Dye Filling to Stain Amphid and Phasmid Neurons

by Michael Koelle, from Beth Sawin

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1. Buy DiO from Molecular Probes, catalog # D-275. DiO fluorescesces green (use FITC filters). If you want red fluorescence, DiI can be substituted; stain exactly the same way but use the Texas red filters on the fluorescence scope. DiI may stain a slightly different set of cells.

2. Stock solution is 2 mg/ml in dimethly formamide, stored at -20deg. in a foil wrapped tube.

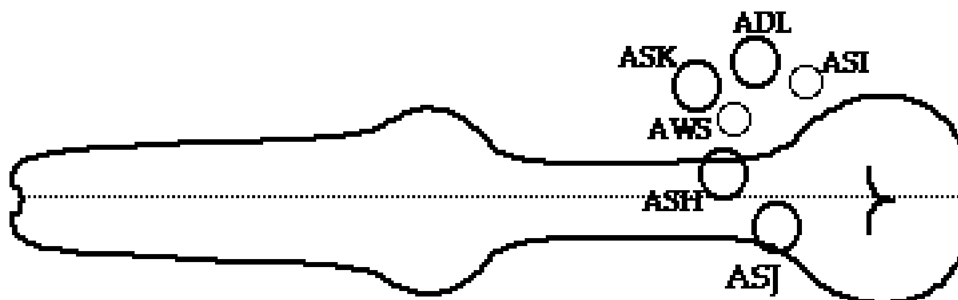
3. Dilute the stock 1:200 in M9. Some dye will precipitate when you do this; don't worry about it.

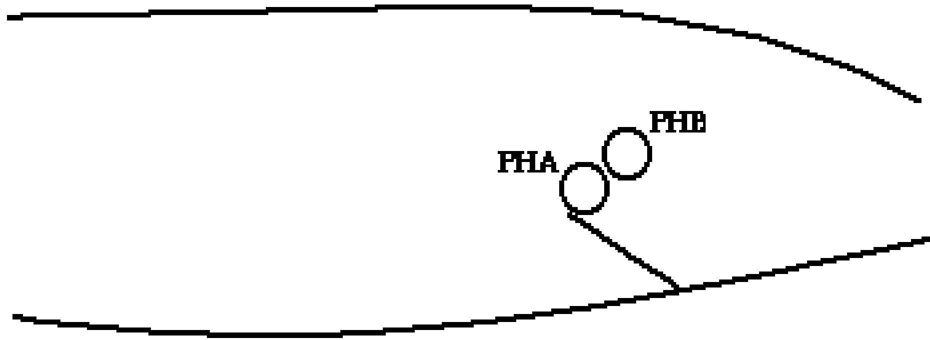
4. Put 150 ul stain in a microtiter well, and use a worm pick to put transfer some worms into the dye. Incubate 2-3 hours at room temperature.

5. Use a mouth pipette to transfer the worms to a fresh plate, and let them crawl on a bacterial lawn for about 1 hour to destain. (Alternatively, wash the worms 3X in M9).

6. Put worms on agar pads with sodium azide and visualize by fluorescence using the appropriate filters.

The following cells stain (one of each bilaterally symmetric pair is diagrammed here, and only the cell bodies are shown, but processes also stain):





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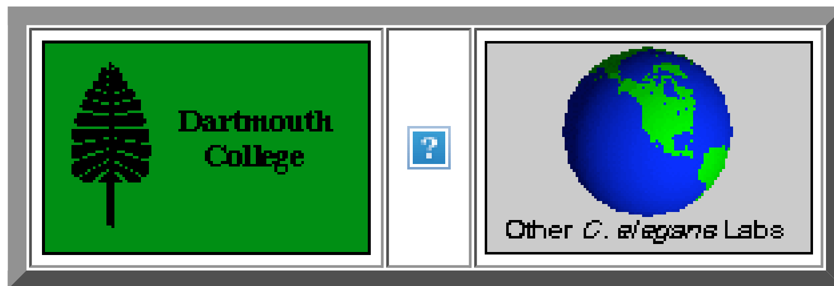

Original file name:dye filling

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