Testing the Aerobic Growth of *Halobacterium* in Light and Dark Conditions: A Student Laboratory Investigation

Question: What environmental conditions cause *Halobacterium* to turn on or off the genes coding for the protein, Bacteriorhodopsin?

Materials per group:		**Classroom Materials:**
3 clear falcon tubes	OR 3 tubes covered in black tape & 1 clear tube	Environmental shaker Spectrophotometer
1 falcon tube containing 5 mL of *Halo*	6 beral pipets (2 for day 1, 4 for day 2)	Lamp with a special full spectrum bulb
1 test tube rack	3 microcentrifuge tubes	Kim wipes
1 tube with 20 mL of CM	Labeling tape	Microcentrifuge
3 cuvettes	Permanent Marker	Distilled water for clean up

Procedure (Day 1):

1. On a clear falcon tube, use a permanent marker to mark the 5 mL line.
2. Pour 5 mL of Complete Media (CM) into the falcon tube. If you overpour, use one beral pipet to remove the appropriate amount of CM.
    a. If your other tubes are clear, repeat the process listed in step 1 with your other tubes.
    b. If your other tubes are covered with electrical tape, pour all of the CM from the clear tube into your dark tube. Repeat the process in step 1 with your other 2 dark tubes.
3. Gently swirl the *Halobacterium* culture. Use your unused beral pipet to remove exactly 1 mL of *Halobacterium* from the tube. Place it into one of your tubes containing 5mL of CM.
4. Place the cap tightly on your sample tube, to the second stop, and gently invert the tube to ensure the sample is evenly mixed.
5. Repeat steps 3 and 4 for your other 2 sample tubes.
6. Using the labeling tape, label your tubes with your group name, the organism's name (*Halo*), the date, the sample number, and the condition being tested (either light or dark). Keep in mind that light will shine through your culture, so do not block it with your label.
7. Take the beginning optical density (OD) of your culture. To do this:
    a. Pour each of your samples into a separate clean cuvette.
    b. Set the spectrophotometer to absorbance and to a wavelength of 600 nm.
    c. Use Kim wipes when handling cuvettes.
    d. To zero the spectrophotometer, insert provided blank, press blank button and **wait** for zero to appear.
    e. Insert cuvette with sample; record absorbance reading.
    f. Pour your sample back into your falcon tube for incubation.
    g. Repeat with each of your samples and record these numbers as the beginning optical density. If your numbers are not nearly identical, describe possible reasons why in your lab notebook.
8. Bring your samples to your teacher to incubate at 37°C and 220 rpm with a special, full spectrum light bulb. Record the time and date your incubation began.

9. In your lab notebook, make a prediction about what will happen to the cell growth and resulting absorbance in the future days. Also, predict how the sample may change in appearance.

(over)

Procedure (Day 2): You will be taking an absorbance reading and spinning down cells.

1. After at least 72 hours, obtain your three samples from the incubator. Record the time and date the incubation stopped.
2. Gently agitate and pour each sample from the tube into a clean, empty cuvette.

To spin down your cells:

a. Using a beral pipet, transfer 1.5 mL from your sample to an empty microcentrifuge tube. You can use the markings on the pipet to first draw out 1 mL, then 0.5 mL.
b. Snap the lid tightly closed.
c. Use a permanent marker to label the outside of your microcentrifuge tube with your group name, the sample number and the sample type (light or dark).
d. Repeat this for each of your samples.
e. Take your samples to the microcentrifuge. Symmetrically place tubes inside a centrifuge so they balance each other out, otherwise the centrifuge may break.
f. Close the lid and turn the centrifuge on for 15 minutes.
g. Remove your tubes from the microcentrifuge and locate the cells (pellet) at the bottom of the tube. Without agitating the cells, use a beral pipet to remove the liquid above the cells.

To measure your cell density:

h. Use the remaining sample in your cuvette to measure the absorbance of each sample.
i. See the procedure in step 7 from Day 1. **Run blank before each sample.**
j. Record data in your data table.

3. **RETURN CUVETTE SAMPLE TO FALCON TUBE - DO NOT DISCARD. Ask your teacher where to keep your liquid samples and your cell pellets. You will need the cell pellets for your next lab activity.**
4. Clean up your lab space and equipment.
    a. Do NOT USE SOAP when cleaning equipment.
    b. Rinse cuvettes thoroughly three times with hot tap water.
    c. Rinse one more time with deionized water.
    d. Return equipment to proper locations designated by the teacher.