WORKSHOP

Trailblazers: Investigating Chemotaxis with *C. elegans*

EDVOTEK®

Designed for the Classroom

SINCE 1987

Background Information *Excerpts from Edvo-Kit #852*

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 1970s, Dr. Sydney Brenner established the nematode *C.elegans* 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.

C.elegans have become important to the study of embryogenesis, morphogenesis, development, nerve function, behavior and aging, and genetics. 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.

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 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! This makes *C.elegans* a valuable model system for neuroscientists (Figure 2).

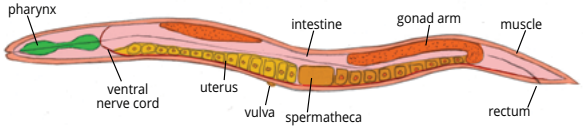


Figure 1: *C.elegans* anatomy

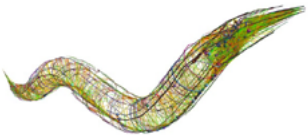


Figure 2: Neural anatomy of *C.elegans*. Source: OpenWorm project. Shared under an MIT License.

Growth and Development of *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.

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. Adults will live for 2-3 weeks, over which time they gradually age and lose vigor.

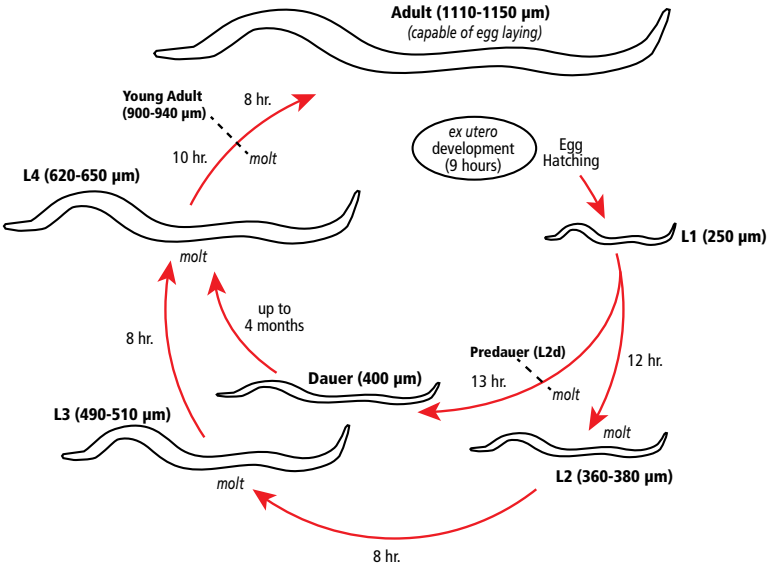


Figure 3: Life Cycle of *C.elegans*

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.

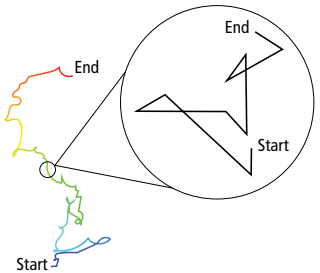


Figure 4: Biased Random Walk and Pirouette movement.

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.

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

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.

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.

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.

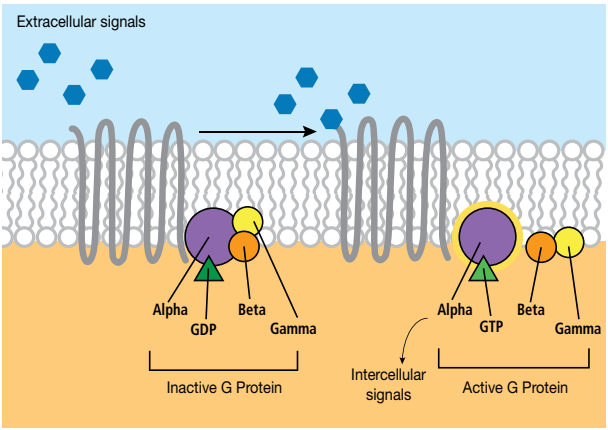


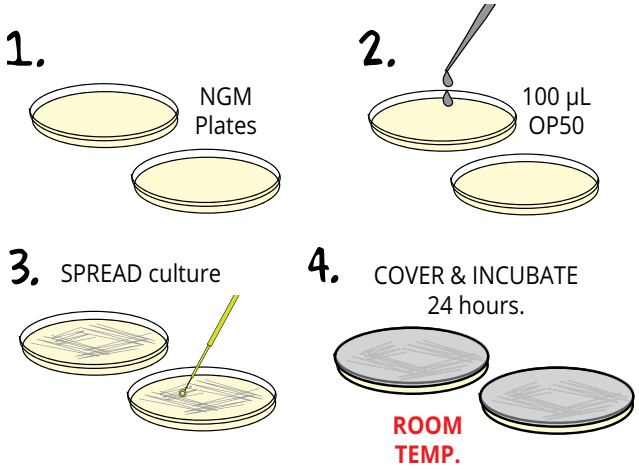
Figure 5: G Proteins

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.

Module I: Preparation of *C.elegans* Food Source ("Seeding" the Plates) *Excerpts from Edvo-Kit #852*

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



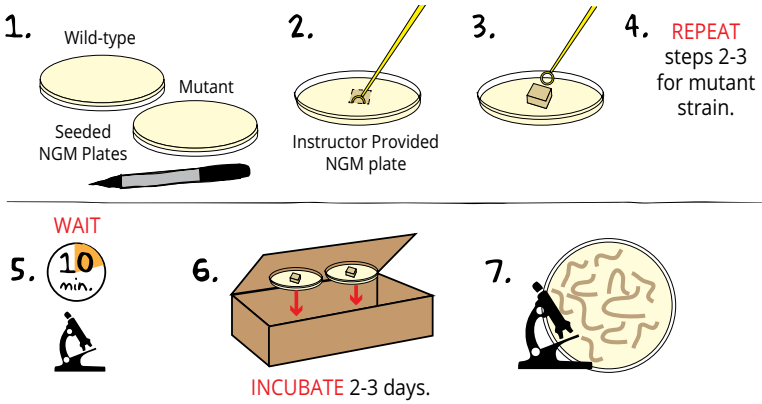
1. **OBTAIN** two Nematode Growth Medium (NGM) plates, the OP50 culture, a small transfer pipet, and a sterile inoculating loop from your instructor. **LABEL** the bottom of both plates with your group number or names.
2. While maintaining sterile technique, **ADD** two drops (100 µL) of OP50 culture 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) *Excerpts from Edvo-Kit #852*

In this module, you will be "chunking" your *C.elegans* strains. This means that you will transfer wild-type and ADR-2 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.



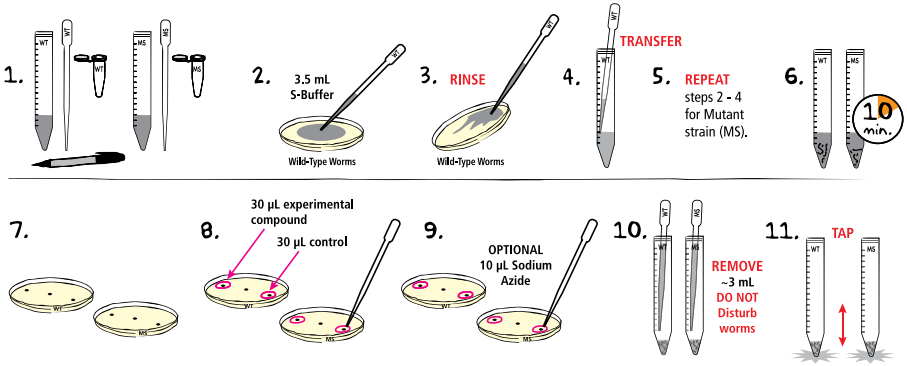
1. **LABEL** the bottom of the seeded NGM plates (from Module I) with "Wild-type" or "Mutant".
2. Your instructor will have NGM plates containing wild-type or mutant *C.elegans*. Using a sterile loop, **CUT OUT** a small portion of the NGM plate containing the wild-type strain (~1 cm square). Make sure 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: Collection of *C.elegans* and Chemotaxis Assay

Excerpts from Edvo-Kit #852



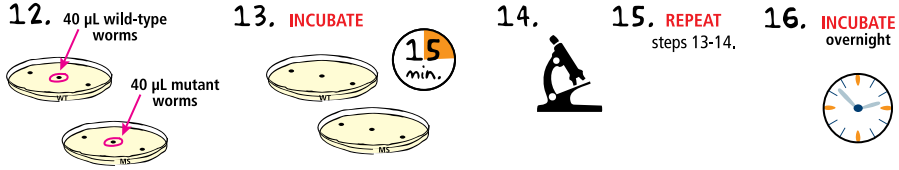
1. **LABEL** one 15 mL tube of S-Buffer, a large transfer pipet, and a snap top tube with "WT" (Wild-Type) and the other 15 mL tube, large transfer pipet, and snap top tube with "MS" (Mutant Strain).
2. Using the WT transfer pipet, **TRANSFER** 3.5 mL of S-Buffer from the WT conical tube to the Petri 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.

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

Excerpts from Edvo-Kit #852



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.

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

16. **INCUBATE** worms overnight.

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.		

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

Excerpts from Edvo-Kit #852

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.

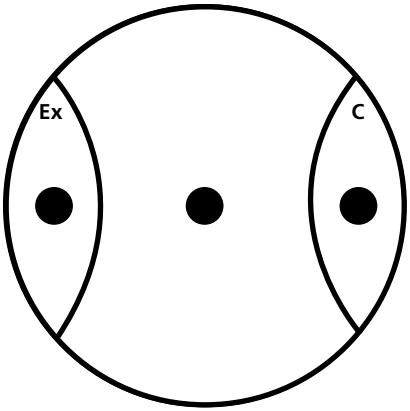
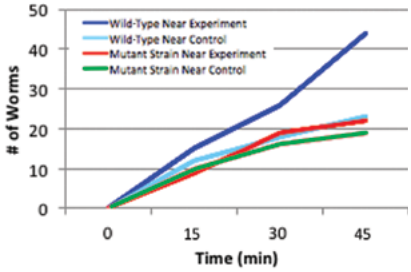


Figure 6:
Petri Template

Experimental Results and Analysis

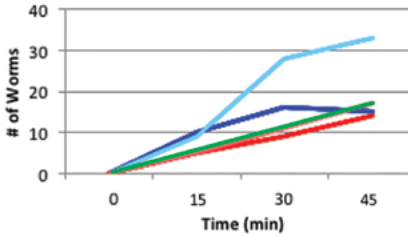
Optional Time Series Data - Vinegar

	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



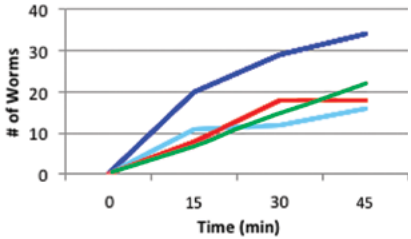
Optional Time Series Data - HCl

	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



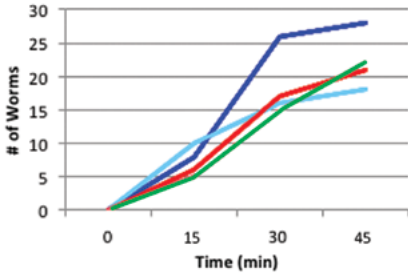
Optional Time Series Data - Bleach

	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



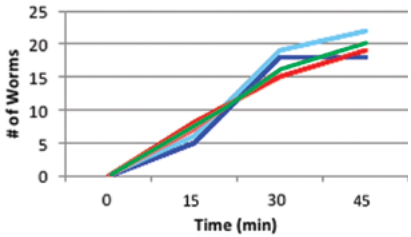
Optional Time Series Data - Imitation Vanilla

	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

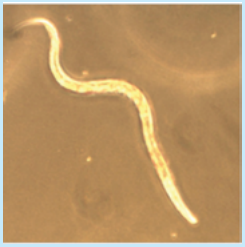
	Wild-Type Near Experiment	Wild-Type Near Control	Mutant Strain Near Experiment	Mutant Strain Near Control
0	0	0	0	0
15	5	6	8	7
30	18	19	15	16
45	18	22	19	20



Related EDVOTEK® Products:

Cat. 852

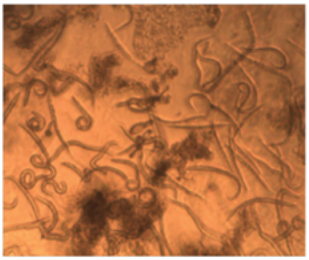
Chemotaxis: The Science of Attraction in *C.elegans*



For 10 Groups. All organisms are affected by "scent" molecules in the environment, including a multicellular organism called *Caenorhabditis elegans.* These worms are composed of 959 somatic cells, of which 300 are neurons comprising organs for taste, smell, temperature and touch. In this experiment, students will observe and record the phenomenon by which normal and mutant strains of *C.elegans* can direct their movement in response to certain chemicals in the environment.

Cat. 856

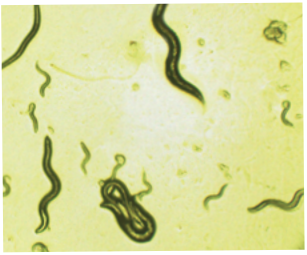
Ennvironmental Toxicity Response in *C.elegans*



For 10 Groups. Caenorhabditis elegans is a soil nematode with great potential for educational research, partly because of its rapid (3-day) life cycle, small size (1.0-mm-long adult), and ease of laboratory growth cultivation. In this experiment, students will observe and compare the effects of heavy metals found in the environment on normal and mutant strains of *Caenorhabditis elegans (C.elegans)*.

Cat. 851

Effects of Alcohol on *C.elegans*



For 10 Groups. You will not believe how similar we are to worms! The genome of the tiny worm, *C.elegans*, was sequenced and found to be 40% similar to the human genome. It is now used as a model system by researchers to address fundamental questions in developmental biology, neurobiology and behavioral biology. The objective of this experiment is to observe and record the effects of alcohol on normal and alcohol mutant strains of *C.elegans*.