

**Paint-filling Technique for Pre and Postnatal Mouse Stages**  
(adapted by Amy E. Kiernan from Martin and Swanson, 1993 and Bissonette and Fekete, 1996)

1. Decapitate the mouse and bisect the head. Remove the brain for stages E18 and above. Fix overnight in Bodian's fixative.
2. Dehydrate the half heads in a series of ethanol washes: 2x 75%, 2x 95%, 2x 100% (minimum, 2hrs each wash).
3. Clear overnight (or several hours for embryonic stages) in 100% methyl salicylate.

**Injection procedure**

1. For injections use a Hamilton syringe filled with heavy mineral oil and attach a pulled glass capillary needle (0.75 mm inner diameter, 1.0 mm outer diameter with a filament) broken to a tip diameter of 20-40 micrometers.
2. Draw several microliters of the paint suspension (1% white gloss paint in methyl salicylate) into the needle (the paint and the oil will mix somewhat but don't worry about it).
3. Use a micromanipulator to hold the syringe and target the needle.
4. Place the half head in a dish of methyl salicylate and inject!

**Injection Targetting**

**For E18 and above** (because of bone you may need to make hole first with a tungsten needle although thicker needles may do the job):

Medial injection

1. Inject into the common crus (thick region of the canals where the anterior and posterior canals join) to fill the vestibule (excluding the saccule)
2. Inject into the cochlea to fill the saccule, cochlea, and sometimes endolymphatic duct.
3. Dissect out the ear now that you can see it!

**E10- E17**

Medial injection

1. remove brain (only if you cannot see the ear as this will probably disrupt the endolymphatic sac in animals E15-E17 so it is best to be avoided at these stages)

2. Inject into the cochlea- should fill the whole ear in animals E10-E15. In animals E16-18, it may fill only the cochlea and saccule. In this case it is best to remove the brain carefully and inject into the common crus

4. Dissect out the ear E16 and above if so desired (gives better pictures).

### **Materials and Equipment Needed:**

#### Bodian's Fixative

75% Ethanol

5% acetic acid

5% formalin

15% water

Methyl Salicylate

White gloss paint

Glass capillary pipets (Thin wall with filament, OD=1mm, ID=0.78mm)

pipet puller

Tygon tubing (B-44-4X)

Micromanipulator

Hamilton syringe (100 microliter)

Dissecting Microscope

Light source

Heavy mineral oil

### **Accessing the chicken ear for paint-filling (addendum to paint-fill protocol for the mouse inner ear provided by Amy Kiernan)(Donna Fekete)**

- hold embryo head with forceps between the eyes throughout the dissection
- remove lower jaw with scissors (work from a ventral view)
- clip off the left and right jaw joints
- remove all soft tissue in a superficial, horizontal plane manner beginning at the palate moving towards the spinal cord
- at this point the lagena should be visible on each side of the midline, but it will be a bit hazy because you are viewing it through some cartilage.
- there is a thin cartilaginous shelf of bone overlying the lagena; carefully remove it by lifting from its posterior edge with sharp forceps. It should come off as one plate from each side. This exposes the soft tissue of the lagena, with the characteristic U-shaped

otoconial mass quite evident. This is now a good target for the insertion of your electrode to fill the ear with paint.

- After each ear is filled, the brain is removed, the soft tissues on the lateral (middle ear) side can be picked away, and the inner ear can be removed from the temporal bone by carefully cracking the bone/cartilage along each major edge of the ear. The more tissue that is cleared away, the better the image for photography.
- We often place the ears in a well dug out of a Sylgard-coated dish to optimally orient the ear for photography from different angles.

**Note:** On older embryos (~E15+), the paint sometimes fills the perilymph space by accident. The goal is to fill only the endolymph space. When perilymph is filled, the cochlear duct appears wider and is less delicate; the characteristic undulations of the tegmentum vasculosum are not visible. See E15 and E17 of Figure 4 of Bissonnette and Fekete, *J. Comp. Neurol.*, 386:620-630.