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Carver et al, Aged Brain Spatial Profiling - Tissue Processing V.1

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We use this protocol and it's working

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Abstract

This protocol provides the steps for mouse brain tissue processing and preparation for use in immunohistochemistry and spatial -omic techniques used in Carver et al., "Senescent- and disease-associated microglia are modifiable features of aged brain white matter"

The steps here should be considered as immediately proceeding the protocol for each separate process to correctly stain and image brain sections in the respective techniques.

Perfusion

1 Euthanize mouse with intraperitoneal injection of 32.5mg/ml pentobarbital

2 Extract blood via inferior vena cava with a syringe

3 Perfuse transcardially with ice-cold PBS

Brain Processing

4 Decapitate mouse and extract brain from skull

5 Bisect the two hemispheres of the brain

6 Submerge right hemisphere in 4% PFA in PBS for 24 hours

7 Microdisect left hemisphere hippocampus from hippocampus adjacent white matter, place each tissue in 1 mL TRIzol in ceramic bead tube for downstream RNA processing

Brain sections for immunohistochemistry

8 Steps for cutting brain slices for traditional immunofluorescence

8.1 In sucrose solutions, brain should initially float and then eventually sink over the time period in each step.
Transfer brain from PFA to 10% sucrose in PBS for 24 hours
Transfer brain from 10% sucrose to 20% sucrose in PBS for 24 hours
Transfer brain from 20% sucrose to 30% sucrose in PBS for 24 hours

8.2 Cryopreserve brain hemisphere in 30% glycerol, 30% ethylene glycol, 40% PBS w/v at -80C

8.3 Embed brain in Tissue-tek O.C.T. compound and freeze the brain block in a cassette on top of dry ice

8.4 Cut sagittal sections at 30 µm thickness on a Leica CM3050 S cryostat at -20C

8.5 Place sections in a well plate containing ice-cold PBS + 0.01% sodium azide and maintain plate in 4C environment prior to further processing

Brain sections for spatial -omics

9 Steps for cutting brain slices for spatial -omics profiling for GeoMx and CosMx

9.1 Transfer PFA-fixed brain to cold 70% ethanol

9.2 Embed brain in paraffin wax block within 24 hours

9.3 Cut sagittal sections at 5 µm thickness with a sliding microtome

9.4 Place cut paraffin section in deionized water bath heated to 40-42C

9.5 Mount the section on a Superfrost Plus glass slide by placing the slide submerged under the section and pulling upward such that the section is within the correct window for GeoMx or CosMx (see attachment)

9.6 Allow water to drain from slide and dry and bake slide for 30 min at 60C