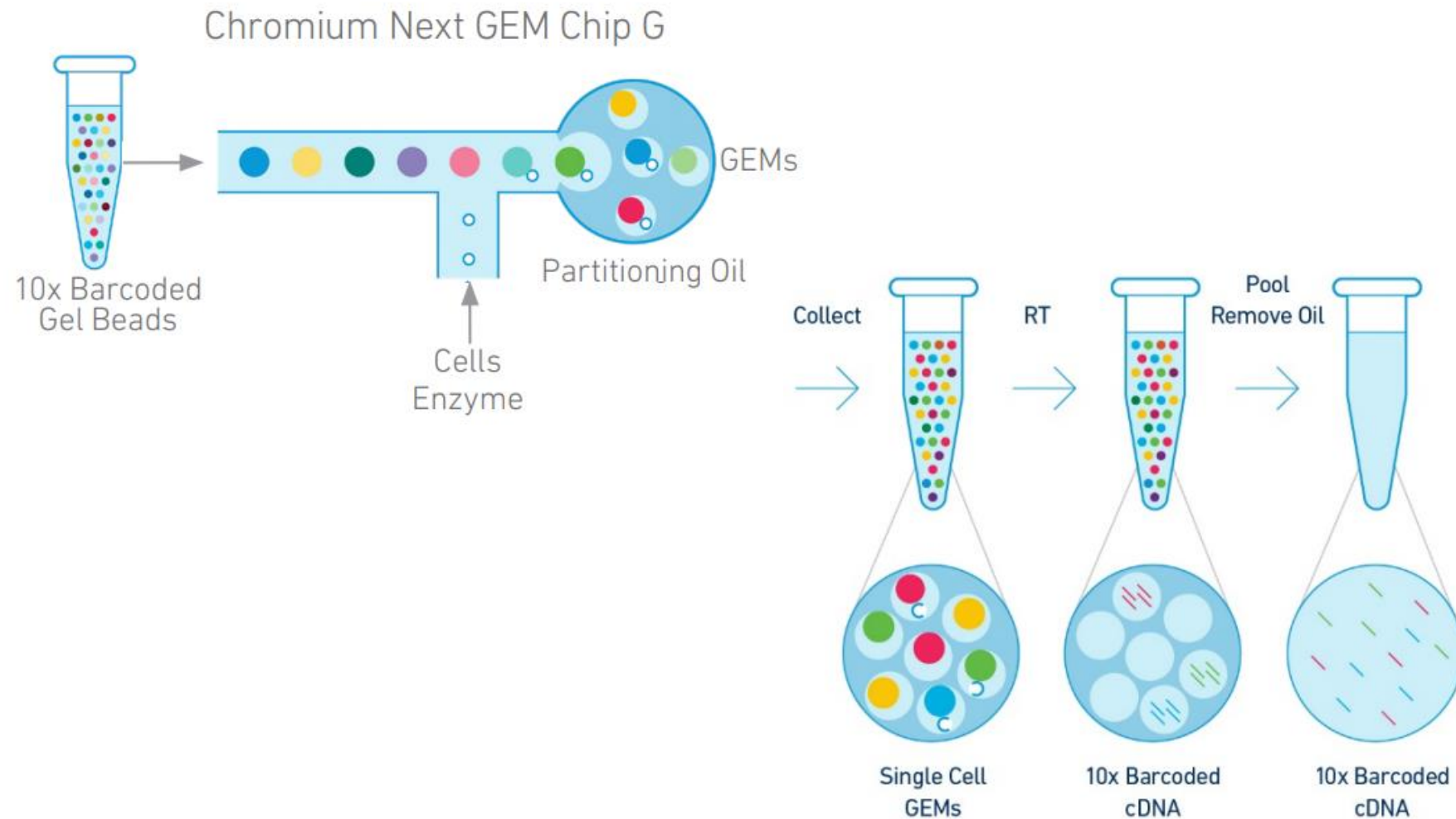
# Gel beads up close

4M Discrete Reagents in One Tube

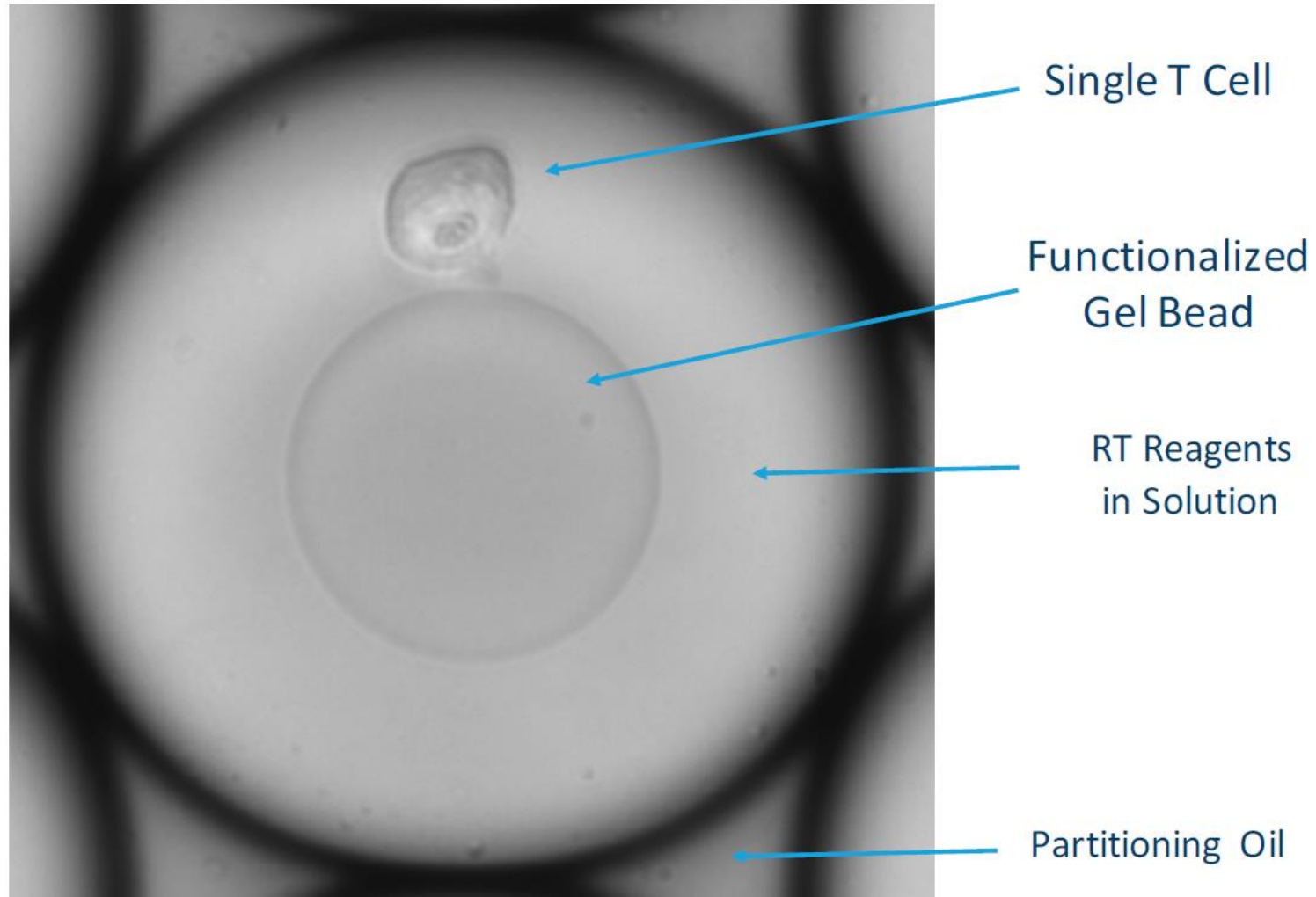


**10x Barcode (16bp): unique for each GemBead**  
**+ UMI (12bp): correct for PCR duplicates**

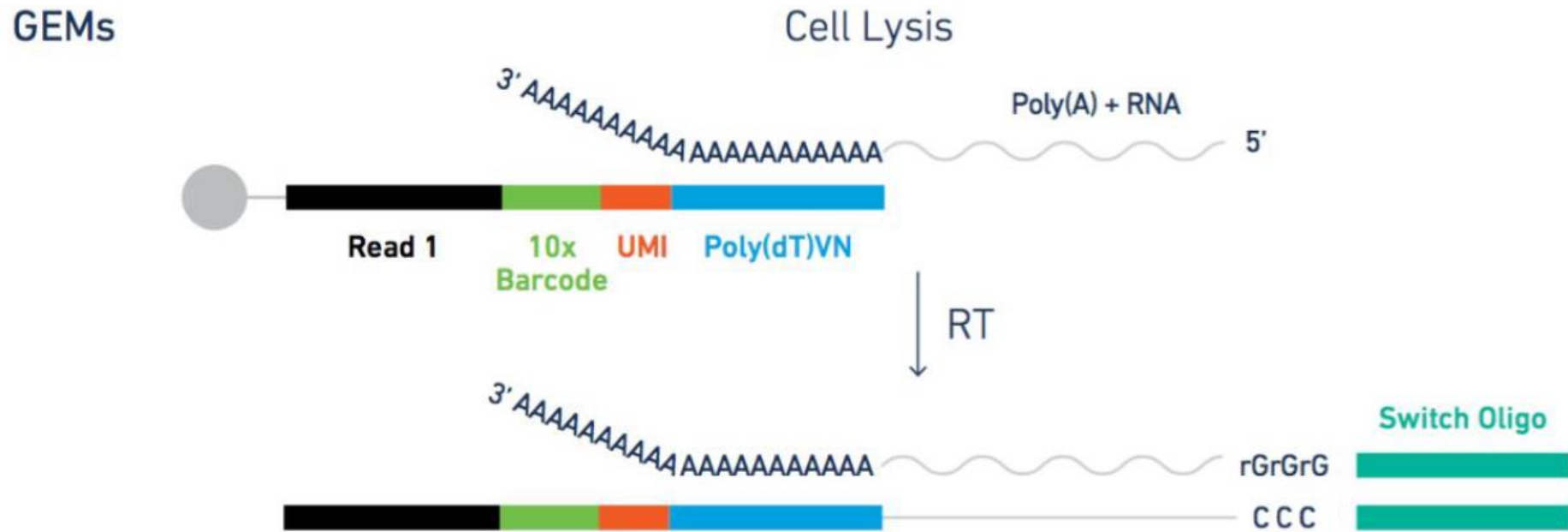
# Gel bead in Emulsion (GEM) technology



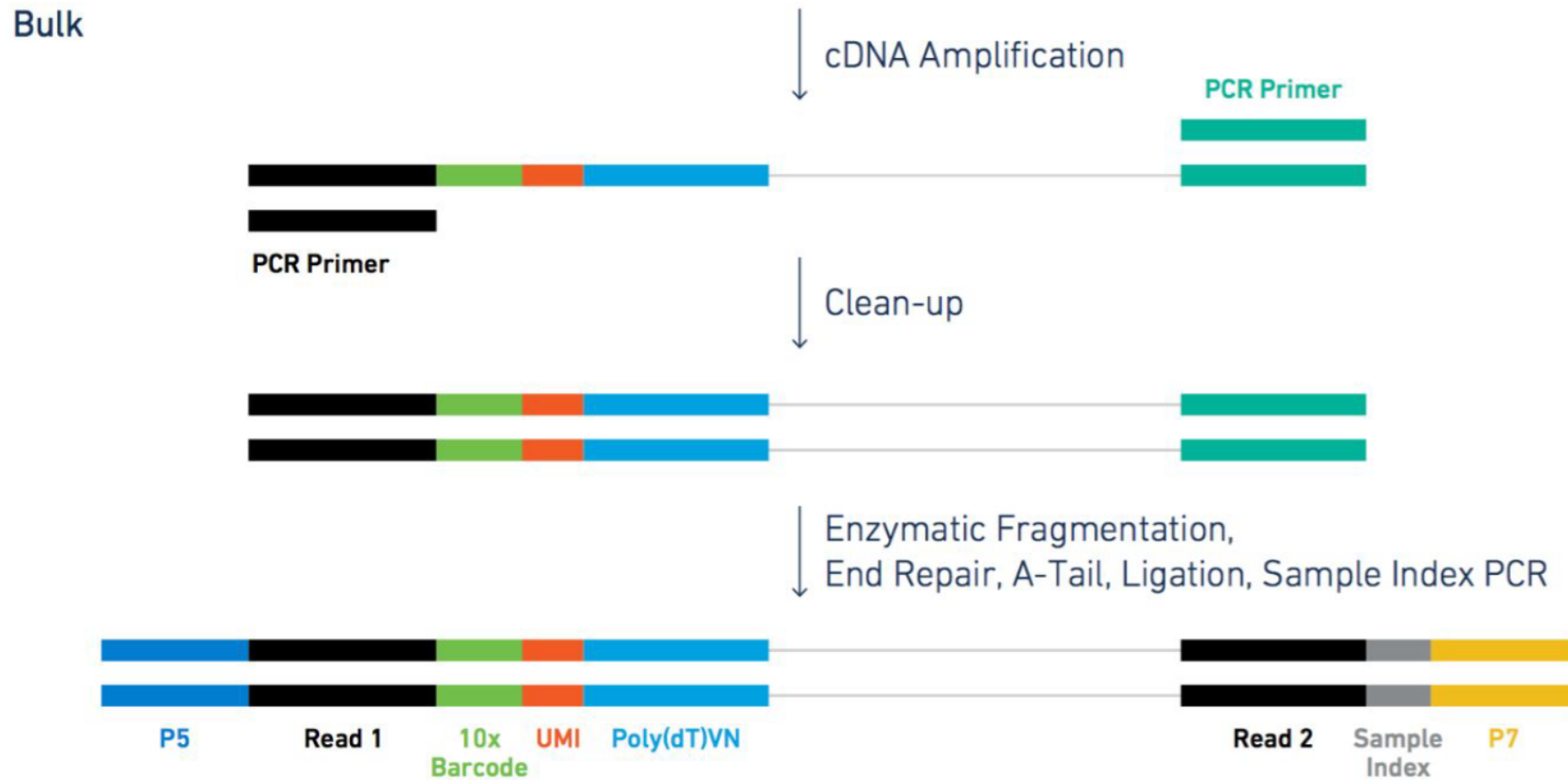
# GEMs up close



# Assay scheme for 3' mRNA sequencing

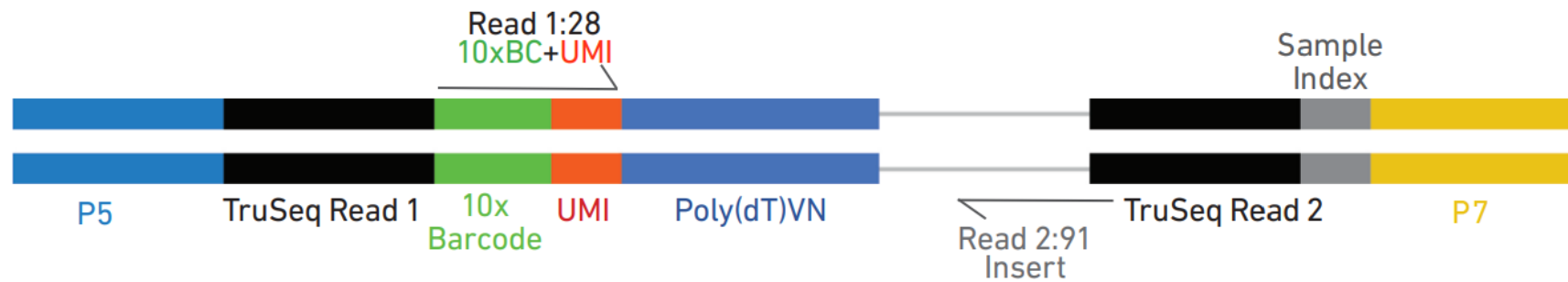


# Assay scheme for 3' mRNA sequencing



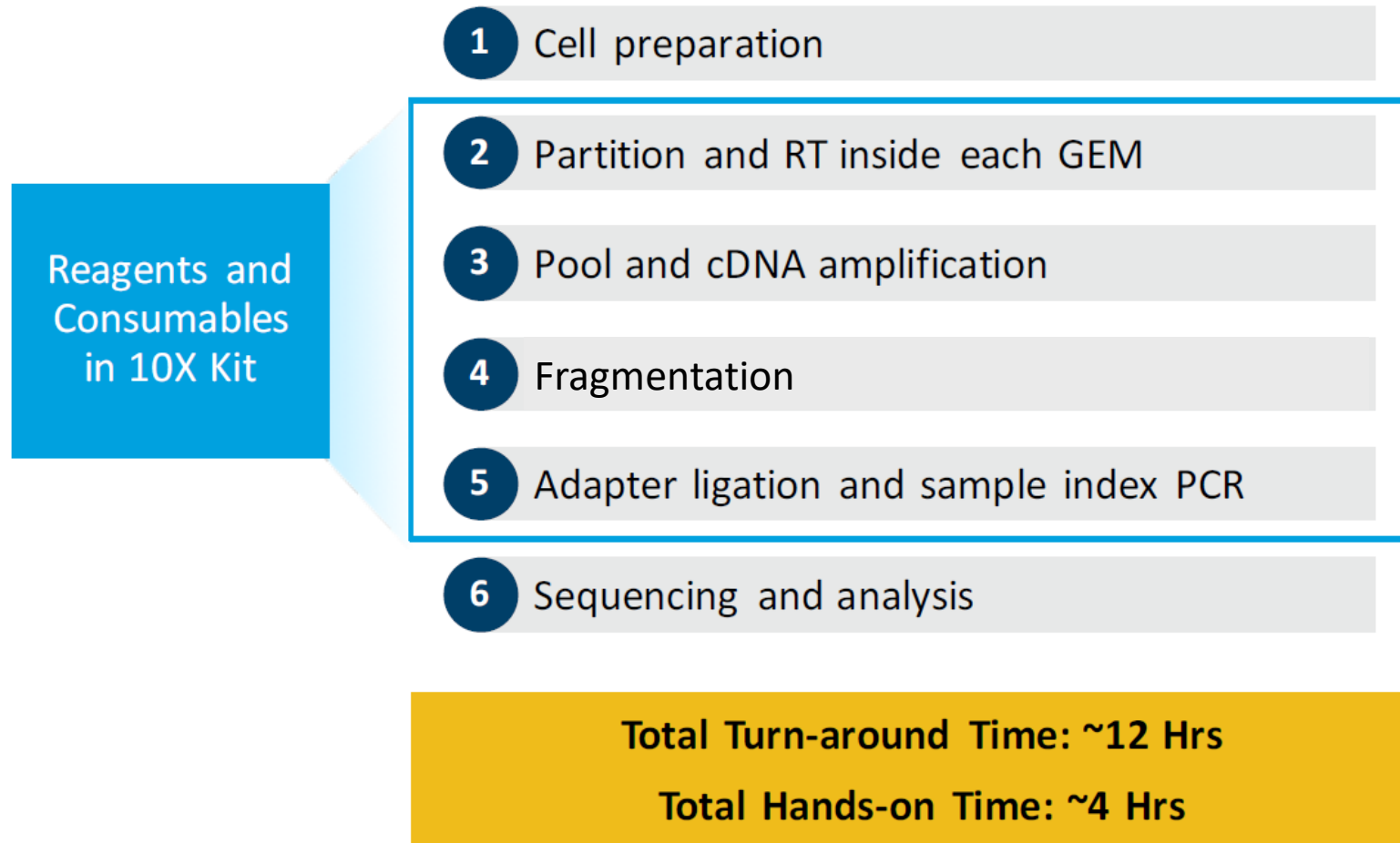
# Final library structure

## Chromium Single Cell 3' Gene Expression Library

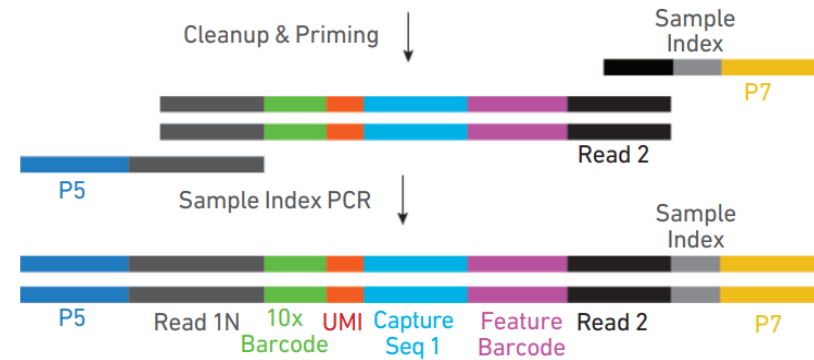
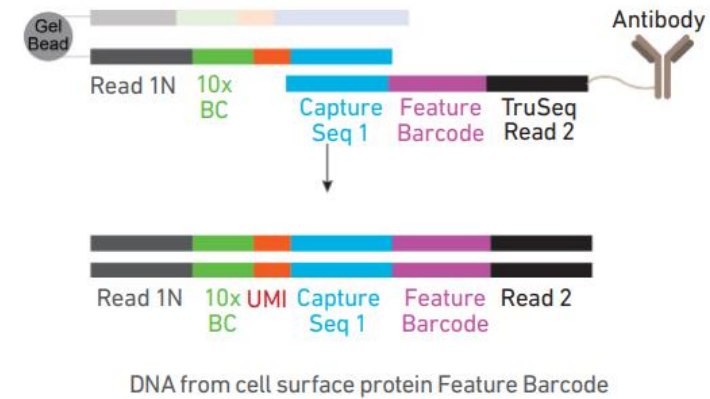
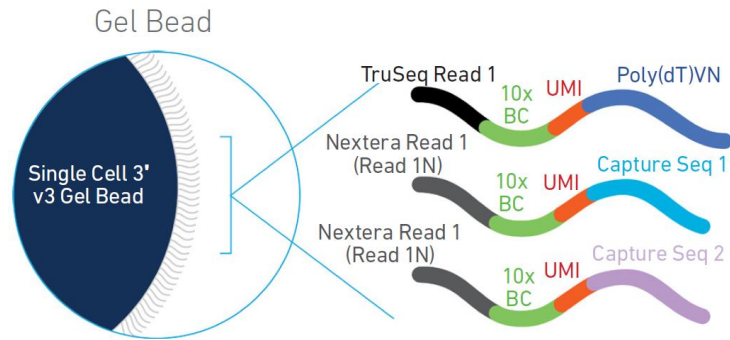




# Single cell 3' end-to-end workflow



# Single cell 3' feature barcoding



# Structure of T and B cell receptors

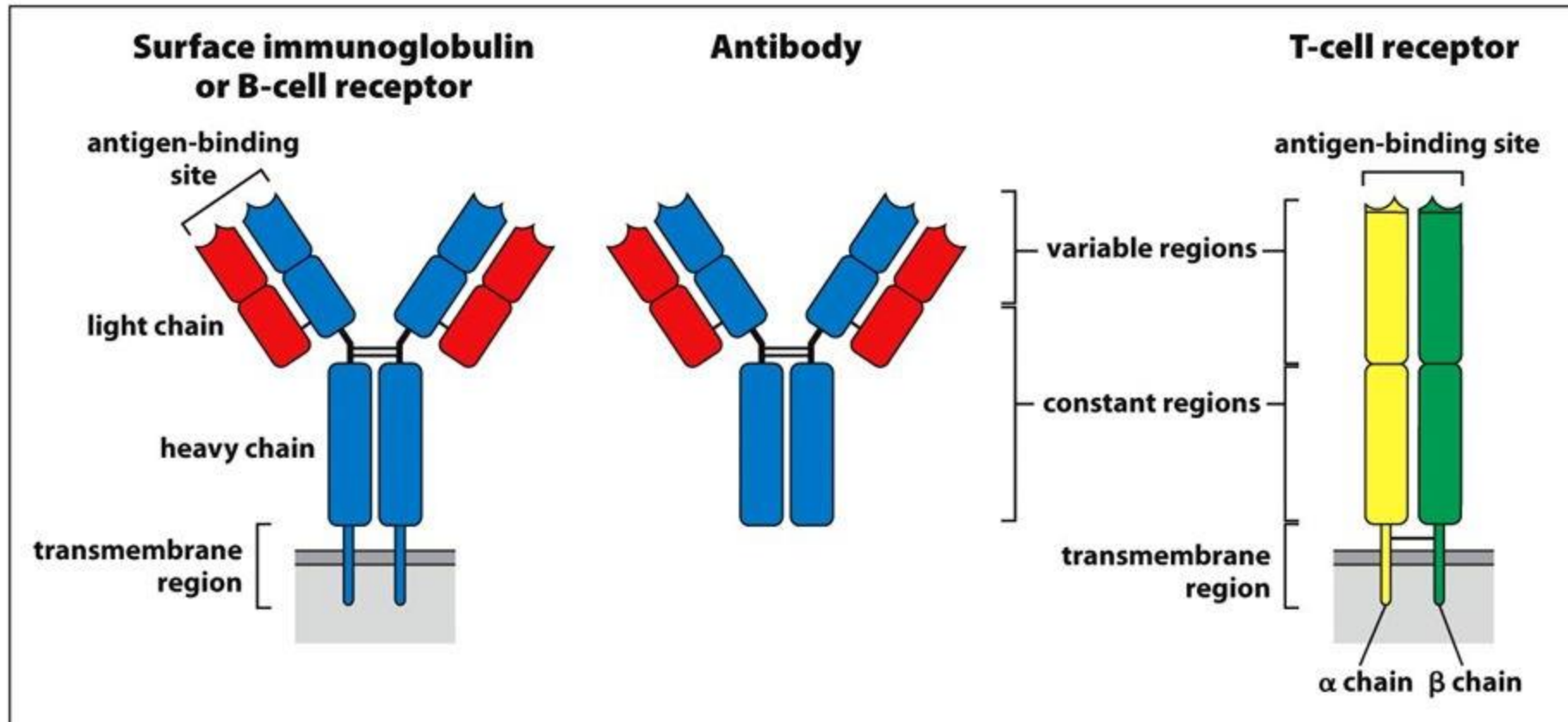
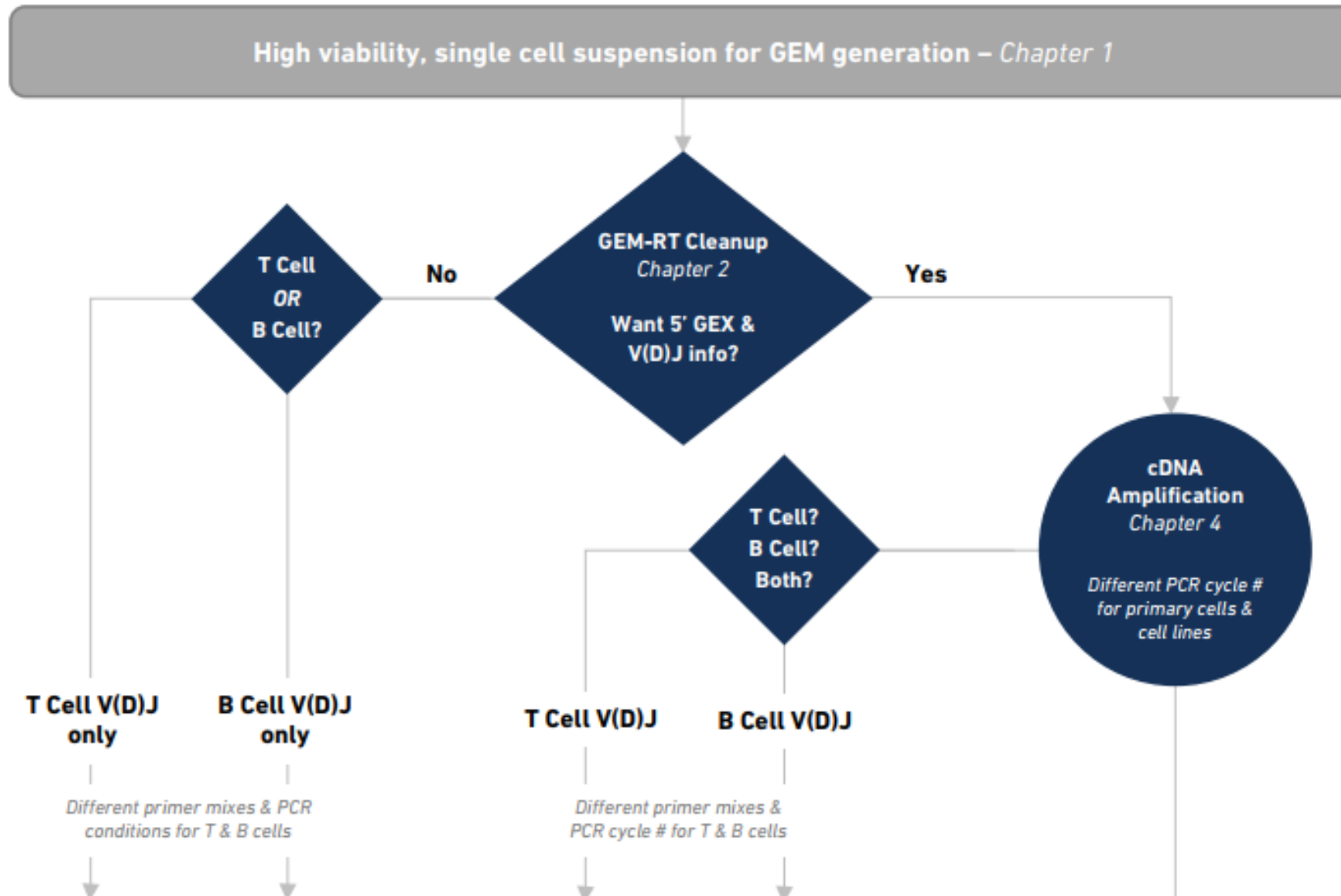


Figure 3.1 The Immune System, 3ed. (© Garland Science 2009)

# General workflow 5' + V(D)J single cell sequencing



# Gel bead oligos

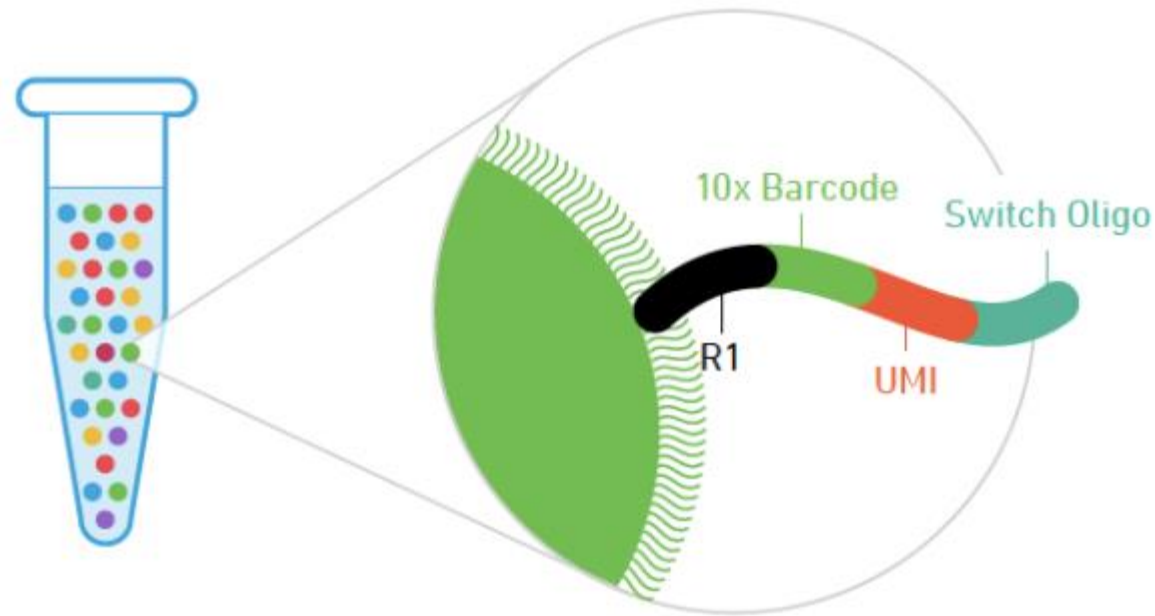
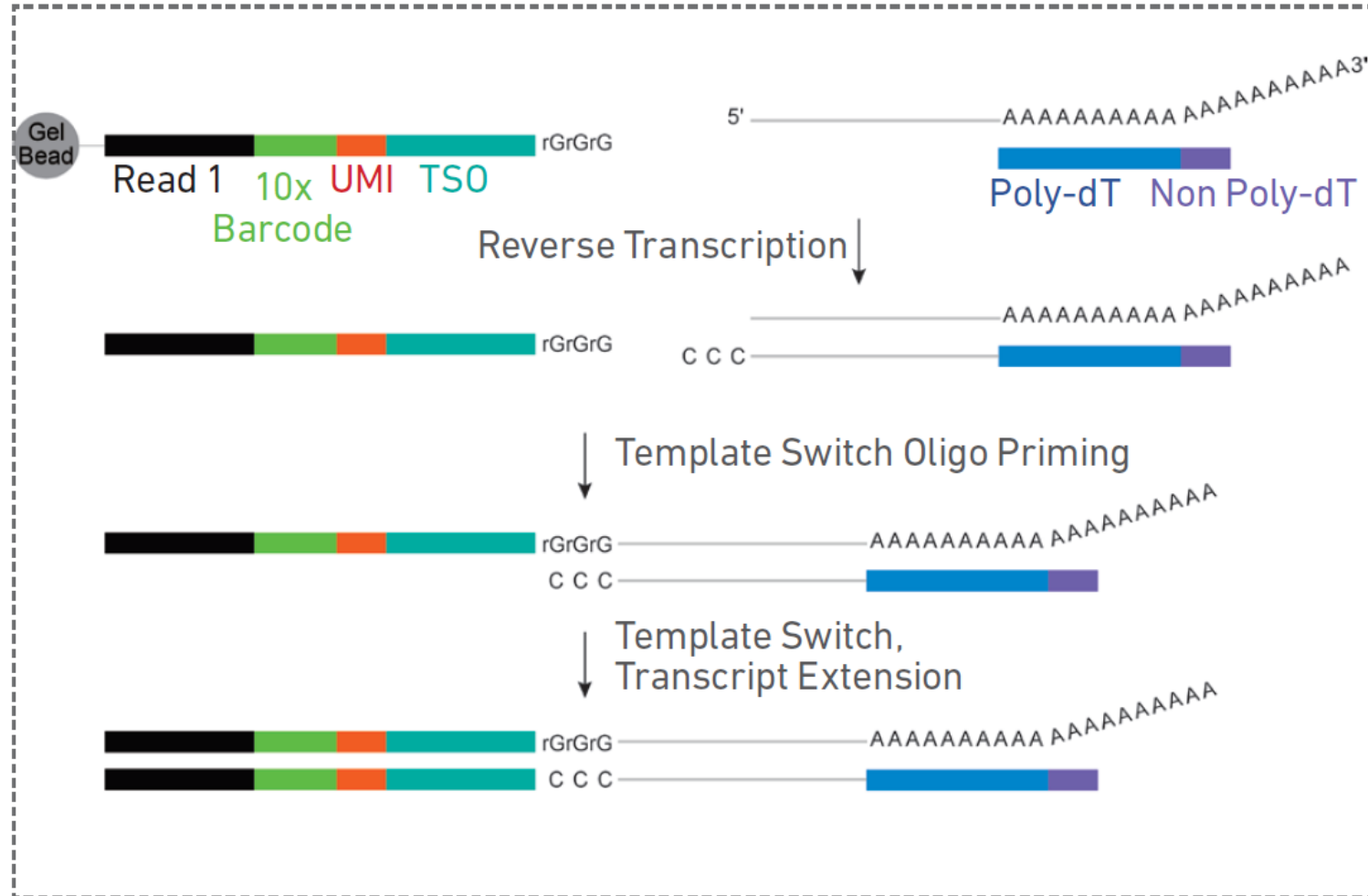


Figure 1. Schematic of a Single Cell 5' Gel Bead oligo primer.

- i. Partial Illumina Read 1 Sequence (22 nucleotides (nt))
- ii. 16 nt 10x™ Barcode
- iii. 10 nt Unique Molecular Identifier (UMI)
- iv. 13 nt Switch Oligo

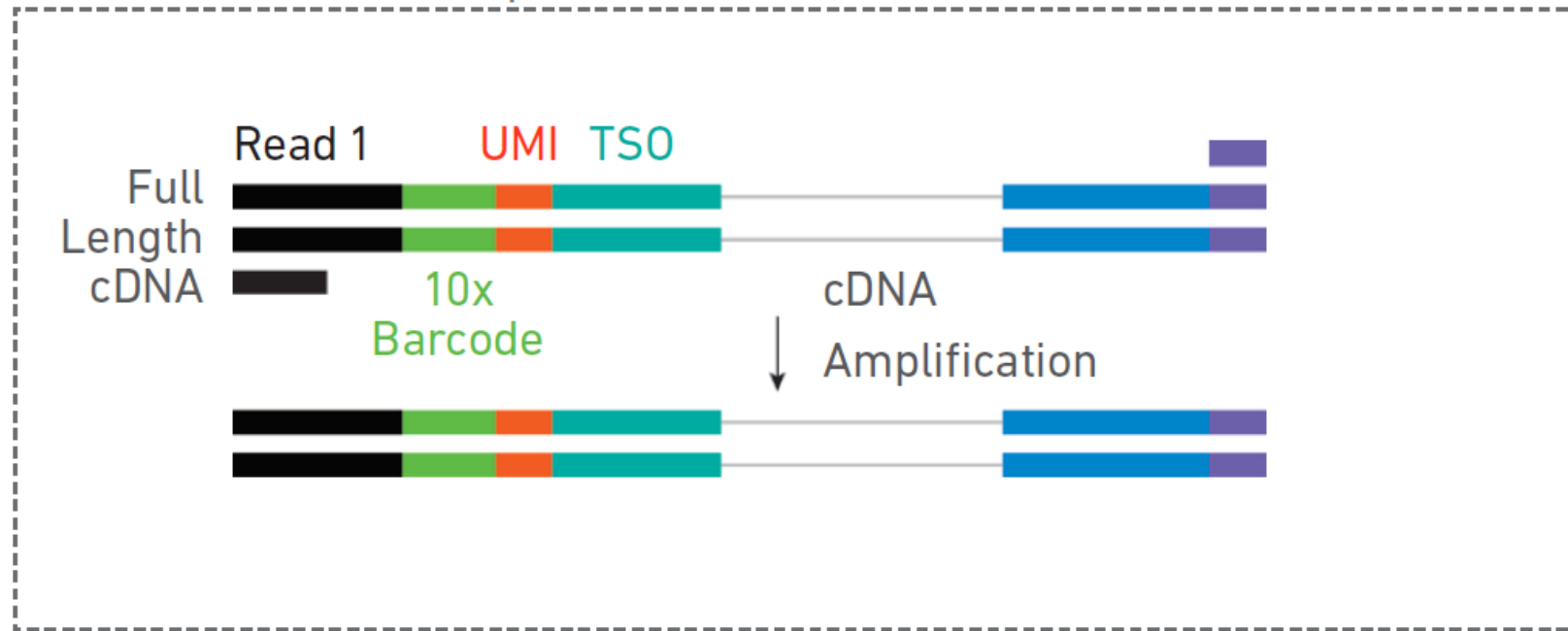
# Assay scheme for 5' scRNA-seq

Inside individual GEMs



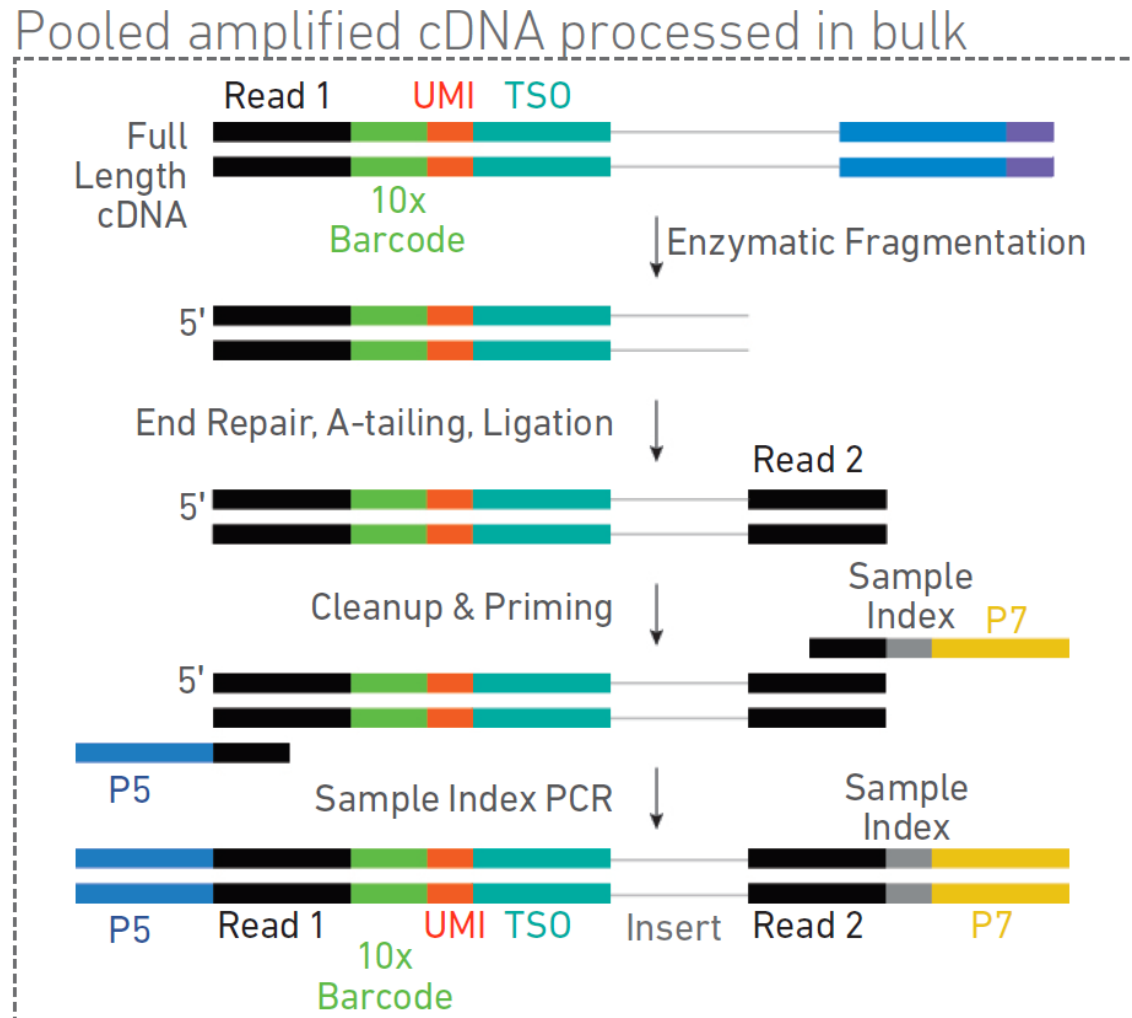
# Assay scheme for 5' scRNA-seq

Pooled cDNA amplification



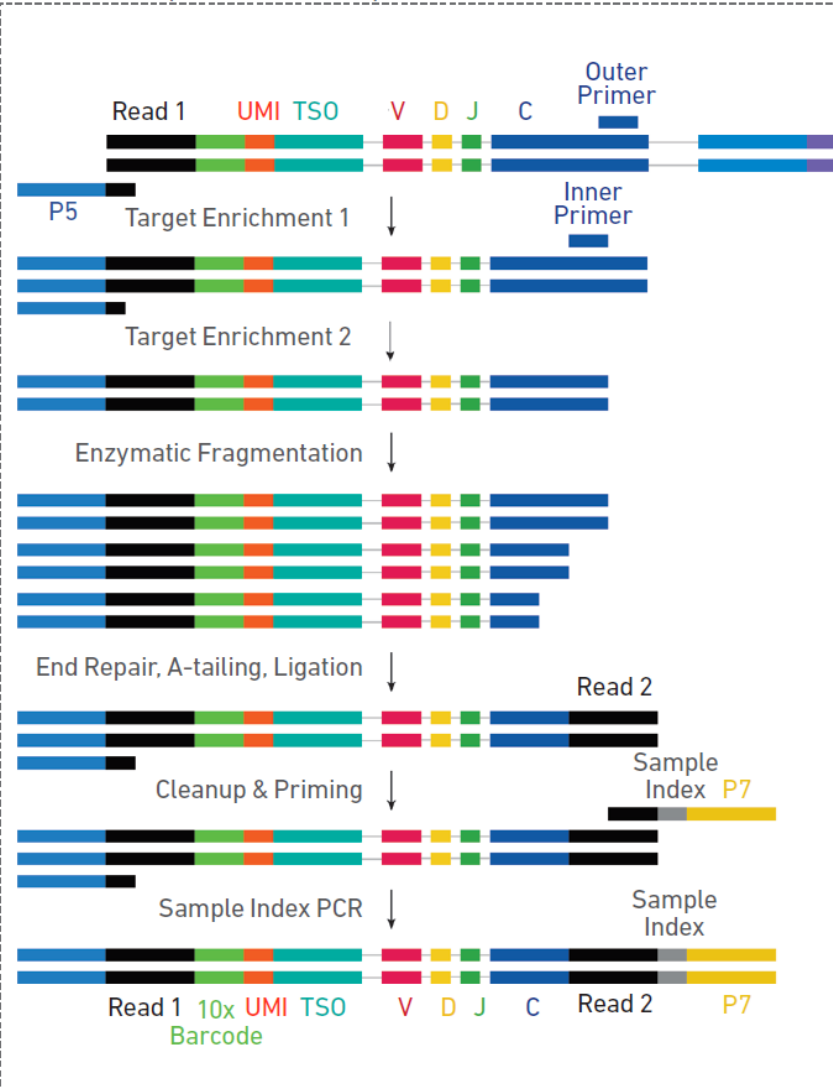


# Assay scheme for 5' scRNA-seq



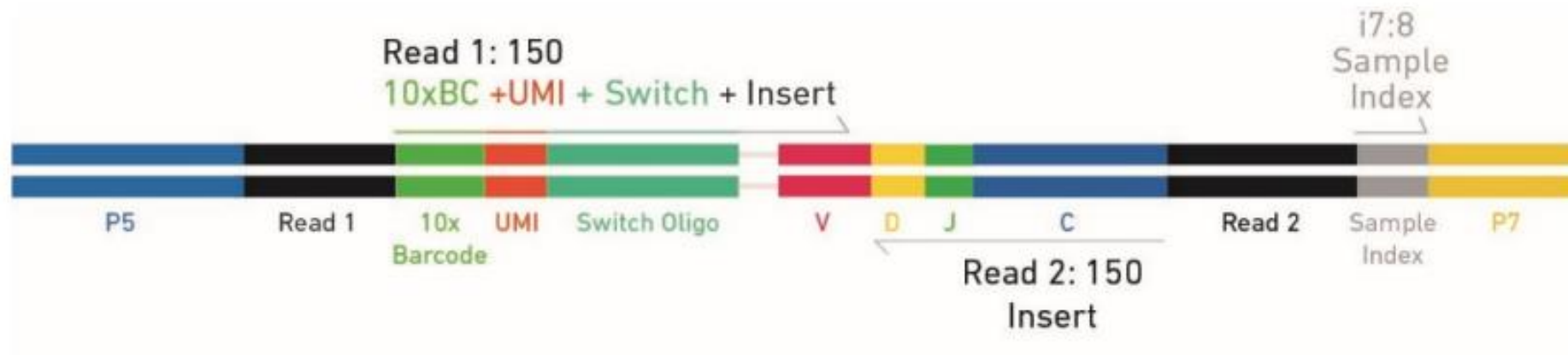
# Assay scheme for 5' VDJ libraries

Pooled amplified cDNA processed in bulk



# Final library structure

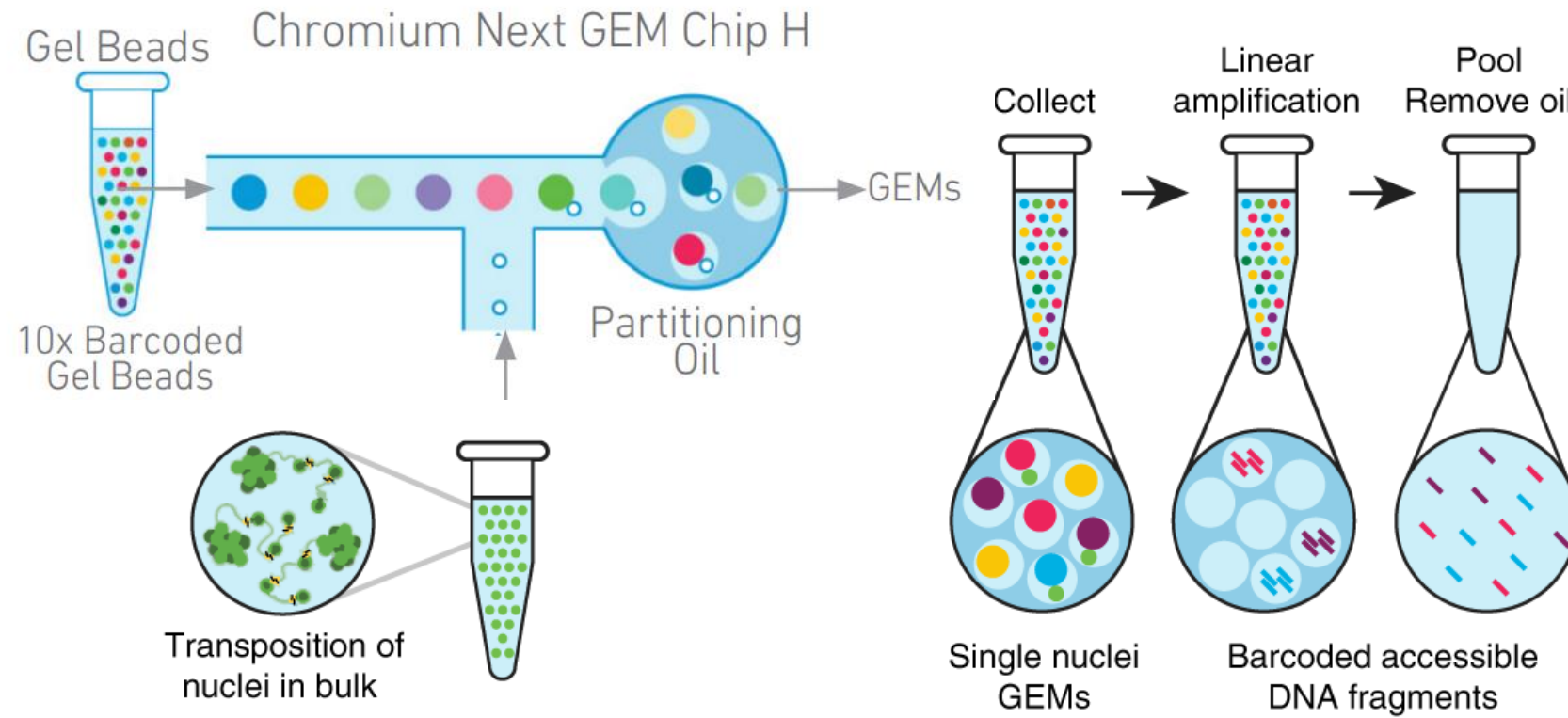
## V(D)J Enriched Library Structure:



## 5' Gene Expression Library Structure:

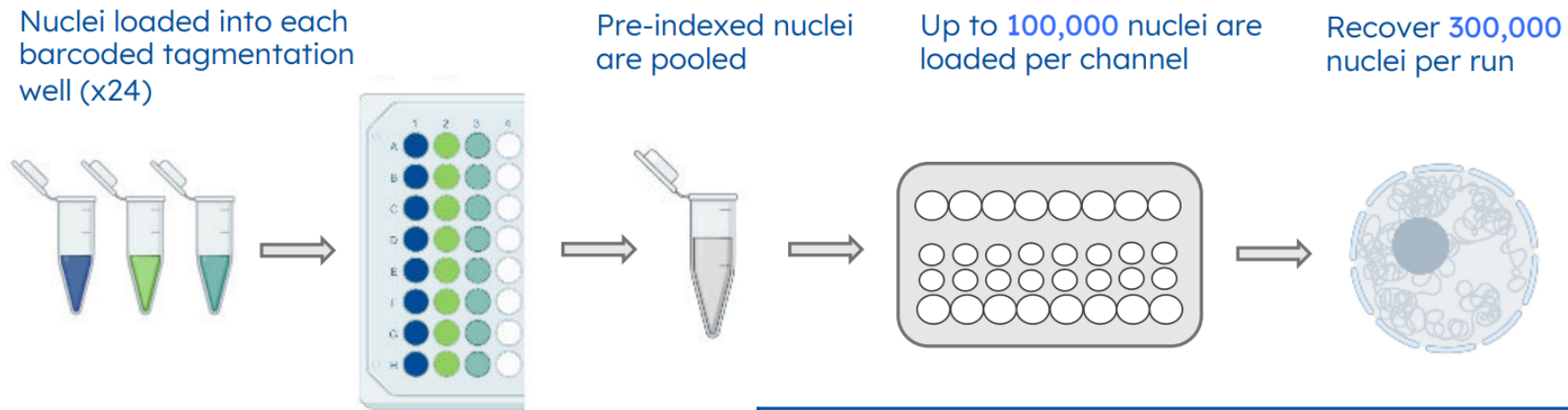


# Single-cell ATAC-seq



# Scale Biosciences

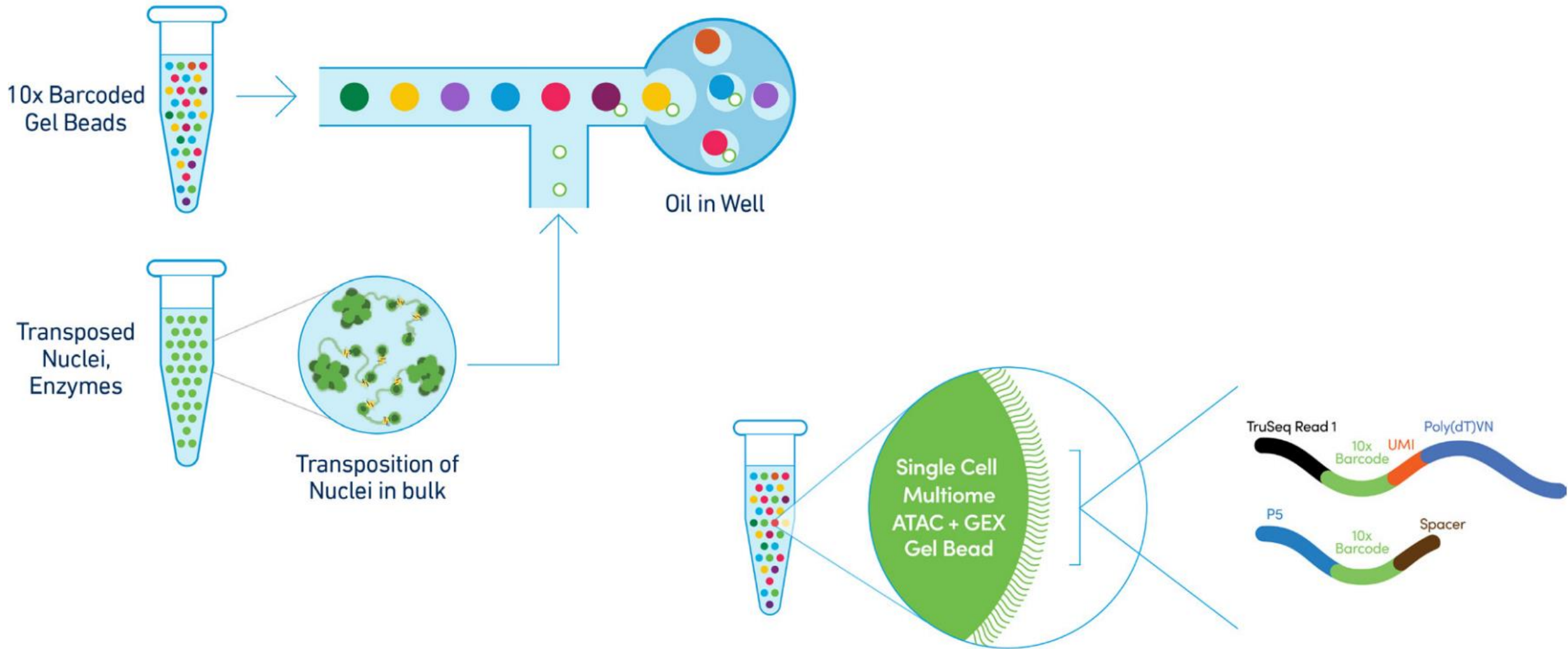
## Pre-indexing kit compatible with 10x Genomics snATAC-seq



ScaleBio's bioinformatics pipeline resolves the combinatorial barcode, rescuing data from multiplets and *maintaining a 3-5% effective doublet rate.*

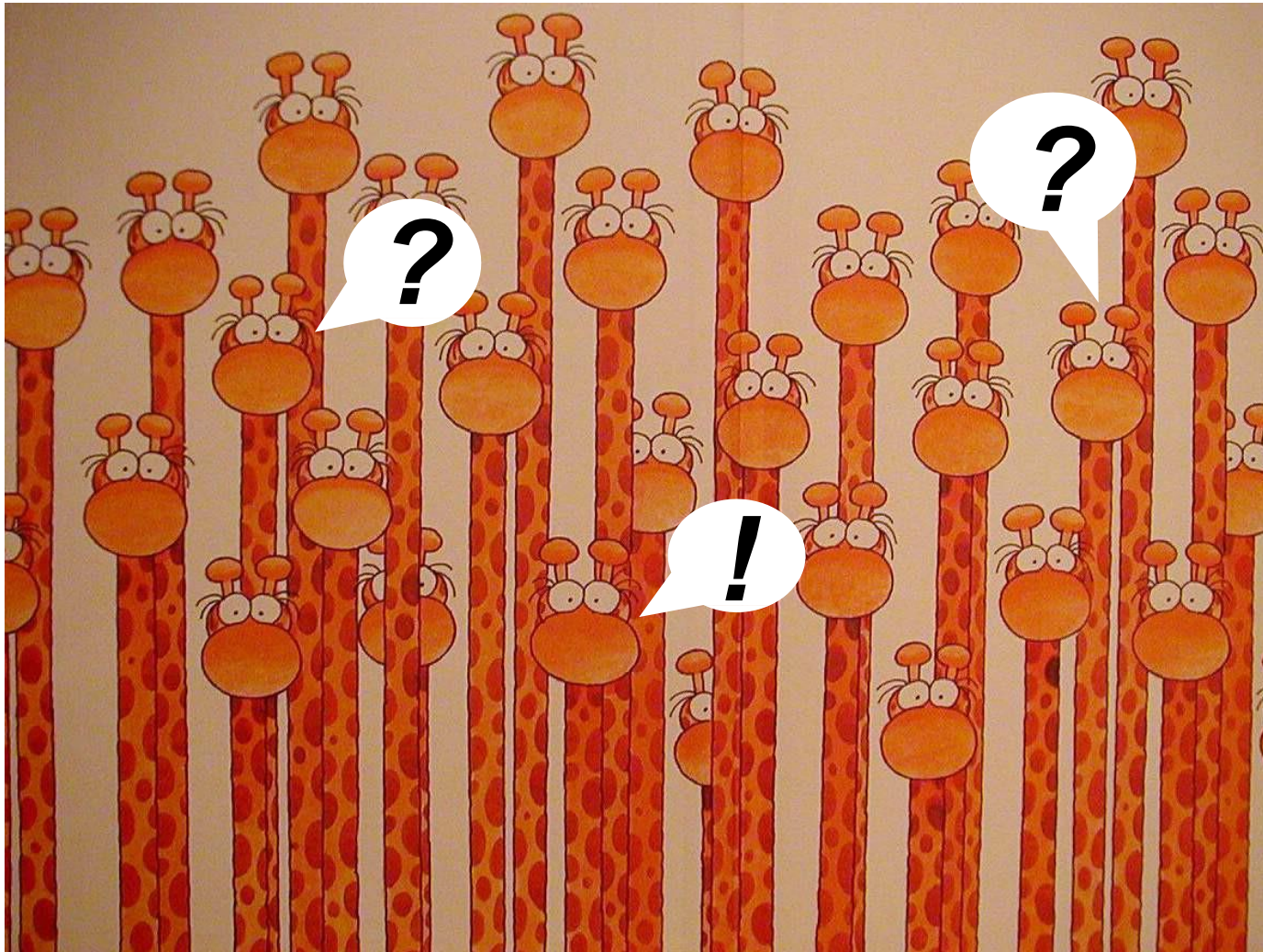
Throughput	
Nuclei loaded into Scale plate	1.2M
Recovery after tagmentation plate	~600K
Nuclei loaded into on-market system	600K
Recovery from sequencing	~300K

# Single-cell multiome (ATAC and RNA)





# Questions or concerns?



[S.L.Kloet@lumc.nl](mailto:S.L.Kloet@lumc.nl)

info@lgtc.nl