



singulator 100

Automated solid tissue dissociation  
for single cell 'omics analyses

Livermore, CA

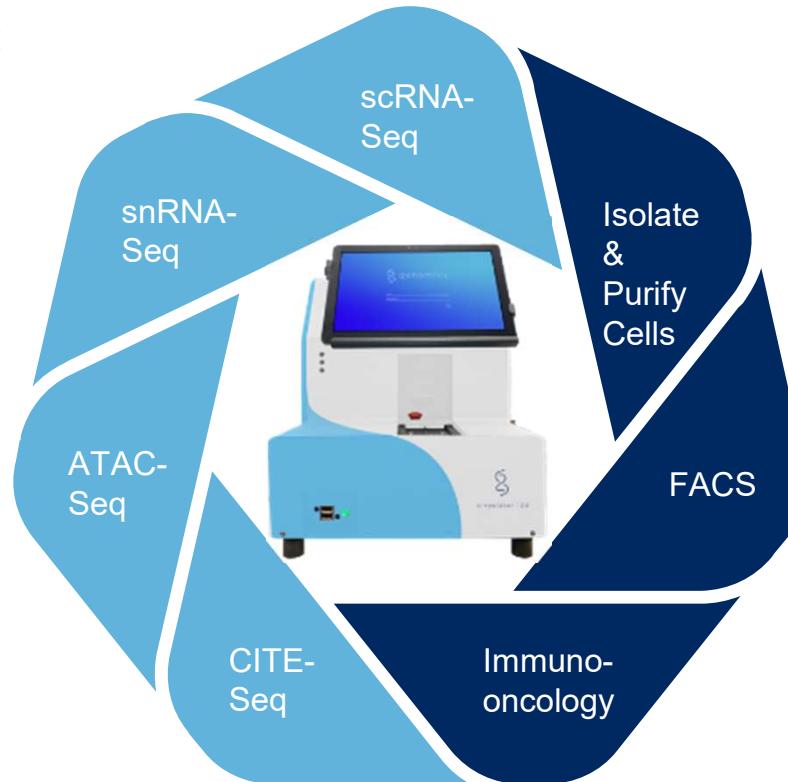
From Samples to Genomics

# One tissue prep platform for omics applications

## Single Cell Genomics:

Genomics Core Labs  
Individual Researchers  
Clinical Research Labs

Genomics



## Stem Cell Biology:

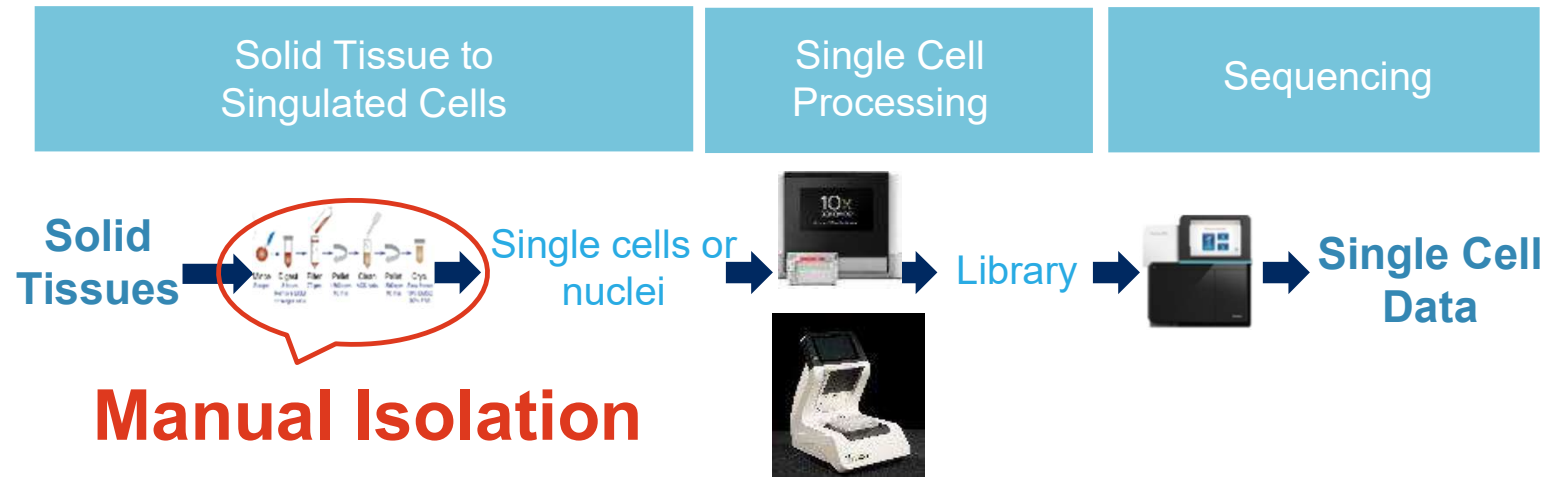
Biotechs  
Clinical Research

Cell Biology

## TILS or Tumor Cells:

Clinical Research Labs  
Personalized Medicine  
Cancer Vaccines

# Manual cell isolation impairs single cell data quality



## Cells

- Requires skilled experts
- No standard protocols
- Isolations affect transcriptome
- Biased cell-type representation

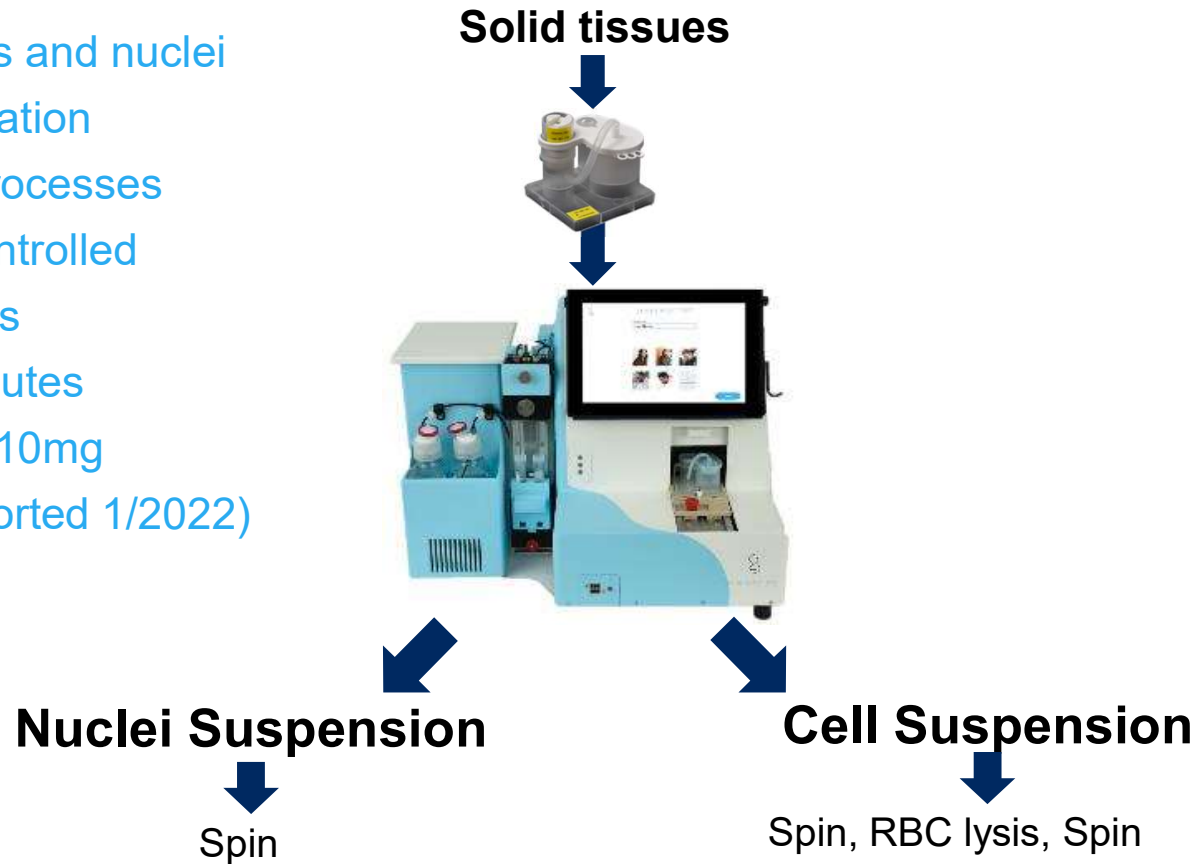
## Nuclei

- Requires skilled experts
- Low, variable yields
- Compromised nuclear membranes
- Degraded chromatin

***Sample Preparation Artifacts In → Data Artifacts Out***

# Simplify and Automate the Isolation Workflow

- High quality cells and nuclei
- Automated operation
- Customizable processes
- Temperature controlled
- Nuclei: 5 minutes
- Cells: 20-60 minutes
- Samples below 10mg  
(2mg supported 1/2022)



## Genomics

- scRNA-Seq
- snRNA-Seq
- ATAC-Seq

## Cell Biology

- FACS
- Organoids
- TILS
- Cell Health

# Simplify and Automate the Isolation Workflow

- Automated operation
- High quality cells and nuclei
- Customizable processes
- Temperature controlled
  - Cold operation for nuclei or cells
- Fast
  - Nuclei: 5 minutes
  - Cells: 20-60 minutes
- Samples below 10mg
  - (2mg supported 1/2022)



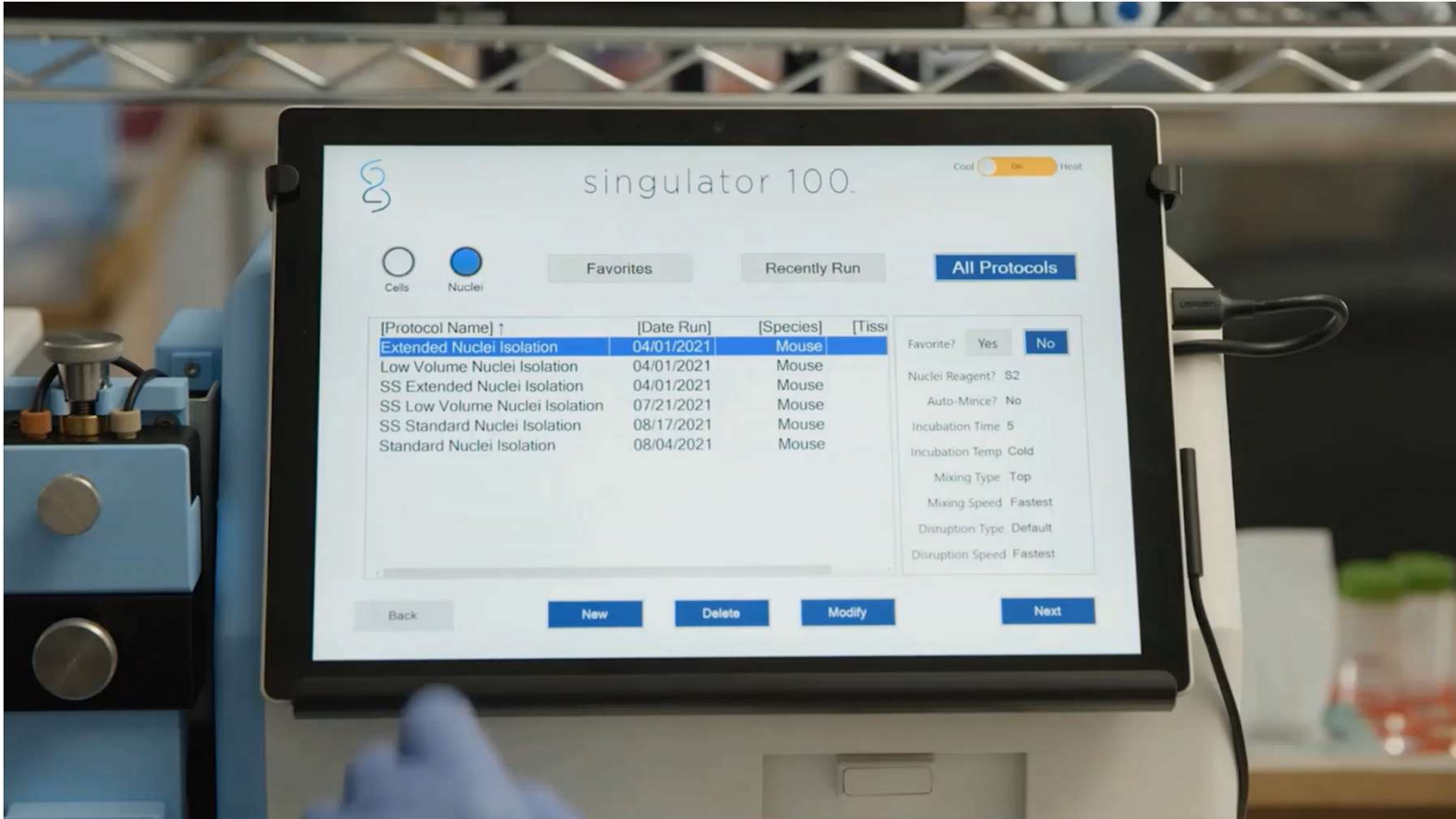
## Genomics

- scRNA-Seq
- snRNA-Seq
- ATAC-Seq

## Cell Biology

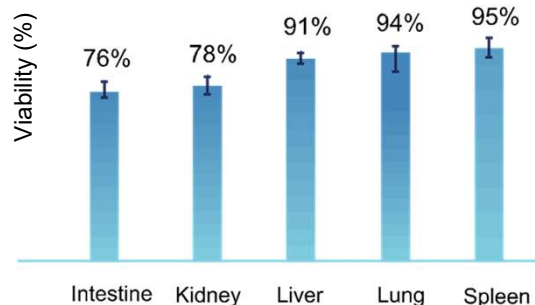
- FACS
- Organoids
- TILS
- Cell Health

# Singulator Operation: Automated & Customizable

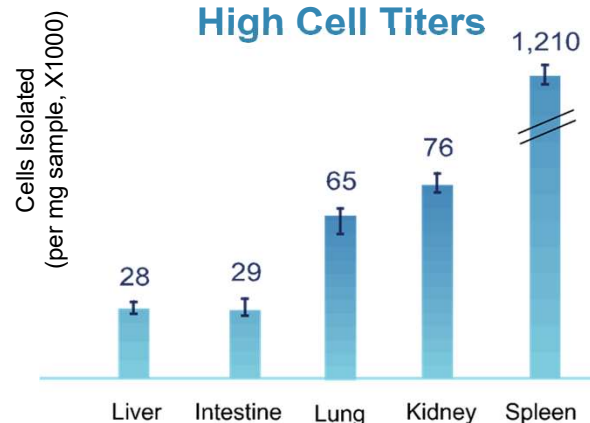


# Benefits of automated tissue processing

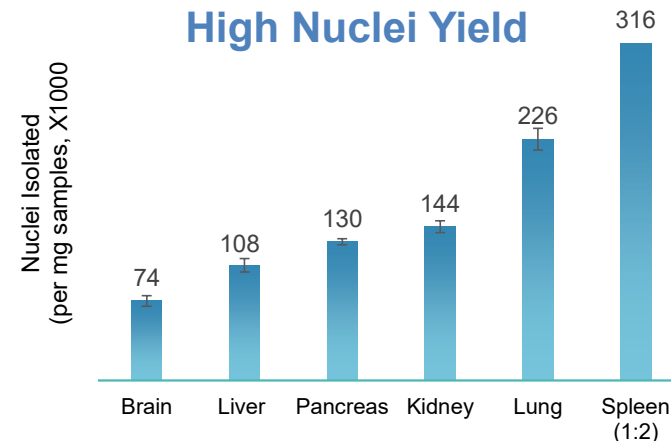
## High Cell Viabilities



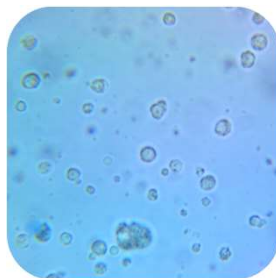
## High Cell Titters



## High Nuclei Yield

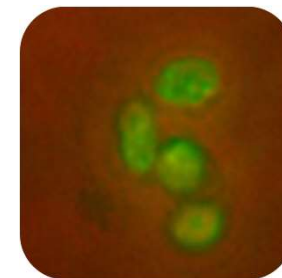


- Improves reproducibility, reduces time/cost
- Outputs single cell suspensions, ready for downstream processing
- 20-60 min



Mouse, lung. Trypan Blue stained.

- Nuclei: transcriptome state
- Cell-type representation
- Fresh, flash frozen, OCT tissue
- Same fast process for most tissues, honeybee to human



Mouse, small intestine. DAPI stained composite image. Courtesy A. Minoda, RIKEN.

## High Quality Cells and Nuclei

Heiko Yang, Franklin Huang, UCSF

Human Bladder Tumor

10x scRNA Seq Data: Singulator vs. Manual Cell Isolations

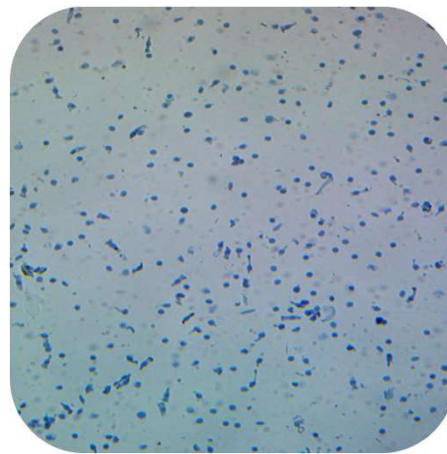
	Singulator	Manual
Initial Dissociation	226,000 cell, 96.8%	135,000 cells, 64.3%
Cells Captured	1185	889
After QC	1013	557
Yield	85.5%	62.7%

nFeature, nCount, % mt, % ribosomal all better with Singulator

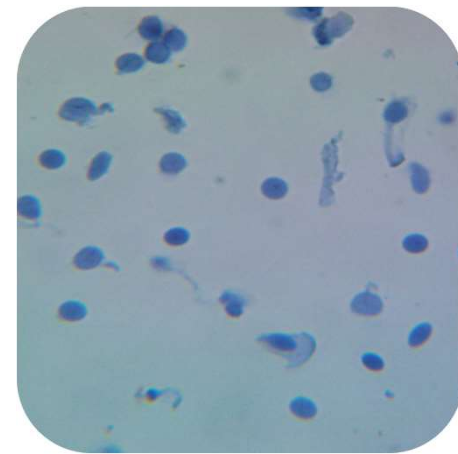


## Nuclei from difficult samples: Human colon

- Success with difficult samples
- Relatively free of debris
- Few clumps



10x

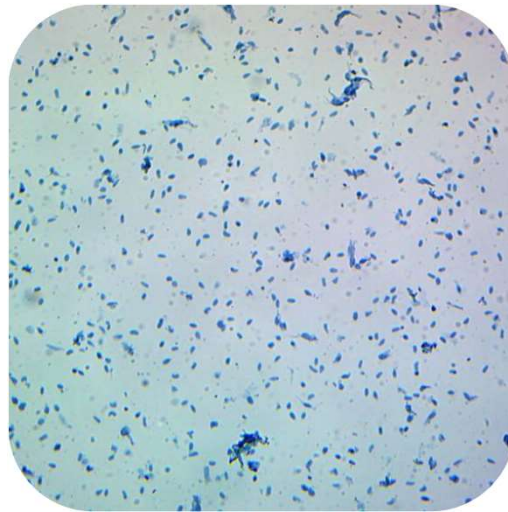


40x

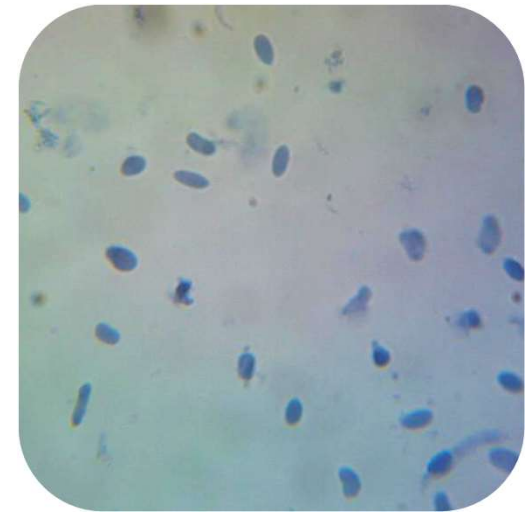
# Infant hemangioma nuclei

2 Samples

- 55,000 nuclei/mg
- 64,000 nuclei/mg

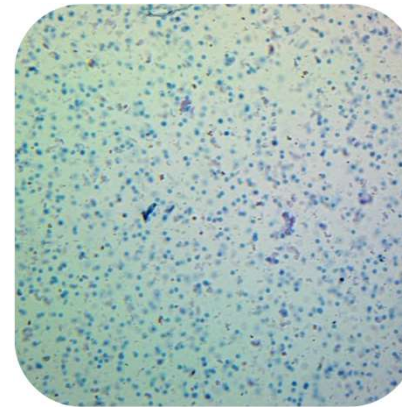
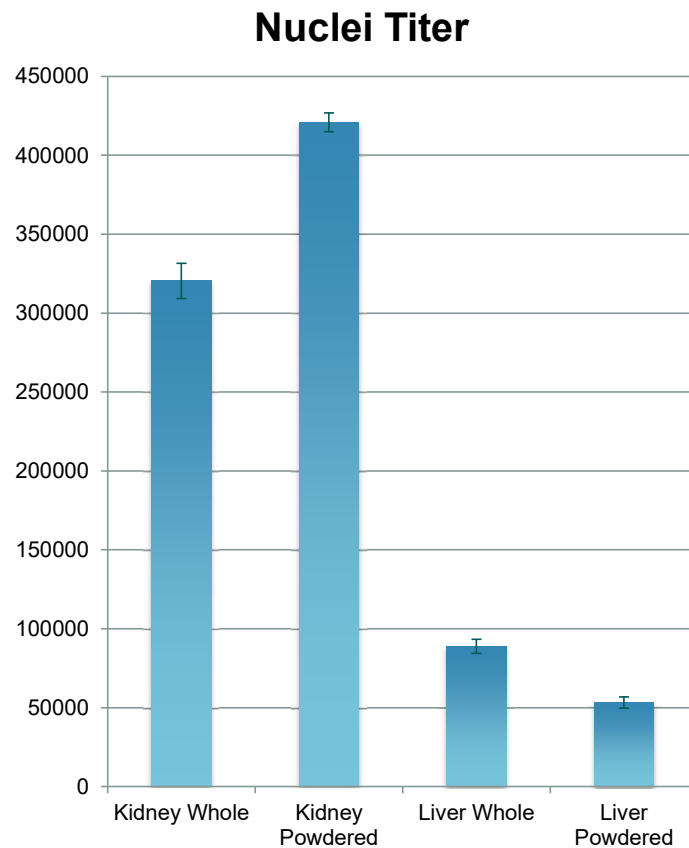


10x

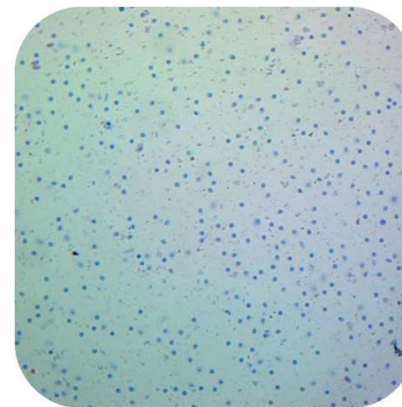


40x

# The Singulator processes frozen and powdered tissue effectively

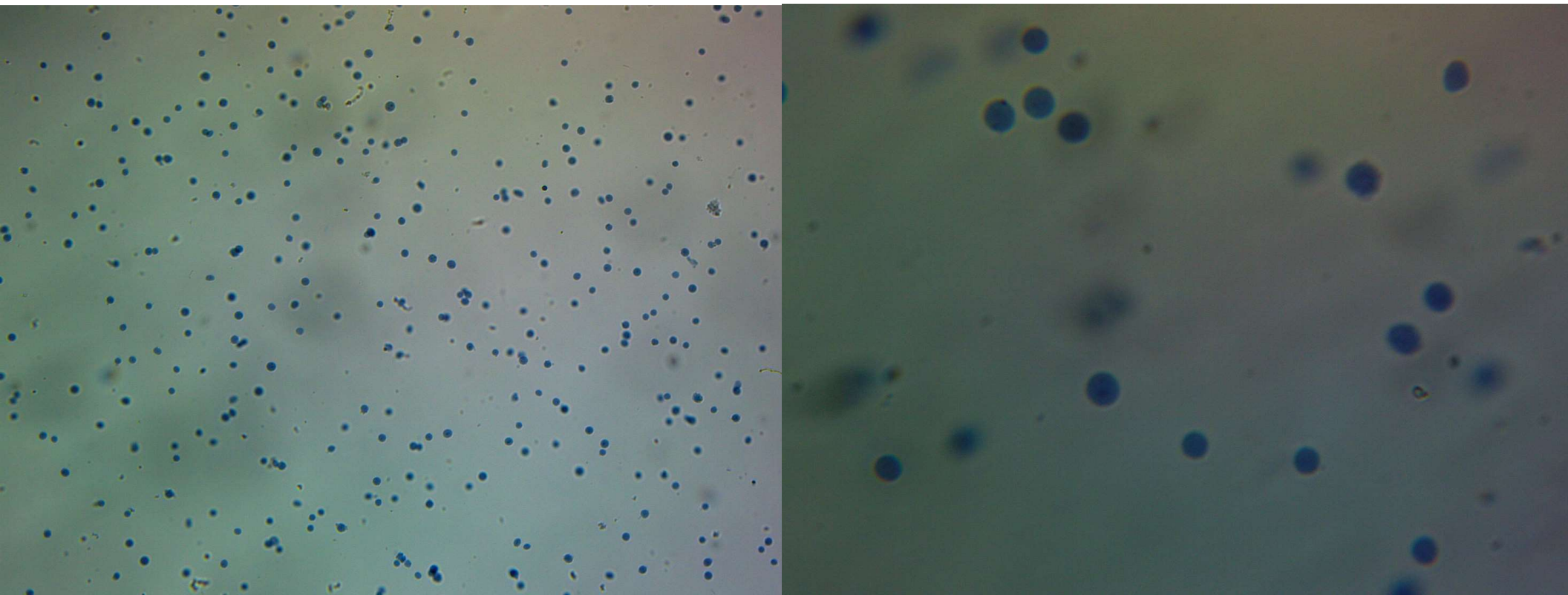


Powdered  
Human  
Kidney 10x



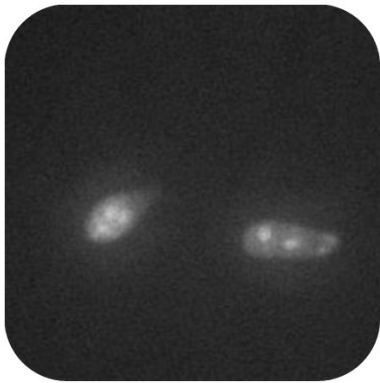
Whole Human  
Kidney 10x

## Mouse Brain: Post 20% Percoll Gradient Spin

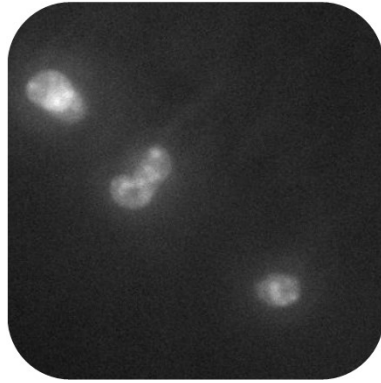


## Intact heterochromatin: high quality nuclei

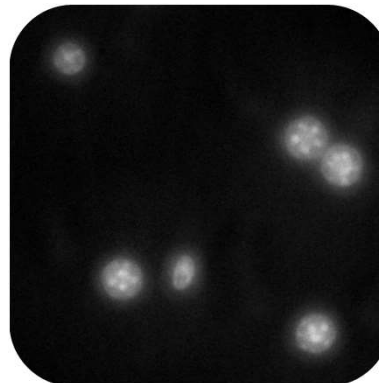
Mouse heart nuclei



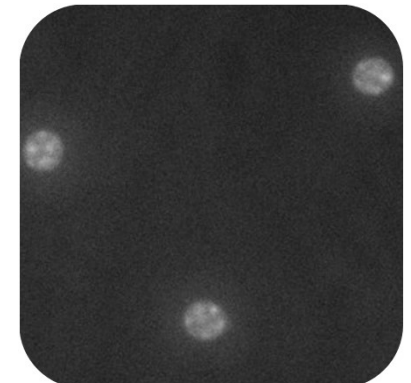
Mouse small intestine



Mouse liver nuclei



Mouse brain nuclei

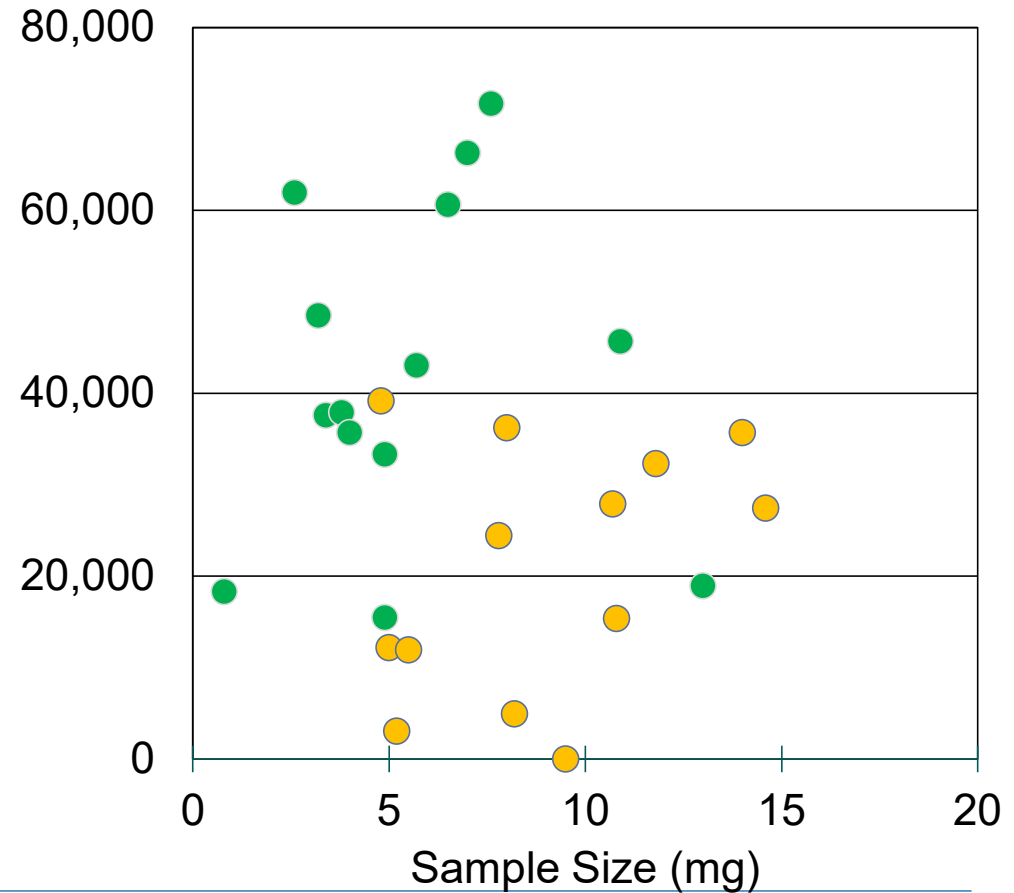
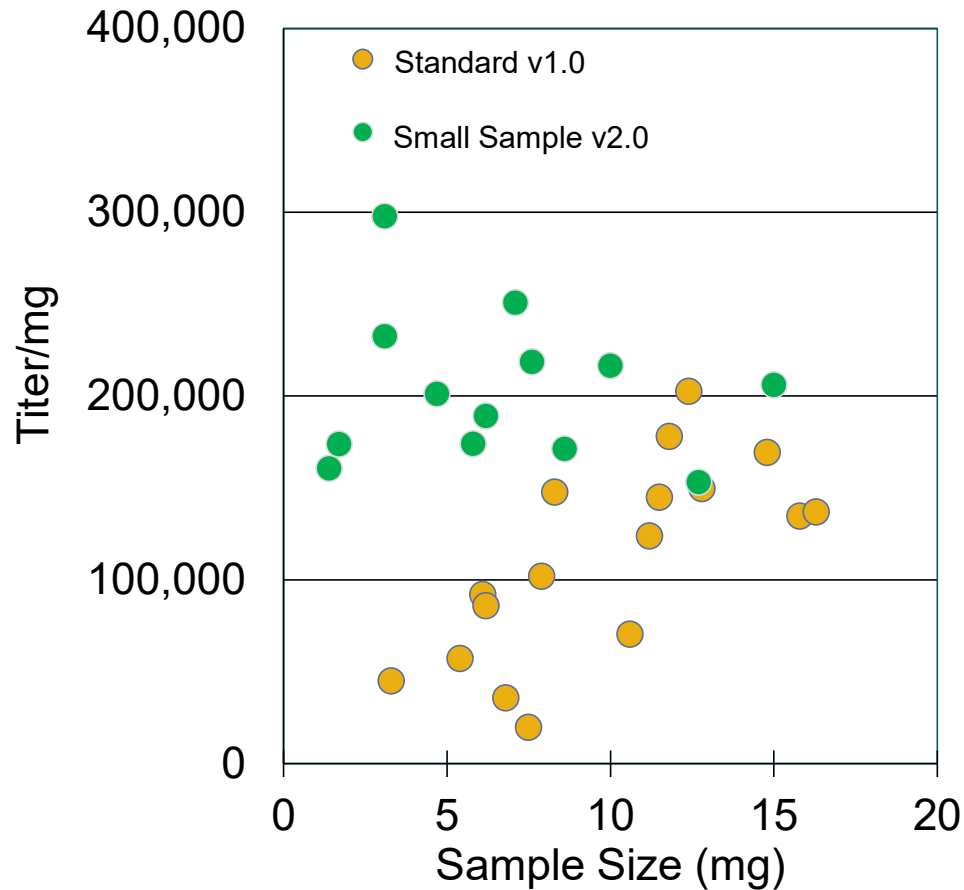


*Images: DAPI stained, Courtesy of Aki Minoda Lab, RIKEN Institute*

# Small Sample Processing

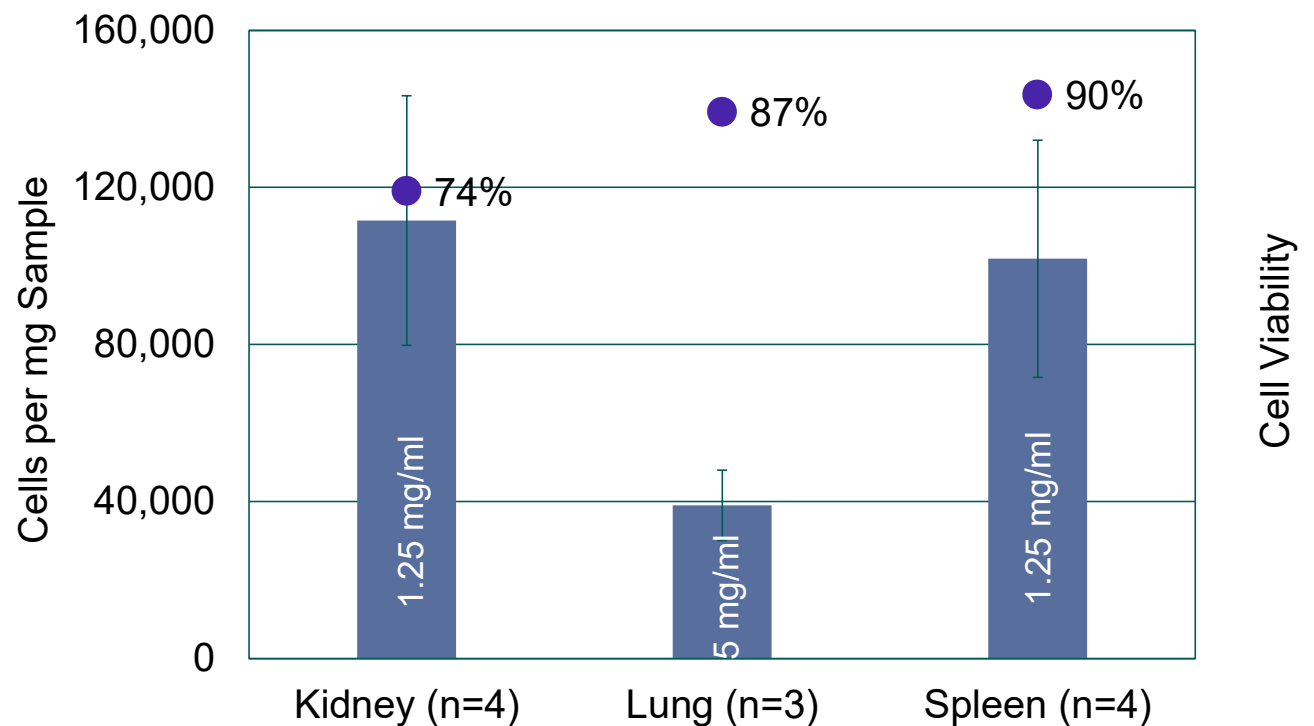
Mouse Kidney Nuclei

Mouse Lung Nuclei



## Temperature Control: Cold Protocols for Cell Isolations

- Cold protocols same duration as 37°C
- Protease from *B. Lichenformis* (Sigma)
- Sequencing to confirm reduction in cell stress genes underway
- Dramatic reduction in enzyme over literature methods

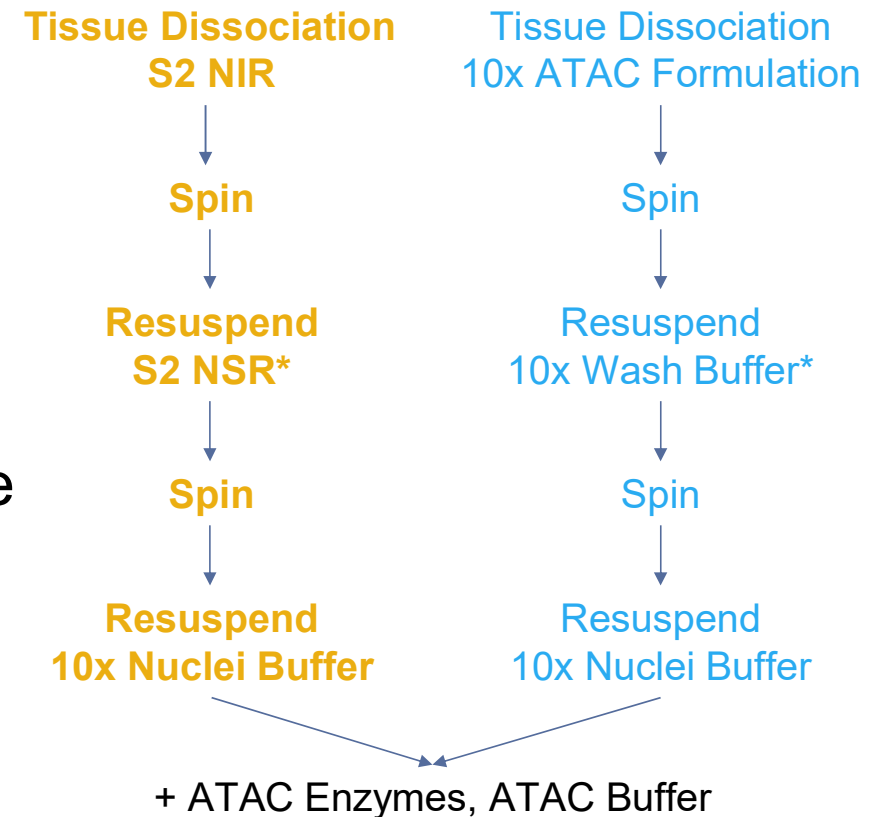




# ATAC SEQ: Impact of Dissociation Reagent

- Isolate Nuclei with the Singulator 100
  - S2 Genomics Reagent (NIR/NSR)
  - 10x ATAC (NP40/TWEEN/Digitonin)
- Compare ATAC data quality
- Fresh Mouse brain\*, lung, kidney tissue

\*Myelin removal in Percoll

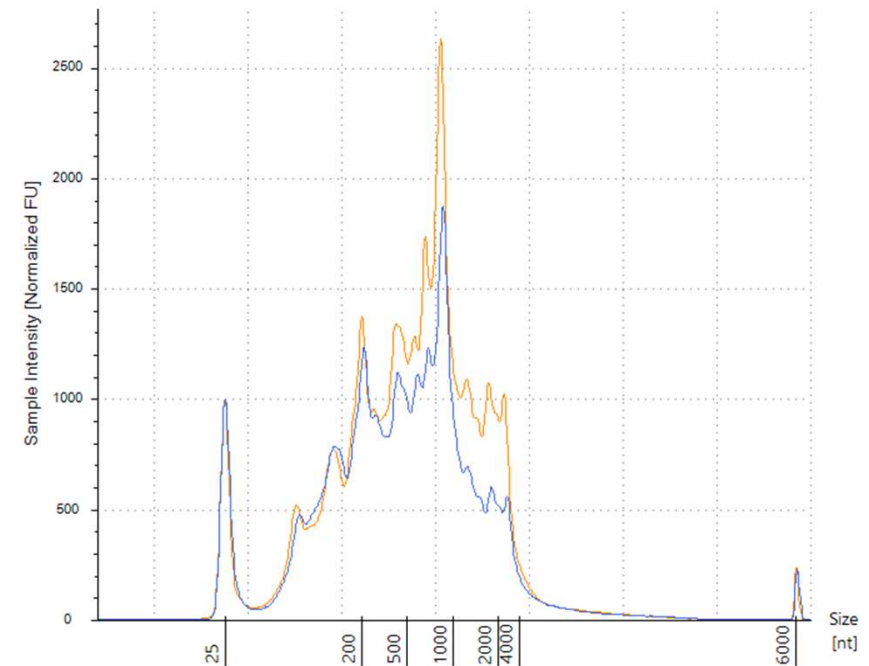
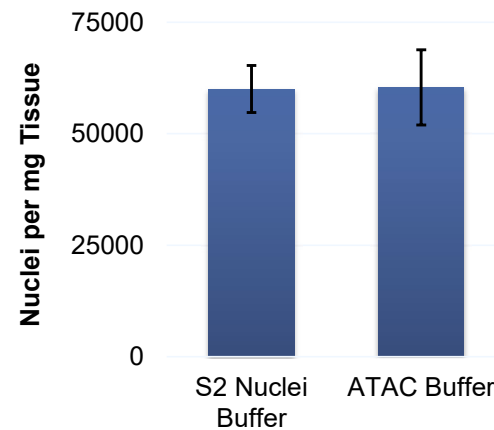




# Nuclei Isolation and RNA Quality: Mouse Lung Tissue

- Standard Singulator Protocol
- +1u/μL Rnase inhibitor
  - 10x ATAC Formulation: 35mg
  - SG2 Genomics Reagents: 47mg

Reagents	DV200
S2	79%
10x	75%

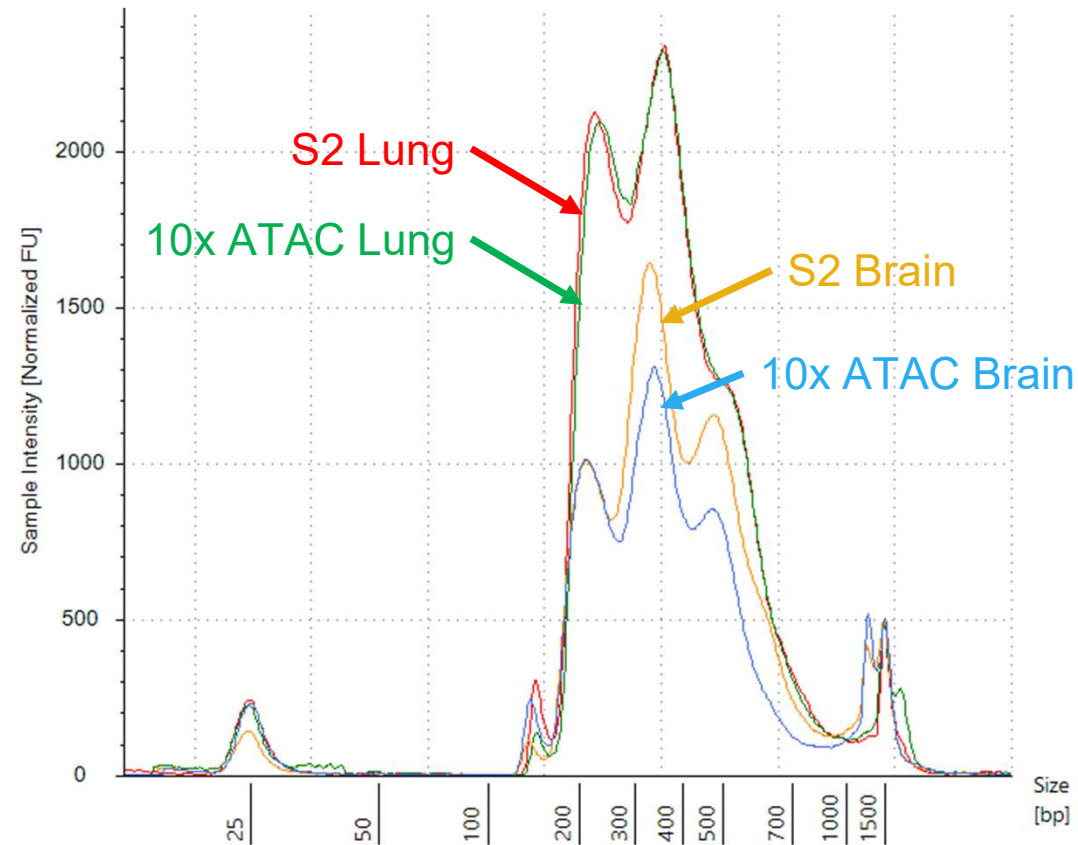


ATAC-Seq Buffer (40x)  
Trypan Blue

# Mouse Brain & Lung Library Quality

- **Comparing**

- S2G Reagents vs. 10x ATAC Formulation
- Tapestation Traces
- S2 formulation has equivalent or better profiles

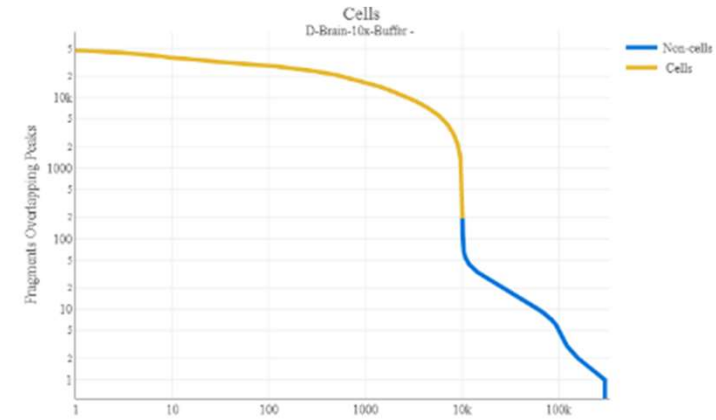
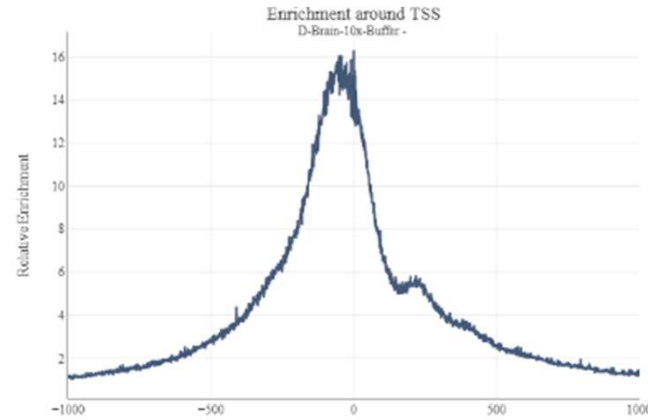
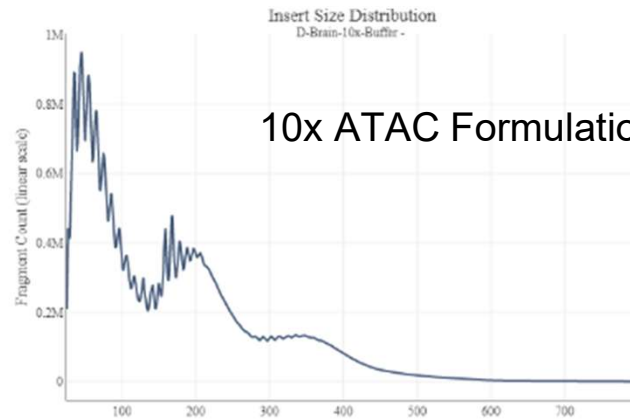


## Mouse Brain & Lung ATAC Data

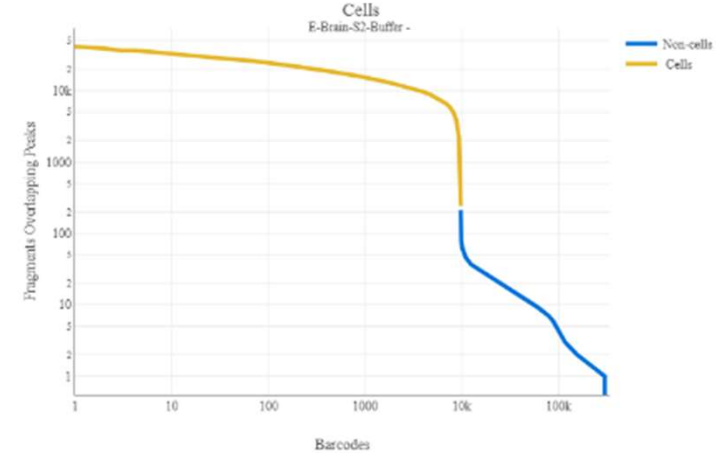
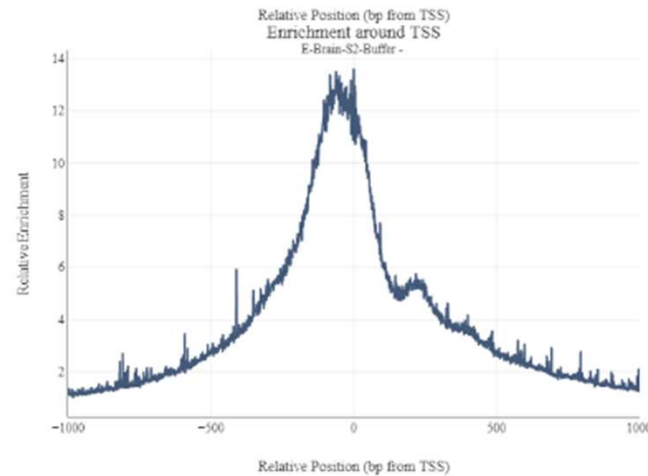
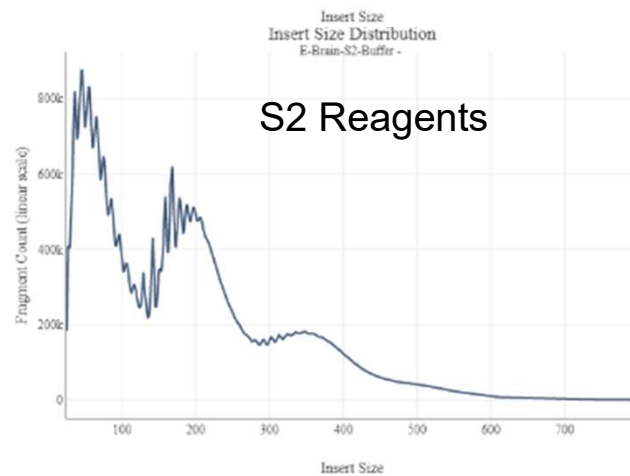
	S2 Brain	10x ATAC Brain	S2 Lung	10x ATAC Lung
Estimated # Cells	9,736	10,110	8,132	8,158
Median HQ Fragments/Cell	13,069	8,758	11,775	10,638
HQ Fragments Overlapping Peaks	56.4%	68.5%	49.1%	46.6%
Confidently mapped read pairs	92.9%	92.6%	88.3%	87.7%
Unmapped read pairs	2.7%	3.4%	2.2%	2.1%
Non-nuclear read pairs	0.3%	0.1%	1.3%	0.8%
Fragments in nucleosome-free regions	44.2%	51.6%	56.5%	57.6%
Fragments flanking a single nucleosome	36.4%	33.4%	31.5%	31.1%
TSS Enrichment Score	13.6	16.3	12.1	10.6
Fraction of HQ fragments overlapping TSS	34.8%	36.1%	31.1%	29.2%
Fraction of HQ fragments overlapping peaks	65.4%	68.5%	49.1%	46.6%

# ATAC Seq Quality Metrics: Mouse Brain Tissue

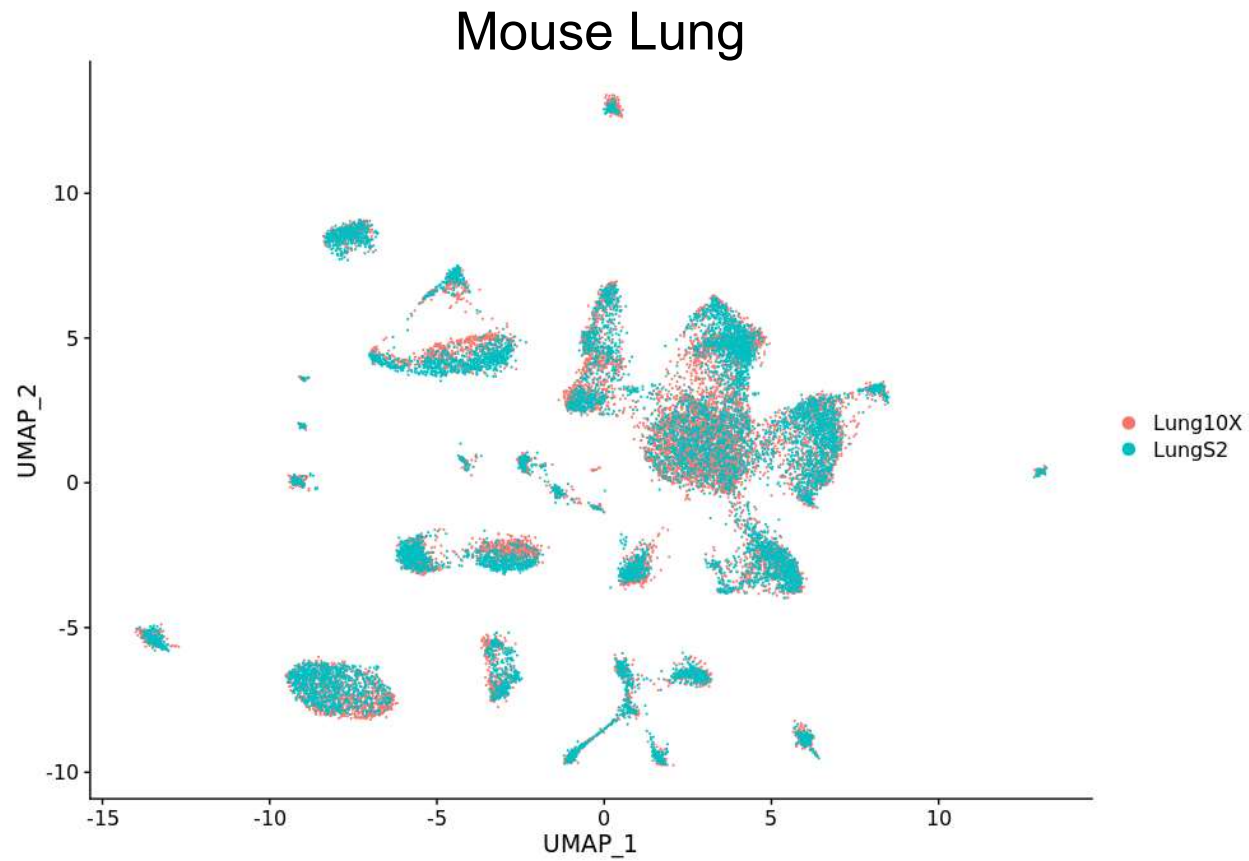
10x ATAC Formulation



S2 Reagents



# Comparative Cell Clustering



# Cells: Success with a wide range of tissues

## Human

*Colon*  
*Colon Polyp*  
*Colon Tumor*  
*Neck Tumor*  
*Colorectal Carcinoma*  
*Bladder Tumor*  
*Lung*  
*Lung Tumor*

## Planaria

*Whole*  
*Cut*

## Mouse

*Brain*  
*Kidney*  
*Liver*  
*Lung*  
*Spleen*  
*Ovaries*

## Snail

*Eye*  
*Embryo*

## Rat

*Brain*  
*Kidney*  
*Liver*  
*Lung*  
*Spleen*

## Zebrafish

*Brain*  
*Liver*

*Italics denotes processing in a customer's lab*

# Nuclei: Success with a wide range of tissues

## Human

*Aorta*  
*Brain: Adult, Infant, Fetal*  
*Breast Tumor*  
*Colon: Normal, Polyps, Tumor*  
*Heart - Adult, Fetal*  
*Hemangioma*  
*Hepatoblastoma*  
*Intestine – Fetal, Adult*  
*Kidney (biopsy)*  
*Lung – Fetal*  
*Muscle: TA and SA Muscles*  
*Organoids: Retinal, Cerebral*  
*Prostate: Normal, Tumor*  
*Spleen – Fetal*  
*Thymus – Fetal*  
*Vascular Abnormality: Arterial, Lymphatic*  
*PBMC's*

## Mouse

*Brain*  
*Colon PDX Tumor*  
*Heart*  
*Intestine*  
*Kidney*  
*Liver*  
*Lung*  
*Muscle*  
*Pancreatic Tumor*  
*Spinal Cord*  
*Spleen*

## Rat

*Brain*  
*Kidney*  
*Liver*  
*Lung*  
*Spleen*

## Acomi

*Kidney*

## Tobacco

*Leaves*

## Honeybee

*Thorax*

## Drosophila

*Brain*  
*Larvae*  
*Ovaries*

## A. Thaliana

*Whole Seedling*  
*Root*  
*Leaves*

*Italics denotes processing in a customer's lab*

# Singulator software is easy to use

- Users can select, edit, and create custom protocols

- Protocol options

- Reagent selection
- Dissociation time
- Dissociation temperature
- Dissociation mixing profiles
- RPM for mechanical disruptions

The screenshot displays the Singulator 100 software interface. At the top, the logo and name 'singulator 100' are visible, along with a 'Cool' (active) and 'Heat' toggle switch. Below this, a text field for 'New Cell Protocol Name' contains 'user\_cell\_7-6-2020 (or enter new name)'. The interface is divided into four main sections: 'Auto Mix', 'Incubation', 'Mixing', and 'Disruption'. The 'Auto Mix' section has 'Yes' and 'No' buttons. The 'Incubation' section includes 'Temperature' (37 °C, Room Temp, 4 °C), 'Time (max 1440 min)' (22), and 'Back' and 'Next' buttons. The 'Mixing' section has 'Mode' (Top, Intermix, Triturate, None) and 'Speed' (high, med, low) options. The 'Disruption' section has 'Mode' (Default, Triturate, None) and 'Speed' (high, med, low) options. A 'Next' button is at the bottom right.

- On-board database to track protocols and log events



# Automation with the Singulator improves results

- Immediate production of high-quality cells or nuclei
  - Increased viability
  - Reduced failures
  - Frozen tissue biobank samples for nuclei
- Automated filtration eases downstream cleanup
- Standardizes front-end process to reduce failures
  - Easy process optimizations
  - Precious samples
  - Costly downstream sequencing
  - Enables access to flash frozen tissue banks
- Works well on small samples
  - ~2mg with v2.0 cartridges
- Temp control for 6°C, RT, 37°C
  - Cold protease protocols
- Open reagent platform



Dr. John Bashkin

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For more information, visit [www.S2Genomics.com](http://www.S2Genomics.com)