



Laboratory 1

Observing Wild-type and Mutant *C. elegans*

## REAGENTS, SUPPLIES & EQUIPMENT CHECKLIST \_\_\_\_\_

This laboratory uses reagents that rely on procedures covered in Laboratory 2 (Culturing *C. elegans*) and Laboratory 3 (Culturing *E. coli*). Refer to these laboratories and the recipes section for details on reagent preparation.

### **Cultures and Media**

- Mutant worms on NGM-lite plates  
(*dpy-10*, *rol-6*, *bli-1*, *unc-22*)
- Wild-type worms on an NGM-lite plate

### **Supplies and Equipment**

- Binocular dissecting microscope

## INTRODUCTION

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A human is a complicated organism, and most molecular genetic experiments would either be technically difficult or unethical to perform on human subjects. For these reasons, biologists often use simpler “model” organisms that are easy to culture and manipulate in the laboratory. Despite obvious physical differences, model organisms and humans share many key biochemical and physiological functions that have been conserved (preserved) during evolution. The nematode worm *Caenorhabditis elegans* is one of several organisms commonly studied by biological researchers today.

*C. elegans* is a microscopic roundworm. Although some roundworms are parasitic, *C. elegans* is a free-living worm that feeds on soil bacteria. These worms grow quickly, developing from embryo to adult in three days. Worms pass through several larval stages, molting between each, to develop into adults – either males or self-fertile hermaphrodites (with both male and female gametes). Thus, scientists can cross males and hermaphrodites to make new worm strains, and then easily maintain these strains by self-fertilization.

Scientists often attempt to study gene function in model organisms by observing the phenotypic consequences of introducing a defined mutation in a single gene. While some mutations may cause obvious physical changes, others may result in subtle behavioral or biochemical differences.

This sort of mutational analysis requires knowledge of the appearance and behavior of wild-type worms. In Part I, the morphology, behavior, and life cycle of wild-type *C. elegans* hermaphrodites is observed. This is followed, in Part II, by observing the abnormal morphology and behavior of mutant worms.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**(1):71-94.

De Melo, J.V., De Souza, W., Peixoto, C.A. (2002). Ultrastructural analyses of the *Caenorhabditis elegans* DR 847 *bli-1(n361)* mutant which produces abnormal cuticle blisters. *J Submicrosc Cytol Pathol.* **34**(3):291-7.

Kramer, J.M., French, R.P., Park, E.C., and Johnson, J.J. (1990). The *Caenorhabditis elegans rol-6* gene, which interacts with the *sqt-1* collagen gene to determine organismal morphology, encodes a collagen. *Mol Cell Biol.* **10**(5):2081-9.

Levy, A.D., Yang, J., Kramer, J.M. (1993). Molecular and genetic analyses of the *Caenorhabditis elegans dpy-2* and *dpy-10* collagen genes: a variety of molecular alterations affect organismal morphology. *Mol Biol Cell.* **4**(8):803-17.

**Silencing Genomes, Laboratory 1: Observing Wild-type and Mutant *C. elegans***

Moerman, D.G., Benian, G.M., and Waterston, R.H. (1986). Molecular cloning of the muscle gene *unc-22* in *Caenorhabditis elegans* by Tc1 transposon tagging. *Proc Natl Acad Sci U S A.* 83(8):2579-83.

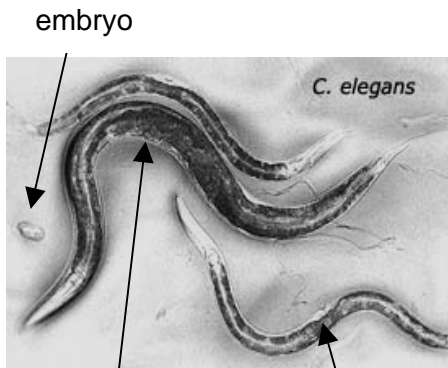
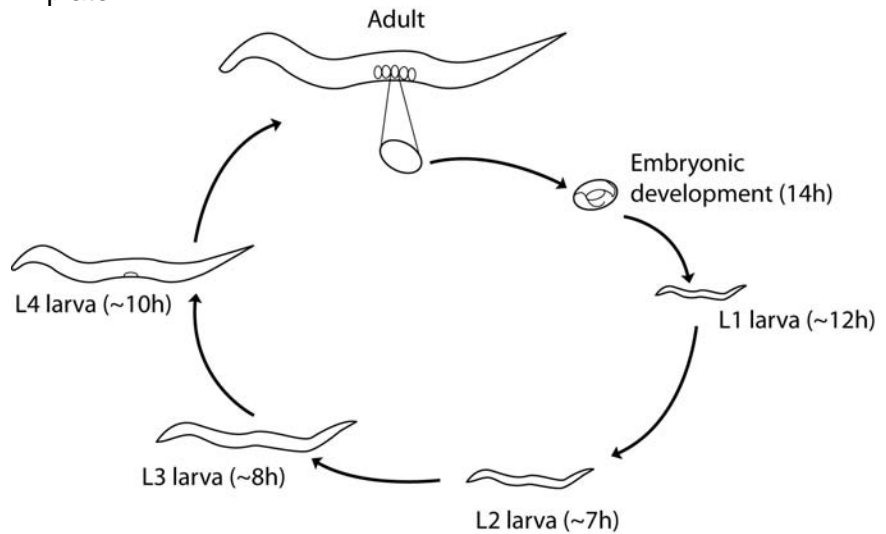
Moerman, D.G., Benian, G.M., Barstead, R.J., Schriefer, L.A., and Waterston, R.H. (1988). Identification and intracellular localization of the *unc-22* gene product of *Caenorhabditis elegans*. *Genes Dev.* 2(1):93-105.

**METHODS**

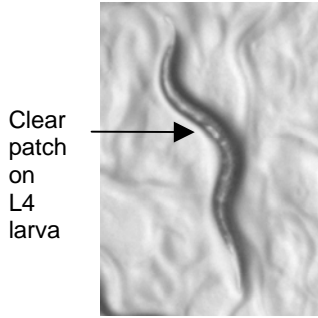
**I. Observe the *C. elegans* Life Cycle**

Culture	Supplies and Equipment
Wild-type worms on NGM-lite plate	Binocular dissecting microscope

1. Obtain a plate with wild-type worms.
2. Observe the worms under a dissecting microscope. Note any physical (morphological) differences between the worms.
3. Note any differences in behavior, paying particular attention to how they move on the plate.
4. Lift the plate several centimeters (about an inch) above the microscope stage, and drop it. Note any changes in worm movement. You may need to tap the plate several times to induce movement.
5. Study the diagram of the *C. elegans* life cycle. Attempt to identify an example of each stage of the worm life cycle on the plate:



- a. The **embryo** is a small, oval object.
- b. An **adult hermaphrodite** is a large worm with embryos inside. (The wild-type N2 strain used in this experiment contains few if any adult males).
- c. An **L1 larva** has recently hatched and is the smallest of the four larval stages.



d. **L2 and L3 larvae** are larger than an L1 worms but not as large as an adult. Examine worms of different sizes to familiarize yourself with these larval stages.

e. The final juvenile stage, an **L4 larva**, is almost as large as an adult hermaphrodite. The lack of internal embryos is one marker that distinguishes an L4 larva from an adult. A clear, crescent-shaped patch near the center of the body is another characteristic of an L4 larva. The egg-laying structure, called the vulva, will develop in this patch when the L4 molts into an adult.

6. Continue to practice identifying L4 hermaphrodites. All of the RNAi experiments in the course begin with identifying L4 hermaphrodites, so it is important to become proficient at identifying them.

**II. Observe *C. elegans* Mutants**

Cultures	Supplies and Equipment
Mutant worms on NGM-lite plates ( <i>dpy-10, rol-6, bli-1, unc-22</i> ) Wild-type worms on NGM-lite plate (from Part I)	Binocular dissecting microscope

1. Obtain plates with mutant and wild-type worms.
2. Observe the worms under a dissecting microscope. Note any physical (morphological) differences between the wild-type and mutant worms. Record your observations and make sketches as needed.
3. Note any differences in behavior, paying particular attention to how the wild-type and mutant worms move on the plate. Gently tap the plates on the microscope stage to induce movement. Record your observations, and make sketches as needed.

## RESULTS & DISCUSSION

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1. Describe the stages of *C. elegans* development. How many stages were you able to identify?
2. Why is it necessary for *C. elegans* to pass through several larval stages, and how is this type of development different from humans?
3. How does a hermaphrodite produce offspring without mating?
4. What physical (morphological) differences did you observe in the mutant worms? What differences in behavior or movement did you notice? Did your classmates identify the same characteristics of the mutant *C. elegans*?
5. For each mutant phenotype you observed, what do you think would be the function of the protein produced by the wild-type gene?

## CONCEPTS AND METHODS

This laboratory can help students understand several important concepts of modern biology:

- The relationship between genotype and phenotype.
- Development and developmental stages.
- The use of model organisms in research.
- Methods to study the function of genes.

The laboratory uses these methods for modern biological research:

- Microscopy.
- Growth and manipulation of a model organism.

## INSTRUCTOR PLANNING, PREPARATION, AND LAB FINE POINTS

The following table will help you to plan and integrate the different parts of the experiment. Parts I and II can all be done concurrently (as they require the same basic preparation and equipment) or on different days.

Part	Day	Time	Activity
	-7	60 min.	<b>Pre-lab:</b> Prepare NGM-lite plates.
	-6	15 min.	<b>Pre-lab:</b> Prepare an overnight culture of OP50 <i>E. coli</i> .
	-5	30 min.	<b>Pre-lab:</b> Seed NGM-lite plates with OP50 <i>E. coli</i> .
	-3	30 min.	<b>Pre-lab:</b> Chunk wild-type and mutant <i>C. elegans</i> strains ( <i>rol-6</i> , <i>bli-1</i> , <i>unc-22</i> , <i>dpy-10</i> ) to OP50-seeded NGM-lite plates.
	-2	30 min.	<b>Pre-lab:</b> Chunk wild-type <i>C. elegans</i> to OP50-seeded NGM-lite plates.
I. Observe Wild-type <i>C. elegans</i>	1	20 min. 45 min.	<b>Pre-lab:</b> Set up student stations. <b>Lab:</b> Examine worms under the dissection microscope. Study the morphology and behavior of wild-type worms and identify the different developmental stages of the <i>C. elegans</i> life cycle.
II. Observe Mutant <i>C. elegans</i>	1	45 min.	<b>Lab:</b> Examine wild-type and mutant worms. Identify differences in morphology or movement.

### I. Observe the *C. elegans* Life Cycle

#### **Pre-lab Preparation**

Prepare an overnight culture of OP50 *E. coli* and seed NGM-lite plates according to Parts I and II of Laboratory 3.

Chunk wild-type *C. elegans* strains according to Part I of Laboratory 2. Be sure to start this process at least a week before the laboratory.

**Pre-lab Setup** (per student station)

Binocular dissecting microscope  
Wild-type worms growing on NGM-lite plate



## II. Observe *C. elegans* Mutants

### **Pre-lab Preparation**

Prepare an overnight culture of OP50 *E. coli* and seed NGM-lite plates according to Parts I and II of Laboratory 3.

Chunk wild-type and mutant *C. elegans* strains according to Part I of Laboratory 2. Be sure to start this process at least a week before the laboratory.

### **Pre-lab Setup** (per student station)

Binocular dissecting microscope  
Wild-type worms growing on NGM-lite plate (from Part I)

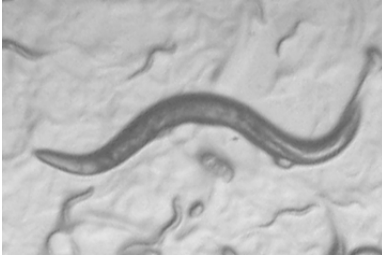
### **Shared Items**

Mutant worms growing on NGM-lite plates (*dpy-10*, *rol-6*, *bli-1*, *unc-22*)

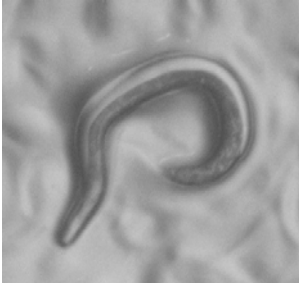
## ANSWERS TO RESULTS & DISCUSSION QUESTIONS \_\_\_\_\_

1. Describe the stages of *C. elegans* development. How many stages were you able to identify? **The *C. elegans* life cycle consists of 6 different stages: embryo, larval (L) 1-4, and adult. Nematodes continue to grow between molts, so each larval stage consists of worms of varying sizes. Although this makes it difficult to clearly distinguish most larval stages, L4s are larger than other larvae but lack the embryos and vulva of adults. Students should be able to distinguish 5 stages: embryo, L1, L2-3, L4, and adult.**
2. Why is it necessary for *C. elegans* to pass through several larval stages, and how is this type of development different from humans? **Most organisms molt because there is a physical limit to growth in each larval phase. However, it is not clear that this is important in *C. elegans* development. Some scientists believe that the larval stages were once important for the ancestors of *C. elegans*, and the molts are remnants from that time. Like many higher organisms, humans do not develop in distinct phases, where each stage has a physical limit to growth. Rather, we grow more or less continuously by adding new cells to existing tissues and organs.**
3. How does a hermaphrodite produce offspring without mating? **A hermaphrodite worm produces both spermatocytes and oocytes, enabling a single worm to self-fertilize within its body to produce embryos.**
4. What physical (morphological) differences did you observe in the mutant worms? What differences in behavior or movement did you notice? Did your classmates identify the same characteristics of the mutant *C. elegans*? **Identifying *C. elegans* phenotypes is subjective, and different students may focus on different aspects of the mutant phenotypes. Comparing different student observations of the same mutant is an interesting way to examine subjectivity in science. The following panel will aid you in leading student discussion.**

### *C. elegans* Phenotypes

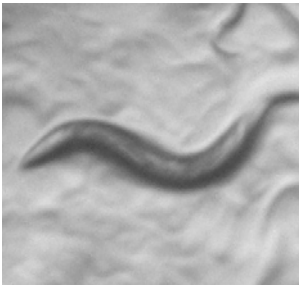
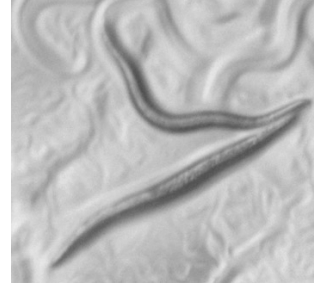


**Wild-type** Very active; graceful serpentine movement and tracks in agar.



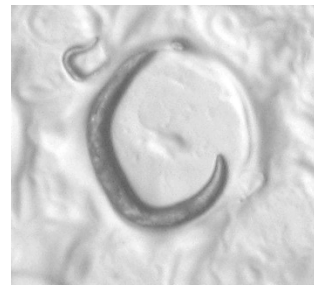
**bli-1** Clear area on the side of the worm is a blister in the cuticle (most obvious in old adults). The blisters may inhibit movement.

**unc-22** Little movement but twitching; body outstretched rather than S-shaped.



**dpy-10** Short and plump.

**rol-6** Body twists like a corkscrew leaving circular tracks in agar.



5. For each mutant phenotype you observed, what do you think would be the function of the protein produced by the wild-type gene? **The shortened body in *dpy-10* mutants suggests the protein product is required to develop full body length. Since *unc-22* and *rol-6* mutations cause movement disorders, these proteins could be components of the nervous or muscle system or be required for the development of these systems. The twisted body shape of *rol-6* mutant worms suggest the protein is required to maintain a straight body. *bli-1* seems to encode a protein that is needed to form the interface between layers of the body wall or to prevent extracellular fluid buildup.**

Laboratory 4, "Inducing RNAi by Feeding," will guide students through a bioinformatics exercise to discover the true function of these proteins. At that time, they will find that *dpy-10*, *rol-6*, and *bli-1* all encode collagens found in the outer cuticle layer while *unc-22* is a muscle protein required for normal muscle morphology and contraction.