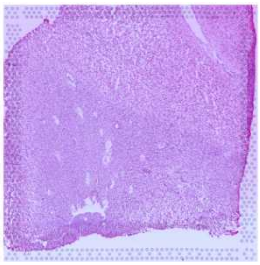
Jan 20, 2023

CUMC-TMC-10X-Gene-Expression



DOI
dx.doi.org/10.17504/protocols.io.3byl4j398lo5/v1

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Cellular Senescence Net...

Ivy Kosater

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Protocol Citation: Shaunice Grier 2023. CUMC-TMC-10X-Gene-Expression. **protocols.io https://dx.doi.org/10.17504/protocols.io.3byl4j398lo5/v1**

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

Created: January 20, 2023

Last Modified: January 20, 2023

Protocol Integer ID: 75641

Keywords: optimal gene expression testing condition, gene expression, cdna sample, tissue optimization, cumc, qpcr with kapa library quantification kit, cortex, gene, kapa library quantification kit, agilent bioanalyzer, qpcr

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.

Troubleshooting

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.