Whole Mount Fluorescent Antibody Staining of *Drosophila* Larvae
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August 11, 2000

DAY ONE

1. Remove wandering third instar larvae from vial or bottle and place into small petri dish filled with 1X PBS. Rinse food particles from larvae.
2. Transfer washed larvae to a clean plastic petri dish filled with fixative (4% paraformaldehyde/1XPBS/0.1% Triton X-100).
3. Using forceps, grasp thin portion of anterior tip of larva and large portion of posterior of larva and pull forceps apart. In the ideal dissection, the brain, imaginal discs, and salivary gland will still be attached to the mouth hooks and the anterior cuticle. The chemosensory neurons will also be intact in this preparation.
4. Transfer dissected larval pieces to a microcentrifuge tube on ice containing 1.5 ml of fixative solution above. Fix larval parts for 1 hour on wet ice.
5. Wash larval parts with 1XPBS + 0.1% Triton X-100 (PBS-Triton), 3 times 10 minutes at room temperature.
6. Block for 30 min., room temperature (1XPBS, 0.1% Triton X-100, 5% heat inactivated normal Goat serum) (to heat inactivate serum, incubate at 55^{o}C for 30 minutes, then sterile filter and freeze in aliquots at –20^{o}C.)
7. Replace blocking solution with primary antibody (Molecular Probes rabbit anti-GFP antibody [#A-6455] is used at 1:1000). Incubate overnight in the refrigerator. Agitation is not necessary. Use only enough diluted antibody to cover the larval pieces. Antibody is diluted in PTS.

DAY TWO

1. Wash tissue 3 times 10 minutes with PBS-Triton.
2. Block as above (DAY ONE, step 6)
3. Replace blocking solution with secondary antibody (Molecular Probes Alexa Fluor 488=FITC, green; Molecular Probes Alexa Fluor 546 or Jackson CY3=CY3, red)(Dilute all secondary antibodies 1:100 in PTS).
4. Incubate in the dark, with tubes sitting vertically in a foil-covered rack on a nutator, 2 hours at room temperature.
5. Wash samples in the dark 3 times 10 minutes with PBS-Triton.
6. Remove last wash and replace with 100 ul Vectashield (Vector Labs). Allow tissue to sink in Vectashield (1 hour room temperature or overnight at 4^{o}C).
7. Dissect preparations further, if desired.
8. Mount on glass slides with bridge coverslips, using additional Vectashield. Coverslip and view in the confocal. Store preparation at 4^{o}C; will be stable for weeks or months.