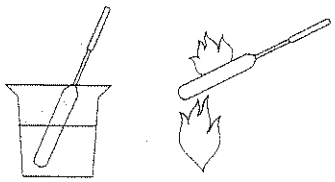
METHODS

I. CHUNK WILD-TYPE AND MUTANT *C. elegans*

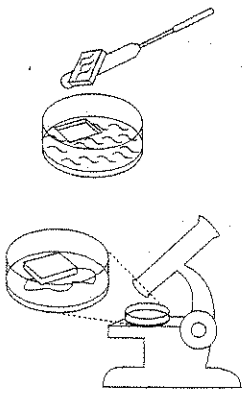
Reagents	Supplies and Equipment
Plate of *bli-1* mutant worms	Stereomicroscope
Plate of *dpy-11* mutant worms	Bunsen burner
Plate of wild-type worms	95% ethanol, in 50- or 100-mL beaker
OP50-seeded NGM-lite plates (3)	Steel spatula



Sterilization prevents cross-contamination with different *C. elegans* strains and non-OP50 bacteria.

1. Two to three days before completing Part II, obtain a plate of wild-type worms.
2. Label the bottom of a fresh OP50-seeded NGM-lite plate with your group name or number, the date, and "wild type."
3. Using your microscope, identify a region of the plate of wild-type worms that is densely populated with worms.
4. Sterilize the metal spatula as follows: dip the end of the spatula into the ethanol beaker, and briefly pass it through a Bunsen flame to ignite the alcohol. Allow alcohol to burn off away from the Bunsen flame; the implement will become too hot if left in the flame.

   CAUTION Be extremely careful not to ignite the ethanol in the beaker. Do not panic if the ethanol is accidentally ignited. Cover the beaker with a petri dish lid or other non-flammable cover to cut off oxygen and rapidly extinguish the fire.
5. Use the sterilized spatula to cut a 1 cm x 1 cm (3/8 in x 3/8 in) piece of agar from the region identified in Step 3.
6. Carefully lift out the cut piece of agar with the worms, and place it on the OP50 lawn of the fresh NGM-lite plate. Ideally, place the piece upside down to make it easier for the worms to crawl into the new bacterial food source.

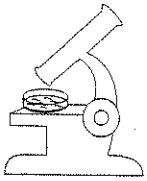


7. Examine the new plate under the microscope to verify that you have successfully chunked the worms. Within a few minutes, worms should crawl from the agar chunk and be visible in the bacterial lawn.
8. Two to three days before you plan to complete Part III, obtain plates of *dpy-11* and *bli-1* mutant worms. Repeat steps 2 through 7 for each mutant strain, labeling the mutant worm strains appropriately.

   Store the plates at +20°C (the temperature of a cool room) until you are ready to continue with Part II or Part III. Choose a place where the plate will not be disturbed or in direct sunlight. Note that you may be able to complete both Parts II and III on the same day.

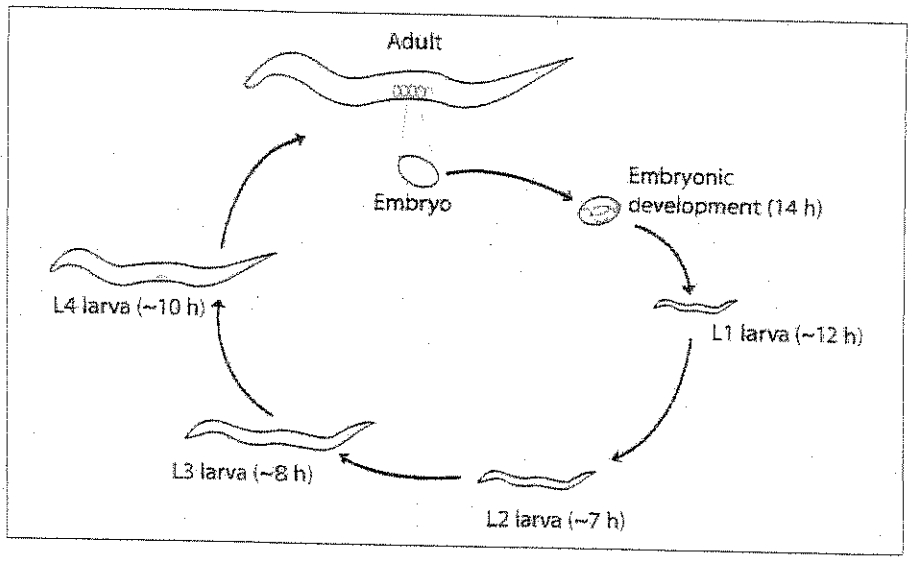
II. OBSERVE THE *C. elegans* LIFE CYCLE

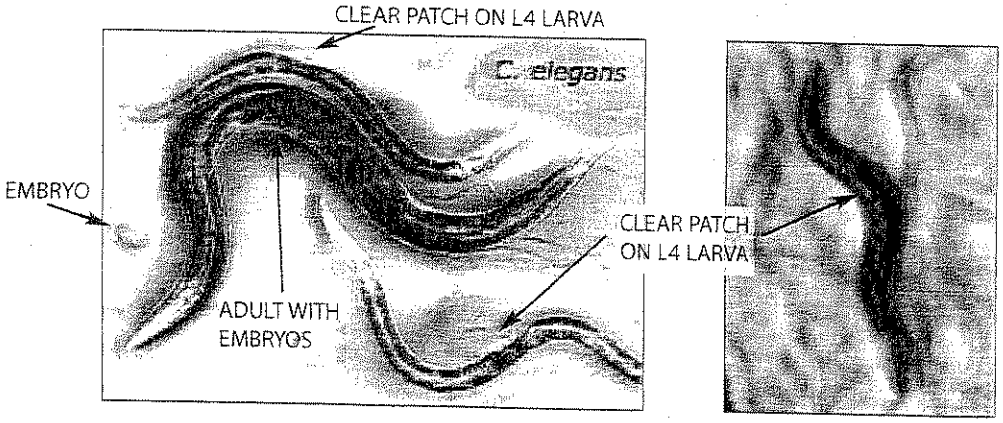
Culture	Supplies and Equipment
Wild-type worms on NGM-lite plate (from Part I)	Stereomicroscope



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. Gently tap the plate on the microscope stage to induce movement. 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. Between each larval stage and after the L4 stage, worms molt. This enables them to continue growing. Identify an example of each stage of the worm life cycle on the plate:

***C. elegans* life cycle**
Numbers in parentheses indicate the approximate number of hours spent in that stage of development.





a. An **adult hermaphrodite** (a worm with both male and female gametes) is a large worm with embryos inside. Hermaphrodites are self-fertile, but can also mate with males. The wild-type strain used in this experiment contains few if any adult males; wild-type *C. elegans* does not have females.

b. The **embryo** is a small, oval object.

c. An **L1 larva** has recently hatched and is the smallest of the four larval stages.

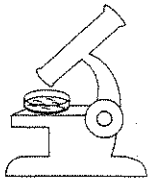
d. **L2 and L3 larvae** are larger than L1 worms, but not as large as an adult. Examine worms of different sizes to familiarize yourself with these larval stages.

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. Practice identifying L4 hermaphrodites and adults.

III. OBSERVE *C. elegans* MUTANTS

Culture	Supplies and Equipment
Mutant worms on NGM-lite plates (*bli-1*, *dpy-11*) (from Part I)	Stereomicroscope



1. Obtain your plates with *bli-1* and *dpy-11* mutant worms.
2. Recall the characteristics of wild-type *C. elegans*. Observe the mutant 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 after observing *C. elegans* nematode worms

1. Check how many stages of C. elegans development you can identify?

_____ embryo _____L1 larva _____L2-3 larva _____ L4 larva _____ Adult worm

2. List 2 characteristics used to distinguish adults from L4 larva?

3. How does a hermaphrodite produce offspring without mating?

4. Summarize your observations in the table below:

	Wild type worms	BLI-1 **mutant** worms	DPY-11 **mutant** worms
Unique structural/behavioral chacteristics			
Diagram			

5. For each mutant you observed, what do you think would be the function of the protein produced?

6. Mutations in both the bli-1 and dpy-11 worms affect the development of the worm's cuticle (outer skin or epidermal layer). The blii-1 gene codes for a structural collagen protein in the skin, while the dpy-11 gene codes for an enzyme that modifies other proteins in the endoplasmic reticulum. How can 2 mutations in skin cells cause such different (phenotype) effects?