Cryosectioning mouse brain

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 4C.
2. Change the solution to 30% sucrose/PBS at 4C until the brain sinks in the bottom (overnight).
3. Change the solution to 60% sucrose/PBS at 4C for 2-5 days (optional).
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" (~20 seconds).
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 -80C until sectioning.
11. Adjust the cryostat to: chamber -22C, specimen -23C, **30 µm**.
12. Place the brains in cryostat when it reaches -22C for half hour to allow the temperature balanced.
13. In 12-wells plate, add 1.5 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 -20C until staining.

	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				