



AQUA-FAANG – Standard Operating Protocol: **Fixation of salmonid embryos for imaging and staging**

Overview: This protocol describes a method used to fix and preserve salmonid eggs at different stages of development, in order to perform retrospective staging of samples, including for imaging. There are no developmental stage specific steps in this protocol.

Consumables:

- 50ml Falcon tube
- 4% PFA
- Watchmaker forceps
- PBS
- 70% ethanol

1.1 Tag 50ml Falcon tube with sample name.

1.2 Add 25ml of 4% PFA to a 50ml Falcon tube.

1.3 Using watchmaker forceps, add 50 eggs to 50ml Falcon tube.

1.4 Leave to fix in the fridge for up to 48 hours.

1.5 In a fume hood, appropriately dispose of the 4% PFA into a waste container. Hold the tube cap over the top of the tube to hold the eggs, leaving a small opening to let the PFA out.

1.6 Add 50ml of PBS, invert several times.

1.7 Empty PBS into PFA waste container as indicated in step 1.4.

1.8 Repeat steps 1.5 and 1.6 another two times (3 times in total).

1.9 Add 50ml of 70% ethanol.

1.10 Edit tube tag to indicate sample is now in 70% ethanol, not PFA.

1.11 Store at -20°C.