Larvae Tracking Protocol
Date: October 24, 2012

Ten days before tracking experiment:

- Vial passing to ensure larvae are available on tracking day
- Adult flies (n >8) of the desired strains including controls are to be placed into fresh vials.
- At least two vials per strain to guarantee enough third instars for testing (may be more depending on the strain).
- Try and avoid large disparities between number of adults in vials to try and prevent effects due to larval crowding.
- Vials should be labeled with strain number (Bloomington's), date added to vial, and optionally the name of the mutant.
- Four or five pairs (8 or 10 animals) seems ideal for avoiding overcrowding of larvae
- If chosen manually, select 4 males and 4 females.
- Vials should be passed into new fresh vials every two days (some strains may require daily passing to avoid overcrowding).
- Wild-type and most mutants will see wandering third instars in about 6 days.
- More larvae are generally seen after the mating pairs have acclimated, i.e., after they have been passed a few times.
- Verify that there are enough 10cm 1.5% agar plates.

Day before tracking: At least 12 hours before experiment: equilibration of all reagents and larvae

- Place glass bottles or plastic vials containing all larvae to be tested in temperature-controlled room. Move also paint brushes and other materials.
- At least two vials or 1 bottle of strains to be tested should be present to guarantee enough larvae.
- Prepare at least 2 plastic repellant rings in tracking room (1.5 cm thick plastic doughnut with 8.5 cm diameter on outside and 6.0 cm diameter for empty space in center)

Day of tracking experiment

Worm Tracker Settings:

- Create a folder that will contain the videos tracked of the day
- Change light as needed. We want white background with mostly black larvae
- Load the configuration for tracking larvae
- In "Directory" click "Browse" to select the folder that you just created.
- Make sure that "add date" box is not checked
- In "Filename", you will type name of the video file
- Set the recording time to 4 minutes:
- Click on the drop-down for recording-time. Select "Time". There will be three text boxes. The middle one is for minutes; type 4 in it.
- Go to File -> Preferences. Set frame rate: 7.5 fps
- Make sure that nothing on "Effects" is selected
- Select the tab (top of window) named "recording". Verify that Tracking-rate is 1. Verify that Tracking-delay is 400
- Select the tab (top of window) named "tracking". Verify that Centroid values are as follows, x = 40, y = 30 (will make fewer stage movements)

- Place 10 cm dish with centering cross lid onto stage
- Click Home to move stage to origin (it may be there already).
- Move the plate together with the transparency until centering cross falls in center of view when stage is at origin.
- Make a note of the temperature and the time.
- Remove lid and place any third instar larva onto plate.
- Focus microscope until the larva has clearly defined edges
- Focus while looking at monitor. Focus may be different than through the eyepieces of the scope.

Plate Preparation

- Find two agar plates with smoothest surfaces
- Plates that are not smooth or that have bubbles should not be used for tracking. They can be used as acclimatization plates
- Place 1.5 cm wide plastic repellant rings on outside of dish

Larval selection

- Experimental larvae are to be taken only from the sides of the glass bottles if they have risen at least 1 cm from the food to ensure they are in fact “wandering” third instars and are not still actively eating.
- Guts should be examined to verify that the larvae has stopped eating. The food is colored (for example, blue) and can be seen by the naked eye in the gut of the worm. While the color of the food will rarely be fully gone from the larvae, if the gut shows clearly the color, it should not be used for tracking. Guts should show a light color tinge or no color at all.
- Larvae must be showing active movement and respond actively to being touched by a paint brush. Wandering third instar are looking for a place to pupate and will slow down as they move closer to that stage. Ideal larvae are still actively moving.
- Larvae will shorten becoming rounder with pronounced “antennae-like” projections as they get closer to pupation. These larvae should not be used for tracking.

Tracking

- After finding an appropriately staged larva, the larva is to be removed using the bristles of a paint brush.
- Care should be taken to not overly agitate the larva and to pick up the larva as quickly as possible. One technique to aid this is to gently roll the larva over and press the bristles onto the underside. The larvae seem to “stick” more easily this way.
- Pre-plate the larva for 60 seconds by placing them on a clean agar plate and waiting
- Figure out the sex of the larvae.
- Record on paper the strain or genotype, gender, and number.
- On the computer, write exactly the same information for the Filename before you place the larvae on the plate on the microscope.
- Remove the larva from the pre-plate and immediately place them onto the center of the centered tracking plate on the microscope. Larva should be visible on monitor
- Hit “start” buttons in the worm tracker program to being stage tracking. Tracker should jump to center larva.
- Start recording only when the larva has completed an entire contraction and straightened out its body.

- If larva walks over plastic and comes too close to the petri dish walls, stop tracking and recording, record in lab notebook that this larva crossed the repellant.
- When a larva goes over the repellent more than two times, consider discarding it and using another larvae
- If the larva has not crossed and the four minutes has elapsed, stop stage tracking and remove the larva gently from agar dish trying not to damage the agar surface.
- If applicable, move the larvae to a well in the recovery plate. Make sure you label the plate accordingly: number of larvae on the lid of the plate.
- Always alternate genotypes in a set cycle with controls. For instance, if we have three genotypes A,B, and C – one of these is the control – they should be run A followed by B followed by C followed by A and so on for the duration of the experiment.

End of Tracking

- Mark vials from which larvae were taken for tracking. These vials should not be used for maintaining fly stocks.