

Fixing *Drosophila* embryos with paraformaldehyde

Materials needed:

- Embryo collection baskets
- 50% bleach
- Paintbrush
- Squirt bottle filled with water
- 2 mL microcentrifuge tubes
- 4% paraformaldehyde in PBS (frozen 5 mL aliquots can be found in the freezer)
- Methanol
- Heptane

Total procedure time: about 40 minutes

Procedure:

1. Collect embryos from fly cross on juice-agar plates. Do not collect embryos from a plate that has been on the laying chamber for 24 hours or more (you will get excessive larvae).
2. Squirt a little water onto the plate, and use the paintbrush to dislodge the embryos from the agar. Avoid gouging the agar with the paintbrush. Get the embryos suspended into the water.
3. Pour the water/embryo mixture into an embryo collection basket. Place any yeast chunks into the embryo basket. Multiple rinses may be necessary to get all of the embryos.
4. Use the squirt bottle to rinse the embryos (in the basket) well, and also to dissolve any yeast chunks in the basket
5. Place the basket into a petri dish filled with 50% bleach
6. Incubate the basket in the 50% bleach for no longer than 3 minutes. During this time, label a 2 mL microcentrifuge tube with the cross or genotype of the embryos that you're fixing. Place 800 μ L of heptane in the 2 mL microcentrifuge tube.
7. Remove the basket from the 50% bleach and place it into a petri dish filled with water
8. Use the squirt bottle to rinse the embryos with water. Try to get as much bleach off of the embryos as possible
9. Blot the embryo baskets dry on a paper towel
10. Disassemble the basket and carefully remove the mesh, taking care to not lose the embryos
11. Using the paintbrush, transfer the embryos to the microcentrifuge tube containing heptane. The embryos should easily come off the paintbrush in the heptane. Be sure to get all of the embryos on the mesh, as well as any that may be stuck on the side of the embryo basket tube.

12. Add 800 μL of 4% paraformaldehyde to the microcentrifuge tube. Be extremely careful with the paraformaldehyde- it is a contact hazard on bare skin!
13. Cap the tube and shake it on a platform shaker (I use the one in the microbiology lab room), at 250 rpm for 20 minutes
14. Remove both layers of liquid (the heptane and the paraformaldehyde) from the tube, taking care to not remove any embryos. You can leave a little liquid behind, just try to remove most of the liquid. Place this liquid mixture in the "Heptane/ Paraformaldehyde waste bottle".
15. Add 800 μL of fresh heptane, and 800 μL of Methanol to the tube.
16. Cap the tube and vortex the tube for 2' minutes on full. This step will dechorionate the embryos, which will allow them to sink to the bottom of the methanol.
17. Examine the tube. You should see a number of embryos at the bottom of the tube, and some embryos trapped at the methanol:heptane interface. The embryos at the bottom are properly dechorionated- these are what we want to keep.
18. Remove as much as the methanol:heptane liquid as you can, taking care to not remove the embryos at the bottom. Place this methanol:heptane waste in the "Methanol:Heptane" waste bottle.
19. Add 1.6 mL of fresh methanol to the tube.
20. Store the tube in the -20°C freezer.