

IMMUNOSTAINING DROSOPHILA OVARIES

1. Dissect 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, 15 minutes
4. Wash 3X 5 minutes in PSN
let ovaries settle between washes
5. Stain with primary antibody, O/N at 4oC in PSN
6. Wash 3X 5 minutes in PSN
7. Stain with secondary antibody, 2H at RT in PSN
8. Wash 3X 5 minutes in PSN
9. 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
10. Wash 1X 50% glycerol in PBS – let settle completely 10 minutes
11. Mount in 50% glycerol in PBS. Use electrician’s tape as feet for coverslip.

Primary Antibodies

anti b-gal is from Cappel, preadsorb to 100 ovaries at 1:100 and use at a further 1:1000 dilution (a lot of background from this antisera)

Secondary Antibodies

Alexa conjugated fluors are used at 1:200

Dyes

DAPI at 1:1000, stain 5’ during step 8

Alexa-conjugated phalloidin at 1:2000, stain 5’ at step 8