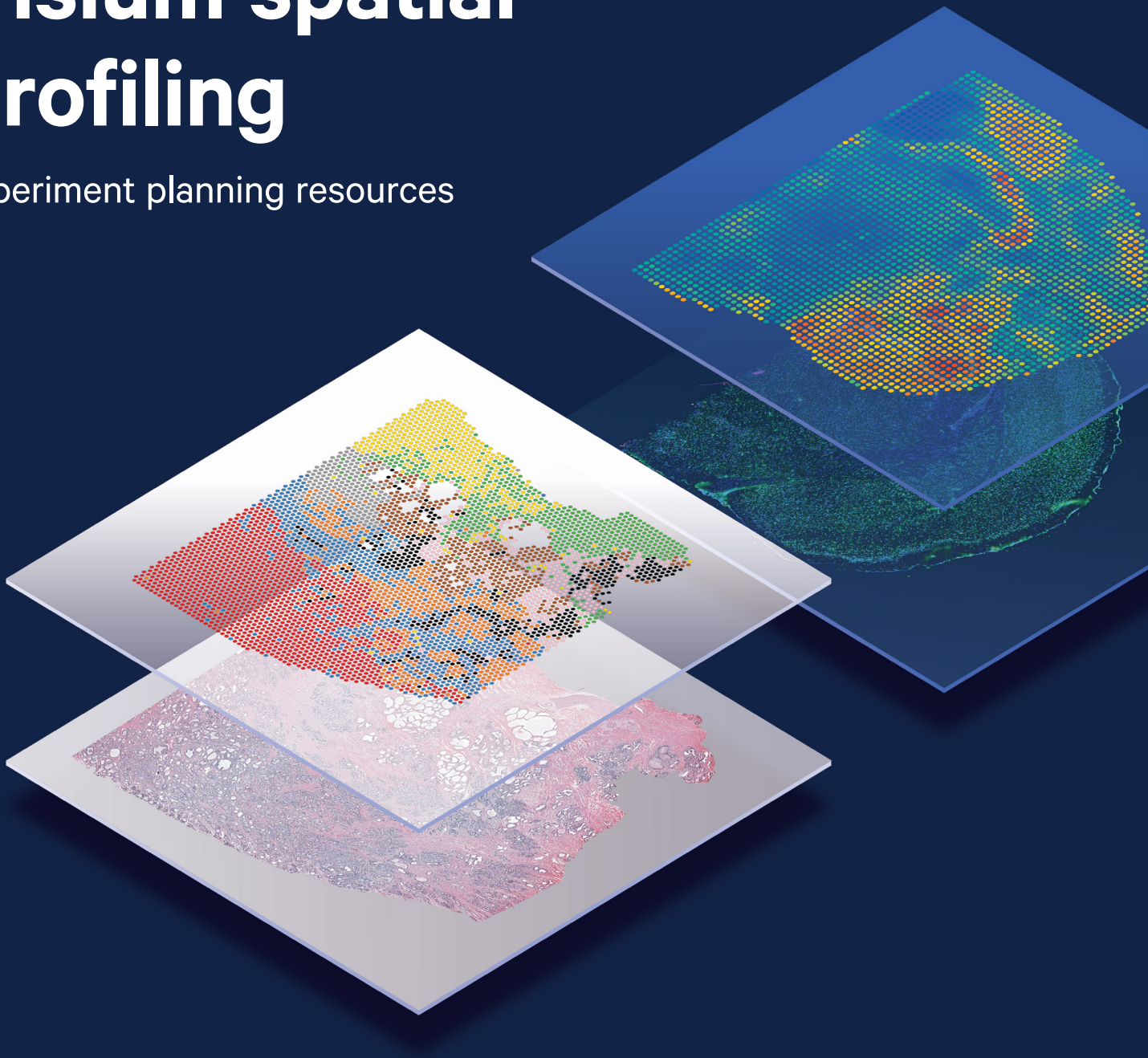
10x GENOMICS

Visium Platform

## The essential guide:

# Visium spatial profiling

Experiment planning resources



10xgenomics.com/spatial-gene-expression

# Planning your experiments

## Measure and visualize gene activity where it is occurring by combining the benefits of traditional histology with the power of whole transcriptome analysis.

Spatial transcriptomics is a groundbreaking molecular profiling method that allows scientists to measure gene activity in a tissue sample and map where the activity is occurring. Visium Spatial Gene Expression spatially resolves gene expression by mapping the whole transcriptome with morphological context in entire formalin-fixed paraffin-embedded (FFPE) or fresh frozen tissues to discover novel insights into normal development, disease pathology, and clinical translational research. This guide should serve as a roadmap to help you design your Visium Spatial Gene Expression experiments, optimize experimental parameters, and identify appropriate computational/analytical tools to analyze your spatial gene expression data.

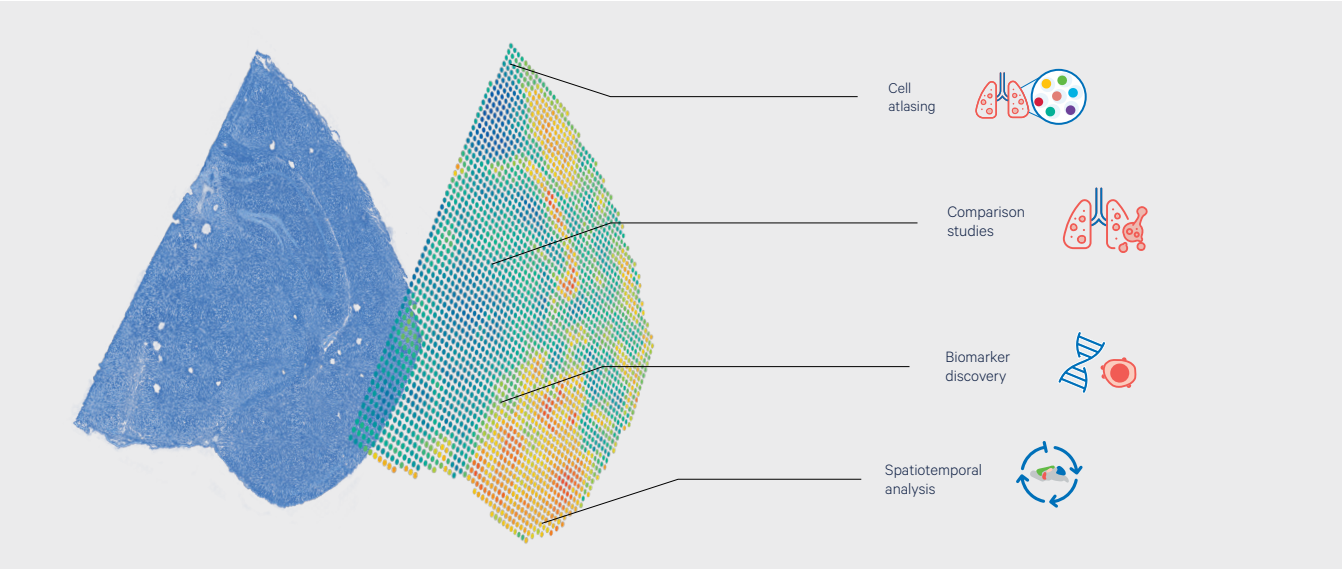



**Figure 1. Gain high-resolution, spatial characterization of gene and protein expression with Visium Spatial Gene Expression.** Discover new biomarkers by examining histology, protein, and mRNA from the same tissue section. Explore the spatial relationship between cells within normal and diseased tissue. Combine with immunofluorescence (IF) for protein co-detection or with hematoxylin and eosin staining (H&E) for morphological context.

Prior to starting your spatial gene expression experiments, we recommend you consider the following questions to help guide your experimental design and determine how to tackle your research aims.

**01 What is the aim of my study?**

**02 What tissue do I have access to?**

**03a Fresh frozen: What are the best practices for preparing and processing my samples?**

**03b FFPE: What are the best practices for preparing and processing my samples?**

**04 How do I analyze and visualize my data?**

**05 What use case examples can I reference?**

**06 How do I prepare my lab?**

# 01 What is the aim of my study?

**Cell atlasing**
Characterize, identify, and catalog tissue while maintaining spatiality

See use case: 1, 3, 6

**Biomarker discovery**
Uncover novel biomarkers and gene signatures of cellular and disease processes

See use case: 2, 5

**Comparison study**
Examine differences between normal and diseased tissue

See use case: 2, 4, 5

**Spatiotemporal analysis**
Track cell populations and gene expression patterns over time and disease progression

See use case: 6, 7

Discover additional applications by viewing a comprehensive listing of peer-reviewed Visium publications.

# 02 What tissue do I have access to?

There are distinct Visium Spatial assays utilizing different gene capture chemistries for fresh frozen and FFPE tissues. The fresh frozen assay utilizes poly(A) capture for whole transcriptome library preparation, while the FFPE assay leverages RNA-templated ligation of probe pairs for highly specific and sensitive detection of the whole transcriptome.

Internally, Visium Spatial Gene Expression has been successfully tested on over 30 different fresh frozen tissues from a variety of different species. Currently, Visium Spatial Gene Expression for FFPE is compatible with a variety of different tissues from human and mouse.

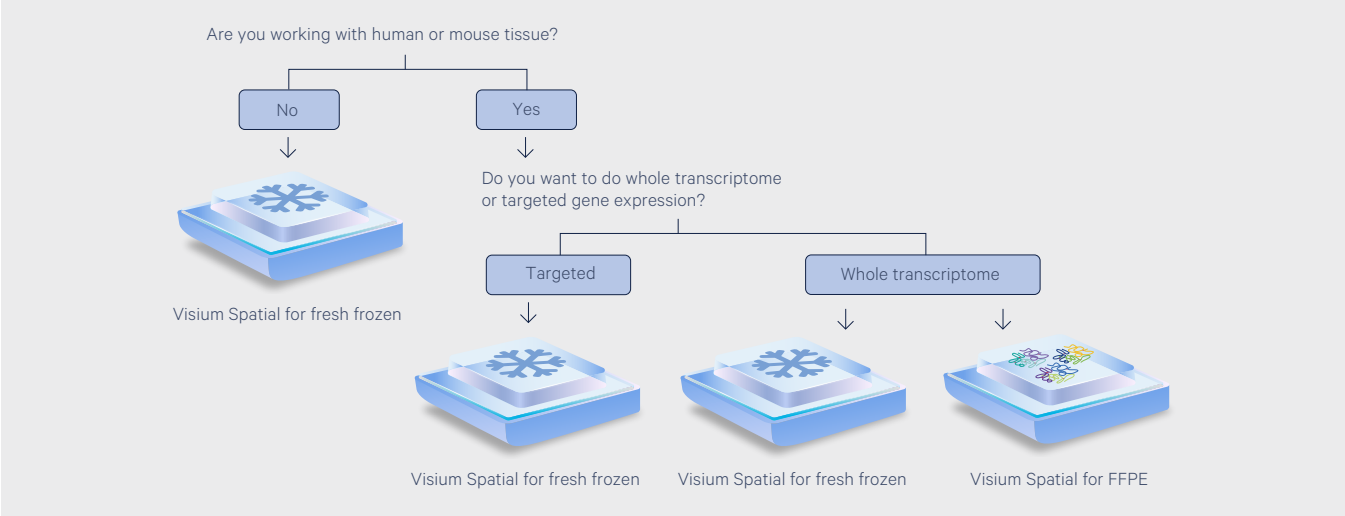
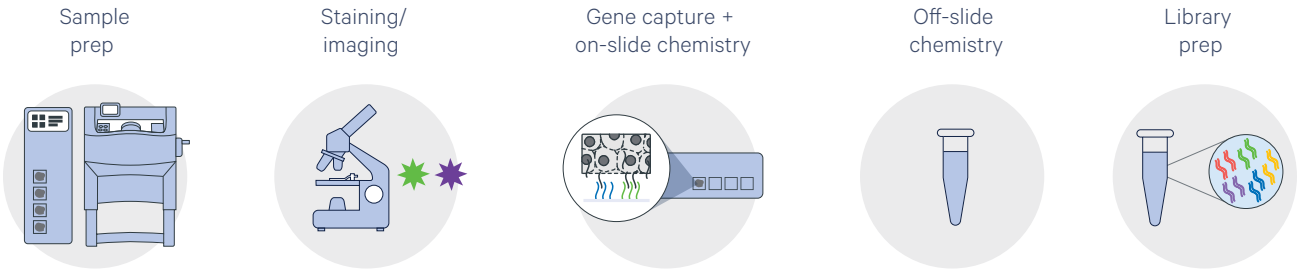



**Figure 2. Visium Spatial Gene Expression tissue compatibility.** The choice of which Visium Spatial workflow to use is dependent on what tissue samples are available to you. Currently, the FFPE assay is compatible with human and mouse tissues. The fresh frozen assay has been tested with human, mouse, rat, and zebrafish, and could be applied to other species. The fresh frozen assay is also compatible with 10x Genomics pre-designed targeted panels allowing you to focus on genes most relevant to your research. Whole transcriptome profiling can be performed on both fresh frozen and FFPE tissue samples.

# 03a Fresh frozen: What are the best practices for preparing and processing my samples?




**Sample prep: Tissue optimization and placement**

Best practices for handling fresh frozen tissues are provided in the fresh frozen Tissue Preparation Guide (CG000240). Since each tissue sample is unique, it is critical to optimize permeabilization conditions for fresh frozen samples using a Visium Spatial Tissue Optimization experiment (Tissue Optimization User Guide CG000238). You can find answers to common questions about sample prep on our FAQs page or view sample prep how-to videos for tips and tricks.

It is crucial that tissue sections are placed on the Visium slide so that they cover the Capture Areas but not the fiducial frames. We highly recommend practicing placement on plain glass slides. While it is possible to reset slides that have been improperly placed this may result in a slight decrease in sensitivity compared to a new slide. Instructions for reset of fresh frozen tissues are provided in the Visium Spatial Slide Reset Demonstrated Protocol (CG000332).

**Sample prep: RNA quality**

Getting high quality sequencing data is highly dependent on the quality of the mRNA transcripts obtained from your tissue sections. For fresh frozen tissues, an RNA integrity number (RIN) of ≧7 is recommended. More information on RNA quality assessment can be found in the fresh frozen Tissue Preparation Guide (CG000240).

**Staining/imaging**

Tissue sections can be stained with H&E (Demonstrated Protocol CG000160) for morphological analysis or immunostained with fluorescently labelled antibodies (Demonstrated Protocol CG000312) for protein co-detection. Successful visualization is highly dependent on good imaging practices and use of compatible reagents. For complete details on the recommended microscope specifications, please refer to the Visium Spatial Gene Expression Imaging Guidelines Technical Note (CG000241).

**Library prep: Sequencing requirements**

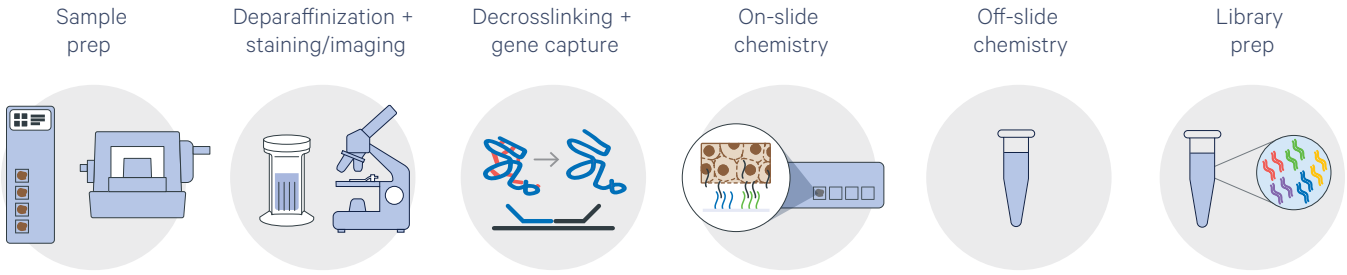
For libraries generated from fresh frozen tissues, a sequencing depth of 50K read pairs per capture spot covered by tissue is recommended. Additional sequencing requirements and performance data for Visium Spatial Gene Expression, can be found on our support site.

### Expedite your experiments by partnering with a Core Service Provider

Looking for help getting your experiments started? Accelerate your Visium Spatial Gene Expression project for fresh frozen or FFPE samples by working with a 10x Genomics Certified Service Provider to quickly and effectively get the results you need. Easily initiate cutting-edge research projects and overcome research roadblocks with our Certified Service Provider Program.

Find a provider near you ⟶

# 03b FFPE: What are the best practices for preparing and processing my samples?




**Sample prep: RNA quality**

Getting high quality sequencing data is highly dependent on the quality of the mRNA transcripts obtained from your tissue sections. RIN is not predictive of assay success in FFPE tissues so we recommend checking blocks for DV200, the percentage of RNA fragments >200 nucleotides. Blocks with DV200 ≧50% are recommended. More information on quality assessment can be found in the FFPE Tissue Preparation Guide (CG000408).

**Sample prep: Tissue adhesion and placement**

Adhesion of FFPE tissues to the surface of Visium Spatial Gene Expression slides may differ from other tissue-based assays. Factors that affect adhesion include fixation and embedding, tissue type, sectioning, and section placement. A Visium Tissue Section Test Slide (FFPE Tissue Preparation Guide CG000408) can be used to screen FFPE blocks for their adhesive properties on Visium slides.

Practicing placing tissues on Visium slides so they cover the Capture Area but not the fiducial frames is highly recommended, especially since slide reset is not supported in the FFPE workflow. You can find answers to common questions about sample prep on our FAQs page.

**Deparafinnization + staining/imaging**

Tissue sections can be stained with H&E (Demonstrated Protocol CG000409) for morphological analysis or immunostained with fluorescently labelled antibodies (Demonstrated Protocol CG000410) for protein co-detection. Successful visualization is highly dependent on good imaging practices and use of compatible reagents. For optimal image quality of FFPE samples, we recommend using a coverslip. For complete details on the recommended microscope specifications, please refer to the the Visium Spatial Gene Expression for FFPE Imaging Guidelines Technical Note (CG000436).

**Library prep: Sequencing requirements**

For libraries generated from FFPE tissues, a minimum sequencing depth of 25K raw reads per capture spot covered by tissue is sufficient for most samples. Additional sequencing requirements and performance data for Visium Spatial Gene Expression for FFPE, can be found on our support site.

# 04 How do I analyze and visualize my data?

During the Visium workflow, two main data types are captured: an H&E or IF tissue image and the sequencing data in FASTQ format. Both data outputs are first processed with Space Ranger analysis software. The results can then be interactively explored with Loupe Browser visualization software. Answers to common questions about Visium data analysis and visualization can be found on our software FAQs page. We also provide sample datasets that will enable you to become familiar with Space Ranger and Loupe Browser prior to starting your own Visium Spatial Gene Expression experiments.

**Space Ranger**

Space Ranger is a set of analysis pipelines that performs sample demultiplexing, image alignment, barcode processing, and gene counting using the Visium Spatial sequencing output and microscope images. It then assigns each detected gene transcript to a spatial location on the tissue image based on the associated spatial barcode. Details of the Space Ranger pipeline operations and instructions for use can be found on our support site.

**Loupe Browser**

Loupe Browser is a point-and-click software that provides interactive visualization functionality to analyze data. For Visium Spatial, it enables users to interrogate significant genes, characterize and refine of clusters, and perform differential expression analysis. Details on Loupe Browser pipeline operations and instructions for use can be found on our support site. You can also view tutorials covering navigation of the browser interface, spatial gene expression analysis capabilities, and manual slide alignment.

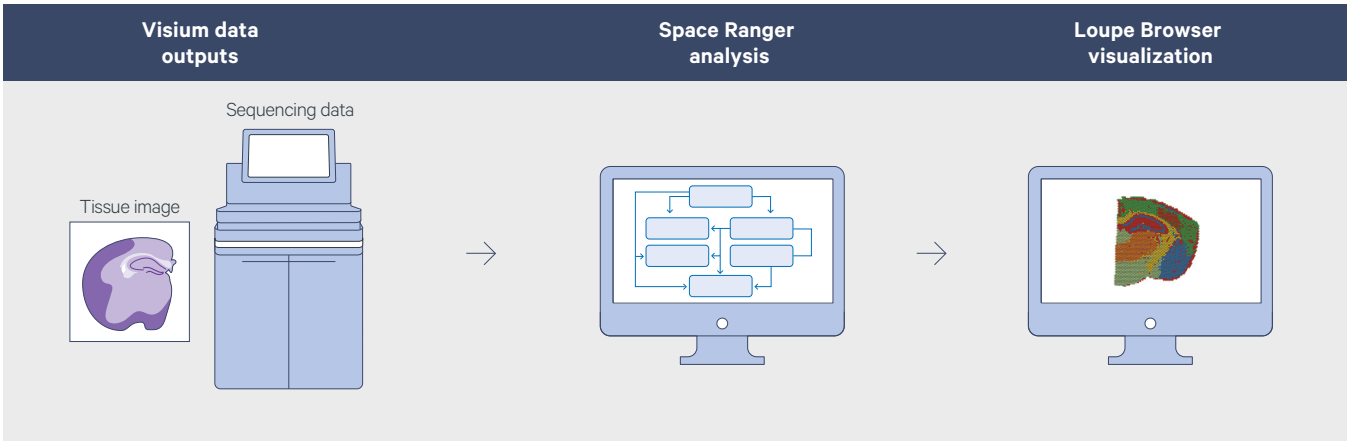



**Figure 3. Visium data analysis workflow.** During the Visium workflow, two main data types are captured: the tissue image and FASTQ files containing the gene expression library sequencing data. Our Space Ranger analysis software automatically overlays spatial gene expression information on your tissue image and identifies clusters of spots with similar transcription profiles. You can then use Loupe Browser visualization software to interactively explore the results.

### Building biological insights by uniting single cell and spatial technologies

Both single cell and spatial data, individually, provide powerful resolution to examine the true complexity of biology. However, the integration of these two analyses enables access to a more refined view of your tissue samples. Gain key insights into tissue function, the spatial relationships between cell types, how cells communicate, and neighboring cell interactions.

Discover how to integrate single cell and spatial data ⟶

# 05 What use case examples can I reference?

| Publications | Experiment snapshot | Aim | Impact |
|---|---|---|---|
| **Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity**<br>1. Berglund E, et al. *Nat Commun* 9: 2419, 2018. | **Research area:** Oncology<br>**Organism:** Human<br>**Tissue type:** Prostate adenocarcinoma | Examine tumor heterogeneity in human prostate cancer microenvironment, including stroma, normal and PIN glands, immune cells and cancer | Generated first-ever spatial gene expression map of prostate cancer. Found distinct cancer expression regions can occur beyond annotated tumor boundaries, indicating this method may be useful for predicting future regions of potential cancer. |
| **Spatial transcriptomics and in situ sequencing to study Alzheimer's disease**<br>2. Chen WT, et al. *Cell* 182(4): 976–991.e19, 2020. | **Research area:** Neuroscience<br>**Organism:** Mouse and human<br>**Tissue type:** Brain | Identify pathways involving dysregulated non-neuronal brain cells in Alzheimer's disease pathogenesis | Characterized two gene co-expression networks resulting from Aβ deposits that span microglia, astroglia, and oligodendrocytes. Implicates non-neuronal cell types and gene pathways as possible drug targets for Alzheimer's disease. |
| **Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex**<br>3. Maynard KR, et al. *Nat Neurosci* 24: 425–436, 2021. | **Research area:** Neuroscience<br>**Organism:** Human<br>**Tissue type:** Brain | Characterize the laminar topography of the six-layered human dorsolateral prefrontal cortex, an area implicated in a number of neuropsychiatric disorders | Identified layer-enriched expression signatures, including genes associated with schizophrenia and autism spectrum disorder, and refined layer-specific associations to known laminar markers. |
| **The orchestrated cellular and molecular responses of the kidney to endotoxin define a precise sepsis timeline**<br>4. Janosevic D, et al. *eLife* 10: e62270, 2021. | **Research area:** Immunology<br>**Organism:** Mouse<br>**Tissue type:** Kidney | Examine the spatial and temporal progression of endotoxin injury to the kidney as a result of sepsis | Provided the first-ever description of spatial and temporal endotoxin-induced transcriptomic changes in the kidney, from early injury to recovery. Determined global cell–cell communication failure may underlie kidney shutdown. |
| **Exuberant fibroblast activity compromises lung function via ADAMTS4**<br>5. Boyd DF, et al. *Nature* 587: 466–471, 2020. | **Research area:** Immunology<br>**Organism:** Mouse<br>**Tissue type:** Lung | Define non-immune structures in the influenza-infected lung that contribute to acute respiratory distress syndrome | Atlased non-immune cells active in infected lung, including previously uncharacterized inflammatory fibroblast populations. Discovered a novel role for ADAMTS4, an enzyme involved in versican degradation, in driving infection severity through modulation of T-cell infiltration into lung tissue. |
| **Spatiotemporal analysis of human intestinal development at single-cell resolution**<br>6. Fawkner-Corbett D, et al. *Cell* 184: 810–826.e23, 2021. | **Research area:** Developmental biology<br>**Organism:** Human<br>**Tissue type:** Intestine and colon | Chart the development of the human intestine to describe origins of key tissue structures and cell types | Tracked epithelial cell differentiation during crypt-villus formation, noting prevalence of immature epithelia may contribute to necrotizing enterocolitis in premature infants. Defined specific cell types and time points of expression for genes linked to genetic defects that are challenging to study in utero. |
| **Spatiotemporal single-cell RNA sequencing of developing chicken hearts identifies interplay between cellular differentiation and morphogenesis**<br>7. Mantri M, et al. *Nat Commun* 12: 1771, 2021. | **Research area:** Developmental biology<br>**Organism:** Chicken<br>**Tissue type:** Heart | Understand the steps of development for the chicken heart from early to late four-chambered heart stage | Mapped the spatial organization and interactions of diverse cellular lineages throughout development. Identified anatomically restricted gene expression programs for genes implicated in congenital heart disease. |

# 06 How do I prepare my lab?

The table below summarizes the key tissue processing equipment and reagents. For a comprehensive list, please refer to the Visium Spatial Gene Expression Protocol Planner (CG000295) and Visium Spatial Gene Expression for FFPE – Protocol Planner (CG000404).

| | |  Fresh frozen |  FFPE |
|---|---|---|---|
| **Sample prep** | Microtome | | ✓ |
| | Cryostat | ✓ | |
| | Methanol for fixation | ✓ | |
| **Staining and imaging** | Xylene | | ✓ |
| | Brightfield imaging system | ✓ | ✓ |
| | Fluorescence imaging system | ✓ | ✓ |
| | Hematoxylin and eosin solutions | ✓ | ✓ |
| | Tris-EDTA for decrosslinking | | ✓ |
| | IF-compatible antibodies | ✓ | ✓ |
| **Off-side chemistry and library construction** | Real-time qPCR system | ✓ | ✓ |
| | Automated electrophoresis system | ✓ | ✓ |
| | Thermal cycler | ✓ | ✓ |

# Resources

Product information
10xgenomics.com/spatial-gene-expression

Spatial gene expression support
support.10xgenomics.com

Spatial gene expression software
support.10xgenomics.com/spatial-gene-expression/software

**Contact us**
**10xgenomics.com | info@10xgenomics.com**


LIT000138- Rev A - The essential guide: Visium spatial profiling Experiment planning resources

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