Grant application resources for Visium Spatial Gene Expression

Summary statement

Visium Spatial Gene Expression from 10x Genomics is a novel assay that combines histology with spatially resolved whole transcriptome gene expression profiling to localize and quantify gene expression in the tissue context. It is based on the Spatial Transcriptomics methodology (1). The assay has been well adopted, being utilized in almost 40 peer-reviewed publications and over 50 pre-prints. Currently, Visium Spatial Gene Expression is compatible with fresh frozen tissue sections from any species. This assay utilizes poly(A) capture and novel spatial barcoding technology for library preparation. 10x Genomics also offers a Visium Spatial Gene Expression assay compatible with human and mouse formalin-fixed paraffin-embedded (FFPE) tissue sections. This assay utilizes RNA-templated ligation of pairs of gene target probes for highly specific and sensitive detection of the whole transcriptome. Both assays leverage the same suite of analysis tools and pipelines (e.g., Space Ranger, Loupe Browser) to process and visualize Visium Spatial data. Additionally, researchers have access to 10x Genomics technical experts who can provide support through scientific and technical consultations, workflow optimization, and methodology troubleshooting.

Overview

The ability to detect and count transcripts by sequencing (RNA-seq) has led to significant advances in our understanding of biology (2), as well as the development of clinical applications. However, traditional RNA-seq suffers from the loss of spatial information. Researchers typically extract RNA from tissue and sequence it in bulk. Data regarding the type of cells expressing a given transcript, the location of these cells within the tissue, and co-expression of transcripts in the tissue geography are lost by this bulk preparation of RNA. Alternatively, researchers can study gene expression from dissociated cells, however, the location of individual cells within the tissue architecture is also lost with this methodology.

10x Genomics has developed a workflow for sequencing mRNA without the loss of spatial information. The Visium Spatial Gene Expression solution allows for the analysis of mRNA using high-throughput sequencing and subsequently maps gene expression patterns in entire tissue sections using high-resolution microscope imaging. The workflow surveys global spatial gene expression in tissue sections, giving researchers the ability to profile the whole transcriptome and a defined set of transcripts via targeted gene panels.

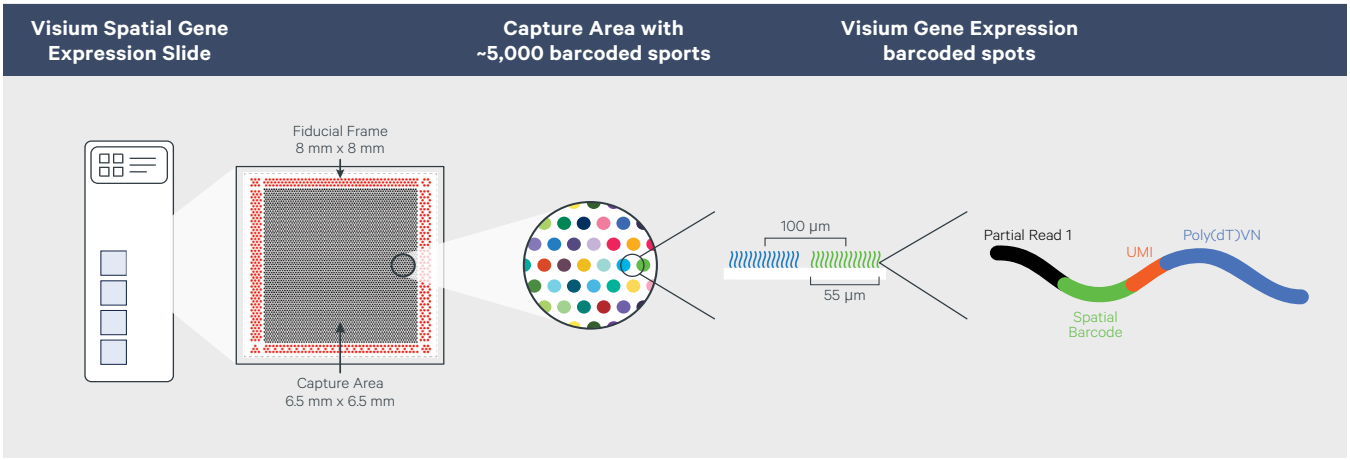
Visium Spatial Gene Expression, commercialized in 2019 and built on the foundation of earlier Spatial Transcriptomics technology (1), has been used in groundbreaking papers that demonstrate the breadth of its applications, including cancer (3,4), neuroscience (5), immunology (6), and developmental biology (7).

Visium Spatial Gene Expression solution

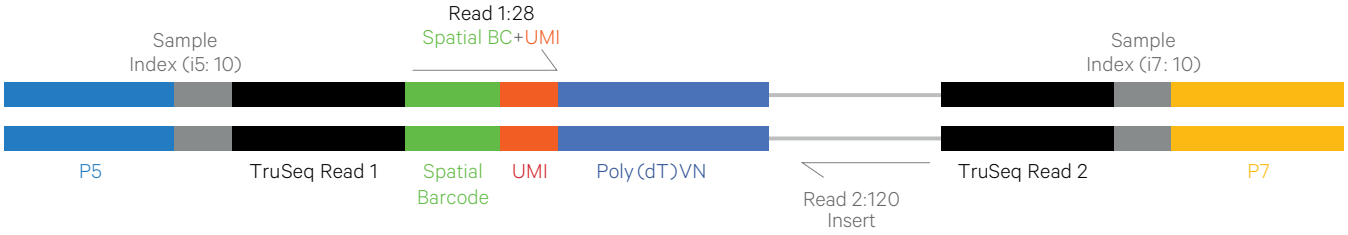
The Visium Spatial Gene Expression workflow allows for whole transcriptome (fresh frozen and FFPE) and targeted analysis (fresh frozen) without the loss of spatial information. This provides gene expression data within the context of tissue architecture, tissue microenvironments, and cell groups.

Spatial capture technology

Visium Spatial Gene Expression relies on the use of Visium slides, each of which has two to four Capture Areas. Each Capture Area is arrayed with ~5,000 capture spots, each containing millions of oligonucleotides with the following features: a 30 nucleotide poly(dT) sequence for the capture of polyadenylated mRNA molecules; a 12 nucleotide unique molecular identifier (UMI) for the identification of duplicate molecules that arise during the library preparation and sequencing process; a 16 nucleotide Spatial Barcode, which is shared by all oligonucleotides within each individual gene expression spot; and a partial TruSeq Read 1 sequence, for use during the library preparation and sequencing portions of the workflow.



Visium Spatial Gene Expression library



Visium Spatial Gene Expression. A Visium Spatial Gene Expression library comprises standard Illumina paired-end constructs which begin and end with P5 and P7. The Visium Spatial Gene Expression Slide includes two to four Capture Areas, each defined by a fiducial frame. Each Capture Area has ~5,000 gene expression spots, each containing millions of oligonucleotides that include a TruSeq Read 1 sequence, Spatial Barcode, UMI, and poly(dT) sequence.

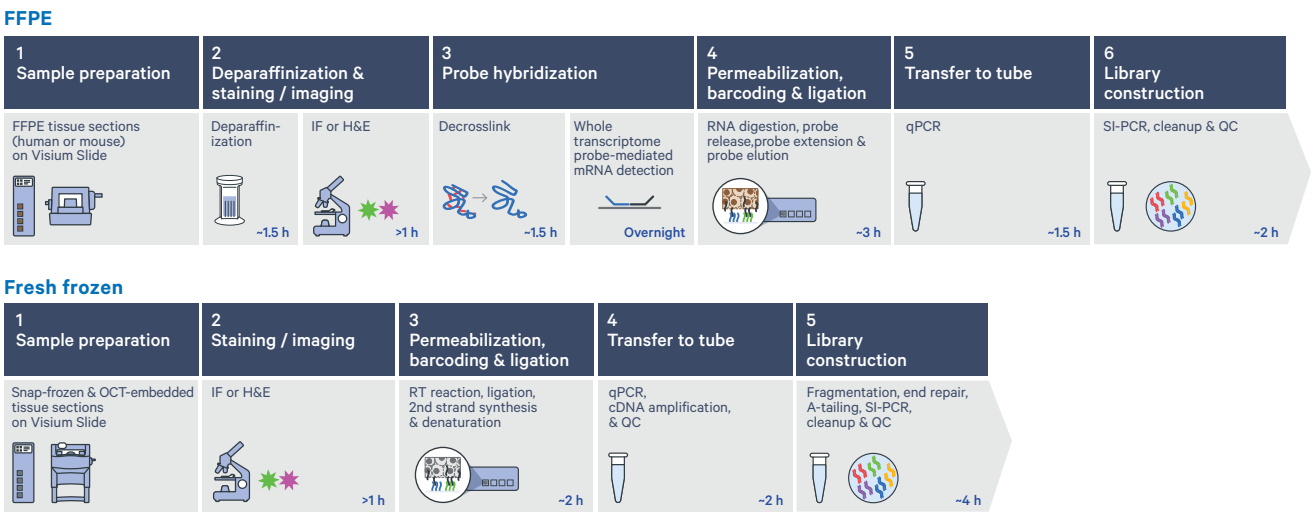
Visium for fresh frozen tissues

Fresh frozen sectioned tissues are placed on each Capture Area of the slide, where they are fixed and stained using either hematoxylin and eosin (H&E) or fluorescently tagged antibodies. Each section is then imaged using the appropriate microscopy technique, the results of which are ultimately used to overlay gene expression patterns onto the image. The tissue sections are then permeabilized, and the mRNA molecules within cells are captured by the poly(dT) sequence on the slide surface. The captured molecule is reverse transcribed by extending the oligo bound on the slide surface, thereby creating a cDNA molecule with a complement of the Spatial Barcode sequence and UMI covalently attached to the slide. The captured mRNA molecule is denatured and removed, which allows for a second-strand copy to be synthesized. The newly synthesized second strand is denatured and PCR amplified using common sequences. The cDNA is further processed into a sequencing library through enzymatic fragmentation, end repair, ligation of sequencing adapters, and enrichment of sequenceable molecules using sample barcoded primers targeting the adapter ends. The final library is sequenced at a recommended depth of 50K read pairs per capture spot covered by tissue.

Visium for FFPE tissues

FFPE-sectioned tissues are placed on each Capture Area of the slide, where they are deparaffinized and stained using either H&E or fluorescently tagged antibodies. Each section is then imaged using the appropriate microscopy technique, the results of which are ultimately used to overlay gene expression patterns onto the image. Stained tissue is then de-crosslinked to release RNA that was sequestered by formalin fixation. Human or mouse whole transcriptome probe panels, consisting of a pair of specific probes for each targeted gene in the transcriptome, are then added to the tissue to capture the free mRNA targets. Together, probe pairs are complementary to a stretch of their target RNA and hybridize to the complementary target RNA. After hybridization, a ligase is added to seal the junction between the probe pairs that have hybridized to RNA, forming a ligation product. The single-stranded ligation products are released from the tissue upon RNase treatment and permeabilization and then captured on the Visium slides. Once ligation products are captured, probes are extended by a polymerase, thereby creating ligated probe products that incorporate a complement of the Spatial Barcode sequence and UMI. The spatially barcoded, ligated probe products are released from the slide and PCR amplified using common sample-indexing primers. The final library is sequenced at a recommended depth of 25k raw reads per capture spot covered by tissue in most FFPE samples. However, for some samples fewer reads will be sufficient, while for more complex samples more reads may be required.

Overview of the Visium Spatial Gene Expression workflows.

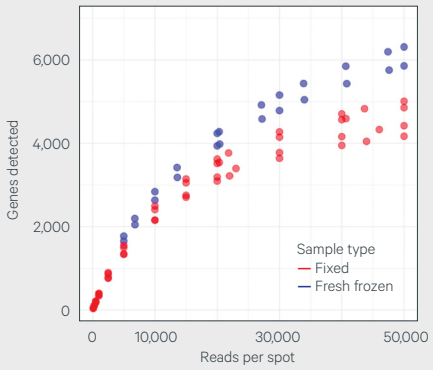


Data analysis

During the Visium workflow, two main data types are captured: a tissue image and sequencing data. 10x Genomics provides two software tools to analyze these Visium data types, Space Ranger and Loupe Browser. Space Ranger processes the input file types to align the Visium sequencing data with the image. Each Spatial Barcode with the associated UMIs captured during the Visium workflow is assigned a spatial location in the tissue image. Space Ranger produces a variety of output files that can be used in Loupe Browser or third-party tools.

Data benchmarking

The Spatial Transcriptomics assay, on which Visium is based, has been validated using laser capture microdissection as well as single molecule fluorescence in situ hybridization (ISH) (2). Comparison to data generated for the Allen Brain Atlas using ISH determined that Spatial Transcriptomics can detect twice as many transcripts (Figure S5 from Ref. 2). Spatial Transcriptomics studies examining gene expression among tissue replicates have found very high reproducibility (r = 0.97; Figure S3, Panel E from Ref. 2). High reproducibility was also observed when compared to RNA in solution (r = 0.94, Figure S3D from Ref. 2).



Comparable sensitivity between Visium for FFPE tissues and Visium for fresh frozen tissues. Expression profiles from human triple-positive breast cancer processed using the Visium for FFPE assay highly correlated with data from the same tumor processed using the Visium for fresh frozen tissue assay.

Applications

Visium Spatial Gene Expression is tissue and species agnostic, allowing for its use in numerous applications in both healthy and diseased tissues. Among many applications, the technology in its current and previous versions has been used to examine:

- Tumor heterogeneity in human prostate cancer (3)
- Spatial architecture in human squamous cell carcinoma (4)
- Spatial topography of the human dorsolateral prefrontal cortex, an area implicated in a number of neuropsychiatric disorders (5)
- Anatomical organization of the fibroblast response to influenza (6)
- Spatiotemporal analysis of human intestinal development (7)

Justification for using Visium Spatial Gene Expression for your research

Visium Spatial Gene Expression offers many advantages, making it an optimal product for spatial transcriptomics. These include:

- **Spatially resolved whole transcriptome detection**—Visium Spatial Gene Expression for fresh frozen tissues captures polyadenylated mRNA molecules, reducing the bias introduced by targeted amplicon sequencing or sequence-specific hybridization techniques. Visium Spatial Gene Expression for FFPE tissues utilizes RNA-templated ligation of pairs of gene target probes for highly specific and sensitive detection of the whole transcriptome in human and mouse FFPE tissue sections.
- **Demonstrated technology**—The Visium Spatial Gene Expression solution has been used as a cornerstone technology in many peer-reviewed papers in high-caliber journals, including *Science*, *Cell*, *Nature Neuroscience*, *Nature Communications*, and *Nature Protocols*.
- **No specialized infrastructure**—Visium Spatial Gene Expression does not require any specialized equipment and can be adopted into labs or core facilities that have standard sectioning equipment, microscopes with image capture capability, and sequencing instruments.
- **Comprehensive data analysis solution**—Visium Spatial Gene Expression includes a data analysis pipeline as well as state-of-the-art software for data visualization. The latter is compatible with most desktop computers and includes tools for differential gene expression analysis.
- **High reproducibility and sensitivity**—Publications using Visium's core technology have determined that data reproducibility between adjacent tissue sections is r = 0.97 (2). Comparison between sequenced mRNA from the Visium workflow and mRNA from traditional RNA-seq found that 95% of transcripts can be found in both assays, highlighting the workflow's sensitivity of detection.
- **High spatial resolution**—Each Capture Area on the Visium slide contains ~5,000 barcoded mRNA gene expression spots, with an average of 1–10 cells captured per spot depending on tissue type. Each Capture Area on the Visium slide is 6.5 x 6.5 mm, providing the flexibility to study many different organisms and tissue types.
- **Optimized conditions for numerous tissues**—The Visium Spatial Gene Expression workflow for fresh frozen tissue has been optimized for healthy and diseased tissues in diverse organisms, including human, mouse, rat, and zebrafish. For an up-to-date list of fresh frozen tissues optimized for the Visium assay, please visit our support website. The Visium Spatial Gene Expression workflow for FFPE tissues does not require individual tissue optimization and has been tested in a number of human and mouse healthy and diseased tissues.
- **Broad support resources**—10x Genomics provides comprehensive support resources, ranging from its technical specialists trained in Visium Spatial Gene Expression to freely available videos and documents that guide new users through the Visium workflow.
- **Certified Service Providers**—Get streamlined access to the complete Visium Spatial Gene Expression workflow through Certified Service Providers, third-party facilities specially trained and verified by 10x Genomics to support spatial gene expression analysis research projects.
- **Certified product quality**—10x Genomics product development and manufacturing processes are ISO 9001:2015 certified.
- **Flexible options for large datasets or proof-of-concept studies**—10x Genomics offers introductory Gateway slides, which enable you to experience the wealth of data provided by spatial transcriptomics on two tissue samples to see how the technology will benefit their research. This gives researchers the flexibility to carry out their proof-of-concept studies without wasting resources. For larger datasets, single and four-slide Visium kits are available, representing four and sixteen samples, respectively.

Product specifications

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. Stark R, Grzelak M and Hadfield J. RNA sequencing: The teenage years. *Nat Rev Genet* 20: 631–656, 2019. doi: 10.1038/s41576-019-0150-2
3. Berglund E, et al. Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity. *Nat Commun* 9: 2419, 2018. doi: 10.1038/s41467-018-04724-5
4. 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
5. 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
6. Boyd DF, et al. Exuberant fibroblast activity compromises lung function via ADAMTS4. *Nature* 587: 466–471, 2020. doi: 10.1038/s41586-020-2877-5
7. 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

Publications

The utility of Visium Spatial Gene Expression is demonstrated in numerous peer-reviewed publications, many in top journals. Visit 10xgenomics.com/publications to see the most current list of Visium publications.

Resources

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

Technology overview
10xgenomics.com/spatial-transcriptomics

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

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