Aug 23, 2024

Methods for xenograft tissue processing and capture for spatial transcriptomics using 10x-Genomics Visium platform

DOI
dx.doi.org/10.17504/protocols.io.4r3l2q1yxl1y/v1

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DOI: **https://dx.doi.org/10.17504/protocols.io.4r3l2q1yxl1y/v1**

Protocol Citation: Sandy S. Pineda, Hongyun Li, YuHong Fu, Glenda Halliday 2024. Methods for xenograft tissue processing and capture for spatial transcriptomics using 10x-Genomics Visium platform. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.4r3l2q1yxl1y/v1**

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

Created: August 23, 2024

Last Modified: August 23, 2024

Protocol Integer ID: 106319

Keywords: ASAPCRN, spatial transcriptomics, 10X Visium, human mouse xenograft model, mouse brain samples for spatial transcriptomic, using 10x genomics visium platform, 10x genomics visium platform, capture for spatial transcriptomic, grafted mouse brain sample, xenograft tissue processing, spatial transcriptomic, genomics visium platform, genomics visium platform this protocol, 10x genomic, mouse brain, tissue processing, using 10x

Funders Acknowledgements:
Michael J Fox Foundation
Grant ID: ASAP-000497

Abstract

This protocol details the process of preparing grafted mouse brain samples for spatial transcriptomics using 10X Genomics Visium platform.

Materials

Consumables

- RNAse AWAY (Invitrogen #10594063)
- RNA/DNAse free plastic ware
- Lo-bind RNAse/DNAse free tubes
- Poly-prep slides (Millipore-Sigma, P0425)
- haematoxylin
- eosin

Hardware

- Rotary microtome - HistoCore MULTICUT R Rotary (Leica Biosystems)
- NovaSeq6000sequencing system (Illumina ´Corporation, USA).

Troubleshooting

Safety warnings

Please refer to the Safety Data Sheets (SDS) for all materials used in this protocol prior to commencing.

Ethics statement

Animal brain tissue samples were obtained in alignment with animal research ethics committees of the University of Sydney (approval number: 2020/1824).

Application of this protocol will need prior approval by the users' institutional review board (IRB) or equivalent ethics committee(s).

Before start

To Note

- All surfaces and equipment were decontaminated in advance with RNAse AWAY, surface decontaminant
- The protocols described below require RNA/DNAse free plastic-ware and Lo-bind RNAse/DNAse free tubes.
- All buffers and water used in these protocols are RNAse/DNAse free.
- Consult all available 10x Genomics guides for detailed equipment models and suggested swaps (CytAssist workflow guides: CG000518, CG000520, CG000521, CG000495). Links in the references tab.

Tissue preparation

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1. Selected 4% PFF-fixed & paraffin-embedded (FFPE) blocks of the xenograft mouse brains at the striatum level were cut in a rotary microtome (HistoCore MULTICUT R Rotary, Leica Biosystems) at 6µm thickness.
2. Four tissue sections were cut and placed on each poly-prep slides (Millipore-Sigma, P0425). Slides were dried in an oven for 3 hours at 42 °C and placed in desiccant, to avoid any moisture forming.
3. Mounted slides were transported to the Garvan Cellular Genomics Platform for further processing (Garvan institute of Medical Research Sydney).
4. Subsequently, tissue sections were deparaffinised, stained with Haematoxylin and Eosin (H&E) following the standard CytAssist workflow (CG000520).
5. Slides were then coverslipped and imaged using a Leica microscope (DM6B, upright) at 10x magnification (CytAssist, Spatial applications imaging CG000521).
6. Images were captured, stitched and exported in low and high-resolution.

RNA hybridisation

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1. Slides were then submerged in water to gently remove the coverslip and were immediately destained and de-crosslinked.
2. RNA probes were then added and hybridised overnight.
3. Slides containing the de-crosslinked and hybridised sections, along with the Visium CytAssist Spatial Gene Expression 11×11mm slides (Visium v5 slide-FFPE v2), were transferred to the CytAssist instrument to continue with the Gene Expression Capture workflow, all steps were followed as described in the 10X user guide GC000495.
4. After capturing the probes, cDNA libraries were pre-amplified and cleaned, followed by a qPCR test, to determine the number of optimal cycles to use to construct the final libraries. Amplification cycles ranged from 13-20, for all the samples processed. cDNA libraries were constructed individually and then pooled in equimolar amounts prior to sequencing.
5. Library pools were sequenced using a NovaSeq6000 sequencing system (Illumina corporation, USA).
6. FASTQ files were inspected using FastQC (v0.12.1) to ensure reads were of consistent high quality, including high Q30 rates.

Image alignment and analysis

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- Images, both in high resolution TIFF + CytAssist TIFF, were loaded into 10X's Loupe browser (v7) using the built-in Visium CytAssist Image Alignment tool and each slide was manually aligned. Since multiple tissue sections were placed in each Visium window, following fiducial alignment, individual tissue sections on each slide were selected one at the time using the Loupe browser's spot selection tool and each tissue section alignment was exported as a separate JSON file.

- Space Ranger v2.1.1 was run once for each tissue section with default settings and using the transcriptomic reference GRCh38-2020-A andVisium_Human_Transcriptome_Probe_Set_v2.0_GRCh38-2020-A.csv probe set.
- The Space Ranger web summaries were inspected for additional quality control metrics, including high rates of valid barcodes and UMIs, high sequencing saturation, and high mapping rates to the probe set.
- Space Ranger filtered matrixes were used for downstream analysis using Seurat v5.1.0 {Hao, Yuhan, 2024}. Briefly, for each sample an individual seurat object was created.

Protocol references

CytAssist workflow guides

CG000518 - CG000518_Demonstrated_Protocol_VisiumCytAssistSpatialProtocolsFFPE_TissuePreparationGuide_RevD.pdf (10xgenomics.com)

CG000520 - CG000520_Demonstrated_Protocol_VisiumCytAssist_Deparaffin_H_E_RevC.pdf (10xgenomics.com)

CG000521 - CG000521_VisiumCytAssistImagingGuidelinesTN_RevD.pdf (10xgenomics.com)

CG000495 - My Document (10xgenomics.com)

Hao et. al. 2024 *Dictionary learning for integrative, multimodal and scalable single-cell analysis*. PMID: 37231261