Unlocking the Potential of Solid Tissue: Precision Single Nuclei Isolation from Fresh, Frozen and FFPE Samples with the Singulator Platform

S2 genomics

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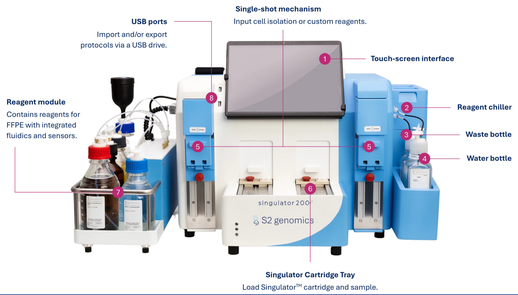
Abstract

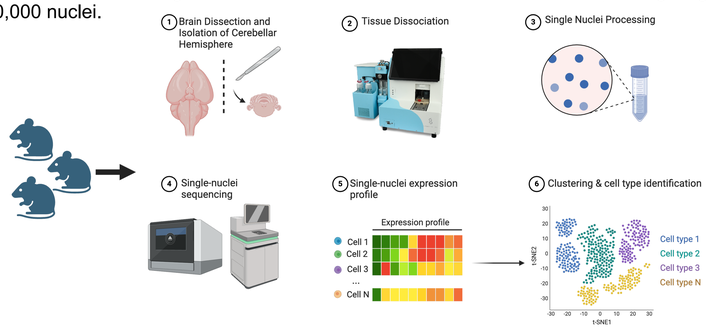
Advances in single-cell research increasingly demand high-quality, reproducible isolation of single nuclei from solid tissues to facilitate downstream applications such as single-nuclei RNA sequencing and epigenomic studies. The Singulator Platform, a groundbreaking tool in tissue dissociation, offers reproducibility and precision in isolating single nuclei from fresh, frozen and formalin-fixed, paraffin-embedded (FFPE) tissues. Key benefits of the Singulator Platform include the ability to isolate reproducible cell populations including rare cell types with minimal bias and sample loss, ensuring consistent results.

Here, we present data demonstrating the Singulator Platform's ability to reproducibly isolate high yields of intact nuclei, from flash frozen samples that are compatible with downstream single cell applications such as single-cell gene expression and single-cell chromatin accessibility (ATAC) profiling. Additionally, we present the development of a fully automated workflow for the deparaffinization and rehydration of FFPE slices in about 40 min and subsequent processing of FFPE slices into single-nuclei suspension on a prototype Singulator 200+ system. Finally, single nuclei sequencing data will be presented on the preparation and analysis of snRNA-Seq libraries from healthy and tumor human FFPE samples including glioblastomas and colorectal cancer.

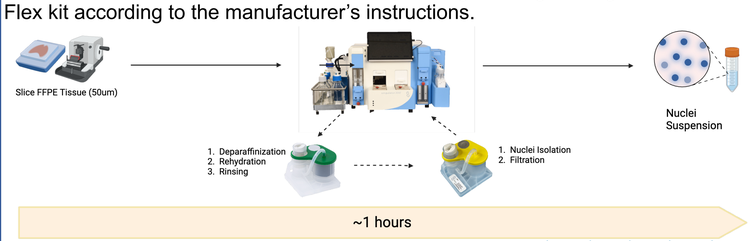


Methods

Nuclei were isolated from three mouse cerebellar hemisphere samples using the Singulator™ Platform following the protocol 'Demonstrated Protocol: Nuclei Isolation and Cleanup from Frozen Mouse Brain Tissue for Single Nuclei Sequencing Applications' with the NIC+ Isolation Bundle with RNase Inhibitor V2. 8-week-old ICR (CD-1®) mice were sacrificed following IACUC-approved protocols, the cerebellum was dissected from the mouse and cut in half along the vermis, and immediately snap frozen on dry ice. Frozen cerebellar hemispheres were loaded into a pre-cooled NIC+ cartridge with RNase Inhibitor V2 and processed using the Low Volume Nuclei Isolation Protocol V2 on a Singulator 100TM instrument. Following nuclei isolation, myelin was removed using Debris Removal Reagents Stock Reagent (S2 Genomics, 100-253-628). Nuclei were counted and the viability was determined using AO/PI (acridine orange/propidium iodide) staining with the Nexcelom K2. Next, nuclei were loaded into the 10x Genomics Chromium GEM-X Single Cell 3' Kit v4 at a concentration of 1,000 nuclei/µL, targeting 10,000 nuclei.



We also adapted a standard workflow onto prototype Singulator systems to fully automate the deparaffinization and rehydration of FFPE samples in approximately 30 minutes using a Reagent Module to deliver a deparaffinization reagent, ethanol washes, and PBS into a processing cartridge. Following deparaffinization and rehydration, tissue was dissociated in a NIC+ (S2 Genomics) cartridge, and nuclei were isolated, cleaned via centrifugation, counted, and approximately 300,000 nuclei were processed for probe hybridization using the single-cell gene expression Flex kit according to the manufacturer's instructions.



Results: Frozen Mouse Cerebellum

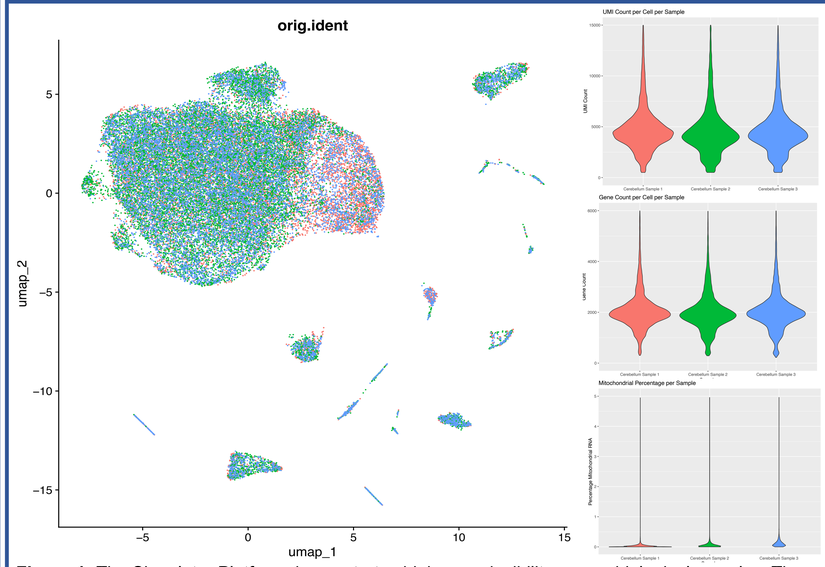


Figure 1: The Singulator Platform demonstrates high reproducibility across biological samples. The left panel shows an integrated UMAP of snRNA-seq data from three adult mouse cerebellar hemispheres, down sampled to an equivalent read depth of 14,150 reads per cell. Consistent clustering across the replicates indicates reliable cell type recovery and gene expression profiles. The right panels display violin plots of sequencing metrics, including UMI counts per cell, gene counts per cell, and mitochondrial RNA percentage for each replicate. All three samples performed reproducibly well, with similar UMI and gene counts per cell, and consistently low mitochondrial RNA percentages, demonstrating the platform's ability to maintain high-quality nuclei suitable for single-nuclei RNA sequencing.

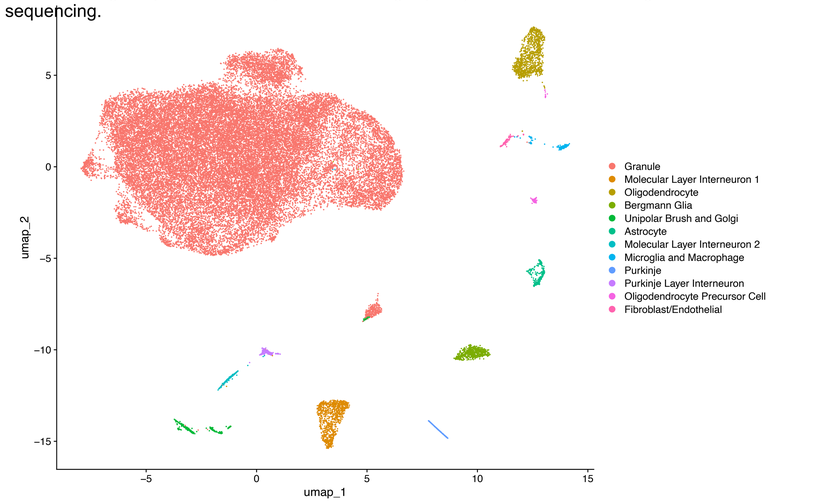


Figure 2: UMAP projection of integrated nuclei from three mouse cerebellum samples, highlighting the ability of the Singulator Platform to isolate nuclei representing the 12 major cell types found in adult mouse cerebellum. The UMAP visualization shows nuclei clustering based on gene expression profiles, with each cluster assigned a different color corresponding to specific cell types2,3.

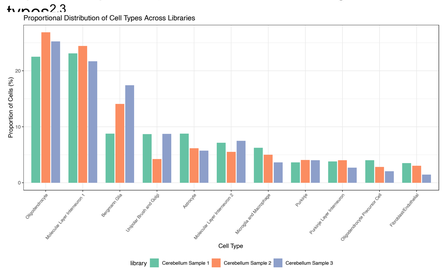


Figure 3. Proportion of rare cell types in three cerebellum samples. Shows reproducible proportions of various rare cell types across biological replicates. Cell proportions were calculated and compared from each cerebellum sample excluding the granule cells. Nearly identical cell type compositions were observed across biological replicates, with only minor expected variations.

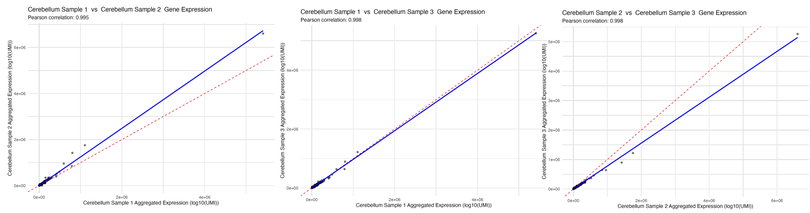
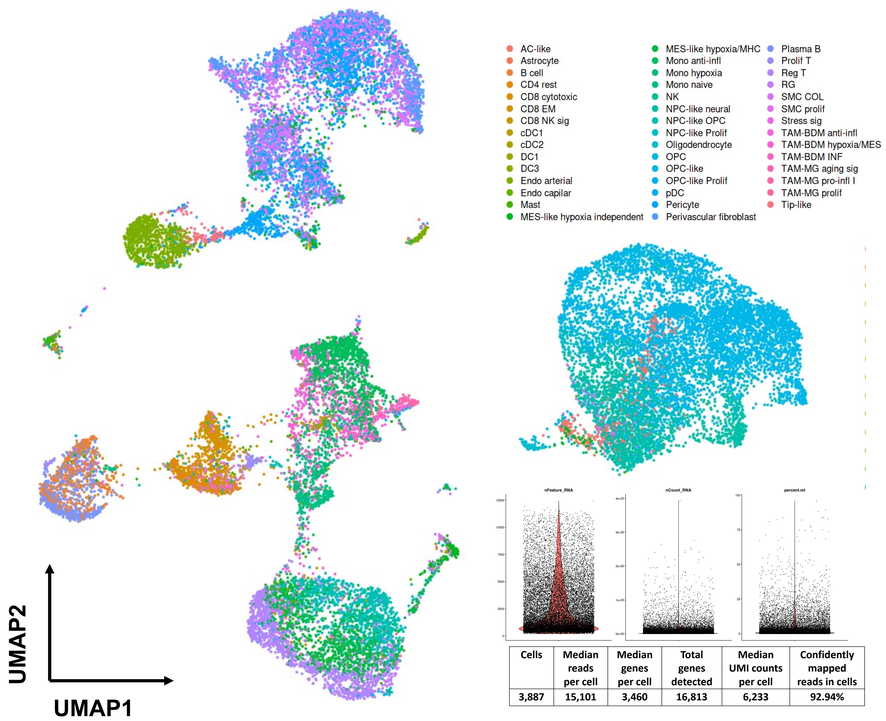


Figure 4: High concordance between nuclei isolations performed on the Singulator Platform by Pearson correlation. Average expression graphs with Pearson correlations demonstrate reproducibility of the nuclei isolations between three down sampled cerebellum samples.

Results: FFPE Processing



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 on a Singulator prototype[1].

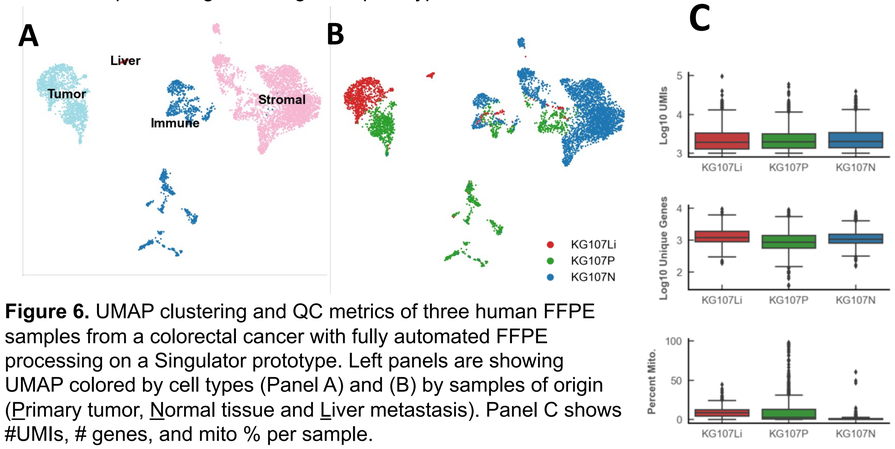


Figure 6. UMAP clustering and QC metrics of three human FFPE samples from a colorectal cancer with fully automated FFPE processing on a Singulator prototype. Left panels are showing UMAP colored by cell types (Panel A) and (B) by samples of origin (Primary tumor, Normal tissue and Liver metastasis). Panel C shows #UMIs, # genes, and mito % per sample.

Conclusion

The Singulator™ Platform provides a reproducible and precise method for isolating nuclei from complex tissues suitable for single-nuclei RNA sequencing. Our results demonstrate consistent nuclei yield and high-quality sequencing metrics across biological replicates, making it a reliable tool for neuroscientific research. The ability to produce high-quality nuclei with minimal variability enhances the accuracy of downstream snRNA-seq analyses, facilitating deeper insights into normal biology and disease. Finally, the ability to identify rare cell types underscores the efficacy of the Singulator Platform in comprehensive cellular analysis, providing a detailed representation of the cellular landscape.

The Singulator 200+ platform has been developed as a fully automated FFPE processing system in addition to processing solid tissues into single cell or nuclei. Initial results show excellent snRNA-Seq performance with well resolved cell types from FFPE Human Glioblatoma sample and high quality sequencing metrics from FFPE Colorectal Samples. The automated ability to quickly and reproducibly isolate nuclei from FFPE samples will significantly enhance the feasibility of single cell genomics studies on archived clinical specimens, allowing researchers to unlock valuable insights from previously inaccessible samples.

References
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3. Sepp, M., Leiss, K., Murat, F. et al. Cellular development and evolution of the mammalian cerebellum. Nature 625, 788– 796 (2023). https://doi.org/10.1038/s41586-023-06884-x