

Skeletal Prep Protocol
Robert Gonzalez-Ryan Gray
Modified 12/16/2016

- 1) Fix tissue in 10% formalin (alternatively, 4% PFA in 1XPBS is fine), leave on low speed shaker overnight to days. *For zebrafish it will work much better if you skin the fish before fixation, also removing the innards makes things much easier and quicker.*
- 2) Transfer to **glass** container (acetone will dissolve plastic, we use scintillation vials); add acetone to dissolve fatty tissue. Leave on shaker overnight.
- 3) Remove acetone and rinse in tap water once.
- 4) Add bone/cartilage stain*, incubate at 37°C overnight.
- 5) Remove stain solution, wash with tap water once quickly and then again for 30 mins.

Either (quicker but more expensive and can dissolve your sample if left too long)

- Place the specimen in 1% trypsin in 2% borax overnight or until cleared. This step is complete when 85% of the soft tissue is dissolved. This step may take several days. Very little further clearing will take place in the steps following this one. For a 15-20 mm long fish, one to two days is usually sufficient.

OR (preferred method but takes a bit longer)

- Add 1% KOH, leave on shaker at room temperature. This step will take up to 2 weeks depending on size of fish.
 - Change out 1% KOH solution after 3 hours on the first day. *Repeat every 2-3 days until the fish is mostly cleared and the spine apparent, scales are mostly removed.*
 - Remove 1% KOH, rinse in RO water.
- 6) Add 25% glycerol (diluted in 1% KOH), leave on shaker for 1 hour at room temperature. *Most of the scales should fall off in this step.*
 - 7) Remove 25% glycerol solution and add 50% glycerol solution (diluted with 1% KOH), leave on shaker for 1 hour at room temperature.
 - 8) Remove 50% glycerol solution and add 80% glycerol solution (diluted with 1% KOH), leave on shaker for one hour at room temperature.
 - 9) Image zebrafish and return to 80-100% glycerol for storage at 4°C. The higher the glycerol concentration the greater chance you will break your fish...so be careful. We find that 80% glycerol is fluid enough to limit this and preserves the prep for months – years (Can add thymol crystals to limit growth of bugs, we find that clean glycerol is just fine).

***1 Liter Solution of Bone/Cartilage Stain**

- 50 mL 0.3% Alcian Blue
- 50 mL 0.1% Alizarin Red
- 50 mL Glacial Acetic Acid
- 850 mL 70% Ethanol

This protocol works well for zebrafish and also can be used for mouse tissues...of course mouse do not have scales.