Spatial biology without limits: Spatially resolve gene expression in FFPE samples

Spatial Gene Expression for FFPE

Break past the barriers that limited spatial analysis of gene expression in formalin-fixed paraffin-embedded (FFPE) tissue sections and unlock what has been hiding in your samples using Visium Spatial Gene Expression for FFPE tissues. Combining the benefits of histological techniques with the massive throughput and discovery power of RNA sequencing in FFPE tissue samples, Visium Spatial Gene Expression for FFPE samples is a groundbreaking solution that complements traditional pathologist-led analysis. Spatially profile RNA expression for over 18,000 genes in human and mouse FFPE samples with high resolution across entire tissue sections. With whole transcriptome analysis and our specialized chemistry for FFPE tissue profiling, you can detect any gene in any pathway, resolve tissue heterogeneity, and reveal the spatial organization of cell types and cell states within a morphological context. Combine with immunofluorescence (IF) for simultaneous visualization of protein and gene expression.

Highlights

- Have confidence in your gene detection with RNA-templated ligation (RTL) probe sets that ensure high sensitivity and specificity
- Revisit archival, biobanked samples for biomarker discovery, or perform retrospective and longitudinal studies to track biological processes over time
- Remove analysis limitations associated with predefined regions of interest by analyzing entire FFPE tissue sections so you don't miss out on important biology
- Combine with immunofluorescence for visualization of protein and gene expression or with H&E for morphological context

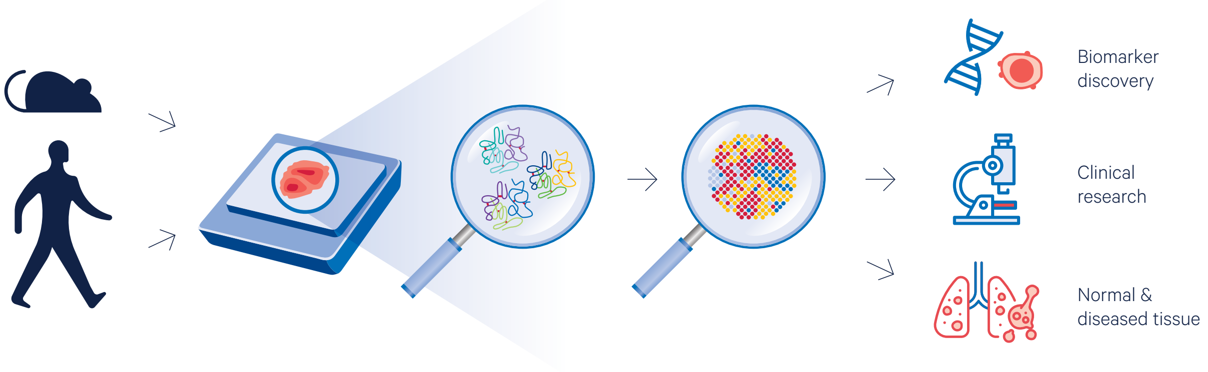

Figure 1. Gain high-resolution characterization of gene expression with morphological context in FFPE tissue sections. Formalin fixation and paraffin embedding (FFPE) is the most common method to preserve tissue, but the processing damages RNA, complicating transcriptomic-level investigations that seek to uncover underlying biological mechanisms. Visium Spatial Gene Expression for FFPE provides unbiased gene expression readout in intact FFPE tissue sections allowing for deeper insights to fuel translational research, including biomarker discovery, performing retrospective and longitudinal studies, and comparisons of normal and diseased tissue.

Product features

- Unbiased, whole transcriptome analysis of FFPE tissue sections allows detection of any gene without the need for predetermined biomarkers, enabling true discovery
- Full tissue section coverage means you are not limited to analyzing regions of interest that cover just a fraction of the section
- Unique RTL probe design for highly specific and sensitive detection of the whole transcriptome in human and mouse FFPE tissue sections
- Compatibility with histological stains, such as H&E and IF, enables morphological context or protein co-detection in combination with whole transcriptome analysis
- All reagents are kitted and ready to use, meaning no specialized instrumentation is required

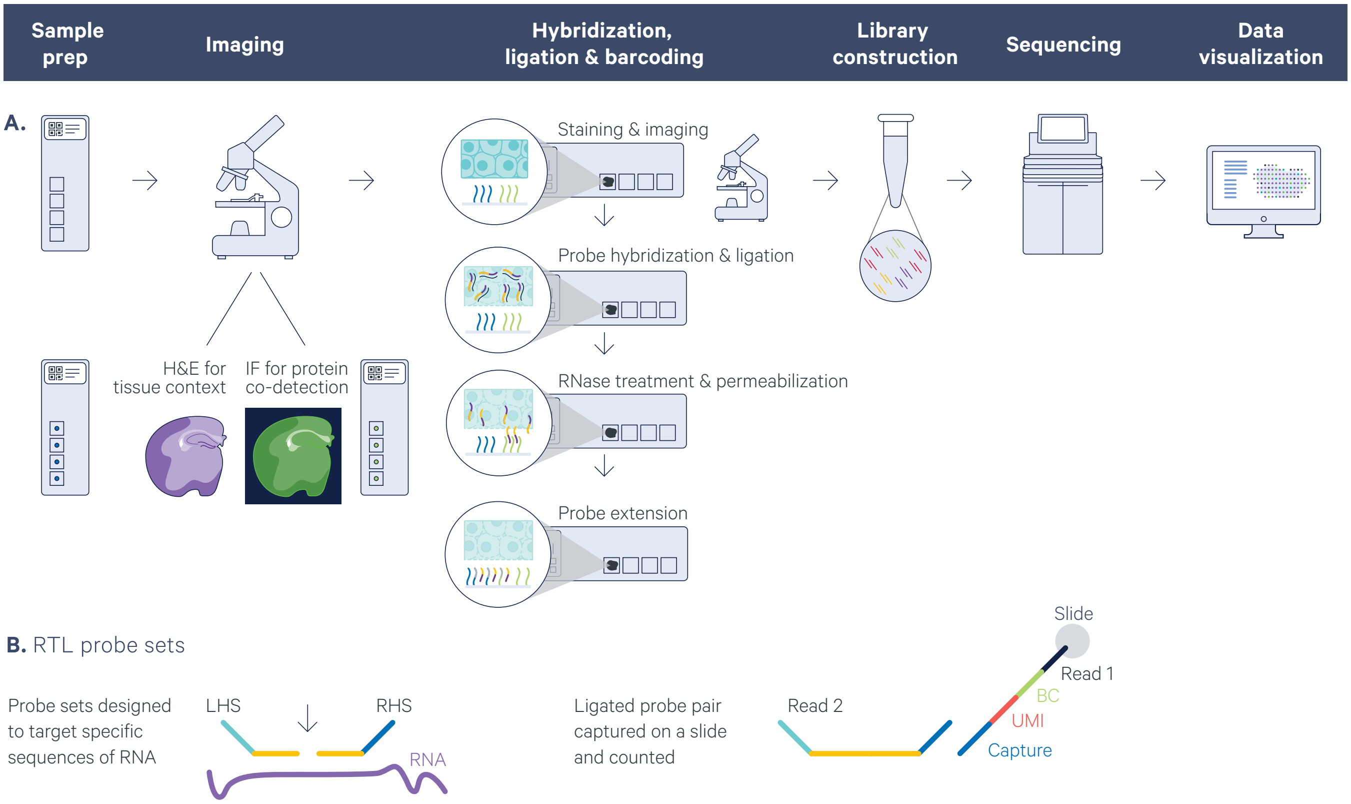

Figure 2. Go from block to data easily with a ready-to-use, robust workflow for whole FFPE tissue section analysis. **A.** An FFPE tissue section is placed onto a Visium gene expression slide and imaged for histological purposes (either H&E for morphological context or IF for protein co-detection). Each Capture Area on a Visium for FFPE slide has an array containing capture probes that bind to RNA. The probe pairs are extended to incorporate complements of the spatial barcodes, and sequencing libraries are prepared. The libraries are then sequenced and data visualized to determine which genes are expressed, and where, as well as in what quantity. **B.** The Visium for FFPE assay utilizes RNA-templated ligation (RTL), in which pairs of probes specific to genes in the protein-coding transcriptome are hybridized to their gene targets and then ligated to one another. The tissue is permeabilized to release ligated probe pairs to bind to capture probes on the slide, allowing for the capture of gene expression information.

Comparable performance to Visium for fresh frozen tissues

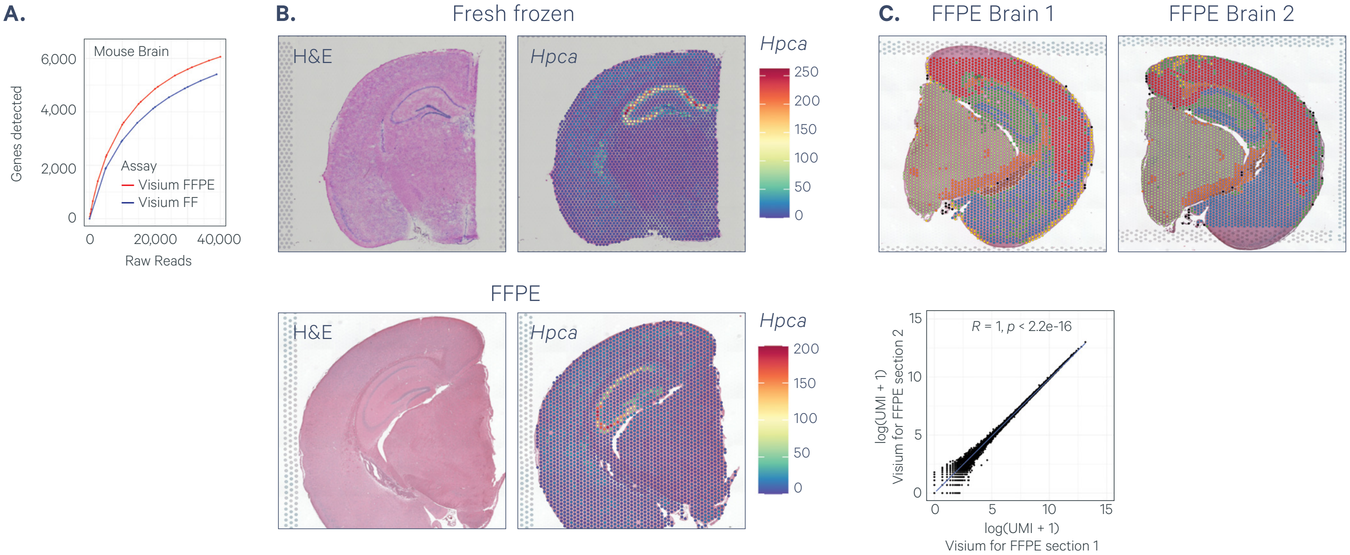

Figure 3. Visium Spatial Gene Expression for FFPE tissues is highly sensitive, specific, and reproducible. Whole transcriptome analysis of fresh frozen or FFPE mouse brain was performed using either Visium Spatial Gene Expression for fresh frozen or FFPE tissue, respectively. **A.** The Visium Spatial for FFPE data shows a high correlation with that of Visium for fresh frozen tissue, demonstrating comparable results between the two assays and high sensitivity. **B.** Spatial mRNA expression data for *Hpca*, demonstrates expression in the hippocampus in both the fresh frozen and FFPE samples and coincides with known expression patterns, demonstrating the specificity of the Visium for FFPE assay. **C.** Serial sections taken from a mouse brain FFPE sample and processed with the Visium Spatial for FFPE assay demonstrate high reproducibility, both in clustering and total unique molecular identifiers (UMIs) detected.

Interrogation of ~18,000 genes in a human breast ductal carcinoma in situ FFPE sample

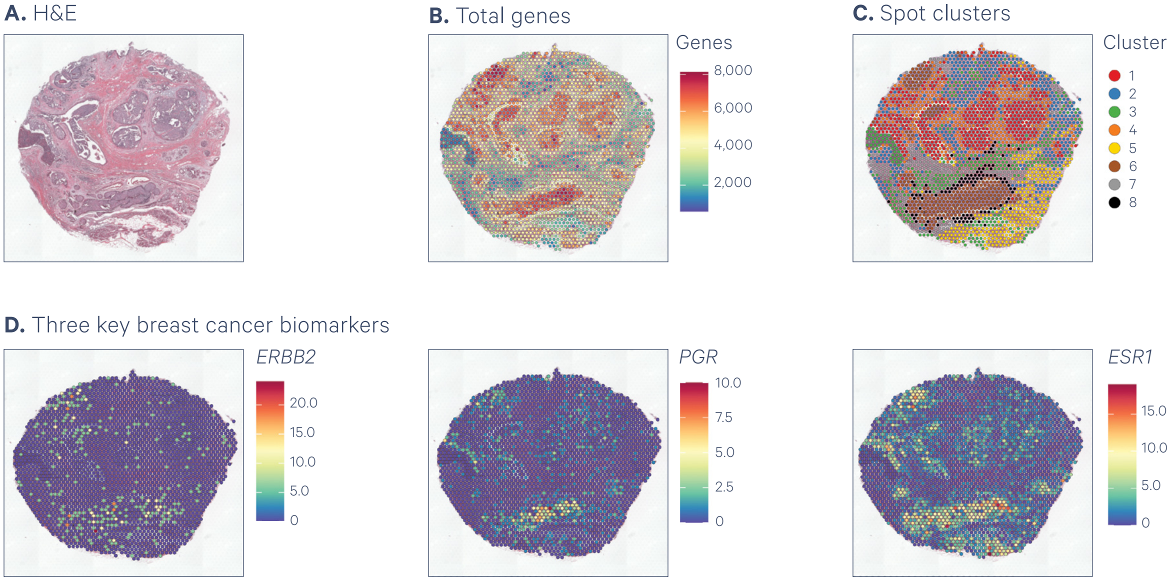

Figure 4. Visium for FFPE profiles the whole transcriptome in archival FFPE tissue samples with full tissue coverage. Visium Spatial Gene Expression for FFPE was used to interrogate approximately 18,000 genes in an FFPE human breast ductal carcinoma in situ sample. An H&E-stained image (**A**) was overlaid with data from the Visium for FFPE whole transcriptome analysis, shown here as total genes (**B**) and spot clustering analysis (**C**). The expression levels and spatial organization of key breast cancer genes (**D**) are shown: *ERBB2* (*HER2*), progesterone receptor (*PGR*), and estrogen receptor (*ESR1*).

Product specifications

- **Configuration:** Visium Spatial Gene Expression Slide & Reagents Kit for FFPE tissue includes all the reagents and slides for 2 (1 slide, 2 capture areas), 4 (1 slide, 4 capture areas), or 16 (4 slides, 16 capture areas) reactions
- **High cellular resolution:** Each capture area (6.5 x 6.5 mm) contains 5,000 barcoded spots that are 55 µm in diameter (100 µm center to center between spots providing an average resolution of 1 to 10 cells)
- **Sensitive detection:** RTL probe chemistry ensures high specificity and sensitivity
- **Whole transcriptome analysis:** Profile the whole transcriptome in entire human and mouse FFPE tissue sections

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Visium Spatial for FFPE Gateway Gene Expression Package, Human Transcriptome	1000342
Visium Spatial for FFPE Gene Expression Kit, Human Transcriptome, 16 rxns	1000336
Visium Spatial for FFPE Gene Expression Kit, Human Transcriptome, 4 rxns	1000338
Visium Spatial for FFPE Gateway Gene Expression Kit, Human Transcriptome, 2 rxns	1000340
Visium Spatial for FFPE Gene Expression Starter Kit, Mouse Transcriptome	1000335
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