

Embryo Fixation

- 1) Collect embryos on grape juice/agar plates
- 2) Rinse embryos with water (a squirt bottle works well) into a collection vial. We use collection scintillation vials with the bottoms cut off. We use nitex mesh to cover the end of the vial that has the threaded neck and hold the mesh in place with a scintillation vial cap that has had a large circle cut out.
- 3) Rinse the embryos well with water making sure to rid them of all yeast and other debris.
- 4) Dechorionate using freshly made 50% bleach solution (~10 ml per scintillation vial) for 3 minutes.

****While the embryos are dechorionating make up the fix solution

- 5) Rinse embryos thoroughly with water. (You can examine the embryos under a dissecting microscope to check to make sure they are completely dechorionated).
- 6) Use water to wash the embryos from the side of the vial and onto the mesh.
- 7) Twist off the cap of the scintillation vial. The mesh will remain in the cap and the embryos will remain on the mesh. Use forceps to pull the membrane out of the cap and dry it out on paper towel.
- 8) Use the forceps to push the embryo containing mesh into fix solution in a clean uncut scintillation vial. Gently pull the membrane up and down with the forceps until most of the embryos fall off, then pull the membrane out of the solution.
- 9) Do this for each set of embryos.
- 10) Place the vials on a rocker for 30 minutes.
- 11) Using a Pasteur pipette, carefully remove as much of the aqueous (lower) solution as you can. If you should suck up embryos, just put them back and try again.
- 12) Remove approximately $\frac{1}{2}$ of the heptane from the organic (top) phase.
- 13) Add 5 mL of methanol and shake the vial vigorously for 30 to 60 seconds in order to split open the vitelline membranes. Devitellinized embryos will fall to the bottom of the methanol and the empty vitelline membranes and the nondevitellinized embryos will stay at the interface of the heptane and methanol
- 14) Unscrew the cap and then swirl the vial in a circular motion to force all of the devitellinized embryos to the center of the vial.

15) Remove embryos from the bottom of the vial with a Pasteur pipette and place them into a clean microcentrifuge tube. Let the embryos settle in the new tube, remove the methanol and repeat until as many embryos as possible are in the collection tube.

16) Wash the embryos 3X 5 minutes in methanol.

17) Store in -20°C

Solutions:

Fix Solution:

Prepare in a clean scintillation vial with a tight-fitting lid.

5 mL Heptane

4.5 mL 1XPBS

0.5 mL 37% Formaldehyde