#### 10x Genomics Platform

# Susan Kloet Leiden Genome Technology Center (LGTC) 19 October 2020



#### 10x Genomics Chromium Controller

Commercial launch early 2016

Microfluidics system for reaction compartmentalization

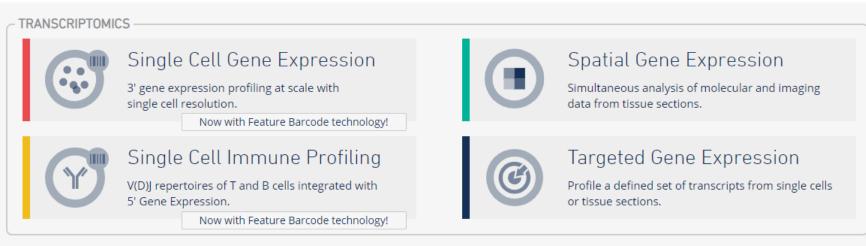
High throughput, up to 80k cells/run High capture rate, ~50%

Single-use microfluidics chip





#### 10x Genomics products



#### SINGLE CELL 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

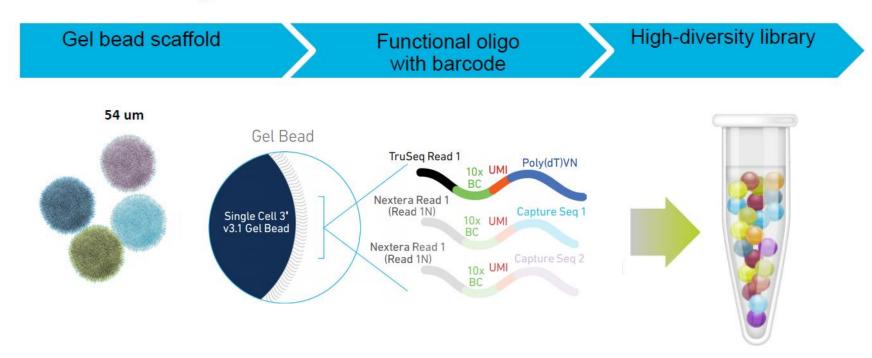


#### Single Cell CNV

Copy number variation and genomic heterogeneity at single cell resolution.

#### Gel beads up close

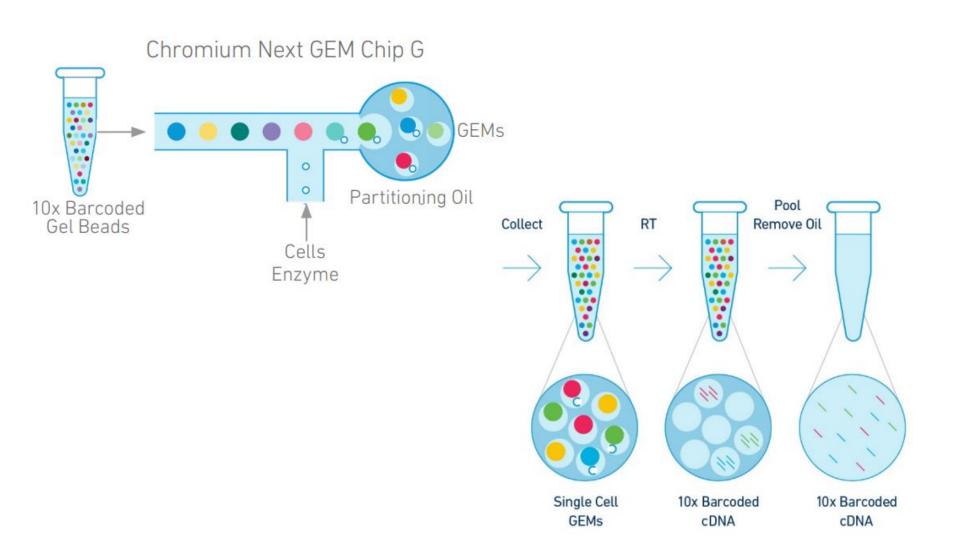
4M Discrete Reagents in One Tube



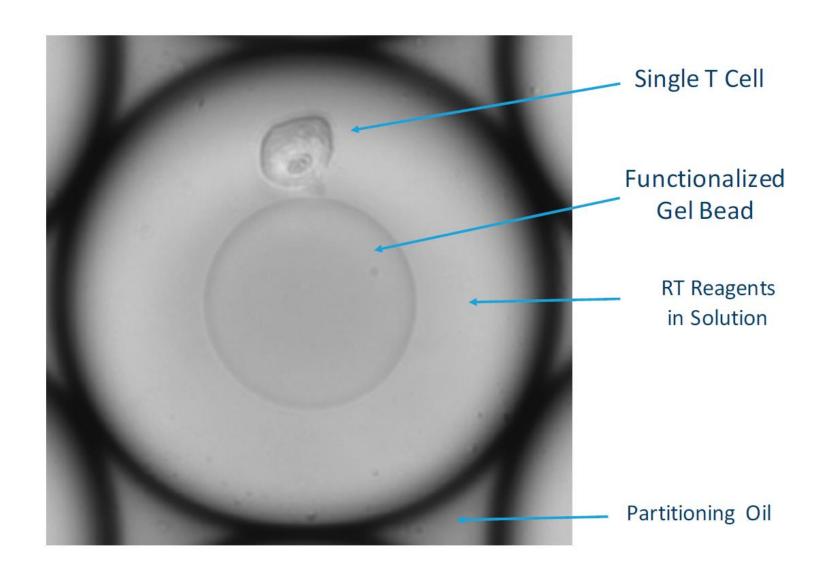
10x Barcode (16bp): unique for each GemBead

**+UMI (12bp): correct for PCR duplicates** 

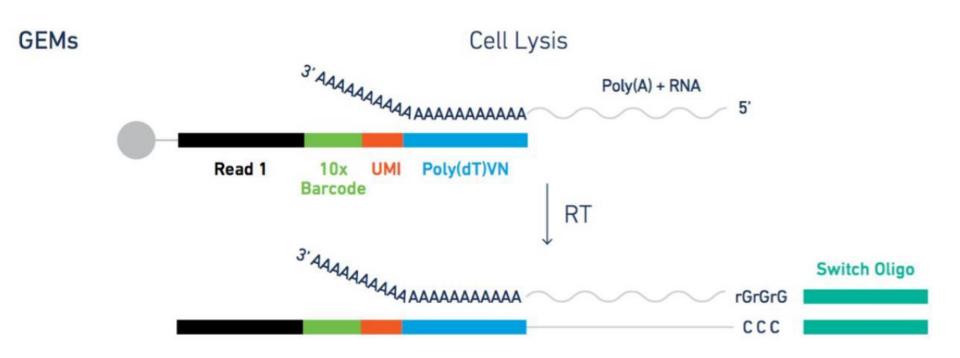
#### Gel bead in Emulsion (GEM) technology



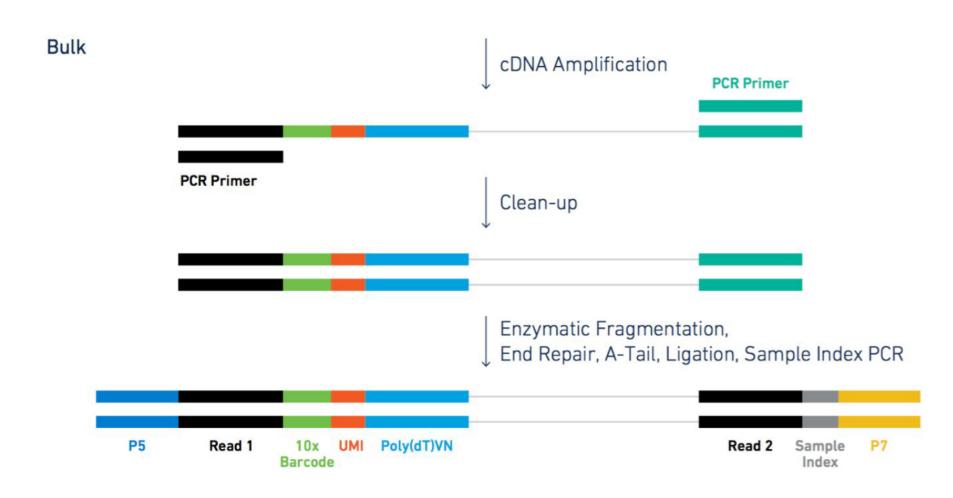
### GEMs up close



#### Assay scheme for 3' mRNA sequencing

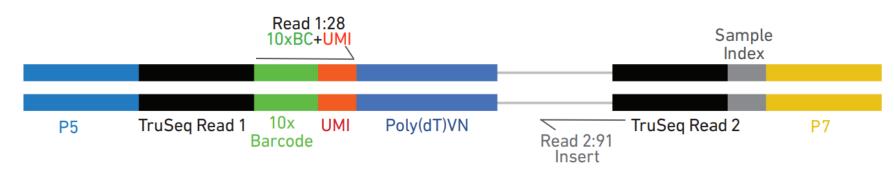


#### Assay scheme for 3' mRNA sequencing



### Final library structure

Chromium Single Cell 3' Gene Expression Library



### Single cell 3' end-to-end workflow

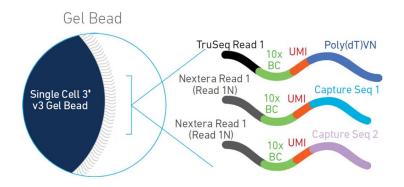
Reagents and Consumables in 10X Kit

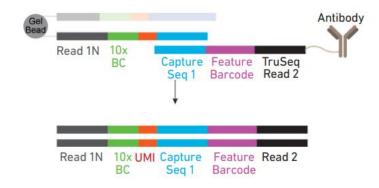
- 1 Cell preparation
- 2 Partition and RT inside each GEM
- 3 Pool and cDNA amplification
- 4 Fragmentation
- 5 Adapter ligation and sample index PCR
- 6 Sequencing and analysis

Total Turn-around Time: ~12 Hrs

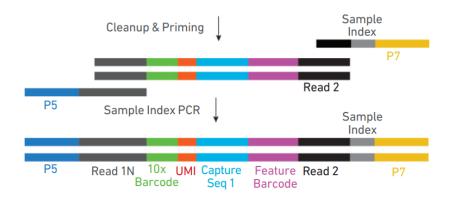
Total Hands-on Time: ~4 Hrs

### Single cell 3' feature barcoding





DNA from cell surface protein Feature Barcode



### Structure of T and B cell receptors

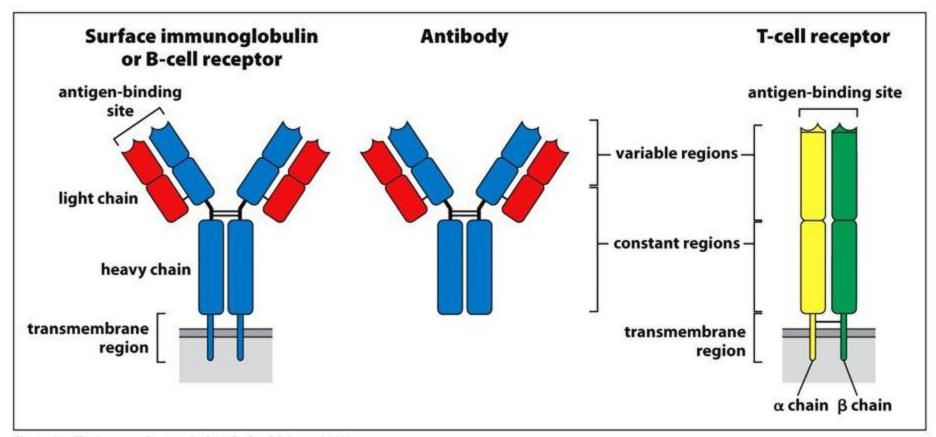
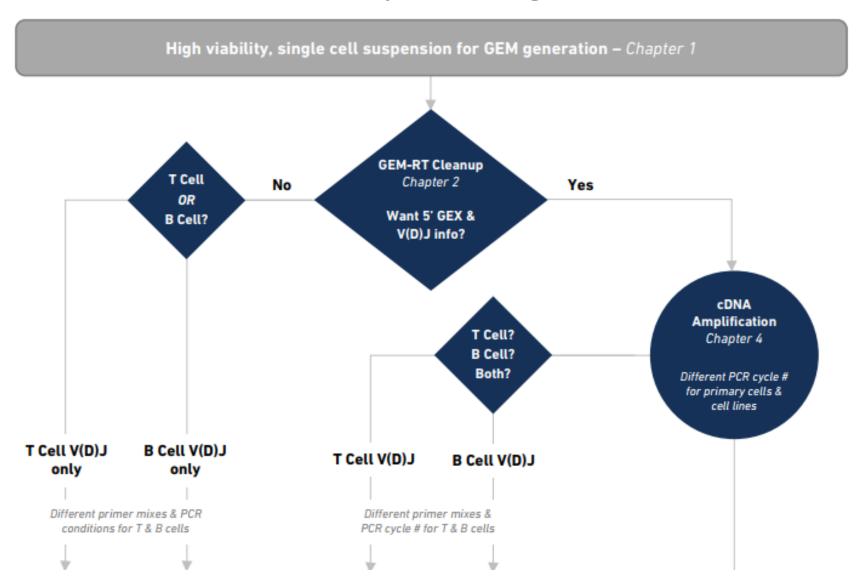


Figure 3.1 The Immune System, 3ed. (© Garland Science 2009)

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



#### Gel bead oligos

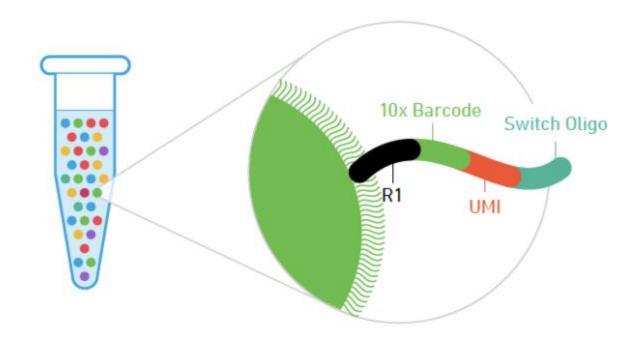
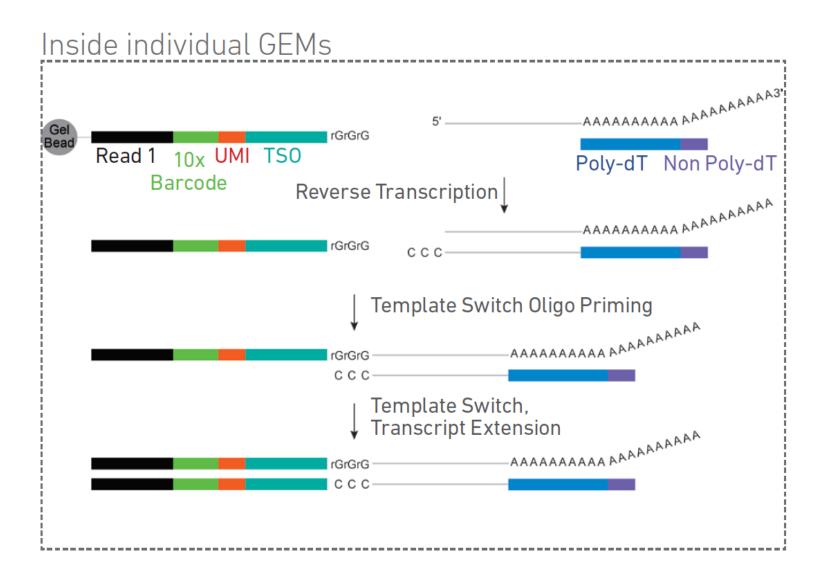


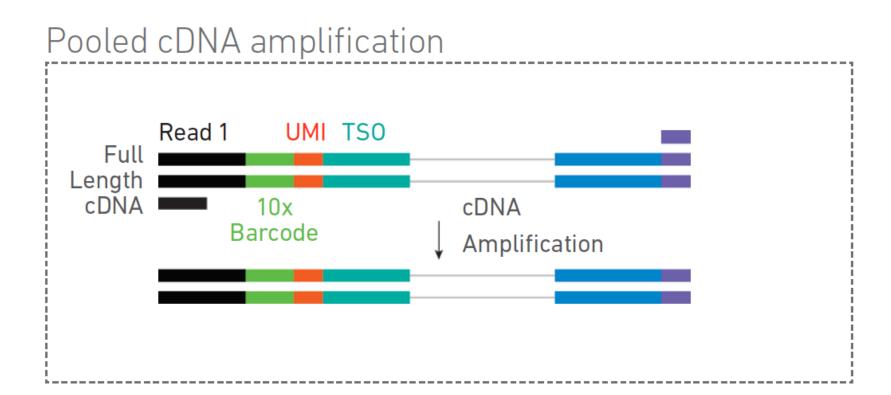
Figure 1. Schematic of a Single Cell 5' Gel Bead oligo primer.

- 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

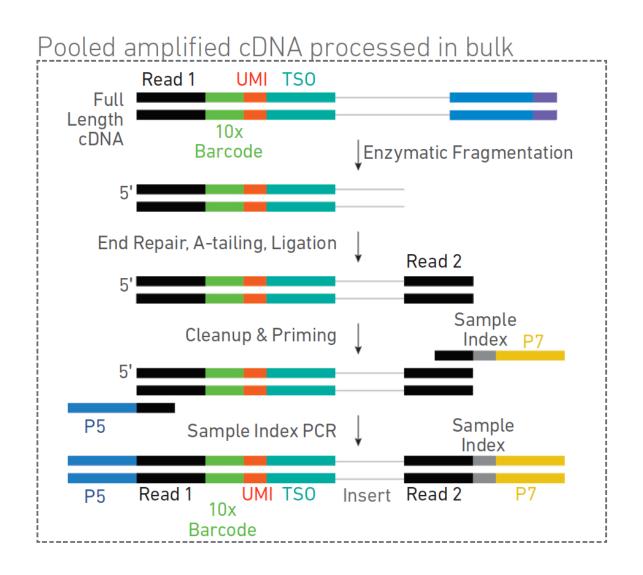
### Assay scheme for 5' scRNA-seq



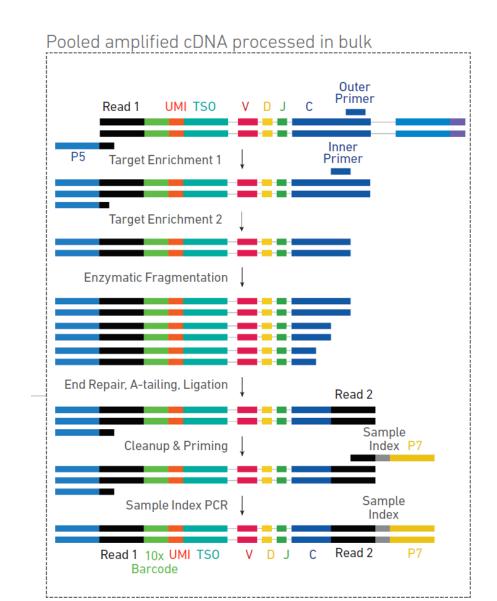
### Assay scheme for 5' scRNA-seq



### Assay scheme for 5' scRNA-seq



### Assay scheme for 5' VDJ libraries

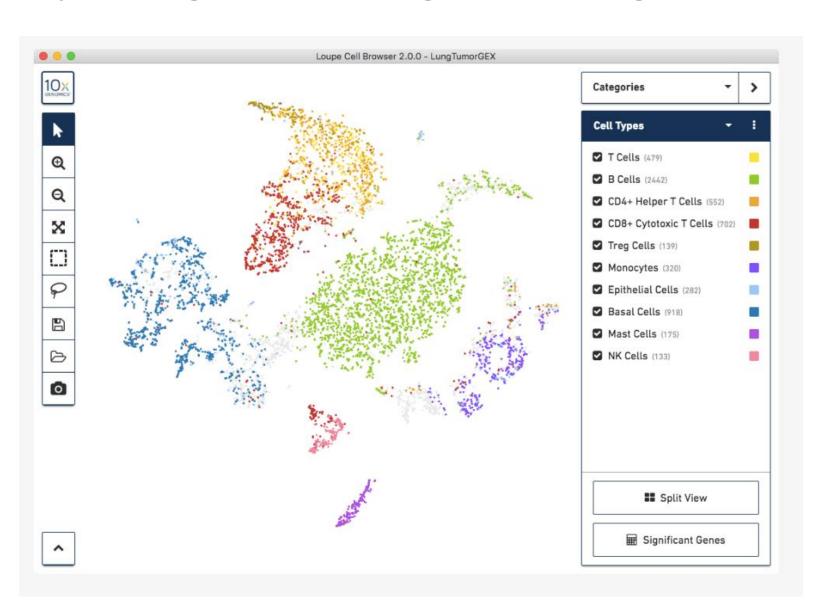


#### Final library structure

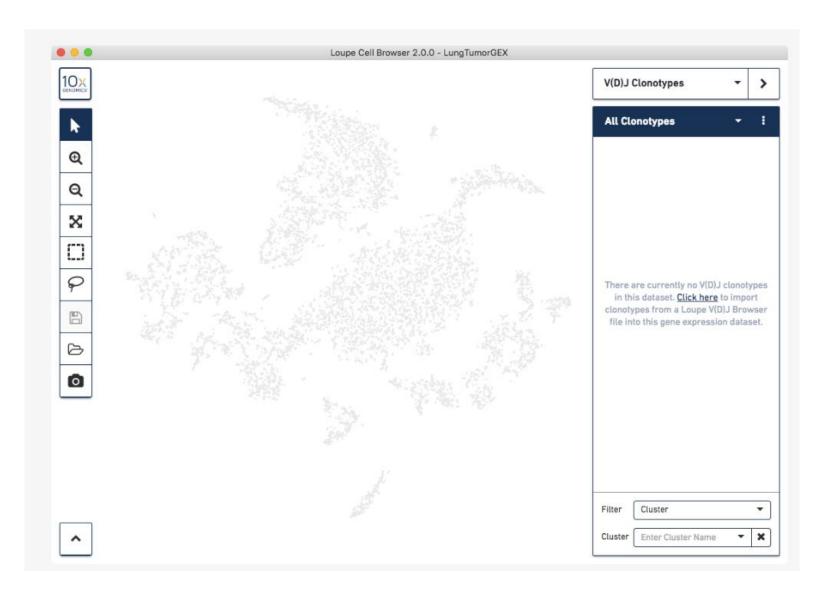
#### V(D)J Enriched Library Structure:



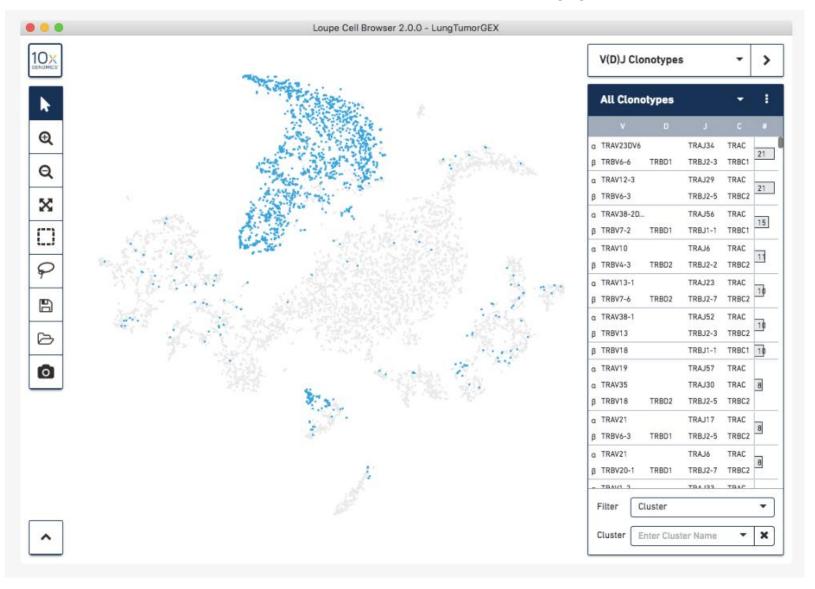
#### Exploring data using Cell Ranger tools



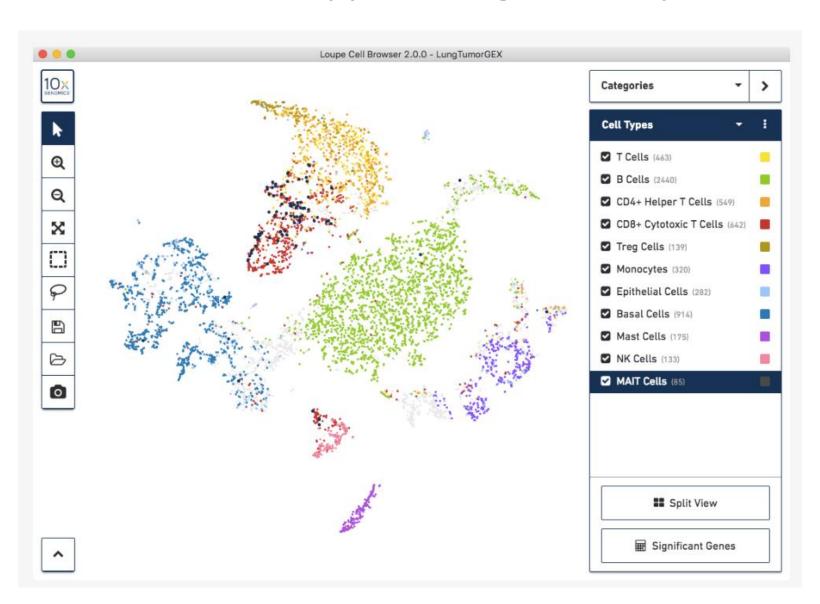
#### Exploring data continued...



#### 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

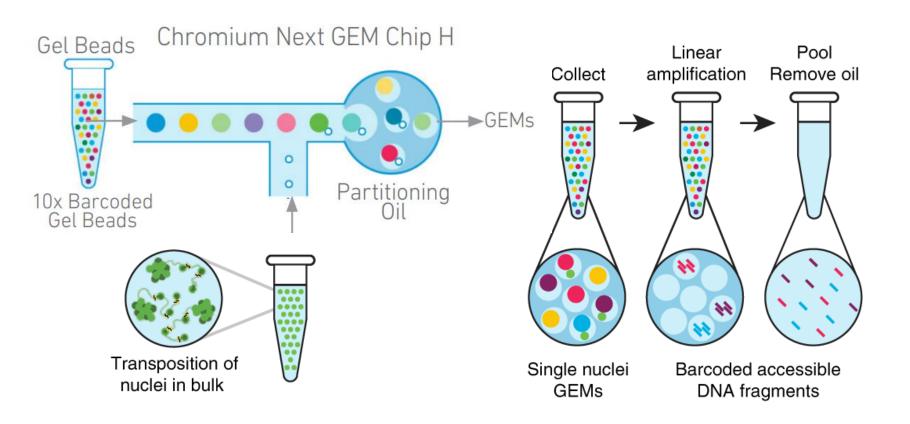
Sequencing BCR @ 2500 reads/cell: 0.02 EUR/cell

Total cost 5' GEX + BCR sequencing

10,000 cells: 0.47 EUR/cell

1,000 cells: 2.72 EUR/cell

### Single-cell ATAC-seq



#### Visium for spatial transcriptomics

