

-GAL STAINING OF ADULT RETINA (RON)

- 1. Cut off heads and slice in half with a razor blade.**
- 2. Fix in PBS + 0.8% Gluteraldehyde for 1 hr at room temp (RT)**
- 3. Dissect retinae out of cuticle in 0.8% Gluteraldehyde (including lenses)**

- 4. Fix in 0.8% Gluteraldehyde for an additional hour at RT**
- 5. Wash 3X10 min in PBS at RT with shaking**
- 6. Incubate in PBS + 0.4% Triton X + 4% Goat Serum for 30 min at RT**
- 7. Incubate in rabbit - gal (1:1000 in PBS + TX + GS) at 4°C overnight**
- 8. Wash 5X10 min in PBS + TX + GS at RT with shaking**
- 9. Incubate in goat rabbit-Avidin (1:200 in PBS + TX + GS) for 3 hours at RT**
- 10. Wash 5X10 min in PBS + TX at RT with shaking.**
Just before starting these washes, start A-B incubation:
15 of A + 15 of B in 1 ml PBS + TX
- 11. Incubate in A-B soln for 1 1/2 to 2 hours at RT**
- 12. Wash 5X10 min in PBS at RT with shaking**
- 11. Color rxn w/NiCl for 10 minutes**
- 12. Wash 3X10 min in PBS at RT with shaking**
- 13. Dehydrate 5-10 min each: 50% EtOH, 70%, 90%, 100%, 100%**
- 14. Incubate 2X10 min in 100% propylene oxide**
- 15. Incubate in 1:1 propylene oxide and plastic overnight**
- 16. Transfer retinal fragments to fresh plastic and dessicate for 2 hours**

17. Make fresh plastic again and embed the fragments in beam capsules so that the rhabdomeres will be tangential to the plane of section

***** To facilitate penetration, fissures should be sliced in large fragments of retina.**