MEDICAL COLLEGE OF WISCONSIN

Linda T. and John A. Mellowes Center for Genomic Sciences and Precision Medicine

Spatial Transcriptomic Sequencing

Test Description

Understanding the localization of transcripts to cells and structures within a tissue is the foundation of Spatial Transcriptomics and will allow investigators to discover localization of cell populations and responses within the tissue. The entire transcriptome can be mapped with morphological context to the tissue to discover novel insights into development, disease pathology, and translational research. Combining the technology of the 10x Genomics Visium platform with the CytAssist instrument for tissue and spot alignment provides a rigorous process for sample preparation and analysis.

Sample Types and Requirements

Unstained tissue section or blocks (human or mouse, FFPE or cryopreserved): Tissue must be on a plain glass slide within allowable area, desired capture area must be clarified by investigator, consultation with the lab is requested

Recommended Sequencing Depth

>25,000 unique reads per tissue spot, as determined with Loupe Browser, paired end, 28bp x 50bp sequencing

Submission Requirements

Sample Intake Form and iLabs request. Contact lab for drop off or shipping requirements.

Turnaround Time

2-3 weeks for fastq files only
2-3 additional weeks for bioinformatics analysis

Deliverables

RNA quality control, if curl is provided (PDF)
Spatial RNA-seq report (that includes feature-spot matrix, spatial cluster detection, spatial enrichment genes)
BAM, fastq.gz files
All annotated NGS data files will be delivered via Mellowes Center portal

Test Methodology

Bulk RNA is isolated from tissue curl to confirm quality, if available. Tissue is sectioned, H&E stained, and area imaged at 10x to 20x magnification. After decrosslinking, the tissue is permeabilized and hybridized with an mRNA panel (human or mouse) of probe pairs that ligate when complementary to the available transcripts. Next, mRNA probes are released and captured by spots on the Visium slide via the 10xGenomics CytAssist instrument. The barcoded and captured cDNA then undergoes a full library preparation according to the 10x Genomics Visium CytAssist Gene Expression kits. The quality and quantity of the cDNA library is checked by fragment analysis and qPCR respectively. Libraries are then pooled and distribution confirmed on the Illumina MiSeq before sequencing is completed on the Illumina NovaSeq.

Bioinformatic Core Analysis

Spatial RNA-seq report includes:

- Quality control, sequencing metrics
- Map and compare gene expression among tissue regions
- Detect spatially enriched genes and domains in the tissue
- Cluster cell types across tissue (t-SNE and UMAP projections)
- Additional analysis can identify differences among tissues and classify cell types and numbers found within a tissue

Contact & Submission

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