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DIO-SPOTlight mice tissue processing

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We use this protocol and it's working

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Abstract

This protocol contains a relatively standard description of transcardially perfusing mice followed by tissue slicing using a cryostat.

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1 Anesthetize mice using the drop jar method containing 3-5% aerosolized isoflurane.

2 Perform a toe pinch to ensure the mouse is fully anesthetized prior to pinning the limbs down.

3 Use scissors to open the thoracic cavity and perform a transcardial perfusion by inserting a needle in to the right ventricle and cut the top of the left lobe, immediately perfuse using ~25 ml ice cold PBS followed by ~30 ml ice cold 4% paraformaldehyde (PFA).

4 Cut off the head and remove the skin behind the ears to behind the eyes.

5 Open the skull and remove the brain.

6 Place the brain in 4% PFA + 20% sucrose in 1x PBS for 24 hours at 4C.

7 The following day transfer the brains to 30% sucrose in 1x PBS for an additional 24 hours at 4C.

8 Embed the tissue in optimal cutting temperature compound (OCT) and freeze.

9 The OCT embedded brains are then sliced cryostat into 40 um sagittal sections and store in 1x PBS with 0.01% sodium azide at 4C until IHC is performed.

10 If spinal cord dissections are performed following the removal of the brain use the following landmarks to dissect the spine: cervical, base of skull to caudal T1 lamina; thoracic, 0.6 cm segment centered on T9 lamina; and lumbar, identified based on L1-L6 nerve roots.

10.1 Repeat steps 6 - 9 with the exception that the spinal cord sections were directly mounted onto microscope sildes.

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