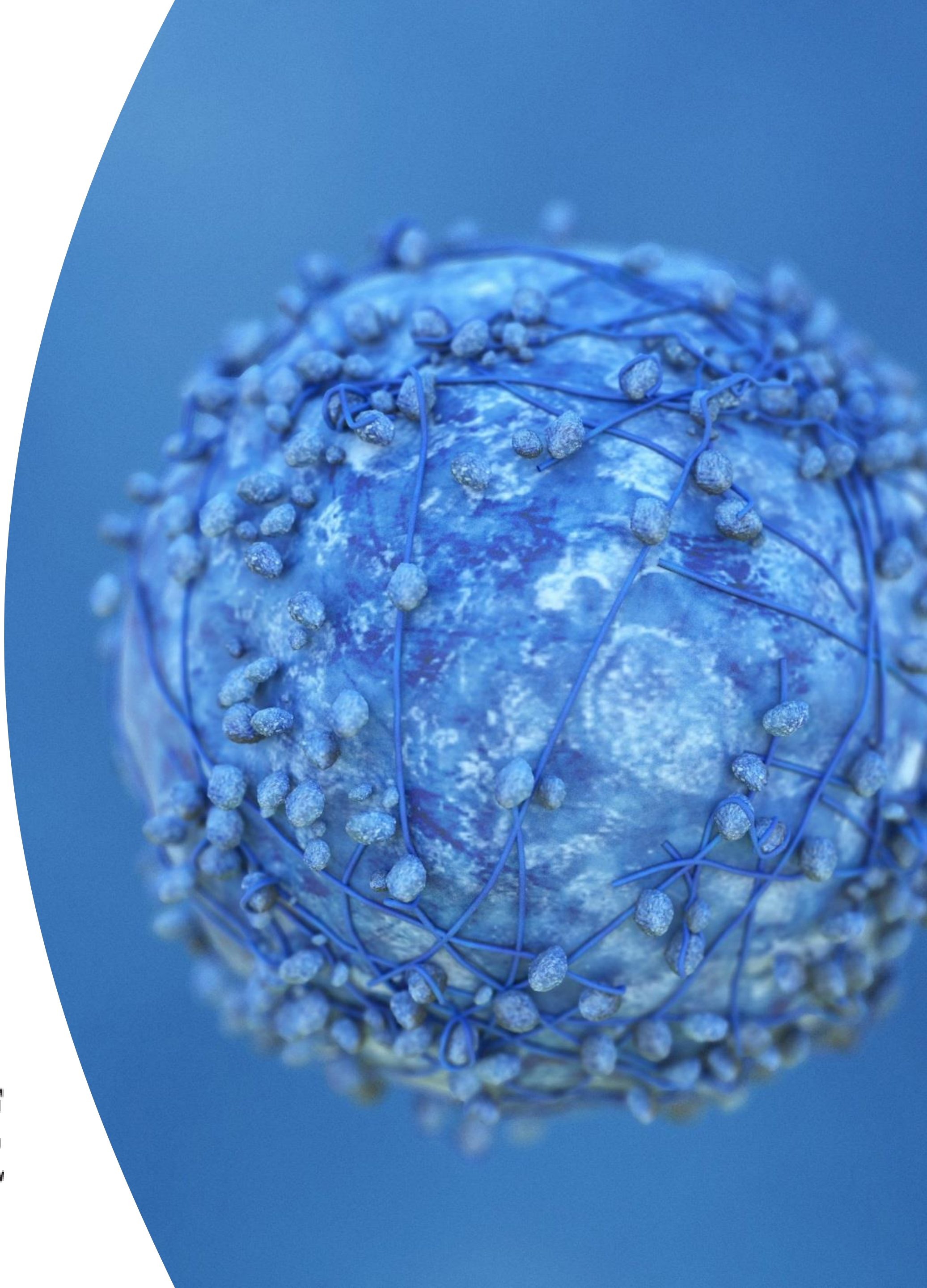
INTRODUCTION TO SINGLE CELL RNA-SEQ

Analysis of single cell RNA-seq data

Katarzyna Kania

1st October 2025



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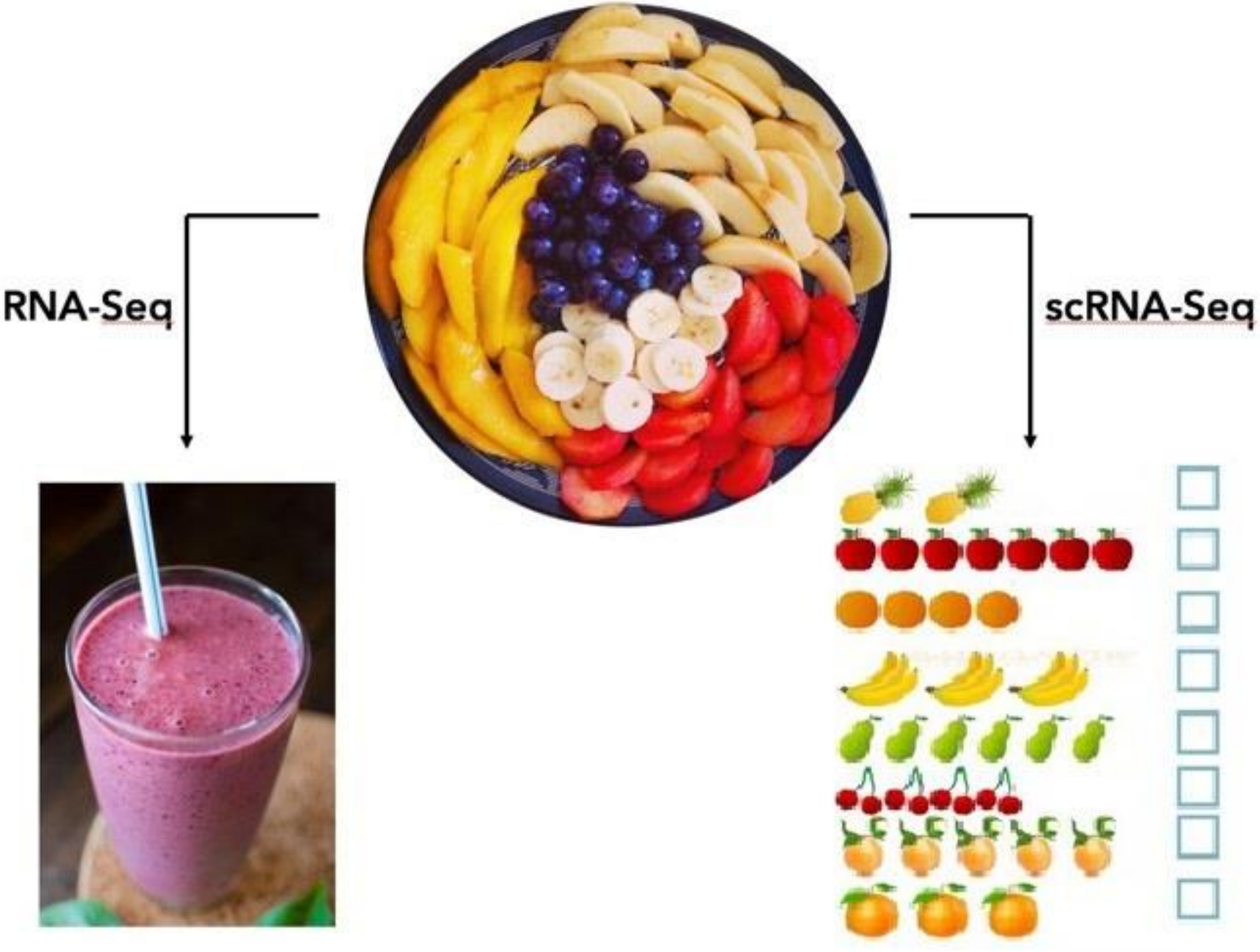
UNIVERSITY OF CAMBRIDGE

Together we will beat cancer

BULK VS SINGLE CELL RNA-SEQ

Average expression level

- Comparative transcriptomics
- Disease biomarker
- Homogenous systems



Separate populations

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

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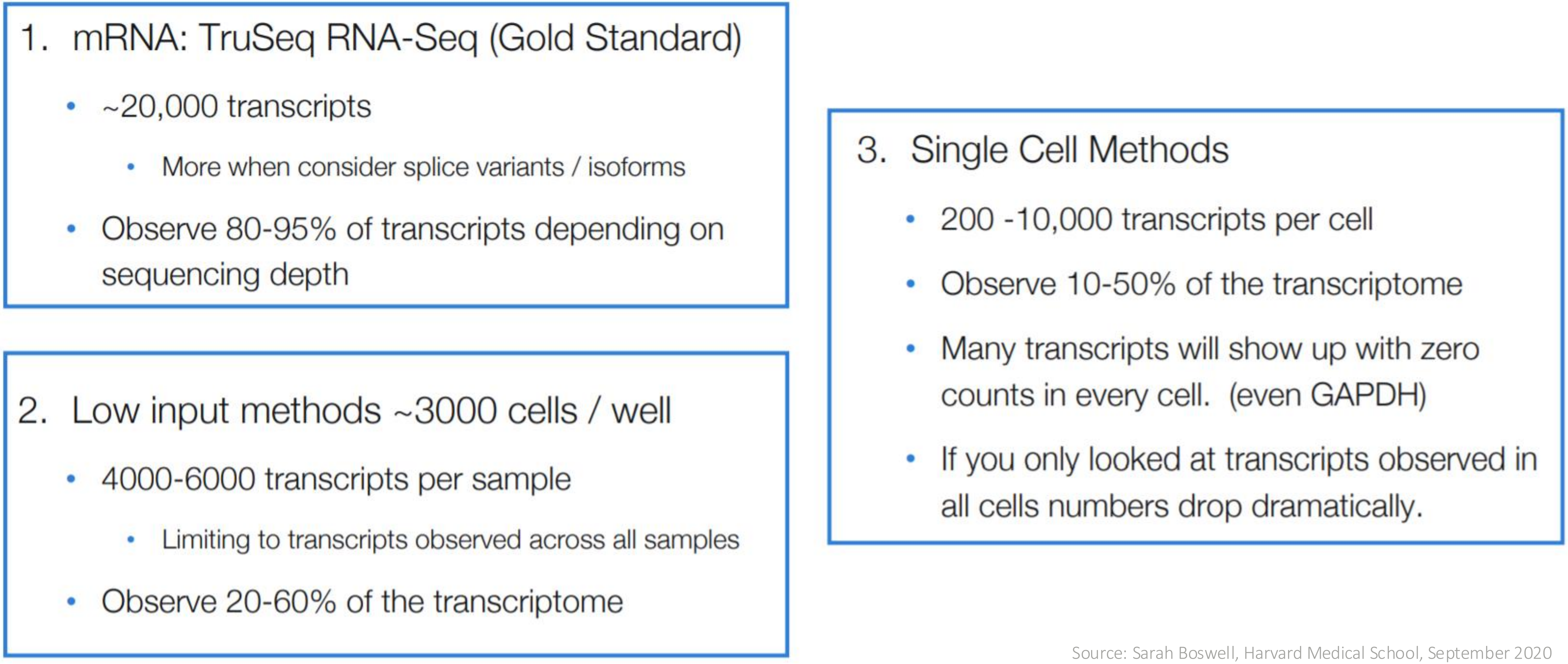
BULK VS SINGLE CELL RNA-SEQ



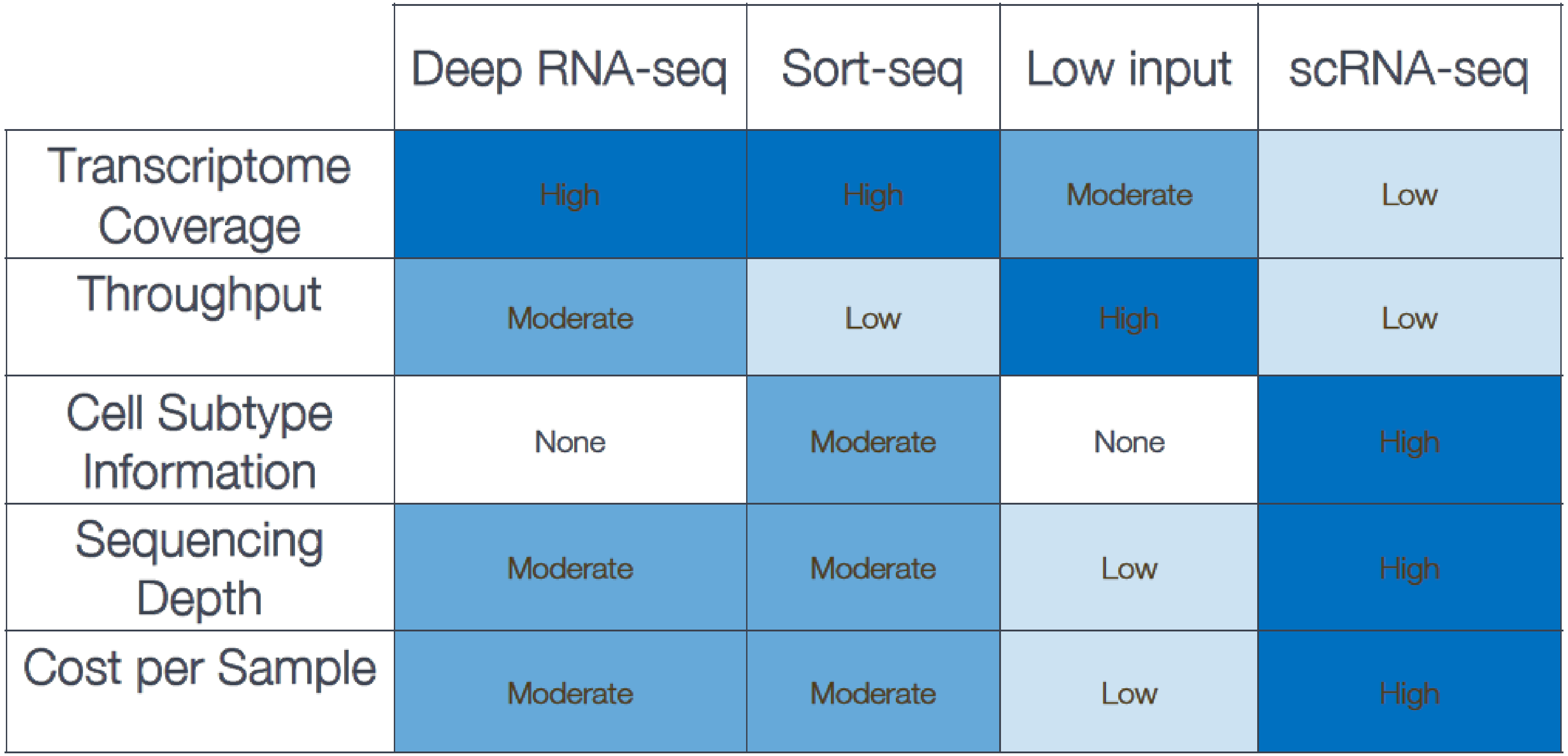
1. mRNA: TruSeq RNA-Seq (Gold Standard)
    - ~20,000 transcripts
        - More when consider splice variants / isoforms
    - Observe 80-95% of transcripts depending on sequencing depth

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.

Source: Sarah Boswell, Harvard Medical School, September 2020

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

Source: Sarah Boswell, Harvard Medical School, September 2020

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

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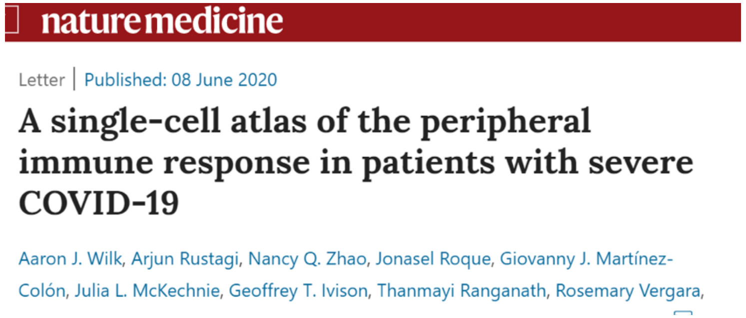
APPLICATIONS

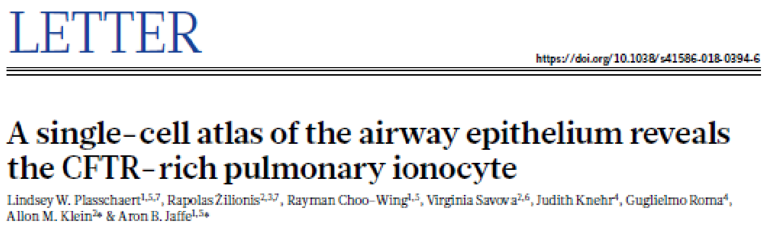


nature medicine

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, Giovanny J. Martínez-Colón, Julia L. McKechnie, Geoffrey T. Ivison, Thanmayi Ranganath, Rosemary Vergara,

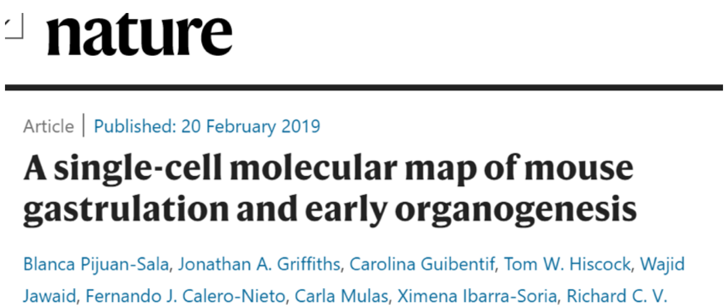


LETTER

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[1,5,7], Rapolas Žilionis[2,3,7], Rayman Choo-Wing[1,5], Virginia Savova[2,6], Judith Knehr[4], Guglielmo Roma[4], Allon M. Klein[2*] & Aron B. Jaffe[1,5*]

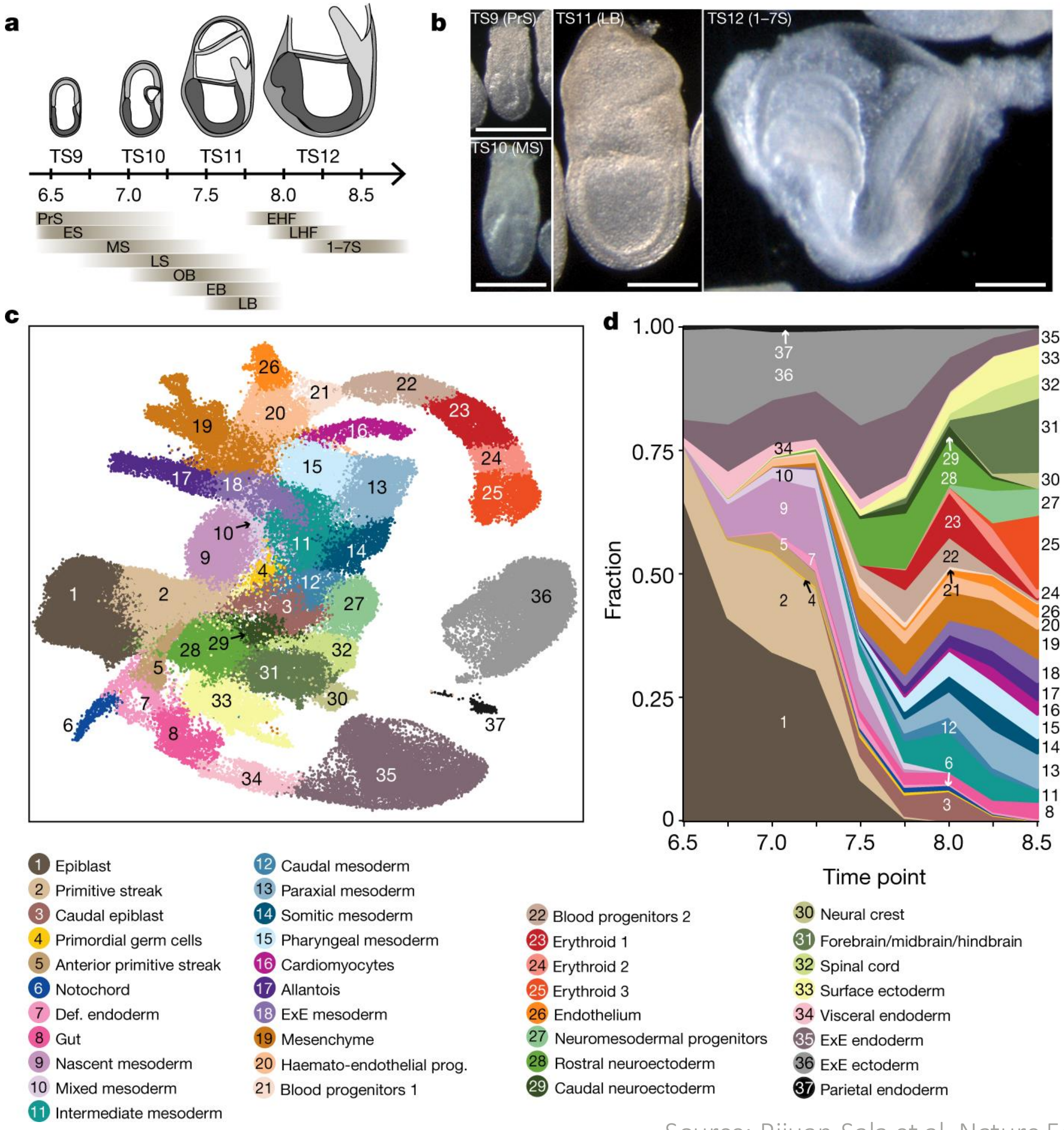


nature

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.



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

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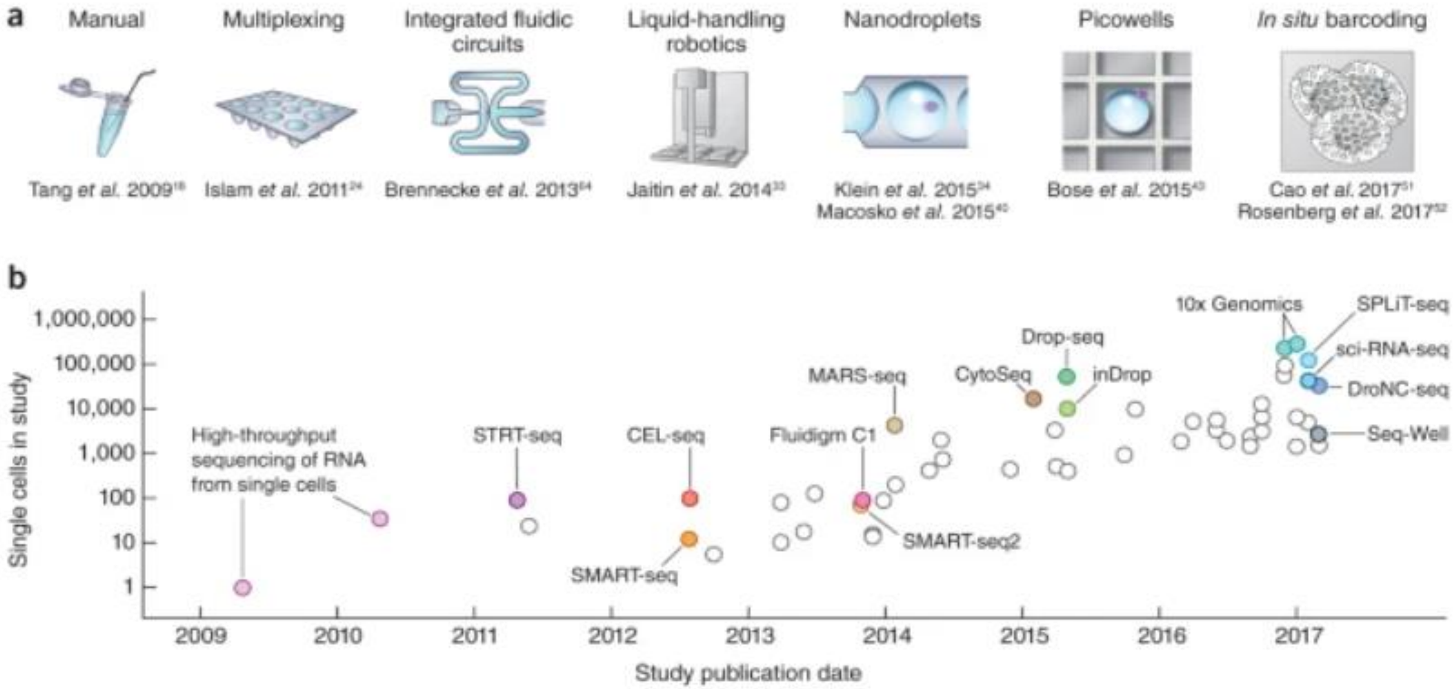
TECHNOLOGIES

Figure 1: Scaling of scRNA-seq experiments.



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

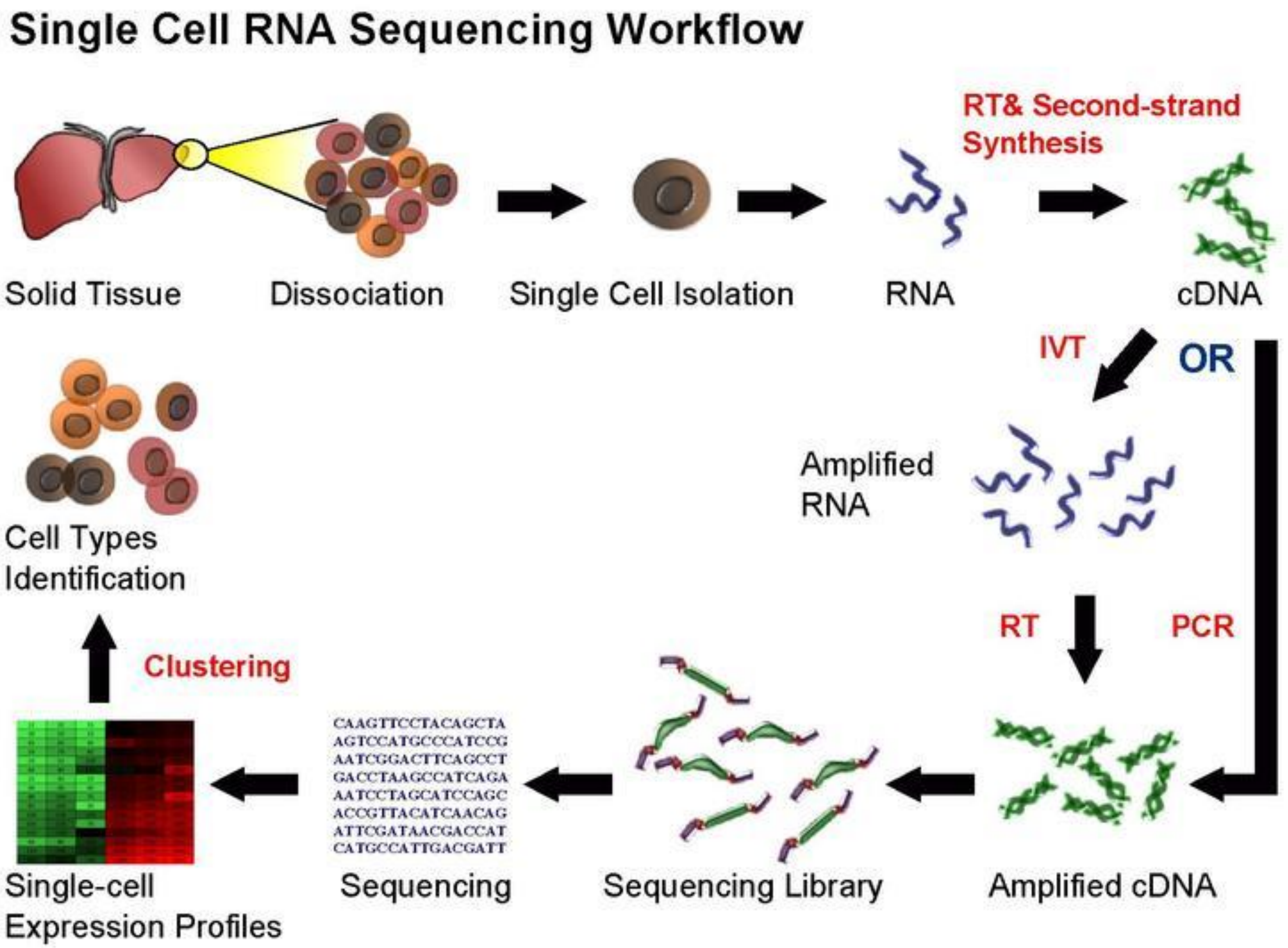
Parse WT Penta – 5mln cells in one experiment

Chromium X

Making 1 million cell experiments routine

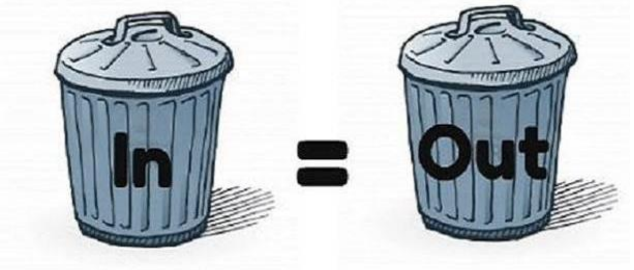
WORKFLOW

Good sample preparation is key to success!



Source: https://en.wikipedia.org/wiki/Single_cell_sequencing

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
4) ‘One tube’ solutions (SciPio Bioscience, Fluent Bioscience, CS Genetics)

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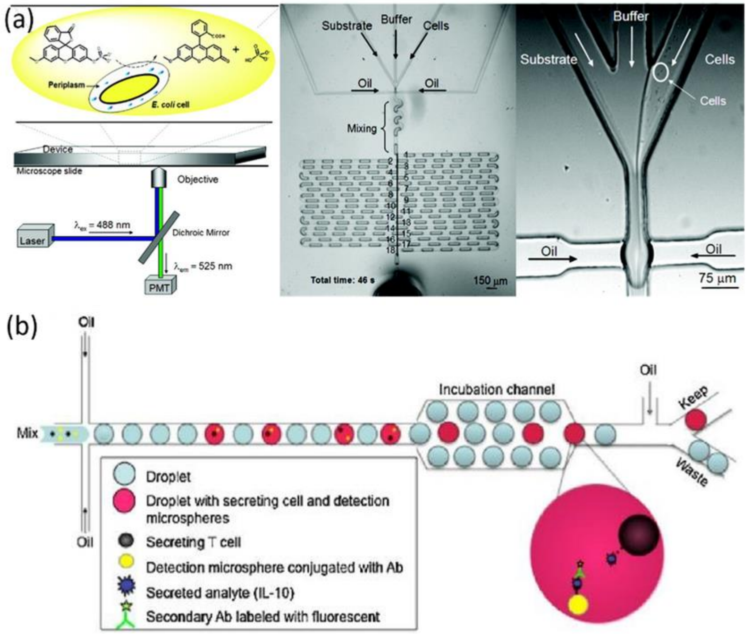
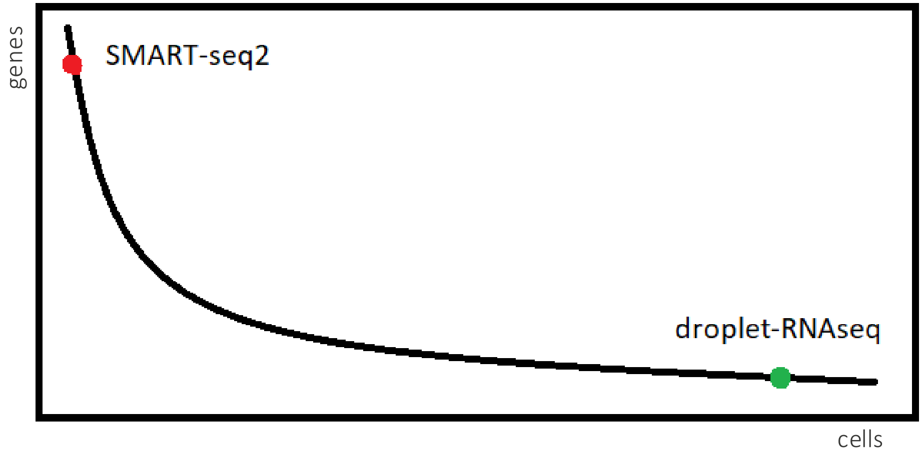
Passive wells

Active pumps and valves



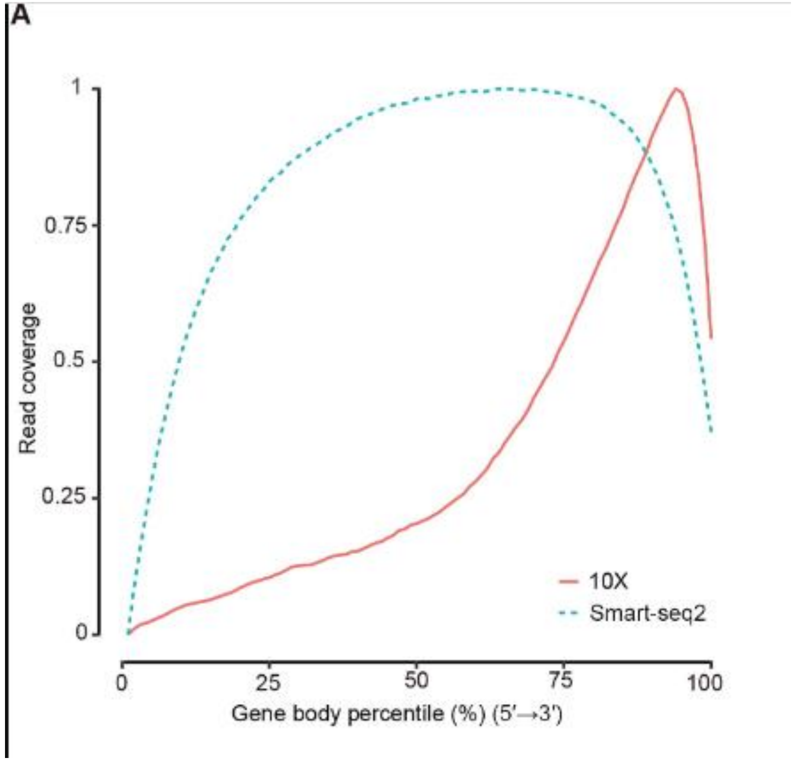
Source: Wen et al. Molecules (2016)

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.

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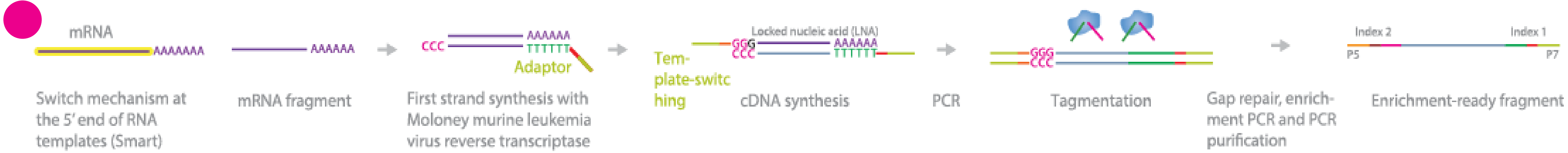
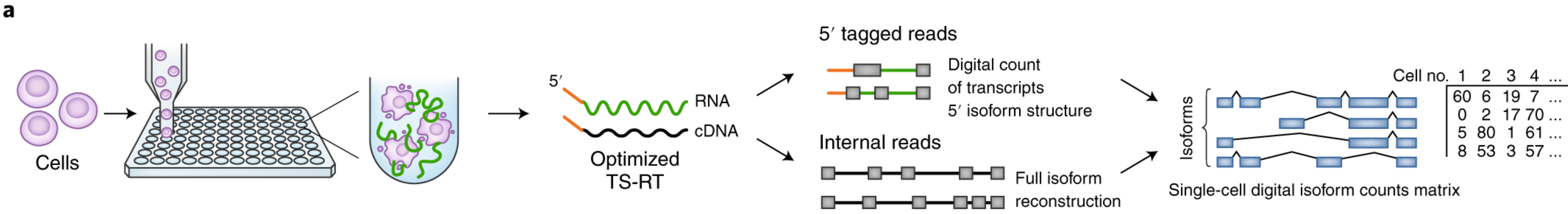
SMART-SEQ2/3/4 OVERVIEW



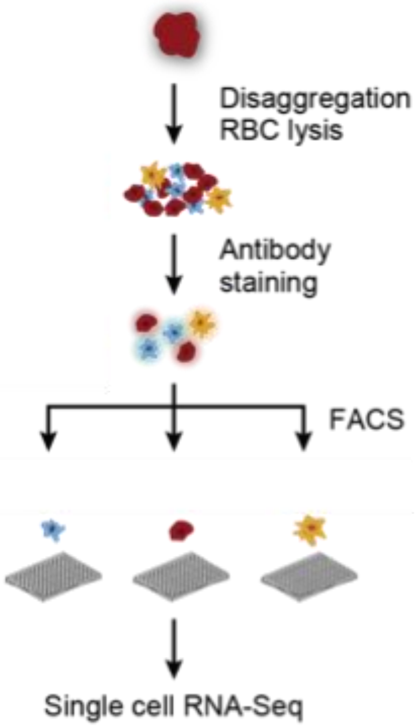
Developed for single cell but can performed using total RNA.

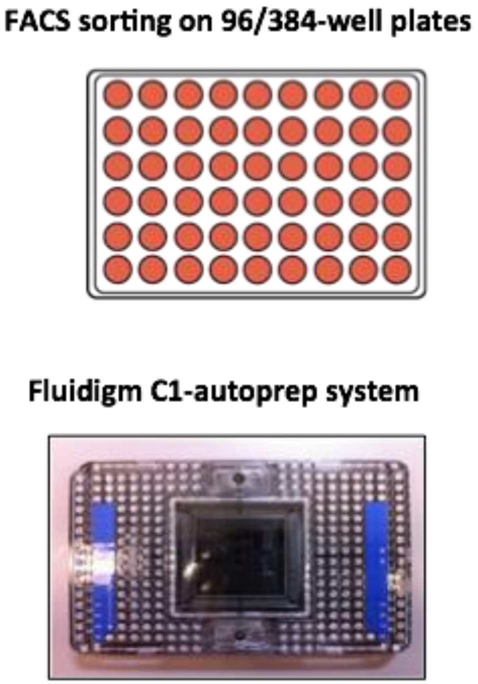
- Selects for poly-A tail.
- Full transcript assay.
- Uses template switching for 5' end capture.
- Standard Illumina sequencing.
- Plate-based solution so labour intensive, slow and costly (~$12/cell)

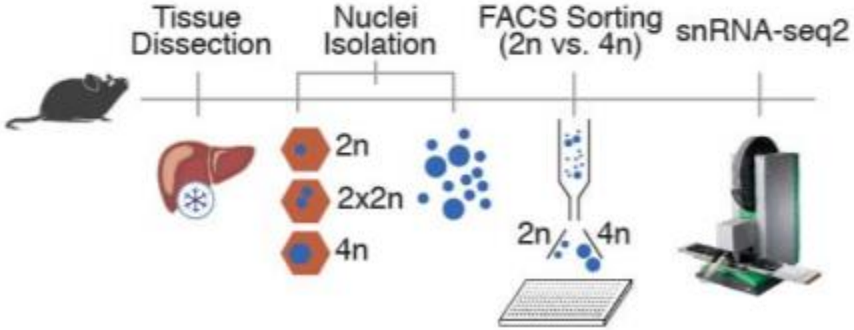


Source: Macosko, Nat Biotechnol 38 (2020).

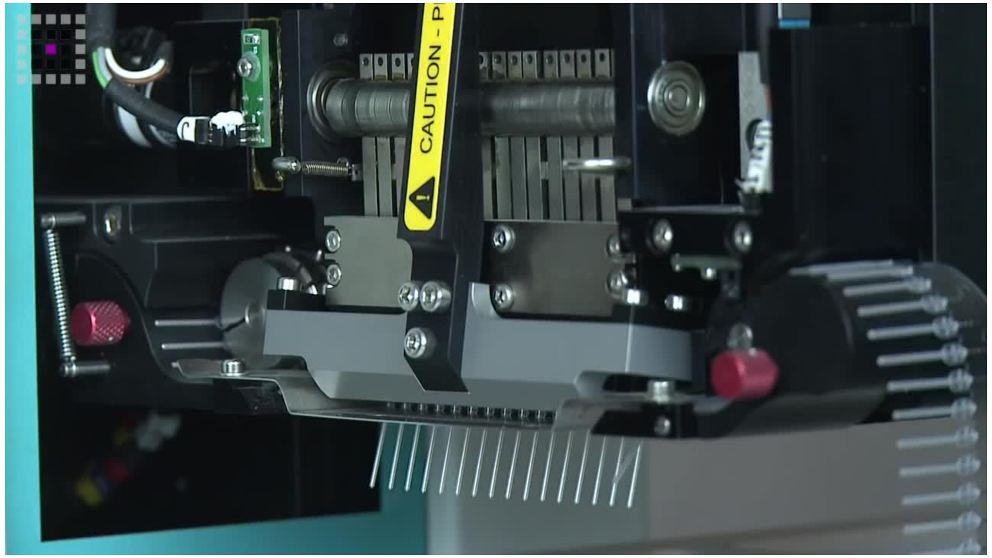
SMART-SEQ2/3/4 + MOSQUITO LV







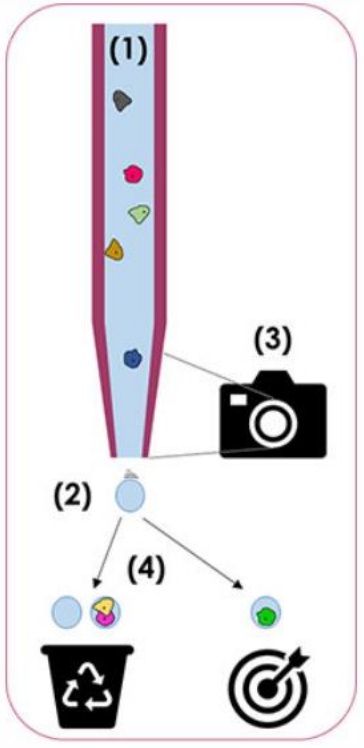
Richter et al. Nat Commun 12, 4264 (2021)



Source: SPT Labtech

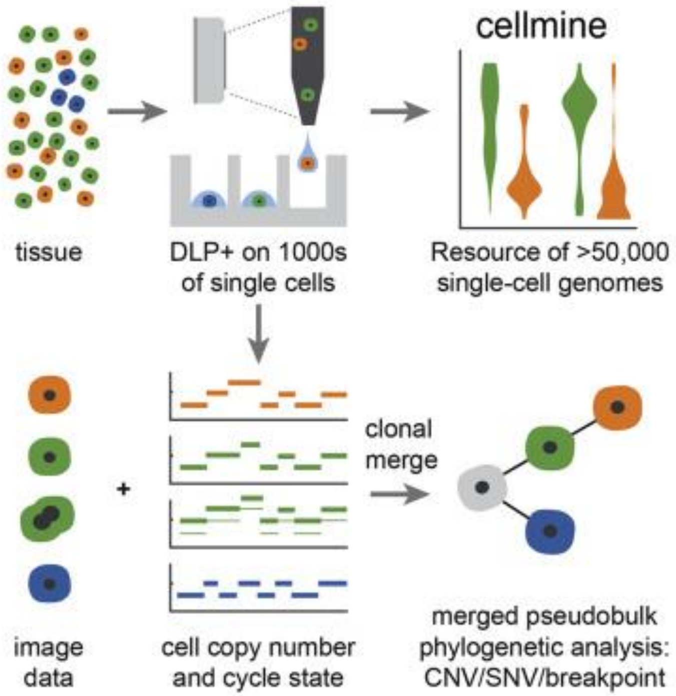
- Mosquito LV makes assay miniaturisation simple, leading to significant savings on precious reagents and time.
- Mosquito LV offers highly accurate and precise multichannel pipetting from 25 nL to 1.2 µL.
- SmartSeq2 cost reduced from $12 to $4 per cell

CELLENONE

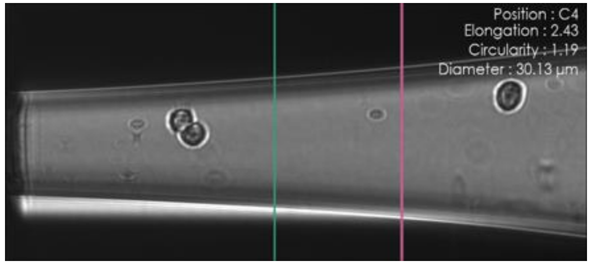


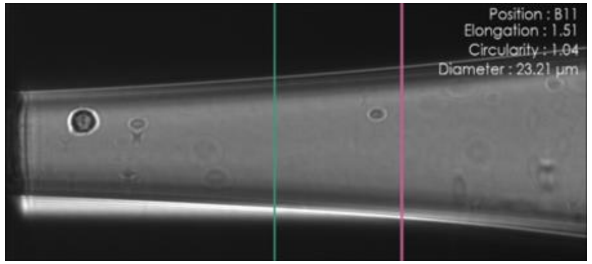
Source: Cellenion

1) Cell suspension is aspirated into a glass capillary
2) Generation of drops on demand, in air
3) Thanks to automated imagining, 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: Laks et al. *Cell*. 179(5):1207-1221.e22. (2019)





Source: Cellenion

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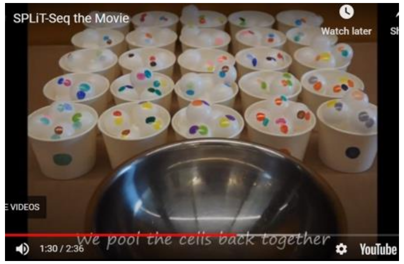
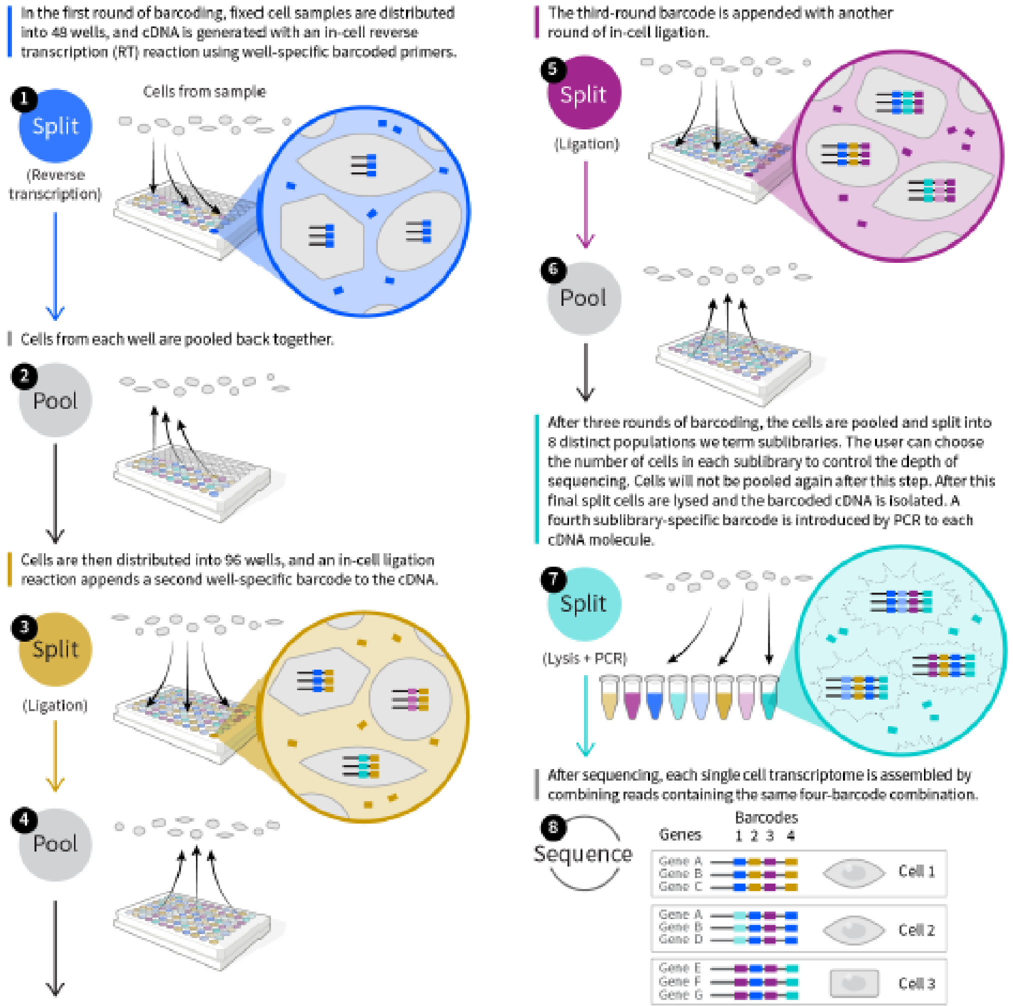
SPLIT-SEQ OVERVIEW (PARSE BIOSCIENCES, SCALE BIO)



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

- Time flexibility – single experiment for samples collected on different dates (up to 6months storage)
- No instrument required for experiment. Computational pipeline available
- Up to 48 samples / 100k cells or 96 samples / 1M cells – kit has to be used at once
- Doublet rate of 0.27% per 1000 cells (3.4% per library)
- Random hexamers method on top of PolyA capture
- Works with any species, any sizes of cells/nuclei & results in lower background noise
- CRISPR, TCR, BCR profiling compatibility



In the first round of barcoding, fixed cell samples are distributed into 48 wells, and cDNA is generated with an in-cell reverse transcription (RT) reaction using well-specific barcoded primers.

Cells from each well are pooled back together.

Cells are then distributed into 96 wells, and an in-cell ligation reaction appends a second well-specific barcode to the cDNA.

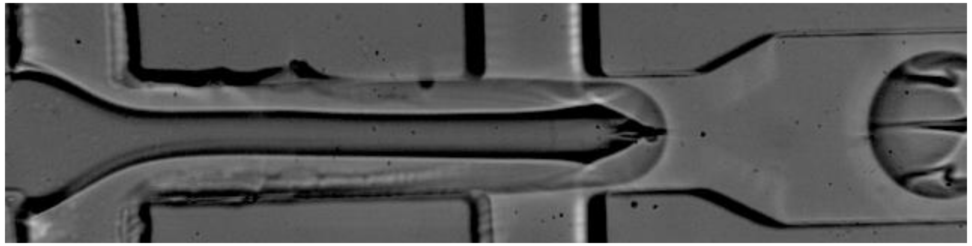
The third-round barcode is appended with another round of in-cell ligation.

After three rounds of barcoding, the cells are pooled and split into 8 distinct populations we term sublibraries. The user can choose the number of cells in each sublibrary to control the depth of sequencing. Cells will not be pooled again after this step. After this final split cells are lysed and the barcoded cDNA is isolated. A fourth sublibrary-specific barcode is introduced by PCR to each cDNA molecule.

After sequencing, each single cell transcriptome is assembled by combining reads containing the same four-barcode combination.

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



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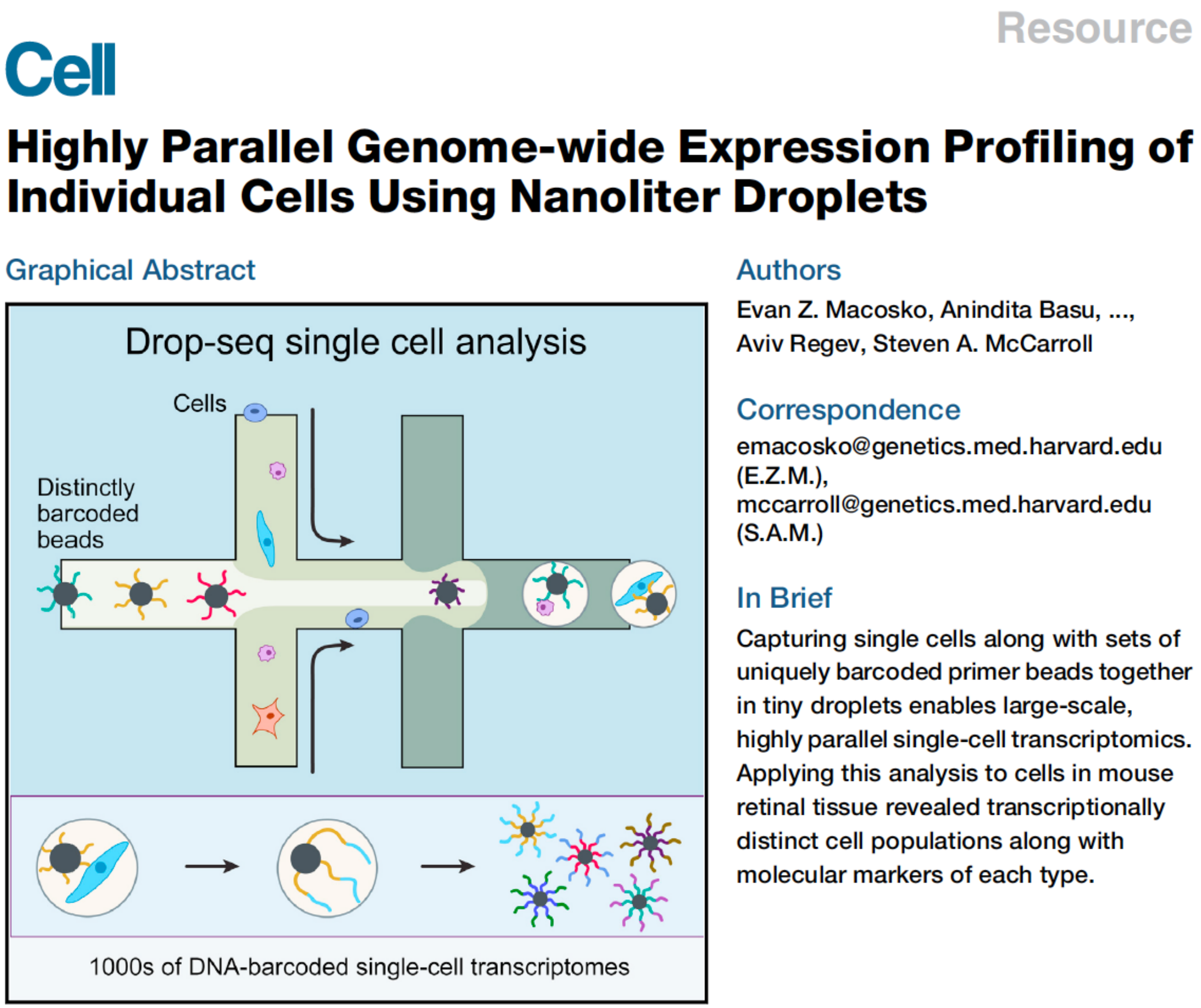

Resource

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

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In Brief

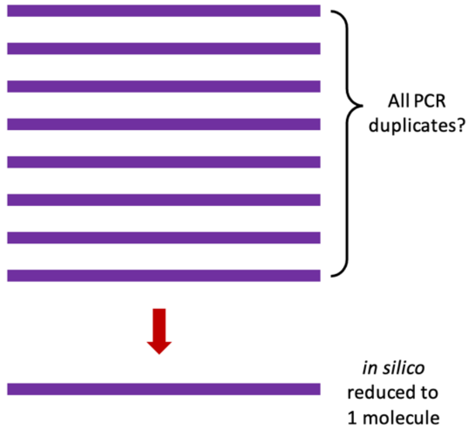
Capturing single cells along with sets of uniquely barcoded primer beads together in tiny droplets enables large-scale, highly parallel single-cell transcriptomics. Applying this analysis to cells in mouse retinal tissue revealed transcriptionally distinct cell populations along with molecular markers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

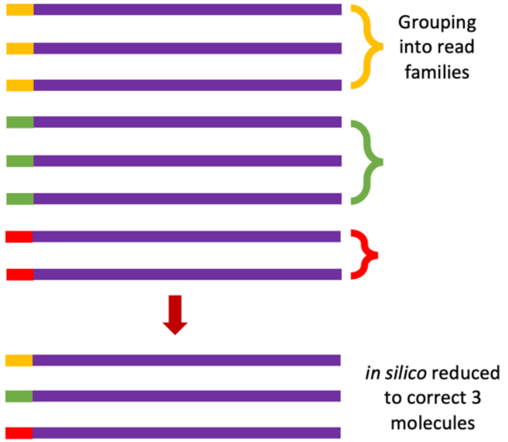
UMI – UNIQUE MOLECULAR IDENTIFIERS

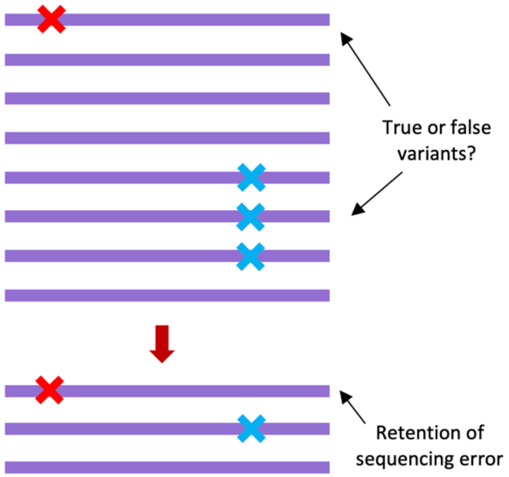
After PCR enrichment, without UMIs, one can not distinguish if multiple copies of a fragment are caused by PCR clones or if they are real biological duplicated.

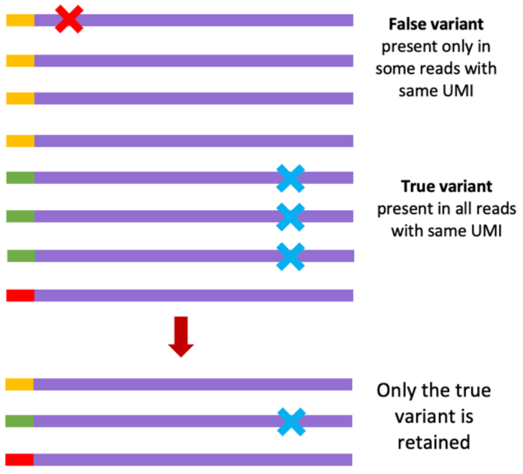
By using UMIs, PCR clones can be found by searching for non-unique fragment-UMI combinations, which can only be explained by PCR clones.

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


PCR duplicate removal **without** UMIs


PCR duplicate removal **with** UMIs


Variant calling **without** UMIs


Variant calling **with** UMIs

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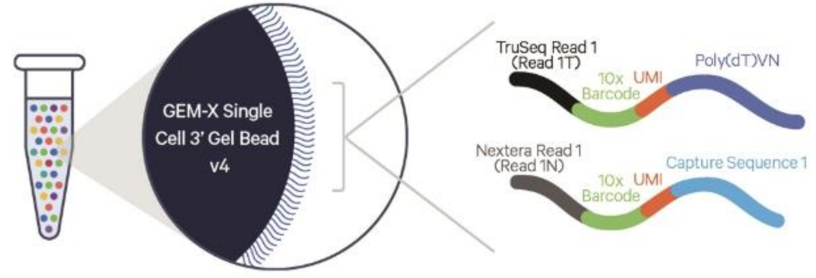
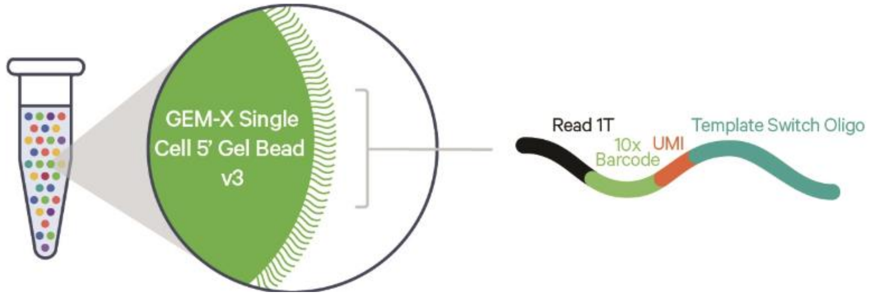
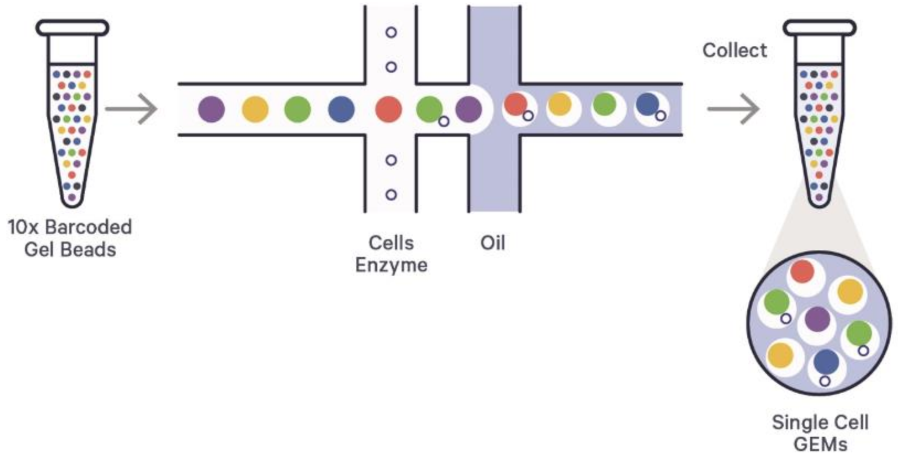
10X GENOMICS OVERVIEW

- 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 - 16 samples can be processed simultaneously with up to 20000 cells captured per sample
- The doublet rate increases with number of cells loaded
- CellRanger and CellLoupe software are available and user friendly
- A lot of additional addons possible (CRISPR, TCR & BCR profiling etc.)







Source: 10x Genomics

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10X GENOMICS OVERVIEW

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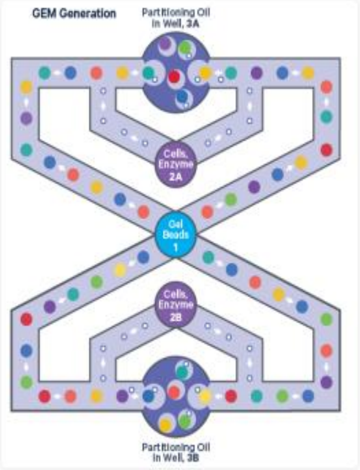
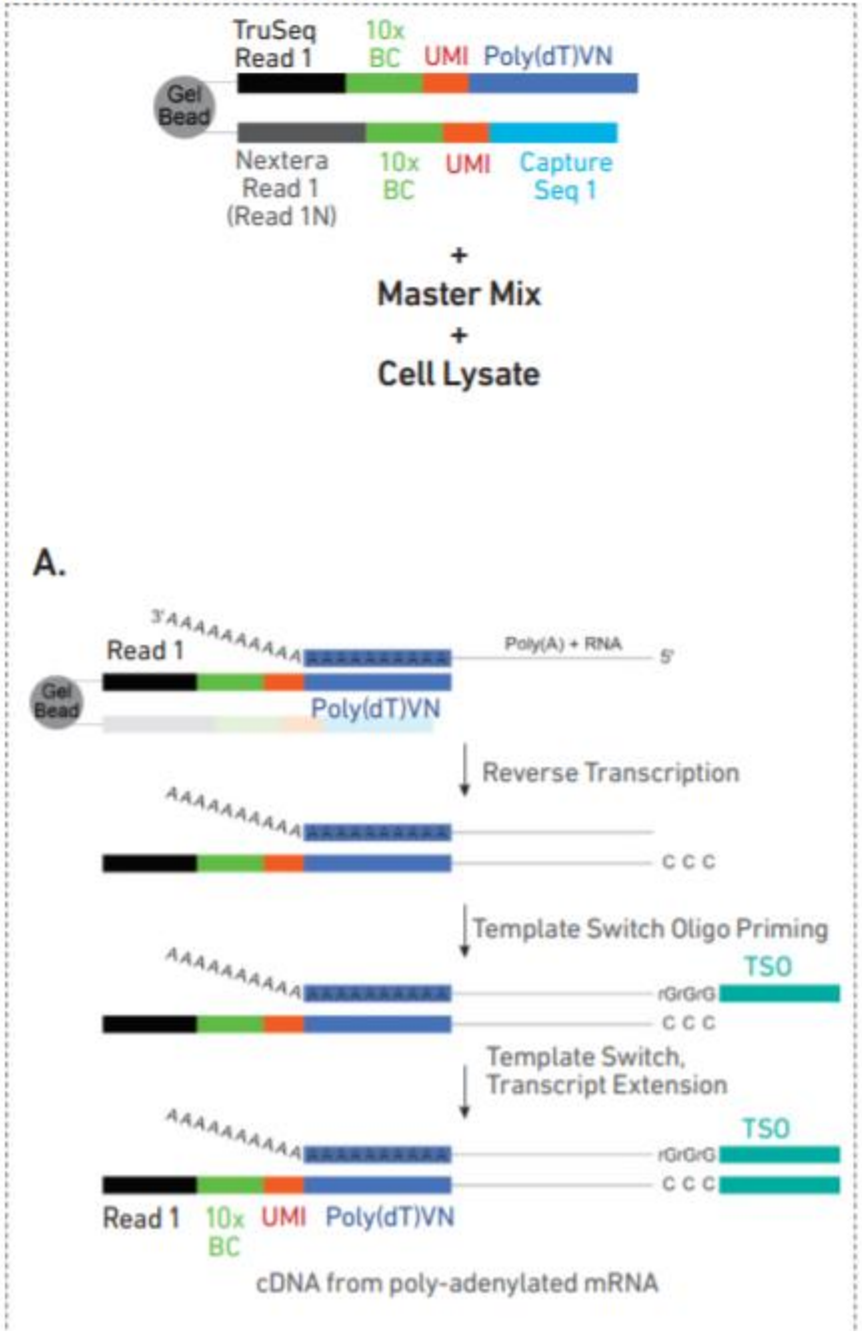


Chromium X

Making 1 million cell experiments routine

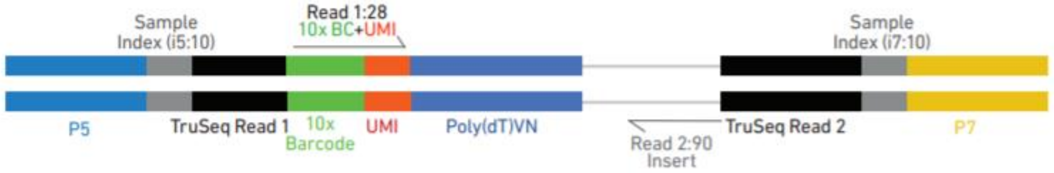
Inside individual GEMs



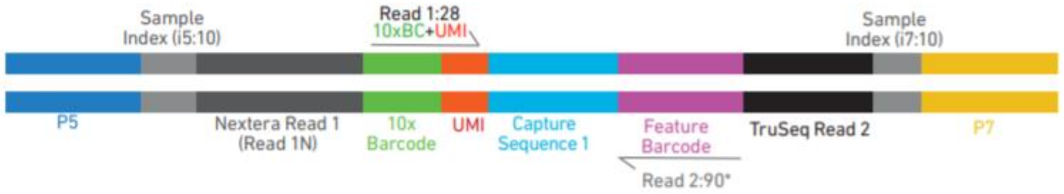
Source: 10x Genomics

10X GENOMICS LIBRARIES

Chromium Single Cell 3' Gene Expression Dual Index Library



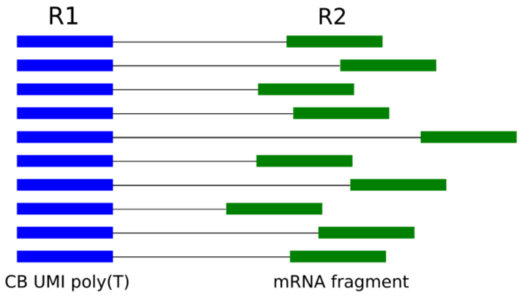
Chromium Single Cell 3' Cell Surface Protein Dual Index Library



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

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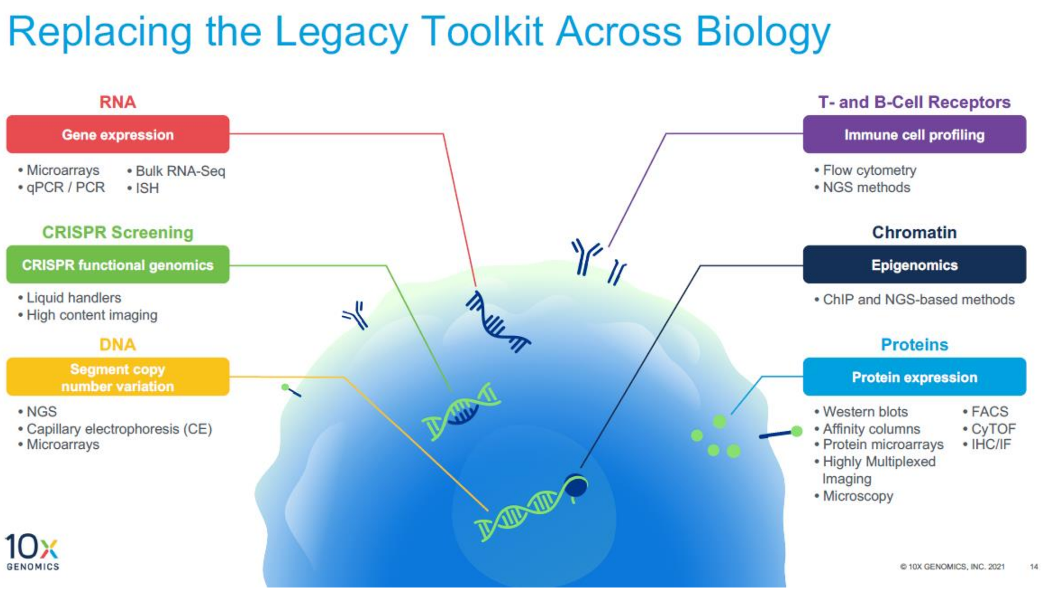
MULTIOMICS AGE



Replacing the Legacy Toolkit Across Biology

RNA

Gene expression

- Microarrays
- qPCR / PCR
- Bulk RNA-Seq
- ISH

CRISPR Screening

CRISPR functional genomics

- Liquid handlers
- High content imaging

DNA

Segment copy number variation

- NGS
- Capillary electrophoresis (CE)
- Microarrays

T- and B-Cell Receptors

Immune cell profiling

- Flow cytometry
- NGS methods

Chromatin

Epigenomics

- ChIP and NGS-based methods

Proteins

Protein expression

- Western blots
- Affinity columns
- Protein microarrays
- Highly Multiplexed Imaging
- Microscopy
- FACS
- CyTOF
- IHC/IF

10x GENOMICS

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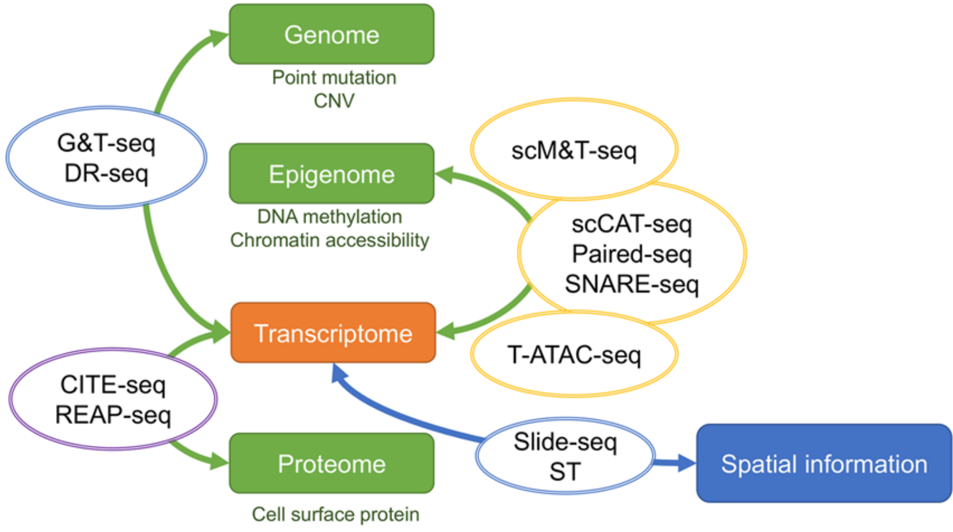
Source: 10x Genomics

TECHNOLOGY FEATURE | 19 July 2021 | Correction 21 July 2021

Single-cell analysis enters the multiomics age

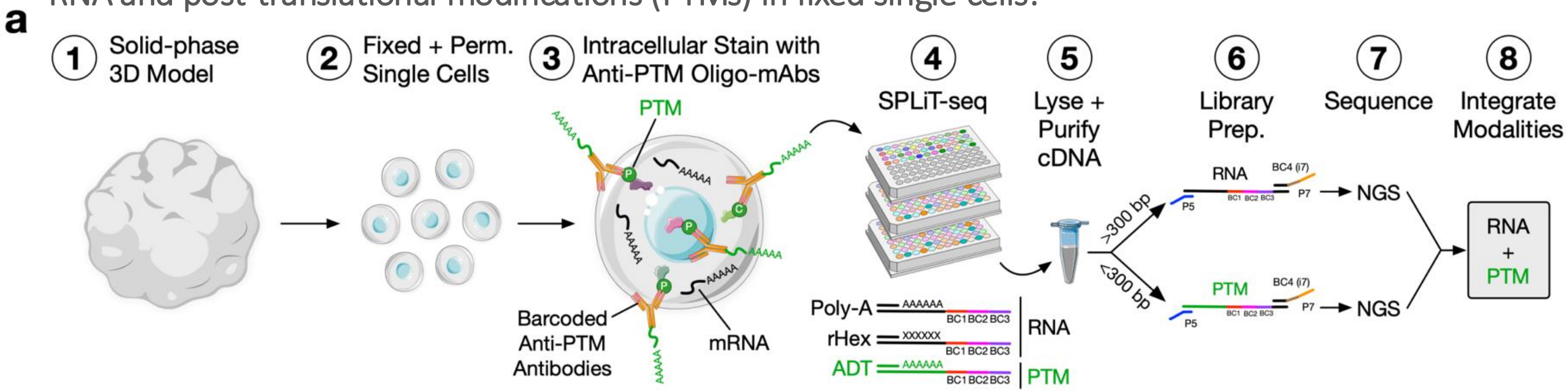
A rapidly growing collection of software tools is helping researchers to analyse multiple huge '-omics' data sets.

Jeffrey M. Perkel



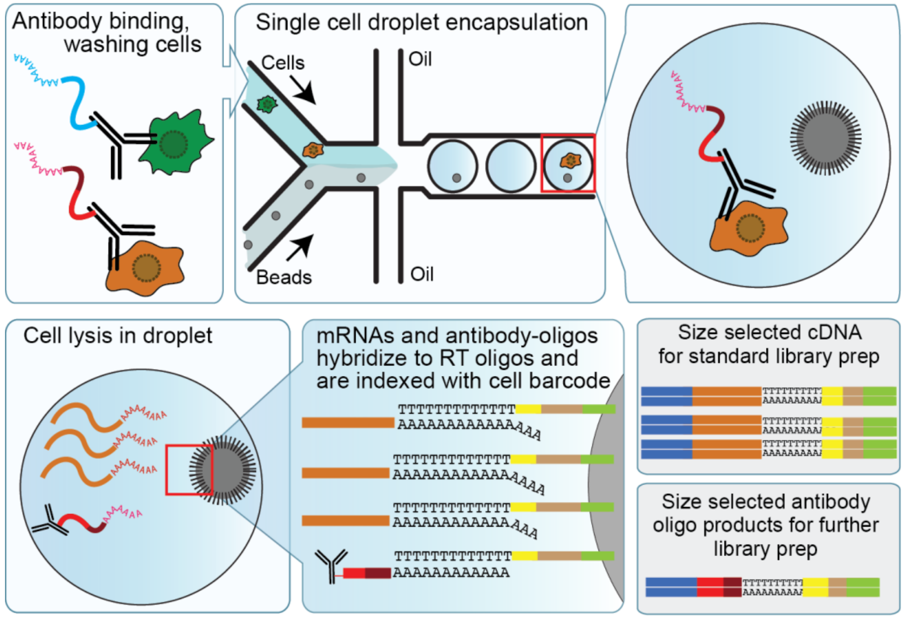
Kashima Y et al. Exp Mol Med 52, 1419–1427 (2020)

SIGNAL-seq: a multiplexed split-pool combinatorial barcoding method that simultaneously measures RNA and post-translational modifications (PTMs) in fixed single cells.



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

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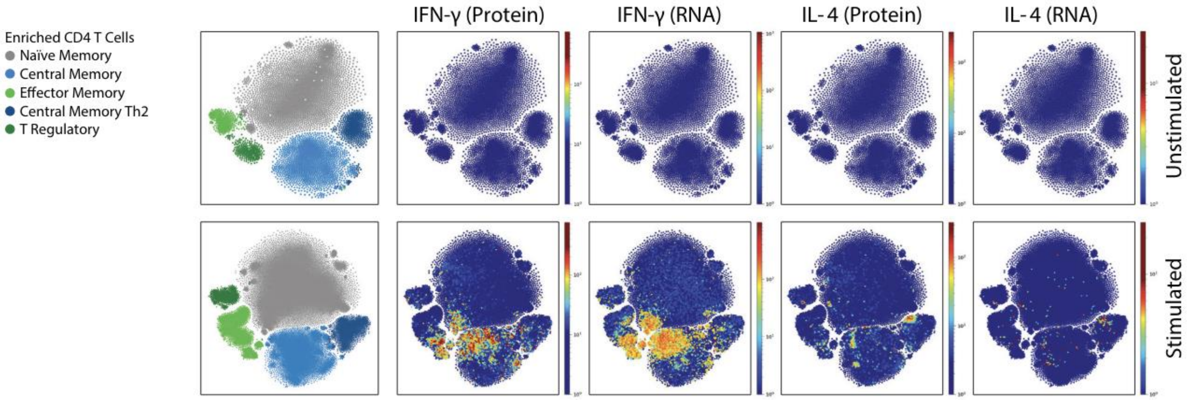
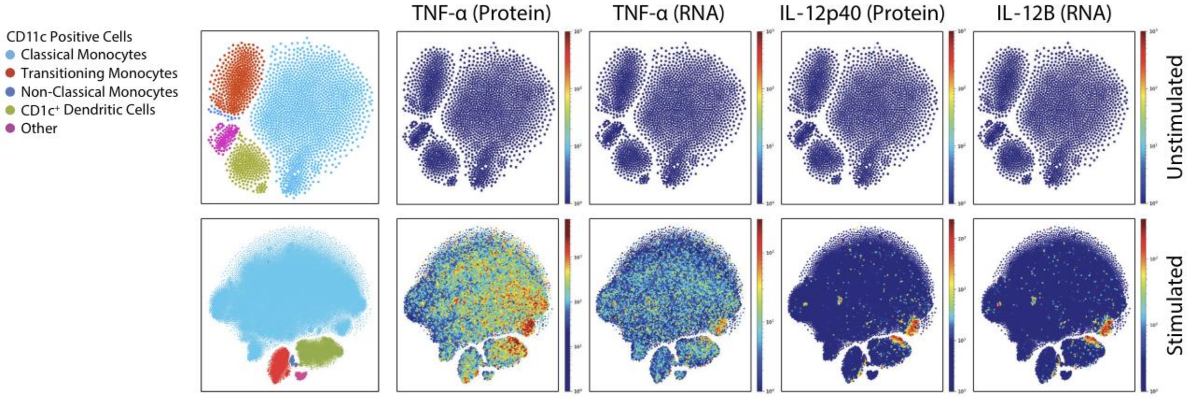
Characterize RNA, Surface, and Intracellular Proteins in the Same Cell

Our TotalSeq-B conjugates offer a wide selection of targets against important secreted and intracellular proteins and complement your single-cell multiomics experiment for comprehensive cellular characterization.



CD4⁺ T cells were magnetically enriched from PBMCs and stimulated with PMA and Ionomycin for six hours in the presence of BFA.



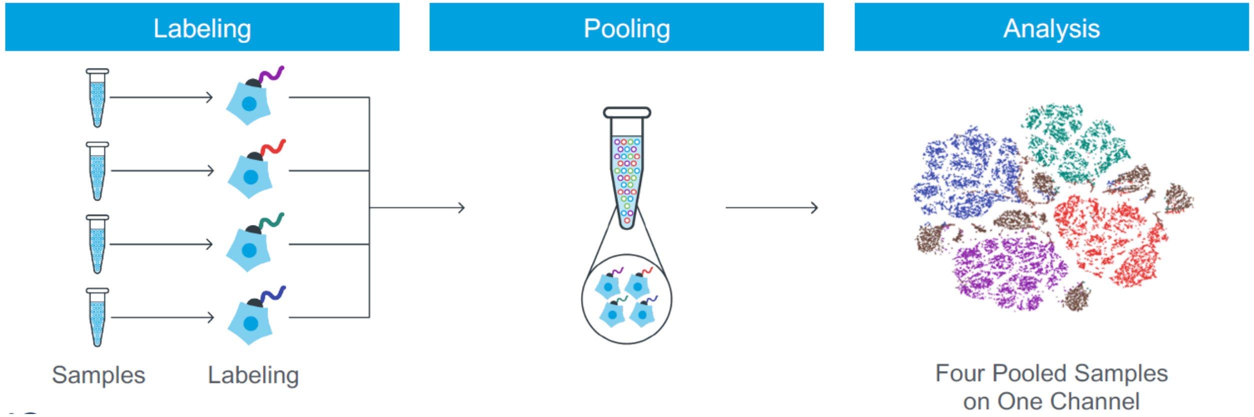
CD14⁺ cells were magnetically enriched from PBMCs and stimulated with GM-CSF overnight in the presence of LPS and R848, and for the final six hours with BFA.

Source: Biolegend, TotalSeq-Intracellular-Targets-Info-Sheet

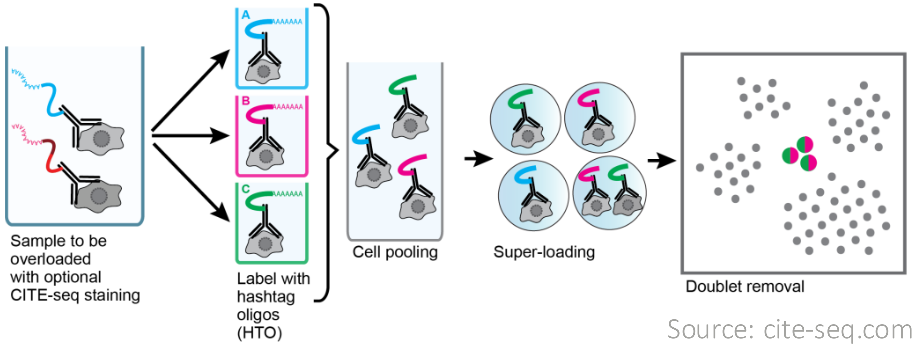
BioLegend solutions:
TotalSeq-A – Poly(dT) based system
TotalSeq-B – 3’ v3.1 Feature barcode
TotalSeq-C – 5’ v2.0

CELL HASHING

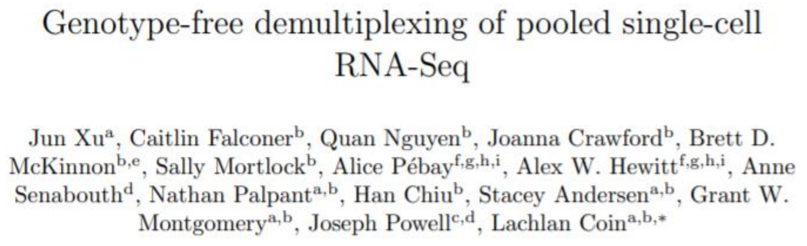
- Reduces cost of running multiple samples by adding hashtag oligos and pooling into single channel of 10x chip (10x CellPlex or TotalSeq antibodies)



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

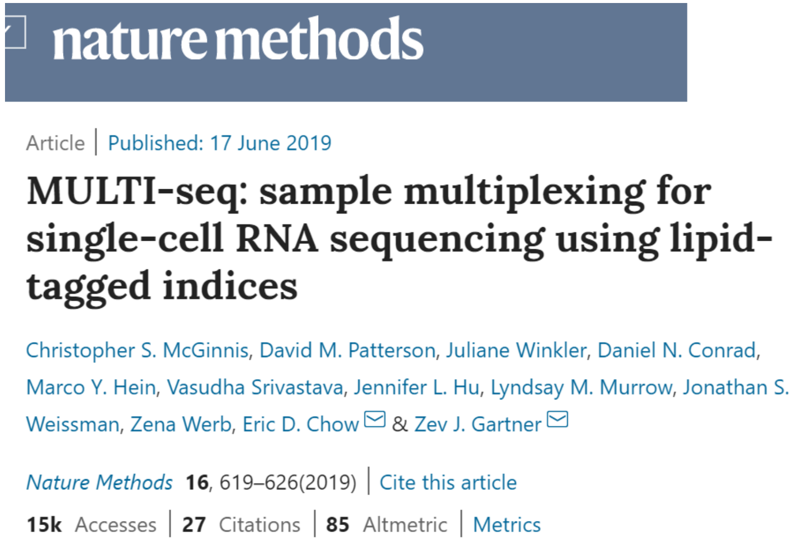


Source: cite-seq.com



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

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



nature methods

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

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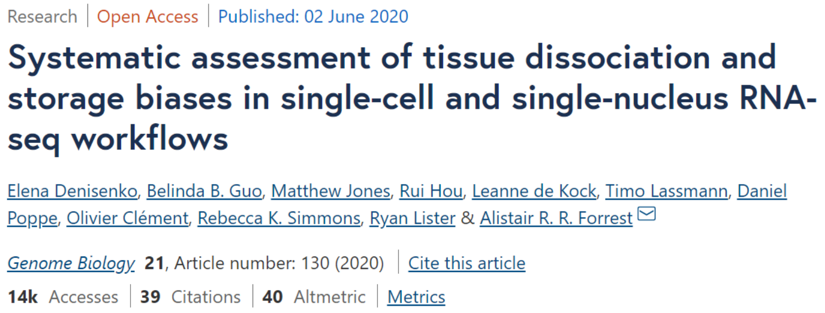
TISSUE PRESERVATION/CELLS FIXATION



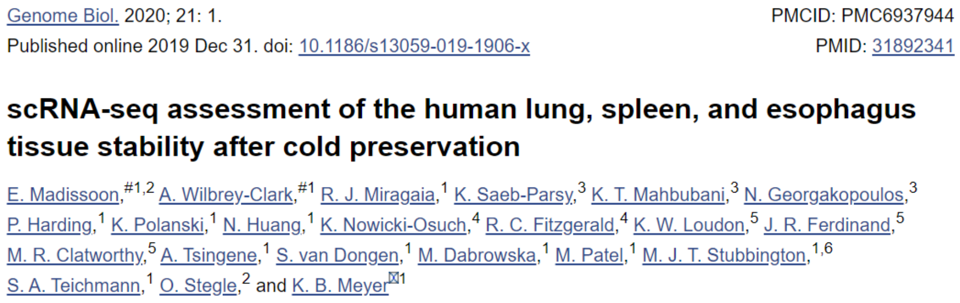
Research | Open Access | Published: 02 June 2020

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

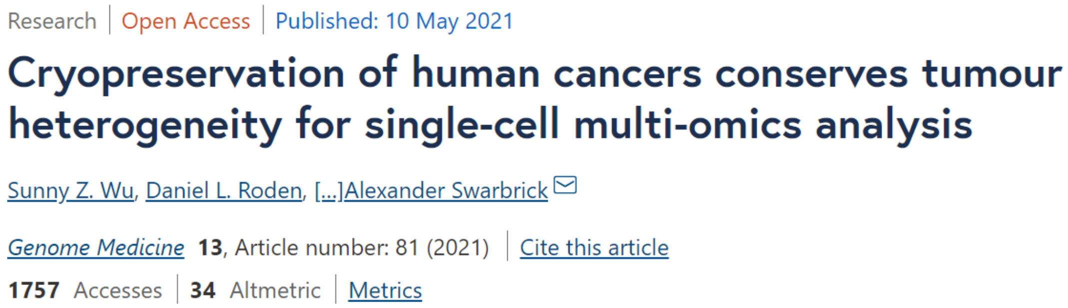


Genome Biol. 2020; 21: 1. PMCID: PMC6937944

Published online 2019 Dec 31. doi: 10.1186/s13059-019-1906-x PMID: 31892341

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

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



Research | Open Access | Published: 10 May 2021

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

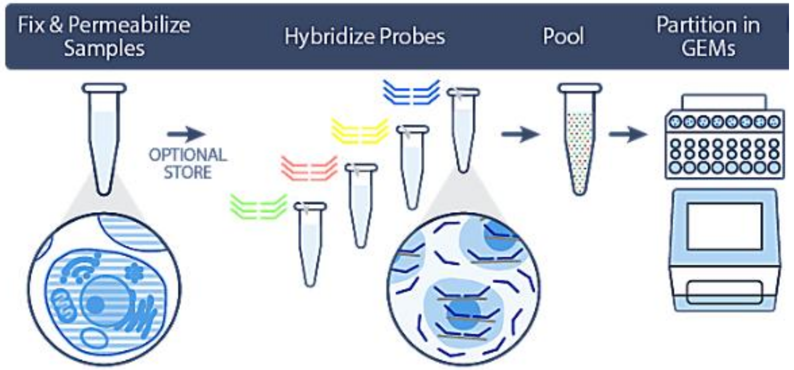
Sunny Z. Wu, Daniel L. Roden, [...]Alexander Swarbrick

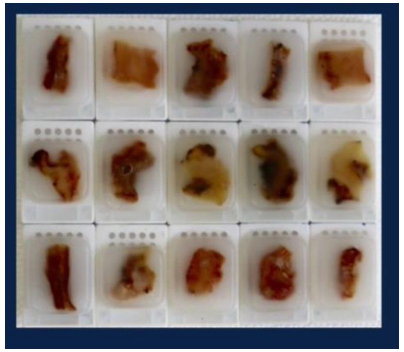
Genome Medicine **13**, Article number: 81 (2021) | Cite this article

1757 Accesses | **34** Altmetric | Metrics

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

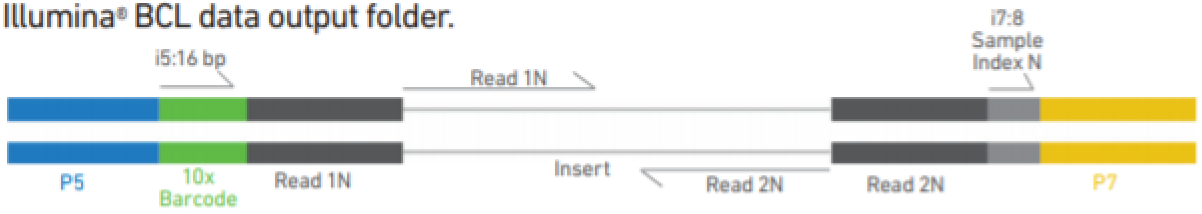




SplitSeq (Parse Bioscience) requires fixation as well

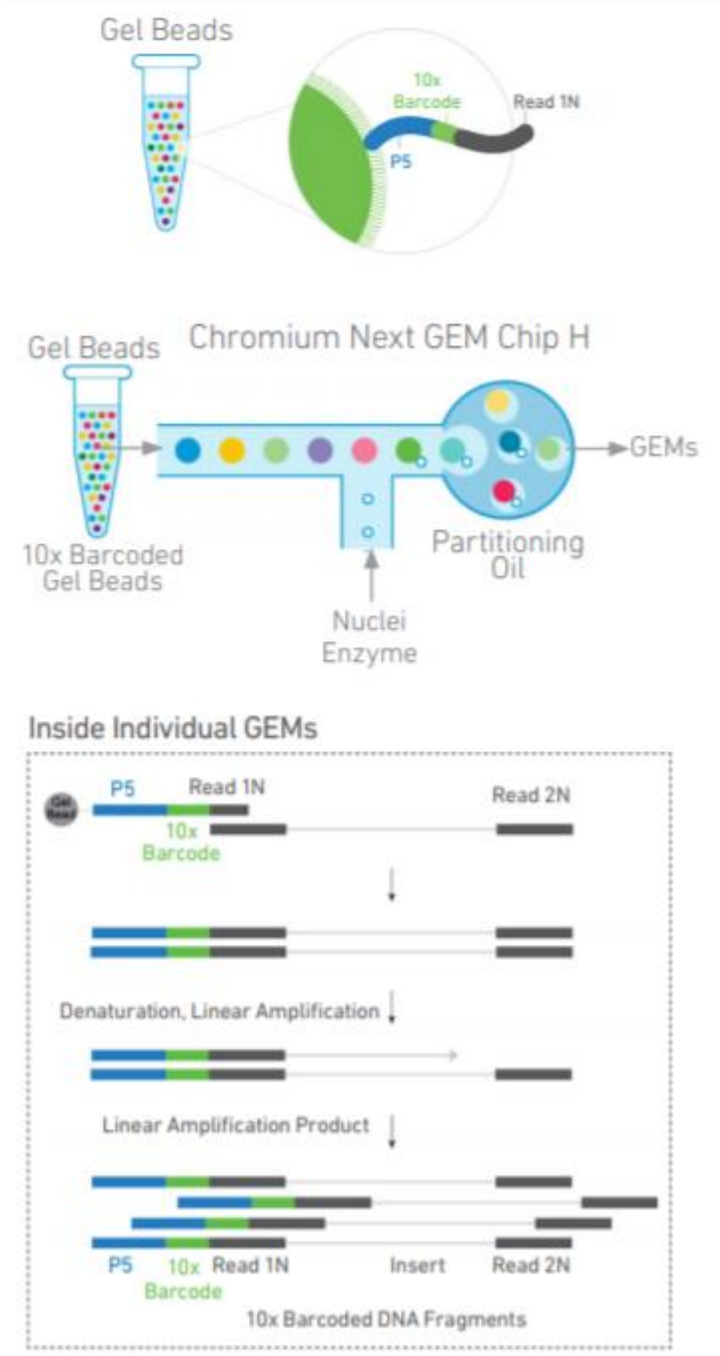
10X ATAC

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.



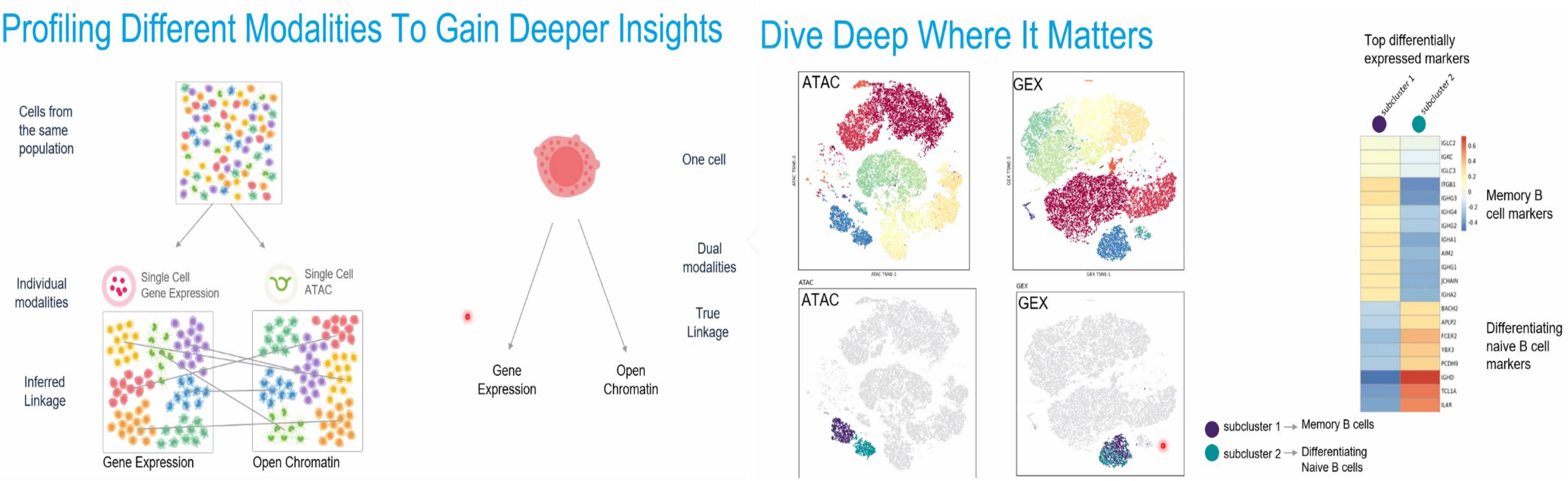
Sequencing Read	Description	Number of cycles
Read1	Insert Sequence 1N	50bp
i7 index	Sample index read	8bp
i5 index	10x Barcode Read (Cell)	16bp
Read2	Insert Sequence 2N (opposite end)	50bp

- **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



Source: 10x Genomics

10X MULTIOME (RNA+ATAC)



Source: 10x Genomics

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

SPATIAL TRANSCRIPTOMICS

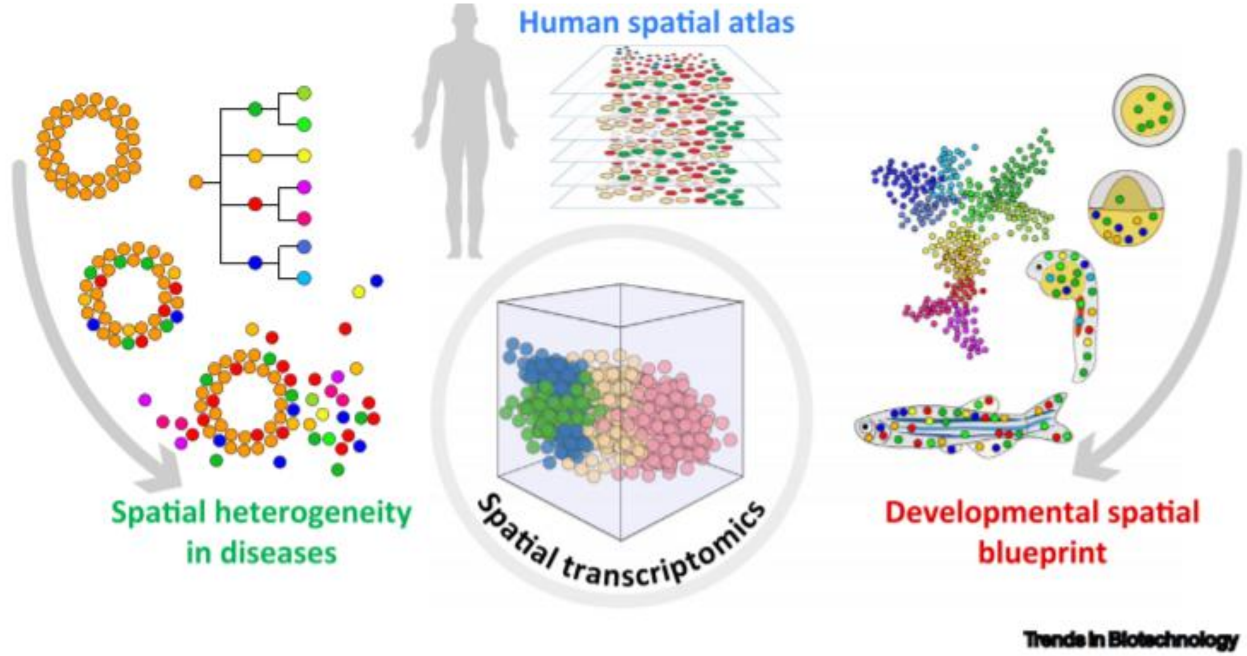
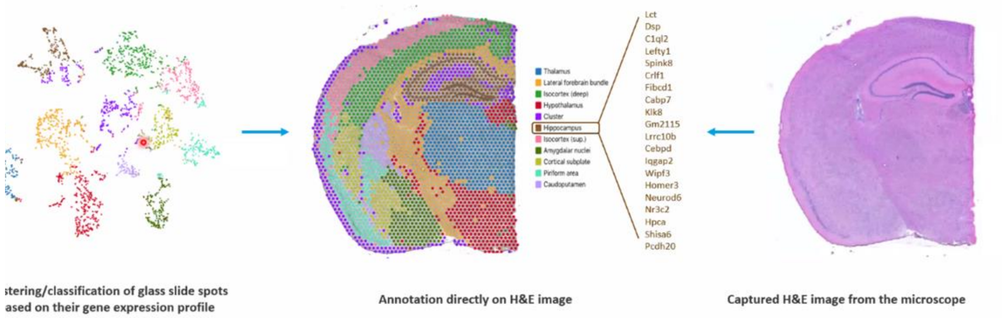


Figure 3. Applications for Spatially Resolved Transcriptomics. Three primary kinds of hot issues can be resolved by spatially resolved transcriptomics: left, discovering spatial heterogeneity of diseases; middle, establishing spatial transcriptome atlases for the human body; and right, delineating an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

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

Visium: 55ul spots -> 1 and 10 cells captured per spot



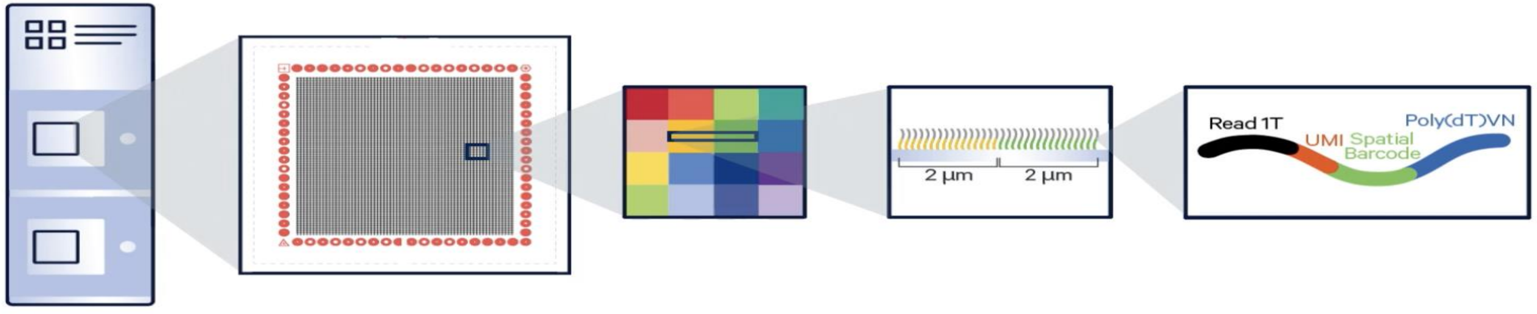
stering/classification of glass slide spots
ased on their gene expression profile

Annotation directly on H&E image

Captured H&E image from the microscope

Source: 10x Genomics

Visium HT:



Visium HD slide, 6.5 mm

Capture area with continuous lawn of oligos, 6.5 x 6.5 mm

Grid of 2 x 2 µm barcoded squares, binned to 8 x 8 µm

Oligo with Spatial Barcode

Source: 10x Genomics

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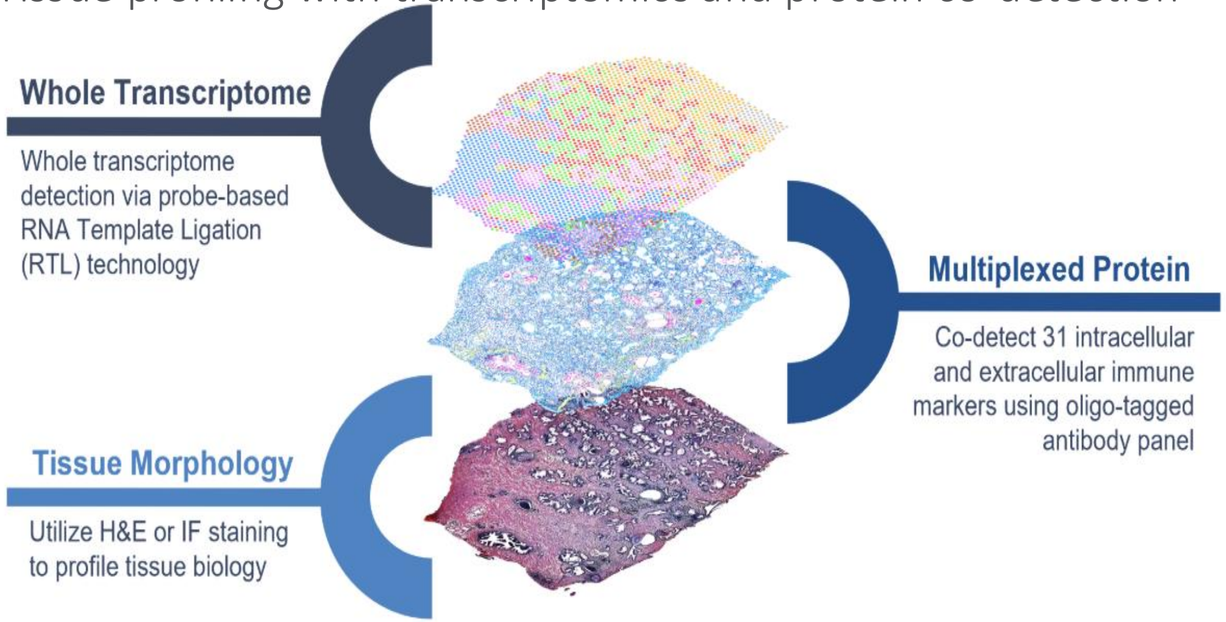
SPATIAL TRANSCRIPTOMICS

Tissue profiling with transcriptomics and protein co-detection



Whole Transcriptome

Whole transcriptome detection via probe-based RNA Template Ligation (RTL) technology

Multiplexed Protein

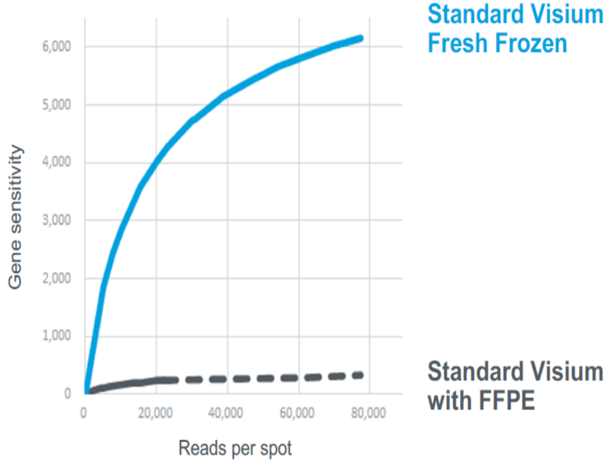
Co-detect 31 intracellular and extracellular immune markers using oligo-tagged antibody panel

Tissue Morphology

Utilize H&E or IF staining to profile tissue biology

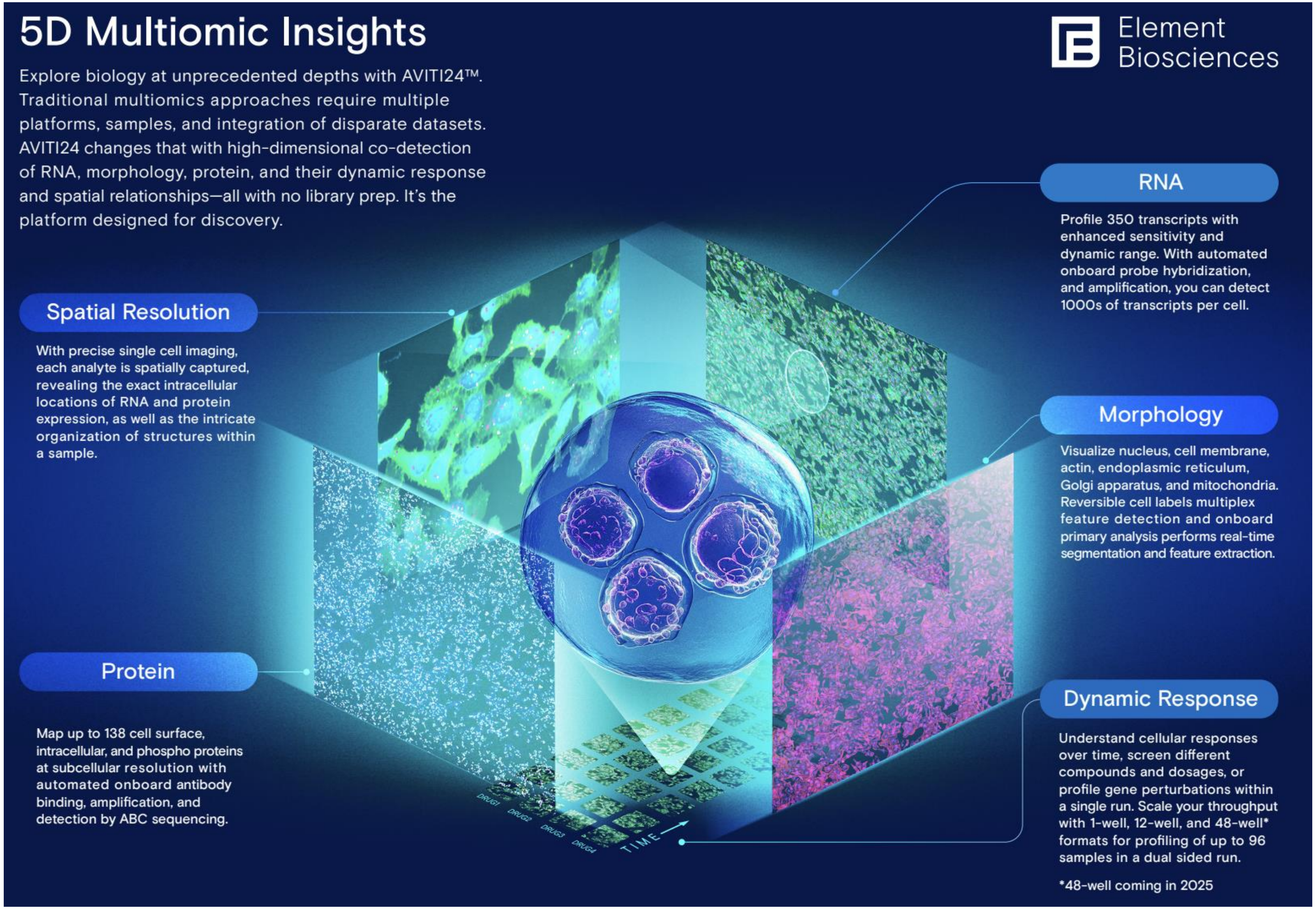
The Challenge of FFPE Samples

FFPE tissue preservation degrades RNA



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

ELEMENT BIOSCIENCES



5D Multiomic Insights

Element Biosciences

Explore biology at unprecedented depths with AVITI24™. Traditional multiomics approaches require multiple platforms, samples, and integration of disparate datasets. AVITI24 changes that with high-dimensional co-detection of RNA, morphology, protein, and their dynamic response and spatial relationships—all with no library prep. It's the platform designed for discovery.

Spatial Resolution

With precise single cell imaging, each analyte is spatially captured, revealing the exact intracellular locations of RNA and protein expression, as well as the intricate organization of structures within a sample.

Protein

Map up to 138 cell surface, intracellular, and phospho proteins at subcellular resolution with automated onboard antibody binding, amplification, and detection by ABC sequencing.

RNA

Profile 350 transcripts with enhanced sensitivity and dynamic range. With automated onboard probe hybridization, and amplification, you can detect 1000s of transcripts per cell.

Morphology

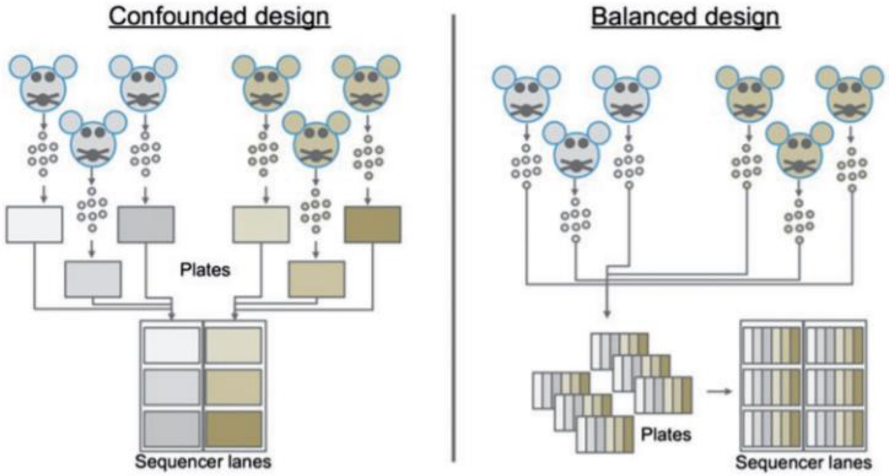
Visualize nucleus, cell membrane, actin, endoplasmic reticulum, Golgi apparatus, and mitochondria. Reversible cell labels multiplex feature detection and onboard primary analysis performs real-time segmentation and feature extraction.

Dynamic Response

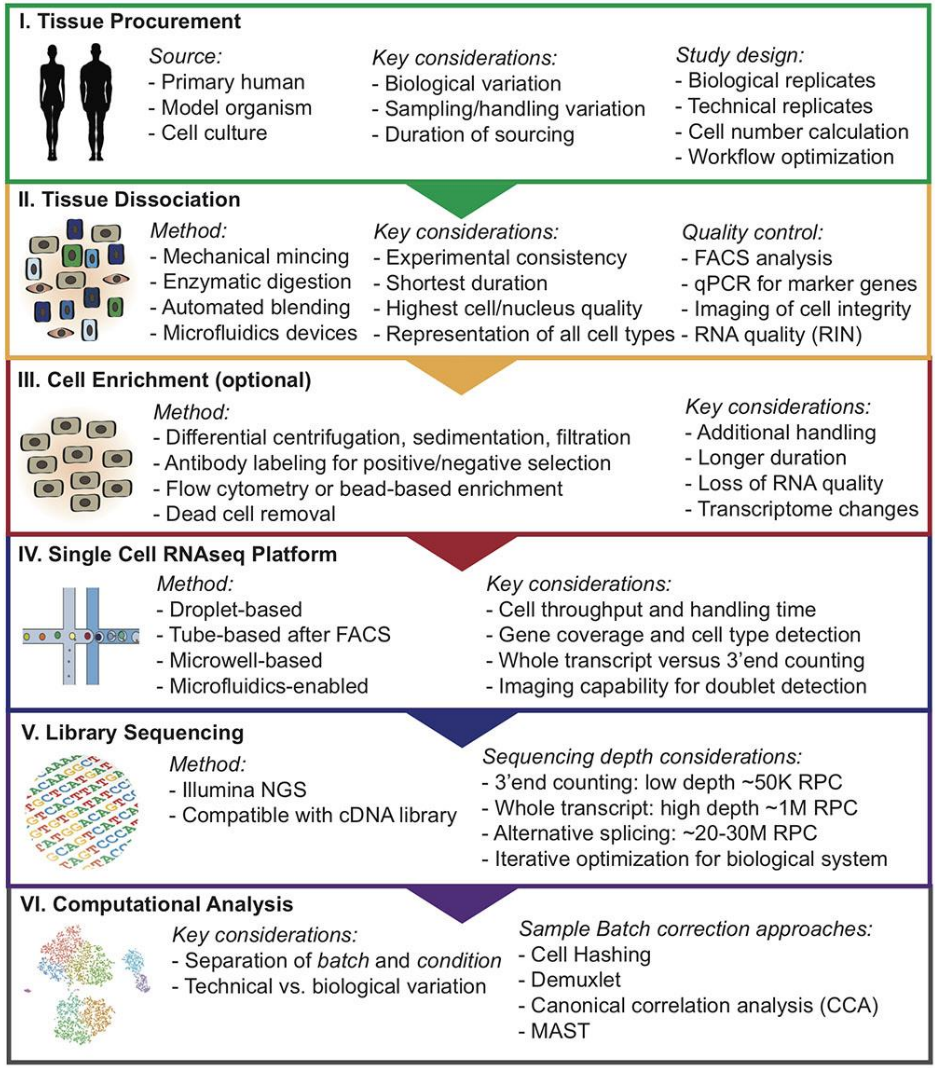
Understand cellular responses over time, screen different compounds and dosages, or profile gene perturbations within a single run. Scale your throughput with 1-well, 12-well, and 48-well* formats for profiling of up to 96 samples in a dual sided run.

*48-well coming in 2025

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)

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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 you 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

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LITERATURE:

- 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
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- A Single-Cell Sequencing Guide for Immunologists. Frontiers in immunology, 9, 2425. (2018).
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- Wang et al. Direct Comparative Analyses of 10X Genomics Chromium and Smart-seq2. Genom Proteom Bioinform Apr;19(2):253-266 (2021).
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- 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).

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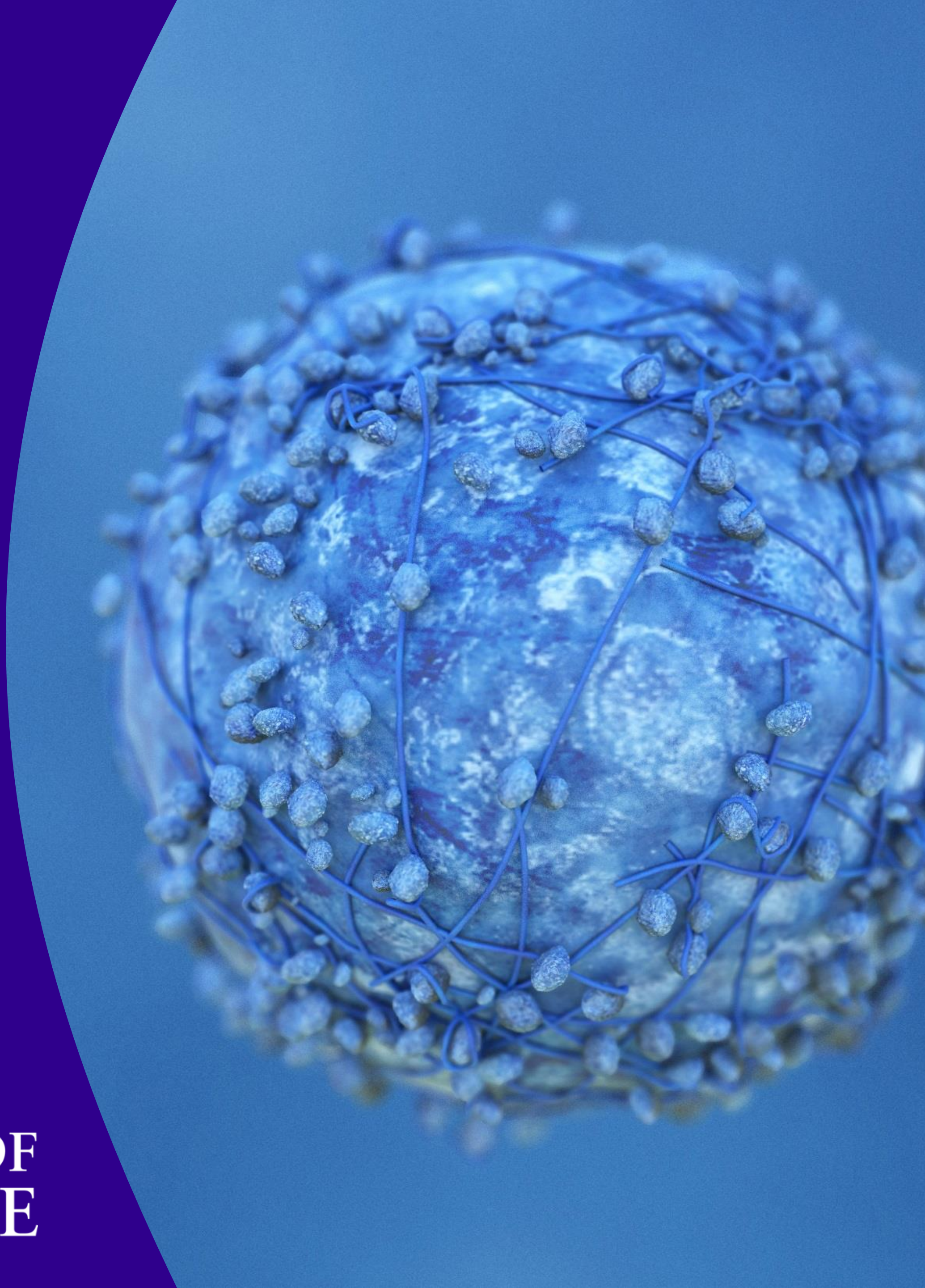
USEFUL RESOURCES:

- A Single-Cell Sequencing Guide for Immunologists. Frontiers in immunology, 9, 2425. (2018).
- Single-cell immune profiling of - gamma-delta T-cells (https://shorturl.at/IXEjc)
- 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)
- 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



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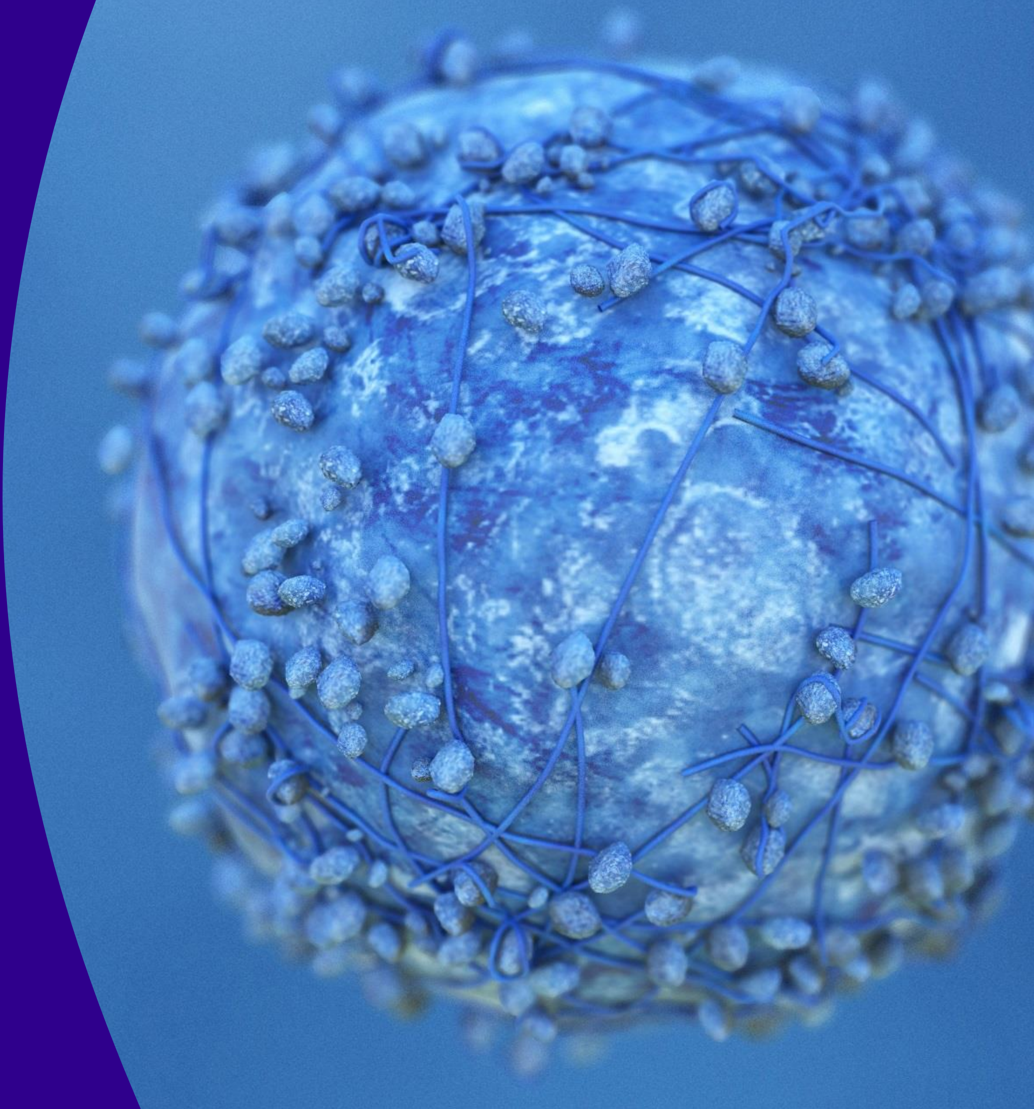
THANK YOU FOR YOUR ATTENTION!



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