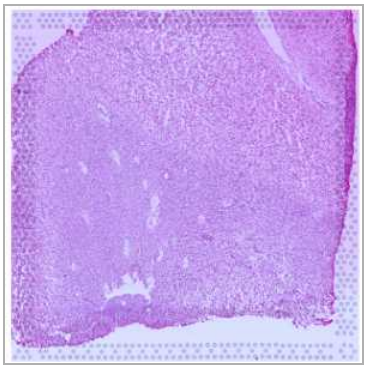

JAN 20, 2023

OPEN ACCESS

Protocol Citation: Shaunice Grier 2023. CUMC-TMC-10X-Gene-Expression. **protocols.io** https://protocols.io/view/cumc-tmc-10x-gene-expression-cm4zu8x6

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

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CUMC-TMC-10X-Gene-Expression

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Cellular Senescence Network (SenNet) Method Development Community

Ivy Kosater

ABSTRACT

Post Mortem Human Fresh Frozen Dorsolateral Prefrontal Cortex tissues are cryo - sectioned onto 10X Visium slides.

Tissue Optimization is performed and determines optimal Gene Expression testing conditions.

cDNA samples are processed and generated into libraries. Libraries are qc'd for size and concentration with the Agilent Bioanalyzer 2100, Qubit Fluorometer and qPCR with KAPA Library Quantification Kit. QC'd libraries are submitted for sequencing.

Tissue QC

1 Section

2 H&E Stain

3 Image

4 Assess

Tissue Optimization

5 Permeabilization & cDNA Synthesis

6 Tissue Removal

Gene Expression

7 cDNA Synthesis

8 Second Strand Synthesis & Denaturation

9 cDNA Amplification & QC

10 Visium Spatial Gene Expression Library Construction

Sequencing

11 Submitted libraries are evaluated for size distribution on the Fragment Analyzer and quantified using Picogreen and qPCR with the Universal KAPA Library Quantification Kit. Library pools are loaded between 1.2 – 1.5 nM using the standard NovaSeq workflow as outlined in Illumina's NovaSeq 6000 system guide.