**Skeletal Prep Protocol**

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1. Fix tissue in 10% formalin (alternatively, 4% PFA in 1XPBS is fine), leave on low speed shaker overnight to weeks.
2. Transfer to glass container; 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, rinse with RO water once quickly and then again for 30 mins.

**Either**

* 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**

* 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.

1. 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*.
2. Remove 25% glycerol solution and add 50% glycerol solution (diluted with 1% KOH), leave on shaker for 1 hour at room temperature.
3. Remove 50% glycerol solution and add 80% glycerol solution (diluted with 1% KOH), leave on shaker for one hour at room temperature.
4. Image zebrafish and return to 80-100% glycerol for storage at 4°C. The higer the glycerol concentration the greater chance your will break your fish...so be careful. We find that 80% glycerol is fluid enough to limit this and preserves the prep for many many months.

\****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.*