For reference when drafting articles, please refer to the final report for accurate analysis methods and parameters.

Methods for Visium Product

Visium Experiment and Sequencing

Sample preparation

Fresh tissues were concurrently frozen and embedded in optical cutting tissue (OCT) compound in liquid nitrogen. The RNA quality of OCT embedded block was assessed by Agilent 2100. RNA integrity number (RIN) of tissues greater than 7 were used for visium spatial gene expression experiments. Cryosections were performed on a Leica CM3050 and brightfield images were taken on a Leica Aperio Versa8 whole-slide scanner at 20× resolution.

Tissue optimization (TO)

The Visium Spatial Tissue Optimization Slide & Reagent kit (10X Genomics) was used to optimize permeabilization conditions for the tissue according to Visium Spatial Tissue Optimization User Guide (CG000238, 10X Genomics).

Briefly, the Visium Spatial Tissue Optimization workflow includes placing tissue sections on 7 Capture Areas on a Visium Tissue Optimization slide. The sections are fixed, stained, and then permeabilized for different times. mRNA released during permeabilization binds to oligonucleotides on the Capture Areas. Fluorescent cDNA is synthesized on the slide and imaged. The permeabilization time that results in maximum fluorescence signal with the lowest signal diffusion is optimal. If the signal is the same at two time points, the longer permeabilization time is considered optimal.

Visium Sequencing Libraries Preparation

The Visium Spatial Gene Expression Slide & Reagent kit (10X Genomics) was used to construct sequencing libraries according to the Visium Spatial Gene Expression User Guide (CG000239, 10X Genomics). A 10um frozen tissue section was placed on one of the Visium gene expression slide capture areas in a slide. After tissue Hematoxylin and Eosin (H&E) staining, bright-field images were acquired as described in the Spatial Transcriptomics procedure. Tissue permeabilization was performed for a optimal minutes, as established in the TO procedure. Then reverse transcription experiment was conducted and sequencing libraries were prepared following the manufacturer's protocol.

Sequencing

Sequencing was performed with a Novaseq PE150 platform according to the manufacturer's instructions (Illumina) at an average depth of 300 million read-pairs per sample.

Primary Bioinformatical Analysis

Data processing

We use in-house script to perform basic statistics of raw data, and evaluate the data quality and GC content along the sequencing cycles. Raw FASTQ files and histology images were processed by sample with the Space Ranger (version spaceranger-1.2.0, 10X Genomics) software with default parameters. The filtered gene-spots matrix and the fiducial-aligned low-resolution image was used for down-streaming data analyses (Seurat).

Seurat analysis

The Seurat package was used to perform gene expression normalization, dimensionality reduction, spot clustering, and differential expression analysis. Briefly, spots were filtered for minimum detected gene count of 100 genes. Normalization across spots was performed with the SCTransform function and 3000 highly variable genes was selected for principal component analysis. For spot clustering, the first 20 PCs were used to build a graph, which was segmented with a resolution of 0.5. Wilcox algorithm was used to perform differential gene expression analysis for each cluster via FindAllMarkers function. Genes with fold change >2 and adjust pvalue <0.05 were defined as significantly differential expressed genes.

Enrichment analysis

The clusterProfiler R package was used to calculate enrichment test for candidate gene sets based on hypergeometric distribution. Pathways with corrected pvalue less than 0.05 were considered as significantly enriched terms. Three pathway classification systems were used as reference databases for human or mouse, including Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome Pathway. GO and Reactome annotations were retrieved from Ensembl BioMart and KEGG pathways was retrieved via KEGG REST API.

Reagent or Resource

1. Visium Spatial Tissue Optimization Slide & Reagents Kit, 4 samples
   10X Genomics Cat#1000193
2. Visium Spatial Gene Expression Slide & Reagents Kit, 16 rxns
   10X Genomics Cat#1000184

Reference

CG000238:https://support.10xgenomics.com/spatial-gene-expression/tissue-optimization/doc/user-guide-visium-spatial-tissue-optimization-reagents-kits-user-guide

CG000239:https://support.10xgenomics.com/spatial-gene-expression/library-prep/doc/user-guide-visium-spatial-gene-expression-reagent-kits-user-guide

Dobin A, Davis C A, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner[J]. Bioinformatics, 2013, 29(1): 15-21

Stuart,T.etal.Comprehensive Integration of Single-Cell Data. Cell. 177, 1888–1902.e21 (2019)

Guangchuang Yu, Li-Gen Wang, Yanyan Han, Qing-Yu He. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS: A Journal of Integrative Biology. 2012, 16(5):284-287

Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes[J]. Nucleic acids research, 2000, 28(1): 27-30.