EMEA 10x Genomics User Day| I2MC, Toulouse

Agenda

09h00 Registrations
9h30-9h45 Présentation Toulouse Single Cell Core Facilities : Carine Valle / Fred Martins / Emeline Lhuillier
10h00 Portfolio 10x Genomics (Christophe Fleury)
10h20 Immudex - *dCODE Dextramer®-Unravel Specificity of T-cell immunity*
10h40 Chervin Hassel - IHAP: Description of lactacting mammary gland immune cells using single-cell RNA-Seq approach.
11h10 Sample prep 10x Genomics: Tips and tricks (Bashir Sadet)
11h40 Aurélie Quilien - CBD : Understanding the development of Left/Right asymmetry in the zebrafish brain by using scRNA-seq.
12h10 Miltenyi : sample prep
12h30 Lunch break

14h00 Jean-Jacques Fournié - CRCT: New Open source tools for single cell multiomics analyses with your laptop computers.
14h30 Biolegend
14h50 Meryem Aloulou – CPTP : scRNA-seq unveils how Foxp3+ Treg programs dendritic cells to promote B cell response.
15h20 Visium Spatial Gene Expression: technology and developments
15h50 Céline Mazzotti / Romain Lannes IUCT : Single-cell : Intégration de données moléculaires des populations plasmocytaires de Myélome Multiple.
16h20 Conclusions et collations/networking

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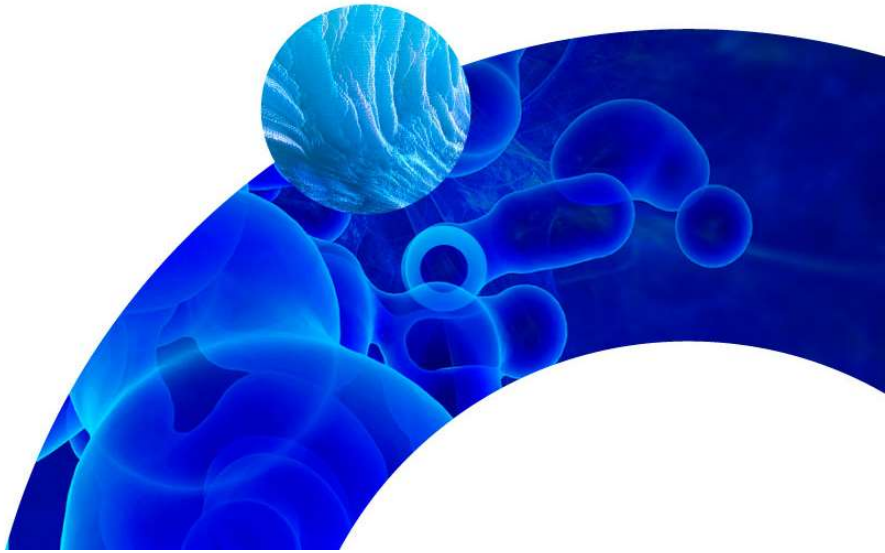
Biology at True Resolution

From single cell transcriptomics to multi-omics to spatial transcriptomics

Christophe Fleury, PhD
Science & Technology Advisor

User day Toulouse – 10/03/2020



“Imagine you were a biologist and didn’t have a microscope — and then I handed you one for the first time. That’s how profound single-cell sequencing is. It lets us see what we haven’t seen before; it gives us a new instruction manual for life.”

Dr. Sam Behjati
Pediatric oncologist and single-cell researcher
WELLCOME SANGER INSTITUTE in Britain

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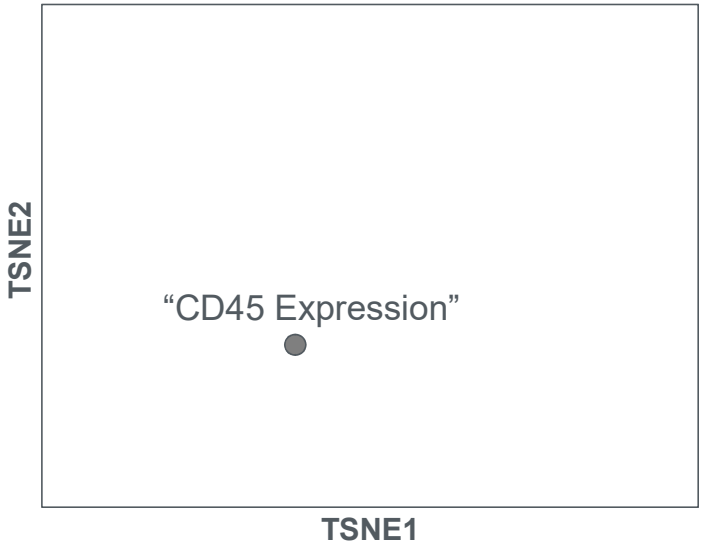
Single Cell Gene Expression – Unbiased and scalable

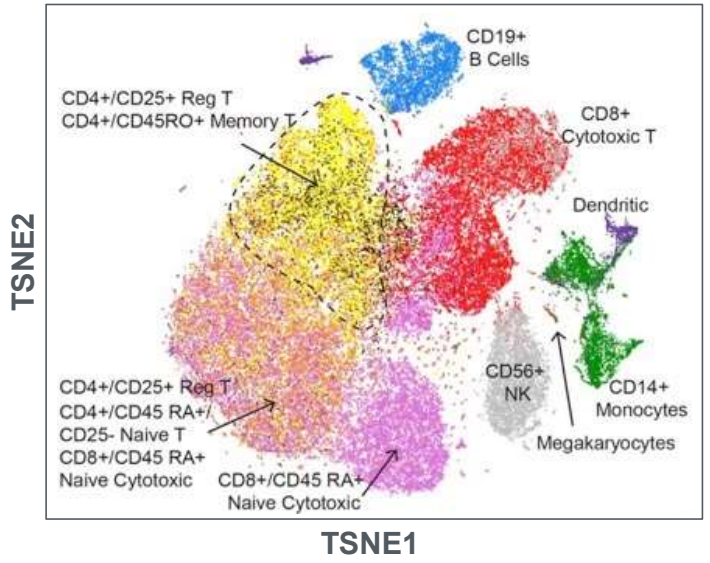
Conventional gene expression



One "average" data point from a mixture of cells

nature COMMUNICATIONS

10x Genomics



Capture cells with:

- Diverse sizes
- Broad transcriptional activity
- Rare frequencies

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Zheng et. al Nature Communications **volume8**, Article number: 14049 (2017)

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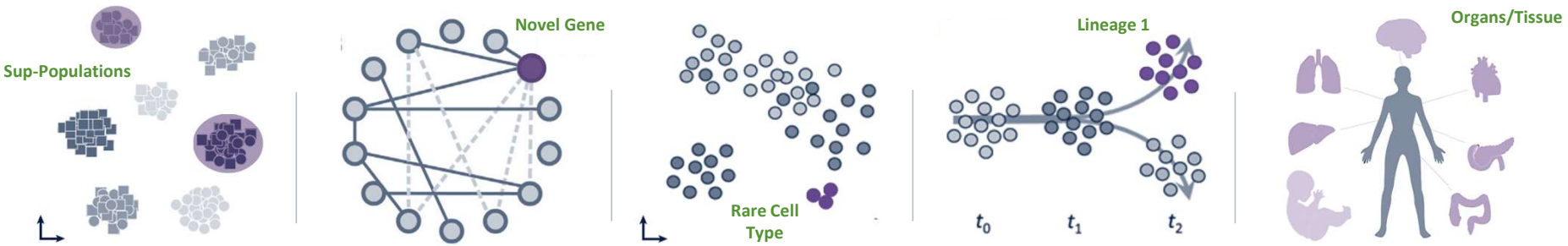
Scientific questions to answer

- 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



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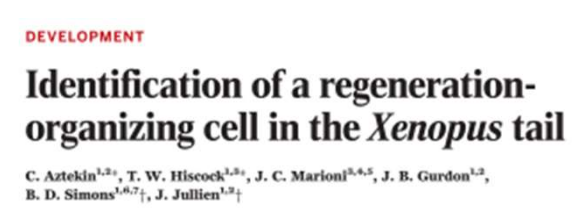
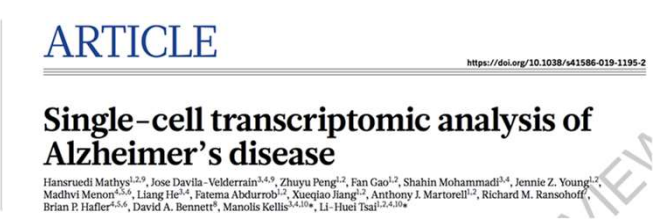
How does single cell sequencing make a difference?



DEVELOPMENT

Identification of a regeneration-organizing cell in the *Xenopus* tail

C. Aztekin[1,2*], T. W. Hiscock[1,3*], J. C. Marioni[3,4,5], J. B. Gurdon[1,2], B. D. Simons[1,6,7†], J. Jullien[1,2†]

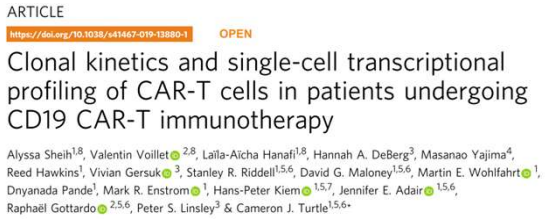


ARTICLE

https://doi.org/10.1038/s41586-019-1195-2

Single-cell transcriptomic analysis of Alzheimer's disease

Hansruedi Mathys[1,2,9], Jose Davila-Velderrain[3,4,9], Zhuyu Peng[1,2], Fan Gao[1,2], Shahin Mohammadi[3,4], Jennie Z. Young[1,2], Madhvi Menon[5,5,6], Liang He[3,4], Fatema Abdurrob[1,2], Xueqiao Jiang[1,2], Anthony J. Martorell[1,2], Richard M. Ransohoff[7], Brian P. Hafler[5,5,6], David A. Bennett[8], Manolis Kellis[3,4,10*], Li-Huei Tsai[1,2,4,10*]



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], Laïla-Aïcha 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*]

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How does single cell sequencing make a difference?

Development

DEVELOPMENT

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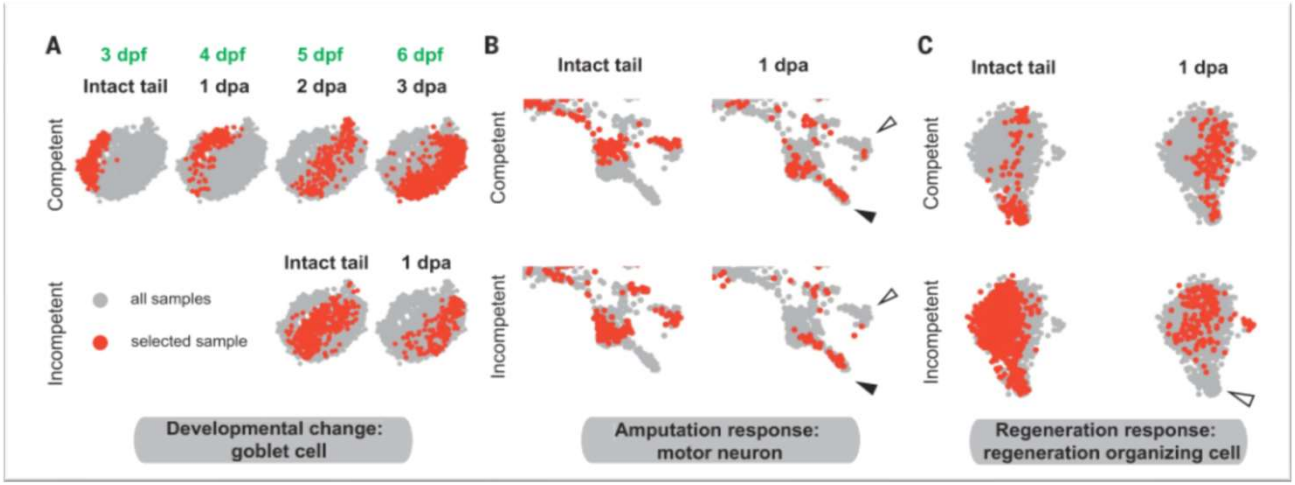
- What are these cells? What genes are involved?
- Discovery of cell progenitors: Regeneration organizing cells (ROC)



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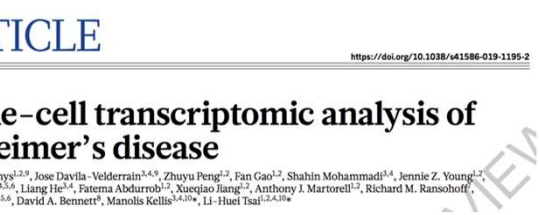
How does single cell sequencing make a difference?

Neuroscience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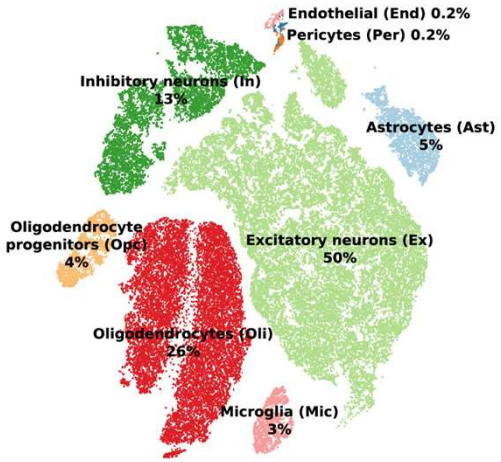
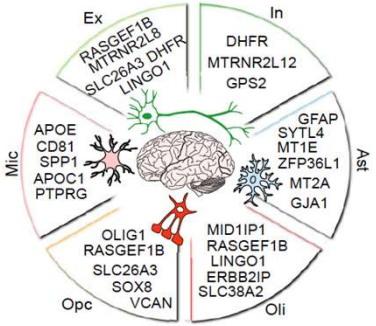
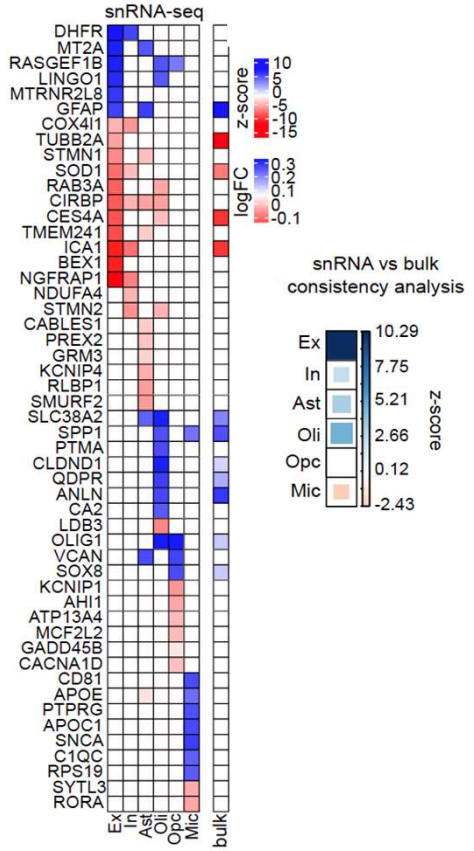
- What are the genes involved in AD?
- Bulk analysis showed 12 genes differentially expressed
- Single cell analysis revealed over 50 genes differentially expressed
- Genes are cell specific







How does single cell sequencing make a difference?

Oncology

ARTICLE
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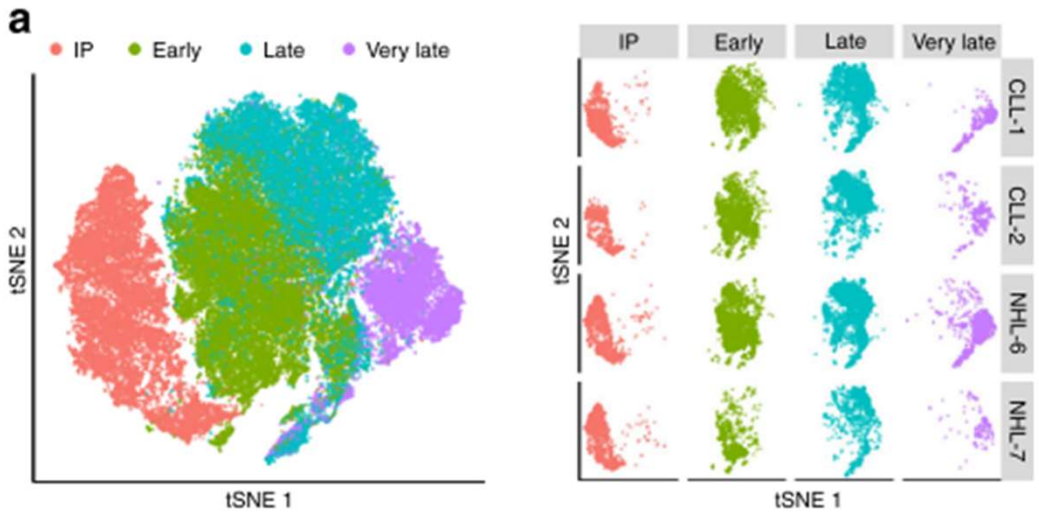
- How do CAR-T cells behave once reinjected?
- Monitored each reinfused clone
- Transcriptional profiles of clones diverge
- Variability between patients



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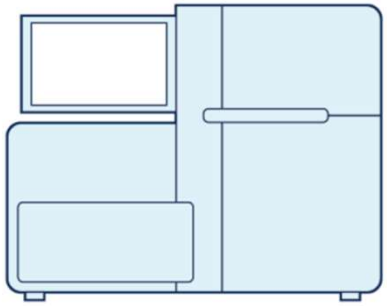
10x Genomics Chromium workflow

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

User-Supplied Cells, Nuclei, Molecules

Molecular barcoding at the single cell level



Sequencing-Ready Libraries

Software

- Analysis Pipelines
- Visualization Software

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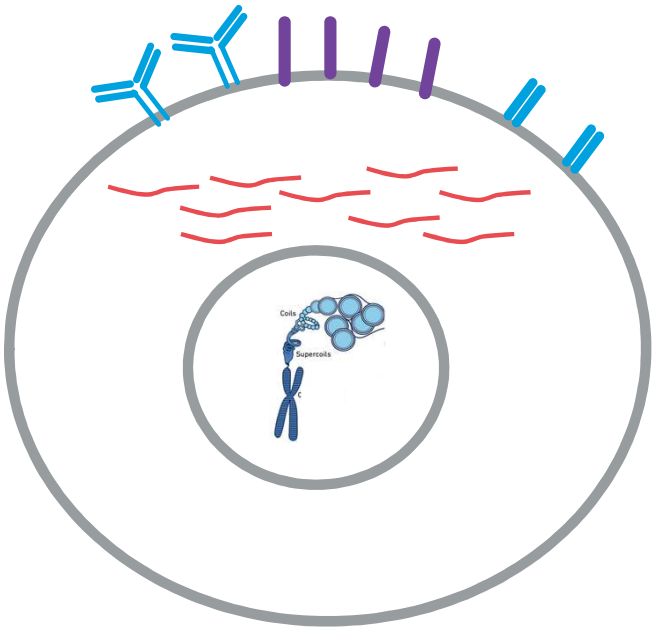
Multiple layers of information at the single cell level



BCR/TCR

Proteins

RNA

DNA/epigenome

	Analysis	Visualisation
Single Cell Immune Profiling	Cell Ranger	Loupe V(D)J Browser & Loupe Browser
Single Cell Gene Expression	Cell Ranger	Loupe Browser
Single Cell ATAC	Cell Ranger ATAC	Loupe Browser

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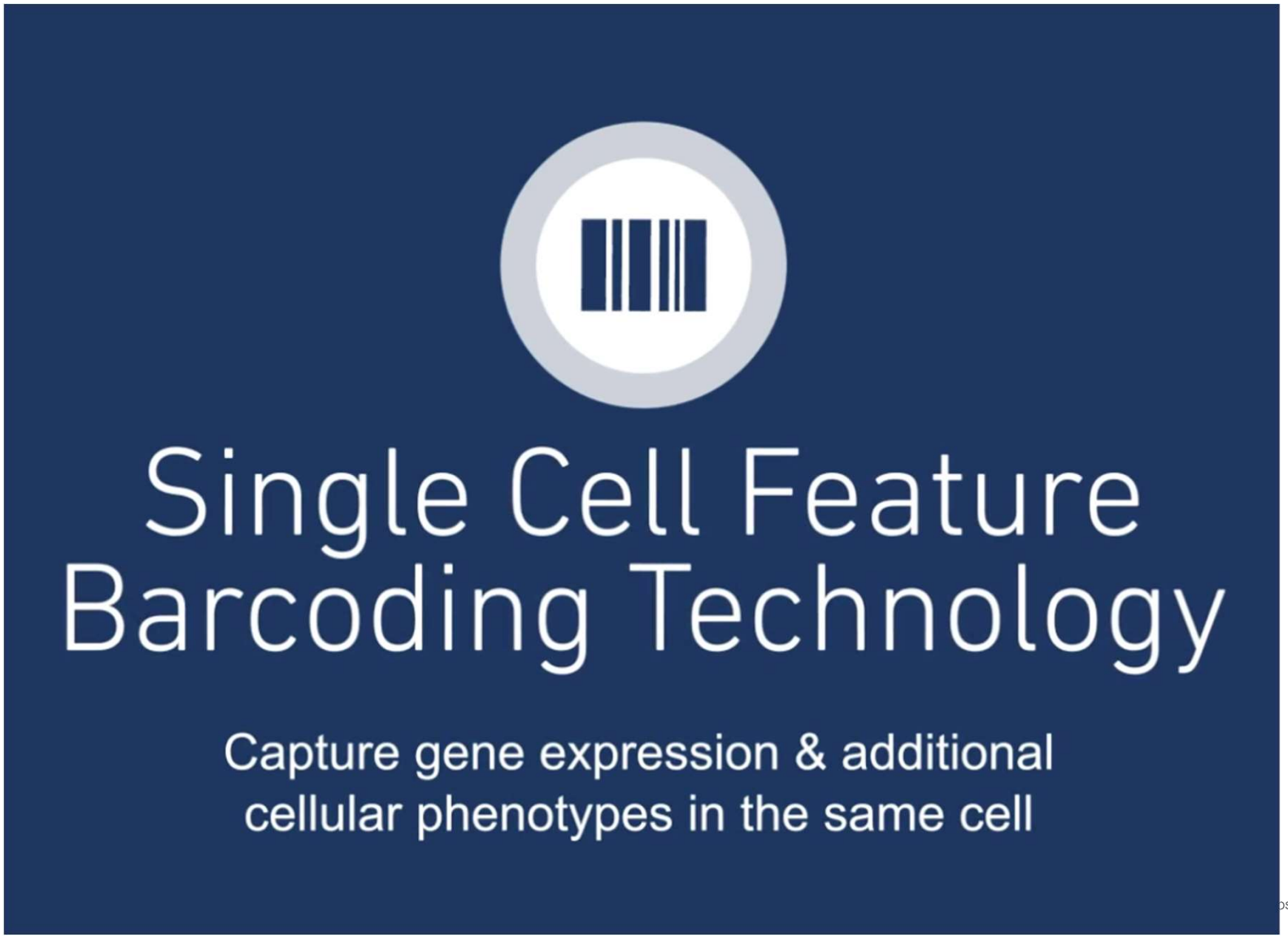

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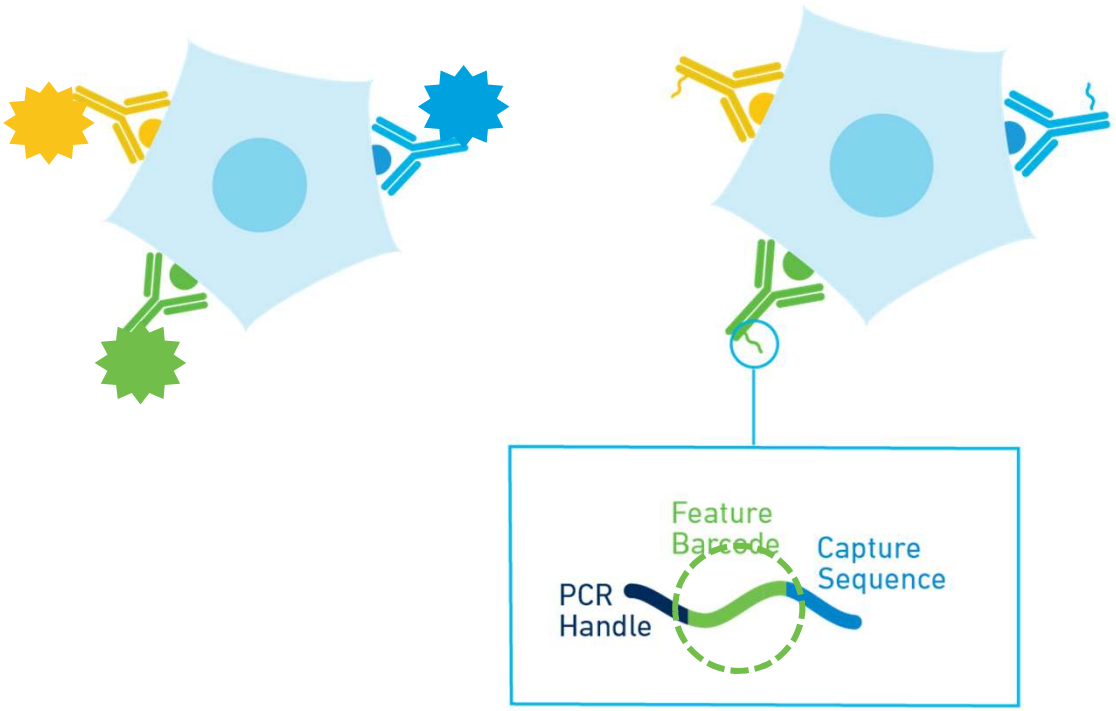
Feature Barcoding Technology: Cell Surface Protein



- A feature barcode is a short nucleic acid sequence, e.g.:
  - AAGGCAGACGGTGCA
  - CGTCCTAGGACATAT
  - ACGAATCGGATACTA
  - Etc,…
- Allow to **identify and quantify** cell surface protein and/or the feature of interest

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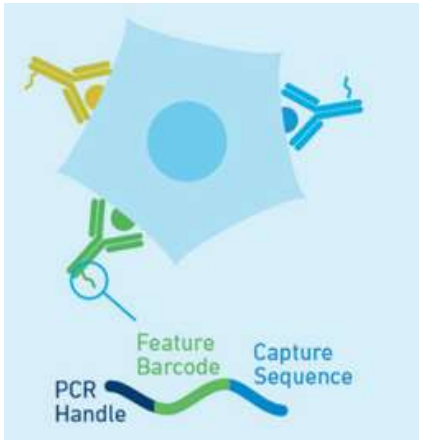
Introduction to Feature Barcode Technology

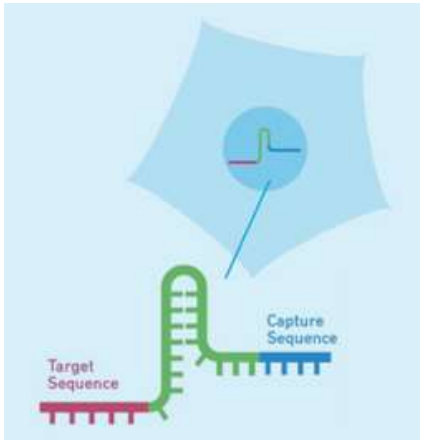
Simultaneously measure interactions and phenotypic information



+ Cell Surface Protein

Antibodies

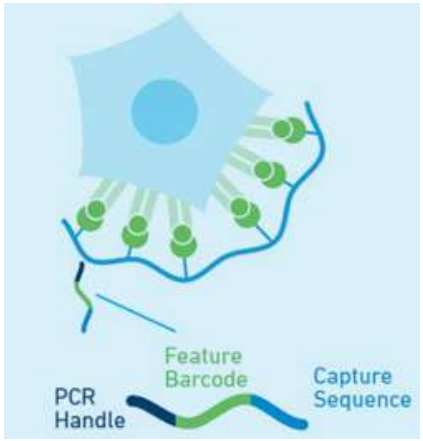
BioLegend®
Enabling Legendary Discovery™



+ CRISPR Screening

gRNA for CRISPR

MilliporeSigma



+ Antigen Specificity

pMHC Multimers

immudex
PRECISION IMMUNE MONITORING

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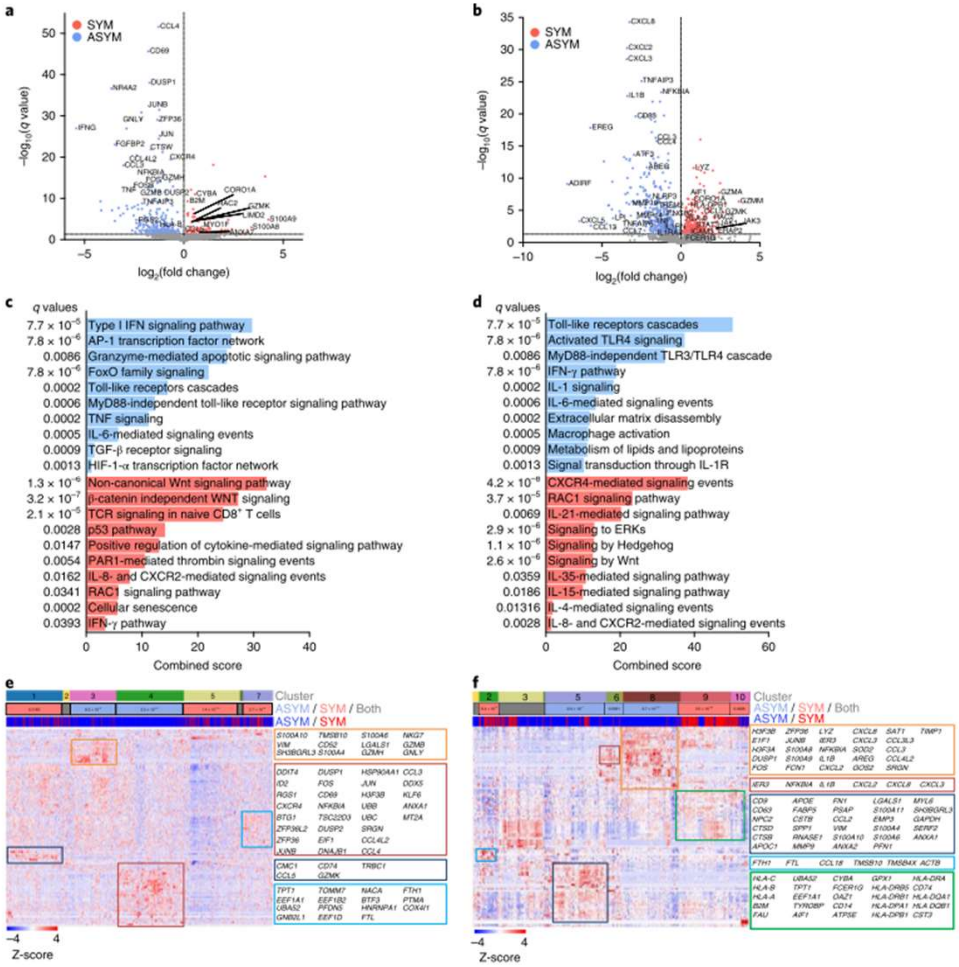
Richer cell phenotypes with scRNA-seq

Fernandez et al., Nature Medicine, 2019

- Single cell gene expression of immune cells (symptomatic Vs asymptomatic patients)
- Confirmed findings by CyTOF. Reconciled CyTOF data and scRNA-seq data by using CITE-seq (21 Ab)
- Discovered more diverse heterogeneity of the lesion macrophages than showed by CyTOF and previously thought
- Refined characterization of T cell clusters by accessing vast number of marker profiles
- Incidental finding of reasons explaining the failure of CANTOS clinical trial



doi:10.1038/s41591-019-0590-4

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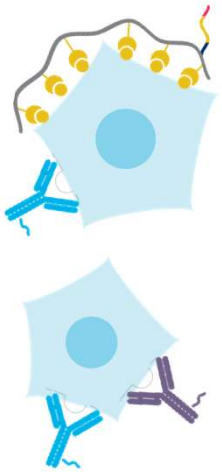
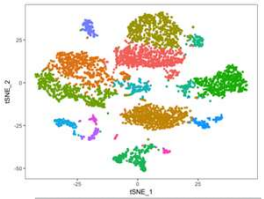
One Sample, several layers of Information

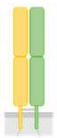


Single-cell Gene Expression



Single-cell T-cell Repertoire Profiling

Paired (TcR α/β) full-length V(D)J clonotypes



Single-cell B-cell Repertoire Profiling

Paired (BcR H/L) full-length V(D)J clonotypes

Cell surface proteins

BioLegend®
Enabling Legendary Discovery™

Cell surface antigen receptors

IMMUDEX

Single cell Immune profiling

Feature Barcoding

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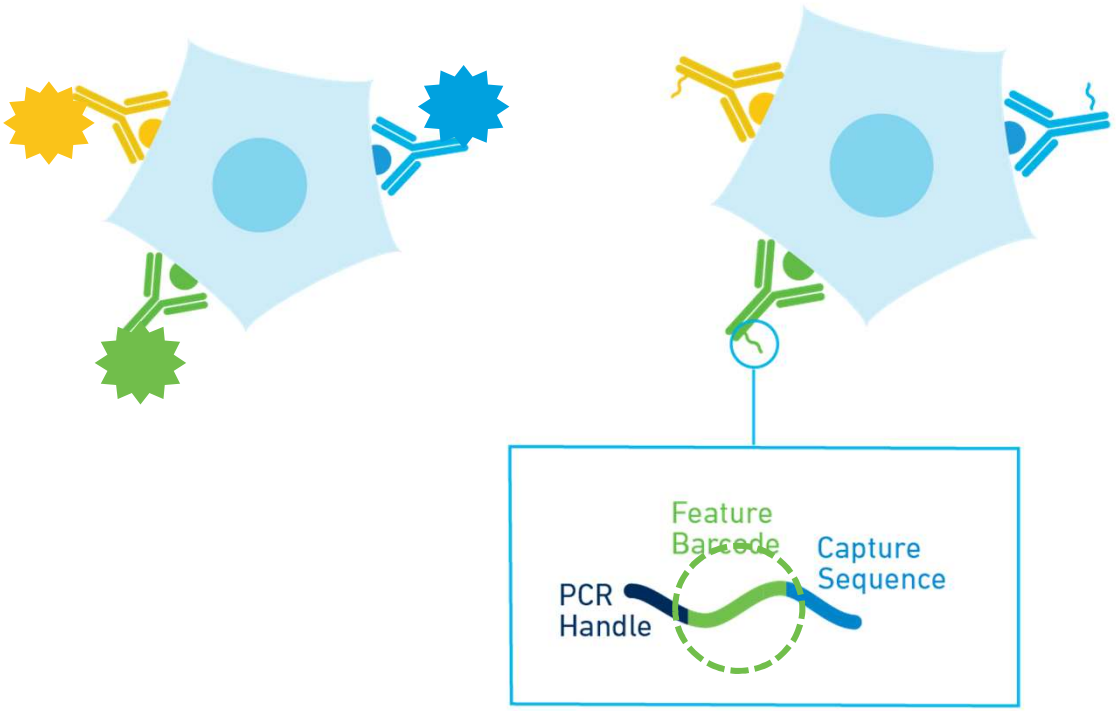
Feature Barcoding Technology: Cell Surface Protein



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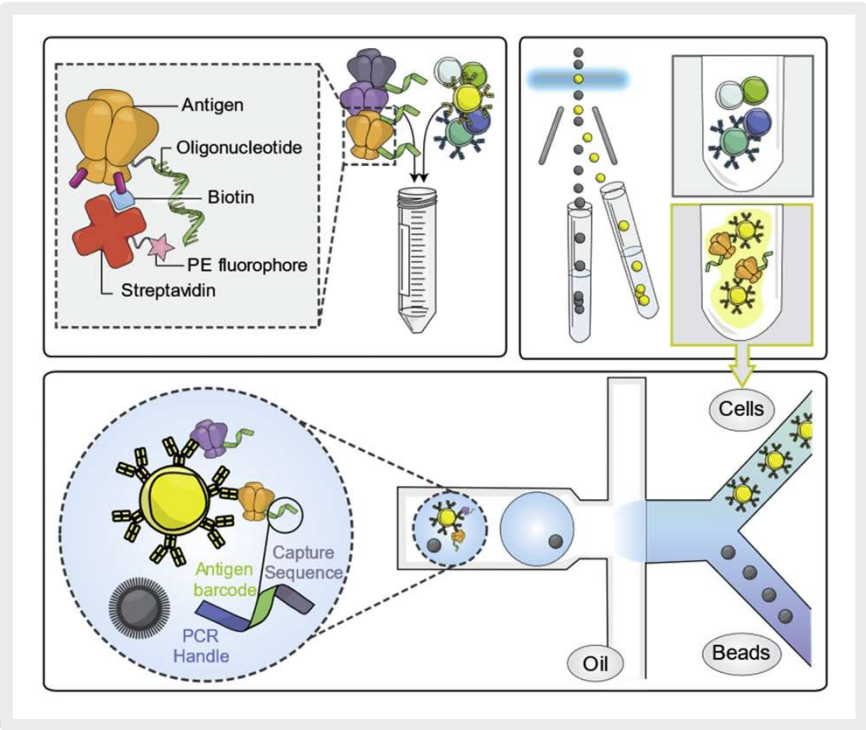
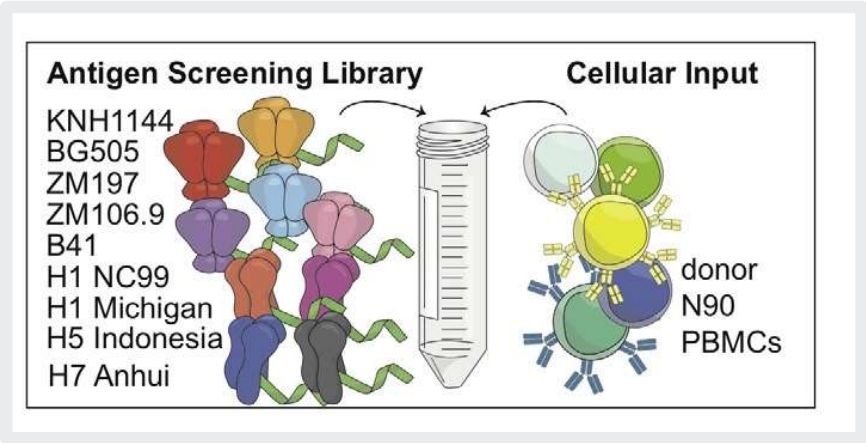
LIBRA-seq enables high-throughput mapping of B cell receptor sequence to antigen specificity at the single-cell level

Setliff et al., Cell, 2019





Cell

High-Throughput Mapping of B Cell Receptor Sequences to Antigen Specificity

Ian Setliff,[1,2,16] Andrea R. Shiakolas,[1,3,16] Kelsey A. Pilewski,[1,3] Amyn A. Murji,[1,3] Rutendo E. Mapengo,[4] Katarzyna Janowska,[5] Simone Richardson,[4,11] Charissa Oosthuysen,[4,11] Nagarajan Raju,[1,3] Larance Ronsard,[7] Masaru Kanekiyo,[8] Juliana S. Qin,[1] Kevin J. Kramer,[1,3] Allison R. Greenplate,[1] Wyatt J. McDonnell,[3,9,17] Barney S. Graham,[8] Mark Connors,[10] Daniel Lingwood,[7] Priyamvada Acharya,[5,6] Lynn Morris,[4,11,12] and Ivelin S. Georgiev[1,3,13,14,15,18,*]

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Profiling the epigenome

Sn ATAC-seq

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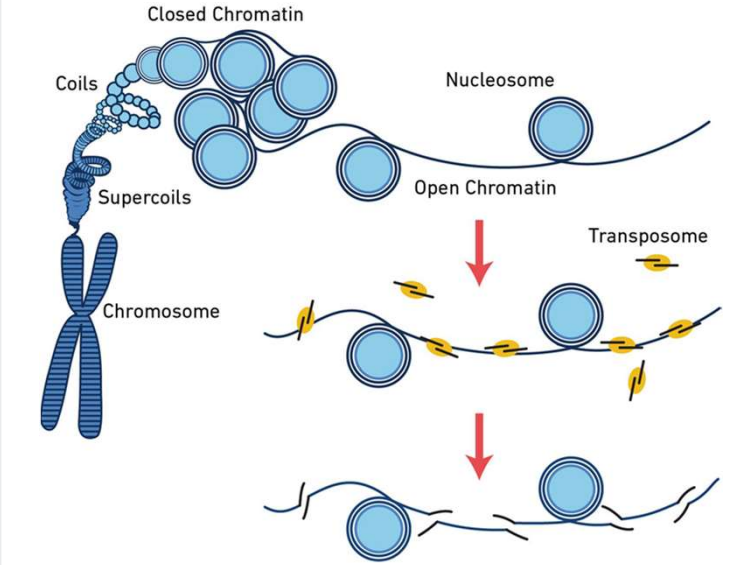
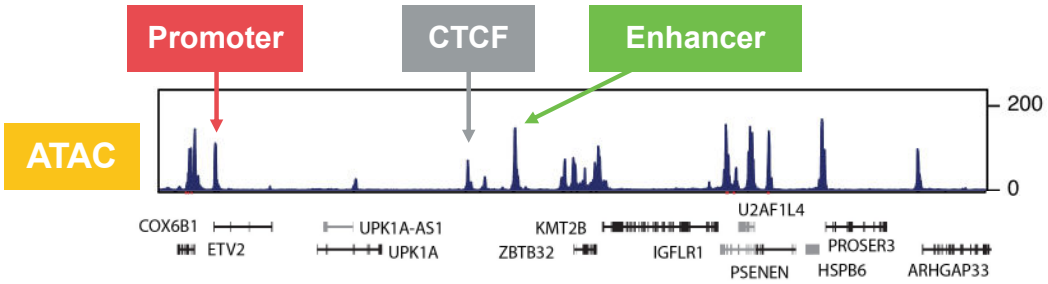
Resolve multiple features using Chromatin Accessibility

Single Cell ATAC

Interrogate open chromatin profiles at single cell resolution



Assay for Transposase Accessible Chromatin (ATAC-seq)



Open chromatin represented as “peaks”

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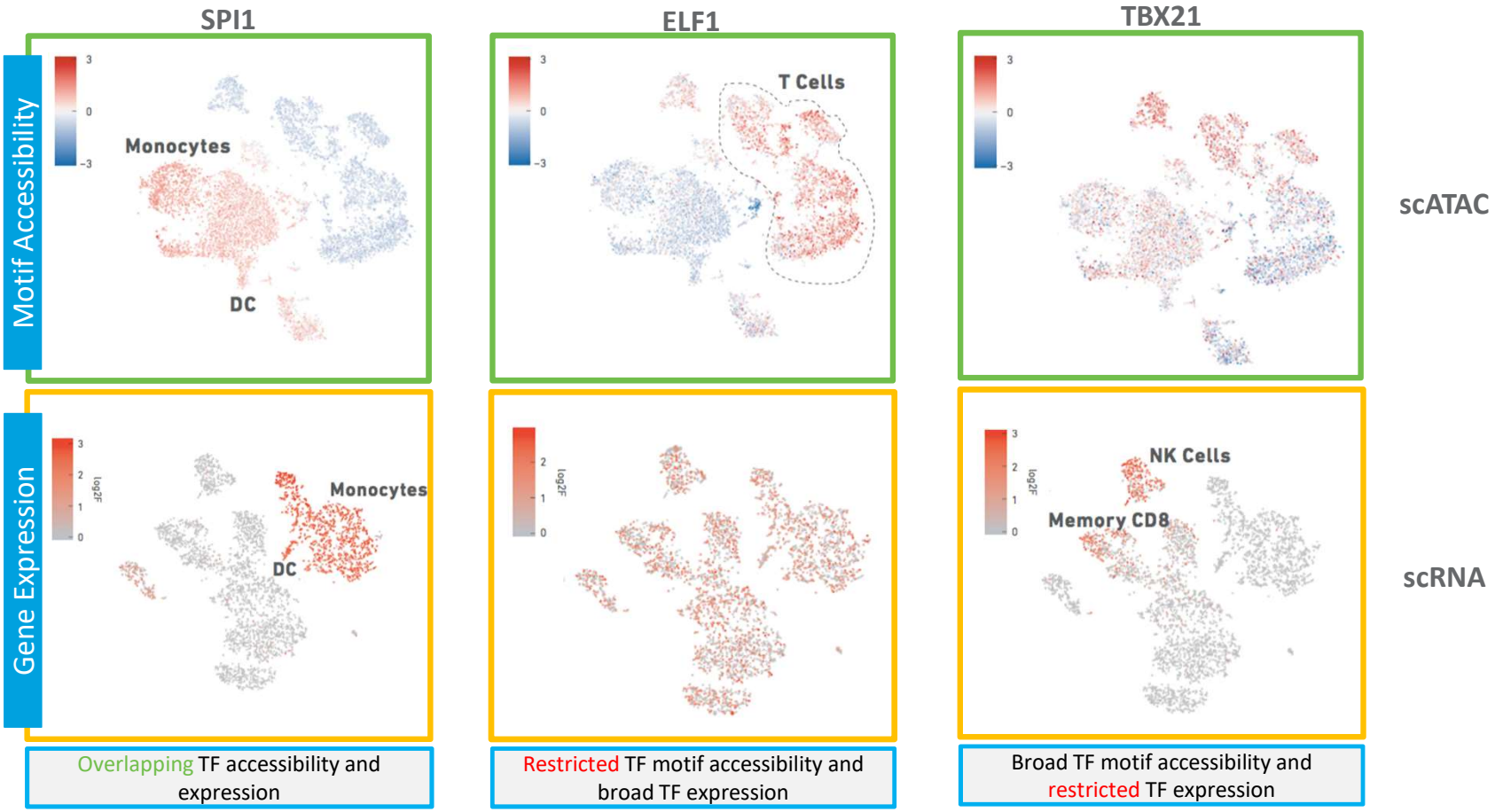
Single Cell ATAC + RNA Expression Reveal Complex Patterns of Transcription Regulation



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Chromium Single Cell ATAC + Gene Expression

- Deeply characterize cell types and states
- Connect regulatory element activity
- Interpret epigenetic pr expression markers

Coming 2nd half of this year

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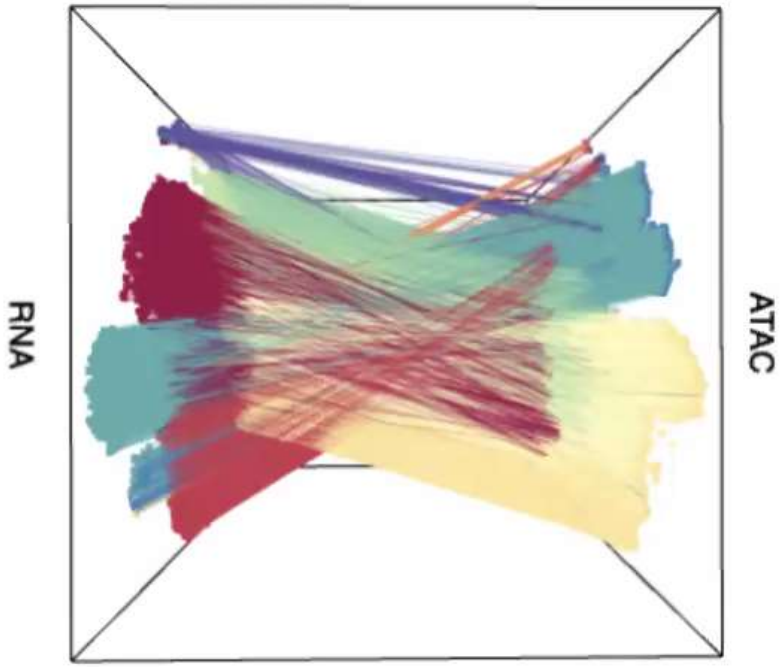
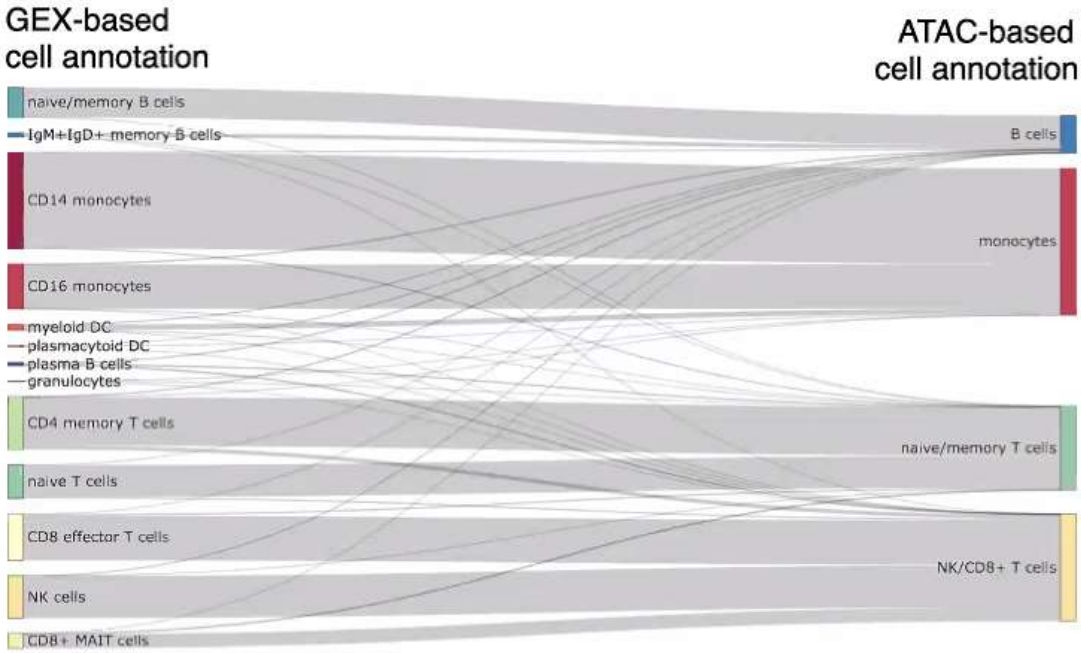
High Concordance Between the Two Read-Outs





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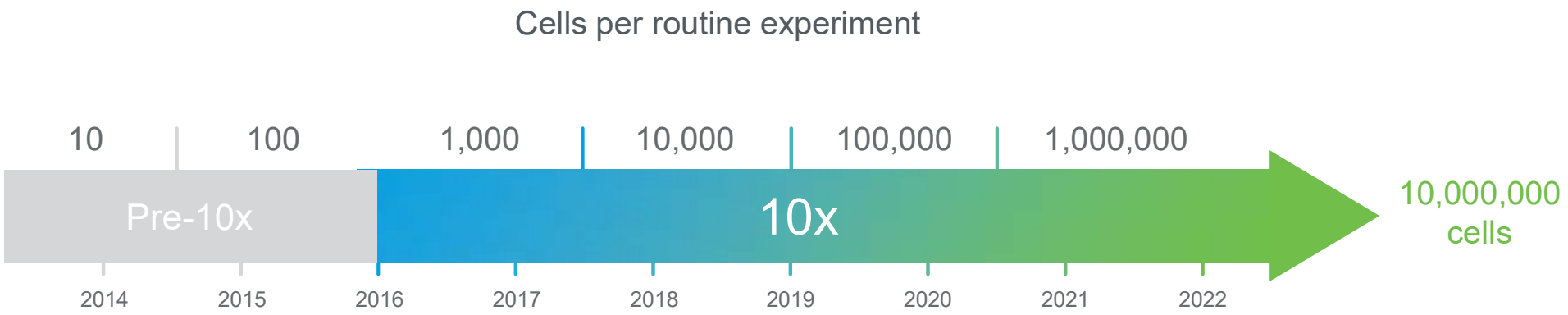
10x Will Enable Routine Million Cells and Beyond



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Targeted Gene Expression

- More flexibility
- More scale
- More applications

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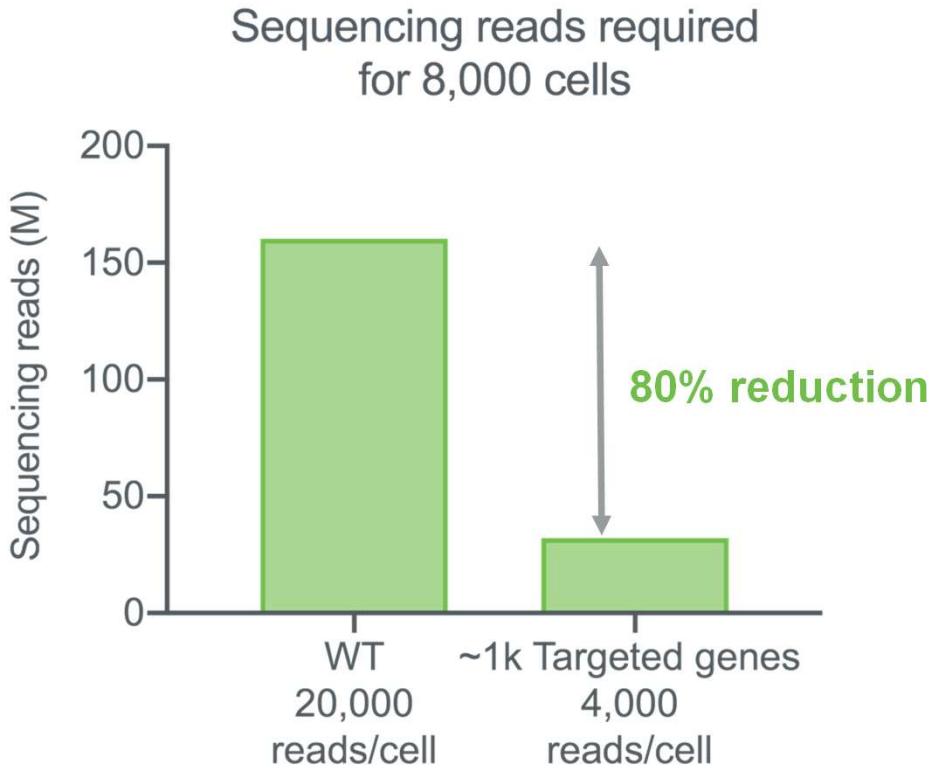
From Discovery to Focused Transcriptomics

Targeted product built for 10x assays

- **Increased experimental efficiency**
- **Reduced sequencing cost**
- **WTA and targeted gene expression from the same cells**
- **Core assay compatibility**
- **Content and customization**



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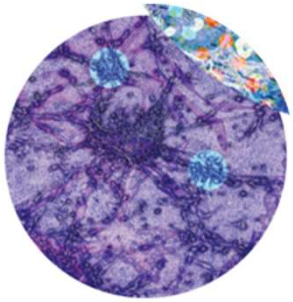
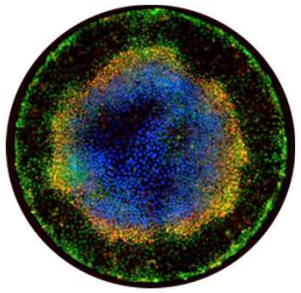
Ready-to-use 10x Genomics Content Offering

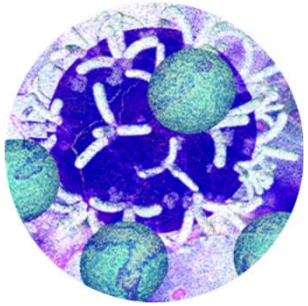
Fixed gene panels with comprehensive content



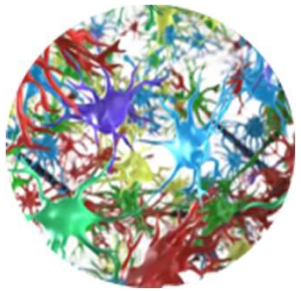
Human Pan-Cancer
~1250 genes



Human Gene Signature
~1140 genes



Human Immunology
~1050 genes



Human Neuroscience
~1150 genes

- Tissue specific marker genes
- Major signaling pathways
- Common biomarkers
- Disease targets for drug discovery

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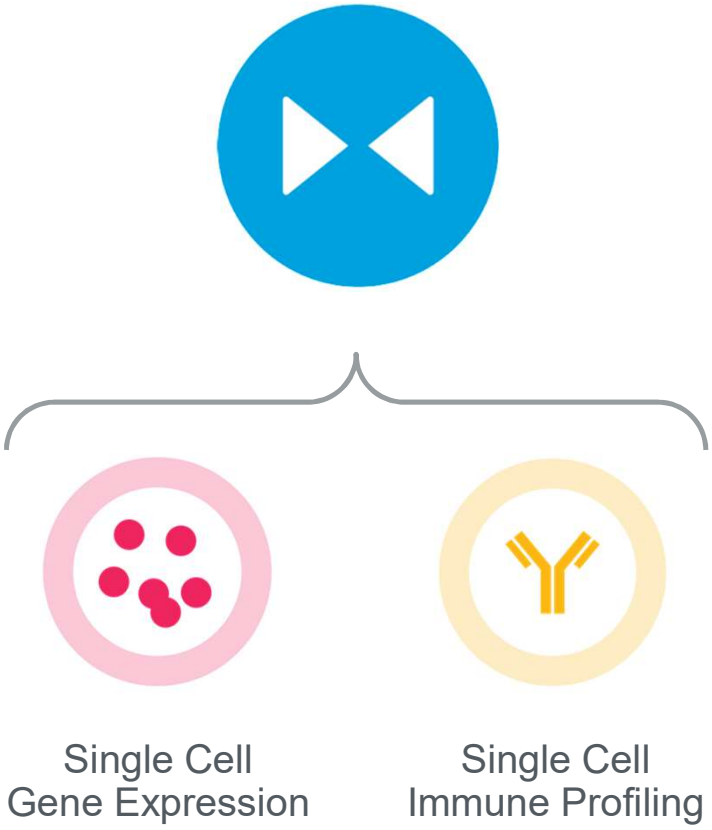
Cell Multiplexing



- Flexible Design
- Higher Throughput
- Species/Cell Type Agnostic
- Nuclei Compatible
- Fully Supported Software

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Cell Multiplexing - Customer Developed Methods

Stoeckius *et al. Genome Biology* (2018) 19:224
https://doi.org/10.1186/s13059-018-1603-1

Genome Biology

METHOD Open Access

Cell Hashing with barcoded antibodies enables multiplexing and doublet detection for single cell genomics

Marlon Stoeckius[1†], Shiwei Zheng[2†], Brian Houck-Loomis[1], Stephanie Hao[1], Bertrand Z. Yeung[3], William M. Mauck III[2], Peter Smibert[1*] and Rahul Satija[2*]

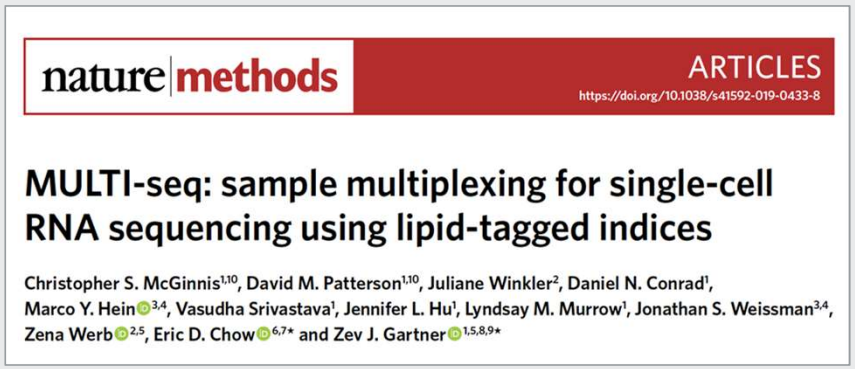
nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-10756-2 OPEN

Nuclei multiplexing with barcoded antibodies for single-nucleus genomics

Jellert T. Gaublomme[1,6,9], Bo Li[1,7,9], Cristin McCabe[1], Abigail Knecht[1], Yiming Yang[2], Eugene Drokhlyansky[1], Nicholas Van Wittenberghe[1], Julia Waldman[1], Danielle Dionne[1], Lan Nguyen[1], Philip L. De Jager[3], Bertrand Yeung[4], Xinfang Zhao[4], Naomi Habib[1,8], Orit Rozenblatt-Rosen[1] & Aviv Regev[1,5]

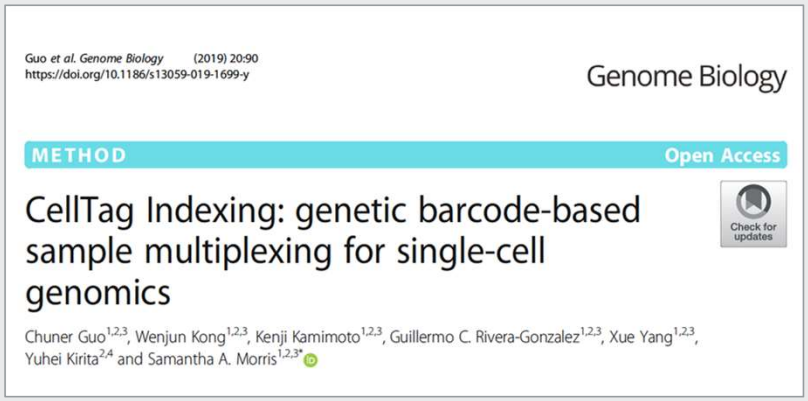


nature methods ARTICLES

https://doi.org/10.1038/s41592-019-0433-8

MULTI-seq: sample multiplexing for single-cell RNA sequencing using lipid-tagged indices

Christopher S. McGinnis[1,10], David M. Patterson[1,10], Juliane Winkler[2], Daniel N. Conrad[1], Marco Y. Hein[3,4], Vasudha Srivastava[1], Jennifer L. Hu[1], Lyndsay M. Murrow[1], Jonathan S. Weissman[3,4], Zena Werb[2,5], Eric D. Chow[6,7*] and Zev J. Gartner[1,5,8,9*]



Guo et al. Genome Biology (2019) 20:90
https://doi.org/10.1186/s13059-019-1699-y

Genome Biology

METHOD Open Access

CellTag Indexing: genetic barcode-based sample multiplexing for single-cell genomics

Chuner Guo[1,2,3], Wenjun Kong[1,2,3], Kenji Kamimoto[1,2,3], Guillermo C. Rivera-Gonzalez[1,2,3], Xue Yang[1,2,3], Yuhei Kirita[2,4] and Samantha A. Morris[1,2,3*]

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Cell Multiplexing, Built The 10x Way

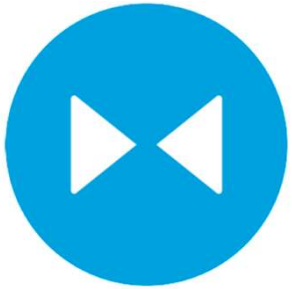
Enabling routine profiling of hundreds of thousands to millions of cells

Lipid based

Species agnostic

Nuclei + Cells

10x product compatible



More cells

20k per channel | 160k per chip

More Samples

12 per channel | 96 per chip

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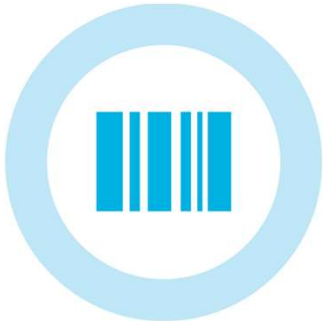
Targeted
Gene Expression

Cell Multiplexing

Intracellular
Protein Detection



10x GENOMICS Compatible Products

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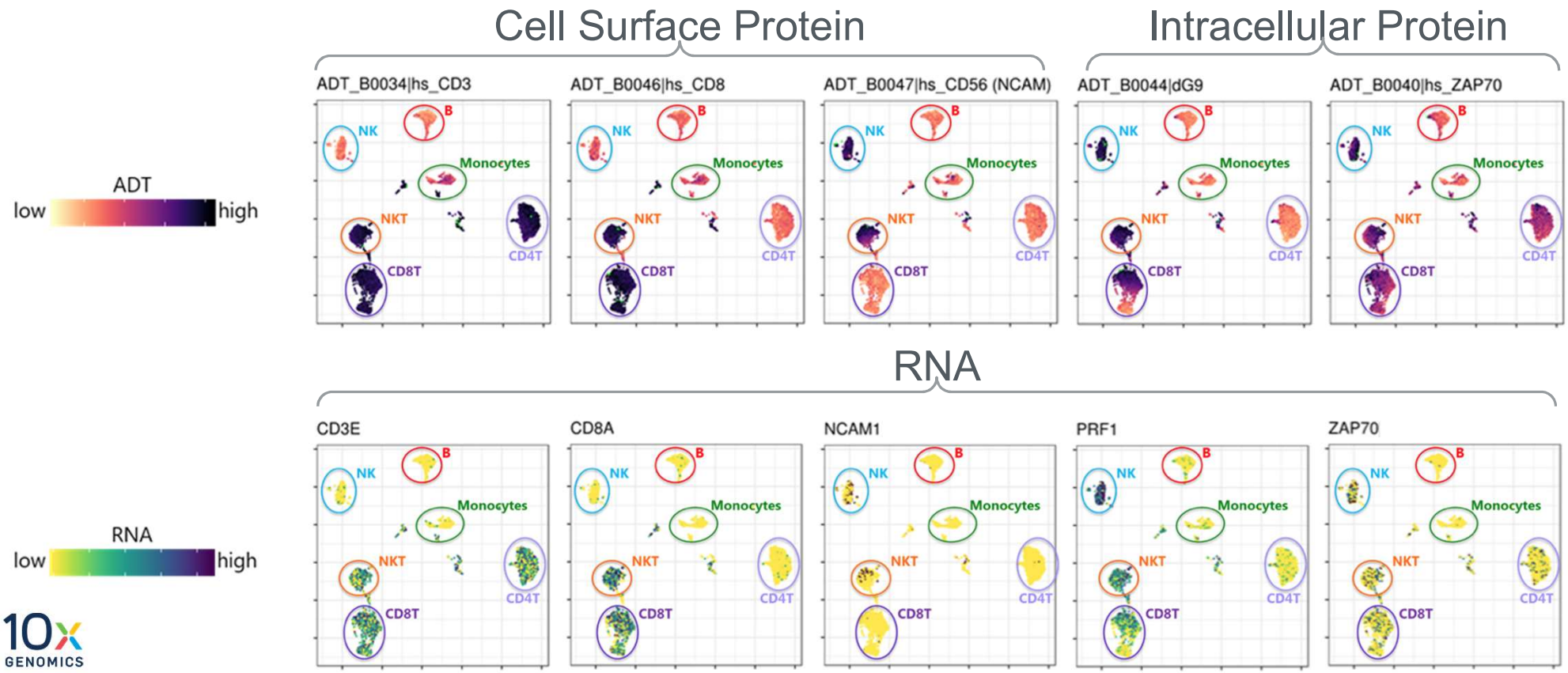
Detecting Intracellular Protein Expression

10x Genomics Workflow + BioLegend Fixation and Antibodies



Detecting Intracellular Protein Expression

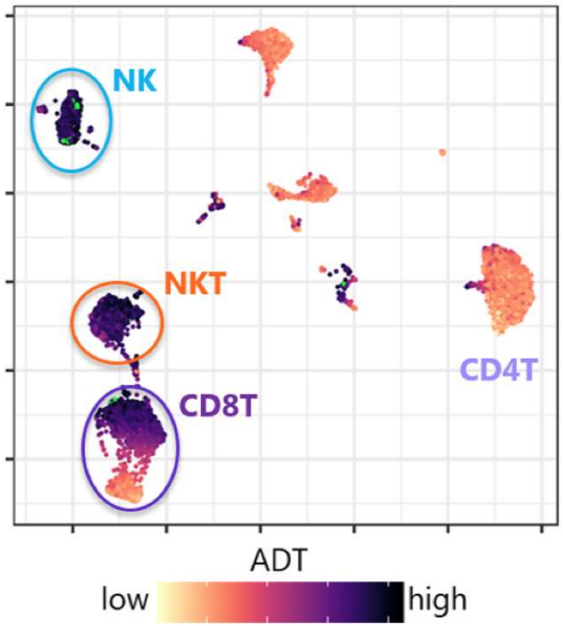
10x Genomics Workflow + BioLegend Fixation and Antibodies

BioLegend®

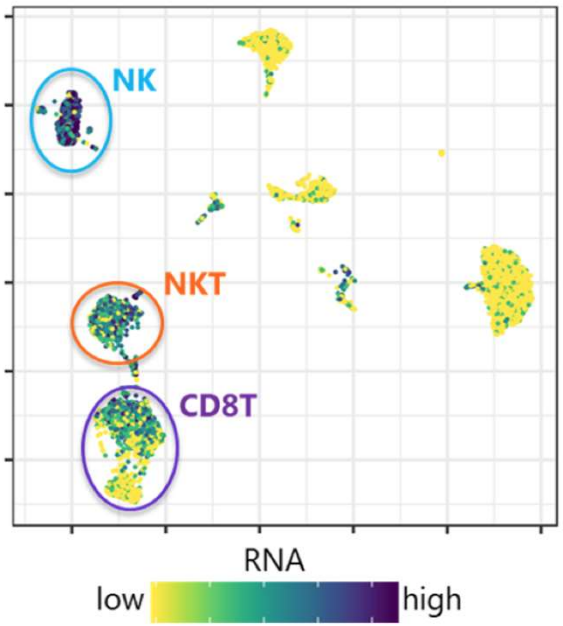
Simultaneous Detection of Perforin Protein & RNA in PBMCs (TotalSeq-B)

Perforin protein detection with BioLegend dG9 antibody

Perforin protein



Perforin RNA



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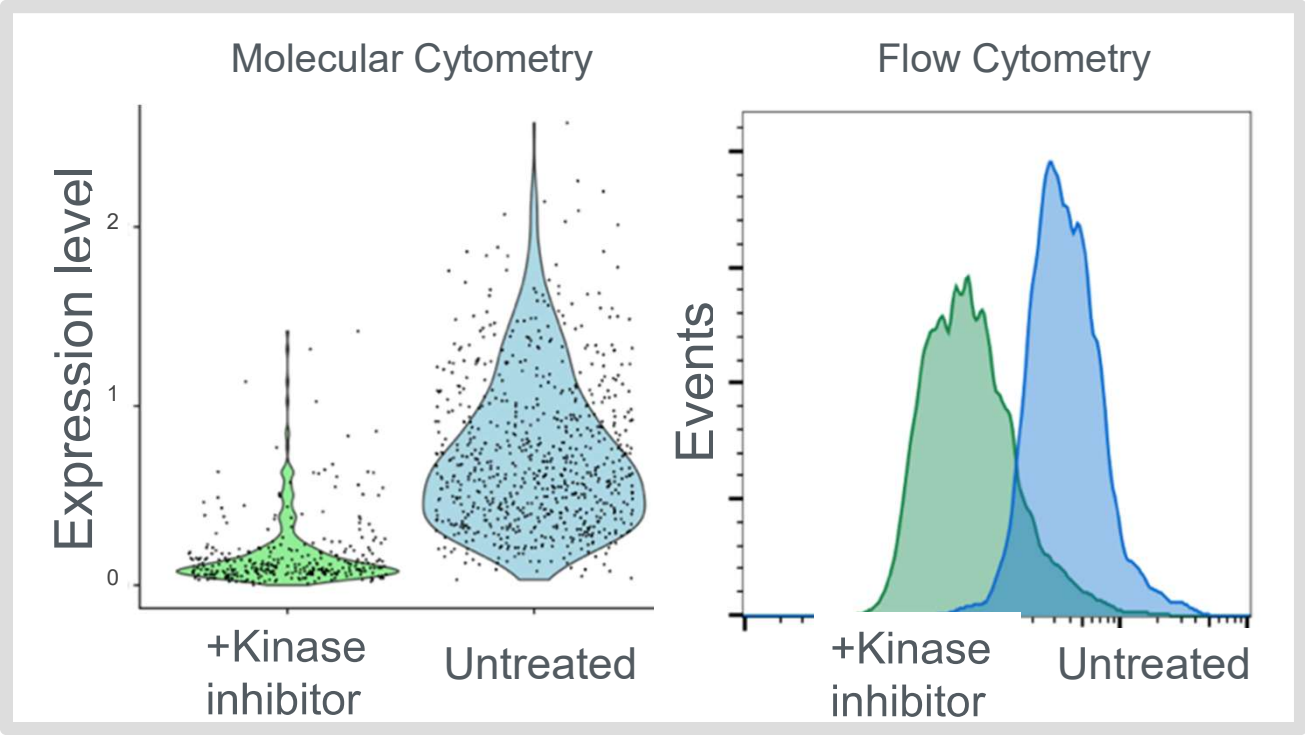
Detecting Changes In Intracellular Protein Expression

10x Genomics Workflow + CST Fixation and Antibodies

Cell Signaling TECHNOLOGY

Jurkat cells treated with kinase inhibitor (↓ phosphorylation)
Detection with CST Phospho 4E-BP1 antibody



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Thank You! Questions?

Christophe Fleury
Science and technology advisor

10x Genomics Team

Jamal FAKIR
Sales Executive

Bashir SADET
Field Applications Scientist

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10x Genomics Scientific Symposium

Copenhagen, Borsen Stock Exchange | 2-3 June

https://10xgenomicsugm-copenhagen.eventbrite.com

- Day 1:
  - Presentations by industry leaders
  - Sample preparation workshop
  - Enjoy an evening social event
  - Book 1:1 meetings with our 10x-perts
  - Visit our troubleshooting stations for expert advice
  - Four juniors researchers (PhD students/early PostDocs) will win a 500 euro travel award to cover their attendance!
  - Enter our Scientific Challenge to win free kits for your projects!
- Day 2 workshops:
  - Getting Started with Chromium Single Cell Gene Expression Data Analysis (by abstract submission)
  - Getting Started with Visium Spatial Gene Expression Data Analysis (by abstract submission)
  - Bioinformatics for Single Cell and Spatial Genomics (bioinformaticians)

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