

## *D. melanogaster* embryo fixation and collection

### Reagents

#### Bleach

Any household bleach

#### 10% formaldehyde

Methanol-free EM grade  
Polysciences, Inc.  
Product #04018  
CAS #50-00-0

#### Methanol

99.9%, spectrophotometric grade  
Sigma M3641

#### Ethanol

200 proof, Aaper

#### Heptane

#### Collection plates

### Collect embryos from population cages

10 mL of flies are grown in population cages. Flies that eclosed on Monday will lay well from Wednesday onwards.

On the morning of the collection, put temporary, well-yeasted collection plates for 1-2 hours. Put yeast paste in stripes. *This allows the flies to lay any retained eggs.*

Leave the remaining collection plates and yeast paste at room temp to warm up.

Put in the actual collection plates for at least 30 minutes.

After taking plates out, allow them to age in the culture room until eggs are the correct age (e.g. 1 hr collection + 3 hr aging = stage 5). *Do not smother the eggs by covering the plates.*

### Dechorinate eggs

Wash eggs with cold tap water into a nitex screen.

Scoop eggs into 50% bleach for 3 minutes. Rinse screen.

Pour embryos in bleach through the screen and wash with cold water until bleach smell is gone. *Eggs will clump together.*

### Fixation

For every 2 mL embryos, prepare a 500mL water-tight glass bottle with 100 mL heptane.

Scoop embryos into heptane.

Add 25 mL 10% formaldehyde.

Shake bottles at an angle for 20 mins at 150 rpm, so that eggs slosh in a monolayer. *This step is time sensitive. No less than 18 minutes, and no more than 25 mins.*

### Remove the vitelline membrane

Aspirate the bottom layer (formaldehyde) into formaldehyde waste.

Add 100 mL 100% MeOH and shake by hand very hard, 1 minute.

Pipet embryos on the bottom of the bottle into a new 50 mL conical.

Wash the embryos 3 times with 25 mL MeOH.

Wash the embryos 1 time with 25 mL EtOH.

Store embryos at -20°C in EtOH  
in a non-defrosting freezer.