Sequencing Lung and Kidney Nuclei: Leveraging the Singulator™ 100 and Parse Biosciences Evercode™ Whole Transcriptome v2 Platform

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Introduction /

Advancements in high-throughput sequencing technologies have revolutionized our understanding of cellular heterogeneity and gene expression patterns. To comprehensively investigate the transcriptional landscapes of specific cell types within complex tissues, researchers have turned to single nucleus RNA sequencing (snRNA-seq) techniques. In this technical note, we demonstrate the compatibility of Singulator produced kidney and lung nuclei with the application of the Parse Biosciences single-cell whole transcriptome technology. The utilization of multiple platforms for single nuclei sequencing is crucial as it allows researchers to overcome the limitations and biases associated with individual technologies. The ability to both fix and run multiple samples simultaneously simplifies the single nuclei sequencing workflow, and combined with the ease of the Singulator system facilitates a wider range of sequencing applications.

Methods /

Singulator Nuclei Isolation

Nuclei suspensions were prepared using the Singulator™ 100 System. Nuclei Isolation Reagent™ (NIR, S2 Genomics, 100-063-396), Nuclei Storage Reagent™ (NSR, S2 Genomics, 100-063-405), Protector RNase Inhibitor (Sigma: 3335399001) and Singulator Nuclei Isolation Cartridge (S2 Genomics, 100-063-287) were used. Sample cartridges were chilled at -20 °C prior to use and the Singulator was set to 'Cool'. For this experiment, lung and kidney tissue samples were isolated from ICR (CD-1®) mice using IACUC-approved protocols. After harvesting, the tissue was placed on ice in cold HBSS until used. Kidney (100 mg) and lung (109 mg) tissue were then placed into the dissociation chamber of pre-cooled Nuclei Isolation Cartridges along with 75 µL of Protector RNase Inhibitor and inserted into the Singulator 100. Tissues were dissociated using the Low Volume Nuclei Isolation protocol. After completion of the automated nuclei isolation protocol in approximately 7 minutes, nuclei from each sample were removed from their respective cartridge with a 1 mL pipette and each placed into a pre-cooled 15 mL conical tube. The suspensions were centrifuged for 5 min at 500 g and 4 °C in a swinging-bucket centrifuge, followed by removal of the supernatant. The pellets of nuclei were each resuspended in 1 mL Nuclei Debris Removal Stock Reagent (S2 Genomics, 100-253-628), diluted to 20% with NSR, and centrifuged at 500 g for 15 minutes. The pellets of nuclei were then resuspended in 500 µL of Loading Buffer (S2 Genomics, 100-257-006) supplemented with 1% BSA and 12.5 µL of Protector RNase Inhibitor. Each nuclei suspension was filtered through a 40 µm Flow-mi strainer and nuclei concentrations were analyzed and counted using a Nexcellom K2 with AO/PI and the cell titer/health assay.

Parse Biosciences Evercode Fixation, Barcoding, and Library Prep

At least 400,000 nuclei were centrifuged at 200 g for 10 minutes at 4 °C. Nuclei were fixed with Evercode Nuclei Fixation ECF2003 and whole transcriptome libraries were created with Evercode WT ECW02030, following Parse Biosciences recommended protocols. Libraries were sequenced on an Illumina Novaseq 6000, and data was processed with Parse Biosciences Analysis Pipeline v1.0.2.

Results /

After cleanup protocols, nuclei isolation yields were 15,300,000 and 3,550,000 for kidney and lung, respectively. After fixation, barcoding, and library prep the resulting 14,097 lung nuclei and 19,779 kidney nuclei were were captured in two sublibraries per sample. For lung nuclei, median transcripts and genes per cell were 3,633 and 1,737 at 26,667 reads per cell. For kidney nuclei, median transcripts and genes per cell were 6,935 and 2,634 at 69,471 reads per cell. Seurat v4.0 was used to cluster and visualize the nuclei as UMAPs[4,5]. Clusters were manually annotated using marker genes from published literature shown below along with QC data including genes per cell, transcripts per cell and mitochondrial contamination (Figure 1)[1,2,3].

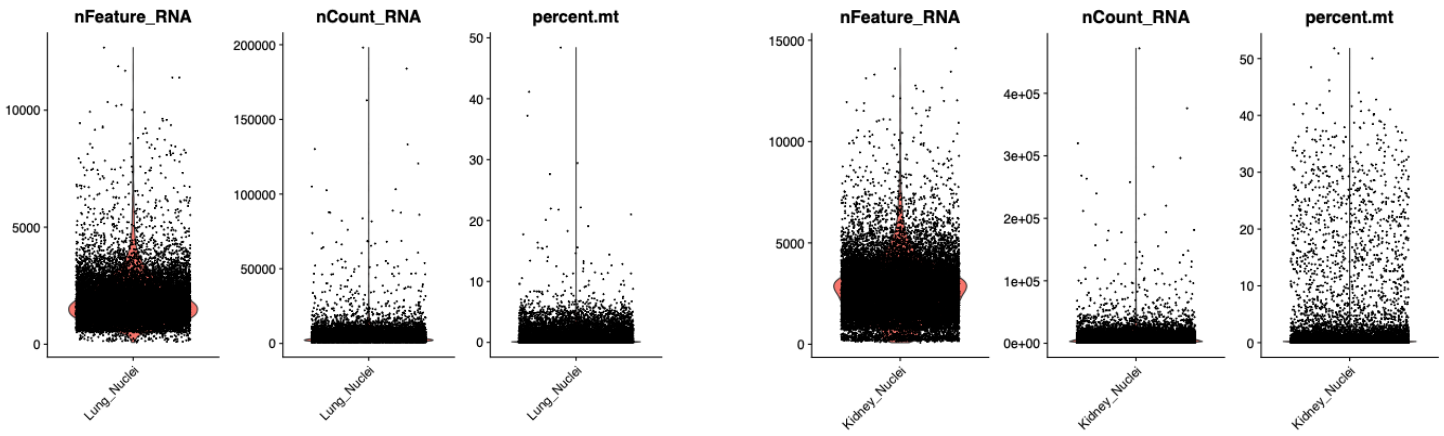

Figure 1. Lung and Kidney samples' QC metrics showing the number of genes detected in each cell (nFeature_RNA), the total number of molecules detected within a cell (nCount_RNA), and a percentage of cell reads originating from the mitochondrial genes (percent.mt).

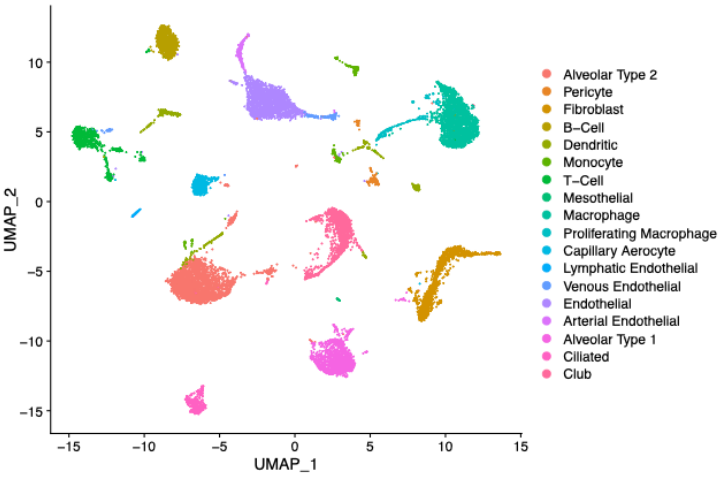

Figure 2. Lung UMAP. Lung nuclei annotated and clustered, showing 19 different cell-types being represented.

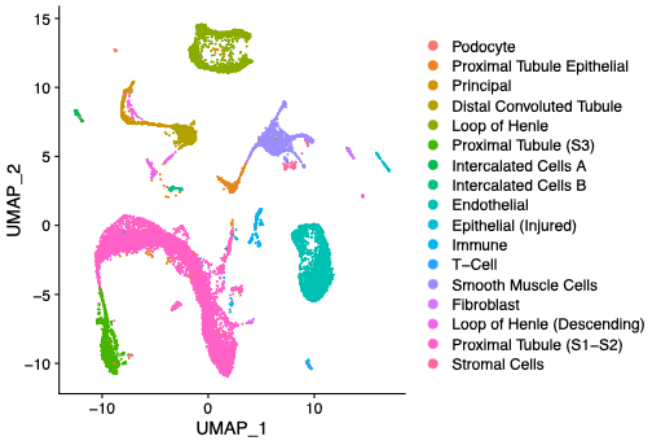

Figure 3. Kidney UMAP. Kidney nuclei annotated and clustered, showing 18 different cell-types being represented.

Discussion /

The integration of the S2 Genomics Singulator for nuclei isolation with the Parse Biosciences platform offers several key benefits that enhance the overall performance and capabilities of the workflow. One major advantage of the Singulator is its compatibility with diverse sample sources for nuclei, including fresh, frozen, or OCT preserved tissues and cell suspensions. This flexibility allows researchers using the Parse Biosciences platform to work with a wide range of biological samples. Another benefit is the ability and ease of optimization of nuclei quality and yield using the Singulator. Changes to the disruption intensity, reagent incubation time, disruption cycles and reagent volumes allow for optimization of nuclei preparations. This is critical for achieving optimal sequencing results, as damaged nuclei can lead to suboptimal data quality.

The Singulator's high-throughput capabilities contribute to the scalability and efficiency of the Parse Biosciences platform. With the ability to process a variety of sample sizes in as little as 6 minutes per sample, the system accommodates batch runs and enables the analysis of multiple samples in parallel. This pairs well with the flexibility offered by the fixation process of the Parse Biosciences platform, allowing samples to be isolated and stored until library generation is performed.

Conclusion /

In conclusion, the compatibility of the Singulator 100 system with the Parse Biosciences Evercode Whole Transcriptome platform offers several notable advantages. Firstly, the ability to fix nuclei before sequencing ensures the preservation of cellular integrity and minimizes the risk of sample degradation, as well as reduces the difficulty of generating and loading multiple samples for sequencing. Secondly, the system's capability to run a small number of samples allows for cost-effective experimentation and faster turnaround times, making it highly suitable for pilot studies or projects with limited sample availability. The reproducibility and robustness of automated sample preparation via the Singulator and compatibility with a range of single sequencing platforms allow researchers to choose the most suitable approach based on their specific research objectives and sample types for sequencing applications.

References:

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