Materials List

What can we learn from worms?

UWGSEO
GENOME SCIENCES EDUCATION OUTREACH

✔	Materials	Lesson needed
	Electronic Equipment	
	Computer and projector, and PowerPoint presentation http://gsoutreach.gs.washington.edu/ (see GEM Instructional Materials)	L1, L2, L3, L4, L5, Assessment
	Video demonstrating chunking technique (found at above URL)	L2
	Student Sheets, 1 per student	
	Student Sheet 1: *Observing Worms*	L1
	Student Sheet 3: *Modeling Worms in Salt*	L3
	Student Sheet 4: *Developing an explanation*	L4
	Student Sheet 5: *Effects of a single nucleotide change*	L5
	Student Data Table, copied one-sided, per student or group *	
	Student Data Table A – D	L2, L3, L4
	Student Resources, 1 per lab group in plastic sleeves for reuse	
	Student Resource: *Student Directions*	L1
	Student Resource: *Worm Rules*	L1, L2
	Student Resource: *C. elegans Life Cycle Stages*	L1, L2
	Student Resource: *Lesson 2 Student Directions*	L2
	Student Resource: *Lesson 3 Student Directions*	L3
	Student Graphs: *Glycerol content of worms; A, B, C and D* *(each group needs only one graph per group, not all four)*	L4
	Student Resource: *Universal Genetic Code*	L5
	Student Resource: *Assessment Instructions*	Assessment
	Student Resource: *Worm Plates (cannot be reused)*	Assessment
	Student Resource: *Claim and evidence card*	Assessment
	Worm Plates, per lab group	
	One plate of ***wild type*** worms per lab group	L1, L2
	One plate of ***mutant*** worms	L1, L2
	Two plates containing ***low salt*** (0.05 M)	L2, L3, L4
	Two plates containing ***high salt*** (0.40 M)	L2, L3, L4
	Lab Supplies, per lab group	
	Dissecting microscope	L1, L2, L3, L4
	Disposable gloves, one pair per student	L1, L2, L3, L4
	Plastic sheet with 4 mm x 4 mm grid	L1

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	Materials, continued	Lesson needed
	Bunsen burner or alcohol burner and lighter	L2
	Square-ended spatula	L2
	Sharpie pen	L2
	Waste container	L2
	2 pieces of serpent skin (or 1" dialysis tubing) each about 5 inches long **	L3
	4 rubber bands	L3
	15 ml Low Glycerol Solution: 1.5% glycerol in 0.05 M NaCl***	L3
	15 ml High Glycerol Solution: 50% glycerol in 0.05 M NaCl	L3
	Crystalline NaCl (table salt)	L3
	2 medium weighing trays (3.5 x 3.5 in)	L3
	Electronic scales	L3
	Colored construction paper, 24 in x 18 in. *or* Manila folders*	Assessment
	Scissors	Assessment
	Copies of extra worm plate shapes and cards, if needed	Assessment

*Assessment materials can be provided per student or per group, depending on teacher wishes.

**Serpent skin tubing, SM-200, is $14.95/roll for 80 inches, can be purchased from:

Educational Innovations, Inc.
5 Francis J. Clarke Circle
Bethel, CT 06801
203-229-0730

***Glycerol is also called glycerin and can be purchased from large animal feed stores. Instructions for making the solutions can be found in Lesson 3.

Wild type worms, mutant worms, and worm plates will be provided for GEM teachers; all other materials must be provided by the teacher. Please contact GSEO to make arrangements to borrow dissecting microscopes if needed.

Appendix

Preparing plates and maintaining worm stocks

Overview

This Appendix contains information about preparing agar plates and maintaining worm stocks. Because teachers have different resources available to them, these instructions cover a number of circumstances.

Maintaining worm stocks and plating worms for your classroom

You have received one or two plates of wild type and mutant worms. Depending on when you will do the Worm unit relative to the time you receive your worms and plates, you may need to chunk your worm strains each week to maintain healthy stocks. You'll also need to chunk plates for your students to use during the unit.

A. *Maintaining worm stocks*

Once a week, transfer a chunk of each stock plate to a new, correctly labeled low salt plate. Use the same sterile techniques your students use when they do their chunking.

a. Label a new low salt plate with the worm strain and the date.
b. Flame the square end of a metal spatula. Allow it to cool for about 4 seconds.
c. Cut out a square of agar from the old stock plate in an area where there are lots of worms. Avoid places where it looks like mold or a different kind of bacteria is growing. Gently slide the spatula under the chunk of agar and lift it out of the plate.
d. Gently drop the piece of agar onto the new labeled plate in an area where there is no *E. coli*, inverting it so it lands worm side down.
e. Holding the plate over a waste container, use the spatula to flick the agar chunk into the waste. Be sure not to touch the spatula to any surface between the time you put the chunk on the new plate and flick it off.
f. Flame the spatula before setting it down.
g. Repeat these steps for each of your stock plates. Be sure to label the new plates correctly and put the right worm strain onto them.

B. *Chunking worms for your students*

If you are planning to start Day 1 of the Worm unit on a Monday, then you'll need to chunk worms for your students on the Thursday and Friday before. You'll need one wild type plate and one mutant plate per student group. Wild type worms grow faster than the mutants, so you may want to chunk the mutants on Thursday and wild type on Friday.

a. Label low salt plates either wild type or mutant, and include the date.
b. Flame the square end of a metal spatula. Allow it to cool for about 4 seconds.
c. Cut out 4-8 squares of agar from the stock plate from an area where there are lots of worms. Avoid places where it looks like mold or a different kind of bacteria is growing.
d. Gently slide the spatula under one chunk of agar and lift it out of the plate.
e. Gently drop the piece of agar onto a new labeled plate, inverting it so it lands worm side down.

f. Holding the plate over a waste container, use the spatula to flick the agar chunk into the waste. Be sure not to touch the spatula to any surface between the time you put the chunk on the new plate and flick it off.
g. Flame the spatula, and then transfer another of the chunks to a new labeled plate.
h. Repeat these steps for each of your two worm strains, making sure that you have one of each worm strain for each of your lab groups. Be sure to label the new plates correctly and put the right worm strain onto them.

Disposing of worm plates
Option 1: Pour a solution of 10% beach over surface of plates. Drain off beach solution and dump plates in trash.

Option 2: Place used plates in autoclave bag provided in kit. Autoclave and throw in trash or return bag of plates to UW for autoclaving and disposal.

Preparing NGM plates for growing *C. elegans*

Per Liter:
3g NaCl (for low salt=51 mM) OR (23.4 g for high salt=400 mM plates)
2.5g Bacto-peptone
20g Difco-agar
Glass-distilled water to 1 liter
Mix in a 2 liter Erlenmeyer flask, and cover mouth of flask with foil.

Autoclave at 250°F and 15.3 psi for 30 minutes.

After autoclaving and cooling to touch-able temperature, add:

1 ml 1M $CaCl_2$ (autoclaved)
1ml 1M Mg_2SO_4 (autoclaved)
1ml 5mg/ml cholesterol (in ethanol)

Once these are fully mixed in, add
25 ml 1.0M potassium phosphate buffer, pH 6.0 (autoclaved)

Pour about 9ml per 6-cm petri dish. Don't let it get too cool before pouring. Re-boiling will cause the phosphate to precipitate, compromising transparency of the solid media.

1 M $CaCl_2$
11.1 g $CaCl_2$
Glass-distilled water to 100 ml

1M $Mg_2SO_4.7H_2O$
24.648 g $Mg_2SO_4.7H_2O$
Glass-distilled water to 100 ml

Autoclave at 250°F and 15.3 psi for 15 minutes.

5mg/ml cholesterol
500 mg cholesterol
100 ml ethanol

1 M potassium phosphate buffer, pH 6.0

Make the following two solutions, and then mix them to obtain the correct pH (6.0)

1 M KH_2PO_4 (monobasic)
136 g KH_2PO_4
Glass distilled water to 1 liter

1 M K_2HPO_4 (dibasic)
87 g K_2HPO_4
Glass distilled water to 500 ml
To the liter of monobasic potassium phosphate, add dibasic until the pH reaches 6.0. (It will start at about pH 4). This will take about 300 ml of dibasic solution.
OR
1 M KPO_4 buffer pH 6.0 (Mix 108.3 g KH_2PO_4, 35.6 g K_2HPO_4, add H_2O to 1 liter). Check pH to make sure that it is 6.

Autoclave at 250°F and 15.3 psi for 15 minutes.

OP50 media (L Broth)
10 g Bacto-tryptone
5 g Bacto-yeast
5 g NaCl
H_2O to 1 liter, pH to 7.0 using 1 M NaOH.
Put 100 ml into 250 ml screw-cap bottles and autoclave.
The bottles of media can be stored at room temperature for several months.
Allow inoculated cultures to grow overnight at 37°C.
The ***E. coli*** OP50 solution is then ready for use in seeding NGM plates.
The ***E. coli*** OP50 streak plate and liquid culture should be stored at 4°C and will remain usable for several months.

Ordering N2 and MT3643 nematodes

Order from:
Caenorhabditis Genetics Center (CGC)
University of Minnesota
Dept of GCD
6-160 Jackson Hall
320 Church St SE
Minneapolis, MN 55455
612-625-2265
http://www.cbs.umn.edu/cgc

Wild type (N2) *C. elegans* ($7)
strain MT3643 of *C. elegans* ($7)

Ordering *OP50 E. coli*

Stock of OP50 can also be ordered from the CGC

For directions for ordering worm strains and *E.coli* OP50, see
http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html

This site also includes detailed directions on preparing worm plates.

Seeding Plates

Seeding Plates General Protocol:

1. Streak and grow fresh bacterial colonies
2. Inoculate OP50 media with a single, fresh bacterial colony
3. Use inoculated OP50 media to seed plates (small, medium, and peptone-rich large)
4. Dry plates on lab bench
5. Pack plates back into their boxes and store

Step 1: Streaking plates

1. Use a small or medium unseeded plate
2. Retrieve an OP50 plate from the 4°C refrigerator that has already been streaked and grown. Make sure that the plate isn't more than 6 months old and that there are distinct, isolated colonies.
3. Turn on Bunsen burner
4. Sterilize streaking stick by flaming it (sterilize every time you streak a new plate)
5. Carefully pick an isolated colony from the old plate
6. Transfer the colony onto the new, unseeded plate and streak
7. Seal plates with parafilm
8. Label new plate with the bacteria strain (OP50), date, and your initials
9. Put new plate into standing 30°C incubator; do not incubate more than overnight
10. Put old plate back into the refrigerator
11. Check for growth of isolated colonies the next day; put plate into refrigerator

Step 2: Inoculating media

1. Obtain as many bottles of OP50 media as you need from the reagent cabinet
2. Obtain a freshly streaked plate with isolated OP50 colonies
3. Turn on Bunsen burner
4. Sterilize streaking stick by flaming it (sterilize every time you inoculate a new bottle)
5. Carefully pick an isolated colony from the old plate
6. Carefully open the OP50 bottle (near the flame), quickly insert the tip of streaking stick (where the colony is) into the media, and swirl. Close the lid of the bottle immediately.
7. On the label of the bottle, add the word "inoculated" with the date
8. Put bottle(s) into standing 30°C incubator, do not incubate more than overnight
9. Put streaked plate back into refrigerator
10. Check for growth (as indicated by cloudiness) the next day; put bottle(s) into refrigerator

Step 3: Seeding plates with OP50 strain in OP50 media

1. Obtain a box of small unseeded plates from the 25°C storage room
2. Sterilize bench by spraying with 70% ethanol and wiping with a paper towel
3. Lay out all the small plates with the lid facing up (be careful not to open any lids); I usually lay them out in stacks of 5 and then rows of 6 stacks (30 plates per row); Give yourself enough space between rows to seed
4. Obtain an OP50 inoculated media
5. Use a micropipette with a sterile tip to transfer 0.1 ml of OP50 to the center of each plate. If you have a pipette that can deliver this volume multiple times, use it, as it will make inoculation easier and help avoid contamination
6. Repeat step 5 until all the plates have been seeded; do not move the plates until after they're dry so that the field of bacteria stays in the middle

Step 4: Drying plates

1. Do not move the plates once they are seeded
2. They take up a lot of bench space, so make sure you have enough room before seeding
3. Allow 3-4 days to dry (always a good idea to seed them on Friday so they can dry over the weekend!)

Step 5: Storing plates

1. Check to see most of the plates are dry
2. Sterilize the box by wiping down with 70% ethanol
3. Pack the plates upside down (with the lid down) in stacks
4. Make label using lab tape with the following information: "seeded small", date packed, your initials
5. After adhering the label onto the box, store the box at 20-25°C

Seeding plate instructions courtesy of Cassie Zhang

Instructions to accompany *Worms in a Changing Environment* kit from Carolina Biological Supply

Kit Components

This list is based on one class consisting of 8 lab groups. The minimum number of plates needed is 32 low salt and 16 high salt. This kit provides enough materials to pour 55 low salt and 25 high salt plates. The extra plates allow for maintaining worm stocks and potential student errors and contamination of plates.

Basic re-fill kit
Caenorhabditis elegans N2 (wild type) plate
Caenorhabditis elegans MT3643 (OSM mutant) plate
Escherichia coli (strain OP-50) culture tube
500 ml low salt (0.05 M NaCl) nematode growth agar (in 8 bottles of 125 ml each)
250 ml high salt (0.4 M NaCl) nematode growth medium (in 4 bottles of 125 ml each)
6 bottles Luria broth (3 ml each)
4 sleeves (20 plates each) of 60 x 15 mm sterile disposable petri dishes
Metal inoculating loop
12 sterile disposable serological 1-mL pipets
Autoclave disposal bag and instructions

Additional materials for 'starter kit'
Shrink-wrapped worm unit (3 hole punched, ready to be put in 3-ringed binder)
10 square-ended metal spatulas

Needed to prepare plates, but not included
Bunsen burner
Disinfectant, such as 70% ethanol or 10% household bleach
Pipet aid

Procedure

Part 1. Medium Preparation

1. Prepare a boiling water bath such that the water level is even with the level of agar in the bottles. To melt agar, loosen the caps, and set the bottles of agar in the boiling water bath for about 30 minutes. Swirl the bottles periodically to make sure that the agar is melted.
2. Cool the agar to about 55°C by setting them in a 55°C water bath for about 30 minutes (the bottles should feel comfortably hot to the touch).
3. While the agar is cooling, clean lab bench and wipe with disinfectant. Wash your hands thoroughly and put on gloves.

4. Unpack 80 petri dishes, being careful not to contaminate them. Label the small side of 55 plates 'Low,' and label 25 plates 'High'. Place plates on the clean bench in a single layer, with the small side of the plate down.
5. Remove the cap and flame the mouth of a low salt agar bottle. Pour about 9 mL agar into each of 12-13 petri plates labeled 'Low', lifting the lid of each plate to pour and then replacing it. The depth should be about 5 mm. Continue with additional bottles of low salt agar until all the 'Low" plates are poured. Then pour the high salt agar into the plates labeled 'High'. Flame the mouth of each agar bottle after opening it.
6. Let the plates stand undisturbed until they solidify (~1 day). Plates can be stored for up to two weeks in a clean plastic lidded container in the refrigerator with agar side of the plate on the top (agar facing down).
7. Dispose of the empty bottles in the autoclave disposal bag.

Part 2. *Escherichia coli* Stock Culture Preparation

1. Disinfect the work surface and wash your hands thoroughly or put on gloves.
2. Loosen the cap of a Luria broth bottle (3 ml). Flame sterilize the mouth of the bottle. Place the cap loosely back onto the bottle. Repeat with the additional two bottles of Luria broth. The three bottles are sufficient to seed all 80 agar plates.
3. Loosen the cap of the *E. coli* culture tube, and flame-sterilize the mouth of the tube. Place the cap loosely on the tube, and set the tube in the test tube rack.
4. Aseptically open a serological pipet, and attach a pipet aid. Remove the pipet from its wrapper, being careful not to touch it to any surface.
5. Insert the pipet into the *E. coli* culture and withdraw 1.0 ml of culture. Dispense about 0.2 ml to each nutrient broth bottle.
6. Dispose of the used serological pipet in the autoclave disposal bag.
7. Flame-sterilize the mouth of each bottle, and replace its cap. Tighten the caps, and then turn back about ¼ turn so the cultures are in an aerobic state.
8. Label each inoculated nutrient broth bottle with the name of the bacterium and the date.
9. Incubate the inoculated nutrient broth bottles at 37°C for 24 hours. After incubation, close the lids tightly. These cultures can be used immediately or stored in the refrigerator for up to one week.

Part 3. Seeding Agar Plates with *E. coli*

1. Disinfect the work surface. Wash hands thoroughly or wear gloves.
2. If agar plates have been stored in the refrigerator, allow them to warm on the lab bench for about one hour prior to seeding them with *E. coli*.
3. Arrange plates in neat stacks of 6, with the agar side toward the bench. Allow enough space between stacks so that you can inoculate the plates without disturbing the neighboring stacks.
4. Remove the cap of each *E. coli* bottle, flame-sterilize the mouth, and replace the cap loosely.

5. Aseptically open a serological pipet, and attach a pipet aid. Remove the pipet from its wrapper, being careful not to touch it to any surface.
6. Withdraw 1 ml of bacterial culture from one of the culture bottles. Lift the lid of an agar plate, and dispense 0.15 ml bacterial culture into the center of the plate, making sure that you do not touch the pipet to any surface. Repeat with the other five plates in the stack. For each stack of six plates, it is helpful to seed the bottom plate first and work up the stack. As you lift the lid of the plate, balance the other plates on top of it.
7. Repeat step 6, inoculating another stack of six plates. If you touch the pipet to any surface, get a clean pipet.
8. Dispose of used pipets in the autoclave disposal bag.
9. Allow plates to sit on lab bench undisturbed for at least two days before using or storing to allow *E. coli* to grow.
10. Plates can be stored in a clean plastic bin with a loose-fitting lid at room temperature for up to two weeks. Store plates with the agar side on top so agar does not collect condensation.

Materials to prepare for 1 class of 8 lab groups

Item	**# for 8 lab groups**	**Maximum # per kit**
Wild type worm plates	8 (on low salt plates)	
Mutant worm plates	8 (on low salt plates)	
Low salt plates	16	55 (need 16 for worm plates and 16 for experiment)
High salt plates	16	25
Square-ended metal spatula	1 per lab group (8 total)	10 in starter kit
Also needed to complete full curriculum but not provided in kit		
Dissecting scopes	1 per lab group (8 total)	
1 inch dia. dialysis tubing (5 inches long)	16 pieces	
Disposable gloves (small, medium, large)	1 pair/student/experimental day	
Small rubber bands	32	
Medium weigh boats	16	
Sodium chloride (table salt)	About 40 grams/group	
Low glycerol solution (15 ml per lab group) 1.5% glycerol, 50 mM NaCl	120 mL	
High glycerol solution (15 ml per lab group) 50% glycerol, 50 mM NaCl	120 mL	
Labeling tape	1 roll	