

10xGenomics technology and future development

The genetic microscope.

Hannes Arnold

Technical Sales Specialist EMEA



What you will hear in this talk.



Introduction into 10x Genomics GEM Technology

Single Cell RNA Assay

Single Cell Immune Repertoire Profiling

Single Cell Copy Number Variation (CNV) Assay

New Applications in 2018

- Single Cell Feature Barcoding
- ATAC sequencing

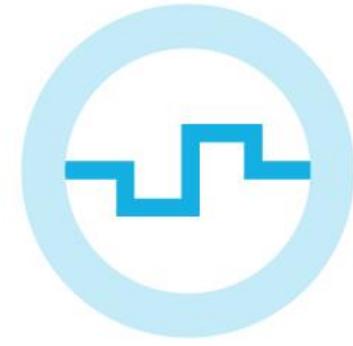
Single Cell Solutions



Single Cell 3'/5'
RNA



Single Cell Immune
Profiling Solution



Single Cell
CNVs

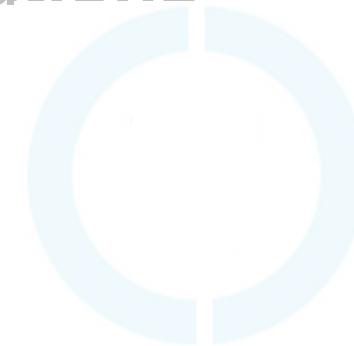


Single Cell
ATAC-seq



Single Cell
Feature Barcoding

Genomic Solutions



de novo

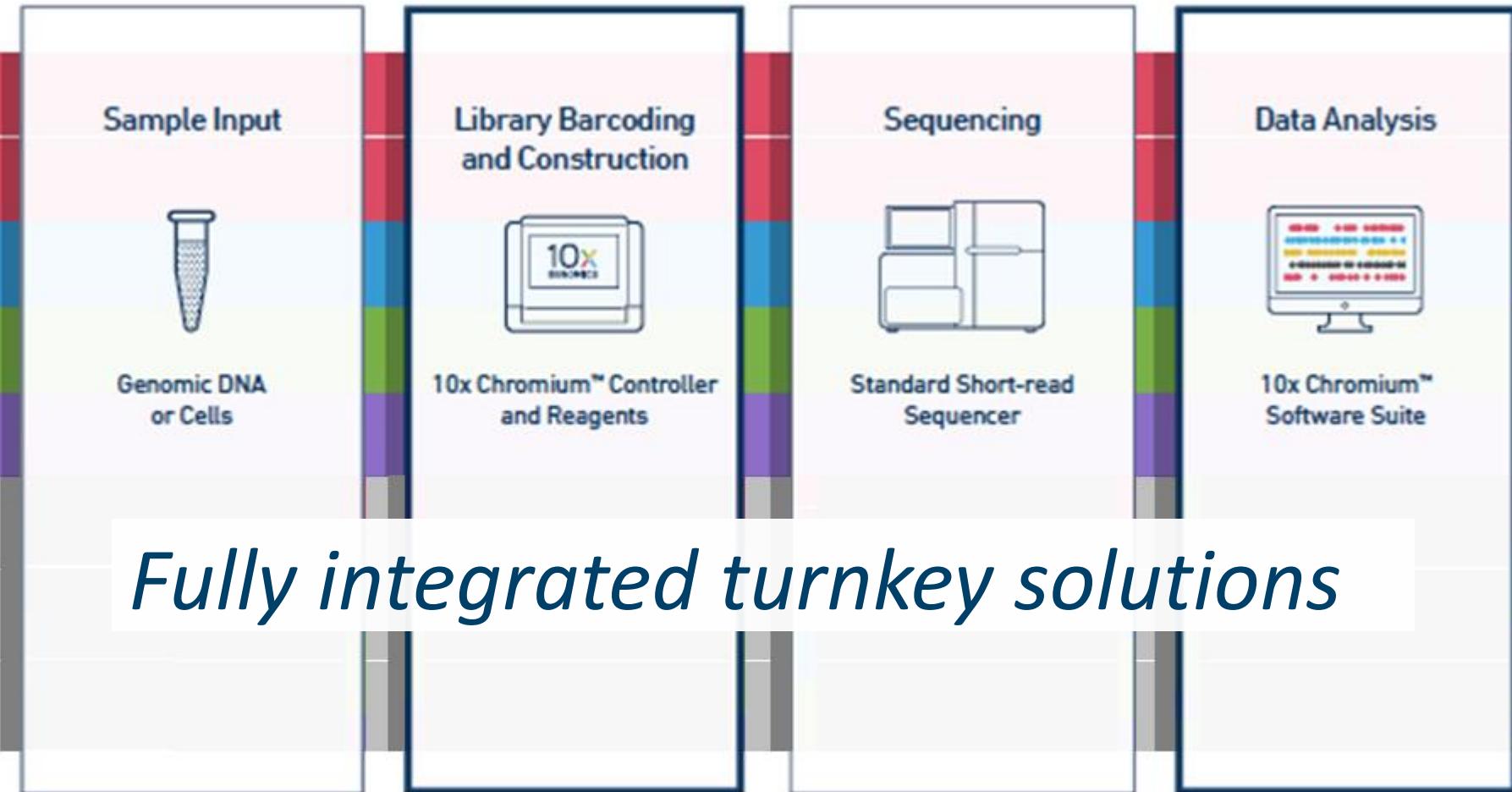


Whole Genome



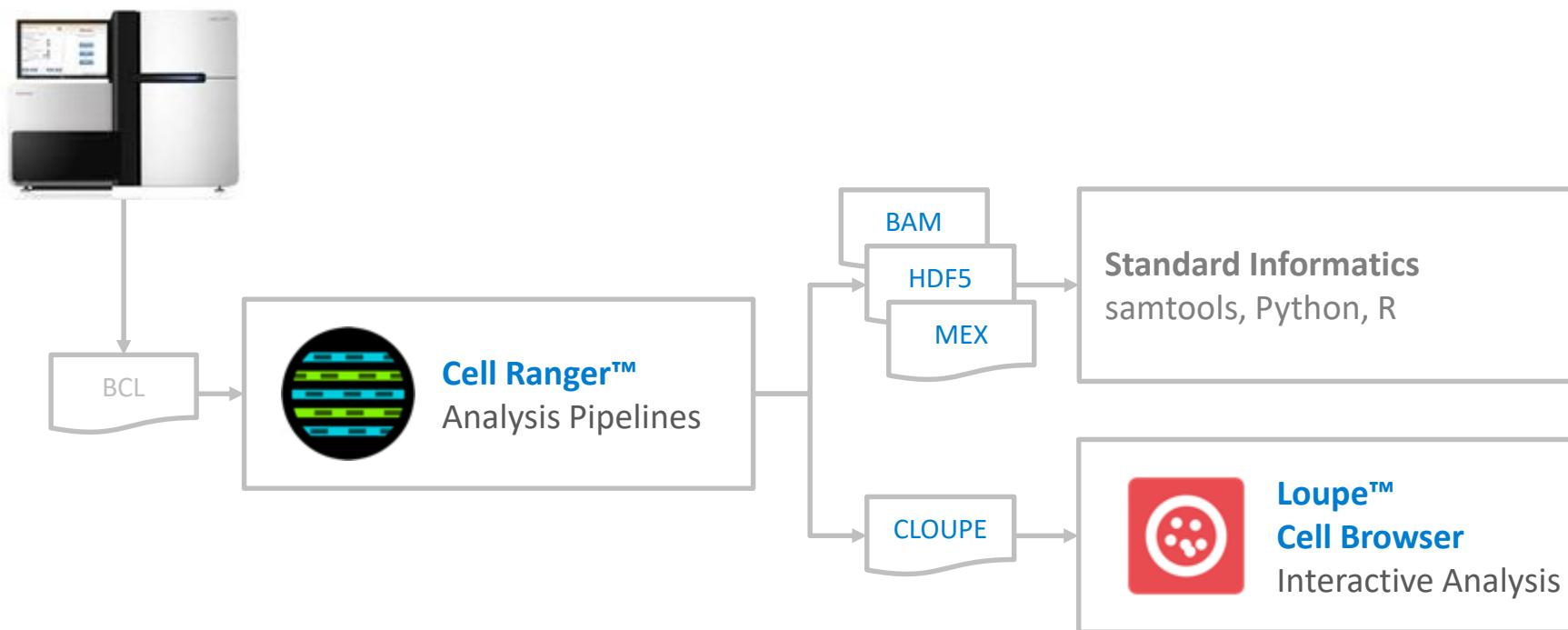
Whole Exome

The Chromium™ System: One Instrument, Multiple Reagent Kits and Software Applications

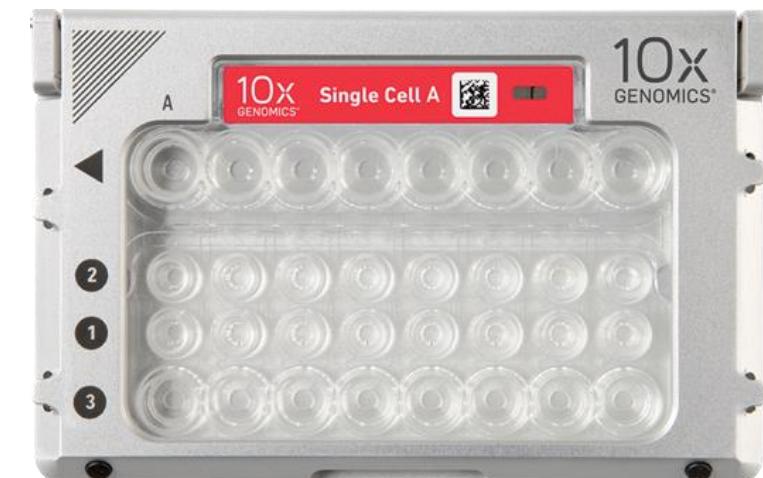


Chromium™ Single Cell 3' Solution Software

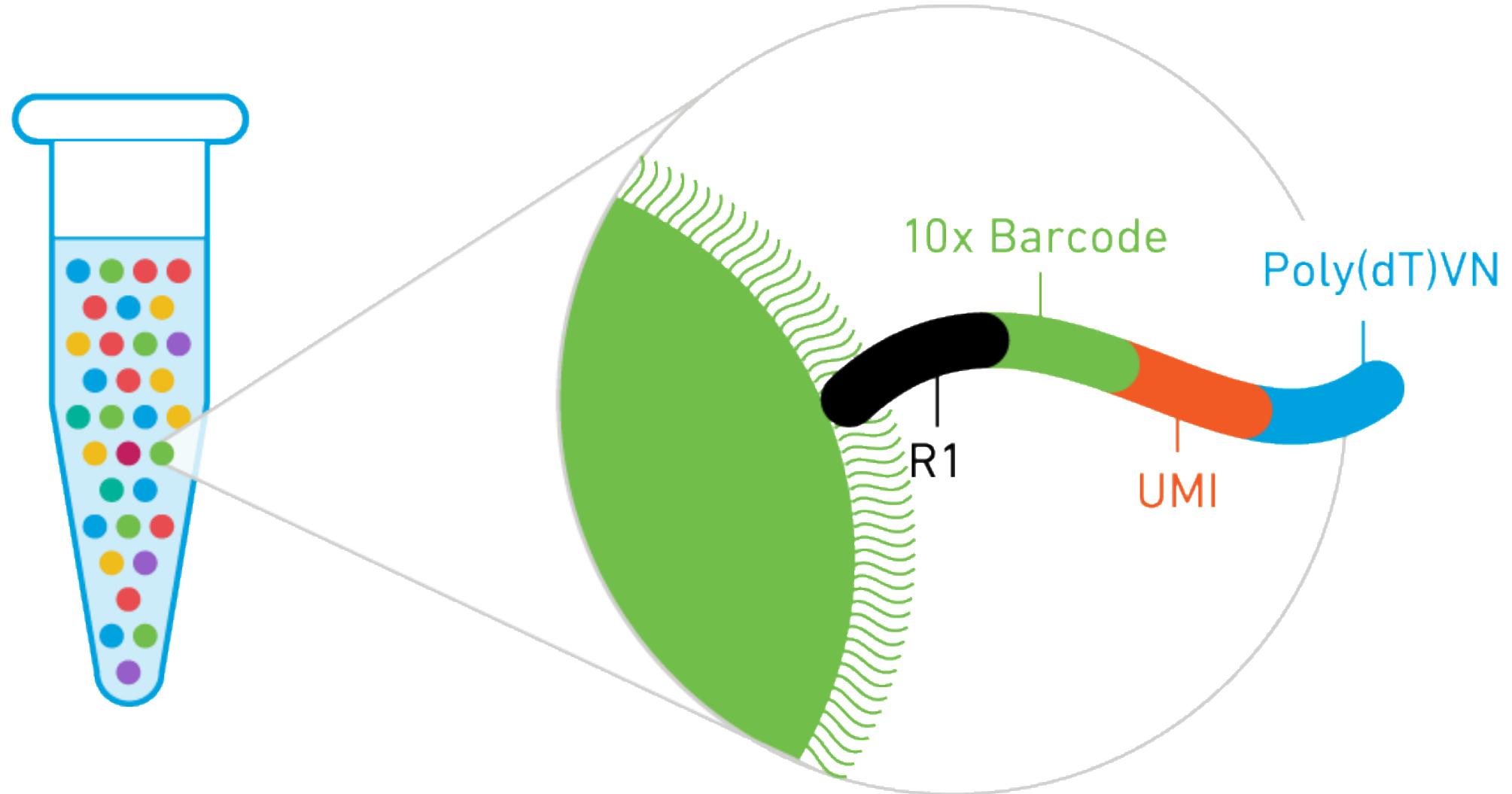
- Sequence Chromium libraries to ~50,000 or more read pairs per cell
- Cell Ranger™ pipeline converts sequence data to single cell gene expression profiles
- Loupe™ Cell Browser enables interactive analysis



Controller, Chip, Reagents...

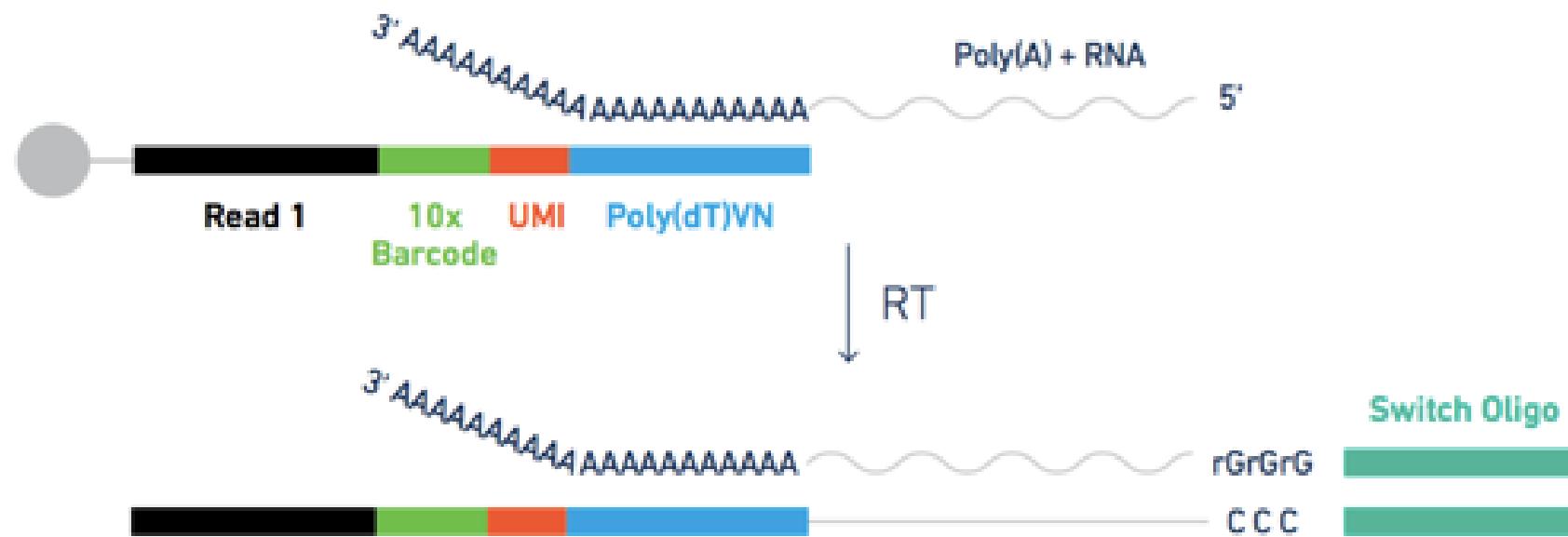


Working Principle of Gelbead Emulsions

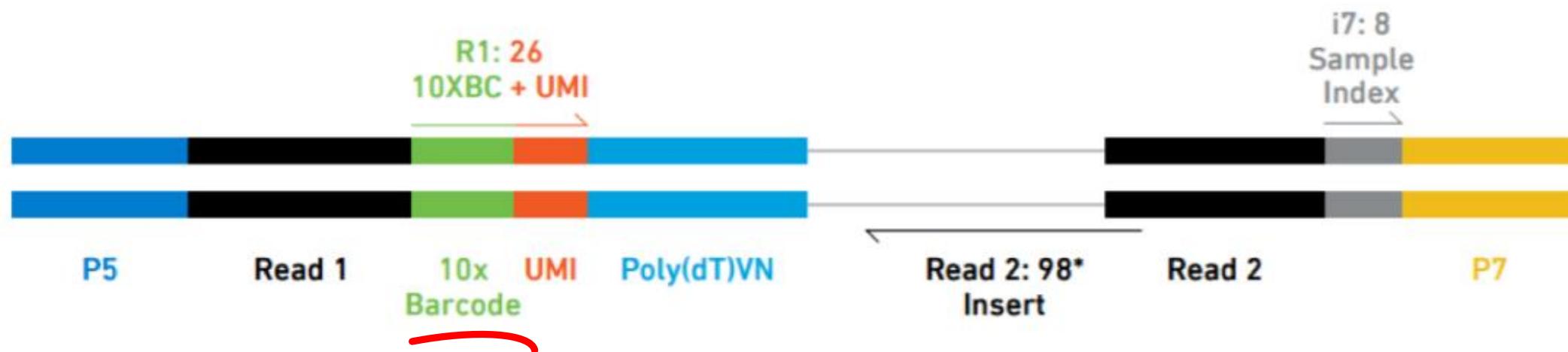


GEMs

Cell Lysis



Final Product



Compatibility with all Illumina Sequencers



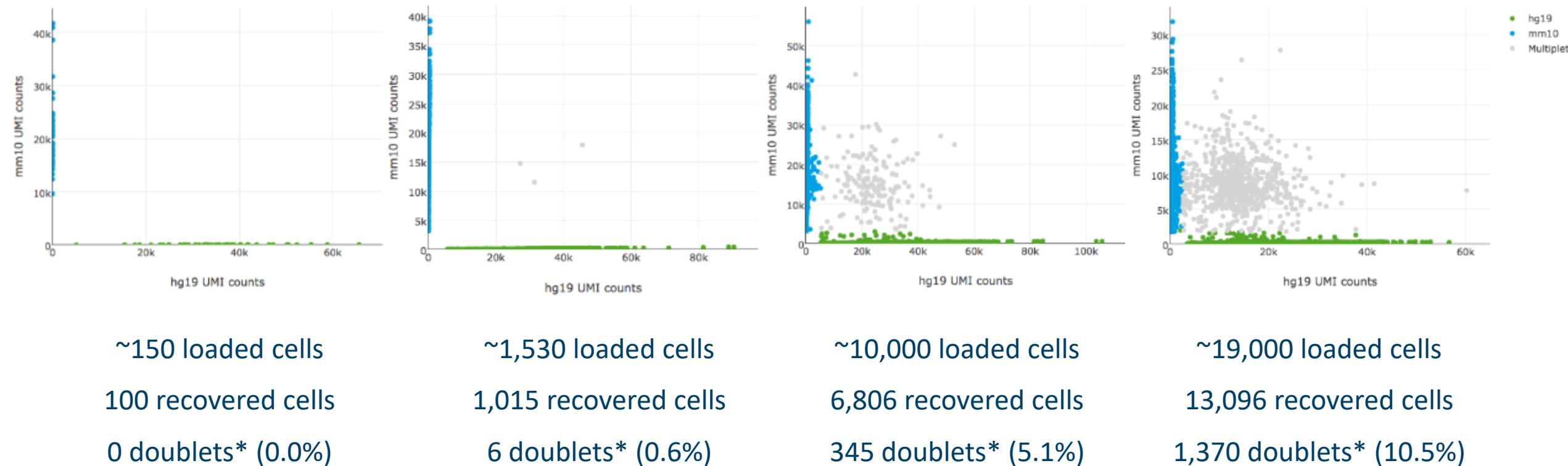
Rapid and Efficient Microfluidics



- Partitions 100 - 10,000+ cells per channel in < 7 minutes
- Recovers ~ up to 65% of all loaded cells
- Low doublet rate: 0.9% per 1,000 cells

Human-Mouse Mixtures Confirm Predicted Doublet Rates

1:1 Mixture of Human (293T) and Mouse (NIH/3T3) Cells, sequenced to ~30-60K reads/cell

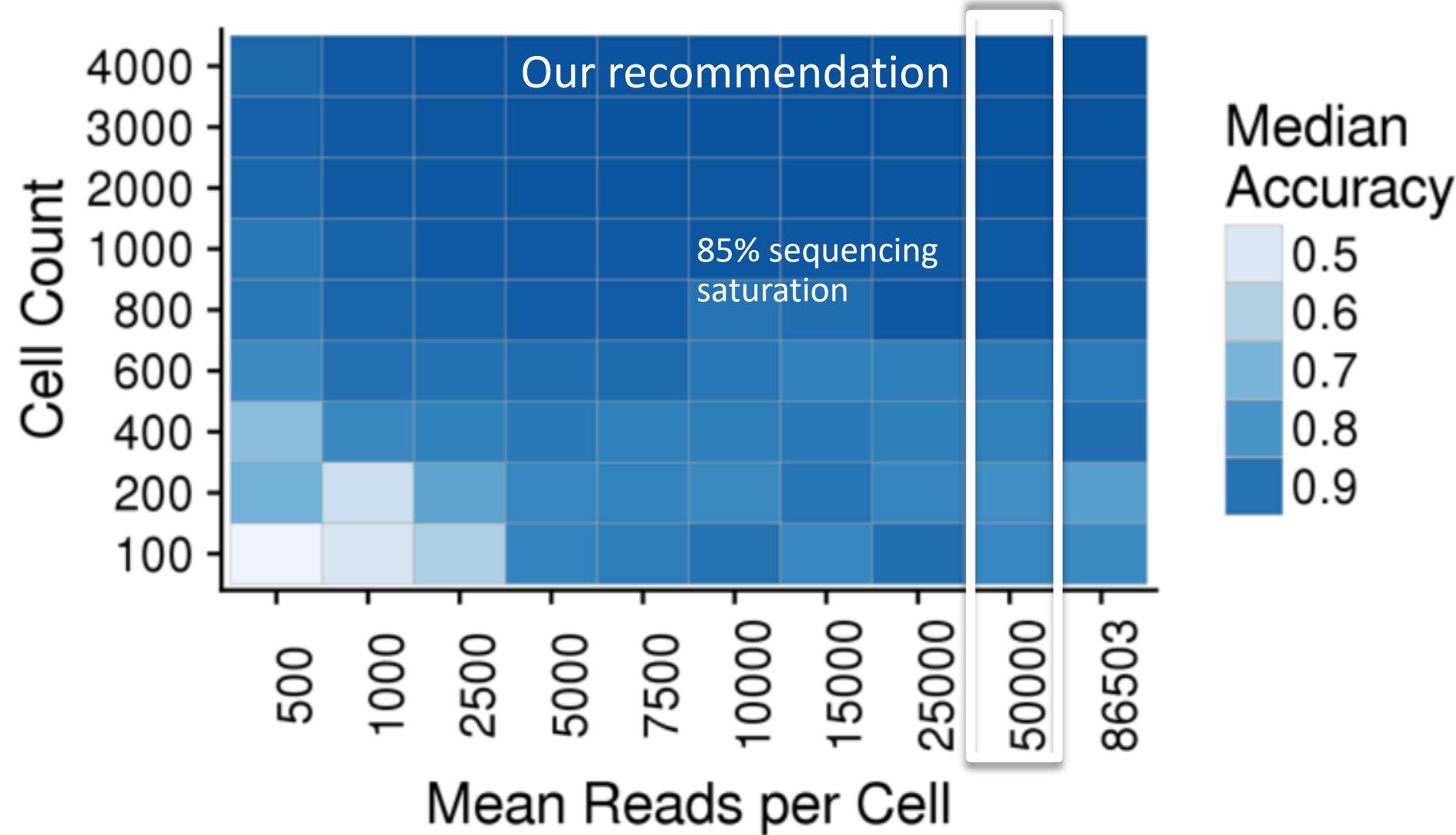


* Includes observed (human-mouse) and inferred (human-human, mouse-mouse) doublets

Raw and processed data freely available from: <http://support.10xgenomics.com/single-cell/datasets>

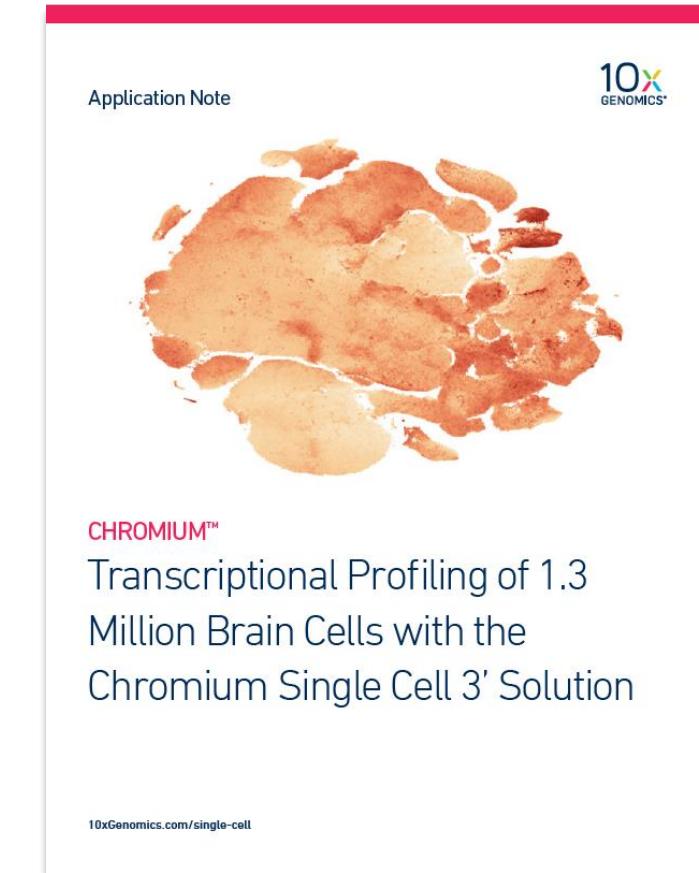
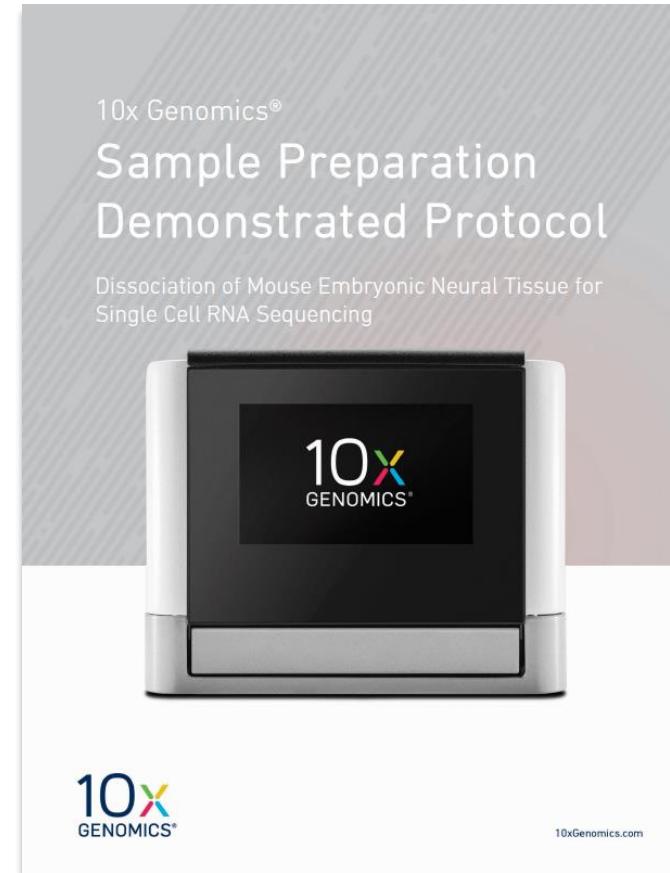
Confidential — Do not distribute

Cell number more relevant for classification than read depth



Example for single cell RNA Analysis

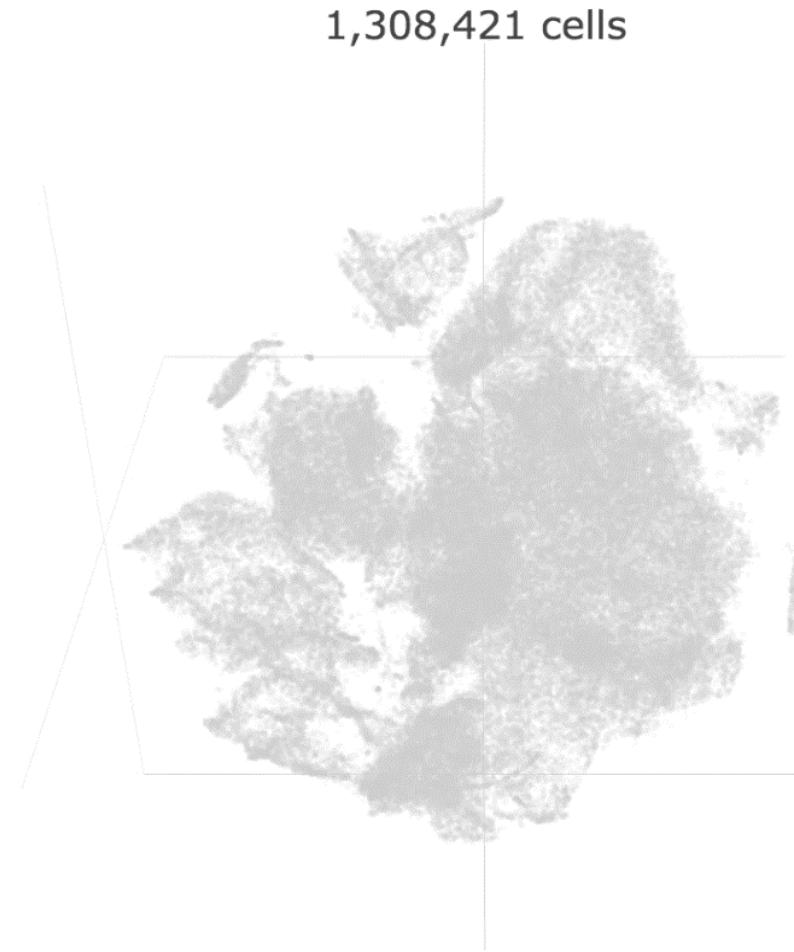
- Combined cortex, hippocampus, and ventricular zones, dissected from 2 embryonic day 18 mice.
- 133 libraries were generated with 17 chips, with each library aiming to recover ~10,000 cells.
- Libraries were sequenced on 11 HiSeq® 4000 flowcells using paired-end sequencing with 26bp (R1), 8bp (i7) and 98bp (R2) run configuration
- Average depth of ~18,500 reads per cell.



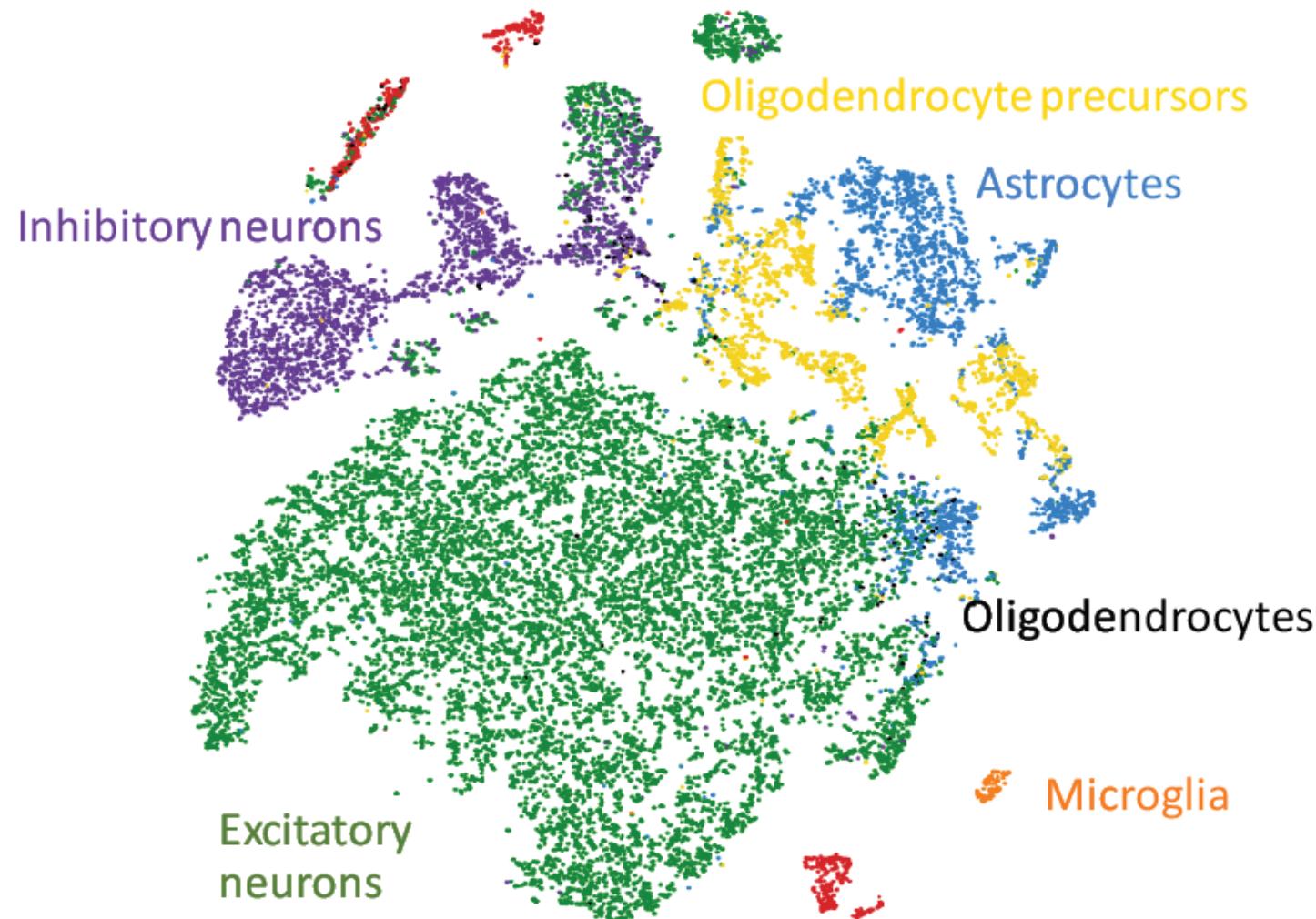
~1.3 million brain cells from two embryonic mice were profiled.

Raw and processed data available from: <http://support.10xgenomics.com/single-cell/datasets>

3D Representation of 1,308,421 Single Cells from Mouse Brain



sc Gene Expression Map of The Mouse Brain



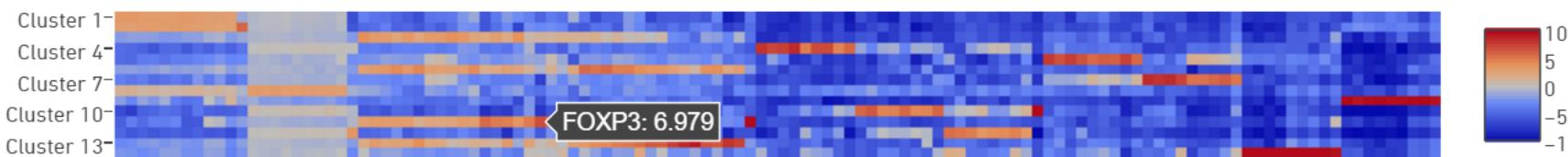
Single Cell RNA



Cell Clustering (NSCLC Sample)



Heatmap of Graph-Based Log2 Fold Changes



Categories



Graph-Based



- Cluster 1 (1362) █
- Cluster 2 (983) █
- Cluster 3 (972) █
- Cluster 4 (935) █
- Cluster 5 (604) █
- Cluster 6 (544) █
- Cluster 7 (451) █
- Cluster 8 (436) █
- Cluster 9 (353) █
- Cluster 10 (341) █
- Cluster 11 (322) █
- Cluster 12 (268) █

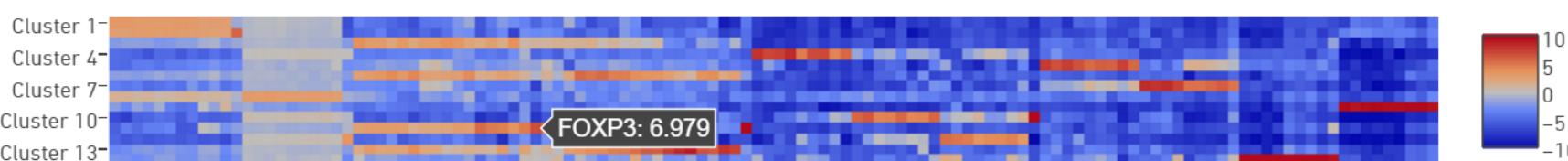
Split View

Significant Genes

Cell Type Categories of Clusters



Heatmap of Graph-Based Log2 Fold Changes



Categories



Cell Types



T Cells (479)



B Cells (2442)



CD4+ Helper T Cells (552)



CD8+ Cytotoxic T Cells (702)



Treg Cells (139)



Monocytes (320)



Epithelial Cells (282)



Basal Cells (918)



Mast Cells (175)



NK Cells (133)



Split View

Significant Genes



bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

New Results

Single cell analysis of quiescent HIV infection reveals host transcriptional profiles that regulate proviral latency

Todd Bradley, Guido Ferrari, Barton F Haynes, David M Margolis, Edward P Browne

doi: <https://doi.org/10.1101/303198>

This article is a preprint and has not been peer-reviewed [what does this mean?].

Abstract

Info/History

Metrics

 Preview PDF

Duke University, Human Vaccine Institute of Medicine, Durham, NC, USA.

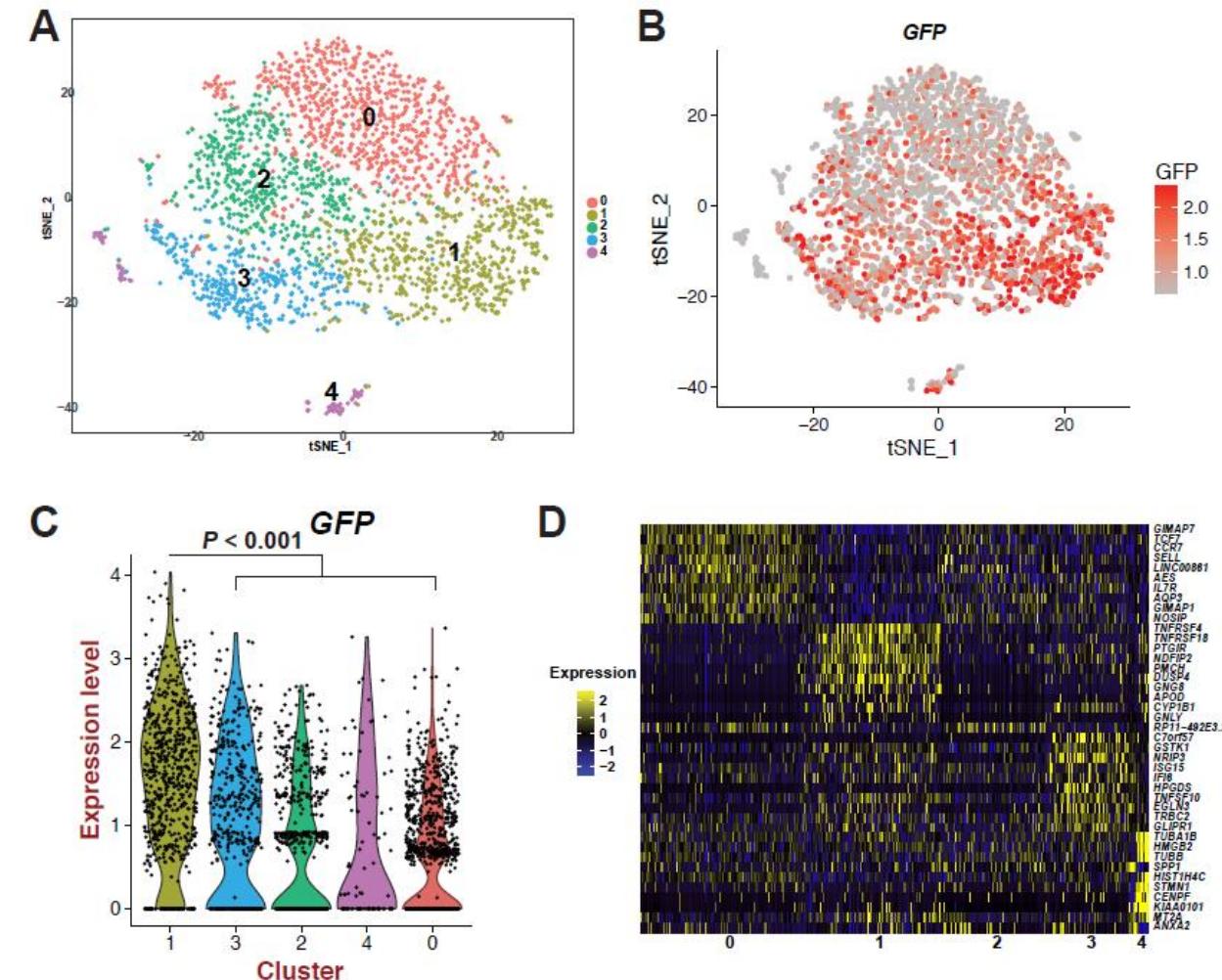
<https://www.biorxiv.org/content/biorxiv/early/2018/04/17/303198.full.pdf>

10x Genomics 3' single cell RNA assay and HIV infection

Striking association between activity of the HIV promoter and the host cell transcriptome within the infected CD4+ cell population.

Clear preference for HIV silencing in cells expressing markers of Tn and Tcm subsets, while effector and activated cells were associated with higher levels of viral gene expression.

These results suggest a role for T cell subset identity and intracellular environment in regulating the outcome of infection.

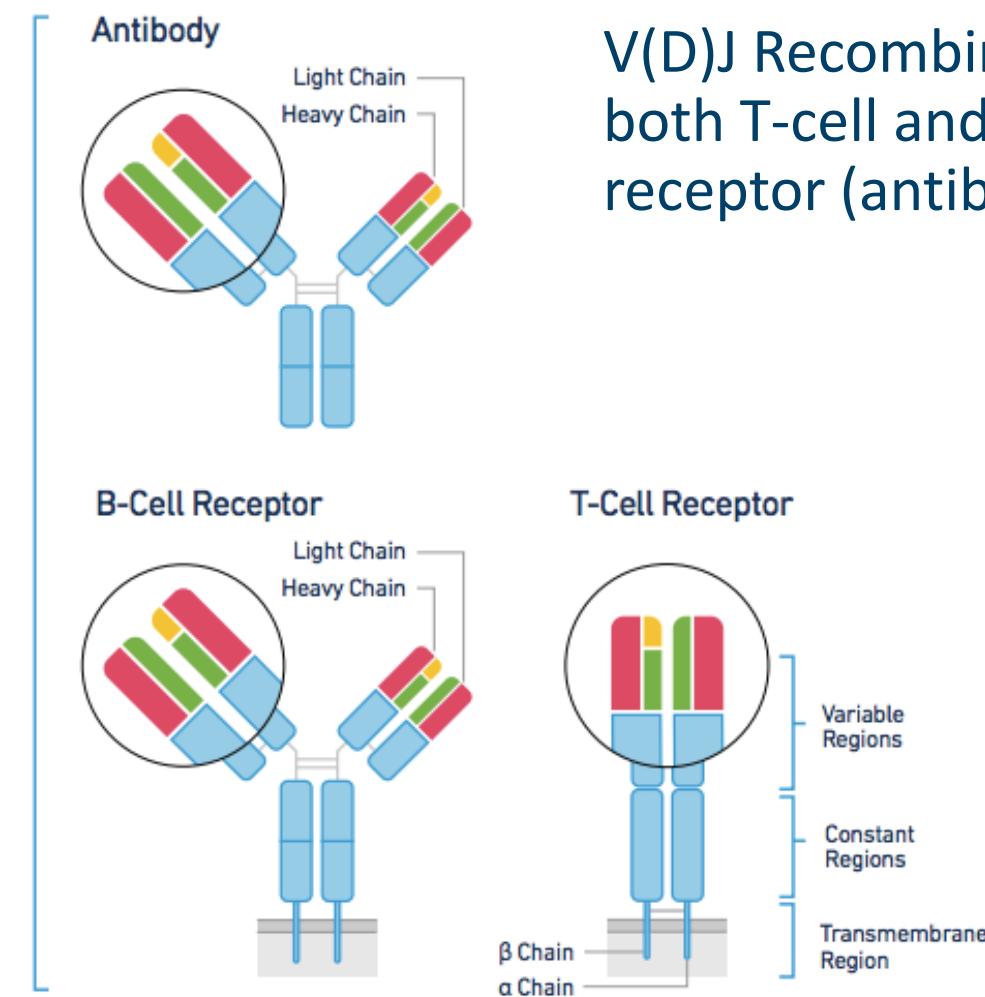
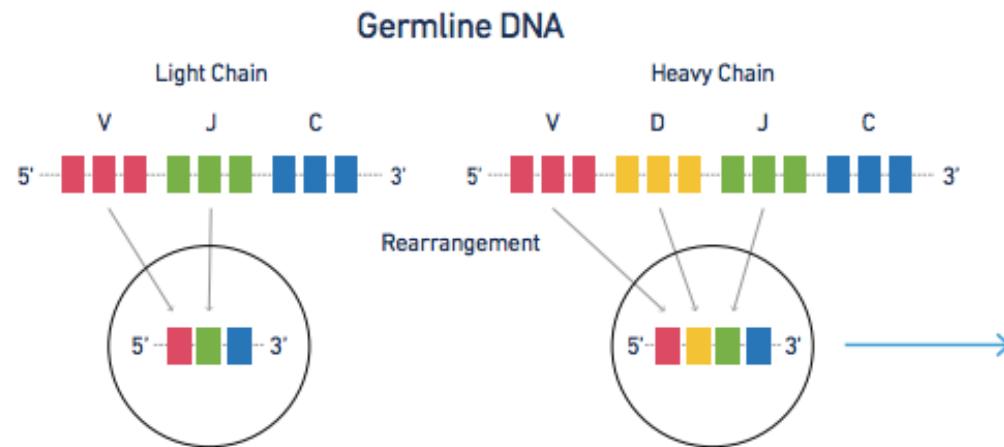


Single Cell V(D)J Assay



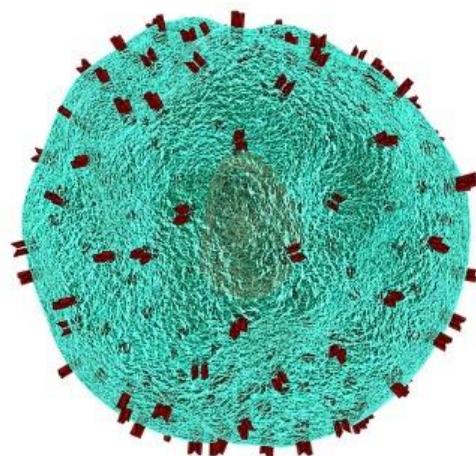
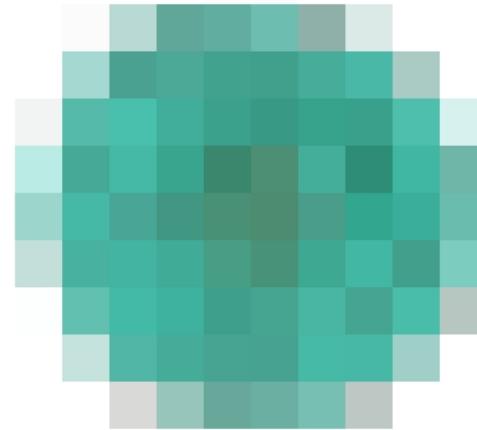
V(D)J Recombination – TCR and BCR

V(D)J Recombination



V(D)J Recombination creates both T-cell and B-cell receptor (antibody) diversity

Immune Repertoire Tools Need To Be High-Resolution



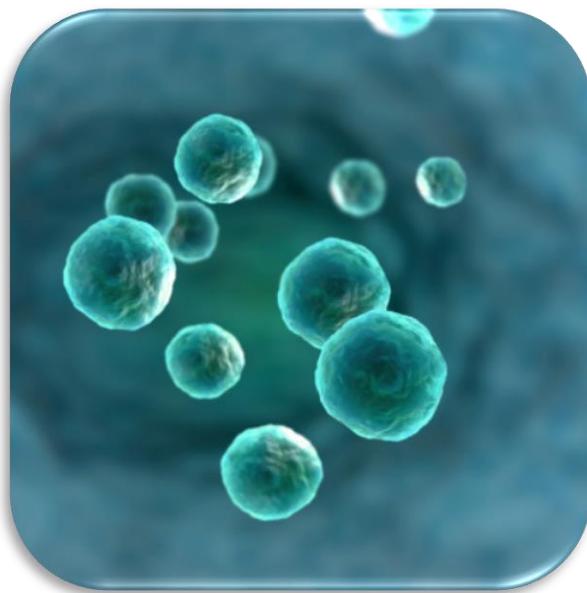
- Shortcomings of current tools:

- Low cell numbers
- No paired TCR/BCR information
- Incomplete data sets, only V(D)J or GEx, not both
- Optimization required, user has to combine prep, assay, reagents, and analysis
- Loss of chain paring information in bulk analysis

- What is really needed:

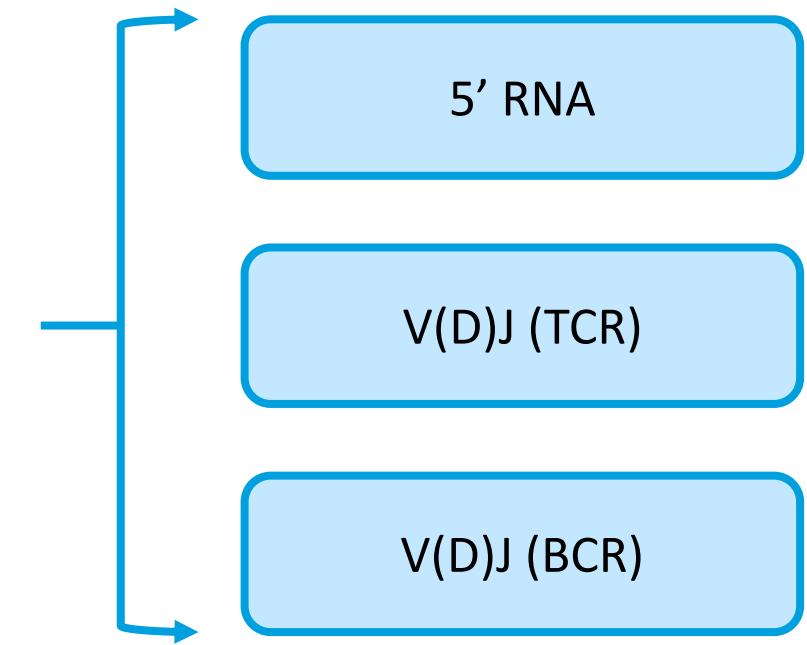
- High cell number for high resolution of populations
- Capture both GEx and paired V(D)J for T and B cells in sample
- Paired chain information
- Robust end to end workflow

10x Chromium V(D)J Single Cell Solution



User-Supplied Cells

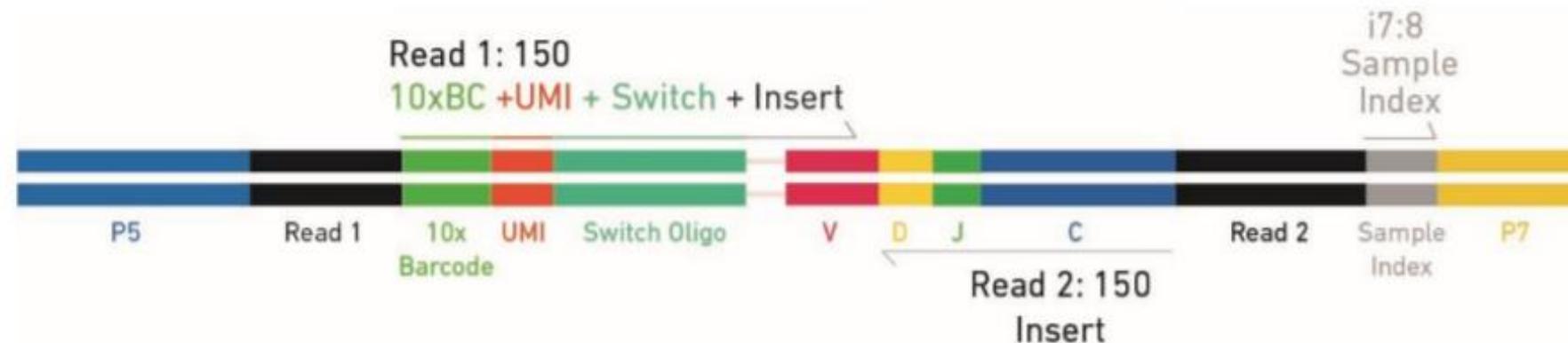
*10x Chromium System,
Reagents, and Software*



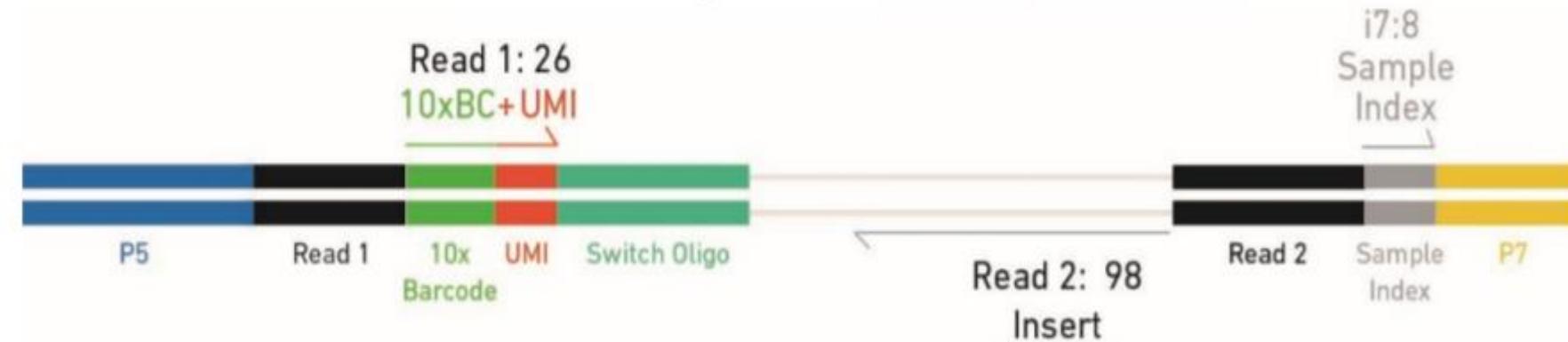
*Multiple data types at
unprecedented resolution*

Parallel Processing of 5'RNA processing and V(D)J Amplification

V(D)J Enriched Library Structure

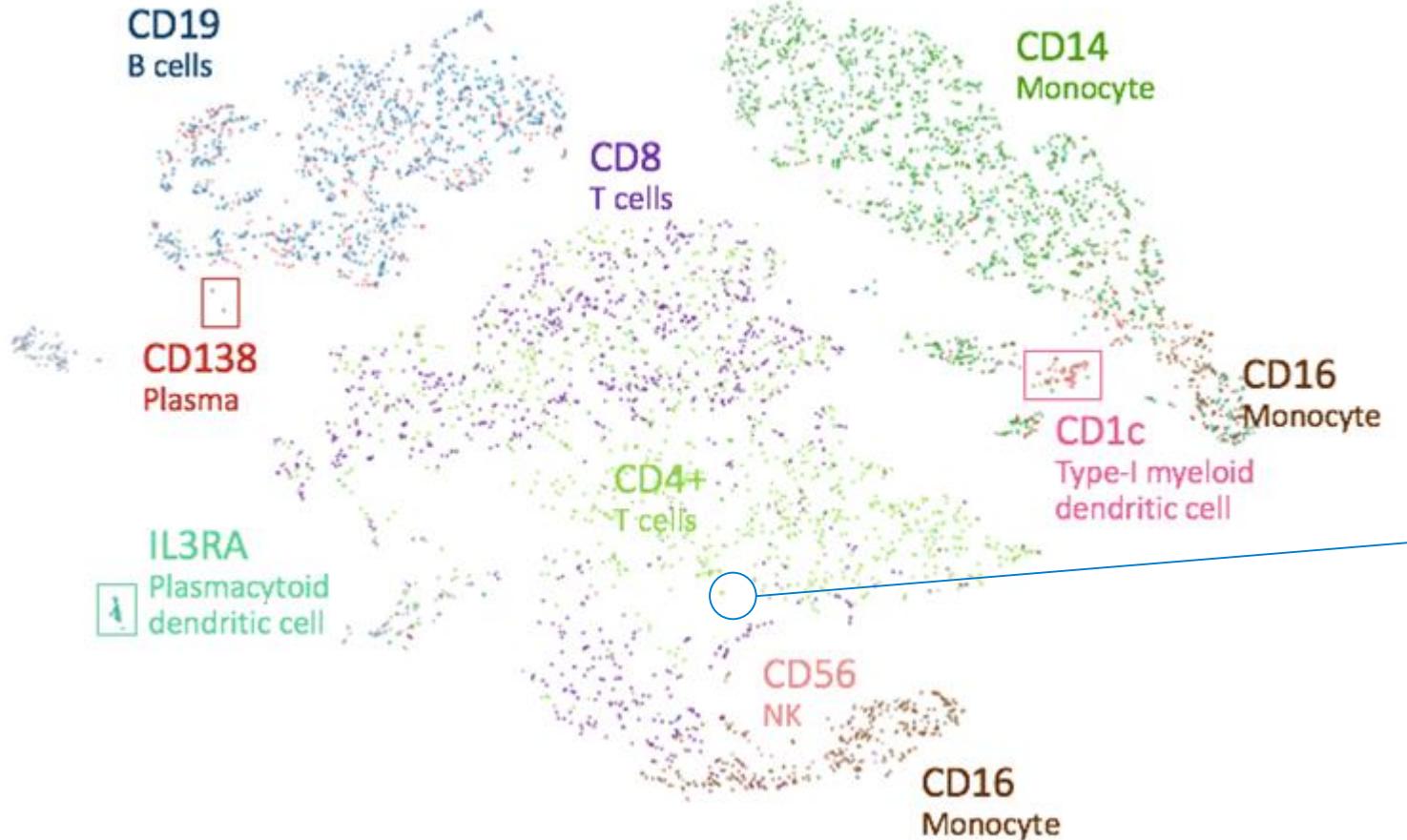


5' Gene Expression Library Structure



Chromium V(D)J Single Cell Solution: Gene Expression and Paired TCR/BCR From The Same Cell

10X
GENOMICS[®]



α chain consensus (682nt) TRAV13-1:J5

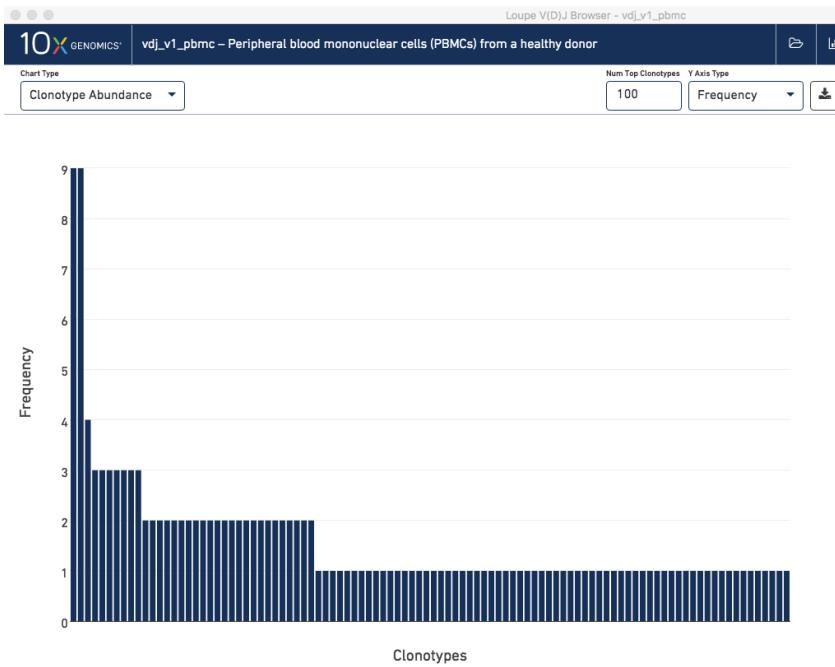
```
TGTCTTATATGGGGATTGGTGGCTACACAGTGTGAGAAACCCCTATGG  
CTGGCCAGGGAGAGAAGAGACAACCTGATGATAGAAGTAACCTTTATAA  
CTGGAGGTTGCAGGCTAATGACTGATCTTAATTGGAAAGAACAGGATG  
ACATCCATTGAGCTGTATTTATTCCTGTGGCTGCAGCTGGACTTGG  
TGAATGGAGAGAATGGAGAGCAGCATCTCAACCCCTGAGTGTCCAGGA  
GGGAGACAGCGCTGTTATCAAGTGTACTTATTCAAGCAGTGCTCAAAC  
TACTTCCCTGGTATAAGCAAGAACTGGAAAAAGACCTCAGCTTATTA  
TAGACATTCGTTCAAATGTGGCGAAAAGAAAGACCAACGAATTGCTGT  
TACATTGAAAGAACAGCCAAACATTCTCCCTGCACATCACAGAGACC  
CAACCTGAAGACTCGGCTGTCTACTTCTGTGCAGCACCCCCGGGGGCCA  
GGAGACCACTACTTTGGGAGTGGAAACAAGACTCAAGTGAACAAA  
TATCCAGAACCTGACCTGCCGTGTACAGCTGAGAGACTCCGGATG  
AAGCTACAAGCGCGAGTGACGTTATGATTGATGGAAGAAATCTGAA  
GCTGGATGAGGGCCAAGCGGAAAGAGGTATGACTCTGCAGACAA
```

β chain consensus (563nt) TRBV27:D1:J1-1

```
TATCTTATATGGGGTTCTGAGGCCAAATAGCTGAAGAGGTGGAGACGTTACA  
GAAACCACTGGAGCCCCAGAACTGGCAGACACCTGCTGATGTCGCATGGG  
CCCCAGCTCTGGCTATGTTGGCTTGGCTTAGGAGCAGGCCCTGGAAAG  
CCCAGTGACCCAGAACCCAAAGATACCTCATCACAGTACTGGAAAGAACGTTAAC  
AGTGACTTGTCTCAGAAATGAAACCATGAGTATATGTCCTGGTATCGACAAGAC  
CCAGGGCTGGCTTAAGGCAGATCTACTATTCAATGAATGTTGGAGGTGACTGATA  
AGGGAGATGTTCTGAAGGGTACAAAGTCTCGAAAAGAGAACAGGAATTCCC  
CCTGATCCTGGAGTCGCCAGCCCCAACAGACCTCTGTACTTCTGCCAGC  
AGTTAGGACAGAACACTGAAGCTTCTGGACAAGGCCAGACTCACAGTTG  
TAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTGCTGTGTTGAGCCATCAGA  
GATCGGAAGAGCA
```

Diverse TCR Repertoire Of A Healthy Individual

Chromium V(D)J data from two aliquots of same blood sample



Majority of clonotypes are observed only 1-2 times per aliquot

Clonotypes unique to Aliquot #1 Clonotypes unique to Aliquot #2

1816

54

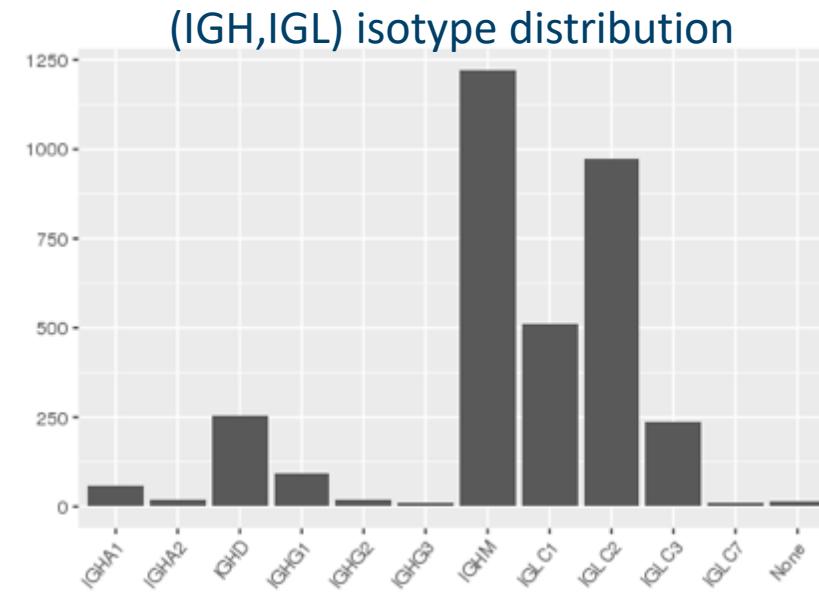
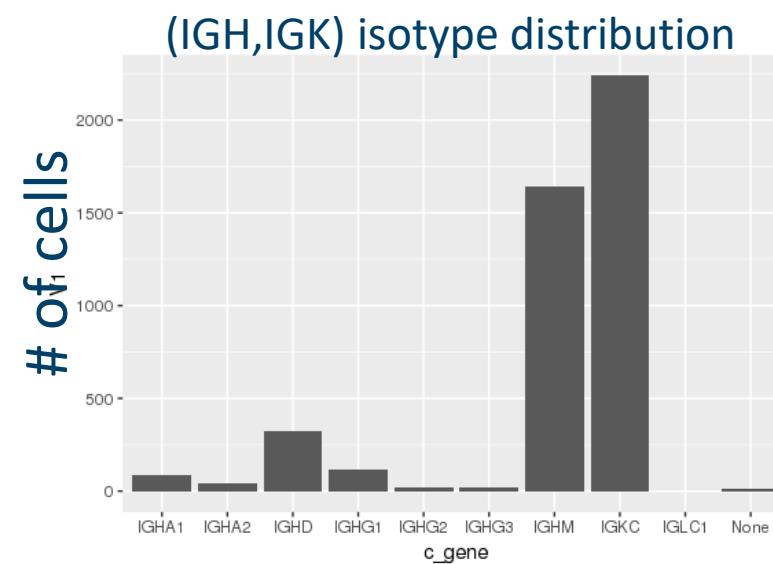
1738

Clonotypes detected in both

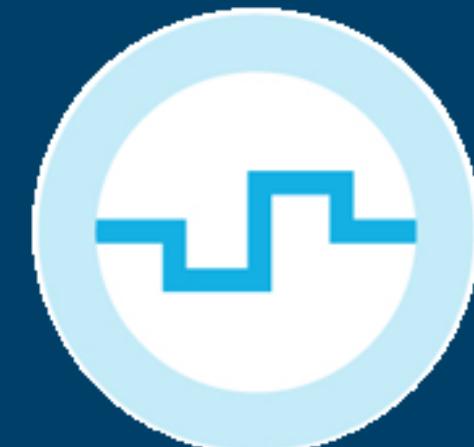
Extremely high unique diversity,
minimal clonotype overlap
among aliquots

VDJ profiling on B cells

Sample	Estimated number of cells loaded	Cells with Ig(s) detected	Productive IGH detected	Productive IGK detected	Productive IGL detected	Productive pair detected	Clonotype diversity
B cells rep 1	~10,440	4,864	88%	58%	47%	85%	3,694
B cells rep 2	~10,440	4,625	89%	58%	44%	86%	3,745
B cells rep 3	~10,440	4,838	89%	57%	44%	86%	3,950



Single Cell CNV Assay



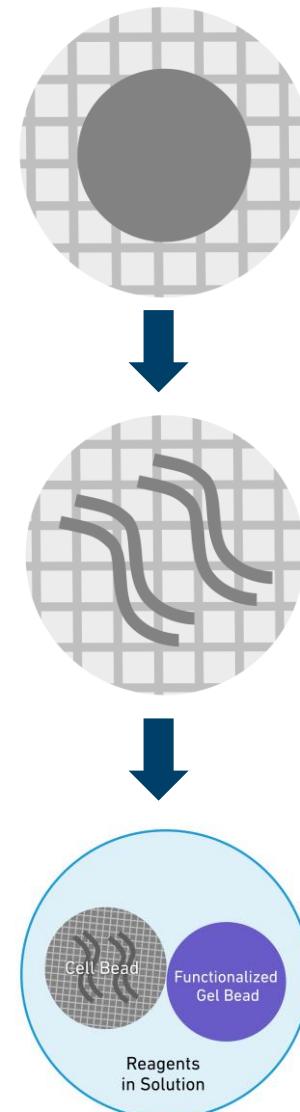
Comprehensive Scalable Solution for Single Cell Genomics



- Reveal genome heterogeneity
- Understand clonal evolution
- Determine pathogenesis and disease progression
- Profile 100s to 1000s of cells
- Demonstrated with cell lines, primary cells, fresh and frozen tissue
- Accurately detect single cell CNV events at 2 Mb resolution
- Detect CNV events down to 100s of Kb on cell clusters
- Analyze data using Cell Ranger DNA and visualize the results with Loupe scDNA Browser

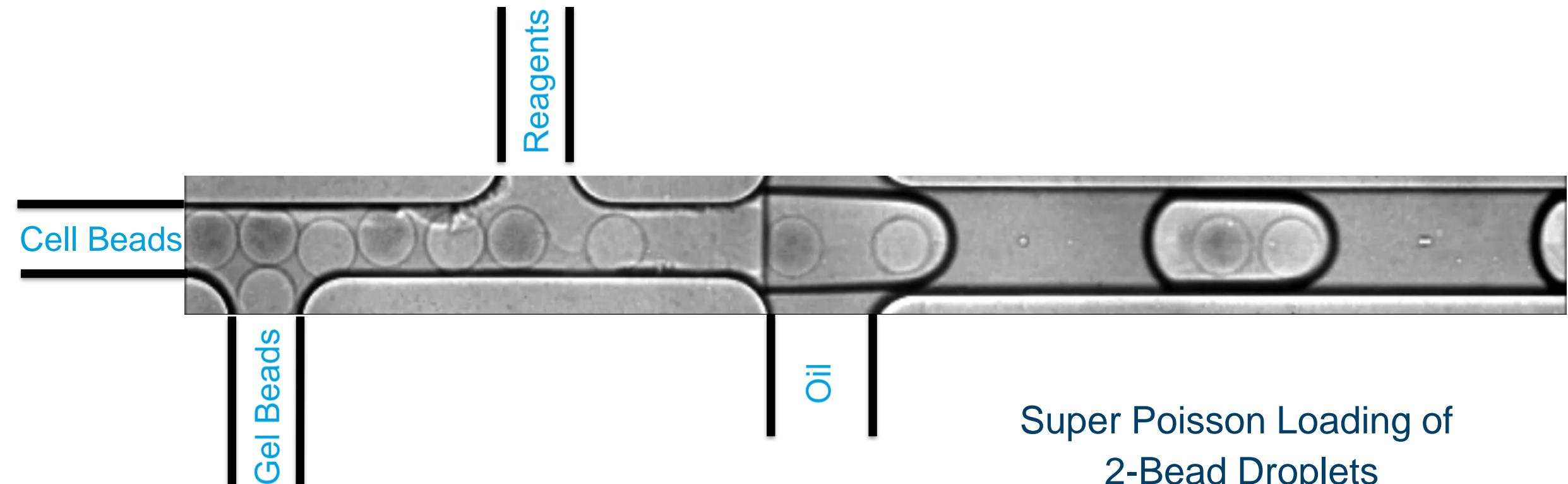
CBGBs Enable Multi-Step Reactions

Trap Cells
in Cell Beads



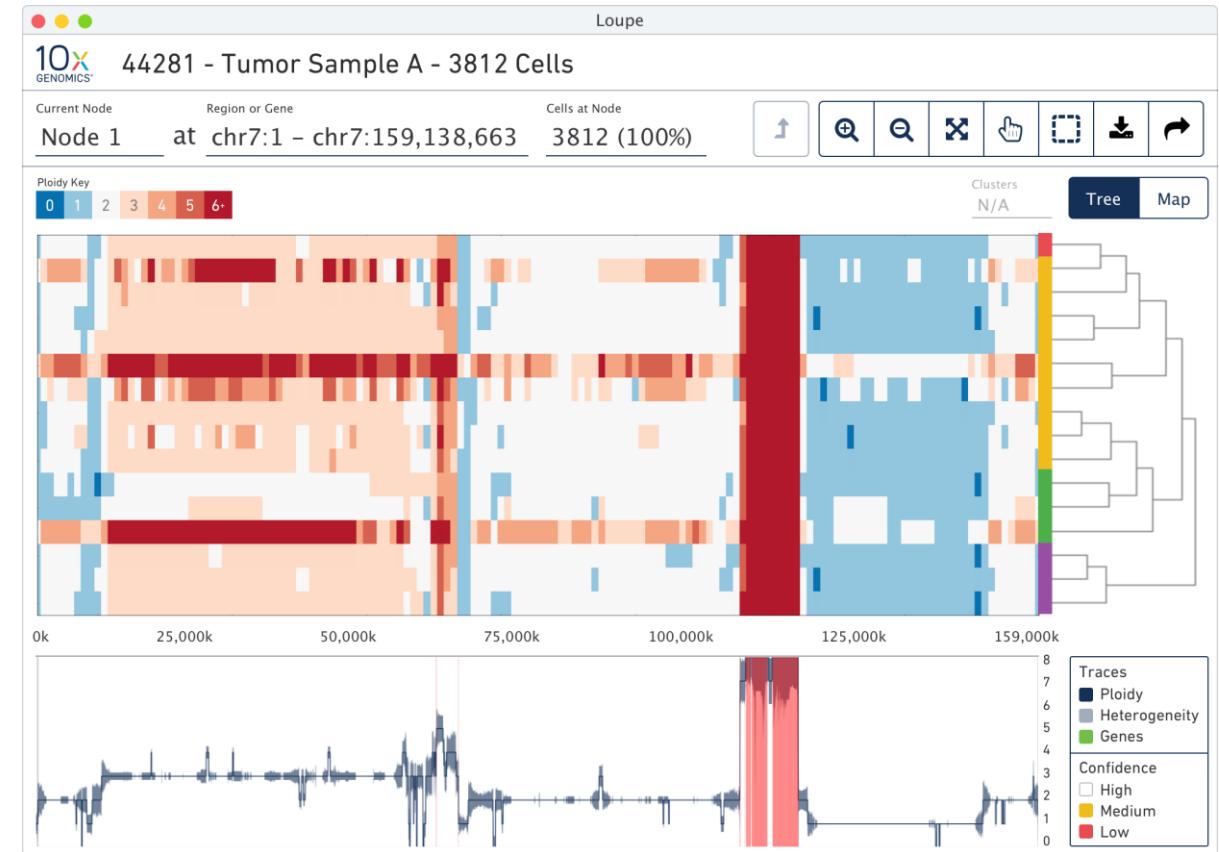
- Separate cell treatment and barcoding reactions enables uniform access to nuclear DNA
- User-friendly workflow with magnetic cell beads
- Works on existing Chromium Controllers
- Additional multi-step reactions possible in future

Uniform CBGB Generation

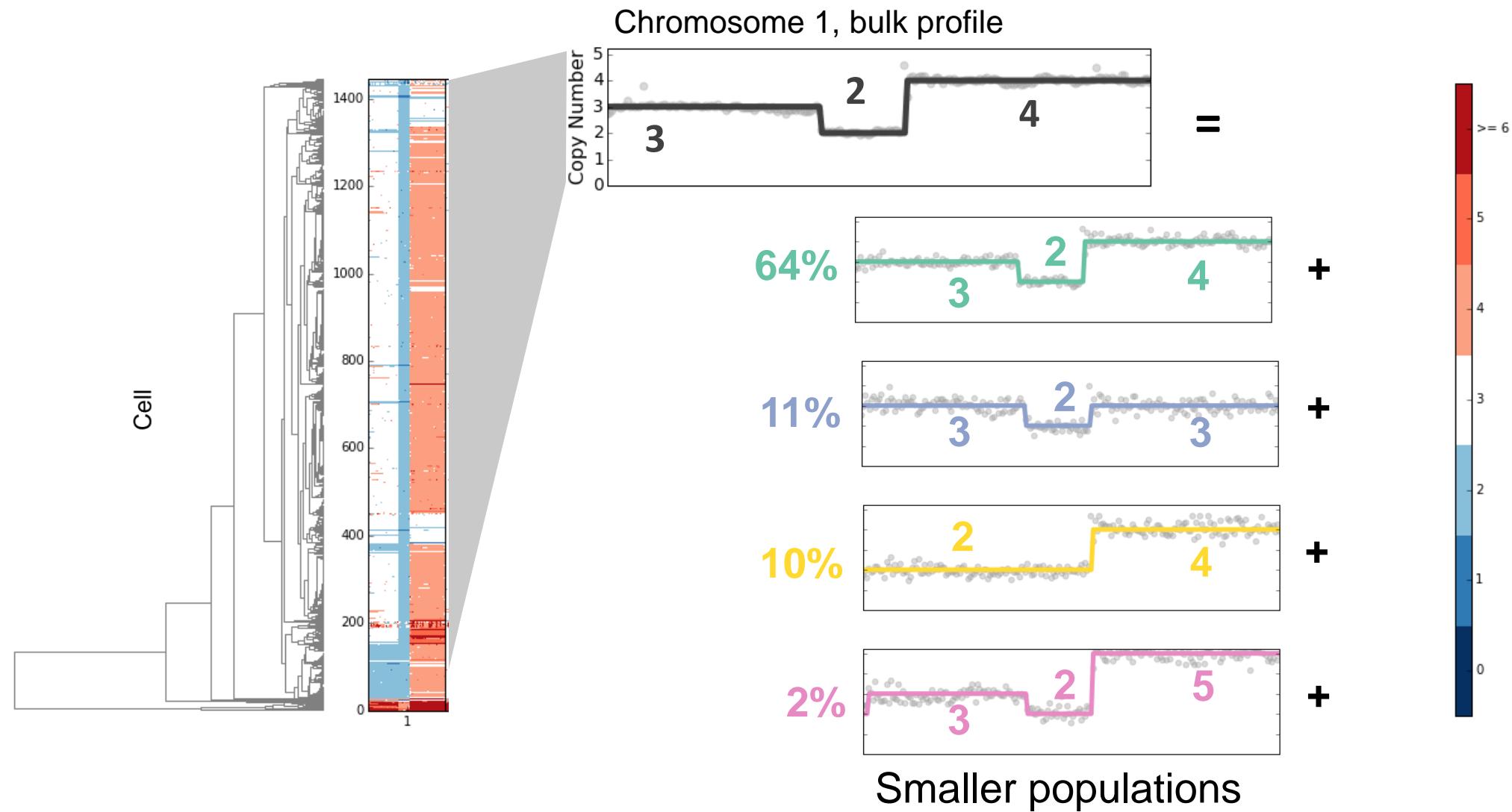


Easy-to-Use Software

- Easy to use analysis software for single cell CNV calling and clustering
- Zoom in to sub-clusters of cells
- Visualization tool to inspect and interrogate genomic regions and cell clusters by coordinates or gene names.
- Demo data and tutorials available
- View heatmap of single cell CNV profiles

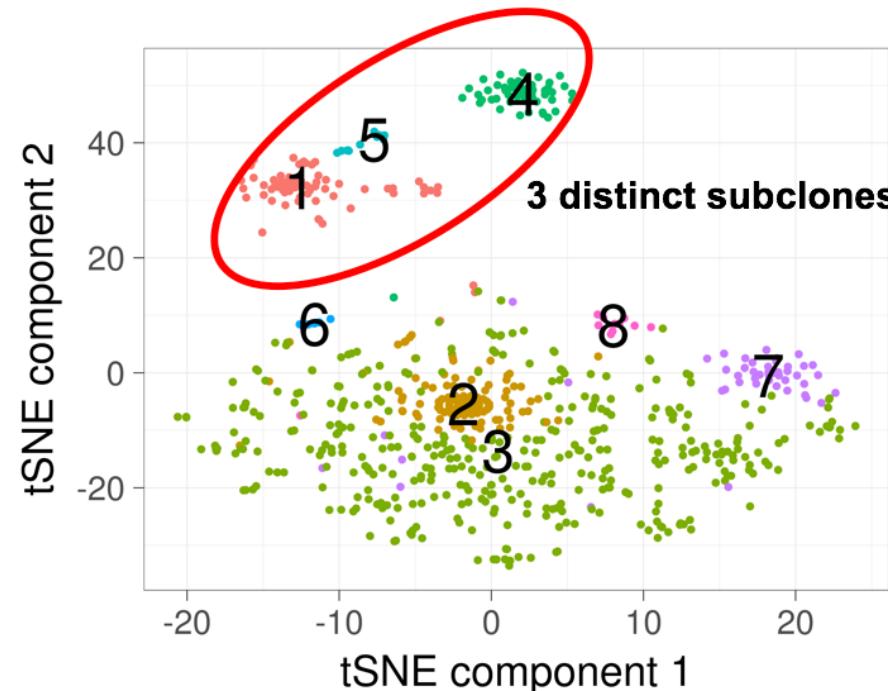


COLO-829: A Mosaic Cell Line



Analysis of tumor heterogeneity in primary cancer samples

Primary gastric cancer sample (N=710)



	N	%
1	75	11%
2	107	15%
3	392	55%
4	56	8%
5	13	2%
6	6	1%
7	48	7%
8	13	2%

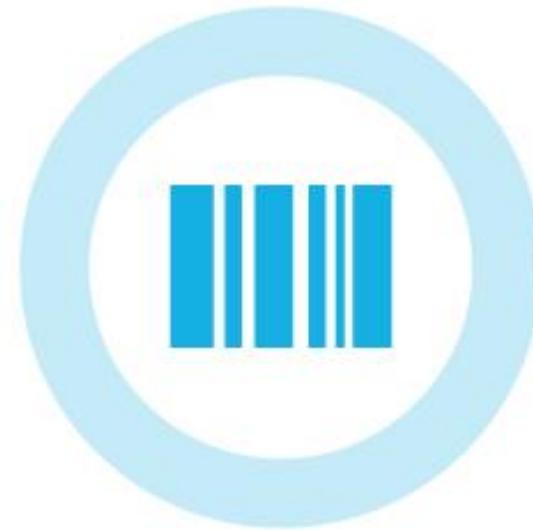
- scDNA-Seq on surgically excised, dissociated tumor cells
- Discovered 3 subclones displaying distinct CNV patterns

Single-cell multi-omic analysis of intratumoral heterogeneity and immune lineages in patient tumors

Billy Lau¹, Noemi Andor², Anuja Sathe², Hanlee P. Ji^{1,2}

¹Stanford Genome Technology Center, Stanford University, Palo Alto, CA, 94304, United States

²Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, 94305, United States

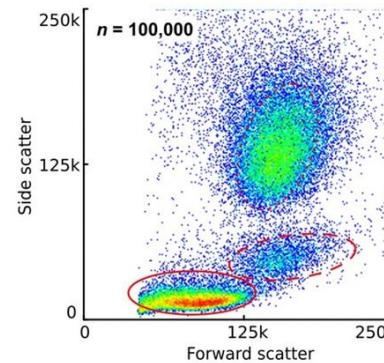


Single Cell
Feature Barcoding

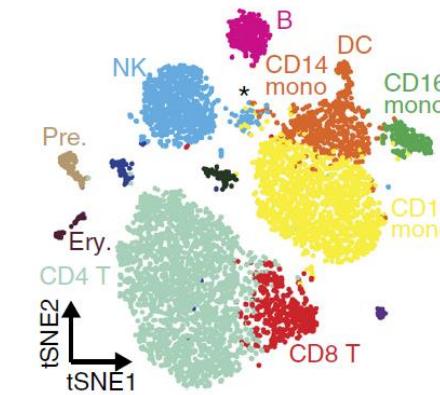


Merging Phenotyping and Gene expression analysis in single cells

10X
GENOMICS



Cytometry
(Flow, Mass, FACS)



scRNA transcriptomics

ADT



Single Cell Protein and Expression Analysis

2 recent publications

nature
biotechnology

Multiplexed quantification of proteins and transcripts in single cells

Vanessa M Peterson^{1,5}, Kelvin Xi Zhang^{2,5},
Namit Kumar¹, Jerelyn Wong³, Lixia Li¹, Douglas
C Wilson³, Renee Moore⁴, Terrill K McClanahan³,
Svetlana Sadekova³ & Joel A Klappennbach¹

Department of Translational
Medicine Merck

REAP Seq

RNA Expression And Protein sequencing

- **82 barcoded antibodies** and >20,000 genes in a single workflow.
- Assessment of the costimulatory effects of a CD27 agonist on human CD8+ lymphocytes
- Identification & characterization of a new CD8+ lymphocyte cell type

NATURE METHODS

Simultaneous epitope and transcriptome measurement in single cells

Marlon Stoeckius¹ , Christoph Hafemeister¹ ,
William Stephenson¹ , Brian Houck-Loomis¹ ,
Pratip K Chattopadhyay² , Harold Swerdlow¹ ,
Rahul Satija^{1,3}  & Peter Smibert¹ 

New York Genome Center

CITE Seq

Cellular Indexing of Transcriptomes and Epitopes by sequencing

- Panel of **13 monoclonal antibodies** targeting cell-surface proteins and routinely used as markers for immune-cell classification

Antibody Derived Sequence Tag

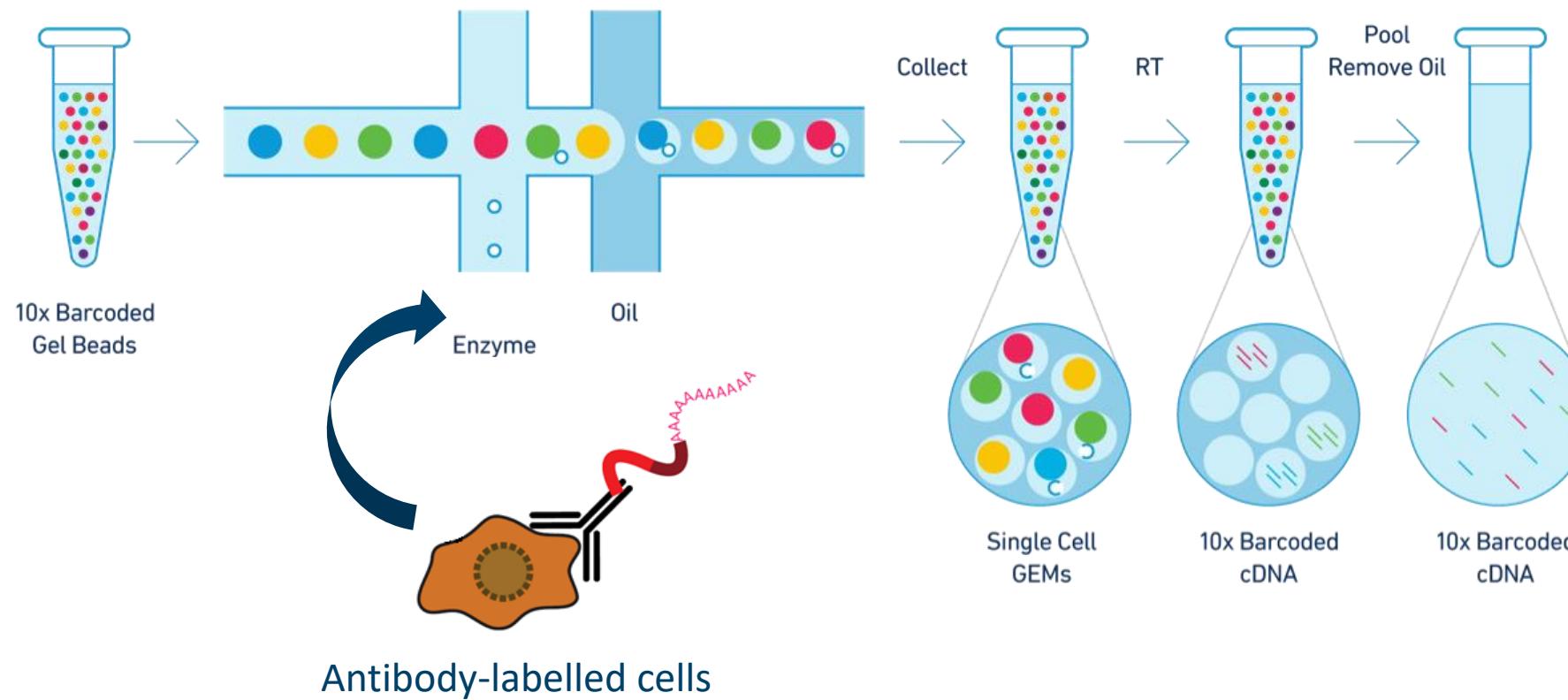


Genetic Barcode polyA tail

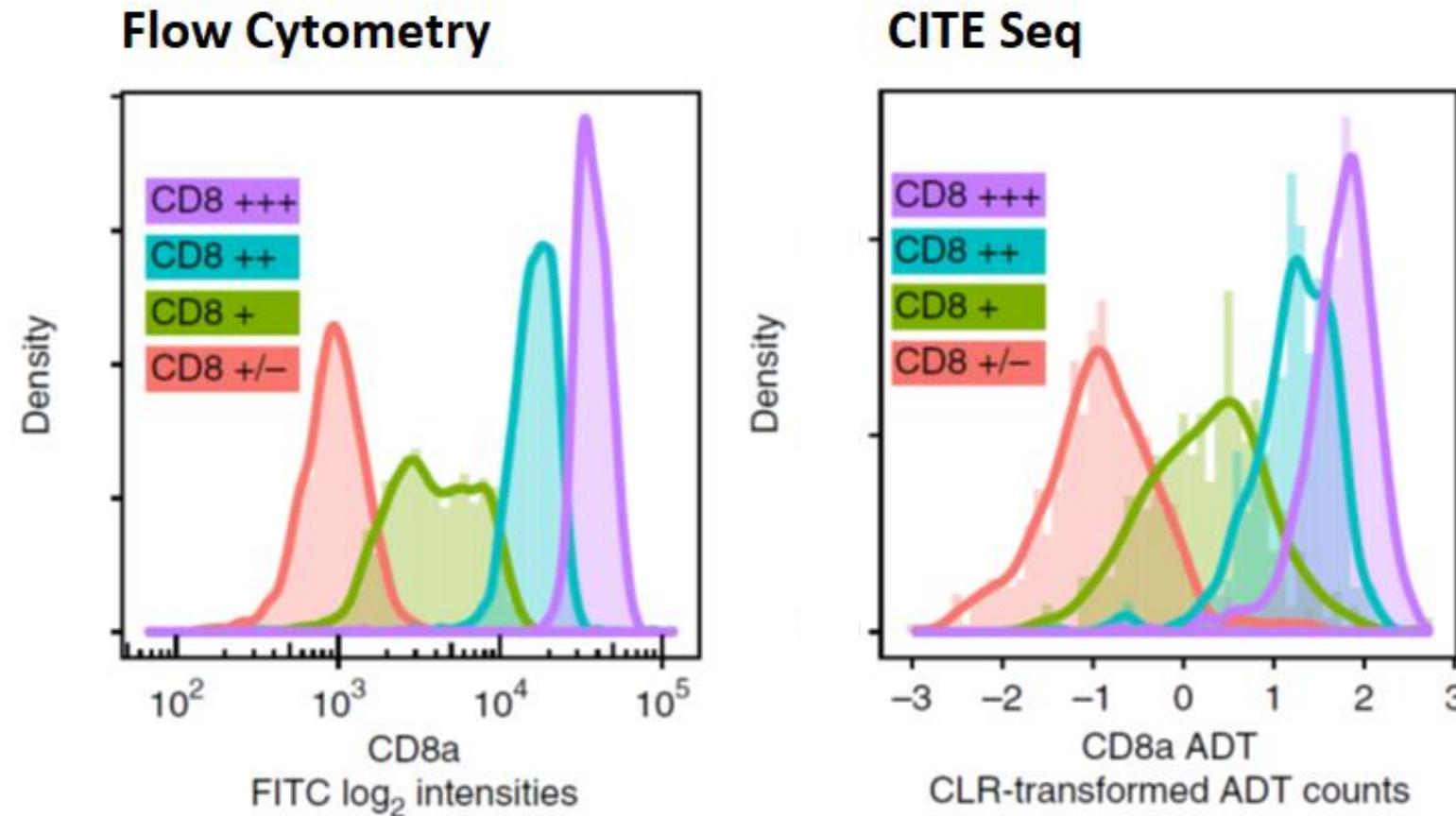
Oligo nucleotide-based barcoding allows virtually unlimited scalability.



No change of off-the-self workflow



Quantitative Comparison: FACS vs. ADT Sequencing

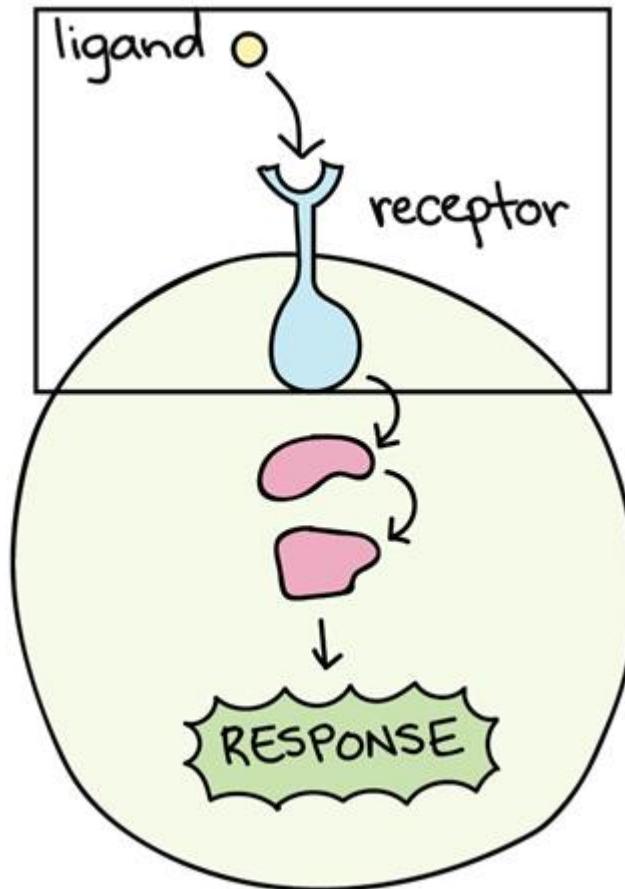




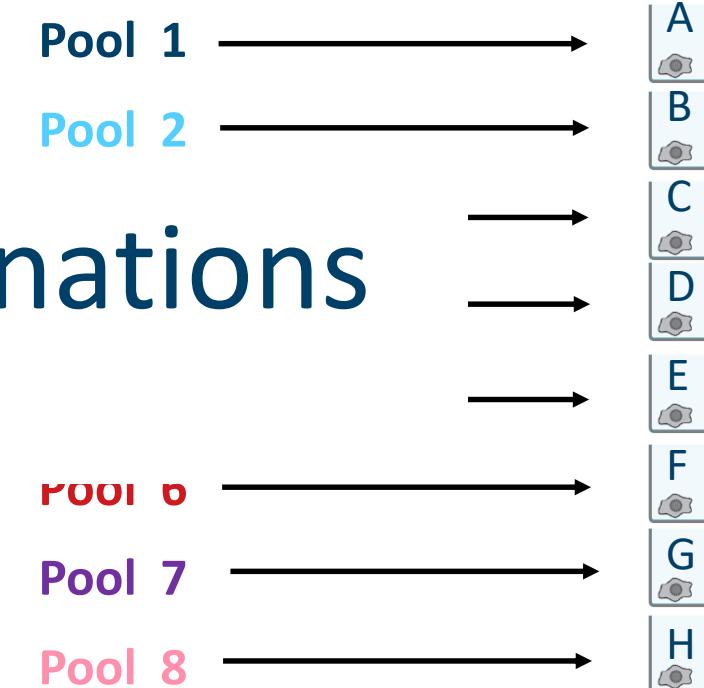
Get exCITED and inspired. “X” can be “anything”.

- **Epitope**
(e.g. screening the effect on cell carrying appropriate receptor)
- **Chemical compound**
(e.g. analysis of effect of drug compound(s) on cells)
- **Polypeptides**
(e.g. screening for immunogenic parts of therapeutic polypeptides)
- **Ligands**
(e.g. for pathway analysis)

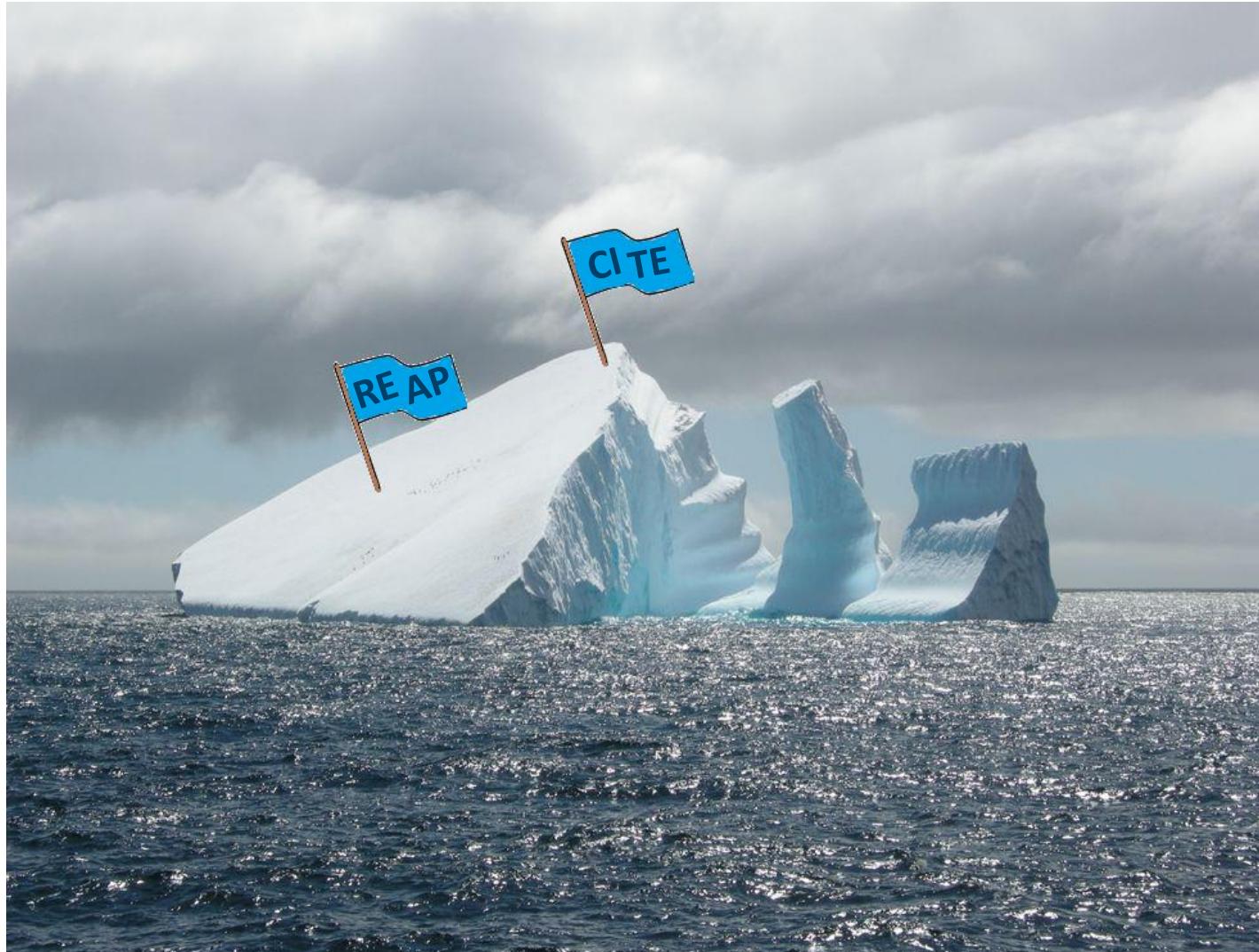
Let our mind wander...



3,072 combinations
1 Chip



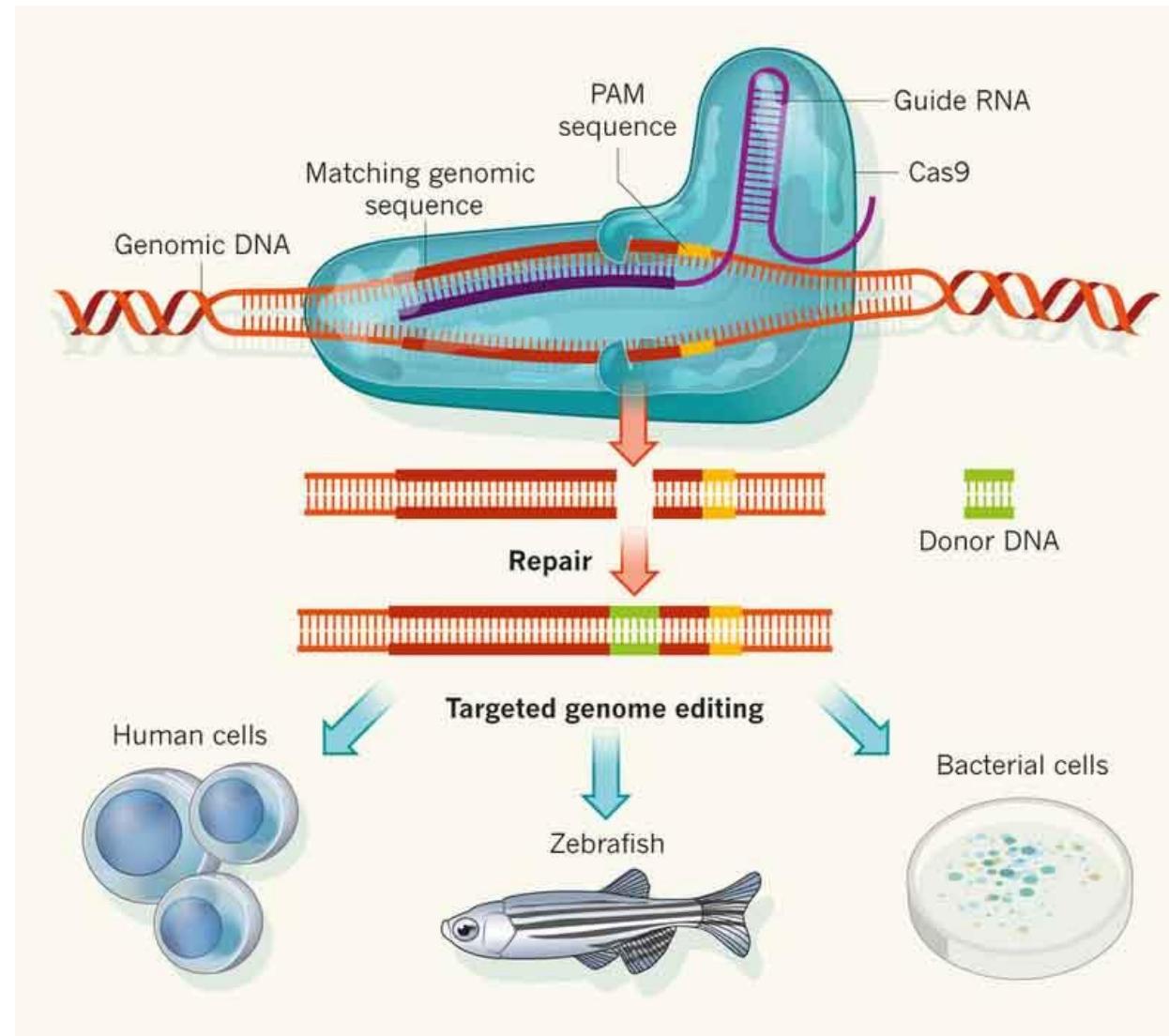
Tip of the Iceberg



Inspired?



CRISPR/Cas9 – guide RNA



Perturb- Seq

Resource

Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens

Atsly Dab¹,² Oran Parnas¹,² Danielle Lyon¹,² Jenny Burns¹,² Charles P. Fuoco¹,² Linat Jerry-Abman¹,² Nemanja D. Matavuljan¹,² Daniele Bini¹,² Yerik Turks¹,² Rakhima Puchaychov¹,² Britt Adams¹,² Thomas M. Fornace¹,² Daniel J. Gitter¹,² Michael S. Lichtenstein¹,² Nir Friedman¹,² and Avi Regev^{1,2,3,4,5,6*}
¹Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139, USA
²Harvard Medical School, Boston, MA 02115, USA
³Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA
⁴Department of Cellular and Molecular Pharmacology, California Institute of Quantitative Biosciences, Center for RNA Systems Biology, University of California San Francisco, San Francisco, CA 94158, USA
⁵Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA
⁶Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02140, USA
Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA
The Weizmann Institute of Science, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
*Correspondence: regev@mit.edu
†Co-first author
‡The Lauder Center for General and Tumor Immunology, the Biomedical Research Institute Israel Canada of the Faculty of Medicine (IMRIC), The Hebrew University Hadassah Medical School, 91120 Jerusalem, Israel
*Lead Contact
<http://doi.org/10.1152/jcb.00115.2018>

第1章 第2节

Genetic screens help infer gene function in mammalian cells, but it has remained difficult to assay complex phenotypes—such as transcription profiles. Here, we develop Perturb-seq, combining single-cell sequencing with perturbation analysis to rapidly interrogate short-palindrome targets (CRISPR)-based perturbations to perform many assays in a pool. We demonstrate Perturb-seq by analyzing 200,000 cells in immune cells from three individuals. Perturb-seq identifies genes regulating the response of dendritic cells to lipopolysaccharide (LPS). Perturb-seq accurately identifies individual gene targets, gene signatures, and cell states affected by individual perturbations, and can predict gene expression changes. We now have a framework for regulators of differentiation, the anti-viral response, and mitochondrial function during immune activation. By decomposing many high-confidence interactions, we can learn more about their interactions, and diverse cell metastasis. Perturb-seq dramatically increases the scope of pooled genomic assays.

INTRODUCTION

Genetic screens systematically analyze gene function in mammalian cells. Such screens are designed in either: (1) an individual ("arrayed") format, where each perturbation

F

Resources

A Multiplexed Single-Cell CRISPR Screening Platform Enables Systematic Dissection of the Unfolded Protein Response

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SUMMARY
Functional genomics efforts face tradeoffs between number of perturbations examined and complexity of phenotypes measured. We bridge this gap with Perturb-seq, which combines droplet-based single-

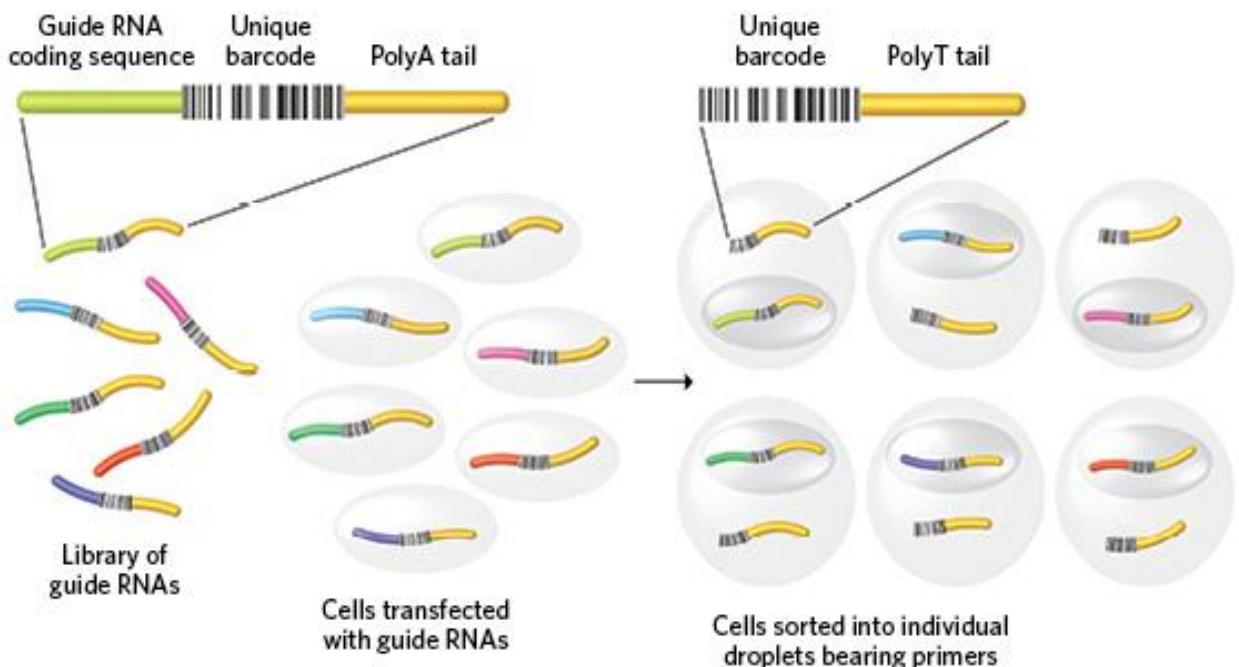
INTRODUCTION
Advances in pooled screening have made it possible to read evaluate mammalian gene function at genome-scale but date have relied on simple phenotypic readouts that average properties of a population, such as the expression of a few exons.

However, the CRISPR-based screening approach has serious requirements or disadvantages. These approaches often require a large number of samples to detect a single gene's response or when a bulk phenotype is driven by a subpopulation. These limitations underscore the need for high-resolution, single-cell screens at the level of hundreds of genes simultaneously. To address this challenge, we developed a platform for single-cell functional genomics, we named the technology with our platform for CRISPR-based phenotypic interference (CRISPRB), which mediates gene inactivation via high-throughput, multiplexed CRISPR-mediated gene targeting. We developed a novel cell barcode strategy that encodes the identity of the CRISPR-mediated perturbation in an expressed transcript, which is captured during single-cell RNA-seq analysis. This platform allows us to screen thousands of genes simultaneously, as well as separate barcode from RNA-seq data, screening with rich phenotypic output from single cells. Moreover, we developed a novel analytical pipeline to parse data.

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Single Cell Gene Expression + CRISPR Perturbations

Massively Parallel Functional Genomics



"Massively Parallel Perturbations." The Scientist. Mar. 2007

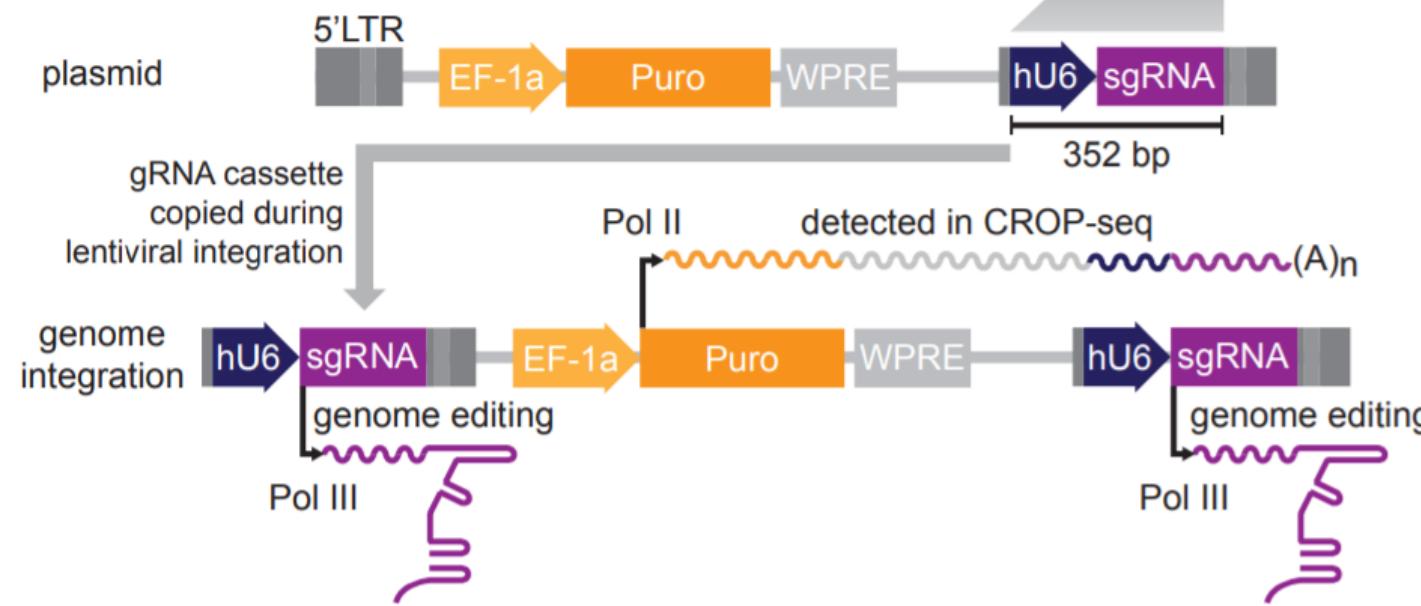
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CROP seq – even easier

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Article

Pooled CRISPR screening with single-cell transcriptome readout

Paul Datlinger, André F Rendeiro, Christian Schmidl, Thomas Krausgruber, Peter Traxler, Johanna Klughammer, Linda C Schuster, Amelie Kuchler, Donat Alpar & Christoph Bock

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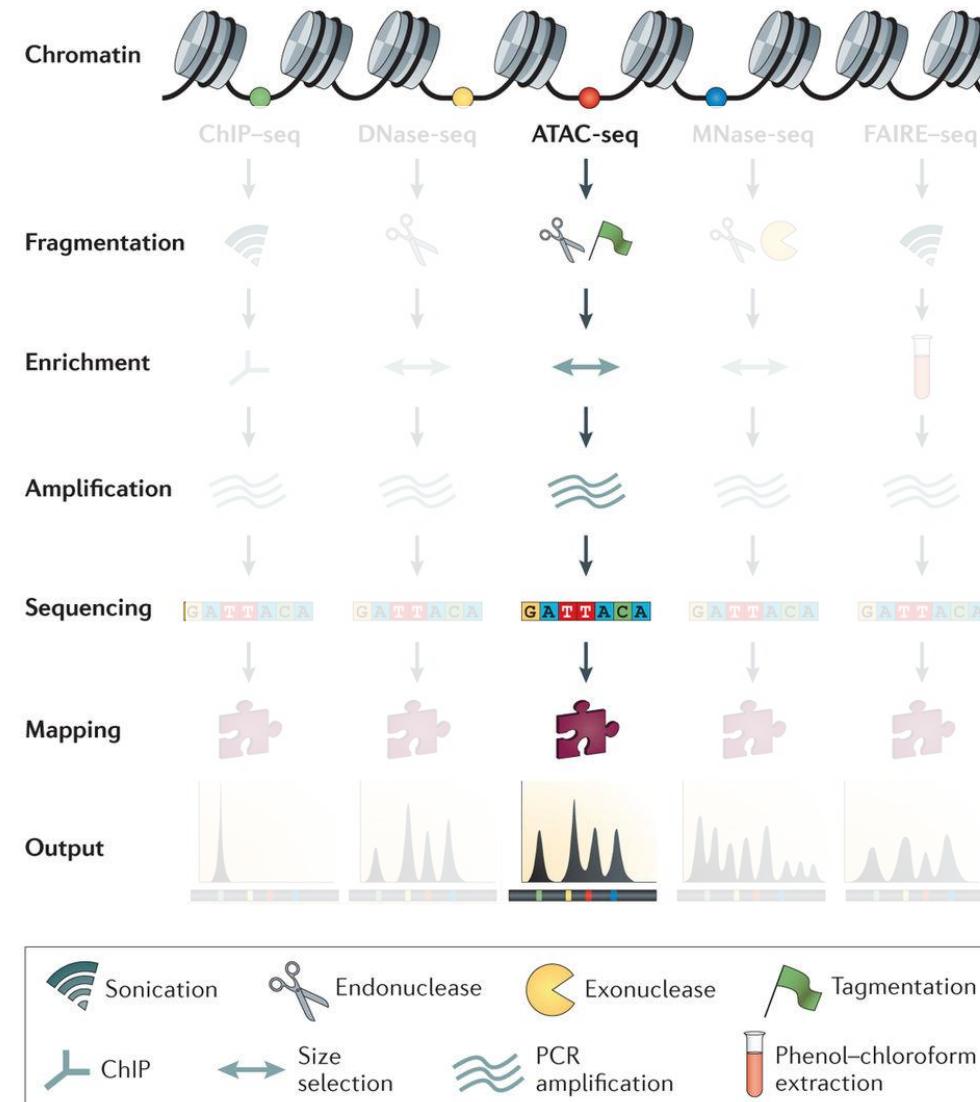
Accepted: 10 January 2017

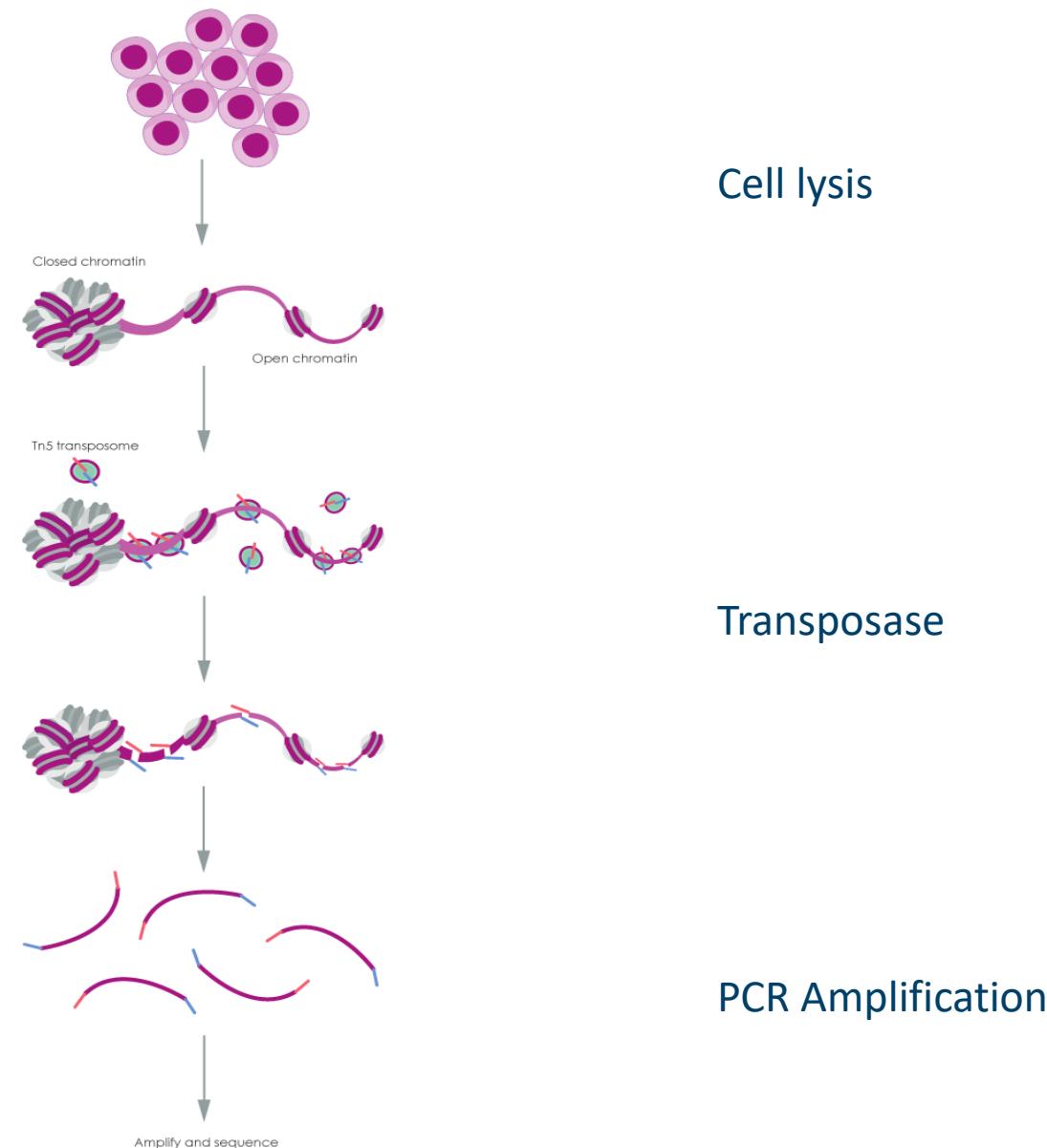
Published: 18 January 2017



Single Cell
ATAC-seq

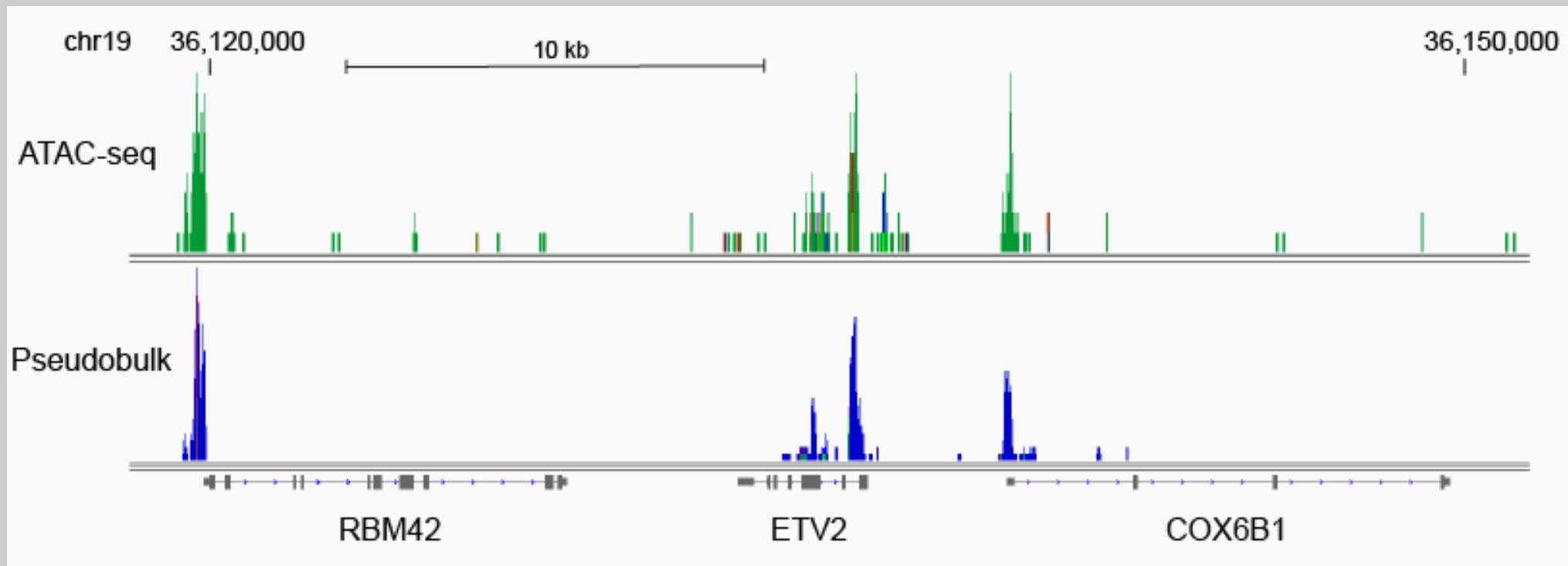
Epigenetic Assays



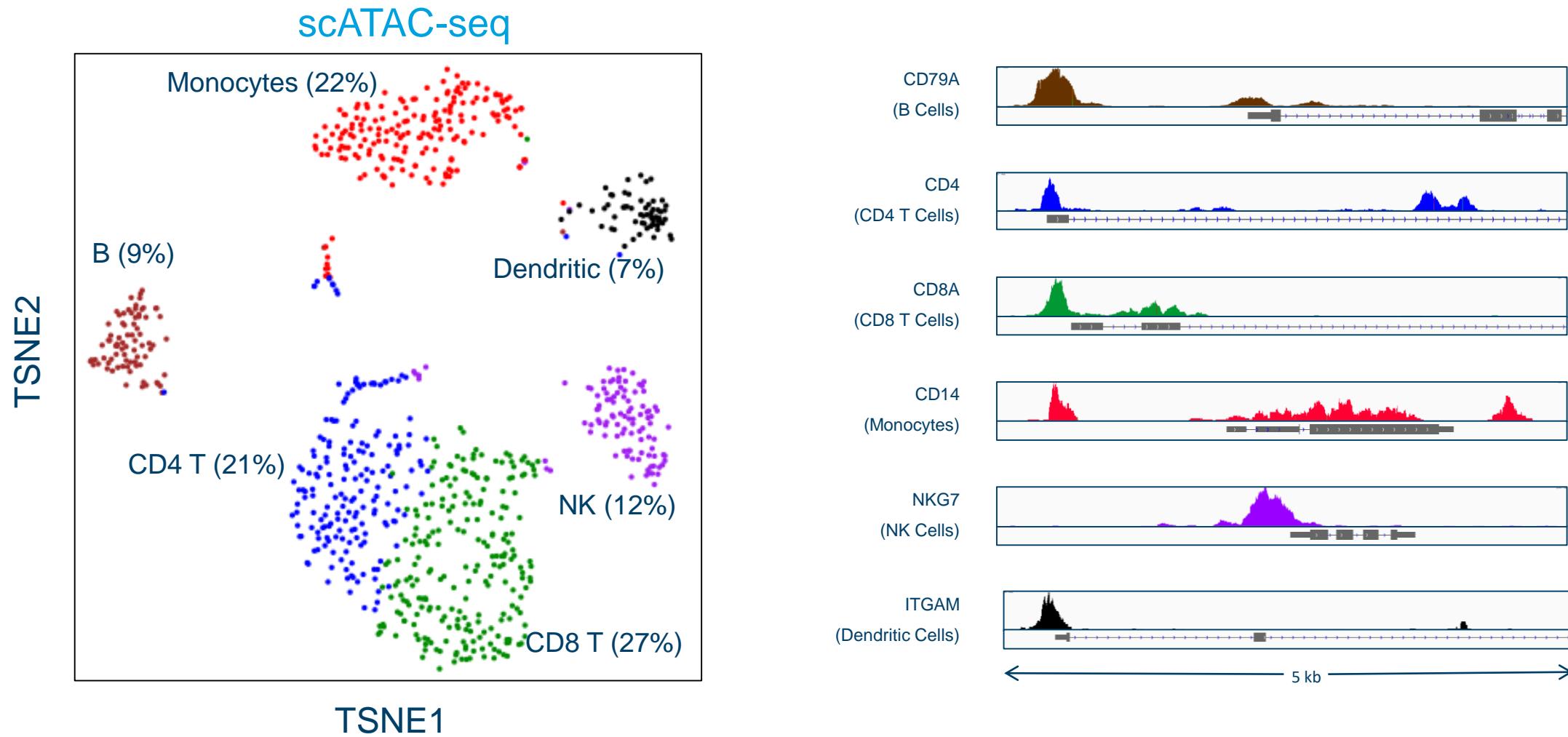


Single Cell ATAC-seq

Genomic Coverage Profile



Single Cell ATAC-seq on 1000 PBMCs



Thank you!

Questions?





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