

# 10x Genomics Platform

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Leiden Genome Technology Center (LGTC)

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# 10x Genomics Chromium Controller

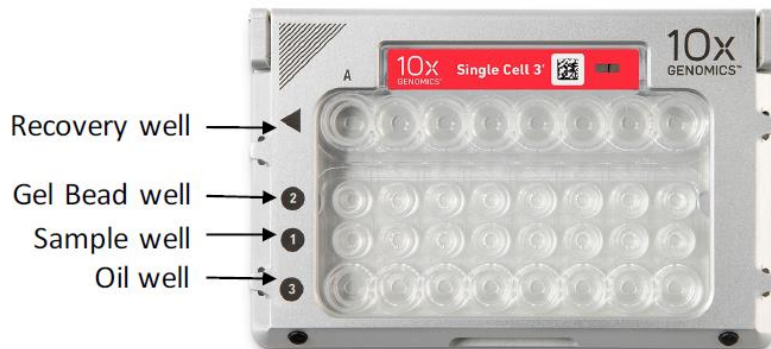
Commercial launch early 2016

Microfluidics system for reaction compartmentalization

High throughput, up to 80k cells/run

High capture rate, ~50%

Single-use microfluidics chip



# 10x Genomics products

## Single Cell Genomics

- Copy Number Variation

## Single Cell Transcriptomics

- Gene Expression Profiling
- Gene Expression CRISPR Screening (Perturb-Seq)
- Gene & Cell Surface Protein (CITE-seq or Cell Hashing)
- Immune Profiling
- Immune Profiling & Cell Surface Protein
- Immune Profiling & Antigen Specificity

## Single Cell Epigenomics

- Chromatin Accessibility (ATAC-seq)

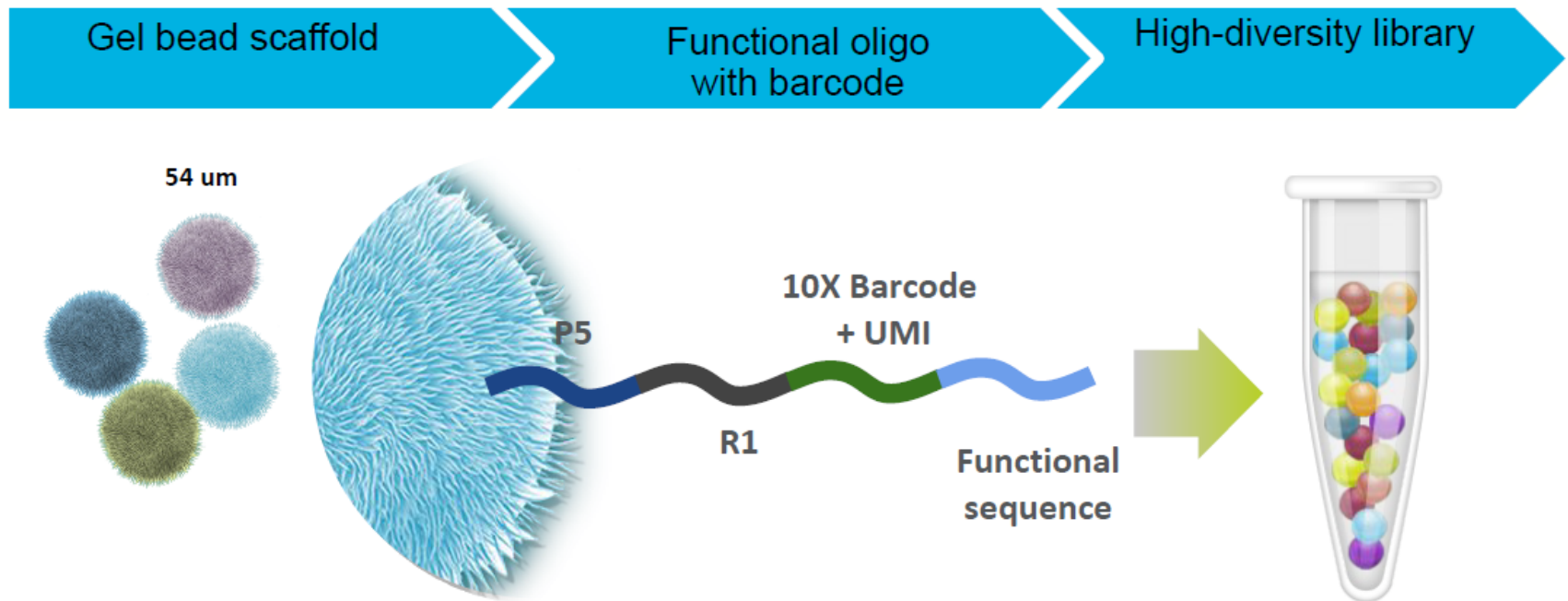
## Linked-Reads Genomics

- Whole Genome Sequencing
- Exome Sequencing
- *de novo* Assembly

## NEW: Spatial transcriptomics

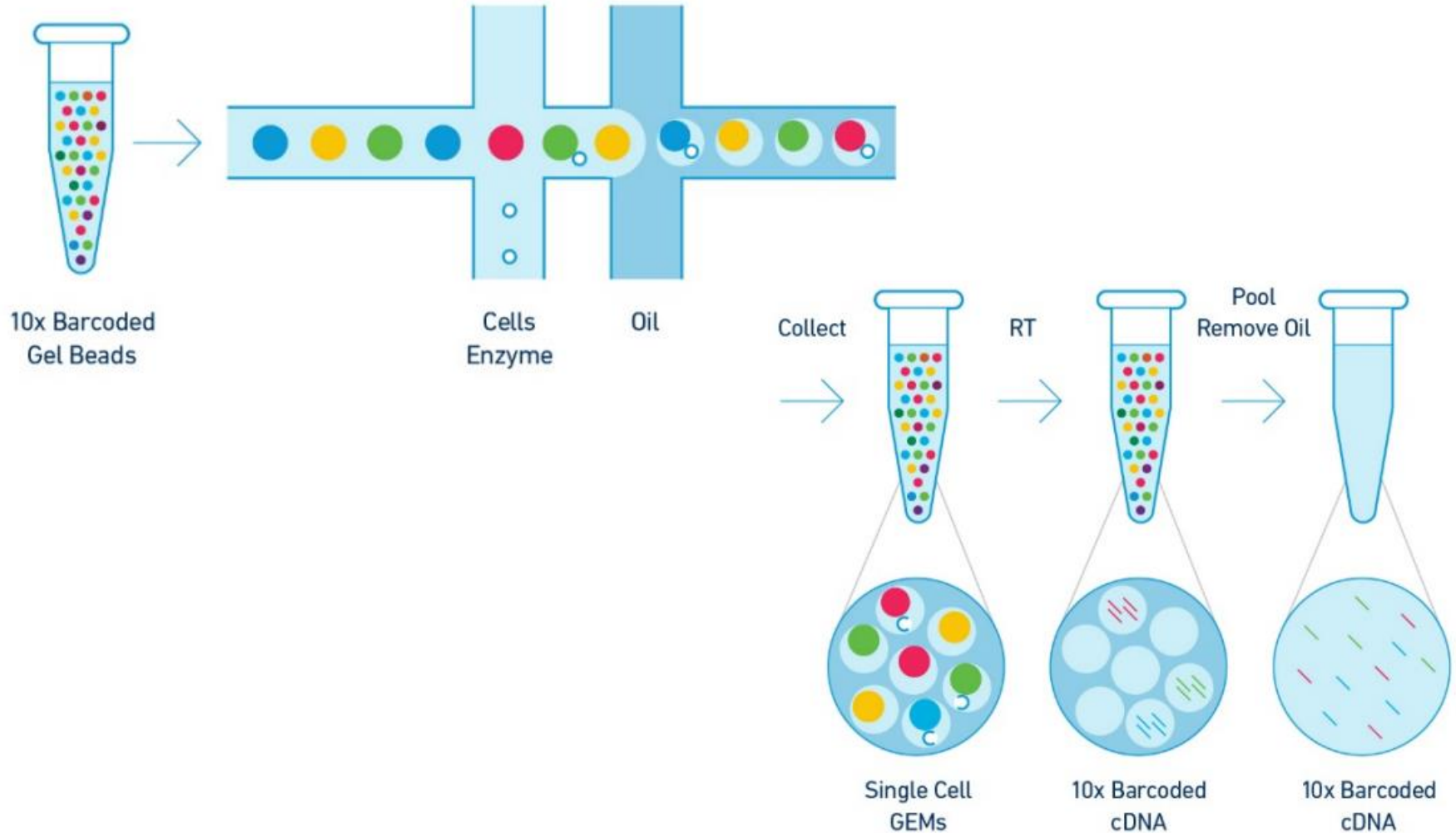
# Gel beads up close

4M Discrete Reagents in One Tube

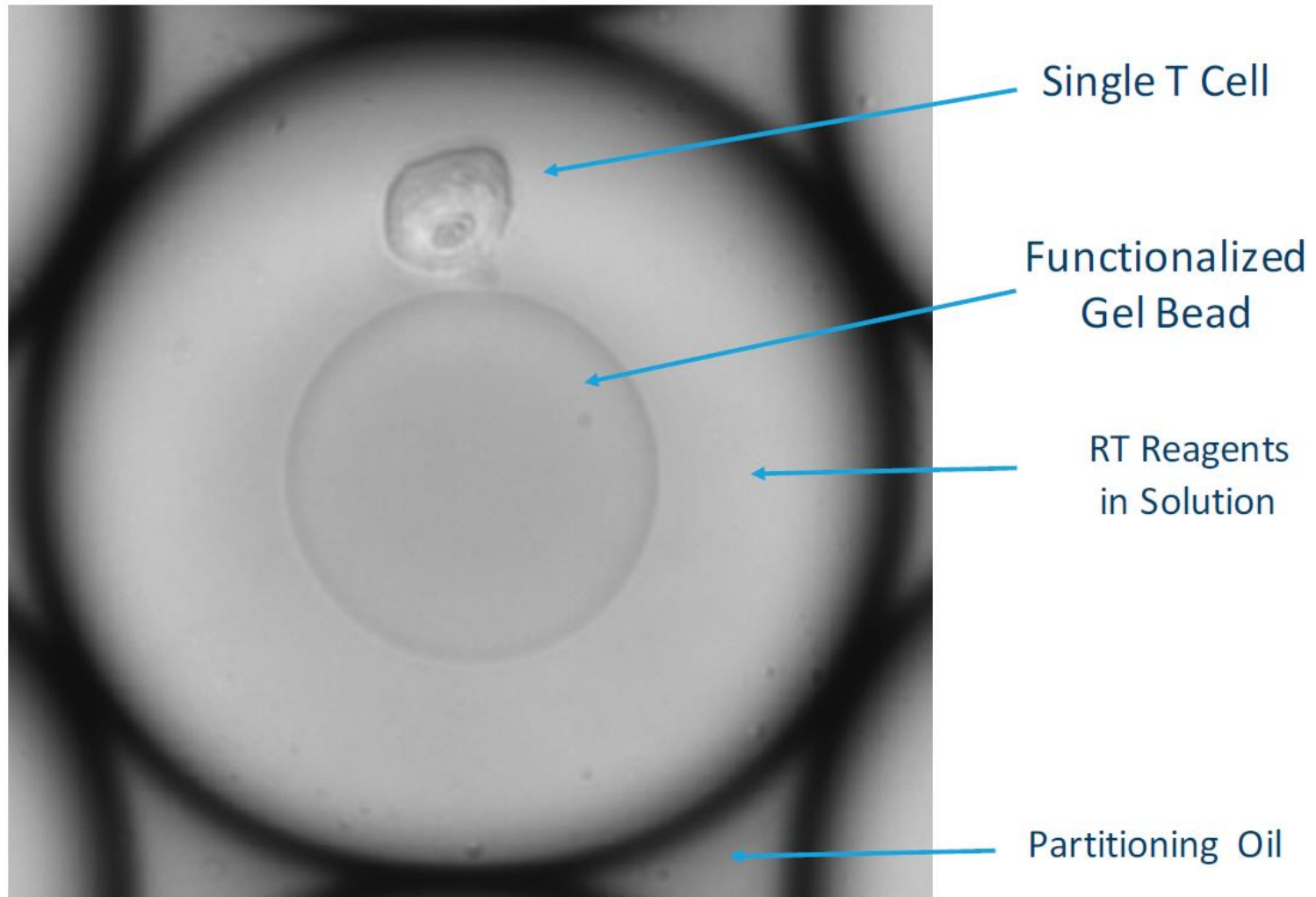


**10X Barcode (16bp):** unique for each GemBead  
**+ UMI (10bp):** correcting PCR duplicates

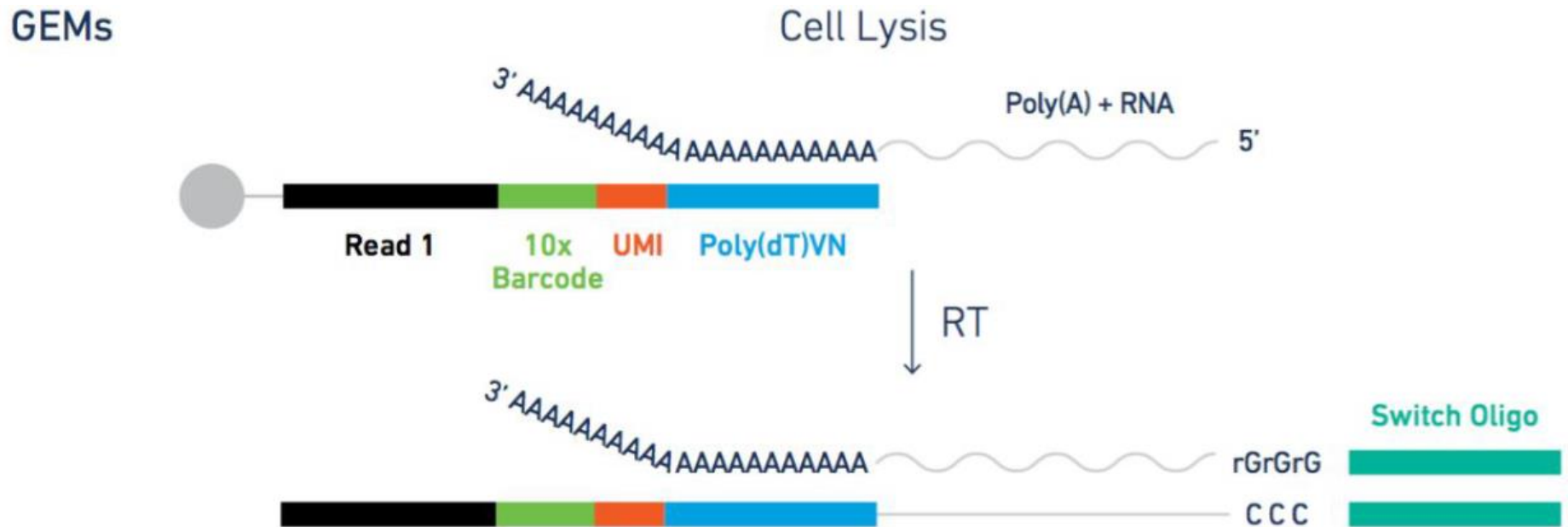
# Gel bead in Emulsion (GEM) technology



# GEMs up close

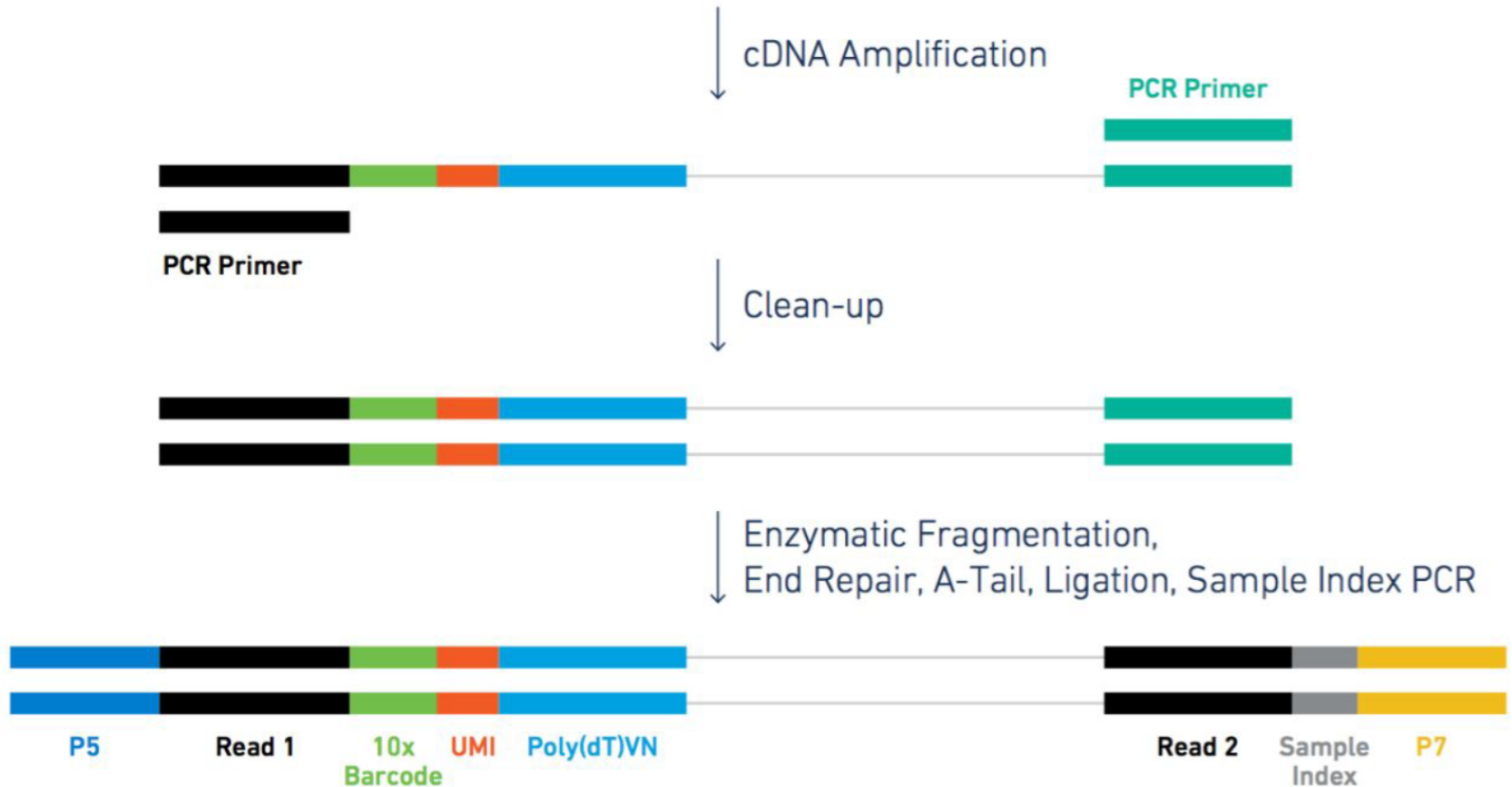


# Assay scheme for 3' mRNA sequencing



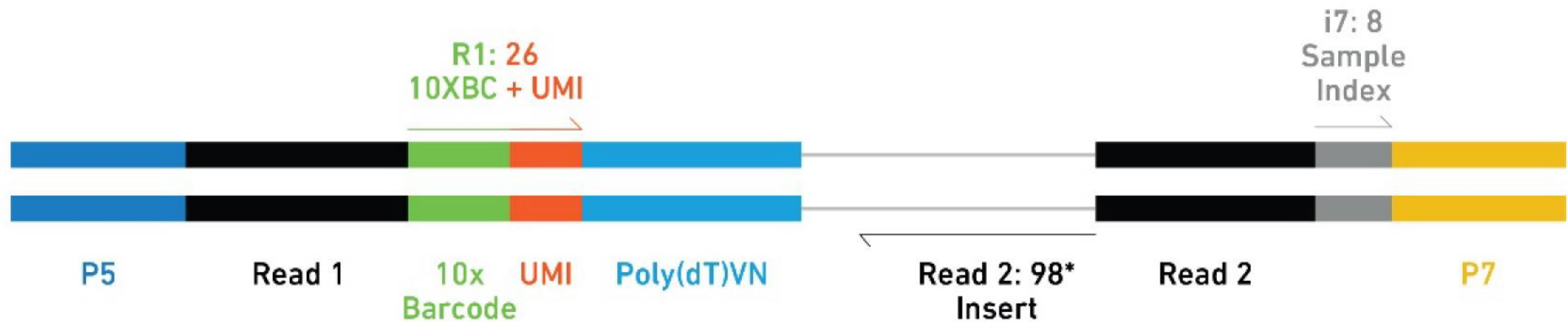
# Assay scheme for 3' mRNA sequencing

Bulk

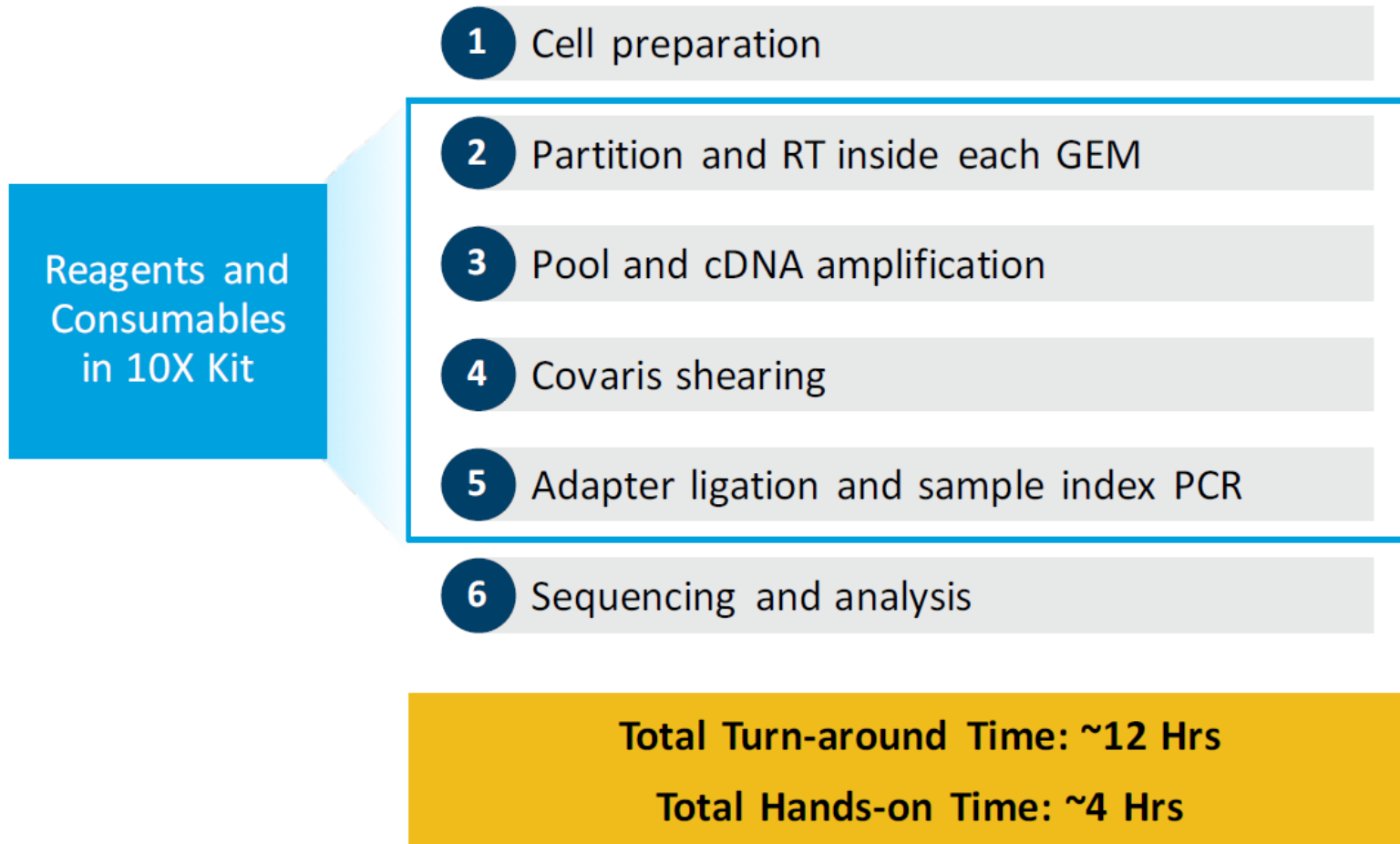




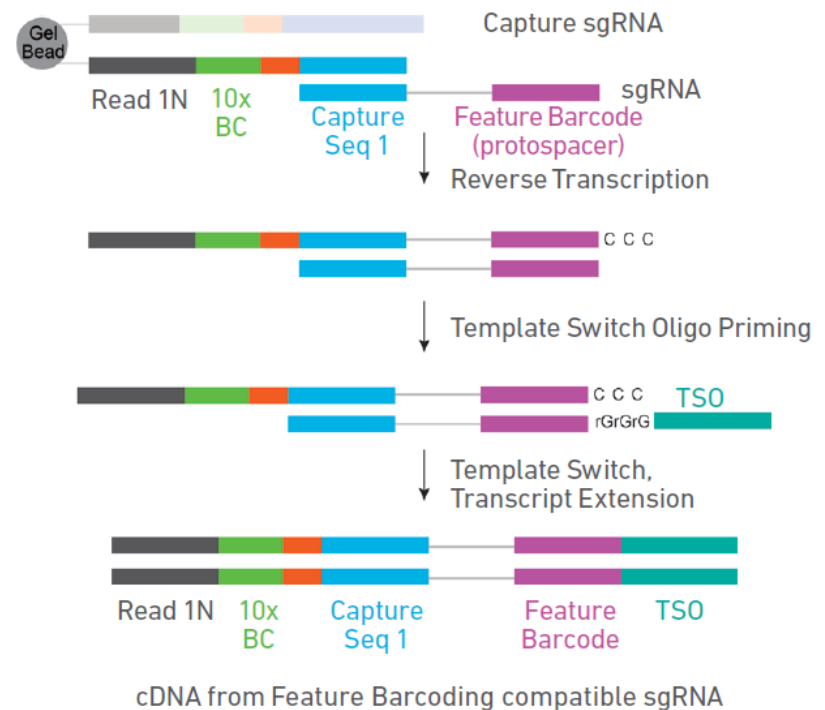
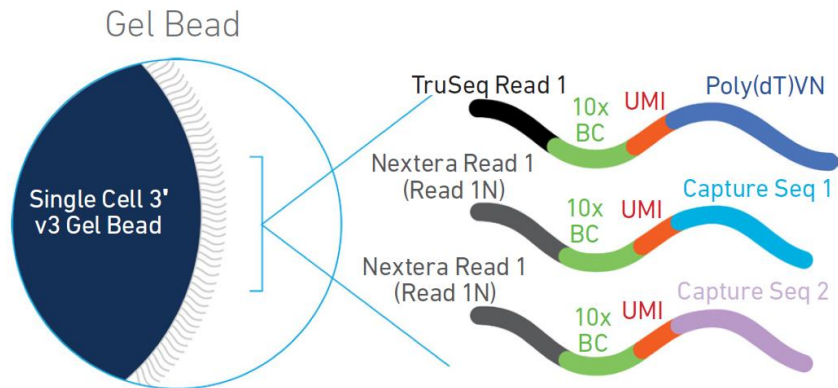
# Final library structure



# Single cell 3' end-to-end workflow



# Single cell 3' feature barcoding



# Structure of T and B cell receptors

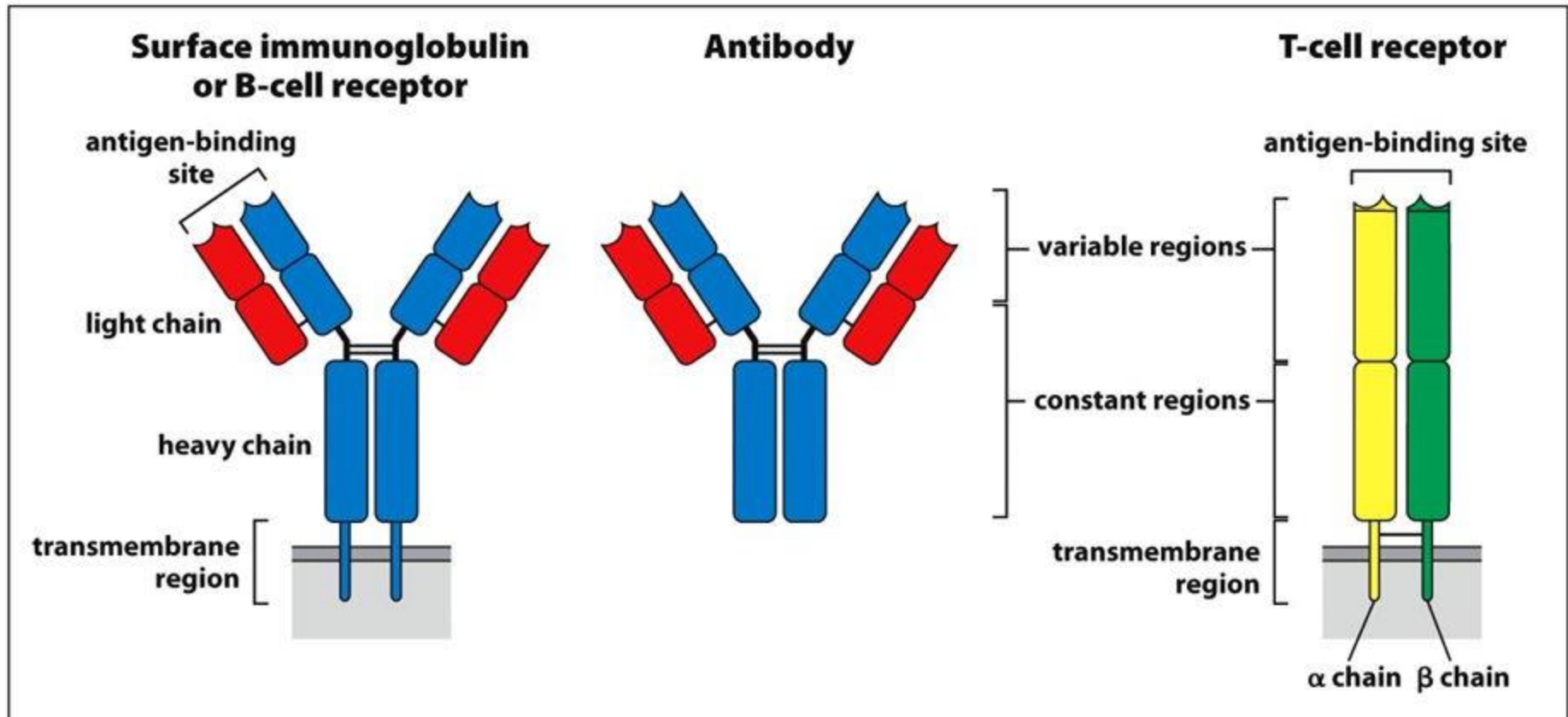
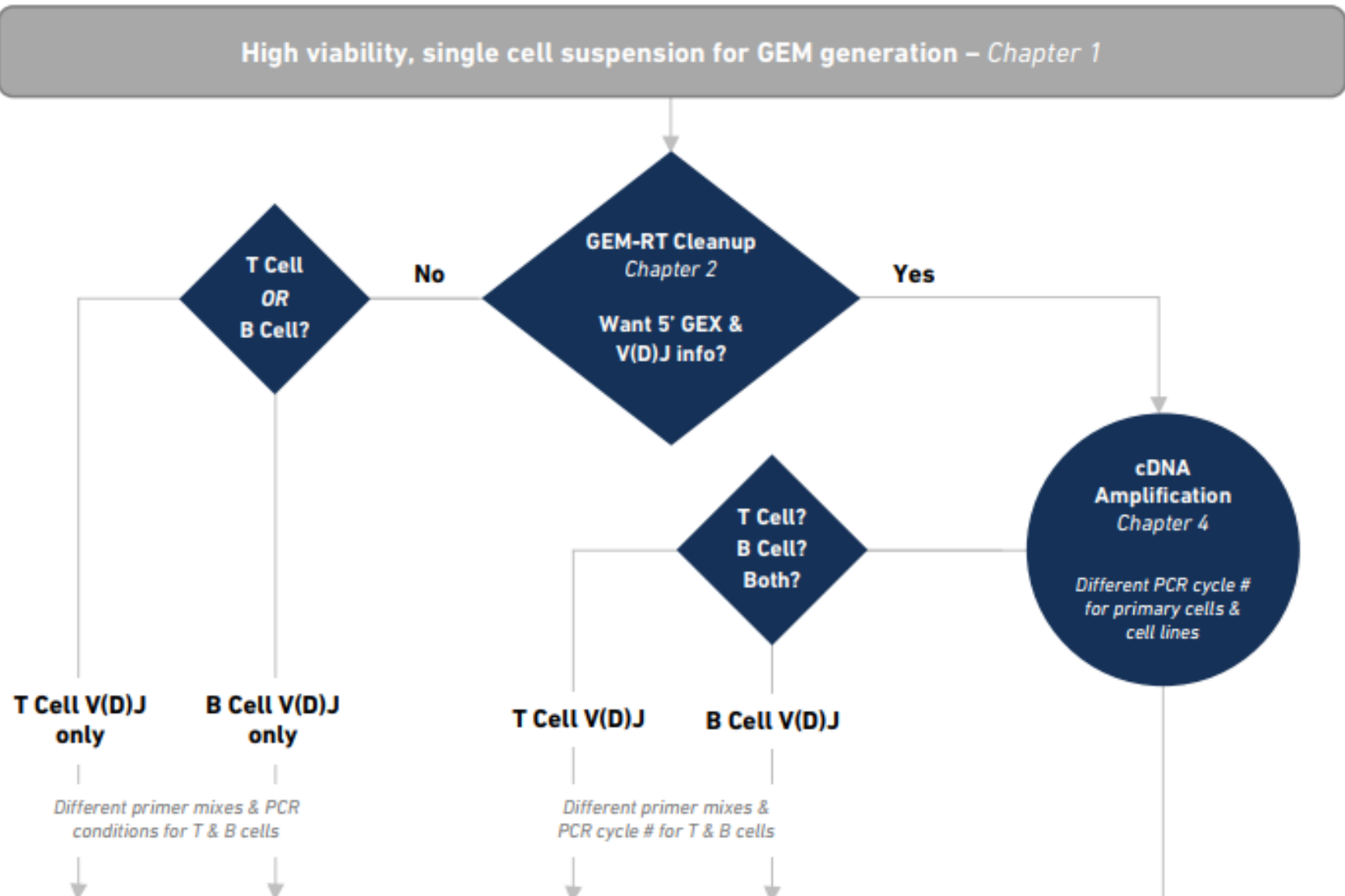


Figure 3.1 The Immune System, 3ed. (© Garland Science 2009)

\*More in lecture from Erik and Indu on Thursday

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



# Gel bead oligos

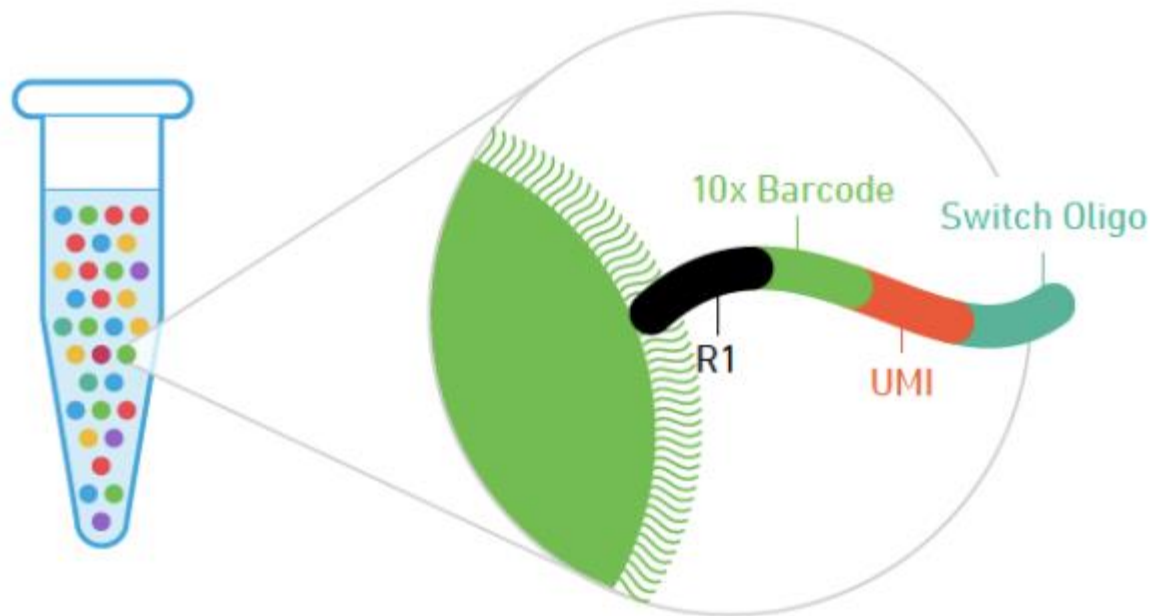
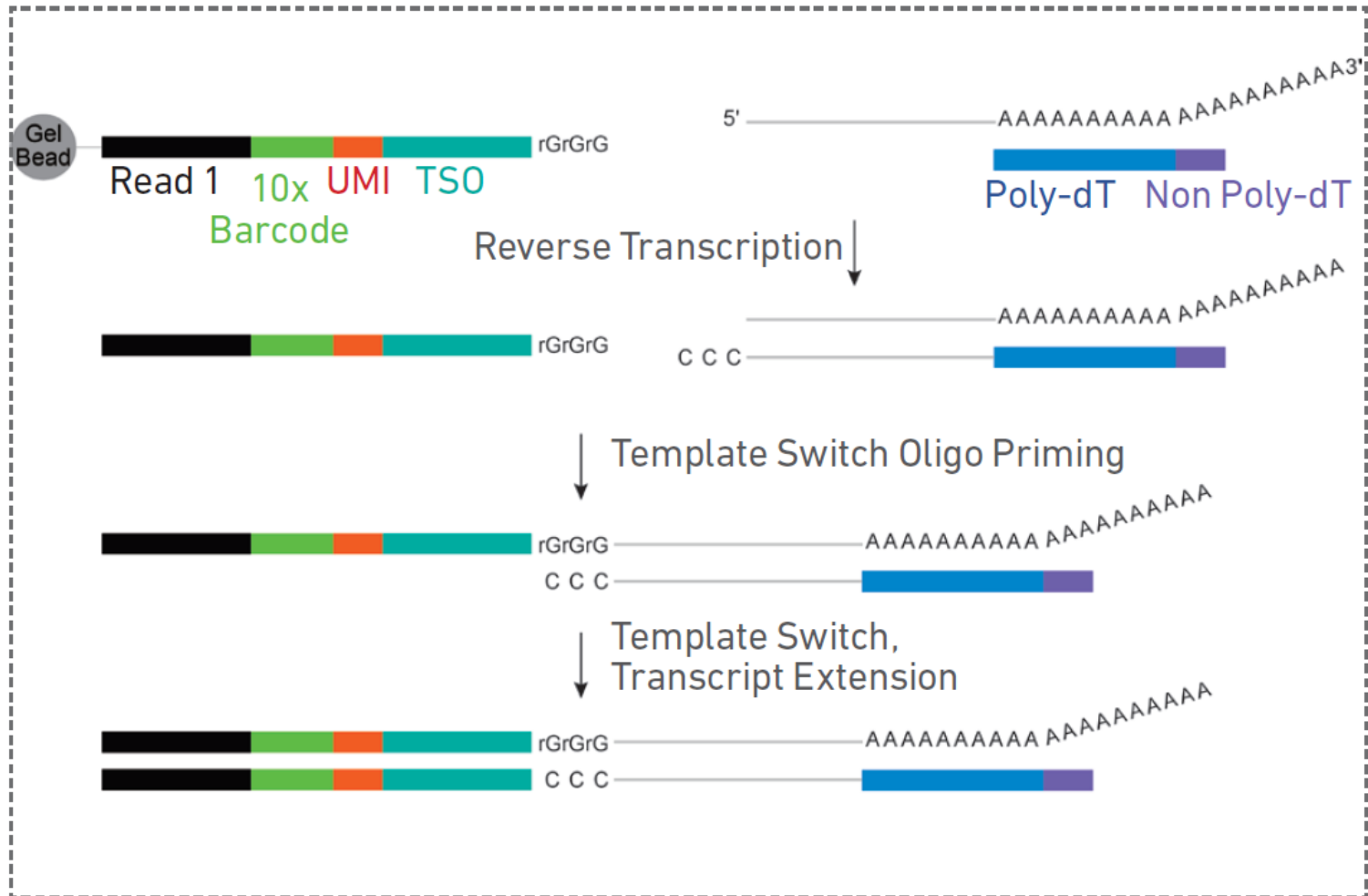


Figure 1. Schematic of a Single Cell 5' Gel Bead oligo primer.

- 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

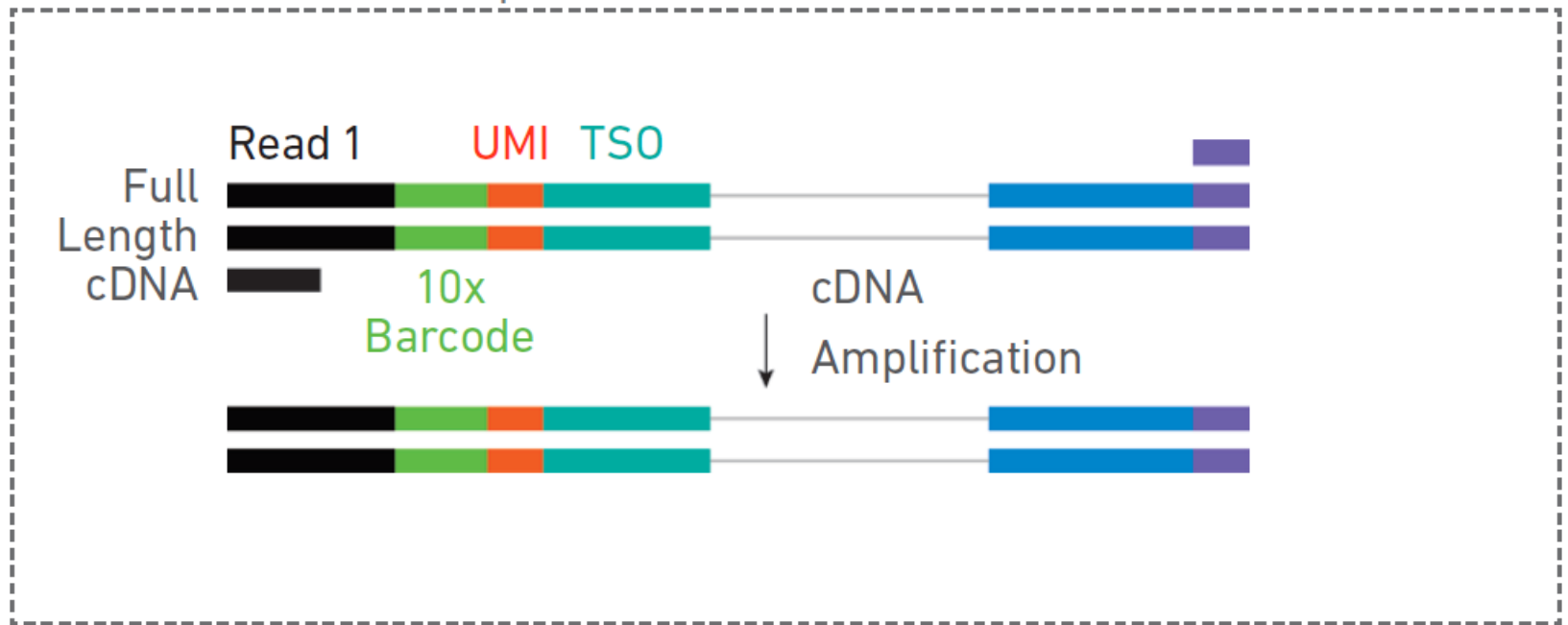
# Assay scheme for 5' scRNA-seq

Inside individual GEMs



# Assay scheme for 5' scRNA-seq

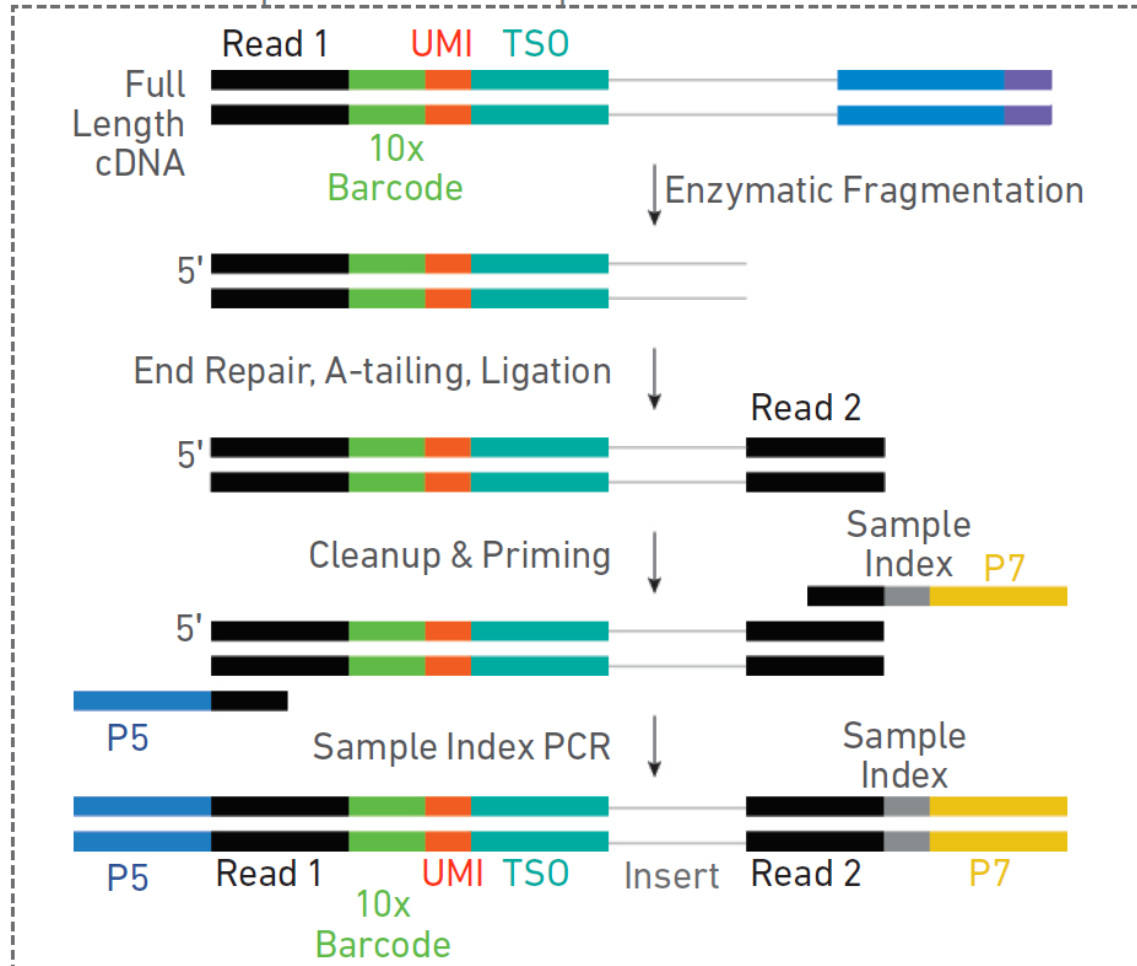
Pooled cDNA amplification





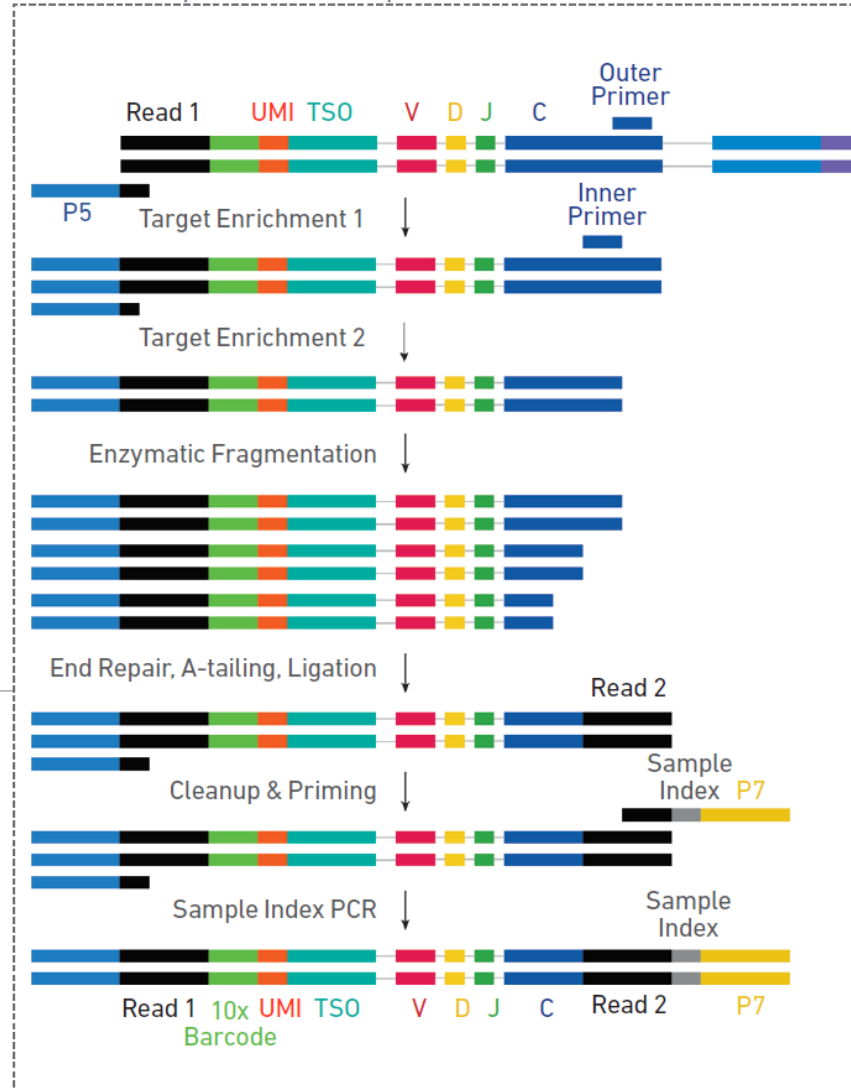
# Assay scheme for 5' scRNA-seq

Pooled amplified cDNA processed in bulk



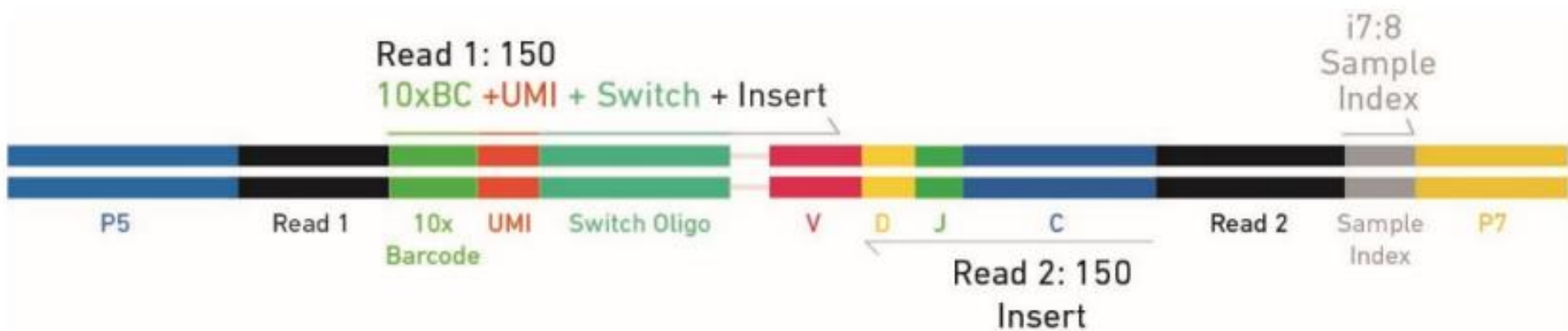
# Assay scheme for 5' VDJ libraries

Pooled amplified cDNA processed in bulk

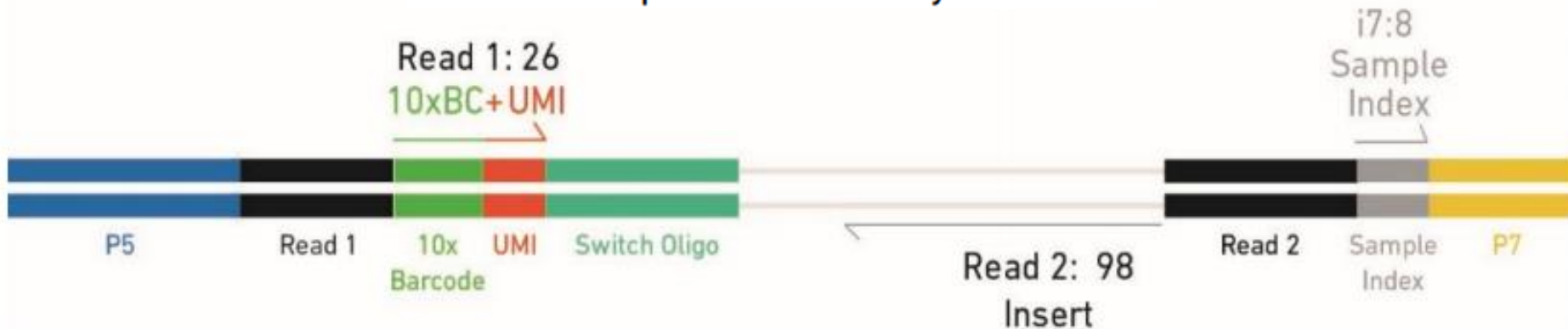


# Final library structure

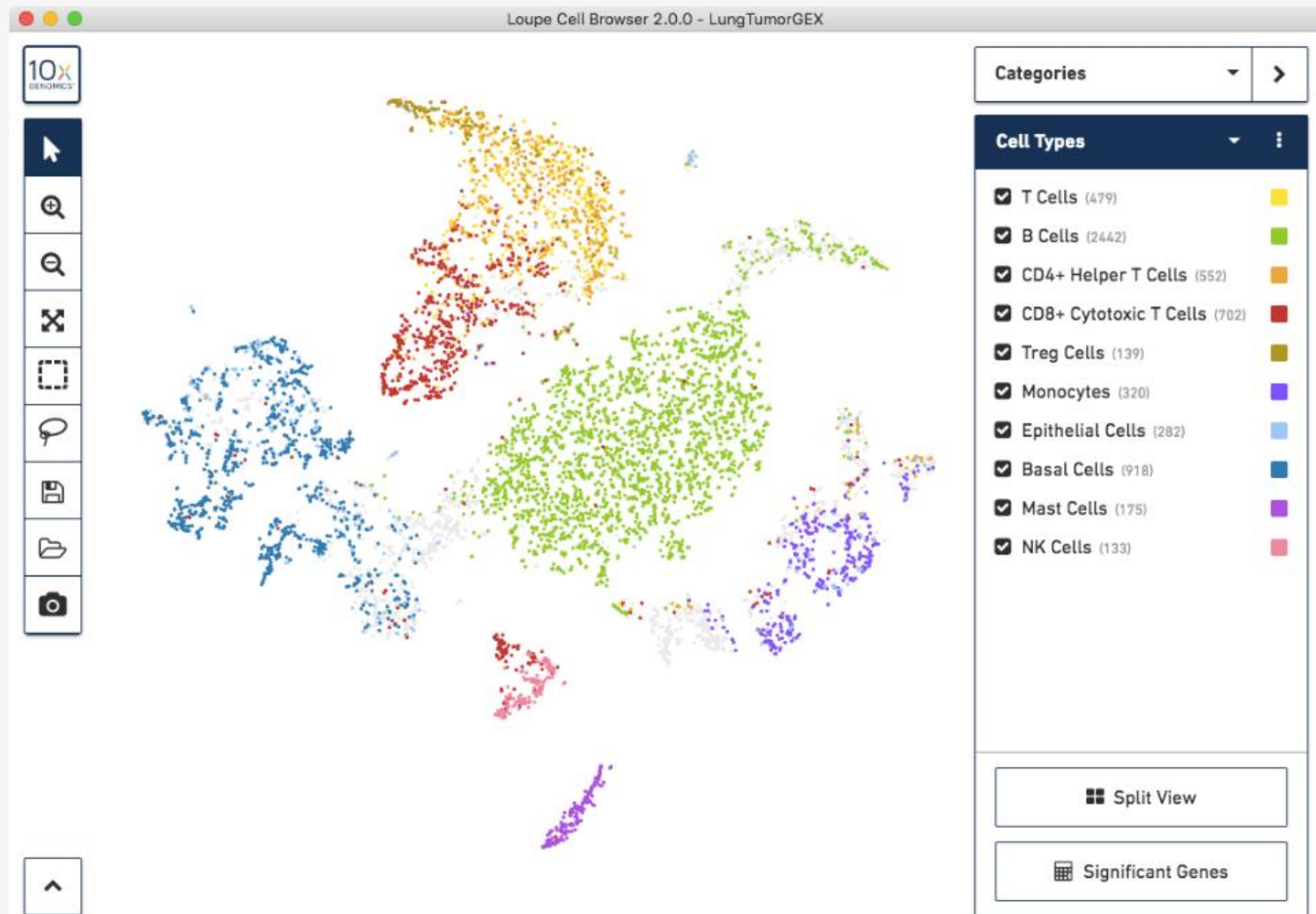
## V(D)J Enriched Library Structure:



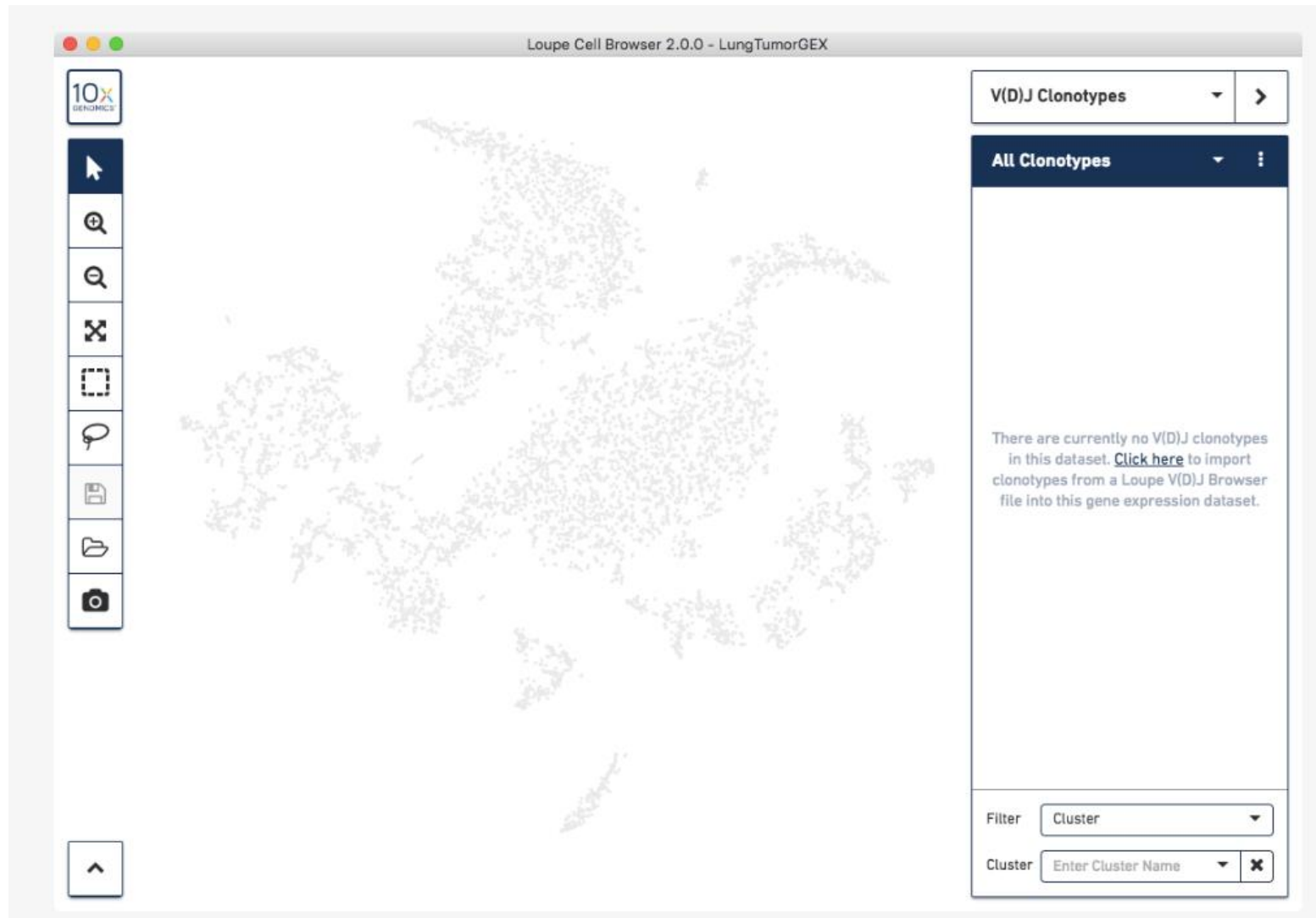
## 5' Gene Expression Library Structure:



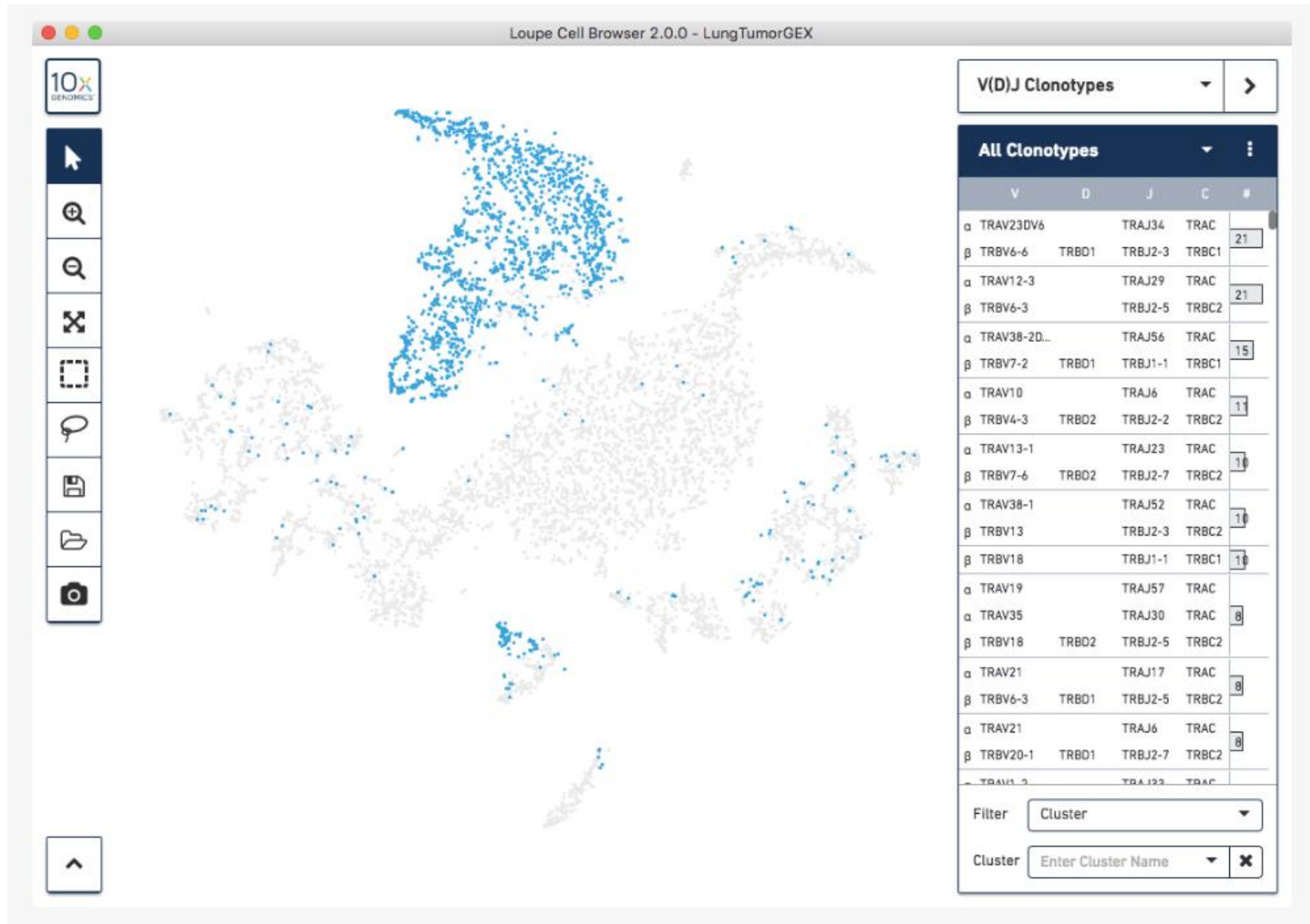
# Exploring data using Cell Ranger tools



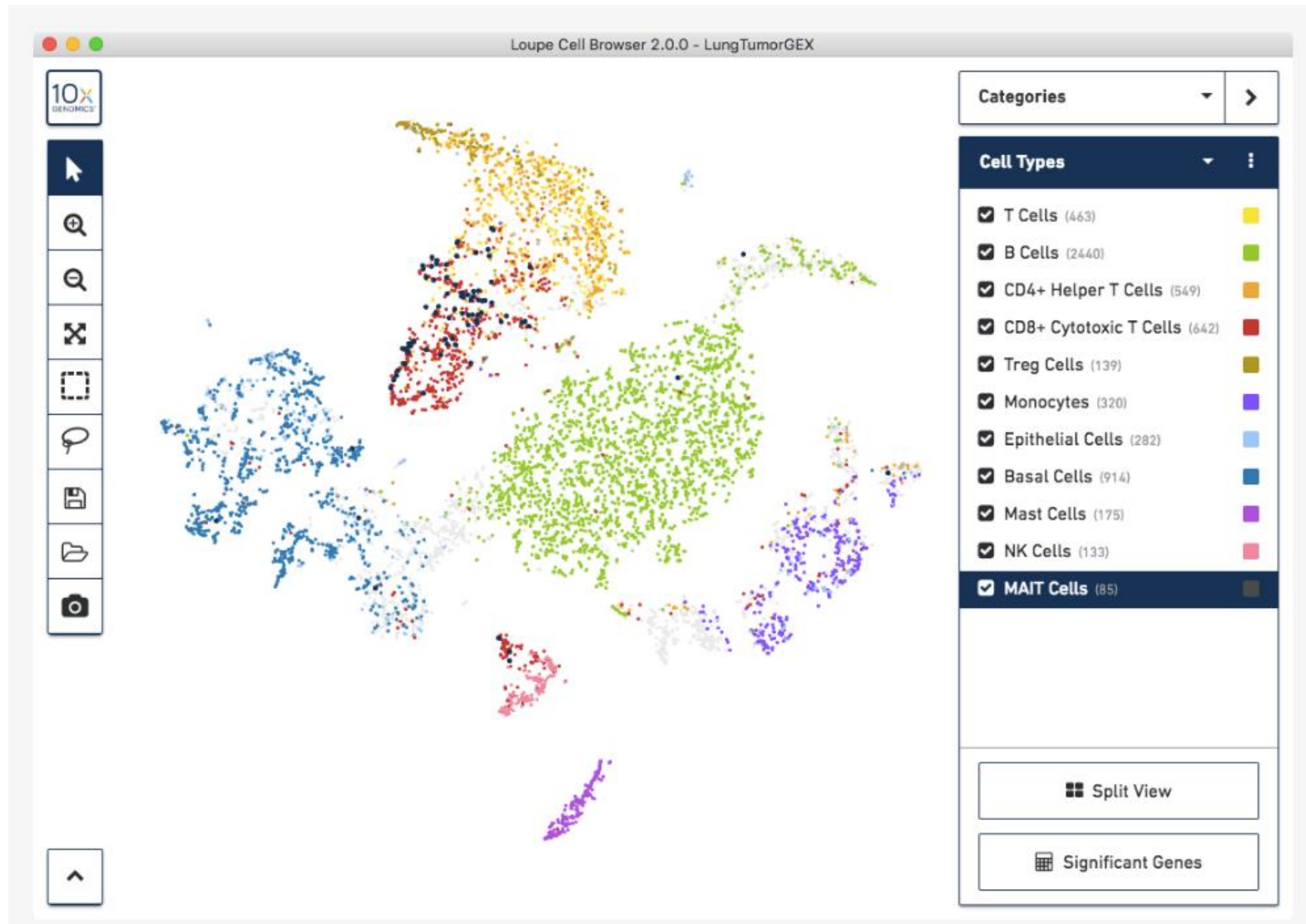
# Exploring data continued...



# 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

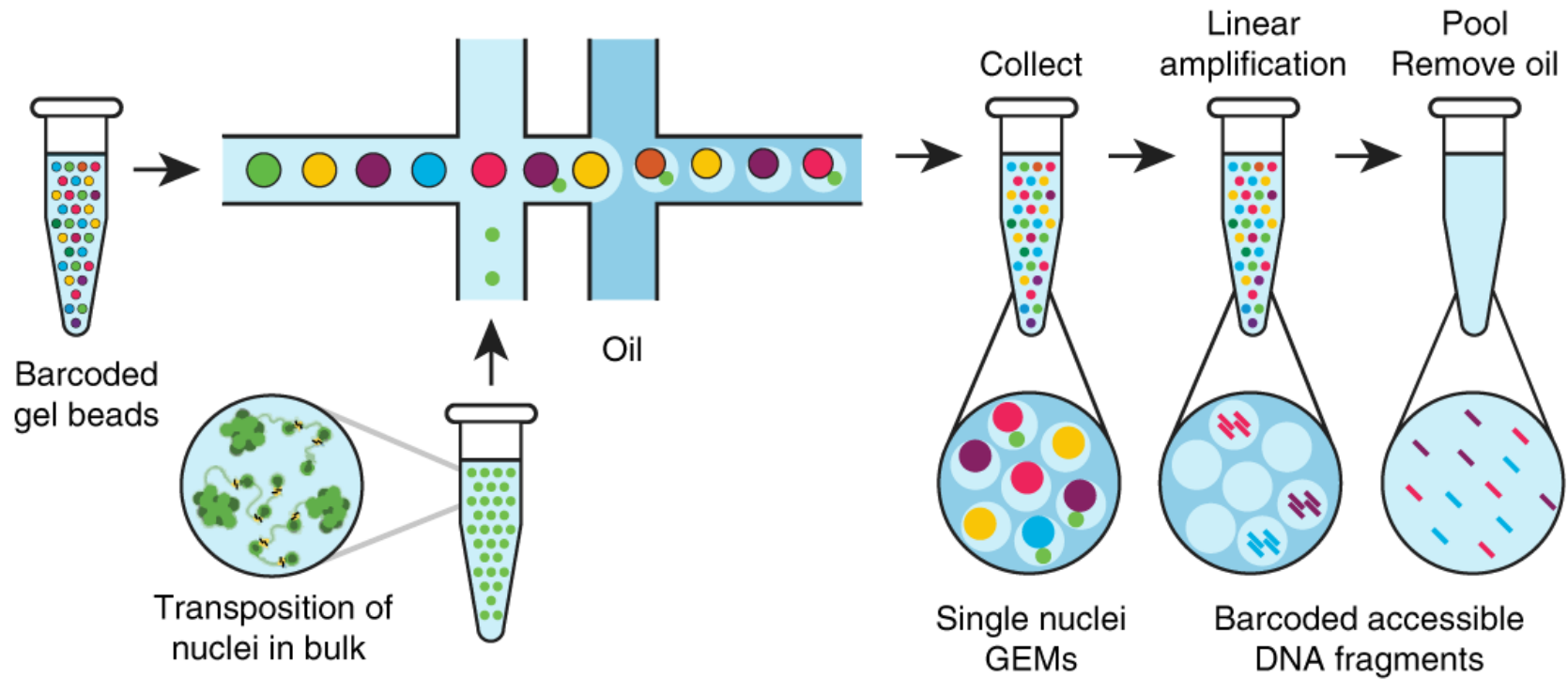
Sequencing BCR @ 2500 reads/cell:  
0.02 EUR/cell

Total cost 5' GEX + BCR sequencing

10,000 cells:	0.47 EUR/cell
1,000 cells:	2.72 EUR/cell



# Single-cell ATAC-seq



# NEW! Spatial transcriptomics

A

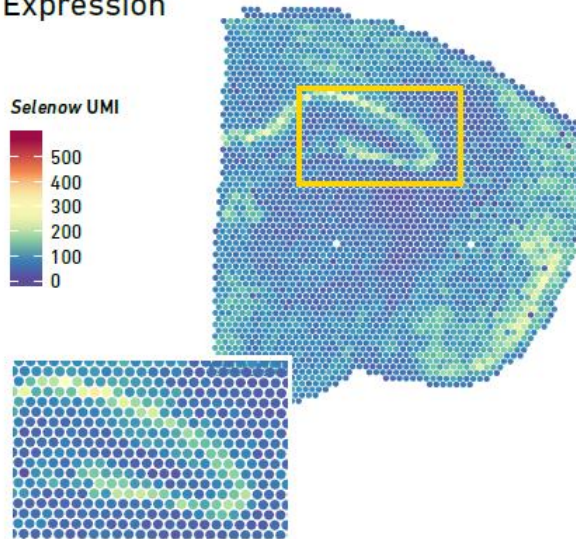
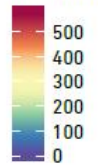
H&E Only



B

*Selenow*  
Expression

*Selenow* UMI



C

*Hpca*  
Expression

*Hpca* UMI

