Tribolium embryo fixation / devitellinization

(Klingler lab 7/02)

1. Collect embryos on fine white flour with 5% yeast powder. Transfer embryos into egg basket (egg baskets are made from polyamide screen 0.10 mm mesh size, fused to a 1 cm section cut from a 15 ml falcon tube: fuse at 200 C on hot plate covered with aluminium foil; at this temperature the tube should melt but not the polyamide fiber)
2. Dechorionate (i.e. get rid of flour sticking to the eggs) by placing basket for 2 min into petri dish with 25% bleach (move basket with tweezers to stir the embryos)
3. Rinse well in deionized H2O
4. Transfer with spatula into glass scintillation vial that contains 6 ml heptane, 2ml PEMS, and 300ul formaldehyde 37%.
5. Fix for 30 min on shaking platform
6. Remove aqueous phase with pasteur pipette; devitellinize by adding 8ml MEOH and shaking vigorously for 30 sek
7. Transfer those embryos that fall to the bottom into an eppendorff vial. Embryos that remain at the interphase still have their vitellin membrane attached. Try to devitellinze these embryos by repeatedly aspirating and violently expelling the remaining fluid (both phases) in and out of the scintillation vial, using a syringe with 0.7 mm needle (19G needle). Transfer all embryos that fall to the bottom after this treatment into the eppendorf vial, discard those that remain at the interphase.

It is difficult to remove the vitellin membrane from post-gastrulation embryos since the serosa sticks tightly to the egg shell. Older stages usually only can be obtained as fragments, or as germ bands separated from yolk and serosa. Care must be taken during washing steps that these fragments are not aspirated with the supernatant, since they require more time to sediment than the intact blastoderm embryos

8. Rinse 2x with MEOH, store at -20°C (indefinitely)

PEMS: 0.1 M Pipes, 2mM MgSO4, 1mM EDTA, pH 6.9. (400ml: 12.08 g Pipes + 800 ul of 1M MgSO4 + 800 ul of 0.5 M EDTA, to pH 6.9 with NaOH)