

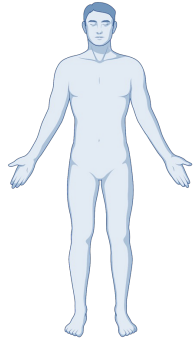
# **RNA, Methylation, and CRISPR, oh my!**

## **New Technologies to Enable Scalable Single Cell Studies**

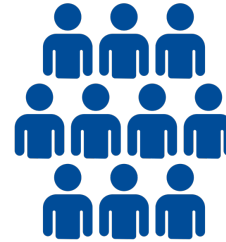
**Mary Arrastia, Ph.D.**  
**Field Application Scientist at Scale Biosciences**

***WACD 2023: The Energy in Synergy***

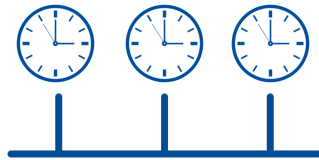
# Many applications need high throughput



Cell Atlasing



Large patient cohorts



Longitudinal studies



CRISPR screens

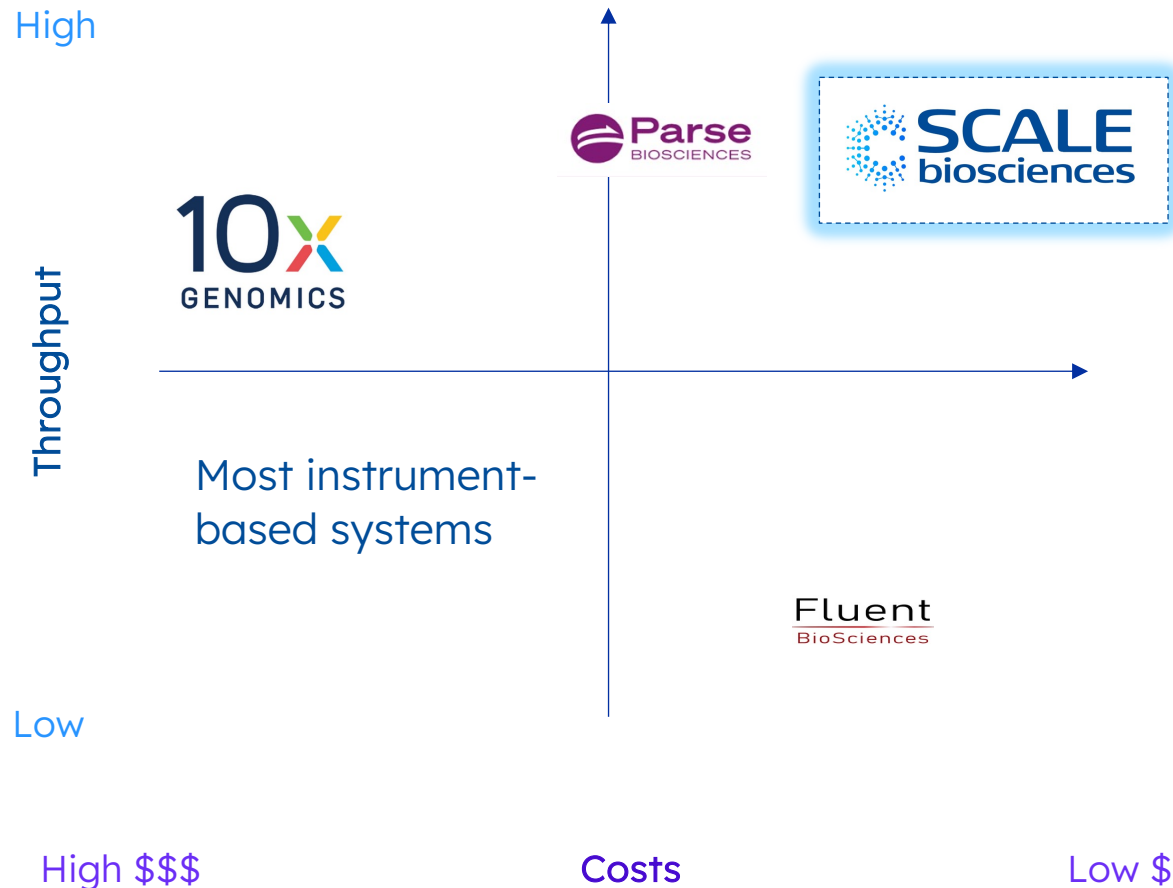


Rare cell profiling



Biomarker/Drug Discovery

# ScaleBio enables flexible, scalable, low-cost single cell studies



High-cost single-cell workflow solutions **constrain** the adoption, scope, and **impact** of single cell research.

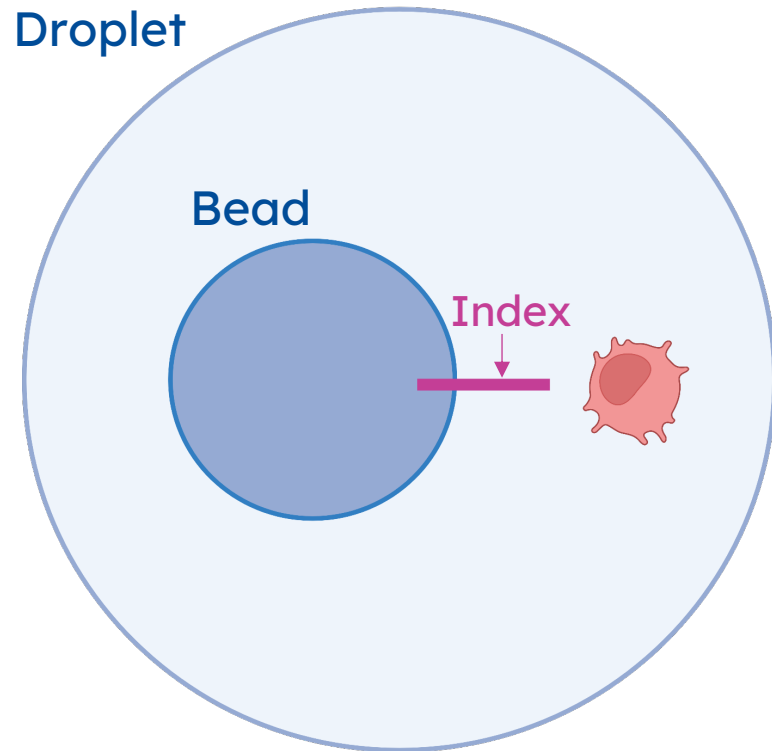


# The cell as the compartment

How combinatorial indexing works

# Traditional capture systems

Physical capture methods can often cause doublets, limiting loading capacity

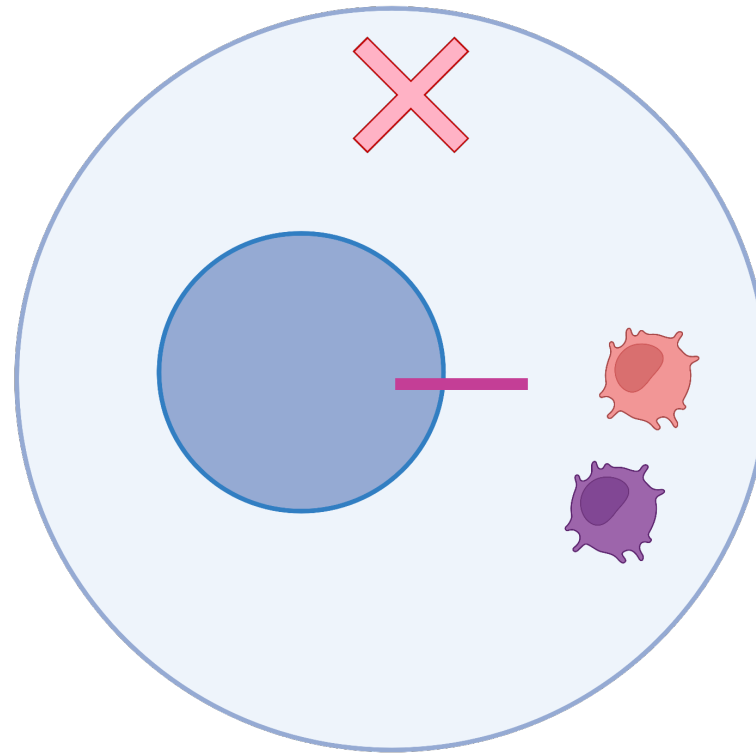
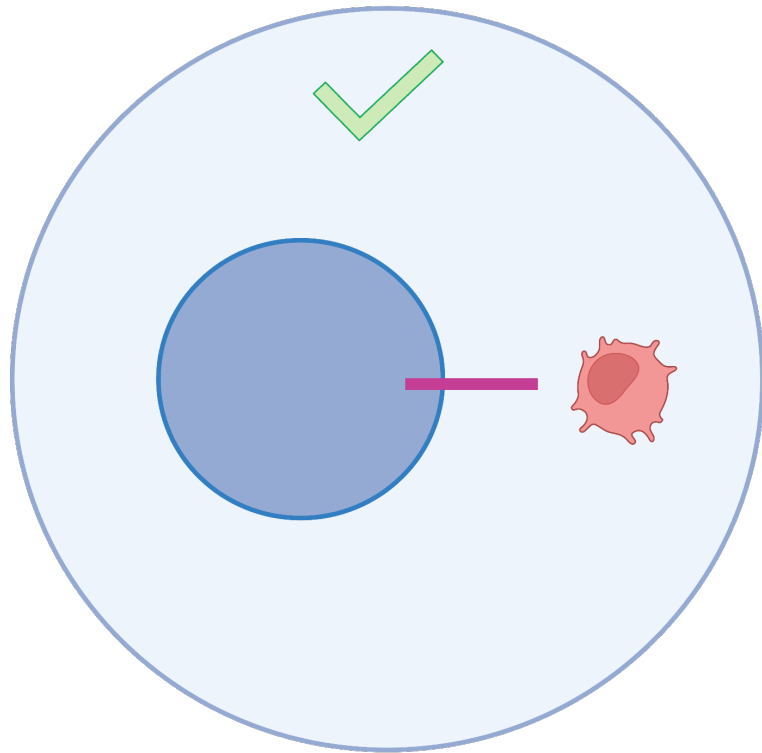


## Single-cell capture in droplet systems

Individual cells are captured in a single droplet. During the initial step, an index is added that identifies the cell.

# Traditional capture systems

Physical capture methods can often cause doublets, limiting loading capacity



If two cells are captured in the same droplet, they will get the same index.

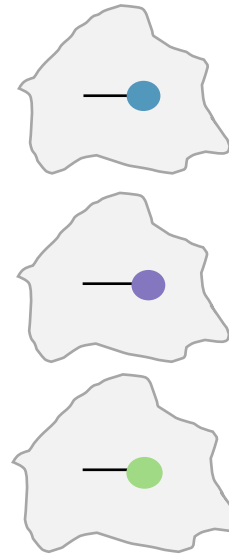
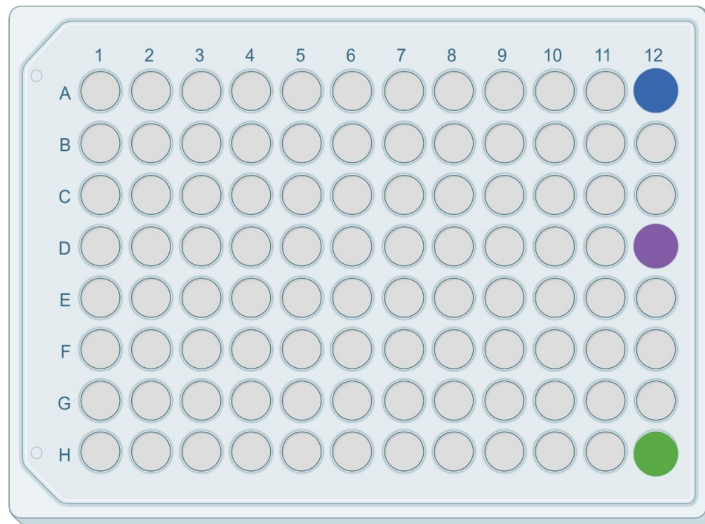
This means they cannot be distinguished during sequencing.

# Combinatorial indexing enables a simple, instrument-free workflow

Single-cell resolution is achieved by barcoding each cell's transcripts with a unique 3-index combination

Level 1

Unique barcodes: 96

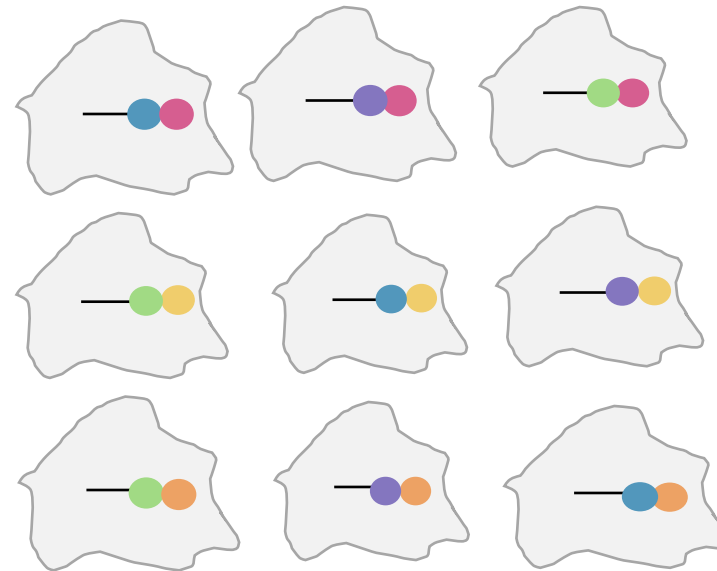
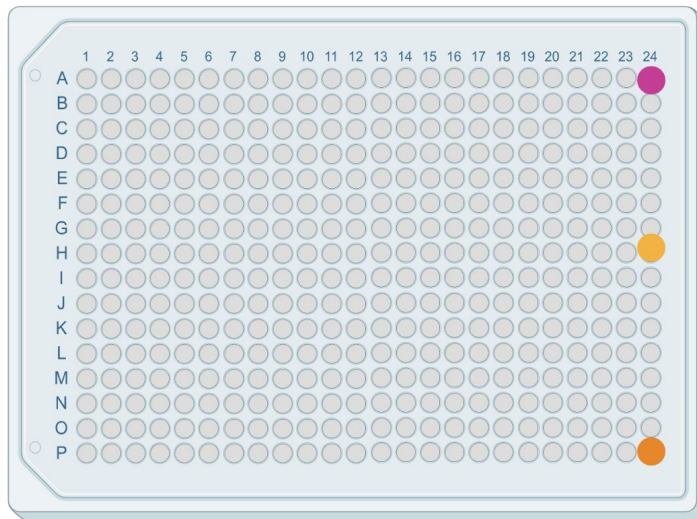


# Combinatorial indexing enables a simple, instrument-free workflow

Single-cell resolution is achieved by barcoding each cell's transcripts with a unique 3-index combination

Level 2

Unique barcodes: 36,864



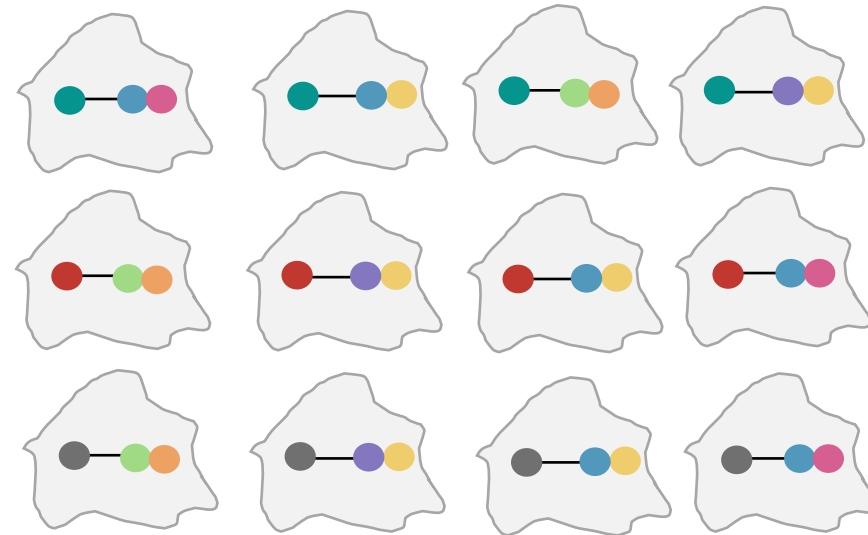
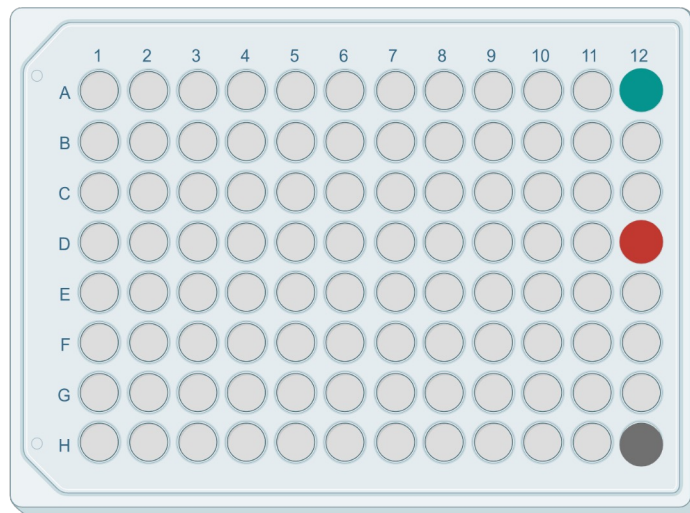


# Combinatorial indexing enables a simple, instrument-free workflow

Single-cell resolution is achieved by barcoding each cell's transcripts with a unique 3-index combination

Level 3

Unique barcodes: 3,538,944



# Our Scientific Co-founders

Key inventors of combinatorial indexing



**Frank Steemers**  
Sequencing



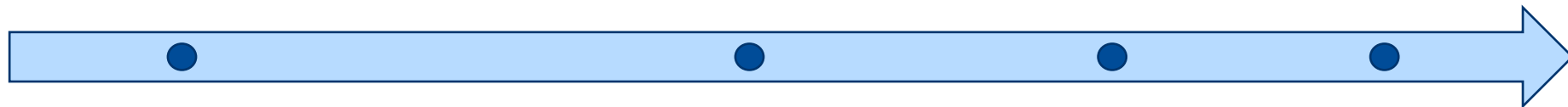
**Garry Nolan**  
Proteomics



**Jay Shendure**  
Genomics



**Cole Trapnell**  
Bioinformatics



**2020**  
ScaleBio is founded

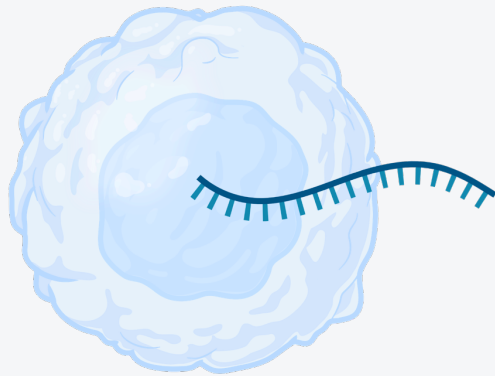
**2022**  
scATAC kit launched

**2023**  
scRNA, scCRISPR,  
scMethylation

**And Beyond**

# Current kits implementing combinatorial indexing for scalable single cell studies

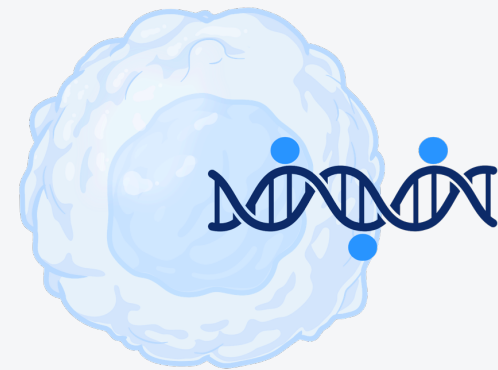
RNA

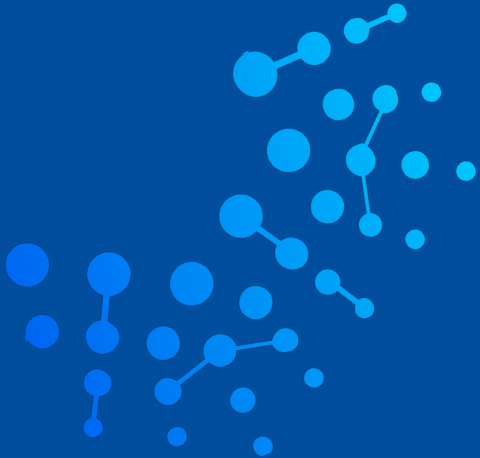


CRISPR



Methylation





# ScaleBio™ Single Cell RNA Sequencing Kit

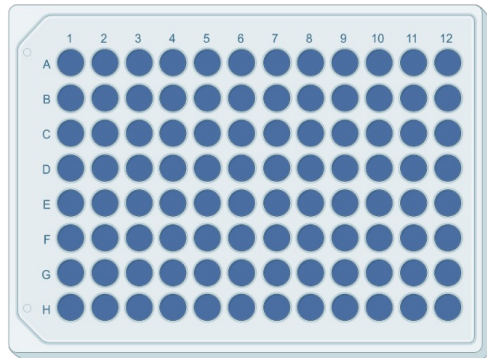
2-day workflow with an output of 125,000 cells

# 2-day workflow with an output of 125,000 cells, nuclei, or both!

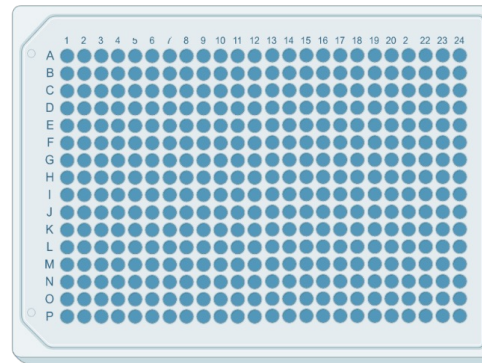
Fixation  
*Enables flexibility*



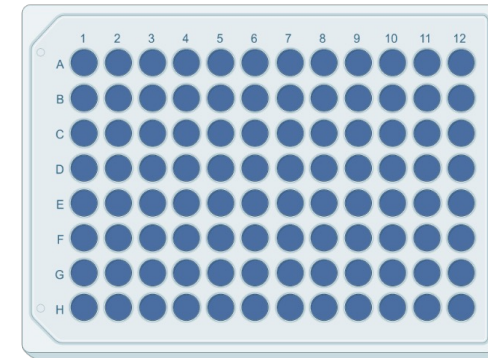
Split 1: Indexed Reverse  
Transcription  
*Input: 2,500-10,000 cells/well*



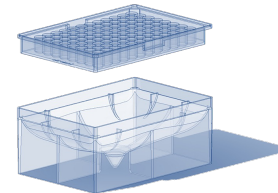
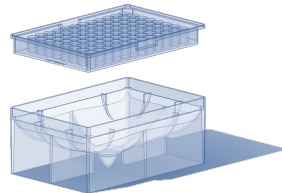
Split 2: Indexed Ligation



Split 3: Indexed PCR



Sequencing  
output of  
125,000 cells

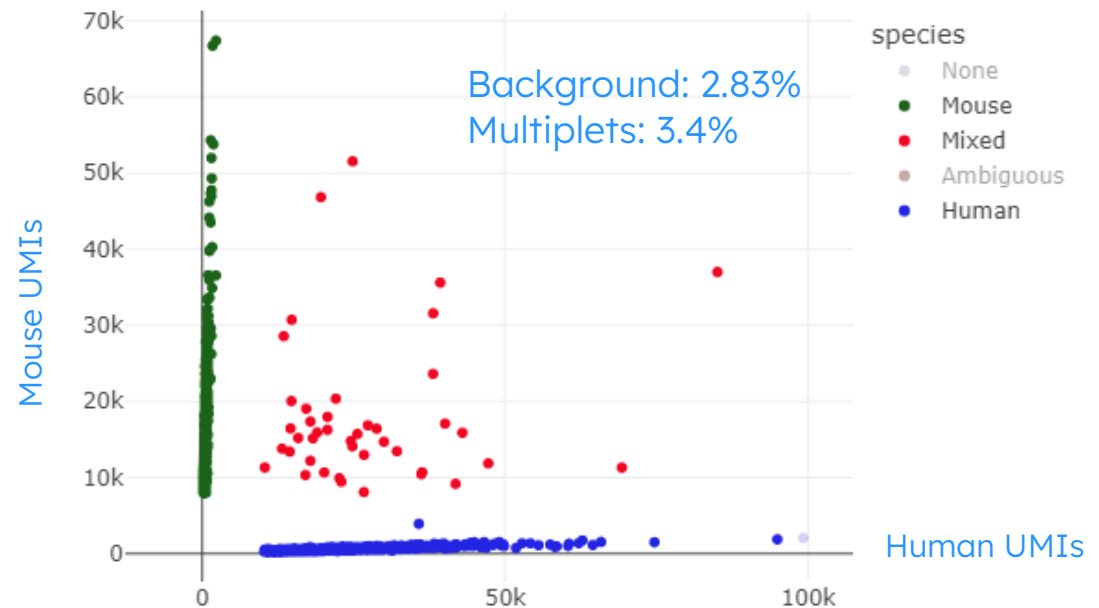
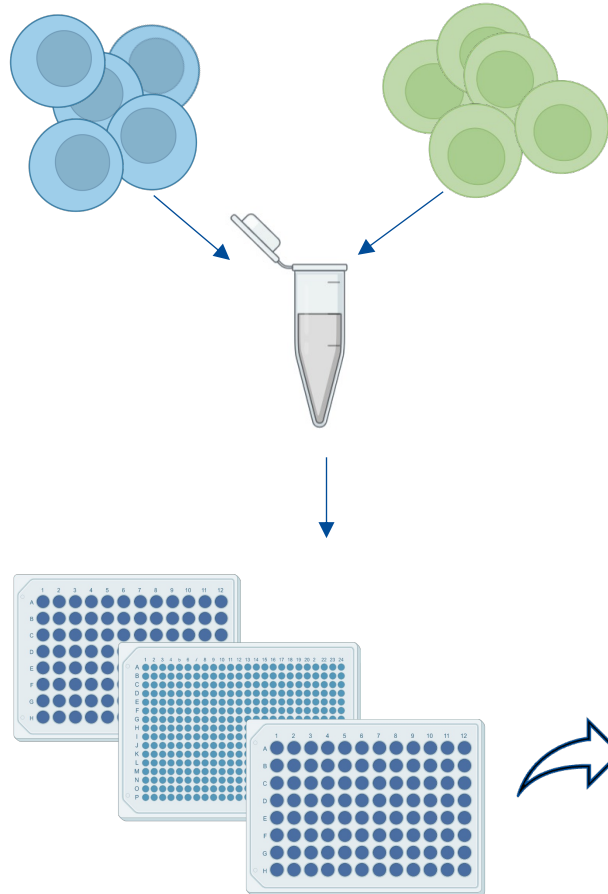


*2-day, instrument-free workflow*

# Increased throughput with low multiplet rates

Human K562 cells

Mouse 3T3 cells

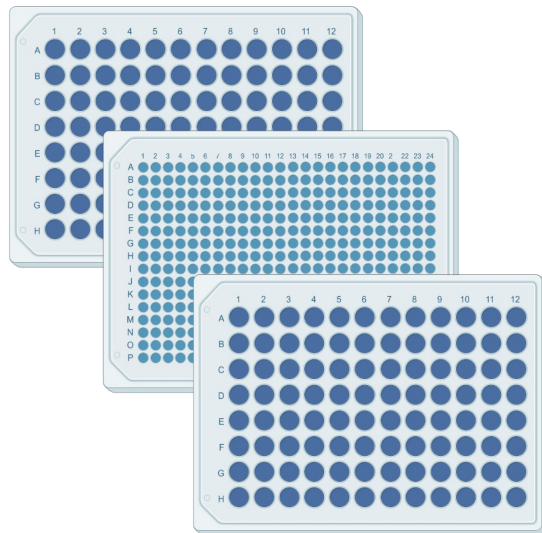


Metric	Value
Mean reads/cell	63,327
Median UMIs/cell	15,195
Saturation	63%



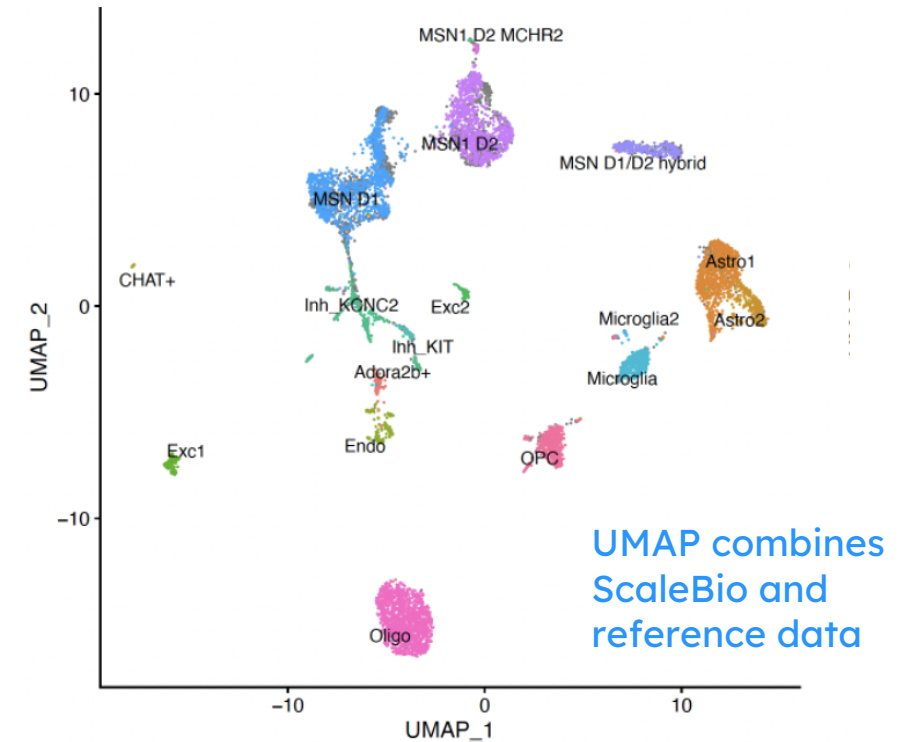
# Macaque brain-derived nuclei show good sensitivity and integration with existing reference data

Cells and nuclei derived from Macaque brain mixed and loaded into ScaleBio scRNA workflow



188,000 cells and nuclei recovered

	Nuclei sample 1	Nuclei sample 2
Mean reads/cell	21,776	19,828
Median UMIs/cell	8,186	7,879
Median genes/cell	2,909	2,747
% reads in cells	85.5%	88.0%
Median Saturation	0.32	0.32





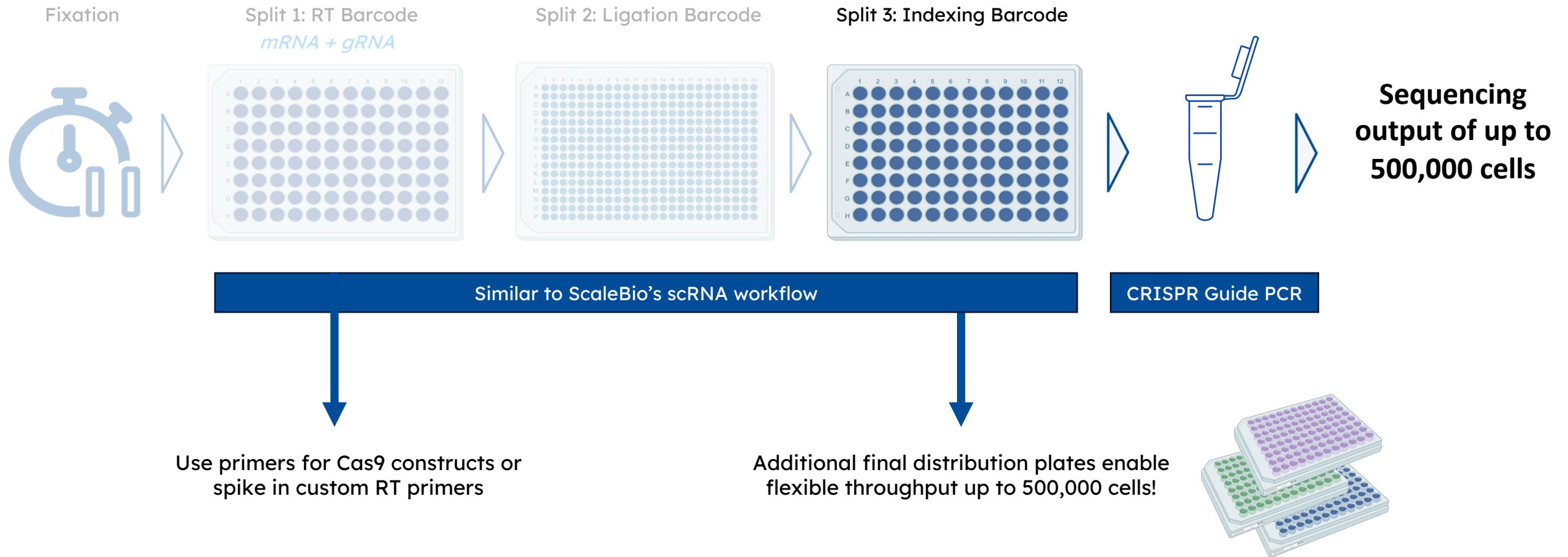


# ScaleBio™ CRISPR Guide Enrichment Kit

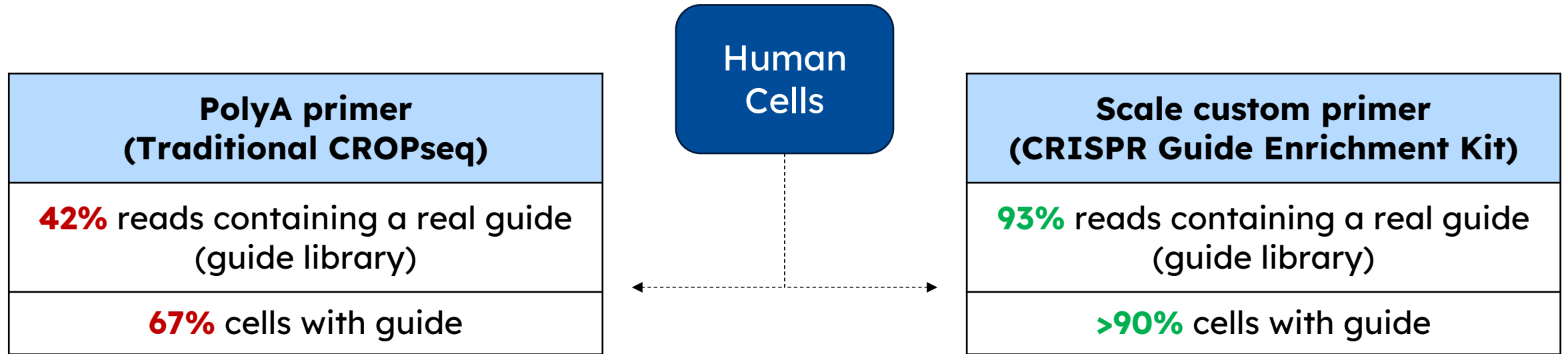
Amplify a CRISPR guide sequence derived from a CROP-style vector alongside your standard RNA Sequencing Library



# ScaleBio's CRISPR Kit workflow with an output of up to 500,000 cells



# ~30% increase in guide detection with Scale's custom primer

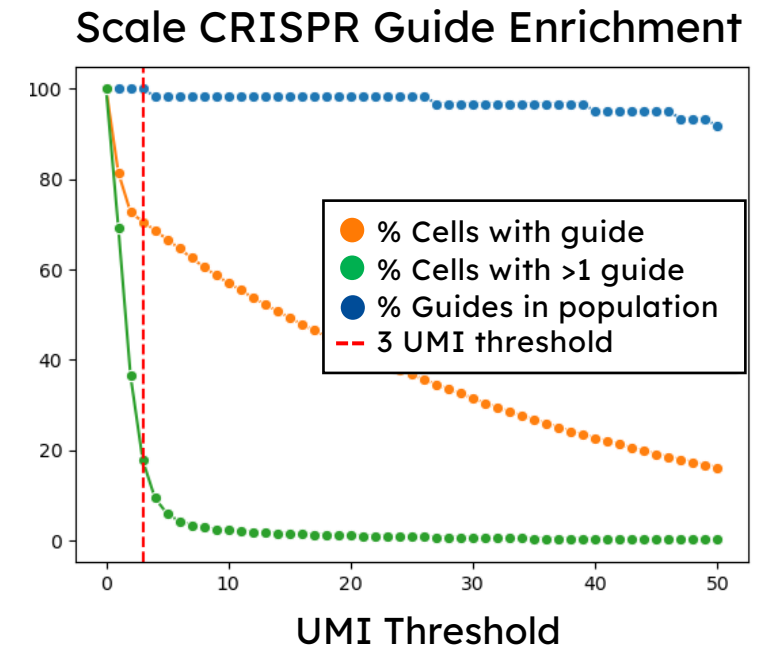
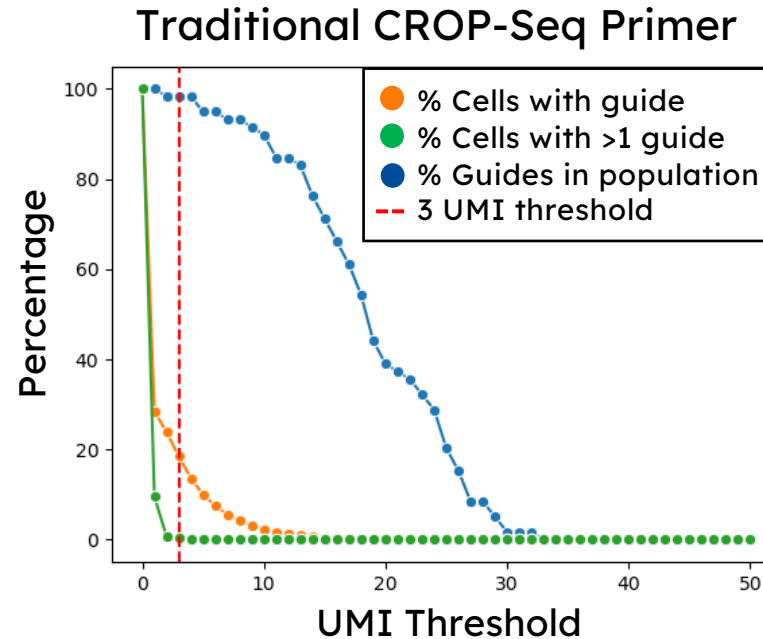


Using Scale's CRISPR Guide Enrichment Kit:

1. **Increases sensitivity** significantly increasing percent cells with a guide
2. **Decreases sequencing costs** by creating an extremely pure guide library

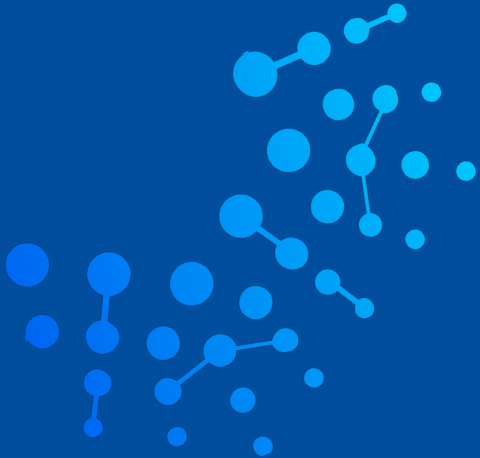
# ScaleBio's CRISPR Kit substantially increases detection rate across cell types

Cell Type	Cells with guide (>3 UMIs)
Cardiac organoids	<b>71.1%</b>
Cell line (human)	<b>95.6%</b>
Cell line (mouse)	<b>93.0%</b>



Increased guide detection with spike-in of Scale CRISPR Primer Signal

**Higher % guides detected in the population**  
**Higher % cells with an identified guide**

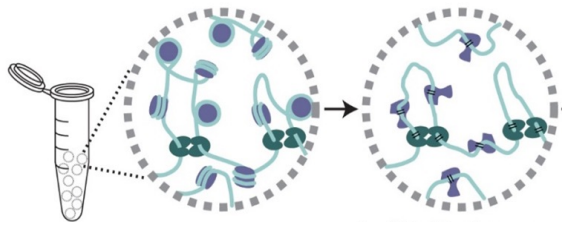


# ScaleBio™ Single Cell Methylation Kit

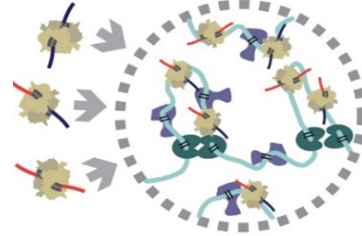
First single-cell DNA methylation kit on the market, which enables profiling single-cell methylation states in tens of thousands of cells

# ScaleBio's scMet workflow can process up to 18,000 nuclei per run

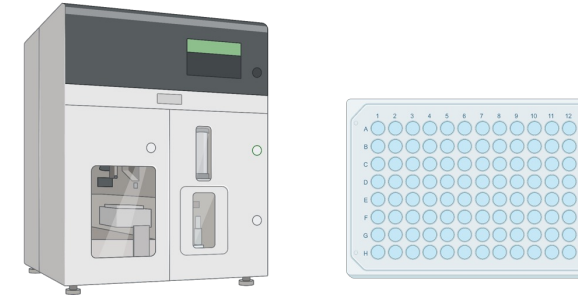
Fix sample,  
nucleosome depletion



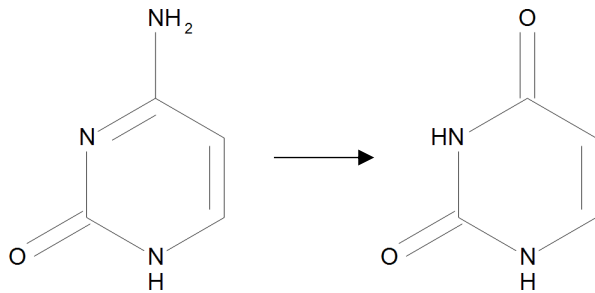
*In situ* tagmentation  
(1st level indexing)



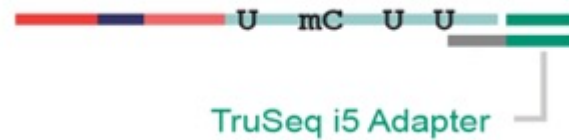
Nuclei sort and distribution to  
96-well plate



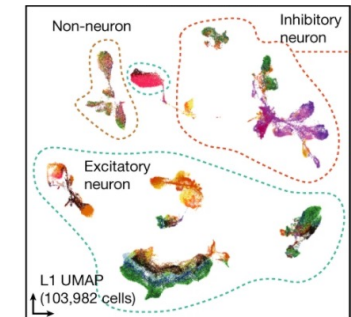
Bisulfite conversion



Library generation  
(Attach adaptor and PCR, 2nd  
level indexing)



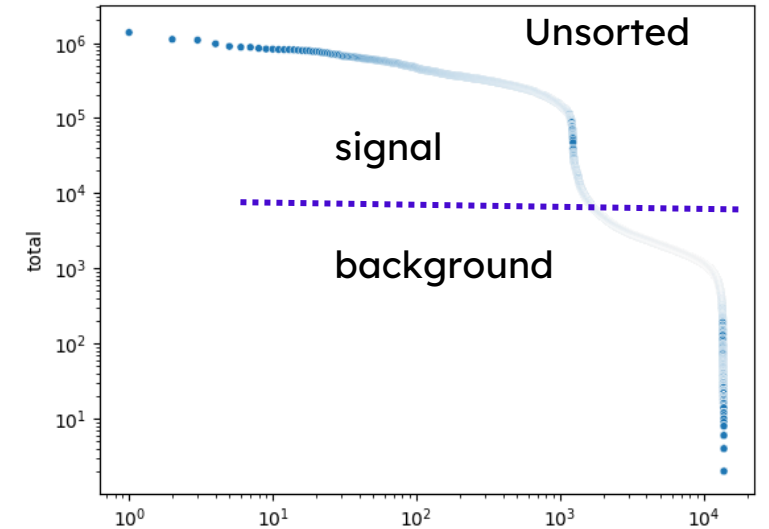
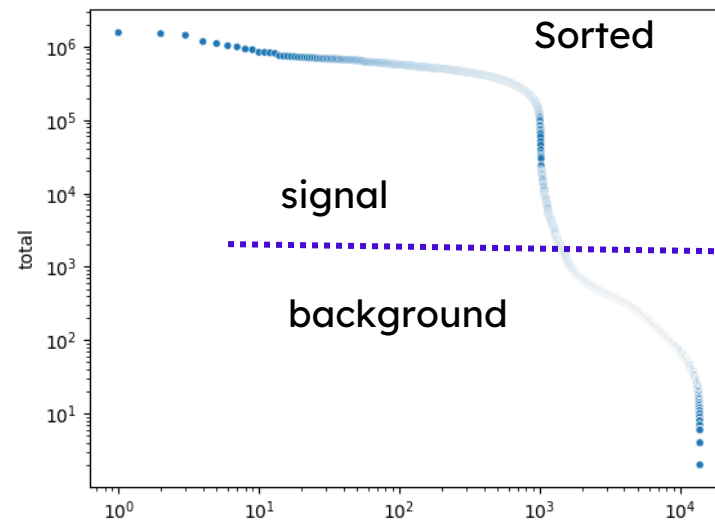
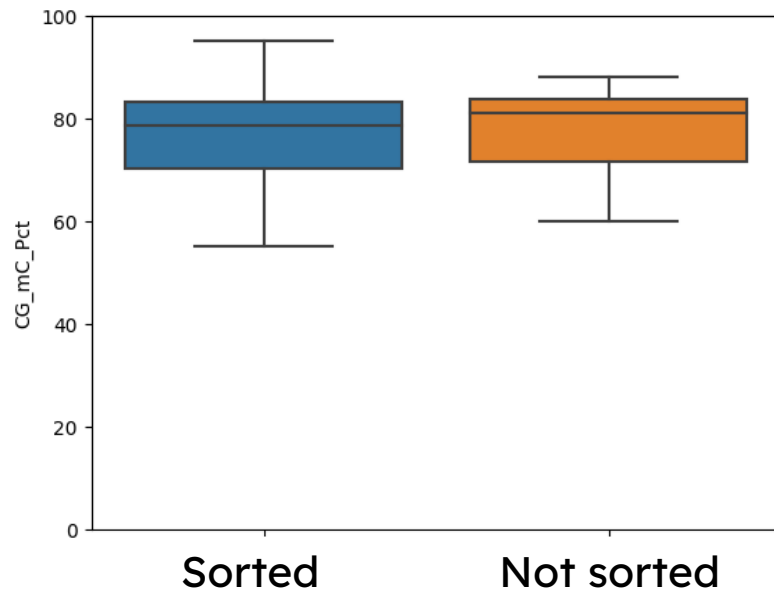
Sequencing and data analysis  
4,600 - 18,300 cells per run



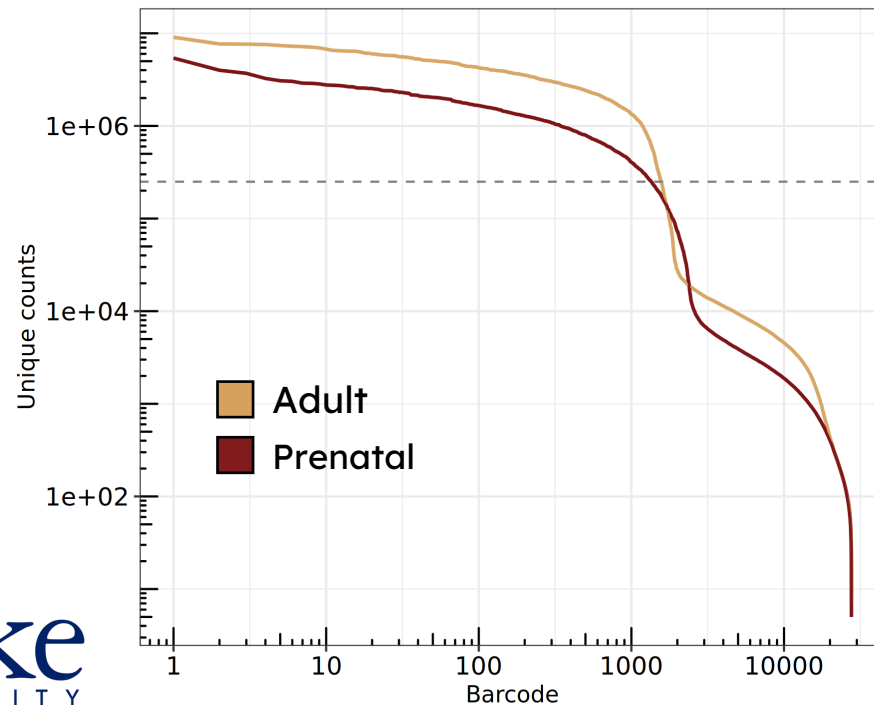
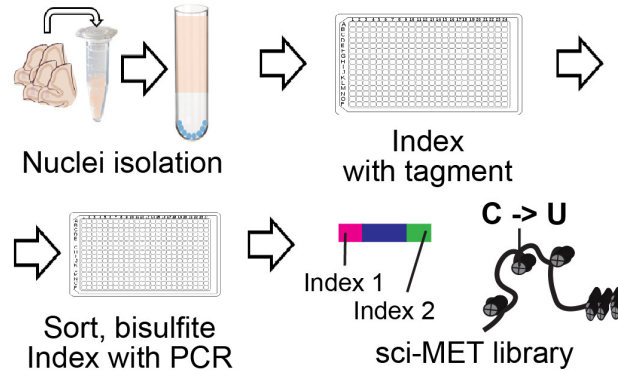
# Methylation produces quality data without cell sorting

Instrument-free no sort method demonstrates higher bisulfite conversion efficiency and comparable CpG site identification, despite a minor increase in background.

% methylated CG



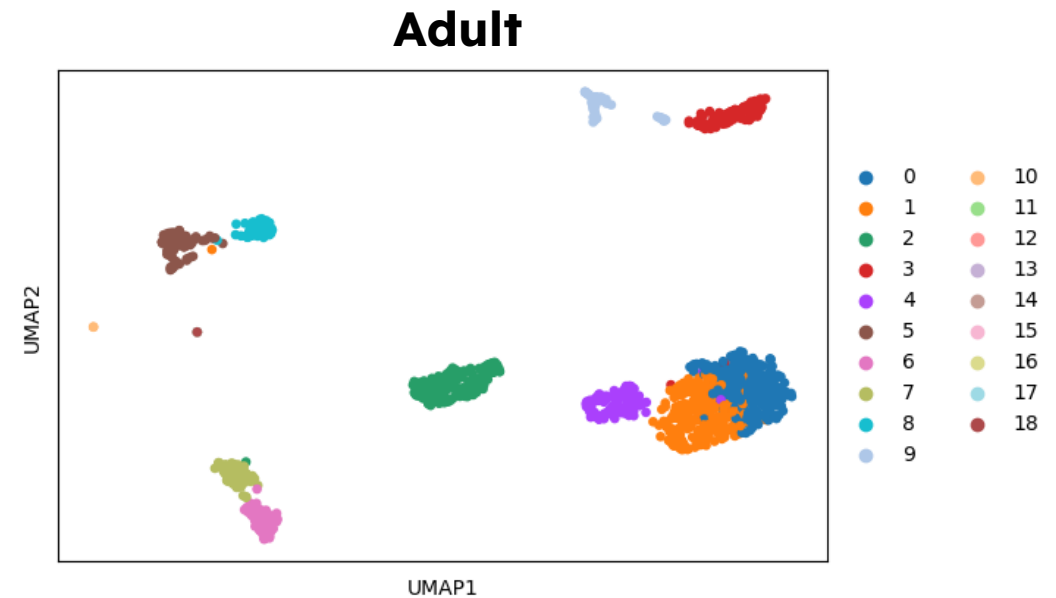
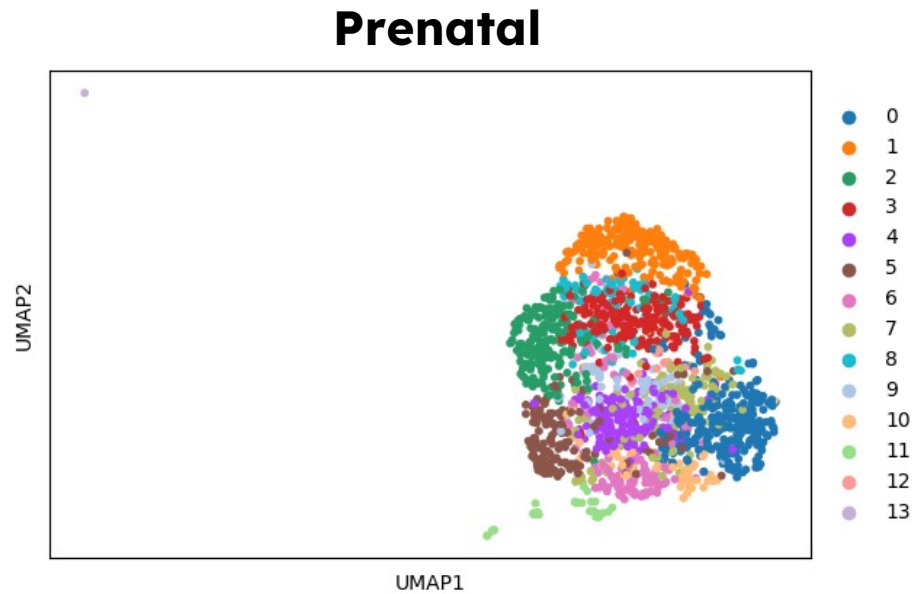
# scMet analysis of prenatal and adult human brain tissue



Input Nuclei	4608
Recoverd Nuclei	4140
Recovery percentage	89.84%
Percentage of reads in nuclei	98.36%
Total_reads	19,526,823,710
Unaligned_reads	935,347,640
Mapped_reads	18,591,476,070
Mapped_percent	95.21%

Recovered nuclei	4140
Median of Total Reads per cell	2,638,331
Median of Reads Pass Filtering per cell	2,413,654
Median Unique Reads per cell	595,769
Median Mitochondrial Reads	n/a
Percentage of Unique Reads of Total Reads	26.00%
Median CG sites covered per cell	246,869
CH_mC_Pct	0.97%
CG_mC_Pct	80.01%

# Unbiased clustering based on scMet profiles





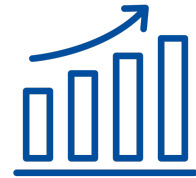
# Enabling single-cell profiling at Scale



Scalable sample indexing



Highly efficient chemistries



Scalable sample throughput

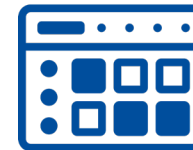


Cost-effective library prep

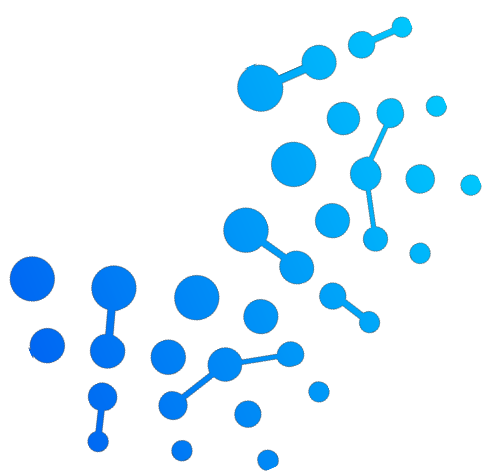


Multi-modal application support

*Genomic, Transcriptomic, Epigenetic and more.*



Instrument agnostic and compatible



**Thank you!**

© SCALE BIOSCIENCES, INC. 2023



