Dissection for spinal cord analysis

Note: this protocol is intended for analysis of commissural axon guidance in the spinal cord. Therefore, we will use tissue from E11.5-E13.5 embryos.

Acronyms and shorthands:

PFA	Paraformaldehyde
PBS	Phosphate-buffered saline
DEPC	Diethyl Pyrocarbonate
Sor	Sorenson´s buffer
RT	Room temperature
O/N	Over night

Prepare ahead of time:

1. A Petri dish with Sylgard and insect pins (Minutiens 0.2mm diameter, Fine Science Tools 26002-20) in it (you need 5 pins per embryo)
2. 1x DEPC-PBS
3. 4% DEPC-PFA in 1x PBS: 10mL of 16% PFA + 4mL 10x PBS up to 40mL total volume with DEPC H_2O
4. 30% Sucrose in 1xPBS**:** 15g Sucrose + 5mL 10xPBS up to 50mL DEPC H_2O

Dissection and embedding

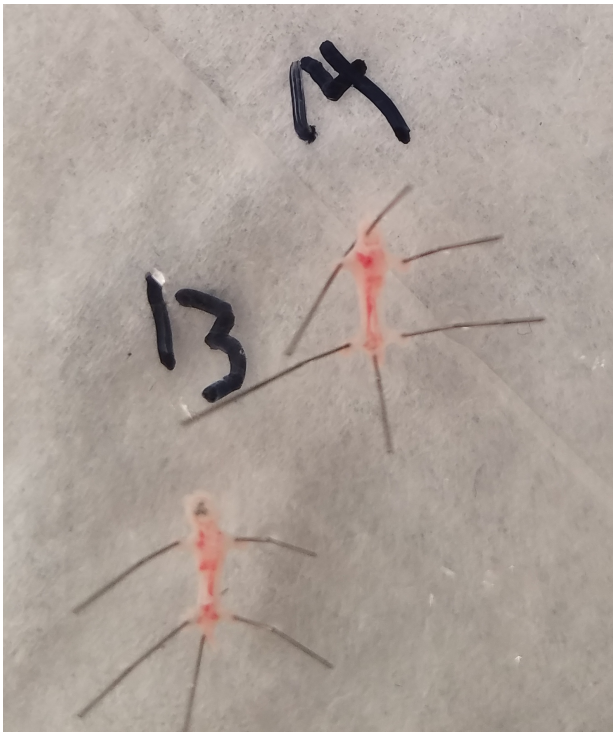
Day 1

1. CO_2 the timed-pregnant female – give CO_2 for 5-10min (or until they are unresponsive), cervical dislocation.
2. Open the abdominal cavity by lifting the skin with forceps (thick, blunt ends) and cut the skin with scissors. Cut the fascia, peritoneum and abdominal musculature. To do this, lift the tissue layers with forceps and cut with scissors to expose abdominal contents.
3. Carefully remove uterine horns and place all the embryonic sacs on a Petri dish.
4. Using forceps, cut each embryonic sac to expose the embryo. They will look like this:

5. Under the binocular and using forceps remove the head. The head can be used to extract proteins or RNA:
    a. **For proteins→** directly submerge in Lysis buffer and homogenize with homogenizer. Incubate rotating in the **cold room** for **10-30min**. Centrifuge at **16,000g** for **20-30min** at **4°C**. Transfer the supernatant to a clean tube, this is the protein extract.
    b. **For RNA→** submerge the <0.5cm tissue into 5x-10x the volume of RNAlater (Invitrogen). 500µL of RNAlater should be enough for one head. RNA sample will be

stabilized and could be stored at room temperature for 1 week without compromising the RNA quality. Store at **4°C overnight** as soon as done with all the sample. Then, transfer to -20°C or -80°C for longer storage. Before freezing, the excess of RNAlater solution can be removed to make the thawing quicker. Whenever ready, follow the RNA extraction protocol from the RNAeasy Qiagen kit.

6. The rest of the body will be used for sectioning, so stretching the tissue is crucial (embryos tend to bend).
7. Use a Petri dish that already has the pins and 1xPBS or 1xSorenson's. Pin down the neck first with the ventral side (the heart side) facing up. Remove all excess of extra embryonic tissue. Then, pin down the tail making sure the embryo stretches so that the spinal cord is semi-flat on the plate. Then pin the arms and finally the legs.



8. Take some tail or heart sample for genotyping and label the embryos!
9. When done pinning down all the embryos, remove the buffer and add **4% PFA** to the plate. Fix for **30min** at **RT**.
10. Wash with buffer 3 times for **10mins** at **4°C**
11. Add 30% sucrose and incubate at **4°C O/N**.

<u>Day 2</u>

12. Embed using embedding molds (the rubber ones that fit 21 blocks, SIPI 2449) by putting NEG first and then transfer each embryo, point tail towards me, lay dorsal side up. Add NEG up to the top and do not allow body to sink to the bottom. Align it well.
13. Cover metal rack with liquid nitrogen in the bucket. Make sure that the $N_2(l)$ bubbles on top of the rack (this means it is making it cold). Also make sure that the $N_2(l)$ has evaporated from the rack before placing the embedding molds (embedding molds should not be in direct contact with $N_2(l)$)

14. Place embedding molds on top of the metal rack until the NEG freezes (becomes white)
15. After cryosectioning, allow sections to fully dry at RT, then transfer to **-80°C** or proceed with staining