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Coronal cryosectioning of mouse brains

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Protocol status: Working

We use this protocol and it's working

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Abstract

Cryostat coronal cryosectioning of mouse brain tissue

Before start

PFA-perfused, 30% sucrose-cryoprotected, and flash-frozen mouse brains are stored at -80C until cryosectioning




Procedure

25m

- 1 Retrieve frozen mouse brain stored at -80 °C .
- 2 Place brain in cryostat. Set chamber temperature to -16 °C to -20 °C . Set brain holder temperature to -20 °C . Let brain equilibrate to temperature for at least 00:20:00 before sectioning. 20m
- 3 Insert chuck and blade into cryostat.
- 4 Apply a drop of OCT on the chuck, and affix brain vertically (olfactory bulbs up).
- 5 Apply another layer of OCT at the base of the brain, turning the brain-holder with the other hand. Cover the cerebellum and the junction between the cerebellum and cortex with OCT. Allow OCT to freeze solid (~ 00:05:00). 5m
- 6 Cut 16 to 30 micron sections. Adjust angle of medio-lateral and ventro-dorsal axis so that sections are free of cutting artifacts, symmetrical, and well-aligned to reference atlas.
- 6.1 Use of anti-roll plate is optional and can help reduce rolling of sections, but must be carefully adjusted to avoid cutting artifacts.
- 6.2 For immunohistochemistry we typically cut 30-micron sections for best results. For RNAscope we typically cut 16 to 20-micron sections.
- 7 Collect free-floating sections in 48-well plate.
- 7.1 Label your plate lid and base with brain/experiment details using lab marker and/or lab tape.
- 7.2 Pre-fill wells with 1 mL 1X PBS containing 0.01% sodium azide (100mg/L).



- 7.3 Can use syringe with blunt needle to collect sections after sectioning. Dip needle into PBS and collect section by gently touching it on the side of the section.
- 7.4 Collect 5 sections per well. Or for experiments that require finer resolution of coordinate location can collect sections serially, adding 1 section per well until the end of the plate and start back at well #A1.
- 8 Use parafilm to seal the lid to the plate (air-tight) and refrigerate at  4 °C until further processing.