Formaldehyde – Manual Devitellinization

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

Fix: 8% PFA in PO$_4$ buffer/PBS

- 1mL 32% PFA
- 3mL PO$_4$ buffer
- 4mL Heptane

$.1M$ NaPO$_4$ Buffer pH 7.4

- 77.4 mL 1M Na$_2$ PO$_4$
- 22.6 mL 1M NaH$_2$ PO$_4$
- Fill to 1L w/ distilled H$_2$O

3) Bleach embryos in plate w/ 50% bleach for 1.5’
4) H$_2$O rinse in a mesh basket
5) Dump embryos in fixative
6) Fix for 30 min on rotator.
7) Take off fixative w/ Pasteur pipet
8) Wash w/ PO$_4$ buffer
9) Suck up embryos in pipet (need to find one they don’t stick in) and transfer to apple juice plate
10) Remove excess liquid
11) Stick embryos to a coverslip with DS tape on it.
12) Place coverslip w/ embryos in a 60mm petri dish lid with DS tape to hold the cover slip.
13) Rinse embryos w/ PO$_4$ buffer and leave them covered
14) Devitellinize w/ 26 ½ gauge needle
15) Add PBT and pipet into eppendorf tube

Notes:
• This is the best fix for phalloidin staining and visualizing fluorescent proteins.