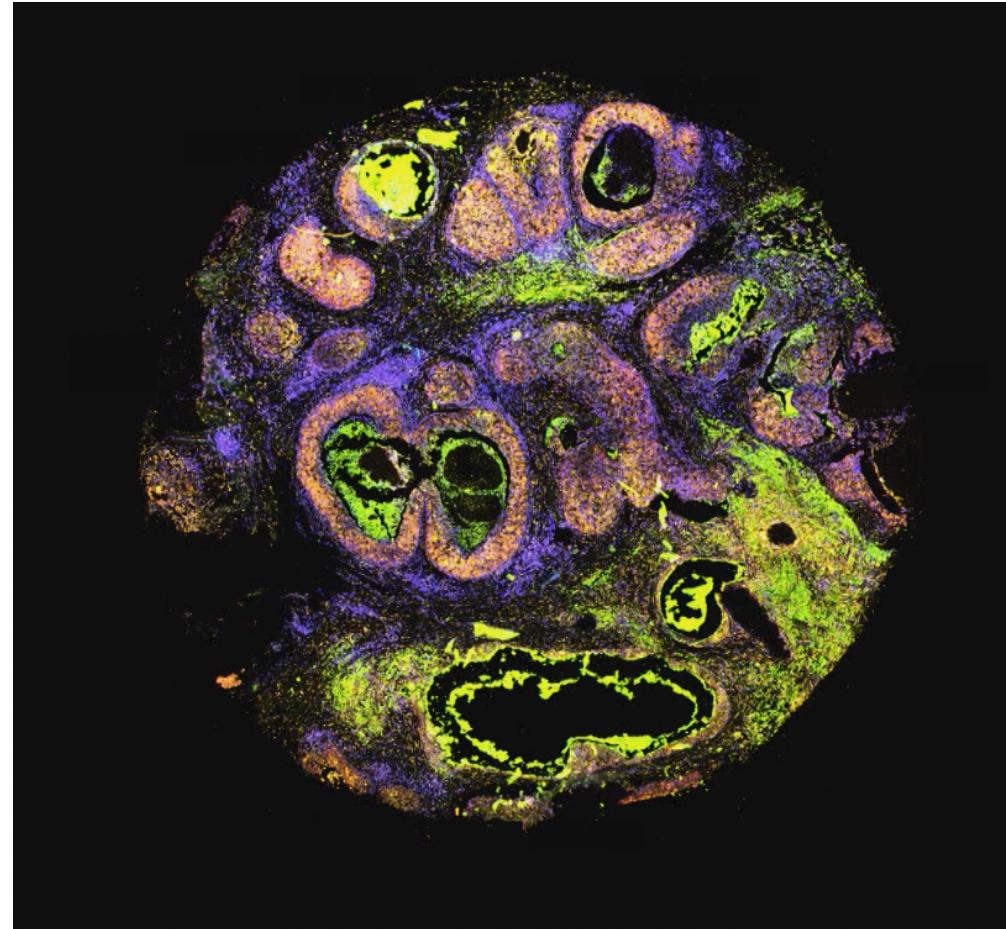
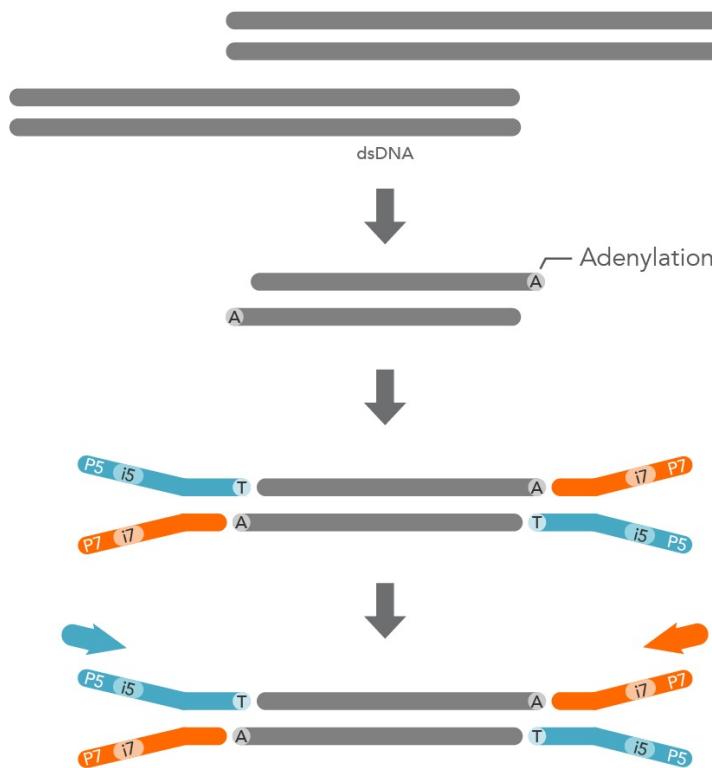
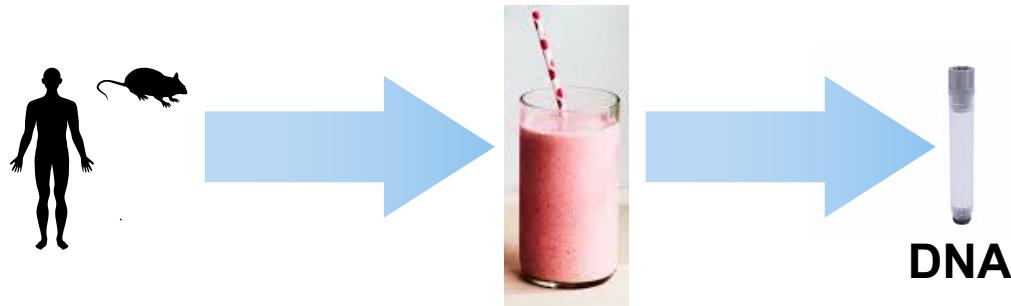


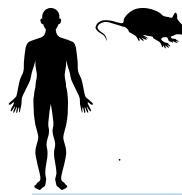
The next genomic frontiers



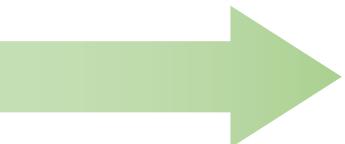
Neeman Mohibullah
Integrated Genomics Operation

Generic overview of Illumina library creation





Bulk



Genome



Epigenome



Transcriptome



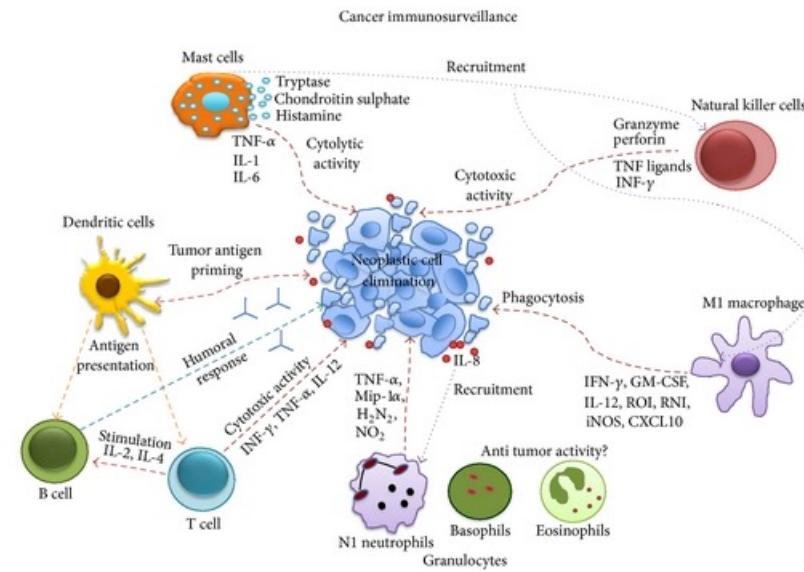
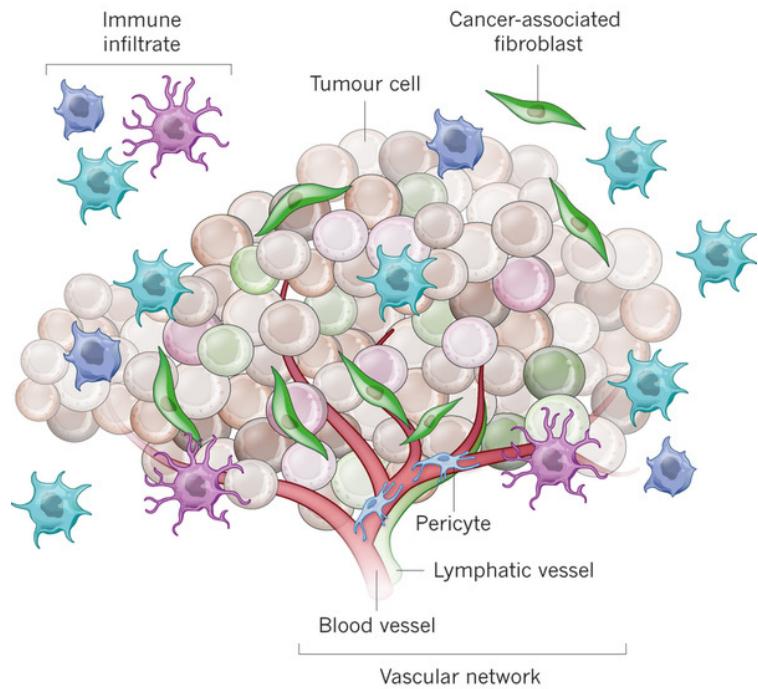
Proteome



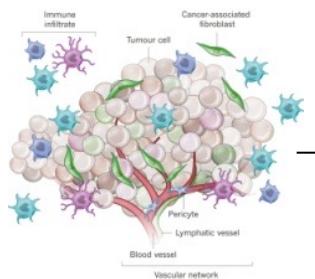
Metabolome



Because tissues (and tumors) are very heterogeneous



Global RNAseq tells a very different story than single cell RNAseq



→ Total RNA → RNAseq

Gene	Expression level
Gene 1	10,000
Gene 2	1,000
Gene 3	5,000
Gene 4	200*
Gene 5	0

*:limit of detection

Gene	Expression level
Gene 1	0
Gene 2	2000
Gene 3	1000
Gene 4	800
Gene 5	0

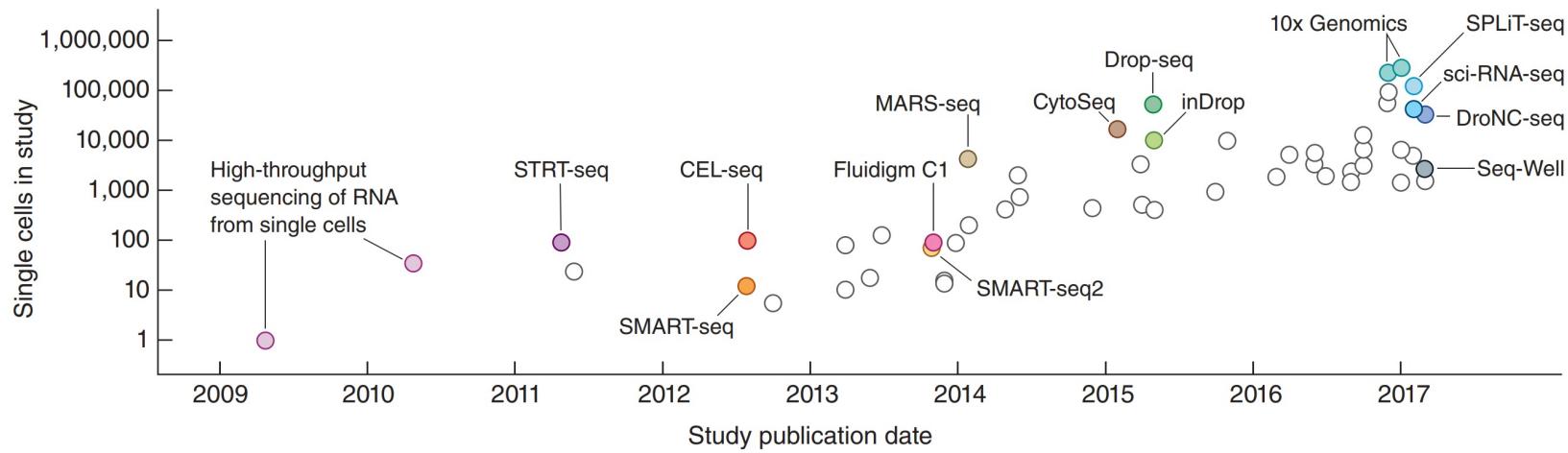


Gene	Expression level
Gene 1	0
Gene 2	2000
Gene 3	1000
Gene 4	0
Gene 5	0

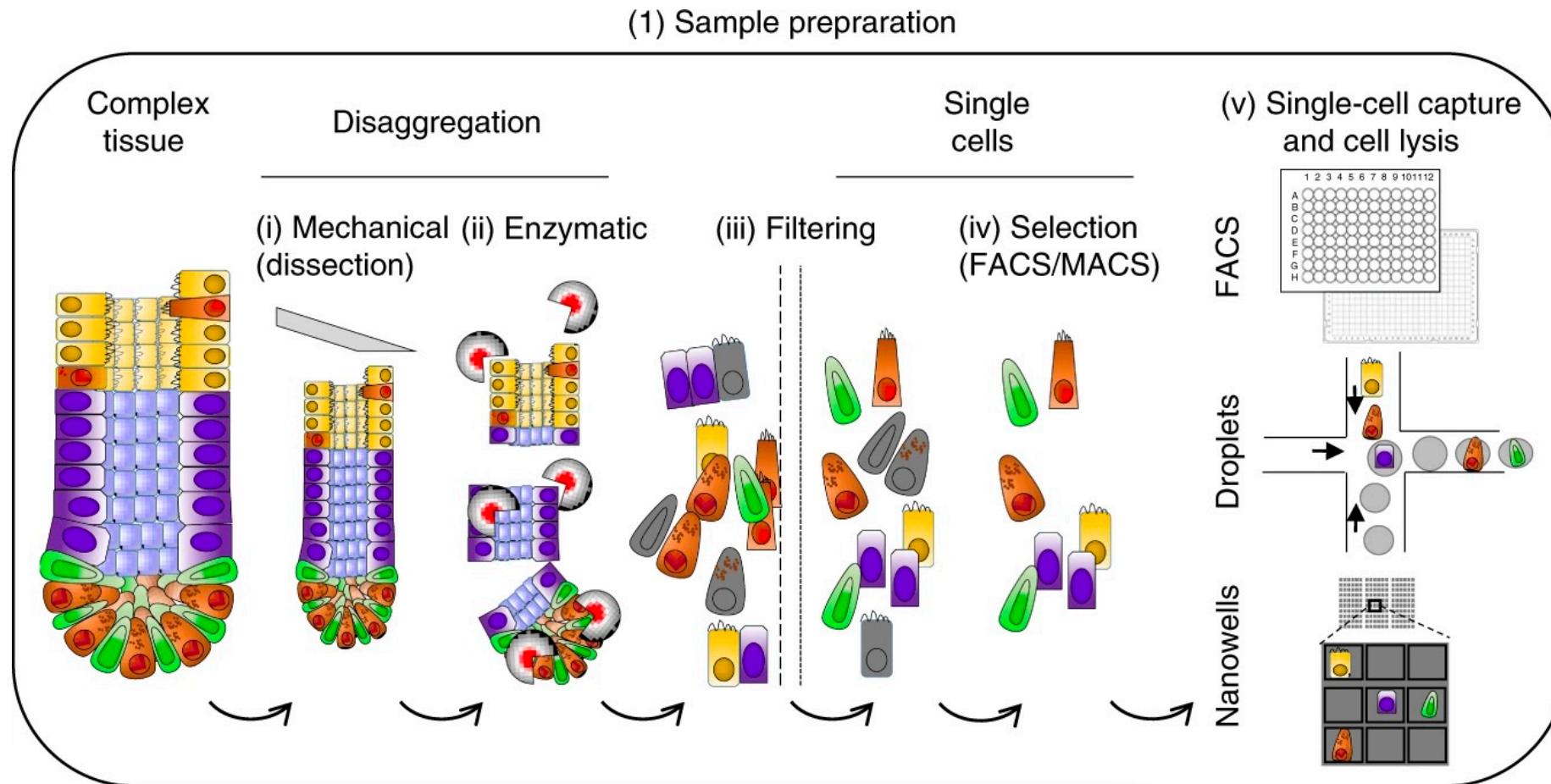


Gene	Expression level
Gene 1	0
Gene 2	0
Gene 3	21 000
Gene 4	0
Gene 5	0

Single cell RNA seq timeline



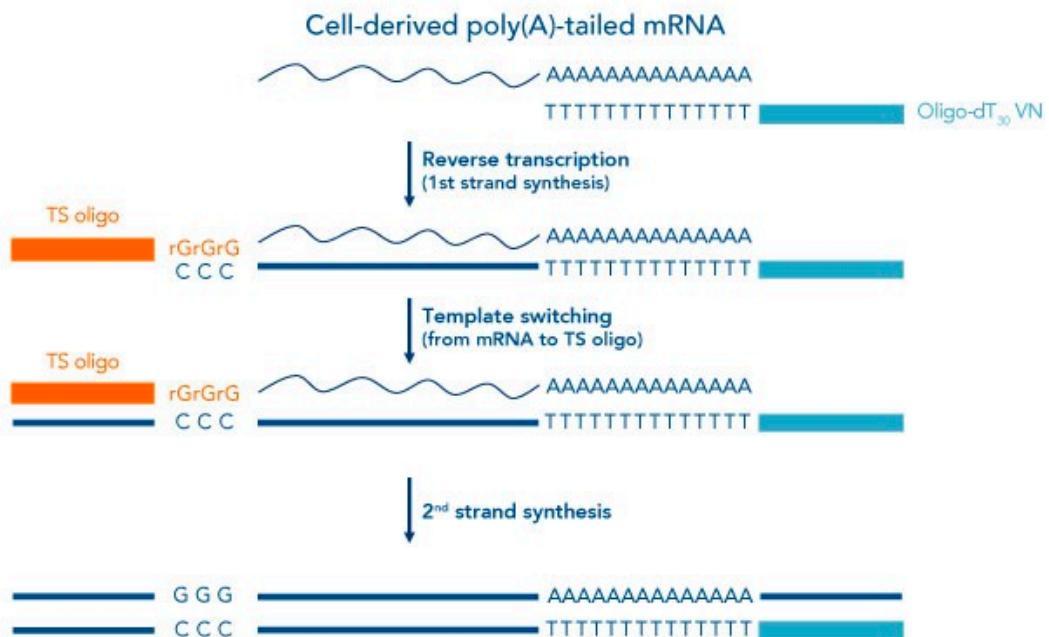
Single cell wet lab workflow



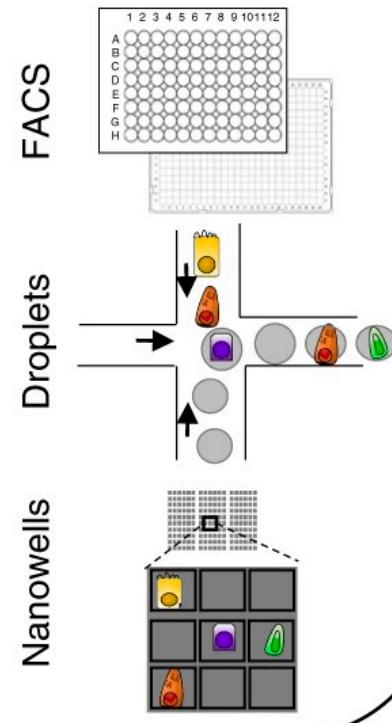
What could go wrong?

Lafzi et al Nature Prot 2018

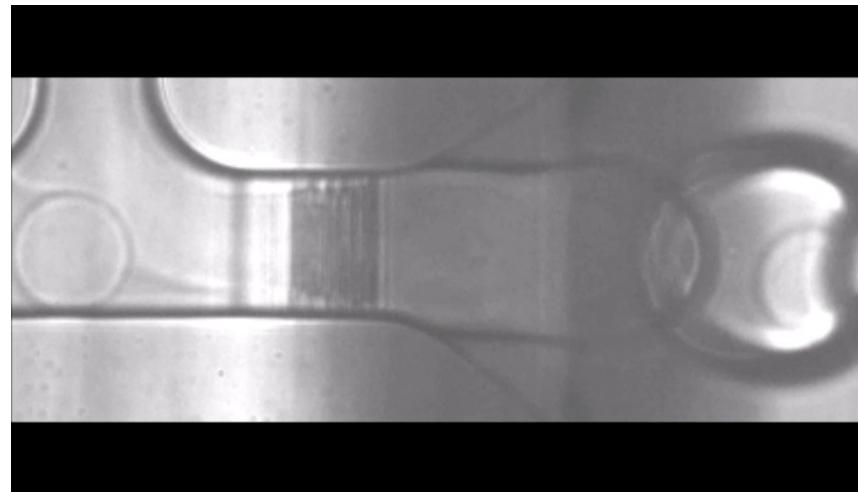
Template-switching



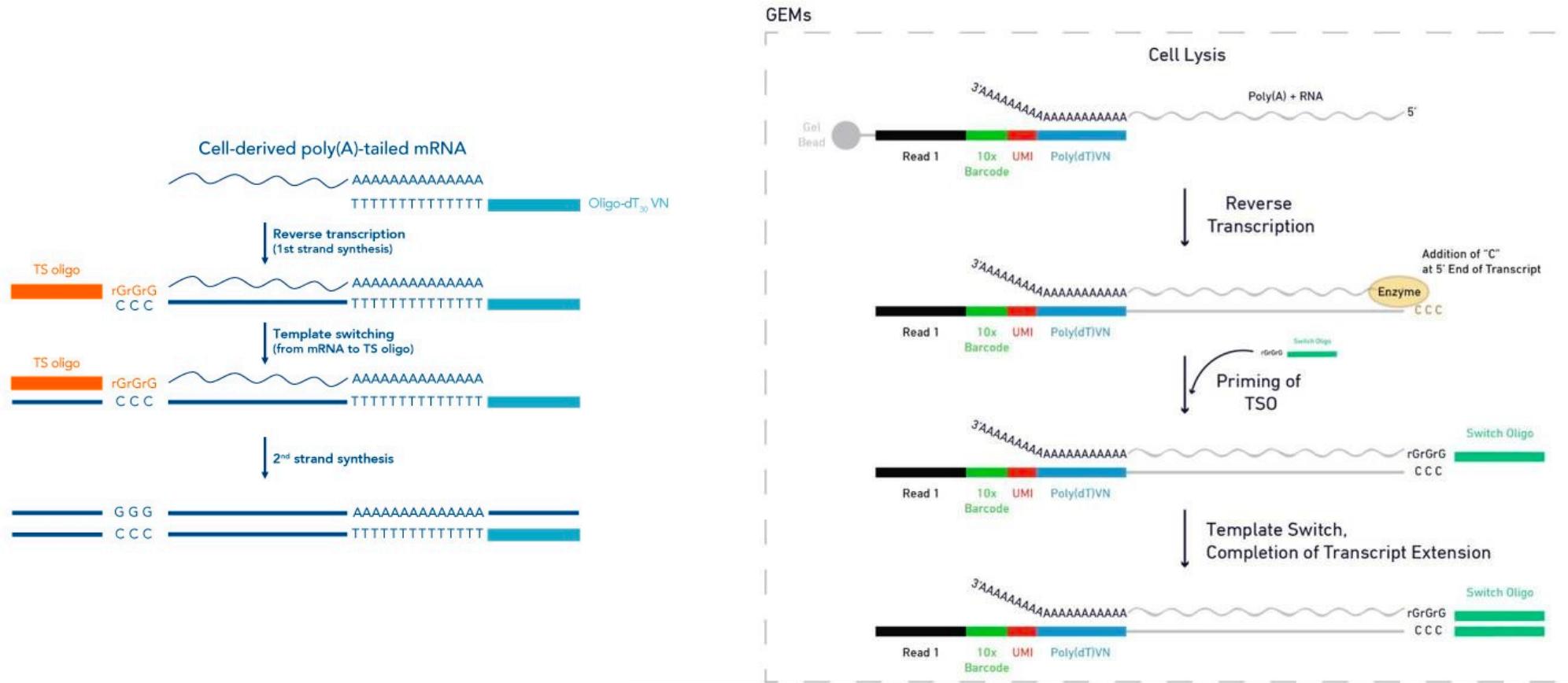
(v) Single-cell capture and cell lysis



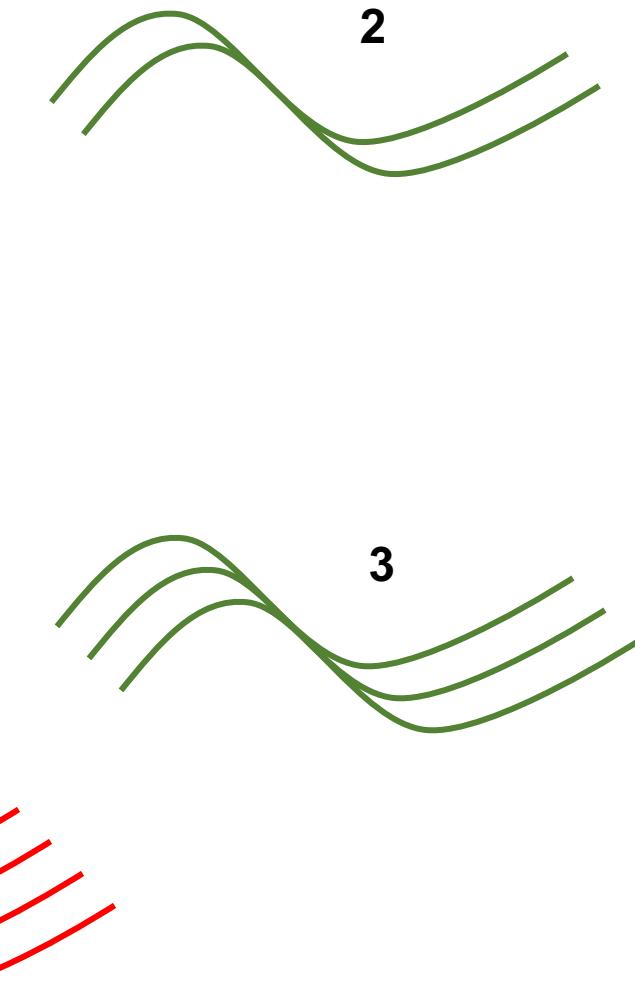
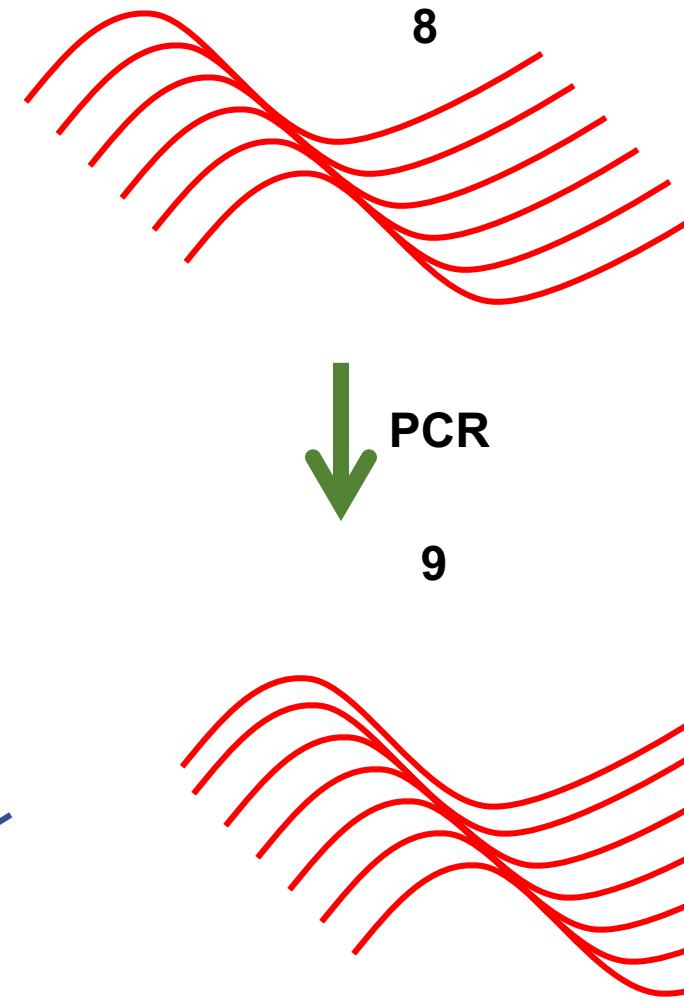
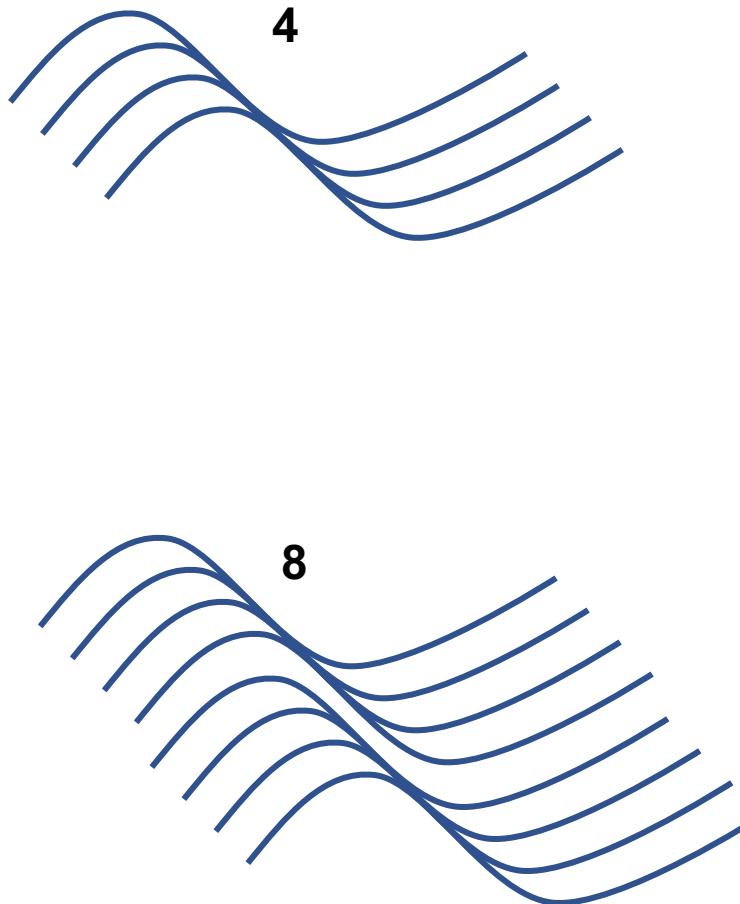
10X genomics



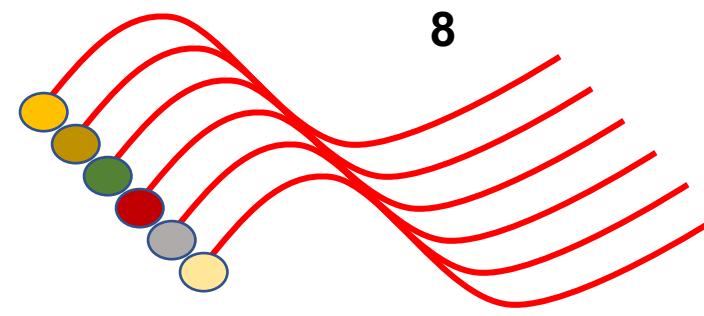
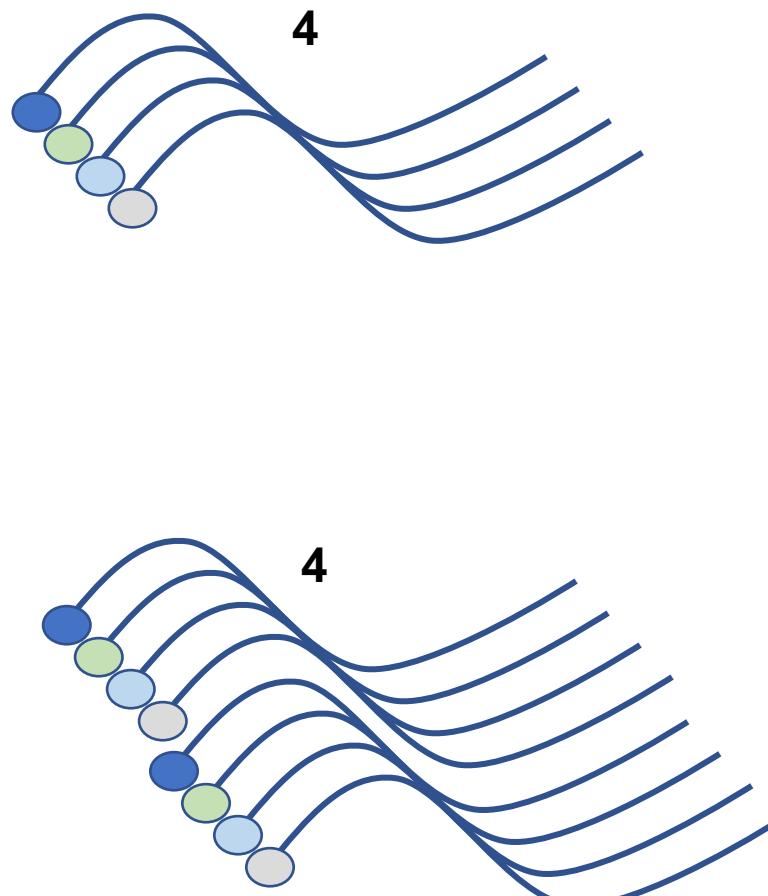
Template-switching with cell barcode



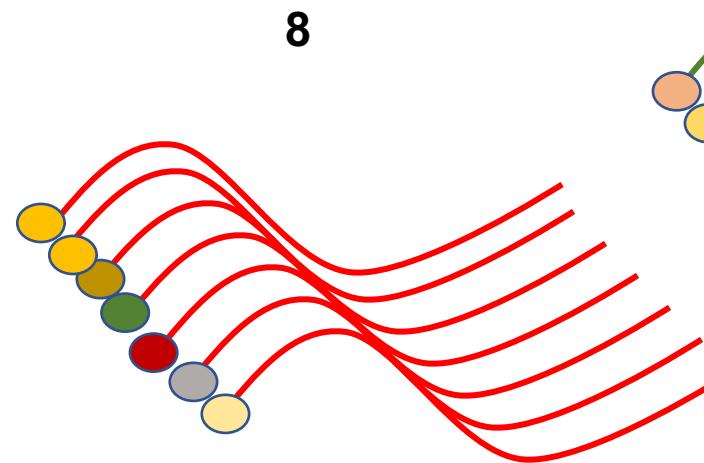
Unique Molecular Identifier



Unique Molecular Identifier

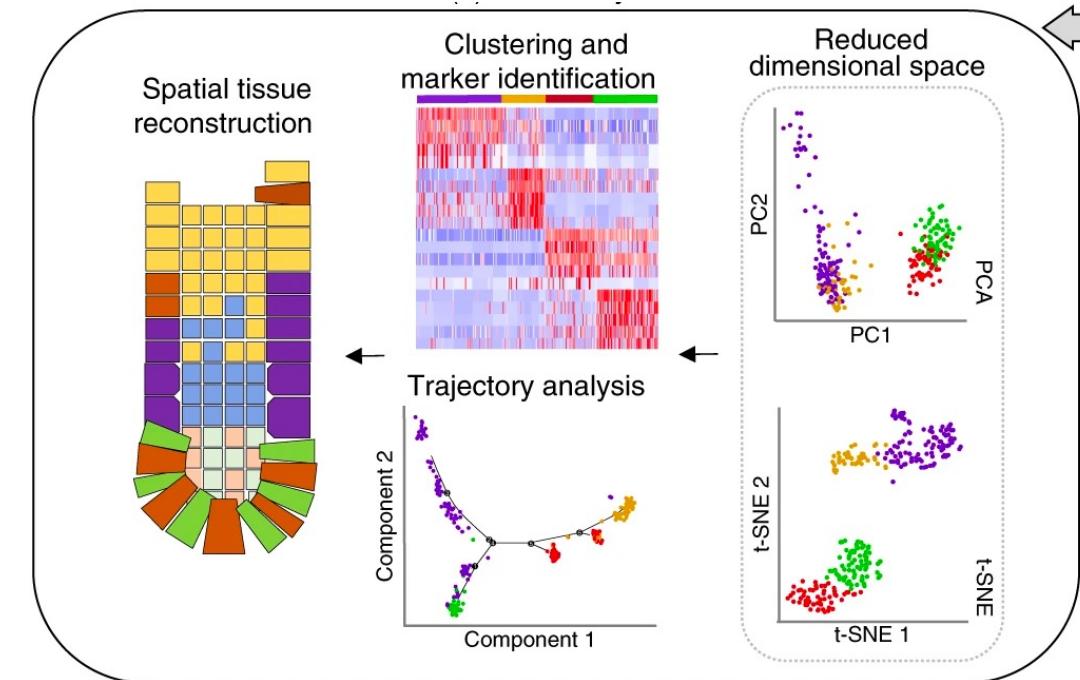
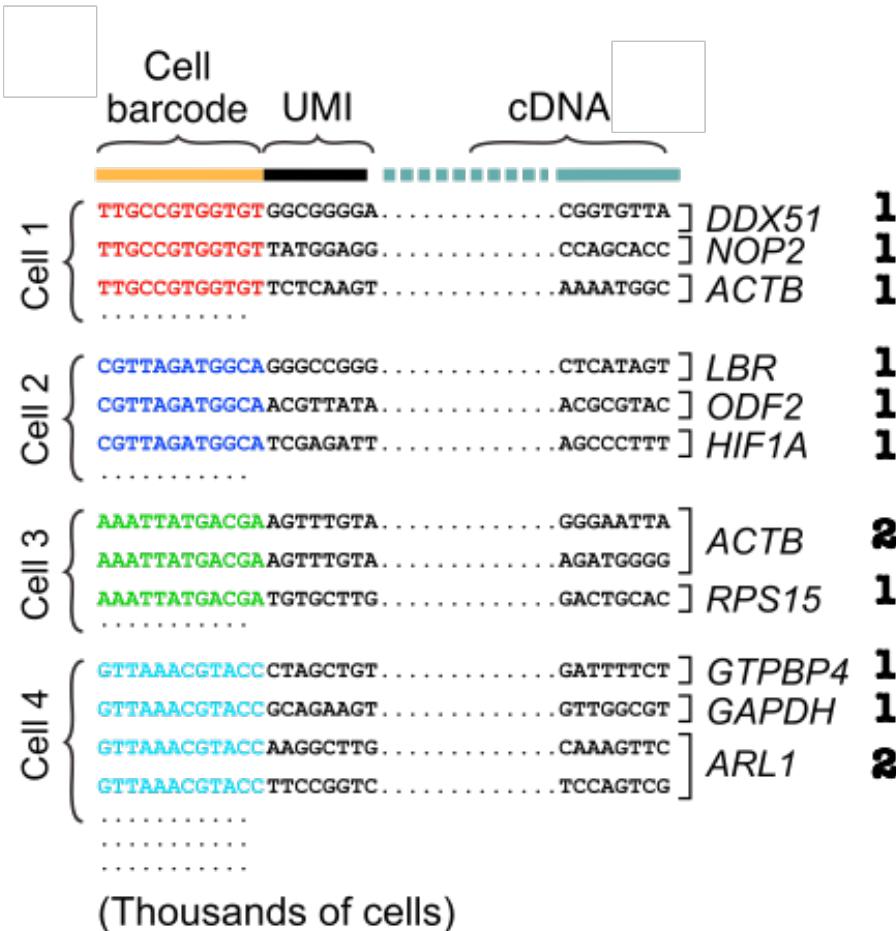


PCR



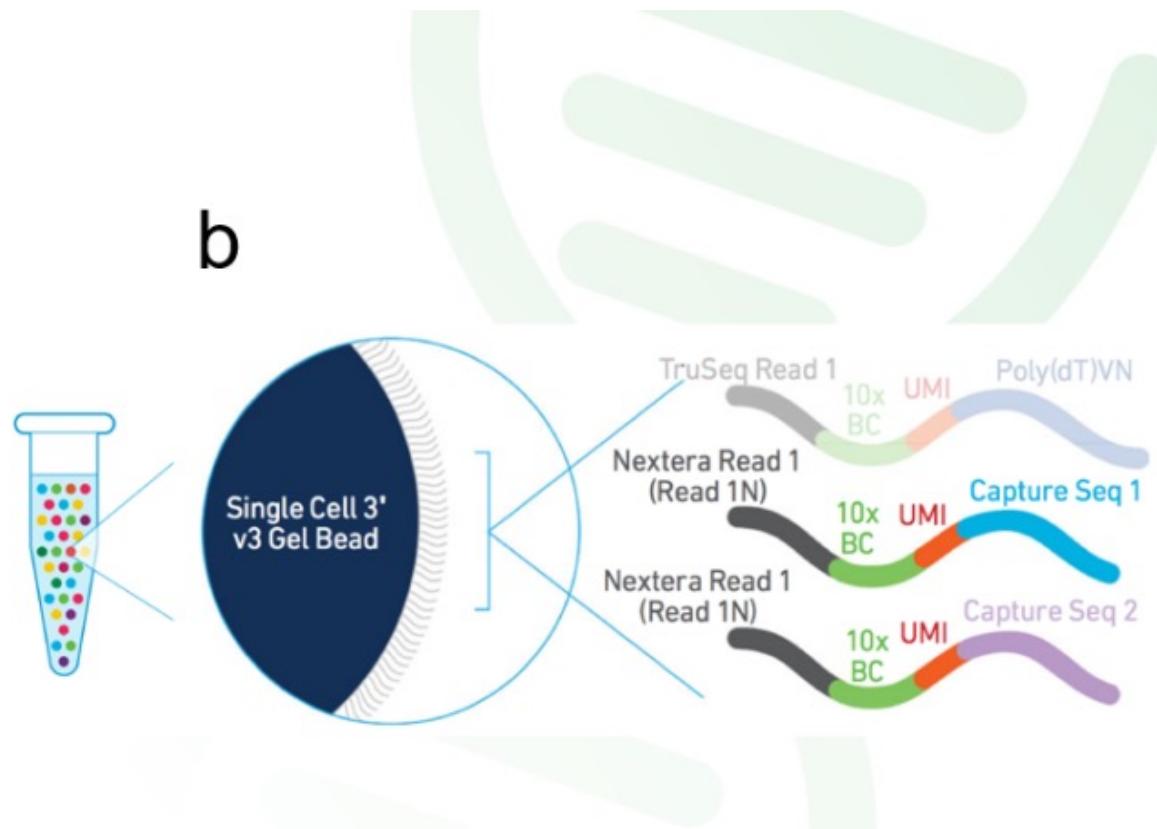
counting UMIs instead of transcripts

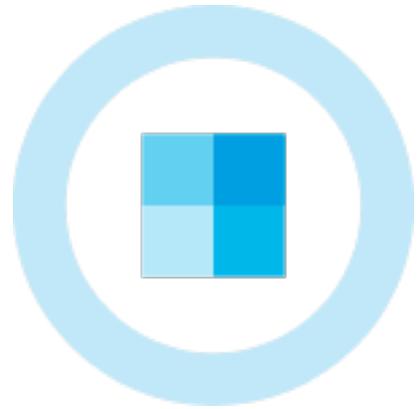
Pipeline to analyze data



Using the same chemistry to perform proteogenomics

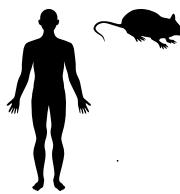
b





SPATIAL GENE EXPRESSION

Visualize Gene Expression within Tissue Organization



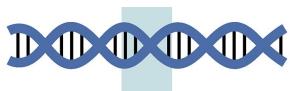
Bulk



Spatial



Genome



Epigenome



Transcriptome



Proteome

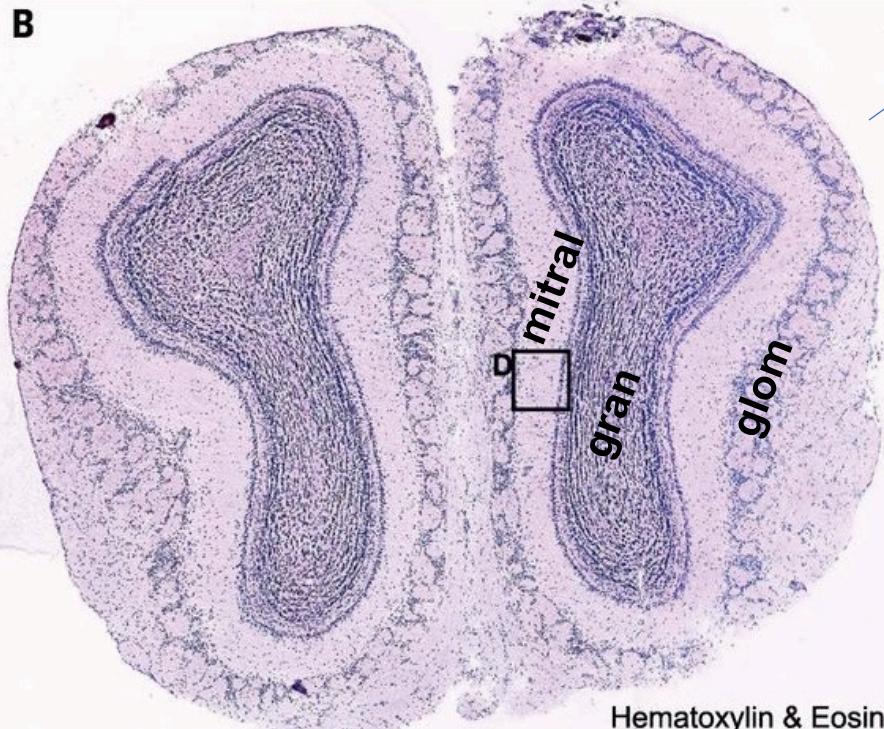


Metabolome



Spatial transcriptomics

Mouse olfactory bulb



Bulk RNAseq

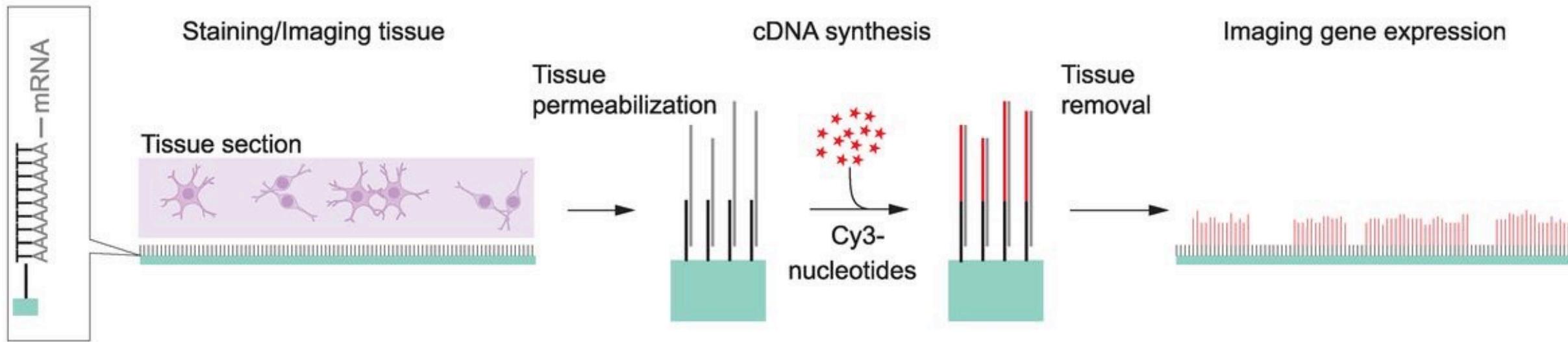
In situ RNAseq
Microdissect and
RNAseq

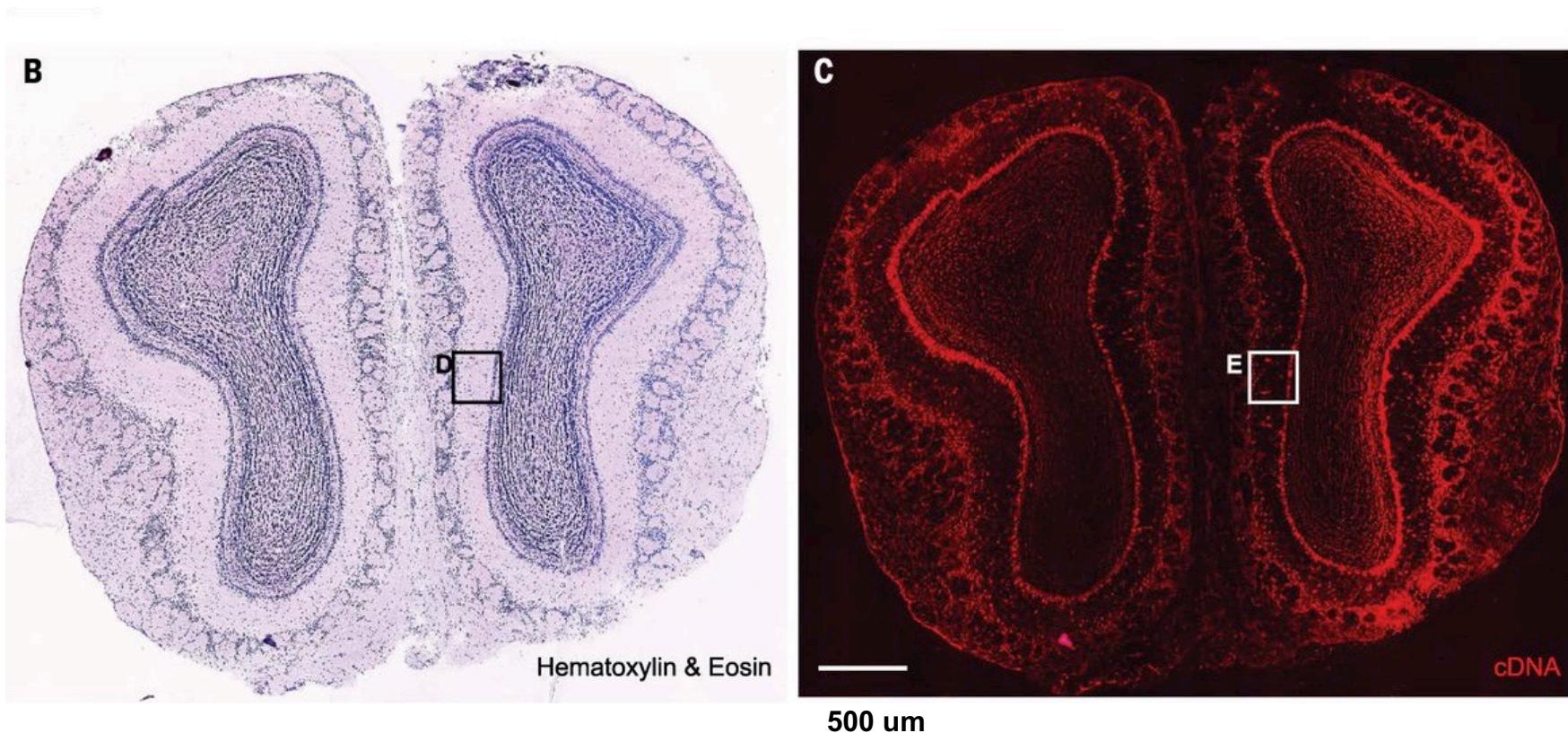
Dissociate tissue and
single cell RNAseq

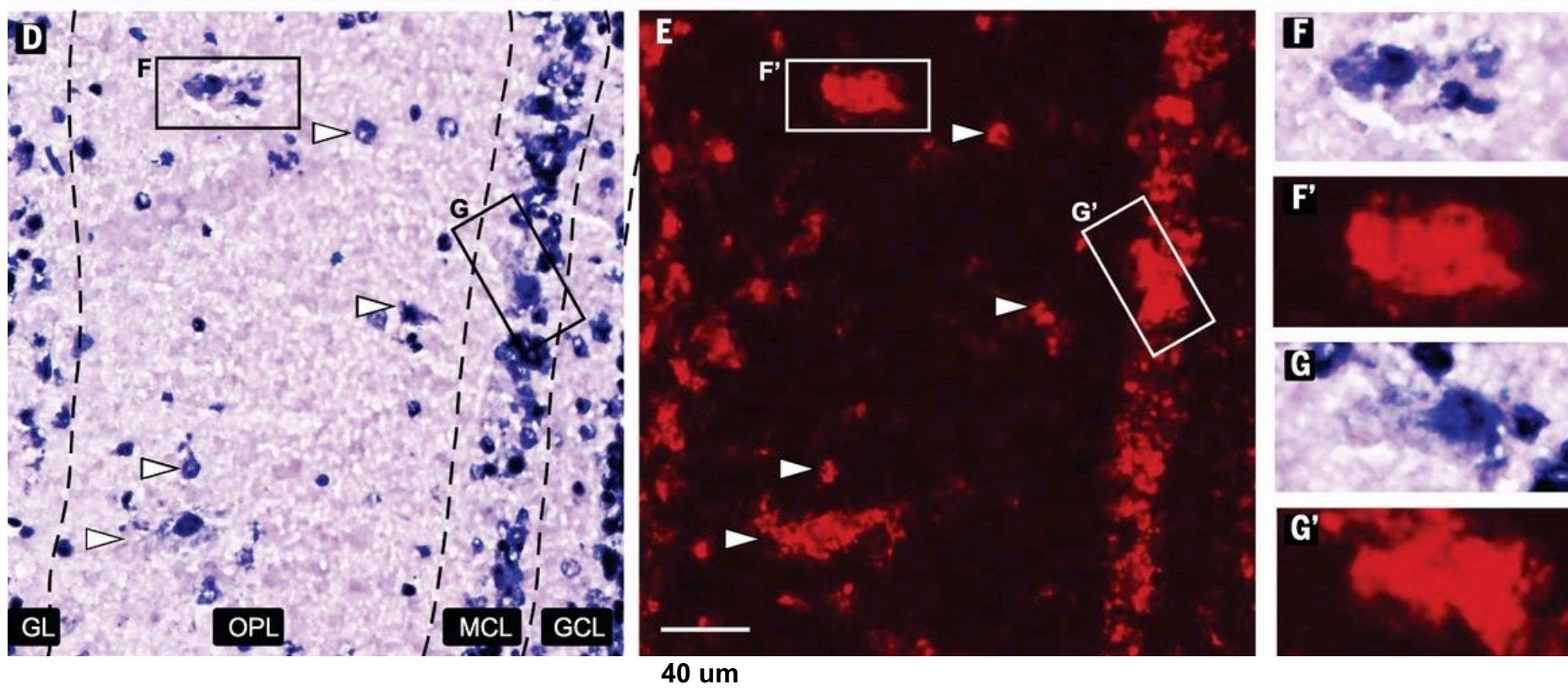


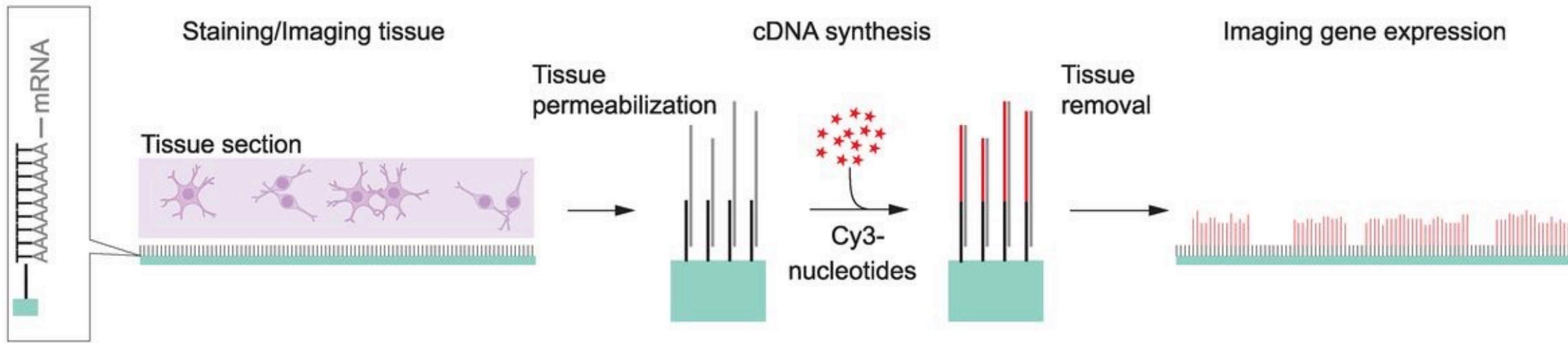
Spatial transcriptomics workflow

A

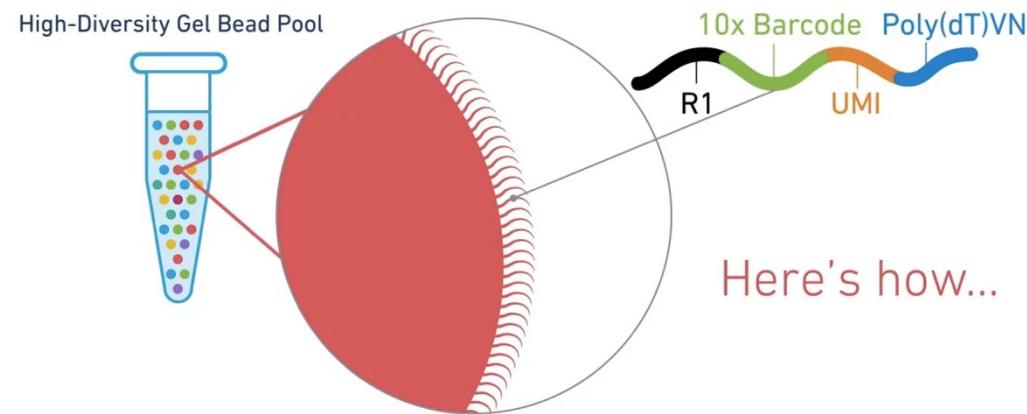
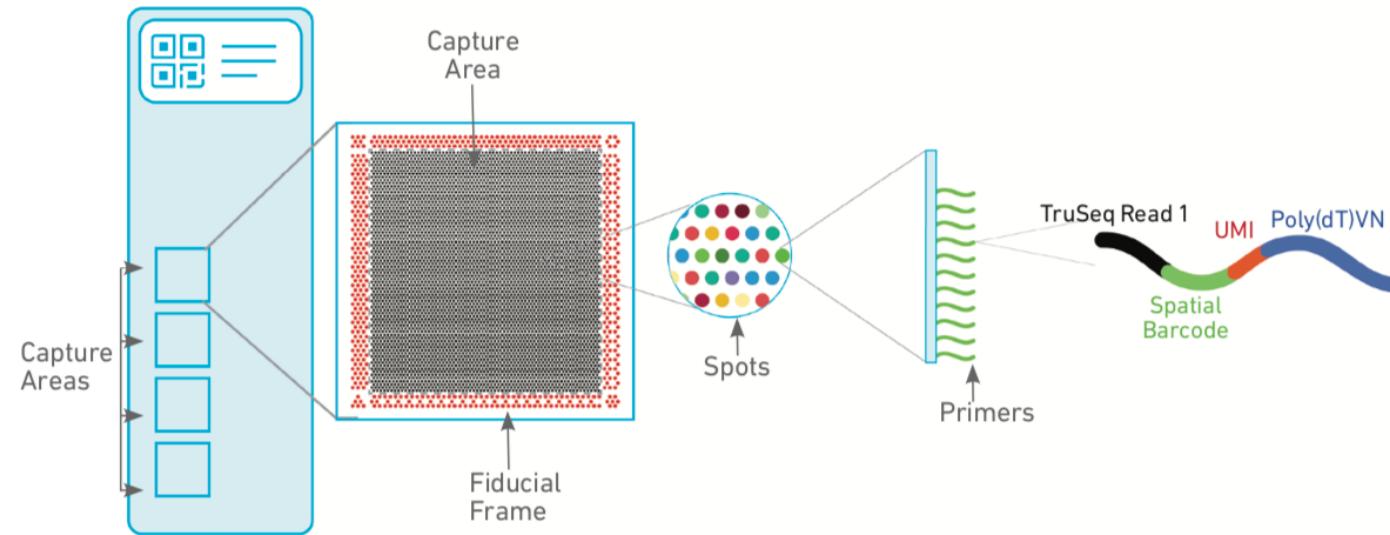






A

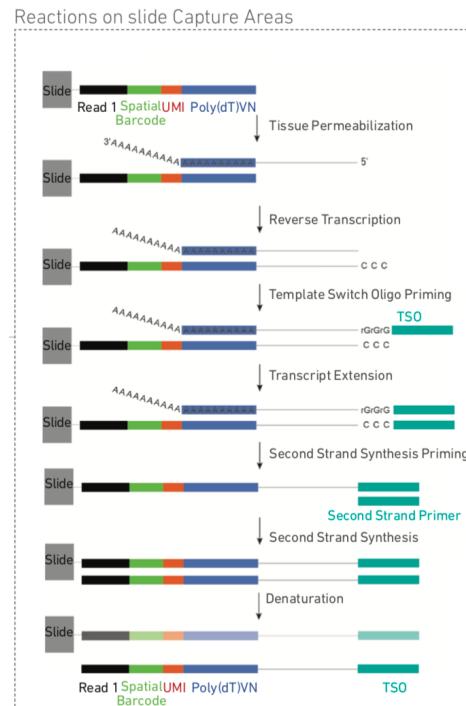
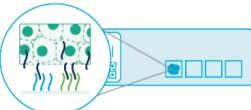
10x genomics Visium



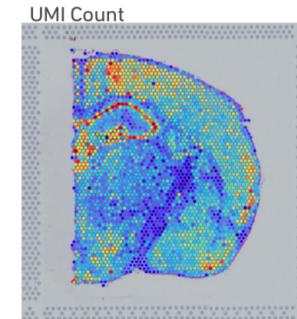
Place tissue on slide H&E stain



Permeabilize and capture H&E stain



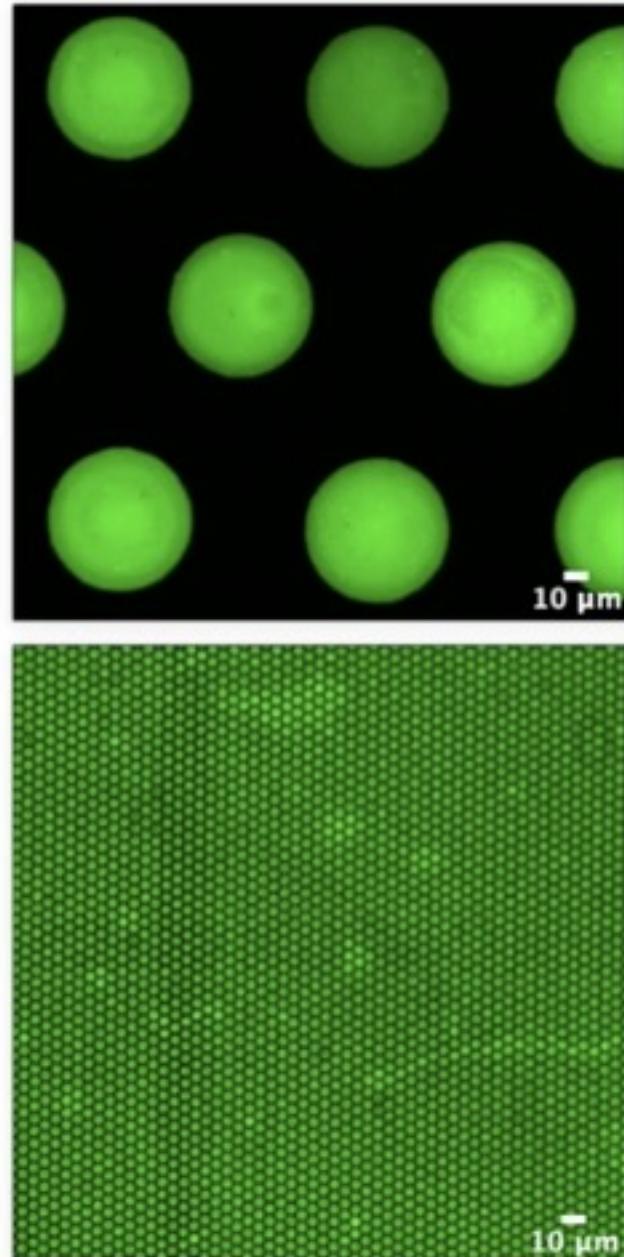
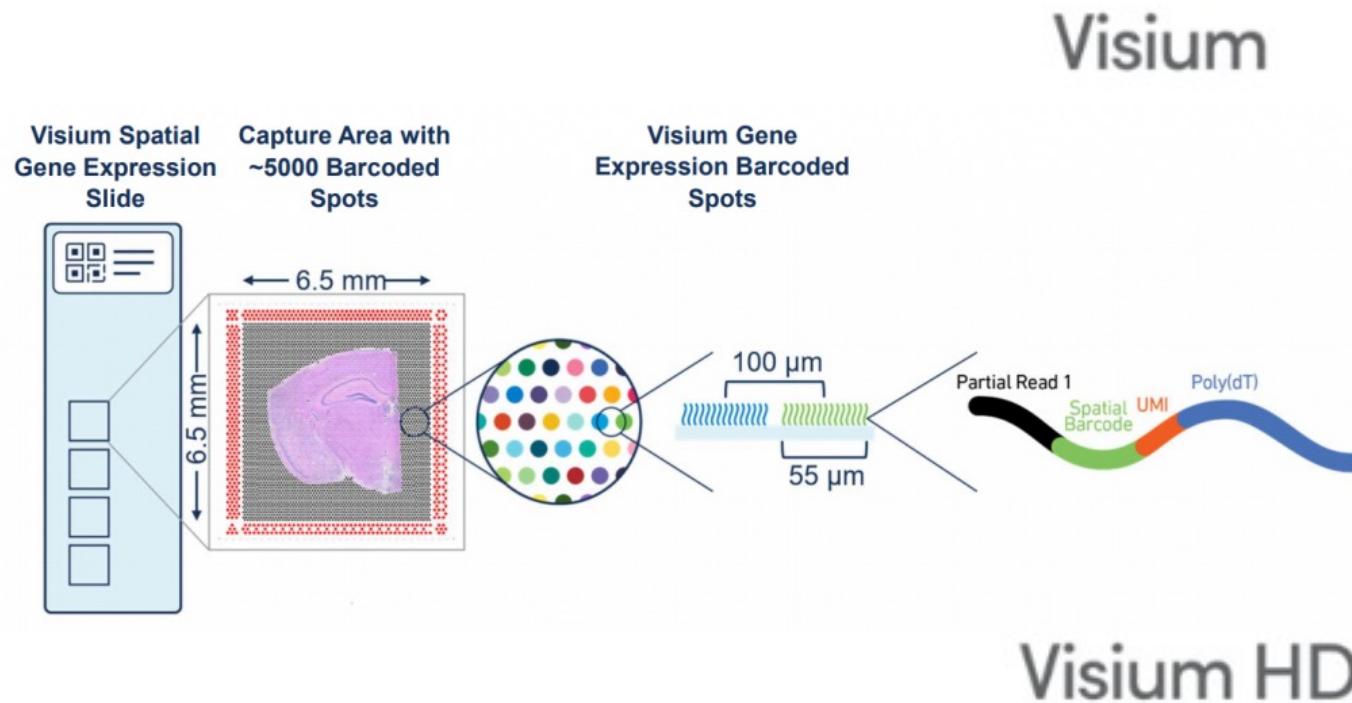
Prepare libraries Sequence



Cell
barcode UMI RNA

ACAGTAATAAGACT	GGCCCCCG
TGACAAATAAGACT	TCTAGCTG
CGTTAGCTTACGTC	GATTATAG
TGACAAAGTTACCTC	ACAATGCT
GTAGCTGATGCCG	CTTTCAT
GTAGCTGATGCCG	TCTCGACT
CGTTAGTGTACCG	CCTCGAGC
ACAGTAGTTACGTC	GTACACATC
TGACAAATGATGCCG	GTACACATC
ACAGTATGATGCCG	TCGACGAT
GTAGCTAAAGACT	ACATGCTG
CGTTAGTGTACGTC	TAGCCAGT

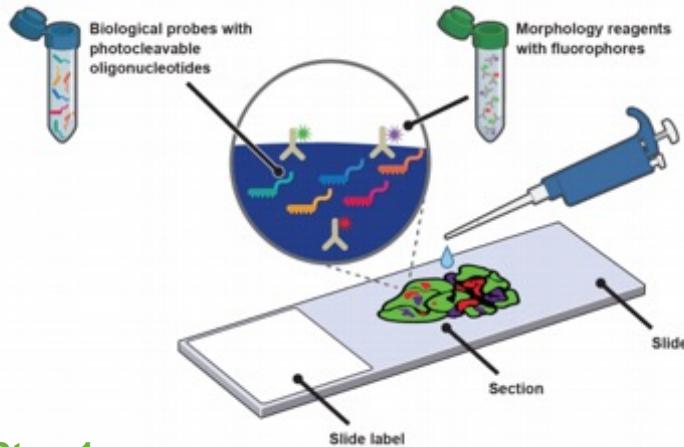
Visium HD | Resolution



How to make this compatible for FFPE?



Nanostring GeoMx



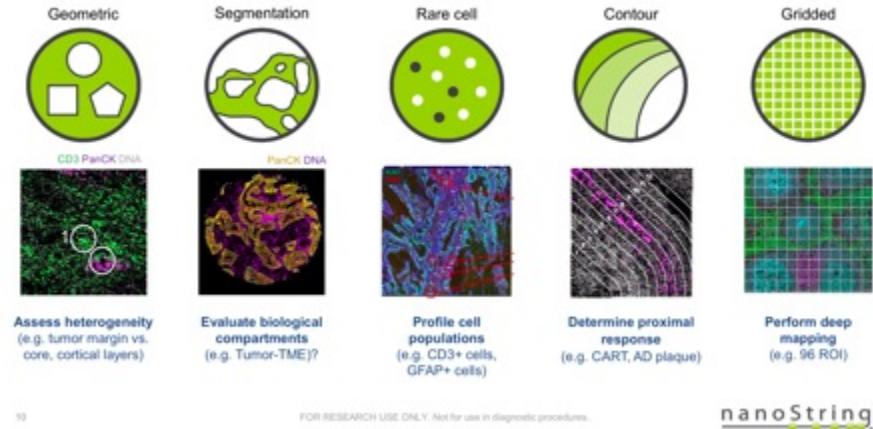
Step 1

IHC+ISH on the same slide

- Panel of oligos (for RNA detection)
- Fluorescent Antibodies

PROS:

Easy selection of ROI
Cheaper Sequencing



Step 2

Regions of Interest (ROI) selection

CONS:

Capital equipment purchase
Expensive (\$140/ROI); ROI 10-700um
Targeted Panels (Whole txtome)
Software in early phases

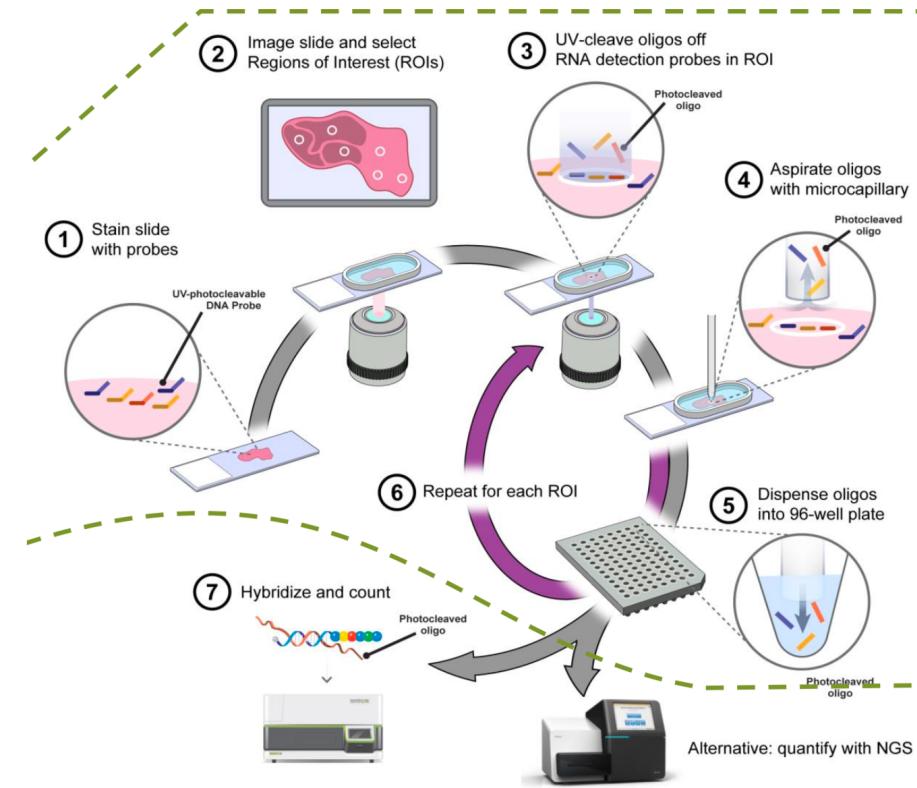
Nanostring GeoMx



GeoMx Digital Spatial Profiler
Your GPS for Immuno-Oncology

8

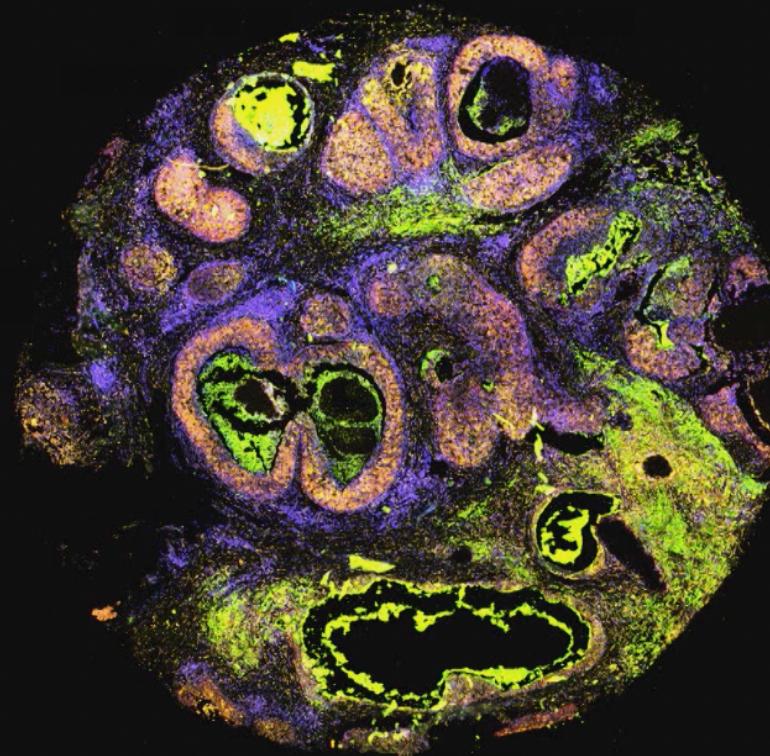
FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.



nanosString
nanosString®

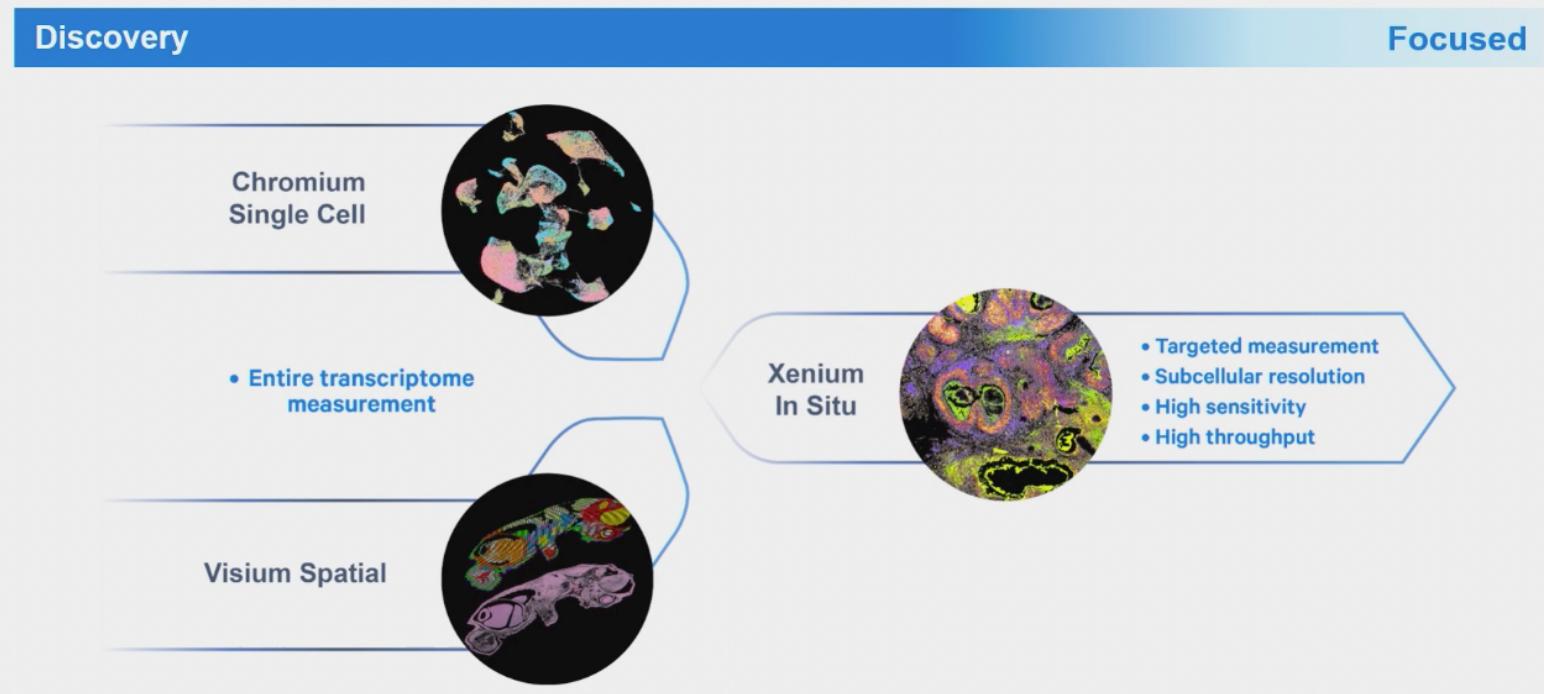
Where do we go from here?

**Xenium
In Situ**

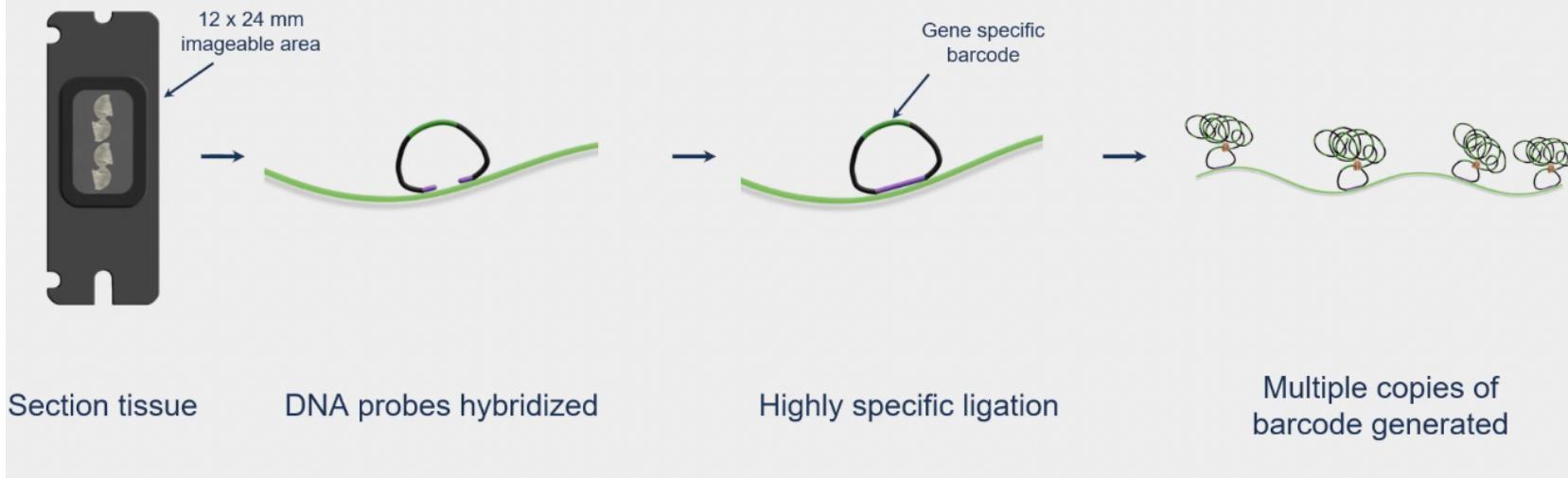


Where do we go from here?

From Discovery to Focused with Complementary Workflows



► Xenium Workflow

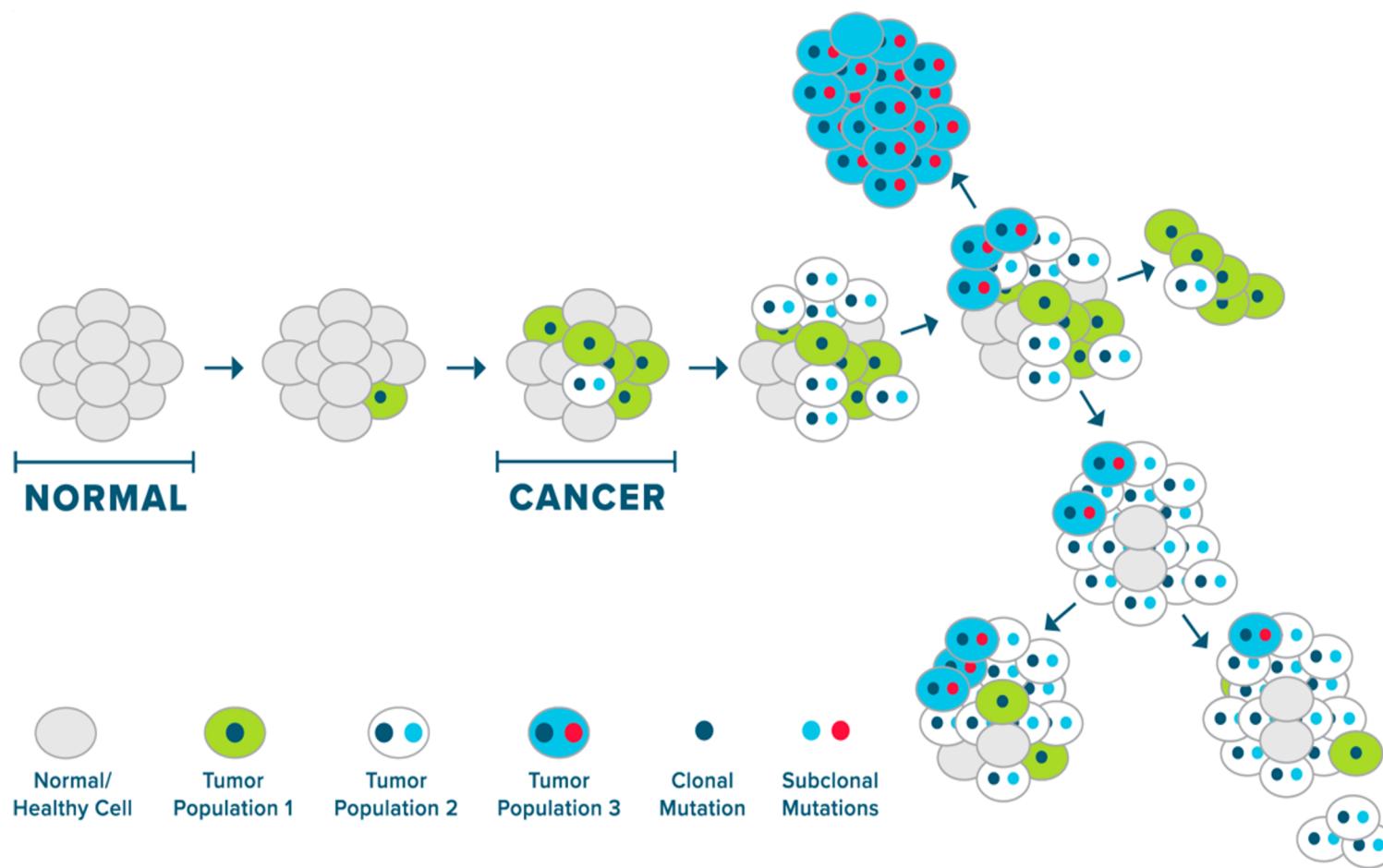




Flexible and Robust Platform to Build Future Capabilities Faster

	Accessible content	More analytes	Throughput & plexy	Software
Now	Curated panels for specific tissues Custom for flexibility	RNA	Up to ~400 genes Up to 6 slides of 12 x 24 mm each/week	On-board analysis Nuclei-based segmentation Xenium Explorer
ROADMAP	Expanded menu of tissue and application panels	Simultaneous protein on same section/slide Expressed SNPs, isoforms, other	Flexible plexy to suit application needs Higher throughput/week	Cell segmentation Analysis features Simplified storage & collaboration with 10x cloud

Single cell DNA studies



Direct Library Prep

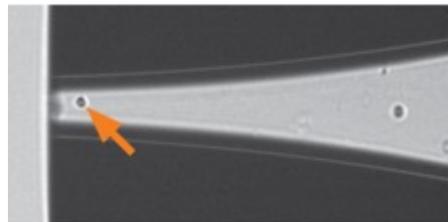


Contactless piezo electric dispenser

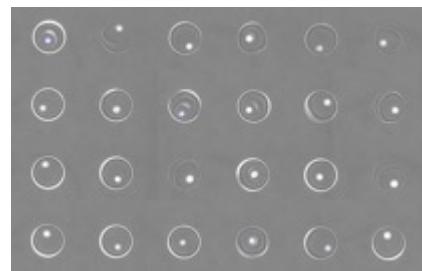
high throughput dispensing of individual cells from cell suspensions 50 pl to 1 ul



Direct Library Prep

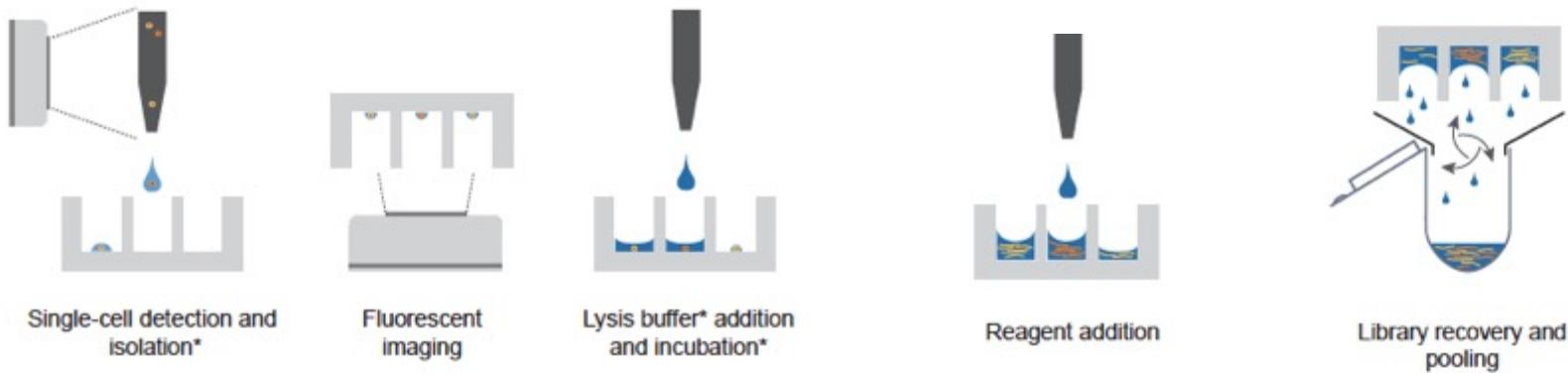


Real-time object recognition during dispensation
On-board fluorescent module



Imaging of cells on chip
Link cell state to sequencing data

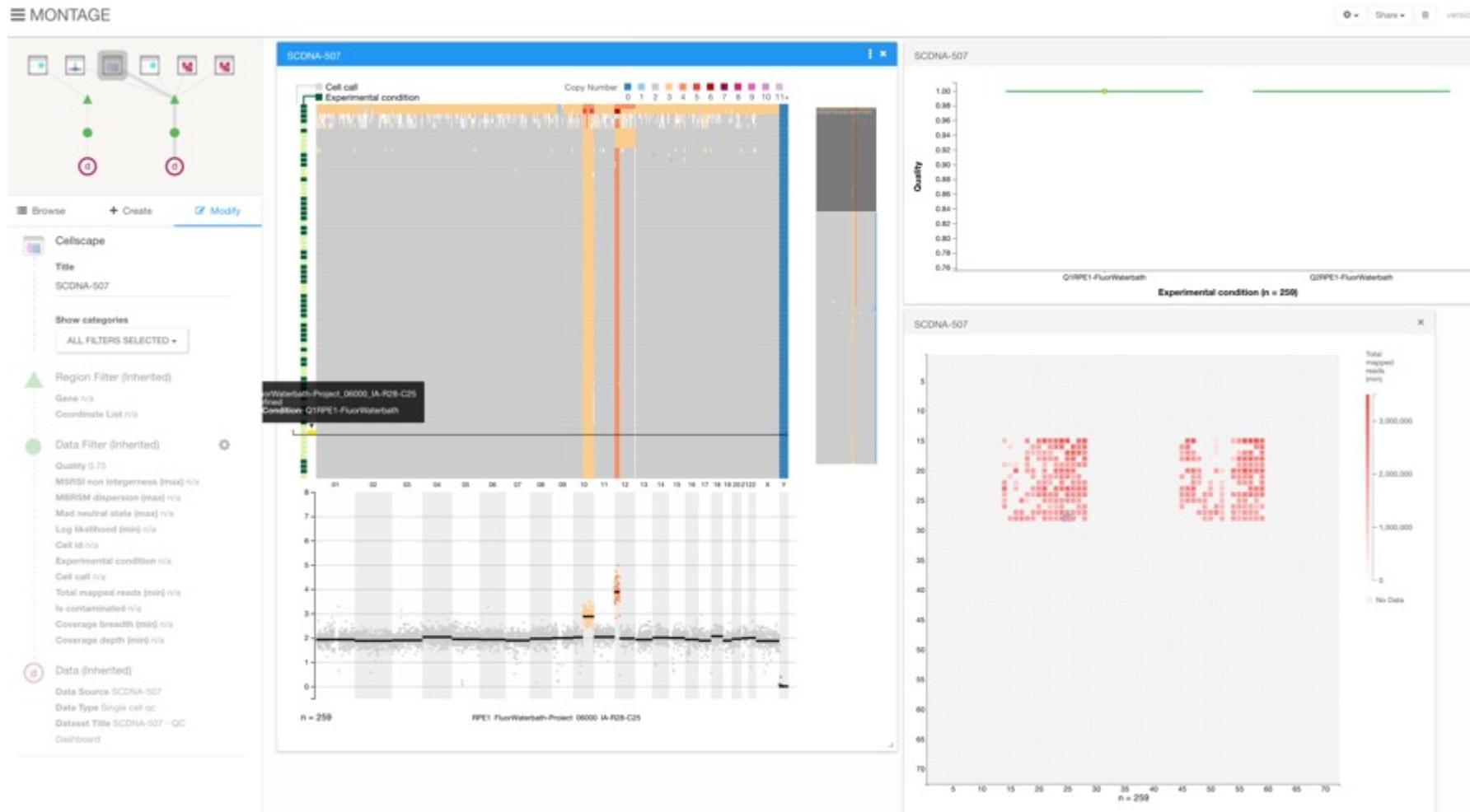
Single cell DNA libraries



Sohrab Shah, Sam Aparicio et al

Scalable whole genome sequencing of 40,000 single cells identifies stochastic aneuploidies, genome replication states and clonal repertoires Cell 2019

Data analysis to look at copy number aberrations



Questions?

This afternoon:

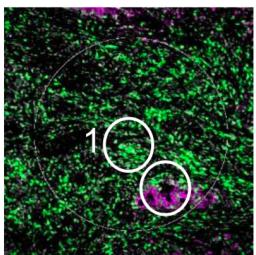
Quantitation of DNA

Meet at ZRC 3rd floor by elevator bank at 2 pm

Geometric



CD3 PanCK DNA

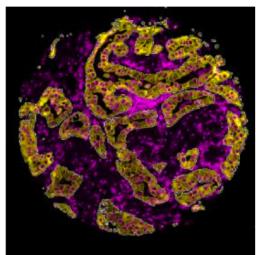


Assess heterogeneity
(e.g. tumor margin vs.
core, cortical layers)

Segmentation

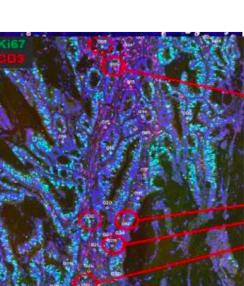
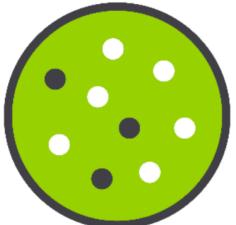


PanCK DNA



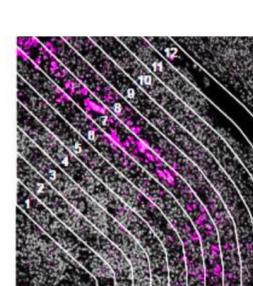
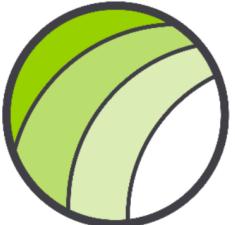
**Evaluate biological
compartments**
(e.g. Tumor-TME)?

Rare cell



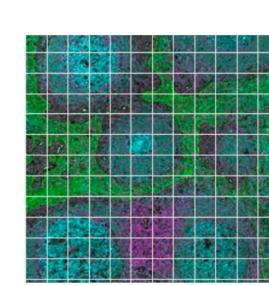
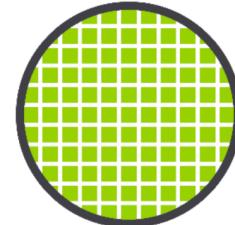
**Profile cell
populations**
(e.g. CD3+ cells,
GFAP+ cells)

Contour



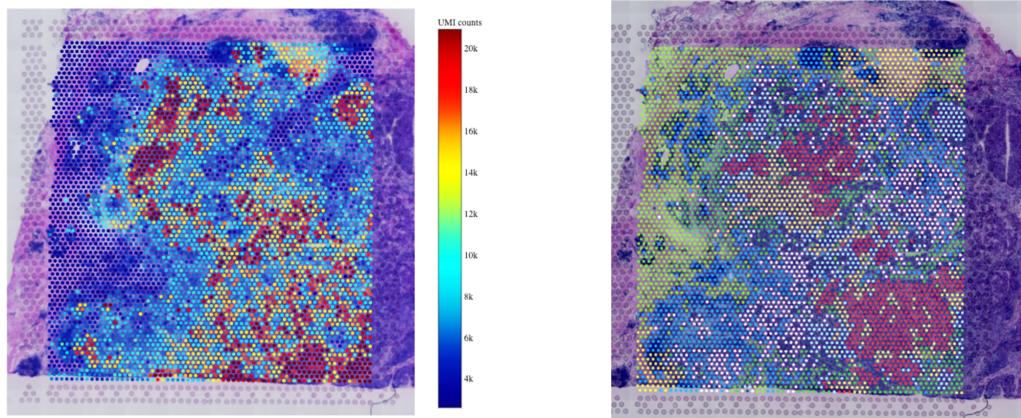
**Determine proximal
response**
(e.g. CART, AD plaque)

Gridded



**Perform deep
mapping**
(e.g. 96 ROI)

Breast tumor sample



Considerations

Frozen tissue only
55 um resolution (not single cell)
No capital equipment purchase