Automated preparation of single nuclei from FFPE samples for snRNA-Seq

genomics

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Abstract

S2 Genomics developed the Singulator™ 100 and 200 Systems to automate the processing of fresh, frozen, or OCT tissues into single cell and nuclei suspensions using disposable cartridges, reagents, and customizable protocols. The systems have prepared cells or nuclei from a wide range of tissues and organisms, including human, mouse, chicken, insects, snails, zebrafish, and plants.

Formalin-fixed, paraffin-embedded (FFPE) tissues are the preferred format for pathologists and as many as one billion FFPE slides are in repositories. FFPE tissue samples have traditionally been difficult to study with molecular techniques due to the chemical modifications caused by the formalin fixation, which can lead to degradation and sequestration of RNA and DNA. Recent advances in snRNA-Seq technology have made it possible to overcome these challenges and generate high-quality genomic data from FFPE samples at the single cell or nucleus level. Singulator 100 and 200 protocols now enable processing of deparaffinized, rehydrated FFPE into single nuclei.

We report here the development of a fully automated workflow for the deparaffinization and rehydration in about 40 min, with optional enzyme treatment of 45 min, and processing of FFPE slices into singulated nuclei or cells on prototype Singulator 200 systems. Data will be presented for the optimization of process parameters using nuclei recovery and RNA quality for six tissues. 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.

Introduction

The commercial Singulator Systems (S2 Genomics) can automatically process fresh tissue samples into single cell suspensions, while nuclei can be isolated from fresh, frozen, OCT preserved tissue, or manually prepared formalin-fixed, paraffin-embedded (FFPE) samples. We have now automated the complete FFPE sample preparation workflow in a Singulator 200+ system (**Figure 1**).

FFPE tissues are the standard samples used by pathologists. It is estimated there are a billion extant FFPE samples.

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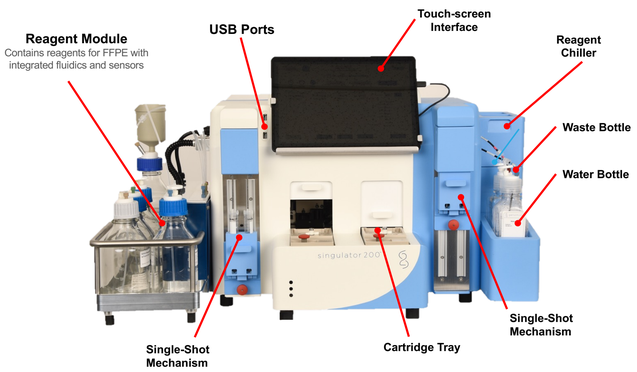


Figure 1. Singulator 200+ System, including the Singulator instrument, single-use cartridge, chiller for nuclei reagents, Single-Shot Mechanism for cell preparation reagents, and Reagent Module for FFPE reagents.

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

Figure 2. Workflow for FFPE preparation: deparaffinization, rehydration, and nuclei production.

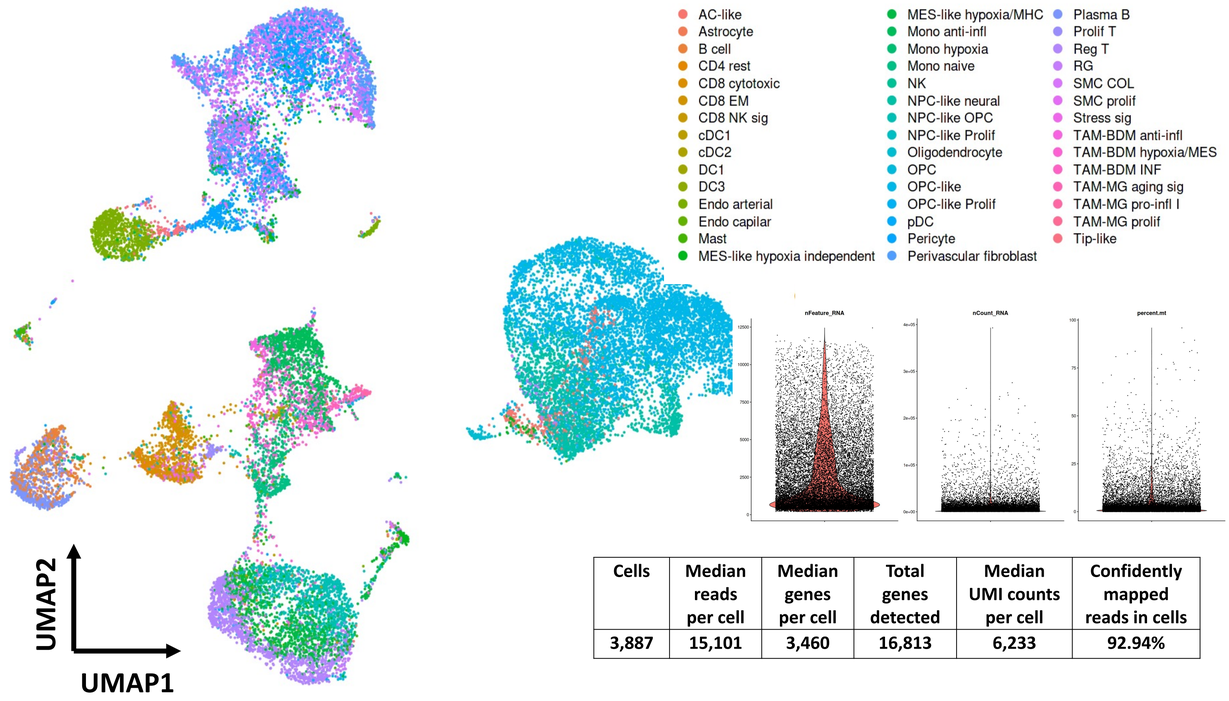
Many FFPE samples are annotated with treatments and patient outcomes. However, these invaluable samples have been inaccessible for single cell analysis because of fragmented nucleic acids and crosslinking.

The Flex scRNA-Seq kit (10x Genomics) has enabled single cell/nuclei library preparation of FFPE samples using probe-based chemistries. However, FFPE samples first need to be properly prepared by deparaffinization, rehydration, and, for some applications, crosslink reversal.

Materials and Methods

We adapted a standard workflow (**Figure 2**) onto prototype Singulator systems to fully automate the deparaffinization and rehydration of FFPE samples in ~40 minutes in cartridges. The Singulator 200+ uses a Reagent Module to deliver a deparaffinization reagent (S2 Genomics), ethanol washes, and PBS to an FPPE processing cartridge to perform the workflow (**Figure 2**).

The deparaffinized and rehydrated tissue was dissociated in a NIC+ (S2 Genomics) cartridge and nuclei isolated. Isolated nuclei were cleaned via centrifugation steps, counted, and ~300,000 nuclei were taken for probe hybridization processing according to manufacture's instructions using the single cell gene expression Flex kit.



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 3. UMAP clustering and QC metrics of a human glioblastoma FFPE sample with fully automated processing using CitriSolv™ as a deparaffinization reagent on a Singulator prototype[1].

Results and Discussion

Figure 3 shows UMAP clustering, QC metrics, and Cell Ranger output of the fully automated processing. Two 50 µm FFPE slices from a CNS WHO Grade 4, IDH-wildtype, glioblastoma tumor were processed using the fully automated nuclei isolation workflow. Cell typing was done using Azimuth, recovering 44 different cell types with a median of 6,233 UMIs per cell at 15K median reads per cell.

We then performed a fully automated processing of a human FFPE trio colorectal samples including a normal (KG107N), tumor (KG107P) and a liver metastasis (KG107Li) sample from the same patient, using the prototype Singulator system. **Figure 4** shows UMAP clustering, number of UMIs and genes for each samples as well as percent of mitochondria reads.

Table 1. Recovery of RNA from Nuclei from Mouse FFPE Samples

Tissue	DV200 (%)	Ave. Size (nt)
Brain	85.55	2,533
Heart	82.79	2,265
Kidney	84.24	2,032
Liver	88.77	2,503
Spleen	83.58	2,436
Tumor	81.60	1,880

The default solvent to deparaffinize FFPE is xylene which is toxic and requires a fume hood. **Table 1** shows that S2 Deparaffinization Reagent produces excellent DV200 scores and long RNAs from nuclei prepared from fresh mouse FFPE blocks. RNA-Seq performance is equivalent or better than the xylene replacement CitriSolv (data not shown) and no toxic fumes are produced.

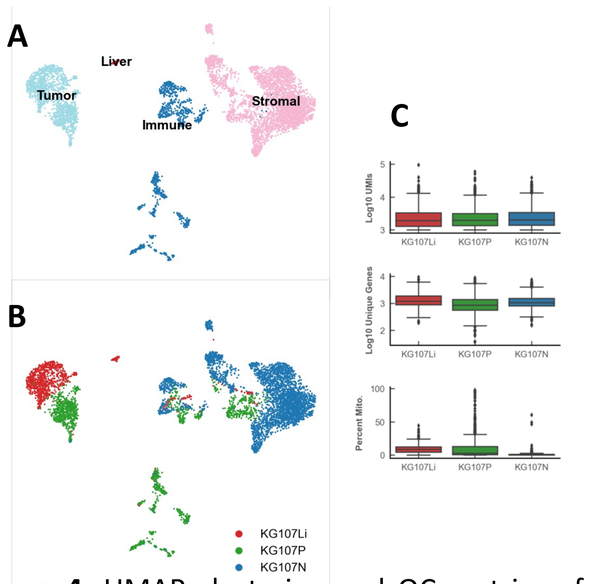


Figure 4. UMAP clustering and QC metrics of three human FFPE samples from a colorectal cancer with fully automated FFPE processing on a Singulator prototype. Left panels are showing UMAP colored by cell types (Panel A) and (B) by samples of origin (Primary tumor, Normal tissue and Liver metastasis). Panel C shows #UMIs, # genes, and mito % per sample.

Summary:

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 no loss of DV200 from the original blocks and excellent snRNA-Seq performance.

[1]D. Haviv, J. Remšík, M. Gatie, C. Snopkowski, M. Takizawa, N. Pereira, J. Bashkin, S. Jovanovich, T. Nawy, R. Chaligne, A. Boire, A.-K. Hadjantonakis & D. Pe'er. The covariance environment defines cellular niches for spatial inference. Nature Biotechnology. April 2024. https://doi.org/10.1038/s41587-024-02193-4.