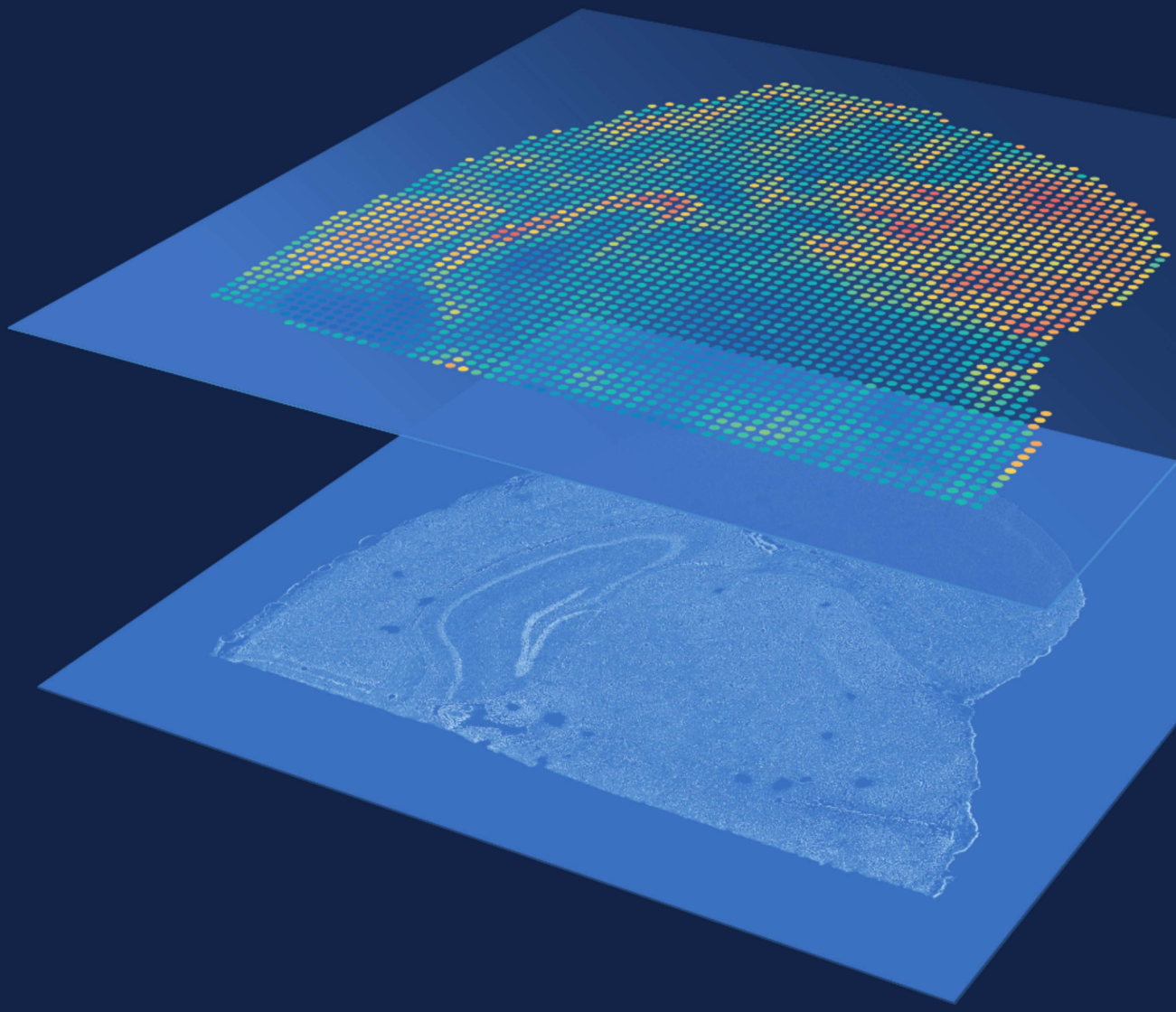
1

Inside Visium spatial capture technology



10xgenomics.com/spatial-gene-expression

The power of two: Mapping and measuring gene expression

Visium Spatial Gene Expression incorporates whole transcriptome analysis for intact tissues sections with morphological context. Bringing these complementary methods together offers a previously inaccessible view of tissue biology.

More than a tissue slide

Map the spatial gene expression of complex tissue samples with slides that utilize poly(A) capture and novel spatial barcoding technology for library preparation.

Visium Spatial Gene Expression slides are powered by spatially barcoded mRNA-binding oligonucleotides (Figure 1). To capture gene expression information in fresh frozen tissue, mRNA is released from processed tissue sections allowing it to bind to capture oligos from a proximal location on the tissue. Reverse transcription occurs while the tissue is still in place, generating a cDNA library that incorporates complements of the spatial barcodes and preserves spatial information. To capture gene expression in formalin-fixed paraffin-embedded (FFPE) tissue, the tissue is permeabilized to release ligated probe pairs which bind to capture probes on the slide. The probe pairs are extended to generate a library that incorporates complements of the spatial barcodes and preserves spatial information.

Barcoded libraries are mapped back to a specific spot on the Capture Area. This gene expression data is subsequently layered over a high-resolution microscope image of the tissue section, making it possible to visualize the expression of any mRNA, or combination of mRNAs, within the morphology of the tissue in a spatially resolved manner.

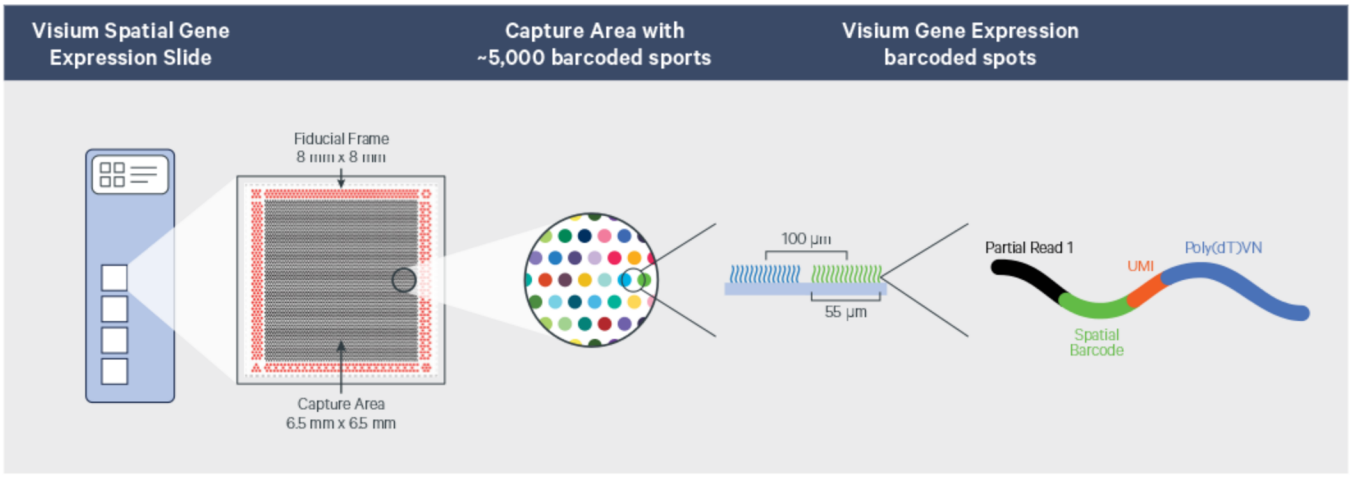


Figure 1. Composition of Visium Spatial Gene Expression Slide. Each slide can contain either two or four Capture Areas with approximately 5,000 barcoded spots, containing millions of spatially barcoded capture oligos. Released tissue mRNA binds to these oligos, enabling capture of gene expression information.

Efficient workflow: Utilize standard laboratory methods

The Visium Spatial Gene Expression workflow makes it easy to implement spatial transcriptomics technology into standard tissue sectioning and staining methods.

Streamlined, ready-to-use assay

Prepare your sample
Embed, section, and place fresh frozen or FFPE tissue onto a Capture Area of the gene expression slide.

Stain and image the tissue
Utilize standard fixation and staining techniques, including hematoxylin and eosin (H&E) staining, to visualize tissue sections on slides using a brightfield microscope or immunofluorescence (IF) staining to visualize protein detection in tissue sections on slides using a fluorescence microscope.

Permeabilize tissue and construct library
Permeabilize the tissue to release mRNA (fresh frozen) or ligated probe pairs (FFPE) from the cells, which bind to the spatially barcoded oligonucleotides present on the Capture Area. Complements of the spatial barcodes are added via a reverse transcription reaction producing cDNA (for fresh frozen tissues) or an extension reaction (for FFPE tissues). The barcoded molecules are then pooled for downstream processing to complete a sequencing-ready library.

Sequence
The resulting 10x barcoded library is compatible with standard NGS short-read sequencing on Illumina sequencers for massive transcriptional profiling of entire tissue sections.

Analyze and visualize your data
Use our Space Ranger analysis software to process your spatial gene expression data and interactively explore the results with our Loupe Browser visualization software.

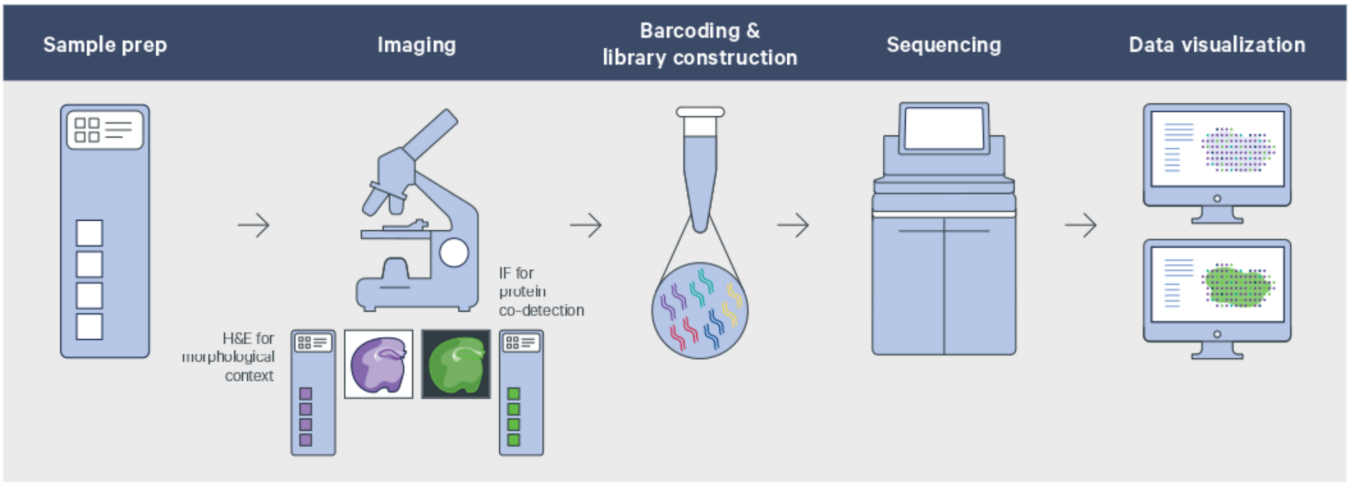


Figure 2. Workflow diagram for Visium Spatial Gene Expression. Fresh frozen or FFPE tissue is sectioned, placed onto a library preparation slide, then fixed, stained with either H&E or immunofluorescence (IF), and imaged, followed by spatial barcoding and library construction. The libraries are then sequenced and data visualized.

Library construction for fresh frozen tissues: How it works

Visium Spatial Gene Expression for fresh frozen tissues utilizes a poly(A) capture method for unbiased detection of the whole transcriptome in a variety of species.

Stepwise library construction

Staining and imaging

Fresh frozen tissue samples are sectioned and placed in the Capture Areas on the Visium Spatial Gene Expression slide. Utilizing standard fixation and staining techniques, including H&E or immunofluorescence staining, tissue sections are visualized.

Permeabilization

The tissue is then permeabilized to release mRNA from the cells. mRNA binds with spatially barcoded oligonucleotides present on the Capture Area. A reverse transcription reaction produces cDNA from captured mRNA.

cDNA synthesis

The second strand of cDNA is then synthesized and denatured. Note, if you're using a fresh frozen tissue for the first time with the Visium solution, you will need to perform tissue optimization beforehand. The barcoded cDNA is then pooled for downstream processing, library preparation, and cDNA amplification. Subsequent steps are taken to fragment and process cDNA to complete a sequencing-ready library. This is followed by a final sample index PCR. The Visium Spatial Gene Expression library is sequenced using standard short-read sequencers, and data is processed and visualized using 10x Genomics software: Space Ranger analysis pipelines and Loupe Browser visualization software.

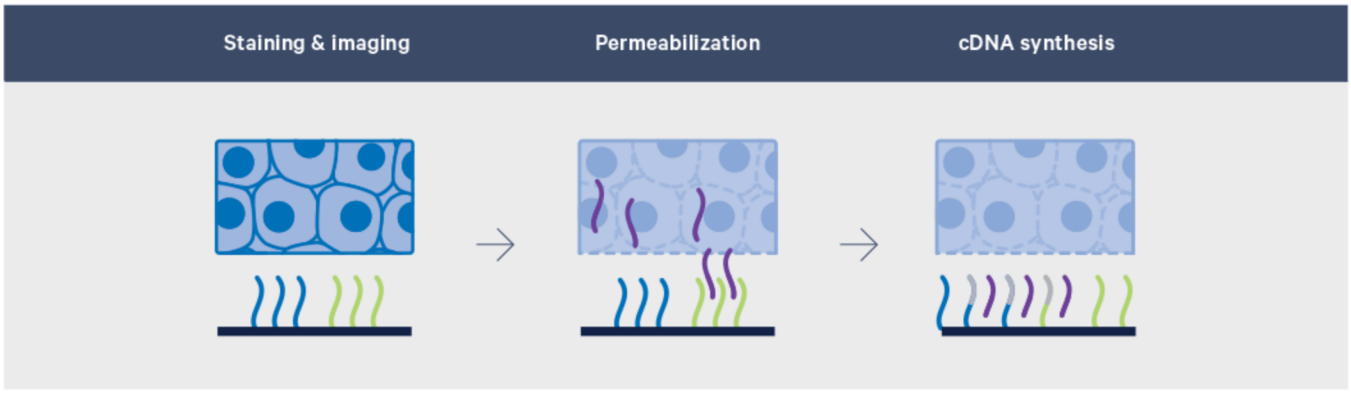


Figure 3. Constructing a sequencing library with Visium Spatial Gene Expression. When ready for library construction, the fresh frozen tissue on the Capture Areas is permeabilized to release mRNA that binds to spatially barcoded capture probes, allowing for the capture of gene expression information. cDNA is synthesized from captured mRNA and then washed off the slide before sequencing library construction. Whole transcriptome or targeted gene expression libraries are then sequenced.

Library construction for FFPE tissues: How it works

Visium Spatial Gene Expression for FFPE tissues utilizes RNA-templated ligation of gene target probe pairs for highly specific and sensitive detection of the whole transcriptome in human and mouse FFPE tissue sections.

Stepwise library construction

Staining and imaging

FFPE tissues are sectioned and placed in the Capture Areas on the Visium Spatial Gene Expression slide. Utilizing standard staining techniques, including H&E or immunofluorescence staining, tissue sections are visualized on the slide.

Probe hybridization & ligation

The tissue is then de-crosslinked to release mRNA that was sequestered by formalin fixation. Human or mouse whole transcriptome probe panels, consisting of a pair of specific probes for each targeted gene, are then added to the tissue. These probe pairs hybridize to their gene target and are then ligated to one another.

RNase treatment and permeabilization

The ligation products are released from the tissue upon RNase treatment and permeabilization. The ligated probe pairs bind with spatially barcoded oligonucleotides present on the Capture Area.

Probe extension

The probe pairs are extended to incorporate complements of the spatial barcodes. The spatially barcoded, ligated probe products are released from the slide and PCR amplified. The probe products are further processed to produce a sequencing library. The Visium Spatial Gene Expression library is sequenced using standard short-read sequencers, and data is processed and visualized using 10x Genomics software: Space Ranger analysis pipelines and Loupe Browser visualization software.

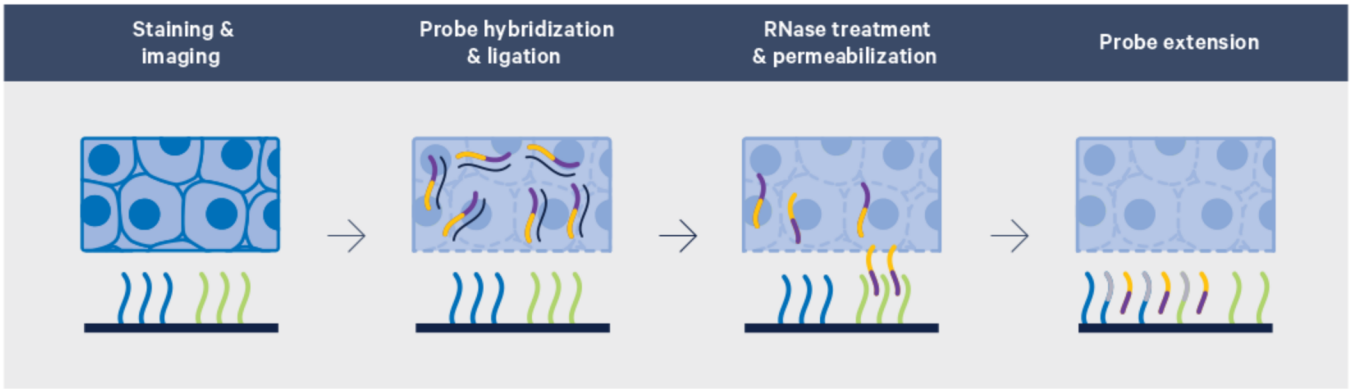


Figure 4. Constructing a sequencing library with Visium Spatial Gene Expression for FFPE. When ready for library construction, the FFPE tissue on the Capture Areas is permeabilized to release ligated probe pairs to bind to capture probes on the slide, allowing for the capture of gene expression information. The probe pairs are extended to incorporate complements of the spatial barcodes and sequencing libraries are prepared.

Data analysis:
Visualize gene expression where it occurs

Analyze and understand gene and protein expression heterogeneity with Space Ranger analysis software to process your data, then interactively explore the results with Loupe Browser visualization software.

Streamlined data analysis

The data generated by Visium Spatial Gene Expression allows you to choose any gene of interest and display its spatially resolved expression on the original tissue section. Since the whole transcriptome is profiled, you are not limited to visualizing only a single gene but can choose any number of genes in any combination to view and analyze together. Furthermore, because gene expression data is captured for the entire section, you can revisit and re-explore different areas of the section for additional insights whenever you want.

During the Visium workflow, two main data types are captured: the tissue image and the sequencing data in FASTQ format. The Space Ranger analysis pipelines use these two data inputs to align the Visium sequencing data with the image by assigning each detected gene transcript captured during the Visium workflow to a spatial location on the tissue image based on the associated spatial barcode. Once the data is processed, you can easily interrogate different views of your spatial gene expression data with Loupe Browser visualization software. Loupe Browser allows you to interrogate significant genes, characterize and refine clusters, and perform differential expression analysis. Alternatively, you can process your data further with third-party tools.

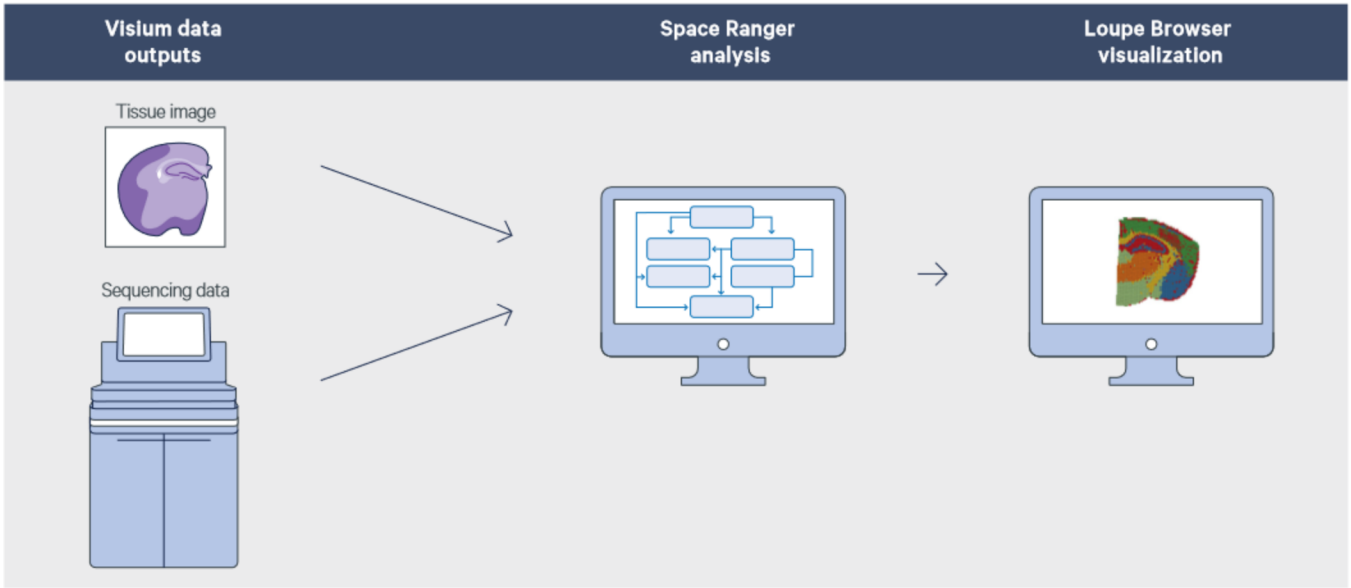


Figure 5. Visium data analysis workflow. During the Visium workflow, two main data types are captured: the tissue image and FASTQ files containing the gene expression library sequencing data. Our Space Ranger analysis software automatically overlays spatial gene expression information on your tissue image and identifies clusters of spots with similar transcription profiles. You can then use Loupe Browser visualization software to interactively explore the results.

Gain additional perspectives: Spatially resolve multiple analytes or targeted genes

Combine whole transcriptome spatial analysis with immunofluorescence protein detection or the ease and breadth of targeted panels to elevate your understanding of human health and disease with a more comprehensive picture of biology captured on a tissue slide.

Co-detect protein

Visualize spatial patterns of gene expression together with protein detection by IF on the same tissue section. The Visium Spatial Gene Expression with Immunofluorescence workflow allows simple incorporation into standard tissue sectioning and IF staining methods using your current antibodies. Get the most out of precious samples by combining protein, total mRNA, and histological analyses on the same tissue section.

A. Immunofluorescence (IF)

B. Visium gene expression

C. IF + Visium gene expression

D. IF + Visium whole transcriptome

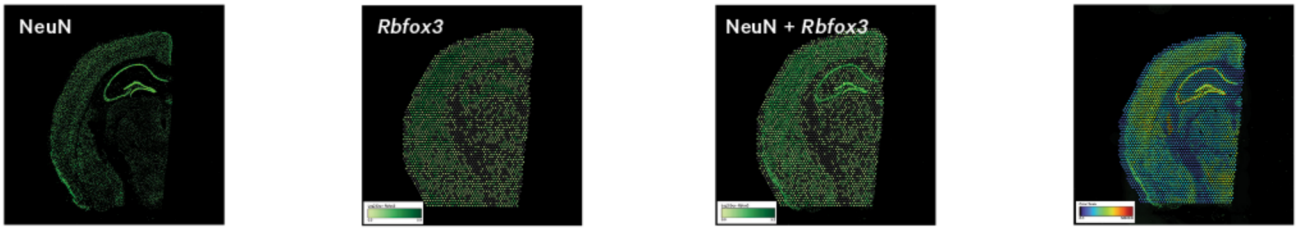


Figure 6. Co-detection of protein and RNA using Visium Spatial Gene Expression with Immunofluorescence. A coronal mouse brain section was stained for NeuN by IF, imaged, and processed through the Visium Spatial Gene Expression workflow. Shown left to right: (**A**) NeuN IF, (**B**) Visium Spatial mRNA expression of *Rbfox3* (gene encoding NeuN), (**C**) overlay of NeuN IF image and Visium Spatial *Rbfox3* mRNA data, and (**D**) NeuN IF image overlaid with total UMI count data obtained by the Visium workflow.

Focus on the genes that matter most

Comprehensively targeting relevant genes lets you view the biology most important to your research. Compatible with Visium Spatial Gene Expression for fresh frozen tissue, profile a defined set of transcripts with pre-designed cancer, immunology, neuroscience, and gene signature panels. Select one of our comprehensive, pre-designed panels, with the option to add up to 200 custom genes, to enrich your Visium Spatial library prior to sequencing.

A. H&E

B. H&E with locomotory behavior genes

C. H&E + *SOD1*

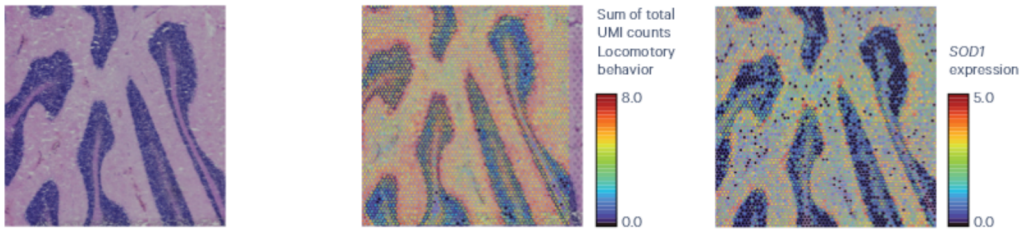


Figure 7. Spatially resolved targeted gene expression profiling with Visium Spatial solutions. A human cerebellum tissue section was H&E stained and processed using the Visium Spatial Gene Expression workflow, then enriched using Targeted Gene Expression with the Human Neuroscience Panel. Shown are (**A**) the H&E image, (**B**) H&E image overlaid with total UMI counts for 36 locomotory behavior genes from the neuroscience panel, and (**C**) H&E image overlaid with *SOD1* expression level.

Publications

1. Ståhl PL, et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science* 353: 78–82, 2016. doi: 10.1126/science.aaf2403
2. Ji AL, et al. Multimodal analysis of composition and spatial architecture in human squamous cell carcinoma. *Cell* 182: 497–514.e22, 2020. doi: 10.1016/j.cell.2020.05.039
3. Maynard KR, et al. Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex. *Nat Neurosci* 24: 425–436, 2021. doi: 10.1038/s41593-020-00787-0
4. Boyd DF, et al. Exuberant fibroblast activity compromises lung function via ADAMTS4. *Nature* 587: 466–471, 2020. doi: 10.1038/s41586-020-2877-5
5. Fawkner-Corbett D, et al. Spatiotemporal analysis of human intestinal development at single-cell resolution. *Cell* 184: 810–826.e23, 2021. doi: 10.1016/j.cell.2020.12.016
6. Janosevic D, et al. The orchestrated cellular and molecular responses of the kidney to endotoxin define a precise sepsis timeline. *eLife* 10: e62270, 2021. doi: 10.7554/eLife.62270
7. Grauel AL, et al. TGFβ-blockade uncovers stromal plasticity in tumors by revealing the existence of a subset of interferon-licensed fibroblasts. *Nat Commun* 11: 6315, 2020. doi: 10.1038/s41467-020-19920-5
8. Chen WT, et al. Spatial transcriptomics and in situ sequencing to study Alzheimer's disease. *Cell* 182: 976–991.e19, 2020. doi: 10.1016/j.cell.2020.06.038

Resources

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

Technology overview
10xgenomics.com/spatial-transcriptomics

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

10x GENOMICS | Authorized Distributor

millennium® science New Zealand
Email: sales@mscience.co.nz
Ph: 0800 MSCIENCE (672 436)

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

LIT000060 - Rev D - Brochure - Inside Visium Spatial Technology

10x GENOMICS