

Introduction and Overview of Single-Cell Platforms

Susan Kloet

Leiden Genome Technology Center (LGTC)

MGC Course on Single-Cell Analysis

10 October 2022

Why single-cell?

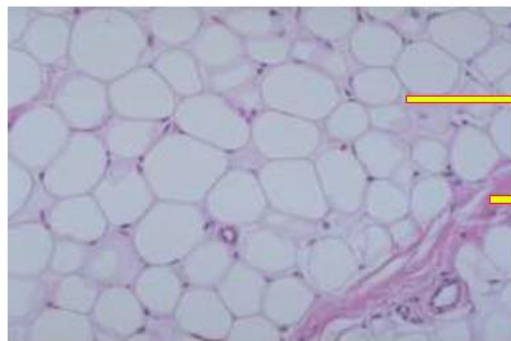
Bulk sample analysis is just like putting a fruit salad into a blender - the taste is an average of all ingredients.



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



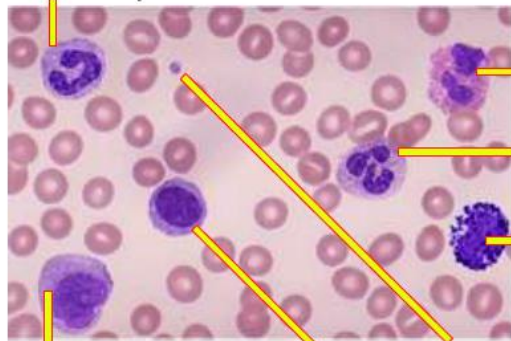
Adipose

Fat

Connective
tissue

Band
Neutrophil

Normal Peripheral Blood



Eosinophil

Segmented
Neutrophil

Basophil

Monocyte

Platelet
Lymphocyte

Small Intestine Mucosa



Epithelial cells

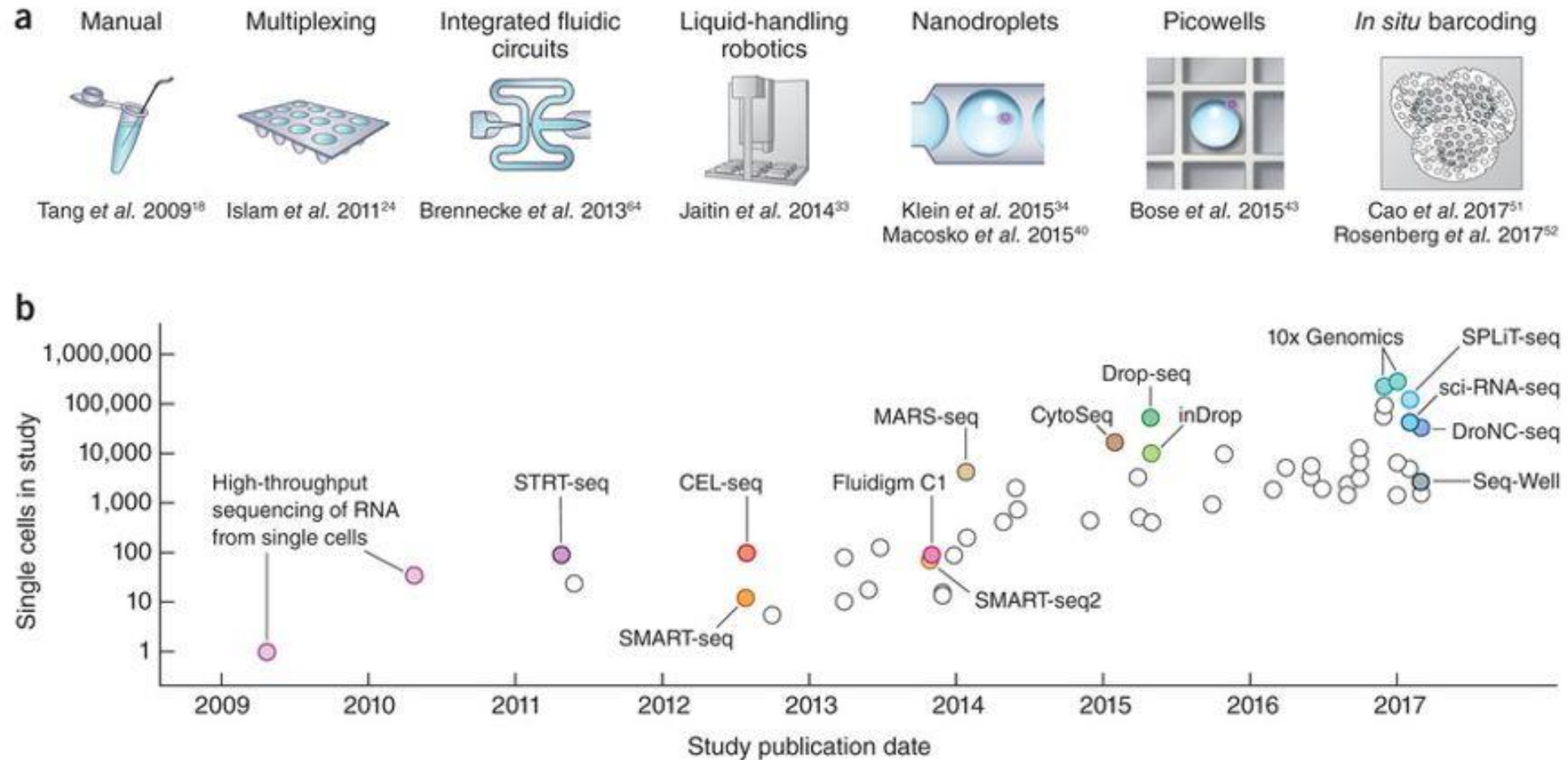
Goblet
cells

Lamina
propria

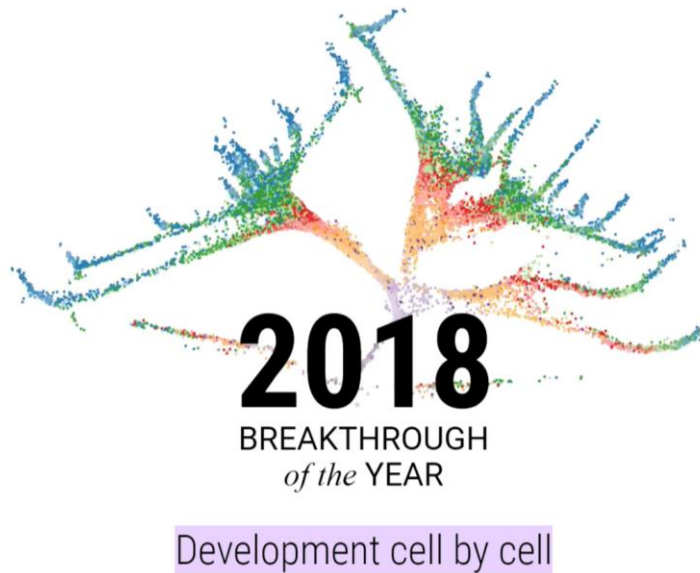
Muscularis mucosa
(smooth muscle)

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

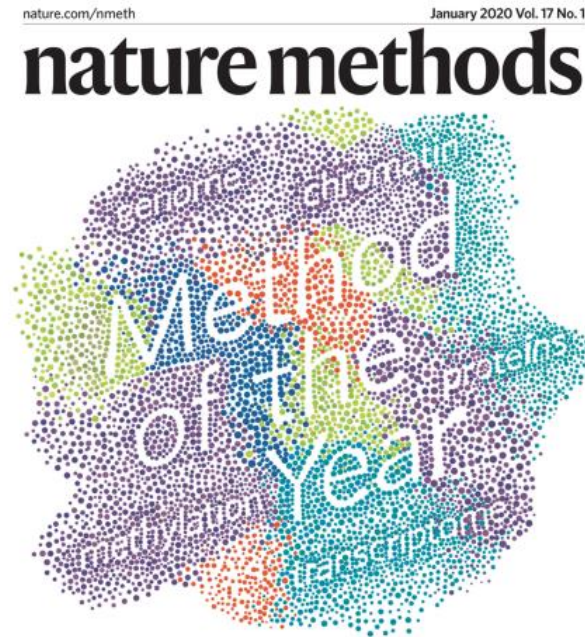
Exponential scaling of single-cell throughput



“The single-cell revolution is just starting.”



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

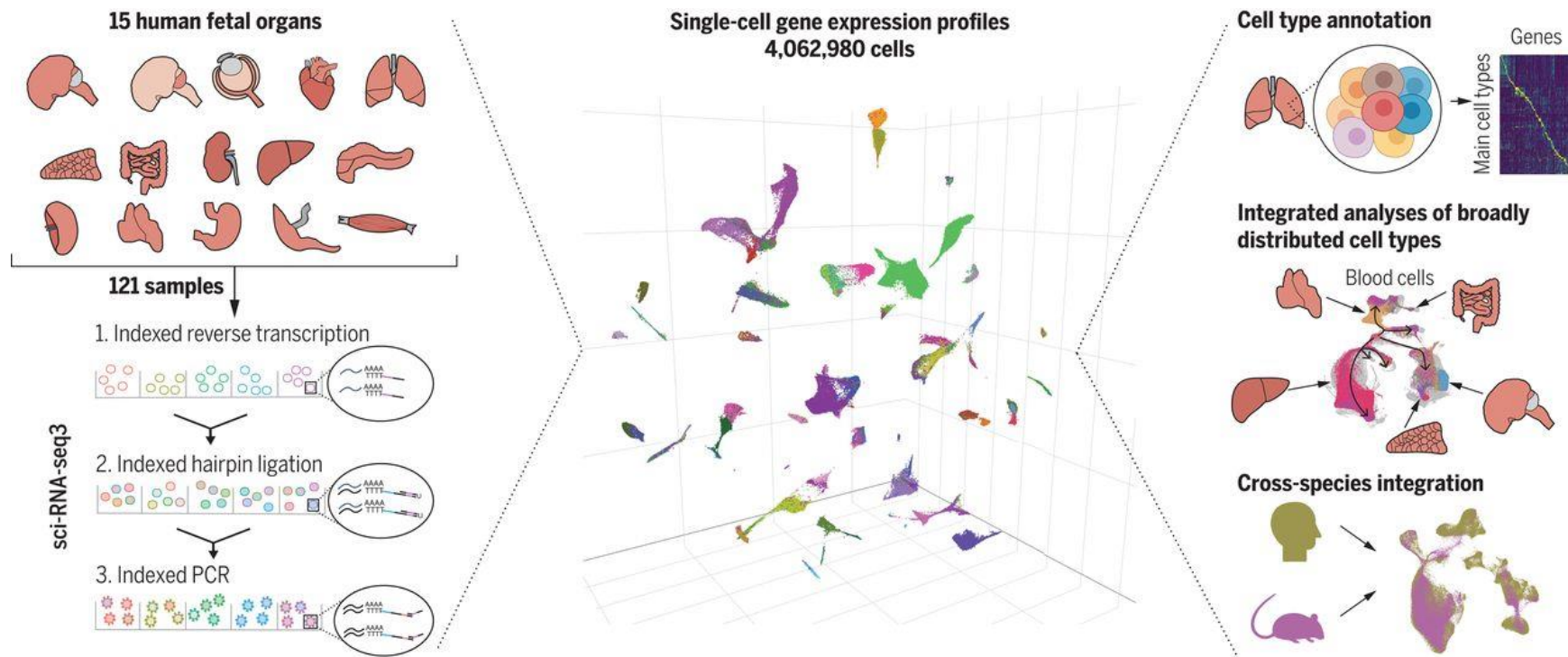


Single-cell multimodal omics
2019 Method of the Year



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
- Data integration
- Trajectory inference
- Differential gene expression

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

WGS (whole genome sequencing)

DIY methods – Miao's multiomics lecture

Chromatin structure and accessibility

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

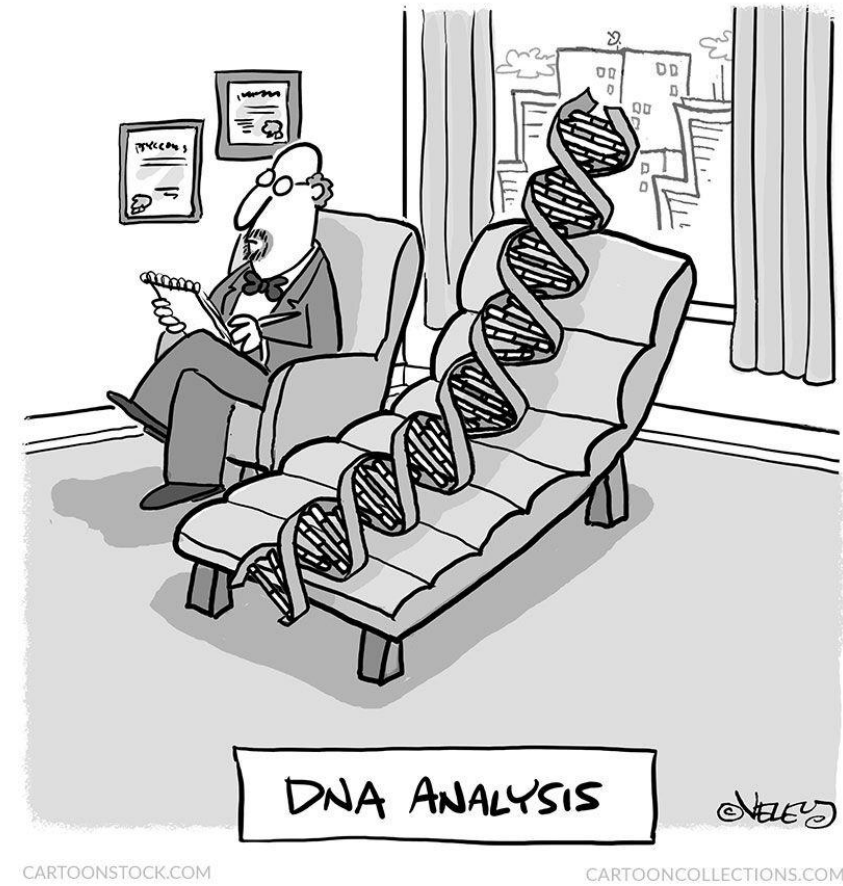
DIY methods – Miao's multiomics lecture

Commercial methods – This lecture

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

DIY methods – Miao's multiomics lecture

Commercial methods – Mission Bio, 10x Genomics (discontinued)



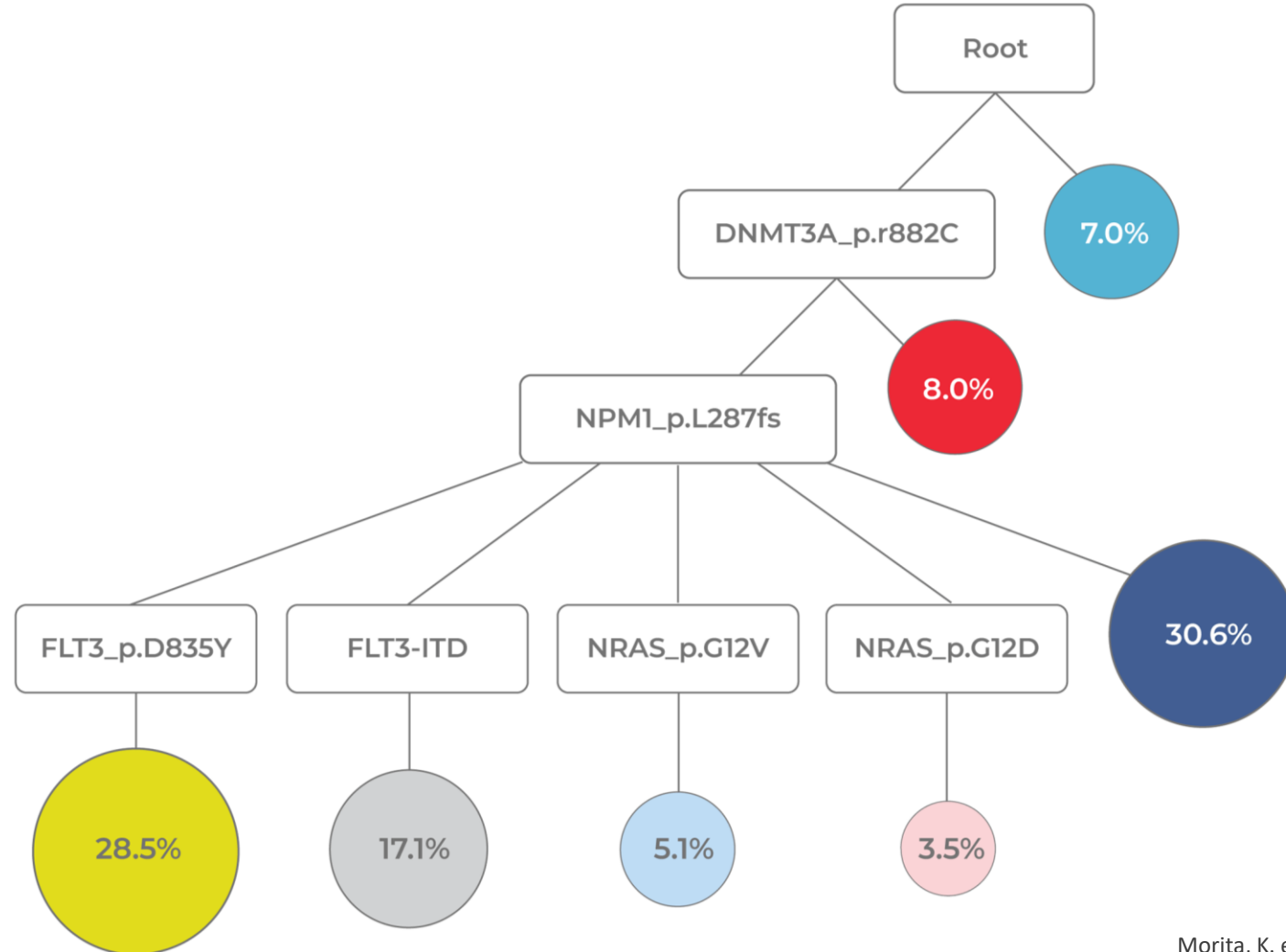
CNV and SNV detection – Mission Bio

Tapestri platform

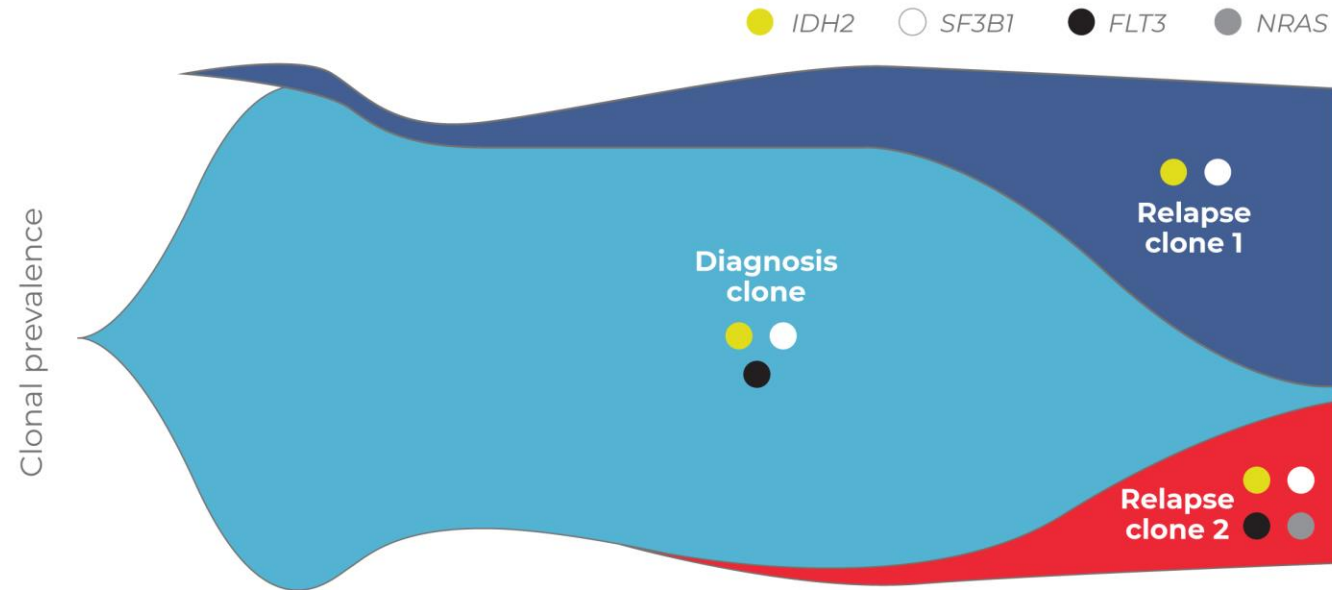
- Droplet-based amplification
- Up to 10k cells
- Panel-based PCR (up to 400 targets)
- Can call both CNVs and SNVs in target regions
- NEW multiomics application: Protein + DNA



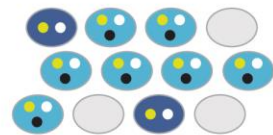
Track clonal evolution (AML)



Reveal therapy resistance

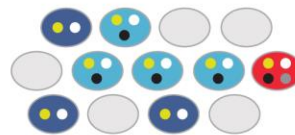


Diagnosis



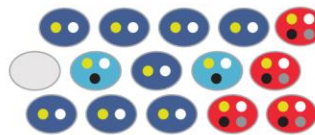
The tumor contains 2 clones. The light blue clone is dominant.

During Therapy



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

Relapse

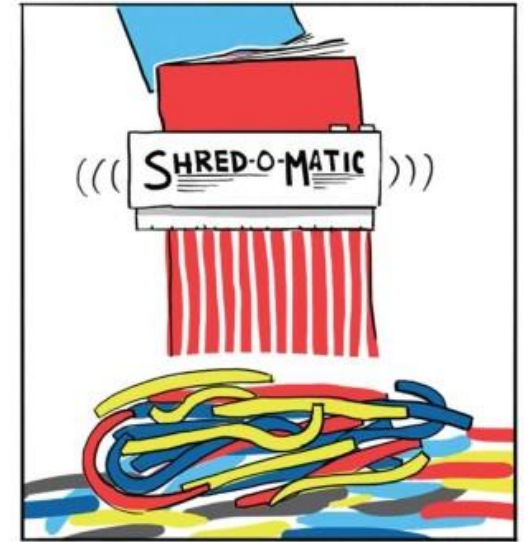


Post-treatment, the red clone & dark blue clone drive relapse.

scRNA-seq

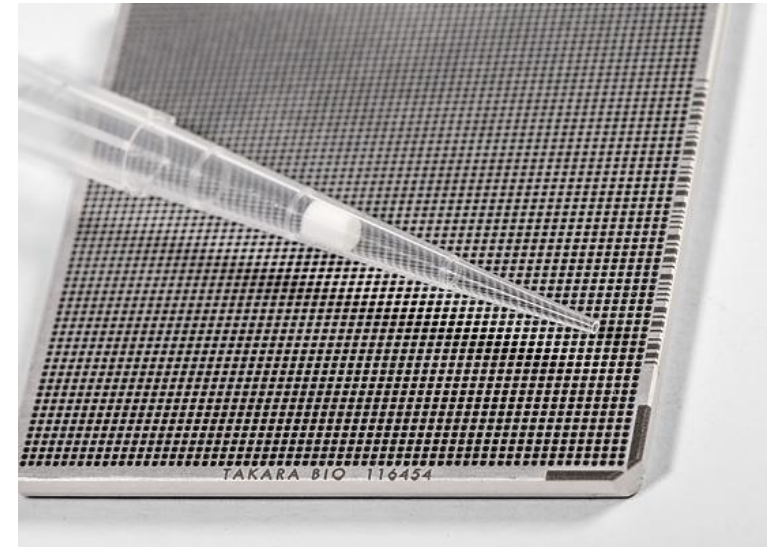
MANY different assays

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

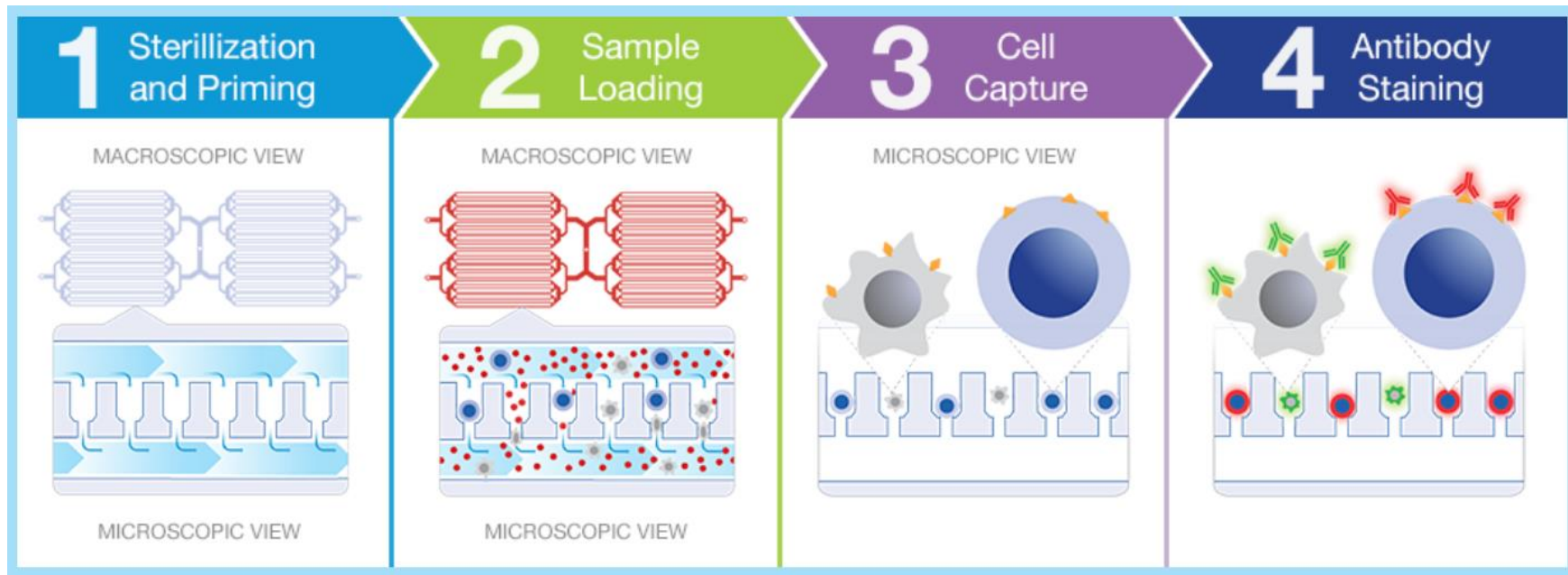


BD Rhapsody

- 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



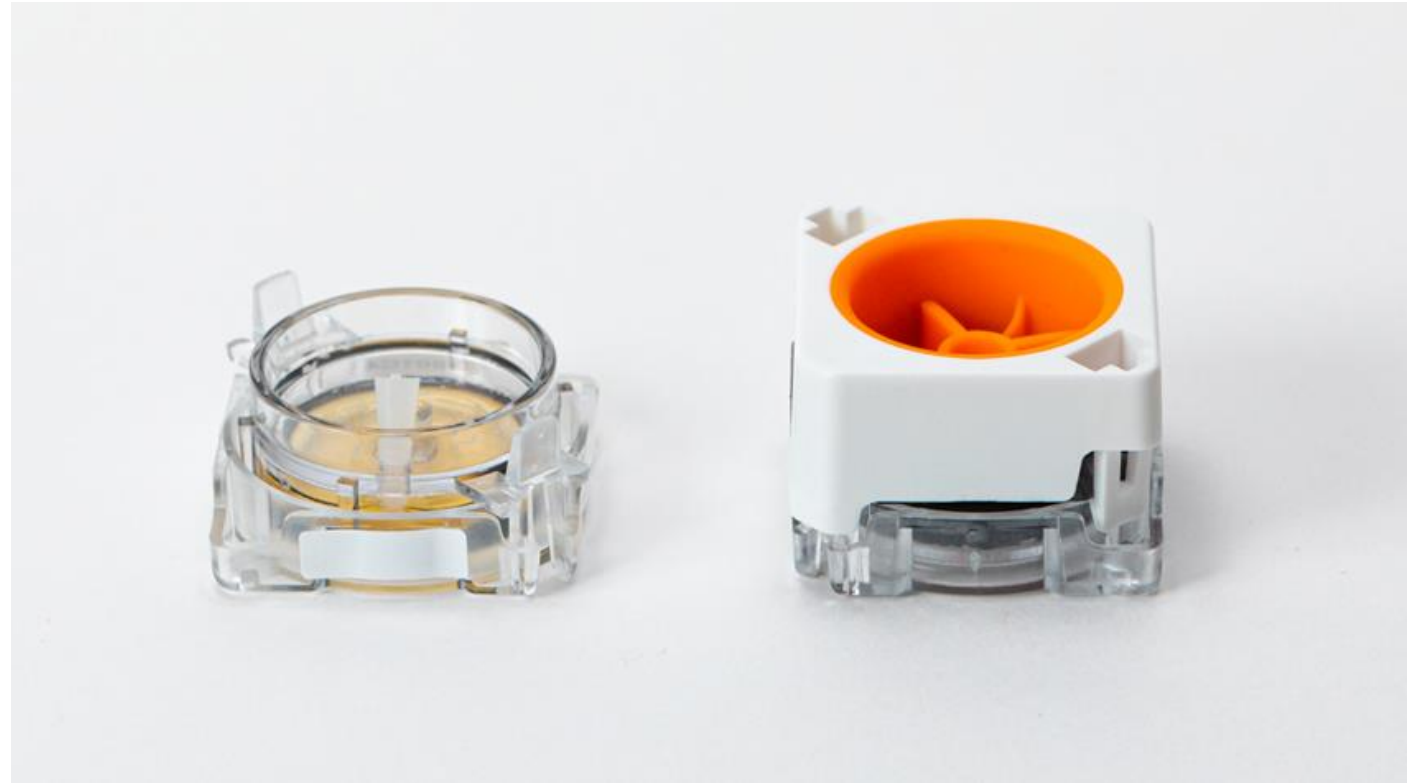
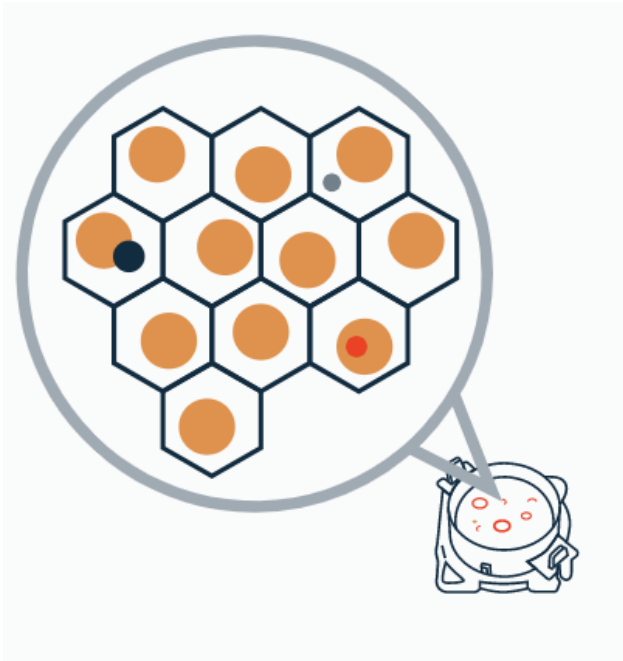
Celsee Genesis platform



Honeycomb

The HIVE - portable, single-use microwell chip

Store samples up to 9 months



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



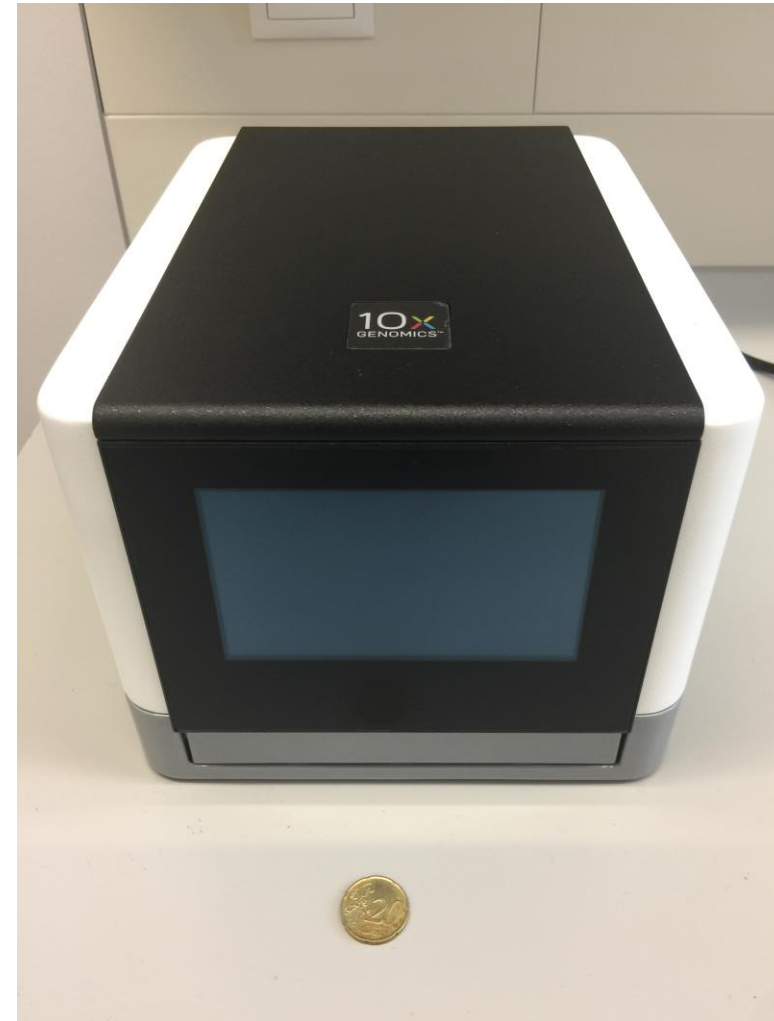
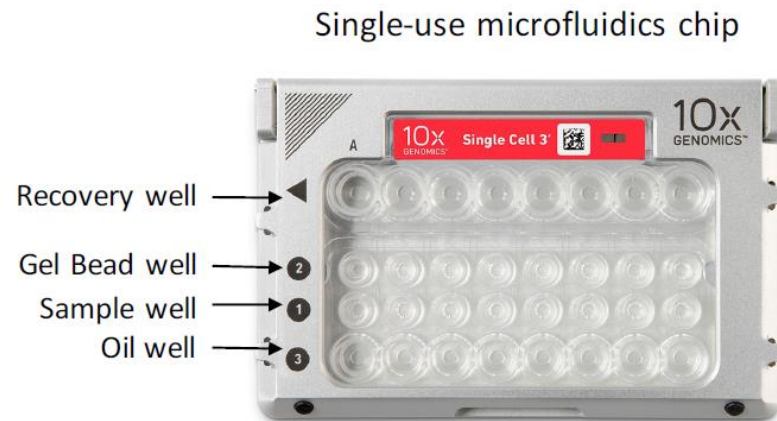
10x Genomics Chromium Controller

Commercial launch early 2016

Microfluidics system for reaction compartmentalization

High throughput, up to 80k cells/run

High capture rate, ~50%



10x Genomics products

TRANSCRIPTOMICS



Single Cell Gene Expression

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

Now with Feature Barcode technology!



Single Cell Immune Profiling

V(D)J repertoires of T and B cells integrated with 5' Gene Expression.

Now with Feature Barcode technology!



Spatial Gene Expression

Simultaneous analysis of molecular and imaging data from tissue sections.



Targeted Gene Expression

Profile a defined set of transcripts from single cells or tissue sections.

SINGLE CELL EPIGENOMICS



Single Cell Multiome ATAC + Gene Expression

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

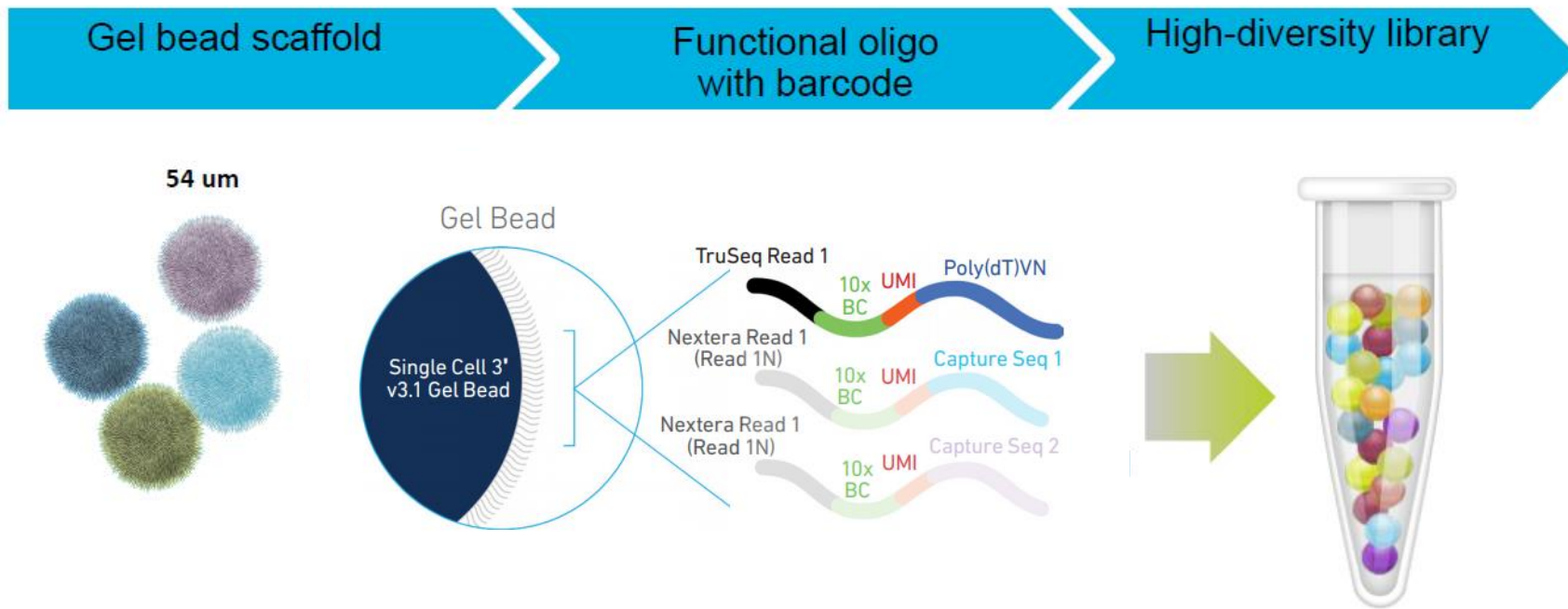


Single Cell ATAC

Chromatin accessibility and transcriptional regulation at the single-cell level.

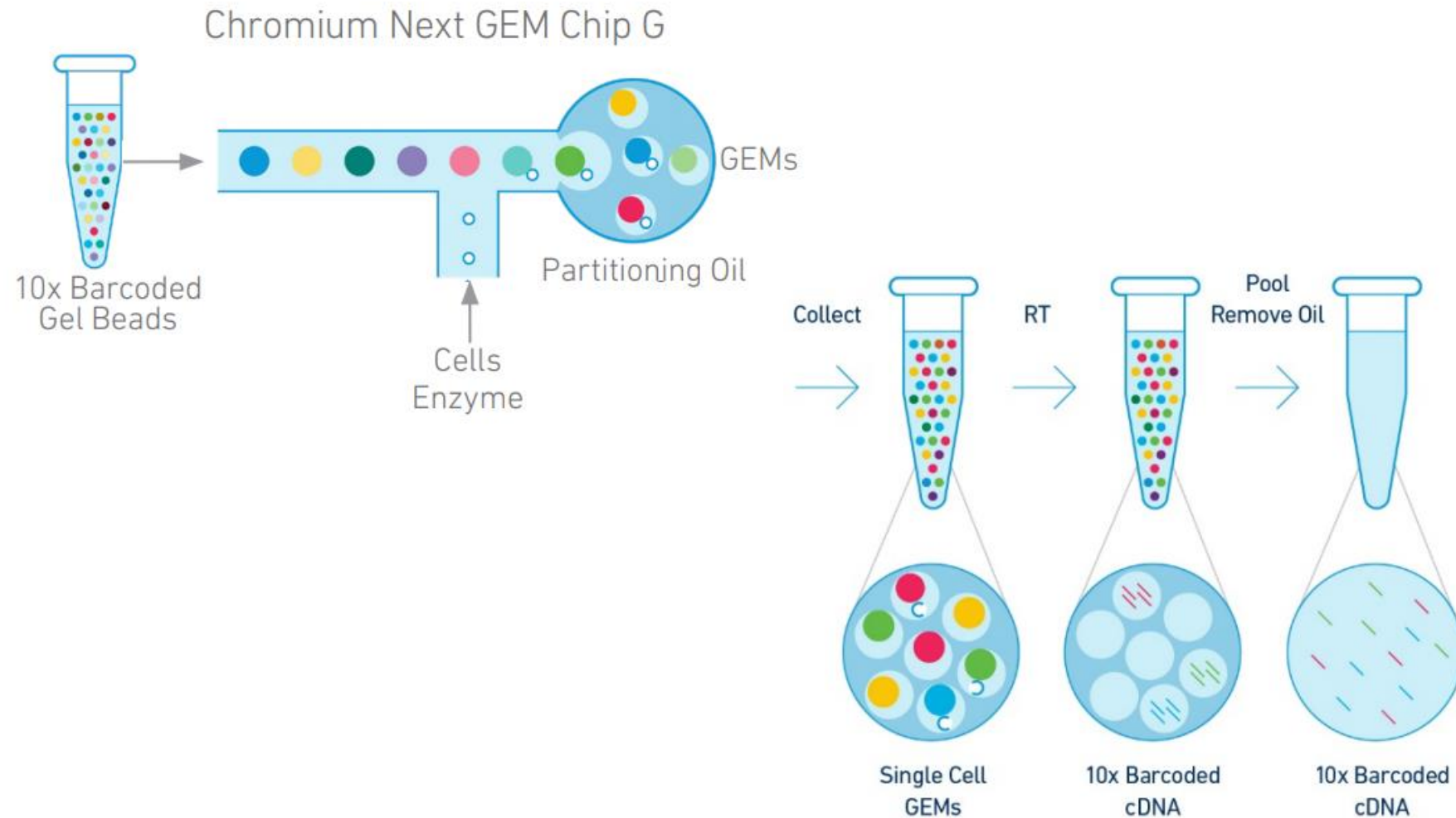
Gel beads up close

4M Discrete Reagents in One Tube

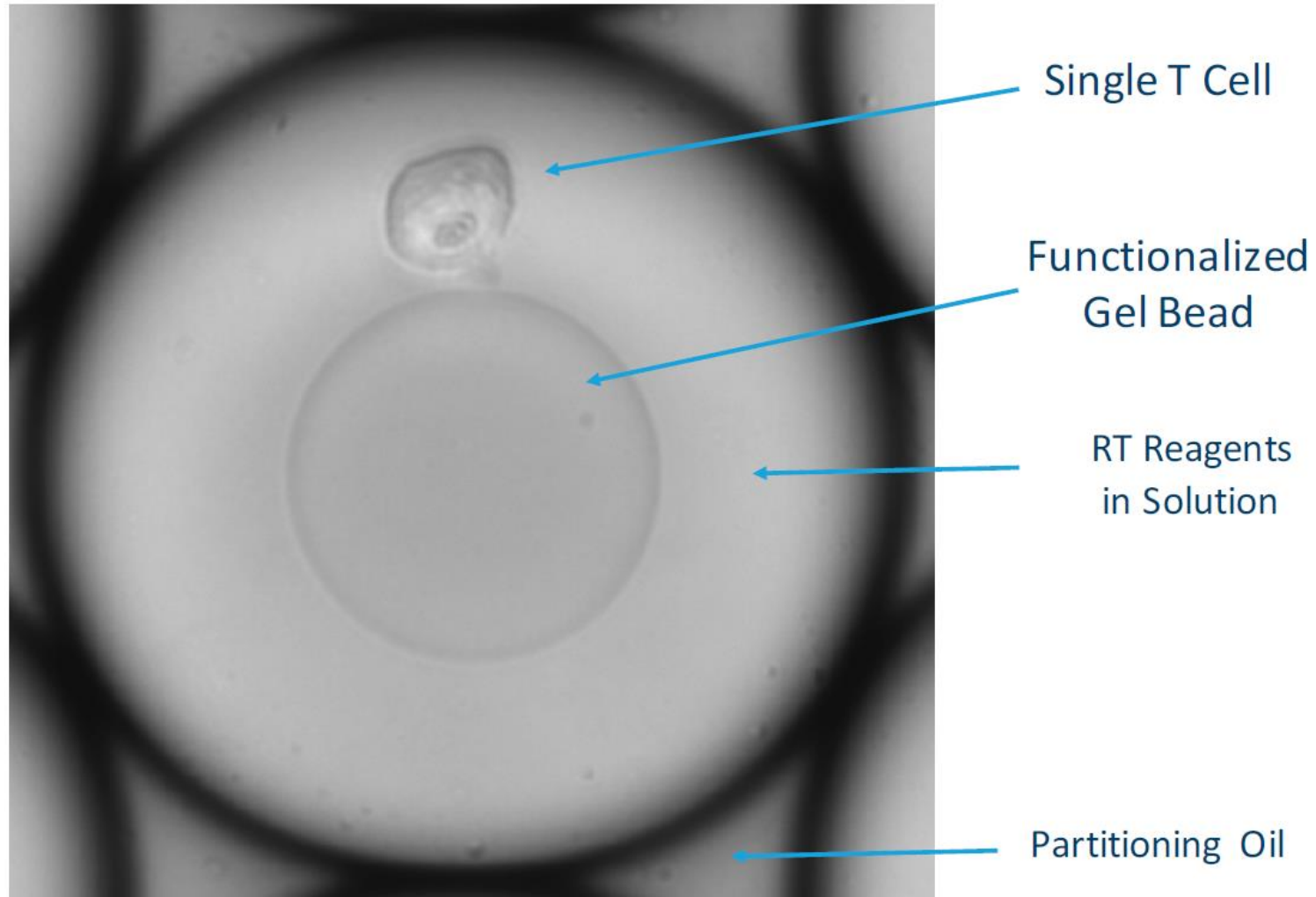


10x Barcode (16bp): unique for each GemBead
+ UMI (12bp): correct for PCR duplicates

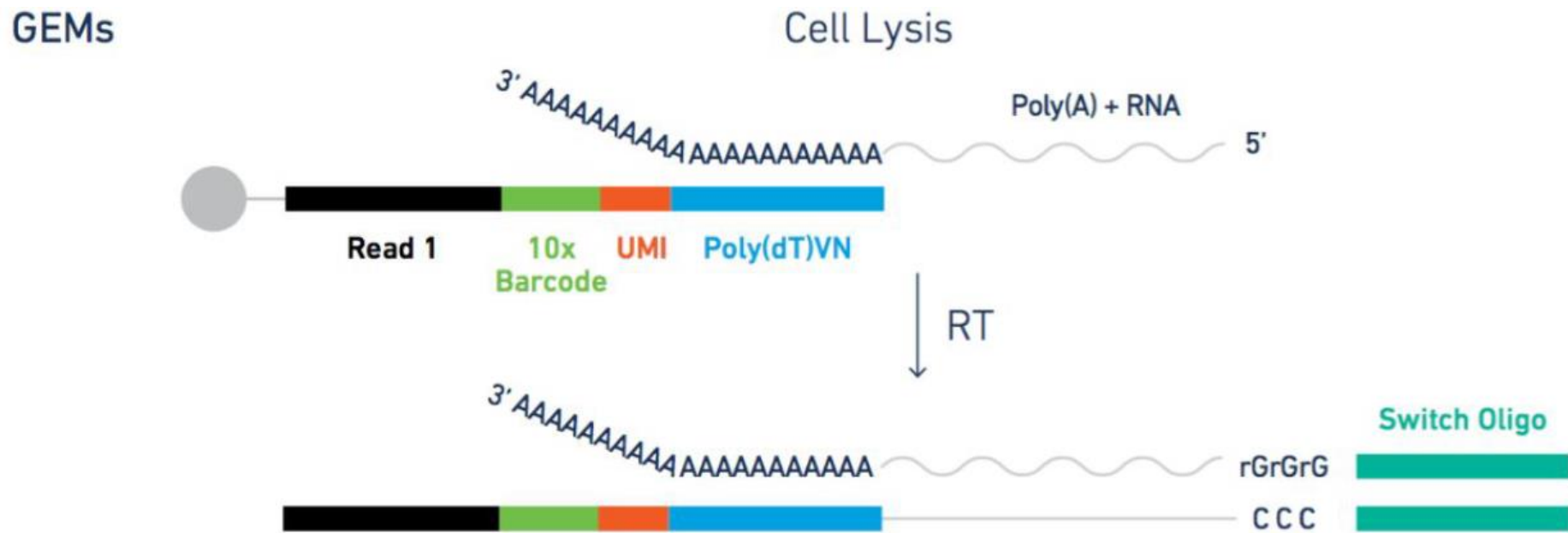
Gel bead in Emulsion (GEM) technology



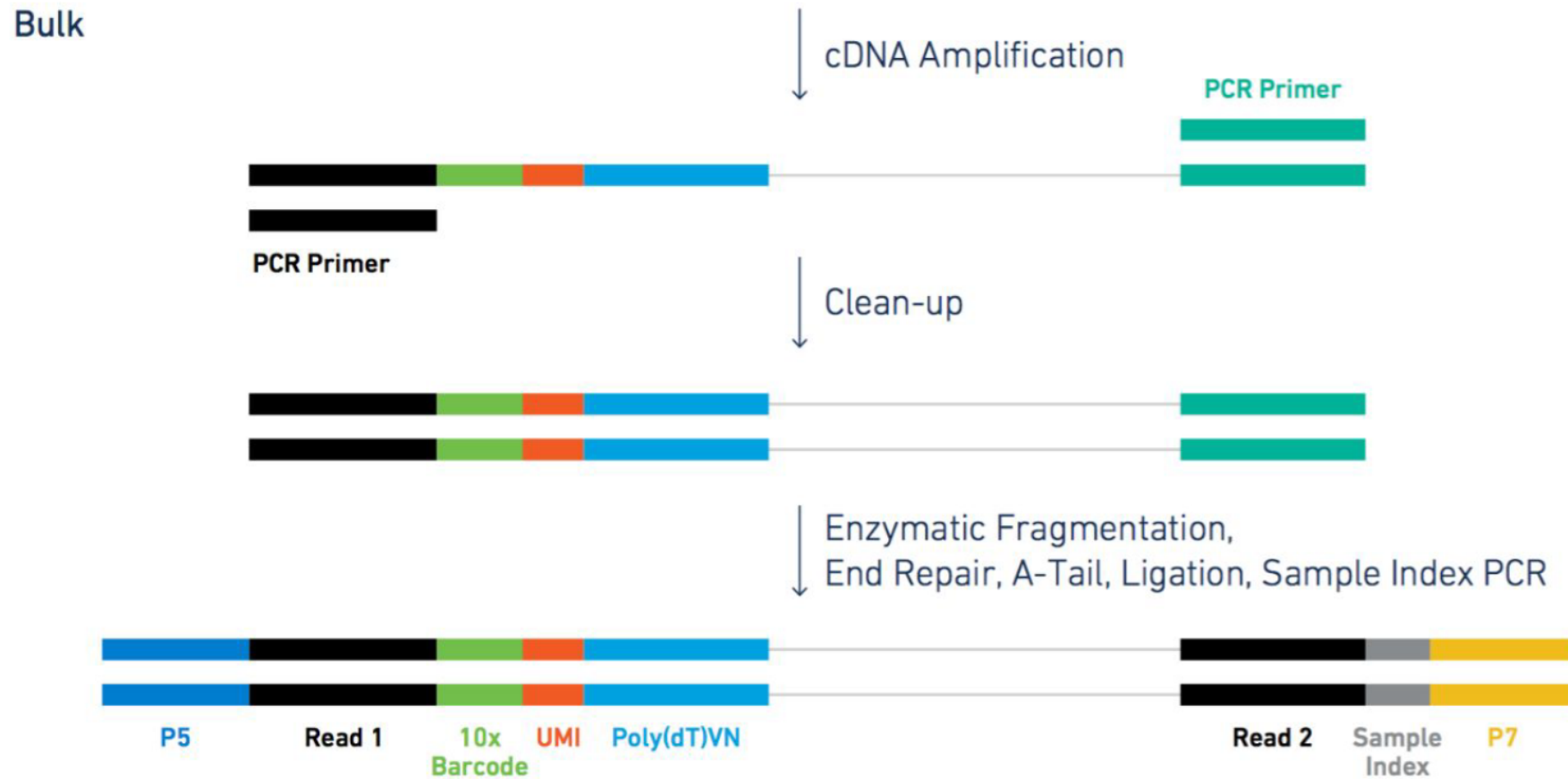
GEMs up close



Assay scheme for 3' mRNA sequencing

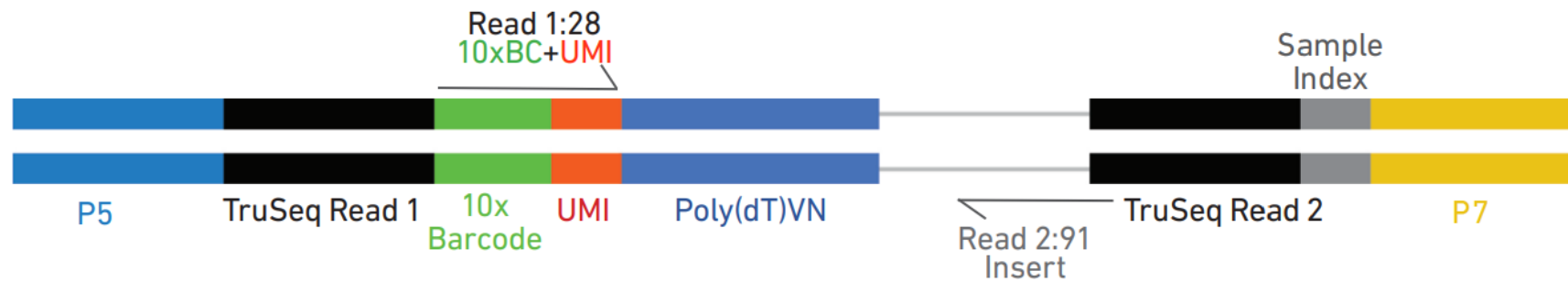


Assay scheme for 3' mRNA sequencing

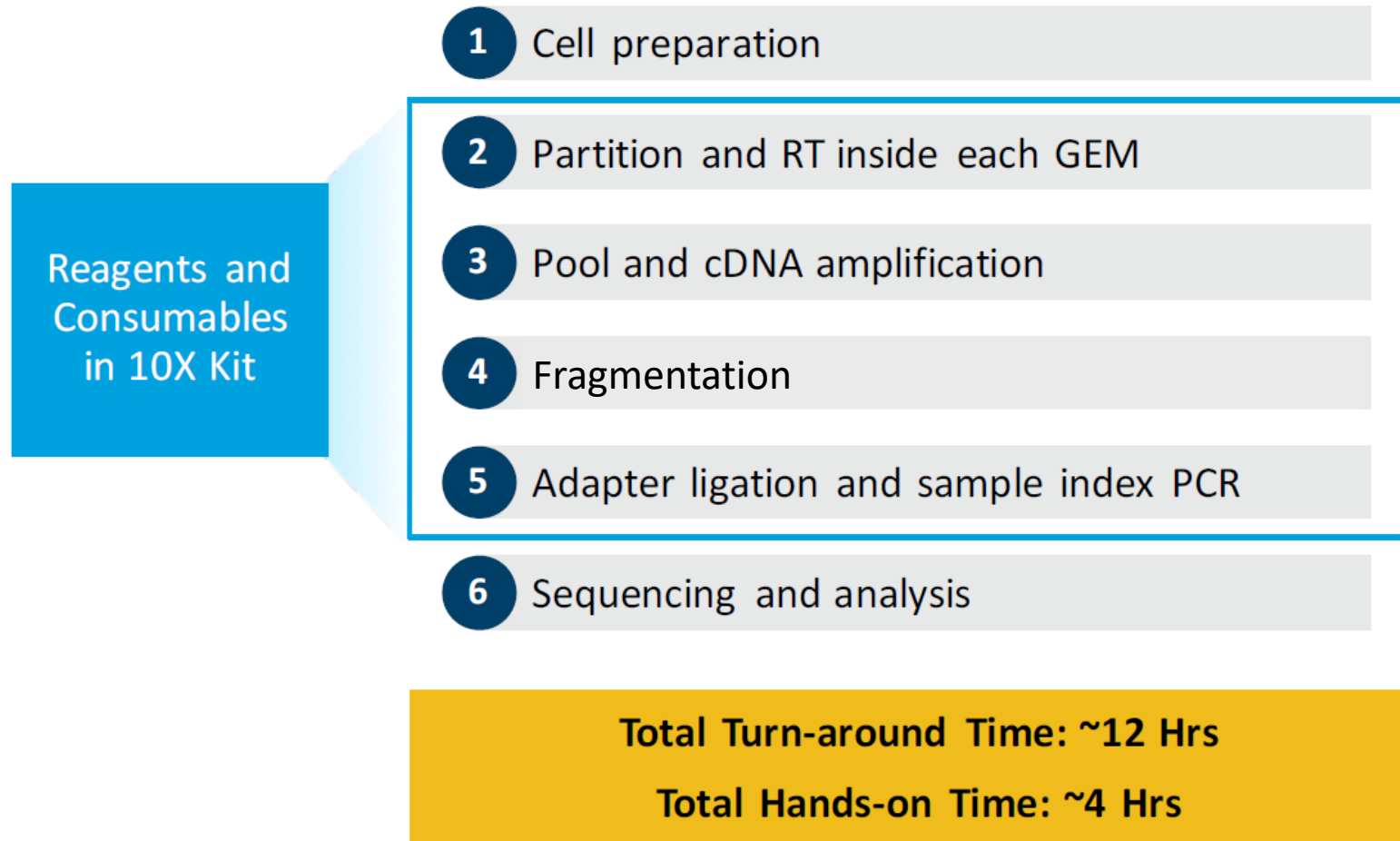


Final library structure

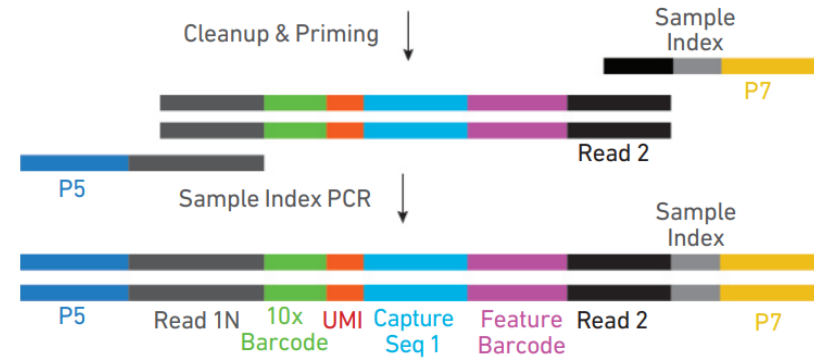
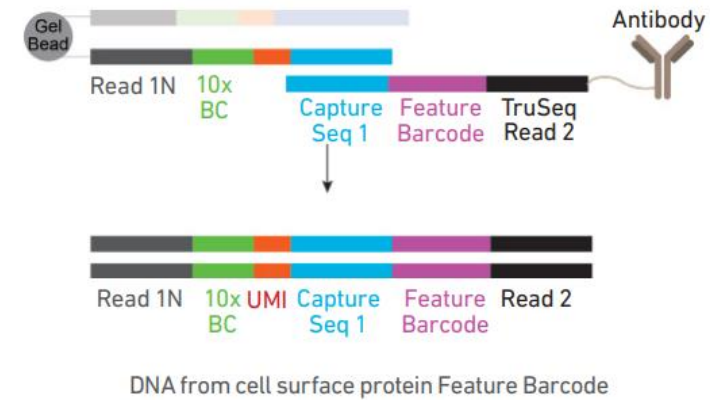
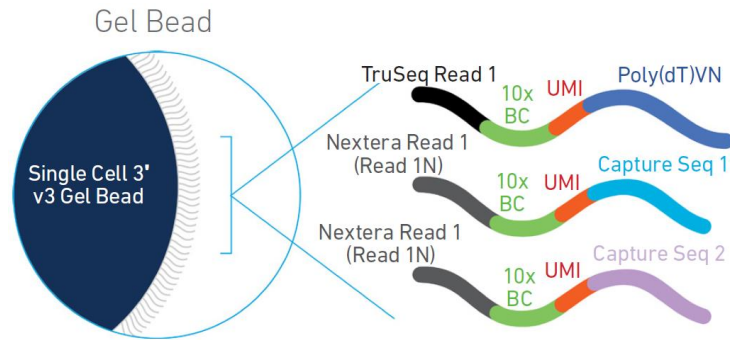
Chromium Single Cell 3' Gene Expression Library



Single cell 3' end-to-end workflow



Single cell 3' feature barcoding



Structure of T and B cell receptors

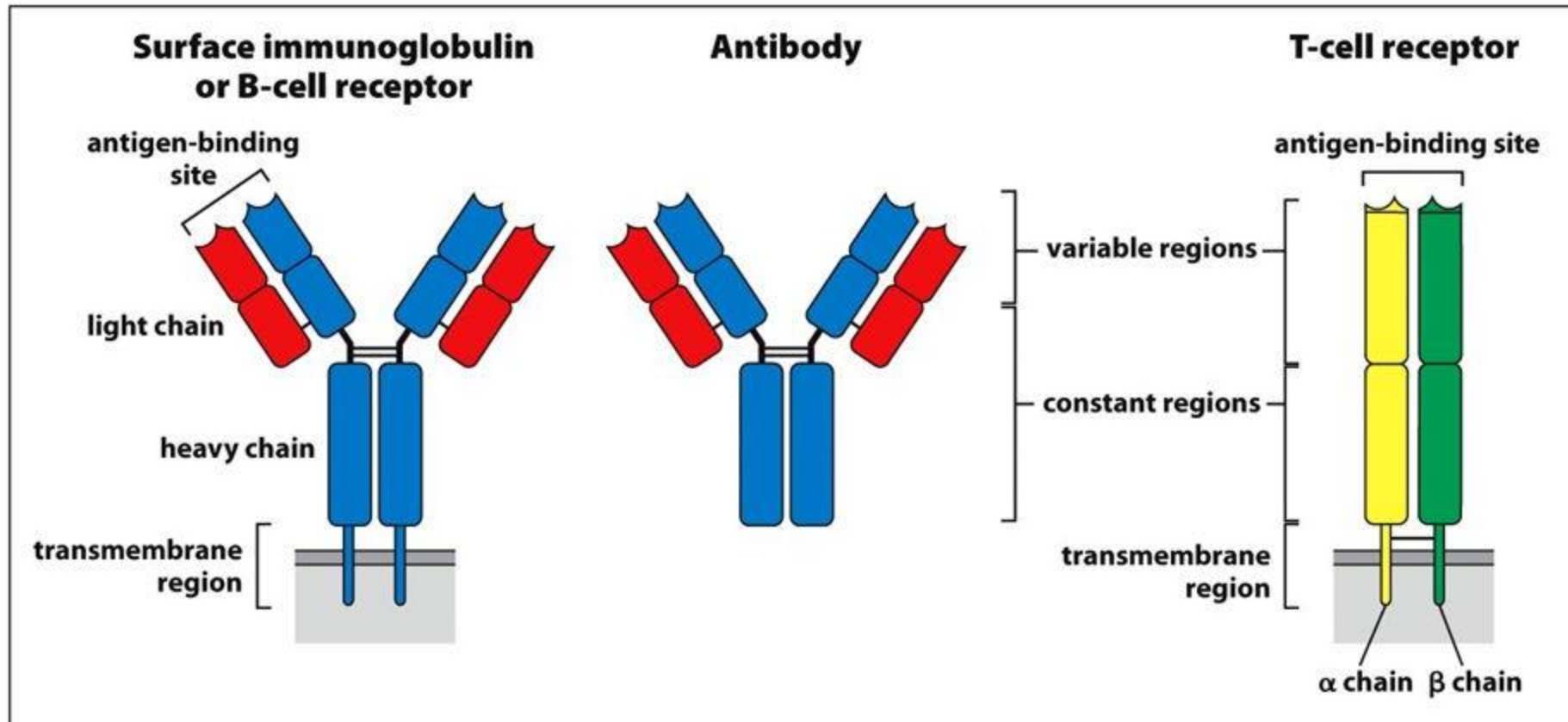
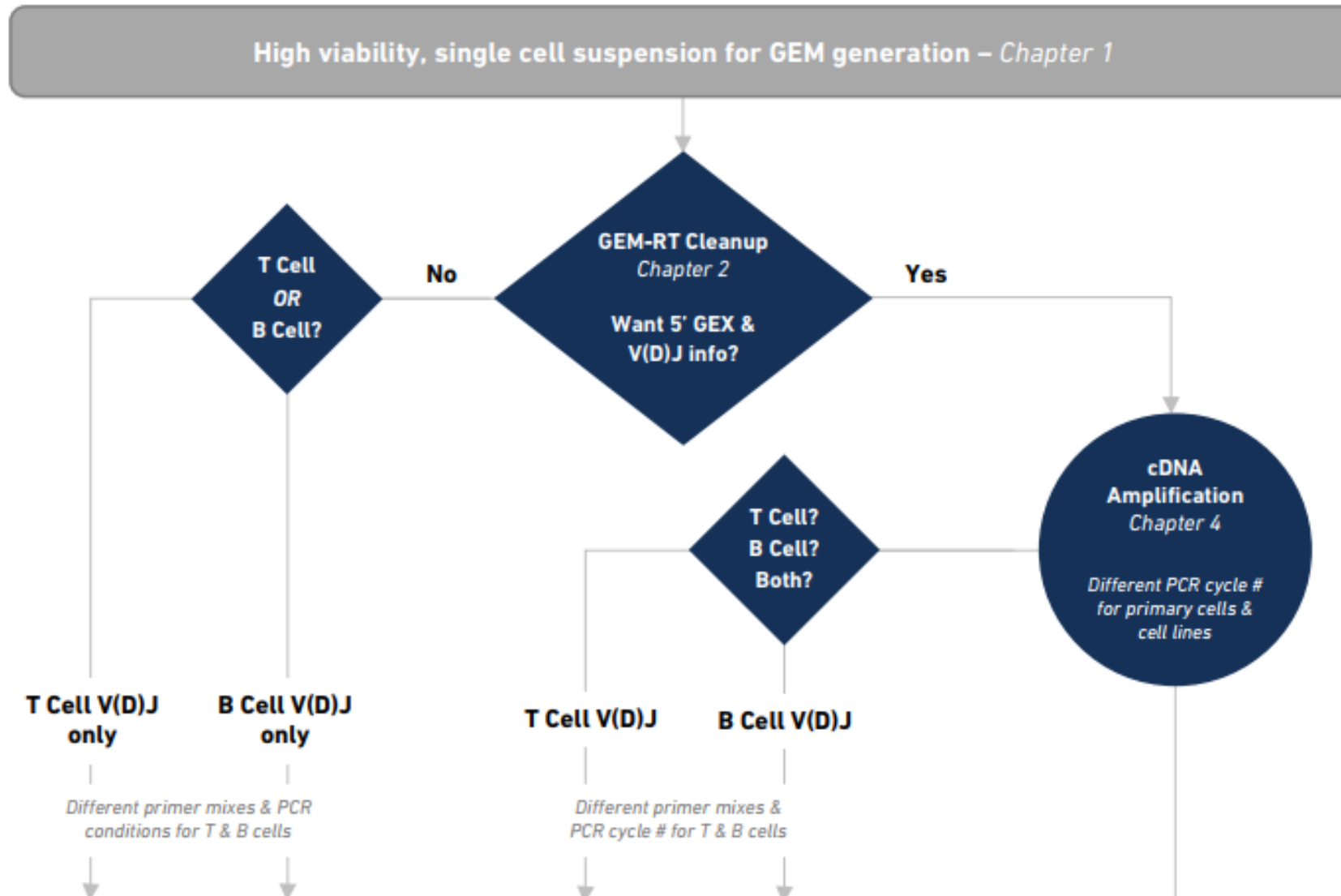


Figure 3.1 The Immune System, 3ed. (© Garland Science 2009)

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



Gel bead oligos

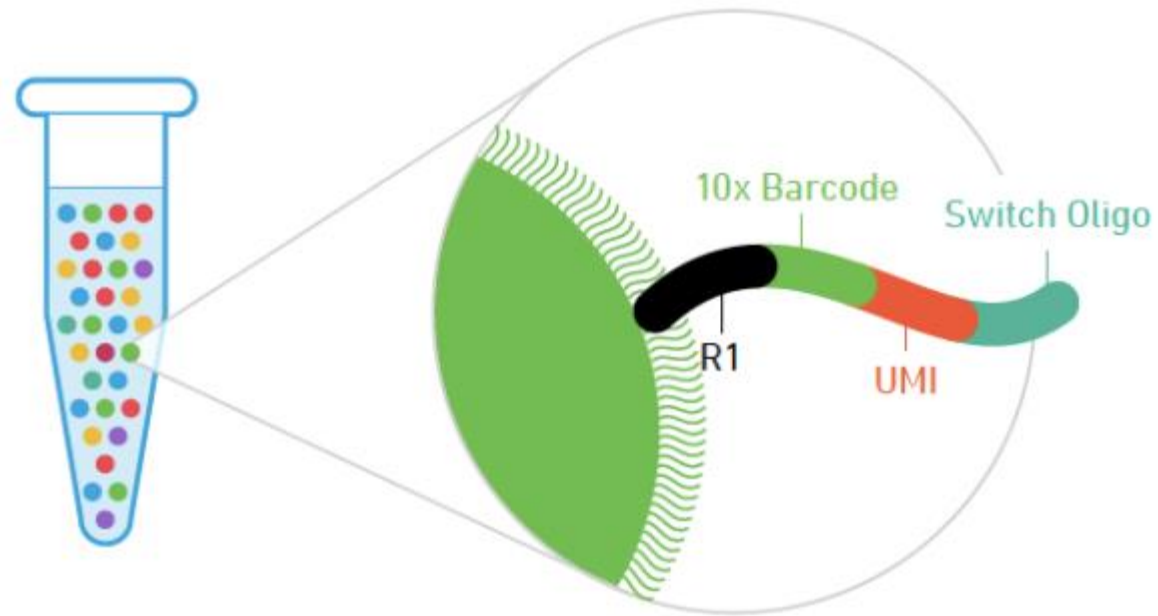
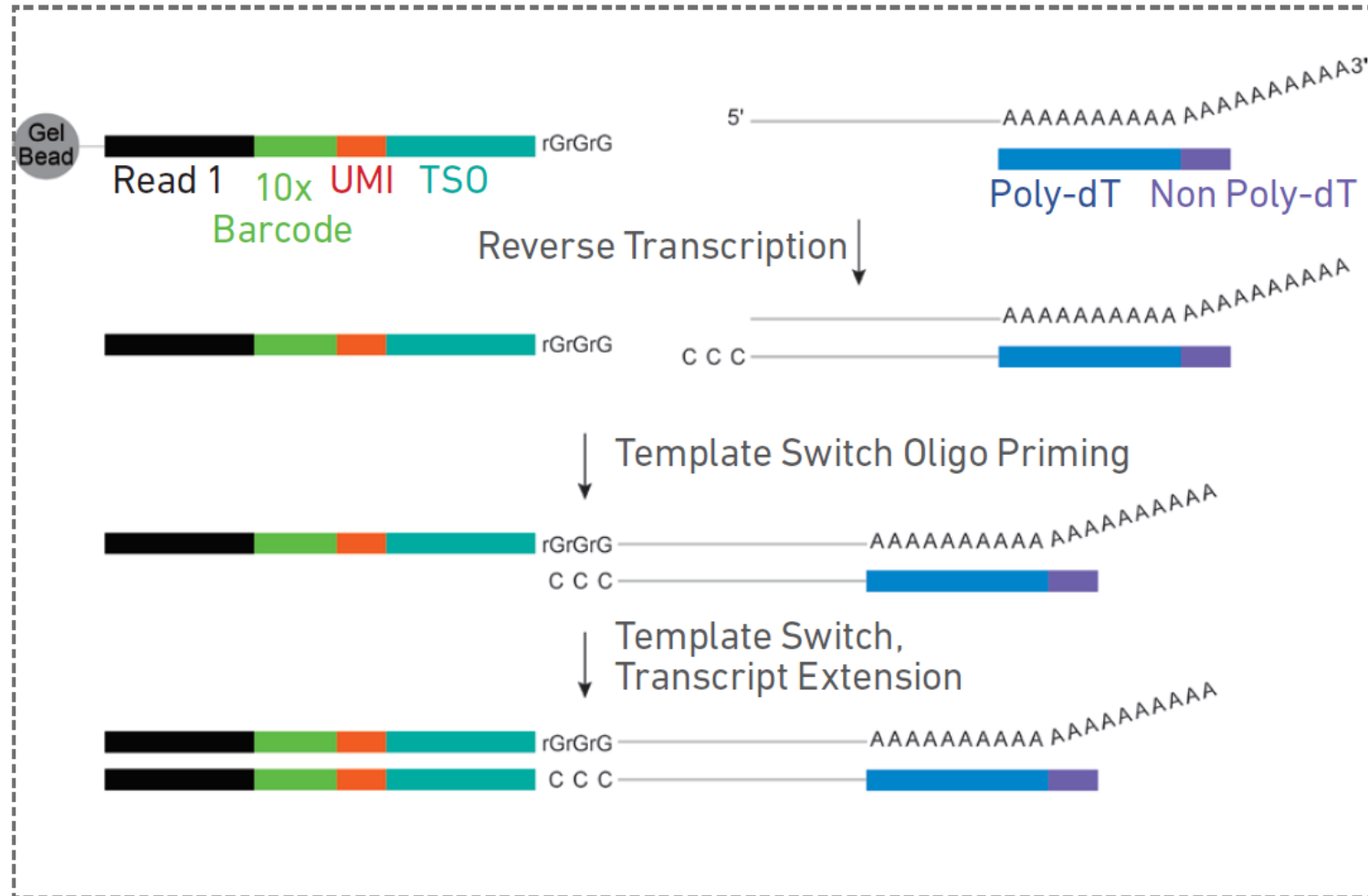


Figure 1. Schematic of a Single Cell 5' Gel Bead oligo primer.

- 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

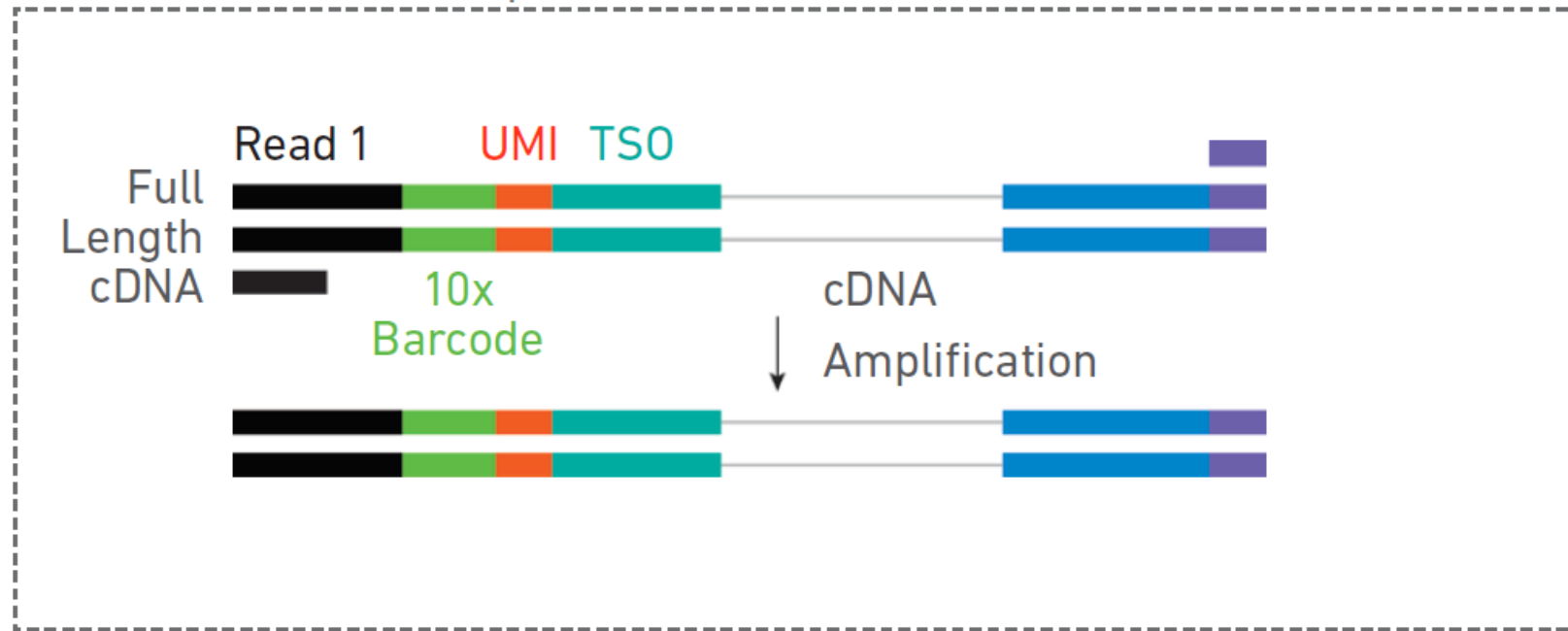
Assay scheme for 5' scRNA-seq

Inside individual GEMs

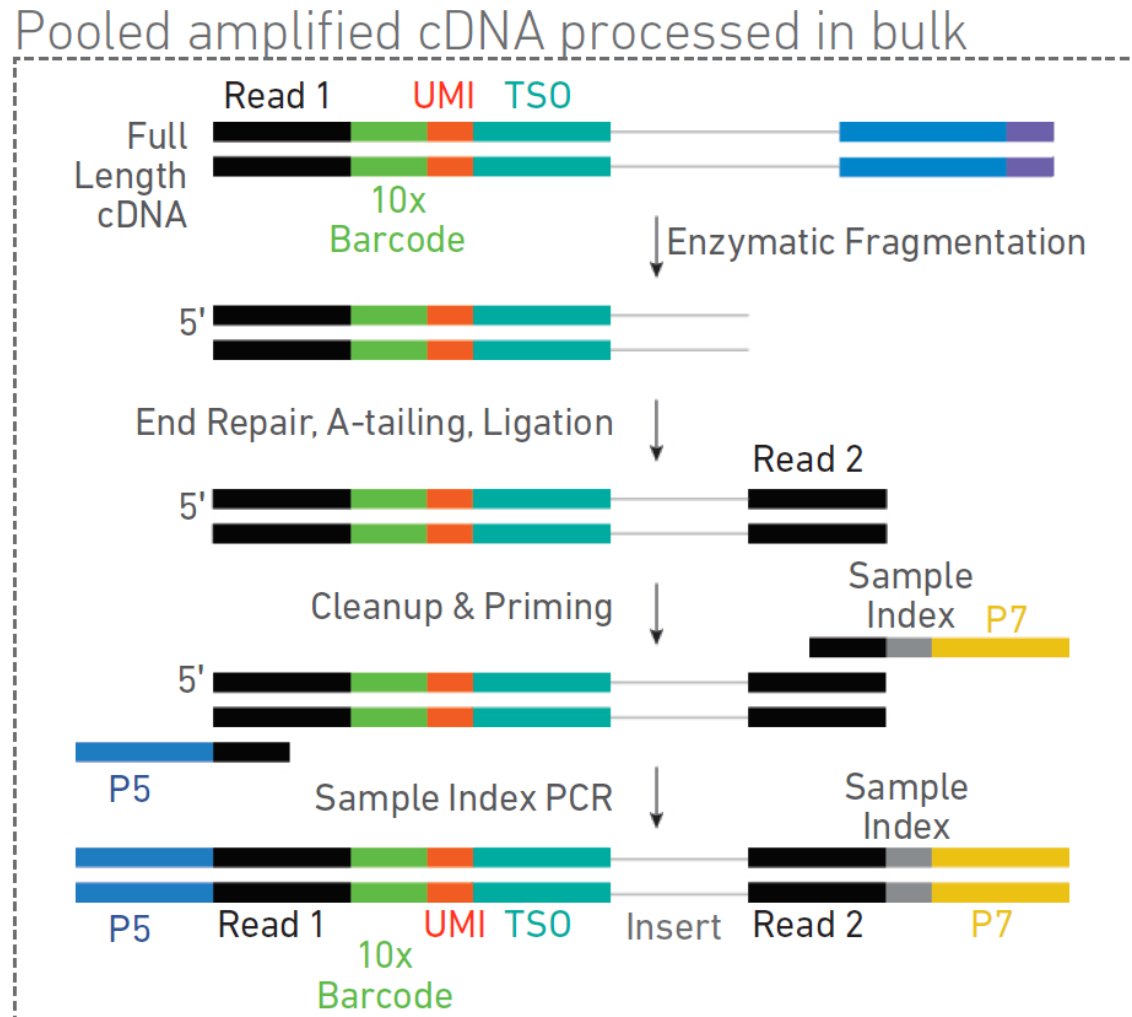


Assay scheme for 5' scRNA-seq

Pooled cDNA amplification

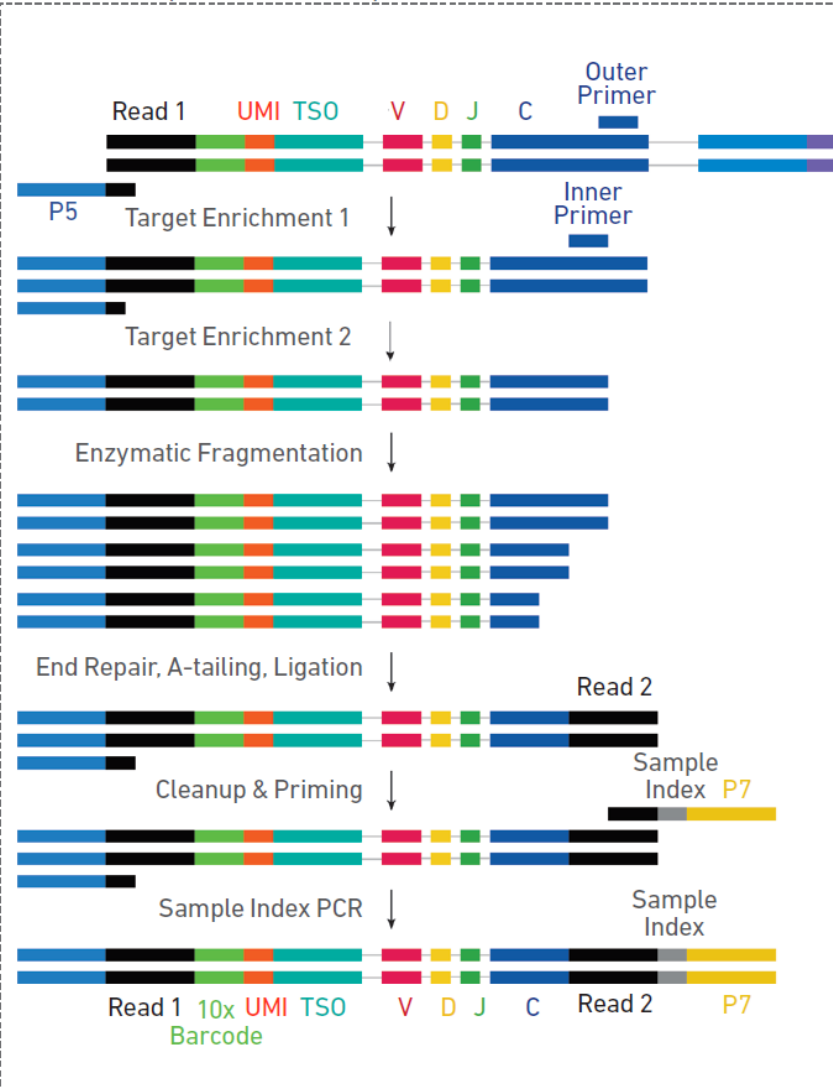


Assay scheme for 5' scRNA-seq



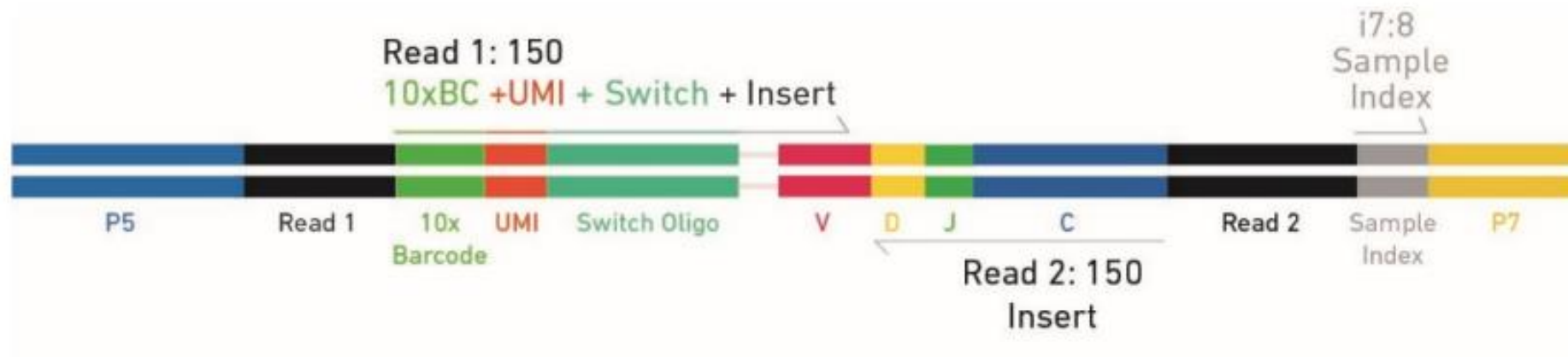
Assay scheme for 5' VDJ libraries

Pooled amplified cDNA processed in bulk

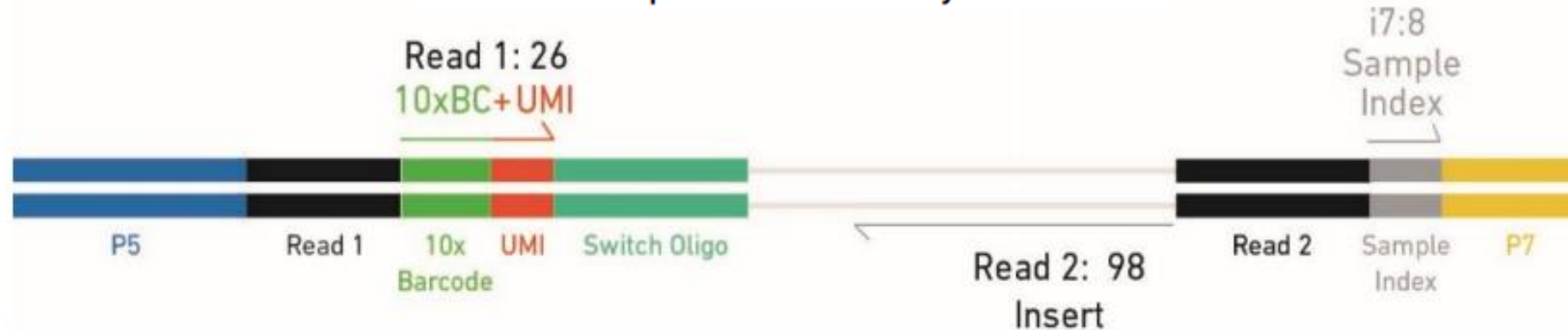


Final library structure

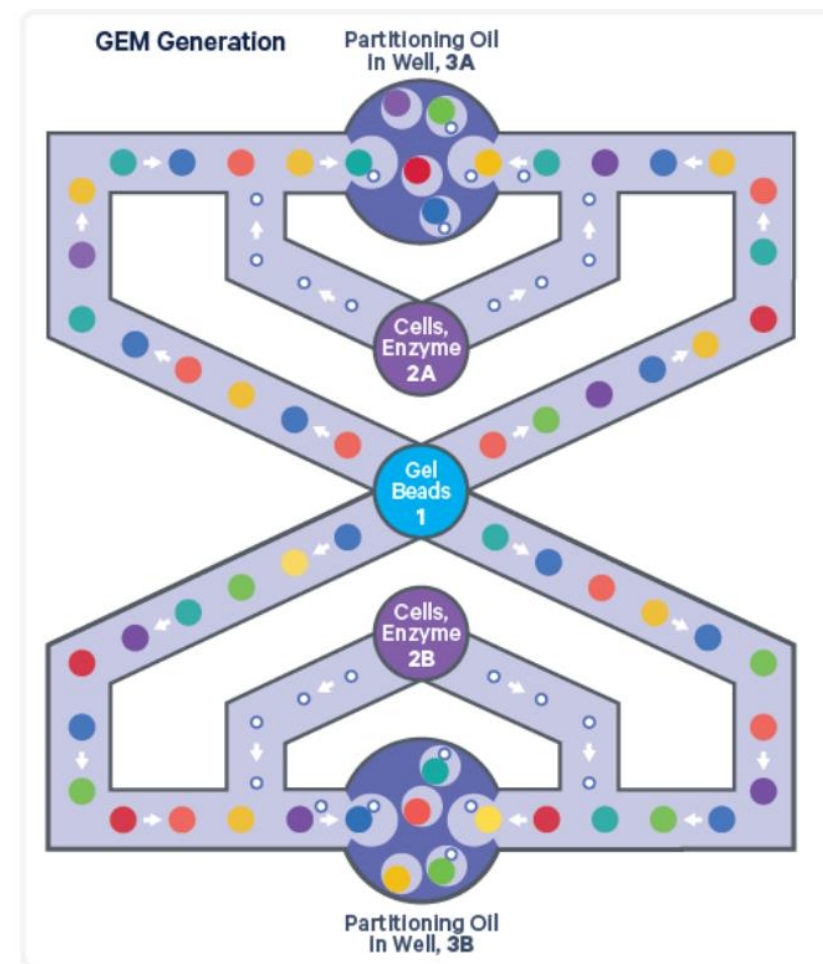
V(D)J Enriched Library Structure:



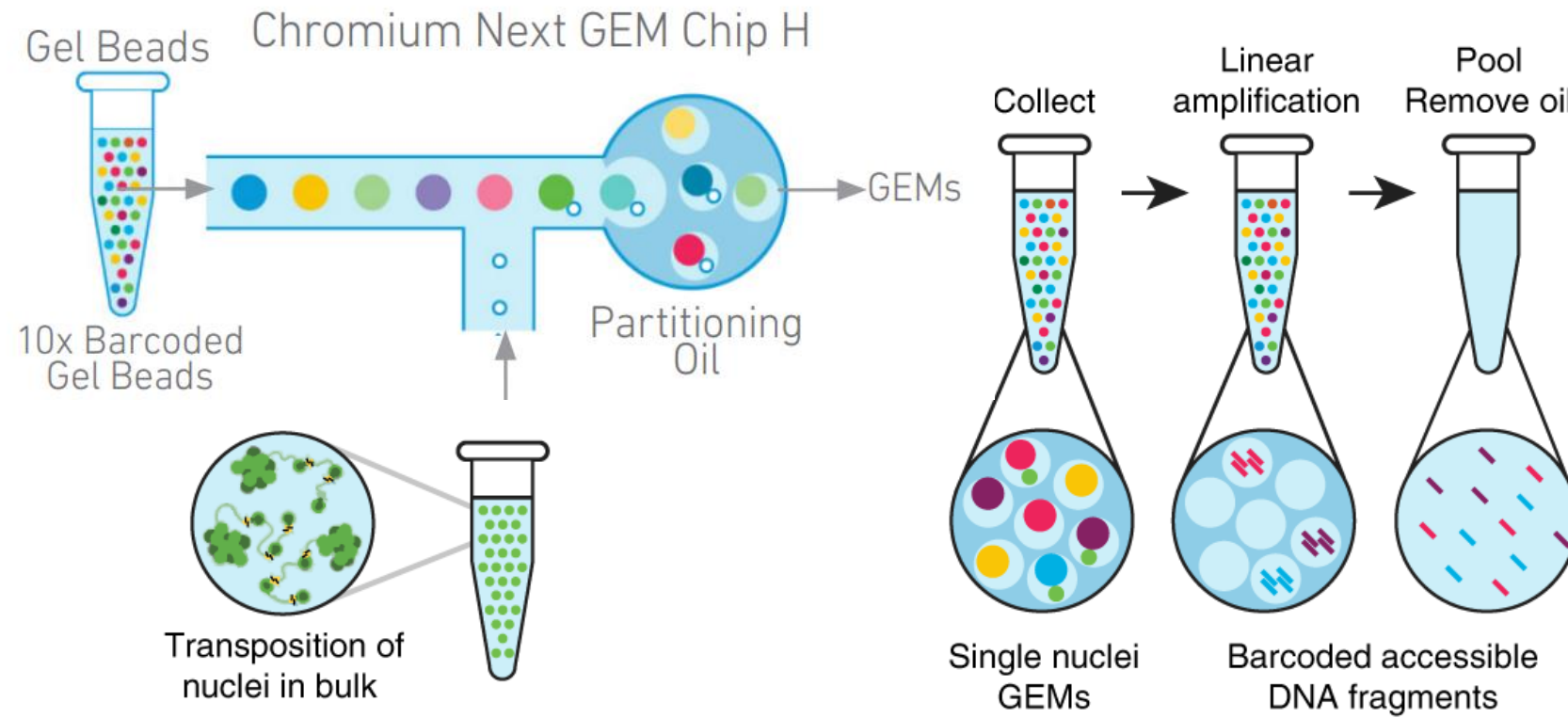
5' Gene Expression Library Structure:



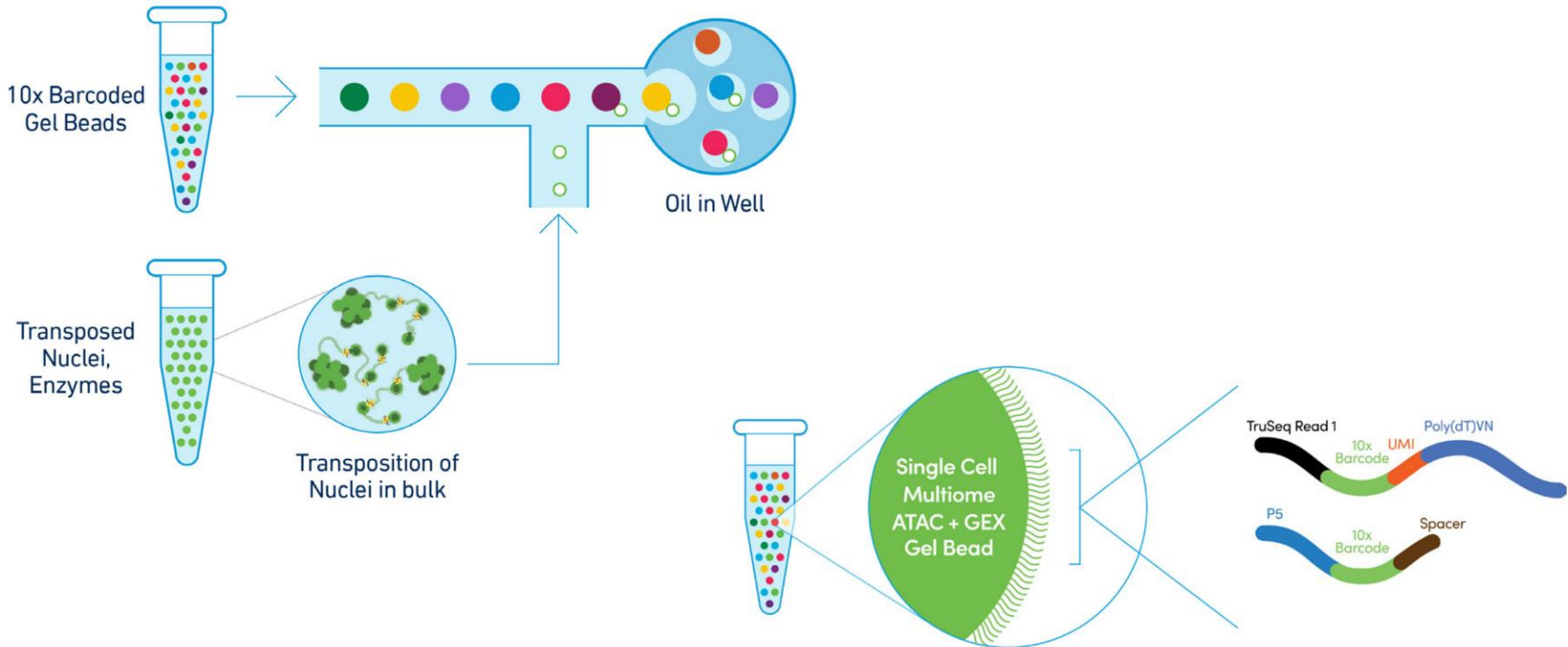
NEW! Chromium X



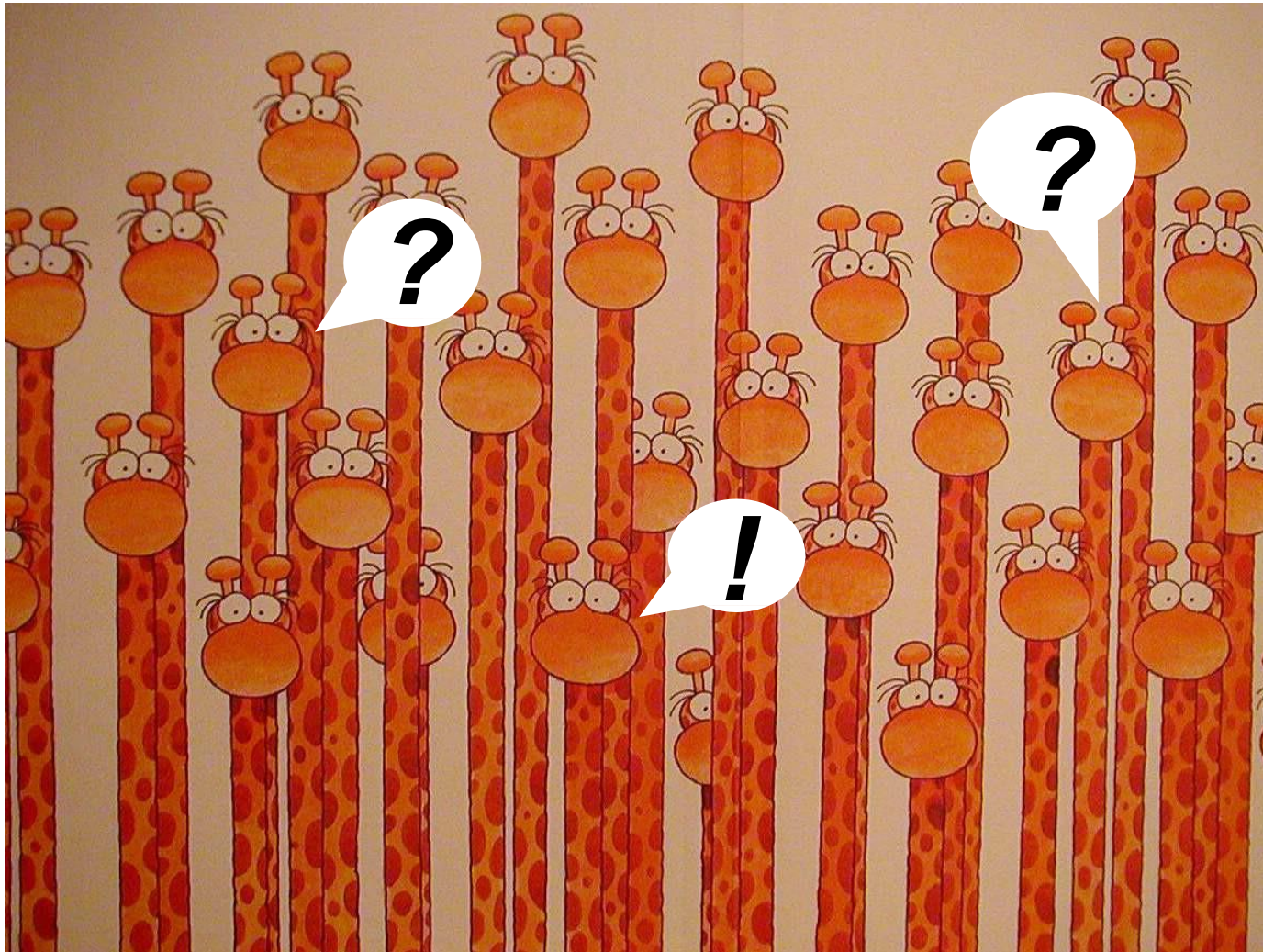
Single-cell ATAC-seq



Single-cell multiome



Questions or concerns?



S.L.Kloet@lumc.nl

info@lgtc.nl