Optimized workflow for isolating nuclei from FFPE tissue sections for single nucleus RNA sequencing

Poster ID: 4088

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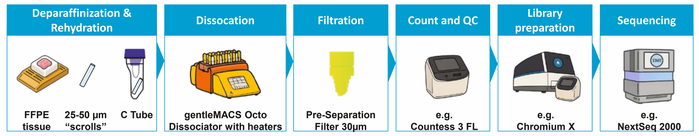
Introduction

Formalin-fixed, paraffin-embedded (FFPE) tissue is an essential resource for long-term sample preservation in both research and clinical settings. It serves as invaluable repository of diverse pathological conditions, often spanning decades of sample collection. As such, FFPE tissues offer unique opportunities for retrospective studies aimed at investigating disease mechanisms, therapeutic responses, and biomarker discovery. Despite their obvious advantages, the fixation and embedding processes introduce chemical crosslinks and compromise RNA integrity.

Here, we present an optimized workflow that addresses these challenges, providing efficient and reproducible nuclei isolation from FFPE tissue sections for high-quality transcriptomic analysis.

Materials & Methods

Figure 1



Dissociation of FFPE tissue scrolls

FFPE tonsil tissue and FFPE lung, colon, and breast tumor tissue blocks (purchased from ProteoGenex) were cut into 25-µm scrolls or 10-µm sections. Scrolls were placed in a gentleMACS™ C Tube and washed three times with 3 mL xylene for 10 minutes each. Rehydration was carried out in an ethanol gradient (2× 100%, 70%, 50%; 30 seconds each), followed by a 30-second rinse in deionized water. An enzyme mix (2.35 mL 1× Buffer S, 100 µL Enzyme D, 65 µL Enzyme P) was added prior to processing on the gentleMACS™ Octo Dissociator with Heaters (program 37C_FFPE_1). The dissociated sample was filtered (30 µm Pre-Separation Filter), centrifuged at 850 g for 5 minutes at 4 °C, and the pellet was resuspended in 0.5 mL resuspension buffer (PBS, RNase-free Tris, RNase-free BSA, RNase inhibitor, RNase-free water). For the 5 µm sections mounted on glass slides, the same protocol was followed. After the final water rinse, a scalpel was used to scrape the tissue into the gentleMACS™ C Tube. The FFPE tonsil tissue was processed and sequenced by the genomics core facility of Cedars-Sinai. Dissociations performed with Liberase TH were done in accordance with 10x Genomics´ recommended protocol (CG000784|RevA).

Bulk RNA sequencing

Sample preparation and bulk sequencing experiments were performed by Lexogen, Inc. Two 25-µm FFPE tonsil tissue scrolls were either dissociated as described above or not, before RNA extraction was performed using the Beckman Coulter FormaPure XL RNA Reagent Kit. After library preparation the Illumina NovaSeq X was used for sequencing.

snRNAseq

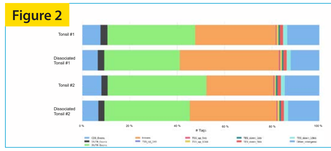
The 10x Genomics Flex Gene Expression Assay was performed using Next GEM or GEM-X consumables in accordance with the manufacturer's instructions. Single-cell suspensions were counted on a Countess™ 3 FL Automated Cell Counter to determine and adjust the cell concentration required for the overnight hybridization step for multiplexing. Following hybridization, a wash and second count was carried out to confirm the target cell number for pooling. Four samples, each targeting 10,000 cells, were combined for GEM generation. After library preparation, the sequencing was performed on a Illumina NovaSeq X. Raw sequencing outputs were processed with Cell Ranger, and downstream analyses, including clustering and differential expression, were performed using Seurat.

Results

1 Dissociation of FFPE tissues results in single nuclei

After UMI collapsing, read counts for the dissociated samples were 152% and 136% of their undissociated counterparts. Next to an absolute overall coding sequence tag count increase, intronic and intergenic reads strongly increased, suggesting a higher fraction of nuclei in the dissociated samples.

Figure 2



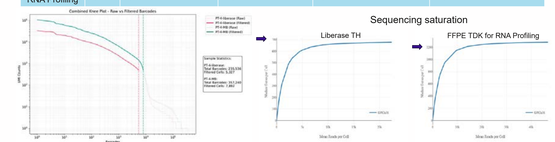
2 Optimized FFPE tissue dissociation improves cell yields and sequencing quality

Scrolls from the same FFPE tonsil tissue blocks used for bulk sequencing were prepared using either 10x Genomics' recommended protocol (CG000784|RevA) with Liberase TH or an optimized approach employing Enzyme P and D in Buffer S. Following the Flex Gene Expression assay, cell calling increased by 48% and 84%, respectively, when using the optimized method (Figure 3). Although the total number of detected genes was only slightly higher, the median genes per cell and median UMI counts per cell rose substantially under the optimized conditions, resulting in deeper sequencing saturation. This improvement can be partly attributed to the higher proportion of confidently mapped reads (81% vs 95%, 87% vs 96%).

Figure 3

FFPE tonsil block #1

	Cells	Median genes per cell	Total genes detected	Median UMI counts per cell	Confidently mapped reads in cells
Liberase TH	5,327	678	16,637	972	81,09 %
FFPE TDK for RNA Profiling	7,892	1,283	17,231	1,928	95,36 %



FFPE tonsil block #2

	Cells	Median genes per cell	Total genes detected	Median UMI counts per cell	Confidently mapped reads in cells
Liberase TH	5,885	741	16,301	1,040	86,70 %
FFPE TDK for RNA Profiling	10,830	1,264	17,230	1,815	95,96 %

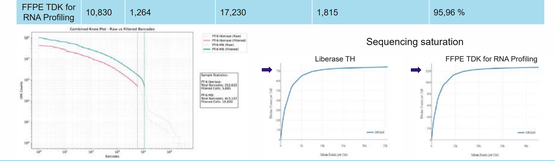
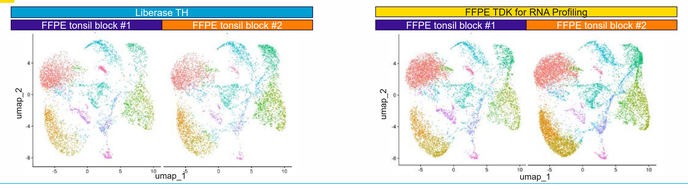


Figure 4 shows UMAP plots for all four tonsil samples. In those plots, where the FFPE scrolls were prepared by using our optimized workflow, more cells are identified. More cells, higher median gene and UMI count detected per cell can reflect greater resolution of transcriptional differences. The deeper coverage allows to obtain more information from the very same sample and experiment.

Figure 4



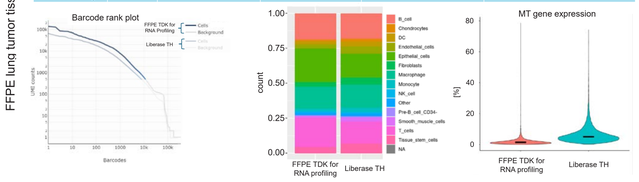
3 Optimized FFPE dissociation results in improved performance across multiple tumor types

To assess the performance of our optimized workflow (Enzyme P and D in Buffer S) on diseased tissues, we applied the Flex Gene Expression Assay to FFPE scrolls from lung, colon, and breast tumors (Figure 5). In lung tumor tissue, the optimized approach yielded 7,892 cells compared to 6,453 with Liberase TH, along with higher median genes per cell (811 vs 669), median UMI counts (1,225 vs 1,049), and confidently mapped reads (74% vs 66%). Similarly, for colon tumor tissue, the optimized method detected 11,821 cells (vs 13,337 with Liberase TH) but registered substantially higher median genes (1,129 vs 757), median UMIs (1,737 vs 1,075), and confidently mapped reads (82% vs 80%). In breast tumor tissue, it recovered 9,766 cells (vs 6,723), with a nearly two-fold increase in median genes (1,965 vs 1,038) and median UMIs (2,913 vs 1,437), alongside a higher percentage of confidently mapped reads (82% vs 71%). In all cases, the relative composition of cell phenotypes remained similar, although the Liberase TH condition consistently showed higher contamination with mitochondrial gene expression. Overall, the results confirm that our optimized FFPE tissue dissociation method boosts cell capture and sequencing depth without altering phenotype distribution.

Figure 5

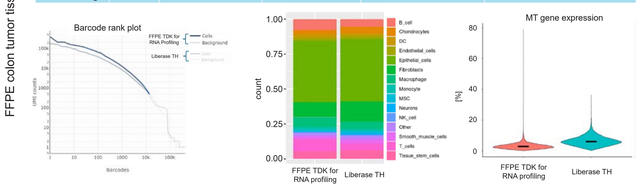
FFPE lung tumor tissue

	Cells	Median genes per cell	Total genes detected	Median UMI counts per cell	Confidently mapped reads in cells
Liberase TH	6,452	669	16,740	1,049	66.01 %
FFPE TDK for RNA Profiling	7,892	811	17,278	1,225	73,91 %



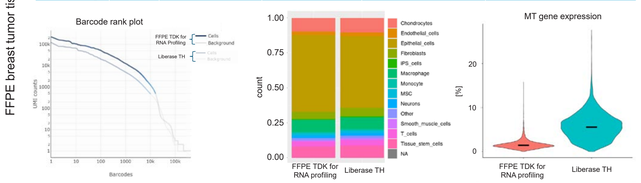
FFPE colon tumor tissue

	Cells	Median genes per cell	Total genes detected	Median UMI counts per cell	Confidently mapped reads in cells
Liberase TH	13,337	757	16,881	1,075	79.89 %
FFPE TDK for RNA Profiling	11,821	1,129	17,174	1,737	82.32 %



FFPE breast tumor tissue

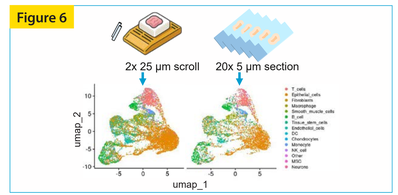
	Cells	Median genes per cell	Total genes detected	Median UMI counts per cell	Confidently mapped reads in cells
Liberase TH	6,723	1,038	17,610	1,437	70.62 %
FFPE TDK for RNA Profiling	9,766	1,965	17,996	2,913	82.24 %



4 Comparable results from 25 µm scrolls and 5 µm sections for Flex Gene Expression

Figure 6 presents UMAP plots based on the sequencing results from either two 25 µm scrolls or twenty 5 µm FFPE colon tumor tissue sections. Although fewer cells were called in the thinner sections, both samples exhibited comparable clustering of distinct cell phenotypes. These findings confirm that 5 µm FFPE sections, prepared with our optimized workflow, serve as a suitable input for the Flex Gene Expression Assay.

Figure 6



Conclusion

Increased cell yields and sequencing quality: The optimized dissociation protocol consistently recovers more cells, detects more genes, and generates higher UMI counts compared to standard methods, resulting in deeper and more informative data.

Higher proportion of confidently mapped reads: Across multiple tissue types, the workflow shows improved alignment metrics and fewer off-target reads, indicating enhanced data quality and more robust gene expression profiles.