TECHNICAL NOTE
SPATIAL TRANSCRIPTOMICS

Resolve the whole transcriptome within tissue architecture

- Leverage Visium Spatial Gene Expression from 10x Genomics for transcriptional profiling of entire tissue sections
- Sequence spatial RNA-Seq libraries on the NovaSeq™ 6000, NextSeq™ 2000, NextSeq 1000, or NextSeq 550 System
- Visualize tissue morphology overlaid with gene activity to understand the spatial relationship between cells within normal and diseased tissues

In collaboration with

10x GENOMICS

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Introduction

To understand the fundamentals of development and disease, researchers need to unlock the architecture of complex, multicellular tissues. Histology and immunofluorescence reveal tissue morphology and biomarkers. Spatial RNA sequencing (RNA-Seq) uses the power of next-generation sequencing (NGS) to profile gene expression with spatial resolution.[1]

Spatial transcriptomics experiments combine molecular and morphological characterization to provide a previously inaccessible view of tissue biology. Tissue-wide whole-transcriptome analysis can enable true exploratory science and help reveal the spatial relationships between cells in both healthy and disease states.[2-7]

This technical note outlines a protocol to map the full transcriptome from tissue sections while retaining morphological context.

Protocol overview

A typical spatial RNA-Seq experiment follows a workflow of tissue sample preparation, imaging, library prep, sequencing, and analysis (Figure 1). The protocol leverages Visium Spatial Gene Expression from 10x Genomics and proven sequencing power from Illumina. Begin with fresh frozen or formalin-fixed, paraffin-embedded (FFPE) tissue, sectioned onto a Visium glass slide. With your stained tissue section, use the Visium reagents to generate a barcoded, spatially aware, whole-transcriptome library. Sequence the library on an Illumina production-scale sequencing system, such as the NovaSeq 6000, NextSeq 2000, NextSeq 1000, or NextSeq 550 System. Data analysis with the Space Ranger pipeline (10x Genomics) links transcriptomic gene expression results to the tissue location with the help of the spatial barcodes from the Visium slide. Loupe Browser software (10x Genomics) makes it easy to visualize and explore spatial gene expression data on top of the morphological data from the tissue image.

Prepare tissue sections

Select the appropriate Visium Spatial Gene Expression protocol depending on your sample type (fresh frozen or FFPE). Demonstrated protocols for Visium sample preparation are available on the 10x Genomics Support website.[8-9] These demonstrated protocols contain instructions for sample handling, including sectioning of tissue samples and placement of sections onto the Visium slides. 10x Genomics has validated the Visium Spatial Gene Expression assay on various human and mouse tissue types.[10]

Proper tissue handling and preparation techniques preserve the morphological quality of the tissue sections and the integrity of mRNA transcripts. This is critical for downstream library preparation and generation of high-quality sequencing data.

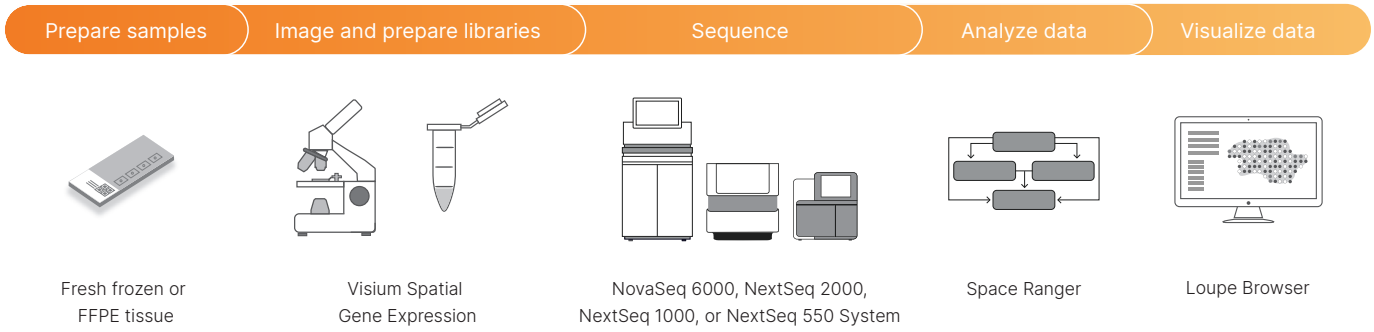

Figure 1: Workflow for a spatial RNA-Seq experiment—Section fresh frozen or FFPE tissue onto a Visium Spatial Gene Expression slide then stain and image the tissue sections. The glass slide captures mRNA with barcoded oligonucleotide probes and cDNA synthesis is followed by library construction. Sequence whole-transcriptome gene expression libraries on Illumina instruments. Connect gene expression location with the tissue morphology using Space Ranger and Loupe Browser software from 10x Genomics.

Stain and image the tissue

Use standard techniques to perform hematoxylin and eosin (H&E) or immunofluorescent staining of the tissue section that has been placed onto the Visium slide. Image stained tissue sections using a microscope. Successful gene expression visualization is highly dependent on good staining and imaging practices.[11]

Permeabilize tissue and construct libraries

After the tissue section has been imaged, it is ready for transcript capture using the Visium Spatial Gene Expression kits for fresh frozen or FFPE tissue. The Visium Spatial Gene Expression slides have four squares, or capture areas, for running up to four tissue sections per slide.* Each capture area has thousands of barcoded spots containing millions of capture oligonucleotides with spatial barcodes unique to each spot (Figure 2).

The transcripts from each small cluster of cells covering a spot is barcoded to track its location on the slide. Tissue is permeabilized to allow cells to release mRNA (for fresh frozen) or ligated probe pairs (for FFPE), which bind to the spatially barcoded probes present on the spots. For fresh frozen tissue, a reverse transcription reaction produces cDNA from the captured mRNA. For FFPE tissue, an RNA-templated ligation method is used to capture and barcode transcripts. The barcoded molecules are then pooled for downstream processing and construction of sequencing-ready libraries.[10]

Sequence with Illumina instruments

To handle the sequencing output required for this application, we recommend sequencing Visium Spatial Gene Expression libraries on the NovaSeq 6000, NextSeq 2000, NextSeq 1000, or NextSeq 550 System (Table 1). Smaller scale instruments like the iSeq™ 100 System can be used for library quality control.[12]

Visium Spatial Gene Expression libraries comprise standard Illumina paired-end constructs that are flanked with i5 and i7 indexes necessary for binding to the Illumina flow cell (Table 2, Figure 3). Read 1 primer is used for sequencing the 16-bp spatial barcode and up to 12-bp unique molecular identifier (UMI). Read 2 primer is used for sequencing the cDNA, or ligated probe, insert. The two 10-bp sample indexes are sequenced in the i5 and i7 reads.[13]

The recommended sequencing depth for the Visium library depends on several factors, including tissue complexity, tissue coverage, and experimental goals. Spatial gene expression libraries need to be sequenced with a paired-end sequencing run.

* Each capture area also gets a sample barcode during library preparation so that multiple libraries from different capture areas can be pooled in a sequencing run. Other Visium slides are available with either two or eight capture areas per slide.

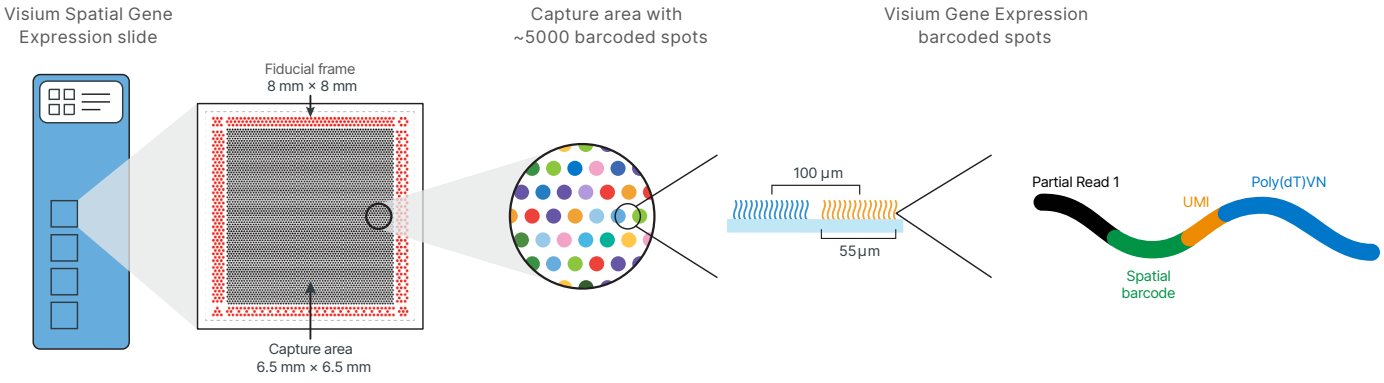

Figure 2: Visium Spatial Gene Expression slide design—Visium gene expression slides contain four capture areas and each capture area has 5000 barcoded spots. Tissue sections are permeabilized, releasing mRNA that binds to spatially barcoded oligonucleotides and allowing for the capture of gene expression information. cDNA is then synthesized from captured mRNA and spatial gene expression sequencing libraries prepared.

Table 1: Example sample throughput for Visium Spatial Gene Expression assay on Illumina sequencing systems

Sample type	Minimum read pairs per tissue spot[a]	Recommended reads per tissue section	No. of capture areas per run[b]						
			NextSeq 550	NextSeq 2000		NovaSeq 6000			
			High output	P2[c]	P3	SP	S1	S2	S4
Fresh frozen tissue	50K[a]	250M	3	3	8	5	10	26	64
FFPE tissue	25K[a]	100-125M	6	6	16	10	20	52	128

a. Minimum read recommendations courtesy of 10x Genomics
b. Calculated based on 125M read pairs (250M reads) per capture area, 5000 tissue spots per capture area, and average 50% capture area covered by tissue section; Visium Spatial Gene Expression slides have four capture areas for running up to four tissue sections per slide
c. P2 flow cells with the same sample throughput also available on the NextSeq 1000 System

Table 2: Recommended read configuration for Visium Spatial Gene Expression library

	Read 1	i7 index	i5 index	Read 2 Fresh frozen	Read 2 FFPE
Purpose	Cell barcode and UMI	Sample index	Sample index	cDNA insert	Ligated probe insert
Length	28 bp	10 bp	10 bp	90 bp	50 bp

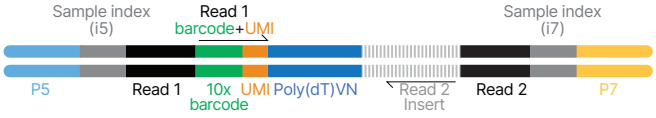

Figure 3: Visium Spatial Gene Expression library configuration—Visium Spatial Gene Expression libraries comprise standard paired-end contructs compatible with Illumina sequencing systems.

10x Genomics recommends a minimum of 50,000 read pairs per tissue-covered spot for fresh frozen tissues and 25,000 read pairs per spot for FFPE tissues.† For spatial gene expression libraries, 10x Genomics recommends a PhiX library spike-in of at least 1%. Example sequencing metrics for the spatial gene expression libraries are available on the 10x Genomics Support website[13] and Illumina BaseSpace™ Sequence Hub public data page.[14]

† The minimum sequencing read recommendation for FFPE tissues is lower because of newer, more efficient chemistry that excludes mitochondrial and ribosomal genes.

Analyze and visualize your data

During the Visium workflow, two main data types are captured: the tissue image and the sequencing data in BCL or FASTQ format. The Space Ranger analysis pipeline uses these two data inputs to align the Visium sequencing data with the image. Each detected gene transcript captured is assigned to a spatial location on the tissue image based on the associated spatial barcode. After data are 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.

Data highlights

Spatial transcriptomic analysis enables high-resolution characterization of gene expression in the tissue context for either fresh frozen tissue (Figure 4)[15] or FFPE samples (Figure 5).[16]

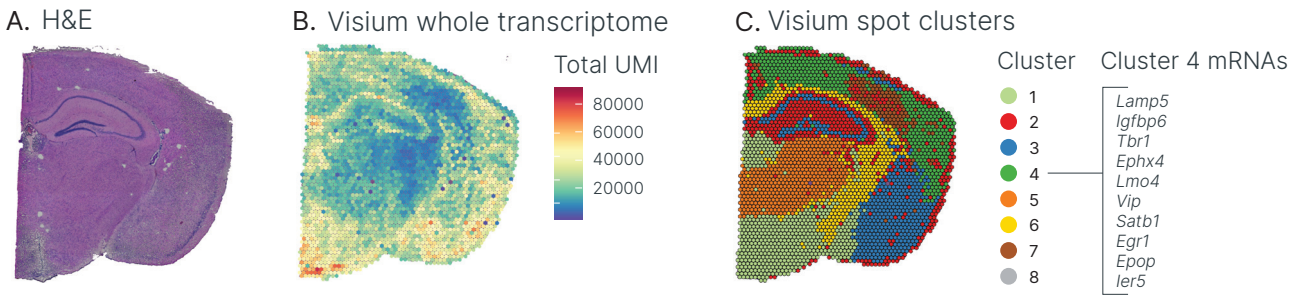

Figure 4: Fresh frozen mouse brain data highlight—(A) H&E image for a coronal mouse brain section. (B) Overlay of Visium data for total UMIs for whole-transcriptome analysis or spatially naïve spot clustering based on total differentially expressed genes. (C) List of the most highly expressed genes in Cluster 4.[15]

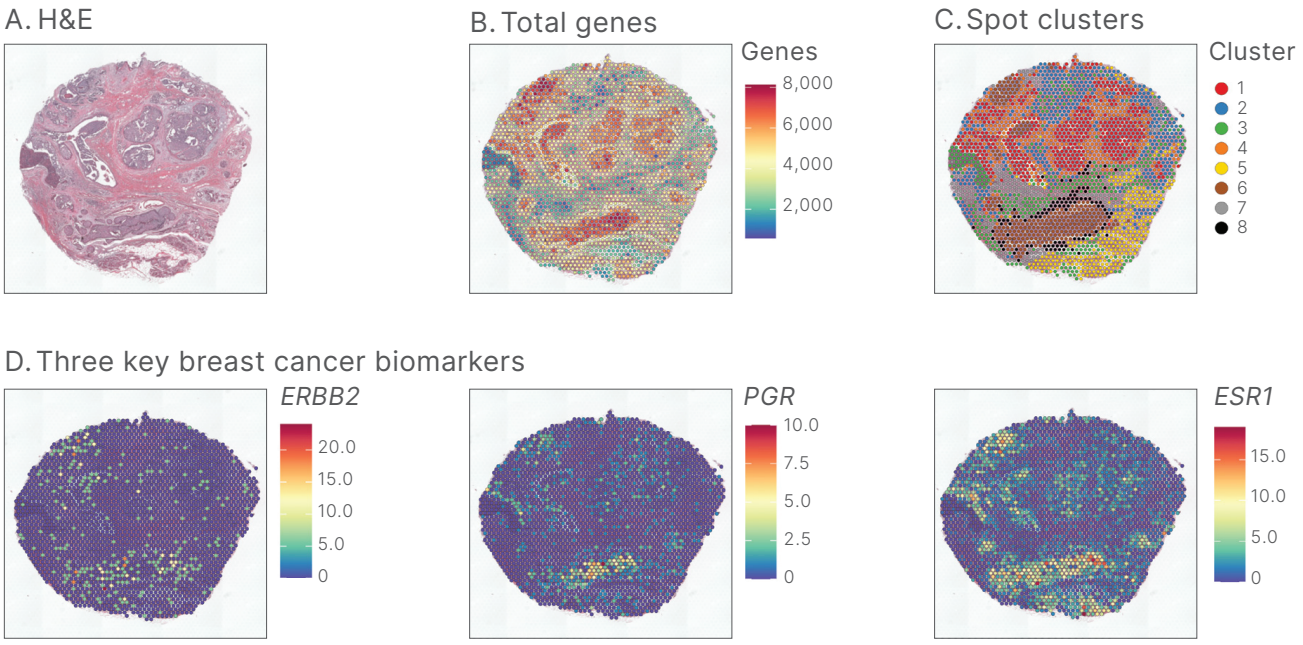

Figure 5: FFPE breast tissue with ductal carcinoma *in situ* data highlight—Visium Spatial Gene Expression for FFPE was used to interrogate approximately 18,000 genes in an FFPE human breast ductal carcinoma *in situ* sample. (A) An H&E-stained image was overlaid with data from the Visium for FFPE whole-transcriptome analysis, shown here as (B) total genes and (C) spot clustering analysis. (D) The expression levels and spatial organization of key breast cancer genes are shown: *ERBB2* (*HER2*), progesterone receptor (*PGR*), and estrogen receptor (*ESR1*).[16]

Access expert support

10x Genomics and Illumina teams collaborate to make sure that you are fully supported throughout the workflow when sequencing Visium Spatial Gene Expression libraries. Contact 10x Genomics Support (support@10xgenomics.com) for assay and analysis questions and Illumina Support (techsupport@illumina.com) for sequencing questions. The teams are also equipped to handle more complex issues together.

Summary

This spatial gene expression protocol incorporates whole-transcriptome analysis for intact tissues sections with morphological context. Bridging the complementary methods of gene expression and tissue morphology can help researchers better understand spatial organization and how it contributes to tissue development, function, and disease state.

Learn more

NovaSeq 6000 System, illumina.com/systems/sequencing-platforms/novaseq.html

NextSeq 1000 and NextSeq 2000 Systems, illumina.com/systems/sequencing-platforms/nextseq-1000-2000.html

NextSeq 550 System, illumina.com/systems/sequencing-platforms/nextseq.html

Visium Spatial Gene Expression, 10xgenomics.com/products/spatial-gene-expression

Ordering information

Sequencing reagents	Catalog no.
NovaSeq 6000 SP Reagent Kit v1.5 (100 cycles)	20028401
NovaSeq 6000 S1 Reagent Kit v1.5 (100 cycles)	20028319
NovaSeq 6000 S2 Reagent Kit v1.5 (100 cycles)	20028316
NovaSeq 6000 S4 Reagent Kit v1.5 (200 cycles)	20028313
NextSeq 1000/2000 P2 Reagents v3 (100 cycles)	20046811
NextSeq 2000 P3 Reagents (100 cycles)	20040559
NextSeq 500/550 High Output Kit v2.5 (150 cycles)	20024907

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1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

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