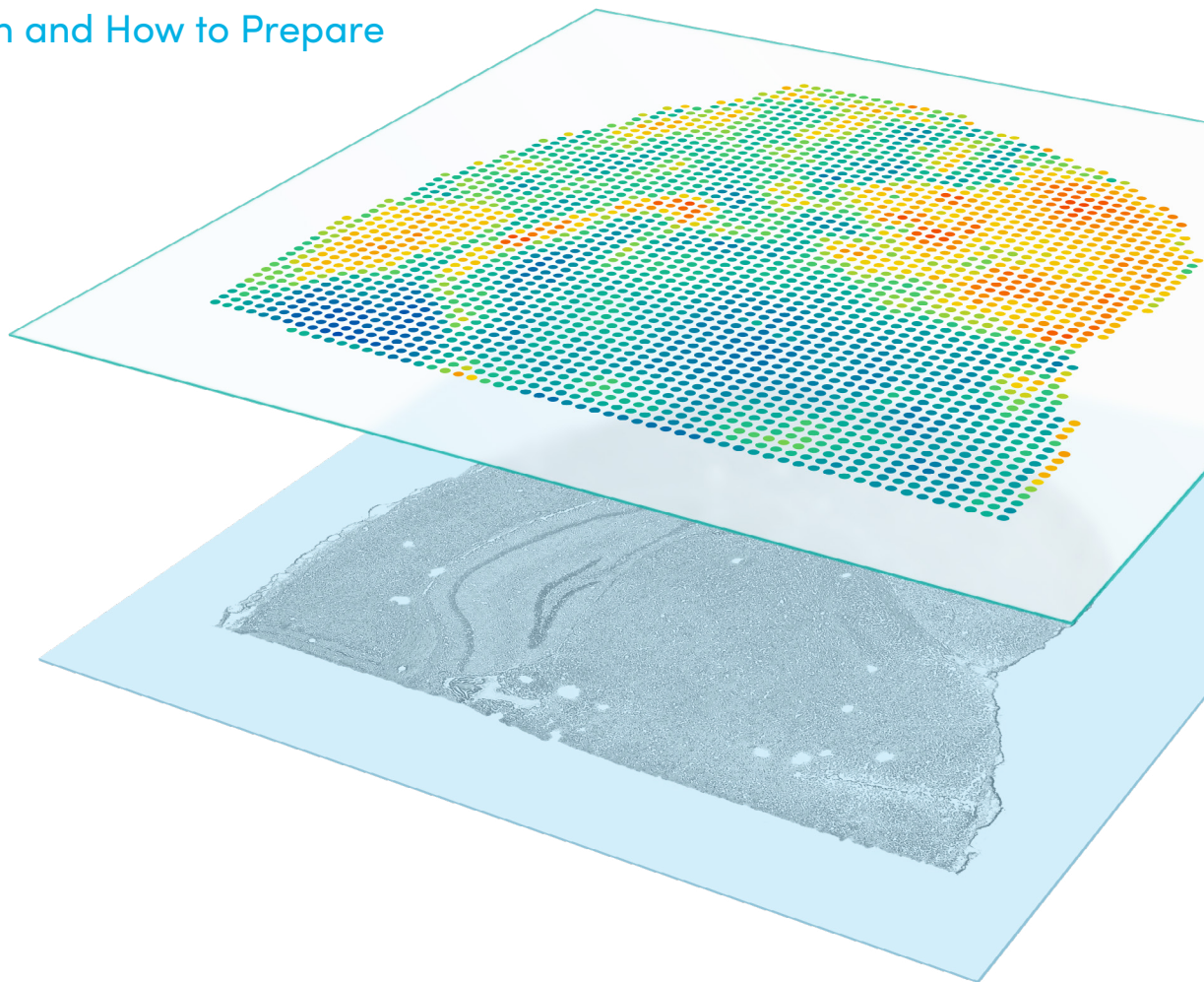# Inside Visium Spatial Technology

Introduction and How to Prepare

10x GENOMICS

# The Power of Two:
Mapping and Measuring Gene Expression

The Visium Spatial Gene Expression Solution incorporates unbiased, high-throughput total mRNA analysis for intact tissue sections with morphological context, offering a previously inaccessible view of tissue biology. These two complementary methods to study tissue come together on Visium Spatial Gene Expression slides.

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.

Fresh-frozen tissue is sectioned and placed onto a slide with thousands of barcoded spots, each containing millions of capture oligonucleotides with spatial barcodes unique to that spot (Figure 1). Once tissue sections are fixed, stained, and permeabilized, they release mRNA which binds to capture oligos from a proximal location on the tissue. A reverse transcription reaction occurs while the tissue is still in place, generating a cDNA library that incorporates the spatial barcodes and preserves spatial information. Barcoded cDNA 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 (Figure 2), 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.
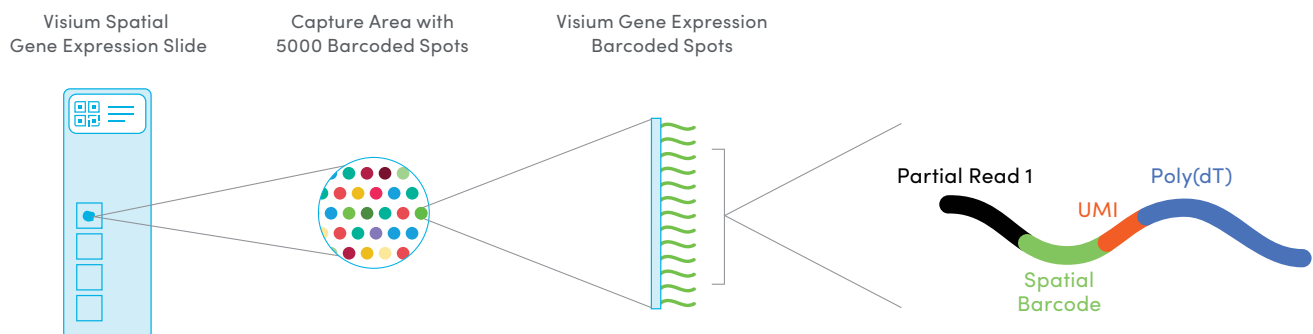
Figure 1. Here we show the composition of the Visium Spatial Gene Expression slide. Each slide contains four Capture Areas with approximately 5000 barcoded spots, which in turn contain millions of spatially-barcoded capture oligonucleotides. Tissue mRNA is released and binds to the barcoded oligos, enabling capture of gene expression information.
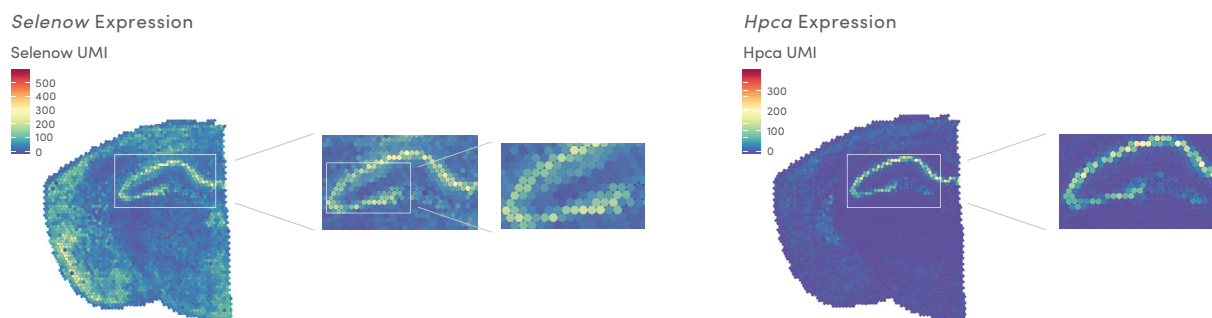
Figure 2. This is a coronal mouse brain section with overlaid spatial gene expression information. The spots correspond to localized mRNA of *Selenow* and *Hpca*, both known to have predominant hippocampal expression.

Library Construction:
How it Works

The Visium Spatial Gene Expression Solution is easily adoptable within existing lab infrastructure, utilizing standard laboratory methods and tools for tissue analysis. From start to finish, complete your spatial gene expression library construction in one day, or pause at strategic points to continue the experiment later.

Stepwise Library Construction

Fresh-frozen tissue samples are sectioned and placed in the four Capture Areas on the Visium Spatial Gene Expression slide.

Utilizing standard fixation and staining techniques, including Hematoxylin and Eosin (H&E) staining, tissue sections are visualized on slides. Microscope recommendations can be found under "Preparing My Lab" on the last page.

The tissue is then permeabilized to release mRNA from the cells. mRNA binds with spatially-barcoded oligonucleotides present on the spots. A reverse transcription reaction produces cDNA from captured mRNA. The second strand of cDNA is then synthesized and denatured. Note, if you're using a 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 is amplified. 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.

Sample Prep	Library Prep		
Tissue Prep	Staining & Imaging	Permeabilization	cDNA Synthesis
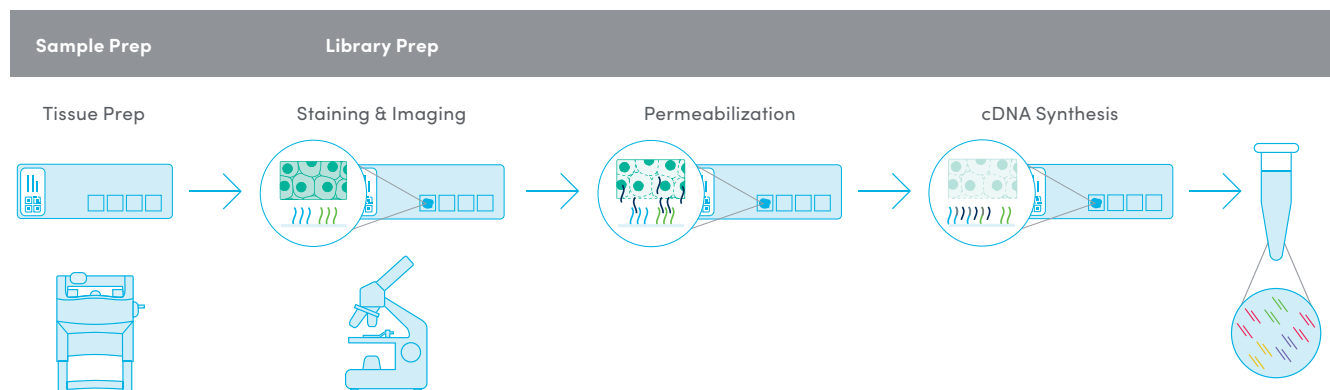
Figure 3. This diagram outlines how to construct a sequencing library using the Visium Spatial Gene Expression solution.

Preparing My Lab

10x Genomics is committed to making adoption of the Visium Spatial Gene Expression Solution as easy and efficient as possible for you and your research team. Here are some microscope considerations to transition seamlessly to the Visium Solution.

Imaging Systems & Specifications	
Microscopes Any equivalent system with the listed features may be used for imaging.	
Nikon	Nikon Eclipse Ti2 with brightfield and fluorescence capacity (TRITC)
Molecular Devices	ImageXpress Nano Automated Cell Imaging System
Microscope Features	
Objectives	• 4X (Plan APO λ; NA 0.20) • 10X (Plan APO λ; NA 0.45) • 20X (Plan APO λ; NA 0.75)
Scanning Stage	Microscope tile scanning functionality is required for imaging tissue sections placed on a Capture Area of a Visium Spatial slide.
Brightfield Features	• Color camera (3 x 8 bit, 2424 x 2424 pixel resolution) • White balancing functionality • Minimum Capture Resolution 2.18 µm/pixel • Exposure times 2–10 milli sec
Fluorescence Features* *Only required for Visium Spatial Tissue Optimization protocol & Visium Spatial Imaging Test Slide verification*	• Light source (or equivalent) with a wavelength range of 380–680 nm • Monochrome camera (14 bit, 2424 x 2424 pixel resolution) • TRITC filter cube (Excitation 542/20, Emission 620/52) (only required for Tissue Optimization protocol) • Minimum Capture Resolution 2.18 µm/pixel • Exposure times 100 milli sec–2 sec
Additional Specifications	
Image Format	Save image in jpeg or tiff format
Computer	Computer with sufficient power to handle large images (0.5–5 GB)
Software	Image stitching software (microscope's software or equivalent, like Image J)

For an entire list of reagents required please see user guide.

Contact Us

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