Booz-o-mat Tracking Protocol
per A. Corl, 2004

Timetable for your fly crosses

Day 0: Set up crosses of fly lines to be screened. Cross 20 males to 20 virgin females in bottles and incubate at 25˚ C.

Day 2: Clear parents from the bottles set up on Day 0.

Day 12: Collect the number of males required for your experiment from each bottle established on Day 0. Store in vials at 25˚ C.

Day 14: Run flies in booz-o-mat, or your preferred tracking apparatus.

Prepare the tracking system

1. Check the water level in the water circulator (labeled "deliverance"). Fill with tap water close to the "full" mark. Turn on the circulator using the switch on the side of the "Fisher Scientific" box atop the water chamber. Be sure the temperature is set to 20˚ C.
2. Using 95% EtOH (dilution made from 200 proof Gold Shield ethanol and filtered water), fill up the alcohol chamber to the chamber lip.
3. Refill the two water humidifier chambers with filtered water so that the water levels are above the aerator sponges.
4. Check the Drierite filters to make sure that they still have absorptive capabilities. (Blue rocks are dry; purple rocks are expended.) If filters are entirely purple, dump out the contents in the trash and refill the containers with new blue Drierite.
5. Turn on the evaporator air flow full blast with the red, four spoked knob.
6. Set the 3 evaporator gauges (EtOH / Air / Air) to your desired ratio (e.g. high dose: 100/50/150, medium dose: 70/80/150, low dose: 50/100/150).
7. Allow the system to equilibrate for 30 minutes to 1 hour.
8. While the tracking system is equilibrating, wash your boozetubes with water, scrubbing them thoroughly with a small brush. Soak the boozetubes in EtOH (95%) in a beaker for 5-10 minutes. Let the tubes air dry on a rack. (In order to save time on the day you do the tracking, you can clean the boozetubes 1-2 days before.)

Setting up the camera system

9. Orient the light box horizontally and turn it on.
10. Place the booz-o-mat on the light box.
11. Check to be sure the camera is on. (It should always be left on.)
12. Check the "bubble" on the camera to be sure it is balanced. The bubble should be in the middle of its field. Adjust camera tripod legs if necessary.
13. Restart the computer, close all programs, and load Adobe Premiere.
14. Click File > New Project > Load > and select your preferred Project Setting (e.g. "00000 Lowest Setting" for lowest quality and frame size).
15. Click File > Capture > Movie Capture. If asked, deactivate Appletalk.

16. Position the booz-o-mat so that it fills the screen. Use the zoom feature of the camera to change magnification. Be sure that all of the booz-o-mat chambers appear straight up and down on the screen. If necessary, adjust the brightness by using the exposure button on the camera.
17. Tape down each corner of the booz-o-mat stage, the camera tripod, and the EtOH and air tubes to ensure stability throughout the filming.
18. Check that the thermometer in the ethanol chamber has stabilized at 20° C.
19. Place a clear plastic ruler across the booz-o-mat and record a brief movie a few seconds long. Save the movie as "Ruler [date]" or similar.

Loading and recording your flies in the booz-o-mat

Optional: Your first loading and recording cycle of the day should be a "mock run" performed with any unimportant set of flies to check the following: a) all boozetubes are being picked up by the camera, b) all equipment is working, and c) file sizes are being generated normally. In addition, the mock run serves as a "flyification" period for your boozetubes. It is not necessary to film the "mock run" if you set up everything correctly.

1. Take your vials of flies from the incubator and transfer these flies into your boozetubes. Then, load the tubes into the booz-o-mat, screwing the tubes in semi-tightly. (Note: All of the boozetubes being filmed must have flies in them, or else the data analysis may go awry.)
2. Hook the booz-o-mat to the air tube and let sit for 10 minutes.
3. After 10 minutes, click "record" on the computer. When the computer starts recording (the delay may be up to 30 seconds), start your timer for nearly the length of your movie.
4. After 2 minutes of air, *quickly* switch over to the EtOH source tube.
5. Stop the recording by clicking the computer mouse when you want the movie to end (e.g. 21 more minutes).
6. Switch the booz-o-mat over to air for 10 more minutes to clear the ethanol vapors.
7. Click File > Save, and save your movie from this run into the computer's hard drive. Close your file. The very first run which should be a mock-run should be saved as "Mock-Run" and subsequent runs should be saved as "Run/Run2". Save files into a folder named with the date.
8. After 10 minutes, remove the boozetubes from the booz-o-mat and dump the flies into a morgue. Repeat this 8-step cycle with new sets of flies as desired. Check mock run in Dias to make sure settings are ok.

Clean-up

1. Turn off the light box. Leave the camera ON (always). Remove your boozetubes, and put the booz-o-mat away. If no one else is using the evaporator and circulator, shut these down.

Data analysis

1. Restart the computer. Turn off file sharing.
2. Proceed with the analysis of your choice.

Computer Analysis of Screen Data

1. Quit out of Adobe Premiere.
2. Figuring out the scale factor.
    a. Open the program "Dias Select".
    b. Within Dias Select, open up the "Ruler file creator for your booz-o-mat run. When prompted, click ok to verify the time-base for the ruler.
    c. Click "Measure> calibrate Scale" : In the calibrate scale screen, type a question mark "?" for the scale factor and leave the unit space blank.
    d. The computer will then prompt you to draw a line of known length. Do this by drawing a horizontal line between two points on the ruler on the screen. E.g., draw a line between the 9 cm and 13cm markings.
    e. When prompted, type in the length of the line (e.g. 40) and the scale you are using (e.g. mm).
    f. In the next screen, note and write down what the scale factor is (e.g. 0.261)
3. Throw out the movie made for the mock run.
4. Open up "Apple – one click> Dias-Aylin"
5. Determining the threshold values.
    a. Through DIAS, open up one of the movie files
    b. Click DIAS>Autotrace by threshold
    c. Enter in the desivet frames (e.g. First frame:1300, last frame:1400)
    d. Choose to "select Region by mouse"
    e. Using the mouse, draw a box that encompasses the entire movie screen
    f. Enter in the settings you wish to test out, settings that have worked for Aylin are "10, 5, 60, 0, 0, 1,1" for the seven settings the program prompts you to enter, resolution high
    g. Click start and watch what objects are highlighted in green, Ideally the flies should all be green while the background should not be colored green.
    h. Press open apple + . on the keyboard to stop the program. Modify the settings if necessary, to encompass more flies and/or less background
    i. When you are happy with the settings you have, save the settings in the folder that the films will be analyzed in . Save as "SET"
    j. Click on "abort" to get out of autotrace by threshold
6. Take the folder you wish to analyze and drag it onto the button labeled "Linus " on the "Dias Aylin" popup
7. Type in the conversion factor you computed in step 2
8. The program will then begin to analyze your movies. Watch for a while to make sure things are proceeding well.
9. For each booz-o-mat ran of 8 boozetubes, the program will require two hours of analysis time.

Analysis of Booz-o-mat data using SumDrop and Excel

1. After the computer "Herb" has finished analyzing your booz-o-mat movies, it will save its analysis output into folders entitled "Run 6.8 Done" and transfer all these "Run Done" folders into your own computer via the Apple-talk network. Make "Run Done Out" folders with date.
2. Take all the "RunDone folders and drag them onto the file "SumDrop DT". MacPerl will then analyze the contents and save files such as "Run6.8 Done Out" within the "Run Done" folders.
3. Open all these "RunDone Out" files by dragging them onto the "Microsoft Excel".
4. Copy Column D from each "Run Done Out" file (Column D corresponds to the speed of the flies at different times (mm/sec) and paste them into the appropriate columns in the EP3 summary template file. Be sure to save the template file under different name.
5. After pasting all the "Run Done Out" column D data into the excel template, type in genotype labels for each of the boozetubes as well as the dates and run numbers.
6. Type in a title for each graph next to each run. Include Run numbers, date and ethanol concentration.

Boozetube construction

1. Select 8 new tubes [Fisherbrand non-sterile plastic tubes, clear polystyrene, 16 x 125 mm. Vendor: Fisher Scientific; Catalog #: 14-956-8G] and a syringe needle (around 21 gauge).
2. Heat the metal needle over a bunsen burner until it is red hot.
3. Melt holes in the convex end of the tube by inserting the hot needle. They must be small enough that an adult fly cannot crawl through.
4. Be sure all of your tubes have the same number of holes of the same size, in order to ensure equal flow rates. You can compare the flow rates for all tubes by performing a quick test measuring the amount of water that pours through each tube in 10 seconds.