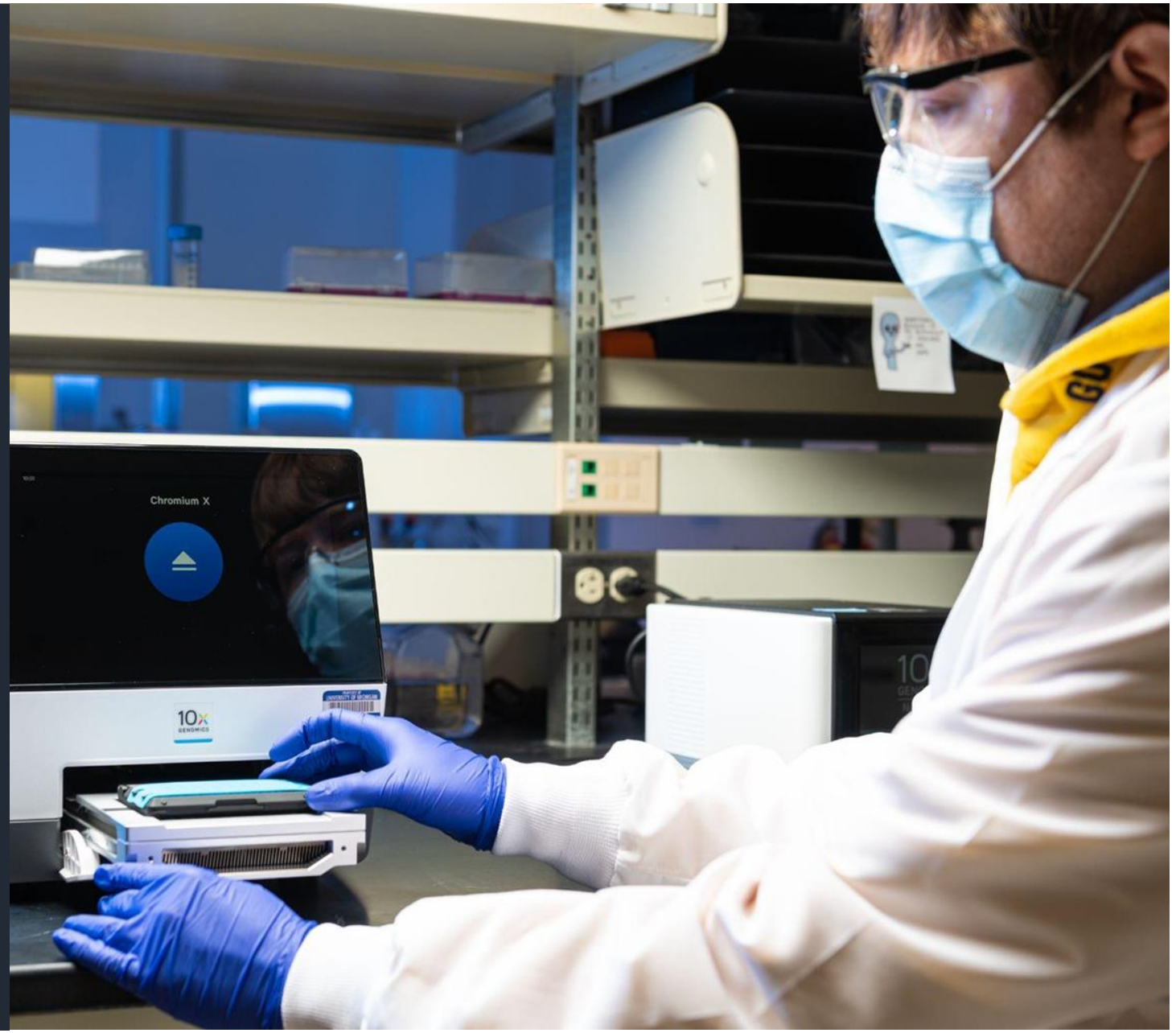
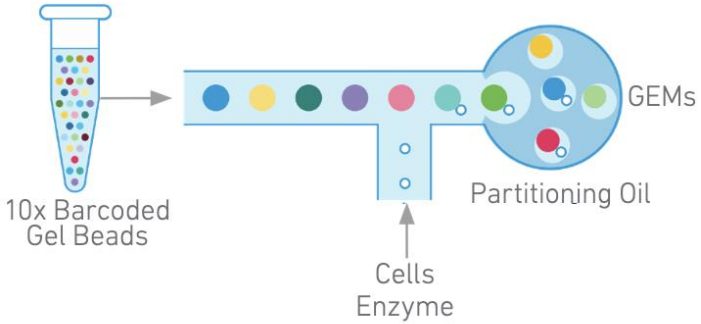
Intro to Single Cell Technologies at the AGC

advanced-genomics@umich.edu



Approaches to Single Cell Sequencing

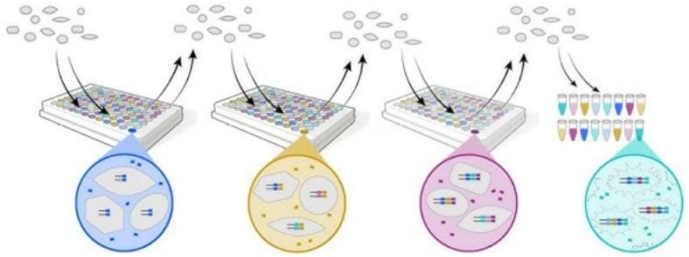
Microfluidic Droplet-Based



Benefits:
- throughput
- performance consistency
- low per cell cost
- multi-modal compatible

Limitations:
- size limitation (<30um)
- 3’ or 5’ bias or targeted

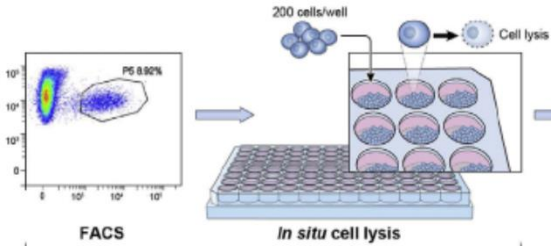
Split-pooling



Benefits:
- throughput
- reduced 3’ bias
- size agnostic

Limitations:
- fixation
- capture efficiency
- labor intensive

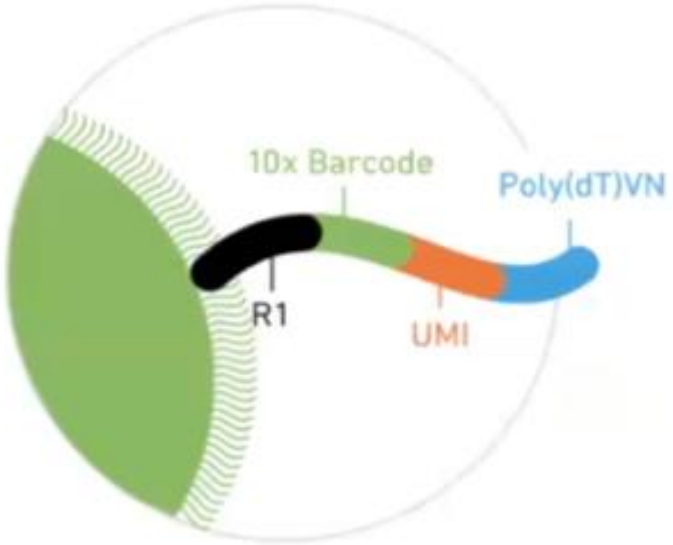
Direct Cell Lysis

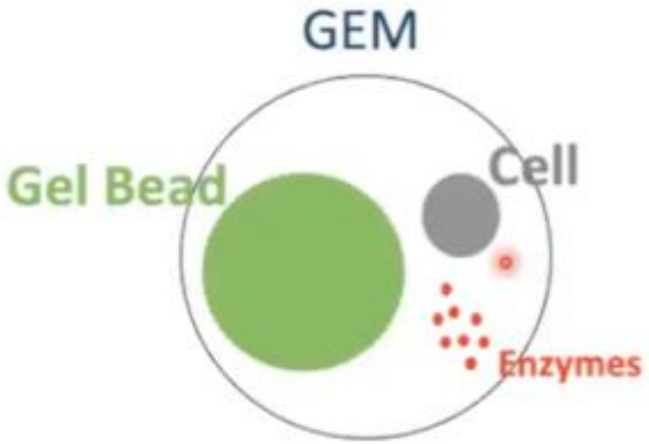


Benefits:
- full-length

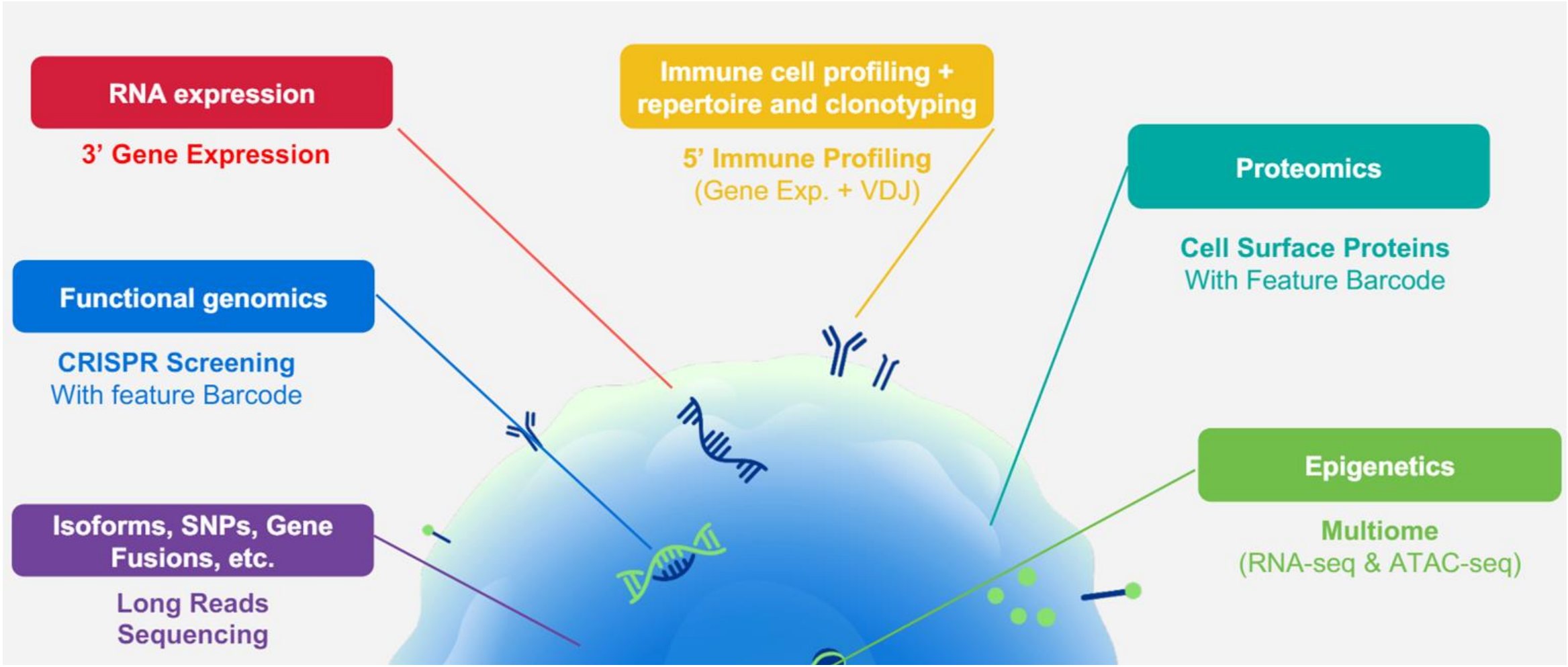
Limitations:
- expense
- throughput

Single Cell RNA Sequencing

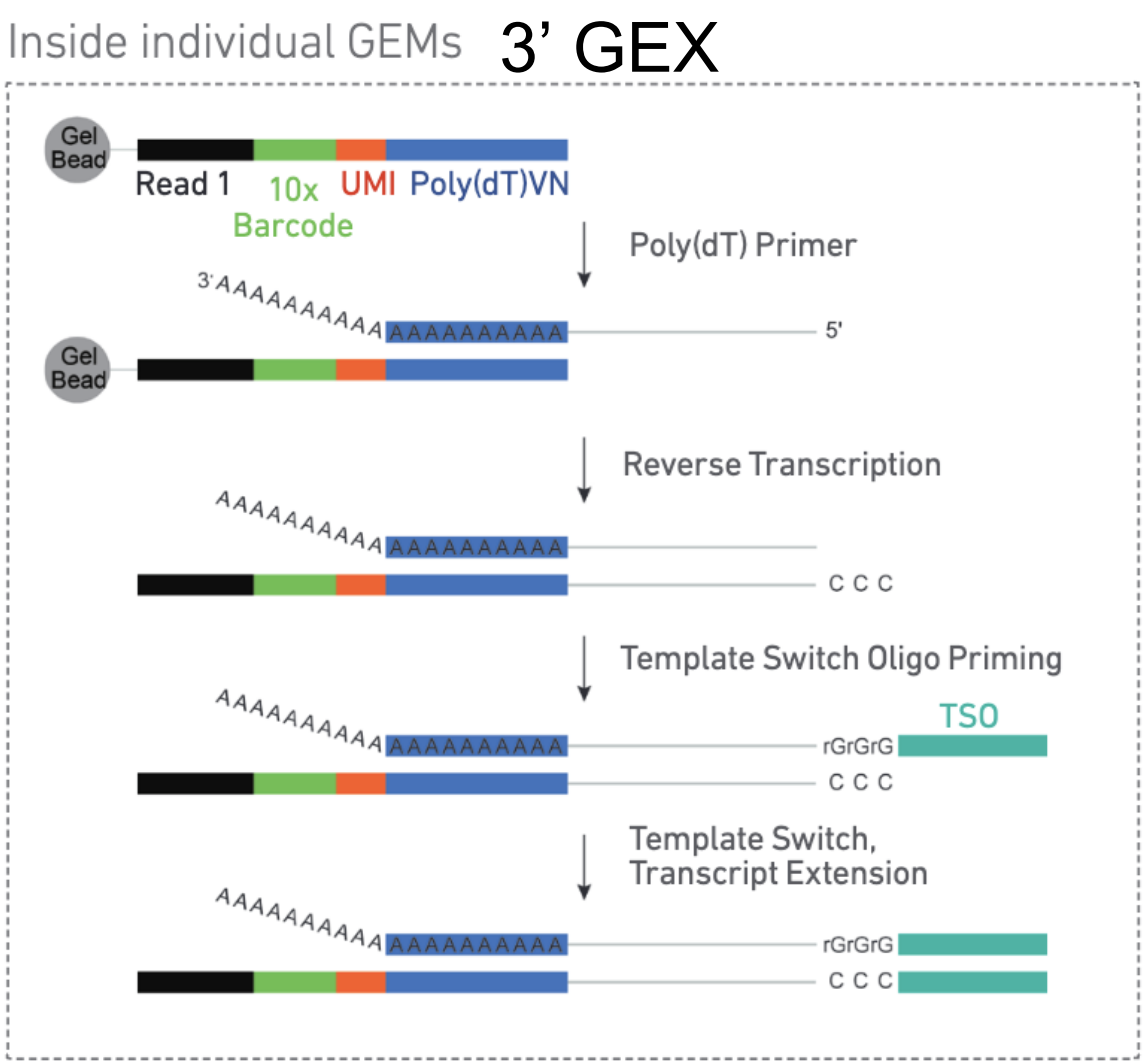


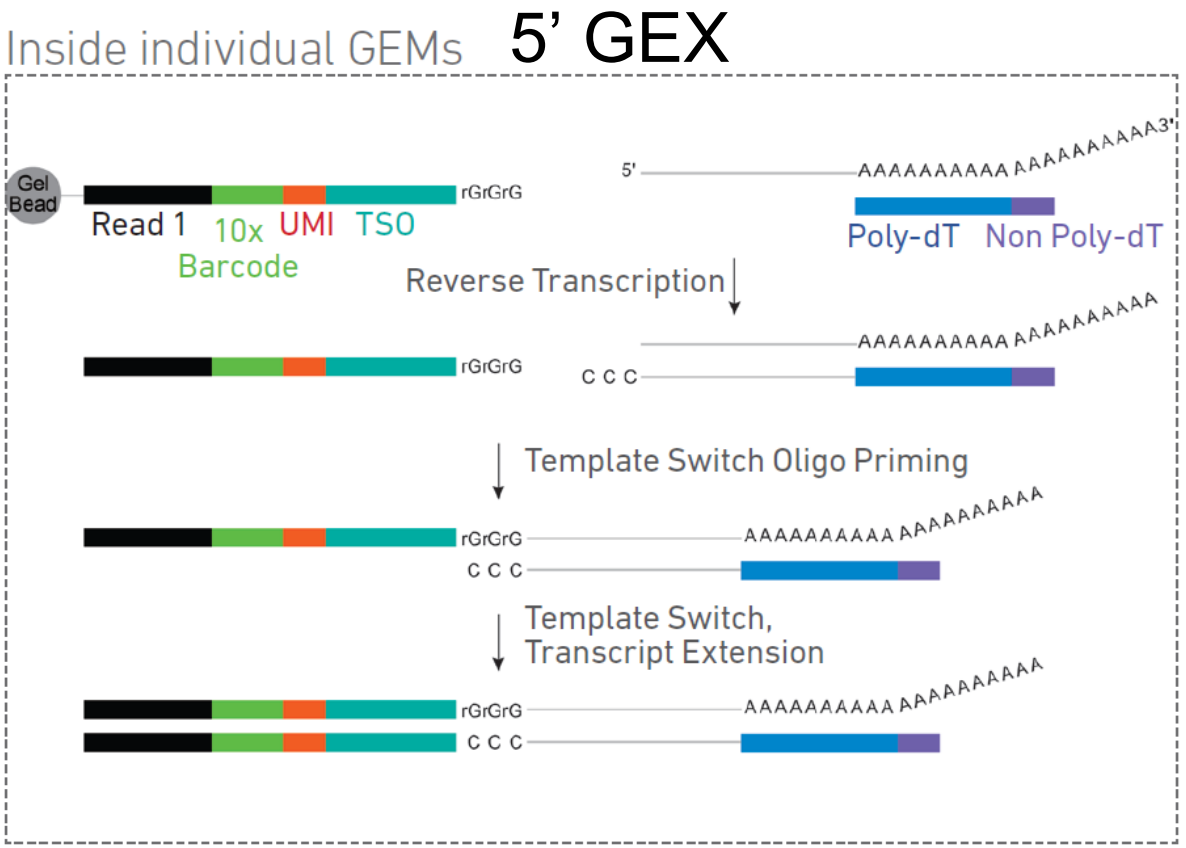


Flavors of Single Cell Sequencing

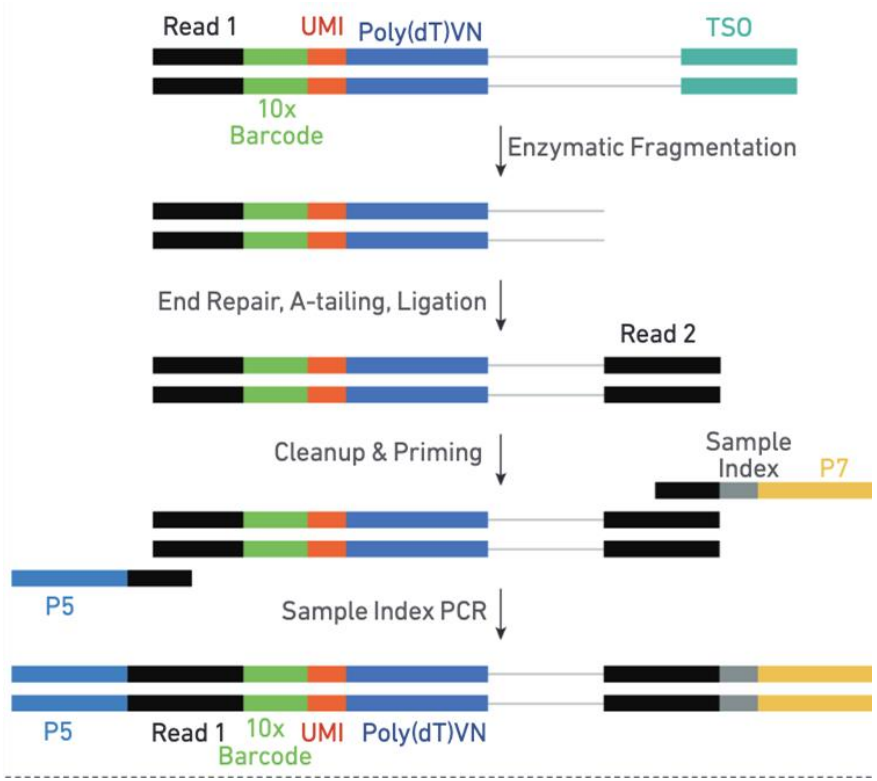


Transcript Capture Mechanisms

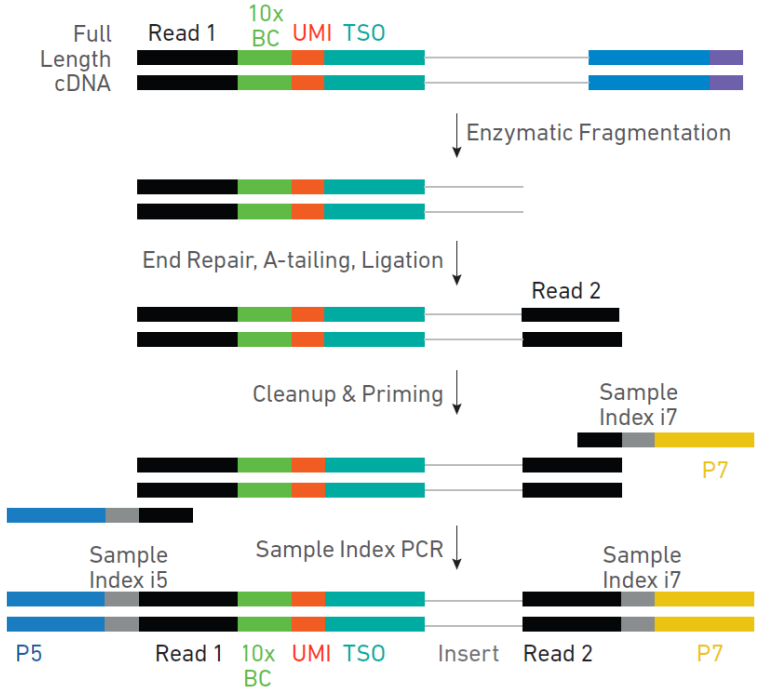




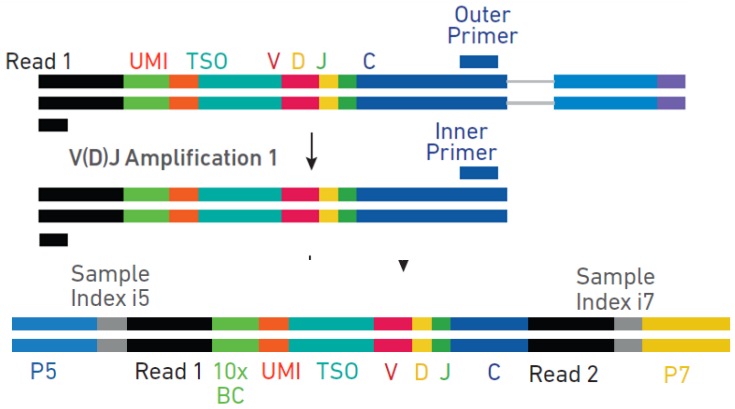
10x Capture Mechanisms



3’ GEX Library



5’ GEX Library



V(D)J Library

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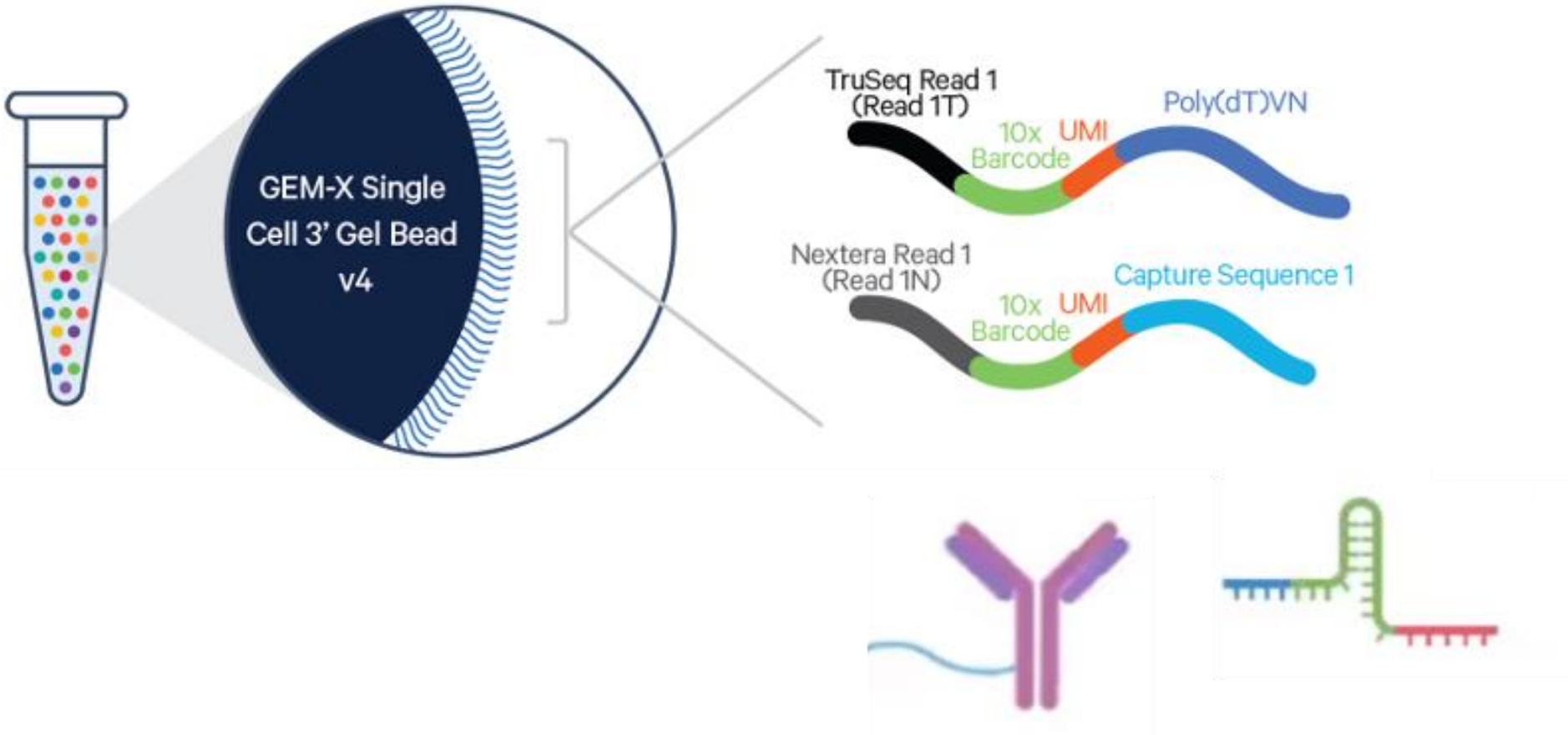
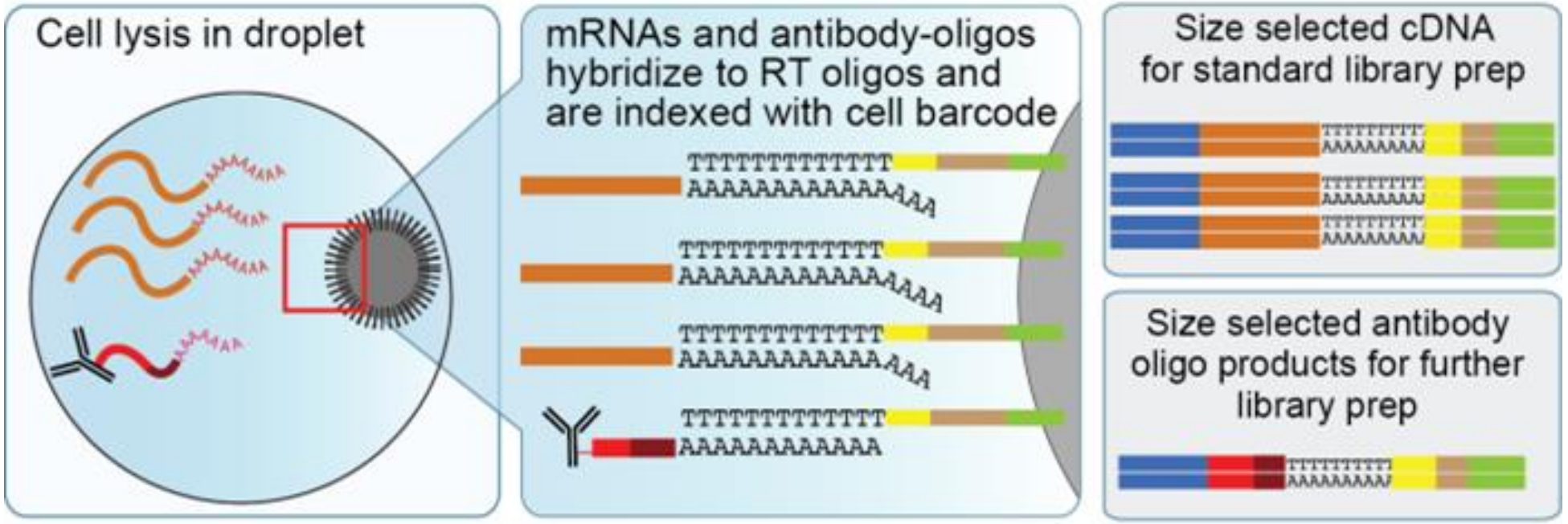
Proteomics

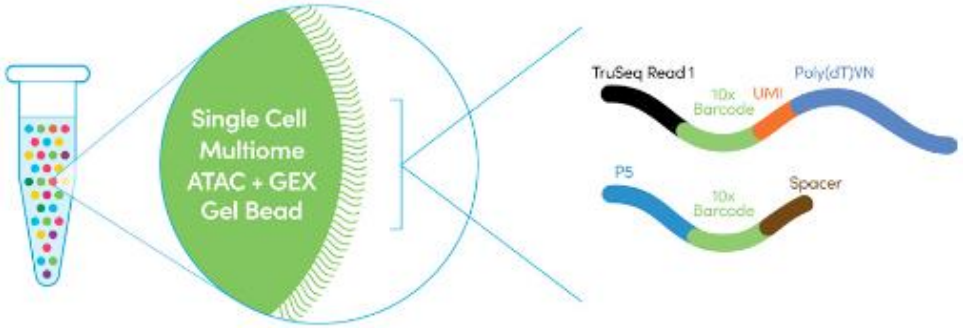

GEM-X Single
Cell 3' Gel Bead
v4
TruSeq Read 1
(Read 1T)
10x
Barcode
UMI
Poly(dT)VN
Nextera Read 1
(Read 1N)
10x
Barcode
UMI
Capture Sequence 1

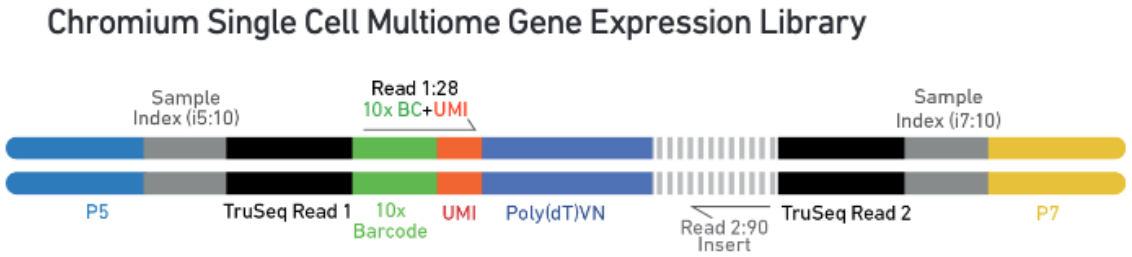
Cellular Indexing of Transcriptomes and Epitopes

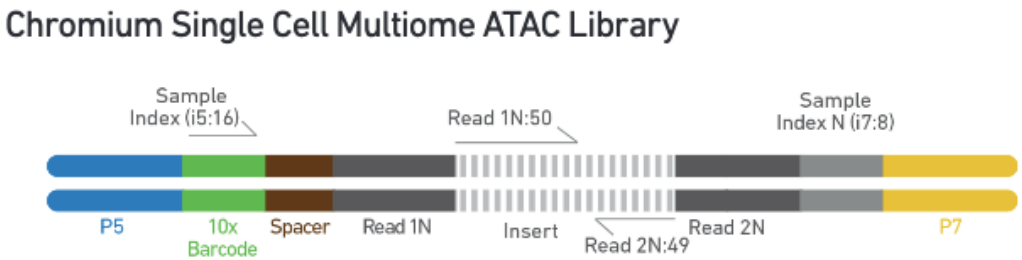


source: cite-seq.com

Epi Multiome (ATAC + Gene Expression)

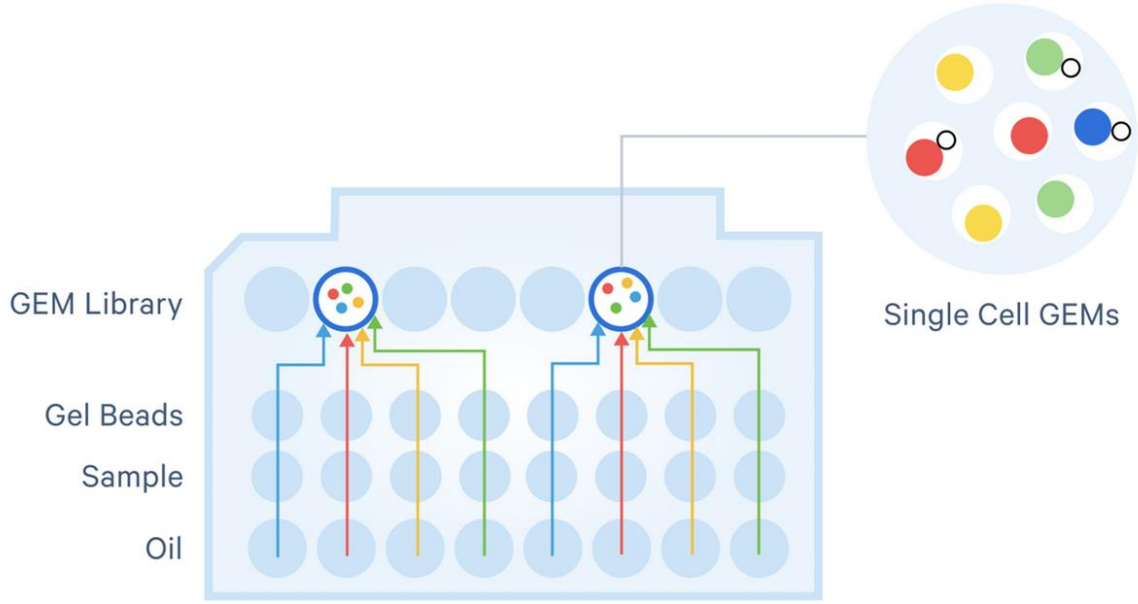






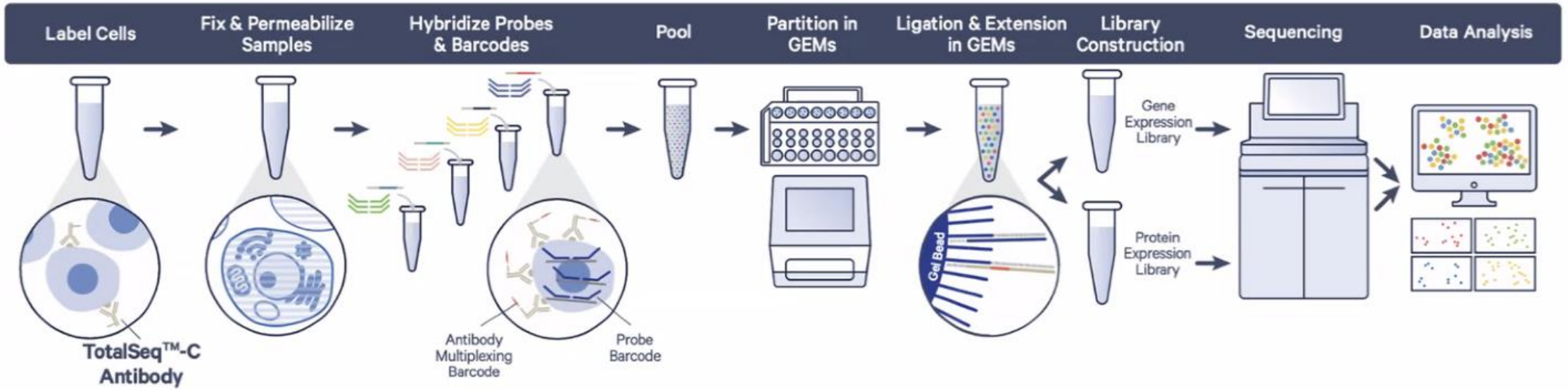
3’ and 5’ OCM: On-Chip Multiplexing

- Pool 4 samples
  - targeting 5000 cells/sample
- Compatible with
  - protein detection
  - TCR/BCR profiling
  - CRISPR screens



Single Cell RNA Flex System

Same workflow - More cost effective and scalable

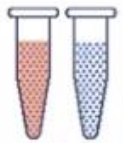


- Sensitive, probe-based **whole transcriptome assay**
- Compatible with Feature Barcode technology for **profiling cell surface and intracellular proteins** using singleplex or multiplex workflows
- Does not depend on polyA capture; covers more than 18,000 **human or mouse** genes

GEM-X Flex Gene Expression – any sample at any scale

Broad sample compatibility

Fresh tissue
Frozen tissue



Cell suspensions
Nuclei suspensions

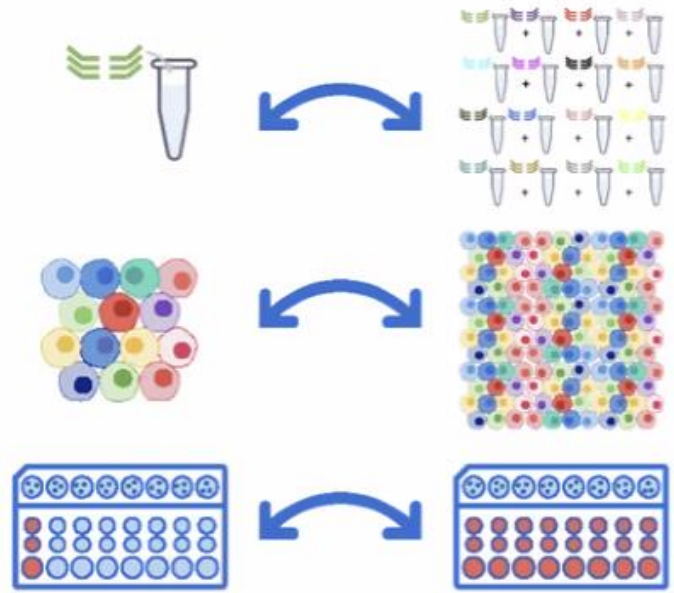
Whole blood

FFPE tissue

In-line multiplexing

- Up to 16 samples per channel
- Up to 128 samples per chip

Flexible project input

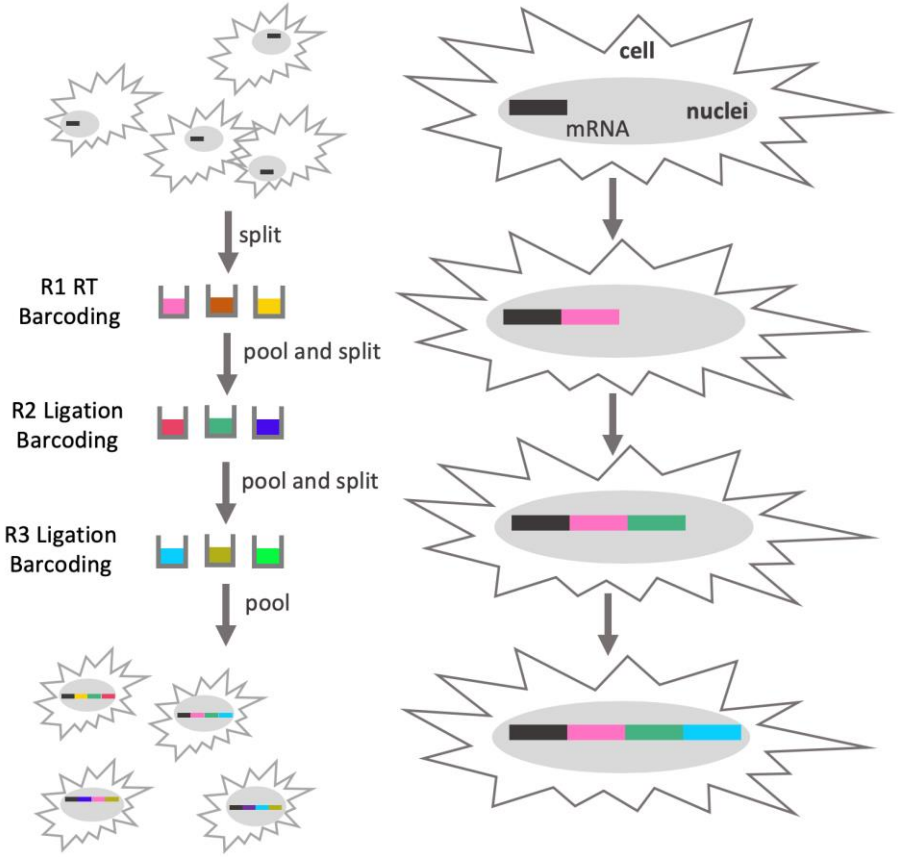


- Up to 2.56M cells per chip
- Up to 80% cell recovery (sample preparation)
- Up to 80% cell recovery (single cell partitioning)
- Low cell input recommendations (25K cells)

SPLiT-seq

PARSE BIOSCIENCES

- Fix 100K-4 million cells or nuclei
- 1 million cells/96 or 384 samples
- RNA with TCR and CRISPR add-ons

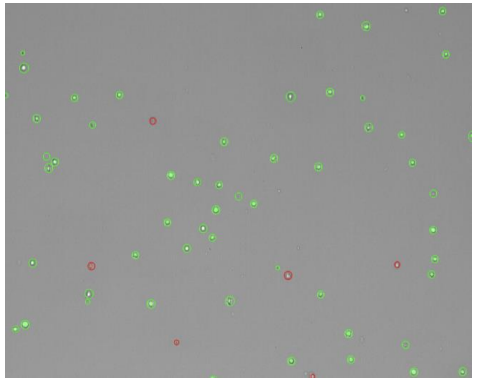


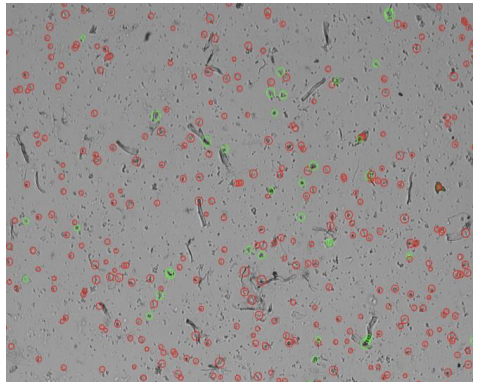
SCALE biosciences

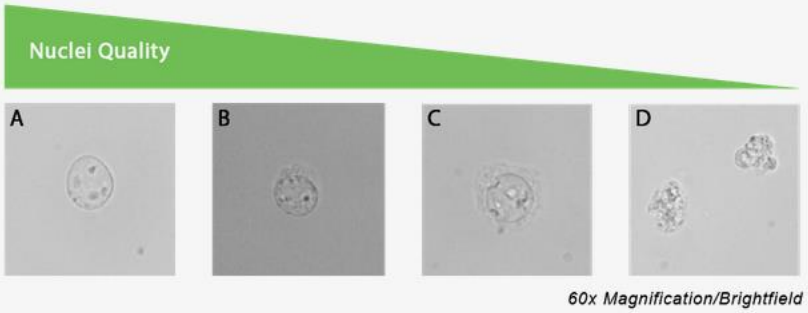
- Fix 100K-2.5million cells or nuclei
- 500K cells/96 samples
- RNA, Methylation, ATAC, CRISPR, Proteomics

Inputs

- 10x 3’/5’ samples
  - We load 2x as many cells as you target
  - Ideally 1000-1500 cells/ul
  - There is a methanol-mediated fixation protocol
  - Most common medias are acceptable – just keep the cells alive!
    - 10% or less FBS
    - 2% or less BSA
    - NO EDTA
- Flex samples
  - 50mg of frozen or fixed/frozen tissue
    - 10X fixation kit
  - 300k cells/500k nuclei into the fixation
  - 50k cells/nuclei into hybridization
  - Two 50um scrolls of FFPE tissue
    - You may need more depending on tissue size/cell density
- Epi Multiome or ATAC
  - Debris-free nuclei suspension
    - 10x Genomics Buffer (Aliquots available at the AGC)
    - Minimum of 3200 nuclei/ul if targeting 10K nuclei for capture
- Split Pooling (Parse/Scale)
  - Fixed cells or nuclei following manufacturer’s protocol







Single Cell Notes

- FACs sorted cells
  - Usually overestimated by 30-60%
  - Sorting first by ruby and then viability greatly reduces error
- Small population of cells?
  - Concentrate into 30ul of buffer
  - Include an unwanted/abundant population as well
- Run a pilot
  - Start with 1-2 samples to check the data/quality
- Proteome does not equal transcriptome
  - You may sort on a protein but not see the transcript
- Cell target numbers are “in a perfect world”
  - Cell size, tissue/cell type, disease state all affect recovery
  - It may take some optimization to get what you need
- Some samples will fail
  - There will be dropouts whether it is the cell recovery, unexpectedly dead, etc.
  - This is especially true of FFPE samples

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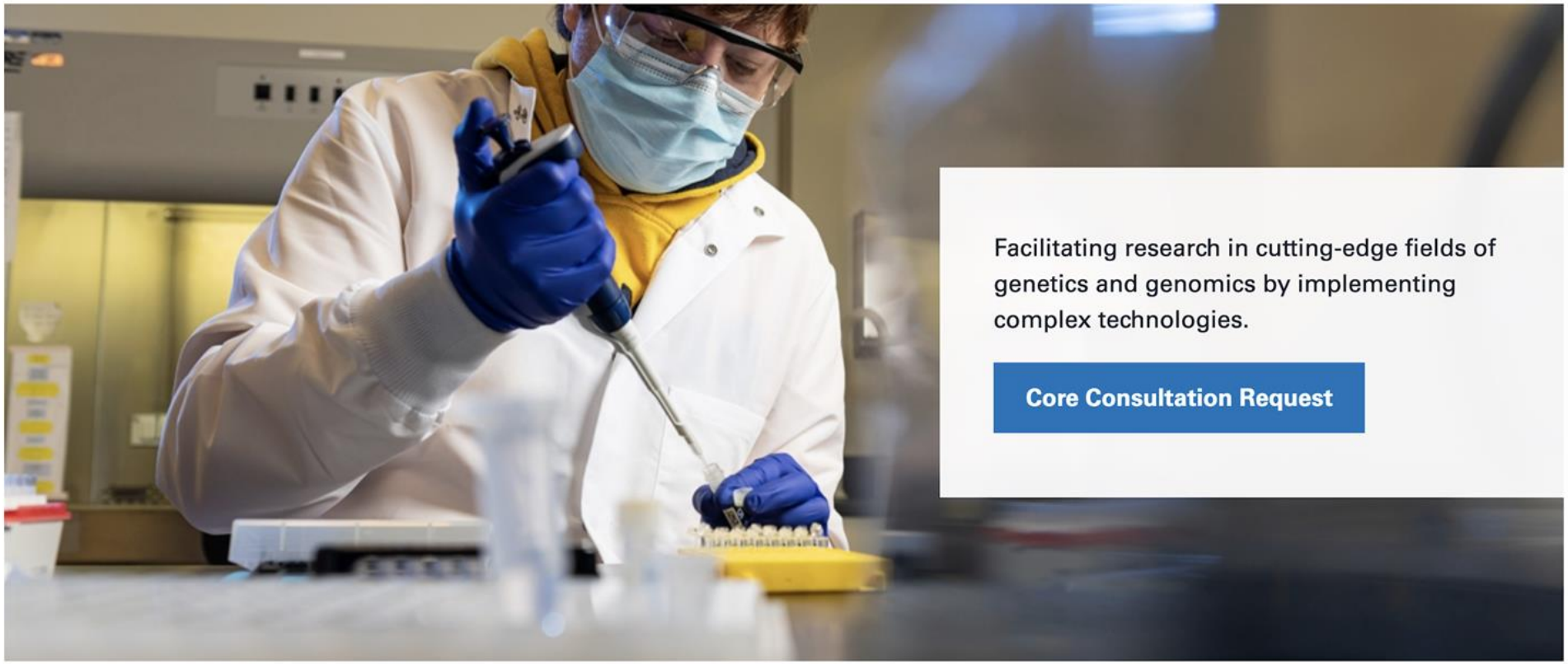

Website: http://michmed.org/agc

Email: advanced-genomics@umich.edu

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