Won's Methods

From labwiki

Contents

C. elegans Experiment Methods (Won's methods) - By Kyla

Overall scope:

There are three experiments, all of which used the model system of C. elegans feeding on E. coli exposed to various temperature series for a generation, to test the hypotheses that: a) variation in temperature has a negative impact on offspring production, b) when the variance is equal, temperature series with higher autocorrelation will have a more detrimental effect on offspring production, c) these effects will vary depending on the mean temperature of the series, and that d) the initial E. coli population density has no impact on C. elegans reproduction during the experimental period. General Experiment Procedures

Preparing Agar Plates

1. Prepare nematode growth medium agar mixture in a flask, following the recipe (makes 2 liters of media). All ingredients listed can be found in the cabinet above the first lab bench (bench with the sink). Use an Erlenmeyer flask from the cabinet and fill it with deionized water to ~200 -100ml less then the volume of media you wish to make. Put a magnetic stir bar in the flask, and place the flask on the hot plate with the heat off and turn the stir on. Place a piece of tin foil wrapped in a piece of autoclave tape on the top of the flask to act as a lid, and write a label on the tape. Using the balance, weigh out the appropriate amount of each ingredient in a weigh boat and add it to the flask, in order.

NOTE: each plate requires 8ml of media, thus to make ~50 plates, ~500ml of media is required (these amounts are to make 2 liters of media, thus divide all ingredients by 4)

4.0g NaCl 6.2g Peptone 6.0g KH_2PO_4 1.3g $K_2HPO_4{:}3H_2O$ 40g Agar

2. Autoclave the mixture along with a bottle of deionized water and the plating machine tubing wrapped in tin foil. Use the autoclave located in the Charles lab in the 3rd floor of B1. Note that you must sign up for a time slot prior to use (there is a sign up sheet on top of the machine). Autoclave these items (can be in plastic bin), at slow exhaust for 1 cycle (setting 2). Be sure to loosen the caps/tin foil on all glassware before autoclaving, and use the orange mitts to transfer hot objects.

3. After autoclaving, let media cool a bit (but not too much), then add:

NOTE: you must make the stock solutions ahead of time. Make them in small bottles, label and store in cupboard.

200mg Streptomycin (found in the fridge) 2.0ml 1M CaCl Stock solution (14.7g CaCl in 100ml H20) 2.0ml MgSO4 Stock solution (24.6g MgSO4 in 100ml H2O) 3.2ml Cholesterol Stock solution (5mg/ml in 100% ethanol, stir overnight) 2.5ml Nystatic Stock solution (10mg/ml in 100% ethanol, keep refrigerated)

4. Then, while media is still warm, pour the plates.

1. Turn on the flow hood.

2. Get 2 and a half-ish sleeves of small plates (about 50 plates), found in the box below the first lab bench. Open them in the flow hood, and lay them out in rows.

3. Also place the autoclaved DI water and hose in the flow hood, and set up the hose in the pourer machine, with both ends of the hose in the DI water. You will also need an empty beaker to serve as a waste bucket.

4. Turn on the plate pourer machine (switch is at the back of the machine). Set the pour settings to manual, 8ml, 150 pouring speed, by pushing the “sel” button several times and altering the settings.

5. Use the “vol. calib” button to run water through the tube (into the waste beaker) press the “stop” button twice. The place the take up end of the tube (the glass end) in the liquid agar, again using the “vol. calib” button, run agar through the tube (into the waste beaker) until only agar is in the tube, press the “stop” button twice.

6. Press the “start” button.

7. Use the foot pedal button to start the agar dispensing into each plate.

8. When all plates have been filled, place the take up end of the hose in the DI water and use the “vol. calib” button to run water through the tube (into the waste beaker). Press the “stop” button twice.

9. Allow the plates to solidify then replace the lids.

10. Plates can be stored in a labeled sleeve in the fridge for a few weeks.

Making standardized E. coli culture

E. coli OP 50-1 (a streptomycin-resistant uracil-auxotroph) is used as a food source for C. elegans on the agar growth media. The E.coli broth is made as followed:

1. Make 500ml of growth media broth in a flask, stirring in the following ingredients found in the cabinet (above the first lab bench):

5g Trypton

2.5g Yeast

2.5g NaCl

2. Autoclave on setting 2 for 1 cycle along with a test tube with a lid. Let media cool to 37 degrees Celsius and pour some media into a test tube.

3. Inoculate the media in the test tube with a loopfull of the previous E. coli culture. To do this, light the Bunsen burner found in the cabinet right of the main sink, flame the loop, and use it to transfer a loopfull of media from the old culture to the new media.

4. Incubate the test tube of new culture on the shaker with 1785 RPM for 18 hours. (If you take an absorbance reading at 650nm, it should be 0.680-0.690A. Dilute/incubate until this desired reading is achieved).

5. The growth media broth can be stored in the fridge to be used to make culture later. NOTE: culture must be renewed every 2 weeks.

Inoculating plates with E. coli

1. Turn on flow hood.

2. Select plates from fridge for inoculation (must be at least 24 hours old), place them in the flow hood in rows.

3. Also place in the flow hood: the standardized E. coli culture, a pipette, pipette tips, the “hockey stick”, the squirt bottle containing ethanol, the ethanol burner and the lighter.

4. Use the pipette to dispense 0.75ml of E. coli culture onto the plates (use the yellow pipette, the numbers will read 075|0).

5. Light the ethanol burner. Use the squirt bottle to cover the hockey stick in ethanol (away from the flame), then light the hockey stick using the flame to sterilize it.

6. Use the hockey stick to spread the E. coli culture on the surface of the plate.

7. Flame the hockey stick again to sterilize it.

8. Allow the plates to dry (1-2 min), and then place them in the incubator (inverted) at 37 degrees Celsius for 24 hours. After 24 hours, plates can be stored at 20 degrees Celsius.

9. Clean the flow hood counter using ethanol and a cloth.

Maintaining Worm Stock plates

The C. elegans population is maintained at all times on 5 or 6 E.coli inoculated stock plates, kept in an incubator with a constant temperature of 20 degrees Celsius. When the C.elegan population exceeds the carrying capacity of the plate (this occurs about every 7 days or so), make new stock plates by transferring ~ 10 worms to each new E. coli inoculated plate, using a metal probe. Be sure that the ethanol burner remains lit for the duration of this process.

Creating Age Synchronization of Worms

1. 24 hours prior, inoculate ~ 8 – 10 plates with E.coli culture, and incubate at 37 degrees Celsius.

2. From a stock plate that is no older than 10 days old (preferably more than 3, less than 7 days old), transfer 5 individual larger adult worms (about 90-96 hours old) from the stock plate to the new plate. Do this for 8-10 plates.

3. Let the plates incubate at 20 degrees Celsius for 5 hours.

4. Remove and burn off the adult worms.

5. Let plates incubate at 20 degrees Celsius for 72 hours. At this point, plates should contain same age adult worms.

Experiment set-up

1. 24 hours prior, inoculate 15 plates with E.coli culture, and incubate at 37 degrees Celsius.

2. Turn on 3 incubators, and set them to the mean temperature being tested in that particular trial.

3. Label the plates into groups of 3 treatments of 5 plates: treatment 1: u#p#a0, treatment 2: u#p#a0, treatment 3: u#p#a0.5. Note the mean # must be constant and the variance # must be constant. Ex: u16p5 (u=mean, p=variance, a=autocorrelation)

4. Transfer 5 adult worms (one at a time) from same age adult plates (that are 72 hours old, from previous step), to a blank, E. coli inoculated plate. Do this to all 15 plates in a random order. Note: the same age adult plates can now be kept as stock plates for another 4-6 days.

5. Place the 3 groups of plates (inverted) into 3 separate Memmert incubators (5 plates per incubator).

6. Set a temperature series to the incubators using the program Celsius to regulate the temperature in the Memmert Incubators. See http://ecotheory.uwaterloo.ca/labwiki/index.php?title=Memmert_Incubators for full details.

7. Run the temperature series for the incubators for 72 hours.

Creating a Temperature series and sending it to Memmert Incubators

Temperature series were created using the MATLAB code “wonAD.m”, which can be found in the computer files found in the Desktop folder “C.E Worm Stuffs”, along with all the other example files mentioned in the steps below. The code should be modified to incorporate the proper mean, variance and autocorrelation values of interest for the particular trial of the experiment you are running.

1. Open MATLAB, open the code “wonAD.m”.

2. Modify the code so that it produces a temperature series with the desired mean, variance and autocorrelation.

3. Run the code by clicking the “Run” bottom (green arrow icon) in the top toolbar of the Editor window.

4. Three time series will be generated in the Command window.

5. Copy and paste the generated time series into a Notepad document. Save this document.

6. Open Microsoft Excel.

7. Import the data. Go to the Data tab ->From Text -> Select the text file you just made -> Import -> Fixed width -> Next -> Next ->Finish.

8. Cut and paste the data to form columns of the 3 time series. Label the columns according to the mean, variance and autocorrelation of the given series (“u#p#a#”).

9. In the same Excel workbook, make a make a sheet (tab), labeled for each of the series.

10. Open the workbook titled “exampletimeseries”, and use it to copy and paste the columns needed for formatting to the separate sheets you just created in your workbook. Copy and paste the appropriate time series column from the first sheet of your workbook into the appropriate column in the individual series sheets.

11. Save each separate sheet as a text delimitated file, calling it by its profile characteristics. NOTE: Once you save one sheet you cannot make changes to the other sheets in the workbook.

12. Open each sheet in Notepad. Also open the file “exampletimeseriestext” and copy and paste the top and bottom sections above and below the time series information into your document. Be sure to change the last “n” in your document to an “e”.

13. Save the text file as a “.cel” file (literally type “.cel” on the filename, it is not a dropdown option). Now the time series can be opened in Celsius

14. Repeat steps 12 and 13 for all the time series text files.

Experiment takedown: Counting

After the temperature series have ran for 72 hours, the C.elegans on all plates for all treatments must be counted.

1. Make a counting grid by tracing a petri dish on a piece of clear plastic sheet, and use a ruler and the microscope (magnification 20x) to make seize appropriate gridlines. The gridlines should be about the width of the field of view in the microscope.

2. Place the sheet under the plates to be counted and use the clicker to add up all worms, as you count all the worms in each box of the grid. Be sure to double count each plate to assure accuracy.

3. NOTE to get the correct magnification on the microscope, you may need to switch out the lens, and then change the settings in the microscope by holding the “Mode” button and adjusting the lens magnification using the wheel.
