Biotechnology Explorer™

C. elegans Behavior Kit

Instruction Manual

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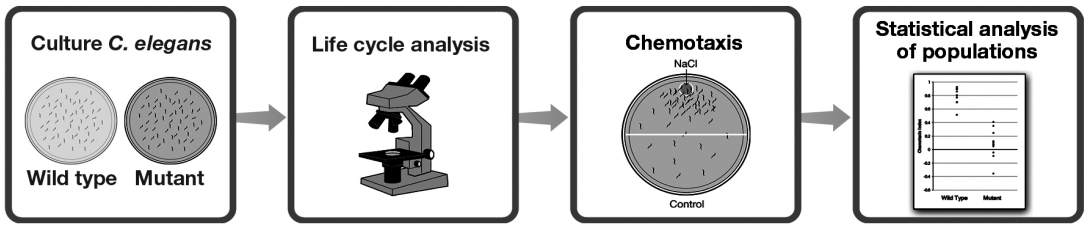
Catalog #166-5120EDU



This kit contains temperature-sensitive reagents.
Open immediately and see individual components for storage temperature.

Please see redemption instructions on how to receive your *C. elegans*.

Please visit explorer.bio-rad.com to access our selection of language translations for Biotechnology Explorer kit curricula.

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Dear Educator,

One of the greatest challenges in studying the biology of how human cells and organ systems function is that experimentation on humans is expensive, complex, and often unethical. It is because of this that scientists worldwide have adopted the use of model organisms that share much of the cellular machinery present in human cells, and study these organisms to help improve our understanding of the nature of our own cellular functions.

In this kit, students will be introduced to one of the most widely used model organisms, the microscopic nematode *Caenorhabditis elegans*. Students will utilize *C. elegans* to understand the function of a conserved protein phosphatase, DAF-18. As with most *C. elegans* genes, *daf-18* has a human homolog, *PTEN*, which is involved in a number of human pathologies such as cancer, autism, and learning disorders. While cancer is not a phenotype that is easily studied in *C. elegans*, because their short life span does not allow for the cellular progression often seen in humans, this kit does explore the role of *daf-18* in learning and behavioral disorders.

Students will be provided with two strains of *C. elegans*, a wild-type strain and a *daf-18* mutant strain. Students will be able to explore the life cycle of *C. elegans* through microscopic examination, observing *C. elegans* as they develop from eggs, through larval stages, and finally as adults. Students will then conduct a brief *C. elegans* subculture lab where they will learn about the importance of culturing, sterile technique, and the purpose of passaging. The lab culminates in a chemotaxis assay where students will assess the learning potential of the *C. elegans* by observing how they react to NaCl and explore how a loss of the protein phosphatase DAF-18 impacts learning capacity. Students will quantify the observed behaviorial phenotype by calculating a chemotaxis index, allowing students to graphically represent the data collected and to apply basic statistical techniques.

The *C. elegans* your students will work with are complex, living creatures. Depending on the focus of your class, you may choose to have your students explore the complex physiology and anatomy of the worms, their genetics, their neuronal connections, or the impact of other variables in the environment on the learning capabilities of the *C. elegans* mutant and wild-type strains. Additionally, this lab offers the possibility of a number of supplementary lab inquiry investigations such as exploring the taste preferences of *C. elegans*, actively changing the *C. elegans* taste preferences, or further studying the cellular function of the *daf-18* gene. Student involvement in additional inquiry activities will result in an increased understanding of the scientific process and the value of model organisms in comprehending human physiology and pathology.

The *C. elegans* behavior kit is a uniquely interactive activity meant to create excitement in your students through the use of a model organism. We hope that this kit is a vehicle for the generation of curiosity and inquiry, and we look forward to hearing your feedback, comments, and suggestions.

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Biotechnology Explorer™
CAPTIVATING SCIENCE EDUCATION

C. elegans Redemption Instructions

Once you have prepared your NGM Lite agar plates, you are ready to receive your *C. elegans* (wild-type and mutant) worms.

1. Call **1-800-424-6723** or 1-800-4BIORAD to speak with a customer service representative in the US or 1-800-268-0213 in Canada.
2. Provide the customer service representative with the original order number or your school's PO number can also be used to reference your original order for the *C. elegans* Behavior Kit.
3. Request a date for shipment of your *C. elegans* wild-type and mutant worms. The *C. elegans* will be shipped overnight on dry ice.
4. It is imperative that the *C. elegans* remain on dry ice or at –70°C until you are ready to plate them on the NGM Lite agar plates.

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Precautions For Handling Your *C. elegans*

C. elegans is a great model organism to study behavior because *C. elegans* behavior is a function of genetic background and environmental conditions. Because environment influences *C. elegans* behavior, proper environmental conditions are essential to obtaining the desired results from your experiment.

***C. elegans* should be maintained at room temperature** between 16 and 25ºC (60–77ºF) and away from direct light.

Excessive heat kills *C. elegans*.
Do not place in a warm or hot environment.
Do not place *C. elegans* in an incubator.

Excessive cold kills *C. elegans*.
Do not place in a cold environment.
Do not place *C. elegans* in a refrigerator or freezer.

Prior to the chemotaxis assay, *C. elegans* should be seeded on a fresh lawn (subculture) of OP50-pBAD *E. coli* — **no older than 3 days old**.

Do not starve *C. elegans*. If *C. elegans* are seeded on an old or dead lawn of OP50-pBAD *E. coli, C. elegans* will display negative chemotaxis instead of positive chemotaxis.

The subculture of *C. elegans* is essential for experimental success. Even though there may be lots of fast moving *C. elegans* on your plate, overcrowded *C. elegans* are unhappy. Unhappy *C. elegans* will learn behaviors that will negatively affect your experiment such as associating salt with overcrowding and demonstrating negative chemotaxis if the subculture is skipped.

Do not refreeze *C. elegans*. They will not survive.

Curriculum Fit

Table of Contents

Timeline

The kit box you receive will not contain the *C. elegans* wild-type and mutant strains but rather a card with instructions for redeeming your worms. It is important that you prepare the NGM (nematode growth medium) Lite agar plates BEFORE you receive your *C. elegans*, since upon receipt, your worms must either be kept frozen at –70ºC, on dry ice, or be plated immediately.

The timeline below incorporates steps that can be performed either by the educator or by the students, depending on your laboratory schedule. Many steps are time dependent and must be performed before the actual laboratory experiment can be run.

Note: Tasks that are shaded in grey are preparatory tasks required for later stages of the experiment and should be conducted when indicated.

Note: Lab activity 2 should be performed no later than 3 days after lab activity 1. The *C. elegans* subculture provides *C. elegans* with an ideal feeding environment that is necessary for proper chemotaxis. Failure to subculture 2–3 days prior to the chemotaxis assay can lead to **overcrowding or starvation** of the *C. elegans*, potentially resulting in negative chemotaxis.

Task	Estimated duration
Instructor's Advance Preparation (1 Week prior to Lab Activity 1)	
1. Pour NGM Lite agar plates	1 hr prep time At least 2 days for drying of plates
2. Pour assay agar plates	1 hr prep time At least 2 days for drying of plates
3. Carry out *C. elegans* redemption instructions	*C. elegans* will be shipped on dry ice overnight
4. Thaw *C. elegans*	15 min
5. Plate *C. elegans* (wild-type and mutant) for 8 student workstations	10 min
6. Allow *C. elegans* to recover and populations to expand	4–7 days

Instructor's Advance Preparation (1–2 Days Prior to Lab Activity 1)	
1. Reconstitute *E. coli* OP50-pBAD, seed 16 NGM Lite agar plates with reconstituted *E. coli* OP50-pBAD, incubate at 37°C (16–24 hr) or room temperature (1–2 days)	15 min *E. coli* reconstitution and spreading 1–2 days *E. coli* growth
2. Prepare 1x *C. elegans* wash buffer	15 min
3. Set up student workstations	45 min

Lab Activity 1: Subculture of *C. elegans* (2–3 Days Prior to Lab Activity 2)	
1. Observe *C. elegans* wild-type and mutant populations using a microscope and record observations	10 min
2. Subculture *C. elegans* wild-type and mutant populations onto the fresh lawns of *E. coli* OP50-pBAD	20 min, then incubate 2–3 days
3. Observe *C. elegans* wild-type and mutant populations using microscope and record observations	45 min

Task	Estimated duration
Instructor's Advance Preparation (1 Day Prior to Lab Activity 2)	
1. Prepare salt gradients on assay agar plates	30 min
2. Allow gradients to diffuse overnight	12–24 hr
3. Set up student workstations	45 min

Lab Activity 2: Chemotaxis (2–3 Days after Lab Activity 1)	
1. Wash *C. elegans*	10 min
2. Perform chemotaxis experiment	40 min
3. Perform mathematical analysis	Variable

Important Notes:

- *C. elegans* must NOT encounter temperatures above –70°C until they are ready to be plated
- Redeem coupon for shipment of the *C. elegans* only when you have poured NGM Lite agar plates
- The vials containing *C. elegans* can be stored on dry ice or in a –70°C freezer until ready to be used
- The *E. coli* OP50-pBAD plates must NOT be plated more than 1–2 days before they are used
- **Lab activity 2 should be performed no later than 3 days after lab activity 1**

Kit Inventory Checklist

This section lists the components provided in the *C. elegans* behavior kit. It also lists required accessories. Each kit contains materials sufficient for eight student workstations of up to four students per workstation. As soon as your kit arrives, open it and check off the listed contents to familiarize yourself with the kit.

Please note that your kit should contain instructions to receive your *C. elegans* in a separate shipment. Prepare the NGM Lite agar plates before you receive your *C. elegans*. Once you receive your worms, **immediately place the bag containing the *C. elegans* in the –70°C freezer. DO NOT store *C. elegans* in a –20ºC freezer. If a –70°C freezer is not available, keep the *C. elegans* on dry ice until you are ready to plate onto NGM Lite agar plates (see Advance Preparation Step 1) and are ready to follow the *C. elegans* thaw procedure.**

Kit Components (included)	Quantity	(✓)
Store at –20°C		
E. coli OP50-pBAD, lyophilized	1 vial	☐
Cholesterol in alcohol, 200 µl	1 tube	☐
Ampicillin, lyophilized, 30 mg	1 vial	☐
Store at room temperature		
NGM Lite agar, 11 g	1 pouch	☐
Assay agar, 4 g	1 pouch	☐
2.5 M NaCl, 0.5 ml	1 tube	☐
C. elegans wash buffer (10x), 30 ml	1 bottle	☐
Microcentrifuge tubes, 2.0 ml	60	☐
Microcentrifuge tubes, 1.5 ml	30	☐
Petri dishes, 60 mm	60	☐
Disposable plastic transfer pipets (DPTPs)	50	☐
Instruction manual	1	☐
Redemption instructions for shipment of *C. elegans*	1	☐
To be redeemed		
C. elegans wild-type, 1 ml	1	☐
C. elegans mutant, 1 ml	1	☐

Required Accessories (not included)	Quantity per Kit	(✓)
Dissection microscopes (10–40x zoom)	1–8 scopes	☐
Container for liquid waste	8	☐
Kimwipes	8	☐
Fine tip marking pen	8	☐
Parafilm	8 sheets	☐
Scissors	8 pairs	☐
100–1,000 µl adjustable-volume micropipet (166-0508EDU, 166-0553EDU)	1–8	☐
100–1,000 µl pipet tips, standard style (223-9350EDU)	1–8 boxes	☐
2–20 µl adjustable-volume micropipet (166-0506EDU, 166-0551EDU) or 10 µl fixed volume micropipet (166-0512EDU)	8	☐
2–200 µl pipet tips, standard style (223-9347EDU)	8 boxes	☐
Distilled water	1 liter	☐
Microwave or magnetic hot plate with stir bar	1	☐
Microwave, hot plate, or autoclave for preparing agar	1	☐
Incubation oven (166-0501EDU)	1	☐
Erlenmeyer flask, 1 L	1	☐
Erlenmeyer flask, 500 ml	1	☐
Graduated cylinder, 500 ml	1	☐

Safety Issues

Some countries outside the U.S. may require a special license to use this kit. In the U.S., Hawaii requires application for a license with the state's Department of Agriculture to obtain permission to import *C. elegans*. Please refer to your state or country's legislative authorities for proper guidelines.

The *Escherichia coli bacteria* OP50-pBAD strain contained in this kit is not a pathogenic organism like the *E. coli* strain O157 H7 that has sometimes been implicated in food poisoning. OP50-pBAD is a non-virulent uracil auxotroph strain of *E. coli* that can grow only on enriched medium. However, handling of the *E. coli* OP50-pBAD strain requires the use of standard microbiological practices. These practices include, but are not limited to, the following:

- Work surfaces are decontaminated once a day and after any spill of viable material
- All contaminated liquid or solid wastes are decontaminated before disposal
- All persons must wash their hands: (i) after they handle material containing bacteria, and (ii) before exiting the laboratory
- All procedures are performed carefully to minimize the creation of aerosols
- Mechanical pipetting devices are used, mouth pipetting is prohibited
- Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Wearing protective eyewear and gloves is strongly recommended

The following are general guidelines for handling and disposing of *C. elegans* waste. You should consult your local environmental health and safety specialist for local rules and regulations.

If an autoclave is not available, all solutions and components (DPTPs and pipet tips) that come into contact with bacteria and *C. elegans* can be placed in a fresh 10% bleach solution for at least 20 min for sterilization. A shallow pan of this solution should be placed at a common workstation. Whichever method you choose, all used DPTPs and pipet tips should be collected for sterilization. Sterilize petri dishes by covering the agar with 10% bleach solution. Let the plate stand for 1 hr or more, and then pour excess plate liquid down the drain. Once sterilized, the agar plates can be double bagged and treated as normal trash. Safety glasses and gloves are recommended when using bleach solutions.

Ampicillin may cause allergic reactions or irritation to the eyes, respiratory system, and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Ampicillin is a member of the penicillin family of antibiotics. Those with allergies to penicillin or to any other member of the penicillin family of antibiotics should avoid contact with ampicillin.

Lesson Points to Highlight

This section describes experimental and conceptual points that may prove challenging to students. These points are extremely important to the overall outcome of the activity. Instructors should direct their students' attention to these points and, when possible, demonstrate the technique before the students attempt the procedure.

The most important thing for students to do is to put the correct components in the correct tubes and onto the correct plates. Marking the tubes clearly and being prepared and organized is crucial for a smooth execution of the experiment. The Quick Guide is provided to organize the activity. This graphic laboratory protocol provides visual depictions of all laboratory steps used in the subculture and chemotaxis assay procedures.

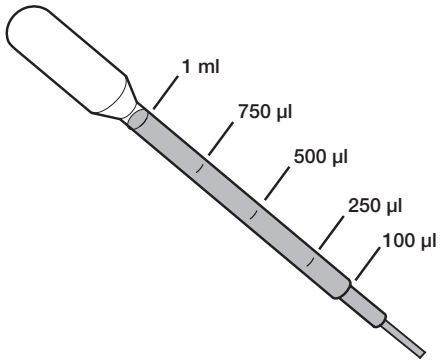
General Laboratory Skills

Sterile Technique

With any type of microbiology technique (such as working with and culturing bacteria), it is important not to introduce contaminating bacteria (or mold or fungi) into the experiment. Because contaminating bacteria are ubiquitous and are found on fingertips, benchtops, etc., it is important to avoid these contaminating surfaces. When students are working with the pipets and agar plates, you should stress that the tip of the pipet and the surface of the agar plate should not be touched or placed onto contaminating surfaces. While some contamination will likely not ruin the experiment, students would benefit from an introduction to the idea of sterile technique. Using sterile technique is also an issue of human cleanliness and safety.

Use of the Pipet

Before beginning the laboratory sessions, point out the graduations on the disposable plastic transfer pipet (DPTP) to the students. To properly use a DPTP, depress the bulb to expel air from the DPTP prior to inserting the tip into the solution to be transferred. Slowly release the bulb to draw the desired volume of solution up and depress slowly to expel the solution.



If available, p1000 micropipets and sterile tips are the preferred option for this activity, as they are easier to use and provide more consistent results than DPTPs.

Incubation

This guide is written to reflect the use of a 37°C incubator. Growing *E. coli* OP50-pBAD in preparation for the *C. elegans* subculture laboratory activity can be done without an incubator. However, the number of days required to grow an *E. coli* lawn to the optimum size depends on the ambient temperature.

Generating *E. coli* Lawns for Subculturing *C. elegans*

The optimum temperature for growing *E. coli* is 37°C (98.6°F); lower temperatures will result in a slower growth rate. At 28°C (82°F), 2 days of incubation are required to obtain optimum lawn size. At 21°C (70°F), 3 days of incubation are required to obtain optimum lawn size. Adjust the advance preparation lead times and laboratory schedule according to your incubation temperature. Incubation of *E. coli* is required as a preparatory activity immediately prior to Lab Activity 1. DO NOT incubate *C. elegans* as this will kill them.

The *C. elegans* feed on the lawn of *E. coli*. The *C. elegans* prefer a lawn of fresh and actively growing bacteria. Therefore, it is important to adjust your advance preparation of the plates with *E. coli* lawns used for subculturing so that you do not need to place the plates in the refrigerator before use. Plating 1–3 days before use, depending on whether you use incubators, is critical.

Instructor's Advance Preparation

The timeline provided with this instruction manual is general because there are many variables that can affect the exact time frame in which tasks should be performed in order to obtain optimal results. Certain tasks, such as pouring and drying the NGM Lite agar and assay agar plates can be done well in advance, with the plates stored in their sleeves at 4°C until ready to be used. Other tasks require careful observation of your *C. elegans* cultures to determine readiness to move to the next step.

Your NGM Lite agar plates must have cured for at least 2 days before you thaw and plate the initial tubes of *C. elegans*.

You must have plated your *E. coli* and have lawns prior to subculturing *C. elegans*.

Assay agar plates must be poured at least 2 days prior to the chemotaxis experiment, and salt gradient plates must be prepared 12 to 24 hours prior to the experiment.

1 WEEK PRIOR TO LAB ACTIVITY 1

Objectives:
- Pour 40 NGM Lite agar plates
- Pour 20 assay agar plates
- Redeem *C. elegans* coupon
- Thaw *C. elegans*, plate, and expand population
- Prepare *E. coli* OP50-pBAD bacterial lawns

Materials Needed for Advance Preparation	Quantity	(✓)
NGM Lite agar	1 pouch	☐
Distilled water	1 liter	☐
Erlenmeyer flask, 1 liter	1	☐
Microwave	1	☐
Graduated cylinder, 500 ml	1	☐
Petri dishes	60	☐
Ampicillin	1 vial	☐
Cholesterol	1 tube	☐
Assay agar	1 pouch	☐
Erlenmeyer flask, 500 ml	1	☐
100–1,000 µl adjustable-volume micropipet and tips	1	☐
C. elegans wild-type, 1 ml	1 tube	☐
C. elegans mutant, 1 ml	1 tube	☐
E. coli, OP50-pBAD	1 vial	☐
Incubator set to 37°C (optional)	1	☐
Water bath set to 50°C (optional)	1	☐
Marking pen	1	☐

1. Pouring NGM Lite agar plates

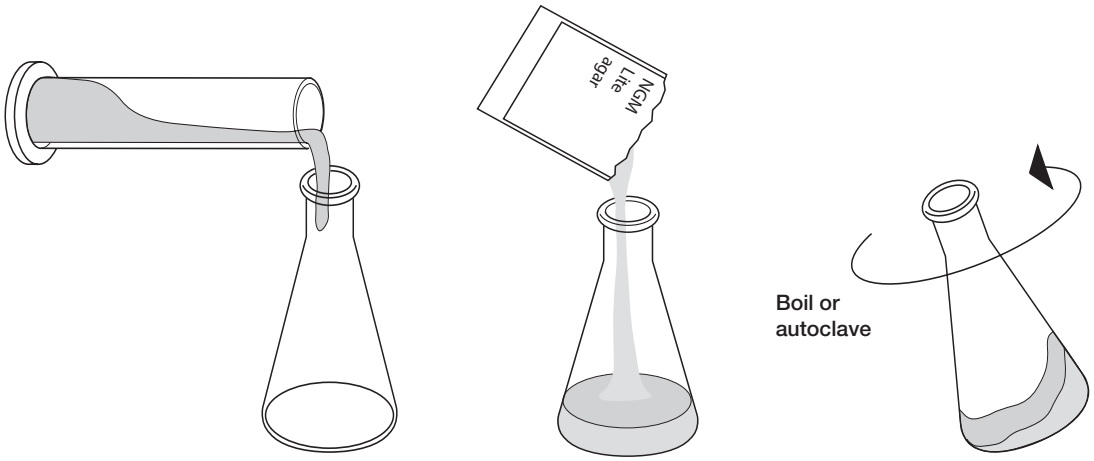
The agar plates should be prepared at least 2 days before the *C. elegans* are thawed. The poured NGM Lite agar plates should be left out at room temperature for 2 days and then refrigerated in a sealed sleeve until they are to be used. It is important that the plates sit out for 2 days on the benchtop as it allows the agar to dry out (cure) sufficiently to readily take up the excess liquid after thawing and plating the *C. elegans*.

A. Prepare NGM Lite agar

Option 1: Microwave or hot plate boiling

To prepare the NGM Lite agar, add 400 ml of distilled water to a 1,000 ml or larger Erlenmeyer flask. Do not use a beaker. Add the entire contents of the NGM Lite agar pouch. Swirl the flask to dissolve the NGM Lite agar, and heat to boiling in a microwave oven or on a hot plate. You must monitor the agar to ensure it does not boil over.

If the agar boils over, reduce the microwave power or hot plate heat until a simmer is reached. Agar should not boil over as this will create an inappropriate amount of solution for the number of plates needed and the concentration of the components in the NGM Lite agar may be changed, creating inadequate growing conditions. Simmer the boiling agar for 2 min. When all of the agar is dissolved cover the flask and allow the NGM Lite agar to cool so that the outside of the flask is just comfortable to hold. While the NGM Lite agar is cooling, prepare the ampicillin and label the plates as outlined below in steps B and C. Be careful not to let the NGM Lite agar cool so much that it begins to solidify. Keeping the flask with molten NGM Lite agar in a water bath set to 50ºC can help prevent the agar from cooling too quickly.



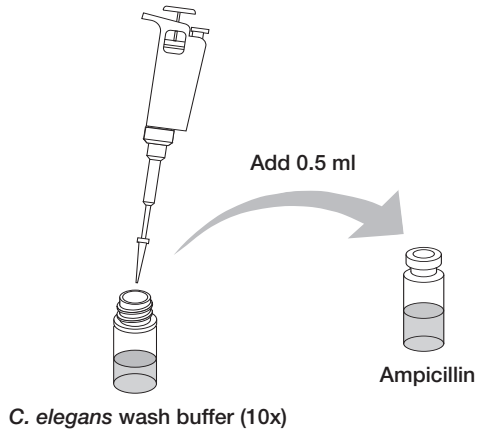
Option 2: Autoclave

To prepare the NGM Lite agar, add 400 ml of distilled water to a 1,000 ml or larger Erlenmeyer flask. Do not use a beaker. Add the entire contents of the NGM Lite agar pouch. Swirl the flask to dissolve the NGM Lite agar. Loosely cover the flask with aluminum foil and autoclave at 121ºC on a liquid cycle for 30 min. Allow the NGM Lite agar to cool so that the outside of the flask is just comfortable to hold.

While the NGM Lite agar is cooling, prepare the ampicillin and label the plates as outlined below in steps B and C. Be careful not to let the NGM Lite agar cool so much that it begins to solidify. Keeping the flask with molten NGM Lite agar in a water bath set to 50ºC can help prevent the agar from cooling too quickly.

B. Prepare ampicillin

Ampicillin is shipped dry in a small vial. With a new pipet tip and a 100–1,000 µl adjustable-volume micropipet, add 0.5 ml of *C. elegans* wash buffer (10x) directly to the vial to rehydrate the antibiotic. (Sterile water would work just as well.)



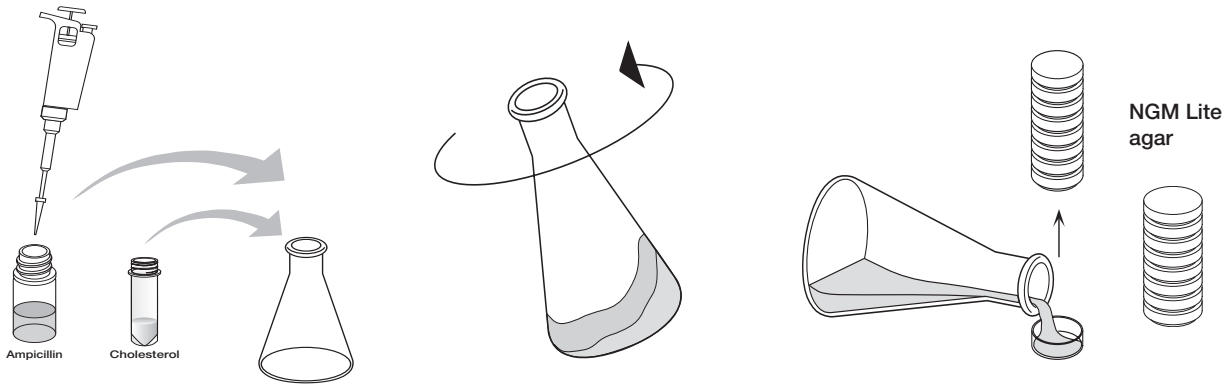
C. Label plates

Label 40 plates "NGM" with a permanent marker on the bottom, close to the edge. Do not label the lids.

D. Pour NGM Lite agar plates

Note: Excessive heat (>60°C) will destroy the ampicillin, but the nutrient agar solidifies at 40°C. So carefully monitor the cooling of the agar and then pour the plates from start to finish without interruption. Keeping the flask with molten agar in a water bath set to 50°C can help prevent the agar from cooling too quickly.

Using a 100–1,000 µl adjustable-volume micropipet and new tips, add the contents of the cholesterol solution tube (200 µl) and the hydrated ampicillin (0.5 ml) to the NGM Lite agar. Swirl briefly to mix. Pour approximately 10 ml of solution into each of the 40 labeled plates. After the plates are poured do not disturb them until the agar has solidified. Pour excess agar in the garbage, not the sink. Wipe any agar drips off the sides of the plates.



E. Plate storage

Ampicillin is light sensitive, therefore the NGM Lite agar plates should be cured in the dark at room temperature. After the plates have cured for 2 days they may be either used or stored in their original plastic sleeve bag. Place the plates in the sleeve, tape the bag closed, invert, and store at 4ºC in the dark until you are ready to use them. By covering the plates with the plastic bag, the plates will stay hydrated. Invert the plates to prevent condensation on the lid, which may drip onto the agar.

2. Pouring assay agar plates

The assay agar plates should be prepared at least 2 days before the student chemotaxis experiment is performed. They should be left out at room temperature for two days and then refrigerated until they are to be used. The two days on the benchtop allows the agar to dry out (cure) sufficiently to readily take up NaCl and vehicle solutions.

A. Prepare assay agar

Option 1: Microwave or hot plate boiling

To prepare the assay agar, add 180 ml of distilled water and **20 ml of *C. elegans* wash buffer (10x)** to a 500 ml or larger Erlenmeyer flask. Do not use a beaker. Add the entire contents of the agar pouch. Swirl the flask to dissolve the agar, and heat to boiling in a microwave oven or on a hot plate. You must monitor the agar to ensure it does not boil over.

If the agar boils over, reduce the microwave power or hot plate heat until a simmer is reached. Simmer the boiling agar for 2 min. When all the agar is dissolved, cover the flask and allow the agar to cool so that the outside of the flask is just comfortable to hold. While the agar is cooling, label the plates as described in step B. Be careful not to let the agar cool so much that it begins to solidify. Keeping the flask with molten agar in a water bath set to 50ºC can help prevent the agar from cooling too quickly.

Option 2: Autoclave

To prepare the assay agar, add 180 ml of distilled water and **20 ml of *C. elegans* wash buffer (10x)** to a 500 ml or larger Erlenmeyer flask. Do not use a beaker. Add the entire contents of the agar pouch. Swirl the flask to dissolve the agar. Cover the flask with aluminum foil and autoclave at 121ºC on a liquid cycle for 30 min. Allow the assay agar to cool so that the outside of the flask is just comfortable to hold. While the agar is cooling, label the plates as described in step B. Be careful not to let the agar cool so much that it begins to solidify. Keeping the flask with molten agar in a water bath set to 50ºC can help prevent the agar from cooling too quickly.

B. Label plates

Label 20 petri dishes “Assay” with a permanent marker on the bottom, close to the edge. Do not label the lids.

C. Pour assay agar plates

Pour approximately 10 ml of solution into each plate. After the plates are poured do not disturb them until the agar has solidified. Pour excess agar in the garbage, not the sink. Wipe any agar drips off the sides of the plates.

D. Plate storage

After the plates have cured for 2 days at room temperature they may be either used or stored in their original plastic sleeve bag. Place the plates in the bag, tape the bag closed, invert, and store at 4ºC until you are ready to use them. By covering the plates with the plastic bag the plates will stay hydrated. Invert the plates to prevent condensation on the lid, which may drip onto the agar.

3. *C. elegans* redemption

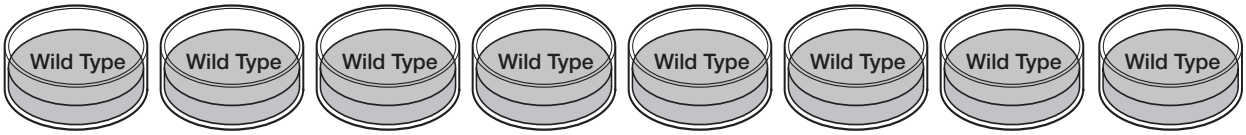
You should not request shipment of *C. elegans* wild-type and mutant strains until you have poured your NGM Lite agar plates. Follow the instructions on the redemption card to have your *C. elegans* strains shipped overnight on dry ice to your location.

4. Thaw *C. elegans*

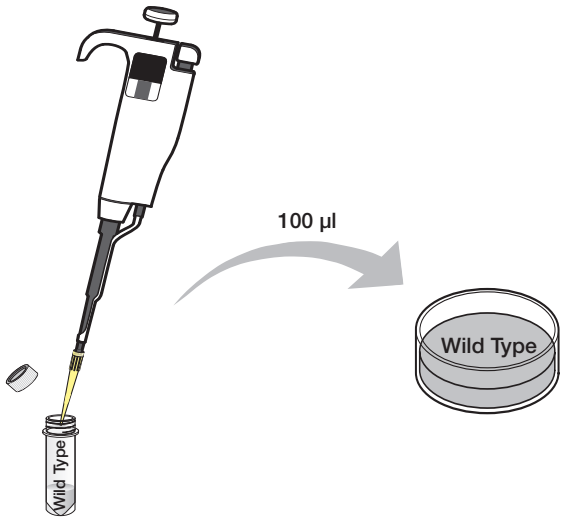
Remove the tubes containing the *C. elegans* wild-type and mutant strains from the dry ice container. Place the tubes on a lab bench in a location out of the sun and away from a heater or air vent. Allow the tubes to thaw until all ice has turned into liquid (approximately 15 min). Do not warm the tubes using your hands!

5. Plate *C. elegans* (wild type and mutant) for 8 student workstations

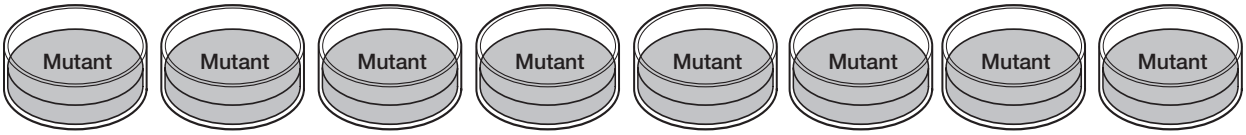
A. Label 8 NGM Lite agar plates “Wild Type” on the bottom of the plate (not on the lid).



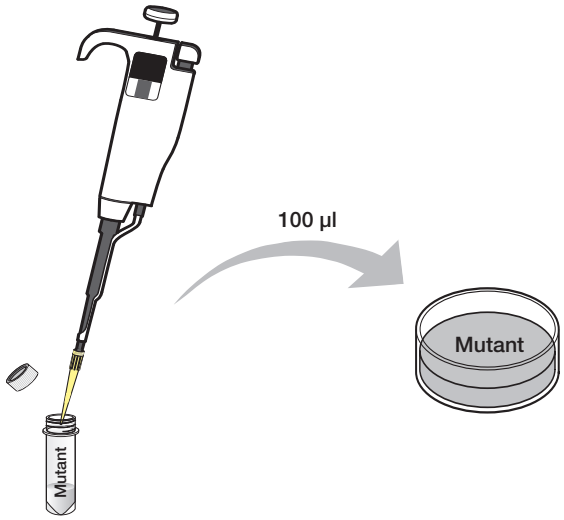
B. Gently invert the tube of ***C. elegans, wild type*** five times to mix *C. elegans* into solution. Using a 100–1,000 µl adjustable-volume micropipet and a new tip, transfer 100 µl of the wild-type *C. elegans* to each of the NGM Lite agar plates labeled “Wild Type.”



C. Label 8 NGM Lite agar plates “Mutant” on the bottom of the plate (not on the lid).



D. Gently invert the tube of **mutant *C. elegans*** five times to mix *C. elegans* into solution. Using a 100–1,000 µl adjustable-volume micropipet and a new tip, transfer 100 µl of the mutant *C. elegans* to each of the NGM Lite agar plates labeled “Mutant.”



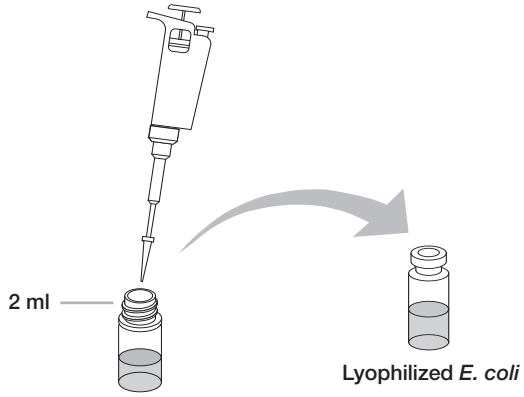
E. Examine plates under a dissection microscope to verify the transfer of *C. elegans* from the tube to the plates. Place the plates at room temperature out of direct sunlight and away from heaters or vents.

6. Allow *C. elegans* to recover and populations to expand

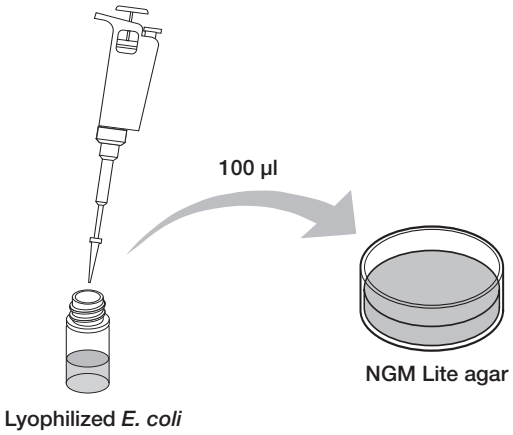
Allow the *C. elegans* to grow on the NGM Lite agar plate for 5–7 days. *C. elegans* will come to life after several hours. The surviving *C. elegans* tend to be the small L1 larvae and may be difficult to see. The initial culture contains bacteria for the worms to eat. You will notice bacterial growth before you may observe *C. elegans*. The *C. elegans* should become apparent 2–3 days after thawing and plating. You should see worm trails in your bacterial lawn. This is evidence that *C. elegans* is feeding.

7. Once your culture is established you are ready to subculture. Reconstitute *E. coli* OP50-pBAD and spread 16 NGM Lite agar plates with reconstituted *E. coli* OP50-pBAD

A. Using a 100–1,000 µl adjustable-volume micropipet and a new tip, rehydrate the lyophilized *E. coli* OP50-pBAD by adding 2 ml of *C. elegans* wash buffer (10x) directly to the vial. Recap the vial and shake it gently to ensure all bacteria are rehydrated.



B. Transfer 100 µl of rehydrated *E. coli* OP50-pBAD to the center of each of the 16 NGM Lite agar plates. Swirl gently to disperse the solution. Allow the solution to fully absorb, and then incubate the plates at 37°C for 16–24 hr. If an incubator is not available, the plates can be left at room temperature for 24–48 hrs.



1 Day Prior to Student Lab Session 1 — Instructor's Advance Preparation for Laboratory

Session 1

Objectives: Dilute and dispense *C. elegans* wash buffer

Set up student and common workstation for laboratory session

Materials Needed for Advance Preparation	Quantity	(✓)
Distilled water	18 ml	☐
C. elegans wash buffer (10x)	2 ml	☐
Sterile container that can hold 20 ml of solution	1	☐
100–1,000 µl adjustable-volume micropipet tips	1	☐
Graduated cylinder	1	☐
Microcentrifuge tubes, 2.0 ml	8	☐

1. Dilute and dispense *C. elegans* wash buffer

A. To prepare the *C. elegans* wash buffer, add 18 ml of distilled water and 2 ml of *C. elegans* wash buffer (10x) to an appropriately size sterile container to make a 1x *C. elegans* wash buffer. Cap and invert to mix.

B. Label eight 2.0 ml microcentrifuge tubes "Wash."

C. Dispense 2 ml of 1x *C. elegans* wash buffer into each 2 ml microcentrifuge tube labeled "Wash." Do not discard the remaining *C. elegans* wash buffer. Some student groups may require extra *C. elegans* wash buffer.

2. Set up student workstations

Student workstation

Materials	Quantity	(✓)
NGM Lite agar plate with *E. coli* OP50-pBAD lawn	2	☐
Microcentrifuge tube, 1.5 ml	2	☐
NGM Lite agar plate with *C. elegans* wild type	1	☐
NGM Lite agar plate with *C. elegans* mutant	1	☐
C. elegans wash buffer (1x), 2 ml	1	☐
Disposable plastic transfer pipet (DPTP)	2	☐
2–20 µl adjustable-volume micropipet and tips	1	☐
Marking pen	1	☐
Scissors	1	☐
Dissection microscope (10–40x magnification)	1	☐

1 Day Prior to Student Lab Session 2 — Instructor's Advance Preparation for Laboratory

Session 2

Objectives: Dilute *C. elegans* wash buffer
Prepare salt gradients on assay agar plates
Allow gradients to diffuse overnight
Set up student workstations

Materials Needed for Advance Preparation	Quantity	(✓)
Distilled water	36 ml	☐
C. elegans wash buffer (10x)	4 ml	☐
Sterile container that can hold 40 ml of solution	1	☐
100–1,000 µl adjustable-volume micropipet and tips	1	☐
Graduated cylinder	1	☐
Microcentrifuge tubes, 2.0 ml	16	☐
Marking pen	1	☐
Assay agar plates	16	☐
2.5 M NaCl solution	1 tube	☐

1. Prepare and aliquot *C. elegans* wash buffer

A. To prepare the *C. elegans* wash buffer, add 36 ml of distilled water and 4 ml of *C. elegans* wash buffer (10x) to an appropriately sized sterile container to make a 1x *C. elegans* wash buffer. Cap and invert to mix.

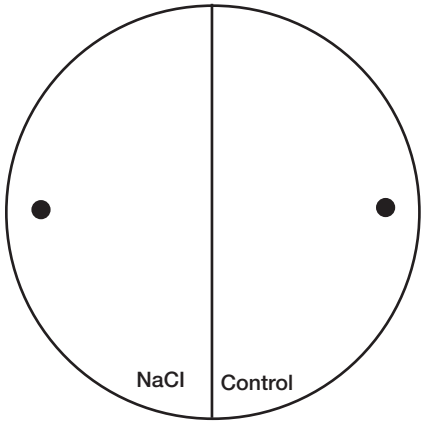
B. Label 16 microcentrifuge tubes (2.0 ml) "Wash."

C. Dispense 2 ml of 1x *C. elegans* wash buffer into each 2 ml microcentrifuge tube labeled "Wash." Do not discard the remaining *C. elegans* wash buffer. Some student groups may require extra *C. elegans* wash buffer.

2. Prepare salt gradients on assay agar plates

A. Using a fine tipped marking pen, draw a straight line down the center of the bottom of 16 assay **agar plates**. Label one side of each plate "NaCl" and the second side "control."

B. Draw 2 dots about 0.5 cm from the edge of each plate (see figure below). Flip the plates right side up and, using a sterile micropipet, transfer 10 µl of the 2.5 M NaCl solution to the spot on the NaCl half of each plate and 10 µl of 1x *C. elegans* wash buffer (the control) to the spot on the control half of each plate. Cover plates.



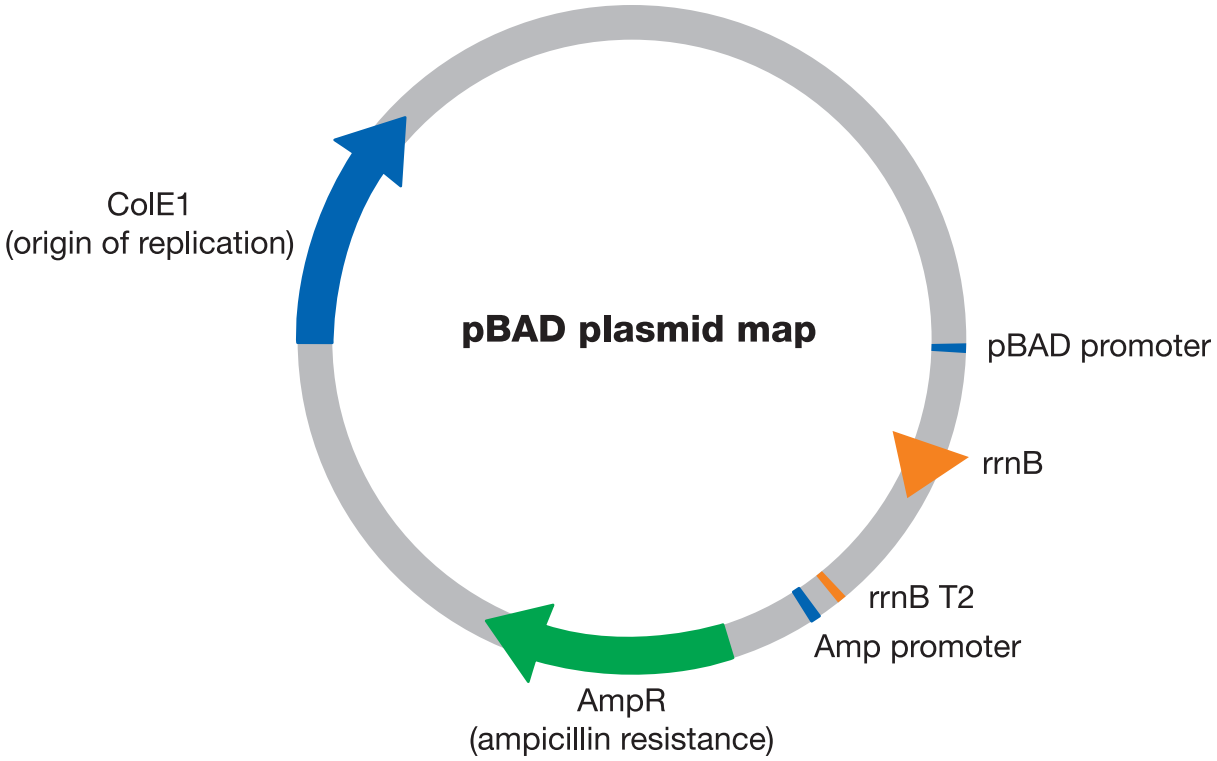
3. Incubate Assay agar plates at room temperature overnight.

Let salt gradient form by allowing diffusion to occur for 12–24 hours.

4. Set up student workstations

Student workstation

Materials	Quantity	(✓)
Microcentrifuge tube, 1.5 ml	2	☐
Assay agar plate with salt gradient	2	☐
DPTPs	4	☐
C. elegans wash buffer (1x), 2 ml	2	☐
2–20 µl adjustable-volume micropipet and tips	1	☐
Microcentrifuge tubes, 2.0 ml	1	☐
Waste container	1	☐
Marking pen	1	☐
Dissection microscope (10–40x magnification)	1	☐



Plasmid map of pBAD, which is transformed into the E. coli OP50 to confer ampicillin resistance on the bacteria. Ampicillin resistance reduces the chance of contamination by unwanted bacteria during the experiment.

Background

C. elegans: A Simple Multicellular Model Organism

Scientists worldwide conduct basic research to address gaps in our knowledge in the hopes that this information can serve humanity in the future. Basic biological research seeks to answer questions of such elementary cellular and organismal activities as how cells grow, divide, die, move, store and use energy, and communicate.

Scientists use model organisms in basic research to answer these questions because model organisms offer simplified cellular systems that reproduce quickly, are easy to maintain, and are cost efficient. For example, if a DNA mutation is known to result in a neurological disorder, more data can be generated using a model organism such as *C. elegans*, which reproduces and matures every 2–3 days, rather than waiting for a human child to mature and show symptoms. Commonly used basic model organisms include *S. cerevisiae* (yeast), *C. elegans* (nematode), *D. melanogaster* (fruit fly), and *M. musculus* (mouse).

Despite the seeming lack of a relationship to human beings, these model organisms have helped researchers understand the basic cellular machinery underlying a host of human pathologies such as cancer, neurological disorders, and aging. Furthermore, research studies utilizing model organisms have helped develop powerful tools used by scientists in applied research that include RNA interference (RNAi), DNA microarrays, and genome sequencing.

Combined, the scientific knowledge and breakthroughs that have been gleaned through the use of model organisms have helped to significantly advance the knowledge of basic biology as well as human function and pathologies. Studies that utilize model organisms often provide the data to both justify and direct cutting-edge therapeutic technologies.

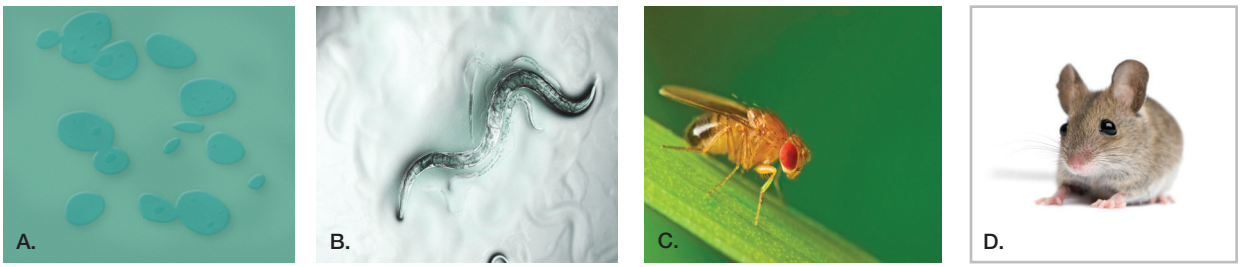


Fig. 1. Commonly used model organisms. A, *S. cerevisiae* (yeast); **B**, *C. elegans* (nematode); **C**, *D. melanogaster* (fruit fly); **D**, *M. musculus* (mouse).

Research on model organisms from prokaryotic (bacteria) to single cell eukaryotic (yeast), multicellular eukaryotic (fruit flies and worms), and mammalian models (mice and rats) have shown that basic cellular operating principles are shared between all living things. A finding made in bacteria can shed light on a biological principle in eukaryotes, while a finding made in *C. elegans* may shed light on a biologic process in humans.

Generally, the underpinnings of a basic biologic principle are studied in the simplest organism possible, and as scientific knowledge is generated, the principles learned are studied in increasingly complex organisms until we finally examine these principles in humans. The scientific knowledge acquired by studying model organisms often could not have been acquired by studying humans or other complex mammals due to ethical considerations, long lifespan, high cost, or, in the case of humans, the lack of a uniform genetic background and environmental conditions.

C. elegans was selected as an experimental model organism in the early 1960s by the Nobel Prize–winning researcher Sydney Brenner. *C. elegans* is a multicellular eukaryotic organism that, like humans, is made up of specialized cells and complex systems such as neurons, muscle cells, a digestive and excretory system, and a reproductive system.

C. elegans was the first multicellular organism to have its genome completely sequenced. As a result, its genetics are well understood. And since the human genome has also been sequenced, we now know that *C. elegans* and humans share a large number of conserved genes and cellular mechanisms.

C. elegans: An Introduction

C. elegans is a barely visible, free-living (not parasitic), microscopic roundworm (nematode) that reaches approximately 1 mm in size when fully grown. These particular nematodes have been found and isolated in temperate soil environments worldwide. In nature, they feed on the bacteria that help to decay decomposing plant matter. In the laboratory, however, *C. elegans* is typically fed lab strains of *E. coli*, most commonly *E. coli* OP50. *C. elegans* is not disease-causing and is not known to cause harm to any living organisms other than its prey, bacteria.

C. elegans is a transparent nematode, thus facilitating the study of the cellular differentiation and developmental fate of each of its cells. *C. elegans* can be found in two sexes, hermaphrodites and males. Hermaphrodites have 959 somatic cells while males have 1,031 cells. The developmental precursors of every *C. elegans* cell have been fully mapped by researchers. This deep knowledge of the cellular developmental fate of the organism facilitated a detailed study of the genetics of organ development and programmed cell death for which Sydney Brenner, H. Robert Horvitz, and John Sulston were awarded the Nobel Prize in Physiology or Medicine in 2002.

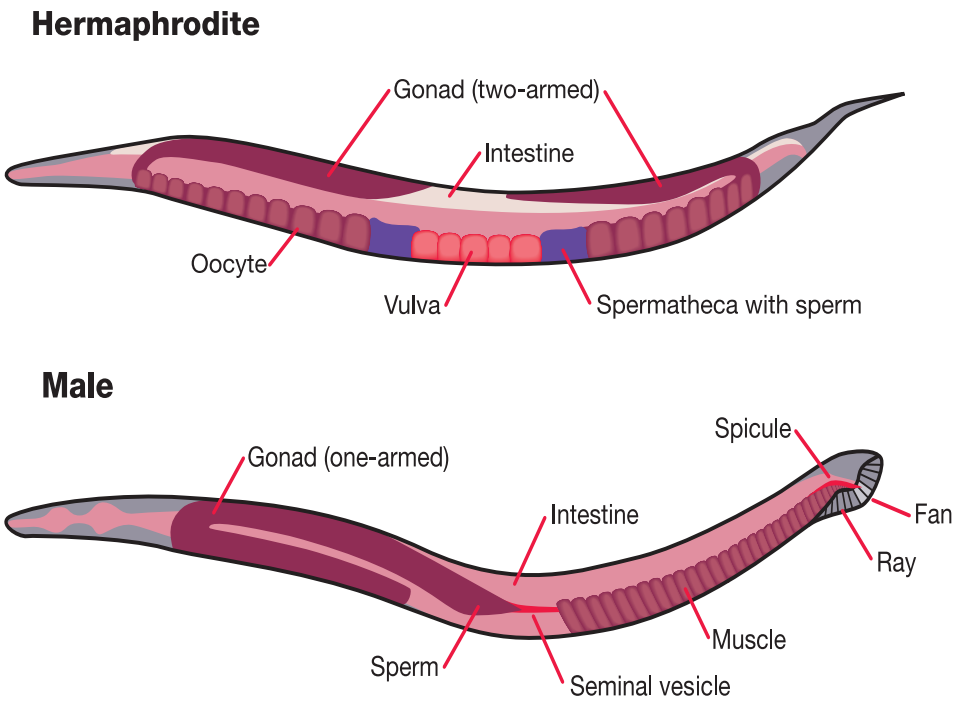


Fig. 2. *C. elegans* anatomy.

C. elegans: Reproduction

As previously stated, *C. elegans* can be found in one of two sexes: hermaphrodites or males. *C. elegans* hermaphrodites contain both male and female sex organs and are capable of self-fertilizing to produce viable progeny in the absence of other *C. elegans*. When self-fertilized, *C. elegans* will typically lay 300–400 eggs. Hermaphroditic *C. elegans* are convenient for laboratory use because no husbandry is required to maintain the laboratory strains.

On the other hand, *C. elegans* males contain only male sex organs and typically comprise just 0.05% of the population. Males can inseminate a hermaphrodite, which could then go on to lay as many as 1,000 eggs. The ability to mate males with hermaphrodites is important to researchers studying the function of specific genes and genetic interactions as they are able to crossbreed *C. elegans* strains. Males can be visually distinguished from hermaphrodites by the presence of a specialized tail that is used during mating.

C. elegans: Lifecycle

One of the greatest advantages of working with *C. elegans* is its short life span of 2–3 weeks. After hatching from an egg, *C. elegans* pass through four distinct juvenile developmental stages (L1–L4) before reaching a fully mature adult stage. Between developmental stages, *C. elegans* undergo a molting process in which the old cuticle exoskeleton is shed, revealing a newly synthesized cuticle. Additionally, *C. elegans* increase in size between each developmental stage, getting progressively larger in a stepwise fashion as the worms develop from L1 to L4 larvae and finally to adults. Growth progresses from approximately 250 µm to approximately 1,100 µm.

When they are crowded or don't have enough food, L1 and L2 *C. elegans* larvae can enter an alternative specialized larval stage called *dauer*. Dauer larvae can be morphologically distinguished from other developmental stages as they are thinner than normal and their oral orifice is plugged, preventing the organism from feeding. The dauer larvae are highly stress-resistant and live much longer than regular *C. elegans*.

Because the dauer larvae have an extended lifespan they have been extensively used to study aging and are being investigated for longevity-promoting factors. Additionally, dauer larvae allow *C. elegans* to be stored on plates for over a month, as long as the plates are sealed to prevent moisture loss. Once favorable conditions are encountered, such as a rich feeding environment, dauer larvae reenter development in the L4 stage, where they continue to develop normally and can live for 2 more weeks.

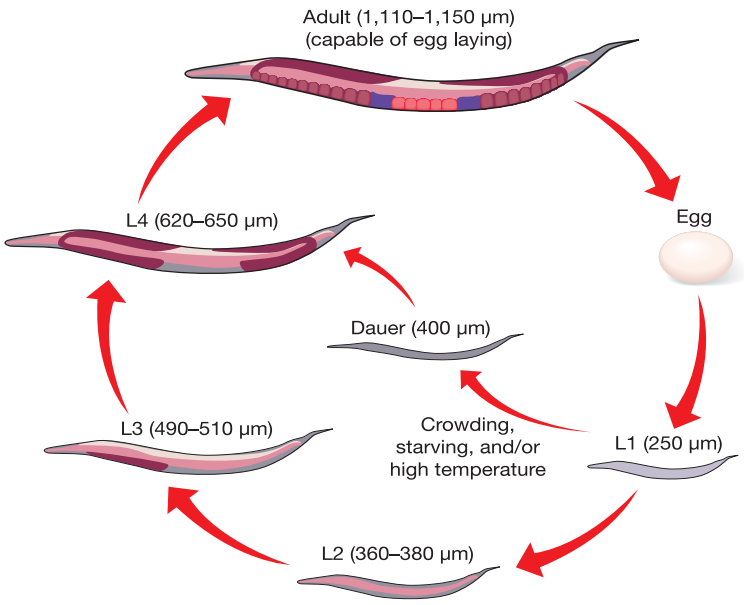


Fig. 3. *C. elegans* life cycle.

C. elegans: Genetics

C. elegans have six chromosomes; five pairs of autosomes (chromosomes I, II, III, IV, and V) and the sex chromosome, X (the letter, not the roman numeral). Hermaphrodites have a pair of X chromosomes (XX) while males have just one X chromosome (XO). Because males have a chromosome that is not paired, it is considered to be hemizygous X (a single chromosome).

C. elegans was the first multicellular organism to have its entire genome completely sequenced. The full genome was first published in October 2002 and consisted of approximately 100 million base pairs. As of 2012, we know that the *C. elegans* genome contains approximately 26% introns, 20,470 protein coding genes, and approximately 16,000 RNA coding genes (involved in gene expression control). However, as new classes of noncoding RNAs continue to be discovered, these numbers may change to reflect future discoveries.

C. elegans: Neurology and Behavior

C. elegans is one of the simplest organisms with a nervous system. Of the 959 cells making up a hermaphrodite, 302 (31%) are neurons. The organism's neural circuit is extremely primitive when compared to the approximately 100 billion neurons that make up the average human brain and the 60 trillion synapses that these neurons form. However, as a result of the transparent nature of *C. elegans*, and the well characterized cell lineages, the entire pattern of neuron connections, or connectome, has been completely mapped.

Despite the relatively simple neuronal system present within *C. elegans*, it displays many primitive and complex behaviors. Primitive behaviors include feeding, locomotion, and reproduction. Complex behaviors include learning, mating, and social behaviors. Ultimately, all behaviors reflect nervous system activity.

Researchers are currently using the known *C. elegans* genome and connectome to try to understand how simple neuronal circuits yield such complex behaviors. Investigators are deconstructing the genetics and neurology behind some of these behaviors in *C. elegans* in the hope of better understanding human neurology and how it translates into our complex function and behavior.

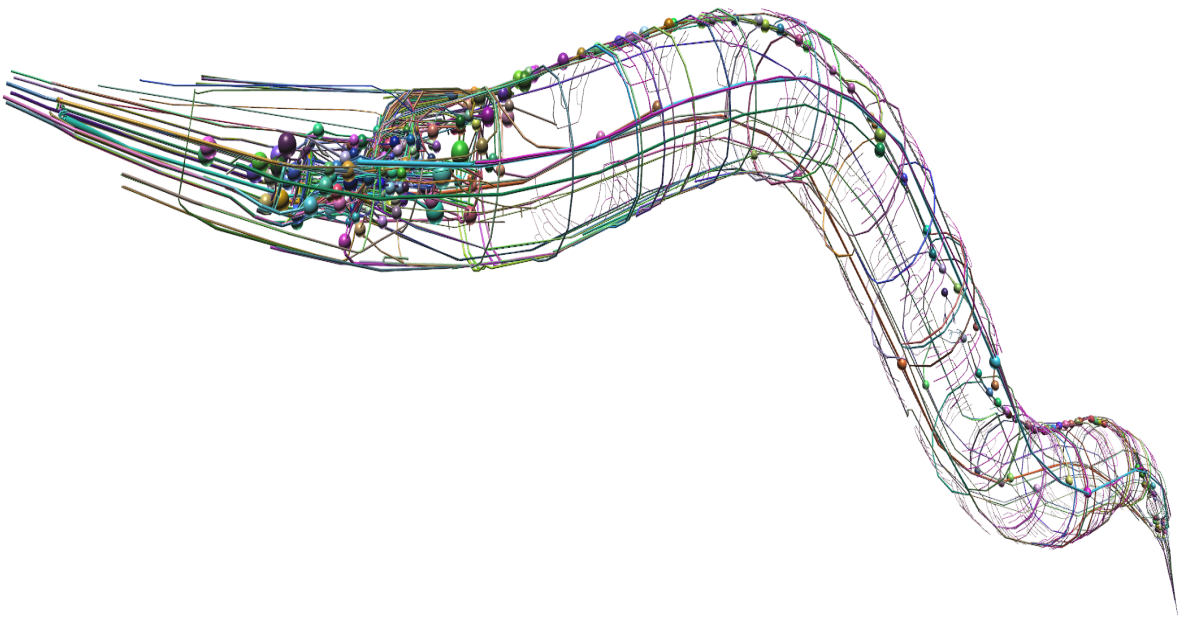


Fig. 4. *C. elegans* connectome.

The *C. elegans* behavior kit is a laboratory activity that explores the role of a single gene, *daf-18*, which regulates associative learning behavior (Adachi et al. 2010). The *daf-18* gene encodes a phosphatase, a class of enzyme that catalyzes the removal of phosphate groups from molecules that are typically involved in intracellular signaling. These molecules are involved in the modulation of a host of cellular activities, such as the regulation of cell proliferation, migration, adhesion, and development.

In *C. elegans*, deletion of the *daf-18* gene or inhibition of its expression results in altered intracellular neural signaling as well as altered neuronal development. These mutant *daf-18* cellular phenotypes translate into a *C. elegans* that presents a defect in associative learning behavior.

In 1902, the Russian physiologist Ivan Pavlov performed a series of famous experiments on conditioning. Pavlov repeatedly presented a dog with food following the ringing of a bell. After some time of conditioning the dog in this manner, when the dog heard the sound of a bell ringing, the dog would respond as if food were present by salivating. The dog in these famous set of experiments had learned to associate the sound of the ringing bell with food (Nobelstiftelsen, 1964). This is called associative learning.

In a similar manner, when *C. elegans* are fed bacteria in the presence of salt, which is an ingredient in NGM Lite agar plates, the *C. elegans* learn to associate salt with food. If the *C. elegans* are then collected, washed of *E. coli*, and transferred to an assay agar plate that contains no nutrients but instead has a point source of salt, the *C. elegans* will migrate toward the salt in search of food.

Associative learning can also work in the opposite manner. If *C. elegans* run out of bacterial food while on NGM Lite agar plates with salt, the *C. elegans* will then associate salt with starvation. If starved *C. elegans* are transferred to assay agar plates with a point source of salt, *C. elegans* will move away from the salt. Movement toward a chemical is called positive chemotaxis while movement away from a chemical is called negative chemotaxis.

C. elegans that have a *daf-18* mutation, however, have an associative learning behavior defect. This mutant strain of *C. elegans* is unable to make associations between a good feeding environment and salt. Thus, when *daf-18*–deficient *C. elegans* are transferred to a salt chemotaxis assay, they will migrate randomly across the plate, without direction, as they are not taking into account the point source of salt in their hunt for bacterial food.

It is important to note that *daf-18* mutants can still sense NaCl. While *daf-18* is a gene that is expressed in the NaCl sensing neurons, its protein, DAF-18, is not required for NaCl sensing. The phenotype observed in this laboratory is due to *C. elegans*' inability to learn rather than its inability to sense.

The human homolog to the *daf-18* gene is the *PTEN* gene. Human *PTEN*, like *daf-18*, encodes a protein phosphatase. In a manner similar to *C. elegans*, *PTEN* mutations have been associated with a number of pathologies, including cancer, autism, and learning disoders (Shi et al. 2010, Zhou and Parada 2012, Conti et al. 2012). These similarities in gene coding and associated phenotypes between *daf-18* and *PTEN* underscore the value of utilizing model organisms such as *C. elegans*.

Expected Results

Thawing of *C. elegans*

Frozen *C. elegans* are provided as a mixed culture containing all stages of *C. elegans* development (L1–L4, adults, and eggs) along with *E. coli* OP50-pBAD. Once fully thawed, it is important to fully resuspend *C. elegans* through gentle inversion as *C. elegans* are denser than the freezing liquid and will quickly settle to the bottom of the tube. Typically, adult *C. elegans* do not survive the freezing process as well as L1–L2 larvae. Thus, the majority of surviving *C. elegans* will be smaller in size and may be difficult to see under low magnifications. *C. elegans* will typically start to reanimate after about 15–30 min but may take up to several hours, depending on the temperature. Keep *C. elegans* away from direct sunlight during this period.

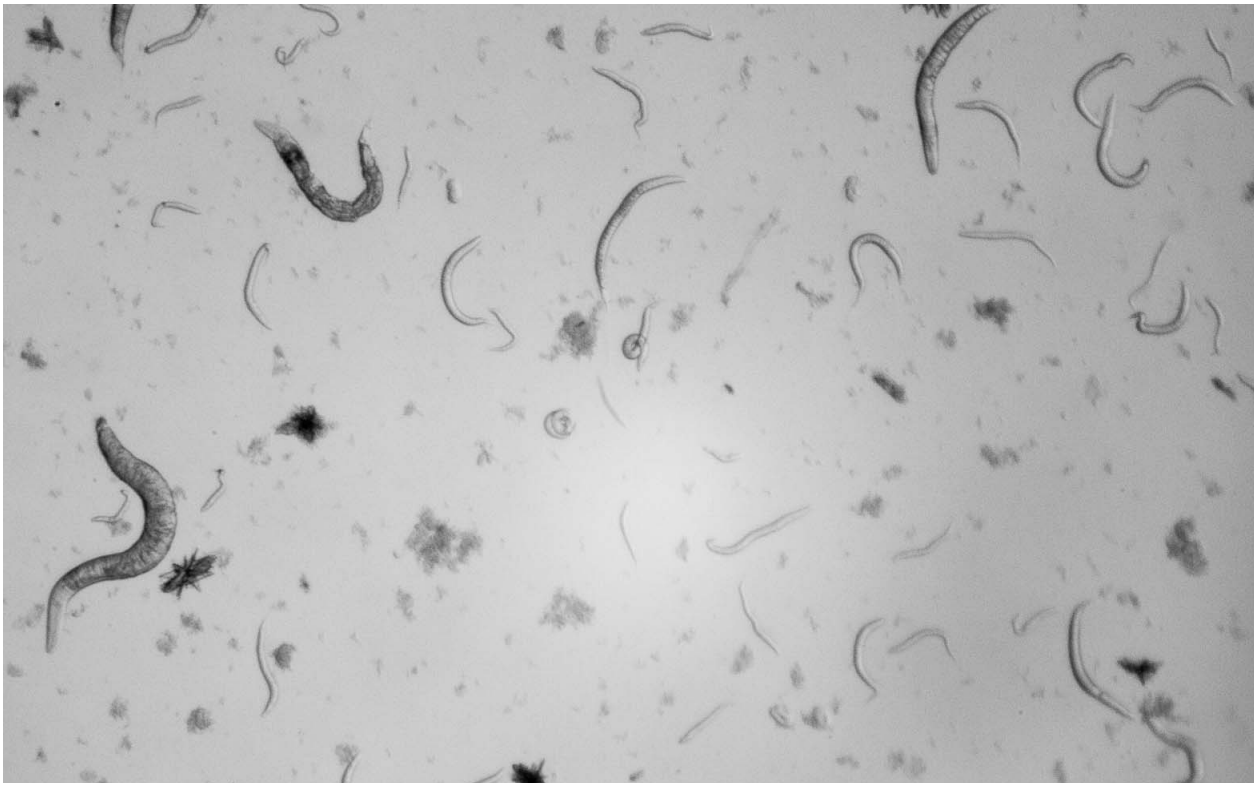


Fig. 5. 10x image of wild-type *C. elegans* after thawing.

Growth after Thaw

Frozen vials of *C. elegans* contain low quantities of *E. coli* OP50-pBAD in addition to the *C. elegans* supplied in the tube. While the frozen *C. elegans* can be readily observed after thawing, the supplied *E. coli* OP50-pBAD may not be apparent initially due to the low concentration provided and their extremely small size. Growth of *C. elegans* occurs after thawing at room temperature. While the growth of *E. coli* is greatest at 37°C, *E. coli* will still grow at a faster rate than *C. elegans* at room temperature. Thus, in the days following the initial thaw of *C. elegans*, the *E. coli* lawn will grow thick, potentially obscuring easy visibility of the *C. elegans*.

A very thick lawn of *E. coli* is desirable in the days after the thaw to support the nutritional needs of the *C. elegans* population as it expands. Since *C. elegans* will be covered in *E. coli* OP50-pBAD during this period, and *C. elegans* may explore the plate, they will tend to spread *E. coli* OP50-pBAD throughout the plate as they move. This seeding of the plate with bacteria is normal and desirable since the growth of *E. coli* OP50-pBAD will provide additional food for the *C. elegans* on the plate. As the *C. elegans* consume the *E. coli*, the *E. coli* lawn will become thin and the growing *C. elegans* population will become apparent.

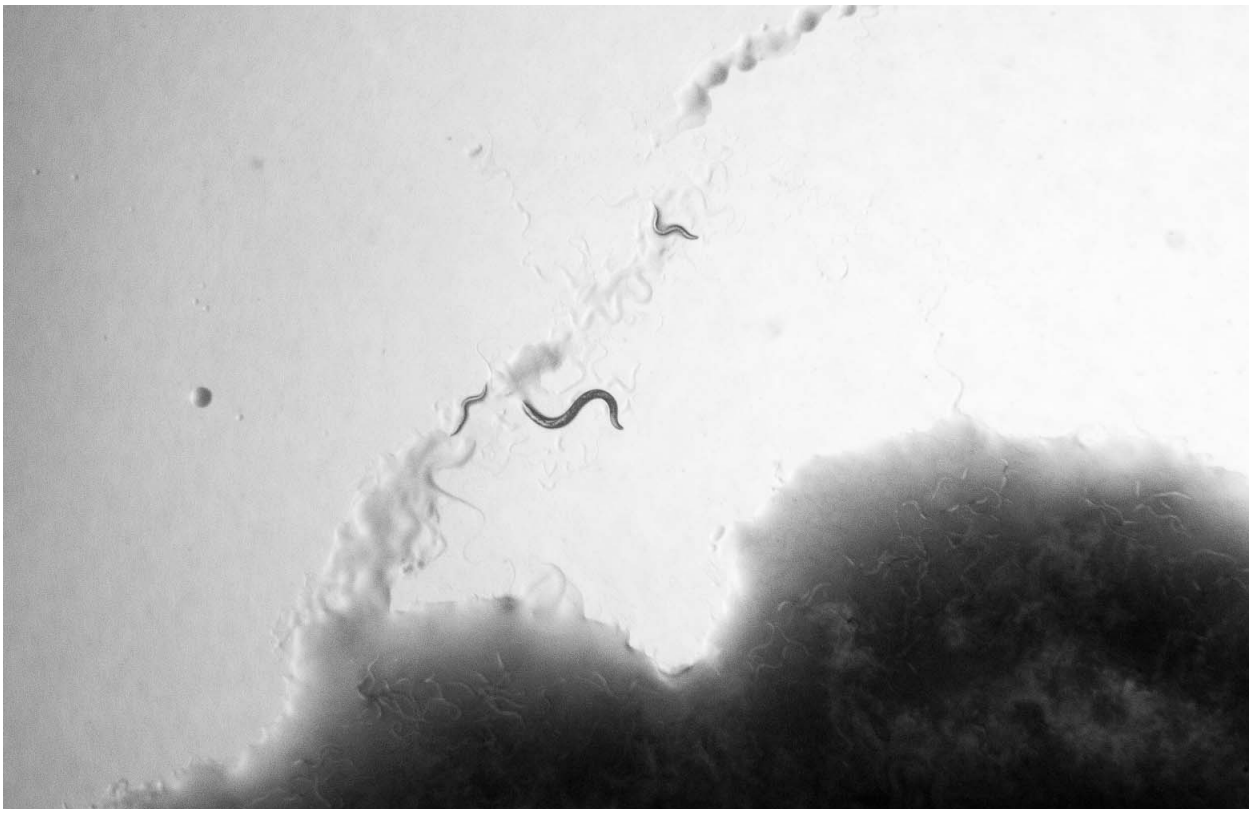


Fig. 6. 10x image of wild-type *C. elegans*. The nematodes are seen in a thick *E. coli* OP50-pBAD lawn 3 days after thaw.

C. elegans Subculture

C. elegans will be overcrowded and may potentially exhaust their food supply after one week in culture. *C. elegans* are subcultured prior to the chemotaxis assay to ensure that the *C. elegans* population is not overcrowded and has an optimal feeding environment with fresh *E. coli* OP50-pBAD. Fresh *E. coli* OP50-pBAD on NGM Lite agar plates, which contain NaCl, ensures that *C. elegans* make a positive association between NaCl and food.

The *E. coli* OP50-pBAD lawn that is prepared from the lyophilized vial supplied in the kit will be very thin lawns that may be difficult to see. Thin lawns are preferred by researchers studying *C. elegans* as they facilitate visualization. Additionally, thin lawns are useful prior to the chemotaxis assay as they will minimize the number of washes necessary to remove the *E. coli* OP50-pBAD from the *C. elegans* during the assay.

Fig. 7. 20x image of wild-type *C. elegans* after subculture. The thin lawn of *E. coli* OP50-pBAD allows for easier viewing of *C. elegans*. As *C. elegans* move through the thin *E. coli* lawns, s-shaped tracks are left on the plate.

C. elegans Washing

As *C. elegans* move through a lawn of *E. coli* OP50-pBAD during feeding they become covered in food. If *C. elegans* are transferred to a new plate while covered in food, the *C. elegans* will find little reason to migrate away from where they were dropped to other areas of the plate since they already find themselves with a good supply of food. Thus, it is necessary to wash the *C. elegans* prior to the chemotaxis assay to remove all *E. coli* OP50-pBAD from the *C. elegans*.

C. elegans are washed in 1x *C. elegans* wash buffer, a gentle isotonic wash solution that does not contain NaCl. When transferred to a fresh tube in 1x *C. elegans* wash buffer, gravity will ensure the dense *C. elegans* sinks to the bottom of the tube where it will form a loose pellet, while most of the *E. coli* OP50-pBAD will remain in the supernatant.

Washing *C. elegans* by gravity is a gentle process that minimizes physical harm done to the *C. elegans* while separating the nematodes from *E. coli* OP50-pBAD based on density. Adult *C. elegans* will precipitate out of solution faster than L1–L3 larvae, and some larvae may still be present in the supernatant when discarding. Discarding the larvae is acceptable as long as a visible pellet is still present in the bottom of the tube, indicating that enough worms remain to perform the chemotaxis assay. Discarding the small *C. elegans* while keeping the larger *C. elegans* in the pellet may help during the experiment, as larger *C. elegans* are easier to observe.

Chemotaxis Assay

After transferring *C. elegans* to the chemotaxis assay plate and wicking away the excess liquid from the worms with a kimwipe, the *C. elegans* will be able to move freely across the whole plate. While most *C. elegans* will begin migrating away from the seed site within 15 minutes, any that were damaged during the transfer process will not migrate. Do not count the worms that have not migrated away from the seed site. Count the *C. elegans* that have migrated away from the seed site and are found on the NaCl or the control side of the plate.

The chemotaxis index calculated at the end of the class is a numerical representation of the attraction of *C. elegans* to the NaCl. The attraction of *C. elegans* to NaCl, however, is not absolute but, rather, depends on associative learning that is influenced by a number of environmental factors experienced by the *C. elegans*.

Even when being extremely careful and when it may seem that all *C. elegans* have experienced the same environment, *C. elegans* may respond differently to the NaCl from one plate to the next. Individual *C. elegans* may experience different microenvironments, even when grown on the same plate. Therefore, it is common to see the majority of wild-type *C. elegans* moving toward NaCl while a few individuals show the opposite response and migrate away from NaCl.

The images below show the location of wild-type and mutant *C. elegans* allowed to migrate for 30 minutes. Most of the wild-type *C. elegans* can be seen at the very top of the plate where the NaCl solution was placed the previous night. However, a few individuals can be seen roaming the control side of the plate. When taking into account all of the wild-type *C. elegans*, the chemotaxis index for the entire plate was 0.84, a positive chemotaxis response.

On the other hand, the mutant *C. elegans* are expected to be located primarily at the center of the plate or evenly dispersed across the plate.. While there is a slightly higher population of mutant *C. elegans* on the NaCl side of the plate, a large number of *C. elegans* can also be seen on the control side of the plate. Importantly, very few mutant *C. elegans* can be seen in the area near where the NaCl solution was placed the previous night. When taking into account all of the mutant *C. elegans*, the chemotaxis index for the entire plate was 0.15. This is a slightly positive NaCl chemotaxis response, yet much lower than that of the wild-type response.

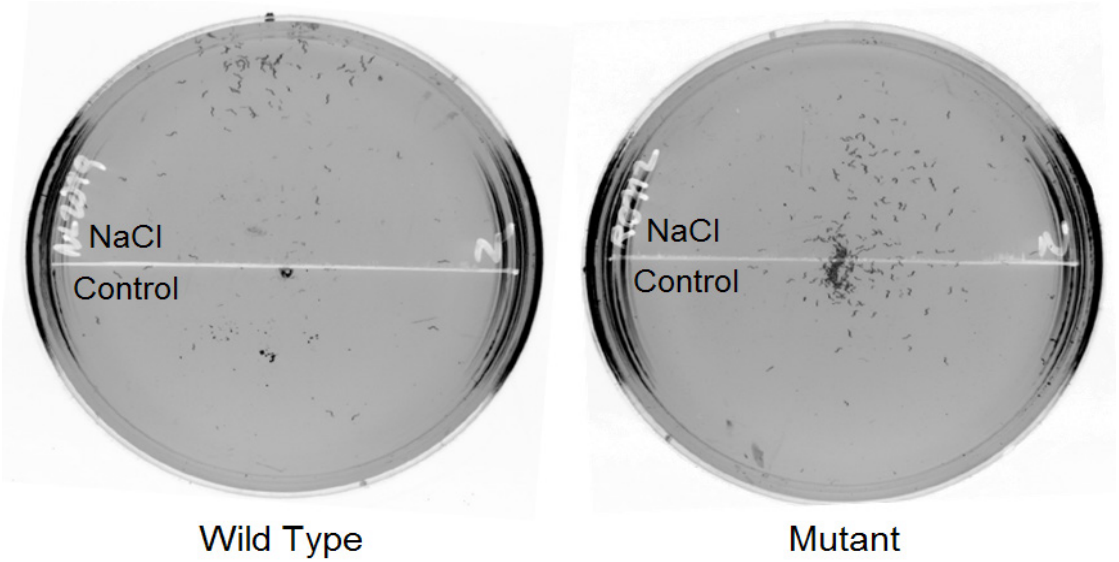


Fig. 8. Illustration of expected chemotaxis assay results.

Rarely will a chemotaxis experiment display perfect results. It is common, rather, to encounter a range of results within a classroom. The graph below shows sample individual group chemotaxis averages obtained from 10 classroom groups. While no group attained a perfect wild-type positive chemotaxis index of 1, which would indicate that all wild-type *C. elegans* observed and counted migrated toward the NaCl side of the plate, the results were overwhelmingly positive.

The wild-type *C. elegans* data ranged from 0.51 to 0.92, with the average chemotaxis index being 0.81. An average of 0.81 indicates a strongly positive NaCl chemotaxis response. Additionally, no group obtained a perfectly random chemotaxis index of 0, which would indicate that all mutant *C. elegans* observed and counted were evenly distributed between the NaCl and control sides of the plate. The mutant data ranged from –0.35 to 0.34 with the chemotaxis index being 0.08. An average chemotaxis index of 0.08 is a good indicator of random movement.

Additionally, if *C. elegans* experience an environment that is not optimal while being cultured on the NGM Lite agar, *C. elegans* will display negative chemotaxis in response to NaCl. It is important to keep good notes on the *C. elegans* culture conditions, such as the age and size of the *E. coli* lawn, room temperature, and exposure to direct sunlight, to make sense of negative results that may occur in the classroom. These observations will help troubleshoot the experiment and allow students to hypothesize about how the culture conditions affect NaCl chemotaxis.

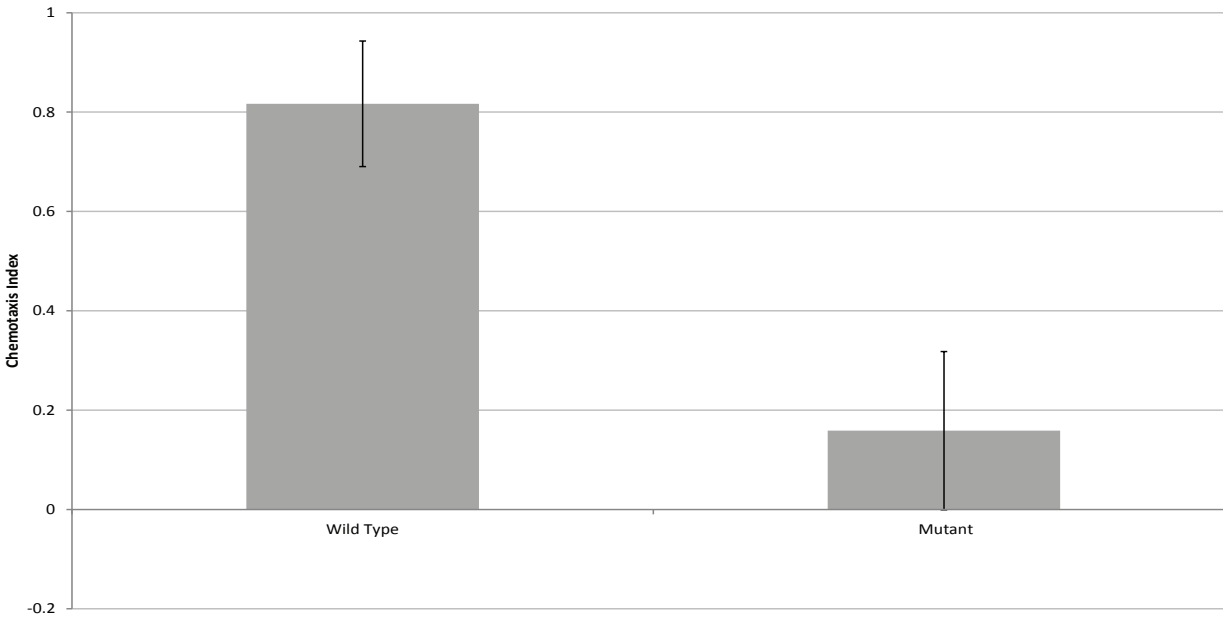


Fig. 9. Example of chemotaxis assay results.

Lesson 1: Subculture

Dr. Ivan Pavlov was a Russian physiologist studying digestion in dogs when he first noticed that his canine subjects would begin to salivate before being fed even when they had not seen or smelled food. It turns out that the canine subjects of his salivary studies were reacting to the lab coats that his assistants were wearing. Every time that the dogs were fed, the person delivering the food wore a white lab coat.

This anticipatory canine response piqued Dr. Pavlov's curiosity and he decided to see whether he could take a neutral stimulus, such as the ringing of a bell and, by pairing it with food, cause a dog to salivate in response to the sound of a bell. Dr. Pavlov first rang the bell many times and was able to demonstrate that the dog did not naturally salivate in response to the bell. Dr. Pavlov then conditioned his dog by ringing the bell just prior to feeding. After some time, Dr. Pavlov was able to ring the bell without feeding the dog, and the dog would salivate. Dr. Pavlov's dog had been conditioned to anticipate food and salivate upon hearing the ringing of the bell (Nobelstiftelsen 1964).

Like Dr. Pavlov's dog, *C. elegans* can be conditioned. *C. elegans* are a nematode measuring at most 1 mm in length and with a nervous system that has just 302 neurons. Yet *C. elegans* can learn to associate their environmental conditions with food (associative learning behavior). *C. elegans* naturally feed on bacteria, and in this laboratory activity *C. elegans* will be fed a laboratory strain of *E. coli* bacteria called OP50-pBAD. *C. elegans* will be fed OP50-pBAD on NGM (nematode growth medium) Lite agar plates that contain 34 mM NaCl. NaCl is typically a neutral stimulus to *C. elegans*. But by pairing food with NaCl-containing plates, wild-type *C. elegans* will be conditioned to seek out NaCl environments.

This laboratory activity will also use a *daf-18* mutant strain of *C. elegans* that is unable to produce a working DAF-18 protein. The DAF-18 protein is required for associative learning behavior, and without this protein *C. elegans* cannot make a positive association between NaCl and food. This laboratory activity will culminate by testing the associative learning ability of wild-type and mutant *C. elegans* through the use of an NaCl chemotaxis assay.

In the first laboratory activity you will observe your *C. elegans* and then subculture the wild-type and mutant *C. elegans* to NGM Lite agar plates containing fresh lawns of *E. coli* OP50-pBAD. The lucky *C. elegans* that have made it onto the new plates will encounter ideal feeding conditions (plenty of food and no overcrowding) while being constantly exposed to NaCl.

In the second activity you will test the ability of the wild-type and mutant strains of *C. elegans* to associate NaCl with food. You will harvest and wash the *C. elegans* of *E. coli* (no food must be transferred) and place the *C. elegans* in the center of an agar plate containing a gradient of NaCl on one half and a control solution on the other half. You will allow the *C. elegans* to move throughout the agar plate for 30 minutes and then count how many *C. elegans* are on each side of the plate. With these data in hand, you will then be able to calculate a chemotaxis index as a numerical representation of the ability of *C. elegans* to associate its food with its environment.

Let's see how smart your *C. elegans* are!

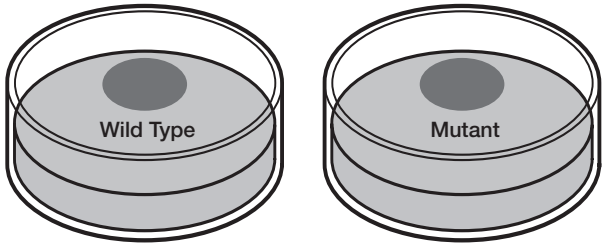
Student workstation

Materials	Quantity	(✓)
NGM Lite agar plate with *E. coli* OP50-pBAD lawn	2	☐
Microcentrifuge tube, 2 ml	2	☐
NGM Lite agar plate with *C. elegans* wild type	1	☐
NGM Lite agar plate with *C. elegans* mutant	1	☐
C. elegans wash buffer (1x), 2 ml	1	☐
Disposable plastic transfer pipet (DPTP)	2	☐
2–20 µl adjustable-volume micropipet and tips	1	☐
Marking pen	1	☐
Scissors	1	☐
Dissection microscope (10–40x magnification)	1	☐
Microcentrifuge tubes, 2.0 ml	1	☐

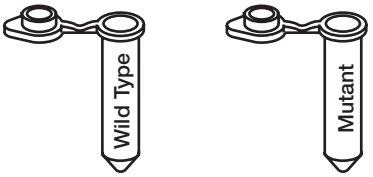
Quick Guide

Lesson 1: C. *elegans* subculture

1. Label one NGM Lite agar subculture plate with *E. coli* OP50-pBAD lawn "Wild Type" and the other "Mutant."



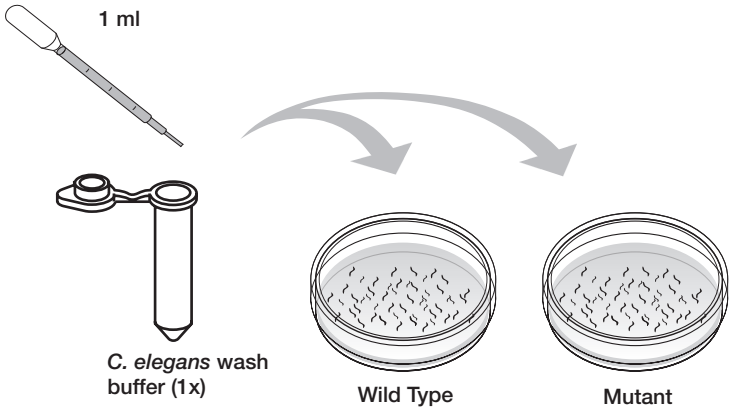
2. Label one microcentrifuge tube "Wild Type" and the other "Mutant."



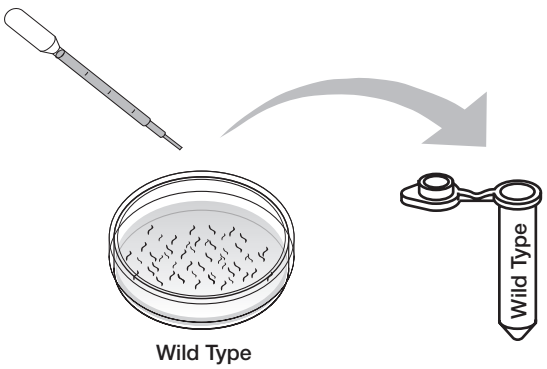
3. Record your observations of what the *E. coli* OP50-pBAD lawns look like.
   - Continuous?
   - Color?
   - Transparent/translucent?
   - Plate coverage?

4. Using a dissection microscope, look at the plates containing your wild-type and mutant *C. elegans* strains. Record your observations.
   - Number of worms on each plate
   - Amount of *E. coli* remaining on each plate
   - Larval stage of worms on each plate
   - Relative percentage of different larval stages on each plate
   - Are eggs present on either plate?
   - Do the mutant and wild-type worms look the same?

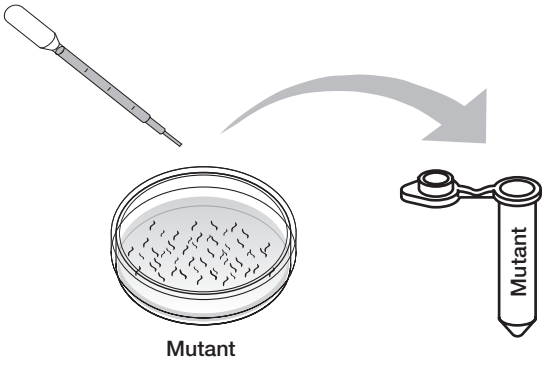
5. Using a sterile DPTP, transfer 1 ml of *C. elegans* wash buffer (1x) to each of the NGM Lite agar plates containing wild-type and mutant *C. elegans*. Rotate the plate to cover the entire surface with *C. elegans* wash buffer. Incubate at room temperature for 30 sec. Save the DPTP in its original wrapper for step 6.



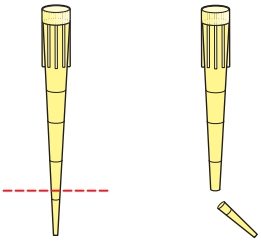
6. Tilt the plate containing the wild-type *C. elegans* at a 45° angle to allow the worms in solution to collect on one side of the plate. Transfer the wild-type *C. elegans* in solution into the microcentrifuge tube labeled “Wild Type” using the DPTP from step 5.



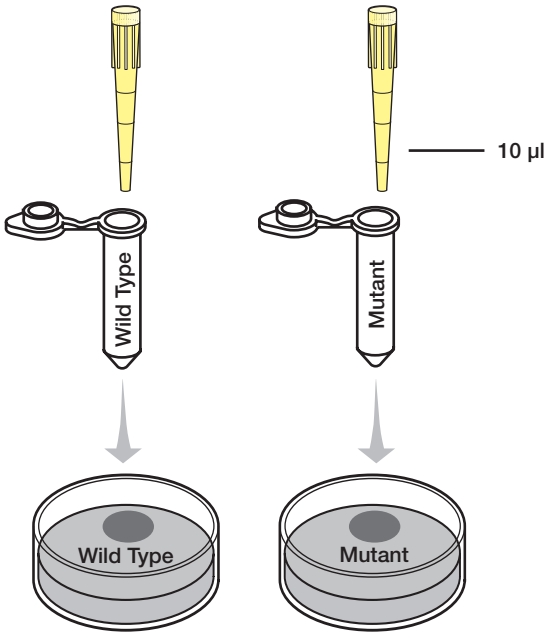
7. Tilt the plate containing the mutant *C. elegans* at a 45° angle to allow the worms in solution to collect on one side of the plate. Using a fresh sterile DPTP, transfer the mutant *C. elegans* in solution into the microcentrifuge tube labeled “Mutant.”



8. Allow *C. elegans* in the microcentrifuge tubes to settle for 2 min. While the *C. elegans* settle, cut the ends off two 20 µl tips to make a larger opening.



9. Transfer 10 µl of each *C. elegans* pellet to the appropriately labeled plate containing a lawn of *E. coli* OP50-pBAD bacteria for food.



10. Cover plates and incubate at room temperature.

Lesson 1: Focus Questions

1. List three reasons why a researcher would want to use a model organism.

2. List the stages of the *C. elegans* life cycle.

3. What are two characteristics that make *C. elegans* a good model organism?

4. Where are *C. elegans* found in nature and what do they eat?

5. Are there any visible physiologic differences between the wild-type and mutant strains of *C. elegans*?

6. What is the purpose of subculturing *C. elegans* prior to the chemotaxis assay?

Lesson 2: Chemotaxis

The *C. elegans* subcultured during Lesson 1 have been allowed to feed on *E. coli* OP50-pBAD for several days. Like Pavlov's dog, the *C. elegans* have fed while subject to a second stimulus, in this case NaCl. Enough time has passed to condition the *C. elegans* to associate the NaCl in the NGM Lite agar plates with the OP50-pBAD food. You have received two assay agar plates on which you will test the degree of NaCl association that your *C. elegans* have made.

The plates you've received have been divided in half. Ten microliters of *C. elegans* wash buffer has been placed on one half of each plate while 10 µl of 2.5 M NaCl diluted in *C. elegans* wash buffer has been placed on the opposite half of the plate. *C. elegans* wash buffer is a solution that has been formulated to contain no NaCl while also providing an isotonic solution that will not harm *C. elegans* during washing or during the chemotaxis assay.

In this laboratory exercise you will harvest the *C. elegans* from the NGM Lite agar plates in *C. elegans* wash buffer and wash the worms thoroughly. It is important to wash the *C. elegans* thoroughly because if any residual *E. coli* remains with the worms when seeded onto the assay agar plate, the *C. elegans* will not move from the center of the plate. After washing, you will place *C. elegans* in the center of the assay agar plate where they will then need to choose which direction to move in. The *C. elegans* may move toward the NaCl half of the plate, toward the control half of the plate, or they may decide to move randomly over the plate. You will mark the position of the *C. elegans*, count them, and numerically calculate a chemotaxis index. Remember, both the *C. elegans* wild-type and mutant strains are fully capable of sensing NaCl. Any phenotypes observed are due solely to the *C. elegans'* ability or inability to associate NaCl with food.

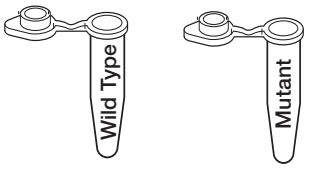
Student workstation

Materials	Quantity	(✓)
NGM Lite agar plate with *E. coli* OP50-pBAD lawn	2	☐
Microcentrifuge tube, 2 ml	2	☐
NGM Lite agar plate with *C. elegans* wild type	1	☐
NGM Lite agar plate with *C. elegans* mutant	1	☐
C. elegans wash buffer (1x), 2 ml	1	☐
Disposable plastic transfer pipet (DPTP)	2	☐
2–20 µl adjustable-volume micropipet and tips	1	☐
Marking pen	1	☐
Scissors	1	☐
Dissection microscope (10–40x magnification)	1	☐
Microcentrifuge tubes, 2.0 ml	1	☐

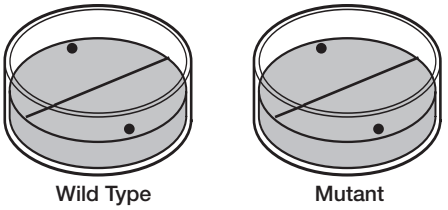
Quick Guide

Lesson 2: Chemotaxis

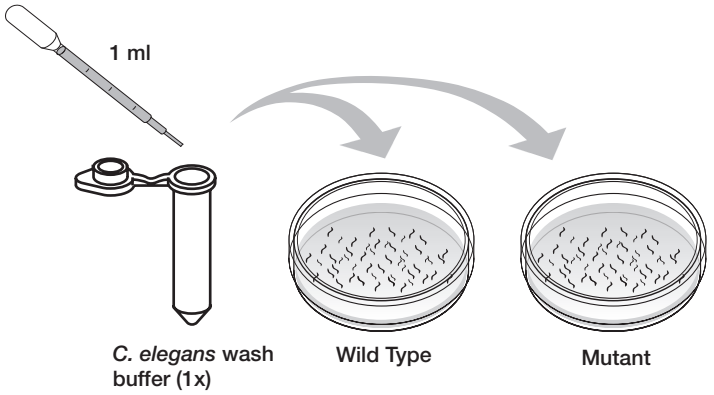
1. Label one 1.5 ml microcentrifuge tube “Wild Type” and another “Mutant.”



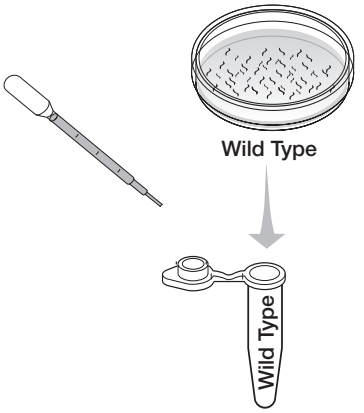
2. Label the bottom of one **assay agar plate** “Wild Type” and the other “Mutant.”



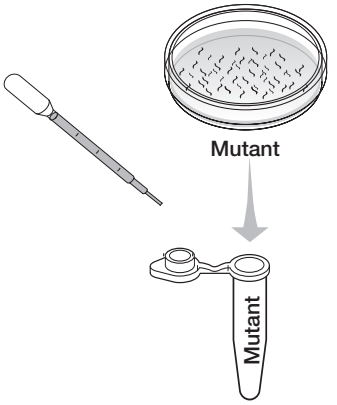
3. Using a sterile DPTP, transfer 1 ml of *C. elegans* wash buffer to each of the two **NGM Lite agar plates containing wild-type and mutant *C. elegans*** (from Lesson 1). Rotate the plate to cover the entire surface with *C. elegans* wash buffer. Incubate at room temp for 30 sec–1 min. Save the DPTP in its original wrapper for use in Step 4.



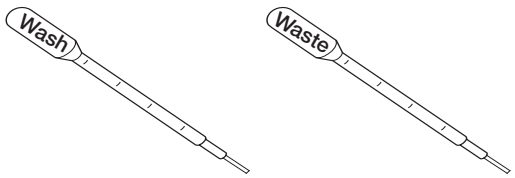
4. Tip the NGM Lite agar plate labeled “Wild Type” at a 45º angle and allow the solution to collect in the bottom of the plate. Using the DPTP from Step 3, collect the solution containing the wild-type *C. elegans* and transfer the *C. elegans* in solution into the microcentrifuge tube labeled “Wild Type.”



5. Tip the NGM Lite agar plate labeled “Mutant” at a 45º angle and allow the solution to collect in the bottom of the plate. Using a new sterile DPTP, collect the solution containing the mutant *C. elegans* off the plate and transfer the *C. elegans* in solution into the microcentrifuge tube labeled “Mutant.”

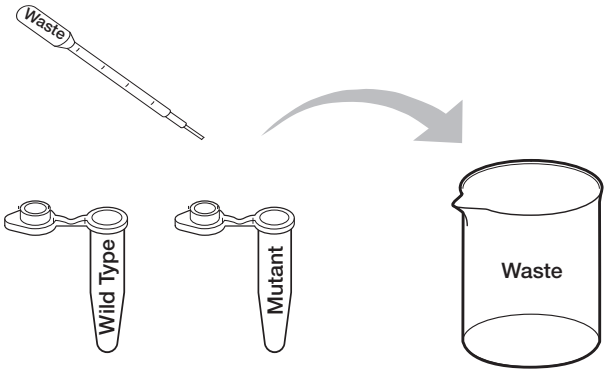


6. Label a new DPTP “Wash” and another “Waste.”

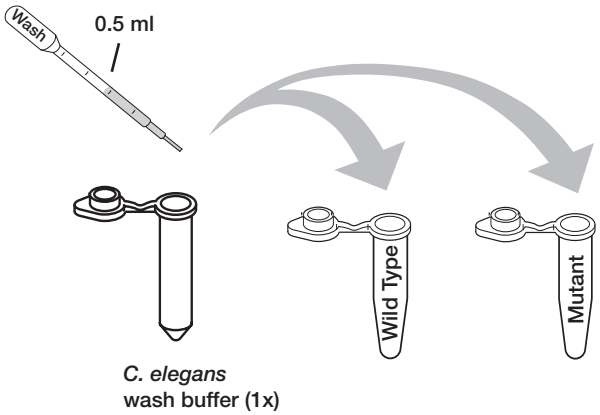


7. Let the *C. elegans* settle to the bottom of the tube for 2 min.

8. Being careful not to disturb the *C. elegans* pellet, use the DPTP labeled “Waste” to remove the supernatant from each microcentrifuge tube and transfer it to the waste container.

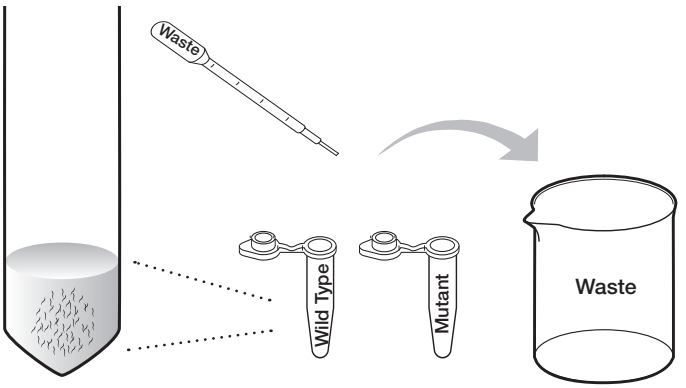


9. Using the DPTP labeled “Wash”, transfer 0.5 ml of *C. elegans* wash buffer to the wild-type and mutant microcentrifuge tubes containing the *C. elegans.*



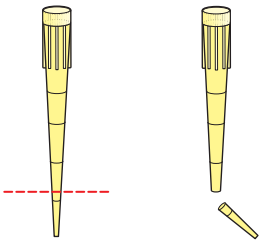
10. Cap and invert both microcentrifuge tubes containing the *C. elegans* 5x to mix. Make sure that the entire pellet has been resuspended.

11. Repeat Steps 8–10 one more time for a total of 2 washes. During the last wash, visually examine the clarity of the *C. elegans* resuspended in the *C. elegans* wash buffer. If the resuspended *C. elegans* are not dispersed in a clear liquid, obtain additional wash buffer from the instructor and continue washing until the liquid is completely clear.



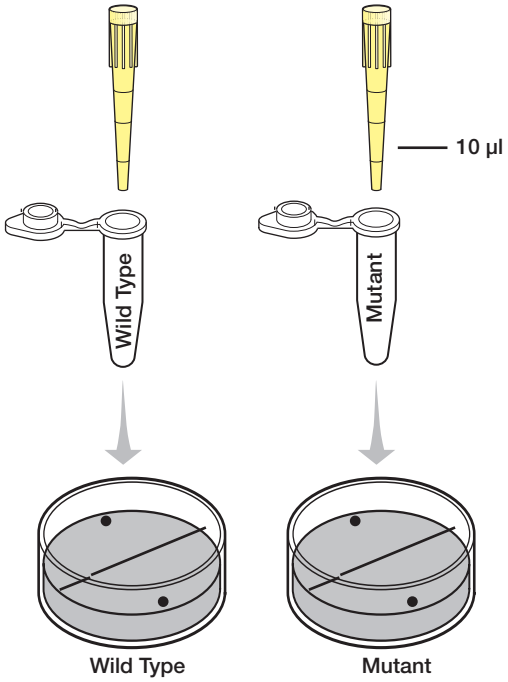
12. After the last wash, discard the supernatants, leaving approximately 50–100 µl of liquid above the pellet.

13. Cut the end of a 20 µl pipet tip to make a large opening.

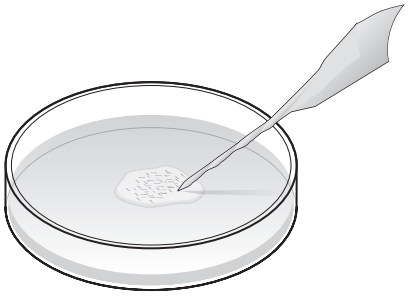


14. Transfer 10 µl of concentrated *C. elegans* pellet to the center of the appropriately labeled assay agar plate.

15. Repeat steps 12–13 with the second *C. elegans* strain.



16. Twist the end of a kimwipe to a fine tip. Gently insert the twisted kimwipe into the *C. elegans* to wick away excess fluid. Hold the kimwipe in place for approximately 5 sec to fully absorb the liquid.



17. Allow the *C. elegans* to migrate across the plate for 30 min.

18. At the end of 30 min, invert the plate and, using a dissection microscope and a fine tip marking pen, mark the locations of *C. elegans* across the plate. Do not mark *C. elegans* that have not moved from the center of the plate as these may have been damaged during the washes.

19. For the wild-type *C. elegans*, record the number of worms on the NaCl side. Record the number of worms on the control side. Repeat for the mutant *C. elegans*.

Wild-type *C. elegans*

NaCl side _____

Control side _____

Mutant *C. elegans*

NaCl side _____

Control side _____

Statistical Analysis

Data

Wild-type *C. elegans*

C. elegans on NaCl side _____

C. elegans on Control side _____

Total number of wild type *C. elegans* counted _____

Mutant *C. elegans*

C. elegans on NaCl side _____

C. elegans on Control side _____

Total number of mutant *C. elegans* counted _____

Calculation of Chemotaxis Index

1. Calculate the chemotaxis index as follows for the assays for both the wild-type and the mutant *C. elegans*:

$$\text{Chemotaxis index} = \frac{(\#\ \textit{C. elegans}\ \text{on NaCl side} - \#\ \textit{C. elegans}\ \text{on control side})}{(\#\ \textit{C. elegans}\ \text{on NaCl side} + \#\ \textit{C. elegans}\ \text{on control side})}$$

Chemotaxis index for wild-type *C. elegans*: _____

Chemotaxis index for mutant *C. elegans*: _____

The chemotaxis index is a calculation of the movement of *C. elegans* specifically toward a chemical relative to just random movement.

- If the index is positive, then more worms moved toward the chemical (NaCl, in this case)
- If the index is zero, then the same number of worms moved toward the chemical as moved away from the chemical
- If the index is less than zero, more worms moved away from the chemical than toward it

The more positive or more negative the chemotaxis index is, the stronger the effect. For example, if the chemotaxis index was 0.8 for wild-type worms but 0.1 for the mutant worms, then it can be said that the wild-type worms were much more strongly attracted to the salt than the mutant worms.

Lesson 2: Focus Questions

1. In what way are the solutions placed at the NaCl and the Control spots on the assay agar plate similar or different?

2. Is *daf-18* an essential gene for *C. elegans*' survival? Explain.

3. What is the benefit of having a functional *daf-18* gene in *C. elegans*?

4. Why are NGM Lite agar plates not used for the chemotaxis assay?

5. What would happen during the chemotaxis assay if *C. elegans* were starved rather than fed prior to the chemotaxis assay?

6. Design an experiment to test this hypothesis.

Effect of Population on Results

Most medications include warning labels with statistical information on the chances of a side effect occurring when you take the medication. Have you ever wondered where these data came from? How many people need to be tested to determine that a medicine is not only effective, but also safe? How many of what groups of people were tested to determine whether there are side effects, what they are, and how likely an individual is to experience them? Or what about opinion polls during elections? When a candidate is said to be leading by 2%, how many people were questioned to arrive at that percentage? What subgroups of people were chosen to be polled? How many of the chosen individuals in those subgroups responded?

These sorts of questions help determine which populations should be tested in order to determine a "true" result. Ideally, we would be able to test entire populations for side effects of potential new medicines, or ask everyone who voted in the U.S. for their opinions. But this is not only unrealistic, it is impossible. Therefore, we must choose a smaller population to represent the whole when doing studies. How this population is chosen and the number of individuals tested can have a huge impact on the results.

Let's start with a simple example. If you wanted to find the average height of people in the U.S., what population would you select to include and how many people would you need to measure to feel that your results are representative of the entire country? By using data from your classroom, we can determine the impact both of the subgroup you choose to measure and of the number in your subgroup.

1. Record the heights of all the people in your class.
2. Calculate the average height of your entire class.
3. Calculate the average height of the first five individuals on your list.
4. Calculate the average height of the first ten girls on your list.
5. Calculate the average height of the first ten boys on your list.

Do you see any differences in the average height results calculated above?

In general, a larger population will give better results than a smaller population. A larger pool of data will be less affected by any outliers while a small population will be more impacted. For example, if one extremely tall student is among five students randomly chosen for a study designed to find the average height of the whole class, then the average will be higher than if the whole class is included in the study (1/5 is greater than 1/25). However, it is not always possible, due to expense or time, to study a very large population.

Choosing a subpopulation to represent the whole population can also be tricky. Did the first five individuals on your list contain an equal number of boys and girls? What impact on the average height did having a subpopulation of just girls or just boys have relative to the average height of the class as a whole?

A Closer Look at Your Data

In these experiments, all students started with wild-type and mutant *C. elegans* grown from the same original population of *C. elegans* that your instructor plated out and allowed to propagate in number. Compare the chemotaxis results that your laboratory group calculated with the values other laboratory groups calculated. Are the values exactly the same? Did all laboratory groups count the same number of *C. elegans* to calculate their chemotaxis index?

Impact of Population Size

Think about the impact on your data if you counted only a small number of *C. elegans*, and there was one outlier — a *C. elegans* that behaved differently than was expected. For example, ten total *C. elegans* for the wild-type experiment on the assay agar plates, including the one outlier.

Wild-type *C. elegans*

C. elegans on NaCl side: 9

C. elegans on Control side: 1

Total number of wild-type *C. elegans* counted: 10

Calculate the chemotaxis index for the assay as follows for the wild-type *C. elegans*:

$$\text{Chemotaxis index} = \frac{(\#\ \textit{C. elegans}\ \text{on NaCl side} - \#\ \textit{C. elegans}\ \text{on control side})}{(\#\ \textit{C. elegans}\ \text{on NaCl side} + \#\ \textit{C. elegans}\ \text{on control side})}$$

Chemotaxis index for wild-type *C. elegans*: (9 – 1)/10 = 0.80

In the above example, there was one outlier *C. elegans* for the wild-type strain that did not learn to associate salt with food. The chemotaxis index resulting from a population with one outlier for the wild-type *C. elegans* was 0.8.

Now, let us consider the impact of a single outlier if 100 *C. elegans* were counted.

Wild-type *C. elegans*

C. elegans on NaCl side: 99

C. elegans on Vehicle side: 1

Total number of wild-type *C. elegans* counted: 100

Calculate the chemotaxis index for the assay as follows for the wild-type *C. elegans*:

$$\text{Chemotaxis index} = \frac{(\#\ \textit{C. elegans}\ \text{on NaCl side} - \#\ \textit{C. elegans}\ \text{on control side})}{(\#\ \textit{C. elegans}\ \text{on NaCl side} + \#\ \textit{C. elegans}\ \text{on control side})}$$

Chemotaxis index for wild-type *C. elegans*: (99 – 1)/100 = 0.98

The single outlier has much less impact on the chemotaxis index when the number of *C. elegans* counted is higher. But it is much more difficult to count 100 *C. elegans* than 10 *C. elegans* in the experiment!

Impact of Subpopulations

If having a larger population of *C. elegans* decreases the impact of any outliers, is it possible to combine data from the whole class and, more important, is this valid to do? All of the *C. elegans* are the offspring of a single population of the original mutant and wild-type *C. elegans* that your instructor plated out and grew for the laboratory experiments. However, once these populations were divided to be part of each laboratory group's experiment, it is possible that they experienced slightly different growth environments, which could have affected their development and behavior.

Factors that might influence their growth could be things as subtle as being subjected to more or less harsh pipetting, having more or less food (relative size of *E. coli* lawn), being washed more or fewer times, or growing in a slightly warmer or colder location. While these differences may seem insignificant, they can still have an impact since the *C. elegans* are a complex multicellular organism and hence can — and do — respond to their environment.

If there are large differences in the chemotaxis indices from group to group, try to determine what some of the causes might have been. Could there have been some systematic errors introduced such as using the same pipet for both the wild-type and mutant *C. elegans* and mixing the populations by mistake?

When performing measurements and experiments, it is critical for measurements to be both accurate and reproducible. Accuracy refers to how close to the expected value the results fall. As an analogy, imagine an archer shooting arrows at a target; how often the arrows fall within and around the center circle is an indication of accuracy.

Reproducibility refers to how tightly clustered the data points are. It is possible to be accurate but not reproducible. To take our archery example again, an archer whose arrows fall in a tight cluster has high reproducibility. But if that cluster is on the edge of the target, the archer has low accuracy. Ideally, data will be reproducible and accurate — arrows tightly packed in the center of the target.

Look at the chemotaxis indices for both the mutant and wild-type *C. elegans* for all the different laboratory groups in your class. How widely spread are the data from expected chemotaxis indices of 0.6–0.8 for the wild-type *C. elegans* and 0–0.3 for the mutant *C. elegans*? Did most of the class get data in this range? Were the class's data sets both accurate and reproducible from one group to the next? If so, it might be possible to combine data from all the groups. But it might not be necessary since the smaller single groups' data sets were accurate.

Appendix A: Glossary

Assay agar — the plates used for the chemotaxis assay in this kit. This plate contains agar and a 1x *C. elegans* wash buffer. The *C. elegans* wash buffer is included when this plate is poured to provide the *C. elegans* with an isotonic environment that does not contain Na+ ions that would interfere with the chemotaxis assay.

Auxotroph — an organism that has lost the ability to synthesize a substance critical for its growth and survival.

C. elegans — a nonsegmented transparent nematode about 1 mm in length that lives in temperate soil environments and is used as a model organism in research labs worldwide.

Chemotaxis — a phenomenon where cells or an organism direct their movement in relation to a chemical stimulus. In positive chemotaxis the orientation of movement is directed toward the chemical stimulus while in negative chemotaxis the movement occurs away from the chemical stimulus.

Chemotaxis index — a numerical representation of the affinity of an organism or cell to move toward or away from a chemical substance.

Connectome — a complete map of neural connections in an organism or a specific organ.

Culture — the growing of cells or organisms in a specifically prepared nutrient medium.

Cuticle — the tough but flexible outer covering of some types of organisms.

daf-18 — a *C. elegans* gene encoding a protein phosphatase that is homologous to human PTEN. This gene plays critical roles in metabolism, development, and longevity as well as associative learning behaviors.

Dauer — an alternative developmental stage of nematode worms characterized by a type of metabolic stasis that allows the nematode to survive harsh conditions for an extended period of time.

Diploid — term used to describe a cell that has two homologous copies of each chromosome.

DPTP — disposable plastic transfer pipet.

Genotype — a reference to the genetic makeup of a cell or organism with respect to a single gene or trait.

Hemizygous — a diploid organism that possesses a single member of a pair of chromosome rather than the usual two.

Hermaphrodite — an organism that has both male and female sex organs.

Husbandry — the practice of breeding an organism.

Isotonic — (isosmotic) having the same osmotic pressure as the surrounding solution, so water is not pushed to cross membranes into or out of whatever is in suspension.

L1–L4 — developmental larval stages of *C. elegans*. Each stage is marked by a molt and an increase in the size of the organism.

Lyophilization — a freeze-drying process utilized to preserve perishable material.

Model organism — a nonhuman species that is studied to understand a biological phenomenon.

Molting — the process of synthesis and secretion of a new cuticle underneath an old one, and the shedding of the old cuticle to expose the newly synthesized cuticle.

NGM Lite agar — a minimal version of nematode growth media used to culture *C. elegans* in the lab. The NGM Lite agar included in this kit contains 34 mM NaCl.

OP50-pBAD — the uracil auxotroph strain of *E. coli* used in this kit as a food source for *C. elegans*. OP50 is a preferred strain of *E. coli* to feed *C. elegans* since it grows as a very thin lawn that maximizes *C. elegans* visualization.

Phenotype — the observable or measureable manifestation of a genotype.

Phosphatase — a class of enzymes characterized by their ability to remove phosphates from molecules.

PTEN — (protein and tensin homolog) is a human gene that encodes a phosphatase. *PTEN* mutations have been implicated in cancers and learning disabilities.

Somatic cell — any cell forming the body of a multicellular organism. Somatic cells do not include gametes.

Subculture — the process of transferring some or all organisms (or cells) from one culture plate to a culture plate with fresh nutrients.

Synapse — a structure that allows for the passage of an electrical or chemical signal from one cell to another.

X (1x, 2x, etc.) — the relative concentration of a substance. 1x is full strength, 2x is twice as strong, etc.

Appendix B: Answers to Focus Questions

Lesson 1 Focus Questions

1. List three reasons why a researcher would want to use a model organism.
   Model organisms reproduce quickly, are easy to maintain, and are cost effective.

2. List the stages of the *C. elegans* life cycle.
   Egg, L1, L2, L3, L4, adult with an alternate stage called dauer if environmental conditions are challenging.

3. What are two characteristics that make *C. elegans* a good model organism?
   No need for husbandry, easy to culture, cheap, transparent, amenable to technologies such as RNAi, many scientific resources are available (such as genome, connectome), eukaryotic.

4. Where is *C. elegans* found in nature and what do they eat?
   ***C. elegans* are found in temperate soils where they feed on bacteria present on decomposing plant matter.**

5. Are there any visible physiologic differences between the wild-type and mutant strains of *C. elegans*?
   No, *daf-18* mutant phenotypes are not physiological. The phenotypes are in development, longevity, and learning associative behaviors.

6. What is the purpose of subculturing *C. elegans* prior to the chemotaxis assay?
   To provide *C. elegans* with an ideal feeding environment so that the *C. elegans* will make positive associations with NaCl present on the NGM Lite agar plate.

Lesson 2 Focus Questions

1. What is similar or different between the solutions placed at the NaCl and the control spots on the assay plate?
   The NaCl and the control spots contain the same solution except for the NaCl. The NaCl is diluted in 1x *C. elegans* wash buffer; 1x *C. elegans* wash buffer without NaCl is used as the control.

2. Is *daf-18* an essential gene for *C. elegans*' survival? Explain.
   ***C. elegans* can survive without a functional *daf-18* gene. This is apparent in this experiment since the mutant *daf-18 C. elegans* appear healthy and able to reproduce.**

3. What is the benefit of having a functional *daf-18* gene in the organism?
   Having the *daf-18* gene would allow *C. elegans* to better hunt for environments that will be food rich since it will be able to associate environmental conditions with the presence of food.

4. Why are NGM Lite agar plates not used for the chemotaxis assay?
   NGM Lite agar plates contain NaCl. NaCl would interfere with the chemotaxis assay.

5. What would happen during the chemotaxis assay if *C. elegans* were starved rather than fed prior to the chemotaxis assay?
   ***C. elegans* would create an association between starvation and NaCl. Negatively associating with NaCl would mean that *C. elegans* would move away from salt during the chemotaxis assay (negative chemotaxis).**

6. Design an experiment to test this hypothesis.
   ***C. elegans* could be subcultured onto an NGM Lite agar plate with no food present.**

Appendix C: References

1. Adachi T et al. (2010). Reversal of salt preference is directed by the insulin/PI3K and Gq/PKC signaling in *Caenorhabditis elegans*. Genetics 186, 1309–1319.

2. Nobelstiftelsen (1964). Physiology or medicine. (Amsterdam: Published for the Nobel Foundation by Elsevier Pub. Co.)

3. Shi Y et al. (2012). PTEN at a glance. J Cell Sci 125, 4687–4692.

4. Zhou J and Parada LF (2012). PTEN signaling in autism spectrum disorders. Curr Opin Neurobiol 22, 873–879.

5. Conti S et al. (2012). Phosphatase and tensin homolog (PTEN) gene mutations and autism: literature review and a case report of a patient with Cowden syndrome, autistic disorder, and epilepsy. J Child Neurol 27, 392–397.

Appendix D: Additional Resources

Bio-Rad Supplementary Materials

To access supplementary neurobiology, cell signaling and bioinformatics materials, please visit www.bio-rad.com/celegansbehaviorkit

Online Video Supplement

To view a video summary of lesson information referenced in this manual, please visit www.openworm.org/educators.html

C. elegans daf-18 gene sequence information

To access the gene sequence of the wild-type *daf-18* gene and the mutant *daf-18* gene please visit www.wormbase.org. The *daf-18* mutant *C. elegans* is strain OK480.

Online *C. elegans* Biology Resources

3D virtual reconstruction of *C. elegans*
www.openworm.org

Online bioinformatics database of *C. elegans*
www.wormbase.org

A database of behavioral and structural anatomy
www.wormatlas.org

Nomenclature

C. elegans genes are given names that consist of three or four italicized letters, a hyphen, and an italicized Arabic number (*daf-18*). The protein product of a gene is given a nonitalicized capital name that references the gene name (DAF-18).

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