Collection and Fixation of *Drosophila* Embryos for ISH or IF

Collect Embryos

1. Set up fly cross for collection in a vial and let flies acclimate overnight up to 2 days.
2. Prepare yeast paste by mixing dry baker's yeast and milipore water together in a small beaker or container. Add enough water to create a peanut butter like consistency.
3. Using a spatula, place a small amount of yeast paste on an apple juice plate.
4. Transfer flies to a clean crossing bottle (with small holes in the sides) and close the bottle by placing an apple juice plate with yeast at the opening.
5. Tape down the plate and cover the juice plate and opening of the bottle with foil. This will prevent flies from escaping or other flies from getting into the bottle.
6. Place bottle plate side down on the bench.

Note: Embryo collections periods can be timed to obtain embryos at specific stages. Always allow flies to acclimate in the bottle with an apple juice plate for several hours before beginning collection experiments. For synchronous embryos, change out the AJ plate one hour before the planned collection period and again when the collection period begins.

Fix embryos

1. Use a spatula to scrape embryos from the apple juice cap and rinse into embryo strainer with 120µm mesh.
2. Rinse embryos well with distilled H_2O to remove the yeast paste.
3. Fill small dish or plastic container with a small amount 100% bleach and place the embryo strainer into the dish. Make sure that bleach covers all of the embryos. Leave for 4 min. Do not over bleach!
4. While embryos are in bleach, prepare a fix solution in a 1.5 mL tube with 500 µl heptane and 500 µl 4% formaldehyde/PBS.
5. Rinse well with distilled H_2O for several minutes to remove all bleach.
6. Remove embryos from the strainer by removing the mesh circle with forceps and place in the 1.5 mL tube containing fix solution. Gently move the mesh up and down in the solution until all of the embryos fall off of the mesh.
7. Remove mesh and place the tube on a shaker for 20 min.
8. Remove lower aqueous phase (formaldehyde) and replace with an equal volume of methanol (i.e. 500 µl).
9. Shake (manually) vigorously for 1 min, and then allow embryos to settle.
10. Remove the upper heptane phase and embryos at the interphase. These embryos are not high quality. Good quality embryos will sink to the bottom of the lower methanol phase.
11. Remove the remaining methanol.
12. Wash 3x quickly in 1 mL methanol by gently inverting the tube.
13. Add fresh methanol. Store embryos at -20°C.