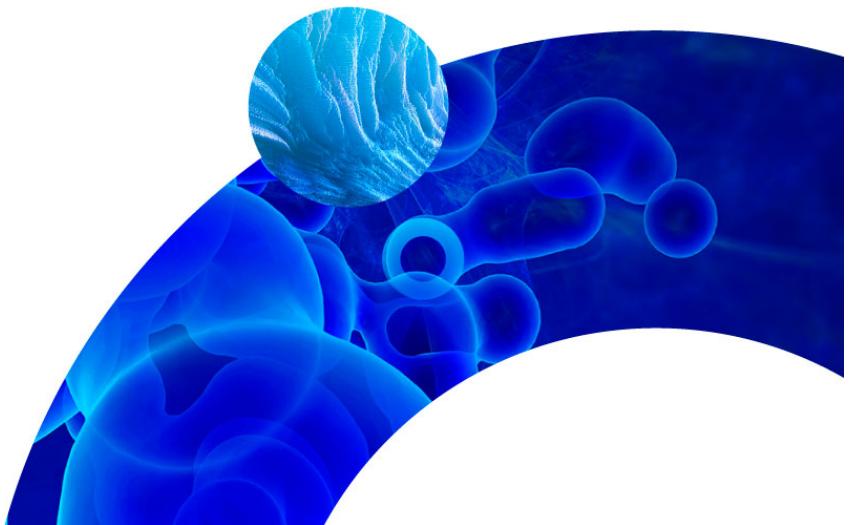


Biology at True Resolution

Nicole Abreu, PhD
Science & Technology Advisor
nicole.abreu@10xgenomics.com



Biology at true resolution

10x Genomics mission

Accelerate the mastery of biology to advance human health

A comprehensive view of biological systems at needed resolution and scale

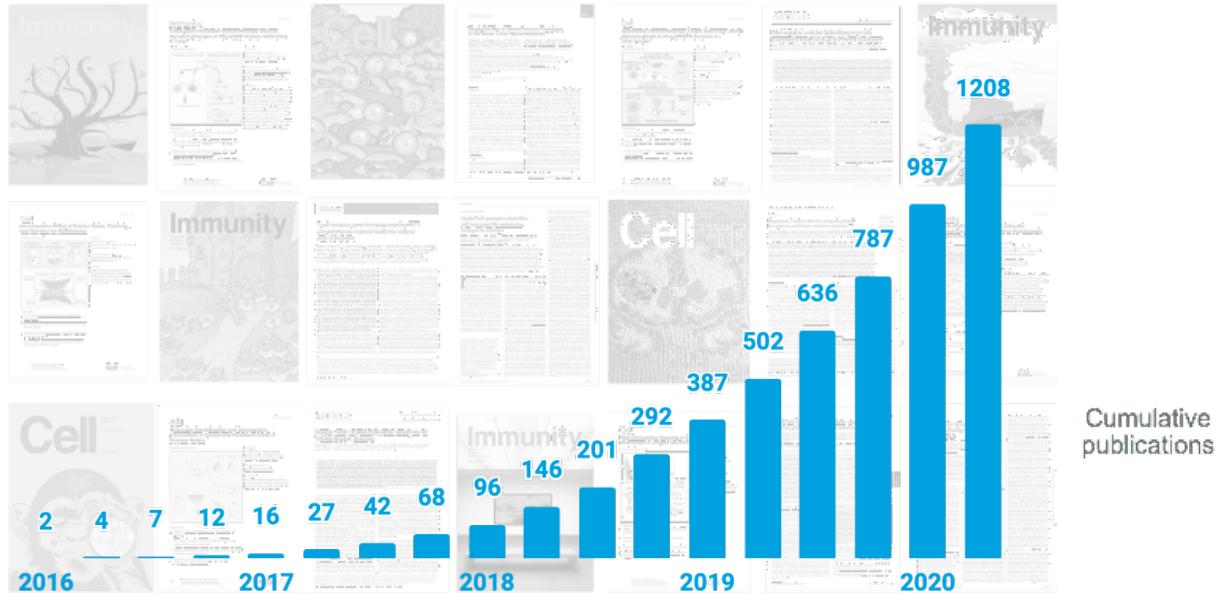
Measurement, understanding and mastery of the Immune System

Functional Genomics



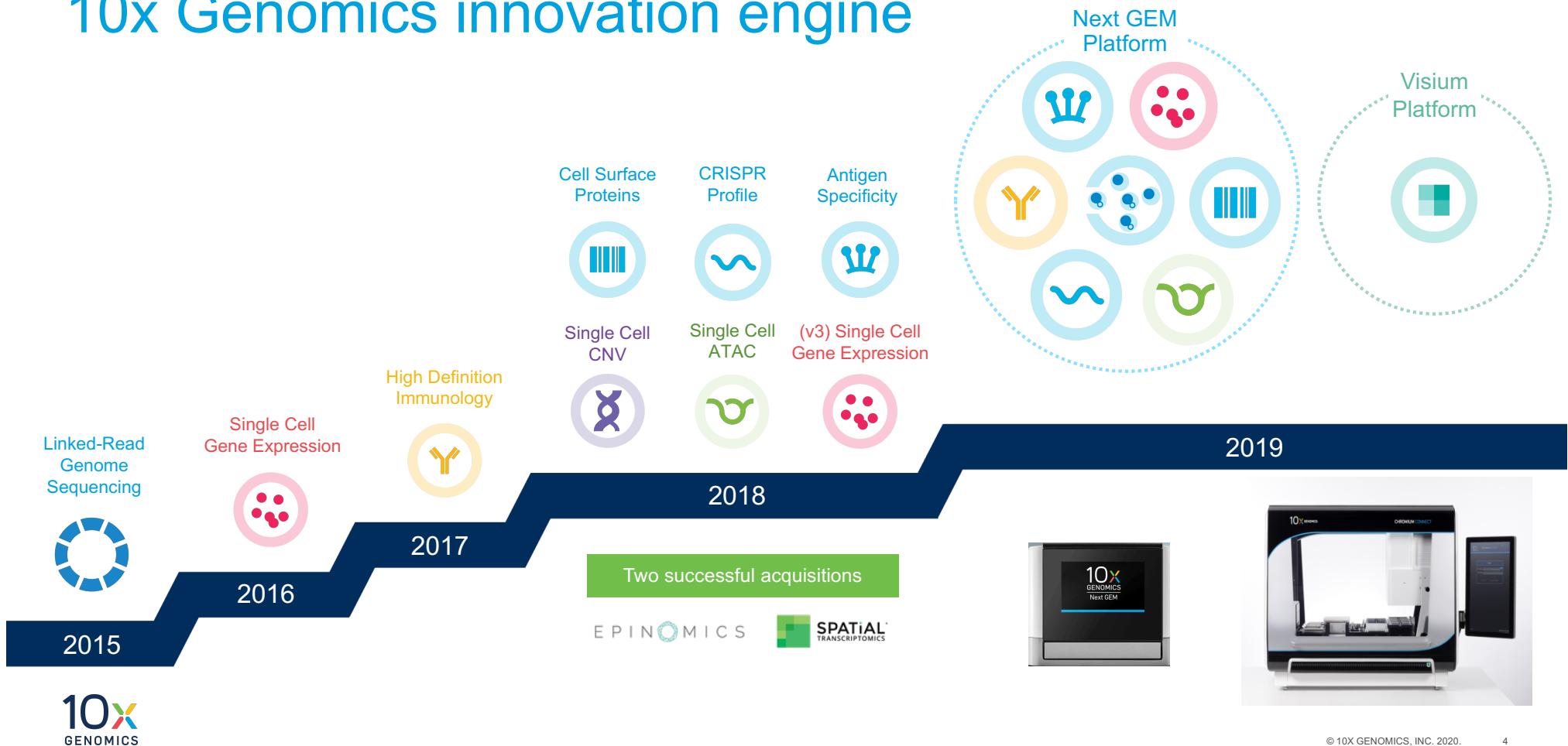
Biology at True Resolution

10x Genomics publications continue to scale



1,200+
publications

10x Genomics innovation engine



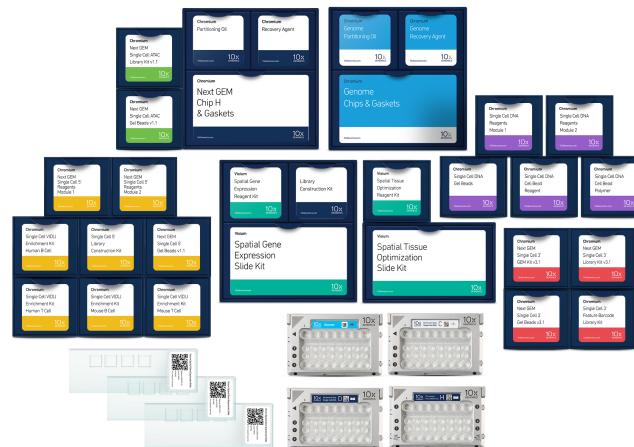
Solving Problems With Continued Innovation

10x Genomics provides complete solutions

Instruments



Consumables

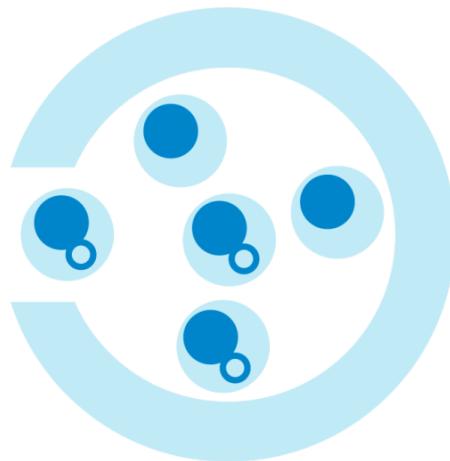


Software

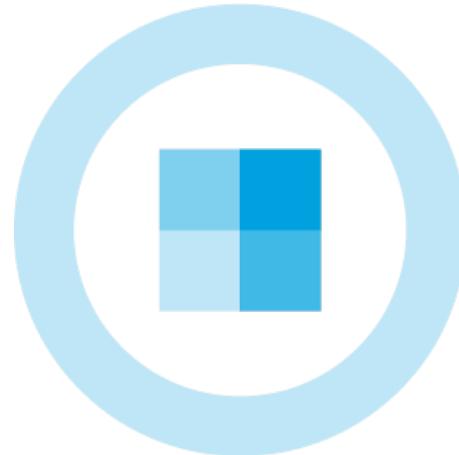


Agenda

Chromium
Platform

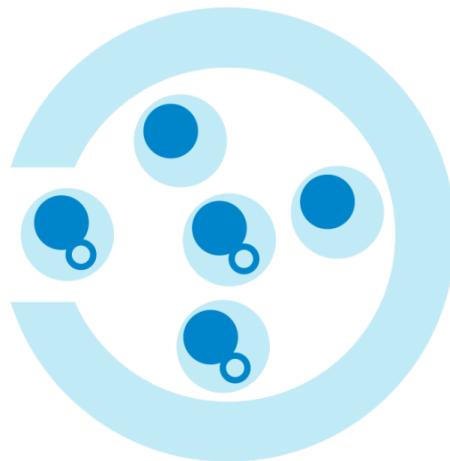


Visium
Platform



Advances in Single Cell Genomics

Chromium
Platform

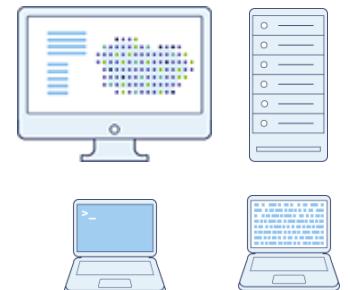
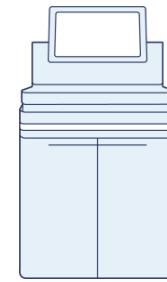
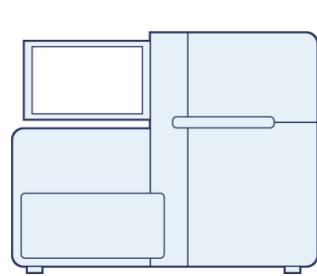
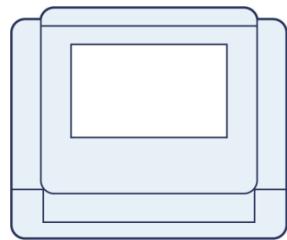
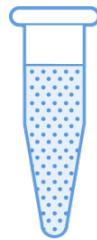


Visium
Platform



Chromium Single Cell Solutions

A transformative and easily integrated technology



Flexible input

Single cell suspensions of 100s to 1000s of cells

Flexible throughput

Automated barcoding, then library construction

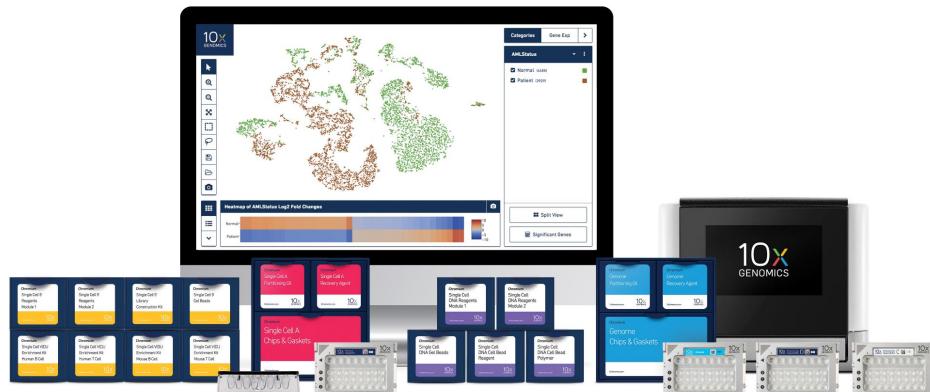
Single cell sequencing

Use existing short-read technologies

Turnkey analysis

Robust, secure, maintained, open-source software

10x Genomics Single Cell Solutions



Single Cell Gene Expression



Single Cell Immune Profiling



Single Cell ATAC

- Flexible cell input: capture 100s to 1,000s of cells per channel
- Recover up to 65% of cells loaded
- Compatible with whole cells or nuclei* across range of cell sizes
- Enabled with Feature Barcoding technology (Gene Expression & Immune Profiling)

Chromium Single Cell Solutions

Efficient and scalable cell capture



Easy loading

Recovery Well →

Gel Bead Well →

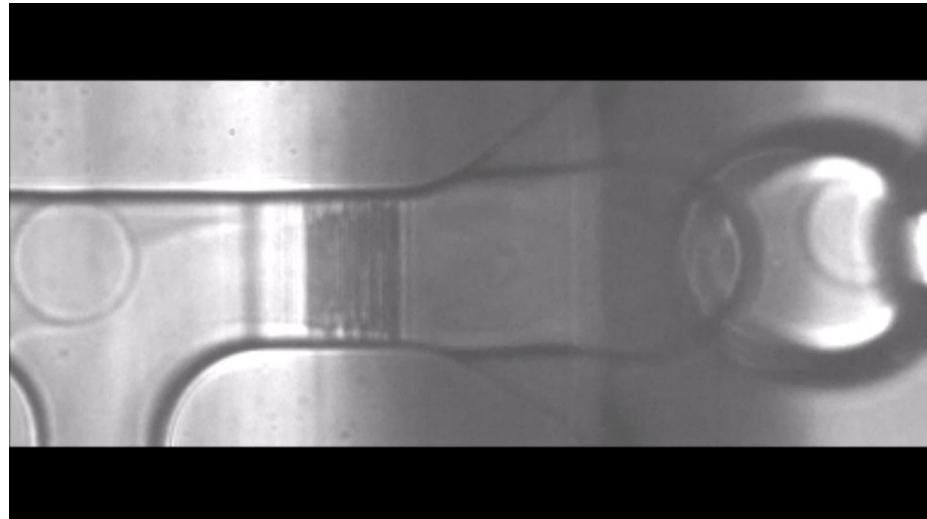
Sample Well →

Single-use microfluidics chip



Chromium Single Cell Solutions

Efficient and scalable cell capture

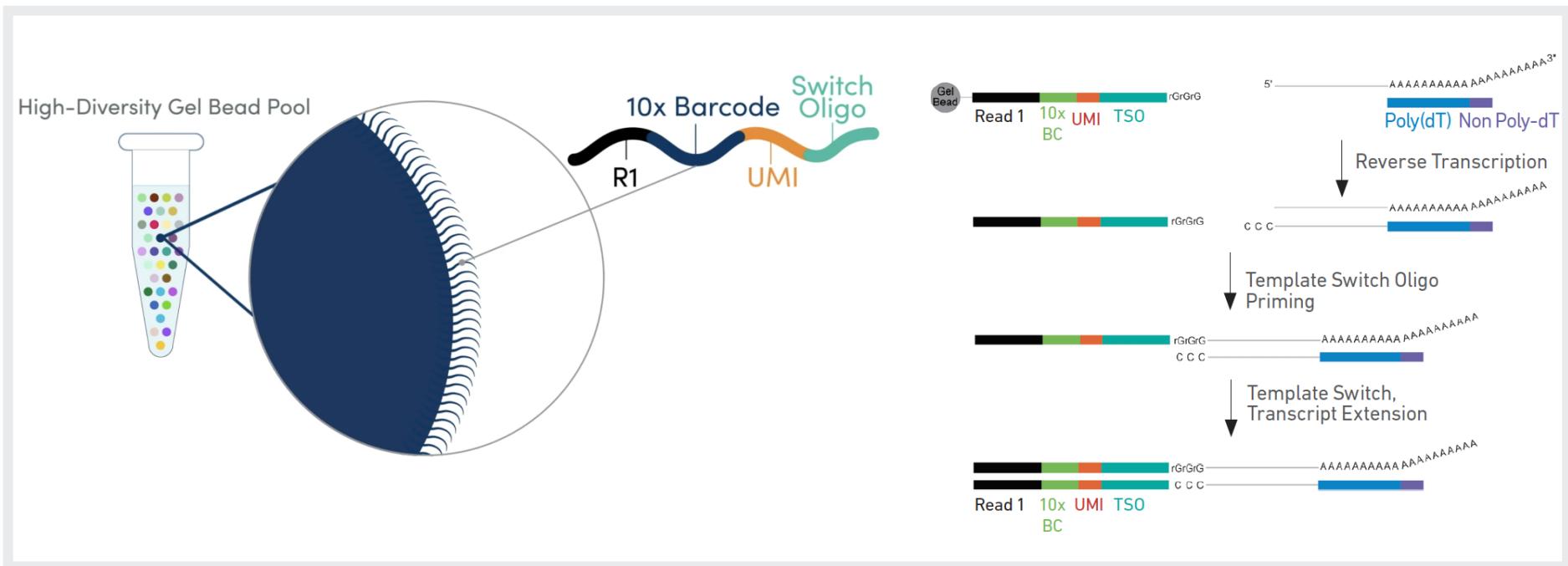


Single Cell Immune Profiling



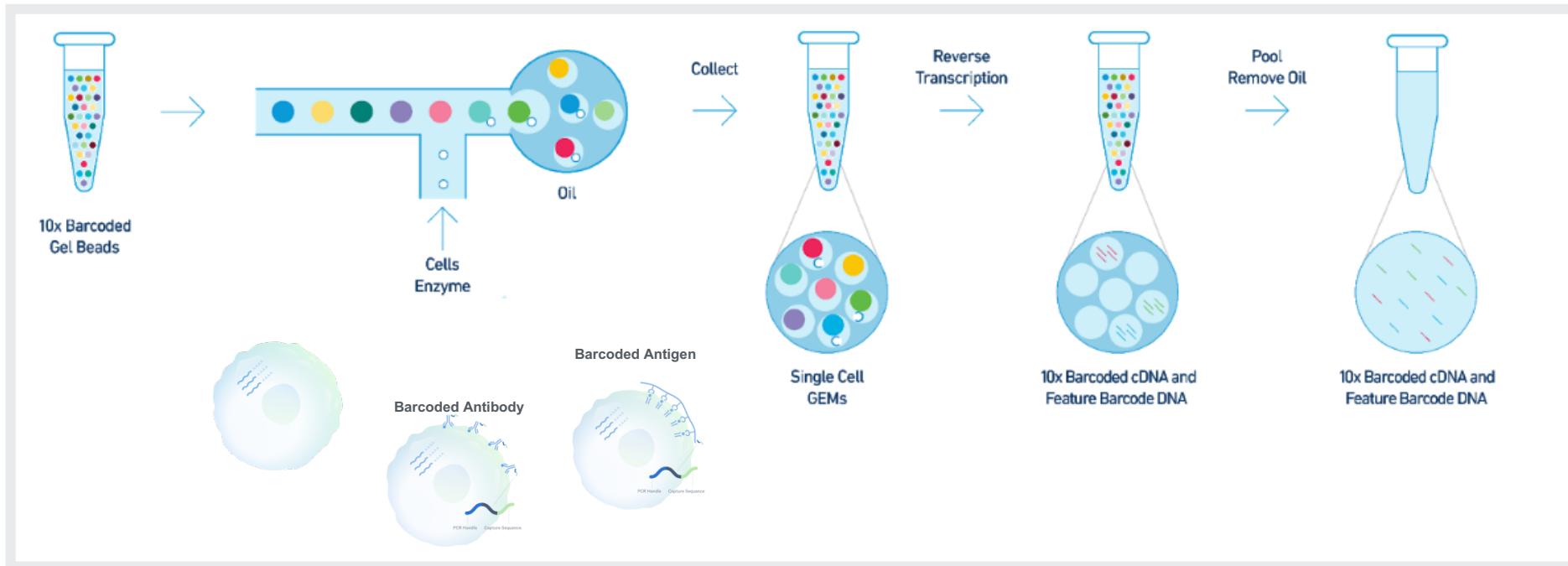
10x Genomics single cell technology

Barcoding single cell transcriptomes and other readouts

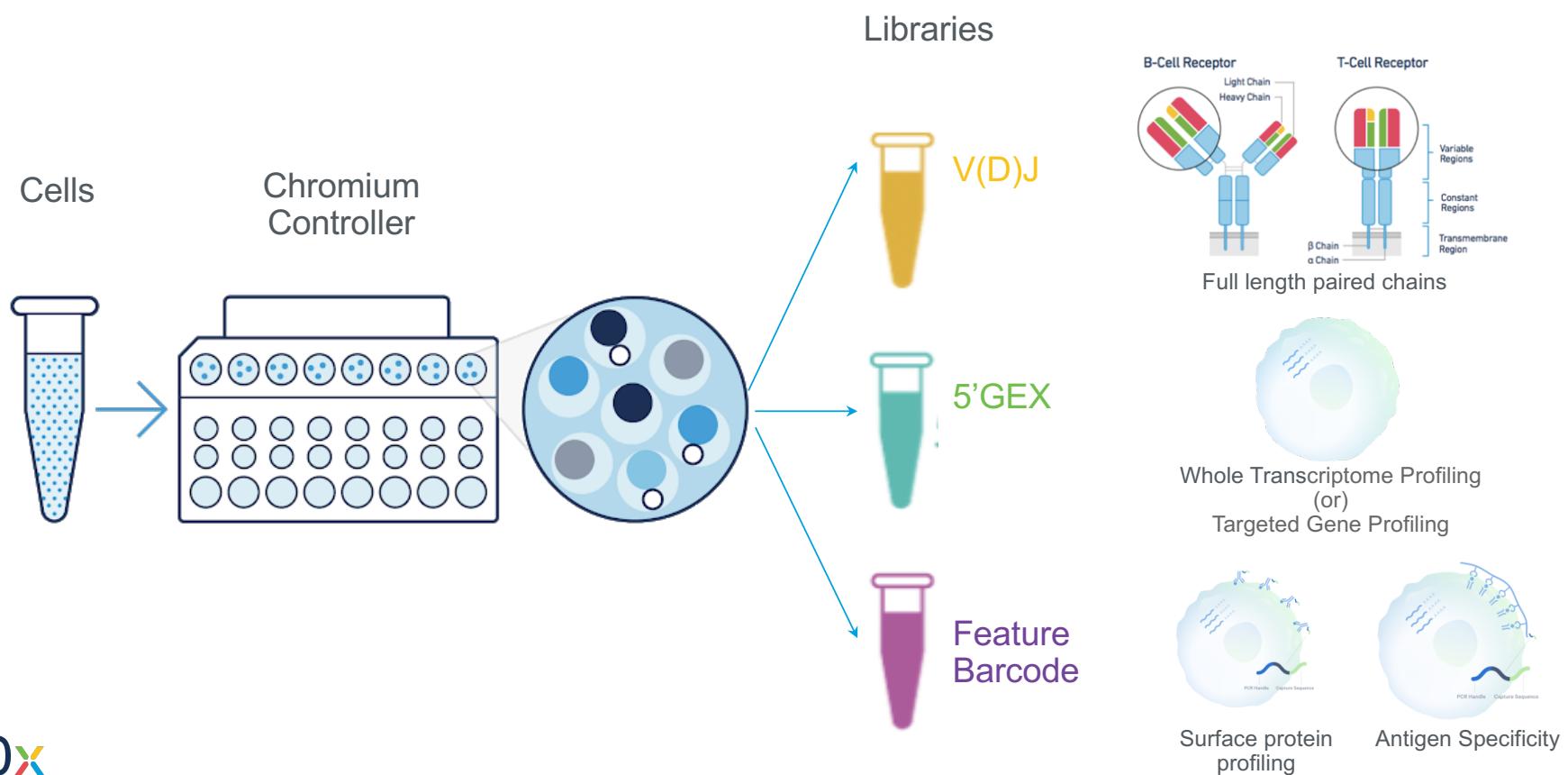


10x Genomics core technology for molecular barcoding and partitioning

Millions of parallel reactions



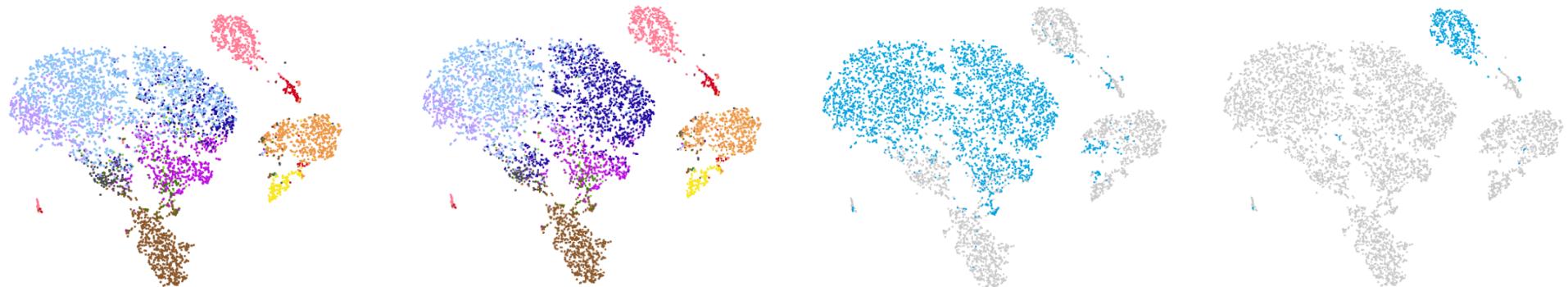
Single cell immune profiling library output



Multiomic profiling provides true resolution

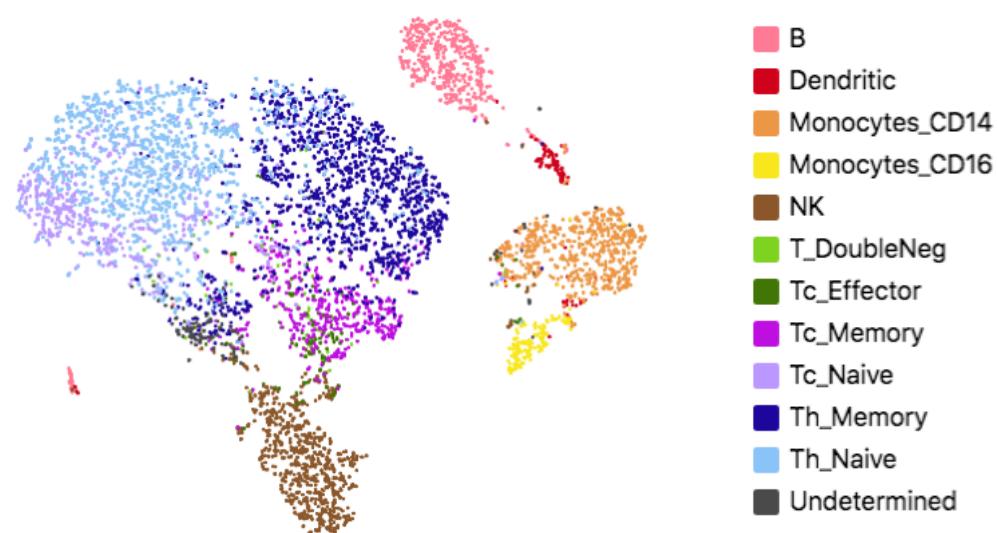
Combined gene expression, cell surface protein and TCR / Ig clonotyping
for every single cell

Targeted gene expression + Cell surface protein + TCR clonotypes + Ig clonotypes



Intersection of cellular phenotyping with paired TCR repertoire profiling

TCR clonotypes can be assigned on a per cell basis



Cell type annotation based on
Gene expression **and** the cell surface
antibody profile



Cells with TCR clonotypes identified

TCR sequences are full length and paired

>clonotype2_consensus_1_alpha

```
AGATCAGAAAGAGGAGGCTTCACCCCTGCAGCAGGGACCTGTGA
GCATGGCATGCCCTGGCTTCTGTGGGCACTTGTGATCTCCACCT
GTCTTAATTAGCATGGCTCAGACAGTCACTCAGTCTCAACCAG
AGATGTCTGTGCAGGAGGAGACCGTGACCCGTGAGCTGCACA
TATGACACCAAGTGAAGAGTATTATTCTGGTACAAGCAG
CCTCCCAGCAGGAGATGATTCTCGTTATTGCAAGAACAGCTTAT
AAGCAACAGAAATGCAACAGAATGTTCTGTGAACTCCAG
AAAGCAGCAAATCCTCAGTCTCAAGATCTCAGACTCACAGCTG
GGGGATGCCCGATGTATTCTGTGTTAGGAGCGTGTATTCA
GGAGGAGGTGCTGACGGAACCTTGGCAAAGGGACTCATCT
AATCATCCAGCCCTATATCCAGAACCCGTGACCCGTGCCGTGACCA
GCTGAGAGACT
```

>clonotype2_consensus_1_beta

```
AGTGACACTGATCTGGTAAAGGCCCTCATCCTGCTCTGACCCGCC
ATGGGCACCAAGTCTCTATGCTGGGTGGTCTGGGTTCTAGGG
ACAGATCACACAGGTGCTGGAGTCTCCAGTCTCCAGTACAAA
GTCACAAAGAGGGGACAGGATGTAGCTCTCAGGTGTGATCCAAT
TTCGGGTCTGATATCCCTTATTGGTACCGACAGGCCCTGGGCA
GGGCCAGAGTTCTGACTTACTCAATTATGAAGCCAACAAGA
CAAATCAGGGCTGCCAATGATCGGTTCTGCAAGAGAGGCTGA
GGGATCCATCTCCACTCTGACGATCCAGCGCACAGAGCAGCGGG
ACTCGGCGATCTGCTGTGCCAGCAGCTTAGCGGGACAGGGGG
GCACCAAGAGACCCAGTACTCGGGCAGGCACGCCGCTCTGG
TGCTCGAGGACCTGAAAACGTGTCGCCACCCGAGGTGCTGTGT
TTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACA
CTGGTGTGCCCTGGCCACAGGCTCTACCCGACCGTGGAGCTG
AGCTGGTGGGTGAATGGGAAGGAGGTGCAAGTGGGGTCAAGCAC
AGACCGCAGCCCCCTCAAGGAGCAGCCCCTCAATGACTCCA
GATACTGCCCTG
```



	V	D	J	C	#
α	TRAV27			TRAJ36	TRAC
β	TRBV12-3	TRBD1	TRBJ2-3	TRBC2	3
α	TRAV21			TRAJ17	TRAC
β	TRBV6-3	TRBD1	TRBJ2-1	TRBC2	2
α	TRAV12-3			TRAJ24	TRAC
β	TRBV15	TRBD1	TRBJ2-2	TRBC2	2
α	TRAV17			TRAJ29	TRAC
β	TRBV14			TRBJ2-7	TRBC2
α	TRAV8-2			TRAJ23	TRAC
β	TRBV12-3	TRBD1	TRBJ1-3	TRBC1	2
α	TRAV8-1			TRAJ27	TRAC
β	TRBV12-3	TRBD2	TRBJ1-1	TRBC1	2
α	TRAV38-2...			TRAJ17	TRAC
α	TRAV8-2			TRAJ8	TRAC
β	TRBV25-1			TRBJ1-1	TRBC1
α	TRAV12-1			TRAJ48	TRAC
β	TRBV7-2	TRBD2	TRBJ2-7	TRBC2	2
α	TRAV38-2...			TRAJ53	TRAC
β	TRBV6-6	TRBD2	TRBJ2-4	TRBC2	2
α	TRAV2			TRAJ8	TRAC
β	TRBV4-3	TRBD1	TRBJ1-2	TRBC1	2

Filter

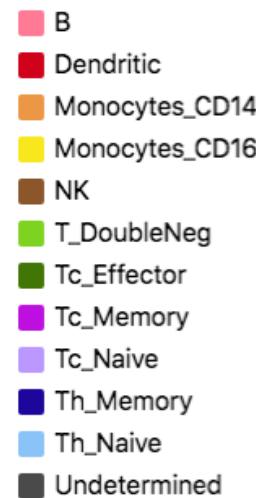
Cluster X

Intersection of cellular phenotyping with paired Ig repertoire profiling

B cell compartment mapped to Ig clonotypes



Cell type annotation based on
Gene expression **and** the cell surface
antibody profile



Cells with Ig clonotypes identified

Paired, full length Ig sequences

Paired, full length Ig sequences for B cell compartment

```
>clonotype1_consensus_1_H
TGGGGACCAAAACACACCCCTCCTGGGAGAACCTTAGATTACAGCTC
CTCACCATGGACTGGACCTGGAGCATCCCTTTCTGGTGGCAGCAGCACAGG
TGCCCCACTCCAGGTTCAGCTGGCAGTCTGGAGCTGAGGTGAAGAACGCT
GGGGCCTCAAGGTCTCTGCAAGGCTCTGGTACACCTTACAGCAGCTC
CATCTCTGGTATTACCTGGTGCAGAGGCCCTGGACAAGGCTTGAAGTGG
TGGGATGGGTAGCGGGTACAATGGTGACACAAAGTATGCACAGAACGTTCCAG
GGCAGAGTCACCATGACCACAGACATCCACGAGTACAGGCTACATGGAGCT
GAGGAGCCTGAGATCTGACGACACGGCCGTATTACTGTTGAGAGACGTC
GTCCCCACTGGGCCAGGGAACCTGGTACCCCTCAGCACCCACCAA
GGCTCGGGATTTACATGGGTGAGACACCCAAAGGATAACA
GCCCTGTGGTCTGGCATGTTGATAACTGGTACCCACCGTCCGTGACT
GTCACCTGGTACATGGGACACAGAGCCAGCCCCAGAGAACCTCCCTGAGAT
ACAAGACGGGACAGCTACTACATGACAAGCAGCCAGCTCTCACCCCCCTCC
AGCAGTGGGCCAAGGGAGTACAATGCGTGGTCCAGCA
```

```
>clonotype1_consensus_1_K
GCCCCCTCAGGGCACAGCAGCTGGGATTTATAAACGGGCCGTTGCAT
TGTGAACCTGAGCTAACACAGGCAGGGCAGCAAGATGGTGGCAGAC
CCAGGCTCTCATTTCTCTGGCTCTGGATCTCTGGTGCCTACGGGGACATCGT
GATGACCCAGTCTCCAGACTCCCTGGCTGTCTCTGGCGAGAGGGCCACC
ATCAACTGCAAGTCCAGGAGCTGGTATACAGCTCCAACAATAAGGAGTAC
TTAGCTTGTGACAGCAGAAACAGGAGCTCCCTAAGCTGCTATTACTGG
GCATCCCACCCGGGAATCGGGGTCCTGACCGATTCACTGGCAGCGGGCTG
GGACAGATTCACTCTCACCATCAGCGGCTGAGGCTGAAGATGTCAGTT
TATCCAGAGAGGGCAAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCGG
TAACCTCCAGGAGAGTGTCAACAGAGCAGGAGCAGCAAGGACAGCACCTACAGC
CTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAA
```



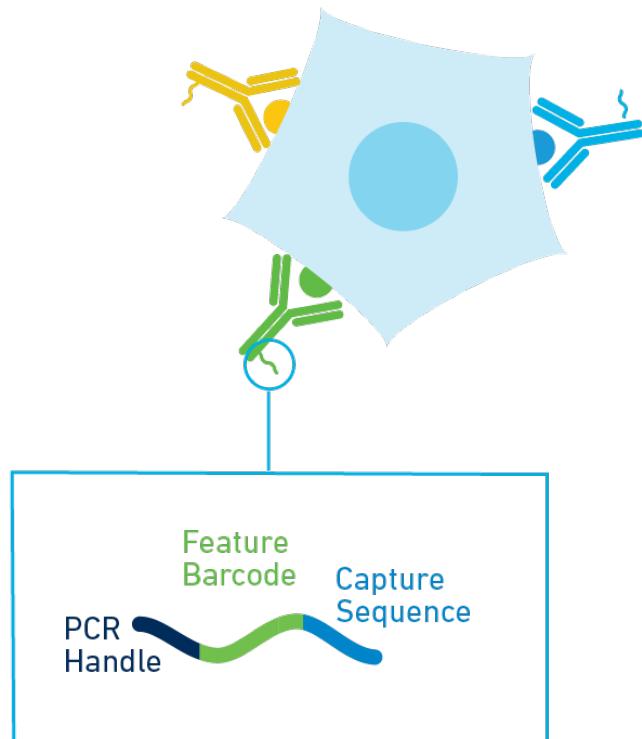
V	D	J	C	#
H IGHV1-18	IGHD2-2	IGHJ4	IGHD	4
K IGKV1-9		IGKJ1	IGKC	
K IGKV4-1		IGKJ2	IGKC	
H IGHV3-72	IGHD3-9	IGHJ4	IGHM	2
K IGKV1-16		IGKJ4	IGKC	
H IGHV3-43	IGHD6-13	IGHJ4	IGHM	2
λ IGLV2-14		IGLJ2	IGLC2	
H IGHV4-39	IGHD3-10	IGHJ4	IGHM	
H IGHV5-51	IGHD2-8	IGHJ6	IGHM	
λ IGLV1-44		IGLJ3	IGLC2	
λ IGLV3-19		IGLJ2	IGLC2	
H IGHV3-11	IGHD1-1	IGHJ4	IGHM	
λ IGLV1-51		IGLJ2	IGLC2	
H IGHV3-30	IGHD3-22	IGHJ6	IGHM	
λ IGLV3-1		IGLJ2	IGLC2	
H IGHV3-33	IGHD6-6	IGHJ6	IGHA1	
λ IGLV2-14		IGLJ3	IGLC3	
H IGHV2-5	IGHD6-19	IGHJ4	IGHG2	
H IGHV3-72	IGHD6-19	IGHJ4	IGHG2	

Filter Cluster ▾

Cluster Identity: B ▾ X

Single Cell Immune Profiling

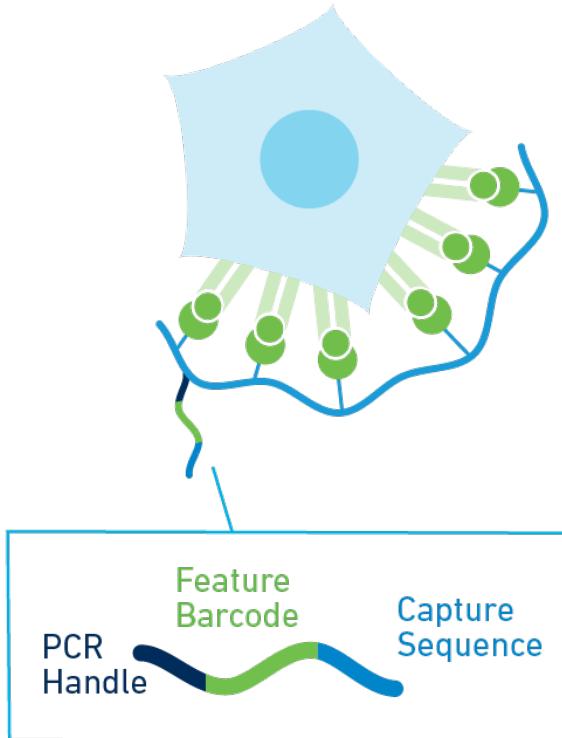
Feature Barcoding Technology: Cell Surface Protein



- **TotalSeq™- C Oligo Conjugated Antibodies** specific for the Chromium Single Cell Immune Profiling Solution with Feature Barcoding Technology
- Menu focus on antibodies for immune profiling in human, custom also available
- Enables highly multiplexed, simulations profiling of cell surface proteins
- For more info:
<https://www.biolegend.com/totalseq>

Single Cell Immune Profiling

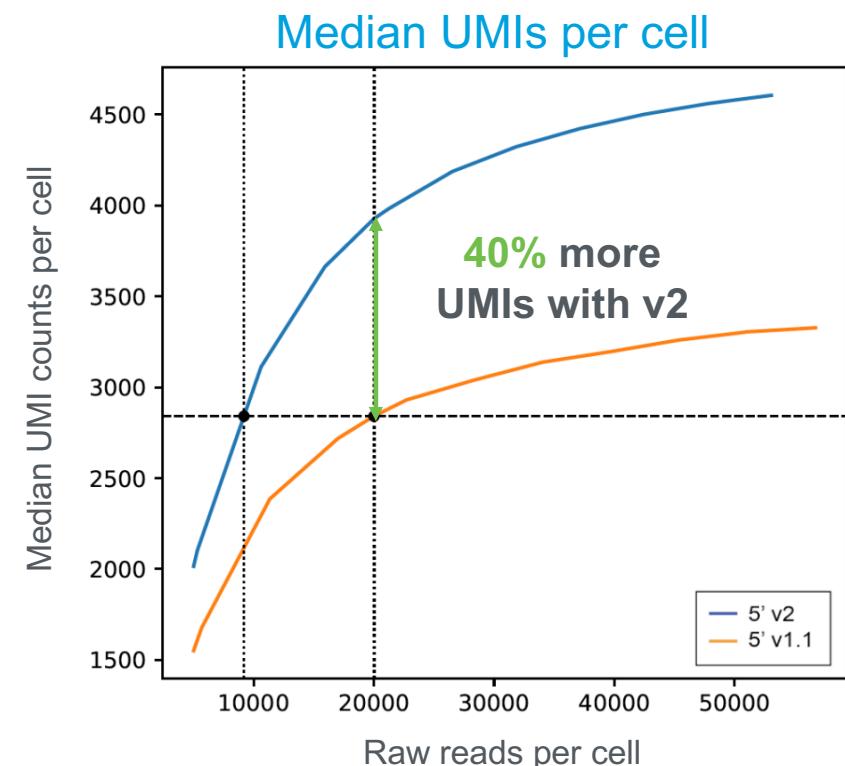
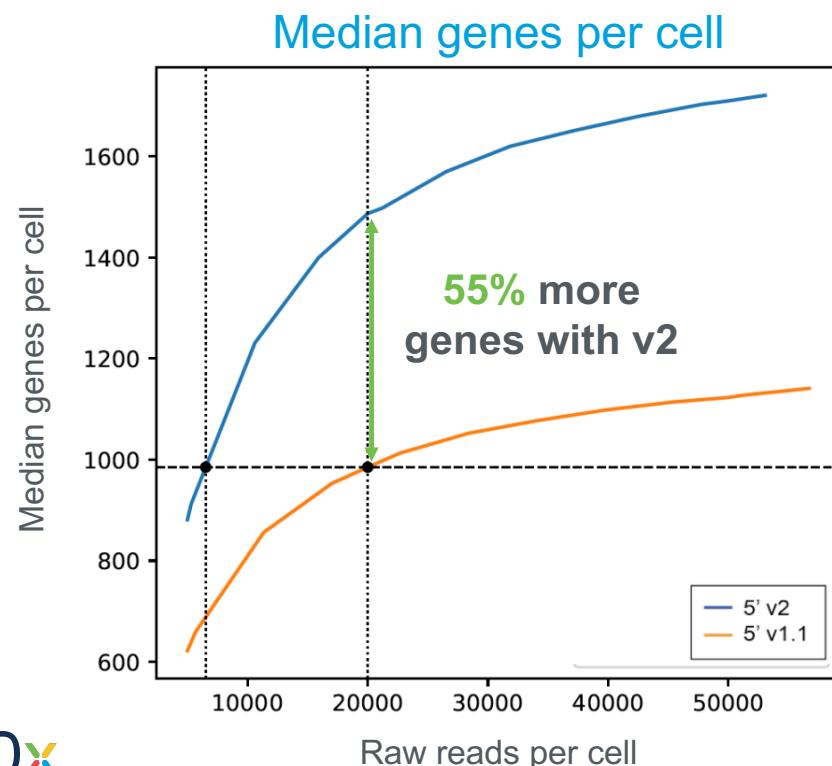
Feature Barcoding Technology: Antigen Mapping



- **dCODE™ Dextramer® Oligo Conjugated**
Dextramers specific for the Chromium Single Cell Immune Profiling Solution with Feature Barcoding Technology
- Menu focus on conventional T cells (MHC I + MHC II)
- Enables highly multiplexed TCR pMHC antigen specificity
- For more info:
<http://www.immudex.com/products/dcode-dextramer.aspx>

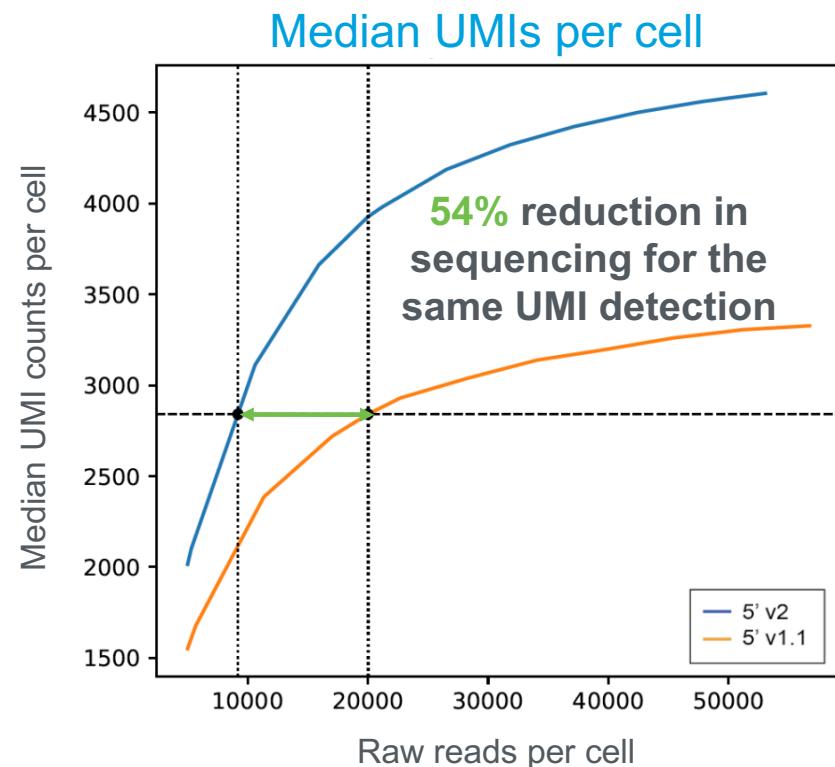
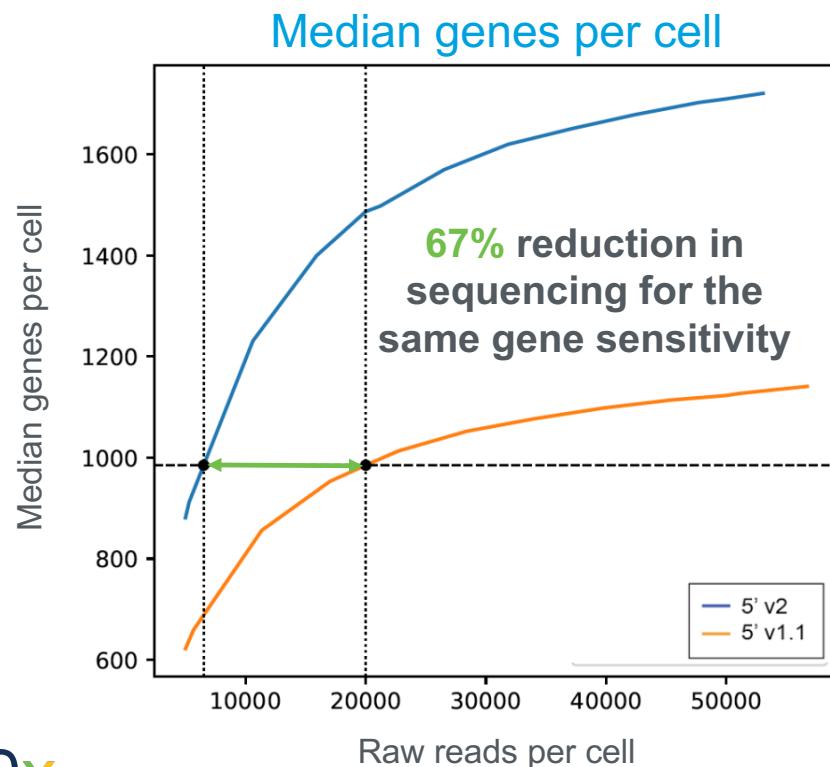
Huge gains in gene expression sensitivity in v2

1,000 Human PBMCs



...allows for reduced sequencing depths

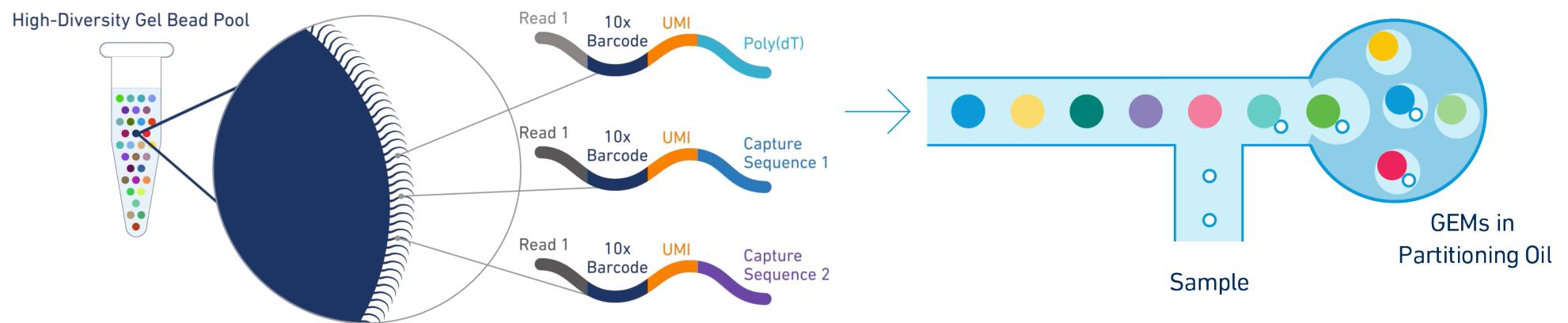
1,000 Human PBMCs



Single Cell Gene Expression



How it works

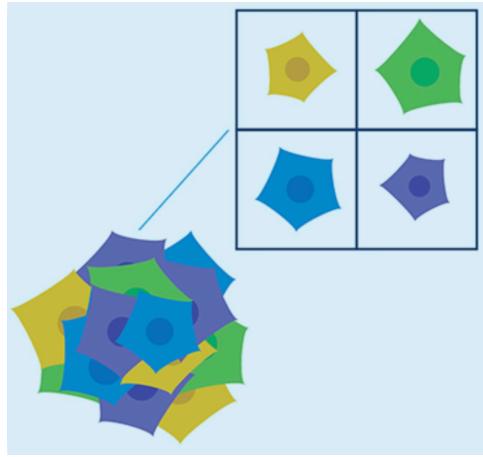


10x Barcode = Cell ID

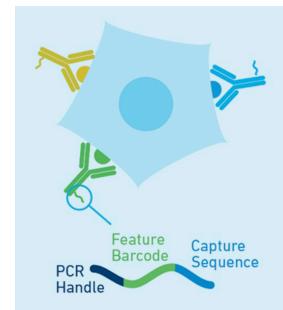
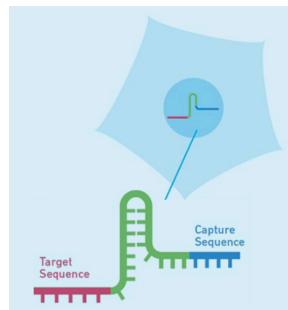
GEMs = Gel beads in EMulsions

Multimodal measurements from the same cell

Feature Barcoding technology



+

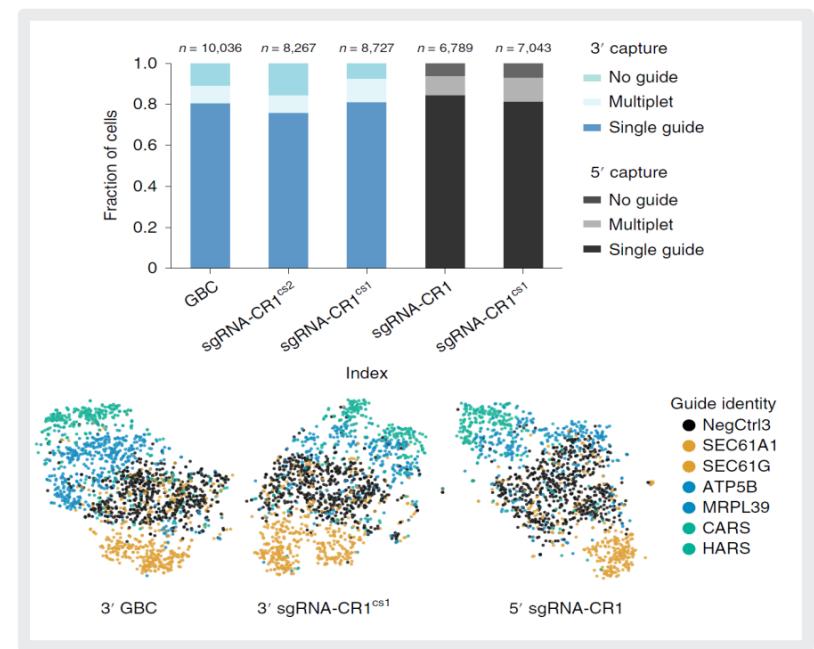


Combinatorial single-cell CRISPR screens by direct guide RNA capture and targeted sequencing

nature
biotechnology

Direct guide RNA capture improves efficacy of pooled CRISPR interference screens

- Presentation of direct capture of guide RNAs for pooled single cell CRISPR screens using 10x Genomics Chromium Single Cell Gene Expression 3'RNA and 5'RNA solutions.
- Greater versatility demonstrated by direct priming of guide RNAs versus indirect identification by index transcripts (Perturb-Seq)
- The authors demonstrated the utility of the direct capture approach for high-throughput investigation of interactions between the cellular processes involved in cholesterol biogenesis and DNA repair.
- Targeted gene expression panels based on hybridization-based capture approach enabled the cost-effective analysis of focused panel of genes at single cell resolution.

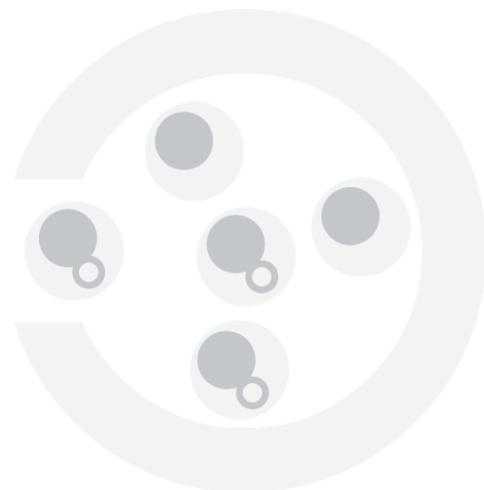


Reprogle et al 2020, nature biotechnology, <https://doi.org/10.1038/s41587-020-0470-y>

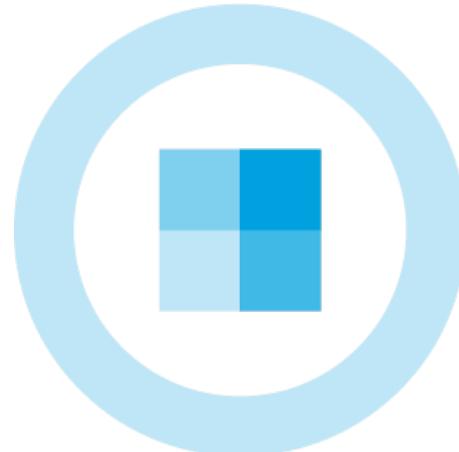
© 10X GENOMICS, INC. 2020. 28

Advances in Spatial Genomics

Chromium
Platform

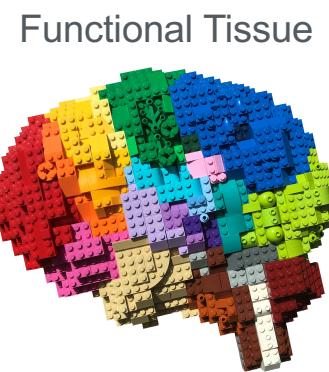
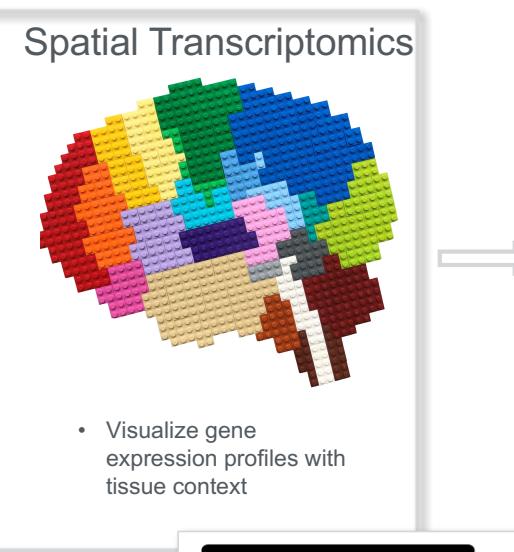
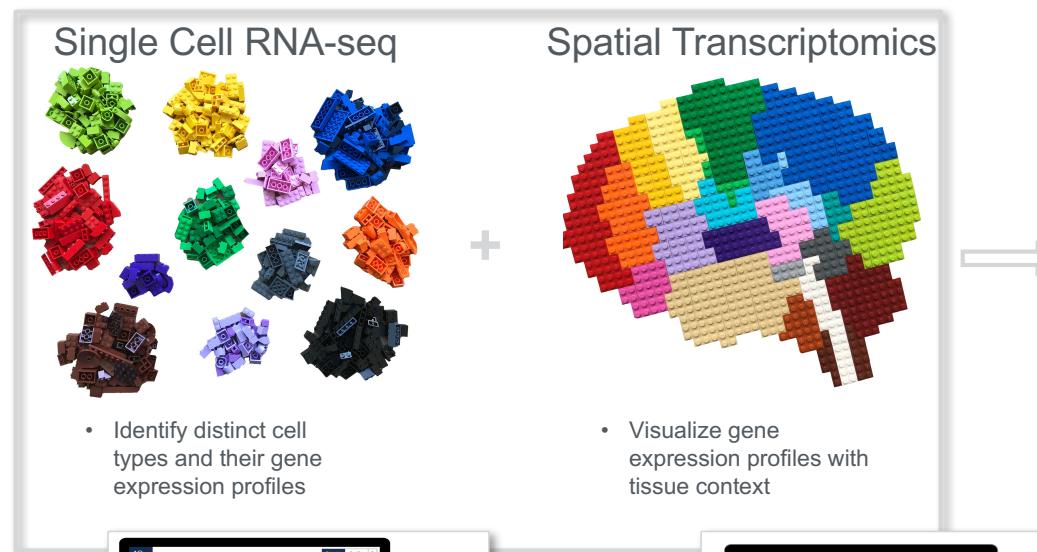
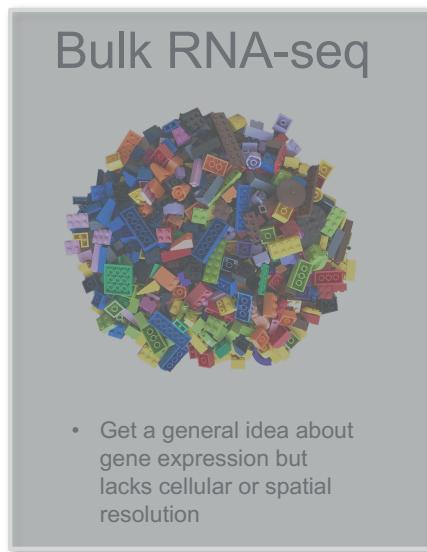


Visium
Platform



Gain a Complete View of Biology with Single Cell and Spatial Analysis

Complementarity of single cell and spatial methods from 10x Genomics



- Single cell plus spatial analysis provides the spatial organization of distinct cell types and their gene expression profiles

Chromium Single Cell Gene Expression Products

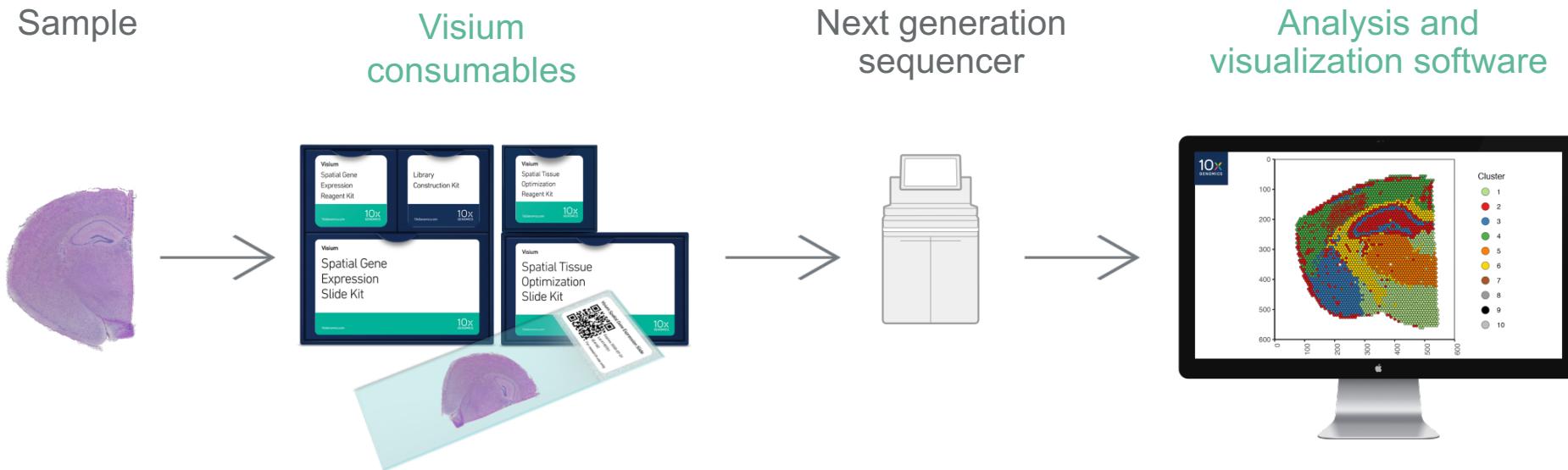


Images and concept courtesy of Bo Xia, NYU School of Medicine



Visium Spatial Gene Expression Products

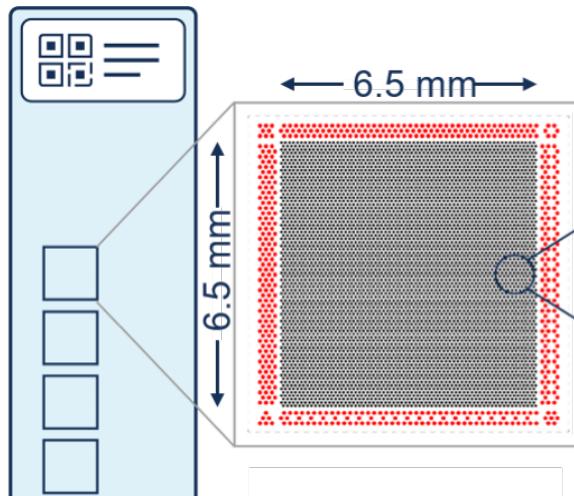
Visium Platform: Rich Spatial Characterization



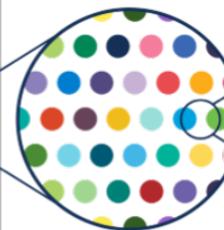
Unbiased gene expression at high spatial resolution

Utilizing poly-A capture and unique spatial barcodes

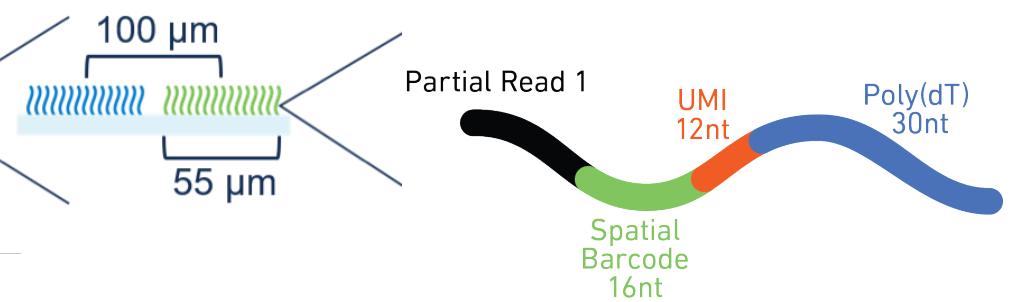
Visium Spatial Gene Expression Slide



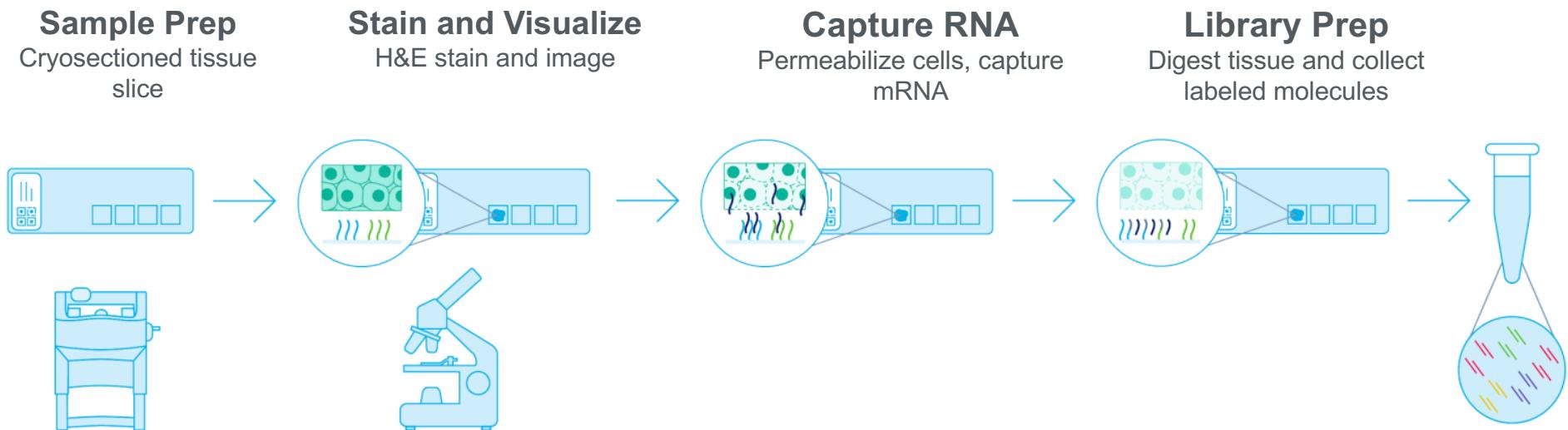
Capture Area with ~5000 Barcoded Spots



Visium Gene Expression Barcoded Spots



Visium Sample & Library Prep Workflow

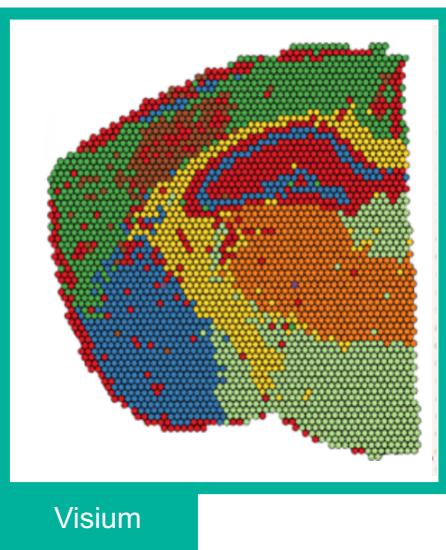
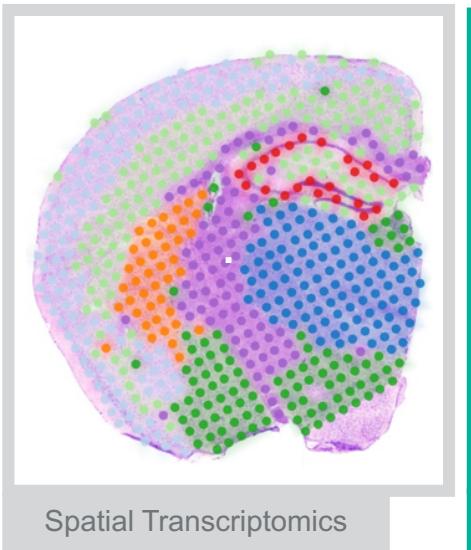


< 1 Day (many available stopping points)

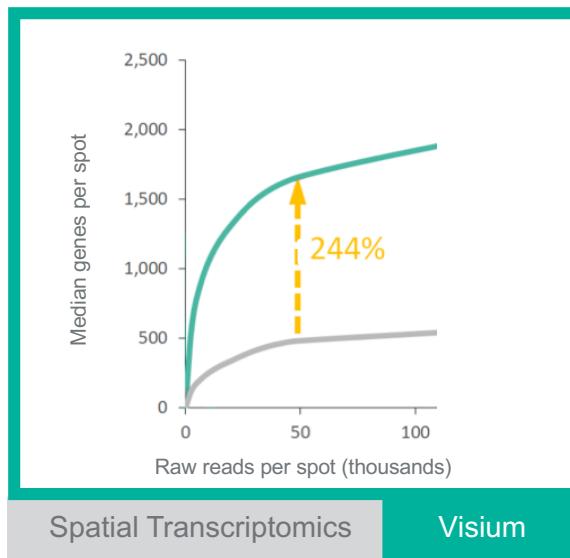
From sectioned tissue to sequence-ready libraries

Visium Improvements

Increased resolution



Increased sensitivity



Shortened
workflow

Spatial Transcriptomics

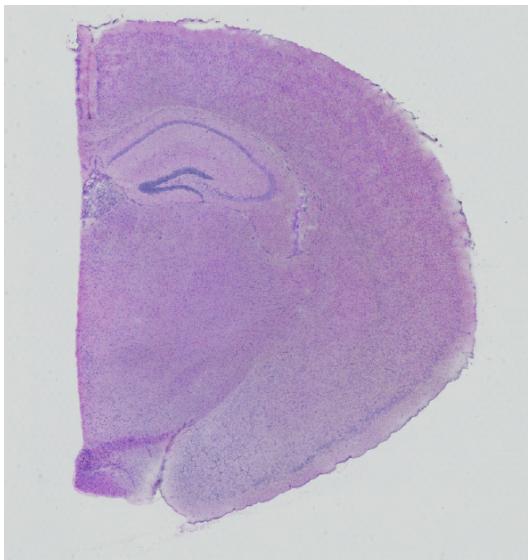
Visium

1 DAY

3 DAYS

Visium Spatial Gene Expression

Mouse Brain



H&E Tissue Section

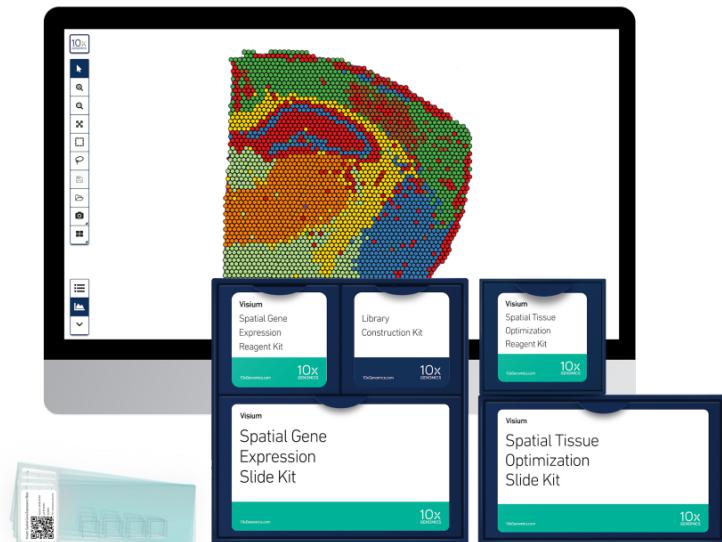
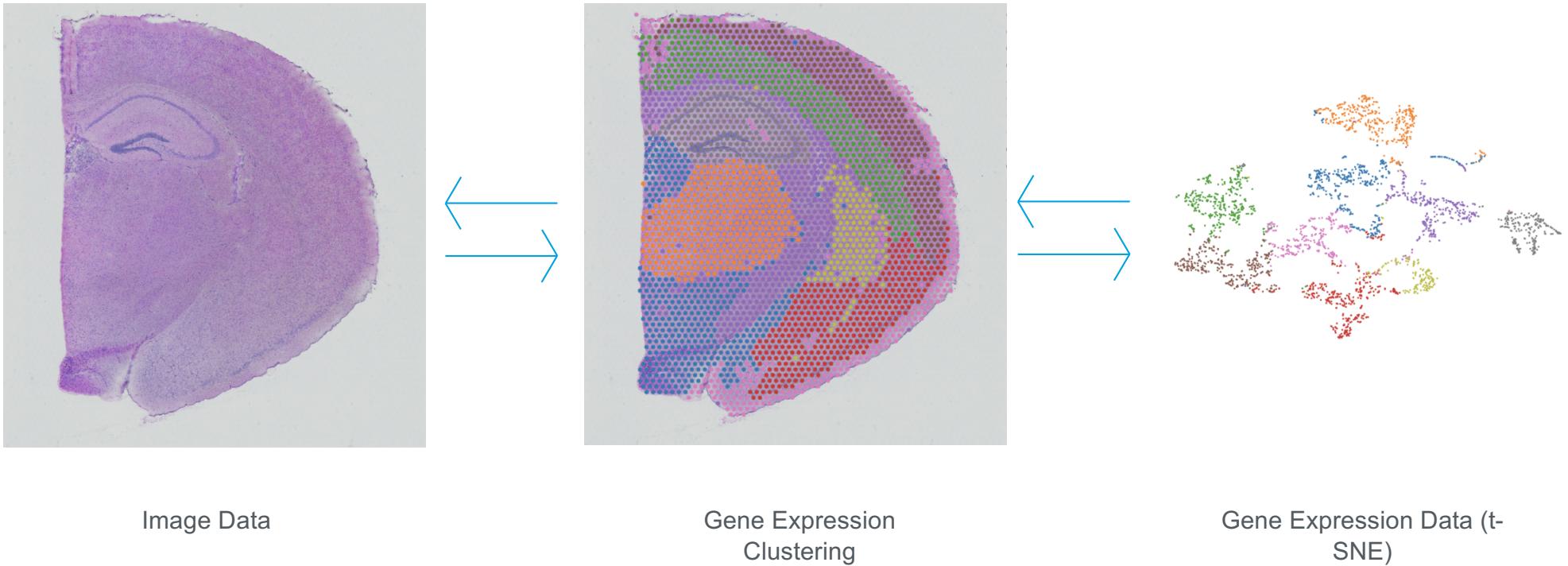


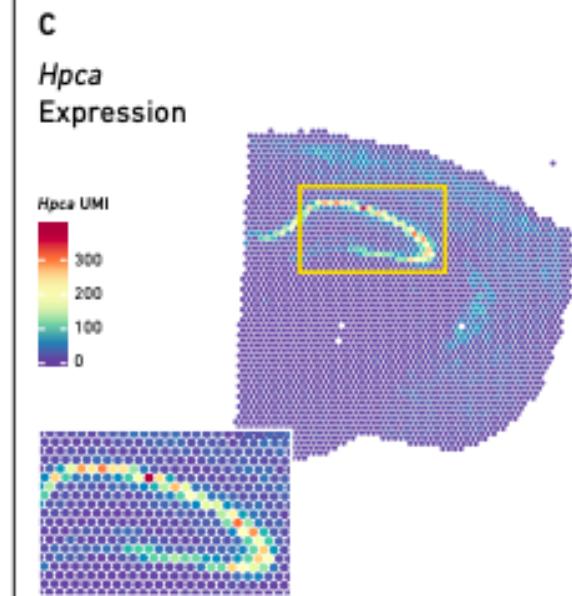
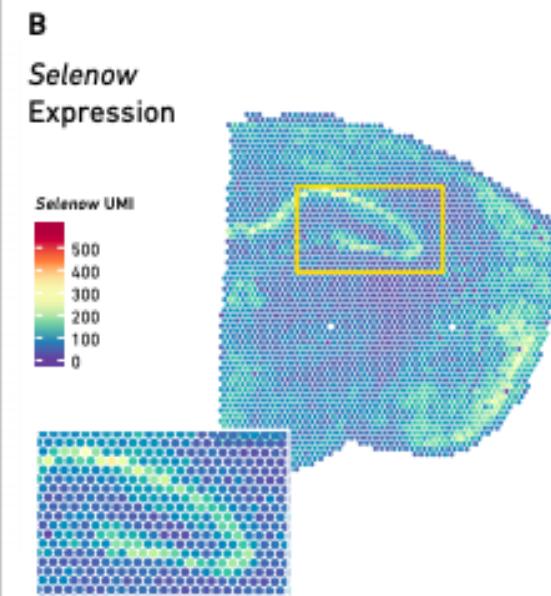
Image or Gene Expression Driven Analysis of Spatial Data

Start with the Gene Expression Data or Image data of the Same Section



Spatially Resolved Expression in the Mouse Brain

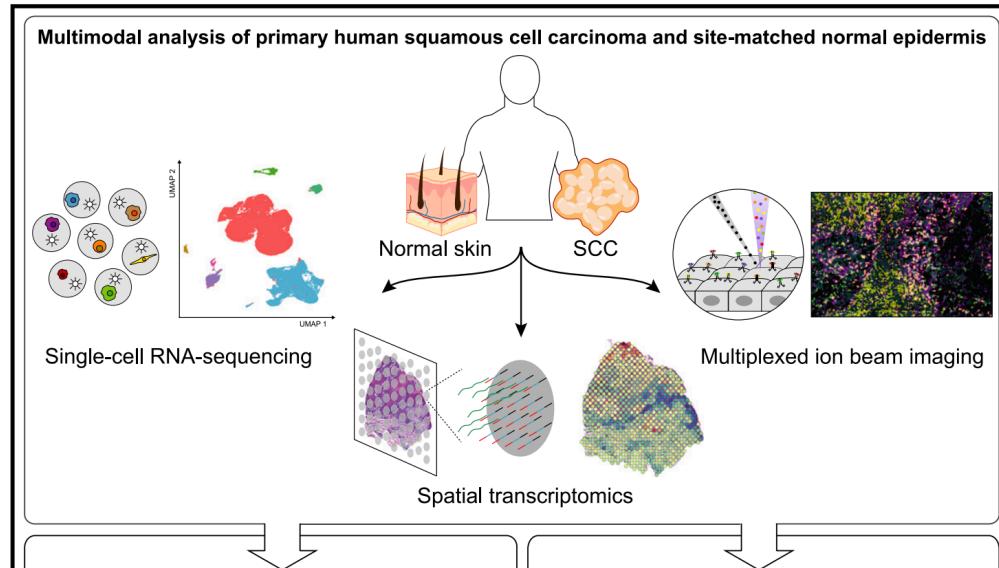
Hippocampal Gene Expression Coincides with Known Gene Expression Patterns



Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma

CellPress

Graphical Abstract



Authors

Andrew L. Ji, Adam J. Rubin,
Kim Thrane, ..., Garry P. Nolan,
Joakim Lundeberg, Paul A. Khavari

Correspondence

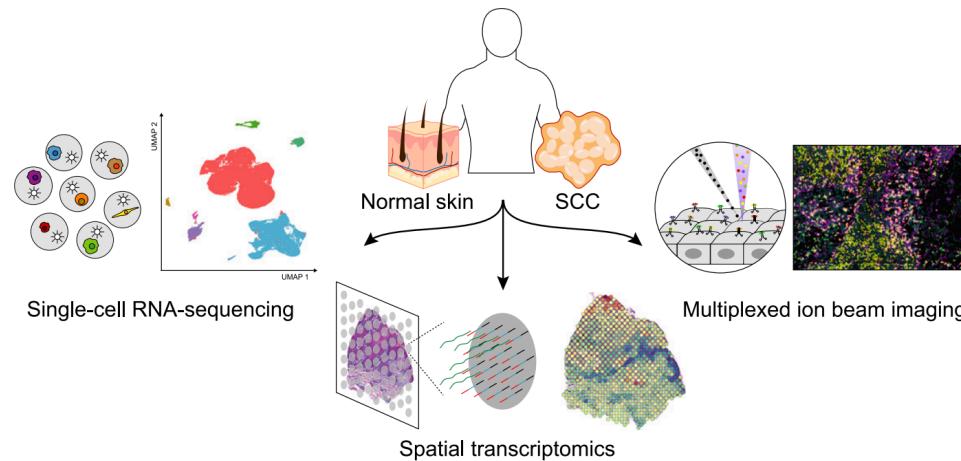
khavari@stanford.edu

In Brief

Integration of high-dimensional multi-omics approaches to characterize human cutaneous squamous cell carcinoma identifies a tumor-specific keratinocyte

Integration of Single Cell and Spatial Gene Expression

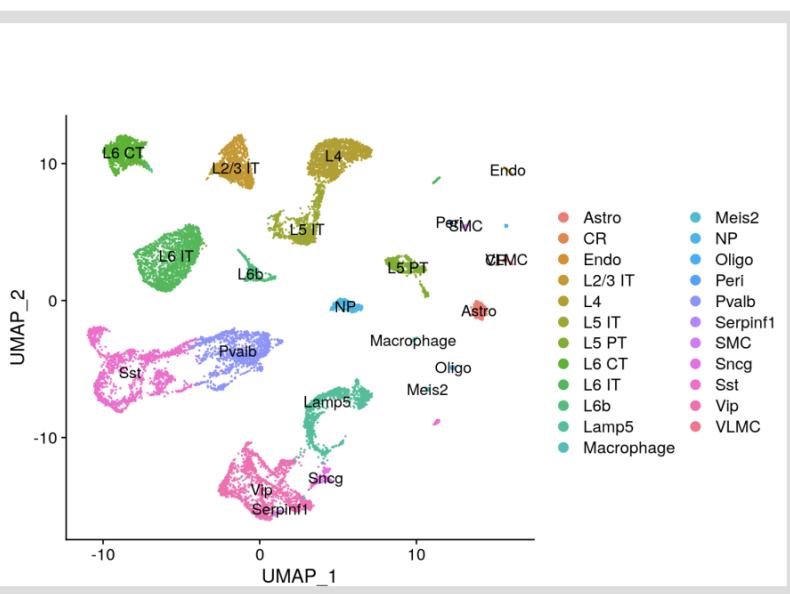
Ji et al., 2020, Cell



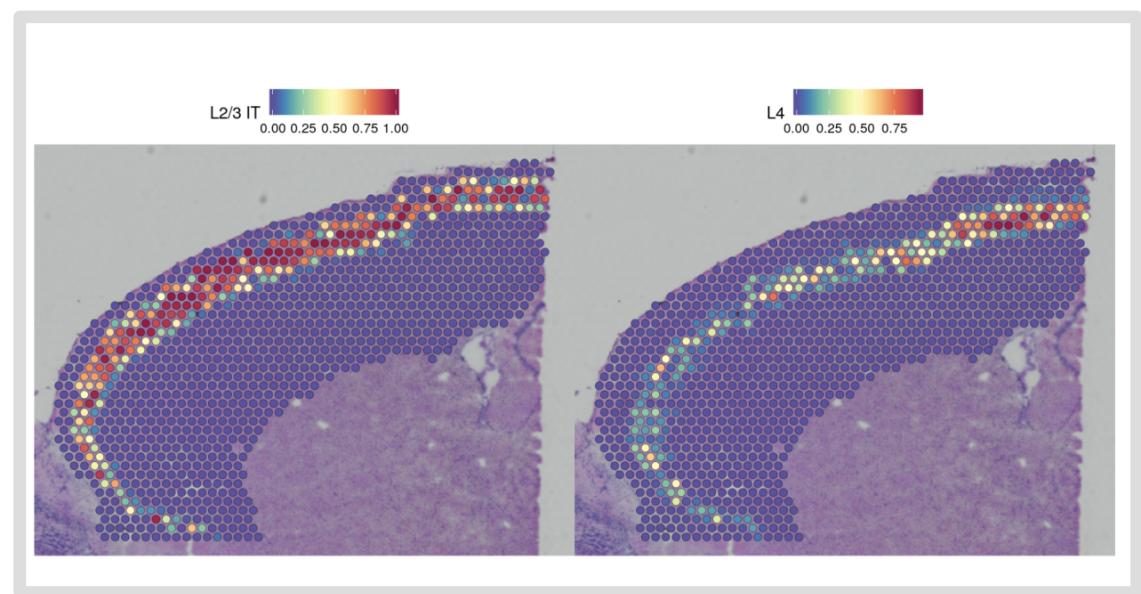
- Profiling of 10 human skin SCCs and matched normal skin via scRNA-seq, ST, and MIBI
- Tumor-specific keratinocytes (TSKs) reside within a fibrovascular niche at leading edges of tumor tissue
- Distinct ligand-receptor and spatial niche associations for tumor and stromal cells
- Subpopulation essential tumorigenic gene networks defined by *in vivo* CRISPR screening

Spatial Analysis Tools: Single Cell Integration

Seurat Support for Spatial Analysis



Single Cell Neuron Annotations



Gene Signatures Mapped into Spatial GEX Clusters

SATIJA LAB

HOME NEWS PEOPLE RESEARCH PUBLICATIONS SEURAT JOIN/CONTACT



SINGLE CELL GENOMICS DAY



About Install Vignettes Extensions FAQs Contact Search

Analysis, visualization, and integration of spatial datasets with Seurat

Compiled: 2020-01-03

Overview

This tutorial demonstrates how to use Seurat v3.2 to analyze spatially-resolved RNA-seq data. While the analytical pipelines are similar to the Seurat workflow for [single-cell RNA-seq analysis](#), we introduce updated interaction and visualization tools, with a particular emphasis on the integration of spatial and molecular information. This tutorial will cover the following tasks, which we believe will be common for many spatial analyses:

- Normalization
- Dimensional reduction and clustering
- Detecting spatial-variable features
- Interactive visualization
- Integration with single-cell RNA-seq
- Working with multiple slices

For our first vignette, we analyze [technology](#) from 10x Genomics, additional data types in the [neuro](#) and [MERFISH](#).

Installation

Seurat v3.2 is currently in a pre-release beta stage and is available for installation through Github.

```
devtools::install_github("satijalab/seurat", ref = "spatial")
```

First, we load Seurat and the other packages necessary for this vignette.

```
library(Seurat)
library(SeuratData)
library(ggplot2)
library(cowplot)
library(dplyr)
```

Dataset

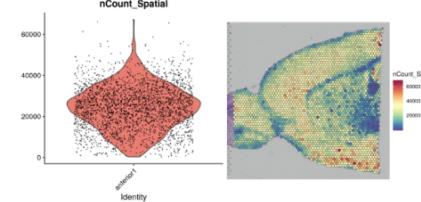
Here, we will be using a recently released dataset of sagittal mouse brain slices generated using the Visium v1 chemistry. There are two serial anterior sections, and two (matched) serial posterior sections.

You can download the data [here](#), and load it into Seurat using the `Load10X_Spatial` function. This reads in the output of the `spaceranger` pipeline, and returns a Seurat object that contains both the spot-level expression data along with the associated image of the tissue slice. You can also use our [SeuratData package](#) for easy data access, as demonstrated below. After installing the dataset you can type `?satxBrain` to learn more



heterogeneity here, which requires effective normalization.

```
plot1 <- VlnPlot(brain, features = "nCount_Spatial",
pt.size = 0.1) + NoLegend()
plot2 <- SpatialFeaturePlot(brain, features =
"nCount_Spatial") + theme(legend.position = "right")
plot_grid(plot1, plot2)
```

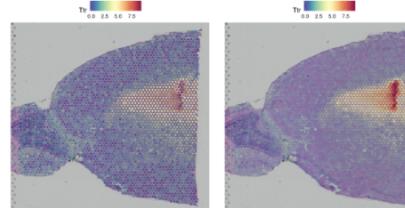


These plots demonstrate that the variance in molecular counts across spots is not just technical in nature, but also is dependent on the tissue anatomy. For example, regions of the tissue that are depleted for neurons (such as the cortical white matter), reproducibly exhibit lower molecular counts. As a result, standard approaches (such as the `LogNormalize` function), which force each data point to have the same underlying 'size' after normalization, can be problematic.

As an alternative, we recommend using `sctransform` (Hafemeister and Satija, In Press), which builds regularized negative binomial models of gene expression in order to account for technical artifacts while preserving biological variance. For more details on `sctransform`, please see

with lower expression

```
p1 <- SpatialFeaturePlot(brain, features = "Ttr",
pt.size.factor = 1)
p2 <- SpatialFeaturePlot(brain, features = "Ttr",
alpha = c(0.1, 1))
plot_grid(p1, p2)
```

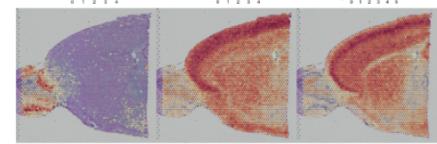


Dimensionality reduction, clustering, and visualization

We can then proceed to run dimensionality reduction and clustering on the RNA expression data, using the same workflow as we use for scRNA-seq analysis.

```
brain <- RunPCA(brain, assay = "SCT", verbose = FALSE)
brain <- FindNeighbors(brain, reduction = "pca", dims
= 1:30)
brain <- FindClusters(brain, verbose = FALSE)
brain <- RunUMAP(brain, reduction = "pca", dims =
```

```
ge_markers <- findmarkers(brain, ident.i = 4, ident.z
= 6)
SpatialFeaturePlot(object = brain, features =
rownames(de_markers)[1:3], alpha = c(0.1, 1), ncol =
3)
```



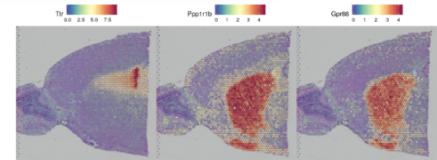
An alternative approach, implemented in `FindSpatiallyVariables`, is to search for features exhibiting spatial patterning in the absence of pre-annotation. The default method (`method = "markvariogram"`), is inspired by the `Trendsceek`, which models spatial transcriptomics data as a mark point process and computes a 'variogram', which identifies genes whose expression level is dependent on their spatial location. More specifically, this process calculates $\gamma(r)$ values measuring the dependence between two spots a certain r distance apart. By default, we use an r -value of 5 in these analyses, and only compute these values for variable genes (where variation is calculated independently of spatial location) to save time.

We note that there are multiple methods in the literature to accomplish this task, including `SpatialDE`, and `Splotch`. We encourage interested users to explore these methods, and hope to add support for them in the near future.

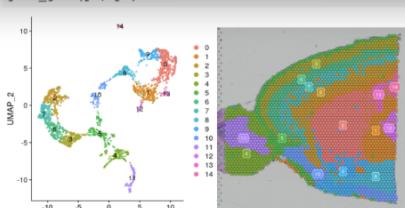
```
(brain, assay =
brain[1:1000,
])
Features(brain,
```

`selection.method = "markvariogram"`, 6)

```
SpatialFeaturePlot(brain, features = top.features,
ncol = 3, alpha = c(0.1, 1))
```

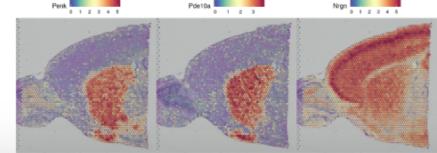


plot_grid(p1, p2)



As there are many colors, it can be challenging to visualize which voxel belongs to which cluster. We have a few strategies to help with this. Setting the `label` parameter places a colored box at the median of each cluster (see the plot above), and the `do.hover` parameter of `SpatialDimPlot` allows for an interactive viewing of the current spot identities.

```
# move your mouse
SpatialDimPlot(brain, do.hover = TRUE)
```



Support through your experimental workflow

- 10x customer publications
- 10x-pert & customer webinars
- Email 10x Support Team
- Open-source community tools
- 3rd party analysis services and products

Advanced Analysis
and Results



Experimental Design

- Discussion with local Science & Technology Advisor (STA), Field Application Scientist (FAS)



Cell Ranger
Data Processing

- Email 10x Support Team
- Software training from FAS
- Support site documentation and software tutorials

support@10xgenomics.com

- Email 10x Support Team
- Support site documentation and software tutorials

Data Visualization
with Loupe Browser



Data QC

- Data QC Technical Note
- Email 10x Support Team

Thank you!

Your Local 10x Genomics Team



Paul Scott
Sales Executive
paul.scott@10xgenomics.com



Nicole Abreu, PhD
Science & Technology Advisor
nicole.abreu@10xgenomics.com



Christine Kao
Senior Inside Sales Specialist
christine.kao@10xgenomics.com



Adam Bemis, MS
Field Application Scientist
adam.bemis@10xgenomics.com

Compatible Tissues and Species with More to Come

https://support.10xgenomics.com/docs/Spatial_GEX_tissues

Human Tissue (Healthy/Diseased)
Brain, Cerebellum (Healthy)
Brain, Cerebral Cortex (Healthy, Glioblastoma Multiforme)
Breast (Healthy)
Breast (Invasive Ductal Carcinoma)
Breast (Invasive Lobular Carcinoma)
Breast (Triple Negative Breast Cancer)
Heart (Ventricle, Healthy, Myocardial Infarction)
Kidney (Healthy, Nephritis)
Large Intestine (Colorectal Cancer)
Lung (Healthy, Papillary Carcinoma)
Lymph node (Healthy, Inflamed)
Ovaries (Tumor)
Spinal Cord (Healthy)
Spleen (Healthy, Inflamed)

Mouse Tissue (Healthy/Diseased)
Brain (Healthy)
Brain, Embryonic (Healthy)
Eyes (Healthy)
Heart (Healthy)
Kidney (Healthy)
Large Intestine (Healthy)
Liver (Healthy)
Lungs (Healthy)
Ovary (Healthy)
Quadriceps (Healthy)
Small Intestine (Healthy)
Spleen (Healthy)
Stomach (Healthy)
Testes (Healthy)
Thyroid (Healthy)
Tongue (Healthy)