Automated Dissociation of FFPE Samples for Single Nuclei RNA-Seq.

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Abstract

S2 Genomics has developed the Singulator™ 100 and 200 Systems to automate the isolation of singulated cells or nuclei from fresh, frozen, or OCT 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.

We extended the applications to the automated processing of FFPE slices into singulated nuclei on modified Singulator systems. The prototype systems can perform all deparaffinization, rehydration, and (optionally) crosslink reversal steps. Isolation of nuclei is then performed on commercially available Singulator systems. The nuclei are then processed using a commercially available probe-based kit (Chromium Single Cell Gene Expression Flex, 10X Genomics) to create single nuclei gene expression libraries. Sequencing of a brain library made from FFPE showed equivalent results between automated and manual deparaffinization and rehydration processing.

Presented here are data on the preparation and analysis of snRNA-Seq libraries from human glioblastoma tumor clinical samples prepared from FFPE slices.

The commercial Singulator Systems can automatically process fresh tissue samples into single cell suspensions, while nuclei can be isolated from fresh, frozen, or OCT preserved tissue. **Figure 1** shows the Singulator 100 for processing a single sample. **Figure 2** shows the Singulator 200 capable of processing two samples.

Formalin-fixed, paraffin-embedded (FFPE) tissues are the standard samples used by pathologists. It is estimated there are a billion extant FFPE samples.

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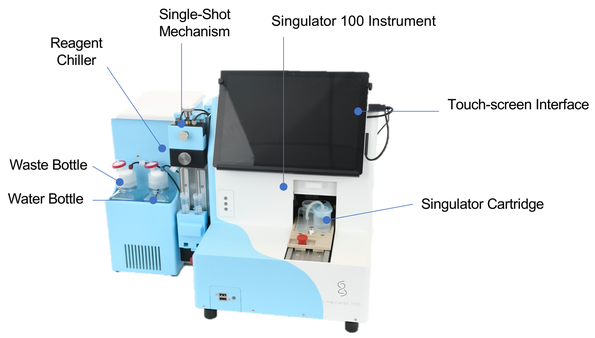


Figure 1. Singulator™ 100 System, including the Singulator instrument, single-use cartridge, chiller for nuclei reagents, and Single-Shot Mechanism for cell preparation reagents.

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.

1. CitriSolv incubation, 3x, 10 min@
2. 100% EtOH wash, 3x, 1 min@
3. 70%, 50%, 30% EtOH washes, 1 min@
4. PBS wash, 3x, 1 min@
5. Sample transfer to NIC+ cartridge
6. Tissue dissociation in Singulator 100, Extended Nuclei Isolation Protocol, 10 min

Figure 3. Workflow for FFPE preparation: deparaffinization, rehydration, and nuclei production.

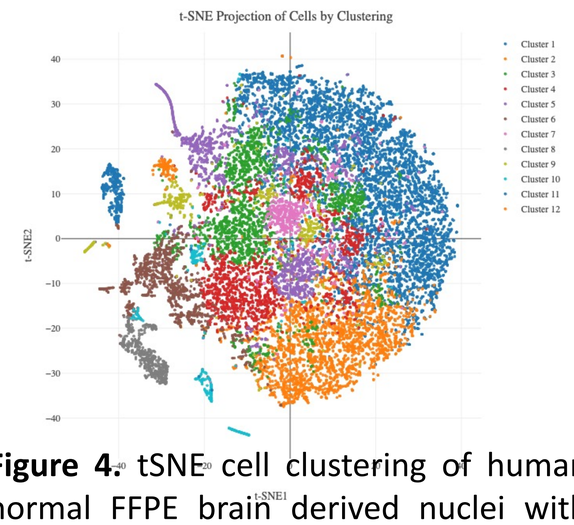
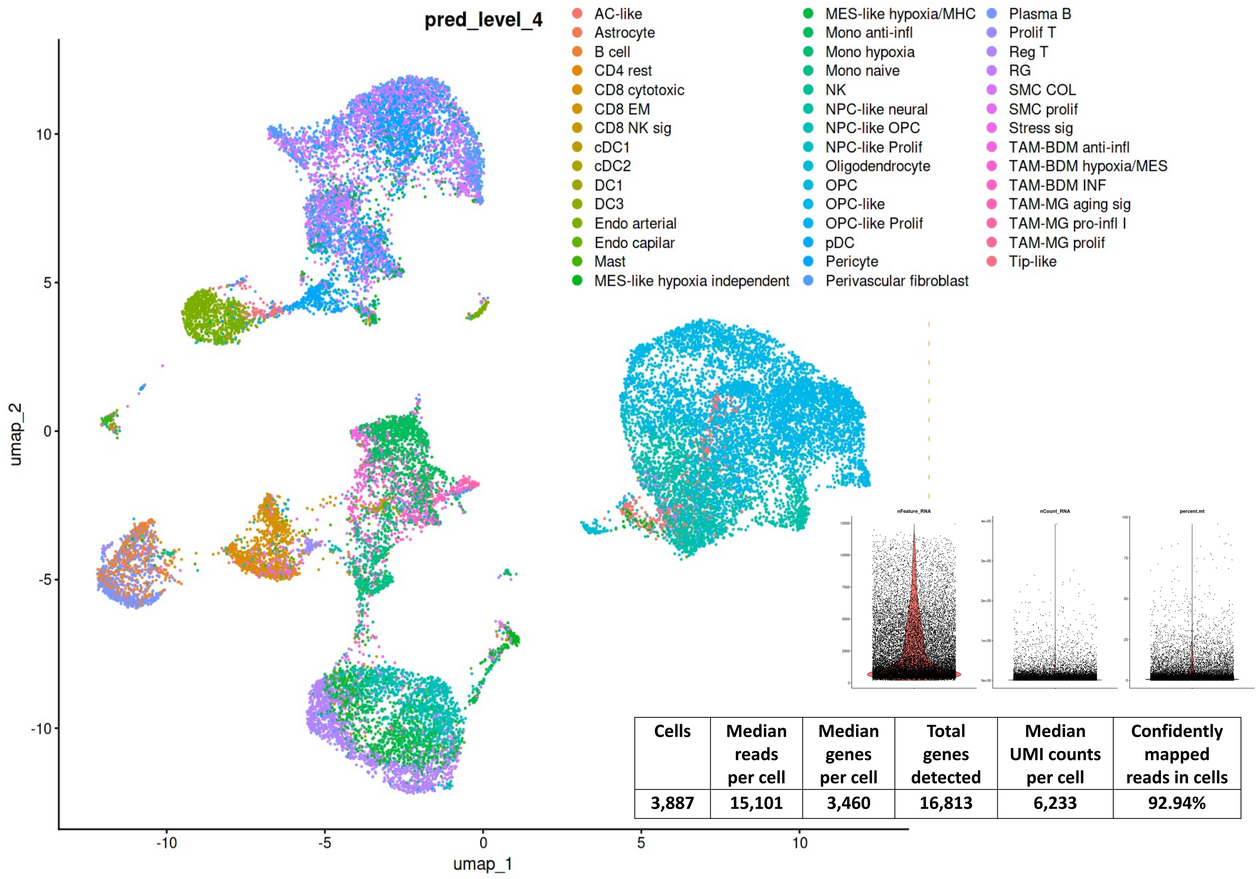


Figure 4. tSNE cell clustering of human normal FFPE brain derived nuclei with manual FFPE processing and automated nuclei isolation.



Cells	Median reads per cell	Median genes per cell	Total genes detected	Median UMI counts per cell	Confidently mapped reads in cells
3,887	15,101	3,460	16,813	6,233	92.94%

Figure 5. UMAP clustering and QC metrics of a human glioblastoma FFPE sample with fully automated processing.

Many FFPE samples are annotated with treatments and patient outcomes. These valuable samples have been inaccessible for single cell analysis due to the fragmented nucleic acid and molecular crosslinking. The Flex kit has enabled single cell/nuclei library preparation once samples have been properly prepared.

First, a standard workflow was used (**Figure 3**) to manually deparaffinize and rehydrate two 70 μm slices of normal brain FPPE samples before nuclei production on a commercial Singulator system using newly developed protocols. **Figure 4** shows the tSNE of the sequenced brain nuclei via Cell Ranger output with 12 cell type clusters.

Fully automated FFPE processing was developed using a prototype system. Using two 50 μm FFPE slices from a CNS WHO Grade 4, IDH-wildtype, glioblastoma tumor, a fully automated nuclei isolation workflow was demonstrated. Deparaffinization and rehydration, using the **Figure 3** workflow**,** was automated in a Singulator cartridge, taking ~50 minutes.

The deparaffinized and rehydrated tissue was added to a NIC+ (S2 Genomics) cartridge and nuclei isolated via the new *Extended nuclei isolation* protocol on a commercial Singulator 100. Isolated nuclei were cleaned via centrifugation steps, counted, and ~1,000,000 nuclei were taken for probe hybridization processing according to manufacture's instructions using the single cell gene expression Flex kit.

Figure 5 shows the UMAP clustering results, QC metrics, and Cell Ranger output of the fully automated processing with 44 Azimuth-predicted cell types with 3,460 median genes per cell at only 15,101 median reads per cell.

Summary: The commercial Singulator 100 and 200 Systems now automate production of cells or nuclei from manually processed FFPE samples. Fully automated processing is under development. Collaborators and potential beta testers are invited to discuss their needs and interests.