Preface

The DNA Learning Center's effort to develop lab experiments for high school and colleges dates to 1985, when Dave Micklos teamed with Greg Freyer to develop a sequence of experiments to make and analyze a recombinant DNA molecule. The initial testing was done in Rich Roberts' lab, well before he won the Nobel Prize, and incorporated key insights on inserting DNA into bacteria from the dean of transformation, Doug Hanahan. The lab sequence was initially a slim lab manual called "Recombinant DNA for Beginners," whose title was derived from the Graham Nash album "Songs for Beginners." It expressed perfectly our ideal of making complicated gene technology accessible to novices.

Within a year, we were training teachers across the country on summer tours of one, then two, Vector vans. These were customized Ford Econoline vans that packed enough equipment, reagents, and supplies to convert any general science lab into a molecular genetics lab. Along the way, we developed a complementary minitext that presented the concepts behind the labs, as well as extensions of recombinant DNA technology in basic and applied research. The much expanded work was formally published in 1991 as the Cold Spring Harbor Laboratory Press book *DNA Science*. That name, which Jim Watson threw out over lunch one day, seemed to capture the excitement of a new science based on the ability to manipulate and understand the DNA molecule.

DNA Science, now in its second edition, has sold more than 90,000 copies and is credited with helping to catalyze the movement to bring hands-on experiments with DNA into high school and beginning college classrooms. Two experiments found their way into the Advanced Placement biology curriculum, giving these experiments a nationwide audience. Stand-alone kits, developed with Carolina Biological Supply Company, reach well over 100,000 students per year.

The experiments in *DNA Science* are based exclusively on bacterial genetic systems. Now, *Genome Science* aims to take students to a higher level of biological and technological integration—to study the function of eukaryotic genes and genomes. Nineteen laboratories focus on four revolutionary technologies—polymerase chain reaction, DNA sequencing, RNA interference (RNAi), and bioinformatics—across three eukaryotic systems: humans, plants, and *Caenorhabditis elegans*. All labs stress the modern synthesis of molecular biology and computation, integrating in vitro experimentation with in silico bioinformatics. In addition to well-tested biochemical methods, *Genome Science* introduces *DNA Subway*, an intuitive bioinformatics platform that makes easy work of gene and genome analysis.

The four major techonologies are organized into stand-alone chapters with extensive text introductions that place related labs into a common historical and conceptual framework. This modular approach provides options to develop new courses or to integrate labs into existing courses or student research. We especially hope that these protocols will help educators to extend research to classroom settings and distribute experiments in which multiple classes analyze and contribute to common data sets. DNA barcoding is especially amenable to "campaigns" in which many students contribute to understanding diversity within a common biogeographical unit.

Genome Science borrows many user-friendly features from its predecessor, including flow charts, marginal notes, reagent recipes, and extensive instructor information. To ease implementation, most labs are available as ready-to-use kits from Carolina Biological Supply Company. In addition, like its predecessor, *Genome Science* aims to help beginners use modern tools to explore the unseen world of genes and genomes.

In contemplating the cosmos in 1927, the great mathematical geneticist J.B.S. Haldane famously said, "My own suspicion is that the universe is not only queerer than we suppose, but queerer than we can suppose." Had he been alive today, Haldane would almost certainly have the same suspicion about the genomes of higher organisms. In this sense, genome scientists are the new cosmologists of biology, uncovering the strange and beautiful structure of the genetic material that runs through all life.

Happy explorations.

David Micklos
Bruce Nash
Uwe Hilgert

Cold Spring Harbor, New York
March 2012

LABORATORY
4.1

Culturing and Observing *C. elegans*

▼ OBJECTIVES

This laboratory demonstrates several important concepts of modern biology. During the course of this laboratory, you will

- Learn about the use of model organisms in research.
- Observe development and identify specific developmental stages in *Caenorhabditis elegans*.
- Explore the relationship between genotype and phenotype.

In addition, this laboratory utilizes several experimental methods in modern biological research. You will

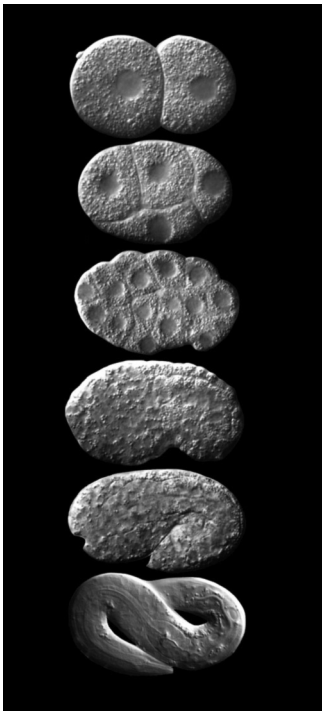
- Use sterile technique to isolate and grow pure cultures of bacteria and *C. elegans*.
- Use antibiotic selection to maintain a recombinant bacterial culture.
- Use dissecting microscopes to observe and analyze cultures of bacteria and *C. elegans*.

INTRODUCTION

Sydney Brenner won a Nobel prize for establishing *C. elegans* as a model organism.
(Photo courtesy of Matthew Meselson.)

A human is a complicated organism, and most molecular genetic experiments would be either 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 *C. 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 3 d. *C. elegans* is a simple animal with only ~1000 cells, and scientists know exactly how each of those cells develops from the fertilized egg. *C. elegans* was the first multicellular organism to have its entire genome sequenced, with the surprising finding that 40% of its genes have human matches. Mating animals, isolating genes, and introducing foreign DNA are much easier in *C. elegans* than in more complicated animals. All of these features make *C. elegans* a great model for understanding how cells divide, develop, and take on specialized tasks in higher (eukaryotic) organisms. Recently, the discovery that any of the organism's genes can be "silenced" using a technique called RNA interference (RNAi) has made *C. elegans* an ideal organism to quickly determine the functions of genes identified by sequencing the genome.



Embryonic development in *C. elegans*. This series of images shows different stages during embryonic development. Fertilization to hatching takes just 14 h.

(Reprinted, with permission, from O'Rourke M, Bowerman B. 2005. *Nature* 434: 444–445; ©Macmillan.)

This laboratory introduces *C. elegans* and describes methods required for its culture; these techniques and familiarity with *C. elegans* are prerequisites for RNAi experiments. Included are techniques for growing *Escherichia coli* cells and preparing plates to feed *C. elegans*. The most common strain of bacteria used to feed worms is the *E. coli* strain OP50, which is grown on standard LB plates or in LB broth. For RNAi experiments, specialized strains of bacteria, each containing a plasmid that expresses a gene-specific double-stranded RNA (dsRNA), are fed to worms to trigger gene silencing.

A technique for spreading bacteria onto standard plates to isolate single cells from one another is described. Each cell then reproduces to form a visible colony composed of genetically identical clones. Small-scale suspension cultures of *E. coli* are then grown by overnight incubation using cells derived from a single colony, which minimizes the chance of using a cell mass contaminated with a foreign microorganism. These overnight cultures are used to inoculate, or seed, specialized "NGM (nematode growth medium)-lite" agar plates on which the worms are grown. *E. coli* OP50 is seeded to NGM-lite plates. In addition, RNAi strains expressing dsRNA corresponding to three genes, *dpy-11, bli-1*, and *unc-22*, are seeded to NGM-lite plates with ampicillin and isopropyl-β-D-thiogalactopyranoside (IPTG). Ampicillin selects for bacteria carrying the RNAi plasmid, and IPTG triggers dsRNA expression from the plasmid in the bacteria. In Laboratory 4.2, the function of these three genes is assayed when RNAi is induced by culturing worms on these plates.

Two methods to propagate worms are described. Worms growing on an NGM-lite plate are transferred by cutting out a section of the medium and placing it on the surface of a new plate. This "chunking" method is a rapid way to move multiple worms from a plate where the *E. coli* food source has been consumed to a plate with a fresh

lawn of bacteria. Individual worms can be transferred to fresh plates using the flattened tip of a platinum wire; this technique for "picking" individual worms is the starting point for genetic crosses and RNAi experiments.

Finally, wild-type *C. elegans* hermaphrodites are observed using microscopy, and their morphology, behavior, and life cycle are analyzed. Abnormal morphology and behavior of mutant worms are also observed.

FURTHER READING

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PLANNING AND PREPARATION

The following table will help you to plan and integrate the different experimental methods.

Experiment	Day	Time		Activity
Stage A: Culturing *E. coli*				
	2 or more d before Part I	90 min	Prelab:	Prepare LB, LB/amp, NGM-lite, and NGM-lite/amp + IPTG plates.
	1–2 d before Part I	30 min	Prelab:	Streak starter plates for OP50 and RNAi feeding strains (*dpy-11*, *bli-1*, *unc-22*).
I. Streak *E. coli* to obtain single colonies	1	20 min	Prelab:	Set up student stations.
		30 min	Lab:	Streak plates.
		15–20 h	Postlab:	Incubate plates.
II. Grow *E. coli* overnight cultures	2	15 min	Prelab:	Aliquot LB and LB/amp.
				Set up student stations.
		15 min	Lab:	Prepare overnight cultures.
		12–48 h	Postlab:	Incubate cultures.
III. Seed NGM-lite and NGM-lite/amp + IPTG plates with *E. coli*	4	30 min	Lab:	Seed NGM-lite and NGM-lite/amp + IPTG plates.
		24–36 h	Postlab:	Incubate plates.[a]
Stage B: Culturing *C. elegans*				
I. Chunk wild-type *C. elegans*	6	30 min	Prelab:	Chunk wild-type worms to OP50-seeded NGM-lite plates.[b]
	8	20 min	Prelab:	Set up student stations.
		15 min	Lab:	Chunk worms to OP50-seeded NGM-lite plates. Incubate for 48 h.
II. Pick individual *C. elegans*	10	20 min	Prelab:	Make worm picks.
				Set up student stations.
		30 min	Lab:	Pick L4 worms to fresh OP50-seeded NGM-lite plates.
Stage C: Observing Wild-Type and Mutant *C. elegans*[c]				
I. Observe the *C. elegans* life cycle	12–13	20 min	Prelab:	Set up student stations.
		45 min	Lab:	Study the morphology, behavior, and life cycle of wild-type worms under a dissecting microscope.
II. Observe *C. elegans* mutants	9	30 min	Prelab:	Chunk wild-type and mutant *C. elegans* strains (*rol-6*, *bli-1*, *unc-22*, *dpy-11*) to OP50-seeded NGM-lite plates.
				Set up student stations.
	12–13	45 min	Lab:	Examine wild-type and mutant worms; identify differences in development rate, morphology, or movement.

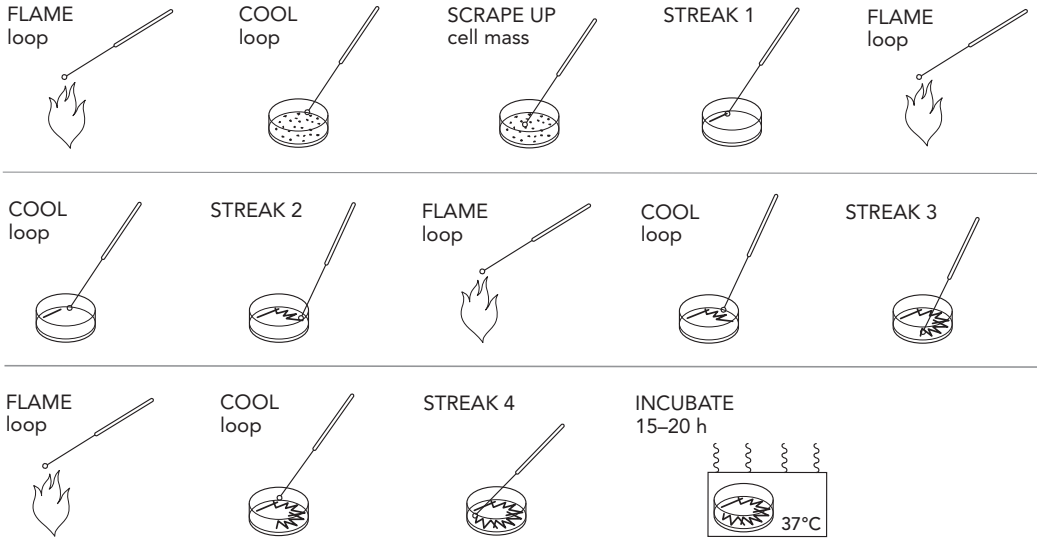
[a]After incubation, seeded plates from Part III of Stage A can be stored in sealed containers for several weeks at 4°C.

[b]If enough plates for each student are available, this extra round of chunking may be skipped. (It is a good idea to prepare extra plates, because some may become contaminated during preparation or student work.)

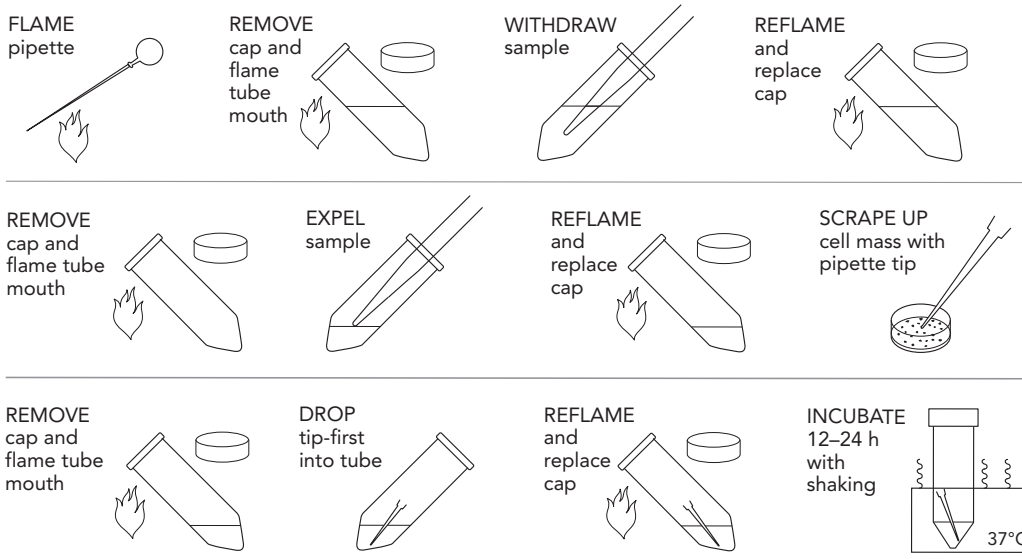
[c]Both procedures in Stage C require wild-type worms from Parts I or II of Stage B. If you are not completing Parts I or II of Stage C immediately after Stage B, transfer small chunks of wild-type worms to OP50-seeded NGM-lite plates 2 d before completing Stage C.

OVERVIEW OF EXPERIMENTAL METHODS IN STAGE A: CULTURING *E. COLI*

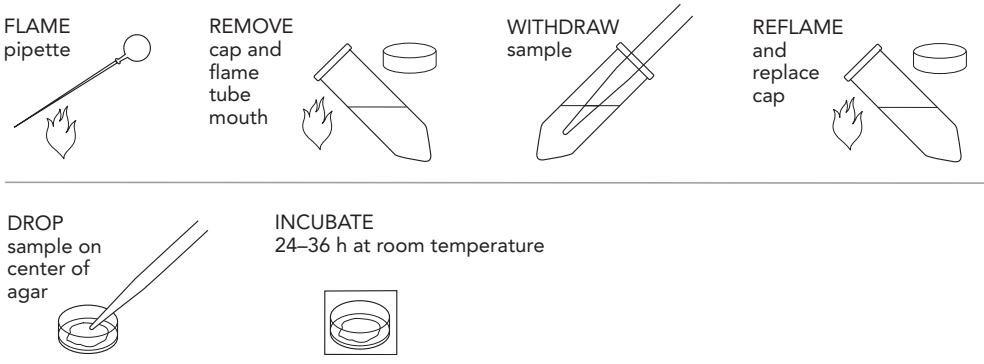
I. STREAK *E. COLI* TO OBTAIN SINGLE COLONIES



II. GROW *E. COLI* OVERNIGHT CULTURES



III. SEED NGM-LITE AND NGM-LITE/AMP + IPTG PLATES WITH *E. COLI*



STAGE A: CULTURING *E. COLI*

I. Streak *E. coli* to Obtain Single Colonies

REAGENTS, SUPPLIES, & EQUIPMENT

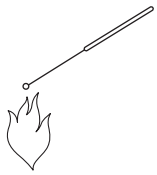
For each group

Bunsen burner
Inoculating loop
LB agar plate
3 LB + ampicillin <!> (LB/amp) plates
Permanent marker (red)
Bleach (10%) <!> or disinfectant (e.g., Lysol)
E. coli OP50 culture
E. coli RNAi feeding strain cultures (*dpy-11*, *bli-1*, and *unc-22*)
Incubator set at 37°C

To share

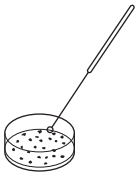
"Bio bag" or heavy-duty trash bag

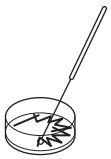
See Cautions Appendix for appropriate handling of materials marked with <!>.



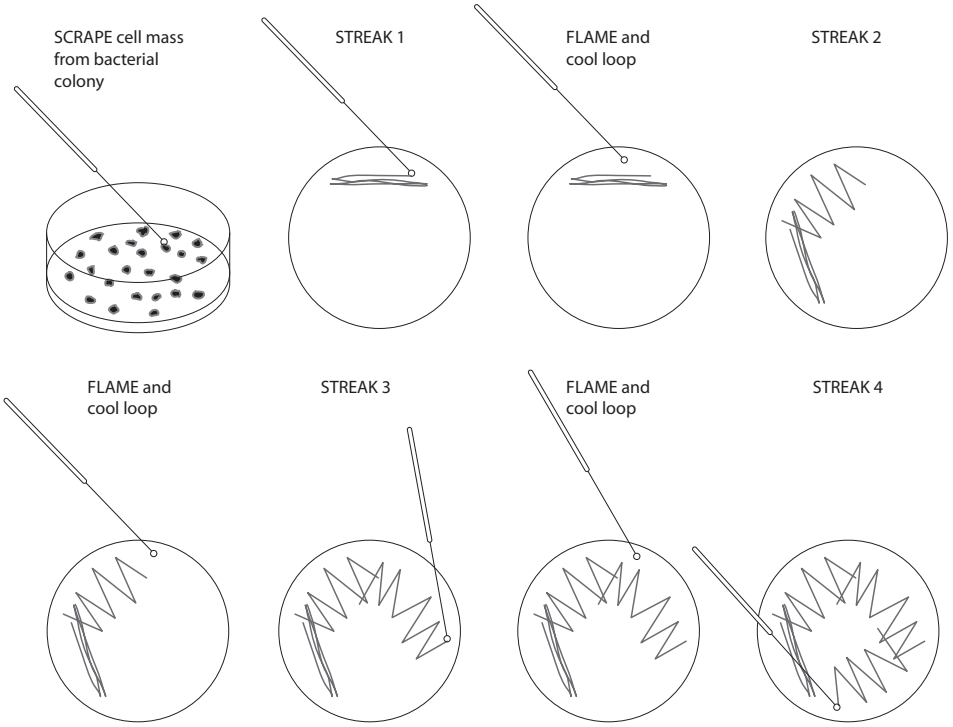
CAUTION! When using an open flame, take appropriate precautions. Make sure that any loose clothing is secured and tie long hair back. Do not lean over the flame.

1. Use a red permanent marker to label the *bottom* of the LB agar plate with your group number, the date, and "OP50."
2. Hold the inoculating loop like a pencil and sterilize the loop in the Bunsen burner flame until it glows red hot.
3. Remove the lid from the *E. coli* OP50 culture plate with your free hand. Do not place the lid on the lab bench; hold the lid face down just above the culture plate to help to prevent contaminants from falling on the plate or lid.
4. Stab the inoculating loop into a clear area of the *E. coli* OP50 culture plate several times to cool it.
5. Use the loop tip to scrape a visible cell mass from a bacterial colony on the *E. coli* OP50 culture plate. Do not gouge the agar. Replace the lid on the *E. coli* OP50 culture plate.
6. Lift the lid of the new LB agar plate just enough to perform streaking as described below. The object is to serially dilute the bacteria with each successive streak, so that individual cells are separated in at least one of the streaks. Do not place the lid on the lab bench; replace the plate lid after each streak.
   i. Streak 1: Glide the loop tip back and forth across the surface of the LB agar to make a streak across the top quarter of the plate. Avoid gouging the agar.
   ii. Streak 2: Reflame the inoculating loop and cool it by stabbing it into the agar away from the first (primary) streak. Draw the loop tip through the end of the primary streak and, without lifting the loop, make a zigzag streak across one quarter of the agar surface.
   iii. Streak 3: Reflame the loop and cool it in the agar. Draw the loop tip *once* through the end of the previous streak and make another zigzag streak in the adjacent quarter.
   iv. Streak 4: Reflame the loop and cool it. Draw the tip *once* through the end of the previous streak and make a final zigzag streak in the remaining quarter of the plate.
   v. Reflame the loop and allow it to cool before placing it on the lab bench.
7. Label the *bottom* of three LB/amp plates with your group number, the date, and the appropriate *E. coli* RNAi feeding strain ("*dpy-11* RNAi," "*bli-1* RNAi," or "*unc-22* RNAi").

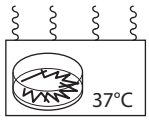




Make it a habit to always flame the loop one last time.



8. Follow the procedures outlined in Steps 2–6 to streak each feeding strain onto a separate LB/amp plate.
9. Place all four plates upside down in a 37°C incubator. Incubate them for 15–20 h, until optimal growth of well-formed colonies is achieved. At this point, colonies should range from 0.5 to 3 mm in diameter.



Plates are inverted to prevent condensation that collects on the lids from falling onto the agar, causing the colonies to run together.

10. Take time for responsible cleanup.
    i. Segregate unwanted bacterial cultures into a "bio bag" or heavy-duty trash bag for proper disposal.
    ii. Wipe the lab bench with soapy water and 10% bleach or disinfectant at the end of the lab.
    iii. Wash your hands before leaving the lab.

II. Grow *E. coli* Overnight Cultures

REAGENTS, SUPPLIES, & EQUIPMENT

For each group

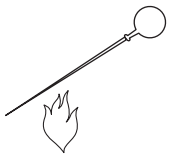
Bunsen burner
4 Culture tubes (15 mL) (sterile)
E. coli OP50 culture plate from Part I
E. coli RNAi feeding strain cultures (*dpy-11*, *bli-1*, and *unc-22*) on LB/amp plates from Part I
Inoculating loop (optional)
LB/amp broth (10 mL)
LB broth (5 mL)
Micropipette tips (10–100 µL, sterile)
Permanent marker (red)
Pipette aid or bulb
2 Pipettes (5 mL) (sterile)
Test tube rack

To share

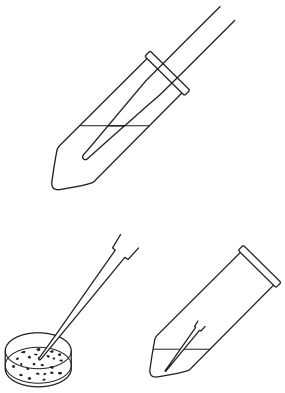
"Bio bag" or heavy-duty trash bag
Bleach (10%) <!> or disinfectant (e.g., Lysol)
Shaking water bath or incubator set at 37°C

See Cautions Appendix for appropriate handling of materials marked with <!>.

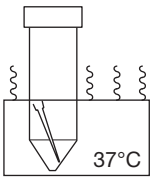
If working in a team, one partner should handle the pipette and the other should handle the tubes and caps.



Pipette flaming can be eliminated if individually wrapped pipettes are used.



Loop flaming can be eliminated if an individually wrapped, sterile plastic loop is used.



1. Use a red permanent marker to label a *sterile* 15-mL culture tube with your group number, the date, and "OP50."
2. Use a 5-mL pipette to *sterilely* transfer 2 mL of LB broth into the culture tube as follows:
   i. Make sure that the culture tube cap is unscrewed to the "loose" position.
   ii. Attach a pipette aid or bulb to a 5-mL pipette. Briefly flame the pipette cylinder.
   iii. Remove the cap of the bottle containing LB broth using the little finger of your hand holding the pipette bulb. Flame the mouth of the LB bottle.
   iv. Use the pipette to withdraw 2 mL of LB. Reflame the mouth of the bottle and replace the cap.
   v. Remove the cap of the labeled culture tube from Step 1. Expel the LB into the tube, reflame, and replace the cap.
3. Use a *sterile* micropipette tip to scrape a visible cell mass from a selected colony on your *E. coli* OP50 culture plate (from Part I) and drop it tip-first into the culture tube. Reflame and replace the tube cap in the loose position. Alternatively, use an inoculating loop as follows:
   i. Sterilize the loop in the Bunsen burner flame until it glows red hot. Cool the loop by stabbing it several times into a clear area near the edge of your *E. coli* OP50 culture plate (from Part I).
   ii. Use the loop to scrape a visible cell mass from a selected colony on your *E. coli* OP50 culture plate. Immerse the cell mass in the LB broth and agitate the loop to dislodge the cell mass.
   iii. Replace the tube cap in the loose position. Reflame the loop before setting it on the lab bench.
4. Label three *sterile* 15-mL culture tubes with your group number, the date, and one of the *E. coli* RNAi feeding strains ("*dpy-11* RNAi," "*bli-1* RNAi," or "*unc-22* RNAi").
5. Use a 5-mL pipette to *sterilely* transfer 2 mL of LB/amp broth into each of the three labeled culture tubes as described in Step 2.
6. Use a *sterile* pipette tip (or a flamed and cooled inoculating loop) to transfer a single colony of RNAi feeding bacteria from each of the *dpy-11*, *bli-1*, and *unc-22* LB/amp plates (from Part I) into the appropriate culture tubes as described in Step 3.
7. Incubate the tubes for 12–24 h in a 37°C shaking water bath (with continuous shaking) or for 24–48 h in a 37°C incubator (without shaking). To allow air flow, do not seal the tube lids during incubation.
8. Take time for responsible cleanup.
   i. Segregate unwanted bacterial cultures and tubes, pipettes, and micropipette tips that have come into contact with cultures into a "bio bag" or heavy-duty trash bag for proper disposal.
   ii. Wipe the lab bench with soapy water and 10% bleach or disinfectant at the end of lab.
   iii. Wash your hands before leaving the lab.

III. Seed NGM-Lite and NGM-Lite/Amp + IPTG Plates with *E. coli*

REAGENTS, SUPPLIES, & EQUIPMENT

For each group

Bunsen burner
E. coli OP50 overnight culture from Part II
E. coli RNAi feeding strain (*dpy-11*, *bli-1*, and *unc-22*) overnight cultures from Part II
3 NGM-lite + ampicillin <!> + isopropyl-β-D-thiogalactopyranoside <!> (NGM-lite/amp + IPTG) plates
4 NGM-lite plates
Permanent markers (black and red)
Pipette aid or bulb
4 Pipettes (5 mL)
Test tube rack

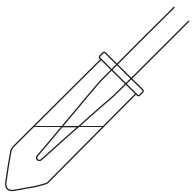
To share

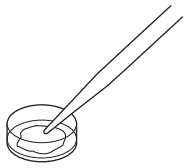
"Bio bag" or heavy-duty trash bag
Bleach (10%) <!> or disinfectant (e.g., Lysol)

See Cautions Appendix for appropriate handling of materials marked with <!>.

1. Label the *bottom* of four NGM-lite plates with your group number and the date with a *black* marker. Use a *red* marker to label the *bottom* of the plates "OP50."
2. Use a 5-mL pipette to *sterilely* seed each of the NGM-lite plates with OP50 as follows:
   i. Attach the pipette aid or bulb to the 5-mL pipette. Briefly flame the pipette cylinder.
   ii. Remove the cap from the OP50 overnight culture (from Part II) using the little finger of your hand holding the pipette bulb. Flame the mouth of the OP50 overnight culture.
   iii. Use the pipette to withdraw 1 mL of overnight culture. Reflame and replace cap.
   iv. Add one to two drops of overnight culture to the center of the surface of each NGM-lite plate. The drops should occupy most of the plate surface but *should not touch the edge* of the dish.
3. Use a *red* marker to label the *bottom* of three NGM-lite/amp + IPTG plates with your group number, the date, and one of the *E. coli* RNAi feeding strains ("*dpy-11* RNAi," "*bli-1* RNAi," or "*unc-22* RNAi").
4. Use a 5-mL pipette to *sterilely* transfer one to two drops of each feeding strain overnight culture to the appropriate NGM-lite/amp + IPTG plate as described in Step 2.
5. Grow the seeded plates *face-up* for 24–36 h at room temperature.
6. Take time for responsible cleanup.
   i. Segregate unwanted bacterial cultures and tubes, pipettes, and micropipette tips that have come into contact with cultures into a "bio bag" or heavy-duty trash bag for proper disposal.
   ii. Wipe the lab bench with soapy water and 10% bleach or disinfectant.
   iii. Wash your hands before leaving the lab.

Seed the plates in a clean area to avoid contamination.





Avoiding the edges of the dish ensures that the worms will remain in the center of the plate.

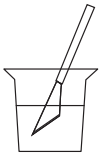
The bacterial lawn should be confluent and dry before any worms are added.

OVERVIEW OF EXPERIMENTAL METHODS IN STAGE B: CULTURING *C. ELEGANS*

I. CHUNK WILD-TYPE *C. ELEGANS*

OBSERVE worms

DIP implement in ethanol



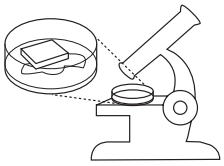
IGNITE ethanol

CUT chunk with worms

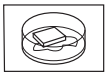
PICK UP chunk

TRANSFER chunk to new plate

VERIFY worm transfer

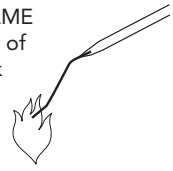


INCUBATE 48 h at room temperature

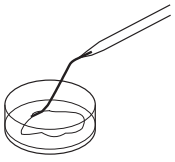


II. PICK INDIVIDUAL *C. ELEGANS*

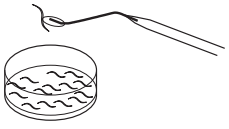
FLAME end of pick



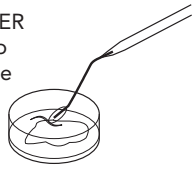
ATTACH glob of bacteria



PICK large worms



TRANSFER worms to new plate



VERIFY worm transfer and health

STAGE B: CULTURING *C. ELEGANS*

I. Chunk Wild-Type *C. elegans*

REAGENTS, SUPPLIES, & EQUIPMENT

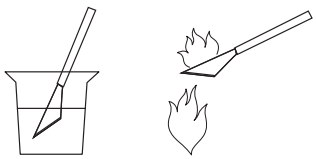
For each group

Binocular dissecting microscope
Bunsen burner
Ethanol (95%) <!> in a 50- or 100-mL beaker
Metal spatula or forceps
OP50-seeded NGM-lite plate from Part III of Stage A
Permanent marker (black)
Wild-type worms on NGM-lite plate

To share

"Bio bag" or heavy-duty trash bag
Bleach (10%) <!> or disinfectant (e.g., Lysol)

See Cautions Appendix for appropriate handling of materials marked with <!>.

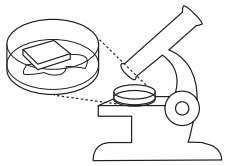


Sterilization prevents cross-contamination with different C. elegans *strains and non-OP50 bacteria.*

CAUTION! Be extremely careful to avoid igniting the ethanol in the beaker. Do not panic if the ethanol is accidentally ignited. Cover the beaker with a glass Petri dish lid or other non-flammable cover to cut off oxygen and rapidly extinguish the fire.

To feed worm strains, it is easier to transfer a chunk of worm-filled agar from a well-grown plate to a new plate rather than to pick individual worms.

Placing the agar piece upside down makes it easier for the worms to crawl into the new bacterial food source.



1. Obtain a fresh OP50-seeded NGM-lite plate (from Part III of Stage A) and a plate with wild-type worms.
2. Examine both plates under the dissecting microscope for signs of bacterial or mold contamination—any growth of a different color or morphology (shape) from the OP50 lawn. Obtain a new plate if you detect any contamination.
3. Use a black permanent marker to label the bottom of the fresh OP50-seeded NGM-lite plate with your group number, the date, and "wild type."
4. Use your dissecting microscope to identify a region of the plate of wild-type worms that is densely populated with worms and eggs.
5. Sterilize a metal spatula or forceps by dipping the end of the implement into the beaker of ethanol and then briefly passing it through a Bunsen burner flame to ignite the ethanol. Allow the ethanol to burn off away from the Bunsen flame; the implement will become too hot if left in the flame.
6. Use a sterilized spatula or forceps to cut a 1-cm (~3/8-in) square chunk of agar from the worm- and egg-dense region of the plate identified in Step 4.
7. Carefully remove the piece of agar with worms from the wild-type plate and place it upside down on the lawn of the fresh OP50-seeded NGM-lite plate.
8. 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.
9. Store the new plate lid-side down for ~48 h at room temperature before continuing with Part II of Stage B (or Parts I or II of Stage C). Choose a place where the plate will not be disturbed.
10. Take time for responsible cleanup.
    i. Segregate any bacterial cultures that need to be discarded into a "bio bag" or heavy-duty trash bag for proper disposal.
    ii. Wipe the lab bench with soapy water and 10% bleach or disinfectant.
    iii. Wash your hands before leaving the lab.

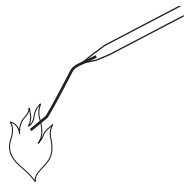
II. Pick Individual *C. elegans*

Repeat this procedure until you can efficiently pick worms to a new plate. Once you feel accomplished, try to pick several worms at once.

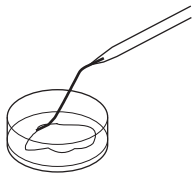
REAGENTS, SUPPLIES, & EQUIPMENT

For each group	*To share*
Binocular dissecting microscope	"Bio bag" or heavy-duty trash bag
Bunsen burner	Bleach (10%) <!> or disinfectant (e.g., Lysol)
Forceps	
OP50-seeded NGM-lite plate from Part III of Stage A	
Permanent marker (black)	
Wild-type worms on NGM-lite plate from Part I	See Cautions Appendix for appropriate handling of materials marked with <!>.
Worm pick	

1. Examine the plate of wild-type worms you chunked in Part I under the dissecting microscope. Confirm that no contaminants have grown since chunking. Obtain a new plate of worms if necessary.
2. Use a black permanent marker to label the bottom of the fresh OP50-seeded NGM-lite plate (from Part III of Stage A) with your group number, the date, and "wild type."
3. Examine a worm pick. The flattened end of the platinum wire should be bent at roughly a 45° angle. Adjust the pick with the forceps if necessary. You may need to adjust your pick from time to time during the course of this exercise.

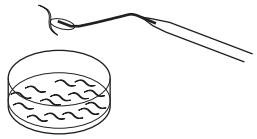


4. Hold the worm pick like a pencil and sterilize the tip in a Bunsen burner flame until it glows red hot.
5. Attach a glob of bacteria to the worm pick by wiping the flat head across the lawn of bacteria on the fresh OP50-seeded NGM-lite plate, as shown to the left. The bacteria will act like double-stick tape when you pick worms.

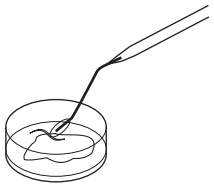


Bacteria from a plate that has aged 2–3 wk serve as a better source of sticky bacteria.

6. Open the lid of your plate of worms and identify a large worm.
7. Gently tap the top of the worm with the glob of bacteria on the bottom of the flattened pick. The glob of bacteria will attach the worm to the pick.
8. To transfer the worm, gently wipe the bottom of the pick in the lawn of the fresh OP50-seeded plate. Make sure to avoid using too much force or you may tear the agar surface and possibly crush the worm.



9. Examine the new plate under the microscope to confirm that the worm has survived picking. If it is visibly damaged or fails to move within several minutes, transfer another worm.
10. For most experiments, it is important to use only one stage of worms. To avoid later confusion, "burn" any embryos or other smaller larval stages that are accidentally transferred to the new plate by heating the worm pick in a Bunsen burner flame until it glows red hot and then immediately touching the flattened end to any unwanted worm. (Alternatively, carefully pick each unwanted worm and flame it in the Bunsen burner.)



11. Store the plate at room temperature. Choose a place where the plate will not be disturbed.
12. Take time for responsible cleanup.
    i. Segregate any bacterial cultures that need to be discarded into a "bio bag" or heavy-duty trash bag for proper disposal.
    ii. Wipe the lab bench with soapy water and 10% bleach or disinfectant.
    iii. Wash your hands before leaving the lab.

STAGE C: OBSERVING WILD-TYPE AND MUTANT *C. ELEGANS*

I. Observe the *C. elegans* Life Cycle

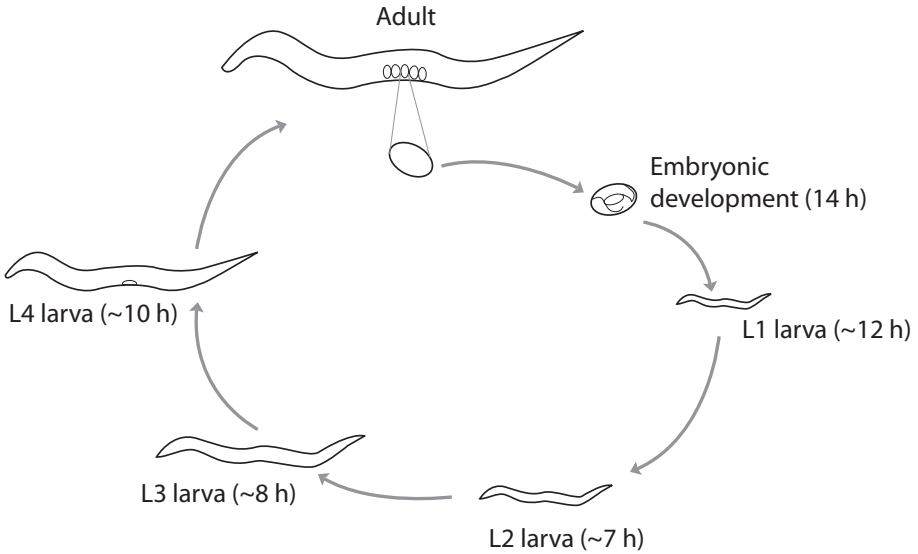
REAGENTS, SUPPLIES, & EQUIPMENT

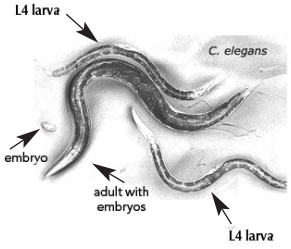
For each group

Binocular dissecting microscope

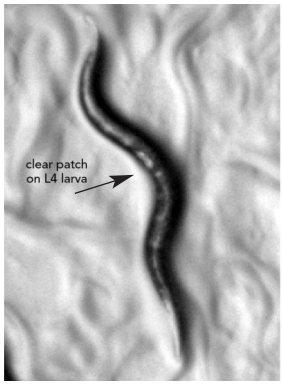
Wild-type worms on NGM-lite plate from Part I or II of Stage B

1. Obtain a plate with wild-type worms.
2. Observe the worms under a dissecting microscope. Note any physical (morphological) differences among the worms.
3. Note any differences in behavior, paying particular attention to how they move on the plate.
4. Lift the plate several centimeters (~1 in) 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 above and attempt to identify an example of each stage of the worm life cycle on the plate.
    i. An adult hermaphrodite is a large worm with embryos inside. (The wild-type strain used in this experiment produces few if any adult males.)
    ii. The embryo is a small, oval object.



All RNAi experiments in subsequent labs begin with identifying L4 hermaphrodites, so it is important to become proficient at identifying them.

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

iv. 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.

v. 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.

II. Observe *C. elegans* Mutants

REAGENTS, SUPPLIES, & EQUIPMENT

For each group

Binocular dissecting microscope

Mutant worms on NGM-lite plates (*dpy-11*, *rol-6*, *bli-1*, and *unc-22*)

Wild-type worms on NGM-lite plate from Part I or II of Stage B

1. Obtain plates with mutant and wild-type worms.
2. Observe the worms under a dissecting microscope. Note any physical (morphological) differences among 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 AND DISCUSSION

1. How many stages of *C. elegans* development were you able to identify? Describe each stage.
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. Based on each mutant phenotype that you observed, what do you think would be the function of the protein produced by the wild-type gene?
6. The mutant *bli-1* and *dpy-11* strains contain mutations that affect the cuticle, the outer layer of the worm that is secreted by the epidermal (skin) cells. These two very different phenotypes show how the nature of the mutation in a strain (the genotype) affects the phenotype.
   i. *bli-1* encodes a collagen. What is a collagen? How can mutations in a collagen affect the cuticle?
   ii. *dpy-11* encodes an enzyme. What do enzymes do? How can mutations in enzymes affect the cuticle?

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