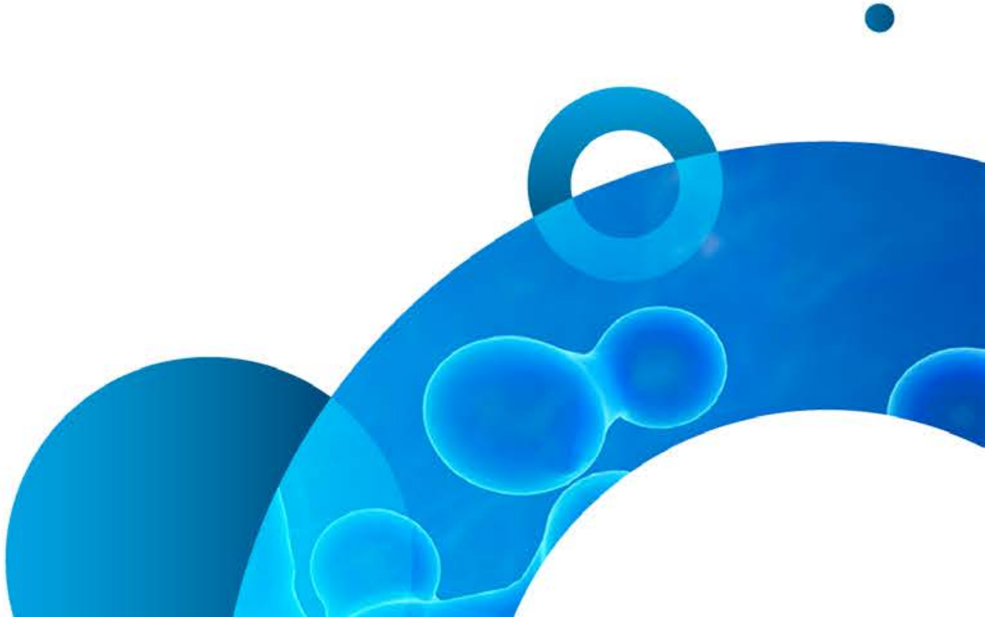
Latest Advances in 10x Genomics’ Single Cell and Visium Spatial Gene Expression Solutions



August 2020

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Today's speaker

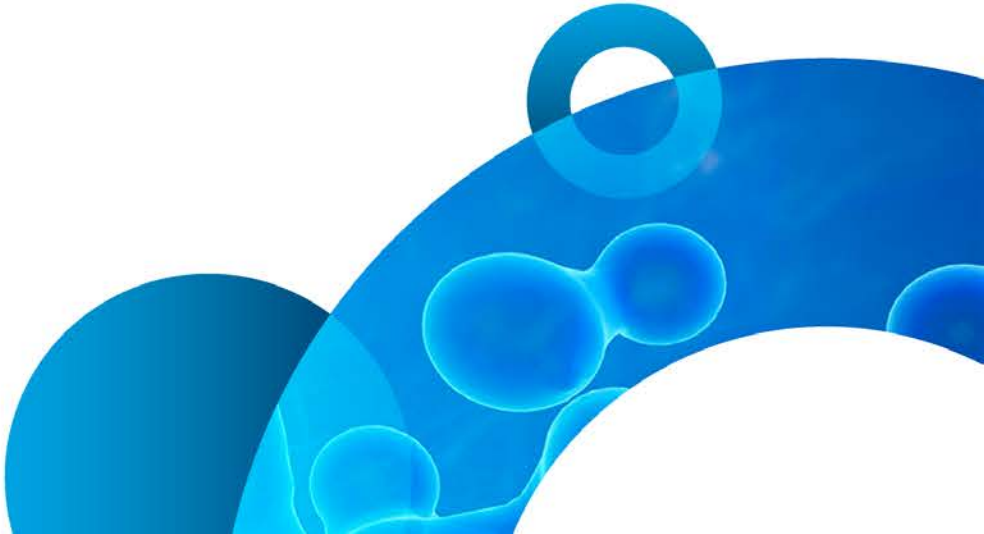
Leo Chan, PhD

Science & Technology Advisor, APAC Lead

10x Genomic

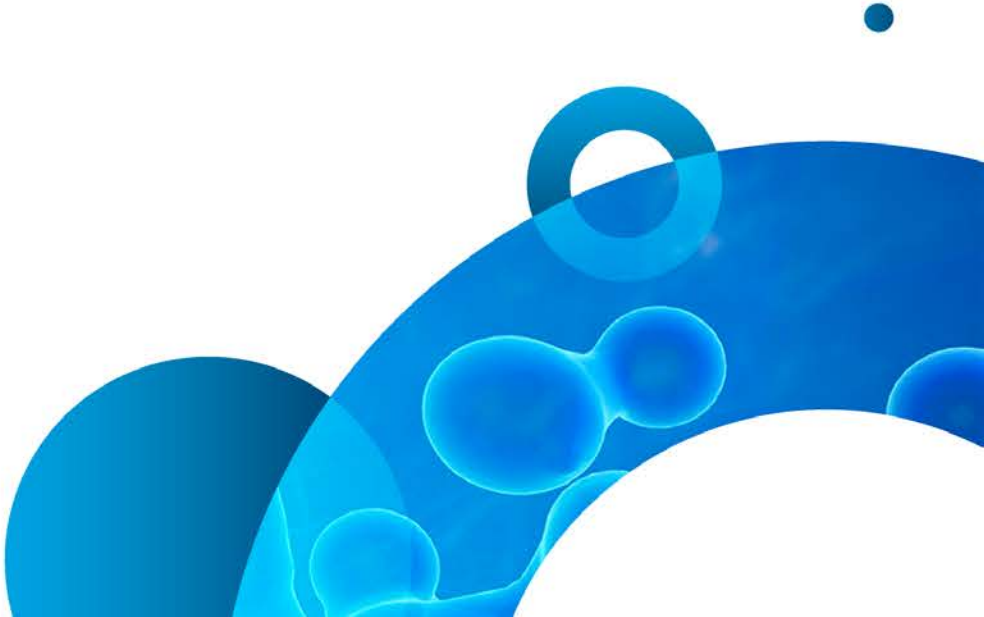
Latest Advances in 10x Genomics’ Single Cell and Visium Spatial Gene Expression Solutions

Leo Chan, PhD
Science & Technology Advisor, APAC Lead



August 2020

Chromium Single Cell Multiome ATAC + Gene Expression



Chromium Single Cell Multiome ATAC + Gene Expression

Multiply your power of discovery

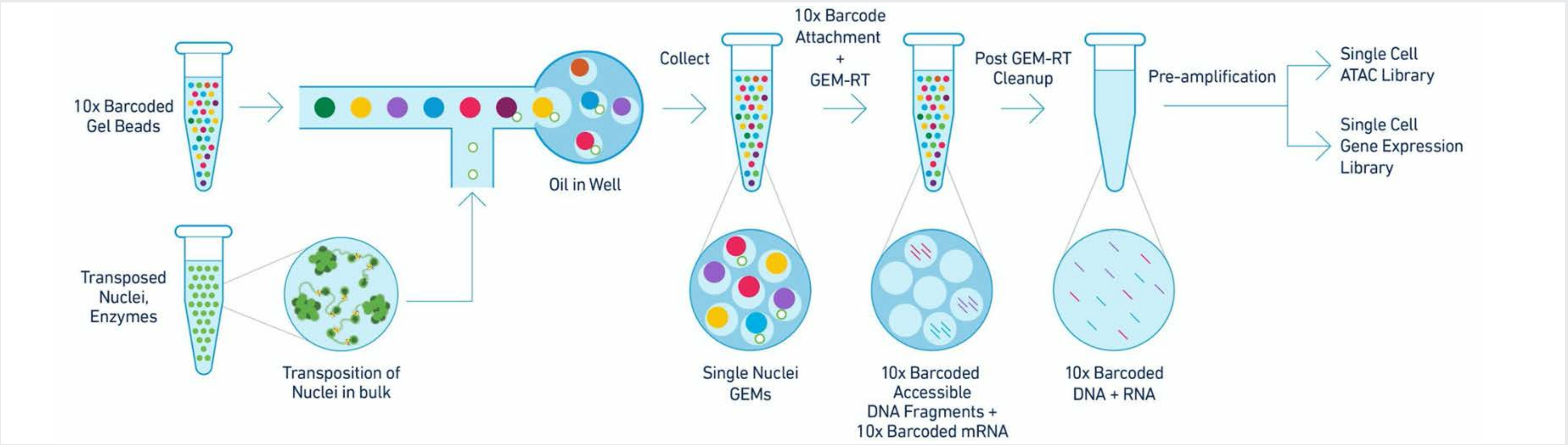
- Simultaneously profile gene expression and chromatin landscape from the same cell, across thousands of cells
- Deeply characterize cell types and states with linked transcriptomic and epigenomic analyses
- Discover new gene regulatory interactions
- Easily interpret epigenetic profiles with key expression markers
- Maximize precious samples with multiple readouts from the same cell

Chromium Single Cell Multiome ATAC + Gene Expression

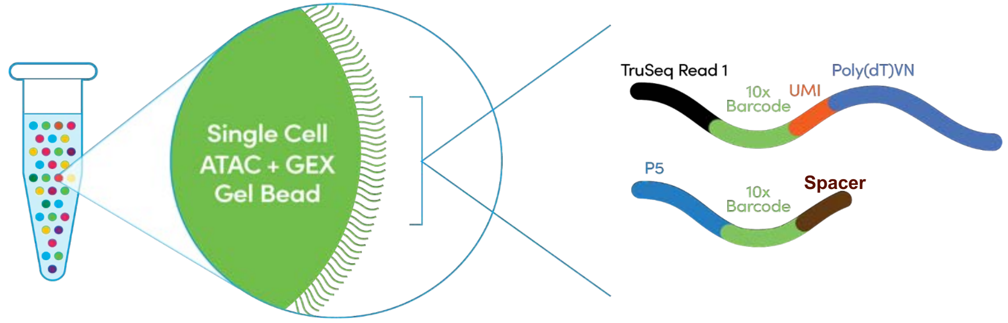
System features

- High sensitivity; similar performance to standalone single cell ATAC or gene expression assays performed on nuclei
- Efficiently partition 500-10,000 nuclei per channel, for up to 80,000 nuclei per run
- Recover up to 65% of loaded nuclei
- Low microfluidic multiplet rate (<1% per 1000 nuclei)
- Demonstrated with cell lines, primary cell, cryopreserved samples, fresh and flash-frozen tissue
- Easy-to-use software for data analysis and visualization

How it works



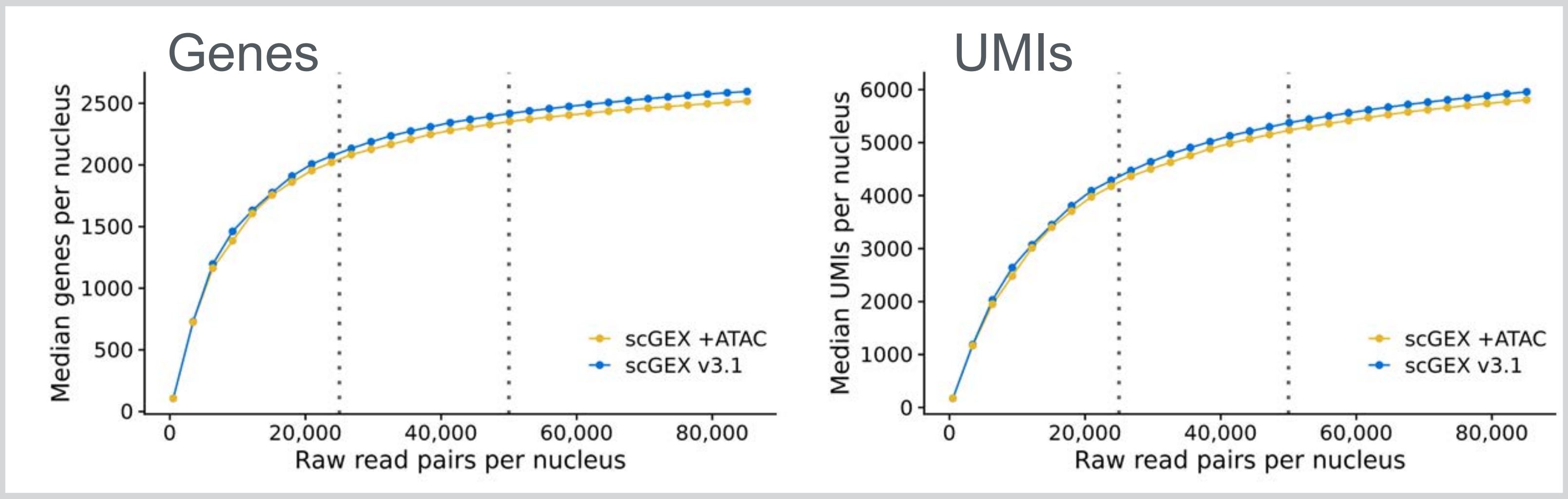
Multiome ATAC + GEX Gel Beads



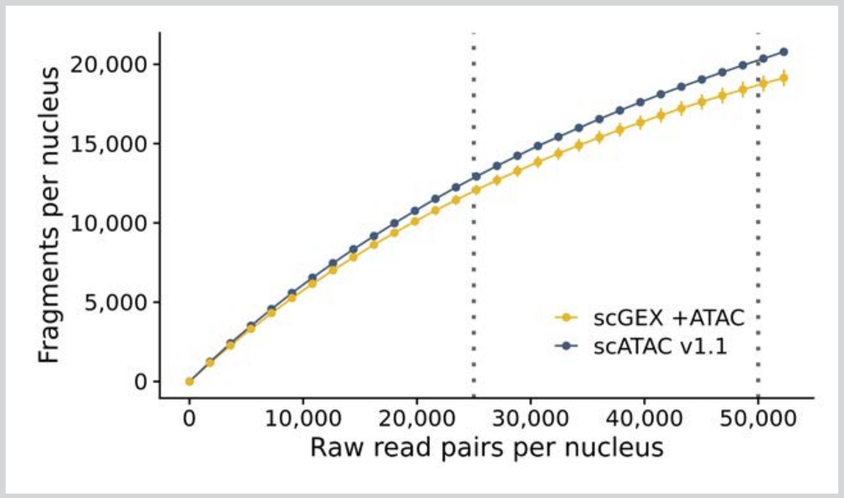
Redesigned gel beads combine oligos for capturing 3’ ends of mRNA & ATAC fragments

Profile gene expression and ATAC modalities at high sensitivity from nuclei

Gene Expression

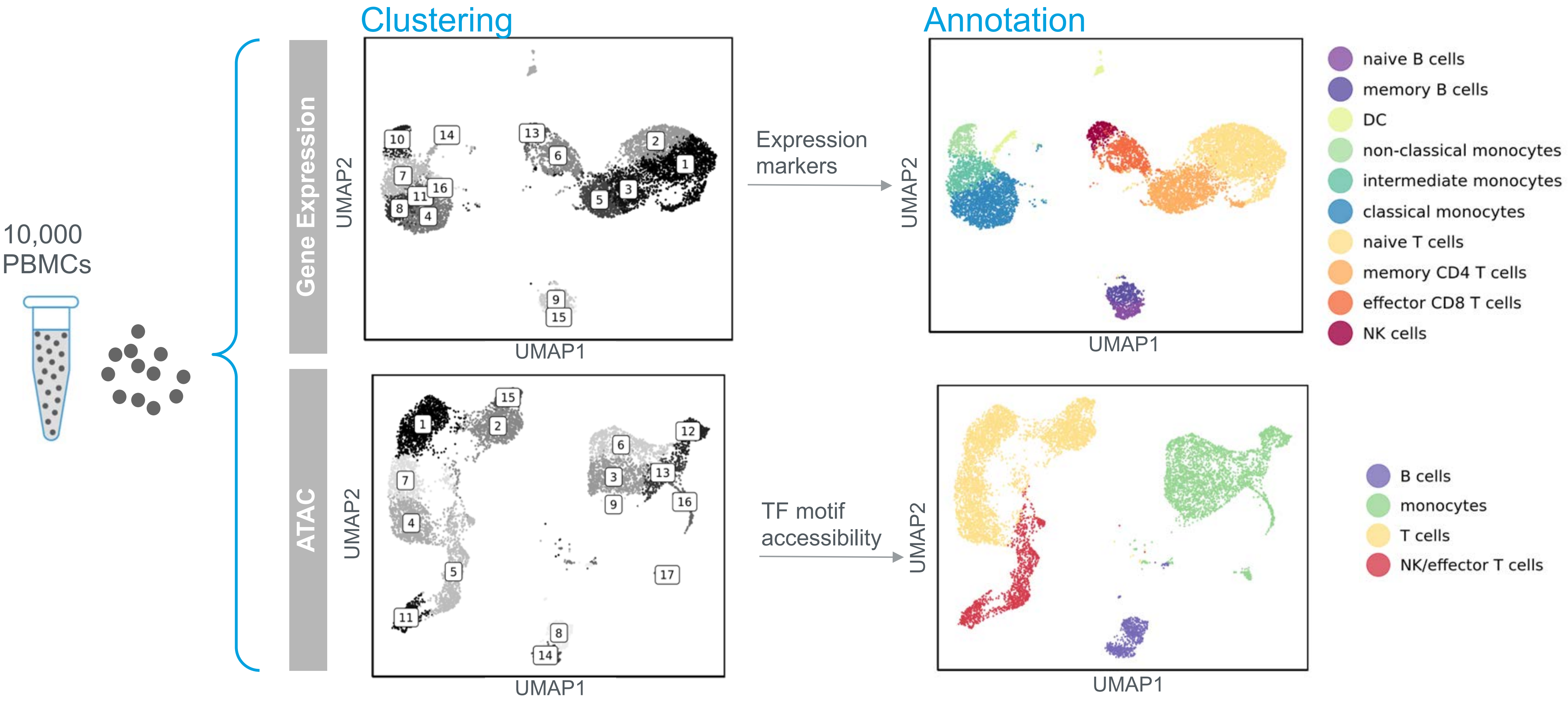


ATAC



Mouse embryonic
E18.5 brain nuclei

Simultaneously detect gene expression and ATAC profiles from single cells



10,000 PBMCs

Gene Expression

ATAC

Clustering

Annotation

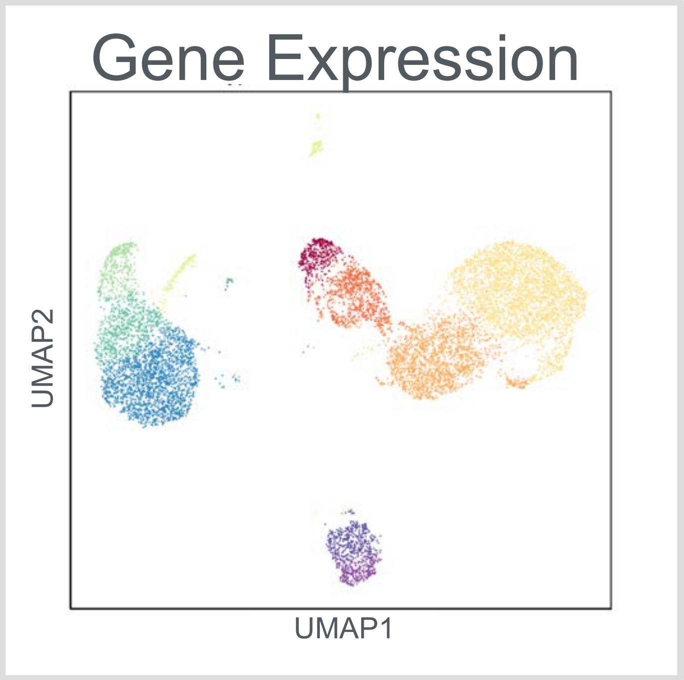
Expression markers

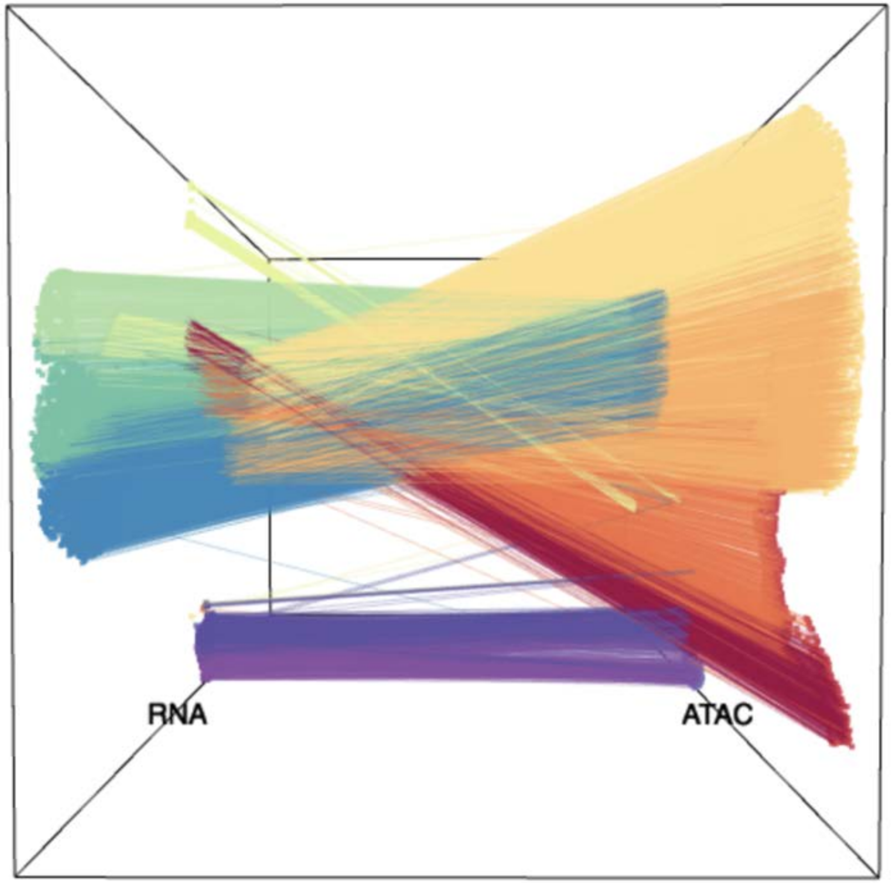
TF motif accessibility

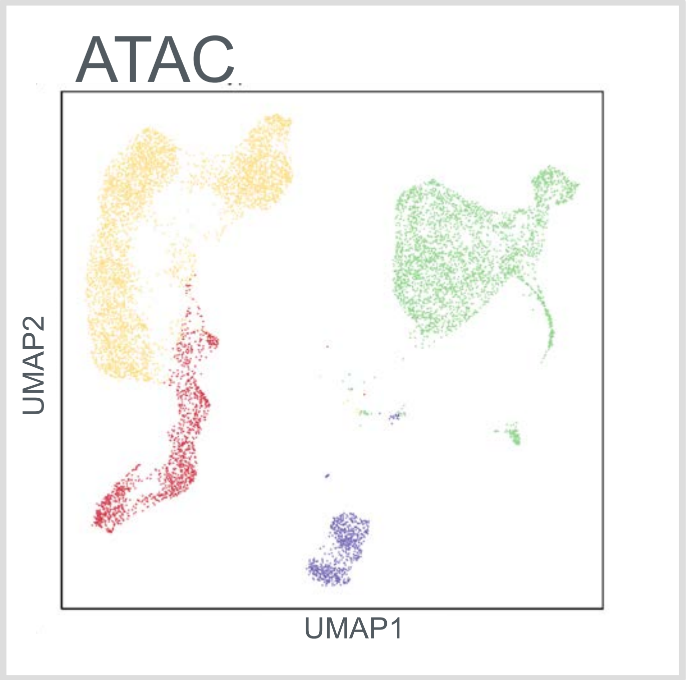
- ● naive B cells
- ● memory B cells
- ● DC
- ● non-classical monocytes
- ● intermediate monocytes
- ● classical monocytes
- ● naive T cells
- ● memory CD4 T cells
- ● effector CD8 T cells
- ● NK cells

- ● B cells
- ● monocytes
- ● T cells
- ● NK/effector T cells

Directly link gene expression and ATAC modalities, cell by cell







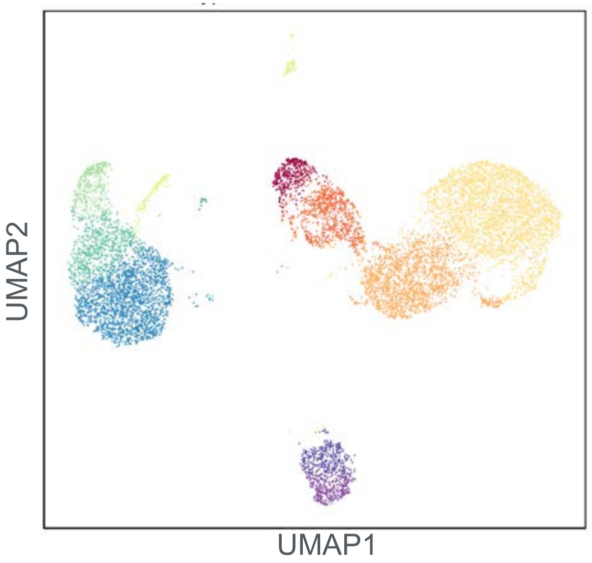
10,000 PBMCs

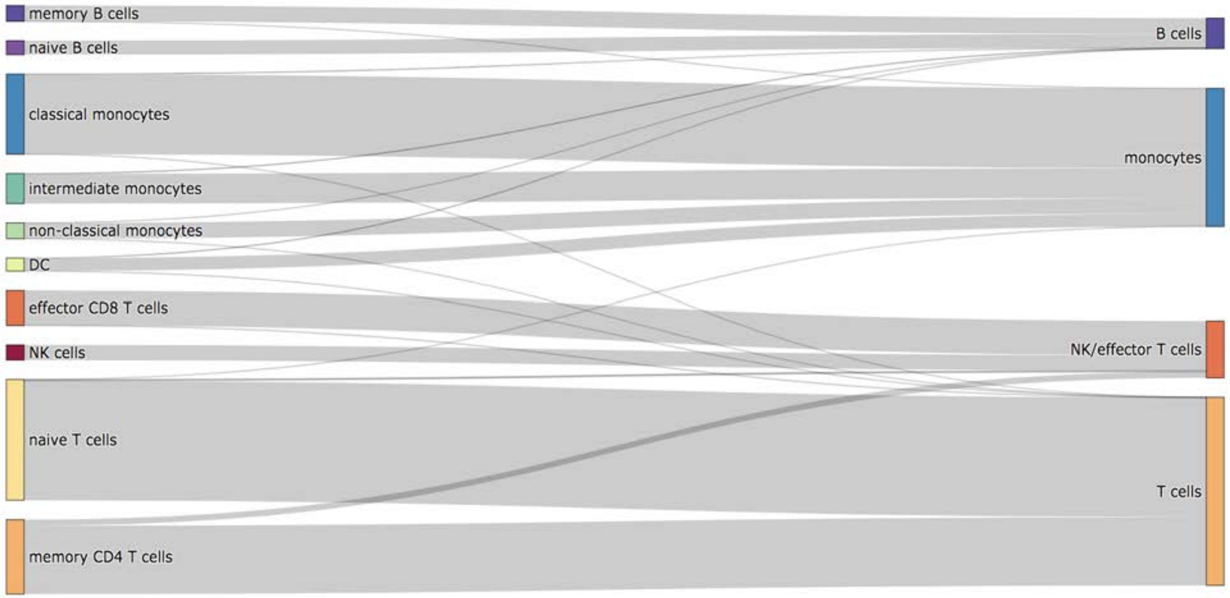
Inference-free linkage of the transcriptome and epigenome across thousands of cells

10x GENOMICS

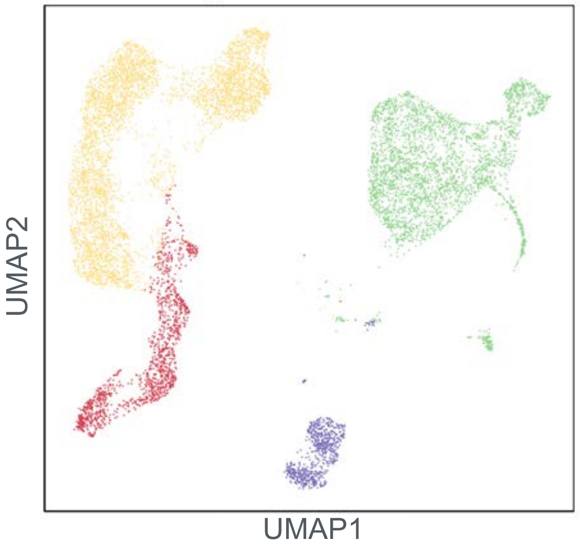
Gene expression and ATAC capture consistent and biologically relevant cellular populations

GEX-defined clusters





ATAC-defined clusters



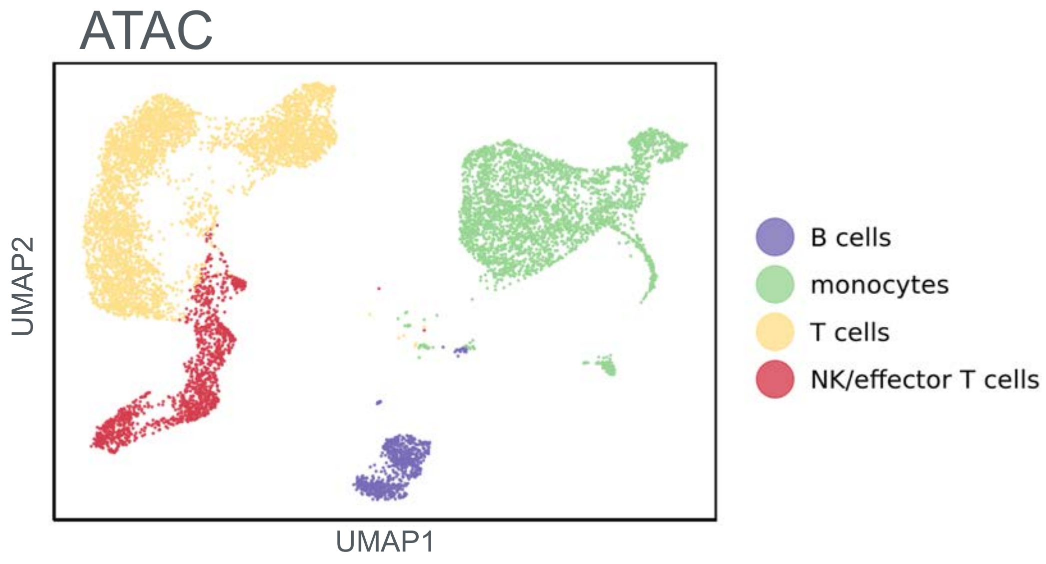
98% concordance of cell annotations

10,000 PBMCs

Refine your annotation of ATAC cell populations

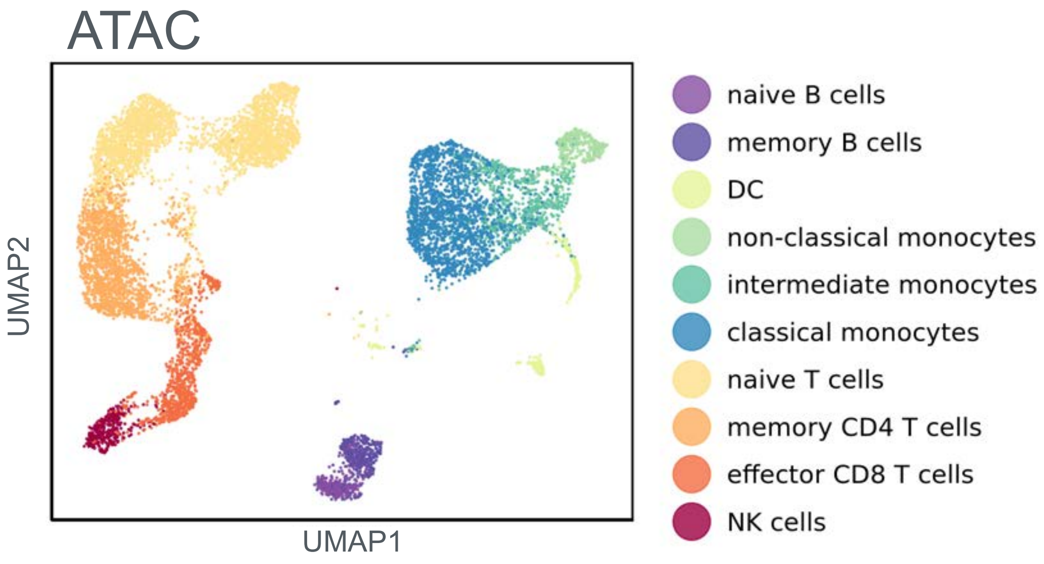
Transfer gene expression marker-derived annotation into ATAC populations

Open chromatin annotation using TF accessibility



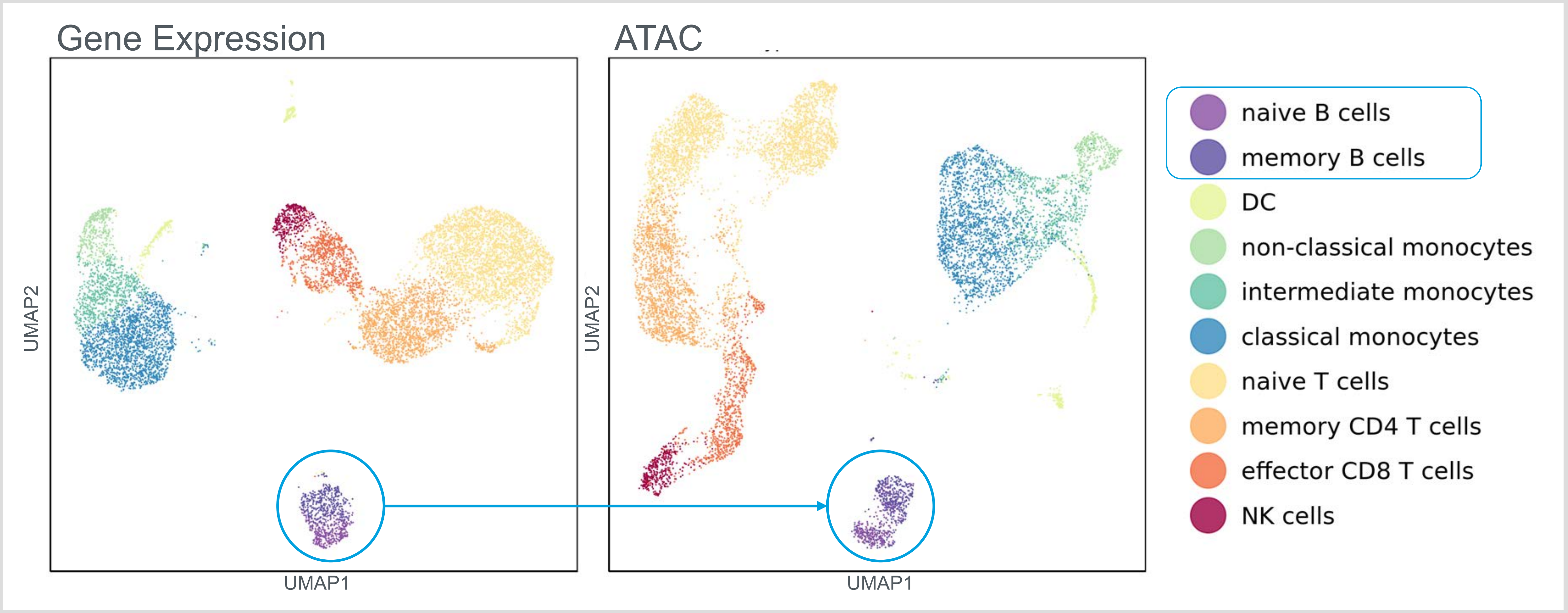
→

Open chromatin annotation using gene expression markers

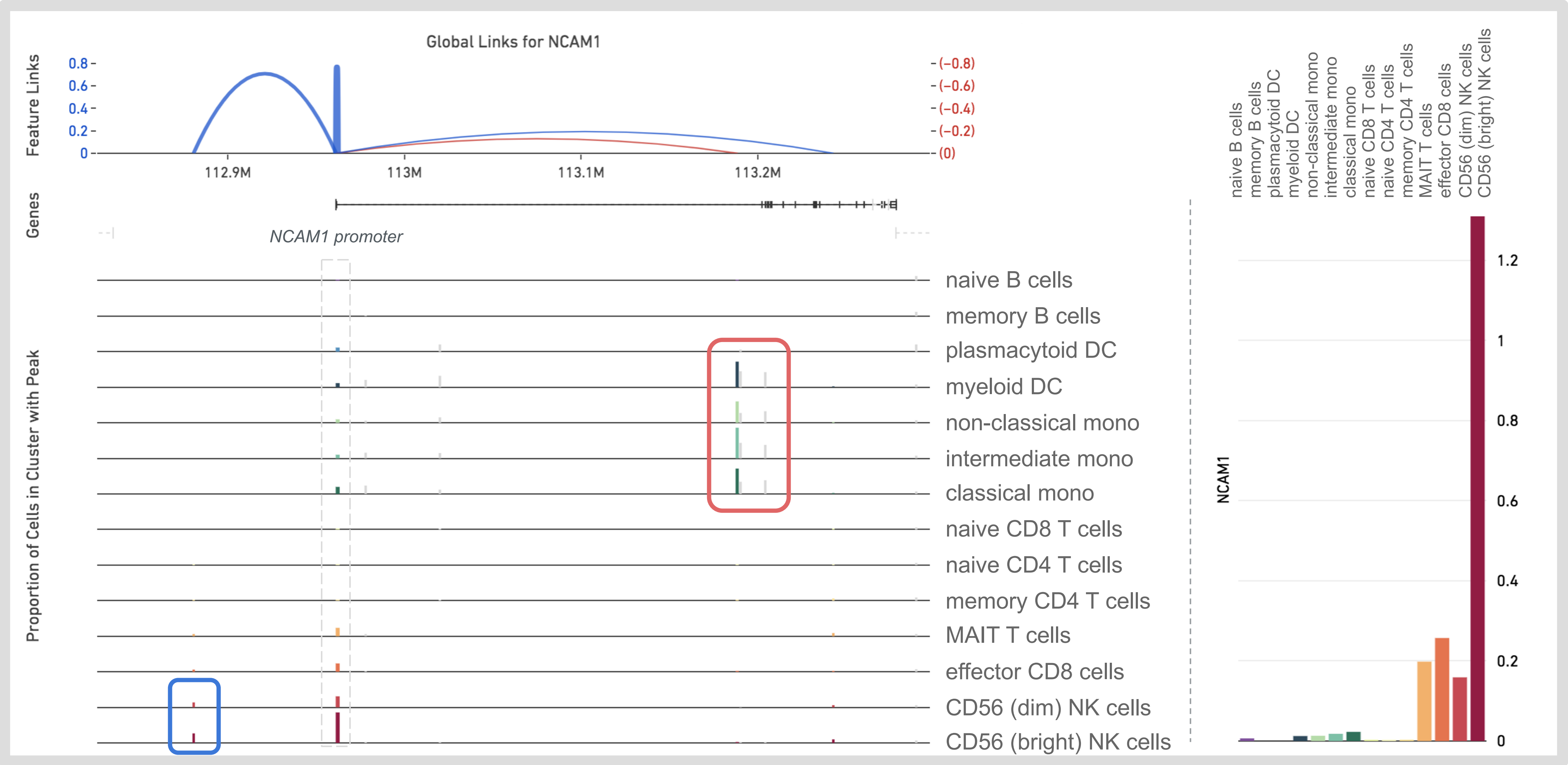


10,000 PBMCs

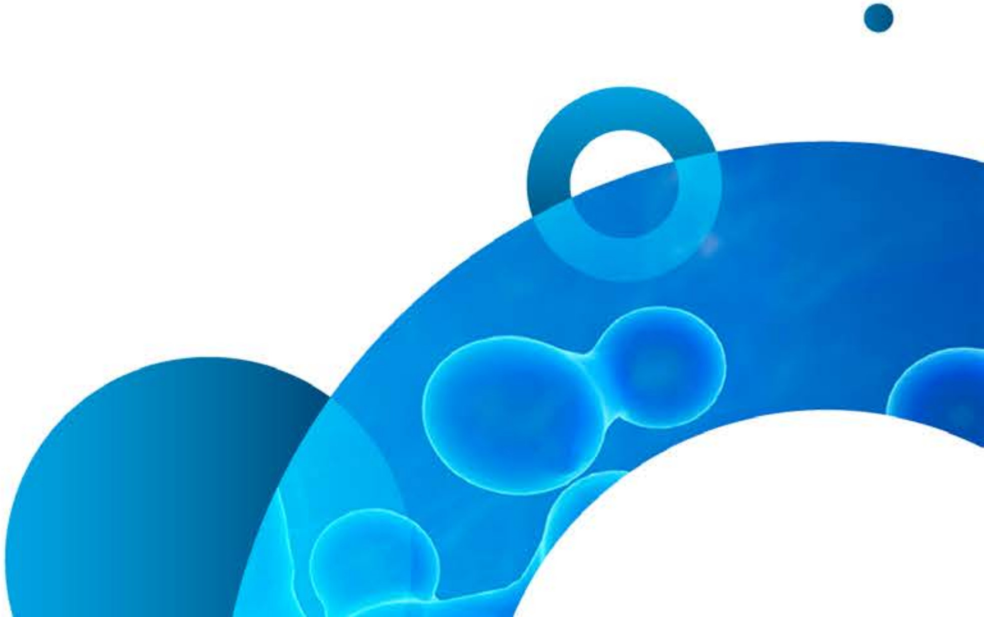
Better separate PBMC populations on ATAC space



Identify putative regulatory elements linked to a gene of interest



High Resolution Characterization of the Immune System with Single Cell Immune Profiling v2

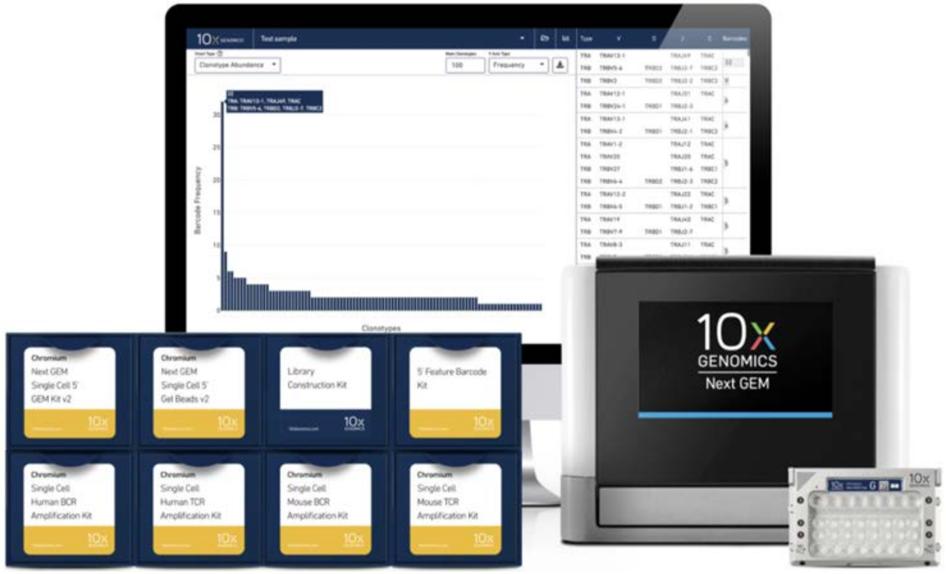


Introducing Single Cell Immune Profiling v2

V(D)J
Higher detection rate

Gene Expression
Higher sensitivity

Library preparation
Dual Indexed library

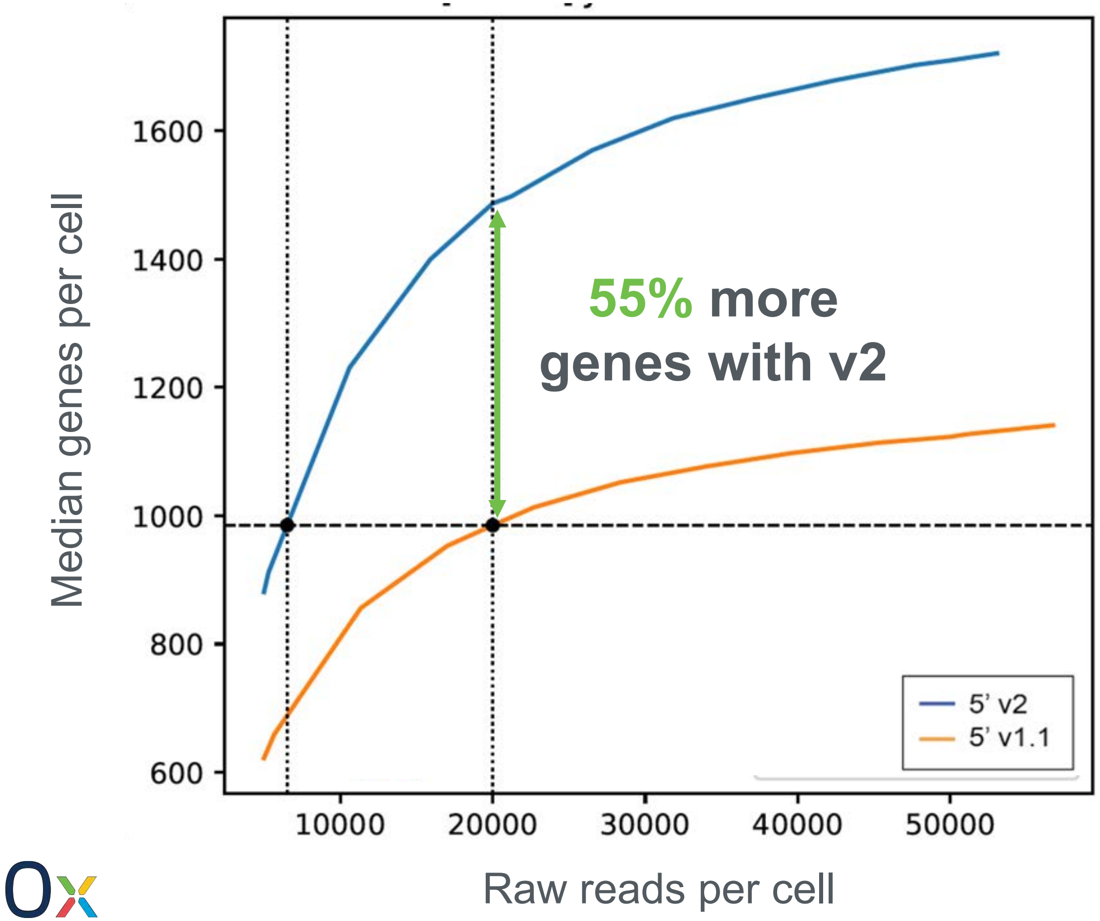


Targeted Gene Expression
Higher on-target reads

Cell Ranger 4.0
Faster turnaround time

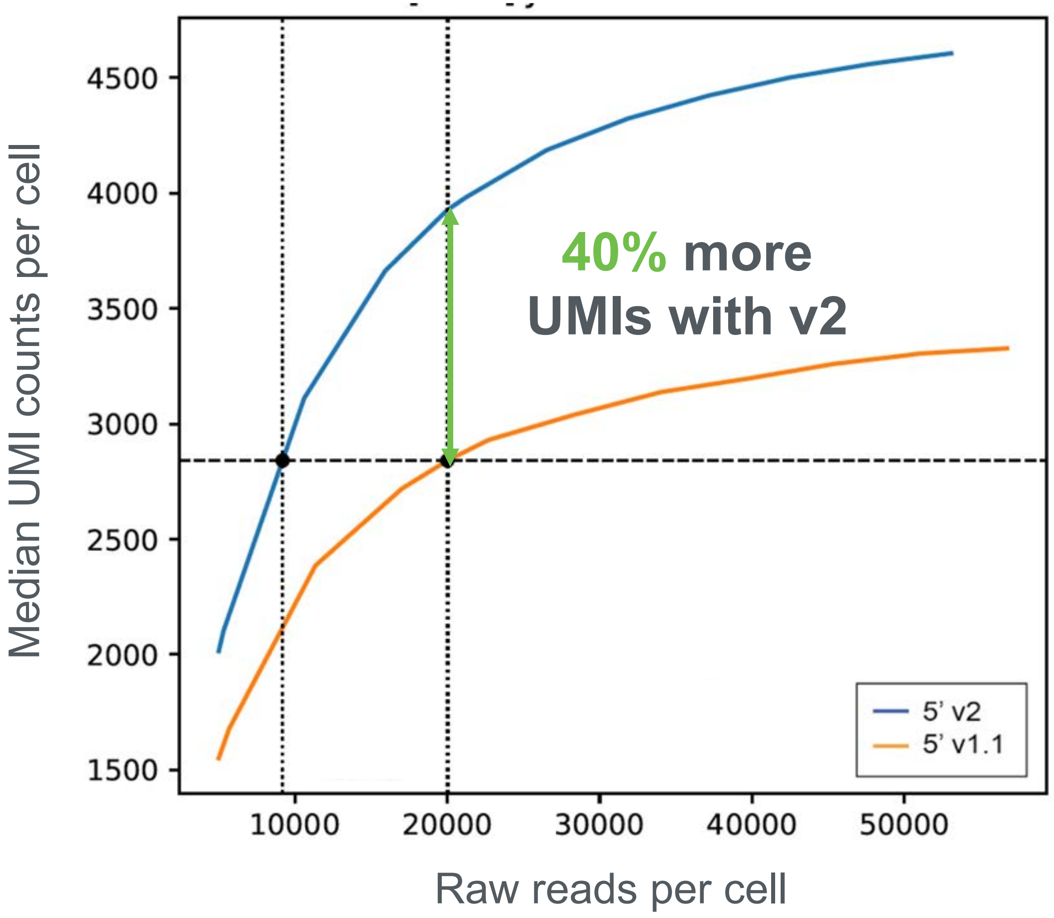
Huge gains in gene expression sensitivity in v2

1,000 Human PBMCs



Median genes per cell

55% more genes with v2



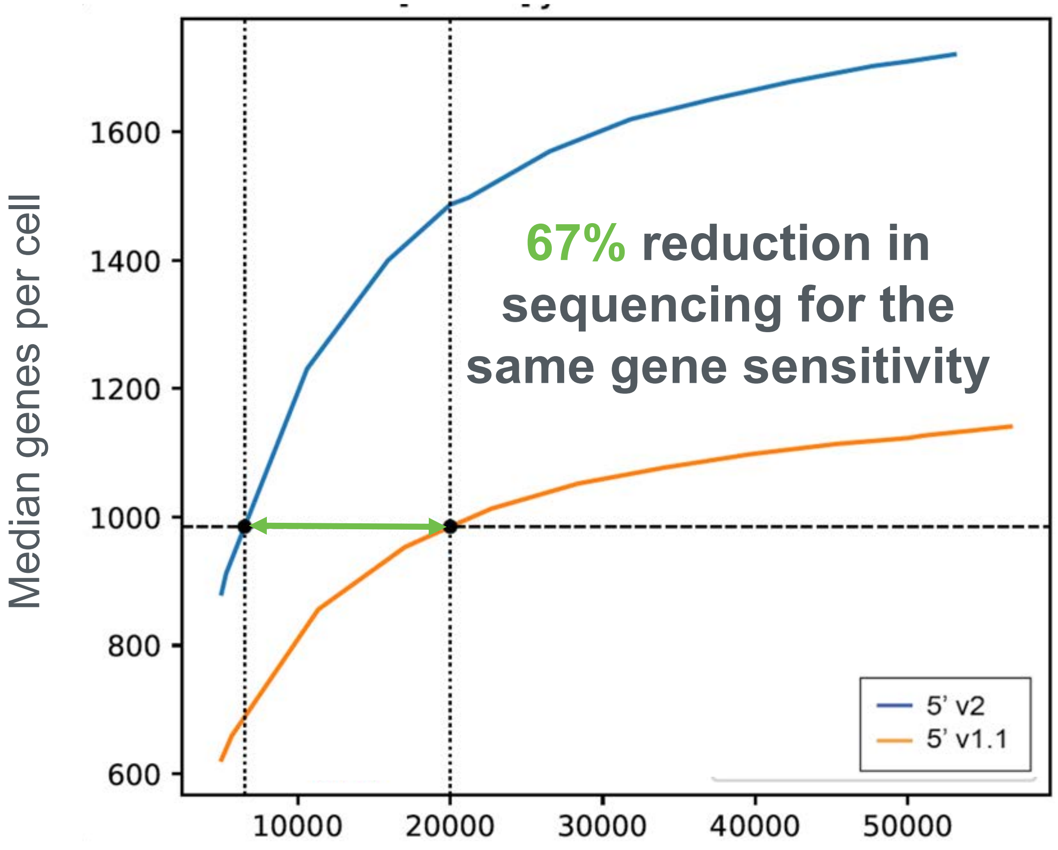
Median UMIs per cell

40% more UMIs with v2

...allows for reduced sequencing depths

1,000 Human PBMCs

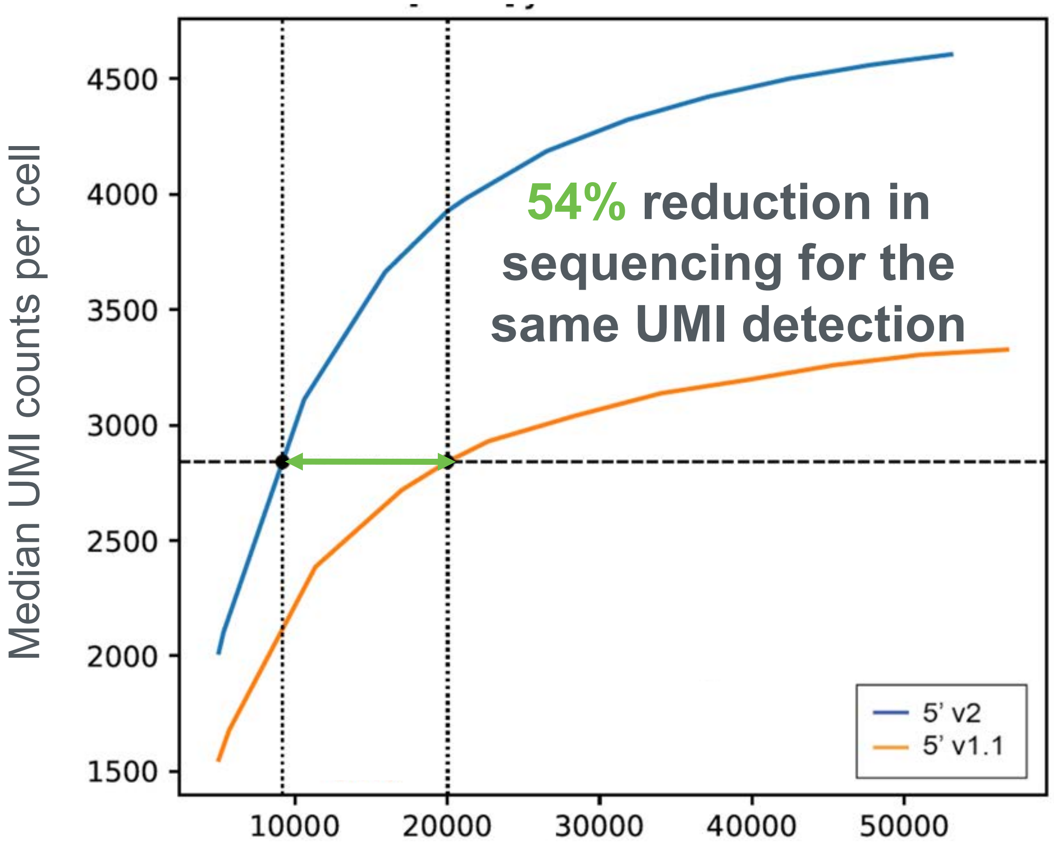
Median genes per cell



67% reduction in sequencing for the same gene sensitivity

Raw reads per cell

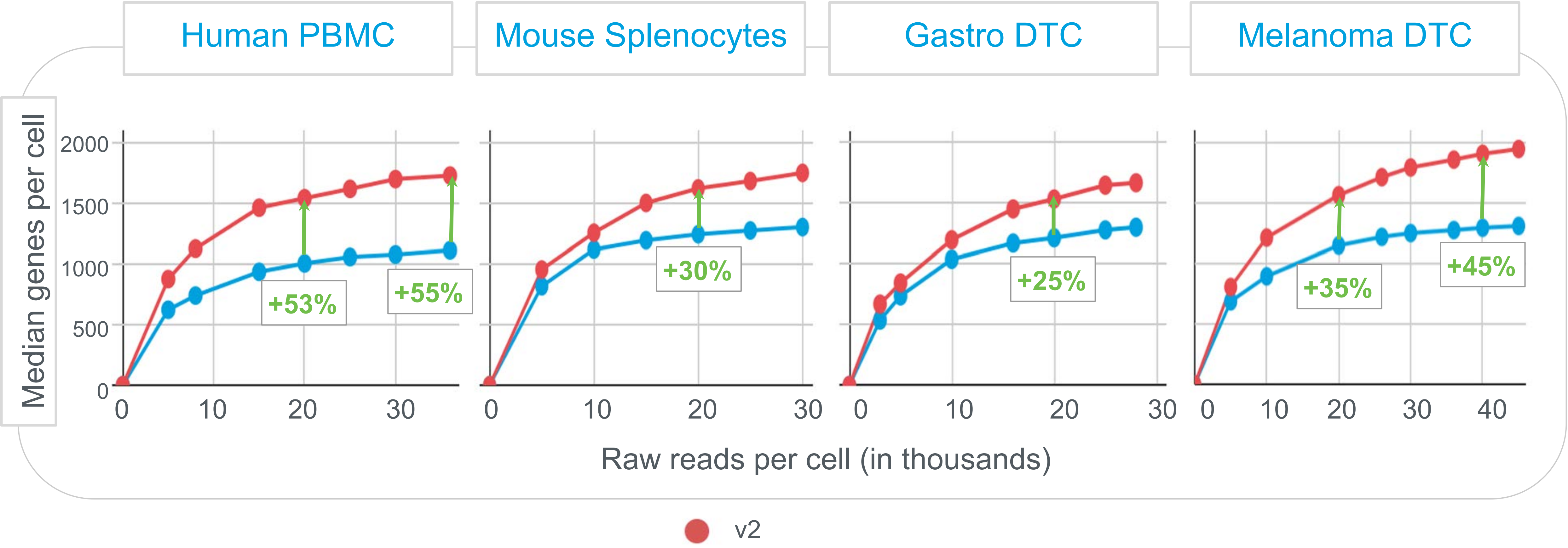
Median UMIs per cell



54% reduction in sequencing for the same UMI detection

Raw reads per cell

Sensitivity improvements are seen across sample types

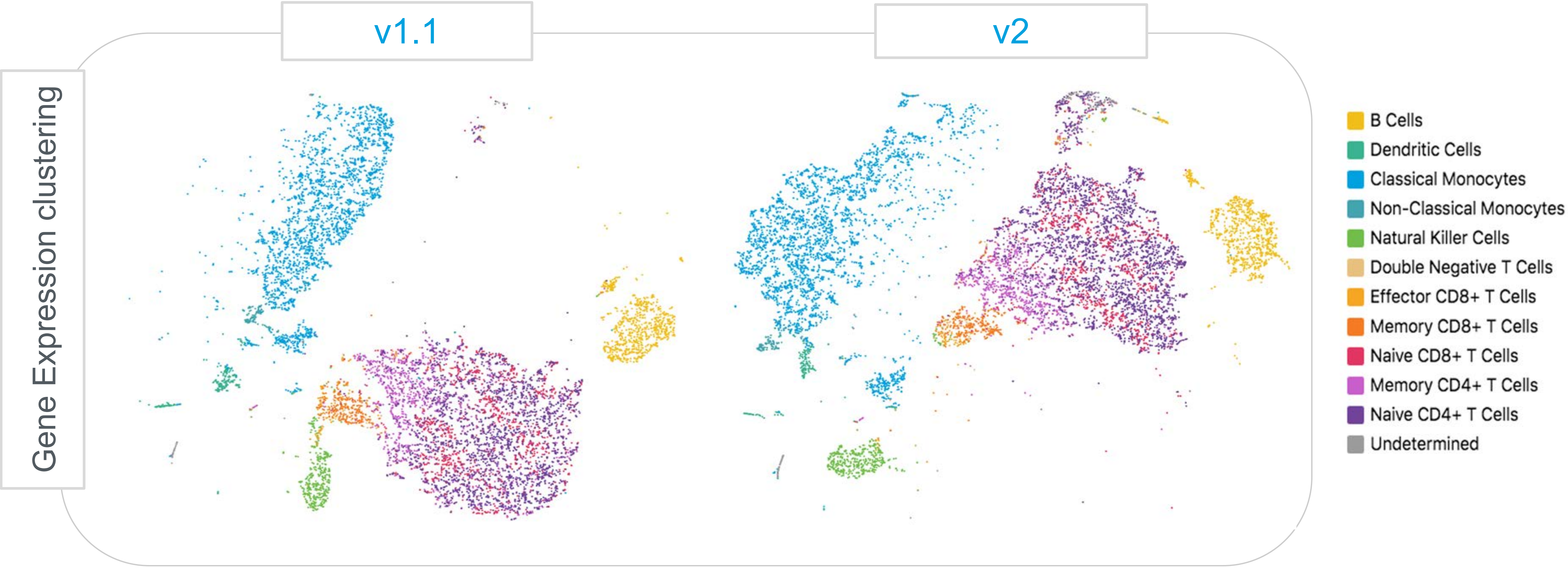


● v2

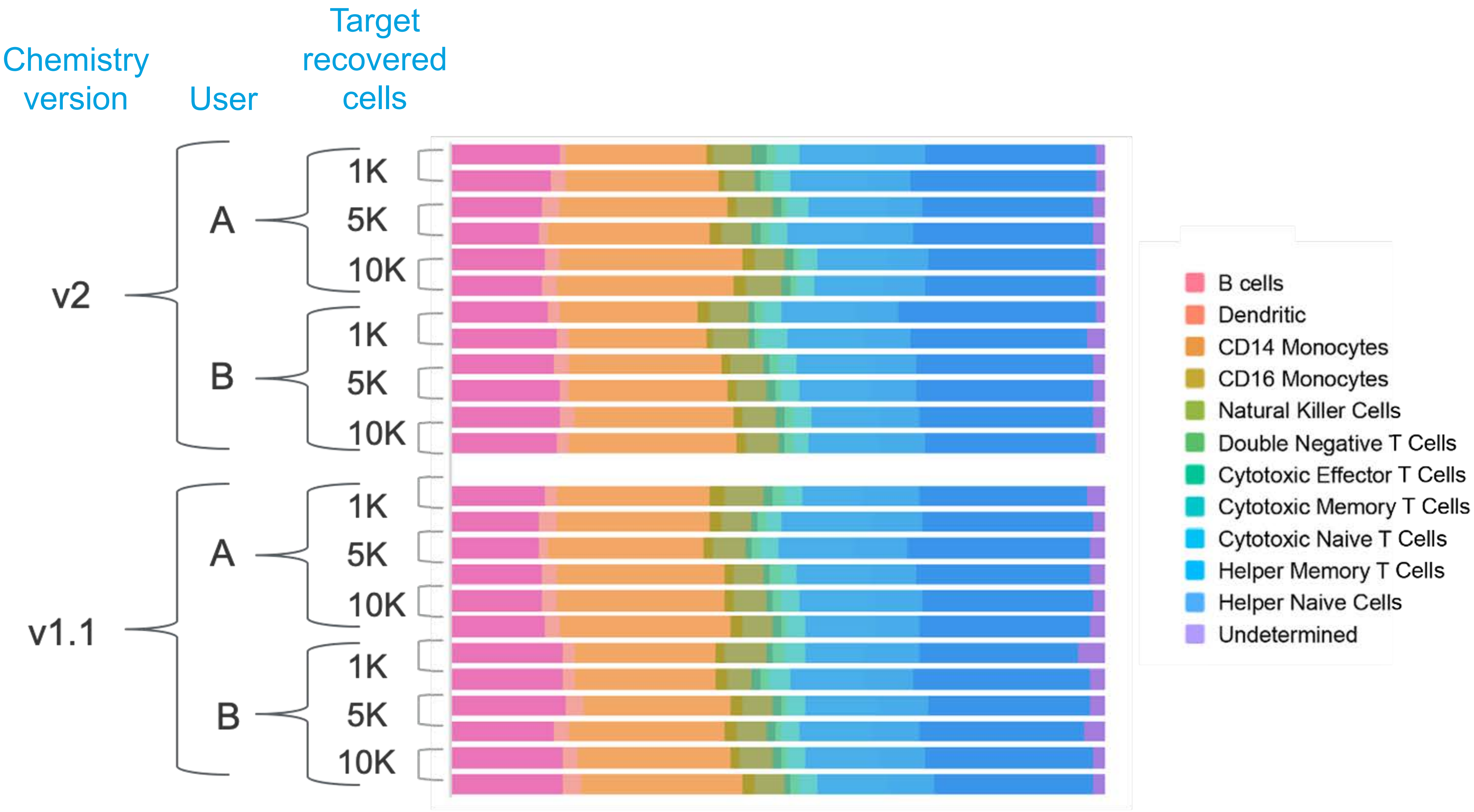
● v1.1

Gene expression based cell type classification is comparable between v1.1 and v2

10,000 Human PBMCs

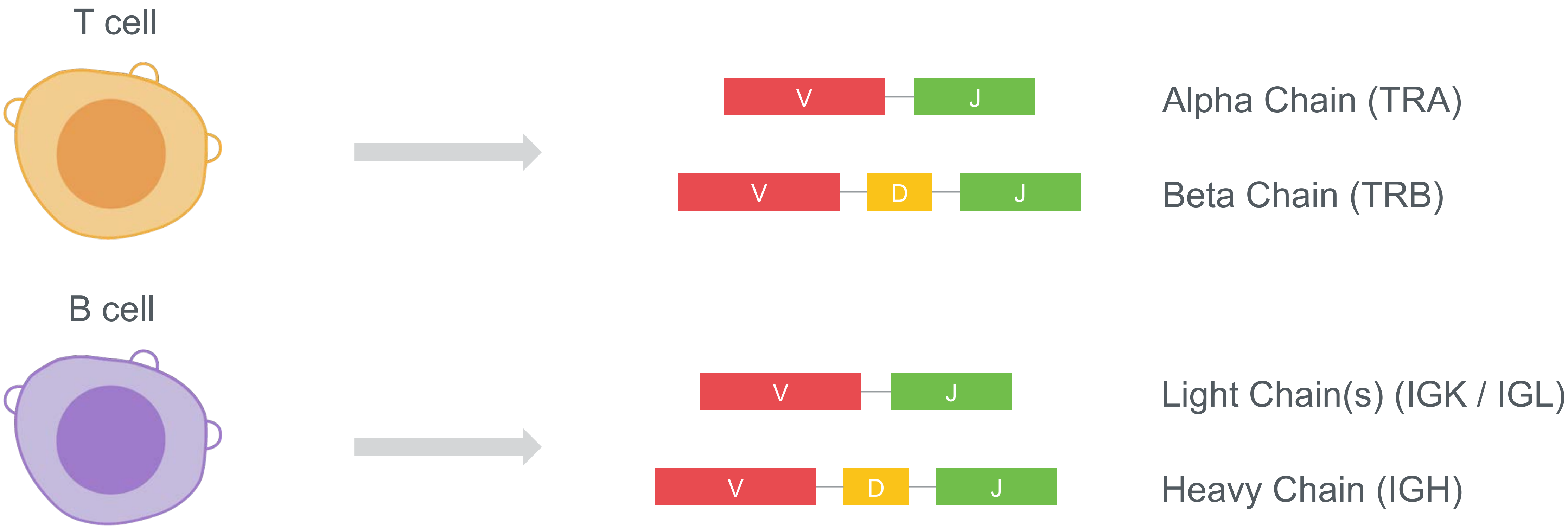


Cell type detection is robust across a range of conditions



Using the Single Cell Immune Profiling v2 assay to examine receptor repertoires

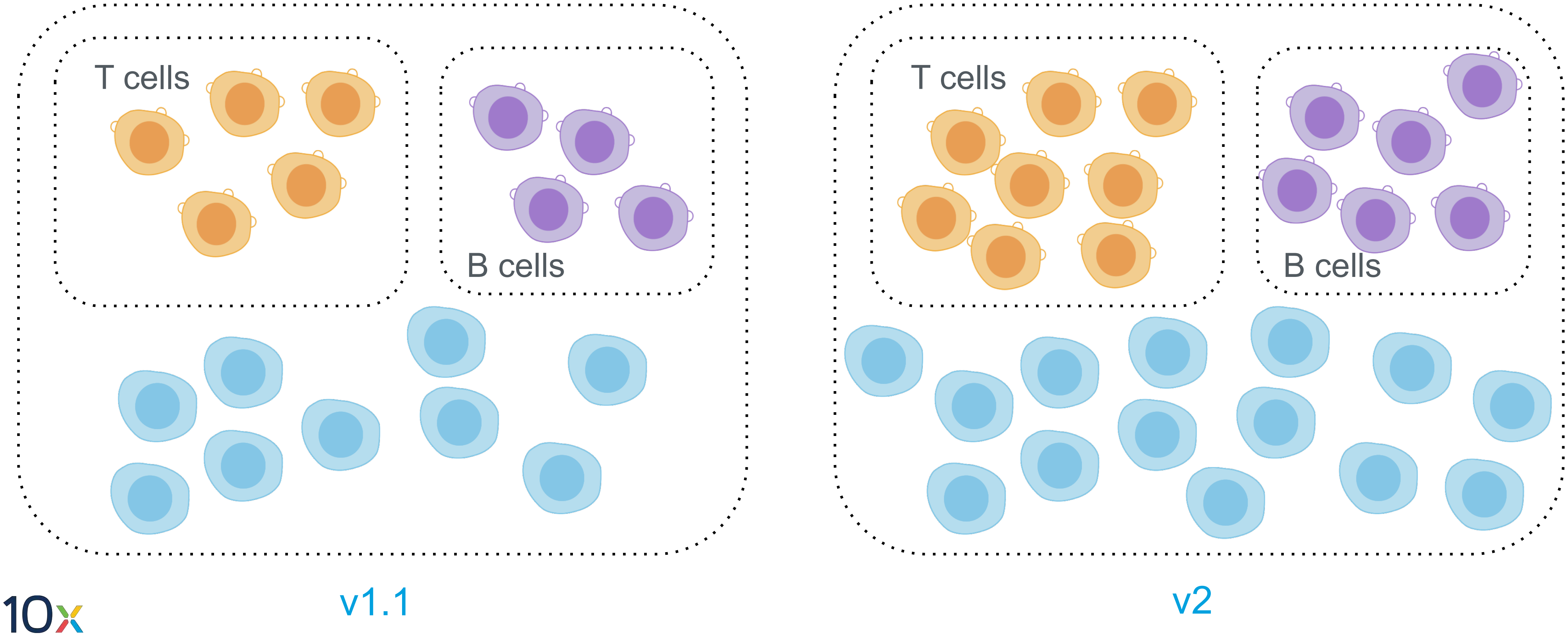
Capture paired full length, sequences from T / B cell receptor transcripts



% paired cells = no. cells with a pair / total number of cells

A typical sample contains a mixture of cell types

v2 sensitivity increases improves detection of ALL cell types



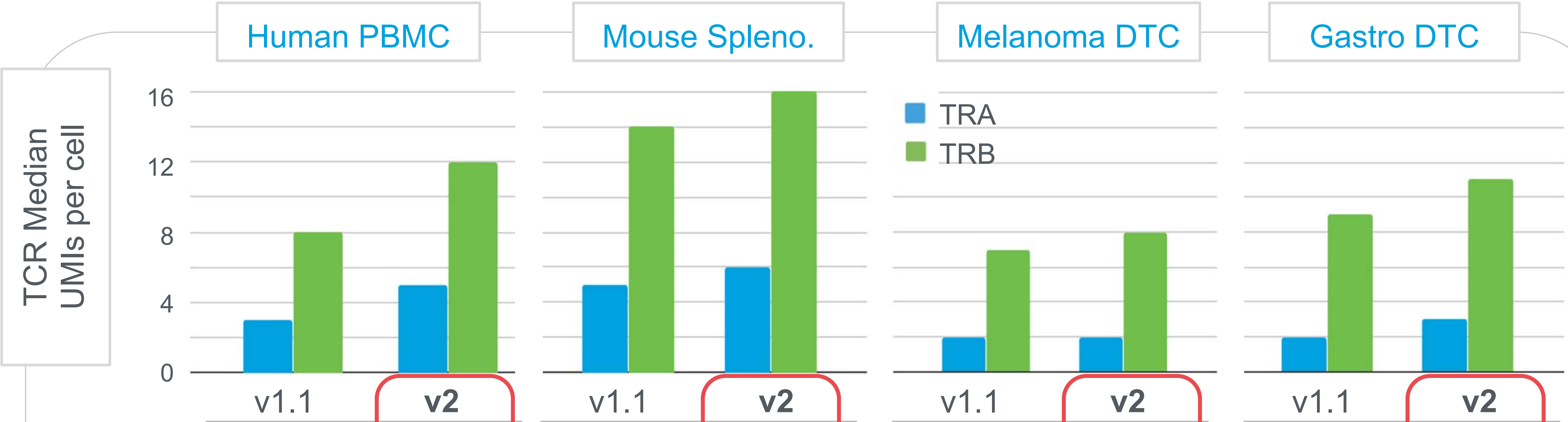
Sensitivity gains result in improved TCR detection

...even in the most challenging samples

	Human PBMC v1.1	Human PBMC v2	Mouse Spleno. v1.1	Mouse Spleno. v2	Melanoma DTC v1.1	Melanoma DTC v2	Gastro DTC v1.1	Gastro DTC v2
Pairing Rate	83.5%	86.3%	81.8%	87.5%	68.3%	70.8%	61.5%	62.0%
No. of cells	339	416	121	175	1,064	1,462	919	1,141

Sensitivity gains result in improved TCR detection

Many more cells with productive TCR pairs are identified using v2

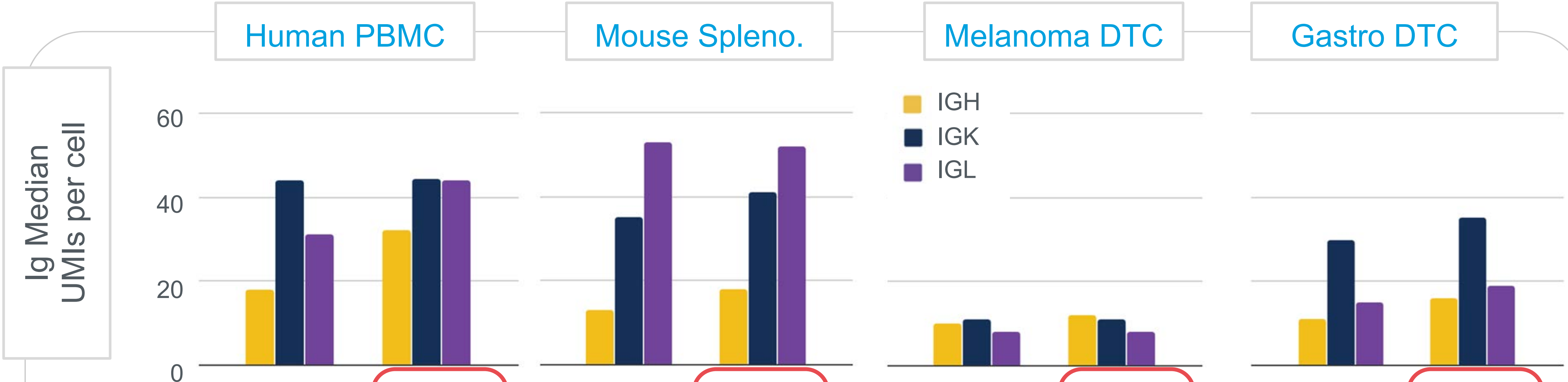


	Human PBMC		Mouse Spleno.		Melanoma DTC		Gastro DTC	
	v1.1	**v2**	v1.1	**v2**	v1.1	**v2**	v1.1	**v2**
Pairing Rate	83.5%	**86.3%**	81.8%	**87.5%**	68.3%	**70.8%**	61.5%	**62.0%**
No. of cells*	339	**416**	121	**175**	1,064	**1,462**	919	**1,141**

*Raw number. of cells with a productive V-J spanning pair

Sensitivity gains result in improved Ig detection

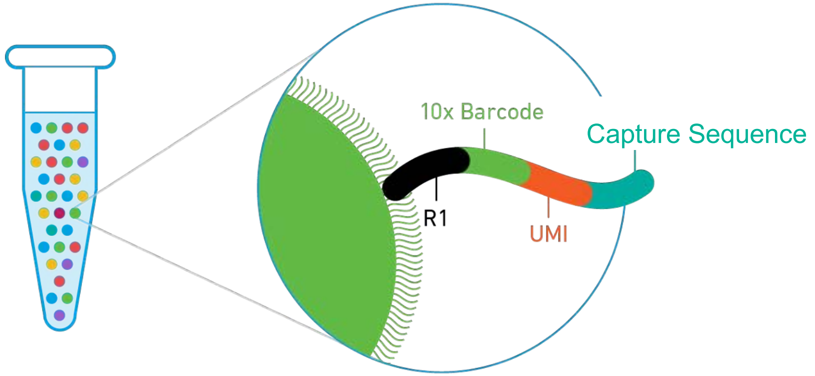
Many more cells with productive Ig pairs are identified using v2



	Human PBMC		Mouse Spleno.		Melanoma DTC		Gastro DTC	
	v1.1	**v2**	v1.1	**v2**	v1.1	**v2**	v1.1	**v2**
Pairing Rate	96.7%	**97.6%**	84.1%	**91.2%**	92.8%	**93.8%**	90.7%	**90.8%**
No. of cells	58	**81**	589	**703**	3,903	**5,293**	4,268	**5,038**

Exploring cell surface protein expression and antigen specificity with Feature Barcode technology

Gene expression, adaptive immune receptors, cell surface epitopes, and antigen specificity from the same single cells

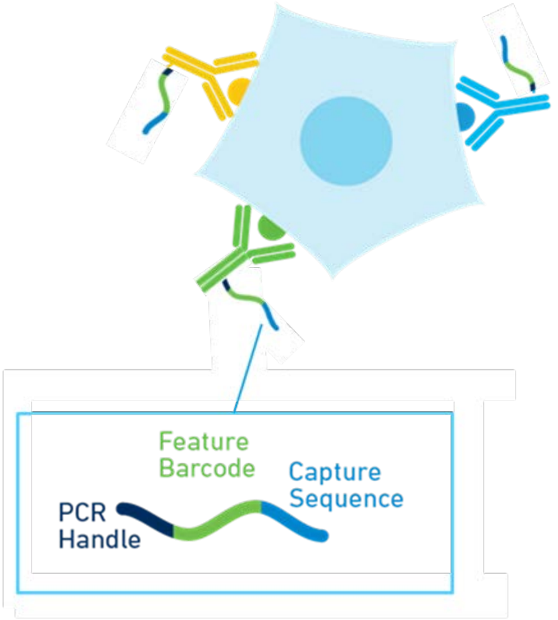


10x Barcode

Capture Sequence

R1

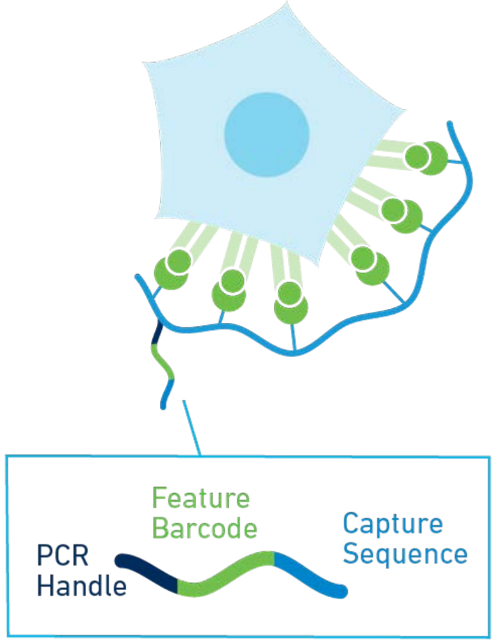
UMI



Feature Barcode

Capture Sequence

PCR Handle



Feature Barcode

Capture Sequence

PCR Handle

Compatible PRODUCTS | 10x GENOMICS

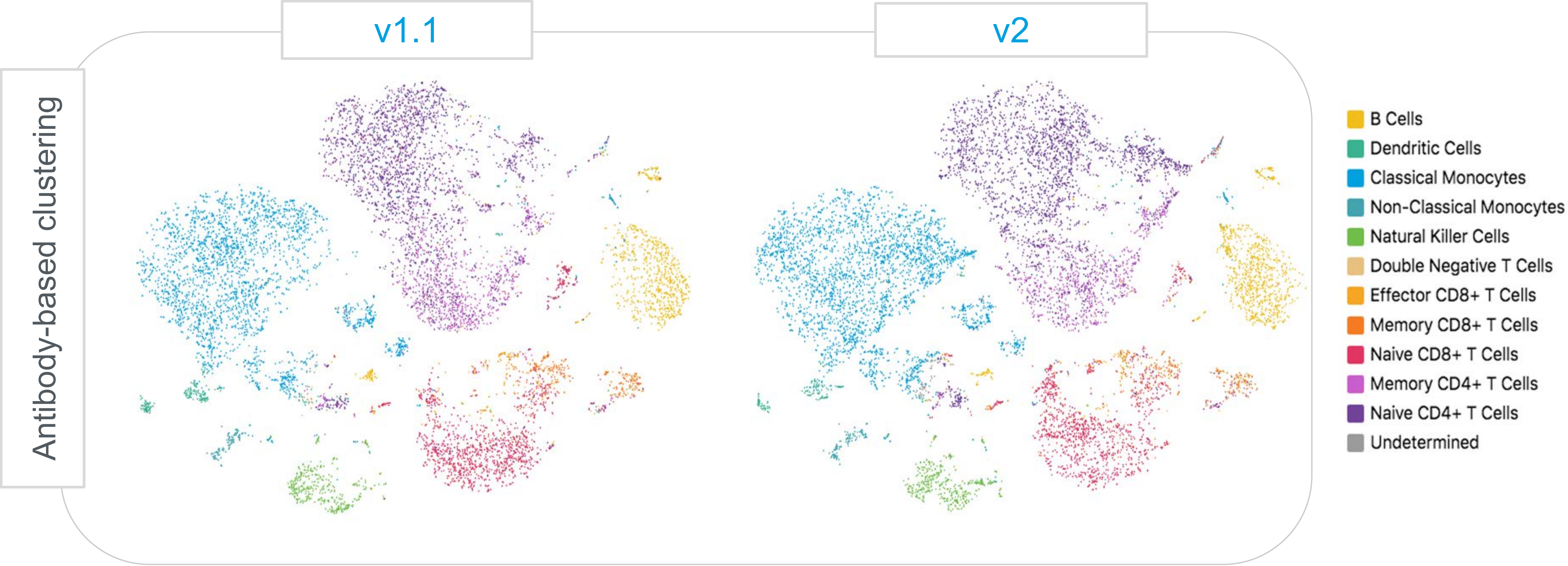
BioLegend®
Enabling Legendary Discovery™

IMMUDEX

10x GENOMICS

Cell surface protein based cell type classification is comparable between v1.1 and v2

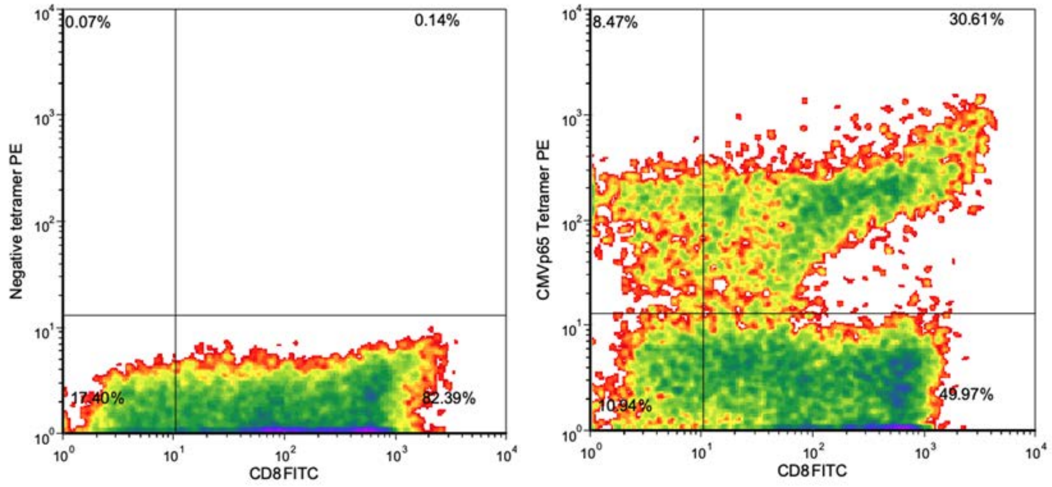
10,000 Human PBMCs



Examining antigen specificity with v2

Anti-CMV T cells

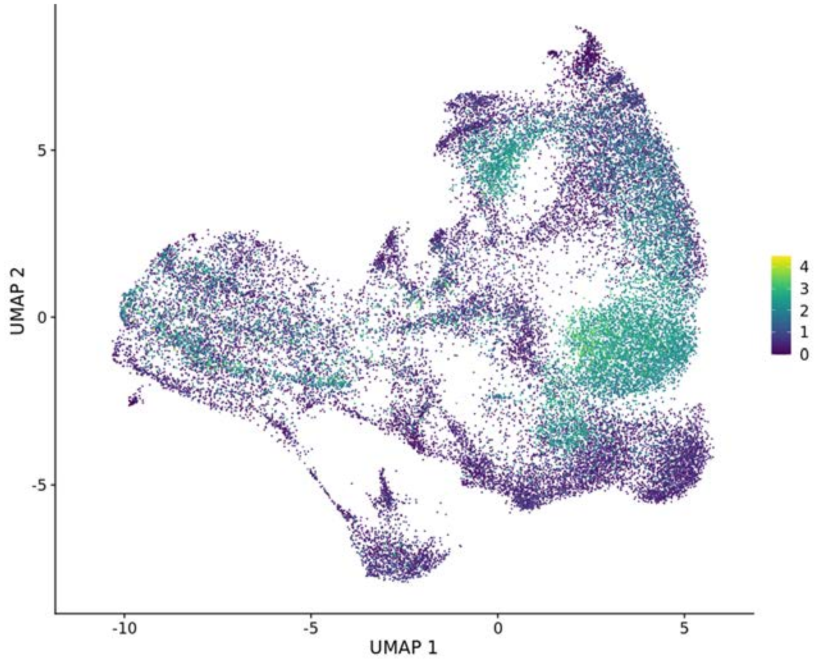
Tetramer staining for CMV+ cells by flow cytometry (from vendor)



Neg tetramer PE = HLA-A*0201-PE iTag MHC Tetramer (MBL International, Woburn, MA); CMV A2 tetramer = CMV pp65 peptide (NLVPMVATV)/ HLA-A*0201-PE iTag MHC Tetramer (MBL International, Woburn, MA)

30.6% of cells are CMVpp65 positive

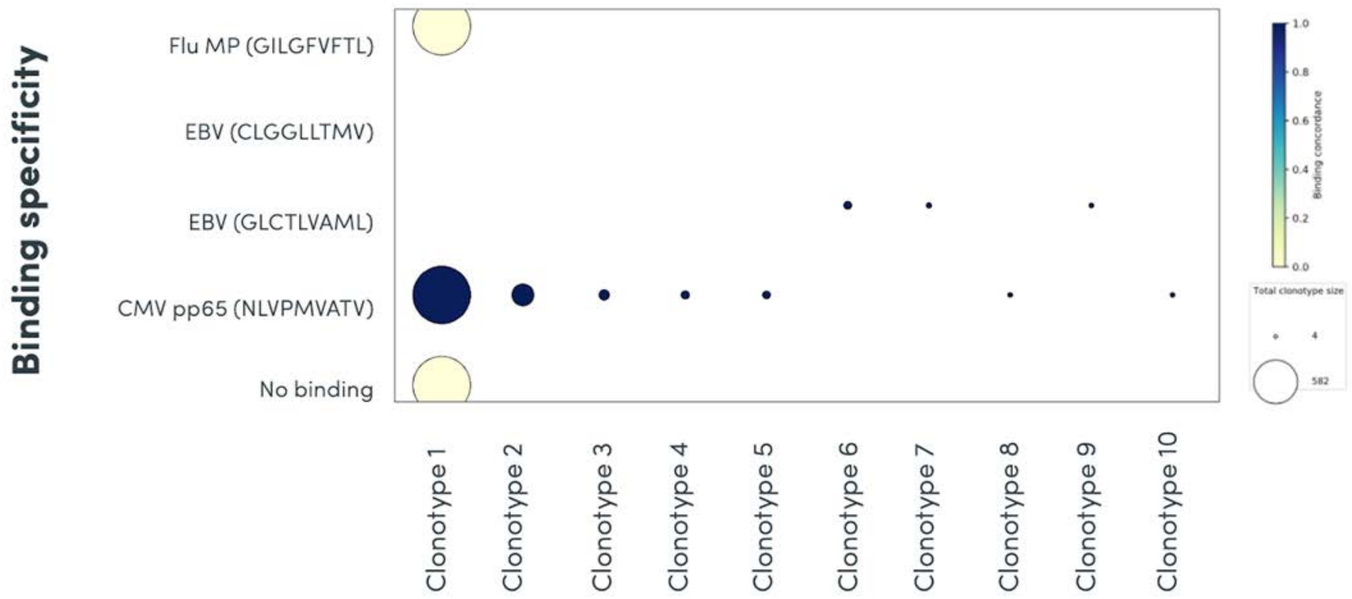
Dextramer counts for CMV+ cells by Feature Barcode technology with v2



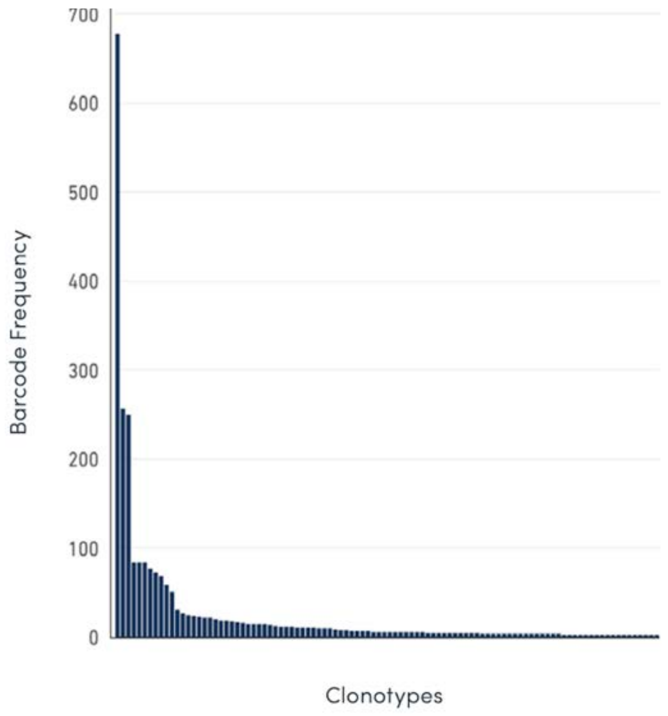
31.8% of cells are CMVpp65 positive

Tying antigen specificity to TCR clonotypes

Antigen Binding Specificities



TCR Clonotype Frequencies



The top CDR3s identified have been previously reported as CMV pp65 binders

Targeted Gene Expression: Focus on the Genes that Matter Most

In Single Cell Suspensions or Tissue Sections

From discovery to focused transcriptomics

Targeted product built for 10x assays

Increased experimental efficiency

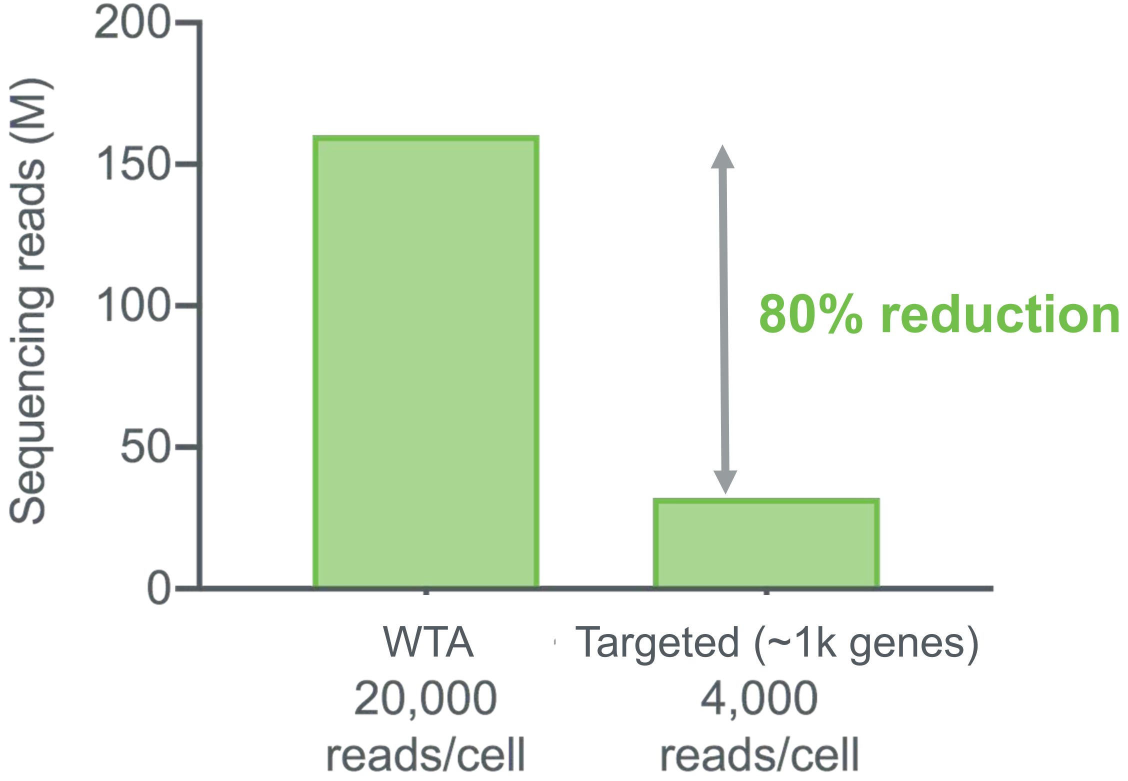
Reduced sequencing cost

WTA and targeted gene expression from the same cells

Core assay compatibility

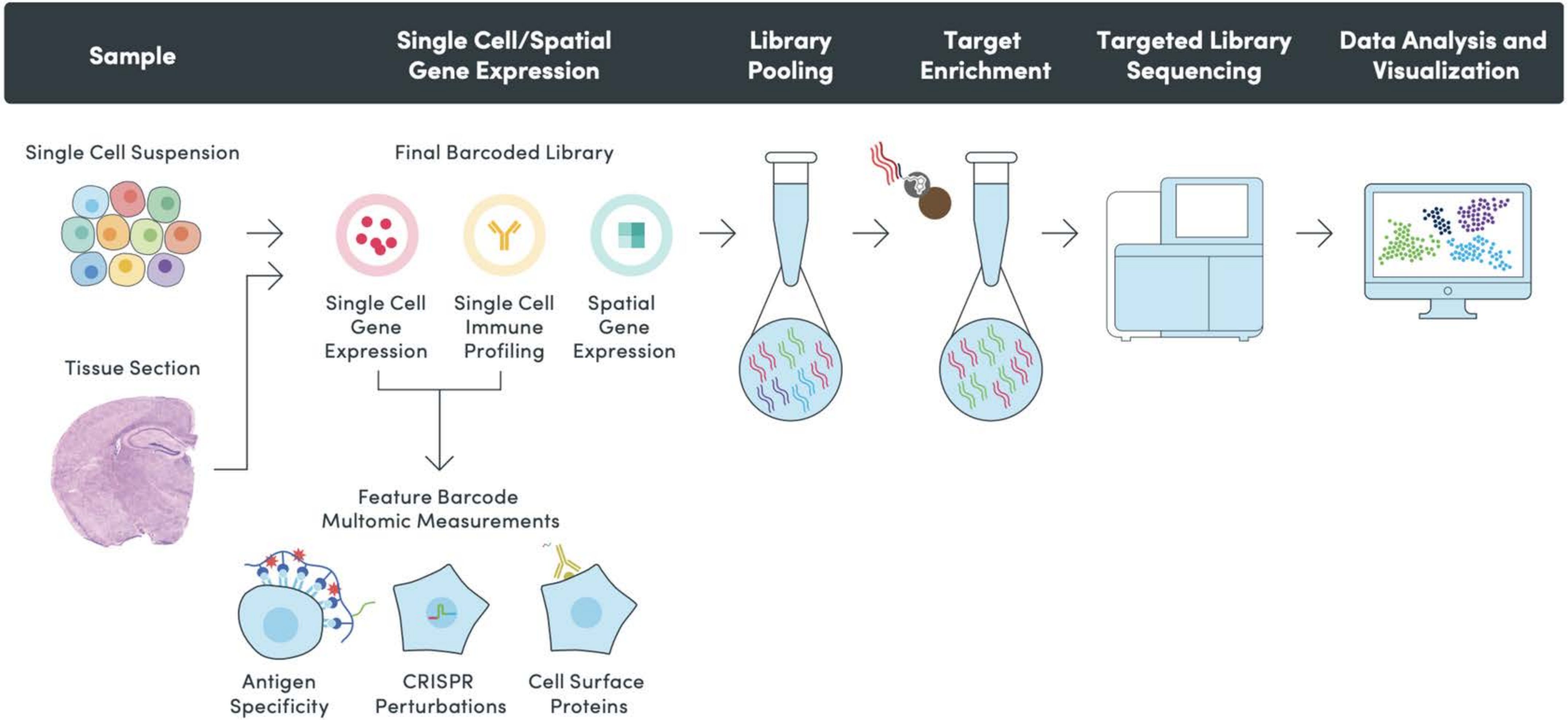
Content and customization

Sequencing reads required for 8,000 cells



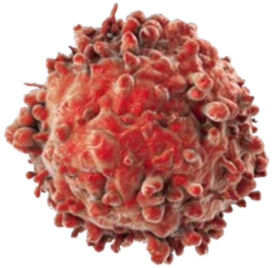
General Workflow

Hybrid capture enrichment for versatile, sensitive, customizable targeting



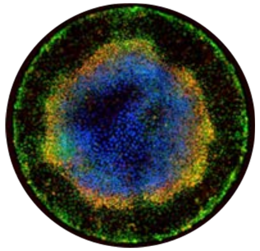
Comprehensive pre-designed panels

Accelerate research in 4 major areas



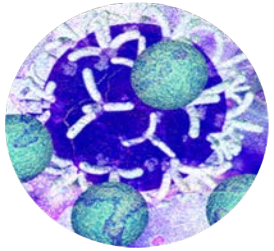
Human Pan-Cancer
1,253 genes

- **33 cancer types**, key biomarkers, pathways, and cellular processes
- Profile tumor microenvironment and heterogeneity, and tumor immune status in a wide variety of tumors



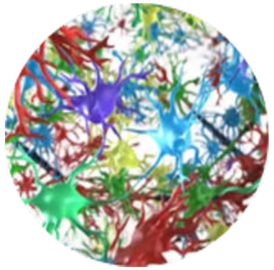
Human Gene Signature
1,142 genes

- **Disease and drug targets,** including kinases, GPCRs, cell cycle/checkpoint genes
- Analyze the **activation or inhibition** of important signaling pathways, and discover mechanism of action of small molecules



Human Immunology
1,056 genes

- Covers **innate and adaptive immunity, inflammation, and immuno-oncology**
- Comprehensively profile the immune response in cells and tissues

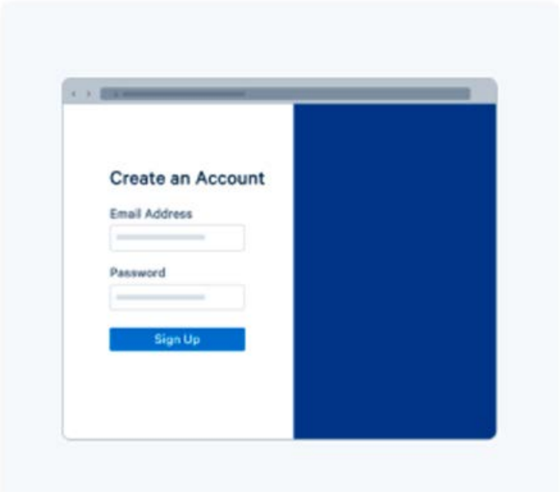


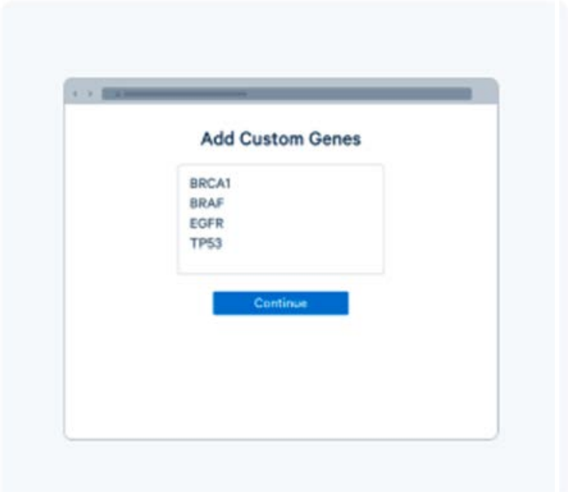
Human Neuroscience
1,186 genes

- Covers **neural development, neurogenesis, neurodegenerative diseases** and **neuro-oncology**
- Characterize changes in gene expression in brain injury and disease

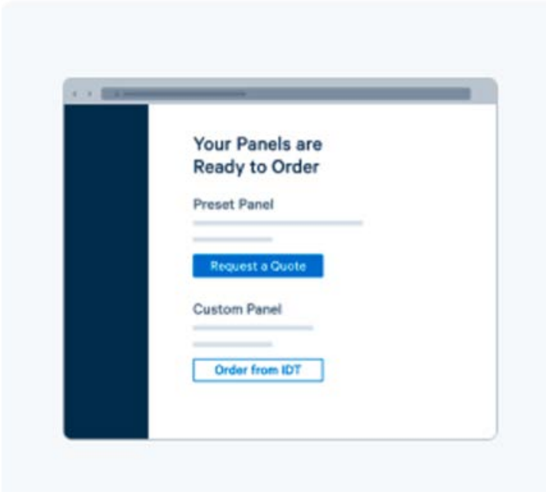
Add genes or create fully custom panels

Focus on the genes that matter most





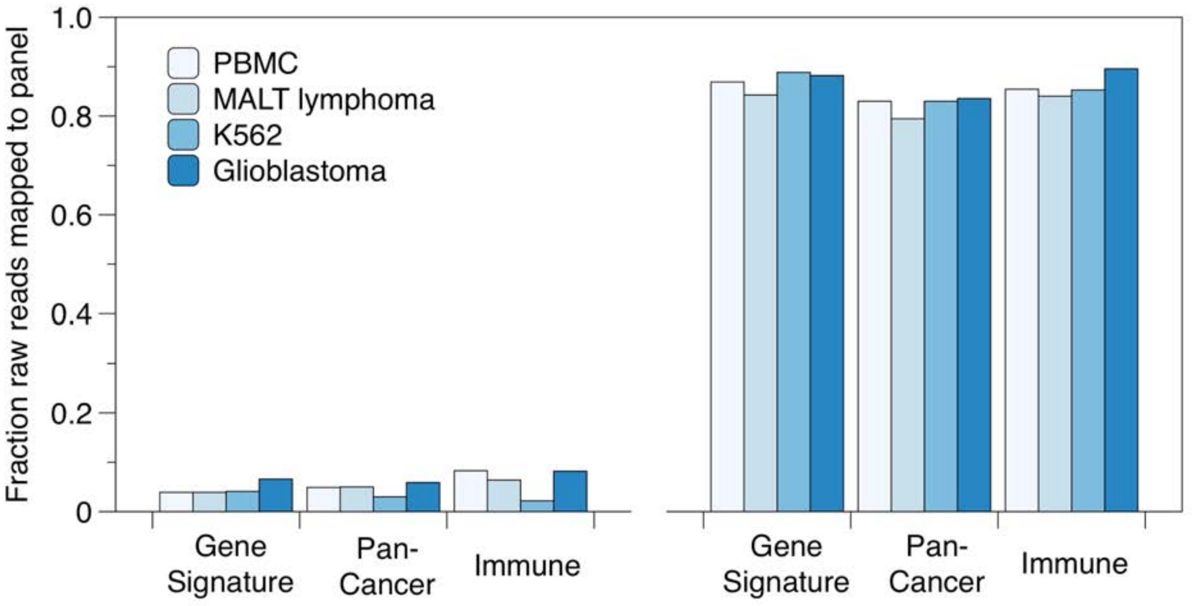
BRCA1
BRAF
EGFR
TP53



- Custom Panel Designer coming soon
- Add **up to 200 genes** on to a pre-designed panel
- Create a fully custom panel of **10 - 1,500 genes**
- Add up to **10 exogenous sequences**

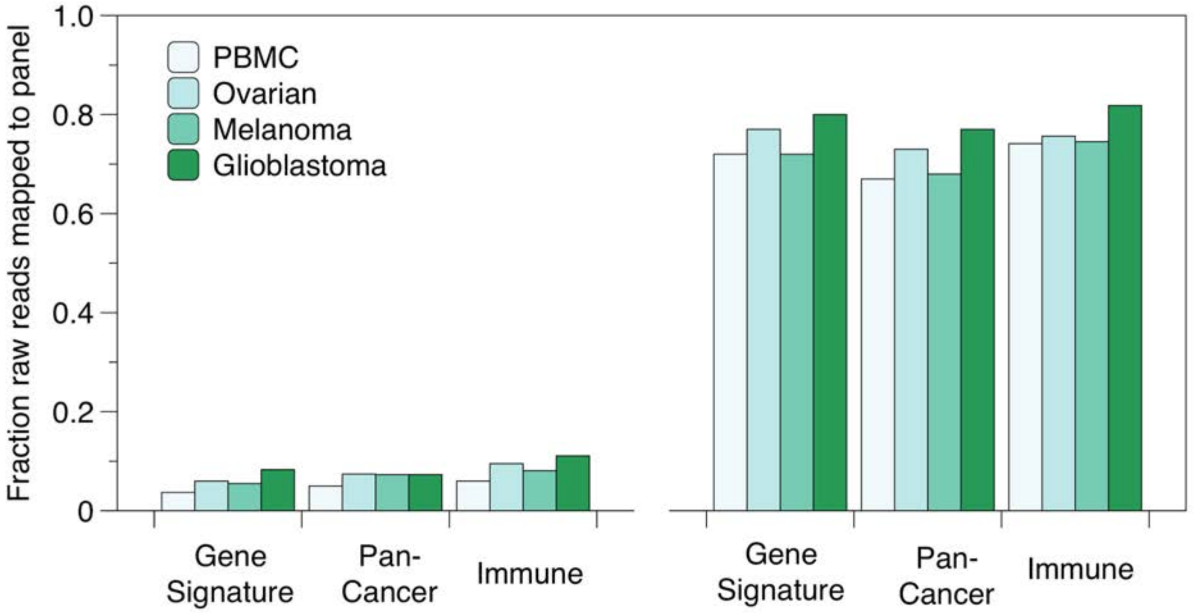
Target enrichment: focus on the genes you care about

Single Cell 3’v3



Whole Transcriptome Targeted

Single Cell 5’ Immune Profiling



Whole Transcriptome Targeted

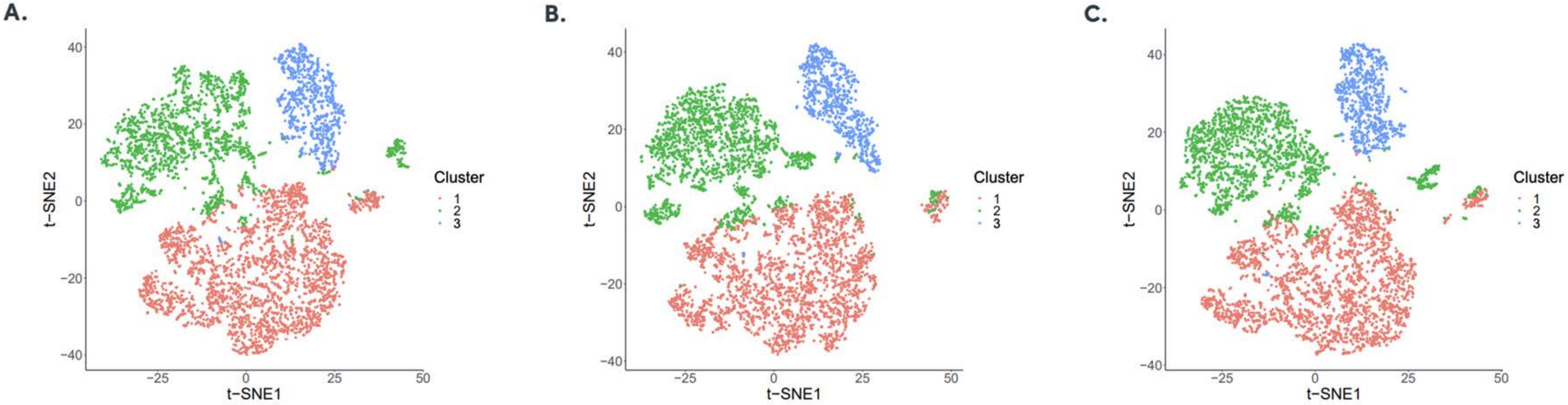
Comparing WTA and Targeted Libraries

Human Pan-Cancer Panel: 1,253 genes to accelerate cancer research

Whole Transcriptome
70,000 reads per cell (~60% saturation)

Clustering based on *in silico* subset of genes in panel

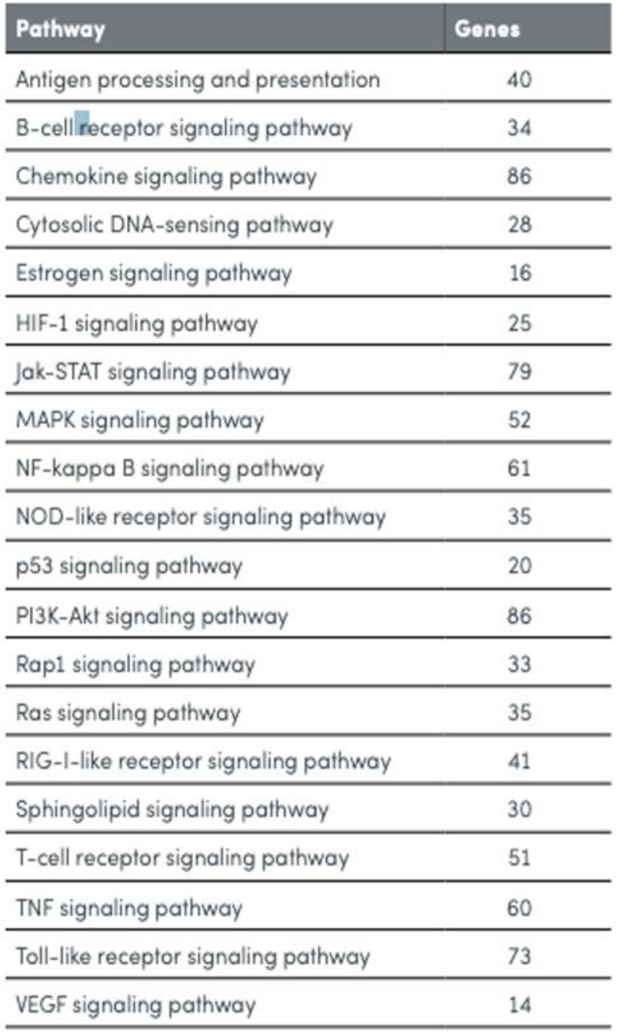
Targeted library
2,000 reads per cell



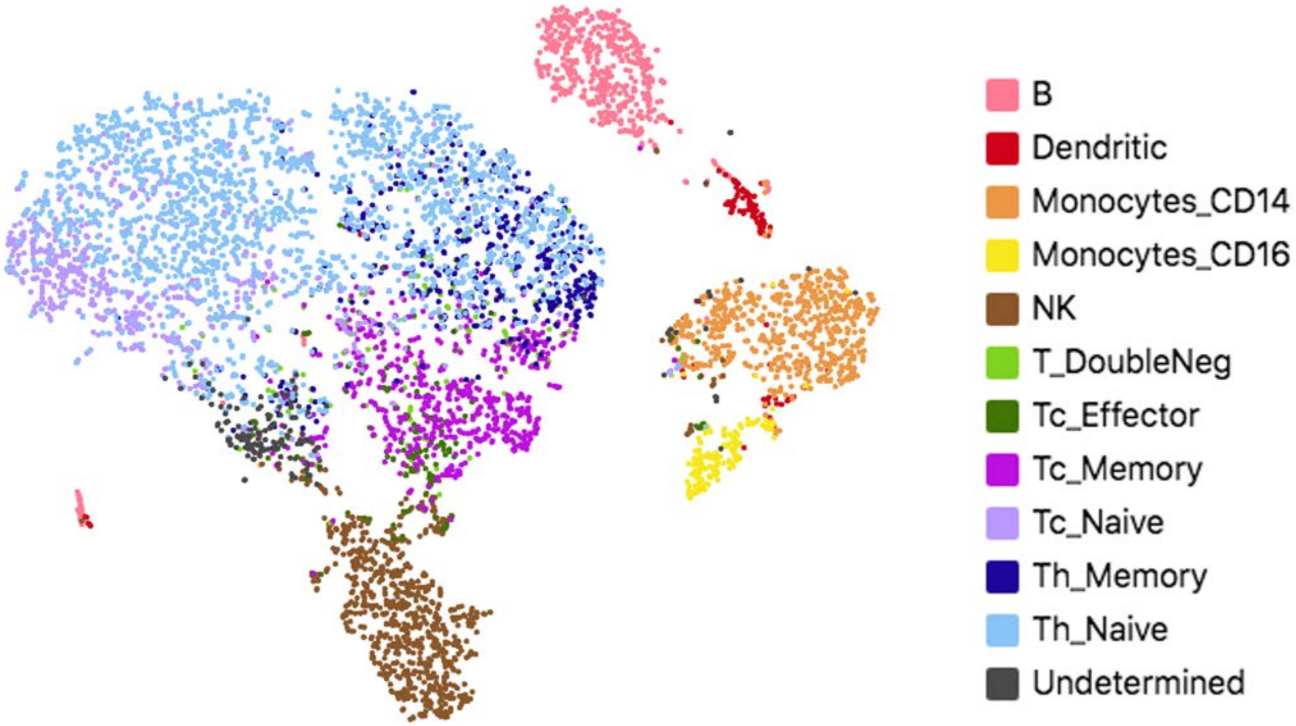
6,000 glioblastoma cells run with Chromium Single Cell Gene Expression 3’ v3 workflow

The targeted immunology panel detects all key cell types in PBMC samples

Immunology Panel ~1050 genes



Pathway	Genes
Antigen processing and presentation	40
B-cell receptor signaling pathway	34
Chemokine signaling pathway	86
Cytosolic DNA-sensing pathway	28
Estrogen signaling pathway	16
HIF-1 signaling pathway	25
Jak-STAT signaling pathway	79
MAPK signaling pathway	52
NF-kappa B signaling pathway	61
NOD-like receptor signaling pathway	35
p53 signaling pathway	20
PI3K-Akt signaling pathway	86
Rap1 signaling pathway	33
Ras signaling pathway	35
RIG-I-like receptor signaling pathway	41
Sphingolipid signaling pathway	30
T-cell receptor signaling pathway	51
TNF signaling pathway	60
Toll-like receptor signaling pathway	73
VEGF signaling pathway	14



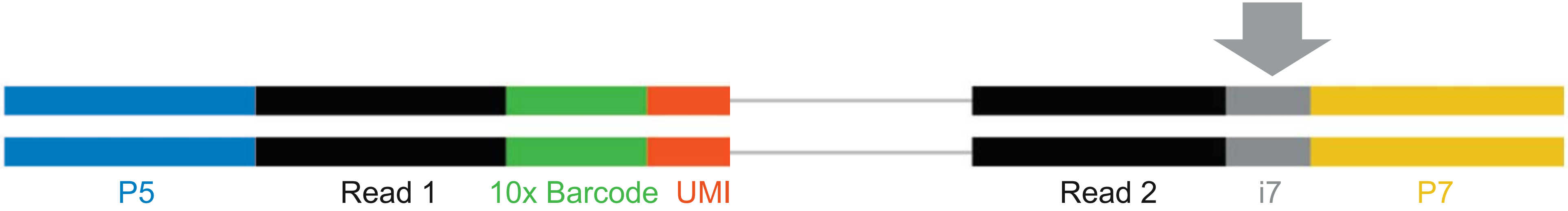
Cell types annotated using genes in the targeted gene expression: Human Immunology panel

Introduction to dual indexing

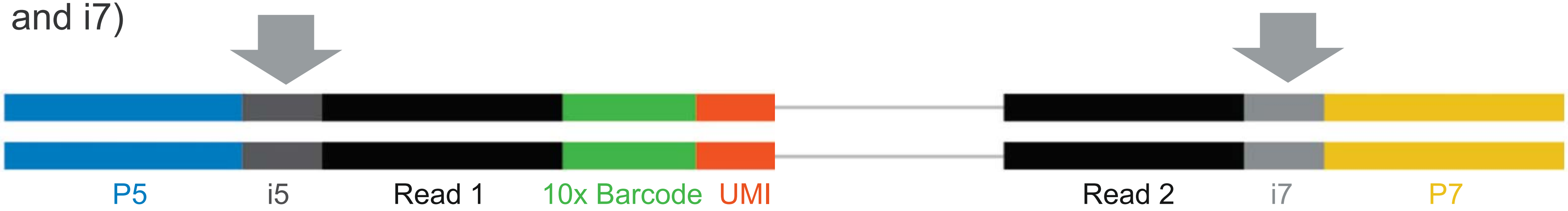
Indexing strategies

Single index versus dual index libraries

Single index (i7)



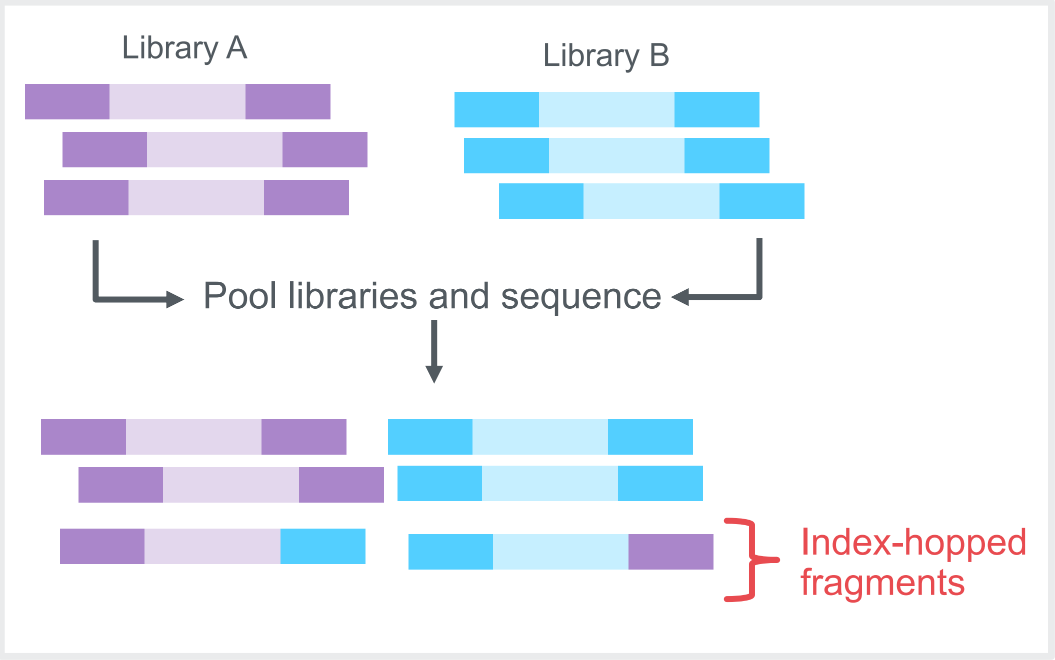
Dual index (i5 and i7)



Index hopping

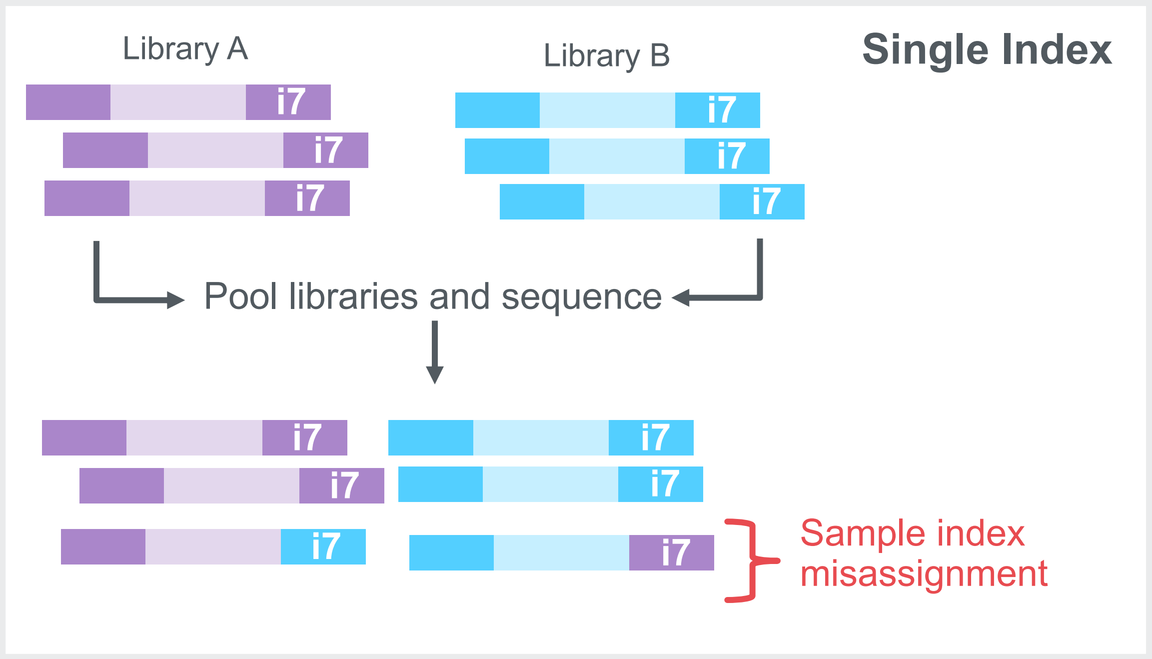
Can occur on Illumina instruments

- During Illumina sequencing, a small fraction of library fragments undergo molecular recombination
- Occurs more frequently on instruments utilizing patterned flow cells and exclusion amplification chemistry



Dual indexing enables filtering of index-hopped fragments

- If index hopping occurs in single-indexed libraries, reads may be assigned to the incorrect sample index
- With dual-indexed libraries, index-hopped fragments can be identified due to mismatched i5/i7 index pair, and can be excluded from downstream bioinformatic analysis



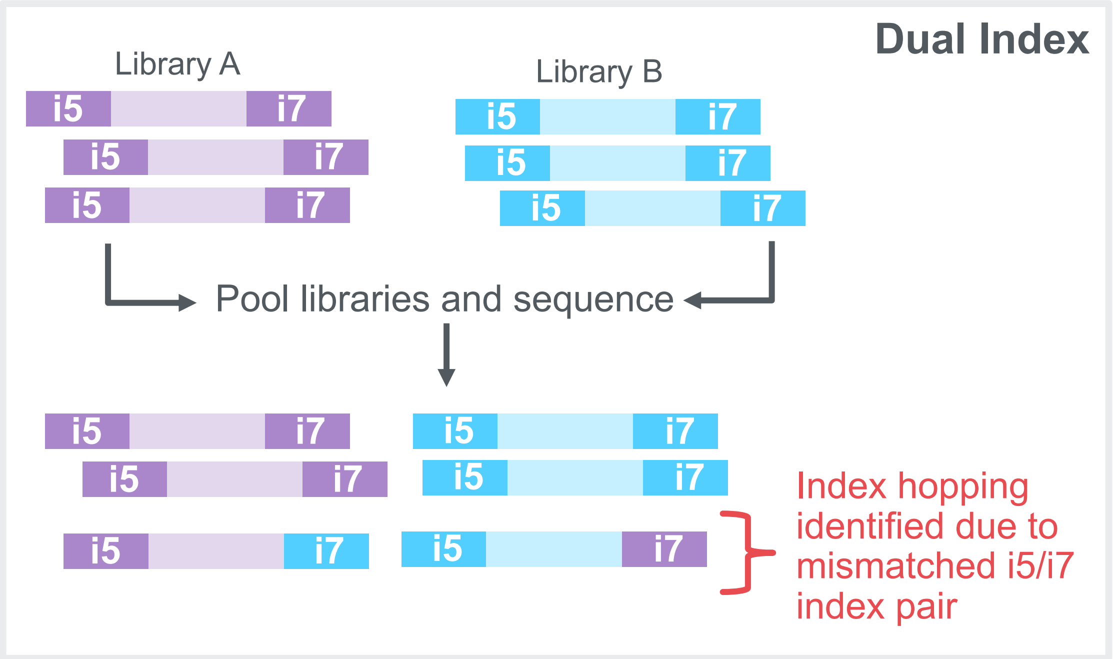
Single Index

Library A

Library B

Pool libraries and sequence

Sample index misassignment



Dual Index

Library A

Library B

Pool libraries and sequence

Index hopping identified due to mismatched i5/i7 index pair

Compatibility of index plates across 10x products

Select appropriate index plate based on assay and library type

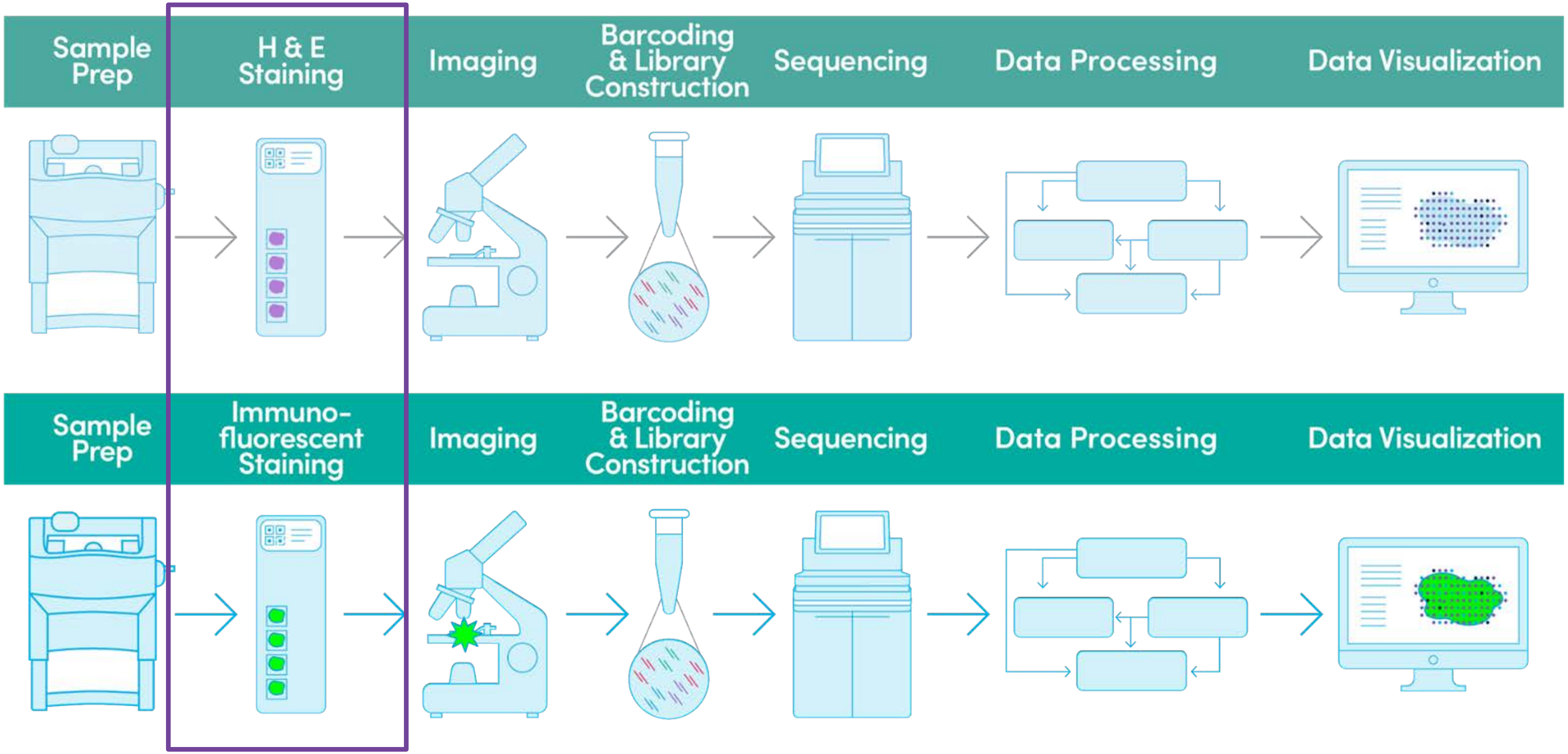
Index plate	Assay
Dual Index Plate TT Set A	• 3’ v3.1 Dual Index Gene Expression Library • 5’ v2 Gene Expression Library • 5’ v2 V(D)J Enriched Library • Spatial Gene Expression Library
Dual Index Plate NT Set A	• 3’ v3.1 Dual Index Cell Surface Protein Library • 3’ v3.1 Dual Index CRISPR Library
Dual Index Plate TN Set A	• 5’ v2 Dual Index Cell Surface Protein Library

Visium with Immunofluorescence

Expanding Visium Capabilities with Protein Detection

Visium with H&E Workflow

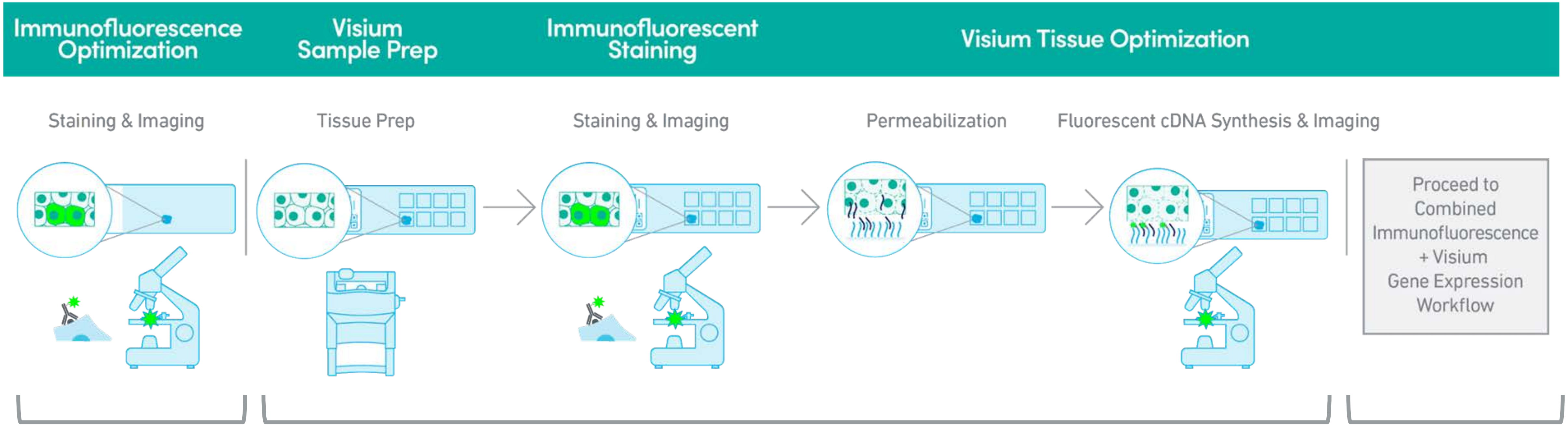
Visium with IF Workflow



***Choose between H&E or IF staining**

Optimization is key for Visium with IF

“Treat each antibody like it’s brand new to your lab”



Proceed to Combined Immunofluorescence + Visium Gene Expression Workflow

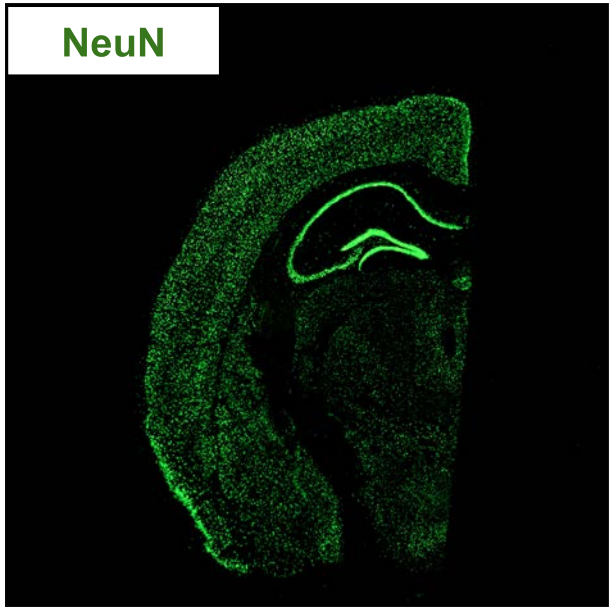
Regular glass slides

Visium TO slides

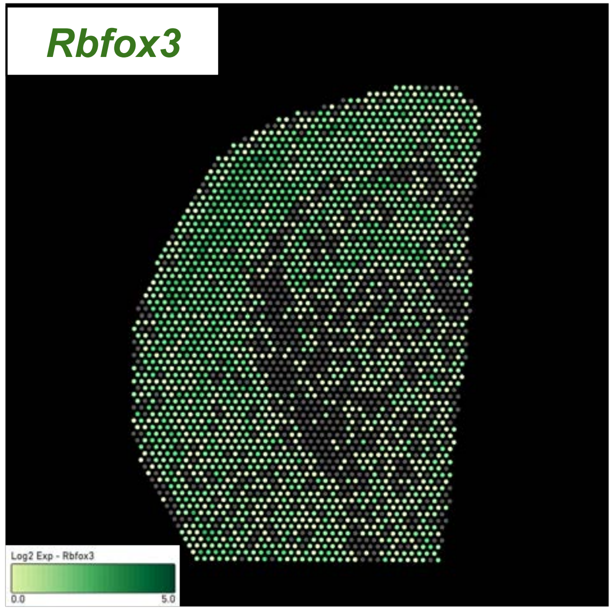
Visium GEx slides

Visium with IF:
Protein Detection Paired with Gene Expression

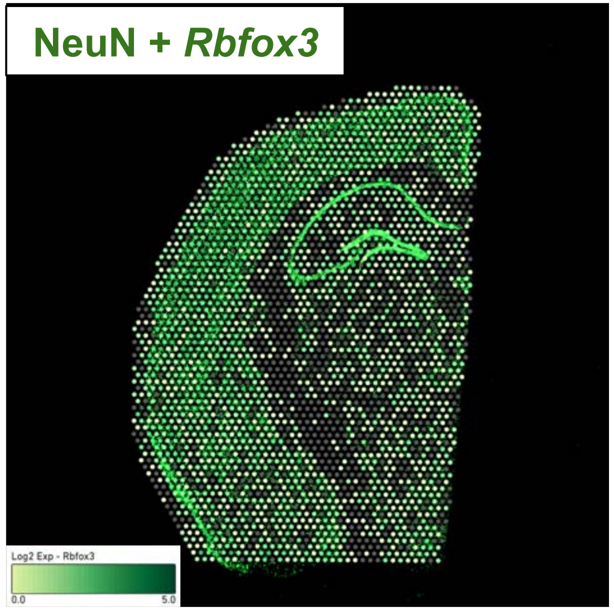
Immunofluorescence (IF)



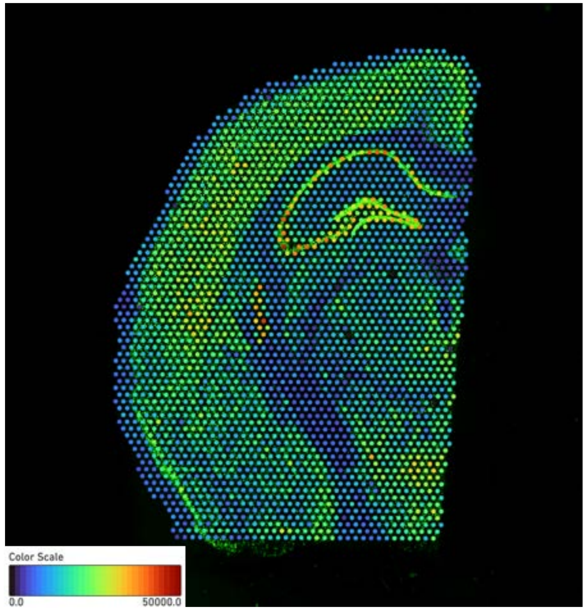
Visium Gene Expression



**IF + Visium
Gene Expression**



**IF + Visium
Whole Transcriptome**



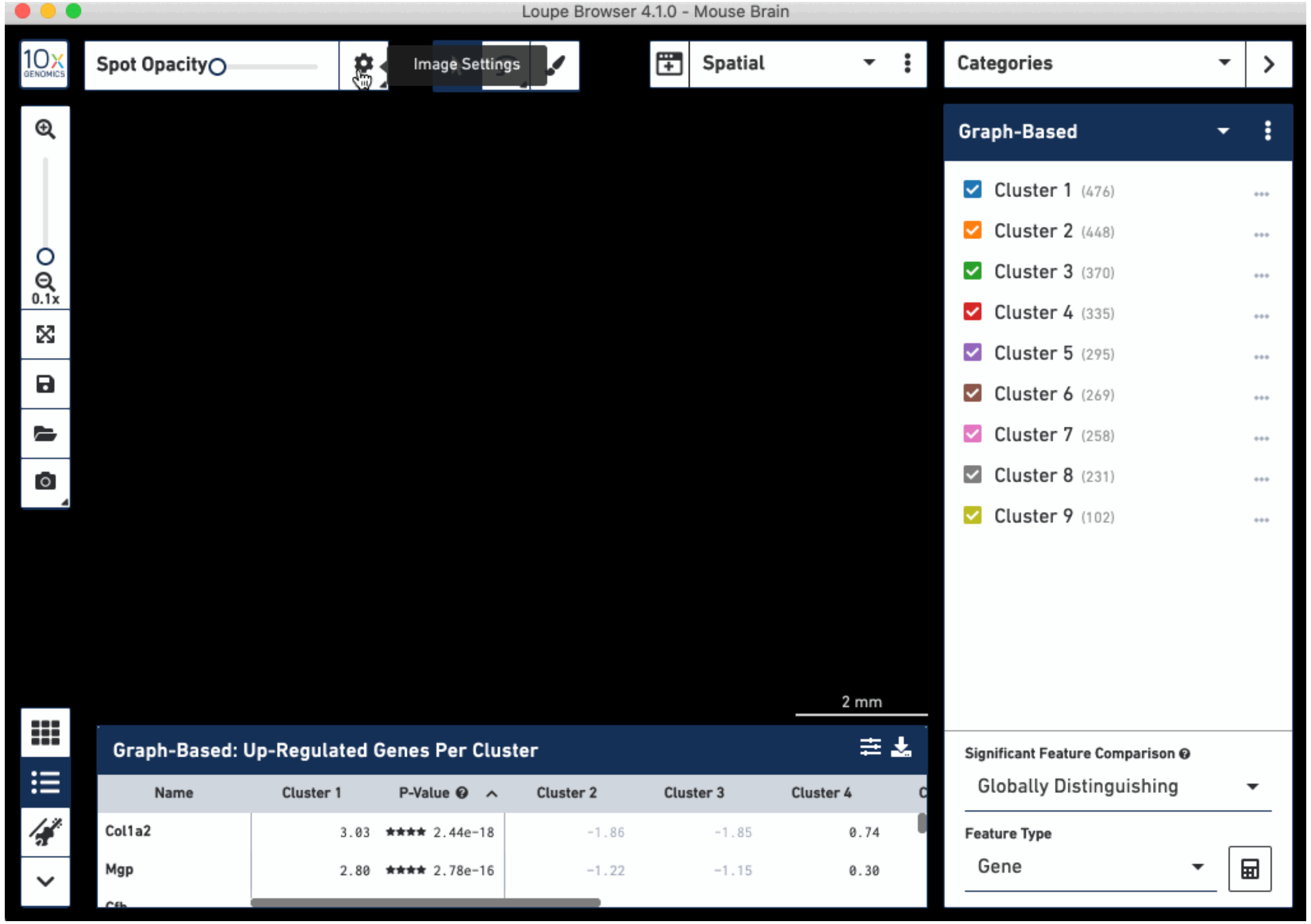
Microscope Imaging Readout

Sequencing Readout

Microscope Imaging + Sequencing Readout

Visualize Multiple Proteins at the Same Time

- Space Ranger and Loupe Browser enables mapping of IF (up to 6 colors) to gene expression
- Fluorescent markers (such as NeuN and DAPI) can be toggled on and off for easy visualization



Graph-Based

☑ Cluster 1 (476)
☑ Cluster 2 (448)
☑ Cluster 3 (370)
☑ Cluster 4 (335)
☑ Cluster 5 (295)
☑ Cluster 6 (269)
☑ Cluster 7 (258)
☑ Cluster 8 (231)
☑ Cluster 9 (102)

Significant Feature Comparison: Globally Distinguishing

Feature Type: Gene

Graph-Based: Up-Regulated Genes Per Cluster

Name	Cluster 1	P-Value	Cluster 2	Cluster 3	Cluster 4
Col1a2	3.03	★★★★ 2.44e-18	-1.86	-1.85	0.74
Mgp	2.80	★★★★ 2.78e-16	-1.22	-1.15	0.30

Overlay Protein Detection with Gene Expression

- Visium gene expression analysis can be overlaid on top of immunofluorescence image
- Spot clusters representing gene expression shown on the right
  - Similar to cell clusters identified by single cell RNAseq

Graph-Based

☑ Cluster 1 (476)
☑ Cluster 2 (448)
☑ Cluster 3 (370)
☑ Cluster 4 (335)
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Significant Feature Comparison: Globally Distinguishing

Feature Type: Gene

Features and Benefits of Visium with IF

Gain a new perspective on tissue complexity with simultaneous gene expression and protein profiling

Protein and Whole Transcriptome Co-Detection

Spatially resolve protein and total mRNA with morphological context in the whole tissue section

Streamlined Data Analysis

Easy-to-use analysis software that combines immunofluorescence images and gene expression data

Antibody Flexibility

Utilize your current antibodies to optimize with our demonstrated protocol

Efficient

End-to-end, seamless workflow from section to sequencing-ready library, including IF staining and imaging

High Cell Resolution

1-10 cells on average per spot depending on tissue
Spot size 55 µm diameter
~5000 spots per capture area

Kitted and Ready to Use

All Visium reagents and slides are ready to use with your current antibodies

10x GENOMICS

Visium Slide Reset

Slide Reset

Incorrectly place the tissue on your Visium slide? No problem, just reset!

Problem

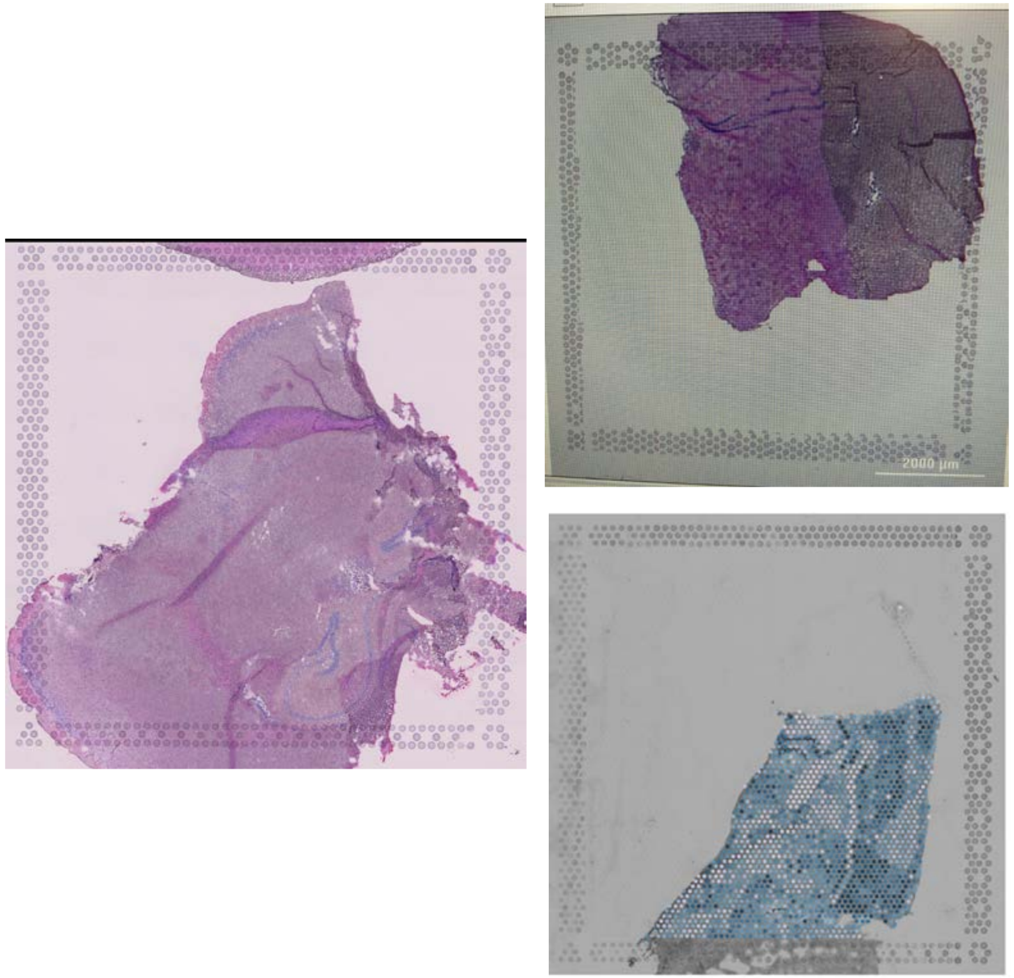
- Incorrect placement of tissue on the Tissue Optimization or Gene Expression slide, can lead to suboptimal analysis of the tissue

Solution

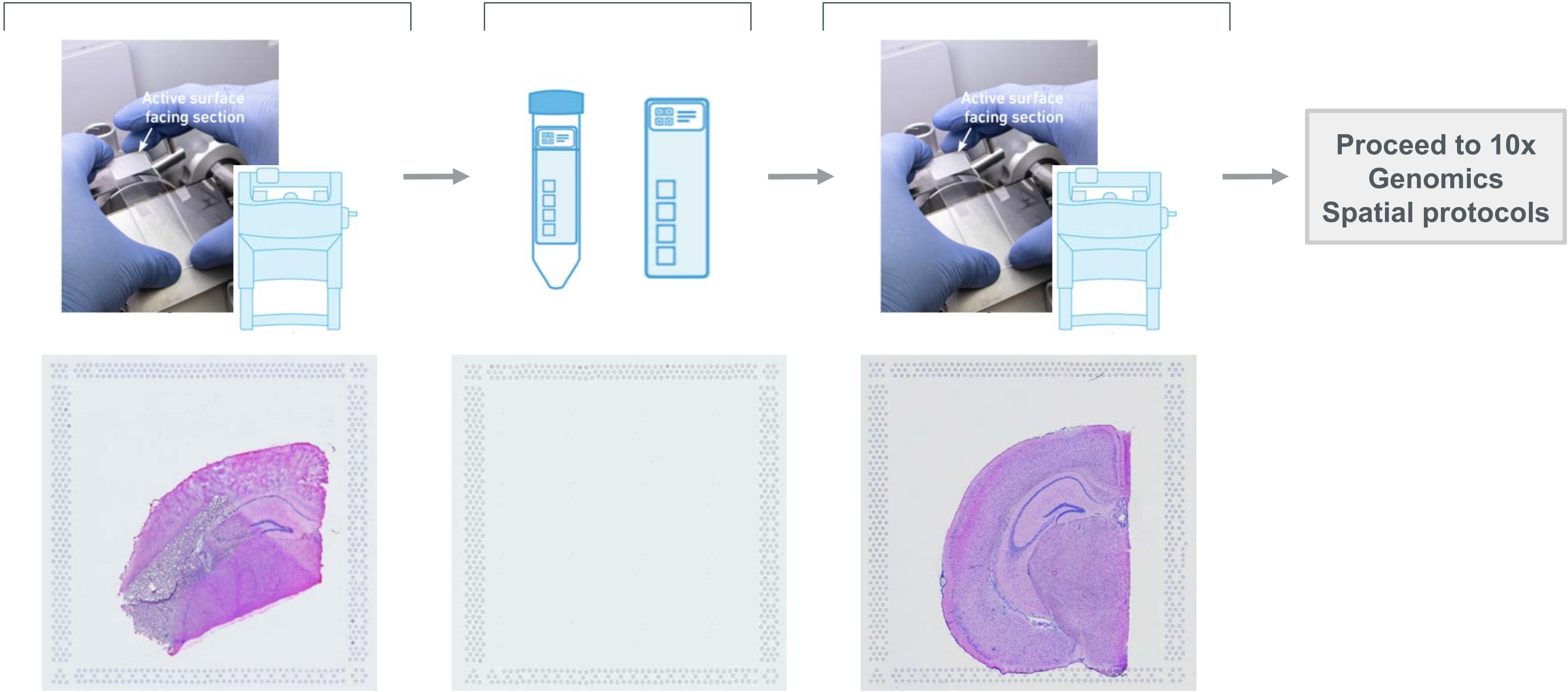
- Slide Reset Demonstrated Protocol
  - Remove tissue to “reset” the slide
  - Use easily sourced reagents to perform the reset protocol
  - Cassette-free → all steps done in 50mL falcon tubes

Benefit

- Don’t waste a valuable GEX or TO slide because tissue was placed incorrectly



Correcting tissue placement



Incorrect tissue placement

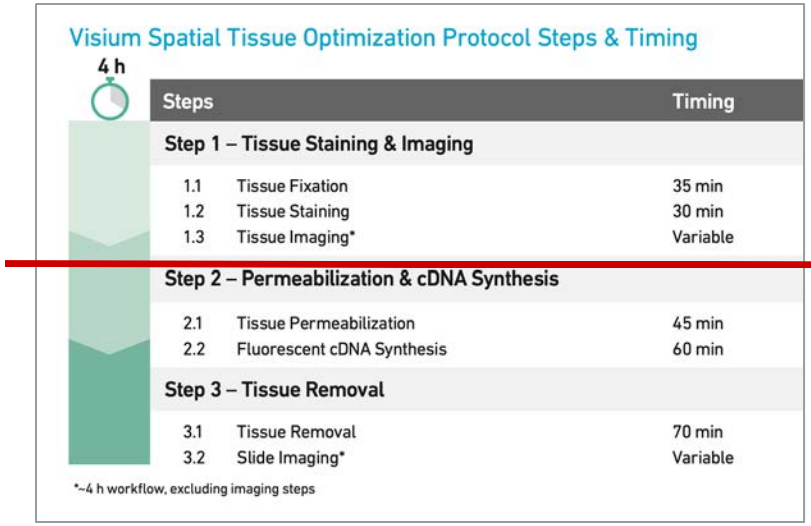
Resetting the slide

Correct tissue placement

Proceed to 10x Genomics Spatial protocols

When Can I Perform Slide Reset?

- Slide reset on Visium TO slides or GEX slides prior to permeabilization
  - Resetting can only be done **prior to permeabilization/RT**→ once RT has started, the slide cannot be reset as the barcodes have been extended into the transcript

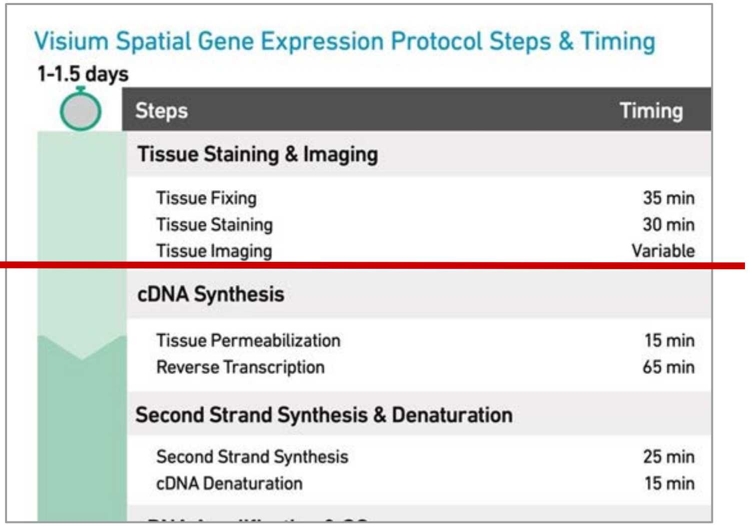


Visium Spatial Tissue Optimization Protocol Steps & Timing

4 h

Steps		Timing
Step 1 – Tissue Staining & Imaging		
1.1	Tissue Fixation	35 min
1.2	Tissue Staining	30 min
1.3	Tissue Imaging*	Variable
Step 2 – Permeabilization & cDNA Synthesis		
2.1	Tissue Permeabilization	45 min
2.2	Fluorescent cDNA Synthesis	60 min
Step 3 – Tissue Removal		
3.1	Tissue Removal	70 min
3.2	Slide Imaging*	Variable

*~4 h workflow, excluding imaging steps



Visium Spatial Gene Expression Protocol Steps & Timing

1-1.5 days

Steps	Timing
Tissue Staining & Imaging	
Tissue Fixing	35 min
Tissue Staining	30 min
Tissue Imaging	Variable
cDNA Synthesis	
Tissue Permeabilization	15 min
Reverse Transcription	65 min
Second Strand Synthesis & Denaturation	
Second Strand Synthesis	25 min
cDNA Denaturation	15 min

Slide Reset can be performed either before or after:

- Tissue Fixation
- Tissue Staining
- Tissue Imaging

As outlined in the Tissue Optimization or Gene Expression Protocols shown at the left

Thank you!

Our collaborators & team 10x