Student: Brian Lohman
Host Lab: Dr. Michael Bell
Dates of Visit: 19 May – 30 Sept.
Title: Whole-Mount Antibody Staining With Trypsin Clearing

Rationale: To visualize collagen condensations as precursors to bone development.

Antibody Fixing Protocol

5. Euthanize with MS-222
6. Fix with paraformeldehyde for 2 hours
7. Wash with 100% methanol
8. Place in 100% methanol for storage

Antibody Staining Protocol

15. Remove entrails of larger fish
16. Immerse in trypsin solution for 12 hours
    a. .1% trypsin in 30% sodium borate
17. Wash twice with 1% potassium hydroxide for five minutes
18. Bleach for 3 to 12 hours
    a. 15% - 3% hydrogen peroxide
    b. 85% - 1% potassium hydroxide
19. Wash twice with 1% potassium hydroxide for five minutes each
20. Treat with Pro K 10µg/ml
    a. 1.3 minutes per mm of sample
21. Remove Pro K with 2 washes of phosphate buffered saline + 0.1% Tween 20 (PBT), 10 minutes each
22. Treat with blocking solution
    a. 5% normal serum in PBT for 1 hour
23. Quick wash with PBT to remove excess blocking solution
24. Primary antibody
    a. Transfer to new tube with primary antibody diluted in PBT 1:100
    b. Incubate while rocking at room temperature overnight
25. Wash with PBT 2 times, 10 minutes each
26. Treat with blocking solution
    a. 5% normal serum in PBT for 1 hour
27. Quick was with PBT to remove excess blocking solution
28. Secondary antibody
    a. Transfer to new tube with secondary antibody diluted in PBT 1:200
    b. Incubate overnight at 4°C
29. Carefully wash with BPT 5 times, 5 minutes each
30. Transfer through PBT:glycerol series to visualize/store (3:1,1:1,1:3 at one hour each. Shortened for smaller samples. Specimen sinks when equilibrated)

31. Store in 100% glycerol

References:

G. Dingerkus and L.D. Uhler. *Stain Technology* 52: 229 (1977)
L.P. Hernandez *et al., Anat. Embryol.* 309: 323 (2005)