



# INTRODUCTION TO SINGLE CELL RNA-SEQ

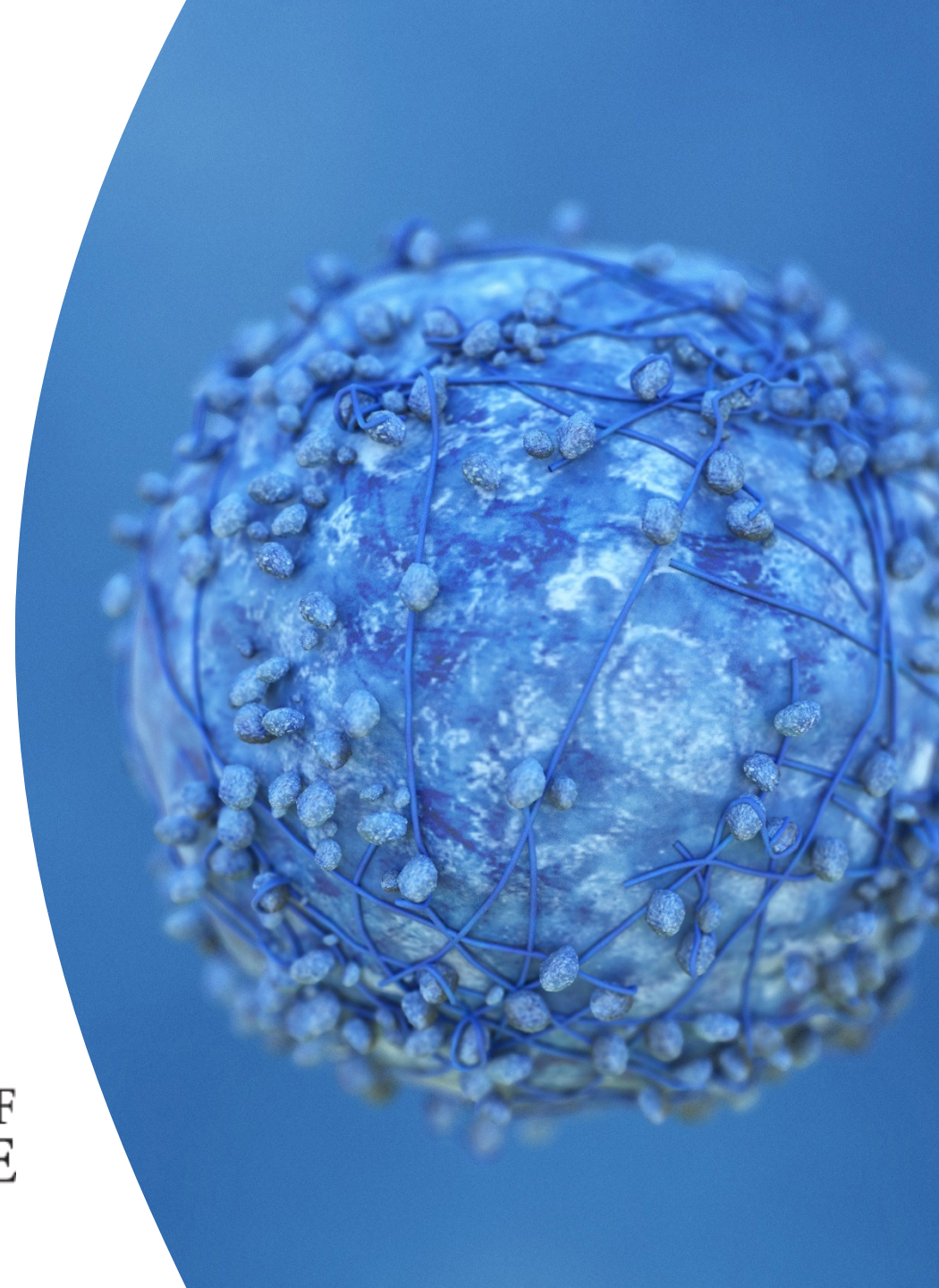
Analysis of single cell RNA-seq data

Katarzyna Kania

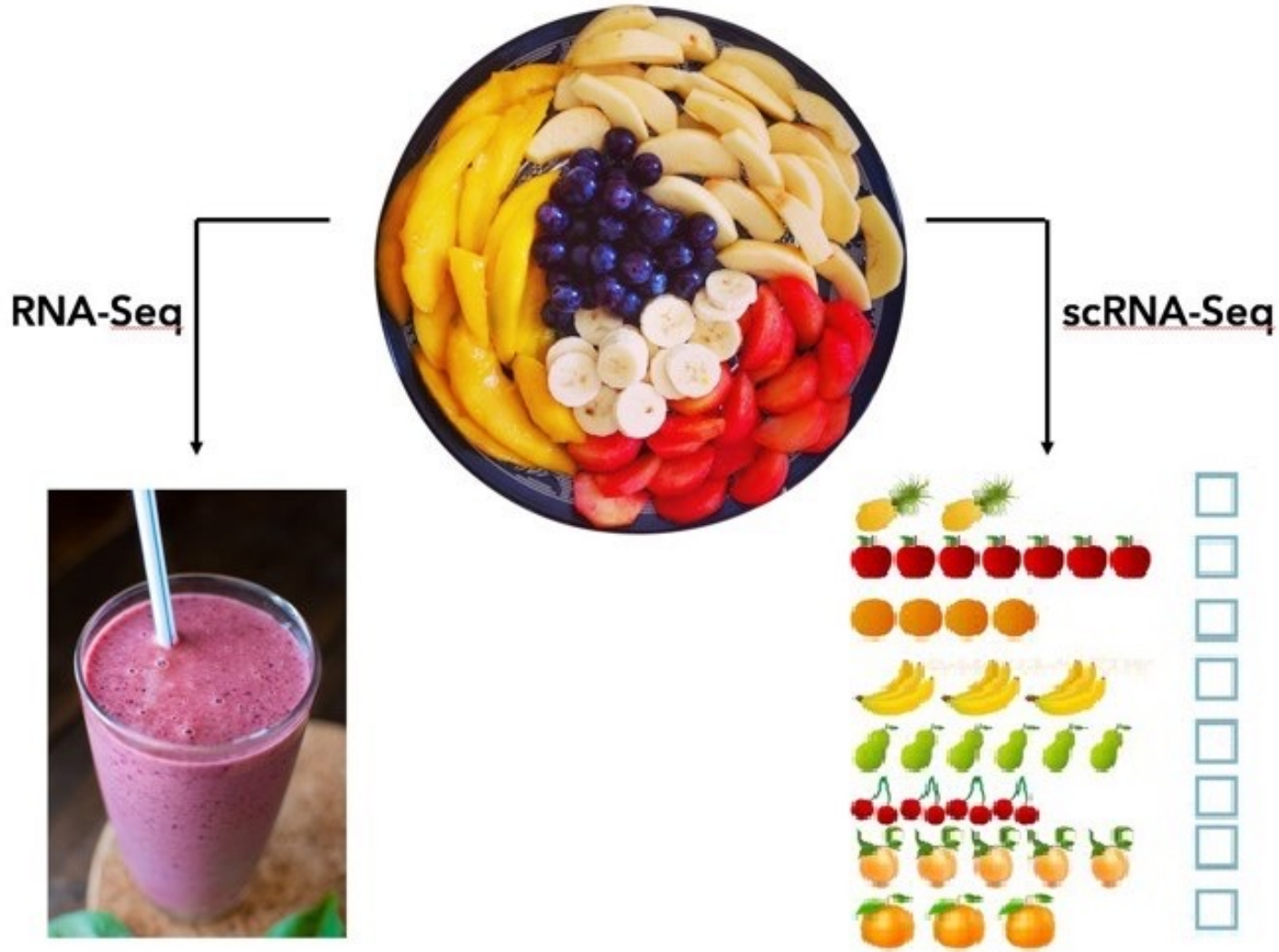
18<sup>th</sup> May 2023



CAMBRIDGE  
INSTITUTE



# BULK VS SINGLE CELL RNA-SEQ



RNA-Seq

scRNA-Seq

Separate populations

- Define heterogeneity
- Identify rare cell populations
- Cell population dynamics

Average expression level

- Comparative transcriptomics
- Disease biomarker
- Homogenous systems

# BULK VS SINGLE CELL RNA-SEQ

## 1. mRNA: TruSeq RNA-Seq (Gold Standard)

- ~20,000 transcripts
  - More when consider splice variants / isoforms
- Observe 80-95% of transcripts depending on sequencing depth

## 2. Low input methods ~3000 cells / well

- 4000-6000 transcripts per sample
  - Limiting to transcripts observed across all samples
- Observe 20-60% of the transcriptome

## 3. Single Cell Methods

- 200 -10,000 transcripts per cell
- Observe 10-50% of the transcriptome
- Many transcripts will show up with zero counts in every cell. (even GAPDH)
- If you only looked at transcripts observed in all cells numbers drop dramatically.

# BULK VS SINGLE CELL RNA-SEQ

	Deep RNA-seq	Sort-seq	Low input	scRNA-seq
Transcriptome Coverage	High	High	Moderate	Low
Throughput	Moderate	Low	High	Low
Cell Subtype Information	None	Moderate	None	High
Sequencing Depth	Moderate	Moderate	Low	High
Cost per Sample	Moderate	Moderate	Low	High

## Disadvantages of scRNA-seq

- Dropouts and noisy data
- Lowly expressed genes might be undetected
- Samples will contain doublets
- Replicates without batch effect are unlikely
- Expensive

Source: Sarah Boswell, Harvard Medical School, September 2020

# APPLICATIONS

## naturemedicine

Letter | Published: 08 June 2020

### A single-cell atlas of the peripheral immune response in patients with severe COVID-19

Aaron J. Wilk, Arjun Rustagi, Nancy Q. Zhao, Jonasel Roque, Giovanni J. Martinez-Colón, Julia L. McKechnie, Geoffrey T. Ivison, Thanmayi Ranganath, Rosemary Vergara,

## LETTER

<https://doi.org/10.1038/s41586-018-0394-6>

### A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte

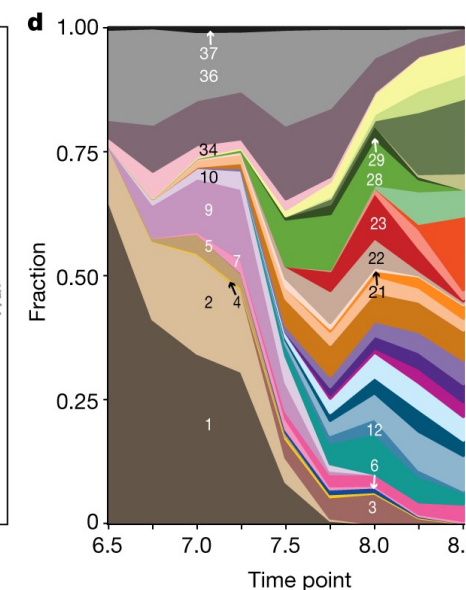
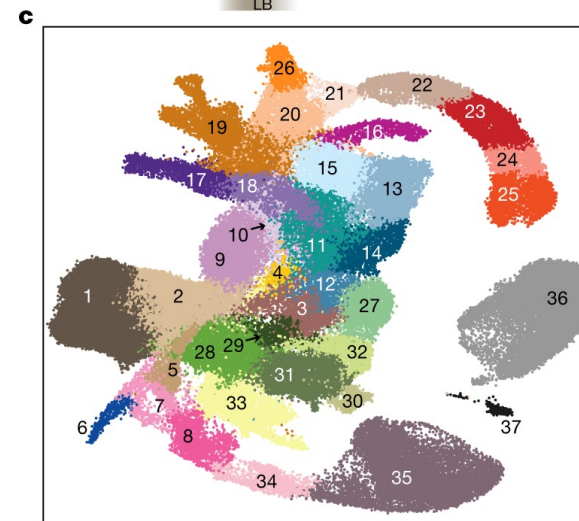
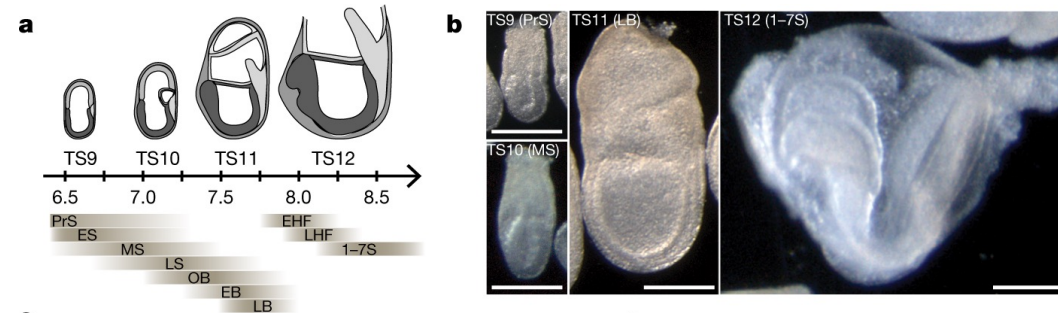
Lindsey W. Plasschaert<sup>1,3,7</sup>, Rapolas Zilionis<sup>2,3,7</sup>, Rayman Choo-Wing<sup>1,5</sup>, Virginia Savova<sup>2,6</sup>, Judith Knehr<sup>4</sup>, Guglielmo Romaz<sup>4</sup>, Allon M. Klein<sup>2\*</sup> & Aron B. Jaffe<sup>1,3\*</sup>

## nature

Article | Published: 20 February 2019

### A single-cell molecular map of mouse gastrulation and early organogenesis

Blanca Pijuan-Sala, Jonathan A. Griffiths, Carolina Guibentif, Tom W. Hiscock, Wajid Jawaid, Fernando J. Calero-Nieto, Carla Mulas, Ximena Ibarra-Soria, Richard C. V.



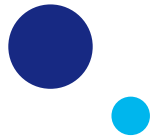
- 1 Epiblast
- 2 Primitive streak
- 3 Caudal epiblast
- 4 Primordial germ cells
- 5 Anterior primitive streak
- 6 Notochord
- 7 Def. endoderm
- 8 Gut
- 9 Nascent mesoderm
- 10 Mixed mesoderm
- 11 Intermediate mesoderm
- 12 Caudal mesoderm
- 13 Paraxial mesoderm
- 14 Somitic mesoderm
- 15 Pharyngeal mesoderm
- 16 Cardiomyocytes
- 17 Allantois
- 18 ExE mesoderm
- 19 Mesenchyme
- 20 Haemato-endothelial prog.
- 21 Blood progenitors 1
- 22 Blood progenitors 2
- 23 Erythroid 1
- 24 Erythroid 2
- 25 Erythroid 3
- 26 Endothelium
- 27 Neuromesodermal progenitors
- 28 Rostral neuroectoderm
- 29 Caudal neuroectoderm
- 30 Neural crest
- 31 Forebrain/midbrain/hindbrain
- 32 Spinal cord
- 33 Surface ectoderm
- 34 Visceral endoderm
- 35 ExE endoderm
- 36 ExE ectoderm
- 37 Parietal endoderm

Source: Pijuan-Sala et al. Nature 566, 490–495 (2019)



CANCER  
RESEARCH  
UK

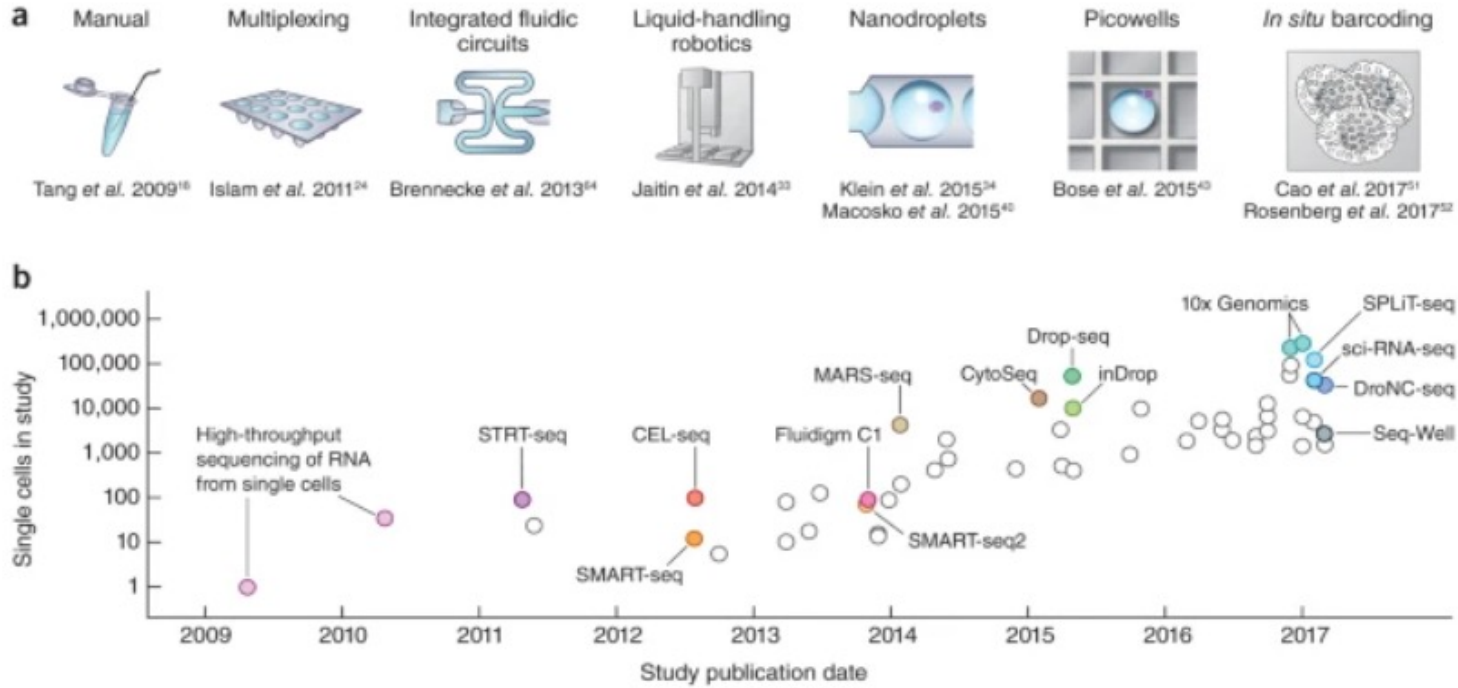
CAMBRIDGE  
INSTITUTE



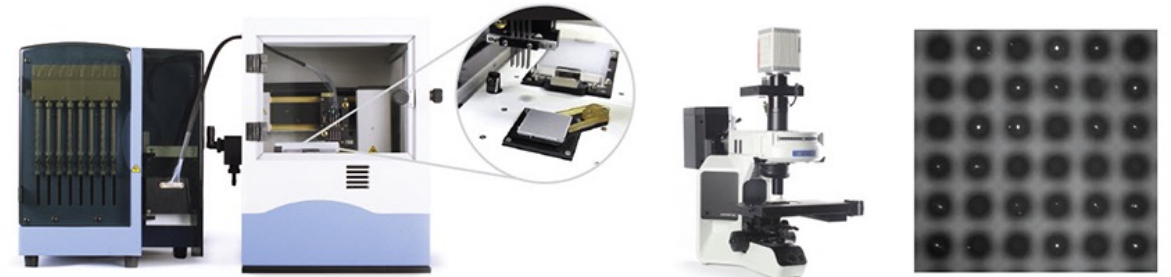
# TECHNOLOGIES



Figure 1: Scaling of scRNA-seq experiments.



Source: Svensson et al. *Nat Protoc* 13, 599–604 (2018)

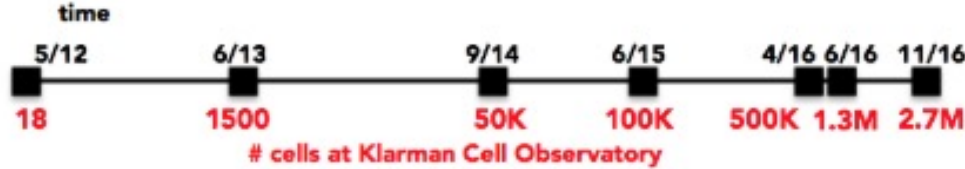


# HISTORY AND PROGRESS

## LETTER

Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells

2013, 18 cells



## ARTICLE

Single-cell RNA-seq reveals dynamic paracrine control of cellular variation

2014, 1700 cells



Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

2015, 45,000 cells

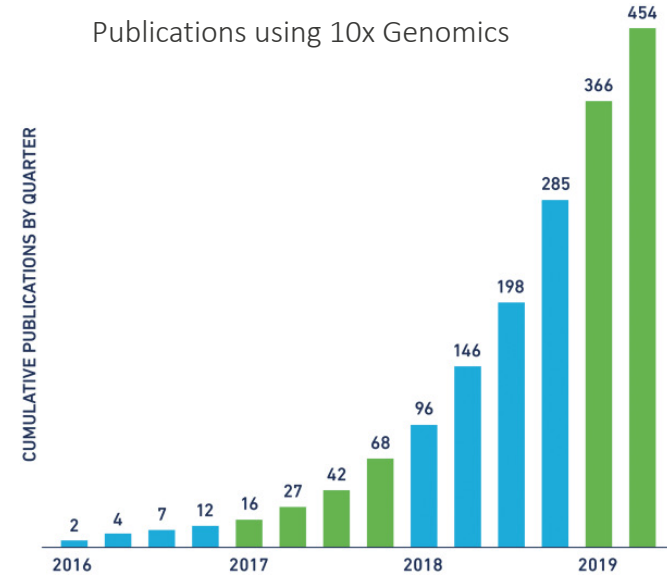


Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens

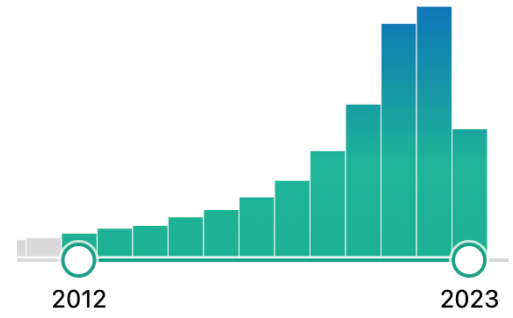
2016, 200,000 cells

2017, 1.3 million cells (10X genomics)

Publications using 10x Genomics



PubMed search for 'scRNA-seq'



Source: Introduction to scRNASeq, Timothy Tickle & Brian Haas, Broad Institute, 2017



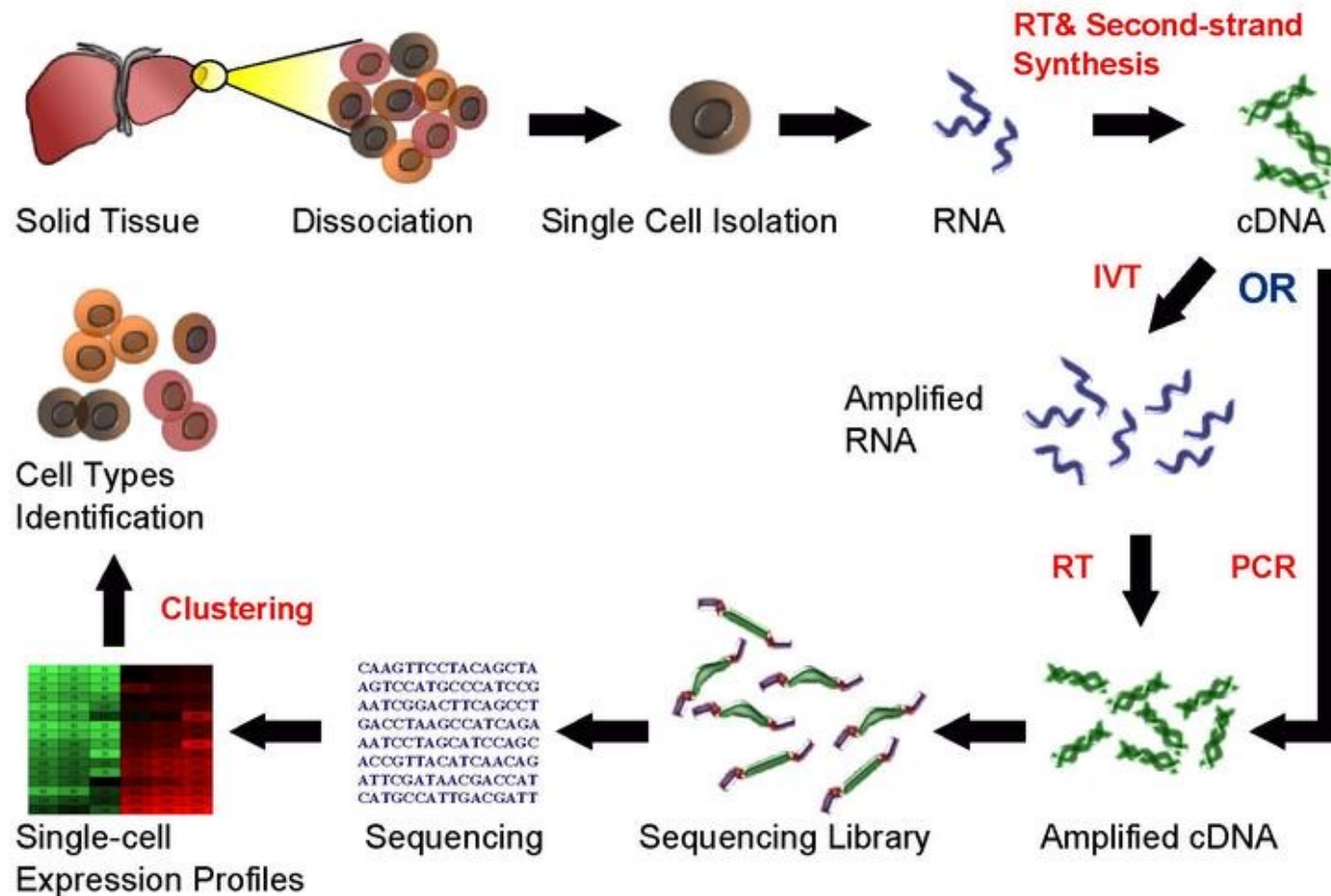
CAMBRIDGE INSTITUTE

# WORKFLOW

## Single Cell RNA Sequencing Workflow

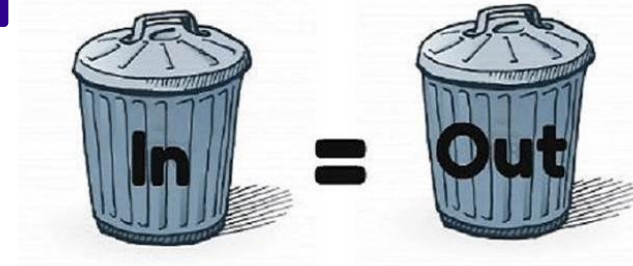


Good sample preparation is key to success!





# SAMPLE PREPARATION



- Understand well the nature of the sample (sampling conditions, preparation, purity)
- Identify the source of technical difficulties in order to resolve them first
- Practice your sample preparation, optimise the protocol well, do not rush to the final experiment
- A well planned pilot experiment is essential for evaluating sample preparation and for understanding the required number of cells.
- You need your cells to be highly viable (>90-95%), have no clumps and no debris. Cell membrane integrity is a must!
- Free-floating RNA will make analysis more challenging
- Be cautious about FACS (especially with more fragile cells). If FACS necessary for enrichment, remember that time is crucial factor
- Count with haemocytometer or cell counter (Countess II Automated Cell Counter) – do not trust sorter counts
- Fixation and cryopreservation are not compatible with many techniques



# METHODS

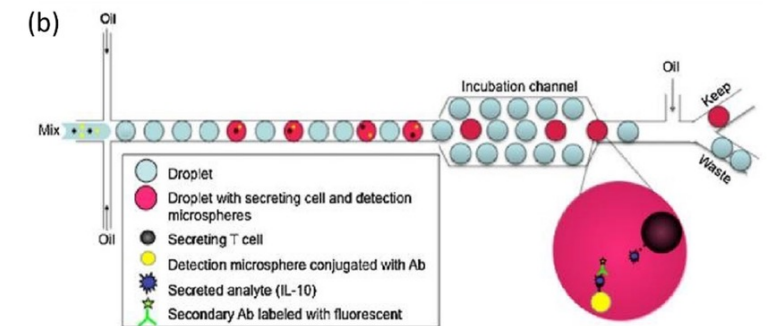
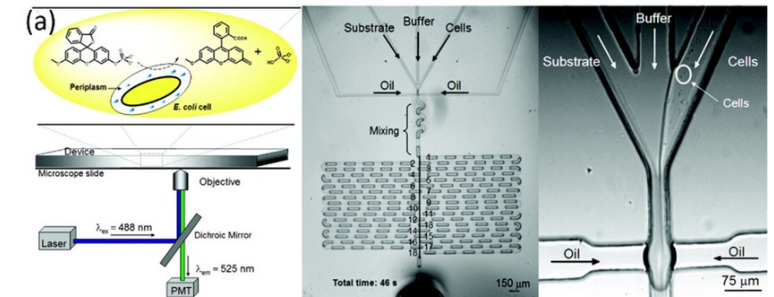
- 1) Cells in wells, traps and valves (nanowell, Flow sorting, CellenOne, Fluidigm C1, SmartSeq, plexWell/seqWell)
  - Screen for and retrieve single cells of interest
  - Enrich for rare cells with decided properties
  - Control the cellular microenvironment
  - Monitor and control cell-cell interactions
  - Precise/extensive manipulation of single cells
  
- 2) Droplets (Drop-seq, 10x Genomics)
  - Introduce distinct 'packets' of reagents to single cell (e.g. barcodes)
  - Perform amplification on individual cells
  - Sort large population of single cells
  
- 3) Combinatorial indexing (SCI-seq, SPLiT-seq)
  - Economic use of reagents for cell separation
  - Efficiency of handling larger population than Drop-seq
  - Maintain complexities of population without bias from droplet or well



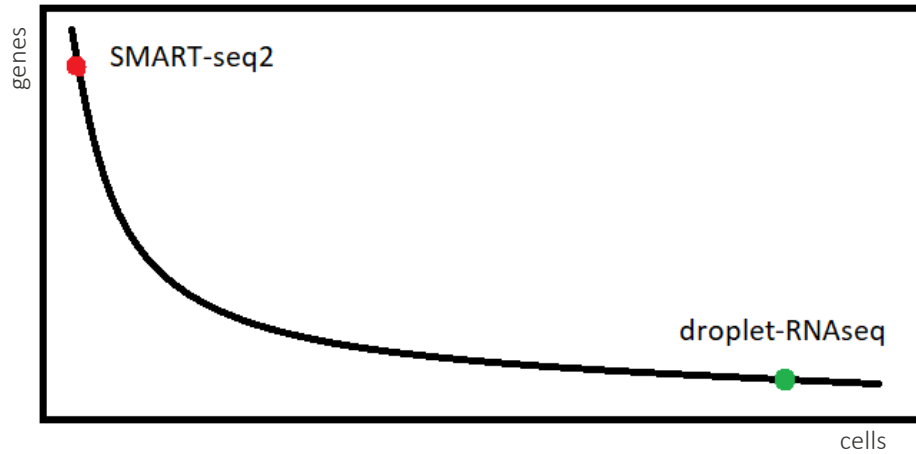
Passive wells



Active pumps and valves



# MORE CELLS OR MORE GENES?

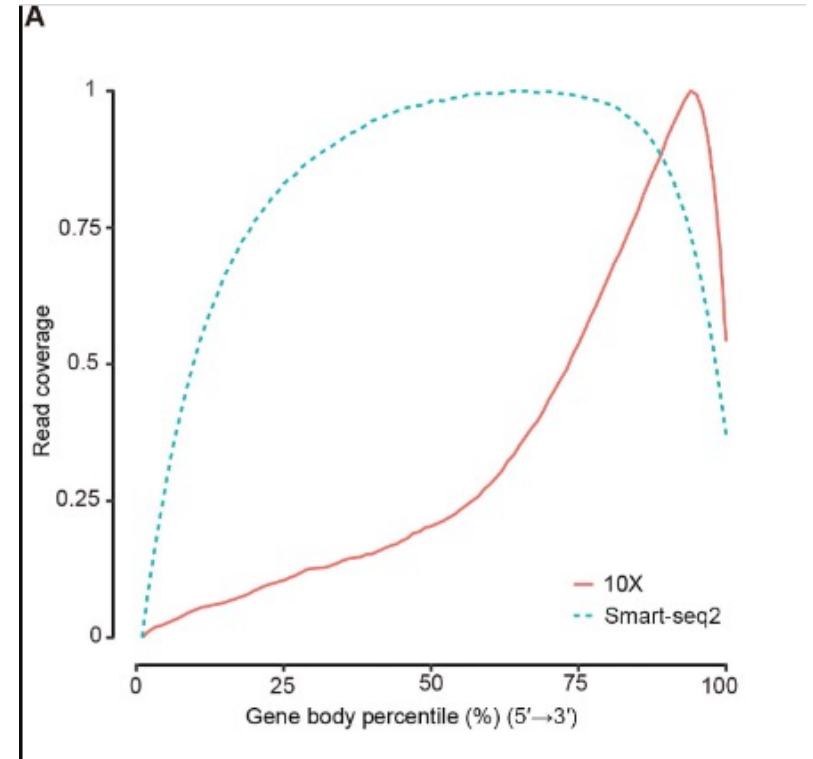


## SMART-seq2

- 100 cells
- Full-length libraries
- 1M reads per cell

## Droplet-RNAseq

- 10000 cells
- 50k reads per cell
- 3'/5' bias



Source: Wang, et al. Genom. Proteom. Bioinform. 19(2), 253-266 (2021).

- Required number of cells increases with complexity of the sample.
- Number of reads will depend on biology of sample
- Cell-type classification of a mixed population usually requires lower read depth
- You can always re-sequence your samples.



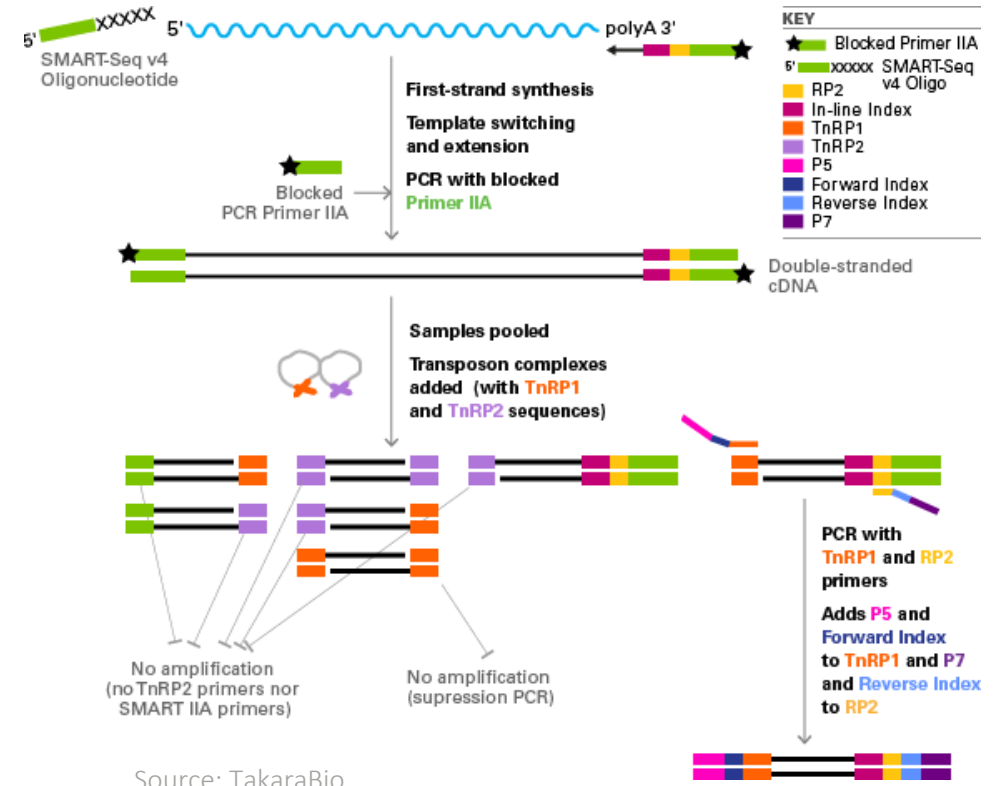
CANCER  
RESEARCH  
UK

CAMBRIDGE  
INSTITUTE

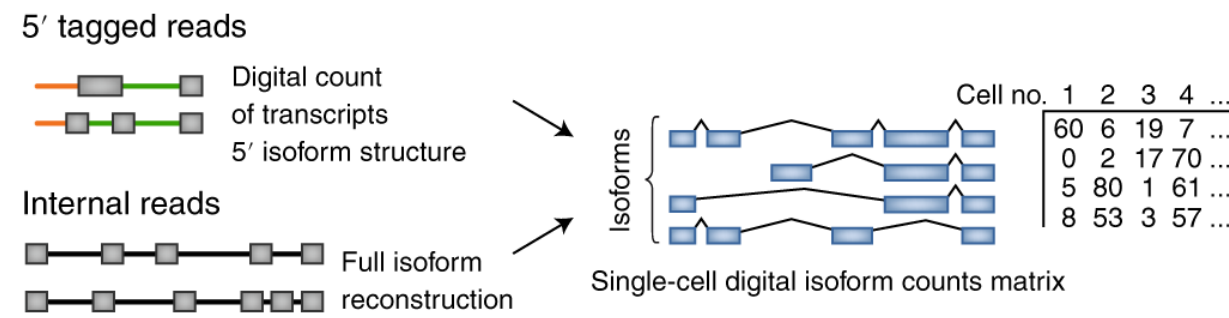
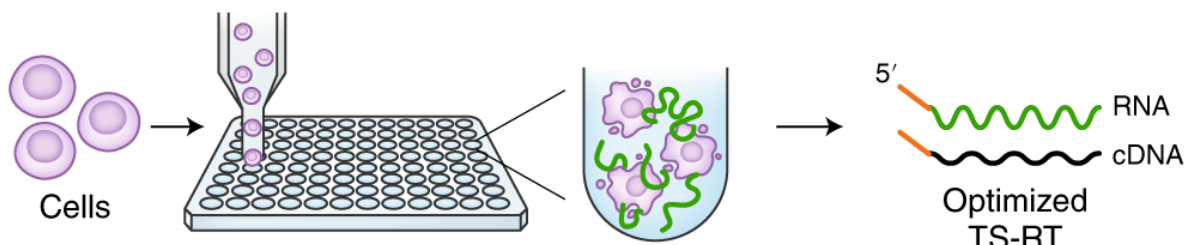
# SMART-SEQ2/3/4 OVERVIEW

Developed for single cell but can be performed using total RNA.

- Selects for poly-A tail.
- Full transcript assay.
- Uses template switching for 5' end capture.
- Standard Illumina sequencing.
- Plate-based solution so labour intensive, slow and costly (~\$12/cell)

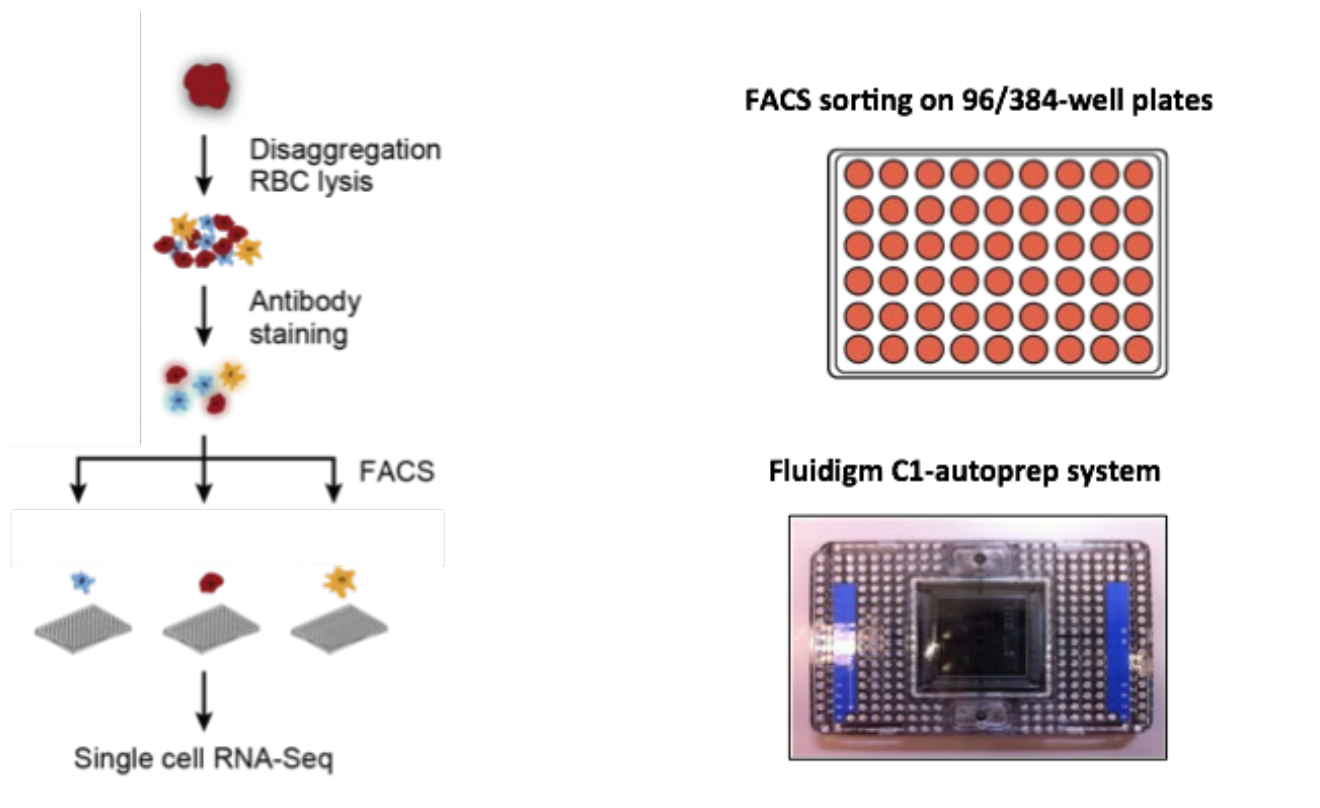


a

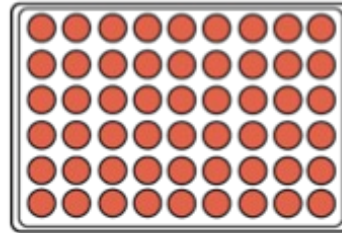


Source: Macosko, Nat Biotechnol 38 (2020).

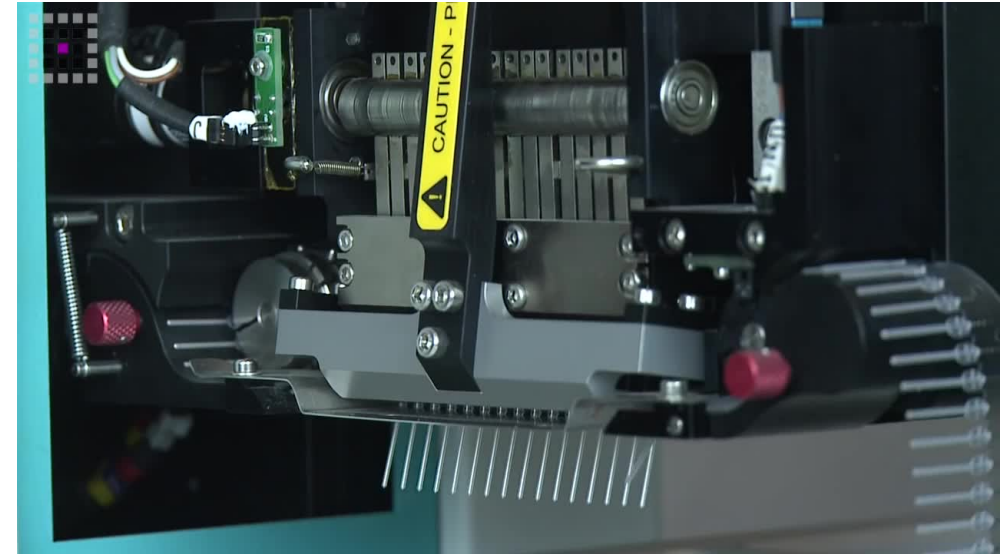
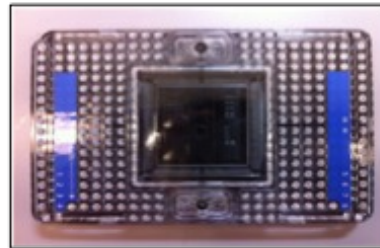
# SMART-SEQ2/3/4 + MOSQUITO LV



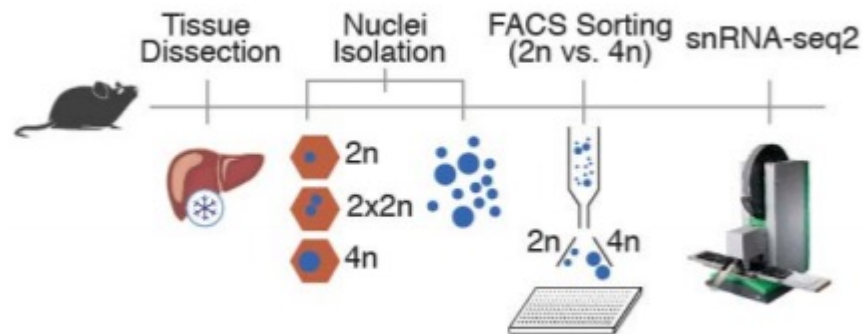
FACS sorting on 96/384-well plates



Fluidigm C1-autoprep system



Source: SPT Labtech

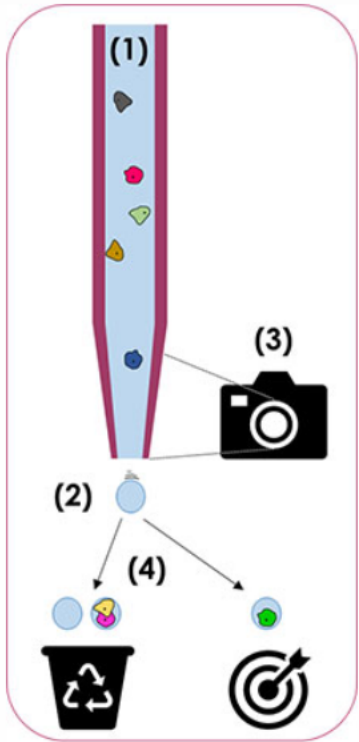


Richter et al. Nat Commun 12, 4264 (2021)

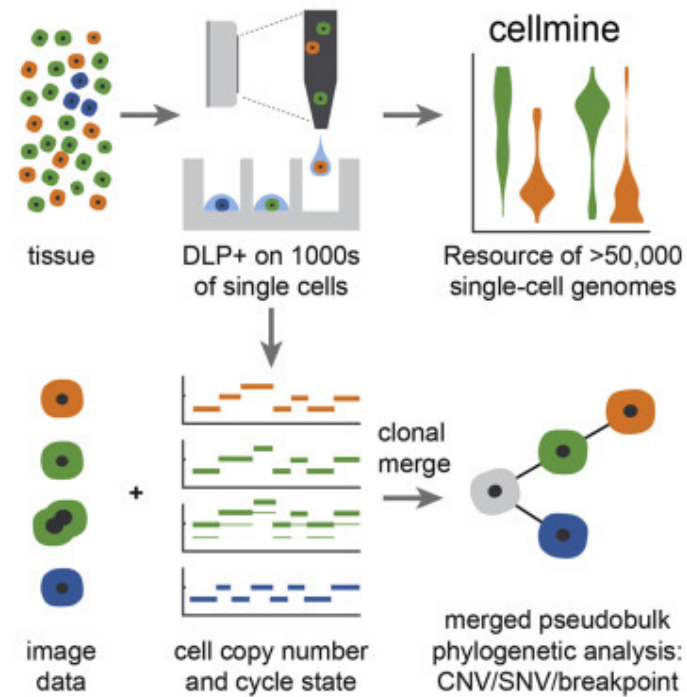
- Mosquito LV makes assay miniaturisation simple, leading to significant savings on precious reagents and time.
- Mosquito LV offers highly accurate and precise multichannel pipetting from 25 nL to 1.2  $\mu$ L.
- SmartSeq2 cost reduced from \$12 to \$4 per cell

# CELLENONE

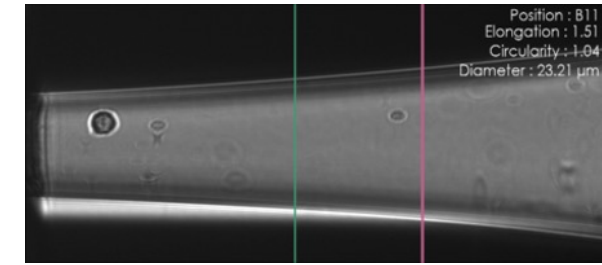
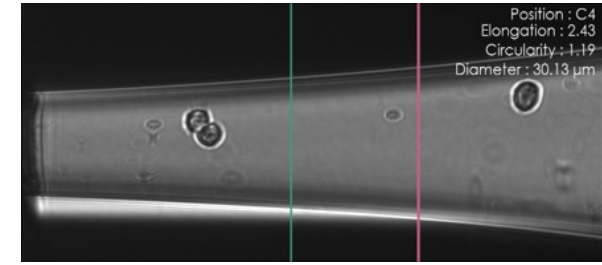
- 1) Cell suspension is aspirated into a glass capillary
- 2) Generation of drops on demand, in air
- 3) Thanks to automated imaging, cellenONE tracks cells and determines if upcoming drops will contain or not a single cell
- 4) Drops containing single cells are dispensed into selected targets, drops without cells or with more than one cells are dispensed into recycling tube



Source: Cellenion



Source: Laks et al. *Cell*. 179(5):1207-1221.e22. (2019)

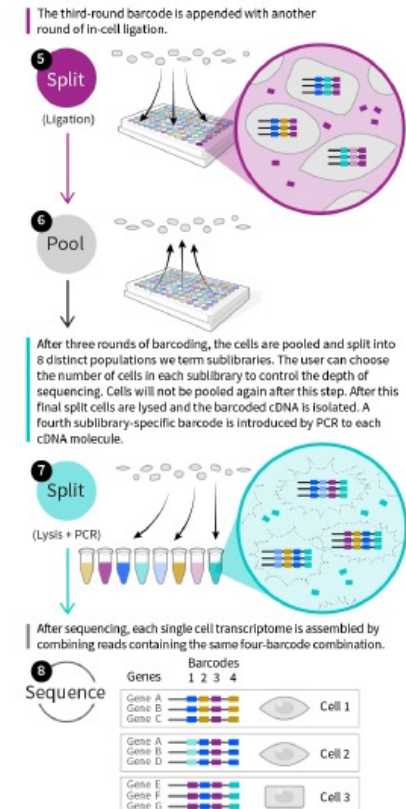
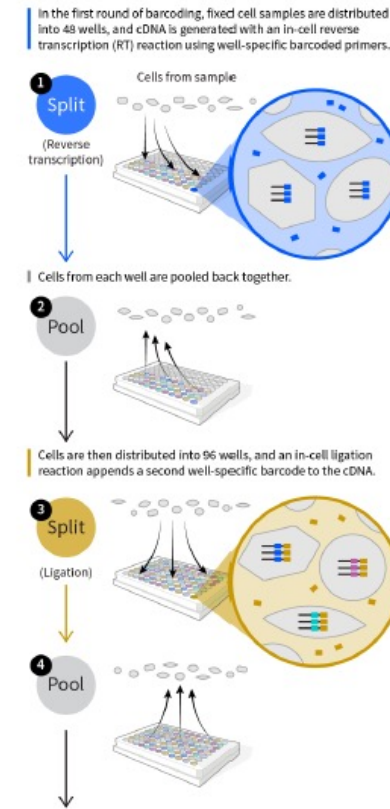
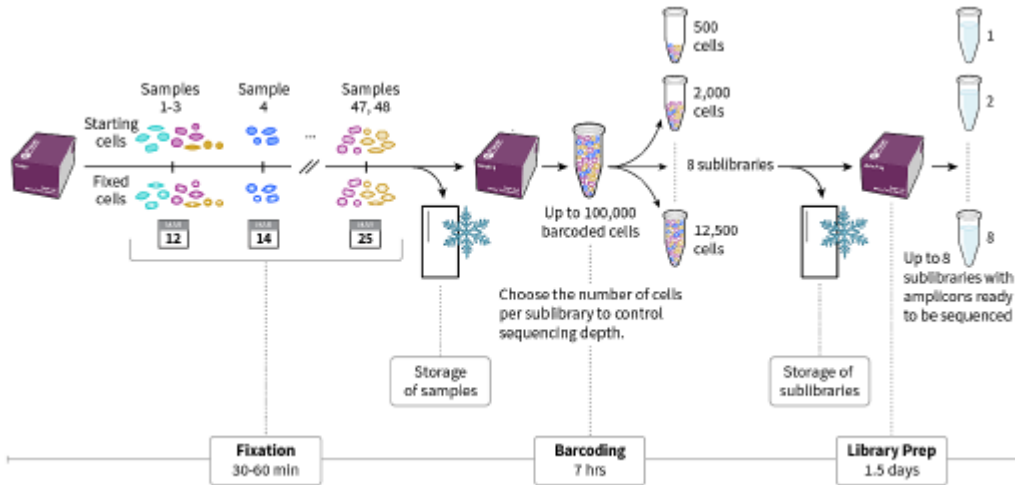


Source: Cellenion

# SPLIT-SEQ OVERVIEW (PARSE BIOSCIENCES, SCALE BIO)



Video available at: <https://www.youtube.com/watch?v=WqaeZe7mKUc>

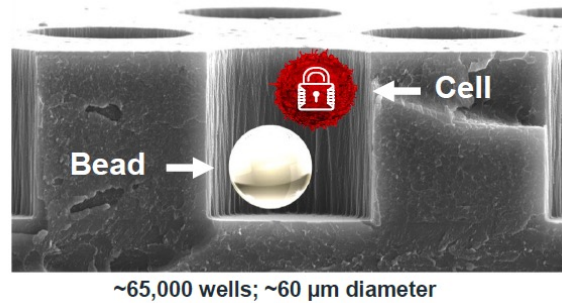


- Time flexibility – single experiment for samples collected on different dates (up to 6 months storage)
- No instrument required for experiment. Computational pipeline available
- Up to 48 samples / 100k cells in total – kit has to be used at once
- Retail price of \$9,800 per 100k cells or \$16,700 per million cells (+fixation kits)
- Doublet rate of 0.27% per 1000 cells (3.4% per library)
- No 3'/5' bias – random hexamers method
- Median genes detection of about 12,000 genes
- Works with any species, any sizes of cells/nuclei & results in lower background noise

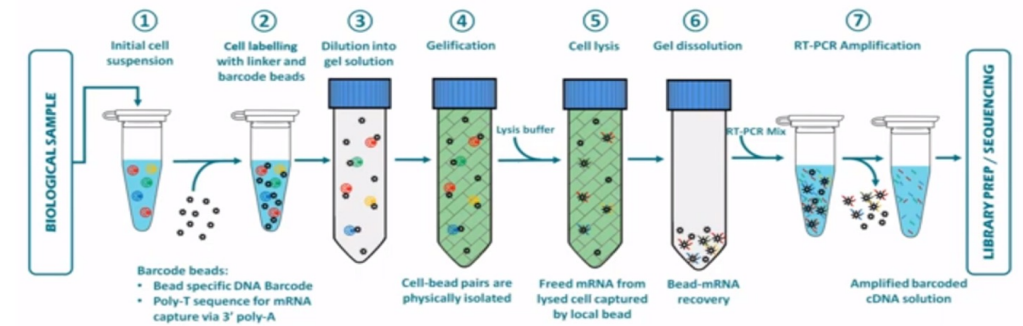
# NEW PRODUCTS (HIVE, SCIPIO, FLUENT, SINGLERON)

## Honeycomb HIVE

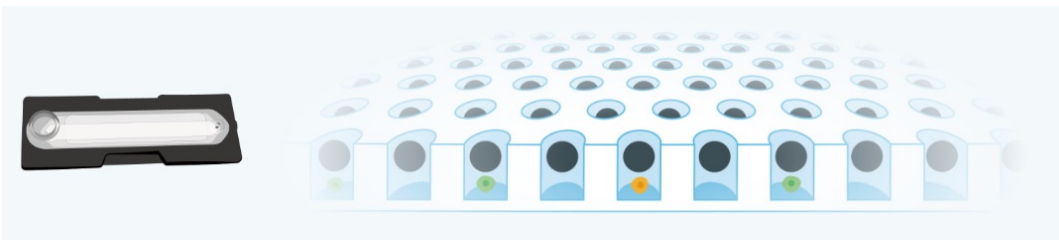
- Capture Cells - Load cells into the HIVE and allow single cells to settle gently into HIVE picowells containing barcoded mRNA-capture beads
- The HIVE Difference: Store Or Ship - With cells in a stable environment, store HIVES in the freezer and/or ship when ready to process



Asteria (SciPIO Bioscience) - hydrogel technology, a new era of instrument-free, ready-to-use scRNA-seq experiments



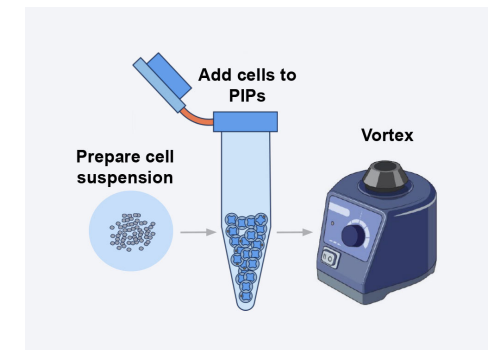
Singleron (SCOPE-chip) - captures single cells by partitioning single cells into hundreds of thousands of microwells on the chip and can analyse 500-30000 cells simultaneously



## Fluent BioSciences

- during sample preparation, cell suspension of interest is mixed with core template particles and segregated into Pre-templated Instant Partitions (PIPs) by simple vortexing

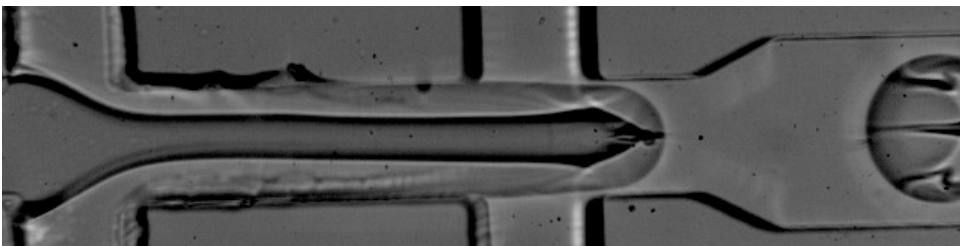
- Great scalability depending on experiment needs (2K cells= \$300, 20k cells=\$900)





# DROP-SEQ OVERVIEW

- Moved throughput from hundreds to thousands.
- Droplet-based processing using microfluidics
- Nanoliter scale aqueous drops in oil.
- 3' End
- Bead based (STAMPs).
- Single-cell transcriptomes attached to microparticles.
- Cell barcodes use split-pool synthesis.
- Uses UMI (Unique Molecular Identifier)
- Chance to have two cells within one droplet

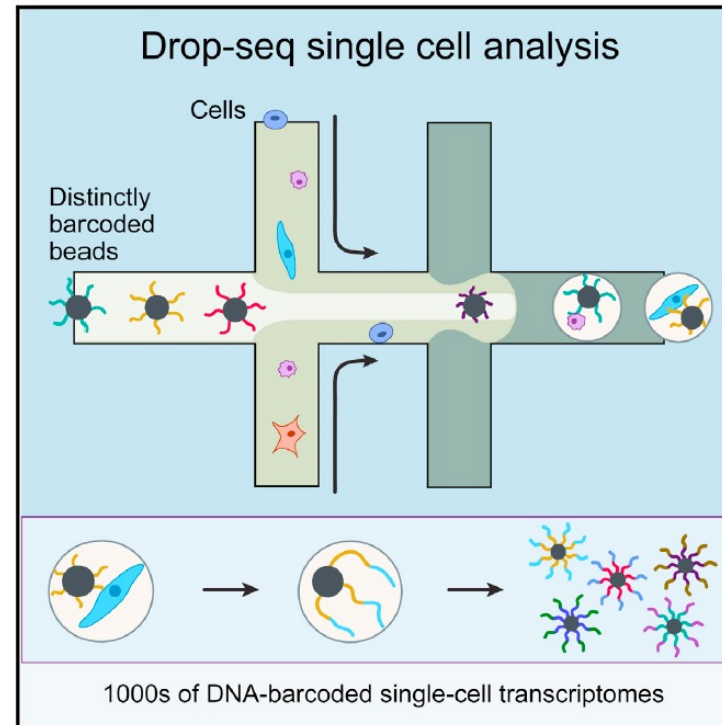


Resource

## Cell

### Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

#### Graphical Abstract



#### Authors

Evan Z. Macosko, Anindita Basu, ..., Aviv Regev, Steven A. McCarroll

#### Correspondence

emacosko@genetics.med.harvard.edu (E.Z.M.),  
mccarroll@genetics.med.harvard.edu (S.A.M.)

#### In Brief

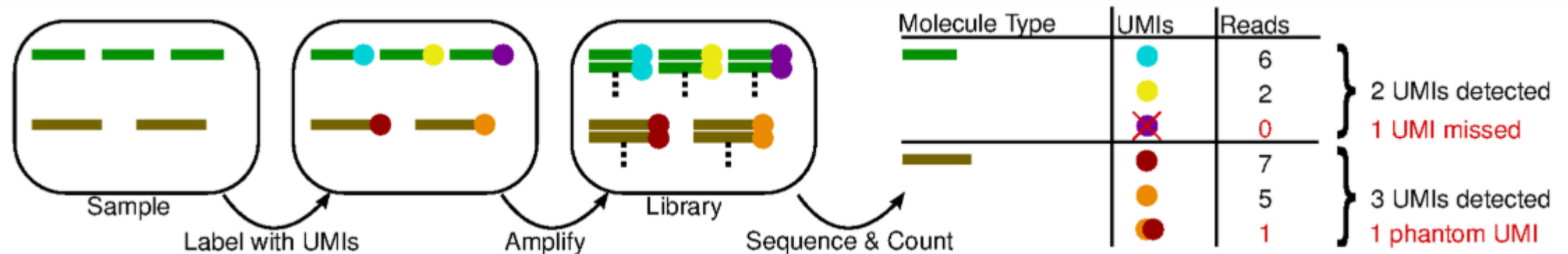
Capturing single cells along with sets of uniquely barcoded primer beads together in tiny droplets enables large-scale, highly parallel single-cell transcriptomics. Applying this analysis to cells in mouse retinal tissue revealed transcriptionally distinct cell populations along with molecular markers of each type.

# UMI – UNIQUE MOLECULAR IDENTIFIERS

After PCR enrichment, without UMIs, one can not distinguish if multiple copies of a fragment are caused by PCR clones or if they are real biological duplicated.

By using UMIs, PCR clones can be found by searching for non-unique fragment-UMI combinations, which can only be explained by PCR clones.

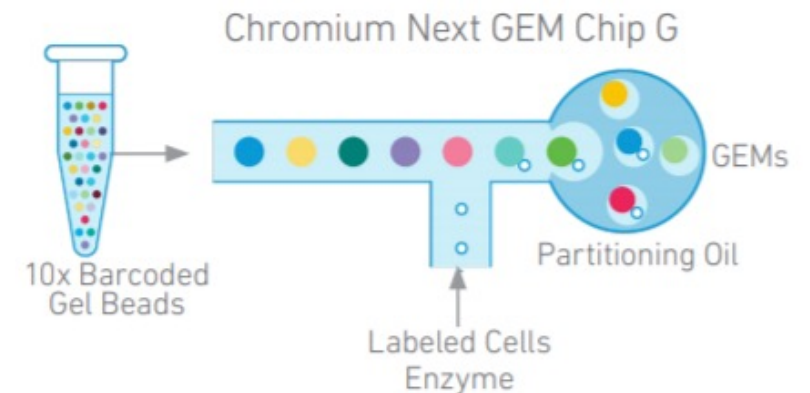
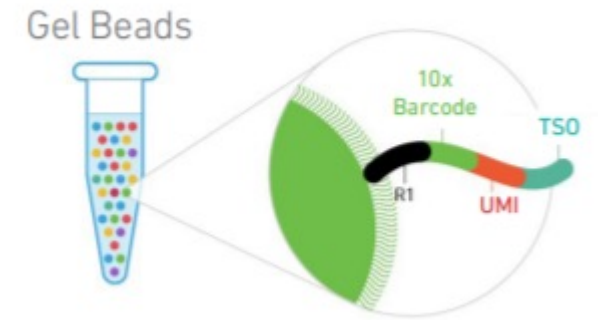
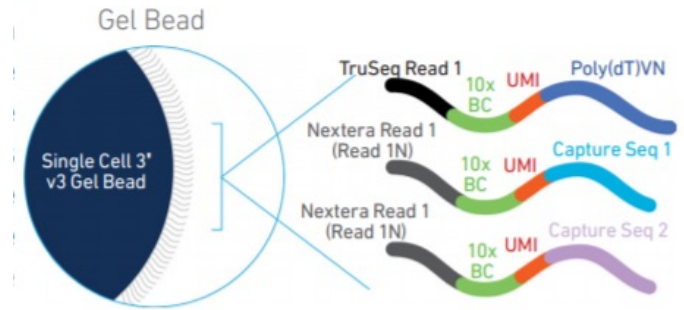
When performing variant analyses, these falsely overrepresented fragments can result in incorrect calls and thus wrong diagnostic findings



Source: Pflug et al. Bioinformatics (2018)

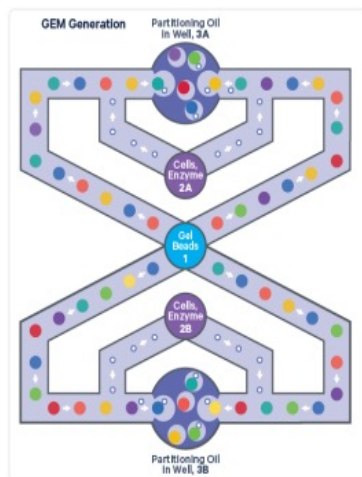
# 10X GENOMICS OVERVIEW

- Droplet-based similar to Drop-Seq, 3' or 5' mRNA
- In contrast to Drop-seq, where solid beads are used for RNA capture, 10X uses soft hydrogels containing oligos. These enable “single Poisson loading” leading to capture of >60% of input cells.
- Standardized instrumentation and reagents (unhackable so no customisation or control)
- Very easy to use and less processing time
- More high-throughput scaling - 8 samples can be processed simultaneously with up to 10000 cells captured per sample
- The doublet rate increases with number of cells loaded
- CellRanger and CellLoupe software are available and user friendly



Source: 10x Genomics

# 10X GENOMICS OVERVIEW

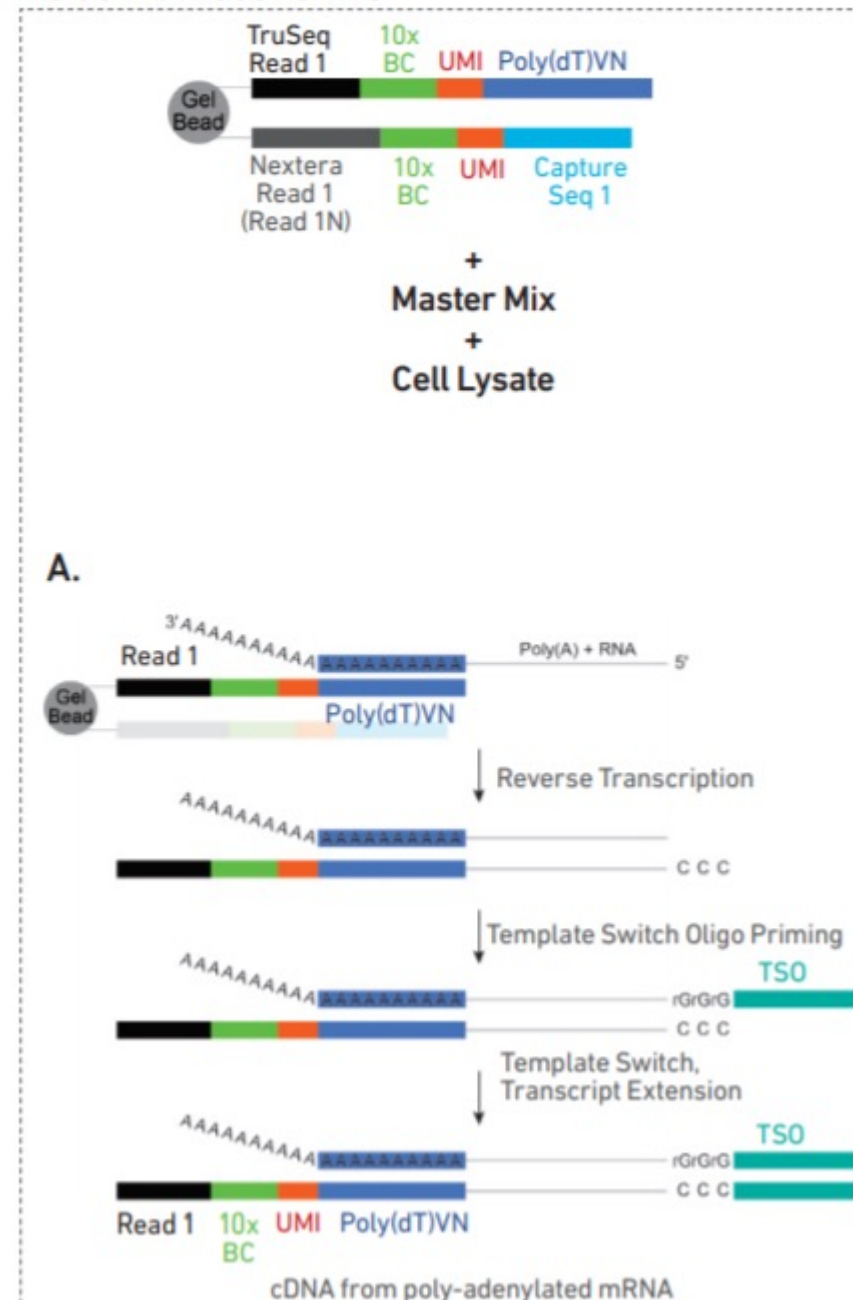


Chromium X



Making 1 million cell experiments routine

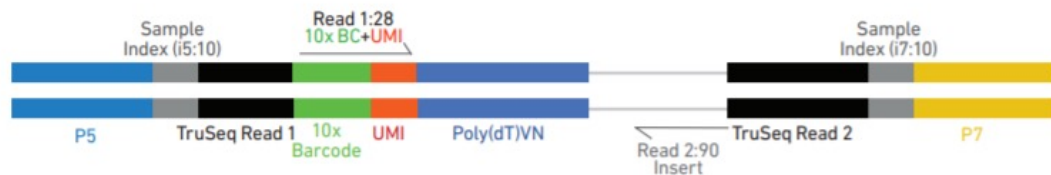
## Inside individual GEMs



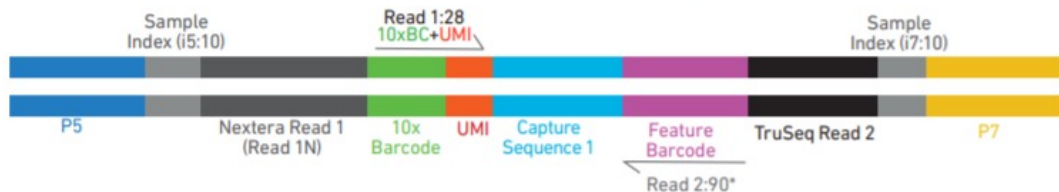
CAMBRIDGE INSTITUTE

# 10X GENOMICS LIBRARIES

Chromium Single Cell 3' Gene Expression Dual Index Library



Chromium Single Cell 3' Cell Surface Protein Dual Index Library



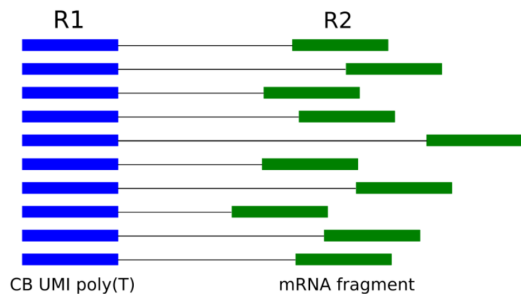
Chromium Single Cell V(D)J Dual Index Library



Chromium Single Cell 5' Gene Expression Dual Index Library



Source: 10x Genomics



Sequencing Read	Description	Number of cycles
Read1	10x Barcode Read (Cell) + Randomer Read (UMI)	28bp
i7 index	Sample index read	10bp
i5 index	Sample index read	10bp
Read2	Insert Read (Transcript)	90bp

# MULTIOMICS AGE

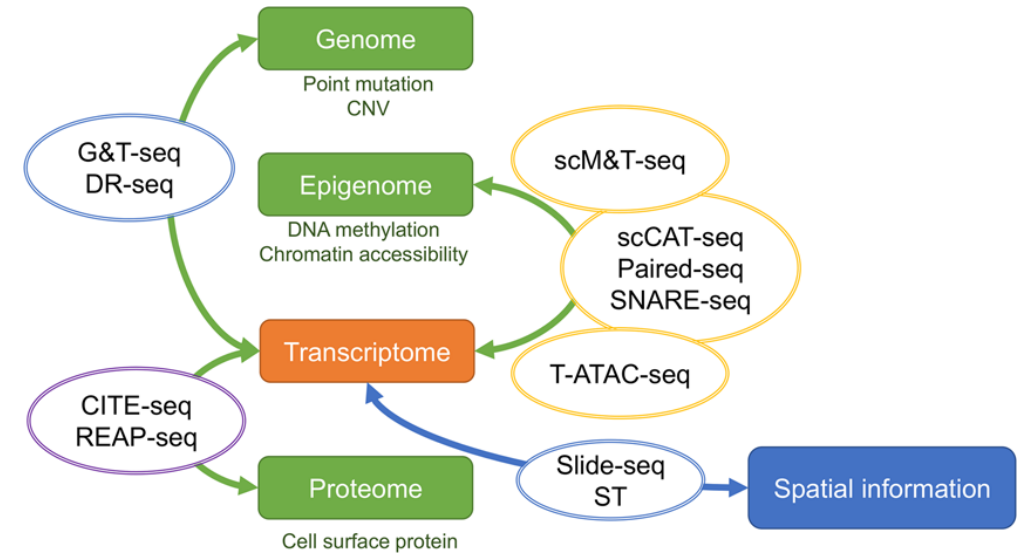
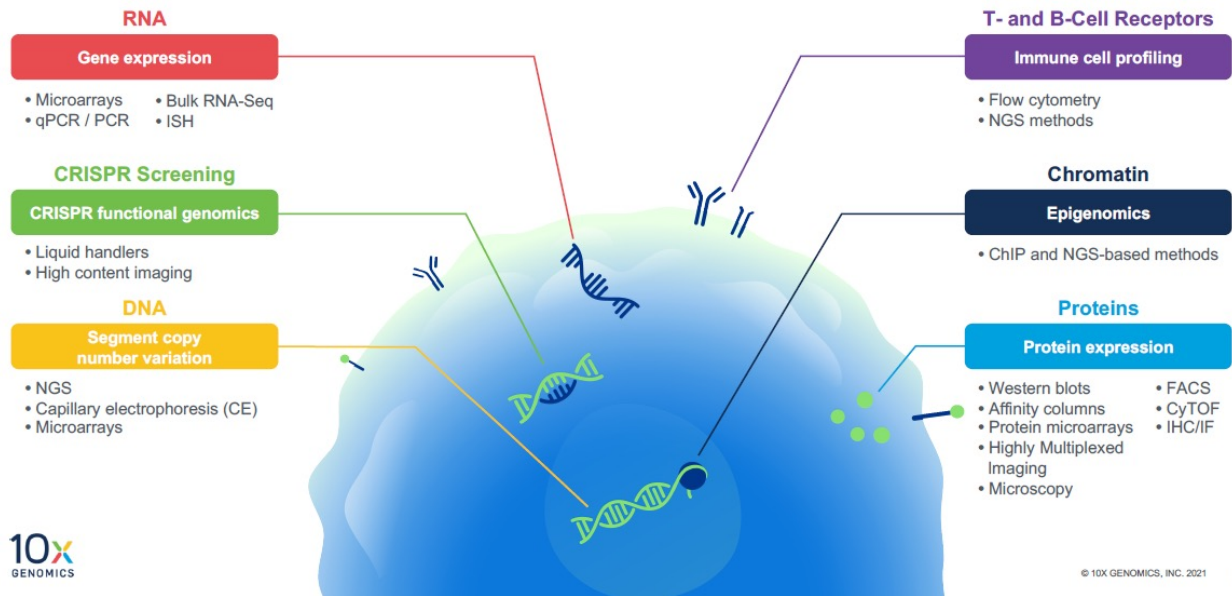
TECHNOLOGY FEATURE | 19 July 2021 | Correction 21 July 2021

## Single-cell analysis enters the multiomics age

A rapidly growing collection of software tools is helping researchers to analyse multiple huge ‘-omics’ data sets.

Jeffrey M. Perkel

### Replacing the Legacy Toolkit Across Biology



Kashima Y et al. Exp Mol Med 52, 1419–1427 (2020)

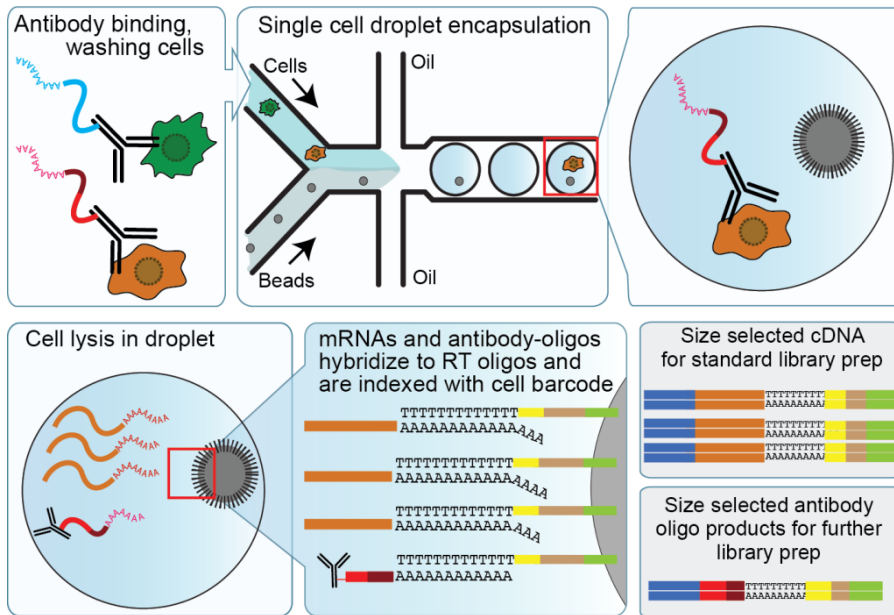
10X  
GENOMICS



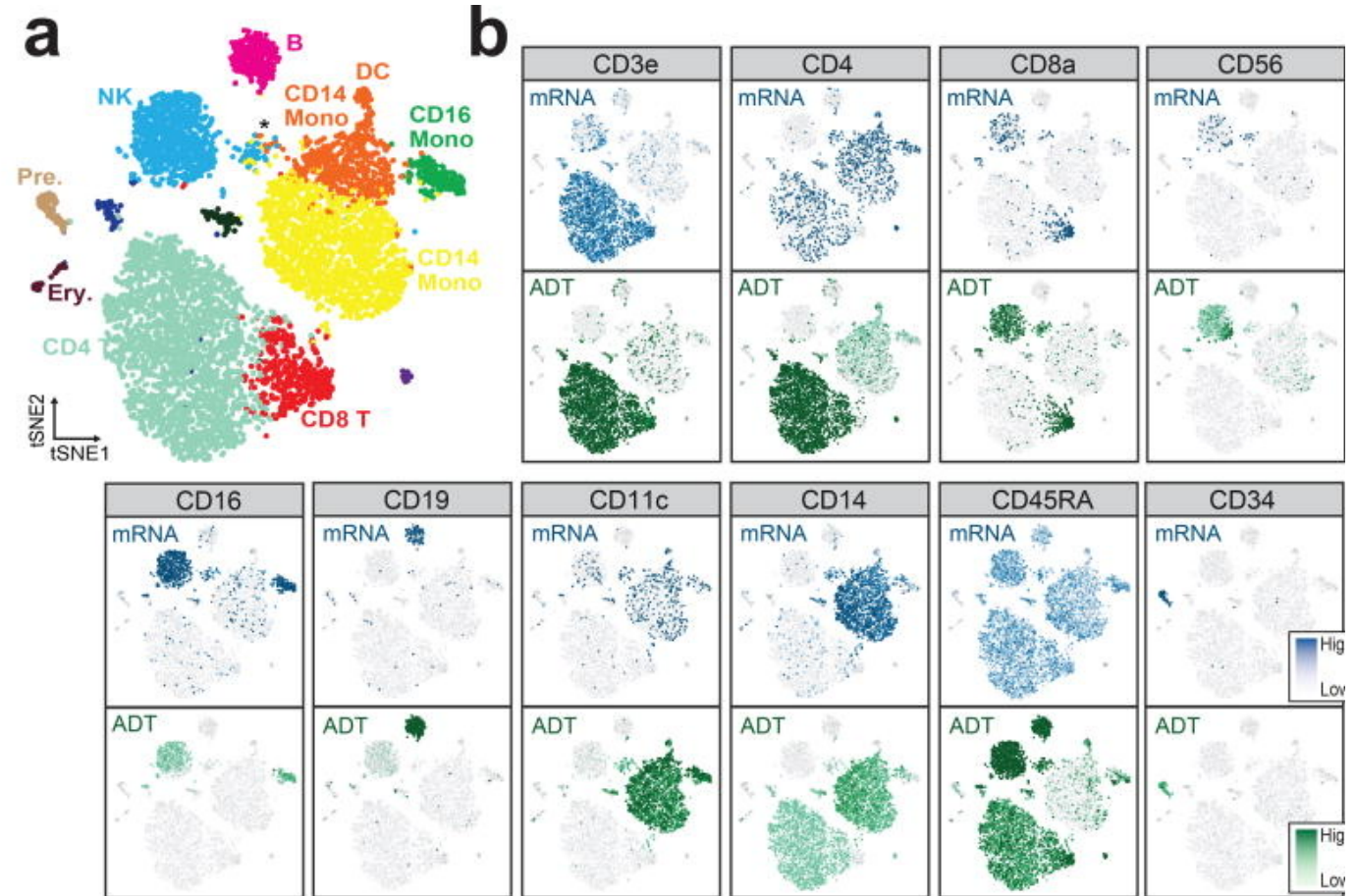
CAMBRIDGE  
INSTITUTE

# CITE-SEQ

- Cellular Indexing of Transcriptomes and Epitopes by Sequencing
- CITE-seq uses DNA-barcoded antibodies to convert detection of proteins into a quantitative, sequenceable readout

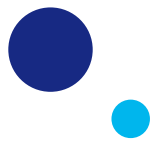


Source: cite-seq.com



BioLegend solutions:  
 TotalSeq-A – Poly(dT) based system  
 TotalSeq-B – 3' v3.1 Feature barcode  
 TotalSeq-C – 5' v2.0

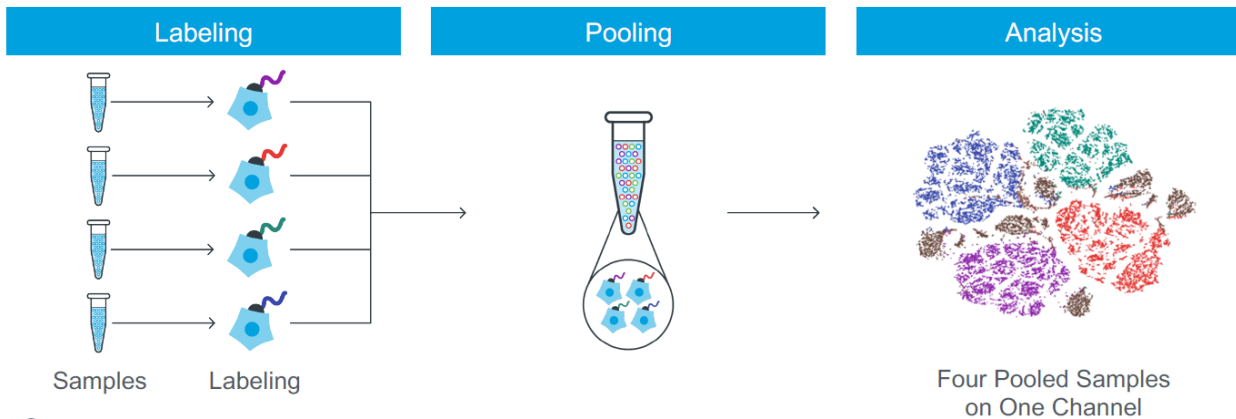
Source: Stoeckius et al. *Nat Methods.* (2017)



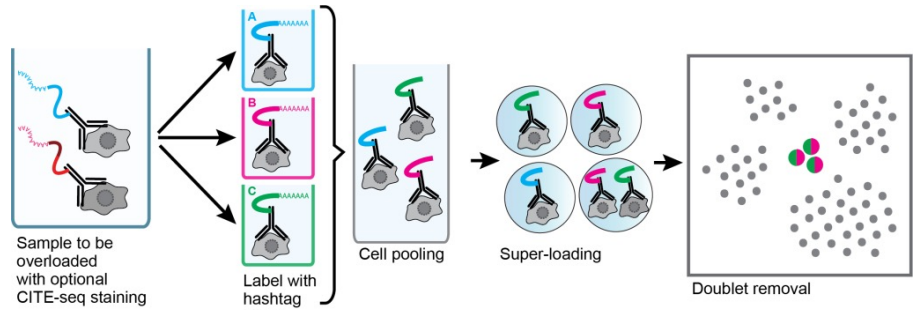
# CELL HASHING



Reduces cost of running multiple samples by adding hashtag oligos and pooling into single channel of 10x chip (10x CellPlex or TotalSeq antibodies)



Allows overloading as by sequencing tags alongside the cellular transcriptome, we can assign each cell to its sample of origin, and robustly identify doublets originating from multiple samples



Source: cite-seq.com

## Genotype-free demultiplexing of pooled single-cell RNA-Seq

Jun Xu<sup>a</sup>, Caitlin Falconer<sup>b</sup>, Quan Nguyen<sup>b</sup>, Joanna Crawford<sup>b</sup>, Brett D. McKinnon<sup>b,c</sup>, Sally Mortlock<sup>b</sup>, Alice Pébay<sup>f,g,h,i</sup>, Alex W. Hewitt<sup>f,g,h,i</sup>, Anne Senabouth<sup>d</sup>, Nathan Palpant<sup>a,b</sup>, Han Chiu<sup>b</sup>, Stacey Andersen<sup>a,b</sup>, Grant W. Montgomery<sup>a,b</sup>, Joseph Powell<sup>c,d</sup>, Lachlan Coin<sup>a,b,\*</sup>



Article | Published: 17 June 2019

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

Christopher S. McGinnis, David M. Patterson, Juliane Winkler, Daniel N. Conrad, Marco Y. Hein, Vasudha Srivastava, Jennifer L. Hu, Lyndsay M. Murrow, Jonathan S. Weissman, Zena Werb, Eric D. Chow & Zev J. Gartner

Nature Methods 16, 619–626(2019) | Cite this article

15k Accesses | 27 Citations | 85 Altmetric | Metrics



CAMBRIDGE INSTITUTE



# TISSUE PRESERVATION/CELLS FIXATION

Research | [Open Access](#) | Published: 02 June 2020

## Systematic assessment of tissue dissociation and storage biases in single-cell and single-nucleus RNA-seq workflows

[Elena Denisenko](#), [Belinda B. Guo](#), [Matthew Jones](#), [Rui Hou](#), [Leanne de Kock](#), [Timo Lassmann](#), [Daniel Poppe](#), [Olivier Clément](#), [Rebecca K. Simmons](#), [Ryan Lister](#) & [Alistair R. R. Forrest](#) ✉

*Genome Biology* 21, Article number: 130 (2020) | [Cite this article](#)

14k Accesses | 39 Citations | 40 Altmetric | [Metrics](#)

*Genome Biol.* 2020; 21: 1.

Published online 2019 Dec 31. doi: [10.1186/s13059-019-1906-x](https://doi.org/10.1186/s13059-019-1906-x)

PMCID: PMC6937944

PMID: [31892341](https://pubmed.ncbi.nlm.nih.gov/31892341/)

## scRNA-seq assessment of the human lung, spleen, and esophagus tissue stability after cold preservation

[E. Madisson](#),<sup>#1,2</sup> [A. Wilbrey-Clark](#),<sup>#1</sup> [R. J. Miragaia](#),<sup>1</sup> [K. Saeb-Parsy](#),<sup>3</sup> [K. T. Mahbubani](#),<sup>3</sup> [N. Georgakopoulos](#),<sup>3</sup> [P. Harding](#),<sup>1</sup> [K. Polanski](#),<sup>1</sup> [N. Huang](#),<sup>1</sup> [K. Nowicki-Osuch](#),<sup>4</sup> [R. C. Fitzgerald](#),<sup>4</sup> [K. W. Loudon](#),<sup>5</sup> [J. R. Ferdinand](#),<sup>5</sup> [M. R. Clatworthy](#),<sup>5</sup> [A. Tsingene](#),<sup>1</sup> [S. van Dongen](#),<sup>1</sup> [M. Dabrowska](#),<sup>1</sup> [M. Patel](#),<sup>1</sup> [M. J. T. Stubbington](#),<sup>1,6</sup> [S. A. Teichmann](#),<sup>1</sup> [O. Stegle](#),<sup>2</sup> and [K. B. Meyer](#)<sup>#1</sup>

Research | [Open Access](#) | Published: 10 May 2021

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

[Sunny Z. Wu](#), [Daniel L. Roden](#), [...][Alexander Swarbrick](#) ✉

*Genome Medicine* 13, Article number: 81 (2021) | [Cite this article](#)

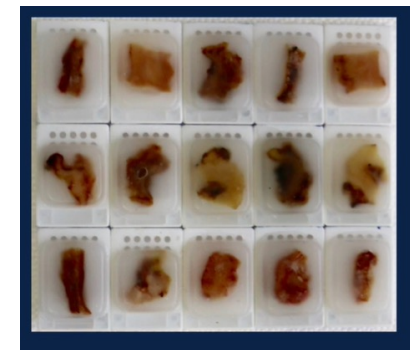
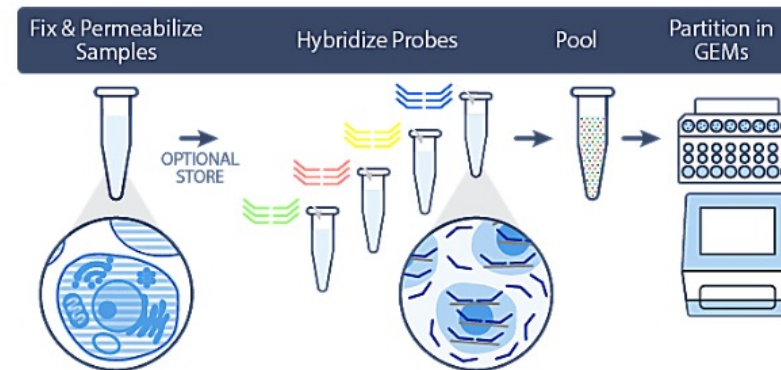
1757 Accesses | 34 Altmetric | [Metrics](#)



CAMBRIDGE  
INSTITUTE

## 10x Fixed RNA Profiling

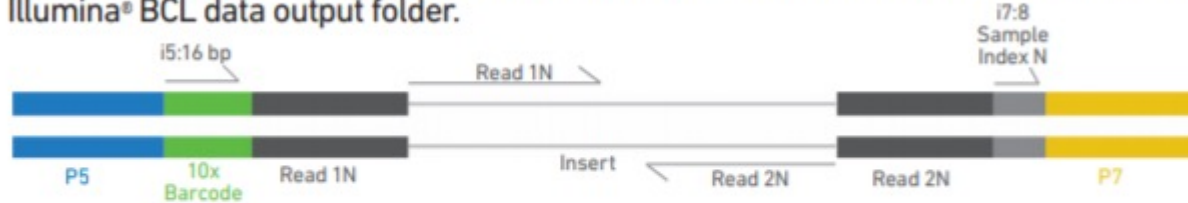
- new chemistry, compatible with formaldehyde fixed samples
- RNA is captured using probes, not poly(d)T like in 3' solution
- Available for human (~18k genes) and mouse (~20k genes) only
- Probes contain barcodes so no additional staining needed for cell hashing
- Kit potentially opens the door to archival material (FFPE blocks)
- pre-print: snPATHO-seq: unlocking the FFPE archives for single nucleus RNA profiling



SplitSeq (Parse Bioscience) requires fixation as well

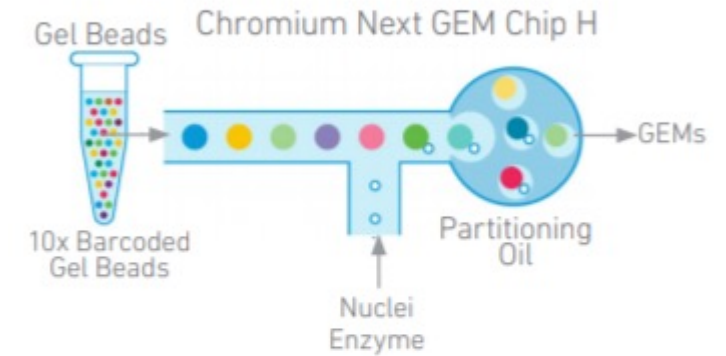
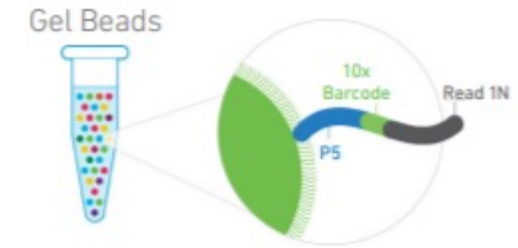
# 10X ATAC

Chromium Single Cell ATAC libraries comprise double stranded DNA fragments which begin with P5 and end with P7. Sequencing these libraries produces a standard Illumina® BCL data output folder.



Sequencing Read	Description	Number of cycles
Read1	Insert Sequence 1N	50bp
i7 index	Sample index read	8bp
i5 index	10x Barcode Read (Cell)	16bp
Read2	Insert Sequence 2N (opposite end)	50bp

- ASAP-seq is to scATAC-seq what CITE-seq is to scRNA-seq.
- Scale Biosciences – ‘pre-indexing of nuclei through tagmentation’ = 100k nuclei per 10x channel with low number of doublets



## Inside Individual GEMs

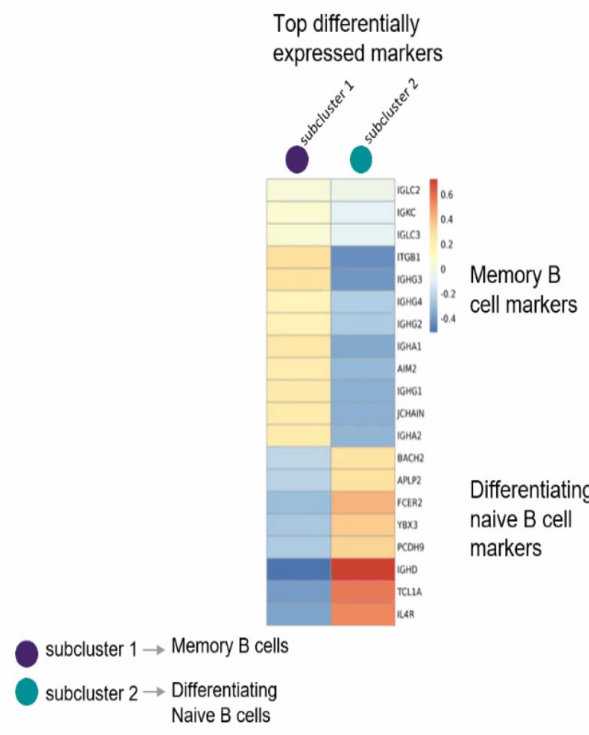
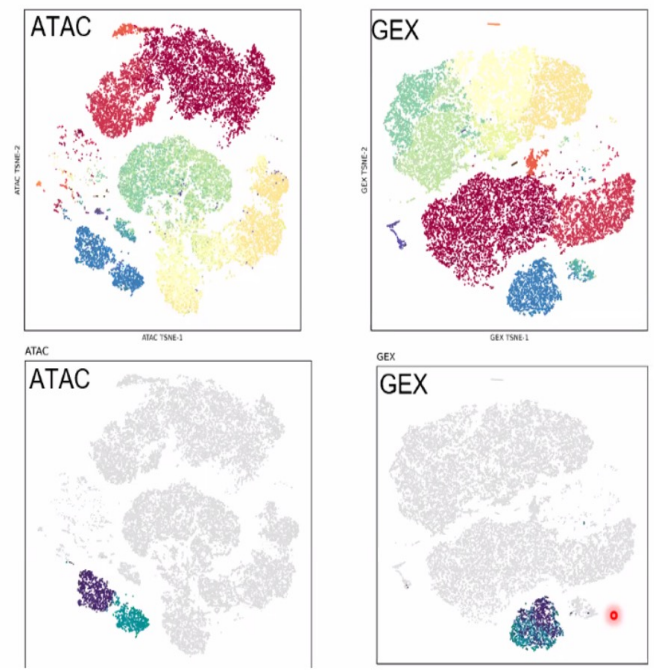
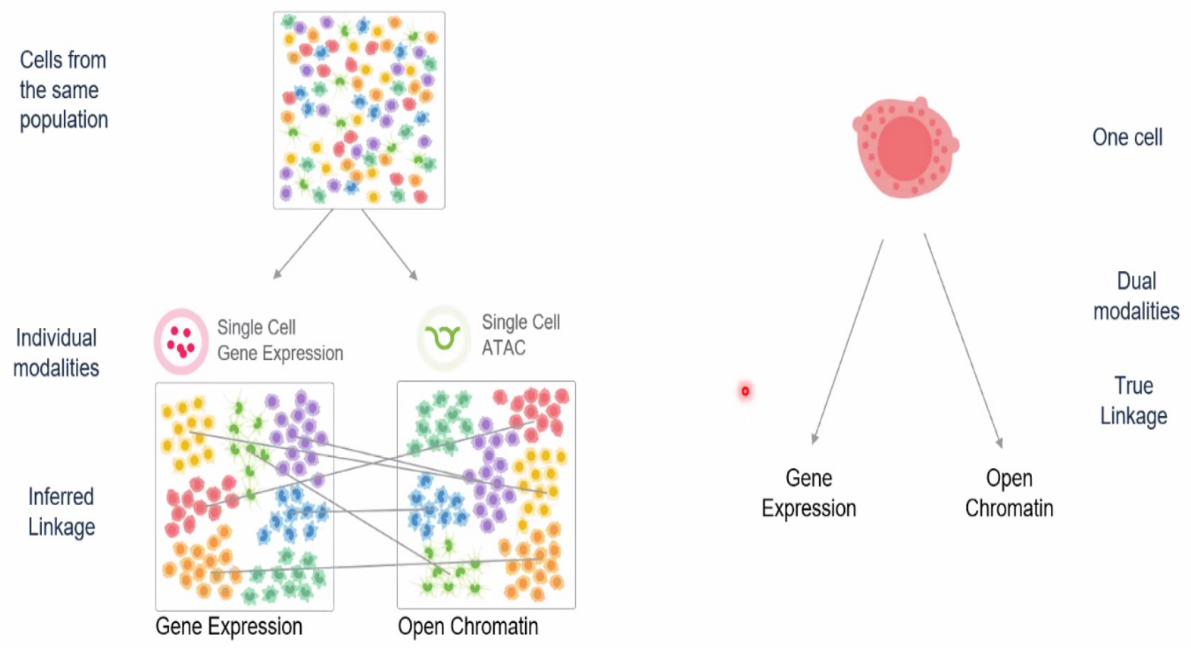


Source: 10x Genomics

# 10X MULTIOME (RNA+ATAC)

Profiling Different Modalities To Gain Deeper Insights

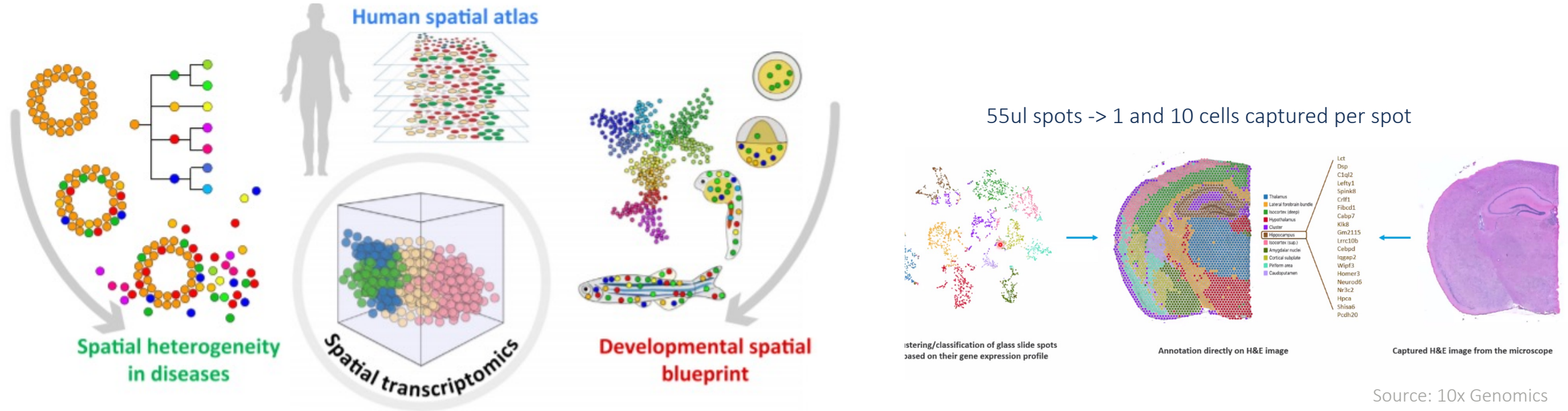
Dive Deep Where It Matters



Source: 10x Genomics

-TEA-seq (Transcription, Epitopes, and Accessibility) = Multiome with permabilised cells & CITEseq

# SPATIAL TRANSCRIPTOMICS

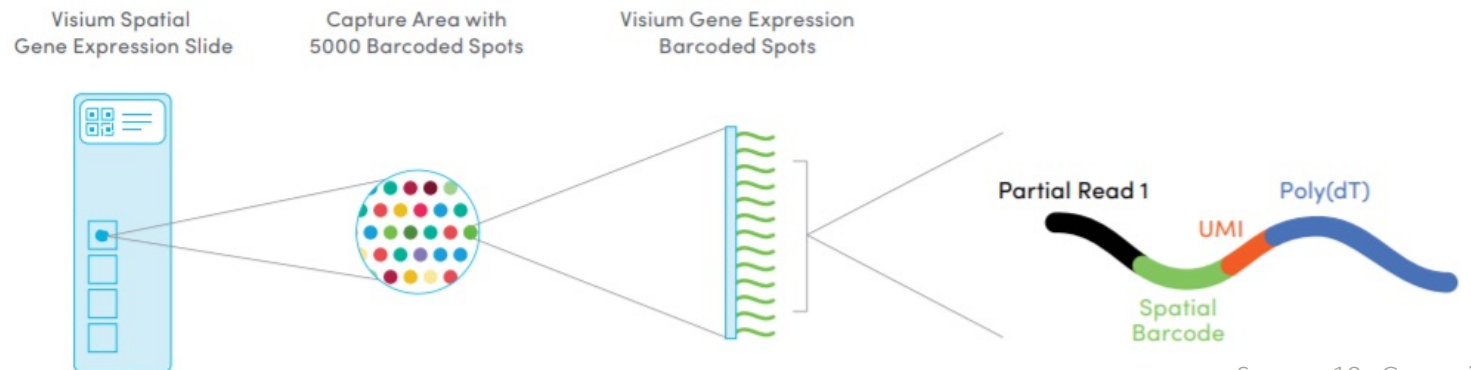


Source: 10x Genomics

## Trends in Biotechnology

Figure 3. Applications for Spatially Resolved Transcriptomics. Three primary kinds of hot issues can be resolved by spatially resolved transcriptomics: left, discovering spatial heterogeneity of diseases; middle, establishing spatial transcriptome atlases for the human body; and right, delineating an embryonic developmental and spatial blueprint.

Source: Liao et al. Trends in Biotechnology. (2020)

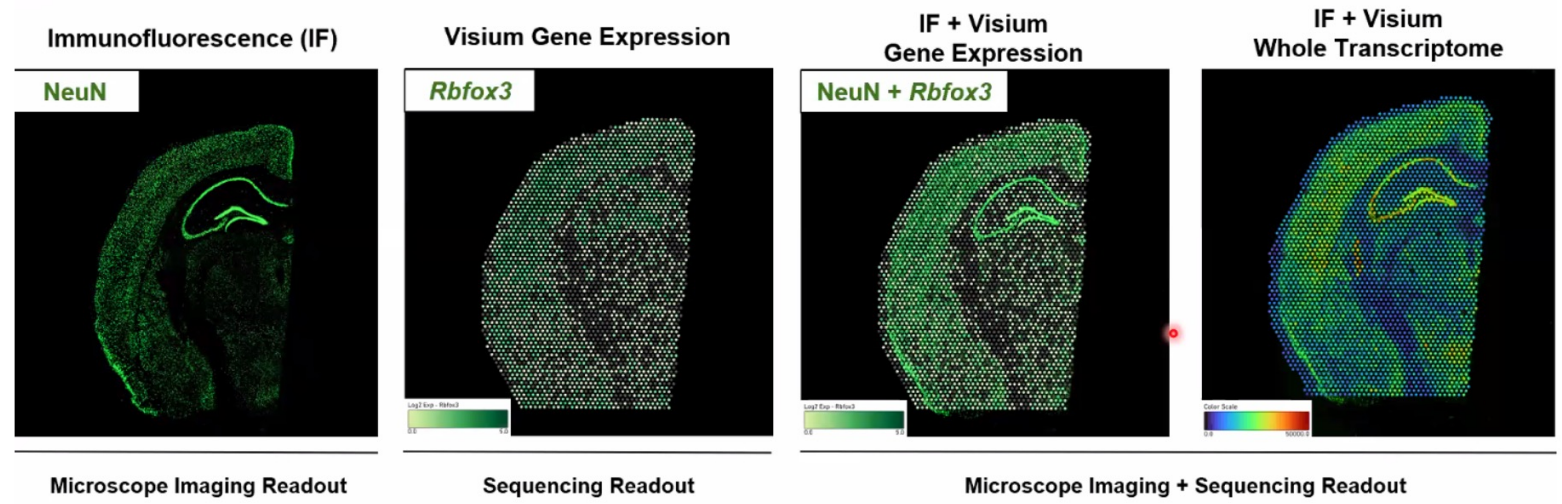


Source: 10x Genomics



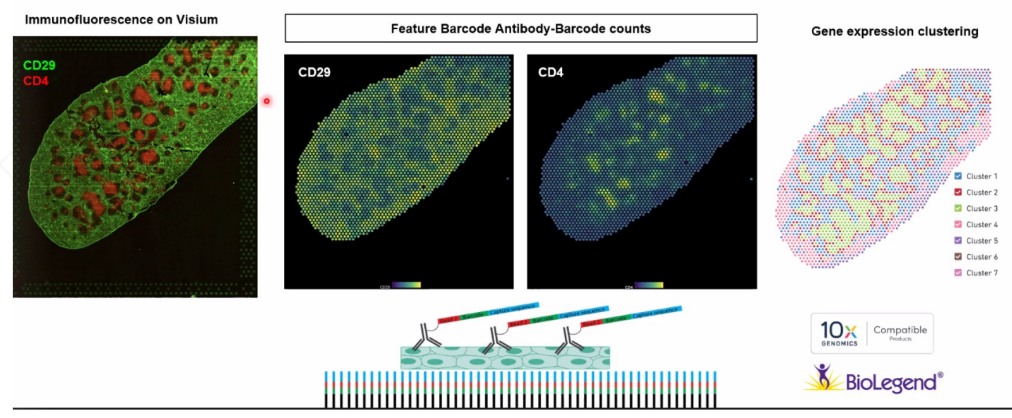
CAMBRIDGE  
INSTITUTE

# SPATIAL TRANSCRIPTOMICS

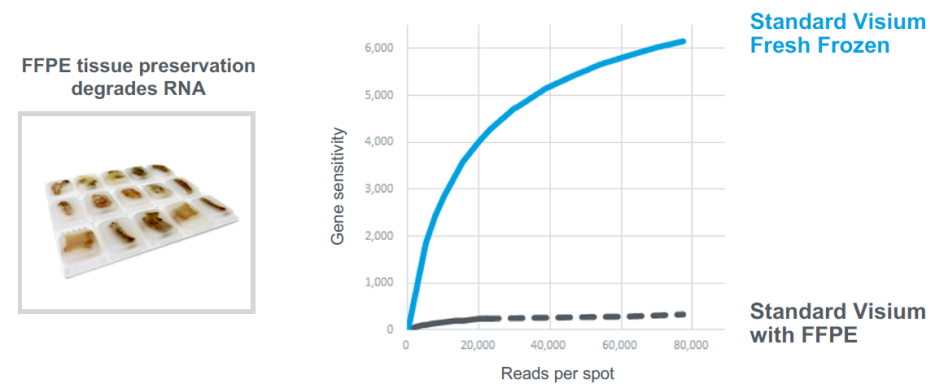


Source: 10x Genomics

## Feature Barcode Correlates with Immunofluorescence

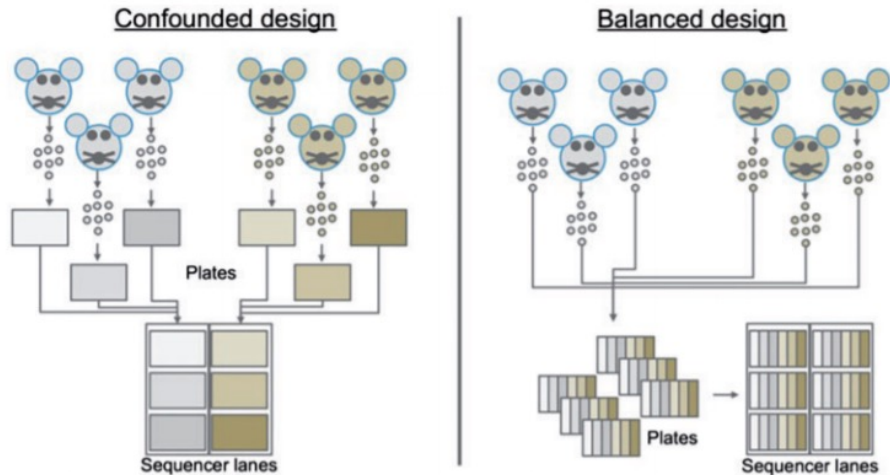


## The Challenge of FFPE Samples



- Visium FFPE uses probe based chemistry similarly to Fixed RNA profiling for single cell

# EXPERIMENTAL DESIGN



Source: Baran-Gale et al. *Brief Func Genomics*. 17 (4):233–239. (2018)

## I. Tissue Procurement



**Source:**

- Primary human
- Model organism
- Cell culture

**Key considerations:**

- Biological variation
- Sampling/handling variation
- Duration of sourcing

**Study design:**

- Biological replicates
- Technical replicates
- Cell number calculation
- Workflow optimization

## II. Tissue Dissociation



**Method:**

- Mechanical mincing
- Enzymatic digestion
- Automated blending
- Microfluidics devices

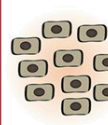
**Key considerations:**

- Experimental consistency
- Shortest duration
- Highest cell/nucleus quality
- Representation of all cell types

**Quality control:**

- FACS analysis
- qPCR for marker genes
- Imaging of cell integrity
- RNA quality (RIN)

## III. Cell Enrichment (optional)



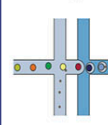
**Method:**

- Differential centrifugation, sedimentation, filtration
- Antibody labeling for positive/negative selection
- Flow cytometry or bead-based enrichment
- Dead cell removal

**Key considerations:**

- Additional handling
- Longer duration
- Loss of RNA quality
- Transcriptome changes

## IV. Single Cell RNAseq Platform



**Method:**

- Droplet-based
- Tube-based after FACS
- Microwell-based
- Microfluidics-enabled

**Key considerations:**

- Cell throughput and handling time
- Gene coverage and cell type detection
- Whole transcript versus 3' end counting
- Imaging capability for doublet detection

## V. Library Sequencing



**Method:**

- Illumina NGS
- Compatible with cDNA library

**Sequencing depth considerations:**

- 3' end counting: low depth ~50K RPC
- Whole transcript: high depth ~1M RPC
- Alternative splicing: ~20-30M RPC
- Iterative optimization for biological system

## VI. Computational Analysis



**Key considerations:**

- Separation of batch and condition
- Technical vs. biological variation

**Sample Batch correction approaches:**

- Cell Hashing
- Demuxlet
- Canonical correlation analysis (CCA)
- MAST

Source: Nguyen QH et al. *Front Cell Dev Biol* 6:108. (2018)



CANCER  
RESEARCH  
UK

CAMBRIDGE  
INSTITUTE

# WHAT PLATFORM SHOULD I USE?

## Choose protocol based on:

- Throughput (number of cells per reaction)
- Sample of origin
- Cost / Labour / Time limitations
- Gene body coverage: 5' / 3' biased or full-length?
- UMI vs no-UMI
- Sequencing depth per cell

## Examples:

- If your sample is fairly homogeneous – bulk RNAseq
- If your sample is limited in cell number – plate-based method
- If you want re-annotate the transcriptome and discover new isoforms – full-length coverage (SMART-seq2, seqWell)
- If you are looking to classify all cell types in a diverse tissue - high throughput
- If you have only archival human samples – nuclei isolation or 10x fixed RNA profiling

# LITERATURE:

- [https://hbctraining.github.io/scRNA-seq/slides/Single\\_Cell\\_2\\_27\\_20.pdf](https://hbctraining.github.io/scRNA-seq/slides/Single_Cell_2_27_20.pdf).
- <https://www.slideshare.net/TimothyTickle/introduction-to-singlecell-rnaseq>
- <https://www.decibio.com/insights/10x-genomics-single-cell-dominance-is-it-sustainable>
- Arzalluz-Luque et al. A. Single-cell RNAseq for the study of isoforms—how is that possible?. *Genome Biol* 19, 110 (2018).
- Clark, I.C., Fontanez, K.M., Meltzer, R.H. *et al.* Microfluidics-free single-cell genomics with templated emulsification. *Nat Biotechnol* (2023)
- Ding et al. Systematic comparison of single-cell and single-nucleus RNA-sequencing methods. *Nat Biotechnol* 38, 737–746 (2020).
- Haque et al. A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications. *Genome Med* 9, 75 (2017).
- Hwang et al. Single-cell RNA sequencing technologies and bioinformatics pipelines. *Exp Mol Med* 50(8):96. (2018).
- Baran-Gale et al. Experimental design for single-cell RNA sequencing. *Briefings in Functional Genomics*, Volume 17, Issue 4, Pages 233–239 (2018).
- Kashima, Y., Sakamoto, Y., Kaneko, K. *et al.* Single-cell sequencing techniques from individual to multiomics analyses. *Exp Mol Med* 52, 1419–1427 (2020).
- Liao et al. Uncovering an Organ’s Molecular Architecture at Single-Cell Resolution by Spatially Resolved Transcriptomics. *Trends in Biotechnology*. (2020).
- Laks et al. Clonal Decomposition and DNA Replication States Defined by Scaled Single-Cell Genome Sequencing. *Cell*. 179(5):1207-1221.e22. (2019).
- Macosko et al. Single-cell RNA sequencing at isoform resolution. *Nat Biotechnol* 38, 697–698 (2020).
- McGinnis et al. MULTI-seq: sample multiplexing for single-cell RNA sequencing using lipid-tagged indices. *Nat Methods* 16, 619–626 (2019).
- Nguyen et al. Experimental Considerations for Single-Cell RNA Sequencing Approaches. *Front Cell Dev Biol* 6:108. (2018).
- Pijuan-Sala et al. A single-cell molecular map of mouse gastrulation and early organogenesis. *Nature* 566, 490–495 (2019).
- Plasschaert et al. A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature* 560, 377–381 (2018).
- Richter, M.L *et al.* Single-nucleus RNA-seq2 reveals functional crosstalk between liver zonation and ploidy. *Nat Commun* 12, 4264 (2021).
- See et al. A Single-Cell Sequencing Guide for Immunologists. *Frontiers in immunology*, 9, 2425. (2018).
- Stoeckius et al. Simultaneous epitope and transcriptome measurement in single cells. *Nat Methods*. (2017).
- Svensson et al. Power analysis of single-cell RNA-sequencing experiments. *Nat Methods* 14, 381–387 (2017).
- Svensson et al. Exponential scaling of single-cell RNA-seq in the past decade. *Nat Protoc* 13, 599–604 (2018).
- Vallejo et al. snPATHO-seq: unlocking the FFPE archives for single nucleus RNA profiling. *bioRxiv* 2022.08.23.505054 (2022).
- Wang et al. Direct Comparative Analyses of 10X Genomics Chromium and Smart-seq2. *Genom Proteom Bioinform* Apr;19(2):253-266 (2021).
- Wen et al. Development of Droplet Microfluidics Enabling High-Throughput Single-Cell Analysis. *Molecules*. 21. (2016).
- Wilk et al. A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nat Med* 26, 1070–1076 (2020).
- Xu et al. Genotype-free demultiplexing of pooled single-cell RNA-seq. *Genome Biol* 20, 290 (2019).
- Ziegenhain et al. Comparative Analysis of Single-Cell RNA Sequencing Methods. *Mol Cell*. 65(4):631-643.e4. (2017).



CANCER  
RESEARCH  
UK

CAMBRIDGE  
INSTITUTE

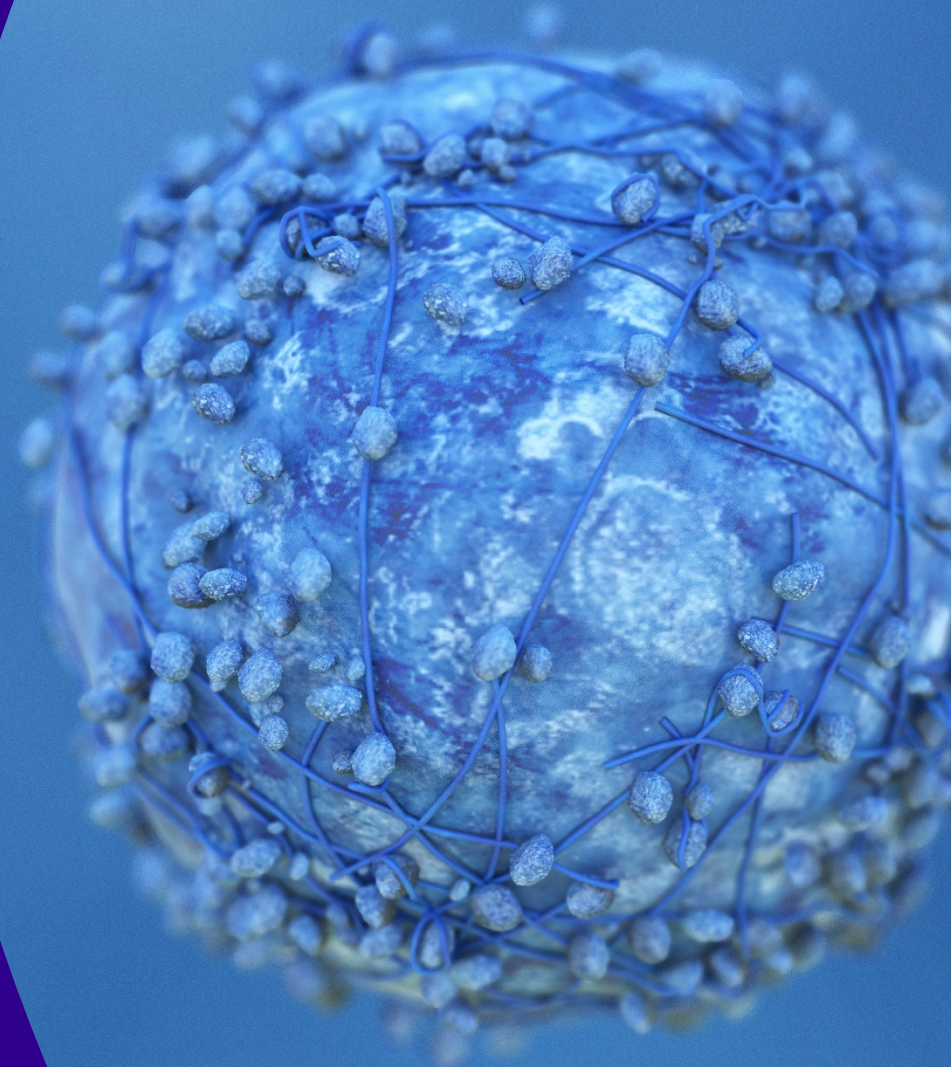


UNIVERSITY OF  
CAMBRIDGE



# USEFUL RESOURCES:

- Van de Sande *et al.* Applications of single-cell RNA sequencing in drug discovery and development. *Nat Rev Drug Discov* (2023).
- Haque et al. A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications. *Genome Med.* 2017;9(1):75.
- Single cell course by Hemberg Lab, Wellcome Sanger Institute (<http://hemberg-lab.github.io/scRNA.seq.course/index.html>)
- Tabula Muris (<https://tabula-muris.ds.czbiohub.org/>)
- Human Cell Atlas (<https://www.humancellatlas.org/>)
- Worthington Tissue Dissociation Guide (<http://www.worthington-biochem.com/tissuedissociation/default.html>)
- Broad Institute Single Cell Portal ([https://singlecell.broadinstitute.org/single\\_cell](https://singlecell.broadinstitute.org/single_cell))
- List of software packages for single cell data analysis (<https://github.com/seandavi/awesome-single-cell>)
- SPLIT-seq (<https://www.youtube.com/watch?v=WqaeZe7mKUc>)
- CITE-seq (<https://cite-seq.com/>)
- Biolegend TotalSeq (<https://www.biolegend.com/en-us/totalseq>)
- **Can chatGPT do single-cell bioinformatic analysis?** <https://www.youtube.com/watch?v=fkuLFIC2ZWk>



THANK YOU FOR  
YOUR ATTENTION!



CANCER  
RESEARCH  
UK

CAMBRIDGE  
INSTITUTE

