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**Protocol status:** Working  
We use this protocol and it's working

**Created:** Sep 01, 2023

## KAPP-Sen TMC: 10x Visium CytAssist FFPE

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Cellular Senescence Network (SenNet) Method Development Community

KAPP-Sen TM



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### ABSTRACT

The Visium CytAssist Spatial Gene Expression workflow assays RNA levels in intact formalin-fixed paraffin embedded (FFPE) tissue sections. It uses probes targeting the whole transcriptome. Each Visium CytAssist Spatial Gene Expression Slide contains Capture Areas with barcoded spots that include oligonucleotides required to capture gene expression probes.

Tissue sections are processed with Visium Spatial Gene Expression assay performed by the **Single Cell Biology Core** at **The Jackson Laboratory for Genomic Medicine**.

## Deparaffinization, H&E, Decrosslinking

- 1 Visium CytAssist Spatial Gene Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking: CG000520 Rev B

 CG000520\_Demonstrated\_Protocol\_VisiumCytAssist\_Deparaffin\_H\_E\_RevB.pdf

## Library Preparation

- 2 Visium CytAssist Spatial Gene Expression Reagent Kits User Guide: CG000495 Rev E

 CG000495\_VisiumCytAssist\_GeneExpressionUserGuide\_RevE.pdf

Library concentration was quantified using a TapeStation High Sensitivity DNA ScreenTape (Agilent) and fluorometry (Thermofisher Qubit) and verified via KAPA qPCR.

## Library Sequencing

- 3 Libraries were pooled for sequencing on an Illumina NovaSeq 6000 200 cycle flow cell using a 28-10-10-90 read configuration, targeting 100,000 read pairs per spot covered by tissue.

 1000000019358\_17\_novaseq-6000-system-guide.pdf

## FASTQ Generation

- 4 Illumina base call files for all libraries were converted to FASTQs using bcl2fastq v2.20.0.422 (Illumina).

<https://support.illumina.com/downloads/bcl2fastq-conversion-software-v2-20.html>