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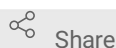
# Cryosectioning mouse brain

Pranay Srivastava<sup>1</sup>, Waijiao Cai<sup>2</sup>, Xiqun Chen<sup>1</sup>

<sup>1</sup>Department of Neurology Massachusetts General Hospital Harvard Medical School Charlestown MA 02129 USA, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network Chevy Chase MD 20815 USA;

<sup>2</sup>Department of Neurology Massachusetts General Hospital Harvard Medical School Charlestown MA 02129 USA, Department of Neurology Longhua Hospital Shanghai University of Traditional Chinese Medicine Shanghai 200032 China

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ktdonahue

## ABSTRACT

This protocol details the cryosectioning of mouse brain.

## ATTACHMENTS

[ds8vbjtc7.pdf](#)

## DOI

[dx.doi.org/10.17504/protocols.io.e6nvw54jdvmk/v1](https://dx.doi.org/10.17504/protocols.io.e6nvw54jdvmk/v1)

## PROTOCOL CITATION

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## KEYWORDS

Cryosectioning, Mouse brain, ASAPCRN

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Jun 16, 2021

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Jul 15, 2022

## OWNERSHIP HISTORY

Jun 16, 2021  Urmilas




Jun 21, 2021  ktdonahue

## PROTOCOL INTEGER ID



50814




### Cryosectioning mouse brain 1w 0d 0h 0m 20s

1 

Place the mouse brain in the mouse brain slicer (  **On ice** ). Cut the hinder of the brain and put it in 4% PFA  **Overnight** at  **4 °C** .

2 

Change the solution to 30% sucrose/PBS at  **4 °C** until the brain sinks in the bottom (  **Overnight** ).


3 Change the solution to 60% sucrose/PBS at  **4 °C** for  **48:00:00** -  **120:00:00** (optional).

1w


4 Cut 1/2 to 2/3 of the cerebellum to make a flat seat.

5 Cut the pieces of foil for the tissue wrapping and label the pieces with chemical resistant marker.

6 Place a small beaker containing 2/3 2-methylbutane, at least half buried in dry ice. Allow it to cool enough (when you see it turn white and thick at the bottom of the container).

7 Gently place the mouse brain into 2-methylbutane and allow it to sit for a while until it no longer "smoking" (~  **00:00:20** ).

20s

- 8 Place a layer of OCT on the foil on dry ice. Wait a few seconds until OCT turns to half opaque.
- 9 Place the brain on the layer of OCT and place another layer of OCT to cover the brain.
- 10 Wrap the brain with the labeled foil and store the brain at  $-80^{\circ}\text{C}$  until sectioning.
- 11 Adjust the cryostat to: chamber  $-22^{\circ}\text{C}$  , specimen  $-23^{\circ}\text{C}$  ,  $30\text{ }\mu\text{m}$  .
- 12 Place the brains in cryostat when it reaches  $-22^{\circ}\text{C}$  for half hour to allow the temperature balanced.
- 13   
In 12-wells plate, add  $1.5\text{ mL}$  cryo-protective solution (30% Glycerol, 30% Ethylene Glycol in PBS) per well.
- 14 For stereological dopaminergic neurons counting, start to collect the sections when it close to substantial nigral (midbrain is apple-ish like shape. Hippocampus spread to 2/3 of the cortex). Collect ~10-12 sections per well. Store plates at  $-20^{\circ}\text{C}$  until staining.

A	B	C	D	E
	1	2	3	4
A Mouse# 1	Section 1,5,9,13,17,21,25, 29	Section 2,6,10,14,18, 22, 24, 30	Section 3,7,11,15,19,23,25, 31	Section 4,8,12,16,20,24,28, 32
B Mouse# 2				
C Mouse# 3				