# Automated processing of solid tissues into single cells or nuclei for sequencing.

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## ABSTRACT

Single cell sequencing is transforming our knowledge of cells and tissues, revealing new cell types and states, and increasing our understanding of what cells are present, what they are doing, and how cells and tissues function in normal and diseased states. Single-cell sequencing technology, including targeted sequencing, single cell or nuclei RNA-Seq, ATAC-Seq and other sequencing application, is now routinely providing un-paralleled insights into the genomics of tens of thousands or more single cells and nuclei per experiment. The single cell workflow *after* the generation of single-cell suspensions is well established with multiple commercial systems available to prepare single cell libraries.

The development of standardized solid tissue dissociation processes into single cells is critical to the development of single cell biology. Automated systems remove user variability and deskill the production of high-quality single cell and nuclei suspensions, helping democratize single cell biology. Reproducible methods with simple to use instrumentation will be required for future clinical applications.

S2 Genomics has developed the patent-pending Singulator™ System to automatically dissociate solid tissues into single cell or nuclei suspensions. The system quickly processes solid mouse, rat, human, or patient-derived xenograph tumors (or other) solid tissues in disposable cartridges into single cell suspensions using tissue-specific enzymatic, or into single nuclei suspensions using almost universal chemical formulations. The system was used process a wide range of fresh mouse, rat, or human solid tissues, *e.g.*, 3 to >600 mg, into highly viable single-cell suspensions in 20 to 60 min with high yields or to process fresh, frozen, or OCT tissue samples into nuclei in seven min.

Ten pre-production prototype instruments were developed, and six pre-production prototype systems have been deployed and tested in an Early Technology Access program. Single cell and single nuclei RNA-Seq analyses from Singulator-prepared samples and controls from solid tissues were sequenced by SmartSeq II and nanodroplet single cell library preparation. The automated system gave comparable sequencing metrics to manually prepared samples. Mouse brain nuclei samples prepared by the automated Singulator clustered with the samples prepared by the gold standard Dounce.

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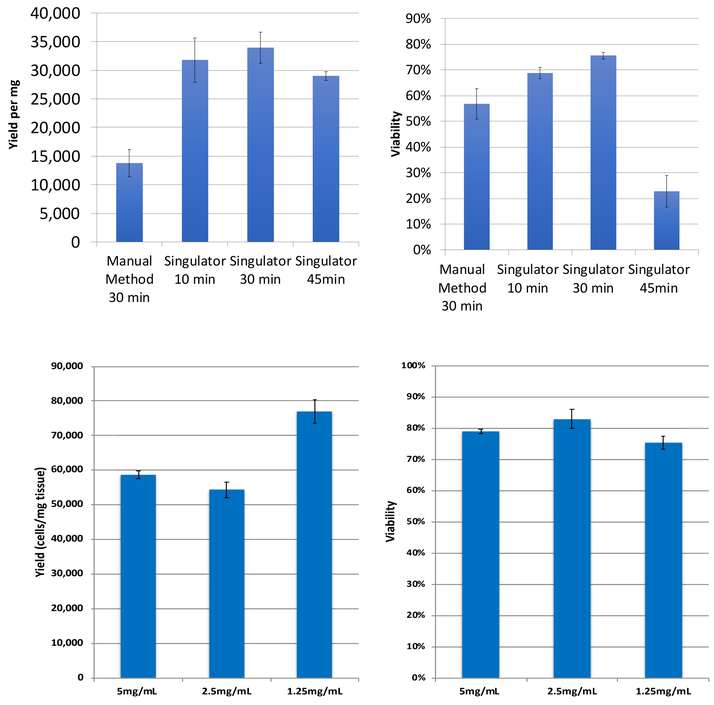



## Isolation of TILS and tumor cells from solid tumors with high yields and viability in 30 min.

***Figure 5.*** *Yield (left) and viability (right) of single cells from a mouse pancreatic tumor processed by the Singulator or manually using Tumor Dissociation Reagent (BD).*

## Automated low temperature dissociation

***Figure 6.*** *Dissociation of mouse kidney at 10°C with cold active protease on the Singulator. Yield (left) and viability (right).*

## Single-cell and nuclei sequencing workflow with the Singulator

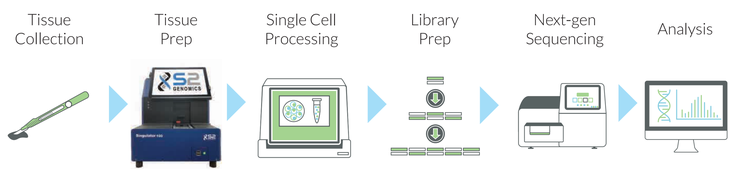



***Figure 1.*** *NGS single cell workflow for solid tissues. Tissue samples are dissociated into singulated cells before single cell processing typically into cDNA when samples can be pooled for library preparation, sequencing, and analysis.*

## Isolate cells from solid tissues with high yield and viability in ~30 min

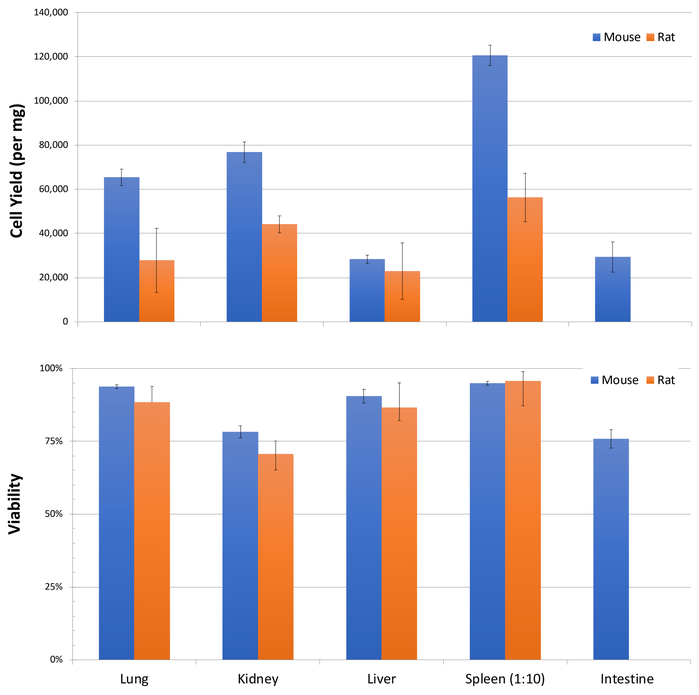
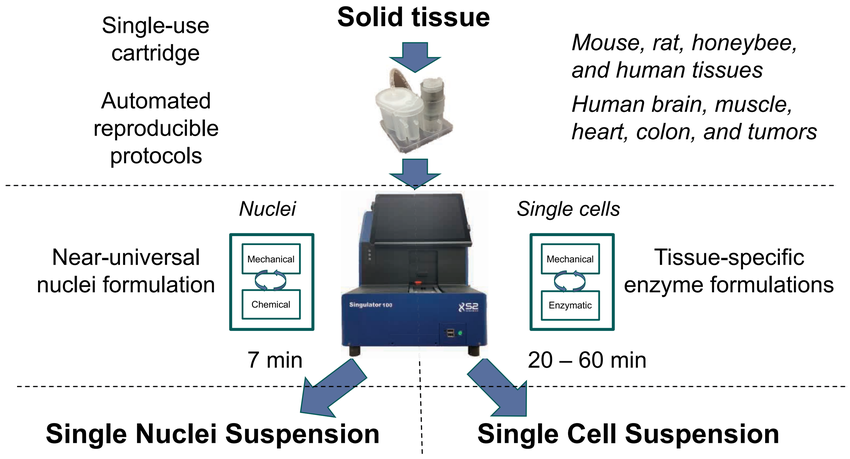



***Figure 2.*** *Automated dissociation of fresh mouse and rat tissues into single cells using the Singulator prototypes with tissue-specific reagents. On-instrument processing time was 20–60 min. Yields were tissue-specific (top) with greater than 70% viabilities for these tissues (bottom). Spleen shown at 1:10 dilution. Samples ranged from 80-250 mg.*

## Singulator™ System processes solid tissues into single cell and nuclei suspensions in single-use cartridges




## Isolate nuclei from solid tissues with high yields in 7 min

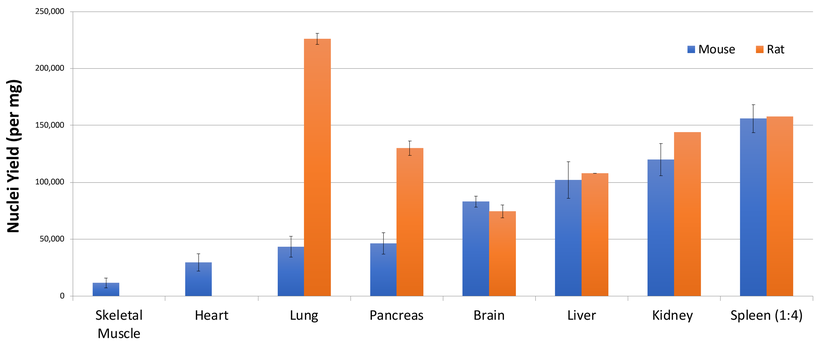



***Figure 3.*** *Automated preparation of nuclei of mouse and rat samples (50-200 mg).*

## Efficient cell or nuclei isolations from biopsy-sized samples

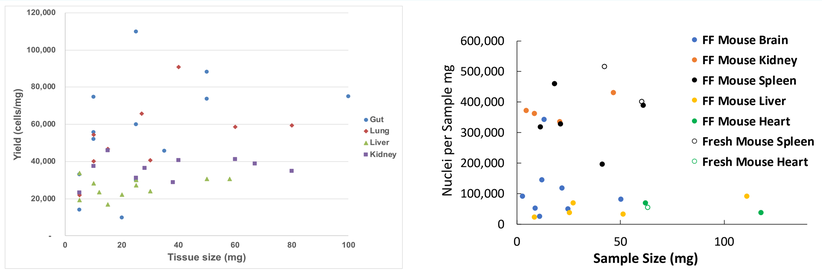



***Figure 4*** *Nuclei yield vs. input mass. Single cells (left) were isolated from fresh tissues. Nuclei (right) were isolated from fresh or flash frozen (FF) tissues.*

## SmartSeq II single cell and nuclei sequencing

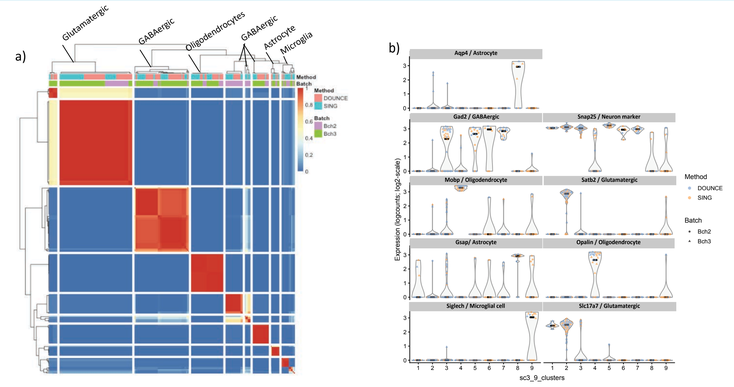



***Figure 7. Cluster heatmap for the best clustering solution for mouse brain nuclei.*** *Single nuclei transcriptomes were clustered using SC3. Optimal results (silhouette score = 0.92) were obtained with k =9. Annotations along the dendrogram in (a) are manually derived from assessing marker gene expression from SC3 and literature. Violin plots for relevant markers are shown in (b).*

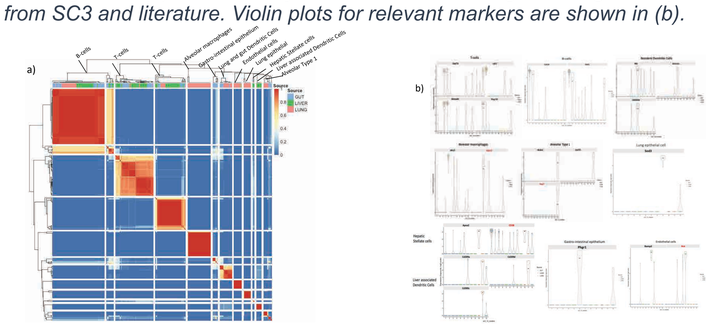



***Figure 8. Cluster heatmap for the best clustering solution for mouse gut, liver, and lung cells.*** *Single cell transcriptomes were clustered using SC3. Optimal results (silhouette score = 0.83) were obtained with k =12. Annotations along the dendrogram in (a) are manually derived from assessing marker gene expression from SC3 and literature. Violin plots for relevant markers are shown in (b) where names given in black are derived from the data while names in red are from the literature.*

## SUMMARY

- Fast standardized automated production of highly viable single cell suspensions from fresh mouse, rat, and human solid tissues in ~30 minutes.
- Fast standardized automated production of nuclei suspensions from fresh or flash frozen mouse, rat, and human solid tissues in 7 minutes.
- Production of TILS and tumor cells from tumors was demonstrated using the Singulator.
- SMARTSeq2 prepared single cells and nuclei sequenced well.