Isolation of Viable Cells from Mouse Heart Tissue with the Singulator™ 100

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Introduction /

Research into cardiovascular diseases can benefit greatly from single-cell approaches to genomics and cell biology analyses. However, traditional methods for viable cell isolations from heart tissue are complex, time-consuming, and technically challenging, especially so for cardiomyocytes due to their fragility. Single-cell sequencing libraries from heart cells are also difficult to generate on droplet-based microfluidics platforms due to cell size restrictions. Here, we demonstrate the use of the Singulator™ 100 for the isolation of viable mouse heart cells with high yields, using S2 Genomics' Heart Cell Reagent and Large Cell Isolation Cartridge. In addition, we have connected the workflow to Parse Biosciences' Evercode WT v2 kit for generation of scRNA-Seq data, showing that the Parse technology can be used to generate sequencing libraries with cardiomyocyte cell-type representation.

Experimental Design /

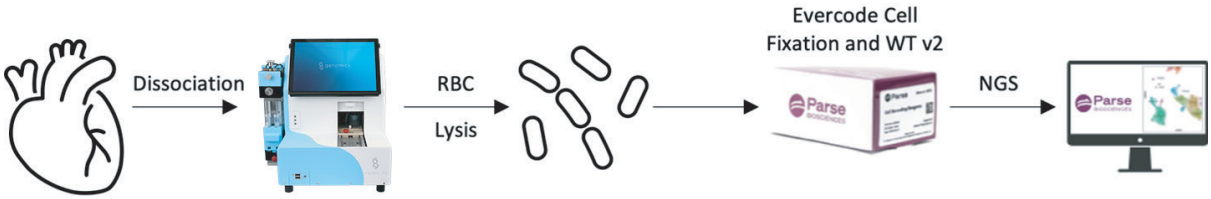

Figure 1. Experimental Design. Freshly harvested mouse heart tissue was dissociated into a single-cell suspension with a Singulator™ 100. The sample was then strained, and red blood cells (RBCs) lysed. The yield and viability of the cells were measured, a target number of cells were fixed with Parse Biosciences Evercode Cell Fixation v2, and the sample was shipped to an independent laboratory for further processing with Evercode WT v2. Sequencing data were analyzed with Parse Biosciences' analysis pipeline and Seurat.

Methods /

Sample Processing

Heart tissue was excised from a 6-week-old CD-1 mouse, cut into four roughly symmetrical pieces, and the blood washed away. Approximately 150 mg of fresh tissue was immediately processed in the Singulator™ 100 using the Mouse Heart Cell Isolation protocol, Mouse Heart Cell Isolation Reagent (100-253-846), and Large Cell Isolation Cartridge (100-258-668). Red blood cells (RBCs) were lysed with RBC Lysis Buffer (G-Biosciences) and cell counts and viability were then determined (AOPI staining, Nexcelom K2). The sample was processed using the Evercode Cell Fixation v2 kit and fixed cells were then shipped to an independent laboratory for barcoding and library preparation with Evercode WT v2.

Sequencing and Data Analysis

The heart tissue dissociation yielded approximately 23,000 cells per mg of tissue, with 68% viability. Images of the cells at 10x and 40x magnification are shown in Figure 2. The single cell library was sequenced on an Illumina Novaseq™ 6000 by a third-party service provider. The data were analyzed with the Parse Biosciences v1.0.2 analysis pipeline and quality control filtering metrics were set to the manufacturer's default values. The data were independently clustered with Seurat v4.0 and the clusters were manually annotated using marker genes described in the literature.

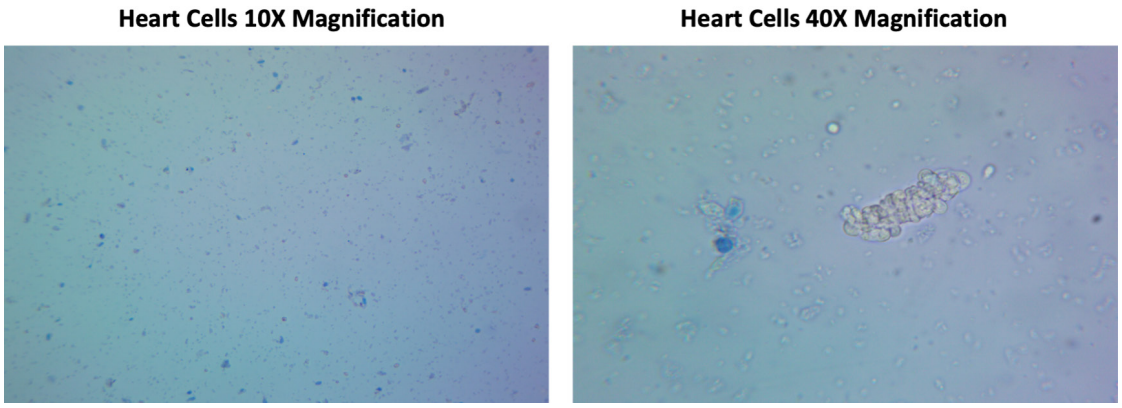

Figure 2. Heart Cells. Images of the cells at 10x and 40x magnification stained with typhan blue for visual assessment of sample quality prior to sequencing library preparation.

Results /

10,264 mouse heart cells were analyzed and independently clustered with Seurat, annotated, and visualized as UMAPs. Approximately 1,643 genes and 3,822 transcripts per cell were detected with 68,365 mean reads per cell. High quality isolations were confirmed with 94% of the cells showing less than 10% mitochondrial contamination. Endothelial cells accounted for approximately 39% of the cells, while fragile cell types such as endocardial cells and cardiomyocytes accounted for 3-4% (Table 1). Clustering of the cell types is shown in Figure 3.

CLUSTER	NUMBER OF CELLS
Endothelial Cells	4,013
Fibroblasts	2,257
Macrophage/DCs	1,061
Pericytes	674
Smooth Muscle Cells	514
Endocardial Cells	362
T-Cells	356
Cardiomyocytes	332
B-Cells	274
Lymphpatic Endothelial Cells	175
Erythocytes	110
Epithelial Cells	77
Neuronal Cells	59

Figure 3. Number of Cells per Cluster. Total number of cells based on the different cell types clustered from 10,264 total heart cells.

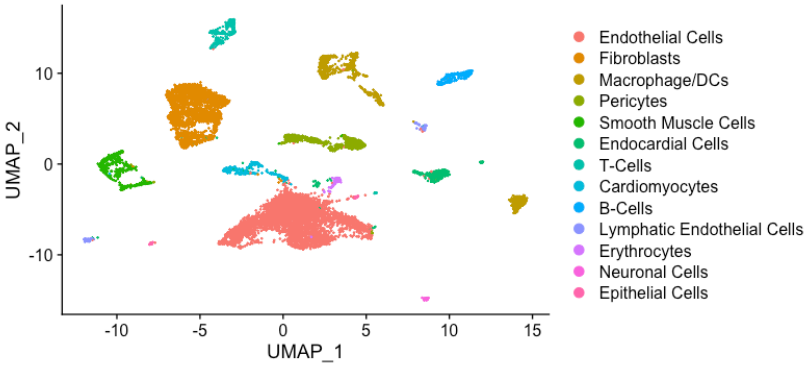

Figure 4. UMAP. UMAP of heart cells from a 6-week-old CD-1 mouse using Parse Biosciences' Evercode WT v2 kits with the Singulator™ 100.

Conclusion /

Using the Singulator™ 100 system along with Parse Biosciences' Evercode WT v2, we were able to successfully isolate viable heart cells and obtain single-cell sequencing data with broad cell-type representation. The Singulator™ 100 eliminates the technical biases, time-consuming protocol development, and technical challenges associated with traditional cell isolation methods, allowing for improved cluster resolution and cell-type representation, including large and fragile cell types.

References:

1. Wang et al., Construction of a cross-species cell landscape at single-cell level, Nucleic Acids Research, 2022; gkac633, https://doi.org/10.1093/nar/gkac633.