STOCKER FLY BRAIN IMMUNOFLUORESCENCE PROTOCOL
January 8, 2001
Vosshall Lab

1. Anesthetize flies under CO2. Remove heads with forceps and transfer to fixative. For each line, decapitate 10-15 animals.

2. FIXATIVE:

4% paraformaldehyde
1X PBS
0.2% Triton X-100

3. Fix in microcentrifuge tubes filled with 1.5 ml of FIXATIVE on wet ice for 3 hours. Make sure that fly heads are submerged in fixative.

4. Remove fixative and replace with 1XPBS/0.2% Triton X-100. Rinse twice quickly with this Stocker Wash solution to remove residual fixative.

5. Wash at least 2 x 30 minutes or overnight at 4 degree C. in 1XPBS/0.2% Triton X-100. Fly heads can be stored in Stocker Wash solution in the refrigerator for weeks and not lose morphological integrity or antigenicity.

6. Replace Stocker Wash solution with 500 ul of Stocker Block solution (P/T/S):

1X PBS
0.2 % Triton X-100
5 % HINGS (heat inactivated normal goat serum)

7. Transfer heads to a Sylgard dissecting dish filled with Stocker Block. Gently remove cuticle to expose the brain, starting with the removal of the proboscis. Gently peel away the cuticle at the back of the fly head, being careful not to damage the antennal lobe, which lies next to the midline, just below the esophagus (hole in brain). Once all the cuticle has been removed, gently remove the fat body and trachea (air sacs) that cover the brain. The retina may be attached to the brain and does not need to be dissected away. Do a minimum of manipulation to ensure that the brains do not float, but not so much manipulation that you damage the brain. As long as the brain does not float, it is acceptable for a few trachea to remain.

8. Use a wide bore pipet tip to transfer the dissected brain to a glass spot well filled with Stocker Block. Rinse the inside of the pipet tip with Stocker Block before transferring the fly brain, or else it will stick to the inside of the pipet. Have the tip partially filled with Stocker Block before picking up the brain, and make sure the brain is never in contact with air, or it will be destroyed. Continue dissecting the rest of the brains.

9. Once all brains of a genotype are finished and collected in the glass spot plate, inspect them to determine if any are floating. If so, remove more fat body, trachea, or air bubbles

until the brains sink. Using the same wide bore pipet tip, transfer all brains gently back to the original microcentrifuge tube containing Stocker Block.

10. Once you have finished dissecting all genotypes, and all brains are collected in the microcentrifuge tubes, make up the primary antibody, by diluting appropriately in Stocker Block:

mouse anti-nc82 (stains all central brain neuropil; non-commercial antibody from Stocker)

use at 1:10

rabbit anti-GFP (Molecular Probes commercial antibody; Catalog #A-6455)

use at 1:1000

Minimize the volume of antibodies you use, to use nc82 sparingly!

For each tube of brains, use:

2 ul nc82
0.02 ul anti-GFP

scale up according to the number of tubes you will have, e.g. 5 tubes:

10 ul nc82
0.1 ul anti-GFP
89.9 ul Stocker Block

11. Carefully remove most of the Stocker Wash from the dissected brains, leaving a small volume covering the brains. Be careful not to remove so much that the brains dry out! Add the primary antibody and incubate overnight at room temperature, with brains sitting in a rack vertically on the nutator.

12. Next day, wash brains with Stocker Wash 3 x 30 minutes or longer, at room temperature. For each wash, use ~1 ml Stocker Wash.

13. Make up secondary antibodies:

Goat anti-mouse Cy3 (red; Jackson; Catalog #115-165-100)

Use at 1:100

Goat anti-rabbit Alexa488 (green; Molecular Probes; Catalog #A-11034)

Use at 1:100

TOTO-3 (blue; nuclear dye; Molecular Probes; Catalog #T-3604)(stored in DMSO—allow to thaw before use)

Use at 1:1000

For each sample, make up 100 ul of secondary antibody. As for the primary, remove most of the Stocker Wash, but not so much that the brains dry out. Add secondary antibody and incubate brains for 4 hours at room temperature while sitting vertically in a foil-covered box on a nutator. FLUORESCENT ANTIBODIES AND TOTO-3 ARE LIGHT SENSITIVE!! KEEP COVERED WITH FOIL.

14. Wash brains with Stocker Wash 3 x 30 minutes or longer, at room temperature. For each wash, use ~1 ml Stocker Wash

15. Remove most of last Stocker Wash, leaving only a small volume to cover the brains, and add ~100ul of Vectashield mounting medium to brains. Allow brains to equilibrate a few minutes at room temperature or overnight at 4 degrees C. in the Vectashield. Mount brains on glass slides with bridge coverslips and store in the dark at 4 degrees C. Photograph in the confocal.