Grant Application Resources for Visium Spatial Gene Expression

Summary

The ability to detect and count transcripts by sequencing (RNA-seq) has led to significant advances in our understanding of biology (1), 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 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 the 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 a transcript's expression pattern in tissue sections using high-resolution microscope imaging. The workflow surveys spatial global 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, has been used in groundbreaking papers that demonstrate the breadth of applications for this technology, including cancer (2), neuroscience (3), and neuropsychiatric disorders (4).

Visium Spatial Gene Expression Solution

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

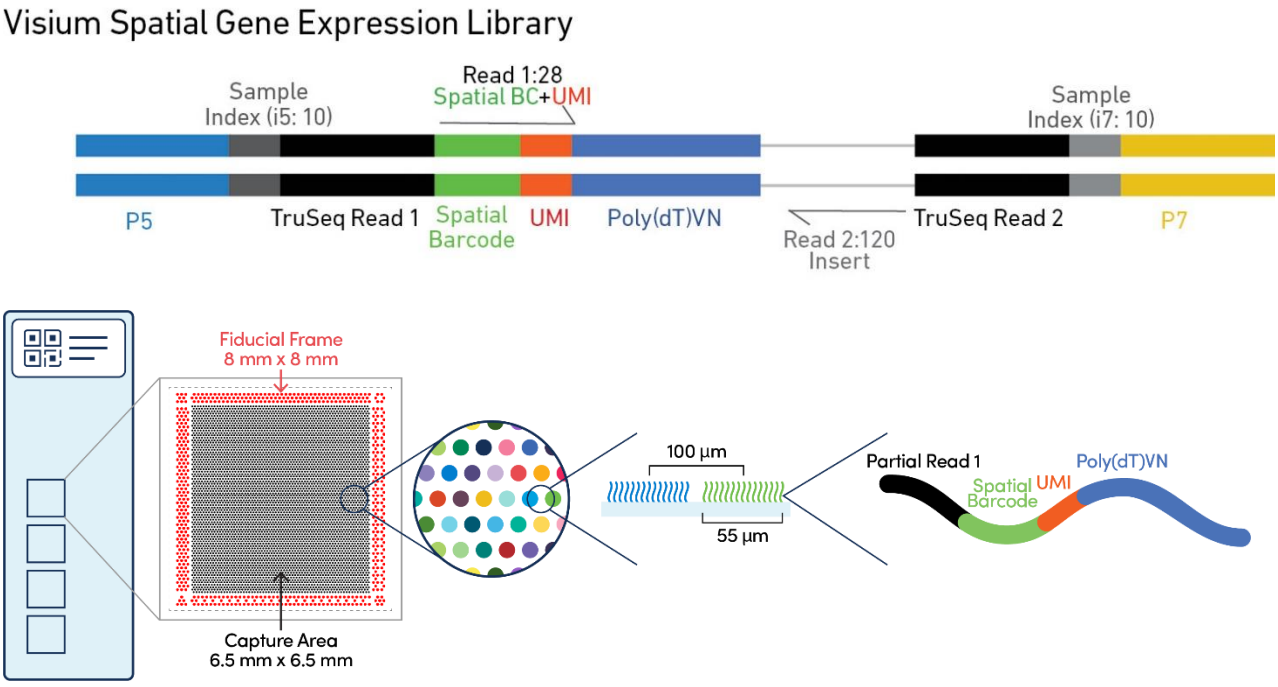
The Visium Spatial Gene Expression workflow relies on the use of Visium slides, each of which has four 6.5 x 6.5 mm Capture Areas. Each Capture Area is arrayed with ~5,000 capture spots, each containing millions of oligonucleotides with the following features: a 30 nucleotide poly(dT) sequence for the 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.

Sectioned tissues are placed on each Capture Area of the slide, where they are fixed and stained using either hematoxylin and eosin 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 that is bound on the slide surface, thereby creating a cDNA molecule that has the Spatial Barcode sequence as well as UMI and is 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.

Data benchmarking

The Spatial Transcriptomics assay has been validated using laser capture microdissection as well as single molecule fluorescence in situ hybridization (ISH) (3). Comparison to data generated for the Allen Brain Atlas using ISH determined that spatial transcriptomics can detect twice as many transcripts (Figure S5) (3). Spatial transcriptomics studies examining gene expression among tissue replicates have found very high reproducibility (r=0.97, Figure S3E) (3). High reproducibility was also observed when compared to RNA in solution (r=0.94, Figure S3D) (3).



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 4 Capture Areas, each defined by a fiducial frame. The 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..

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:

- Spatial expression profiles in *Arabidopsis thaliana* (5);
- Spatial distribution of markers in cardiac biopsies from patients with heart failure (6);
- Identification of clusters of gene expression in periodontitis-affected gingival tissue, and identification of genes that are upregulated in inflamed tissue (7);
- An examination of tissue heterogeneity in prostate cancer, which suggested that spatial gene exploration can better delineate cancerous tissue boundaries than pathologist annotations, while studying gene expression gradients in tissues surrounding tumors (2).

Visium Spatial Gene Expression Advantages

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

- No specialized infrastructure—The Visium Solution does not require any specialized equipment and can be adopted into labs or core facilities that have standard cryosectioning equipment, microscopes with image capture capability, and sequencing instruments.
- Comprehensive data analysis solution—The Visium Solution 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 (3). 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, which highlights 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 1–10 cells captured per spot dependent on tissue type. Initial studies suggest that this resolution can be used to delineate cancerous regions analogous to pathology annotations (2).
- 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.
- Unbiased transcript detection—The Visium Solution captures polyadenylated mRNA molecules, reducing the bias introduced by targeted amplicon sequencing or sequence-specific hybridization techniques.
- 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 Communications, and Nature Protocols.
- Optimized conditions for dozens of tissues—The Visium Spatial Gene Expression workflow has been optimized for healthy and diseased tissues in diverse organisms including human, mouse, rat, and zebrafish.
- Broad support resources—10x Genomics provides comprehensive support resources, ranging from its technical specialists trained in the Visium Spatial Gene Expression Solution to freely available videos and documents that guide new users through the Visium workflow.
- 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 single slide or four slide Visium kits, representing four and sixteen samples, respectively. This gives researchers the flexibility to carry out their proof-of-concept studies without wasting resources.

References

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