

<p style="text-align: center;">Inebriometer Use Protocol Per D. Guarnieri / A. Corl, 2003</p>

Timetable for your fly crosses:

Day 0: Set up crosses of fly lines to be screened. Cross 20 males by 20 virgin females in bottles. Also set up several bottles of y-control males by X⁺X virgin females to use for standardization of the columns. Incubate the bottles at 25° C.

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

Day 12: Collect ~ 110 males from each bottle established on Day 0. Store in vials at 25° C. Collect 3 vials of y-control x X⁺X males for each inebriometer you will be using.

Day 13: Check the tops of the inebriometers you wish to use to make sure the silicone seal is still intact. If the seal is broken, apply fresh silicone gel. This silicone needs 24 hours free from EtOH vapors in order to dry and set properly.

Day 14: Run you flies in the inebriometer.

Set up the inebriometers and computers

1. Check the water levels in the water bath circulators you wish to use. Each water bath modulated the temperature in two inebriometers. If the water level is below the “low” mark, add tap water up to the “full” mark. Turn on the circulators, and be sure they are set to 20° C.
2. Check the ethanol levels in the EtOH chambers. If the EtOH levels are below the “flare” at the top of the chamber, add 95% EtOH (950 ml 200 proof Gold Shield EtOH + 50 ml filtered H₂O) up to the flare.
3. Refill the 250 ml water humidifier chambers with dH₂O so that the water levels are higher than 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 airflow.
6. Set the vapor gauges of the inebriometers (EtOH / Air) to your desired dose for testing. Set the vapor gauges of the inebriometers you won't be using down to 0 / 0.
7. Check the column gear to make sure all the tubes are connected properly.
8. Let the system equilibrate for 1.5 hours.
9. Uncover the collection bottles at the bottom of the inebriometers and position them under the Drosophila monitors. Be sure there is sufficient EtOH in each bottle.
10. Turn on the two computers:
 - a. For the computer on the left, turn on the monitor by pressing the round button, then click “Restart”. After restarting, push in the floppy disc containing the DFM26.P Mac Program.
 - b. For the computer on the right, press the “on” button on the tip right of the keyboard.
11. Create a folder for yourself on the Hard Drive of each computer. Within your folder, create a second folder labeled with the date.

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12. On each computer, double-click on the DFM26.P Mac Program.
13. On the left-hand computer, click: Command > Monitors 1-4 to hide those monitor screens. On the right-hand computer, click: Command > Monitors 5-8 to hide those monitor screens.
14. For the monitors you intend to use (e.g. 5 & 6 for Napa and Sonoma, 3 & 4 for Gin and Tonic), fill in all blank spaces (i.e. date, Start time, Researcher, description, Comment, Fly line, EtOH / Air, Column, and Reg pressure) with “XXXX”. Then click “start” on each of the monitors and wait 3 minutes to make sure zeros come up after the first 3-minute bin.
15. If the zeros come up properly, click “Stop”, then “Save”, then “Cancel”.
16. Using a straightened paperclip, ensure that the funnel at the base of each inebriometer is not clogged.

Loading flies into the inebriometer

1. For the first and last runs of the day, run y-control x X^X males to make sure that the columns are running normally. “Normally” = MET between 18-22 minutes.
2. Loading flies into syringes (AC method):
 - a. Note that the plunger of each syringe is labeled “N”, “S”, “G”, or “T”, corresponding to the 4 “good” columns: Napa, Sonoma, Gin, and Tonic.
 - b. For each syringe: Pull the black rubber off the plunger, and take out the grey plunger. Replace the black rubber onto the plunger, and insert a tan sponge into the cut off end of the syringe. Push in the sponge so that it doesn’t stick out.
 - c. Remove vials of flies to be loaded from the 25° C incubators and place one next to each syringe.
 - d. To transfer flies from vial to syringe: Place syringe, sponge side down, into a test tube rack. Place a plastic funnel into the syringe. “Flip” flies from vial to syringe, much as you would flip flies from vial to vial. The funnel and rack will help prevent flies from escaping. Remove funnel and replace with the plunger. Only insert the black tip of the plunger into the syringe. Carefully pull the sponge out halfway – don’t let the flies escape.
 - e. Repeat with the next syringe.
3. Allow flies to sit in the syringes for 5 minutes.
4. Loading the inebriometer:
 - a. Climb up the stepstool with a syringe of flies and set the syringe down on the top shelf.
 - b. Remove the green stopper from the top of the inebriometer column.
 - c. Holding the syringe, tap the flies firmly down to the bottom (plunger end) of the syringe.
 - d. Quickly remove the sponge, and insert the plunger into the column. Let the plunger sit in the column for about 5 seconds.
 - e. Press the plunger smoothly until all flies are out. Shake the plunger, then quickly remove the syringe and replace the green stopper, being sure that you support the back of the funnel with your other hand to avoid damaging the column.
5. Quickly click “start” on the appropriate monitor screen on the computer.

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Ending a run

1. After the monitor counts 3 consecutive zero readings at 3 time points and at least 80 flies have eluted out of the inebriometer, click “stop” on the appropriate monitor screen.
2. Click “save” to save the file in the folder you created earlier. The file name will look something like this: M3T132. (M3 = Monitor 3; T1532 = time you started the run, in this case 15:32 or 3:32 pm.)
3. On the right-hand computer, saved files can be opened later for viewing; you’ll need to drag the file onto the “Excel” icon on the desktop to open it. On the left-hand computer, you cannot save files (broken hard drive); simply double-clicking the file will allow you to open the file using Simpletext.

Preparing for the next run

1. Load the next batch of flies into syringes.
2. Copy down the elution numbers from your last runs onto an Inebriometer Data Form.
3. Check the mean elution times of your y-control x X^X flies to make sure they are between 18-22 minutes. Record your Mean Elution Times (METs) and flow rates in the lab notebook.
4. If your y-control x X^X METs don’t fall within 18-22 minutes, adjust the columns minutely. Per DG: Down 2 with air = Up 1 with EtOH = 1-2 minute difference in MET. Try to keep EtOH + Air = 95. Allow the system to reequilibrate for about 30 minutes before loading your next set of flies.
5. Load your next batch of flies into the columns.

Turning off the columns

1. Make sure no one else is using the columns!
2. Turn off the circulators, computer, air pressure, and close the ethanol collection jars.
3. Turn off the computers: Finder > Special > Shut down.