Cartilage Staining of Mouse Embryos
(Rivera lab)
(Adapted from Jegalian and De Robertis, 1992. Cell 71, 901)

Used with good results in embryos between 13.5 – 16.5 days.

Dissect embryos in PBS and remove extra-embryonic membranes (use them for genotyping if necessary).

1. Fix in Bouin’s solution for two hours.

2. Rinse with a solution of 70% ethanol plus 0.1% NH₄OH over a course of 24 hours until embryos appear white. Six to eight changes may be required.

3. Equilibrate with 5% acetic acid. Two changes, each for one hour.

4. Stain for two hours with 0.05% Alcian Blue 8GX (Fisher) in 5% acetic acid.

5. Rinse twice with 5% acetic acid for one hour each.

6. Dehydrate embryos by incubating twice in methanol one hour each.

7. Clear in 1:2 benzylalcohol:benzyl benzoate (BABB). Use glass containers when using BABB.

* In older embryos (15.5-16.5) evisceration may be required.

** Staining has been shown to last at least two years in BABB. It is advisable to document the results immediately if they are relevant.