Formaldehyde Fixation – Methanol Devitellinization

1) Collect properly staged embryos
2) Make fix early in order to saturate the heptane w/ pfa

Fix: 8% PFA in PO₄ buffer/PBS
-1mL 32% PFA
-3mL PO₄ buffer
-4mL Heptane

.1M NaPO₄ Buffer pH 7.4
-77.4 mL 1M Na₂ PO₄
-22.6 mL 1M NaH₂ PO₄
-Fill to 1L w/ distilled H₂O

3) Bleach embryos in plate w/ 50% bleach for 1.5’
4) H₂O rinse in a mesh basket
5) Dump embryos in fixative
6) Fix for 30 min on rotator.
7) Take off fixative (bottom layer) w/ Pasteur pipet. This will leave the Heptane layer.
8) Add 4 ml of methanol (1 push)
9) Vortex 30 sec.
10) Remove all of the Heptane and most of the methanol. NB: Most embryos fall to the bottom but some embryos remain at the interface. Embryos that remain at the interface should be discarded.
11) Rinse with 4 ml of Methanol. Suspend embryos in the methanol and transfer embryos to an eppendorf 1.7 ml tube in a final volume of 500 ul of Methanol.
12) Embryos can be stored in Methanol at -20C.