Examination of Mouse Embryos

Most embryos may be frozen in OCT blocks, sectioned sagittally, and stained with hematoxylin and eosin (H&E) for viewing morphologic landmarks. Comparisons of wildtype and mutant embryos are possible if multiple sections are viewed. Alternatively, coronal sections (from the crown to the rump) will also demonstrate abnormalities. These frozen sections can then be used in immunostaining assays.

Paraffin sections provide good morphology; thus, it is really important to fix larger embryos ***very well***. It may be necessary to open the abdomen a little to allow the fixative to penetrate.

Fixing in Bouin's solution allows rapid fixation and processing. However, do not let them sit in Bouin's for too long – the tissue will harden and become challenging to process and section.

Procedure

Fixation (P. Mellon lab, UC San Diego)

1. Prepare 10-15 mL of fresh fixative of absolute alcohol, 37% formaldehyde, and glacial acetic acid in a 6:3:1 ratio.
    a. Note: it is important that this mixture is ***prepared fresh*** every time
2. Use 10 mL of fixative for small embryos and 15 mL for larger embryos.
3. Place on a rotator and incubate overnight at 4°C.
4. If samples ***are not white*** the next day, fix again in fresh fixative for another 24 h. Otherwise, proceed to next step.
5. Wash with 70% alcohol until the acetic acid smell is gone.

Embedding and orienting in Agarose

6. Make 1% agarose in water
7. Place the specimen in the mold (Biopsy Mold: Tissue-Tek, cat. #4565, disposable vinyl specimen molds 10 mm x 10 mm x 5 mm)
8. Pour the hot agarose on the specimen and very quickly orient the specimen under the microscope (the agarose will solidify quickly)
9. Pop out the little formed square and place in the plastic embedding cassette (Fisher cat. # 15-197-700E) appropriately labeled with either a #2 soft graphite pencil or with an organic solvent resistant pen (Secureline: Fisher, cat. #450-20-FSC)
10. Drop into 70% alcohol and get it processed and embedded for paraffin sections.

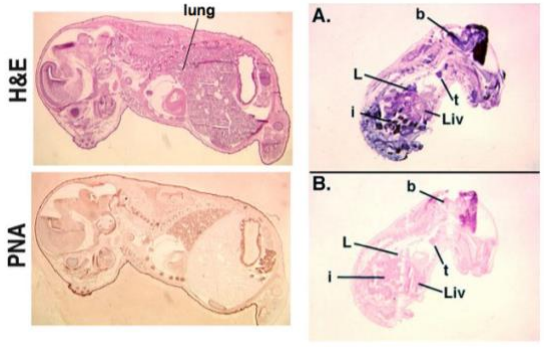

Figure 1. Immunohistochemistry on sections of mouse embryos. Panels on left side show sections that have been frozen well. Panels on the right side show sections that have been frozen poorly.