***Drosophila* embryo collection and fixation**

Embryogenesis in *Drosophila* takes around 24 hours at 25°C. When collecting embryos, try to collect at approximately the same time every day, and your collection will include embryos at all stages of development.

Set up an egg collection cage containing males and females of the desired genotype(s). Ideally, set up a vial cross a few days ahead of time, then transfer the cross to a cage. Add yeast paste to the apple juice agar plate and secure it to the cage beaker using lab tape or rubber bands. Be sure to label the plate!

Embryo collection procedure

Before beginning, add yeast paste to enough new apple juice plates for all of your cages (use juice plates that have been pre-warmed to room temp if possible).

1. Remove the apple juice plate from the cage and replace with a fresh plate.
2. Dechorionate embryos in 50% bleach (squirt directly onto egg lay plate; no need to remove yeast) for 2-5 min.
3. Prepare fixative in glass scintillation vials:

H_2O	4.5ml
37% formaldehyde	0.5ml
Heptane	5ml

4. Wash dechorionated embryos into collection net with H_2O. Rinse embryos and sides of collection tube well.
5. Unscrew collection cap, pick out net with forceps, blot off H_2O, dunk into fix vial and shake off embryos. If desired, collection tube/cap may be rinsed a second time onto net to transfer any remaining embryos.
6. Screw cap onto scintillation vial and fix on nutator for 12-15 min (longer fixation up to 20 min produces stiffer/more durable embryos, but these may be harder to dissect).
7. Remove fix (aqueous bottom phase) with glass pasteur pipet. Remove as much as you can without aspirating embryos. Keep separate beaker for heptane/formaldehyde waste; dispose of in waste container in hood.
8. Add 10ml 100% methanol, or until scintillation vial is nearly full. Devitellinize ("crack") embryos by shaking for 30 sec. Devitellinized embryos fall to bottom; undevitellinized embryos and membranes stay at interphase. (If you add enough methanol there won't be an interphase and all will fall to bottom.)
9. Aspirate upper phase (heptane), interphase and most of methanol.
10. Wash embryos twice with methanol in scintillation vial, then transfer to 1.5ml tube for storage. Be sure to label your tubes so that you know what cross or stock was used to produce the embryos.

Fixed embryos can be stored indefinitely at -20°C in 100% methanol, or rehydrated immediately for antibody staining.