

## X-GAL STAINING DROSOPHILA OVARIES

1. Dissect 10-20 ovaries in 1X PBS + a pinch of BSA.  
(BSA is necessary to keep tissue from sticking to plastic or glass)
2. Wash in 1X PBS  
make sure that ovaries are clean of other organs
3. Fix in 0.4ml PBS + 4% paraformaldehyde, 5 minutes
4. Wash 3X 5 minutes in PBT  
let ovaries settle between washes
5. Add 0.4ml Embryo Stain Solution/10ul XGAL solution
6. O/N at RT with rocking.
7. Wash 3X 5 minutes in PBT
8. Break up the ovaries into individual egg chambers by repeatedly pipeting through a pipet tip - they should become "snow" – overdoing this step will destroy egg chambers
9. Wash 1X 50% glycerol in PBS – let settle completely 10 minutes
10. Mount in 50% glycerol in PBS. Use electrician's tape as feet for coverslip.

### Embryo Stain solution (50ml)

1M Na <sub>2</sub> HPO <sub>4</sub>	0.684ml
1M Na <sub>2</sub> PO <sub>4</sub>	0.316ml
5M NaCl	1.5
1M MgCl <sub>2</sub>	0.05
K <sub>4</sub> Fe(II)(CN) <sub>6</sub>	0.065g (yellow crystals)
K <sub>3</sub> Fe(II)(CN) <sub>6</sub>	0.051g (orange)
Triton X 100	0.15ml
H <sub>2</sub> O	47ml

### Paraformaldehyde

16% Polysciences CAT#18814