Procedure

Materials Needed:

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| #1. [Bioluminescent Dinoflagellates](http://www.botany.utexas.edu/infores/utex.) *(pyrocystis lunula, noctiluca, fusiformis, ceratium)*- $15.00-20.00 | #2. Various Medias: Miracle Gro, Algae Gro, Saltwater, Distilled, etc. |
| #3. Vials, and stand: May need small ones if use spectrometer, some come w/ algae gro | #4. Centrifuge: Don't need to buy, have one in classroom, your choice to use |
| #5. Pipettes: Recommend disposable, but you could get measurable ones, may also want petri dishes, your choice | #6. Some way of measuring light output: Spectrophotometer without light, industrial light measuring device, your choice to use etc. |
| #7. A light source: fluorescent only!! | #8. A timer for light: 12 hrs. light, 12hrs. dark |

Step #1: **Medias**- Gather at least five different medias. These medias need to differ in types of nitrogen's, minerals, and phosphates that can affect the growth or behavior of a Dinoflagellate. For examples I chose the following with ingredients:

* Erdschreiber- (How to make also buy)Prepare separate tubes to contain 0.2 NaNO3/10ml H2O and .03g Na2HPO4.7H2O/10 ml H2O, alutoclave and store at 10'C. For solution add following to filtered sea water:
  + NaNO3 stock solution - 1 tube  
    Na2HP04.7H2O stock solution- 1 tube  
    Vitamin B12 (15 x 10-6 g/100 ml H2O)- 1ml  
    PIV metal solution- 12 ml  
    Soil water (GR+) (w/calcium) supernatant- 50 ml
* Algae Gro Concentrate(10 drops)- Prepared media from Carolina Biological Supply Co. Phone #(800) 547-1733. High levels of phosphates and nitrogens. Unknown exact company product.
* Liquid Miracle Gro(2 or 4 drops)- Media for soil houseplants available any hardware store.
  + Total Nitrogen 8%: 1.2% Ammoniacal, 1.2%Nitrate,5.6%Urea  
    Phosphate(P2O5)7%  
    Soluble Potash (K2O) 6%  
    Chelated Iron 0.1%  
    H2O 78.9%
* Instant Ocean(0.5g)- enriched sea-salt available at any Fish Retailer (Wet Pets)
* Kent Freshwater Plant Nutrients(10 drops)- micronutrient supplement for bottom dwellers

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| * + Deionized Water | * + Manganese | * + Zinc |
| * + Iron | * + Magnesium | * + Molybdenum |
| * + Salts of potassium | * + Copper | * + EDTA |
| * + Boron | * + Cobalt | * + Inorganic Stabilizers |

Step #2: **Diluting the Medias and Combinations**- Take each of your medias, combinations or the examples and mix in Distilled (DH2O) Water of 20 ml. To add the noctiluca cultures I simply pipetted them but you can also but them in a centrifuge to ensure that you have some. The list below shows what I combined with what (make sure to label the test tubes):

**Saltwater Mediums**

Erdschreiber--> nothing was my control w/culture of noctiluca

Algae Gro(10 drops)--> 20ml of distilled H2O + 0.5g of Instant Ocean + 18 drops of noctiluca culture

Instant Ocean(0.5g)--> 20ml of distilled H2O + 18 drops of noctiluca culture

Kent Nutrients(10 drops)--> 20ml of distilled H2O + 0.5g of Instant Ocean + 18 drops of noctiluca culture

Instant Ocean(0.5g)--> 20ml of **tap** H2O+ 18 drops of noctiluca culture

Miracle Gro(2 drops)--> 20ml of distilled H2O + 0.5g of Instant Ocean + 18 drops of noctiluca culture

Miracle Gro(4 drops)--> 20ml of distilled H2O + 0.5g of Instant Ocean + 18 drops of noctiluca culture

Del Valle Creek(10 drops)--> 20 ml of distilled H2O + 0.5g of Instant Ocean + 18 drops of noctiluca culture

**Freshwater Mediums**

Algae Gro(10 drops)--> 20 ml of distilled H2O + 18 drops of noctiluca culture

Miracle Gro(2 drops)--> 20 ml of distilled H2O + 18 drops of noctiluca culture

Miracle Gro(4 drops)--> 20 ml of distilled H2O + 18 drops of noctiluca culture

Algae Gro(10 drops)--> 20ml of **tap** H2O + 18 drops of noctiluca culture

Distilled H2O(20ml)--> 18 drops of noctiluca culture

Tap H2O(20ml)--> 18 drops of noctiluca culture

Del Valle Creek(10 drops)--> 20 ml of distilled H2O + 18 drops of noctiluca

Step #4: **Storage-** To keep the Dinoflagellates from dying or not illuminating from other causes to falter your results follow these instructions:

#1. Keep them all in the same area

#2. Avoid extreme heat or coldness

#3. Have a vial rack that won't absorb heat, I recommend Styrofoam

#4. Make sure the light source is fluorescent and all are getting equal amounts of the light

#5. Keep the vials at the same pH 8.5 for saltwater mediums and 7.0 pH for freshwater mediums

Step #5: **The Actual Testing-** For the testing it's up to your resources. One type of testing is with a professional photometer however Dinoflagellates don't give off that much light and only last for seconds. Another way is using a spectrometer reversing it to measure the light total not spectrums. Physics for the math lovers is also a way but the light may be so low and so fast you may not even get a reading plus the math is a little complicated if interested see Mr. Hall. The last test to measure the intensity and the easiest is to have a scale 1-10, 10 dependent upon the Dinoflagellates in the control and rate them compared to this control. After you get the method you want to determine the intensity and all the mixtures done and Dinos have gone through their cycle of 12 hrs. of light you can begin. After all this the testing is very easy. Simply go into a very dark room, tip the test tubes horizontally and look for the blue light. **A warning the Dinoflagellates bioluminescence is not bright and usually very minuet and lasts only seconds.** This is why I recommend the noctiluca because of it's size but it's still only 2 mm long. If your working with machinery I don't know how it will come out. I simply used the scale method.

[-Back Home-](http://docs.google.com/contents.htm)