Worms and Mutations

Objectives:
- Students will observe several varieties of C. elegans and make observations. Each group of students will be getting a different variety of C. elegans. It will be up to each group to determine the characteristics of their variety.

State of Florida Next Generation Sunshine State Standards:
- SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.
- SC.912.N.1.6 Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.

Important:
- **This lab uses ethanol which is a flammable liquid!** Take great care when using and be sure to review the Safety Data Sheet for ethanol.

Materials:
- Pre-grown worm plates
- Inoculated plates
- Dissecting microscope
- One of the following: Lab spatulas, scalpels, toothpicks or cotton swabs.
  If using spatulas or scalpel:
  - Ethanol
  - Lighters or flame source

Procedure:

Spatula or Scalpel Procedure:
1. Obtain one pre-grown plate from the instructor. Each group of students will be getting a different plate.
2. Obtain a small amount of ethanol in a beaker. Be sure to label your beaker as having ethanol.
3. Obtain one lab spatula, dip and stir it in your ethanol.
4. Without waiting too long, use a lighter or open flame to ignite the ethanol on the spatula. This will burn off all of the ethanol and sterilize the spatula.
5. Using the sterilized spatula, cut into the agar making a circle around the visible ring on the agar itself. Then cut the circle into four small pieces.
6. Meanwhile, have one of the group members remove the lid from an inoculated plate.
7. Using the spatula, lift up one of the four pieces of agar.
8. Now place the piece of agar onto the inoculated plate. Replace the lid of the plate.
9. Label your plate with your group number, the date, and the worm type.
10. Record your initial observations of the plates.
11. Go to another group and observe their plate. Do this one more time to observe two other plates besides your own.
12. Observe the plates again after 24-48 hours. Record your observations.
Toothpick or Cotton swab procedure:
1. Obtain one pre-grown plate from the instructor. Each group of students will be getting a different plate.
2. Using a toothpick or cotton swab, gently drag the tip across a small portion of the circle where the worms are growing. Several worms will now be on the tip.
3. Remove the lid from your new plate and drag the tip (with the worms) across the new plate. Replace the lid.
4. Label your plate with your group number, the date, and the worm type.
5. Record your initial observations of the plates.
6. Go to another group and observe their plate. Do this one more time to observe two other plates besides your own.
7. Observe the plates again after 24-48 hours.
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<th>Plate Name</th>
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<th>Written Observations (provide three)</th>
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Observations after 24-48 hours

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Worms and Mutations

Contact Dr. Brock Grill to obtain worm samples: http://www.scripps.edu/grill/resources.html

Overview:
The students will be put into groups. Each group will receive a different strain of C. elegans. They will then compare their strains to other group’s strains and determine which group has which.

- Be sure to read over the “How to Grow Worms” protocol first, since it will be used in this lab.
- The three strains include N2 (wild type), unc-42, and unc-51. N2 is active and motile. It reproduces very quickly. Unc-42 has a gene mutation (in the unc-42 gene) that alters the neurons of C.elegans. Simply put, unc-42 is less active and will reproduce more slowly. Thus the name unc for uncoordinated. Unc-51 also has a gene mutation that effects neurons. This mutation so severe that unc-51 have trouble laying eggs and are almost paralyzed.
- Students will be able to view these three types of worms under a dissecting microscope and observe their behavior to conclude that they each received a different type.
- It may be easier to prepare the plates beforehand and have the students observe them. This lab was created so students would learn some basic biotechnology techniques.
- Depending on the teacher’s preference, students may want to be given information on each strain of C. elegans beforehand, so they know what characteristics to look for. Or the instructor can allow the students to make their own observations and categorizations.

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Important:
- This lab uses ethanol which is a flammable liquid! Take great care when using and be sure to review the Safety Data Sheet for ethanol.
- Even though the plates contain biological materials, they can be disposed of in any trash. The worms are found naturally in soil so there is no concern when it comes to disposal.
Materials:
- Pre-grown worm plates
- Inoculated plates
- Dissecting microscope
- One of the following: Lab spatulas, scalpels, toothpicks or cotton swabs.

If using spatulas or scalpel:
- Ethanol
- Lighters or flame source

Teacher Prep:
1. Prepare 6 cm agar plates with E. coli (worm food).
   - 4-7 days before lab, grow OP-50 E. coli strain in LB liquid broth overnight at 37°C.
   - 3 days prior to lab pour 6 cm plates. Fill plates with 10 mL of molten agar. Let sit 24 hours to solidify (plates are good for as long as 2 weeks after pouring at room temp and 1-3 months after pouring if refrigerated).
   - 2 days prior to lab inoculate the center of each plate with E. coli.

   Note: Alternatively, you can obtain a set of plates from Dr. Grill’s lab.

2. Using ordered wild type N2 strain of C. elegans (or obtained from Dr. Grill’s lab) transfer several large worms and place them onto a plate. 10-20 will suffice. This procedure needs to be repeated so each group of students will receive one of these plates.
   - This can be done using the chunking technique, with toothpicks, or cotton swabs.
   - You may want to label the plates with the date and as follows:
     - Group A (which will be the N2 strain)
     - Group B (which will be the unc-42 (e270))
     - Group C (which will be the unc-51 (e369))

3. Let the plates sit at room temperature for 24 hours. This will allow the worms to multiply.

4. Prepare the ethanol. Depending on your initial ethanol concentration, you can use the formula below.
   \[ V_i \times C_i = V_f \times C_f \]
   Where \( C_i \) is the initial concentration of ethanol. \( V_f \) is the final volume you want to make up. \( C_f \) is the final concentration requested (70% for this lab). \( V_i \) will be the needed volume of your original solution.
   - As an example, in the case of a 96% solution, to make 200ml of 70% ethanol.
     \( C_i = 96\%; \ V_i = x; \ C_f = 70\%; \ V_f = 200ml \)
     \[ x = (70x200)/96 = 145.8ml \] (bring to 200ml with distilled H2O)

5. Note: 70% ethanol is the norm. You may use different concentrations. Between 70-90% is recommended.

For more resources go to: [http://www.scripps.edu/grill/resources.html](http://www.scripps.edu/grill/resources.html) and click on “Resources for Teachers.”

**Please send comments or suggestions to:**
edwin.meagher@palmbeachschools.org
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| **C. elegans type A** | ![Image](image1.png) | - Worm is dark and slender.  
- It is moving very slowly.  
- Only its head appears to be moving. |
| Worm count: 1 | | |
| **C. elegans type B** | ![Image](image2.png) | - Worms are dark, but shorter and fatter than A.  
- One of them is covered in eggs.  
- They are hardly moving. |
| Worm count: 2 (adults) | | |
| **C. elegans type C** | ![Image](image3.png) | - Worms are dark and slender.  
- They are moving actively.  
- They leave lots of tracks. |
| Worm count: 2 (adults) | | |

Include eggs? -