AQUA-FAANG – Standard Operating Protocol: **Total RNA preservation from salmonid embryos using Trizol**

Overview: This protocol describes a method used to preserve total RNA from salmonid embryos. There are no developmental stage specific steps or adjustments in this protocol.

Consumables:

- 1.5ml Eppendorf tubes with safety lock
- Trizol (or equivalent)
- Sterile homogenization beads (e.g. tungsten or zirconium beads)
- Tissuelyser or equivalent homogenization device.
- Water from egg trays

1.1 Label the required number of 1.5ml Eppendorf tubes depending on the required number of biological replicates. No more than 3 eggs per tube should be used in salmonids.

1.2 Fill each tube with the required amount of homogenization beads.

The amount of beads is dependent on the type of beads used. We initially used two 3mm tungsten beads, later ~500ul of 1mm Zirconium beads, and finally around the same volume of 1mm glass beads. It may be necessary to monitor the homogenate after homogenization and use more beads/homogenization time if necessary.

1.3 In a flow hood, add 1,000ul of Trizol to each tube. Store tubes in fridge while collecting embryos.

The number of tubes used will depend on the sampling strategy for each species.

1.4 Place the required number of eggs for all replicates in water from egg trays within a Petri dish on ice.

Using water from egg trays to temporally keep the eggs while sampling allows for easy identification of dead/dying eggs, which will go white and should not be sampled. If eggs are kept in PBS they will not go white when they die, instead the chorion will turn more transparent and the yolk/embryo mass will clump into a ball. This is more difficult to distinguish than eggs going white.

1.5 Using watchmaker forceps, transfer each egg to an empty petri dish, and puncture it with a pair of closed watchmaker forceps, while holding the egg with a second pair of watchmaker forceps. Do not remove forceps from puncture, instead, keep moving forceps

inside the egg until they come out through the other side. Transfer egg over a tube while held by the closed watchmaker forceps, and open them over the tube, dechorionating and depositing egg in the tube at the same time. Add no more than three punctured eggs per tube*. **Make sure tubes close tightly.**

**Salmonid specific, this is to avoid overfilling of tubes due to egg size.*
Egg puncturing is not necessary if using heavy 3mm tungsten beads.

1.6 Homogenize using a Tissuelyser at a setting of 30 Hz for 6min.

1.7 Store tubes at -80^{o}C until RNA extraction