Clearing And Staining For Larval Fish Cartilage And Bone

(Source: the Parichy Lab from Zebrafish Book 5th Edition)

1. Fixation

Fix fish in 4% paraformaldehyde in PBS for 2 x overnight at 4°C.

2. Dehydration

For larvae less than 20 mm SL:
  a. Wash 2 x 5 minutes in PBS.
  b. Dehydrate in 50% EtOH / 50% nano water for 24 hours.
  c. Dehydrate in 100% EtOH for 24 hours.

For larvae more than 20 mm SL:
  a. Wash 2 x 5 minutes in PBS.
  b. Dehydrate in 50% EtOH / 50% nano water for 48 hours.
  c. Dehydrate in 100% EtOH for 48 hours with one intermediate solution change.

3. Staining cartilage

Cartilage staining solution:

For 100 ml final volume:
  70 ml 100% EtOH
  30 ml acetic acid
  20 mg Alcian blue

  a. Transfer specimens to staining dish.
  b. Incubate in cartilage staining solution for 24 hours at room temperature with mild agitation.

4. Neutralization

Wash in saturated sodium borate solution for 9-12 hours.

5. Bleaching (optional)

Bleaching solution:

For 100 ml final volume:
  15 ml 3% hydrogen peroxide
  85 ml 1% potassium hydroxide

  Incubate in bleaching solution for:
  20 minutes for specimens less than 20 mm SL
  40 minutes for specimens more than 20 mm SL

6. Trypsin digestion

Digestion solution:

For 100 ml final solution:
  35 ml saturated sodium borate
  65 ml ddH2O
  1 g trypsin

  Incubate in solution at room temperature with mild agitation until specimens are 60% clear.

7. Staining bone

Bone staining solution:

1% potassium hydroxide with
0.1 g/100 ml Alizarin red

  Incubate in bone staining solution for 24 hours at room temperature with mild agitation.

8. Destaining

For specimens less than 20 mm SL:
  Incubate in trypsin digestion solution for 40-48 hours.

For specimens more than 20 ml SL:
  Incubate in trypsin digestion solution until specimen is clear and solution remains unstained. Change to fresh solution every 2 days.

9. Preservation
a. Transfer to 30% glycerol/70% of 1% potassium hydroxide. Incubate 2-3 days at room temperature with mild agitation.
b. Transfer to 60% glycerol/40% of 1% potassium hydroxide. Incubate 2-3 days at room temperature with mild agitation.
c. Transfer to 100% glycerol with thymol.

Reference