

SOP Dissection and freezing of E14.5 mouse embryos

Materials

Freezing cooler stored at -80°C

Copper base freezing chambers

Stereomicroscope with guide for positioning of the freezing chambers and graticule in eyepiece to orient embryos

Dissection tools (small scissors, 2 watchmaker forceps, blunt curved forceps, preparation needle)

Stereomicroscope for dissection

PBS, sterile, RNase free

OCT freezing medium from Sakura

Dry ice

Procedure

Before beginning make sure

- that the freezing cooler is cold (-60°C), use a little ethanol and dry ice to cool it down
- the graticule in the stereomicroscope is oriented to coincide with the engraved crosshair at the bottom of the freezing chambers
- OCT bottles are kept upside down to minimize the formation of air bubbles



- (1) Sacrifice the mouse using either cervical dislocation or isoflurane and CO_2 (dry ice).
- (2) Spray the mouse with 70% Ethanol.
- (3) Lift the skin over the belly and make a small incision.



(4) Enlarge the incision and pull the string of embryos out and transfer to a Petri dish on ice filled with ice-cold PBS.



(4) Dissect the embryos out of the uterus under a stereomicroscope in a Petri dish with fresh pre-cooled (4°C) PBS, make sure to remove all the membranes and transfer the cleaned embryo to an empty Petri dish on ice



(5) Using strips of filter paper dry off as much of the PBS on and around the embryo.

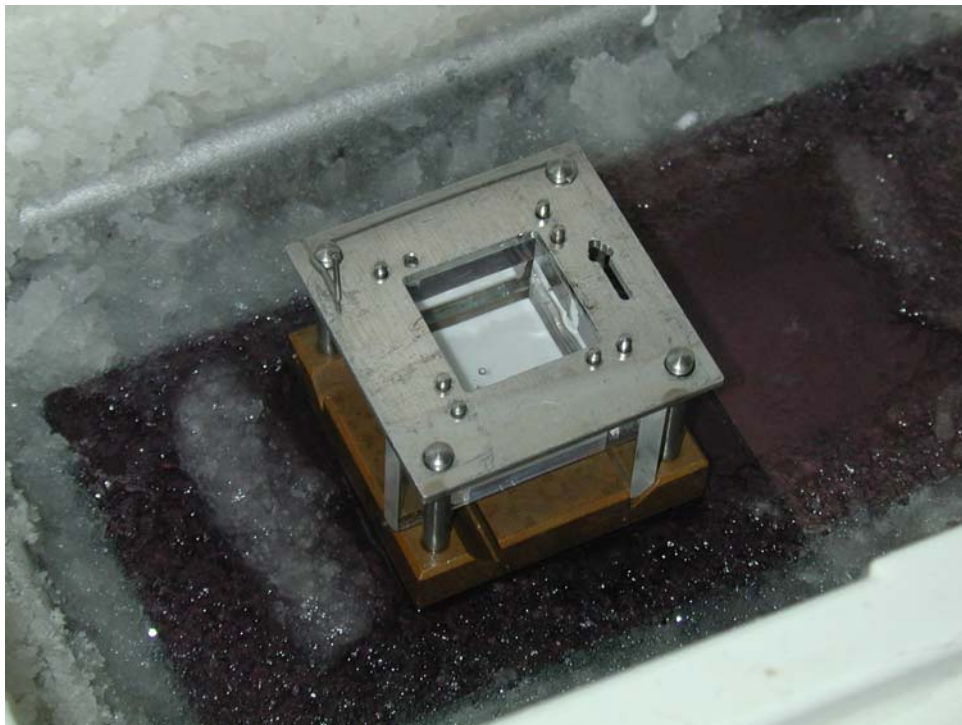


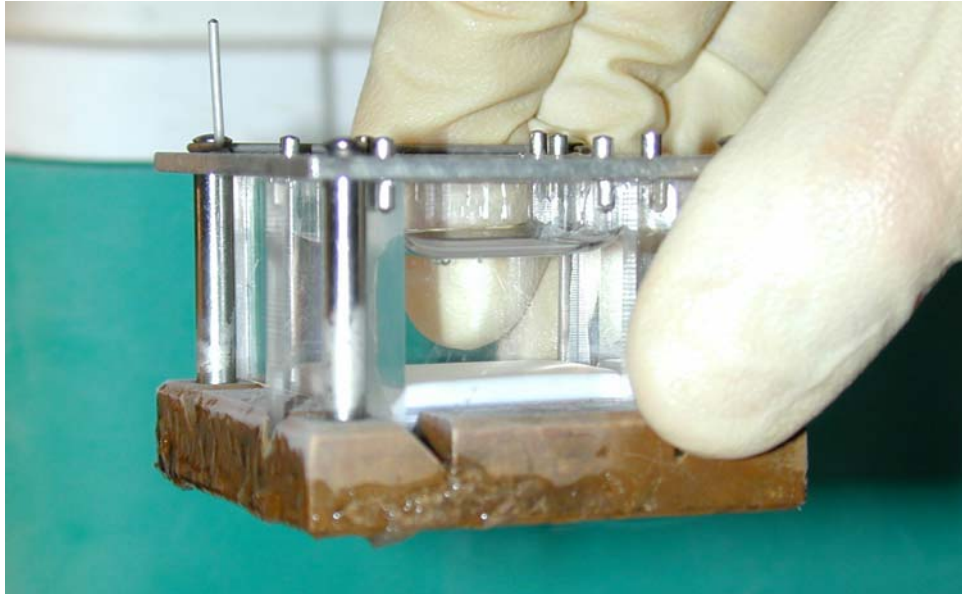
(6) Cover the dried embryo with OCT, then turn the embryo over to ensure the OCT comes in contact with all of the embryo's surfaces (use the curved forceps to gently manipulate the embryo).



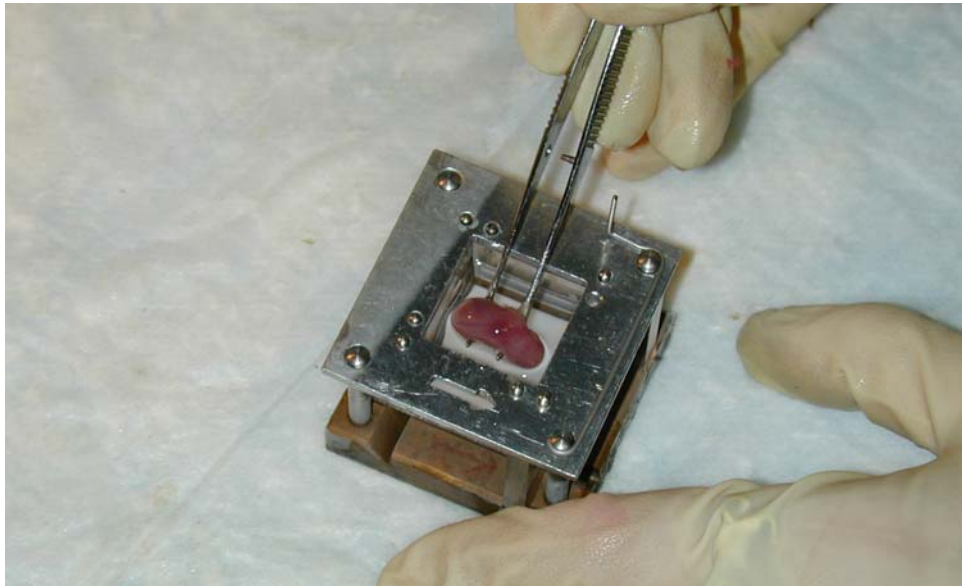
(7) Let the embryo sit in OCT on ice for at least 10-15 minutes (this helps to ensure better adhesion of the OCT to the tissue during sectioning and does not compromise the quality of the tissue and the RNA).

(8) Fill a freezing chamber with OCT to about 3 mm below the upper edge and place in the cold freezing cooler until the OCT just begins to become solid above the copper base.

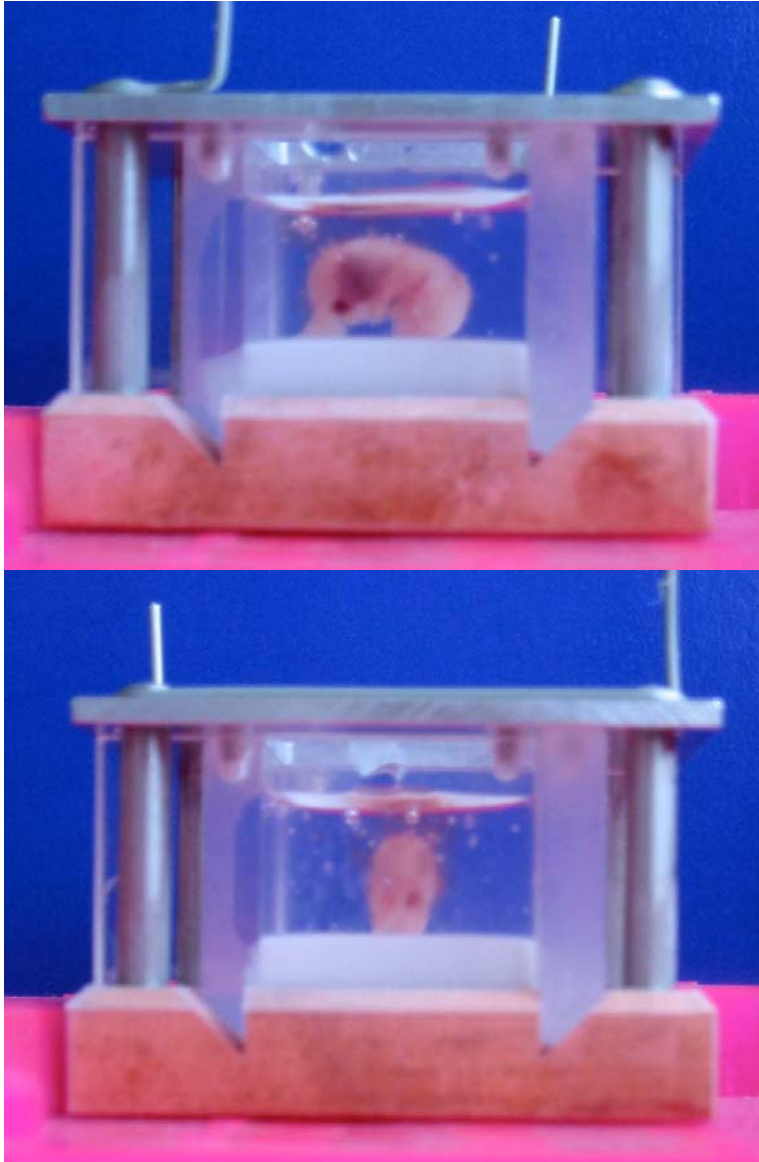




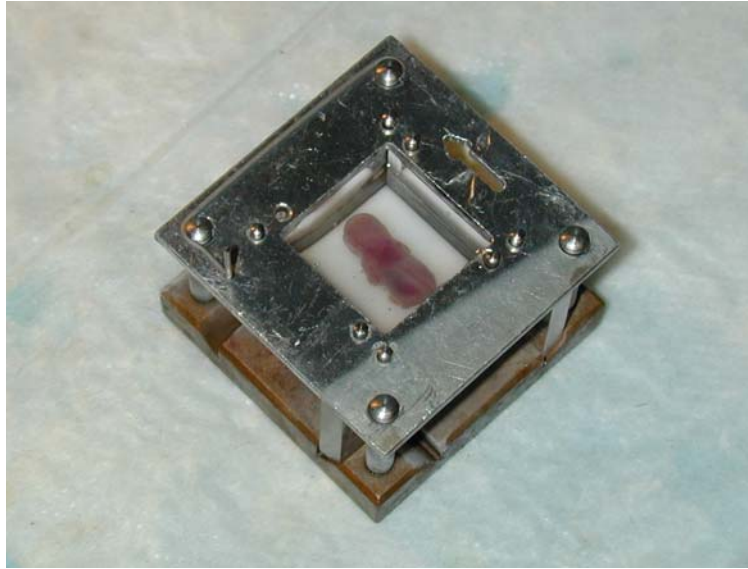
- (9) Remove freezing chamber from cooler and using the curved forceps transfer an embryo into the freezing chamber
(10) Orient it belly side down and with the anterioposterior axis parallel to the arrow engraved on the freezing chamber, head at the tip of the arrow.



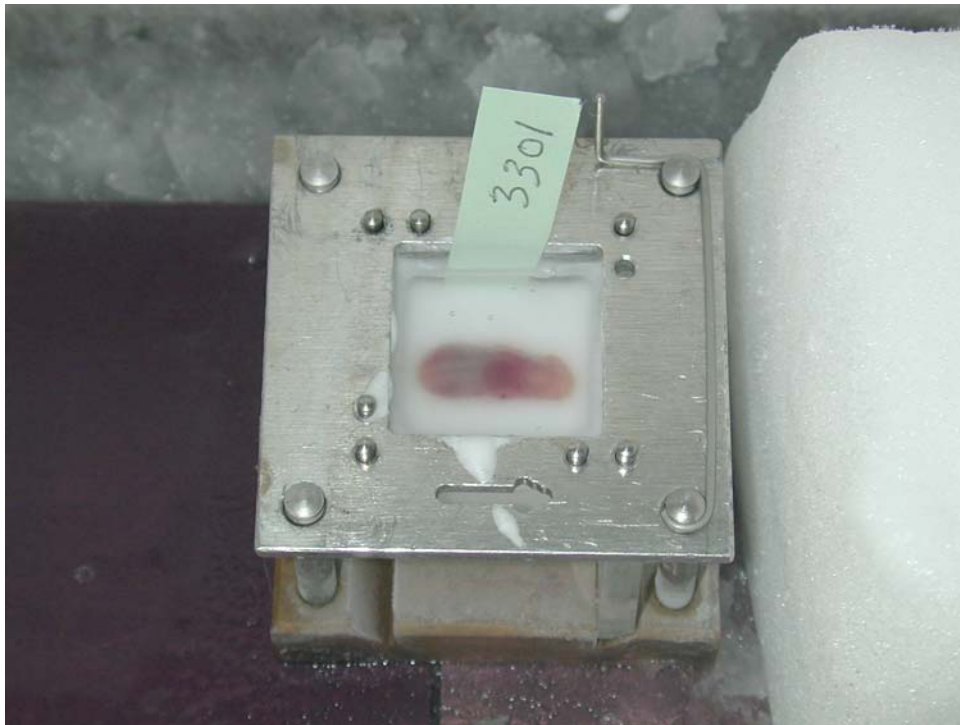
- (11) Looking from the side make sure the embryo is ~3mm below the meniscus of the OCT otherwise gently push it down with the curved forceps.



(12) Position the freezing chamber under the stereomicroscope and use the preparation needle to orient the embryo so that the midline, marked by the vertebrate column is parallel to the lines of the graticule in the eyepiece. Check orientation by looking from the side onto the chamber, The embryo has to be straight in the horizontal direction, correct if necessary and check orientation in the microscope again.



(13) Place freezing chamber with the oriented embryo carefully in the freezing cooler. The OCT should freeze evenly from the bottom up. Place a paper strip with the specimen number in the still liquid portion of the OCT, use pencil or graphite not a fiber pen. Wait until the embryo is fixed in the place at its ventral side by frozen OCT.



(14) Then remove the freezing chamber from the cooler and place in a -20C freezer on a metal plate or block. (A minimum time for freezing has not yet been determined, but is probably 1 hour).

(15) Before disassembling the freezing chamber, mark the direction of the arrow and the specimen number on the now solid OCT. Artist's charcoal or graphite work fine

for this. Labeled OCT blocks are collected in small sandwich bags that are collected in a lidded plastic box in the freezer.



(16) Clean freezing chamber parts in running water and rinse in distilled water, if OCT adheres clean with soft brush. Rinse briefly in 95% ethanol (careful, Plexiglas becomes brittle if left in ethanol for any length of time. Spread out on a diaper to dry and then spray with silicon spray to avoid OCT from attaching to Plexiglas and copper base.

(17) If you expect to section the specimen within the next few weeks store it at -20°C . For long term storage (months, years) store in -80°C but move from -80°C to -20°C at least a few days before sectioning

(18) Complete the “specimen/sectioning sheet”, and also enter the data in the inventory Excel form.