Integrated Sample Preparation Pipeline for scRNA-Seq and snRNA-Seq Utilizing the S2 Genomics Singulator™ Tissue Dissociation Platform and Parse Biosciences Evercode™ Whole Transcriptome Assay

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Introduction

S2 Genomics has developed the Singulator™ 100 and 200 Systems to automate the isolation of singulated cells or nuclei from fresh, frozen, OCT, or FFPE samples using disposable cartridges, reagents, and customizable protocols. The systems have been applied to prepare cells or nuclei from a wide range of tissues and organisms, including human, mouse, rat, chicken, pigs, insects, snails, zebrafish, *Drosophila*, honeybee, planaria, and plants.

Parse Biosciences has developed combinatorial barcoding technology that enables scalable single cell analyses without the limitations of microfluidic droplet approaches. With the Parse Evercode technology, it is possible to profile 10,000 to 1 million cells in a single experiment.

Single-cell sequencing workflows for multiple applications require reproducible generation of high-quality single-cell or nuclei suspensions, while combinatorial approaches to library preparation can, in turn, provide superior data quality to droplet-based methods. Here, we demonstrate the utility of combining automated dissociation of solid tissue samples with combinatorial library preparation and show results for a variety of tissue types, including snRNA-Seq data for adult mouse lung and kidney, and scRNA-Seq data for adult mouse kidney and skin.

Figure 1: Integrated workflow. Tissue excision, tissue dissociation, tissue fixation, sample barcoding and library preparation, NGS sequencing, and data analysis.

Figure 2. Singulator™ 200 System for processing two samples in single-use cartridges, with integrated chiller for nuclei reagents, and dual Single-Shot Mechanisms for delivering cell preparation and other reagents.

Methods

Nuclei suspensions were prepared using the Singulator 100. Lung and kidney tissue samples were isolated from ICR (CD-1®) mice using IACUC-approved protocols. After harvesting, each tissue was dissociated in a Nuclei Isolation Cartridge with RNase inhibitor using the Low Volume Nuclei Isolation protocol. After completion of the dissociation, resulting suspensions were centrifuged and purified further by gradient centrifugation and pellets resuspended in 500 µL of S2 Genomics Loading Buffer supplemented with BSA and RNase inhibitor. Each nuclei suspension was filtered through a 40 µm strainer and nuclei concentrations were determined using a Nexcelom K2 with AO/PI and the cell titer/health assay. Lung and skin cells were prepared using S2 tissue-specific enzyme formulations and dissociation protocols, followed by centrifugation and RBC lysis. At least 400,000 cells or nuclei were centrifuged at 200 g for 10 minutes at 4°C. Nuclei or cells were fixed with Evercode Nuclei Fixation v2 and whole transcriptome libraries were created with Evercode WT v2, following Parse Biosciences recommended protocols. Libraries were sequenced on an Illumina NovaSeq 6000, and data were processed with Parse Biosciences Analysis Pipeline v1.0.2.

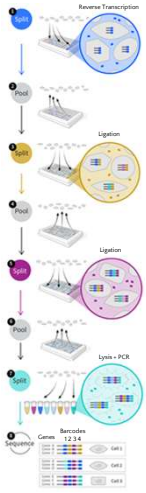

Figure 3: Parse Evercode combinatorial barcoding technology. Samples are dispensed into a 96-well plate. A reverse transcription step introduces a different initial barcode into each well. Samples are then pooled and re-dispensed into a new 96-well plate. Subsequent barcodes are sequentially introduced through ligation, sample pooling, and splitting. Resulting transcripts from each cell are tagged with a unique pattern of barcodes and sequenced.

Results

Tissue	Est # Cells	Median Transcripts/Cell	Median Genes/Cell	Mean Reads/Cell
Kidney Nuclei	19,799	6,935	2,634	69,471
Kidney Cells	18,417	9,135	2,899	77,849
Lung Nuclei	14,097	3,633	1,737	26,667
Skin Cells	15,117	3,392	1,351	28,930

Mouse Kidney: A key experimental consideration for single cell analyses is whether to analyze cells or nuclei. **Figures 4** and **5** show side by side and overlay UMAP projections, respectively, of cell type clusters obtained from snSeq and scSeq of fresh mouse kidney tissue samples. The UMAPs illustrate the substantial concordance of the cell type representations of the two sample types for this tissue.

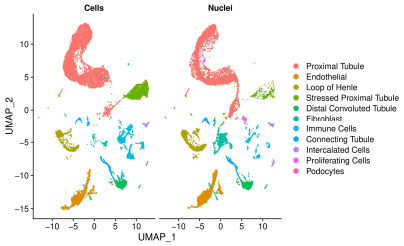

Figure 4. Comparison of UMAP cell clusters for cells (left) and nuclei (right) with the major cell types annotated.

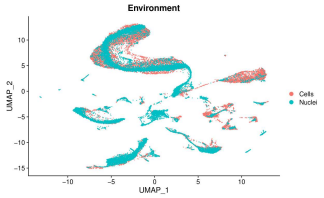

Figure 5. Overlay of UMAP cell clusters for cells and nuclei showing concordance of the two data sets.

Mouse Lung Nuclei: **Figure 6** shows the violin plots of sequencing quality metrics for the data on mouse lung, showing a high number of genes per cell detected and low percentage of mitochondrial contamination. **Figure 7** shows the annotated UMAP representation of cell type clusters.

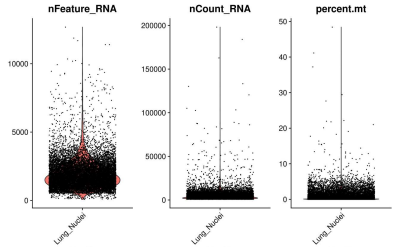

Figure 6. Violin plots of sequencing quality metrics for data from mouse lung.

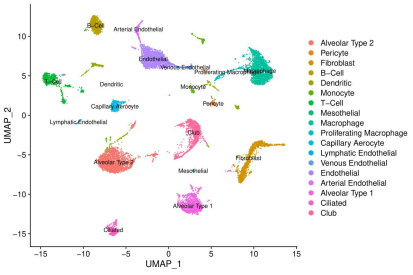

Figure 7. UMAP clustering of cell types detected in mouse lung tissue, annotated.

Mouse Skin Cells: **Figure 8** shows the violin plots of the sequencing quality metrics for the data on mouse skin, showing a high number of genes per cell detected and low percentage of mitochondrial contamination. **Figure 9** shows the annotated UMAP representation of cell type clusters.

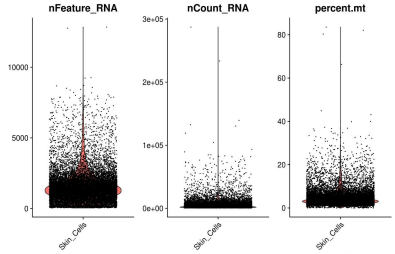

Figure 8. Violin plots of sequencing quality metrics for data from mouse skin.

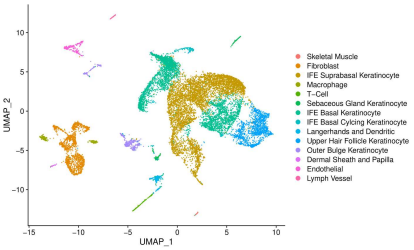

Figure 9. UMAP clustering of cell types detected in mouse skin tissue, annotated.

Summary

The automated Singulator 100 and 200 Systems produce high quality cells and nuclei from a variety of tissues and organisms. These can be used directly in combination with the Parse Evercode combinatorial barcoding technology to generate high-quality single cell and single nuclei sequencing data.