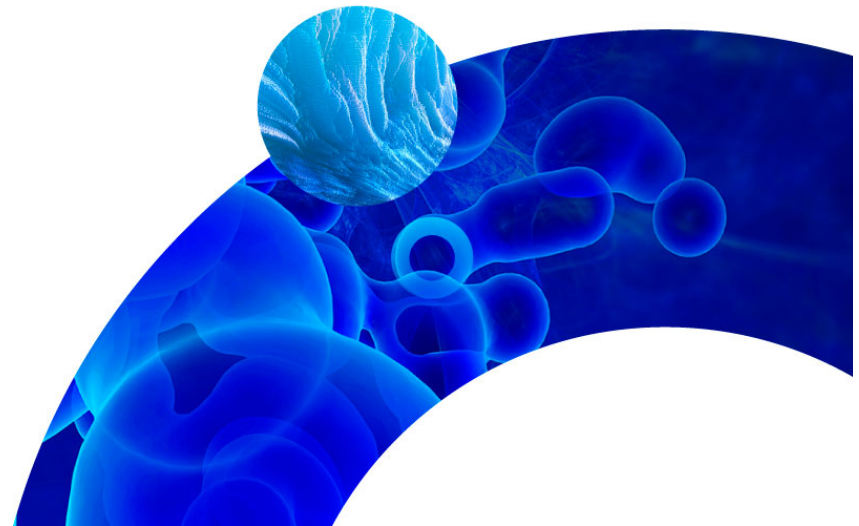


Biology at True Resolution

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

 [@10xgenomics](https://twitter.com/10xgenomics)



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

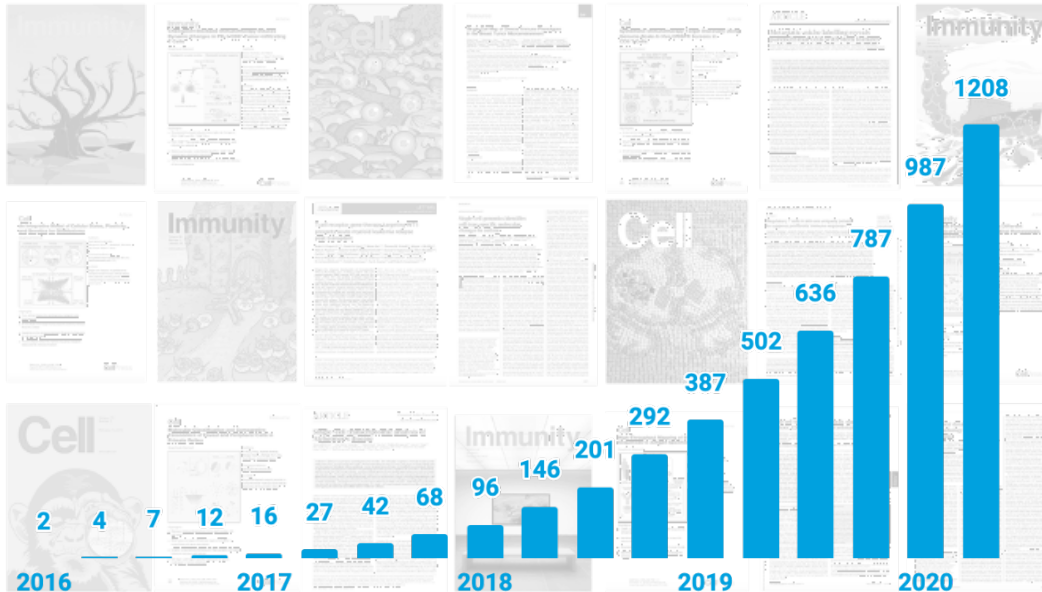
Measurement, understanding and mastery of the Immune System

Functional Genomics



Biology at True Resolution

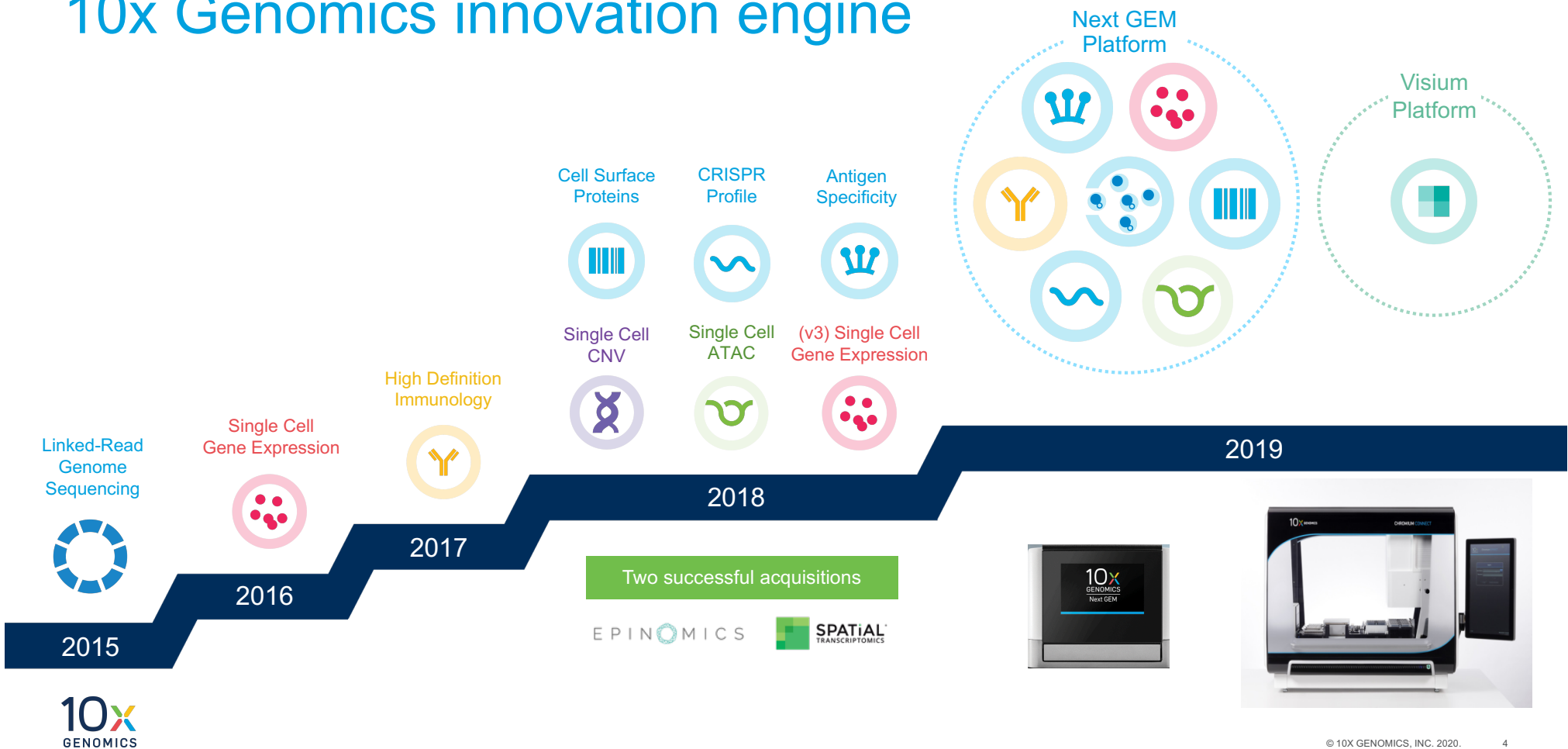
10x Genomics publications continue to scale



Cumulative publications

1,200+
publications

10x Genomics innovation engine



Solving Problems With Continued Innovation

10x Genomics provides complete solutions

Instruments



Consumables

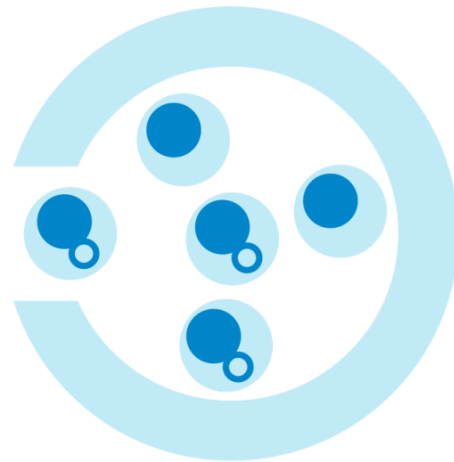


Software



Agenda

Chromium
Platform

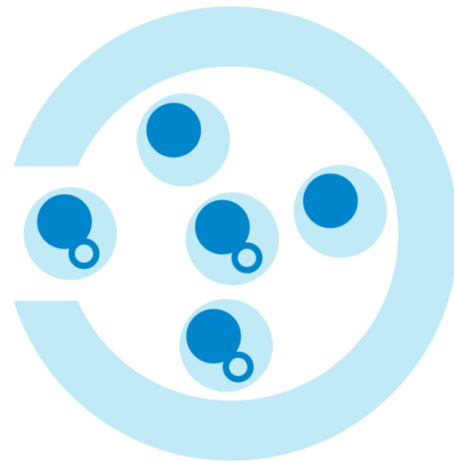


Visium
Platform



Advances in Single Cell Genomics

Chromium
Platform

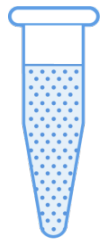


Visium
Platform

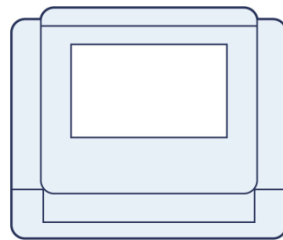


Chromium Single Cell Solutions

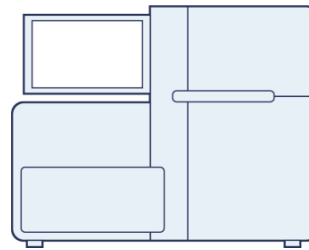
A transformative and easily integrated technology



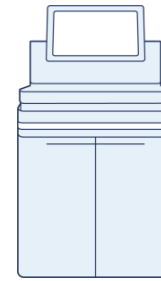
Flexible input
Single cell suspensions of 100s to 1000s of cells



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



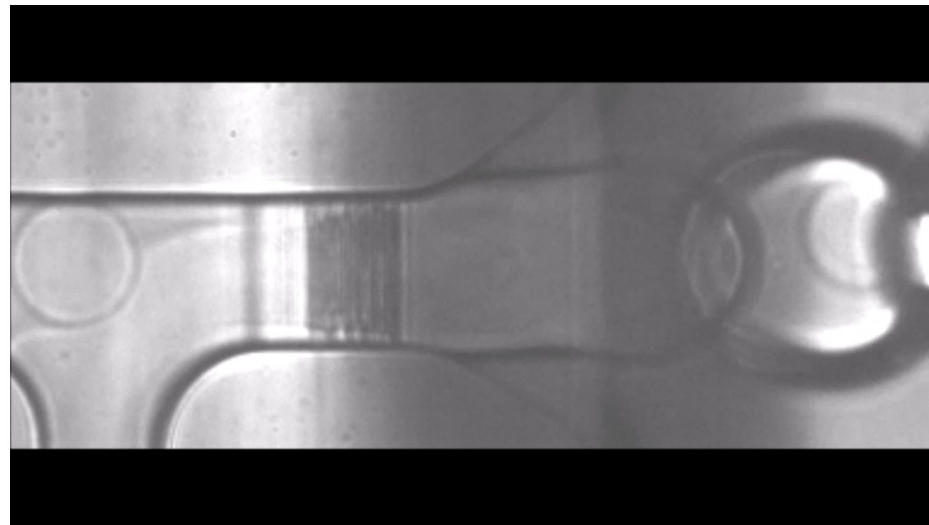
Single-use microfluidics chip

Recovery Well →
Gel Bead Well →
Sample Well →



Chromium Single Cell Solutions

Efficient and scalable cell capture

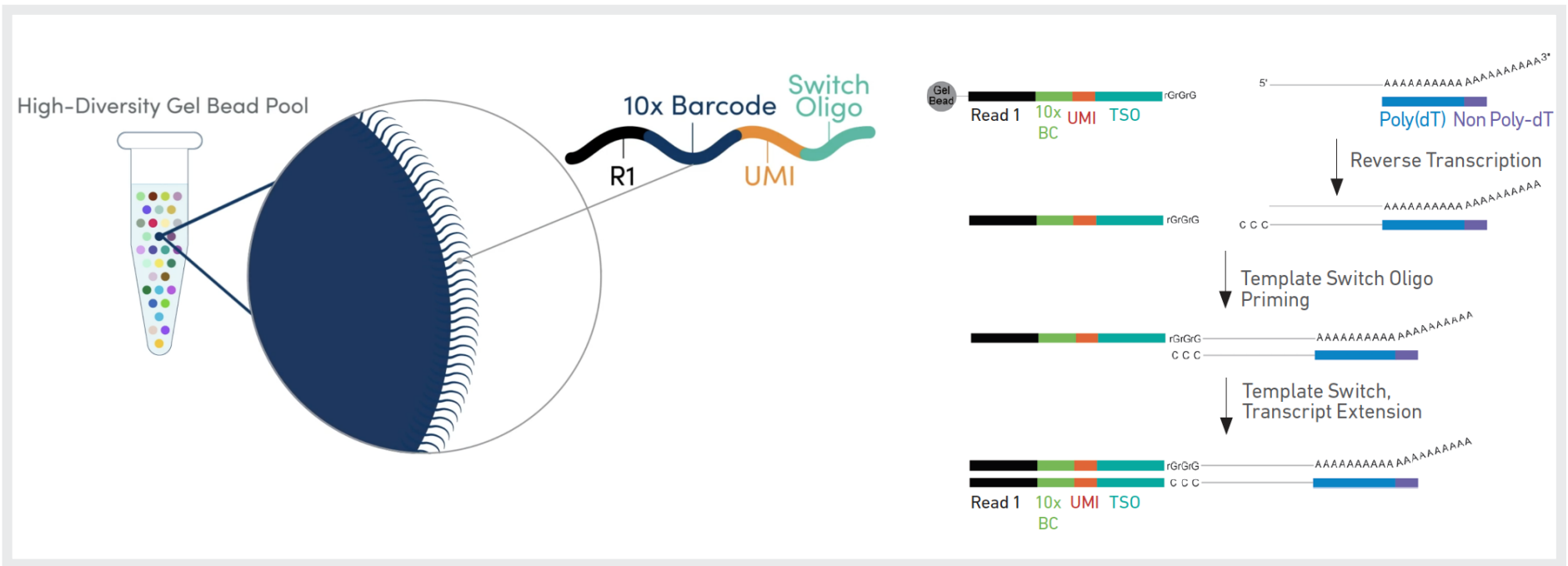


Single Cell Immune Profiling



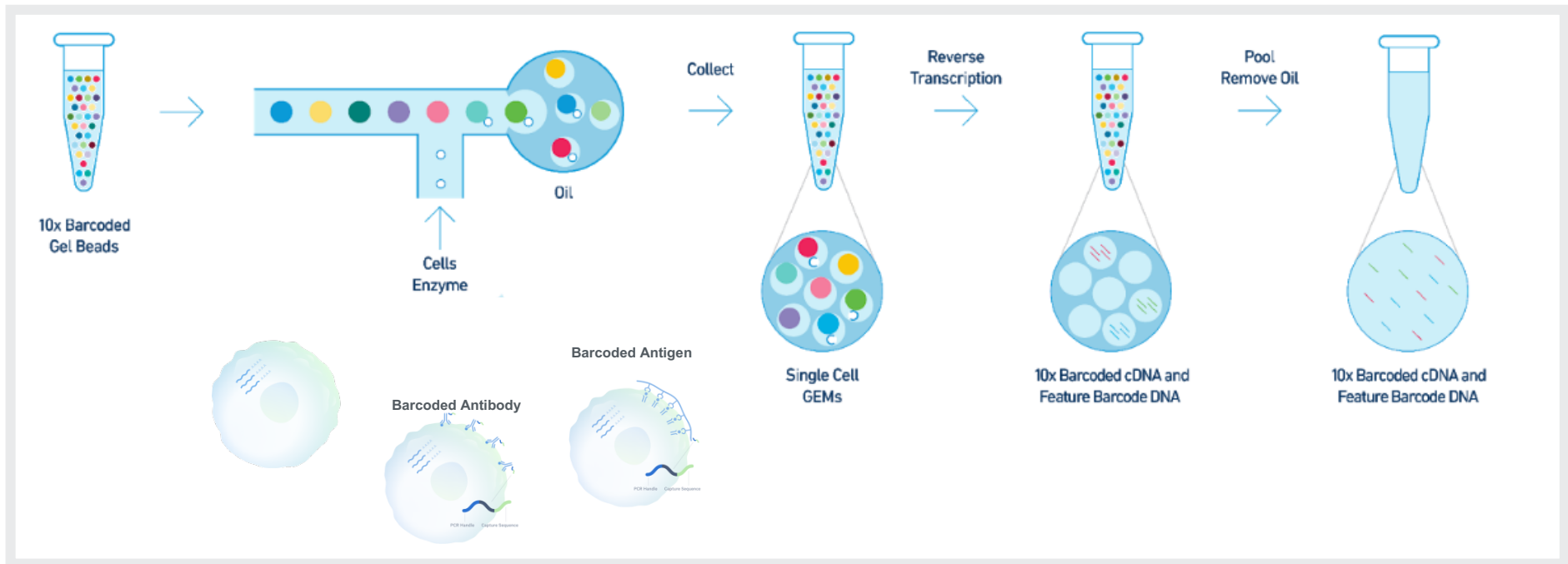
10x Genomics single cell technology

Barcoding single cell transcriptomes and other readouts

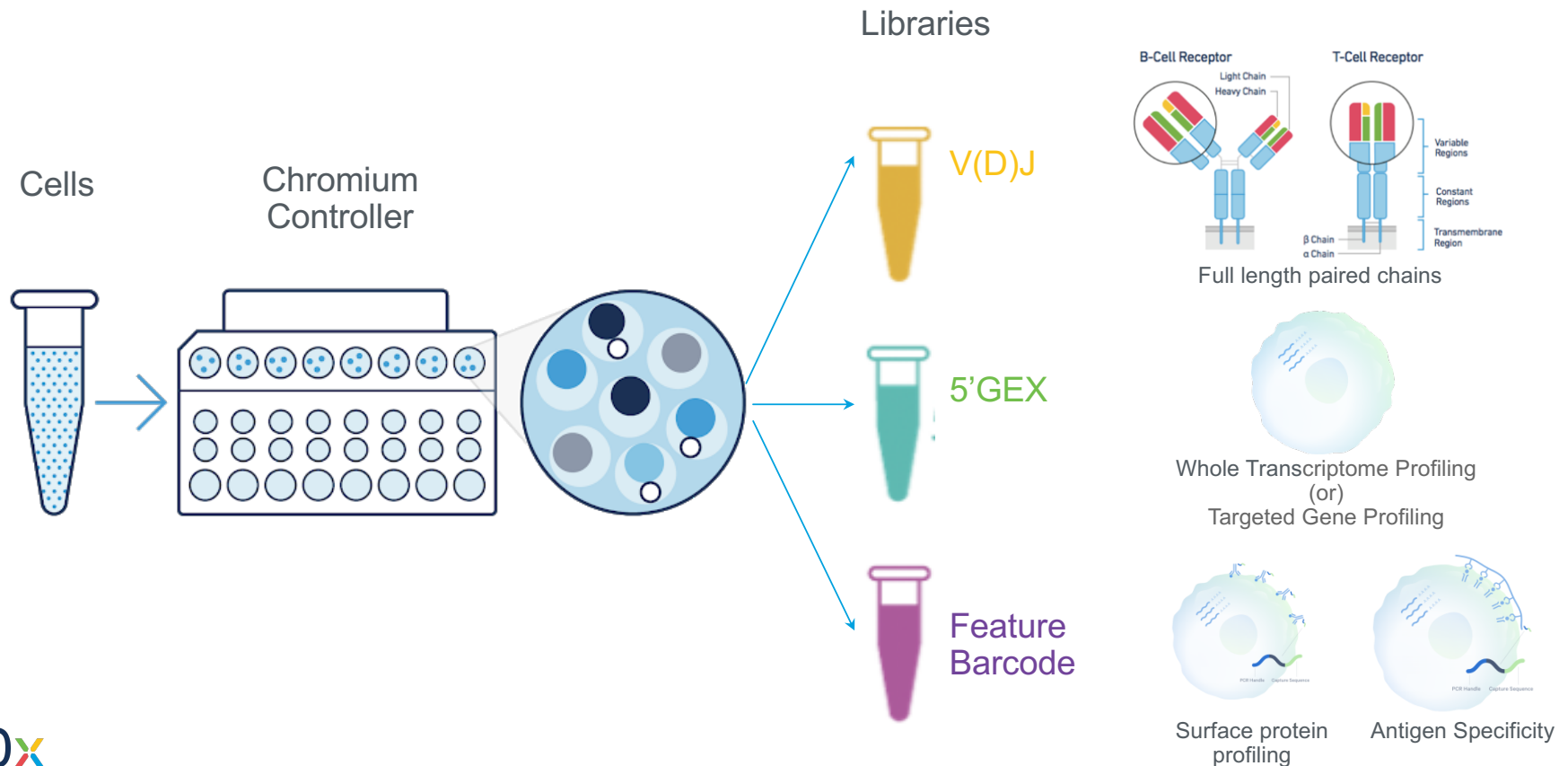


10x Genomics core technology for molecular barcoding and partitioning

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

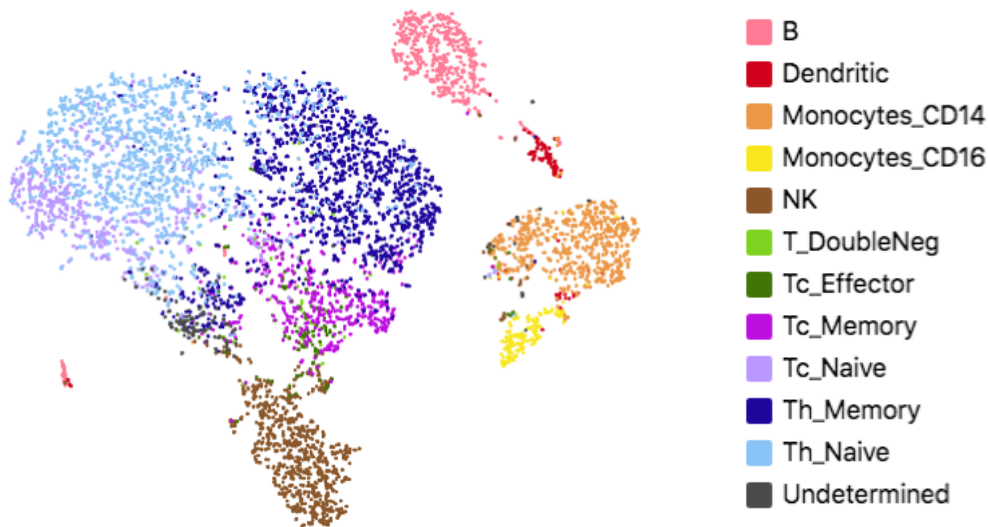
+

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

```
AGATCAGAAGAGGAGGCTTCTCACCTGCAGCAGGGACCTGTGA
GCATGGCATGCCCTGGCTTCTGTGGGCACTTGTGATCTCCACCT
GTCTTGAATTTAGCATGGCTCAGACAGTCACTCAGTCTCAACCAG
AGATGTCTGTGCAGGAGGCAGAGACCTGACCCTGAGCTGCACA
TATGACACCAGTGAGAGTGATTATTATTATTCTGGTACAAGCAG
CCTCCAGCAGGCAGATGATTCTCGTTATTCGCCAAGAAGCTTAT
AAGCAACAGAATGCAACAGAGAATCGTTTCTCTGTGAACTTCCAG
AAAGCAGCCAAATCCTTCAGTCTCAAGATCTCAGACTCACAGCTG
GGGGATGCCGCGATGATTTCTGTGCTTATAGGAGCGTGATTCA
GGAGGAGGTGCTGACGGACTCACCTTTGGCAAAGGGACTCATCT
AATCATCCAGCCCTATATCCAGAACCCTGACCCTGCCGTGTACCA
GCTGAGAGACT
```

>clonotype2_consensus_1_beta

```
AGTGACACTGATCTGGTAAAGCCCTCATCCTGTCCTGACCCTGCC
ATGGGACACAGTCTCCTATGCTGGGTGGTCTGGGTTTCCTAGGG
ACAGATCACACAGGTGCTGGAGTCTCCAGTCTCCAGGTACAAA
GTCACAAAGAGGGGACAGGATGTAGCTTCAGGTGTGATCCAAT
TTCGGGTCAATGATCCCTTTATTGGTACCGACAGGCCCTGGGGCA
GGGCCAGAGTTTCTGACTTACTTCAATTGAAGCCCAACAAGA
CAAATCAGGGCTGCCAATGATCGGTTCTTGCAGAGAGGCCTGA
GGGATCCATCTCCACTCTGACGATCCAGCGCACAGAGCAGCGGG
ACTCGCCATGTATCGCTGTGCCAGCAGCTTAGCGGGACAGGGG
GCACCAAGAGACCCAGTACTTCCGGCCAGGCACGCGGCTCCTGG
TGCTCGAGGACCTGAAAAACGTGTTCCACCCGAGGTCGCTGTGT
TTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACA
CTGGTGTGCCTGGCCACAGGCTTACCACCGACCACTGGAGCTG
AGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCAC
AGACCCGAGCCCTCAAGGAGCAGCCCGCCCTCAATGACTCCA
GATACTGCCTG
```



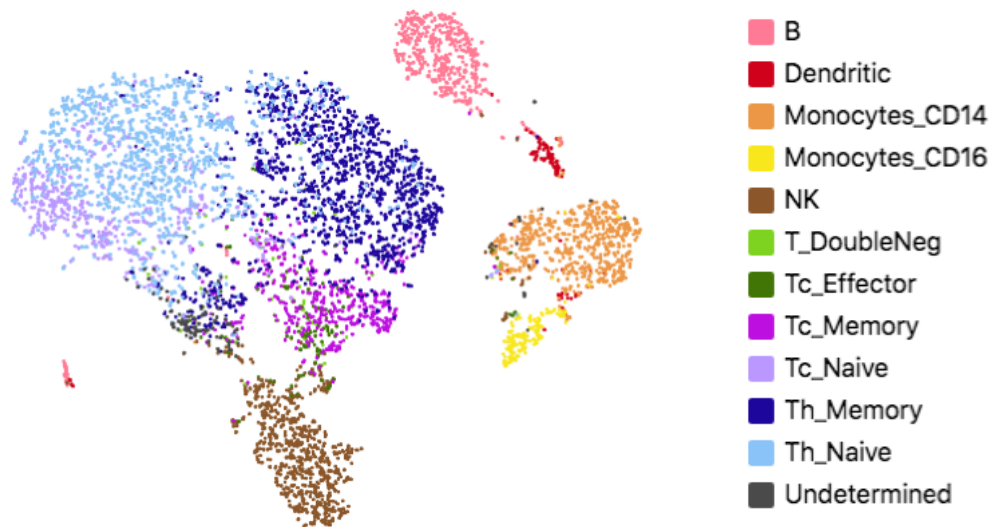
	V	D	J	C	#
α	TRAV27		TRAJ36	TRAC	3
β	TRBV12-3	TRBD1	TRBJ2-3	TRBC2	
α	TRAV21		TRAJ17	TRAC	2
β	TRBV6-3	TRBD1	TRBJ2-1	TRBC2	
α	TRAV12-3		TRAJ24	TRAC	2
β	TRBV15	TRBD1	TRBJ2-2	TRBC2	
α	TRAV17		TRAJ29	TRAC	2
β	TRBV14		TRBJ2-7	TRBC2	
α	TRAV8-2		TRAJ23	TRAC	2
β	TRBV12-3	TRBD1	TRBJ1-3	TRBC1	
α	TRAV8-1		TRAJ27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

Filter

Cluster

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

```
TGGGGACCCAAAAACACACCCCTCCTTGGGAGAATCCCCTAGATTACAGCTC
CTCACCATGGACTGGACCTGGAGCATCCTTTTCTGGTGGCAGCAGCAACAGG
TGCCCACTCCAGGTTACAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAAGCCT
GGGGCCTCAGTGAAGGTTCTCCTGCAAGGCTTCTGTTACACCTTTACCAGCTC
CATCTCTGGTATTACCTGGGTGCGACAGGCCCTGGACAAGGGCTTGAGTGGG
TGGGATGGGTGAGCGGTGACAATGGTGACACAAAGTATGCACAGAAGTCCAG
GGCAGAGTCACCATGACCAACAGACACATCCACGAGTACAGGCTACATGGAGCT
GAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTTTCGAGAGACGTCA
GTCCCCACTGGGGCCAGGGAACCTGGTACCCGTCTCCTCAGCACCCACCAA
GGCTCCGGATGTGTTCCCATCATATCAGGGTGCAGACACCCAAAGGATAACA
GCCTGTGGTCTGGCATGCTTGATAACTGGGTACCAACCCAAAGTCCGTGACT
GTCACCTGGTACATGGGGACACAGAGCCAGCCCCAGAGAACCTTCCCTGAGAT
ACAAAGACGGGACAGCTACTACATGACAAGCAGCCAGCTTCCACCCCTCC
AGCAGTGGCCCAAGGCGAGTACAAATGCGTGGTCCAGCA
```

>clonotype1_consensus_1_K

```
GCCCTCCAGGCCACGACAGCTGTTTGGATTTTATAAACGGGGCGTTTGCAT
TGTGAACTGAGCTACAACAGGCAGGCAGGGGCAGCAAGATGGTGTTCAGAC
CCAGGTCTTCATTCTCTGTGCTCTGGATCTCTGGTCCCTACGGGACATCGT
GATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGGCAGAGGGCCACC
ATCAACTGCAAGTCCAGCCAGAGTGTATACAGCTCCAACAATAAGGAGTAC
TTAGCTTGGTACCAGCAGAAACCAGGACAGTCCCTAAGCTGCTCATTACTGG
GCATCCACCCGGGAATCCGGGGTCCCTGACCGATTAGTGGCAGCGGGTCTG
GGACAGATTTCACTCTCACCATCAGCGGCTGCAGGCTGAAGATGTGGCAGTT
TATTACTGTCAAGCAATATTCTAGTACTCCGTACACTTTTGGCCAGGGGACCAAG
CTGGAGATCAAACGAAGTGTGGCTGCACCATCTGTCTTATCTTCCCGCCATCT
GATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGCTGCTGAATAACTTC
TATCCAGAGAGGGCAAAGTACAGTGGAAAGTGGATAACGCCCTCCAATCGGG
TAACTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGC
CTCAGCAGCACCTGACGCTGAGCAAGCAGACTACGAGAA
```



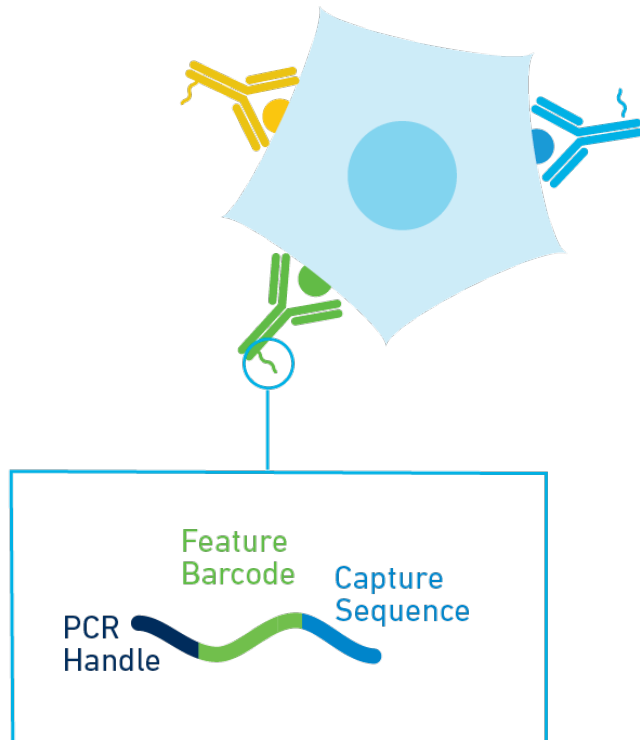
	V	D	J	C	#
H	IGHV1-18	IGHD2-2	IGHJ4	IGHD	
κ	IGKV1-9		IGKJ1	IGKC	4
κ	IGKV4-1		IGKJ2	IGKC	
H	IGHV3-72	IGHD3-9	IGHJ4	IGHM	2
κ	IGKV1-16		IGKJ4	IGKC	
H	IGHV3-43	IGHD6-13	IGHJ4	IGHM	2
λ	IGLV2-14		IGLJ2	IGLC2	
H	IGHV4-39	IGHD3-10	IGHJ4	IGHM	
H	IGHV5-51	IGHD2-8	IGHJ6	IGHM	
λ	IGLV1-44		IGLJ3	IGLC2	
λ	IGLV3-19		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 ✕

Single Cell Immune Profiling

Feature Barcoding Technology: Cell Surface Protein

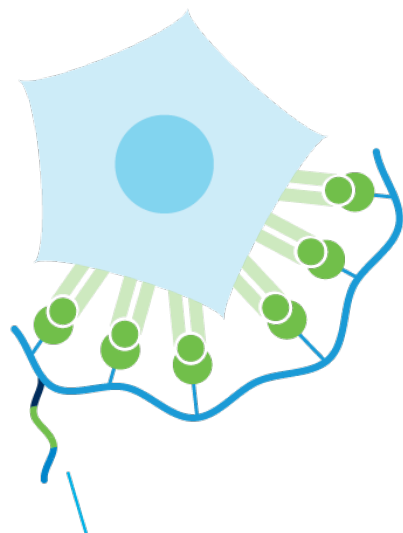
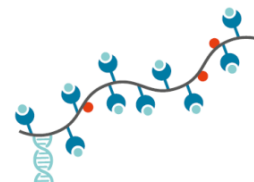


- **TotalSeq™- C Oligo Conjugated Antibodies** specific for the Chromium Single Cell Immune Profiling Solution with Feature Barcoding Technology
- Menu focus on antibodies for immune profiling in human, custom also available
- Enables highly multiplexed, simultaneous profiling of cell surface proteins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

Single Cell Immune Profiling

Feature Barcoding Technology: Antigen Mapping

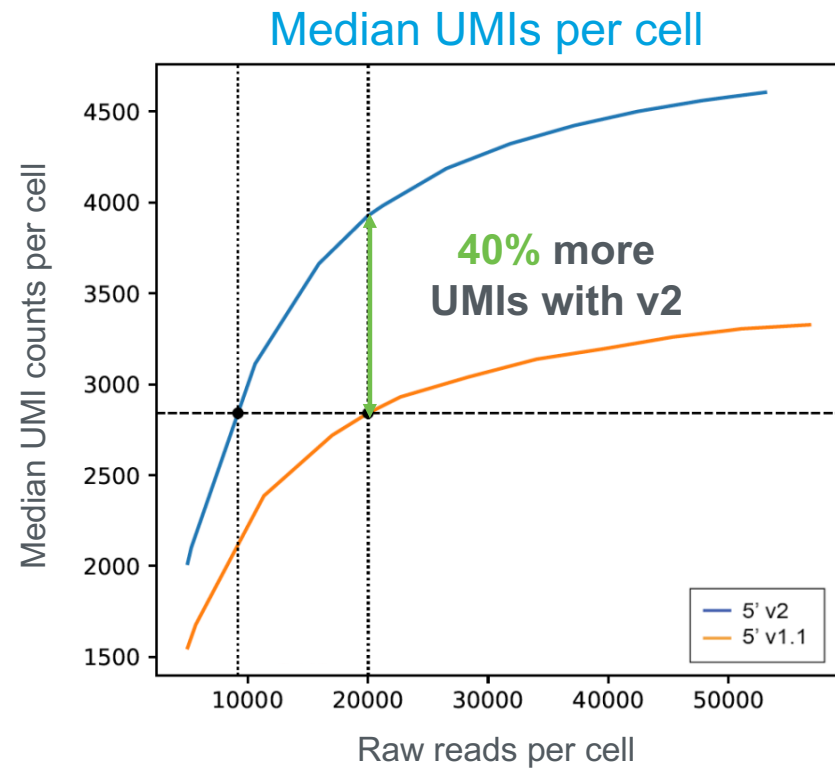
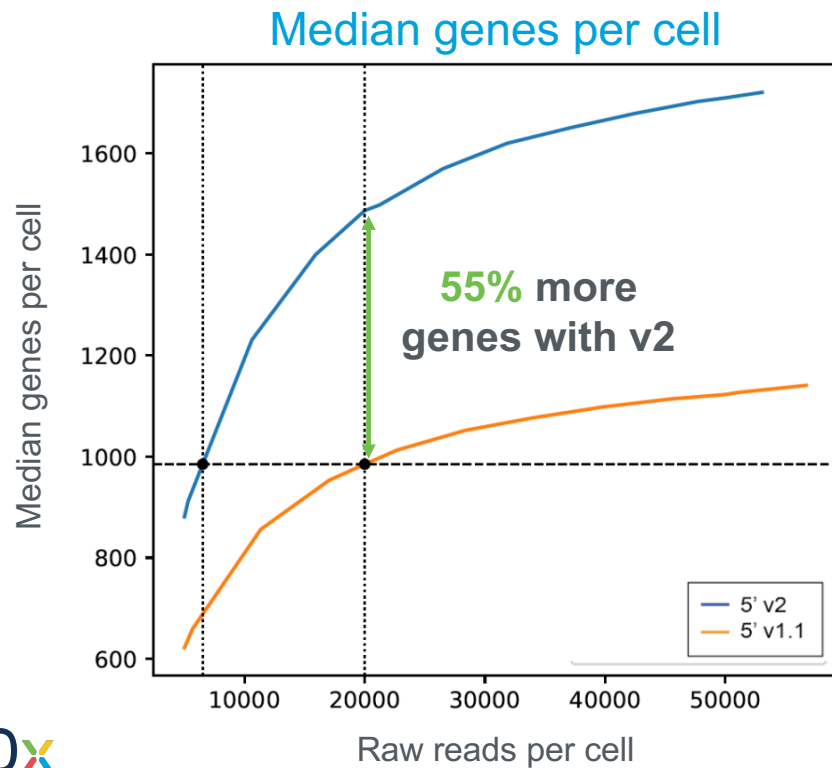
IMMUDEx
PRECISION IMMUNE MONITORING



- **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

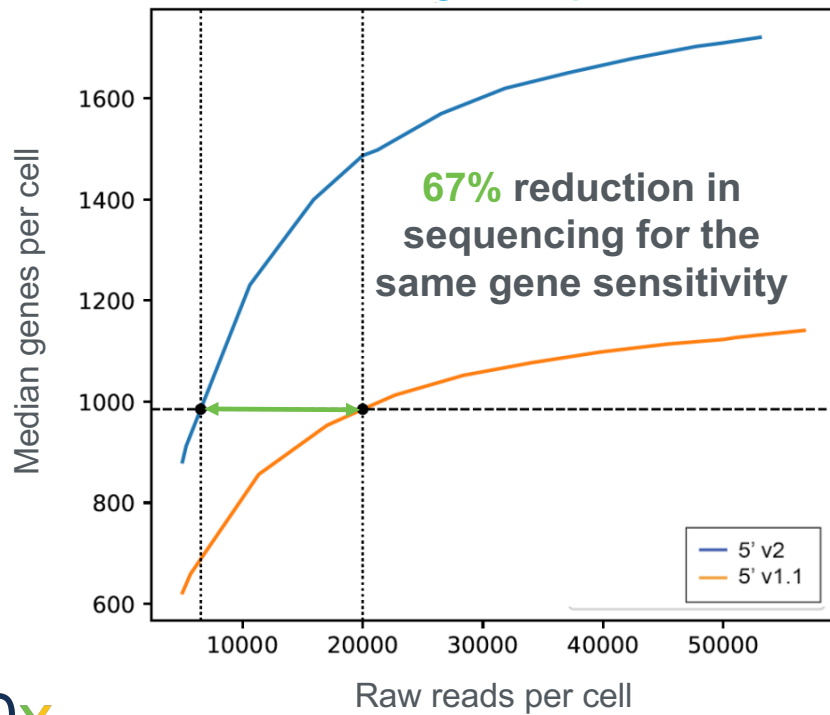
1,000 Human PBMCs



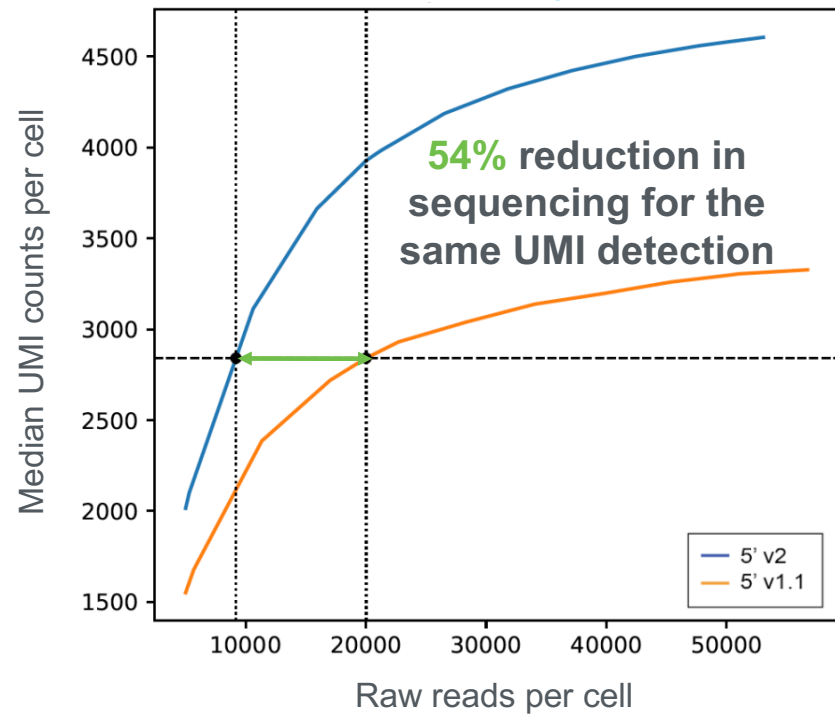
...allows for reduced sequencing depths

1,000 Human PBMCs

Median genes per cell



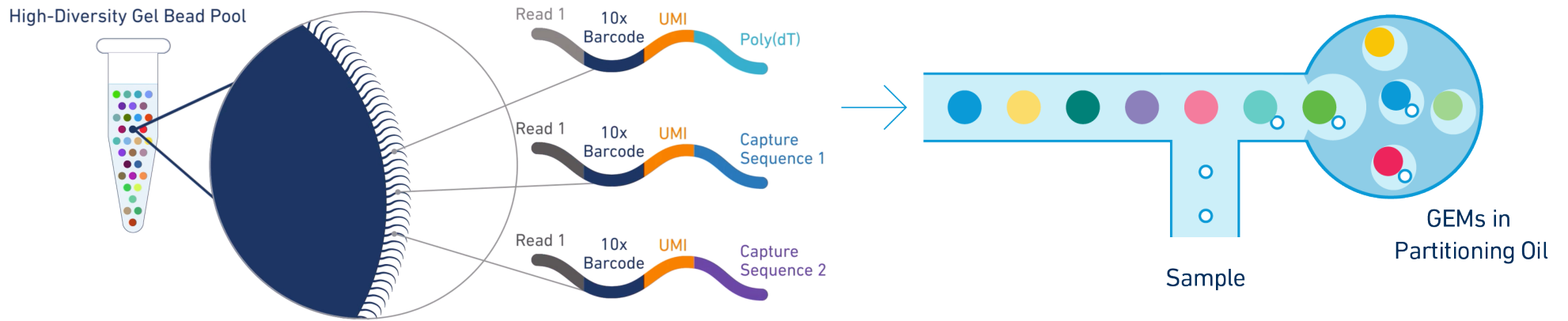
Median UMIs per cell



Single Cell Gene Expression



How it works

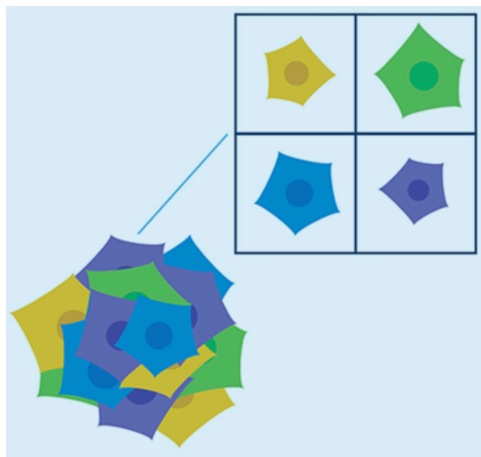


10x Barcode = Cell ID

GEMs = Gel beads in Emulsions

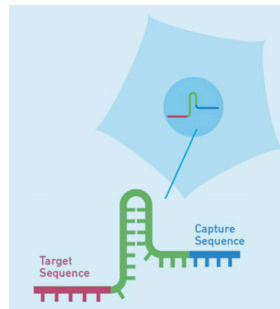
Multimodal measurements from the same cell

Feature Barcoding technology

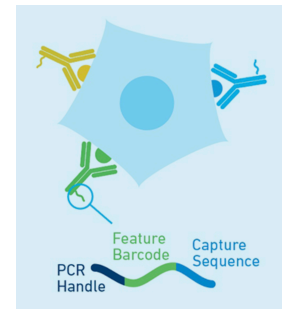


Gene Expression

+



CRISPR Screening

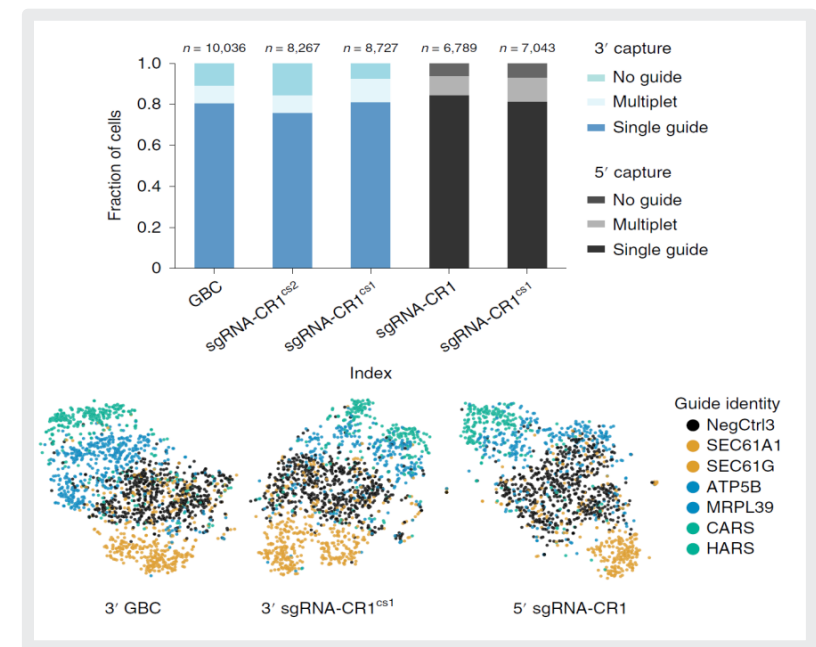


Cell Surface Protein

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

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.



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

Advances in Spatial Genomics

Chromium
Platform

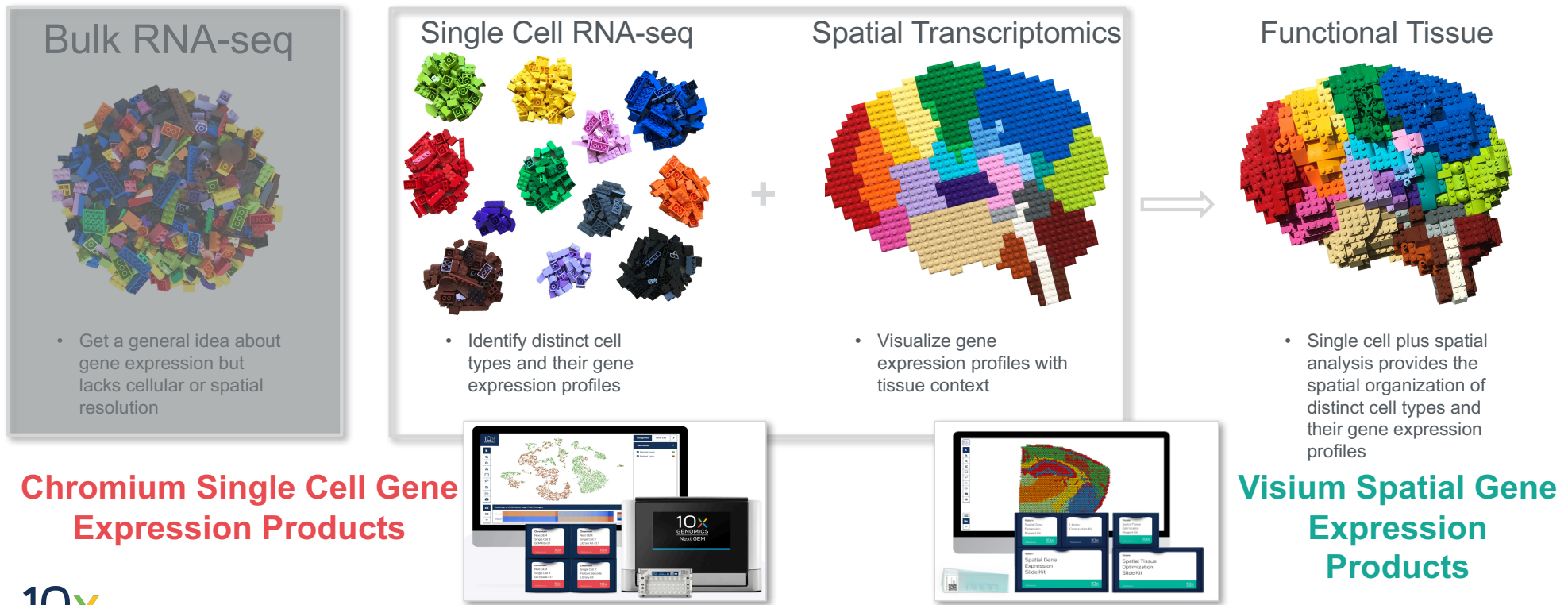


Visium
Platform



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

Complementarity of single cell and spatial methods from 10x Genomics



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

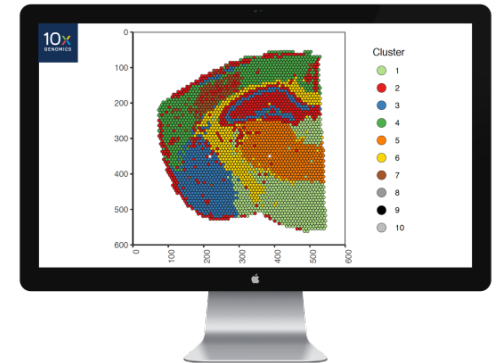
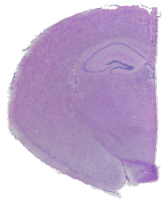
Visium Platform: Rich Spatial Characterization

Sample

Visium
consumables

Next generation
sequencer

Analysis and
visualization software



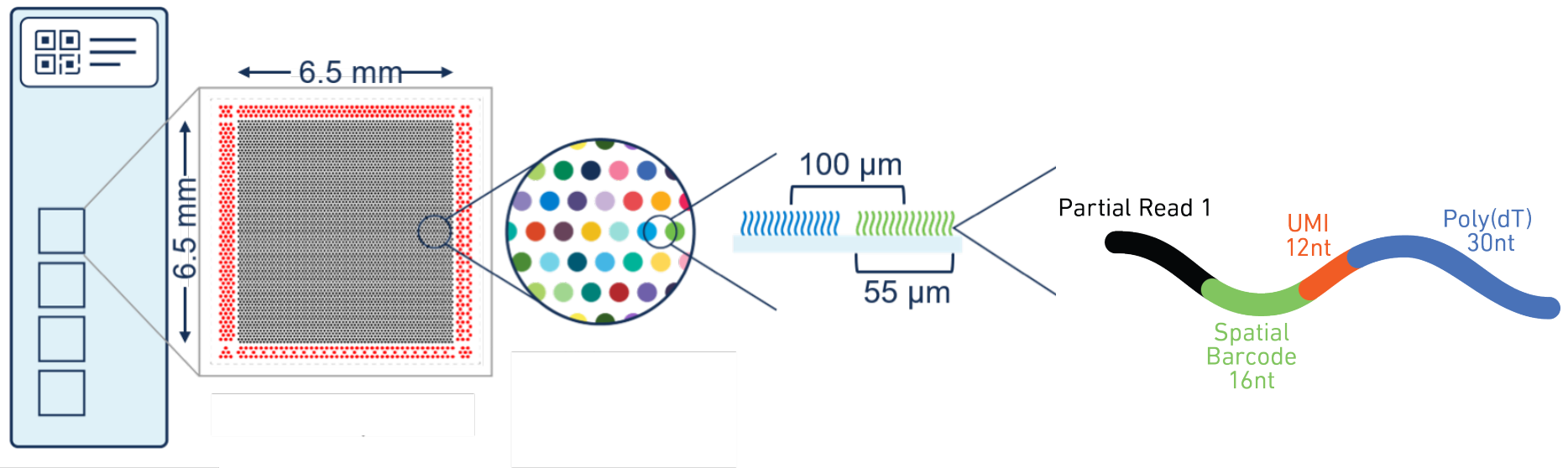
Unbiased gene expression at high spatial resolution

Utilizing poly-A capture and unique spatial barcodes

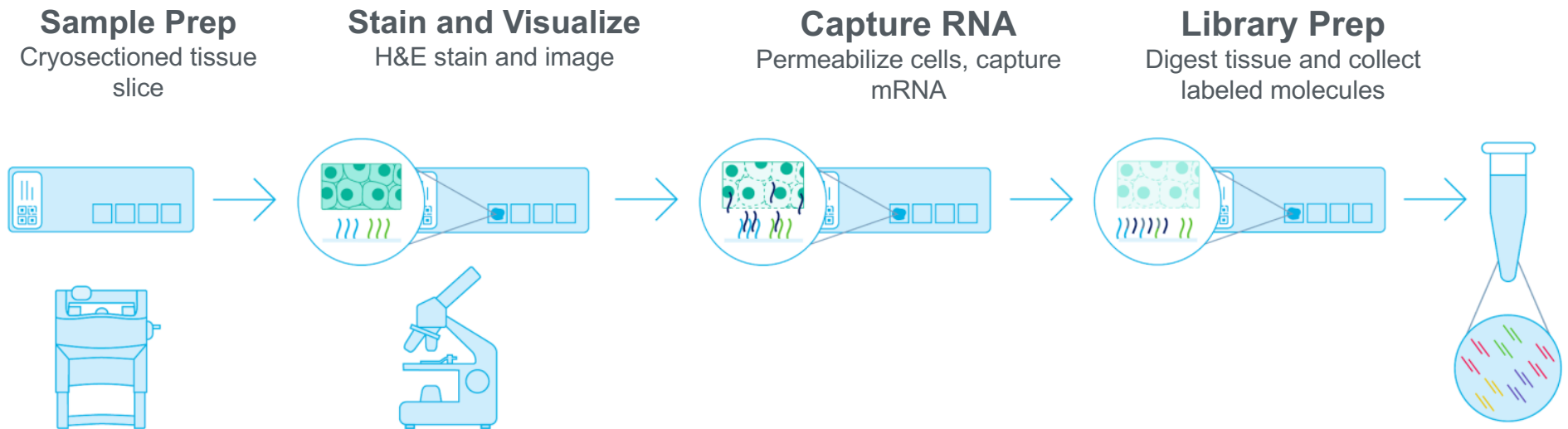
Visium Spatial Gene Expression Slide

Capture Area with ~5000 Barcoded Spots

Visium Gene Expression Barcoded Spots



Visium Sample & Library Prep Workflow

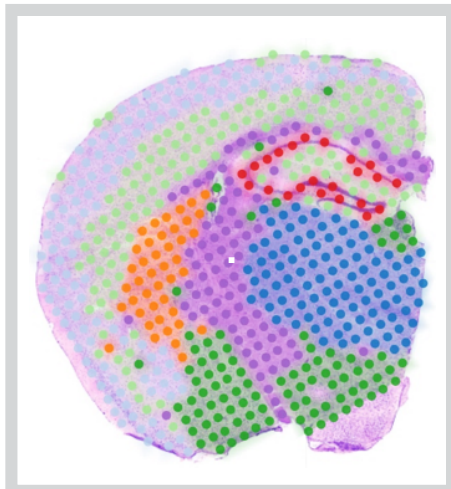


< 1 Day (many available stopping points)

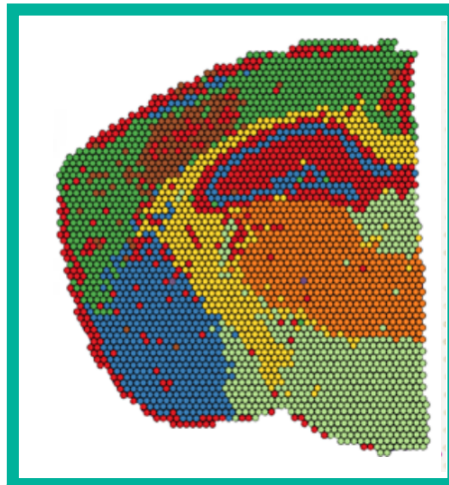
From sectioned tissue to sequence-ready libraries

Visium Improvements

Increased resolution

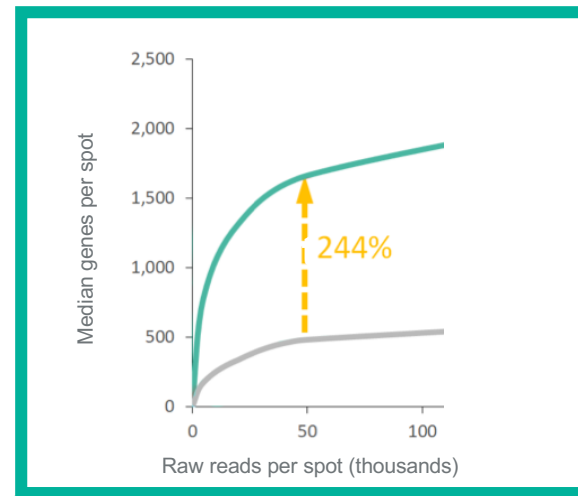


Spatial Transcriptomics



Visium

Increased sensitivity



Spatial Transcriptomics

Visium

Shortened workflow

Spatial Transcriptomics

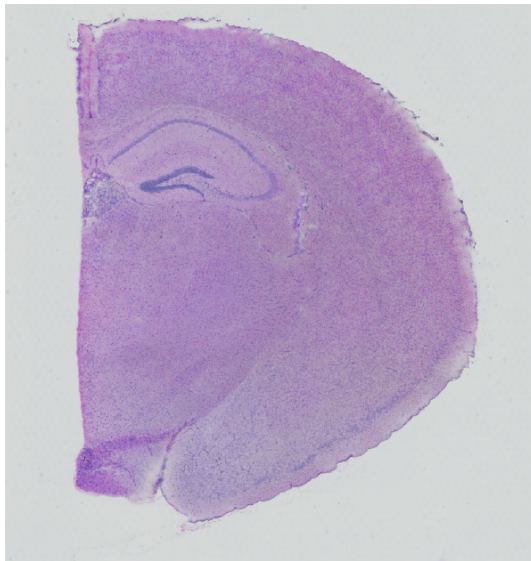
3 DAYS

Visium

1 DAY

Visium Spatial Gene Expression

Mouse Brain



H&E Tissue Section

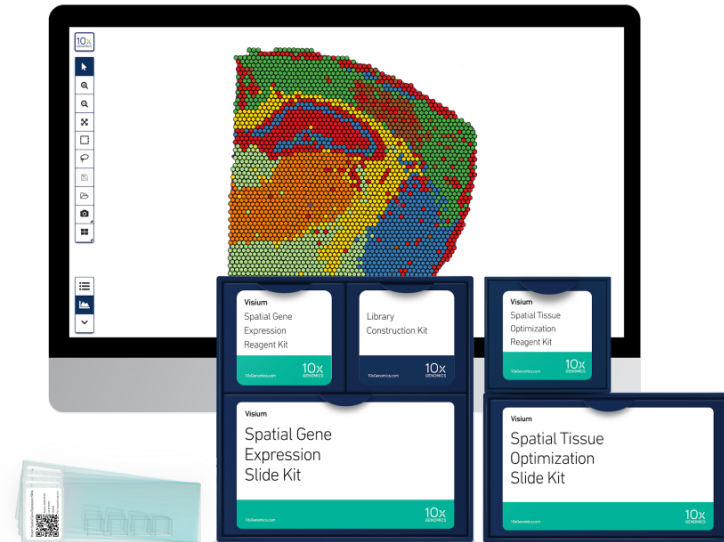


Image or Gene Expression Driven Analysis of Spatial Data

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

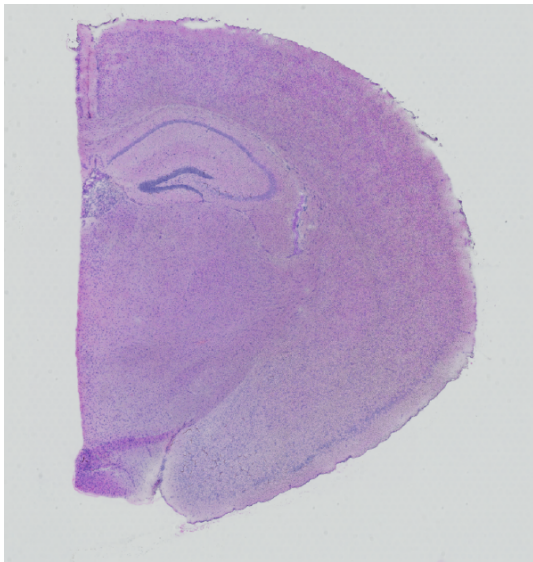
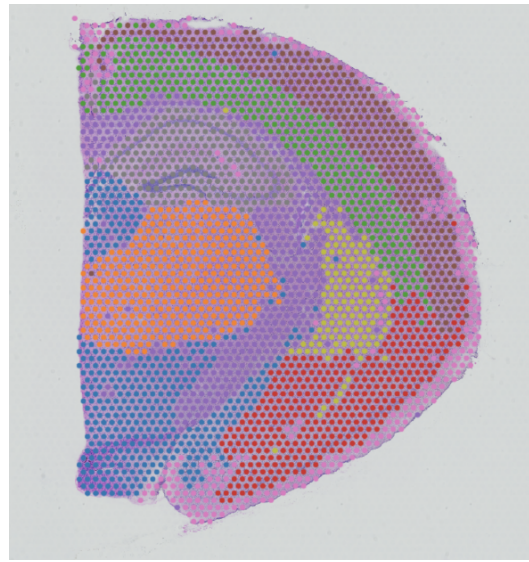
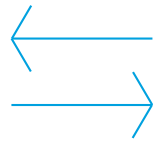
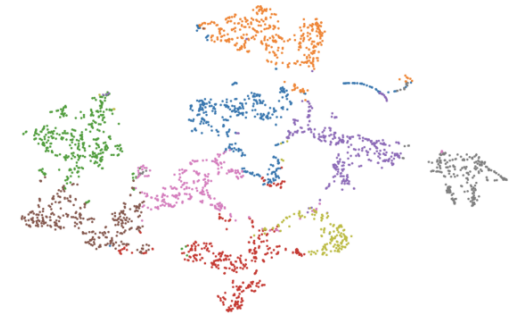
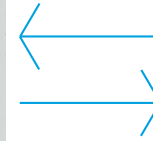


Image Data



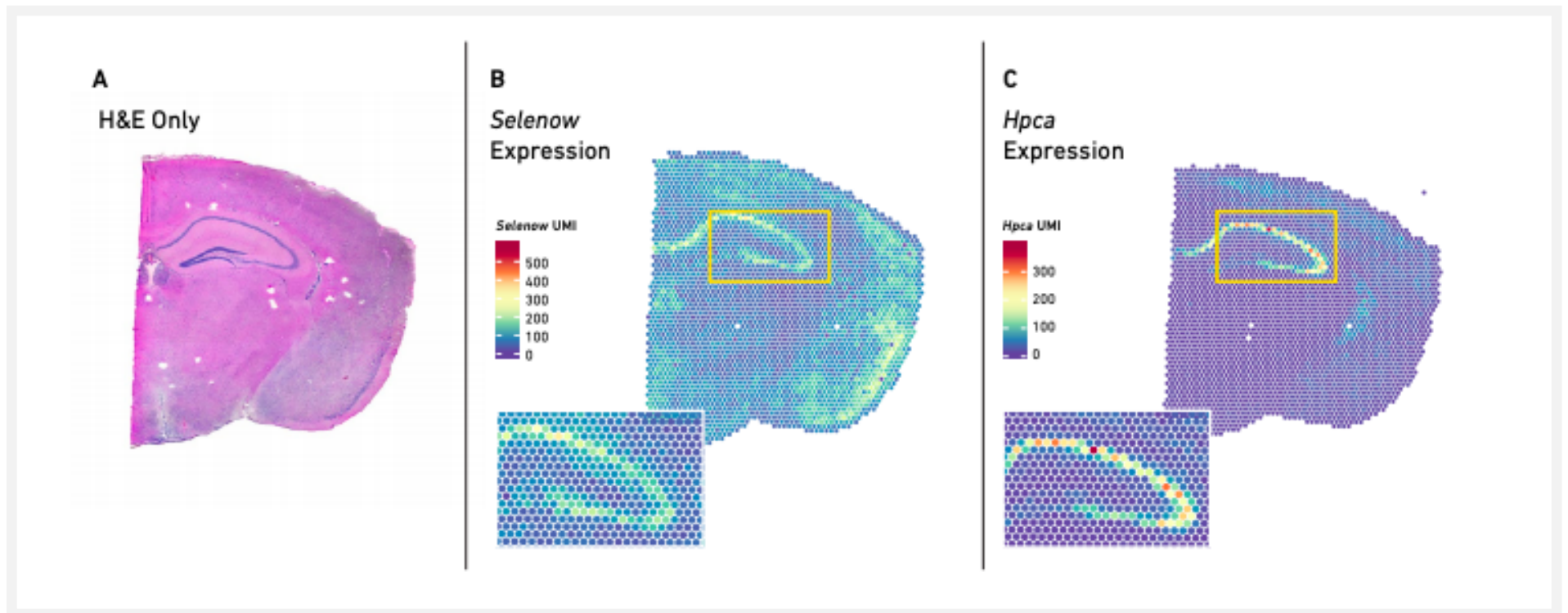
Gene Expression Clustering



Gene Expression Data (t-SNE)

Spatially Resolved Expression in the Mouse Brain

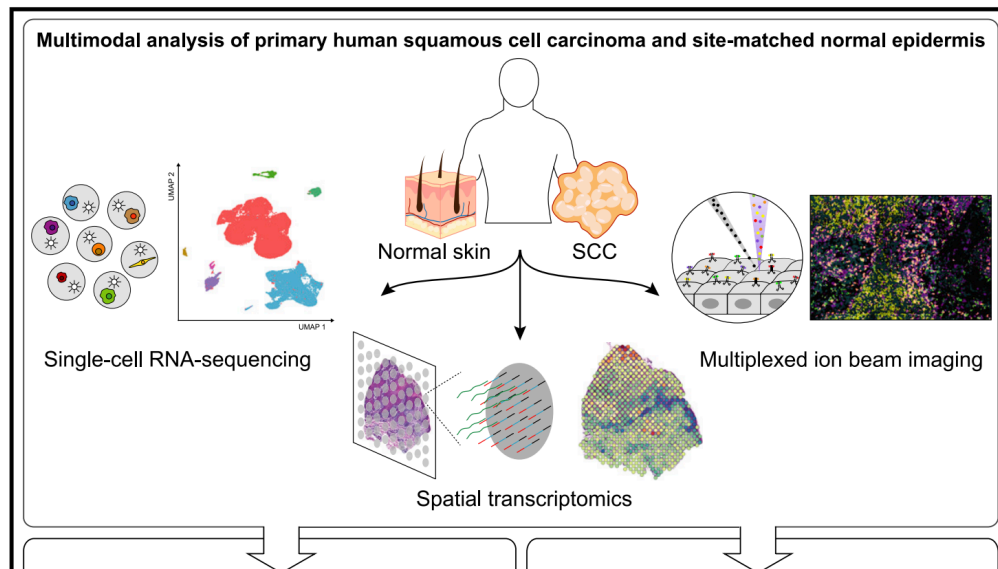
Hippocampal Gene Expression Coincides with Known Gene Expression Patterns



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



Graphical Abstract



Authors

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

Correspondence

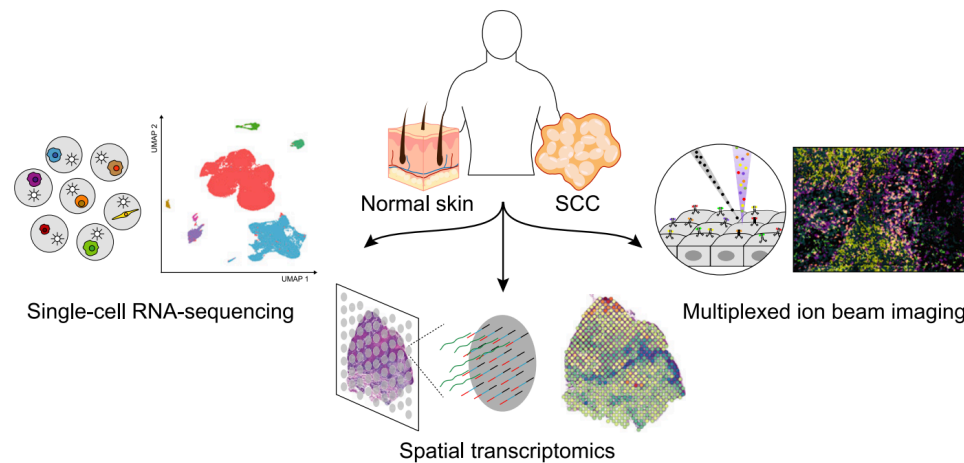
khavari@stanford.edu

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

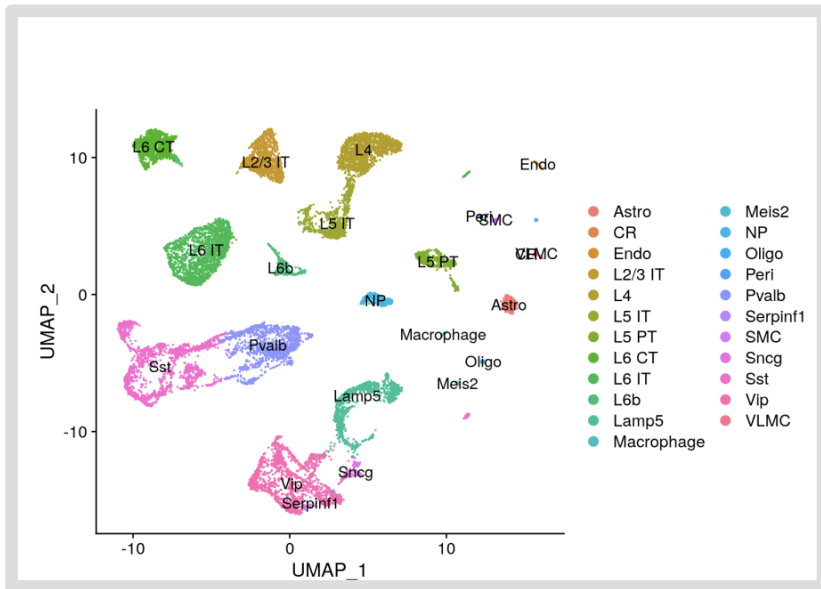
Ji *et al.*, 2020, Cell



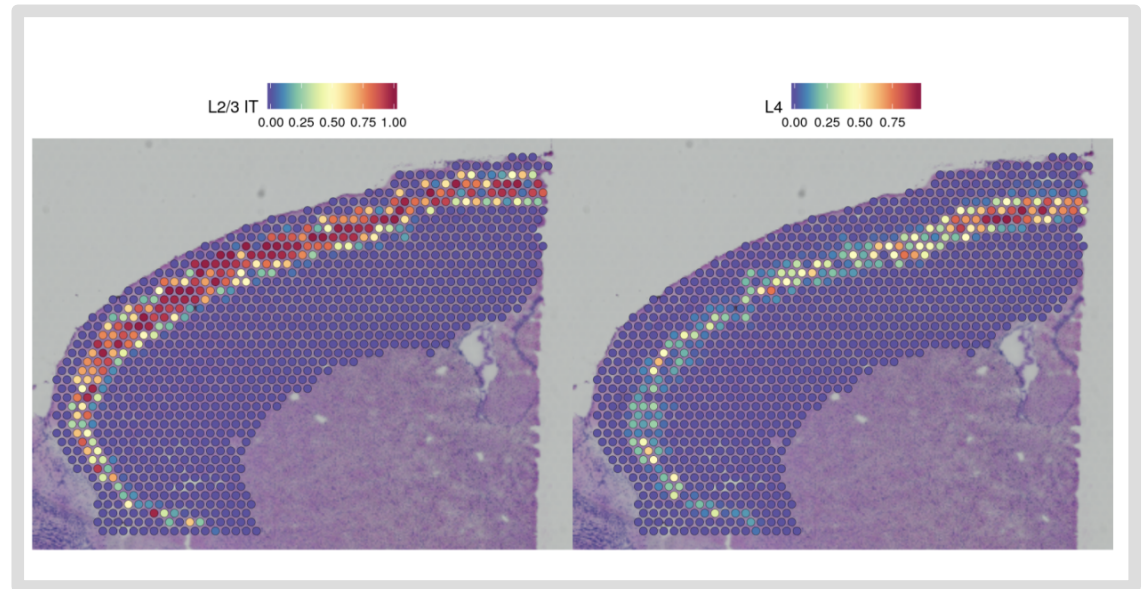
- Profiling of 10 human skin SCCs and matched normal skin via scRNA-seq, ST, and MIBI
- Tumor-specific keratinocytes (TSKs) reside within a fibrovascular niche at leading edges of tumor tissue
- Distinct ligand-receptor and spatial niche associations for tumor and stromal cells
- Subpopulation essential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

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Analysis, visualization, and integration of spatial datasets with Seurat

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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 spatially-variable features
- Interactive visualization
- Integration with single-cell RNA-seq
- Working with multiple slices

For our first vignette, we analyze [10x Genomics technology](#) from 10x Genomics additional data types in the new [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)
```

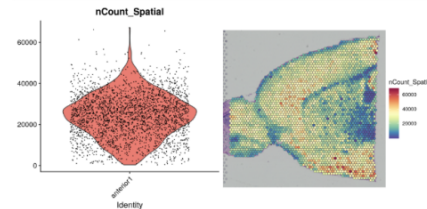
Dataset

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 `getBrain` 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)
```

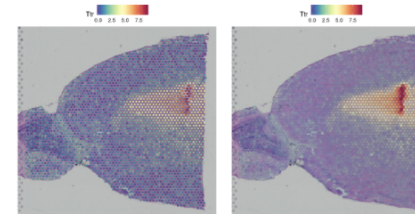


These plots demonstrate that the variance in molecular counts across spots is not just technical in nature, but also is dependent on the tissue anatomy. For example, regions of the tissue that are depleted for neurons (such as the cortical white matter), reproducibly exhibit lower molecular counts. As 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 [this tutorial](#).

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)
```

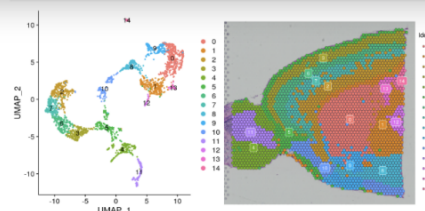


Dimensionality reduction, clustering, and visualization

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 =
```

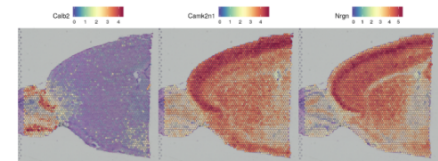
plot_grid(p1, p2)



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.param` 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)
```

```
de_markers <- FindMarkers(brain, id.ent.1 = 4, id.ent.2
  = 6)
SpatialFeaturePlot(object = brain, features =
  rownames(de_markers)[1:3], alpha = c(0.1, 1), ncol =
  3)
```

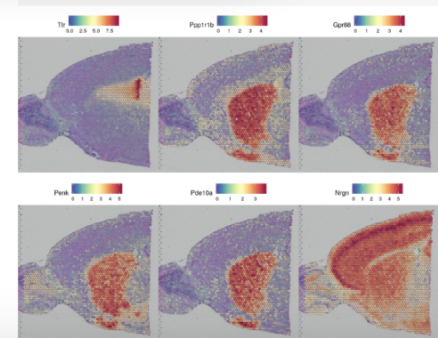


An alternative approach, implemented in `FindSpatiallyVariables`, is to search for features exhibiting spatial patterning in the absence of pre-annotation. The default method (method = "markvariogram"), is inspired by the [Trendsseek](#), which models spatial transcriptomics data as a mark point process 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 [Splootch](#). We encourage interested users to explore these methods, and hope to add support for them in the near future.

```
FindSpatiallyVariables(brain, assay =
  "SCT", n.vars = 1000,
  r = 5)
rownames(FSV)
# features identified by this
```

```
FindSpatiallyVariables(brain,
  selection.method = "markvariogram", 6)
SpatialFeaturePlot(brain, features = top.features,
  ncol = 3, alpha = c(0.1, 1))
```

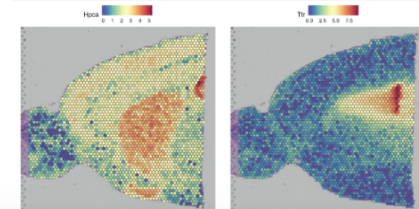


https://satijalab.org/seurat/v3.1/spatial_vignette.html

Gene expression visualization

In Seurat v3.2, we have included new functionality to explore and interact with the inherently visual nature of spatial data. The `SpatialFeaturePlot` function in Seurat extends `FeaturePlot`, and can overlay molecular data on top of tissue histology. For example, in this data set of the mouse brain, the gene `Hpca` is a strong hippocampal marker and `Ttr` is a marker of the choroid plexus.

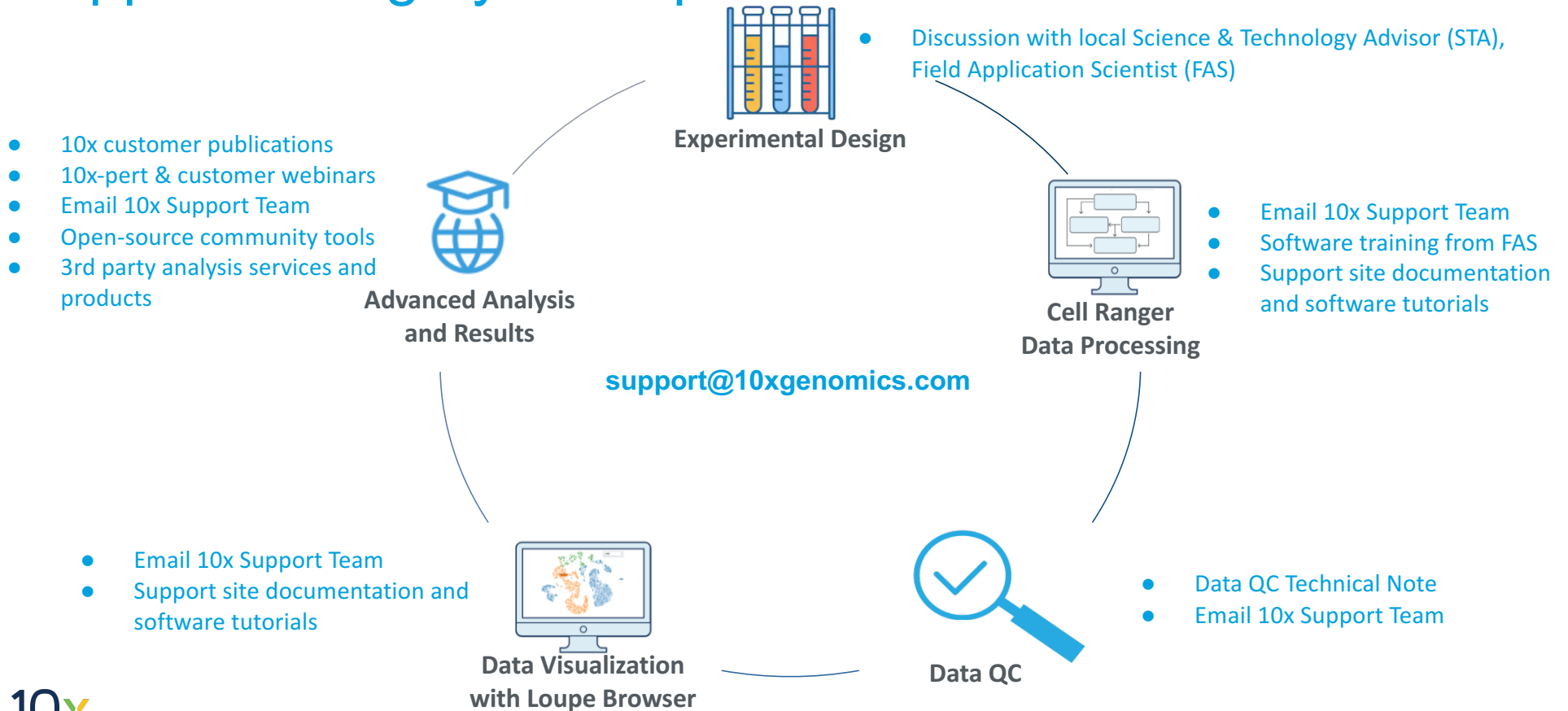
```
SpatialFeaturePlot(brain, features = c("Hpca", "Ttr"))
```



The default parameters in Seurat emphasize the visualization of molecular data. However, you can also adjust the size of the points (and their



Support through your experimental workflow



Thank you!

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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)