Embryo Fixation Protocol:

1) Collect embryos on grape juice agar plate and age to appropriate stage.

2) Using a paint brush, move embryos from the collection plate to a basket in embryo wash solution (7% NaCl, 0.7% Triton X-100).

3) Dechorionate by incubated basket of embryos in 50% bleach for 2 min.

4) Wash embryos in basket again.

5) With a squirt bottle, wash embryos down to one side of the basket, and blot dry with a kimwipe from underneath.

6) Transfer embryos to an eppendorf (or other tube) by squirting heptane on the embryos from the bottom of the basket.

7) Add an equal volume of fix solution to the heptane. You want 1:1 heptane:fix and your embryos floating at the interface.

8) Fix by rocking on a Nutator for the appropriate time (see below).

9) Remove the fix by pipetting or aspiration, leaving the heptane behind. Make sure you remove all or most of the fix, otherwise they won't "pop" at the next step.

10) Add an equal volume of MeOH (1:1 MeOH:Heptane) and immediately shake vigorously. The devittelinized embryos will sink to the bottom, and the ones that don't pop will remain at the interface.

11) Aspirate off everything except the embryos at the bottom.

12) Wash 2 X with MeOH and 2 X with EtOH, and store at -20°C in EtOH.

FIX: straight 37% formaldehyde for 5 min
OR
7% formaldehyde in PBS for 15-25 min depending on application.