

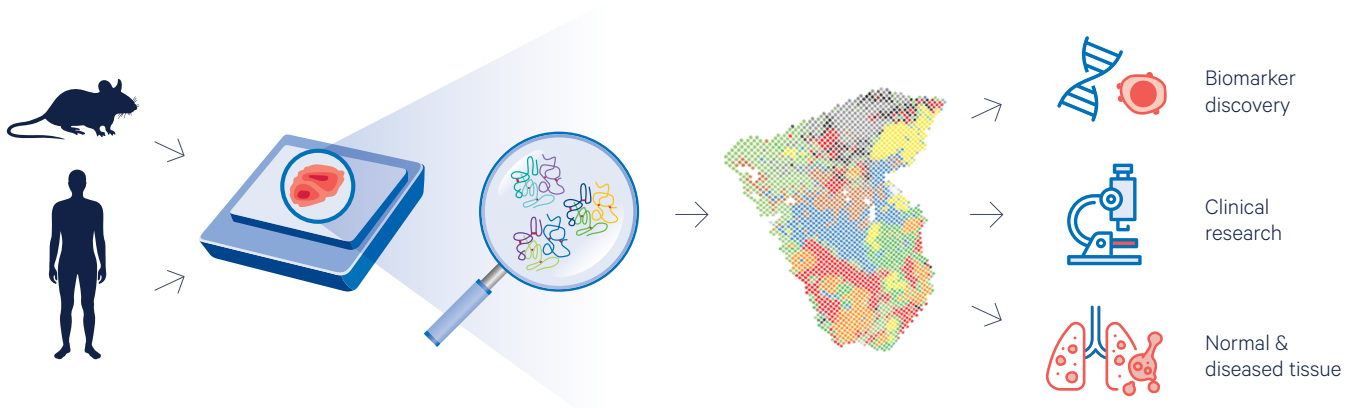
# Spatial biology without limits: Spatially resolve gene expression in FFPE samples

## Spatial Gene Expression for FFPE

Move 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. 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 complements traditional pathologist-led analysis. Spatially profile RNA expression for more than 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. The Visium CytAssist for FFPE instrument allows for a simplified Visium workflow by facilitating the transfer of transcriptomic probes from standard glass slides to Visium slides, enabling spatial profiling insights to be gained from an expanded range of FFPE samples.

## Highlights

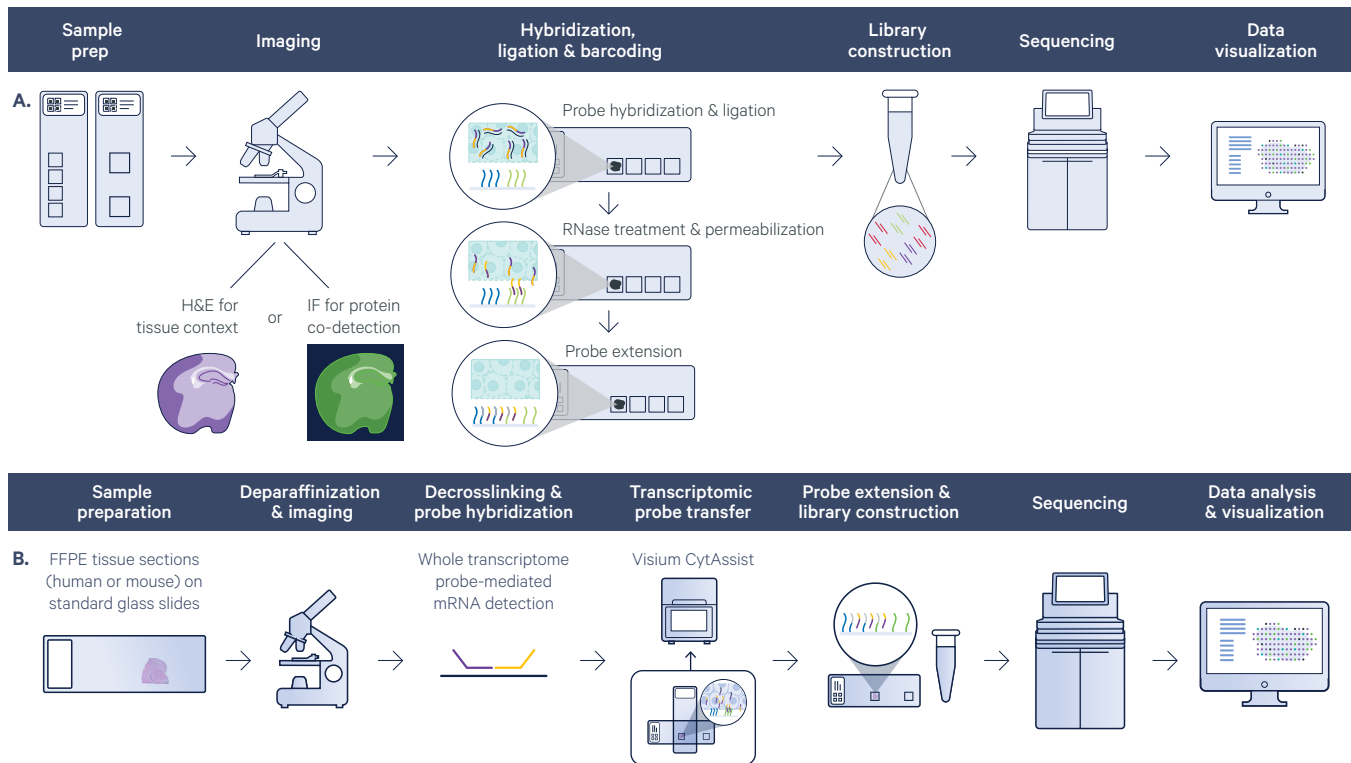
- Revisit archival, biobanked samples for biomarker discovery, or perform retrospective and longitudinal studies to track biological processes over time
- Take advantage of flexible workflow options enabling you to profile pre-sectioned FFPE tissues and select the most biologically significant sections
- Perform whole-tissue profiling on tissues of varying sizes 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.** 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.

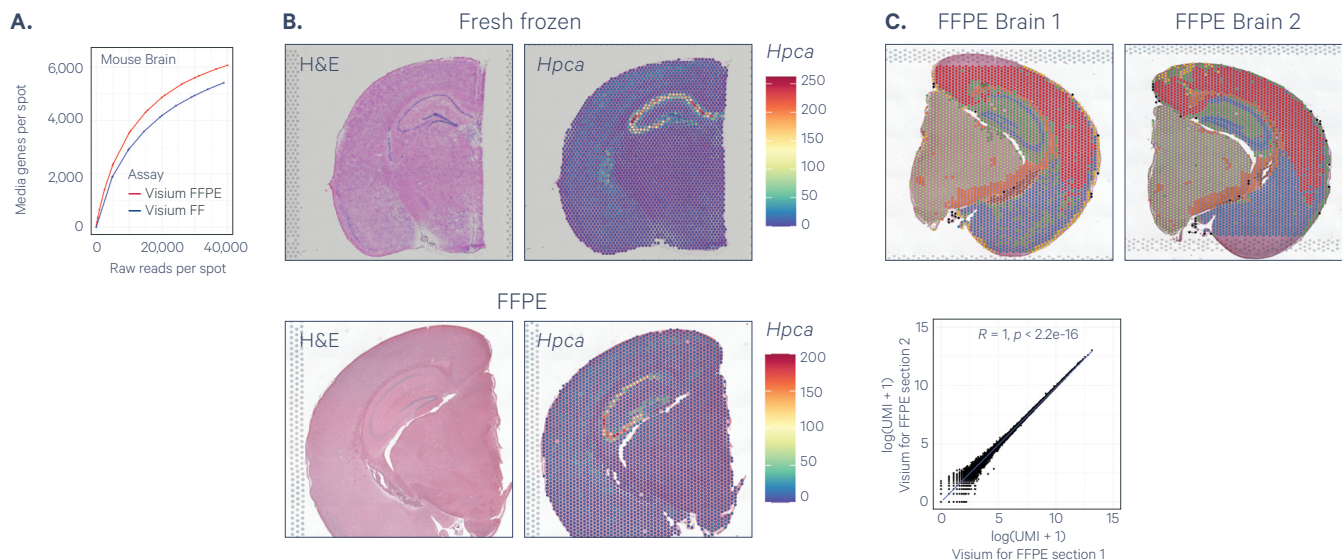
## Product features

- Unbiased, whole transcriptome analysis of FFPE tissue sections enables true discovery by allowing detection of any gene without the need for pre-determined biomarkers
- Visium slides with two capture area sizes (6.5 x 6.5 mm and 11 x 11 mm) expand your analysis across your entire tissue section without the need to choose subregions of interest
- Unique RNA-templated ligation (RTL) probe design gives you highly specific and sensitive detection of the whole transcriptome in human and mouse FFPE tissue sections
- Compatibility with histological stains, such as hematoxylin and eosin (H&E) and immunofluorescence (IF), enables morphological context or protein co-detection in combination with whole transcriptome analysis
- The Visium CytAssist capture workflow allows for gene expression profiling of pre-sectioned FFPE tissues on standard glass slides, expanding access to more samples



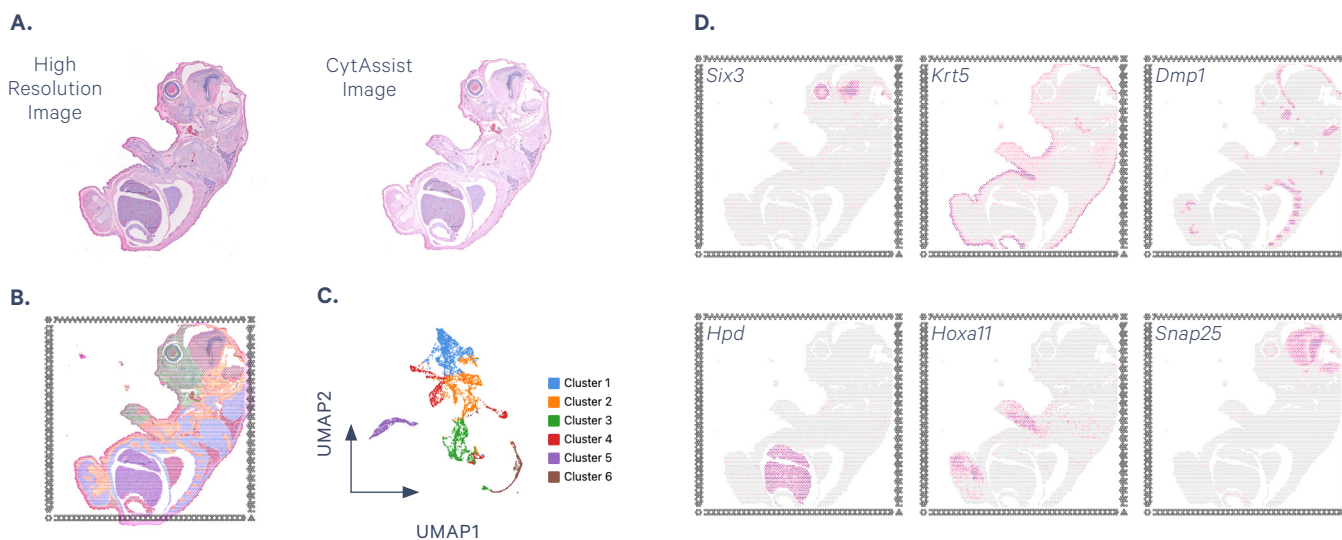
**Figure 2. Go from block to data easily with a ready-to-use and robust workflow for whole FFPE tissue section analysis.** **A.** In the direct placement workflow, an FFPE tissue section is placed onto a Visium gene expression slide and imaged for histological purposes (either H&E for morphological context, 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.** In the Visium CytAssist capture workflow, human or mouse FFPE tissue sections are placed onto standard glass slides. Slides are deparaffinized and imaged with H&E or IF as in a typical FFPE workflow, then de-crosslinked and hybridized to probe pairs. The Visium CytAssist then transfers transcriptomic probes from the glass slide to the Capture Area on a Visium for FFPE slide and probe extension, library construction, and sequencing proceed as described in **A.**

### 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.

### Expanded sample access with Visium CytAssist for FFPE workflow



**Figure 4. Visium CytAssist for FFPE profiles the whole transcriptome in HE stained whole mouse embryo on a standard glass slide.** (A) FFPE samples were processed for RNA profiling on a 11 x 11 mm Visium CytAssist capture area format. (B) Unbiased RNA clustering superimposed on the H&E image shows distinct clustering patterns in different organs. (C) UMAP analysis shows the corresponding gene expression clustering projected onto the spatial RNA profile map (D). Visium CytAssist provided organ- and tissue-specific localization of genes associated with embryonic development corresponding to RNA expression in the eyes, skin, bone, liver, limbs, and brain.

## Product specifications

- **Compatibility:** Human or mouse FFPE blocks or pre-sectioned tissues on glass slides
- **High cellular resolution:** Each Capture Area contains either 5,000 (6.5 x 6.5 mm) or 14,000 (11 x 11 mm) barcoded spots that are 55 µm in diameter (100 µm center to center between spots, providing an average resolution of 1 to 10 cells per spot)
- **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 (18,000 human and 21,000 mouse genes)

Visium for FFPE direct placement products	Product code
Visium Spatial for FFPE Gene Expression Starter Kit, Human Transcriptome	1000334
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 Gene Expression Starter Kit, Mouse Transcriptome	1000335
Visium Spatial for FFPE Gene Expression Kit, Mouse Transcriptome, 16 rxns	1000337
Visium Spatial for FFPE Gene Expression Kit, Mouse Transcriptome, 4 rxns	1000339
Visium Accessory Kit	1000194
Dual Index Plate TS Set A, 96 rxns	1000251

Visium for FFPE CytAssist capture products	Product code
Visium CytAssist Spatial Gene Expression for FFPE, Mouse Transcriptome, 6.5mm, 4 rxns	1000521
Visium CytAssist Spatial Gene Expression for FFPE, Mouse Transcriptome, 6.5mm, 16 rxns	1000445
Visium CytAssist Spatial Gene Expression for FFPE, Mouse Transcriptome, 11mm, 2 rxns	1000523
Visium CytAssist Spatial Gene Expression for FFPE, Mouse Transcriptome, 11mm, 8 rxns	1000446
Visium CytAssist Spatial Gene Expression for FFPE, Human Transcriptome, 6.5mm, 4 rxns	1000520
Visium CytAssist Spatial Gene Expression for FFPE, Human Transcriptome, 6.5mm, 16 rxns	1000443
Visium CytAssist Spatial Gene Expression for FFPE, Human Transcriptome, 11mm, 2 rxns	1000522
Visium CytAssist Spatial Gene Expression for FFPE, Human Transcriptome, 11mm, 8 rxns	1000444
Visium CytAssist Training Kit	1000458
Visium CytAssist Calibration Kit	1000459
Visium CytAssist Reagent Accessory Kit	1000499
Visium CytAssist, 24 months warranty	1000442

Related products	Product code
Space Ranger <a href="https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/what-is-space-ranger">support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/what-is-space-ranger</a>	Download
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LIT000128 - Rev C - Product Sheet - Spatial biology without limits: Spatially resolve gene expression in FFPE samples

