Retina Dissection Protocol for postnatal eyes (Fixation method)

Special conditions: Postnatal eyes
With fixation

Acronyms and shorthands:

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

Recipes:

Note: use either PBS or Sorenson´s buffer depending on the antibodies that will be used later. Use RNAse-free water (DEPC-treated or filtered MilliQ water) in case we use the tissue for *in situ* hybridization.

4% PFA in 1xPBS: 10mL 16% PFA + 4mL 10x PBS, up to 40mL total volume with DEPC H_2O
4% PFA in 1xSor: 10mL 16% PFA + 20mL 2x Sorenson's, up to 40mL total volume with DEPC H_2O
30% Sucrose in 1xPBS: 15g Sucrose + 5mL 10xPBS, up to 50mL DEPC H_2O
30% Sucrose in 1xSor: 15g Sucrose + 25mL 2x Sorenson's, up to 50 mL DEPC H_2O

Mouse euthanasia:

CO_2 mice P0-P11 – give CO_2 for 5-10min (or until they are unresponsive), cervical dislocation, decapitate, and dissect. Mice younger than P7 – can be anesthetized with cold followed by decapitation

Dissection and embedding:

Day 1

1) Remove eyes from head and place them in the 48-well plate that has 4% PFA in them. Let them sit for **5min** at **RT**. Then transfer to Sylgard plate and dissect in 1xPBS or 1xSorenson's. Remove extraocular tissue, cornea and lens.
2) Return to multiwell and fix in **4%PFA** DEPC (in 1xPBS or 1xSorenson´s buffer) for **20min** at **RT** or **2h on ice**
3) Wash 3x, **10min** each at **4°C** with 1xPBS or 1xSorensons's DEPC
4) Add 30% Sucrose DEPC in 1xPBS or Sorenson's, sit in this for at least **2h** or **O/N** at **4°C**
   - This should make eyes to go to the bottom of the well – means that they absorbed the sucrose
5) Add NEG-50 until the eyes are covered, leave in NEG-50 for a minimum of 2h, **O/N** is most desirable. Keep in **cold room** during this time, shaking.

Day 2 (or 3)

6) Label flags for eyes to be embedded, keep 5mL Eppendorf tubes in the freezer for eyes to go in once they are embedded (do not want to put eyes in warm tube or they may melt)
7) 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 $N_{2(l)}$)
8) Embed using embedding molds (the rubber ones that fit 21 blocks, SIPI 2449) by putting NEG first and then transfer each eye, one by one grabbing by optic nerve. Optic nerve should face

upwards. Transfer embedding molds to cold rack in the bucket and leave for **5min** or until the NEG-50 becomes white.

9) Bring embedding mold to **-20°C** for a **few minutes** (until the mold is flexible again to be able to remove the blocks)
10) Put blocks in 5mL Eppendorf tubes and store in **-80°C** freezer

Embryonic Brain Dissection Protocol for retinal sections (Fixation method)

Special conditions: Embryonic eyes
With fixation

Acronyms and shorthands:

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

Recipes:

Note: use either PBS or Sorenson´s buffer depending on the antibodies that will be used later. Use RNAse-free water (DEPC-treated or filtered MilliQ water) in case we use the tissue for in situ hybridization.

4% PFA in 1xPBS: 10mL of 16% PFA + 4mL 10x PBS up to 40mL total volume with DEPC H_2O
4% PFA in 1xSor: 10mL of 16% PFA + 20mL 2x Sorenson's up to 40mL total volume with DEPC H_2O
10% Sucrose in 1xPBS: 5g Sucrose + 5mL 10xPBS up to 50mL DEPC H_2O
10% Sucrose in 1xSor: 5g Sucrose + 25mL 2x Sorenson's up to 50 mL DEPC H_2O
30% Sucrose in 1xPBS: 15g Sucrose + 5mL 10xPBS up to 50mL DEPC H_2O
30% Sucrose in 1xSor: 15g Sucrose + 25mL 2x Sorenson's up to 50 mL DEPC H_2O

Mouse euthanasia:

Sacrifice pregnant dam by giving CO_2 for 5-10min (or until they are unresponsive), cervical dislocation, decapitate, and dissect

Dissection and embedding:

Day 1

1) Dissect in 1xPBS or 1xSorenson's DEPC. Open the abdominal cavity and remove the embryos. Cut their heads off and place them in 48-well plates with cold PBS or Sorenson´s.
2) Fix in Fix in 4%PFA (PBS or Sorenson's) **O/N** at **4°C**.

Day 2

3) Rinse with PBS or Sorenson´s 3 times, **10min** each at **4°C**.
4) Put in 10% Sucrose DEPC **O/N**, shaking at **4°C** (or until they have sunk, younger tissue will sink faster)
5) Put in 30% Sucrose DEPC **O/N**, shaking at **4°C** (or until they have sunk, younger tissue will sink faster)

Day 3 (or 4)

6) Put in 50:50 Sucrose/NEG (30% DEPC sucrose) for **~2 hours** at **4°C**.
7) When embedding put the divets in the mold (cubic plastic mold) on the right side, point nose towards me, lay dorsal side down. This is for **CORONAL** sections, so we can have both eyes in

one section (if aligned properly). Add NEG up to the top and do not allow head to sink to the bottom.