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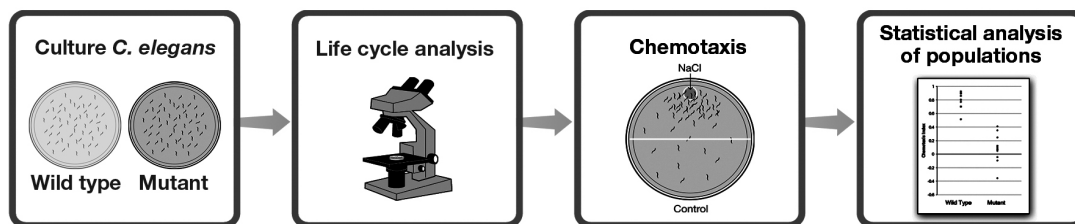
# Biotechnology Explorer™

## *C. elegans* Behavior Kit

### Planning Guide

[explorer.bio-rad.com](http://explorer.bio-rad.com)

Catalog #166-5120EDU



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## Overview

The intention of this planning guide is to provide an overview of the *C. elegans* behavior kit, including details of timelines, materials included, and equipment requirements, so that instructors may prepare their curriculum and estimate budgetary requirements in advance. These are estimates based on current information available, and may change in the final version of the kit.

The wild-type and mutant worms are shipped on dry ice and must be kept on dry ice or at  $-70^{\circ}\text{C}$  until ready to plate. The kit will be shipped prior to the shipment of worms to allow for preparation of NGM Lite agar plates. Wild-type and mutant *C. elegans* will be shipped overnight at date specified by customer.

If you have further questions about the kit, please contact [biotechnology\\_explorer@bio-rad.com](mailto:biotechnology_explorer@bio-rad.com).

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## ***C.elegans* Behavior Kit Components**

The manual provided with the kit comes with complete curricula and appendices with basic information to help you think about further investigations with your *C. elegans*.

### ***C.elegans* Behavior Kit (catalog #166-5120EDU)**

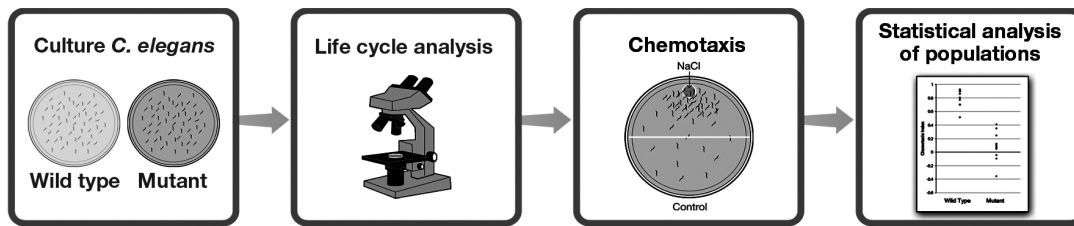
Kit contains sufficient materials for 8 students workstations (2–4 students per workstation)

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

**Note:** The wild-type and mutant worms are shipped on dry ice and must be kept on dry ice or at –70°C until ready to plate. The kit will be shipped prior to the shipment of worms to allow for preparation of NGM Lite agar plates. Wild-type and mutant *C. elegans* will be shipped overnight at date specified by customer.

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

## Series Overview



## Kit Summary

The *C. elegans* behavior kit allows students to work with and learn about *C. elegans*, one of the most studied model organisms.

With this kit, students are able to:

- Get hands-on experience with a model eukaryotic organism
- Observe and study the life cycle of *C. elegans*
- Utilize their microscopic skills
- Learn how to subculture
- Learn about genetics and its effect on behavior
- Discover the connection between learning, the *daf-18* gene, and the AIY and ASE neurons

*C. elegans* was the first multicellular organism to have its complete genome sequenced. The Nobel Prize in Physiology or Medicine was awarded to *C. elegans* researchers in 2002 (genetics of organ development and apoptosis), 2006 (RNA interference), and 2008 (GFP expression), emphasizing the importance of research on this model organism. This microscopic nematode is ideal for students to learn about subculturing so that they can observe the life cycle and different stages of development of the worms.

Following life cycle observations, students will get the chance to carry out a chemotaxis assay and study how *C. elegans* can learn to associate salt with their food. If salt is part of the environment in which wild-type *C. elegans* feed, they learn to associate the salt with their food. When *C. elegans* then sense salt in their environment they will migrate toward the salt in search of food. A mutation in the *daf-18* gene (and subsequently the AIY and ASE neurons) results in a worm that is not able to learn to associate salt with food. While the mutant *C. elegans* are able to sense salt, their inability to associate food with salt in their environment results in no migration toward salt, which will affect the chemotaxis assay.

**Note:** The wild-type and mutant worms are shipped on dry ice and must be kept on dry ice or at  $-70^{\circ}\text{C}$  until ready to plate. The kit will be shipped prior to the shipment of worms to allow for preparation of NGM Lite agar plates. Wild-type and mutant *C. elegans* will be shipped overnight at date specified by customer.



## Timeline for the Laboratory Course

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  $-70^{\circ}\text{C}$ , 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.

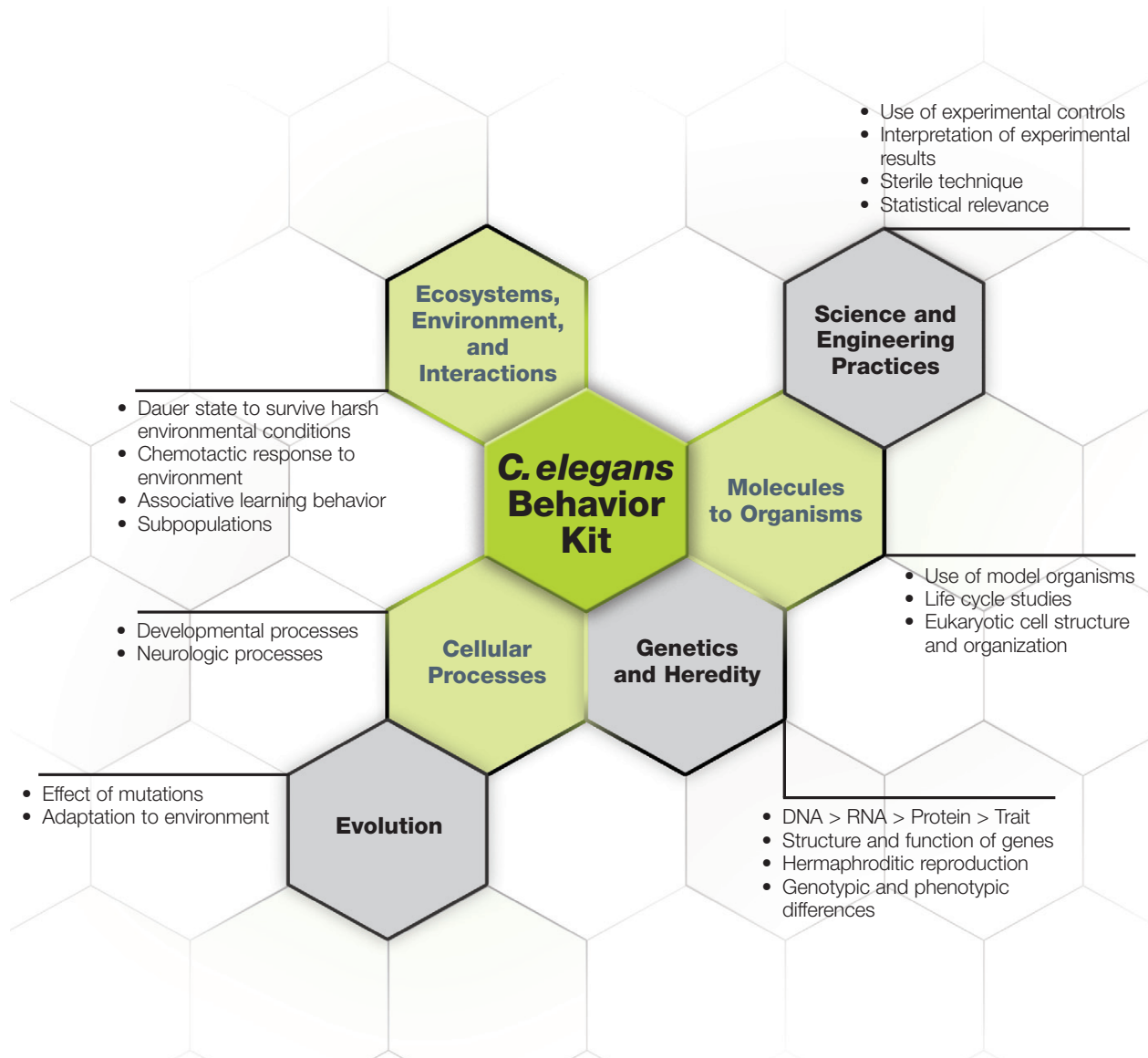
Task	Estimated duration
<b>Instructor's Advance Preparation</b> (1 Week prior to Lab Activity 1)	
1. Pour NGM Lite agar plates	1 hr prep time At least 2 days for drying of plates
2. Pour assay agar plates	1 hr prep time At least 2 days for drying of plates
3. Carry out <i>C. elegans</i> redemption instructions	<i>C. elegans</i> will be shipped on dry ice overnight
4. Thaw <i>C. elegans</i>	15 min
5. Plate <i>C. elegans</i> (wild-type and mutant) for 8 student workstations	10 min
6. Allow <i>C. elegans</i> to recover and populations to expand	4–7 days
<b>Instructor's Advance Preparation</b> (1–2 Days Prior to Lab Activity 1)	
1. Reconstitute <i>E. coli</i> OP50-pBAD, seed 16 NGM Lite agar plates with reconstituted <i>E. coli</i> OP50-pBAD, incubate at $37^{\circ}\text{C}$ (16–24 hr) or room temperature (1–2 days)	15 min <i>E. coli</i> reconstitution and spreading 1–2 days <i>E. coli</i> growth
2. Prepare 1x <i>C. elegans</i> wash buffer	15 min
3. Set up student workstations	45 min
<b>Lab Activity 1: Subculture of <i>C. elegans</i></b> (2–3 Days Prior to Lab Activity 2)	
1. Observe <i>C. elegans</i> wild-type and mutant populations using a microscope and record observations	10 min
2. Subculture <i>C. elegans</i> wild-type and mutant populations onto the fresh lawns of <i>E. coli</i> OP50-pBAD	20 min, then incubate 2–3 days
3. Observe <i>C. elegans</i> wild-type and mutant populations using microscope and record observations	45 min

Task	Estimated duration
<b>Instructor's Advance Preparation</b> (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
<b>Lab Activity 2: Chemotaxis</b> (2–3 Days after Lab Activity 1)	
1. Wash <i>C. elegans</i>	10 min
2. Perform chemotaxis experiment	40 min
3. Perform mathematical analysis	Variable

**Important Notes:**

- *C. elegans* must NOT encounter temperatures above  $-70^{\circ}\text{C}$  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  $-70^{\circ}\text{C}$  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**

# Curriculum Fit



## 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 25°C (60–77°F) and away from direct light.



ROOM TEMPERATURE



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



NO HEAT



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



NO FREEZING



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.**



SUBCULTURE



**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.



NO STARVING



**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.



NO OVER CROWDING



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



NO FREEZING



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