Multi-tissue scRNA seq atlasing of mouse nuclei

Introduction

Although single cell profiling of tissue-derived nuclei can unlock profound insights into cellular heterogeneity and function, achieving high-quality data from these samples can be challenging. Single-nuclei isolation and debris removal before the single-nuclei workflow can lead to substantial nuclei loss, which in turn decreases chances of seeing rare cell populations in these heterogeneous tissues. Nuclei quality can also vary across these samples, which can be difficult to capture or particularly sensitive to shear forces.

To investigate the performance of these samples on the ScaleBio platform, we isolated nuclei from multiple mouse tissues and profiled them using the ScaleBio scRNA Kit. Nuclei from brain, liver, lung, and kidney showed uniformly high-quality libraries with strong sample recovery and sensitivity and good diversity in cell type detection. Together, these results demonstrate the quality of data that can be obtained with the ScaleBio scRNA Kit.

Highlights

Quality data at scale: High-quality methylomes were generated from 8,049 glioma nuclei using ScaleBio's Single Cell DNA Methylation workflow

Single cell resolution: Single cell DNA methylation enabled detection of tumor and nontumor cell types

High sensitivity: Differential methylation analysis revealed cancer-specific hyper- and hypomethylated regions, including known prognostic biomarkers

High cell recovery from multiple tissues: Excellent cell recovery and sensitivity from tissue-derived nuclei from mouse brain, liver, lung, and kidney

High-quality libraries from debris-heavy samples: Streamlined workflow produces clean libraries with low background and high usable reads from tissues

Multiple sample type multiplexing: Four different mouse tissue types with disparate RNA content successfully captured and profiled in a single experiment

Methods

Sample preparation

Approximately 100 mg samples removed from flash-frozen mouse tissues (Jackson Laboratories- C57BL/6/J, 12 weeks) were dissociated using the Miltenyi gentleMACS Octo Dissociator. High-debris samples (brain and liver) were further taken through anti-nucleus microbead enrichment, while kidney and lung tissues were taken directly into ScaleBio fixation without enrichment.

Single cell workflow

Fixed nuclei from each tissue were multiplexed onto the first plate of the ScaleBio workflow, as shown in **Figure 1**. The ScaleBio scRNA protocol was followed with no modifications, and nuclei were distributed in two final distribution plates based on recovery after ligation. Libraries were sequenced on a NovaSeq 6000 S4 flow cell and analyzed with the ScaleBio pipeline.

Results

Sample preparation

After dissociation, nuclei were counted and imaged. Nuclei were subsequently taken through enrichment (brain and liver) or filtering (lung and kidney) to further remove debris. Resulting nuclei **(Figure 2)** were taken through the ScaleBio scRNA workflow.

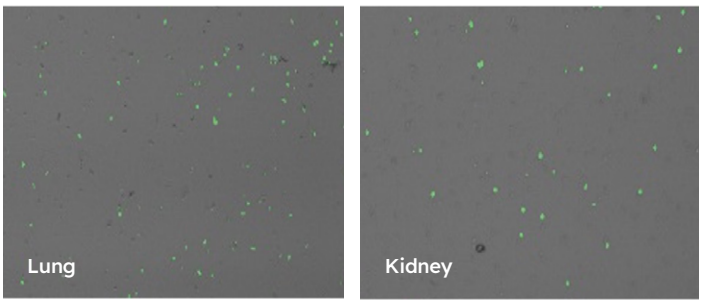


Figure 2. Example images of dissociated and filtered nuclei going into the assay.

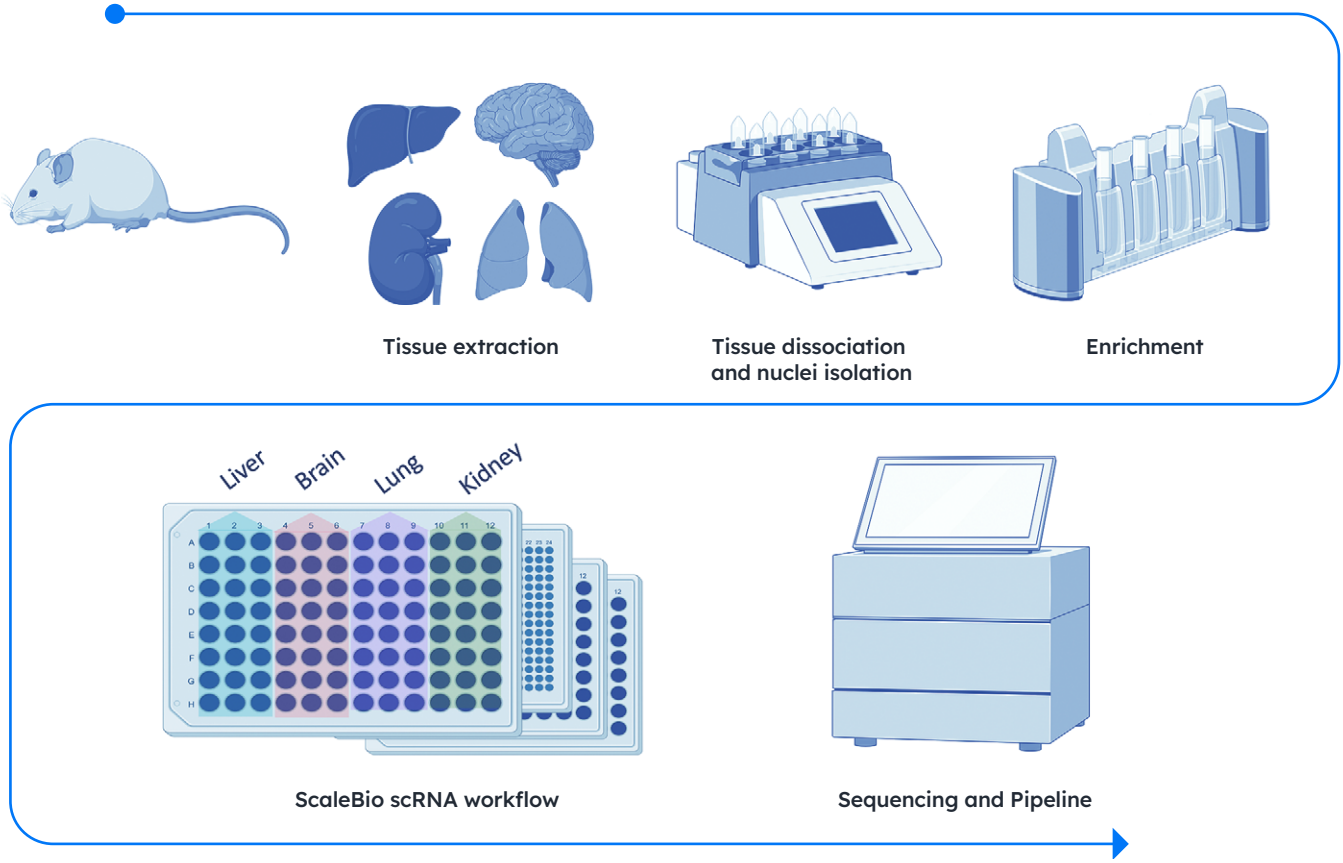


Figure 1. Nuclei from four different mouse tissues (liver, brain, lung, and kidney) were isolated using the Miltenyi gentleMACS Octo Dissociator. Brain and liver nuclei samples were further enriched using Miltenyi Anti-Nucleus MicroBeads. Nuclei were fixed and loaded onto the same RT plate for simultaneous processing using the ScaleBio Single Cell RNA Kit. Following library generation and sequencing, a total of 468,081 nuclei were analyzed using the ScaleBio Seq Suite.

Library and sample quality

The resulting libraries were sequenced and analyzed using the ScaleBio Seq Suite pipeline. QC metrics showed 86% of reads contained full cell barcodes with high mapping rates to the genome and transcriptome **(Figure 3, left and middle)**. Notably, all samples showed a high percentage of reads in cells **(Figure 3, right)** and less than 1% mitochondrial reads, further emphasizing that sample integrity is maintained throughout the ScaleBio scRNA workflow. Together, these data demonstrate that the ScaleBio workflow produces clean libraries from tissue-derived nuclei.

Sensitivity and cell recovery

To further examine data quality, we analyzed cell recovery and sensitivity across tissue samples. QC metrics showed high cell recovery across all samples, with a total recovery of 468k nuclei from this single experiment. Of note, recovery from the final distribution plates was >200k per plate (higher than the expected 125k), which could reflect challenges with counting; however, this does not appear to have impacted overall sensitivity, suggesting robust performance of the kit across a range of cell inputs. At shallow sequencing depth (25-30% saturation across samples), we observed strong transcript and gene recovery across samples, with differences consistent with disparate RNA content across these tissues **(Figure 4A)**. A UMAP projection of all samples together showed clean distinction between different tissues, as well as extensive cellular subclusters within each **(Figure 4B)**.

Tissue analysis

To better understand cell-type-specific heterogeneity and recovery from the ScaleBio workflow, UMAPs were generated with nuclei from each individual tissue. Differentially expressed genes taken from gene lists from public datasets were then used to manually annotate cell clusters. Analysis of brain, liver, lung, and kidney identified 8, 13, 15, and 10 populations, respectively **(Figure 5)**. Notably, in addition to expected major cell populations such as neurons, hepatocytes, lung epithelial, and renal cells, the throughput and sensitivity of this assay also enabled identification of more rare cell subsets, such as glial populations in the brain; small populations of immune cells infiltrating various tissues, such as Kupffer cells in the liver; and small but important effector populations in all tissues. Collectively, these findings highlight the platform's versatility, sensitivity, and robustness in capturing cellular diversity across different tissue types in the same workflow.

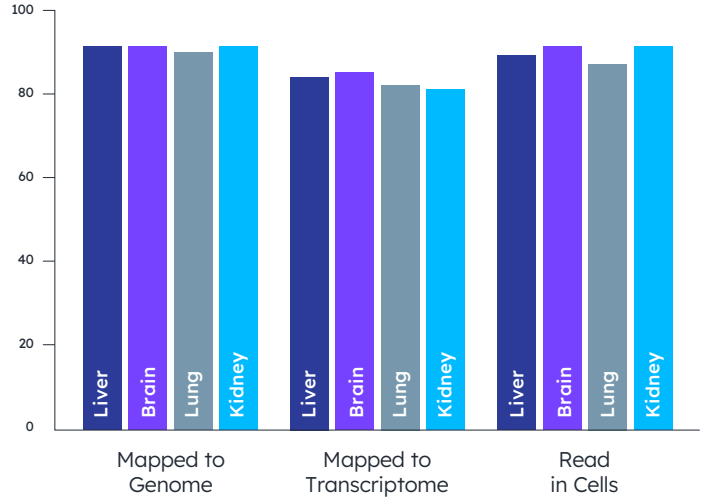


Figure 3. Usable reads observed in all samples.

	Liver	Brain	Lung	Kidney
Cells	76,104	81,310	164,886	145,766
Reads/cell	18,694	31,881	3,378	5,360
Saturation	28%	31%	24%	25%
Transcripts	6,889	10,940	1,444	2,404
Genes	2,937	3,440	997	1,472

Figure 4A. Table showing sensitivity and sequencing depth metrics across samples.

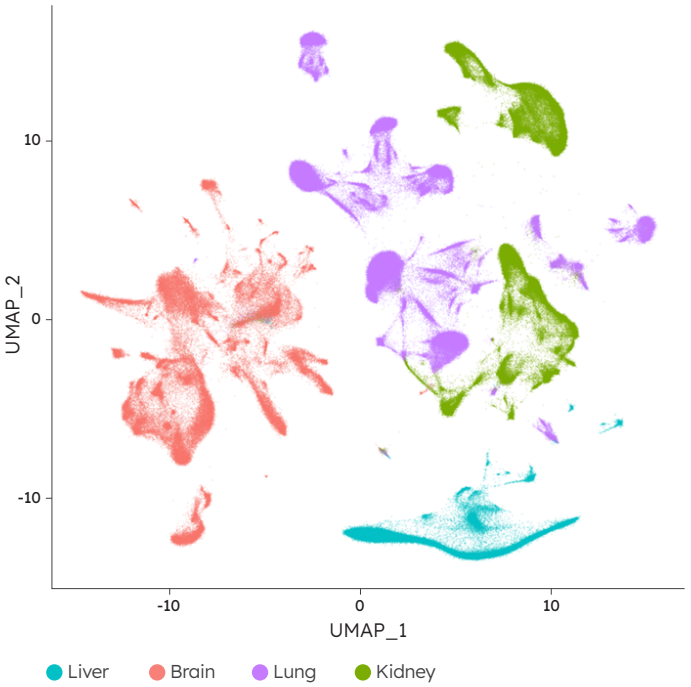
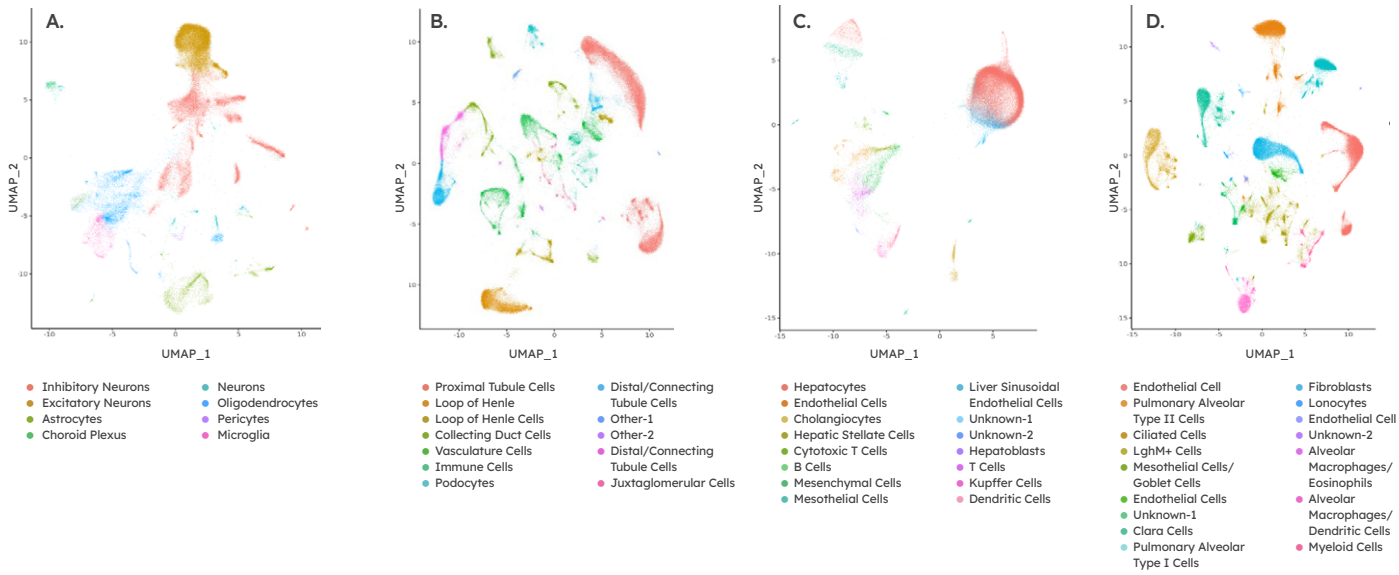


Figure 4B. UMAP projection of all nuclei recovered.

Figure 5: Tissue-specific UMAP plots. A.) Brain sample identified 8 distinct clusters. **B.)** Kidney sample identified 10 distinct clusters. **C.)** Liver sample identified 13 distinct clusters. **D.)** Lung sample identified 14 distinct clusters.



Conclusion

The ScaleBio Single Cell RNA Sequencing Kit enables users to profile nuclei from complex tissues simultaneously on the same workflow, yielding robust data that highlights tissue-specific cell type heterogeneity. Here, we demonstrate the capture of 468k nuclei isolated from mouse brain, kidney, lung, and liver, recovering major and minor cell populations and providing a comprehensive snapshot of cellular diversity within these tissues. Our single cell sequencing platform combines exceptional cell recovery, sensitivity, and high-quality data output with an efficient workflow, making it an ideal solution for advanced biological research and cellular analysis.

Product Code	Product Description
2020001	ScaleBio Sample Fixation Kit
950884	ScaleBio Single Cell RNA Sequencing Kit v1.1
936360	ScaleBio Single Cell RNA Extended Throughput Kit v1.1

To learn more about ScaleBio's Single Cell RNA Sequencing Kit, visit scale.bio/single-cell-rna-sequencing-kit/

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