

Introduction and Overview of Single-Cell Platforms

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Leiden Genome Technology Center (LGTC)

MGC Course on Single-Cell Analysis

23 October 2023

Why single-cell?

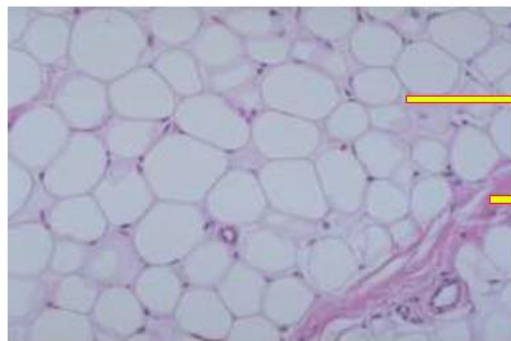
Bulk sample analysis is just like putting a fruit salad into a blender - the taste is an average of all ingredients.



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



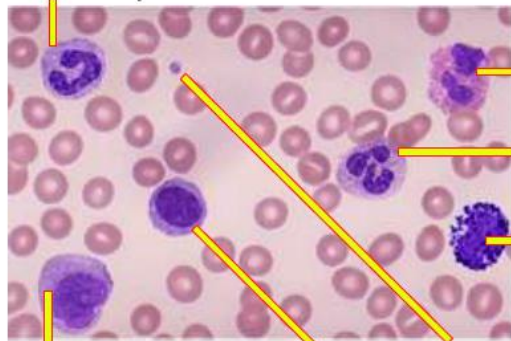
Adipose

Fat

Connective
tissue

Band
Neutrophil

Normal Peripheral Blood



Eosinophil

Segmented
Neutrophil

Basophil

Monocyte

Platelet
Lymphocyte

Small Intestine Mucosa



Epithelial cells

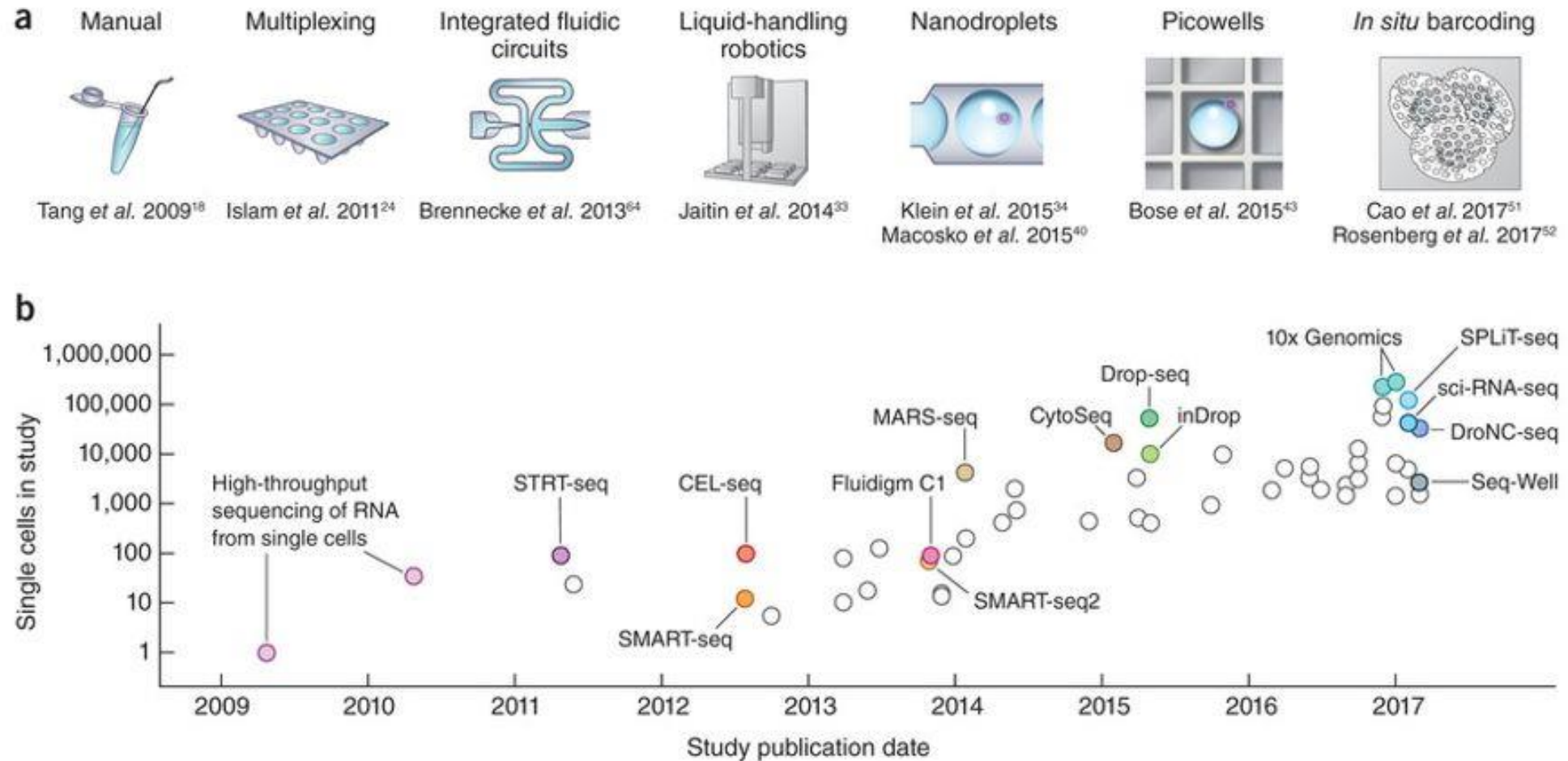
Goblet
cells

Lamina
propria

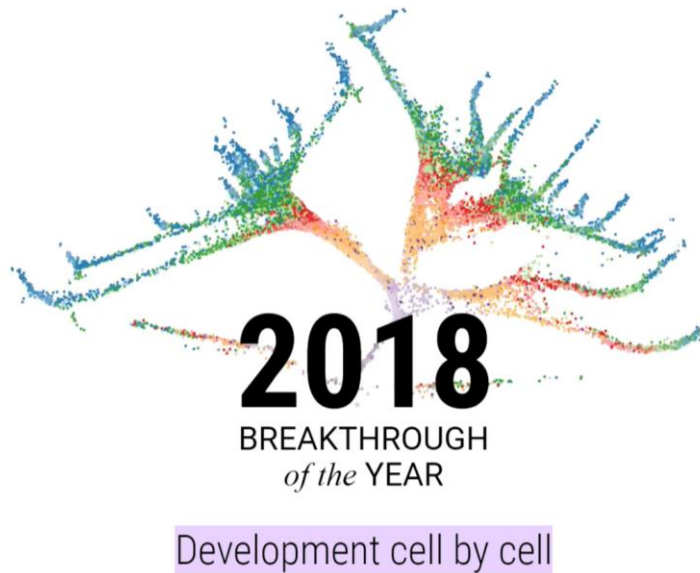
Muscularis mucosa
(smooth muscle)

Created with figures from library.med.utah/WebPath/HISTHTML/HISTO.html

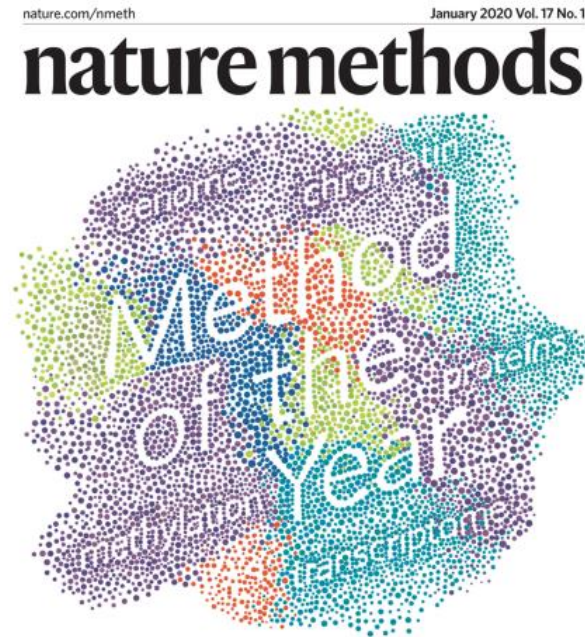
Exponential scaling of single-cell throughput



“The single-cell revolution is just starting.”



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

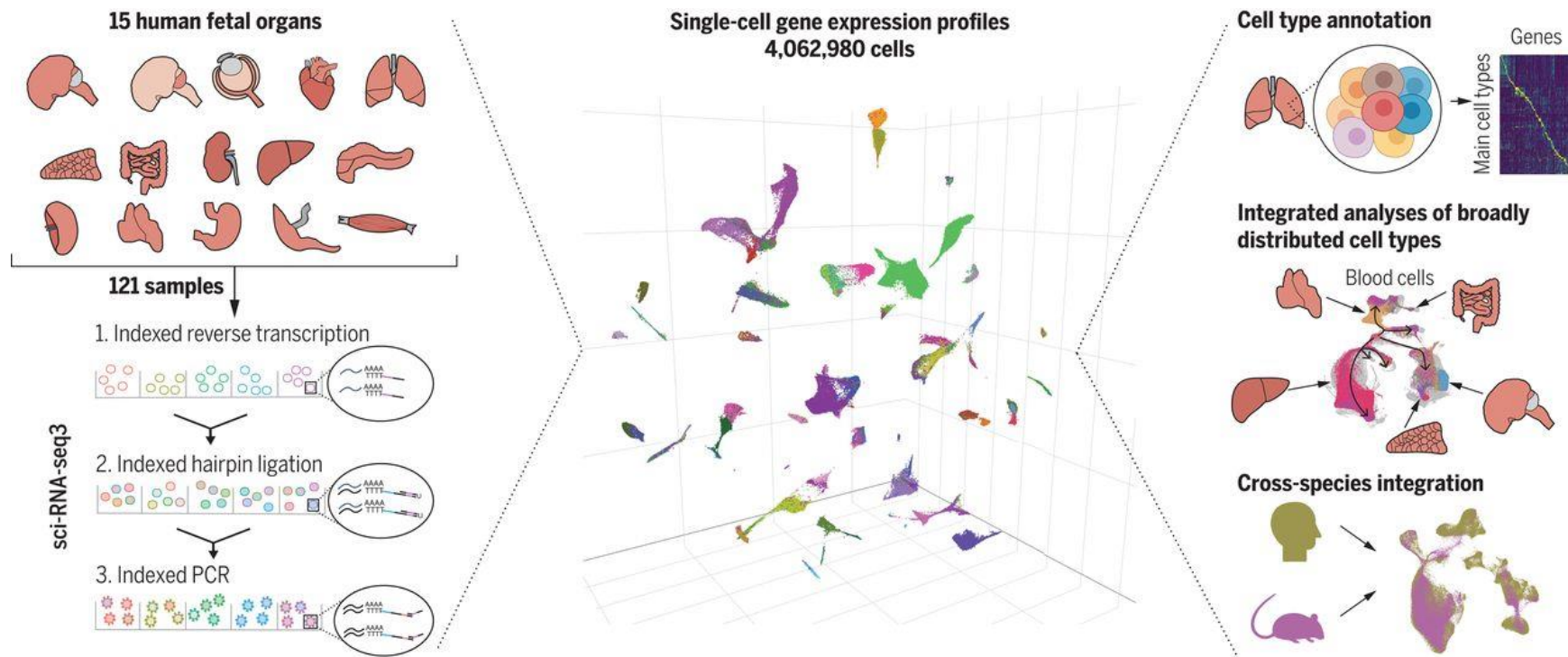


Single-cell multimodal omics
2019 Method of the Year



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?

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.

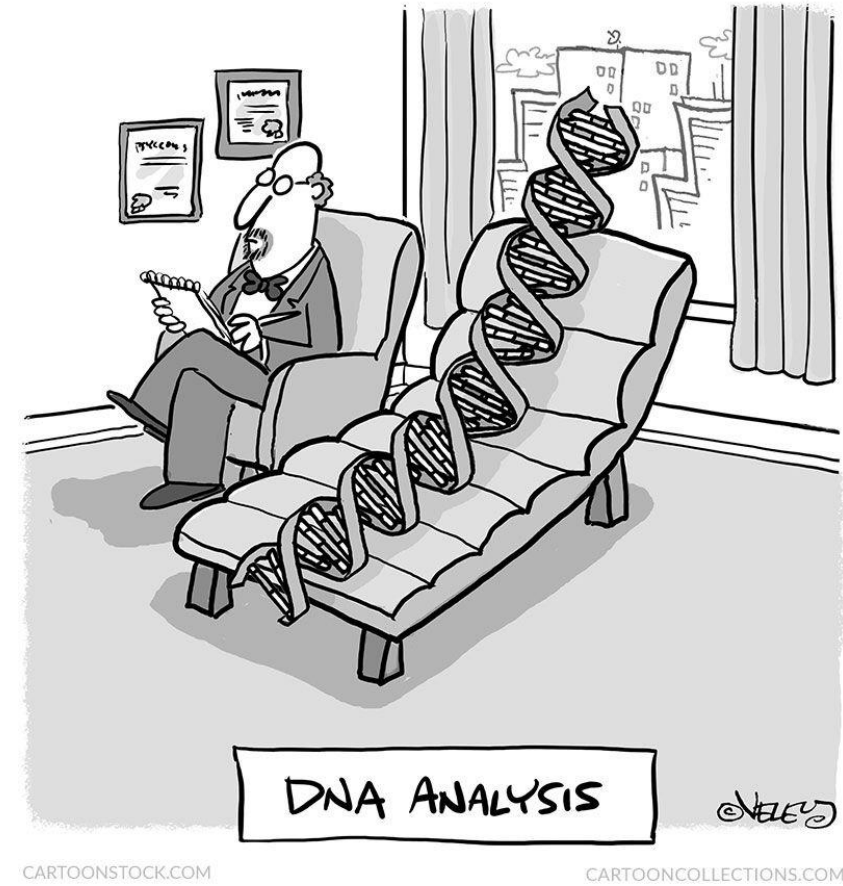
DIY methods – Miao's multiomics lecture

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

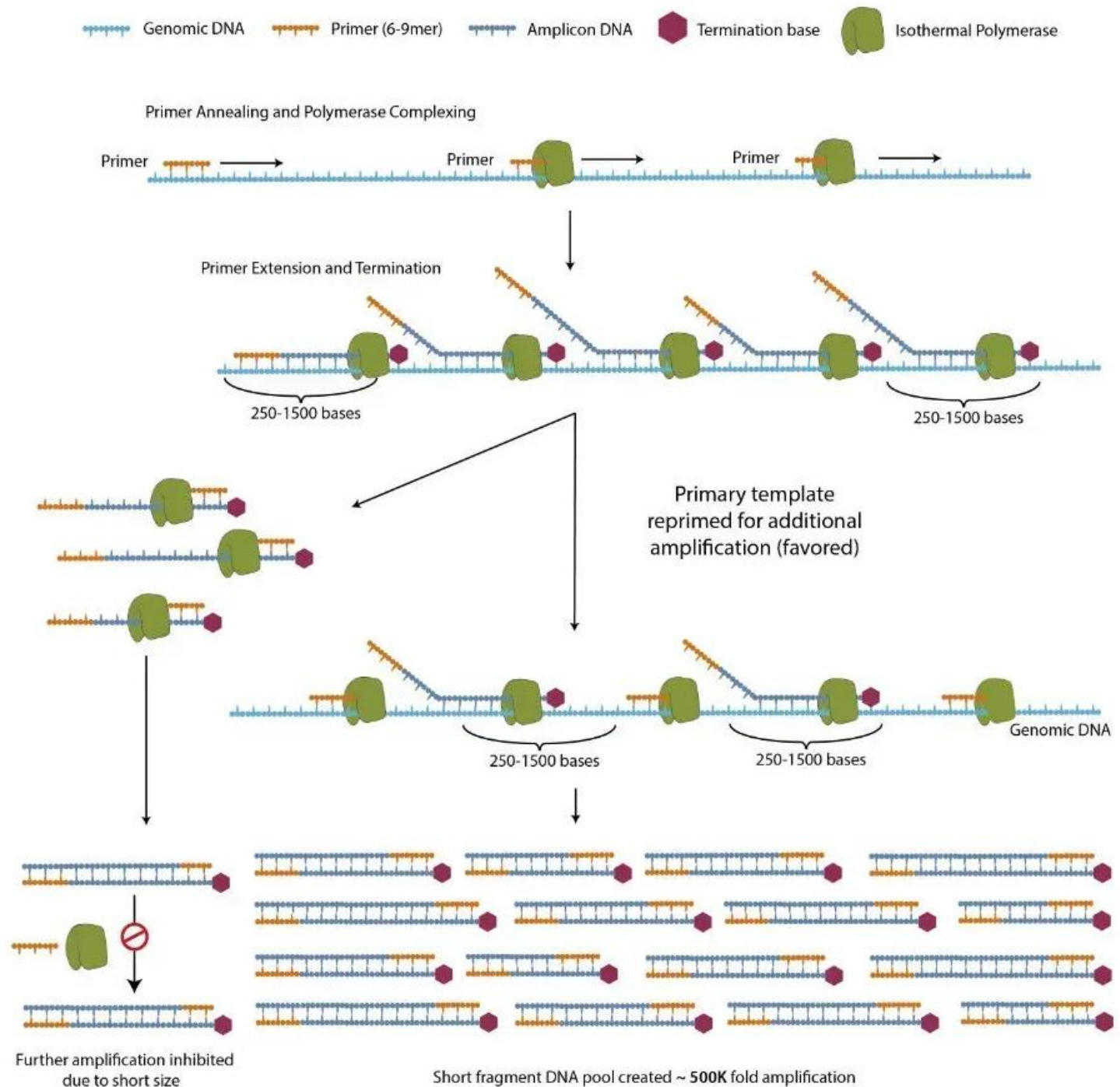


BioScryb

No instrument required!

WGS with PTA
(primary template-directed
amplification)

BioScryb
GENOMICS



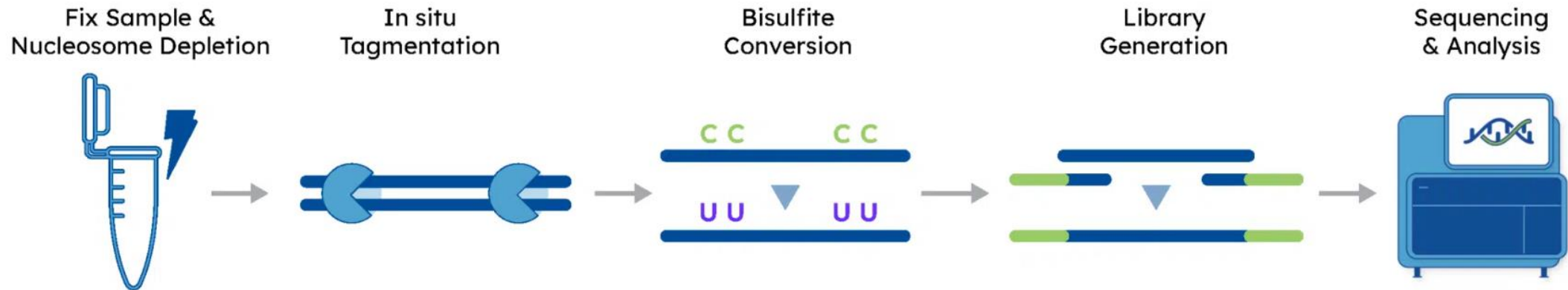
Scale Biosciences



NEW single-cell methylation kit

Up to 18k cells / run

Capture-compatible



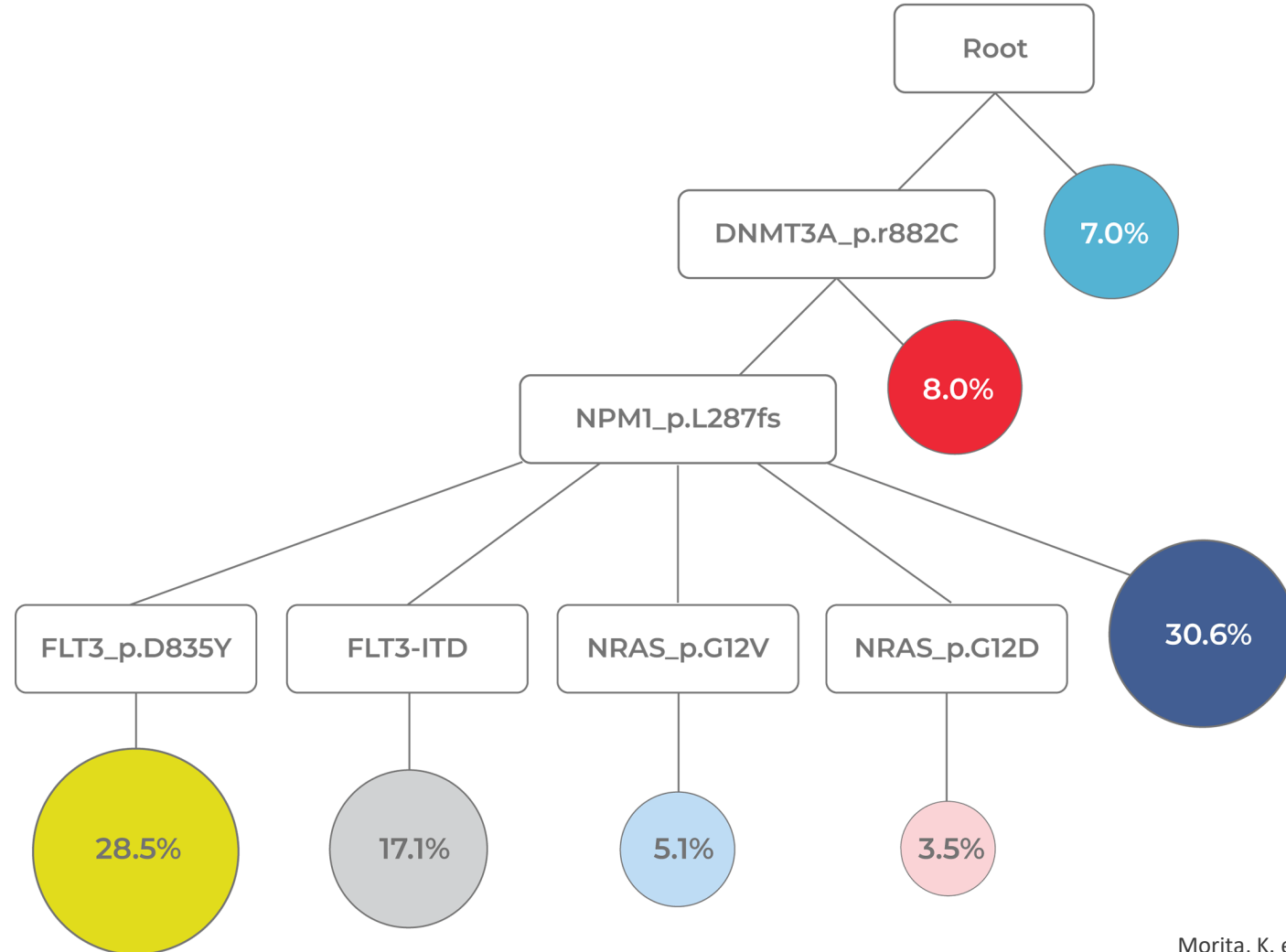
CNV and SNV detection – Mission Bio

Tapestri platform

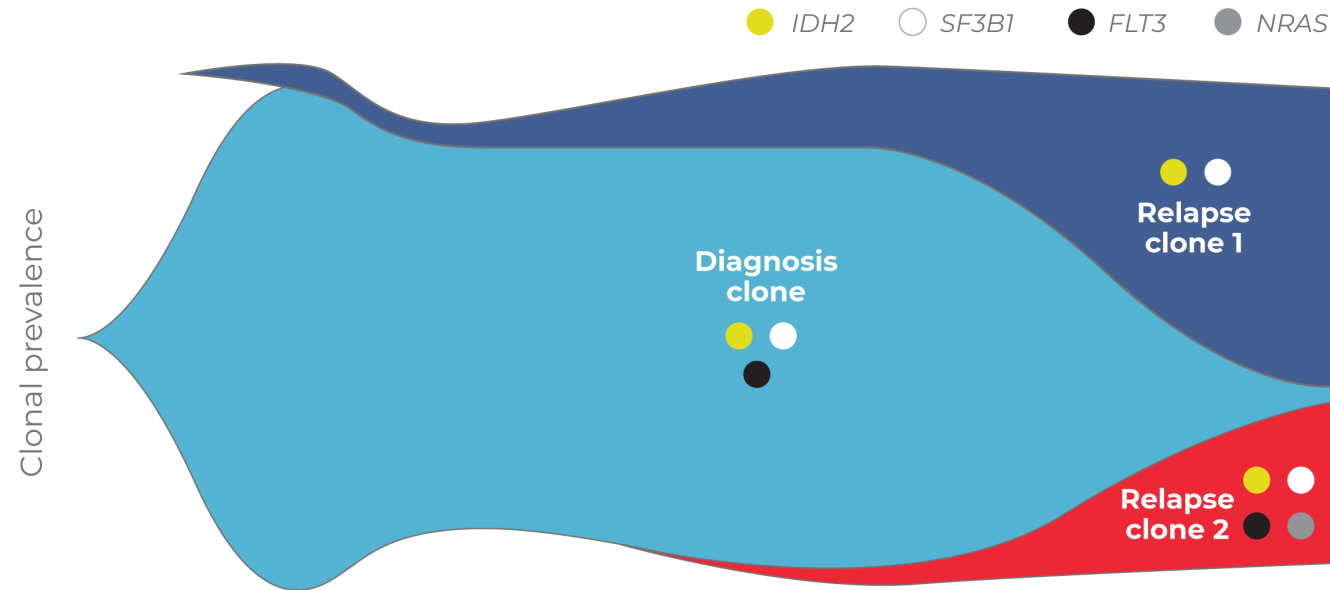
- Droplet-based amplification
- Up to 10k cells
- Panel-based PCR (up to 400 targets)
- Can call both CNVs and SNVs in target regions
- NEW multiomics application: Protein + DNA



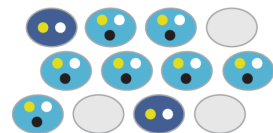
Track clonal evolution (AML)



Reveal therapy resistance

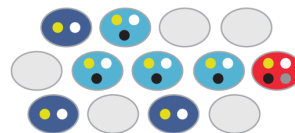


Diagnosis



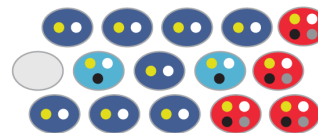
The tumor contains 2 clones. The light blue clone is dominant.

During Therapy



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

Relapse

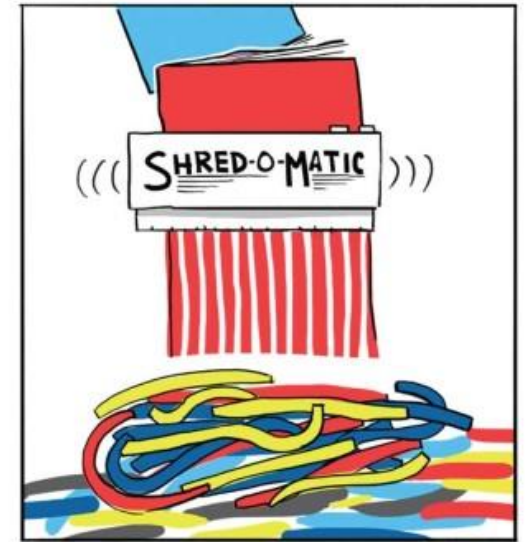


Post-treatment, the red clone & dark blue clone drive relapse.

scRNA-seq

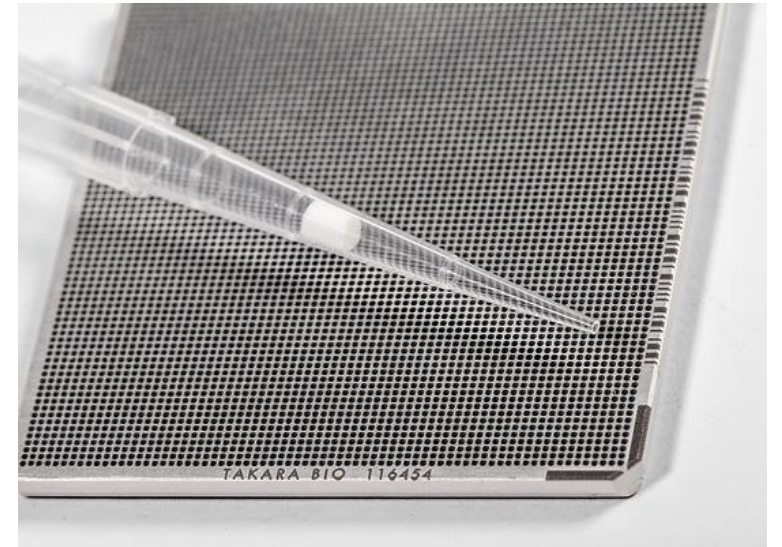
MANY different assays

- 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 (Biomics facility)
- 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

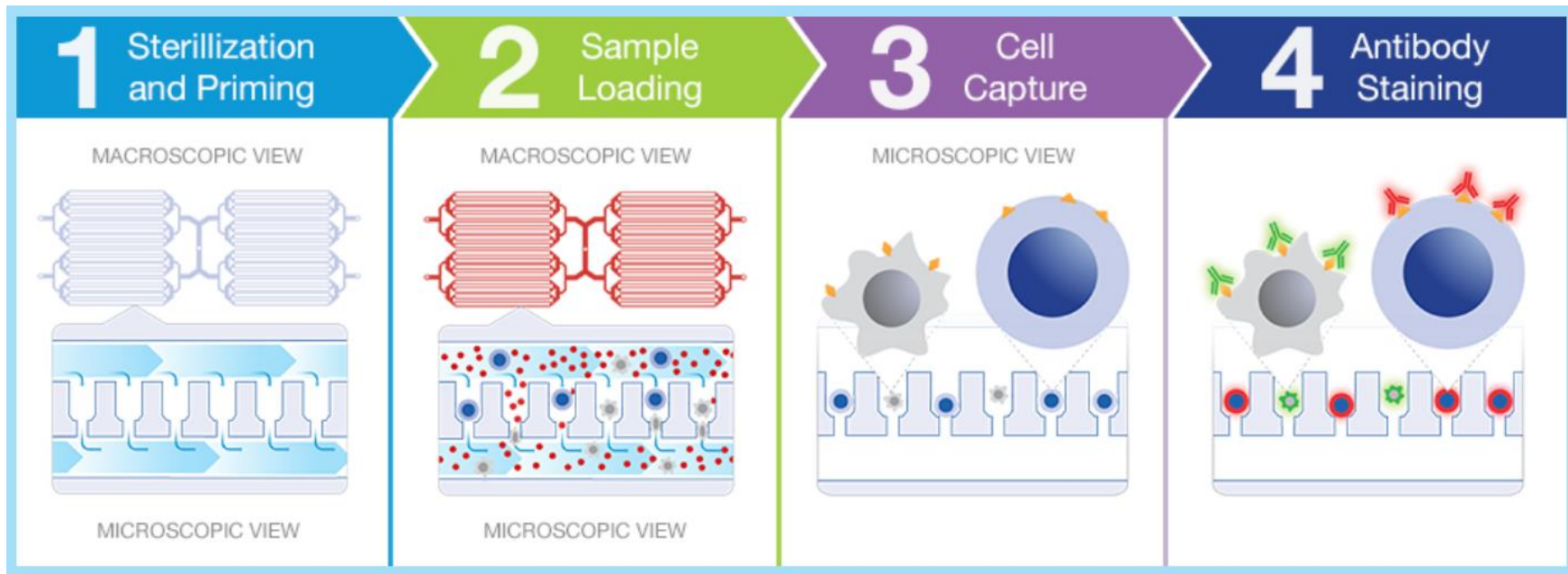


BD Rhapsody

- 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



Celsee Genesis platform

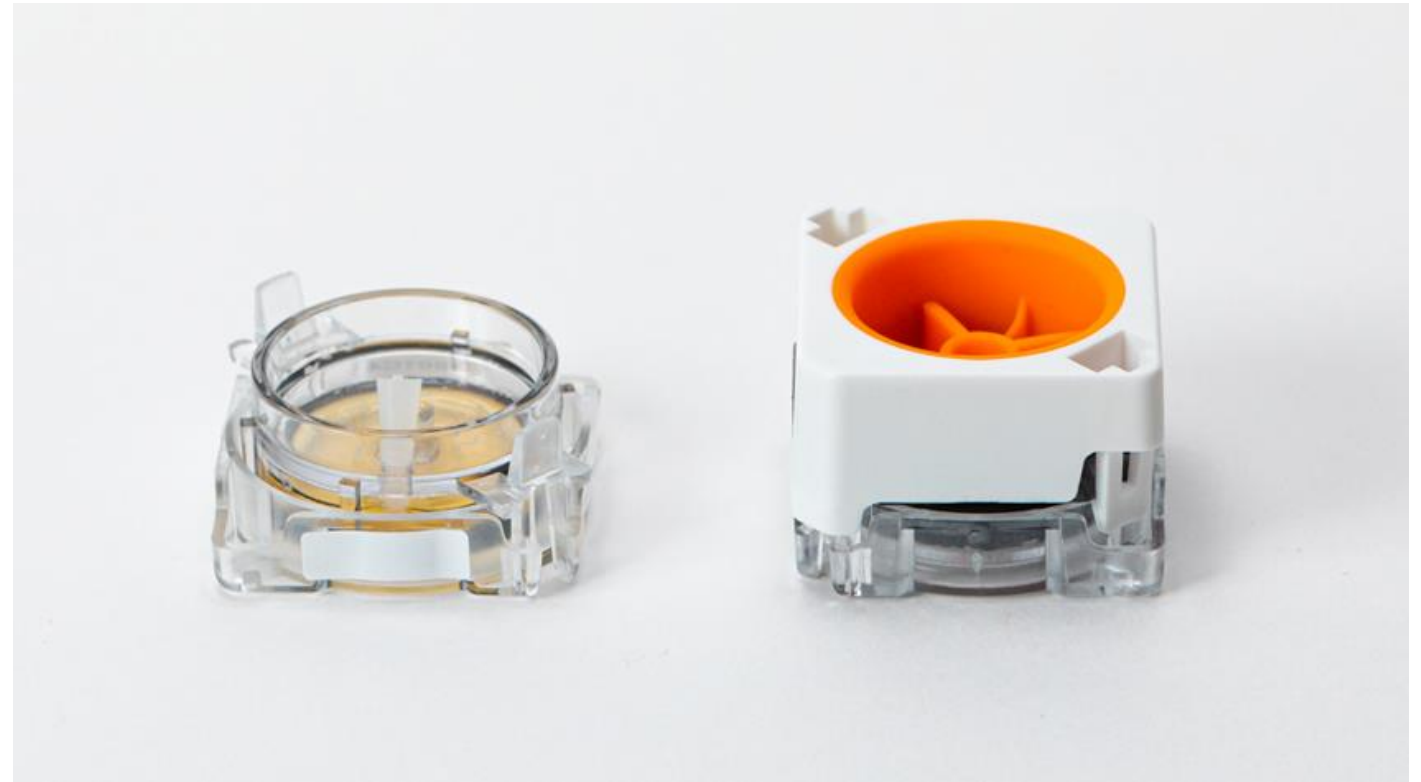
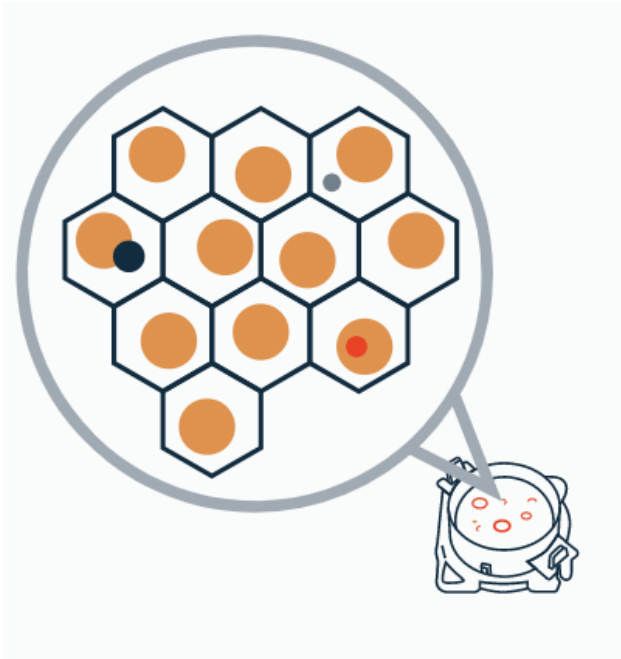


Honeycomb

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



HONEYCOMB



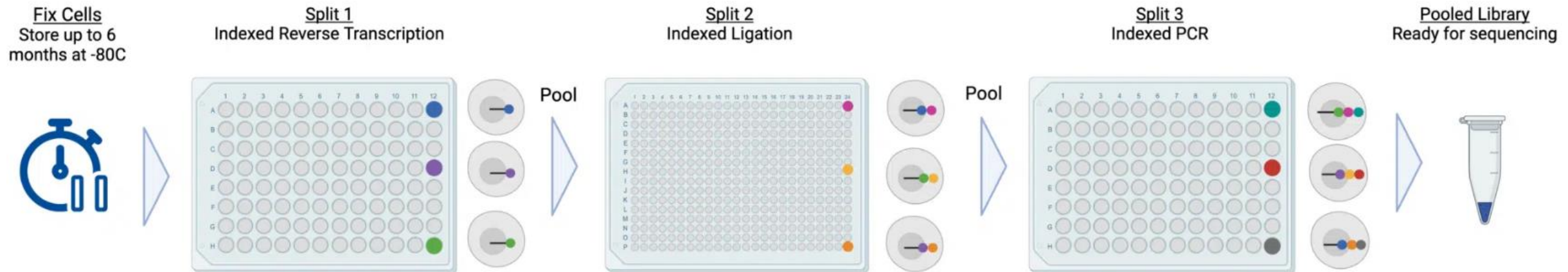
Scale Biosciences



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

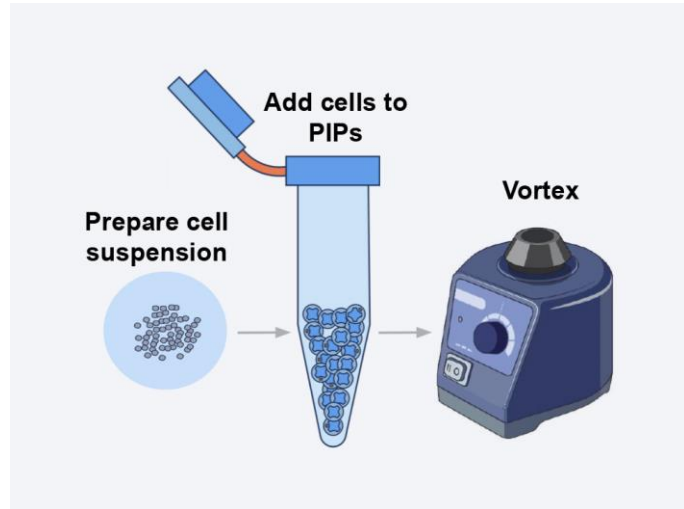


Fluent Biosciences

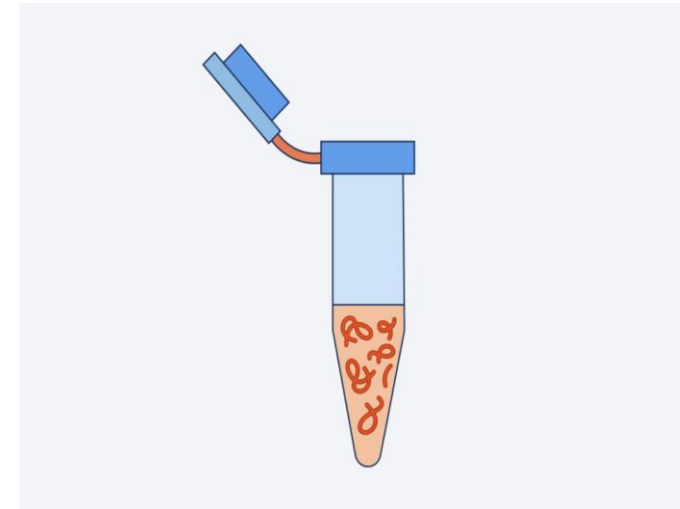


No instrument required!

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



~5 min for encapsulation
Thermocycler does the rest

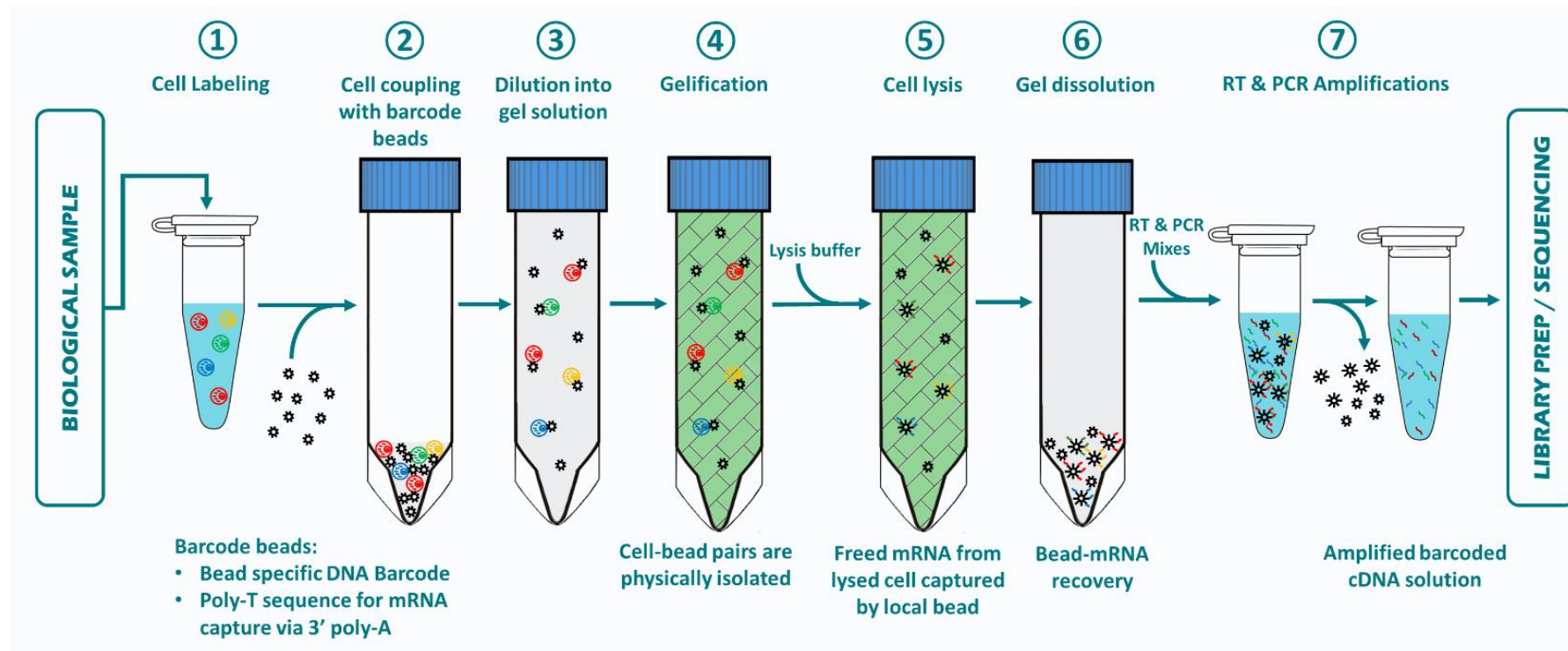
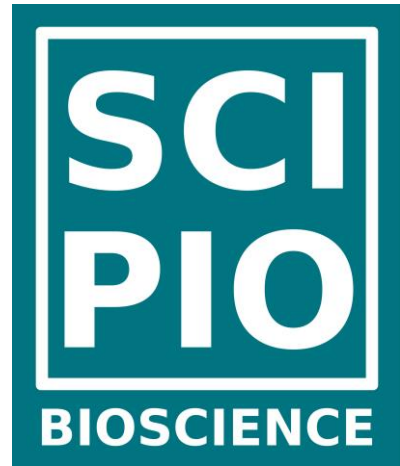


1 tube library prep based on
standard Illumina workflows

Scipio Bioscience

No instrument required!

Based on hydrogels for reaction compartmentalization



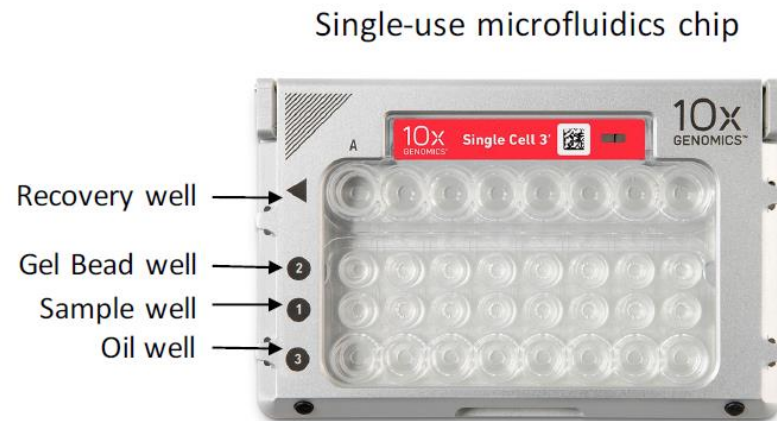
10x Genomics Chromium Controller

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

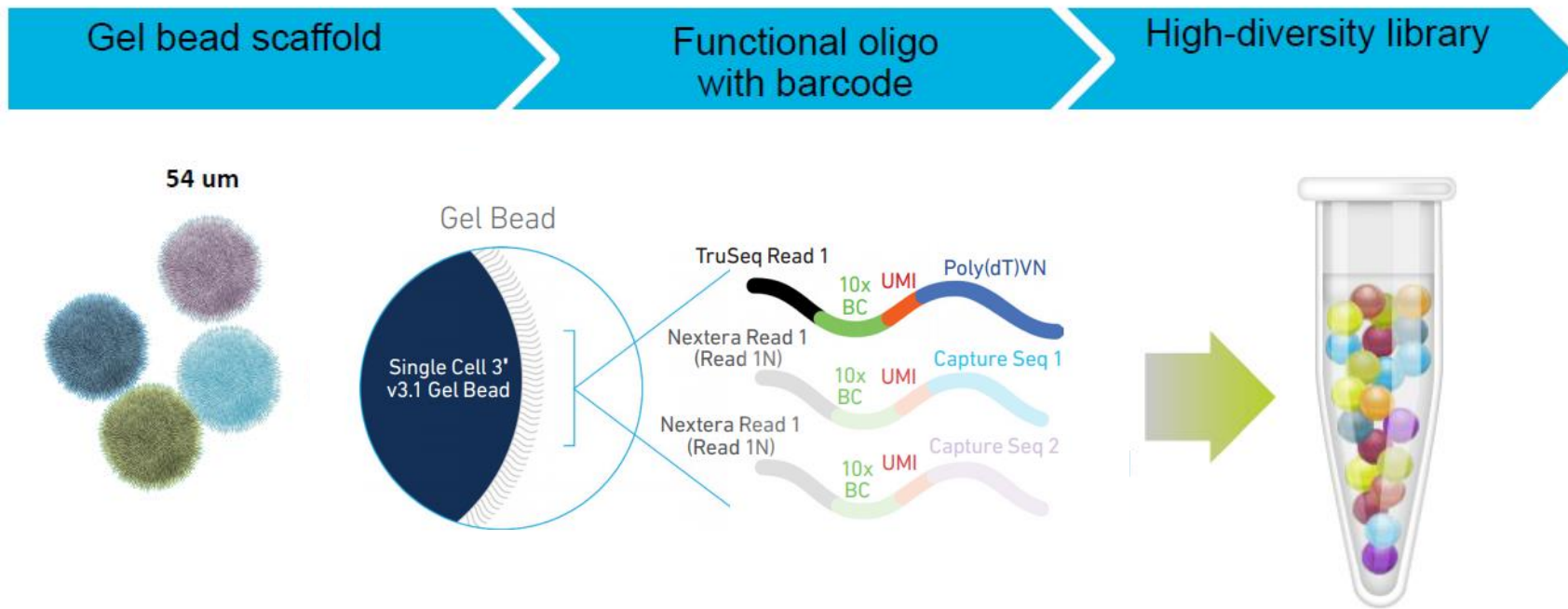
5' gene expression alongside V(D)J repertoire profiling and antigen specificity of T and B cells.

Single Cell Multiome ATAC + Gene Expression

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

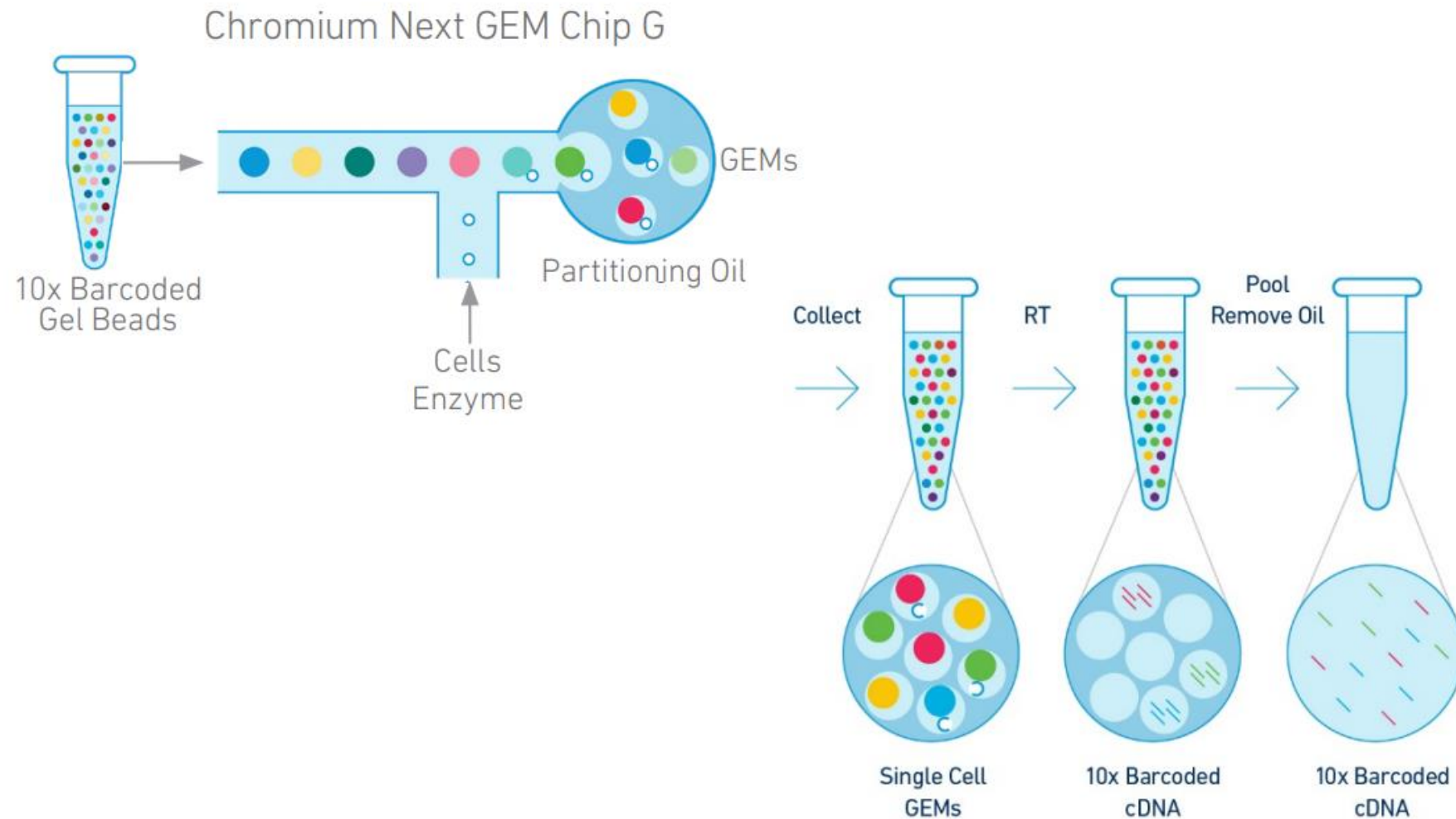
Gel beads up close

4M Discrete Reagents in One Tube

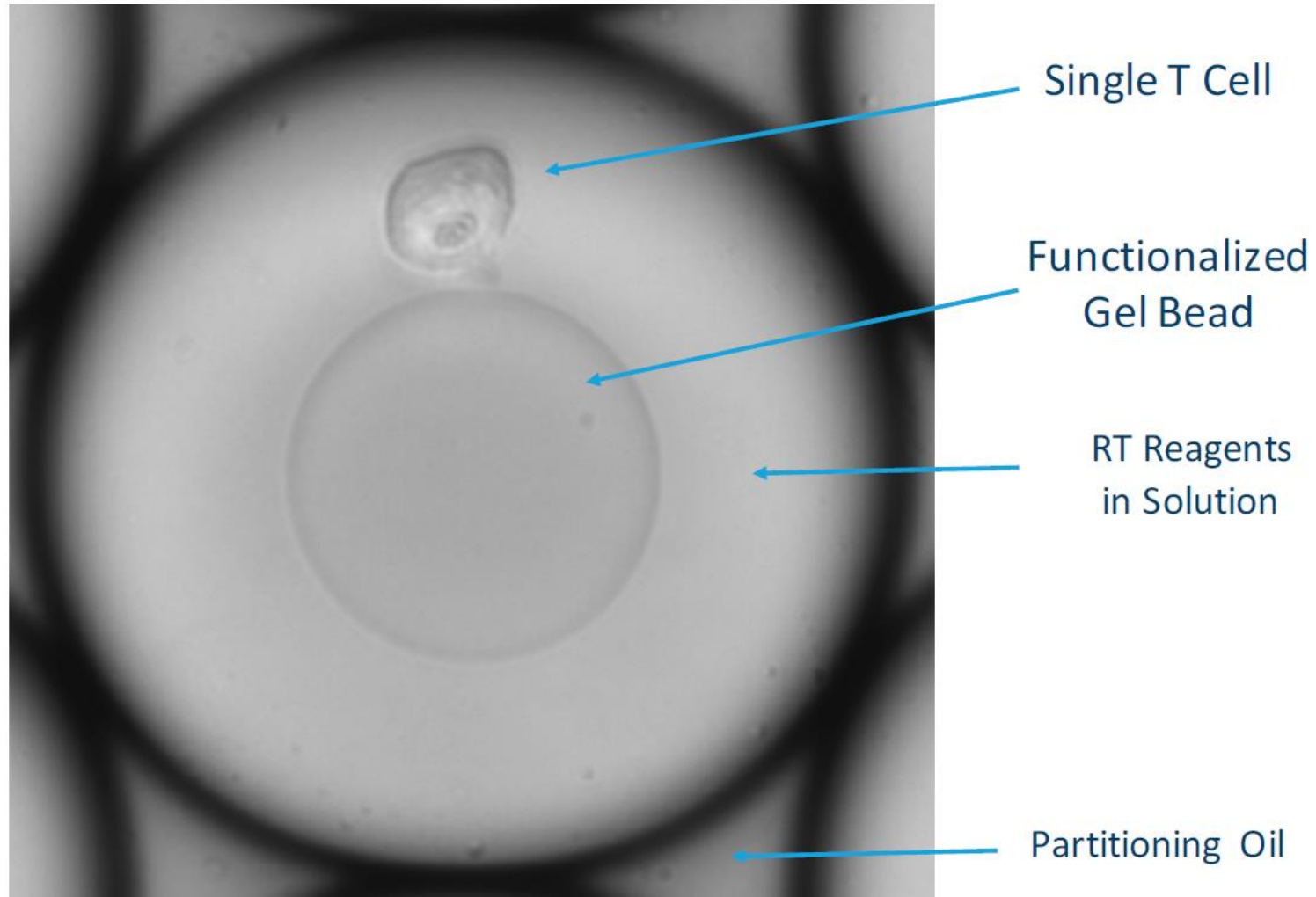


10x Barcode (16bp): unique for each GemBead
+ UMI (12bp): correct for PCR duplicates

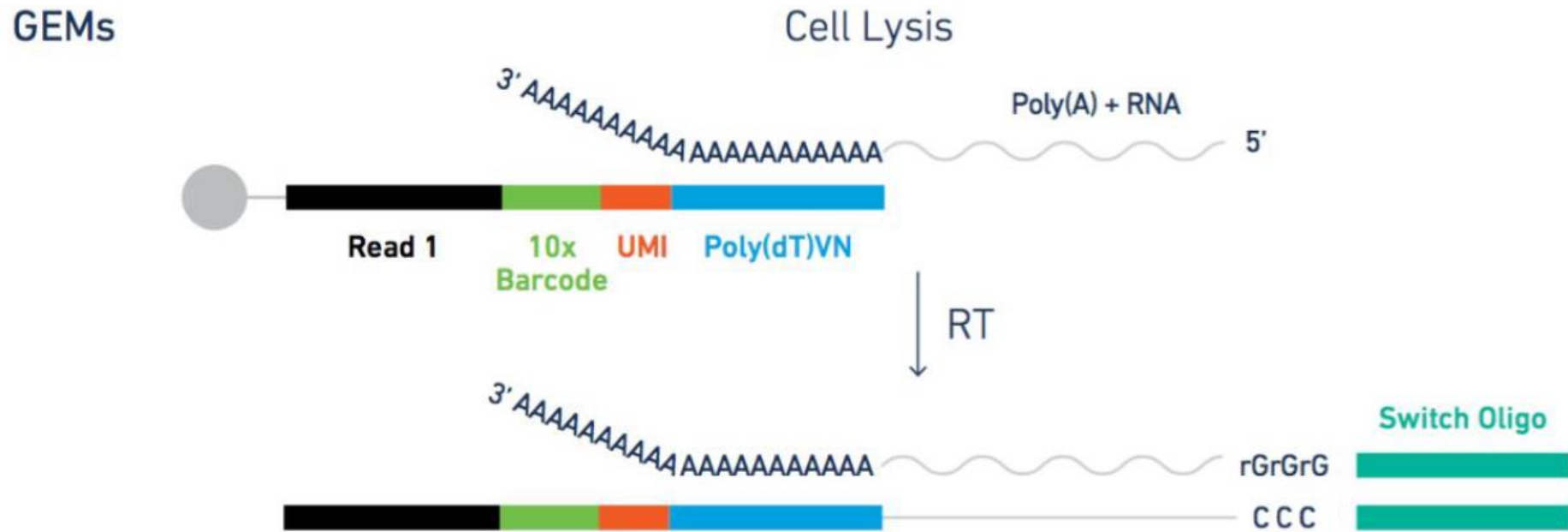
Gel bead in Emulsion (GEM) technology



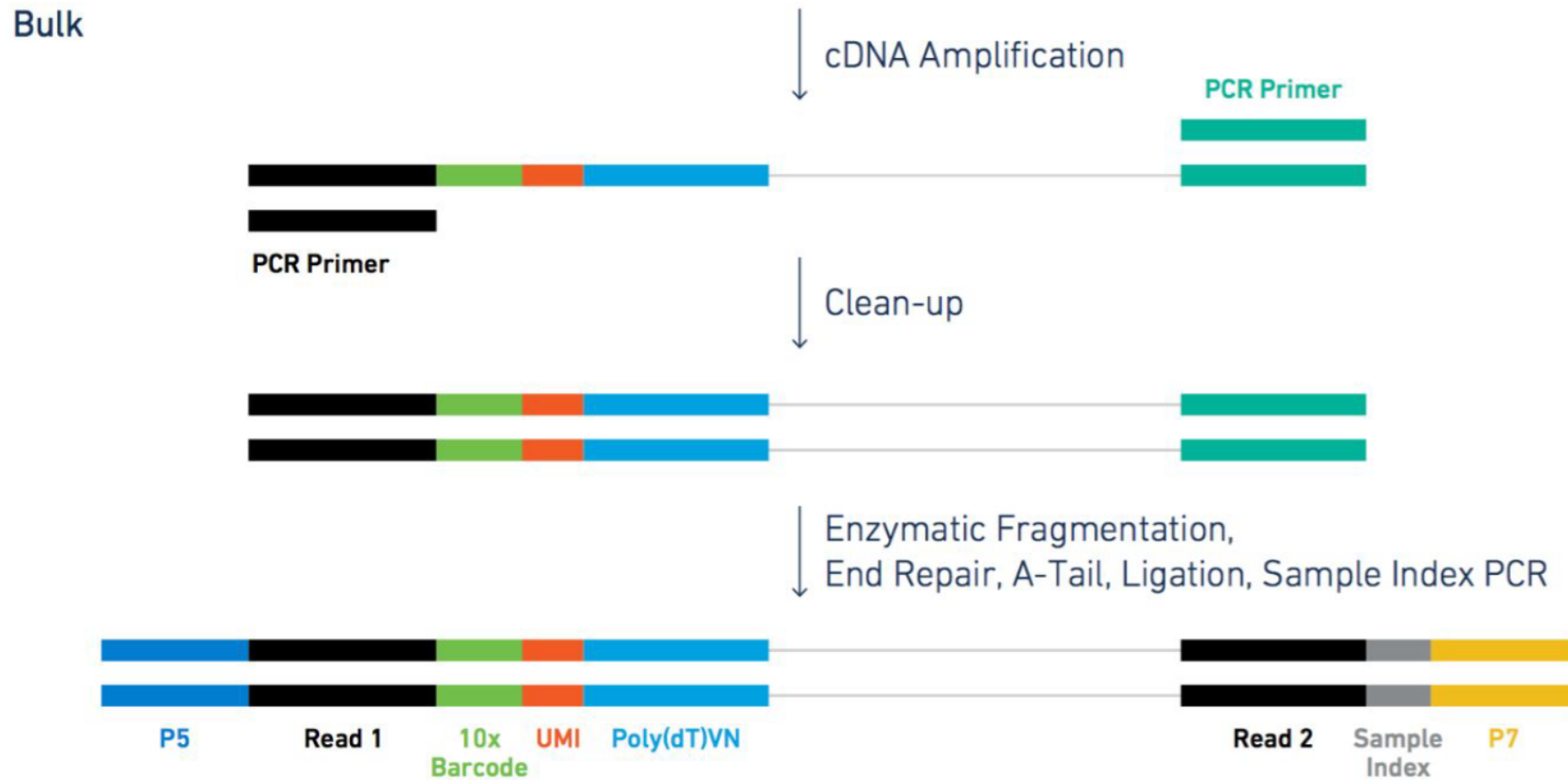
GEMs up close



Assay scheme for 3' mRNA sequencing

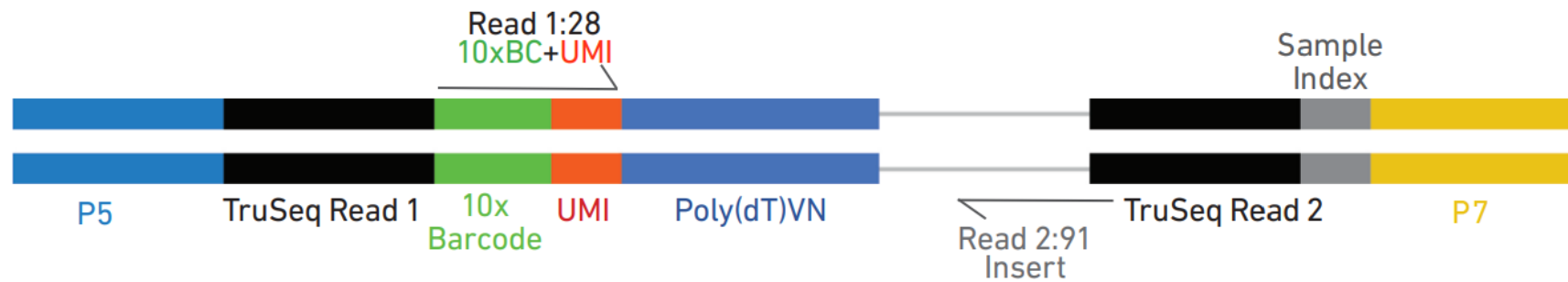


Assay scheme for 3' mRNA sequencing

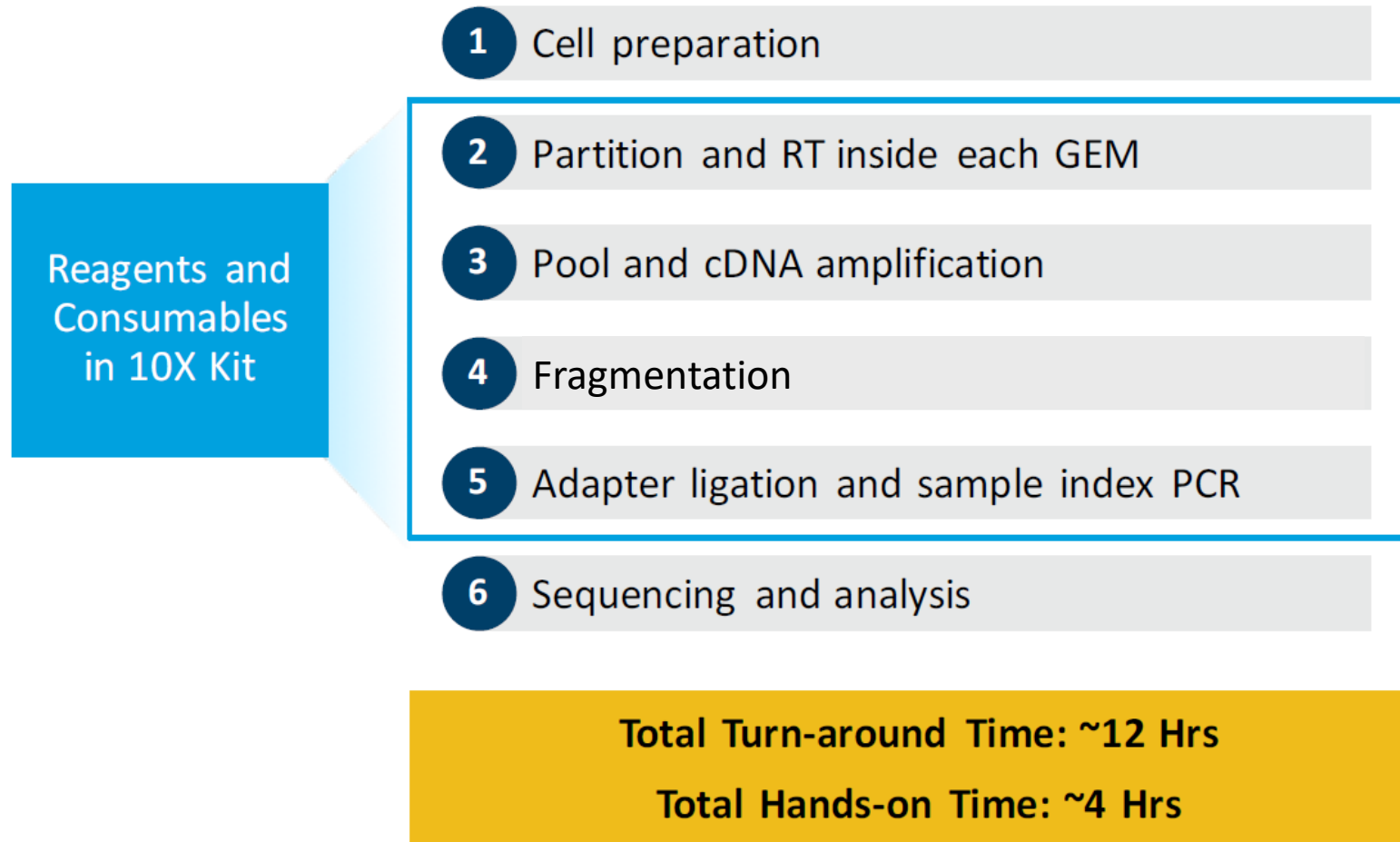


Final library structure

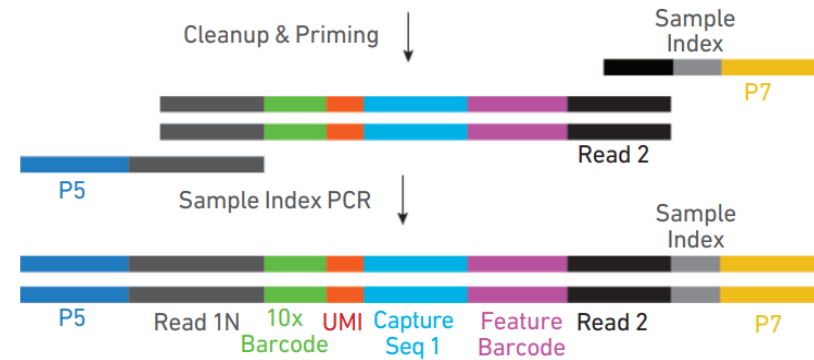
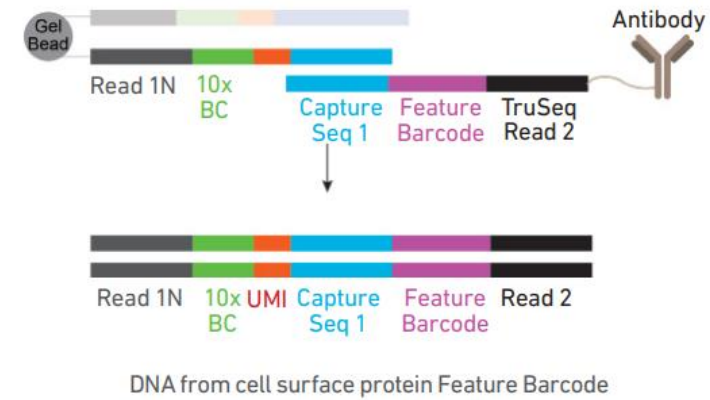
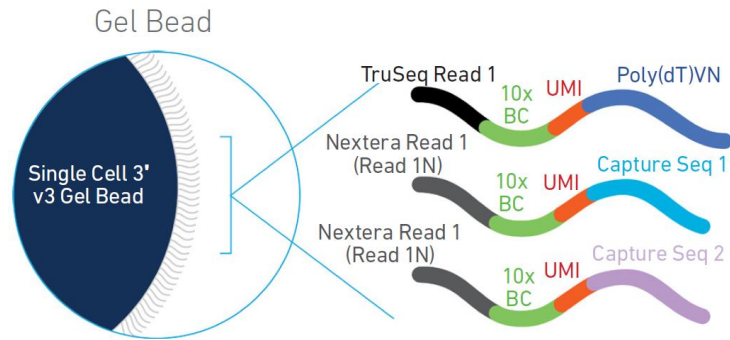
Chromium Single Cell 3' Gene Expression Library



Single cell 3' end-to-end workflow



Single cell 3' feature barcoding



Structure of T and B cell receptors

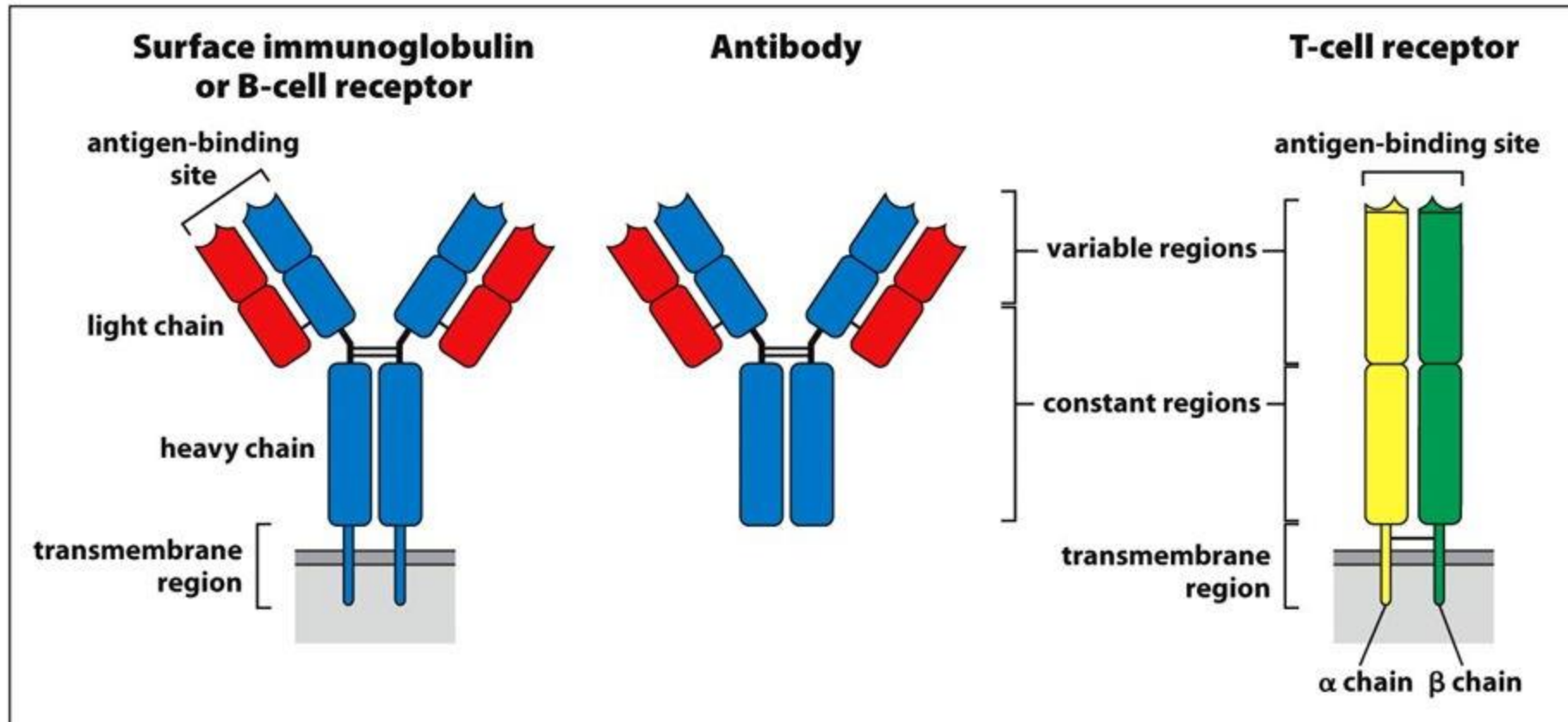
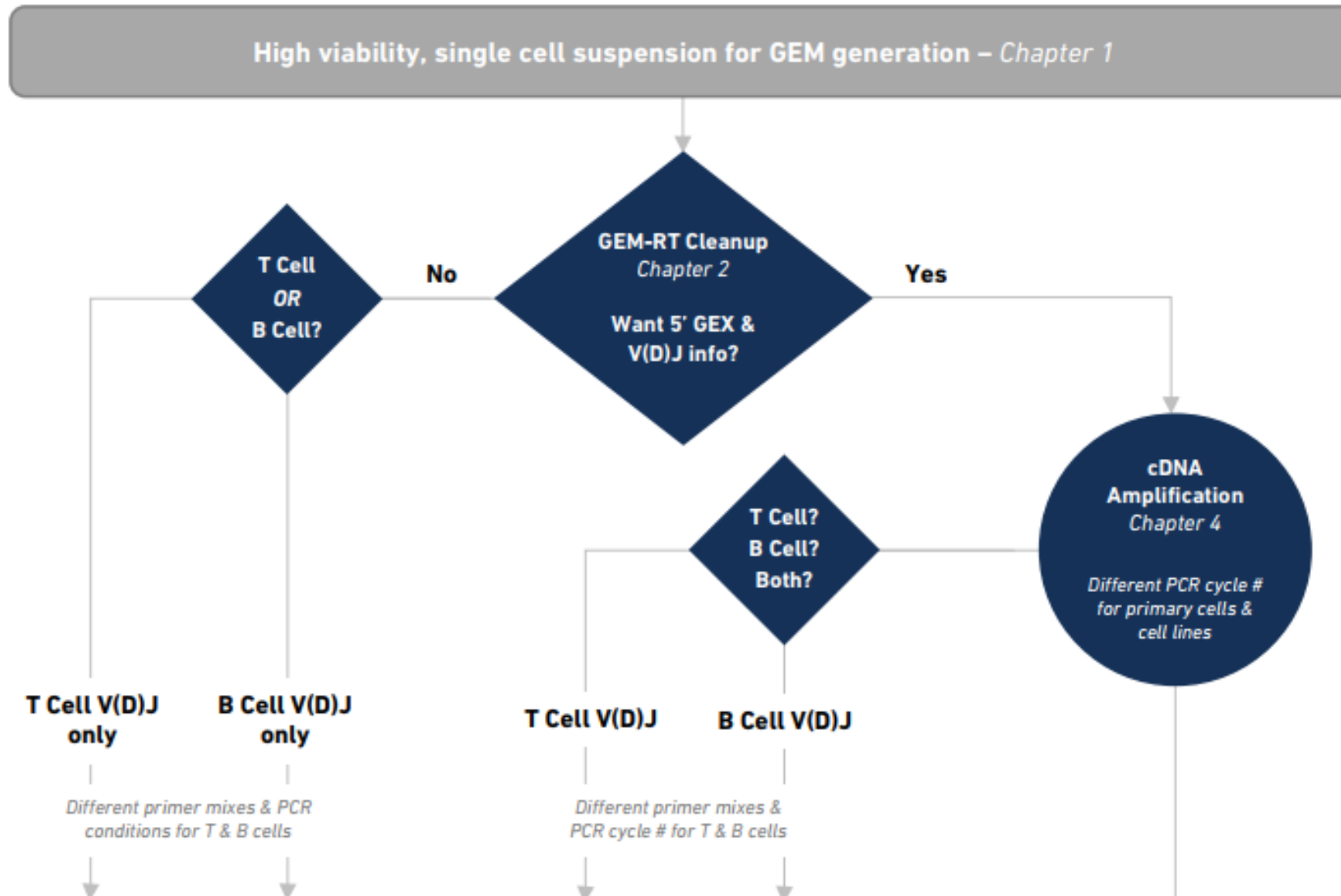


Figure 3.1 The Immune System, 3ed. (© Garland Science 2009)

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



Gel bead oligos

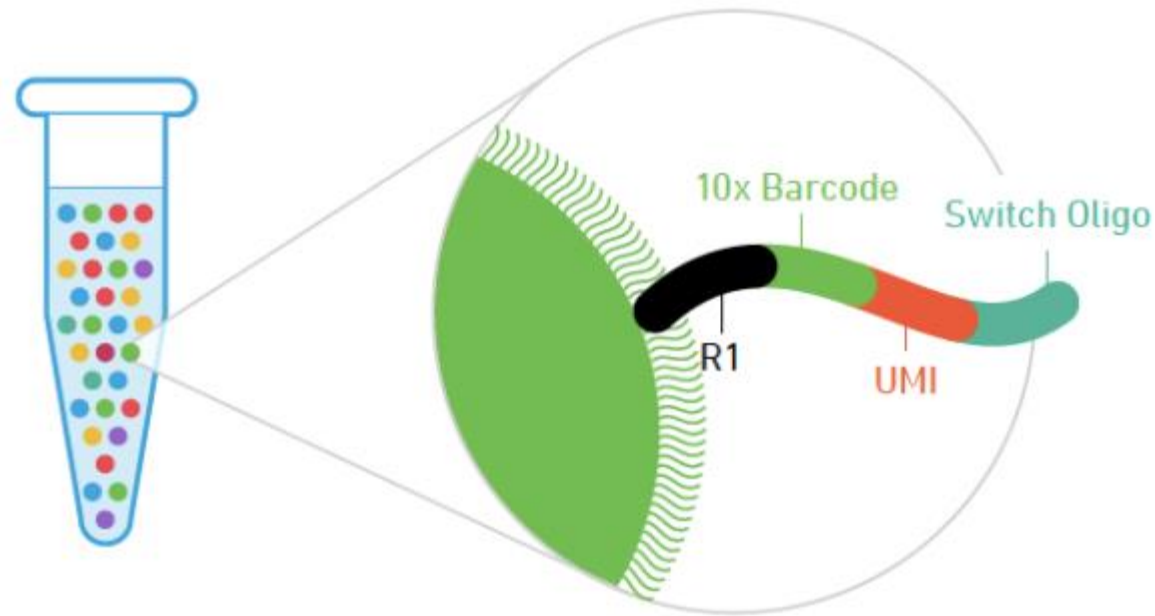
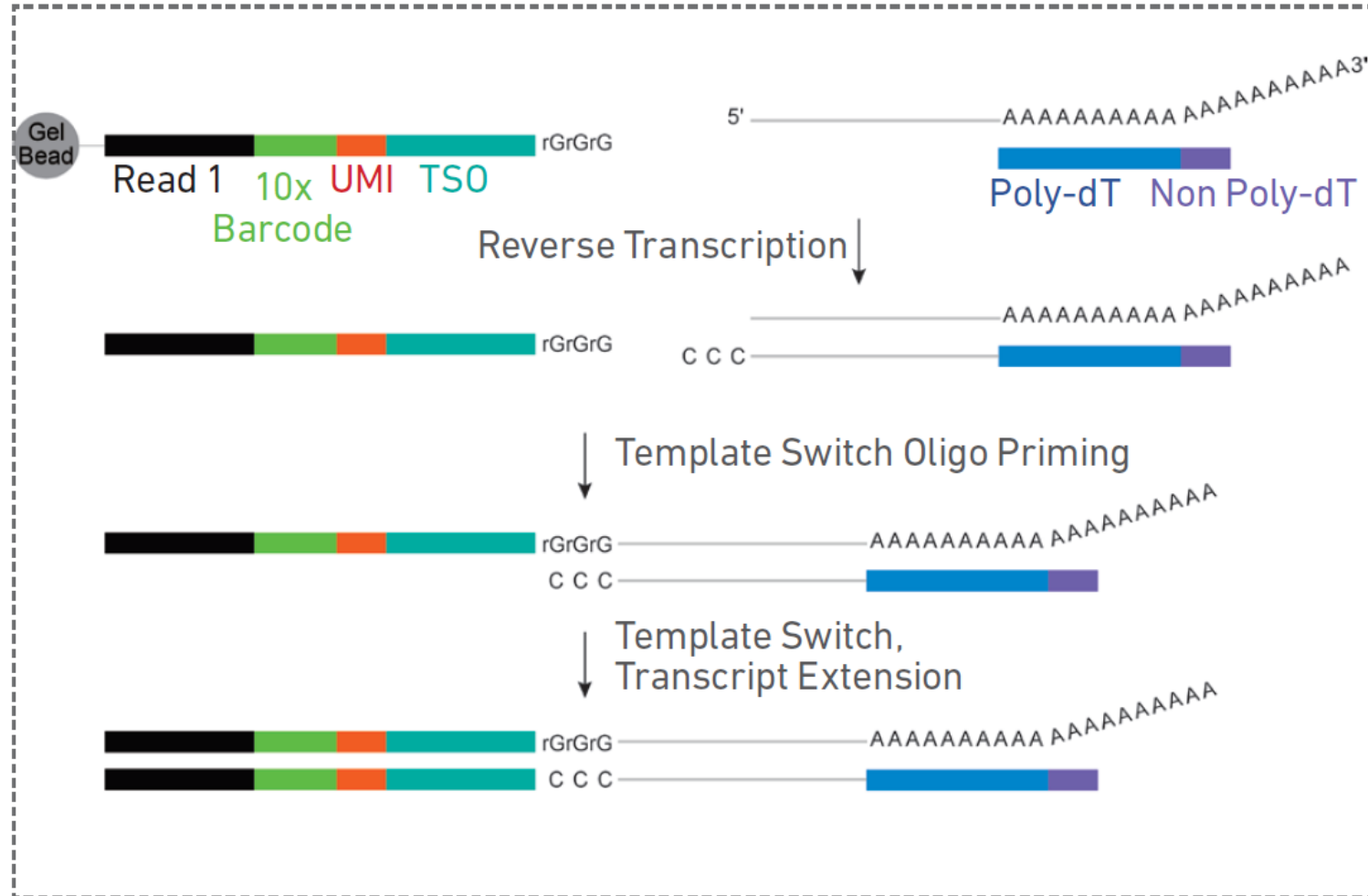


Figure 1. Schematic of a Single Cell 5' Gel Bead oligo primer.

- 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

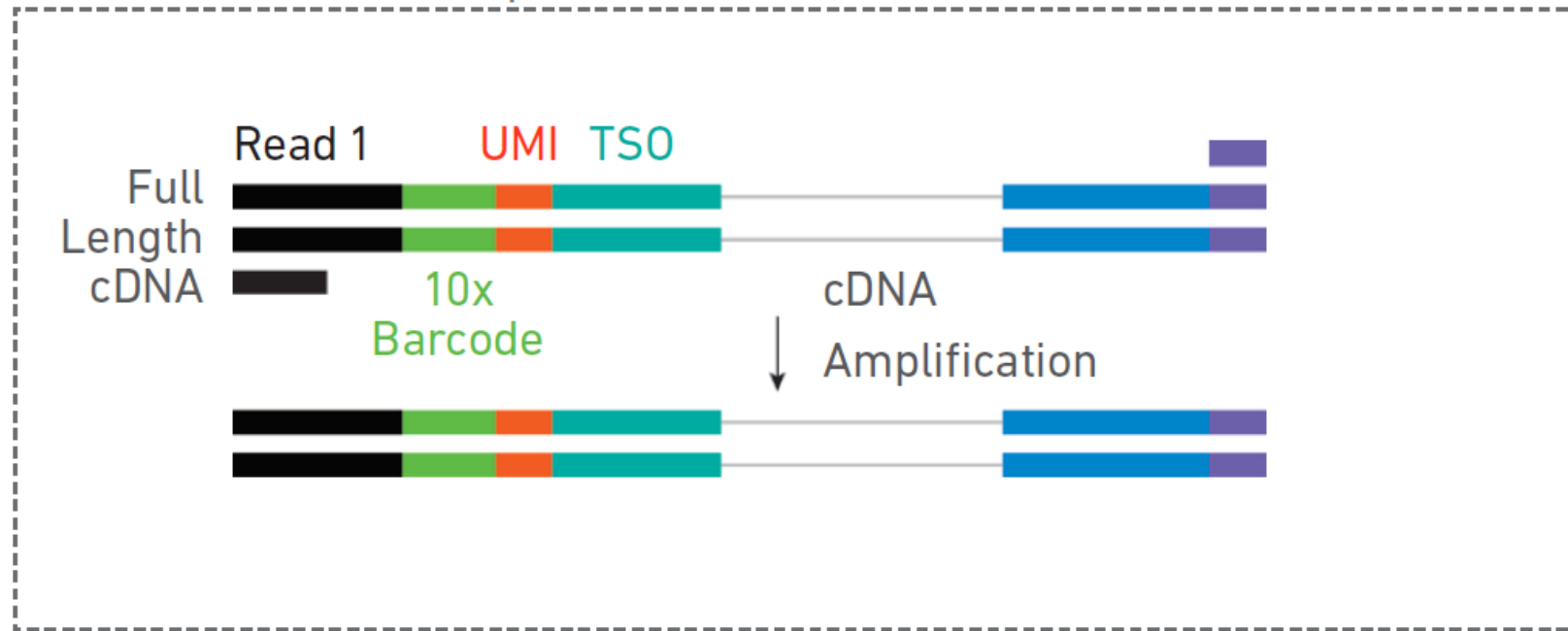
Assay scheme for 5' scRNA-seq

Inside individual GEMs

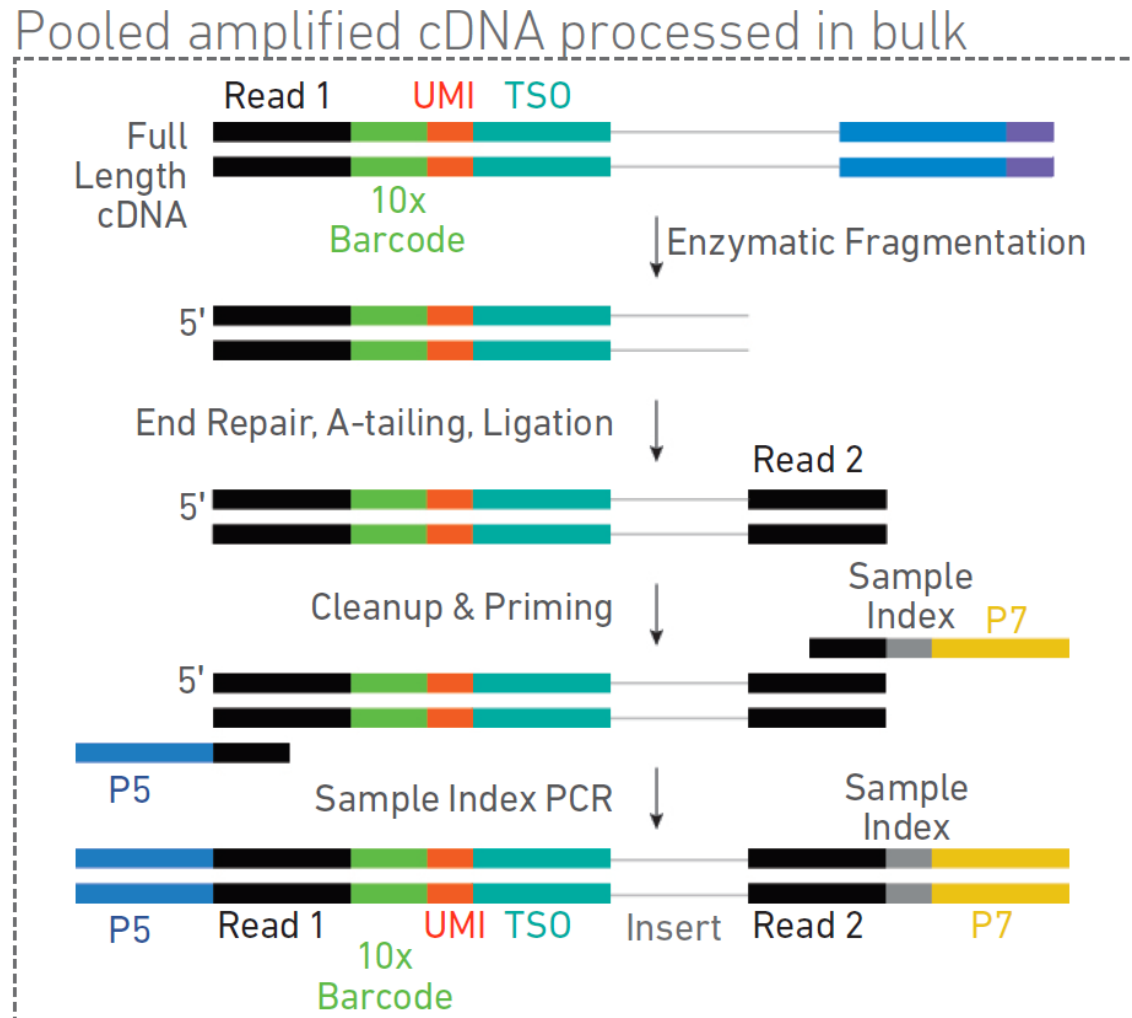


Assay scheme for 5' scRNA-seq

Pooled cDNA amplification

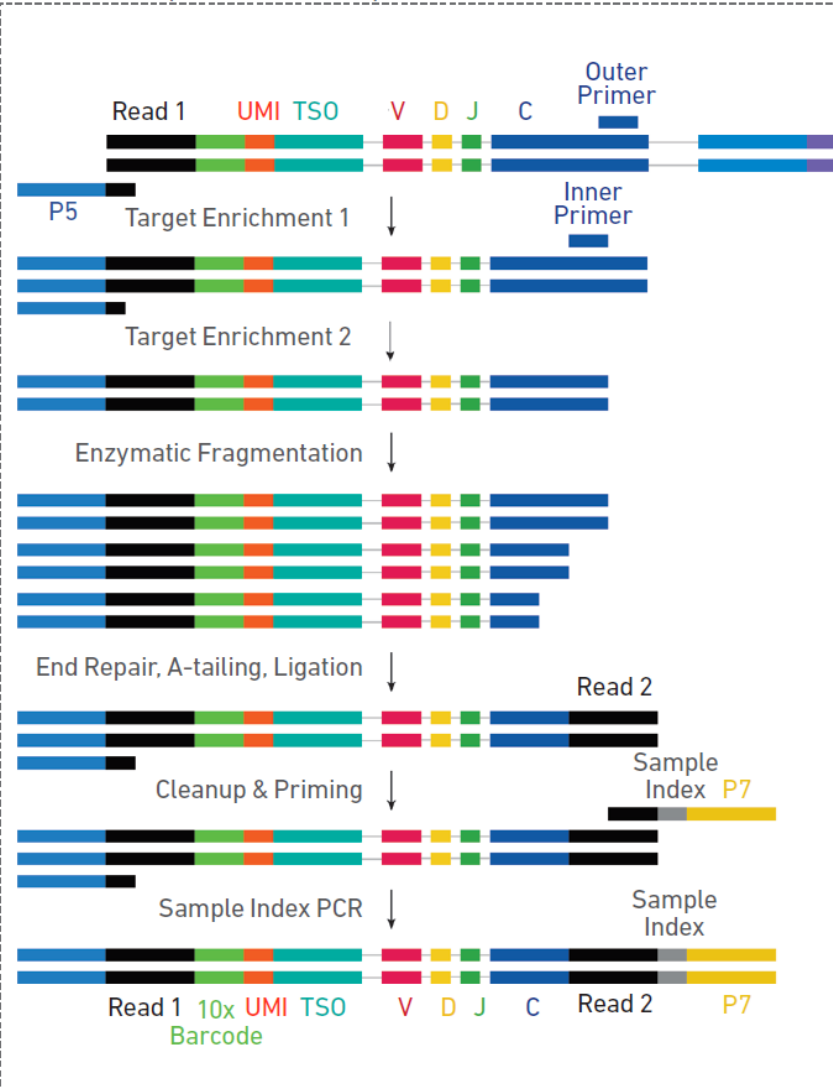


Assay scheme for 5' scRNA-seq



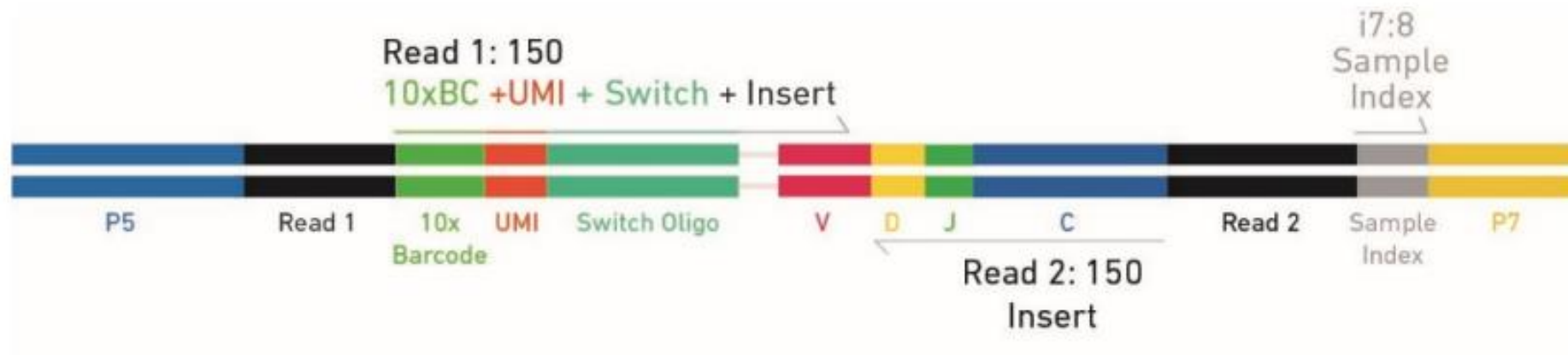
Assay scheme for 5' VDJ libraries

Pooled amplified cDNA processed in bulk



Final library structure

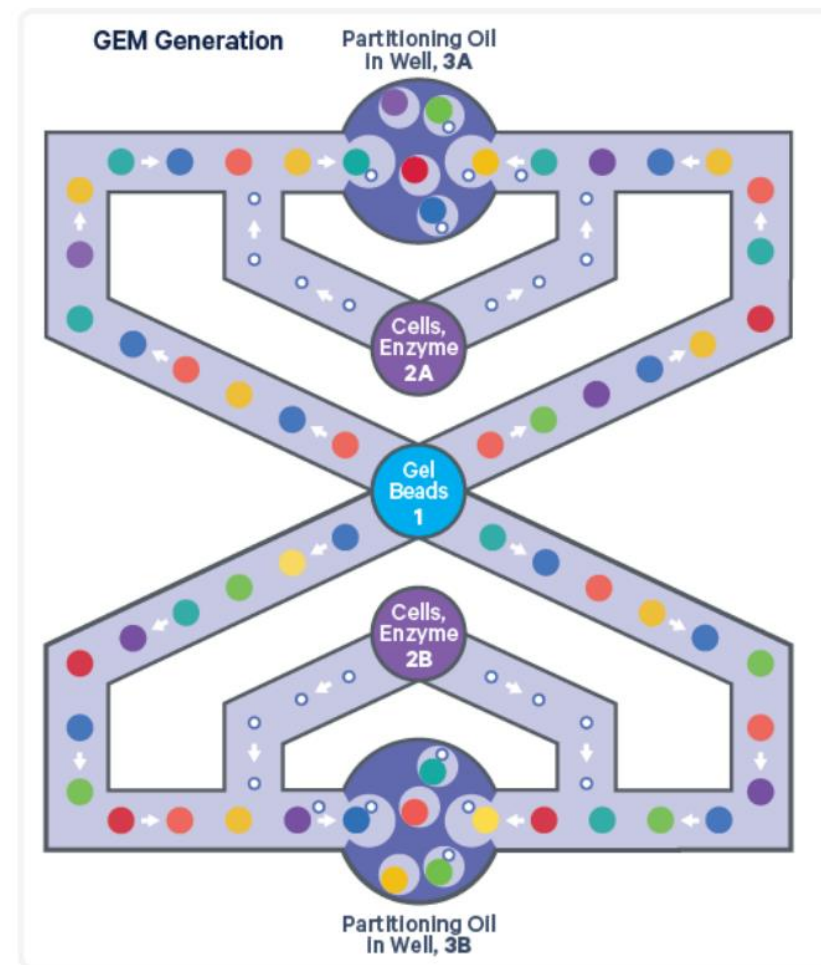
V(D)J Enriched Library Structure:



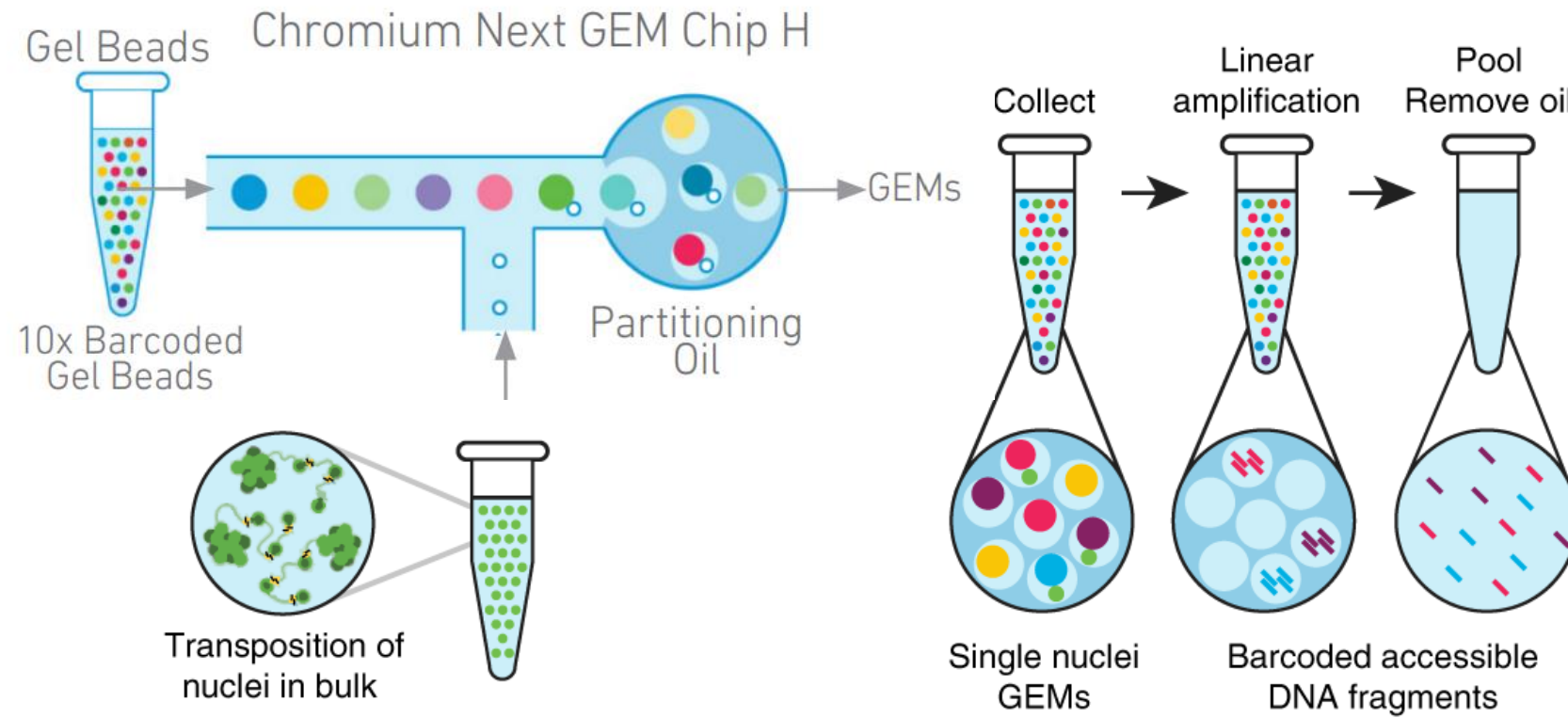
5' Gene Expression Library Structure:



NEW! Chromium X

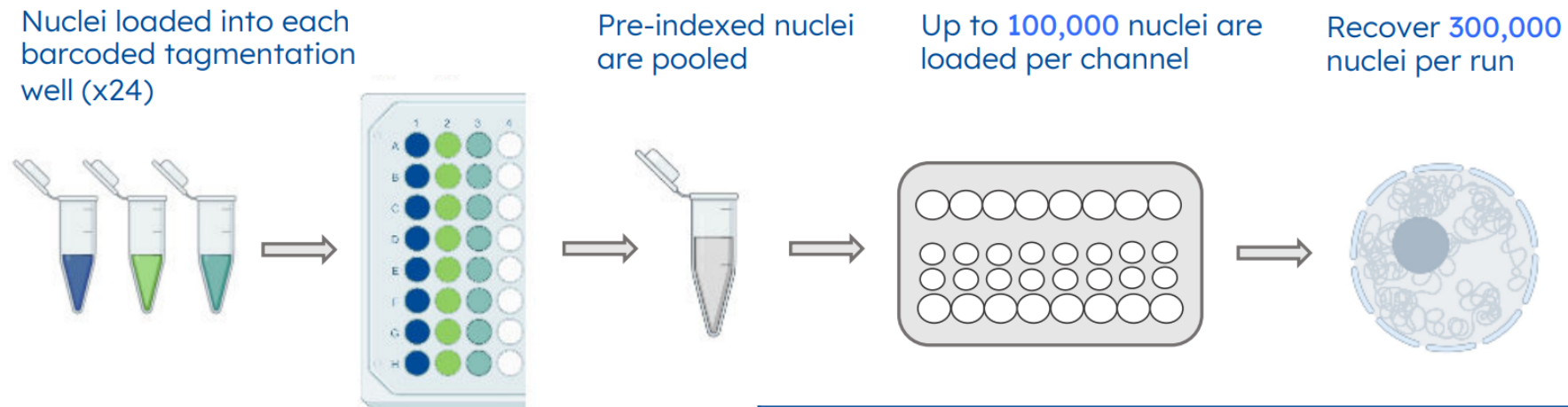


Single-cell ATAC-seq



Scale Biosciences

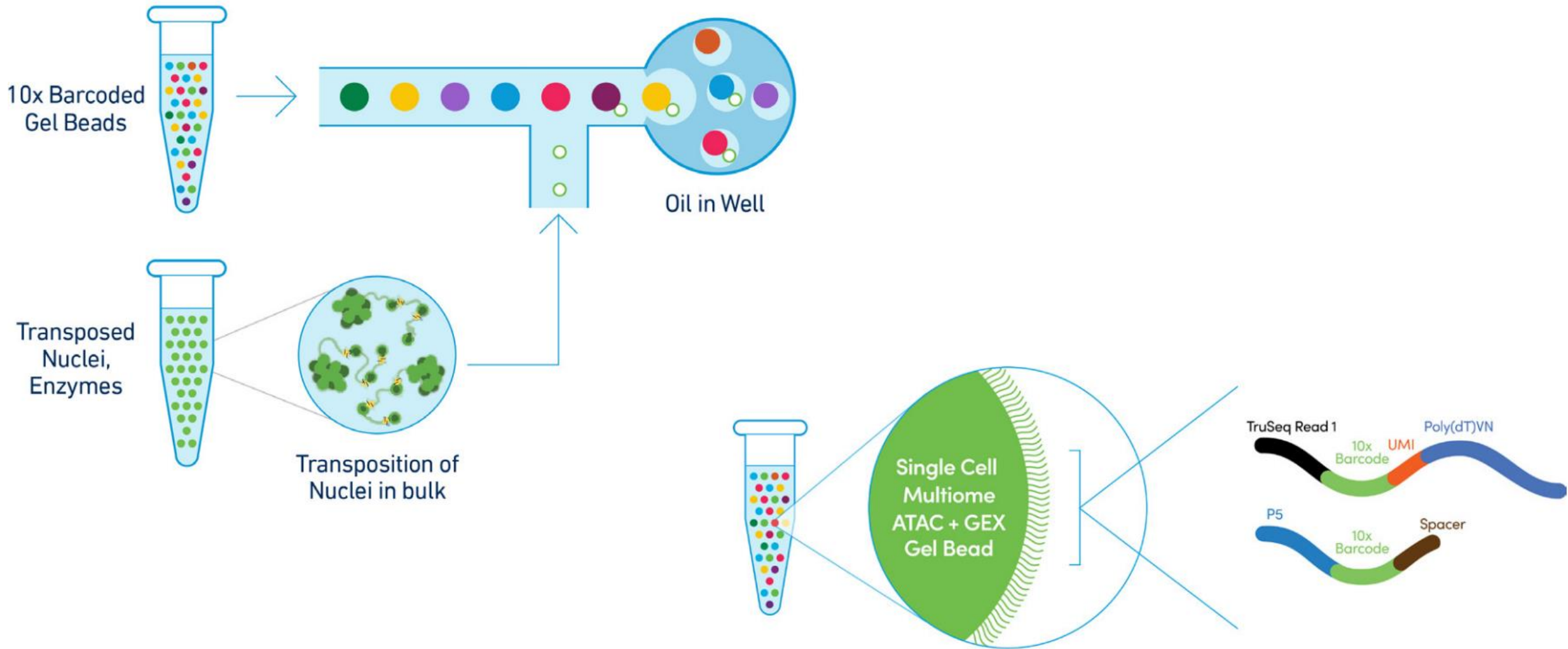
Pre-indexing kit compatible with 10x Genomics snATAC-seq



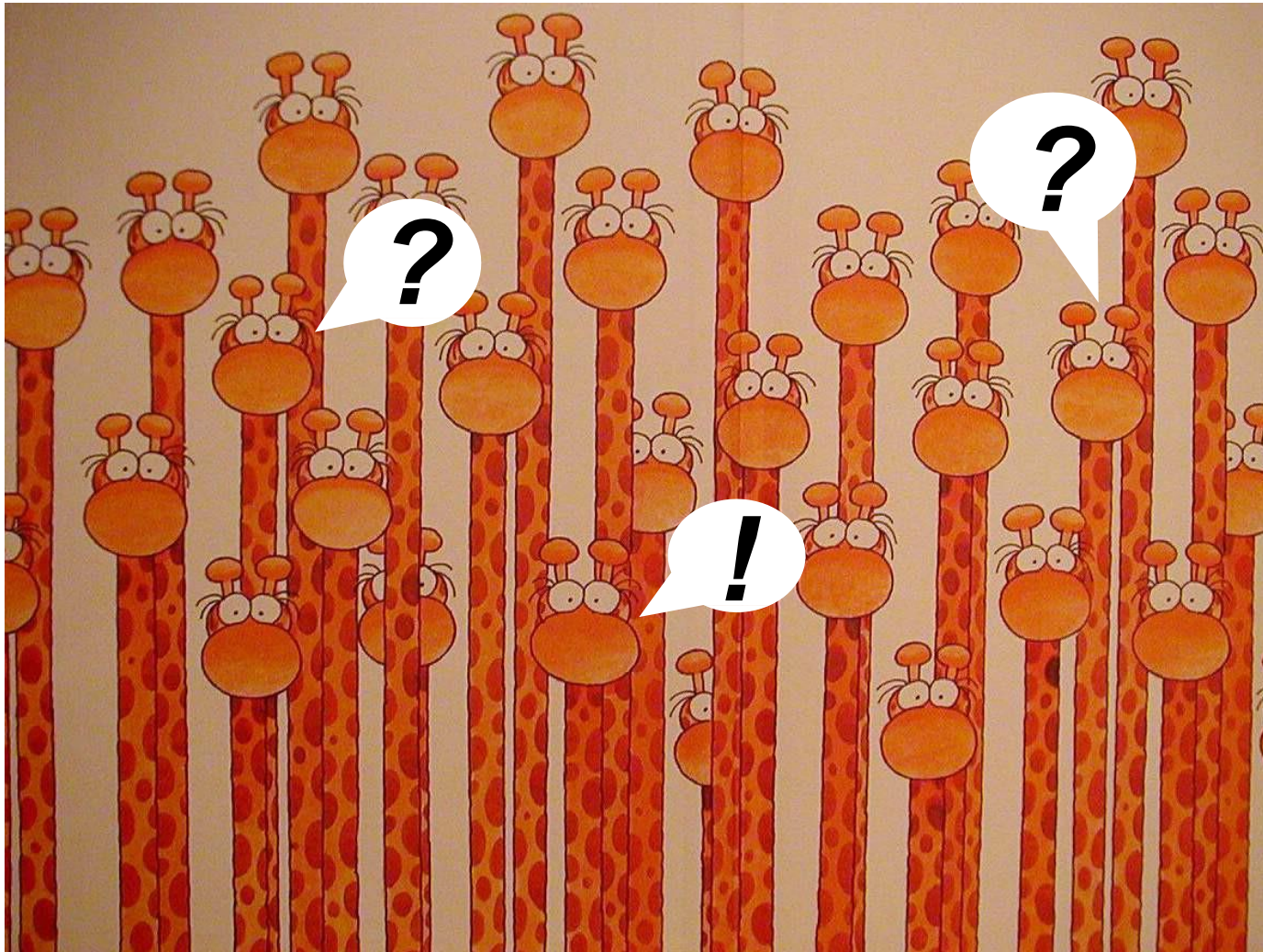
ScaleBio's bioinformatics pipeline resolves the combinatorial barcode, rescuing data from multiplets and *maintaining a 3-5% effective doublet rate.*

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?



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