**Embryo staining**

1. With small artists brush and NaCl-TX, gently collect embryos and transfer them to a watchglass. Aspirate liquid.
2. Cover the embryos with 50% bleach for 2 min. to dissolve chorion.
3. Rinse 2-3 times in NaCl-TX and once in ddH2O
4. Pick up embryos in a Pasteur pipette and allow them to settle to the tip. Transfer them (with a little fluid as possible) to a 20 ml scintillation vial containing 5ml of fix and 5 ml n-Heptane. Fix 20 min. at room temperature on a shaker platform.
5. Pipette off the lower phase (fix). The embryos should be floating on tip of the fix at the fix/heptane interface.
6. Add 5 ml 100% Methanol and shake vigorously for about 10 sec. This should rupture the vitelline membrane. The embryos will fall from the interface to the bottom of the vial and the membranes and any membraned embryos will remain in the interface.
7. Aspirate upper phase and most of the methanol. Rinse once in methanol and transfer the embryos with the pipette to a 24 well tissue culture plate (Fisher).
8. Rinse 1-2 times in methanol, 3 times in 95 % Ethanol and rehydrate with half volumes of ddH2O.
9. Wash in PBT 4 times, 15 min each.
10. Block in PBTB for 1 hour at room temperature.
11. Nutate overnight with primary antibodies.
12. Wash in PBT 4 times, 15 min each
13. Block in PBTB for 1 hour at room temperature.
15. Wash in PBT for 15 min
16. Add DapI to the next PBT wash for 5-10 min.
17. Wash in PBT 2 times more, 15 min each.
18. Mount embryos on the slide with glycerol+NPG

**Solutions:**

1. 0.7% NaCl, 0.3% Triton X100 (NaCl-TX)
2. 5.25 % Sodium Hypochloride (Bleach)
3. 4% Formaldehyde, 0.1M Sodium Phosphate Buffer pH 7.2 (Fix)
4. 100 % Metanol
5. 100 % n-Heptane
6. 95 % Ethanol