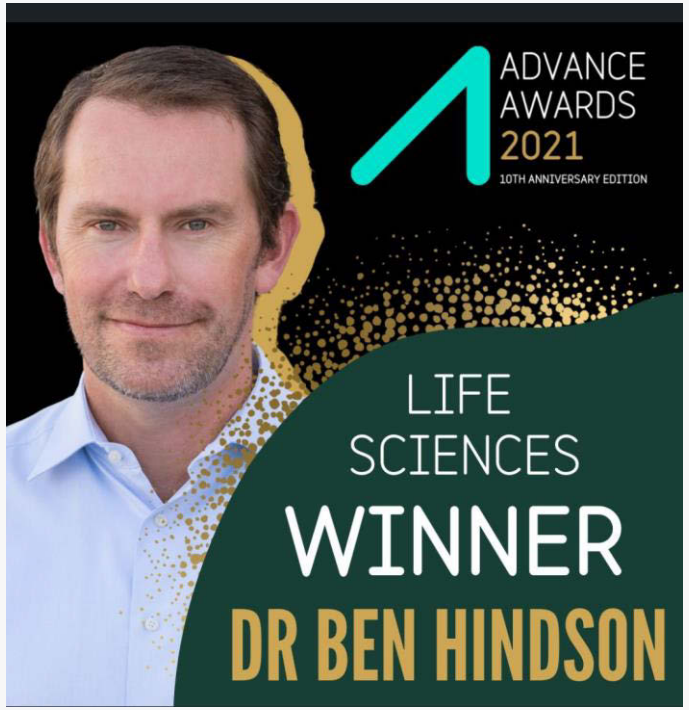
Explore the full richness of biological insights with single cell applications

Agnieszka Ciesielska PhD

Science and Technology Advisor 10x Genomics,
CEE & Israel & Russia, Distributors

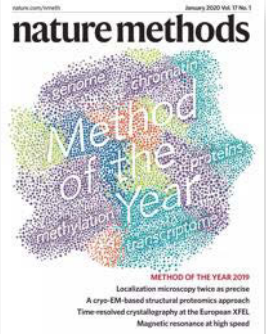
We are 10x Genomics



TheScientist
TOP 10
INNOVATIONS

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

2018
Single Cell Genomics

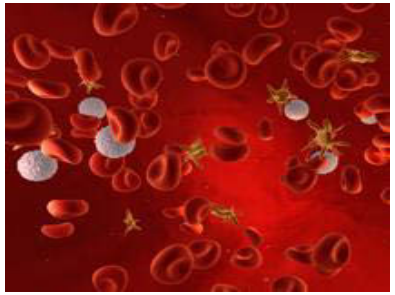


2019
Single Cell
Multimodal Omics

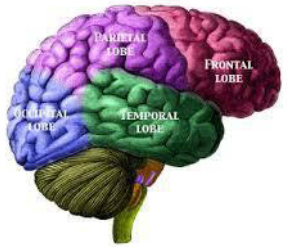
Mastering Biology to Advance Human Health

Tissues are Complex Mixtures of Distinct Cellular Populations

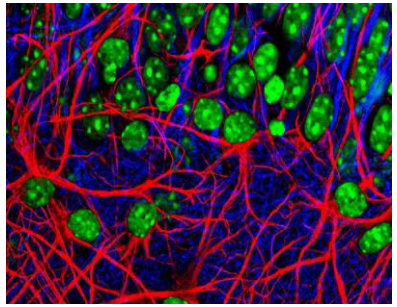
"Blood"



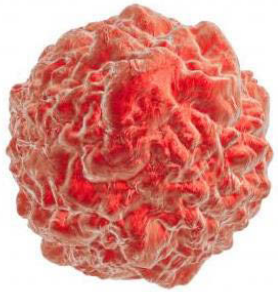
- Platelets and red blood cells
- Immune cells
- Circulating tumor cells



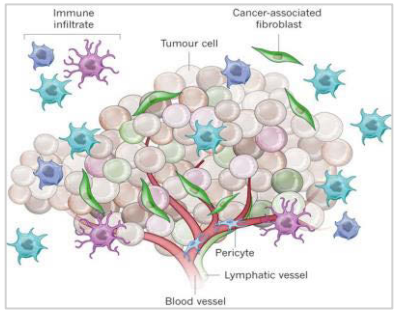
"Brain"



- Excitatory neurons
- Microglia
- Astrocytes

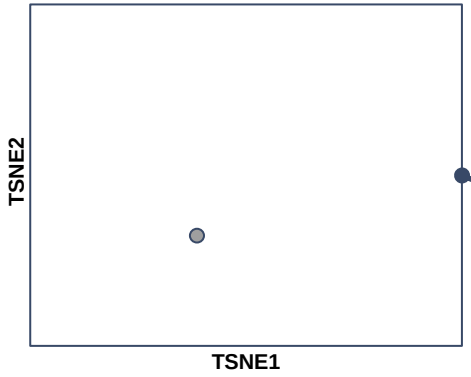


"Tumor Microenvironment"

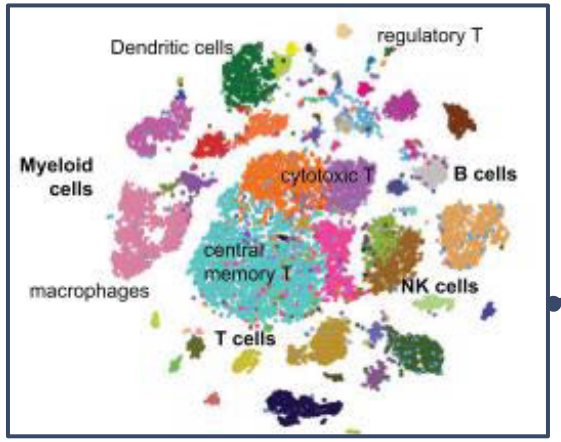


- Tumor infiltrating lymphocytes
- Stromal cells
- Tumor cells

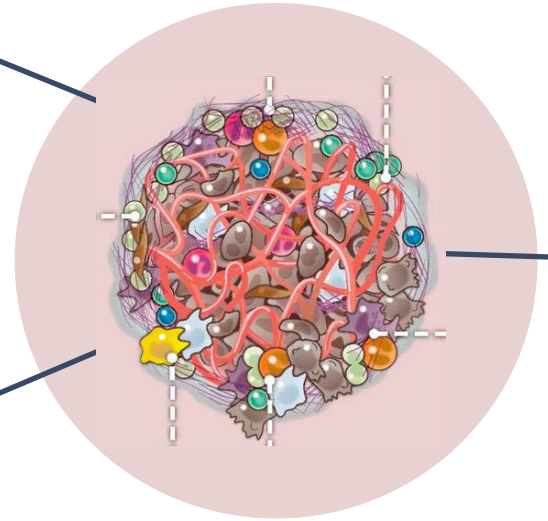
Building your understanding of biological samples – cell by cell



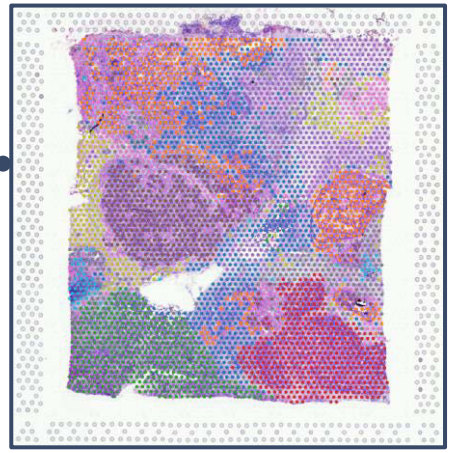
Bulk analysis (qPCR, ddPCR, NGS)



Single Cell Chromium solution

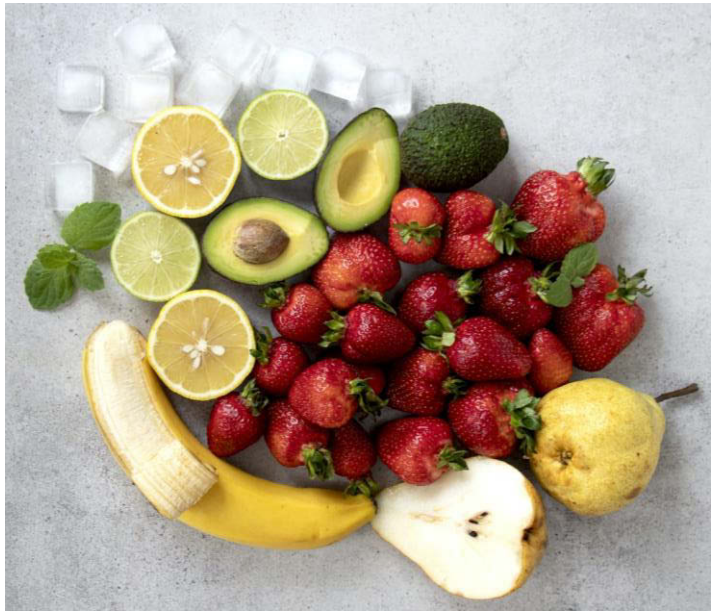


Visium Spatial Transcriptomics



Adapted from Joyce & Fearon, Science, 2015 doi: 10.1126/science.aaa6204

Why Single-cell Analysis?

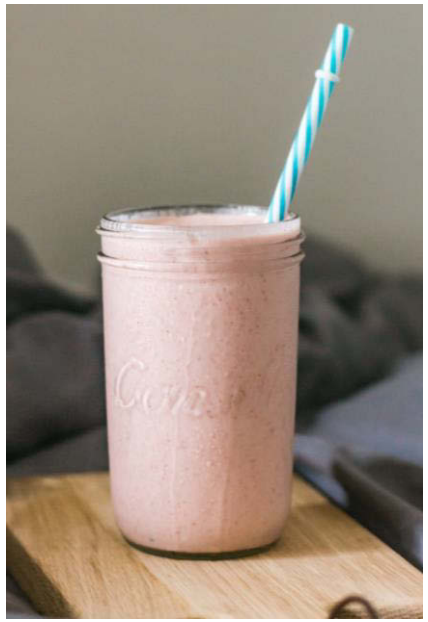


Single component analysis

Photo by Tetiana Bykovets on Unsplash

Your sample

Photo by Blendtopia Smoothies on Unsplash

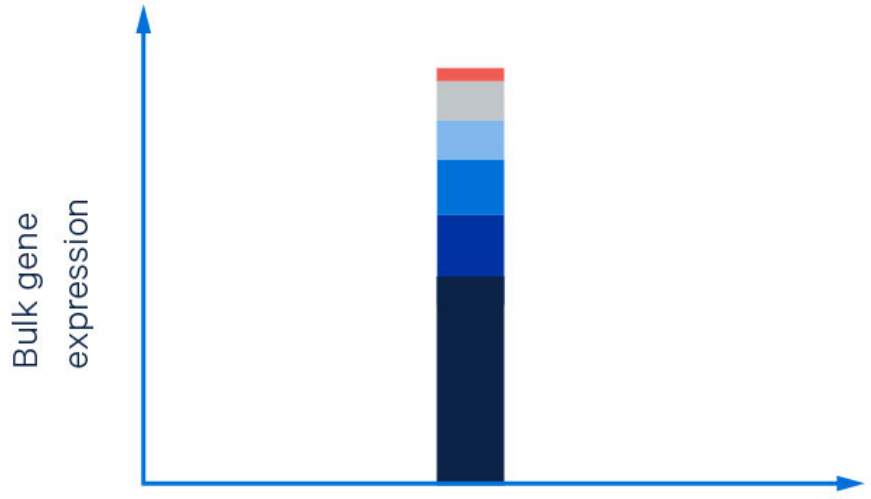


Bulk Analysis

Photo by Sincerely Media on Unsplash

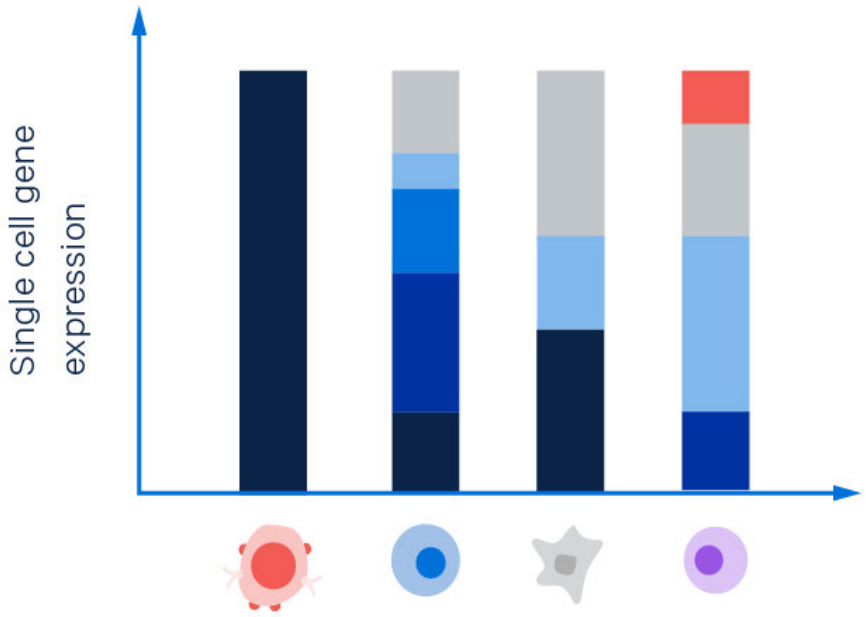
What is single cell sequencing?

Bulk RNA-sequencing



Average gene expression from all cells

Single cell resolution



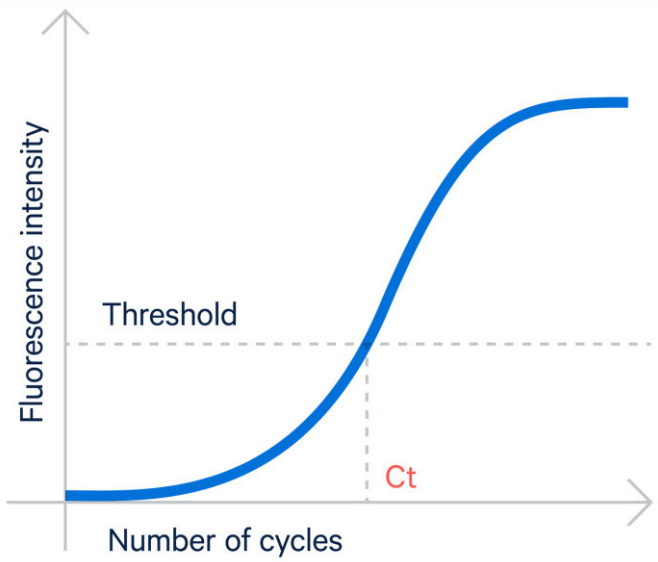
Each cell type has a distinct expression profile

Bulk RNA seq

Single cell sequencing shares steps with other techniques

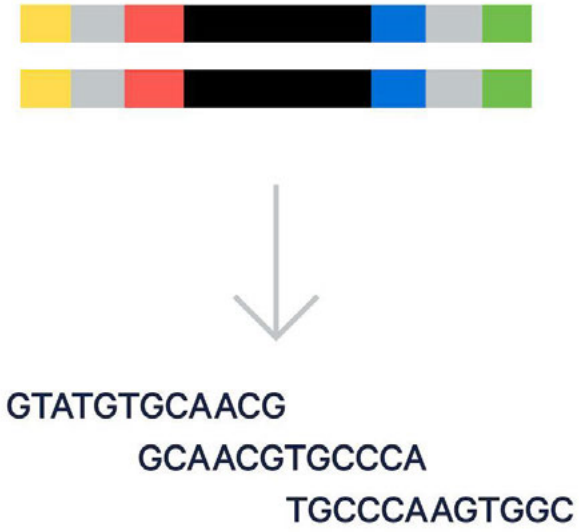
Familiar steps, with deeper insights

qPCR



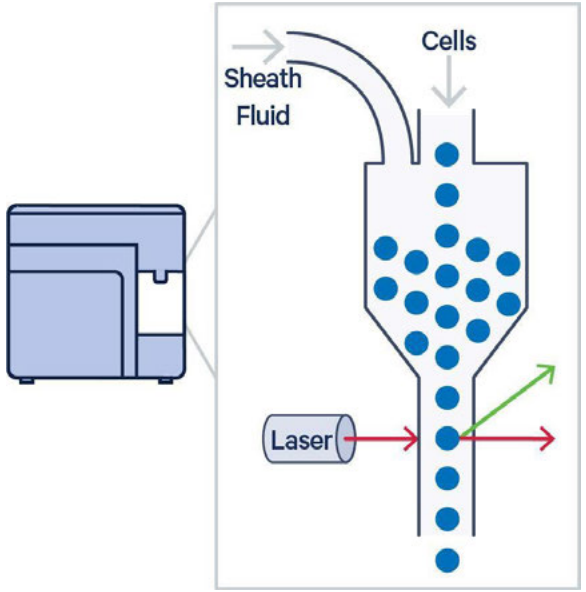
RNA released from lysed cells is converted to cDNA for analysis

Bulk RNA-seq



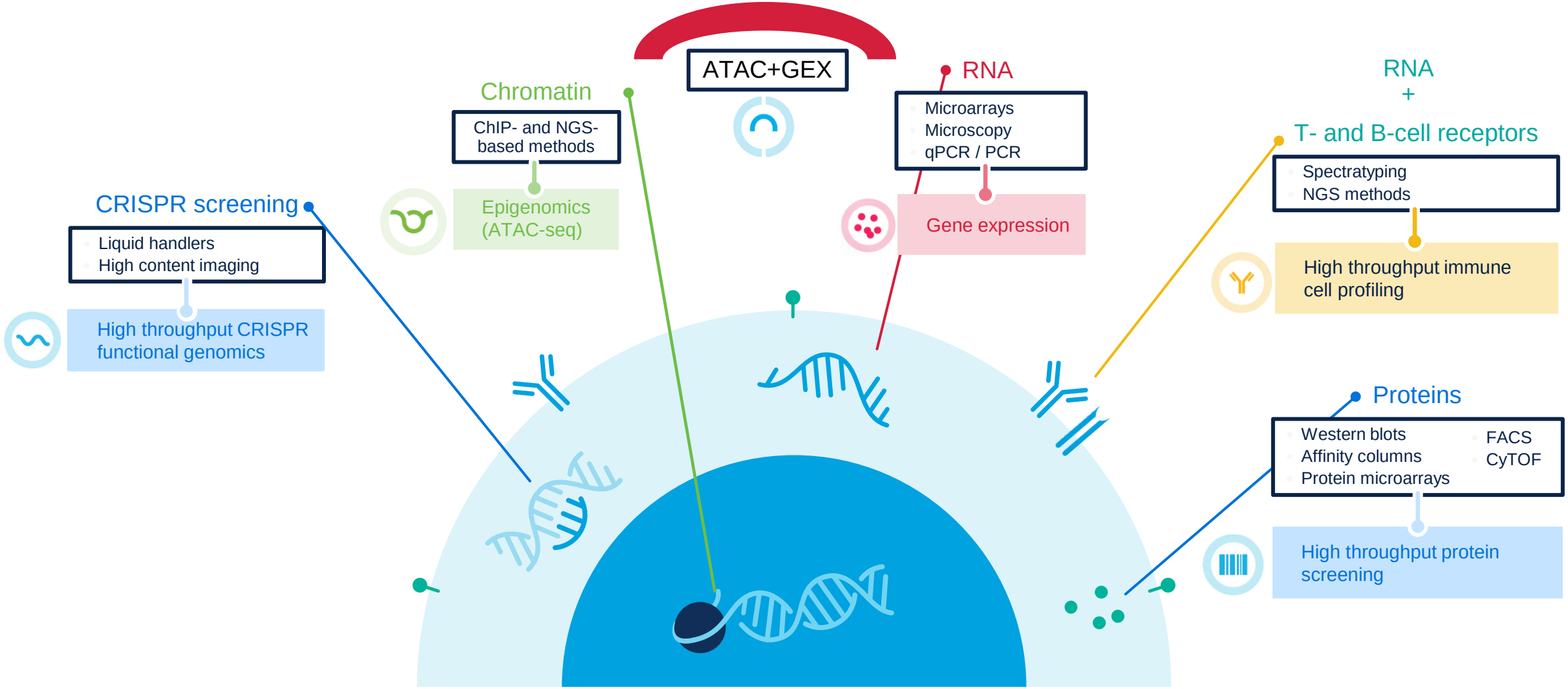
NGS library preparation and sequencing

Flow cytometry



Requires single cell suspensions as input

Next generation molecular profiling solutions



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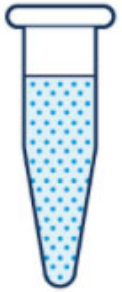
Single Cell RNA Sequencing

How does it work?

Chromium Single Cell Gene Expression Workflow

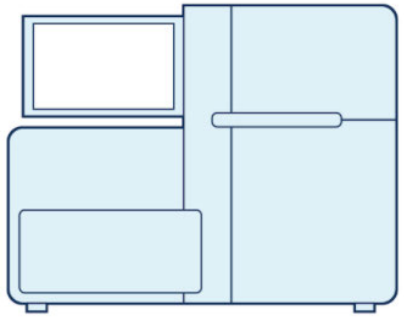
Input → **Library Creation** → **Sequencing** → **Data Analysis and Visualization**



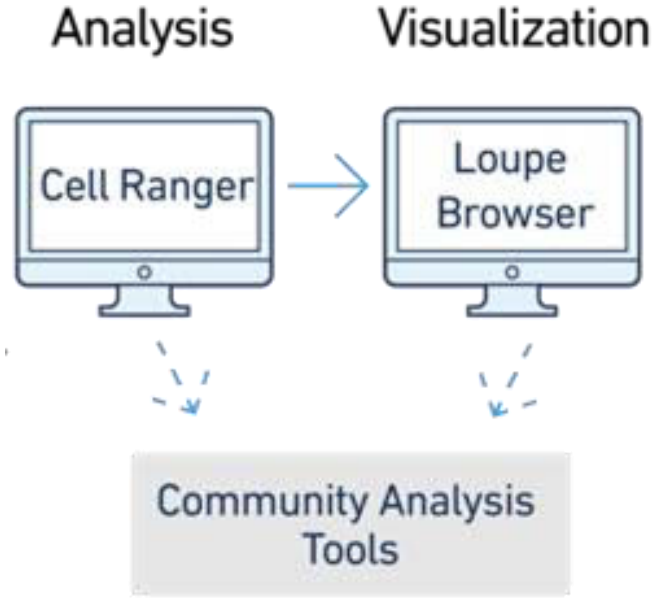
Suspension of dissociated single cell/nuclei

Cell partitioning and molecular barcoding

- **8 channels/chip**
- **500-10 000 cells recovered per channel**
- **40-65% cells recovered**



Sequencing



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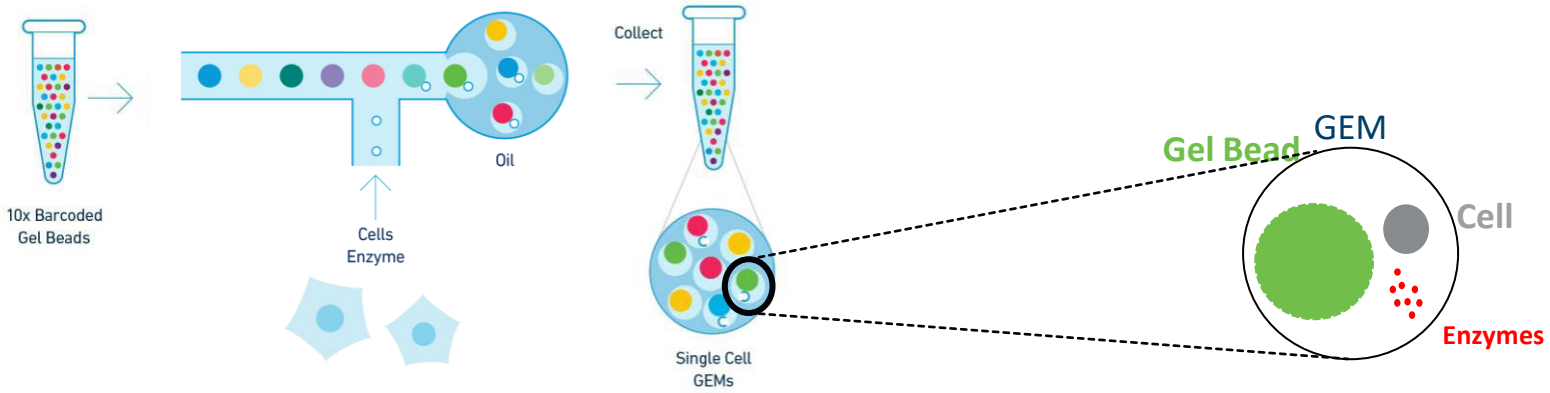
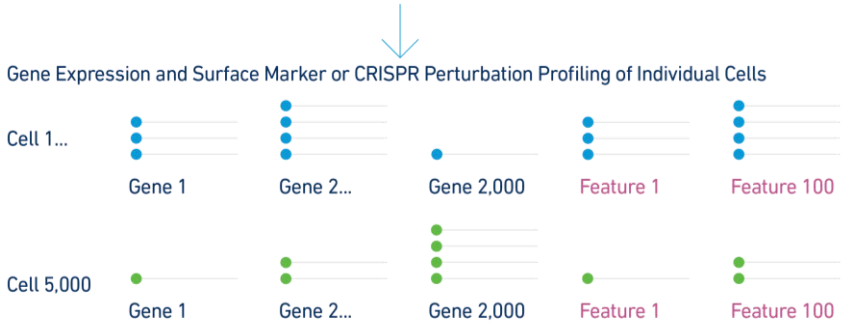
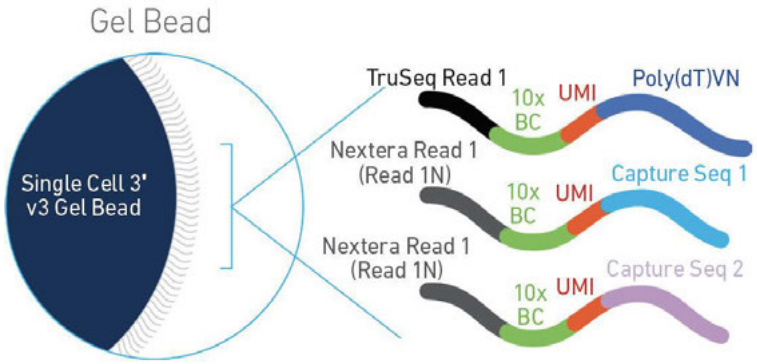
Chromium Single Cell Gene Expression Workflow

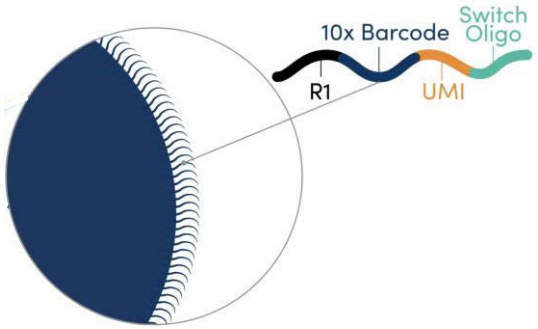
Microfluidic system & Next GEM Technology





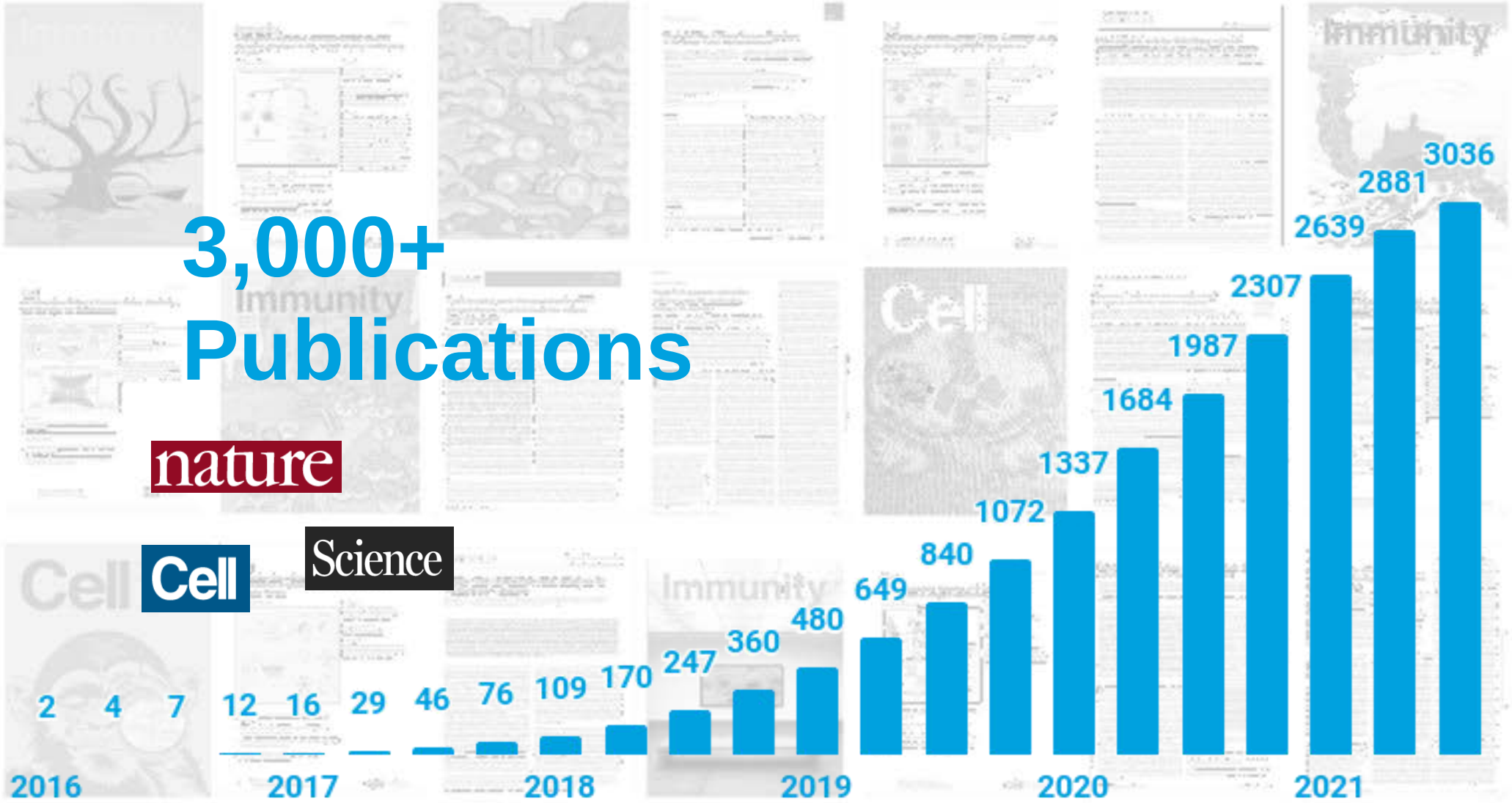


3' single cell gene expression



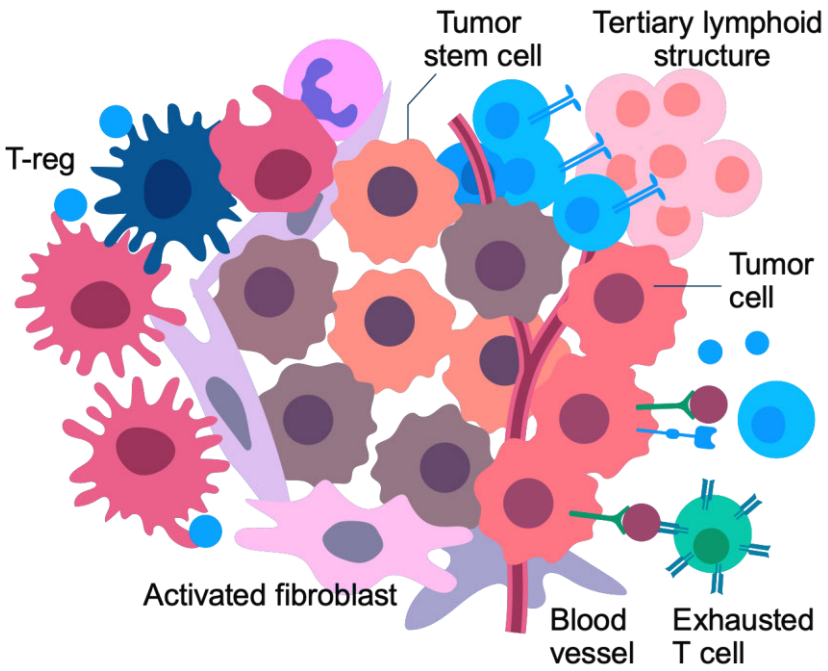
5' immune profiling

Customer publications citing 10x Genomics continue to rise



10x GENOMICS

Understanding and Fighting Cancer



Cell — Article

Single-Cell Analyses Identify Brain Mural Cells Expressing CD19 as Potential Off-Tumor Targets for CAR-T Immunotherapies

Cancer Cell — Article

Single-cell dissection of cellular components and interactions shaping the tumor immune phenotypes in ovarian cancer

nature medicine — BRIEF COMMUNICATION
https://doi.org/10.1038/s41591-021-01564-7

Neurocognitive and hypokinetic movement disorder with features of parkinsonism after BCMA-targeting CAR-T cell therapy

Cancer Cell — Article

Tumor and immune reprogramming during immunotherapy in advanced renal cell carcinoma

CellPress — Cancer Cell

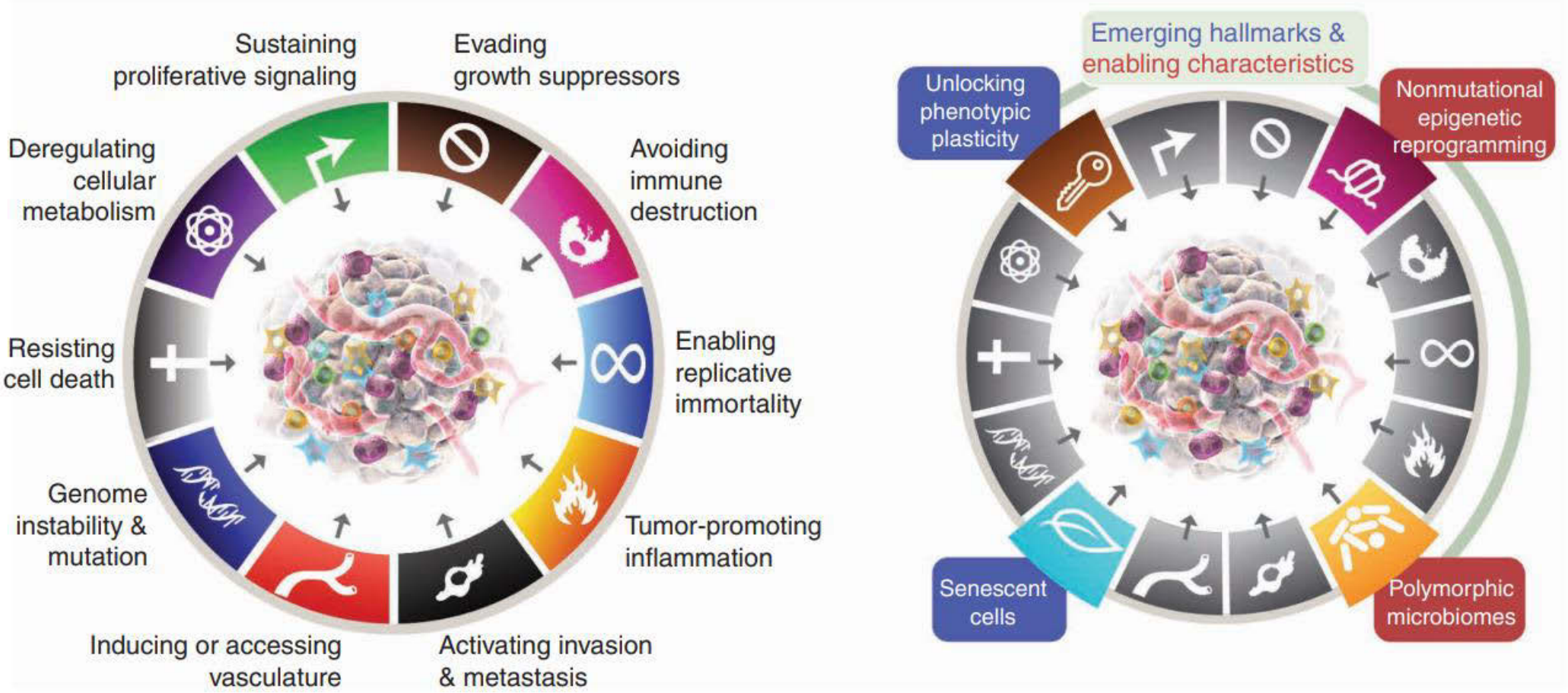
Letter

Expression of chimeric antigen receptor therapy targets detected by single-cell sequencing of normal cells may contribute to off-tumor toxicity

nature medicine — ARTICLES
https://doi.org/10.1038/s41591-021-01323-8

A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer

Hallmarks of Cancer: New Dimensions



DOI: 10.1158/2159-8290.CD-21-1059 Published January 2022

Understanding (Auto) immunity and (Auto)immune Therapies

Systemic sclerosis

TRANSLATIONAL SCIENCE

Single-cell transcriptome analysis identifies skin-specific T-cell responses in systemic sclerosis

Alyxzandria M Gaydosik,[1] Tracy Tabib,[1] Robyn Domsic ,[1] Dinesh Khanna ,[2] Robert Lafyatis,[1] Patrizia Fuschiotti [1]

Article

Interpreting type 1 diabetes risk with genetics and single-cell epigenomics

https://doi.org/10.1038/s41586-021-03552-w
Received: 21 June 2020
Accepted: 14 April 2021
Published online: 19 May 2021

Joshua Chiou, Ryan J. Geusz, Mei-Lin Okino, Jee Yun Han, Michael Miller, Rebecca Melton, Elisha Beebe, Paola Benaglio, Serina Huang, Katha Korgaonkar, Sandra Heller, Alexander Kleger, Sebastian Preissl, David U. Gorkin & Maike Sander & Kyle J. Gaulton

SCIENCE ADVANCES | RESEARCH ARTICLE

DISEASES AND DISORDERS

Gene expression signatures of target tissues in type 1 diabetes, lupus erythematosus, multiple sclerosis, and rheumatoid arthritis

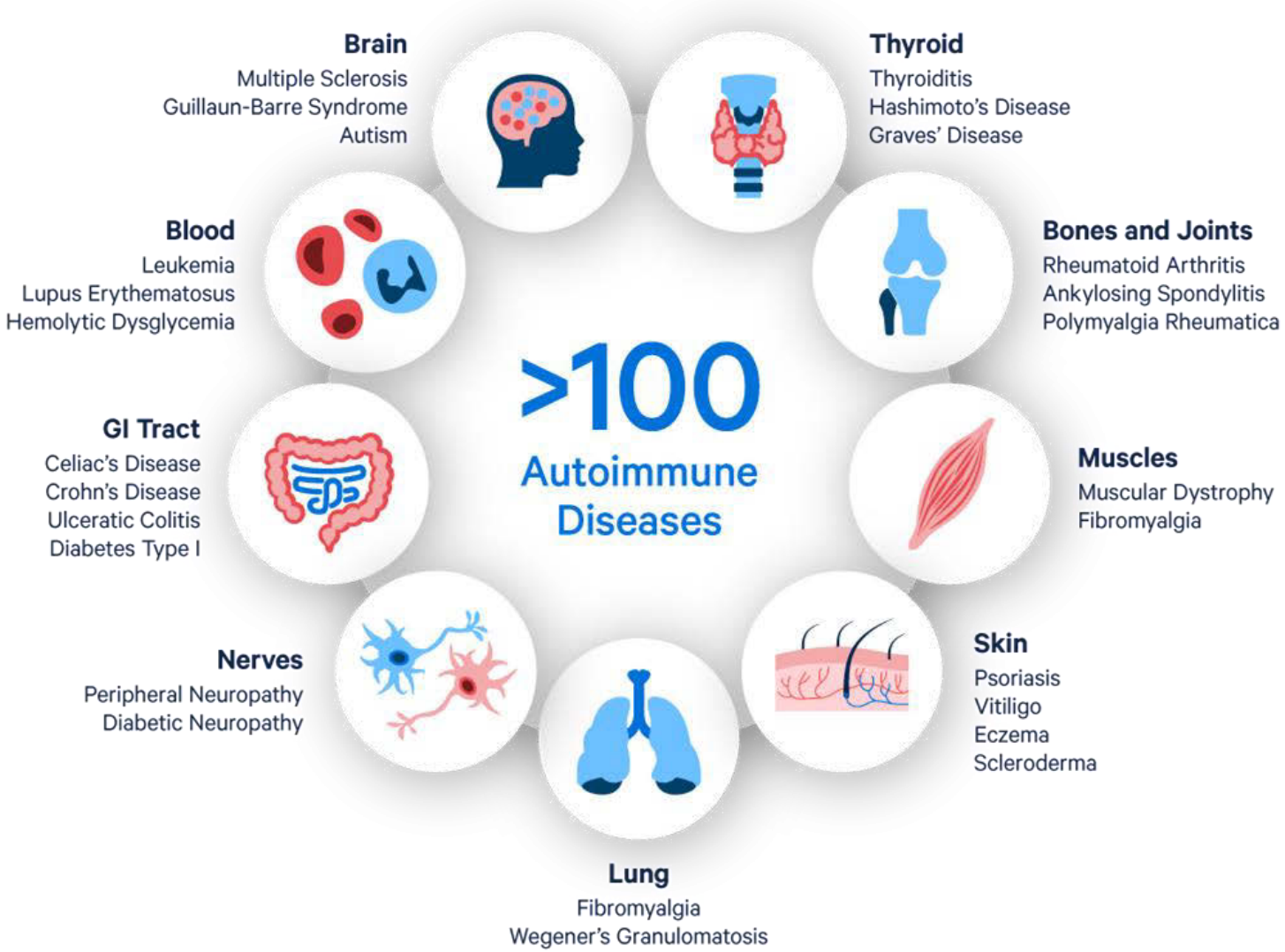
F. Szymczak[1,2*], M. L. Colli[1*†], M. J. Mamula[3], C. Evans-Molina[4], D. L. Eizirik[1,5†]

Article

A myeloid–stromal niche and gp130 rescue in NOD2-driven Crohn's disease

https://doi.org/10.1038/s41586-021-03484-5
Received: 8 May 2020
Accepted: 23 March 2021

Shikha Nayar, Joshua K. Morrison, Mamta Giri, Kyle Gettler, Ling-shiang Chuang, Laura A. Walker, Huaibin M. Ko, Ephraim Kenigsberg, Subra Kugathasan, Miriam Merad, Jaime Chu & Judy H. Cho



Elucidating the Biology of Neurodegeneration

"We were blind to this complexity. Things looked simpler than they really are."

—Richard Hodes
Director, National Institute of Aging

ARTICLES
https://doi.org/10.1038/s41590-021-00913-5
nature immunology

Microglia use TAM receptors to detect and engulf amyloid β plaques

nature genetics
ARTICLES
https://doi.org/10.1038/s41588-021-00894-z

Single-nucleus chromatin accessibility and transcriptomic characterization of Alzheimer's disease

ARTICLES
https://doi.org/10.1038/s41588-020-00721-x
nature genetics

Single-cell epigenomic analyses implicate candidate causal variants at inherited risk loci for Alzheimer's and Parkinson's diseases

Science
RESEARCH ARTICLES

Cite as: D. Gate *et al.*, *Science* 10.1126/science.abf7266 (2021).

CD4+ T cells contribute to neurodegeneration in Lewy body dementia

Cell
Resource

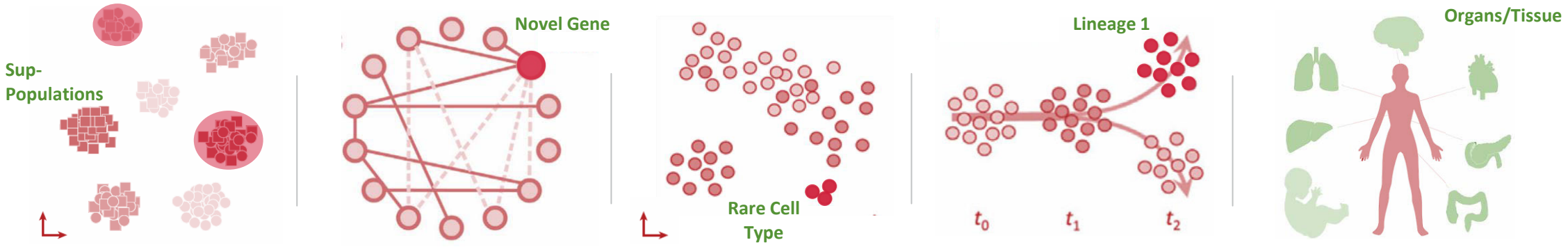
Spatial Transcriptomics and *In Situ* Sequencing to Study Alzheimer's Disease

Acta Neuropathologica
https://doi.org/10.1007/s00401-021-02263-w
ORIGINAL PAPER

Distinct amyloid-β and tau-associated microglia profiles in Alzheimer's disease

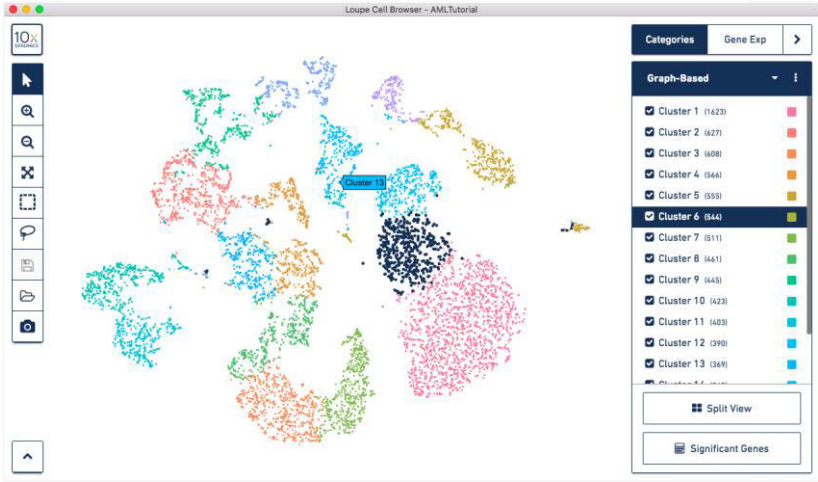
How can single cell data be applied to your research?

- Characterize & identify heterogeneous cell populations
- Discover new cell markers & regulatory pathways
- Uncover novel cell types, cell states & rare cell types
- Reconstruct developmental hierarchies and reveal lineage relationships
- Profiling healthy and diseased tissue and organs

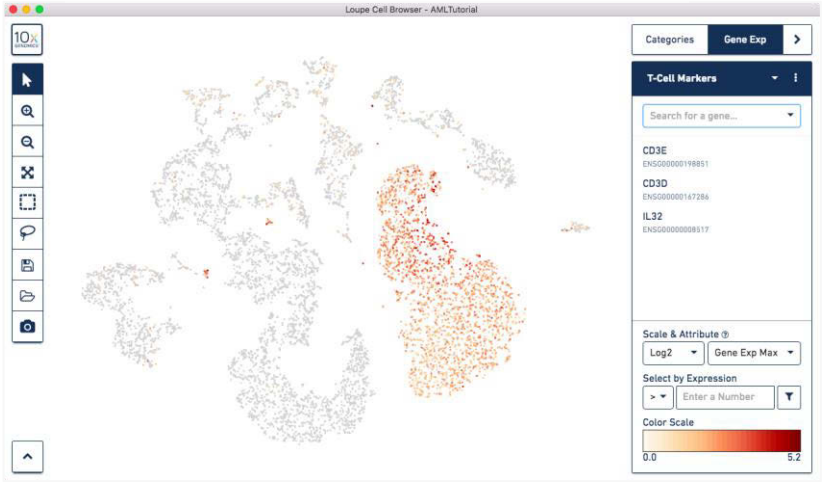


Loupe Cell Browser – Analysis for Everyone

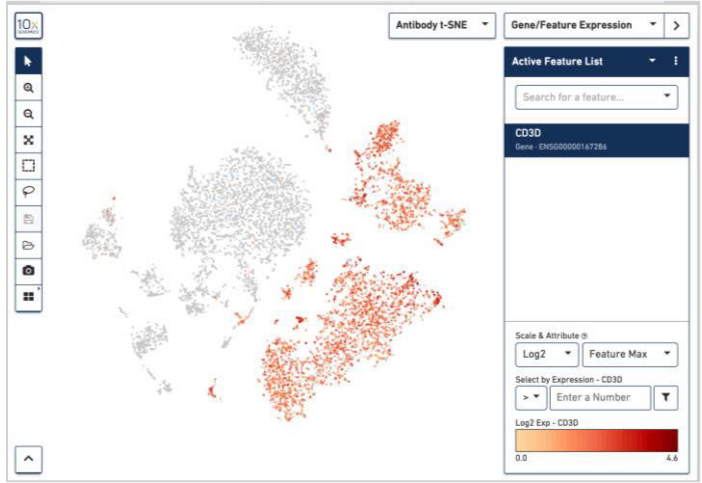
Precomputed GEX Clusters



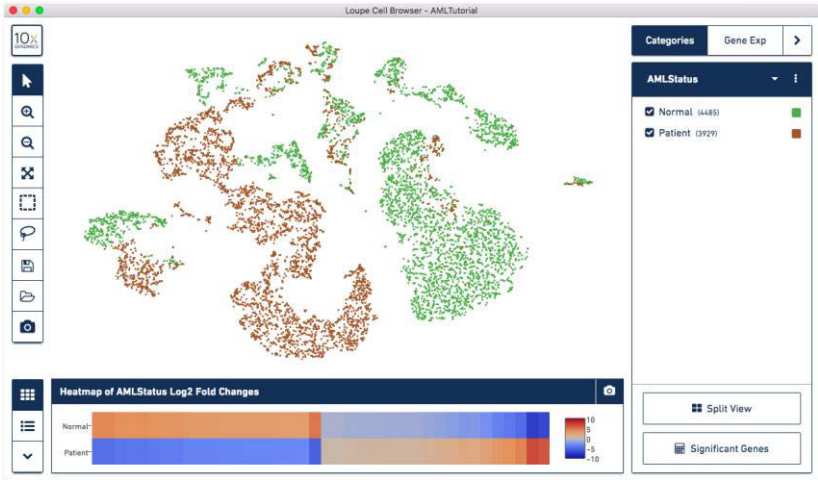
Gene Expression Level



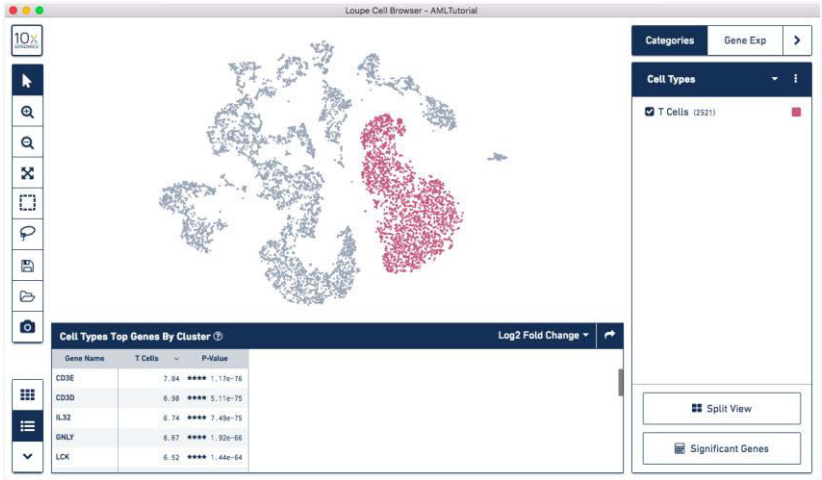
Protein Expression Level



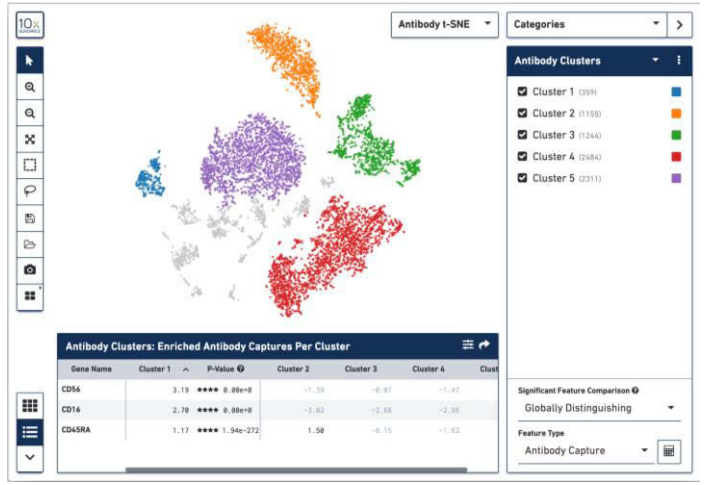
Experimental Conditions



Custom Groups

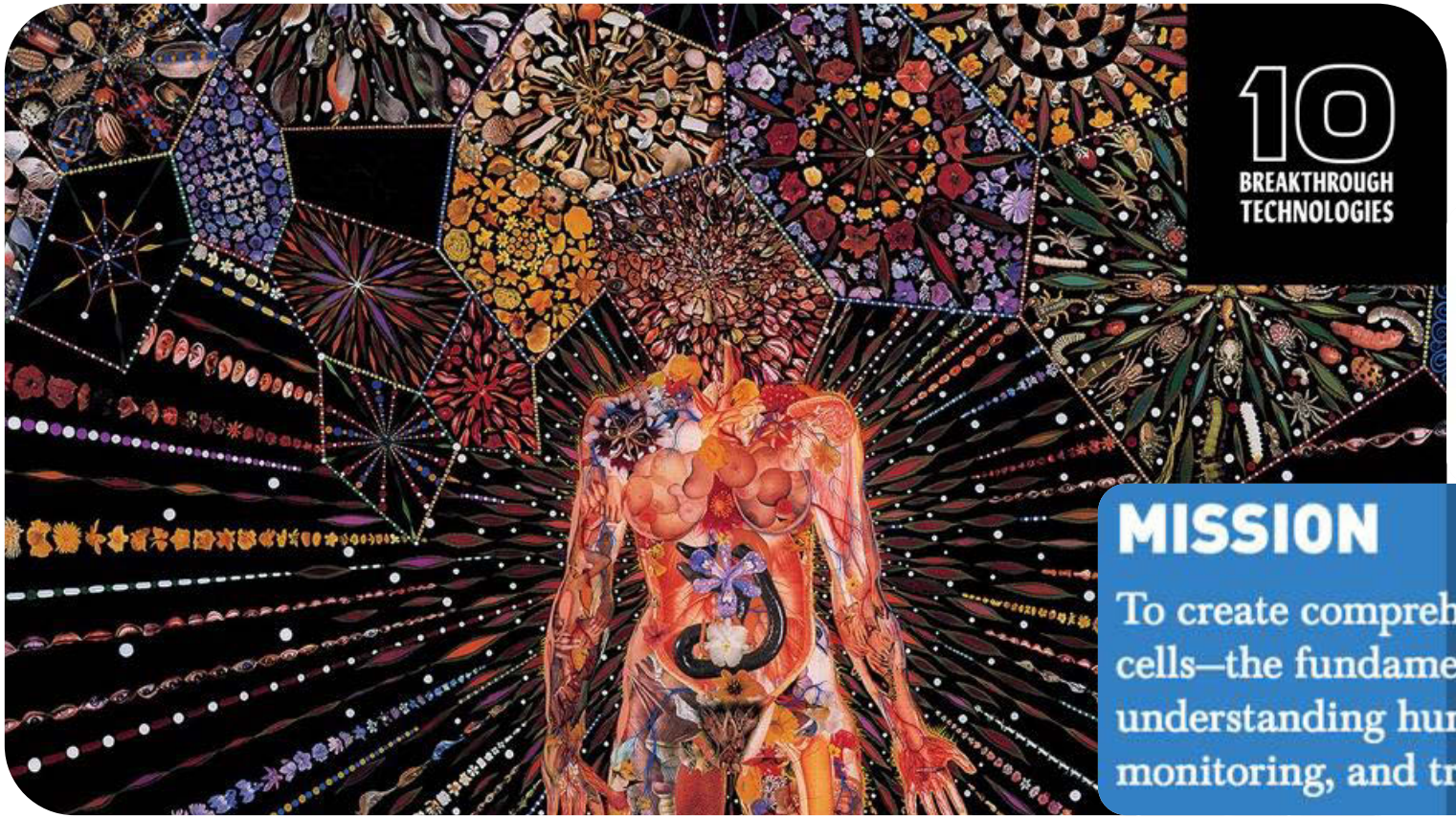


Precomputed Protein Clusters



Chromium Single Cell Gene Expression Solution Chosen by the HCA

"Recent advances in single-cell technology have allowed us to look at cells with a clarity and depth of analysis that we have never been able to achieve before, making this ambitious project a reality within reach." – Aviv Regev



HUMAN CELL ATLAS

MISSION

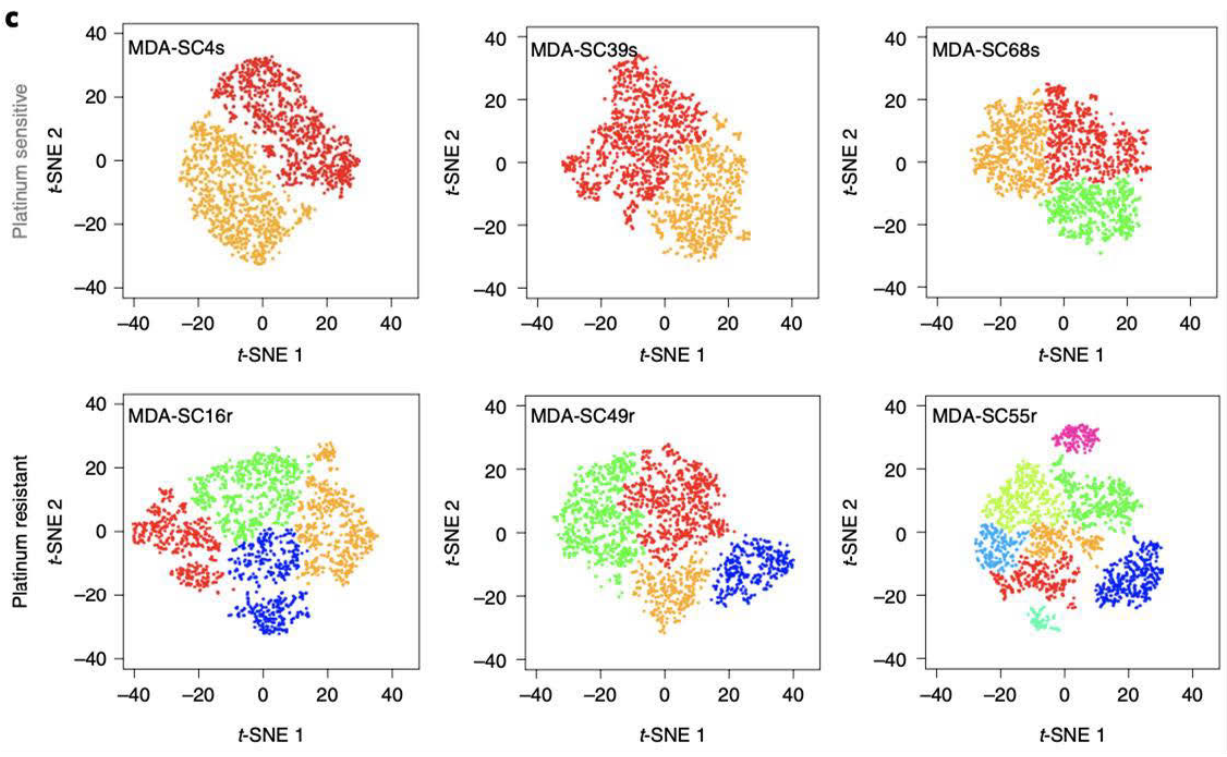
To create comprehensive reference maps of all human cells—the fundamental units of life—as a basis for both understanding human health and diagnosing, monitoring, and treating disease.

Single cell RNA seq –resolution at the scale

Increased intratumoral heterogeneity after onset of therapy resistance in small-cell lung cancer

Stewart et al. Nature Cancer 2020

- SCLC is known to have a robust response to treatment followed by a relapse
- Authors used single cell RNA sequencing to look at tumor heterogeneity after treatment
- Authors observed globally increased intratumoral heterogeneity (ITH) post-treatment, including expression of therapeutic targets and resistant pathways following treatment resistance



DOI: https://doi.org/10.1038/s43018-019-0020-z

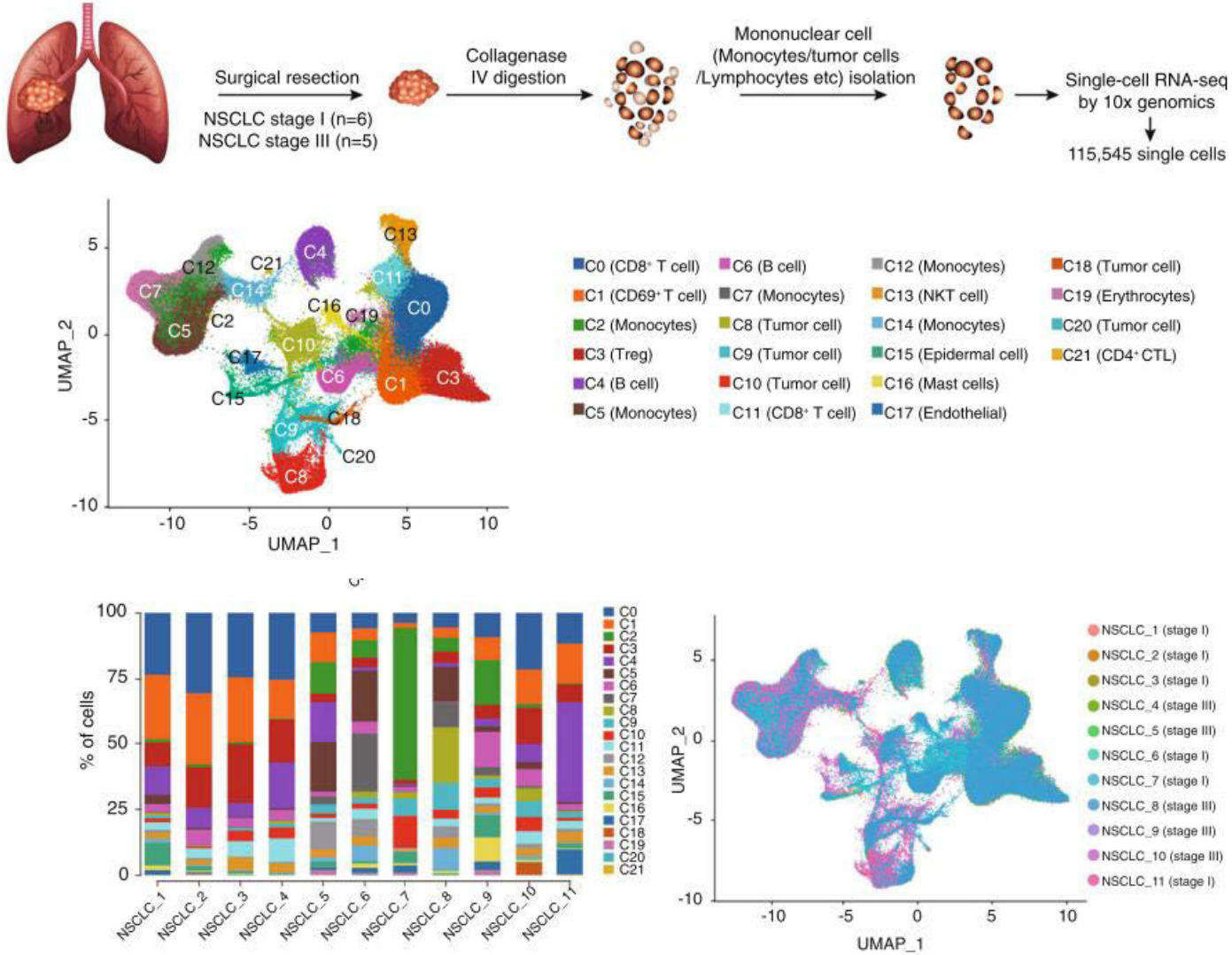
Single-cell transcriptome and antigen-immunoglobin analysis reveals the diversity of B cells in non-small cell lung cancer

Malignant transformation and progression of cancer are driven by the coevolution of cancer cells and their dysregulated tumor microenvironment (TME).

Recent studies on immunotherapy demonstrate the efficacy in reverting the anti-tumoral function of T cells, highlighting the therapeutic potential in targeting certain cell types in TME. However, the functions of other immune cell types remain largely unexplored.

The naïve-like B cells are decreased in advanced NSCLC, and their lower level is associated with poor prognosis.

Plasma-like B cells in the advanced-stage NSCLC promote tumor cell proliferation while those in the early-stage NSCLC partially inhibit the proliferation.



10X GENOMICS

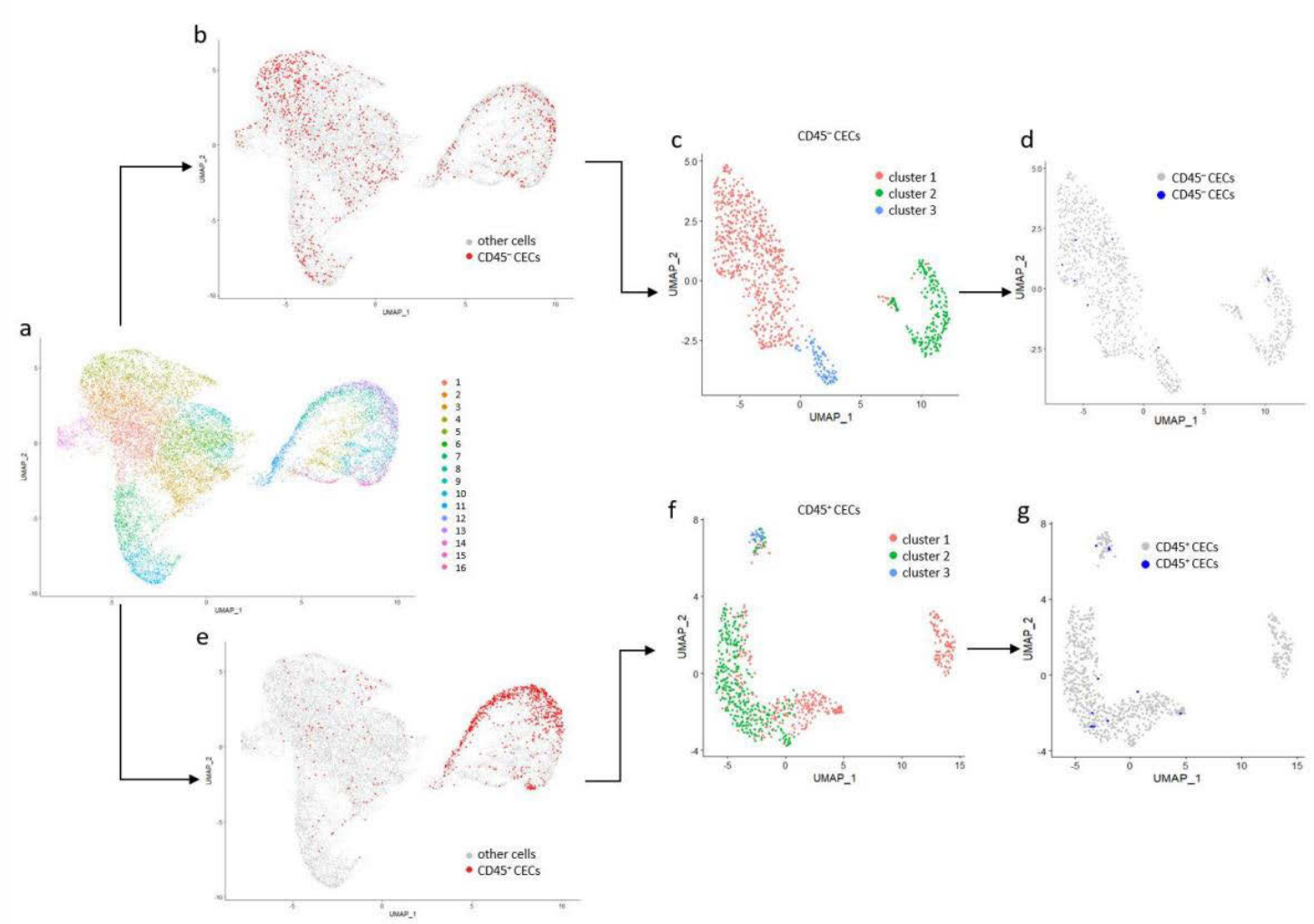
Deciphering heterogeneity of circulating epithelial cells in breast cancer patients

Circulating tumor cells (CTCs) and tumor hybrid cells, being the leading players in metastasis, have prognostic relevance and are potential antimetastatic targets.

CTCs are identified as epithelial-positive and CD45 (leukocyte)-negative cells, whereas tumor hybrid cells usually have epithelial and leukocyte components. However, epithelial and hybrid cells are also observed in healthy subjects that complicate the detection of CTCs and tumor hybrid cells in cancer patients.

This study evaluated the diversity of CD45-negative and CD45-positive circulating epithelial cells (CECs) in breast cancer patients (n=20) using single-cell RNA sequencing.

CD45⁻ and CD45⁺ CECs are highly heterogeneous in breast cancer patients and consist of transcriptionally-distinct cell populations



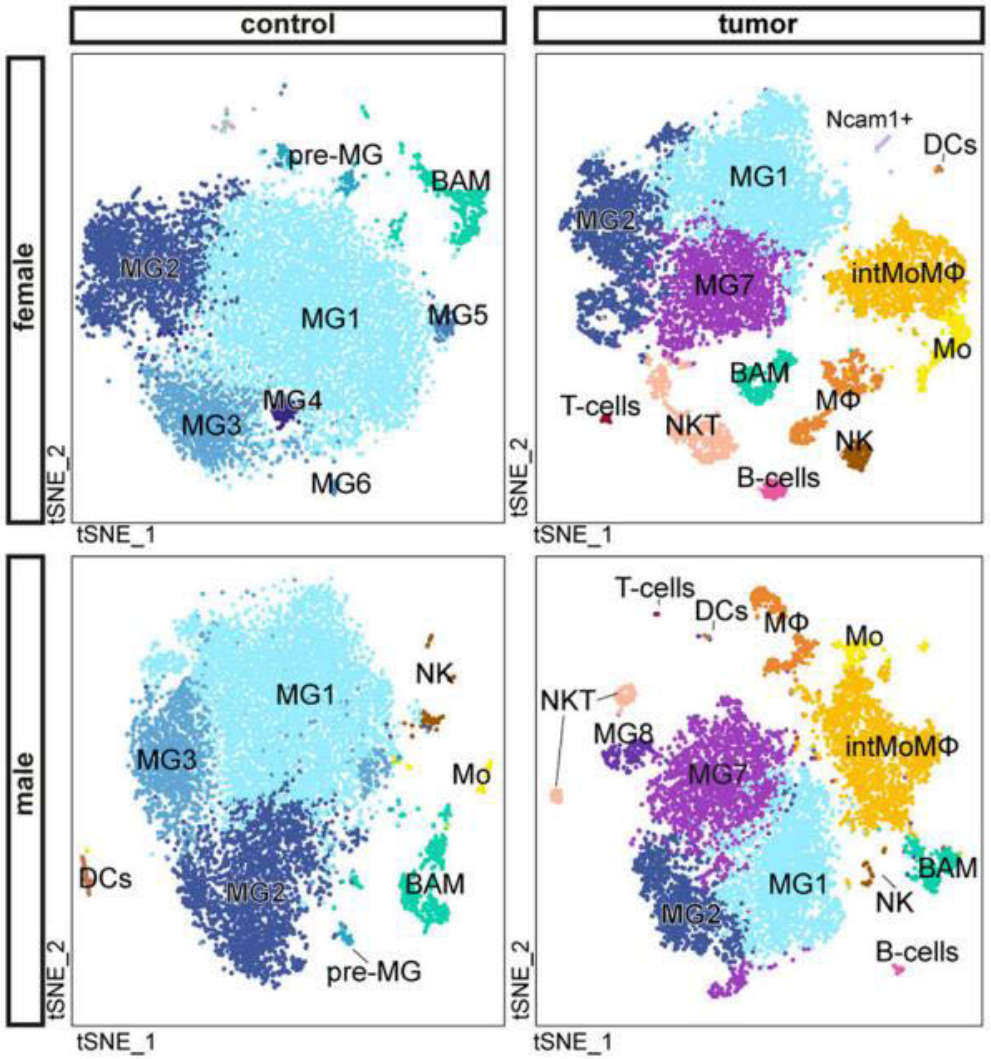
doi: https://doi.org/10.1101/2021.11.24.469962

Single-cell RNA sequencing reveals functional heterogeneity of glioma-associated brain macrophages

Microglia and peripheral myeloid cells accumulate and adapt tumor supporting roles in human glioblastomas that show prevalence in men.

Cell heterogeneity and functional phenotypes of myeloid subpopulations in gliomas remain elusive. Here is shown scRNA-seq of CD11b+ myeloid cells in naïve and GL261 glioma-bearing mice that reveal distinct profiles of microglia, infiltrating monocytes/macrophages and CNS border-associated macrophages.

It is demonstrated an unforeseen molecular heterogeneity among myeloid cells in naïve and gliomabearing brains, validate selected marker proteins and show distinct spatial distribution of identified subsets in experimental gliomas.



10X GENOMICS

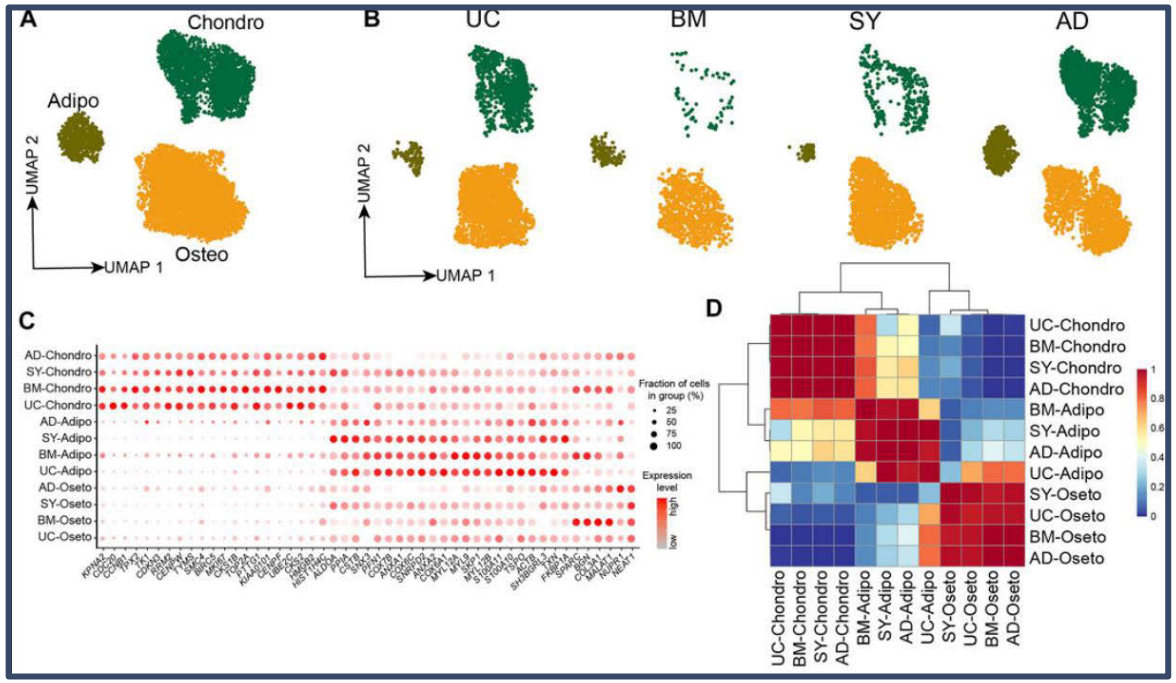
https://doi.org/10.1038/s41467-021-21407-w

Cross-Tissue Characterization of Heterogeneities of Mesenchymal Stem Cells and Their Differentiation Potentials

Mesenchymal stem/stromal cells (MSCs) are multipotent stromal cells that can differentiate into a variety of cell types including chondrocytes, osteocytes, myocytes, and adipocytes *in vivo* and *in vitro*.

Mesenchymal stem/stromal cells (MSCs) are promising cell sources for regenerative medicine and the treatment of autoimmune disorders. Comparing MSCs from different tissues at the single-cell level is fundamental for optimizing clinical applications.
Here was analyzed scRNA-seq data of MSCs from four tissues, : **umbilical cord, bone marrow, synovial tissue, and adipose tissue.**

Was identified three major cell subpopulations, namely osteo-MSCs, chondro-MSCs, and adipo/myo-MSCs, across all MSC samples. MSCs from the umbilical cord exhibited the highest immunosuppression, potentially indicating it is the best immune modulator for autoimmune diseases

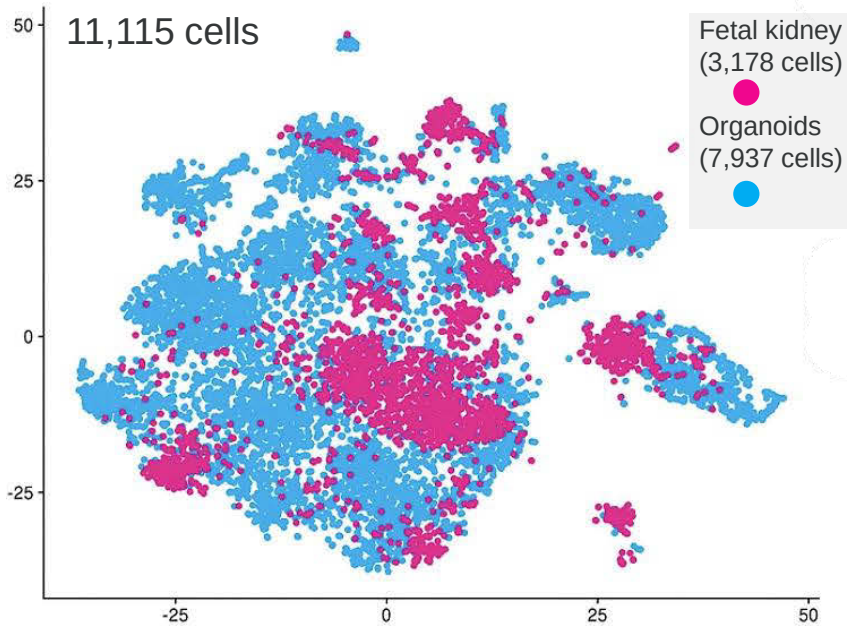


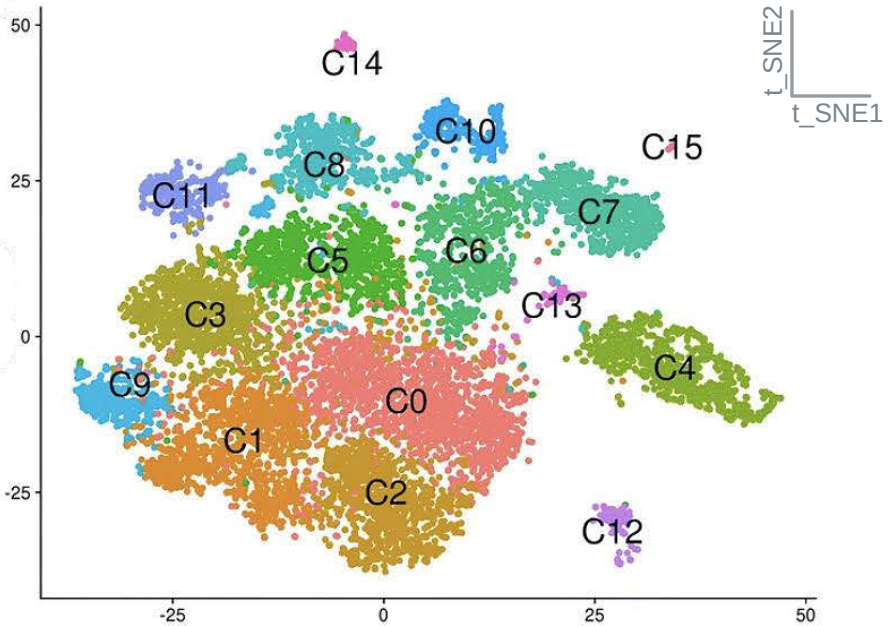
scRNA-Seq Showed Consistent MSC Subpopulations Across Tissues

10X GENOMICS

https://doi.org/10.3389/fcell.2021.781021

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





Stroma (C0, C1, C2, C3, C9)

Endothelium (C4)

Cell cycle (C5, C8)

Committing nephron progenitor (C6)

Podocyte (C7, C15)

Nephron (C10)

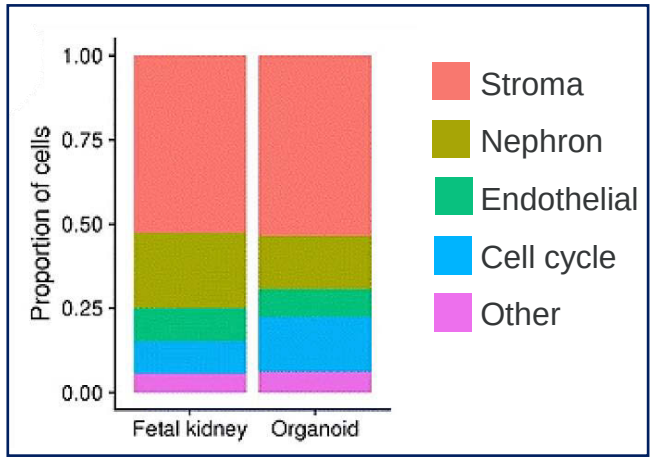
Glial (C11)

Immune (C12)

Blood (C13)

Neural progenitor (C14)

- 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



10X GENOMICS

Genome Medicine

DOI: https://doi.org/10.1186/s13073-019-0615-0

Single-Cell Analyses Identify Brain Mural Cells Expressing CD19 as Potential Off-Tumor Targets for CAR-T Immunotherapies

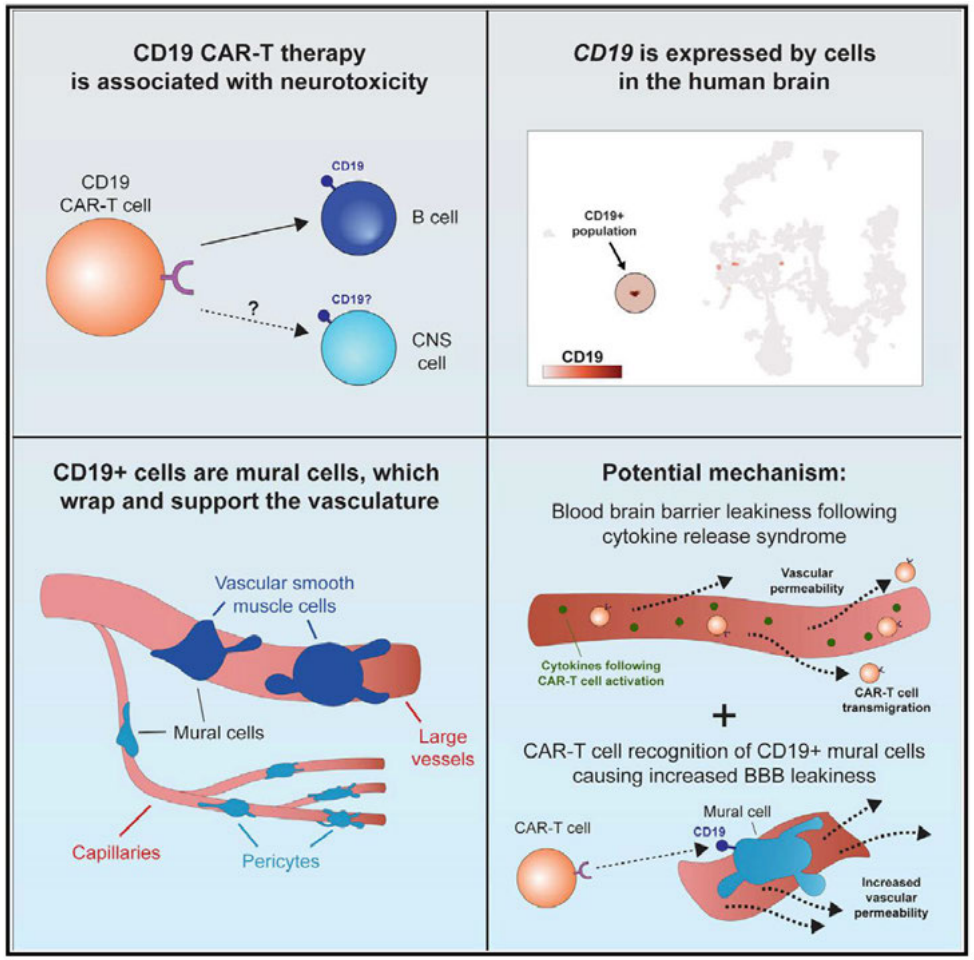
Cell

Chromium single-cell RNA sequencing analysis showed that CD19, primarily considered as a B cell-specific surface antigen, is expressed in human brain mural cells that are critical for blood-brain-barrier integrity.

This cell population may contribute to the neurotoxicity of CD19-directed immunotherapy including CAR-T.

Mouse mural cells demonstrated lower levels of Cd19 expression, suggesting limitations in preclinical animal models of neurotoxicity.

These data highlight the utility of human single-cell atlases for designing immunotherapies.



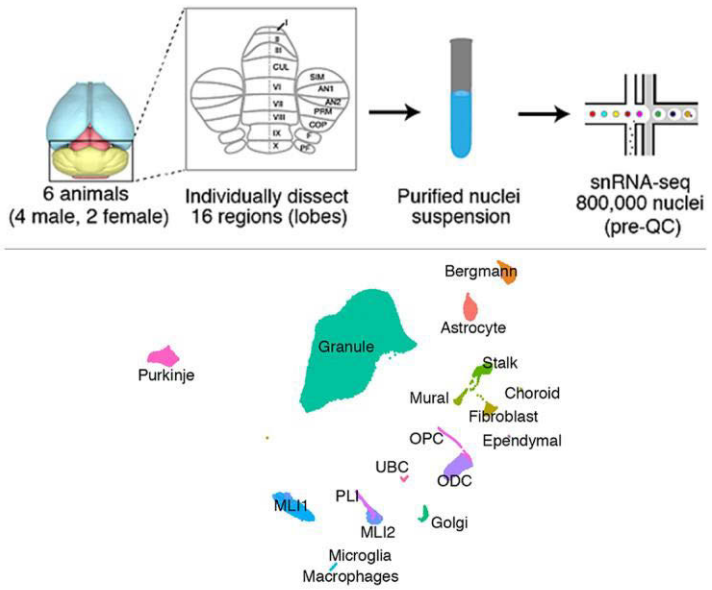
10X GENOMICS

Parker et al., 2020, Cell, https://doi.org/10.1016/j.cell.2020.08.022

Detection of rare cell types/states

Neuroscience

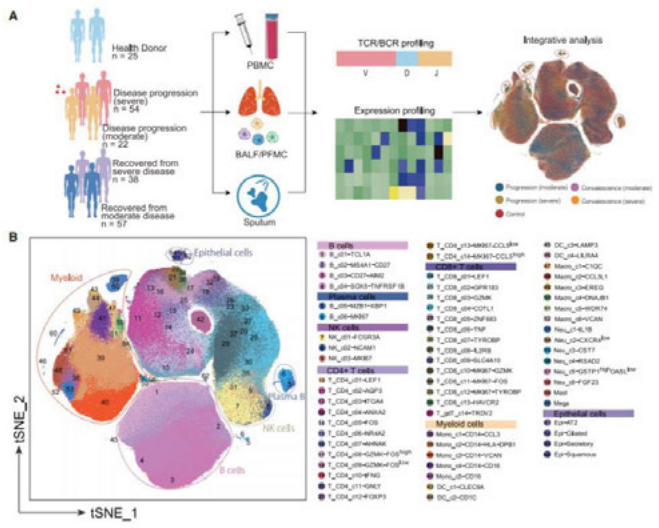
Kozareva *et al*, Nature, 2021



>600.000 nuclei profiled.
A transcriptomic atlas of the mouse cerebellum comprehensively defines cells types.

Infectious Disease

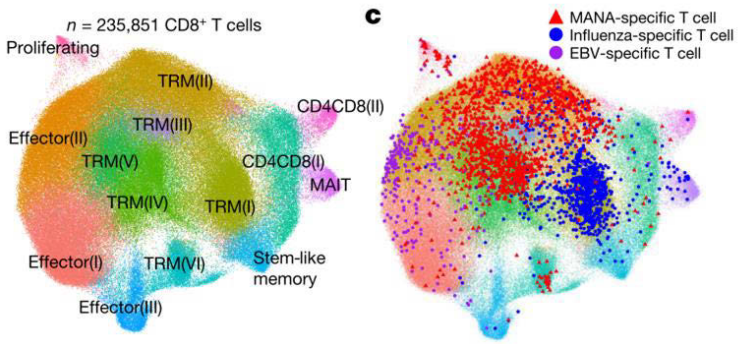
Ren *et al*, Cell, 2021



Detailed COVID-19 immune landscape depicted by integrated **1.46 million single cells**.
Association of immune subtypes with biological and clinical features.

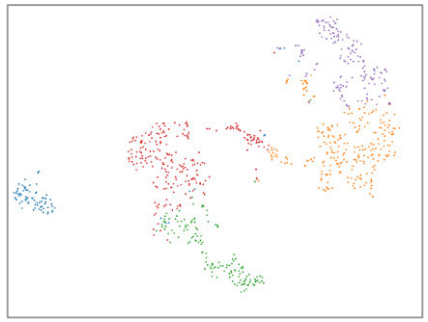
Immune-Oncology

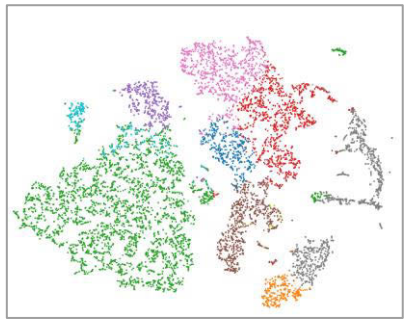
Caushi *et al*, Nature, 2021

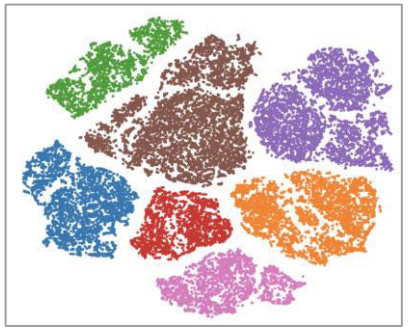


>560.000 cells profiled in total including 235.000 T cells.
1350 **M**utation **A**ssociated **N**eo**A**ntigens-specific TCR detected in anti-PD1-treated lung cancers.

Match the scale you need for your Single Cell Gene Expression studies







Small scale

100-1,000 cells

Single Cell Gene Expression LT

Standard scale

500-10,000 cells

Single Cell Gene Expression

Large scale

2,000-20,000 cells

Single Cell Gene Expression HT

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Chromium Controller

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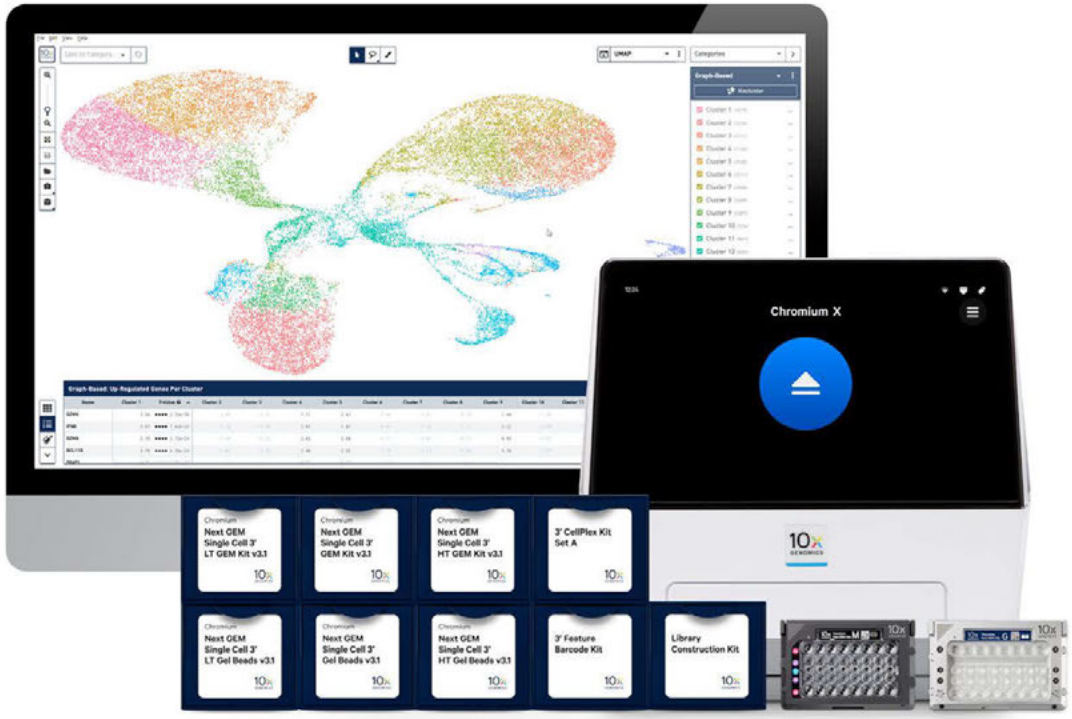
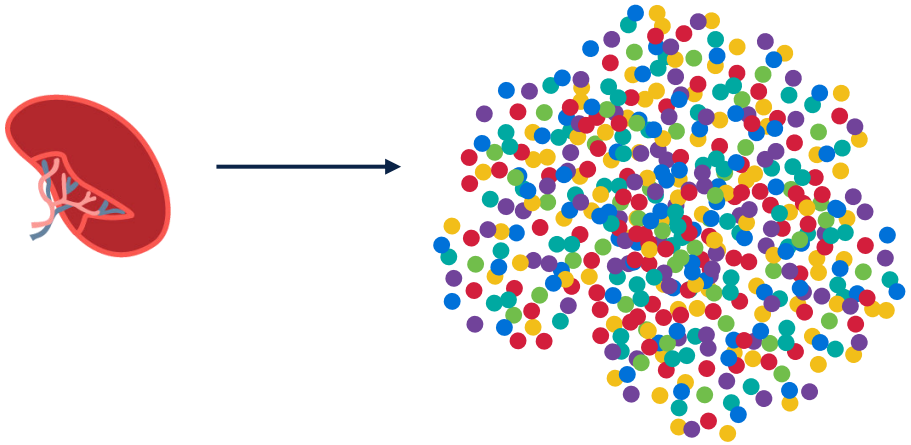


Standard Throughput

High Throughput

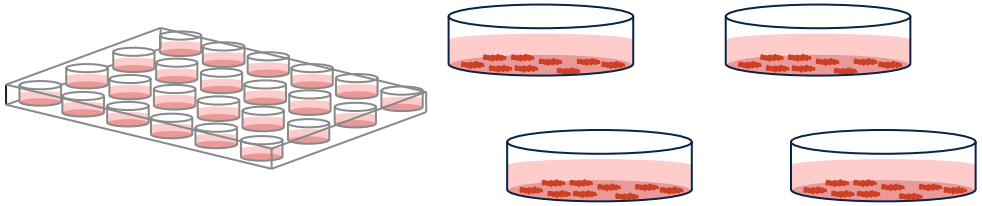
Two types of high throughput

Large number of cells



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

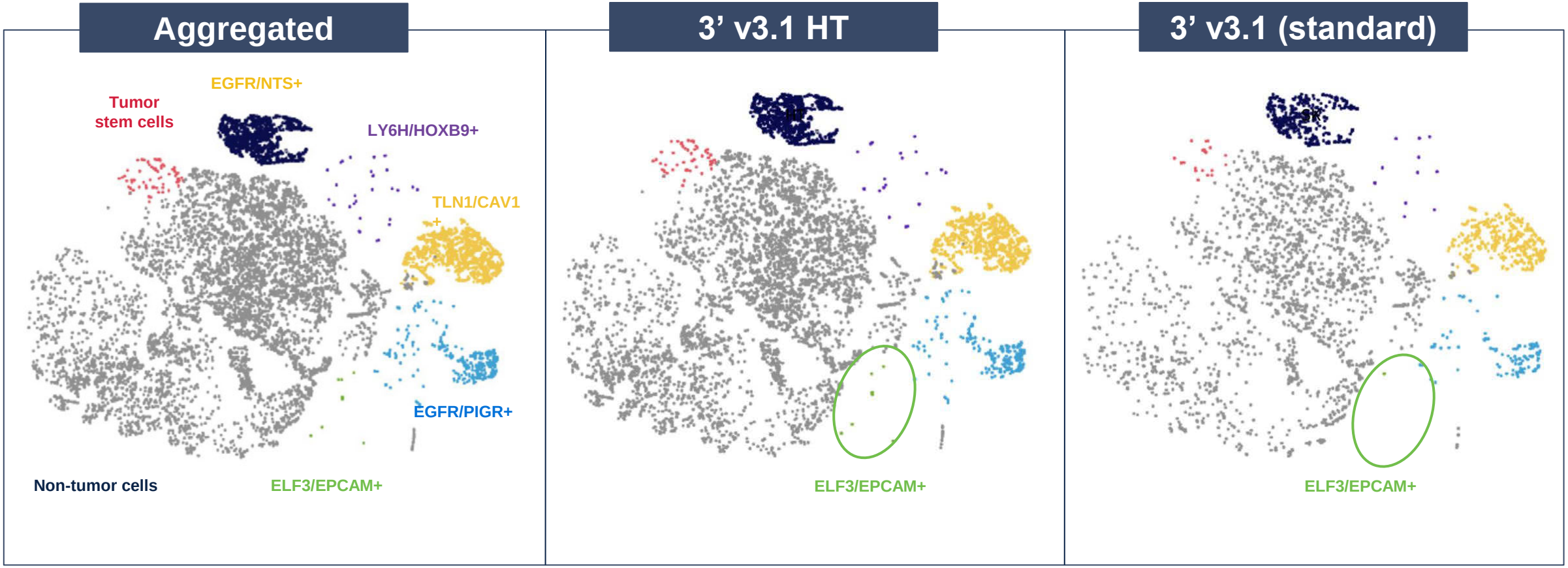
Large number of samples



More samples, easily prepared at the same time

HT enables identification of rare tumor clusters - NCSLC

Deeper look at donor 6



Powering Translational and Clinical Research

Genentech
A Member of the Roche Group

Article

Peripheral T cell expansion predicts tumour infiltration and clinical response

Thomas D. Wu, Shravan Madireddi, Patricia E. de Almeida, Romain Banchereau, Ying-Jiun J. Chen, Avantika S. Chitre, Eugene Y. Chiang, Hina Iftikhar, William E. O'Gorman, Amelia Au-Yeung, Chikara Takahashi, Leonard D. Goldstein, Chungkee Poon, Shilpa Keerthivasan, Denise E. de Almeida Nagata, Xiangnan Du, Hyang-Mi Lee, Karl L. Banta, Sanjeev Mariathasan, Meghna Das Thakur,

GlaxoSmithKline

Original Article

Lymphocyte Activation Gene (LAG)-3 Is Associated With Mucosal Inflammation and Disease Activity in Ulcerative Colitis

Stephanie M. Slevin, Lucy C. Garner, Conor Lahiff, Malcolm Tan, Lai Mun Wang, Helen Ferry, Borgel Greenaway, Kate Lynch, Alessandra Geremia, Stephen Hughes, Karen Leavens, David Krull, Daniel J. B. Marks, Katherine Nevin, Kevin Page, Naren Srinivasan, Ruth Tarzi, Paul Klenerman, Simon Travis, Carolina V. Arancibia-Cárcamo, Satish Keshav

ALECTOR
THERAPIES TO REMEMBER

Washington University in St. Louis

ARTICLE

Anti-human TREM2 induces microglia proliferation and reduces pathology in an Alzheimer's disease model

哈爾濱工業大學
HARBIN INSTITUTE OF TECHNOLOGY

Single cell RNA and immune repertoire profiling of COVID-19 patients reveal novel neutralizing antibody

Fang Li, Meng Luo, Wenyang Zhou, Jinliang Li, Xiyun Jin, Zhaochun Xu, Liran Juan, Zheng Zhang, Yuou Li, Renqiang Liu, Yiqun Li, Chang Xu, Kexin Ma, Huimin Cao, Jingwei Wang, Pingping Wang, Zhigao Bu, Qinghua Jiang

Sackler Faculty of Medicine
Tel Aviv University

RESEARCH ARTICLE

Multi-clonal SARS-CoV-2 neutralization by antibodies isolated from severe COVID-19 convalescent donors

Michael
C. Lee,
E. Clark
Hila Sh
Sandra
Moshe
T. Freu

UNIVERSITY of WASHINGTON

Article

Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19

Yapeng Su, Daniel Chen, Dan Yuan, Christopher Lausted, Jongchan Choi, Chengzhen L. Dai, Valentin Voillet, Venkata R. Duvvuri, Kelsey Scherler, Pamela Troisch, Priyanka Baloni, Guangrong Qin, Brett Smith, Sergey A. Kornilov, Clifford Rostomily, Alex Xu, Jing Li, Shen Dong, Alissa Rothchild, Jing Zhou, Kim Murray, Rick Edmark, Sunga Hong, John E. Heath, John Earls, Rongyu Zhang, Jingyi Xie, Sarah Li, Ryan Roper, Lesley Jones, Yong Zhou, Lee Rowen, Rachel Liu, Sean Mackay, D. Shane O'Mahony, Christopher R. Dale, Julie A. Wallick, Heather A. Algren, Michael A. Zager, the ISB-Swedish COVID19 Biobanking Unit, Wei Wei, Nathan D. Price, Sui Huang, Naeha Subramanian, Kai Wang, Andrew T. Magis, Jenn J. Hadlock, Leroy Hood, Alan Aderem, Jeffrey A. Bluestone, Lewis L. Lanier, Philip D. Greenberg, Raphael Gottardo, Mark M. Davis, Jason D. Goldman, and James R. Heath

NOVARTIS

TGFβ-blockade uncovers stromal plasticity in tumors by revealing the existence of a subset of interferon-licensed fibroblasts

Angelo L. Grauel, Beverly Nguyen, David Ruddy, Tyler Laszewski, Stephanie Schwartz, Jonathan Chang, Julie Chen, Michelle Piquet, Marc Pelletier, Zheng Yan, Nathaniel D. Kirkpatrick, Jincheng Wu, Antoine deWeck, Markus Riester, Matt Hims, Felipe Correa Geyer, Joel Wagner, Kenzie MacIsaac, James Deeds, Rohan Diwanji, Pushpa Jayaraman, Yenyen Yu, Quincey Simmons, Shaobu Weng, Alina Raza, Brian Minie, Mirek Dostalek, Pavitra Chikkegowda, Vera Ruda, Oleg Iartchouk, Naiyan Chen, Raphael Thierry, Joseph Zhou, Iulian Pruteanu-Malinici, Claire Fabre, Jeffrey A. Engelman, Glenn Dranoff & Viviana Cremasco

Penn Medicine

RESEARCH ARTICLE SUMMARY

CLINICAL TRIALS

CRISPR-engineered T cells in patients with refractory cancer

Edward A. Stadtmauer*†, Joseph A. Fraietta*, Megan M. Davis, Adam D. Cohen, Kristy L. Weber, Eric Lancaster, Patricia A. Mangan, Irina Kulikovskaya, Minnal Gupta, Fang Chen, Lifeng Tian, Vanessa E. Gonzalez, Jun Xu, In-young Jung, J. Joseph Melenhorst, Gabriela Plesa, Joanne Shea, Tina Matlawski, Amanda Cervini, Avery L. Gaymon, Stephanie Desjardins, Anne Lamontagne, January Salas-Mckee, Andrew Fesnak, Donald L. Siegel, Bruce L. Levine, Julie K. Jadlowsky, Regina M. Young, Anne Chew, Wei-Ting Hwang, Elizabeth O. Hexner, Beatriz M. Carreno, Christopher L. Nobles, Frederic D. Bushman, Kevin R. Parker, Yanyan Qi, Ansuman T. Satpathy, Howard Y. Chang, Yangbing Zhao, Simon F. Lacey*, Carl H. June*†

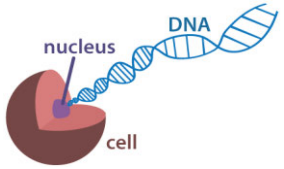
Chromium Single Cell Multiome ATAC + GEX

Two modalities, same cell

Mechanisms of transcriptional regulation

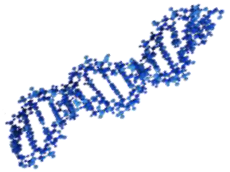
Chromatin remodeling

- **Chromatin structure**
- Chromatin exists in complex hierarchical structures within each cell.
- Rearrangement of chromatin from a condensed state (heterochromatin) to an accessible state (euchromatin) **determines DNA accessibility to DNA binding proteins**



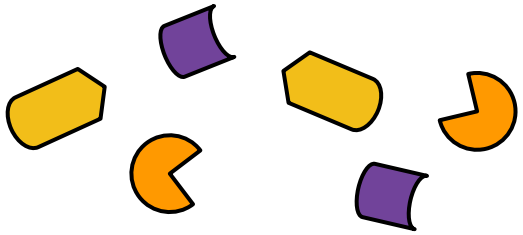
Epigenetic modifications

- Epigenetic modifications
- Modifications on histone proteins can change chromatin conformation.
- Modifications on DNA can prevent or promote protein binding.
- Epigenetic alterations are dynamic and potentially reversible, unlike genetic mutations.



DNA binding proteins

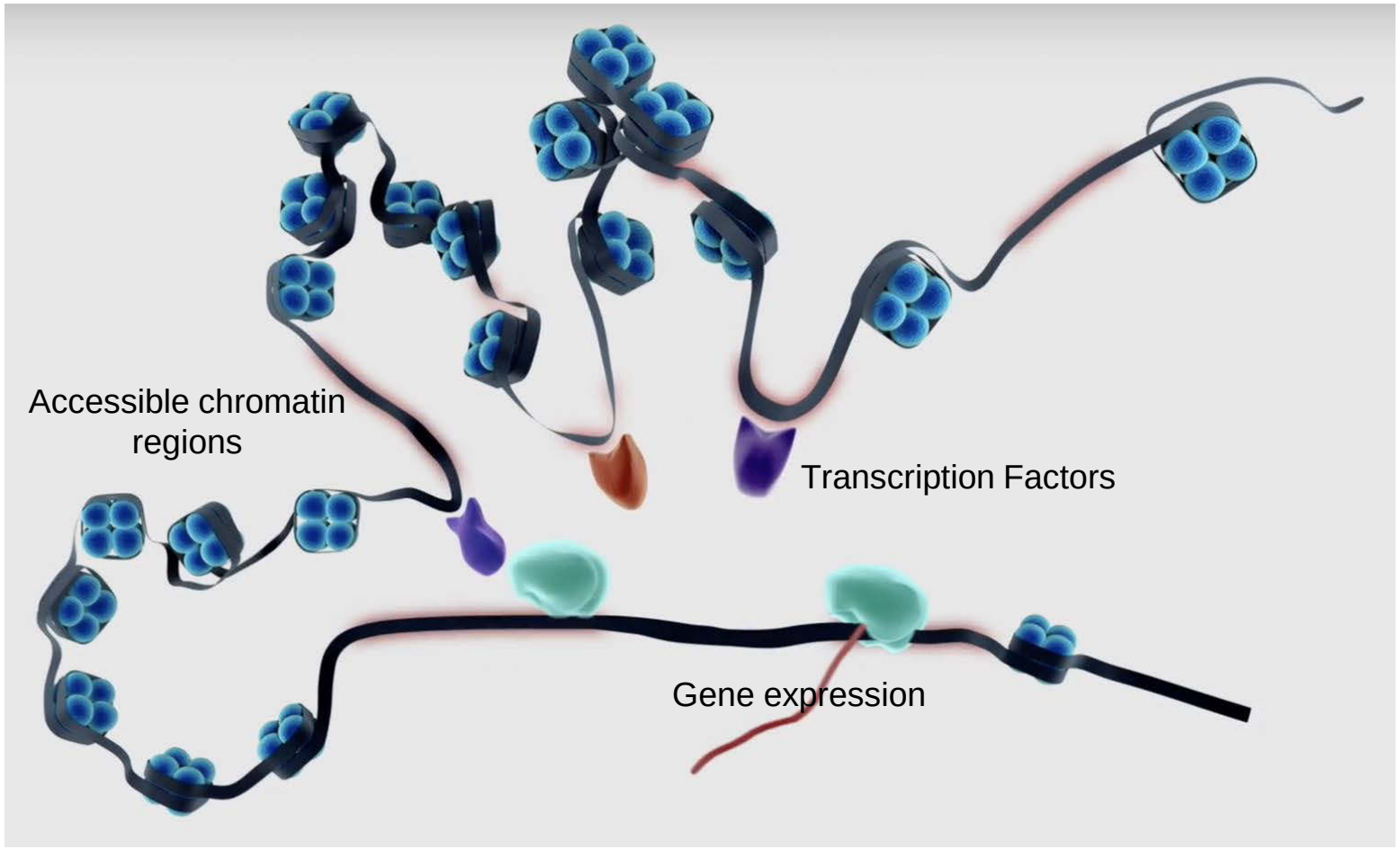
- Transcription factors (TFs) modulate the process of transcription
- TFs can promote (activators), or block (repressors) the recruitment of RNA polymerase to the DNA site



Interplay Between Epigenetic Programs and Gene Expression - ATAC-seq + GEX

Epigenome

Transcriptome



analyse function of cells

cell atlasing

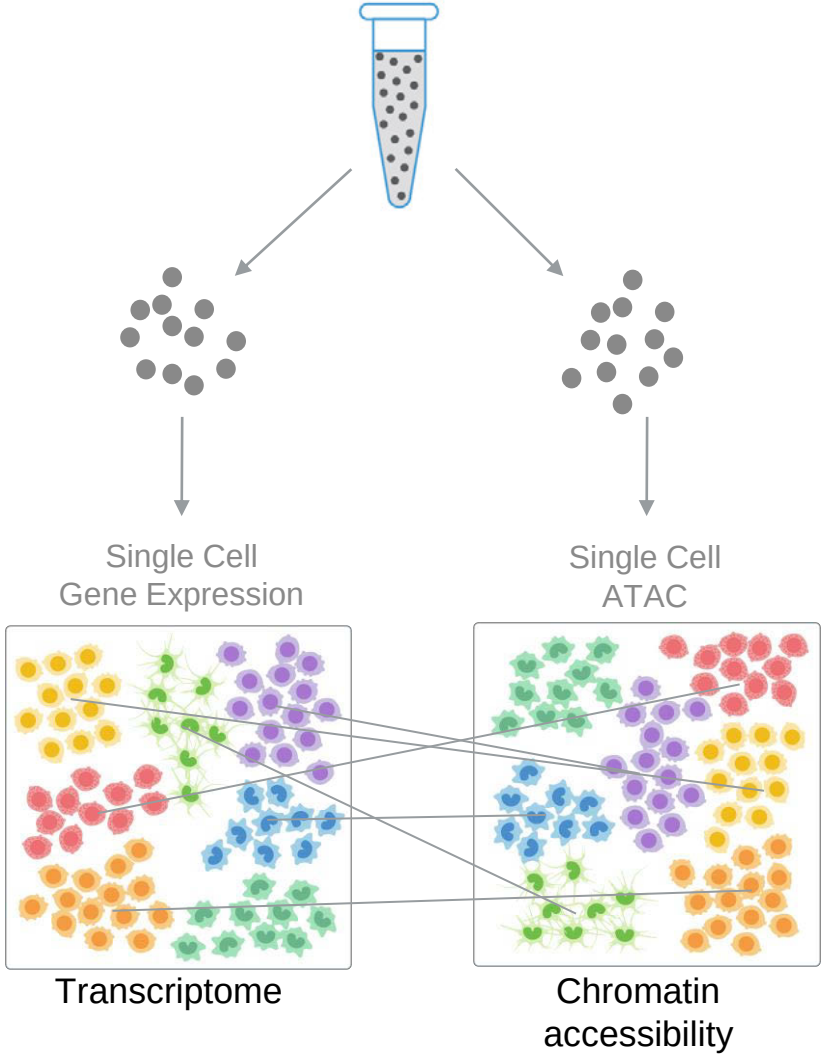
discovery tool

subpopulation detection

understanding of biological processes and pathways

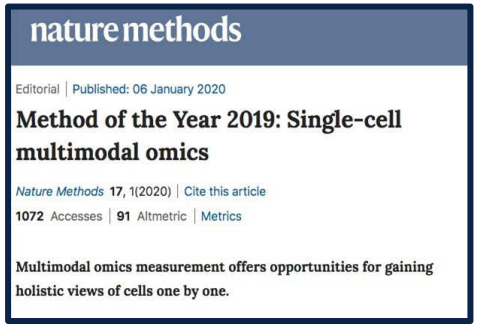
Multiomic approach

Split sample



Inferred Linkage

Every cell


nature methods

Editorial | Published: 06 January 2020

Method of the Year 2019: Single-cell multimodal omics

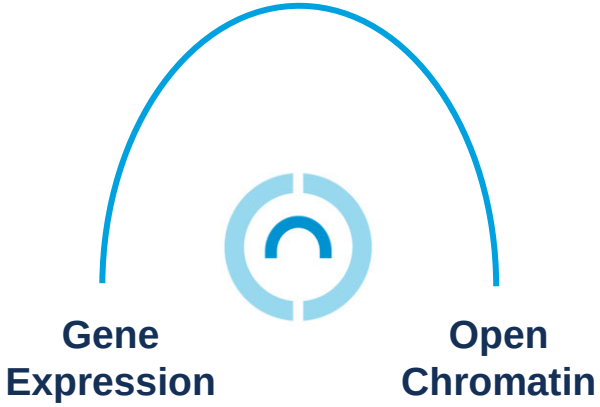
Nature Methods 17, 1(2020) | Cite this article

1072 Accesses | 91 Altmetric | Metrics

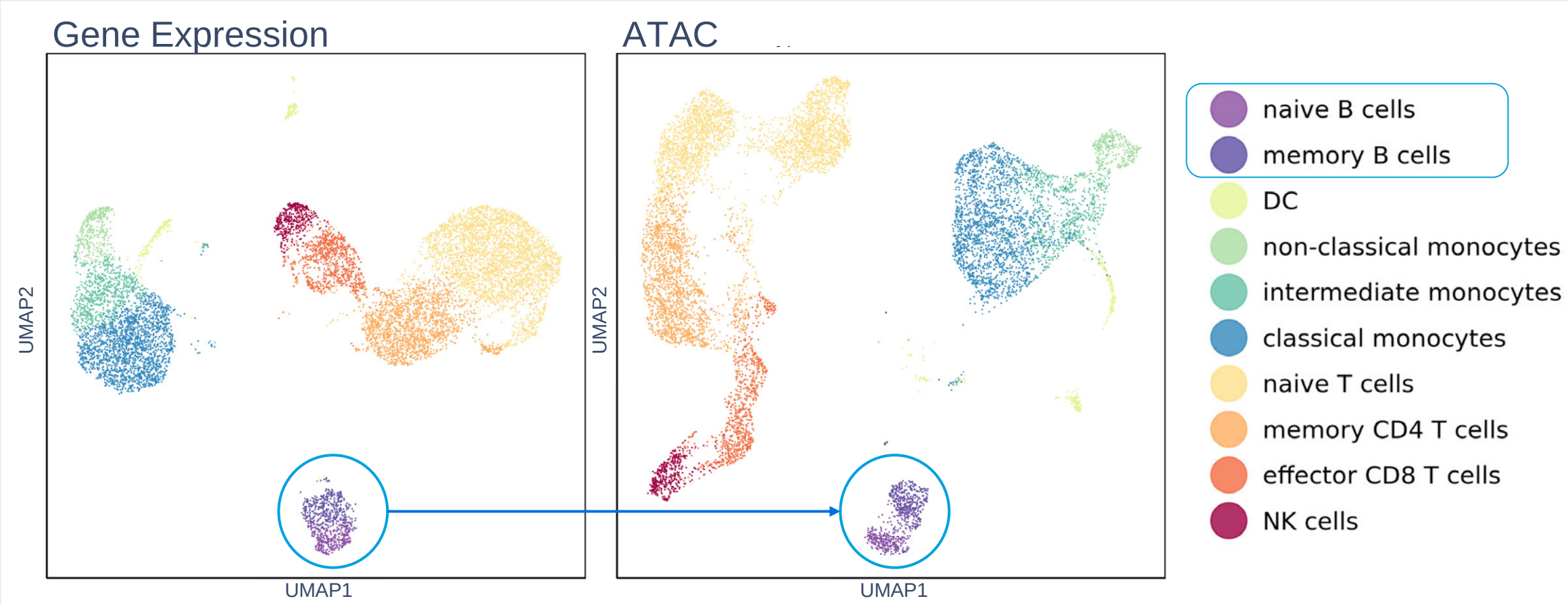
Multimodal omics measurement offers opportunities for gaining holistic views of cells one by one.

Dual modalities

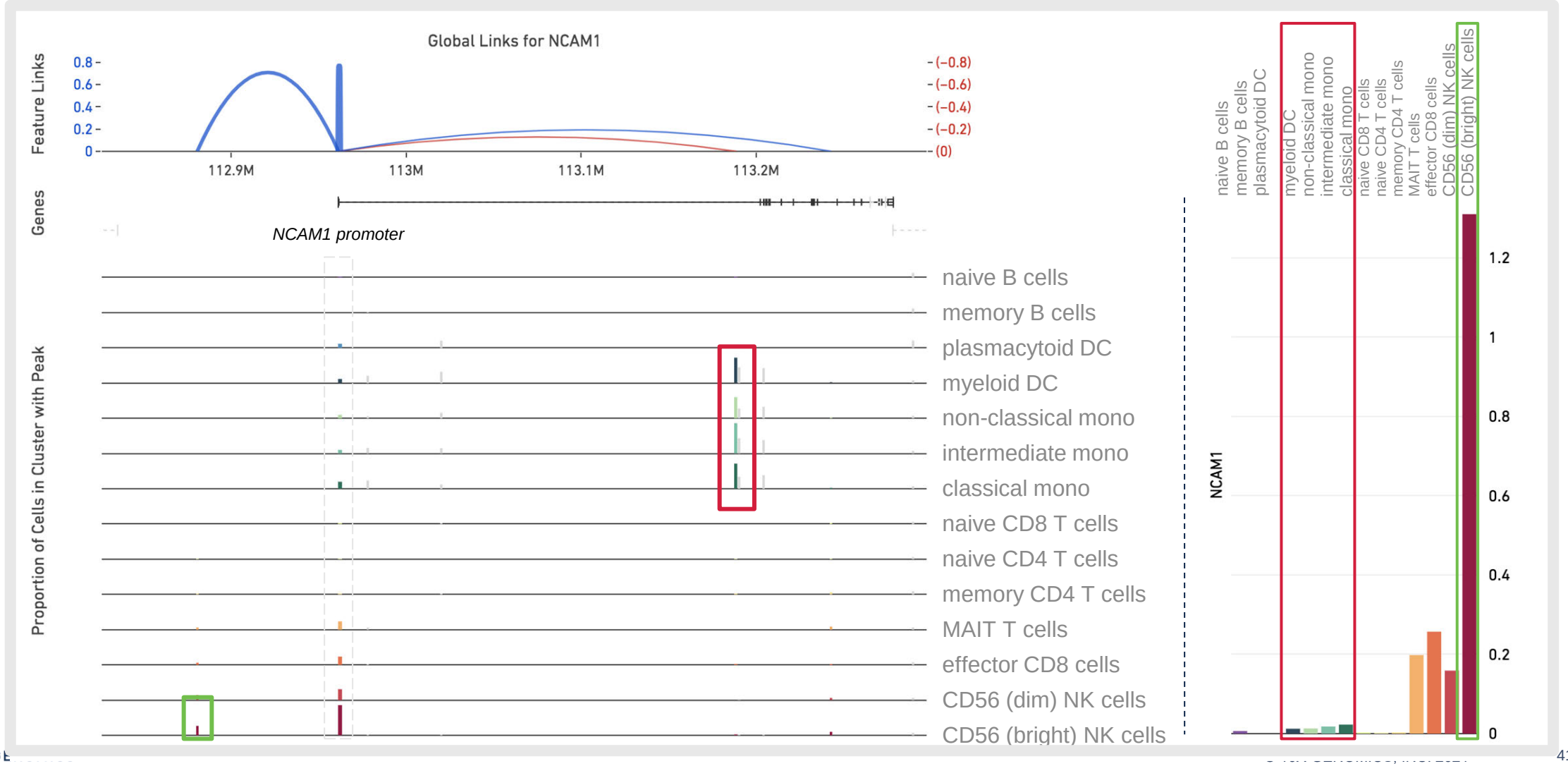
True Linkage



Better separate PBMC populations on ATAC space



Link putative regulatory elements to target genes (CD56)



Emergence of a High-Plasticity Cell State (HPCS) during Lung Cancer Evolution

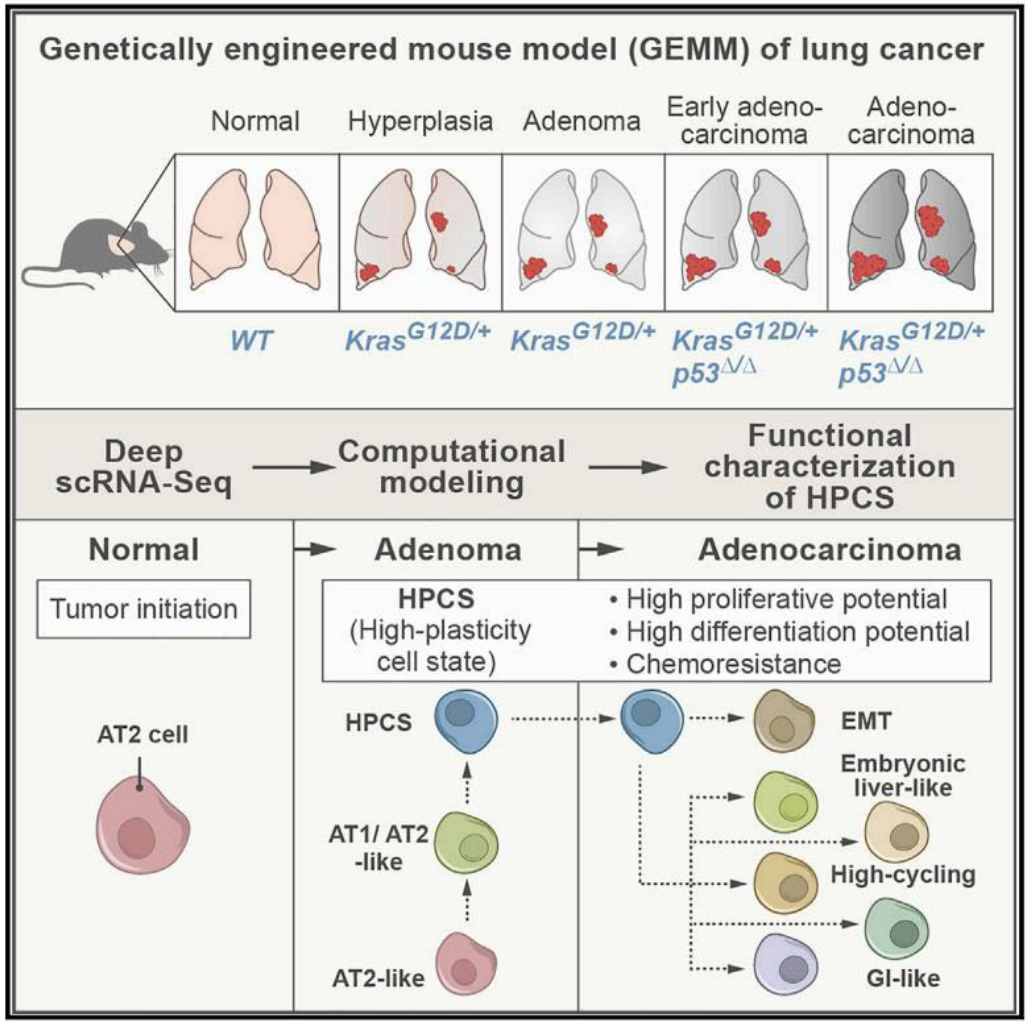
Authors used scRNA-seq to study cell state changes during tumor evolution in a mouse model of LUAD (lung adenocarcinoma) mimicking the oncogenic transformation processes observed in human disease.

Transcriptional heterogeneity grew dramatically during tumor progression.

Identification of a Highly Plastic Cell State (HPCS) with a Distinct Chromatin Accessibility Profile (ATAC-seq)

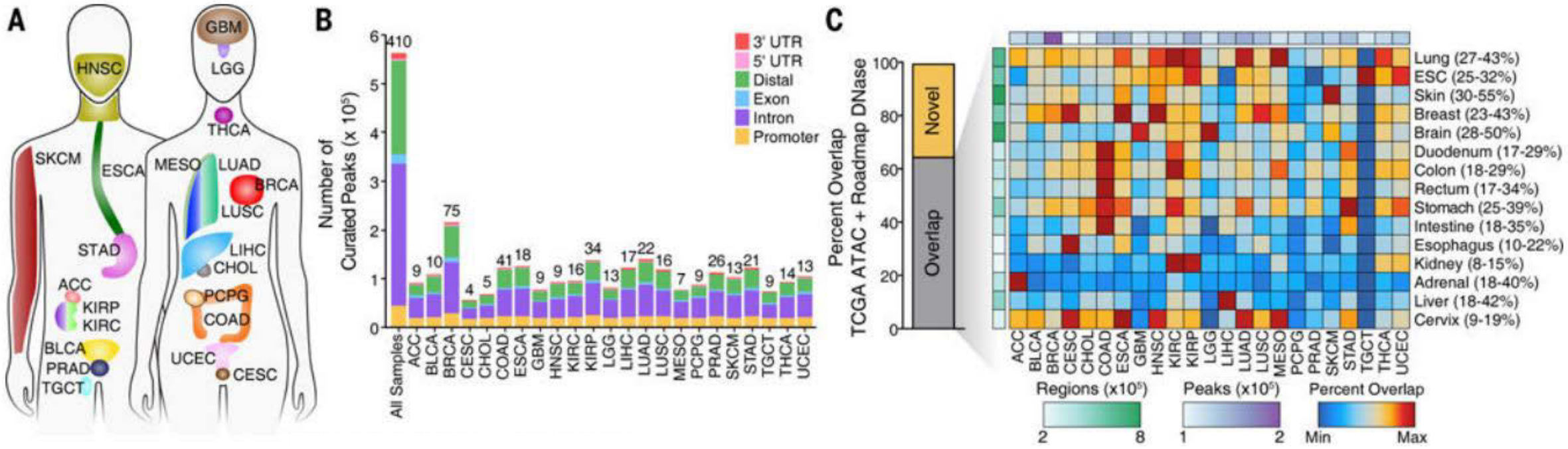
State transitions occur via an HPCS harboring high differentiation and growth capacity

The HPCS is drug resistant and potends poor patient survival across all cancers. Targeting the HPCS may enable therapeutic strategies to suppress tumor heterogeneity and treatment resistance



Marjanovic et a 2020, Cancer Cell, https://doi.org/10.1016/j.ccell.2020.06.012

The chromatin accessibility landscape of primary human cancers

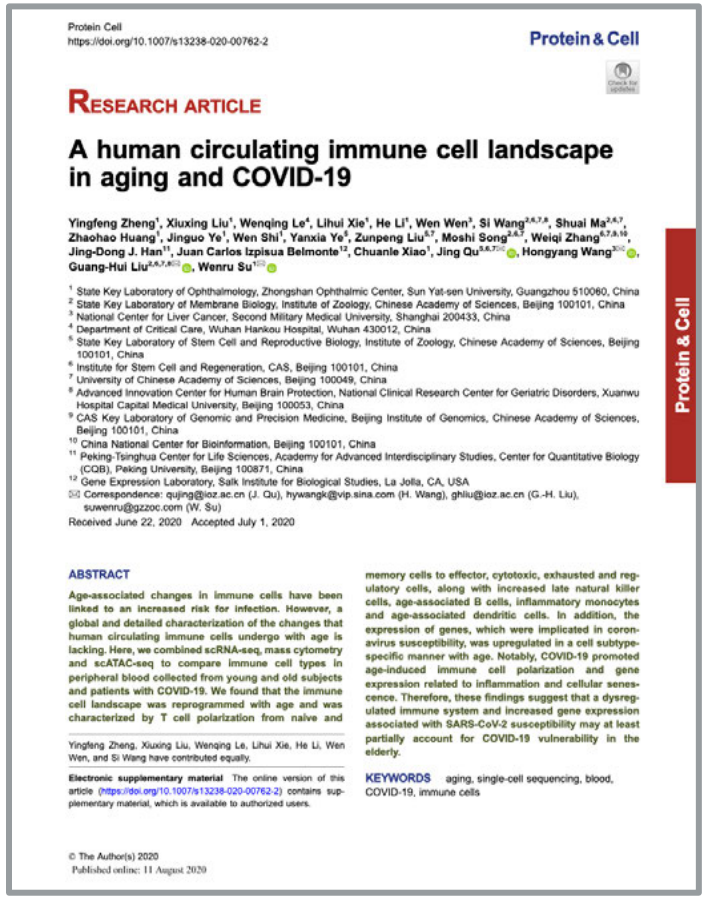


Pan-cancer ATAC-seq of TCGA samples identifies diverse regulatory landscapes.
Genome-wide chromatin accessibility profiles of 410 tumor samples spanning 23 cancer types from The Cancer Genome Atlas (TCGA). Were identified 562,709 transposase-accessible DNA elements that substantially extend the compendium of known cis-regulatory elements.

doi:10.1126/science.aav1898.

Assaying the molecular genetic regulation of immunology

Combined gene expression and chromatin accessibility measurements



Protein Cell
https://doi.org/10.1007/s13238-020-00762-2

Protein & Cell

RESEARCH ARTICLE

A human circulating immune cell landscape in aging and COVID-19

Yingfeng Zheng[1], Xiuxing Liu[1], Wenqing Le[4], Lihui Xie[1], He Li[5], Wen Wen[3], Si Wang[2,6,7,8], Shuai Ma[2,6,7], Zhaohao Huang[1], Jinguo Ye[1], Wen Shi[1], Yanxia Ye[9], Zunpeng Liu[5,7], Moshi Song[2,6,7], Weiqi Zhang[6,7,8,10], Jing-Dong J. Han[11], Juan Carlos Izpisua Belmonte[12], Chuanle Xiao[1], Jing Qu[5,6,7], Hongyang Wang[3], Guang-Hui Liu[2,6,7], Wenru Su[1]

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[3] National Center for Liver Cancer, Second Military Medical University, Shanghai 200433, China
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[5] State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
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[9] CAS Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100101, China
[10] China National Center for Bioinformation, Beijing 100101, China
[11] Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Center for Quantitative Biology (CQB), Peking University, Beijing 100871, China
[12] Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA
✉ Correspondence: qujing@ioz.ac.cn (J. Qu), hywangk@vip.sina.com (H. Wang), ghliu@ioz.ac.cn (G.-H. Liu), suwenru@gzzoc.com (W. Su)

Received June 22, 2020 Accepted July 1, 2020

ABSTRACT

Age-associated changes in immune cells have been linked to an increased risk for infection. However, a global and detailed characterization of the changes that human circulating immune cells undergo with age is lacking. Here, we combined scRNA-seq, mass cytometry and scATAC-seq to compare immune cell types in peripheral blood collected from young and old subjects and patients with COVID-19. We found that the immune cell landscape was reprogrammed with age and was characterized by T cell polarization from naive and memory cells to effector, cytotoxic, exhausted and regulatory cells, along with increased late natural killer cells, age-associated B cells, inflammatory monocytes and age-associated dendritic cells. In addition, the expression of genes, which were implicated in coronavirus susceptibility, was upregulated in a cell subtype-specific manner with age. Notably, COVID-19 promoted age-induced immune cell polarization and gene expression related to inflammation and cellular senescence. Therefore, these findings suggest that a dysregulated immune system and increased gene expression associated with SARS-CoV-2 susceptibility may at least partially account for COVID-19 vulnerability in the elderly.

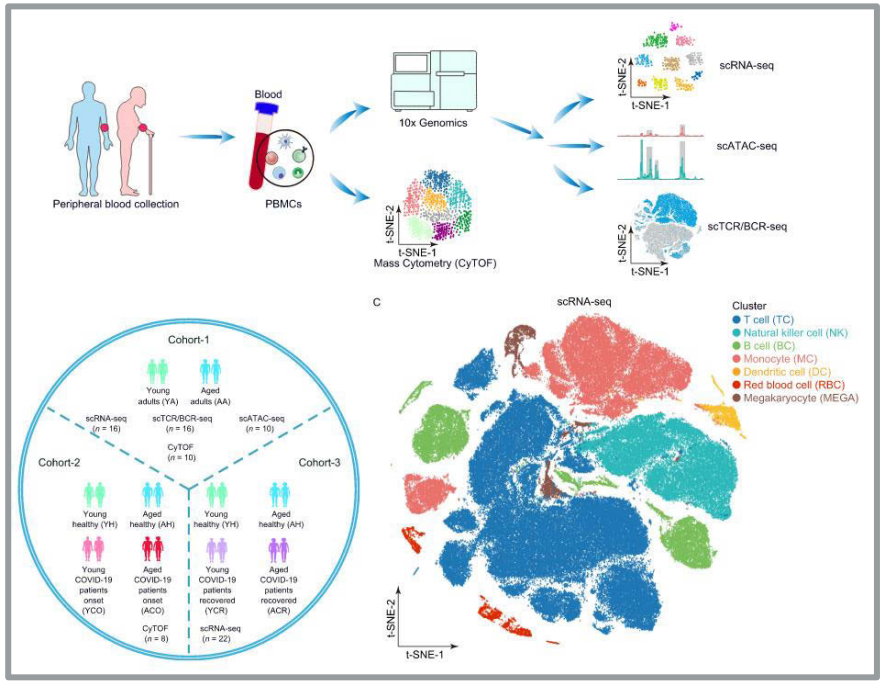
Yingfeng Zheng, Xiuxing Liu, Wenqing Le, Lihui Xie, He Li, Wen Wen, and Si Wang have contributed equally.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13238-020-00762-2) contains supplementary material, which is available to authorized users.

KEYWORDS aging, single-cell sequencing, blood, COVID-19, immune cells

Published online: 11 August 2020

Protein & Cell



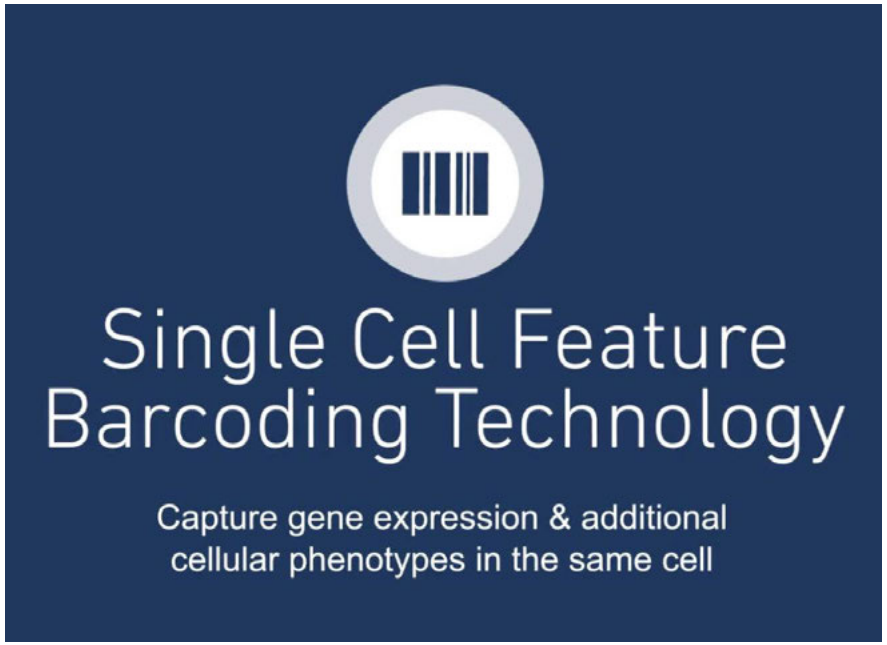
Single-cell chromosomal accessibility profiles of immune cells shows that the **AP-1 family TFs are the most affected by ageing across all cell types and subtypes and are further upregulated in COVID-19**

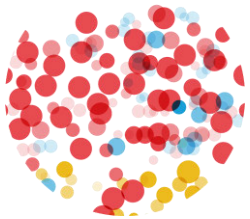
- Identification of aging-related cell-type-specific transcriptional and chromosomal accessibility changes
- **Aging-associated heterogeneous changes in clonality and diversity of TCRs and BCRs**
- **Aging increases expression of COVID-19 susceptibility genes**
- **COVID-19 enhances upregulation of aging-induced inflammatory genes**

doi.org/10.1007/s13238-020-00762-2

Feature Barcode Technology

Measuring multi-modalities from the same cell



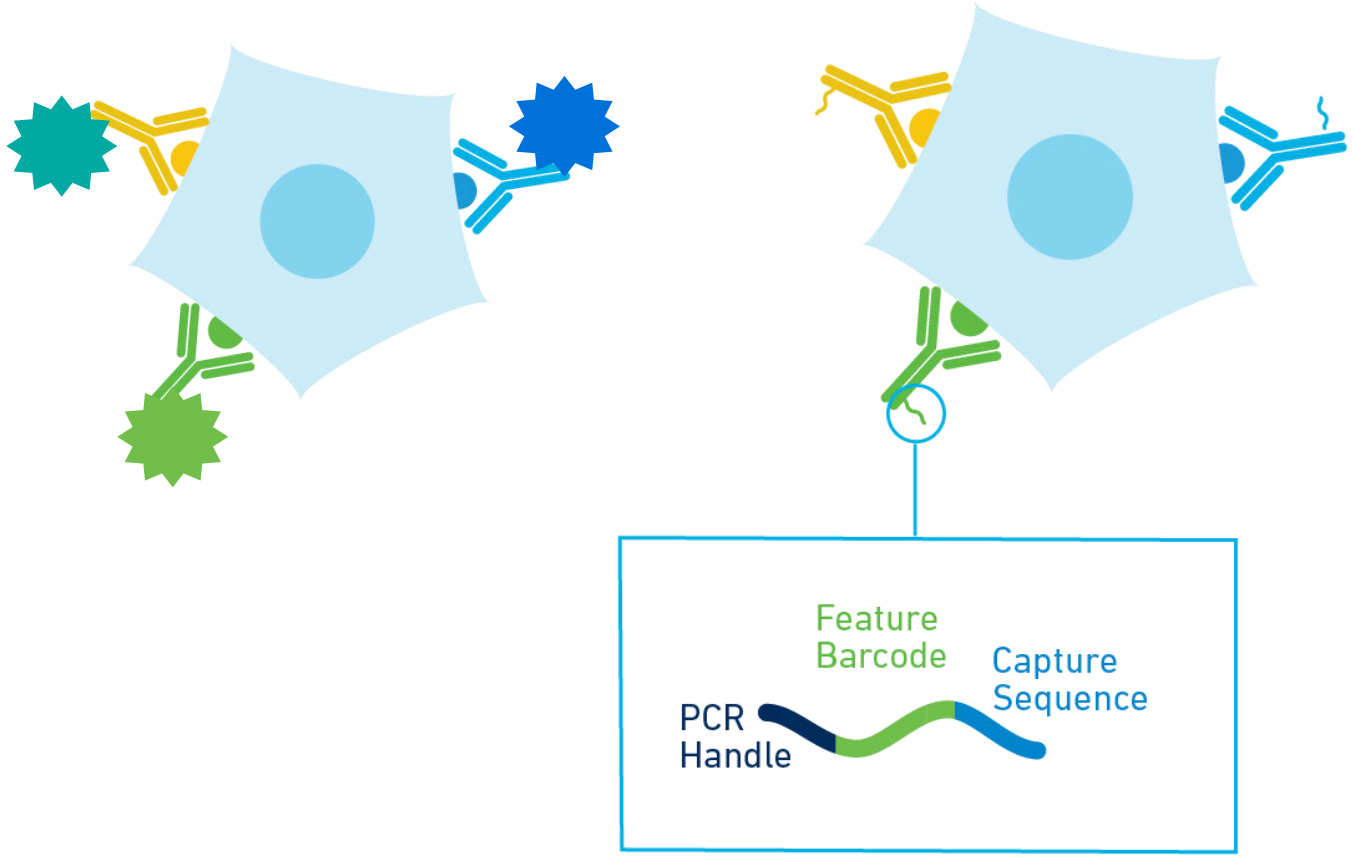


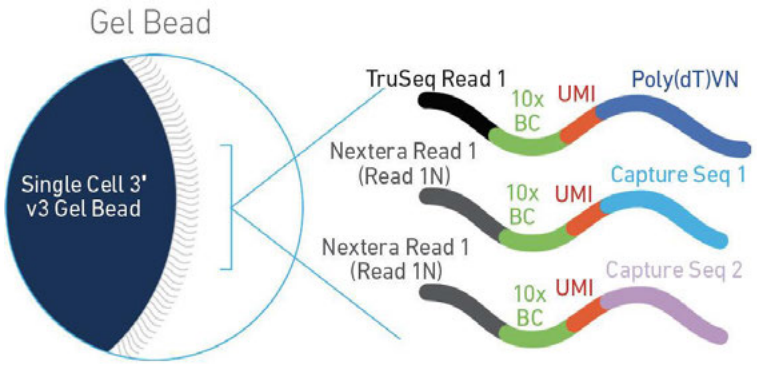
+ X + Y + Z...

Gene Expression

Moving beyond transcriptome ...

Feature Barcode Technology: Molecular (digital) cytometry

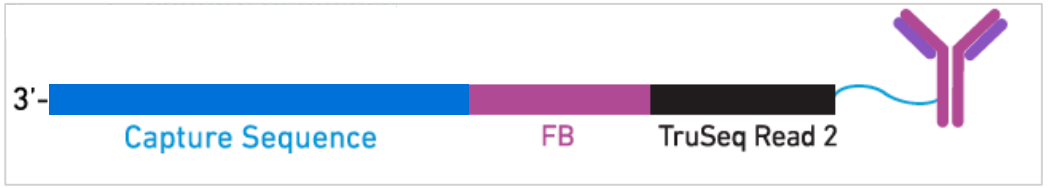




Potential thousands tags to detrmine cell surface proteins

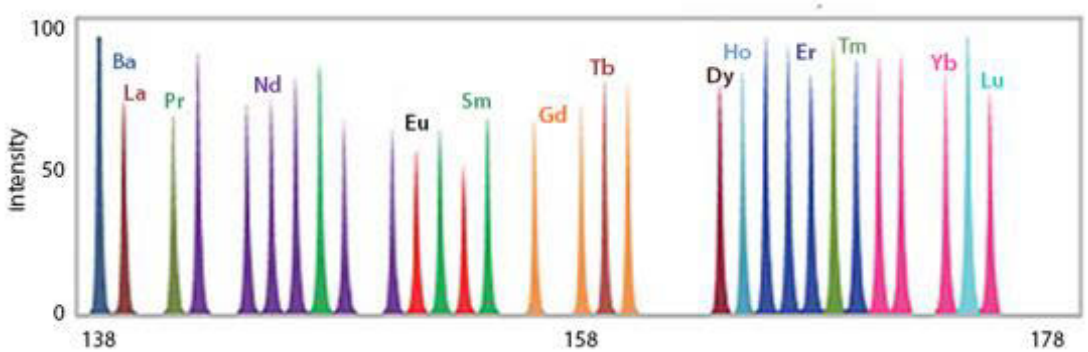
Scale

Feature Barcoding Technology
(Distinct 15nt Barcodes)



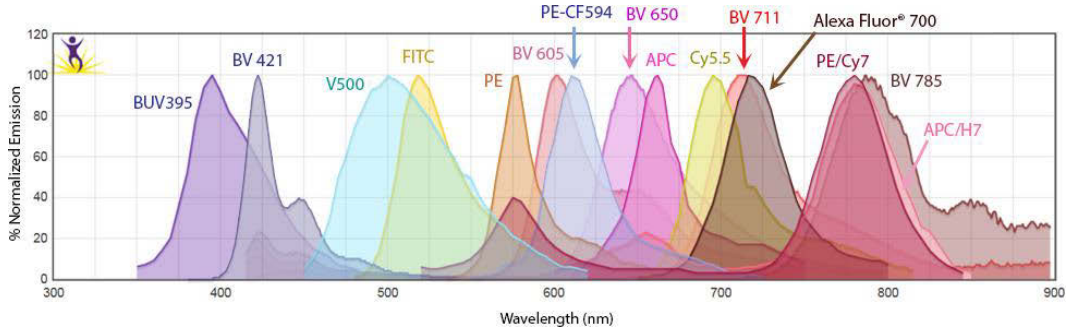
> 1 billion parameters (4^{15})

CyTOF
(Distinct Mass Isotopes)



~70 parameters

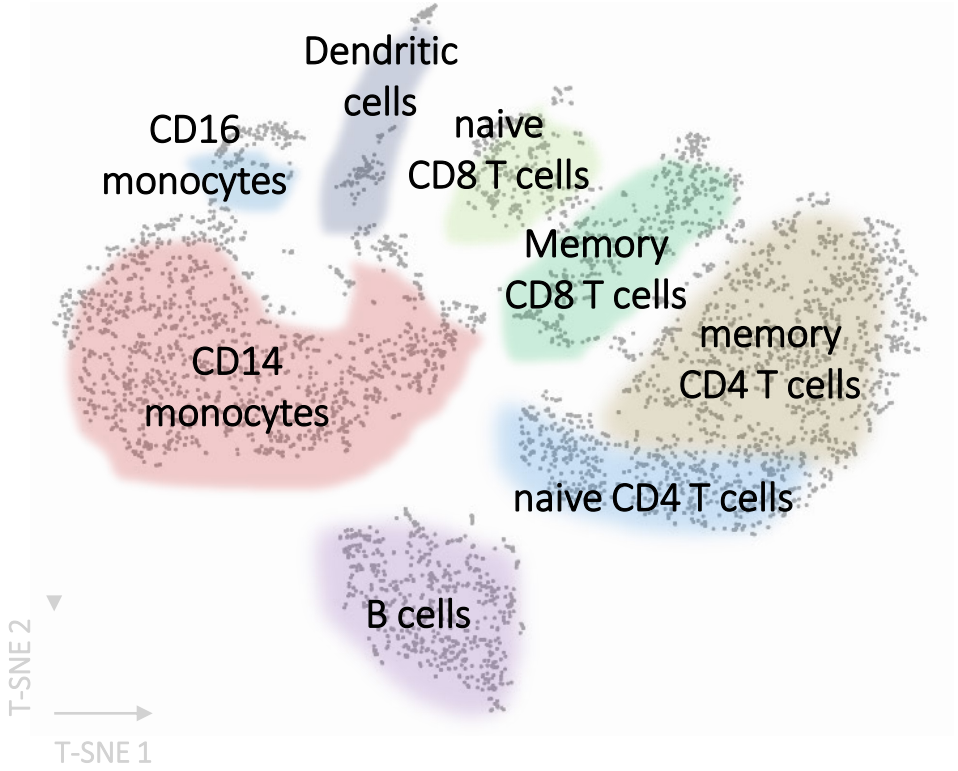
Flow
(Distinct Emissions)

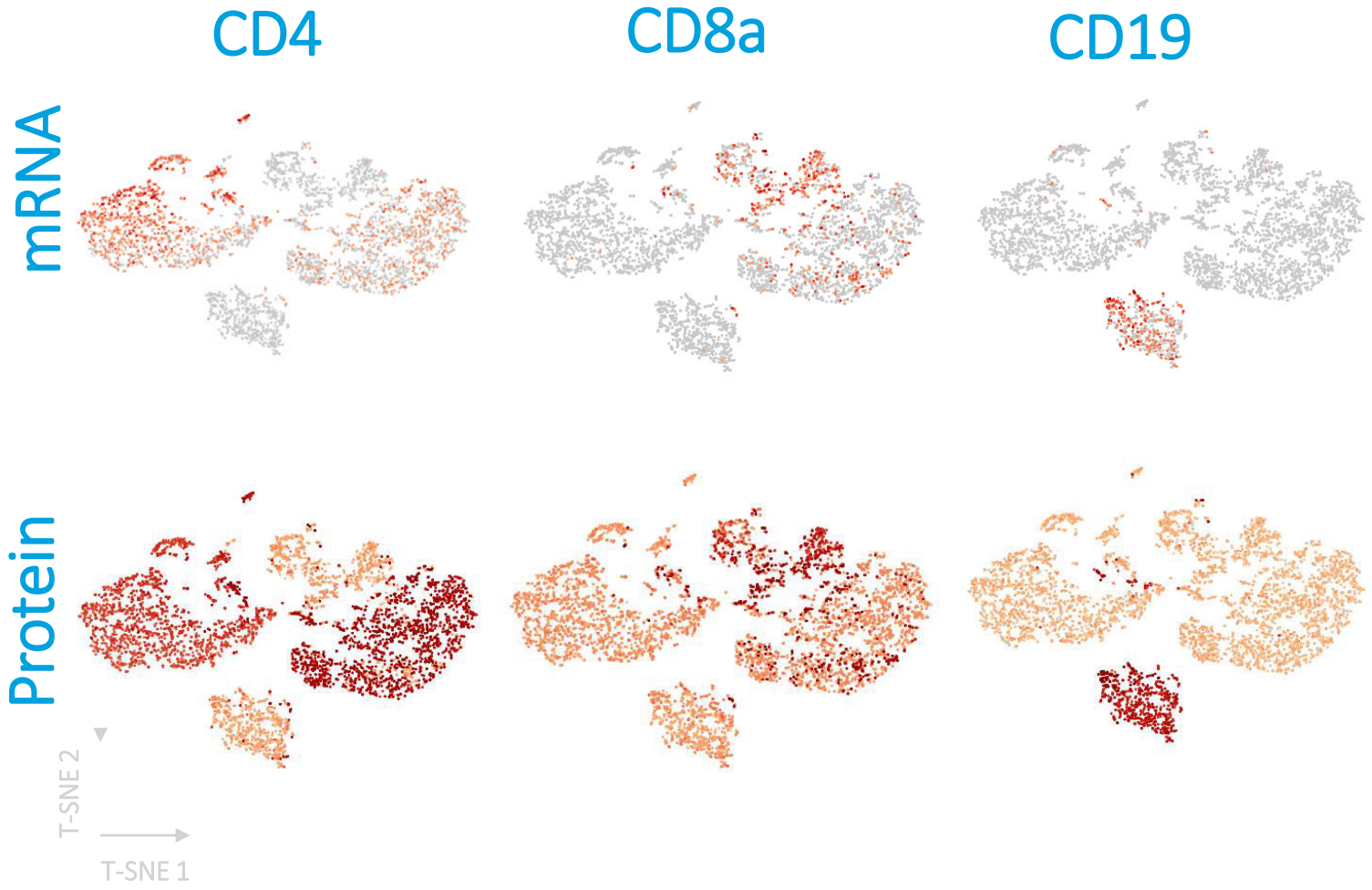


15-20 parameters

Single cell multi omics

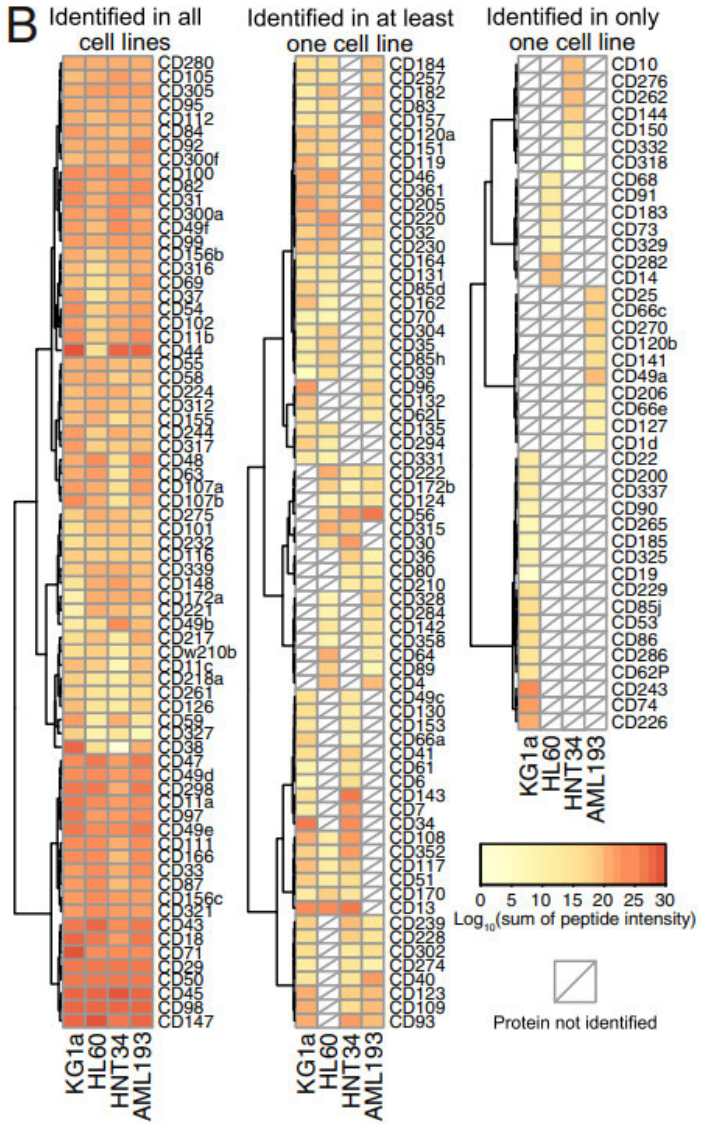
Detecting Low Expressed Biomarkers with Feature Barcoding





Multiomics of azacitidine-treated AML cells reveals variable and convergent targets that remodel the cell-surface proteome

- Acute myeloid leukemia (AML) is a disease of abnormal hematopoietic differentiation with aberrant epigenetic alterations.
- **Azacitidine (AZA) is a DNA methyltransferase inhibitor** widely used to treat MDS and AML
- Yet the impact of AZA on the cell-surface proteome has not been defined. **To identify potential therapeutic targets for use in combination with AZA** in AML patients, we investigated the effects of AZA treatment on four AML cell lines representing different stages of differentiation.
- **One gene encoding a surface protein, TRPM4, was found to be commonly up-regulated by AZA treatment** in all four cell lines and may represent a **potential novel therapeutic target for AML in combination with AZA.**



PNAS | January 8, 2019 | vol. 116 | no. 2 | 695–700

A pan-cancer blueprint of heterogeneous tumor microenvironment revealed by single–cell profiling

J Qian. *Cell Research,* 2020

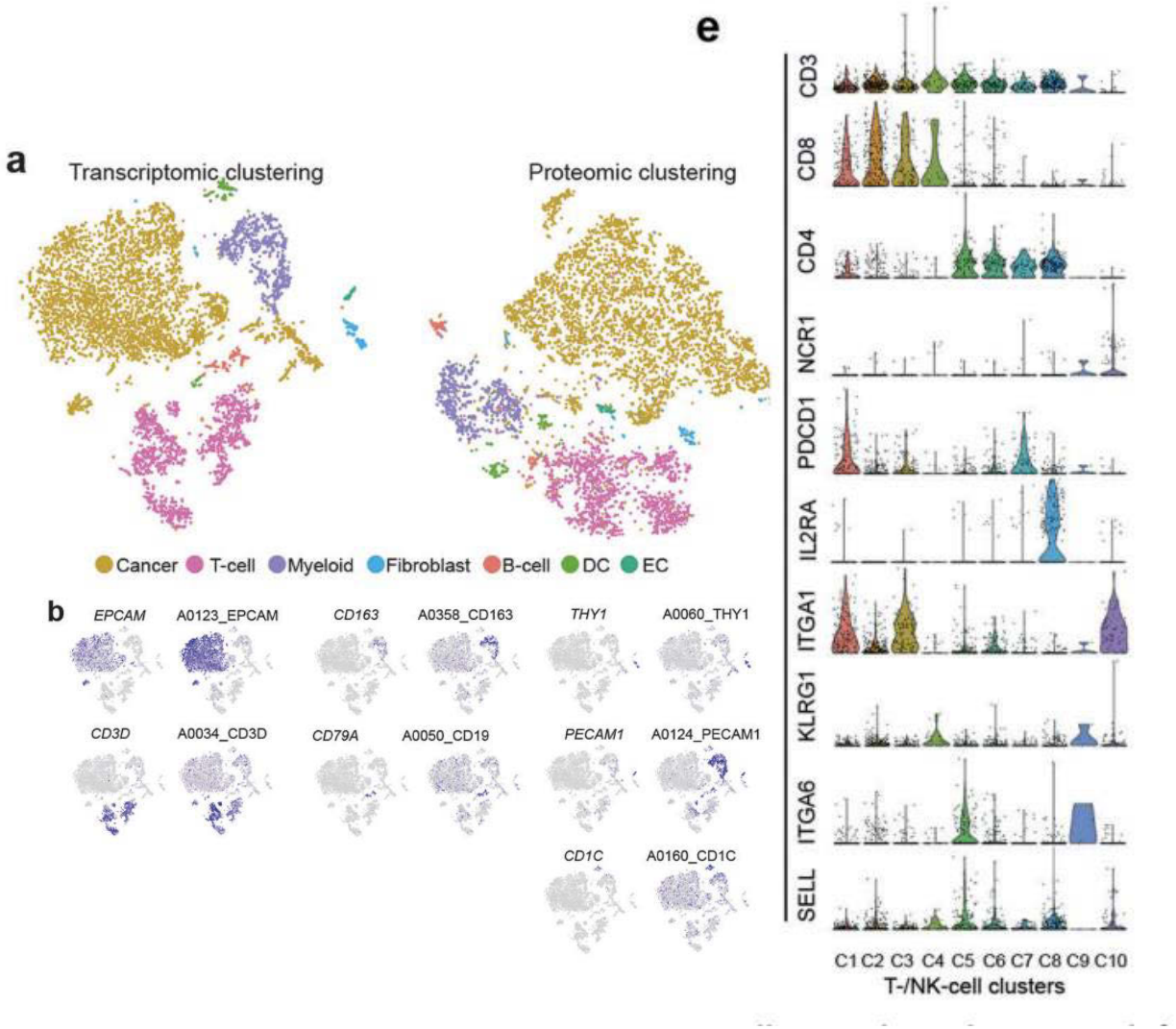
Profiled the transcriptomes of more than 233,000 cells cancer (n=36) *(lung, colorectal, ovary, breast)*

An outstanding question is to what extent thisTME heterogeneity is similar between cancers affecting different organs.

Majority of cell phenotypes have previously not been characterized in detail at single-cell level.

Pooled T-/NK-cells with both RNA and protein data together. Selected marker genes amongst the **198 antibodies** and explored protein expression per cluster.

Were identified 68 stromal cell populations, of which 46 are shared between cancer types and 22 are unique.



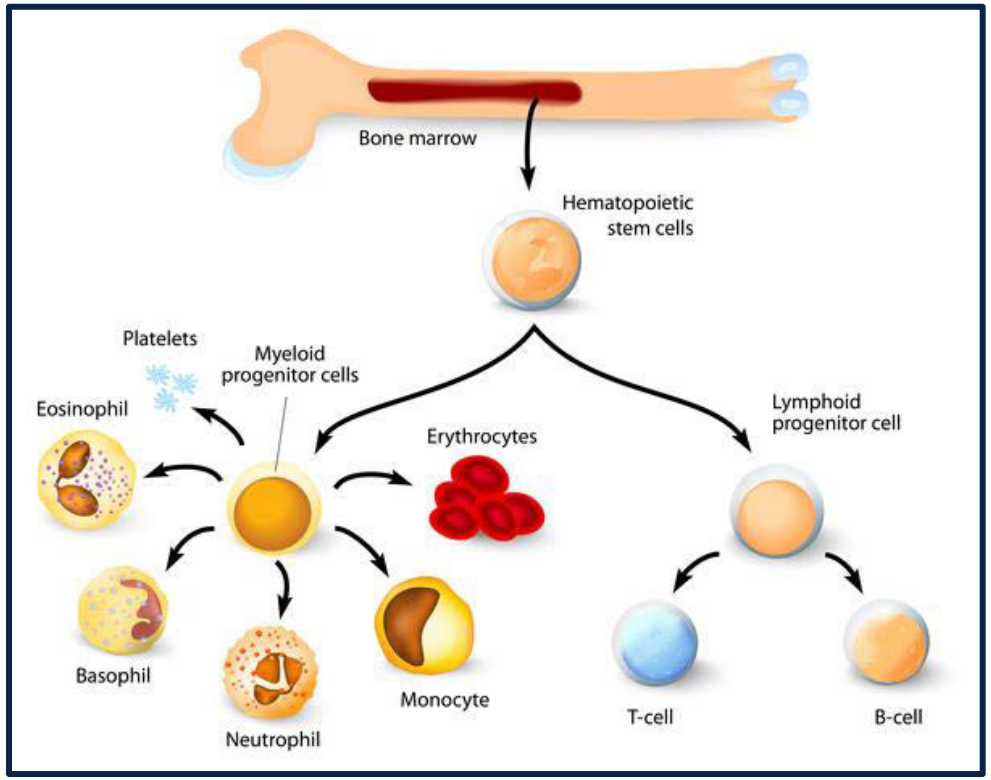
https://doi.org/10.1038/ s41422-020-0355-0.

10X GENOMICS

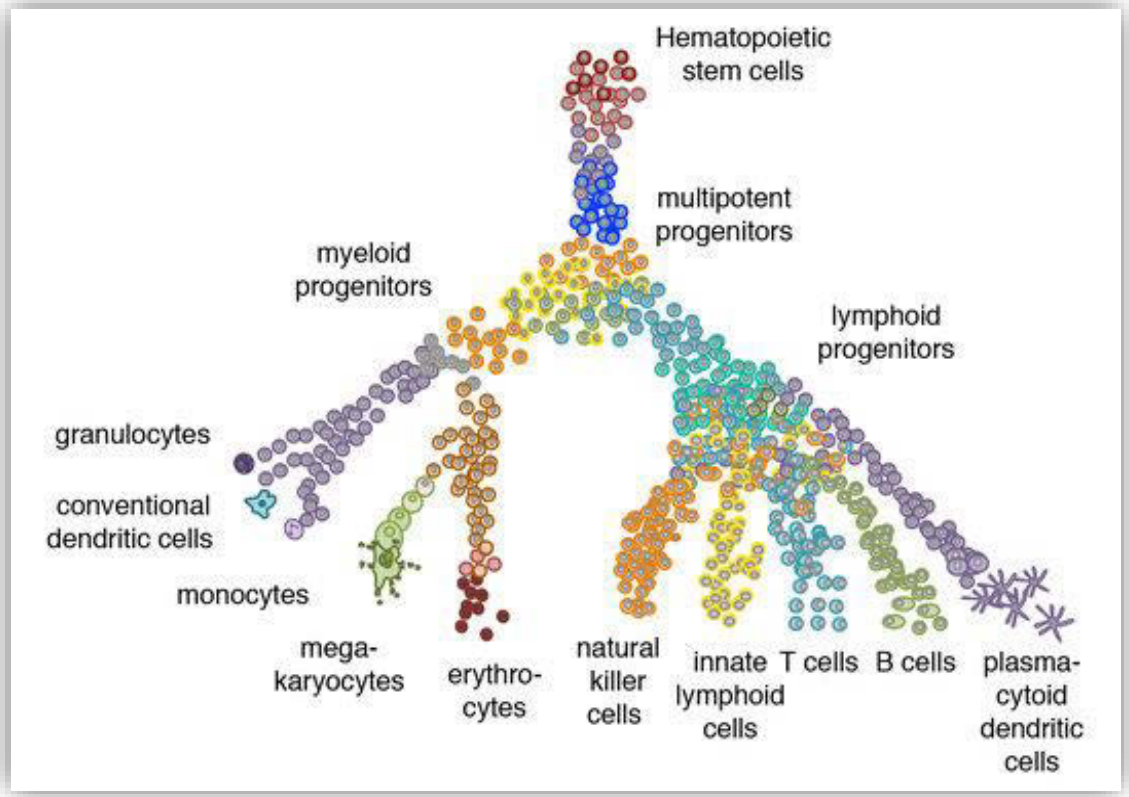
Single Cell Immune Profiling

A „Rosetta Stone” for immunology

Heterogeneity is a common factor in disease – environment matters



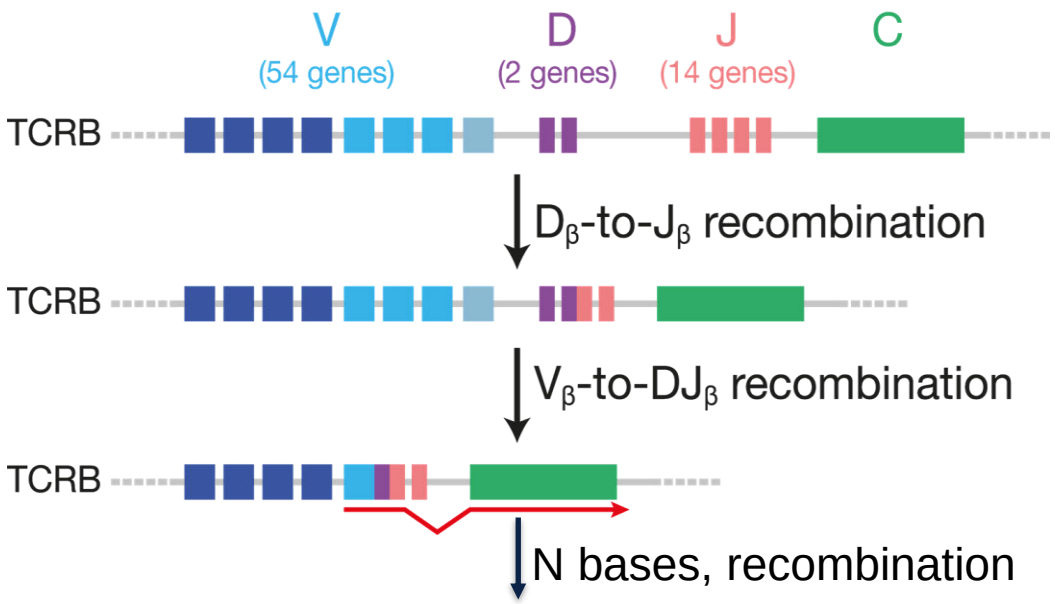
Evaluate Cell States and Progression



***Adapted from* © Dominic Grün.**
https://www.ie-freiburg.mpg.de/4836851/immuncell_differenciation

Generating Immune Repertoire Diversity (TCR/BCR)

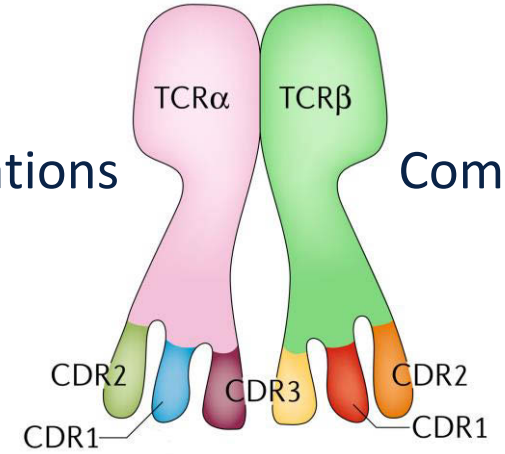
V(D)J Recombination



10^{14} Combinations

Paired rearranged TCRs

10^{7} Combinations



10^{14} Combinations

10^{21} Paired Possibilities

Paired V(D)J TCR sequences

Cells with TCR clonotypes identified

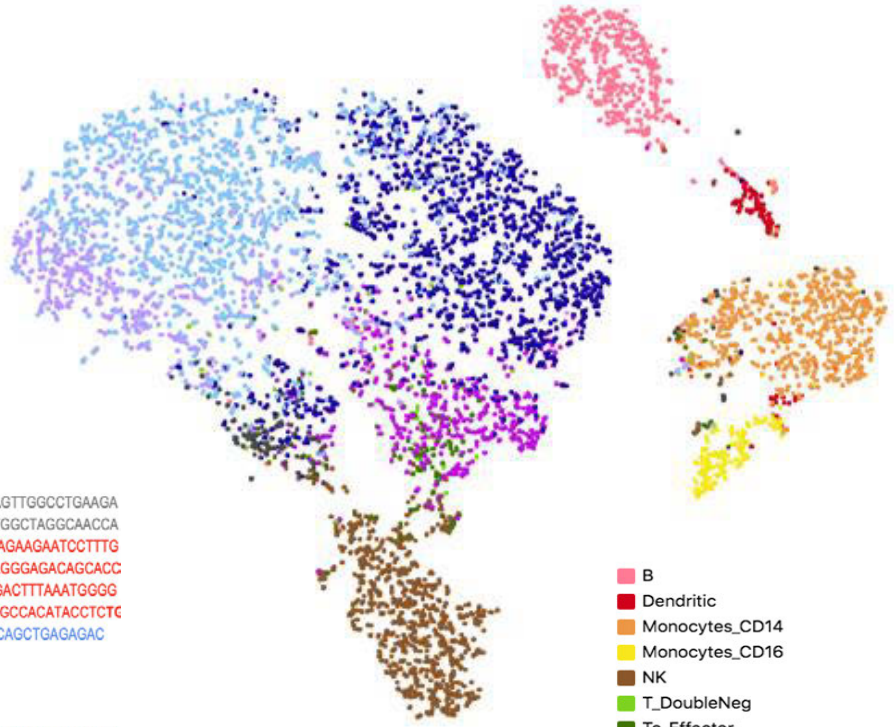
Drill down to exact paired V(D)J sequence of any T Cell

TCR Alpha

TGGGGAGTCCACAGTTAGGCAGCGCACTTCTGAAAGGGTGTCAATTCTCGTTGTGTTCCACACTCATGGGGGATTTCCTCAAAGTTTATGAAGTTGGCCTGAAGA
AAGCAGTTACTTCGACCCCTCTTTGTGAACCACAGGAAACTGAACATAGGGCAGAGCGTTTCTAGGACATTTATGGGAGCTGGTCTCTGATTGGCTAGGCAACCA
GATGCAGAATCCTGTTTTCTGAAGCAGAATATTTCCACAGATTTTGGCTGCAAAACGTTTTTCTGCTGTGGGTACGTGAGCAGGAAACATGGAGAAGAATCCTTTG
GCAGCCCCATTACTAATCCTCTGGTTTCATCTTGACTGCGTGAGCAGCATACTGAACGTGGAACAAAGTCCTCAGTCACTGCATGTTCAGGAGGGAGACAGCACC
AATTTCACCTGCAGCTTCCCTTCCAGCAATTTTTATGCCTTACACTGGTACAGATGGGAAACTGCAAAAAGCCCCGAGGCCTTGTTTGTAATGACTTTAAATGGGG
ATGAAAAGAAGAAAGGACGAATAAGTGCCACTCTTAATACCAAGGAGGGTTACAGCTATTTGTACATCAAAGGATCCCAGCCTGAAGACTCAGCCACATACCTCTC
TGCCTTCATCACCGGTAACCAGTTCTATTTTGGGACAGGGACAAGTTTGACGGTCATTCCAAATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGAC

TCR Beta

GAGAGTCCTGCTCCCCTTTCATCAATGCACAGATACAGAAGACCCCTCCGTCATGCAGCATCTGCCATGAGCATCGGCCTCCTGTGCTGTGCAGCCTTGTCTCTC
CTGTGGGCAGGTCCAGTGAATGCTGGTGTCACTCAGACCCCAAAATTCCAGGTCCTGAAGACAGGACAGAGCATGACACTGCAGTGTGCCCAGGATATGAACCA
TGAATACATGTCCTGGTATCGACAAGACCCAGGCATGGGGCTGAGGCTGATTCATTACTCAGTTGGTGCTGGTATCACTGACCAAGGAGAAGTCCCCAATGGCTA
CAATGTCTCCAGATCAACCACAGAGGATTTCCCGCTCAGGCTGCTGTCGGCTGCTCCCTCCCAGACATCTGTGTACTTCTGTGCCAGCAGCCTGGTTACCGGGA
CAGGGGCATACGGCTACACCTTCGGTTCGGGGACCAGGTTAACCGTTGTAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGA



	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
α	TRAV8-2		TRAJ8	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	

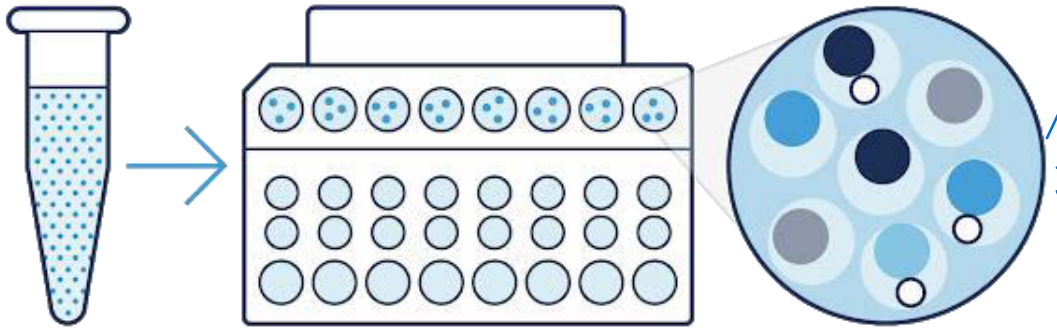
Filter: Cluster

Cluster: Identity: Th_Memory

Single cell immune profiling library output

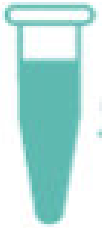
Cells

Chromium Controller



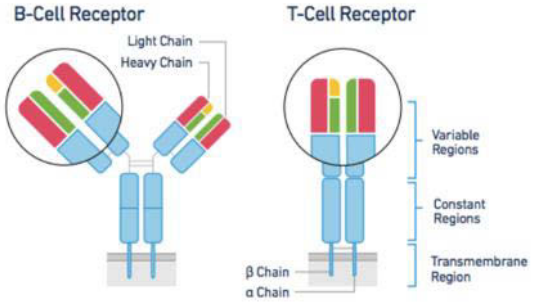
Libraries

V(D)J

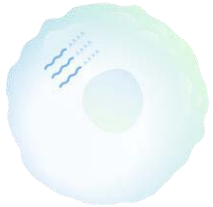


5’GEX

Feature Barcode

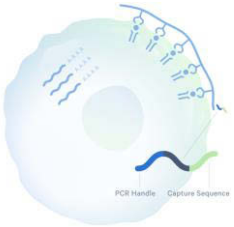


Full length paired chains



Whole Transcriptome Profiling
(or)
Targeted Gene Profiling

Surface protein profiling



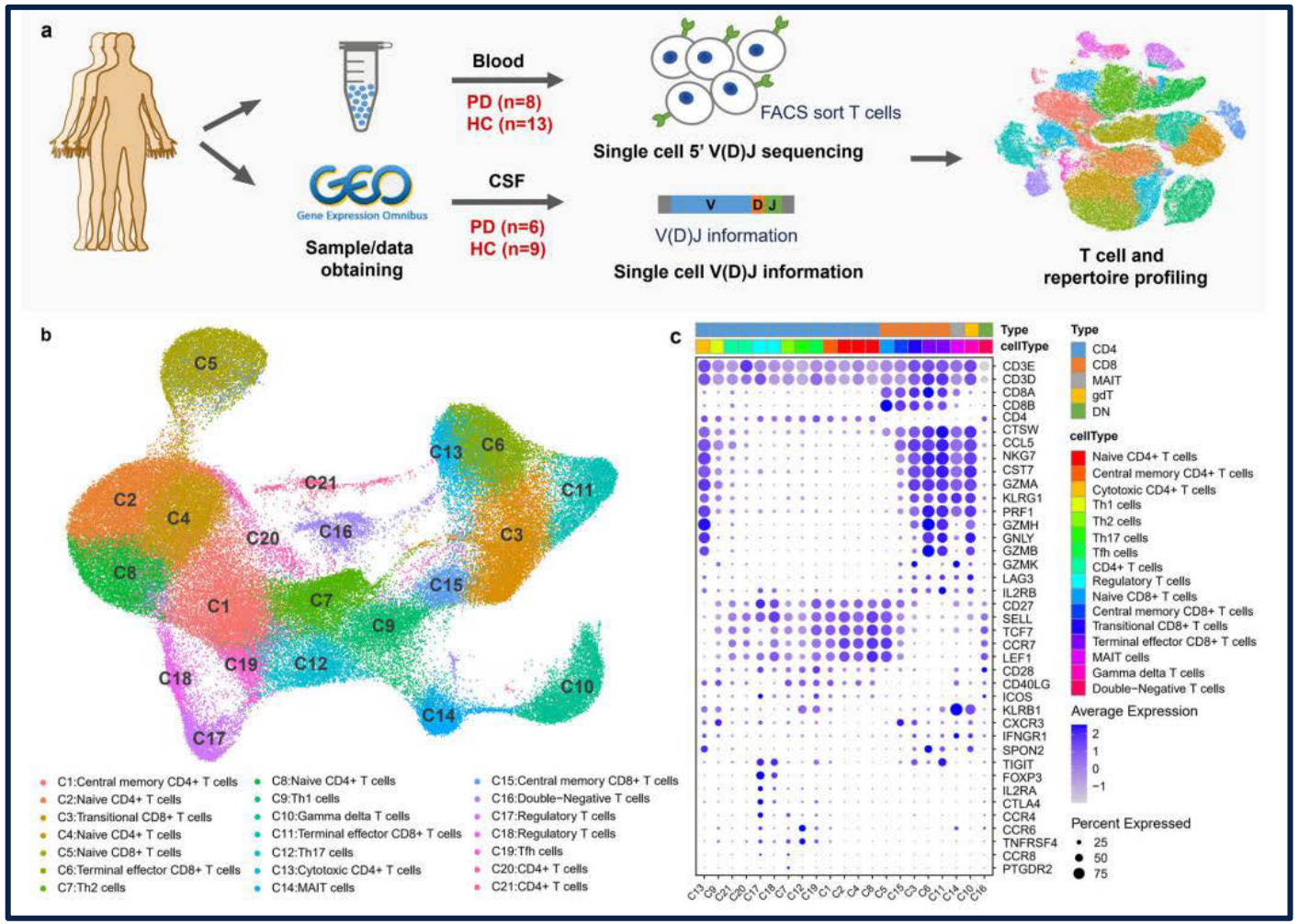
Antigen Specificity

Single-cell transcriptome and TCR profiling reveal activated and expanded T cell populations in Parkinson's disease

Increasing studies suggest that immune system dysfunction plays important roles in the pathogenesis of PD.

Given the chronic inflammatory nature of Parkinson's disease (PD), T cell immunity may be important for disease onset.
It was performed single-cell transcriptome and TCR sequencing, and conducted integrative analyses to decode composition, function and lineage relationship of T cells in the **blood and cerebrospinal fluid of PD.**

In total, **21 T cell subsets** with distinct functions were identified from 103,365 T cells. Integrative analyses of single-cell gene expression and TCRs revealed connectivity and potential differentiation trajectories of these subtypes and provided novel evidence of clonal expansion of T lymphocytes patrolling in the blood and cerebrospinal fluid of PD

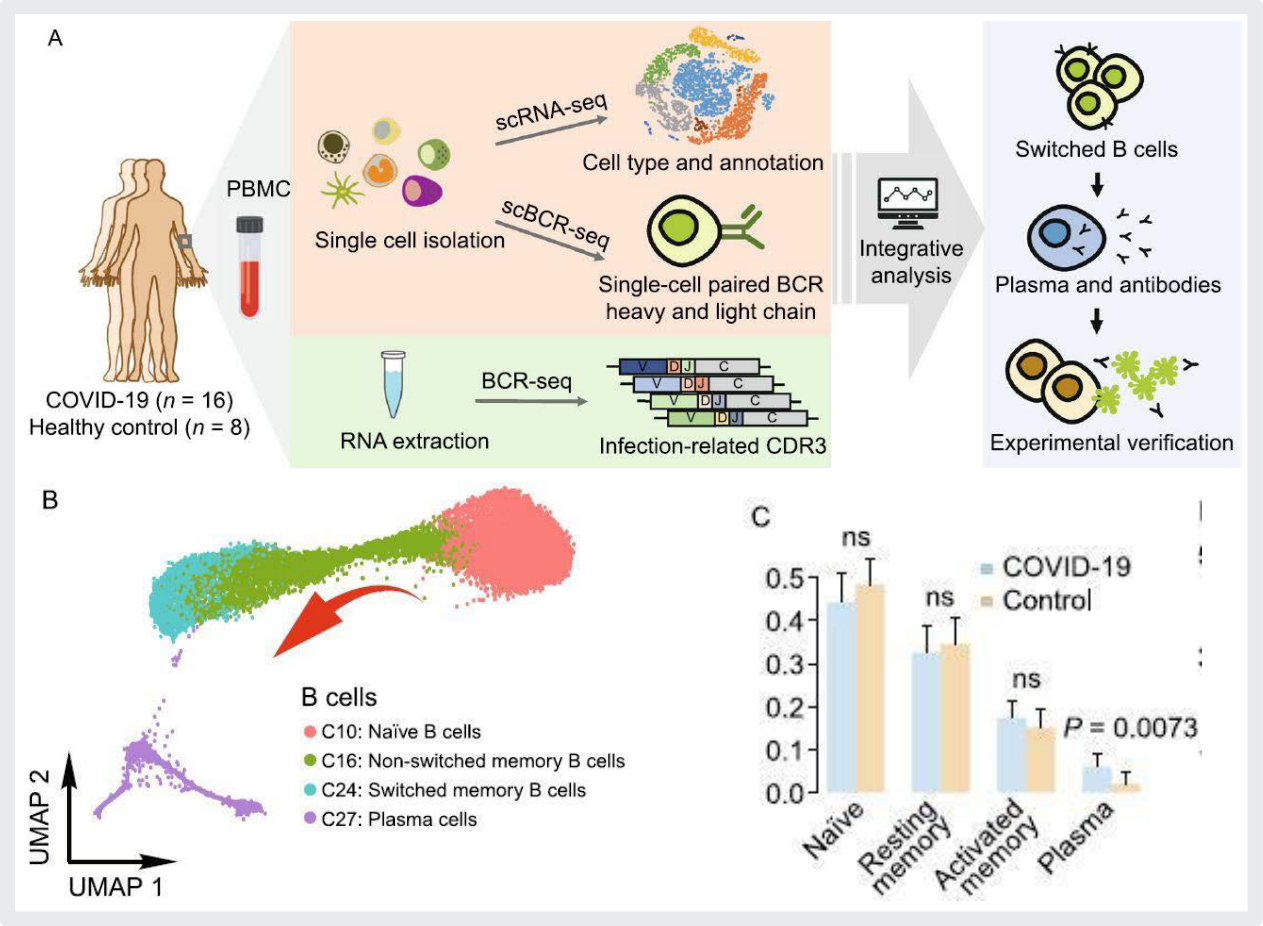


https://www.nature.com/articles/s41421-021-00280-3.pdf

Novel neutralizing antibody against SARS-CoV-2 revealed by single cell immune profiling

Li et al., 2020, Protein & Cell

- Chromium Single Cell Immune Profiling and BCR sequencing **to identify therapeutically relevant neutralizing antibody in early-stage recovered COVID-19 patients**
- Authors observed **a gradient of transcriptional states from naïve B cells to activated memory B cells then to plasma cells** (*fig. b*).
- COVID-19 patients showed lower BCR diversity, indicating **widespread clonal expansion upon likely antigen recognition**
- 347 BCR groups were selected for further study, of which 14 novel antibodies binding to S protein were identified (ELISA) and one (GD1-69) showed the highest neutralizing activity



DOI: https://doi.org/10.1007/s13238-020-00807-6

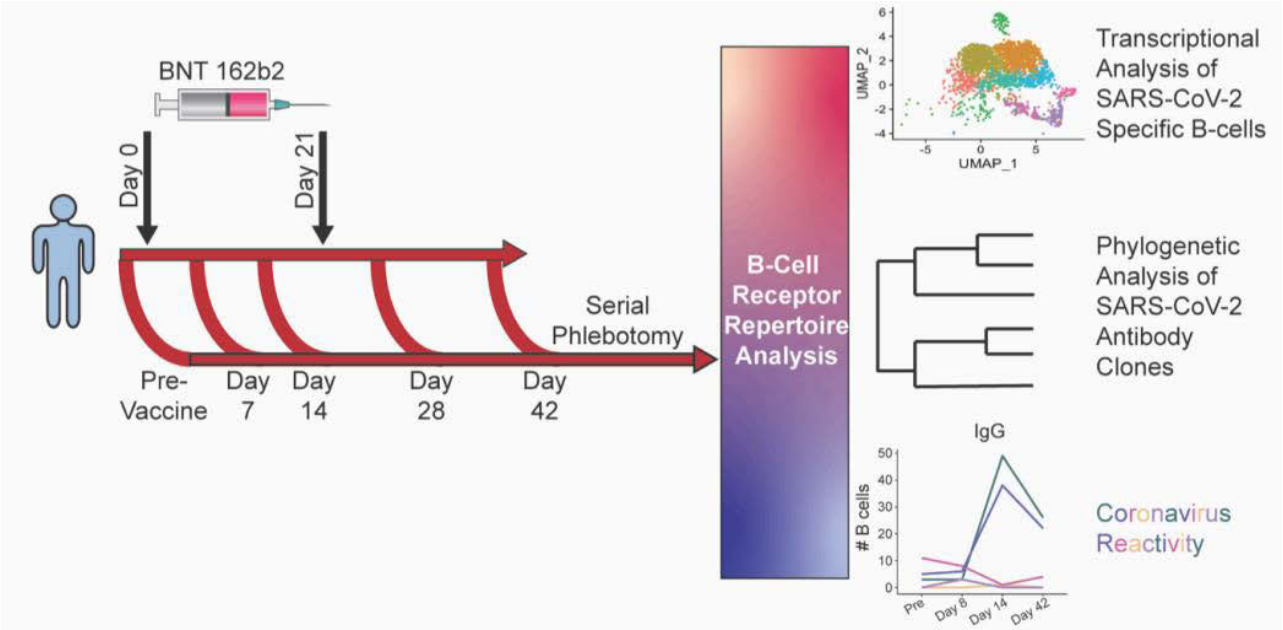
Single-Cell Profiling of the Antigen-Specific Response to BNT162b2 SARS-CoV-2 RNA Vaccine

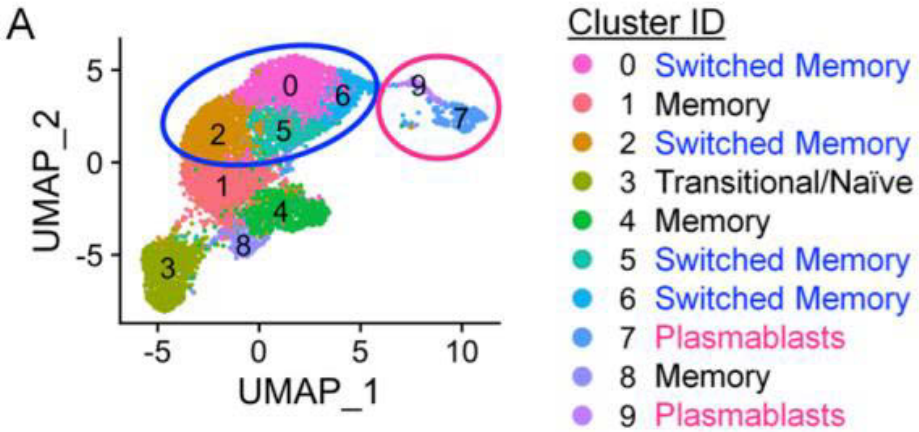
RNA-based vaccines against SARS-CoV-2 are critical to limiting COVID-19 severity and spread.

Cellular mechanisms driving antigen-specific responses to these vaccines, however, remain uncertain.

B cells, T cells, and other leukocytes undergo significant shifts upon SARS-CoV-2 infection that may contribute to anti-viral immunity and protective antibodies

Here was used single-cell technologies to identify and characterized antigen-specific cells and antibody responses to the RNA vaccine BNT162b2 in longitudinal samples from a cohort of healthy donors.



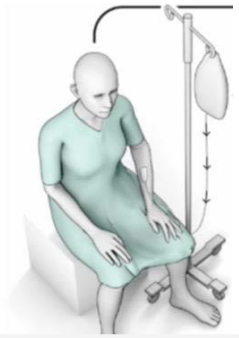


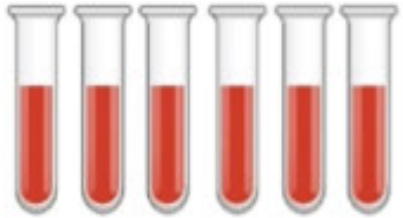
doi: https://doi.org/10.1101/2021.07.28.453981
Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville,

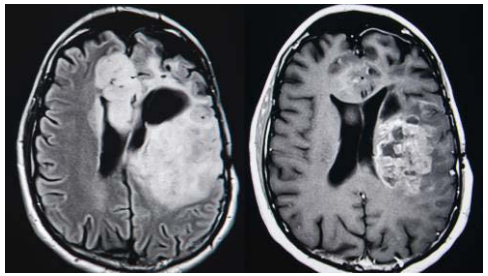
Applying single cell siquencing to advance cell therapies

Leveraging the 10x toolkit across pre-clinical and translational clinical applications

Target Discovery → **Characterization of Infusion Product** → **Response Monitoring** → **Resistance Profiling**







Resource — Cell

Pooled Knockin Targeting for Genome Engineering of Cellular Immunotherapies

Theodore L. Roth,[1,2,3,4,5,16,*] P. Jonathan Li,[3,4,5,15] Franziska Blaeschke,[3,4,5,15] Jasper F. Nies,[3,4,5,15] Ryan Apathy,[3,4,5,15] Cody Mowery,[1,2,3,4,5,15] Ruby Yu,[3,4,5] Michelle L.T. Nguyen,[3,4,5] Youjin Lee,[3,4,5] Anna Truong,[3,4,5] Joseph Hiatt,[1,2,3,4,5] David Wu,[1,2] David N. Nguyen,[3,4,5,6] Daniel Goodman,[3,4,5] Jeffrey A. Bluestone,[6,7,8] Chun Jimmie Ye,[7,8,10,11,12,13] Kole Roybal,[3,8,9,14] Eric Shifrut,[3,4,5] and Alexander Marson[3,4,5,6,7,8,14,16,17,*]

Cell — CellPress

Article

Single-Cell Analyses Identify Brain Mural Cells Expressing CD19 as Potential Off-Tumor Targets for CAR-T Immunotherapies

Kevin R. Parker,[1,2,18] Denis Migliorini,[3,4,5,18,*] Eric Perkey,[5,7] Kathryn E. Yost,[1,2] Aparna Bhaduri,[8,9] Puneet Bagga,[10,20] Mohammad Haris,[10,11,12] Neil E. Wilson,[10] Fang Liu,[4] Khatuna Gabunia,[4] John Scholler,[4] Thomas J. Montine,[13] Vijay G. Bhoj,[3,14] Ravinder Reddy,[10] Suyash Mohan,[10] Ivan Maillard,[7] Arnold R. Kriegstein,[8,9] Carl H. June,[3,4,14] Howard Y. Chang,[1,2,19] Avery D. Posey, Jr.,[3,4,16,17,19] and Ansuman T. Satpathy[2,3,18,21,*]

nature medicine — ARTICLES

https://doi.org/10.1038/s41591-020-1061-7

Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas

Qing Deng[1,2], Guangchun Han[2,5], Nahum Puebla-Osorio[1], Man Chun John Ma[1], Paolo Strati[1], Beth Chasen[1], Enyu Dai[2], Minghao Dang[2], Neeraj Jain[1], Haopeng Yang[1], Yuanxin Wang[2], Shaojun Zhang[2], Ruiping Wang[2], Runzhe Chen[2], Jordan Showell[1], Sreejoyee Ghosh[1], Sridevi Patchva[1], Qi Zhang[1], Ryan Sun[4], Frederick Hagemeister[1], Luis Fayad[1], Felipe Samaniego[1], Hans C. Lee[1], Loretta J. Nastoupil[1], Nathan Fowler[1], R. Eric Davis[1], Jason Westin[1], Sattva S. Neelapu[1,2], Linghua Wang[2] and Michael R. Green[1,2]

nature COMMUNICATIONS — ARTICLE

https://doi.org/10.1038/s41467-019-13880-1 OPEN

Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunotherapy

Alyssa Sheih[1,8], Valentin Voillet[2,8], Laila-Aicha Hanafi[1,8], Hannah A. DeBerg[3], Masanao Yajima[4], Reed Hawkins[1], Vivian Gersuk[3], Stanley R. Riddell[1,5,6], David G. Maloney[1,5,6], Martin E. Wohlfahrt[1], Dnyanada Pande[1], Mark R. Enstrom[1], Hans-Peter Kiem[1,5,7], Jennifer E. Adair[1,5,6], Raphaël Gottardo[2,5,6], Peter S. Linsley[3] & Cameron J. Turtle[1,5,6+]

Science — RESEARCH ARTICLES

Cite as: E. A. Stadtmauer *et al.*, *Science* 10.1126/science.aba7365 (2020).

CRISPR-engineered T cells in patients with refractory cancer

Edward A. Stadtmauer,[1,2*†] Joseph A. Fraietta,[2,3,4,5*] Megan M. Davis,[2,6] Adam D. Cohen,[1,2] Kristy L. Weber,[2,7] Eric Lancaster,[8] Patricia A. Mangan,[1] Irina Kulikovskaya,[4] Minnal Gupta,[4] Fang Chen,[4] Lifeng Tian,[9] Vanessa E. Gonzalez,[4] Jun Xu,[4] In-young Jung,[4,7] J. Joseph Melenhorst,[2,3,4] Gabriela Plesa,[2] Joanne Shea,[2] Tina Matlawski,[2] Amanda Cervini,[2] Avery L. Gaymon,[2] Stephanie Desjardins,[2] Anne Lamontagne,[2] January Salas-Mckee,[2] Andrew Fesnak,[2,4] Donald L. Siegel,[3,4] Bruce L. Levine,[1,4] Julie K. Jadlowsky,[2] Regina M. Young,[2] Anne Chew,[5] Wei-Ting Hwang,[9] Elizabeth O. Hexner,[1,2] Beatriz M. Carreno,[2,5,6] Christopher L. Nobles,[5] Frederic D. Bushman,[4] Kevin R. Parker,[10] Yanyan Qi,[10] Ansuman T. Satpathy,[10,11] Howard Y. Chang,[10,12] Yangbing Zhao,[1,4] Simon F. Lacey,[1,4] Carl H. June[2,3,4,6+†]

Article

c-Jun overexpression in CAR T cells induces exhaustion resistance

https://doi.org/10.1038/s41586-019-1805-z
Received: 7 September 2018
Accepted: 17 October 2019
Published online: 04 December 2019

Rachel C. Lynn[1!], Evan W. Weber[1!!], Elena Sotillo[1!!], David Gennert[1], Peng Xu[1], Zinaida Good[1,3,4], Hima Anbunathan[1], John Lattin[1], Robert Jones[1], Victor Tieu[1], Surya Nagaraja[1], Jeffrey Granja[2], Charles F. A. de Bourcy[4,5], Robbie Majzner[1], Ansuman T. Satpathy[2,6], Stephen R. Quake[4,5], Michelle Monje[1&], Howard Y. Chang[2,4,5] & Crystal L. Mackall[1,4,7!!!*]

10X GENOMICS

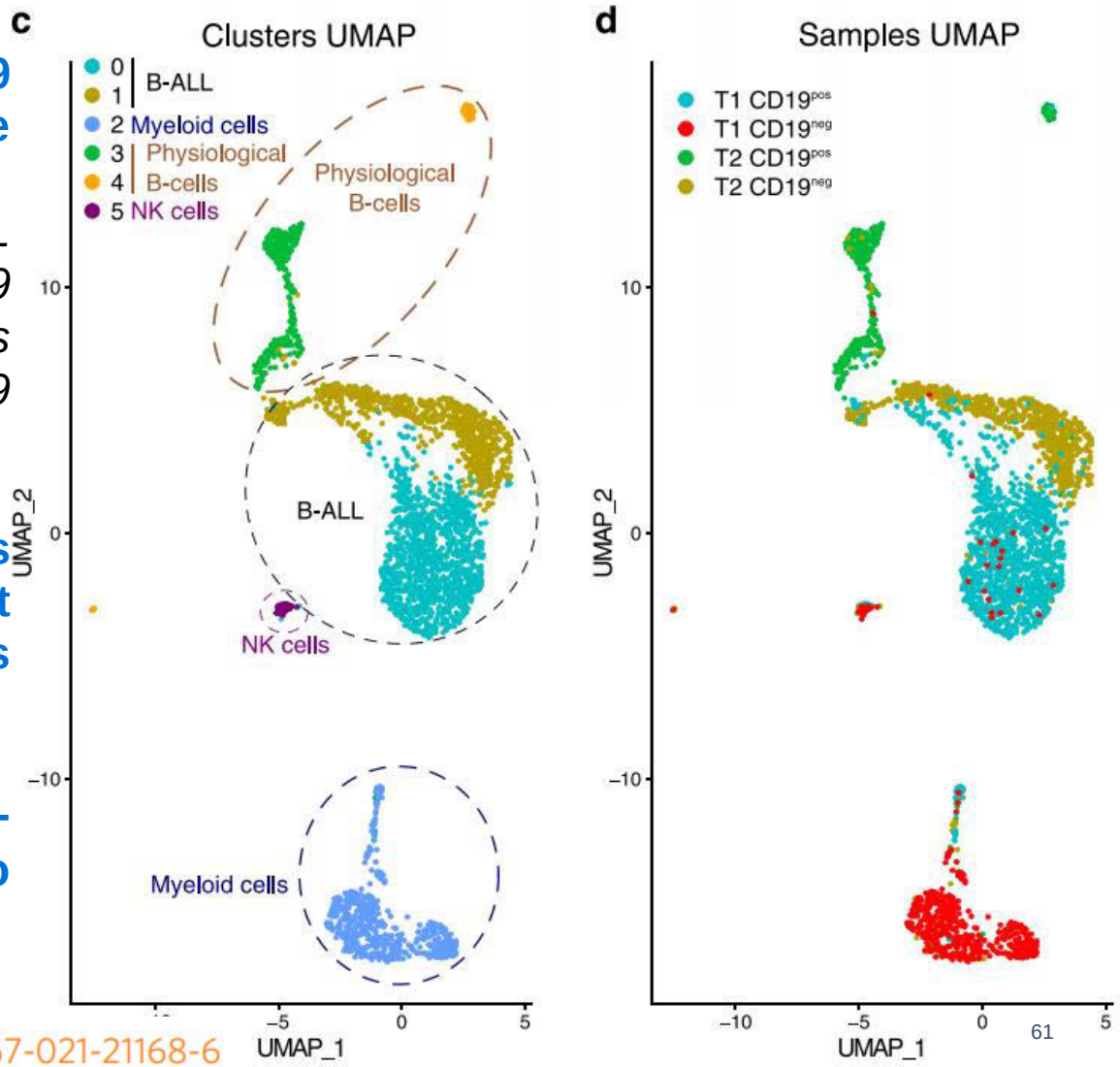
Single-cell profiling identifies pre-existing CD19-negative subclones in a B-ALL patient with CD19-negative relapse after CAR-T therapy

Around half of relapsing CD19 CAR-T patients develop CD19 negative (CD19neg) B-ALL allowing leukemic cells to evade CD19-targeted therapy.

Herin, were investigated leukemic cells of a relapsing B-ALL patient, at two-time points: before (T1) and after (T2) anti-CD19 CAR-T treatment. It was shown that at T2, the B-ALL relapse is CD19 negative due to the expression of a non-functional CD19 transcript retaining intron 2.

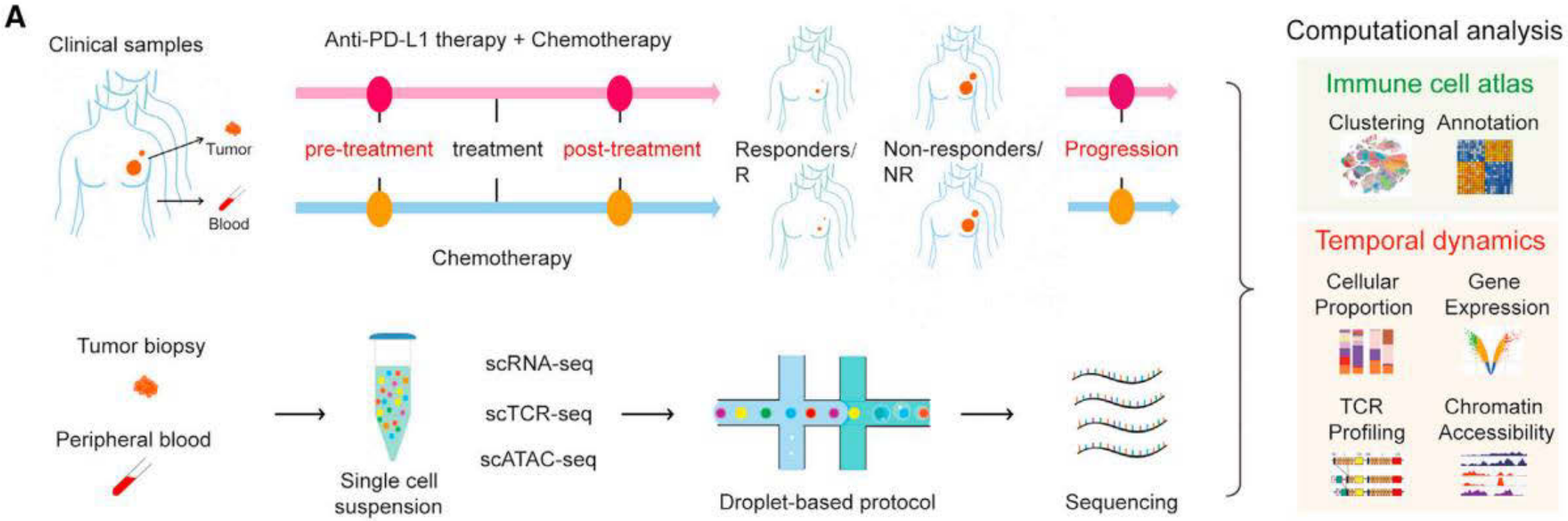
Using single-cell RNA sequencing (scRNAseq) approach, was demonstrated that CD19neg leukemic cells were present before CAR-T cell therapy and thus that the relapse results from the selection of these rare CD19neg B-ALL clones.

Study shows that scRNAseq profiling can reveal pre-existing CD19neg subclones, raising the possibility to assess the risk of targeted therapy failure.



NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-021-21168-6

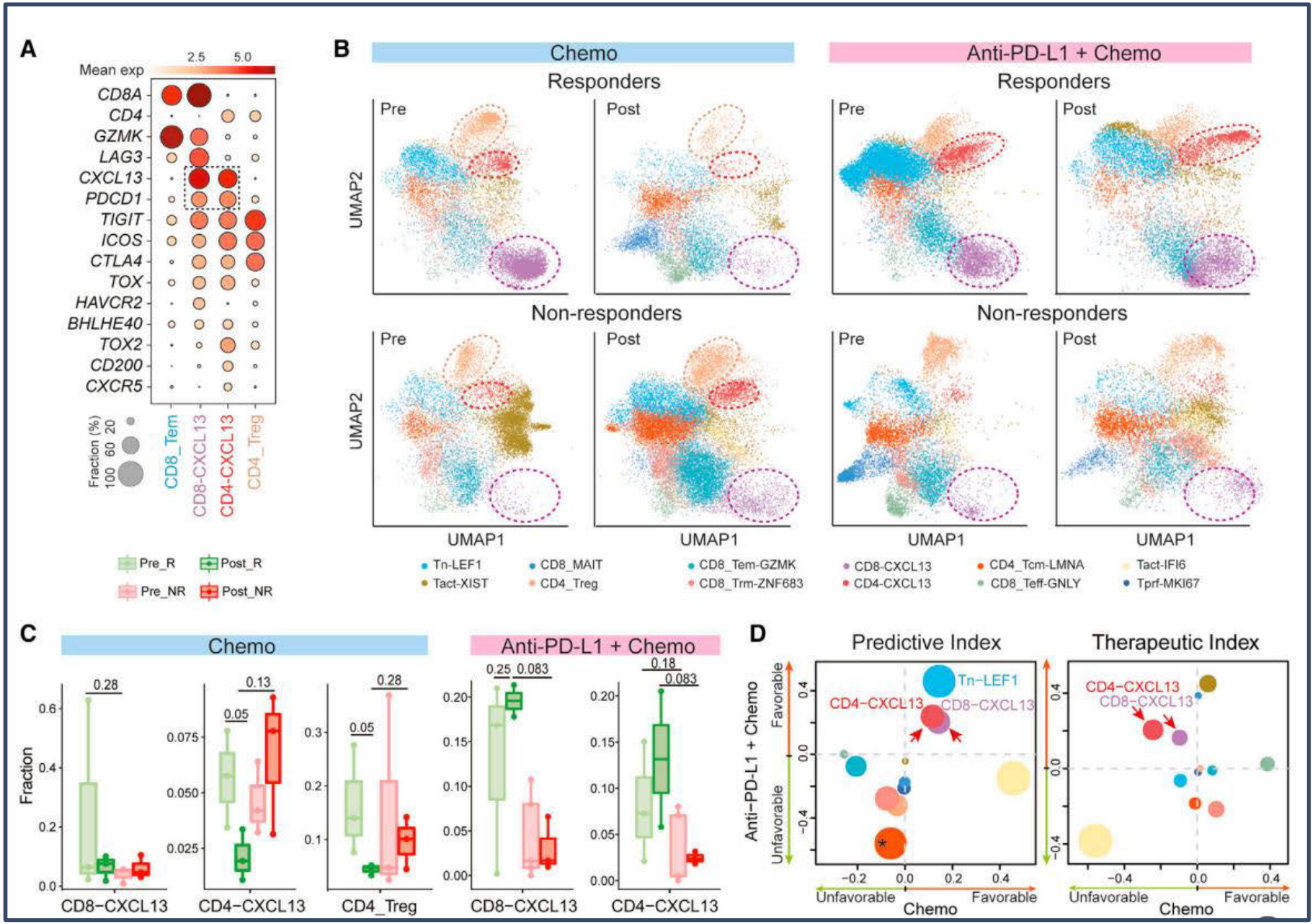
Single-cell analyses reveal key immune cell subsets associated with response to PD-L1 blockade in triple-negative breast cancer



Zhang et al. combine single-cell RNAseq, TCR-seq, and ATAC-seq to investigate immune cell dynamics in the tumor microenvironment and peripheral blood of patients with TNBC treated with paclitaxel or paclitaxel plus atezolizumab, revealing immune features of responders and nonresponders, the mechanisms and intertwined effects of paclitaxel and atezolizumab in TNBC treatment.

https://doi.org/10.1016/j.ccell.2021.09.010

Temporal dynamics of tumor-infiltrating T cell subsets



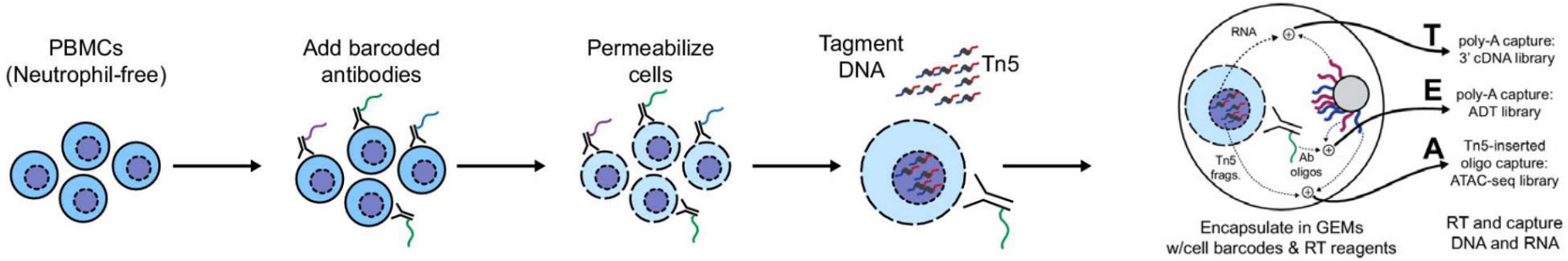
https://doi.org/10.1016/j.ccell.2021.09.010

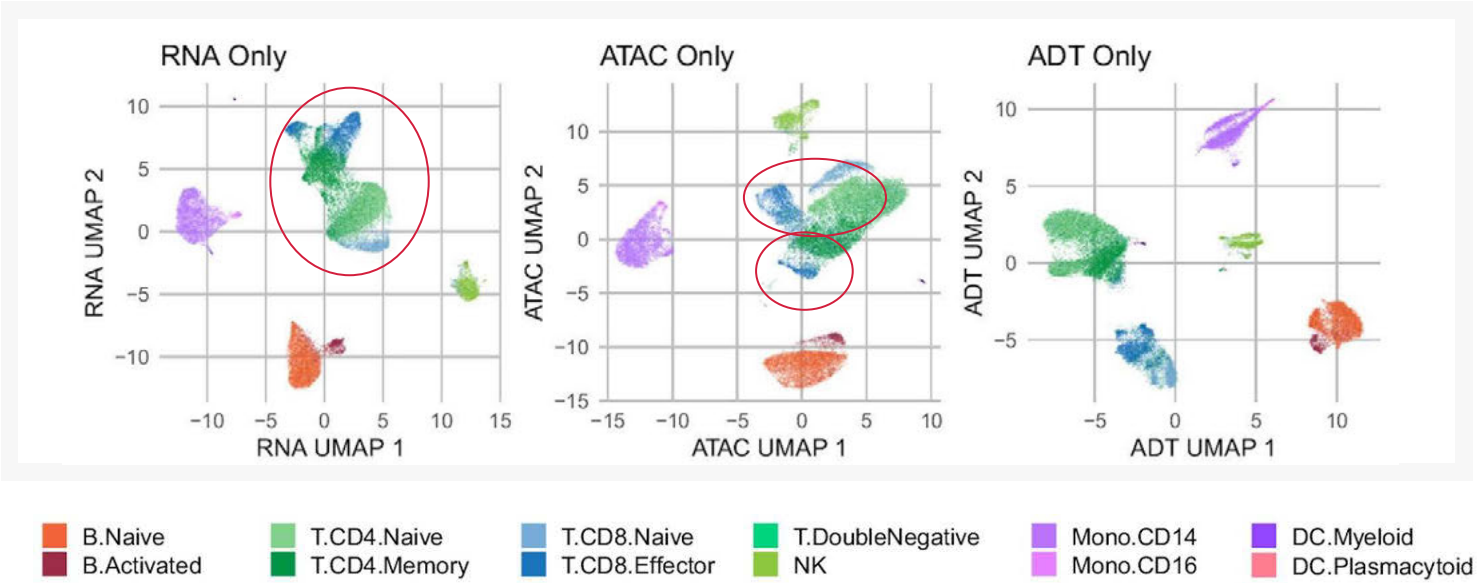
10X GENOMICS

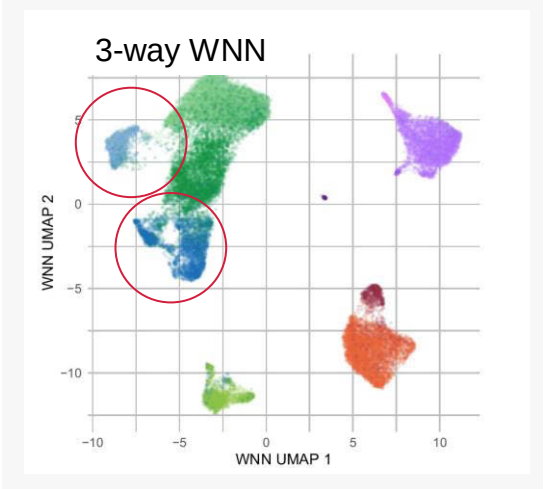
What’s more?

Measuring additional modalities increases power to separate cell states

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





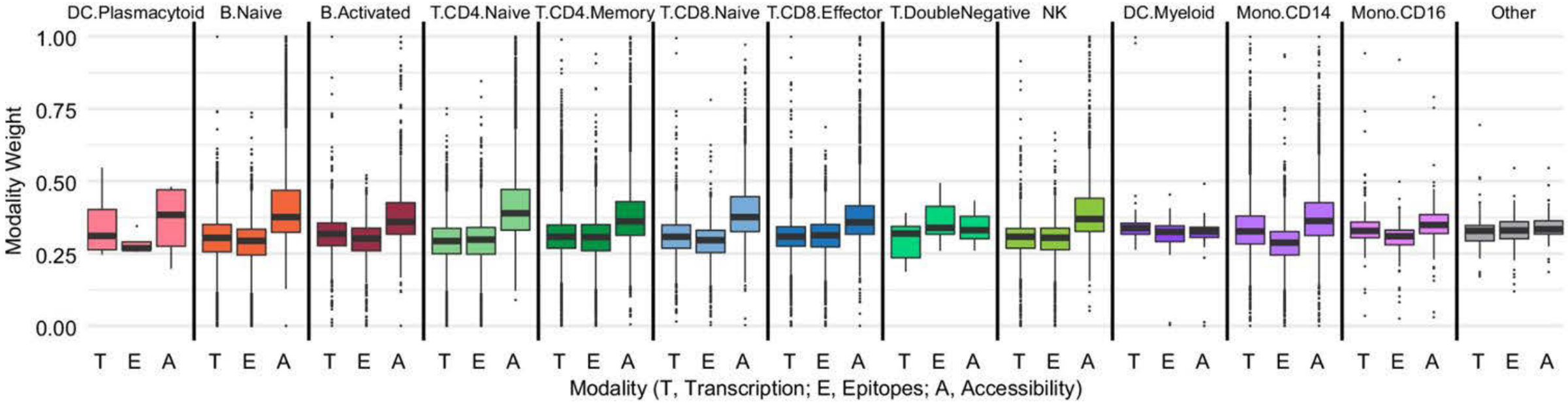


Improved resolution of CD4/CD8 T cell states when using RNA, ATAC, and protein data

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

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

Using MULTIOME 10x



Simultaneous trimodal single-cell measurement of transcripts, epitopes, and chromatin accessibility using TEA-seq

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

Learn more about TEA-seq from the authors

"Deep immune profiling at scale: Efficient pipelines and TEA-seq for simultaneous trimodal measurements"

Watch this webinar on-demand:

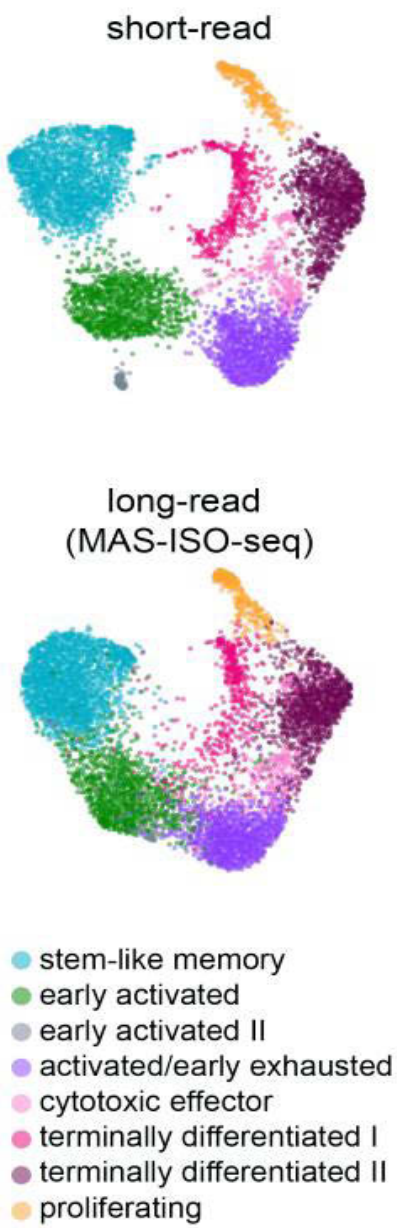
https://pages.10xgenomics.com/2021-multiomics-week.html

High-throughput RNA isoform sequencing using programmable cDNA concatenation

Alternative splicing is a core biological process that enables profound and essential diversification of gene function. Short-read RNA sequencing approaches fail to resolve RNA isoforms and therefore primarily enable gene expression measurements - an isoform unaware representation of the transcriptome.

Conversely, full-length RNA sequencing using long-read technologies are able to capture complete transcript isoforms, but their utility is deeply constrained due to throughput limitations. Here it's introduced **MAS-ISO-seq.**

This approach was used for single-cell RNA sequencing analysis of tumor-infiltrating T cells (based on 5'GEX and CSP analysis). PacBio sequencing was used.



https://www.biorxiv.org/content/10.1101/2021.10.01.462818v1.full.pdf

Coming soon

ATAC v2

- Measure chromatin accessibility at single cell resolution
- Improved signal to noise ratio
- Reduced sequencing costs

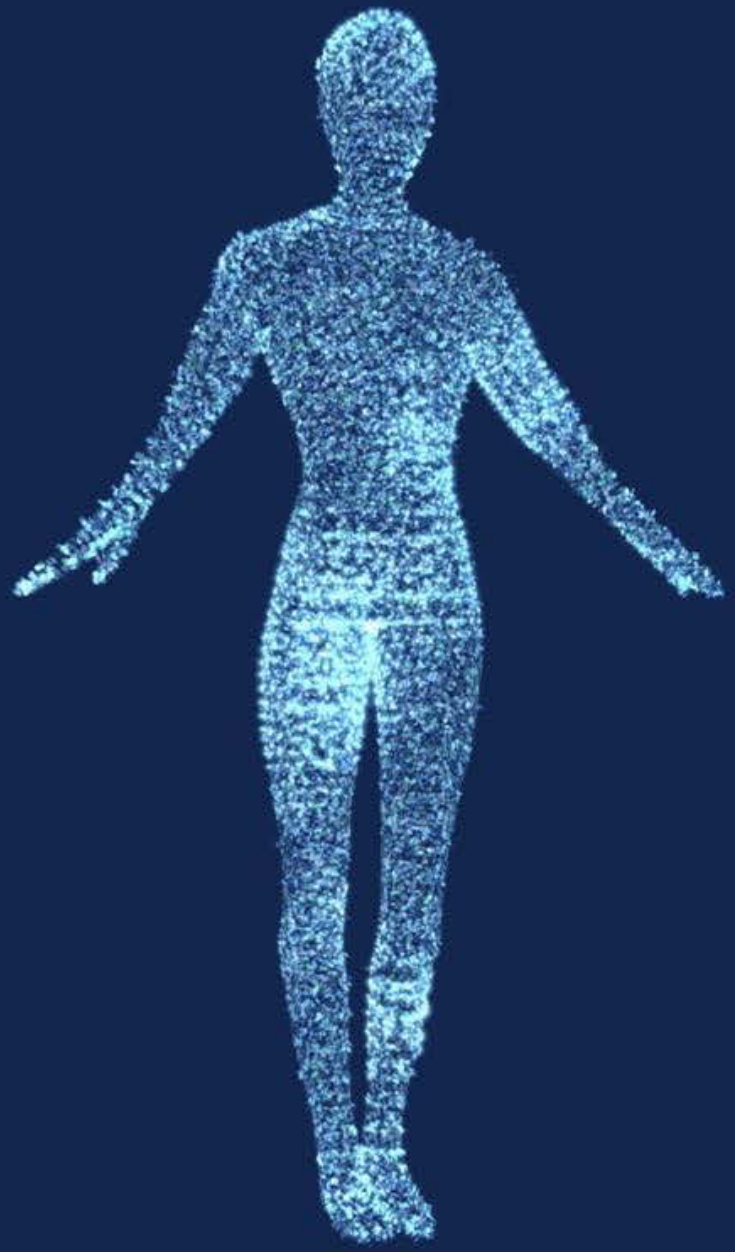
Expected **Mid 2022**

5′ CRISPR

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

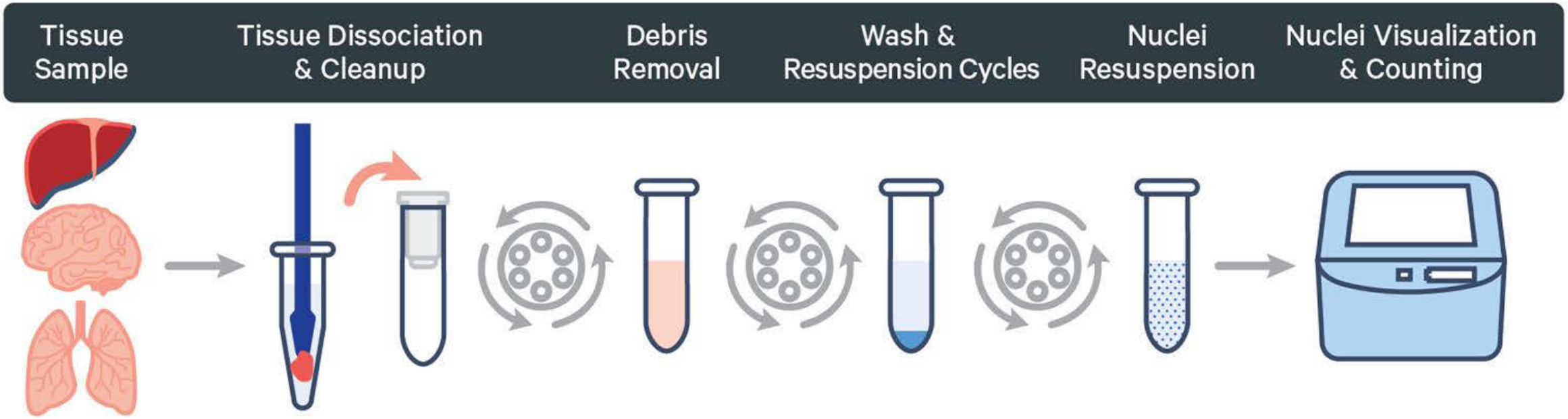
Expected **Early 2022**

Nuclei Isolation



Nuclei Isolation Kit

Streamlined sample preparation workflow

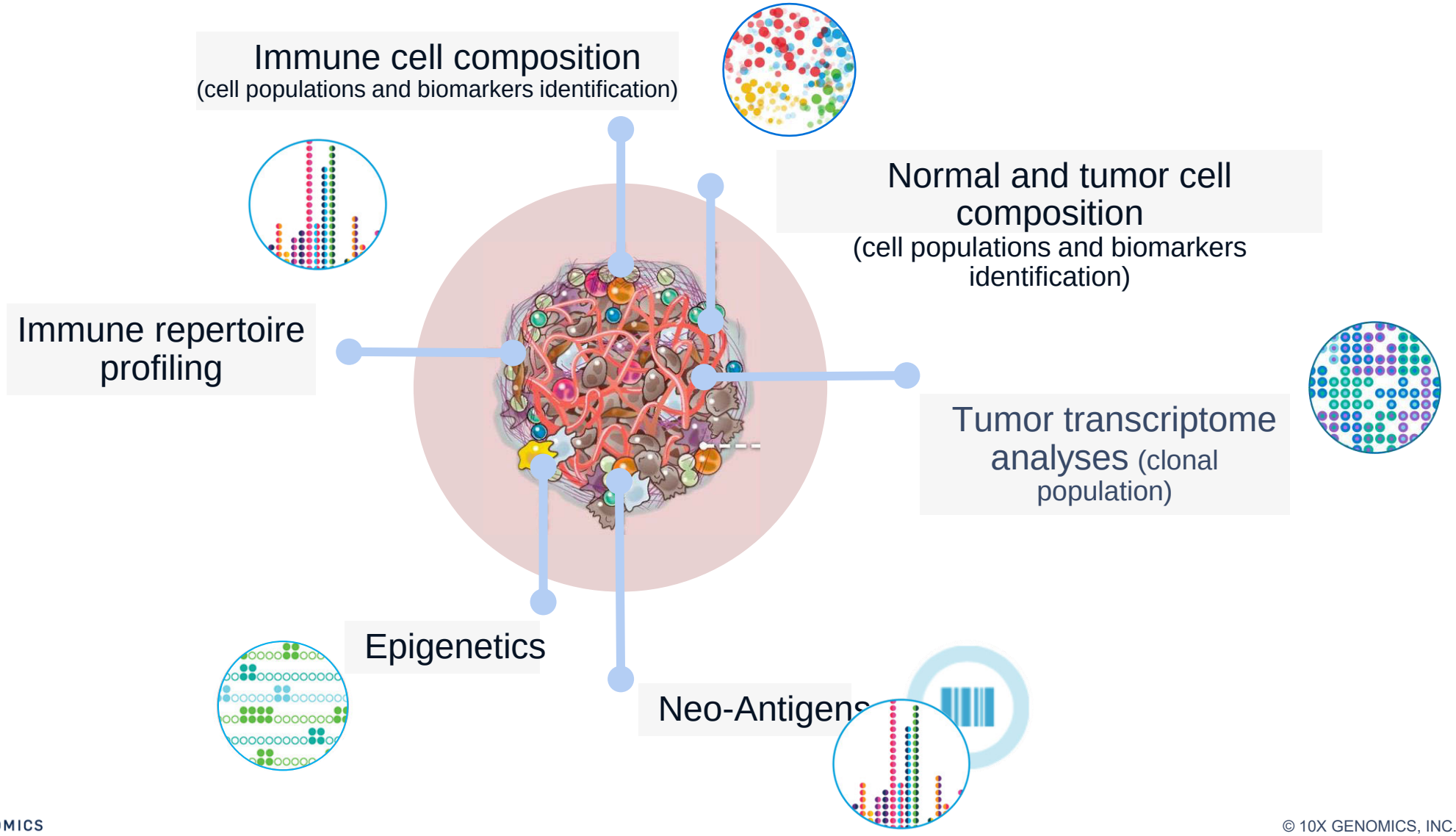


All you need is an hour of lab time, a benchtop centrifuge, and an interesting frozen sample!

Become one of single cell Ninjas ☺

https://www.singlecellninjas.com/

Summary
Integration of sample Information with 10x Genomics



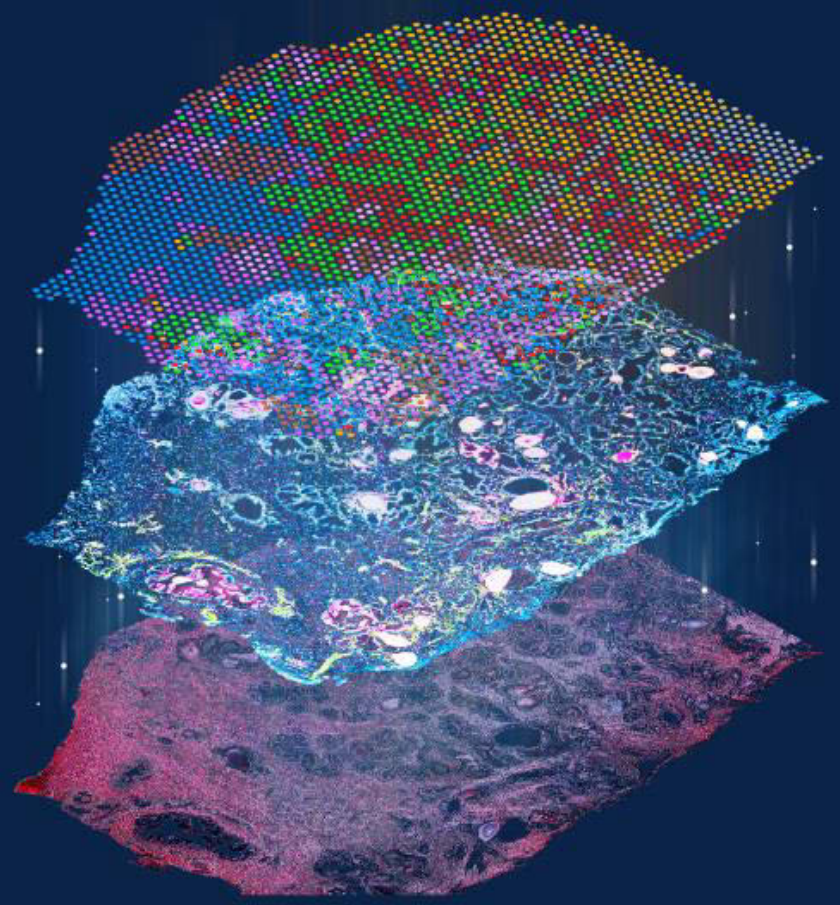
Adapted from Joyce & Fearon, Science, 2015 doi: 10.1126/science.aaa6204

Thank You from the 10x Team & our Collaborators

Agnieszka Ciesielska

Science & Technology Advisor

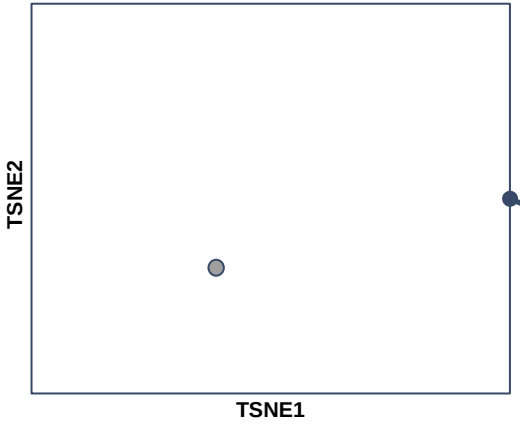
Enter the next level of complexity – spatial transcriptomics approaches



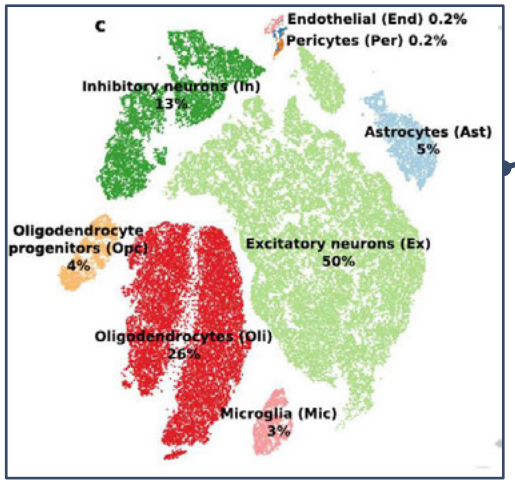
Agnieszka Ciesielska PhD

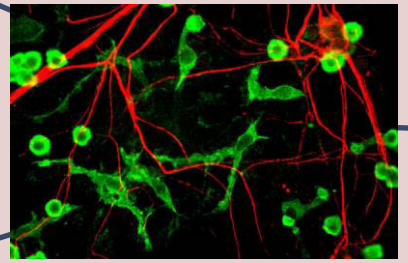
Science and Technology Advisor 10x Genomics,
CEE & Israel & Russia, Distributors

Building your understanding of biological system – slice by slice

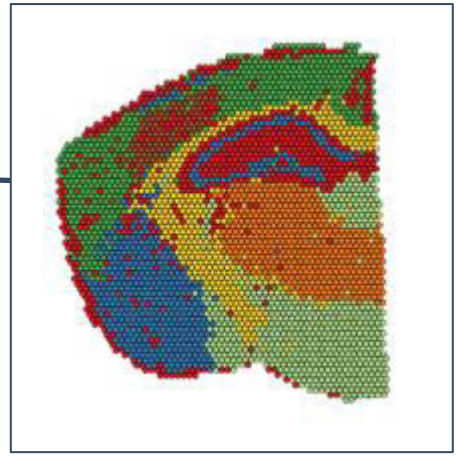


Chromium Single Cell





Visium Spatial Transcriptomics



Adapted from Joyce & Fearon, Science, 2015 doi: 10.1126/science.aaa6204

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

- Complementarity of single cell and spatial methods from 10x Genomics

Bulk RNA-seq

- Get a general idea about gene expression but lacks cellular or spatial resolution

Single Cell RNA-seq

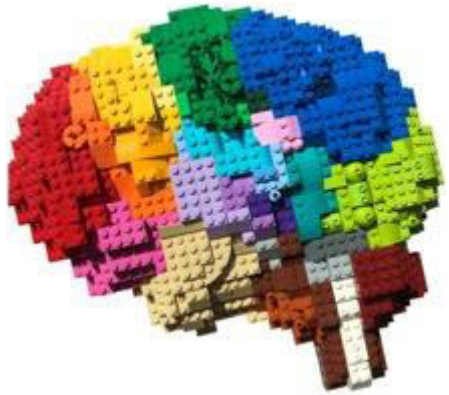
- Identify distinct cell types and their gene expression profiles

+

Spatial Transcriptomics

- Visualize gene expression profiles with tissue context

Functional Tissue



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

Chromium Single Cell Gene Expression Products

Visium Spatial Gene Expression Products

10X GENOMICS

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

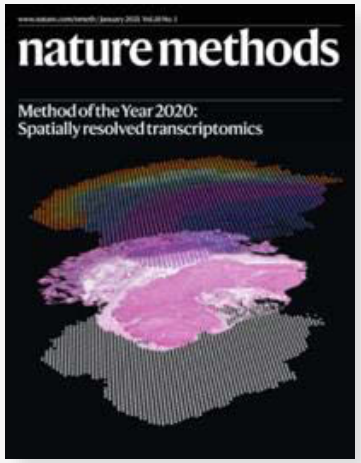
Spatial

http://www.StuckInCustoms.com

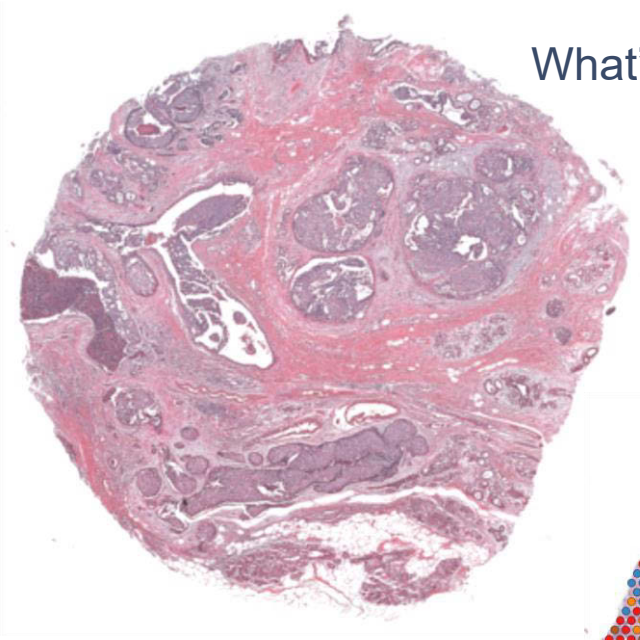
Entering digital histology era. TOP 10 Innovations

TheScientist
TOP 10
INNOVATIONS

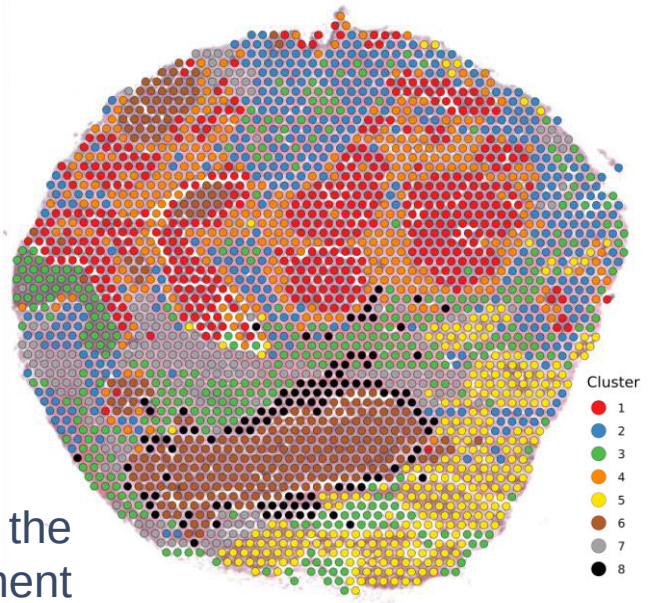
2020
Visium Spatial Gene Expression



2020
Spatially Resolved Transcriptomics



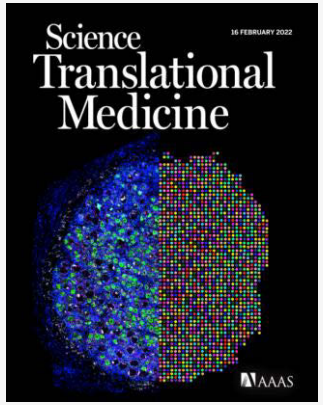
What's hiding in this tissue?



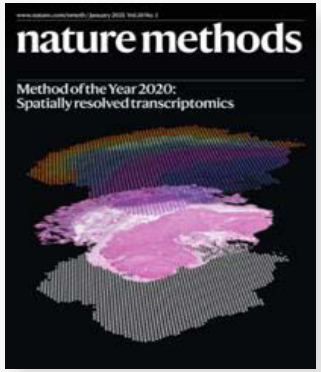
Visium reveals the complex environment

Advancing spatial biology

2022
Spatial Multiomics



2022
1st Visium Cover

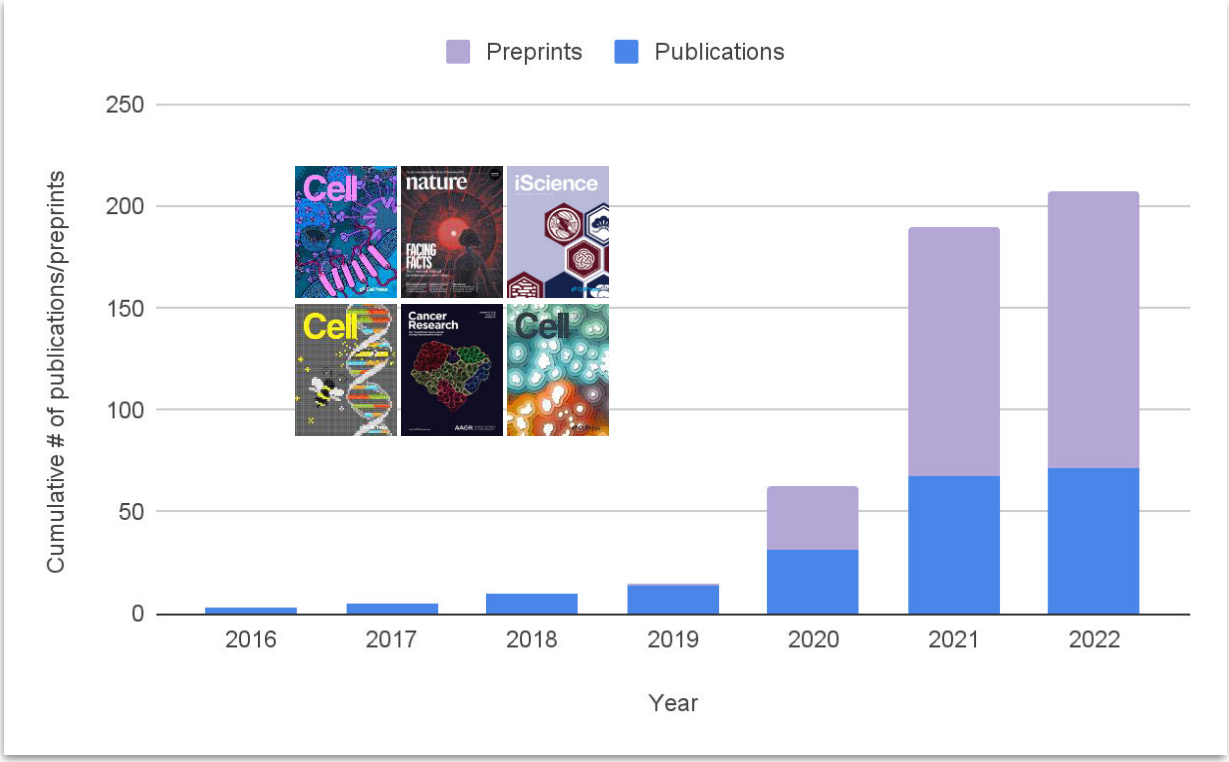


2020
Spatially Resolved Transcriptomics

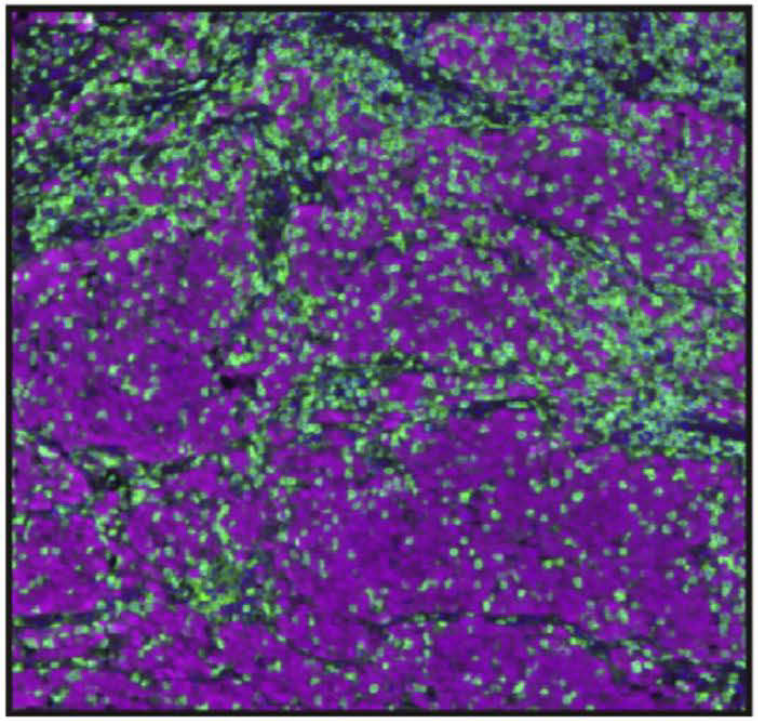
TheScientist
TOP 10
INNOVATIONS

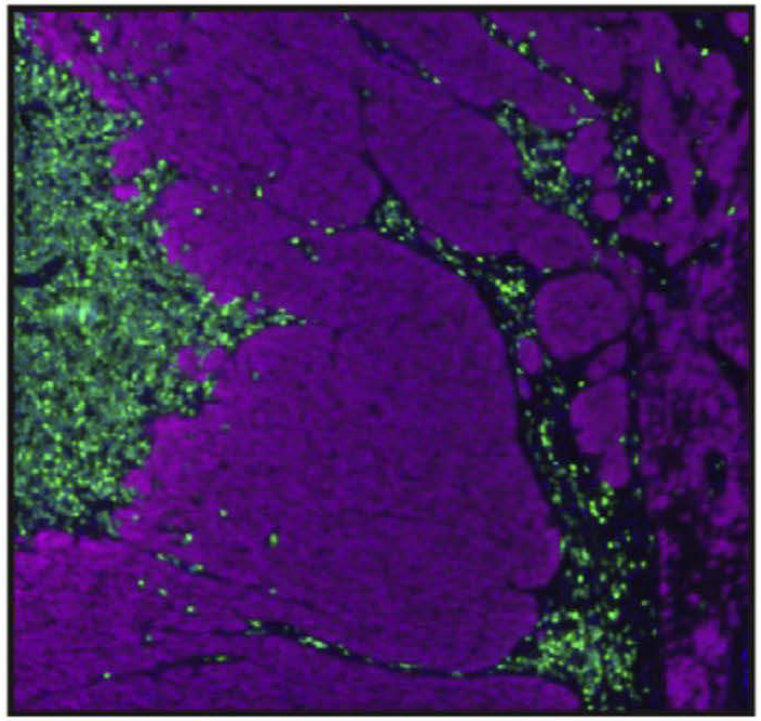
2020
Visium Spatial Gene Expression

200+ Visium Publications and Preprints



Why Spatial Analysis? Location Matters!





Van der Woude *et al.*, 2017

Tumor cell
Immune cell

“Hot tumor”
Lymphocytes infiltrating tumor

“Cold tumor”
Lymphocytes stopped at tumor boundary. “non-inflamed”, „non-immunogenic” tumors

The level of infiltration of tumors by lymphocytes can be a prognostic factor.

Visium Spatial Gene Expression

Whole Transcriptome Analysis in FFPE and Fresh Frozen Tissues

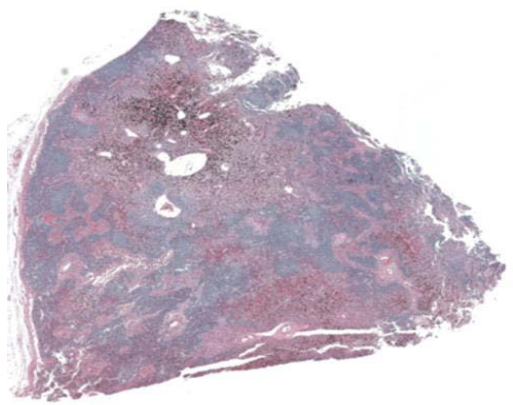
Spatially resolve mRNA across FFPE and fresh frozen tissue sections

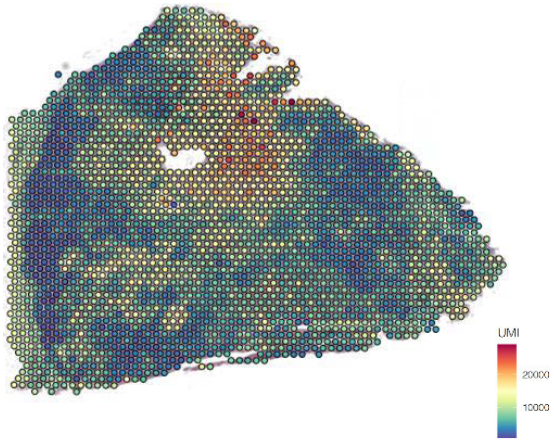
FFPE tissues

~18,000 genes targeted in human

Human lymph node

~20,000 genes targeted in mouse

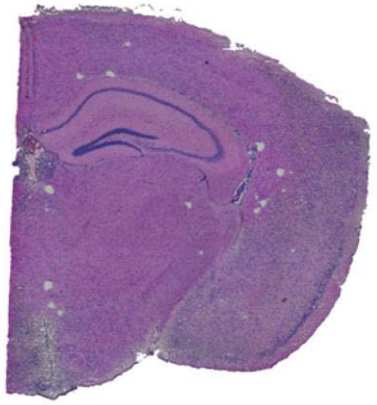


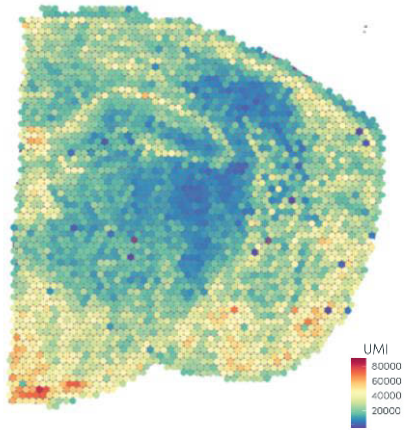


Fresh frozen tissues

Applicable for a variety of species

Mouse brain

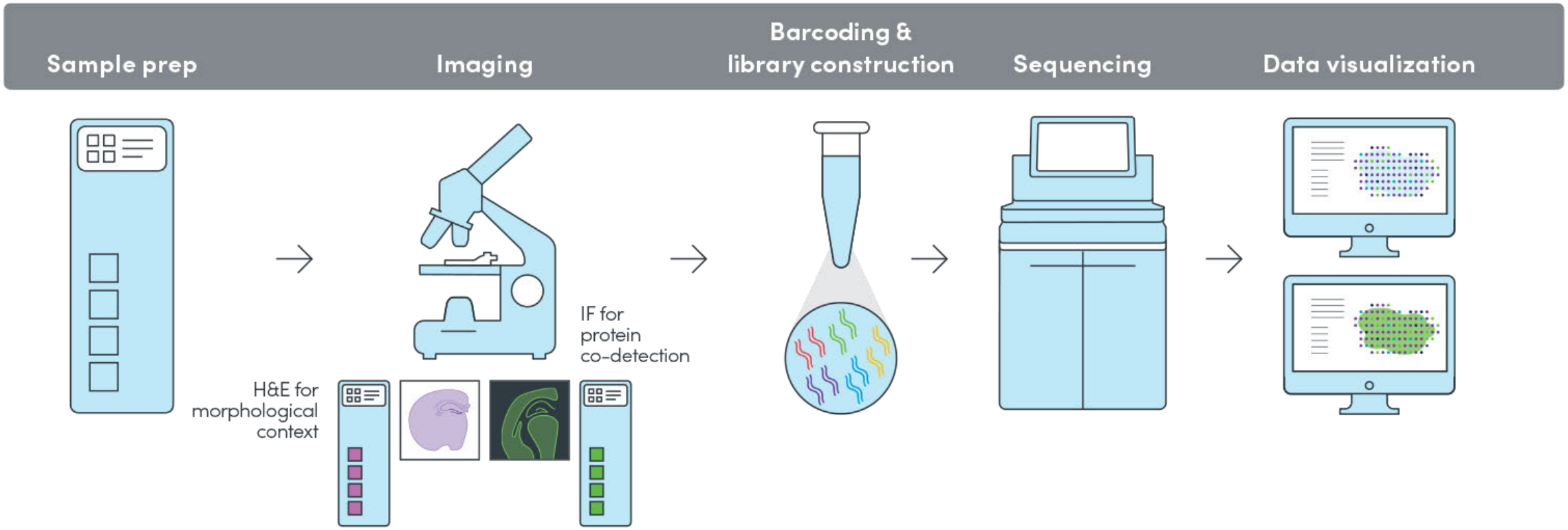




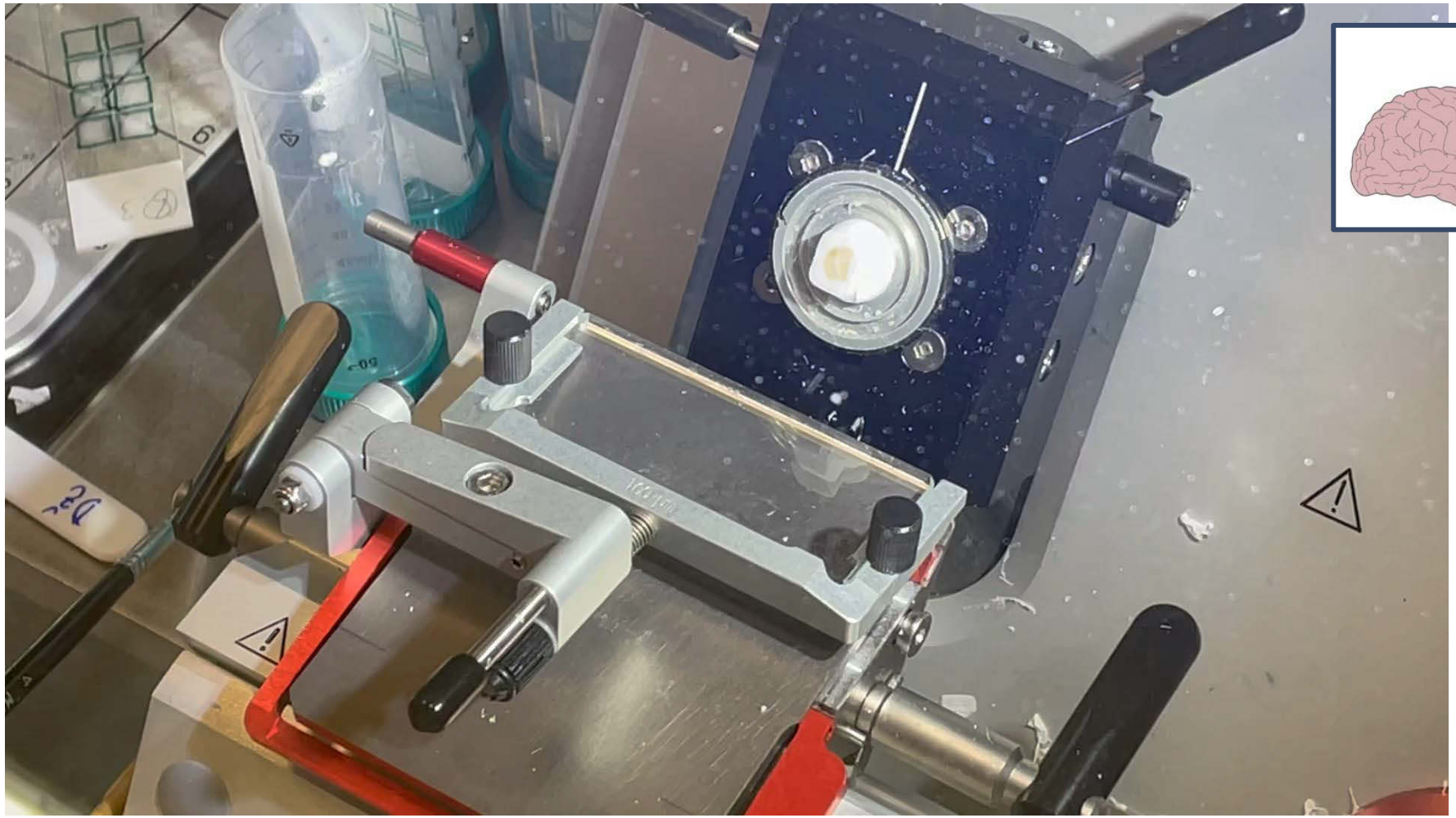
Visium Spatial Gene Expression workflow

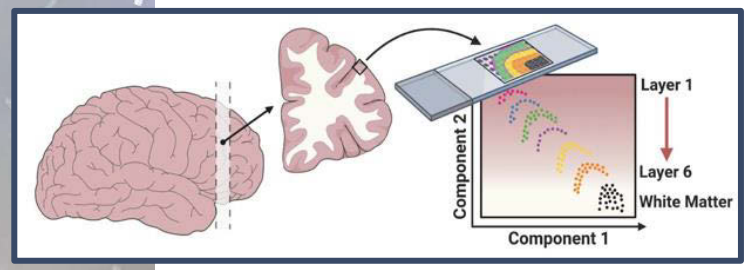
Streamlined workflow with the choice of H&E or Immunofluorescence

Provides whole transcriptome or targeted gene expression with protein co-detection



Visium Fresh - Frozen samples hands on procedure

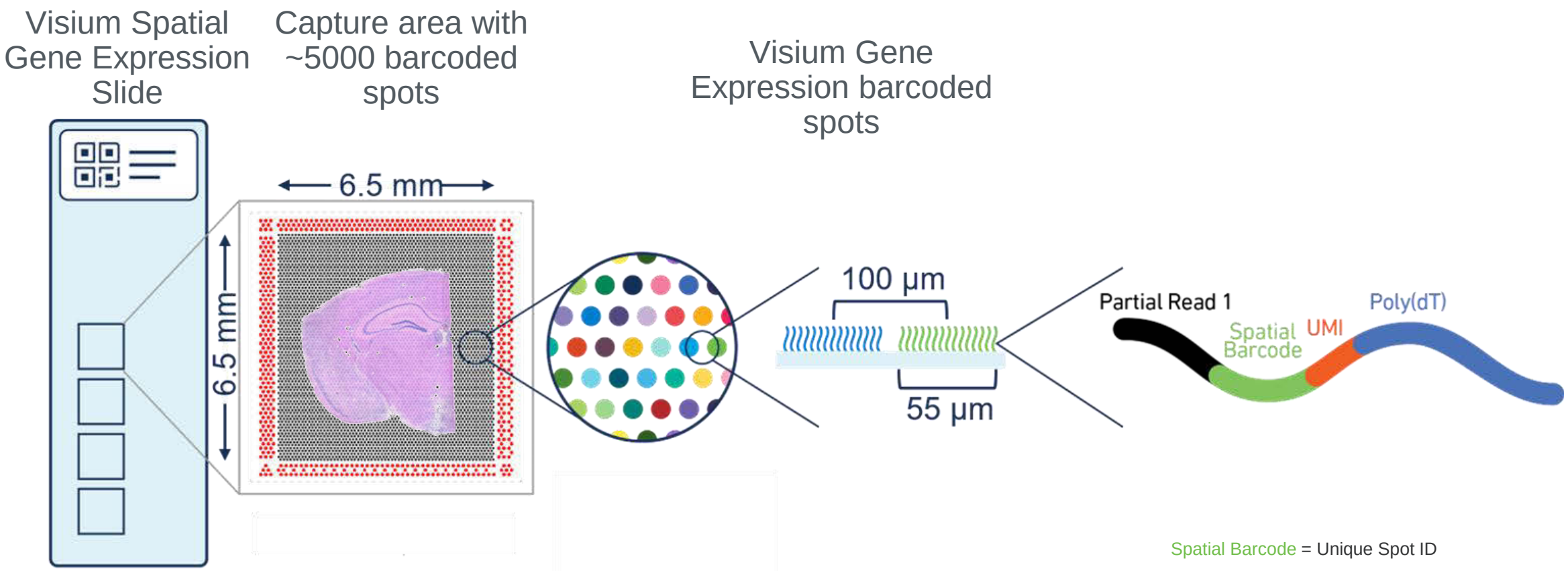




@ Daniel Zucha, IEM CAS Prague

Unbiased gene expression at high spatial resolution

Utilizing Poly-A Capture and unique spatial barcodes

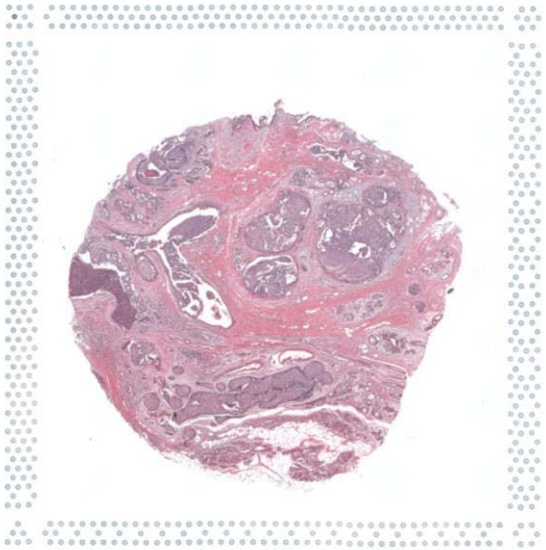


Spatial Barcode = Unique Spot ID

Cluster or image driven analysis of spatial data

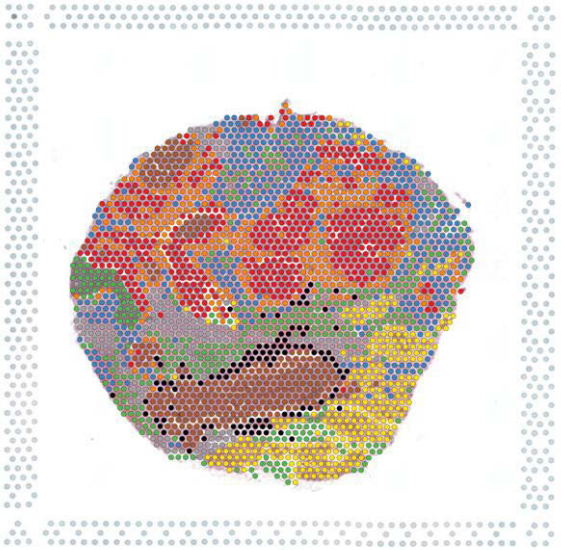
Start with the gene expression data or microscopy images of the same section

Image



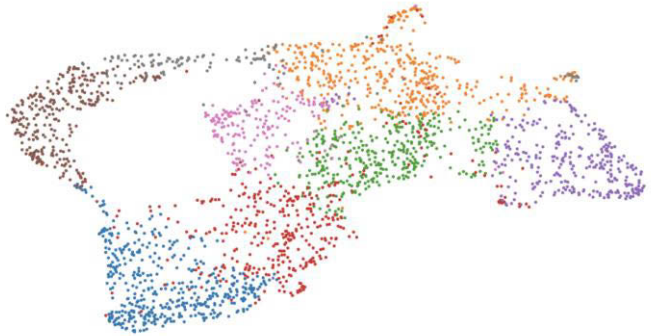
Tissue morphology
Breast ductal carcinoma in situ

Visium Data



Annotation directly on H&E image

Whole Transcriptome Data



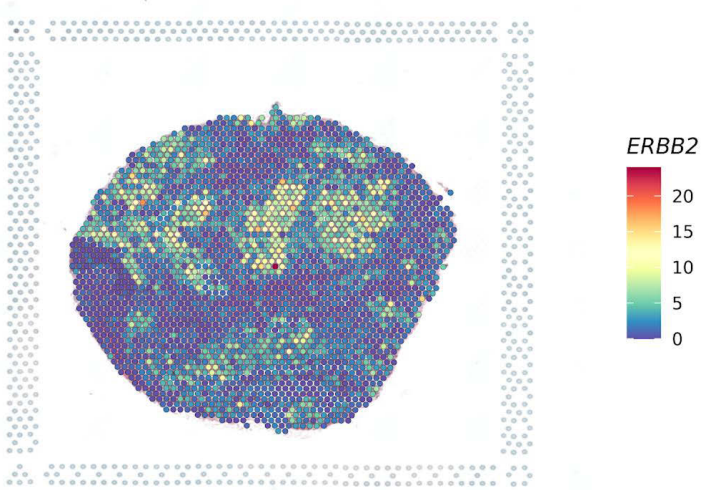
Clustering of
glass slide spots based on
their GEX profile

Explore Ductal Carcinoma In Situ of the Breast Cancer

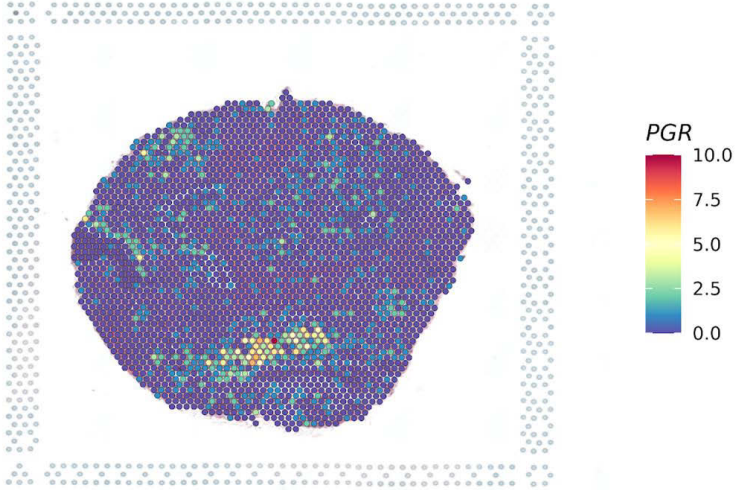
Start with gene expression

Key breast cancer biomarkers

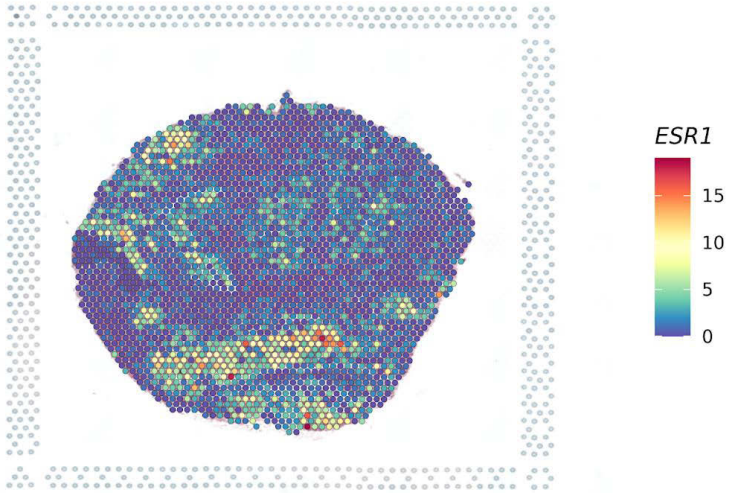
ERBB2



PGR



ESR1



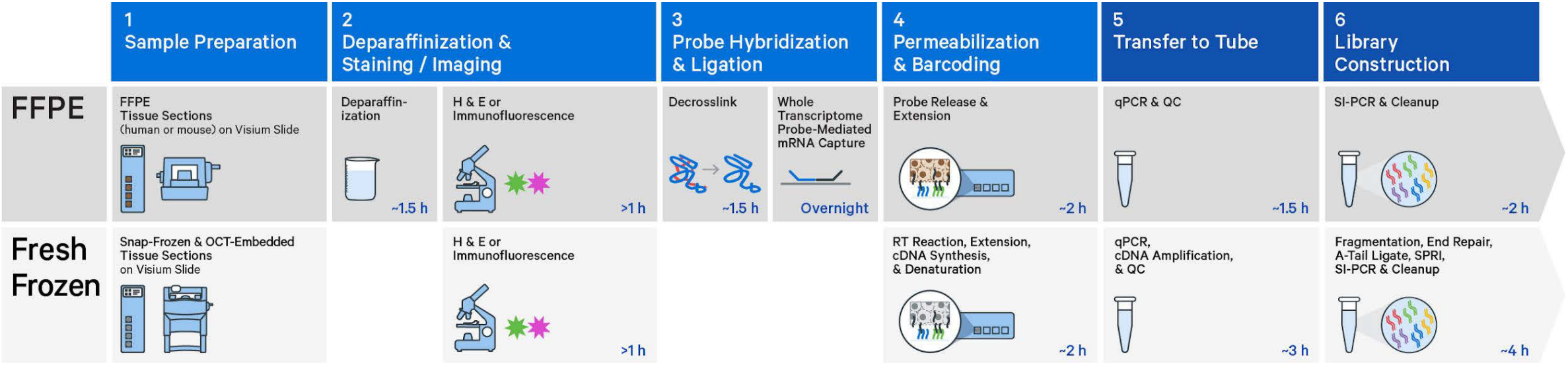
Formalin is most common sample preservation methos

- Fresh tissue with methanol fixation is the only processing currently compatible with Visium
- Formalin Fixation and Paraffin Embedding (FFPE) is the primary method of clinical sample preservation
  - Excellent for preservation and tissue stability

Current problem:

- **FFPE leads to the sequestration of analytes (due to crosslinking) and nucleic acid degradation**
  - Makes Next Generation Sequencing (NGS) analysis particularly challenging

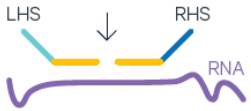
Fresh- Frozen and FFPE samples workflow comparison



Visium for FFPE utilizes new chemistry to detect mRNA

RNA Templated Ligation (RTL) for sensitive, specific RNA detection in FFPE samples

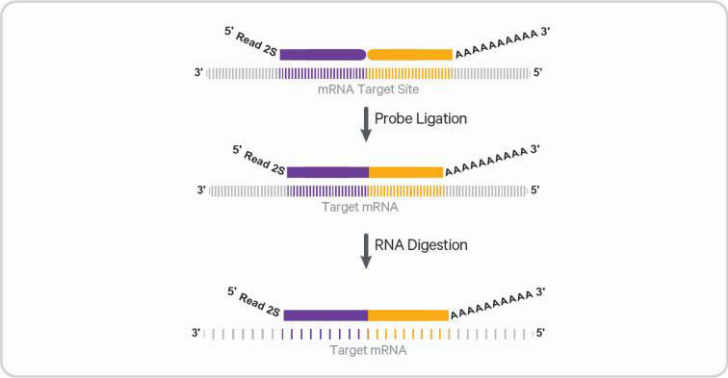
Probe pairs designed against the protein-coding transcriptome, one pair per gene



Split two probe chemistry reduces nonspecific signal

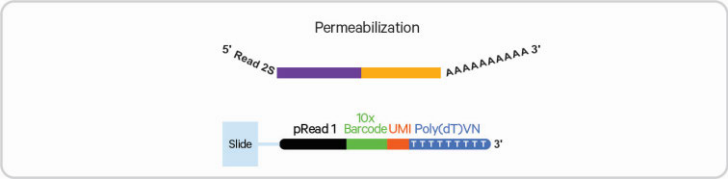
Probe hybridization and ligation

Ligated probe pair released



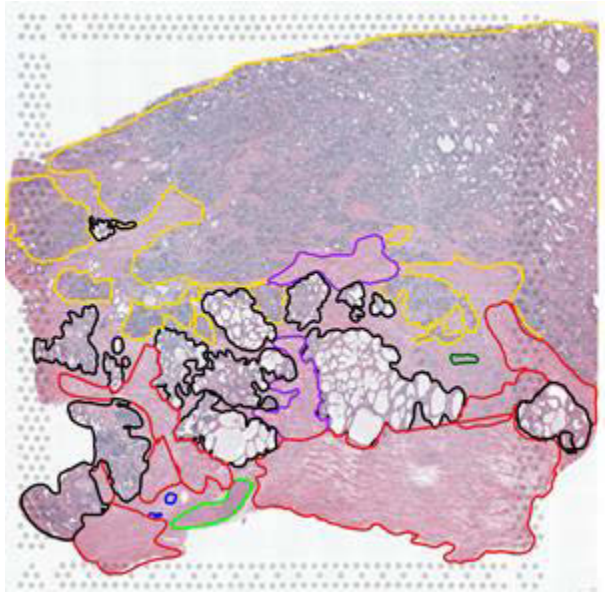
~18,000 genes targeted in human

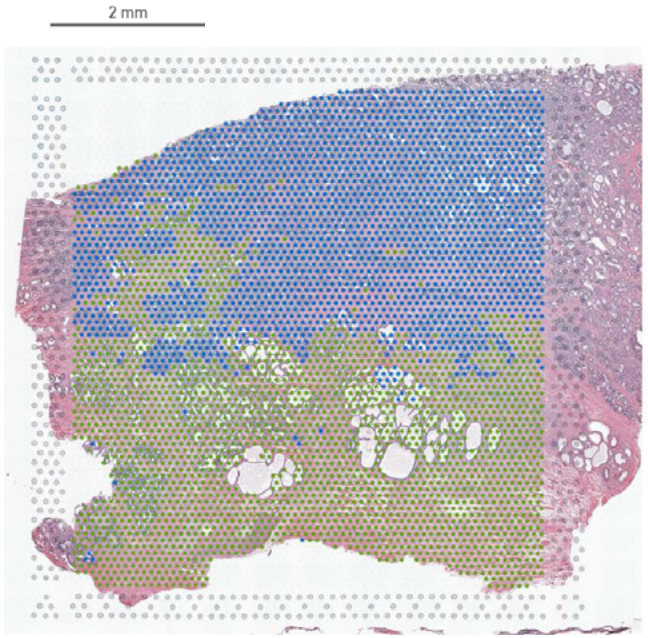
Tissue permeabilization and ligated probe pair capture



~20,000 genes targeted in mouse

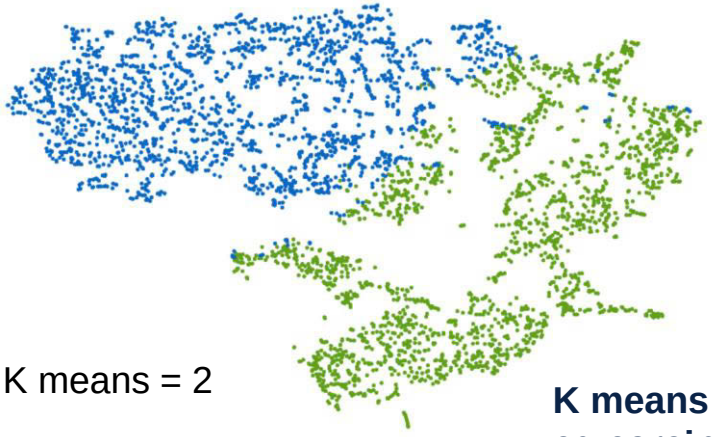
Prostate Tumor and Markers



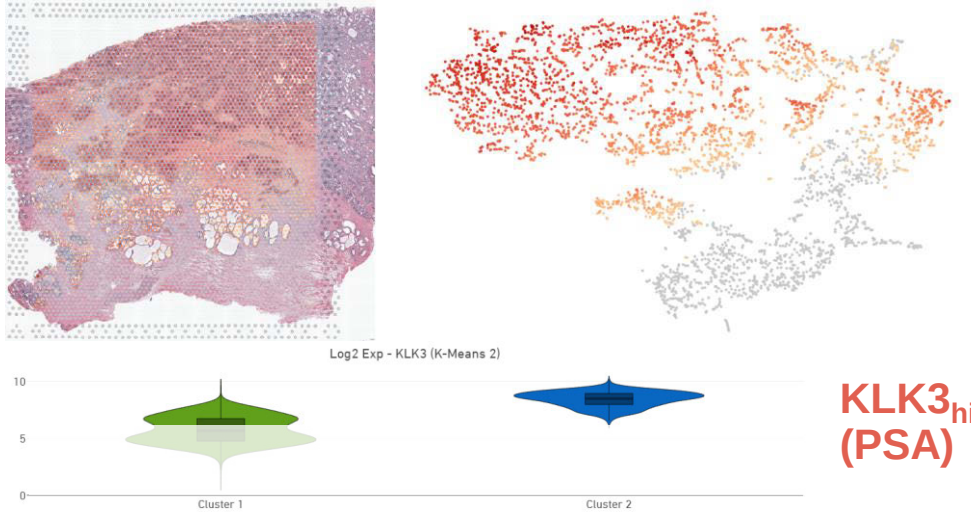


Classification

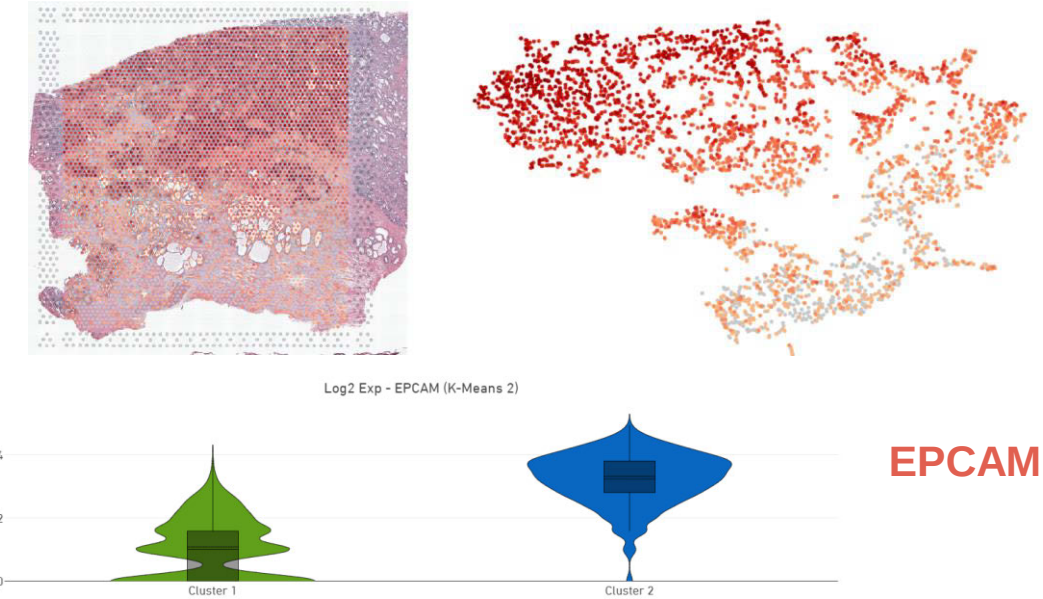
- Blood Vessel
- Fibro-Muscular Tissue
- Fibrous Tissue
- Immune Cells
- Invasive Carcinoma
- Nerve
- Normal Gland



K means = 2



$KLK3_{high}$ (PSA)

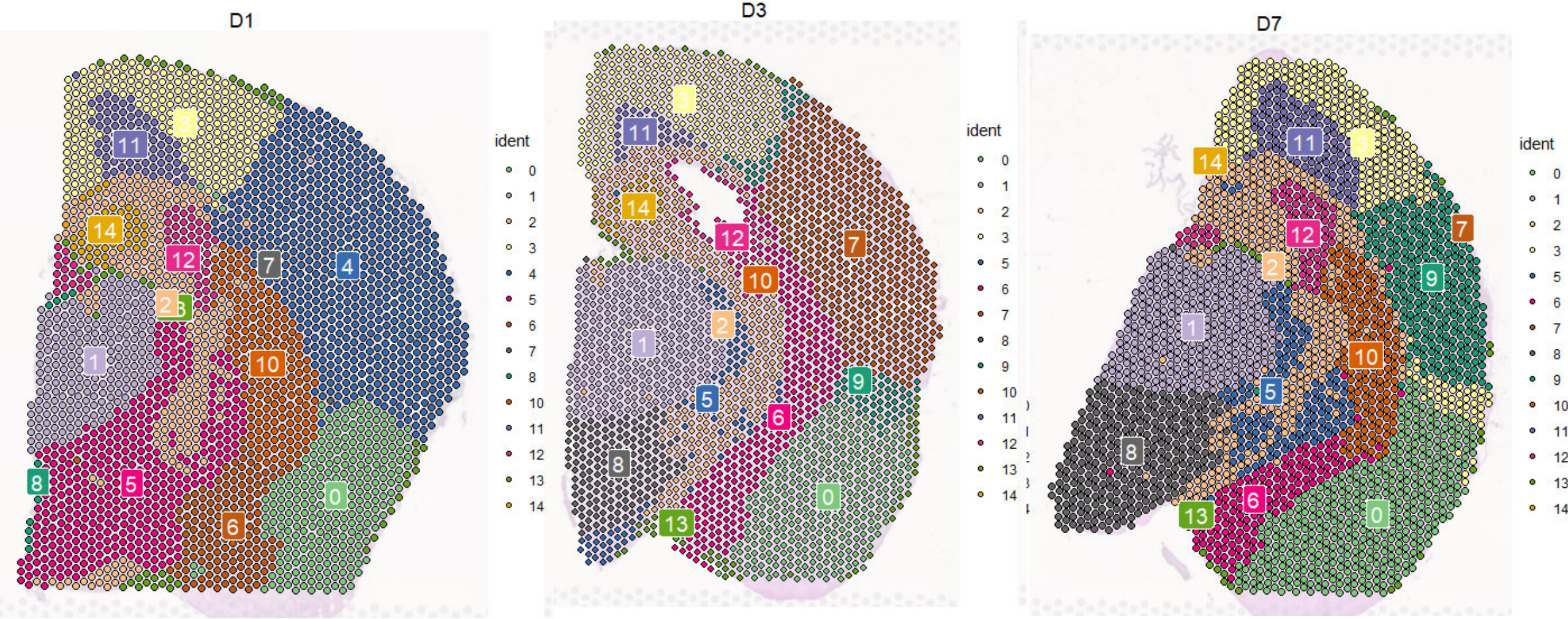


EPCAM

K means clustering finds strongest pattern in cell clusters.
eg carcinoma vs noncarcinoma if we define to find 2 strongest clusters

10X GENOMICS

Inschemia in colour, by Daniel Zucha, Institute of Biotechnology, CAS



Day 1

Day 3

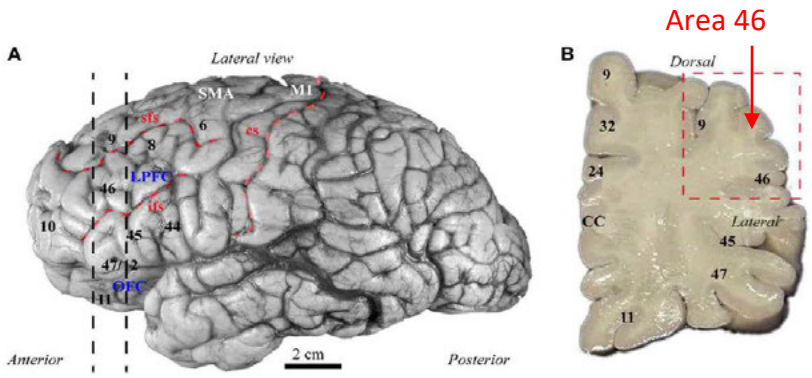
Day 7

94

Spatial Gene Expression in Human Prefrontal Cortex

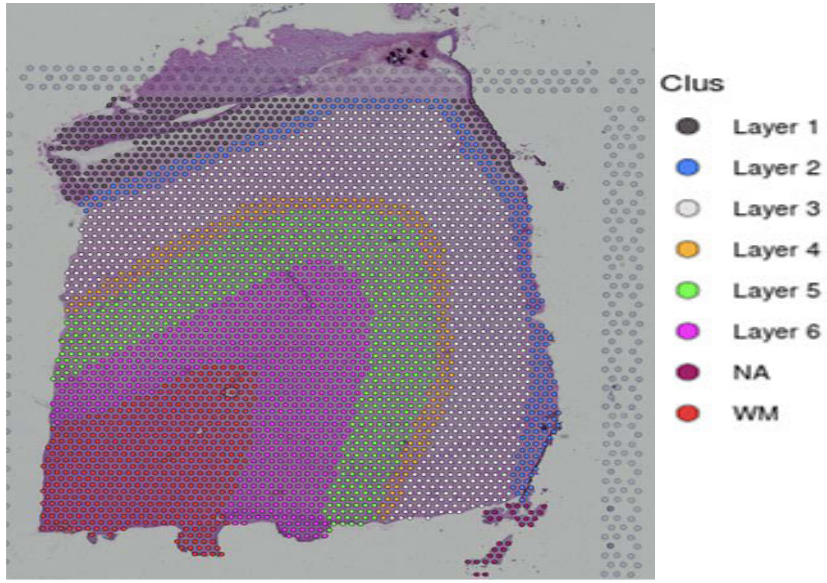
- Visium platform was used to define the spatial topography of gene expression in the **six-layered human dorsolateral prefrontal cortex**

Human brain prefrontal cortex



Zikopoulos and Barbas, 2013

Cortical layers revealed by Visium



nature neuroscience

Maynard et al. 2021, Nature Neuroscience,, https://doi.org/10.1038/s41593-020-00787-0

Spatial Gene Expression in Human Prefrontal Cortex

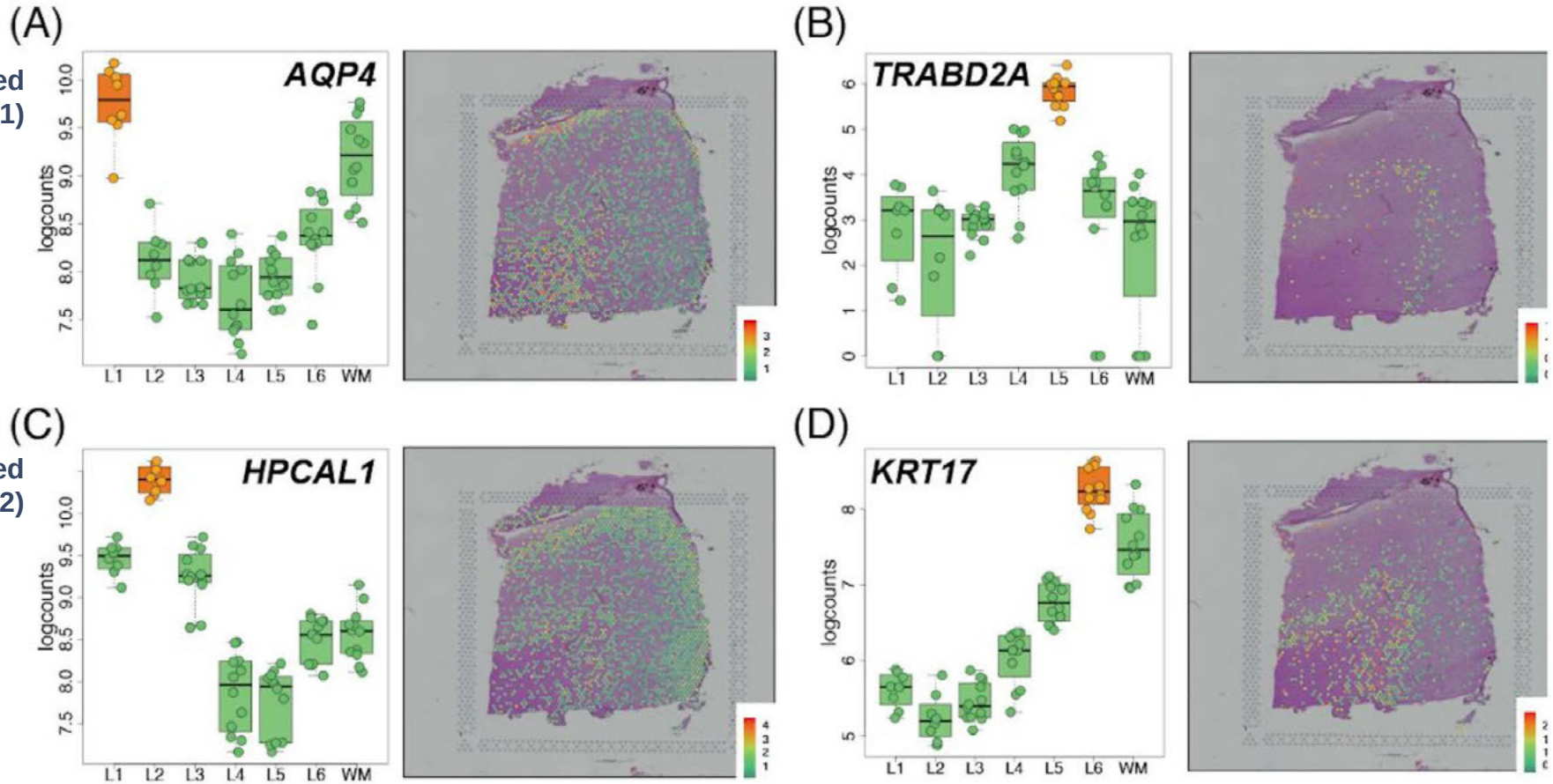
- **Visium discovers novel cortical layer-enriched genes, associated with schizophrenia and autism spectrum disorder, highlighting the clinical relevance of spatially defined expression.**

AQP4 **is enriched in Layer 1 (L1)**

TRABD2A **is enriched in Layer 5 (L5)**

HPCAL1 **is enriched in Layer 2 (L2)**

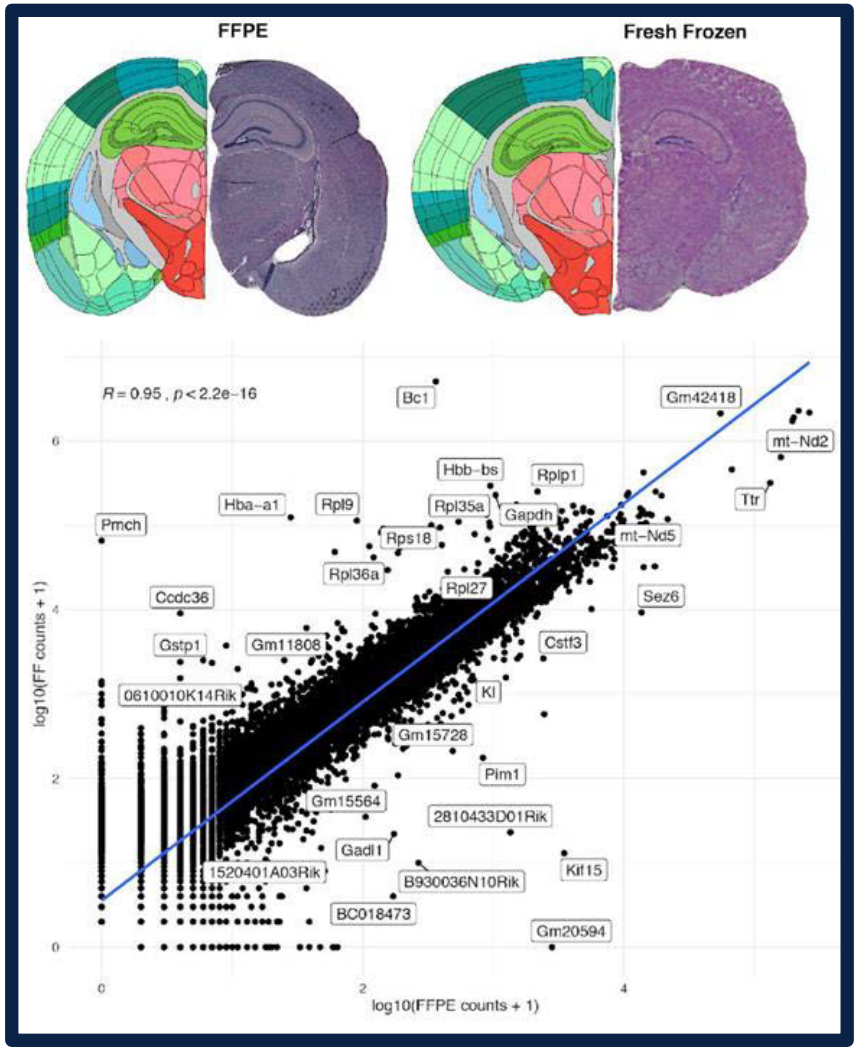
KRT17 **is enriched in Layer 6 (L6)**



Maynard et al. 2021, Nature Neuroscience,, https://doi.org/10.1038/s41593-020-00787-0

Genome-wide Spatial Expression Profiling in FFPE Tissues

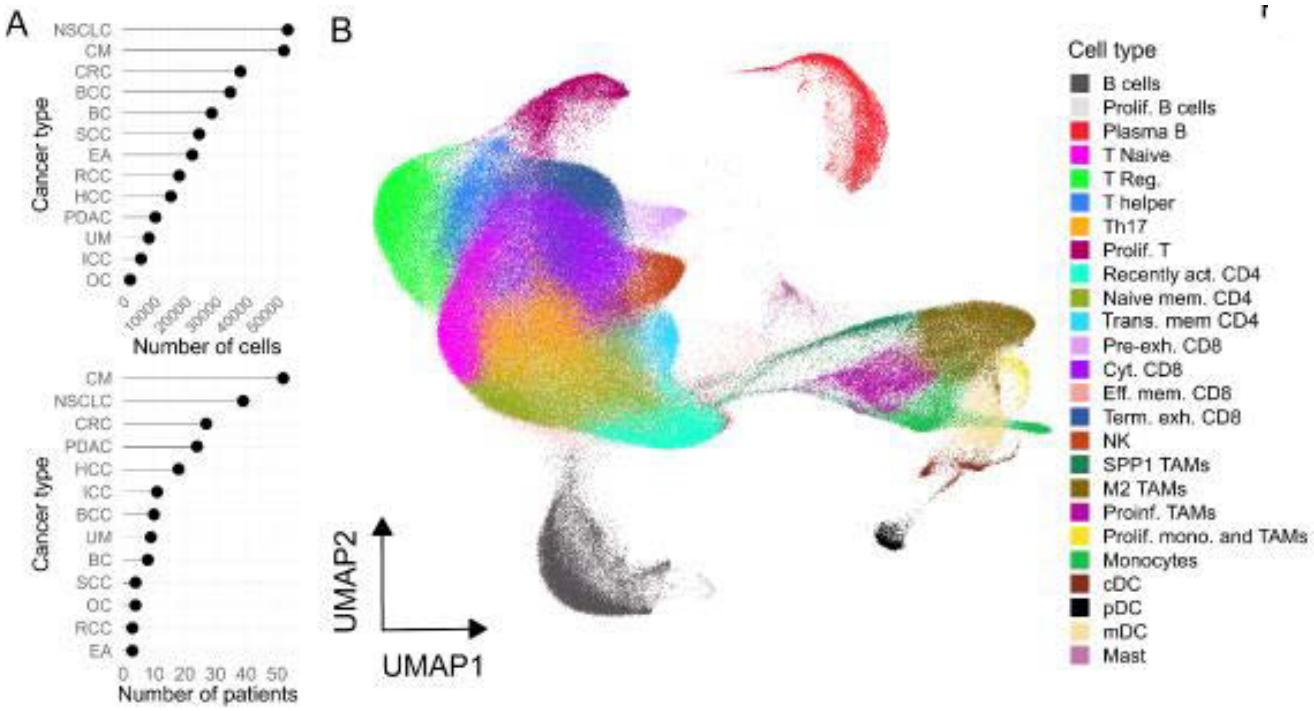
- Authors present a procedure to perform genome-wide spatial analysis of mRNA in FFPE tissue sections using *Visium Spatial*.
- Authors conducted expression profiling and cell type mapping in **coronal sections from the mouse brain** to demonstrate the method's capability to delineate anatomical regions from a molecular perspective.
- They further explored the spatial composition of transcriptomic signatures in **ovarian carcinosarcoma** samples using data driven analysis methods, exemplifying the method's **potential to elucidate molecular mechanisms in heterogeneous clinical samples.**
- Comarison with data from Allen Brain Atlas



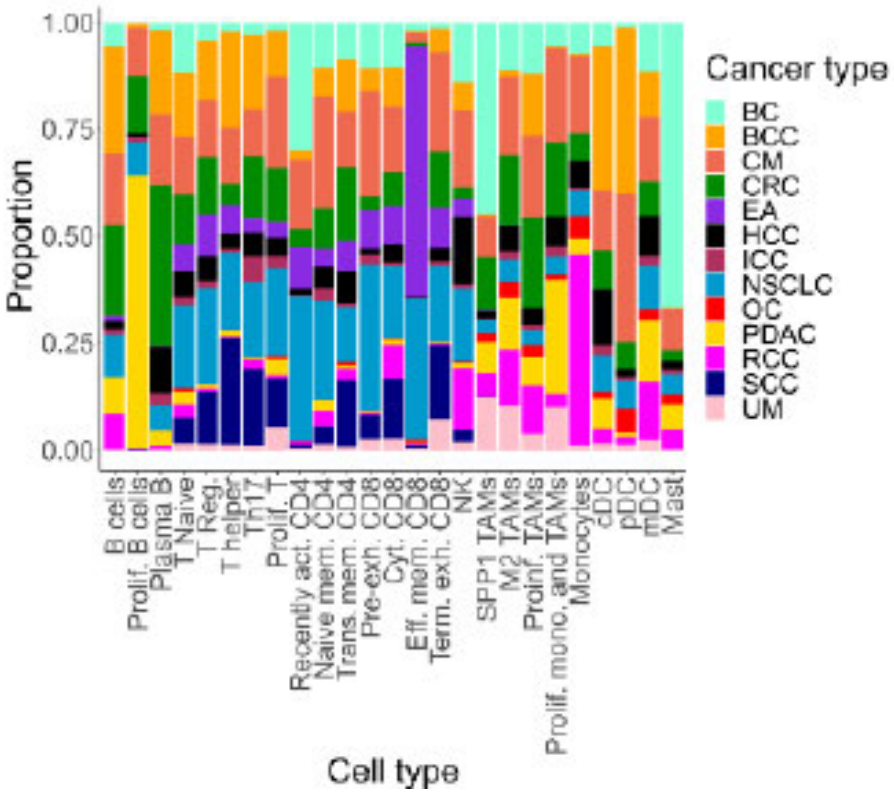
Villacampa et al 2020, bioRxiv, https://doi.org/10.1101/2020.07.24.219758

A single-cell tumor immune atlas for precision oncology

Built and tested a cancer immune atlas for patient stratification using single cell and spatial gene expression data



Identified shared immune signatures across patients and cancer types

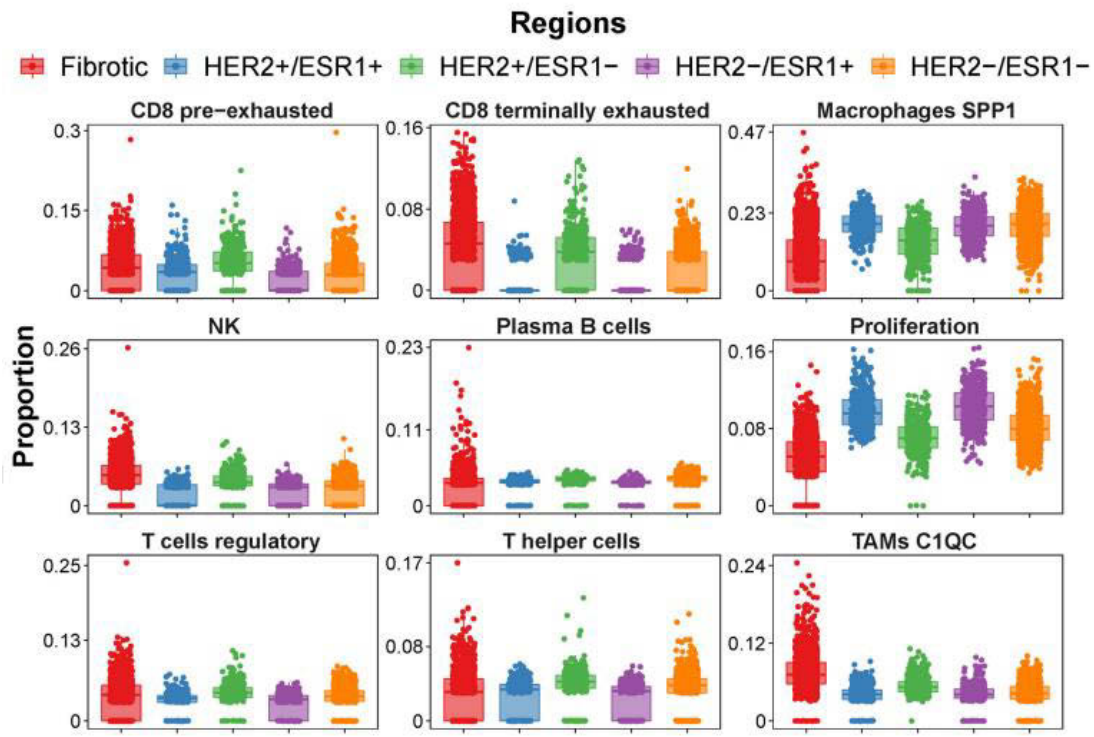


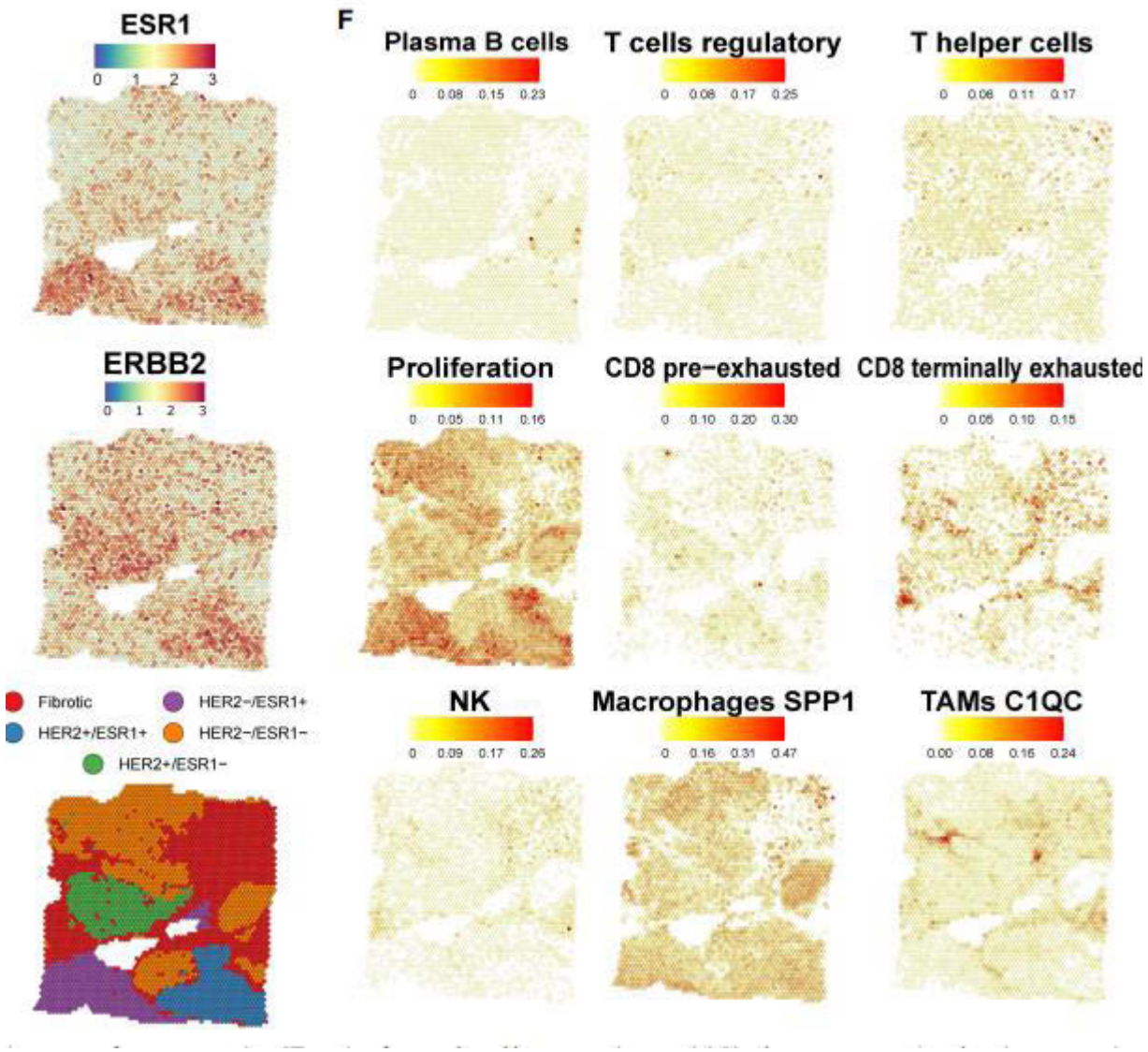
https://www.genome.org/cgi/doi/10.1101/gr.273300.120

A single-cell tumor immune atlas for precision oncology

- **Integrated single cell and spatial data to explore tumor immune composition across cancer types**

- **Found differences in immune architecture across tumor types which could contribute to unraveling immuno-therapy responses**





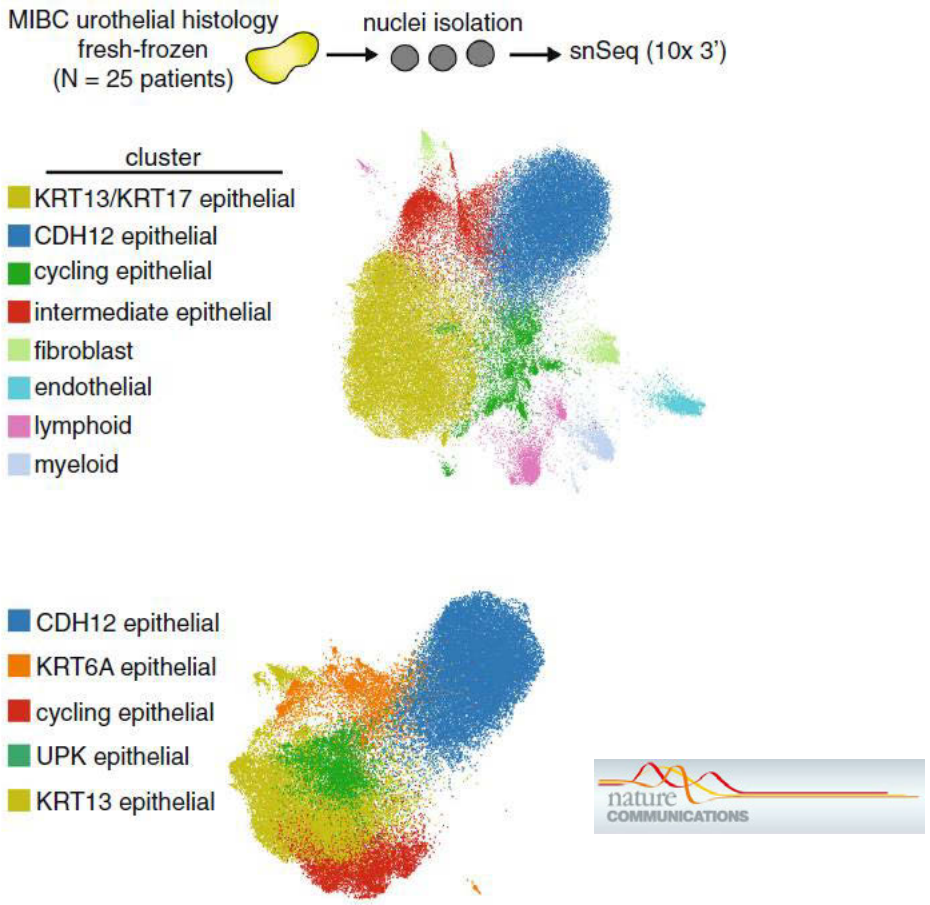
https://www.genome.org/cgi/doi/10.1101/gr.273300.120

An N-Cadherin 2 expressing epithelial cell subpopulation predicts response to surgery, chemotherapy and immunotherapy in bladder cancer

The authors combined *Chromium* single nuclei RNA sequencing with *Visium* spatial transcriptomics and single-cell resolution spatial proteomic analysis of human bladder cancer to identify an epithelial subpopulation with therapeutic response prediction ability.

These cells expressed Cadherin 12 (CDH12, N-Cadherin 2), catenins, and other epithelial markers.
CDH12-enriched tumors defined patients with poor outcome following surgery with or without neoadjuvant chemotherapy.

In contrast, CDH12-enriched tumors exhibited superior response to immune checkpoint therapy.



Gouin et al., 2021, Nature Communications, https://doi.org/10.1038/s41467-021-25103-7

Single-cell ATAC and RNA sequencing reveal pre-existing and persistent subpopulations of cells associated with relapse of prostate cancer

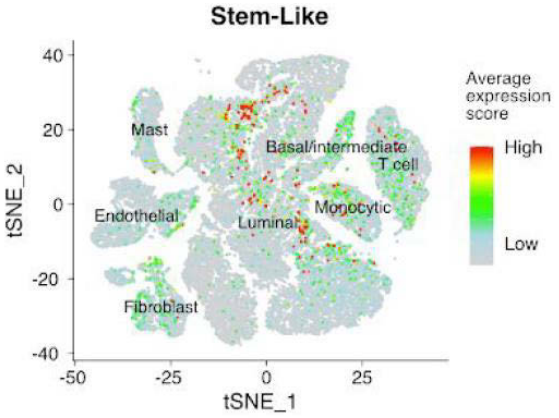
The authors employed *Chromium* single-cell assay for transposase-accessible chromatin (ATAC) and RNA sequencing in prostate cancer models of early treatment response and resistance to enzalutamide (ENZ).

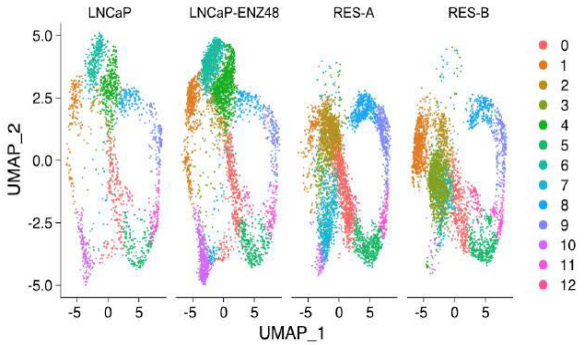
They identified pre-existing and treatment-persistent cell subpopulations that possess transcriptional stem-like features and regenerative potential.

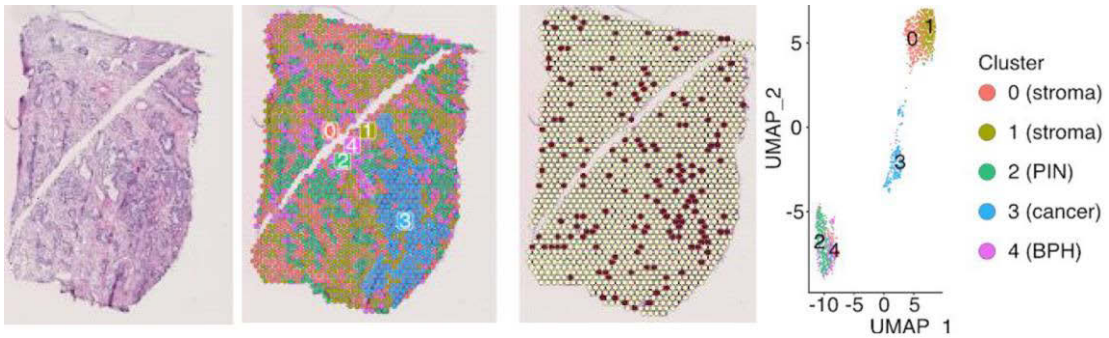
Distinct chromatin landscapes were associated with ENZ treatment and resistance.

Transcriptional profiles characteristic of persistent stem-like cells were able to stratify the treatment response of patients.

Using *Visium Spatial*, the presence and location of the signatures of the stem-like cells within two sections of primary untreated prostate cancer were assessed.

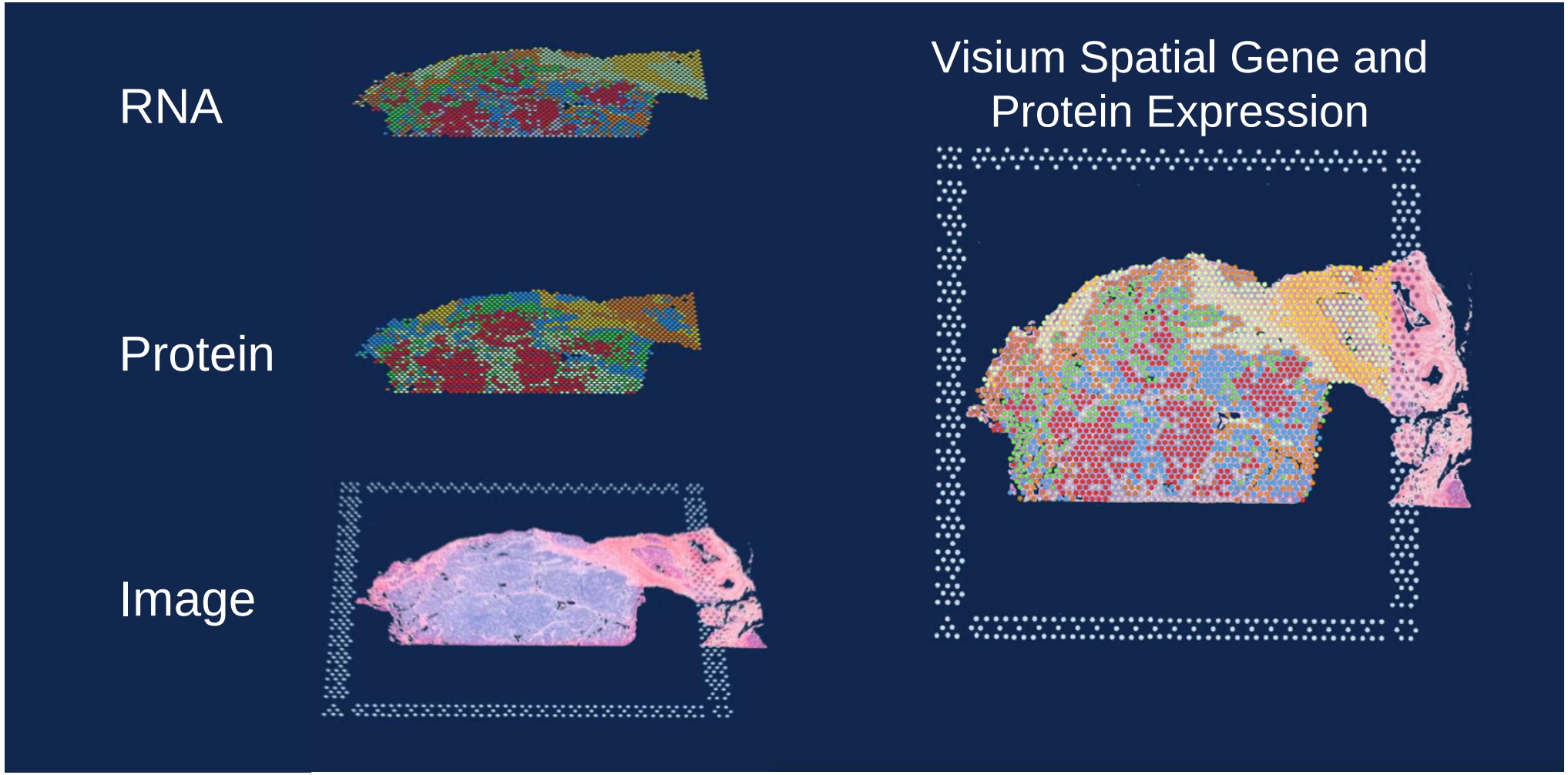






Taavitsainen et al 2021, https://www.nature.com/articles/s41467-021-25624-1

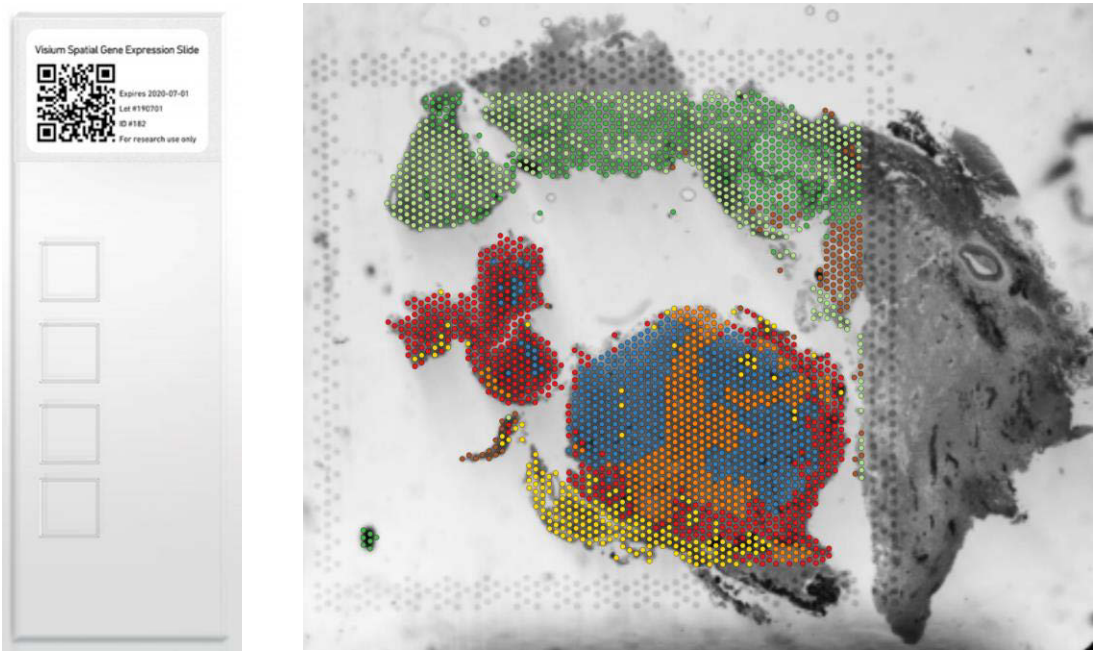
Simultaneous RNA and Protein detection for deeper insights



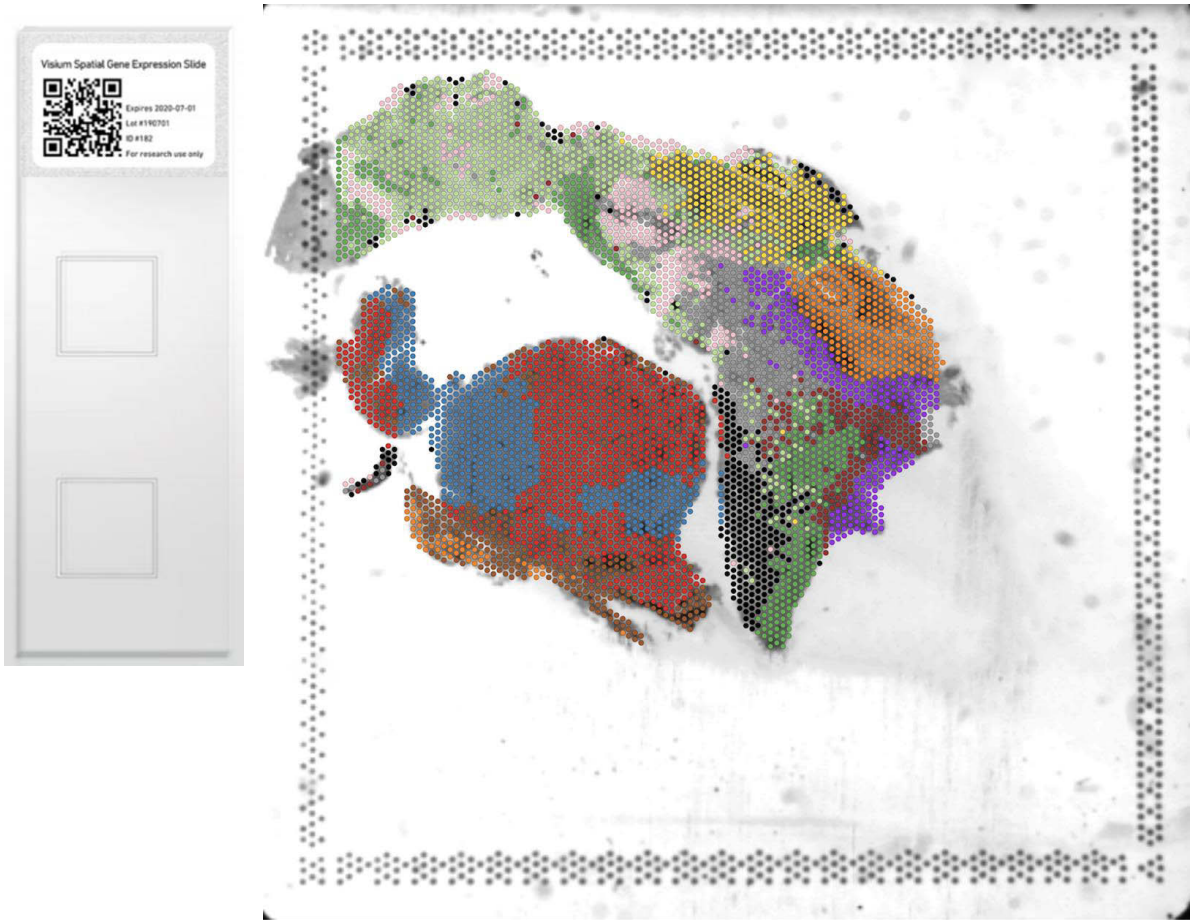
Visualize larger tissue sections without compromising block integrity

FFPE Human Brain Cancer

Visium GEx slide, 6.5mm

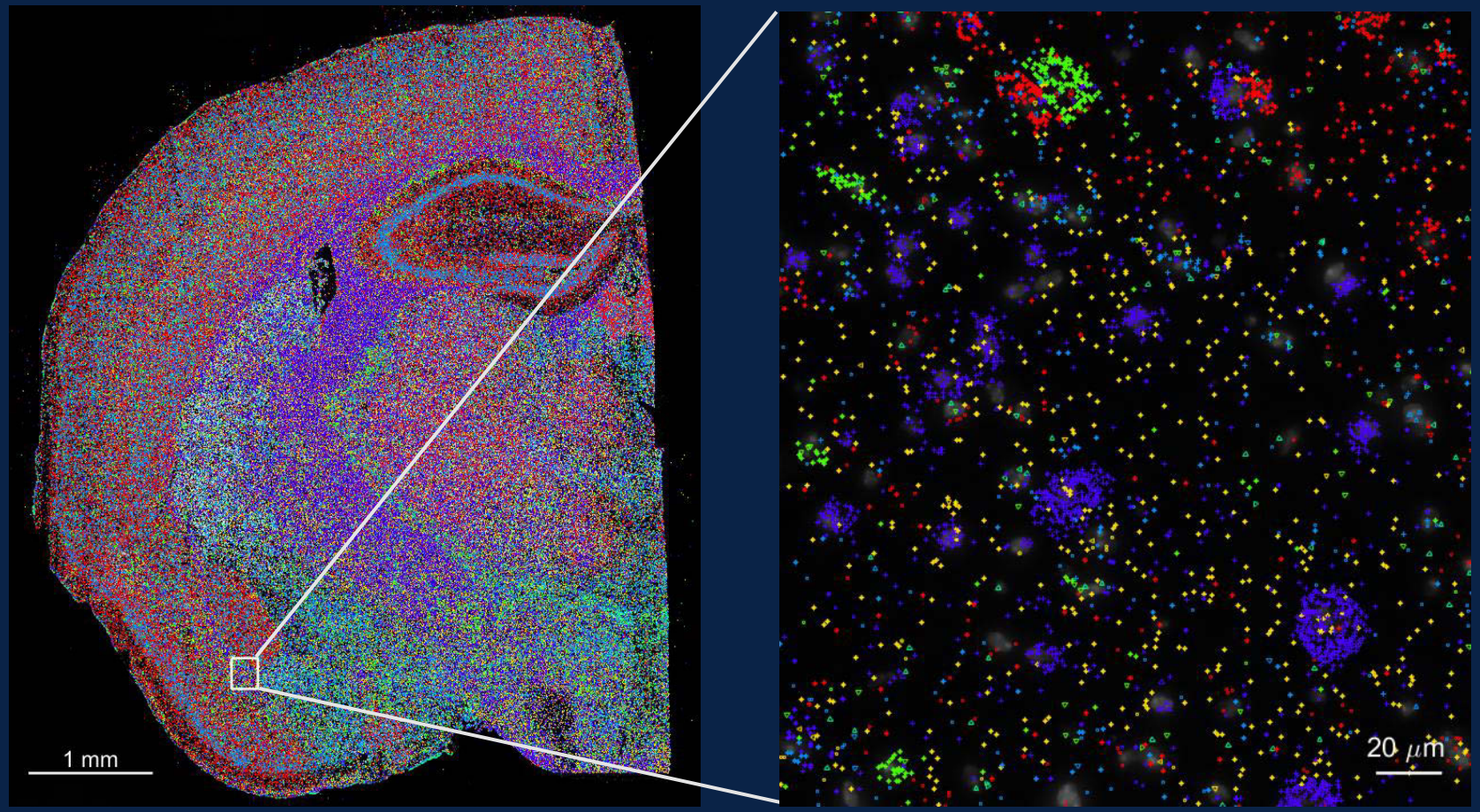


Visium GEx slide, 11mm



10X GENOMICS

Profile an entire tissue section with subcellular resolution – In Situ solution



Oligodendrocytes
Somatostatin+ interneurons
Glutamatergic neurons

Xenium

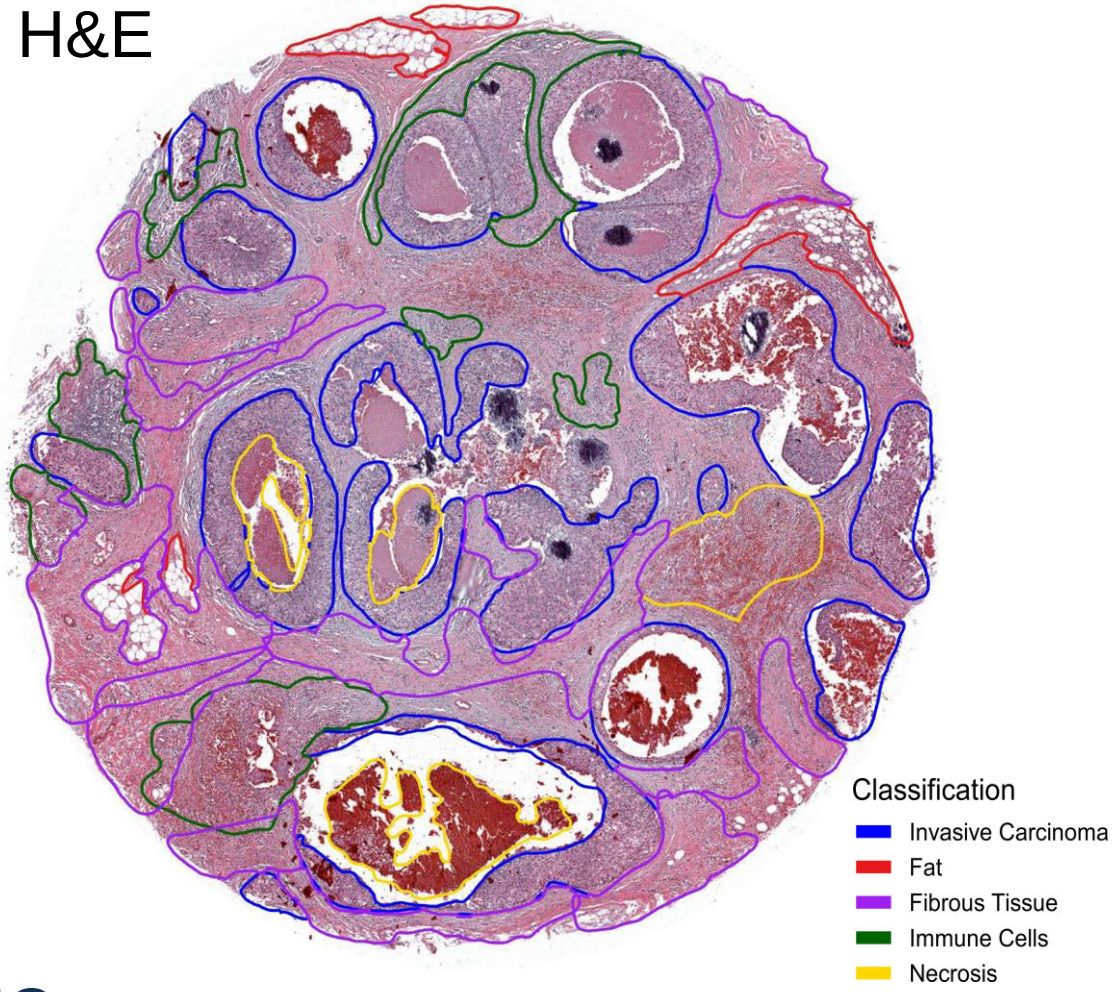
In Situ Platform

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

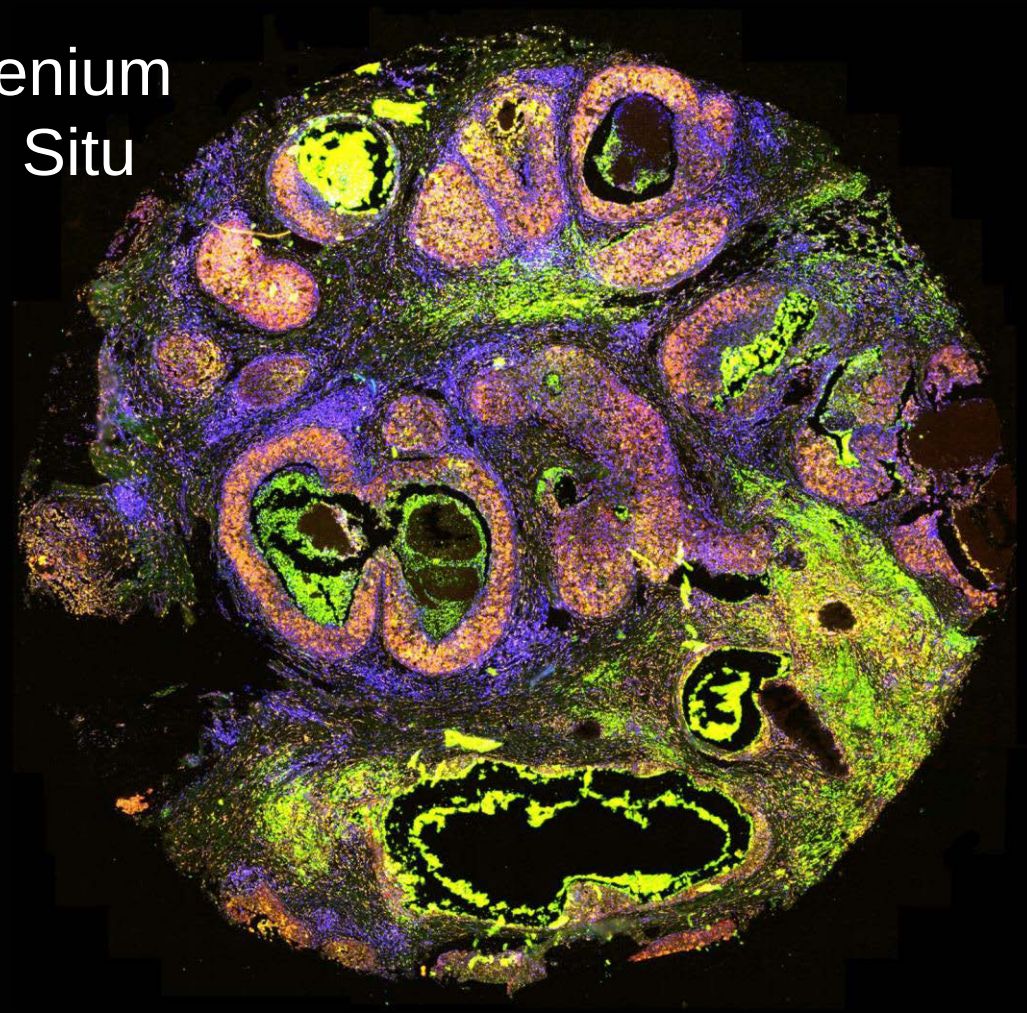
10X GENOMICS

In situ analysis of human FFPE breast cancer

H&E



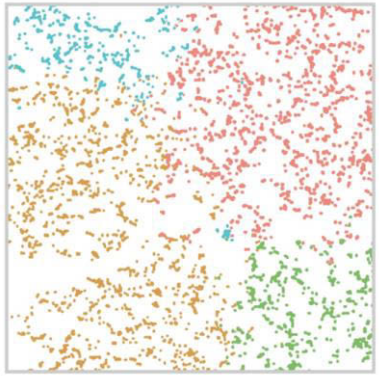
Xenium
In Situ



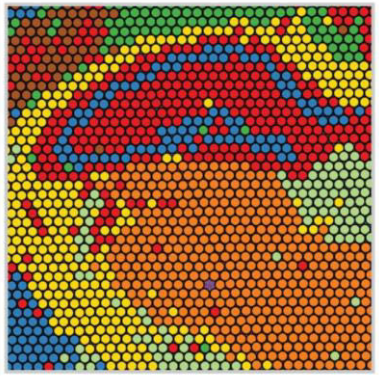
10X GENOMICS

From discovery to clinical with three complementary workflows

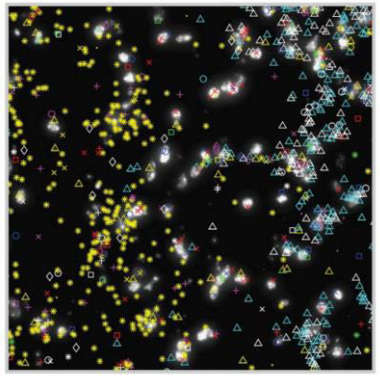
Chromium Single Cell



Visium Spatial



In Situ



Discovery

Translational

Clinical

10x GENOMICS

Sample Preparation for 10x Genomics

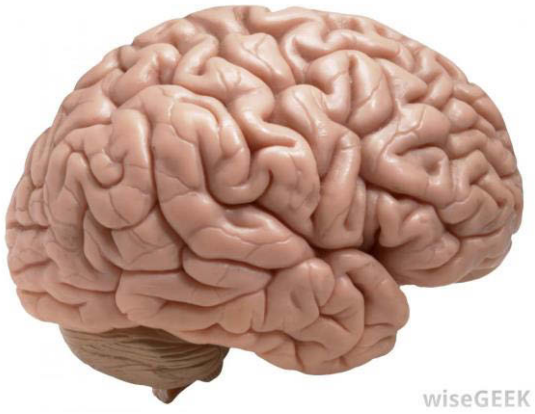
A How To Guide: Considerations and Best Practices

Agnieszka Ciesielska PhD, STA 10x Genomics

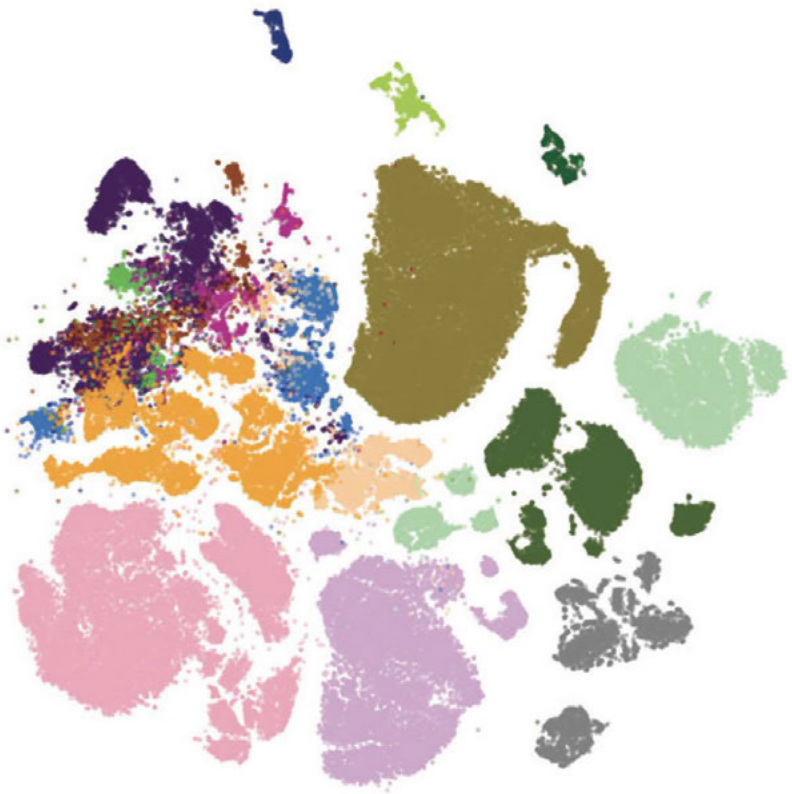
From averages to high resolution

Whole Tissue/Organs

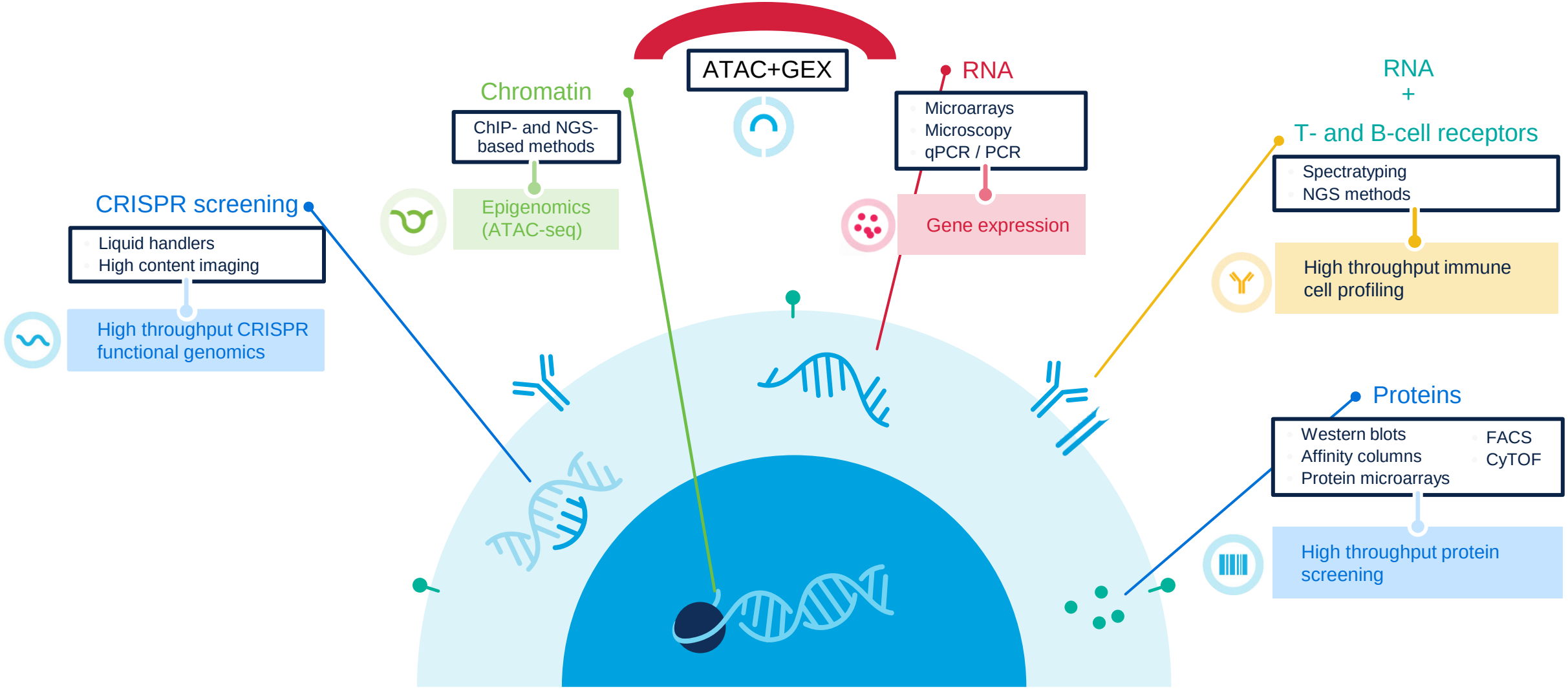
(Genetic) Disease Model



Resolve Cell Type-Specific Data



Next generation molecular profiling solutions



How We Think About Sample Preparation

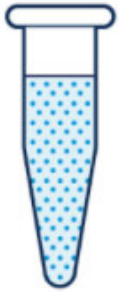
It’s what you bring to the experiment.

It’s a workflow. A set of decisions.

Quality is critical.

Chromium Single Cell Gene Expression Workflow

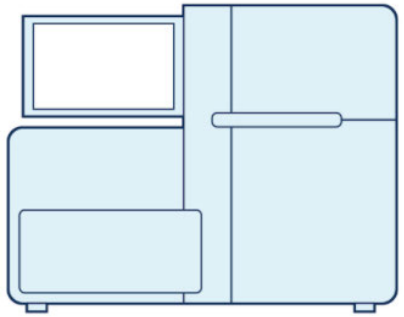
Input → **Library Creation** → **Sequencing** → **Data Analysis and Visualization**



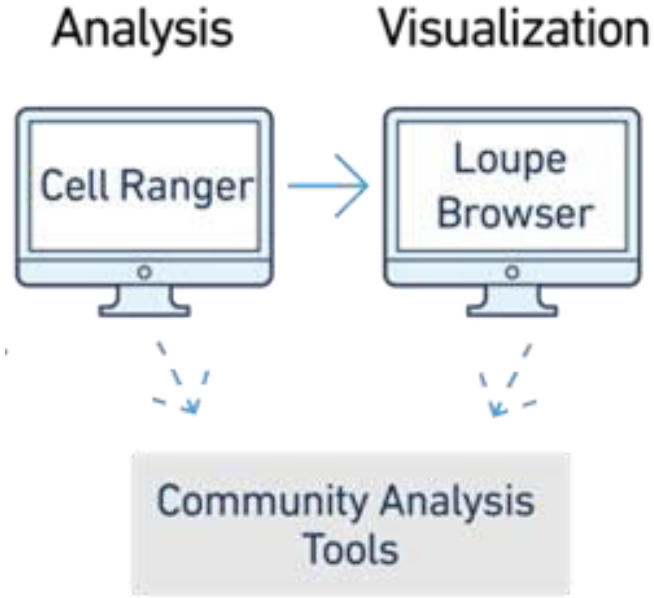
Suspension of dissociated single cell/nuclei

Cell partitioning and molecular barcoding

- **8 channels/chip**
- **500-10 000 cells recovered per channel**
- **40-65% cells recovered**



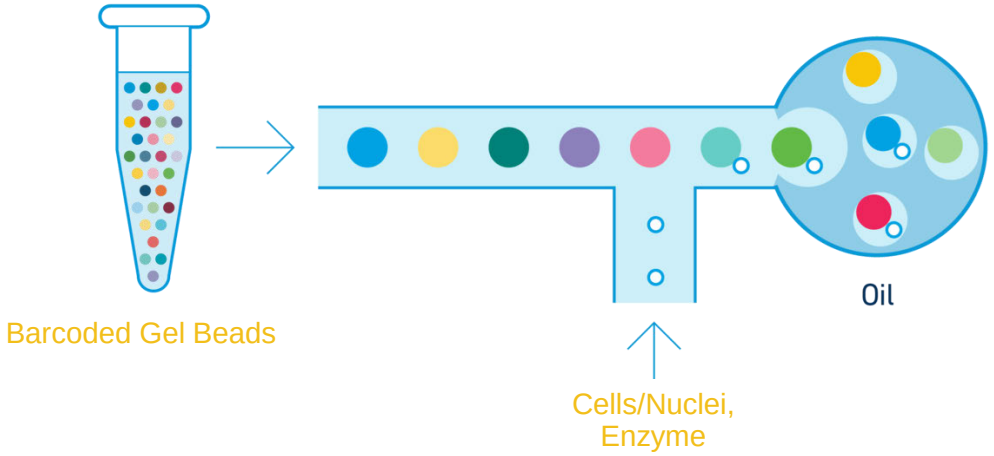
Sequencing

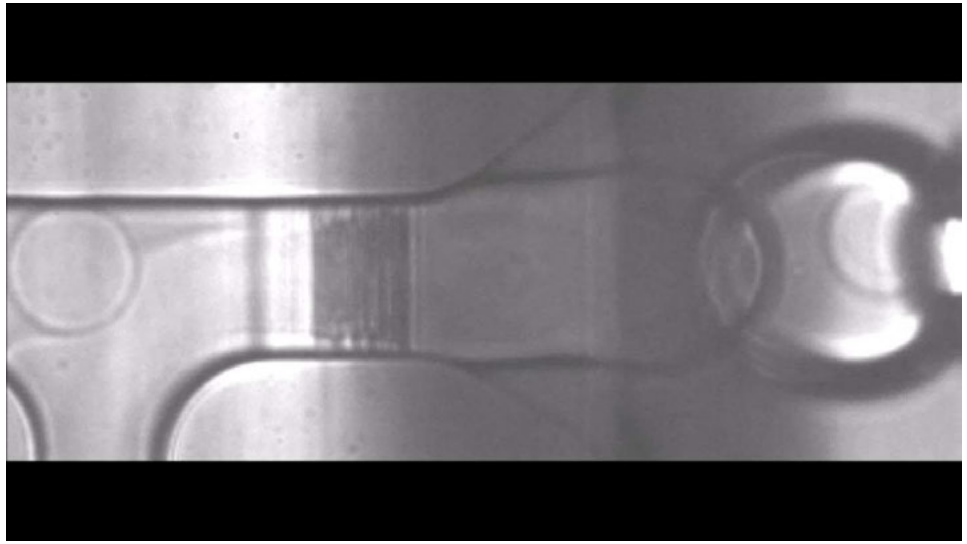


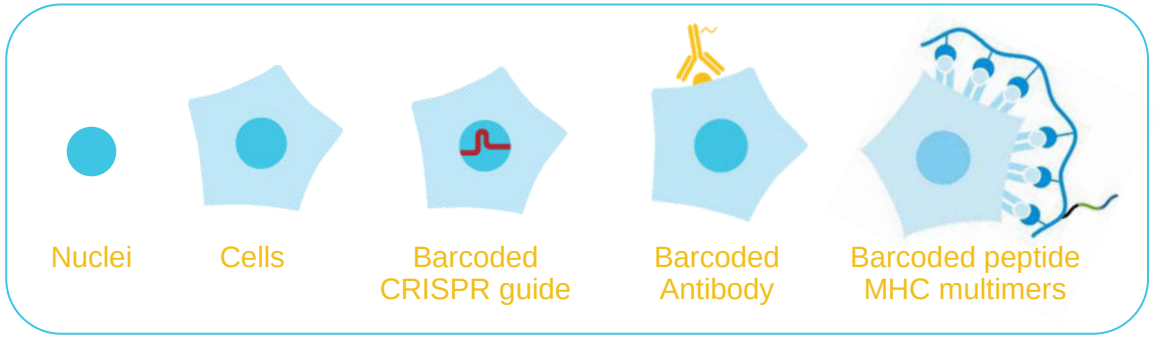
10x GENOMICS

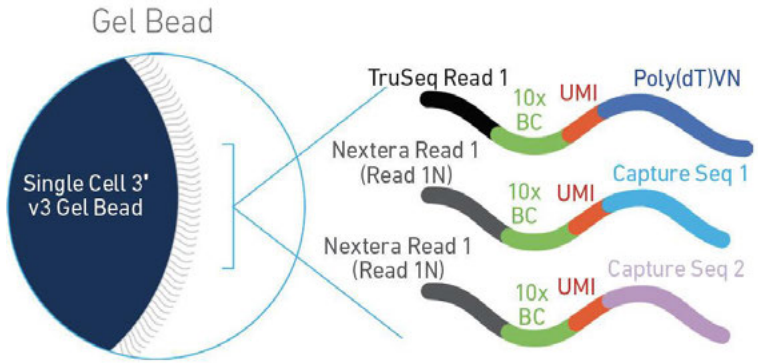
Technology

Partitioning and molecular barcoding millions of parallel reactions









10x GENOMICS

Single cell sample prep resources from 10x Genomics

- https://support.10xgenomics.com/
- Protocols are free to download

General sample preparation guidelines

- Guidelines for optimal sample preparation
- Guidelines for accurate target cell counts
- General cell preparation guide
- Preparation of single cell suspensions from cultured cell lines
- Isolation of nuclei

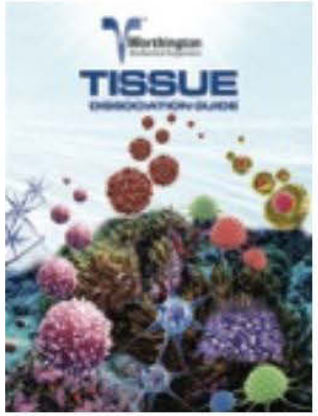
Preparation of specific sample types

- Fresh frozen human-mouse cell line mixtures
- Fresh frozen human peripheral blood mononuclear cells
- Dissociation of mouse embryonic neural tissue
- Tumor dissociation
- Methanol fixation of cells
- Moss protoplast suspensions

Sample improvement

- Enrichment of CD3+ T cells from dissociated tissues
- Removal of dead cells from single cell suspensions

General Cell Handling Recommendations



Worthington Tissue Dissociation Guide

Introduction

Tissue dissociation/primary cell isolation and cell harvesting are principal applications for enzymes in tissue culture research and cell biology studies. Despite the widespread use of enzymes for these applications over the years, their mechanisms of action in dissociation and harvesting are not well understood. As a result, the choice of one technique over another is often arbitrary and based more on past experience than on an understanding of why the method works and what modifications could lead to even better results.

Tissue Tables (references, grouped by tissue type and species)

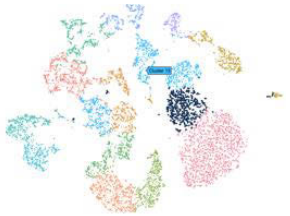
Adipose/Fat	Adrenal	Bone	Brain
Cartilage	Colon	Endothelial	Epithelial
Eye	Heart	Intestine	Kidney
Liver	Lung	Lymph nodes	Mammary
Miscellaneous	Muscle	Neural	Pancreas
Parotid	Pituitary	Prostate	Reproductive
Scales	Skin	Spleen	Stem
Thymus	Thyroid/Parathyroid	Tonsil	Tumor

https://www.worthington-biochem.com/tissuedissociation/

General Cell Handling Recommendations

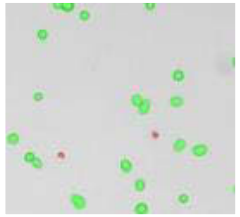
Analysis of Single Cell Transcriptomes

- Requires a fully dissociated, **single cell suspension.**
- Minimizing the presence of cellular aggregates, dead cells, non-cellular nucleic acids and potential inhibitors of reverse transcription is critical to obtaining high quality data.
- Suspension cell lines, bead-enriched and flow-sorted cells can be used directly after washing



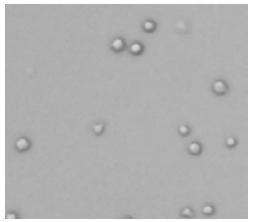
Importance of Input Cell Quality

- Ideally, input cell suspensions should contain more than **90% viable cells**.
- **The presence of a high fraction of non-viable or dying cells may decrease recovery.**
  - **The presence of ambient RNA and cellular debris may impact application performance and negatively impact quality metrics reported by Cell Ranger.**



Cell Handling

- It is important to **treat cells *gently*** to minimize cell lysis and loss:
  - **When cells lyse, the released ambient mRNA will contaminate other GEMs**
  - **Wash cells twice** using a wide-bore pipette tip to remove ambient RNA and contaminants.
  - **Wash and resuspend in PBS + 0.04% non-acetylated BSA to minimize cell loss during handling.**



General Cell Handling Recommendations

Debris/Aggregate Removal

- **Use a cell strainer to remove aggregates or debris from washed cells**
- **The presence of cell aggregates, debris and/or fibers can result in inaccurate cell counts**
- GEM generation occurs in microfluidic channels that are narrower than the typical human hair (i.e. < 100 µm) and the presence of cell debris or large aggregates **may clog or wet the chip**

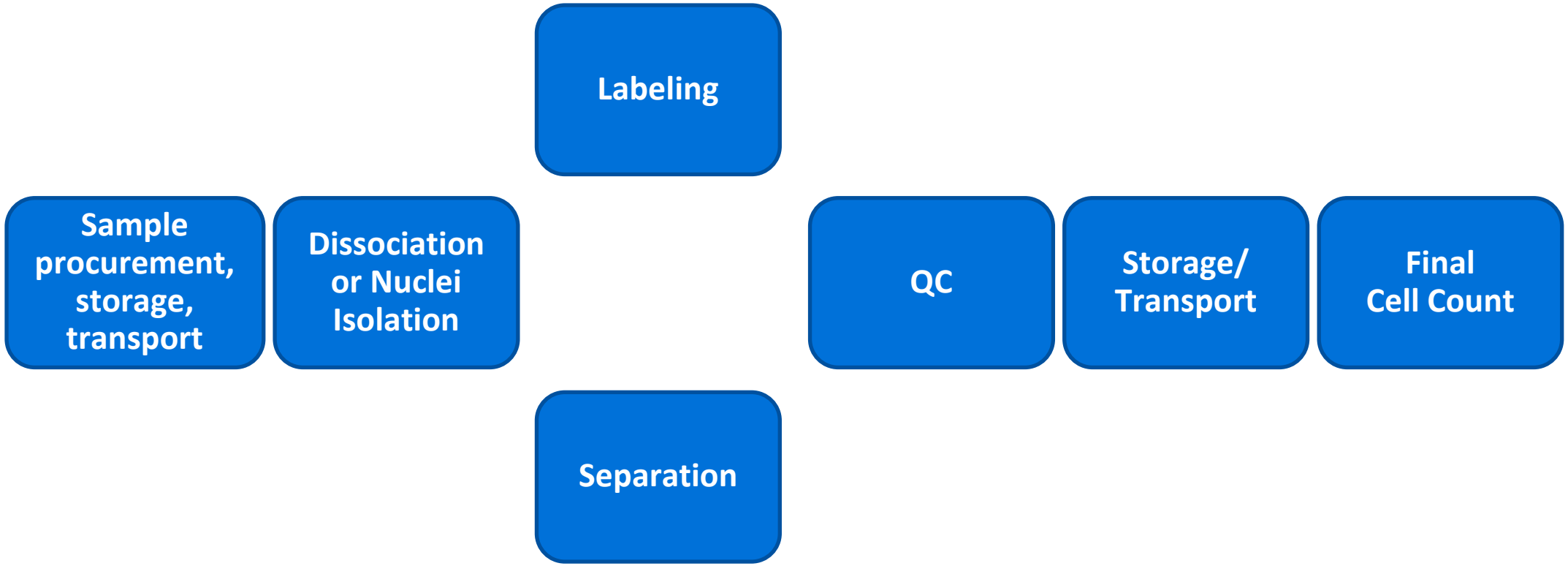
Cell Counting

- Quantitate cells accurately before loading into the system
  - **Approximately 65% loaded cells will be recovered**
  - To maximize the likelihood of achieving the desired recovery target, the optimal input **cell concentration is 700-1200 cells/µl**
  - **Recommended range: 500 to 10,000 recovered cells**
  - **Under- or over-loading may impact application performance**

Storage of Single Cell Suspensions

- **Cell suspensions should always be kept on ice and where possible proceed with cell loading immediately after sample preparation**
  - Ideally incubation time should be kept to a minimum (< 30 min)
- **Some cell types are more fragile and cell viability may decrease significantly if not processed and loaded immediately**

It’s a Workflow. A Set of Decisions.



Choosing a Single Cell Assay

Gene Expression

Provides **transcriptome +/- immune receptor profiling**

Considerations:

- Interested in **cellular mRNA**?
- Interested in **feature barcode** or **cellplexing**?
- Interested in **targeted gene** expression?
- Interested in **automation**?
- High sensitivity

Multiome ATAC + Gene Expression

Provides nuclear transcriptome with **paired** chromatin accessibility profiling

Considerations:

- Interested in multimodal cell phenotyping?
- Pair gene expression with regulatory activity?
- **Limited sample** type?
- Interested in **nuclear** mRNA only?

ATAC

Provides **chromatin** accessibility profiling

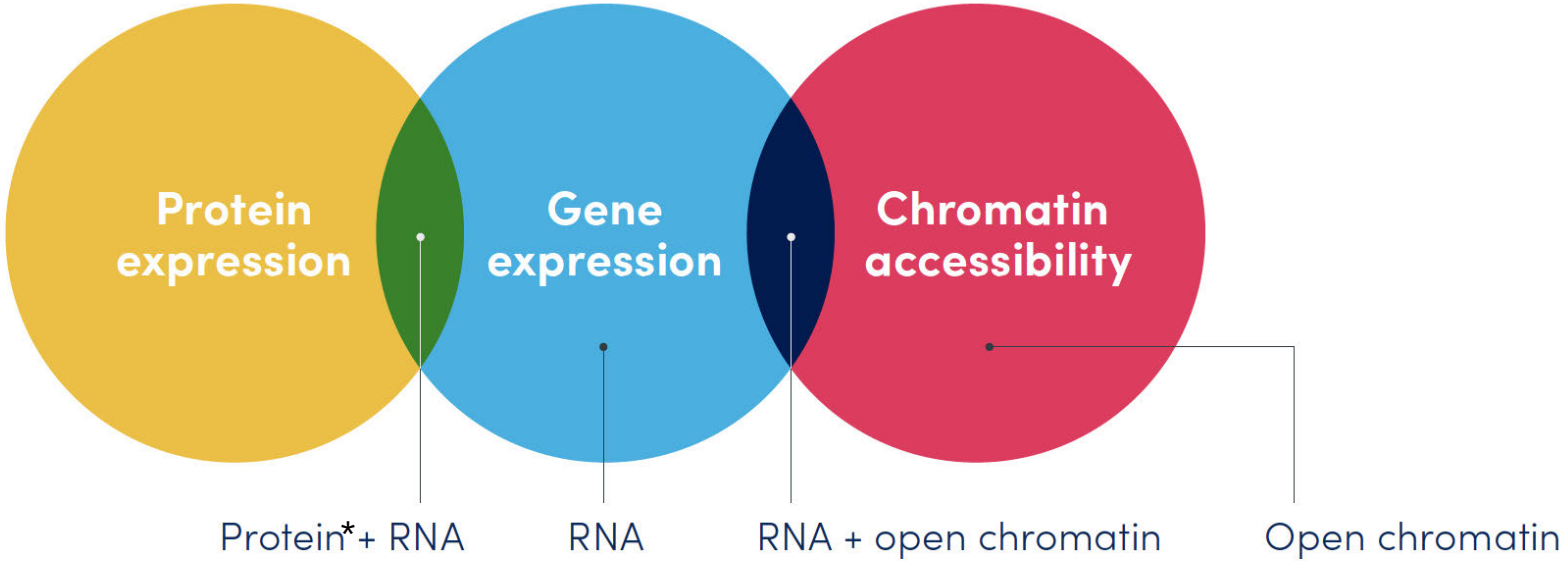
Considerations:

- Interested in **open chromatin & TF binding** only?
- Sample types with **unknown or low mRNA integrity**?
- Cost sensitive?

Integrate data with third party tools using Single Cell Multiome ATAC + Gene Expression as bridge

Different assays require different input materials

What are you measuring?

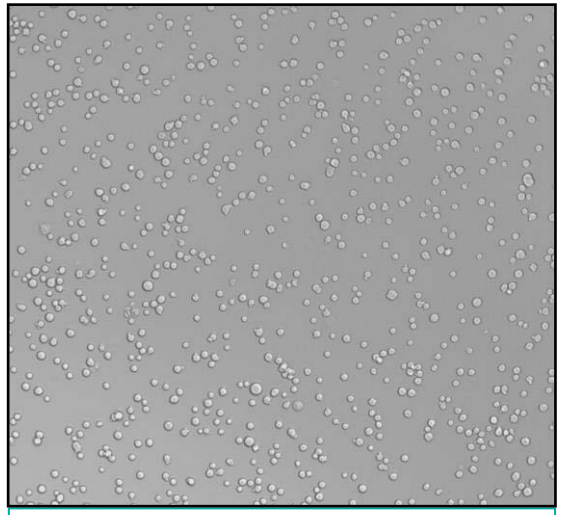
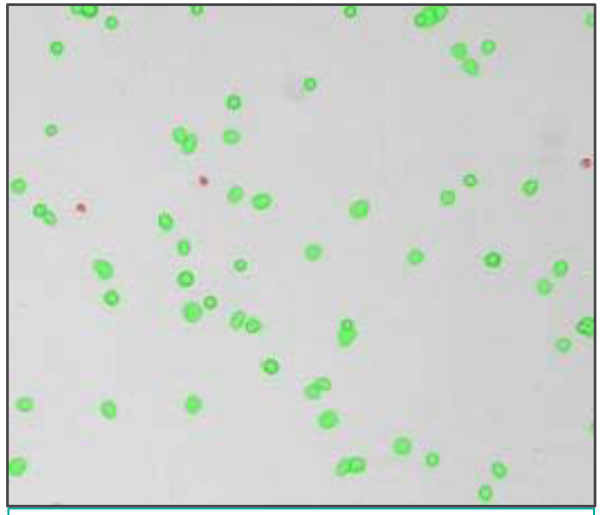


Input required	Whole cells	Whole cells or nuclei	Nuclei

**Cell Surface protein*

Quality is Critical

Goal is to minimize

Clean	Healthy	Intact
		
• Aggregates/clumps • Subcellular debris • Free-floating RNA/DNA	• Biological decomposition • RNA leakage (background) • RNA degradation (signal)	• Physical decomposition • RNA leakage (background) • RNA degradation (signal)

Sample Procurement, Storage, and Transport

SCIENTIFIC REPORTS

RESEARCH Open Access

Cryopreservation of human cancers conserves tumour heterogeneity for single-cell multi-omics analysis

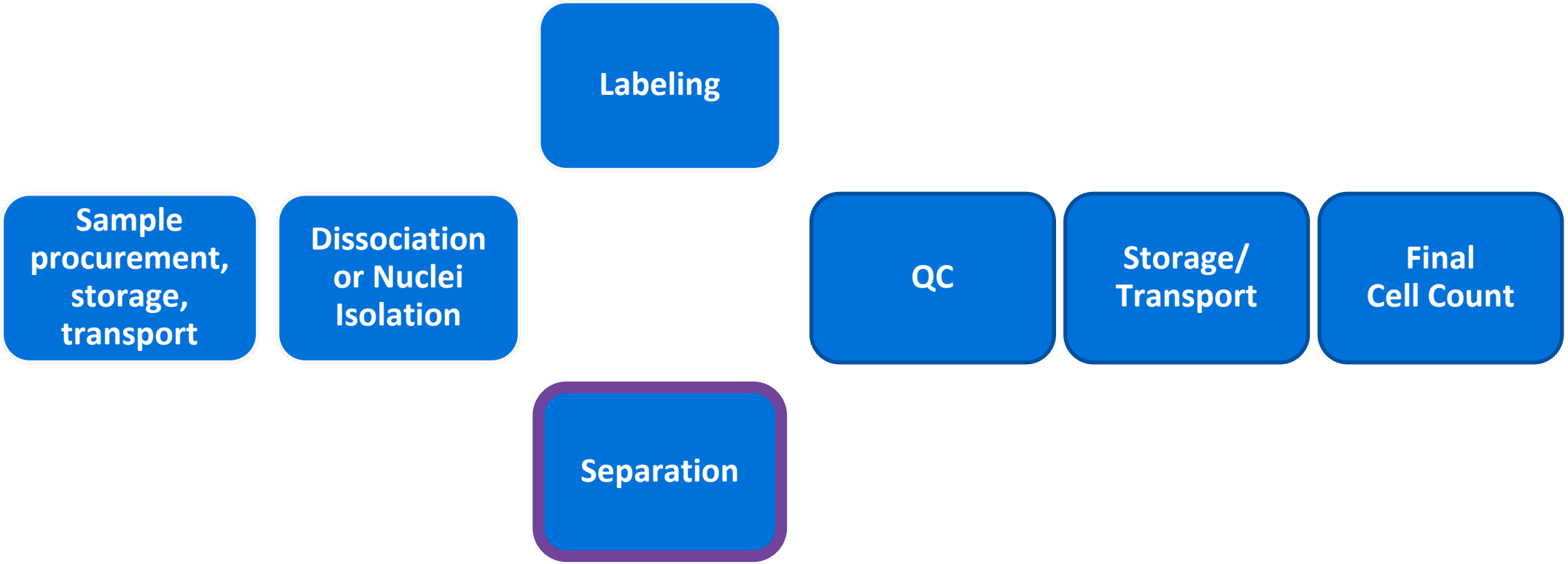
Sunny Z. Wu[1,2], Daniel L. Roden[1,2], Ghamdan Al-Eryani[1,2], Nenad Bartonicek[1,2], Kate Harvey[1], Aurélie S. Cazet[1,2], Chia-Ling Chan[1,3], Simon Junankar[1,2], Mun N. Hui[1,4], Ewan A. Millar[5,6,7], Julia Beretov[5,8], Lisa Horvath[1,4,9], Anthony M. Joshua[1,10], Phillip Stricker[10], James S. Wilmott[11,12], Camelia Quek[11,12], Georgina V. Long[11,12,13], Richard A. Scolyer[11,12,14], Bertrand Z. Yeung[15], Davendra Segara[10], Cindy Mak[4], Sanjay Warrier[16,17], Joseph E. Powell[3,18], Sandra O'Toole[1,2], Elgene Lim[1,2,10] and Alexander Swarbrick[1,2*]

Received: 1 March 2
Accepted: 4 July 201
Published online: 23

Syst
diss
cell

Elena Der
Olivier Clement[1], Rebecca K. Simmons[1,3], Ryan Lister[1,3] and Alistair R. R. Forrest[1]

Detailed decisions:
Single Cell GEX sample separation



Sample Separation

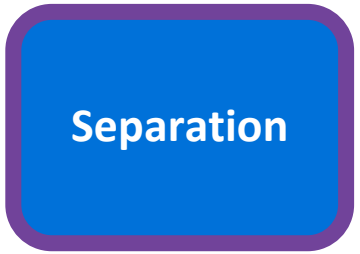
Separate intact cells and nuclei from

- Aggregates/clumps
- Debris
- Free-floating mRNA
- Dead Cells
- Enrichment/Depletion

Challenges with separation

- Samples are fragile
- Physical stress
- Buffers
- Time
- Yield

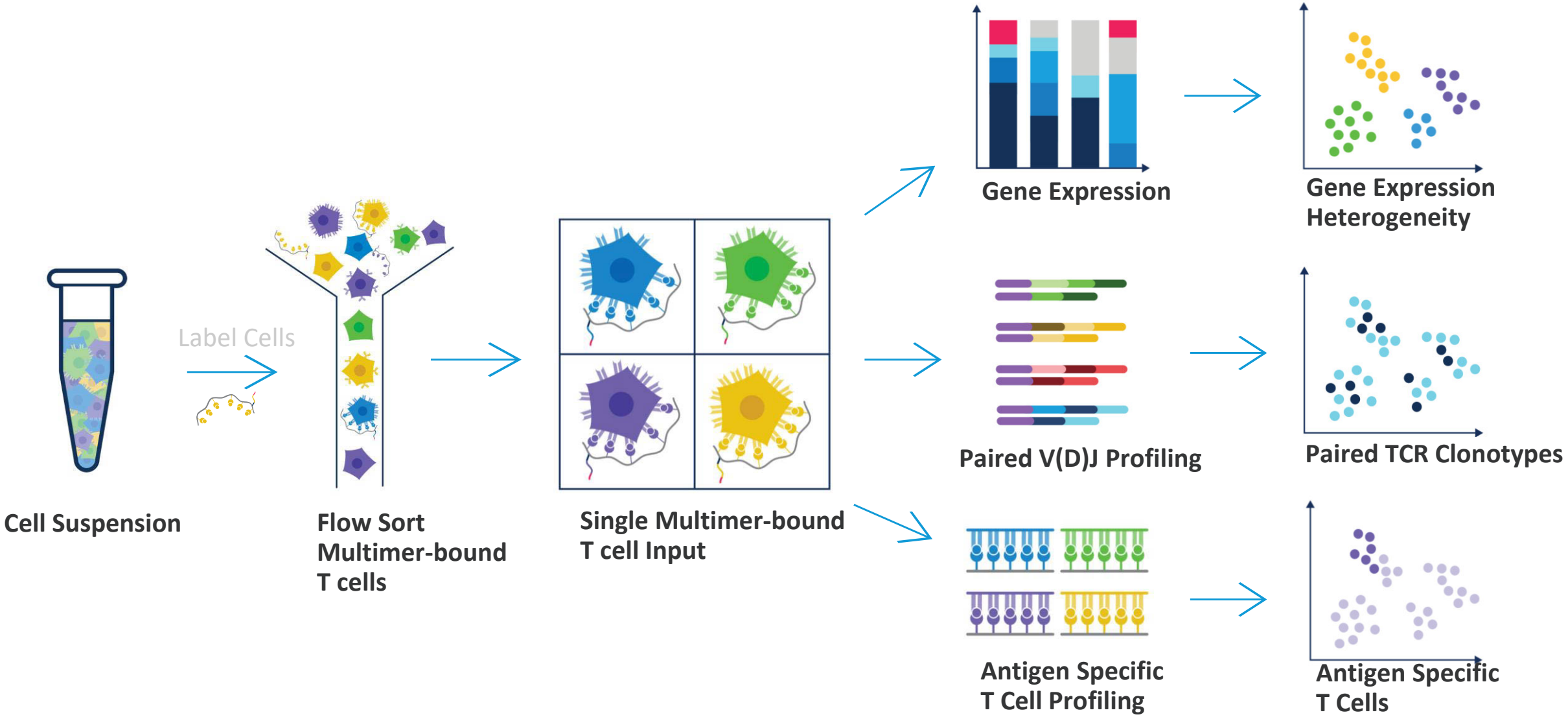
Want the minimum handling necessary. Maintain sample integrity.



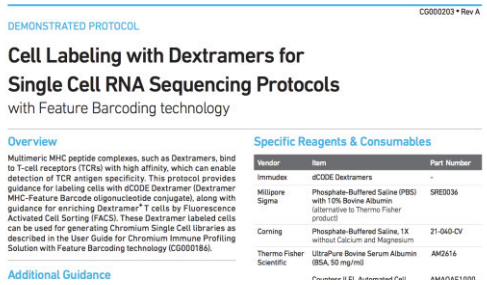
Basic Methods for Sample Separation

Method	Thorough centrifugation (e.g. 3x with PBS + 0.04% BSA)	Gentle centrifugation (e.g. 1x with media)	Magnetic beads	Density Gradient	FACS
10x Protocol Example	PBMC (CG000039)	Cell Prep Guide (CG000053)	Dead Cell Removal (CG000093)	Nuclei Isolation (CG000124)	Customer Developed Protocol (Martelotto)
Sample Size	Abundant	Limited	Abundant	Abundant	Limited
Benefits	Thorough	Gentle	Specific, easily accessible, scalable	Removes Debris	Versatile, quick
Possible Challenges	Yield, Harsh	Less thorough	Yield	Yield, Harsh, Time	Expensive, Harsh

Reveal Antigen Specificity with Feature Barcoding Technology



Sample Prep Workflow: Rare Cell Population


CG000203 • Rev A

DEMONSTRATED PROTOCOL

Cell Labeling with Dextramers for Single Cell RNA Sequencing Protocols

with Feature Barcoding technology

Overview

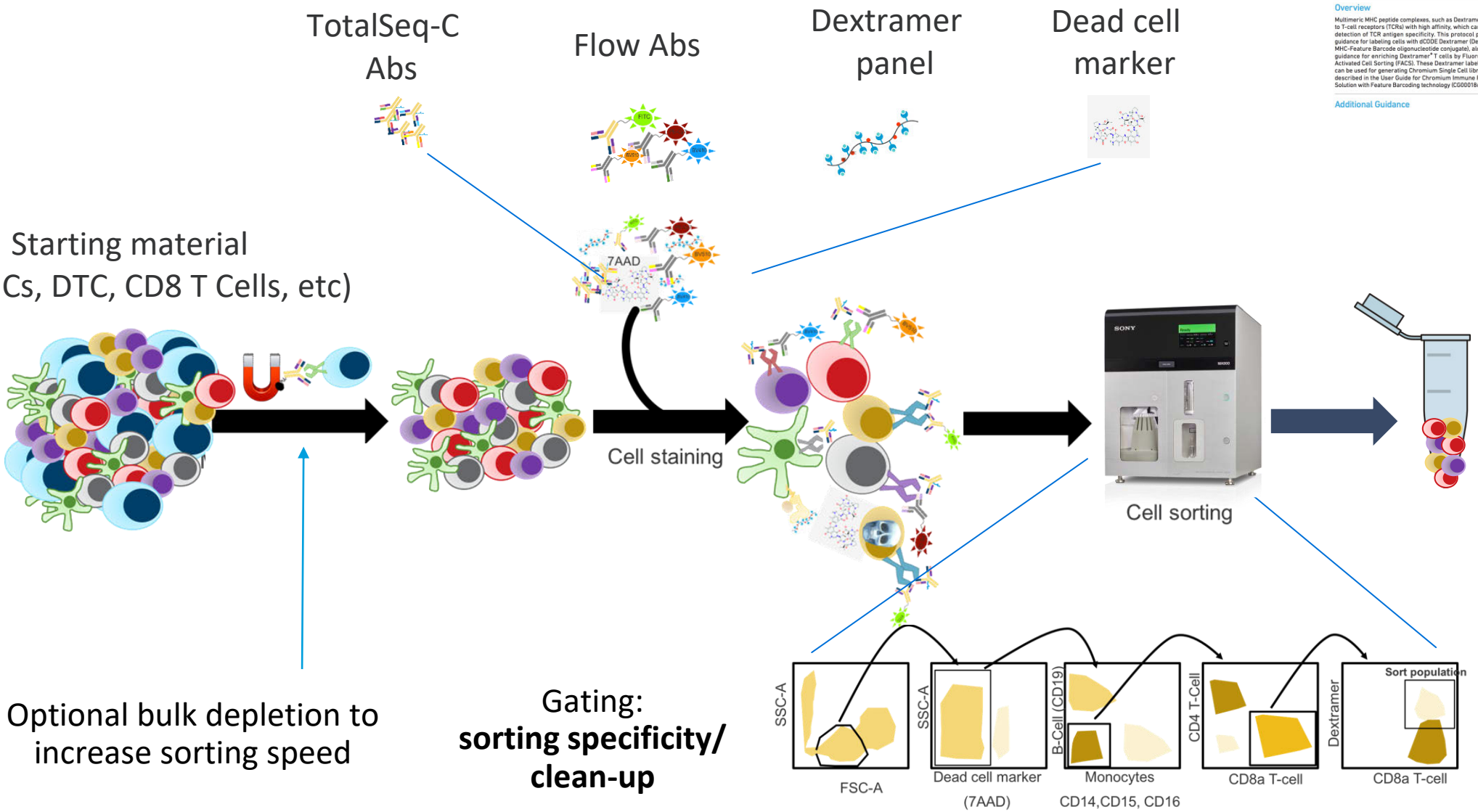
Multimeric MHC peptide complexes, such as Dextramers, bind to T-cell receptors (TCRs) with high affinity, which can enable detection of TCR antigen specificity. This protocol provides guidance for labeling cells with dCODE Dextramer (Dextramer MHC-Feature Barcode oligonucleotide conjugate), along with guidance for enriching Dextramer^{+} T cells by Fluorescence Activated Cell Sorting (FACS). These Dextramer labeled cells can be used for generating Chromium Single Cell libraries as described in the User Guide for Chromium Immune Profiling Solution with Feature Barcoding technology (CG000186).

Additional Guidance

Specific Reagents & Consumables

Vendor	Item	Part Number
Immudex	dCODE Dextramers	-
Millipore Sigma	Phosphate-Buffered Saline (PBS) with 10% Bovine Albumin (alternative to Thermo Fisher product)	SRE0036
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magnesium	21-040-CV
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616

CG000203



PBS + 2% FBS: **cell viability**

Summary of Key Lessons

When our standard guidance isn't applicable:

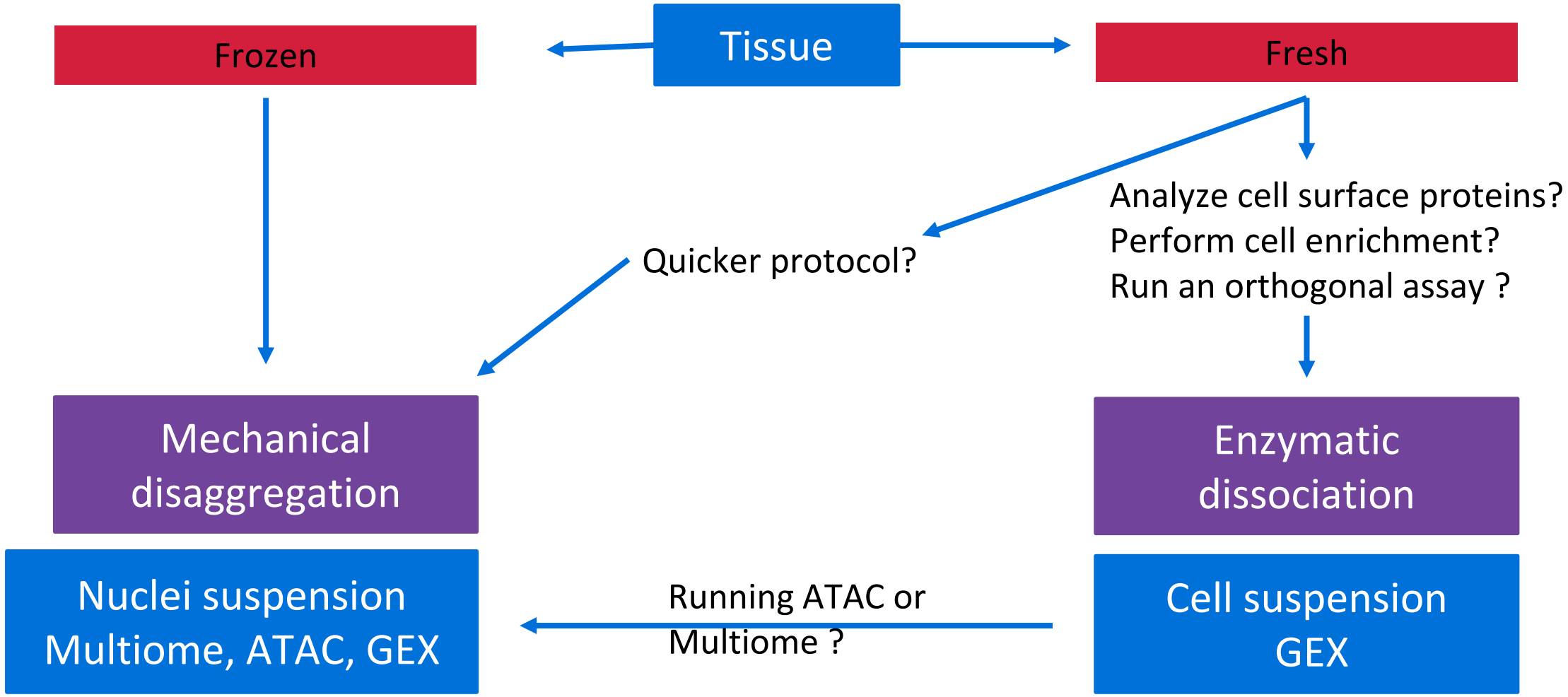
- Treat cells gently and minimize decomposition
  - Use gentl(er) lysis conditions
  - Reduce wash steps*
  - Use a swinging bucket centrifuge
  - Keep cells in media + FBS instead of PBS**
- Work quickly
  - Consider sorting, it is a versatile tool for sample prep
  - Minimize unnecessary handling steps
- *Consider the benefits and drawbacks of every different technique*

*Cell surface protein analysis requires thorough washing
**ATAC has specific buffer formulation

10X GENOMICS

Isolation of Nuclei for Single Cell Sequencing

Prepare a Cell or Nuclei Suspension



Why Use Nuclei?

A clean, viable single cell suspension is necessary for optimal results in scRNA sequencing. However, there are times when getting a good cell suspension is difficult and nuclei is an alternative option.

- **When cells are large and exceed the limits for the microfluidic chip**
  - Hepatocytes
  - Neurons with significant extensions
- **When cells are of a challenging shape**
  - Cardiomyocytes
- **When cells are difficult to get into a single cell suspension**
  - Sample contains a lot of debris
  - Neurons are highly interconnected and may not efficiently dissociate into single cells after enzymatic treatment
  - Dissociation-resistant tissue samples such as complex tissues/ organs where nuclei (but not whole intact cells) can be isolated

Why Use Nuclei?

- ***Possible*** **solution for archival (cryopreserved) or damaged samples in which the cell wall is breaking down**
  - Laser capture microdissection will physically damage whole cells (cell wall)
  - Nuclei isolation will not rescue damaged cells that are already dying or undergoing apoptosis
- ***Possible*** **solution for experiments aiming to reveal molecular genetic regulatory mechanisms specific to the nucleus**
- **Sample types that have a cell wall that does not lyse in our assay**
  - Various plants, yeast
- **For ATAC and Multiome**

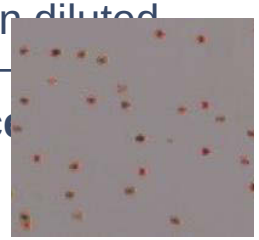
General Handling Recommendations

Starting Sample Requirements

- Tissues or cell suspensions
- Dissociate tissues when possible, some tissues will require going straight into nuclei isolation
- If starting with low viability cell suspension, sorting prior to nuclei isolation may help reduce ambient DNA and cellular debris
  - **Sorting after nuclei isolation is not recommended as it may damage nuclear membrane**

Nuclei Isolation

- Refer to Demonstrated Protocols for Nuclei Isolation for Single Cell ATAC Sequencing:
  - Nuclei Isolation from mouse brain tissue
  - Nuclei Isolation from cell lines and PBMCs
  - **Isolation of Nuclei for Single Cell RNA Sequencing demonstrated protocol decreases single cell ATAC assay performance**
- Resuspend nuclei in diluted Nuclei Buffer (1X)—**assay performance**



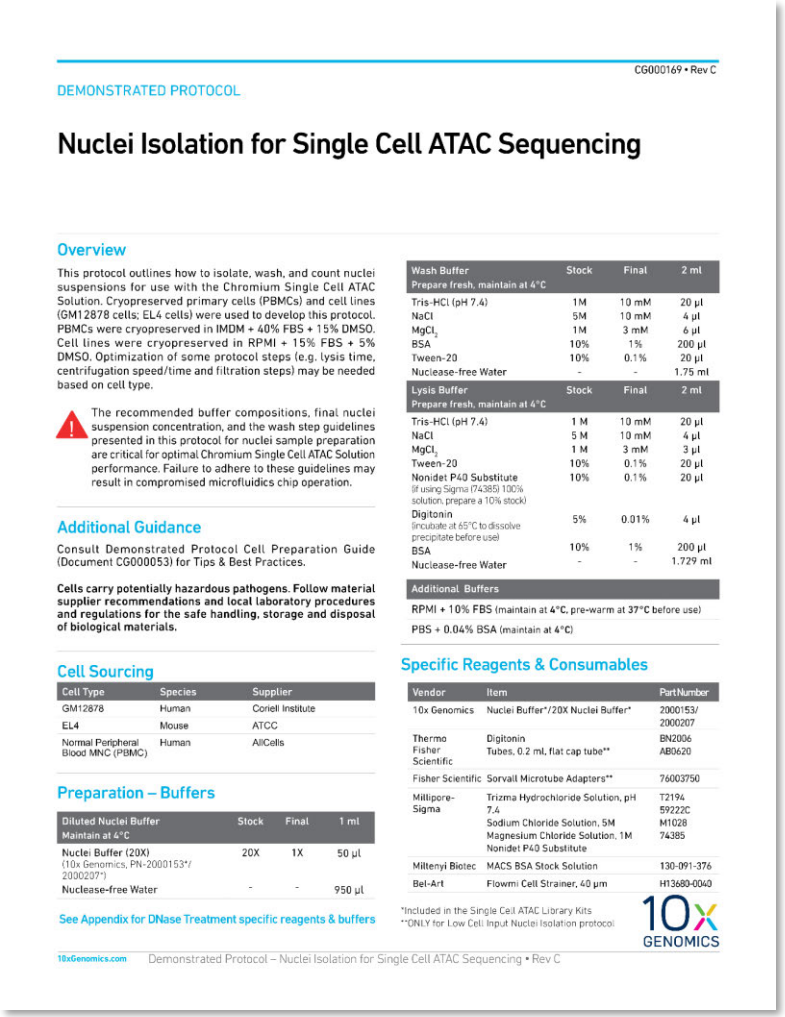
Nuclei Isolation

- It is important to **treat nuclei gently** to minimize lysis and loss
- Count Nuclei using Countess and trypan blue, ideal viability should be <5% live
  - Counting may also be done using ethidium homodimer and fluorescence microscope or Countess II FL.
- Visualization under the microscope may give further indications of nuclear membrane quality

Demonstrated Protocol Available from 10x Genomics

Nuclei Isolation for Single Cell ATAC Sequencing (From Cell Lines and PBMCs)

- Demonstrated protocol includes recommendations/tips for preparing nuclei from PBMCs and from cell lines (GM12878:EL4 mix), fresh and cryopreserved
- Low sample input protocol in appendix for limited samples
- Protocol can be adapted for other cell types with optimization
- Demonstrated Protocol is available on 10x support website
- **Note: Not all demonstrated protocols on our website will be compatible with the Chromium Single Cell ATAC Solution**



CG000169 • Rev C

DEMONSTRATED PROTOCOL

Nuclei Isolation for Single Cell ATAC Sequencing

Overview

This protocol outlines how to isolate, wash, and count nuclei suspensions for use with the Chromium Single Cell ATAC Solution. Cryopreserved primary cells (PBMCs) and cell lines (GM12878 cells; EL4 cells) were used to develop this protocol. PBMCs were cryopreserved in IMDM + 40% FBS + 15% DMSO. Cell lines were cryopreserved in RPMI + 15% FBS + 5% DMSO. Optimization of some protocol steps (e.g. lysis time, centrifugation speed/time and filtration steps) may be needed based on cell type.

The recommended buffer compositions, final nuclei suspension concentration, and the wash step guidelines presented in this protocol for nuclei sample preparation are critical for optimal Chromium Single Cell ATAC Solution performance. Failure to adhere to these guidelines may result in compromised microfluidics chip operation.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG000053) for Tips & Best Practices.

Cells carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.

Cell Sourcing

Cell Type	Species	Supplier
GM12878	Human	Coriell Institute
EL4	Mouse	ATCC
Normal Peripheral Blood MNC (PBMC)	Human	AllCells

Preparation – Buffers

Diluted Nuclei Buffer Maintain at 4°C	Stock	Final	1 ml
Nuclei Buffer (20X) (10x Genomics, PN-2000153*/ 2000207*)	20X	1X	50 µl
Nuclease-free Water	-	-	950 µl

See Appendix for DNase Treatment specific reagents & buffers

Wash Buffer Prepare fresh, maintain at 4°C	Stock	Final	2 ml
Tris-HCl (pH 7.4)	1 M	10 mM	20 µl
NaCl	5 M	10 mM	4 µl
$MgCl_2$	1 M	3 mM	6 µl
BSA	10%	1%	200 µl
Tween-20	10%	0.1%	20 µl
Nuclease-free Water	-	-	1.75 ml

Lysis Buffer Prepare fresh, maintain at 4°C	Stock	Final	2 ml
Tris-HCl (pH 7.4)	1 M	10 mM	20 µl
NaCl	5 M	10 mM	4 µl
$MgCl_2$	1 M	3 mM	3 µl
Tween-20	10%	0.1%	20 µl
Nonidet P40 Substitute (if using Sigma (74385) 100% solution, prepare a 10% stock)	10%	0.1%	20 µl
Digitonin (incubate at 65°C to dissolve precipitate before use)	5%	0.01%	4 µl
BSA	10%	1%	200 µl
Nuclease-free Water	-	-	1.729 ml

Additional Buffers
RPMI + 10% FBS (maintain at 4°C, pre-warm at 37°C before use)
PBS + 0.04% BSA (maintain at 4°C)

Specific Reagents & Consumables

Vendor	Item	Part Number
10x Genomics	Nuclei Buffer*/20X Nuclei Buffer*	2000153/ 2000207
Thermo Fisher Scientific	Digitonin Tubes, 0.2 mL, flat cap tube**	BN2006 AB0620
Fisher Scientific	Sorvall Microtube Adapters**	76003750
Millipore-Sigma	Trizma Hydrochloride Solution, pH 7.4 Sodium Chloride Solution, 5M Magnesium Chloride Solution, 1M Nonidet P40 Substitute	T2194 59222C M1028 74385
Miltenyi Biotec	MACS BSA Stock Solution	130-091-376
Bel-Art	Flowmi Cell Strainer, 40 µm	H13680-0040

*Included in the Single Cell ATAC Library Kits
**ONLY for Low Cell Input Nuclei Isolation protocol

10x GENOMICS

10xGenomics.com Demonstrated Protocol – Nuclei Isolation for Single Cell ATAC Sequencing • Rev C

Nuclei Isolation for Single Cell ATAC Sequencing

Required Reagents and Buffer Composition

Reagents

Vendor	Item	Part Number
10x Genomics	Nuclei Buffer*/20X Nuclei Buffer*	2000153/ 2000207
Thermo Fisher Scientific	Digitonin Tubes, 0.2 ml, flat cap tube**	BN2006 AB0620
Fisher Scientific	Sorvall Microtube Adapters**	76003750
Millipore-Sigma	Trizma Hydrochloride Solution, pH 7.4 Sodium Chloride Solution, 5M Magnesium Chloride Solution, 1M Nonidet P40 Substitute	T2194 59222C M1028 74385
Miltenyi Biotec	MACS BSA Stock Solution	130-091-376
Bel-Art	Flowmi Cell Strainer, 40 µm	H13680-0040

*Included in the Single Cell ATAC Library Kits
**ONLY for the Low Cell Input Nuclei Isolation protocol

Buffers

Diluted Nuclei Buffer Maintain at 4°C	Stock	Final	1 ml
Nuclei Buffer (20X) (10x Genomics, PN-2000153*/ 2000207*)	20X	1X	50 µl
Nuclease-free Water	-	-	950 µl

Wash Buffer Prepare fresh, maintain at 4°C	Stock	Final	2 ml
Tris-HCl (pH 7.4)	1M	10 mM	20 µl
NaCl	5M	10 mM	4 µl
$MgCl_2$	1M	3 mM	6 µl
BSA	10%	1%	200 µl
Tween-20	10%	0.1%	20 µl
Nuclease-free Water	-	-	1.75 ml

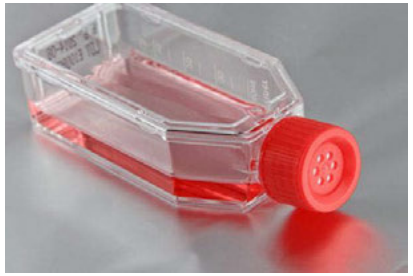
Lysis Buffer Prepare fresh, maintain at 4°C	Stock	Final	2 ml
Tris-HCl (pH 7.4)	1 M	10 mM	20 µl
NaCl	5 M	10 mM	4 µl
$MgCl_2$	1 M	3 mM	3 µl
Tween-20	10%	0.1%	20 µl
Nonidet P40 Substitute (if using Sigma (74385) 100% solution, prepare a 10% stock)	10%	0.1%	20 µl
Digitonin (incubate at 65°C to dissolve precipitate before use)	5%	0.01%	4 µl
BSA	10%	1%	200 µl
Nuclease-free Water	-	-	1.729 ml

Additional Buffers
RPMI + 10% FBS (maintain at **4°C**, pre-warm at **37°C** before use)
PBS + 0.04% BSA (maintain at **4°C**)

Supplied in Chromium Single Cell ATAC Library Kits. The tube contains enough to make ~32 ml of working dilution for final nuclei resuspension. Typical usage is up to 1 ml per sample.
Once prepared, maintain diluted Nuclei Buffer on ice while isolating nuclei.

Validated with Nuclei Isolated from Multiple Sample Types

Cell Lines

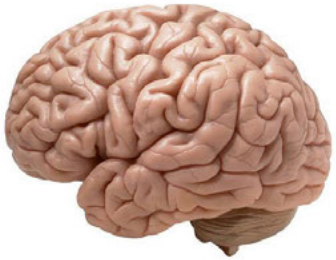


- Suspension: GM12878, A20, EL4, K562
- Adherent: A549

Primary Immune Cells

- Human Peripheral Blood Mononuclear Cells
- Human Bone Marrow Mononuclear Cells

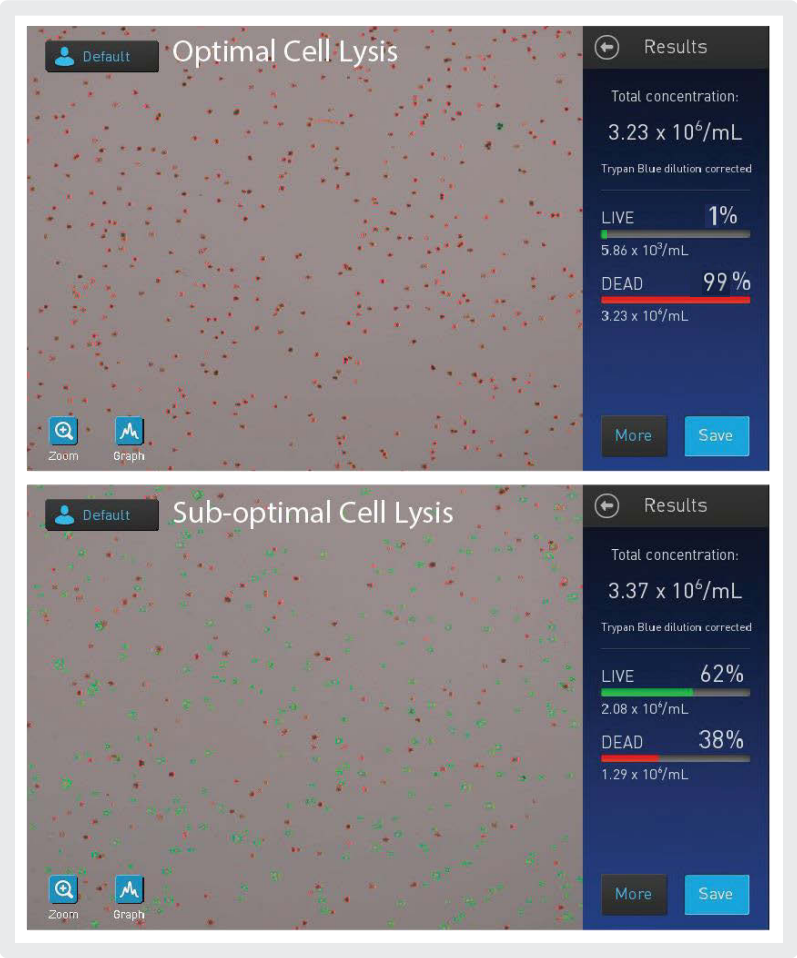
Dissociated Primary Tissues



- Embryonic Mouse Brain Tissue
- Adult Mouse Brain Tissue
- Mouse Splenocytes

Nuclei isolation for Single Cell Multiome ATAC + Gene Expression sequencing

Lysis can be assessed using a cell viability stain



When counting look for:

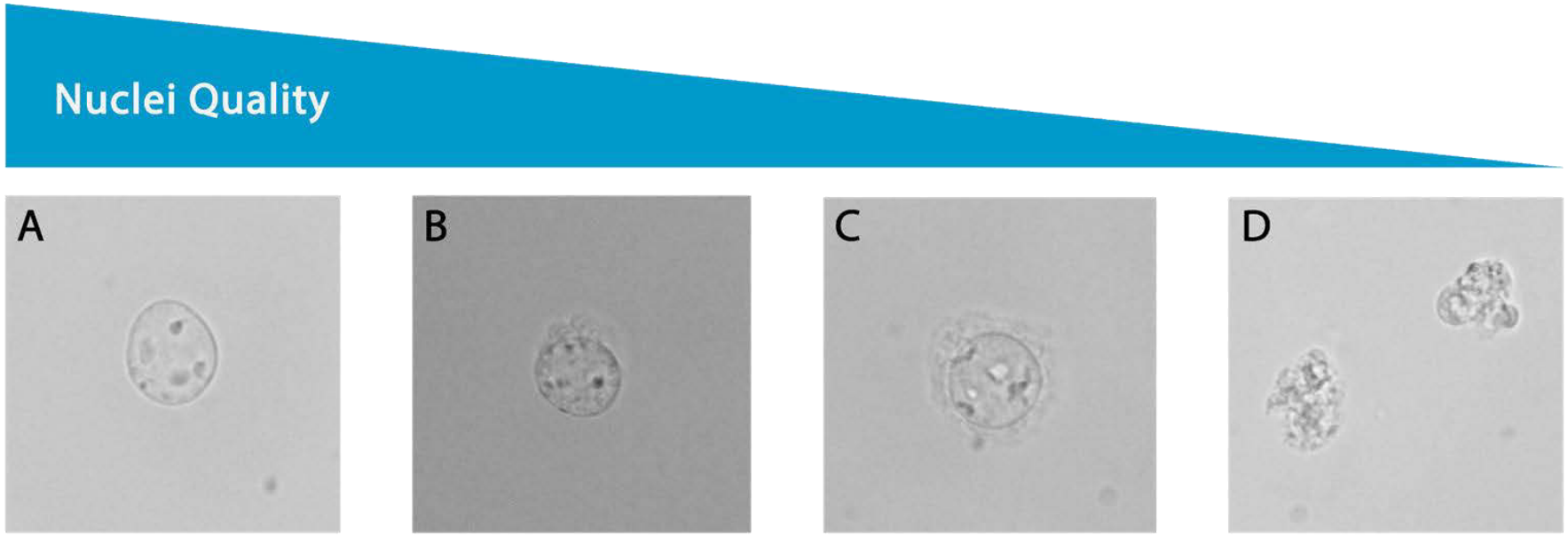
- <5% Live Cells (or >95% dead)
  - Nuclei will stain as dead
  - Lysis time will be cell-type dependent
  - Lysis time course may be required to determine optimal lysis time
- Clean, clump free nuclei
  - Filtering may help break clumps and remove debris

This provides a yes/no answer as to whether the cell membrane was lysed. Resolution is enough to assess clumping and debris but may not be enough to evaluate nuclear membrane integrity.

For hard to count cells (small size, lots of debris), use a fluorescent stain like Ethidium Homodimer-1. An automated counter with fluorescent capability is needed.

Nuclei isolation for Single Cell ATAC sequencing

Nuclear morphology can indicate nuclei quality



60x Magnification/Brightfield

A: High-quality nuclei have well-resolved edges. Optimal quality for single cell ATAC libraries.
B: Mostly intact nuclei with minor evidence of blebbing. Quality single cell ATAC libraries can still be produced.
C: Nuclei with strong evidence of blebbing. ***Proceed at your own risk.***
D: Nuclei are no longer intact. ***Do not proceed!***

Data Review – Cells vs Nuclei

Gene Expression Levels Are Well Correlated Between Cells and Nuclei

nature biotechnology

ANALYSIS

https://doi.org/10.1038/s41587-020-0465-8

Check for updates

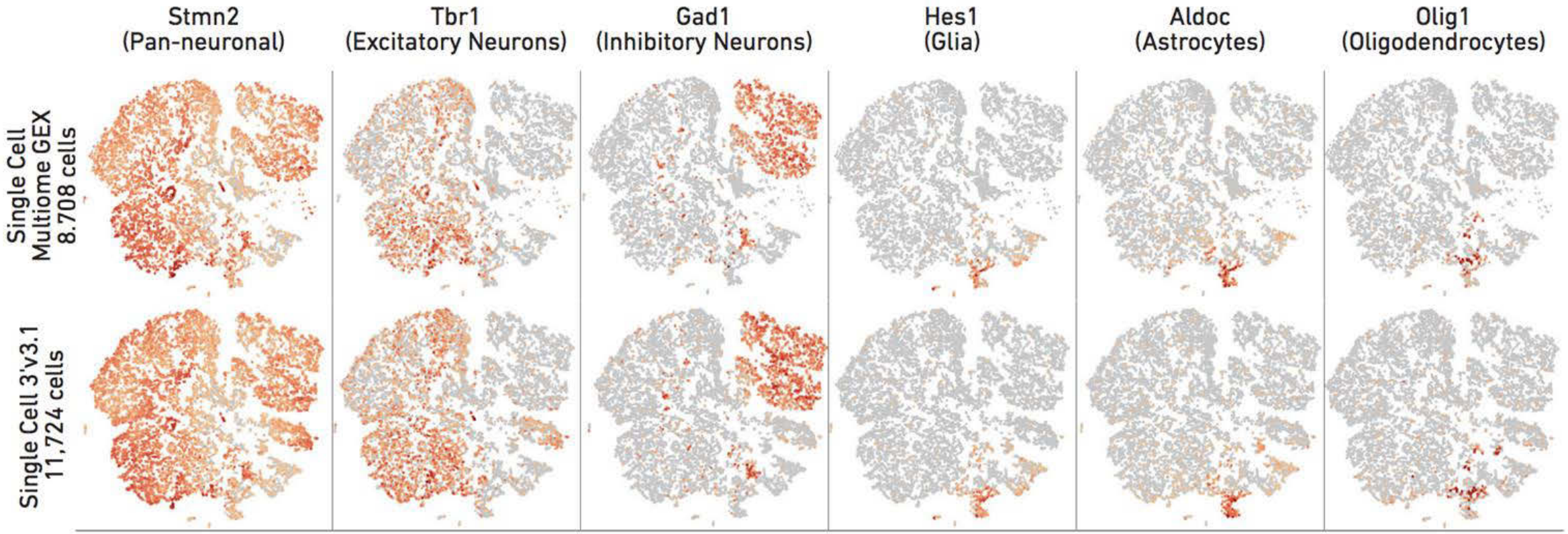
Systematic comparison of single-cell and single-nucleus RNA-sequencing methods

Jiarui Ding[1], Xian Adiconis[1,9], Sean K. Simmons[1,9], Monika S. Kowalczyk[1], Cynthia C. Hession[1], Nemanja D. Marjanovic[1], Travis K. Hughes[1,2,3,4], Marc H. Wadsworth[1,2,3,4], Tyler Burks[1], Lan T. Nguyen[1], John Y. H. Kwon[1], Boaz Barak[5], William Ge[1], Amanda J. Kedaigle[1], Shaina Carroll[1,2,3,4], Shuqiang Li[1], Nir Hacohen[1,6], Orit Rozenblatt-Rosen[1], Alex K. Shalek[1,2,3,4], Alexandra-Chloé Villani[1,6,7], Aviv Regev[1,4,8] and Joshua Z. Levin[1]✉

Cell type specific markers are conserved

Between Single Cell Multiome Gene Expression and 3’ v3.1

Gene Expression

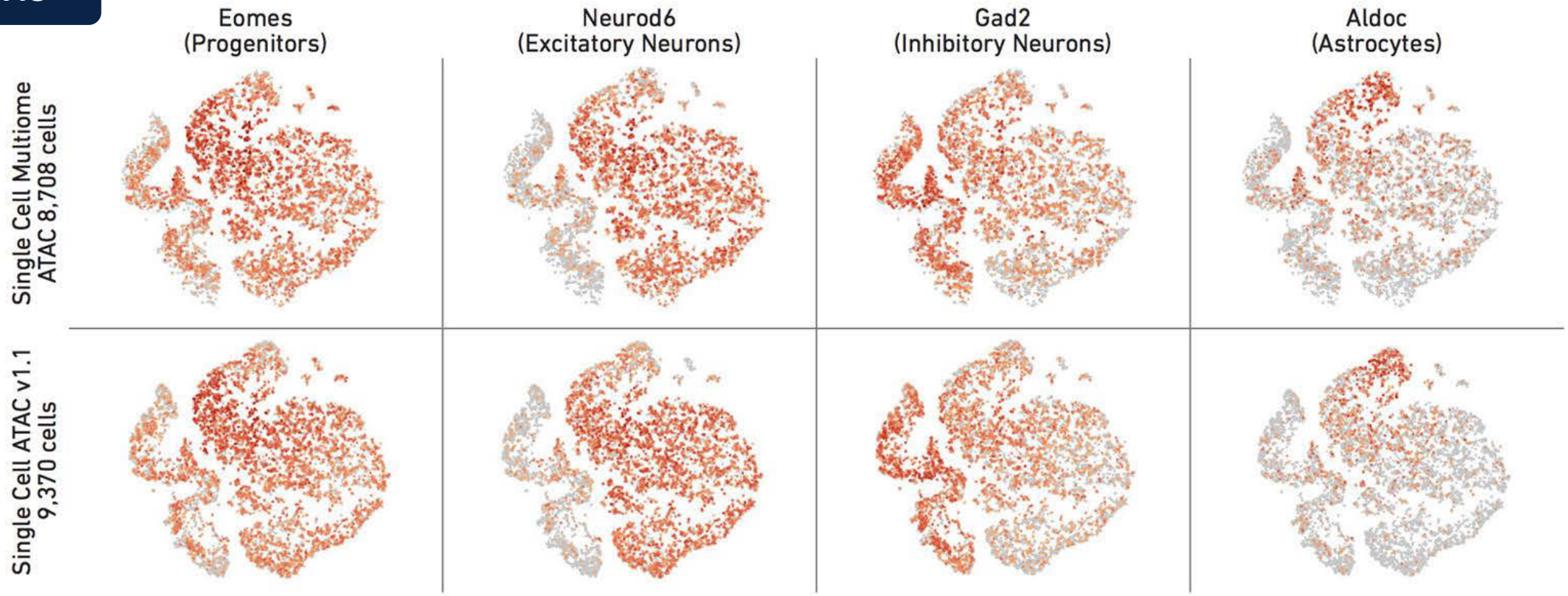


Mouse embryonic E18.5 brain nuclei

Cell type specific markers are conserved

Between Single Cell Multiome ATAC and ATAC v1.1

ATAC

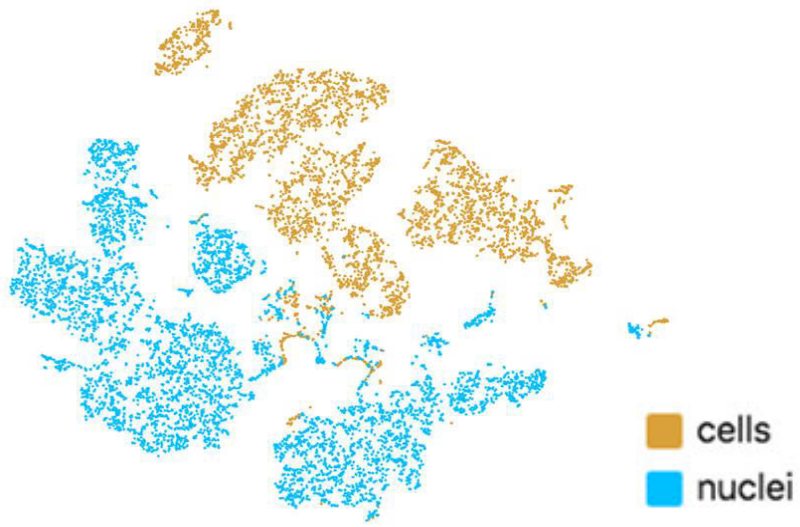


Mouse embryonic E18.5 brain nuclei

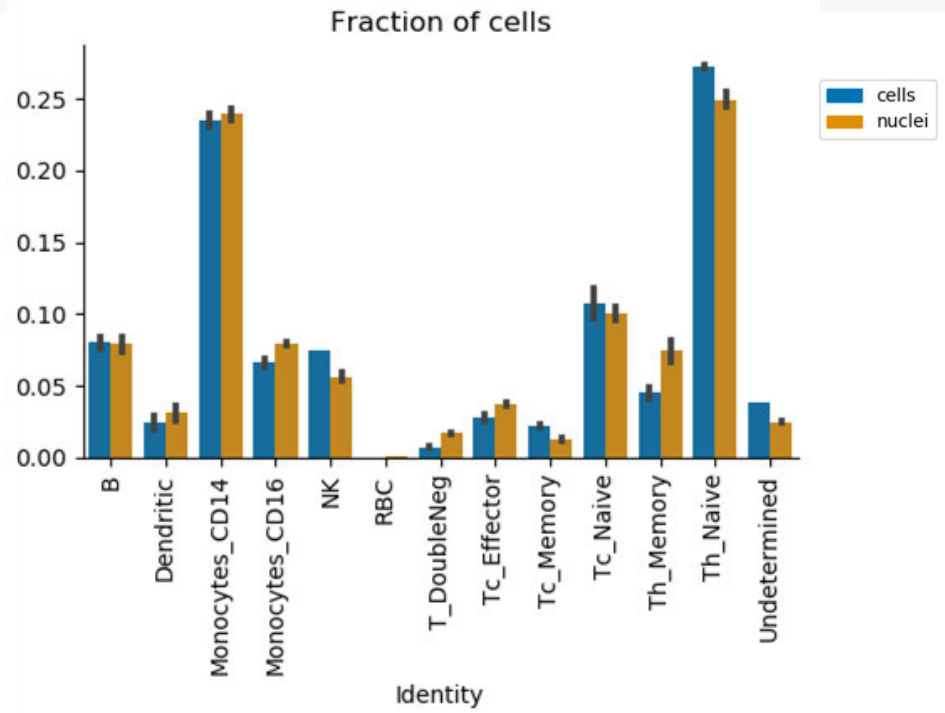
How does information from nuclei compare to cells?

Clusters do not overlap but biological information is conserved

PBMCs



- Cells run on SC3'v3.1 capture mostly mRNA
- **Nuclei run on Multiome ATAC+GEX capture mostly pre-mRNA (unspliced mRNA)**

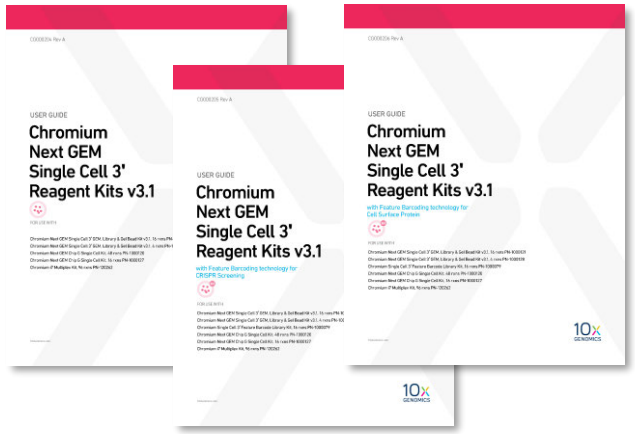


- **Cell populations can still be identified**

Resources

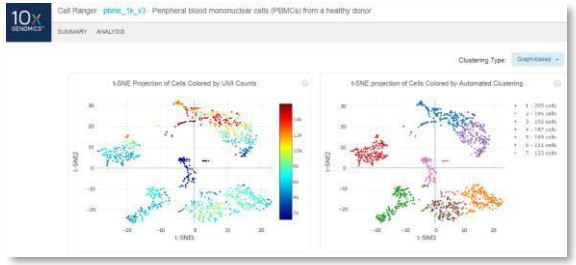
Demonstrated Protocols

User Guides



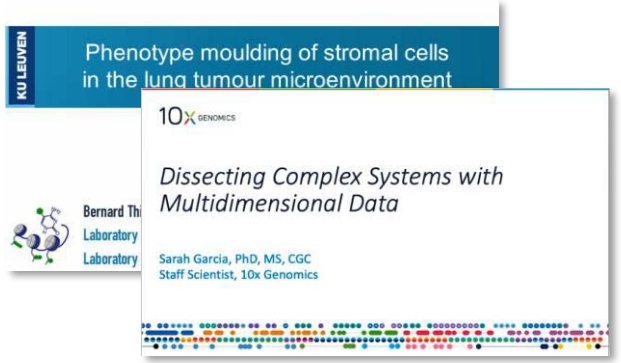
Application Notes

Public Data Sets



How-To Videos

Scientific Seminars



https://support.10xgenomics.com/
https://www.10xgenomics.com/10x-university/

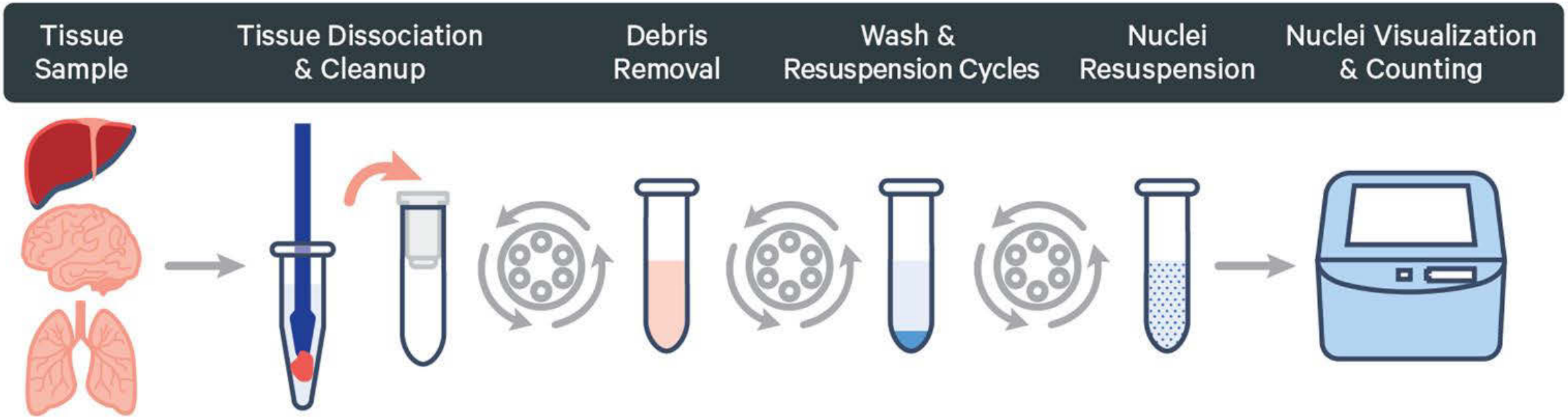
Customer Developed Protocols

https://community.10xgenomics.com/t5/Customer-Developed-Protocols/ct-p/customer-protocols

1. High Molecular Weight Genomic DNA Extraction from Grape LeavesContributed by: Xia Xu,Lance Cadle-Davidson USDA-ARS, GGRU
2. CTAB Protocol for Isolating DNA from Plant TissueContributed by: Allen Van Deynze, Van Deynze Lab, UC Davis
3. Cell dissociation and crypt isolation of the mouse small intestineContributed by: Aviv Regev, Regev Lab, Broad Institute
4. Tissue dissociation and single cell preparation of breast cancer patient-derived xenoglaftsContributed by: Ioannis Ragoussis and Morag Park
5. Isolation of single cell suspensions from epidermisContributed by: Samuel Lukowski
6. Generation of single cell suspension from E8.25 mouse embryosContributed by: Bertie Gottgens
7. Preparation of non-myocyte cardiac single cell suspensionsContributed by: Galen Squiers & Alex Pinto, Pinto Lab, The Jackson Laboratory, Bar Harbor
8. 'Frankenstein' protocol for nuclei isolation from fresh and frozen tissueContributed by: Luciano Martelotto, Ph.D., Melbourne, Centre for Cancer Research, Victorian Comprehensive Cancer Centre

Nuclei Isolation Kit

Streamlined sample preparation workflow



All you need is an hour of lab time, a benchtop centrifuge, and an interesting frozen sample!

Thank You from the 10x Team & our Collaborators

Agnieszka Ciesielska

Science & Technology Advisor