BLUE MOON™ BIOLUMINESCENT PRODUCTS

Bioluminescence (n) Light generated by living systems, a property of some animals, some fungi, and some protozoa.

Blue Moon (n) 1. The second of two full moons occurring within a single calendar month, 2. A Protein Solutions, Inc. (PSI) product line that incorporates *bioluminescence* into innovative science education products.

Protein Solutions' Blue Moon™ product line consists of packages of the bioluminescent marine organisms, *Pyrocystis lunula*, along with other items that will allow you to perform interesting explorations. (Use by children under the age of 12 requires supervision.) If maintained under appropriate, life-sustaining conditions, the organisms will survive in their sea water environment for 3-6 months without the addition of any external input other than light.

In order to enjoy them fully and make use of *P. lunula's* unique properties which invite scientific investigation, we've provided information about them and the principles they can be used to illustrate.

Galaxsea® - Bring home the exciting world of bioluminescence with tiny phytoplankton in a sealed ecosystem. This living product includes a beautiful color inset with information about bioluminescence and instructions on care. This product cannot be wrapped or covered for long periods of time. Galaxsea® must be exposed to normal room light for 8-14 hours every day, with temperatures in the range of 50-75° F.

Night-life® is Blue Moon Products' **Science in the Dark** education kit. It contains actual lab components - tissue culture flask with 30ml of nutrients, Petri dish, pipet, blotter paper and clear, easy-to-follow instructions. Recommended for ages 10-16 as well as for teachers and other adults and ideal for Science Fair projects, it contains a mail-in coupon for one *P. lunula* colony.

Lunula Nutrients solution is 30ml of ready-mixed medium in which you culture your *P. lunula* colony. 30ml is sufficient to culture one bag of colony.

Bioluminescent Colonies of *P. lunula* each contain 30ml. With a purchase of ten or more colonies you will receive additional information useful for class demonstration or discussion.

Colony with Dry Nutrient Concentrate is a RECYCLE/CULTURING KIT with which you can grow your own bioluminescent colony using a recycled clear soda bottle or translucent polyethylene milk container. Kit includes salt/nutrient packet to make your own sea water, starter colony and instructions.

Science Explorations with Bioluminescent Organisms consist of a variety of related explorations and are designed for Middle School students. They can be adapted to serve a younger age group if desired. Each exploration is designed for groups of 2 to 4 students, and will take roughly, half an hour to complete.

Our four kits are entitled *Microworlds, Salinity, Temperature, and Color of Light*.

Microworlds covers such topics as Observation; Operation of a microscope; Internal structure of single cells; Function of cellular structure; Life cycle of single-celled organisms and Photosynthesis at the cellular level.

Salinity addresses Solubility; Density; Buoyancy; Evaporation; Vapor pressure and Environmental influences on cell function and survival.

Global Warming introduces concepts such as Temperature; Heat and heat sources; Heat conduction, convection and radiation; World climate, Temperature dependence of chemical reactions and cell processes and the Effects of Temperature (climate changes) on the total Earth environment.

Color of Light is a whole spectrum of ideas exploring energy consumption capabilities of unicellular *Pyrocystis lunula.* This exploration touches on Waves - their elements and nature; Dark adaptation capabilities of the eye (humans and other species); Waves and the particle nature of light; Light absorption capabilities of plants and the Electromagnetic spectrum.

Blue Moon Products

a division of Protein Solutions, Inc.
Salt Lake City, Utah

Retail Price List/Order Form

PRODUCT	PRICE	QTY	TOTAL
Galaxsea® (30mL bag of *P. lunula* colony with Literature)	$9		
*Night Life® (Materials for science explorations with *P. lunula*)	$22		
Lunula Nutrients™ solution (30mL)	$4		
Lunula Nutrients™ solution and Flask (30mL)	$6		
Dry Nutrient Concentrate (enough to make 500mL of Nutrient solution)	$5		
Bioluminescent Colonies of *P. lunula* (30mL)			
Single Colony (bag)	$6		
10 or more Colonies (bags)	$5 each		
Colony with Dry Nutrient Concentrate	$10		
Science Explorations with Bioluminescent Organisms			
*Microworlds (Life Under the Microscope)	$28		
*Salinity (Salty Solutions for Enlightened Organisms)	$28		
*Temperature (Effects of Global Warming on Phytoplankton)	$28		
*Color of Light (A Whole Spectrum of Ideas)	$28		
"Glow in the dark" **Seastar** T-shirt (sizes Large or X-large)	$19		
		SUBTOTAL	
	SHIPPING&HANDLING:	$7 for 1st item $2 for each additional item	
		TOTAL	

*Coupon provided with product– redeemable for bag(s) of Colony

(We ship on Mondays and Tuesdays. Please plan for delivery later in the week.)

NAME: _____

ADDRESS: _____ **Note: We can not ship to P.O. boxes!!**

CITY:_____ STATE:_____ ZIP: _____

PHONE: ( ) _____

Special shipping instructions: Please ship during the week of _____.

Please make check payable to: Protein Solutions, Inc.
PO Box 58093
Salt Lake City, UT 84158-0093
Phone/FAX: (801)583-9301

Prices subject to change.
Please call for wholesale pricing.

rev. 8/95

Everything you ever wanted to know about *Pyrocystis lunula* ..... but were afraid to ask.

Enlightening information for maintaining healthy colonies of Blue Moon Products' Bioluminescent Organisms.

1) How much light do colonies of *Pyrocystis lunula* require?

They should have at least 6 hours of light a day in a bright, well-lit room. *P. lunulae* will tolerate several days of dim light, but they won't thrive or luminesce optimally unless they get 10-12 hours of bright light every day. In general, the health of a culture can be judged by how brightly it luminesces (the brighter the better).

Remember- the organisms require *light* photons, not heat, so be sure that you don't put them near a hot lamp or any other heat source (including direct sunlight) which would cause them to heat up. A cool fluorescent light source placed at least several feet away from the colony is ideal.

2) How often do my dinoflagellates need to be fed?

Cultures will live in their bags for a long time without feeding if the bag remains completely sealed, adequately lit, and is not exposed to temperature extremes. We've had cultures survive in their sealed plastic microenvironments for over 3 years, although the normal lifetime in the bags is 3 to 6 months. They will also live in a sterile open flask or bottle for a long time. To keep these experimental cultures healthy and bright they should be "fed" every 6-8 weeks. "Feeding" consists of replacing about 2/3 of the culture volume with Lunula Nutrients, a solution of salt, minerals and other nutrients described below.

The 2/3 of the culture that you remove can be safely discarded in your toilet - or, it can be used to start a new culture.

3) What Temperature Range do the Dinos prefer?

Here Dinos are very fussy. They don't tolerate high or low temperatures. It is best to keep them at temperatures in the range of 55°-75°F where they are happiest and will exhibit their bioluminescence. Remember..... they normally live in the temperate zones near the ocean surface. They are healthiest in environments that are similar to their native habitat.

4) Are there any Safety Concerns?

In addition to *P. lunula*, the culture may contain low concentrations of organisms such as algae and marine bacteria. These could be dangerous in high concentrations, although we've never known concentrations to reach toxic levels. Handle the organisms with care, and only under adult supervision. Avoid contacting the culture to your skin, and do not ingest.

If the cells splash on your clothing or contact your skin, wash with warm water and mild soap. Wash your hands after handling the culture.

Ingestion is unpleasant, similar to swallowing sea water. It should not have any health effects. Remember- the organisms aren't poisonous or pathogenic, and they only survive in salt water environments.

5) Can I change their night and day cycle?

P. lunula follow a circadian rhythm emitting light only during their *"dark"* phase. They can be induced to change their night and day cycle very easily. All you have to do is put the culture on whatever 24 hr. light/dark cycle you want, and after a day or two the cells will synchronize their activities to the new cycle. For brightest luminescence during *P. lunula's* night cycle, be sure that your culture gets roughly 12 hours of cool light a day. The rest of the time it should be placed in darkness or near darkness.

In our lab, the cultures receive light during our night, and dark during our day. This procedure allows us to experiment with organisms in their luminescent phase during daytime (working, classroom) hours.

6) *P. lunula* sex life

P. lunula reproduces by cell division. However, its reproductive cycle is intermediate between those processes seen in eukaryotic and prokaryotic cells. The traditional division phases of interphase, prophase, anaphase, metaphase, and telophase do not exist. Nonetheless, dividing cells are apparent and exhibit distinct phases of division.

The process of asexual reproduction is readily visualized while observing *P. lunulae*. Because mitosis in dinoflagellates is simple, they are good organisms for demonstrating this means of reproduction. Contrast their division with that of other organisms as an advanced assignment.

For further information, please call the BLUE MOON lightline: (801) 583-9301

PO Box 58093
Salt Lake City,UT 84158-0093

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I Saw The Light

(or ..... Fun Things to Do with and Know about *P. lunula*)

1) Okay, so what's in the Lunula Nutrients? (food for thought... and for the Dinos!)

Lunula Nutrients is a special medium that has been optimized for the growth of your dinoflagellate species, *P. lunula*. It contains dissolved salts, minerals, and nutrients in a concentration that is very close to those found in *P. lunula*'s natural sea water habitat.

2) Can I vary the Dinos' Diet?

If you live near an ocean you can feed your colony normal sea water. Or, you may wish to serve artificial sea water mixes from a marine aquarium supply store. You can buy these in fairly small quantities and prepare them by following the directions on the packages.

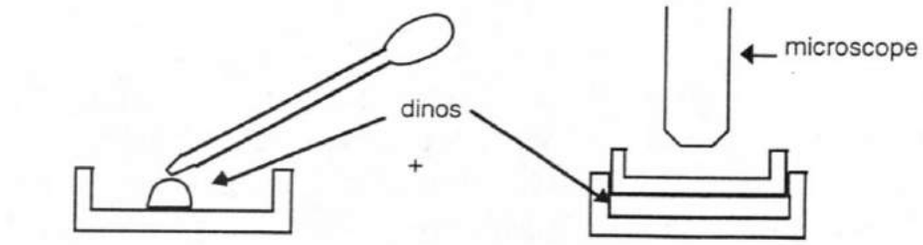
Because *P. lunulae* are similar to plants and live via photosynthesis, a conventional liquid indoor plant food (such as Miracle-Gro) can be used to supplement the minerals found in the artificial sea water. Use only a single drop of liquid plant food to a quart of the sea water medium. Do not add more than this, as excess nutrients can be toxic! This formulation has worked well for us, and should work equally well for you.

3) Can I see them under a microscope?

Yes! Dinos are very photogenic. You need about 100X total magnification. If your culture is contained in a sealed bag, let the culture settle to the bottom of the bag, mount the bag in such a way that it isn't disturbed, and focus on the orange/brown concentrated colony right through the bag. Although the polyethylene bag is a little rough and scratched, you can generally get a reasonable idea of the appearance of individual *P. lunula* cells. Most of them look like crescent moons, about 1/5 of a millimeter long.

If your culture is contained in a flask or other open container, you can use the pipet and petri dish provided with

your kit to prepare a sample of the concentrated suspension for observation. Draw some of the concentrated sample from the bottom of the container up into your pipet. Place a small drop of the cells on the petri dish as shown (left, below.)



Top of dish, upside down: now put bottom of dish, right side up, on top:

Then, carefully place the bottom of the petri dish on top of the upside-down top containing the Dinos (above, right). This smears the drop between the two dish parts and makes a great microscope sample. You can view this mounted sample of *P. lunula* under the microscope.

If you like, you can attach a home camcorder to the top of the microscope to record the Dinos in action. This will take a little patience and finesse, but it works. Trust us...You'll like it.

4) Are there other organisms like the Dinos?

There are over 2,000 species of dinoflagellates and most of them have never been studied by biologists or other scientists. They represent a relatively unknown scientific area.

We do know that some dinoflagellates secrete toxins, and that only a few are bioluminescent. We've selected *P. lunula* for science demonstrations and experiments because it's tough, non-toxic, and emits bright blue bioluminescent light. *P. lunulae* are found in the oceans .... especially in the temperate regions.

5) Why is the light blue?

Not all bioluminescence is blue. Some fireflies and worms produce green and yellow light. But almost all bioluminescence produced by organisms in the sea is blue. Scientists think that is because, under water, blue light travels a greater distance than

the other colors. Underwater bioluminescent organisms have evolved to be maximally efficient with their light emission.

6) Why do Dinos only light up at night?

Many bioluminescent organisms including *P. lunulae* follow circadian rhythms. They have a built-in "biological clock" telling them when it is night and day. These organisms are used by biologists to study this biological clock.

The biologic advantage afforded by light production isn't known, but is believed to be related to predator aversion. When a fish swims up to eat some of the bioluminescent dinoflagellates, it produces disturbances in the water (waves). The pressure changes and agitation produced by the waves trigger the bioluminescence, which some scientists believe serves to scare the fish away. By not lighting up during the day when their blue light cannot be seen, *P. lunulae* conserve their energy for nightly luminescence.

7) Where do dinoflagellates live?

There are thousands of different kinds of dinoflagellates. Some live in fresh water, but most live in salt water. Your bioluminescent dinos are found in salt water. They generally live near the surface of the ocean, within the top 30 meters (100 feet) or so. They are found in oceans all over the world, although certain species or types prefer specific regions. In some areas at particular times of the year, the conditions are just right for very efficient growth and multiplication of the organisms. Under these conditions, they are said to "bloom". Blooms sometimes result in concentrated populations over a large area, forming "red tides".

Dinoflagellates and their cousins the diatoms, which are not bioluminescent, play a very major role in the gas balance of the planet. By consuming carbon dioxide and producing oxygen, they help regulate the oxygen/carbon dioxide balance of our atmosphere. Scientists are now studying dinoflagellates to learn more about their possible role in minimizing the greenhouse effect.

8) What are "red tides?"

Red tides are high local concentrations of dinoflagellates or other microalgae that occur in nature when nutrient conditions, temperature, and other variables are optimal for a particular population of organisms to grow to large numbers. Sometimes the organisms are toxic. If this is the case, then the fish that eat this organism can concentrate the toxin in their own tissues and, when these fishes are eaten by other fishes, or by humans, the toxin is carried along with the food and can affect the consumer.

Secondary effects of red tides can cause rapid changes in the populations that inhabit the infested waters. Species that are unaffected by the red tide rapidly reproduce, and may consume nutrients and in other ways alter the conditions of their marine habitat. The habitat can be changed to the extent that the rapidly reproducing species may become dominant, forcing the previous inhabitants to seek a more hospitable location.

9) How old are the *P. lunula*?

That is really hard to say, because dinoflagellates generally multiply by mitosis or cell division. So, you could say that the first Dino is still alive. Dinoflagellates are primitive organisms that have probably been on the earth for hundreds of millions of years. They were on Earth before the dinosaurs arrived, and are still living now!

10) Is *P. lunula* a plant or an animal?

Most of the dinoflagellates are called animals by zoologists, and most of them are called plants by botanists. (If you're a carpenter, everything looks like a nail...) In the more modern biological classifications they are assigned to the kingdom Protista, which consists of single-cell organisms, many of which exhibit both animal- and plant-like characteristics.

Because *P. lunulae* are photosynthetic, we commonly refer to them as "plants".

11) Where are their flagella?

Dinoflagellate means "whirling tail" (from the Greek *dinos*, *flagellata*). Most dinoflagellates have two flagella, or tails, which they "wag" for transportation. Most move with a characteristic spiraling motion using a longitudinal flagellum that extends posteriorly (that is, a tail present at one end that extends away from the organism) and a transverse flagellum that encircles the cell at its midsection. Some dinoflagellates, like *Pyrocystis lunulae*, are uniflagellar (one flagellum). Not only that, but your dinoflagellate loses its only flagellum shortly after cell division. So much for the flagellum! It's a sad, short tail...

12) Why do they look red and brown in the light?

Because different phytoplankton have different types of chlorophyll, they appear as different colors. Examples are red algae and blue-green algae. Your *Pyrocystis lunulae* are a red algae; they reflect red light. Their red chlorophyll absorbs green and blue light.

13) Why do the cultures sometimes turn green?

If green algae begin to grow in the medium the culture will turn green. Green algae growth in the open flasks can be minimized by feeding and diluting your dinos on a regular basis.

The eventual dominance of green algae in your culture is a good example of what occurs in nature when environmental conditions change to favor a different organism or set of organisms.

Although dinoflagellates initially dominate the culture, they eventually change the culture environment. The new environment favors the growth of green algae, and they begin to dominate.

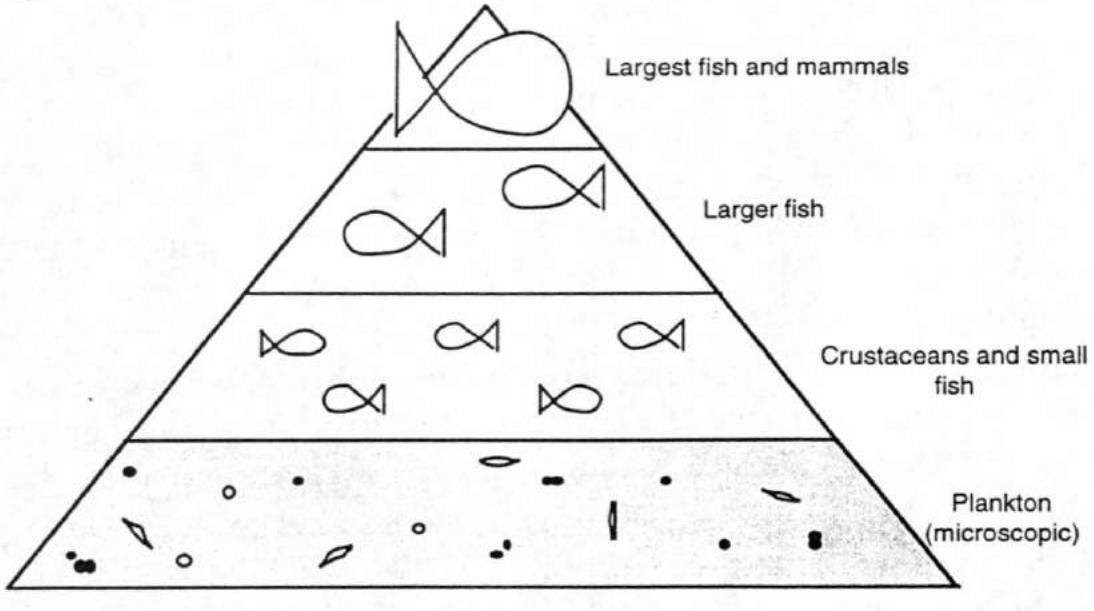
You can keep observing your culture even after it begins looking green. You will be able to see bioluminescence from the surviving dinoflagellates for a long time. You may want to follow the changes in your culture as the population shifts from dinos

to algae (Dino Dimming). However, if you are more interested in observing bright bioluminescence, then the best thing to do is to discard the culture completely and order a replacement.

To safely discard your organisms, flush them down the toilet. Dino Death. Lights Out!

14) Feeding the planet

Dinoflagellates and other phytoplankton are very small; they are also extremely important. They are important because they are the beginning of the food chain for most of the planet. They carry out the initial transfer of light energy to chemical energy by photosynthesis. Phytoplankton, like other plants, are able to form food from sunlight and carbon dioxide. This food - in this case, the phytoplankton themselves - is consumed by non-photosynthetic organisms (zooplankton, fish, etc.) Smaller fish are consumed by larger fish, and sometimes by humans. All of the consumers of food are directly dependent on the primary producers for their survival. So, phytoplankton may be small - but they are extremely valuable! All other organisms depend on the energy transfer of primary producers for their existence. Without these primary producers, we would have to learn how to convert light energy into stored chemical energy (food).



Ocean Food Pyramid

15) Phytoplankton as chemical sensors (Earth watch)

Phytoplankton grow best when their environment provides optimum conditions. Different types of plankton require slightly different conditions. Scientists can determine conditions in different parts of the ocean by looking at the type (color) of phytoplankton in a given area. Cell population distributions indicate where pollutants poison the ocean and prevent plant growth, and where changes in the climate occur to affect phytoplankton growth. Since phytoplankton require specific conditions for growth, they are a first indicator (an indicator species) of a change in their environment. You could say that they are the canaries of the ocean.

16) P. lunulae ... oxygen tanks.

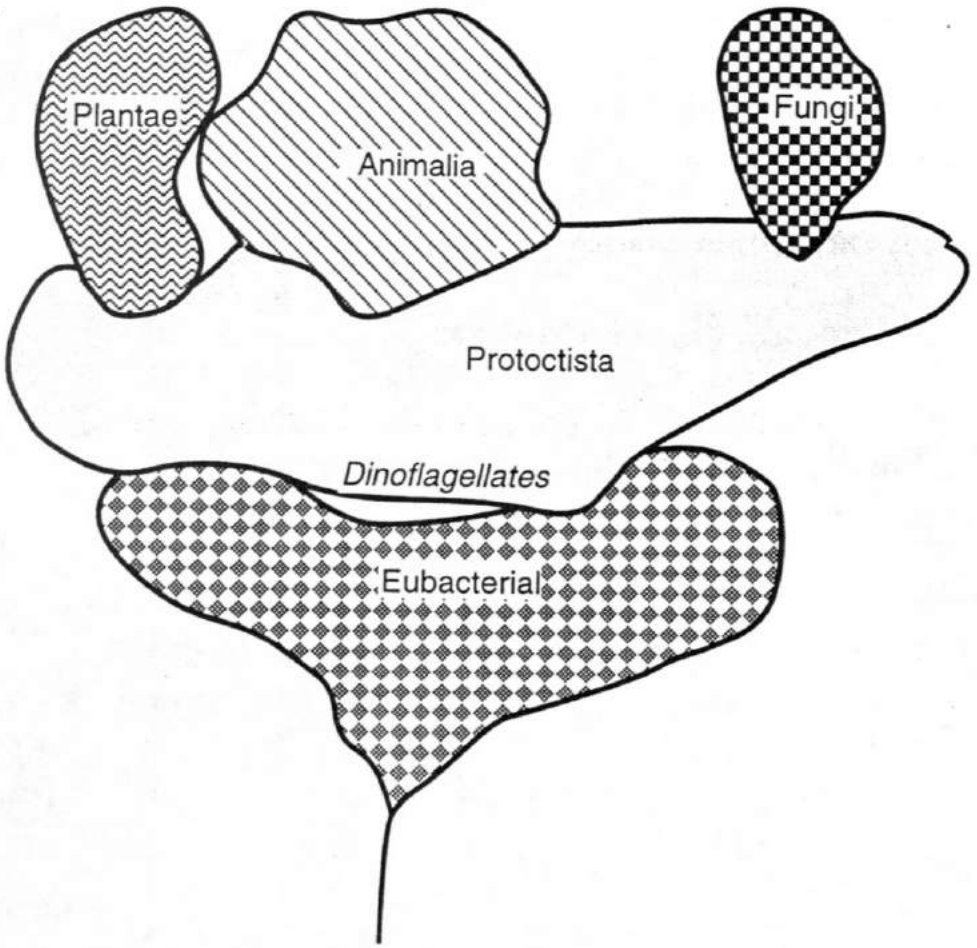
One consequence of the photosynthesis carried out by phytoplankton is the production of oxygen. The phytoplankton consume dissolved carbon dioxide in the ocean and give off oxygen. This process moderates the amount of carbon dioxide and oxygen in the atmosphere. If we had fewer phytoplankton our atmosphere would have more carbon dioxide and less oxygen. So, you see, phytoplankton like other plants, help provide us with oxygen. Their health is important to the health of our planet.

17) Where do we fit in?

The classification of dinoflagellates is a tricky business. Some species possess plant-like properties; others possess animal-like properties. Because dinoflagellates aren't plants and they aren't animals, scientists did what anyone would do.... they created a new kingdom for creatures like the dinoflagellates, called Protoctista (protozoa).

Your organism fits into the following classification (incomplete):

Kingdom	Protoctista
Phylum	Dinoflagellata (Greek *dinos*, whirling and *flagella*, tail)
Genus	Pyrocystis (*pyrrhos*, flame colored and *cyst*, cell)
Species	lunula (*lunula*, moon)



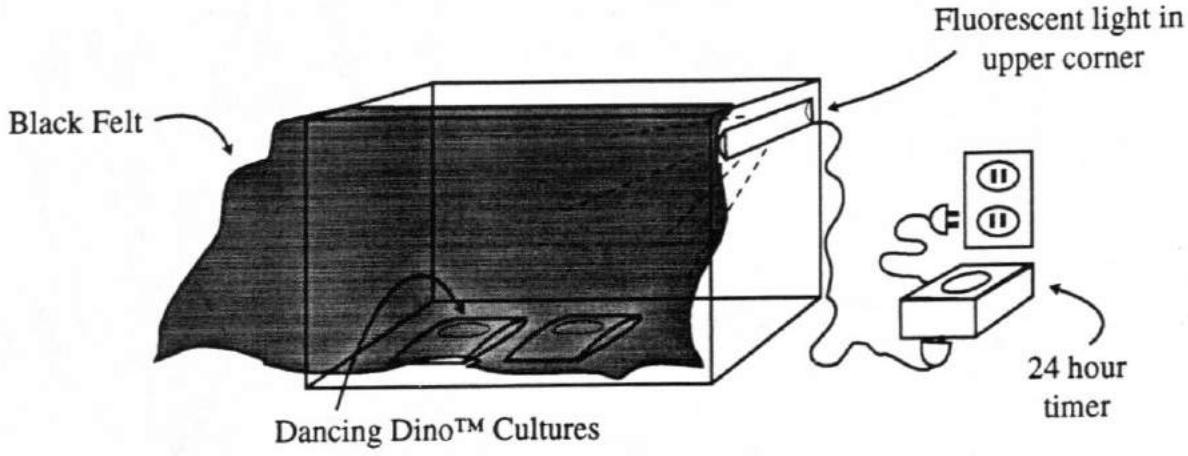
The Five Kingdoms of Life.

For further information, please call the BLUE MOON lightline: (801) 596-2675

NIGHT LAB™ KIT

Build your own culture area. Ideal for adjusting the Dinos for "daytime" luminescence. If you want, we will supply all materials; all you will need is a utility knife or scissors. If you prefer to use your own materials (available at hardware stores), here's what you do:

Collect Supplies:
- 1 yard of black felt
- Stapler
- Small (4-10 watt) fluorescent lamp
- 24 hour timer
- Extension cord
- White paint
- Empty paper box (approximately 1 X 1 X 2 feet)



Assembly:

1) Remove the box top and use pieces of it to seal any light holes in the box. The open top of the box is now the front of your Night Lab™ where you will access and view the Dinos.
2) Paint the inside of the box white to increase light efficiency.
3) Cut a hole in the side of the box near the top that is big enough for the lamp plug to fit through.
4) Cut enough felt to double cover the front of the box. Doubling is required to prevent room light from entering the Night Lab™.
5) Staple the felt to the box along the top side. Now your box is sealed from light except for the small hole you cut for the lamp plug.
6) Plug the lamp into the timer through the hole in the box. Keep the timer outside the box so you can easily adjust the light/dark schedule. Here you may need to use some extra felt to seal the plug hole.
7) Set the timer for your desired light cycle and plug it in the wall.
8) Place your cultures in the Night Lab™ and observe their luminescence by peeking under the felt cover during their dark cycle.
9) Check the box carefully to be sure it isn't getting warm. If it feels warm, your light is too large or your box is too well sealed. Leave the felt flap partially open to permit air circulation.
10) **Caution!** AC electricity is very dangerous! Be careful. Adult supervision required!

#800 Night Lab™ Kit, with construction materials and isntructions $50.00
Night Lab Instructions Only Free

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Protein Solutions, Inc. (PSI)
Science Education Innovators

DRY NUTRIENT CONCENTRATE

INSTRUCTIONS ON HOW TO RECYCLE CLEAR, CLEAN SODA OR POLYETHYLENE MILK BOTTLES AND MAKE YOUR OWN MINIATURE OCEAN

Materials Required:

Two empty soda or milk bottles (plastic)
500 mL (2 cups) of spring water, (available in grocery or drug stores)
Packet of Lunula Nutrients™ mix
Dino culture bag or other active *Pyrocystis lunula* culture

First obtain your clear soda or polyethylene milk bottles.

(Optional) You may want to remove the label and the base. Most labels and bases are attached by a heat-sensitive glue so you can use a hair-drier set at low or you can quarter fill the bottle with very warm water. Tilt the bottle until the label responds to the heat and can be peeled off, then stand the bottle upright until the base of the bottle can be twisted off.

Wash your bottles inside and out with detergent and warm water. Be sure to rinse at least 10 times to remove all traces of detergent.

Add the Lunula Nutrient™ mix to two cups (500mL) of spring water which should be at room temperature. Mix or shake until the powder is completely dissolved. This is your culture solution.

Add half of your culture solution to each bottle.

Cut one corner of the bag containing the *Pyrocystis lunula* Dinos and add them to one bottle.

Put a lid on the other bottle or cover it with aluminum foil, and save it in the refrigerator until you are ready to dilute your new colony, in about four weeks.

Keep the culture loosely covered by placing the lid on with only one half of a turn or by covering with aluminum foil. Swirl the bottle every day or two.

To dilute and expand your culture, pour all of the culture into the bottle of fresh nutrients and swirl gently. Pour half of the solution back into the original bottle. You now have two cultures and in about four weeks they will be ready to divide again.

If you want to keep the cultures optimally healthy, you'll need to continue to dilute and expand them every four weeks or so.

You can also order premixed Lunula Nutrients™ from PSI.

To discard your cultures, add about 1 ounce (20-30mL) of bleach, swirl gently, and carefully pour into a toilet.

Jul/95.RJS

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