Dec 20, 2024

Spatial RNA sequencing of human ganglia

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlk8jb6l5r/v1

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PRECISION Human Pain ...

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Protocol Citation: Stephanie Shiers, Diana Tavares-Ferreira, Ishwarya Sankaranarayanan, Khadijah Mazhar, Theodore Price 2024. Spatial RNA sequencing of human ganglia. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlk8jb6l5r/v1

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

Created: September 16, 2024

Last Modified: December 20, 2024

Protocol Integer ID: 107725

Keywords: human, Visium, DRG, spatial, sequencing, human dorsal root ganglia section, spatial rna, human ganglia, human ganglia this protocol, 10x visium spatial gene expression kit, rna, sequencing assay, gene expression, based mrna, mrna, gene, human dorsal root ganglia

Funders Acknowledgements:
National Institutes of Health
Grant ID: U19NS130608
National Institutes of Health
Grant ID: NS111929
National Institutes of Health
Grant ID: NS065926

Abstract

This protocol covers spatial RNA sequencing of human dorsal root ganglia sections from fresh frozen samples using the 10X Visium Spatial Gene Expression kits, a poly-A tail based mRNA sequencing assay. The final raw data output are count matrices of gene expression.

Materials

Tissue preparation and sectioning

1. Fresh frozen human ganglia
2. Metal cryomold (Ted Pella, 27276-5)
3. OCT (Fisher, 23-730-571)
4. Visium spatial gene expression slide (10X Genomics, PN-2000233)

Fixation, Hematoxylin and Eosin Staining

1. 100% methanol (Fisher Chemical, A456-212)
2. Hematoxylin (Agilent Tech, S330930-2)
3. Eosin (Sigma, HT110216)
4. Tris base (Thermo Fisher Scientific, BP152-500)
5. Nuclease-free water (Invitrogen, AM9937).
6. 100% Acetic Acid (Millipore Sigma, A6283).
7. 0.2 µm Corning 250mL Vacuum System (Corning, 430771)
8. Bluing Buffer (Agilent Tech, CS70230-2)
9. Glycerol (Sigma Aldrich, G9012)

Library construction

1. Visium Spatial Gene Expression Slide & Reagent Kit, 16 rxns PN-1000184

Equipment:

1. Cryostat for sectioning
2. Bio-Rad T100 Thermal Cycler with Thermocyler Adaptor
3. Microscope for brightfield imaging
4. Sequencing system

Safety warnings

Safety training courses on Bloodborne Pathogens, Biological Safety, Chemical Hygiene, Compressed Gases, Biological Waste Management, Personal Protective Equipment, Autoclave and Media Kitchens, General Laboratory Safety, and Cryogen Safety are required by UTD and can be taken through its BioRAFT system.

Safety Training related to Biomedical Research with Human Subjects is required by UTD and can be taken through its CITI program.

Ethics statement

All human tissue recovery, research and data handling is performed in accordance with the University of Texas at Dallas Institutional Review Board (IRB) regulations.

Before start

Ensure proper personal protective equipment (PPE) is worn and institutionally required safety guidelines are followed.

Tissue preparation and sectioning

1 Human ganglia are procured fresh frozen and stored at -80 C.

2 Tissue is embedded in OCT for cryosectioning. For a detailed embedding protocol, see steps 37-42 of **dx.doi.org/10.17504/protocols.io.kqdg32qr1v25/v1**.

3 Embedded tissue is sectioned onto slides provided for 10X Genomics Visium spatial gene expression assay (Visium Spatial Slides), following **Demonstrated Protocol CG000240** (section 2). Sections are 10 µm thick and trimmed before being adhered to the slide to fit capture areas.

Fixation, Hematoxylin and Eosin (H&E) staining, and imaging

4 Slides are fixed as described in **Demonstrated Protocol CG000160** (section 1.2).

5 Slides are H&E stained as described in **Demonstrated Protocol CG000160** (section 1.3).

6 Slides are coverslipped as described in **Demonstrated Protocol CG000160** (Appendix) using a glycerol-based mounting medium.

7 Slides are mosaically imaged at 10X using default brightfield settings on an Olympus vs120 slide scanner

8 After imaging, the coverslip is removed by soaking slides in 3X SSC buffer as described in **Demonstrated Protocol CG000160** (Appendix). Slide is then immediately processed for Library Construction.

Library Construction and Sequencing

9 Library construction is performed following **User Guide CG000239** for the Visium Spatial Gene Expression Reagent Kit.

9.1 Permeabilization & Reverse Transcription: Permeabilization time needs to be optimized for each tissue type used. Optimized time for human DRG tissue is 12 minutes as published in **10.1126/scitranslmed.abj8186**.

9.2 Second Strand Synthesis & Denaturation: no changes or optimization to protocol

9.3 cDNA Amplification & QC: cDNA amplification steps are performed with the optimal cycle numbers as determined per run, per protocol instructions. Amplification and QC are performed by UTD Genome Center as described in protocol.

9.4 Spatial Gene Expression Library Construction: no changes or optimization to protocol. These steps are performed by UTD Genome Center

9.5 Sequencing: sequencing is performed at UTD Genome Center.

Analysis

10 Raw sequencing files are processed with the 10X Genomics SpaceRanger pipeline to generate count matrices of gene expression per Visium barcode.

11 Visium sections are examined on Loupe Browser (10X Genomics) to select for barcodes of interest

12 Gene expression analysis of barcodes is done using R (version 4.3.3) for downstream processing of the SpaceRanger output.