# BIOGLO®

## Discover Bioluminescence

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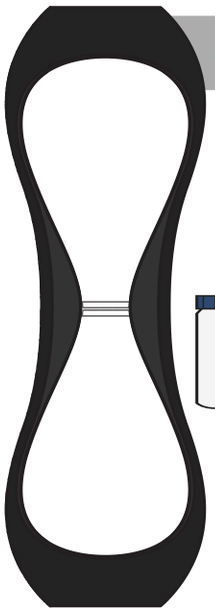
# BIOGLO COMPONENTS

## BioGlo Standard Components



1X Aquarium

2X Nutrient Vials

1X Salt Mix

1X Carrying Pouch


*Note: You'll need 1 Liter (34 fl. oz.) of spring or distilled water.*

## BioGlo Explorer Components

2X Salt Mix

4X Nutrient Vials

6X Test Tubes

6X Pipettes

1X Magnifier

## Where Are Your Dinos?

Your living Dinos need special treatment to survive shipping, so they must be shipped separately from the other BioGlo components. Find the "Ship Your Dinos" Card and enter the 7 digit "Control Code" into the website located on that card. This will set your Dinos on their journey to you!

# GETTING STARTED

1. First, pick out the perfect location to keep your BioGlo. We recommend placing it on a nightstand so that you have easy access to it as you go to bed. You'll want a place with moderate temperatures of 18°-25°C (64°-78°F) and no direct sunlight (away from windows).

2. Using the instructions on the next page, create your own "growth medium" (that's Dino food).

3. Once your Dinos have arrived, pour them gently into the BioGlo aquarium. Then, slowly pour your Growth Medium in to the BioGlo until the bottom half of your aquarium is about 3/4 full.

4. The microbes' recovery from shipping or jet lag (seriously!) may take a week or more. Check for glowing each night and enjoy the magic of bioluminescence (instructions on page 9) once they've recovered!

Dinos do not need much light to thrive. Avoid placing your BioGlo in direct sunlight or near a window because it risks overheating the water and killing the Dinos.

# MAKING GROWTH MEDIUM

## Ingredients

Dinos' favorite food is called "growth medium" and it's a tasty mix of sea salts, inorganic nutrients (e.g. nitrates and phosphates), trace elements, and vitamins! To make growth medium, you will need the following items:

- 1 Liter (34 fl. oz.) of spring or distilled water from an unopened bottle (not included, but can be bought at any pharmacy or grocery store)
- Salt mix pack (included)
- Nutrient vials A and B (included)

1. Gather the ingredients listed above and begin by pouring out a small splash of water (e.g. 1-2 tablespoons) from your water bottle; just enough to make room for the remaining ingredients.

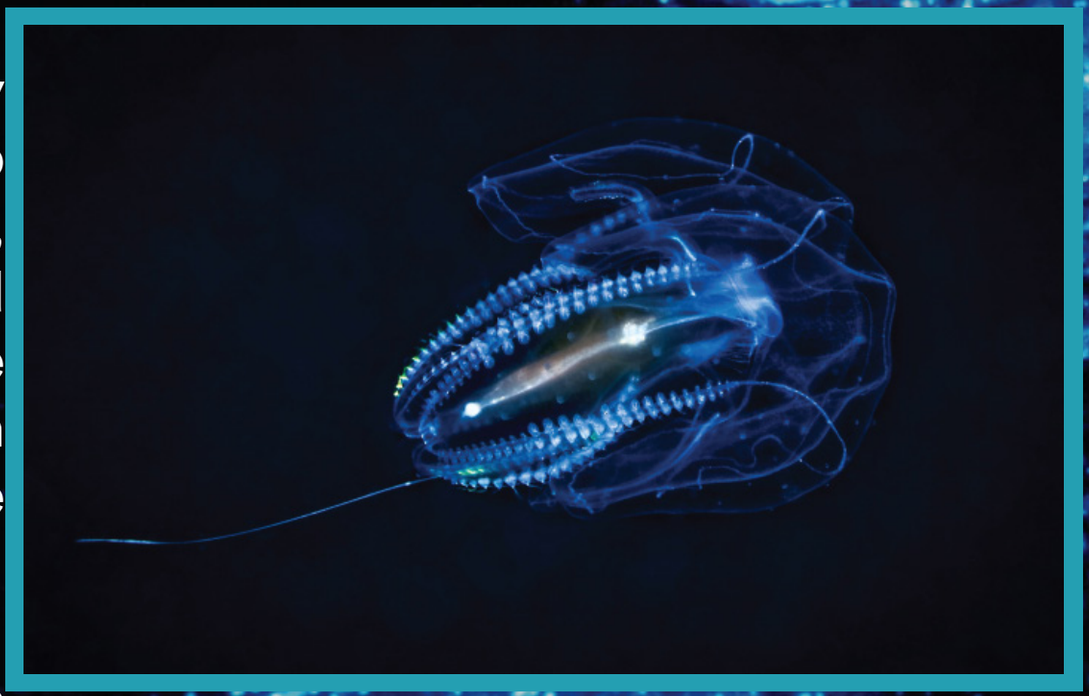
2. Pour all of the contents from the salt mix pack into the bottle of water. Mix vigorously. Let the salt dissolve for 15 minutes before continuing.

3. Add the entire contents of the nutrient vial A into the bottle. Mix vigorously. This vial contains concentrated nutrients.

4. Add the entire contents of the nutrient vial B into the bottle. Mix vigorously. This vial contains trace metals and vitamins. That's it, you're done! This fresh growth medium will help sustain a healthy Dino culture and a brighter glow!

# BIOLUMINESCENCE

Bioluminescence is defined as the emission of light (”luminescence”) by a living organism (“bio”), and is the result of a chemical reaction between light-generating molecules created by the organism. For creatures that lack the ability to produce their own light (like us), bioluminescence may seem unreal, but this incredible spectacle is surprisingly common in nature.

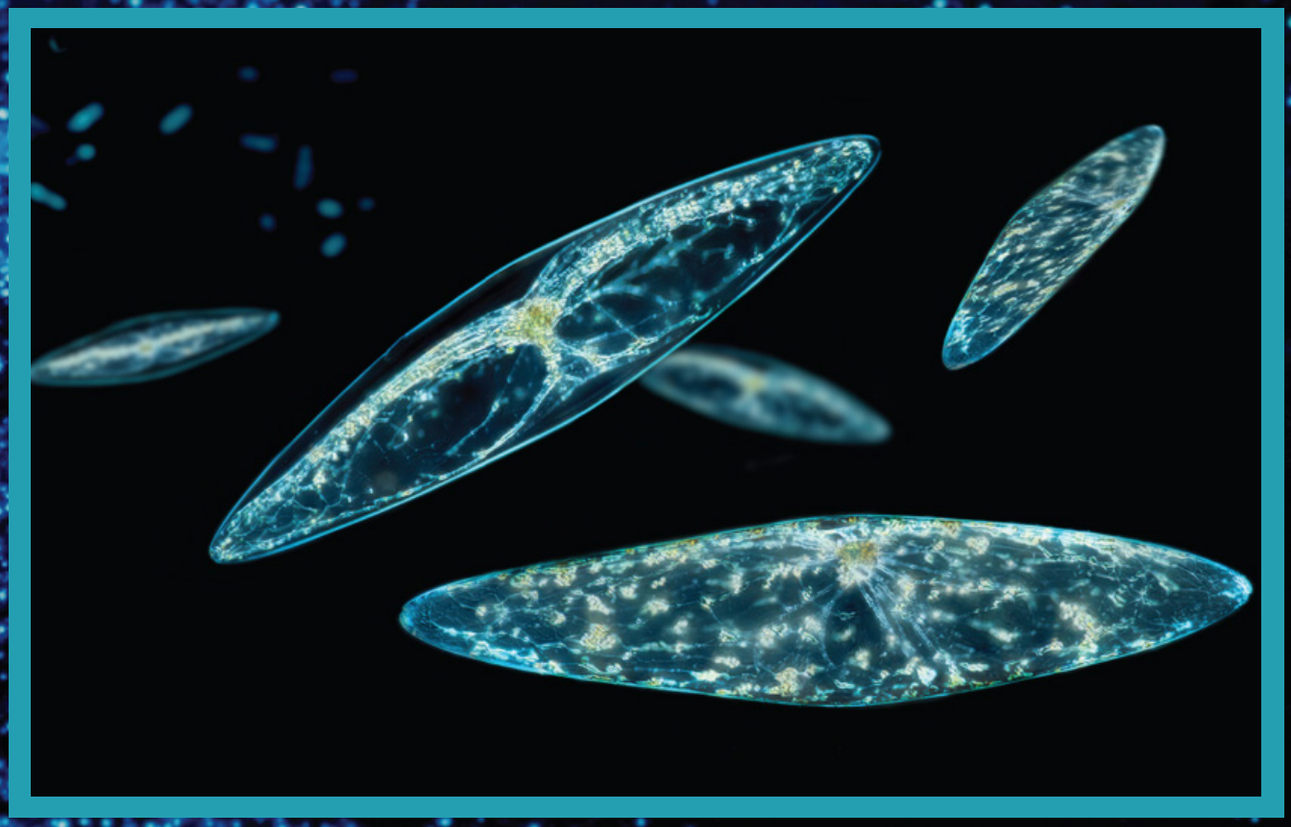


From the humble firefly to the magnificent comb jellyfish shown to the right, bioluminescence is all around us. In fact, scientists estimate that more than 75% of known ocean organisms have the ability to bioluminesce.

For many creatures, the purpose of this unusual ability is still not fully understood since it is used in so many different ways. The angler fish, for example, famously luminesces to lure prey while other animals may use it to attract a mate, distract predators, or simply communicate with each other.

# DINOS IN NATURE

*Pyrocystis fusiformis*, shown below, is a Dino which inhabits ocean waters all over the world, and just happens to be the species of Dino that will inhabit your new BioGlo aquarium! Because Pyrocystis fusiformis is part of the phylum Dinoflagellata, many people prefer to call them “Dinos” for short.



Dinos are typically found at depths of 60-100 meters beneath the ocean surface in temperate and tropical waters. They derive their energy from the sun during the day, but can release it as a beautiful blue glow of light at night. Scientists still aren’t exactly sure why Dinos glow in the first place. The leading hypothesis is that Dinos use luminescence in order to illuminate their predators so that their predators are seen and eaten by even larger predators, leaving the Dinos free to live in peace. Why do you think they glow?

# CIRCADIAN RHYTHMS

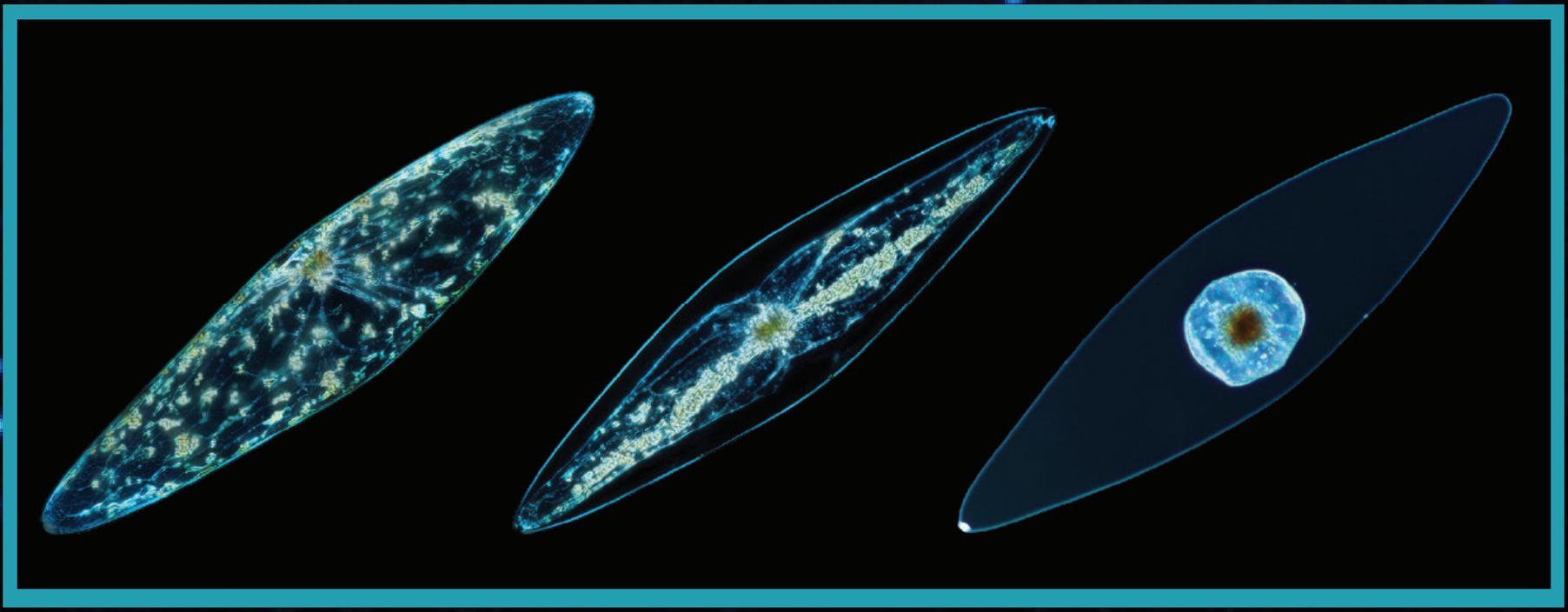
In most living things, internal biological clocks enable organisms to anticipate daily environmental changes corresponding with the 24-hr solar cycle on Earth. These internal clocks are called “circadian rhythms.” In humans, circadian rhythms heavily influence sleepiness, hunger, mental alertness, mood, and much more.

*Pyrocystis fusiformis* also maintains a circadian rhythm. In a 24-hr period, the microbes prefer approximately 12 hours of light and 12 hours of darkness. During the light portion of this cycle they do not luminesce, and significant disruptions to this circadian rhythm can cause them to stop luminescing altogether.

# DINO LIFE CYCLE

A typical single *Pyrocystis fusiformis* microbe lives for only 5-7 days, but the cycle can be much longer depending on environmental conditions. During this time several morphological stages can be observed as the microbes reproduce and multiply (shown below).

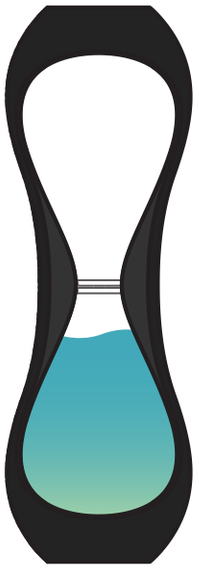
The name "*Pyrocystis*" is derived from two Latin roots — "*pyro*", meaning fire, and "*cystis*", meaning cavity. As you can see below, the name "fire cavity" is fitting for these Dinos.



Your dino culture may appear to be the same familiar Dinos lighting up night after night, but in reality your BioGlo is home to a dynamic and bustling community of new and old Dinos. Over the course of several weeks, you will observe the life and times of several generations of Dinos as they get old and give birth to new life!

# KEEPING YOUR DINOS HAPPY

It turns out, taking care of your Dinos is very similar to taking care of an indoor plant:



**Food** - Feed your Dinos every 3-4 weeks by pouring out 1/4 of your total BioGlo's liquid volume (this is a perfect opportunity to use those dumped Dinos for experiments!) and then replenishing that volume with new growth medium.

**Light** - You'll want to expose your Dinos to ambient lighting from indirect sunlight or indoor lighting. Avoid direct sunlight!

**Temperature** - Dinos prefer moderately cool temperatures, 64° - 78° F (18° - 25° C). Dinos don't like rapid temperature changes, so don't put them near a heating or A/C vent.

If your Dinos stop glowing at night, they may still be alive! Before feeding them new nutrients, first give them a few days of the right lighting and temperature conditions and they may bounce back!

With the BioGlo BaseLight accessory, you can provide optimal lighting conditions for your Dinos. BaseLight's mobile app lets you control the circadian rhythm and provides temperature warnings when the Dinos get too hot or too cold.

**Available at www.magicalmicrobes.com**

# BIOGLO MODES

With BioGlo, you can enjoy bioluminescence in different ways. It takes energy for Dinos to luminesce, so when their glow begins to fade, they'll need to rest and absorb more light the next day to be ready to show off again the next night.

**Bubble & Lightning Mode** - Simply turn your BioGlo upside down and enjoy the calming light bubbles and the streaks of light down the base of your aquarium. You can do this a few times before the Dinos get tired and need to rest for the night. This mode maximizes your observation time.

**Tornado Mode** - Turn your BioGlo upside down and gently swirl in one direction to form a tornado. Once the tornado has appeared you can set your BioGlo down until the Tornado is finished. Note that your Dinos will get tired quickly in this mode.

## Viewing Tips:

1) Dinos will only glow in complete darkness, so be sure to view them at night or in a completely dark room (if you've changed their circadian rhythm) with no light seeping in. Give your eyes 1-2 minutes to adjust to the darkness in order to see the full effect.

2) Dinos glow brightest about 1 hour into their dark cycle, so let them adjust to the darkness before viewing them.

# ART OF EXPERIMENTATION - PART I

## Scientific Method

The framework below will guide you through the process of designing an experiment. This framework, called the “Scientific Method,” is used by scientists and engineers to investigate and further our understanding of the world around us:

**Purpose** - What do you want to learn about or find the answer to? What do you already know about this subject?

**Testable Question** - Pose your idea in the form of a question that can be answered through experimentation.

**Independent Variables** - What conditions are you going to change in this experiment? These are the independent variables and include conditions like light and temperature.

**Dependent Variables** - What are you going to measure? These are the dependent variables and can include outcomes like circadian rhythm timing and brightness.

**Hypothesis** - What do you think will happen?

**Materials** - List all the materials and supplies you’re going to need.

**Procedure** - Write out each step in your experiment in enough detail that someone else could do the same experiment from your directions.

# ART OF EXPERIMENTATION - PART II

**Note**: Before you begin, it's important to design your experiment by determining which "independent" variables you'd like to change and "dependent" variables you'd like to measure. Be sure to check out our suggested experiments in this booklet if you're not sure where to begin!

**Independent Variables**

- Temperature
- pH Level
- Light Intensity
- Timed Lighting
- Nutrients
- Contaminants
- What else?

**Dependent Variables**

- Glow Brightness
- Population Count
- Circadian Timing
- Glow Duration
- Microbe Size
- Microbe Lifetime
- What else?

Example: How does sunlight exposure affect the growth of a plant?

To answer that question we could vary the amount of sunlight exposure (independent variable) and perhaps measure the height of the plant (dependent variable) to learn more about how sunlight exposure affects growth.

# ART OF EXPERIMENTATION - PART III

## Data Collection & Analysis

So you've selected your variables and developed an experimental plan? You can now begin to collect and analyze the results. So let's glow!

**Data Collection** - Record your observations of the dependent variables you selected. It's a good idea to include units, the time of measurement, and a brief description of what you've observed.

**Data Visualization** - Depending on the type of data measured, graphing or plotting the data can be helpful. Consider bar graphs, line graphs, scatter plots, or even a series of sketches.

**Analysis** - Examine your data, and describe any patterns, trends, and changes you see.

**Conclusion** - Were you able to answer your testable question from your results? What did you find out? Were your results what you expected? Why or why not?

**Next Steps** - If something didn't work as well as you expected, note anything you'd do differently if you repeated the experiment. Also record new ideas or questions that came up after performing the experiment.

# SUGGESTED EXPERIMENTS

**How do farm fertilizers affect *Pyrocystis fusiformis*?**

It turns out your Dinos are good indicators of environmental health. The healthier their environment, the brighter they will glow. Can you use your Dinos to show how run-off from farm fertilizers impacts ocean health?

**What stimulates the microbes to luminesce?**

As explained previously, vibrations in water are known to cause *Pyrocystis fusiformis* to luminesce, and is believed to be a self defense mechanism. Can you identify another way to stimulate (e.g. chemical) the microbes?

**Can microbes get too much or too little light?**

Like plants, *Pyrocystis fusiformis* cells contain chloroplasts to harness light energy. How do you think light exposure and intensity affect luminescence brightness?

**How does the amount of light exposure impact glowing brightness? (This one is made easy by the BioGlo BaseLight accessory)**

Dinos absorb energy from sunlight during the day. But how much is enough to survive? How much will cause them to glow brightest? Find out by exposing Dinos to different durations of light and see how it impacts their brightness at night. (See Page 15 for a step-by-step guide)

## Experiment #1 - Modeling Farm Run-off

Dinos can serve as an indicator organism to tell you about the health of the environment they live in. The healthier the environment, the denser and brighter the luminescent glow will be.

Unfortunately, almost half of all coastal waters in the United States are too polluted to swim or fish in. One of the reasons for this is fertilizer run-off from agriculture. Some fertilizers contain chemicals that are poisonous to sea life. Other fertilizers are perfect foods for microbes, and will cause algae to thrive and "bloom". This can be equally dangerous, as those "algae blooms" can create their own toxins or simply suck up all the oxygen in the water, thus suffocating other life around it.

A simple experiment will allow you to see first-hand the impact that different fertilizers or other chemicals have on Dinos:

- [ ] **Step 1** - Pipette 9mL of your dino culture into 2 test tubes. Label one "C" for Control, and label the other "T" for Test.

- [ ] **Step 2** - Add a drop of the plant fertilizer or other liquid you want to test into the "T" test tube and observe the difference in glowing over the next 3 nights. What do you see?

## Experiment #2 - Circadian Rhythms

These instructions walk through a sample experiment testing how changes to the length of light exposure affect bioluminescence brightness. The instructions are written for the BioGlo Explorer model, but can also be performed with your own lab supplies. Allow your Dinos at least 1 week to adjust to their new home before beginning this experiment.

- [ ] **Step 1** - Wash your hands. Your microbes are sensitive to foreign microbes, so it's best to avoid contamination whenever handling them.
- [ ] **Step 2** - With a permanent marker, label one test tube on its cap "C", to indicate that it is the control. This tube will be used to keep a sample of your existing culture for reference and comparison.
- [ ] **Step 3** - Label a second test tube "T" (also on the cap) to indicate that it is the test sample. Note that the labels should be placed on the caps so as not to interfere with light exposure.
- [ ] **Step 4** - Pipette 9mL of microbe culture into each test tube, gently shaking the culture beforehand to ensure a more even distribution. Note that this can be done using the starter culture or a culture that has already been mixed with growth medium.

# SAMPLE EXPERIMENTS

- [ ] **Step 5** - Next you will need a means of timing light cycles for the microbes. This can be accomplished using cool white lamps (e.g. fluorescent or LED) and timers or the BaseLight system (see below).

- [ ] **Step 6** - Place both test tubes in separate dark spaces with no ambient light. Each test tube should have its own light and timer. Set up each timer to turn the light on when the sun rises in your region and stay on for a period of 12 hours.

- [ ] **Step 7** - Allow both samples to establish the same circadian rhythm with the timed artificial light. Ideally, you should wait roughly 1 week for this to happen.


## BaseLight System

Designed to help you take care of your Dinos with more confidence and ease, BaseLight is a bluetooth connected lighting system for BioGlo.

With its free app you can easily schedule light cycles, automate circadian adjustments, receive nutrient purchasing reminders, monitor the environmental temperature and much more!

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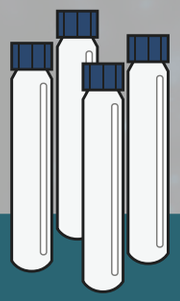
☐ **Step 8** - Once both samples have established the same circadian rhythm, maintain that lighting schedule for the control sample. For the test sample start shortening the light duration by turning on the light 1 hour later than the previous day (or stopping it 1 hour earlier) as shown in the following timetable:

| | Control Sample | Test Sample |
|---|---|---|
| Day 1 | Start: 08:00; End: 20:00 | Start: 08:00; End: 20:00 |
| Day 2 | Start: 08:00; End: 20:00 | Start: 09:00; End: 20:00 |
| Day 3 | Start: 08:00; End: 20:00 | Start: 10:00; End: 20:00 |
| Day 4 | Start: 08:00; End: 20:00 | Start: 11:00; End: 20:00 |
| Day 5 | Start: 08:00; End: 20:00 | Start: 12:00; End: 20:00 |
| Day 6 | Start: 08:00; End: 20:00 | Start: 13:00; End: 20:00 |
| Day 7 | Start: 08:00; End: 20:00 | Start: 14:00; End: 20:00 |

☐ **Step 9** - Compare the bioluminescence each night by flipping both test tubes upside down next to each other. Do you see a difference? How much light do the Dinos need to maximize their brightness at night?



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