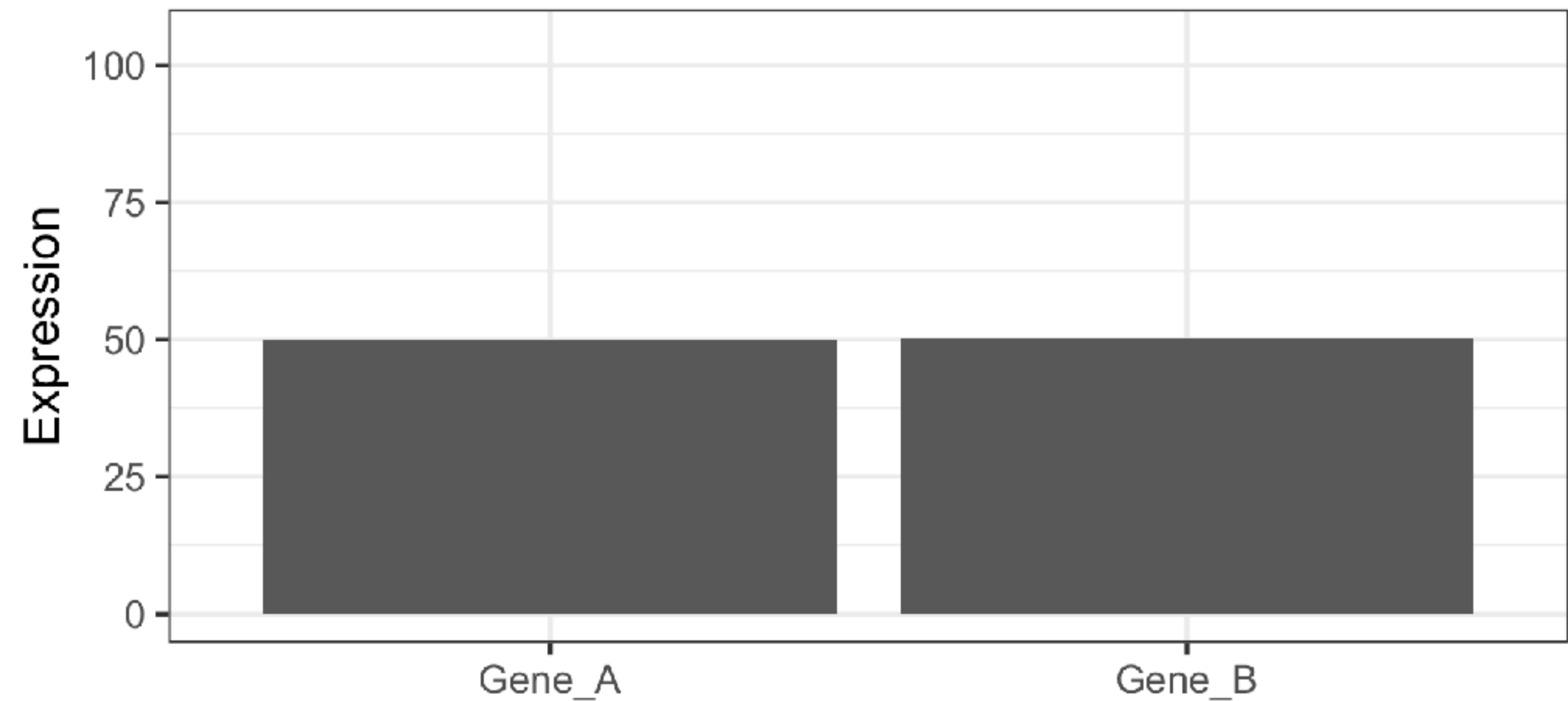
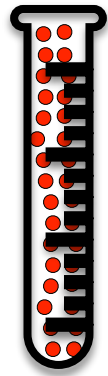


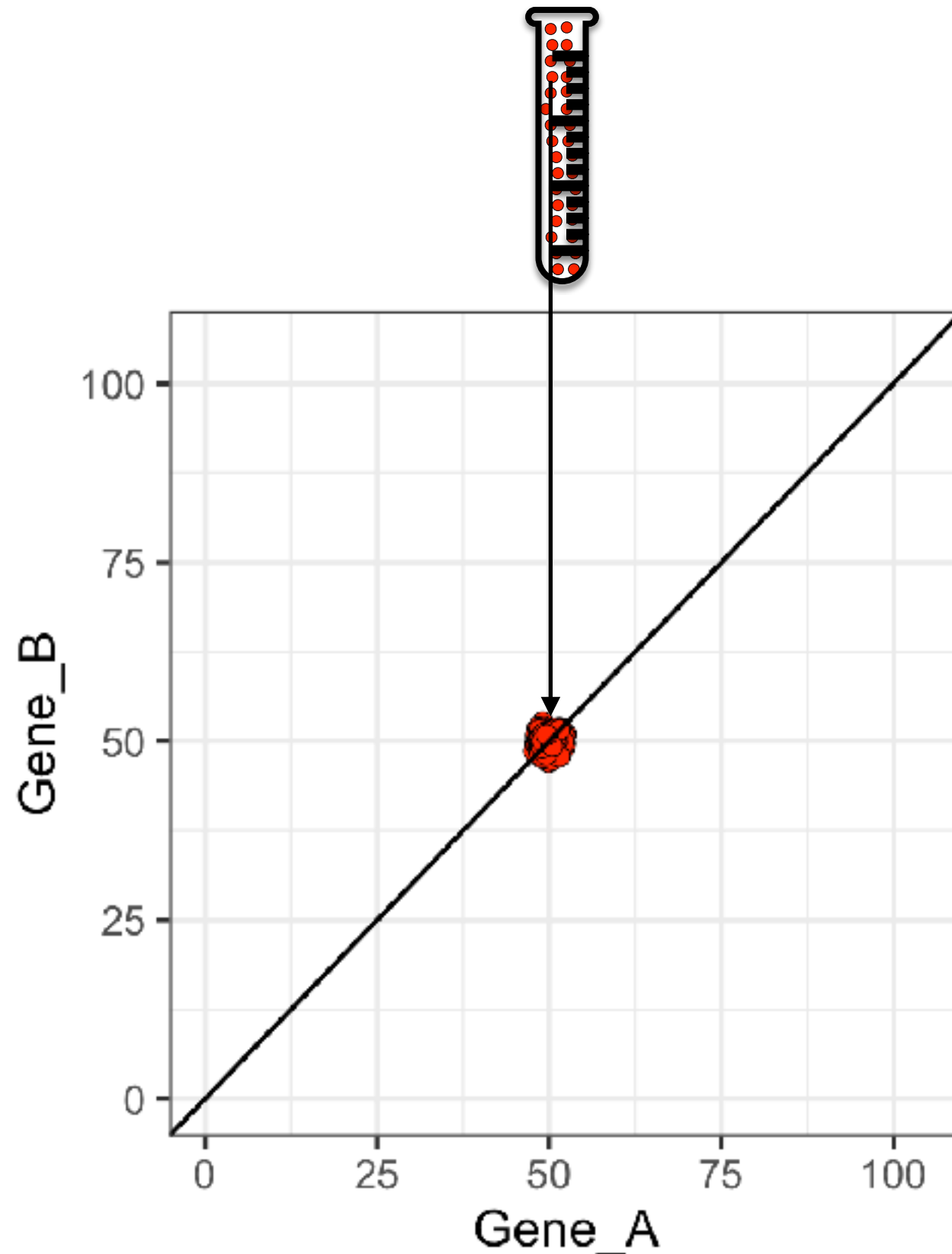
scRNA-Seq Background and Theory

Josh Granek

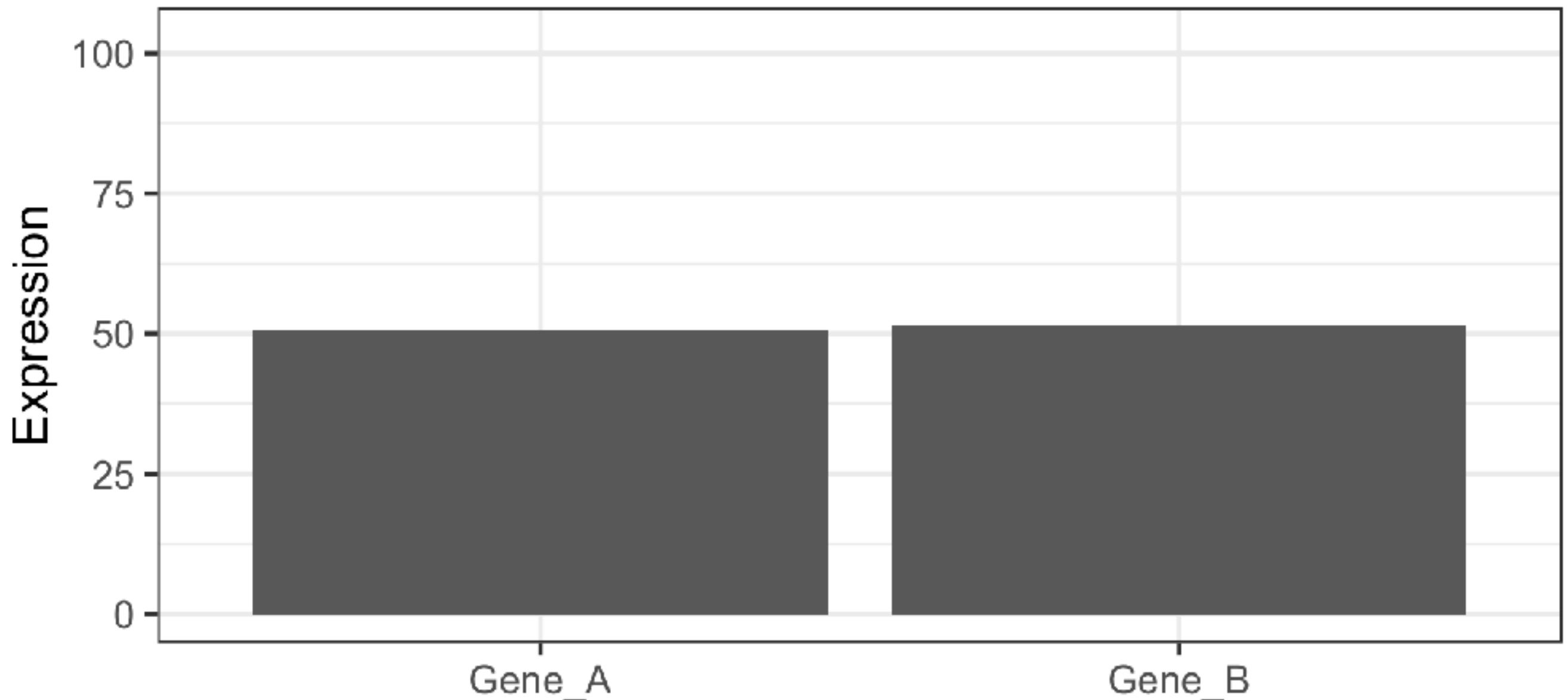
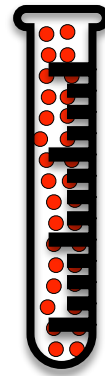
Bulk Gene Expression



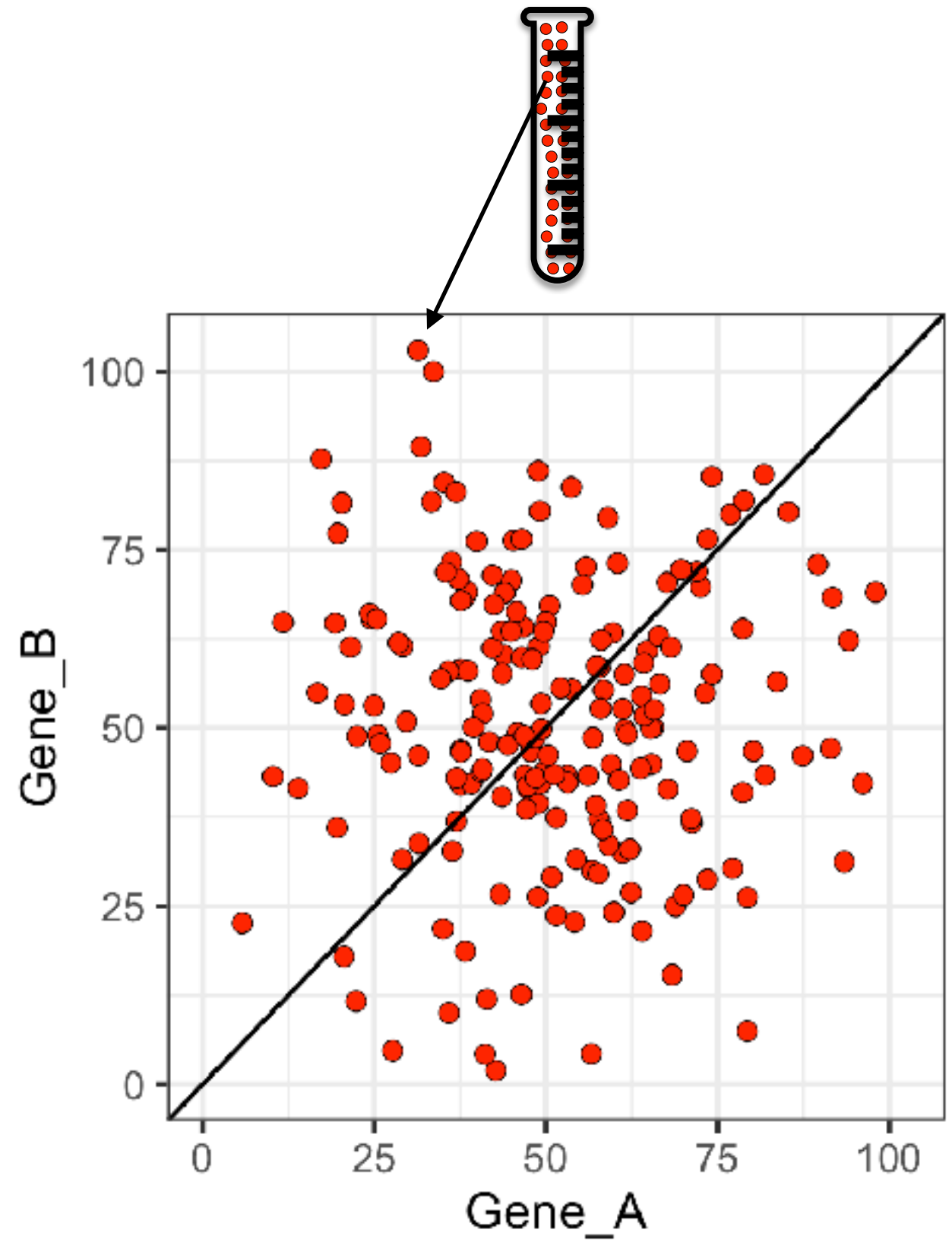
Single-Cell Gene Expression



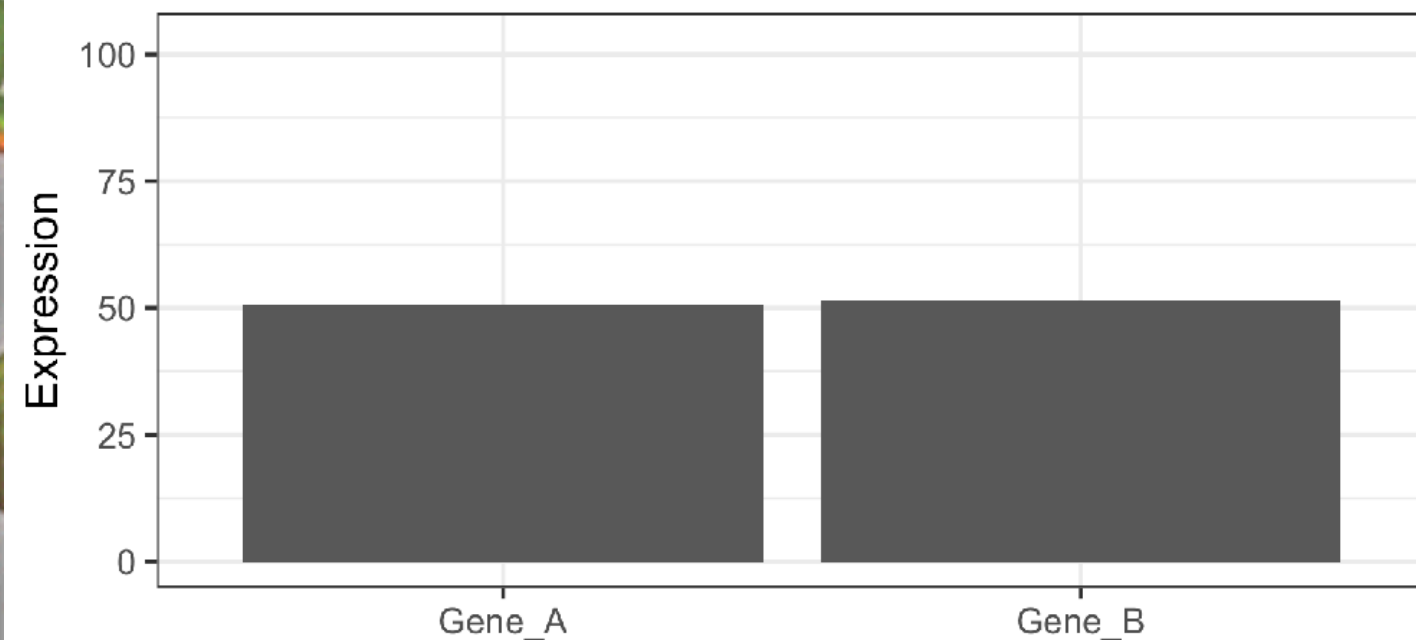
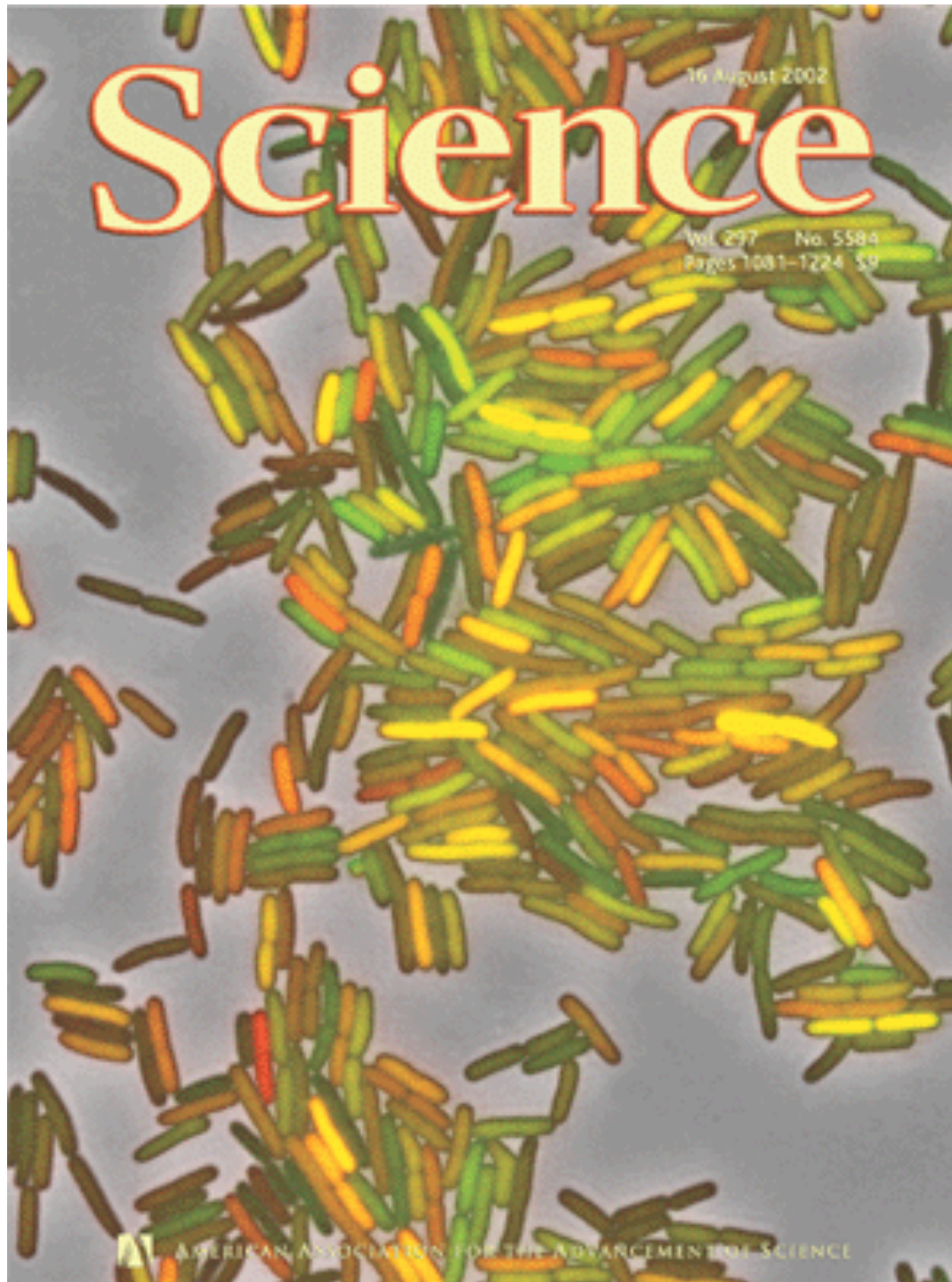
Bulk Gene Expression



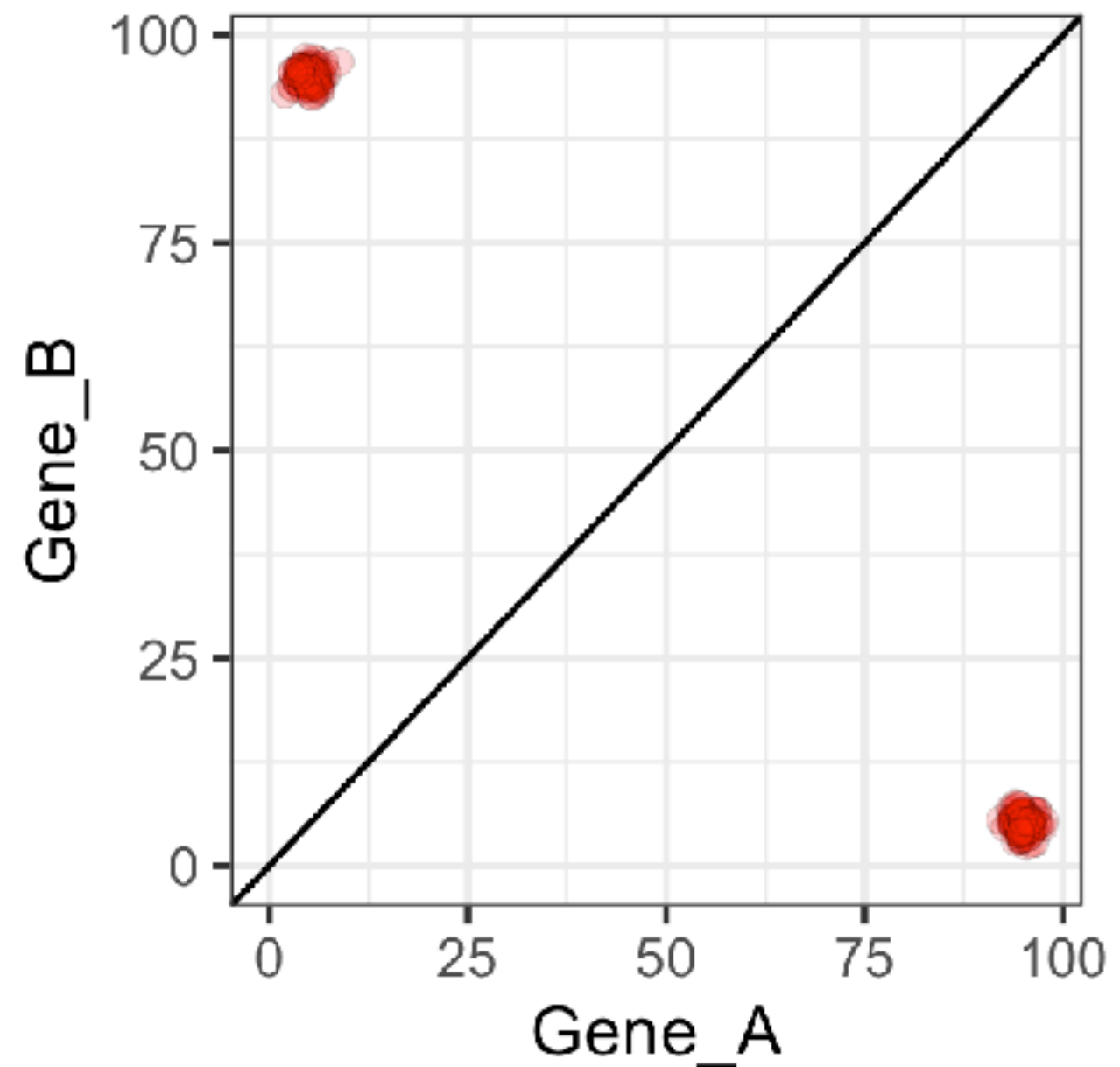
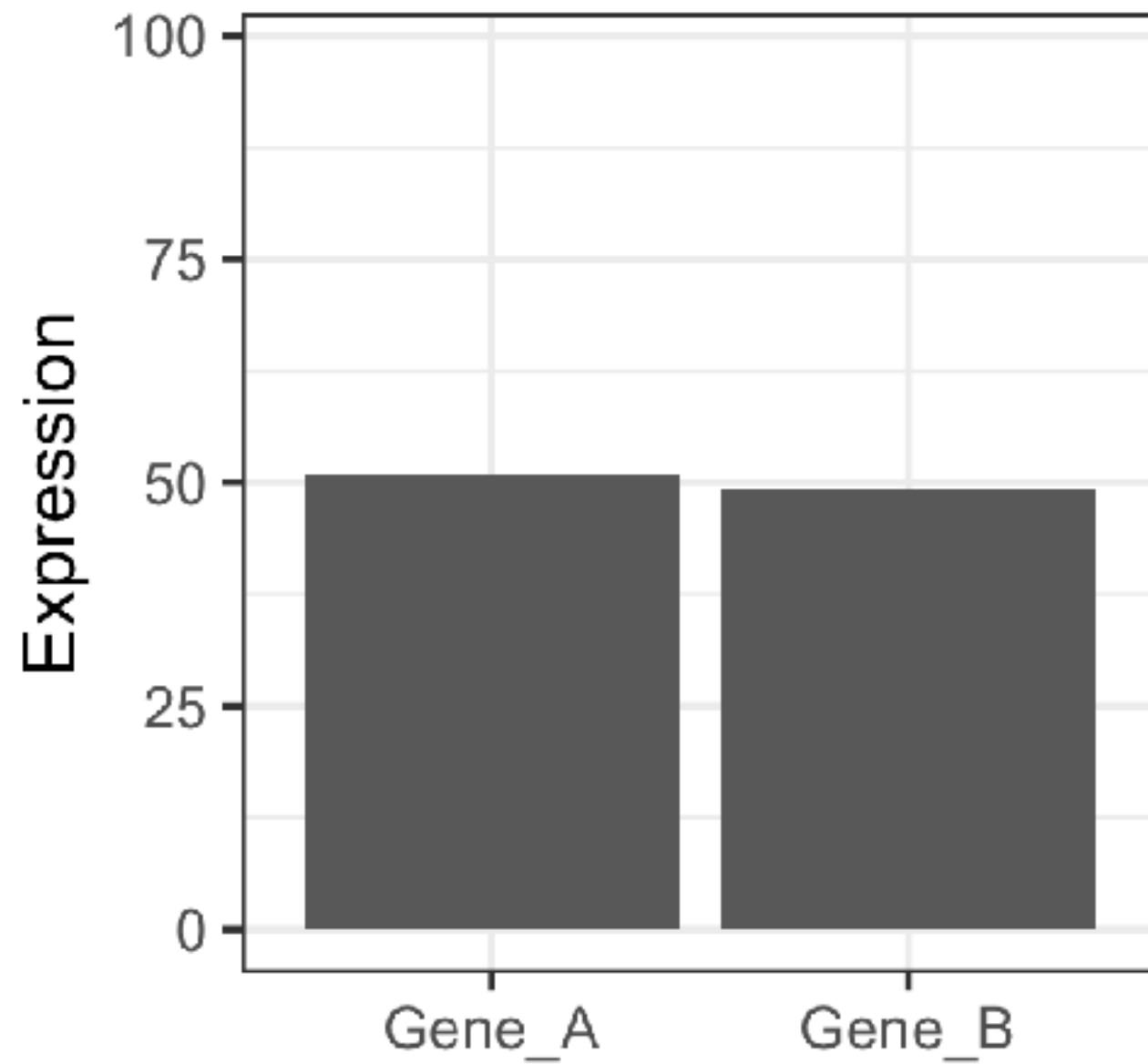
Single-Cell Gene Expression



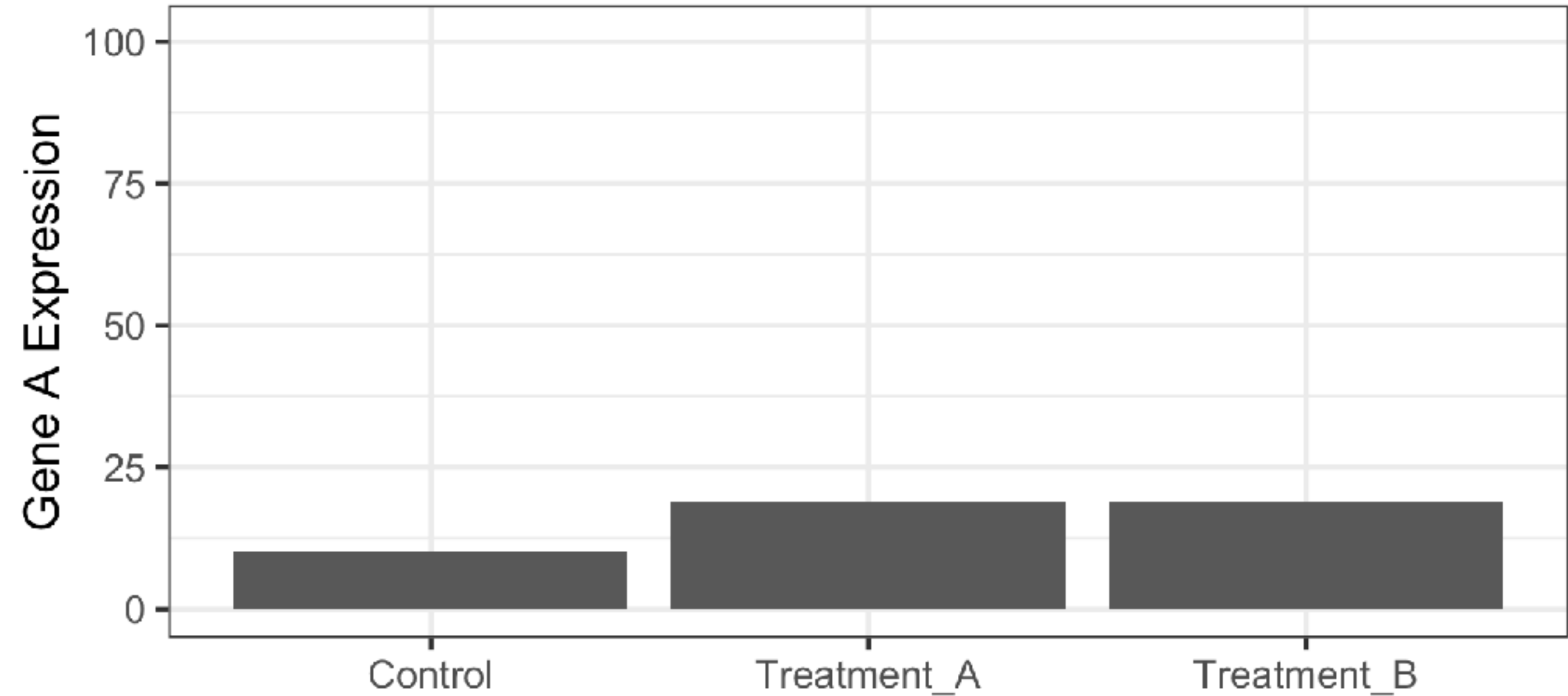
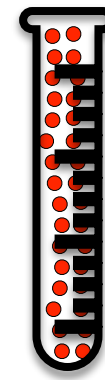
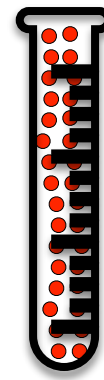
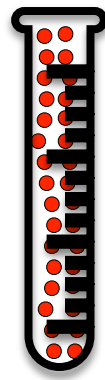
Single-Cell Gene Expression



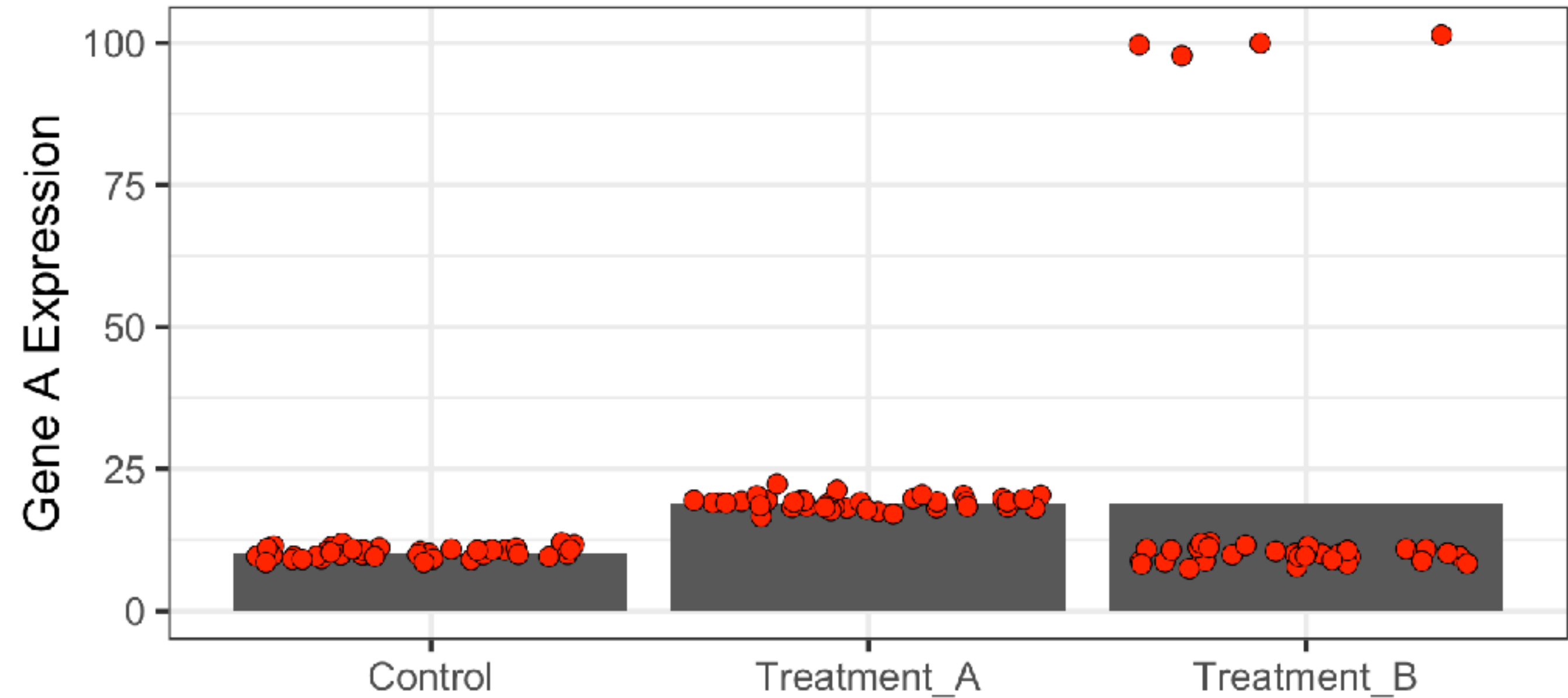
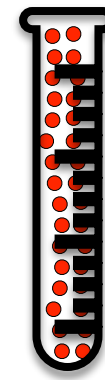
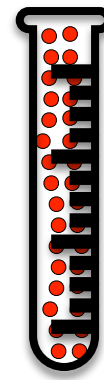
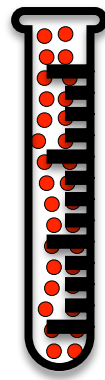
Two Cell Types



Bulk Gene Expression



Single-Cell Gene Expression



How?

“One person, one vote”

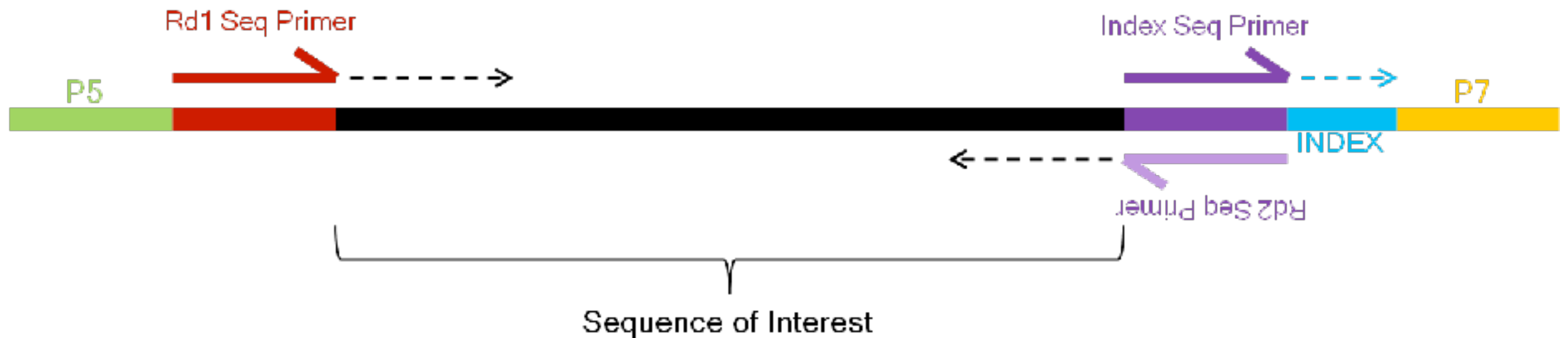
- Earl Warren, 14th Chief Justice of the United States

“One cell, one barcode”

- Josh Granek

Multiplexing (Barcodes)

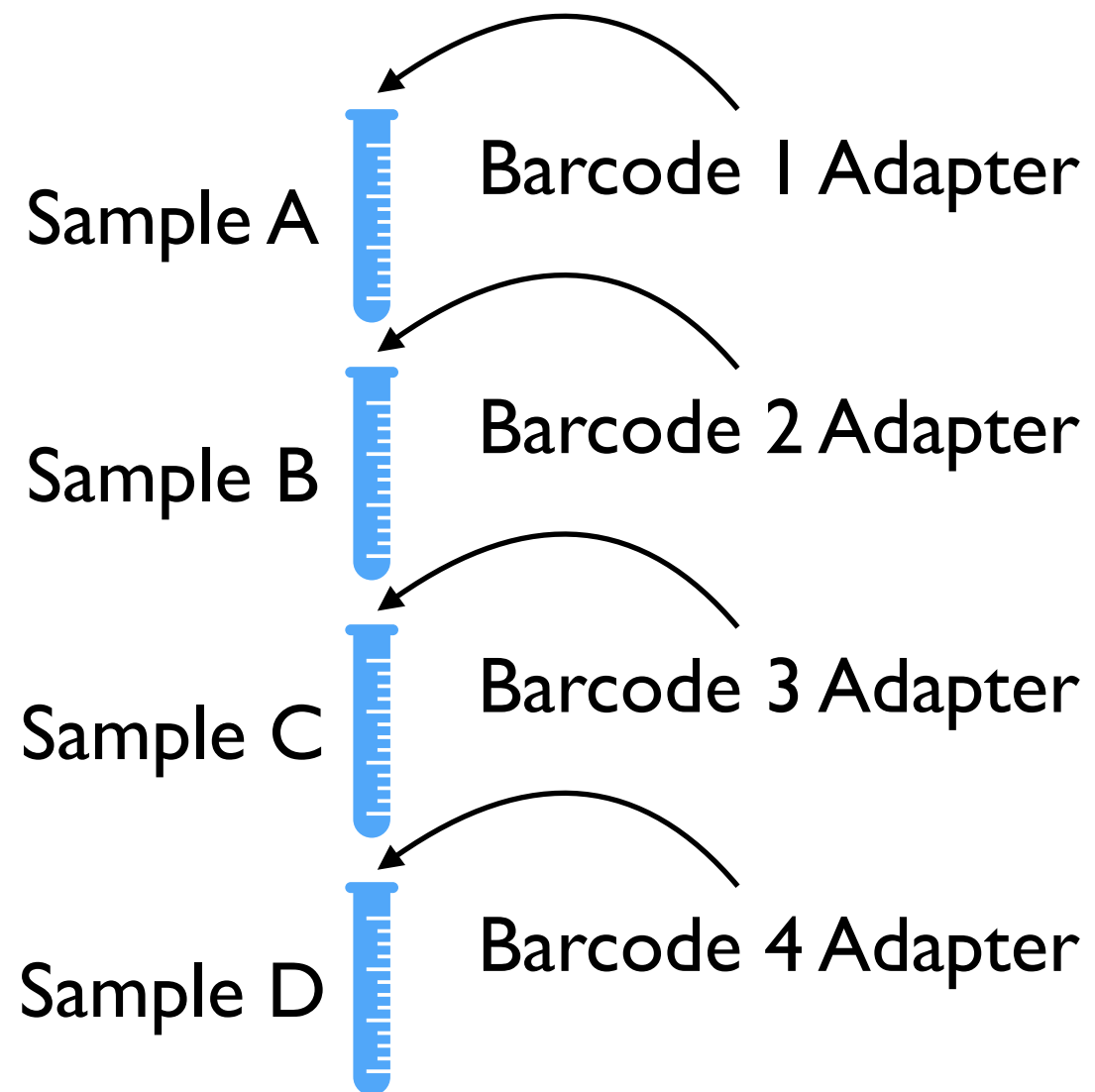
STRUCTURE DETAILS



Barcodes

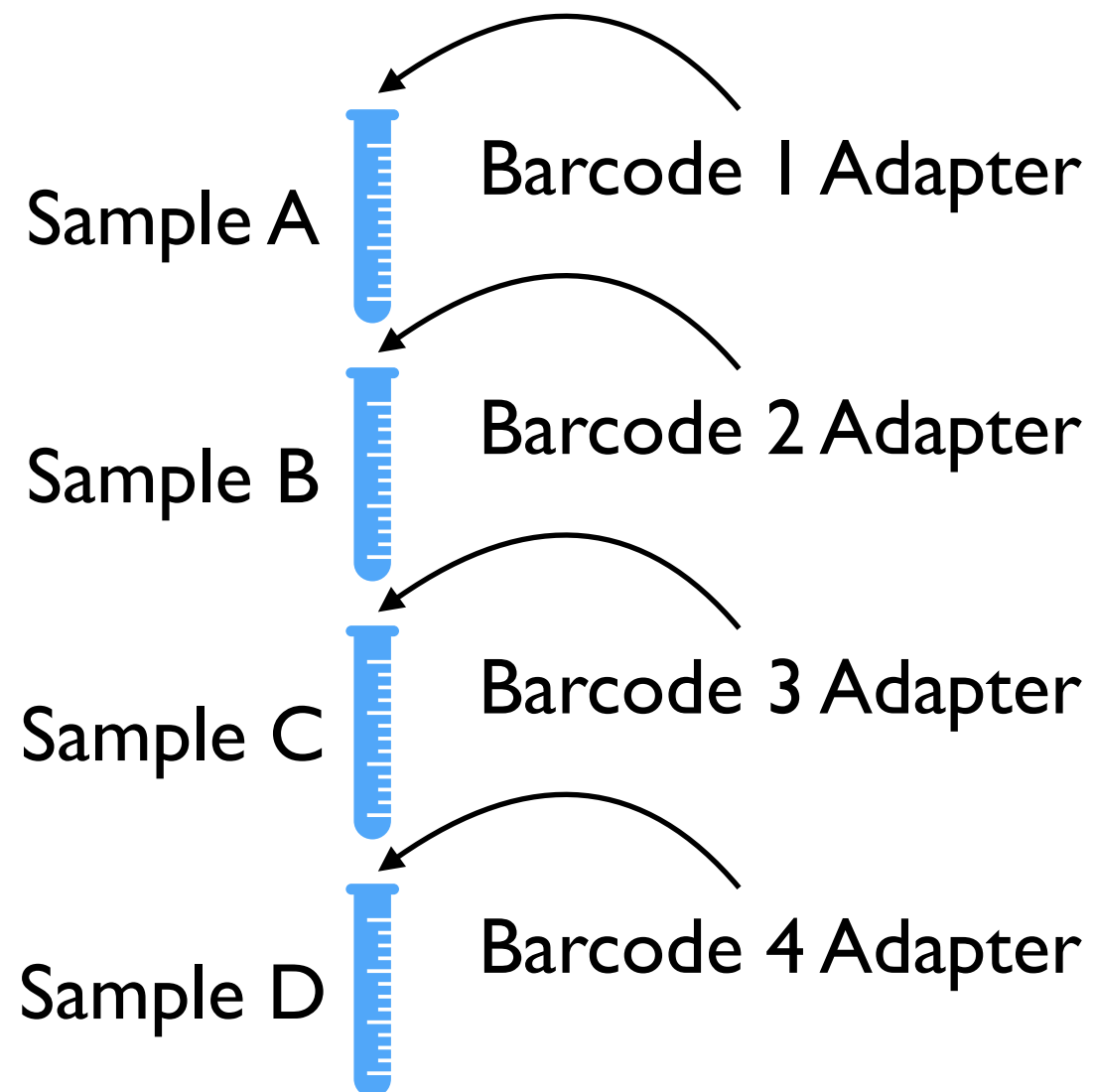
| Sample_Name | index |
|--------------|----------|
| control_1 | AAGACCGT |
| control_2 | TTGCGAGA |
| control_3 | GCAATTCC |
| treatmentA_1 | GAATCCGT |
| treatmentA_2 | CCGCTTAA |
| treatmentA_3 | TACCTGCA |
| treatmentB_1 | GTCGATTG |
| treatmentB_2 | TATGGCAC |
| treatmentB_3 | CTCGAACA |

Barcodes



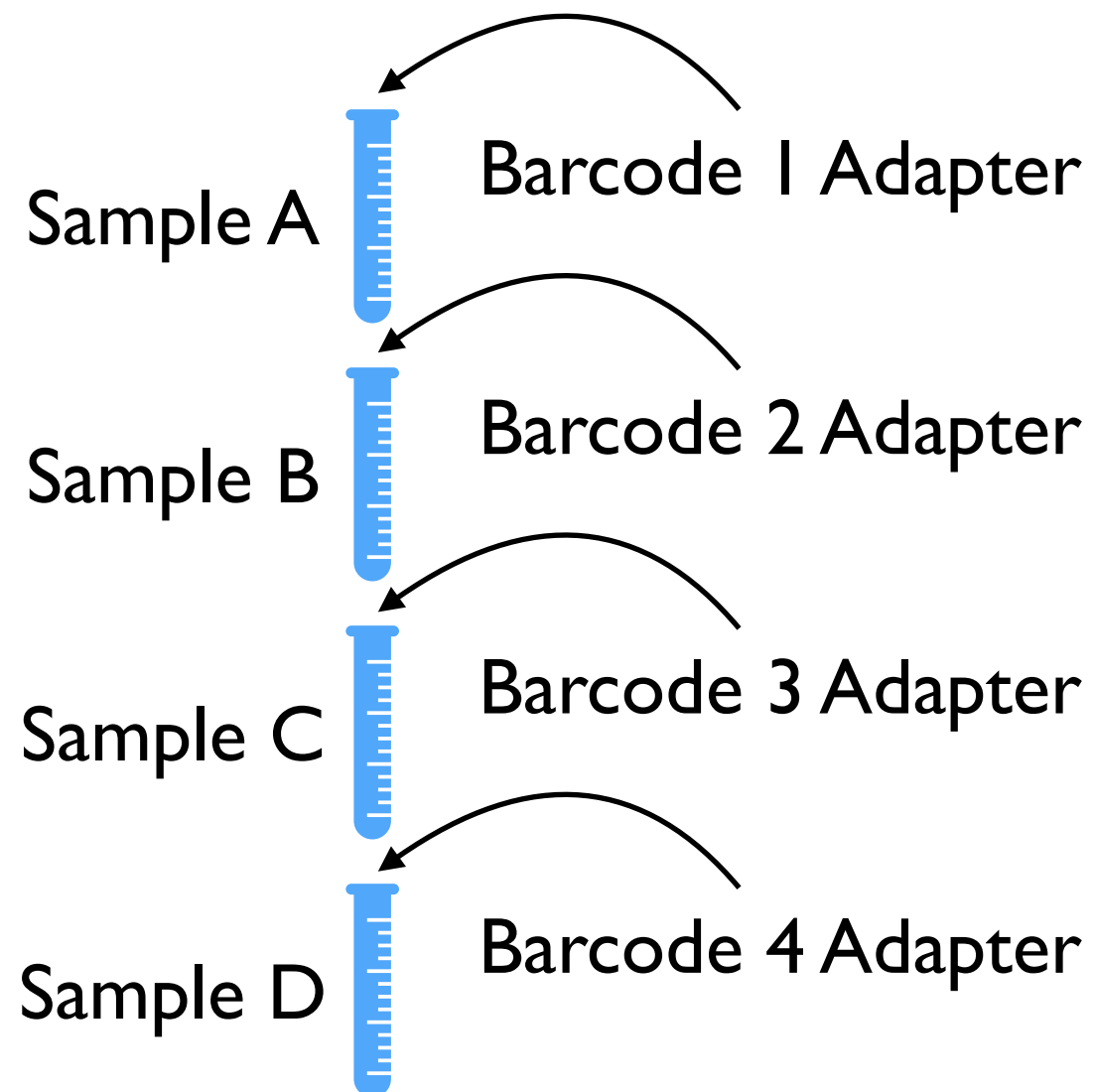
Barcodes

Bulk HTS



Barcodes

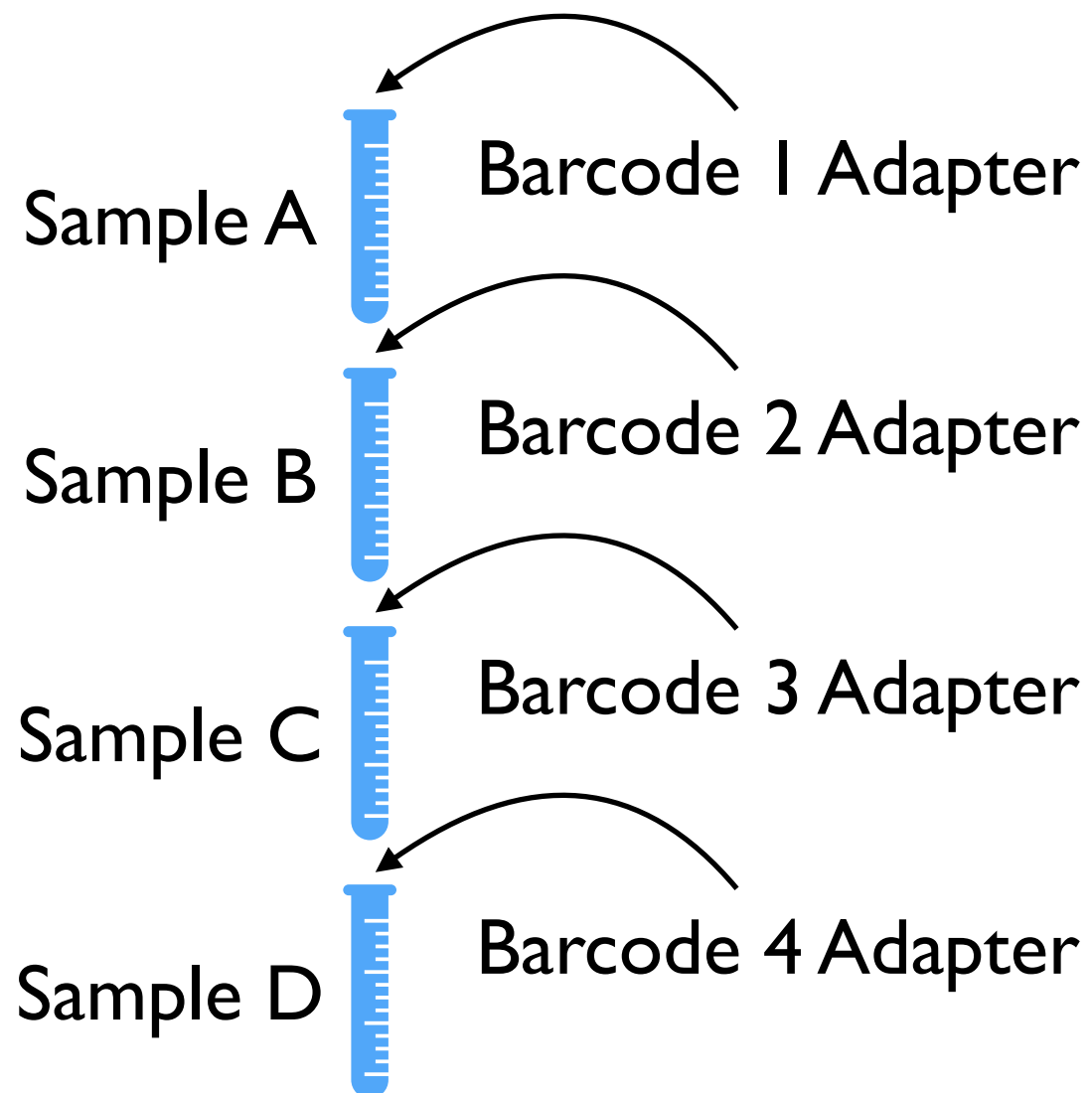
Bulk HTS



Single-Cell HTS

Barcodes

Bulk HTS

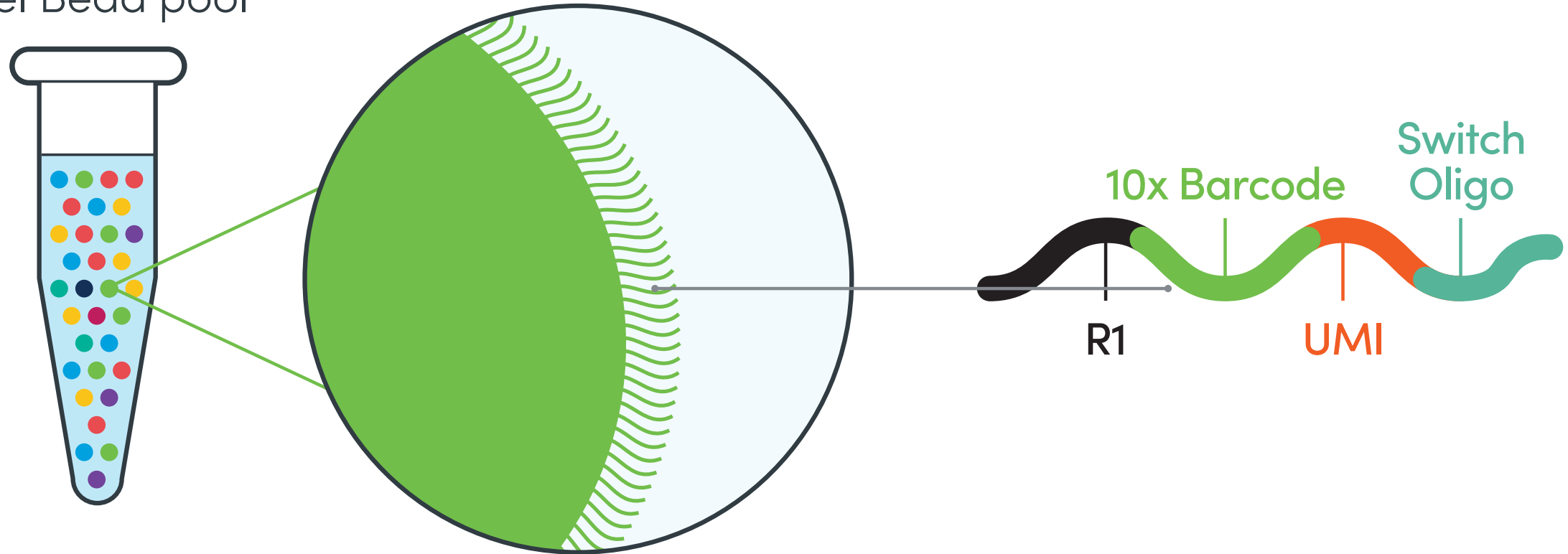


Single-Cell HTS

- More Barcodes!
- More Test Tubes!

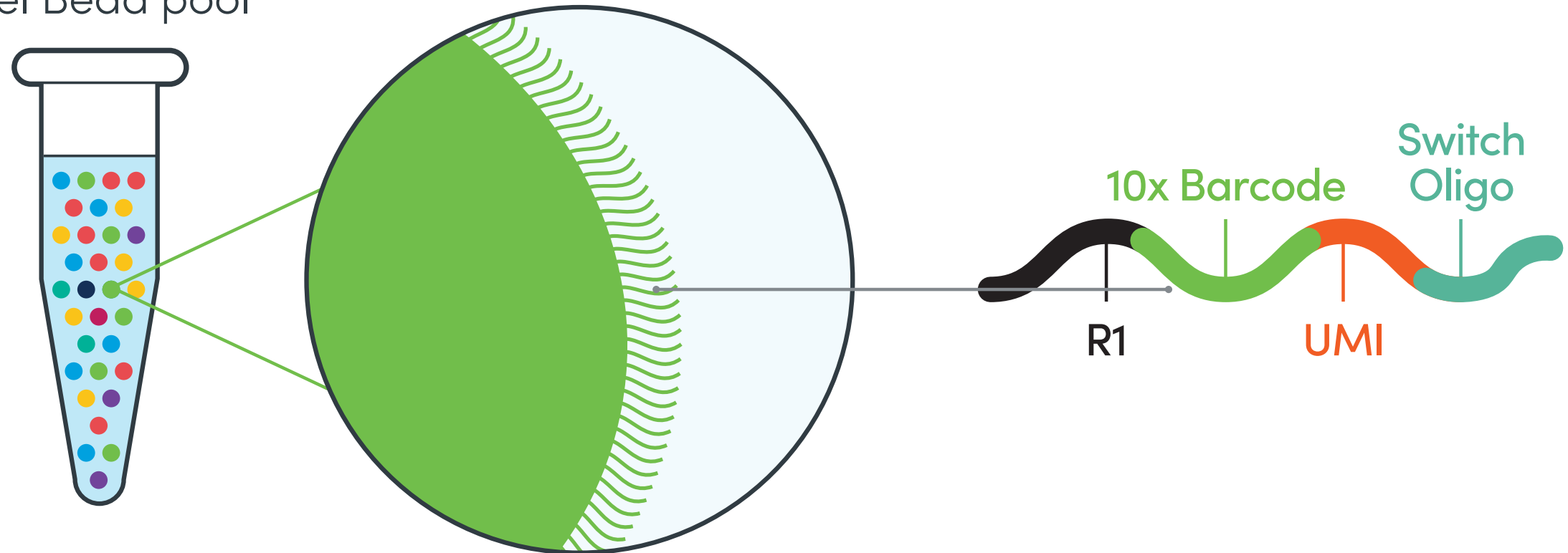
More Barcodes

High-diversity
Gel Bead pool



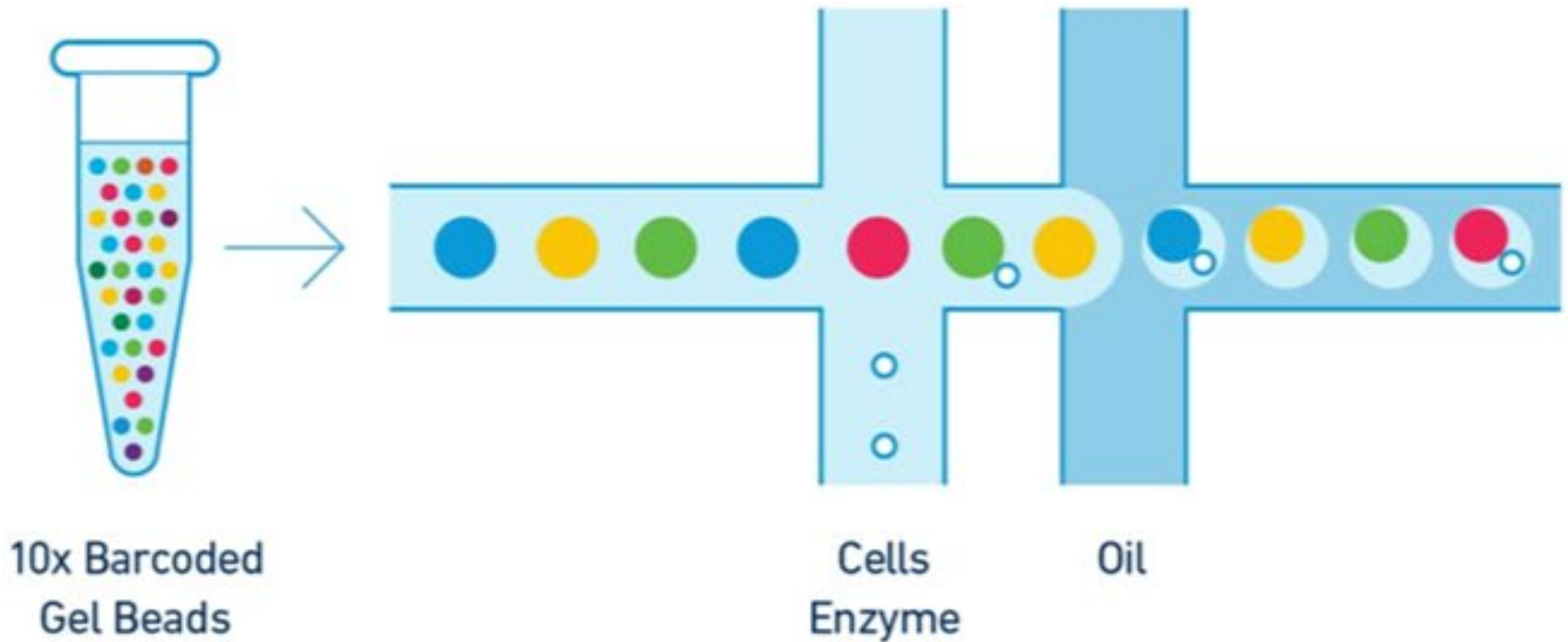
More Barcodes

High-diversity
Gel Bead pool



| | | Length | Unique Barcodes (Theoretical) |
|--------------------|----------|--------|--------------------------------|
| 10x Barcode | Per Cell | 16bp | 4.3×10^9 (4^{16}) |
| UMI | Per RNA | 10bp | 1.0×10^6 (4^{10}) |

More Test Tubes



More Test Tubes



Single-Cell Applications

- RNA-Seq (Gene Expression)
 - Cell Typing
 - Differential Expression
- ATAC-Seq (Chromatin Accessibility)
- Cell Surface Proteins
- BCR-Seq
- TCR-Seq
- TCR-Specificity
- Spatial Gene Expression

Why Not Single-Cell

- Expensive
- Noisy
- Small Populations