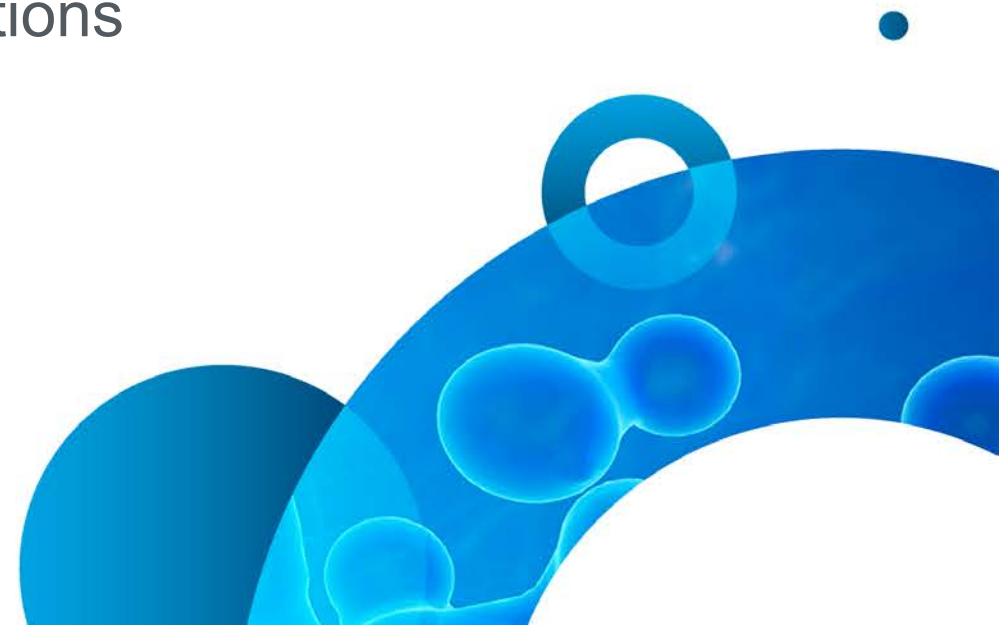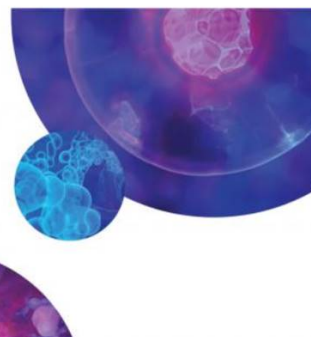# Biology at true resolution

Resolve biological complexities with single cell solutions

Australian National University Pilot Project Support for 10x Genomics Single Cell Projects

10x Genomics and Millennium Science are pleased to announce a special 10x Genomics single cell pilot project program for ANU. The Millennium Science and 10x Genomics pilot support program is a one-off opportunity for researchers to receive support for getting started with 10x single cell technology or support data generation for grant applications for further work.

About the Program:

Millennium Science and 10X Genomics support the program by offering 50% off either 3' SC V3.1, 5' SC V2 or SC ATAC V1.1 10X Genomics reagents for approved pilot studies. This offer is limited to 4 reactions per Lab Group and is for new customers.

How to Apply:

All interested researchers are required to complete a registration form including the summary and aims of their project and how it will support further work.

About the Program:

All applications must be received by 13th November 2020. All Consumables must be purchased by 4th December with delivery to be taken prior to end of 2020.

Submit Application

ACRF Biomolecular Resource Facility

Photo: Tim Levy from the ACRF

Sales in Australia and New Zealand

James Miller, PhD

Business Development Manager

jmiller@mscience.com.au

Millennium Science Pty Ltd

Marissa Doherty

Territory Manager NSW/ACT

mdoherty@mscience.com.au

Millennium Science Pty Ltd

Support in Australia and New Zealand

Paul Gooding PhD

Field Applications Scientist

Australia and New Zealand

pgooding@mscience.com.au

Millennium Science Pty Ltd

Catherine King

Senior Field Applications Scientist
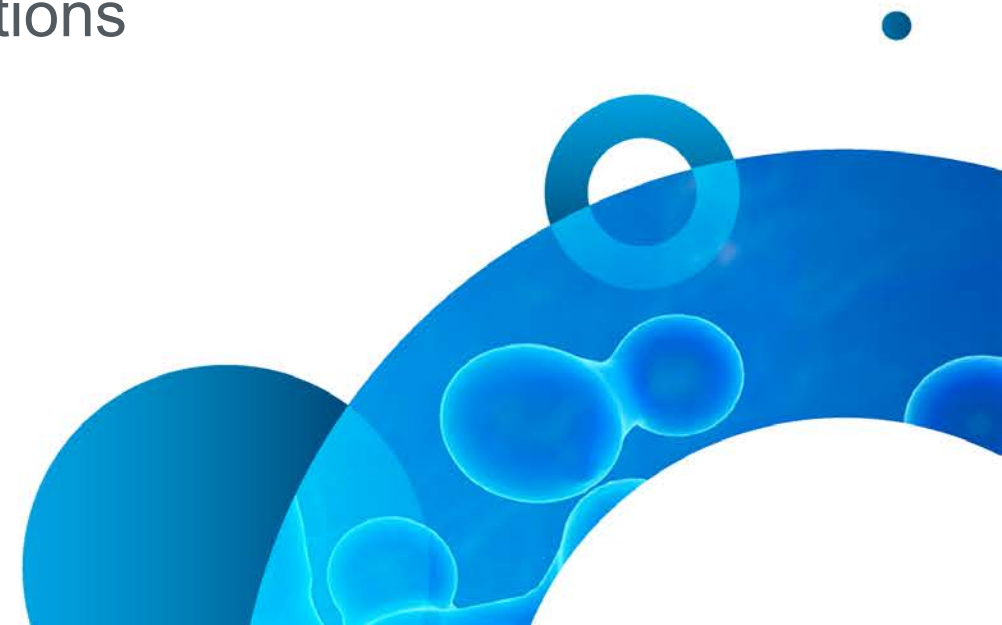
Australia and New Zealand

10x Genomics

Biology at true resolution

Resolve biological complexities with single cell solutions

Leo Chan, PhD
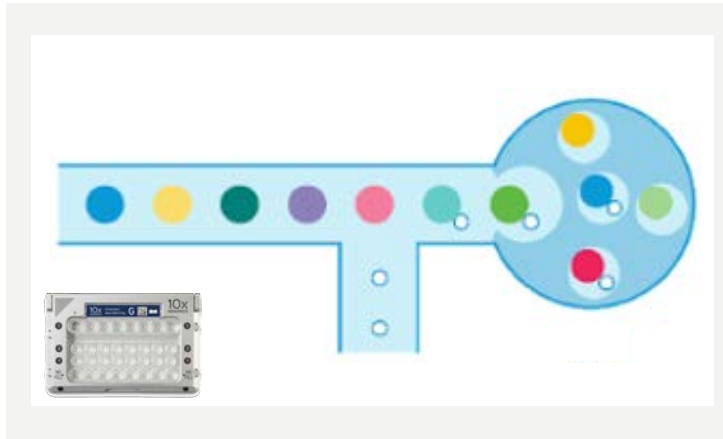Science & Technology Advisor, APAC Lead
Oct 13, 2020

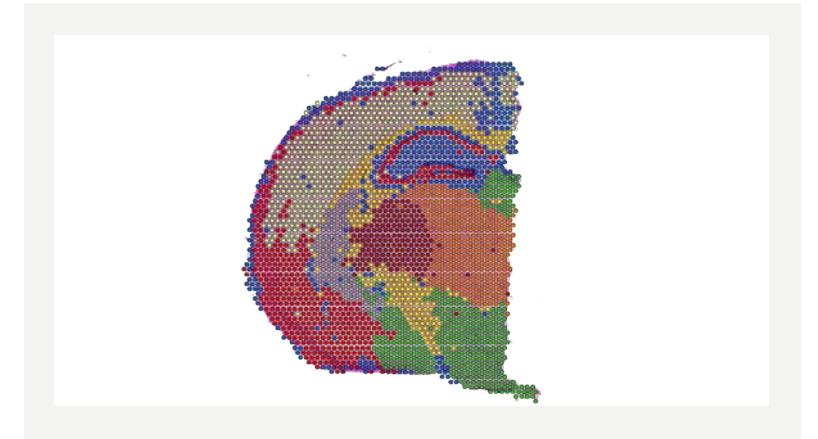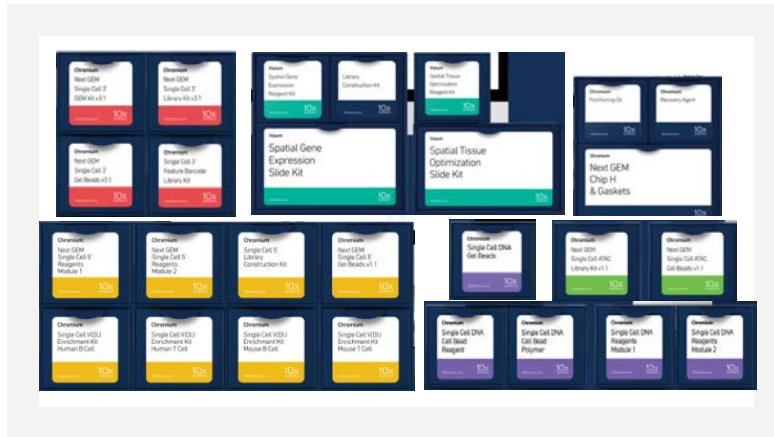# 10x develops tools to resolve complexity

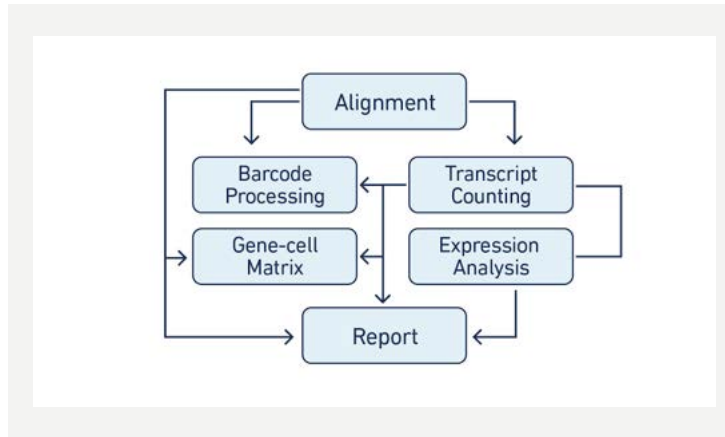### Instruments

Microfluidic Partitioning & Barcoding
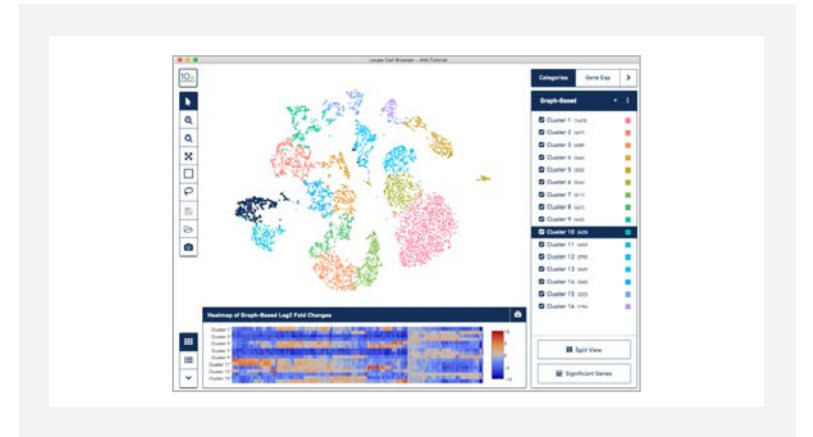

Spatial Barcoding
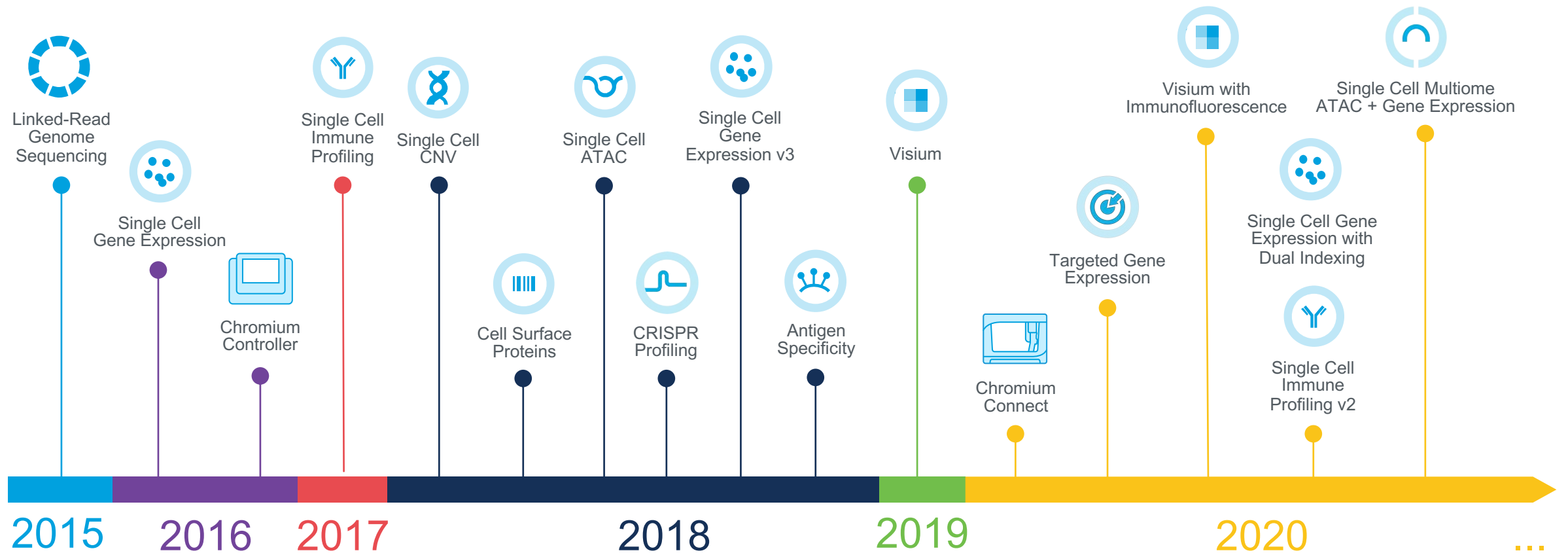

Novel Assays & Biochemistry
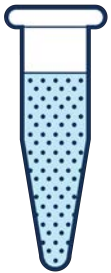

Analysis Software

Visualization Software

10x Innovation Engine

2015 — Linked-Read Genome Sequencing; Single Cell Gene Expression

2016 — Chromium Controller

2017 — Single Cell Immune Profiling

2018 — Single Cell CNV; Single Cell ATAC; Single Cell Gene Expression v3; Cell Surface Proteins; CRISPR Profiling; Antigen Specificity

2019 — Visium; Chromium Connect

2020 — Visium with Immunofluorescence; Single Cell Multiome ATAC + Gene Expression; Single Cell Gene Expression with Dual Indexing; Single Cell Immune Profiling v2; Targeted Gene Expression

...

Chromium Single Cell Gene Expression workflow

Sample

Chromium Controller and consumables

Next generation sequencer
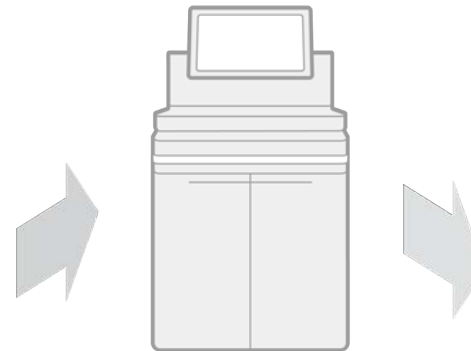
Analysis and visualization software

Less than 25% of customers own a DNA sequencer

Chromium Controller and Single Cell Gene Expression Reagents

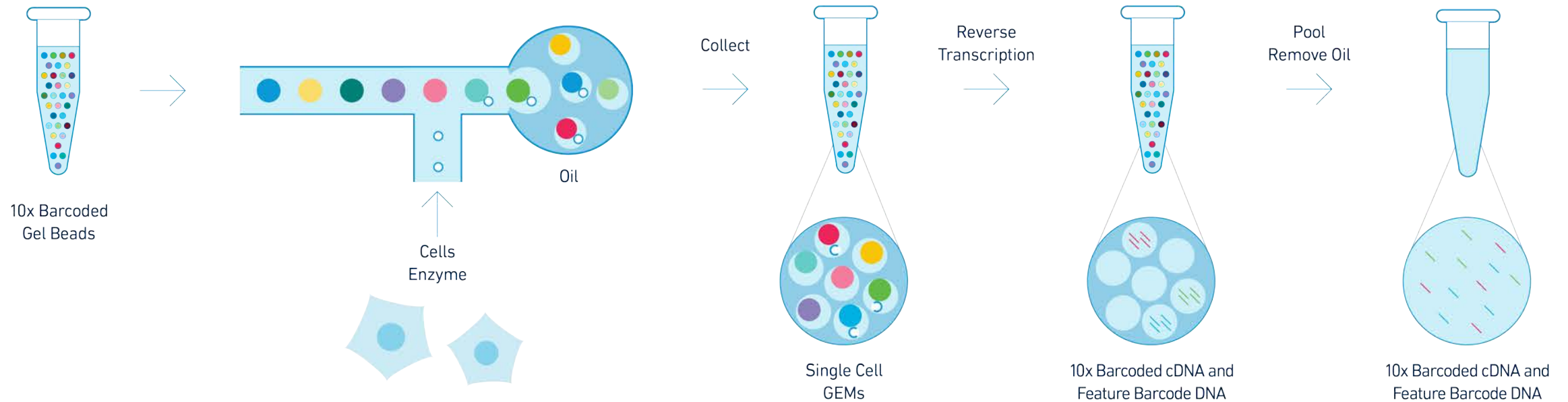• Gel Bead and Library Reagents
• Feature Barcoding Library Kit
• Chip and Sample Indexes

Cell Ranger and Loupe Browser

• Generate gene-cell matrix
• Evaluate actionable QC metrics
• Explore, annotate cell clusters
• Compare multiple samples

The 10x technology

10x Barcoded
Gel Beads

Cells
Enzyme

Oil

Collect

Single Cell
GEMs

Reverse
Transcription

10x Barcoded cDNA and
Feature Barcode DNA

Pool
Remove Oil

10x Barcoded cDNA and
Feature Barcode DNA

Single Cell Gene Expression with Feature Barcode technology

Feature Barcode technology Enabled via Single Cell 3′ Gel Beads
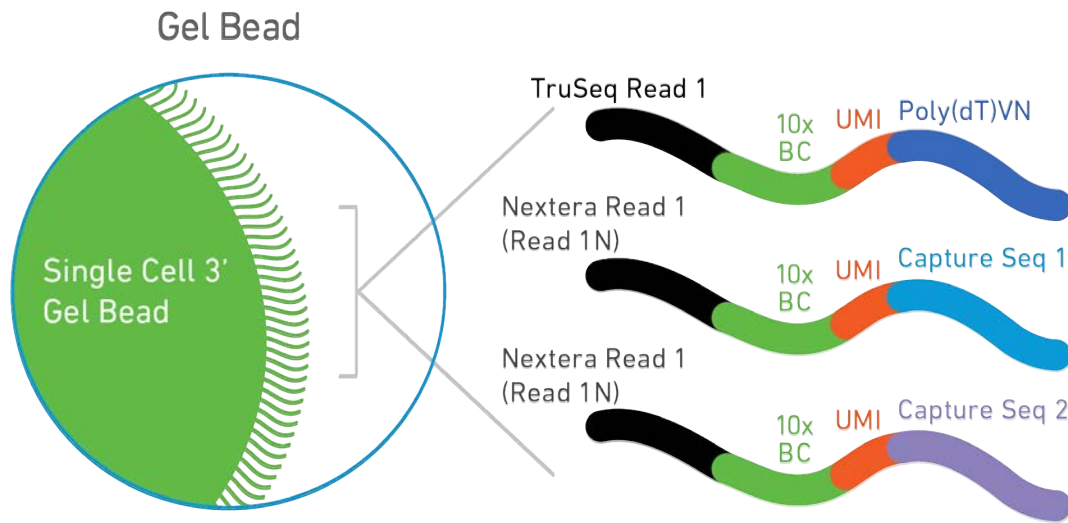
i. **TruSeq Read 1**

22 nt Partial Illumina TruSeq Read 1 sequence

ii. **10x Barcode**
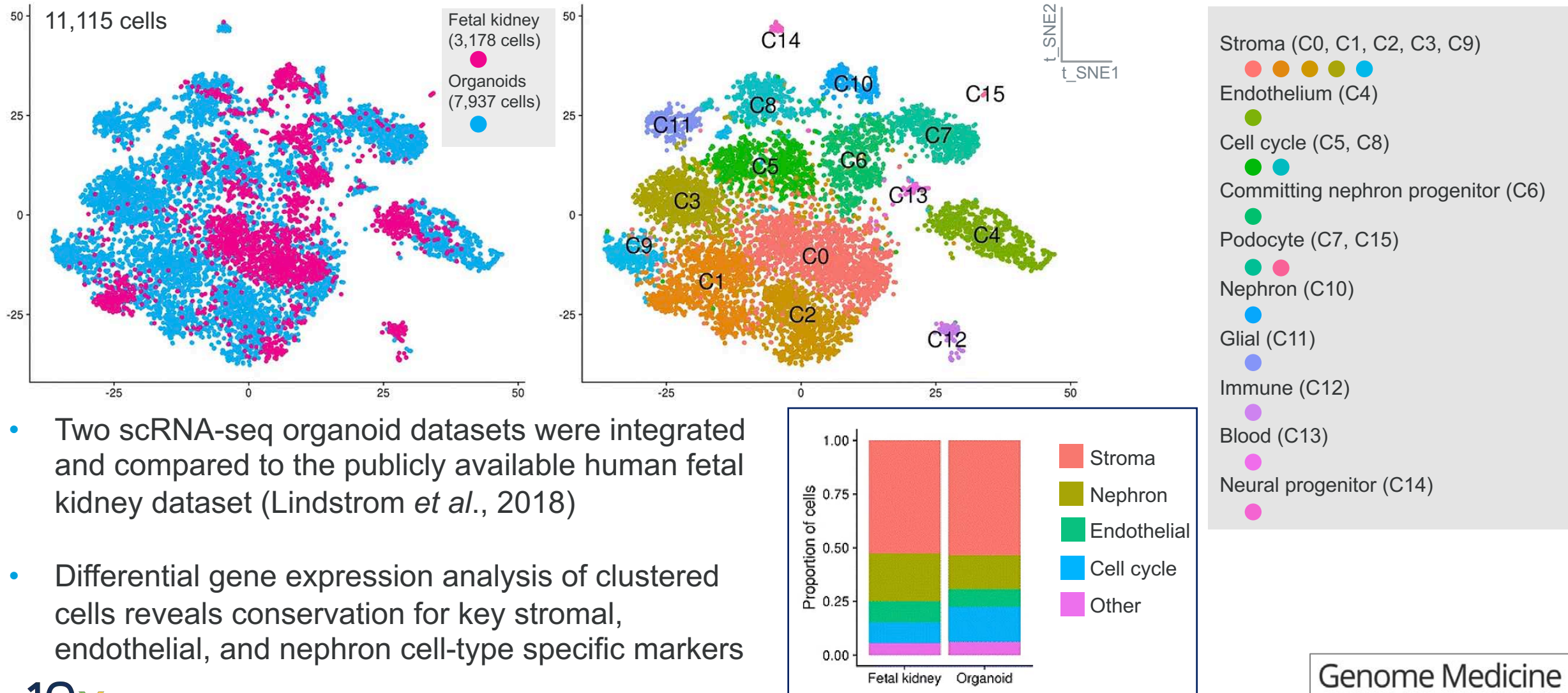
16 nt 10x Barcode
~3.6 M defined barcode sequences

iii. **UMI**

12 nt Unique Molecular Identifier

iv. **Poly(dT)VN**

30 nt Poly(dT) sequence
Enables capture of poly-adenylated mRNA molecules

Cell Type Congruency in Organoids and Fetal Kidney Affirms the Fidelity of Human Kidney Organoids as Models

- Two scRNA-seq organoid datasets were integrated and compared to the publicly available human fetal kidney dataset (Lindstrom *et al*., 2018)

- Differential gene expression analysis of clustered cells reveals conservation for key stromal, endothelial, and nephron cell-type specific markers

Single-cell transcriptomic analysis of Alzheimer's Disease

- Authors used the Chromium Single Cell 3' Gene Expression System to profile transcriptional signatures of ~80k nuclei across 6 major cell types.

- Differential gene expression disease-associated perturbations were largely cell-type specific, specifically at early stages.

- Single cell transcriptomic profiling resolution unmasked differential expression profiles with opposite directionality in different cell types.
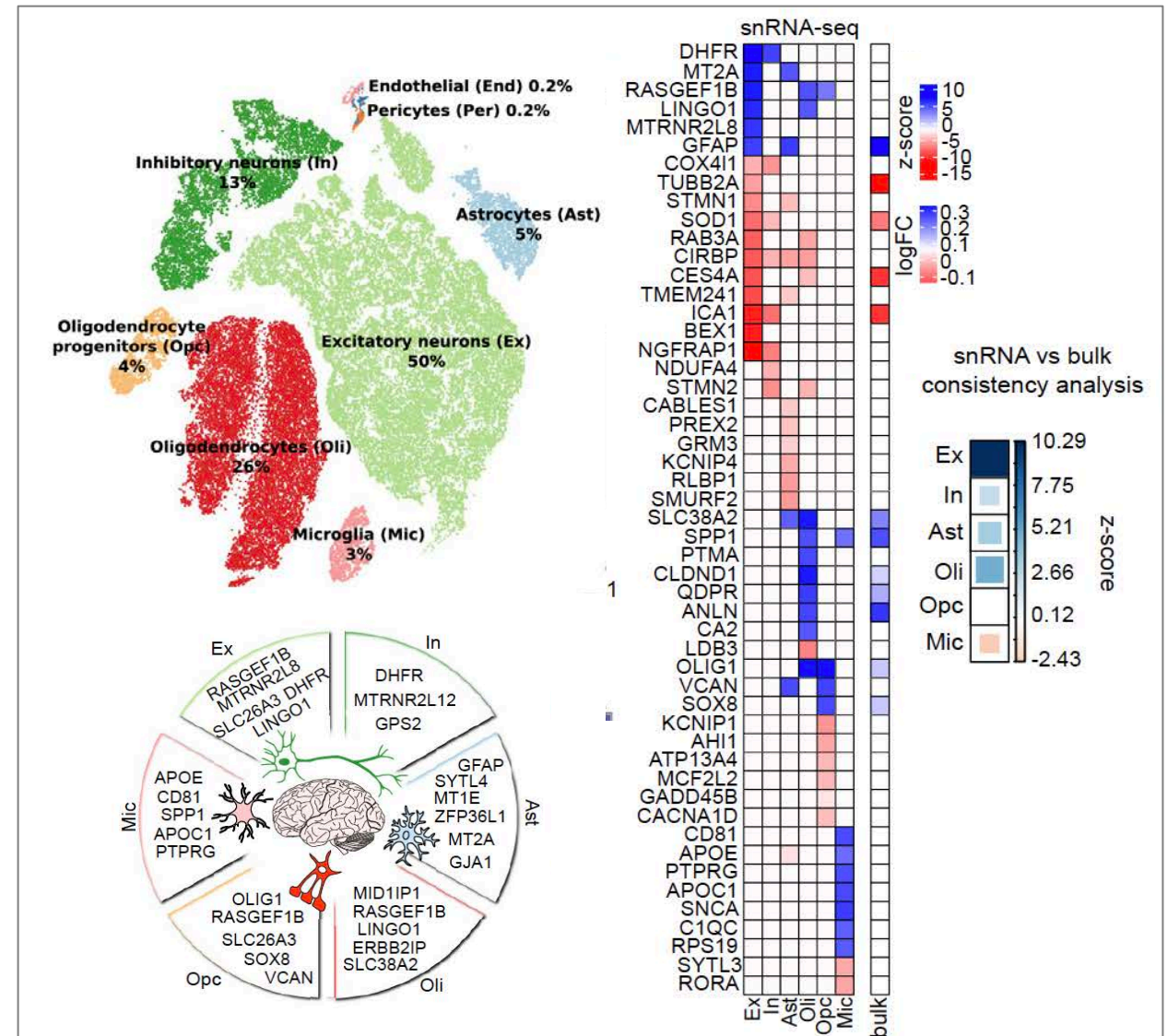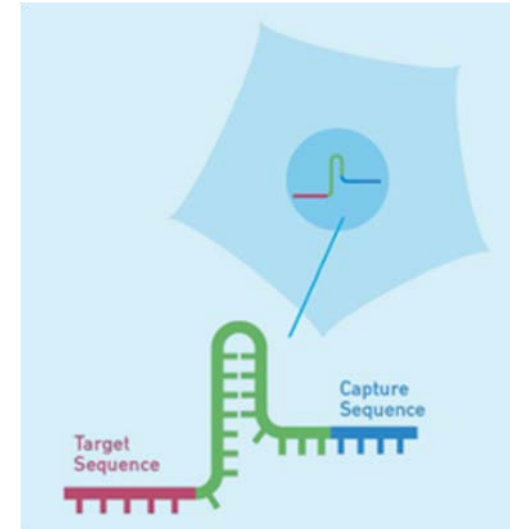
Introduction to Feature Barcode technology

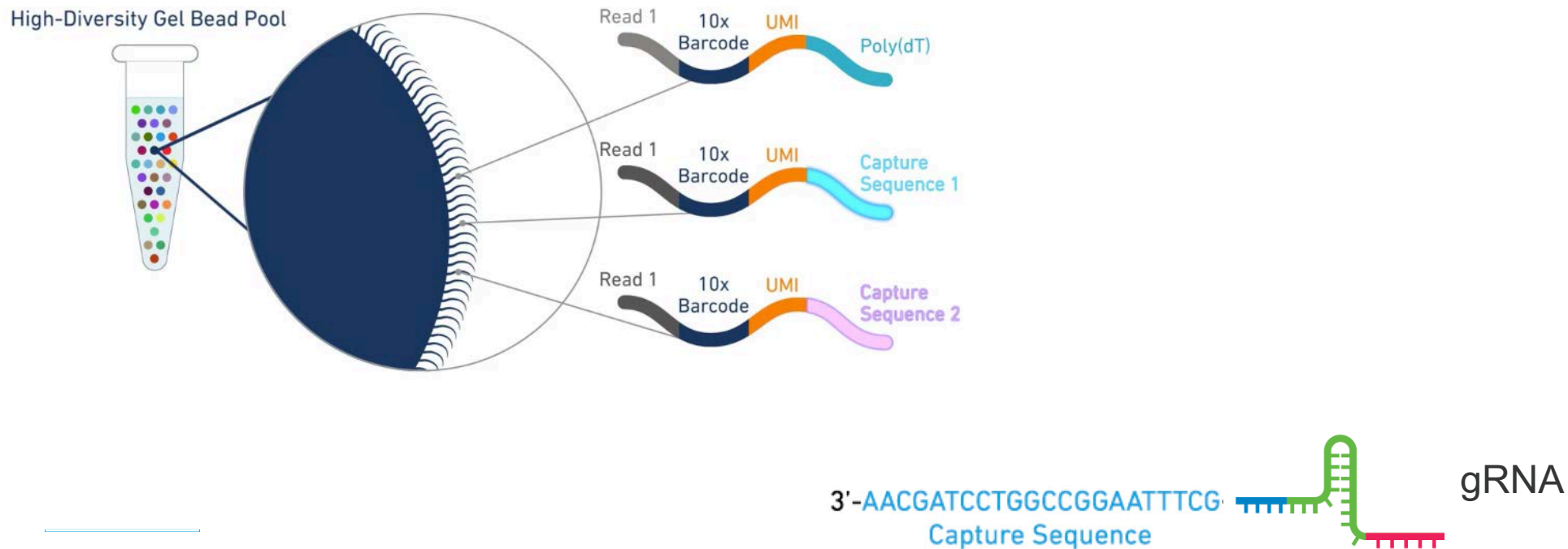Simultaneously measure mRNA-based gene expression and CRISPR screening
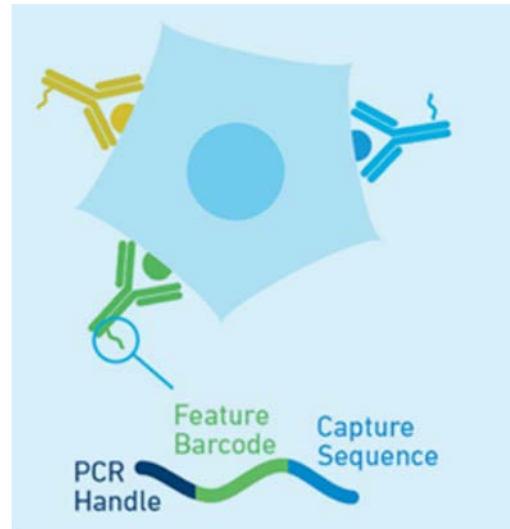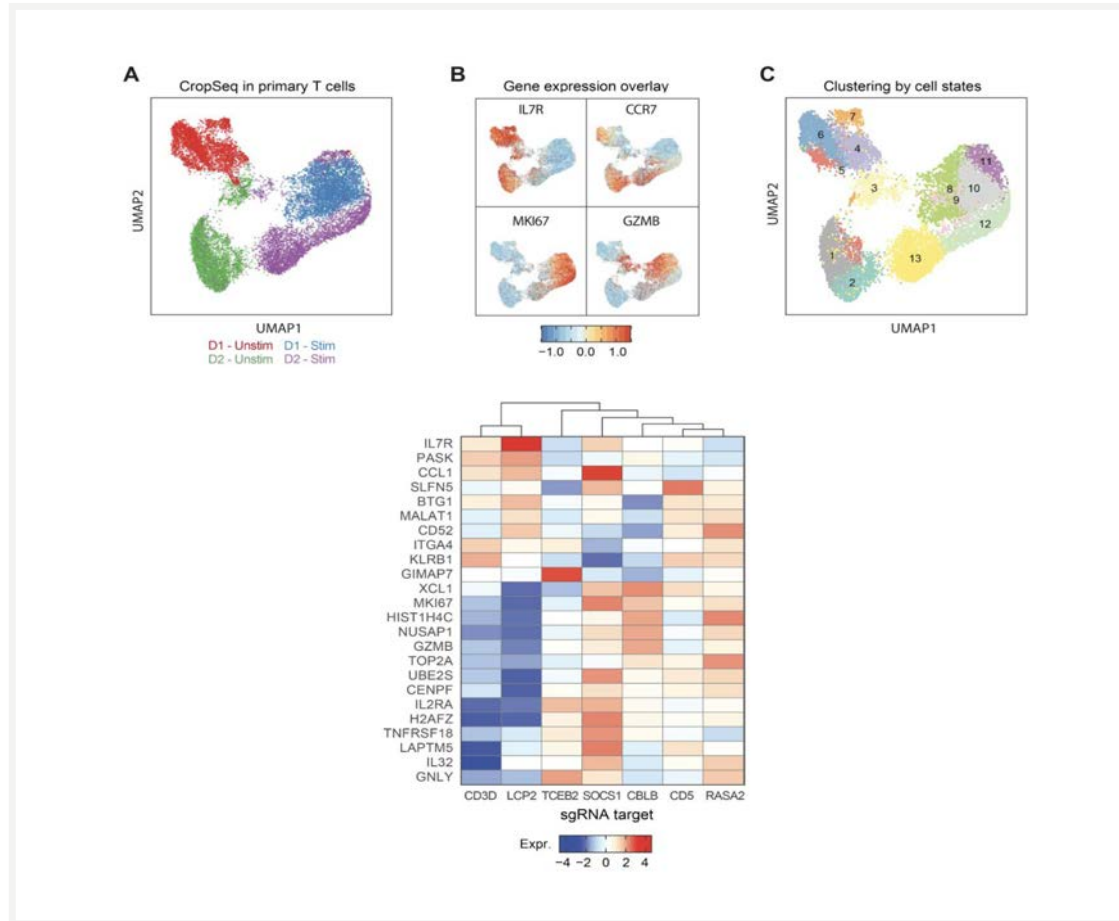

+ Cell Surface Protein

+ CRISPR Screening

Detect multiple analytes with Feature Barcode technology

Direct capture of gene expression and barcoded CRISPR / Cas9 sgRNAs using a single Gel Bead

gRNA

3'-AACGATCCTGGCCGGAATTTCG
Capture Sequence

How CRISPR is being leveraged in cancer immunotherapy

Cell

**Genome-wide CRISPR Screens in Primary Human T Cells Reveal Key Regulators of Immune Function**

- A method for genome-wide CRISPR screens in primary human T cells

- Screens identify regulators of T cell stimulation and immunosuppression

- Candidate hits can boost T cell activation and in vitro cancer cell killing

- Pooled perturbations with Single Cell RNA-seq validated affected gene programs

UCSF

DOI: https://doi.org/10.1016/j.cell.2018.10.024

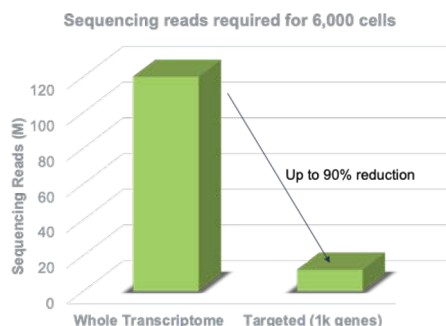# Introducing Single Cell Immune Profiling v2
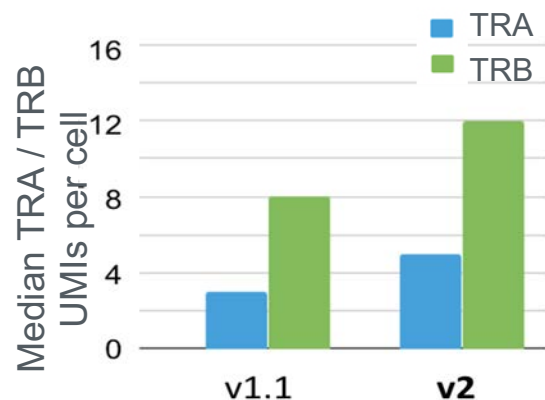
V(D)J
Up to 30% Higher detection rate
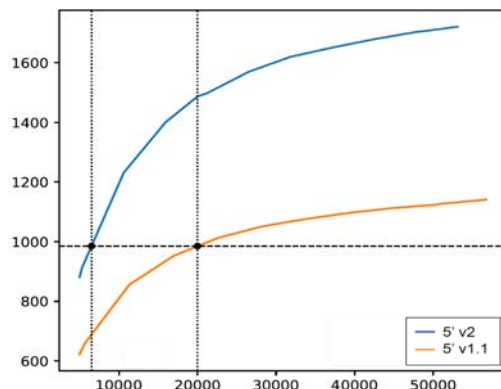
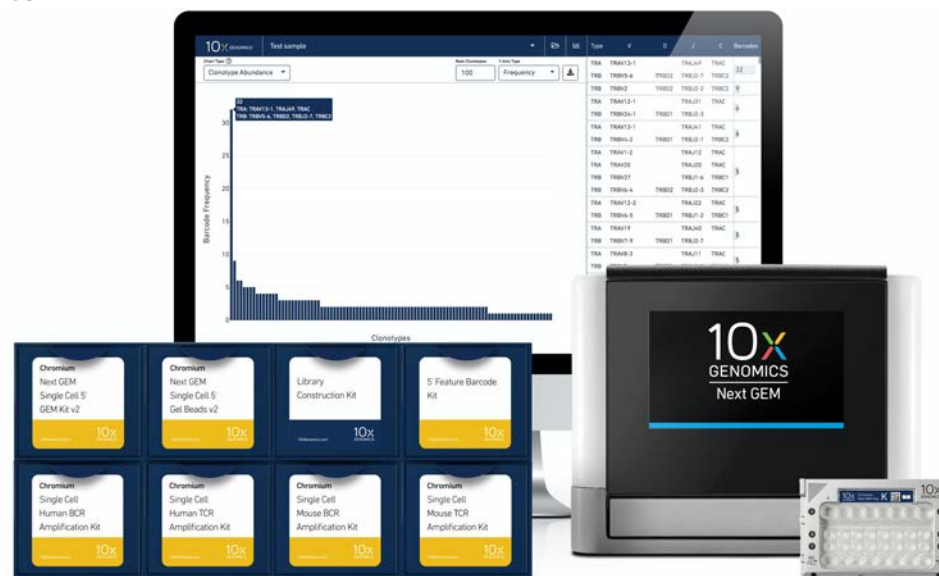
Gene Expression
Up to 60% Higher sensitivity

Library preparation
Dual Indexed library
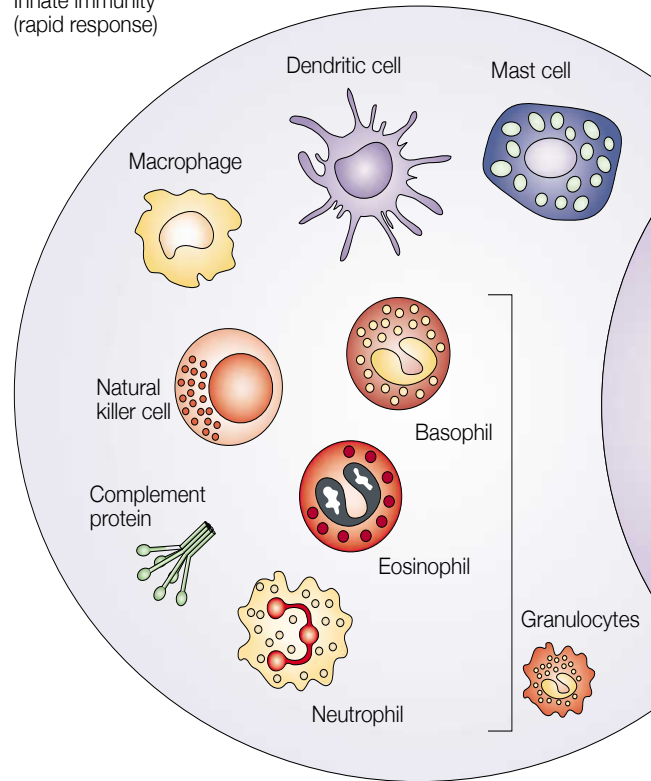

Targeted Gene Expression
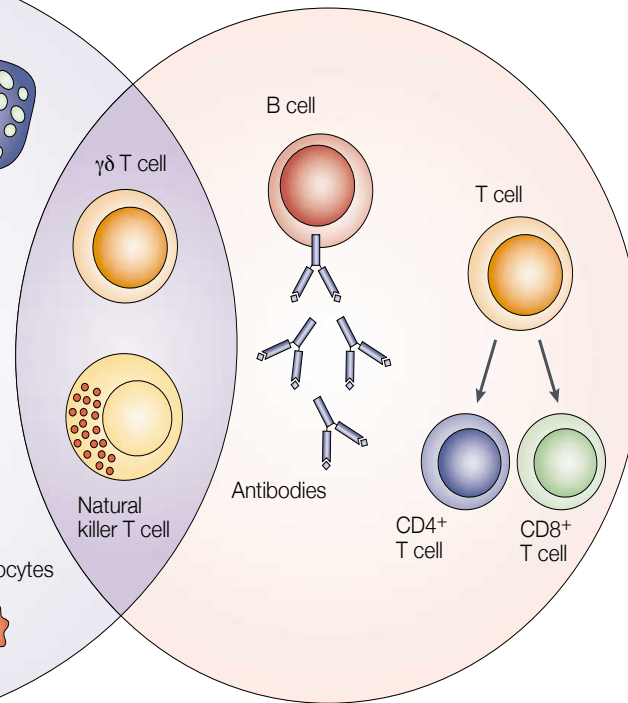*Higher on-target reads*

Up to
4X
faster

Cell Ranger 4.0
Faster turnaround time

Immune cell diversity and antigen receptor diversity

From Dranoff (2004) Nature Reviews Cancer 491), 11–22
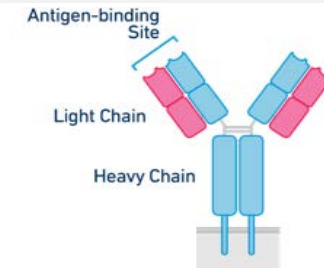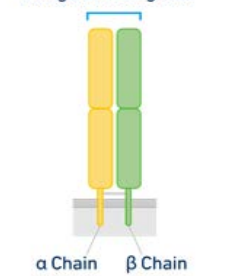
Assess cell type heterogeneity and the immune repertoire

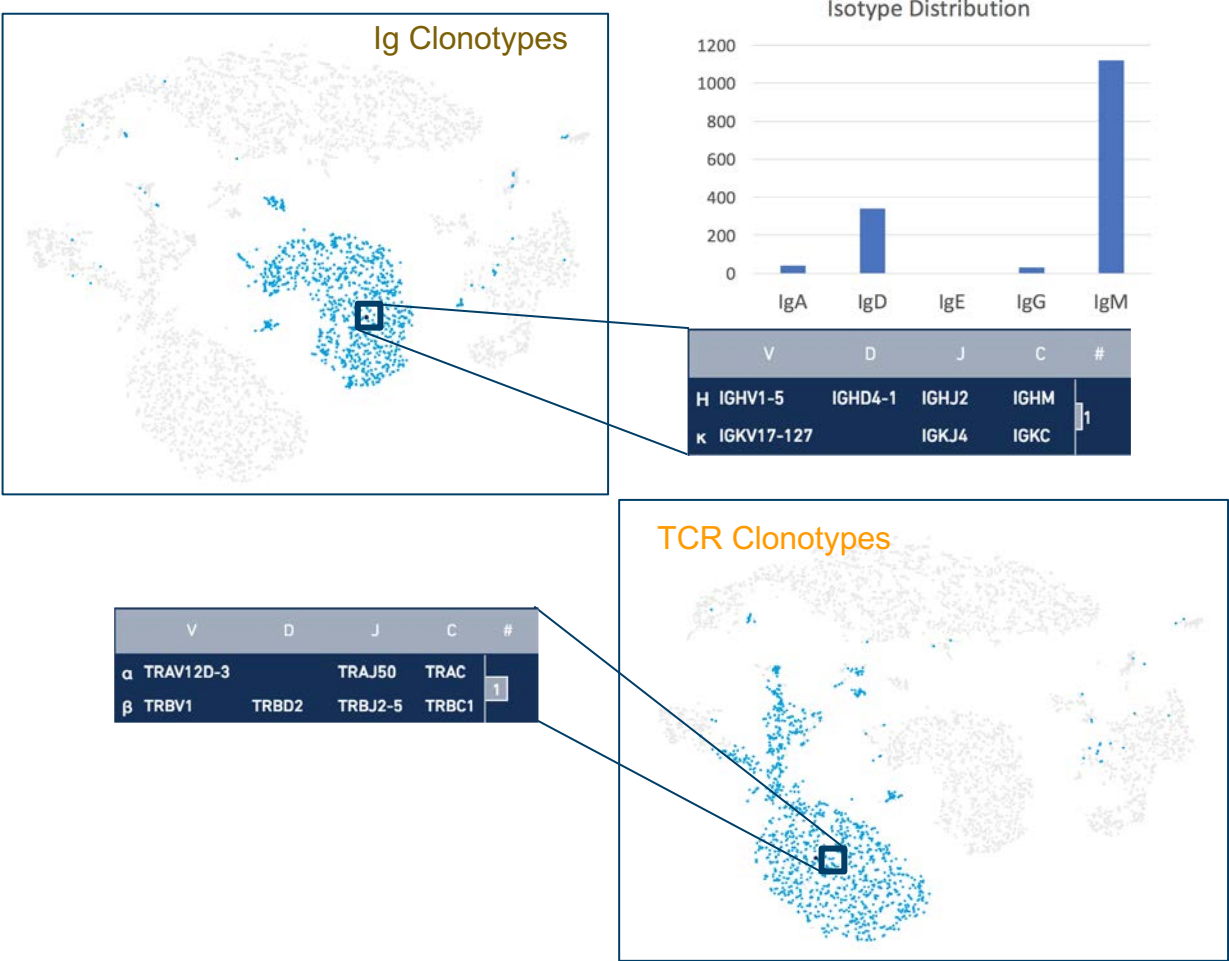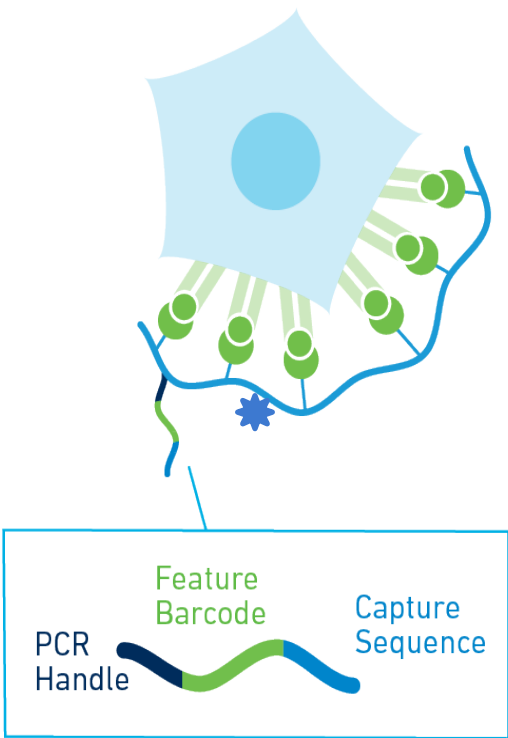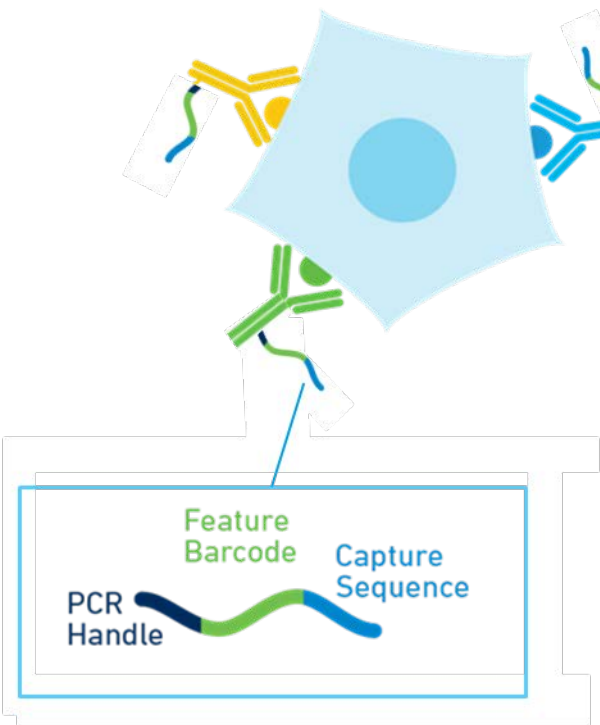5' Gene Expression
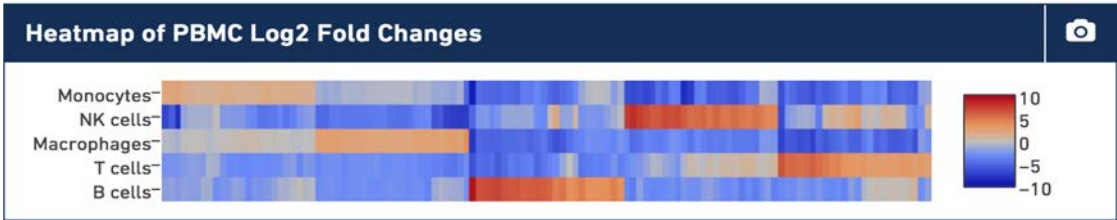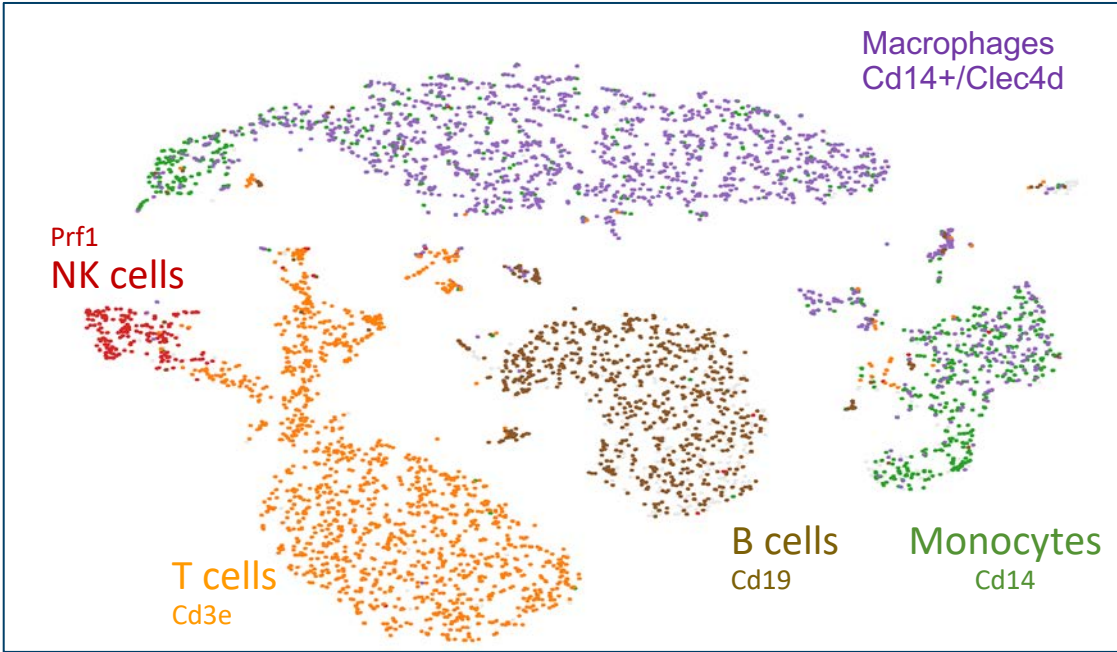
V(D)J clonotypes overlapped with GEX

Mouse PBMCs

Feature Barcode technology

Simultaneous Multiomic Analyses on the Same Single Cells:
Gene Expression, Adaptive Immune Receptors, Cell Surface Epitopes, and Antigens

Immune cell profiling of COVID-19 patients in the recovery stage by single cell sequencing

- Peripheral Blood Mononuclear Cells (PBMCs):
  - After filtering, a total of 128,096 cells were analyzed
  - 10 patients divided into early-recovery stage (ERS) group and late-recovery stage (LRS) group
  - And 5 healthy controls (HC)

- Single cell analysis reveals immune cells interaction characterized by increasing of a subset of CD14++ IL1β+ monocytes in ERS of patients

- The composition of the T and NK cells subset differed significantly among HCs and COVID-19 patients, and the patients' T cells express higher levels of inflammatory genes, such as, JUN, FOS, JUNB, KLF6
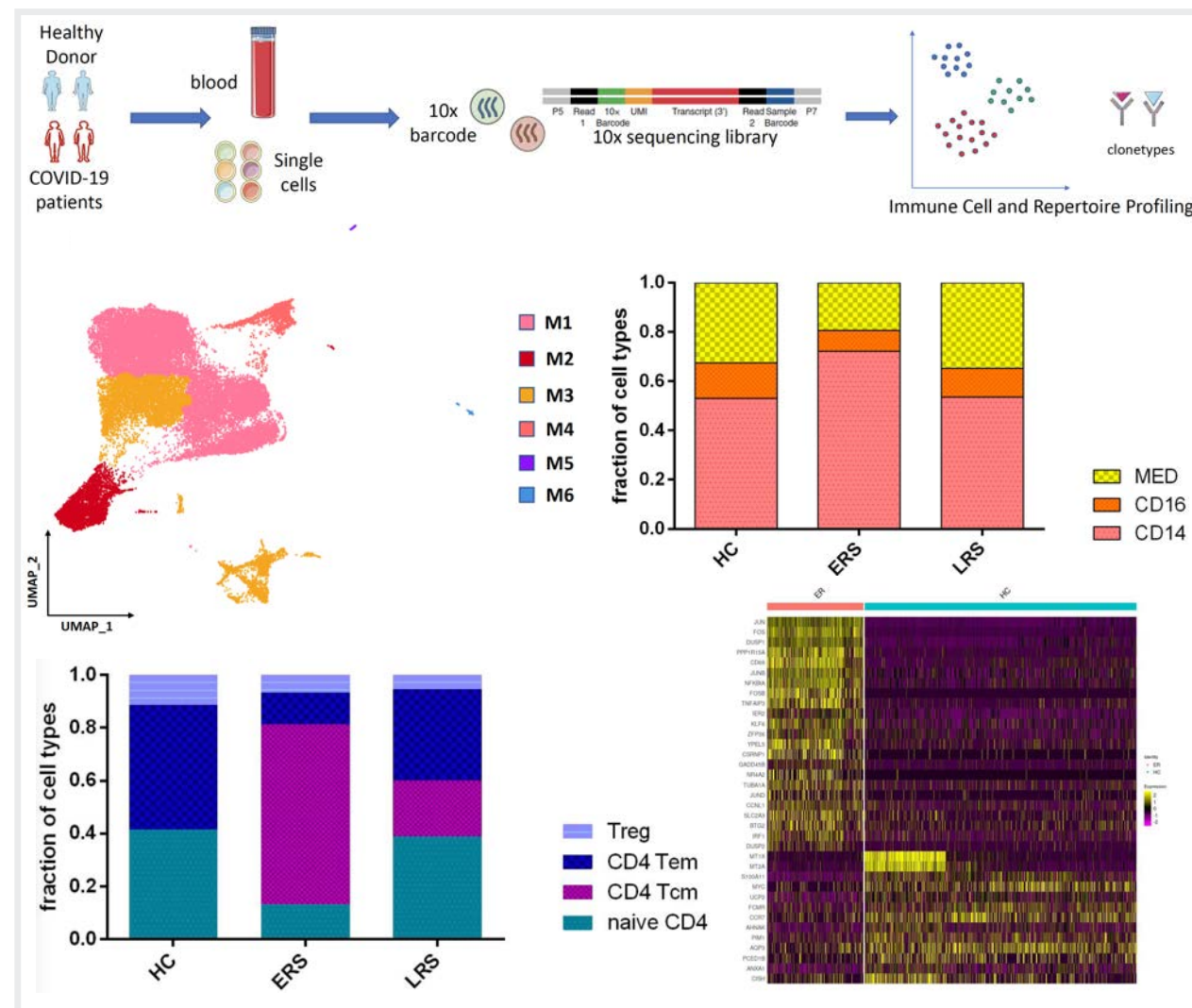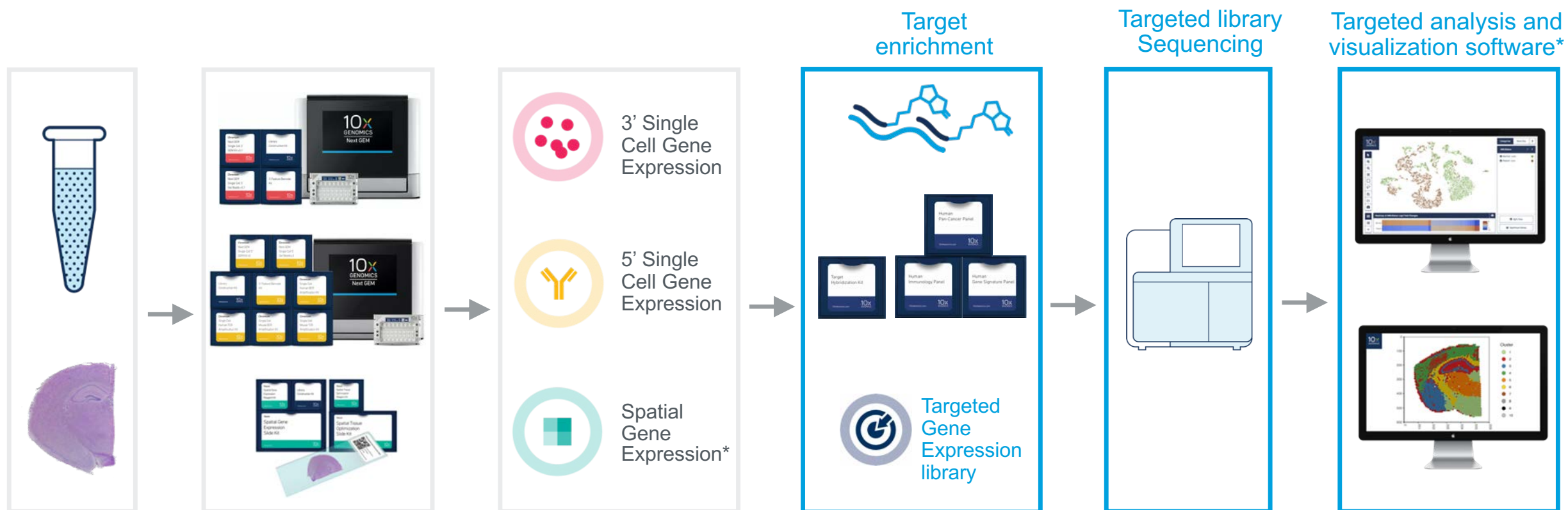
Immune cell profiling of COVID-19 patients in the recovery stage by single cell sequencing

- Percentage of plasma cells are increased in patients, supporting that B cells had experienced unique clonal VDJ rearrangements

- In ERS, IL-1β and M-CSF are discovered as novel mediators for inflammatory cytokine storm, and might be potential candidate targets in COVID-19 treatment. In LRS, TNFSF13, TNFSF13B and IL4, IL18 are discovered as novel mediators for recovery mechanisms

- Provides the first evidence of inflammatory immune signature in early recovery stage, suggesting that the COVID-19 patients are still vulnerable after hospital discharge. The identification of novel BCR signaling may lead to the development of vaccine and antibodies for the treatment

DOI 10.1101/2020.03.23.20039362
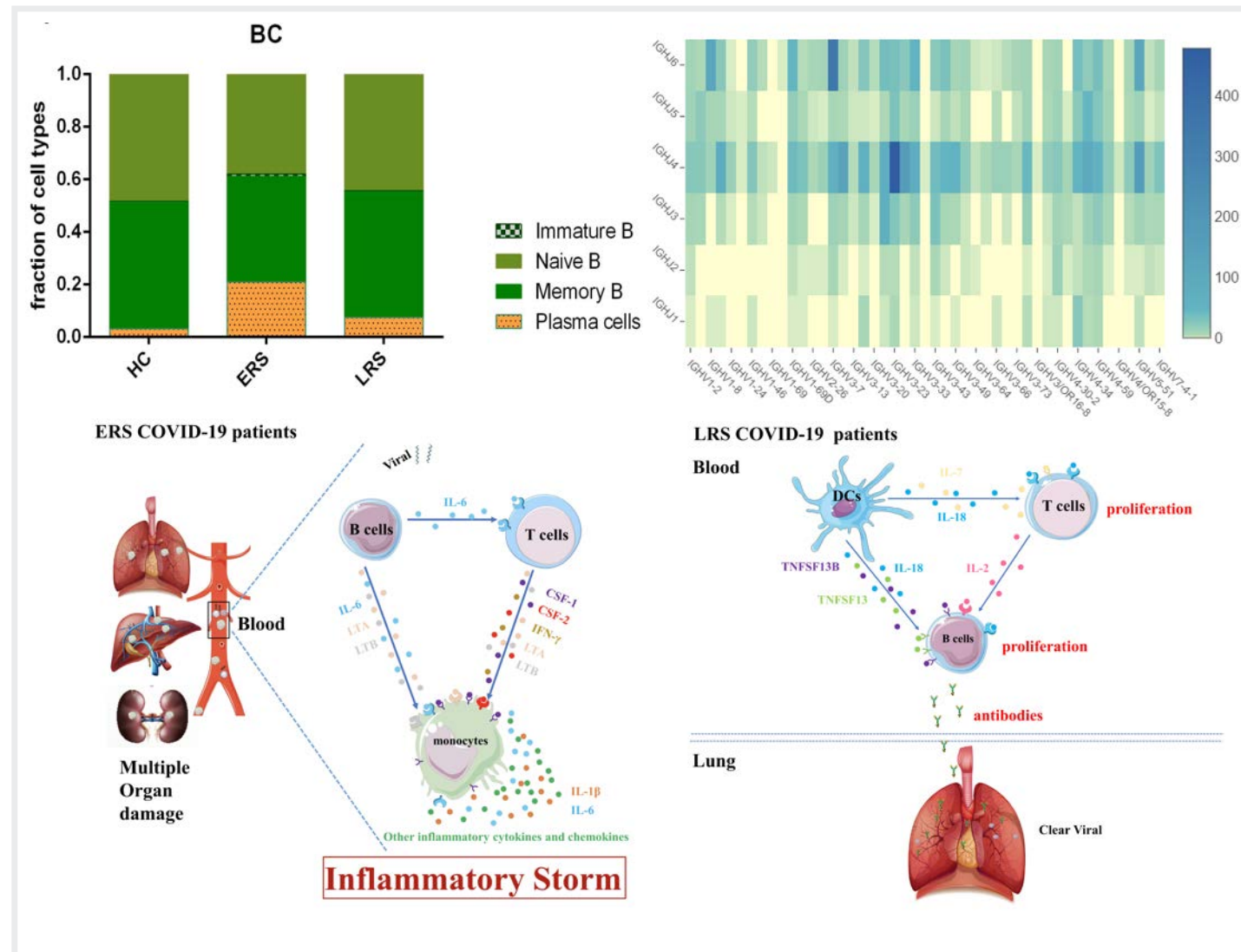
Targeted Gene Expression

Compatible with existing 10x Genomics workflows

Target enrichment

Targeted library Sequencing

Targeted analysis and visualization software*

3' Single Cell Gene Expression

5' Single Cell Gene Expression

Spatial Gene Expression*

Targeted Gene Expression library
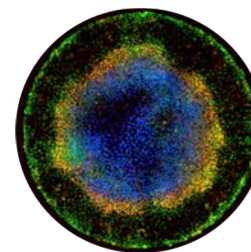
10x
GENOMICS

Comprehensive pre-designed panels

Accelerate research in 4 major areas
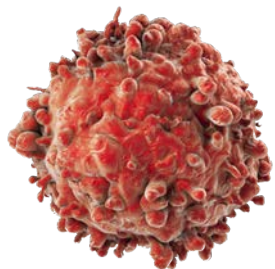
Human Pan-Cancer
1,253 genes

- **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
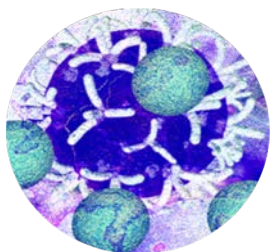
Human Immunology
1,056 genes

- Covers **innate and adaptive immunity, inflammation, and immuno-oncology**
- Comprehensively profile the immune response in cells and tissues
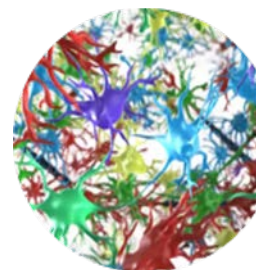
Human Neuroscience
1,186 genes

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

10x Genomics Custom Panel Designer

Design add-on or fully custom panels

https://cloud.10xgenomics.com/custom-panel-designer
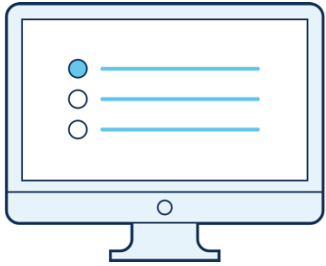
- Add-on panels
  - Add up to 200 genes to a 10x pre-designed panel
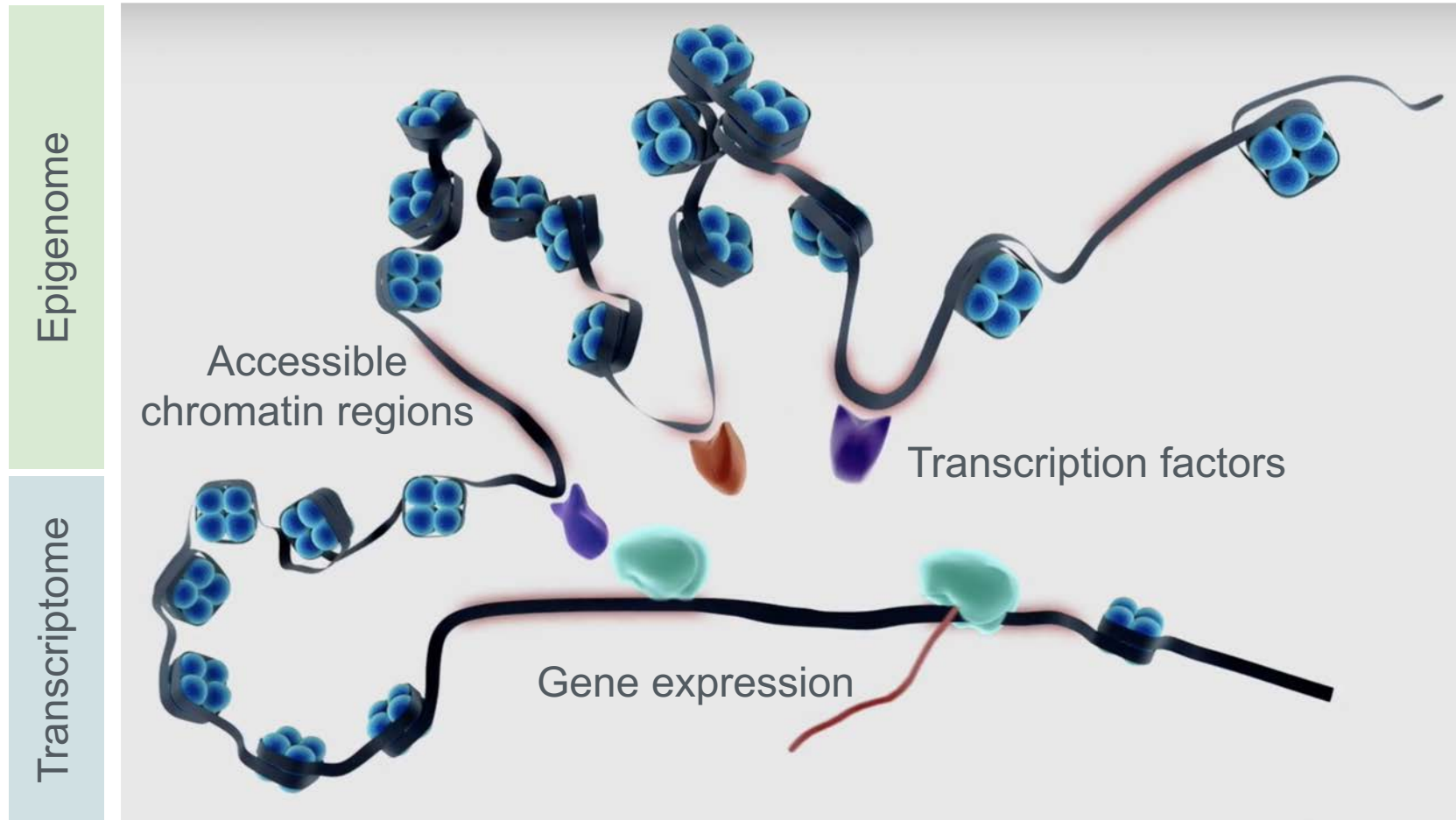- Fully custom panels
  - 10 – 1,500 genes

- **Input:**
  - Human gene names or Ensembl IDs
  - FASTA sequences for up to 10 exogenous genes
    - eg. reporter alleles, viral transcripts

- **Downloadable output files for:**
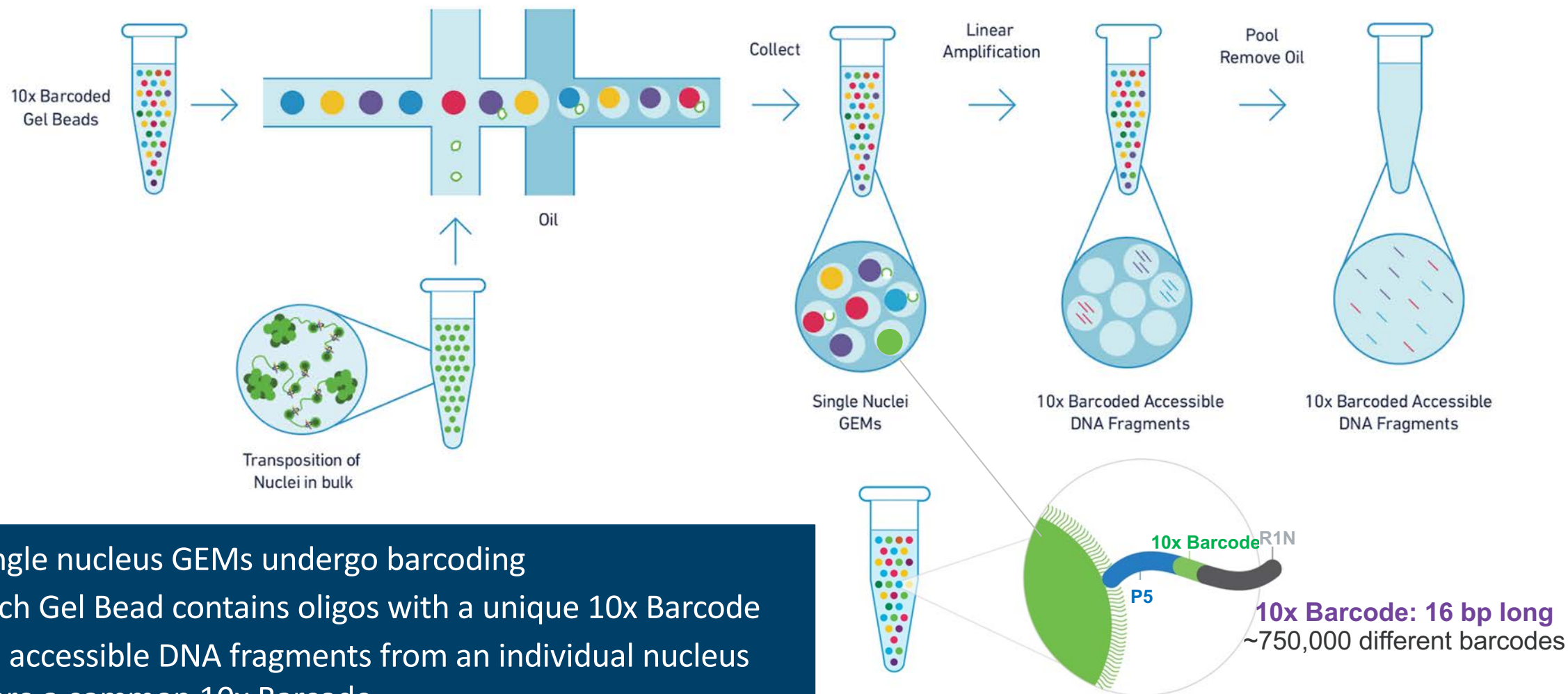 - Ordering the custom panel
    - from a 10x Genomics Compatible Partner
  - Inspection of the custom panel
    - via third-party genome browsers
  - Data analysis
    - Analysis of Targeted Gene Expression data with custom panels using Cell Ranger and Space Ranger*

10x
GENOMICS

Interplay between epigenetic programs and gene expression

Epigenome

Transcriptome

Accessible chromatin regions

Transcription factors

Gene expression

10x GENOMICS

The Chromium Single Cell ATAC Solution
Workflow Overview

10x Barcoded Gel Beads

Oil

Transposition of Nuclei in bulk

Collect

Single Nuclei GEMs

Linear Amplification

10x Barcoded Accessible DNA Fragments

Pool Remove Oil

10x Barcoded Accessible DNA Fragments

10x Barcode

R1N

P5

10x Barcode: 16 bp long
~750,000 different barcodes

- Single nucleus GEMs undergo barcoding
- Each Gel Bead contains oligos with a unique 10x Barcode
- All accessible DNA fragments from an individual nucleus share a common 10x Barcode
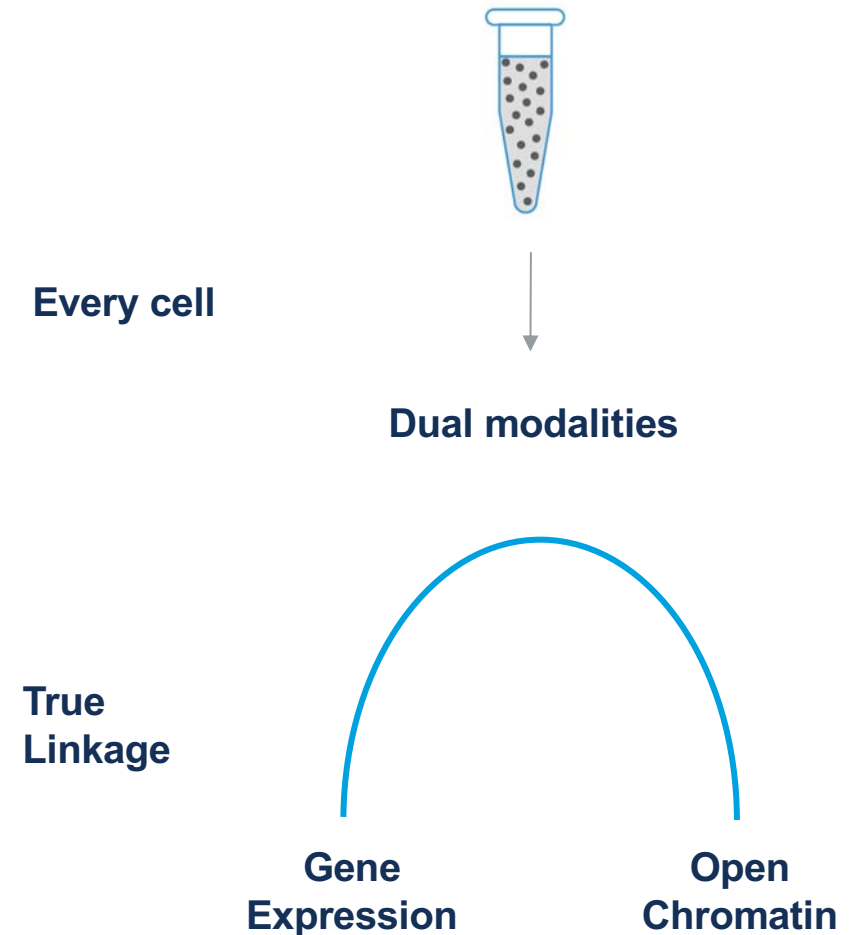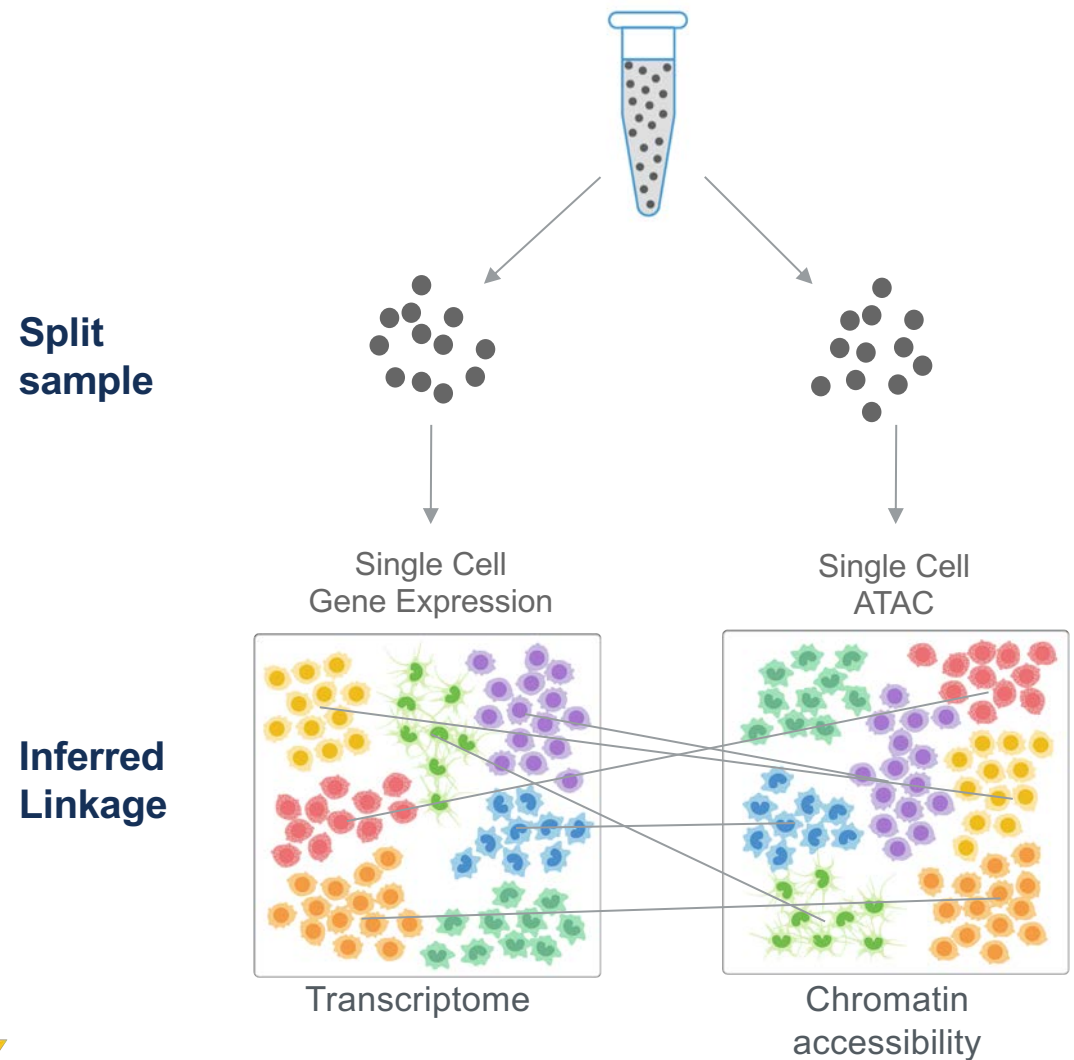
Chromium Single Cell Multiome ATAC + Gene Expression
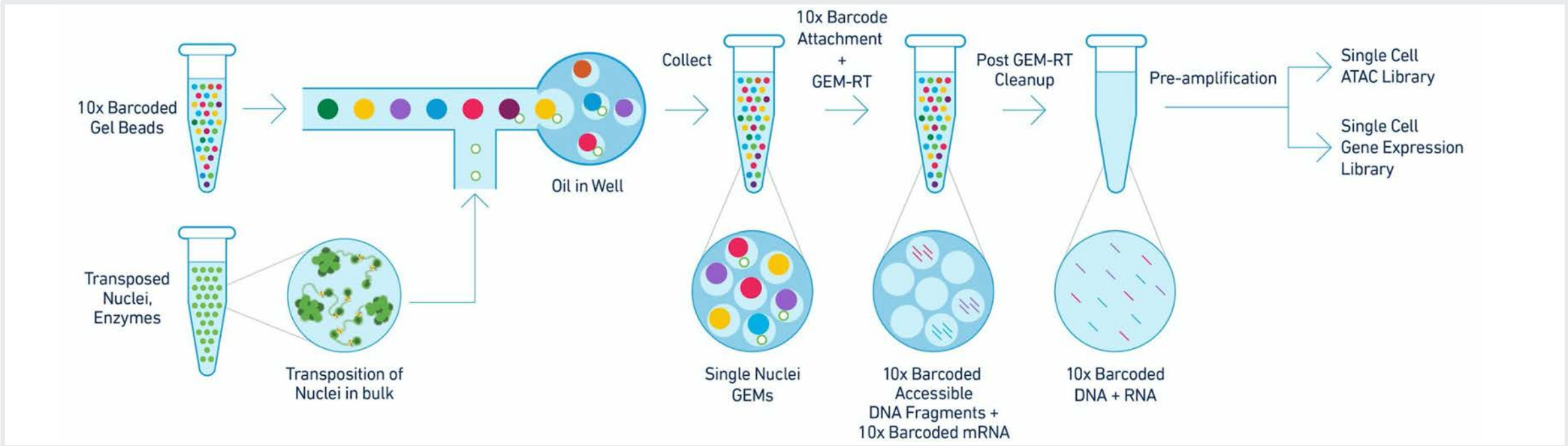
Multiply your power of discovery

- 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
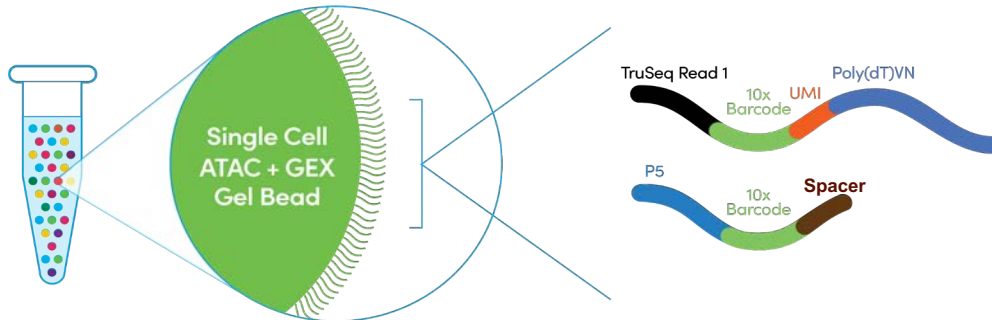- Maximize precious samples with multiple readouts from the same cell

# New approach to capture transcriptomic and epigenomic data from the same single cell

Split sample

Single Cell Gene Expression

Single Cell ATAC

Inferred Linkage

Transcriptome

Chromatin accessibility

Every cell

Dual modalities

True Linkage

Gene Expression
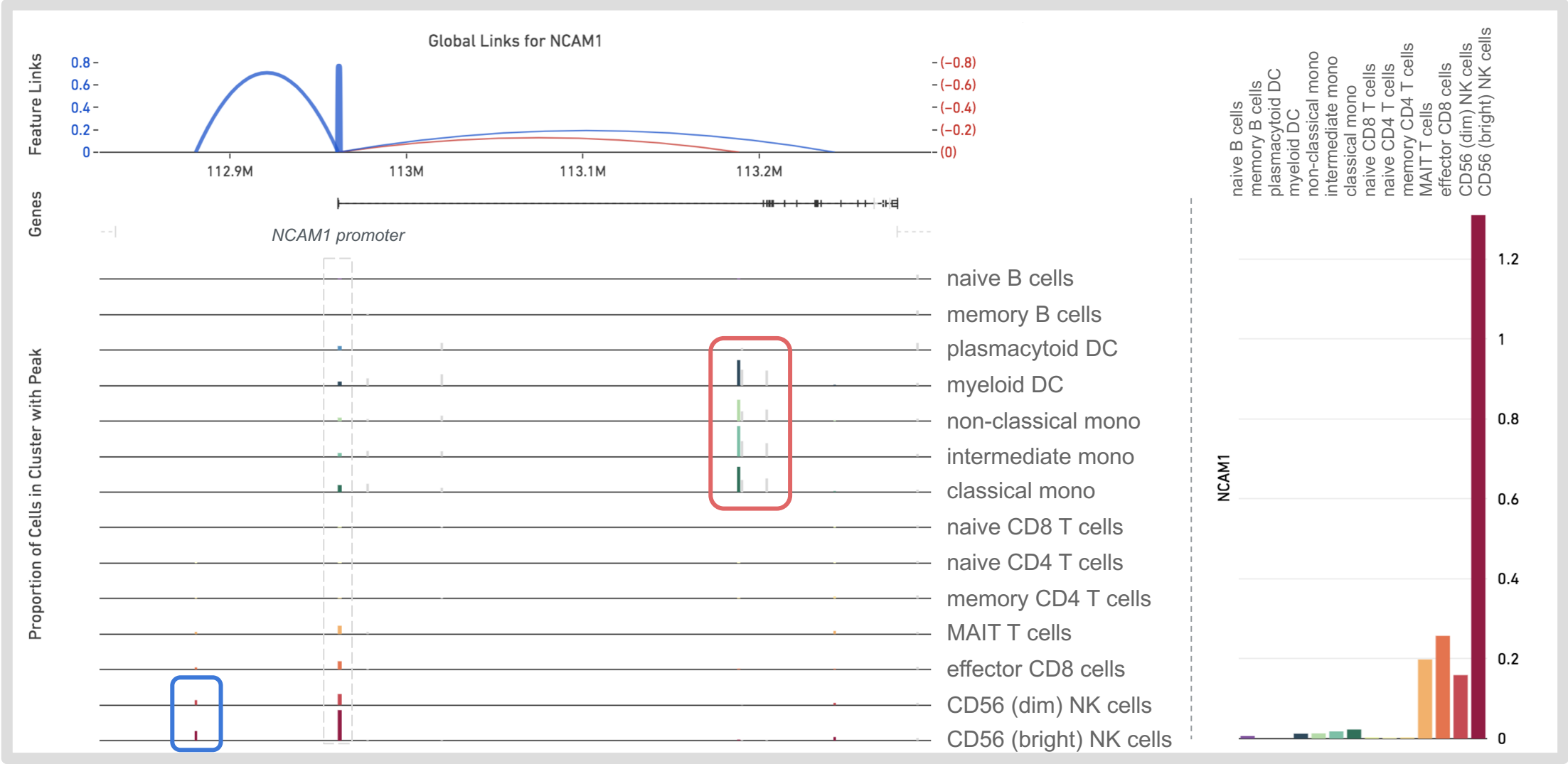
Open Chromatin

How it works

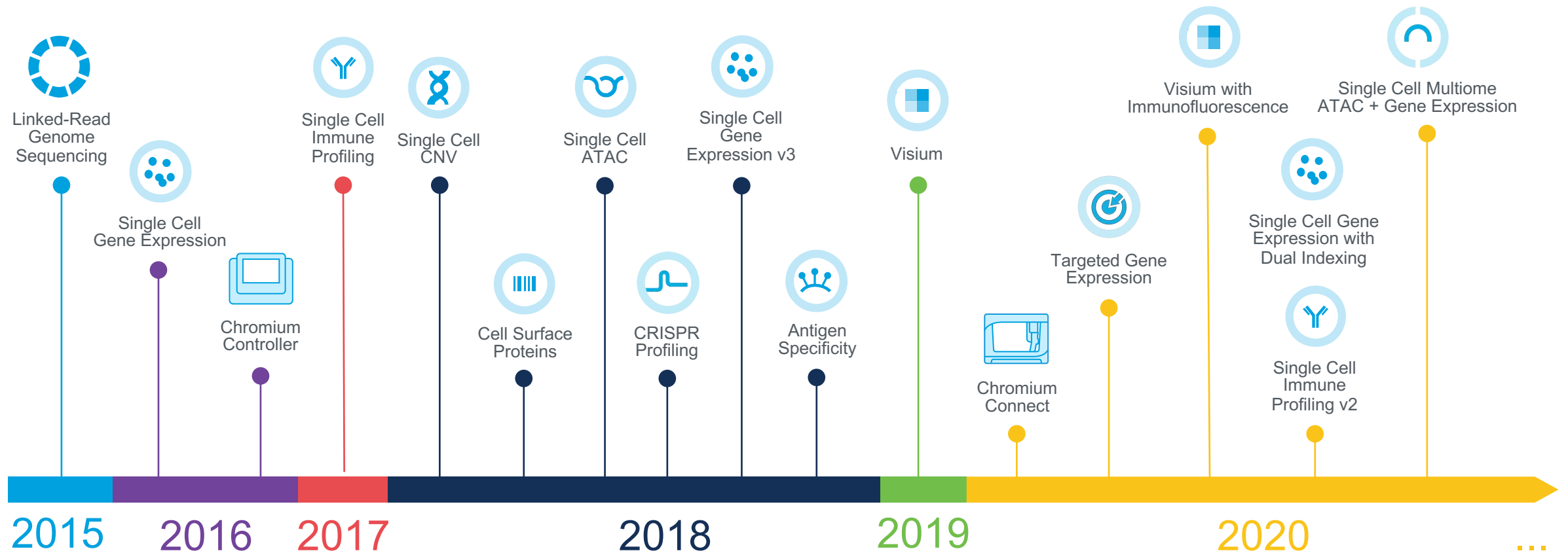Redesigned gel beads combine oligos for capturing 3' ends of mRNA & ATAC fragments

Simultaneously detect gene expression and ATAC profiles from single cells
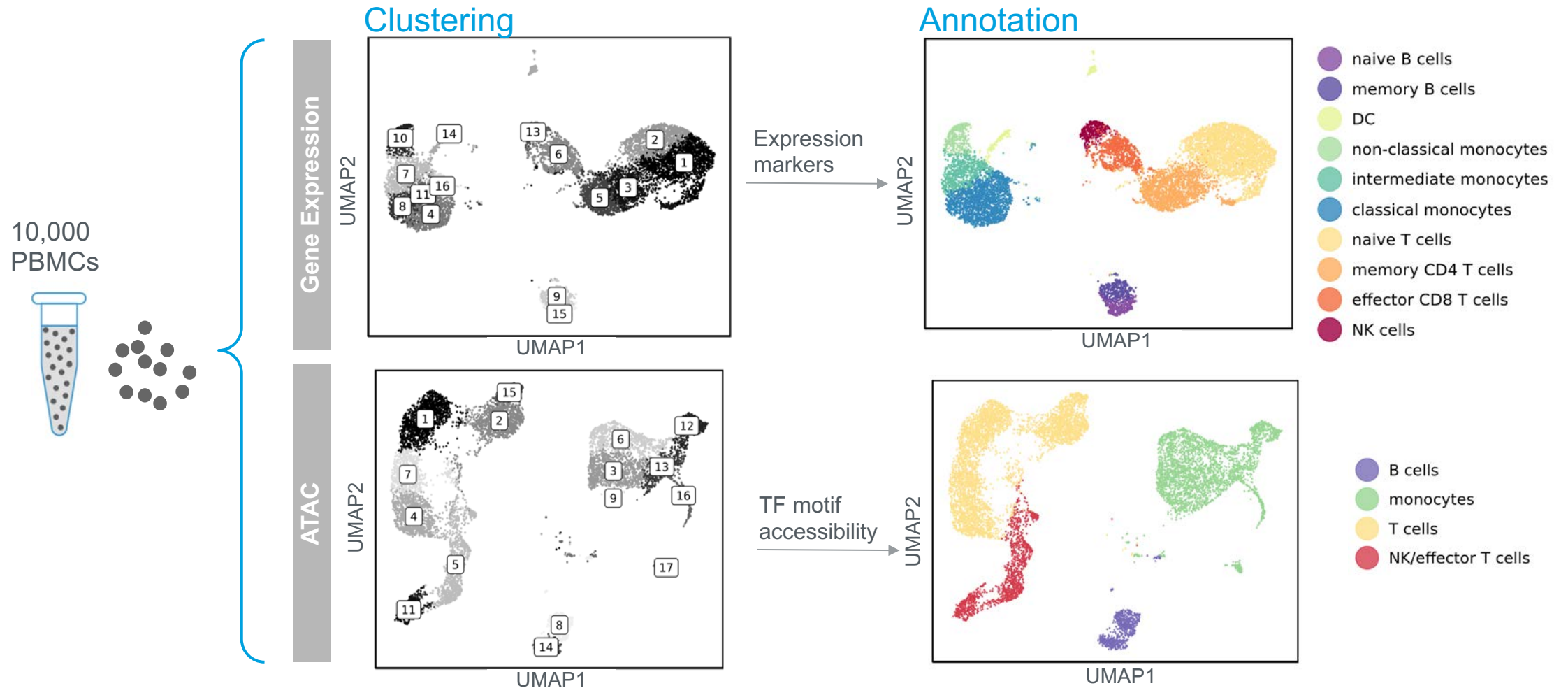
Identify putative regulatory elements linked to a gene of interest

10x Innovation Engine

Thank you!

Our collaborators & team 10x