Edvo-Kit # 852

Edvo-Kit #852

Chemotaxis: The Science of Attraction in *C. elegans*

Experiment Objective:

In this experiment, students explore the cellular, molecular and genetic mechanisms behind the *Caenorhabditis elegans* sensory nervous system by performing a chemotaxis assay in wild-type and mutant strain populations.

See page 3 for storage instructions.

Version 852.210812

EDVOTEK®

Table of Contents

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

	Component	Storage	Check ✓
A	Wild-type *C. elegans**	Room Temperature	❑
B	ODR-3 Mutant *C. elegans**	Room Temperature	❑
C	*E. coli* OP50 BactoBeads™	4 °C Refrigerator	❑
D	S-Buffer	4 °C Refrigerator	❑
E	NGM Salts	4 °C Refrigerator	❑
F	Sterile Water	4 °C Refrigerator	❑
G	Red Wine Vinegar	4 °C Refrigerator	❑
H	HCl	4 °C Refrigerator	❑
I	Imitation Vanilla	4 °C Refrigerator	❑
J	Pure Vanilla	4 °C Refrigerator	❑
K	Bleach	4 °C Refrigerator	❑
L	Sodium Azide	4 °C Refrigerator	❑
•	ReadyPour™ NGM Agar	4 °C Refrigerator	❑

Experiment #852 is designed for 10 groups.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

**Components A&B are live items that cannot be stored. Call 1-800-EDVOTEK to request these two weeks before the experiment's start date. Review page 18 for additional instructions.*

Reagents & Supplies *(included with this experiment)*

Store all components below at room temperature.

- Petri Dishes ❑
- Large Transfer Pipets (Sterile) ❑
- Small Transfer Pipets ❑
- 1.5 mL Microcentrifuge Tubes (Snap-top) ❑
- Counting Chambers ❑
- Sterile Loops ❑
- 15 mL Conical Tubes ❑
- 10 mL Pipet ❑

Experiment Requirements *(NOT included with this experiment)*

- Incubator, covered plastic box, or cardboard box (kit box will work as an incubator)
- Microscopes (10-40X magnification)
- Pipet pump or bulb
- Adjustable micropipets and tips (10-100 µL, 100-1000 µL) (optional)
- Marking pens
- Microwave or hot plate
- Distilled or deionized water
- Bleach solution or laboratory disinfectant
- Small spatulas or flat edge toothpicks
- Disposable laboratory gloves

Background Information

If you've ever forgotten to take out the garbage or driven by a landfill then you've experienced your chemosensory system in action. This system: (1) obtains detailed information about the environment by detecting molecules of diverse chemical structure and (2) determines an appropriate response to this mixture of signals. Our sense of smell and taste drives our food choices, warns us about potential dangers, and can even influence our memories and emotions.

Several systems within our body also rely on the ability of single cells to move toward or away from a chemical cue - a phenomenon known as chemotaxis. For example, white blood cells accumulate at the site of injuries and infections by tracking chemicals released by injured tissue and invading bacteria. Impaired chemotaxis has been linked with several health issues including multiple sclerosis, male infertility, Hodgkin disease, AIDS, Chediak-Higashi syndrome, and cancer. Scientist hoping to treat these and others conditions use the simple nervous system of *C. elegans* to explore the cellular, genetic, and molecular basis of chemotaxis.

WHY STUDY *C. elegans*?

A model organism is any plant, animal or microorganism that allows us to study fundamental questions in biology that may be hard to study directly in complex organisms like humans. In the 1970's, Dr. Sydney Brenner established the nematode *Caenorhabditis elegans* (see-no-rab-DITE-iss el-leh-GANS) as a model organism because they have a simple genome, a fast generation time, and are easy and inexpensive to maintain. While characterizing the worm, Brenner, along with Drs. John Sulston and Robert Horvitz, discovered that the developmental fate of every cell in the worm is invariable between animals. They also discovered key genes involved in organ development and programmed cell death. For this work, Brenner, Sulston and Horvitz were awarded the Nobel Prize in Physiology or Medicine in 2002.

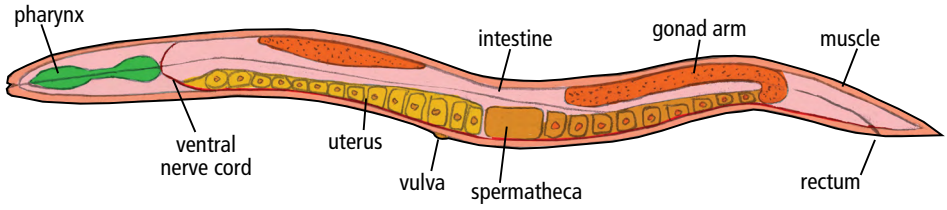


Figure 1: *C. elegans* anatomy

C. elegans have become important model organisms for the study of embryogenesis, morphogenesis, development, nerve function, behavior and aging, and how genes regulate these processes. The *C. elegans* genome has been completely sequenced, and several thousand genetic mutants are available for study. This allows scientists to correlate changes at the DNA level with changes in phenotype. Notably, by comparing DNA sequences, it was determined that over 35% of worm genes have human homologs. Many of these genes are important for human health and development.

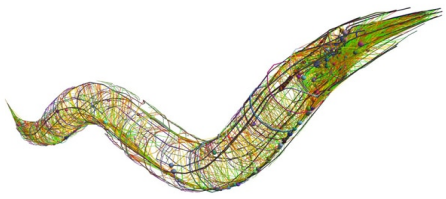


Figure 2: Neural anatomy of *C. elegans*. Source: OpenWorm project. Shared under an MIT License.

C. elegans is a free-living, non-parasitic nematode that lives in temperate soil, where it feeds on microbes that are found in decaying organic matter. Adult worms measure approximately one millimeter (mm) in length. The outer cuticle of *C. elegans* is transparent, making it easy to visualize the growth and development of internal structures like the pharynx, the intestine, the gonads and the muscles (Figure 1). The worm also has an extensive nervous system – in fact, the nervous system comprises almost 1/3 of the worm's 959 somatic cells (Figure 2)!

GROWTH & DEVELOPMENT IN *C. elegans*

There are two naturally occurring sexes in *C. elegans*. The vast majority of worms are self-fertile hermaphrodites, meaning that they produce both the sperm and the eggs used for reproduction. Free-living males represent <1% of the total nematode population. However, free-living males plus a hermaphrodite can produce over 1000 offspring in a generation; in contrast, self-fertilized hermaphrodite worms will produce about 300. Because their sperm will preferentially fertilize a hermaphrodite's eggs and produce more offspring, free-living males are often used to introduce specific genetic mutations into a worm population to be studied.

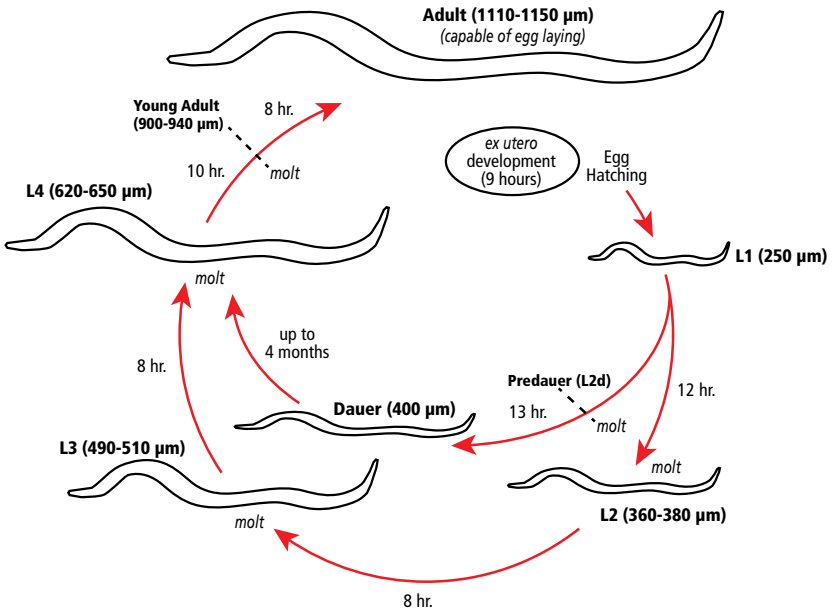


Figure 3: Life Cycle of *C. elegans*

C. elegans develop from embryo to adult in four days, allowing for rapid studies in the laboratory (Figure 3). The worms are grown on agar plates or in liquid culture, and they feed on *E. coli*. After being laid, the worm embryo will develop for approximately 14 hours before hatching. Juvenile worms progress through four larval stages (L1-L4) over the next two days, increasing in size with each stage. After the fourth larval molt (L4), the worms are reproductively mature, meaning that they can be used for further genetic studies.

SNIFFER WORMS - THE CHEMOSENSORY SYSTEM OF *C. elegans*

C. elegans have sophisticated sensory capabilities that allow them to navigate chemically diverse environments. They also use chemical cues to temporarily survive harsh conditions by going into an alternate low energy larval state. When finding food and avoiding danger, *C. elegans* maneuver using a biased random walk or pirouette model (Figure 4). Rather than moving directly toward or away from a chemical an individual will alternate between long forward movements and random changes in directions. In the presence of attractants, much more time is spent moving forward. In the presence of a repellent, more time is spent changing direction. While this movement may appear slow, it is actually well suited to the nematode's noisy and turbulent soil environments.

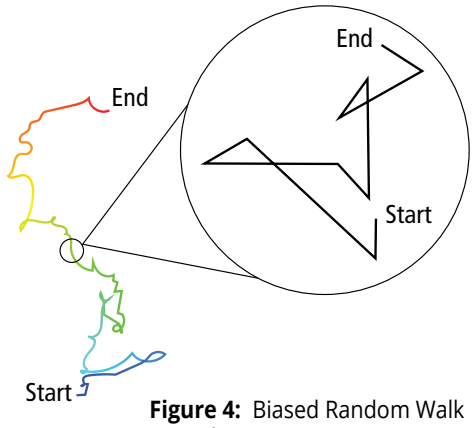


Figure 4: Biased Random Walk and Pirouette movement.

As soil-dwelling organisms, *C. elegans* have adapted to live in a dynamic habitat that is sometimes aquatic and other times terrestrial. Consequently, they can detect and respond to both volatile and water-soluble molecules. The latter (water soluble molecules) are used mainly for short-range navigation and particularly avoidance. While the former (volatile molecules) are used for longer range food searching. In general, *C. elegans* recognize a wider variety of volatile odorants and are more sensitive to volatile signals. Still, in experiments, *C. elegans* have shown chemotaxis in modest gradients of both types of cues and overall scientists have documented unique responses to at least 40 different chemicals (Table 1). Scientists have also observed changes in chemical preferences that suggest imprinting, associative learning, and long-term memory. All these behaviors are controlled by the 302 neurons that make up the *C. elegans* nervous system.

Each neuron within *C. elegans* has a defined role, shape, and position. Moreover, these features are invariant – they are the same between individuals of different populations and generations. Over the last 30 years scientists have determined the locations of every neurons as well as the roughly 7,000 connections between them. This has been translated into a comprehensive neural map, or connectome (Figure 2). Investigators have also classified each neuron by task: (1) sensory neurons that collect information from the environment, (2) motor neurons that control muscles, and (3) interneurons that connect sensory and motor neurons. While all three neural types are involved in chemotaxis, most research has focused on the biology of sensory neurons.

TABLE 1: Chemicals recognized by C. elegans

	Volatile	Water Soluble
Attractant	Alcohol, Ketones, Diketones, Esters, Pyrazines, Thiazoles, Aldehydes, Aromatics, Ethers.	Basic pH, Lysine, Histidine, Cysteine, Serotonin, Biotin, cAMP, cGMP, Cl-, SO4-, NO3-, Br-, I-, Na+, K+, Li+, Ca++, Mg++
Repellant	Heptanol, Octanol, Nonanol, Benzaldehyde, Thrimethylthiazole, Ethyl Hepanoate	Acidic pH, Heavy Metals, SDS, Tryptophan

C. elegans has 129 sensory neurons out of which 32 focus primarily on chemical stimuli. These chemosensory (CS) neurons are primarily organized into left/right pairs that cluster around two small openings at the head (the amphid and labial) and one at the tail (the phasmid). Here, molecules from the outside environment attach to receptors in the neuron's exposed tip. This activates ion channels that in turn change the neuron's membrane potential and eventually triggers the release of neurotrasmitters. This process is known as signal transduction. The morphologies of CS neurons are adapted to their function. Most CS neurons have many hair-like organs, called cilia, at one tip in order to maximize environmental contact and a high concentration of presynaptic connections at the other tip in order to send out multiple chemical messages.

ODR-3, G PROTEINS, AND THE GENETICS BEHIND CHEMOSENSATION

Compared to other organisms *C. elegans* has a small number of CS neurons and yet is able to detect a diversity of chemicals and respond in complex ways. This is because each neuron expresses a large and unique subset of signal transductions molecules. Signal transduction molecules enable the transmission of a molecular signal from a cell's exterior to its interior and include transmembrane receptors, g proteins, ion channels, and regulators. Genomics studies suggest that the *C. elegans* genome contains over 1,500 genes that code for signal transduction molecules and other chemosensory related proteins. In this experiment, you will examine one of these – the *odr-3* gene.

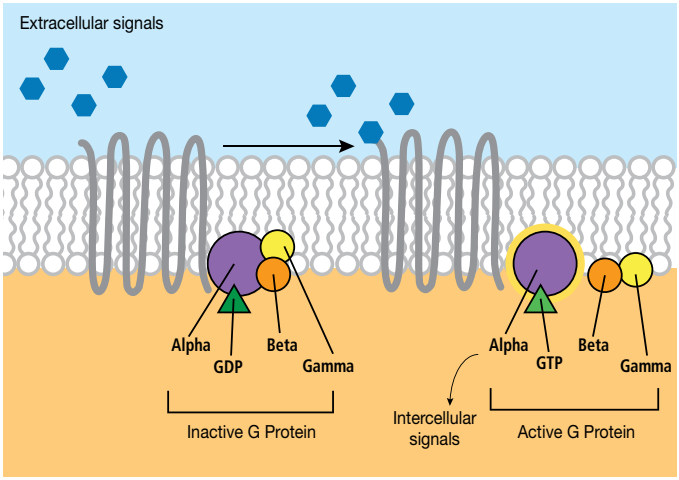


Figure 5: G Proteins

The *odr-3* gene encodes part of a Guanine nucleotide-binding (G) protein (Figure 5). G proteins consist of three subunits (alpha, beta, and gamma) that together act as molecular switches within cells. When "off" the three subunits plus a GDP molecule are bound together. When "on" the GDP is exchanged for a GTP molecule and the beta and gamma units separate from the alpha unit. An "on", or active, G protein can trigger a cell to produce thousands of secondary messenger molecules that in turn create a large and diverse cellular response. Eventually the GTP degrades into GDP, and this switches the G protein back to its "off" configuration.

Expressions studies in *C. elegans* indicate that the protein ODR-3 is active in several CS neurons including AWC, AWA, AWB, ASH, and ADF. Worms with *odr-3* mutations show reduced responses to volatile and waters soluble odorants as well as dysfunctional osmotic and touch avoidance. In addition, the shape of certain neurons or neural cillia are malformed in worms with *odr-3* mutations.

In this experiment, you will use both volatile (olfactory) and water-soluble (gustatory) chemical cues to compare the responses of *C. elegans* mutants with a partial loss of function odr-3 gene and wild-type *C. elegans*. To do this you will use a two-quadrant chemotaxis assay. In this type of assay, a population of worms is introduced to the center of an agar plate that has been previously spiked with a test compound and a control compound at polar ends. (In some cases these compounds will be mixed with an undetectable anesthetic like Sodium Azide to immobilize individuals once they have navigated towards a side.) The response of each strain to the test chemical will be quantified by calculating the difference between how many worms move towards the attractant/repellent versus the control.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students explore the cellular, molecular and genetic mechanisms behind the *Caenorhabditis elegans* sensory nervous system by performing a chemotaxis assay in wild-type and mutant strain populations.

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. Answer the prelab questions (below).
3. Write a hypothesis that reflects the experiment and predicts experimental outcomes.

Module I:
Preparation of *C. elegans* Food Source

Time Requirements:
10 minutes plus
1 day incubation.

Module II:
Cultivation of *C. elegans*

Time Requirements:
15 minutes plus
3+ day incubation.

Module III:
Collection of *C. elegans* and Chemotaxis Assay

Time Requirements:
70-95 minutes plus
overnight incubation.

PRELAB QUESTIONS

1. How do you expect individuals with the *odr-3* mutation to perform in the chemotaxis assay as compared to wild-type individuals?

2. Brainstorm ways that this experiment is similar to and different from conditions that worms would experience in a natural soil environment.

3. Predict how wild-type individuals might react to a solution that was a mixture of attractants and repellents. What about mutant individuals?

LABORATORY NOTEBOOKS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before Starting the Experiment:
- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:
- Record (draw) your observations, or photograph the results.

After the Experiment:
- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment was repeated.
- Write a hypothesis that would reflect this change.

Laboratory Safety

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!


Wear gloves and safety goggles

1. Wear gloves and goggles while working in the laboratory.
2. Exercise caution when working in the laboratory – you will be using reagents that can be dangerous if used incorrectly.
3. Neither the *E. coli* nor the *C. elegans* used in this experiment are considered pathogenic. Regardless, it is good practice to follow simple safety guidelines in handling and disposing of materials contaminated with living organisms.

   A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.

   B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with living organisms should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:

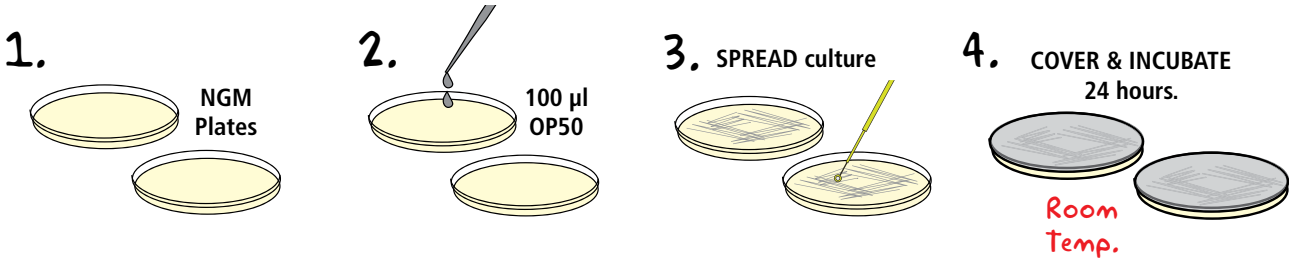
      - Autoclave at 121 °C for 20 minutes.
        Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.

      - Soak in 10% bleach solution.
        Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves, goggles, and a lab coat when working with bleach.

   C. Wear gloves, and at the end of the experiment, wash hands thoroughly with soap and water.

Module I: Preparation of *C. elegans* Food Source ("Seeding" the Plates)

In this module, you will seed two petri plates with a favorite *C. elegans'* food (*E. coli* OP50 strain bacteria).



1. **OBTAIN** two Nematode Growth Medium (NGM) plates, the OP50 culture, a small transfer pipet, and a sterile inoculating loop from your instructor. **LABEL** the bottom of both plates with your group number or names.

2. While maintaining sterile technique, **ADD** two drops (100 µL) of OP50 culture to each plate.

3. Using the loop, **SPREAD** the culture over the entire surface of the NGM plates. **COVER.**

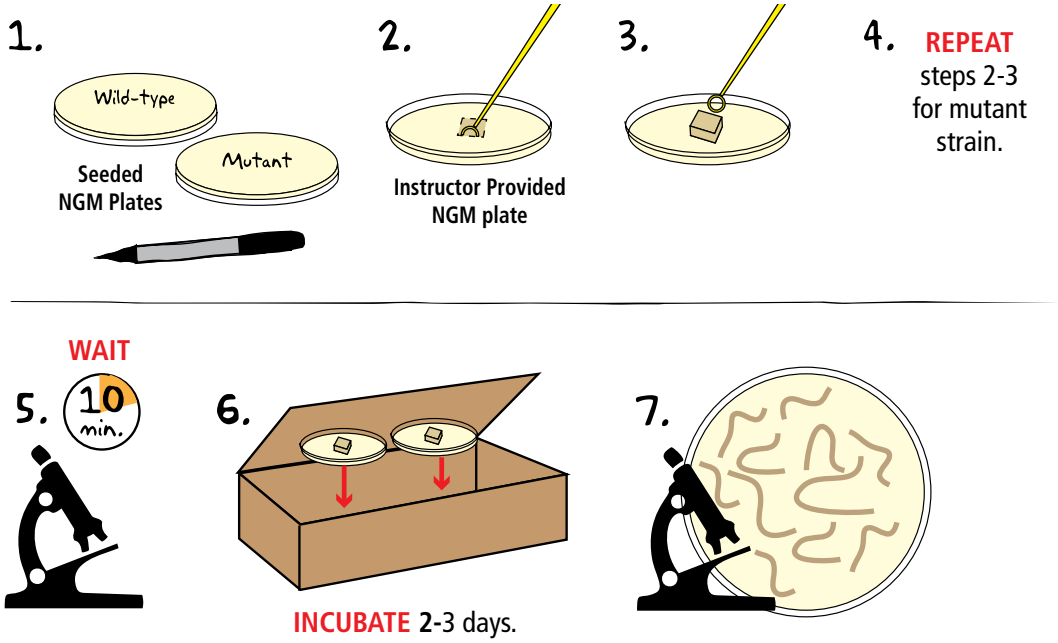
4. **INCUBATE** the plates inverted and at room temperature for 24 hours.
NOTE: Seeded OP50 plates can also be prepared overnight (~12 hours) by incubating at 37 °C.

HINTS for Step 3:

- Avoid gouging or scratching the agar surface as this can affect visibility as well as worm movement.
- Widely spreading the bacteria creates a larger lawn for the worms but stop just before the plate's edge. This discourages the worms from crawling up the plate's sides and drying out.

Module II: Cultivation of *C. elegans* ("Chunking" the Plates)

In this module, you will be "chunking" your *C. elegans* strains. This means that you will transfer wild-type and ODR-3 mutant *C. elegans* to the plates you prepared in Module I. Over the next few days, both strains will establish new populations in their individual plates. Once these populations have expanded to at least 50 individuals you will continue to Module III.



1. **LABEL** the bottom of the seeded NGM plates (from Module I) with "Wild-type" or "Mutant".
2. Your instructor will have NGM plates containing wild-type or mutant *C. elegans*. Using a sterile loop, **CUT OUT** a small portion of the NGM plate containing the wild-type strain (~1 cm square). Make sure to completely cut the agar by pushing the loop all the way to the bottom plate.
3. **REMOVE** the "chunk" from the plate. **PLACE** the chunk, worm side down, in the center of the Module I Wild-type plate.
4. With a new sterile loop, **REPEAT** step 2 and 3 for the mutant strain.
5. After 5-10 minutes, use a microscope to **CONFIRM** the presence of *C. elegans* on the "chunked" plates.
   NOTE: Occasionally, a "chunk" is transferred nematode side up. Worms in this position will eventually migrate to the plate. If the "chunk" is incorrectly positioned, keep the plate but wait 1 hour before confirming the presence of C. elegans and continuing on to Step 6.
6. **COVER** and **PLACE** the plates into a cardboard box. **INCUBATE** at room temperature for 2-3 days.

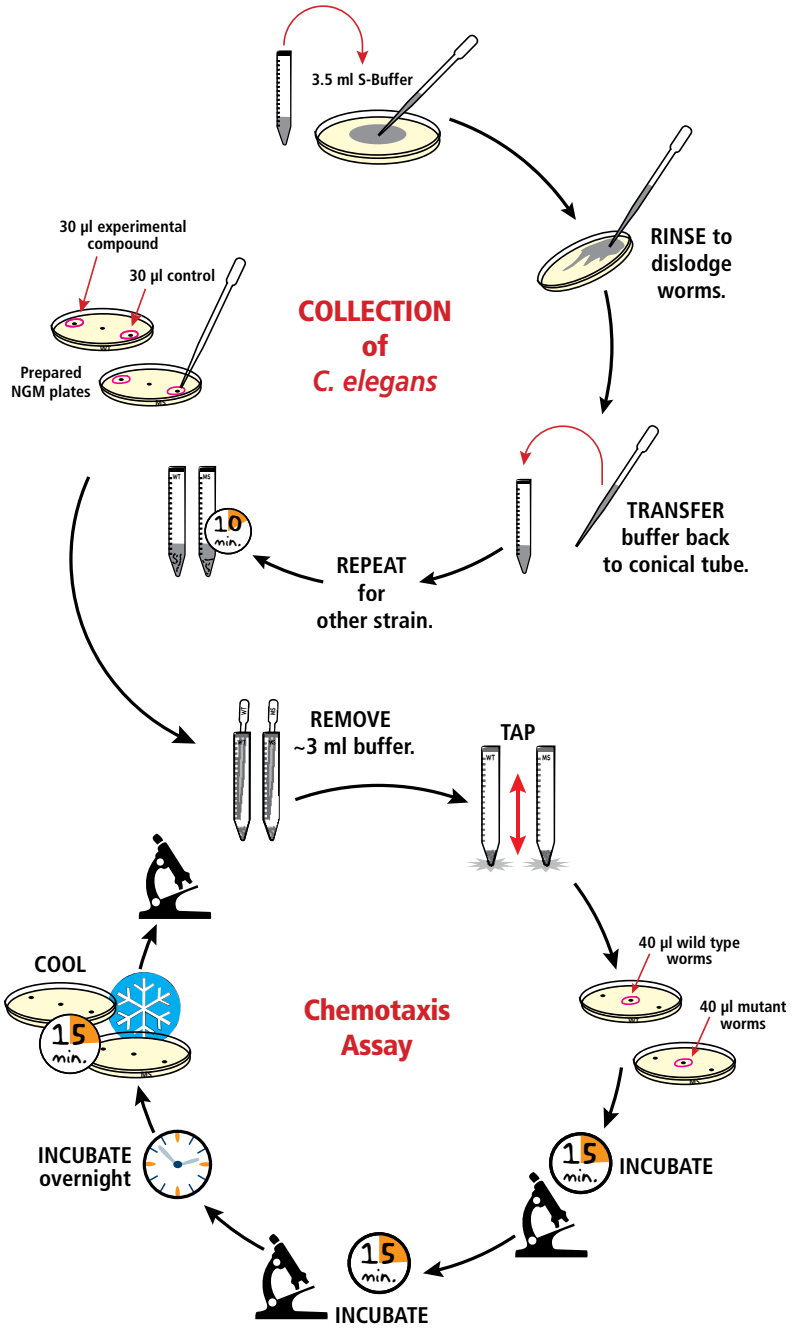
OPTIONAL STOPPING POINT:
Plates may be stored for up to a week but need extra OP50 to avoid drying and to feed the growing population. See Appendix A.

7. **CHECK** growth of *C. elegans* under a microscope. If the plate contains 50 or more worms, proceed with Module III. If the plate contains fewer than 50 worms, continue incubating at room temperature.

HINT for Step 7:
To quickly confirm worm numbers, divide the plate into quarters. If you see 12 or more in the first quarter, the plate is ready.

Module III Overview

In this module, you will first harvest your *C. elegans* populations and prepare a concentrated solution of worms and buffer. You will also prepare chemotaxis plates. Next, you will observe both strains' movement responses to potential attractants/repellents as well as to a neutral control.



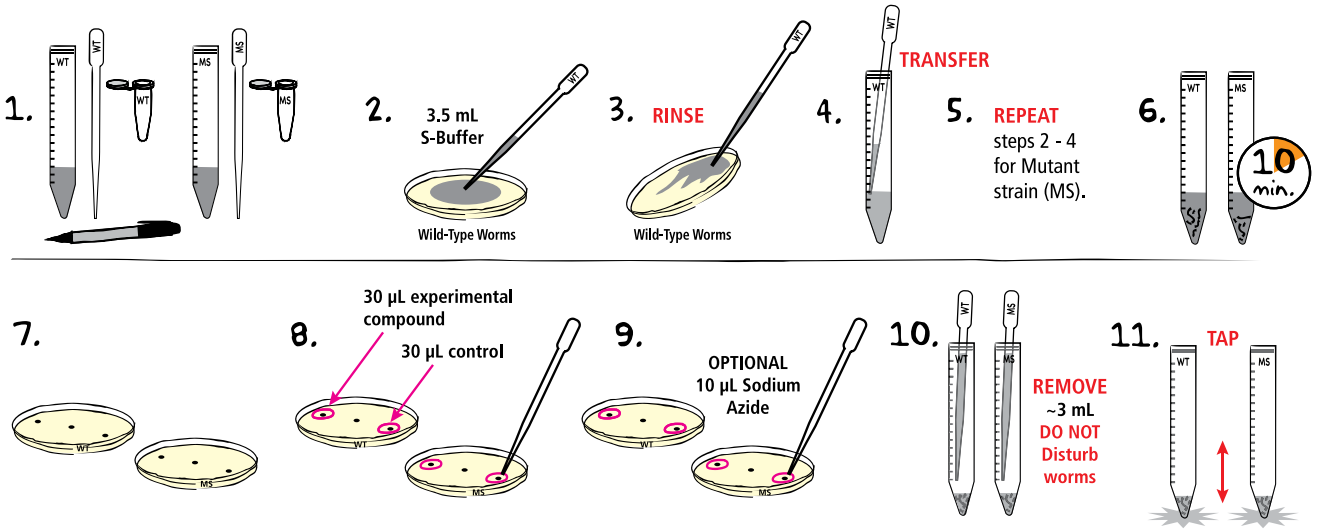
Harvesting and Plate Preparations
Time Requirements: 25 minutes

Assay Set-Up and Optional Time Series
Time Requirements: 15 - 60 minutes

Incubation
Time Requirements: Overnight

Final Count
Time Requirements: 30 minutes

Module III: Collection of *C. elegans* and Chemotaxis Assay



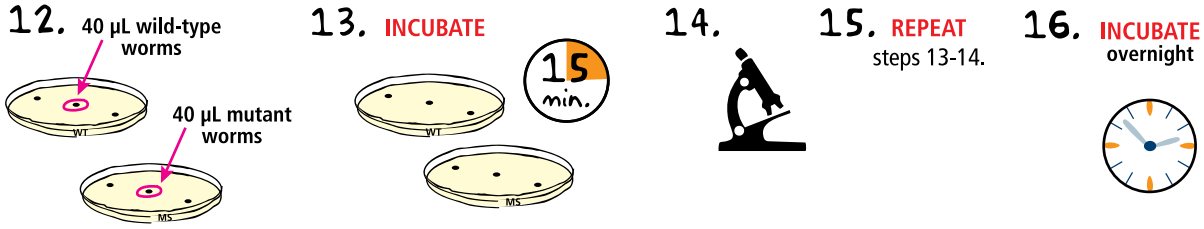
1. **LABEL** one 15 mL tube of S-Buffer, a large transfer pipet, and a snap top tube with "WT" (Wild-Type) and the other 15 mL tube, large transfer pipet, and snap top tube with "MS" (Mutant Strain).
2. Using the WT transfer pipet, **TRANSFER** 3.5 mL of S-Buffer from the WT conical tube to the Petri dish containing the wild-type *C. elegans*.
3. **DISLODGE** worms by rinsing the dish several times. Rinse the dish by either (a) swirling the plate or (b) holding the plate at a slight angle and allowing the buffer to collect near the bottom. Next suck up the buffer using the transfer pipet and then expel the buffer near the top so that it runs down the plate.
4. Once most worms are suspended in the buffer, **TRANSFER** the worms and buffer back to the WT 15 mL conical tube using the WT transfer pipet.
5. **REPEAT** steps 2-4 for mutant *C. elegans* using the MS labeled items.
6. Keep the conical tubes still and upright to **ALLOW** the worms to settle to the bottom of the tubes (~10 minutes). While you wait, **CONTINUE** to steps 7, 8, and 9.
7. **PREPARE** two NGM plates for the assay using the template provided (see Figure 6, page 15).
   a. **TRACE** the two inner lines and then use a well cutter to punch the three holes.
   b. **REMOVE** the NGM plugs from each hole with a flat edged toothpick or spatula.
   c. **LABEL** each plate with either "WT" or "MS" on the side and on the lid.
8. To both assay plates, **ADD** 30 µL of your experimental compound to the left well and 30 µL of the control solution to the right well. **RECORD** which chemical compound was used here and in your lab book.

   Experimental Compound: _____.

9. (OPTIONAL) **ADD** 10 µL of Sodium Azide to the two outer wells of both plates. ***BE CAREFUL AND WEAR GLOVES WHEN HANDLING BOTH THE SODIUM AZIDE TUBE AND THE PLATES WITH SODIUM AZIDE.***
10. Using the appropriately labeled transfer pipets, slowly **REMOVE** ~3 mL of the cleared S-Buffer supernatant without disturbing the worms that have settled to the bottom of the tubes. Between 300 µL and 500 µL of buffer with worms should remain at the bottom of both tubes.
11. **TAP** both tubes several times to resuspend the worms.

continued

Module III: Collection of *C. elegans* and Chemotaxis Assay, continued



12. **ADD** 40 µL of the wild-type worms to center hole of the wild-type plate. With a new small transfer pipet or pipet tip **REPEAT** for the mutant strain.

13. Allow the plates to **INCUBATE** face up on your bench at room temperature for 15 minutes.

14. **OBSERVE** the movement of the worms under the microscope and count the number of worms in the Experimental (left) and Control (right) areas. **RECORD** these numbers in Table 2.

    NOTE: By 15 minutes, worms should be visible and moving across the plate surface. If there are no worms on the NGM agar, examine the central well. Surface tension may be keeping the worms caught within this well. If so, carefully use a kimwipe to touch the edge of the drop and wick away excess liquid.

Table 2

Wild-Type

	# of Worms in Experimental Area	# of Worms in Control Area
15 min.		
30 min.		
45 min.		

Mutant Strain

	# of Worms in Experimental Area	# of Worms in Control Area
15 min.		
30 min.		
45 min.		

15. (Optional) **CREATE** a time series by repeating step 13 and 14 two more times.

16. **INCUBATE** worms overnight.

continued

Module III: Collection of *C. elegans* and Chemotaxis Assay, continued

17. **COOL** both plates to 4 °C by placing in fridge for 15 minutes. This will further slow worms and help with counting.

18. **COUNT** the final number of worms in the experimental and control areas. Also count the worms in the middle section of the plate. Worms can be counted under the microscope. Alternatively the location of each worm on the plate can be marked with a permanent marker under the microscope and the dots counted by eye at a later point.

    Number of wild-type worms near experimental compound: _____

    Number of wild-type worms near control: _____

    Number of wild-type worms in middle section: _____

    Number of mutant strain worms near experimental compound: _____

    Number of mutant strain worms near control: _____

    Number of mutant strain worms in middle section: _____

19. **CALCULATE** a chemotaxis index (CI) for each plate:

    $$CI = \frac{(\#\text{ of worms in experimental area} - \#\text{ of worms in control area})}{(\#\text{ of worms in experimental area} + \#\text{ of worms in control area} + \#\text{ of worms in middle section})}$$

    CI Index for Wild Type worms: _____

    CI Index for Mutant Strain worms: _____

    NOTE: A chemotaxis index close to 1 indicates that the chemical is a strong attractant while a chemotaxis index close to -1 indicates that it is a strong repellent.

20. (Optional) **SHARE** your group's results from steps 18 and 19 with the class in order to generate a classroom data set of *C. elegans* responses to multiple chemicals.

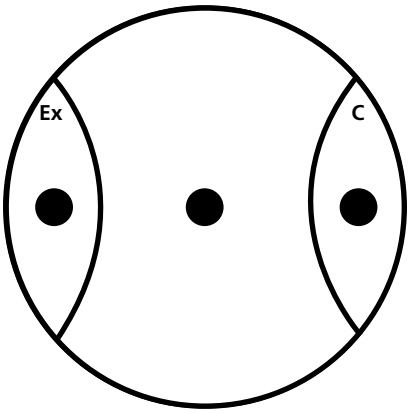


Figure 6:
Petri Template

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. What is a model organism?
2. What are some advantages to using *C. elegans* as a model system?
3. What is chemotaxis? Provide an example of chemotaxis at the organism level and at the cellular level?
4. How might a mutation in a gene coding for a G protein interrupt signal transduction? And how might this effect chemotaxis in *C. elegans*?

Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION:

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity.

Preparation For:	What to do:	When:	Time Required:
IMPORTANT: Arrange for live item delivery of C. elegans approximately 2 weeks before Module I.			
MODULE I	Prepare NGM Agar Plates	Up to a week before the class period of Module I	45 min.
	Prepare OP50 culture	Up to a week before the class period of Module I	15 min.
	Distribute plastics and reagents	The day of the class period of Module I	10 min.
MODULE II	Prepare microscopes with 20-40X magnification	Before Module II	Varies
	Distribute plastics and reagents	Up to 2 days before the class period of Module II	10 min.
MODULE III	Prepare microscopes with 20-40X magnification	Before Module III	Varies
	Aliquot and distribute reagents	Up to 2 days before the class period of Module III	15 min.

Suggested Implementation Schedule

Two Weeks Before Module I: Call Edvotek to request that live items be delivered. (This is also a great time to run through your experiment's time line with one of our scientists!)

Before Module I: Inspect and monitor *C. elegans* plates. Prepare NGM plates and OP50 culture.

Day 1: Students "seed" plates (Module I).

Day 2: Students "chunk" *C. elegans* (Module II).

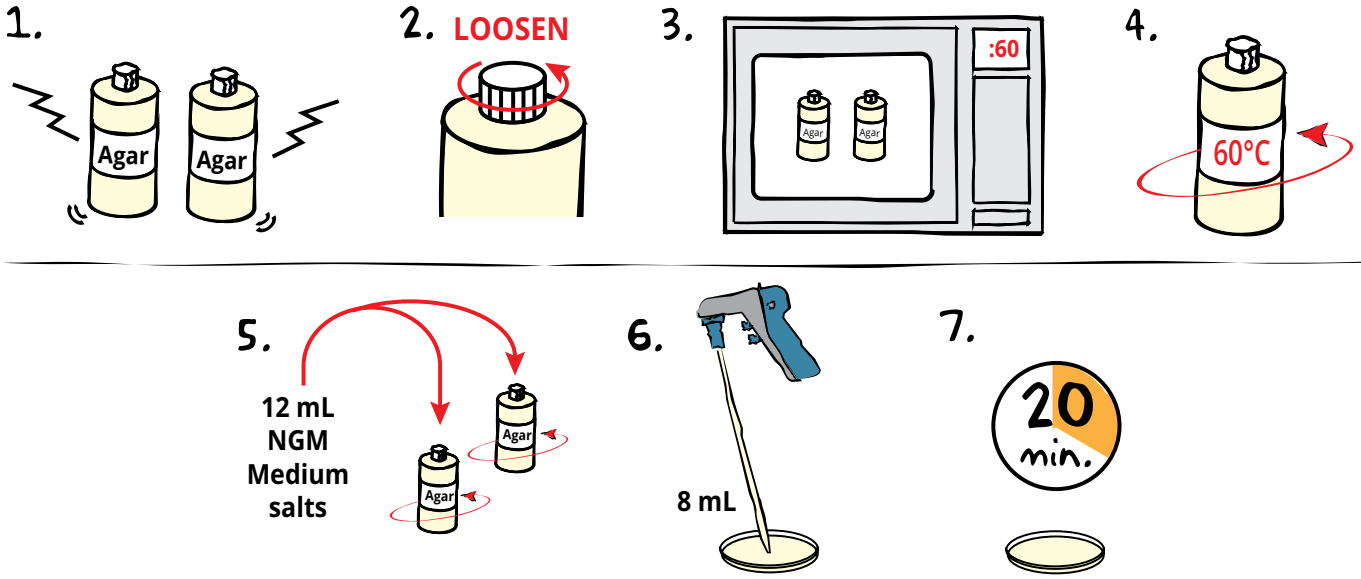
Day 4/5*: Students set up chemotaxis assay, observe initial worm movement, and incubate overnight (Module III).

Day 4/5*: Students count the final number of individuals in the experimental and control areas (Module III).

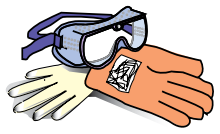
**This experiment works best when each plate has a population of ~50 C. elegans. This usually takes 2-3 days following chunking (Module II). If this does not work with your class schedule you can store chunked plates for up to a week but will need to add extra OP50 to feed the growing populations and to delay plate drying. Alternatively, Module I and II can be incorporated into the prelab. In this case, students will only need a single class period for this experiment.*

Pre-Lab Preparations

POURING NGM PLATES



1. **BREAK** solid ReadyPour™ medium into small chunks by vigorously squeezing and shaking the two plastic bottles.
2. **LOOSEN**, but DO NOT REMOVE, the caps on the ReadyPour™ medium bottles. This allows the steam to vent during heating. *CAUTION: Failure to loosen the cap prior to heating may cause the bottle to break or explode.*
3. **MICROWAVE** the ReadyPour™ medium on high for 60 seconds to melt the agar. Carefully **REMOVE** the bottles from the microwave and **MIX** by swirling the bottles. Continue to **HEAT** the solution in 30-second intervals until the agar is completely dissolved (the solution should be free of small particles).
4. **COOL** the ReadyPour™ to 60 °C with careful swirling to promote even dissipation of heat.
5. **ADD** 12 mL of NGM Medium Salts (Component E) to each bottle. **RECAP** the bottles and **SWIRL** to mix the reagents. ONLY ADD REAGENTS TO COOLED MEDIUM.
6. Using a fresh 10 mL pipet, **POUR** 8 mL of the medium into 44 small petri plates. **COVER**. If you have additional petri plates around the lab consider pouring extra plate. These can be used for additional projects (see Openings for inquiries). One bottle of ReadyPour™ NGM Agar can make ~28 plates.
7. **WAIT** at least twenty minutes for the agar to solidify. For optimal results, leave plates at room temperature overnight.
8. If you are using plates within 2 days, **STORE** them at room temperature. Otherwise, **STORE** plates in the refrigerator (4 °C) inverted and in a sealable plastic bag in order to prevent drying out. You will need 20 plates for Module I and an additional 20 plates for Module III.



Wear Hot Gloves and Goggles during all heating steps.

Pre-Lab Preparations

TAKING CARE OF *C. elegans* LIVE CULTURES

1. **CALL** and **ORDER** components A and B (Wild-type and Mutant *C. elegans*). Strains are sent on NGM petri plates with the worms still feeding on *E. coli* OP50. Plates are sealed with parafilm and with strain information attached to each plate. **KEEP** plates sealed for as long as possible as this minimizes the dual risks of contamination and drying. It takes 2-5 days for a *C. elegans* request to be processed, filled, and delivered.
2. Upon arrival **STORE** plates at room temperature. *C. elegans* can be maintained at wide range of temperatures (16 °C to 25 °C). However, temperature effects growth rate and food consumption - populations at 25 °C grow twice as fast than those at 16 °C! In this experiment, time estimates and feeding instructions assume a ~20 °C environment.
3. Before Module II, **INSPECT** both plates under a microscope to confirm that there are at least 50 *C. elegans* on each plate. **KEEP** plates covered as much as possible to reduce contamination risks.

PREPARE AND ALIQUOT REAGENTS FOR MODULE I

NOTE: The OP50 culture can be prepared ahead of time and stored in the refrigerator for several days.

1. **PREPARE** 0P50 culture by combining 3 mL of Sterile Water (Component F) with three OP50 BactoBeads™ (Component C) in a 15 mL conical tube.
2. **MIX** and **INCUBATE** at room temperature for 10 minutes.
3. **LABEL** ten snap-top tubes "OP50 culture" and aliquot 250 µL of the classroom OP50 culture to each group.
4. If NGM plates were refrigerated, **REMOVE** from the refrigerator and let sit for at least 30 minutes at room temperature.
5. **DISTRIBUTE** an OP50 tube, two NGM plates, a sterile loops, and a small transfer pipet or fixed volume pipet to each group.

PREPARE AND ALIQUOT REAGENTS FOR MODULE II

1. **PROCURE** and set up microscopes. We suggest top illumination or bright field microscopes with 20-40X magnification. (These microscopes will also be required for Module III.)
2. **INSPECT** wild-type and mutant strain plates.
3. **PREPARE** incubation oven or box(es) for student's plates.
4. **DISTRIBUTE** two sterile loops to each group.

PREPARE AND ALIQUOT REAGENTS FOR MODULE III

1. **ALIQUOT** 3.5 mL of S-buffer (Component D) to twenty 15 mL conical tubes. These tubes will also be used to hold collected *C. elegans* so remind students not to dispose of them once the S-buffer is used.
2. **DISPENSE** 70 µL of Red Wine Vinegar (Component G) into two snap top tubes and **LABEL**. **REPEAT** for HCl (Component H), Imitation Vanilla (Component I), Pure Vanilla (Component J), and Bleach (Component K). These ten tubes can be distributed at random to each student group. Alternatively, enough solution is provided that each group can request a specific chemical compound. ***NOTE: This kit provides five different odorants but the experiment set up allows for any safe chemical or combination of chemicals to be tested. Encourage students to choose and then bring in their own experimental compound.***
3. **DISPENSE** 120 µL of S-buffer into ten snap top tubes and **LABEL** "Control".
4. (Optional) **DISPENSE** 50 µL of Sodium Azide (Component L). ***CAUTION: Sodium Azide in an anesthetic that slows C. elegans movement and helps with counting. However, even in dilute form, Sodium Azide is toxic. Students must wear gloves when handling this chemical.***
5. **DISTRIBUTE** two additional NGM plates, 1 well cutter, micropipets or 2 large and 5 small transfer pipets, the chemical and control solutions, and Sodium Azide (optional).

Pre-Lab Preparations

ADJUSTABLE PIPETTE ALTERNATIVES

Students in this experiment will need to aliquot solutions in the following volumes: 10 µL, 30 µL, 40 µL, 100 µL, 3 mL, and 3.5 mL. Adjustable pipettes can provide easy transfer and are by far the most accurate option. However, absolute precision is not required for this bioassay. An alternative is for students to use small and large transfer pipets (Figure 7). Students can use the highlighted volumes to estimate an approximate height for their experimental volumes. Have students mark these heights with a permanent pen. Similarity between sample measurements is still very important.

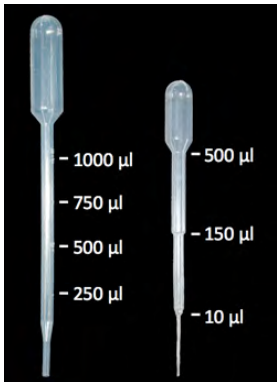


Figure 7: Transfer pipets.

Openings For Inquiry

This experiment is designed for each student group to have similar experimental conditions. An alternate approach is for each student group to design and perform their own investigation. The material and information provided here can be used as a starting block for such open-ended experiments. Examples of possible investigations are:

- Use Table 1 in the background to find and test additional chemicals. Or have students propose potential attractants and repellents based on *C. elegan's* primary food choice (bacteria) and the potential dangers of a soil environment.
- Add additional wells and test what happens when individuals must respond to multiple signals from different directions.
- Assign worms on the chemotaxis plate to a size category and use this to approximate if chemotaxis varies across different age groups.
- Add a small amount of an attractant/repellent to the petri dish in Module II in order to test if worms cultured in the presence of the chemical become desensitized.

A full inquiry lab may require additional time for students to formulate their question, redesign the experiment, and interpret the results. Additional material may also be needed. If you have any questions, please contact us!

Please refer to the kit insert for the Answers to Study Questions

Experimental Results and Analysis

Below are representative results from in-house testing after overnight incubation. This test was performed without Sodium Azide. Your results will vary based on a number of factors.

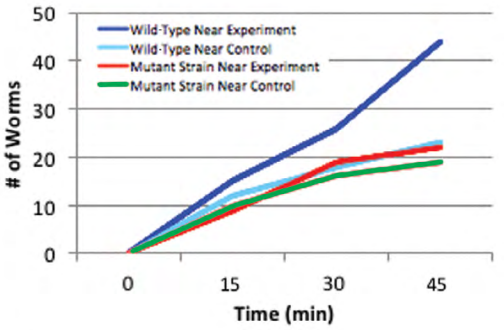
		# of Worms in Experimental Area	# of Worms in Control Area	# of Worms in Middle Section	Chemotaxis Index (CI)
Red Wine Vinegar	Wild-Type	54	10	11	0.59
	Mutant Strain	35	25	19	0.13
HCl	Wild-Type	12	45	17	-0.45
	Mutant Strain	15	33	30	-0.23
Imitation Vanilla	Wild-Type	33	15	28	0.24
	Mutant Strain	25	10	38	0.21
Pure Vanilla	Wild-Type	20	19	35	0.01
	Mutant Strain	17	18	38	-0.01
Bleach	Wild-Type	43	12	20	0.41
	Mutant Strain	19	21	34	-0.03

Red wine vinegar is a strong attractant for the Wild-type and a weak attractant for the Mutant Strain due to the fermented alcohol and aromatics in the solution. In contrast, the other provided acid, HCl, will be a strong repellent for the wild-type and a weak repellent for the mutant strain because of its low pH. Imitation vanilla has aromatics, alcohols and aldehydes which makes it attractive to the wild-type but not the mutant strain. Pure vanilla has a complex array of attractive and repellent odors. As a result wild-type worms tend to show no net movement toward the sample. The mutant strain cannot detect most of these chemicals and so also display neutral movement. Bleach is a strong attractant to the wild-type because of its high pH. However, the mutant strain is unable to detect this signal and so has a neutral response.

Experimental Results and Analysis

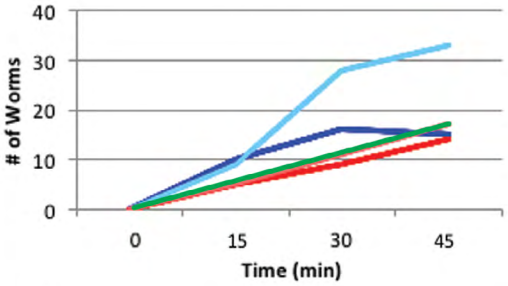
Optional Time Series Data - Vinegar

	Wild-Type Near Experiment	Wild-Type Near Control	Mutant Strain Near Experiment	Mutant Strain Near Control
0	0	0	0	0
15	15	12	9	10
30	26	18	19	16
45	44	23	22	19



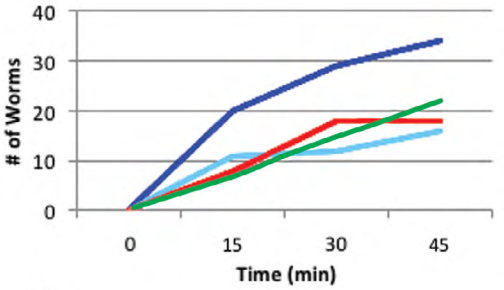
Optional Time Series Data - HCl

	Wild-Type Near Experiment	Wild-Type Near Control	Mutant Strain Near Experiment	Mutant Strain Near Control
0	0	0	0	0
15	10	9	5	5
30	16	28	9	11
45	15	33	14	17



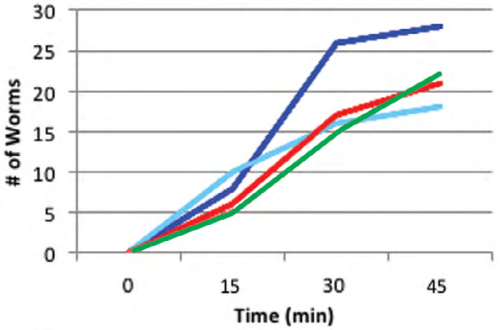
Optional Time Series Data - Bleach

	Wild-Type Near Experiment	Wild-Type Near Control	Mutant Strain Near Experiment	Mutant Strain Near Control
0	0	0	0	0
15	20	11	8	7
30	29	12	18	15
45	34	16	18	22



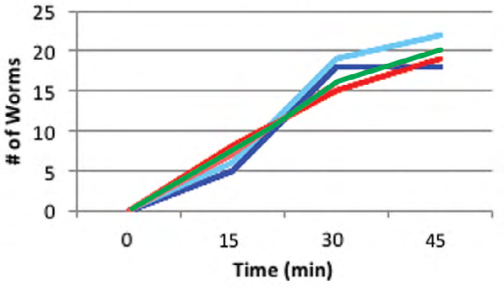
Optional Time Series Data - Imitation Vanilla

	Wild-Type Near Experiment	Wild-Type Near Control	Mutant Strain Near Experiment	Mutant Strain Near Control
0	0	0	0	0
15	8	10	6	5
30	26	16	17	15
45	28	18	21	22



Optional Time Series Data - Pure Vanilla

	Wild-Type Near Experiment	Wild-Type Near Control	Mutant Strain Near Experiment	Mutant Strain Near Control
0	0	0	0	0
15	5	6	8	7
30	18	19	15	16
45	18	22	19	20



Please refer to the kit insert for the Answers to Study Questions

Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
OP50 growth is not visible on a plate after incubation.	An OP50 lawn on agar can be hard to see.	Examine under the plate under the microscope OR use a sterile loop to gently scrape the top of the agar for growth OR smell plate for bacterial odor. If no growth is still observed re-seed the plate with OP50 liquid culture.
While transferring the nematodes to a new plate the agar was flipped in the wrong orientation.	It is difficult to transfer the small piece of agar.	Nematodes will leave the chunk and move towards their food regardless of orientation, but allow extra time for migration.
No nematodes are visible on chunked plates when observed through a microscope.	Nematodes haven't migrated from the chunk onto plate.	Wait for an additional 5-10 minutes and observe under the microscope again.
	Chunk did not contain enough live nematodes.	Make new chunk of agar from the source plate and repeat the transfer process.
Nematodes on chunked plates do not move when observed through a microscope.	Nematodes have become dormant.	Add 200 µl of additional 0P50 solution to plate.
	Nematodes are dead.	Examine initial plates or a classmate's plates for living nematodes and re-chunk.
Nematode population is growing slowly.	Inhospitable conditions are slowing nematode development.	This is most likely a food supply issue. Add 200 µL of additional OP50 culture. Also check that classroom temperatures are between 20 °C and 25 °C.
The plates containing the nematodes show a white/pink growth, white threads, or brown/green/ yellow spots.	The plates are contaminated with bacteria, yeast, or mold.	Most contamination will not harm the nematodes but can make observation more difficult. If necessary, prepare a new NGM plate and transfer a chunk from a clean region of the contaminated plate.

Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
NGM agar is too thin and does not cover the full plate.	Incorrect volume was poured into the plates.	It is important to have the correct thickness of agar in the plates. Be sure to pour the amount recommended by the protocol.
	The plates are dry.	Keep plates sealed and inside of a box or bag. If they are in a high temperature environment cover the edges with parafilm or saran wrap. Dry plates can be partially rehydrated with 200 µL of OP50 solution.
More OP50 liquid culture is needed.	Experiment involves extra steps that require OP50.	Large (>10 mL) OP50 solution can be created from a single BactoBead™ or from a small amount of old solution. Incubate the new solution at room temperature overnight, ideally on a shaking incubator. Alternatively, order new OP50 Bactobeads.
Worms are not visible on the experimental plate.	Too few nematodes in the experimental solution.	Check the 15 mL collection tube. If nematodes are visible thoroughly re-mix this solution and then re-make the experimental solutions. If nematodes are not visible they may not have settled properly or may have been accidently aspirated with the buffer. In this case use a classmate's collection.
	Worms are stuck in the central well.	Disrupt the surface tension by inserting the tip of a kimwipe or paper towel.
Need to delay the experiment after chunking student plates.	Life happens.	Nematode populations can be maintained for a week or more. Feed these populations with 200 µL of OP50 liquid culture every 3-4 days. If a longer delay is expected prepare additional NGM plates and chunk the nematodes onto a new plate every two weeks. Alternatively incubate plates at 16 °C for up to a month with weekly feeding.