



INTRODUCTION TO SINGLE CELL RNA-SEQ

CRUK Bioinformatics Summer School 2021

Katarzyna Kania (CRUK CI Genomics Core Facility)

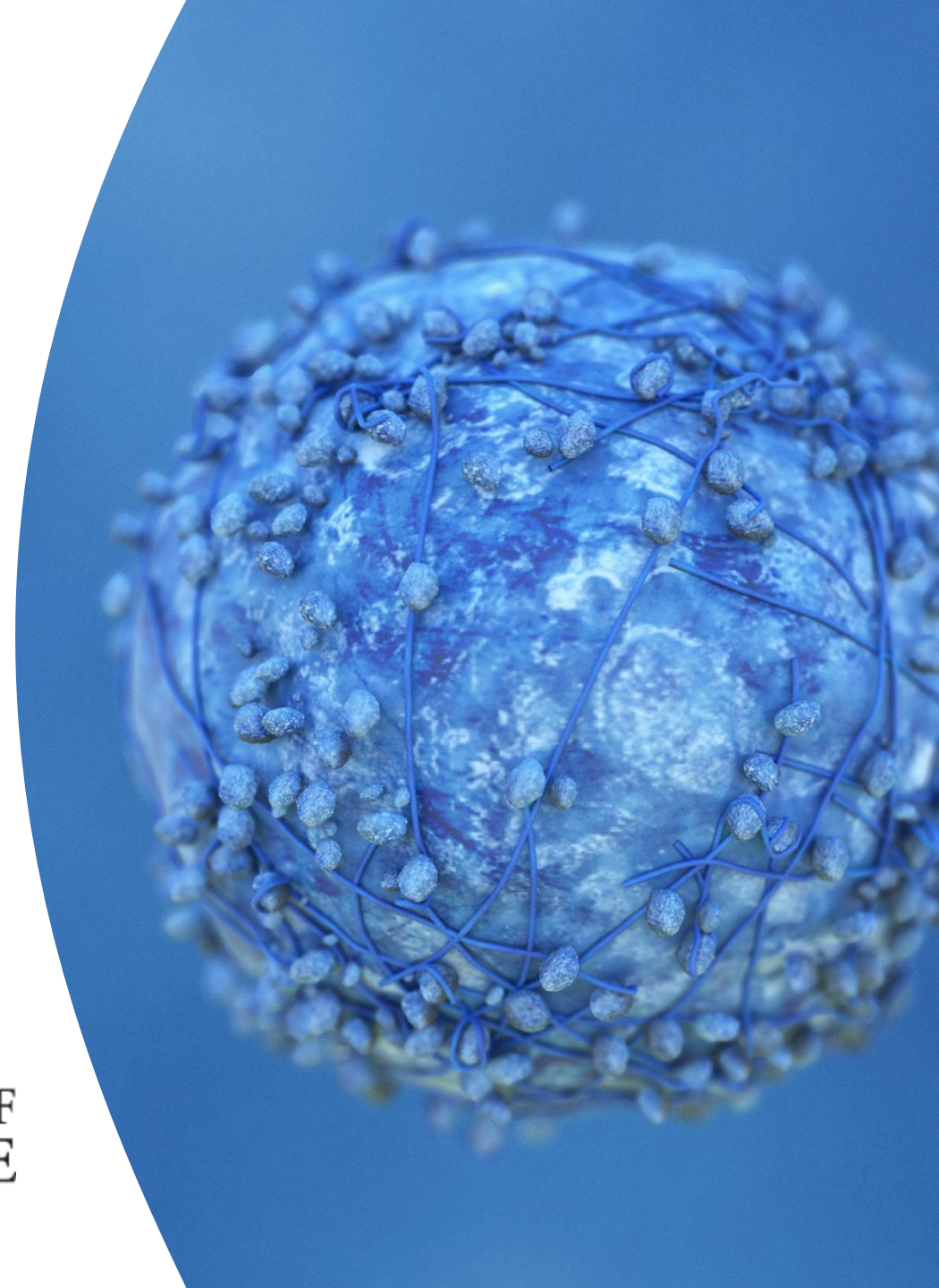
23rd July 2021



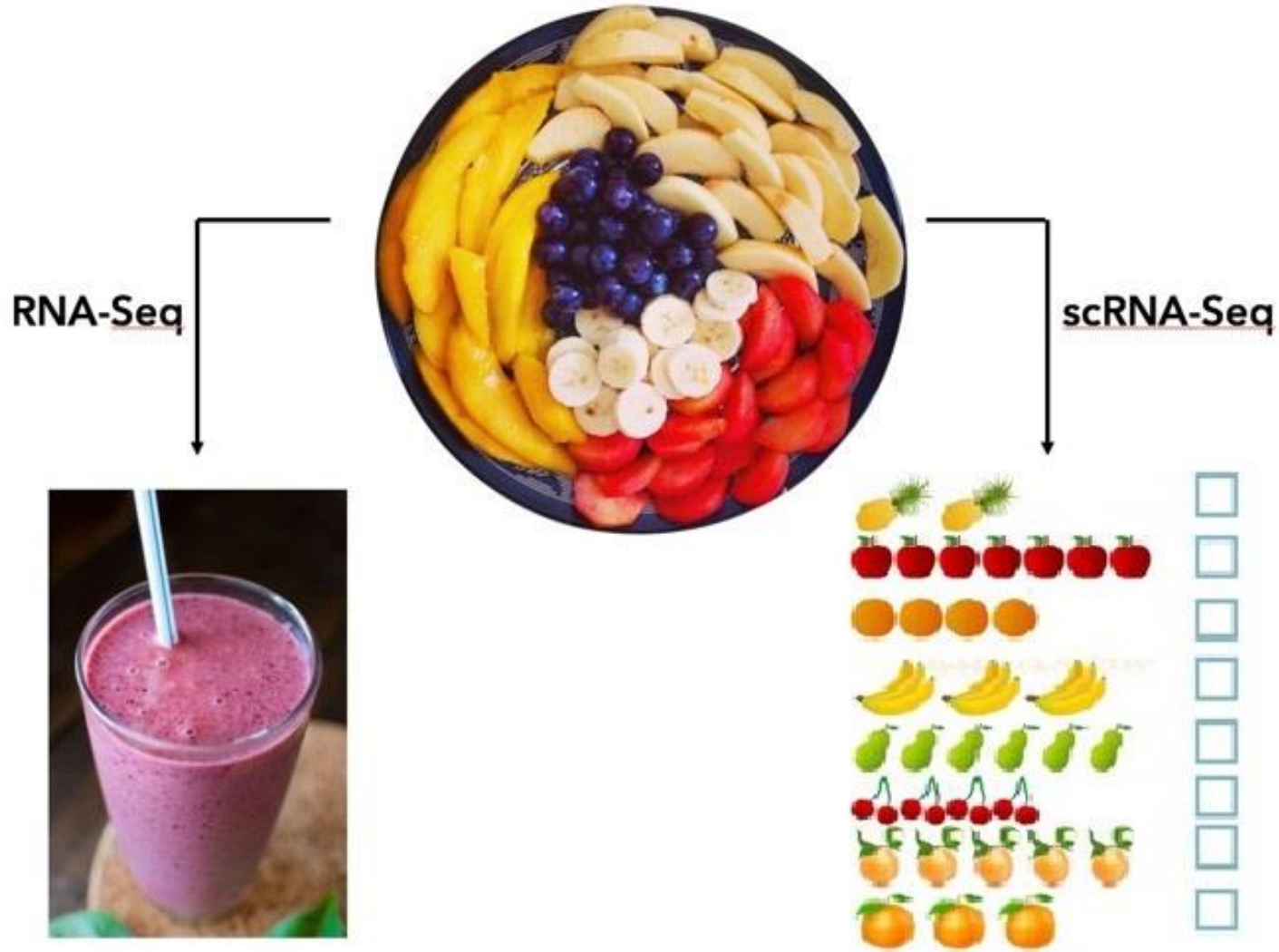
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BULK VS SINGLE CELL RNA-SEQ



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



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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, Giovanni 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

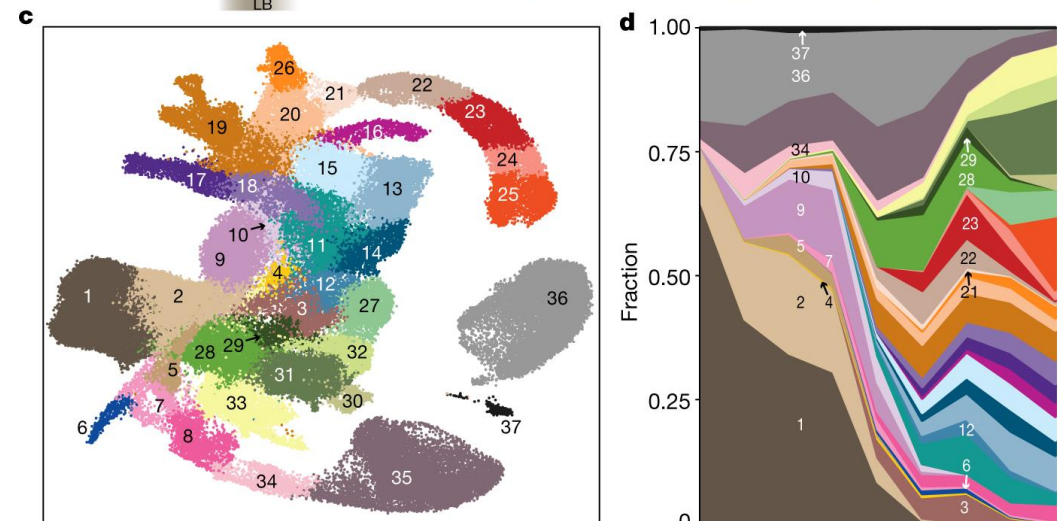
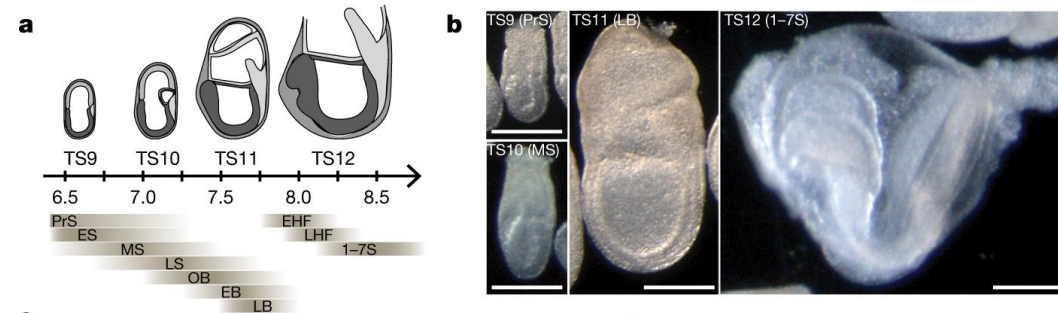
Lindsey W. Plasschaert^{1,3,7}, Rapolas Zilionis^{2,3,7}, Rayman Choo-Wing^{1,5}, Virginia Savova^{2,6}, Judith Knehr⁴, Guglielmo Romà⁴, Allon M. Klein^{2*} & Aron B. Jaffe^{1,3*}

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.



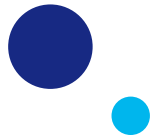
- | | | | |
|-----------------------------|------------------------------|--------------------------------|---------------------------------|
| 1 Epiblast | 12 Caudal mesoderm | 22 Blood progenitors 2 | 30 Neural crest |
| 2 Primitive streak | 13 Paraxial mesoderm | 23 Erythroid 1 | 31 Forebrain/midbrain/hindbrain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

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



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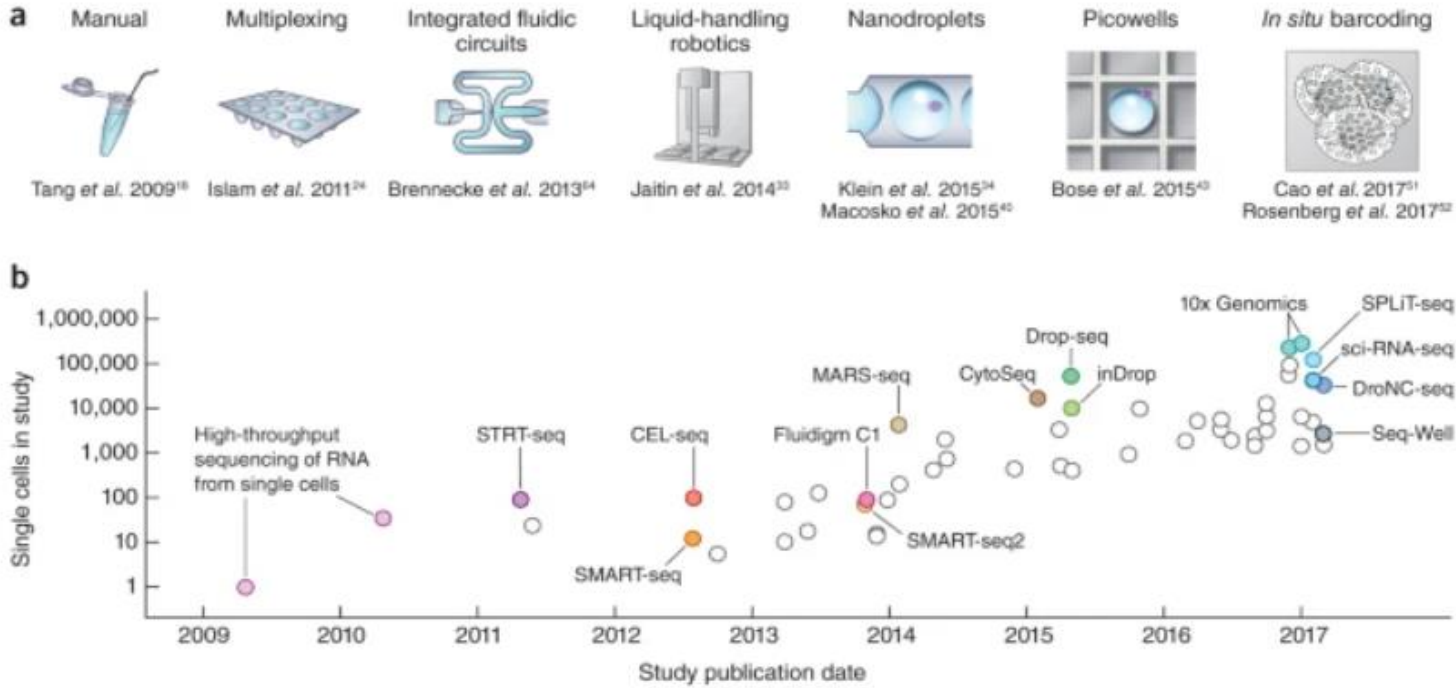
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TECHNOLOGIES



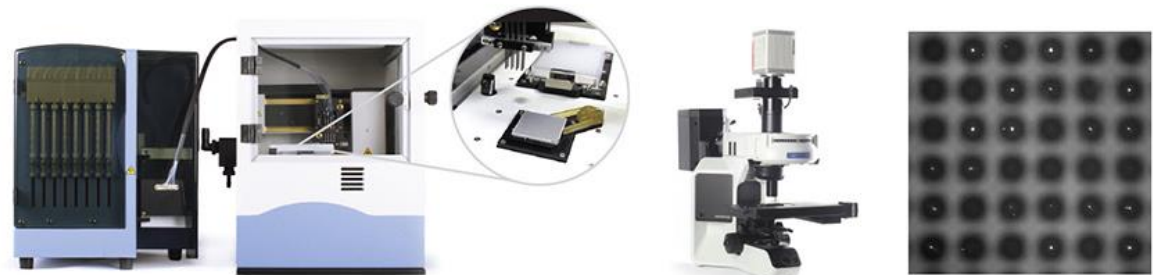
Figure 1: Scaling of scRNA-seq experiments.



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



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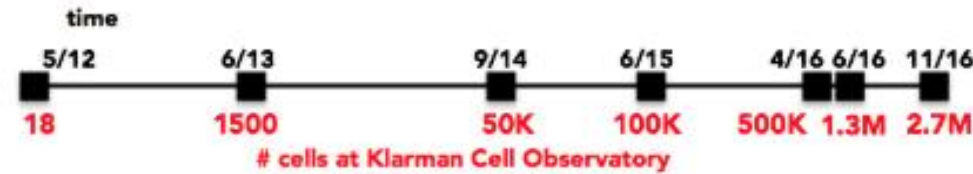


HISTORY AND PROGRESS

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



Resource

2015, 45,000 cells

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



Resource

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

2016, 200,000 cells

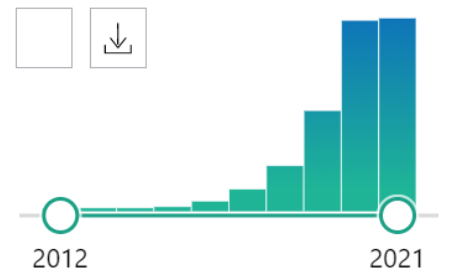
2017, 1.3 million cells (10X genomics)

Publications using 10x Genomics



PubMed search for 'scRNA-seq'

RESULTS BY YEAR



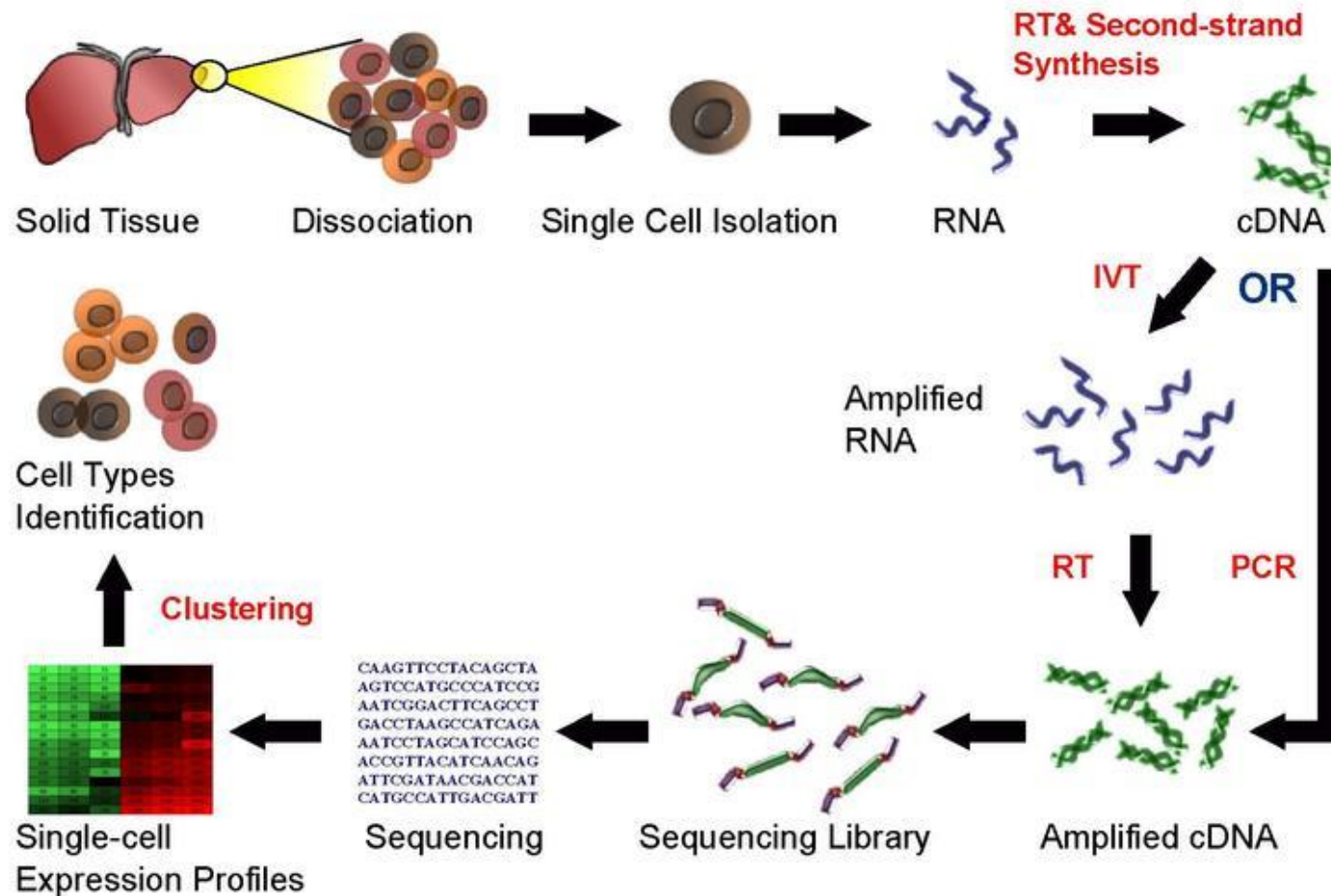
Source: Introduction to scRNASeq, Timothy Tickle & Brian Haas, Broad Institute, 2017

WORKFLOW

Single Cell RNA Sequencing Workflow

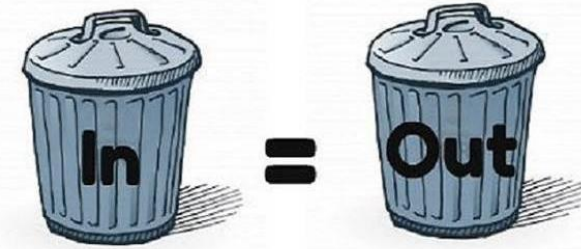


Good sample preparation is key to success!



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 – and generally should be avoided if possible (Nuc-seq might be a solution for frozen samples)



TISSUE PRESERVATION

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](#)

[Immune Netw.](#) 2020 Aug; 20(4): e34.

Published online 2020 Jul 15. doi: [10.4110/in.2020.20.e34](https://doi.org/10.4110/in.2020.20.e34)

PMCID: PMC7458795

PMID: [32895621](https://pubmed.ncbi.nlm.nih.gov/32895621/)


Effects of Cryopreservation and Thawing on Single-Cell Transcriptomes of Human T Cells

[Jeong Seok Lee](#),^{1,†} [Kijong Yi](#),^{1,†} [Young Seok Ju](#),^{1,2} and [Eui-Cheol Shin](#)^{✉1,2}

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Article | [Open Access](#) | Published: 23 July 2019

DMSO cryopreservation is the method of choice to preserve cells for droplet-based single-cell RNA sequencing

[Christian T. Wohnhaas](#), [Germán G. Leparo](#), [Francesc Fernandez-Albert](#), [David Kind](#), [Florian Gantner](#), [Coralie Violet](#), [Tobias Hildebrandt](#) & [Patrick Baum](#) 

Scientific Reports **9**, Article number: 10699 (2019) | [Cite this article](#)

14k Accesses | 20 Citations | 49 Altmetric | [Metrics](#)



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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](#)

[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](#),^{#1,2} [A. Wilbrey-Clark](#),^{#1} [R. J. Miragaia](#),¹ [K. Saeb-Parsy](#),³ [K. T. Mahubani](#),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 article | [Open Access](#) | Published: 09 November 2020

Single-cell transcriptome conservation in a comparative analysis of fresh and cryopreserved human skin tissue: pilot in localized scleroderma

[Emily Mirizio](#), [Tracy Tabib](#), [Xinjun Wang](#), [Wei Chen](#), [Christopher Liu](#), [Robert Lafyatis](#), [Heidi Jacobs](#) & [Kathryn S. Torok](#) 

Arthritis Research & Therapy **22**, Article number: 263 (2020) | [Cite this article](#)

2256 Accesses | 4 Citations | 4 Altmetric | [Metrics](#)

Fixed RNA Profiling Kit

Coming 2H 2021



METHODS

1) Cells in wells, traps and valves (nanowell, Flow sorting, CellenOne, Fluidigm C1, SmartSeq)

- 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



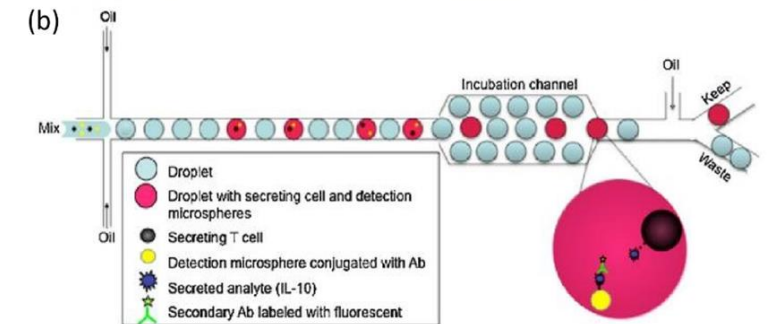
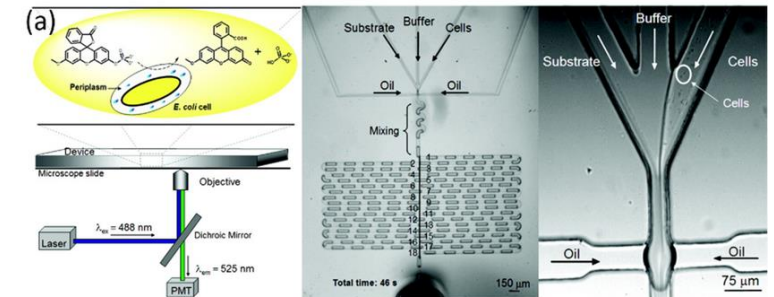
Passive wells



Active pumps and valves

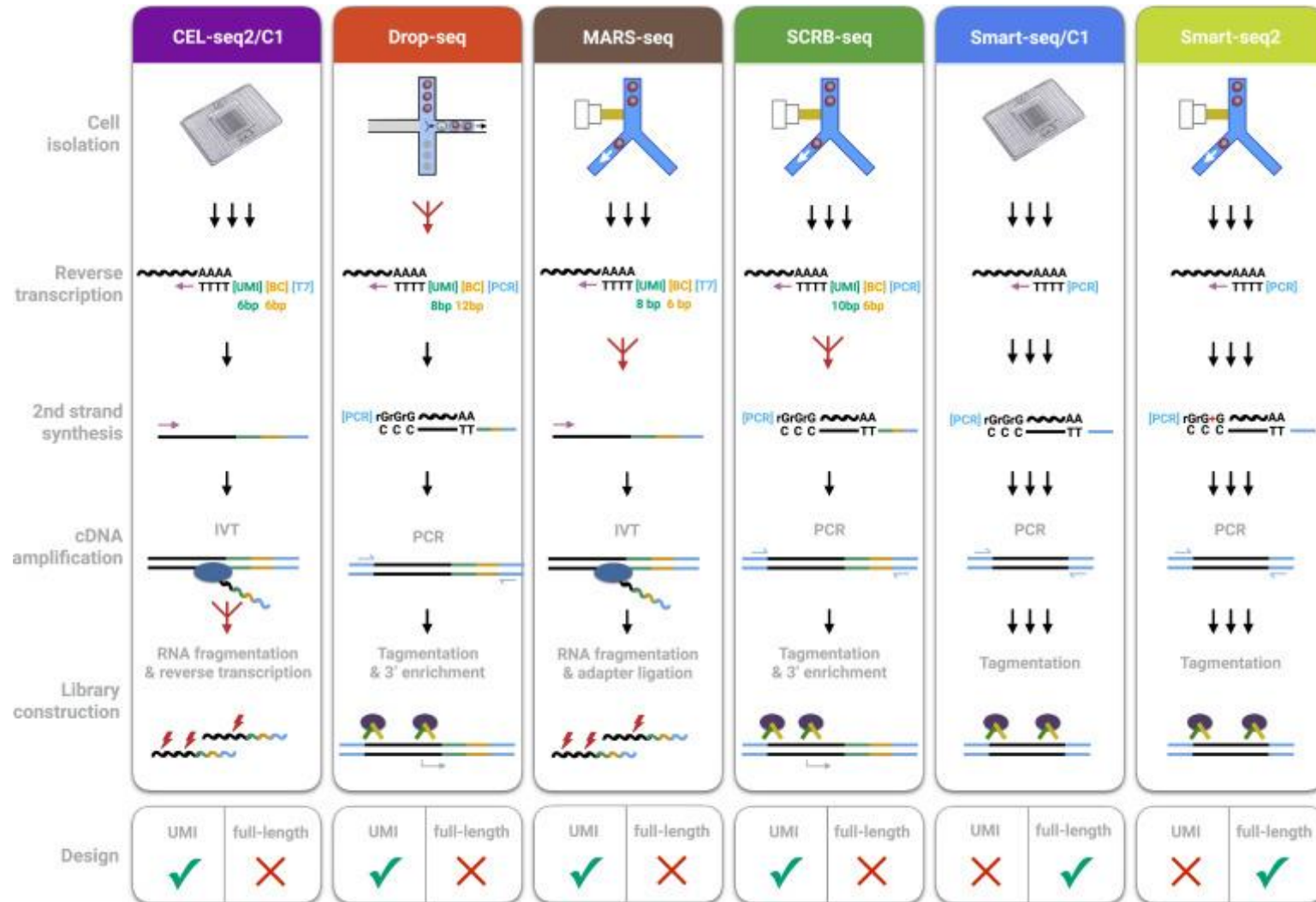
2) Droplets (Drop-seq, 10x Genomics)

- Introduce distinct 'packets' of reagents to single cell (e.g. barcodes)
- Perform amplification on individual cells
- Sort large population of single cells



Source: Wen et al. Molecules (2016)

COMPARISON OF METHODS

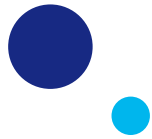


COMPARISON OF METHODS

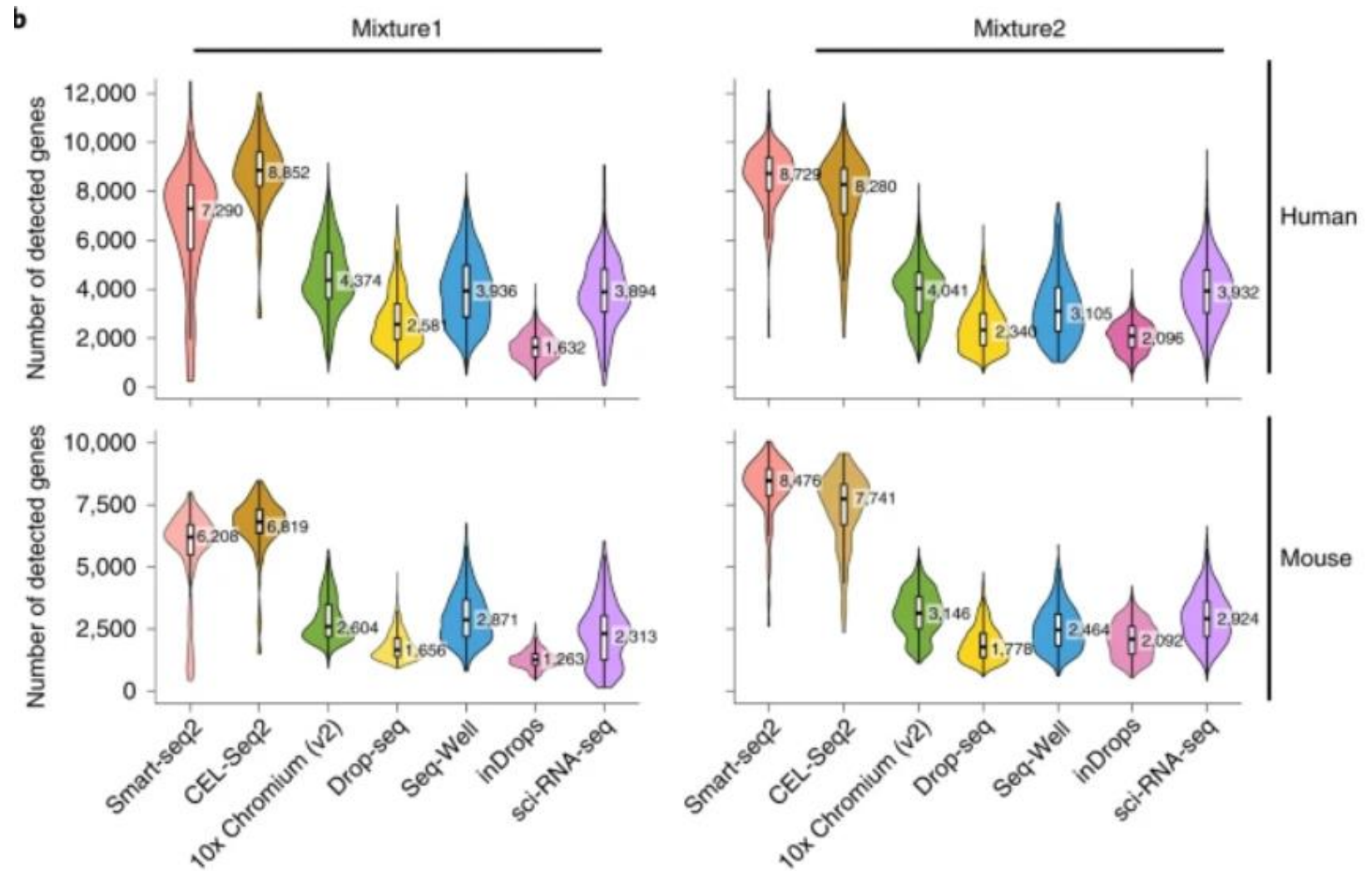
Method	Fluidigm C1 system (SMART-seq)	Fluidigm C1 system (mRNA Seq HT)	SMART-seq2	10X Genomics Chromium system	MARS-seq
cDNA coverage	Full-length	3' counting	Full-length	5'/3' counting	3' counting
UMI	No	No	No	Yes	Yes
Amplification technology	Template switching-based PCR	Template switching-based PCR	Template switching-based PCR	Template switching-based PCR	<i>in vitro</i> transcription
Multiplexing of samples	No	Yes	No	Yes	Yes
Single cell isolation	Fluidigm C1 machine	Fluidigm C1 machine	FACS	10X Genomics Chromium single cell controller	FACS
Cell size limitations	Homogenous size of 5-10, 10-17, or 17-25 μ M	Homogenous size of 5-10, 10-17, or 17-25 μ M	Independent of cell size	Independent of cell size	Independent of cell size
Required cell numbers per run	$\geq 10,000$	$\geq 10,000$	No limitation	$\geq 20,000$	No limitation
Visual quality control check	Microscope examination	Microscope examination	No	No	No
Long term storage	No, must process immediately	No, must process immediately	Yes	No, must process immediately	Yes
Throughput	Limited by number of machines	Limited by number of machines	Limited by operator efficiency	Up to 8 samples per chip	Process is automated
Cost	++++	+++	++++	+	++
Sample Preparation Scenario 1 (~5000 single cell)	Targeted cell No: 4992 cells	Targeted cell No: 4800 cells	Targeted cell No: 4992 cells	Targeted cell No: 5000 cells	Targeted cell No: 4992 cells
	26 rounds of 2 runs (2 C1 machines; concurrent)	3 rounds of 2 runs (2 C1 machines; concurrent)	26 rounds of 2 96-well plates	1 run	13 runs of 1 384-well plate
	~26 weeks	~3 weeks	~26 weeks	~2-3 days	~7 weeks
Sample Preparation Scenario 2 (~96 single cell)	Targeted cell No: 96 cells	Targeted cell No: Minimum 800 cell	Targeted cell No: 96 cells	Targeted cell No: Minimum 500 cells	Targeted cell No: 96 cells
	1 run (1 C1 machine)	1 run (1 C1 machine)	1 run of 96-well plates	1 run	1 run of 384-well plate
	~1 week	~1 week	~1 week	~2-3 days	~2-3 days



PERFORMANCE



Distribution of the number of genes in human or mouse cells in the two mixture experiments.



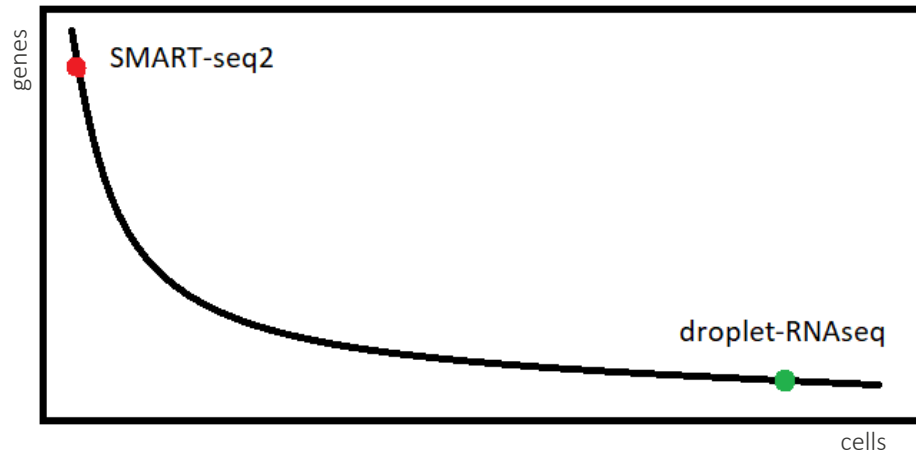
Source: Ding et al. *Nat Biotechnol* 38, 737–746 (2020).



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MORE CELLS OR MORE GENES?

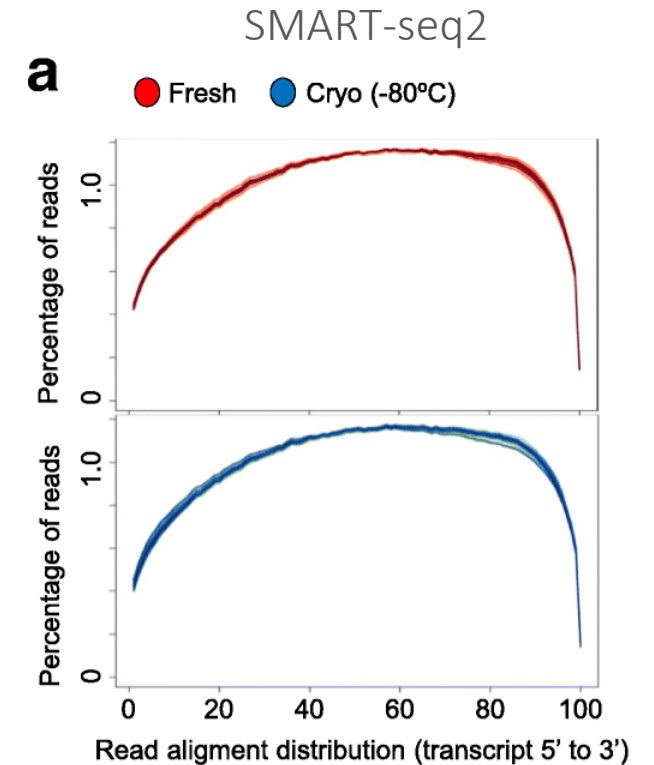
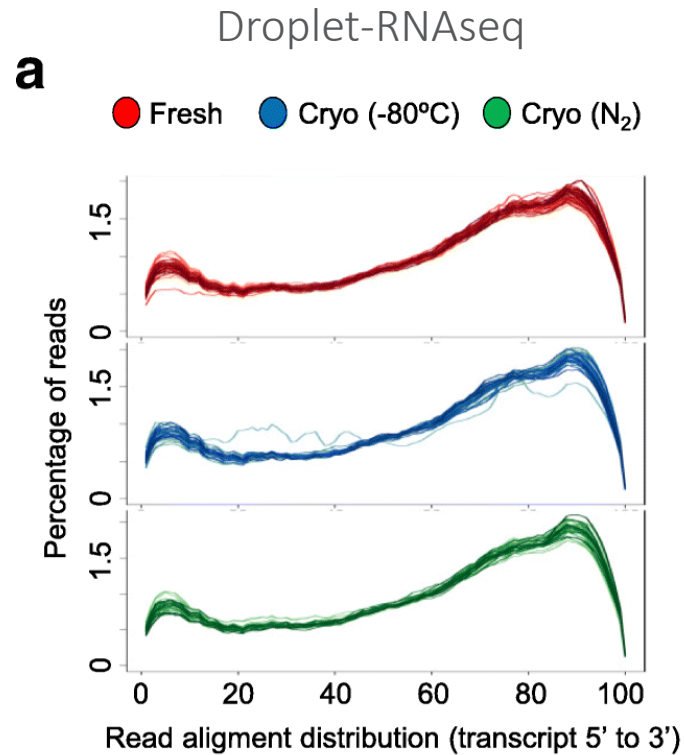


SMART-seq2

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

Droplet-RNAseq

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



Source: [Guillaumet-Adkins, et al. Genome Biol 18, 45 \(2017\).](#)

- 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.



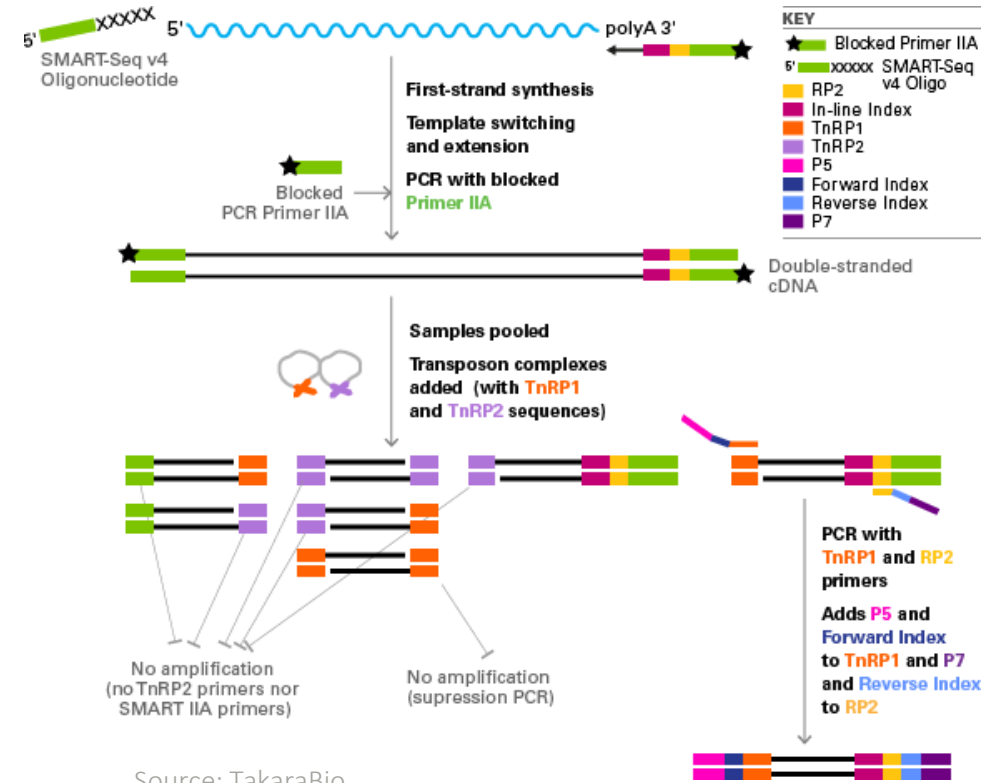
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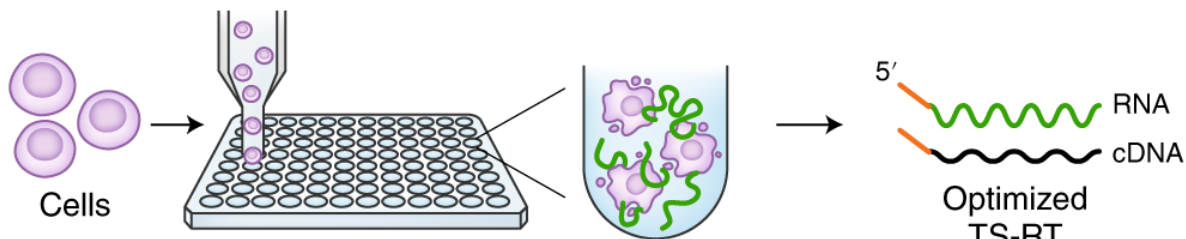
SMART-SEQ2/3/4 OVERVIEW

Developed for single cell but can 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



5' tagged reads

Digital count of transcripts
5' isoform structure

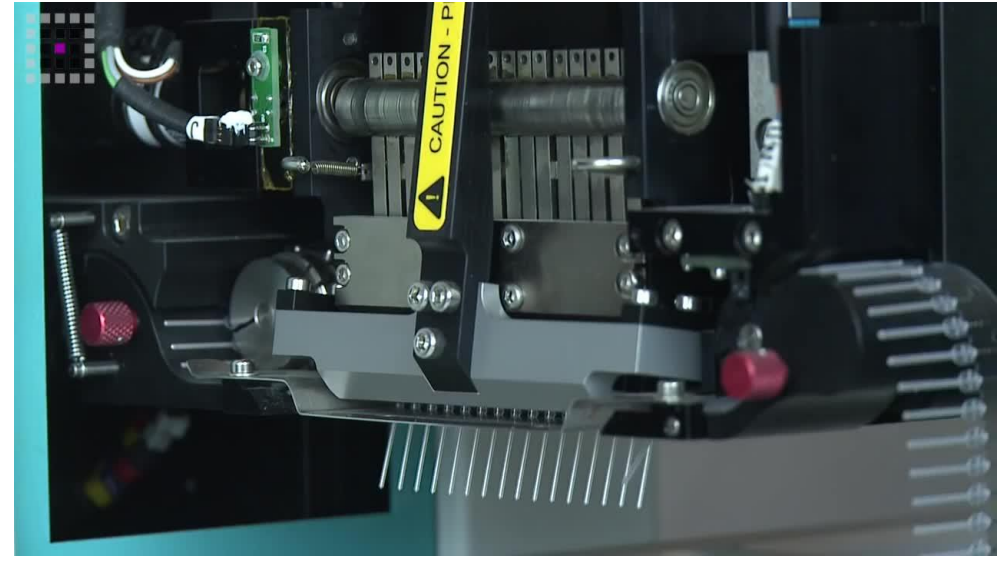
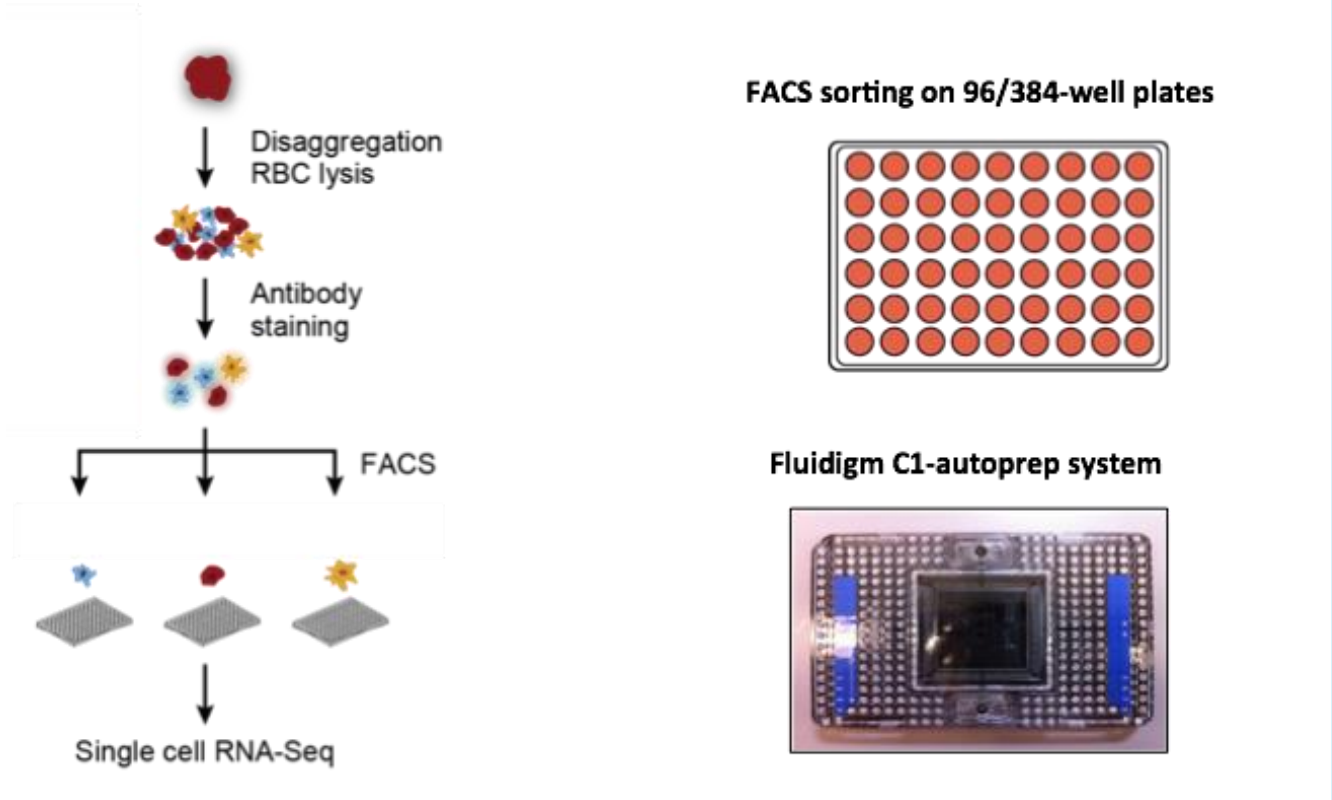
Internal reads

Full isoform reconstruction

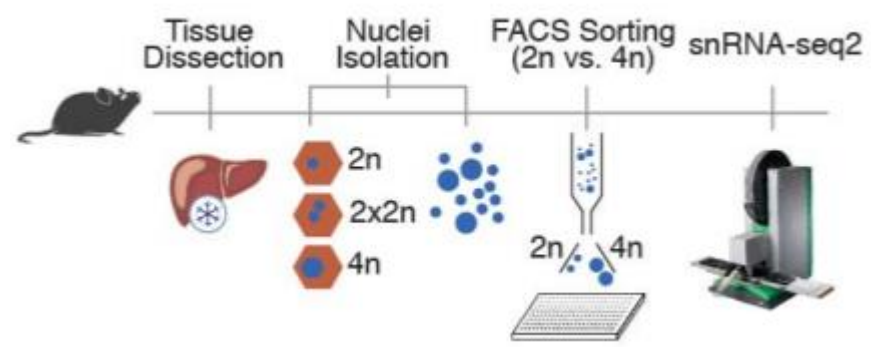
Single-cell digital isoform counts matrix

Isoforms	Cell no.	1	2	3	4	...
		60	6	19	7	...
		0	2	17	70	...
		5	80	1	61	...
		8	53	3	57	...

SMART-SEQ2/3/4 + MOSQUITO LV



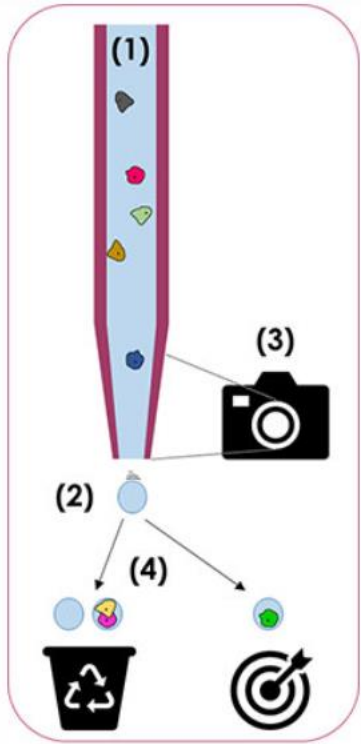
Source: SPT Labtech



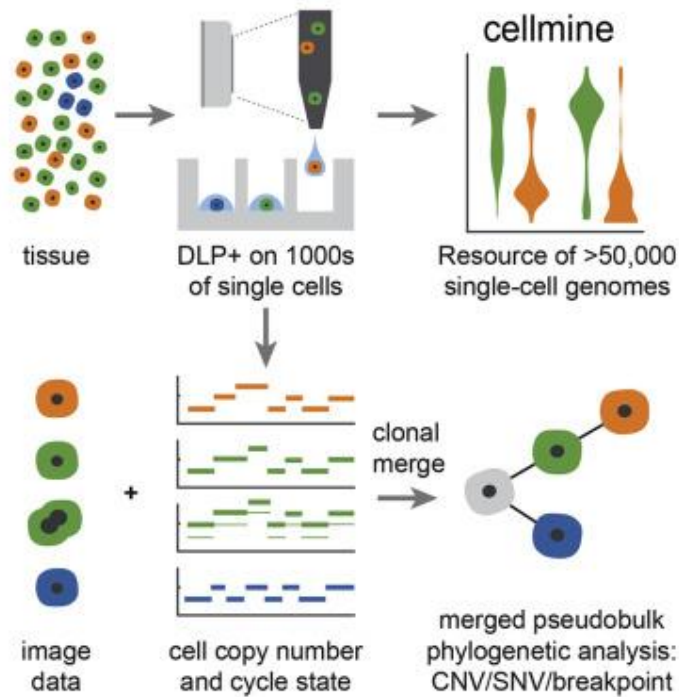
- Mosquito LV makes assay miniaturisation simple, leading to significant savings on precious reagents and time.
- Mosquito LV offers highly accurate and precise multichannel pipetting from 25 nL to 1.2 μ L.
- SmartSeq2 cost reduced from \$12 to \$4 per cell

CELLENONE

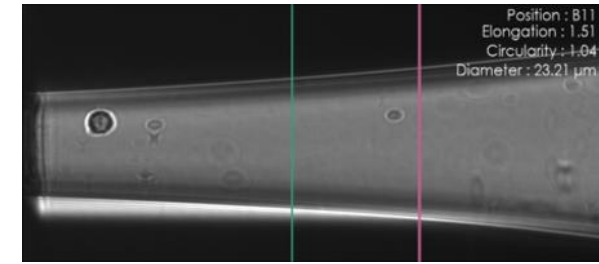
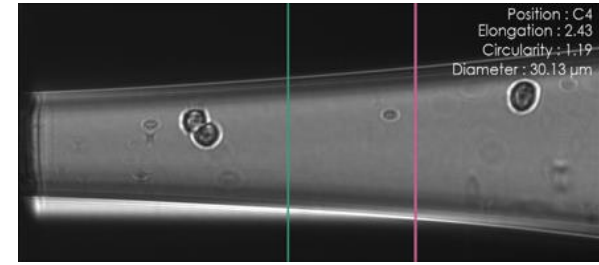
- 1) Cell suspension is aspirated into a glass capillary
- 2) Generation of drops on demand, in air
- 3) Thanks to automated imaging, cellenONE tracks cells and determines if upcoming drops will contain or not a single cell
- 4) Drops containing single cells are dispensed into selected targets, drops 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)

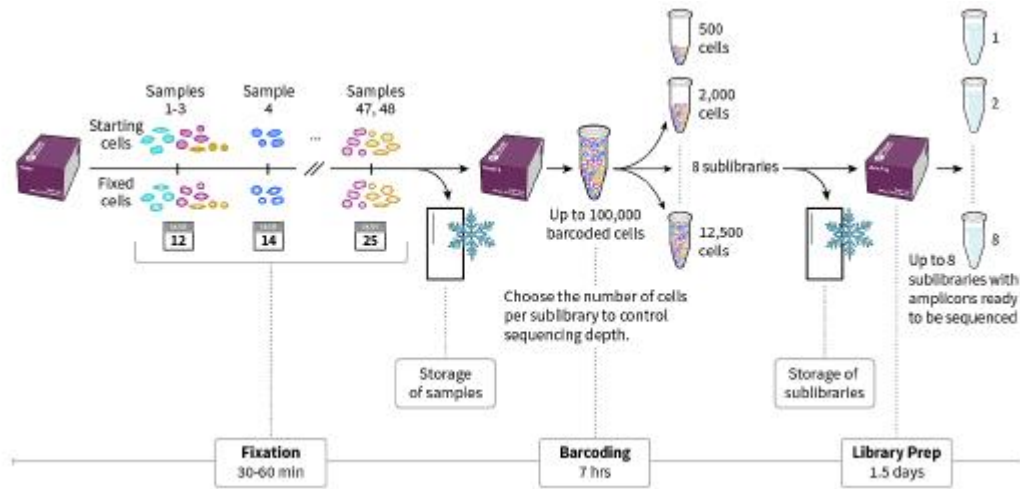


Source: Cellenion

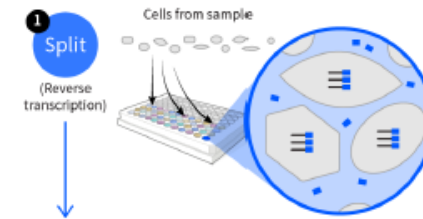
SPLIT-SEQ OVERVIEW (PARSE BIOSCIENCES)



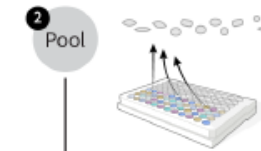
Video available at: <https://sites.google.com/uw.edu/splitseq>



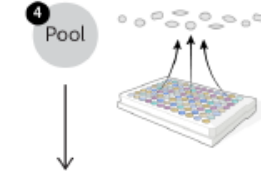
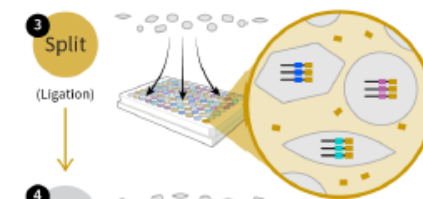
In the first round of barcoding, fixed cell samples are distributed into 48 wells, and cDNA is generated with an in-cell reverse transcription (RT) reaction using well-specific barcoded primers.



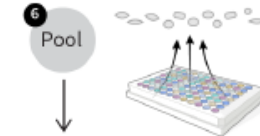
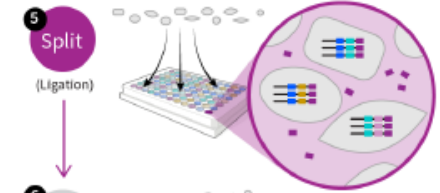
Cells from each well are pooled back together.



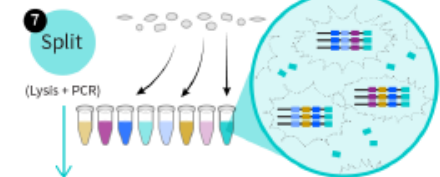
Cells are then distributed into 96 wells, and an in-cell ligation reaction appends a second well-specific barcode to the cDNA.



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.



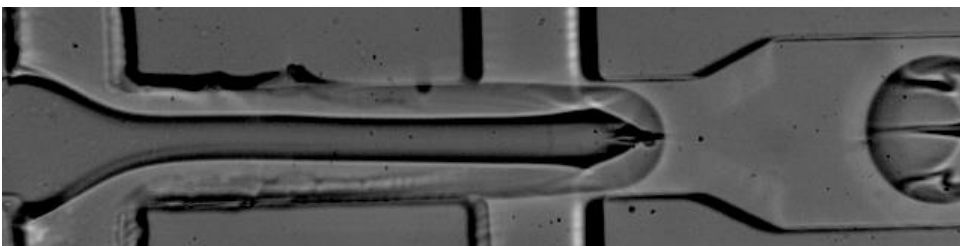
After sequencing, each single cell transcriptome is assembled by combining reads containing the same four-barcode combination.



- Time flexibility – single experiment for samples collected on different dates
- No instrument required
- Up to 48 samples / 100k cells in total
- Retail price of \$8,800 per kit*
- doublet rate of 3.4 percent per 100,000 cells*
- detection of about 12,000 genes / 100,000 unique molecular identifiers per cell*

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

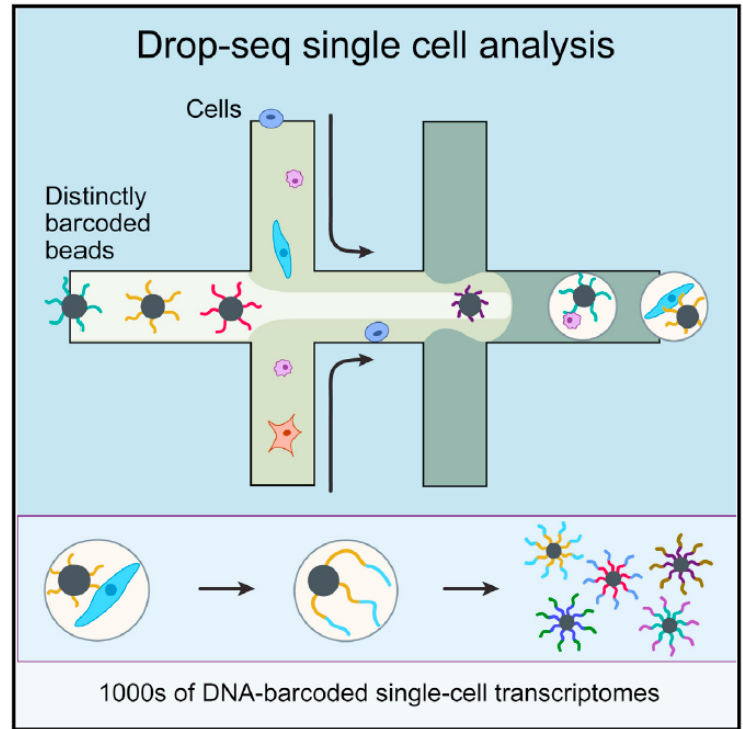


Resource

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

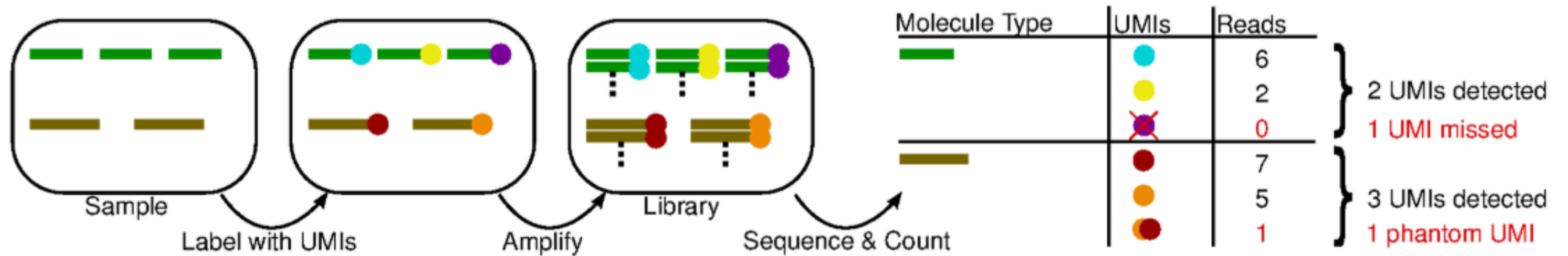
Capturing single cells along with sets of uniquely barcoded primer beads together in tiny droplets enables large-scale, highly parallel single-cell transcriptomics. Applying this analysis to cells in mouse retinal tissue revealed transcriptionally distinct cell populations along with molecular markers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

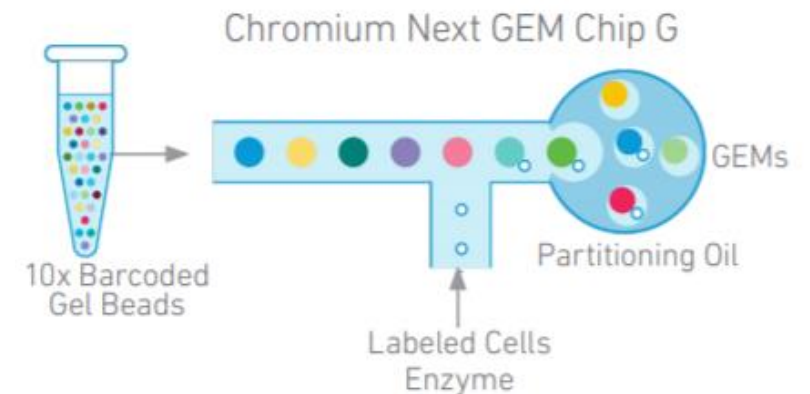
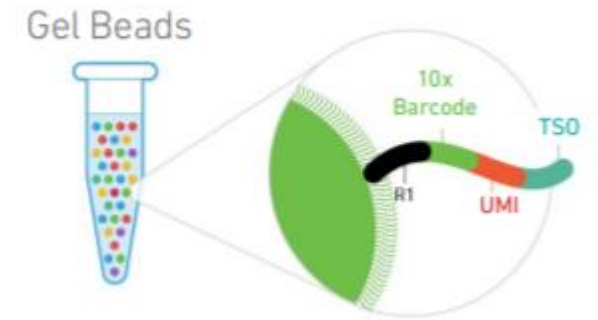
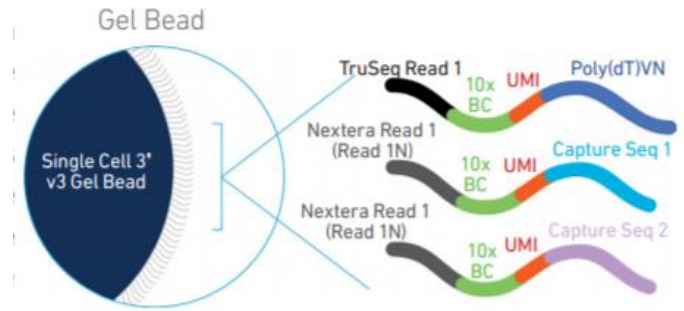
When performing variant analyses, these falsely overrepresented fragments can result in incorrect calls and thus wrong diagnostic findings



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
Coming 2H 2021



Making 1 million cell experiments routine

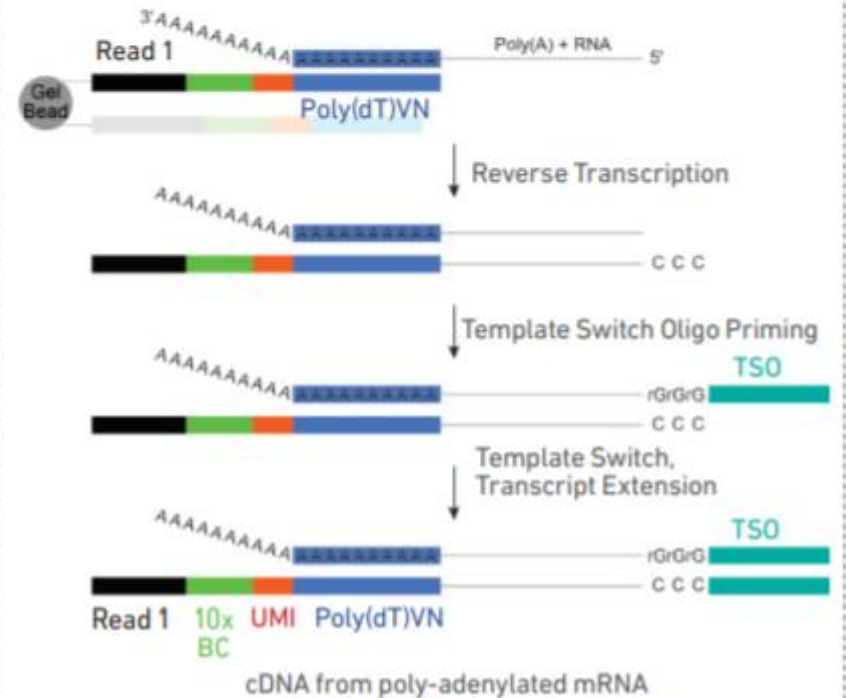


Inside individual GEMs



+
Master Mix
+
Cell Lysate

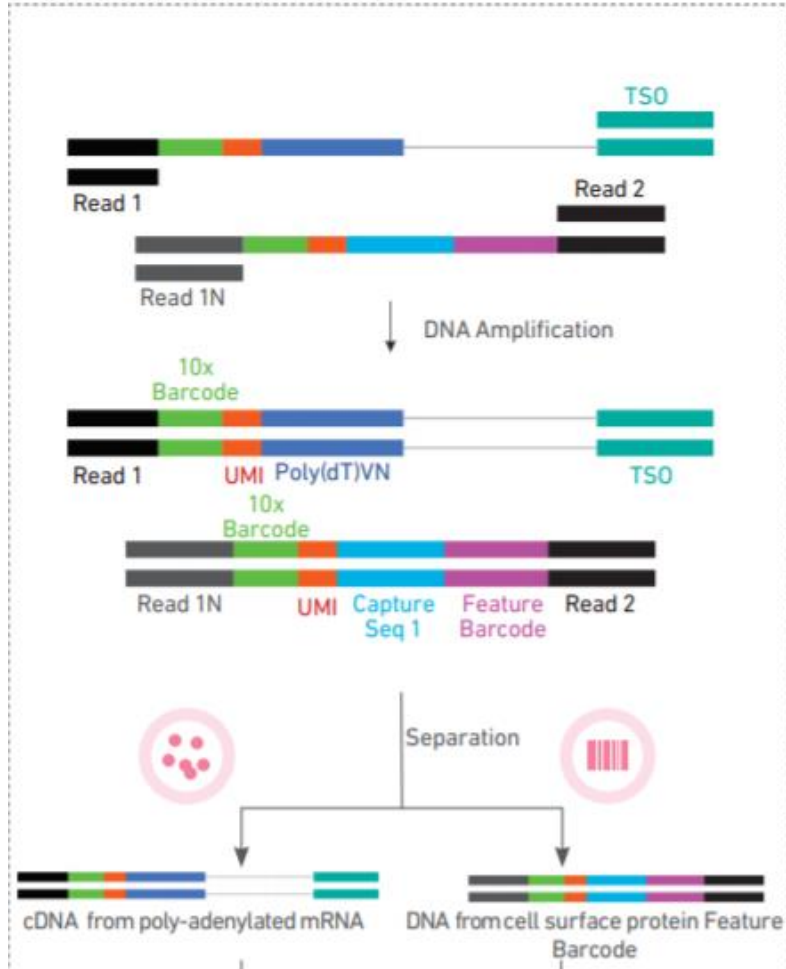
A.



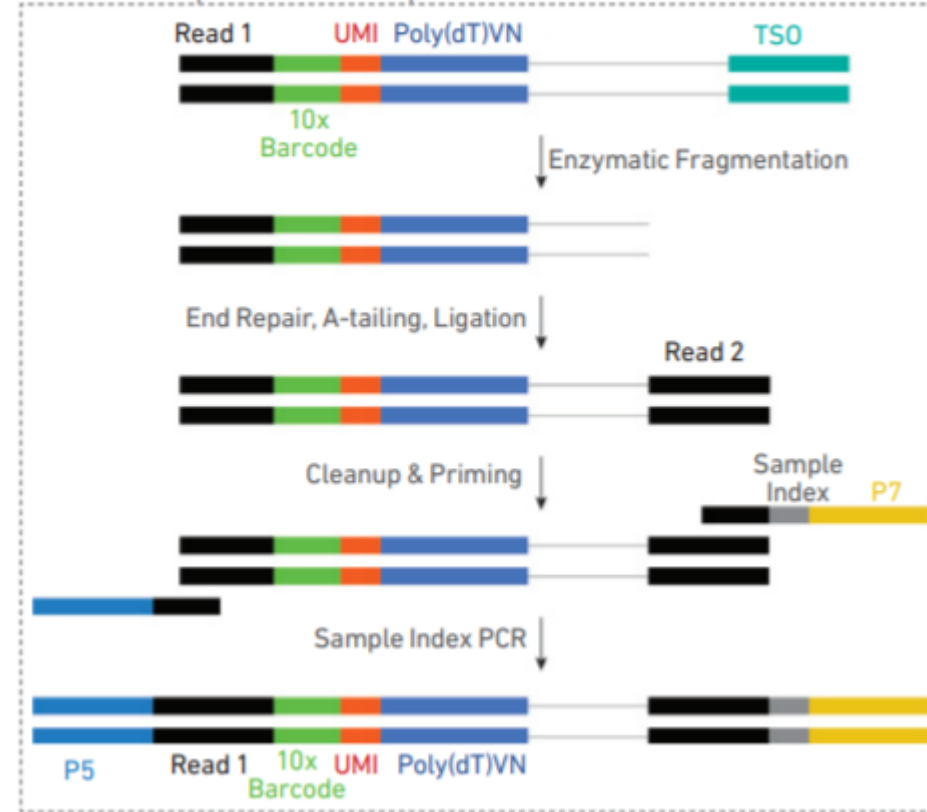
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10X GENOMICS OVERVIEW

Pooled cDNA amplification



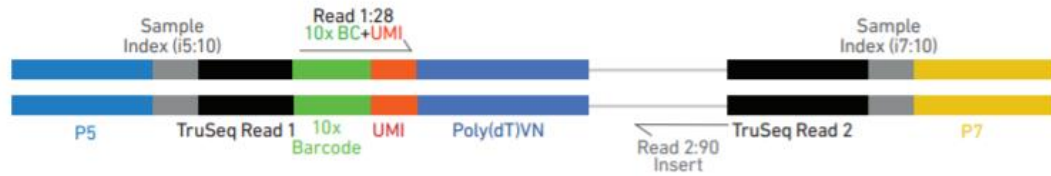
Pooled amplified cDNA processed in bulk



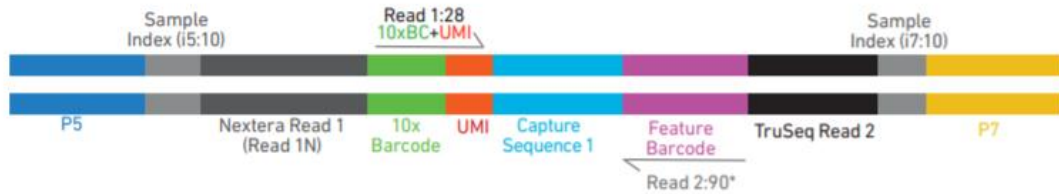
Source: 10x Genomics

10X GENOMICS LIBRARIES

Chromium Single Cell 3' Gene Expression Dual Index Library



Chromium Single Cell 3' Cell Surface Protein Dual Index Library



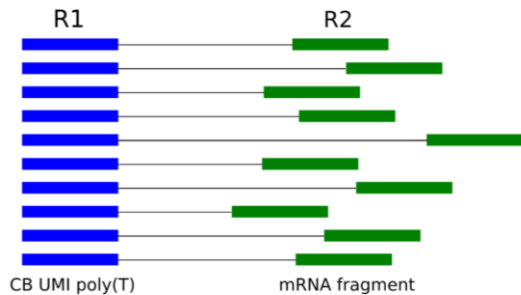
Chromium Single Cell V(D)J Dual Index Library



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

MULTIOMICS AGE

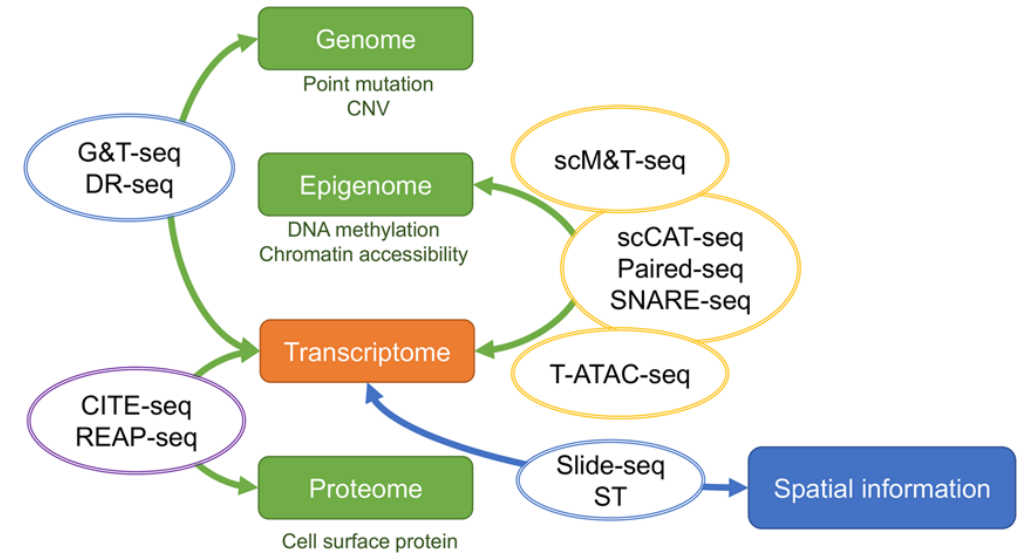
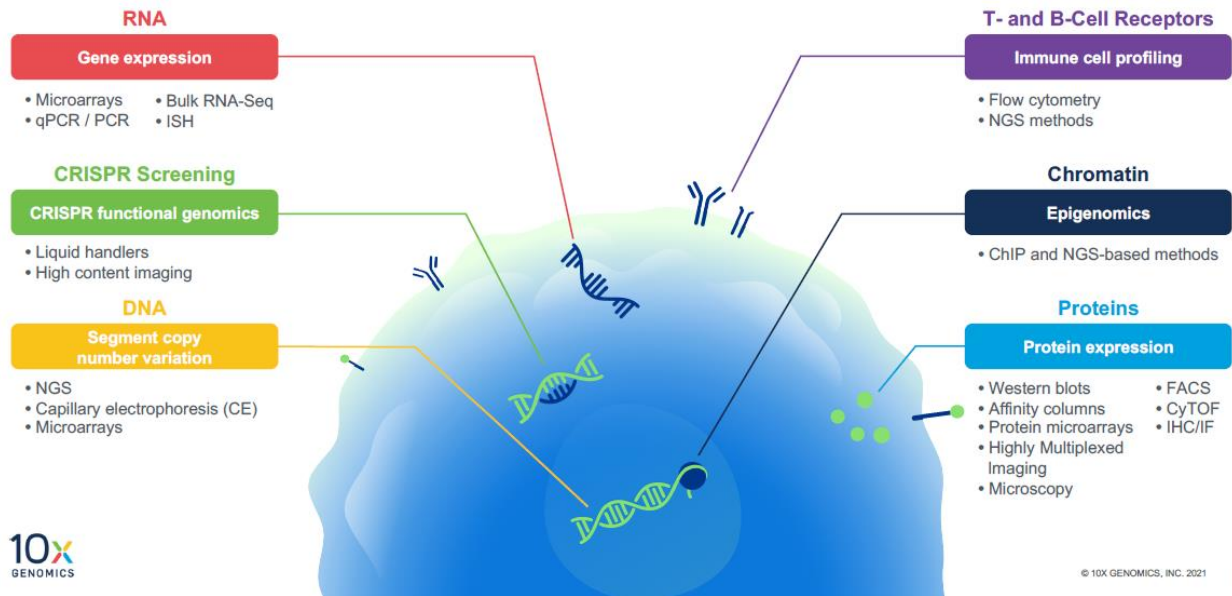
TECHNOLOGY FEATURE | 19 July 2021 | Correction 21 July 2021

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

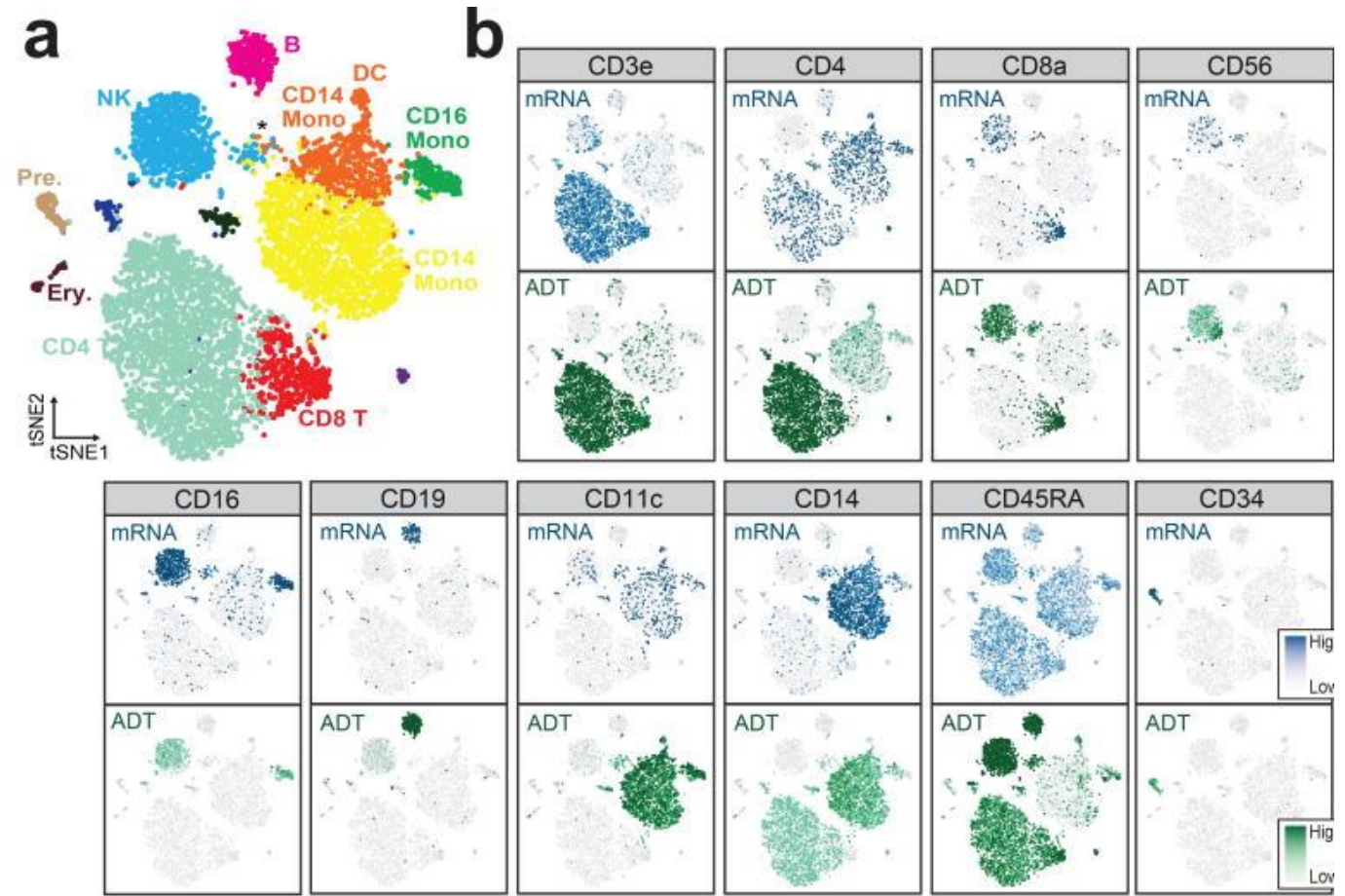
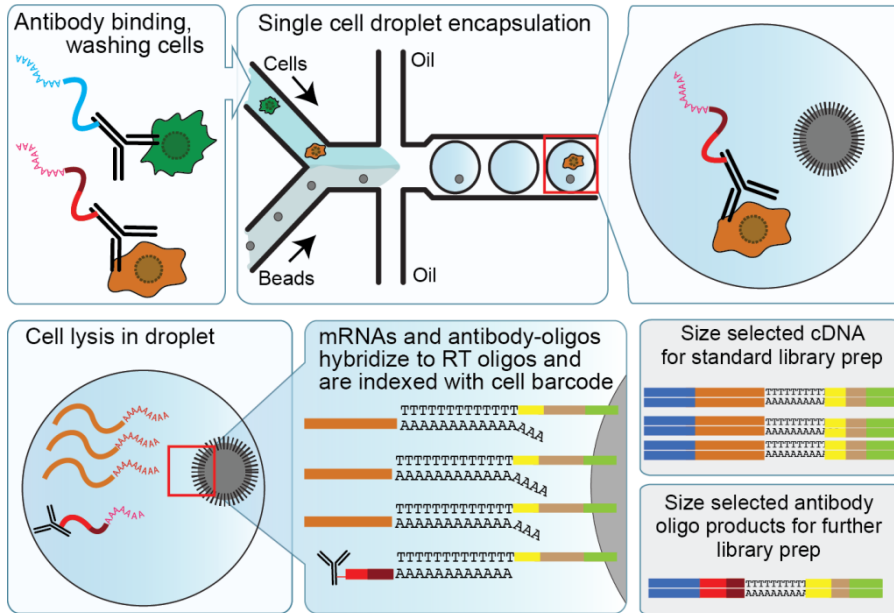
Replacing the Legacy Toolkit Across Biology



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

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

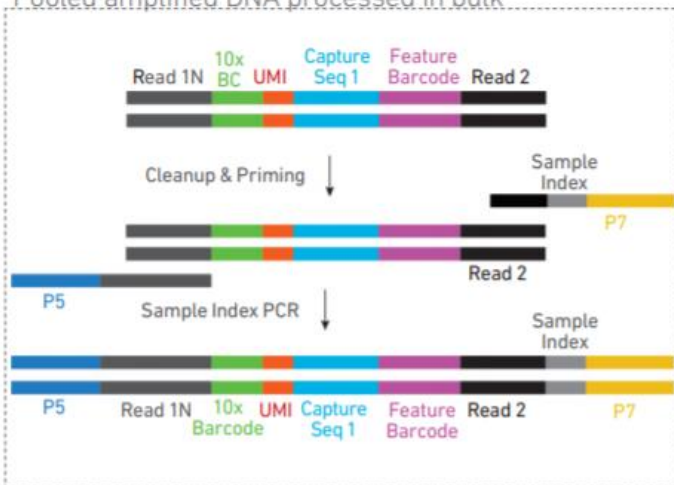


Source: Stoeckius et al. *Nat Methods*. (2017)

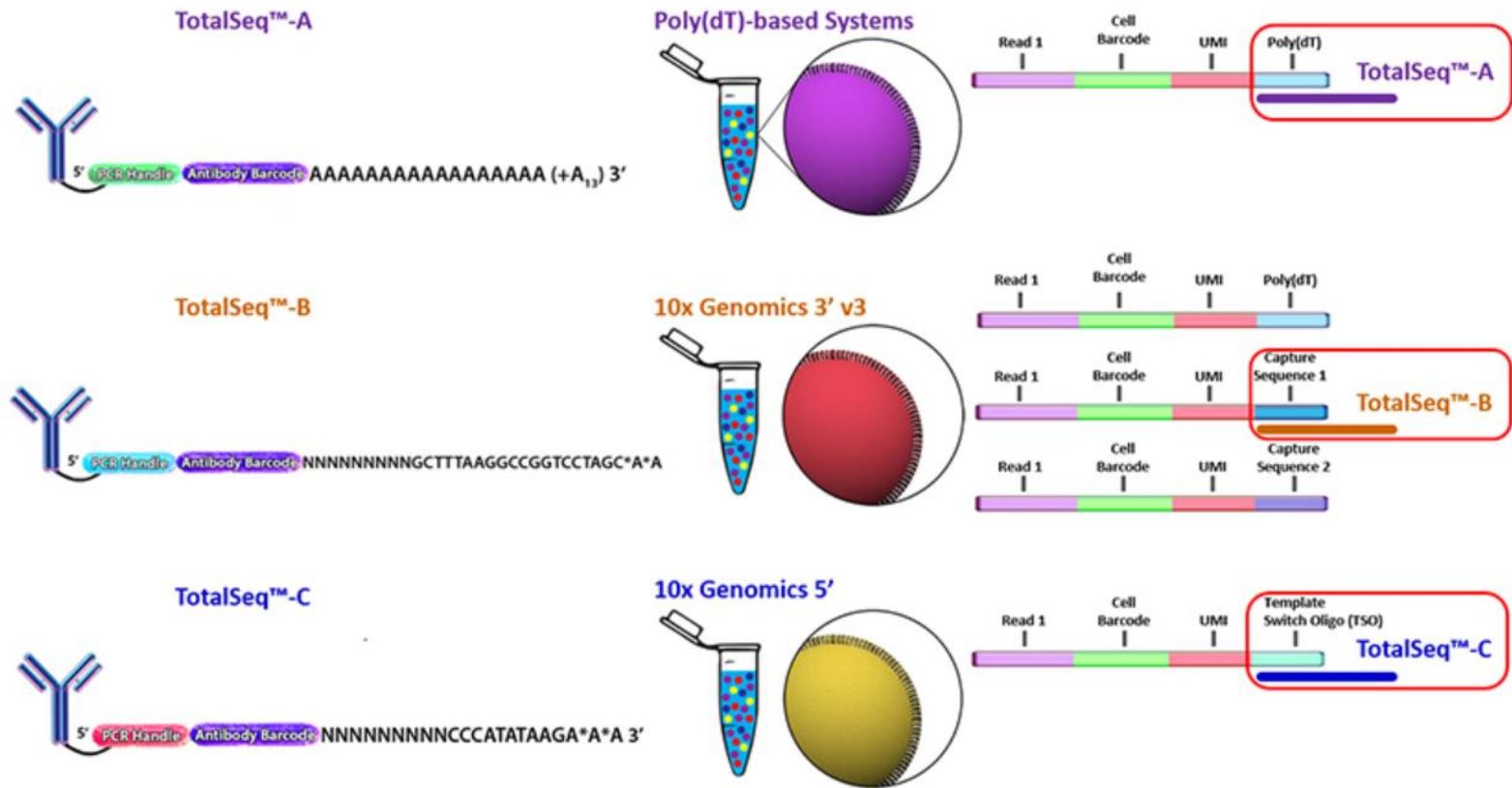
CITE-SEQ



Pooled amplified DNA processed in bulk



Source: 10x Genomics

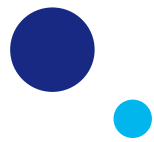


Source: BioLegend



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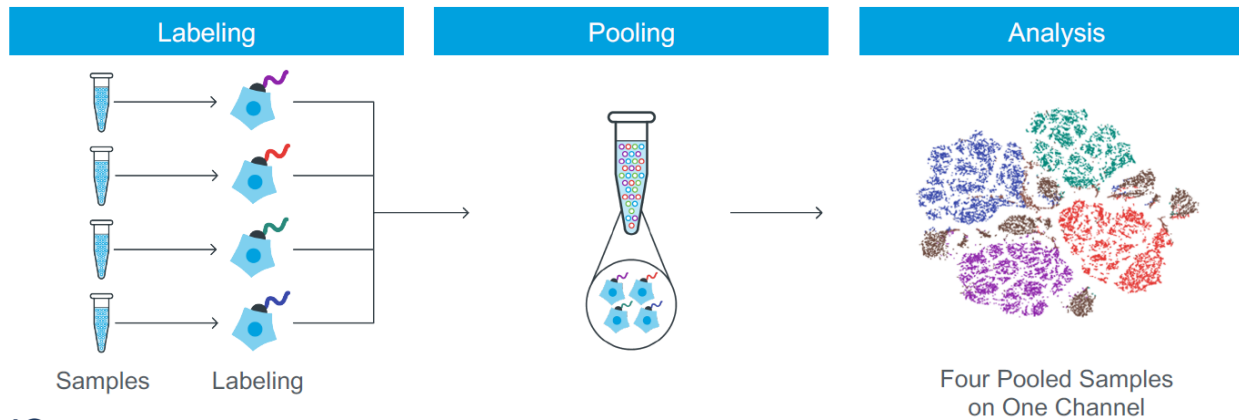
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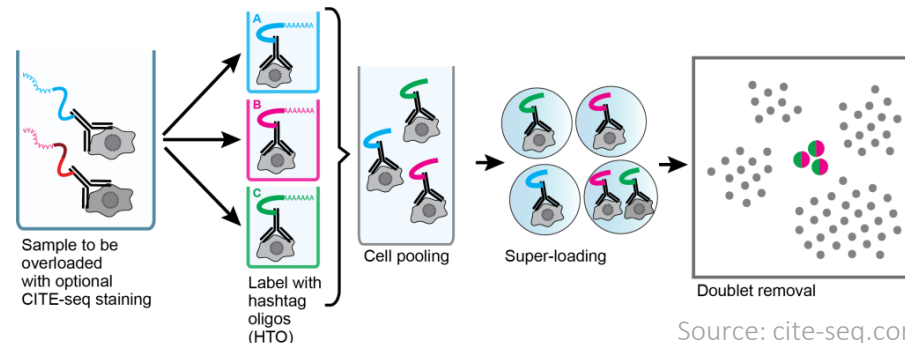
CELL HASHING



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,*}



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](#)

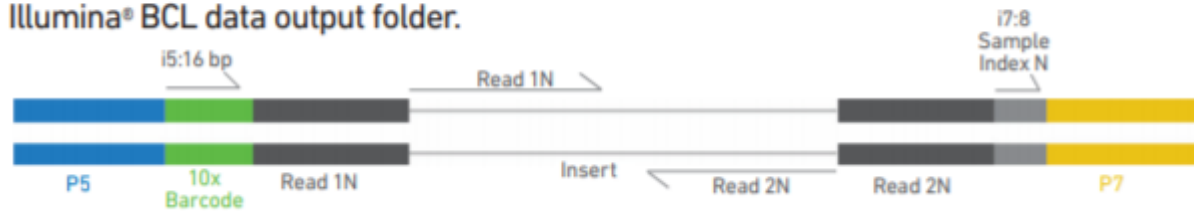
15k Accesses | **27** Citations | **85** Altmetric | [Metrics](#)



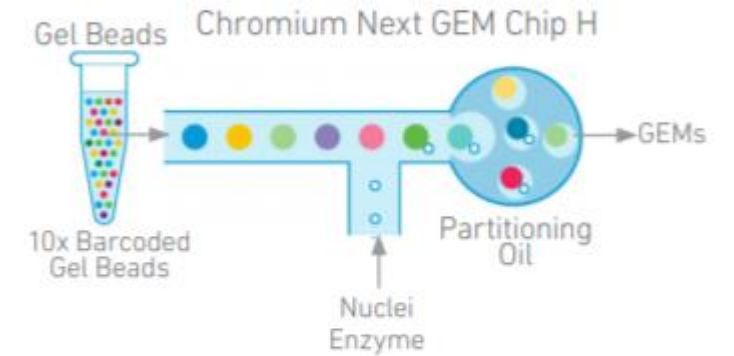
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10X ATAC

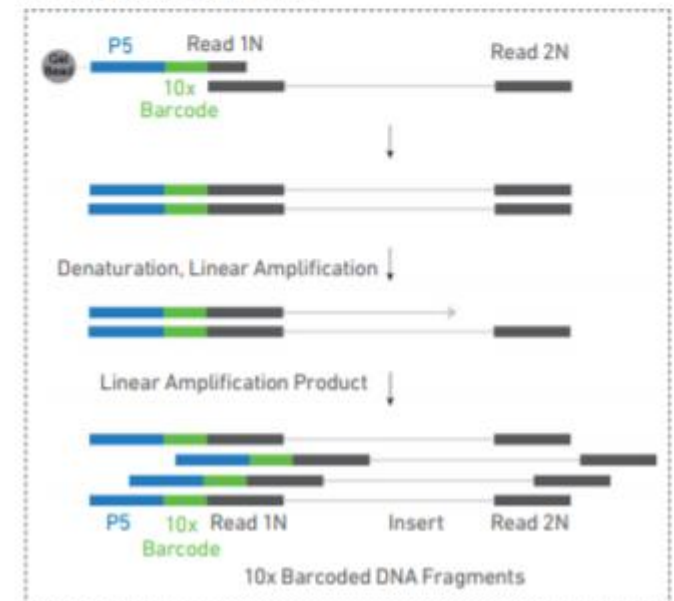
Chromium Single Cell ATAC libraries comprise double stranded DNA fragments which begin with P5 and end with P7. Sequencing these libraries produces a standard Illumina® BCL data output folder.



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



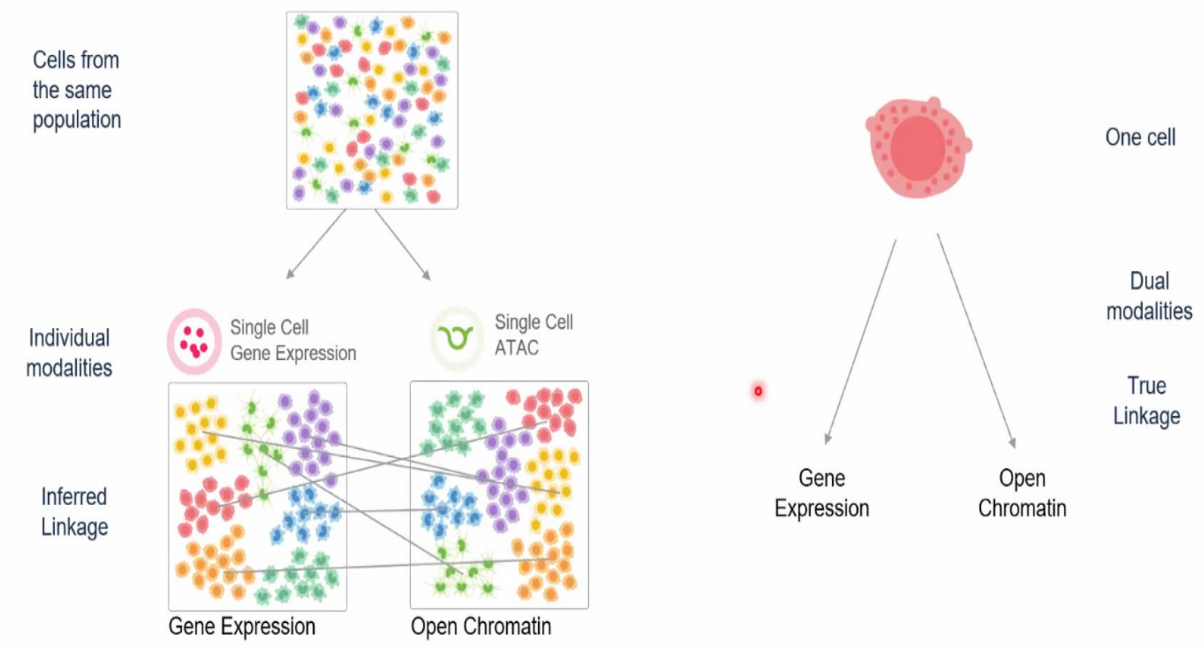
Inside Individual GEMs



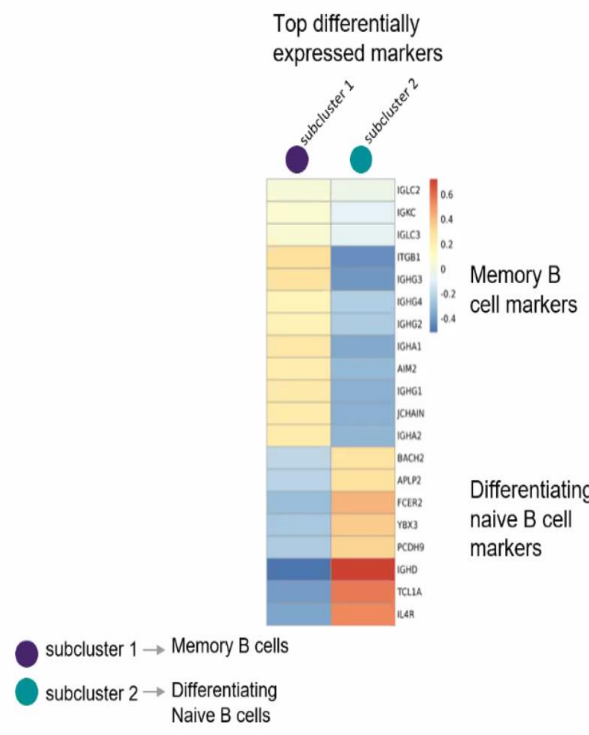
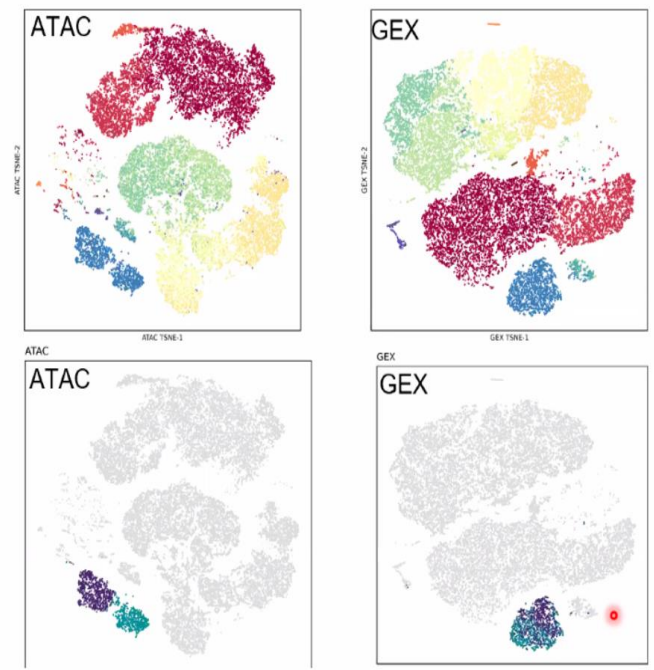
Source: 10x Genomics

10X MULTIOME (RNA+ATAC)

Profiling Different Modalities To Gain Deeper Insights

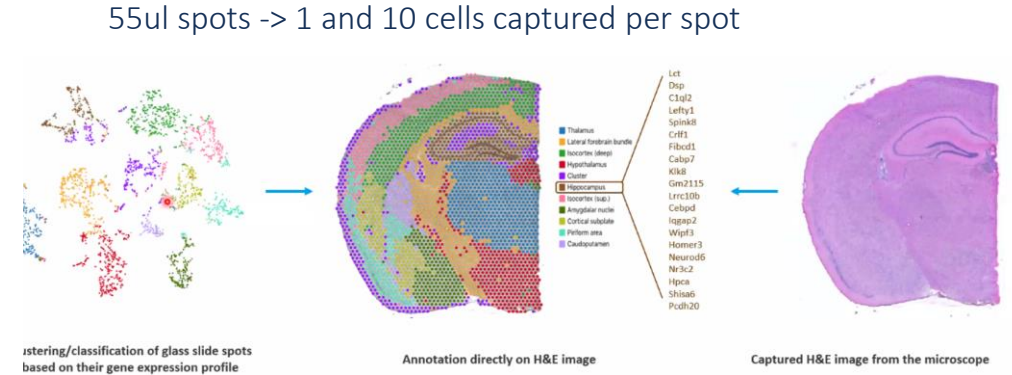
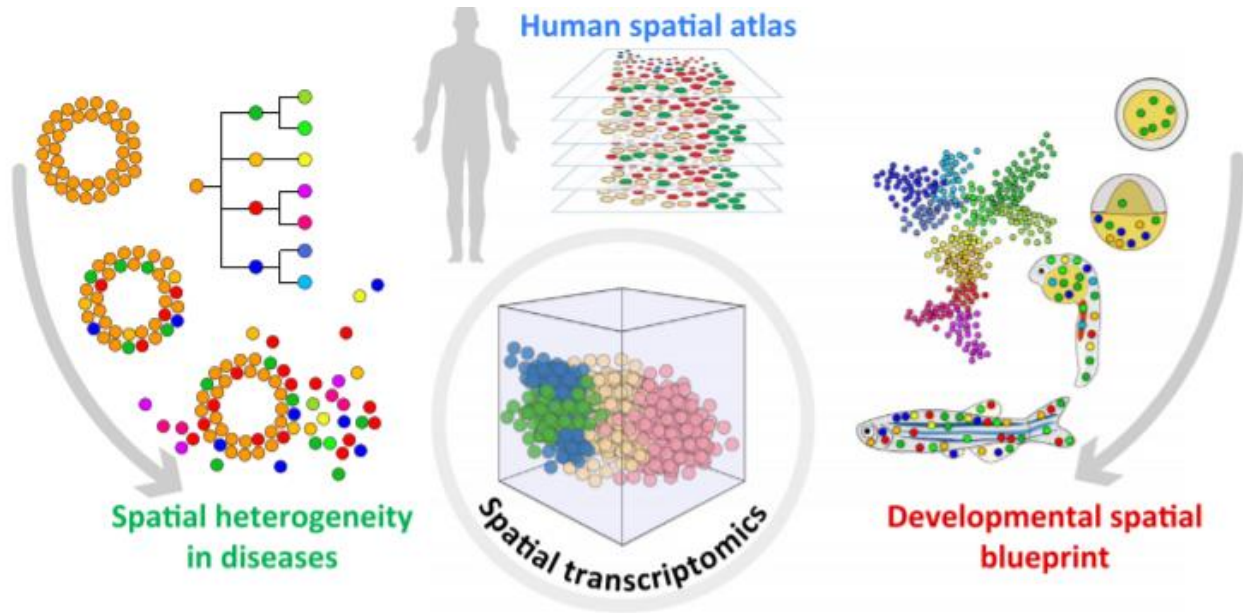


Dive Deep Where It Matters



Source: 10x Genomics

SPATIAL TRANSCRIPTOMICS



Source: 10x Genomics

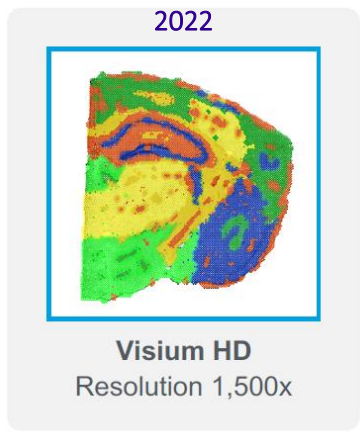
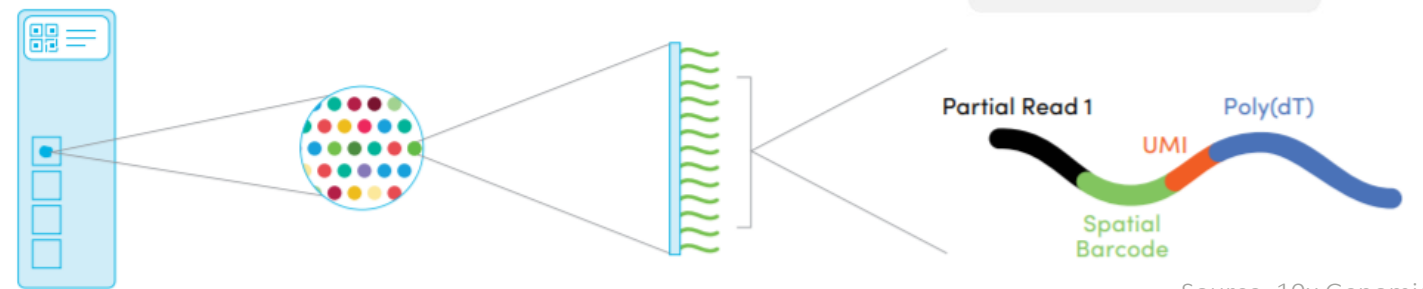


Figure 3. Applications for Spatially Resolved Transcriptomics. Three primary kinds of hot issues can be resolved by spatially resolved transcriptomics: left, discovering spatial heterogeneity of diseases; middle, establishing spatial transcriptome atlases for the human body; and right, delineating an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

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

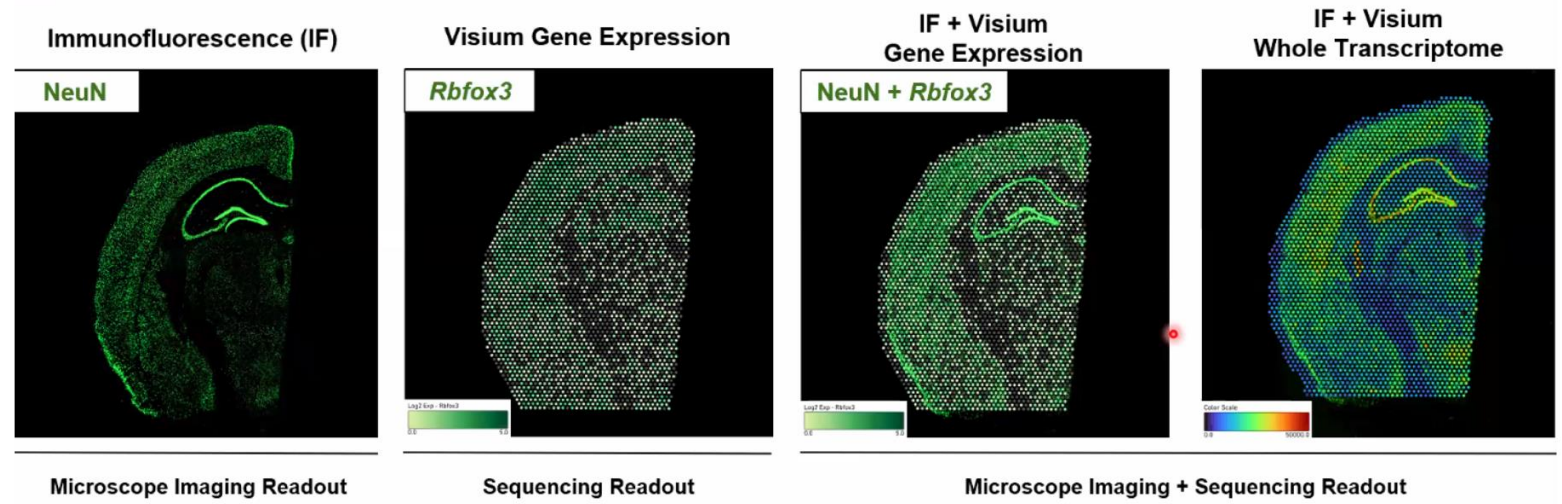
Trends in Biotechnology

Visium Spatial Gene Expression Slide Capture Area with 5000 Barcoded Spots Visium Gene Expression Barcoded Spots

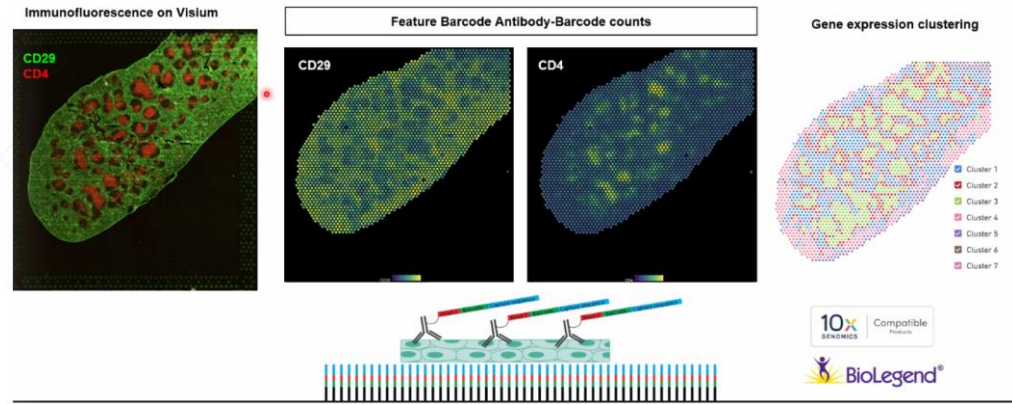


Source: 10x Genomics

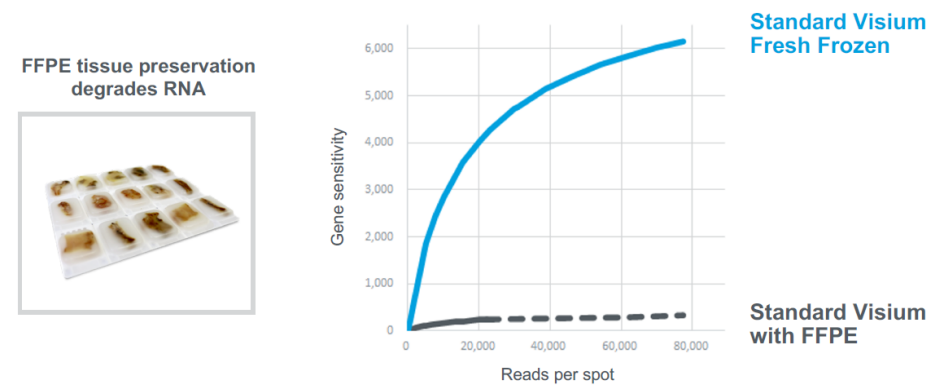
SPATIAL TRANSCRIPTOMICS



Feature Barcode Correlates with Immunofluorescence

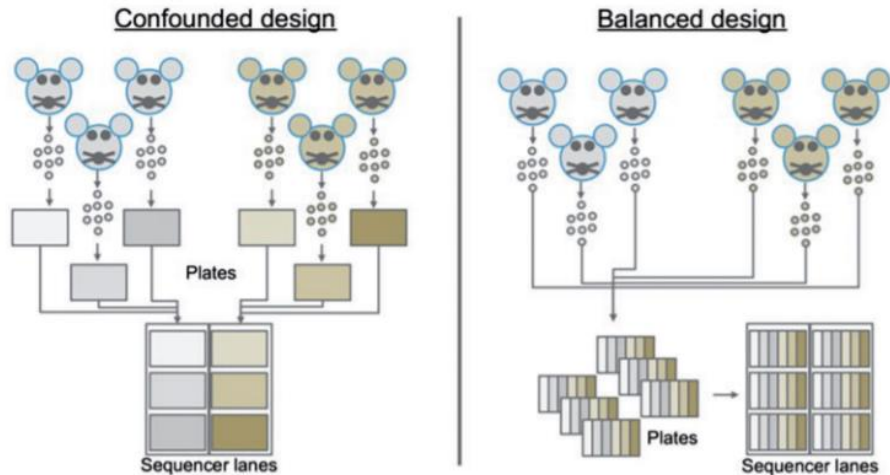


The Challenge of FFPE Samples



Source: 10x Genomics

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)



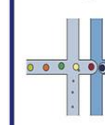
Method:

- 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



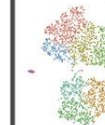
Method:

- 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 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)
- If you are looking to classify all cell types in a diverse tissue - high throughput
- If you have only archival human samples – nuclei isolation

LITERATURE:

- https://hbctraining.github.io/scRNA-seq/slides/Single_Cell_2_27_20.pdf.
- <https://www.slideshare.net/TimothyTickle/introduction-to-singlecell-rnaseq>
- Arzalluz-Luque et al. A. Single-cell RNAseq for the study of isoforms—how is that possible?. *Genome Biol* 19, 110 (2018).
- Ding et al. Systematic comparison of single-cell and single-nucleus RNA-sequencing methods. *Nat Biotechnol* 38, 737–746 (2020).
- Guillaumet-Adkins et al. Single-cell transcriptome conservation in cryopreserved cells and tissues. *Genome Biol* 18, 45 (2017).
- 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).
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- 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).
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- Pijuan-Sala et al. A single-cell molecular map of mouse gastrulation and early organogenesis. *Nature* 566, 490–495 (2019).
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- Richter, M.L et al. Single-nucleus RNA-seq2 reveals functional crosstalk between liver zonation and ploidy. *Nat Commun* 12, 4264 (2021).
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- Svensson et al. Exponential scaling of single-cell RNA-seq in the past decade. *Nat Protoc* 13, 599–604 (2018).
- 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).



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USEFUL RESOURCES:

- 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/>)
- 10x Genomics demonstrated protocols for sample preparation (<https://support.10xgenomics.com/single-cell-gene-expression/sample-prep>)
- 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://sites.google.com/uw.edu/splitseq>)
- CITE-seq (<https://cite-seq.com/>)
- Biolegend TotalSeq (<https://www.biolegend.com/en-us/totalseq>)

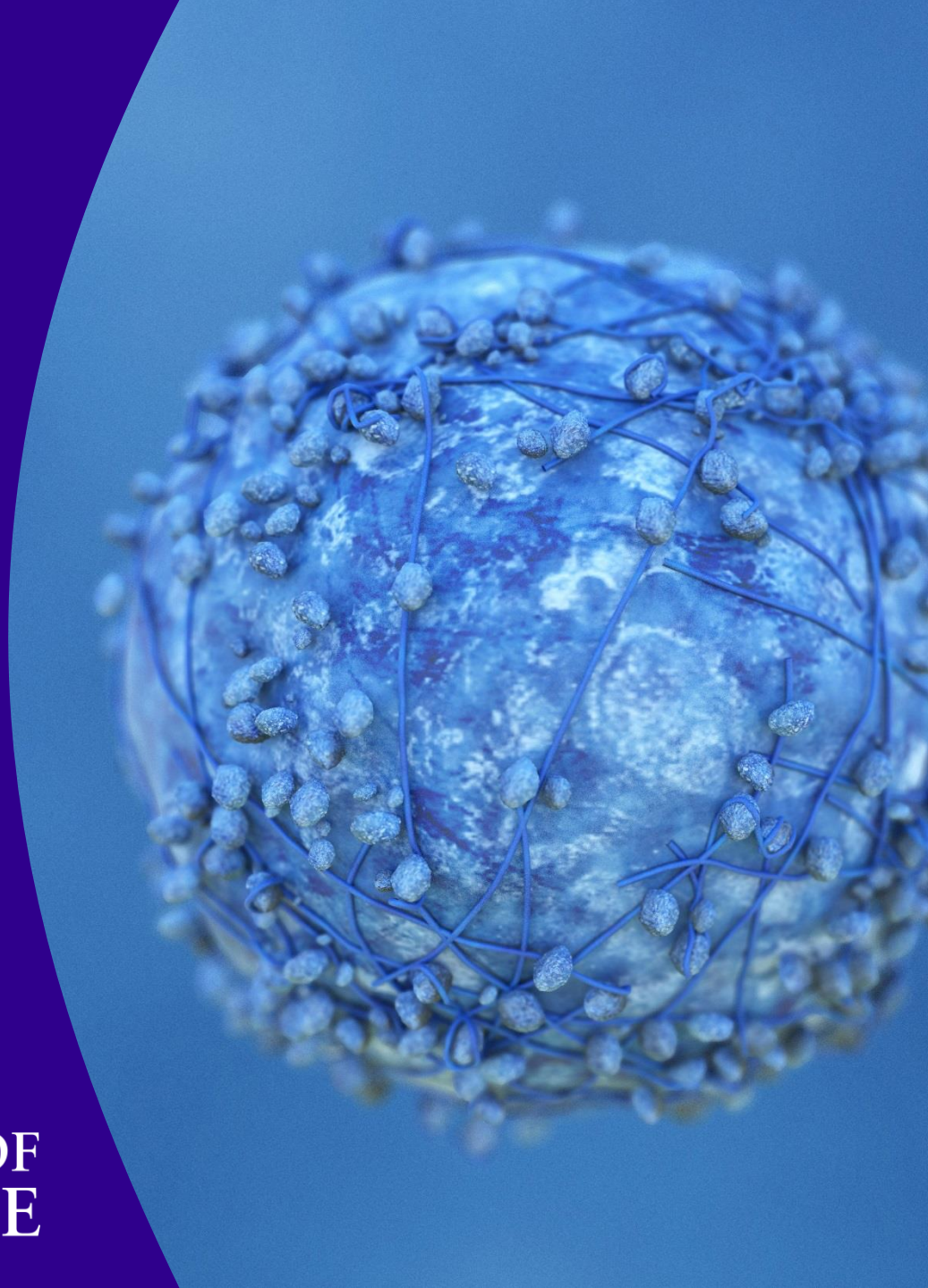


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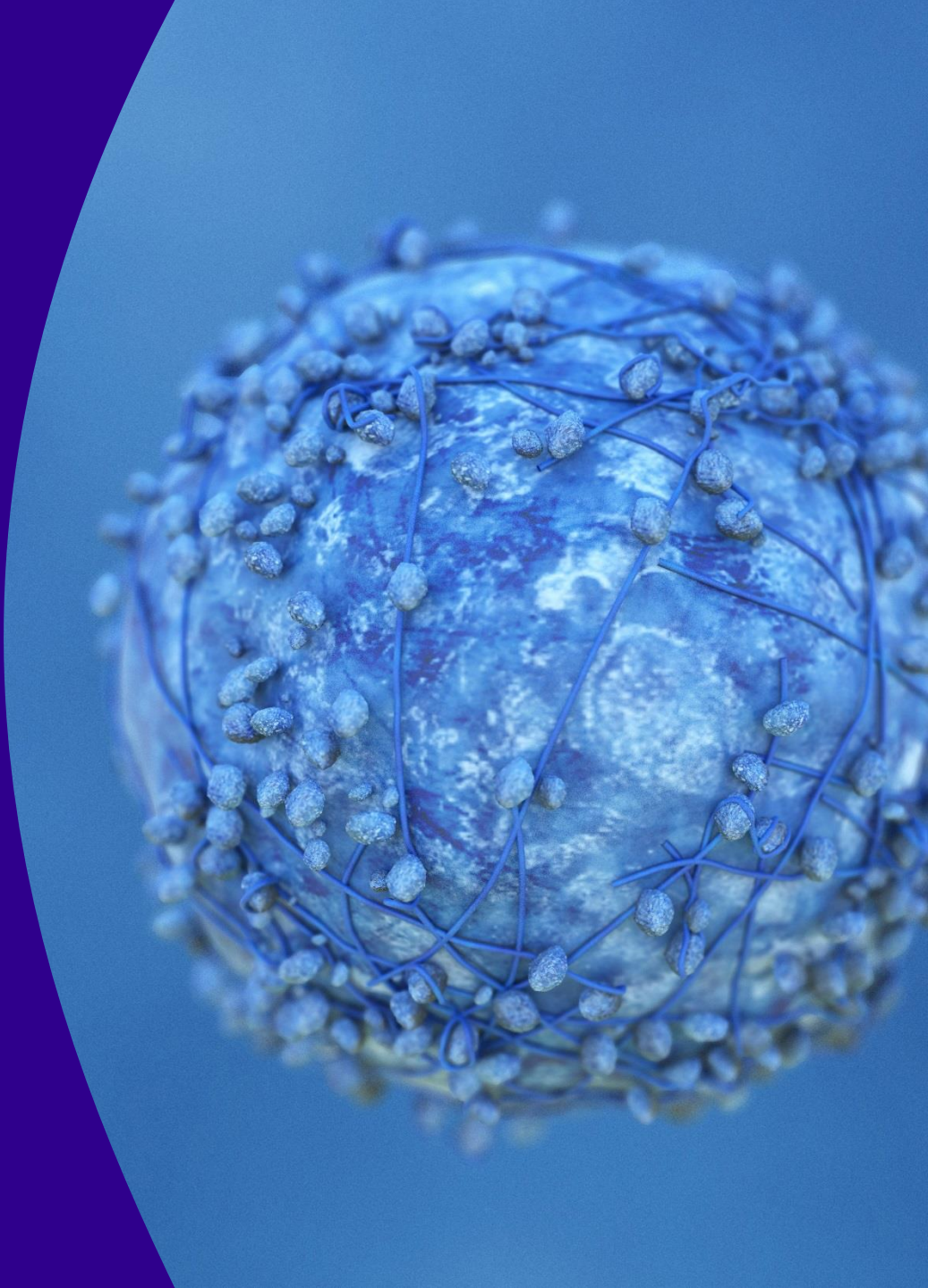
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