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FFPE Human Brain Tissue Quality Control for Xenium

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CASCADE

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We use this protocol and it's working

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Abstract

The Xenium In Situ Gene Expression platform from 10x Genomics enables high-plex, targeted RNA detection at subcellular resolution, supporting spatially resolved cell typing and molecular profiling. While an optimized workflow has been provided by 10x, formalin-fixed paraffin-embedded (FFPE) post-mortem human brain tissue presents unique challenges due to its heterogeneity and lipid-rich composition. In particular, the quality and integrity of FFPE tissue blocks vary considerably across donors and brain regions. Rigorous quality control prior to Xenium assays is necessary.

In this protocol, we describe a standardized pre-assay QC and region of interest (ROI) selection workflow for FFPE human brain tissue. Tissue sections undergo deparaffinization followed by histological and molecular quality checks, including hematoxylin and eosin (H&E) staining to assess morphology, DAPI staining to evaluate nuclear integrity, and immunofluorescent staining to identify ROI regions. These QC steps enable the identification of regions with preserved morphology and targeted protein markers, ensuring that selected ROIs are biologically relevant and suitable for high-quality Xenium analysis.

Guidelines

This protocol is for quality check of FFPE tissue blocks and selection of regions of interest (ROIs) for Xenium experiments.

Materials

1. CitriSolv (d-limonene) Clearing Agent – Decon Laboratories, Inc.
CN: 1601H
2. 100% Ethanol – Fisher Chemical
CN: A4094
3. 70% Ethanol – Decon Laboratories, Inc.
CN: 2405
4. Citrate Buffer pH 6 – Sigma-Aldrich
CN: C9999
5. 10X Phosphate-Buffered Saline – Quality Biological
CN: 119-069-131
6. Bovine Serum Albumin – Sigma-Aldrich
CN: A7906
7. TrueBlack Lipofuscin Autofluorescence Quencher – Biotium
CN: 23007
8. ProLong Gold DAPI Mountant – Invitrogen
CN: P36931
9. ProLong Glass Mountant – Invitrogen
CN: P36984
10. Cover Glass – ThorLabs Inc.
CN: CG15KH
11. Glass Slides – Globe Scientific Inc.
CN: 1358W
12. Harris Hematoxylin Solution – Sigma-Aldrich
CN: HHS32
13. Eosin Solution – Sigma-Aldrich
CN: 1098442500
14. Shandon Bluing Reagent – Epridia
CN: 6769001
15. Microscope Slides – Globe Scientific
CN: 1358W

Safety warnings

N/A

Ethics statement

All brain specimens were derived from two longitudinal clinico-pathological cohort studies, that is, the ROS and MAP. In both cohorts, participants did not have known dementia at the time of enrollment. Participants agreed to receive clinical evaluation each year and to donate their brain at the time of death. Each study was approved by the institutional review board of Rush University Medical Center. All participants signed a written informed consent, Anatomical Gift Act and repository consent.

Before start

Prior to the quality check steps in this protocol, prepare a microscope slide mounted with an intact tissue section from your interested block. Refer to the **Block Facing, Rehydration and Sectioning** sections from "**FFPE Human Brain Tissue Serial Sectioning for Xenium**" protocol for specific procedures.

Tissue Deparaffinization

1 Incubate the tissue sections at **60°C** for **30 min**

2 Cool down the slides at room temperature (RT) for **7 min**

3 Incubate the tissue sections in **Xylene** for **10 min**. Repeat once

4 Incubate the tissue sections in **100%** Ethanol for **3 min**. Repeat once

5 Incubate the tissue sections in **96%** Ethanol for **3 min.** Repeat once

6 Incubate the tissue sections in **70%** Ethanol for **3 min**

7 Wash the tissue sections with Milli-Q **water** for **20 sec.** Immediately proceed to H&E staining.

H&E Staining

8 Incubate the tissue sections in Milli-Q water for **2 min**

9 Incubate the tissue sections in **Hematoxylin** solution for **20 min**

10 Wash the tissue sections with Milli-Q **water** for **1 min.** Repeat twice

11 Incubate the tissue sections in **Bluing** Solution for **2 min**

12 Wash tissue with Milli-Q **water** for **2 min**

13 Incubate the tissue sections with **70%** Ethanol for **3 min**

14 Incubate the tissue sections with **95%** Ethanol for **3 min**

15 Incubate the tissue sections with **Eosin** Solution (400:1 mixture of Eosin and 0.1M Hydrochloric acid) for **3 min**

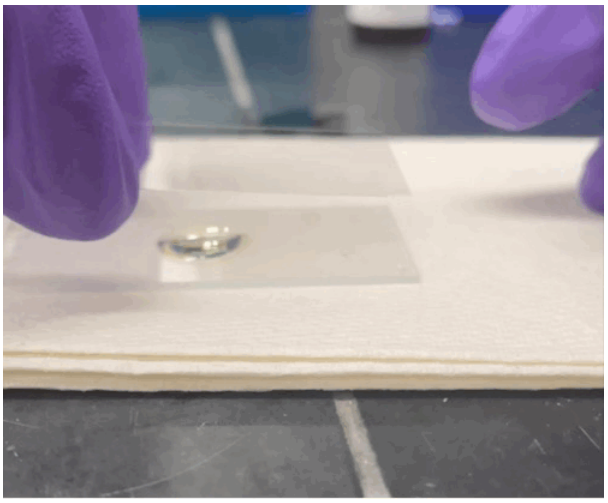
16 Incubate the tissue sections in **95%** Ethanol for **30 sec.** Repeat once

17 Incubate the tissue sections in **100%** Ethanol for **30 sec.** Repeat once

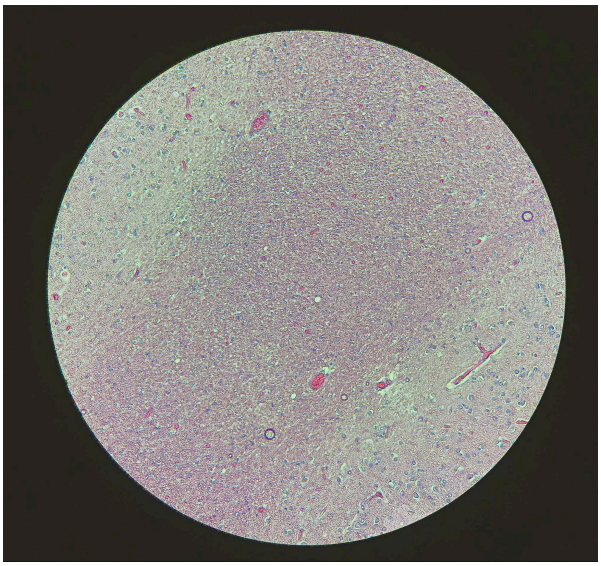
18 Incubate the tissue sections in **Xylene** for **3 min.** Repeat once

19 Let the tissue sections **dry** completely. Mount the tissue slide by adding ~100 uL of **Prolong Glass**, depending on tissue size, on the tissue section.

20 Coverslip tissue slide by taking a cover glass and placing one finger on one side of the cover
glass. Then, slowly and gently lower the cover glass using your other hand to guide the coverglass down until the entire slide is coverslipped. Avoid bubbles.



Cover glass placement



H&E staining of FFPE human brain tissue at 20X.

Note

There are two suggested options for performing the incubation steps:
- A glass staining jar or 50 mL conical tube can be used to insert slides and submerge tissue in the solution.
- Another option is adding approximately 250 uL (may vary depending on tissue size) of solution directly on the tissue ensuring the entire tissue section is covered. This option requires more thorough washing using a wide mouth wash bottle.

DAPI and Immunofluorescent Staining for Region of Interest (ROI) Selection

21 Deparaffinize the tissue sections with a **clearing agent** for **20 min**. This protocol uses d-limonene as a non-toxic Xylene alternative.

22 Immediately rehydrate tissue with graded ethanol washes starting with **100%** Ethanol **twice**
and then **70%** Ethanol

23 Wash the tissue slide with 1X phosphate-buffered saline (**PBS**) for **3 times** at RT

24 Perform heat-induced antigen retrieval using **citrate buffer** in a **microwave** (800W, 30% power) for **25 min**

25 Wash the tissue sections with 1X **PBS** for **3 times** at RT

26 Add 3% bovine serum albumin (**BSA**) for **30 min** as a blocking agent atRT

27 Incubate the tissue sections with **primary antibodies** diluted in 1% BSA with 1X PBS **overnight** at **4°C**

Note

The antibodies used in the immunofluorescent staining will depend on the project's objectives.

28 Wash the tissue sections slide with 1X **PBS** for **3 times** at RT

29 Incubate the tissue sections with Alexa Fluor **secondary antibodies** diluted in 1% BSA with 1X PBS for **1 hr** at RT

30 Wash the tissue sections with 1X **PBS** for **3 times** at RT

31 Treat the tissue sections with 1X **TrueBlack lipofuscin quencher** for **2 min** at RT to minimize autofluorescence

32 Wash the tissue sections with 1X **PBS** for **3 times** at RT

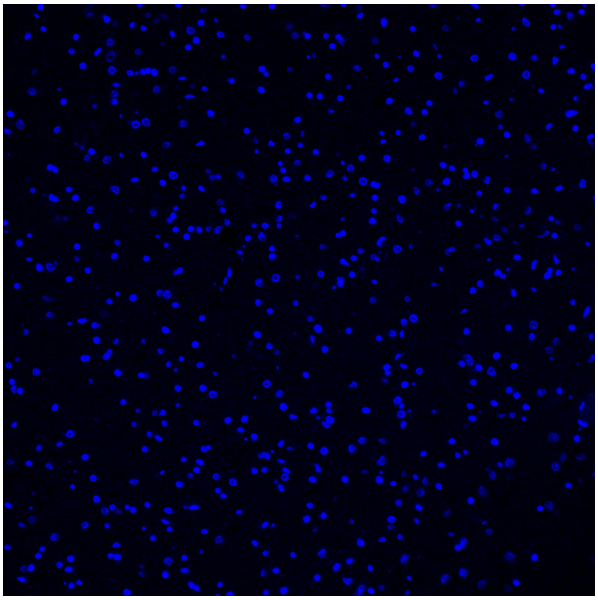
33 Mount slide by adding ~100 uL of an **anti-fading reagent** containing DAPI to visualize nuclei

34 Coverslip slide by slowly and gently releasing the cover glass over the tissue slide

Note

When coverslipping, ensure the tissue is not too dry to prevent air bubbles and to allow proper adhesion of the cover glass to the tissue slide. If air bubbles form, use a 10 uL pipette tip on top of the cover glass to gently guide the bubbles away from the tissue toward the edge of the slide.

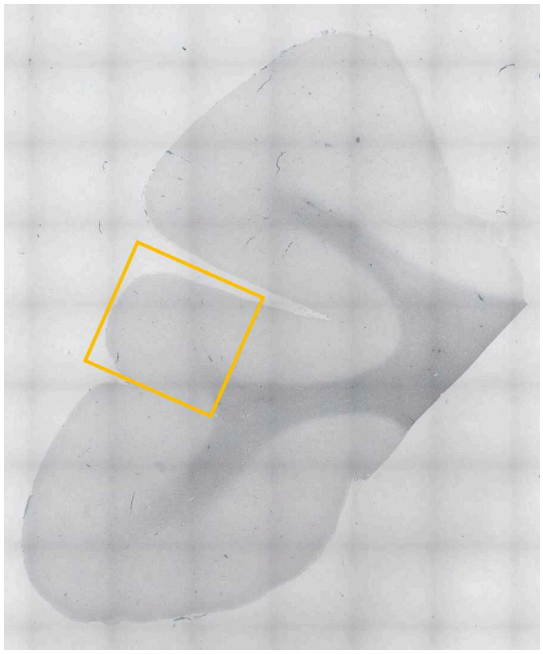
35 Visualize the staining using a fluorescent microscope to determine an appropriately sized tissue area for ROI selection



DAPI staining of FFPE human brain tissue at 20X.

Note

The ROI selected for this protocol is approximately 8 mm × 6 mm in size to accommodate three sections within the capture area. ROIs will be selected according to the experiment objectives.



4X Brightfield image of whole tissue section with ROI annotated in yellow box