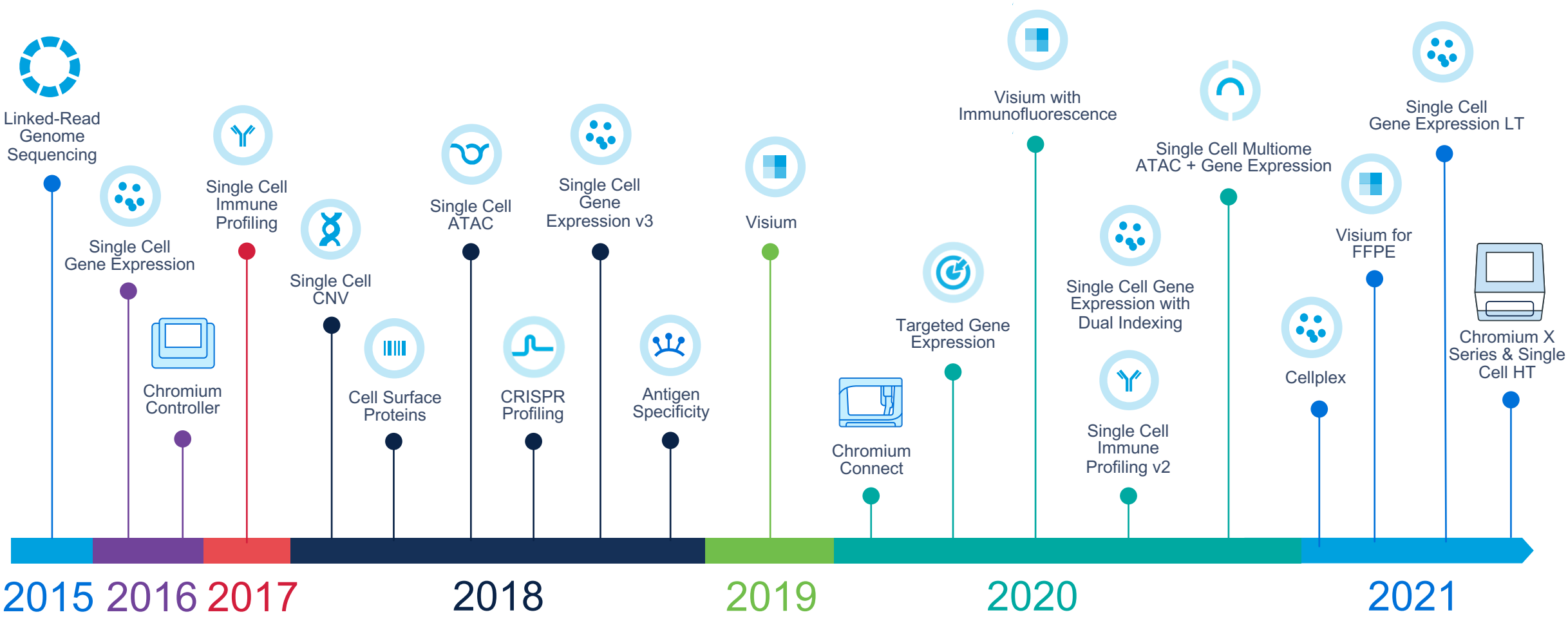
10x GENOMICS

Reaching new Levels of Biological Insights with Single Cell Genomics Solutions

University of Minnesota – March 3rd, 2022

Egon J. Ranghini, PhD
Science & Technology Advisor - North Central
egon.ranghini@10xgenomics.com

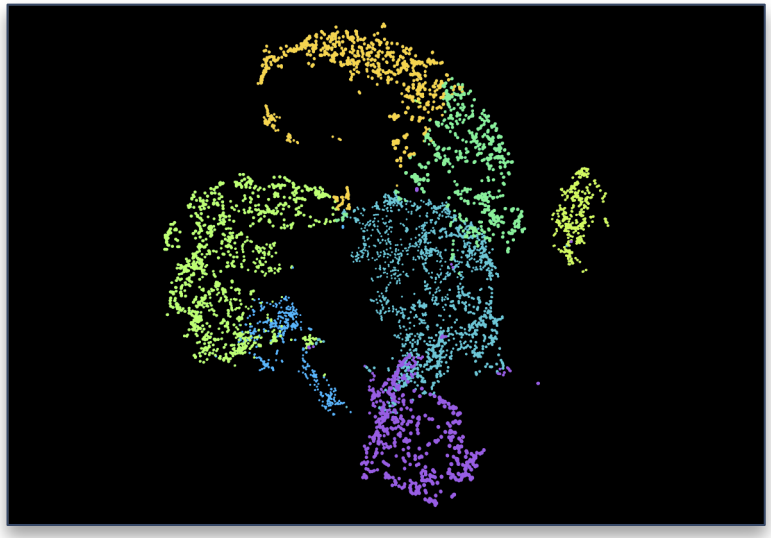
10x Genomics Innovation Engine

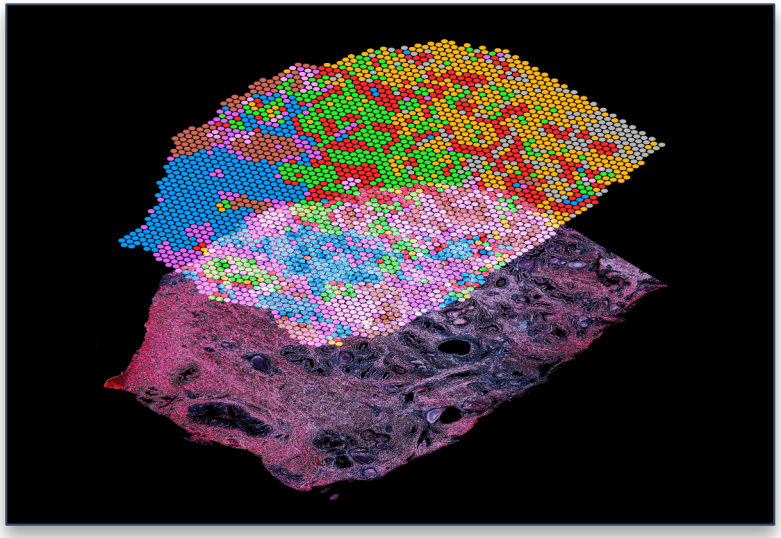


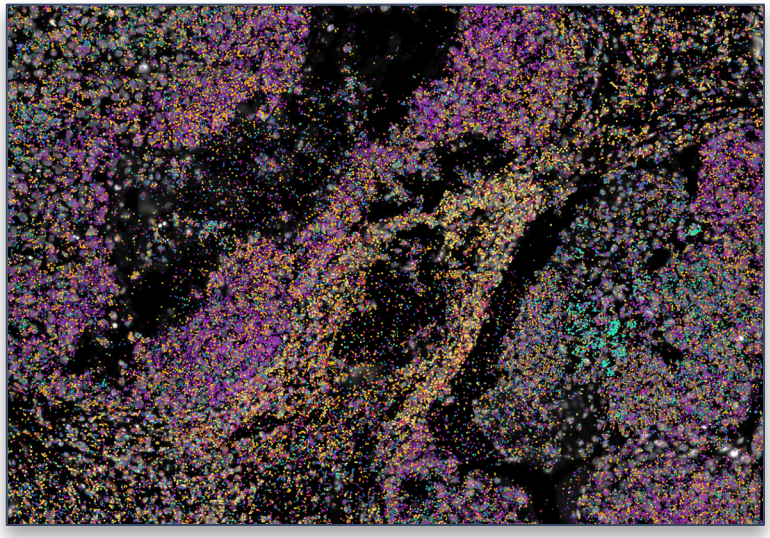
10X GENOMICS

From discovery to focused with three complementary workflows

Chromium Single Cell	Visium Spatial	Xenium In Situ







Discovery

Focused

10x GENOMICS

Chromium Single Cell Platform

3,250+
publications

TheScientist
TOP 10
INNOVATIONS

2021 Chromium X
2020 Single Cell Multiome, Total Seq C
2019 Single Cell ATAC
2018 Single Cell Immune Profiling
2017 Single Cell Gene Expression
2015 Gemcode

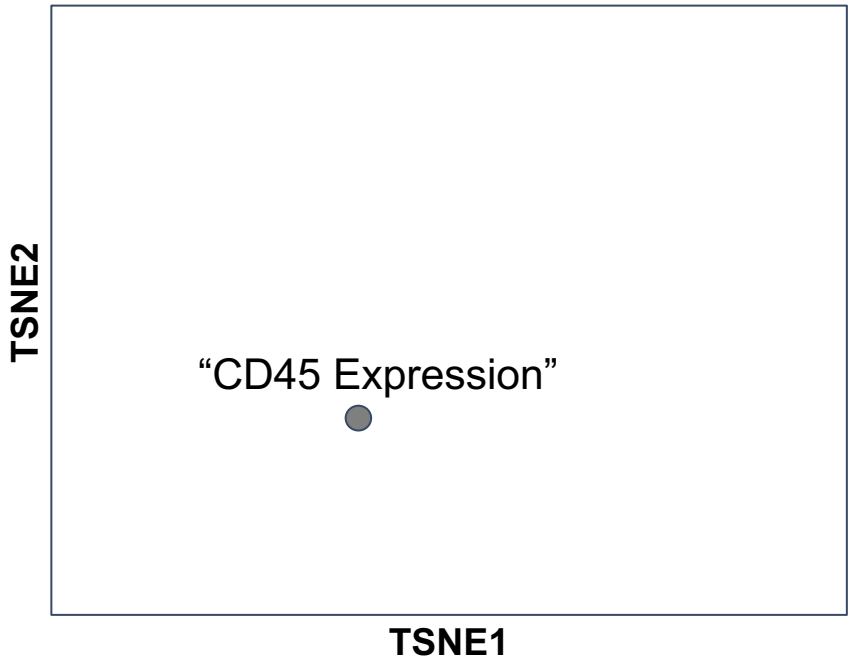
2018
Single Cell Genomics

2019
Single Cell
Multimodal Omics

10X GENOMICS

Single Cell Gene Expression – Unbiased and scalable

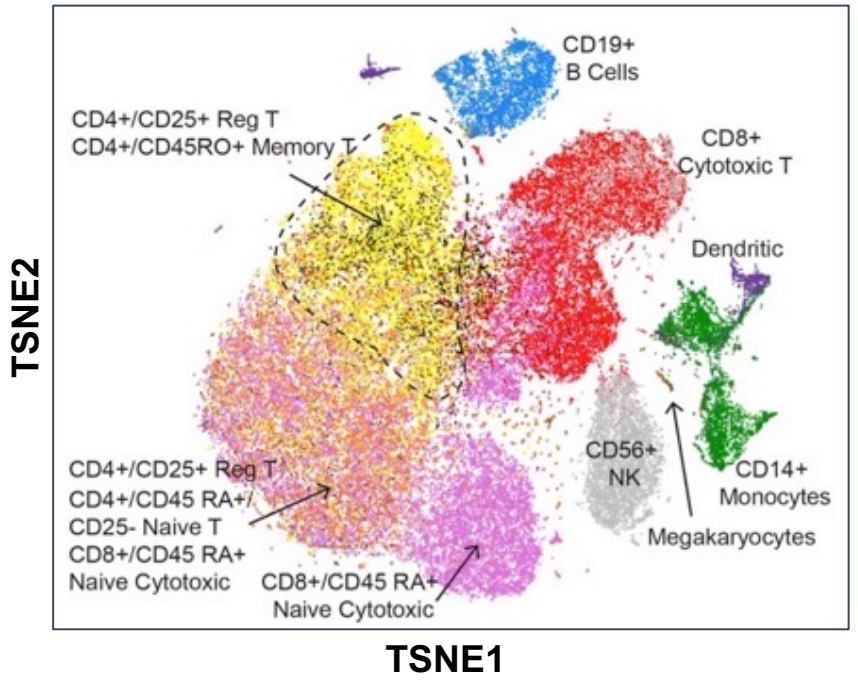
Conventional gene expression



One “average” data point from a mixture of cells

nature COMMUNICATIONS

10x Genomics

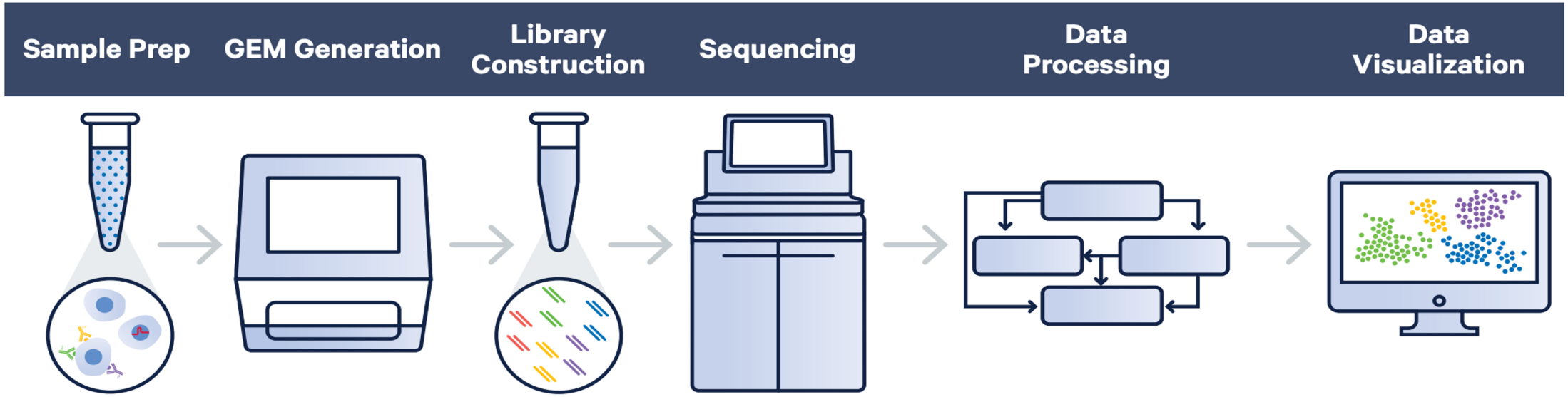


Capture cells with:

- Diverse sizes
- Broad transcriptional activity
- Rare frequencies

Zheng et. al Nature Communications **volume8**, Article number: 14049 (2017)

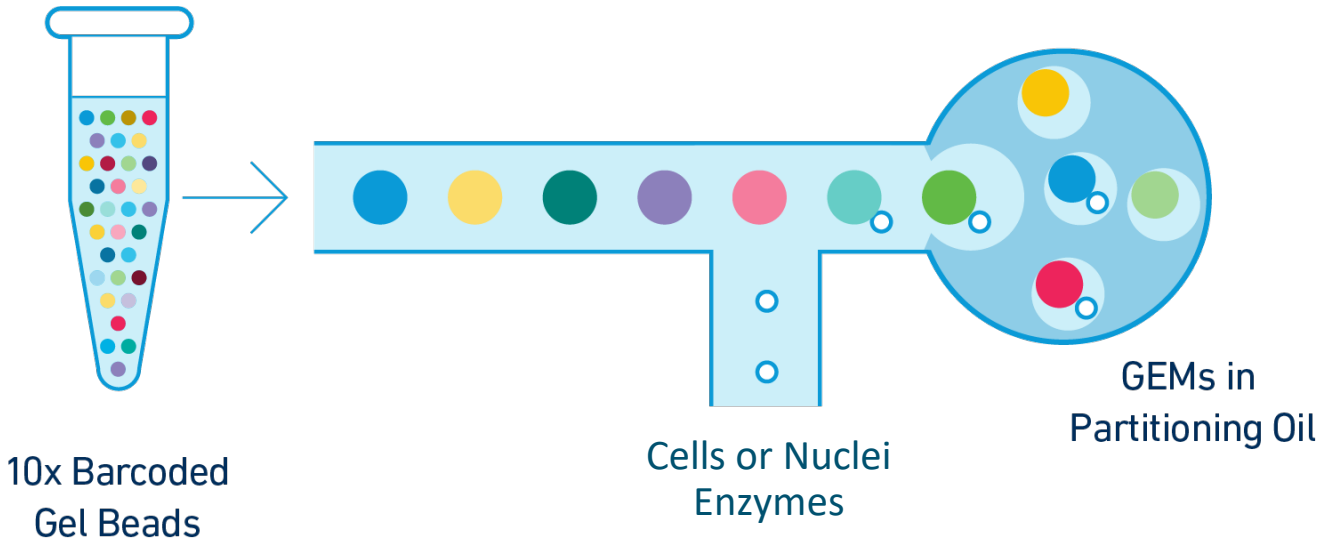
Streamlined and robust workflow for your experiment

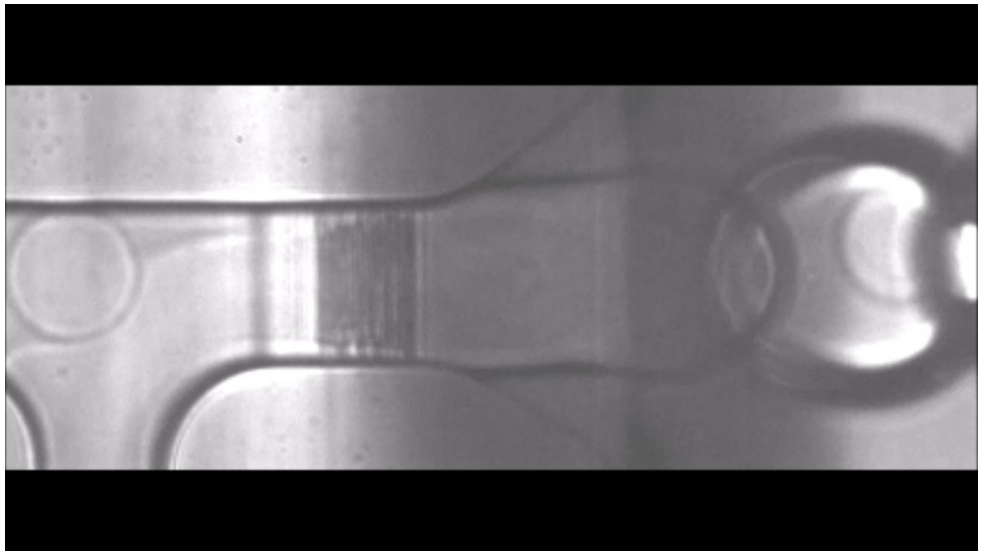


Cell Ranger Cloud Analysis (US-only)

Loupe Browser

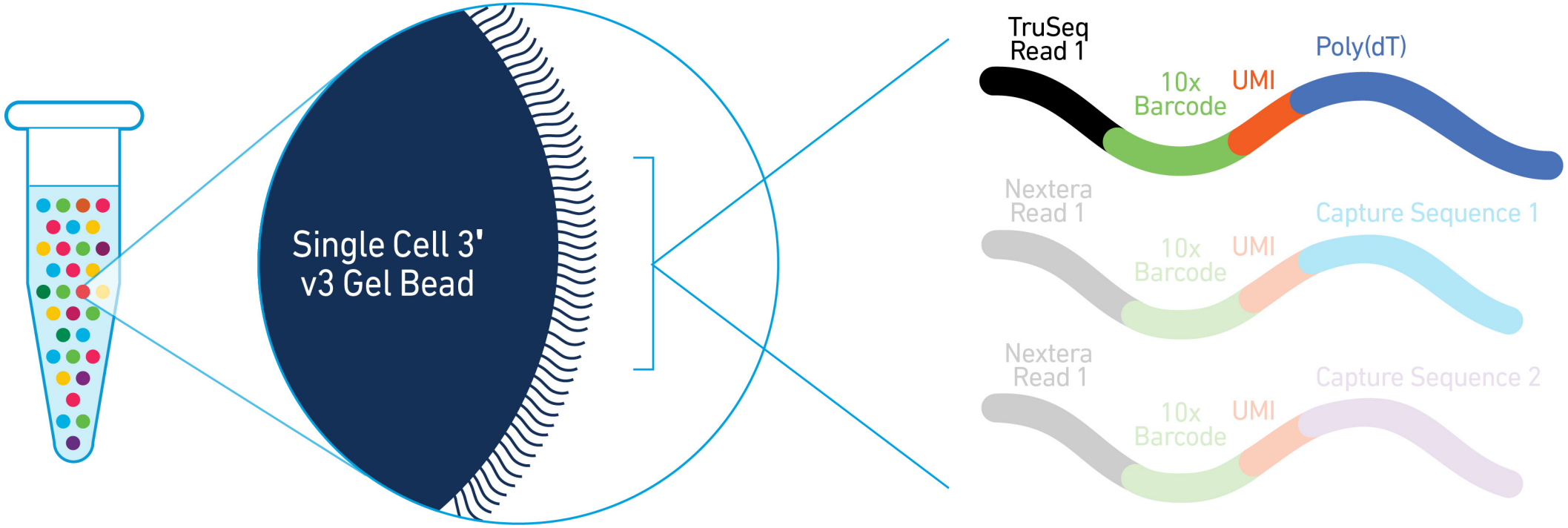
Next GEM technology – creating Gel Beads in EMulsions (GEMs)





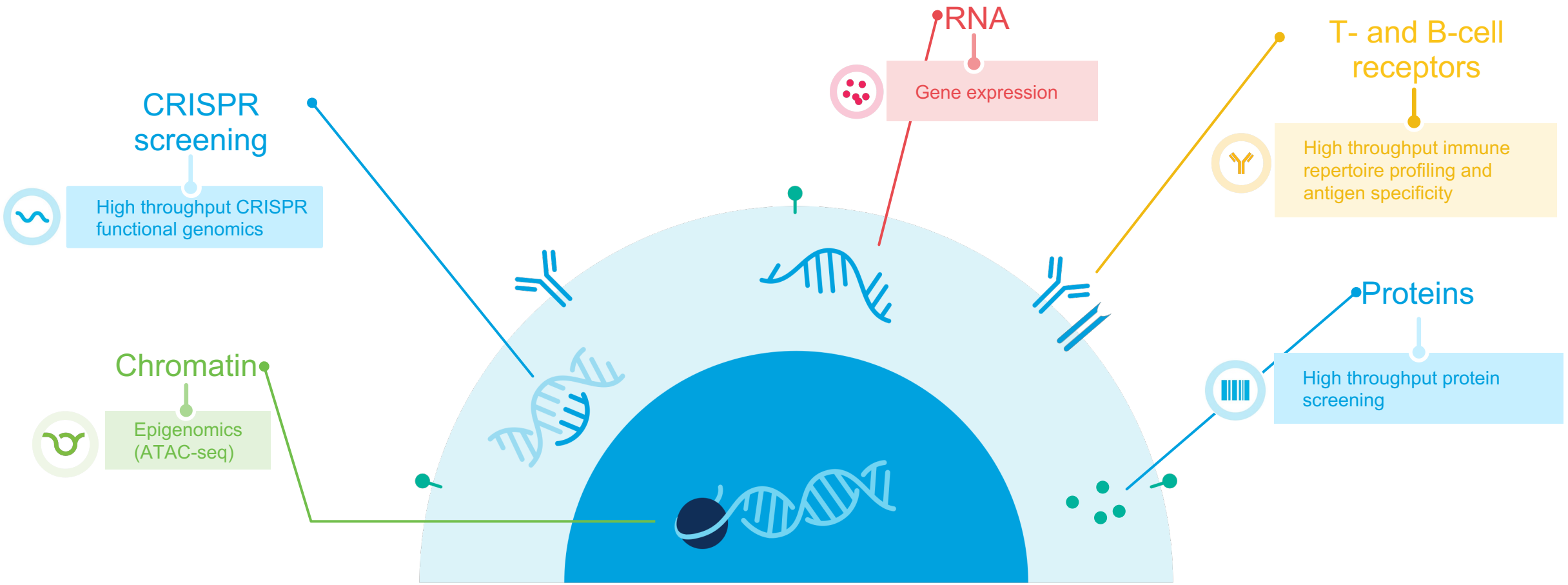
10x Genomics single cell technology

Barcoding single cell transcriptomes and other readouts



Single cell multiomic cytometry

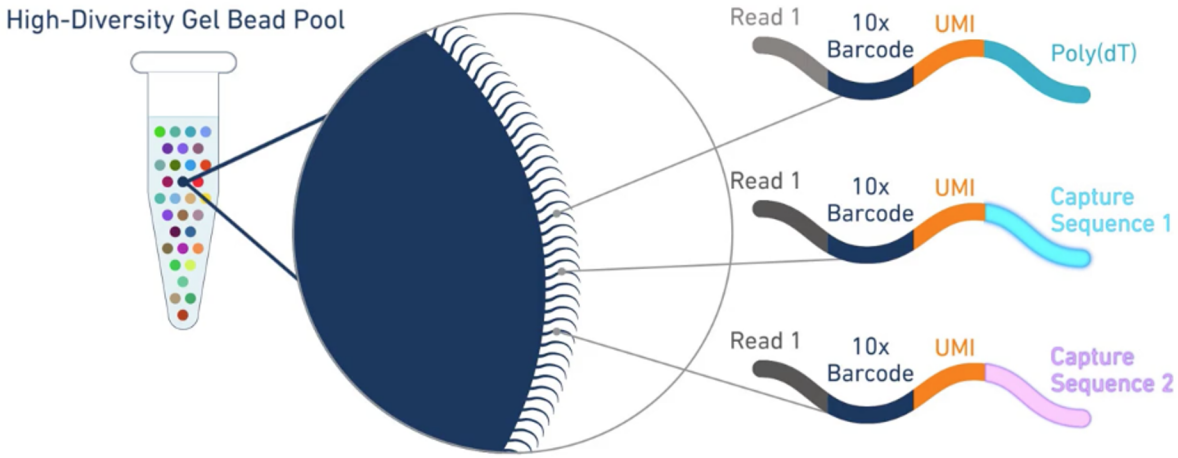
Simultaneous measurements of different cellular analytes



10X GENOMICS

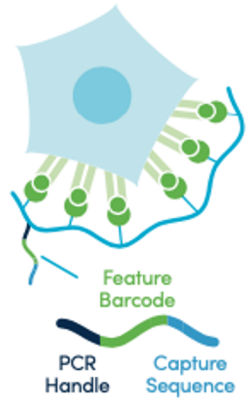
Multiomic cytometry with Feature Barcode technology

Barcoding biological analytes beyond mRNAs in single cells



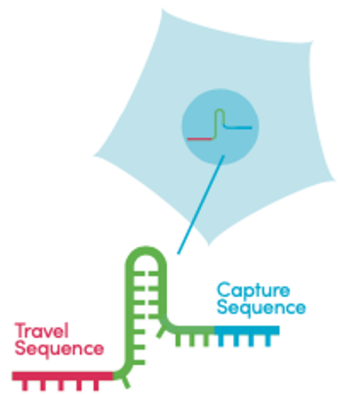
Antibodies for cell surface protein analysis

BioLegend®
Enabling Legendary Discovery™



Peptide-MHC multimers to measure antigen specificity

IMMUDEX



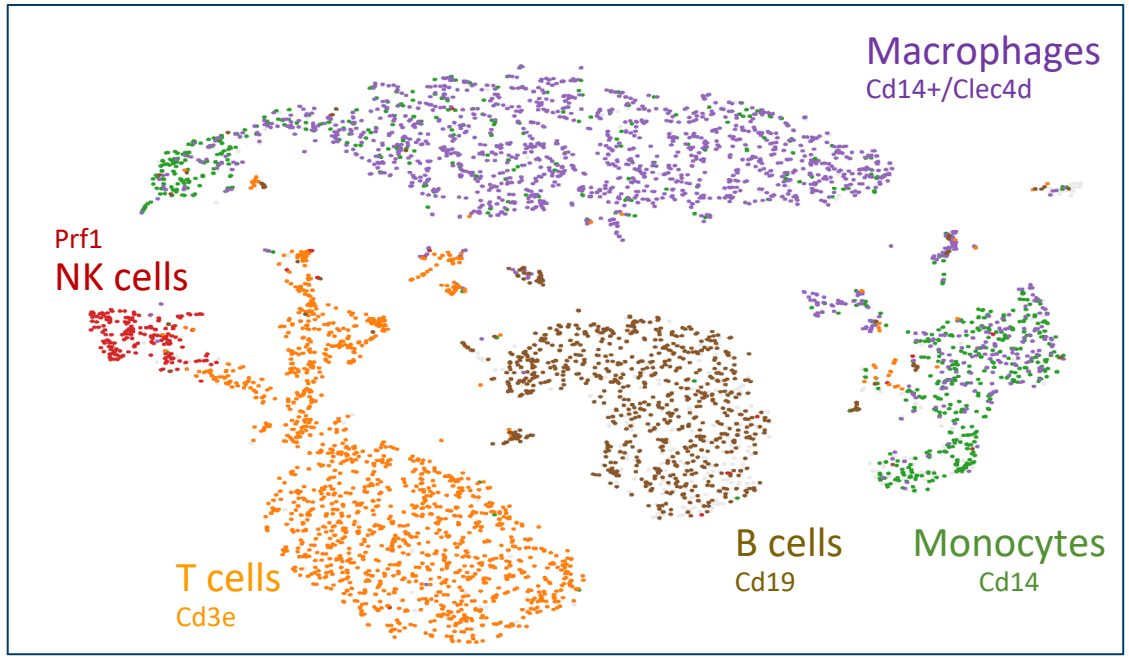
Specific CRISPR perturbations for functional genomics screens

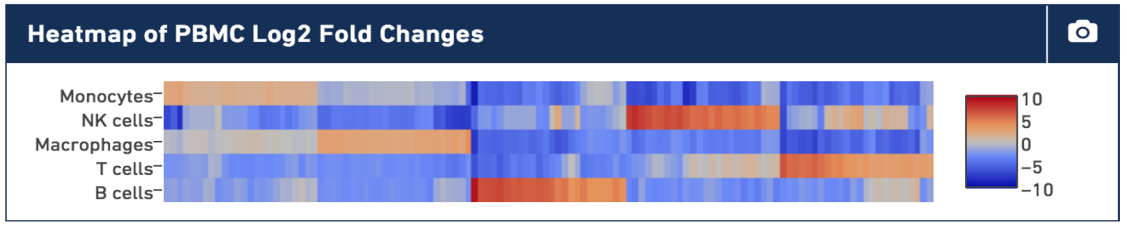
MilliporeSigma

Compatible PRODUCTS | 10x GENOMICS

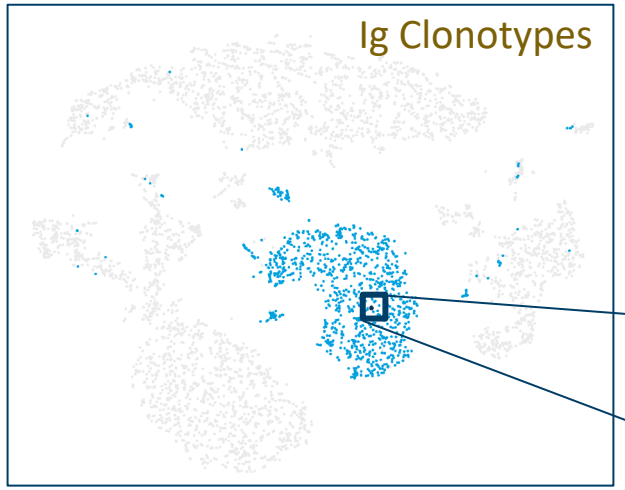
Assess Cell Type Heterogeneity and the Immune Repertoire Mouse PBMCs

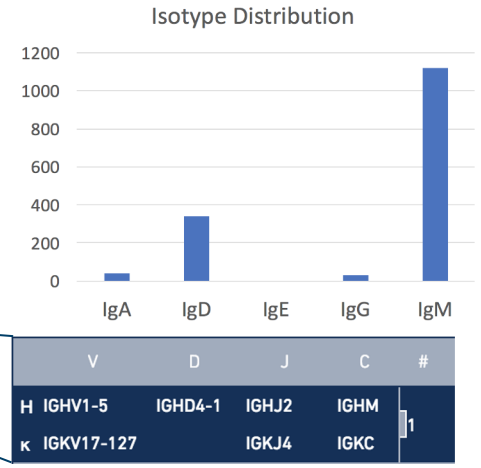
5’ Gene Expression

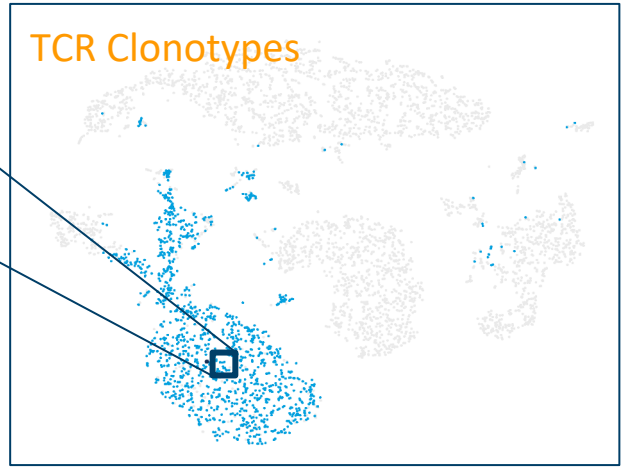




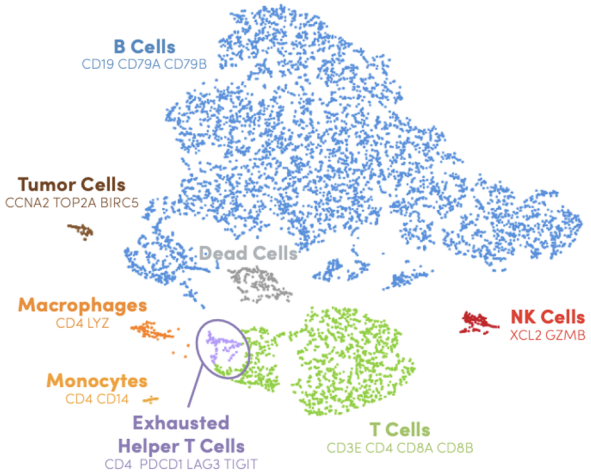
V(D)J clonotypes overlapped with GEX

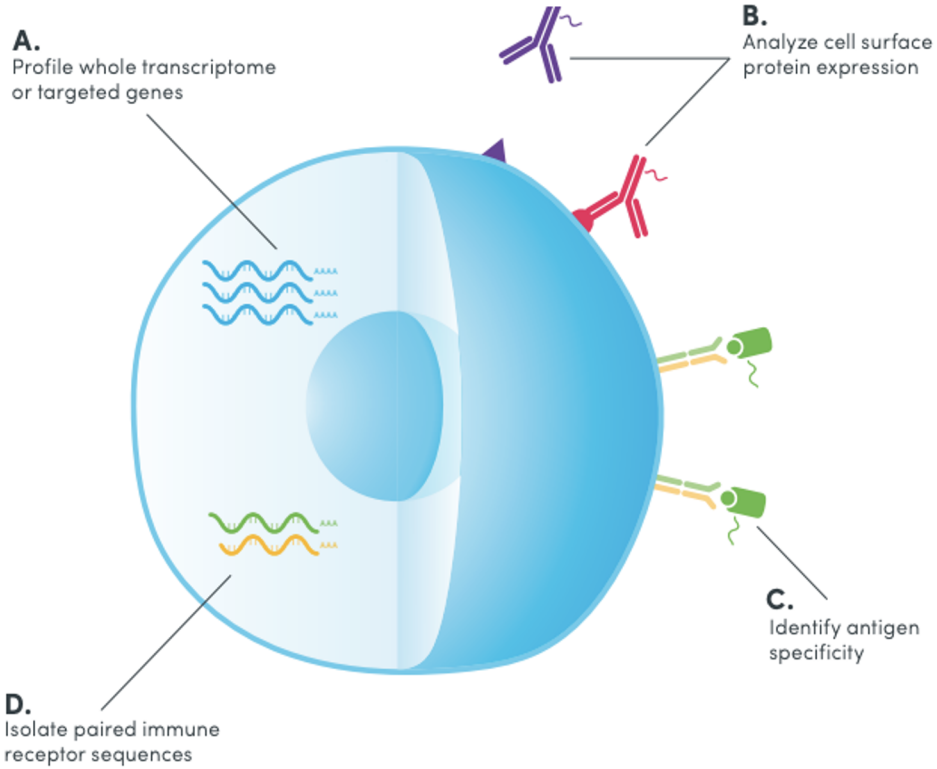


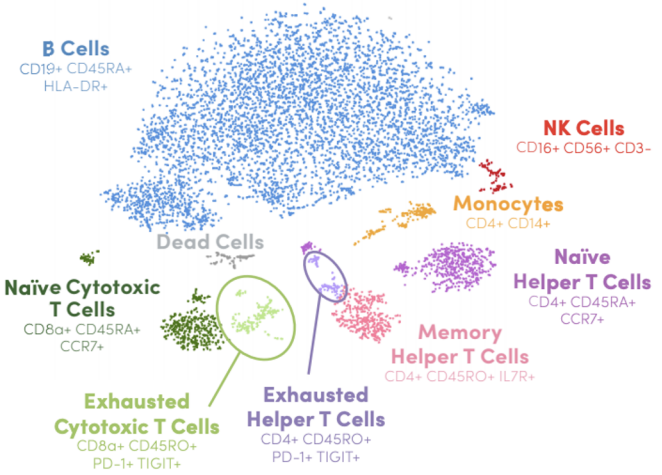


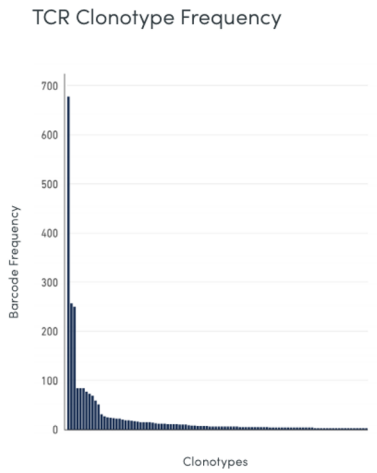


Multiomic solution to study immunology







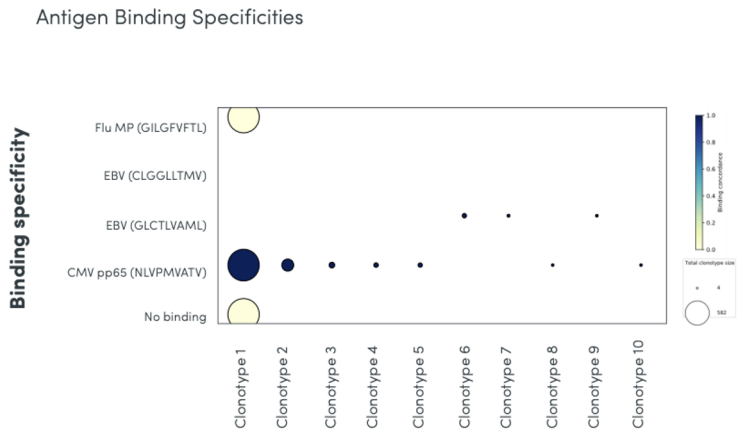


TCR Alpha

TGGGGAGTCCACAGTTAGGCAGCGCACTTCTGAAAGGGTGTCAATTCTCGTTGTGTTCCACACTCATGGGGGATTTCCTCAAAGTTTATGAAGTTGGCCTGAAGA
AAGCAGTTACTTCGACCCCTCTTTGTGAACCACAGGAAACTGAACATAGGGCAGAGCGTTTCTAGGACATTTATGGGAGCTGGTCTCTGATTGGCTAGGCAACCA
GATGCAGAATCCTGTTTTCTGAAGCAGAATATTTCCACAGATTTTGGCTGCAAAACGTTTTTCTGCTGTGGGTACGTGAGCAGGAAACATGGAGAAGAATCCTTTG
GCAGCCCCATTACTAATCCTCTGGTTTCATCTTGACTGCGTGAGCAGCATACTGAACGTGGAACAAAGTCCTCAGTCACTGCATGTTCAGGAGGGAGACAGCACC
AATTTCACCTGCAGCTTCCCTTCCAGCAATTTTATGCCTTACACTGGTACAGATGGGAAACTGCAAAAAGCCCCGAGGCCTTGTTTGTAATGACTTTAAATGGGG
ATGAAAAGAAGAAAGGACGAATAAGTGCCACTCTTAATACCAAGGAGGGTTACAGCTATTTGTACATCAAAGGATCCCAGCCTGAAGACTCAGCCACATACCTCTG
TGCCTTCATCACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGTCATTCCAAATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGAC

TCR Beta

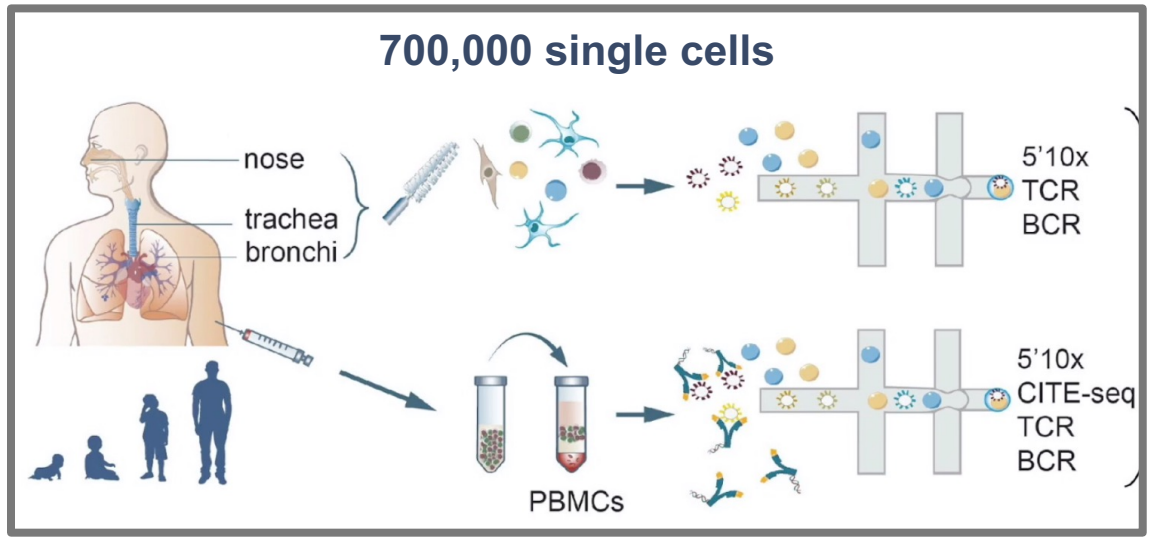
GAGAGTCCTGCTCCCCTTTCATCAATGCACAGATACAGAAGACCCCTCCGTCATGCAGCATCTGCCATGAGCATCGGCCTCCTGTGCTGTGCAGCCTTGTCTCTC
CTGTGGGCAGGTCCAGTGAATGCTGGTGTCACTCAGACCCCAAAATTCCAGGTCCTGAAGACAGGACAGACATGACACTGCAGTGTGCCCAGGATATGAACCA
TGAATACATGTCCTGGTATCGACAAGACCCAGGCATGGGGCTGAGGCTGATTCATTACTCAGTTGGTGCTGGTATCACTGACCAAGGAGAAGTCCCCAATGGCTA
CAATGTCTCCAGATCAACCACAGAGGATTTCCGGCTCAGGCTGCTGTCGGCTGCTCCCTCCAGACATCTGTGTACTTCTGTGCCAGCAGCCTGGTTACCGGGA
CAGGGGCATACGGCTACACGTTGGGTTCGGGGACCAGGTTAACGGTTGTAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGA

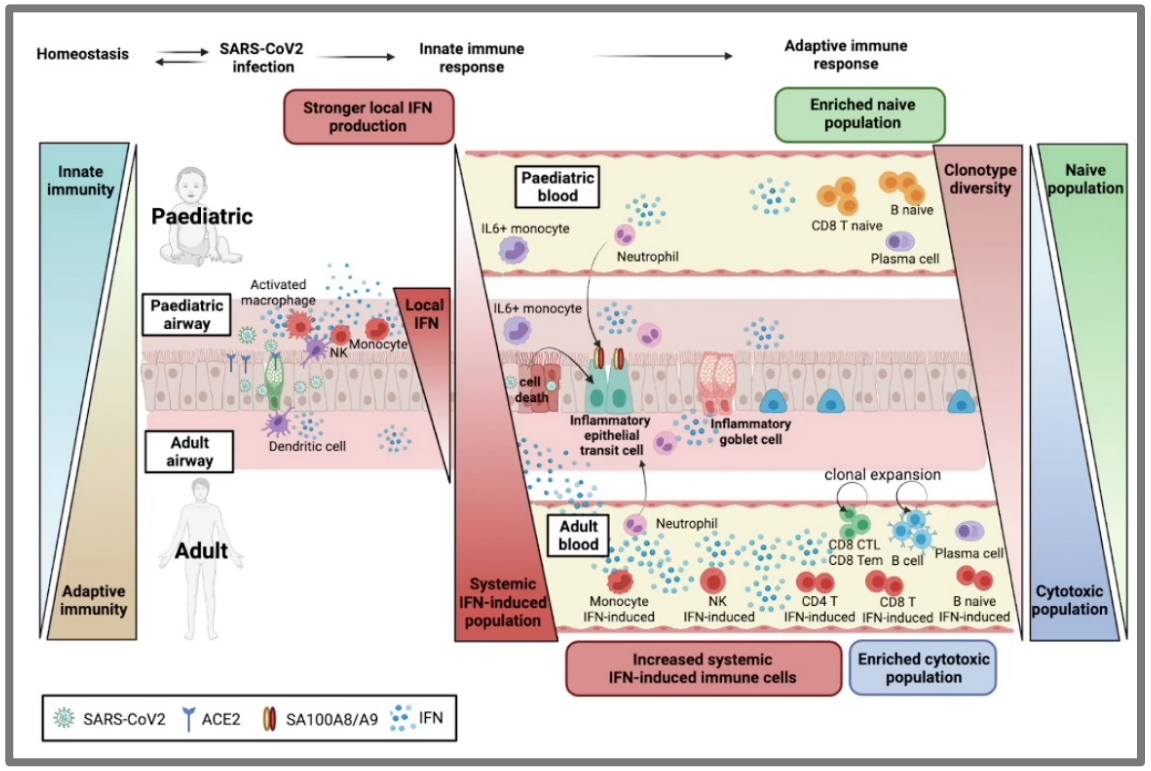


10X GENOMICS

The local and systemic response to SARS-CoV-2 infection in children

Yoshida et al., Nature, 2021



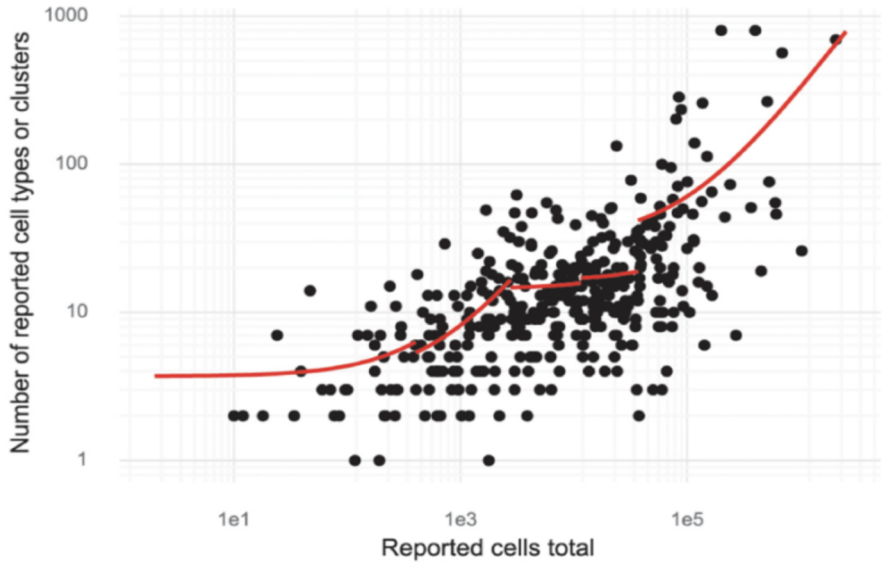


Pediatric-specific COVID19 response that prevent severe disease:

- Protective pre-activated IFN state of airway
- Higher immune repertoire diversity
- Naive and less cytotoxic immune compartment
- Absence of systemic interferon stimulation

https://doi.org/10.1038/s41586-021-04345-x

Paradigm-shifting studies require increased scale

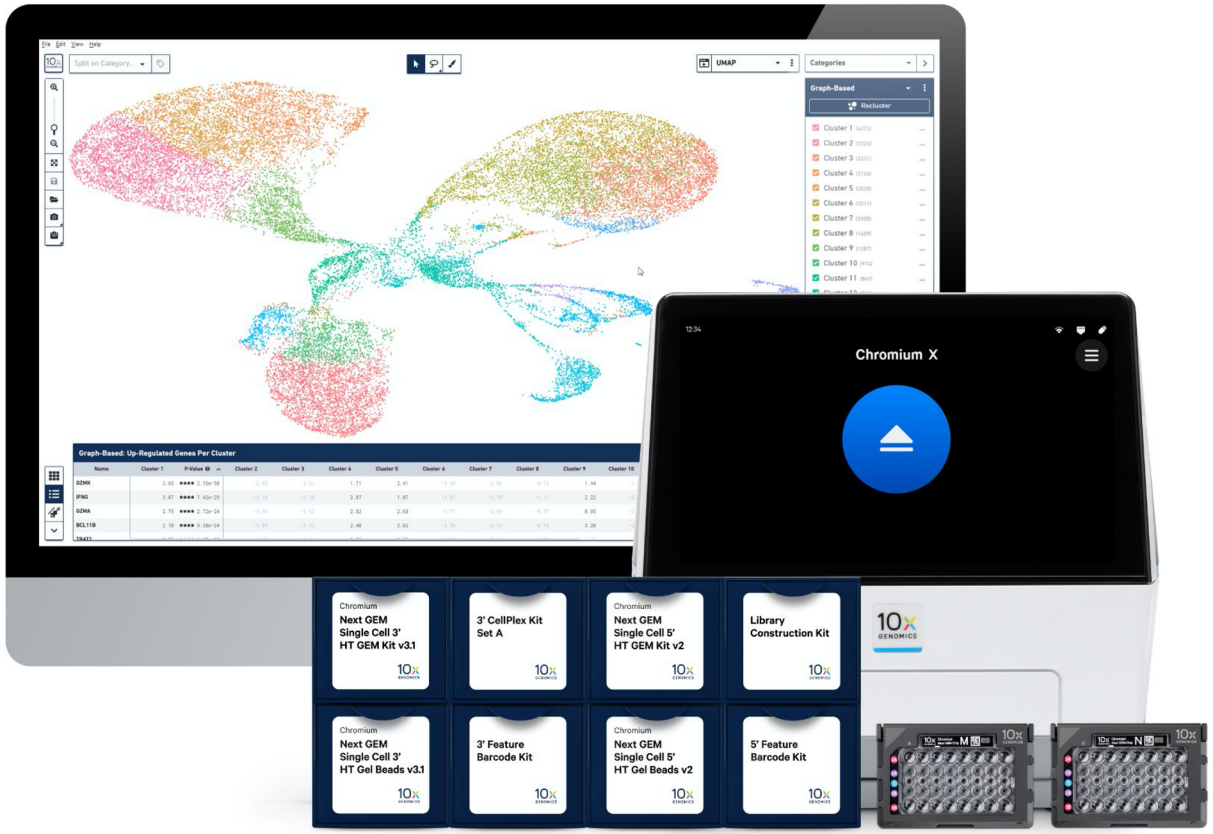


- Single cell publications have increased in cell number
- More cells > greater insights > more biology
- However, getting to very high cell numbers is challenging:
  - Profiling millions of cells is prohibitively expensive
  - Existing workflows for scaling are cumbersome, time-consuming and difficult to execute
- Robust methods for scaling up in a cost-effective manner are needed for science to move forward

A curated database reveals trends in single cell transcriptomics
Valentine Svensson, Eduardo da Veiga Beltrame, & Lior Pachter
Database, Volume 2020, 2020, baaa073; doi: 10.1093/database/baaa073

Introducing...the Chromium X and Single Cell HT

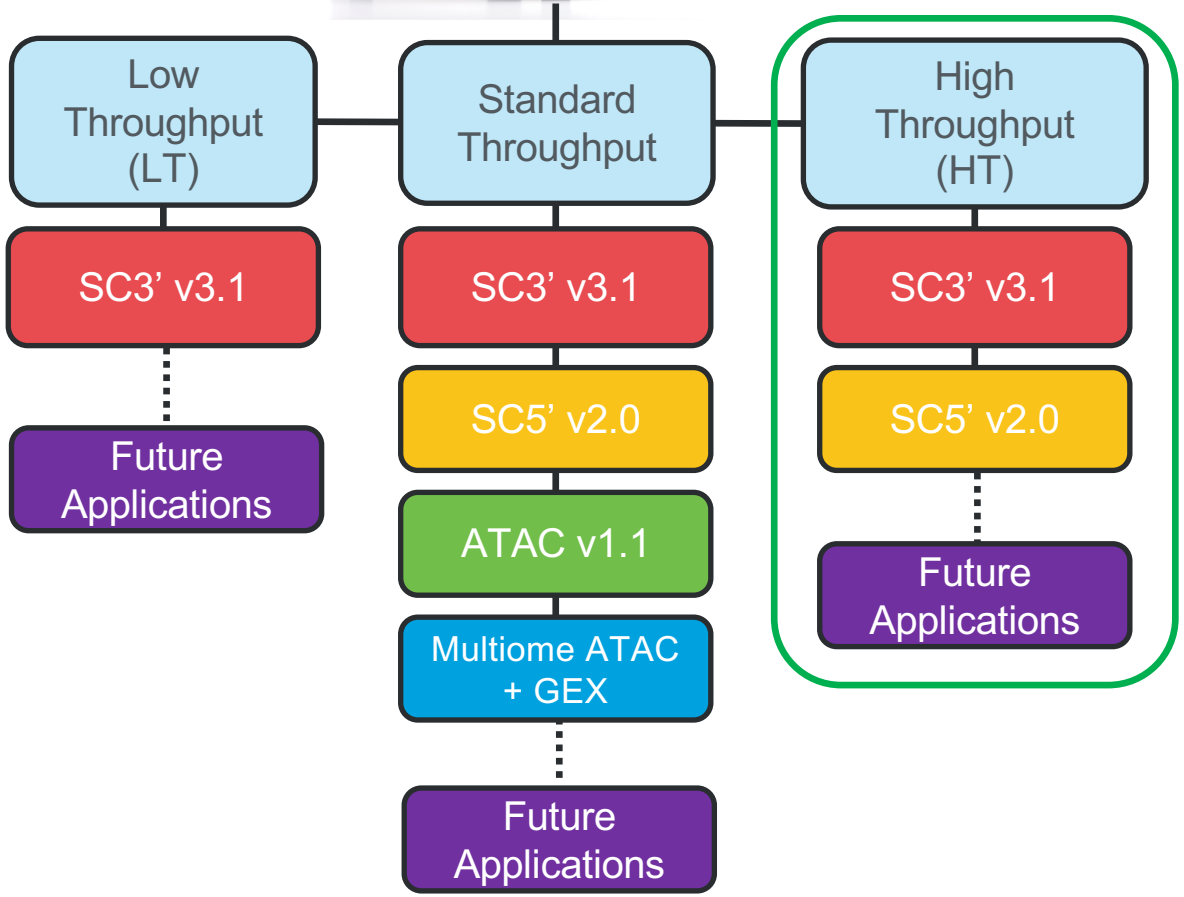
TheScientist TOP 10 INNOVATIONS



- Chromium X + high-throughput (HT) assays offer:
  - Single cell experiments at scale, with an up to 7-fold reduction in cost per cell
  - Profiling transcripts of hundreds of thousands to millions of cells with high-dimensional multiomic outputs
  - Robust, fully supported, end to end workflow solutions
- Available now!

10x GENOMICS

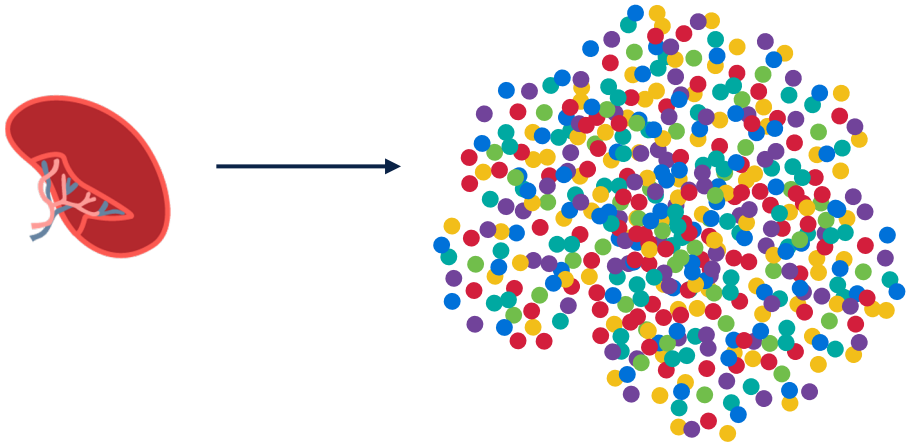
Our most flexible single cell instrument to date



10X GENOMICS

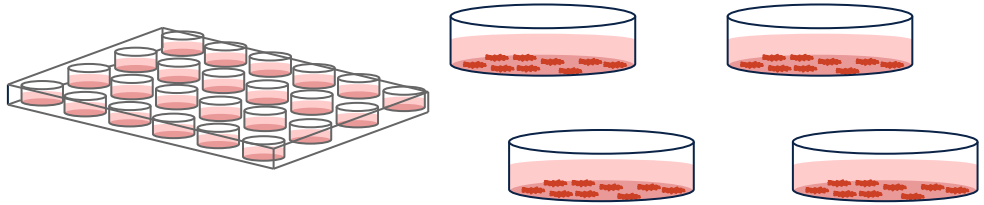
Two types of high throughput

Large number of cells



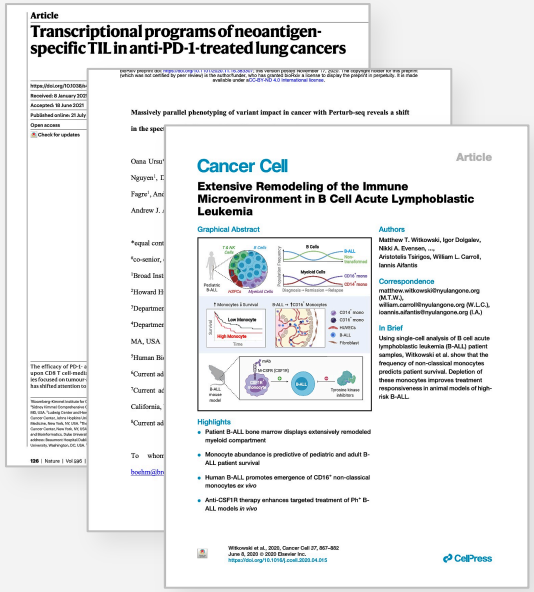
A few samples at a time, focus on high cell recovery

Large number of samples

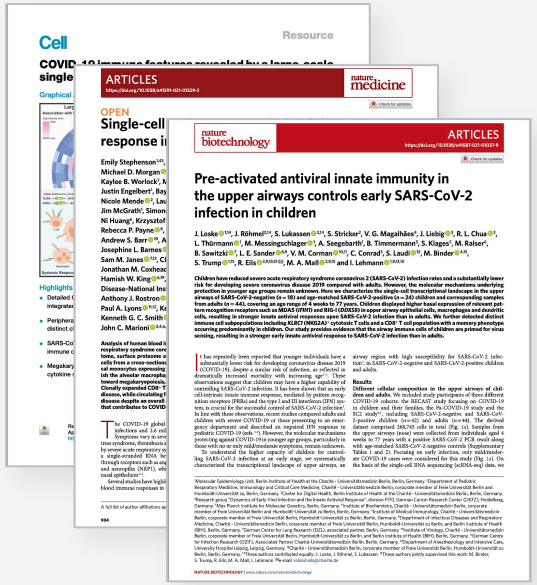


More samples, easily prepared at the same time

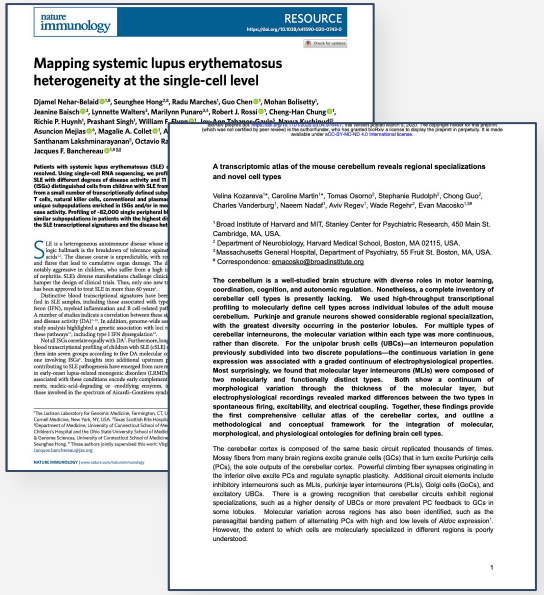
Application segment



Oncology



Infectious disease

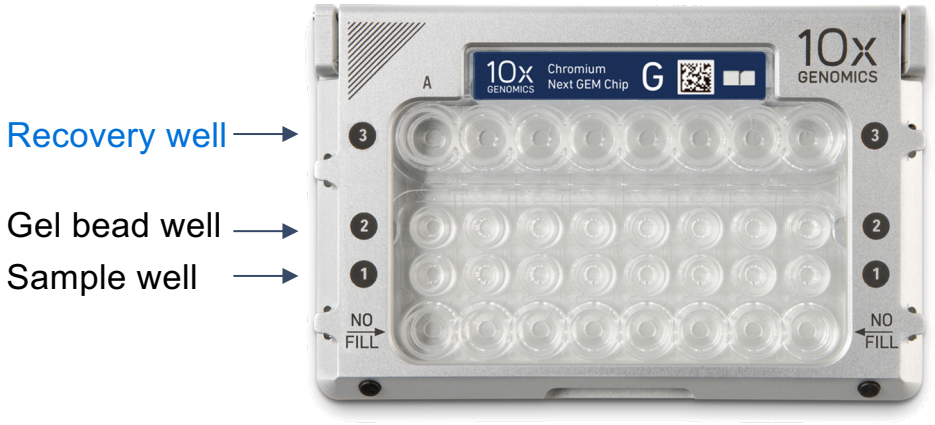


Immune disorder

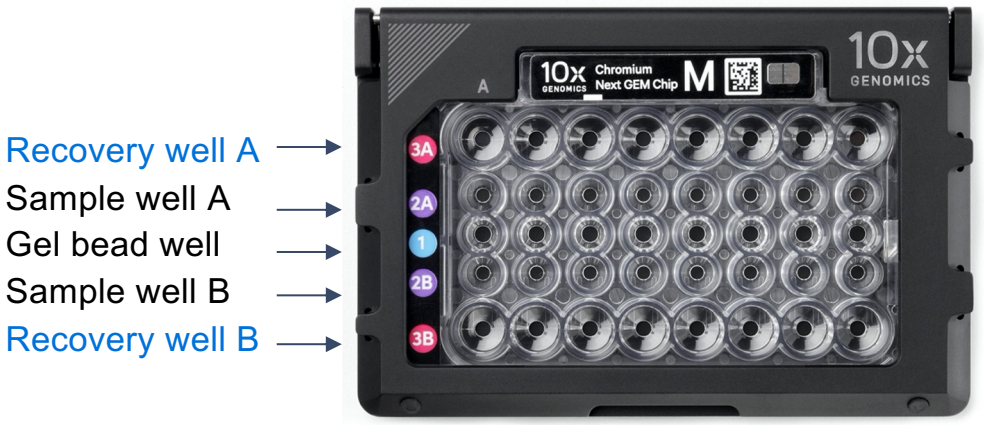
Neuroscience

New HT chip allows for significant increase in throughput

Chromium Next GEM Chip G (standard 3’ assay)

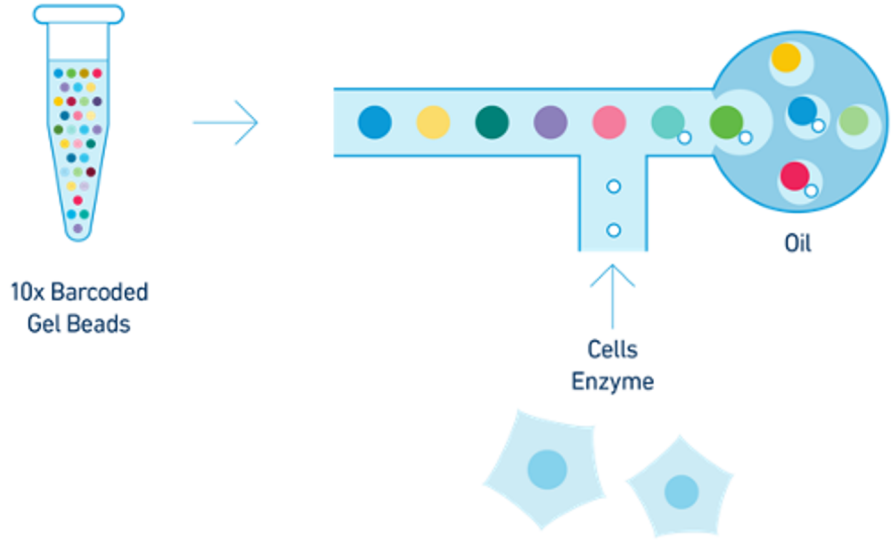


Chromium Next GEM Chip M (HT 3’ assay)



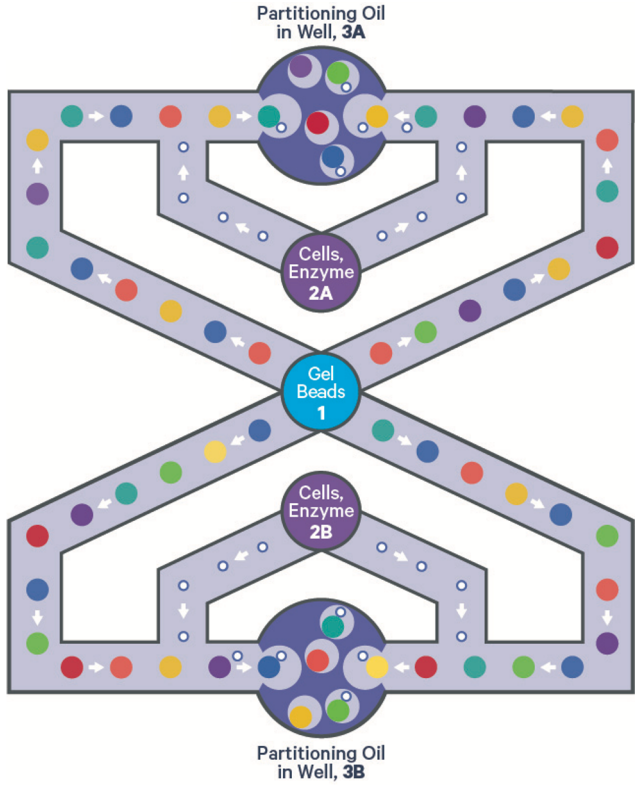
Comparing standard and HT single cell assays

Standard GEM Generation



Inputs:
10x Genomics gel beads, reagents and single cells in suspension

HT GEM Generation



Comparing standard and HT single cell assays

Assay-level specifications without sample multiplexing

Sensitivity, mapping rates, gene length distributions, library quality, etc. are similar

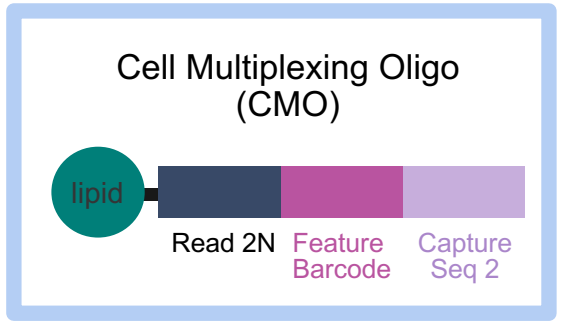
	Standard assay		HT assay	
Supported Dynamic Range (per sample input well)	500 – 10,000		2,000 – 20,000*	
Optimal Cell Stock Concentration (cells/µL)	700 - 1,200			
Cell Multiplet Rate per 1,000 cells	~0.8%		~0.4%	
Cell Recovery Efficiency	Up to 65%			
Number of GEMs per recovery well	~100k		~200K	
Number of reactions per kit	4 rxn	16 rxn	8 rxn	48 rxn
Number of chips per kit	2	6	1	5

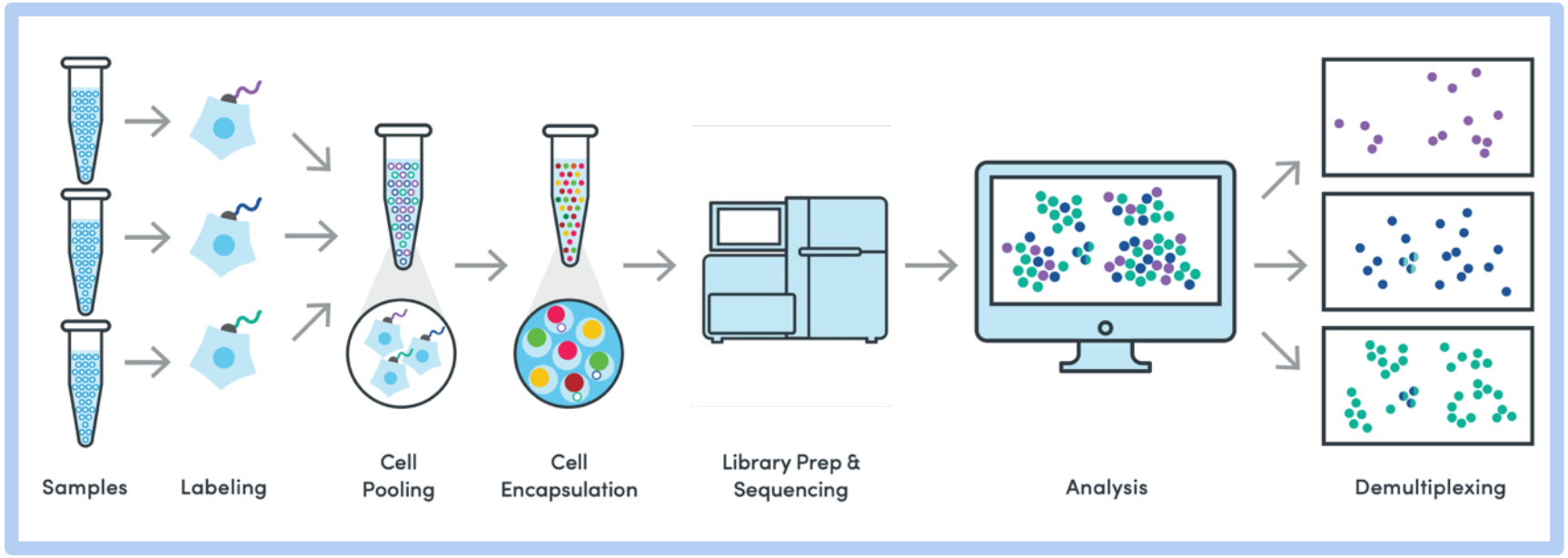
Up to 320,000 cells/chip

Cell multiplexing

Increase experimental efficiency

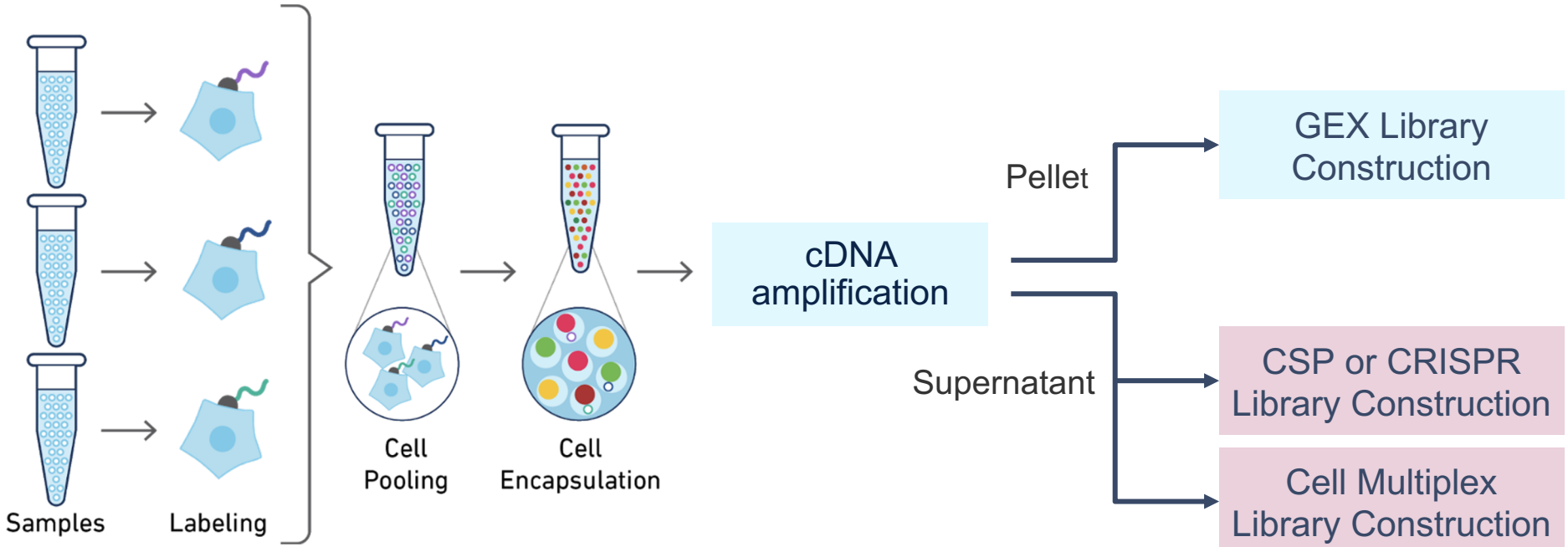
- Label cells (or nuclei) with lipids conjugated to Feature Barcode oligos (Cell Multiplexing Oligos)
- Pool labeled cells/nuclei together to run in a single well of a 10x Genomics chip (pool up to 12 samples)
- Load more cells in a single well, bioinformatically filtering out cell multiplets (target up to 60,000 cells with 3' HT v3.1)





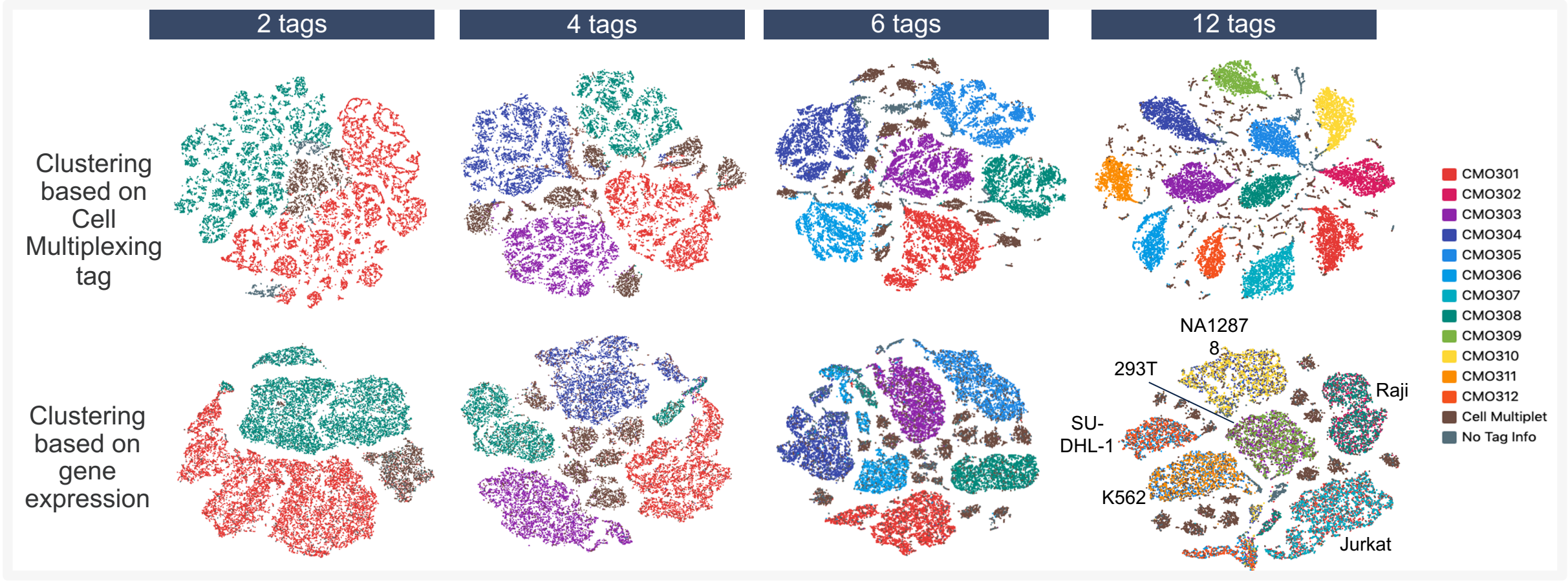
3’ CellPlex workflow

Highly similar to existing Feature Barcode workflows



Cell multiplexing demonstration

12 CMO tags with 6 human cell lines

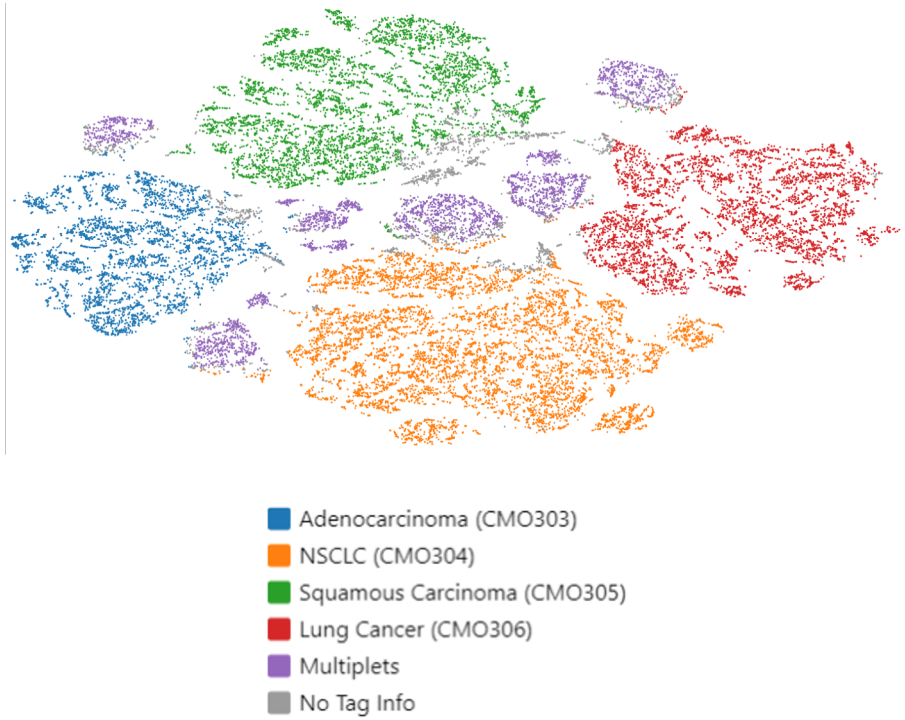


Colored based on tag assignment
Targeted cell recovery = 30,000

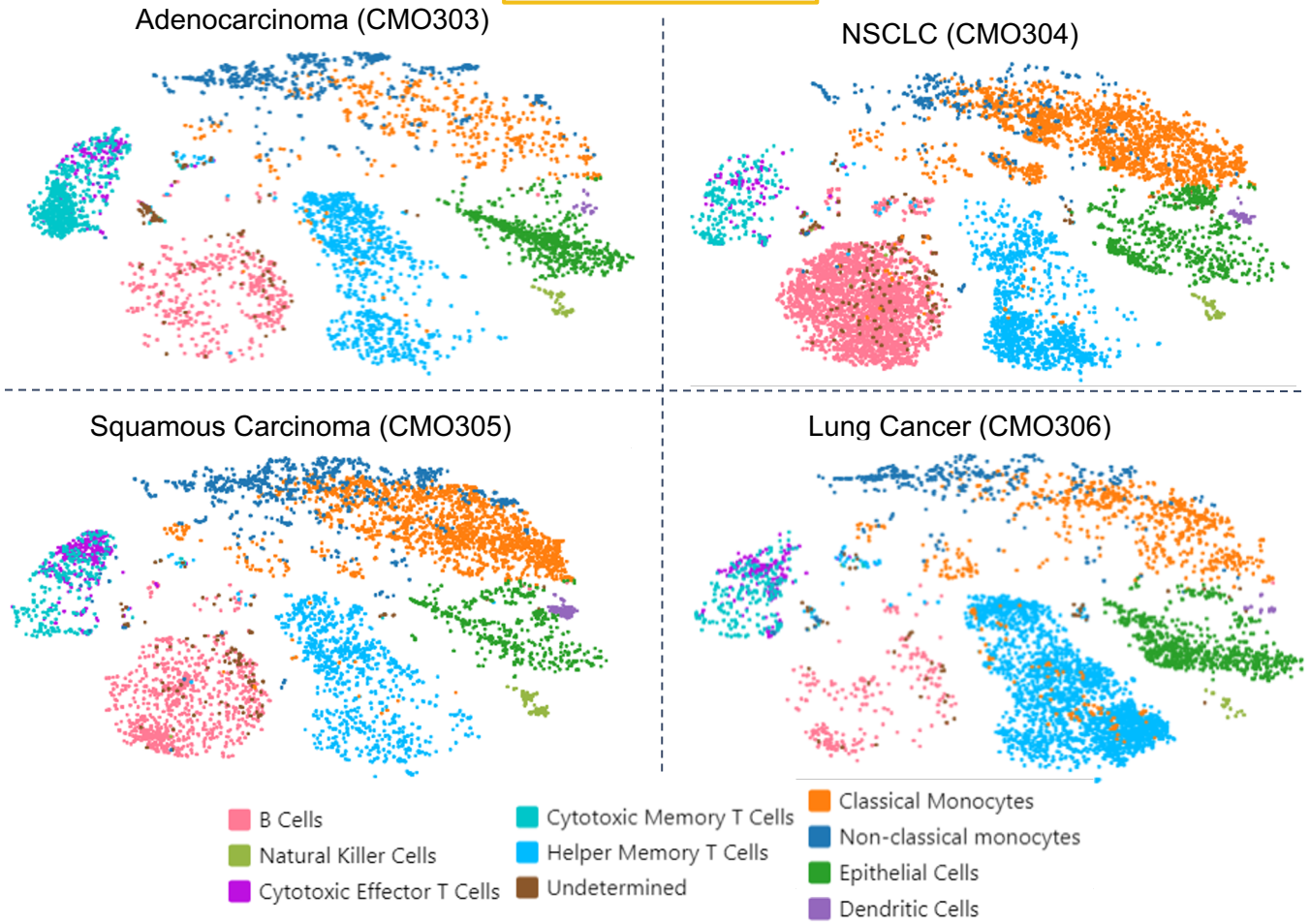
Compare differences in sample composition with multiomic data

3’ CellPlex enables multiplexing with multiomic workflows

Tag Clustering

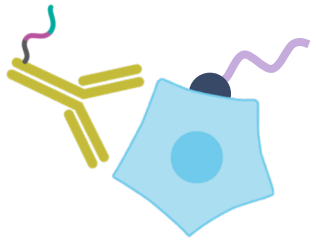


Antibody Clustering

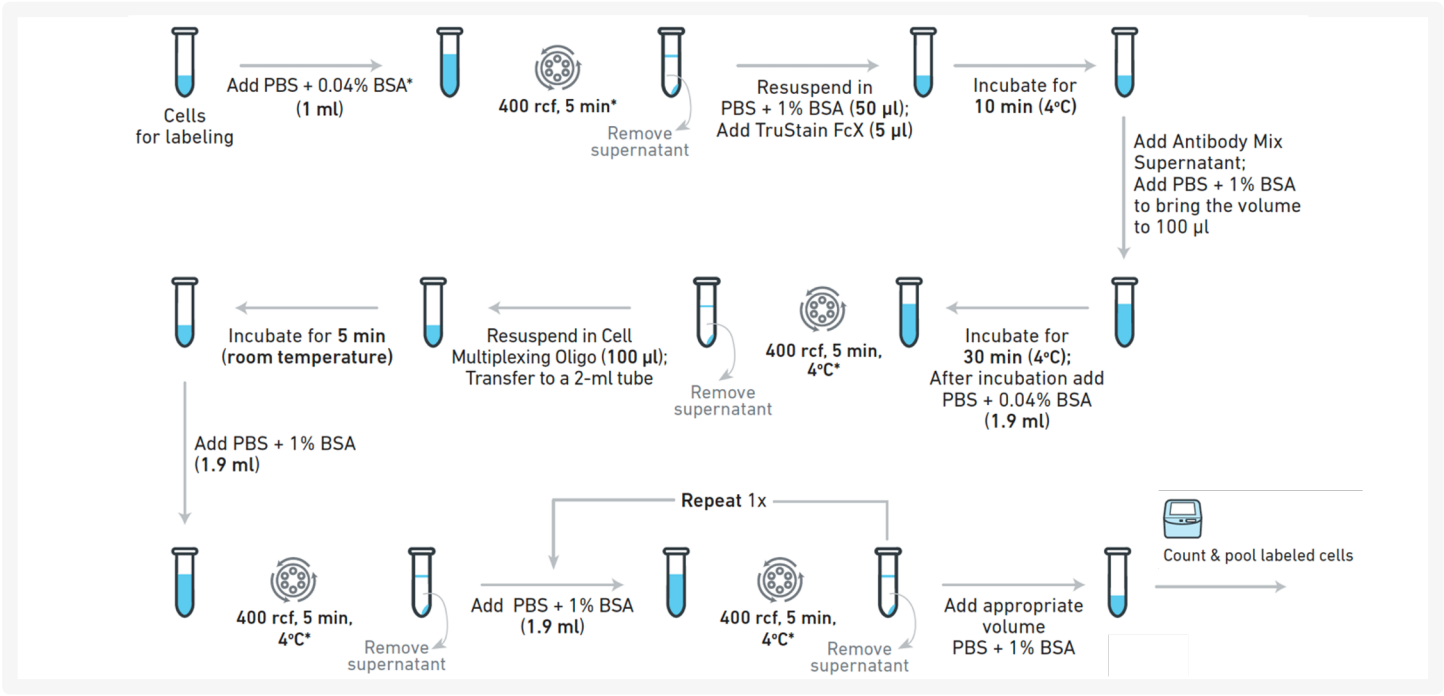


Demonstrated protocol for CMO labeling

Labeling cells with CMO & antibodies

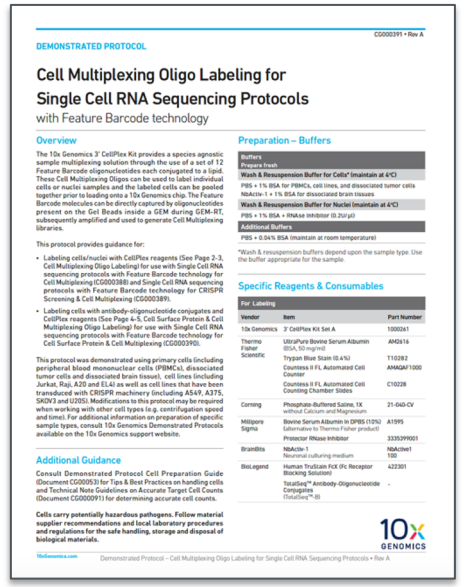


- Label cells with oligo-conjugated antibodies for 30 min
- Label cells in CMO for 5 min
- Wash to remove unbound antibodies and CMO



CG000391

Cell Multiplexing Oligo Labeling for Single Cell RNA Sequencing Protocols


DEMONSTRATED PROTOCOL

Cell Multiplexing Oligo Labeling for Single Cell RNA Sequencing Protocols

with Feature Barcode technology

Comparing standard and HT single cell assays

Assay-level specifications with sample multiplexing

Sensitivity, mapping rates, gene length distributions, library quality, etc. are similar

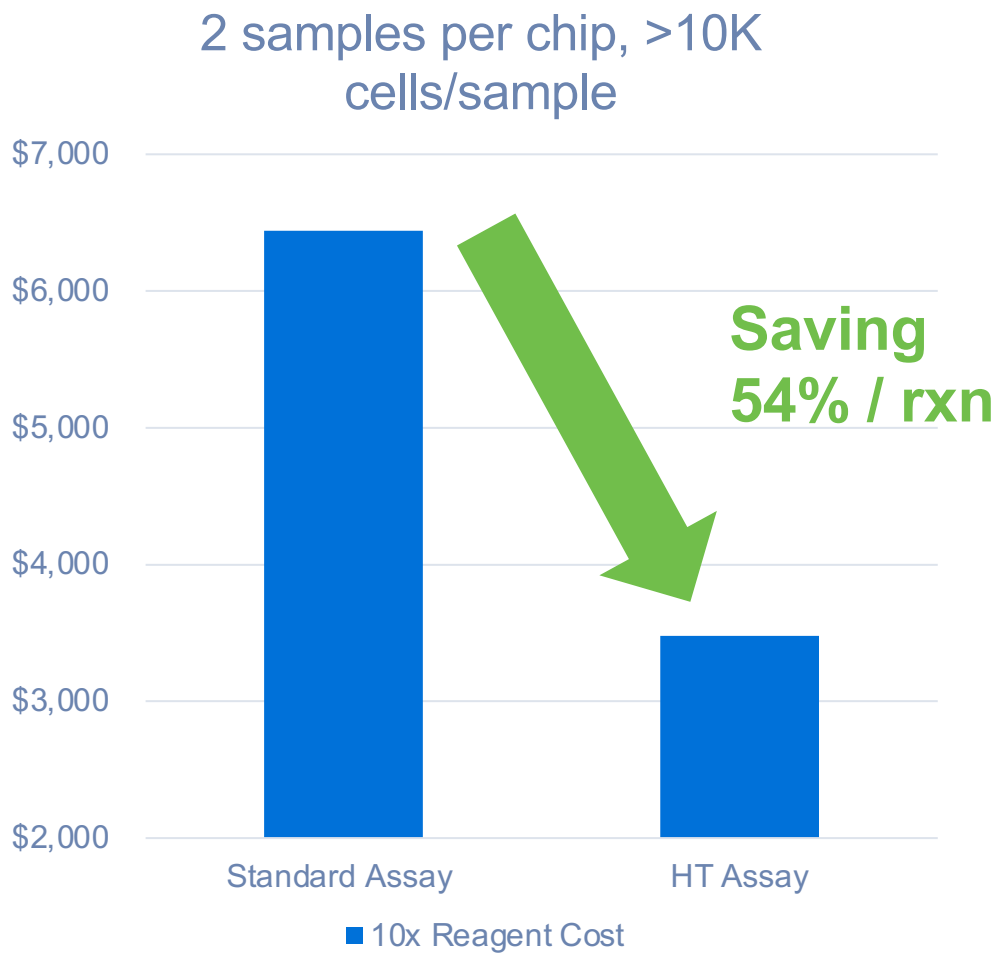
	Standard assay		HT assay	
Supported Dynamic Range (per sample input well)	500 – 30,000		2,000 – 60,000*	
Singlets per sample input	~500 - 17,700		~1,980 - 45,600	
Optimal Cell Stock Concentration (cells/µL)	1,300 - 1,600			
Cell Multiplet Rate per 1,000 cells	~0.8%		~0.4%	
Cell Recovery Efficiency	Up to 65%			
Number of GEMs per recovery well	~100k		~200K	
Number of reactions per kit	4 rxn	16 rxn	8 rxn	48 rxn
Number of chips per kit	2	6	1	5

Up to 960,000 cells/chip

10X GENOMICS

Break-even point between HT and Standard Assays

- Start saving approximately in 10x reagents cost when either scenario occurs:
  - Scenario #1 (see graph)
    - Running 2 samples/chip *and*
    - >10,000 cells sample
  - Scenario #2
    - Running 4 samples/chip *and*
    - Any number of cells/sample
- Plus, 2X cells compared to ST
- These scenarios *do not* require combining samples with CellPlex



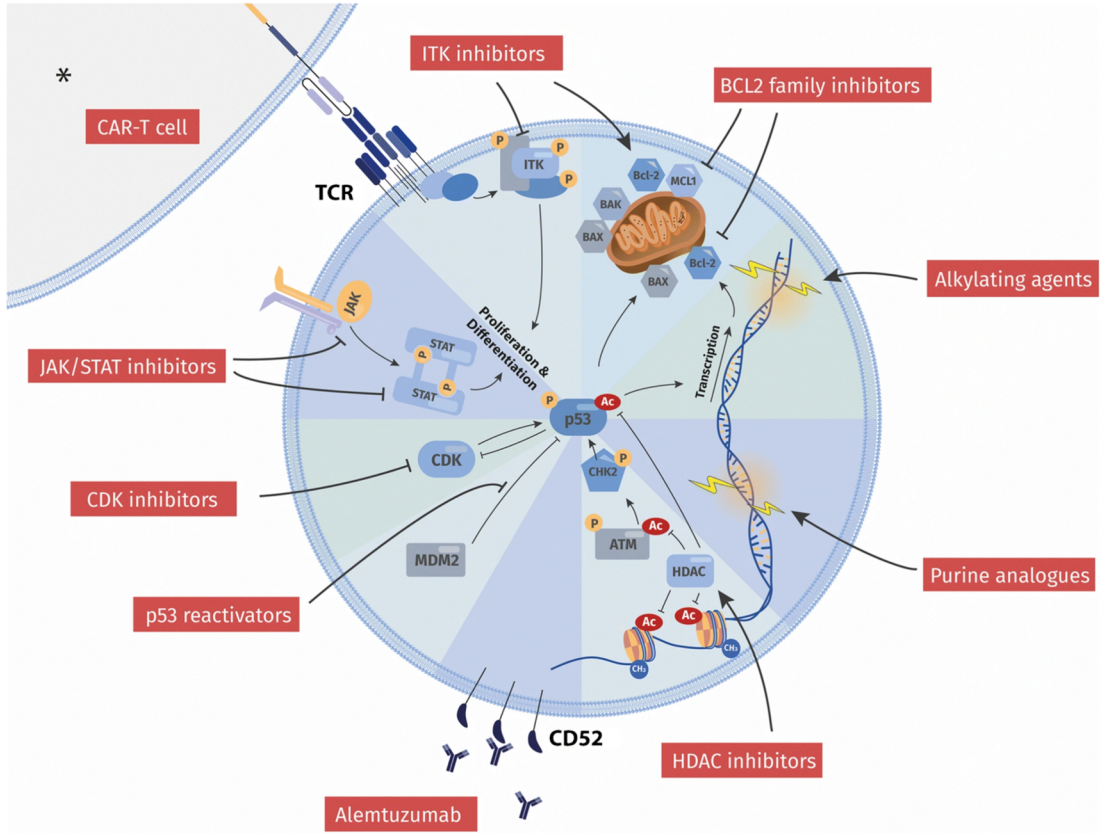
Maximizing multiomic insights with Single Cell HT

Highlighting Chromium Next GEM Single Cell 5' HT v2

10x GENOMICS

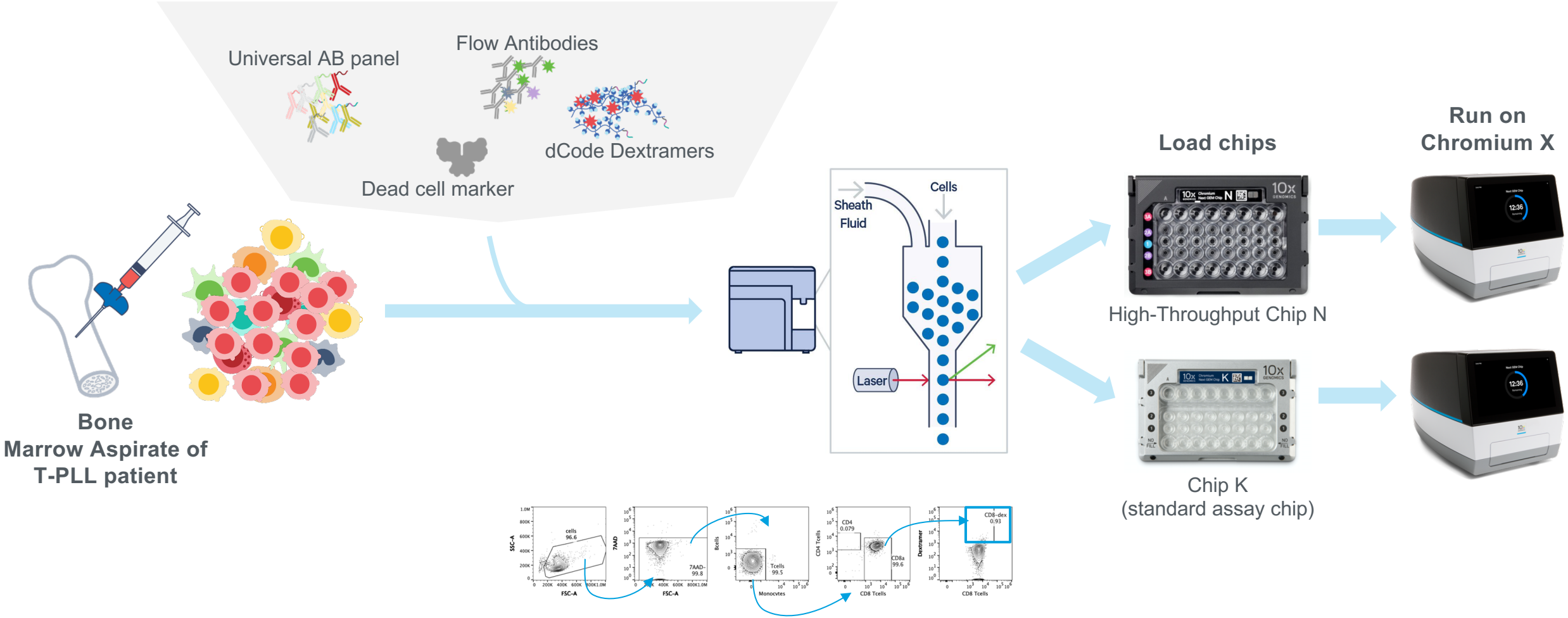
Why study T-PLL?

- T-cell prolymphocytic leukemia (T-PLL) is a rare and aggressive cancer that is characterized by the out of control growth of mature T-cells
- Only 10-20% of patients treated with standard treatments reach long-term remission
- Median survival after diagnosis is < 3 years
- Novel strategies are needed in order to improve clinical outcomes



Braun, T., von Jan, J., Wahnschaffe, L. *et al.* Advances and Perspectives in the Treatment of T-PLL. *Curr Hematol Malig Rep* 15, 113–124 (2020).

Using Single Cell Immune Profiling with Feature Barcode technology to detect TCR-antigen specificity

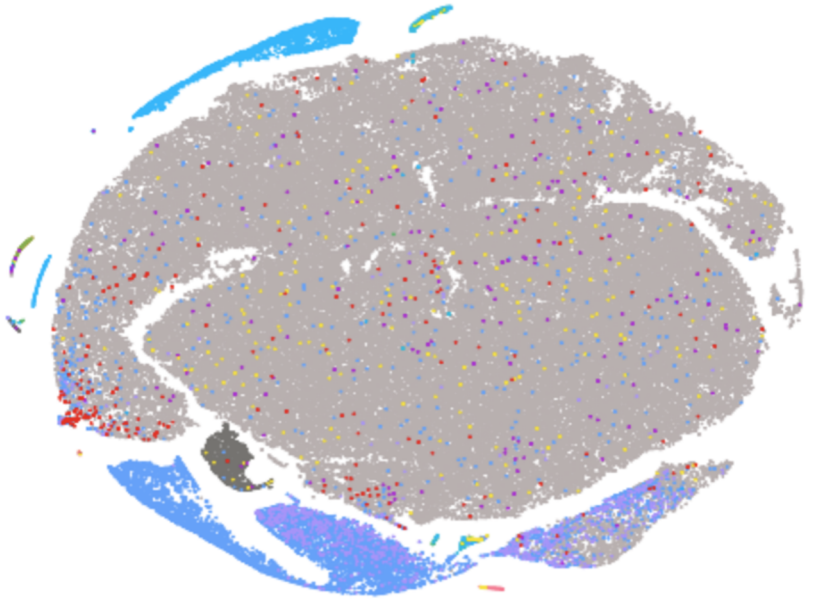


Patients with T-PLL suffer from uncontrolled T cell growth

B Memory (262)
B Naive (1715)
Dendritic classic (405)
Dendritic plasmacytoid (72)
Doublets (2321)
HSC (25)
MAIT (253)
Monocytes CD14 (2491)
Monocytes CD16 (1090)
NK Cytotoxic (2330)
NK Immunoregulatory (407)
T gamma-delta (160)
Tc Effector (1002)
Tc Memory (1013)
Tc Naive (1067)
Th Effector (571)
Th Memory (7490)
Th Naive (3108)
CMP (11)
GMP (7)
MEP (27)
MLP (18)
MPP (5)

Healthy Bone Marrow

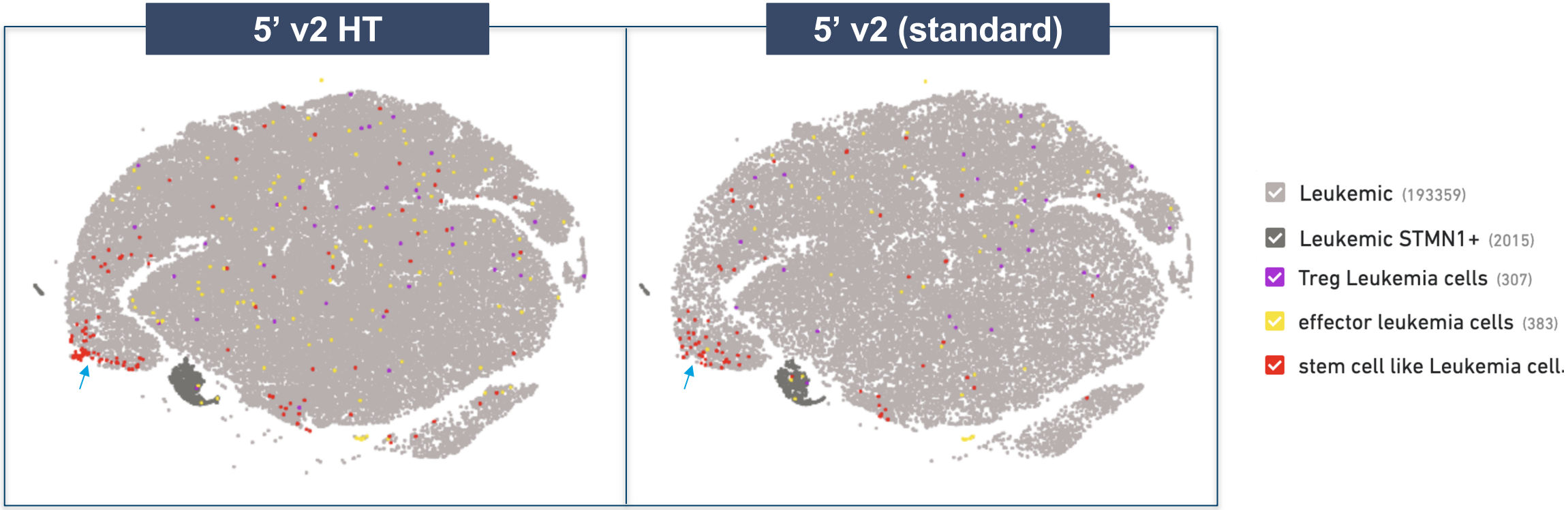
T-PLL sample



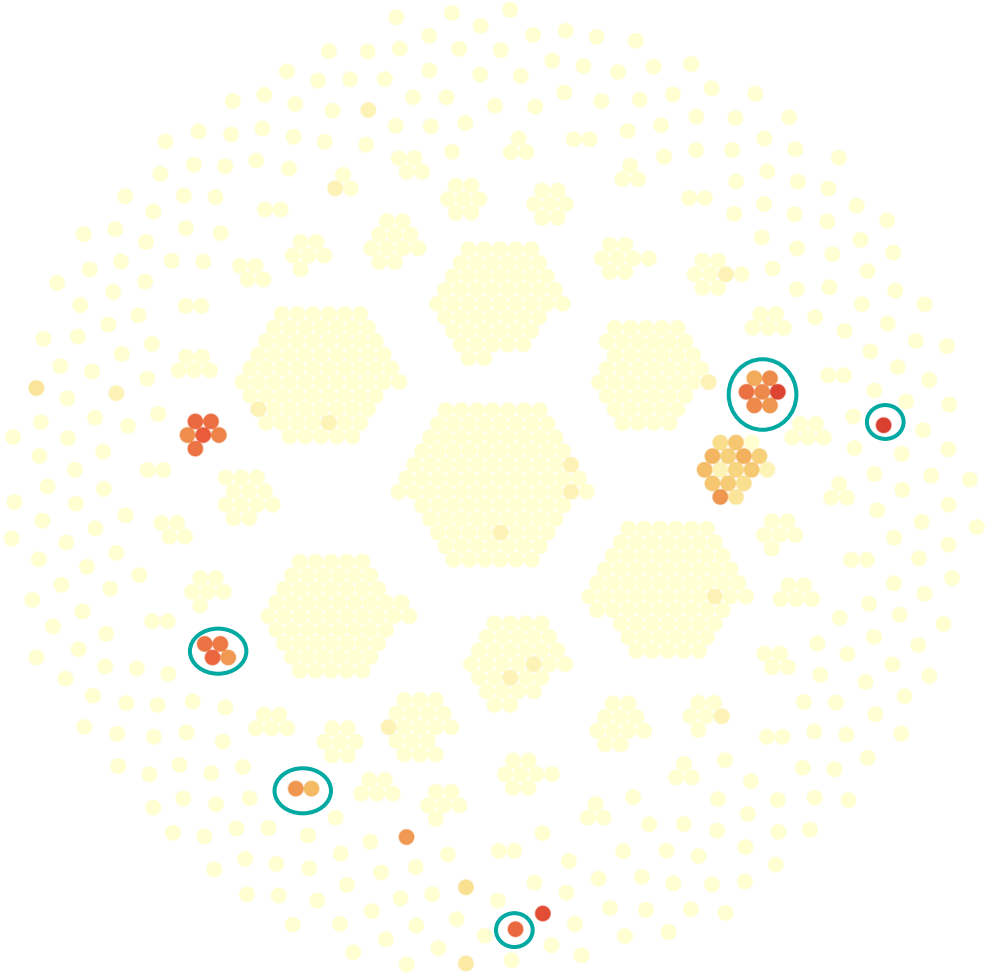
B (167)
Dendritic (69)
Leukemic (193359)
Leukemic STMN1+ (2015)
Monocytes_CD14 (245)
Monocytes_CD16 (66)
NK (332)
RBC (22)
T_DoubleNeg (106)
Tc_Effector (1040)
Tc_Memory (3284)
Tc_Naive (32)
Th_Memory (10271)
Th_Naive (1417)
Treg Leukemia cells (307)
effector leukemia cells (383)
stem cell like Leukemia cell

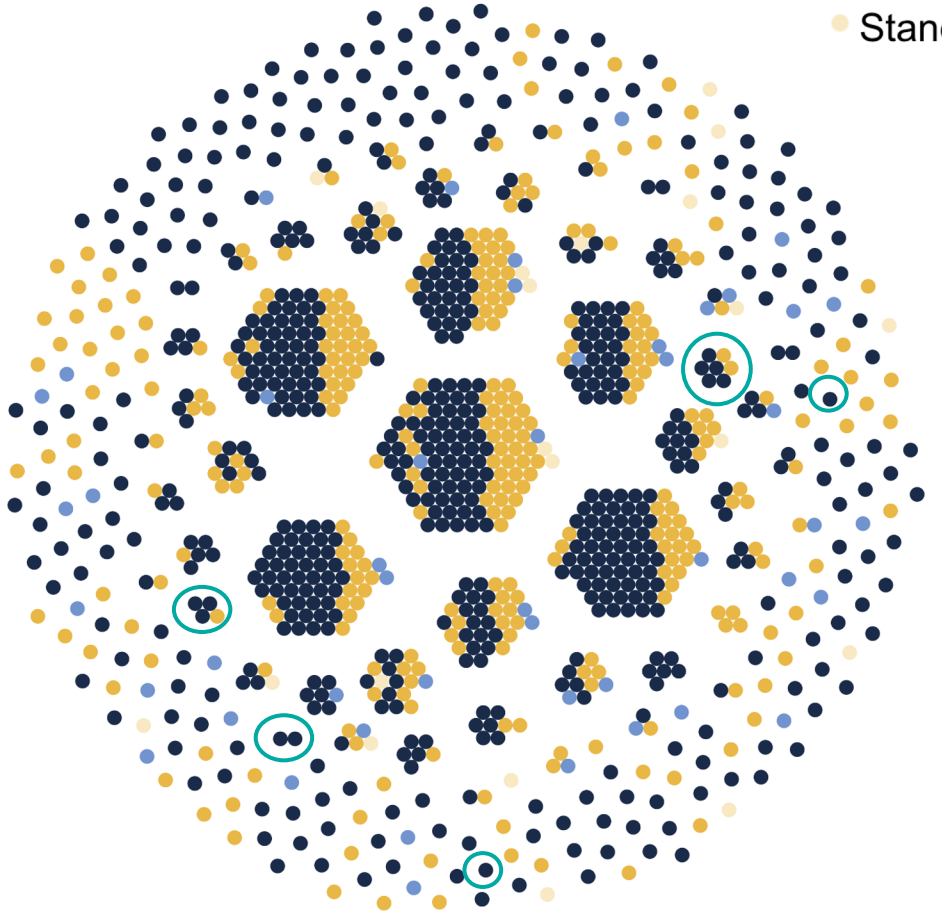
10X GENOMICS

Better detection of minor leukemia clones in HT



Higher cell throughput from the HT assay enables detection of rare TCR clonotypes



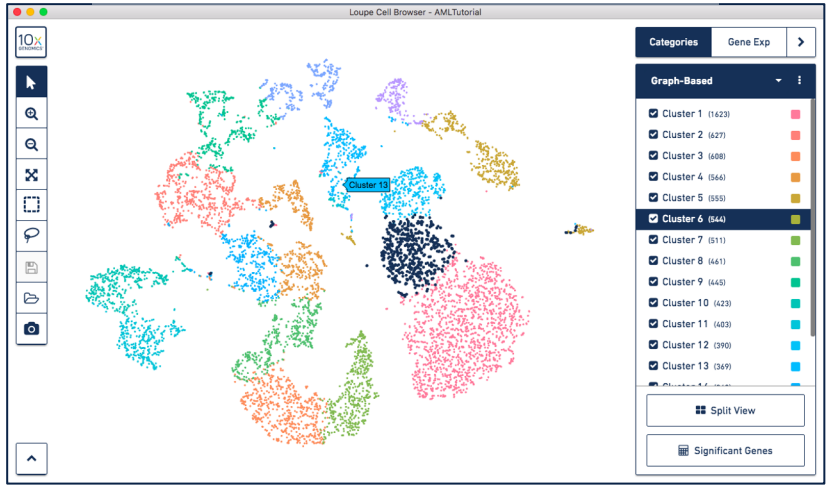


Circled cells/clonotypes are only identified (or are more confidently assigned as expanded and specific) in the HT data

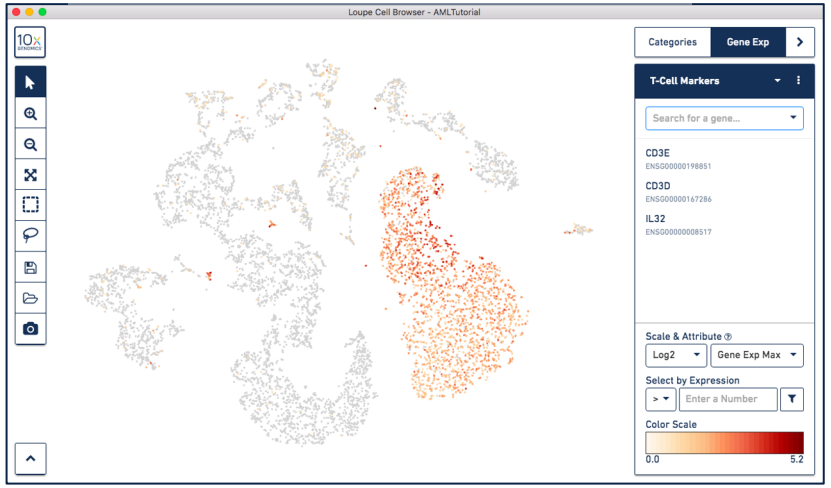
10X GENOMICS

Loupe Browser – analysis for everyone

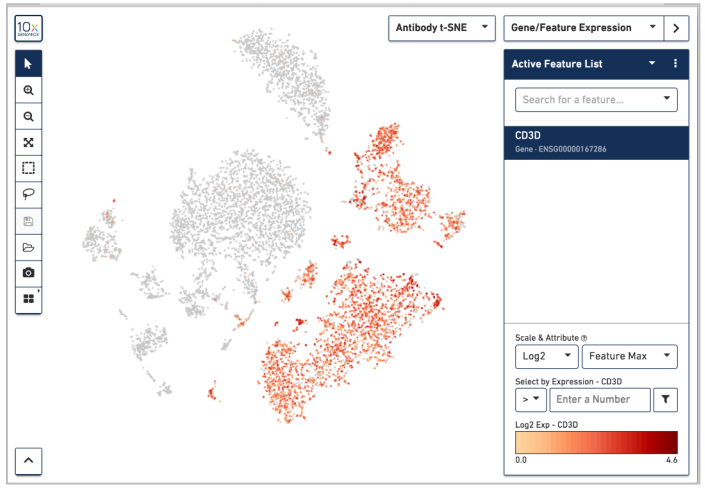
Precomputed GEX clusters



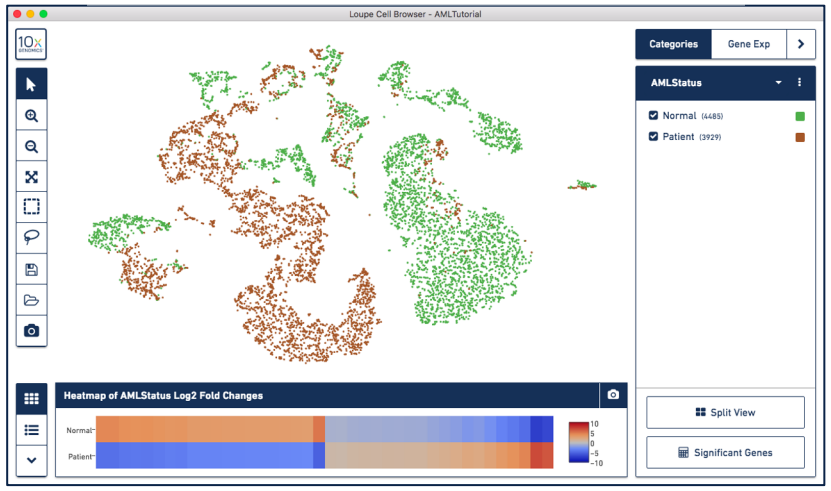
Gene expression level



Protein expression level

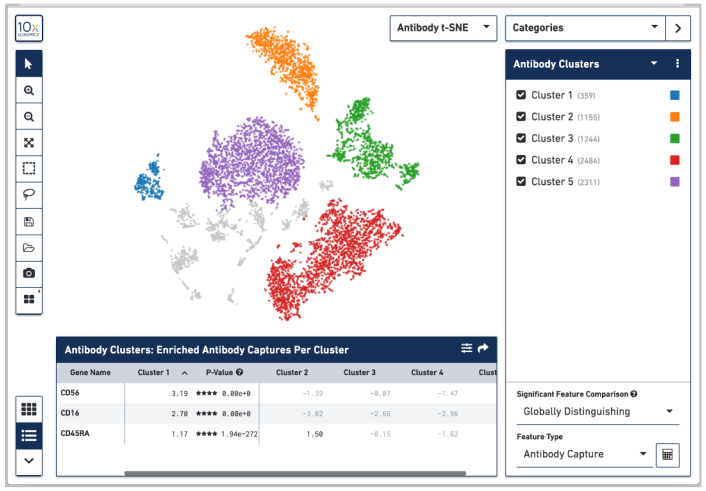


Experimental conditions



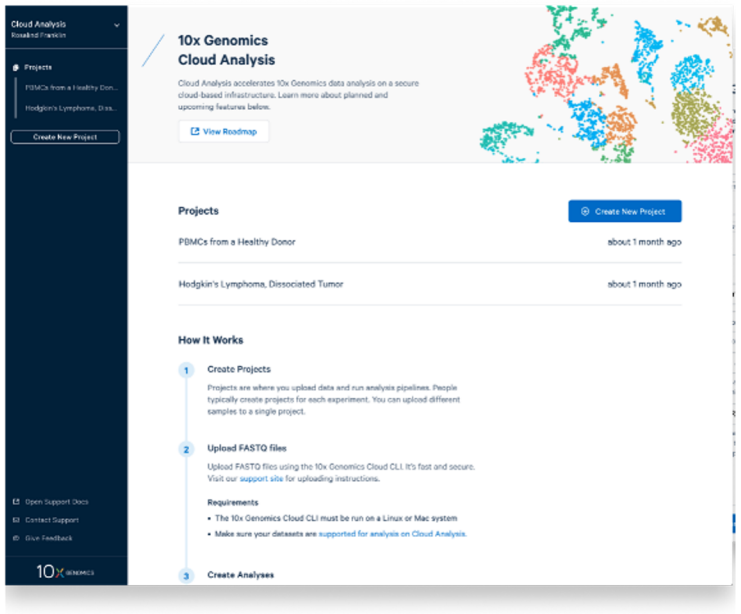
CRISPR guide Clusters

Precomputed protein clusters



10x Genomics Cloud Analysis

Accelerate your data analysis



- Quickly get started analyzing 10x data
- Standard set of analysis per sample at no additional cost[1]
- Scale your data analysis

Now available to U.S Customers

Visit the product page at

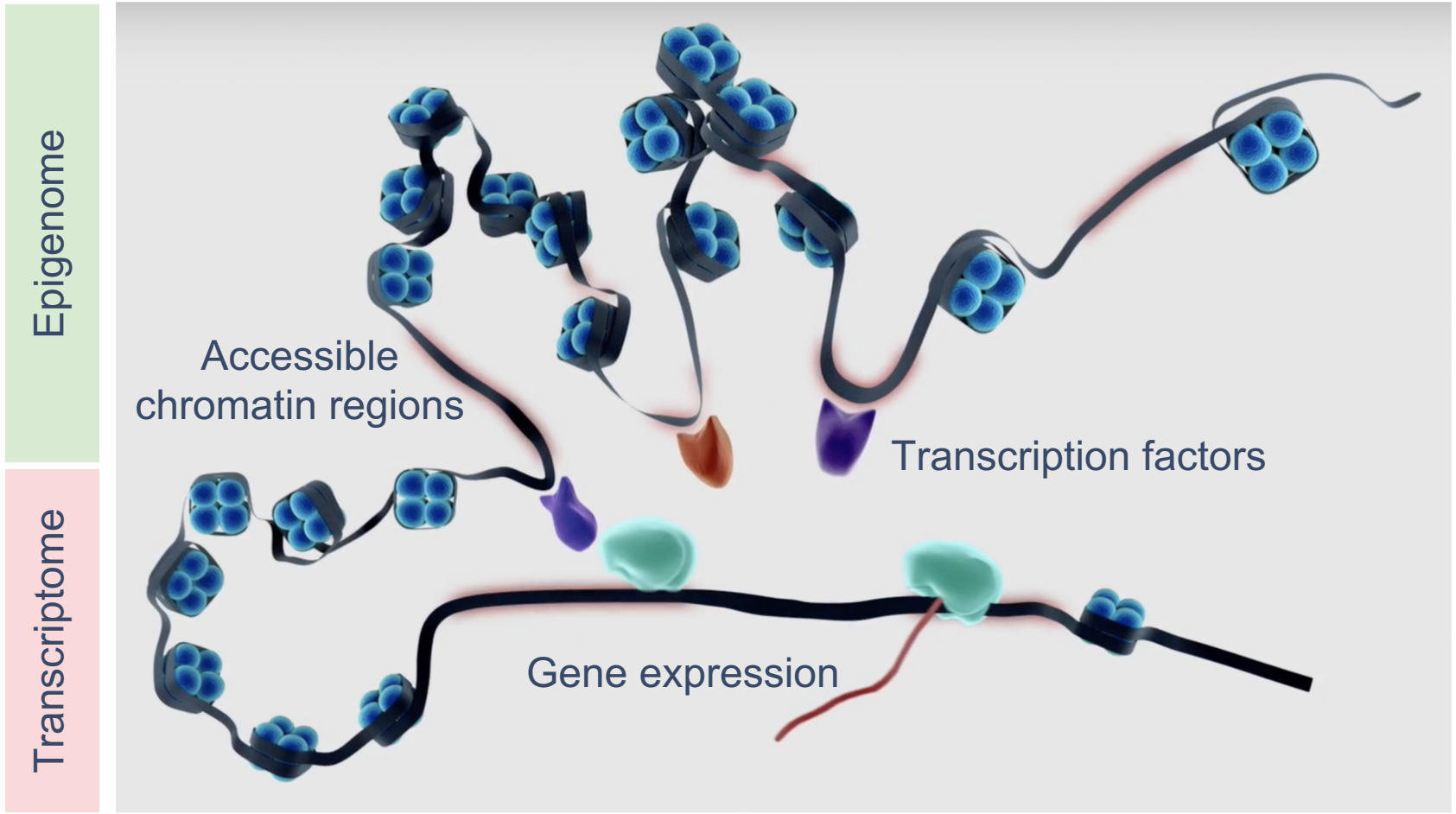
10xgenomics.com/products/cloud-analysis

[1]See 10x Genomics Cloud Terms of Use for restrictions and details

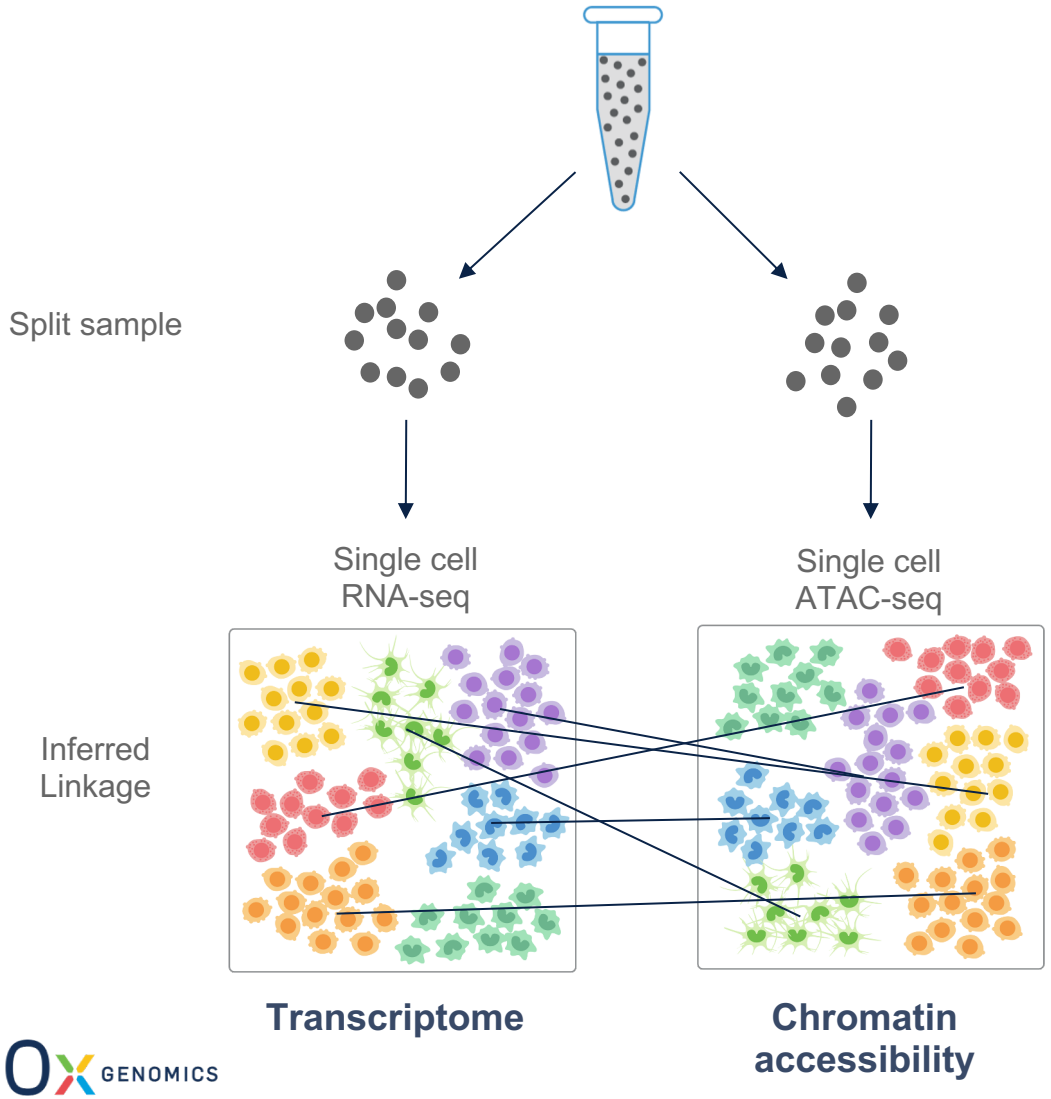
Single Cell Multiome ATAC + Gene Expression

10X GENOMICS

Interplay between epigenetic programs and gene expression



Computational approaches to integrate single cell transcriptomic and epigenomic data



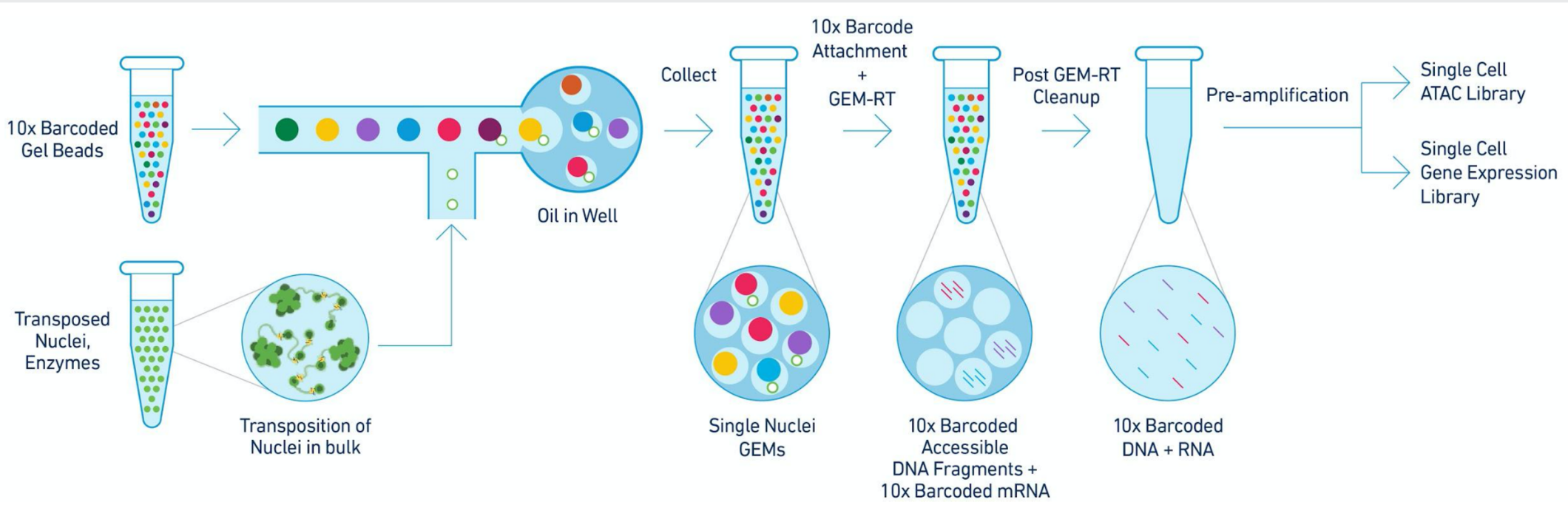
Potential downsides:

- Chromatin accessibility is assumed to be positively correlated with gene expression
- Chromatin changes can proceed gene expression (lineage priming)
- Open chromatin peaks not associated with promoters is discarded (rich in cell type information)

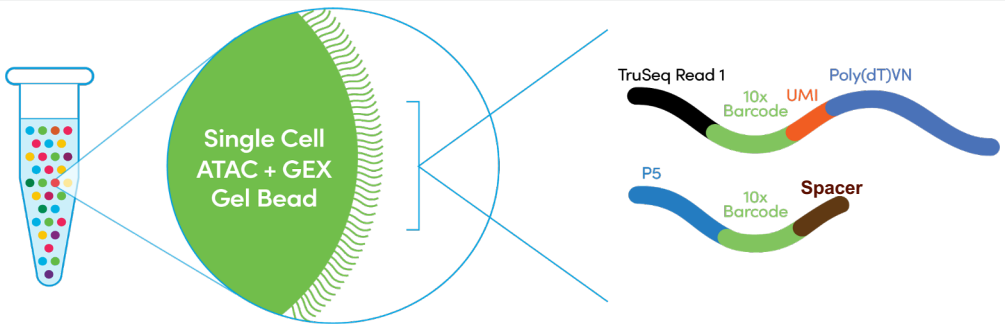
Cell types may be misassigned
Rare cells may be difficult to link

10X GENOMICS

How it works

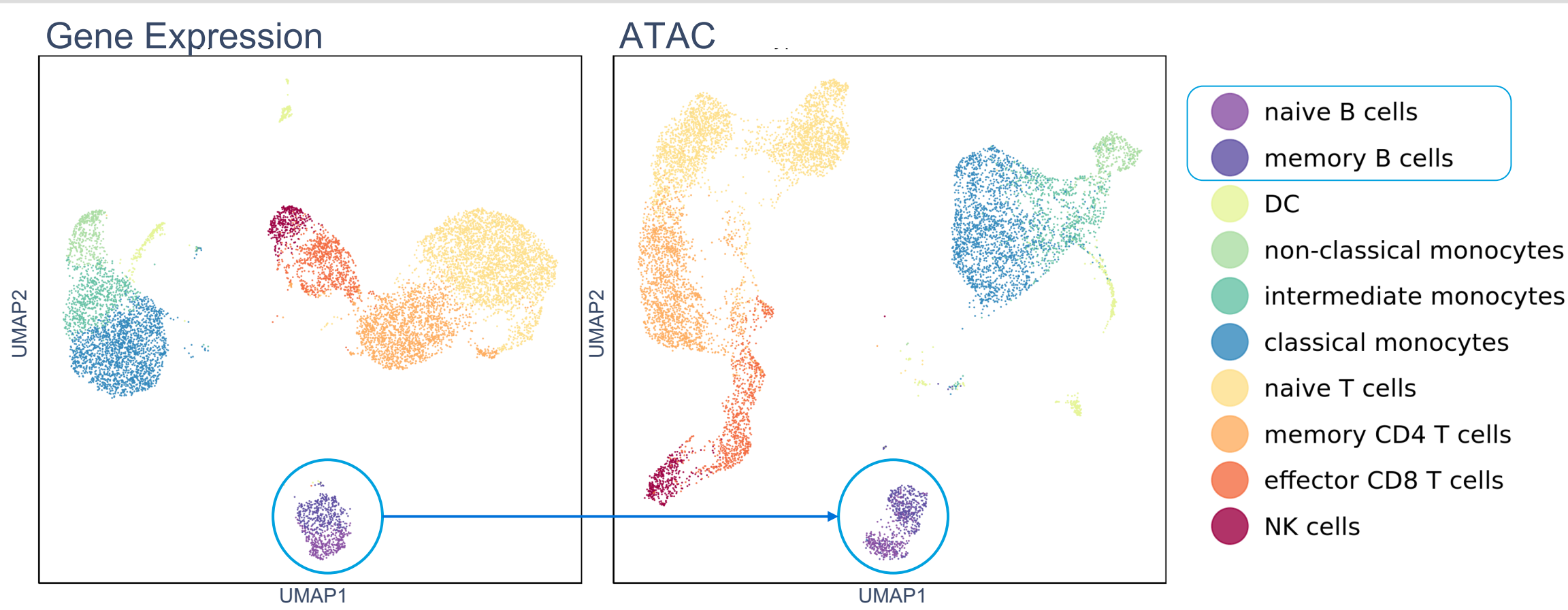


Multiome
ATAC + GEX
Gel Beads

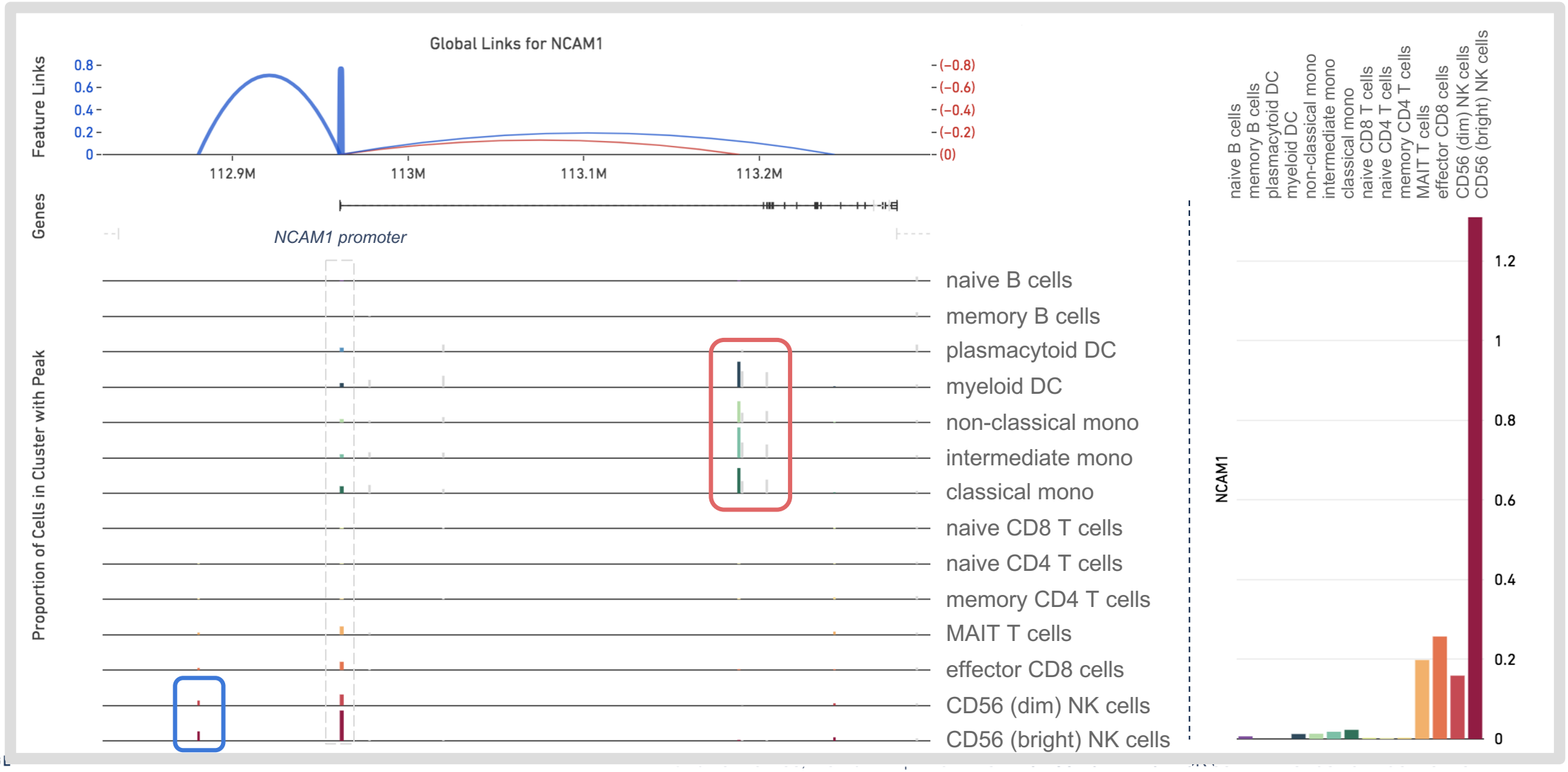


Redesigned gel beads combine oligos for capturing 3’ ends of mRNA & ATAC fragments

Better separate PBMC populations on ATAC space

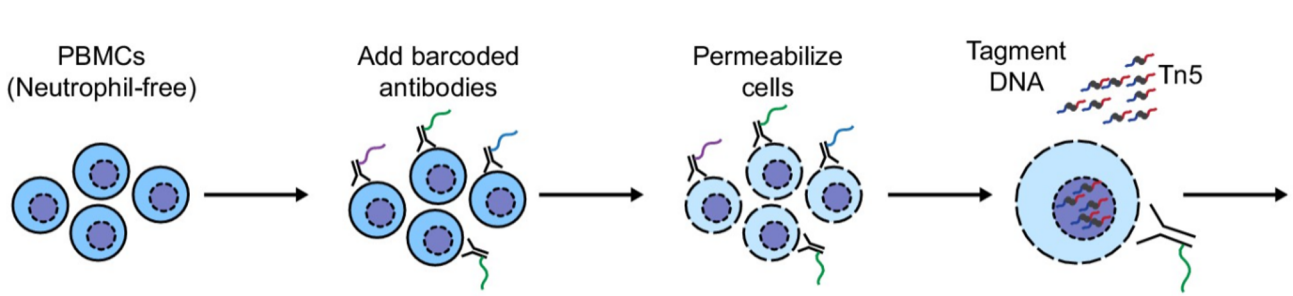


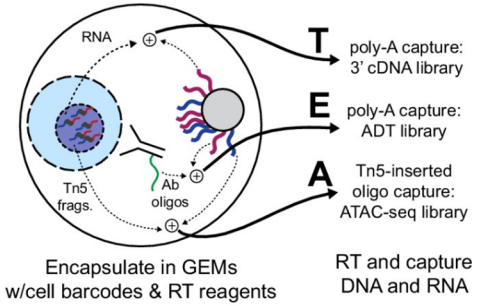
Identify putative regulatory elements linked to a gene of interest

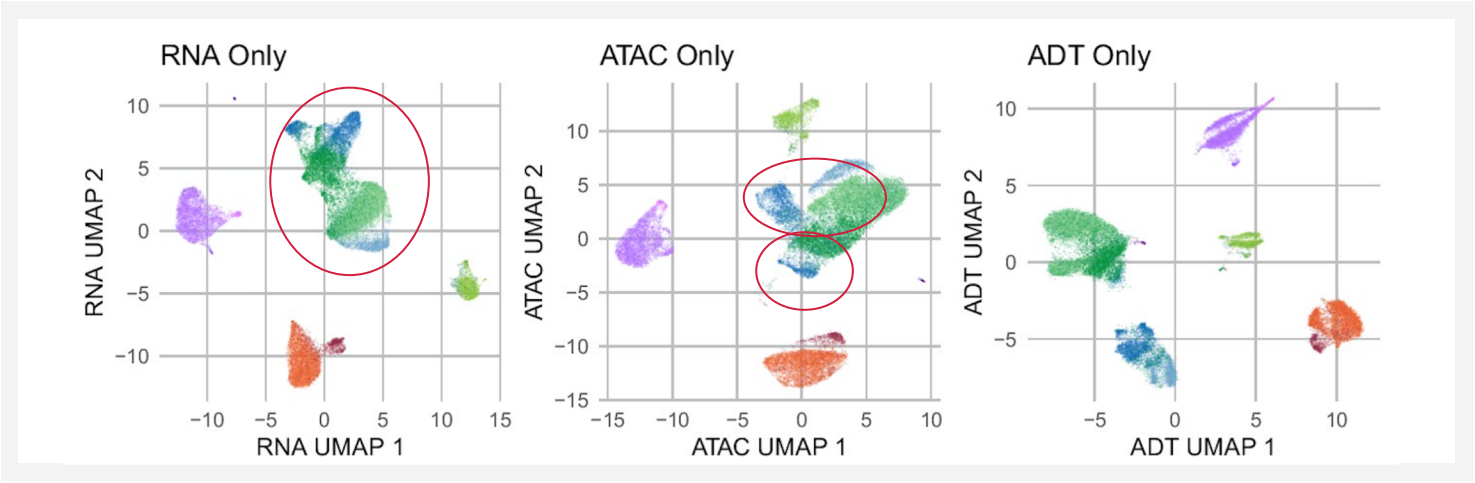


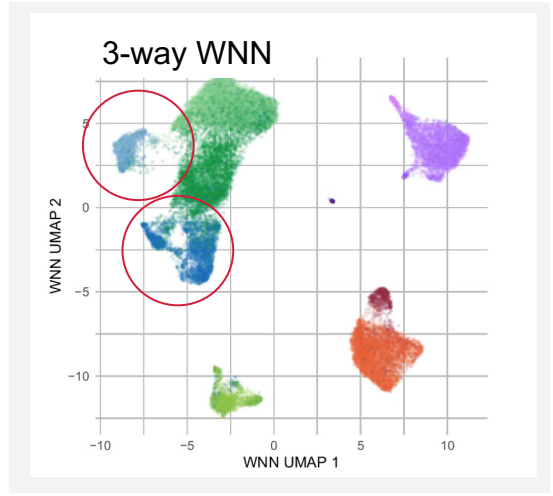
Measuring additional modalities increases power to separate cell states

TEA-seq: Simultaneous detection of transcriptome, epitopes, and epigenome





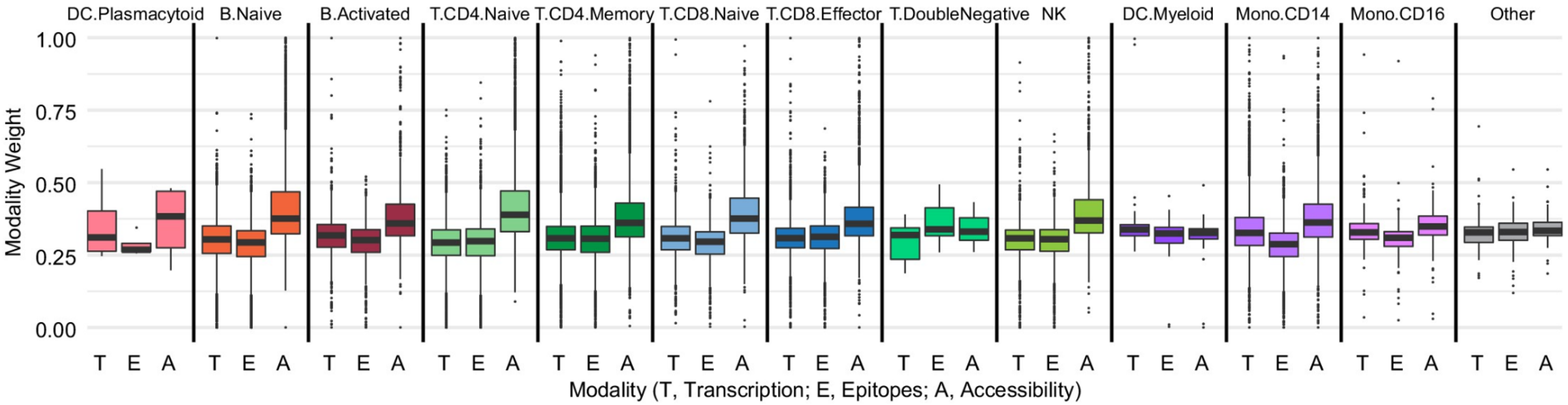




Improved resolution of CD4/CD8 T cell states when using RNA, ATAC, and protein data

Swanson et al, 2021 *eLife* doi: 10.7554/eLife.63632

For many cell types, scATAC-seq largest contributor to improved cell state resolution



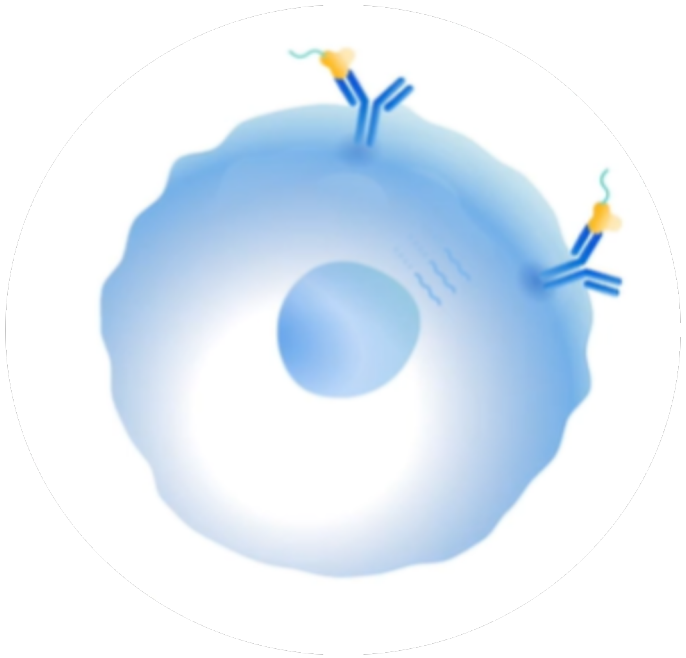
Swanson et al, 2021 *eLife* doi: 10.7554/eLife.63632

Coming soon

Antibody and T-cell Receptor Discovery at Massive Scale

BEAM-Ab

Expected in 2H 2022



Single experiment on SARS-CoV-2 screened:

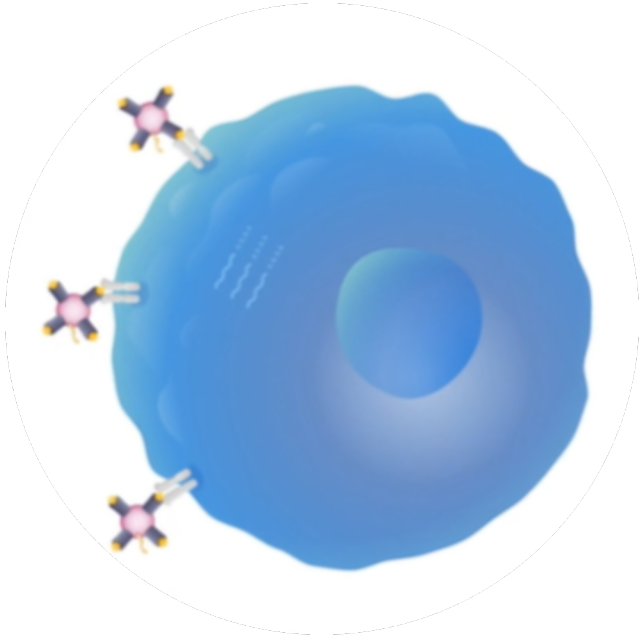
4M+ B-cells in less than a week to yield

55 human broadly neutralizing antibodies with pM affinity

2 pan-coronavirus antibodies

BEAM-T

Expected in 2H 2022



Find optimal T Cells for
hyper-personalized cancer cell therapy

5′ CRISPR

- Measure perturbation effects with multiomic readouts
- Increased flexibility for functional genomics studies
- Rapidly deploy existing Cas9 RNA libraries

Expected **Early 2022**

Fixed RNA Profiling Kit

- Preserve biological states
- Flexible study designs
- New assay design for additional readouts in the future

Expected **Mid 2022**

Chromium Nuclei Isolation Kits

- Standardize nuclei isolation
- Little to no optimization on most samples
- Built and optimized for 10x Genomics assays

Expected **Mid 2022**

Xenium

In Situ Platform

- Hundreds of gene targets
- Subcellular resolution
- Microscopy based read-out
- Fresh Frozen and FFPE
- Simultaneous RNA and proteins
- Throughput for larger cohorts

Expect **Late 2022**

10X GENOMICS

Thank you! Questions?

Your local 10x Team

Eve-Marie Walter, Sales Executive
eve-marie.walter@10xgenomic.com

Heather Eckart, Sales Associate
heather.eckart@10xgenomics.com

Egon Ranghini, Science & Technology Advisor
egon.ranghini@10xgenomics.com

Kara Pivarski, Field Application Scientist
kara.pivarski@10xgenomics.com

Joseph Ipe, Sr. Field Application Scientist - Tissue Specialist
joseph.ipe@10xgenomics.com

Leilani Marty Santos, Spatial Science & Technology Advisor
leilani.martysantos@10xgenomics.com

10x GENOMICS