Let There Be Light!

Introduction: Its Alive!

Watch Short Clip!

Materials per Student:
- 1 “canvas”
- 1 wooden “paint brush”
- 1 tube of “paint”

The first day at SEA you were asked to draw a picture with mystery paint on a nutrient rich Petri plate. The plates have been incubated in darkness overnight at 28°C (82.4 °F).

**Attention: Please See Cover Pages for Culture Ordering information, media prep, and safety measures to be taken when handling microbial cultures.**

A1. What did your plate look like before the lights were turned off? And after? (Draw a picture.)

<table>
<thead>
<tr>
<th>Before:</th>
<th>After:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Students should draw the white colonies in whatever pattern they may have drawn</td>
<td>Students should draw the same pattern but color the colonies blue/green.</td>
</tr>
</tbody>
</table>

A2. What is your observation about your painting?

_The “spots”/colonies/growth glow when the lights are turn off._

A3. What do you think your mystery paint was?

_Answers will vary based off previous experiences and knowledge._

**NOTE: Here you will reveal the true identity of mystery paint as bacteria. (The scientific name is either Photobacterium leiognathi or Vibrio fisheri, depending on what starter culture you have chosen to work with.) Here you may want to explain that this bacterium is not harmful to humans.**

http://web.mst.edu/~microbio/BIO221_2006/P_leiognathi.htm is a website you can visit to gain background information on P. leio. For Enviroment Science classes, these video will explain a biological phenomenon called the milky seas.

http://www.lifesci.ucsb.edu/~biolum/organism/milkysea.html
This is the GFP Mice video to show http://youtu.be/jFTyUpw2Zgw.

A4. What was your mystery paint? Is it alive? What about the mice in the video?

_Bacteria, Yes, Yes_
A5. Our Investigative question for the day!
How do you think the living ______Bacteria________ produced ______Light________? 

*Answers will vary based on current misconceptions and many may have no clue. (There is no right or wrong answers here)*

**Exploration 1: Shining Star**

**Exploration A : Prisms and Spectrometers**

**Materials required for groups of 2-4 students**

- Ocean Optics Visible Spectrometer
- Multiple lights
- Color Pencils
- Spectroscope
- Computer (w/ Ocean Optics software)

*NOTE: Ocean optics is not needed for the activities spectrometers will work. Spectroscope ordering information can be found in the cover pages.*

**Experimental Procedures**

1. Use the spectroscope to measure 2 different light sources.  
   *NOTE: Light Sources should include a white, green, yellow, red, and/or blue. Provide at least 3 different colored lights.*

2. Record the light type and color pattern of each light below.

![Figure 1: Measured spectrum from different light sources.](image)
B3. View other groups spectrums. Did all the light bulbs give the same color spectrum? Compare and Contrast your chosen light source spectrum to others you have observed. (Use complete sentences)

*No, Answers will vary for the compare and contrast section.*

**Concept Development 1 : ROY G BIV**

C1. Are the different colors of white light always in the same order? What is the order? (Hint: Look at the title)

*Yes, Violet, Indigo, Blue, Green, Yellow, Orange, Red.*

C2. Where does the energy for the light bulb come from?

*Electricity, the light socket, etc.*

**Exploration C: Energy Waves**

**Materials needed for a group 2-4 students**
- Rope
- Camera

D1. A wavelength is defined as maximum to maximum (X-X or Y-Y). Below are two waves which wave has a shorter wavelength? *The Y-Y Wave.*

![Figure 2: Wavelength 1](image-url)
Figure 3: Wavelength 2
D1. Making Waves Activity. Your group will need get one rope and head outside. There you will attempt to make a wave with a long wavelength and then a wave with a short wavelength. Allow everyone a turn to make waves.

**NOTE:** The ropes that create the best waves are 15 ft or longer and ½ inch thick or thicker. One student will hold each end, but only one will be moving to make the waves (thicker ropes can cause rope burn so wearing gloves is a good idea). You may want to demo how to make wave with a simple straight up and down motion of your arm(s).

http://youtu.be/1vmEHmJJO00 Here is a video for those who not capable of demo-ing for their students.

Concept Development 2: Light is Energy

E1. From the previous activity which wavelength was “easier” to make?

*Long Wavelength*

E2. From the previous activity which wavelength has lower energy?

*Long Wavelength*

The energy of light is calculated with the following equation:

\[
\text{Energy} = \frac{\text{Constant} \times \text{Speed of Light}}{\text{Wavelength}} \Rightarrow E = \frac{hc}{\lambda}
\]

\[h = 6.6 \times 10^{-34} \text{ J} \cdot \text{sec}, \quad c = 3 \times 10^8 \text{ m/sec}\]

Remember that in Exploration B: Figure 2 each individual color has an individual wavelength that stays consistent from one spectrum to the next.

E3. Looking again at Figure 2. What color of light had the shortest wavelength and the longest wavelength? What is the wavelength?

*Violet* = Shortest = approx. 400x10^-9

*Red* = Longest = approx. 650x10^-9

**Note:** http://eosweb.larc.nasa.gov/EDDOCS/Wavelengths_for_Colors.html Is a good website with a great image of wavelength and color association.

E4. What color of light has the highest energy and what color has the lowest energy?

*Highest* = Violet

*Lowest* = Red

E5. Using the spectrometer data from Exploration B: Figure 2. Calculate the amount of energy for the colors you listed above. (Show your work and be mindful of units). Did the equation support the answer you provided in E4? If No, revisit the previous question.

*Violet* = 4.95x10^-19 J/s

*Red* = 3.05x10^-19 J/s

**NOTE:** Remind the students of the proper units. Wavelength is in Meters = 10^-9 number not just “400” which is nanometers.
Concept Application 1: Fluorescence

Materials need for a group of 2-4 students

- A beaker with 300mLs tonic water (CONTAINS: Quinine)
- UV Light

F1. Expose the tonic water to the ultraviolet (UV) Light. Record your observations.

*The solution emits a blue light.*

F2. Expose the tonic water to white light and any of the other lights you used earlier? Record your observations

*The solution no longer emits light.*

F3. Draw the spectrum of the UV light.

WARNING!!!! DO NOT STARE AT THE UV LIGHT FOR MORE THAN A FEW MOMENTS! THIS CAN DAMAGE YOUR EYES!

NOTE: The purple light may still be visible and but this is where you should also introduce the invisible spectrum. Point out the meaning of the names Ultra Violet, Infra Red.

Figure 4: UV Spectrum

F4. Draw the spectrum of the glowing tonic water.

Figure 5: Tonic Water Spectrum
F5. Is the light source (UV) and the light being emitted (tonic water) the same color? Same energy? Use the Spectrum as help to explain.

*No, No, The wavelength of the UV light is shorter therefore, higher in energy. The tonic water is lower in energy because the wavelength is longer.*

F6. Is the tonic water’s emitting light (glow) lower or higher energy than the UV? How do you know?

*Lower, because of the wavelengths.*

F7. Where did the tonic water get the energy to emit light?

*The UV light*

F8. Using our new knowledge of light, characterize *P. loei*’s color, wavelength, and energy. Draw a predictive spectrum. Is the wavelength shorter or longer than Red? Is it emitting energy? How do you know?

*Shorter, Yes, because it is emitting light.*

![Figure 6: P. loei Light](image)

**Concept Application 2: Flame Test Chemistry**

**Exploration – Energy, Color, Wavelength, and Material**

**Materials required for groups of 2-4 students**

- 6 – Pyrex watch glasses
- 1g – Strontium chloride
- 1g - Calcium chloride
- 1g – Copper II chloride
- Methyl alcohol (methanol)

- 1g - Potassium chloride
- 1g – Lithium chloride
- Safety goggles
- Flame source

**Figure 7. Setup for Flame Test**

1. Place approximately one teaspoon of the desired chemical onto the watch glass. Add between 5-10 mL of methanol (note that the more methanol used, the longer the flame will burn).
NOTE: Do not stare directly into the flame for any length of time).

WARNING: Never add methanol to a watch glass that has already been burned. Even if you think the flame is out, it may still be burning. It may not be visible to the naked eye.

NOTE: Do not handle the watch glasses for at least ten minutes after then demonstration is over. This should be adequate time for the watch glasses to cool sufficiently. To clean the watch glasses simply wait until they have cooled and then wash them in water; the salts are water soluble and should easily rinse off. The watch glasses can be reused again and again.

**TEACHER NOTE:** Dissolving the chemicals first then moving the slurry to the watch glass aid in a better flame color development

2. Use a flame source to carefully ignite the chemical.

3. It should take a couple of seconds for the full intensity of the flame color to be visible.

**Table 2 Chemical Chart**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Flame Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strontium chloride</td>
<td>Red</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>Orange</td>
</tr>
<tr>
<td>Copper II chloride</td>
<td>Green</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>Purple/White</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>Red/Pink</td>
</tr>
</tbody>
</table>

G1. Choose two chemicals from Table 2 and draw the color spectrum for each and calculate the energy.

**Chemical 1**

*Answers Will Vary Just check the color placement and math.*

<table>
<thead>
<tr>
<th>Wavelength</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calculated Energy: __________

**Chemical 2**

*Answers Will Vary Just check the color placement and math.*

<table>
<thead>
<tr>
<th>Wavelength</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calculated Energy: __________

Figure 8: Flame Readings
G2. Previously, we identified the energy source for the light bulbs as **Electricity**. Is the same energy source used here? *No* How do you know? *It's not plugged in to a light socket.*

G3. What is the energy source for the flame test?

*The Flame.*

G4. When the color changes is the light energy emitted different? Explain. (HINT: Think about wavelengths of different colors and their associated energy)

*Yes, because different colors means different wavelengths, which mean different energies.*

G5. What make each flame a different color?

*The Chemicals*

OBSERVE LUMINOL DEMO!!!

**NOTE:** Purchasing information can be found in the cover pages. It is more cost effective/time effective to just use the Luminol as a demo, but in small AP classes could perform the demo on their own. Be sure to explain each solution you add into the mix. (Luminol solution, Hydrogen Peroxide solution) **NOTE:** The higher percentage of Hydrogen Peroxide solution the more intensive the glow. (25% is recommended!!! USE GLOVES!!!!!!) It is also helpful to gather the students as close as possible because the glow is short lived. (Glow sticks will work as well to prove the point that chemicals can independently produce light. Just you will need to explain the design and composition of a glow stick.)

G6. What did you observe in the luminol demo?

*Chemical without heat produced light.*

**NOTE:** Teachers may need to question the student about the difference between this experiment and the flame experiment. In order for the students to put the without heat section.


*Yes, Yes, I observed the light production and light is energy.*

G7. Thinking about the *P. leio* and its light production, where do you think the energy to produce the light is coming from?

*Answers will vary. But, guide the students to realize that the luminol and *P. leio* both produce the same color and reiterate that chemicals produced the light in the luminol demo.*

**Let’s Explore some possibilities!**

G8. Do light bulbs produce heat while producing light? *YES* Does the flame produce heat while producing light? *YES* What about the sun? *YES* In order for our organism to grow we had to heat it up just a bit. So do, you think heat might be contributing to the light production in *P. loei*? Why?

*Answers will vary.*
Let’s heat it up and find out.

G9. What do you think will happen to *P. loei*’s glow after being heated?  
*NOTE: USE LIQUID CULTURES. AGAR PLATES WILL MELT IN THIS SECTION. You will need to heat it for at least 5 mins at 60 degrees C. Moving on to the next activity while it heats up is a feasible option or splitting the class in two groups work as well. If you do not have an incubator/ heat block available then a microwave designated for lab use should work at full power for one minute, WARNING: COULD CAUSE BOILING. ALLOW LIQUID TO COOL IN MICROWAVE AND STUDENTS SHOULD WEAR PROTECTIVE EYEWARE. DO NOT MICROWAVE ANYTHING WITH A CAP. PRESSURE COULD BUILD INSIDE.*

G10. What happened to *P. loei*’s glow after being heated?  
*P. loei* no longer glows after being heated. It died.  
*NOTE: Teachers can use the example of if we heated up most living things they would not survive. Glowing is an indicator of *P. loei*’s life.*

G11. Do you think heat is responsible for *P. loei*’s ability to produce light? Provide Evidence.  
*NO, The students should use the previous experiment as evidence.*

G12. Let’s think of a few other things that we know produce light in the dark. Glow in the dark stars and solar powered lights. They work much like the tonic water, but they are able to store the light. Let’s take a glowing sample outside and attempt to “charge” the cultures and compare to culture that was not charged. What do you think will happen?  
*Answers will vary.*  
*NOTE: PLATES OR LIQUID MEDIA MAY BE USED HERE. Also, you will want to have the students compare the two samples before they go outside to make sure they are of the same brightness. Use the plates they made in the first activity.*

G13. What happened after we “charged” the non-glowing cultures?  
*Nothing*

G14. How did the “charged” glowing culture compare to the “non-charged” glowing cultures?  
*They are still the same.*

G15. Do you think *P. loei* can be “charged” by another light source? Provide Evidence.  
*No, They should use the previous experiment as evidence.*

G16. Return to question A4 on Page 1. Using your new knowledge of chemistry and light production. What is your new hypothesis?  
*Answers will vary. But look for a use of their new chemistry knowledge. Preferably that there must be chemicals in *P. loei* making it glow.*

**Exploration 2: Lets Break It Down; Biochemistry**

**Question:** Does your mouth “water”? Is the saliva in your mouth and water the same thing?
Materials needed for a group of 2-4 students

- 6 glass tubes
- 1 Ruler
- 1 Marker
- 1 roll labeling tape
- Starch solution
- Iodine solution
- 2 Drovers (plastic or glass)

Part A:

1. Using labeling tape and a marker label all 6 of your glass test tubes. The tubes should be labeled as follows: Water, water + starch, saliva + starch (your initials), saliva + starch (your partner’s initials), Body Builder+starch, Bio-Pure Protein+starch.

2. Each partner spit into the tube labeled saliva + starch with their own initials. While collecting saliva try not to laugh, and do not chew gum (gum will skew our results). Collect enough saliva to fill the tube to the lowest mark. Make sure to handle only the tube containing your own saliva.

3. In the two tubes labeled water, and water + starch, add an equal level of distilled water to the highest mark.

4. In the test tube label Body Builder + Starch, add BodyBuilder protein solution to the lowest mark.

5. In the test tube label Bio-Pure Protein+ Starch, add Amylase solution to the lowest mark.

6. Add the starch solution using the provided dropper to the “saliva + starch” tubes, “water + starch” tube, “Body Builder + Starch” tube, “Bio-Pure Protein + Starch” tube add enough solution to bring the liquid up to the highest mark. Swirl to mix. Record your observations in the column labeled “Before Addition of Iodine” in Table 2. Wait 10-15 minutes

7. Add a few drops of the iodine solution using the dropper provided to each tube (Wait to ADD the Iodine to the Body Builder and Bio- Pure till after question F6) and swirl to mix. Record your observations in the column labeled “After Addition of Iodine” in Table 3. Record your color observations.

Table 3 Color Observations

<table>
<thead>
<tr>
<th>Tube</th>
<th>Before Addition of Iodine</th>
<th>After Addition of Iodine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva + Starch (1)</td>
<td>Chalky white</td>
<td>Yellow</td>
</tr>
<tr>
<td>Saliva + Starch (2)</td>
<td>Chalky white</td>
<td>Yellow</td>
</tr>
<tr>
<td>Water + Starch</td>
<td>Cloudy White/Clear</td>
<td>Purple/Blue</td>
</tr>
<tr>
<td>Water (no starch)</td>
<td>Clear No Color</td>
<td>Yellow</td>
</tr>
<tr>
<td>Body Builder + Starch</td>
<td>Will vary with Protein</td>
<td>Purple/Blue</td>
</tr>
<tr>
<td></td>
<td>Powder</td>
<td></td>
</tr>
<tr>
<td>Bio-Pure Protein + Starch</td>
<td>Chalky white</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

Concept Development 3: The Breaking Point

H1. What is starch? What are some sources of starch?

A complex sugar molecule/ a long chain sugar molecule found in potatoes, corn, rice, bread, etc.

H2. Does iodine always result in a blue color? What evidence leads you to this conclusion?
No, Water+Starch=yellow.

H3. Using your data table 2. What compound causes the blue color reaction?

Starch

NOTE: Teachers may want to introduce iodine as a starch indicator.

H4. Using your data table 2. How does the saliva + starch + iodine reaction compare to the water + iodine reaction?

They are the same color, but one has starch and the other does not.

H5. Using your data table 2. How does the saliva + starch + iodine compare to the water + starch + iodine?

They both have starch, but the one with saliva is yellow and the one with water is blue/purple.

H6. Consider your response to the question H4 & H5, how do you explain the reaction you observe in your saliva + starch + iodine tube? (Why is it not blue?)

Something to the effect of, the saliva did something to the starch to make it not react with the iodine. You may get answers that say it inhibited the iodine, point out that it is still yellow and iodine is just a chemical and would not be inhibited.

Let’s try with some “Body Builder” protein and some Bio-Pure protein (Amylase).

A. Return to step 7 on page 9 where you were instructed to wait.

H7. Did the body builder protein break down the starch? How do you know?

NO, the solution still turned blue.

H8. What do you think the function of Body Builder protein is?

Answers will vary. There is no wrong answer, but the most common will be build muscle.

H9. Did the Amylase protein break down the starch? How do you know?

Yes, the solution is yellow.

NOTE: Amylase ordering information can be found in the cover pages. Store amylase in a refrigerator to maintain activity for a year or more. Here is the time to talk about proteins and how
they work in our bodies. (Enzymes that breakdown food, keratin composition of hair and nails) Below is a ribbon diagram of the amylase protein.

What Amylase really looks like! Figure 9

![Wikipedia, Amylase](image)

H10. Do all proteins perform the same function? Provide evidence.

*No, they should use the body builder vs. bio-pure protein as evidence.*

What is Really Going on Figure 10

![Figure 10](image)

H12. Figure 10 Illustrates the chemistry preformed by amylase. In your own words describe what is going on in the figure. (Use Complete Sentences)

*Answers will vary.* Starch enters the active site of the amylase protein and is broken down into smaller sugars.

*NOTE: Figure 10’s resolution has to be decreased for storage purposes. Visit the website provided in the citation for an image that will be easier to read.*

H11. Do you think there are proteins in your saliva? Why (Hint: You just got back from lunch!)? Which one? What evidence do you have to support that?

Yes, *Answers will vary, because we eat starchy foods and our bodies have to break it down, Amylase, the evidence should be the previous experiment.*

**Concept Application 3: Luciferin and Luciferase**

**Materials needed for a group of 2-4 students**
- 1mL Buffer
- 1 Firefly Lantern
- ATP Dropper

*NOTE Purchasing information can be found in the cover pages.*

1. Observer Firefly lantern in dark

11. What did you observe?

*Nothing.*

2. Uncap Firefly Lantern
3. Fill Firefly Lantern with 1mL Buffer.
4. Recap and Shake gently
5. Uncap add 4 drops of ATP
6. Recap and shake gently.
7. Wait for classroom lights to be turned off

I2. Did you observe any reactions? What Reaction, if any?

Yes, *the solution produced a green glow.*

Explain the buffer they added dissolves the proteins of the dried organism. After they observe the glow proceed to explain what proteins were dissolved and the reaction that occurred. (Use Figure 11 as a guide).
I3. Figure 11 shows the **CHEMICAL** reaction occurring within the firefly lantern. Numbers 1 and 2 indicate the two proteins needed for this reaction to occur. The number 3 indicates the two proteins coming together. In your own words interpret this diagram. (Include the terms proteins, energy, and light) BE SURE AND READ THE LABELS ON THE FIGURE THEY **WILL** HELP.

*Answers will vary. Luciferin and Luciferase proteins come together to form oxyluciferin. Oxyluciferin is highly energized and releases energy in the form of light to become a non-energized oxyluciferin.*

**Figure 11 Luciferin Luciferase Reaction**

1. Luciferin
2. Luciferase
3. Oxyluciferin
   - Highly Energized
   - Light!!!
4. Oxyluciferin
   - Non-Energized

I4. Where did the energy come from?

*The proteins/ Luciferin & Luciferase.*

I5. What main two “chemicals” are needed for the light reaction to occur? What is a general name for these “chemicals”? *Luciferin and Luciferase, Protein*

**Authentic Assessment:**

J1. Using our new knowledge about proteins, chemistry, and light, return again to question A4 on page
1. How do you think *P. loei* produces light?

*Answers will vary. But the students should mention protein interactions and walk through the Luciferin/Luciferase reaction figure 13.*

You have the option to make a drawing with captions, a video, a prose, or a screen cast to answer this question. But, you must address light, chemistry, and proteins and how your exploration of them allowed you to make your final conclusion.

J2. Would you agree with each one of these statements? Explain, why or why not.

*I and II are incorrect, but if they are selected true guide them back to part of Student Guide that debunks these misconceptions.*

*III and IV could both be correct, just look for a thorough explanation.*

I. *P. loei* produces light by capturing the energy from an outside light, for example the sun.

II. *P. loei* produces light by moving vigorously and creating excessive heat that leads to its light production.

III. Chemicals inside of *P. loei* interact to produce light in *P. loei*.

IV. Proteins are responsible for the light production of *P. loei* as well as many other biological functions.