Unlocking multiomic analysis in spatial genomics: Integrating spatial ATAC-seq and V(D)J sequencing with gene expression using novel spatial barcoding of single nuclei

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Abstract

While single-cell multiomic analyses such as RNA-seq, ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing), or V(D)J sequencing have advanced our understanding of gene expression, chromatin accessibility, and immune receptor diversity, these methods miss the critical spatial organization of cells within their native environments. Existing spatial methods, whether it's microscopy- or sequencing-based, often lack true single-cell resolution, instead relying on cell segmentation or deconvolution strategies. These methods significantly limit spatial-based exploration of immune receptor diversity and the regulatory landscape of tissues.

To address this limitation, Curio Trekker, based on the Slide-tags technology, offers a novel solution by transforming any single-cell sequencing assay into spatially-resolved single-cell data. This technology utilizes unique DNA barcodes on a tightly-packed bead array to spatially label individual cells. The resulting tagged nuclei are then processed on conventional single-cell sequencing platforms, preserving spatial information while providing true single-cell resolution.

In this study, we applied Curio Trekker to achieve spatial ATAC-seq combined with gene expression analysis, as well as spatial V(D)J sequencing paired with gene expression. These previously difficult-to-achieve combinations enable the exploration of chromatin accessibility and gene regulation within the tissue's native spatial context, as well as spatial mapping of immune receptor diversity and functionality. This integrated spatial multiomics approach provides a more comprehensive view of tissue microenvironments.

We validated these approaches across immune and tumor tissues, demonstrating the robustness and versatility of Curio Trekker in capturing high-resolution spatial data. Our findings highlight how this technology can unlock new insights into cellular regulatory mechanisms and immune cell interactions, advancing research in immunology, oncology, and other fields.

Methods

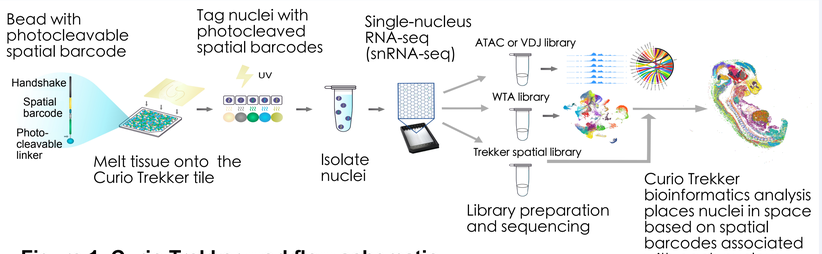

Figure 1. Curio Trekker workflow schematic.

- A 25 µm fresh frozen section was melted onto a 10 mm x 10 mm Trekker tile consisting of a monolayer of uniformly distributed 10 µm beads coated with photocleavable DNA oligos containing spatial barcodes. The spatial barcodes were released upon UV light exposure and diffused into the tissue to tag individual nuclei. The tissue was then detached from the Trekker tile and dissociated into single-nucleus suspension.
- For spatial single-nucleus ATAC-seq (snATAC-seq) experiments, using the BD Rhapsody Single-Cell ATAC-Seq and mRNA Whole Transcriptomic Analysis (WTA) kit, spatially-tagged nuclei were incubated with Tn5 transposase to fragment and insert adaptors into the accessible chromatin regions. Individual nuclei were then captured on the BD Rhapsody system. mRNA, ATAC products, and spatial barcodes were simultaneously captured and separate libraries for each modality were generated. The WTA and Trekker libraries were sequenced on the Illumina® NextSeq® 1000, and the ATAC-seq libraries were sequenced on the Element AVITI.
- For spatial single-nucleus V(D)J (snV(D)J-seq) experiments, we used the BD Rhapsody TCR/BCR Next and mRNA WTA kit to simultaneously capture mRNA, V(D)J sequences, and spatial barcodes, as well as to generate separate libraries for each modality. All libraries were sequenced on the Nextseq 1000.
- A custom informatics pipeline integrated the spatial positions of each nucleus and the transcriptomic, epigenetic, and immune repertoire data.
- For this pilot study, only a subset of isolated nuclei was loaded onto the BD Rhapsody system (**Table 1**).

Sample	Mouse kidney-1	Mouse kidney-2	Mouse brain -1 (- Trekker)	Mouse brain -2	Mouse lymph node	Human breast cancer
Assay	snRNA-seq + snATAC-seq				snRNA-seq + snV(D)-seq	
# of nuclei isolated	2.9x10^5	1.4x10^5	8.9x10^4	5x10^4	1.03x10^5	5.7x10^5
# of nuclei loaded	4x10^4	4.6x10^4	3.1x10^4	2.4x10^4	1.7x10^4	3.2x10^4
# of nuclei captured	11,357	10,494	9,691	7,495	5,603	10,671
% of nuclei positioned	47.1%	60.2%	N/A	76.8%	79.6%	61.48%
# of nuclei positioned	5,346	6,317	N/A	5,758	4,460	6,561

Table 1. Sample metrics

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Results

A	Mouse brain-1 - Trekker	Mouse brain-2 + Trekker
Total nuclei	9,693	7,495
% useful ATAC reads	88.64%	88.54%
Median reads per cell	22,479	20,045
% of fragments in peaks	33.61%	31.65%
TSS score	11.13	10.98
% duplicate fragments	16.86%	14.78%
Total number of peaks identified	305,941	282,434

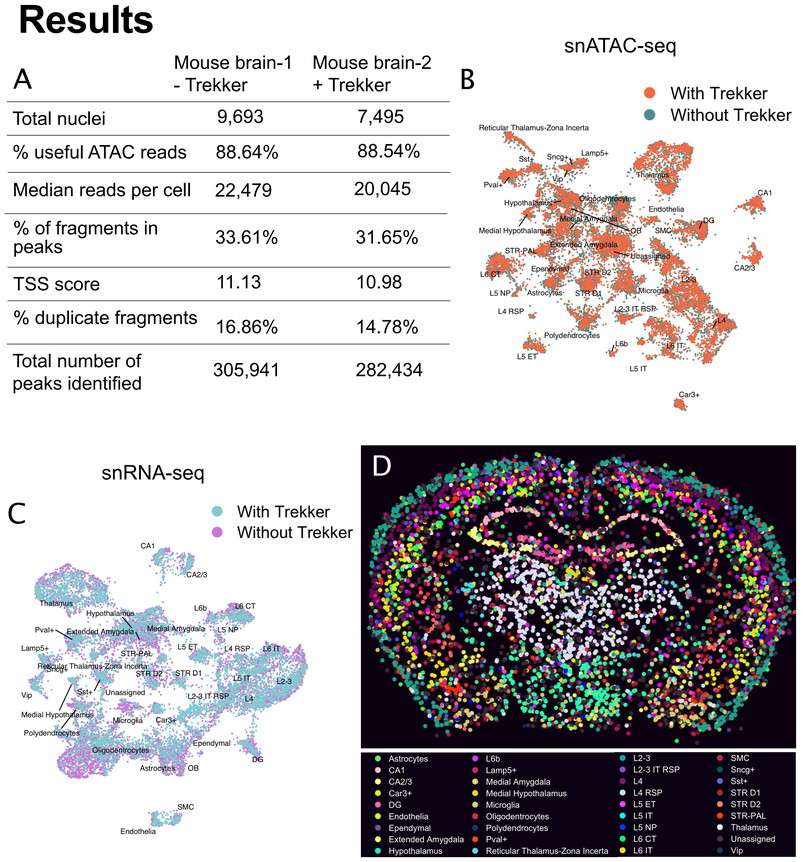

Figure 2. Curio Trekker adds spatial context without compromising WTA and ATAC data quality. Two adjacent 25 µm tissue sections of an adult mouse brain were used to compare snATAC-seq performance with and without the addition of spatial barcoding by Curio Trekker. (**Panel A**) snATAC-seq library metrics show comparable performance between the two samples. UMAP generated by snATAC-seq peaks (**Panel B**) and snRNA-seq (**Panel C**) show high concordance between the two samples. (**Panel D**) Annotated cell types (based on gene expression) of mouse brain-2 projected onto the spatial map.

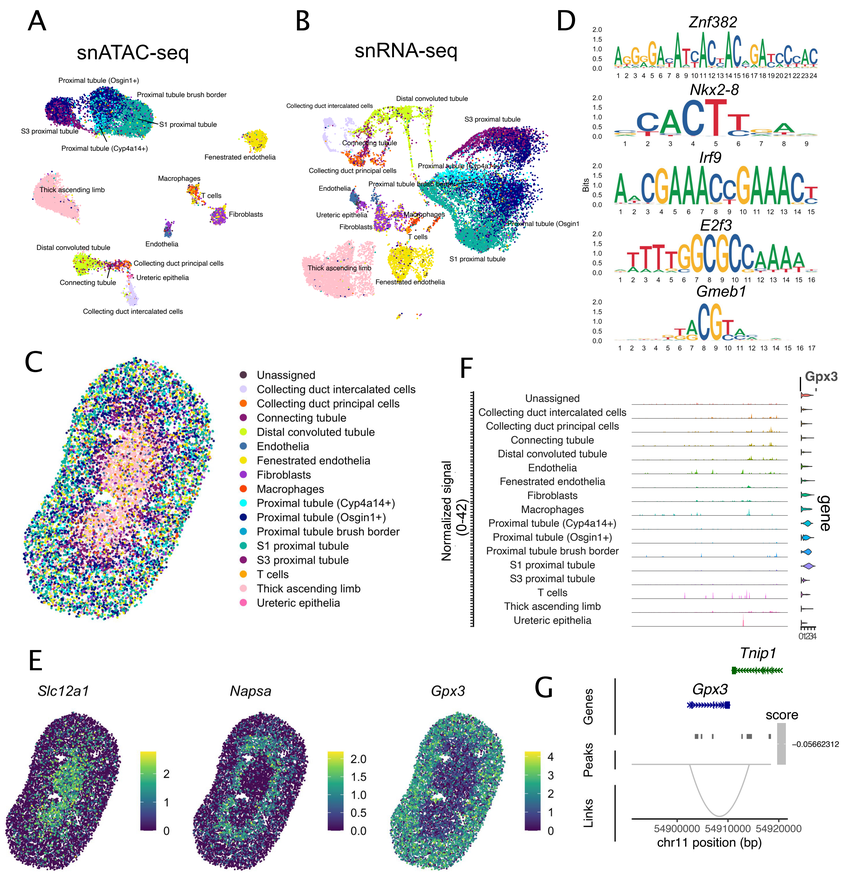

Figure 3. Curio Trekker brings spatial dimension to snRNA-seq and snATAC-seq data. 11,612 nuclei from two adjacent 25 µm sections of an adult mouse kidney were spatially positioned using two 10 mm x 10 mm Curio Trekker tiles. The data from the two tiles were merged. Annotated cell types on UMAP were generated from snATAC-seq peaks (**Panel A**) and snRNA-seq expressions (**Panel B**). (**Panel C**) Spatial projections of annotated cell types. (**Panel D**) Spatial pattern of kidney region-specific genes, *Slc12a1, Napsa* and *Gpx3*. (**Panel E**) Sequence logo plots of top 5 overrepresented DNA motifs in differentially accessible ATAC peaks in S3 vs S1 proximal tubule cells. (**Panel F**) *Gpx3*-linked genomic region accessibility (left) and *Gpx3* expression (right) in annotated cell types. (**Panel G**) **Top:** gene annotation (blue/green represent +/- strand); **Middle:** reference peaks; **Bottom:** peak linkage over *Gpx3*-linked genomic region as in (**Panel F**). Gene-associated genomic region: 10,000 bp up- and downstream of the gene of interest. All cell types were annotated based on snRNA-seq data.

A	Mouse lymph node
Mean useful reads per nucleus - TCR	1,049
% T cells with alpha chain CDR3	13.6%
% T cells with beta chain CDR3	20.9%

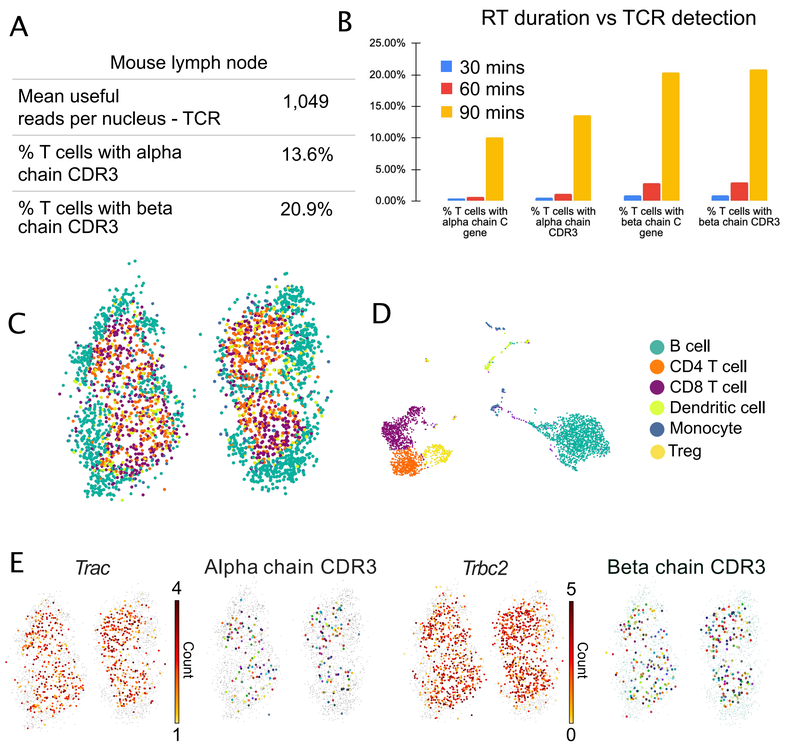

Figure 4. Spatial snTCR-seq and snRNA-seq of the same mouse lymph node section. 4,460 nuclei from a 25 µm mouse lymph node section containing two lymph nodes were spatially positioned on a 10 mm x 10 mm Curio Trekker tile. (**Panel A**) TCR library metrics. (**Panel B**) % T cells with TCR molecules detected increased with longer reverse transcription duration. Spatial (**Panel C**) and UMAP (**Panel D**) projections of annotated cell types based on gene expression. (**Panel E**) Comparing spatial distribution of constant regions vs alpha chain (**left**) or beta chain (**right**) CDR3. *Trac* and *Trbc2* plots are colored by their respective UMI counts. Each color in the alpha and beta chain CDR3 plots represent a unique clonotype.

A	Human breast cancer
Mean useful reads/nucleus - BCR	372.4
Mean useful reads/nucleus - TCR	332.5
% B cells with heavy chain CDR3	39.6%
% B cells with light chain CDR3	52.44%
% T cells with alpha chain CDR3	7.6%
% T cells with beta chain CDR3	14.2%

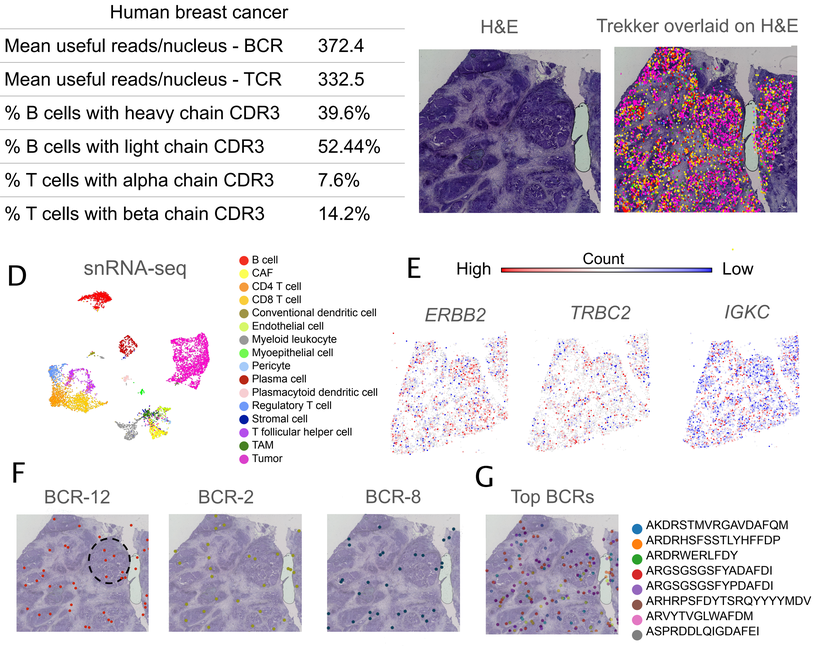

Figure 5. Spatial snTCR/BCR-seq and snRNA-seq of human breast cancer. (**Panel A**) TCR and BCR library metrics. (**Panel B**) H&E staining of an adjacent section. (**Panel C**) Overlay of spatial snRNA-seq data with H&E staining. Each dot is a single nucleus with 1:1 correspondence to the UMAP (**Panel D**) and colored by the same cell type annotations. (**Panel E**) Spatial plots colored by expression level of *ERBB2*, *TRBC2* and *IGKC*. (**Panel F**) Overlay of three BCR clones with H&E staining. The dotted circle highlights a tumor region. (**Panel G**) Spatial projections of the top eight BCR heavy-chain CDR3 clonotypes overlaid with H&E staining.

Summary

In this study, we showcased how Curio Trekker integrates easily upstream of existing single-nucleus assays, enabling previously hard to achieve spatial multiomics analysis. Even with subsampling of nuclei and shallow sequencing, we were able to accurately recapitulate spatial morphology, identify major cell types, and perform meaningful peak analysis (ATAC-seq) and clonotype analysis (V(D)J). These methods provide a comprehensive high-dimensional approach to studying complex tissues and diseases.