**Wiggle Index Protocol**

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See Denecke et. al 2015, PLOS ONE for further details

**Materials Needed**

* 5% sucrose (Analytical Reagent) in dH2O
* 20% Sucrose in dH2O
* Nunc Treated 24-well plate
* Insecticide
* Larvae Picker
* Grape Juice Plates
* LED light pad
* Camera
* Step Pipette
* Normal Pipette

**4 Days Before Assay:**

*A) Obtaining third instar larvae*

1. Put 50-60 virgin females and 15-20 males of selected genotypes into hoff vial sprinkled with dried yeast powder.
2. Allow to acclimate to vial conditions overnight
3. Between 12 and 2PM tip flies into fresh hoff vial sprinkled with dried yeast.
4. Leave flies undisturbed at 25 Degrees for 24 hours
5. Remove flies from vial
   1. Note: For replication flies can be tipped into a fresh hoff vial sprinkled with yeast
6. Leave larvae at 25 degrees for 3 additional days after removal to generate a population of third instar larvae
7. You now have 3rd instar larvae for your assay
   1. Ex: Step 1 on Wednesday; Step 3 on Thursday; Step 5 on Friday; Step7 on Monday

**Day of Assay:**

*B) Preparation of materials*

1. Prepare sucrose solutions, grape juice plates as described by other protocols
   1. Note: All solutions should be at room temperature before beginning
2. Label 24 well plate corresponding to your experimental design
3. Add 200 µL of 5% sucrose solution into the 24 well plate using the step pipette.
4. Dilute insecticides to 5x of the desired concentrations and keep in room temperature dark location until usage.

*C) Sorting third instar larvae*

1. Add 20% sucrose to vial with third instar larvae until it is approximately ¾ of the way up the vial
2. Stir the food gently with a larvae picker or metal rod
3. Let stand for ~5 minutes or until larvae have fully floated to the top and separated from food material
4. Carefully pour onto Larvae-Catcher 3000© mesh. The larvae should pour off first and it is important to minimize the amount of food that gets into the mesh
5. Dry the larvae thoroughly using tissue paper. Flip the larvae ball around to make sure that they are as dry as possible
6. Tip the larvae ball onto a grape juice plate
7. Using the larvae picker, transfer 25 larvae/well into each well of your 24 well plate depending on your experimental design

*D) Filming*

1. Centre the camera on the LED light pad using the black cross drawn on the LED light pad. The centre of the cross should be at the midpoint of the camera view
2. Turn the LED light pad on by holding down the power button
   1. Note: Do not remove finger until the light pad is on maximum intensity
3. Place the 24 well plate on the pad for 15 seconds to acclimate the larvae to light conditions
4. Immediately before filming give the larvae a gentle swirl to center them in the 24 well plate
5. Align four wells corresponding to one treatment with the cross to ensure that the plate is in the same exact position on each film
6. Hit the red button on the top of the camera to start recording. Leave running for 10 seconds and then hit the same button to stop recording. This first film will serve as your 0 minute time point.
   1. Note: VERY IMPORTANT. Any vibrations detected by the camera will be picked up as larval motion and will obfuscate results. Be careful!!
7. Repeat filming at specified time intervals after dosing

*E) Dosing*

1. Once initial filming is done set up your diluted insecticides and 24 well plates to an open location
2. Add in 50µL of 5x insecticide solution to the first 4 wells (corresponding to your first film) to bring the final concentration to the 1x final concentration. (50µL into 200µL = 1/5 dilution)
3. Start the timer immediately after dosing the first 4 wells
4. Every 15 seconds from there on out, dose 4 wells at a time in the order they were filmed
5. After insecticide is added to all the wells, gently shake each plate to ensure insecticide is well
6. Before the next film, remove 50µL of mixed solution to bring the final concentration back to 200µL

*F) Analysis*

1. Download videos onto personal computer using black micro-USB:USB cord
2. Rename videos using Renaming Script and .csv/Excel document containing the names of all of your videos
   1. See Renaming\_Script.R and Rename Example.csv files
   2. Note: Copy all videos to separate folder as backup before renaming them
   3. Note: Follow model exactly
3. Use Free Video-JPG Converter to convert .MTS videos to image sequences. Select All when choosing number of images to extract
4. Upload Image Sequences to Folder on “Shane” Server using WINSCP
   1. IP address: 115.146.93.131
   2. Username: shanedenecke
   3. Password: lucylucy
5. Divide image sequences into 3 folders labled 1 2 and 3
6. Login to “Shane” serer using Moba-X-Term (or putty on a mac) and open Fiji with the command “fiji”
7. Repeat this 11 more times using independent tabs of Moba-X-Term to open independent fiji aplications
8. In each fiji application (12 in total) go into macros and select 1 of the 4 scripts (Bottom Left, Bottom Right, Top Left, Top Right) for each of the three folders
   1. Note: after selecting one of these scripts you will be prompted to choose a folder
9. Let the WI run until finished. 12 excel documents should appear corresponding to each script
10. Transfer these documents into a folder on your local drive
11. Run processWiggleOutput.py (#) on your machine and select the folder that you put all of the output into. This will consolidate all your outputs into one document
12. Optional: You will now have your final dataset in a subfolder called output in a file called output.csv. You may want to relocate this file or rename it but it is not necessary
13. Now run the WI\_Script.R. It will prompt you for several bits of information. After which the script will run and will have your graphs generated for you.