β-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 β-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:
   15λ of A + 15λ 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
13. Color rxn w/NiCl for 10 minutes
14. Wash 3X10 min in PBS at RT with shaking
15. Dehydrate 5-10 min each: 50% EtOH, 70%, 90%, 100%, 100%
16. Incubate 2X10 min in 100% propylene oxide
17. Incubate in 1:1 propylene oxide and plastic overnight
18. Transfer retinal fragments to fresh plastic and dessicate for 2 hours
17. Make fresh plastic again and embed the fragments in beem 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.