Sectioning and HE staining of Mouse Brain and embryo by Cryostat V.1

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Protocol status: Working
We use this protocol and it's working

DISCLAIMER

ABSTRACT

This protocol describes how to use the cryostat to prepare and slice mouse brain and embryo sections for HE staining and following spatial transcriptomic experiments.

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Preparation Methods for E12.5 mouse-embryo-eye

1 E12.5 pregnant female mice was anesthetized with carbon dioxide, the whole uterus was collected and washed 3 times in ice-cold DPBS;

2 Uterus was separated under stereo microscope, each embryo was numbered and photographed with Motorized Fluorescence Stereo Zoom microscope (ZEISS, Axio Zoom V16);

The morphology of E12.5 mouse embryo.

3 The yolk sac was collected to extracted DNA for genotyping (identification of sex);

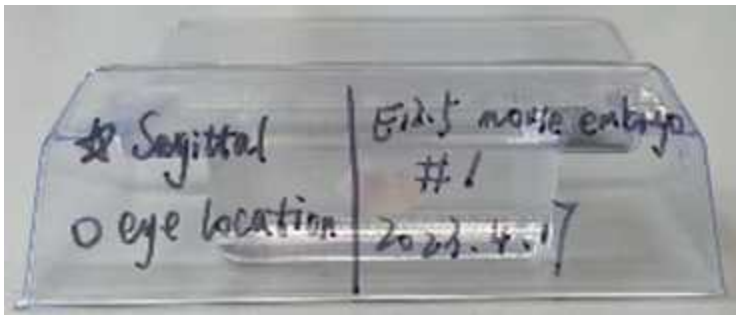
4 Using dust-free paper to gently wiped the liquid on the surface of the embryo, the embryo was rinsed with ice-cold Tissue-Tek OCT (Sakura, 4583), and then moved to encapsulation box with ice-cold OCT;

5 Mouse embryos were collected from pregnant C57BL/6J female mice at embryonic day 12.5

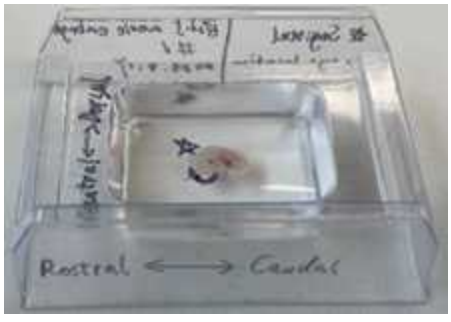
(E12.5). Mouse brain was dissected from 8-week-old C57BL/6J male mice;

6 The air bubbles were carefully removed with the syringe, and the embryo was placed in sagittal position with tweezers;

embedding method



embedding method



embedding method

7 The location of embryonic eye was circled, and marked the orientation of embryo, then tissues was transferred to a -80°C freezer for snap-frozen and storage;

8 Embryos of average size and normal phenotype were selected for subsequent cryosection and sequencing (note: the embryos used in our benchmarking analysis came from a litter of mice);

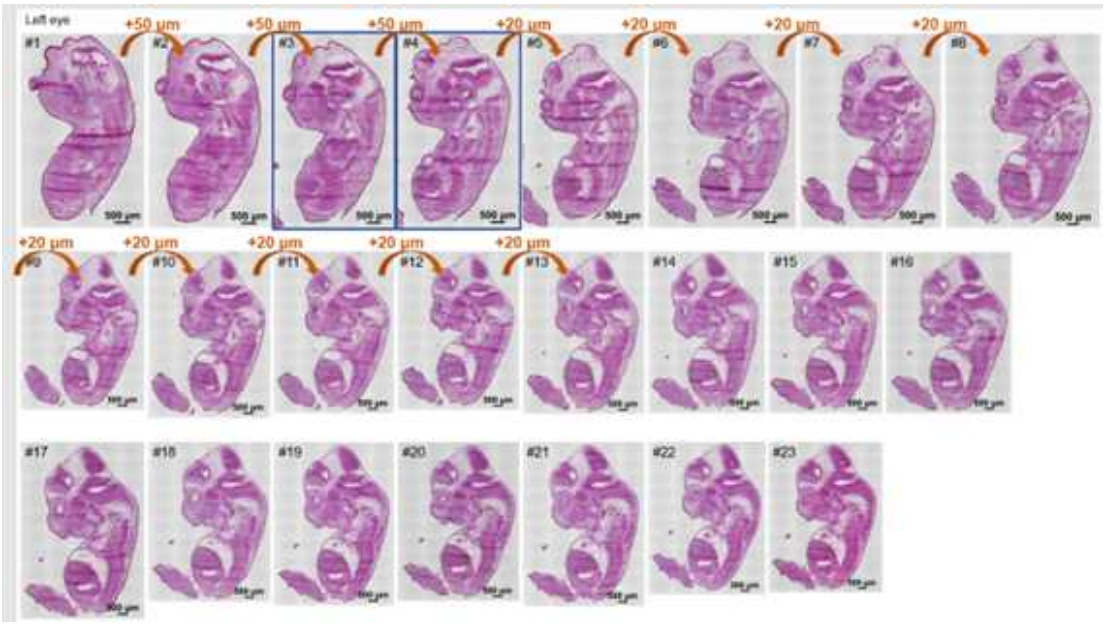
9 Before sectioning, tissue block was took out from -80°C freezer and placed in cryostat (Leica, CM1950) to balance for at least 30 min;

Set the chamber temperature (CT) and object temperature (OT).

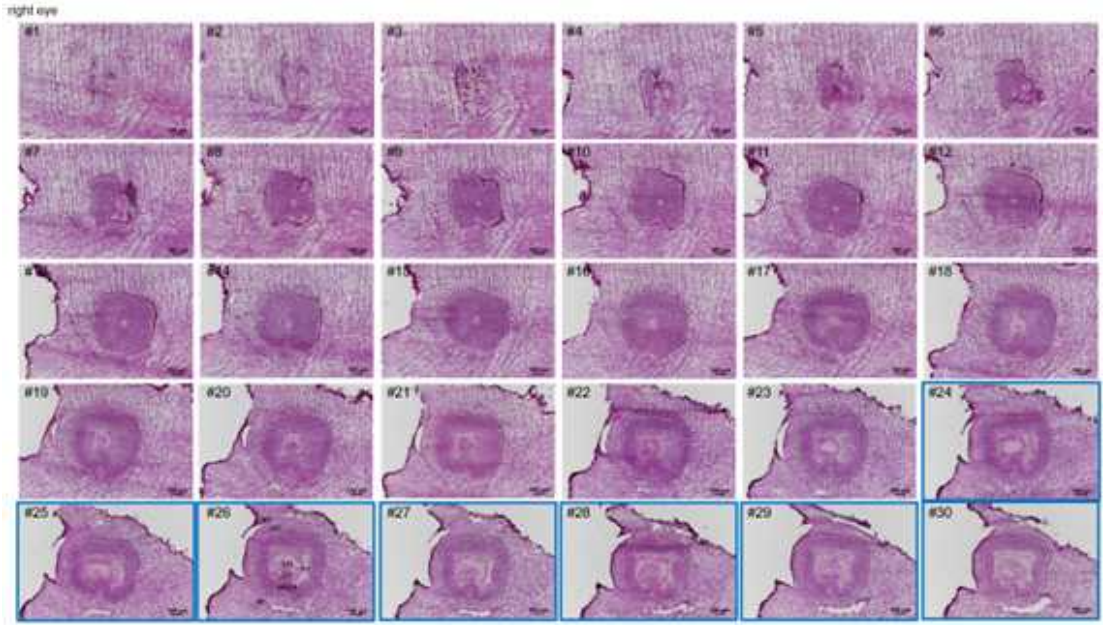
10 The tissue block was smoothly glued to the sample head, so that the embryo was sectioned in sagittal position. If necessary, the angle can be fine-tuned so that the blade section is strictly parallel to the cross-section of the tissue block;

11 Cryosections were cut at a thickness of 10 μm, both left eye and right eye can be collected;

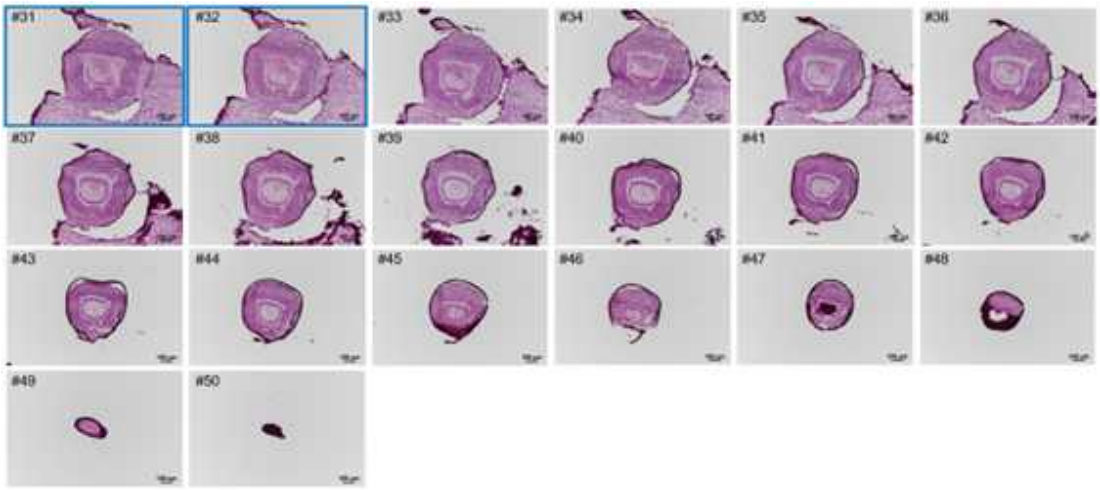
12 The structure of the sequenced cryosections were shown in the followed image:



HE staining of cryosections. The distance of cryosections was marked.



HE staining of cryosections.



HE staining of cryosections.

13 HE staining procedure: cryosections were balanced at room temperature for 30 min, and then fixed with 4% PFA for 3 min. Then, the sections were washed with ddH_2O for 2 min, stained with hematoxylin for 6 min, washed with ddH_2O, stained with eosin for 2 min, washed with ddH_2O. After that, sections were gradient dehydrated (75% ethyl alcohol for 1 s, 85% ethyl alcohol for 1 s, 95% ethyl alcohol for 1 s, 100% ethyl alcohol for 1 s, 100% ethyl alcohol for 1 min), cleared (xylene for twice), and sealed with Permount TM Mounting Medium after airing. Finally, the figure was scanned using Motorized Fluorescence Microscope (Nikon, Ni-E).

Preparation Methods for male mouse brain

14 8-week-old male mice was anesthetized with carbon dioxide and decapitated;

15 The whole brain was rapidly dissected, numbered and photographed with Motorized Fluorescence Stereo Zoom microscope;

The morphology of mouse brain.

16 Using dust-free paper to gently wiped the liquid on the surface of the brain, the brain was rinsed with ice-cold Tissue-Tek OCT (Sakura, 4583), and then moved to encapsulation box with ice-cold OCT;

embedding method

embedding method

17 The air bubbles were carefully removed with the syringe, and the brain was placed properly with tweezers;

18 The location of hippocampus was circled, and marked the orientation of brain, then tissues was transferred to a -80°C freezer for snap-frozen and storage;

19 Brain samples of average size and normal phenotype were selected for subsequent cryosection and sequencing;

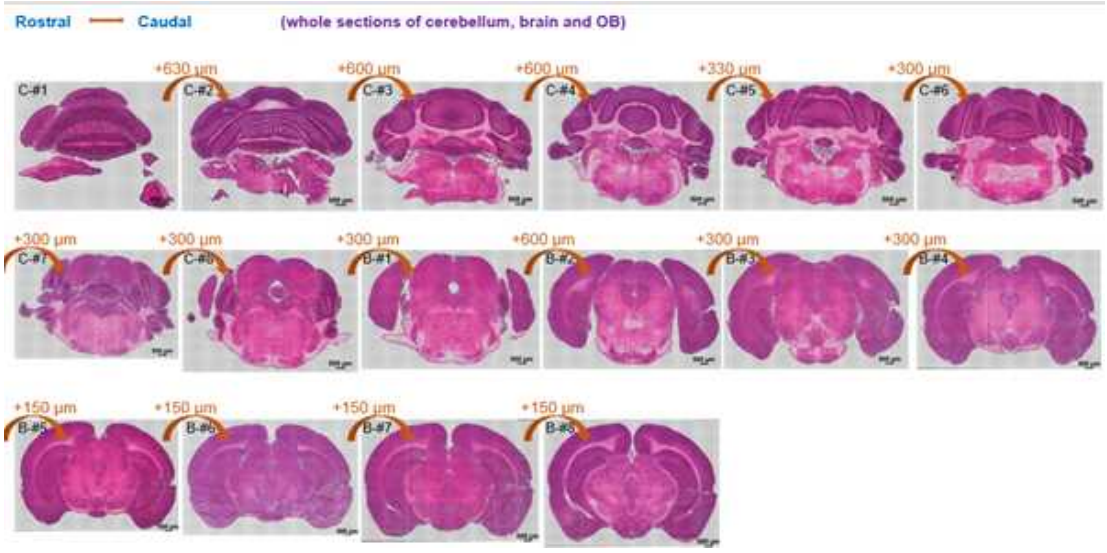
20 Before sectioning, tissue block was took out from -80°C freezer and placed in cryostat (Leica, CM1950) to balance for at least 1 h;

Set the chamber temperature (CT) and object temperature (OT).

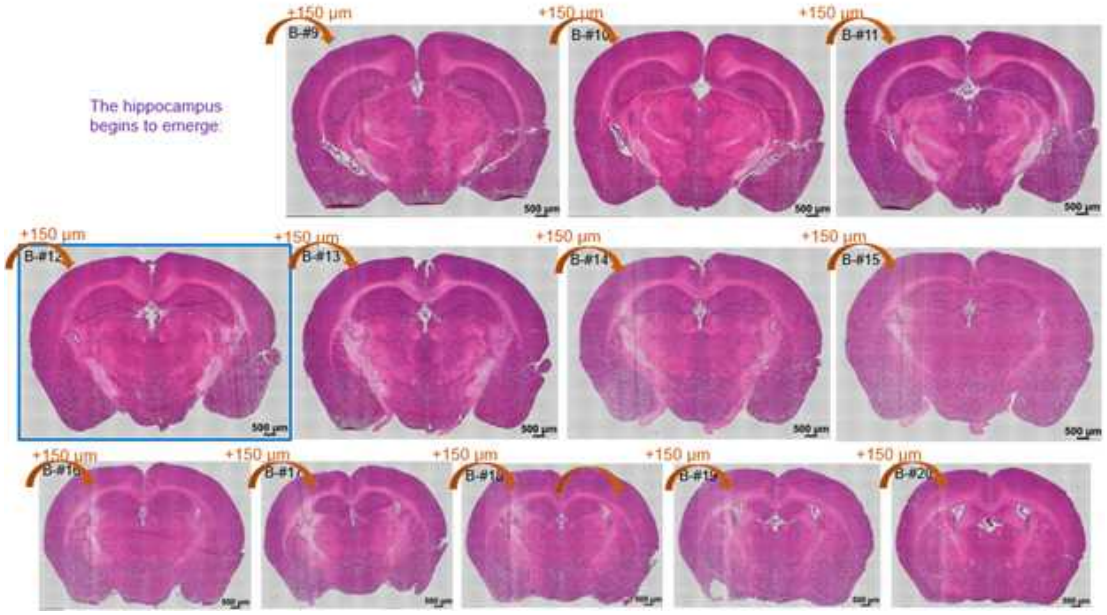
21 The tissue block was smoothly glued to the sample head, and the cerebellum was oriented towards the experimenter, so that the brain was sectioned in coronal position. If necessary, the angle can be fine-tuned so that the blade section is strictly parallel to the cross-section of the tissue block;

22 Cryosections were cut at a thickness of 10 μm;

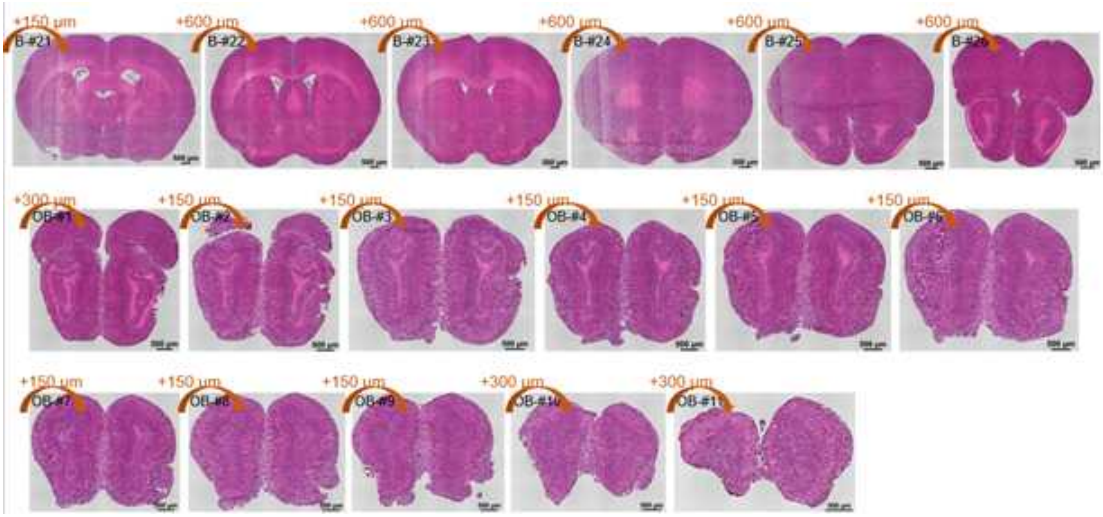
23 The structure of the sequenced cryosections were shown in the followed image:



HE staining of cryosections. The distance of cryosections was marked.



HE staining of cryosections. The distance of cryosections was marked.



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24 HE staining procedure: cryosections were balanced at room temperature for 30 min, and then fixed with 4% PFA for 3 min. Then, the sections were washed with ddH_2O for 2 min, stained with hematoxylin for 6 min, washed with ddH_2O, stained with eosin for 1 min, washed with ddH_2O. After that, sections were gradient dehydrated (75% ethyl alcohol for 1 s, 85% ethyl alcohol for 1 s, 95% ethyl alcohol for 1 s, 100% ethyl alcohol for 1 s, 100% ethyl alcohol for 1 min), cleared (xylene for twice), and sealed after airing. Finally, the figure was scanned using Motorized Fluorescence Microscope (Nikon, Ni-E).