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## Drosophila Locomotion Protocol

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

This protocol is used to assay the locomotion of flies as a screening tool.

## Troubleshooting



## Day -10: Set up crosses

- 1 Set up fly crosses as needed.


## Day 0-6: Collect progeny

- 2 ~10 days after setting up the crosses, progeny will begin to eclose. Once daily, collect those that are desired, discarding any undesired genotypes. Flies should be collected in vials with 9-14 flies per vial.

6 replicates per genotype is desirable for the assay, so once 6 vials (of 9-14 flies each) have been collected, the crosses may be discarded even if they are still producing additional flies.

Note: Flies are sensitive to the effects of anesthesia, so collecting them at the time of eclosion is the only time they should be exposed to CO<sub>2</sub>.

## Day 1-10: Aging flies

- 3 Flies should be flipped (without anesthesia) to new food every 2-3 days while aging and stored at  25 °C in an incubator.

## Day 10\*: Locomotion assay


1m

- 4 Supplies needed:  
Timer  
Empty vials and buzz plugs  
White paper  
iPhone or iPod, or alternate recording device, optional

\*Day 10 is a standard time point but the assay can be performed at any desired timepoint and will show age related decline in locomotion over at least the course of 3-20 days.

- 5 Perform assay in groups of 4-7 vials at a time when possible. For example, if 30 vials are to be assayed on one day, divide them into 5 groups of 6 or 6 groups of 5.
- 6 Flip flies to new polystyrene or polypropylene vials, without food, plugged with a Buzz Plug. Write the genotype or code on the bottom of the vial so that it does not interfere with seeing the flies when the tube is on its side.



- 7 Allow flies to acclimate for  00:01:00 before beginning.
- 8 Start Timer. Briefly but firmly tap the first vial 3 times, strong enough to knock the flies to the bottom on the vial, then immediately lay it on its side on top of a white piece of paper and note the time on the timer. Wait 15 seconds, watching the time count down on the timer.
- 9 After the 15 second delay, record a 3 second video of the flies moving in the vial using an iPhone or iPod or equivalent.  
(As an alternative, counts can be performed manually).
- 10 After the video is recorded, put the vial upright and set aside for now.
- 11 Repeat steps 8-10 for each additional vial, until each vial has been recorded 1 time. Then start back again with vial #1, completing a total of 6 sets of recordings.
- 12 At the end of the assay flies are anesthetized and fixed or frozen as needed for downstream assays.
- 13 Analyze movement data in a blinded fashion.

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