Supplementary Materials for

A Mutant Search—*Caenorhabditis elegans* and Gene Discovery

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This PDF file includes:

Mutant Worm Forward Genetic Screen:
Using *C. elegans* to Study Biological Processes

LABORATORY OBJECTIVE

The goal of this Genetics laboratory module is to emphasize the concept of scientific discovery while teaching a challenging concept- how to identify genes that regulate biological processes. In this ***inquiry-based laboratory project*** students will (a) understand through an experimental approach the relationship between gene, allele, wild-type and phenotype; (b) learn more about the "Forward Genetic Screen" technique- an approach used by geneticist to identify genes that regulate biological processes; (c) gain experience using the genetic model system *Caenorhabditis elegans* (d) gain experience writing a research proposal and (e) present their findings to the class.

With this project students will:

1. Learn and think about a forward genetic screen- an experimental approach geneticist use to identify genes involved with a specific biological process.
2. Work as a team (3-4 students) towards an over arching goal of isolating a genetic mutant with a specific phenotype of interest to the team.
3. Practice general laboratory techniques using the model system *C. elegans*.
4. Read published scientific literature regarding genes regulating a biological process.
    a. Read and discuss methodology used in a forward genetic screen (1,2)
    b. Identify primary article in which a genetic mutant was identified. Discuss and outline the methodology, results and significance of the study.
5. Learn to use the genetic model system databases wormbase.org.
6. Propose and conduct a forward genetic screen given available resources.
7. Write a research proposal to describe the genetic screen and preliminary results.
8. Develop scientific skills by formulating a research question, develop a hypothesis, propose a genetic screen, use methodologies to screen for a mutant phenotype of interest and communicate their preliminary results.

BACKGROUND INFORMATION:

Sydney Brenner and *C. elegans* Genetics: Sydney Brenner, the "father of *C. elegans* Genetics" published a pioneering research article in which he described how he induced mutations in *C. elegans* to gain a greater understanding of specific biological processes (*1*). That is, he conducted a ***forward genetic screen***, which is when one starts with a phenotype of interest and seeks mutants of that phenotype for analysis. He identified over 600 mutants with visible phenotypes clearly demonstrating that mutants could be identified in *C. elegans*. See Table 1 for common phenotypes observed in *C. elegans*. Of interest to Brenner was the genetic regulation of neuronal development. *His work led to the elucidation of genetic pathways that regulate complex processes such as organism development and neuron function.* Brenner was not the first or last scientist to study genetic mutants to understand biology. However, he was the first to use *C. elegans* and through his efforts, along with those of John Sulston and Robert Horvitz, led to their winning of the 2002 Noble Prize in Medicine and Physiology. Today, many scientists continue to use the forward genetic screen technique to identify and study genes and their products (RNA, proteins) involved with specific biological functions (*2*).

The Concept of Forward Genetics: You have learned so far, through your reading and introductory lab experience with *C. elegans,* that these animals develop a certain way, move in

a particular manner, produce offspring and have other *observable characteristics*. Genes (as well as environment- but that is a different subject) regulate phenotypes. Mutations in genes can affect these observable phenotypes. **How does the introduction of a genetic mutation lead to an understanding of a biological process?** To answer this question one has to think more about genes, alleles and phenotypes- beyond the definitions of such but the relationship between these concepts. Imagine now if an individual *C. elegans* inherited a mutation in a gene that is important for muscle function- the resulting phenotype would likely be that the animal is no longer able to move normally. The phenotype could be observably different then that of wild-type animals (the experimental control) or animals that move normally. Now imagine that this individual worm reproduces and its offspring also had the same "abnormal movement phenotype". This indicates that the mutation is inherited since the resulting phenotype is observed in the offspring. Also, consider the phenotypic ratio of the offspring if the mutation is dominant or recessive. Keep in mind that *C. elegans* are either a hermaphrodite or male and for our purposes we will primarily be working with hermaphrodites. The identification of a mutant worm that moves abnormally can lead to the identification of a gene that functions within cells and tissues (e.g. muscles or neurons) involved with body movement. The genetic mutation can be "mapped and DNA sequenced" leading to the *molecular characteristic* of the mutant allele. The mutant allele can be compared to the wild-type allele and the resulting gene products (RNA, protein) can be analyzed. The approach to screen mutant animals for a certain phenotype is called **"Forward Genetics"** because one is first isolating animals with a specific phenotype yet the molecular nature of the mutation is not known until mapping and DNA sequencing is conducted.

The approach of inducing genetic mutations in the germline of organisms and thus studying offspring for specific phenotypes has been extremely important for understanding biological processes at the molecular level. Geneticists use the forward genetic screen approach to identify *genetic pathways* that regulate biological functions. In the case of identifying a mutation that leads to abnormal movement the gene could be part of a genetic pathway that is important for the function of neurons, that signal muscle movement, or muscles, that actually do the work of movement. Thus, a forward genetic screen for a mutant animal with an abnormal phenotype could lead to a greater understanding of genes, proteins and signaling pathways important for tissue and organ function. This forward genetic screen approach has been extensively used with other genetic model systems (Ex: *E. coli*, Yeast, *Drosophila*, *C. elegans*, zebrafish) to understand many biological processes important for life. Processes such as cell division, gene expression, metabolic pathways, organ and tissue development, behaviors, aging, stress responses and much more have been elucidated using the forward genetic approach in model systems. Thus, the reputation of the "Power of Genetics" resulted from the ability to discover and understand gene function relative to biological processes.

Laboratory Exercise Considerations: In this laboratory project, you will be given a population of mutagenized *C. elegans* (P0 generation, hermaphrodites) to maintain and grow to the F2 generation so you can screen the F2 population for a phenotype of interest. There are several things to consider for increasing success of this project. First, the phenotype your team proposes screening for should be observably different than that of wild-type animals. Second, the phenotype you are screening for should be detectible using the resources within the student lab. That is, students will look for phenotypes that are *visible/observable using a stereomicroscope* (e.g. animals that do not move normally can be phenotype that is observable given the resources in class). Note that more complex phenotypes can be produced from the screen but require more precise methodologies (e.g. high-powered microscopes to observe phenotypes within cells). Although as a student you are limited to the resources available in this lab (e.g. you do not have access to a high powered microscope) there are still many phenotypes

the team can screen for. Third, there should be a rationale for the expected phenotype in relation to the biological function. For example, if one is trying to identify a gene that regulates motility one would look for a mutant animal that cannot move normally (e.g. uncoordinated phenotype, an *unc* mutant) as opposed to say perhaps looking for an animal that cannot lay eggs. Fourth, realize that this laboratory lesson involves experimentation with unknown outcomes. Hence, you do not know if the phenotype you are screening for will actually be isolated. If this is the case you can still comment on the other type of mutants you did observe in your screen. It is possible that you will discover a new genetic mutant that may not have been previously identified- reinforcing the idea that discovery is the foundation of science. The nature of this type of project encourages flexibility, teamwork, an understanding of concepts, critical thinking, planning and creativity – ideals that are instrumental for a scientist.

Table 1. Examples of common phenotypes observed in *C. elegans*

Phenotype	**Observed characteristic**	**Gene Alleles**
Roller (Rol)	The animal rotates around its long axis as it moves causing a circular pattern of movement	*rol-9* *rol-9(sc148)*
Dumpy (Dpy)	Worms are shorter and stouter than wildtype at the same developmental stage	*dpy-5* *dpy-5(e565)*
Uncoordinated (Unc)	Deviations in self-propelled movement on a solid medium compared to control	*unc-119* *unc-119(e2498)*
Protruding vulva (Pvl)	Incomplete vulval morphogenesis resulting in the formation of a protrusion at the vulva	*pvl-4* *pvl-4(ga96)*
Lineage variant (Lin)	The descendants of a particular cell exhibits variations in developmental programs	*lin-2* *lin-2(e1309)*
Egg laying defect (Egl)	Variations in the egg laying phenotype; eggs not laid, slower eggs laying rate, stage of eggs laid, or number of eggs laid in response to stimulus	*egl-32* *egl-32(n155)*
Blister (Bli)	Fluid filled blisters appear on the cuticle	*bli-1* *bli-1(e1431)*
Long (Lon)	Body length variant; animals are longer and thinner	*lon-1* *lon-1(wk50)*
Sterile	Animals generate defective gametes and unable to reproduce or generate progeny	*glp-1* *glp-1(e2141)*

In the above table note the nomenclature to designate phenotypes, gene and alleles.

EXPERIMENTAL MATERIALS AND METHODS:

Refer to the previous laboratory exercise "*C. elegans* as a Genetic Model System" for more information regarding worm husbandry. Notes below are just a review.

Review of Basic Worm Husbandry: *C. elegans* (N2 strain) are grown and maintained in the laboratory on Nematode Growth Medium (NGM) that has been aseptically poured into petri plates. NGM plates are "seeded" with *E. coli* bacteria (OP50 strain), which is the food source for the worms (Note: the OP50 *E. coli* strain is a non-pathogenic laboratory strain). Each student team will be provided with a sufficient number of NGM plates to conduct the experiment. Maintaining a worm stock is relatively simple but requires close monitoring of the population. The worms crawl around the plate, eat the bacterial lawn, grow and reproduce. Eventually, the worms will eat all of the *E. coli* and become starved- avoid this situation since starved worms do not develop well and can die. As the food is being consumed worms can be moved to another

NGM plate with *E. coli*. It takes about three days, at 20ºC, for an embryo to develop into a fertile adult. At lower temperatures (15ºC) growth is slower and at higher temperatures (25ºC) growth rate is faster. A thermometer has been placed in the lab to allow you to monitor the ambient lab temperature throughout the experiment (which is typically within a few degrees of 20ºC). Embryogenesis, which takes about 14-16 hours at 20ºC, is followed by four larval stages. Wild-type worms at 20ºC will begin laying eggs 3-4 days into their life cycle. A hermaphrodite can produce on average 250 self-fertilized progeny.

Worm Picking: A worm pick (Figure 1), is used to move an individual worm or a group of worms. The pick is made by adhering an approximately 1.0-inch piece of 32 gauge platinum wire into the tip of a glass Pasture pipet. To make a worm pick: 1) use a glass cutter to remove the tip of the pasture pipet since this is often too long and prone to breakage; 2) with forceps hold the tip of the platinum wire and place other end into the opening of the pasture pipet; 3) hold the glass pipet/platinum wire region (shown at 12cm region on figure below) over Bunsen burner to heat the glass so that the wire is embedded into the glass; 4) be careful with the flame as to not burn oneself. The platinum wire is used because it heats and cools quickly and can be flamed often (between worm transfers) to avoid contaminating the worm stocks. The end of the wire, used for picking up worms, can be flattened slightly with a hammer and then filed with an emery cloth to remove sharp edges; sharp points can poke holes in the worms and kill them or make holes in the agar. The tip of the wire can be fashioned to your liking. Some people prefer a flattened end, while others prefer a slight bend that forms a hook. To pick a worm use the dissecting microscope- slowly lower the tip of the wire onto the NGM plate and collect a bit of *E. coli* OP50 onto the end of the pick before gently touching the pick to the top of the worm you want to move. The worm will stick to the bacteria. Several animals can be picked at once, although worms left too long on the pick will desiccate and die. To place the picked worm onto a fresh plate, slowly lower the tip of the worm pick, to the plate and gently touch the surface of the agar, and allow the worm to crawl off of the pick. It takes some practice to learn to pick a worm.

Figure 1. Shown are platinum-wire worm picks used to move *C. elegans*. These simple worm picks can be made in the lab.

F2 Mutant Screen Protocol and Methods:

Mutagens: A mutagen is a chemical or physical agent that causes DNA mutations. Mutagens can affect DNA in different ways. For example, a mutagen can act as a base analog used as a substrate during DNA synthesis whereas other mutagens react directly with DNA causing structural changes, in either case DNA replication errors can result. Some mutagens act indirectly on DNA and cause the cell to synthesize chemicals such as peroxides that have a direct mutagenic effect. Chemicals such as ethyl methanesulfonate (EMS) add alkyl groups to nucleotides in DNA molecules. Shown below is how EMS altars guanine leading to abnormal base pairing with thymine (Figure 2). **Safety Note:** Students will NOT be handling mutagenic chemicals and will not have any exposure to mutagens. The lab coordinator will conduct the actual mutagenesis.

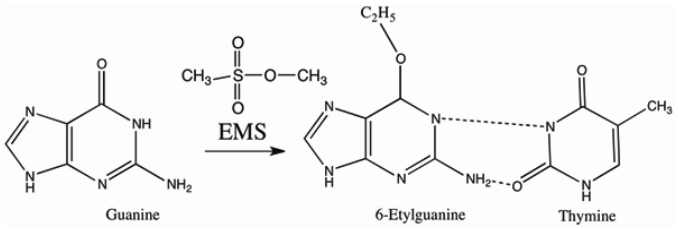

Figure 2. EMS is an efficient mutagen for generating point mutations. Shown is the DNA base Guanine and how it is altered by the mutagen EMS resulting in aberrant hydrogen bonding with Thymine during DNA synthesis. The chemical structure of EMS is also shown.

A lab coordinator followed the protocol below a day before the student lab meets:

1. From a synchronized population of *C. elegans* collect approximately 50 wild-type **L4 Larvae to young adult** hermaphrodite worms and wash with M9 buffer. Resuspend worms in 3 ml of M9 buffer.
2. Wearing gloves and working in a hood add 20 µl EMS to .980 ml of M9 buffer and shake gently. Transfer the 3 ml of worms into the 15 ml conical tube of EMS. Parafilm the top of the closed tube to further reduce potential of leakage. Place the tube on a rocker at 20° for 4 hours. The final concentration of **EMS is 50 mM**.
3. Spin the worms down and remove the supernatant to a plastic centrifuge waste tube used to collect EMS waste for proper disposal. Wash the worms twice with M9 buffer, transferring the supernatants to the waste tube.
4. Transfer the worms, in a few drops of M9 buffer, using a sterile glass pipette, to the edge of the bacterial lawn on an *E. coli* NGM plate. **This plate contains the P0 population of mutagenized worms that students will use to obtain the F2 generation of mutants.**
5. **Let animals recover 4-12 hours before giving to students for collection of F1 population.** The first eggs laid are not collected. These eggs may have arisen from a meiotic cell that completed meiosis at the time of mutagenesis, therefore the mutations may not be present on both strands of the DNA.
6. The collected EMS waste from the washes, must be neutralized by mixing with an equal volume of 0.1M NaOH, 20% w/v $Na_2S_2O_3$ [sodium thiosulfate]. All pipet tips that were used for the EMS treatment also needs to be soaked in the inactivating solution. Leave in the hood for a day before disposal.
7. **Students will be provided with mutagenized P0 animals to collect the F1 population.**

<u>Student Protocol</u>

1. Student teams must meet together to discuss what type of phenotype they will screen for and the rationale for such. How could isolation of this mutant lead to an understanding of a specific biological function of interest to you?
2. Keep a record of the methods you use to screen for the phenotype of interest, results of your screen and the additional phenotypes you observed in the F2 population.
3. Figure 3 diagrams the forward genetic screen for your reference.
4. Your TA will provide you with a stock of P0 mutagenized worms. Pick healthy looking adult hermaphrodites onto fresh NGM plates seeded with *E. coli*. Each group should place 3 P0 adults per NGM plate. You will be provided a number of NGM plates. Label your plates and keep track of the generations (P0, F1, F2).
5. Allow the P0 adults to lay eggs (F1 population) throughout the day.
6. Move the P0 to new plates every 12 hours throughout the next 24 hours (3 P0 per plate).
7. Monitor the F1 generation of plates (several times each day) so that the worms do not starve. Transfer worms to fresh plates with food to avoid starvation. <u>If animals starve they will not produce F2 animals, and your experiment will fail</u>. Within 2-3 days transfer the F1 worms (~7 F1 worms per plate) to fresh NGM plates so that you can collect the F2

generation. **Label your plates and to keep track of the generation (P0, F1, F2), date/time and observations in your lab notebook.**

8. The F1 animals need to be maintained to adulthood (refer to the *C. elegans* life cycle) so that the F2 generation can be collected. It is the F2 generation in which phenotypes may be more easily identified. *Think about why this is the case.*
9. ***Screen the F2 generation for the phenotype your team is looking for.*** Document the phenotype observed. Quantify aspects of the phenotype (discuss among the group and lab instructor how to do such). Describe the phenotype as clearly and precisely as possible. At what stage of development does the phenotype become apparent? Do the F3 generation animals also display the same phenotype? What is the "penetrance" of the phenotype- that is do all or only a percentage of the offspring display the phenotype?
10. You can also collect other mutant worm(s) identified onto separate NGM plate- to share your findings with the class.
11. Discuss in your group and with your lab instructor what type of genes may potentially be affected that would lead to this phenotype in your mutant. (Address this in your proposal!)
12. Discuss in your group and class what additional experiments could potentially be conducted with the mutant you identified. (Address this in your proposal!)
13. Give the mutant(s) your group has identified to the lab instructor for saving and perhaps future work by other researchers. **Example of Log Information to Collect:**

Group Member:			
Date and Time	**Plate Name**	**Generation**	**Observations**

PROPOSAL AND PRELIMINARY RESULTS

In many teaching labs students conduct laboratory procedures with known outcomes. The students document the process in a laboratory report. Such a report is important so students learn about methodologies and how to document findings. Another type of scientific communication is a research proposal. Another type of scientific writing is a research proposal. This type of written communication provides information such as background information, hypothesis, research objectives, preliminary data, and rationale for proposed experiments. Typically this type of communication is used to propose scientific research prior to obtaining support and or funding for research. In your case, it is a means to sharpen your critically thinking skills regarding a research experiment prior to completion of the study.

Your Research Proposal is a complete description of the intended genetic screen experiment. Even though this is an individual student assignment you should discuss the ideas and content of the research proposal with your laboratory instructor and teammates. Through the full proposal, you must communicate how the proposed study will make a contribution to the

understanding of the genetics of a biological process. The full research proposal must contain the following:

- Title
- Brief Introduction
- Background and statement of the problem (this in the light of a literature review)
- Research question
- Hypothesis
- Specific aims
- Research Objectives
- Experimental Approach (Methodology)
- Resources required for the study, including budget if applicable
- Expected Results
- Data Interpretation – if applicable statistical planning must be fully addressed, or you should provide evidence that statistics are not required.
- Timetable for completion of the project
- References

Students will be provided a file with more information regarding the research proposal.

QUESTIONS FOR STUDENTS TO PONDER AND DISCUSS:

What affect is the EMS having on the L4 larvae animals that lead to inherited mutations? Which cells is this occurring in in order to see inheritance?

How can you test if the phenotype you are observing is due to a genetic mutation and not an environmental condition (given the resources you have in the student lab)?

Why would newly isolated mutant strains typically be “backcrossed” to wild-type animals and the desired mutations re-segregated? To answer think of second-site mutations.

REFERENCES

1. S. Brenner, The genetics of Caenorhabditis elegans. *Genetics* **77**, 71 (May, 1974).
2. E. M. Jorgensen, S. E. Mango, The art and design of genetic screens: caenorhabditis elegans. *Nature reviews. Genetics* **3**, 356 (May, 2002).

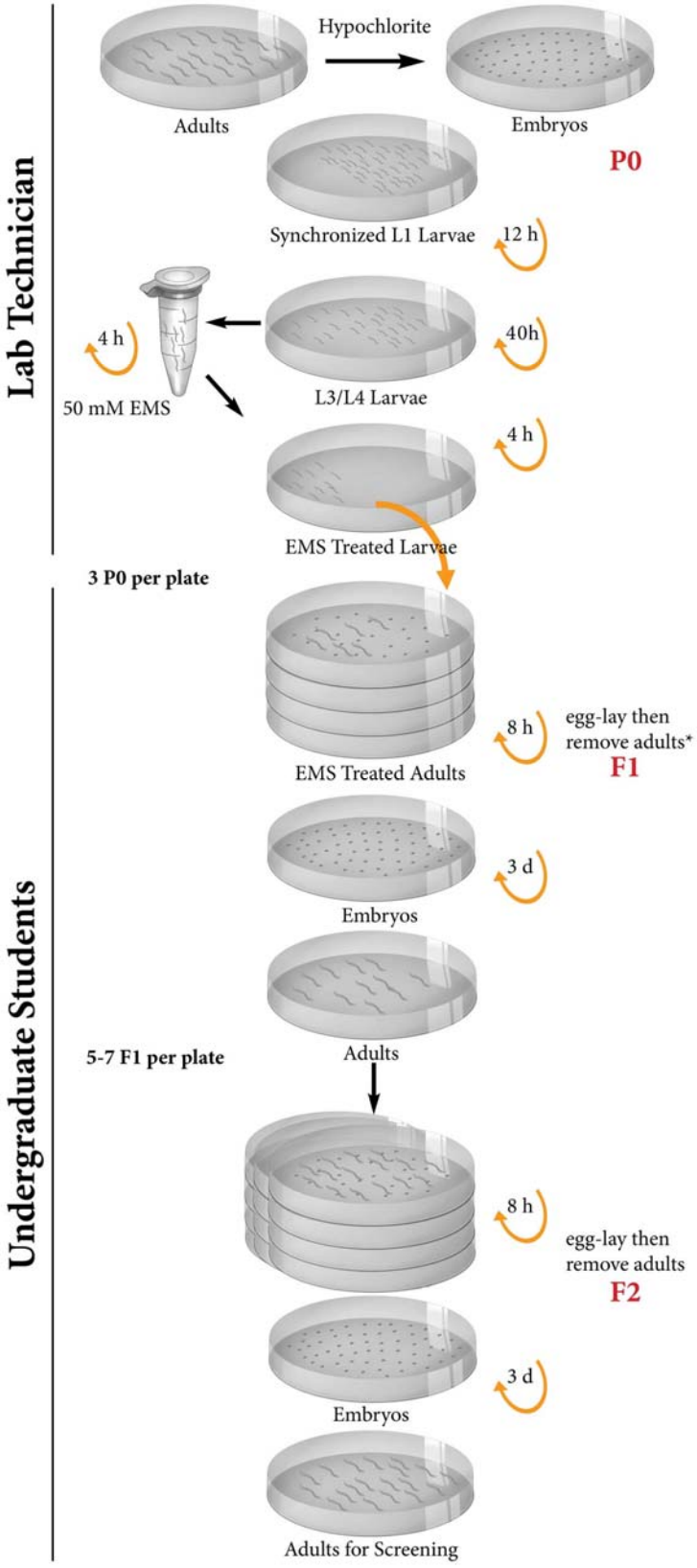

Figure 3. Schematic diagram of forward genetic screen. Animals are mutagenized, and the P0 generation is grown to produce the F1 generation, which is subsequently grown to produce the F2 generation. The F2 adults are screened for the phenotype of interest. Arrows are proximal to the approximate time that each step takes.

Proposal Rubric

Name: _____ Score: _____

Points	Title, Table of Contents	Introduction and Specific Aims	Materials and Methods			Preliminary Results and Discussion	Figures and Tables	References and In-text citations	Grammar, Style, Nomenclature
			Background	Materials	Methods				
5	Title page includes the title of project, student name, TA name and date. A table of contents is included after the title page and lists all sections with correct page numbers.	Sufficient and relevant background information provided. An understanding of a broader context is evident. Hypothesis is clearly stated. Specific aims logically stated. Transitions well into methods.	Relevant sources are included. Sources are connected to the experiment performed.	Description of materials, instruments and equipment in the correct context for their use.	Method procedure clearly stated and reproducible. Methods relate to hypothesis and specific aims. Timeline is logical and easy to follow.	Preliminary results or expected outcomes of screen presented. Hypothesis and specific aims addressed. Discussion and interpretation based on results. Highlights strengths, novelty and significance of study.	Figures and tables are clearly presented and relevant. Figure legends cited in narrative. Figure legend is clear.	All sources are cited in the text, listed in the bibliography, and follow the specified style guide. All non-student derived ideas and facts are referenced.	Written concisely, logically and with a technical tone. Grammatically correct, complete sentences, proper use of punctuation. Appropriate genetic nomenclature followed.
3	Title page and table of contents lacks one or more pieces of required information.	Mostly relevant background provided. Broader context is underdeveloped. Hypothesis and specific aims are vague. Transition to specific aims is disjointed.	Missing relevant sources. Sources not completely connected to experiment performed.	Materials, instruments and equipment are named and described, but correct context is lacking.	Methods clear but are not related to hypothesis and specific aims. Timeline illogical or difficult to follow.	Hypothesis is mentioned, but not discussed in relation to results. Preliminary results show a lack of understanding of the lab objective.	Figures and tables not relevant to narrative or not cited in narrative. Inadequate figure legend.	Most sources are cited in the text, listed in the bibliography, and follow the specified style guide. Most non-student ideas and facts are referenced.	Some wordiness or conversational tone, but generally grammatically correct (small grammatical errors- punctuation etc.). Genetic nomenclature mostly followed.
1	Title page or table of contents in missing.	Broader context ignored. Ideas do not flow logically from introduction to hypothesis to specific aims. Hypothesis or specific aims are missing.	Used only one source or lack of understanding of the sources used. Missing concepts for experiment performed.	Materials, instruments and equipment are named, but not described.	Methods difficult to understand with no clear progression of events. No connection to hypothesis or aims.	Preliminary results missing, not tied to methods or objectives. Conclusions are poorly thought out or unclear.	No clear purpose for figure or table. Figure legend missing.	Sources are not listed in the bibliography, and do not follow the specified style guide. Non-student derived ideas in the text are not referenced.	Almost entirely conversational tone. Grammatical mistakes, but still comprehendible. Inappropriate genetic nomenclature.
0	Both title and table of contents is missing.	No understanding of major concepts. Hypothesis and specific aims are missing.	No background sources for methodology included.	Materials, instruments and equipment are neither named nor described.	Methods not described. Fabricated methods. No connection to hypothesis and aims.	No expected outcomes addressed. No conclusions discussed.	Figures or table absent.	Sources are missing and do not follow specified style guide. No in-text citation is included.	Lack of revision and planning evident. Poor grammar prevents comprehension. Genetic nomenclature not followed.

NOTES FOR FACULTY WHO WANT TO TRY THIS MODULE AT OTHER UNIVERSITIES

There is an abundant amount of information available by the *C. elegans* research community. Below is a list of information on specific websites:
WormAtlas: General information regarding the anatomy http://www.wormatlas.org/
WormBook: Details regarding specific aspects of worm biology http://www.wormbook.org/
C. elegans Genetics Center (CGC): A site where wild-type and mutant strains can be purchased http://www.cbs.umn.edu/CGC/
WormBase: Information regarding *C. elegans* genetics http://www.wormbase.org.
WormClassroom: Educational information is shared on http://www.wormclassroom.org/

Below is some information that may be beneficial for starting a similar module at your university.

Introduction to the nematode *C. elegans* as a genetic model system: *C. elegans* is a small nematode, which grows to about 1 mm in length and can be found as hermaphrodites or males. It is found on rotting fruit and vegetation in the soil, survives by feeding on microbes such as bacteria. In the 1960s Sydney Brenner was interested in identify a genetic model system to understand the genetic mechanisms regulating nervous system development and function (Brenner 1974). He chose *C. elegans* (N2 strain, originally found in Bristol, England) for his studies and since then thousands of scientists throughout the world have followed his lead and use this "simple" animal to understand "complex" biological functions. This nematode is now used as a genetic model system to understand the genetics of neuronal development, embryogenesis, meiosis, behavior, aging, cell death, organism development and much more. In 2002 the Nobel Prize for Physiology and Medicine was awarded to Sydney Brenner, and two other *C. elegans* researchers, John Sulston and Robert Horvitz, for their contribution to the field of genetics. In 2006 Andrew Fire and Craig Mellow were awarded the Nobel Prize for their work in *C. elegans* discovering the mechanisms regulating RNA interference.

***C. elegans* anatomy:** *C. elegans* is an increasingly important genetic model system in biology because it is a genetically and anatomically simple animal, yet it shares many of the essential biological characteristics that are central problems of human biology. The "worm", as it is commonly referred to as, is fairly transparent and has a variety of complex tissues. These animals possess complex organs such as a nervous system, intestines, muscles and a gonad that produces sperm and eggs. *C. elegans* exhibits specific behaviors, mates and reproduces, has a specific life cycle and ages. The two sexes, hermaphrodites and males, differ in appearance. The hermaphrodite produces both oocytes and sperm and can reproduce by self-fertilization. Males arise spontaneously at low frequency (~.05%) in the population and can fertilize the hermaphrodite. Interestingly, the number of somatic cells in the organism is fixed, that is the adult hermaphrodite has only 959 somatic nuclei and the adult male has only 1031 somatic nuclei. Thus the "lineage" of each cell can be tracked throughout development, from zygote to adulthood.

C. *elegans* life cycle: Under laboratory conditions the growth and development of *C. elegans* is quite rapid; time of development is dependent on temperature. At 20°C the life cycle from egg to fertile adult takes about 3.5 days. The *C. elegans* average life span is a mere 2-3 weeks. Development of an embryo (embryogenesis) takes about 14 hours. Post-embryonic development involves four larval stages, referred to as L1, L2, L3 and L4. In the absence of food, an alternative stage, referred to as a dauer, is formed after the L2 stage. This "dauer larvae" can remain viable without food for many months and is highly resistant to stress. The adult hermaphrodite can produce more than 300 embryos over a period of 3-4 days.

C. *elegans* genes and mutations: *C. elegans* is a powerful genetic model organism and many methods have been developed over the year to optimize its use as a tool for learning about disease and cellular function. Furthermore, in 1998 it became the first multicellular organism for which a complete genome sequence was obtained. Annotation of the worm's 100 Mb genome sequence is quite advanced and it is

now becoming more common for researchers to sequence large areas of the genome to identify genetic mutations from forward genetic screens. In terms of genetic analysis, an attractive feature of *C. elegans* is the ease of generating mutations within the genome; that is wild-type genes can be mutated to result in observable phenotypes within the organism. Several mutagens, in additions to EMS (ethyl methane sulfonate), work efficiently for generating mutations. Mutations can also be generated using other methods such as ionizing radiation and transposon hopping. Since *C. elegans*' somatic cells are diploid, deleterious mutations can be propagated without lethality. Also, since the major mode of reproduction is by self-fertilization of hermaphrodite, the effect of making mutation homozygous can be examined (Mendelian segregation analysis). The efficiency of mutagenesis, recessive phenotype screening and ease of preserving mutant lines as frozen stocks has led to multiple mutations existing for many of the genes within the genome.

Nematode Materials and Methods: *C. elegans* is usually grown on "nematode growth media" agar plates (NGM plates) covered with the bacteria *E. coli* (OP50 strain) for food to support the growth and development of the worm. Nematodes can be moved using a platinum wire. Also, a quick and common way to transfer worms to maintain stocks without having to individually pick up a worm is to slice out a piece of the agar from an older plate containing worms and place it on a new plate with fresh food. To collect all of the worms on an individual plate, the worms are washed off with M9 buffer, placed in a 15 ml conical tube and centrifuged to collect a worm pellet which can be transferred to fresh NGM plates.

Worm Picking: This needs to be done to maintain stocks and conduct experiments. To move specific animals a worm pick is used. A worm pick is made by mounting a 1-inch piece of 32 gauge platinum wire into the tip of a Pasture pipet. Platinum wire heats and cools quickly and can be flamed often (between transfers) to avoid contaminating the worm stocks. The end of the wire, used for picking up worms, can be flattened slightly with a hammer and then filed with an emery cloth to remove sharp edges; sharp points can poke holes in the worms and kill them or make holes in the agar. The tip of the wire can be fashioned to your liking. Some people prefer a flattened end, while others prefer a slight bend that forms a hook. It takes a bit of practice with a worm pick to use it with ease. To pick a worm identified under the dissecting microscope, slowly lower the tip of the wire and gently swipe the tip at the side of the worm and lift up. Another method is to get a bit of *E. coli* OP50 on the end of the pick before gently touching it to the top of the chosen worm. The worm will stick to the bacteria. Several animals at a time can be picked by this method, although worms left too long on the pick will desiccate and die. To put a picked worm on a fresh plate, slowly lower the tip of the worm pick, gently touch the surface of the agar, and hold it there to allow the worm to crawl off of the pick. Use a small flame to sterilize the tip of the pick before using it to move a worm. With patience and practice everyone learns to move worms efficiently.

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