APPLICATION NOTE

Single Nuclei Sequencing Using the Singulator™ Platform and S2 Genomics RNase Inhibitor

ABSTRACT /

The Singulator™ 100 and 200 can quickly isolate nuclei from a wide variety of tissue types, and from samples that are fresh, frozen, or OCT preserved. FFPE samples can be dissociated if previously deparaffinized and rehydrated. If the nuclei are intended for downstream single nuclei RNA Seq analysis, it is critical to prevent degradation of the RNA by endogenous RNase enzymes. To help preserve RNA integrity, RNase inhibitor is commonly added to samples during the isolation and purification of nuclei. Here we describe the use of S2 Genomics' RNase Inhibitor combined with the Singulator for nuclei isolation for the purpose of single nuclei RNA sequencing applications.

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Introduction /

When isolating nuclei, one or more types of RNase enzymes may be released, which can cause the rapid degradation of nuclear RNA. RNases are ubiquitous and have the potential to compromise RNA integrity, thereby leading to degraded RNA Seq data. To preserve RNA quality and help ensure the success of cDNA preparations for downstream snRNA Seq analyses, an RNase inhibitor is commonly added to a sample during the nuclei isolation and purification steps. The amount of RNase inhibitor required depends upon the sample tissue type, as RNase activity can vary dramatically from tissue to tissue. For example, the RNase activity in mouse pancreas tissue is >180,000-fold higher than in mouse brain tissue.

The Singulator 100 and 200 systems can rapidly and automatically dissociate solid tissues into filtered suspensions of nuclei or cells. In this study, we demonstrate the use of S2 Genomics reagents, including RNase Inhibitor and Lysis reagents, for the isolation of nuclei from various mouse tissues with the Singulator 100. We show that S2 Genomics' RNase Inhibitor combined with Singulator 100 workflow for nuclei isolation produces high quality single nuclei sequencing libraries and data.

Materials & Methods /

Nuclei suspensions were prepared using the Singulator 100 System. Nuclei Isolation Reagent™ (S2 Genomics, 100-063-396), Nuclei Storage Reagent™ (S2 Genomics, 100-063-405), S2 Genomics RNase Inhibitor (S2 Genomics, 100-255-535) and Singulator NIC+ cartridge (S2 Genomics, 100-215-389) were used. Sample cartridges were chilled at -20 °C prior to use and the Singulator was set to 'Cool'.

For these studies, lung, brain, 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. A 15-50 mg piece of tissue was then placed in the Dissociation Chamber of a pre-cooled NIC+ Cartridge and inserted into the Singulator. Tissue was dissociated using the Low Volume Nuclei Isolation protocol. 75 µL of S2 Genomics RNase Inhibitor was added directly to the Dissociation Chamber along with the sample. After completion of the automated nuclei isolation protocol, taking approximately 7 minutes, nuclei were removed from the cartridge with a 1 mL pipette and placed in a pre-cooled 15 mL conical tube. The suspension was centrifuged for 5 min at 500 g and 4 °C, followed by removal of the supernatant.

The pellet of nuclei was then resuspended in 500 µL of Loading Buffer (S2 Genomics, 100-257-006) supplemented with 1% BSA and 12.5 µL of S2 Genomics RNase Inhibitor. In the case of the brain sample an additional myelin removal step was completed using S2 Genomics Nuclei Debris Removal Stock Solution (S2 Genomics, 100-246-754). The pellet of brain nuclei was resuspended in Nuclei Debris Removal Stock Solution, diluted to 20% with NSR, and centrifuged at 500 g for 15 minutes. The pellet was then resuspended in supplemented Loading Buffer. Each nuclei suspension was filtered through a 40 µm Flowmi strainer and nuclei concentrations were analyzed and counted using a Nexcellom K2 with AO/PI and the cell titer/health assay. The concentration of the nuclei suspensions was adjusted to 1000 nuclei/µL with additional supplemented Loading Buffer. Nuclei were then loaded into a 10x Genomics 3' Gene Expression assay targeting 10,000 nuclei. cDNA was assayed using an Agilent 4150 Tapestation™ and D5000 assay and final library analyzed using D1000 assay. The final libraries were pooled and sequenced on a NovaSeq™ V1.5 SP targeting 20,000 reads per cell.

Results & Discussion /

Nuclei yields from the samples ranged from 60,000 to 135,000 nuclei per milligram of tissue. The 50 mg kidney and lung samples yielded 6,735,000 and 3,035,000 respectively. After the density gradient clean-up, the 35 mg brain sample yielded 3,630,000 nuclei. The brain, lung and kidney samples were sequenced at 22,054, 18,756, and 33,497 mean reads per cell with 1,581, 1,262, and 1,917 median genes per cell, respectively. The data (Figure 1) show the S2 Genomics RNase Inhibitor can successfully be used for single nucleus RNA sequencing experiments for a variety of tissues.

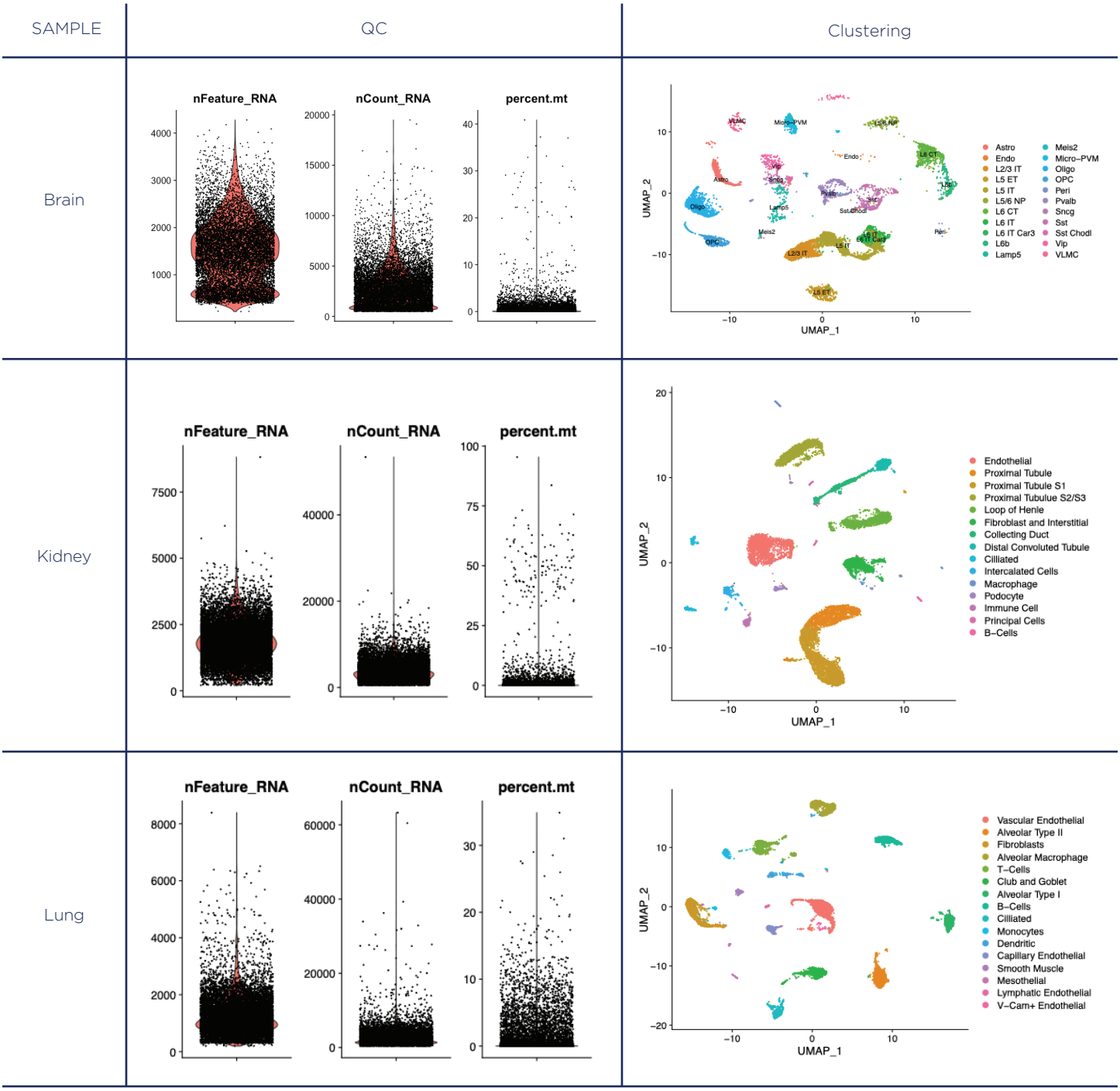

FIGURE 1 - Summary quality metrics and clustering for single nuclei sequencing data from mouse brain (top), kidney (middle), and lung (bottom).

Sequencing Data Analysis /

Data were analyzed using Seurat 4.0. Ambient RNA was removed using SoupX[6] to provide a corrected count matrix. For the brain sample, the corrected count matrix was then integrated with Azimuth mouse cortex reference[5] for reference-based mapping pipeline that performs normalization, visualization, cell annotation, and differential expression[4]. For the lung and kidney samples, the corrected count matrixes were independently clustered with Seurat v4.0 and the clusters were manually annotated using marker genes described in the literature for lung[2] and kidney[1,3] cells.

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