EDVOTEK WORKSHOP:

Environmental Toxicology Using EDVOTEK's new E-Z *elegans*™

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Introduction

Caenorhabditis elegans (C.elegans) senses various environmental stimuli and exhibit rapid responses in its behavior for survival. The nematode is a suitable model for studying animal behavior thanks to its simple yet well-characterized nervous system. The gene encoding the enzyme Alcohol dehydrogenase (ADH) has been studied intensively in a broad range of organisms. Previous studies have demonstrated the effect of alcohol on behaviors of *C.elegans*, similar to alcohol's effect on other organisms. In this workshop, we examine the behavioral effects of alcohol in normal and mutant *C.elegans* strains.

Background Information

Excerpts from Cat. #858

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 system 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.

Today, *C.elegans* continues to be an important model organism for the study of embryogenesis, morphogenesis, development, nerve function, behavior, aging, and how genes regulate these processes. The entire *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 their 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.

Growth and Development of *C.elegans*

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 around one millimeter (mm) in length. The outer cuticle of *C.elegans* is transparent, making it easy to visualize growth and devel-

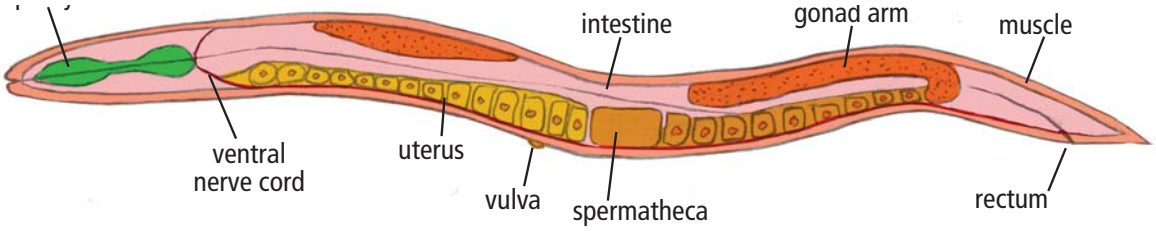


Figure 1: *C.elegans* anatomy.

Background Information

Excerpts from Cat. #851

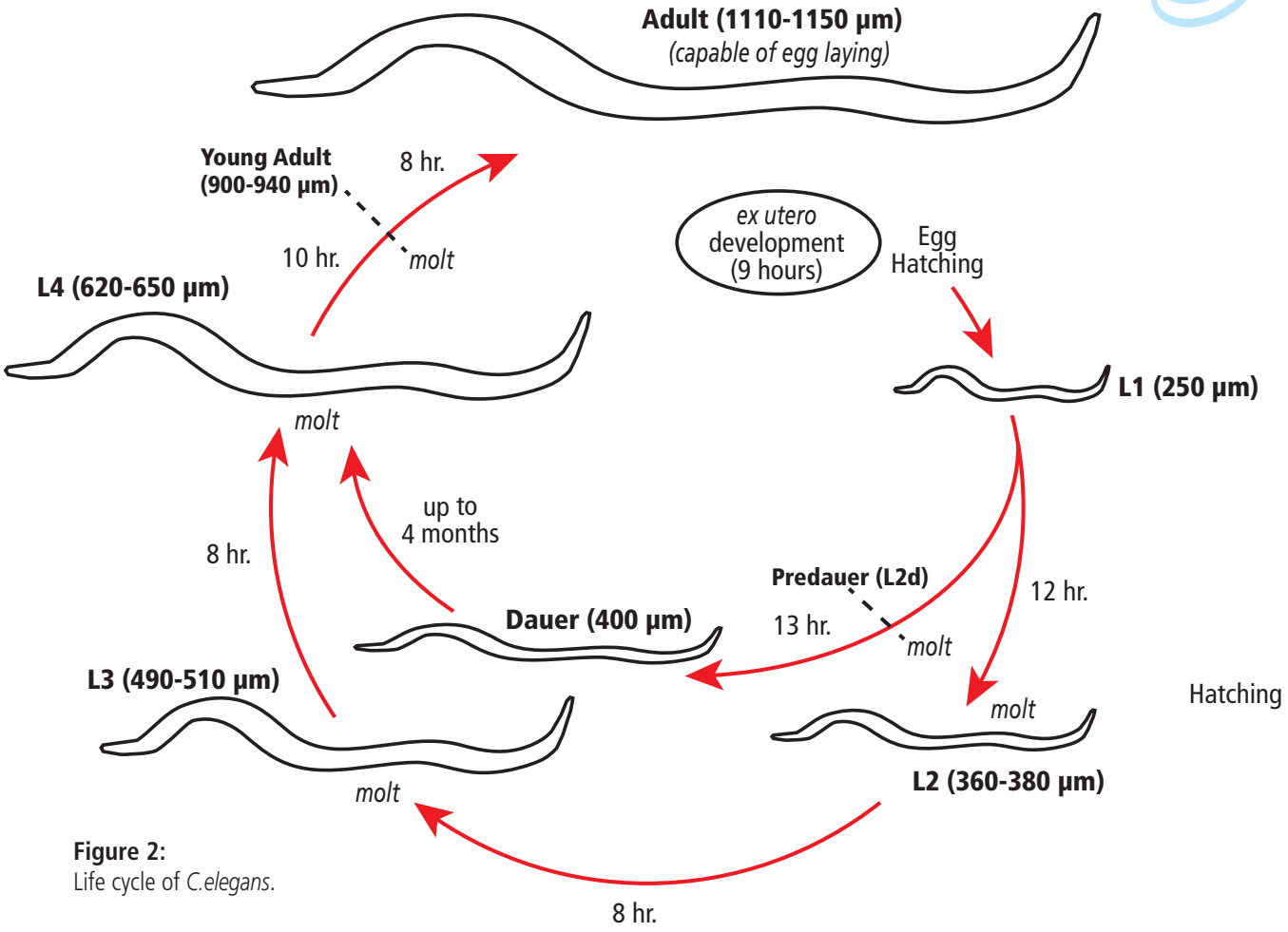


Figure 2:
Life cycle of *C.elegans*.

opment 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.

There are two naturally occurring sexes in *C.elegans*. The vast majority of worms are self-fertile hermaphrodites, meaning they produce both the sperm and the eggs used for reproduction. Free-living males represent <1% of the total nematode population. Notably, the mating of a male with a hermaphrodite can produce over 1000 offspring in a generation; in contrast, a self-fertilized hermaphrodite worm 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 develops from embryo to adult in four days, allowing for rapid studies in the laboratory (Figure 2). 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 about 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 they can be used for further genetic studies. Adults will live for 2-3 weeks, over which time they gradually age and lose vigor.

Background Information

Excerpts from Cat. #858

C.elegans as a Model Organism to Study Toxicoloty

Performing experiments that characterize the human response to toxic chemicals can be unethical and dangerous. To better understand toxicity, scientists perform studies in model organisms. *C.elegans* is often used as a model system for toxicity testing because it is one of the simplest animals with a well-defined neural system. The neural structures include a battery of sense organs in the head, which mediate responses to taste, smell, temperature and touch—and although *C.elegans* has no eyes, they respond slightly to light. The nematode exhibits specific behaviors and is even capable of rudimentary learning. These features have led to an increased use of *C.elegans* in toxicology studies, both in the study of how the toxins affect the nervous system and as a high-throughput model to determine the potential toxicity of substances.

The toxicity of heavy metals like arsenic, chromium, cadmium, copper, and iron can be studied using *C.elegans*. While small amounts of some heavy metals are critical for living organisms, high concentrations of the metals can be toxic. For example, iron is a critical cofactor for the protein hemoglobin, which facilitates gas exchange in the body. However, excessive amounts of iron can lead to nausea, vomiting, brain and liver damage, and even death. Although all of these elements are naturally occurring in the environment, manufacturing and mining have concentrated the levels of heavy metals in certain areas, often increasing the chance of harmful human exposure.

In this workshop, we will use *C.elegans* to study the effects of heavy metals on living organisms. Using an inquiry-based bioassay, we will expose wild-type and mutant *C.elegans* to different heavy metal solutions. The mutation affects the ability of the worms to sense the toxicant solutions, changing their behavior. After collecting data, we will compare the behavior of the two strains and record the effects. Finally, we will use critical thinking and statistics skills to interpret the data.

Laboratory Safety

Important READ ME!

This experiment involves the use of toxic materials. Care should be taken in aliquoting, mixing and handling any solutions containing heavy metals. Always use personal protective equipment when using hazardous materials. These chemicals can be toxic if taken internally.


Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment which is used in conjunction with the heating and/or melting of reagents.
3. Do not mouth pipet reagents - use pipet pumps or bulbs.
4. The *C. elegans* used in this experiment is not considered pathogenic. Although it is rarely associated with any illness in healthy individuals, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with worms.
5. Properly dispose materials after completing the experiment:
   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 worms 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 and goggles when working with bleach.
6. Wear gloves, and at the end of the experiment, wash hands thoroughly with soap and water.

Experiment Overview

Excerpts from Cat. #856

EXPERIMENT OBJECTIVE:

The objectives of this experiment are to observe and compare the effects of heavy metals found in the environment on normal and mutant strains of *Caenorhabditis elegans (C. elegans).*

BRIEF DESCRIPTION OF THE EXPERIMENT:

In this experiment, each group of students will treat *C. elegans* worms with heavy metal solutions and record the movements of an individual worm when exposed. The results for several groups can be compared to illustrate the toxicity of heavy metals.

Experiment Procedure

Excerpts from Cat. #856

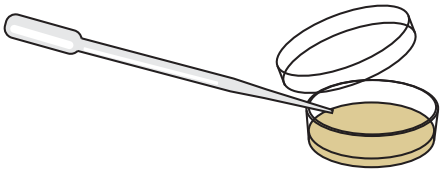


Figure 3

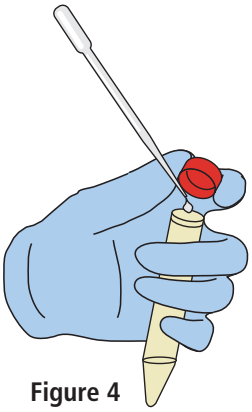


Figure 4

Figure 5

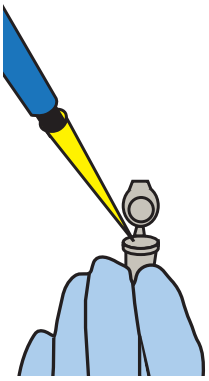


Figure 6

MODULE I: COLLECTION OF *C. elegans*

1. Check growth of worms under a microscope. Worms are visable at 40X magnification. Place on an inverted microscope and observe. If the plate contains 50 or more worms, proceed with the experiment. If the plate contains less than 50 worms, continue incubation. (When using a standard compound microscope, turn the plate upside down on the stage and focus through the agar to view the worms on the surface.)

2. Transfer 3.5 ml S-buffer to the Petri dish with a large transfer pipet (Figure 3).

3. Rinse the dish with the buffer and transfer the worms to 15 ml centrifuge tube with a large pipet (Figure 4).

4. Allow the worms to settle to the bottom of the tube (~15 minutes) and remove 3 ml of the cleared buffer with a large pipet (Figure 5). (Be careful to avoid removing the worms, leave ~500 µl of worms and buffer in the bottom of the tube, check tube graduations.)

5. Mix to resuspend the worms and transfer 1 drop of the worms (~20 µl) into each labeled 1.5 ml tube with a small transfer pipet (Figure 6). Label tubes Control, CU, CD and ZN. (Note: each tube will take ~10 minutes to count) so the number of samples can be adjusted based on the time available. Keep tubes at room temperature.

Experiment Procedure

Excerpts from Cat. #856

MODULE II: EXPOSURE OF *C. elegans* To Heavy Metal Solutions

Important READ ME!

Complete steps 1-4 for each tube individually. Only add the heavy metal solutions to one sample at a time and complete the experiment for that sample before proceeding to the next sample.

1. To a tube add 100 µl sample (S-Buffer or heavy metal solution).
2. Cap the tube and mix by tapping the bottom of the tube.
3. With a small pipet transfer 1 drop to the opening of one chamber on the counting plate. Allow the solution to move into the chamber by capillary action.
4. Observe movement of a single worm under the microscope. Count the number of "thrashes" that occur for 30 seconds. Wait 2 minutes and repeat the count for a total of three counts (use the same worm for each count). For each group one member will observe and count the "thrashes" on the animal and another member will time the count.
5. Check the chambers after all the samples have been counted and note the appearance of the worms.
6. Record all your observations in your notes including the counts and a description of the appearance of the worms.

	Thrash Normal Control	Thrash Mutant Control	Thrash Normal $CdCl_2$	Thrash Mutant $CdCl_2$	Thrash Normal $CuSO_4$	Thrash Mutant $CuSO_4$	Thrash Normal $ZnSO_4$	Thrash Mutant $ZnSO_4$
Trial #1 (30 seconds)								
Wait 2 min								
Trial #2 (30 seconds)								
Wait 2 min								
Trial #3 (30 seconds)								

Experiment Procedure

Excerpts from Cat. #856

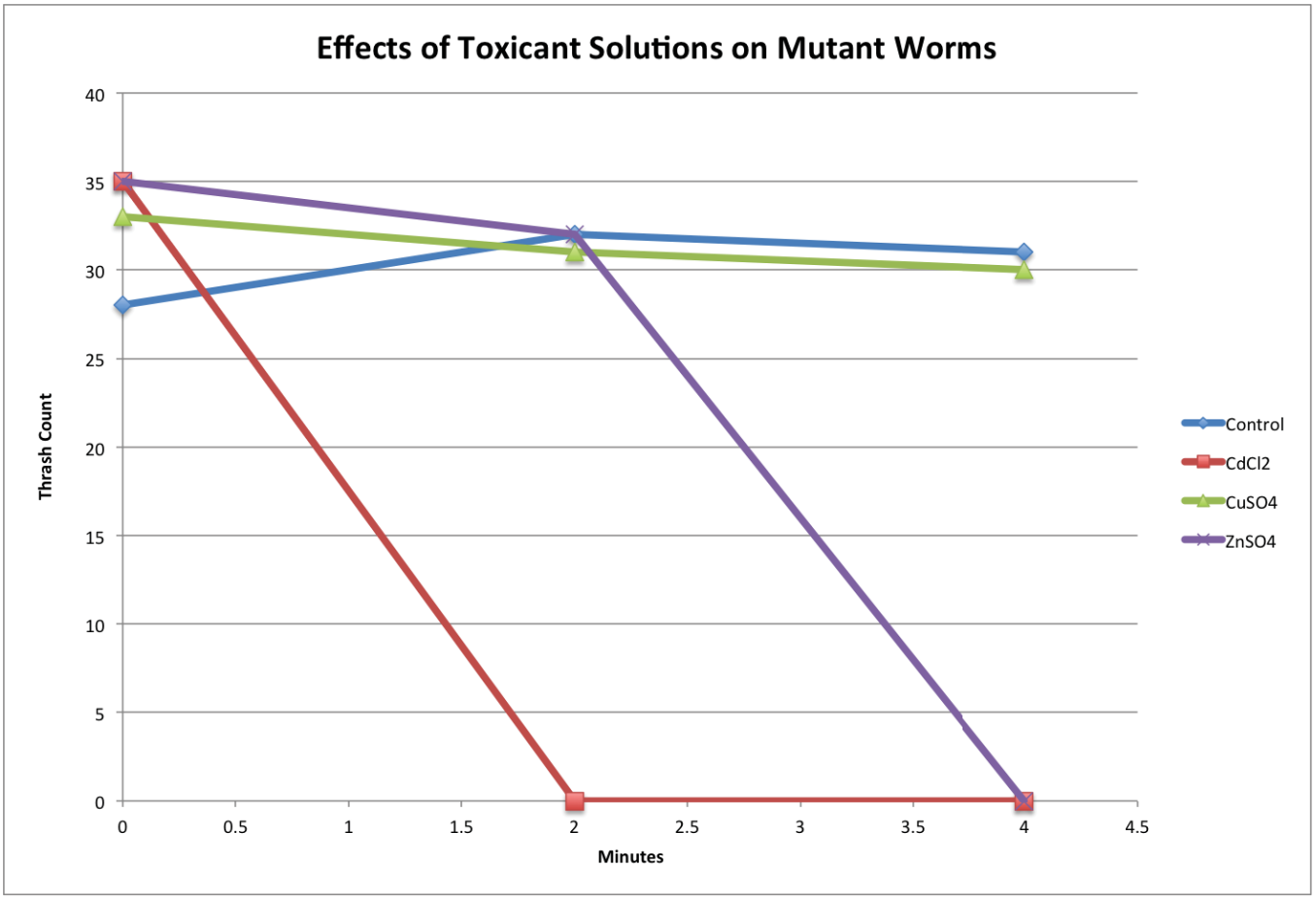
Answer these questions BEFORE analyzing your results.

1. How would you expect the worms to react to the heavy metal solutions? Explain.
2. Which heavy metal solution will have the greatest reaction on the worms? Explain.
3. What factors could affect the worm's reaction to the heavy metal solutions?

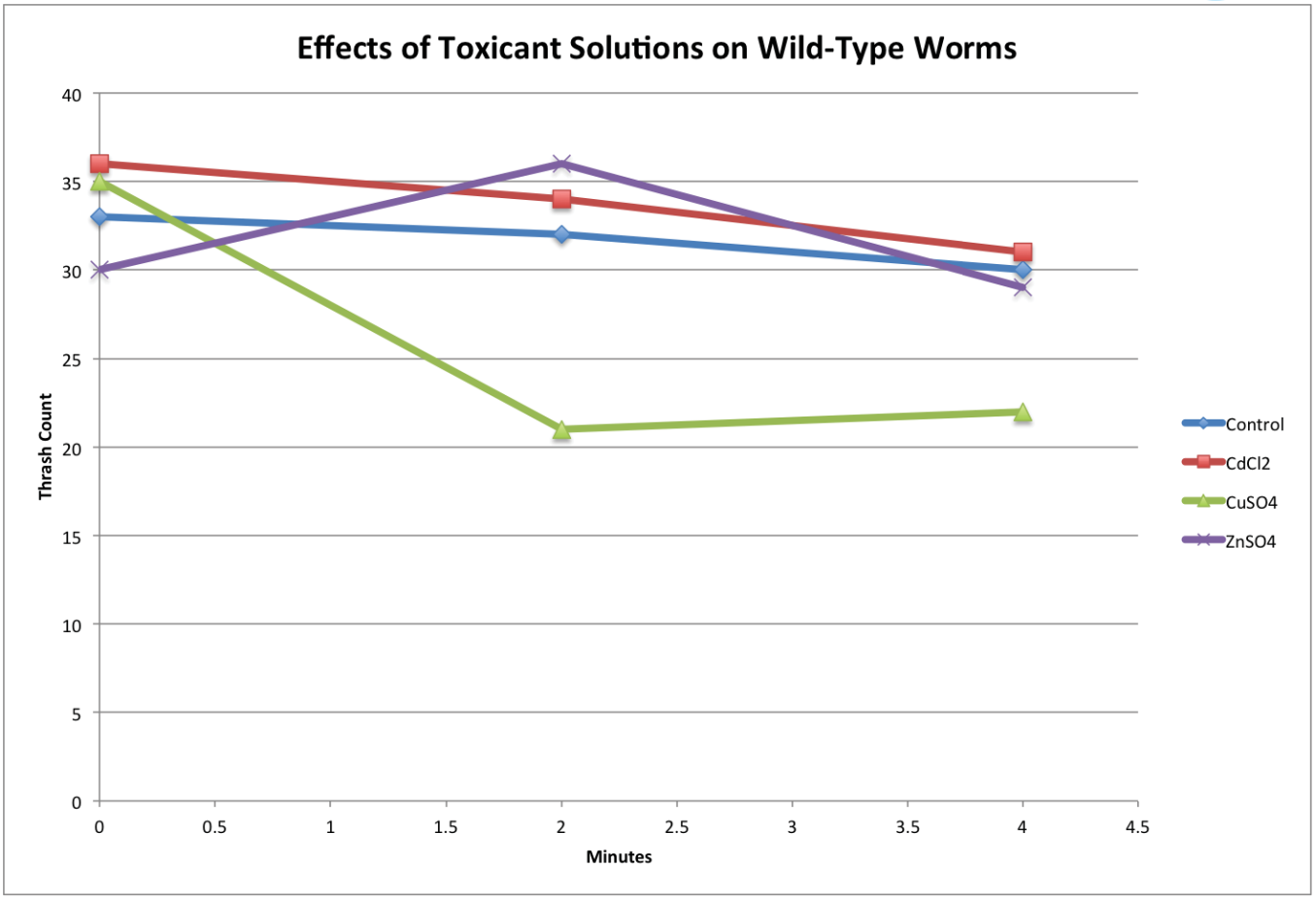
Data Collection

- Compare the movement and behavior of the worms in each chamber.
- What do you notice about how the worms move?
- What do you think is happening to the worms?
- What differences do you observe in the worms reaction to heavy metal solutions (compare large and small worms, different concentrations)?
- What are the effects of each heavy metal solution on the worms? How do these effects change over time?

Results and Data Analysis

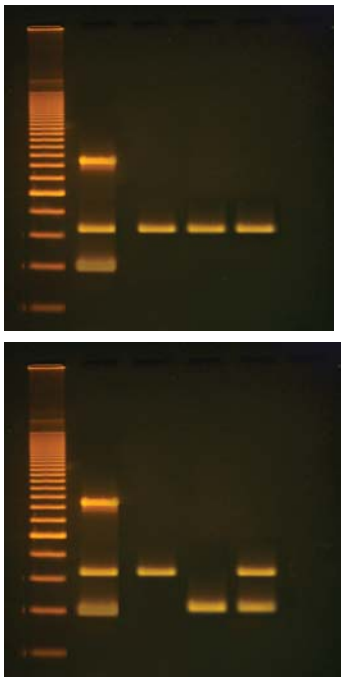


Results and Data Analysis



Related Products

In Search of the Alcohol Gene



The rate of an individual's alcohol metabolism is dependent on several environmental and genetic factors (body weight, food intake, gender, genetics). In this experiment, students will identify a simulated Alcohol Dehydrogenase (ADH) polymorphic gene sequence by PCR amplification and digestion of the PCR products. When the digested DNA samples are analyzed on an agarose gel, students will identify several polymorphic genes.

Kit includes: instructions, DNA template, primer mix, PCR reaction beads, RNAse-free water, DNA size ladder, Eco RI restriction enzyme, restriction enzyme reaction & dilution buffers, agarose, electrophoresis buffer gel, InstaStain® Ethidium Bromide gel stain.

All you need: thermal cycler, electrophoresis apparatus, power supply, automatic micropipet, microwave or hot plate, waterbath, UV transilluminator.

For 6 groups

Cat. #346 **Available only on our website**

NIAAA National Institute on Alcohol Abuse and Alcoholism

Supported by SBIR grant R44 AA 015026 from the National Institute on Alcohol Abuse and Alcoholism.

Effects of Alcohol on *C.elegans*

BactoBeads™

You can not imagine how similar we are to worms! The genome of the tiny worm, Caenorhabditis elegans, was sequenced and its genome was found to be 40% similar to us. This little nematode, that is just 1 mm in length (the smallest division in the foot ruler on your desk), has provided a wealth of information for researchers around the world. It has been used as a model system to address fundamental questions in developmental biology, neurobiology and behavioral biology. In this experiment, students will study the effect of alcohol on the locomotion and health of the worms.

• SCIENCE • TECH • MATH STEM NOLOGY • ENGINEERING •

Kit includes: instructions, *C.elegans*-normal, *C.elegans* Alcohol -resistant, petri dishes, NGM medium, *E.coli* OP50 Bactobeads™, cell counting chambers, buffer, pipets, sterile loops, tubes, and 10% alcohol.

All you need: ethanol, timers, microscopes, covered box.

For 10 Lab Groups

Cat. # 851 **$89**

NIAAA National Institute on Alcohol Abuse and Alcoholism

Supported by SBIR grant R44 AA 015026 from the National Institute on Alcohol Abuse and Alcoholism.

Kit contains LIVE materials which must be requested 1 week prior to lab.

Related Products

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

All organisms are affected by "scent" molecules in the environment. Ants find a picnic lunch, people get attention by the cologne they use, and we all want to go into a bakery that smells like fresh cookies. Allow your students to have the wonderful experience of working with a multicellular organism called Caenorhabditis elegans, which can be attracted to a number of scent molecules. These worms are composed of 959 somatic cells of which 300 are neurons comprising organs for taste, smell, temperature and touch. In this experiment your students will explore positive and negative chemotaxis in *C.elegans* using volatile chemical compounds like vinegar, vanilla, bleach and acids.

Kit includes: instructions, *C.elegans*- normal, *C.elegans* Chemotaxis- mutant, petri dishes, NGM medium, *E.coli* OP50 Bactobeads™, cell counting chambers, buffer, pipets, sterile loops, tubes and chemical compounds.

All you need: ethanol, timers, microscopes, covered box.

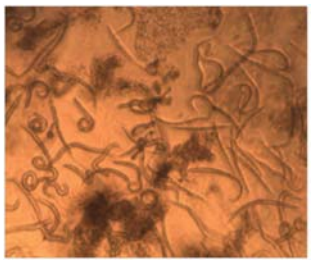
Kit contains LIVE materials
which must be requested 1 week prior to lab.

For 10 Lab Groups

Cat. # 852 $99

C.elegans Ecology Platform

After one week of working with *C.elegans* under a microscope, your students will feel like real scientists! Caenorhabditis elegans is a soil nematode that has 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. Thousands of animals can grow on a single petri dish seeded with a lawn of Escherichia coli as the food source. Students will engage in an environmental toxicity scenario and will use pre-diluted concentrations of heavy metal solution to determine the effect on the worms. Time courses will be assessed and LD toxicity will be determined.



Kit includes: instructions, *C.elegans*-normal, *C.elegans*-Toxicity mutant, petri dishes, NGM medium, *E.coli* OP50 Bactobeads™, cell counting chambers, buffer, pipets, sterile loops, tubes and heavy metal compounds.

All you need: ethanol, timers, microscopes, covered box.

Supported in part by NIH SBIR NCRR Grant.

For 10 Lab Groups

Cat. # 856 $99

Kit contains LIVE materials
which must be requested 1 week prior to lab.

Related Products

EdvoCycler™

Finally, a PCR machine at an affordable price!

BEST SELLER

EdvoCycler™

Holds 25 x 0.2 ml sample tubes.

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MegaCycler™

Taking Classroom PCR One Step Further!

MegaCycler™

Holds 49 x 0.2 ml sample tubes.

Cat. #542 $2,499

The EdvoCycler™ and the MegaCycler™ are stand alone classroom PCR machines that are easy to use! The EdvoCycler™ features a 0.2 ml tube block for up to 25 student samples and the MegaCycler™ has nearly twice that capacity with its 49-place block! Both come pre-programmed with all Edvotek PCR protocols. These programs may be modified or deleted, plus there's extra memory slots for more! The vivid 7-line LCD displays all program parameters on a single screen. A heated lid makes operation a snap. Proudly made in the USA and backed by a 2 year warranty!

Features:

- EdvoCycler™ holds 25 x 0.2 ml PCR tubes
- MegaCycler™ holds 49 x 0.2 ml PCR tubes
- Heated oil-free lid with magnetic latch
- Pre-programmed Edvotek PCR protocols
- Vivid 7-line LCD display with live program information
- Standalone machine - no PC required!
- Temperature Range: 4 to 99° C
- Maximum Ramp Rate: 3° C/sec.
- Dimensions: 16 x 8.5 x 7"

NIH Research supported in part by NIH SBIR NCRR Grant #R44RR18670

M36 HexaGel™ DNA Electrophoresis Apparatus

Cat. #515 $325

DuoSource™ 150 Power Supply

Cat. #509 $179

EDVOTEK® Fixed Volume 40 µl MiniPipet™

Cat. #588 $23