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GENOME SCIENCES EDUCATION OUTREACH

What can we learn from worms?

How the nematode *C. elegans* maintains balance in a changing environment

SEPA SCIENCE EDUCATION PARTNERSHIP AWARD

Supported by the National Institutes of Health

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Overview

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Overview

Goals

The primary goals of the *Genes, the Environment, and Me* (GEM) curriculum are:

- To teach how genes and the environment interact to determine traits in living organisms, from the simplest bacteria to humans
- To teach about homeostasis in living organisms and how body systems interact to maintain internal balance in a changing environment
- To provide students with opportunities to develop models based on their observations, analysis of data, and readings
- To engage students in applying their understanding of science concepts by developing models and call to action projects

The GEM curriculum includes two units: *What can we learn from worms*? which introduces students to the model organism soil nematode *Caenorhabditis elegans* (*C. elegans*), and the Type 2 Diabetes unit which focuses on this complex human disease. The units may be used together or independently.

Unit Introduction

C. elegans is a well-studied model organism used in research on genetics, development, and behavior. In this unit, students conduct an experiment comparing the effect of elevated salt in the environment on wild type worms and a mutant that is resistant to higher salt concentrations (or ***osmolarity***, referred to as an OSM strain). Through this unit, students set up the experiment, make observations, analyze their results and other scientific evidence, and develop a model that explains their results. These activities guide students in learning how worms maintain homeostasis in an unfavorable environment caused by high osmotic stress and help them build an understanding of how genes and environment interact to determine traits.

Target Level

Introductory and advanced high school biology courses

What are Students Looking for?

Students observe behavioral changes of *C. elegans* worms when wild type and mutant worms are moved from a low salt environment to a high salt environment. Students will see some worms thrive and some worms be challenged in response to the environmental change, and will collect and analyze behavioral data such as worm movement, signs of eating, evidence of reproduction and general vigor.

What Happens To the Worms?

At the 15-minute observation time, students should see the worms continue to move and behave normally when transferred to low-salt plates. When transferred to high salt plates, however, most wild type worms will stop moving, change shape, and appear to be dead. The mutant worms will continue to move on the high salt plates and may have migrated to the food source and begun to eat after 15 minutes. At this point, students can see the behavioral effects of the mutation in an obvious way. But that is not the end of the story! After 24 - 48 hours on the high salt

plates, the wild type worms have rebounded and are now moving, eating, and reproducing, thanks to increased glycerol production in response to the environmental challenge. Students experience first-hand how an **environmental factor affects the expression of a trait** (NGSS LS3.B). A completed *Observational Graphic Organizer* detailing the changes in worm behavior can be found on page 7.

Organization of Curriculum

Materials marked **Teacher Pages** include background and procedural information for the teacher. **Student Resource** pages are for students to look at but not write on, so they may be photocopied and re-used with groups of students (or given to individual students at the teacher's discretion). A **Student Sheet** is a lab sheet or worksheet that requires student answers and should be photocopied for each student.

The 5 E Model

The unit is designed around the 5E Learning Cycle Model developed by the Biological Sciences Curriculum Study. The 5E model provides a scaffold for guiding and assessing student inquiry and learning through the following stages: Engage; Explore; Explain; Elaborate; and Evaluate.

Instructional Components

The worm unit consists of five lessons plus an assessment, as described in Table 1 on the next page. The entire unit will take from six to eight class periods.

Assessment

Each lesson provides opportunities to assess student learning through closing activities and questions. In addition, a summative assessment for the instructional unit is included.

Timing

Ideally, this curriculum unit would begin on a Monday or Tuesday so that students could make their 15-minute, 24-hour and 48-hour worm observations on three consecutive school days. If the schedule does not allow for three consecutive days, the third observation can also be done at 72 hours.

If constrained to 50-minute class periods, some teachers recommend inserting a "pre-lab" day prior to starting Lessons One and Two in order to talk about the concepts, background information and laboratory techniques described in the first 20 slides of the PowerPoint presentation, which correspond to Lessons One and Two. This allows for students to jump into the lab itself in Lessons One and Two with minimal explanation from the teacher. The 15-minute observations that students make at the end of Lesson Two are particularly important to building an understanding of how changes in the environment affect the two types of worms. The observations also set the stage for the 24 and 48 hour observations. *Please make sure students have enough class time* to chunk the worms, wait 15 minutes, and then make accurate observations in Lesson Two. Lesson Three may also take up to two 50-minute class periods, as described in the body of the lesson.

Table 1: Lesson descriptions and time to complete

Lesson	Description	Time to present	Nematode activities	Conceptual activities
Lesson 1. Getting to know your worms: Observing wild and mutant *C. elegans*	Students discuss familiar examples of organisms that respond to environmental changes. They learn about nematodes through a PowerPoint presentation and then observe and compare two nematode strains under a microscope.	50 min.	Students observe and draw wild type and mutant worms.	Students learn about *C. elegans* as a model organism and learn "worm facts" through a presentation and observation.
Lesson 2. Worms in a changing environment: How does high salt affect *C. elegans*?	Through a PowerPoint presentation, students learn a few more basics about *C. elegans* and the experiment they will be doing. They "chunk" (transfer) both wild type and mutant worms to low and high salt plates. After 15 minutes, students record their observations for both worm strains.	90 min.	Students transfer wild type and mutant worms to low and high salt plates and observe them after 15 minutes.	Students learn more "worm facts" through direct observation.
Lesson 3: How does *C. elegans* keep from drying up in high salt?	Students use dialysis tubing to model what might be occurring with their worms on low and high salt. Students also make 24 hour observations of the two worm strains on low and high salt.	90 min.	Students make 24 hour observations of worms on low and high salt plates.	Students set up a model system using dialysis tubing and solutions containing low and high glycerol and test the effect of salt.
Lesson 4: Using evidence to develop an explanation for worm observations	Students examine worms after 48 hours and record observations. They analyze data from the scientific literature to develop an explanation for their observations of wild type and mutant worms on low and high salt plates.	50 min.	Students make 48 hour observations of worms on low and high salt plates.	Students examine graphs from the scientific literature comparing glycerol content and production in wild type and mutant worms.
Lesson 5: How does a mutation affect *C. elegans* in low and high salt?	Students learn about the genes involved in worm response to osmotic stress, and how single nucleotide mutations can result in significant changes to how worms respond to the environment.	50 min.	--	Class reviews process of transcription and translation; students translate mRNA from wild and mutant worms.
Final Assessment What can we learn from worms?	Students build a model that describes what is occurring during the experiment, and they provide evidence for their claims.	90 min.	--	Students summarize their worm observations and inferences in a paper model.

Classroom Observational Graphic Organizer -- Teacher Resource

Sample responses are included below. After eliciting student observations for each condition, it may be helpful simplify the chart by recording whether or not the worms appear to be thriving (**T**), challenged (**C**), or some combination (**C/T**) in that situation.

Time	Wild type on low salt	Wild type on high salt	Mutant on low salt	Mutant on high salt
15 min.	Worms may be: • moving • traveling out of the drop site • in the food • eating • making worm tracks Eggs may be visible at the drop site	Worms may be: • not moving, or barely moving • curled up in a C shape • looking like a stick No sign of traveling to the food, eating, or making worm tracks. Worms may appear to be dead. Eggs may be visible at the drop site	Worms may be: • moving • traveling out of the drop site • in the food • eating • making worm tracks Eggs may be visible at the drop site	Worms may be: • moving, but slowly • traveling out of the drop site, but slowly • in the food • eating • making worm tracks Eggs may be visible at the drop site
24 hour	*All of the above, plus:* • More life stages may be visible • Eggs at the drop site have hatched • Eggs may be found outside of the drop site • Food is decreasing • Population is increasing	• Worms may be moving, especially the large worms. • Some adults may have reached the food and begun eating. • Smaller worms may still be curled up/stationary/dead	*All of the above, plus:* • More life stages may be visible • Eggs at the drop site have hatched • Eggs may be found outside of the drop site • Food is decreasing • Population is increasing	*All of the above, plus:* • More life stages may be visible • Eggs at the drop site have hatched • Eggs may be found outside of the drop site • Food is decreasing • Population is increasing
48 hour	*All of the above, plus:* • Food may be almost gone • Plate may be overgrown with worms of all life stages	Worms may be: • moving • traveling out of the drop site • in the food • eating • making worm tracks • reproducing (eggs laid)	*All of the above, plus:* • Food may be almost gone • Population may be increasing, but not at the same rate as the wild type worms	*All of the above, plus:* • Food may be almost gone • Population may be increasing, but not at the same rate as the wild type worms

A blank copy of this organizer can be found in the *Assessment* on page 80.

Correlation to the Next Generation Science Standards

	Lesson One: Getting to know worms	**Lesson Two:** Changing Environment	**Lesson Three:** Worms and Glycerol	**Lesson Four:** Developing Explanations	**Lesson Five:** Mutations	**Final Assessment**
Scientific Practices						
1. Asking Questions		●	●			
2. Developing and Using Models	●	●	●			●
3. Planning and Carrying out Investigations		●	●	●		
4. Analyzing and Interpreting Data		●	●	●	●	●
5. Using Mathematics, Information and Computer Technology, and Computational Thinking			●	●		●
6. Constructing Explanations			●	●		
7. Engaging in Argument from Evidence			●	●	●	●
8. Obtaining, evaluating, and communicating information	●		●	●	●	●
Crosscutting Concepts						
1. Patterns					●	●
2. Cause and Effect: Mechanism and Explanation					●	●
3. Scale, Proportion and Quantity	●			●		●
4. Systems and System Models	●	●	●	●		●
5. Energy and Matter: Flows, cycles, and conservation						
6. Structure and Function	●	●	●	●	●	●
7. Stability and Change	●	●	●		●	●
Core Ideas: Life Sciences						
HS LS1: From Molecules to Organisms: Structures and Processes	●	●	●	●	●	●
HS LS3: Heredity: Inheritance and Variation of Traits	●	●	●	●	●	●
HS LS4: Biological Evolution: Unity and Diversity		●	●	●	●	●

Source: Committee on Conceptual Framework for the New K-12 Science Education Standards, National Research Council. 2011. *A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas.* Washington D.C.: National Academies Press.

Introduction to the nematode *Caenorhabditis elegans*

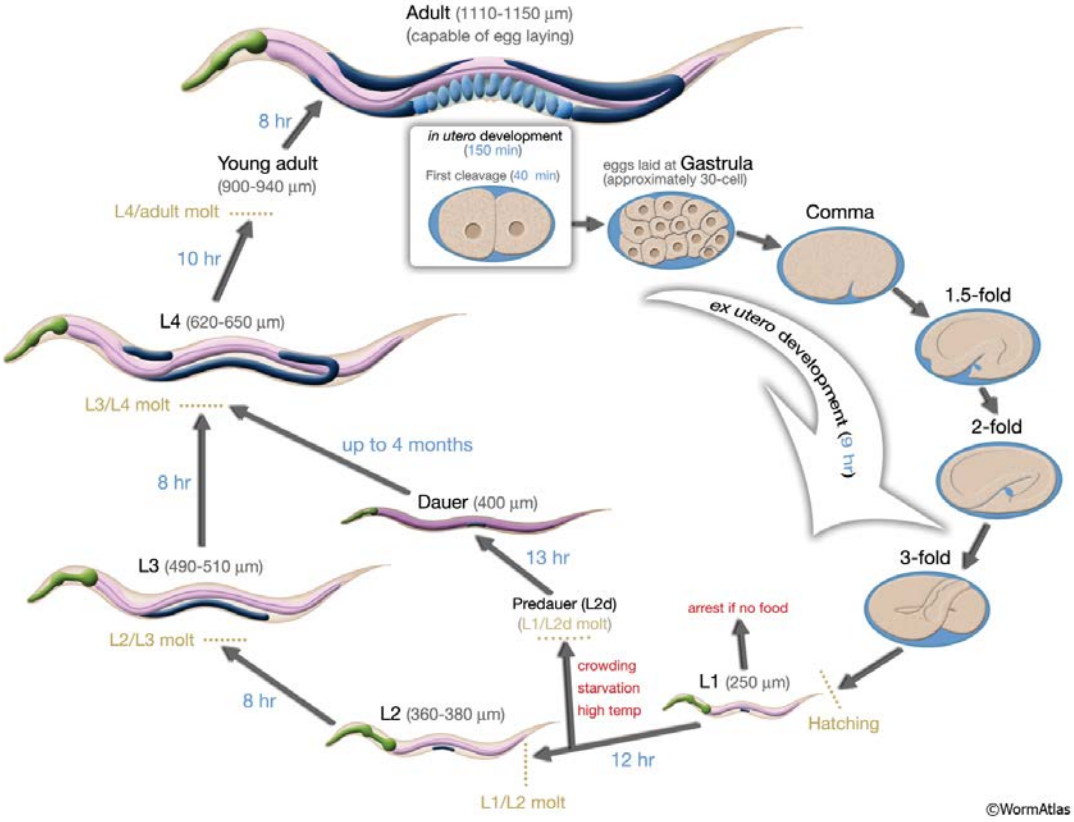
Worm Basics

Nematodes are the most abundant multi-cellular organism in the world. There are an estimated 1-10 million different nematode species, though some estimates go as high as 100 million species. They occupy a wide range of ecological niches and lifestyles. Some are free-living in the soil and decaying plant matter, and others live as parasites in plants, animals (including humans), and insects.

The soil nematode *Caenorhabditis elegans* is a useful model organism for studying gene-environment interactions because it is a multicellular eukaryote that can be easily grown in the lab on agar plates. Geneticists routinely study *C. elegans* due to its short lifespan, relatively simple genetics, transparency, and ease of ***propagation*** (reproduction).

Short Lifespan

Each *C. elegans* worm reaches sexual maturity within three days and lives up to three weeks. *C. elegans* feed on bacteria, such as *Escherichia coli***,** which is placed on the agar plates. Throughout its lifespan, *C. elegans* go through several life stages (***see* Figure 1**). *C. elegans* can endure stressful environmental conditions by entering the ***"dauer" stage*** (a dormant phase), enabling the worm to live four to eight times as long as the typical three-week lifespan.



Reprinted with permission from Z. Altun & C. Crocker (wormatlas.org)

Figure 1. Life cycle of *C. elegans* at 22ºC.

Reproduction

C. elegans has two sexes – hermaphrodites (XX) and males (XO). Hermaphrodites can self-fertilize or mate with males but cannot fertilize each other. Males are less common and arise infrequently by ***spontaneous non-disjunction*** (frequency of 0.1%) in hermaphrodites and at a higher frequency (50%) after mating with males. Hermaphrodites can also be induced to produce a higher rate of male ***progeny*** (offspring) by increasing the temperature during self-fertilization.

Genetics and Genomics

In 1998, *C. elegans* was the very first multicellular organism to have its ***genome*** completely sequenced, essentially launching the field of genomics. The *C. elegans* genome has a hundred million base pairs on six pairs of chromosomes (five pairs of ***autosomes*** and one pair of sex chromosomes). Even though the *C. elegans* genome is about $1/30^{th}$ the size of the human genome, both genomes surprisingly have about the same number of genes (~20,000). The genome database for *C. elegans* is readily available online for all scientists to use.

Transparency

The transparent body of *C. elegans* makes it especially easy to observe their internal structures. Each worm ranges in length from about 0.25 mm after hatching, to about 1 mm as an adult, and can easily be seen through a dissecting microscope. Wild-type hermaphrodites contain 959 somatic cells.

Propagation

Each hermaphroditic worm can produce over 300 offspring in its lifetime, so it is easy for scientists to cultivate these worms in the lab. When mating with a male, hermaphrodites can produce 1200-1400 progeny. An agar plate with just a few worms can become a plate with thousands of worms in just a few days. Scientists routinely manipulate nematode stocks to produce progeny with desired ***genotypes***.

Maintaining Homeostasis

How is C. elegans able to survive in high osmotic stress?

Like all living organisms, *C. elegans* need to be able to respond to environmental changes such as temperature, availability of food, and ***osmolarity***. When exposed to high osmotic stress (high salt or sugar), the nematodes shrivel as water ***diffuses*** out of their bodies into the surroundings. They quickly become inactive and may die because of damage to proteins and DNA. In intermediate levels of salt (~0.5 M), nematode adults and larvae initially become ***desiccated***, inactive, and unresponsive to touch. However, within 24 hours, the worms regain activity, eating food and reproducing like normal.

What happens to allow them to adapt to this severe environmental stress?
In response to high osmotic shock, nematodes quickly produce glycerol inside their cells. Glycerol is an organic solute that binds water, helping cells to restore their normal osmotic strength. When worms that have adapted to high salt conditions are returned to a normal salt environment, they initially swell and then resume their normal diameter. The worms excrete glycerol at 5 times the normal rate until normal levels are reached.

How is glycerol production controlled?
Within three hours of exposure to high salt, there is a dramatic increase in the synthesis of the messenger RNA for the enzyme glycerol 3-phosphate dehydrogenase (GPD), leading to an increase in the amount of the enzyme GPD inside the worm's cells. This enzyme catalyzes the rate-limiting step in the synthesis of glycerol, so increasing the production of GPD results in increased synthesis of glycerol.

How does C. elegans detect an increase in osmotic pressure and then stimulate up-regulation of GPD mRNA synthesis? This is a much more complicated question, and one which is not completely understood. However, genetic studies are providing clues about how this system might work. Mutations in several *C. elegans* genes result in resistance to osmotic stress. Worm strains carrying any one of these mutant genes are active in **0.5 M (high) NaCl**, and they contain a high level of glycerol, even when grown on low salt plates. Some of these genes code for collagen proteins that form furrows that wrap around the worm's cuticle (outer coat); others appear to be signaling molecules. One model that has been proposed to explain these observations is: 1) the cuticle collagens act as stretch sensors that detect pressure on the cuticle; 2) the signaling molecules are associated with the cuticle and relay the stretch signal to other parts of the worm; 3) this signal results in the increase in transcription of the GPD gene and thus increase in glycerol synthesis.

Model Organism

C. elegans is an exceptionally useful model organism for a number of reasons. On a practical level, it is small, relatively inexpensive to house and maintain, reproduces quickly, and its development can be observed due to its transparency. It has been extensively studied, so there is a wide body of knowledge for scientists to refer to and use. Its genome has been completely sequenced, and it can be genetically manipulated on a molecular level. Additionally, there are fewer ethical considerations when using invertebrate animals for research purposes, as compared with higher vertebrate animals.

Glossary

Autosomes: Chromosomes not directly involved in determining the sex of an organism.

Dauer: When food is scarce or colonies become crowded, young worms stop developing normally and enter the ***dauer stage***. In this form they can live, without eating or reproducing, for months - about ten times longer than the worm's normal lifespan. When ideal conditions (including necessary resources) are available, the ***dauer*** finally develops into an adult and resumes its normal aging process.

Desiccated: Freed of moisture; dried out.

Diffusion: The movement of a substance from areas of high to low concentration.

Field of View: the area that is visible (as through an optical instrument like a microscope).

Genome: the complete set of DNA, including all genes and genetic material, present in an organism.

Genotype: Genetic makeup of an organism or combination of genes/chromosomes in an organism.

Larvae: The newly hatched, earliest stage of any of various animals that undergo metamorphosis, differing markedly in form and appearance from the adult.

Mole: The amount of a substance that contains as many atoms, molecules, ions, or other elementary units as the number of atoms in 12 grams of carbon 12. The number is $6.0225 \times 10^{23,}$ or Avogadro's number.

Molt: To shed periodically part or all of a coat or an outer covering, such as feathers, cuticle, or skin, which is then replaced by a new growth.

Nematode: Any unsegmented worm of the phylum Nematoda, having an elongated, cylindrical body; a roundworm.

Osmolarity: Solute (e.g. salt or sugar) concentration expressed as molarity (moles/L).

Osmoregulation: Ability to sense and respond to changes in cell volume.

Spontaneous non-disjunction: Failure of two members of a chromosome pair to separate (disjoin) during meiosis so that both go to one daughter cell and none to the other.

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Wheeler, J.M. and Thomas, J.H. 2006. Identification of a Novel Gene Family Involved in Osmotic Stress Response in *Caenorhabditis elegans*. *Genetics* 174, 1327-1336.

Worm Classroom, Laboratory for Optical and Computational Instrumentation at the University of Wisconsin-Madison, Retrieved August, 2016 from www.wormclassroom.org. 8/2/16), www.wormclassroom.org

Additional resource on the natural history of *C. elegans*:

Félix, M.-A. and Braendle C. 2010. The natural history of *Caenorhabditis elegans*. *Current Biology* 20:22 R965-R969.

Lesson One

Getting to know your worms: Observing wild and mutant *C. elegans*

Overview
Students are introduced to the concept that living organisms, including the soil nematode, *C. elegans*, respond to changes in the environment. They are introduced to *C. elegans* through a PowerPoint presentation and then directly observe two strains by comparing them through a dissecting microscope.

Enduring understanding: Scientists use model organisms like the nematode *Caenorhabditis elegans* to study processes that occur in all living organisms, such as development and growth, transmission of traits from one generation to the next, and interaction with the environment.

Essential question: What does *C. elegans* look like, and how does it behave under laboratory conditions?

Learning objectives
Students will be able to:
- Draw lifecycle stages of the model organism *C. elegans* to scale
- Explain why model organisms are useful in research
- Identify similarities and differences in the two worm strains they are studying

Prerequisite Knowledge
Proper use and handling of a dissecting microscope

Time: 50 minutes

This lesson connects to the Next Generation Science Standards in the following ways:

HS LS1.3 Performance Expectation
Structures and Processes: Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.

HS LS1.A Disciplinary Core Idea
Structure and Function: Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range.

This lesson highlights the Scientific Practice of **Developing and Using Models,** and the Crosscutting Concept of **Scale, Proportion and Quantity**

Materials

Materials	Quantity
Computer and projector	1 per class
PowerPoint presentation found at http://gsoutreach.gs.washington.edu/ (see GEM Instructional Materials)	1 per class
A document camera is useful, but not necessary	1 per class
Student Sheet 1: *Observing Worms*	1 per student
Possible Answers to Student Sheet 1	1 per class
Student Resource: *Student Directions*	1 per lab group, in plastic sleeve
Student Resource: *Worm Rules*	1 per lab group, in plastic sleeve
Student Resource: *C. elegans Life Cycle Stages*	1 per lab group, in plastic sleeve
Dissecting microscope	1 per lab group
Compound microscopes on low power can be helpful for some observations.	As available
Plastic sheet with 4 mm x 4 mm grid	1 per lab group
One plate of ***wild type*** worms	1 per lab group
One plate of ***mutant*** worms	1 per lab group
Disposable gloves	1 pair per student

Lesson Preparation

- Plan to start the unit on a Monday or Tuesday so the experimental portion can be completed within one week. Some teachers recommend introducing the background information on worms and lab techniques from the PowerPoint on the Friday prior to beginning the worm observations to give students enough time for the lab activities.
- Make copies of the student sheet and student resources listed above. The student resource materials may be placed in plastic sleeves for reference at lab stations and reused, or you may prefer to give copies to each student.
- Copy the 4mm x 4mm grid onto transparency paper and cut out individual grids.
- Set up lab stations, each with one dissecting scope, two worm plates (one wild type and one mutant), and the other materials listed above. If available, a compound microscope to be used on low power can be added to each station.

Presenting the Lesson

Part 1 (Engage): How do living organisms react to a changing environment? *(10 min.)*

The goal of this brief activity is to challenge students to consider what they know about the following question: *How do living organisms survive in a changing environment?*

1. Encourage students to consider organisms they are familiar with, like dogs and cats, rodents, birds, reptiles, and plants and to answer the following questions:

   How do these organisms respond to changes in the temperature throughout the day or year, or to extremes of drought or precipitation, or to the availability of food?

   What factors control these changes?

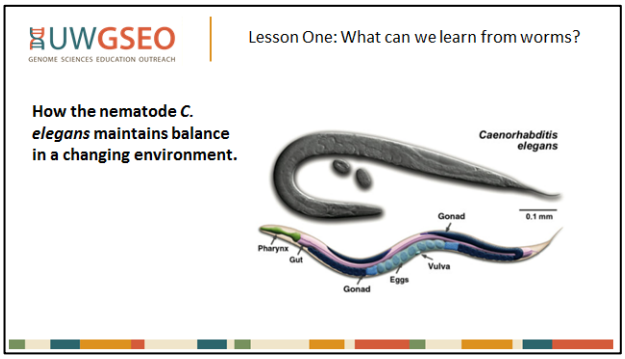
2. This activity can be done using a think-pair-share strategy to give each student a change to consider the question on their own first, then discuss it with a partner or their lab team, and then as a class.

   Possible responses: *1) Students may discuss how mammals like dogs, cats, or coyotes grow heavy fur in the fall in preparation for winter and then shed their extra fur in the spring. They may discuss the migration of certain birds in the fall, or hibernation among rodents and reptiles. Another example is the change in diet of omnivores like coyotes as different foods become more available through the year (a diet of rodents, birds, etc. may be supplemented by berries in the fall). 2) Students may suggest that certain changes are part of the animal's physiology or genetics, like growing a heavier coat in the winter. Other changes may be learned behaviors, like food selection, or may be a combination of both.*

Part 2 (Explain): Learning about the nematode *C. elegans* (PowerPoint Presentation; *15 min.*)

3. Begin this activity by showing Slide 1 of the PowerPoint presentation and letting the students know that for the next few days they'll be doing an experiment using an organism called a ***nematode***. They will be comparing the responses of two different nematode strains to a low salt and high salt environment.

Slide 1



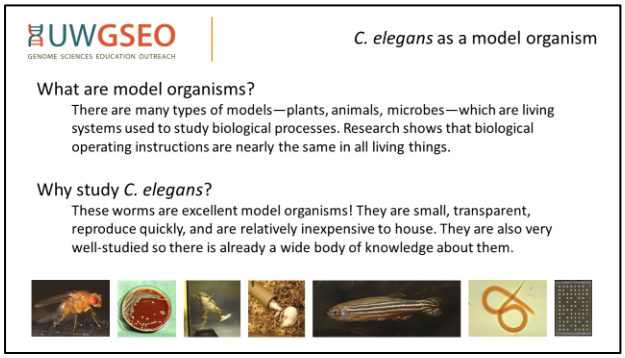
4. Ask students whether they have ever heard of nematodes, and if so what they know about them.

Students may have learned that they are small worms that live in the soil, that they can make people or animals sick, are agricultural pests, etc. They may wonder whether nematodes are the same as earthworms. If so, point out that nematodes are much smaller and are not segmented.

5. Explain to students that they'll be doing some experiments with the nematode *C. elegans*, which is a common ***model organism*** studied in scientific labs**.** Models are important in science, and *C. elegans* is studied as a living system in order to better understand genetics and development.

6. Showing Slide 2, ask students which living things they think might make good model organisms, and why. While there are many types of model organisms (mice being the most common vertebrate), *C. elegans* are widely used because they are small, transparent, reproduce quickly, and are relatively inexpensive to house and care for. Also, because they are so well-studied, there is a wide body of knowledge to which scientists can refer.

Slide 2

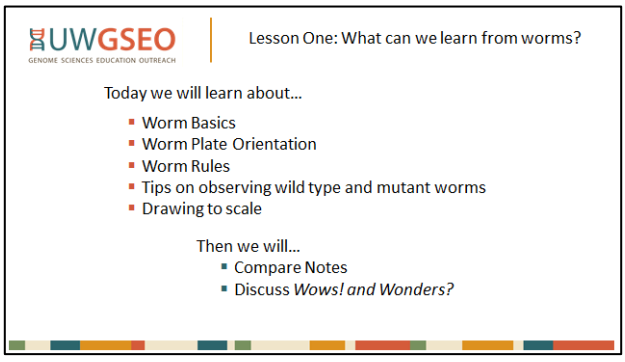


7. Reassure students that these worms are not harmful.

8. Let students know that they can call the *C. elegans* nematodes or simply worms for short.

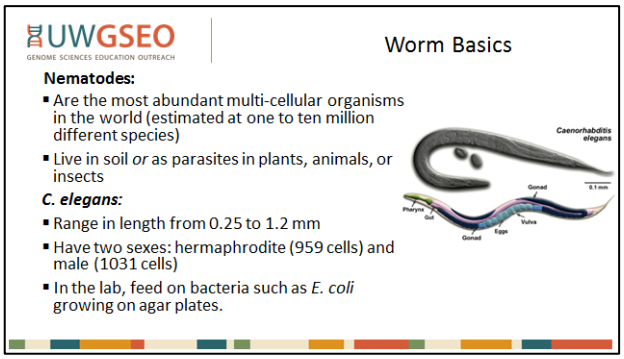
9. Show students Slide 3 of the PowerPoint presentation. The presentation provides basic information about nematodes, describes the worm plates, shows rules for handling the worms, and provides guidelines for observing worms using dissecting microscopes.

Slide 3



10. Use the image and discussion on Slide 4 to talk about some of the features of *C. elegans*.

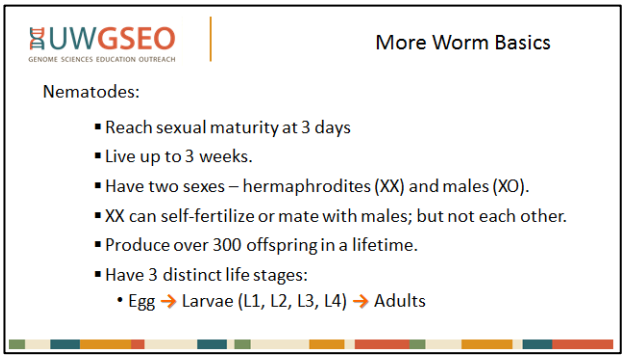
Slide 4



***Note*: OpenWorm.org** is a wonderful resource. The short video *A brief introduction to C. elegans* (2:12) can be found here: http://www.openworm.org/getting_started.html

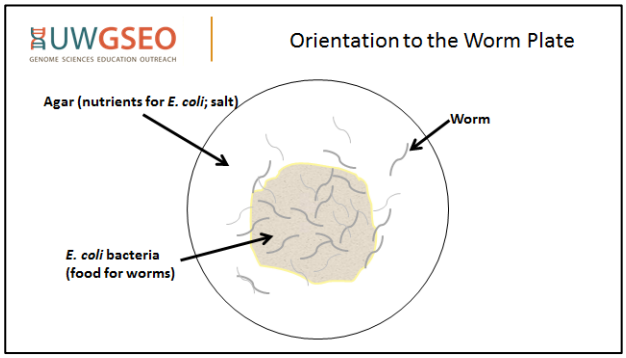
11. Discuss "More Worm Basics" using Slide 5. Students may ask how to distinguish male and hermaphrodite adults. The male has a fan-shaped tail and is smaller in size; the hermaphrodite has a pointed tail. The frequency of males in a population is 0.1% (1/1000), so it is not likely that students will see one. The distinguishing feature of the four larval stages is size. At each larval stage, the worm develops more internal structures, and the larva molts between stages. It is not necessary for students to identify the four stages, although they should recognize that larvae a range in size from about 0.25-0.65 mm.

Slide 5



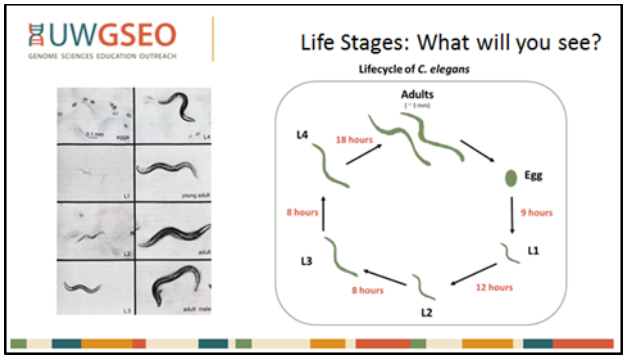
12. Slide 6 shows the plates that worms are grown on in the lab. It is helpful to show an actual plate under a document camera. The dark region of food in the center of the plate may or may not be visible to students, depending on how long ago the worms were transferred to the plate and how much food they have already eaten. Explain to students that this is where the food originally was, which might account for the location of many of the worms.

Slide 6



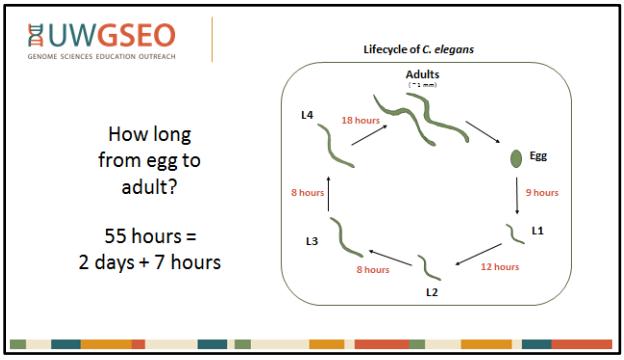
13. Using Slide 7, show students the life stages they may see, from eggs to adult worms.

Slide 7



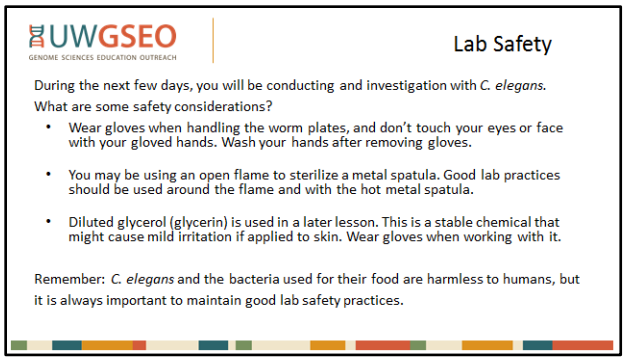
14. Using Slides 8, students can calculate the length of time it takes an egg to develop into a sexually mature adult.

Slide 8

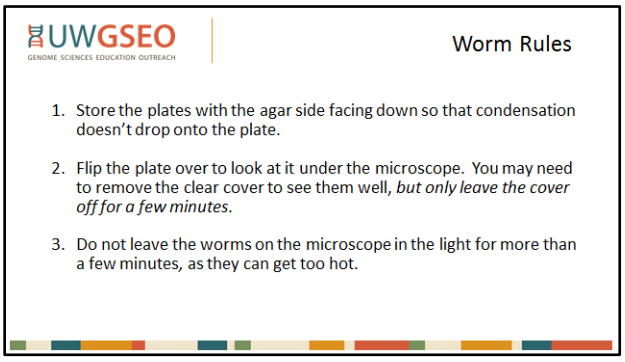


15. Slides 9 and 10 present safety considerations and rules for working with worms.

Slide 9



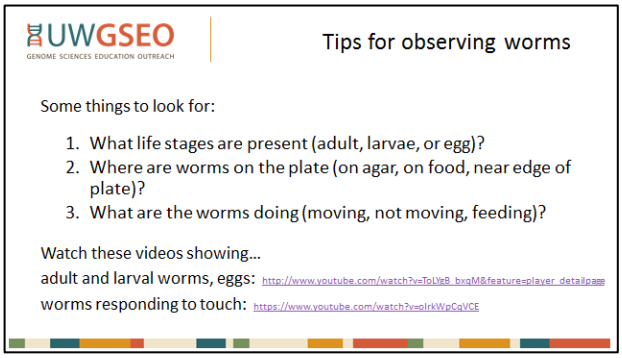
Slide 10



16. Use Slide 11 to discuss the features students should look for and record when they observe the worms. It is helpful to show the YouTube videos or use a microscope with camera to project a worm plate for the class to observe together. Here are the links:

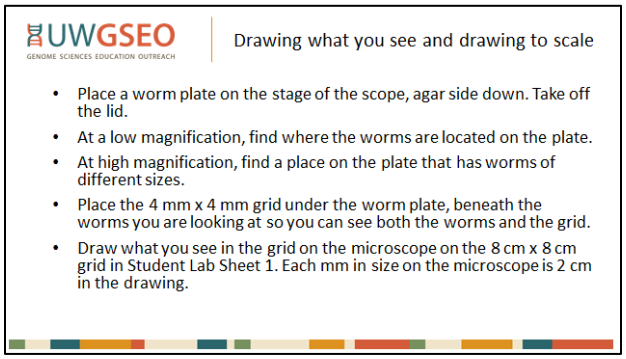
    http://www.youtube.com/watch?v=ToLYgB_bxqM&feature=player_detailpage
    https://www.youtube.com/watch?v=olrkWpCqVCE

Slide 11



17. Students may also need some guidance in drawing worms to scale (Slide 12).

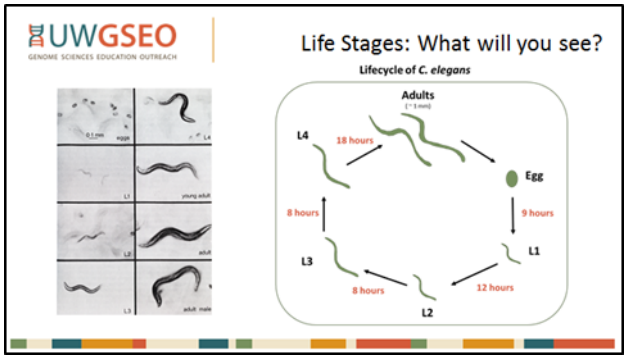
Slide 12



Note: It is also possible to place the worm plate upside down (on its lid) and look at the worms by focusing through the agar. This method decreases the chance of contaminating the plate and would work well for this activity.

18. Go back to Slide 7 to look at the lifecycle stages of *C. elegans*. Students should be able to identify all these stages when they look at their plates.

Slide 7



Part 3 (Explore): Observing wild type and mutant nematodes (*20-30 min.*)

19. Tell students they will be observing two types of C. elegans: ***wild type*** and ***mutant.*** Wild type worms are a typical or "normal" strain that would live in the wild under natural conditions. The mutant worms have a mutation in a gene that gives them different, or atypical, characteristics. Tell students that we will explore the impacts of the mutation in later lessons.

Note: There are over a million different types of mutations in *C. elegans.* Scientists make and study these mutations to better understand how different body systems function.

20. If students have not used a dissecting scope before, show them how to use one, pointing out the focus and magnification knobs. Show students how to turn on the light *under* the platform, which is the one they will use for this activity. You may also want to show them how to carry the scopes.

21. Show students a worm plate on the document camera. Point out that the plate consists of a jello-like substance called ***agar*** that contains some nutrients, and a layer of bacteria in the middle of the agar. The worms eat the bacteria, which are a harmless strain of ***E. coli***.

22. Put the **Lesson 1 Directions** on the document camera and/or read them aloud as a class. Answer student questions.

23. Pass out Student Sheet 1: *Observing Worms* and ask students to go to their lab stations. Point out the different items at each station, including the dissecting scope, two worm plates (one wild type and one mutant), the plastic grid, and the student resource sheets (*Lesson 1 Directions, Worm Rules, and Life Cycle of C. elegans*).

Throughout the curriculum, ***Student Sheets*** are designed to be written on by students and should be copied one per student. ***Student Resources*** are informational and can be reused with groups of students.

24. Ask students to place a worm plate on the microscope platform without removing the lid and with the agar side on the bottom, with the lid on top. Then ask them to turn on the light under the platform, checking from time to time to make sure the plate does not heat up too much.

25. Ask students to take turns looking at what they see on the plate, using different magnifications from lowest to highest. Remind them about how to adjust the focus and the magnification.

26. Tell students to draw the wild type and mutant worms as they appear at the highest magnification on Student Sheet 1: *Observing Worms* or in their lab notebook. They should place the 4 mm x 4 mm grid over the worm plate. Encourage them to draw the different worm life stages to scale. They should use the grid to estimate size of the worms. They may need to focus up and down to find a position where they can see

both the grid and the worms. They should show as much detail as possible, including any internal structures that they see. Ask them to answer the questions on the bottom of Student Sheet 1: *Observing Worms*.

27. Encourage all students to look at both worm strains and draw what they see in the squares provided on Student Sheet 1: *Observing Worms*.

28. Neat the end of class, ask students to share what they observed, referring to PowerPoint Slide 7 to point out the life stages. If you have a microscope camera that can be connected to a computer and projector, you may want to use it to demonstrate and discuss the worms as a class. You may also make and save images of students' plates so they can measure and describe the worms directly from the images.

Closure (Evaluate): How do living organisms react to their environment? *(5 min)*

29. It may be helpful to keep a record of class observations on a sheet of poster paper, as well as students' *Wows and Wonders*.

30. Using Slide 13 as a guide, discuss worm life stages, and how the mutant and wild types may have differed. Lastly, pose questions similar to the one that began class:

    What evidence indicates that the worms react to their environment?

    Students may respond that worms move towards the food as shown by the higher number of worms on the food than off the food, as well as the numerous worm tracks through the food. Worms on the food may appear more sluggish than worms off the food. Students may also say that the worms respond to touch, as shown in the video.

    What kinds of factors might the worms encounter in their environment?

    In the wild, worms encounter changes in temperature, moisture, food sources, light, salinity and many other factors. As living organisms, they need to be able to maintain their internal conditions at certain levels, even as their external environment changes.

    These questions can be answered in the form of an exit ticket, think-pair-share strategy, or class discussion.

31. Elicit from students that the worms must have a way to sense information about their environment (whether or not they are on food, for example) and respond to that information. In other words, as external environmental conditions change, a living system's internal conditions must respond in order to change behavior.

32. Let students know that the wild type and mutant worms may behave differently under different conditions, as students will experience in the next lesson.

Glossary

Agar: Seaweed extract that is used to thicken liquids to a "jello-like" consistency

E. coli: A gram-negative bacteria commonly found in the large intestines of many organisms.

Model organism: An organism studied widely by many scientists to help understanding of all living organisms.

Mutant: A strain of an organism that has one or more genetic differences from the wild type.

Nematode: A phylum of 1 to 10 million different species of small, unsegmented round worms.

Wild Type: The strain of an organism that is most similar to the form that is found in nature.

Lesson One: *Getting to know your worms*

Teacher Pages

Copy master for 4 mm x 4 mm grids

Copy onto transparency sheet and cut out for student use.

Name: _____ Date: _____ Period: _____

POSSIBLE ANSWERS

Student Sheet 1: Observing Worms

Discussion questions:

1. Where did you find most of the worms? Why do you think they are there?

   Students will probably notice that most of the worms are in the center of the plate where the food is. Depending on how old the plate is, they may be able to see the food, which is more opaque that the agar and may have worm tracks through it.

2. Why do you think nematodes make a good model organism for understanding humans?

   Students may point out that nematodes are multicellular organisms, have organ systems (digestive, reproductive, nervous), and have several life stages. They move, eat, and respond to their environment. They are small, reproduce easily and are relatively inexpensive to house. You may also point out that worms have many of the same genes as humans. With over a million different known mutations to the C. elegans genome, research with C. elegans may give researchers insight into diseases and medical conditions that affect humans.

3. Describe 3-4 ways that the wild type and mutant worms are similar.

   For both types of worms there are adults, larvae, and eggs, and they are about the same sizes. Both types have the same kinds of movements.

4. Describe 1-2 ways, if any, that the wild type and mutant worms are different.

   Students may see a different distribution of adults, larvae, and eggs on their two plates. They may notice a difference in the size of the adults or the rate of movement of the two strains. Most differences will be slight.

Lesson One: Observing Worms

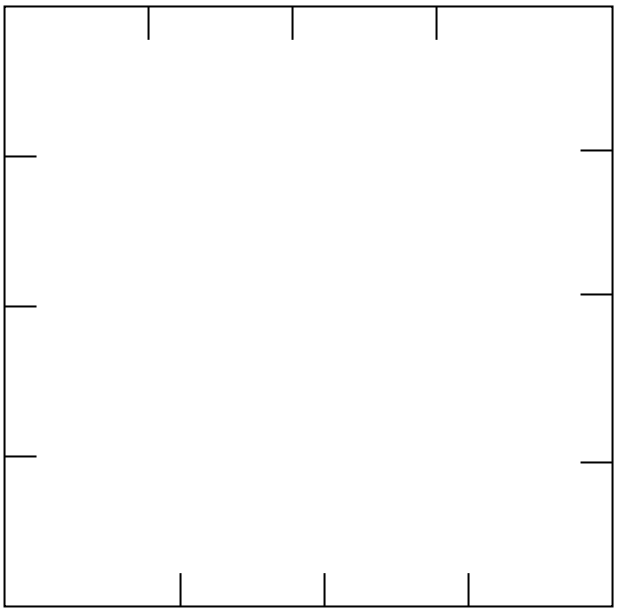
Student Sheet 1

Name: _____ **Date:** _____ **Period:** _____

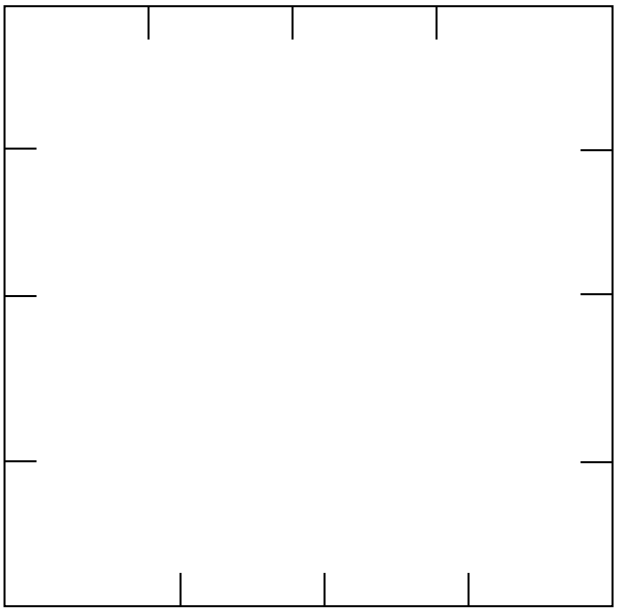
Student Sheet 1: Observing Worms

1. Follow Lesson 1 Directions to complete this activity.
2. Draw what you see under the microscope inside the 4 cm x 4 cm grid. Draw both worm types.

Wild Type



Mutant



Scale of drawing is 20x:
4 mm in microscope field = 8 cm (80 mm) on diagram.

Discussion questions:

1. Where did you find most of the worms? Why do you think they are there?

2. Why do you think nematodes make a good model organism for understanding humans?

3. Describe 3-4 ways that the wild type and mutant worms are similar.

4. Describe 1-2 ways, if any, that the wild type and mutant worms are different.

Lesson One Student Directions

Materials

Materials	Quantity
Student Sheet 1	1 per student
Dissecting scope (and/or other magnifier)	1 per station
Plastic strip with 4 mm x 4 mm grid	1 per station
Plate of **wild type** worms	1 per station
Plate of **mutant** worms	1 per station
Gloves	1 pair per student
Student Resource: *Student Directions*	1 per lab group, in plastic sleeve
Student Resource: *Worm Rules*	1 per lab group, in plastic sleeve
Student Resource: *C. elegans Life Cycle Stages*	1 per lab group, in plastic sleeve

1. Place one of the two worm plates on the stage of the microscope with the part with the agar on the bottom. Turn on the light *under* the stage.
2. Adjust the microscope to the lowest magnification, and focus on a place where there are several worms. You may need to remove the lid to see the worms clearly. If you remove the lid, put it back on in a few minutes so the worms don't dry out.
3. Switch to the highest magnification, and adjust the focus so you can see the worms clearly.
4. Move the plate so you can see some worms that you would like to draw. Place the plastic strip under the plate so you can see the 4 mm x 4 mm square when you look through the scope. Adjust the grid so it is under the worms you want to draw. You may need to adjust the focus slightly so you can see both the worms and the grid.
5. On Student Sheet 1, there are two squares, one for wild type and one for mutant worms. Draw what you see inside the square under your worm plate in one of these squares. The 8 cm x 8 cm grid on the student sheet represents the 4mm x 4mm grid under the microscope, so draw the picture to scale. Draw at least four worms of different sizes. Also draw some eggs if you see any.
6. Place the other plate on the microscope and draw what you see in the other square.
7. Make sure that everyone in your group observes and draws the two strains of worms.
8. Using the pictures of the worm life cycle stages found on the Student Resource, label each worm in your drawing.

Worm Rules

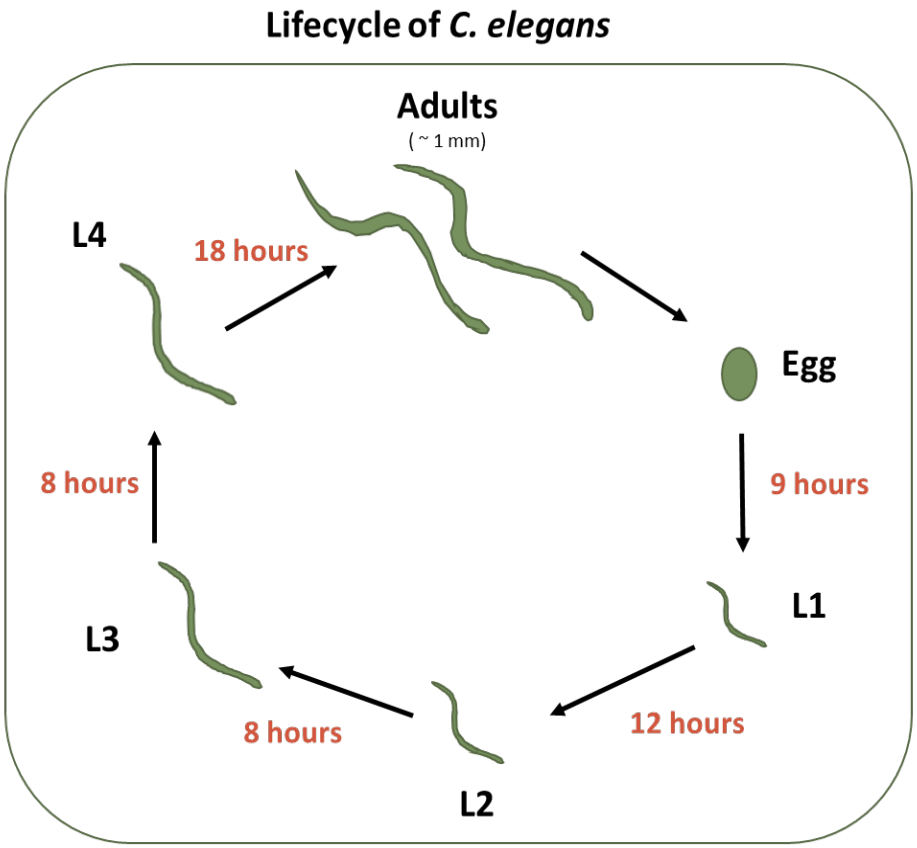
- Always wear gloves when handling the worm plates, and wash your hands after removing gloves.

- Store the plates with the agar side facing down (resting on the lid) so that condensation doesn't drop onto the plate.

- Flip the plate over to look at it under the microscope. You may need to remove the clear cover to see them well, but only leave the cover off for a few minutes.

- Do not leave the worms on the microscope in the light for more than a few minutes, as they will become too hot.

C. elegans Life Cycle Stages



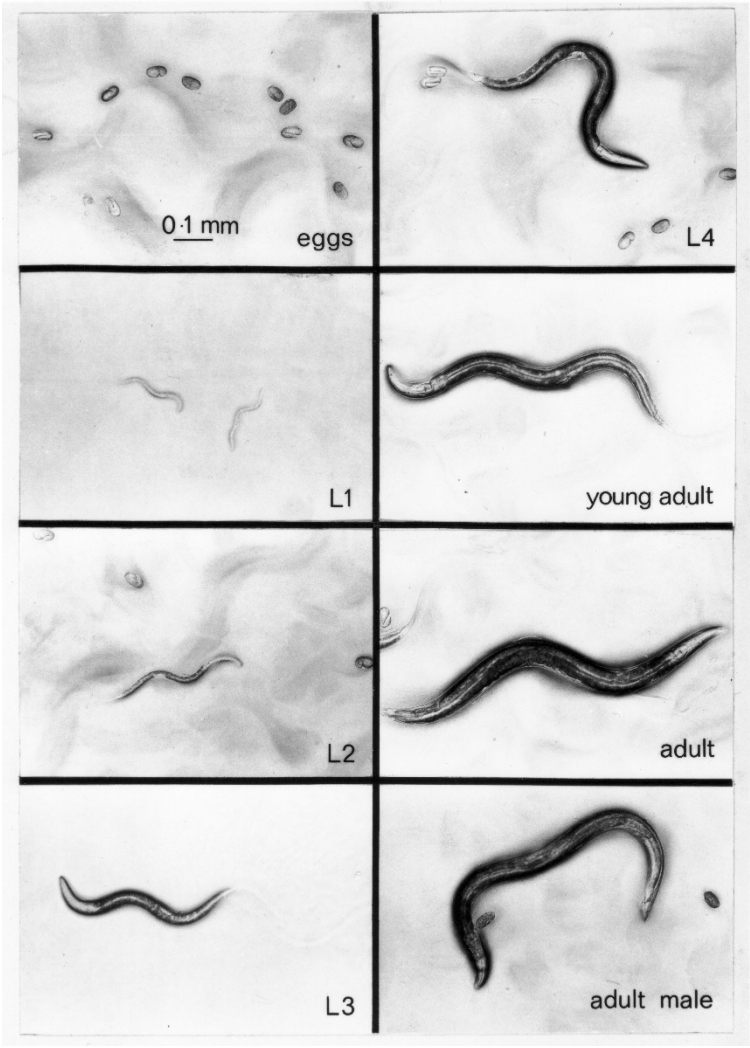


Photo credit J. Sulston. Used with permission.

Lesson Two

Worms in a changing environment: How does high salt affect *C. elegans*?

Overview
Students observe behavioral changes of *C. elegans* worms when wild type and mutant worms are moved from a low salt environment to a high salt environment. Students will see some worms thrive and some worms be challenged in response to the environmental change. Students will collect and analyze behavioral data such as worm movement, signs of eating, and evidence of reproduction and general vigor.

Enduring understanding: The nematode *Caenorhabditis elegans* can be used to study processes that occur in all living organisms, including how it responses to changes in the external environment. Wild type and mutant *C. elegans* have similar responses in a low salt environment, and different responses in a high salt environment.

Essential question: How do wild type and mutant nematodes respond when transferred to low and high salt plates?

Learning objectives
Students will be able to:
- Transfer wild type and mutant worms to low and high salt plates by chunking
- Make observations of worms 15 minutes after transferring to the new plates and accurately record them on the data tables
- Identify similarities and differences in the response of two worm strains to an environmental change after 15 minutes exposure

Prerequisite Knowledge
Proper use and handling of a dissection microscope

Time: 90 minutes

This lesson connects to the Next Generation Science Standards in the following ways:

HS LS1.3 Performance Expectation
Structures and Processes: Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.

Disciplinary Core Ideas
HS LS1.A Structure and Function: Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range.
HS LS3.B Variation of Traits: Environmental factors affect expression of traits.
HS LS4.B Natural Selection: Trait variation leads to differences in performance among individuals.

This lesson highlights the Scientific Practice of **Planning and Carrying out Investigations**, and the Crosscutting Concept of **Stability and Change**.

Materials

Materials	Quantity
Computer and projector	1 per class
PowerPoint presentation found at http://gsoutreach.gs.washington.edu/ (see GEM Instructional Materials)	1 per class
Video demonstrating chunking technique (found at above URL)	1 per class
A document camera is useful, but not necessary	1 per class
Student Data Table A – D, printed one-sided	1 per student, or pair of students
Student Resource: *Lesson 2 Student Directions*	1 per lab group, in plastic sleeve
Student Resource: *Worm Rules* (from Lesson One)	1 per lab group, in plastic sleeve
Student Resource: *C. elegans Life Cycle Stages* (from Lesson One)	1 per lab group, in plastic sleeve
Dissecting microscope	1 per lab group
Bunsen burner or alcohol burner and lighter	1 per lab group
Square-ended spatula	1 per lab group
One plate of ***wild type*** (N2) worms	1 per lab group
One plate of ***mutant*** worms	1 per lab group
Two plates containing ***low salt*** (0.05 M)	1 per lab group
Two plates containing ***high salt*** (0.40 M)	1 per lab group
Sharpie pen	1 per lab group
Waste container	1 per lab group
Disposable gloves	1 pair per student

Lesson Preparation

- Make copies of the student data table and student resources listed above. Print the data tables *one-sided*, as students will be cutting them out for use in the assessment. Some teachers prefer having students fill out the data table in pairs, or groups of four.
- Organize lab stations with all the materials listed above.
- Become familiar with the PowerPoint presentation for this lesson, and consider timing options for the lab (see note below).

Note: The 15-minute observations students make at the end of this lesson are crucial to building an understanding of how changes in the environment affect the two types of worms. The observations also set the stage for the 24 and 48 hour observations. *Please make sure students have enough time* to chunk the worms, wait 15 minutes, and then make accurate observations. Some teachers suggest inserting a "pre-lab" day to talk about concepts in the PowerPoint and prepare students for chunking the worms.

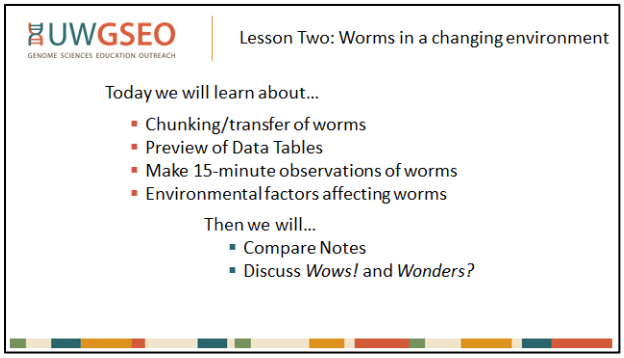
Presenting the Lesson

Entrance activity: Reflect on the following questions: Is it likely that worms in the wild would experience areas of changing salt or sugar concentrations? Why and/or when?

Part 1 (Engage/Explain): Preparing for the Lab (PPT Presentation; *15 minutes*)

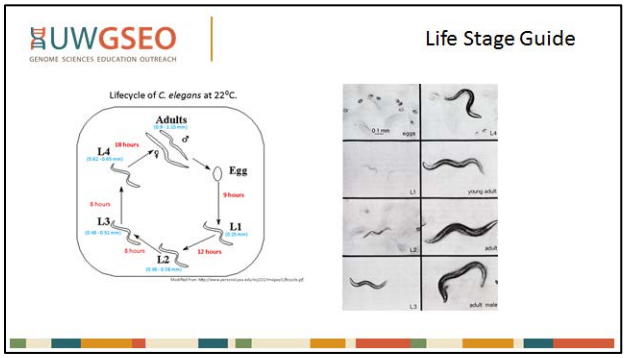
1. Introduce Lesson Two using PowerPoint Slide 14.

Slide 14



2. Use Slide 15 to remind students how the various developmental stages look.

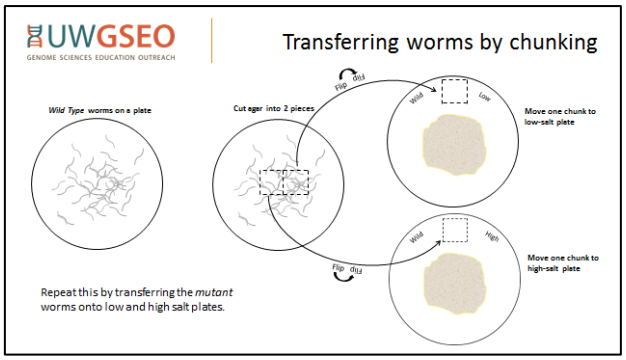
Slide 15



3. Explain that students are going to transfer wild type and mutant worms to low (normal) and high salt plates and will observe the worms over the next 48 hours (at 15 minutes, 24 hours, and 48 hours post transfer).

4. Slide 16 illustrates how to chunk worms. It is helpful to demonstrate chunking under the document camera using a worm plate, spatula, and new agar plate. Make sure students understand that the chunk taken from the worm plate must be flipped upside down before being placed on the new plate so that the worms are in contact with the agar

Slide 16



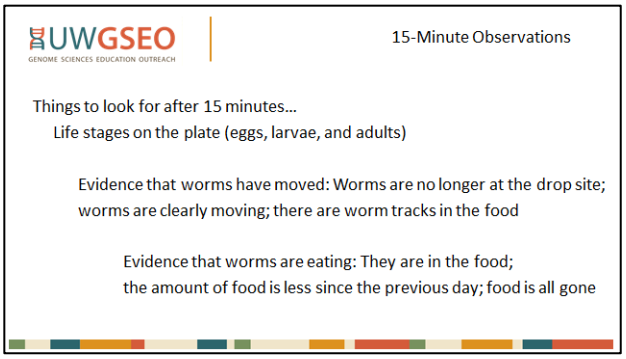
Note: If Bunsen or alcohol burners are not available, teachers can sterilize metal spatulas by wrapping them in foil and putting them in an oven at 350 degrees for about 30 minutes.

A video of a student demonstrating chunking worms can be found at:
https://gsoutreach.gs.washington.edu/instructional-materials/genes-the-environment-and-me/

A visual guide to chunking worms can be found at:
http://exolabs.dozuki.com/Guide/How+to+Transfer+%28Chunk%29+C.+elegans+to+a+New+Plate/35

5. Slide 17 shows some features that students should look out for when making their observations 15 minutes after the transfer, and Slide 18 describes possible observations 24 and 48 hours later.

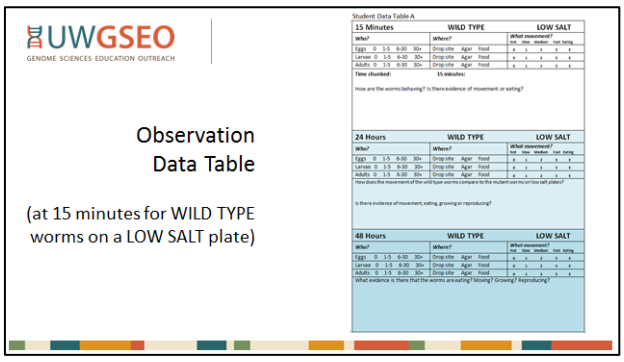
Slide 17



Slide 18

6. Students will record their observations in the data table as shown in Slide 19. Make sure students understand that they will be filling out information on *four data tables* for each time period observation: (A) Wild Type on Low Salt, (B) Wild Type on High Salt, (C) Mutant on Low Salt, and (D) Mutant on High Salt.

Slide 19



Field test teachers recommend color-coding the data tables, if possible. This can be done by using a different color of highlighter for each table, or copying each data table on a different color of paper. An example can be seen on the next page.

Part 2: (Explore): Setting up the experiment and making initial observations *(30 minutes)*

7. Discuss the experimental protocol in **Lesson 2 Student Directions**, and make sure students understand what they're doing at each step before they start the experiment. It may be helpful to refer back to PowerPoint Slide 17, which shows the chunking procedure students will use to transfer each type of worm to a low and high salt plate.

8. You may want to leave up PowerPoint Slide 18 so students are reminded of what to look for as they make their 15 minute observations.

9. While students are carrying out the experiment, circulate through the class to make sure they understand what they are doing, are recording their 15 minute observations for both worm strains, and everyone in the group is taking a turn observing the worms. Remind them to turn off the light and cover the worms when they are not observing them.

10. Students will make 24 and 48 hour observations during Lessons 3 and 4.

11. Encourage students to tidy their lab station when they have completed their observations.

Closure (Evaluate) *(15 minutes)*

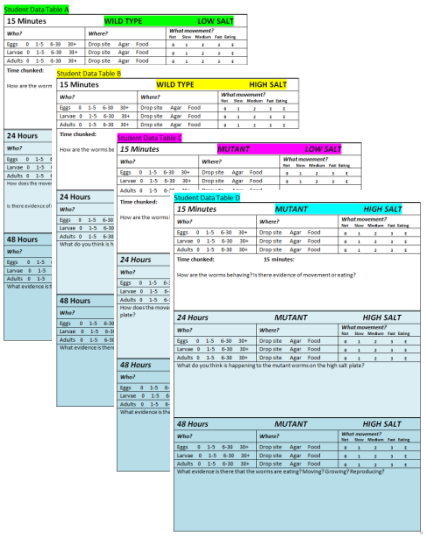
12. Discuss the 15-minute observations made by students. After 15 minutes what are the effects of low and high salt on the wild type and mutant worms? Do wild type and mutant act the same or different?

    Students should find that after 15 minutes on high salt, wild type worms stop moving; however, the mutant worms appear unaffected. On low salt, both wild type and mutant worms continue moving.

13. To reinforce the important point that the wild type worms on high salt are very challenged while worms on the other plates may still be thriving after 15 minutes, teachers may choose to fill out the first row of the **Observational Graphic Organizer** (found on page 80-81 and pictured below) as a class after each observational period, rather than waiting until the *Assessment* to review the classroom data. Color coding the data tables and graphic organizer can be helpful, as shown.

Observational Graphic Organizer

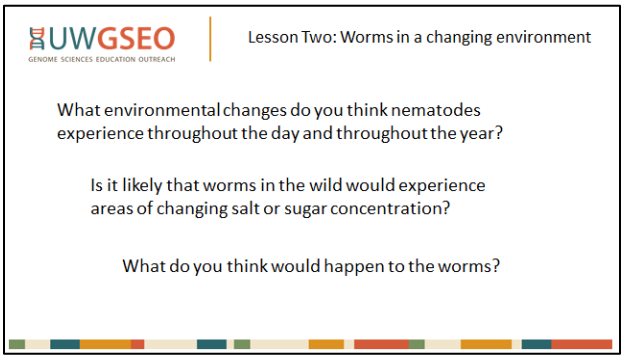
Time	Wild type on low salt	Wild type on high salt	Mutant on low salt	Mutant on high salt
15 min.				
24 hour				
48 hour				



14. Videos showing exemplar behavior from each type of worm in each environment can be found at: https://gsoutreach.gs.washington.edu/instructional-materials/genes-the-environment-and-me/

15. Revisit the entrance question by using Slide 20 to elicit student ideas about changes in the environment encountered by worms in the wild.

Slide 20



Students may mention changes in temperature, amount of moisture, pH of soil, and amount of food available. Encourage them to discuss what effects these changes may have on worms.

16. Ask students what might happen to worms if they moved into an area where the concentration of salt or sugar is higher than the worms are used to. Do they think that this is likely to happen to worms in the wild?

    Worms may encounter areas of high sugar when near rotting fruit, or areas of high salt next to roads that are salted in the winter to control for icy conditions.

17. Lastly, ask groups to share any other observations, including their *Wows! And Wonders?* (see Slide 21). As a think-pair-share, written exercise, or class discussion, address these questions:

    How does the change in environmental salt affect the behavior and function of wild type and mutant nematodes?

    What do you predict about the behavior of the worms at the 24-hour observation?

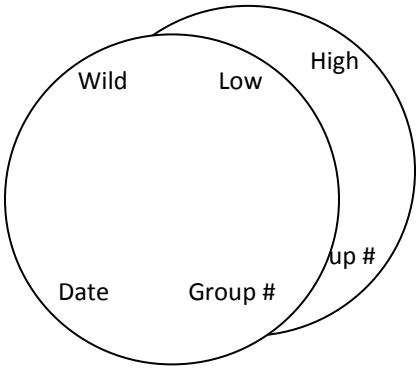
Lesson Two Student Directions

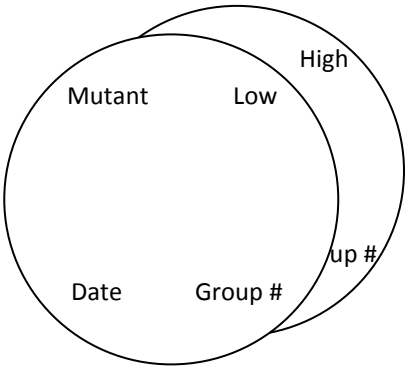
Materials

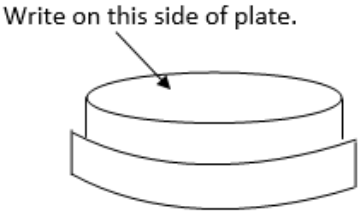
Materials	Quantity
Student Data Table A - D	1 per student
Dissecting microscope	1 per lab group
Bunsen burner or alcohol burner and lighter	1 per lab group
Square-ended spatula	1 per lab group
One plate of ***wild type*** (N2) worms	1 per lab group
One plate of ***mutant*** worms	1 per lab group
Two plates containing ***low salt*** (0.05 M)	1 per lab group
Two plates containing ***high salt*** (0.40 M)	1 per lab group
Sharpie pen	1 per lab group
Waste container	1 per lab group
Disposable gloves	1 pair per student
Student Resource: *Lesson 2 Student Directions*	1 per lab group, in plastic sleeve
Student Resources from Lesson One	1 per lab group, in plastic sleeves

Procedure: Transferring worms to low and high salt plates

1. Label the **four** new plates with: the kind of worms that will be transferred (**wild** or **mutant**), the salt concentration in the plate (**low** or **high**), the **date**, and **your group number**, as shown below.



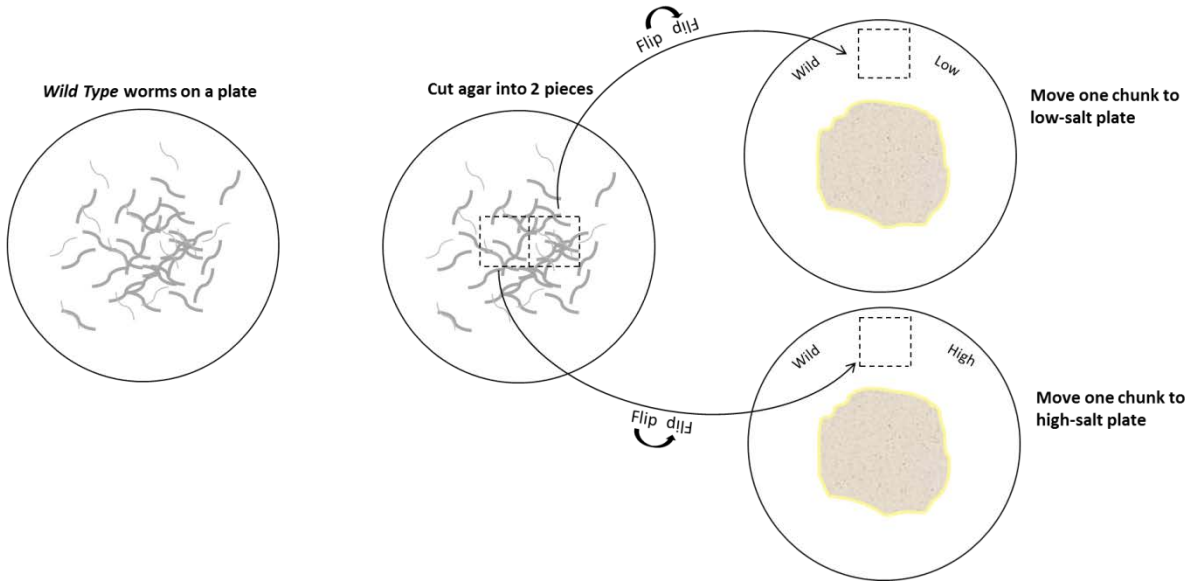




2. Remove the lid from the plate of wild type worms, place the plate under the microscope at low power, and find where most of the worms are.

3. Heat the flat end of the spatula in the Bunsen burner for a few seconds. Let the spatula cool for a few seconds.

4. Use the flat end of the spatula to cut the part of the worm plate that contains most of the worms into two pieces, each with about the same number of worms, as shown below.



5. Heat and cool the end of the spatula again. Slide spatula under one of the two chunks of agar and place the chunk, worm side down, onto the fresh **low salt plate** near the edge of the plate. Use your Sharpie to draw a circle on the outside of the plate at the place where the chunk landed (the "drop site"). Let the chunk of agar sit on the new plate for 3-4 seconds, and then use the clean spatula to flick the chunk of agar into the waste container.

6. Record the time that you transfer the worms on the data table.

7. Look at the plate under the microscope to make sure that you have transferred some worms.

8. Repeat steps 5-7 to transfer wild type worms onto the **high salt** plate.

9. Repeat steps 3-8 with the mutant worms.

10. **Look at the plates and record on the data table what you see *15 minutes*** after you transfer the worms to each plate. Here are some things for you to observe and record:
    - **Who:** How many adults, larvae, and eggs were transferred?
    - **Where:** Are the worms at the drop site or have they moved away? Are they on the food or the agar?
    - **What:** What are the worms doing? Are they moving or still? Have they moved from the drop spot or are they still in the same place? Do they seem to be eating?

11. Record your observations and comments on all four sections the Student Data Table 1A – 1D.

12. Discuss your results for the two worm strains with your team members.

Name: _____ **Date:** _____ **Period:** _____

Student Data Table A

15 Minutes	**WILD TYPE**	**LOW SALT**
Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E

Time chunked: **15 minutes:**

How are the worms behaving? Is there evidence of movement or eating?

24 Hours	**WILD TYPE**	**LOW SALT**
Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E

How does the movement of the wild type worms compare to the mutant worms on low salt plates?

Is there evidence of movement, eating, growing or reproducing?

48 Hours	**WILD TYPE**	**LOW SALT**
Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E

What evidence is there that the worms are eating? Moving? Growing? Reproducing?

Name: _____ **Date:** _____ **Period:** _____

Student Data Table B

15 Minutes	**WILD TYPE**	**HIGH SALT**

Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**

Time chunked: **15 minutes:**

How are the worms behaving? Is there evidence of movement or eating?

24 Hours	**WILD TYPE**	**HIGH SALT**

Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**

What do you think is happening to the wild type worms on the high salt plate?

48 Hours	**WILD TYPE**	**HIGH SALT**

Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**

What evidence is there that the worms are eating? Moving? Growing? Reproducing?

Name: _____ **Date:** _____ **Period:** _____

Student Data Table C

15 Minutes	***MUTANT***	***LOW SALT***

Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**

Time chunked: **15 minutes:**

How are the worms behaving? Is there evidence of movement or eating?

24 Hours	***MUTANT***	***LOW SALT***

Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**

How does the movement of the mutant worms compare to the wild type worms on the low salt plate?

48 Hours	***MUTANT***	***LOW SALT***

Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not **0** Slow **1** Medium **2** Fast **3** Eating **E**

What evidence is there that the worms are eating? Moving? Growing? Reproducing?

Name: _____ **Date:** _____ **Period:** _____

Student Data Table D

15 Minutes	***MUTANT***	***HIGH SALT***
Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E

Time chunked: **15 minutes:**

How are the worms behaving? Is there evidence of movement or eating?

24 Hours	***MUTANT***	***HIGH SALT***
Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E

What do you think is happening to the mutant worms on the high salt plate?

48 Hours	***MUTANT***	***HIGH SALT***
Who?	***Where?***	***What movement?***
Eggs 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Larvae 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E
Adults 0 1-5 6-30 30+	Drop site Agar Food	Not 0 Slow 1 Medium 2 Fast 3 Eating E

What evidence is there that the worms are eating? Moving? Growing? Reproducing?

Lesson Three

How does *C. elegans* keep from drying up in high salt?

Overview
Students explore the effects of environmental change on worms by creating an experimental model using dialysis tubing, high and low glycerol solutions, and salt. Students also complete their 24 hour worm observations and make connections between the role of glycerol in the dialysis model and the *C. elegans* system.

Enduring understanding: Scientists use physical models to test and demonstrate what might be occurring inside a living organism.

Essential questions: What is the effect of glycerol on the diffusion of water from a low salt to high salt condition? What happens to wild type and mutant nematodes after 24 hours in low and high salt?

Learning objectives
Students will be able to:

- Demonstrate that glycerol binds water and prevents it from moving across a dialysis membrane into higher salt
- Observe two worm strains in two salt concentrations and compare their activity with each other and with the observations made on the previous day
- Make connections between the role of glycerol in the dialysis model and the *C. elegans* system

Prerequisite Knowledge
A basic understanding of both osmosis and hydrogen bonding are helpful, although teachers could spend more time introducing these concepts while conducting this lab.

Time: See *Time Required* on the next page.

This lesson connects to the Next Generation Science Standards in the following ways:

HS LS1.3 Performance Expectation
Structures and Processes: Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.

HS LS1.A Disciplinary Core Idea
Structure and Function: Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range.

This lesson highlights the Scientific Practice of **Constructing Explanations**, and the Crosscutting Concept of **Systems and System Models**.

Time Required: Two class periods of 50 minutes, or one longer class period. The dialysis tubing bags may remain in the salt bed anywhere from 30 minutes to 24 hours, which allows for some flexibility in the timing. If time is a constraint, the dialysis lab can be conducted as a classroom demonstration.

Lesson Timing Options		
With two 50-minute class periods:	Day One Day Two	Make 24-hour worm observations Set up the dialysis tubes to rest overnight in the salt bed Make 48-hour worm observations Finish the dialysis tubing experiment
With one longer class period:	Start of class Middle of class End of class	Set up the dialysis tubing experiment Make 24-hour worm observations while tubing is on salt bed Finish dialysis tubing experiment

Materials

Materials	Quantity
Computer and projector	1 per class
PowerPoint presentation at: http://gsoutreach.gs.washington.edu/ (see GEM Instructional Materials)	1 per class
A document camera is useful, but not necessary	1 per class
Student Resource: *Lesson Three Student Directions*	1 per student or group
Student Sheet 3: *Modeling Worms in Salt*	1 per student or group
Class Resource: *Class Data Table for Modeling Worms in Salt*	1 per class
2 pieces of serpent skin (or 1" dialysis tubing) each about 5 inches long	1 per lab group
4 rubber bands	1 per lab group
15 ml **Low Glycerol Solution**: 1.5% glycerol in 0.05 M NaCl	1 per lab group
15 ml **High Glycerol Solution**: 50% glycerol in 0.05 M NaCl	1 per lab group
Crystalline NaCl	1 per lab group
2 medium weighing trays (3.5 x 3.5 in)	1 per lab group
Electronic scales	1 per lab group
Dissecting microscope	1 per lab group
Worm plates from Lesson Two	1 per lab group
Disposable gloves	1 pair per student
Data Tables from Lesson Two	1 per student or group

Getting Ready

- Serpent Skin, available from *Educational Innovations,* is an inexpensive alternative to dialysis tubing. One 12-meter roll can accommodate about 46 groups of students. It can be ordered from: http://www.teachersource.com/category/s?keyword=serpent+skin

- Inexpensive table salt from the grocery store works well for the salt beds. Though not normally used in lab stock solutions, table salt can also be used to make the glycerol solutions, below.

- To make 1 L of the **Low Glycerol Solution**:
  Dissolve 2.9 g NaCl in 500 ml distilled H_2O
  Add 15 ml glycerol
  Add distilled H_2O to a final volume of 1 L

- To make 1 L of the **High Glycerol Solution**:
  Dissolve 2.9 g NaCl in 500 ml distilled H_2O
  Add 500 ml glycerol for a final volume of 1 L

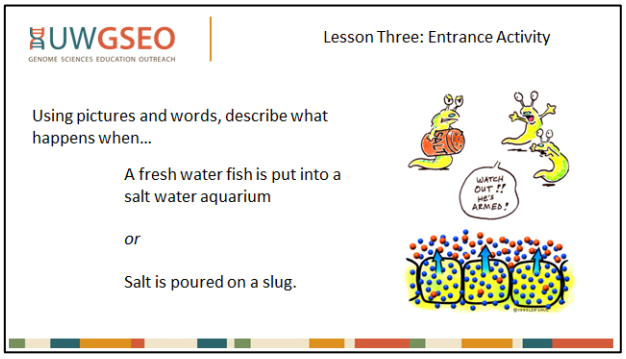
- It is best to have the low and high glycerol solutions premeasured in 15 ml volumes and available at lab stations. Labeled 15 ml conical tubes work well for this and can be rinsed and re-used.

- Set up the microscopes at each lab station and set out each group's plates.

Presenting the lesson
Entrance activity (Engage):

1. Have PowerPoint Slide 22 projected for students to see and complete as they enter.

Slide 22



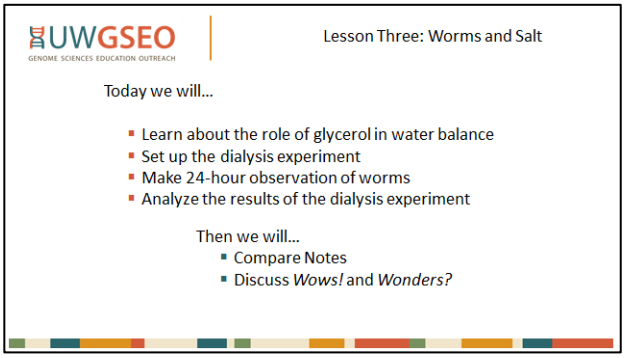
Note: Students may be familiar with the small white bags of ***desiccants*** (usually silica gel) that are packaged with some items such as food products to keep them crisp, or in shoe boxes to maintain dryness.

Students may say that the fish or slug will "dry up" or shrivel because water is being pulled out of the organism by the salt. Students should understand that water will travel from an area of low salt concentration (inside the animal) to an area of high salt concentration (outside the animal) through the skin of the fish or slug until the salt concentration is equalized on both sides. This will, indeed, ***desiccate*** *or dry out the organism and cause it to shrivel. This process of water traveling from an area of low solute concentration to an area high solute concentration across a* ***semi-permeable*** *membrane is called* ***osmosis.***

Part 1: (Explain) Understanding Glycerol (PowerPoint presentation, *15 min.*)

2. Use Slide 23 to introduce the agenda for the day.

Slide 23

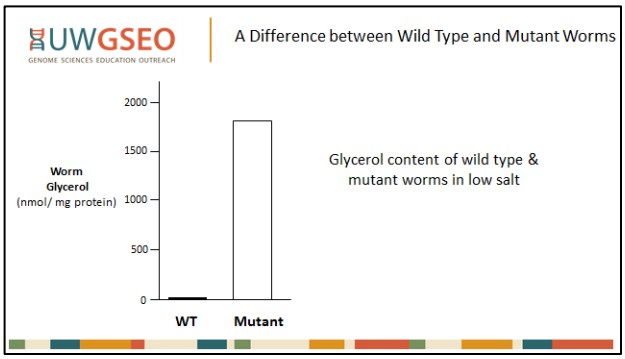


3. Ask student to recall their 15-minute worm observations. What happened to the wild type worms? What happened to the mutant worms?

   Students may recall that the mutant worms seemed more active than the wild type worms in the higher salt environment. The wild type worms may have looked immobile or shriveled. The mutant worms seemed to tolerate the high salt environment better, although they may seem more sluggish on high salt compared to low salt.

4. Show students Slide 24. Give them a minute or so to process the slide, and then turn to a neighbor to share their understanding of the information on the slide.

Slide 24



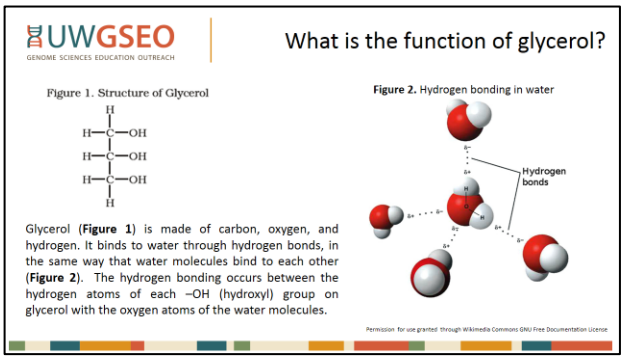
5. Ask students: What does this graph show about the difference between the wild type and mutant worms?

   Explain to students that scientists have measured a difference between wild type and mutant worms and have found that the mutant worms contain much more ***glycerol*** *than the wild type worms when grown on low salt—about 35 times as much.*

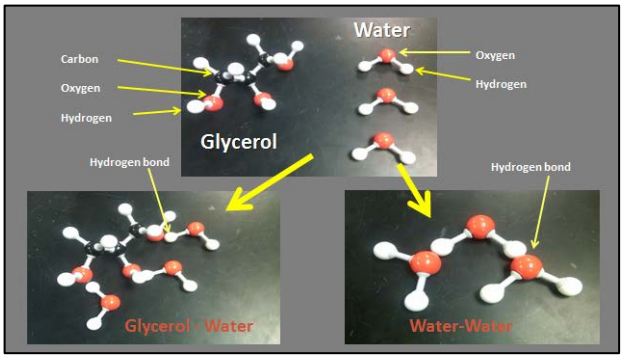
6. Tell students that the class will be exploring what glycerol is and conducting a lab that shows how glycerol can help *C. elegans* in a changing salt environment.

7. Use Slides 25 and 26 to discuss the chemical structure of glycerol and water and the formation of weak bonds between glycerol and water (as well as water and water).

Slide 25



Slide 26



8. Ask students: Since glycerol has the ability to bind water, how do you think the amount of glycerol in a worm may affect it? Is glycerol good or bad?
   Since glycerol can bind to water, a worm with more glycerol should lose less water when put onto salt. In high salt, glycerol is a good thing.

9. Given the answer to #8, ask students if it would be better to be a wild type or a mutant worm and why.
   Just based on the information presented so far, it appears that mutant worms are better than normal worms because they have more glycerol than wild type worms and would therefore lose less water in a high salt environment.

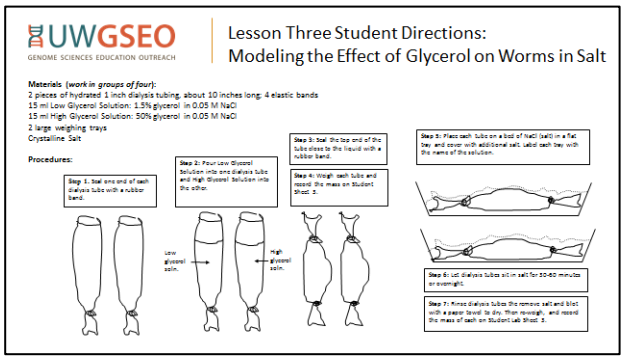
Part 2 (Explore) Preparing for the dialysis tube worm model (PowerPoint presentation and Lab)

10. Give students copies of *Lesson Three Student Directions* and the Student Lab Sheet 3: *Modeling Worms in Salt*.

11. Use Slide 27 to guide students through the procedure for the dialysis tube experiment. Students can follow along with their own directions. Point out that the dialysis tube is permeable to water, just like the worms are.

Note: This activity works well as classroom demonstration if time and/or materials are in short supply.

Slide 27



12. Remind students to weigh each filled dialysis tube before putting onto salt and record the mass in the table on Student Lab Sheet 3.

13. Tell students to label the trays Low Glycerol (wild type) or High Glycerol (mutant) depending on which solution is in each tube.

14. Ask students to predict what will happen to the liquid inside the two membranes and record their predictions on Student Sheet 3.

15. The tubes need to sit on salt for about 30-60 minutes. During this time, students should complete their 24 hour worm observations (Part 3). Alternately, the tubes can sit in the salt bed overnight if necessary.

16. After about 30-60 minutes, ask students to wash the salt off the outside of their tubes, dry them, weigh them again, and record the weights on Student Sheet 3. Then they should calculate the percent change in mass.

$$\frac{\text{final mass} - \text{initial mass}}{\text{initial mass}} \times 100 = \text{Percent Change}$$

17. Have student groups add their data to the *Class Data Table for Modeling Worms in Salt* and project the data table for the class to see. Calculate the classroom average percentage change for each type of tube. Students can use their own data or the classroom averages to answer questions on Student Sheet 3.

18. Allow time for student groups to explain what happened to their dialysis tubes after sitting in salt. As groups share, make sure that their claim (their statement of understanding about what happened to the dialysis tube) is backed up by evidence (such as data, observation, background information) and connected through a reasoned explanation.

Part 3 (Explore): Making 24 hour worm observations

19. Students should make 24 hour observations of their wild type and mutant worms on high and low salt.

20. After students have recorded observations on the Student Data Sheet, prompt the class with some discussion questions:

    After 24 hours on low and high salt, how does the movement of the wild type worms compare to the mutant worms?

    After 24 hours all worms are moving on all plates.

    Were you surprised by what you observed on the high salt plates after 24 hours? What do you think is happening to the worms to account for what you saw?

    Students may express surprise that the wild type worms are active because they appeared dead after 15 minutes on the high salt. They may suggest that the wild type worms made more glycerol after being on high salt.

Closure (Elaborate/Evaluate) How does the production of glycerol help a worm? *(10 min)*

21. Using Side 28 as a prompt, ask students:

    How does glycerol prevent water from moving through the dialysis tubing into the salt? How does this model help explain what is happening in the C. elegans in different environments?

22. Give students a chance to revise the drawing/story they created for the entrance activity.

Glossary

Desiccated: Free of moisture; dried out.

Glycerol: A syrupy, viscous liquid that creates hydrogen bonds with water molecules. Glycerol is colorless, odorless, and has a low toxicity.

Membrane: A thin layer of tissue covering a surface. The *C. elegans* skin is a membrane, as is the dialysis tubing.

Osmosis: The movement of water through a semi-permeable membrane

Permeable: Having a porous quality that allows liquids and gasses to pass through it.

Semipermeable membrane: A membrane that will allow some, but not all, molecules or ions to pass through it. Generally, water may freely pass through a semipermeable membrane, but larger molecules will not.

Student Resource

Lesson Three Student Directions:
Modeling the Effect of Glycerol on Worms in Salt

Complete as a class or in your lab group. Use Student Sheet 3 to record your data and to answer the questions.

Materials

2 pieces of serpent skin or 1 inch dialysis tubing, each about 5 inches long
4 rubber bands
15 ml Low Glycerol Solution: 1.5% glycerol in 50 mM NaCl
15 ml High Glycerol Solution: 50% glycerol in 50 mM NaCl
2 medium weighing trays
Crystalline NaCl

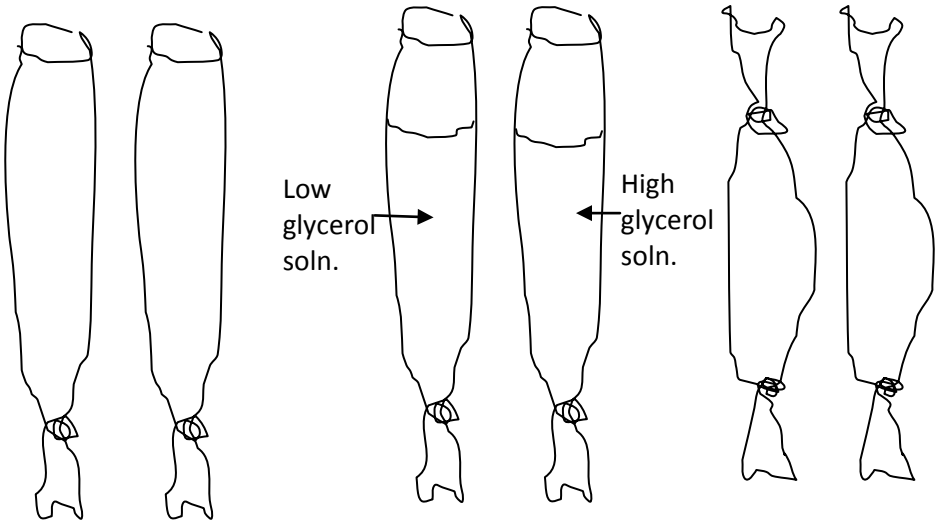
Procedure:

Step 1: Seal one end of each dialysis tube with a rubber band.

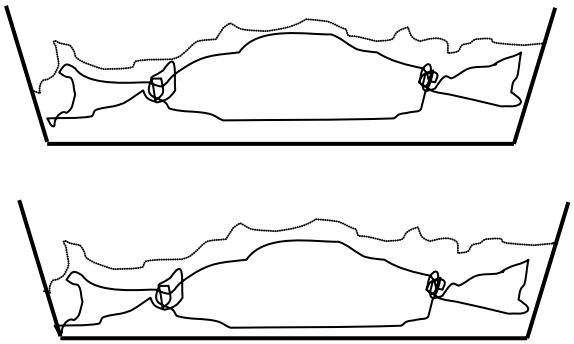
Step 2: Pour Low Glycerol Solution into one dialysis tube and High Glycerol Solution into the other.

Step 3: Seal the top end of the tube close to the liquid with a rubber band.

Step 4: Weigh each tube and record the mass on Student Sheet 3.



Step 5: Place each tube on a bed of NaCl (salt) in a flat tray and cover with additional salt. Label each tray with the name of the solution.



Step 6: Let dialysis tubes sit in salt for 30-60 minutes or overnight.

Step 7: Rinse dialysis tubes to remove salt and blot with a paper towel to dry. Then re-weigh, and record the mass of each on Student Sheet 3.

Name: _____ **Date:** _____ **Period:** _____

Student Sheet 3: *Modeling Worms in Salt*

A. Setting up the Dialysis Tube Lab

1. Set up your dialysis tube experiment according to the directions provided.
2. Before putting the filled dialysis tubes into the tray of salt, weigh them and record the initial mass (in grams) in the table below.

	Initial Mass (grams)	Final Mass (grams)	% Change = (Final – Initial) / Initial x 100
Low glycerol *"Wild type"*			
High glycerol *"Mutant"*			

3. After your group has set up both dialysis tubes, draw what you predict will happen to the two tubes after sitting in salt for a time, and explain why.

Predicted Tube with LOW glycerol	**Predicted Tube with HIGH glycerol**
Drawing:	***Drawing:***
Explanation:	***Explanation:***

Student Sheet 3: *Modeling Worms in Salt*

B. Results of the Dialysis Tube Lab

4. Briefly dip the two dialysis tubes in water and dry them with a paper towel. Weigh each tube and record the masses in the table on page one. Then calculate the percent change in mass for each.

$$\frac{\text{final mass} - \text{initial mass}}{\text{initial mass}} \times 100 = \text{Percent Change}$$

5. In the table below, draw and explain what happened to your dialysis tubes after sitting in salt.

Observed Tube with LOW glycerol	**Observed Tube with HIGH glycerol**
Drawing:	***Drawing:***
Explanation:	***Explanation:***

6. Do your observations agree with the prediction you made before setting up the dialysis? ***Explain.***

7. Prepare to share your findings with your classmates by:
   - stating a **claim** of your understanding of the lab
   - providing **evidence** for the claim by referring to your data, observation, background information or other appropriate evidence
   - providing **reasoning** that explains how the evidence is connected to the claim.

Class Data Table for Modeling Worms in Salt

One member of each group should fill out the initial mass, final mass, and percent change for both the low glycerol and high glycerol tubes. When the data table is complete, find the class average for each type of tube.

		Initial Mass	Final mass	% change	Average % change
Low glycerol ***"Wild type"***	Group 1				
	Group 2				
	Group 3				
	Group 4				
	Group 5				
	Group 6				
	Group 7				
	Group 8				
High glycerol ***"Mutant"***	Group 1				
	Group 2				
	Group 3				
	Group 4				
	Group 5				
	Group 6				
	Group 7				
	Group 8				

Lesson Four

Using evidence to develop an explanation for worm observations

Overview
Students carry out their 48 hour worm observations. Students then analyze data from the scientific literature to develop an explanation for their observations of wild type and mutant worms on low and high salt plates.

Enduring understandings: Scientists use data from previous research to develop an explanation for an observed phenomenon. Living organisms have systems to maintain homeostasis within a range of environmental conditions.

Essential question: How does *C. elegans* maintain homeostasis in a high salt environment?

Learning objectives
Students will be able to:

- Document differences between wild type and mutant worms growing in different environmental conditions.
- Analyze and interpret data from the scientific literature
- Develop an explanation for how *C. elegans* maintains homeostasis based on gene expression

Prerequisite Knowledge
Familiarity with bar and line graphs

Time: 50 minutes

This lesson connects to the Next Generation Science Standards in the following ways:

Disciplinary Core Ideas
HS LS1.A Structure and Function: Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (through positive feedback) or discourage (negative feedback) what is going on inside the living system. (HS-LS1-3)

HS LS3.B Variation of Traits Environmental factors affect expression of traits.

HS LS4.B Natural Selection: Trait variation leads to differences in performance among individuals.

This lesson highlights the Scientific Practices of **Analyzing and Interpreting Data**, and **Engaging in Argument from Evidence**. It also highlights the Crosscutting Concept of **Stability and Change**.

Materials

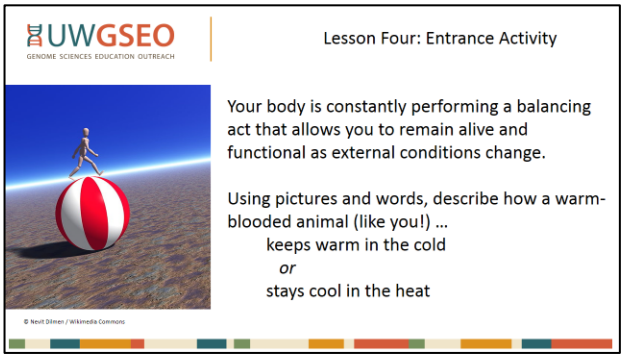
Materials	Quantity
Computer and projector	1 per class
PowerPoint presentation at http://gsoutreach.gs.washington.edu/ (see GEM Instructional Materials)	1 per class
Student Sheet 4: *Developing an explanation*	1 per student
Possible Answers to Student Sheet 4	1 per class
Student Graphs: *Glycerol content of worms; A, B, C and D*	1 set per lab group, in plastic sleeves
Dissecting microscope	1 per lab group
Worm plates from Lesson Two	1 per lab group
Data tables	1 per student
Disposable gloves	1 pair per student

Presenting the Lesson

Entrance activity (Engage): *(5-10 min)*

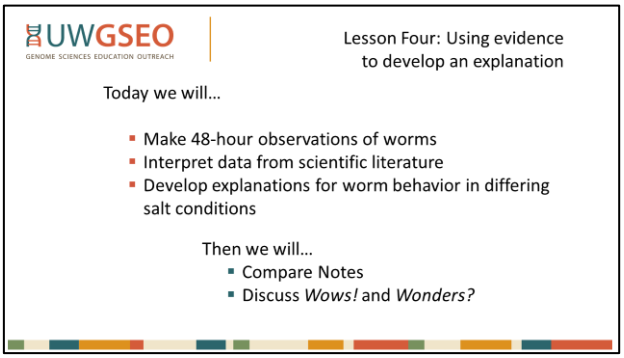
1. Have PowerPoint slide 29 projected for students to see and complete as they enter.

Slide 29



2. Tell students that all living things have feedback mechanisms that allow them to cope with changing environmental conditions. ***Homeostasis*** is the ability of an organism to adjust its internal environment to maintain stability, even as the external environment is changing. For example, some warm-blooded animals produce sweat that evaporates off the skin, thereby cooling them down in warm weather and maintaining a stable internal body temperature. One of the many things all living organisms also need to regulate is the water content of their cells. We will continue to explore this with *C. elegans* today.

3. Show Slide 30



Part 1 (Explore): 48 hour worm observations *(15 min.)*

4. Ask students to go to their lab stations and complete the 48 hour worm observations.

5. Make sure that all students are making the observations and recording their results in the data tables.

6. To dispose of the worm plates when students are finished, the plates may be soaked in a 10% bleach solution and then thrown away. If available, the plates may be autoclaved. Gloves, paper towels and other materials may be thrown away in the garbage.

Part 2: (Elaborate) Using the scientific literature to explain worm observations *(20 min)*

7. Explain to students that the scientists who first did these experiments thought that glycerol might be involved in keeping worms from shrinking in high salt, based on similar experiments in yeast.

8. Pass out Student Sheet 4: *Developing an explanation*. Provide each student group with the four graphs shown in **Figure 4 A-D**, *Glycerol Content of Worms* (student copies can be put in plastic sleeves for reuse). These graphs summarize data from the scientific literature that may help students understand what is going on with their worms.

> ***Note***: This exercise works well using a ***jigsaw*** discussion structure. In a jigsaw, groups of four students first meet with the *same graph* (all students with graph A, for example) to discuss, interpret the graph and fill out the correct section of Student Sheet 4. Students are then rearranged into new groups in which all four students have *different graphs* (A, B, C, and D) and share the information on their graph with the rest of the group.

9. Ask students to discuss the figures with their group and answer the questions on Student Sheet 4: *Developing an explanation*.

10. Discuss student responses as a whole class, supporting students in their explanations as needed with this potentially difficult task. Possible answers to Student Sheet 4: *Developing an explanation* can be found in the resource section. Slides 31 – 34 can be used for the class discussion.

Closure (Evaluate) *(5-10 min)*

11. Use Slide 35 to discuss the following questions:

    How does C. elegans maintain homeostasis in a high salt environment?

    Is it an advantage to always produce high levels of glycerol? Or is it better to be able to control glycerol production?

Note: Students may wish to explore real-life connections to the need for *C. elegans* to control its water content while living in higher salt environments. The ongoing debate about whether or not to salt the streets during snow storms is relevant in many parts of the country. A helpful article can be found here:

De-icing dilemma: Do streets need salt?
Salt saves lives on icy roads, but it can have the opposite effect in nearby ecosystems. Here's a look at the pros and cons of salt and other de-icers.
http://www.mnn.com/earth-matters/translating-uncle-sam/stories/de-icing-dilemma-do-streets-need-salt

Glossary

Homeostasis: Process by which organisms maintain an internal stable condition, even as external conditions change. Feedback mechanisms can encourage (through positive feedback) or discourage (through negative feedback) what is going on inside the living system.

Gene expression: Process by which information from a gene is used to make a functional product, such as a protein.

References

Lamitina, S.T., Morrison, R., Moeckel, G.W., and Strange, K. 2004. Adaptation of the nematode *Caenorhabditis elegans* to extreme osmotic stress. *American Journal of Physiology - Cell Physiology 286*, C785-C791.

Rohlfing, A.-K., Miteva, Y., Moronetti, L., He, L., and Lamitina, T. 2011. The *Caenorhabditis elegans* Mucin-like Protein OSM-8 Negatively Regulates Osmosensitive Physiology Via the Transmembrane Protein PTR-23. *PLoS Genetics* 7, e1001267.

Name: _____ Date: _____ Period: _____

Student Sheet 4: *Developing an explanation for worm observations*

1. Look at **Figure 4A**. What is different about the mutant compared to the wild type worms?

2. Look at **Figure 4B**. How does growing on different salt concentrations affect glycerol levels inside wild type worms?

3. Look at **Figure 4C**, and think about what you observed with your wild type worms when you grew them on **high salt**. How might the glycerol level inside the worms affect their level of activity right after being transferred to high salt? 24 hours after transfer?

4. GPD is the enzyme that carries out the final step in making glycerol inside worm cells. **Figure 4D** shows the amount of GPD in wild type and mutant worms at two salt concentrations. What do you notice about the level of this enzyme in the two worm strains at normal and high salt? How would this affect how much glycerol they produced?

5. The level of GDP enzyme in the worm is controlled by the amount of mRNA made from the *gdp* gene in the DNA. Information from a gene (*gdp*) being used to make a product (the enzyme GDP) is called ***gene expression***. Which type of worm shows heightened gene expression when the worm is moved from a low salt to a higher salt environment?

6. Based on your observations and the results of your experiment(s), is there an advantage or disadvantage to making glycerol **only at high salt** (***not all the time***)? Is there an advantage or disadvantage to making glycerol all the time, as in the **mutants**? *Please be as thorough as possible in your answer.*

POSSIBLE ANSWERS

Student Sheet 4: *Developing an explanation for worm observations*

1. Look at **Figure 4A**. What is different about the mutant compared to the wild type worms?

   In a low salt environment, the wild type worms produce very little glycerol (less than 100 nmol/mg), while the mutant worms produce much more (about 1750 nmol/mg). This is remarkable because the mutant worms are apparently already producing glycerol in a low salt environment, even before they need the extra glycerol to control water content in a high salt environment.

2. Look at **Figure 4B**. How does growing on different salt concentrations affect glycerol levels inside wild type worms?

   This graph shows that the wild type worms can change their internal conditions in response to changes in their environment. When put into a higher salt environment, wild type worms that had not been previously producing much glycerol are able to begin producing glycerol. Worms in an environment of higher salt content will produce more glycerol than worms in an environment of lower salt content.

3. Look at **Figure 4C**, and think about what you observed with your wild type worms when you grew them on **high salt**. How might the glycerol level inside the worms affect their level of activity right after being transferred to high salt? 24 hours after transfer?

   When the wild type worms were originally put on the higher salt plates (time 0, or 15 minutes after transfer) they were not producing much glycerol, which caused them to curl up and not move. When challenged by the environment, however, in the next 24 hours they began production of glycerol, which helped them maintain water balance and survive. The worms did not continue to make even more glycerol, so the 24 hour and 48 hours observations were very similar.

4. GPD is the enzyme that carries out the final step in making glycerol inside worm cells. **Figure 4D** shows the amount of GPD in wild type and mutant worms at two salt concentrations. What do you notice about the level of this enzyme in the two worm strains at normal and high salt? How would this affect how much glycerol they produced?

   The wild type worms are able to ramp up production of glycerol when challenged by the high salt environment by producing more of the enzyme GDP. In wild type worms, GDP production increases about 18 fold in high salt, while in the mutant worms it increases by about 1 1/2 fold compared to the already high level seen on low salt. Although the mutant worms have a much lower increase in GDP production in response to high salt, they produce more glycerol in low salt than the wild type worms do in low salt (compare Figures 4A and 4B), so there is less need to ramp up their glycerol production in high salt.

5. The level of GDP enzyme in the worm is controlled by the amount of mRNA made from the *gdp* gene in the DNA. Information from a gene (*gdp*) being used to make a product (the enzyme GDP) is called ***gene expression***. Which type of worm shows heightened gene expression when the worm is moved from a low salt to a higher salt environment?

   The Wild Type worm shows heightened gene expression when moved to a higher salt environment, as is shown by increased glycerol production.

6. Based on your observations and the results of your experiment(s), is there an advantage or disadvantage to making glycerol **only at high salt** (***not all the time***)? Is there an advantage or disadvantage to making glycerol all the time, as in the **mutants**? *Please be as thorough as possible in your answer.*

   Answers may vary based on observations, and both arguments may be supported by the evidence.

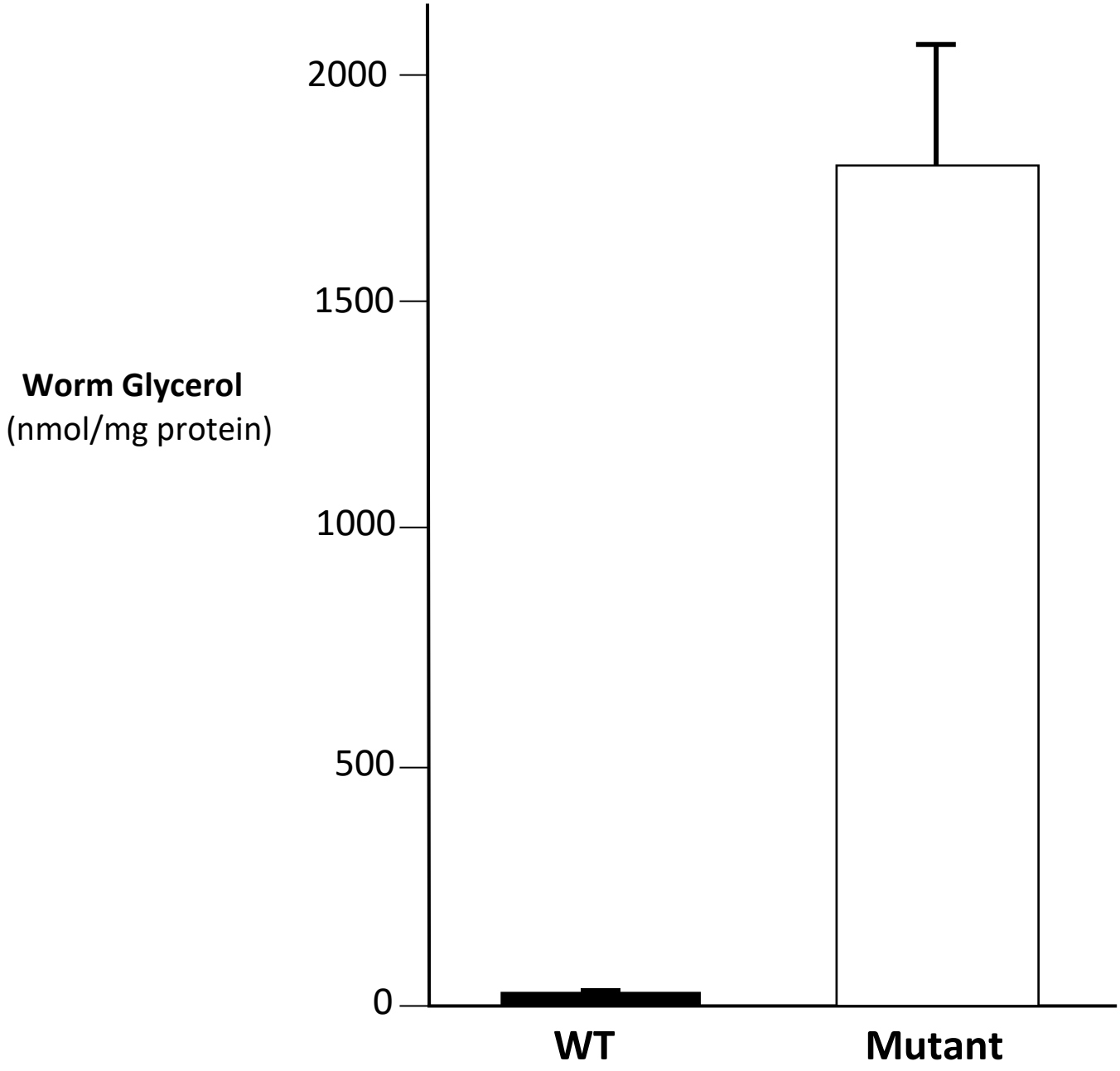
   The advantage to not making glycerol all the time, except when the organism needs it, is that it probably takes energy to make glycerol, so it slows down the worms' growth and development. The wild type worms are bigger, more active, and reproduce more quickly than the mutant worms. In a mixed population in a low salt environment, they would be able to out compete with the slower worms.

   The advantage to making high glycerol all the time is that then the organism is prepared if it suddenly encounters an environment with high osmotic stress like high salt.

   There is probably a trade-off between being able to react quickly to high salt and being able to grow and reproduce quickly. Depending on the environmental conditions, one strain may be favored over the other.

Glycerol Content of Worms

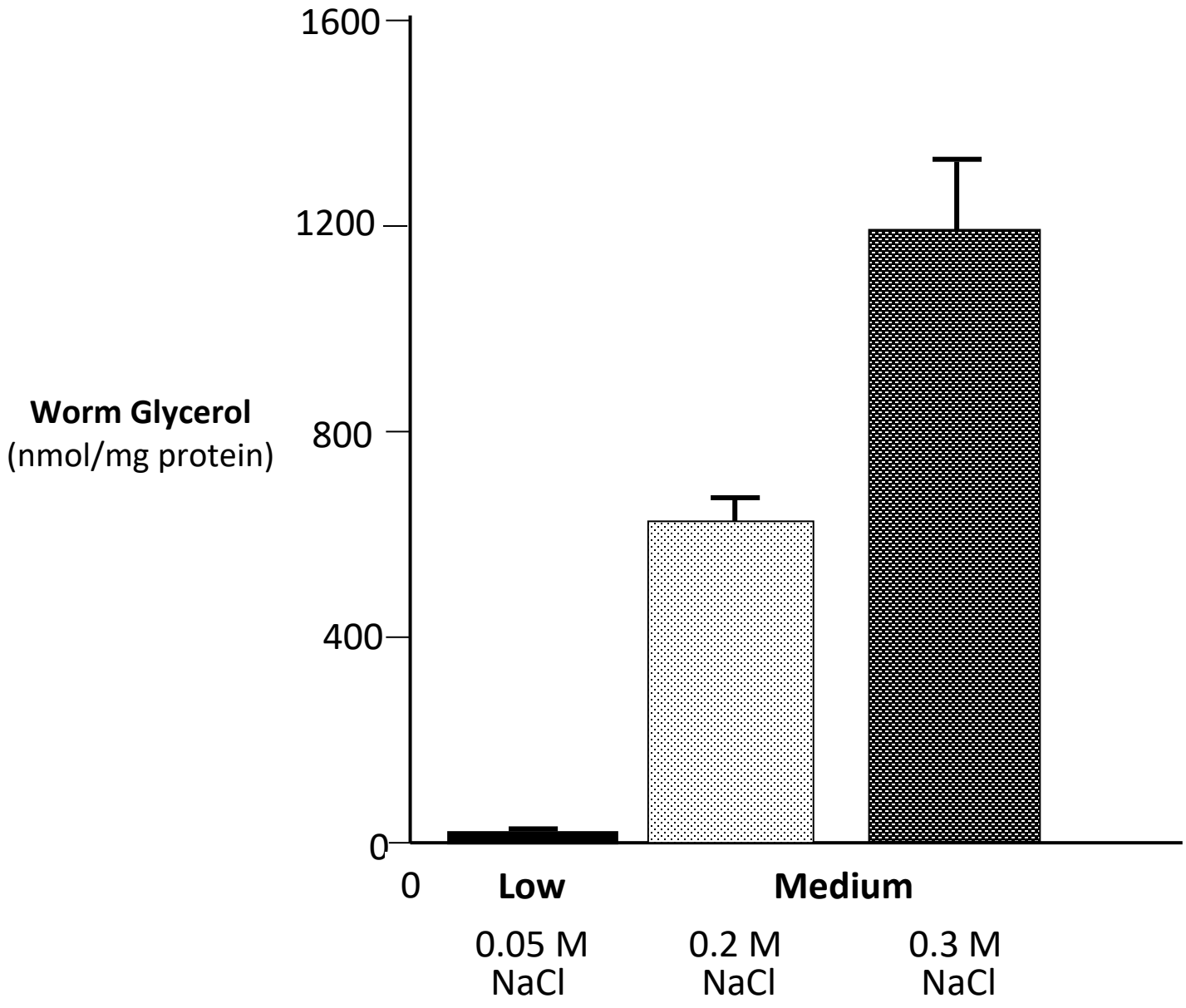
A. Glycerol content of wild type and mutant worms in **low salt** (0.05 M NaCl)



Data from this figure originally published in *PLoS Genetics* in 2011.

Glycerol Content of Worms

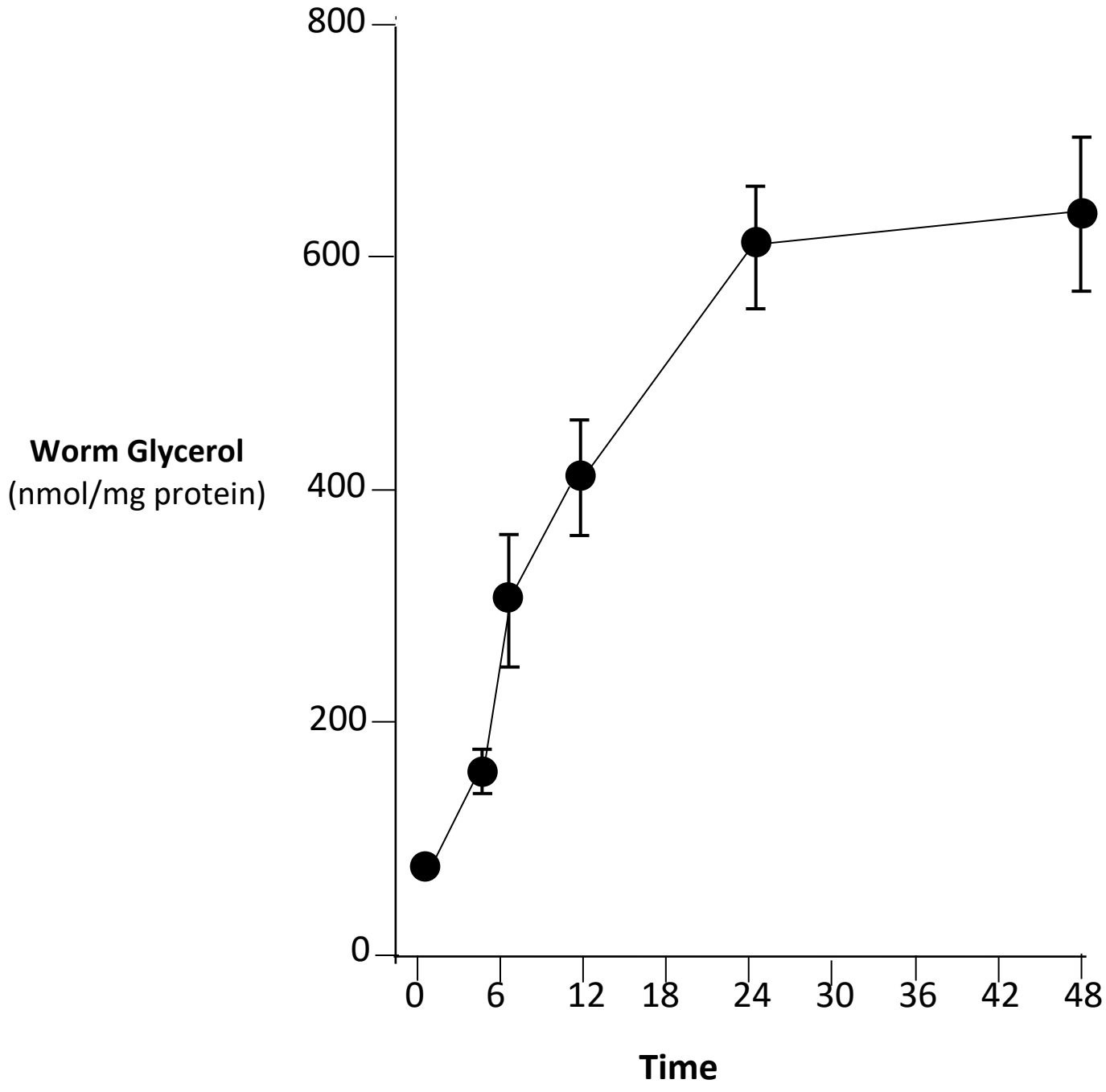
B. Glycerol content of wild type worms grown on **low and medium** salt for **18 hours**.



Data from this figure originally published in the *American Journal of Physiology - Cell Physiology* in 2004.

Glycerol Content of Worms

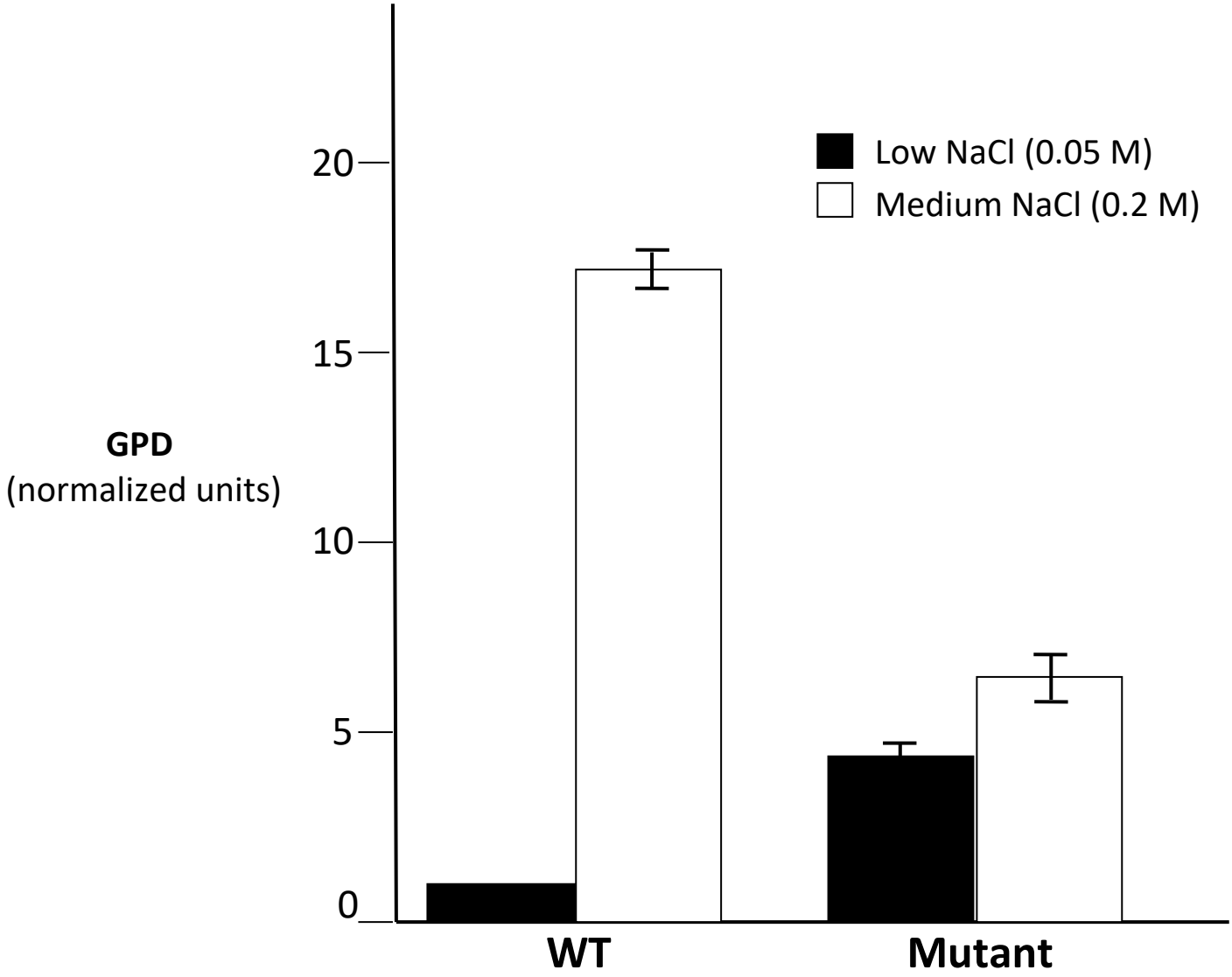
C. Glycerol accumulation over time in wild type worms grown on **medium salt** (0.2 M NaCl).



Data from this figure originally published in the *American Journal of Physiology - Cell Physiology* in 2004.

Glycerol Content of Worms

D. Accumulation of GPD in wild type and mutant worms grown on **low salt** (0.05 M NaCl) or **medium salt** (0.2 M NaCl) for 18 hours.



Note:
GPD is the enzyme that carries out the final step in making glycerol inside worm cells. It is coded for by the *gpd* gene.

Data from this figure originally published in *PLoS Genetics* in 2011.

Lesson Five

How does a mutation affect *C. elegans* in low and high salt?

Overview
Students use their understanding of transcription and translation to understand how a single nucleotide change in *C. elegans* results in a difference in how the wild type and mutant worms respond to osmotic stress.

Enduring understanding: A single nucleotide change to the DNA sequence of a gene can have a dramatic effect on the protein coded for by that gene.

Essential question: How do changes in the nucleotide sequence of a gene affect the protein coded for by that gene?

Learning objectives
Students will be able to:

- Use a chart of the Universal Genetic Code to determine the protein sequence of a gene
- Demonstrate how the DNA sequence of a gene codes for the amino acid sequence of a protein
- Explain the probable effect of a single nucleotide change on the function of the protein

Prerequisite Knowledge

- Gene expression (DNA→RNA→protein→trait): These concepts may have been taught in previous lessons. Resources for reviewing them are provided below.
- Classes of proteins: enzymes, structural protein like collagen; muscle

Time: 50 minutes

This lesson connects to the Next Generation Science Standards in the following ways:

HS LS1.1: Performance Expectation:
Structures and Processes: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins, which carry out the essential functions of life through systems of specialized cells.

HS LS1.A Disciplinary Core Idea
Structure and Function: Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells.

This lesson highlights the Scientific Practice of **Engaging in Argument from Evidence**, and the Crosscutting Concept of **Cause and Effect: Mechanism and Explanation**.

Teacher Background: How does *C. elegans* detect differences in osmotic stress and regulate glycerol production?

The scientific name for one osmotic mutant is JT89. This nematode strain has a single nucleotide change in the *osm-7* gene, which codes for a protein called T05D4.4 (not a very informative name!). The activity on Student Sheet 5 will help your students understand how a single nucleotide change might affect the protein made from that gene. The Universal Genetic Code Chart used in this activity is from the Learn.Genetics website, so if you have just reviewed transcription and translation using that site, it will be familiar to your students.

The single nucleotide change in the mutant gene changes the codon for argentine (Arg) to a stop codon, resulting in a shortened protein being made in the mutant strain. Since the mutation is in the first half of the mRNA, the resulting protein is not functional.

Possible mechanism for control of glycerol synthesis in wild type worms: All of the osm mutations result in glycerol being constitutively synthesized (i.e. it's made all the time). In wild type worms, the level of glycerol inside the worm increases dramatically when the worm is in a high osmotic stress environment. The exact mechanism by which the worm controls the synthesis of glycerol is not entirely understood. However, by analyzing which mutations lead to constitutive production of glycerol, scientists can develop models for control of glycerol production in wild worms. One model is that there is a pathway for detecting osmotic pressure in the environment. In wild type worms, pressure changes are detected on the worm's surface when in a high osmotic pressure environment. This results in the stimulation of signaling molecules, resulting in an increase in the expression of the gene for glycerol-3-phosphate dehydrogenase (GPD). This leads to a dramatic increase in the production of glycerol.

Why don't worms make high glycerol all the time? When wild type worms are moved back to low salt after being exposed to high salt for >24 hours, they quickly get rid of the excess glycerol through defecation. This suggests that having a high internal glycerol concentration at all times is not beneficial to the worms. Your students may notice that the wild type worms replicate more quickly in low salt than the mutant. Under normal environmental conditions, it would be advantageous not to make a high level of glycerol because these worms are able to replicate more quickly and out-grow the osm mutants. However, in the high salt, the situation may be reversed, with the mutants having the advantage.

Materials

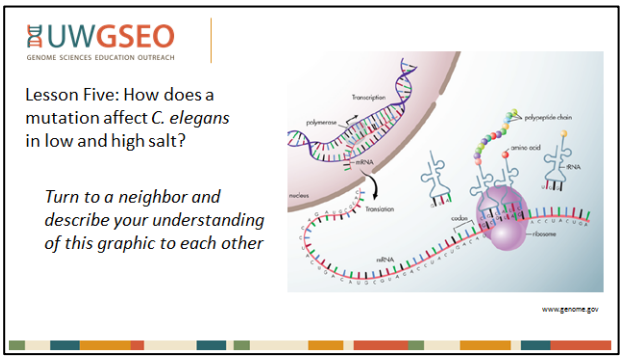
Materials	Quantity
Computer and projector	1 per class
PowerPoint presentation found at http://gsoutreach.gs.washington.edu/ (see GEM Instructional Materials)	1 per class
Student Sheet 5: Effects of a single nucleotide change	1 per student
Possible Answers to Student Sheet 5	1 per class
Student Resource: *Universal Genetic Code*	1 per student or group

Presenting the Lesson

Entrance activity (Evaluate)

1. Have PowerPoint slide 36 projected for students to see and discuss as they enter.

Slide 36



Part 1 (Engage): Consequences of mutations *(10 min.)*

2. Ask students to think about how the term "mutant" is used in popular culture. Show Slide 37 to demonstrate how "mutations" can result in negative, mixed, or positive outcomes for the creature. The results range from the horrifying *(The Fly)*, to the conflicted (*X-Men*) to the heroic (*Teenage Mutant Ninja Turtles*).

Slide 37

3. Tell students that *C. elegans* gives us insight into what being a mutant can mean in the scientific world. Today students will look at the molecular cause of the mutation that differentiates the wild type from the mutant worms.

4. In nature, mutations can be harmful, beneficial, or have no consequence at all. Mutations can also, as in the case of the mutation to the *osm-7* gene, lead to traits that allow different organisms to survive and flourish in different environments. Genetic mutation is a major driver of the evolutionary process; organisms with mutations that contribute to positive survival traits in a particular environment may live to reproduce and pass on their genes, whereas their counterparts without the mutation may die off.

While a mutation to the *osm-7* gene in the mutants allows them to produce glycerol at a higher level on low salt plates, it also appears to contribute to changes in other functions, such as an altered defecation cycle for the worms.

Part 2 (Explain/Explore): Reviewing the Concepts of DNA, Genes, and Proteins *(time varies)*

5. This lesson requires that students have a basic understanding of the processes of transcription and translation. If this is new for students or the entrance activity highlights a lack of understanding, you may want to use the following activities at the Genetic Science Learning Center (http://learn.genetics.utah.edu/) at the University of Utah. This site provides a useful review of DNA, genes, transcription, translation, proteins, and traits:

   Tour the basics: http://learn.genetics.utah.edu/content/begin/tour/

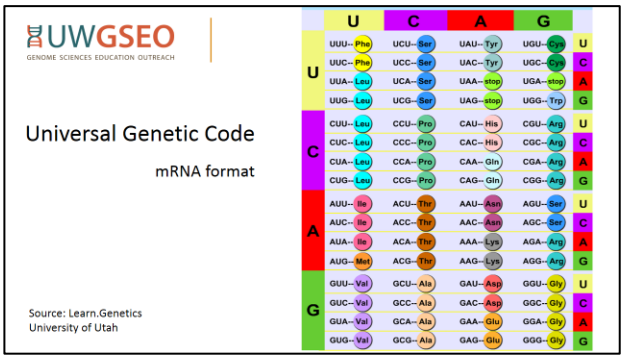
   - What is DNA?
   - What is a gene?
   - What is a chromosome?
   - What is a protein?
   - What is heredity?
   - What is a trait?

6. Additional information on DNA and the process of making proteins can be found at: http://learn.genetics.utah.edu/content/begin/dna/
   - Build a DNA Molecule: an interactive activity that teaches base pairing during DNA replication
   - Transcription & Translation: Students first transcribe a gene into RNA and then translate it into an amino acid chain (a protein)
   - What is a Protein? This section has several activities that demonstrate how proteins work normally and can be disrupted by mutations.

Part 3 (Explore): Translating DNA sequences *(20 minutes)*

7. Hand out Student Sheet 5 and guide students as they work, individually or in groups, to translate the mRNA into amino acid sequences. It may be helpful to show Slide 38 as students work.

Slide 38



8. Make sure that students understand that proteins are functional structures that are essential to all living things. In the case of the *osm-7* mutant, the protein that helps to maintain the necessary balance of salt and water is mutated. While this particular mutation is not lethal for the organism (and may even be beneficial in some circumstances) the mutation does affect how the worm behaves in a high salt environment.

> ***Note:*** A helpful lesson using pipe cleaners and pencils that makes a connection between the structure and function of a protein (and the relative impacts of where a mutation may occur) is described in the article
>
> *Modeling Structure & Function: Pencil Transferase*
>
> found at:
> http://www.nabt.org/websites/institution/File/pdfs/american_biology_teacher/2012/ABT_Online_Oct_2012.pdf

Closure (Evaluate): *(10 minutes)*

9. Probe student understanding by asking some additional questions which may be answered individually, in small groups or using a think-pair-share strategy. These questions can be projected using Slide 39.

- Describe one way in which a single nucleotide change in the DNA can have a dramatic effect on the protein coded for.

  Students may mention that a mutation in the DNA will affect the RNA which will, in turn, either call for the wrong amino acid or instruct the ribosome to STOP making the protein, as happened in this case. Students more familiar with protein structure may note that a substitution in amino acids may have a profound effect on the protein structure if, for example, a hydrophilic amino acid is substituted for a hydrophobic amino acid.

- Describe one way in which a single nucleotide change can have NO effect on the resulting protein.

  Students may have noticed from the Universal Genetic Code sheet that two different mRNA segments may code for the same amino acid. There are also instances in which two amino acids with similar properties may be substituted for each other with no effect on the protein.

10. If time allows, teachers may wish to revisit Slide 32 and once again have students describe any new insights into transcription and translation.

Glossary

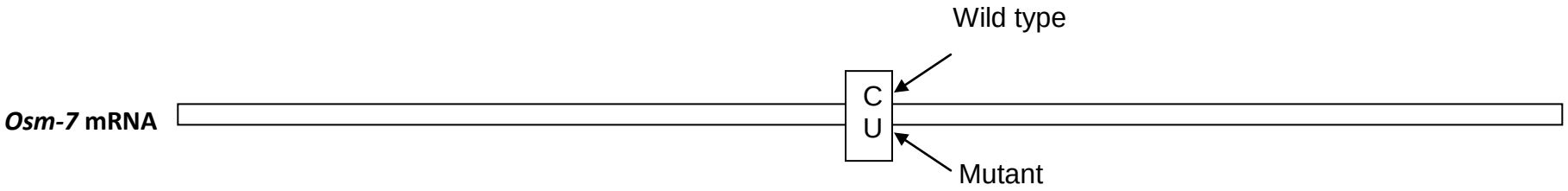
Osmoregulation: The process by which cells or organisms maintain a constant osmotic pressure through fluid and electrolyte balance.

Name: _____ Date: _____ Period: _____

Student Sheet 5: Effects of a single nucleotide change

Background: The mutant worm you have been using is called an OSM mutant because it has a mutation in the *osm-7* gene that regulates ***osmoregulation***, which is the ability to maintain fluid pressure in an organism by controlling water and salt concentrations. There are many OSM mutants that react to high salt in the same way as the mutant you used. One mutant is called JT89, which has a mutation that codes for a protein called T05D4.4 (not a very informative name!). Here are some facts about this strain compared to the wild type:

- The *osm-7* gene of the mutant differs by *one nucleotide* compared to the wild type gene.
- The *osm-7* mRNA is 1700 nucleotides long. The mutation is near the middle of the mRNA.
- The protein made from the wild type mRNA is 562 amino acids long.

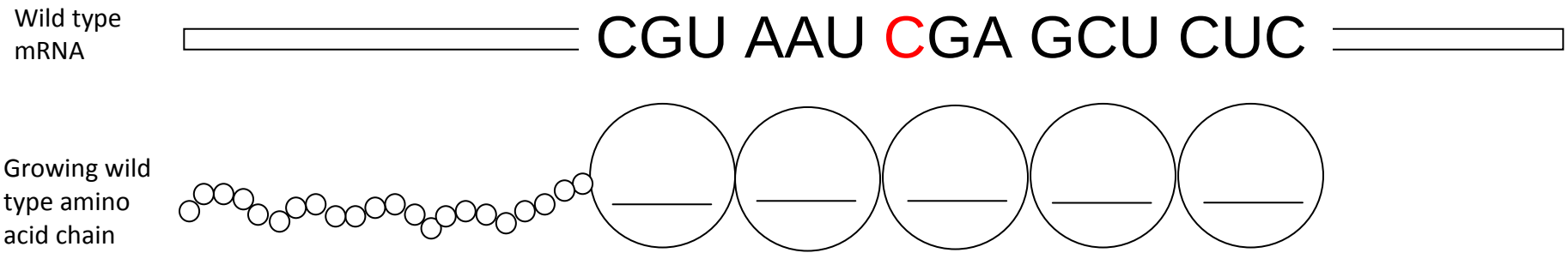


Directions: Complete the following activity to learn how a single base change can affect the protein made from that gene.

1. Look at the two figures on the next page. Each figure shows 15 nucleotides around the mutation site, and the growing amino acid chain below.

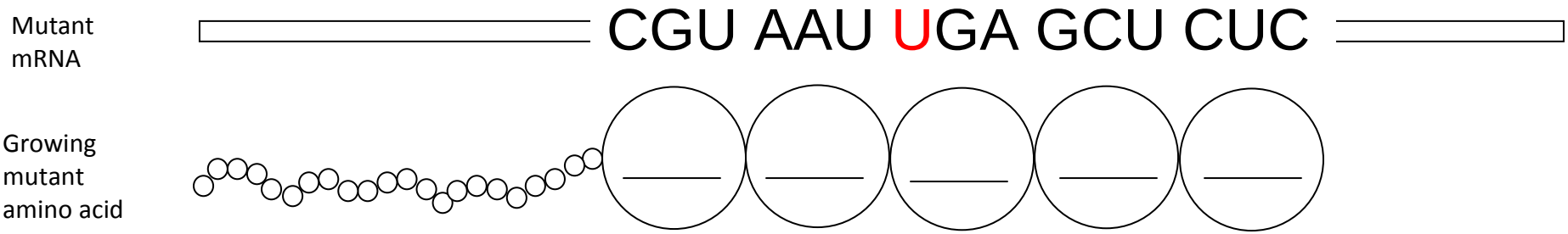
2. Use the Universal Genetic Code to decide which amino acid is coded for by each nucleotide triplet in the mRNA for the wild type mRNA. Using the Student Resource sheet, figure out the name of each amino acid using the three letter abbreviation in the circle below the mRNA sequence. Write your answers in the circles below:

Figure 1: Wild Type



3. Now use the Universal Genetic Code to translate the mutant mRNA gene.

Figure 2: Mutant



Discussion Questions

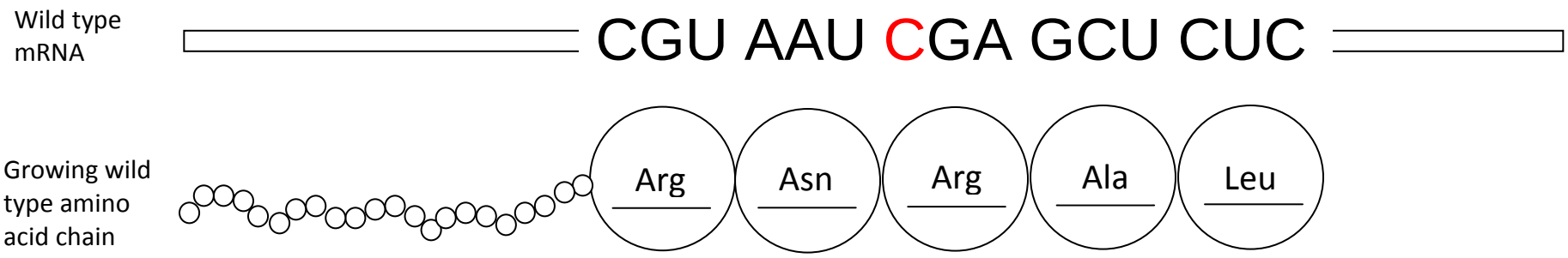
1. How are the two resulting proteins different?

2. How would this difference affect the protein made? Explain whether you think the mutant protein would still function properly.

POSSIBLE ANSWERS to Student Sheet 5: Effects of a single nucleotide change

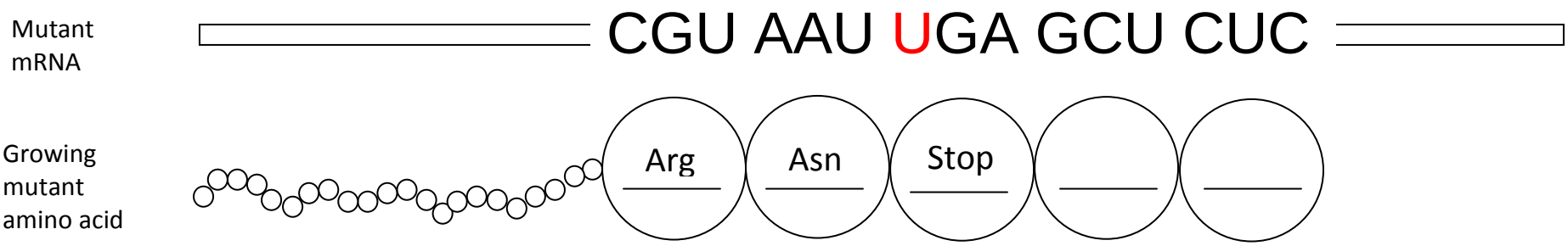
2. Use the Universal Genetic Code to decide which amino acid is coded for by each nucleotide triplet in the mRNA for the wild type mRNA. Using the Student Resource sheet, figure out the name of each amino acid using the three letter abbreviation in the circle below the mRNA sequence. Write your answers in the circles below:

Figure 1: Wild Type



3. Now use the Universal Genetic Code to translate the mutant mRNA gene.

Figure 2: Mutant



Discussion Questions

1. How are the two resulting proteins different?
   At the point of mutation, the wild type mRNA codes for an Argenine amino acid and the mutant mRNA codes for a stop codon.

2. How would this difference affect the protein made? Explain whether you think the mutant protein would still function properly.
   The mutant protein would stop being made about half way through the instructions. It is unlikely to function properly if half of it is missing.

Universal Genetic Code
(mRNA format)

	U	C	A	G	
U	UUU-- Phe	UCU-- Ser	UAU-- Tyr	UGU-- Cys	U
	UUC-- Phe	UCC-- Ser	UAC-- Tyr	UGC-- Cys	C
	UUA-- Leu	UCA-- Ser	UAA-- stop	UGA-- stop	A
	UUG-- Leu	UCG-- Ser	UAG-- stop	UGG-- Trp	G
C	CUU-- Leu	CCU-- Pro	CAU-- His	CGU-- Arg	U
	CUC-- Leu	CCC-- Pro	CAC-- His	CGC-- Arg	C
	CUA-- Leu	CCA-- Pro	CAA-- Gln	CGA-- Arg	A
	CUG-- Leu	CCG-- Pro	CAG-- Gln	CGG-- Arg	G
A	AUU-- Ile	ACU-- Thr	AAU-- Asn	AGU-- Ser	U
	AUC-- Ile	ACC-- Thr	AAC-- Asn	AGC-- Ser	C
	AUA-- Ile	ACA-- Thr	AAA-- Lys	AGA-- Arg	A
	AUG-- Met	ACG-- Thr	AAG-- Lys	AGG-- Arg	G
G	GUU-- Val	GCU-- Ala	GAU-- Asp	GGU-- Gly	U
	GUC-- Val	GCC-- Ala	GAC-- Asp	GGC-- Gly	C
	GUA-- Val	GCA-- Ala	GAA-- Glu	GGA-- Gly	A
	GUG-- Val	GCG-- Ala	GAG-- Glu	GGG-- Gly	G

Source: Learn.Genetics.utah.edu

Overview
In this paper and pencil modeling activity, students work in lab groups to summarize what they observed during the worm experiment and record their understanding of how *C. elegans* maintains balance in a changing environment.

Enduring understanding:
The nematode *C. elegans* can maintain homeostasis within a range of environmental conditions. Changes in the environment can affect the expression of genetic traits.

Essential question: What can we learn from worms?

Learning objectives
Students will be able to:

- Build a model that demonstrates how wild type and mutant worms behave in low and high salt environments over three time periods
- Demonstrate an understanding of the physiological differences between the worm strains, including the role of glycerol production
- Make and defend claims based on data and evidence

Prerequisite Knowledge
Students will need to have completed their 15 minute, 24 hour and 48 hour worm observations as instructed in the unit. A familiarity with engaging in argument from evidence is also helpful.

Time: 90 minutes

This assessment connects to the Next Generation Science Standards in the following ways:

HS LS1.A Disciplinary Core Idea
Structure and Function
Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range.

Scientific Practice: Engaging in Argument from Evidence

- Make and defend a claim based on evidence about the natural world that reflects scientific knowledge and student-generated evidence.
- Construct, use and/or present an oral and written argument or counter-arguments based on data and evidence.

This assessment highlights many Crosscutting Concepts, including **Systems and System Models**.

Materials

Materials	Quantity
Computer and projector	1 per class
PowerPoint presentation at http://gsoutreach.gs.washington.edu/ (see GEM Instructional Materials)	1 per class
Class Resource: *Observational Graphic Organizer*	1 per class
Possible Answers to *Observational Graphic Organizer*	1 per class
Colored construction paper, 24 in x 18 in *or* Manila folders	1-2 per group
Scissors	2 per group
Student Resource: *Assessment Instructions*	1 per group
Student Resource: *Worm Plates*	1 per group
Student Resource: *Claim and evidence cards*	1 set per group
Copies of extra worm plate shapes and cards	As needed

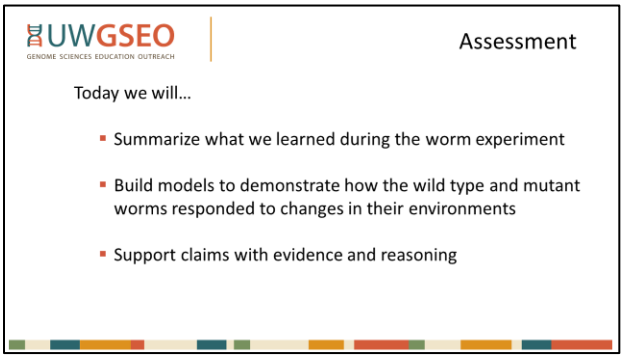
Teachers wishing to conduct individual assessments should make 1 copy of each student resource per student.

Presenting the Assessment *(5 min.)*

1. Tell students that in this assessment activity, they will summarize what they observed during the worm experiment. They will record their explanation of what occurred inside the worms based on the concepts they have learned, and generate 1-3 Claim—Evidence—Reasoning statements that support their finding.

2. Slide 40 gives an overview of the assessment.

Slide 40



Part 1 Review and organize *(40 min.)*

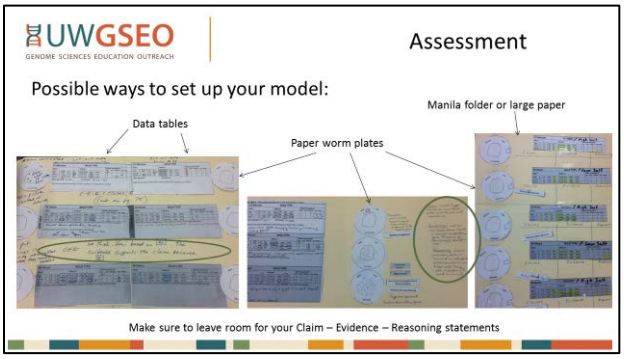
3. The first steps of the assessment allow students to review and organize the data they have collected throughout the unit.

4. If students need additional scaffolding in order to make sense of the data, it can be helpful to use the Class Resource: *Observational Graphic Organizer* found on page 80. As a teacher-directed discussion, the class can compare their individual group observations in order to find similarities between groups and look for patterns over time. The teacher can record this information as a resource for students to use in addition to their own data tables.

5. Show students the paper worm plates and the manila folders or large pieces of construction paper. Students will be using their data tables and paper worm plates in order to 'tell the story' of what happened to both the wild type and mutant worms under different environmental conditions. Students can relate their understanding using drawings, pictures and words.

6. Show students Slide 41, which gives examples of different ways to organize the data.

Slide 41



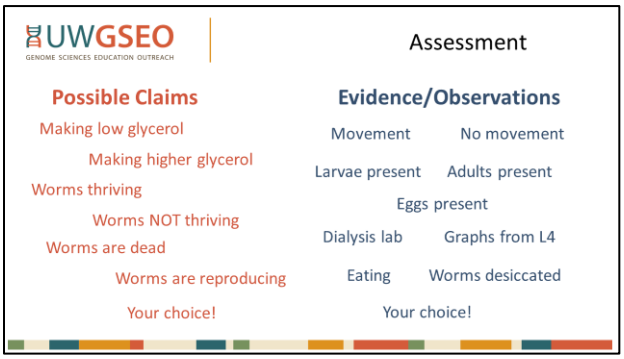
7. Hand out Student Resources *Assessment Instructions* and *Worm Plates.* Answer any questions as needed and give students time to work on their paper models.

Part 2: Claims, evidence and reasoning *(30 min.)*

8. When students have their graphic models organized, ask them to make a statement (claim) about what happened to the worms over the course of the experiment, based on the data they have generated and organized. More than one claim can be made.

9. To support students in generating claims, show Slide 42. Continue through the slide in order to talk about the types of evidence that can be used to support various claims.

Slide 42



Note: Observations can be one type of evidence used to support a claim. Other types of evidence can be the data from the data table, information from the dialysis lab, the data from the research graphs shared in class, or other appropriate background information.

10. Hand out a set of the *Claim and evidence cards* to each group. Students can practice making a claim (using a *claim* card) and then finding evidence to support that claim (using an *evidence* card). Point out that students are using *reasoning* when they are trying to figure out how, or if, a claim is supported by certain type of evidence.

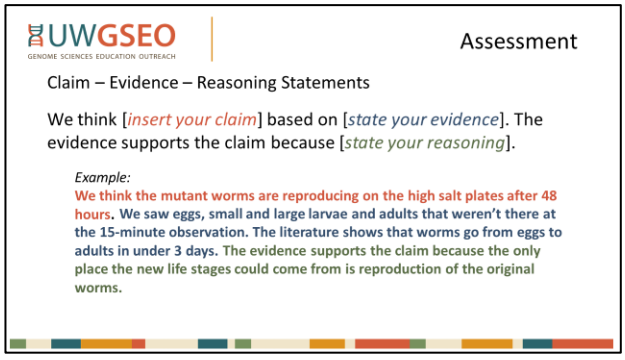
11. Point out that the boundaries between claims and evidence can be blurry. For example, "Worms are eating" can be used as a claim, backed up by the evidence of seeing worm tracks through the food. "Worms are eating" can also be used as evidence to support the claim that the worms are thriving in a certain environment.

12. Tell students that they will be asked to write 1-3 Claim –Evidence—Reasoning statements, based on this unit. They can be in this form:

    We think [*insert your claim*] based on [*state your evidence*]. The evidence supports the claim because [*state your reasoning*].

13. Slide 43 gives an example of a Claim—Evidence—Reasoning (C – E - R) statement.

Slide 43



Note: Reasoning is the *why* that bridges the evidence to the claim. It often involves a "rule" or scientific principle that students have been exploring during the unit.

14. Some examples of C – E – R statements could include:

    We think that the wild type worms make higher glycerol when on high salt plates after about 24 hours. According to our observations, after 15 minutes the wild type worms looked desiccated, but they were moving after 24 hours. The dialysis lab also showed how glycerol "holds on" to water when in a salty environment. Also, other researchers' data shown in Graph C from Lesson 4 showed glycerol levels increase over time. The evidence supports the claim because it shows how the wild type worms can react to their environment.

    We think the mutant worms are reproducing on the high salt plates after 48 hours. We saw eggs, small and large larvae and adults that weren't there at the 15-minute observation. We also learned that worms go from eggs to adults in under 3 days. The evidence supports the claim because the only place the new life stages could come from is reproduction of the original worms.

Part 3: Gallery walk *(15 min.)*

15. When students have completed their models, including Claim—Evidence—Reasoning statements, give them a chance to view each other's work. This could be done through student presentations or through a gallery walk.

16. As a class, highlight some exemplars of thorough and thoughtful work.

Assessment: What can we learn from worms?

Class Resource

Observational Graphic Organizer

Time	Wild type on low salt	Wild type on high salt	Mutant on low salt	Mutant on high salt
15 min.				
24 hour				
48 hour				

Classroom Observational Graphic Organizer -- Teacher Resource

Sample responses are included below. After eliciting student observations for each condition, it may be helpful simplify the chart by recording whether or not the worms appear to be thriving (**T**), challenged (**C**), or some combination (**C/T**) in that situation.

Time	Wild type on low salt	Wild type on high salt	Mutant on low salt	Mutant on high salt
15 min.	Worms may be: • moving • traveling out of the drop site • in the food • eating • making worm tracks Eggs may be visible at the drop site	Worms may be: • not moving, or barely moving • curled up in a C shape • looking like a stick No sign of traveling to the food, eating, or making worm tracks. Worms may appear to be dead. Eggs may be visible at the drop site	Worms may be: • moving • traveling out of the drop site • in the food • eating • making worm tracks Eggs may be visible at the drop site	Worms may be: • moving, but slowly • traveling out of the drop site, but slowly • in the food • eating • making worm tracks Eggs may be visible at the drop site
24 hour	*All of the above, plus:* • More life stages may be visible • Eggs at the drop site have hatched • Eggs may be found outside of the drop site • Food is decreasing • Population is increasing	• Worms may be moving, especially the large worms. • Some adults may have reached the food and begun eating. • Smaller worms may still be curled up/stationary/dead	*All of the above, plus:* • More life stages may be visible • Eggs at the drop site have hatched • Eggs may be found outside of the drop site • Food is decreasing • Population is increasing	*All of the above, plus:* • More life stages may be visible • Eggs at the drop site have hatched • Eggs may be found outside of the drop site • Food is decreasing • Population is increasing
48 hour	*All of the above, plus:* • Food may be almost gone • Plate may be overgrown with worms of all life stages	Worms may be: • moving • traveling out of the drop site • in the food • eating • making worm tracks • reproducing (eggs laid)	*All of the above, plus:* • Food may be almost gone • Population may be increasing, but not at the same rate as the wild type worms	*All of the above, plus:* • Food may be almost gone • Population may be increasing, but not at the same rate as the wild type worms

Assessment Instructions

1. ***Review and Organize:*** Use an opened Manila folder or a piece of colored construction paper that is about 24 inches x 18 inches for the background of your model. You will need the front and back sides, and may need two folders or pieces of paper.

2. Cut out the worm plates given to you by your teacher. Glue the twelve worm plates on your sheet to represent the three observation times (15 minutes, 24 hours, 48 hours) for each of the two worm strains in both high and low salt environments. You may also use additional images or pictures of worm plates if available.

3. Incorporate your data tables into your model. You may cut them out to correspond with the paper worm plates.

4. Draw in worm images to represent what you observed on your plates at the three observation times. You may want to draw additional features on your plates, such as eggs or larvae.

5. Add any other information, observations, drawings, or notes to your model, if appropriate.

6. ***Supporting Claims:*** Use the *Claim and evidence cards* to practice generating claims supported by the appropriate evidence. You are using *reasoning* when trying to figure out how, or if, a claim is supported by certain type of evidence.

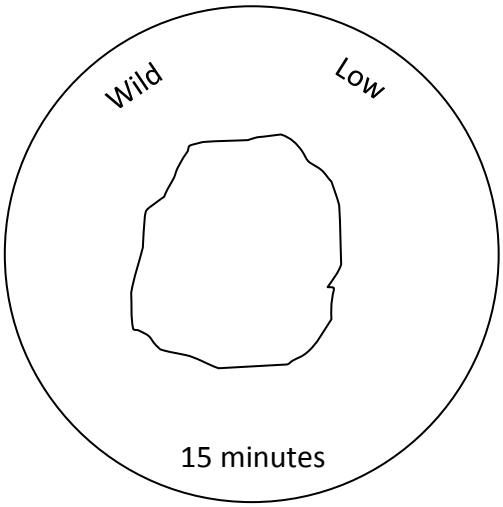
7. Using your model, provide a **C**laim-**E**vidence-**R**easoning (CER) statement for at least 2 things you understand about this lab using the following form:

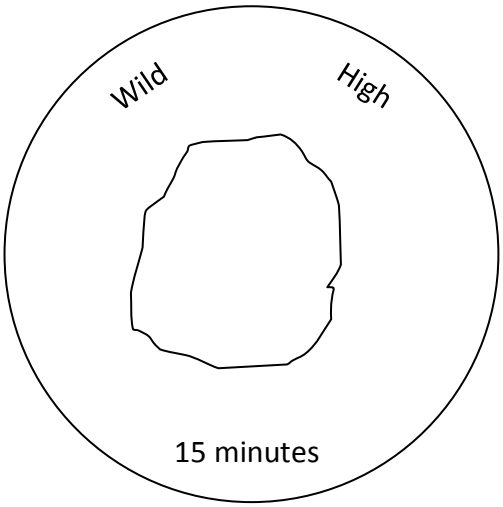
   We think [*insert your claim*] based on [*state your evidence*]. The evidence supports the claim because [*state your reasoning*].

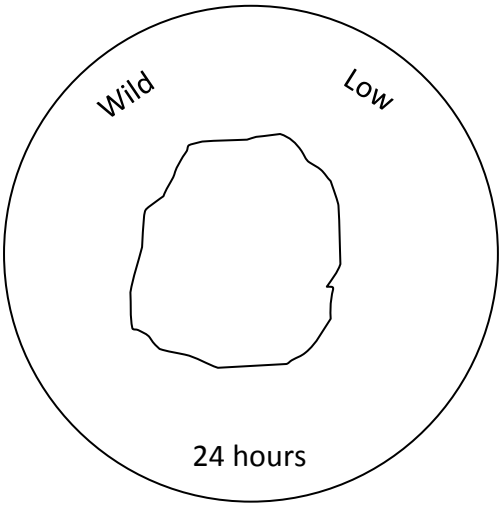
8. ***Sharing***: Prepare to share your model with your classmates.

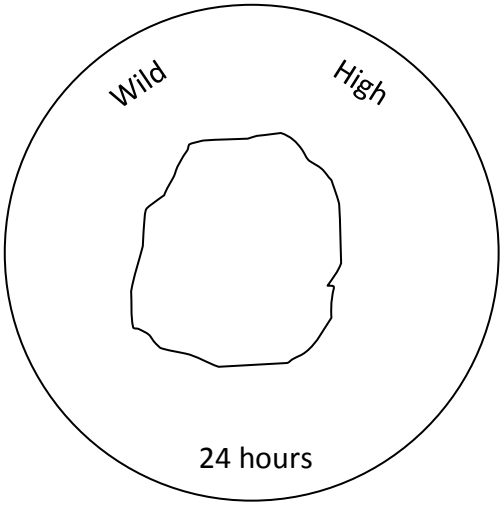
Worm Plates

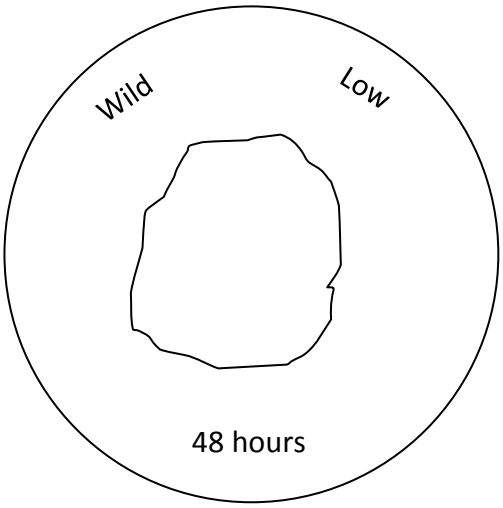
Directions: Cut out all 12 "worm plates" and arrange them on a piece of paper or on your table.

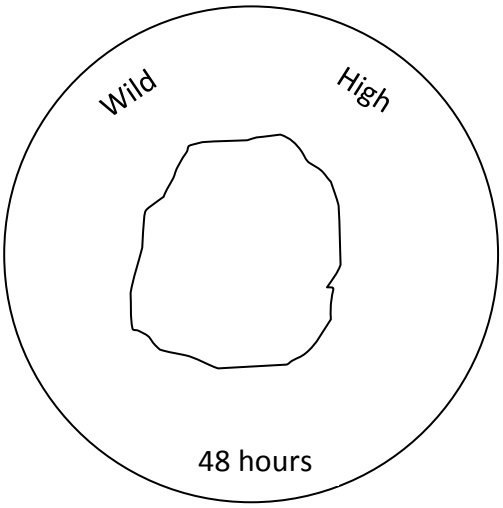


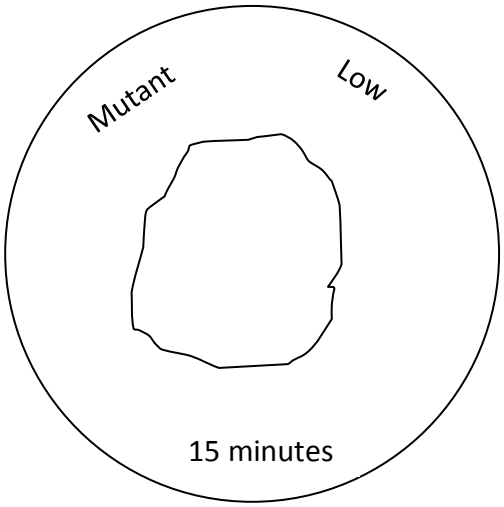


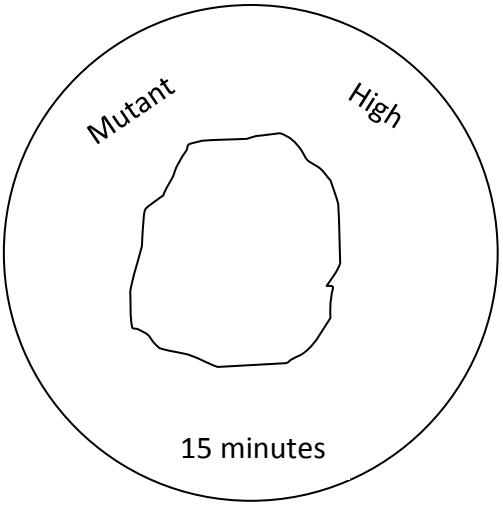


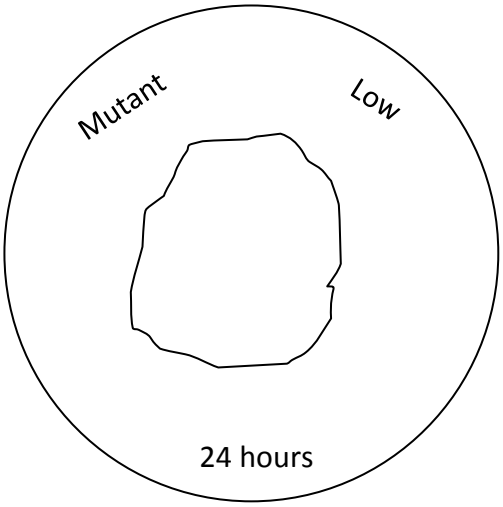


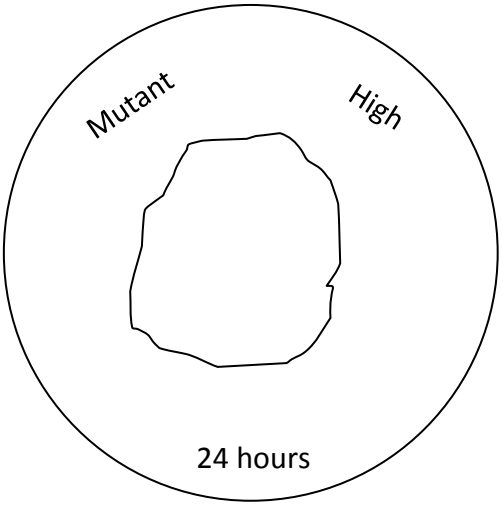


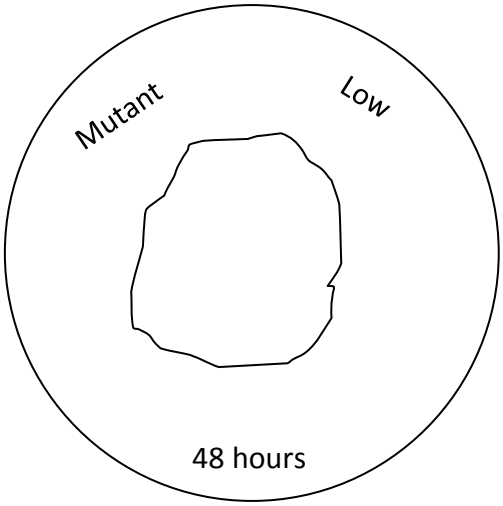


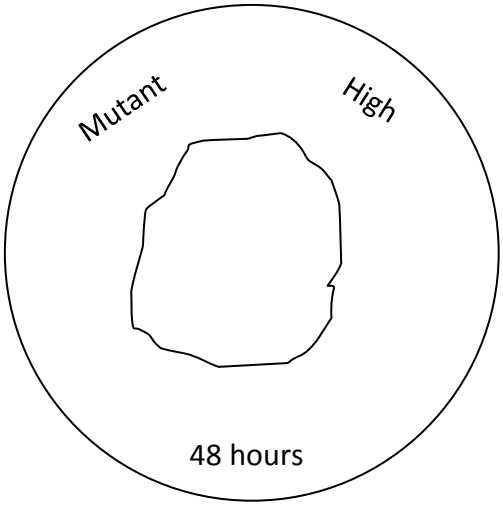












Assessment: What can we learn from worms?

Student Resource

Claim and Evidence Cards

Cut out the cards below. You may reproduce the cards and/or add new cards as you need.

CLAIM Worms are making low glycerol	CLAIM Worms are making higher glycerol	CLAIM Worms are thriving in this environment	CLAIM Worms are NOT thriving in this environment
CLAIM Worms are reproducing	CLAIM Worms are dead	CLAIM Worms are eating	CLAIM Student choice

Assessment: What can we learn from worms?

Student Resource

EVIDENCE Worms are not moving	EVIDENCE Worms are moving	EVIDENCE Data from the graphs in Lesson Four	EVIDENCE In mutant worms, a STOP codon effects the production of a protein involved in glycerol regulation
EVIDENCE Adults present	EVIDENCE Data from the dialysis tubing in salt lab	EVIDENCE Worms are desiccated (dried out)	EVIDENCE In wild type worms, a protein involved in glycerol regulation is functional

Assessment: What can we learn from worms?

Student Resource

EVIDENCE Eggs are present	EVIDENCE Life cycle stages Student Resource	EVIDENCE Worm tracks through the food	EVIDENCE Student choice
EVIDENCE No eggs present	EVIDENCE Food is no longer on plate	EVIDENCE No adults or larvae present	EVIDENCE Student choice

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.

✔	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 [1]	
	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[2]	L1, L2, L3, L4
	Disposable gloves, one pair per student	L1, L2, L3, L4
	Plastic sheet with 4 mm x 4 mm grid	L1

	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[3]	L3
	4 rubber bands	L3
	15 ml Low Glycerol[4] 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

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

2. Dissecting scopes are sometimes found in middle school classrooms, whereas high school classrooms often have compound microscopes. Teachers might consider borrowing scopes from their middle school colleagues, if possible.

3. 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

4. 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.

Safety:

This lab represents a Biosafety Level 1 risk for work involving well-characterized agents not known to consistently cause disease, and presents minimal potential hazard to laboratory personnel and the environment. (https://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_sect_iv.pdf)

Physical hazards include those involved with working with an open flame and hot metal spatulas.

Glycerol could be a mild irritant if applied directly to the skin or open wounds, and students should wear gloves when working with it.

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

Note: Seeded plates can be prepared 2 – 3 months ahead of time and stored in a refrigerator at 4°C until needed. Allow plates to come up to room temperature overnight before using them.

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	

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