

# Overview of Single-Cell Platforms

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MGC Course on Single-Cell Analysis

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# Why single-cell?

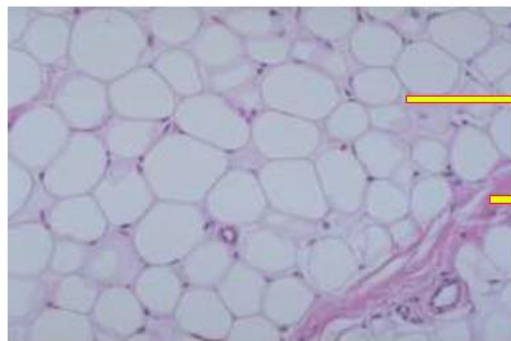
Bulk sample analysis is just like putting a fruit salad into a blender - the taste is an average of all ingredients.



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



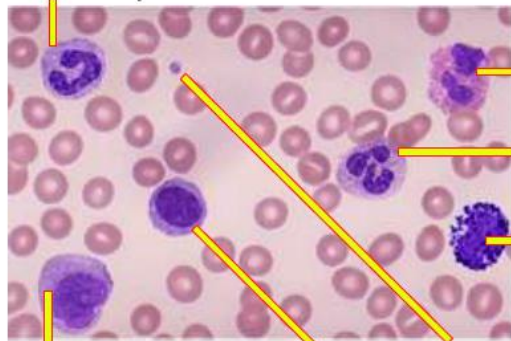
**Adipose**

Fat

Connective  
tissue

Band  
Neutrophil

**Normal Peripheral Blood**



Eosinophil

Segmented  
Neutrophil

Basophil

Monocyte

Platelet  
Lymphocyte

**Small Intestine Mucosa**



Epithelial cells

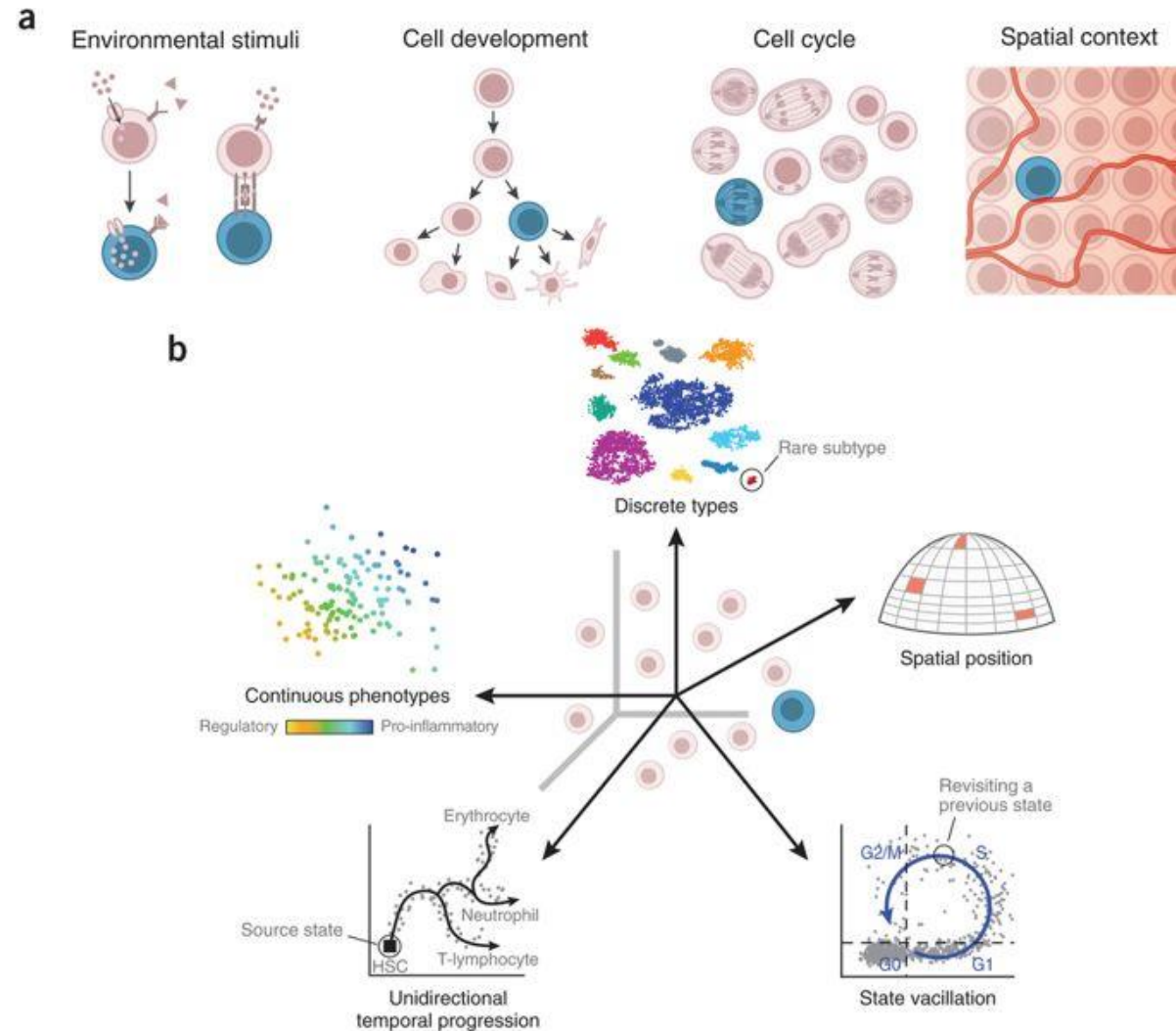
Goblet  
cells

Lamina  
propria

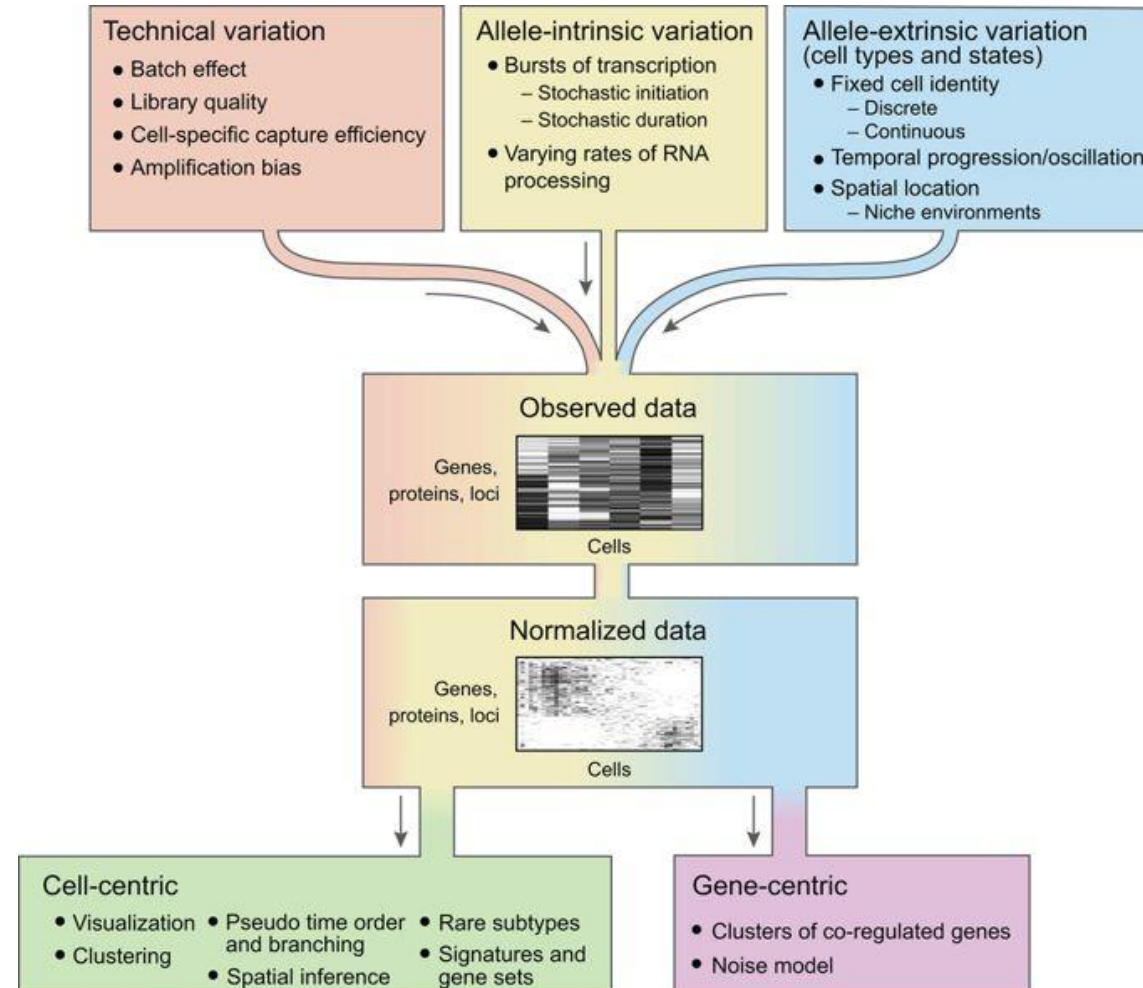
Muscularis mucosa  
(smooth muscle)

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

# Cell identity is more than histopathology



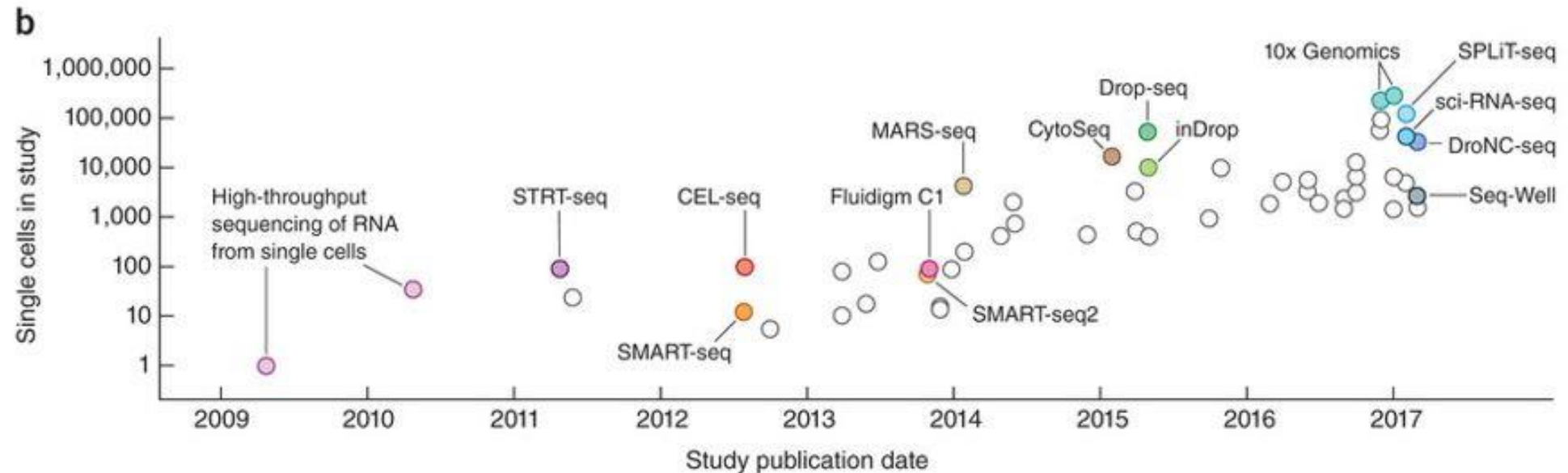
# Biology is messy – computational methods help to clean this up



# We will cover

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

# Exponential scaling of single-cell throughput





# scRNA-seq

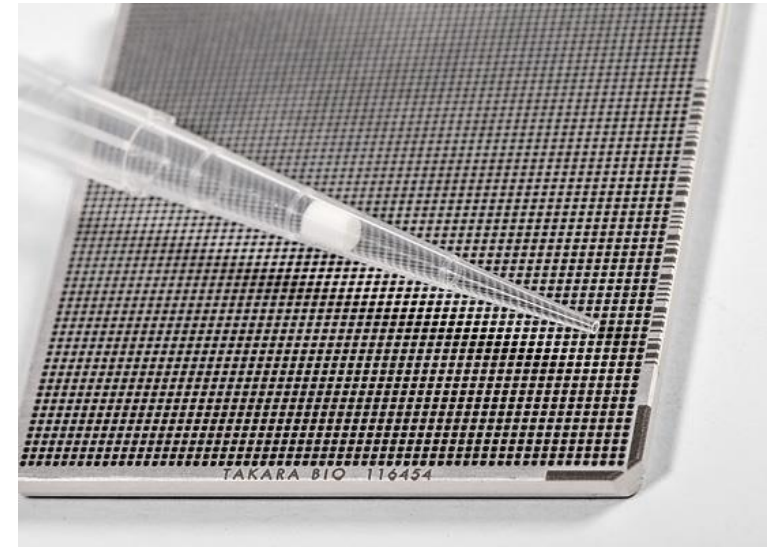
## **MANY** different assays

- 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

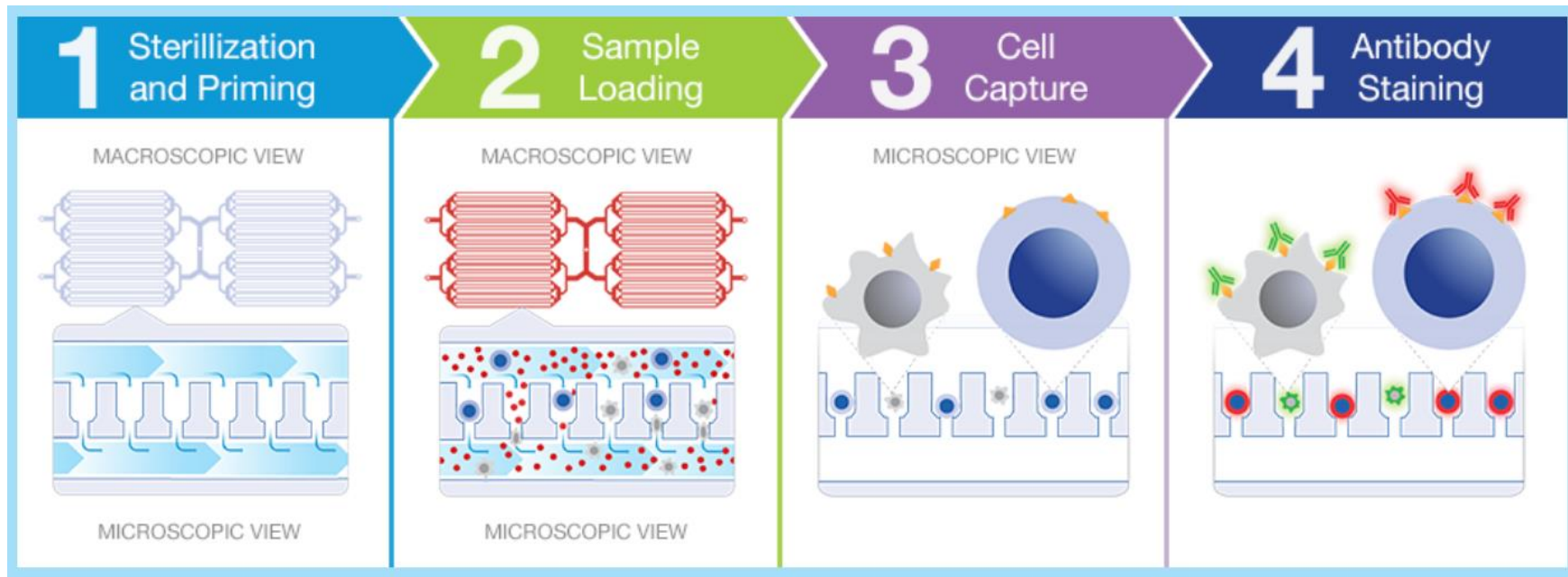


# BD Rhapsody

- 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



# Copy number variation – Droplet based

- Mission Bio Tapestri
  - Uses proteases to break down chromatin
  - Panel-based PCR (up to 400 targets)
  - Can call both CNVs and SNVs in target regions
- Up to 10k cells
- Rare subclone detection, down to ~0.1%



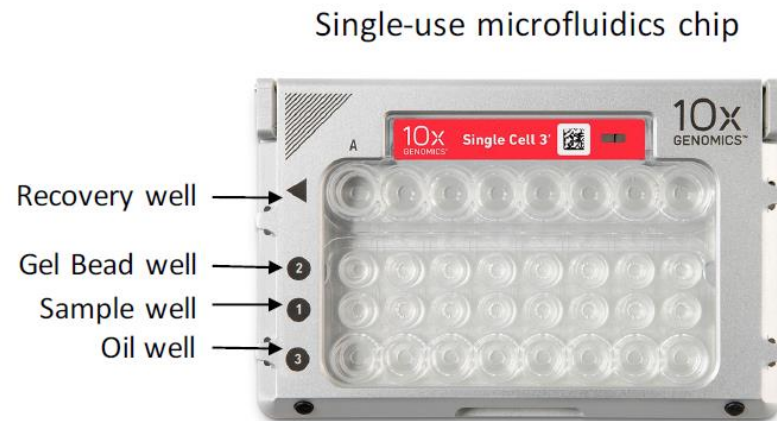
# 10x Genomics Chromium Controller

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.

## AUTOMATED SOLUTIONS



### Automated Single-Cell Gene Expression

3' automated gene expression from cells to sequencing-ready libraries.

## SINGLE CELL GENOMICS



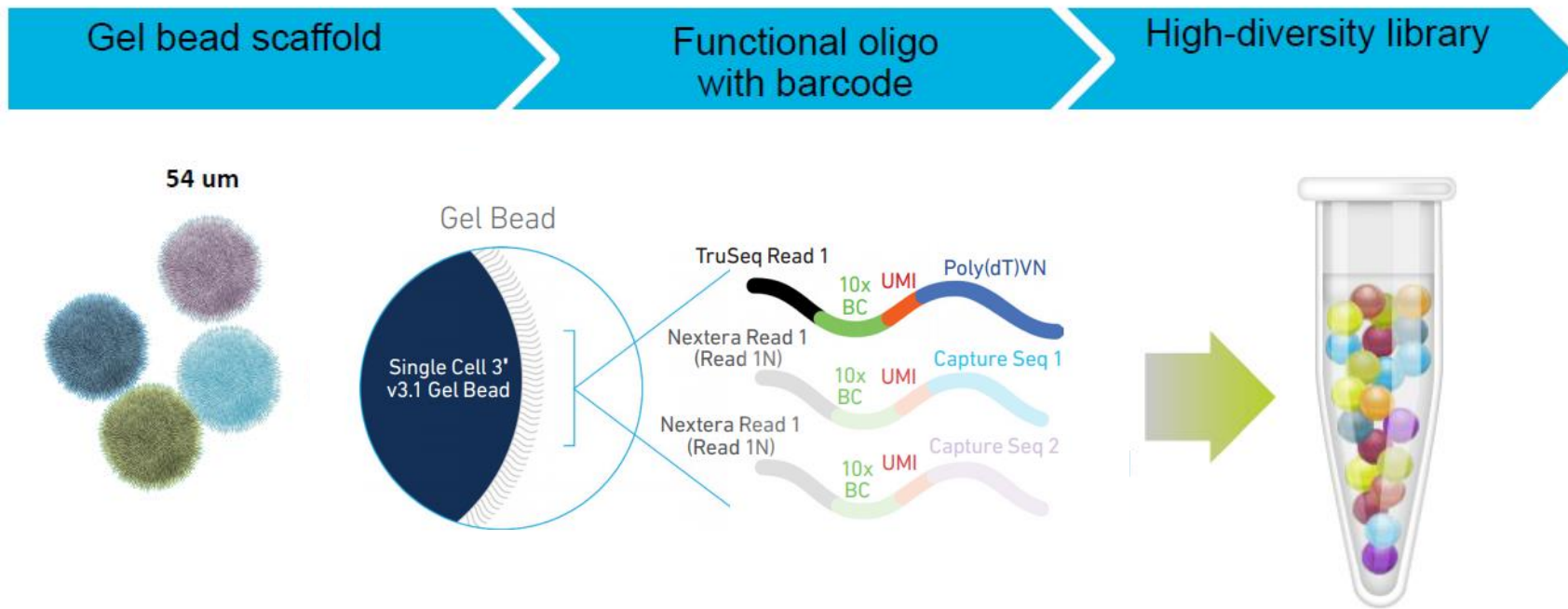
### Single Cell CNV

Copy number variation and genomic heterogeneity at single cell resolution.



# Gel beads up close

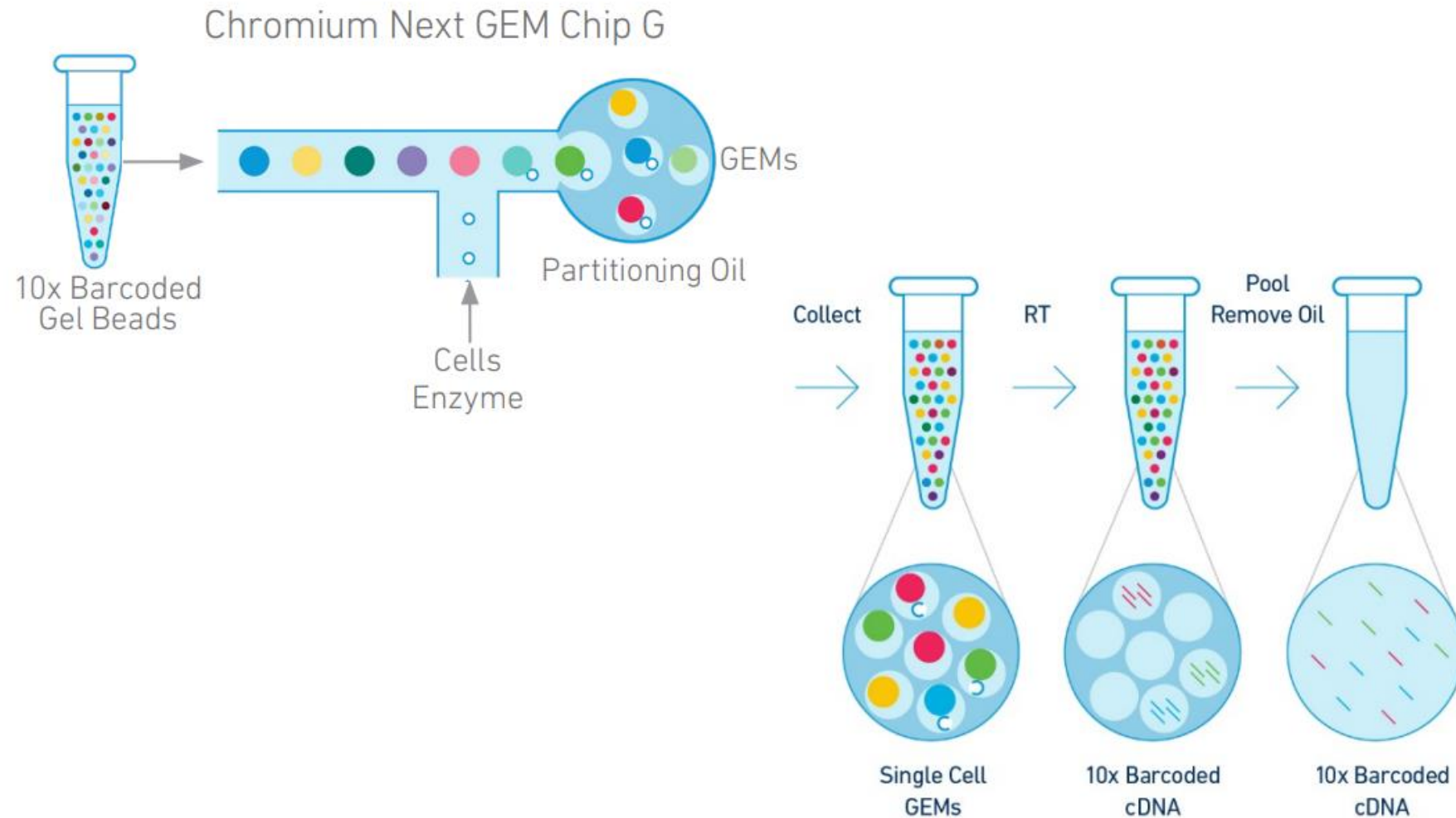
4M Discrete Reagents in One Tube



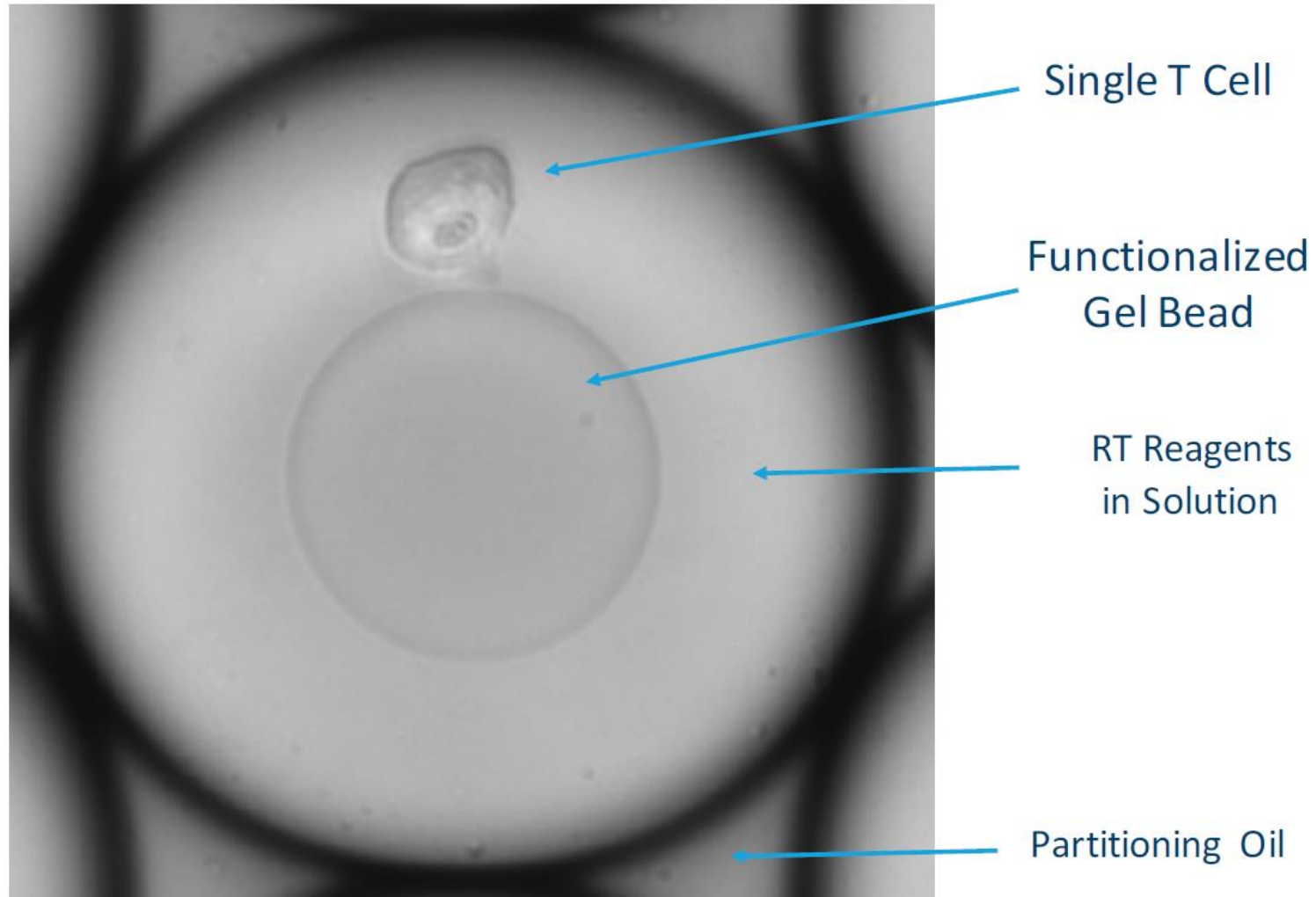
**10x Barcode (16bp): unique for each GemBead**  
**+UMI (12bp): correct for PCR duplicates**



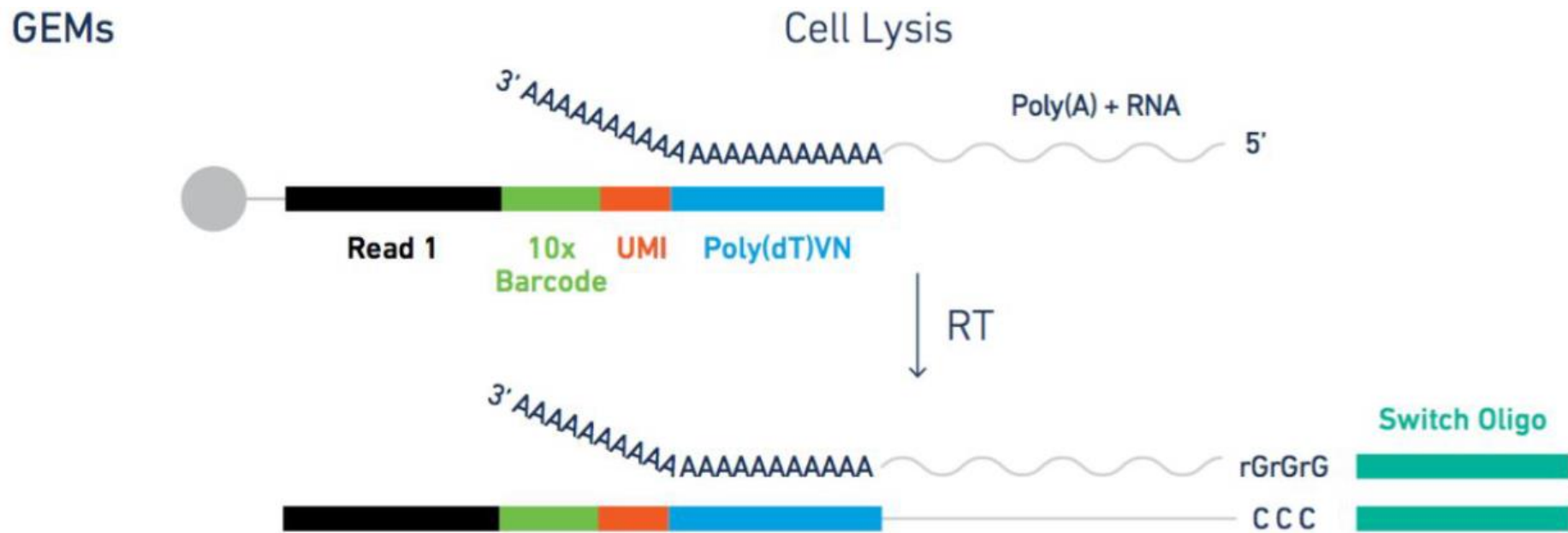
# Gel bead in Emulsion (GEM) technology



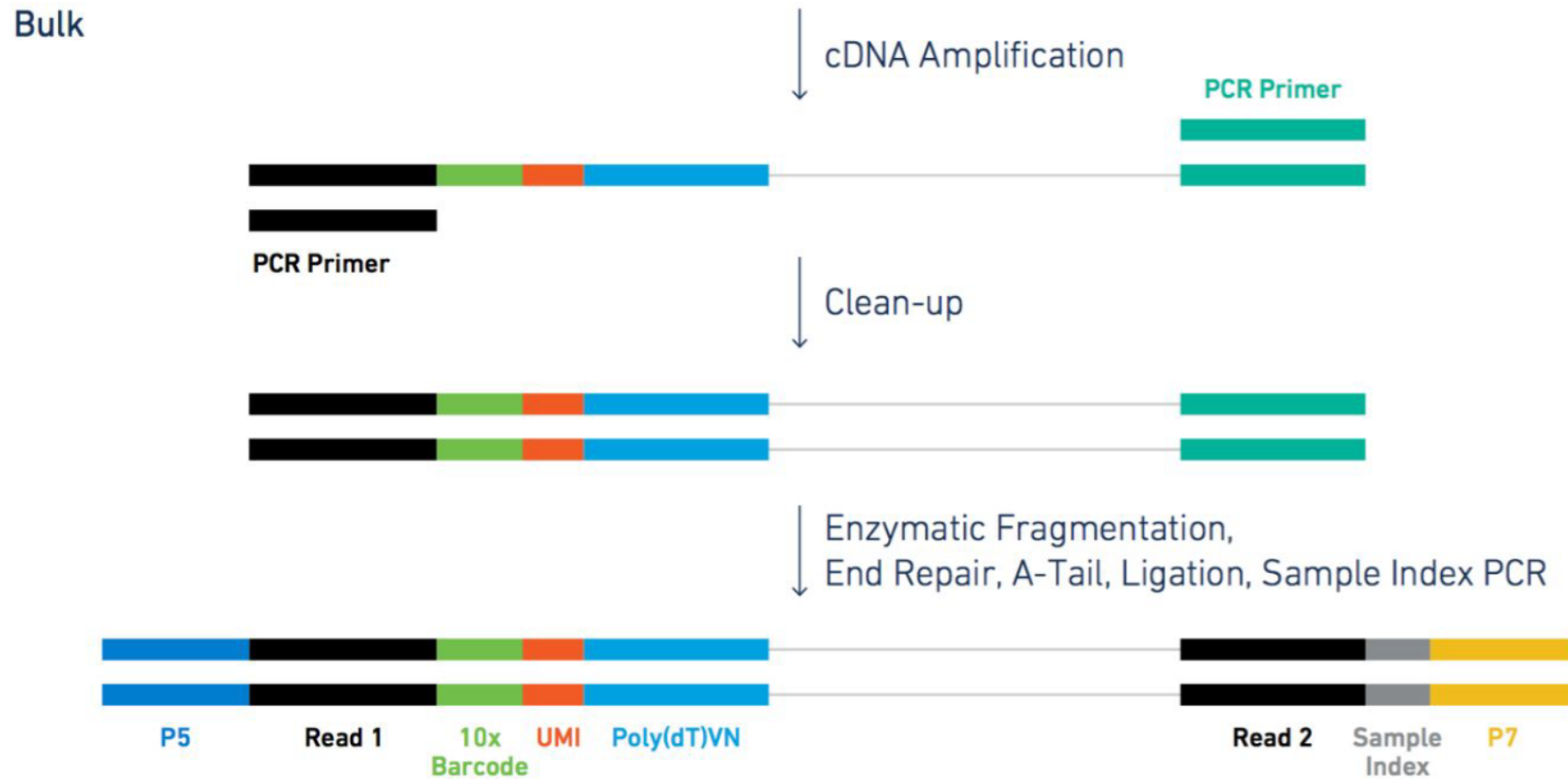
# GEMs up close



# Assay scheme for 3' mRNA sequencing

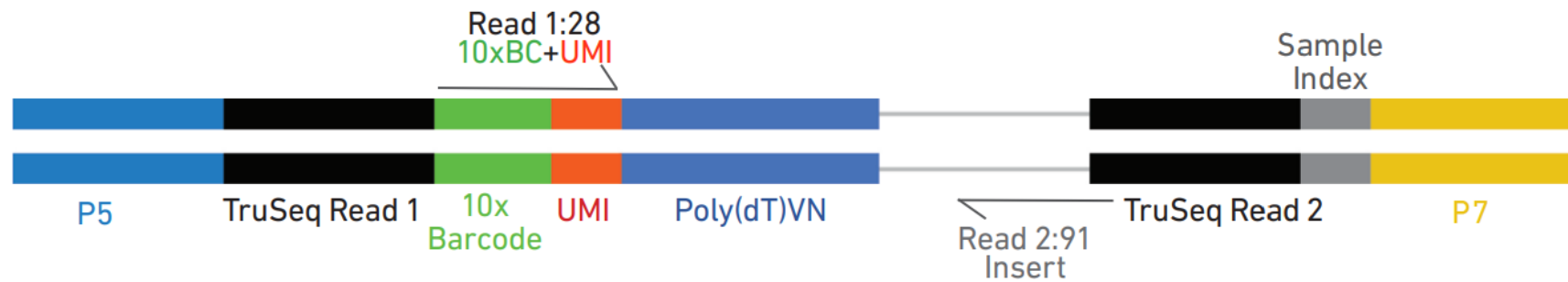


# Assay scheme for 3' mRNA sequencing

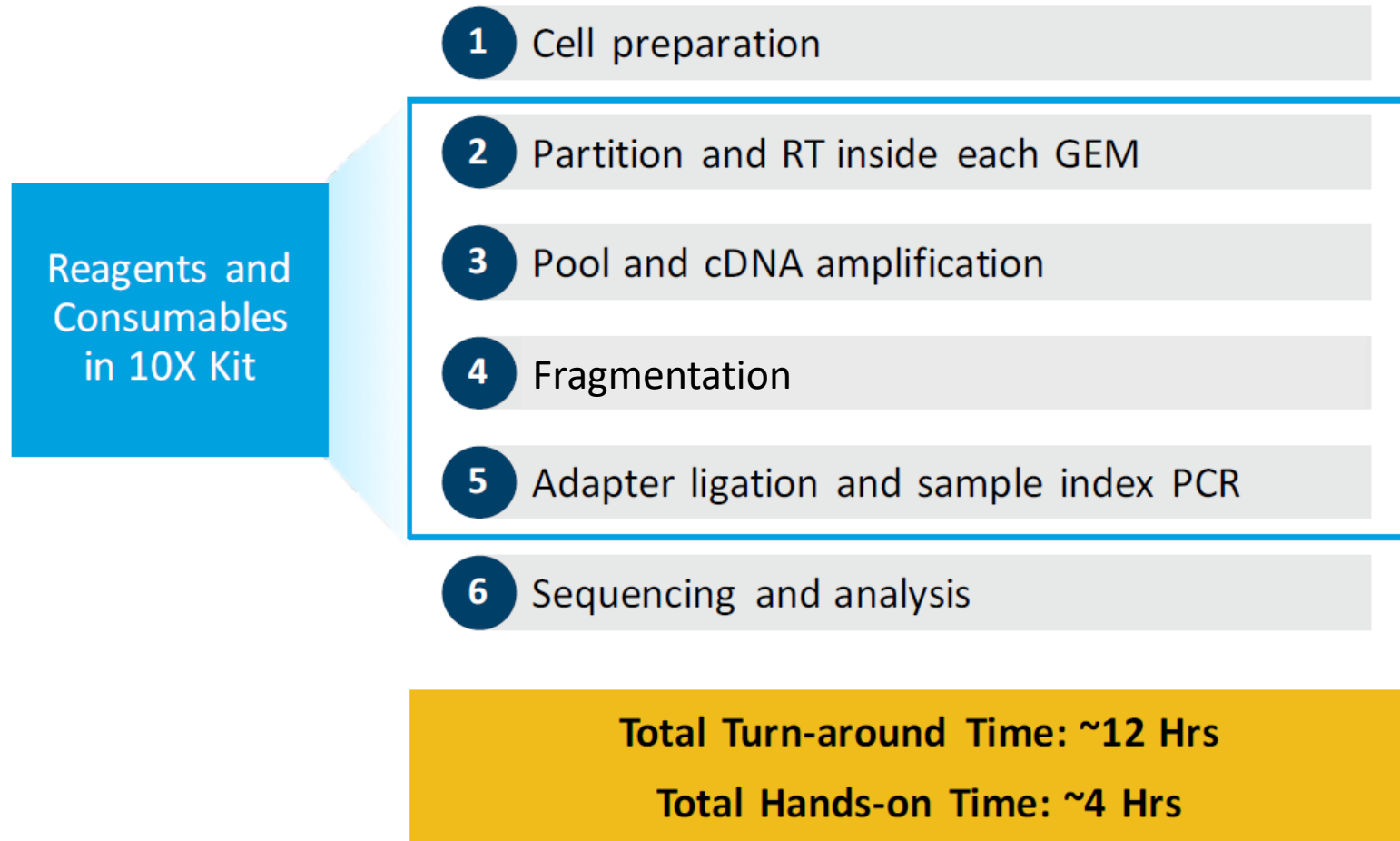


# Final library structure

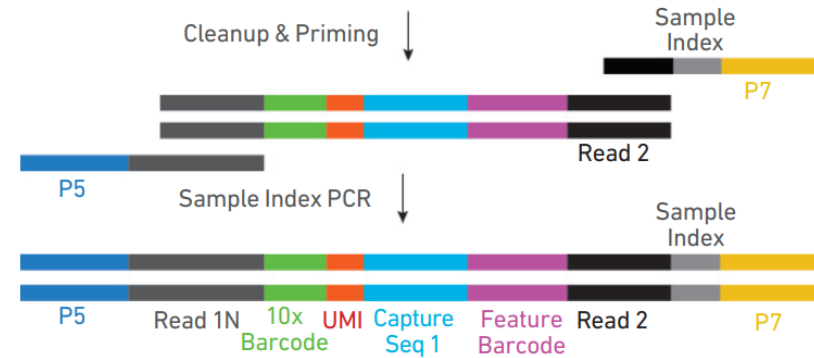
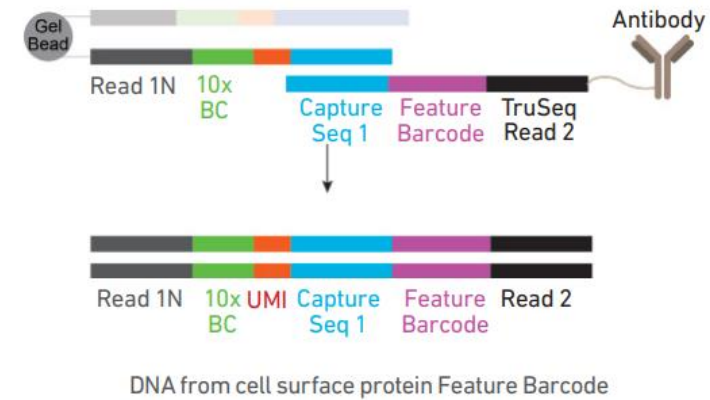
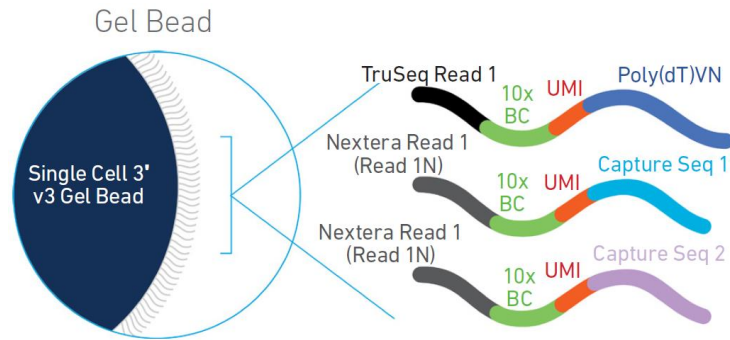
## Chromium Single Cell 3' Gene Expression Library



# Single cell 3' end-to-end workflow



# Single cell 3' feature barcoding





# Structure of T and B cell receptors

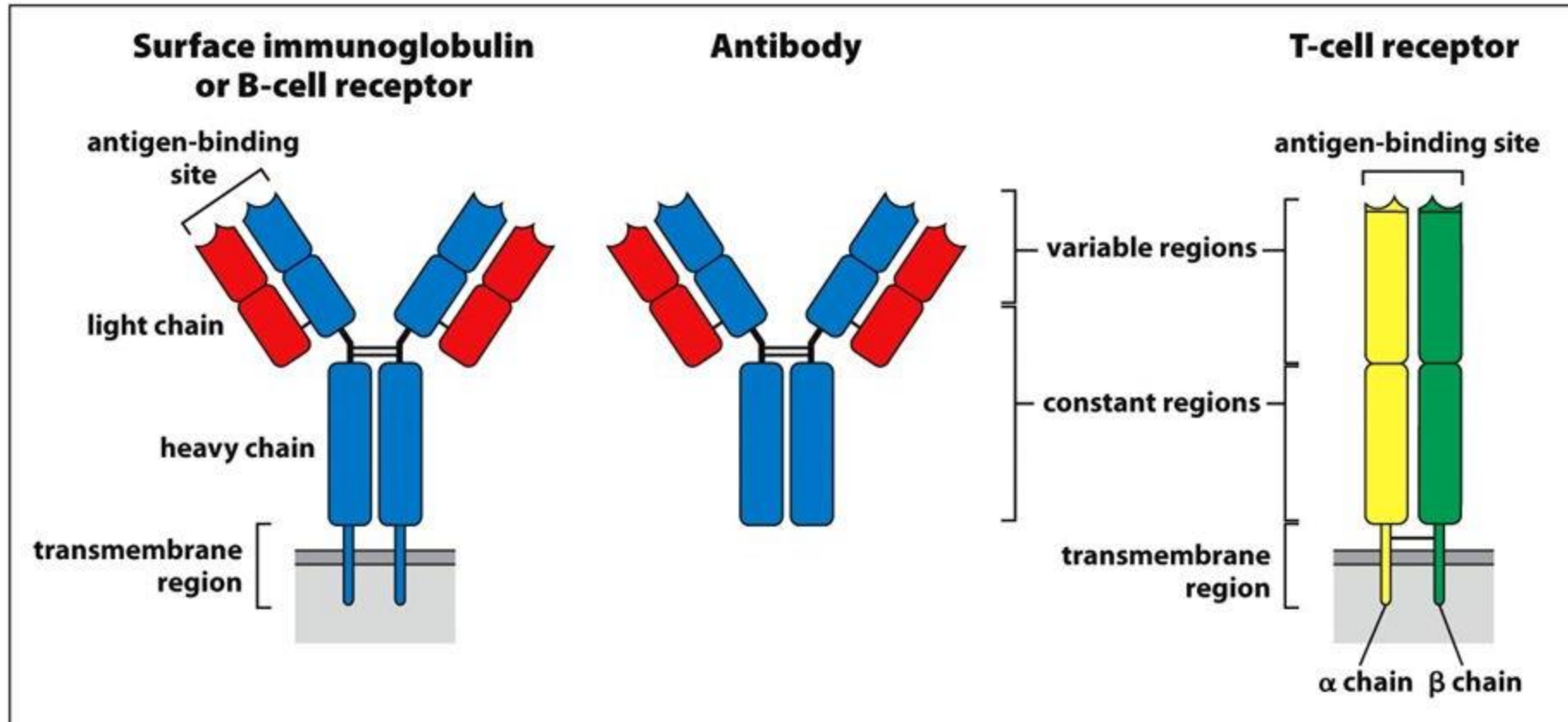
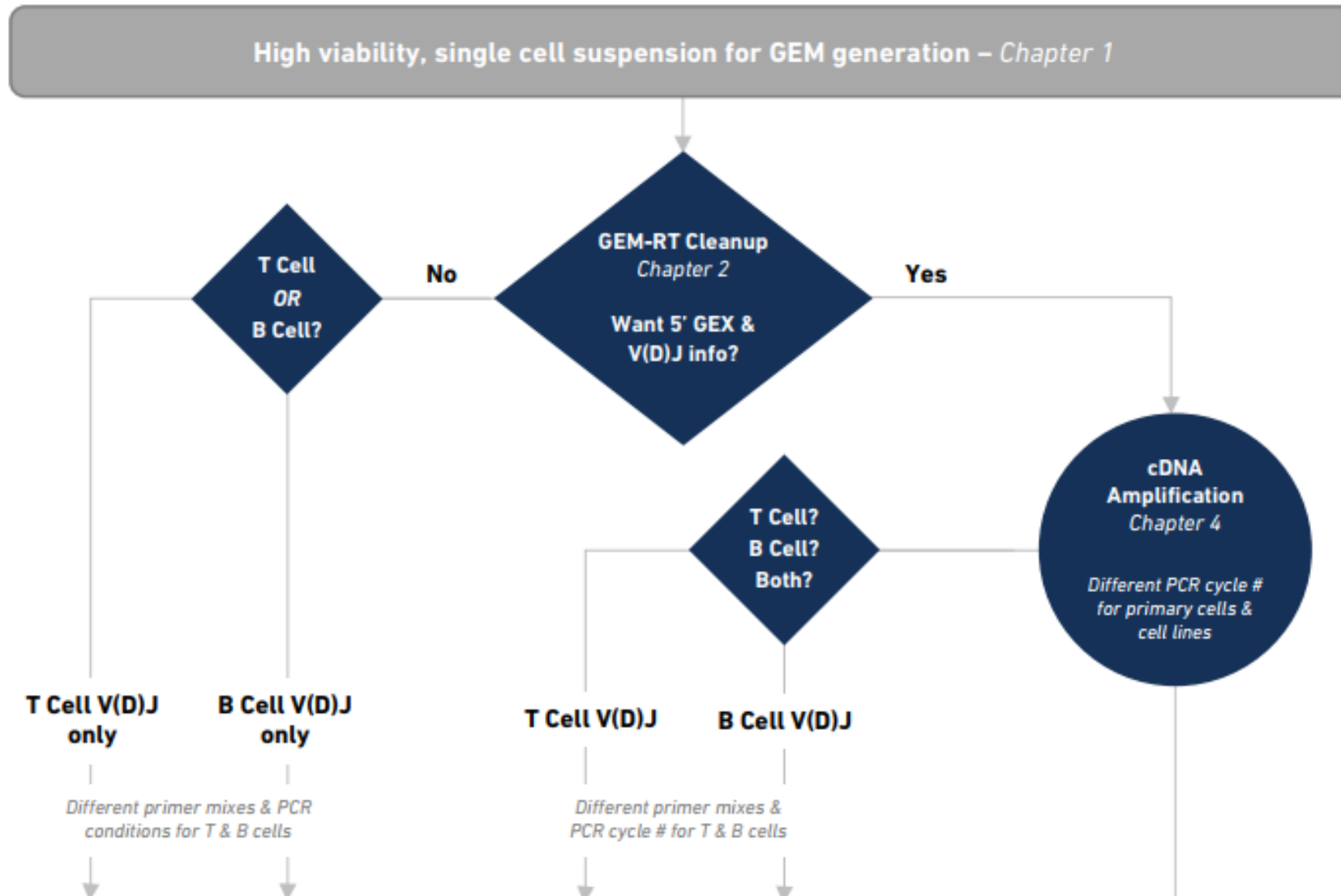


Figure 3.1 The Immune System, 3ed. (© Garland Science 2009)

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



# Gel bead oligos

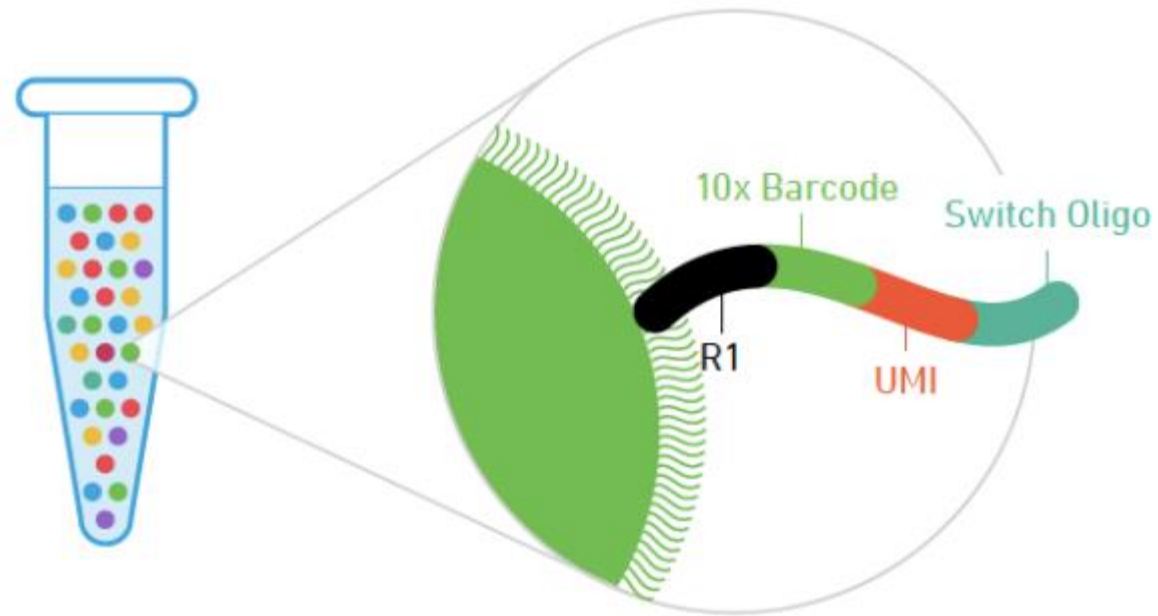
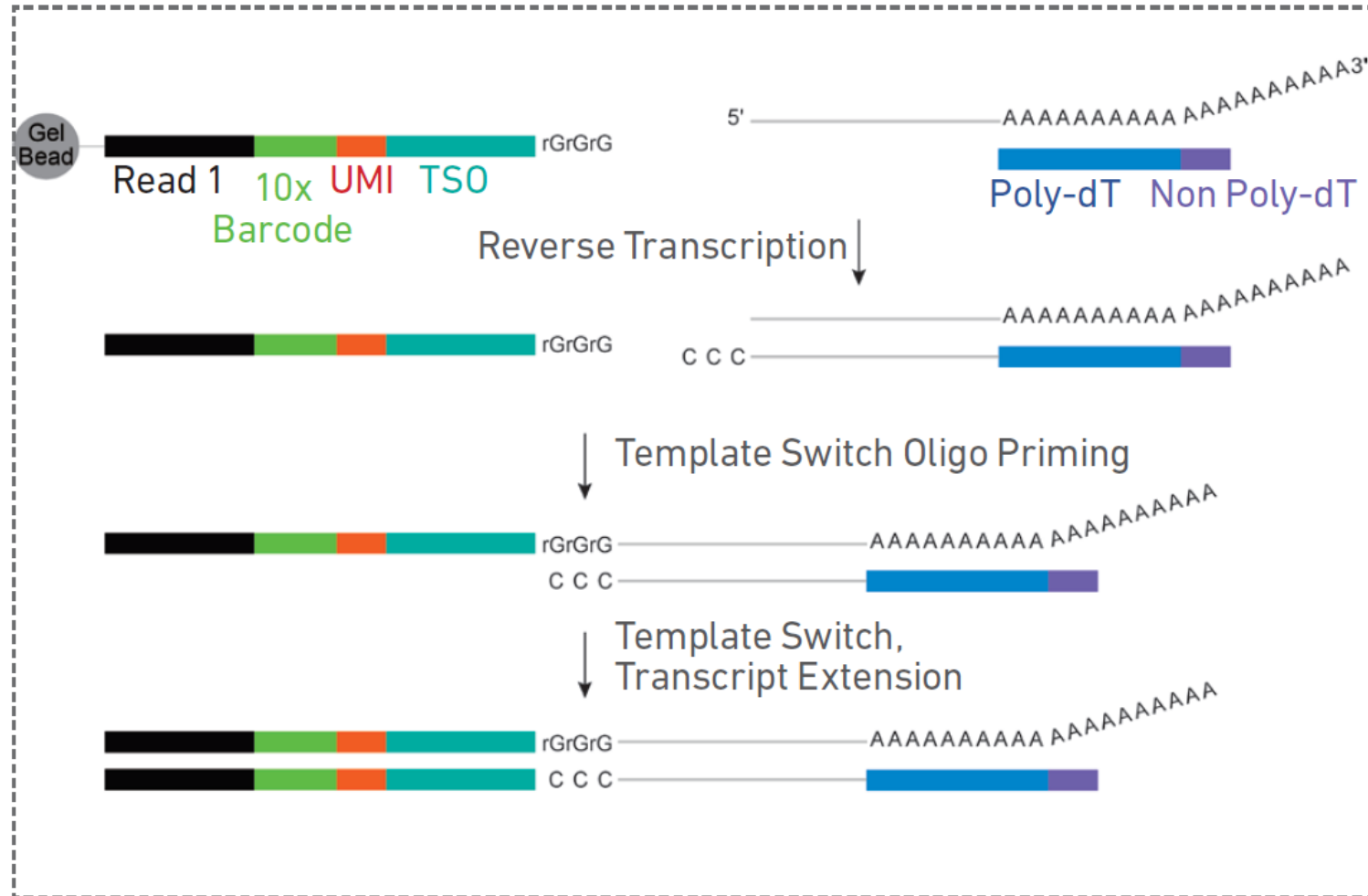


Figure 1. Schematic of a Single Cell 5' Gel Bead oligo primer.

- 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

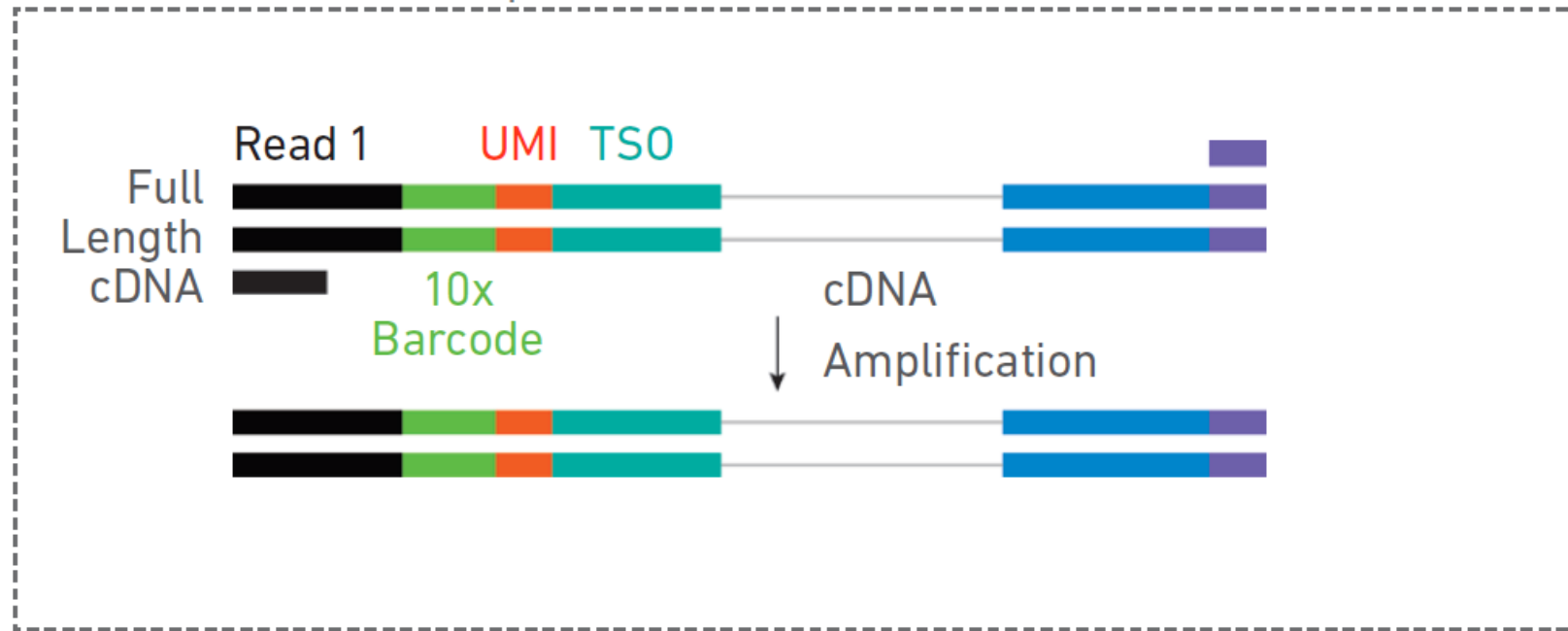
# Assay scheme for 5' scRNA-seq

Inside individual GEMs

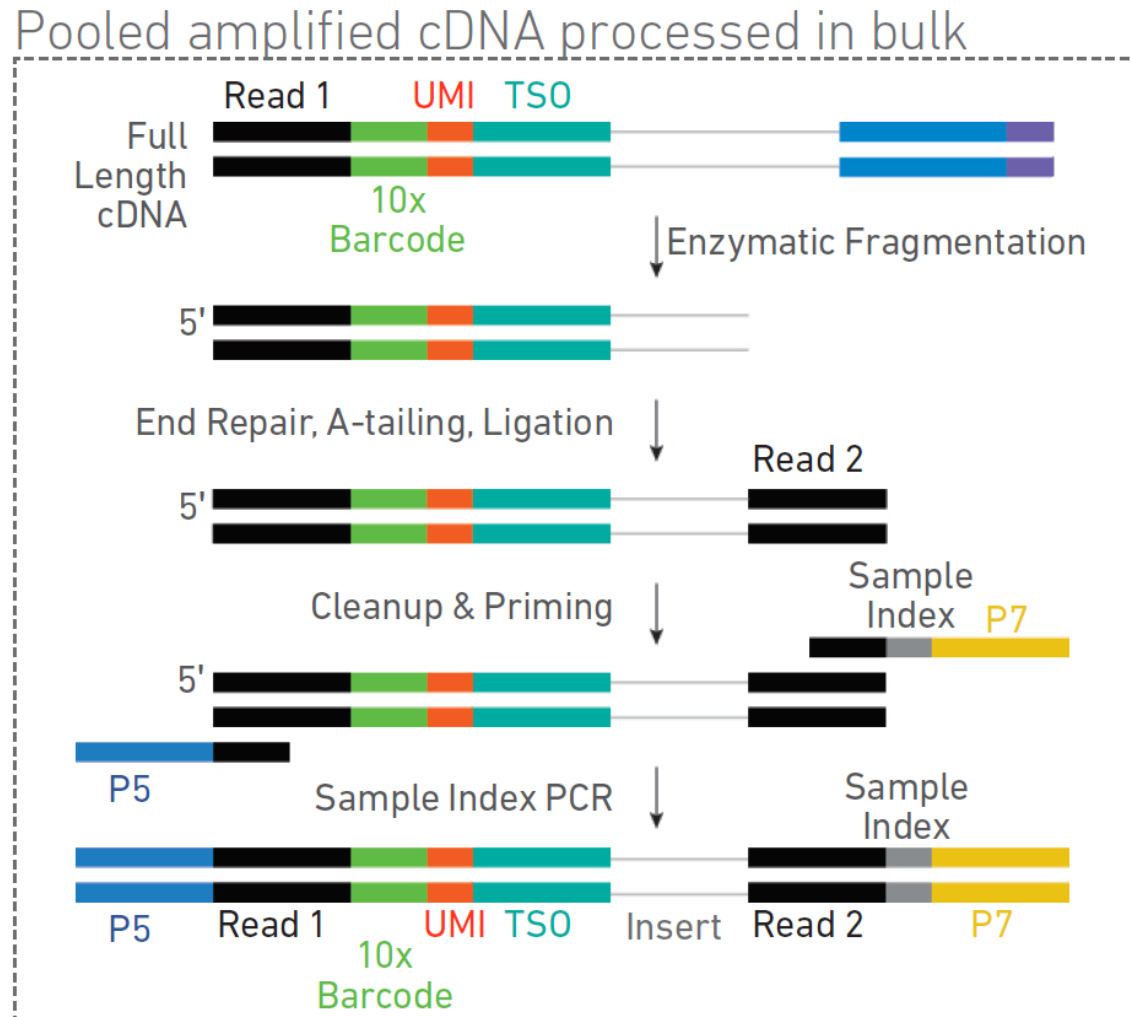


# Assay scheme for 5' scRNA-seq

Pooled cDNA amplification

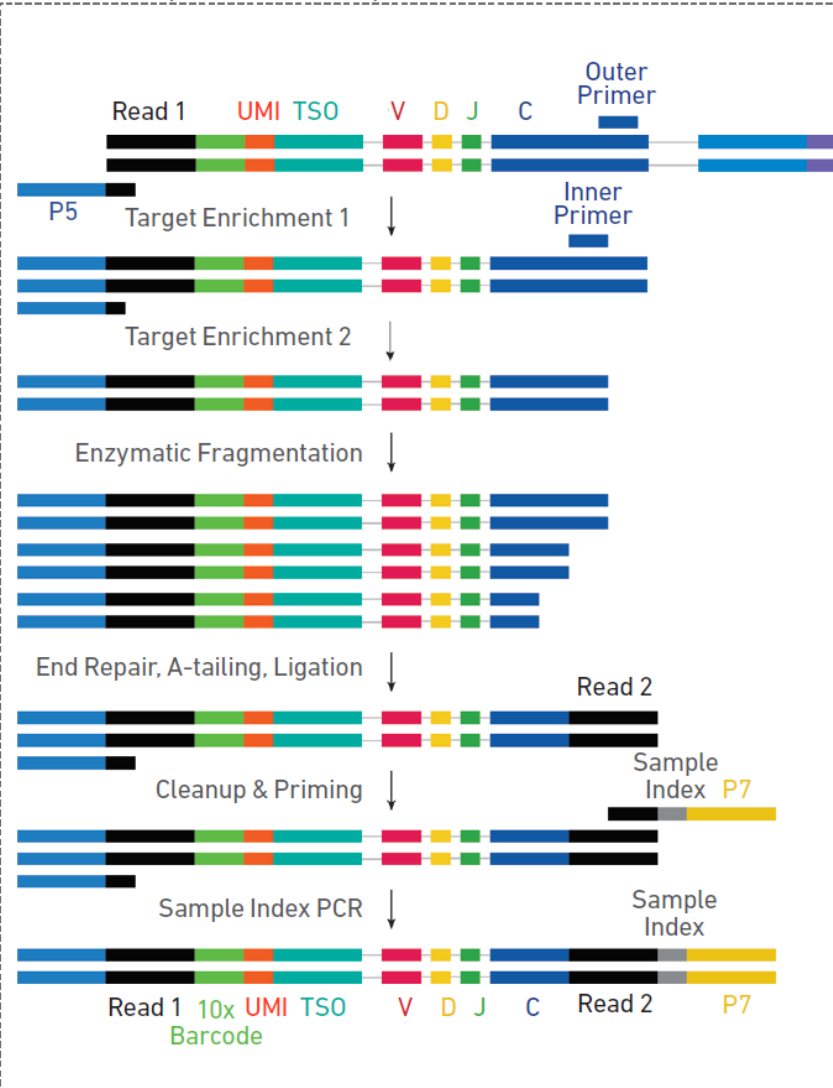


# Assay scheme for 5' scRNA-seq



# Assay scheme for 5' VDJ libraries

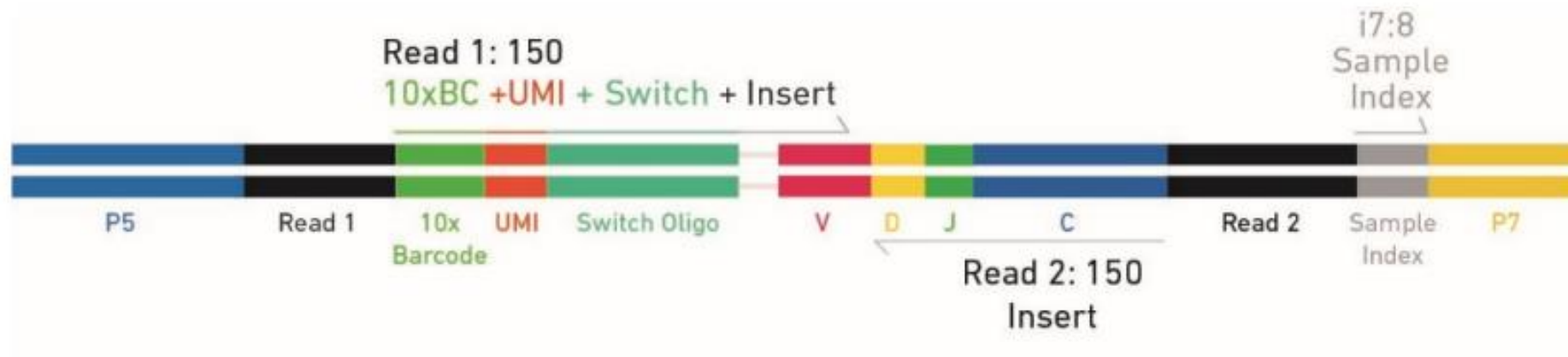
Pooled amplified cDNA processed in bulk



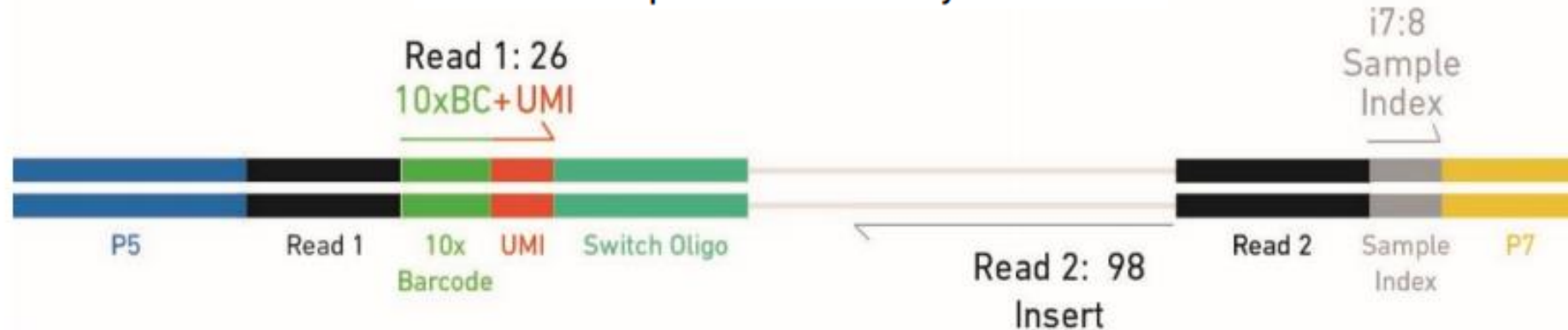


# Final library structure

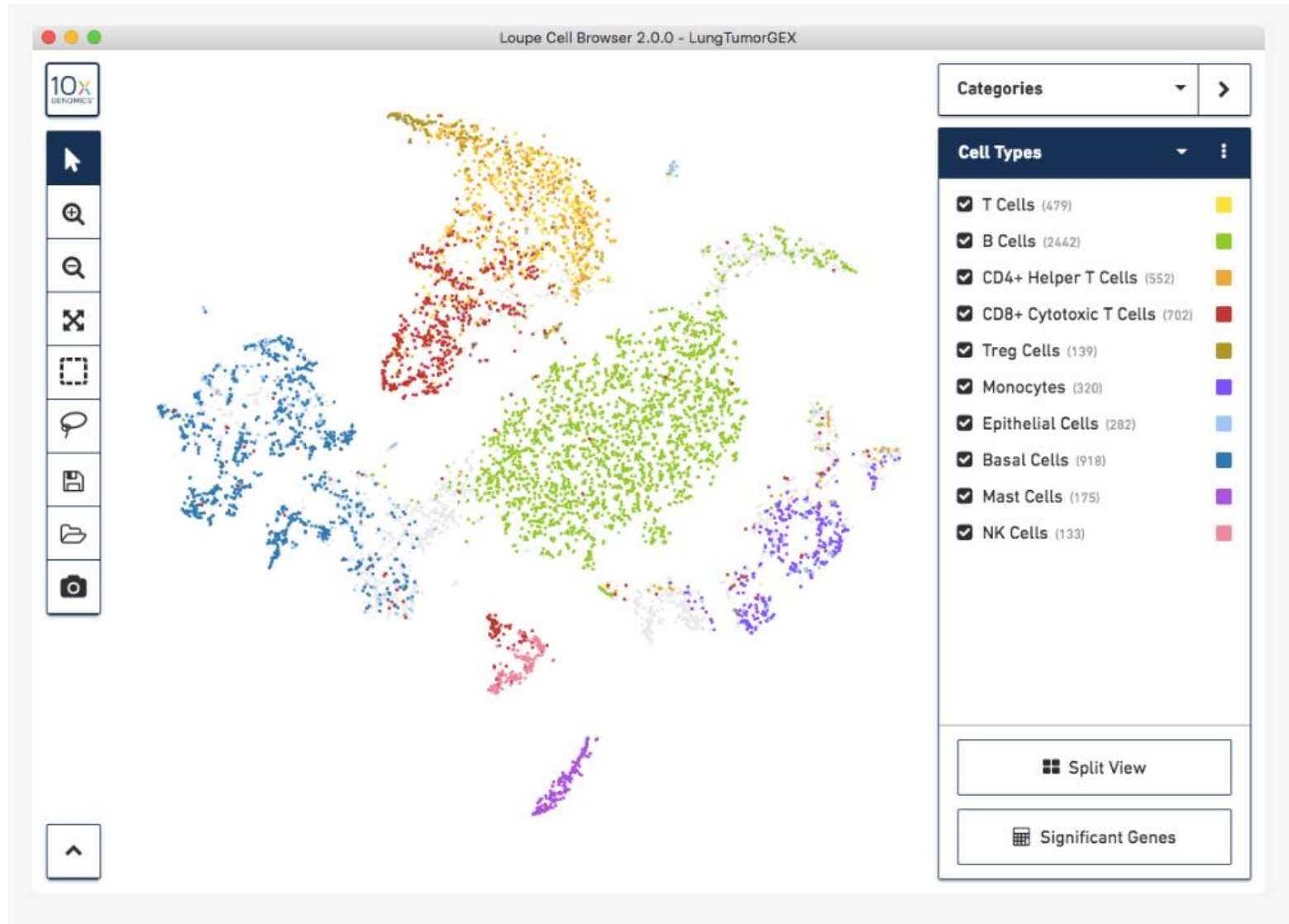
## V(D)J Enriched Library Structure:



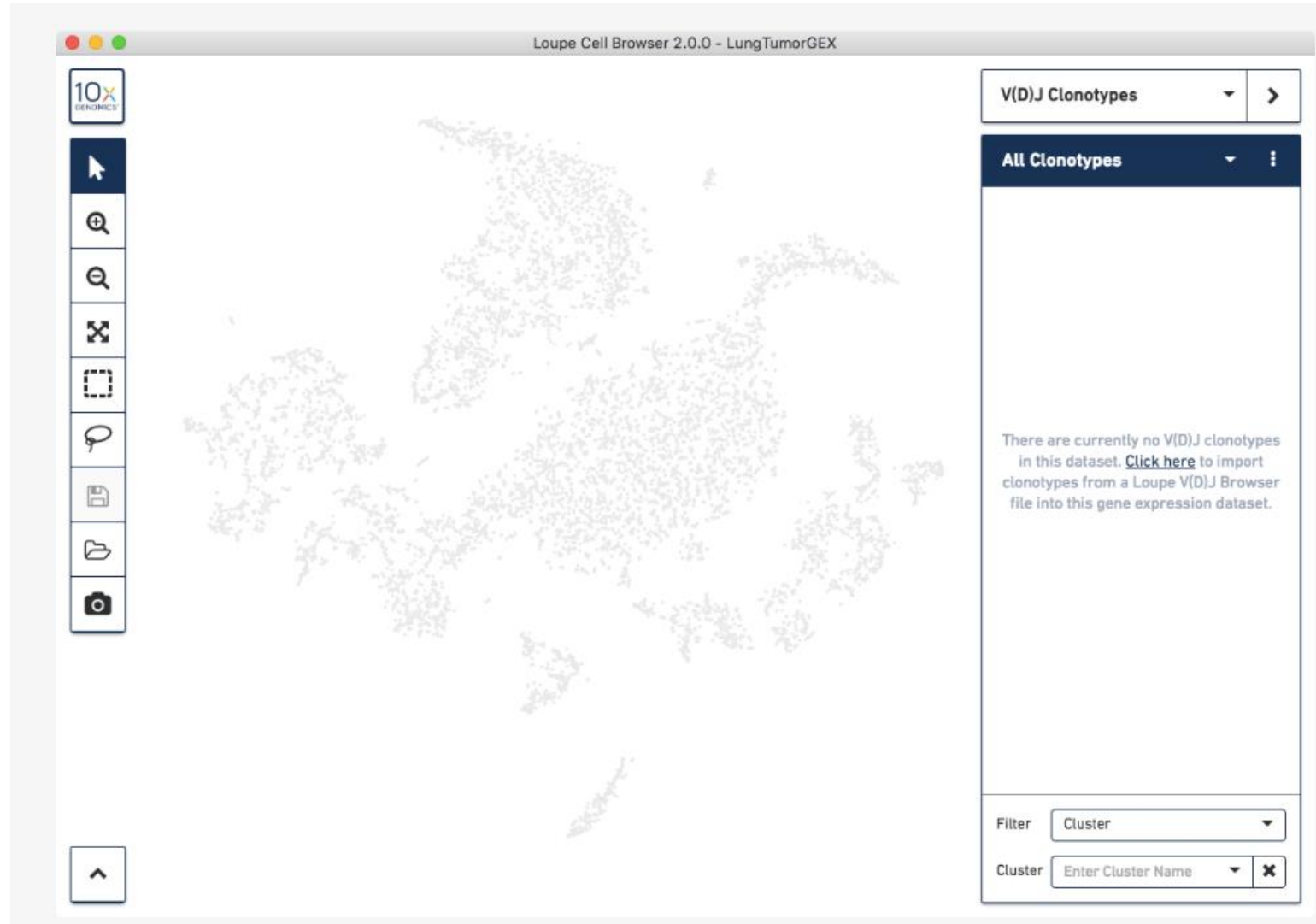
## 5' Gene Expression Library Structure:



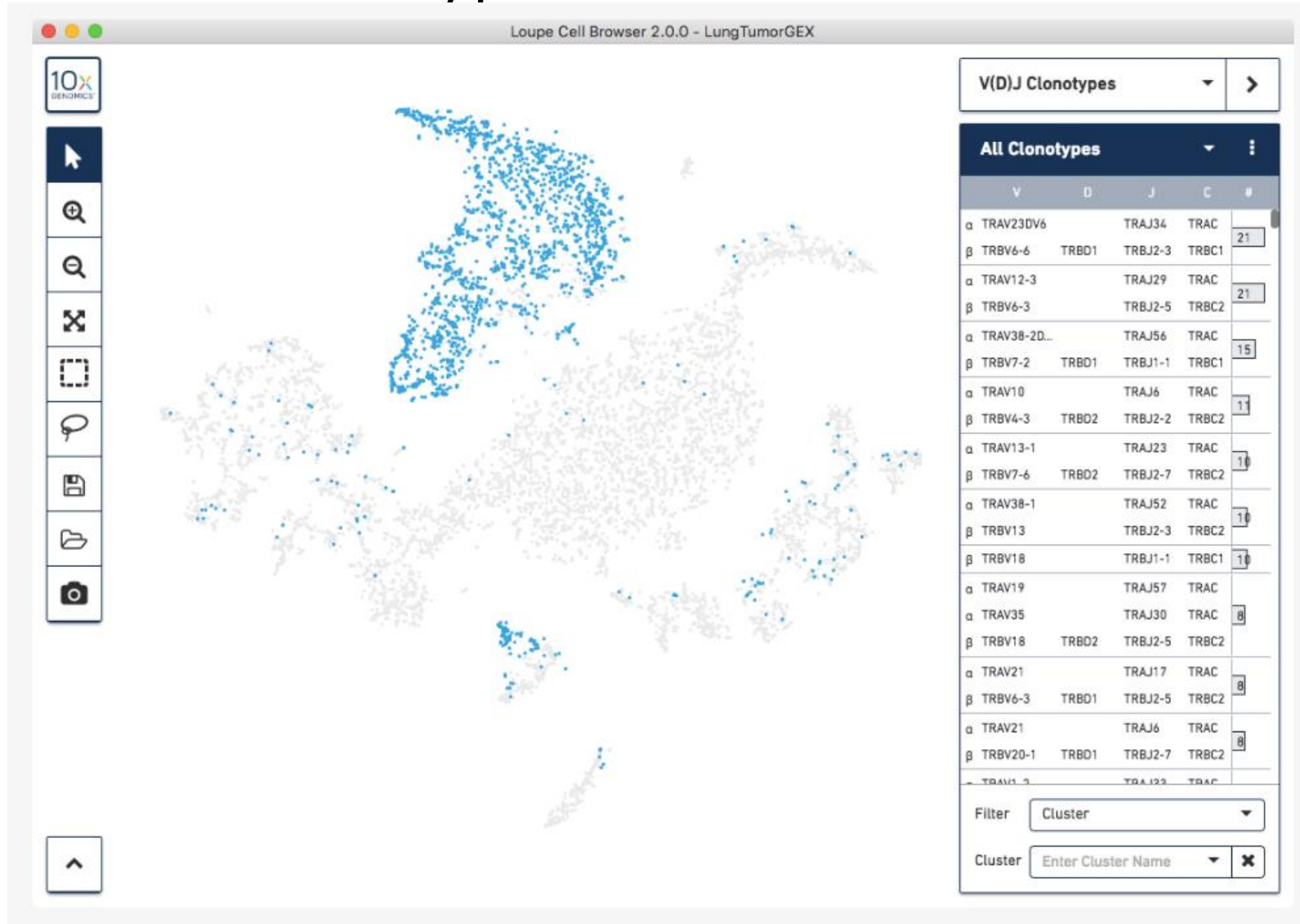
# Exploring data using Cell Ranger tools



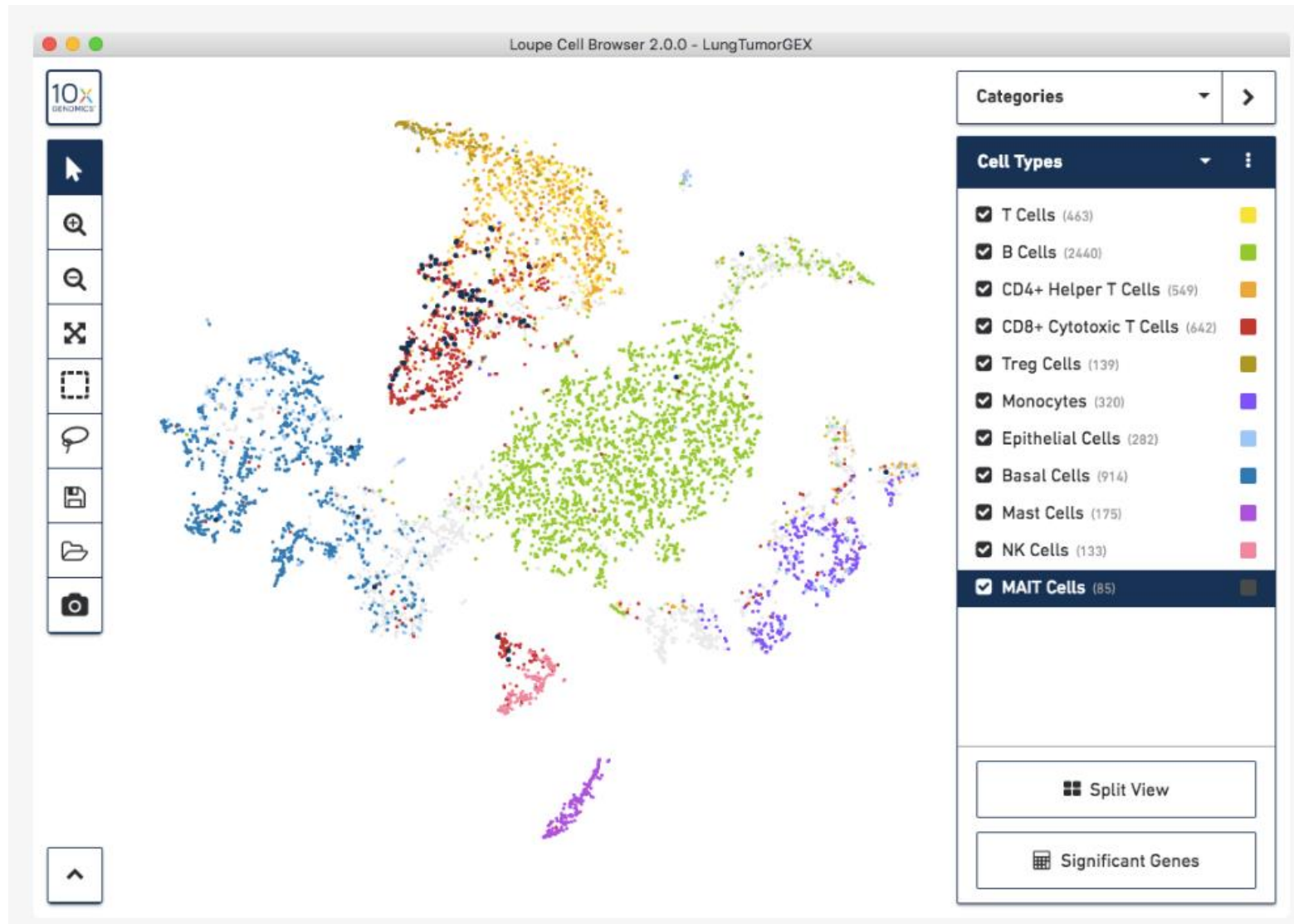
# Exploring data continued...



# Visualize ALL clonotypes



# Combine clonotypes w/gene expression



# Cost estimates

## Library prep

- With 10,000 cells/reaction: 0.25 EUR/cell
- With 1000 cells/reaction: 2.50 EUR/cell

Sequencing 5' expression @ 25,000 reads/cell:  
0.20 EUR/cell

Sequencing BCR @ 2500 reads/cell:  
0.02 EUR/cell

Total cost 5' GEX + BCR sequencing

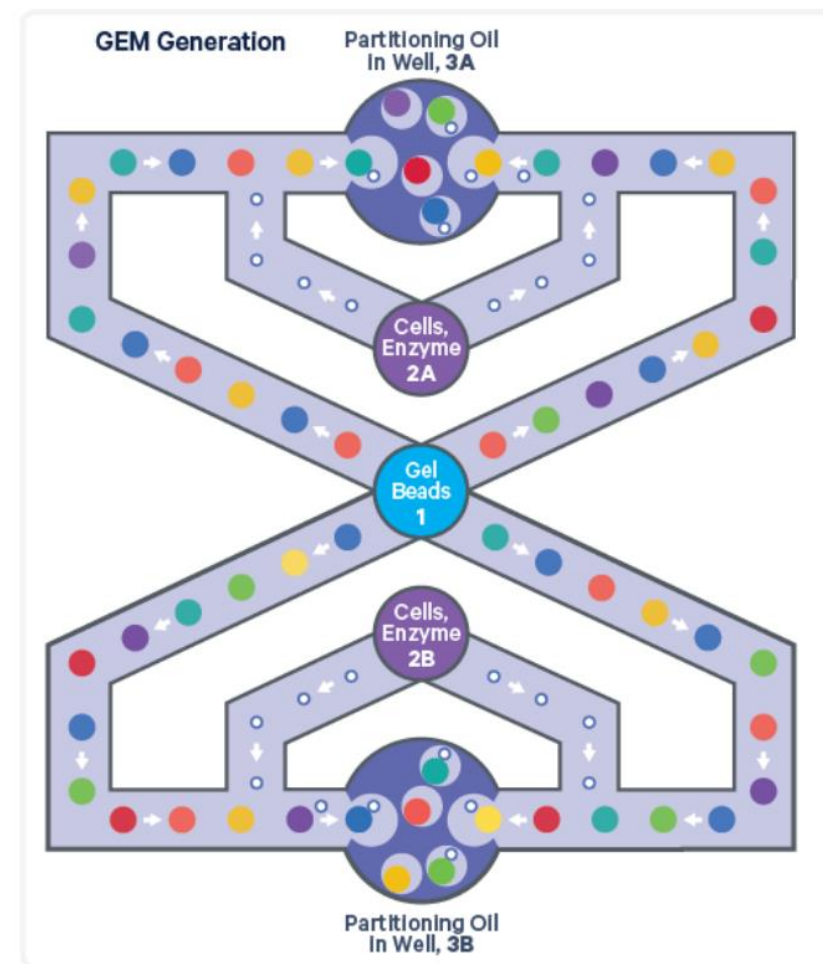
10,000 cells:

0.47 EUR/cell

1,000 cells:

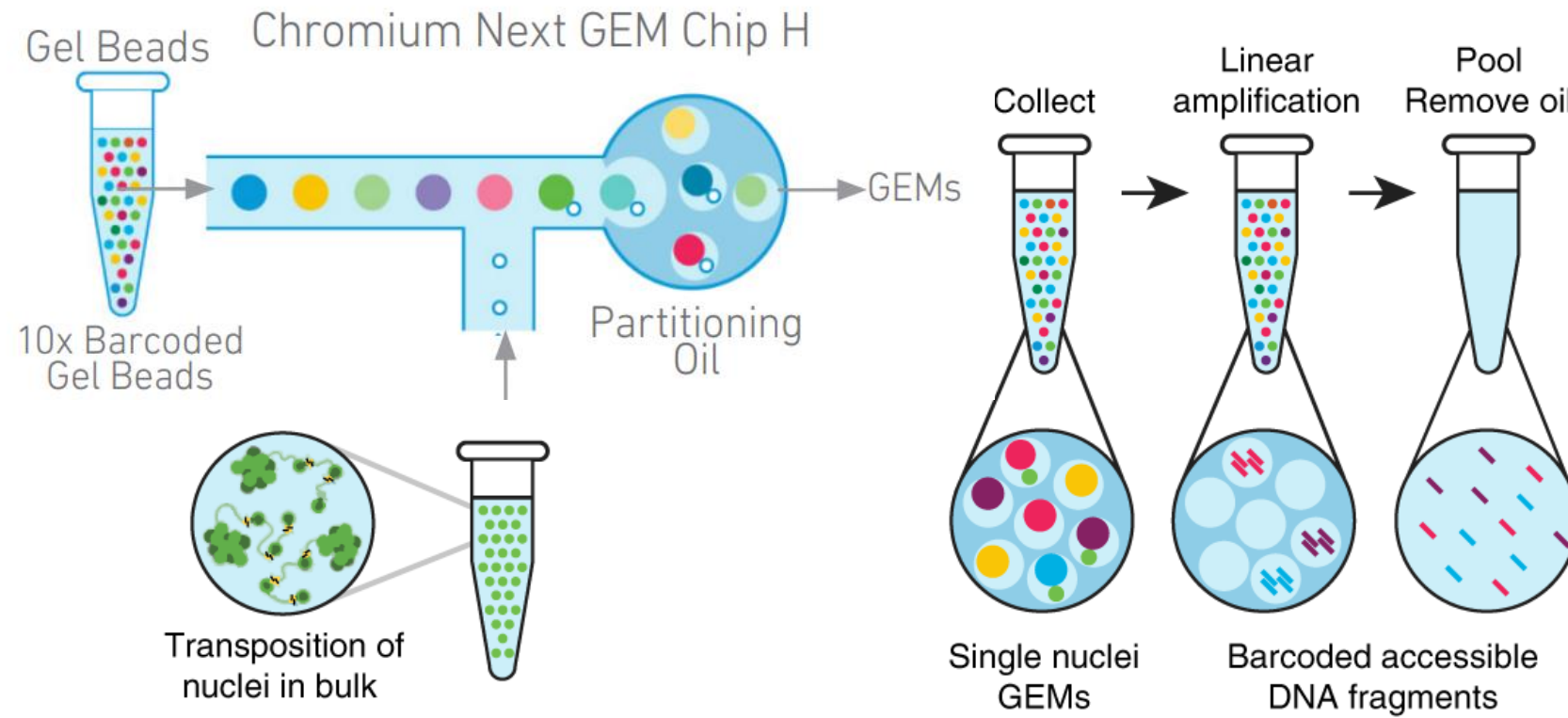
2.72 EUR/cell

# NEW! Chromium X

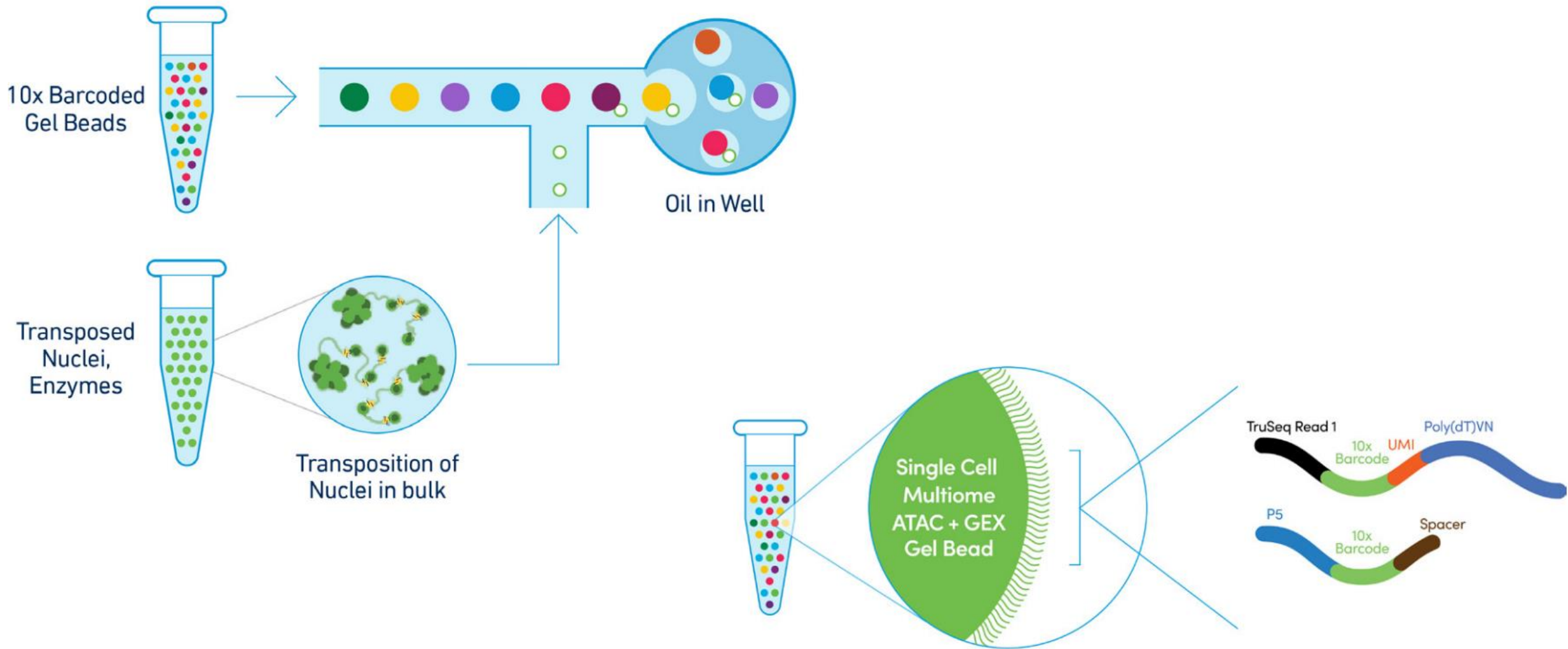




# Single-cell ATAC-seq



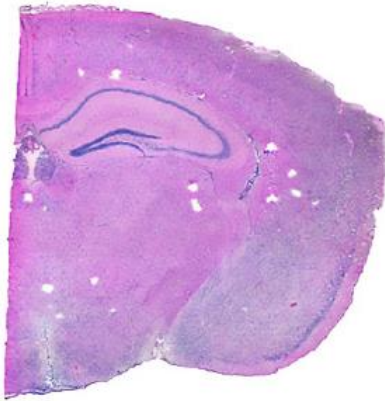
# Single-cell multiome



# Visium for spatial transcriptomics

A

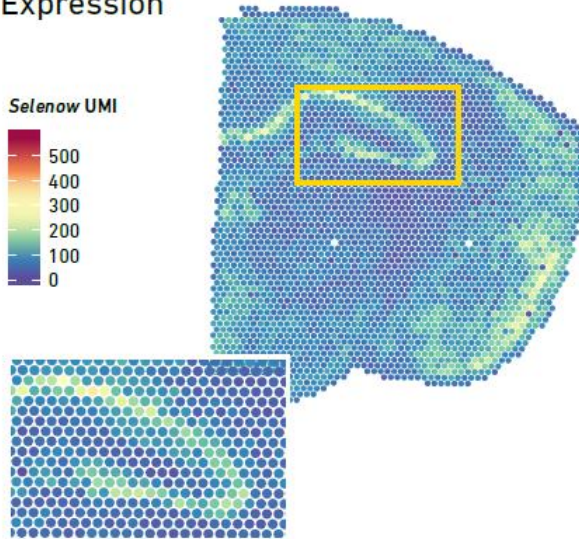
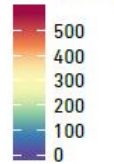
H&E Only



B

*Selenow*  
Expression

*Selenow* UMI



C

*Hpca*  
Expression

*Hpca* UMI

