Sequencing Skin and Kidney Cells: 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-cell RNA sequencing (scRNA-seq) techniques. The availability of multiple platforms for single-cell sequencing is important to allow researchers to select the appropriate technology to address a specific scientific question. In this technical note, we demonstrate the compatibility of Singulator-produced skin and kidney cells with the Parse Biosciences single-cell whole transcriptome technology. The ability of the Evercode technology to both fix cells and process many samples in parallel facilitates a wider range of sequencing applications when combined with the ease of use and reproducibility of the Singulator system.

Methods /

Singulator Cell Isolation

Skin and kidney cell suspensions were prepared using the S2 Genomics Singulator 100 system and reagents. Mouse Skin Reagent (100-254-082), Mouse Kidney Reagent (100-064-631) and Cell Isolation Cartridge (100-063-178) were used. Sample cartridges were maintained at room temperature prior to use and the Singulator 100 was set to 'Heat'. Skin and kidney tissue samples were isolated from ICR (CD-1®) mice using IACUC-approved protocols. After harvesting, the fur was removed from the skin tissue using a scalpel and both tissue samples were placed on ice in cold HBSS until used. Skin (249 mg) and kidney (220 mg) tissue were each finely minced (~1-2mm2) then placed in the Dissociation Chamber of Cell Isolation Cartridges and inserted into the Singulator 100. Tissues were dissociated using the Mouse Skin and Kidney Cell Isolation protocols, respectively. After completion of the automated cell isolation protocols, cells were removed from the cartridge with a 1 mL pipette and placed in a pre-cooled 15 mL conical tube. The suspensions were then centrifuged in a 4 °C swing-bucket centrifuge for 5 minutes at 300 g, followed by removal of the supernatant. The pellet of cells was resuspended in 1 mL of RBC ACK lysis solution and incubated on ice for 3-5 minutes, quenched with 8 mL of DMEM or HBSS, and centrifuged again at 300 g for 5 minutes. The pellets of cells were then resuspended in 1 mL of DMEM or HBSS supplemented with 1% BSA. Each cell suspension was analyzed and counted using a Nexcelom K2 (Revvity, Inc.) with AO/PI to assess cell viabilities and titer.

Parse Biosciences Evercode Fixation, Barcoding, and Library Prep

At least 400,000 cells were centrifuged at 200 g for 10 minutes at 4 °C. Cells were fixed with Evercode Cell Fixation ECF2001 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 were processed with Parse Biosciences Analysis Pipeline v1.0.2.

Results /

Cell isolations yielded approximately 5,300 cells/mg at 89.5% viability and 35,600 cells/mg at 88.5% viability for skin and kidney, respectively. After fixation, barcoding, and library prep, 15,117 skin cells and 18,417 kidney cells were captured from two sublibraries per sample. For skin cells median transcripts and genes per cell were 3,392 and 1,351 at 28,930 reads per cell. For kidney cells median transcripts and genes per cell were 6,935 and 2,634 at 77,849 reads per cell. Seurat v4.0 was used to cluster and visualize the cells as UMAPs[4,5]. Clusters were manually annotated using marker genes from published literature shown below (Figures 2 & 3) along with QC data including genes per cell, transcripts per cell and mitochondrial contamination (Figure 1)[1,2,3].

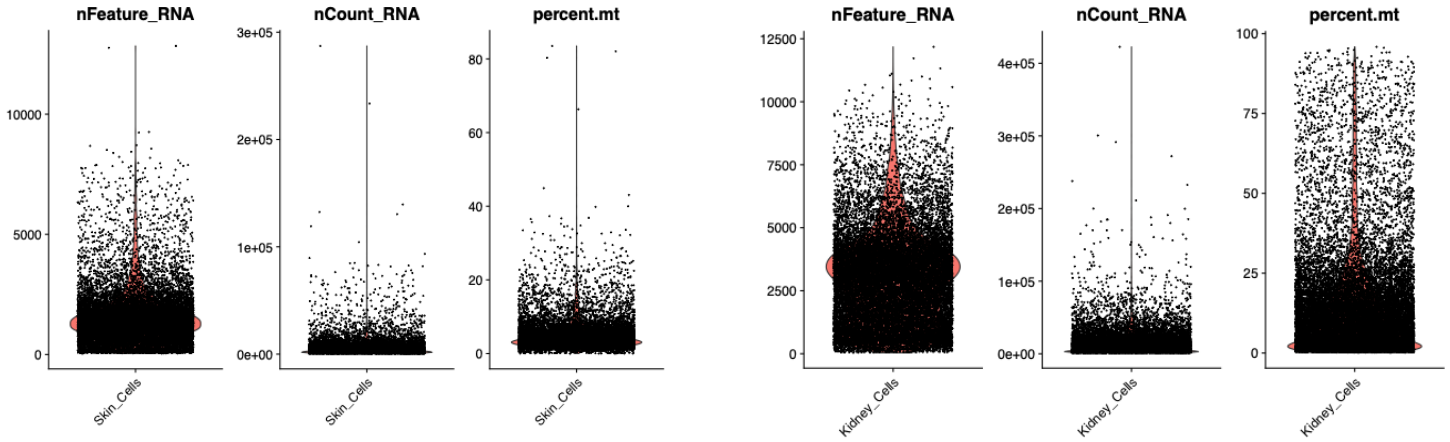

Figure 1. QC Metrics. Skin and Kidney samples' QC metrics showing the number of genes detected in each cell (nFeature_RNA), the total number of molecules detected within a cell (nCount_RNA), and a percentage of cell reads originating from the mitochondrial genes (percent.mt).

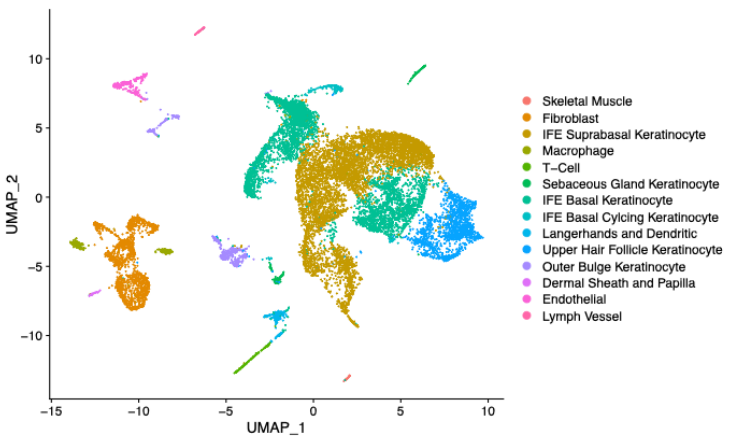

Figure 2. Skin UMAP. Skin cells annotated and clustered, showing 14 different cell-types being represented.

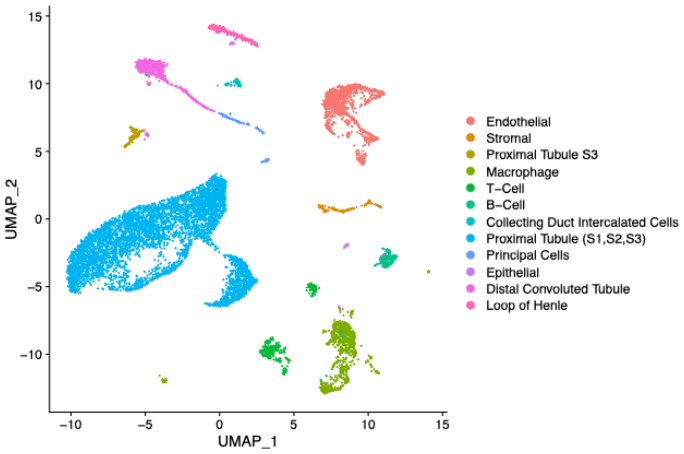

Figure 3. Kidney UMAP. Kidney cells annotated and clustered, showing 12 different cell-types being represented.

Discussion /

The integration of the S2 Genomics Singulator for cell or nuclei isolation with the Parse Biosciences' platform offers several key benefits for researchers. The Singulator is compatible with diverse sample sources, including fresh (for cells or nuclei) or frozen (for nuclei) 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 ease of protocol optimization for cell yield and viability using the Singulator. Changes to dissociation intensity, incubation time, disruption cycles and reagent volumes enable process optimization. This is critical for achieving optimal sequencing results, as low cell viability can lead to suboptimal data quality.

The Singulator 200's high-throughput capabilities contribute to the scalability and efficiency of the Parse Biosciences platform. With the ability to process two samples for nuclei in as little as 6 minutes, and cells in 20-60 minutes, the system accommodates batch runs and enables the analysis of multiple samples in parallel. The 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.

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 cells 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.

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