10x GENOMICS

10x Genomics Chromium Technology: Multiple Features in Single Cells - Biology at True Resolution

10x Genomics Seminar - Toulouse

January 15th

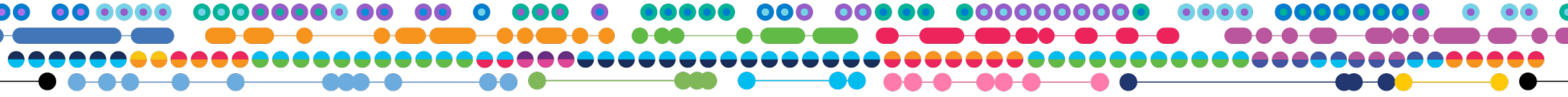
Institut des Maladies Métaboliques et Cardiovasculaires

Hôpital Rangueil, Toulouse

Hannes Arnold, PhD

Technical Sales Specialist

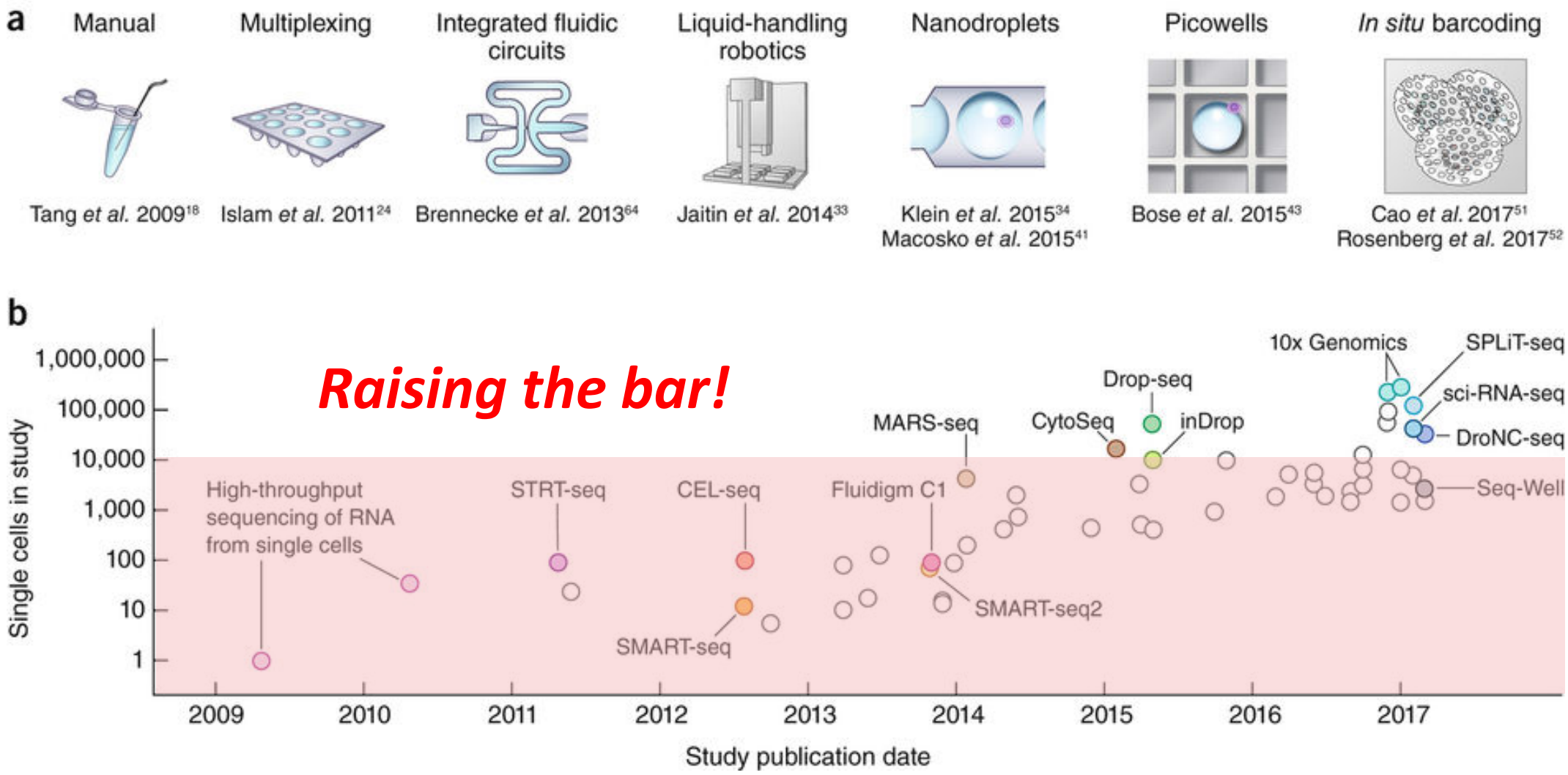
10x Genomics



Do you remember?

Does one still mention NGS in the title or abstract?

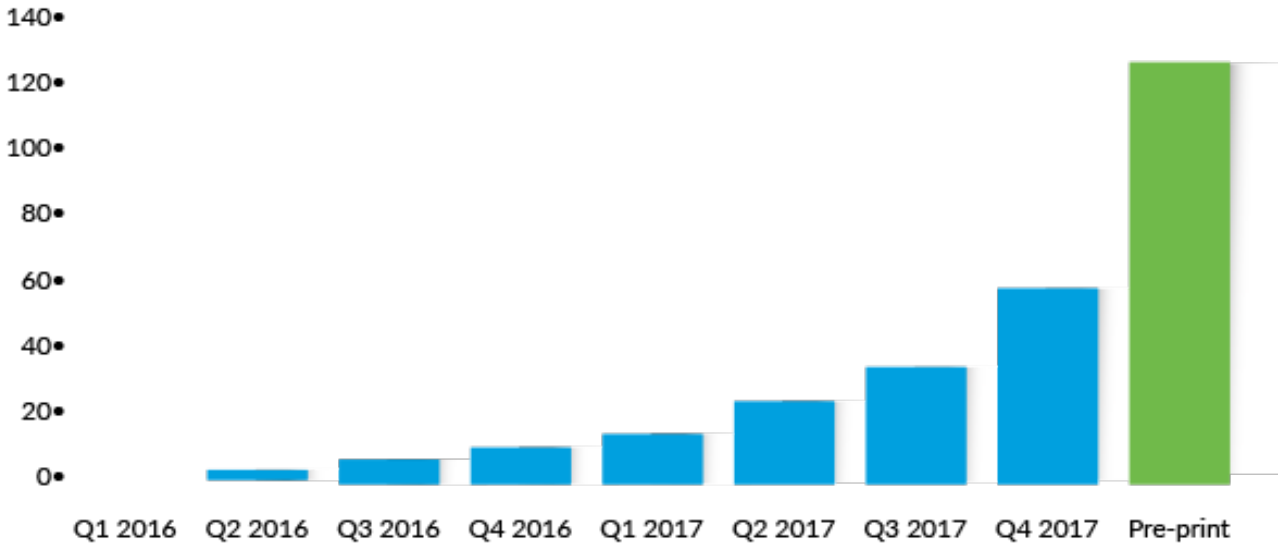
Historical overview about single cell applications



Svensson et al Nature Protocols volume 13, pages 599–604 (2018)

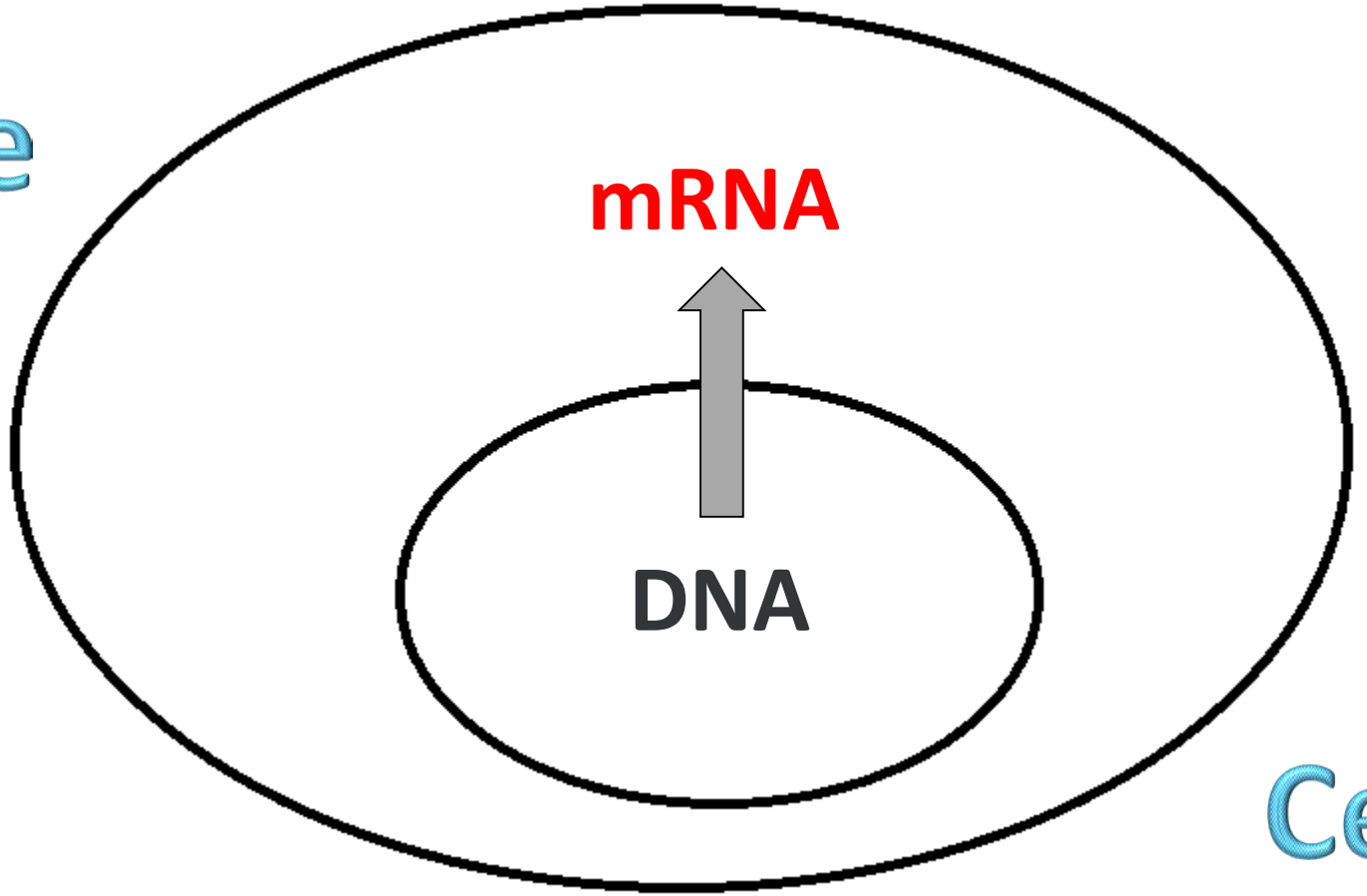
Rapid Growth in Publications

Cumulative Publications by Quarter



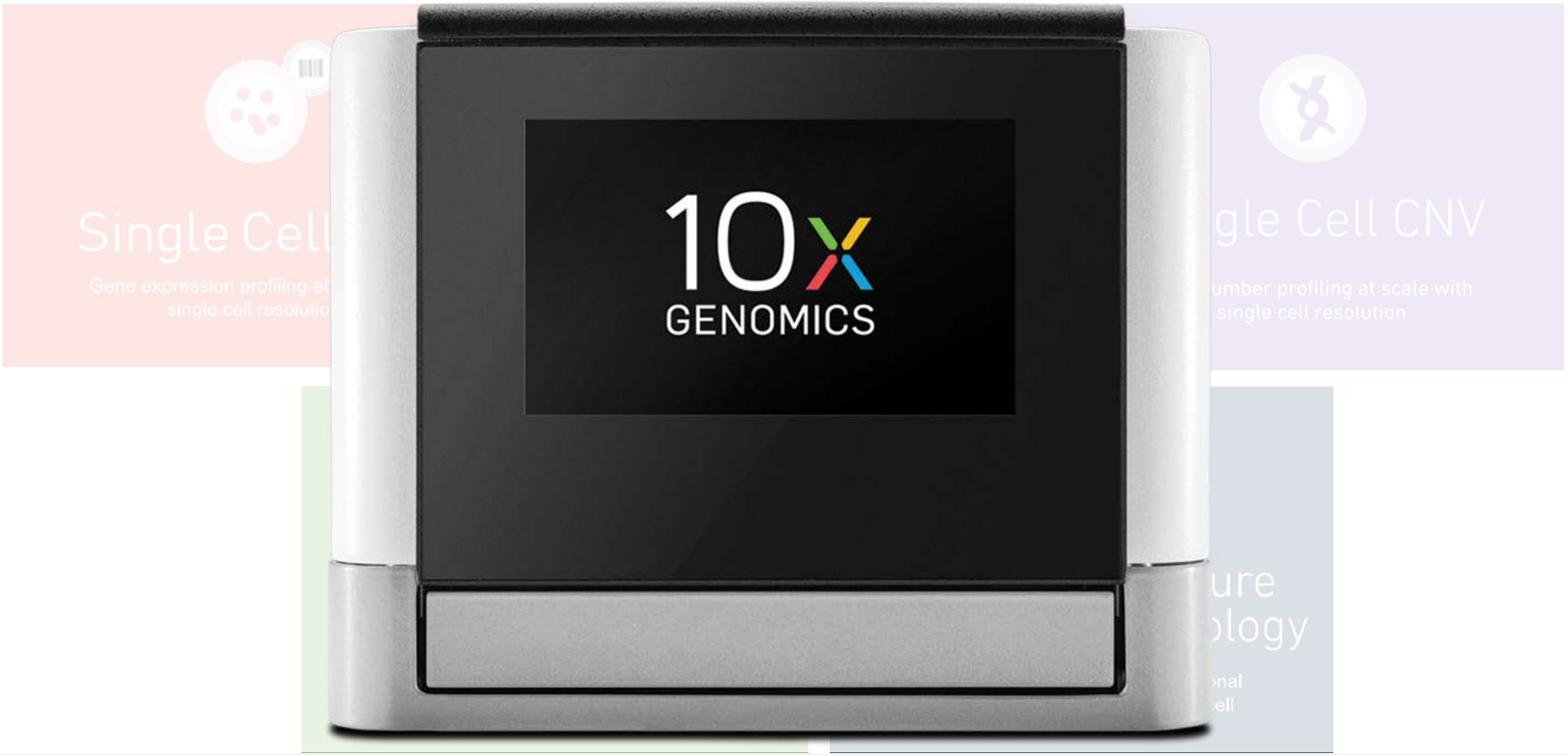
People hear *10x Genomics* and may think…

Cell Type

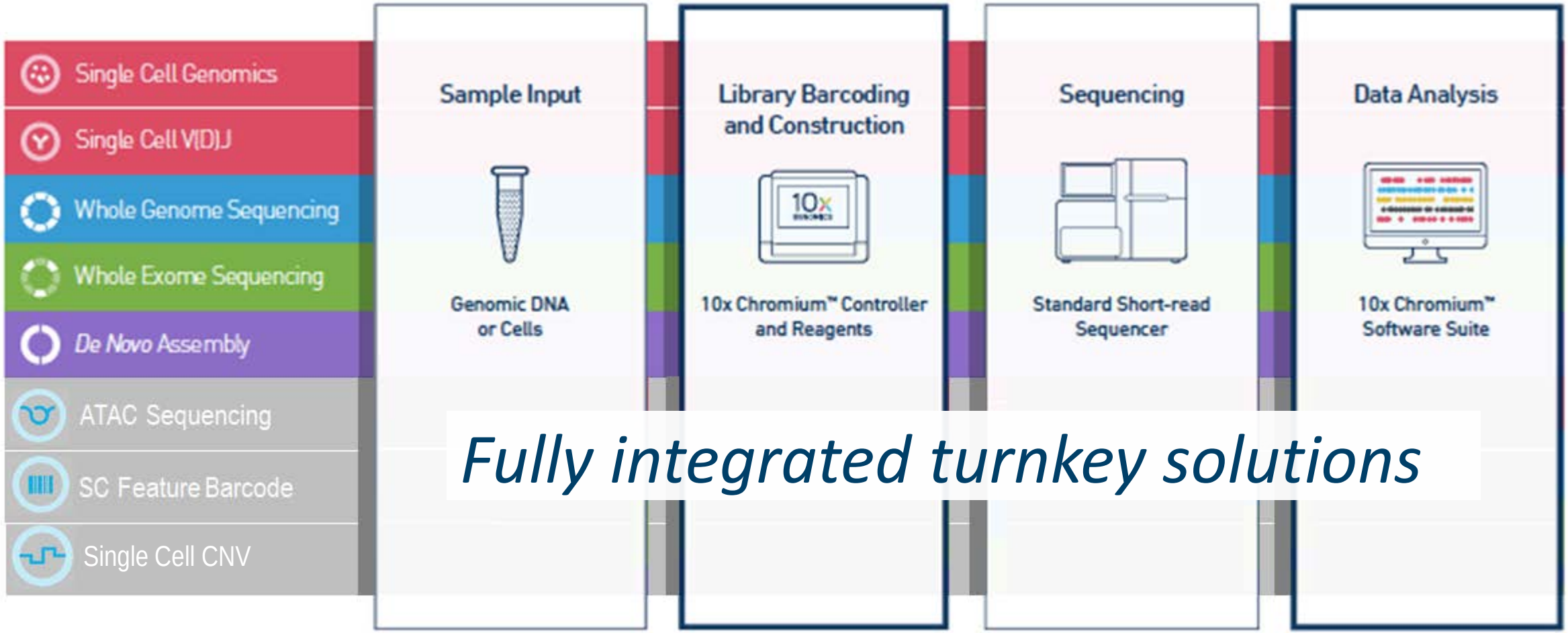


Cell State

10x Genomics Chromium Single Cell Applications - Overview

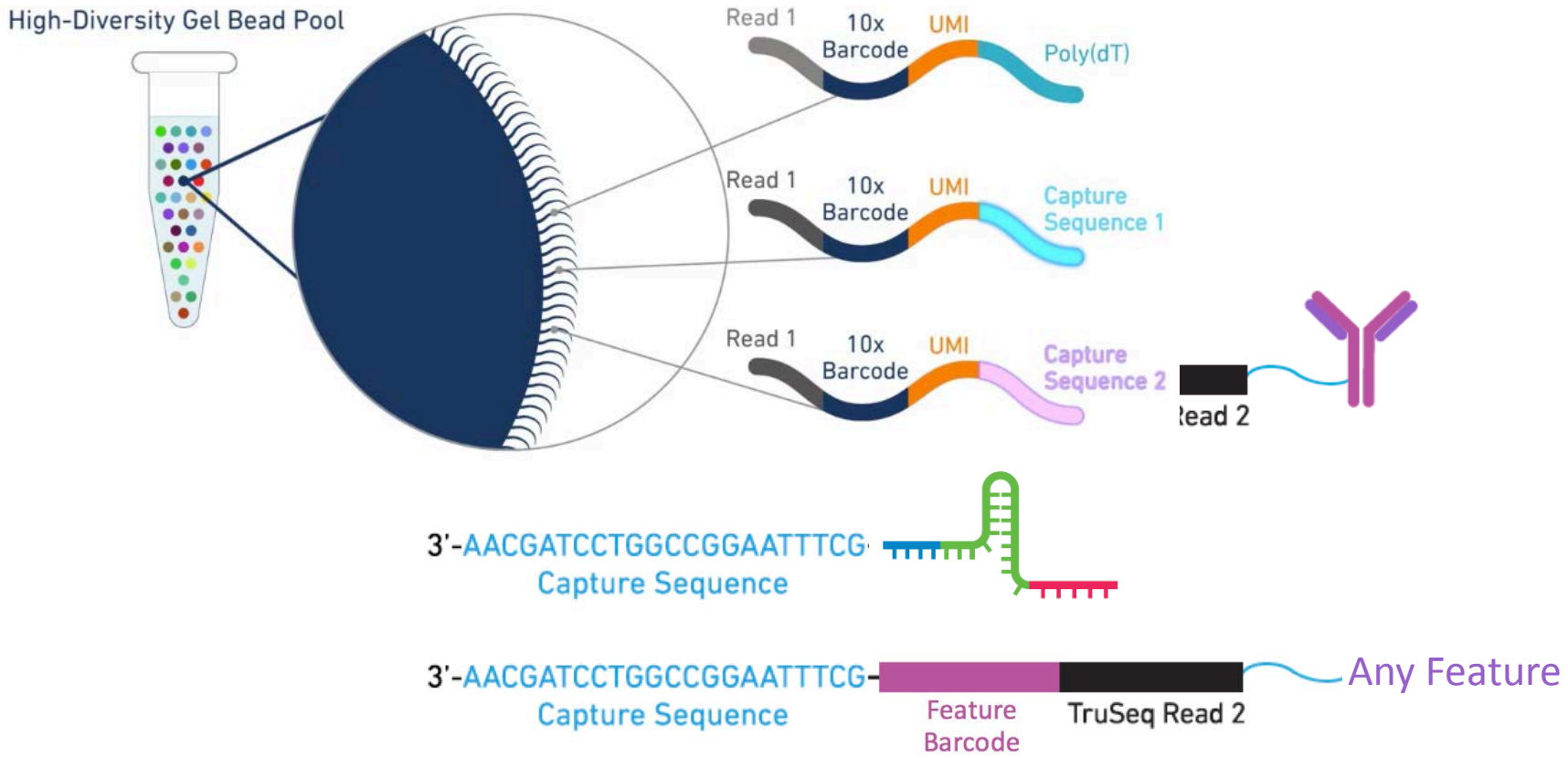


The Chromium™ System:
One Instrument, Multiple Reagent Kits and Software Applications

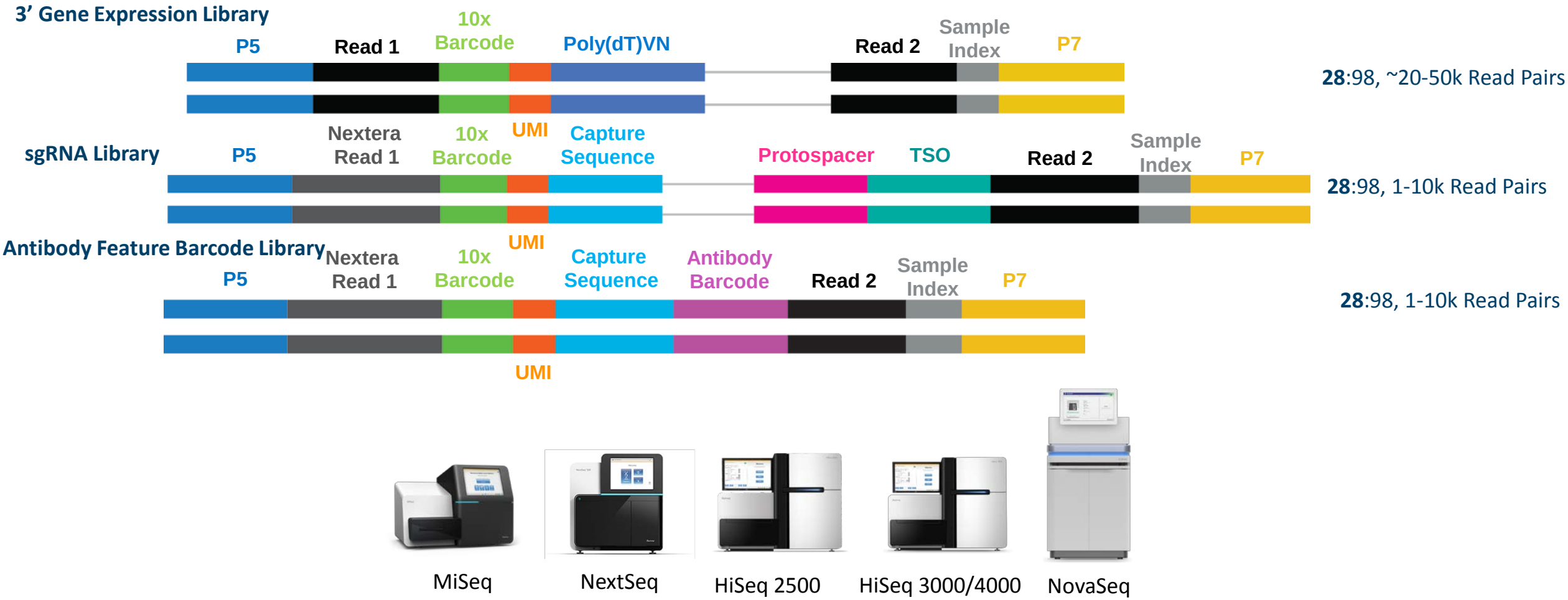


Working Principle of **G**elbead **Em**ulsions (**GEM**)

New Single Cell 3’ Beads
Chromium Single Cell Feature Barcoding

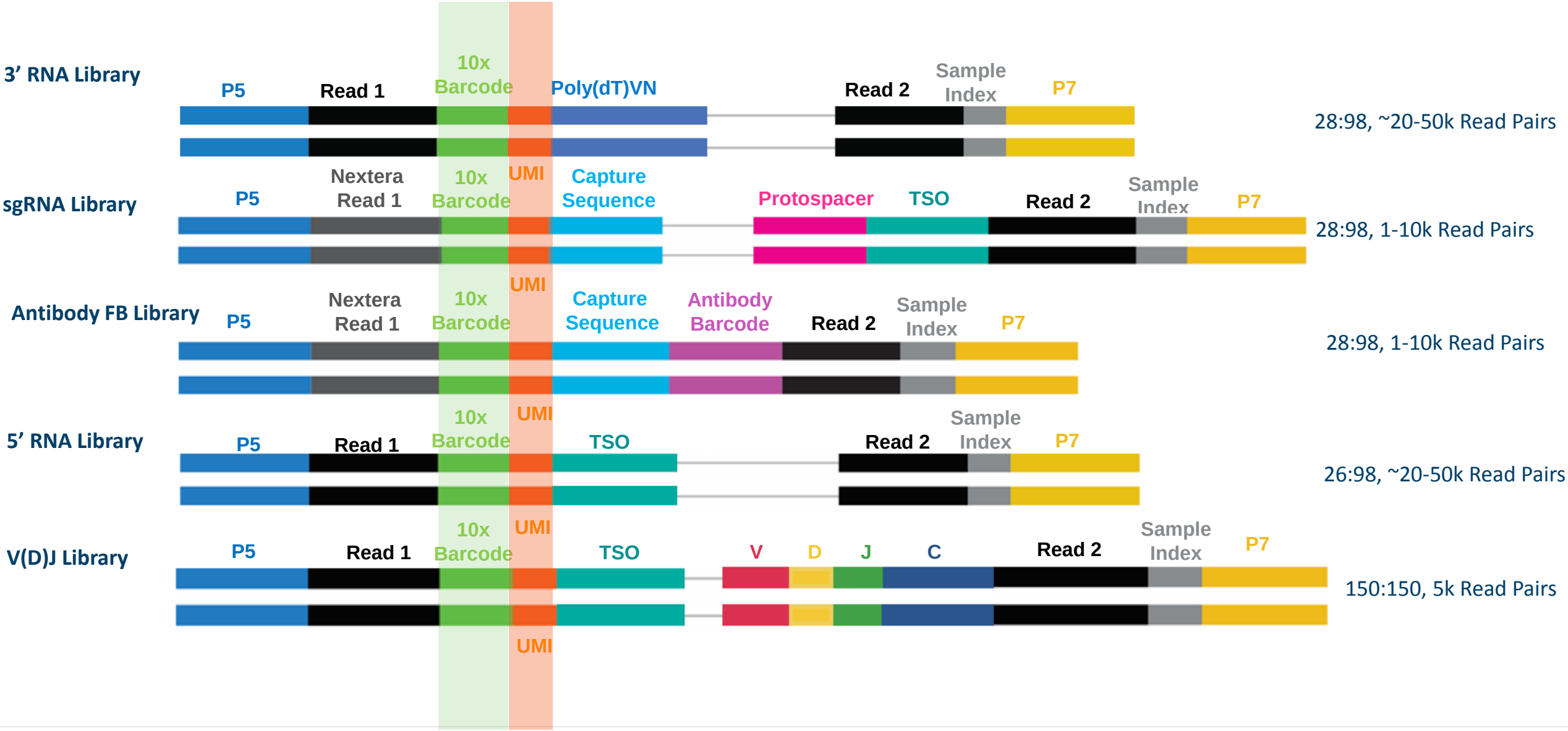


Ready to Sequence Libraries Compatible with Illumina® Sequencers



Supported Sequencers

All Molecules Identifiable and Quantifiable



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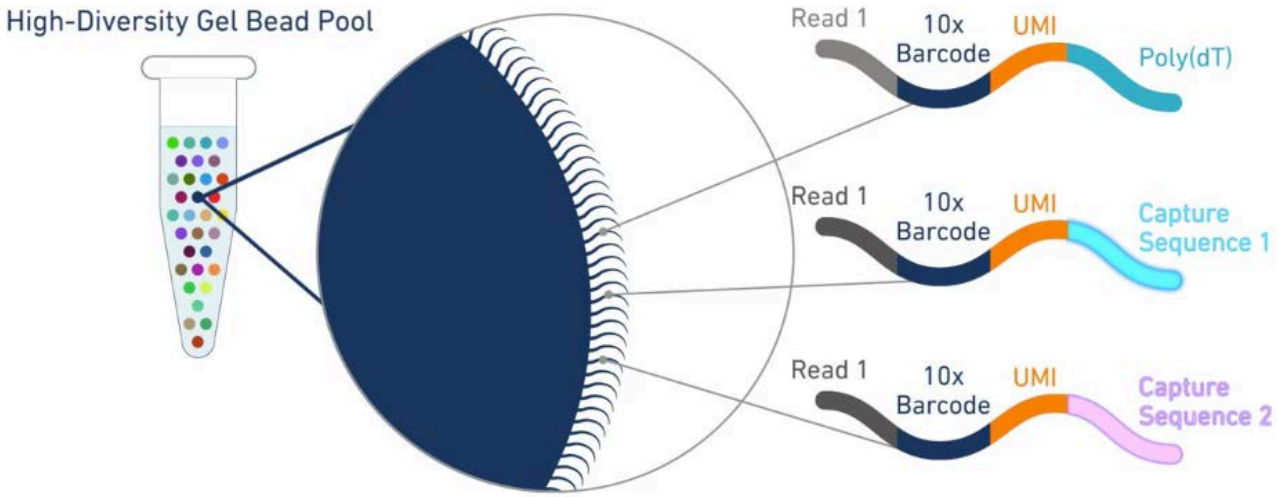
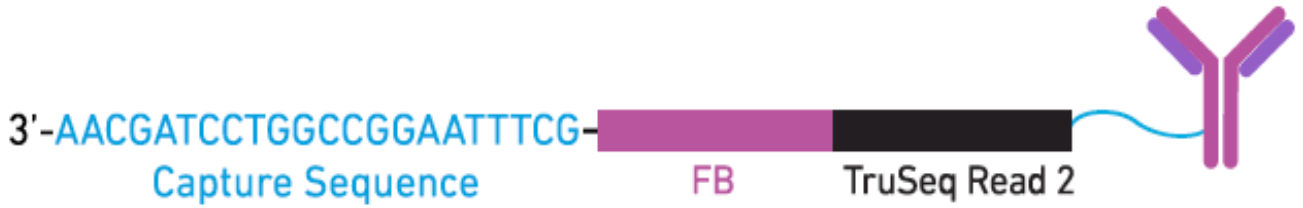
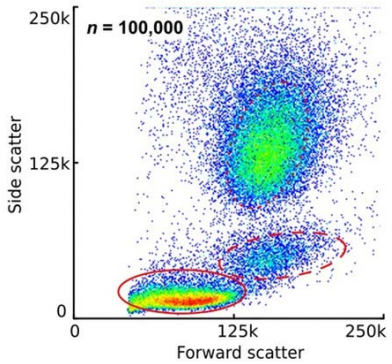
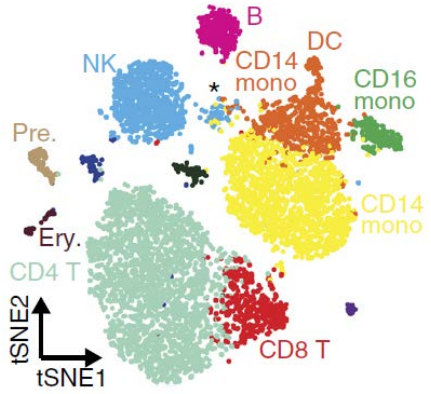




Merging Phenotyping and Gene expression analysis in single cells



Cytometry
(Flow, Mass, FACS)



scRNA transcriptomics

ADT

Single Cell Protein and Expression Analysis

Potential to Tag Thousands of Proteins per Cell and Combine with Gene Expression

What would you do in cytometry with 1,073,741,824 dyes?

Capture sequences can be used to analyze many different features...

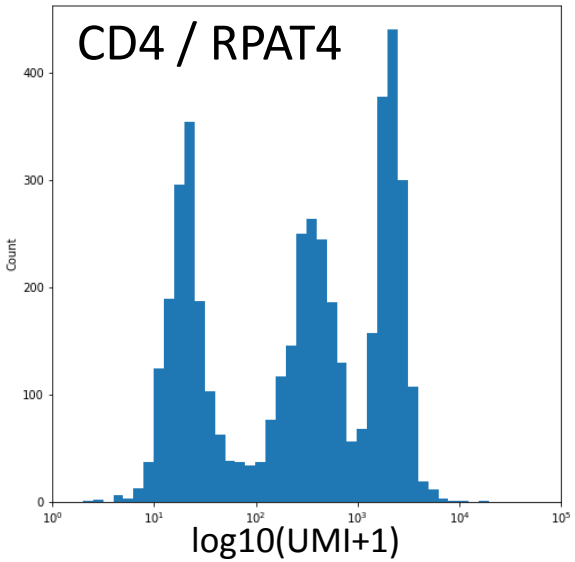
Cell Surface Proteins

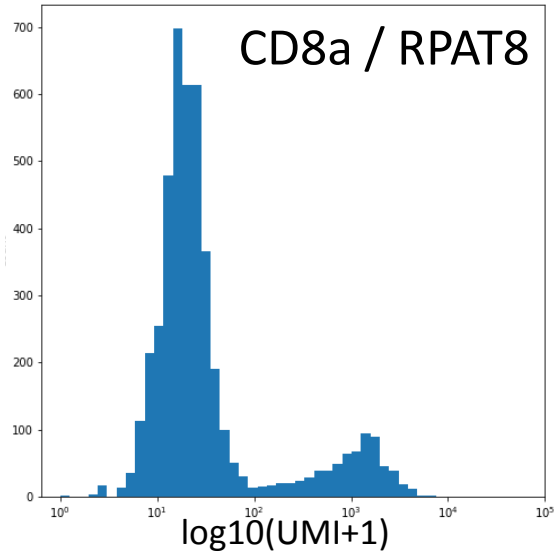
T-Cell Receptor Specificity

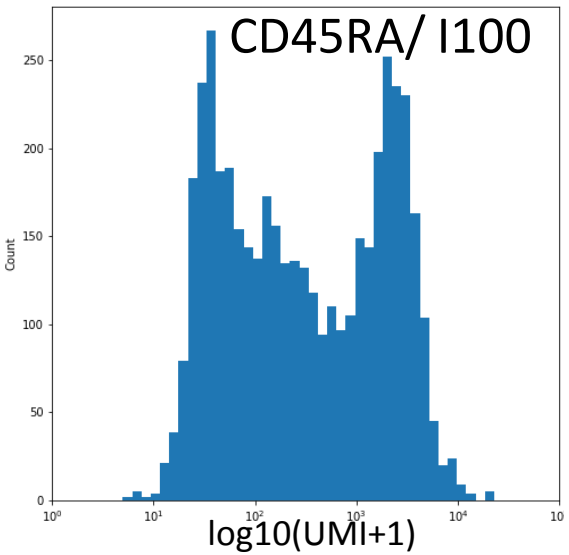
and more...

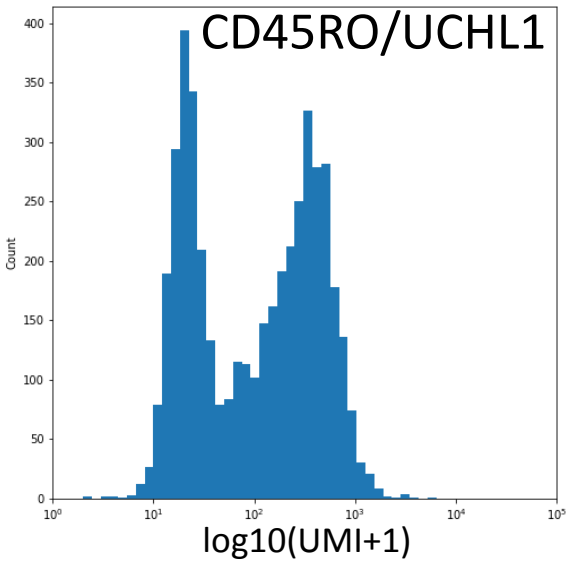
Feature Barcode UMI Counts Confirmed By Flow Cytometry

Feature Barcode

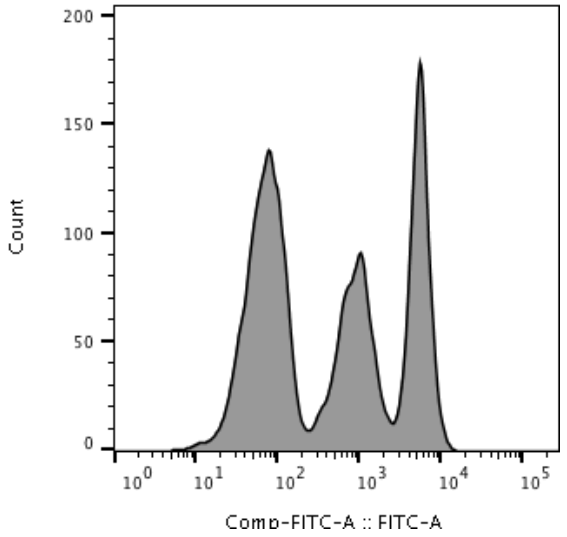


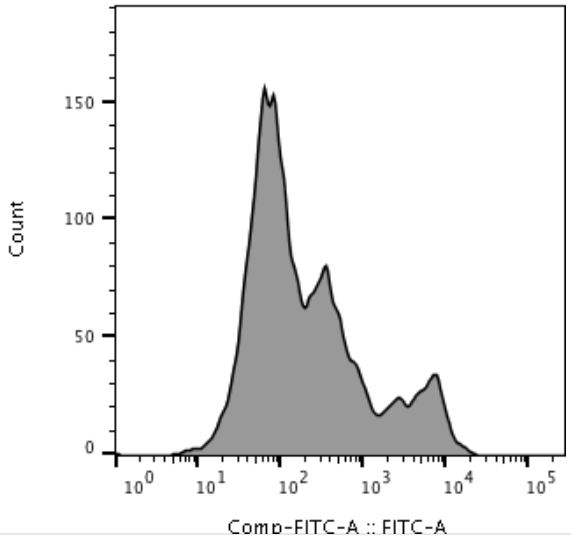


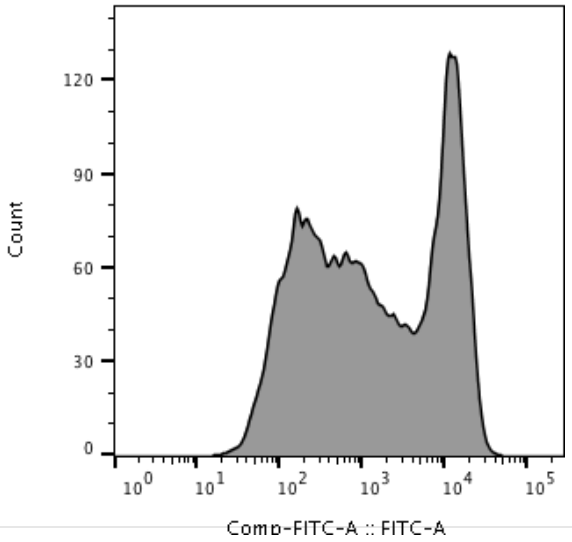


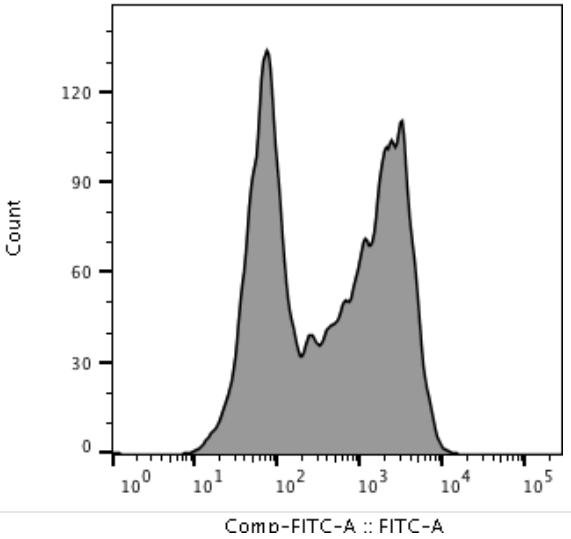


Flow Cytometry



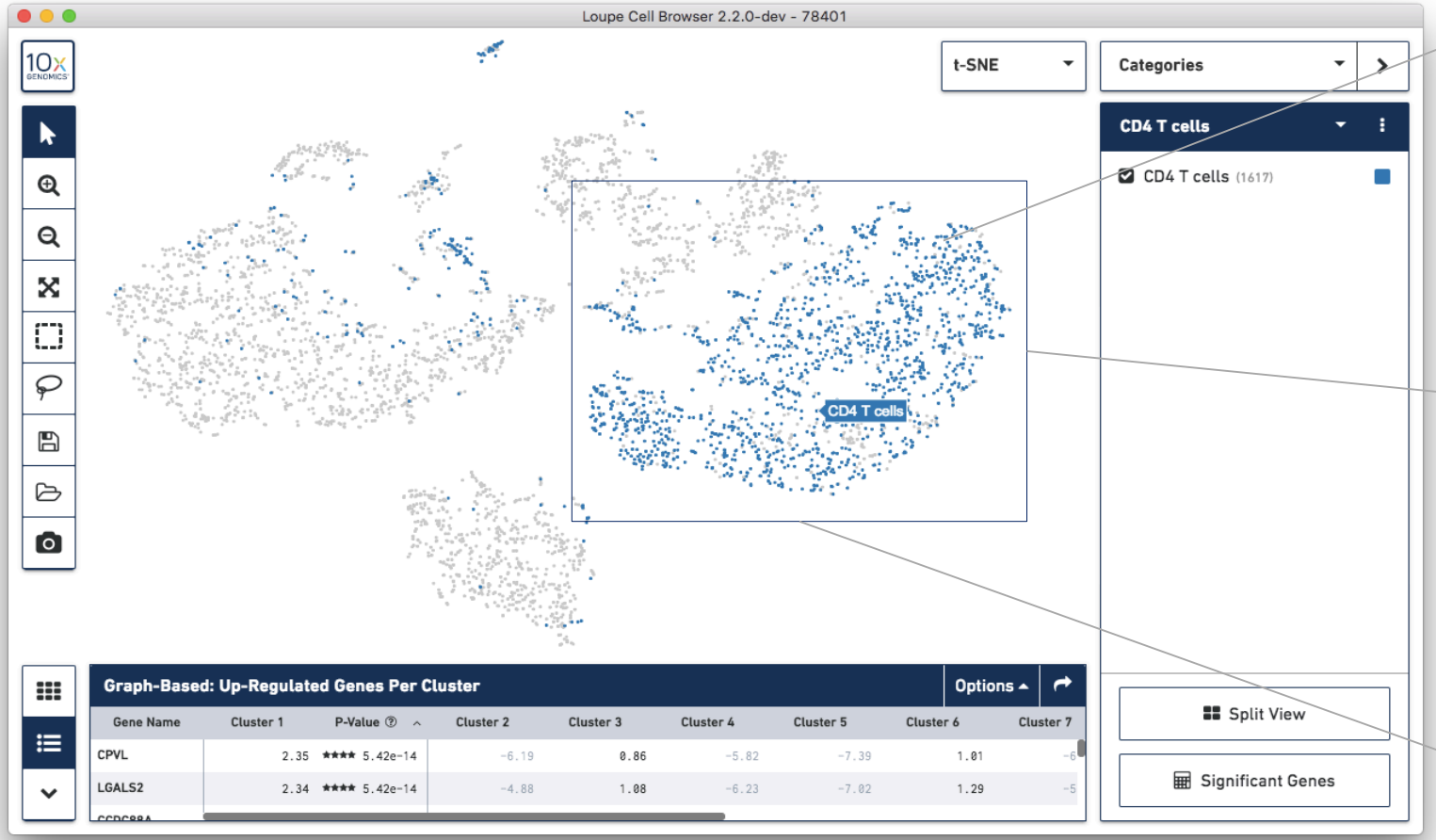


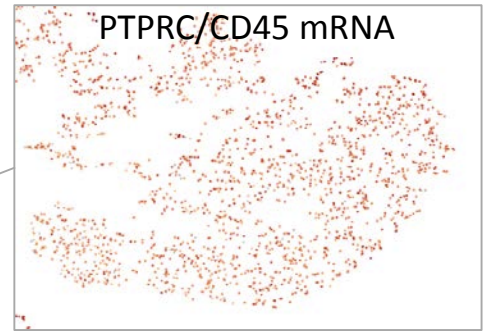


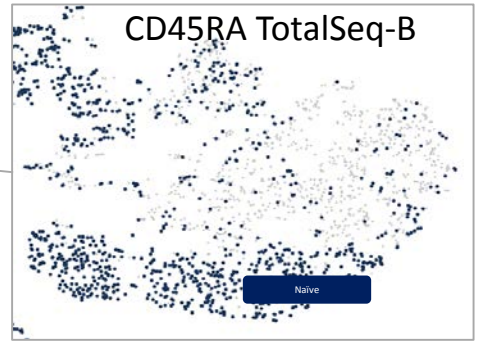


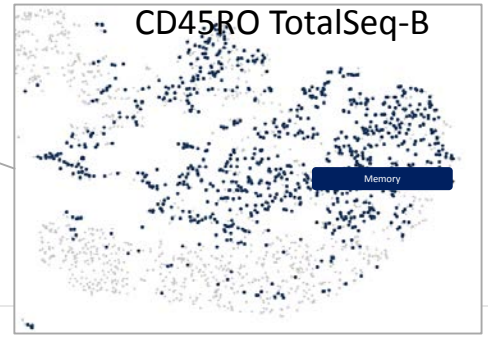
Refine Resolution of Sub-Populations with Protein Information

Detect protein isoforms that are indistinguishable at the transcript level (CD45RA and CD45RO)









BioLegend: Compatible Partner & TotalSeq Co-Development

- Hundreds of tagged antibodies commercially available
- TotalSeq-**B** antibodies compatible with Chromium Feature Barcoding technology (3’ RNA Assay)
- TotalSeq-**C** antibodies compatible with Chromium Feature Barcoding technology (5’ RNA Assay)
- Initial antibody release will focus on immune profiling

https://www.biolegend.com/totalseq

Capture sequences can be used to analyze many different features...

Cell Surface Proteins

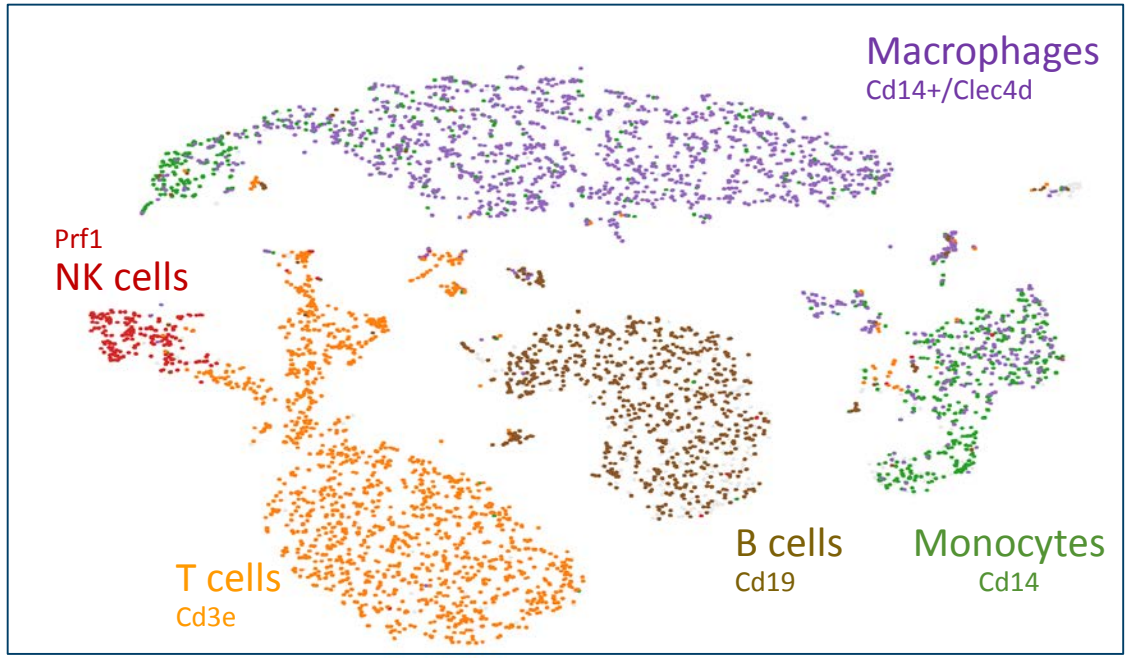
T-Cell Receptor Specificity

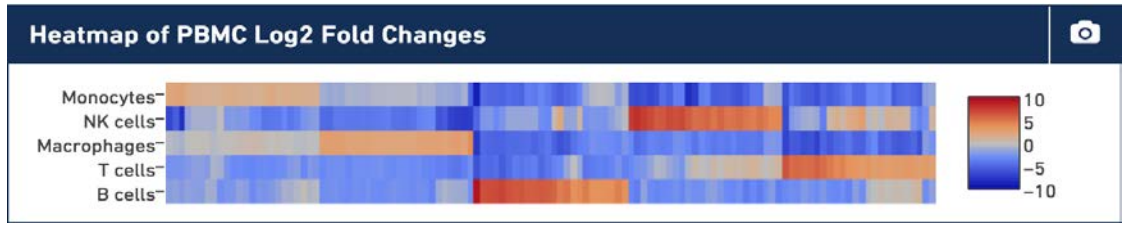
and more...

Reveal Heterogeneity at Scale

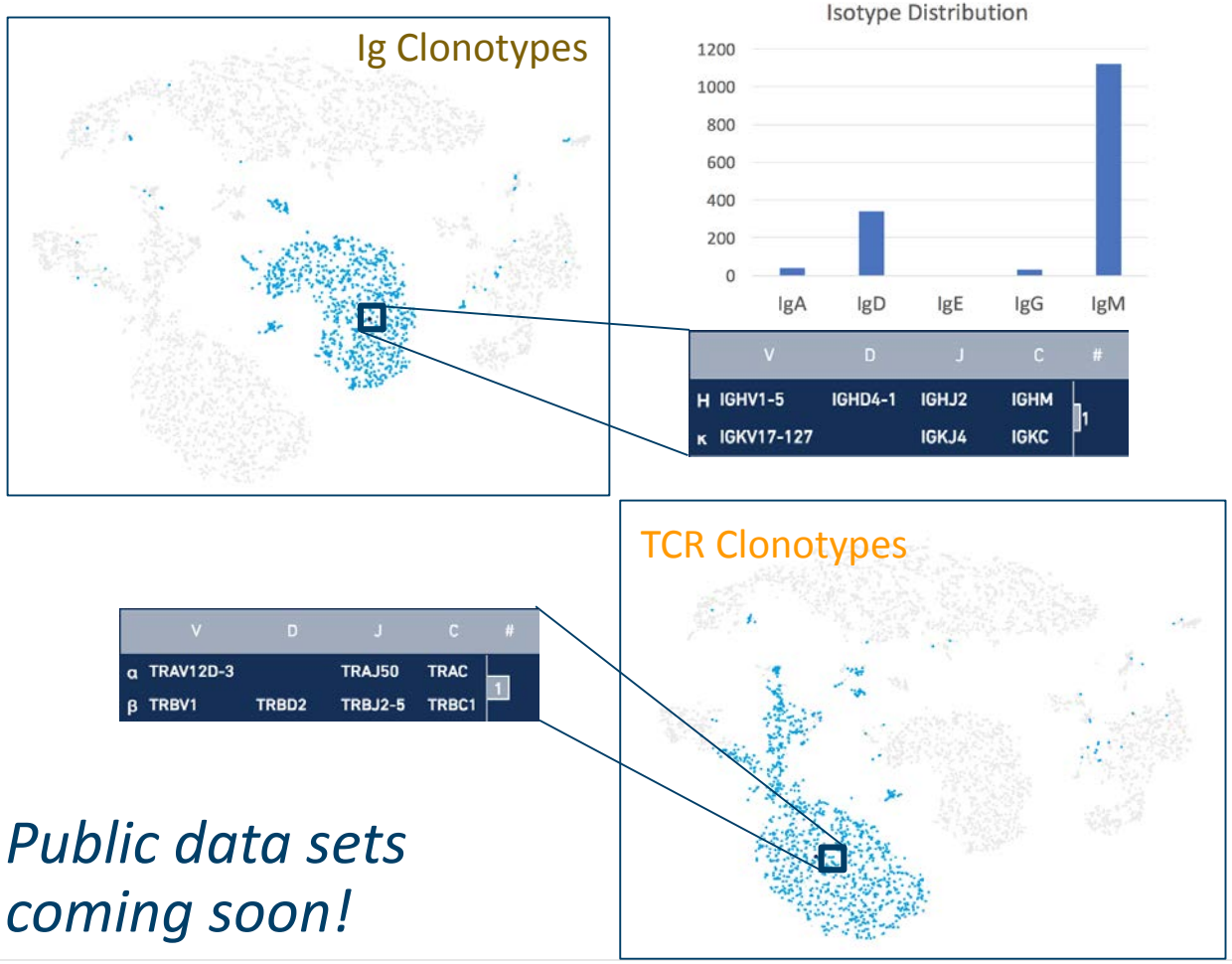
Simultaneously Assess Gene Expression Heterogeneity and the Immune Repertoire

5’ Gene Expression



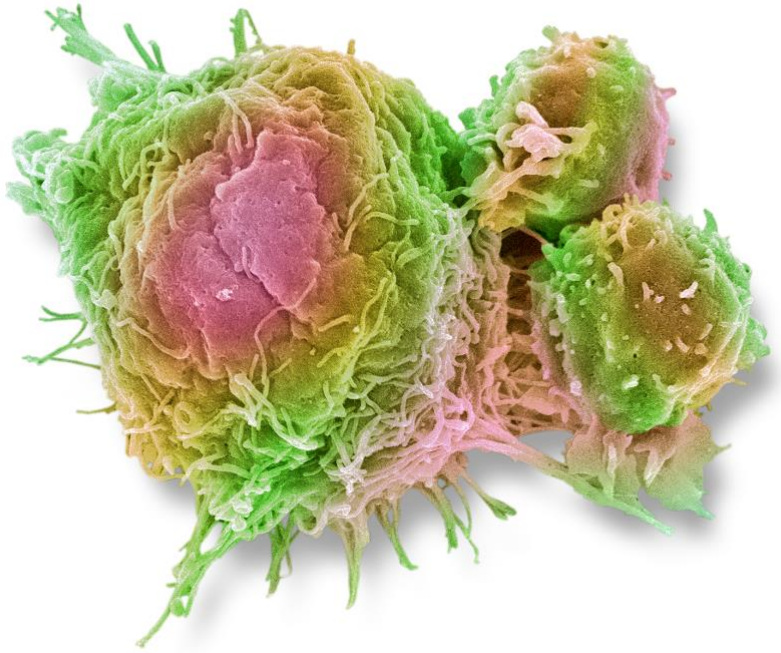


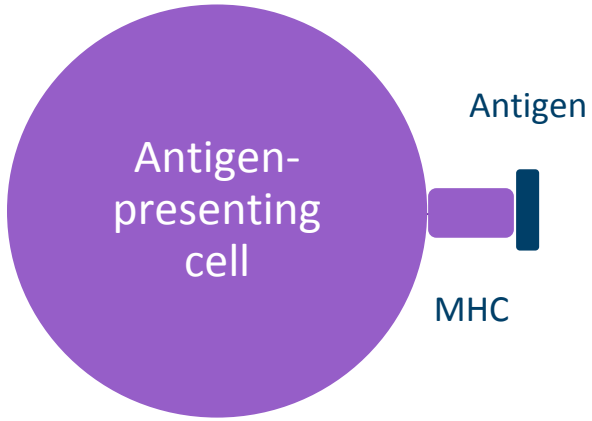
V(D)J clonotypes overlapped with GEX

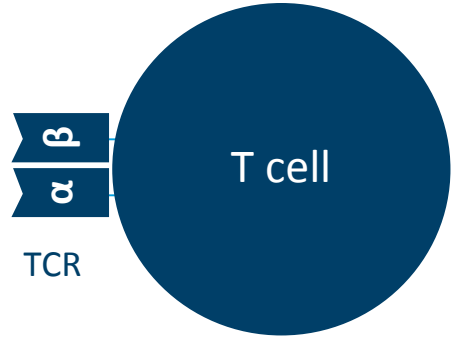


Public data sets coming soon!

New Feature Barcode Application: Mapping TCRs to Antigens







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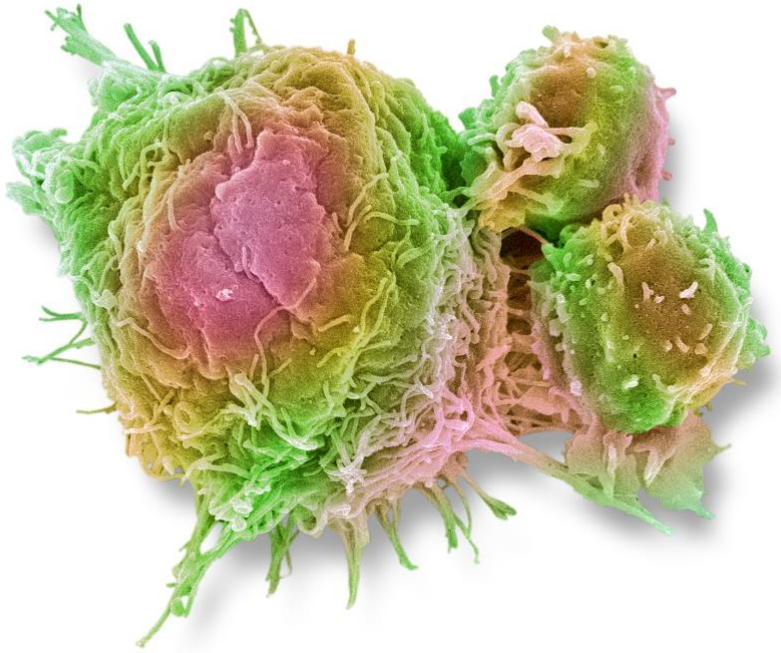
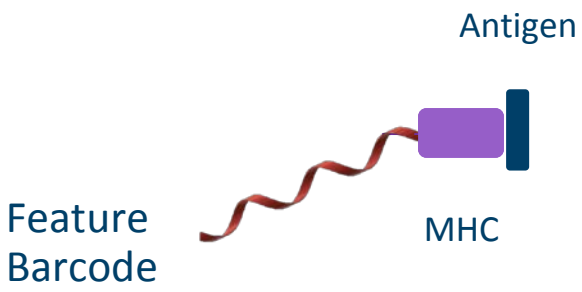
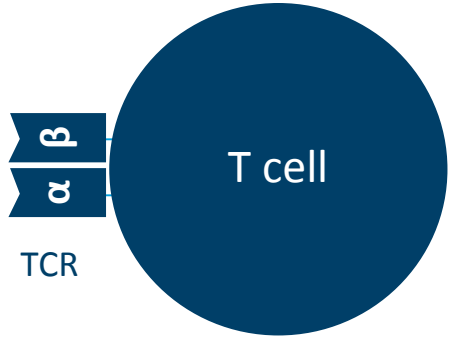
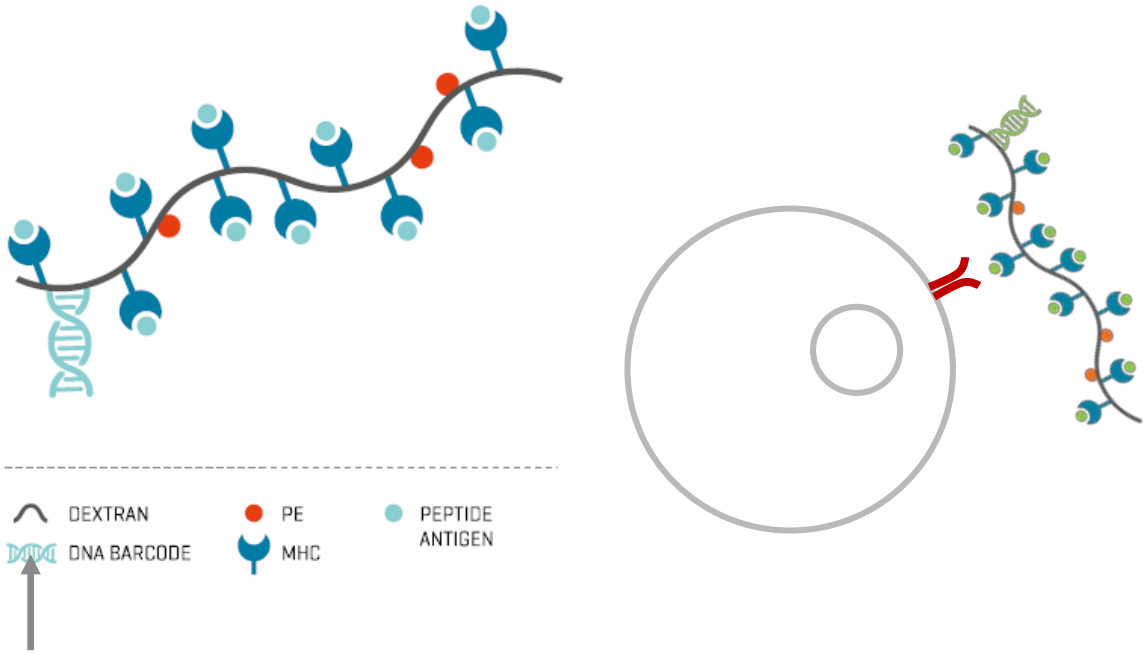






Compatible Product: Immudex's DeCODE ™ Dextramers ®

DeCODE™ Dextramer®



Oligo Conjugated Dextramer compatible with the 5' Assay Template Switch Oligo

- Combine 10x Genomics Immune Profiling Solution with Immudex Dextramer Technology:
  - –Multiplex many MHC Dextramer complexes in one assay
  - –Scale peptide specificity
  - –Study TCR-pMHC binding events *at single cell resolution*

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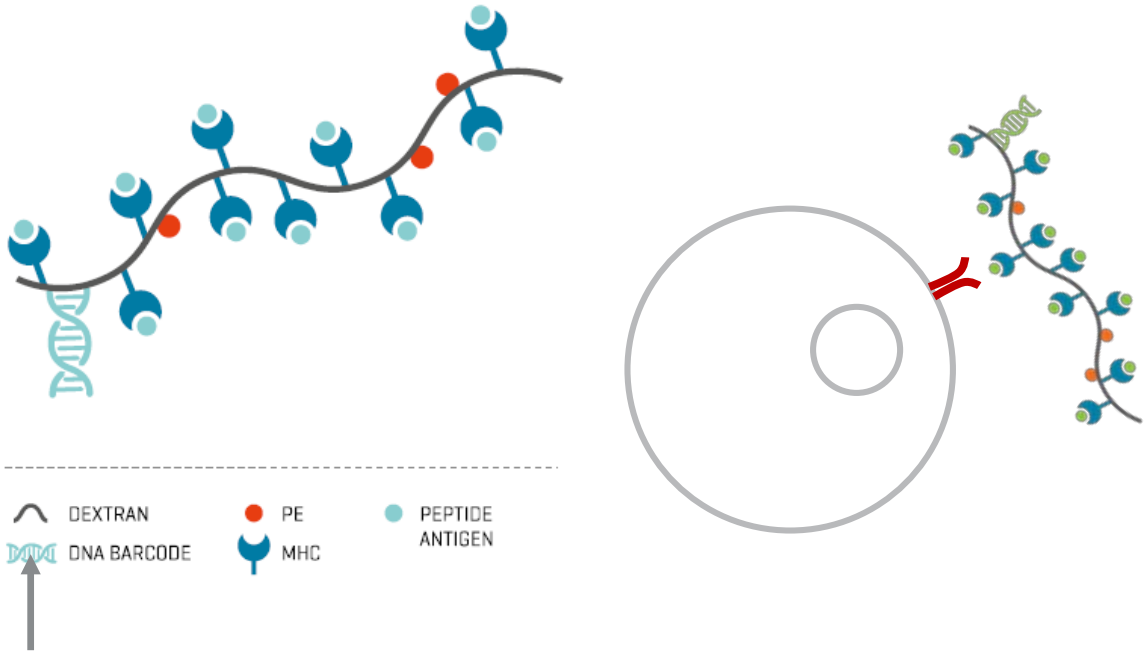
DeCODE™ Dextramer®



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Antigen-Expanded Cells Are Detectable in a Lymphocyte Background

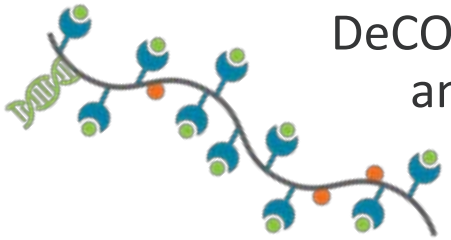
Human PBMC
CMV seronegative

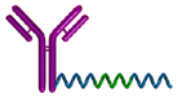
Antigen-expanded CD8+ T cells
HLA-A*0201 NLVPMVATV
(CMV)

2% CMV-expanded

98% PBMC

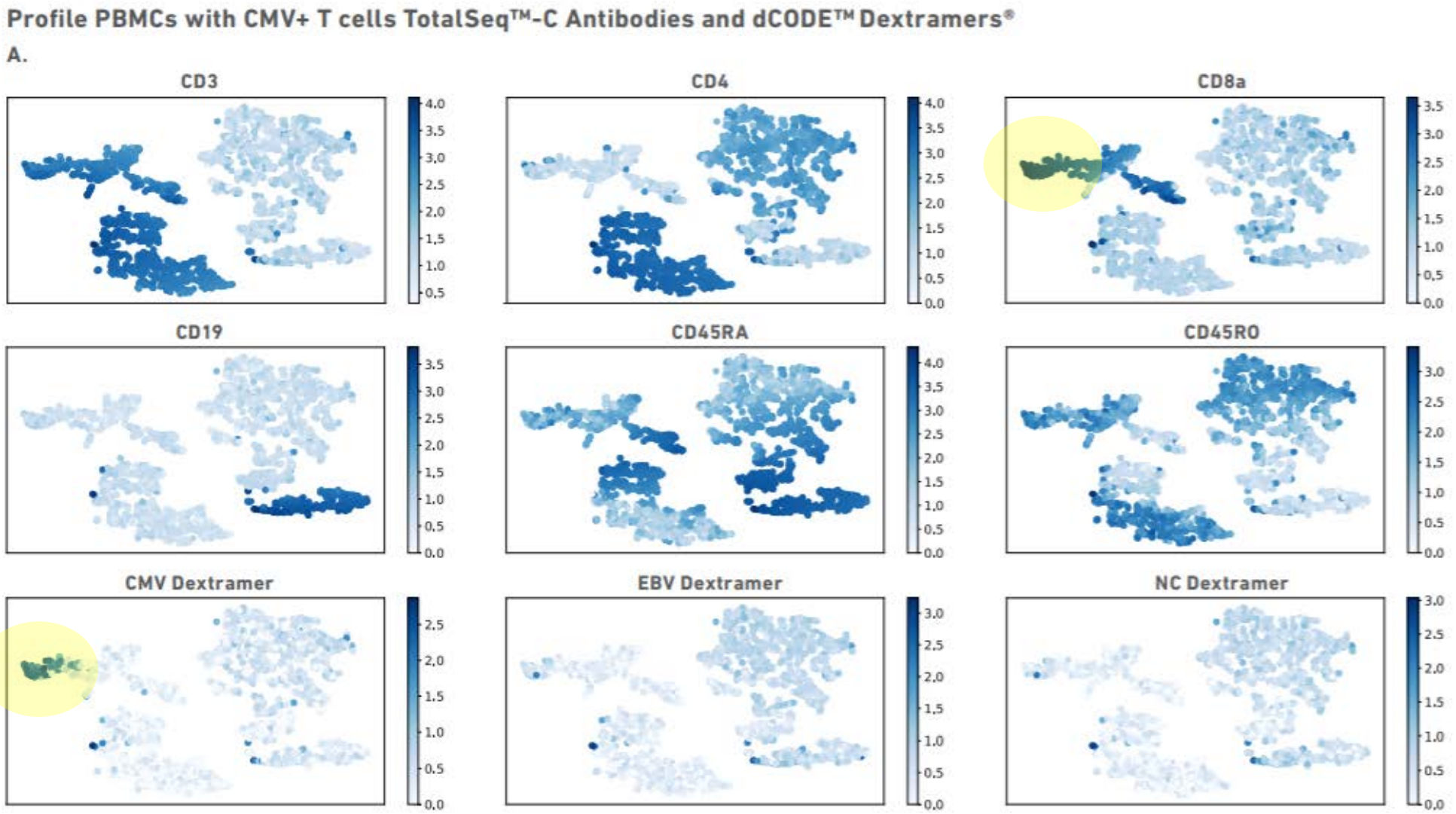
Stain pooled cells with
DeCODE™ MHC Dextramer®
and Totalseq™-C ABs



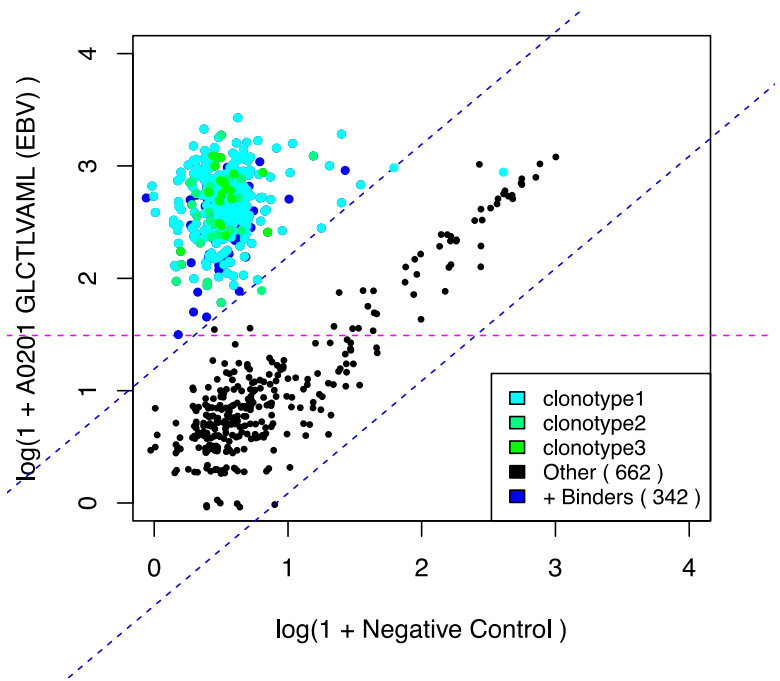


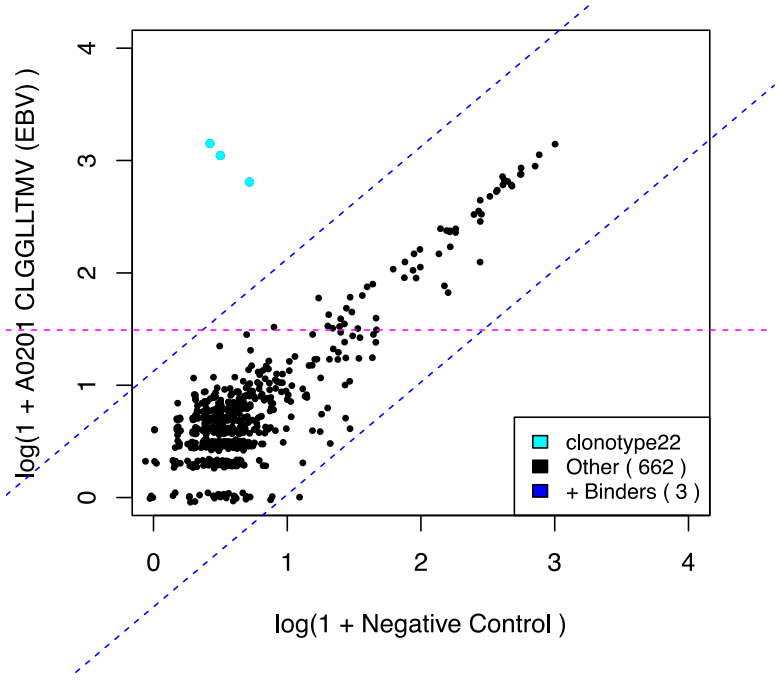
Library Preparation
and Sequencing

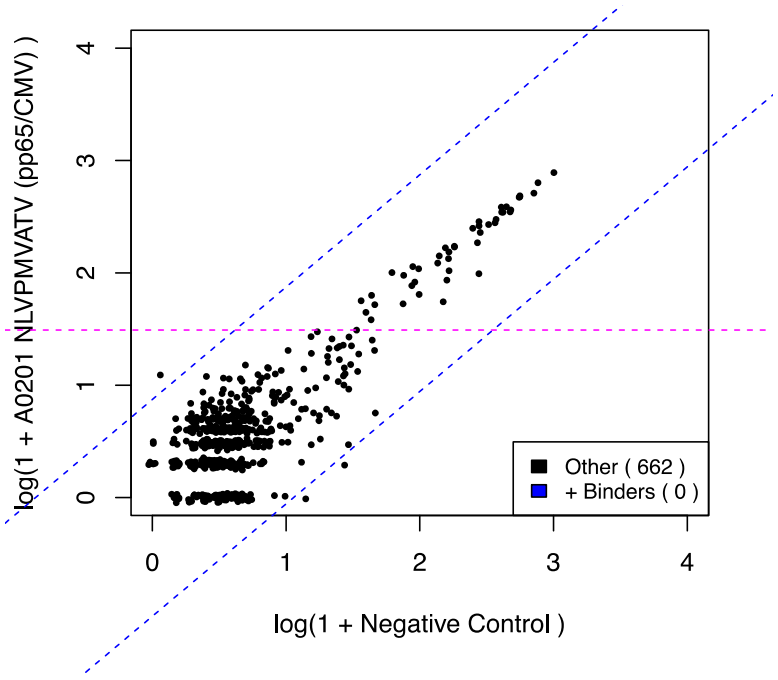
Multi Feature Analysis – An Example



Identification of T-cells with Specific Dextramer Binding Activity







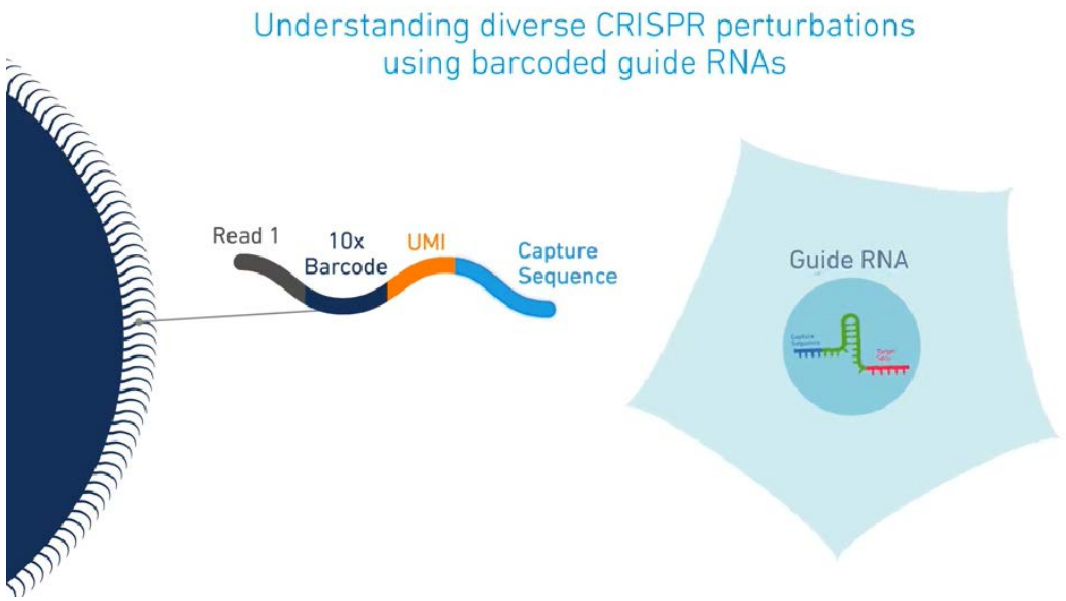
Integration of orthogonal VDJ sequence data is vital for assessment & interpretation

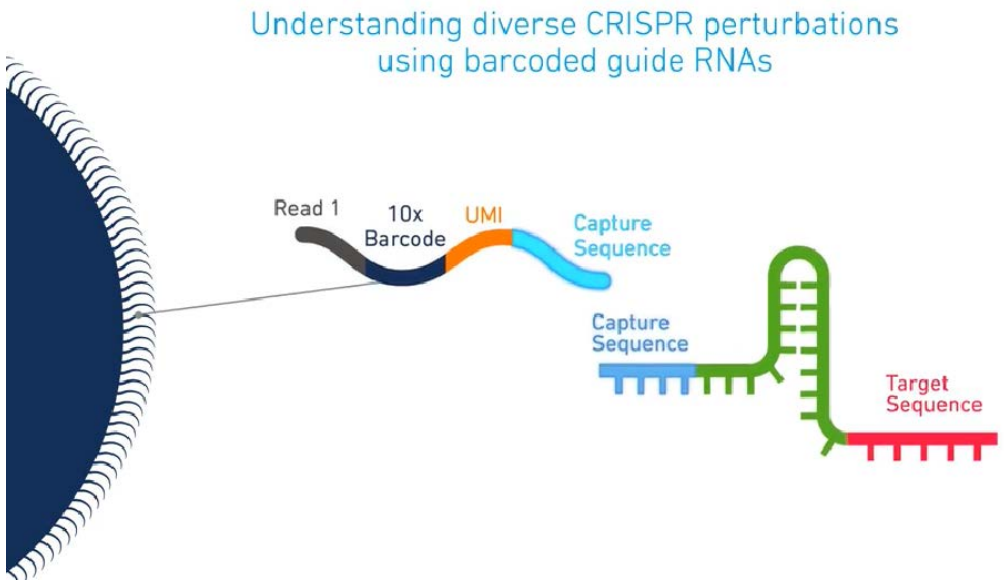
Simple visual assessment of antigens immunogenicity

MHC multimers can be highly multiplexed (10 demonstrated here)

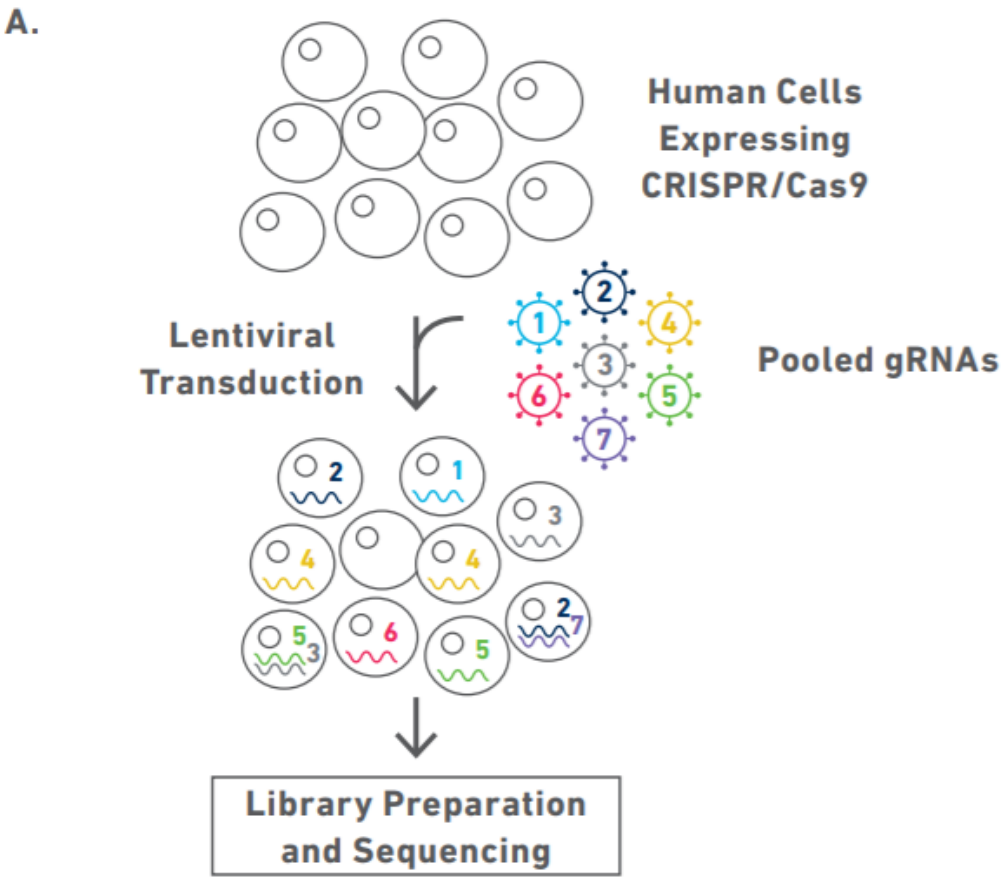
Allows high-throughput mapping of hundreds to thousands of peptide-MHC complexes, identification of rare clonotypes

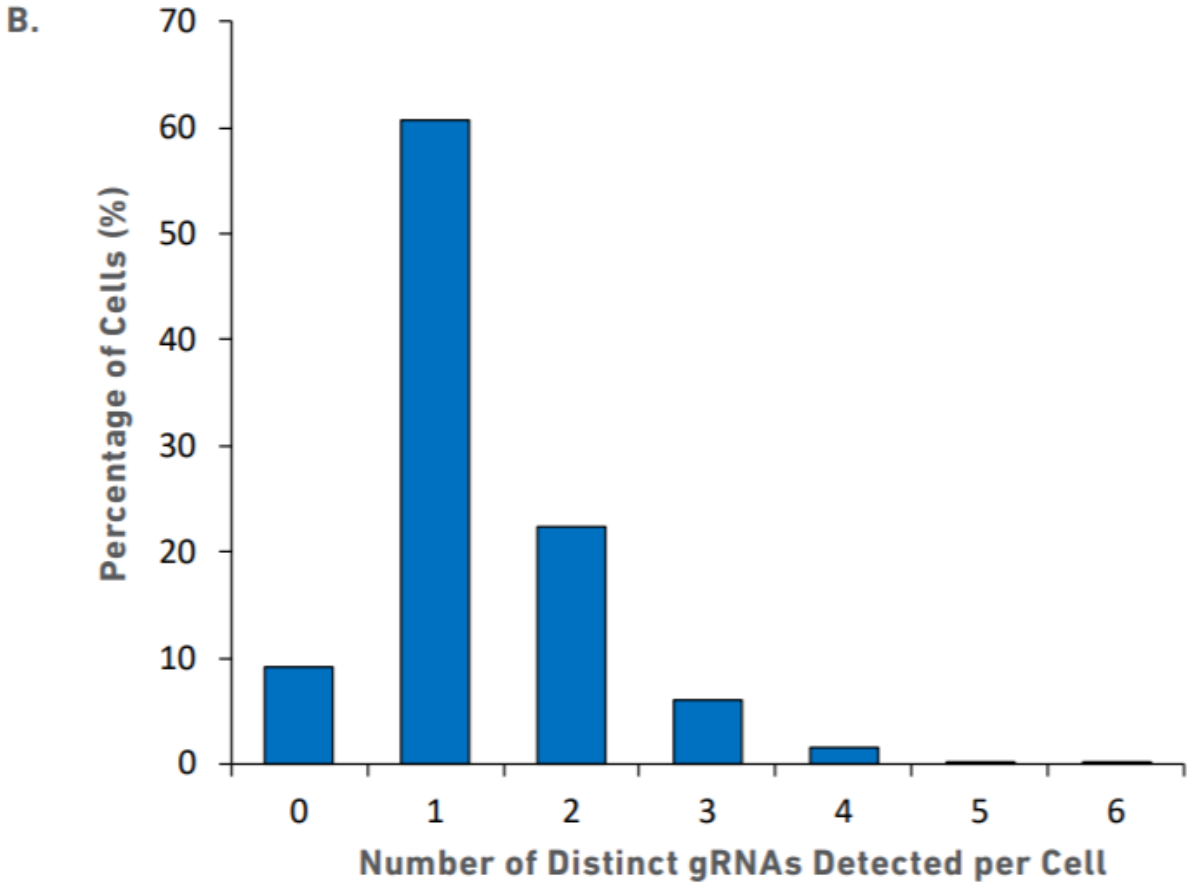
Analysis of Genome Editing Effects in Single Cells





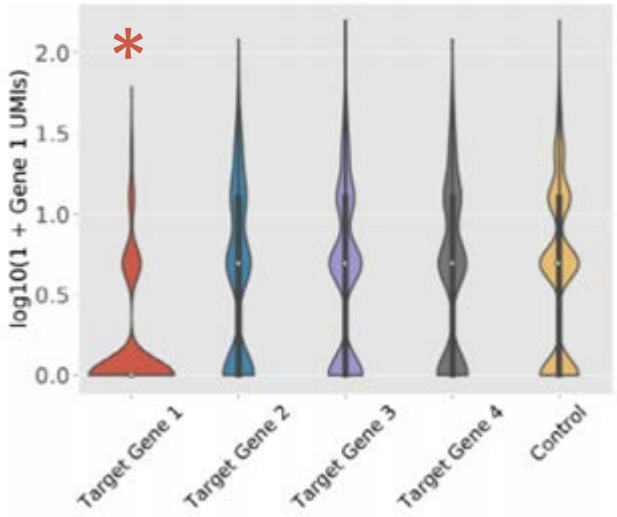
High-throughput Perturbation Studies Enabled via Feature Barcoding Technology



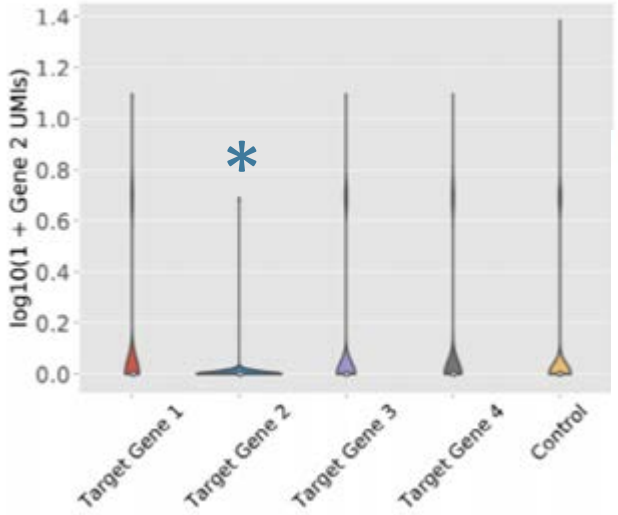


Feature Barcoding Technology Enables Determination of Knockdown Efficiency

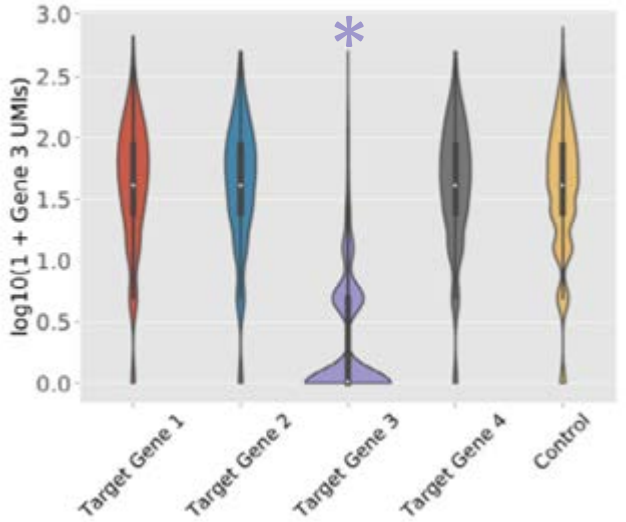
A. Fb gRNAs targeting gene 1



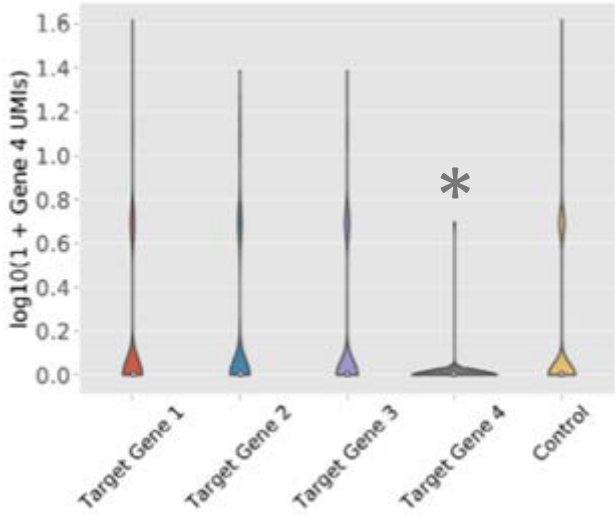
B. Fb gRNAs targeting gene 2



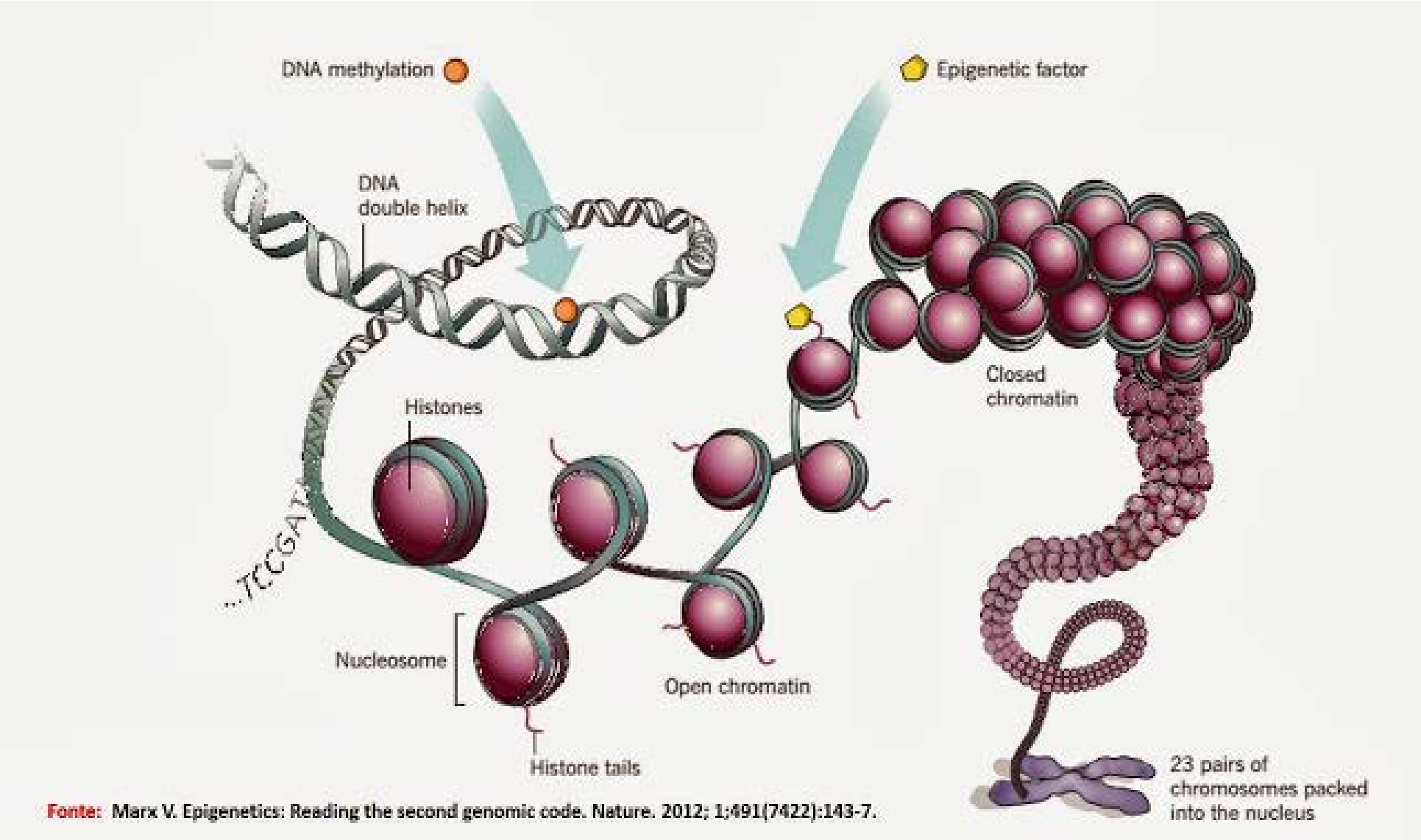
C. Fb gRNAs targeting gene 3



D. Fb gRNAs targeting gene 4

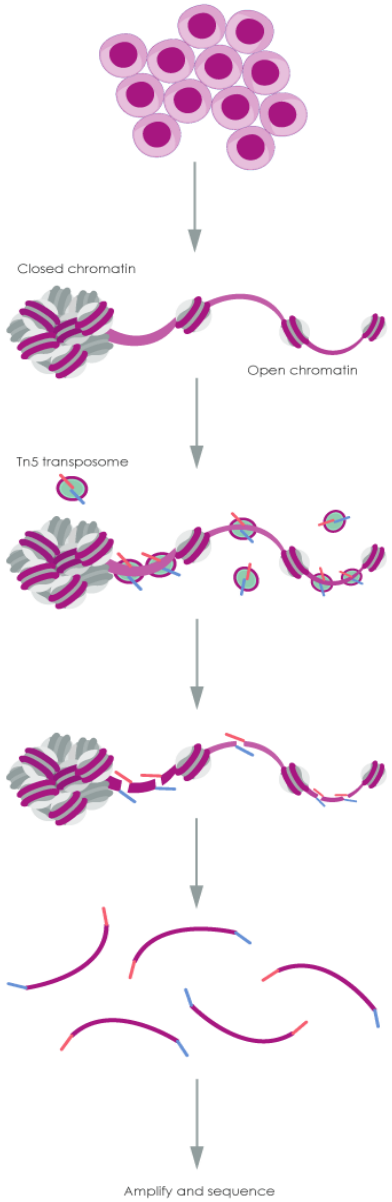


Chromatin Packaging



Fonte: Marx V. Epigenetics: Reading the second genomic code. Nature. 2012; 1;491(7422):143-7.

ATAC-seq – How it works

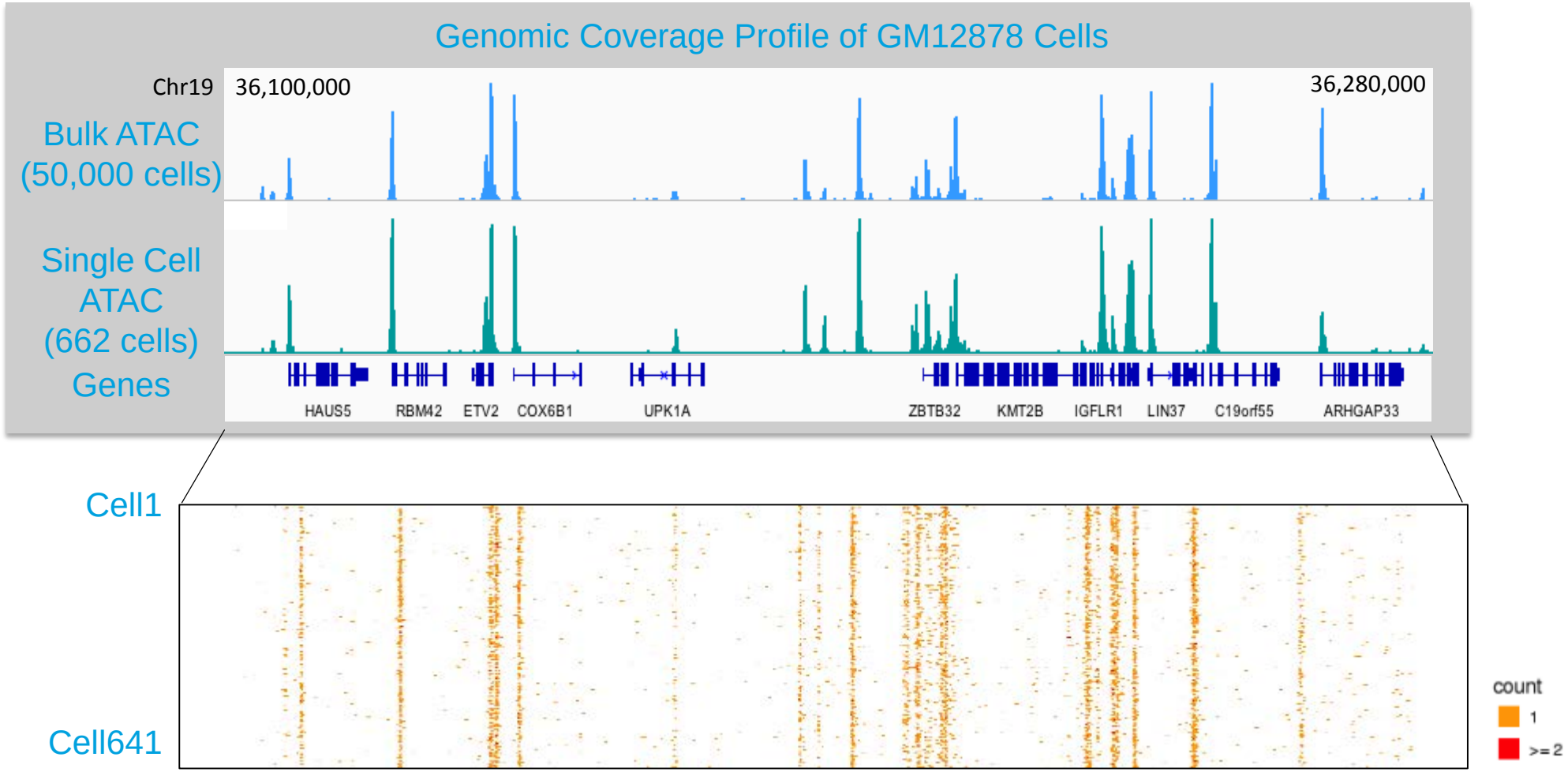


Cell lysis

Transposase

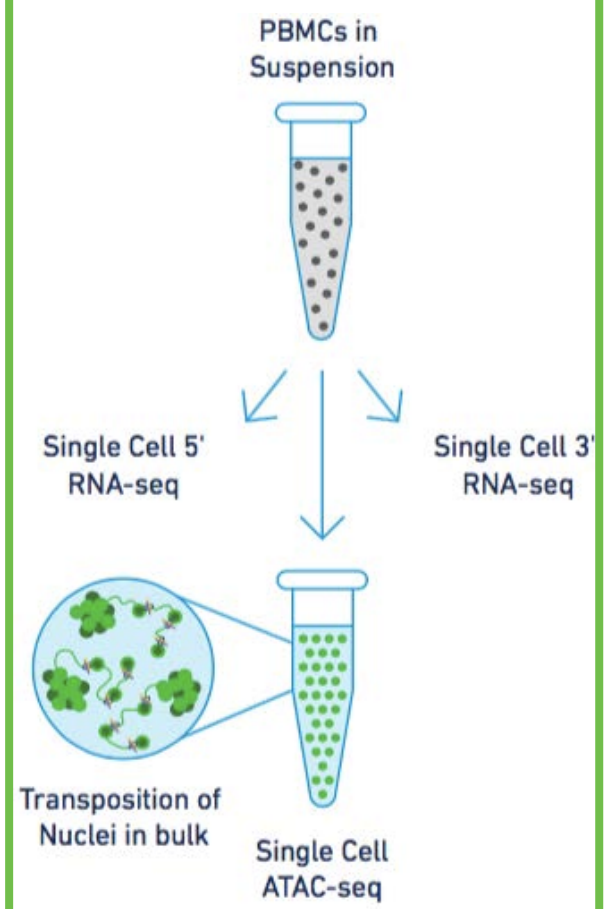
PCR Amplification

Chromatin Accessibility at Single Cell Resolution



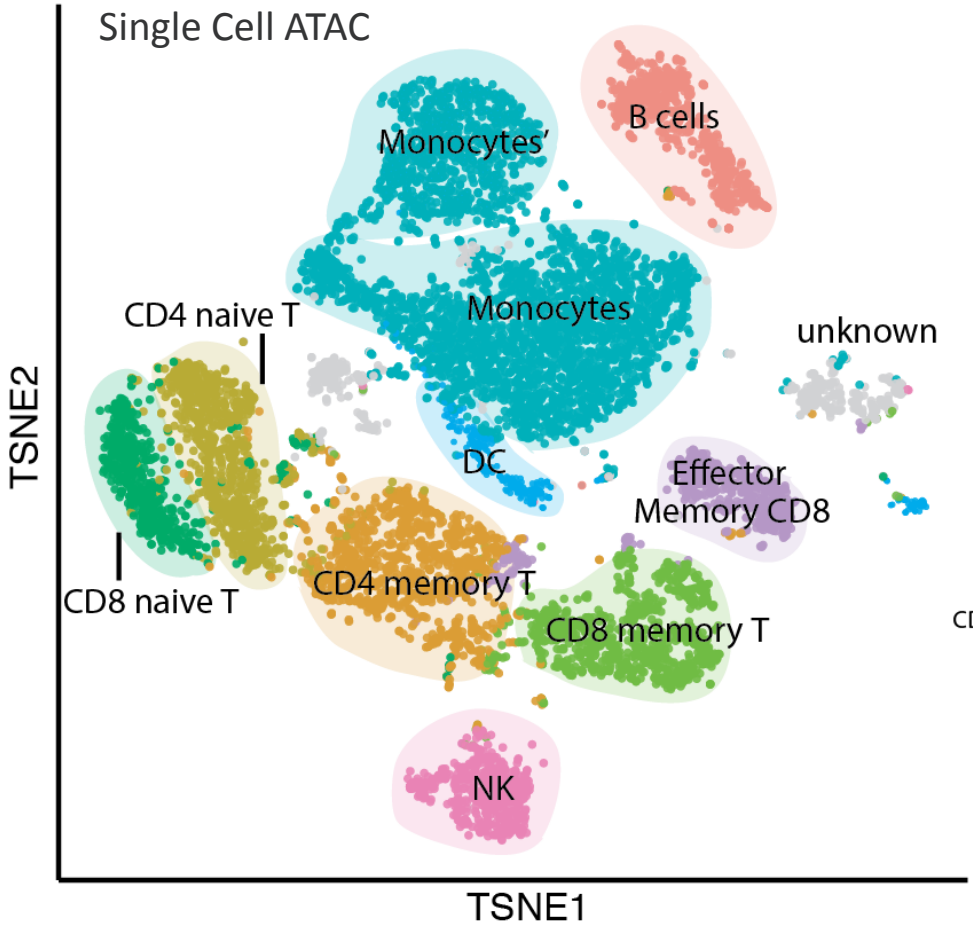
Chromium Single Cell ATAC Solution Provides Complementary Data to Single Cell RNA-Seq

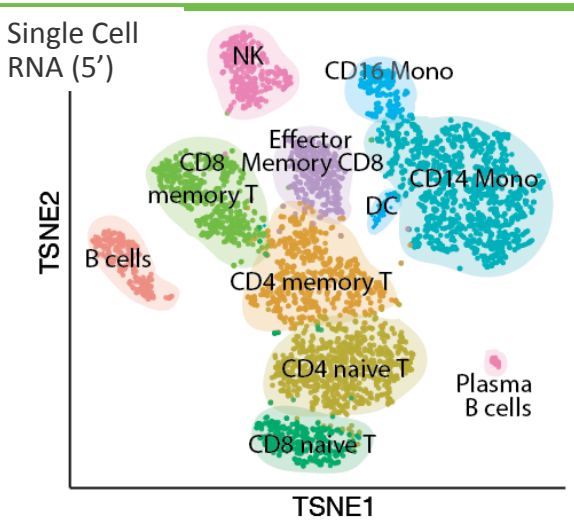
Experimental Setup



PBMCs

Complementary Data

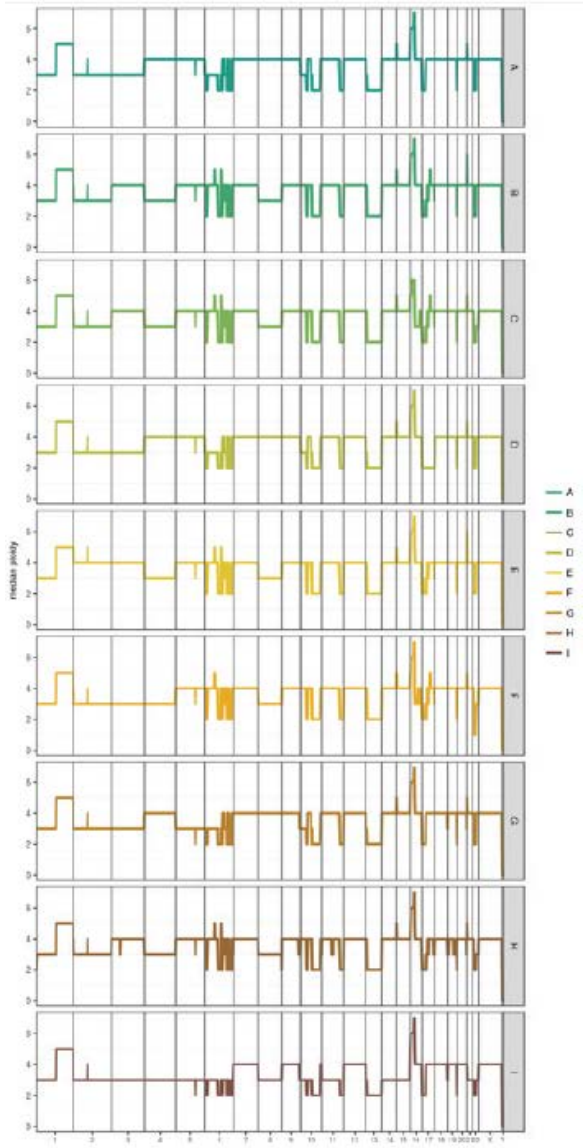


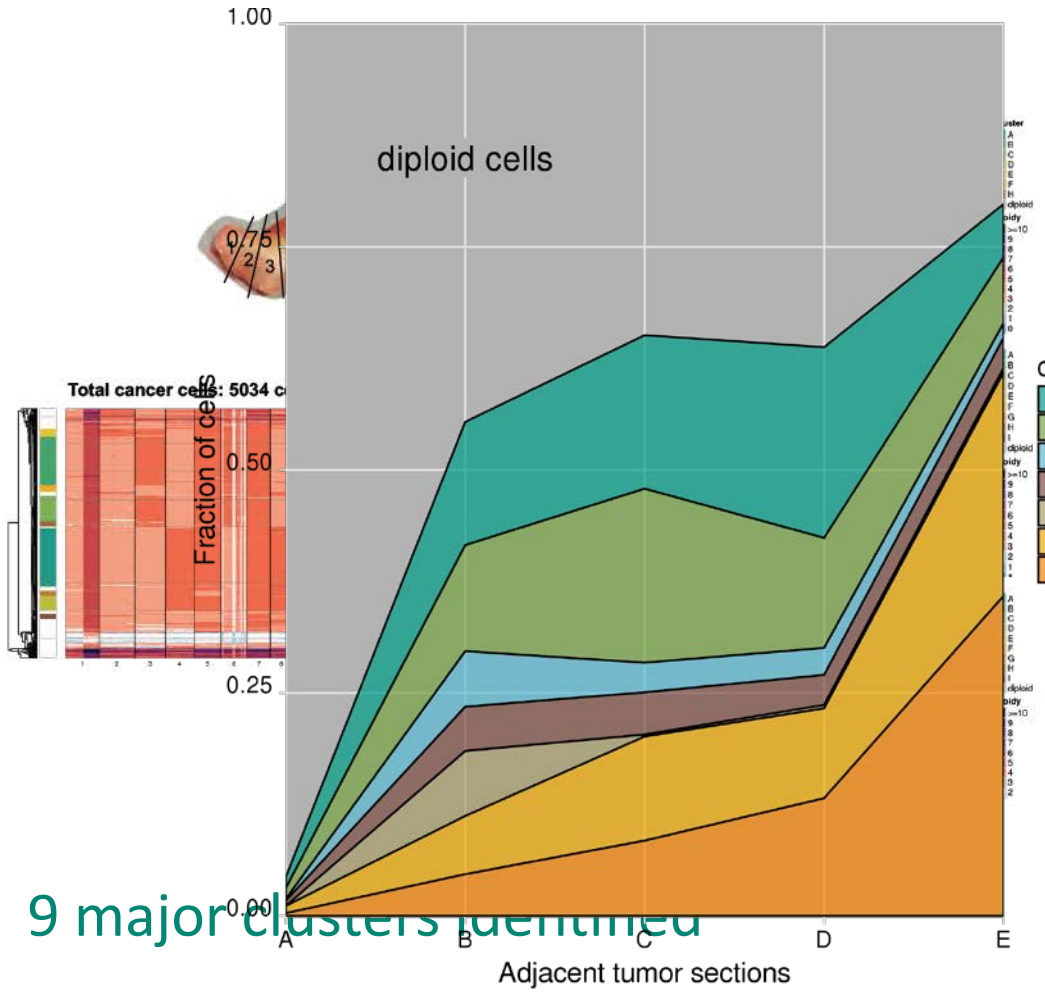


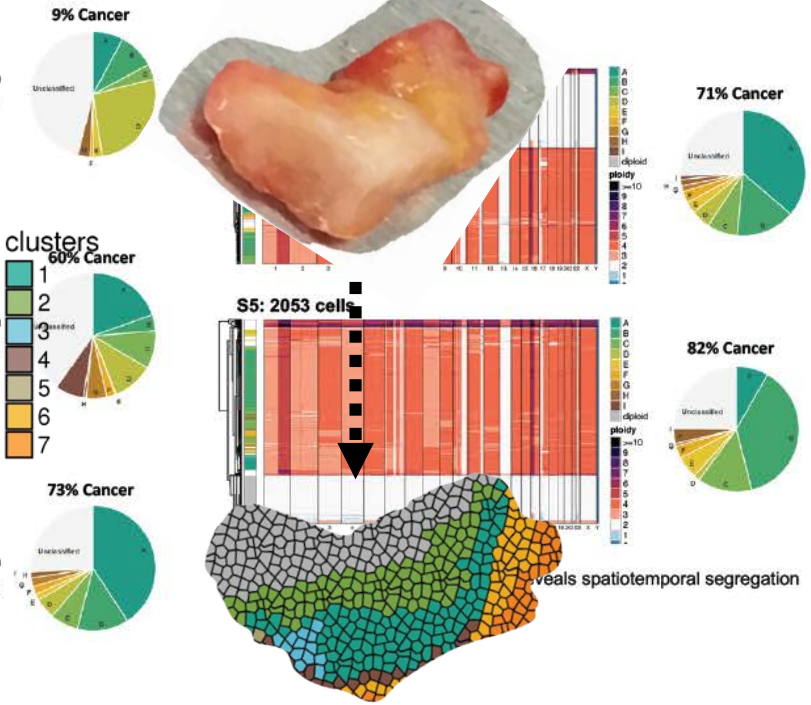
	SC5'	SC3'	scATAC
B cells	247 (5.3%)	158 (6.5%)	687 (6.8%)
CD4 memory T	637 (13.7%)	245 (10.2%)	1,080 (10.7%)
CD4 naive T	794 (17%)	479 (19.9%)	878 (8.7%)
CD8 effector memory T	350 (7.5%)	143 (5.9%)	549 (5.4%)
CD8 memory T	541 (11.6%)	241 (10%)	1,496 (14.8%)
CD8 naive T	320 (6.9%)	–	545 (5.4%)
Monocytes'	181 (3.9%)	70 (2.9%)	918 (9.1%)
DC	73 (1.6%)	63 (2.6%)	323 (3.2%)
Monocytes	1,143 (24.5%)	855 (35.4%)	2,605 (25.8%)
NK	344 (7.4%)	138 (5.7%)	509 (5%)
Plasma B	36 (0.8%)	21 (0.9%)	–
unknown	–	–	498 (4.9%)
Total	4,666	2,413	10,088

PBMCs

Analysis of Spatial Heterogeneity in Breast Cancer





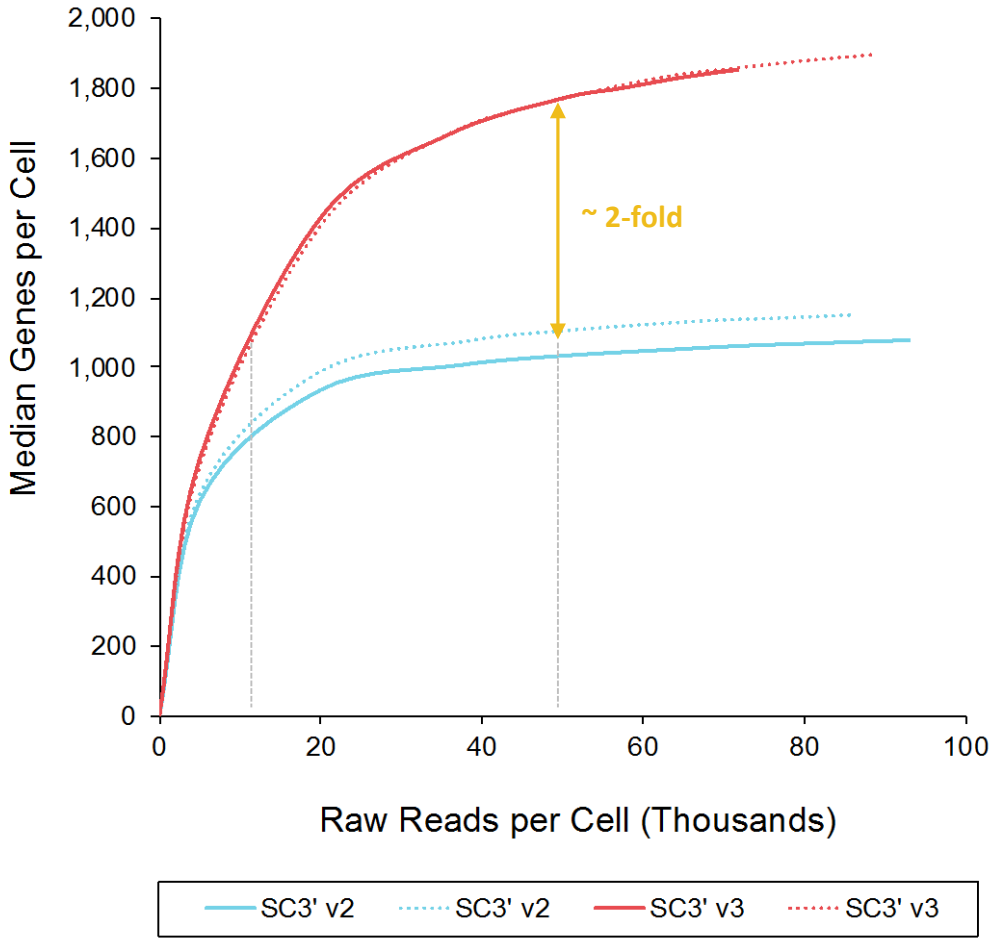


9 major clusters identified

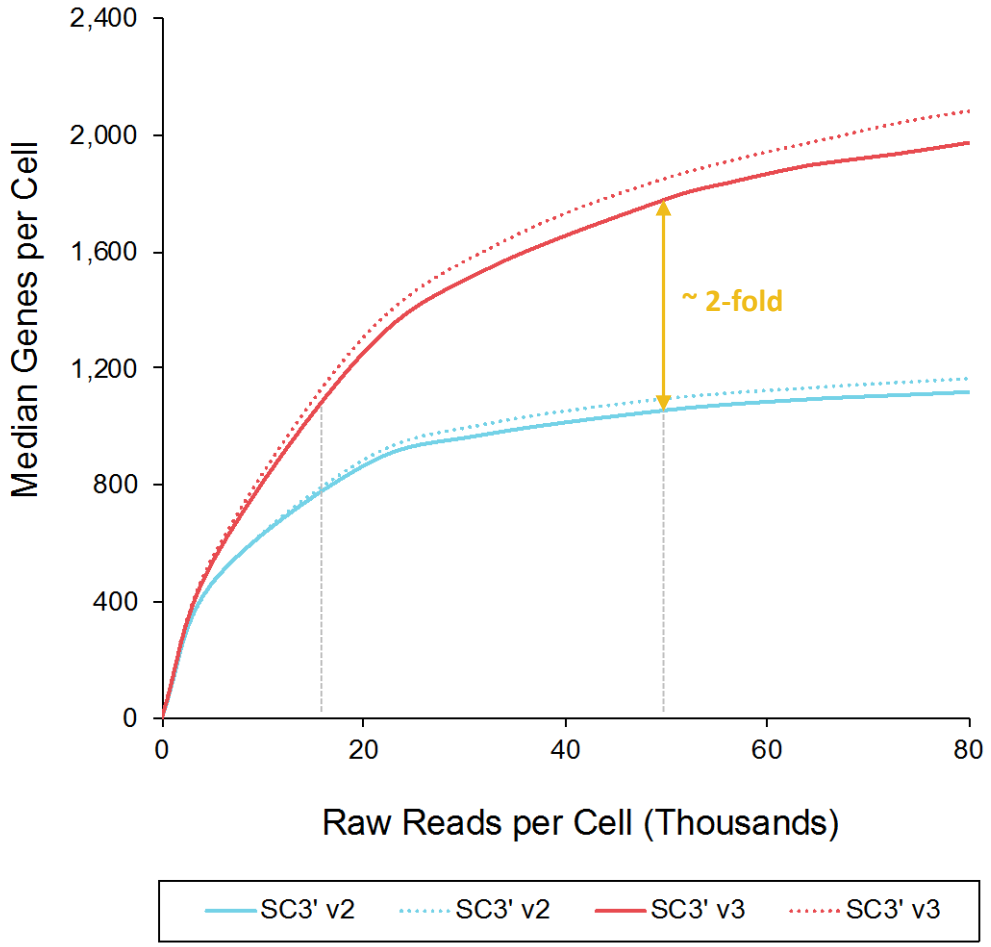
One more thing…

3’ RNA v3: Significant Improvements of Gene Sensitivity

Human PBMCs

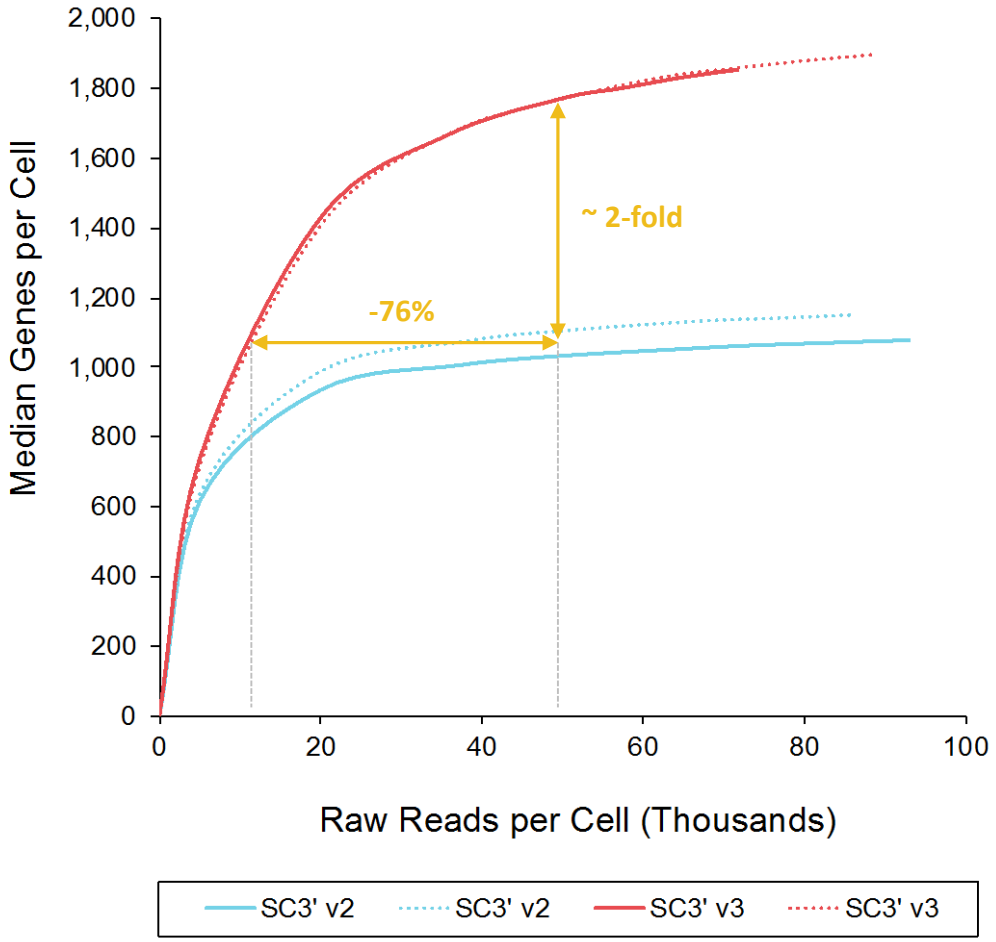


Mouse Embryonic Whole Heart

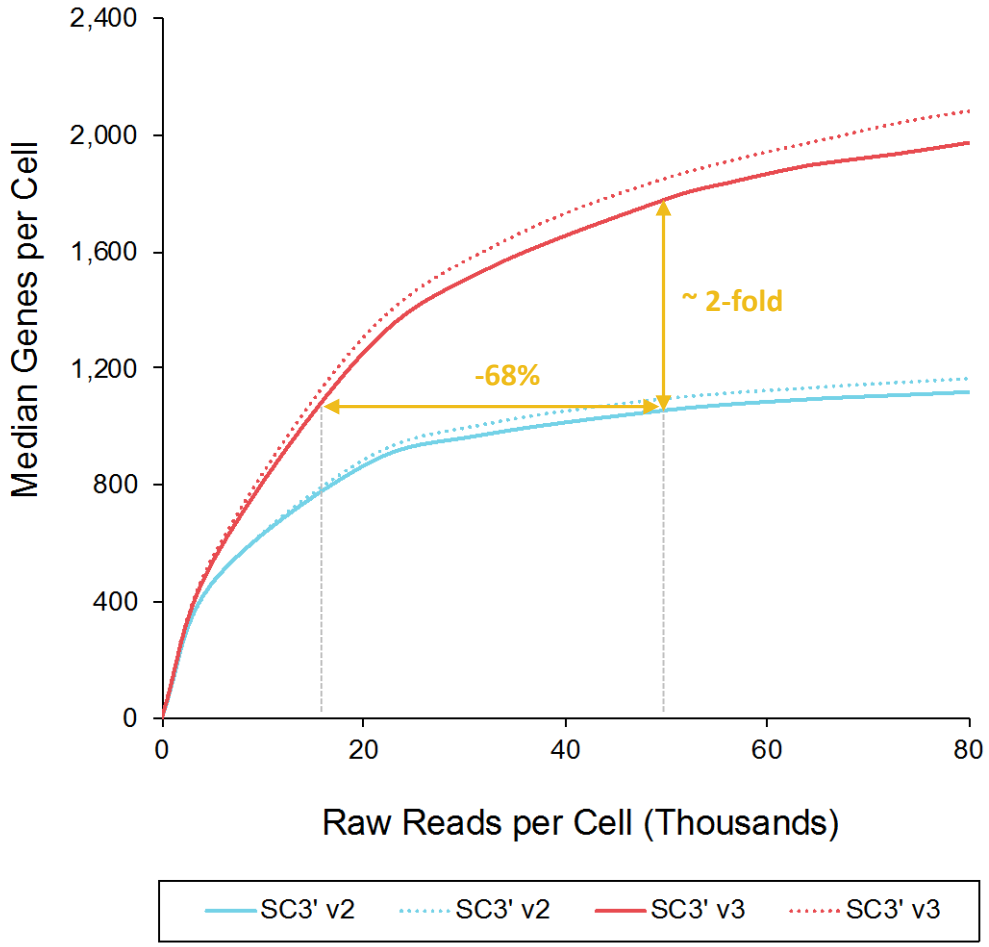


Same Performance – Significantly Lower Sequencing Cost

Human PBMCs



Mouse Embryonic Whole Heart

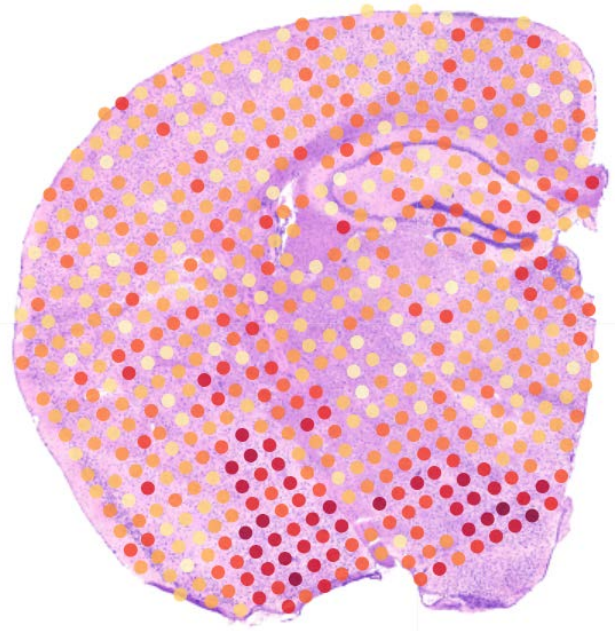


Next to come…

An Analogy for Single Cell Analysis

Spatial Transcriptomics – From *What* to *Where*…

SPATIAL TRANSCRIPTOMICS
Part of 10x Genomics



Unbiased assay of poly-A positive mRNA species in a tissue.

Barcodes used for spatial location of *in situ* RNA expression analysis

10X GENOMICS SOLUTIONS & PRODUCTS RESEARCH & APPLICATIONS EDUCATION & RESOURCES SUPPORT

PRESS RELEASES

The Latest from 10x

PRESS RELEASES IN THE NEWS BLOG EVENTS

10x Genomics Acquires Spatial Transcriptomics

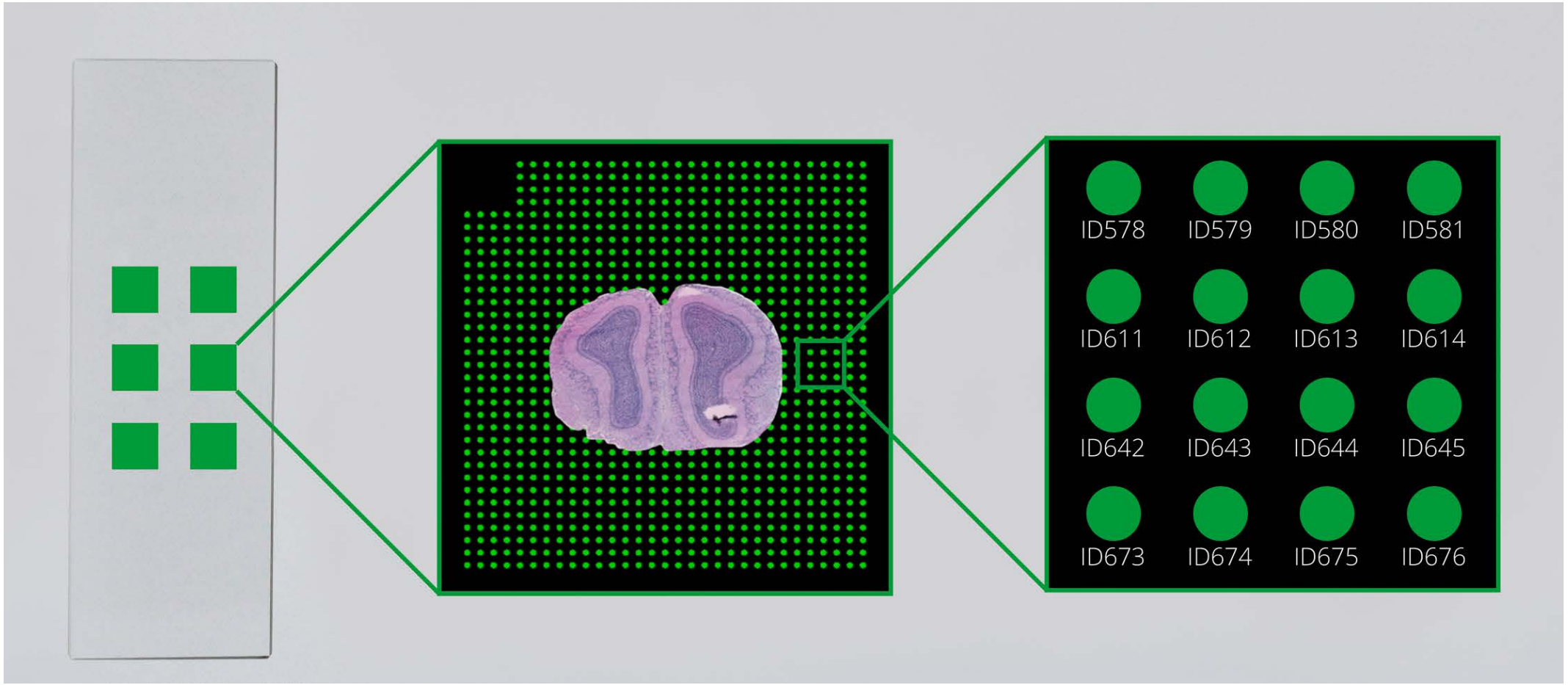
Stakes Claim in Emerging Spatial Genomics Space

PLEASANTON, CA.—December 10, 2018—10x Genomics today announced the acquisition of Stockholm-based Spatial Transcriptomics, a pioneer in the emerging field of spatial genomics. The new field enables researchers to not only see what is in a cell but how cells are organized in relation to one another, offering up invaluable insight into understanding disease by using data that was previously beyond the reach of modern methods. This opens up a field of possibilities within disease areas, such as oncology, neurology and immunology, as well as in the broader area of biology.

Today's announcement builds on a number of recent milestones from 10x Genomics including the acquisition of pioneering epigenetics company Epinomics; introduction of new products; and the expansion of headquarters and manufacturing into Pleasanton, California quadrupling its presence in the city, while creating approximately 200 new jobs.

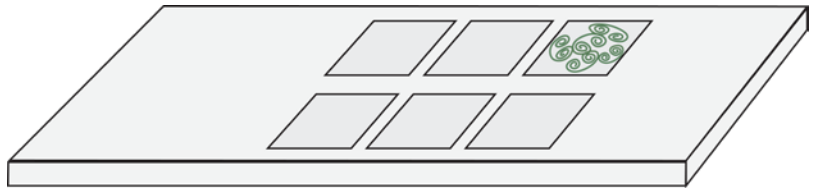
Library Preparation Slide

Introducing spatial barcodes to tissue derived transcripts

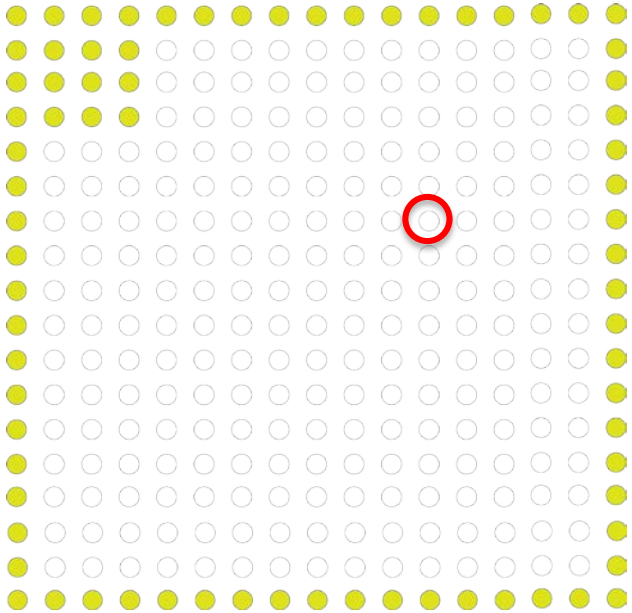


The ST Library Preparation Slide

Glass slide with array of capture probes

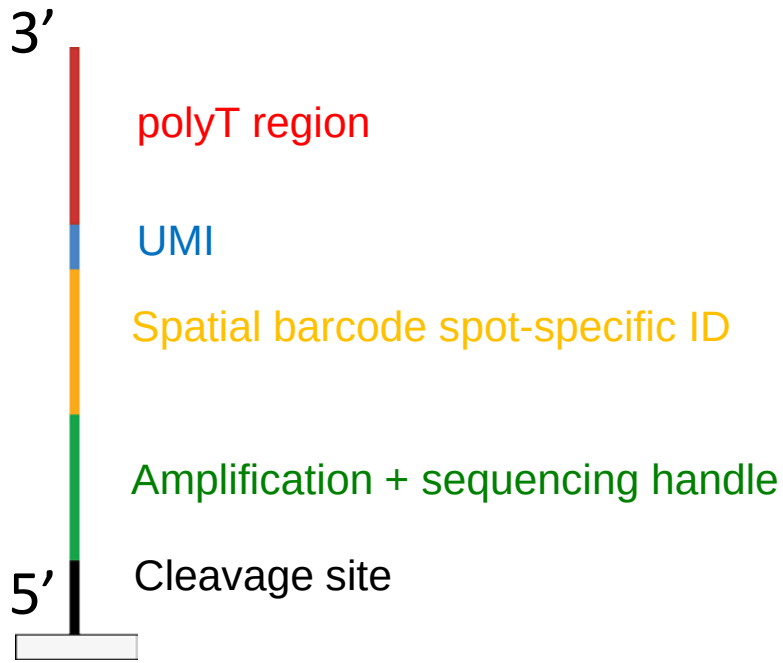


Sub-region with capture probes

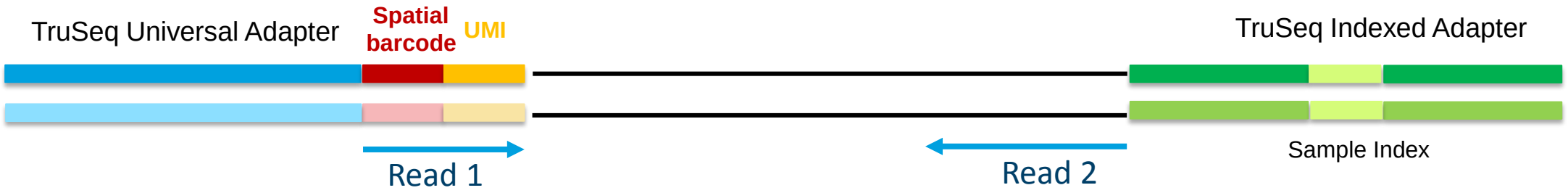


- 1007 spots in total
- 100 µm diameter
- Millions of probes /spot

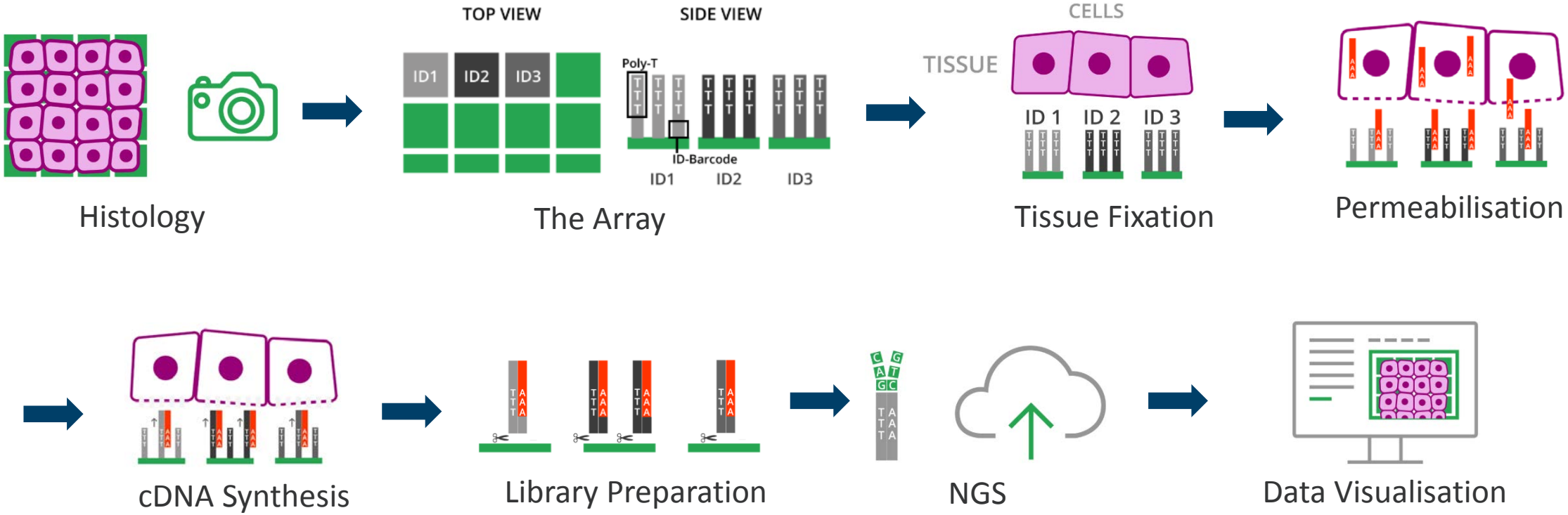
Capture Probe



Final Product

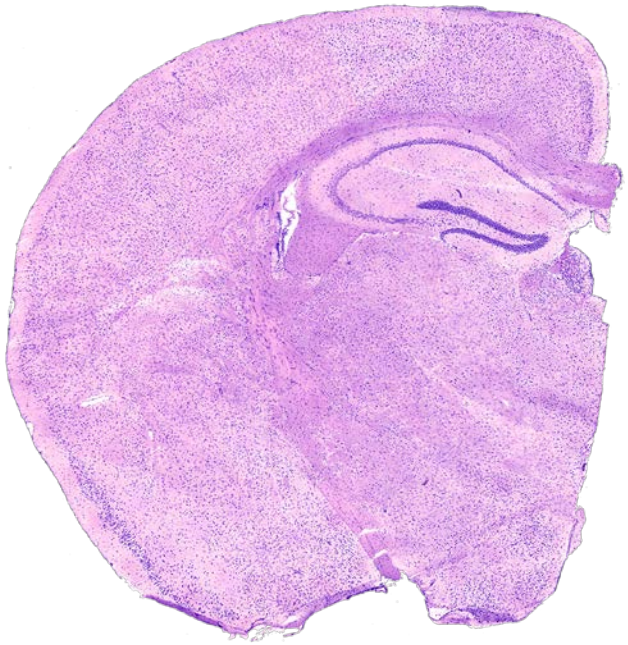


Workflow



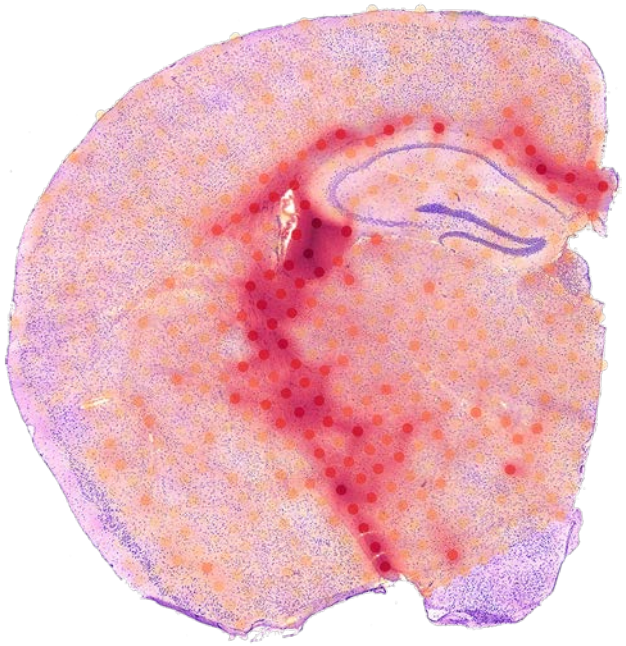
https://spatialtranscriptomics.com/showcase/

Spatial Transcriptomics



Mouse Brain Section

(H&E Stained)



Mouse Brain Section

(overlayed with ST Data)

Inspired?

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Thank You!

Questions?

www.10xgenomics.com