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Preparation post-mortem brain for spatial transcriptomics

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Protocol status: Working

We use this protocol and it's working



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Abstract

This protocol describes the preparation of post-mortem brain tissue for spatial transcriptomics (Xenium and GeoMx). Guidelines provided to work with flash frozen tissue (FF) and formalin-fixed paraffin embedded tissue (FFPE).

Flash frozen tissue (FF)

- 1 **Obtain tissue:** Tissue blocks from brain bank  100 mg
Storage: -80°C in sealed cryovials/ependorf tubes until use.
Transport: dry ice
Work always in RNase-free conditions, keeping samples ice-cold at all times to maintain RNA integrity.
- 2 Collect tissue block from container on dry ice
- 3 Place tissue block on a sterile, RNase-free plastic surface on dry ice
- 4 Cut to desired size using sterile blade, pre-cooled at -20°C
 - *Note: slide area is 10.45 mm x 22.45 mm, so ensure blocks are not larger than this size to avoid difficulty mounting*
- 5 **Embed tissue block in OCT** (in fume hood)
 - a. Place an isopentane bath in a box of dry ice, and allow to cool with the lid on (~5 minutes)
 - *Tip: check isopentane has sufficiently cooled by placing a piece of dry ice in the liquid (using forceps), if it does not bubble, it is sufficiently cool*
 - b. Once cool, fill the base of a cryomold with OCT, place tissue on top (taking careful note of orientation and any key structures), then fully cover tissue with OCT
 - *Tip: take a picture of the tissue to recall orientation within block*
 - c. Place cryomold in the isopentane bath, ensuring isopentane does not overflow into the cryomold
 - d. Place the lid on the box and leave to solidify for ~10 minutes, until OCT is entirely white
 - e. Remove cryomold from isopentane bath, wrap in foil (then if possible into a sealed plastic bag), label then store at -80C
- 6 **Cryosection sample**
 - a. Retrieve the wrapped cryomold from -80C and place at -20C for ~15 minutes to adjust to temperature
 - b. Mount the sample onto cryostat then section at 5 um (up to 10 um) thick sections
 - *When mounting sections onto slides, consider the area of the slide suitable for the technology (practice with mock slides), this may require trimming of surrounding OCT to ensure neither tissue, nor OCT, extends outside of allowed region*

- Xenium: 10.45 mm x 22.45 mm, ensure tissue/OCT does not cover white outline or fiducial area (denoted with + signs inside white line)
- GeoMx: 14.1 mm x 35.3 mm, tissue and OCT can extend outside the area and be removed at a later date, but only the tissue within this area can be analysed
- c. Collect 1-2 sections on standard superfrost slides, and perform H&E to confirm correct region selection and confirm tissue quality
 - *e.g. finding neuromelanin-positive cells in SNpc for midbrain sections or putamen in basal ganglia sections*
- d. Make any necessary adjustments, then collect 5 um section (or as required for specific technology) on a slide suitable for chosen technology
 - Xenium: specific Xenium slide
 - GeoMx: Superfrost Plus or BOND Plus slide
- e. Prepare several adjacent slides from each sample on Superfrost Plus slides, to ensure sufficient sections for additional staining and any desired validation
- f. Once sectioned, store slides at -80C (for up to 4 weeks) in a sealed slide-storage box until use

7 Follow technology-specific protocol

Formalin-fixed paraffin Embedded (FFPE)

8 **Obtain tissue:** FFPE blocks or pre-cut sections can be requested from brain bank (ask your technician about fixation protocol (where possible samples will have been prepared with 10% neutral buffered formalin or 4% PFA, for a duration suitable for the tissue size)

Storage: 4C in sealed box, avoiding exposure to light

Transport: RT (short) or with ice packs (long)

Work always in RNase-free conditions.

9 Section samples using microtome

- a. If needed, FFPE blocks can be rehydrated in a bath of ice and water (nuclease free, including water used to make ice) for 10-30 minutes
- b. Mount the sample onto microtome then section at 5 um
- c. Collect sections into a 38C water bath, and leave to float for at least 1 minute, then mount onto slides and leave to air dry ~10 minutes at RT
 - *When mounting sections onto slides, consider the area of the slide suitable for the technology (practice with mock slides), this may require trimming of surrounding paraffin to ensure neither tissue, nor paraffin, extends outside of allowed region*
 - Xenium: 10.45 mm x 22.45 mm, ensure tissue/paraffin does not cover white outline or fiducial area (denoted with + signs inside white line)
 - GeoMx: 14.1 mm x 35.3 mm, tissue and paraffin can extend outside the area and be removed at a later date, but only the tissue within this area can be analysed



- d. Collect 1-2 sections on standard histology slides, and perform H&E to confirm correct region selection
 - *e.g. finding neuromelanin-positive cells in SNpc for midbrain sections or putamen in basal ganglia sections*
- e. Make any necessary adjustments, then collect 5 um section (or as required for specific technology) on a slide suitable for chosen technology
 - Xenium: specific Xenium slide
 - GeoMx: Superfrost Plus or BOND Plus slide
- f. Prepare several adjacent slides from each sample on Superfrost Plus slides, to ensure sufficient sections for additional staining and any desired validation
- g. Once sectioned, store slides at 4C (short/long term) in slide-storage box

10 Follow technology-specific protocol (most protocols begin with deparaffinisation)