

# Characterizing the tumor microenvironment using spatially barcoded archival FFPE tissue: Converting single-nucleus RNA-seq into spatial transcriptomics



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## Abstract

Identifying the contribution and the role of each individual cell in the spatial context of the tumor microenvironment (TME) is crucial in determining tumor progression, examining the extent of immune infiltration, and identifying potential therapeutic targets. While single-cell sequencing-based techniques help reveal cellular heterogeneity in a cell population, they do not provide spatial context. In turn, recently emerged spatial transcriptomics techniques are challenged by the difficulty in pinpointing the spatial measurement to single cells, requiring either complex cell segmentation or deconvolution algorithms in the data processing.

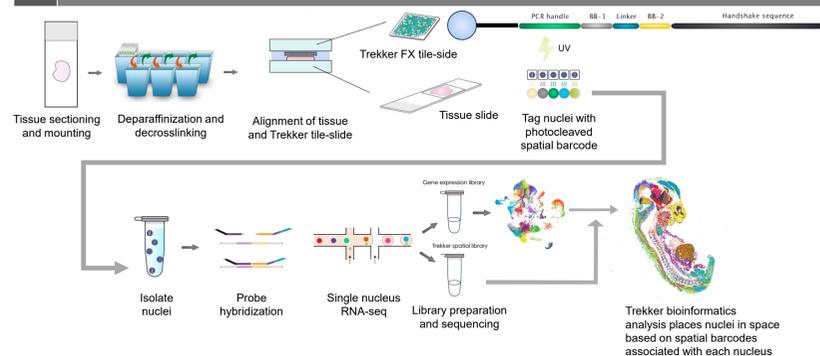
To address these limitations, we introduce the Trekker™ single-cell spatial mapping kit, a unique platform that turns a single-cell experiment into a spatial experiment, essentially providing a spatial measurement that has true single-cell resolution. It is based on the Slide-tags methodology (Russel *et al*, 2023) that spatially tags each nucleus within its native tissue environment. This approach utilizes existing single-nuclei (sn) sequencing infrastructure to generate high-resolution spatial transcriptomics data. Using archived FFPE samples, we demonstrate the effectiveness of Trekker by aligning 25–30 μm tissue sections to spatially barcoded tiles. Spatial barcodes are cleaved and hybridized to cells and nuclei in the intact tissue, followed by tissue dissociation and nuclei isolation. The tagged nuclei are processed with established snRNA-seq workflows (e.g., 10x Chromium FLEX), producing gene expression and spatial libraries for the individual nuclei in the tissue section. This spatial tagging allows each nucleus to be bioinformatically mapped back to its original tissue location.

We applied this solution to breast cancer biospecimens from different patients using whole transcriptome probes (10x Genomics). The resulting data allowed for detailed characterization of the TME, confidently identifying epithelial-malignant, stromal, and immune cells from each biospecimen. This enabled marker-guided regional selection and differential gene expression analysis based on both cell type and spatial location. Overall, the Trekker platform provides a streamlined, high-resolution solution for profiling cellular heterogeneity and interactions within tumors, seamlessly integrating with existing workflows to generate rapid insights into disease mechanisms and therapeutic development.

## Methods

- Individual 25 μm FFPE sections were floated onto a standard microscope slides and allowed to dry. The tissue was then trimmed to fit the Trekker tile area (10 mm x 10 mm), deparaffinized, then mildly decrosslinked to enable spatial-tag attachment and transcript access.
- The tissue was then aligned with the 10x10 Trekker tile using a custom alignment fixture. The Trekker tile consists of a monolayer of uniformly distributed 10 μm beads with photocleavable DNA oligos containing spatial barcodes. Following assembly, the spatial barcodes were released upon UV light exposure and diffused into the tissue to tag individual nuclei.
- After spatial tagging, the fixture was disassembled, and the tissue was treated with a stabilization buffer to improve spatial tagging retention. Subsequently, the tissue was scraped from the slide and dissociated into single-nuclei suspensions for loading into a single-cell assay.
- For spatial snRNA-seq experiments using the 10x Genomics Single-Cell GEM-X Flex Kit for Multiplex samples with Feature Barcode technology for Protein Expression, the isolated nuclei were incubated overnight with the multiplex human WTA and feature barcode probes. For the pilot study, a subset of isolated nuclei were then captured and barcoded on the Chromium GEM-X system, and separate mRNA and spatial libraries were generated. The WTA and Trekker libraries were sequenced on the Illumina® NextSeq® 1000 (28;12;12;90).
- The Cell Ranger pipeline was implemented for gene expression analysis, and a custom informatics pipeline was used to integrate the spatial positions of each nucleus and transcriptomic data.

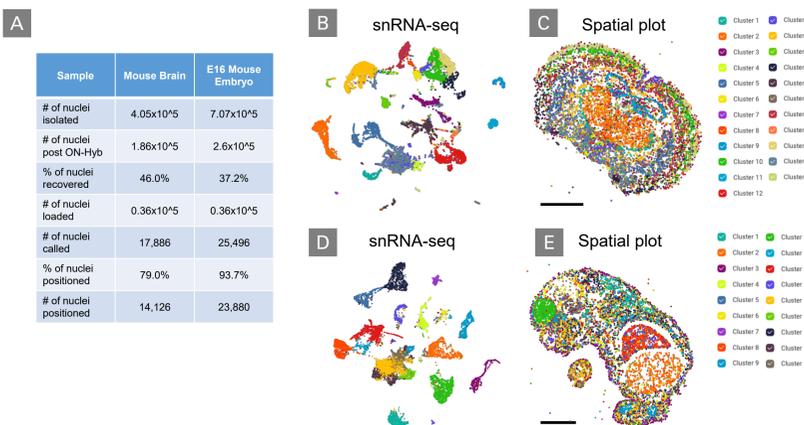
## 1 FFPE Trekker workflow



**Figure 1. FFPE Trekker workflow upstream of snRNA-seq assay.** The FFPE Trekker integrates single-nucleus spatial tagging with standard tissue processing and snRNA-seq assay.

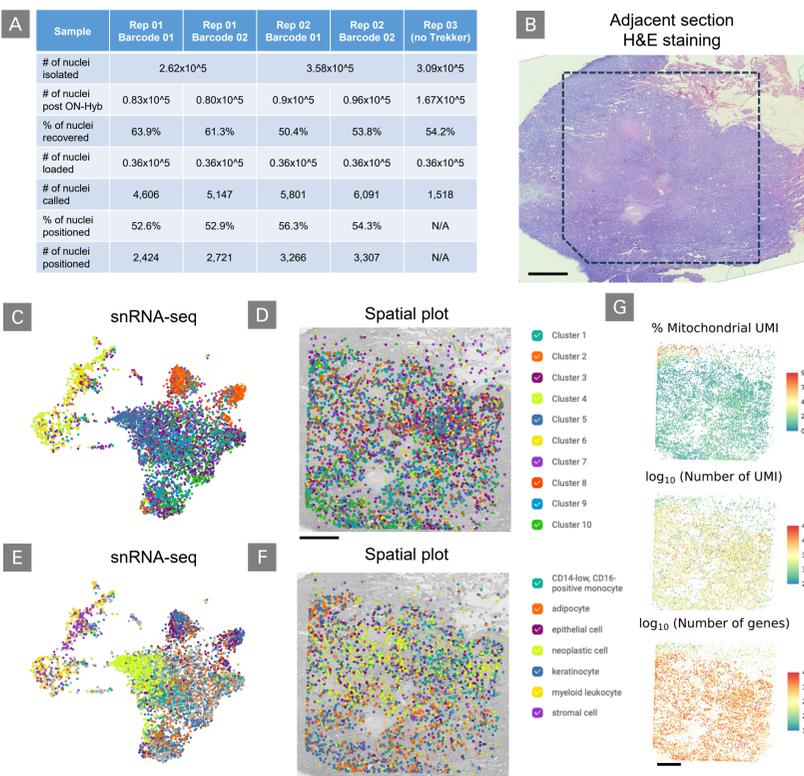
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## 2 FFPE Trekker validation with mouse brain and embryo



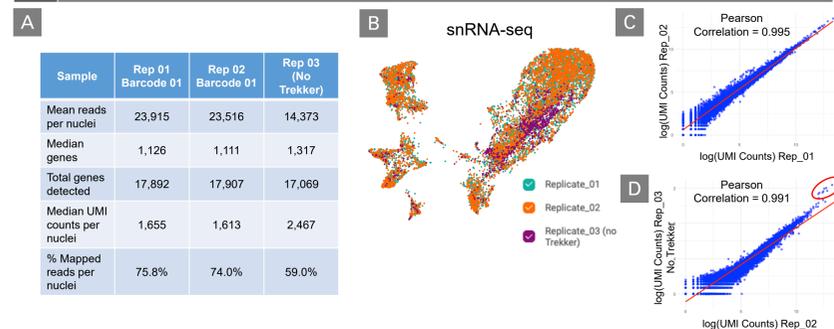
**Figure 2: FFPE Trekker performance validation with adult mouse brain and E16 mouse embryo.** Individual 25 μm coronal sections of the mouse brain and sagittal sections of an E16 mouse embryo. **Panel A.** Summary table of sample metrics of sections used in the validation study. Mouse brain UMAP (**Panel B**) and spatial plot (**Panel C**) demonstrating unbiased clustering of different cell populations aligning with the morphology. Mouse embryo UMAP (**Panel D**) and spatial plot (**Panel E**) demonstrating unbiased clustering of different cell populations and tissue types aligning with the morphology. Scale bar = 2 mm

## 3 FFPE Trekker adds spatial context without compromising gene expression quality in breast cancer samples



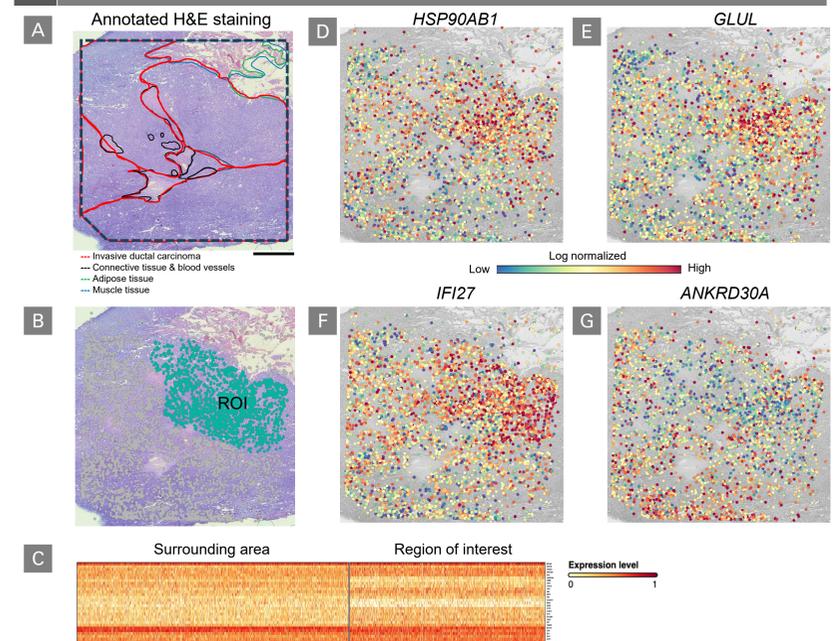
**Figure 3: FFPE Trekker adds spatial context to snRNA-seq data on breast cancer biospecimen.** Individual 25 μm tissue sections taken from a 74-year-old breast cancer patient with Stage IIIB invasive ductal carcinoma (ER+/PR+/Her2-) was used to demonstrate the performance of the FFPE Trekker. **Panel A.** Summary table of sample metrics of sections used in pilot study. **Panel B.** Representative H&E staining of an adjacent 5 μm section demonstrating Trekker tile position and coverage. Replicate 01 UMAP (**Panel C**) and spatial plot (**Panel D**) demonstrating unbiased clustering of different cell populations. UMAP (**Panel E**) and spatial plot (**Panel F**) following cell type prediction exhibiting notable detection of immune, cancer, stromal, and epithelial subpopulations. **Panel G.** Quality metrics for single-nuclei mitochondrial counts, number of molecules, and number of genes across the tissue section. Scale bar = 2 mm

## 4 Spatial tagging is reproducible and does not impact gene expression data quality



**Figure 4. Gene expression data is reproducible between technical replicates and replicates without spatial tagging.** **Panel A.** Summary table of gene expression library and mapping metrics from the replicates in the pilot study. **Panel B.** UMAP plot of the combined replicates demonstrates overlap. Correlation plots from (**Panel C**) two technical replicates of two different sections, and (**Panel D**) between sections with or without Trekker spatial tagging, which excludes mild decrosslinking and stabilization buffer treatment necessary for tagging. In the absence of Trekker spatial tagging, there was a higher representation of mitochondrial genes (red oval).

## 5 Differential gene expression analysis between different tumor regions



**Figure 5. Annotation-guided tumor region selection and identification of gene markers.** **Panel A.** Annotation of H&E-stained adjacent section highlighting regions of invasive ductal carcinoma, connective tissue, blood vessels, adipose tissue, and muscle tissue. **Panel B.** Regional section of nuclei from a high-density area of the tumor. **Panel C.** Execution of local differential gene expression analysis displaying the top 20 genes between the region of interest (ROI) and the surrounding area. Spatial gene expression patterns of *HSP90AB1* (**Panel D**), *GLUL* (**Panel E**), and *IFI27* (**Panel F**) demonstrate elevation within the ROI and correlate with tumor progression. **Panel G.** *ANKRD30A* conversely exhibited reduced expression within the ROI, which is typically associated with triple-negative breast cancer. Scale bar = 2 mm

## Conclusions

- In this study, we showcased the application of FFPE Trekker for spatially tagging single nuclei and its integration into an existing single-cell assay
- Validation on both mouse and human breast cancer biospecimens demonstrate high nuclei recovery, gene expression quality, and the generation of snRNA-seq and spatial profiles
- With spatial context, the assay enables for regional-based examination of snRNA-seq data and spatial gene expression patterns to understand the tumor microenvironment
- Overall, the FFPE Trekker platform provides a streamlined, high-resolution solution for profiling cellular heterogeneity and interactions within tumors, while integrating with existing workflows



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