Name: _____ MOLE BIO/BIOCHEMISTRY

MOLEBIO LAB #25: Fluorescent *E. coli* MM294 ART SHOW!

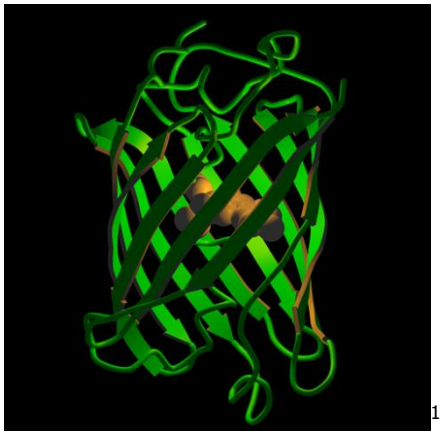
Introduction:

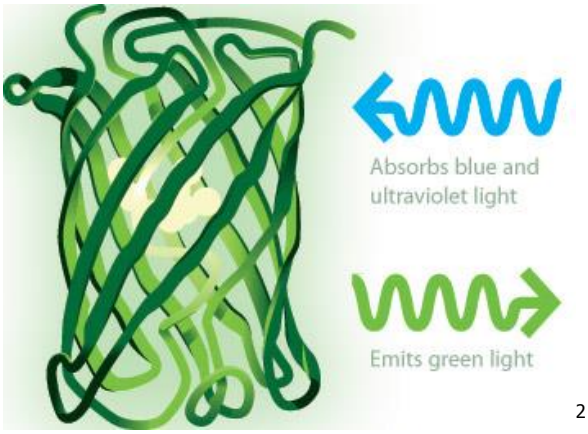
Green fluorescent protein (GFP) was the first fluorescent protein to be discovered and manipulated for use in biotechnology. The protein was actually isolated in Princeton's Department of Biology in 1961 by Osamu Shimomura, who won the 2008 Nobel Prize in Chemistry. Shimomoura and his colleagues isolated GFP from the jellyfish *Aequorea victoria*, whose outer edge glows green when the jellyfish is disturbed. The cells that make the GFP are called "photo-organs" and are located in the outer ring of the jellyfish.

The protein is made up of 238 amino acids and was crystallized in 1996. It turns out to have a very interesting barrel shape (sometimes referred to as a soda can). The barrel is made up of 11 beta sheets. The region of the protein that is responsible for the fluorescence is called the chromophore and is located in the middle of the barrel. The GFP chromophore consists of three adjacent amino acids (serine, tyrosine, and glycine at positions 65 – 67) which react to form a ring structure that absorbs blue light and emits fluorescent green light. The chromophore is attached to an alpha helix that runs through the cylinder of the barrel and cyclized forming an imidazolidone ring. You can watch an animation of this reaction at the following website:

http://www.olympusconfocal.com/java/fpfluorophores/index.html

Figure 1: Molecular Structure of GFP

 [1]



[2]

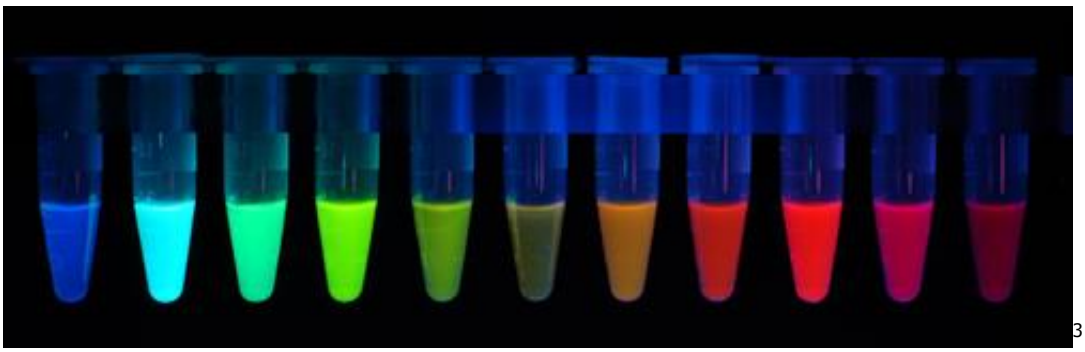
The gene for GFP protein was cloned in 1992 and shortly after was expressed in bacterial. Scientists quickly realized the GFP gene could be attached to just about any gene and the resulting protein (called a fusion protein) would be visible because it would glow green. This allowed scientists to visually detect when and where different proteins are made and to analyze the biological processes in which they are involved without having to disturb the cells. After the GFP gene was cloned scientists started looking for additional fluorescent proteins in other animals (mainly jellyfish and corals). This resulted in the identification of a wide variety of fluorescent proteins that can be used for biological imaging.

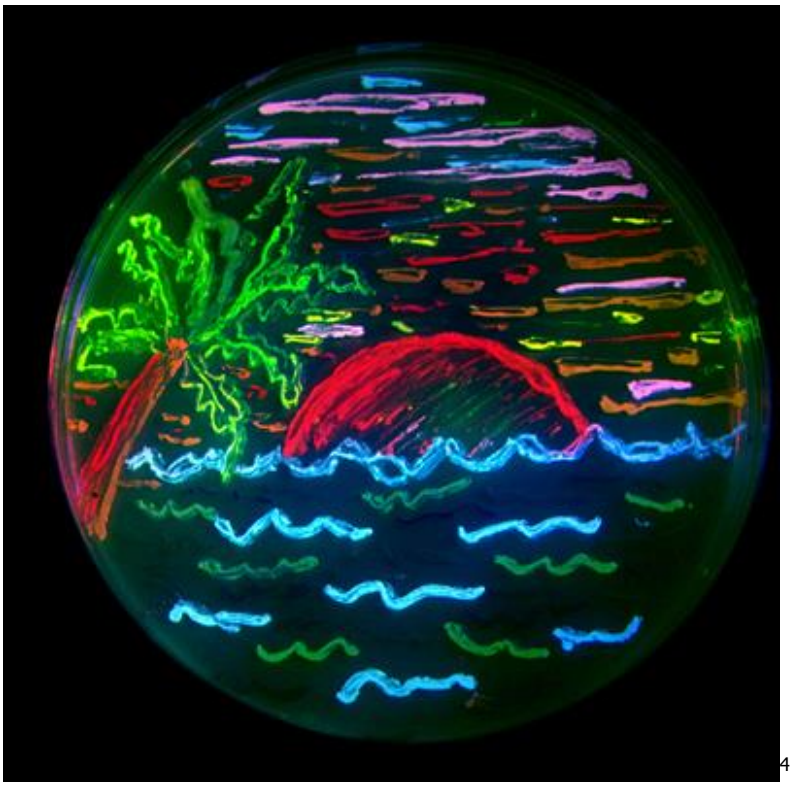
[1] http://www.nigms.nih.gov/news/results/nobel_chemistry20081008.htm

[2] http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2008/illpres.html

Roger Tsein, a neurobiologist at the University of California at San Diego who shared the 2008 Nobel prize for his work on fluorescent proteins. Tsien and his colleagues in addition to isolating fluorescent proteins from corals, created mutations in the genes for fluorescent proteins that allowed them to isolate an entire palate of colors that glowed brighter and longer than the original proteins. He playfully named them as part of an mFruit motif, m Plum, mCherry, m Strawberry, mTangerine, etc. Some of these fluorescent proteins are shown in **Figure 2** (unfortunately, without the dazzling color - sorry). His work has helped us understand molecularly how fluorescent proteins work.

Figure 2: Fluorescent Proteins

 [3]

 [4]

Micklos, David A. Freyer, Greg A. *DNA Science*. 2nd edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2003. 122-124; 385-398. Print.

BioBridge protocol; HHMI Summer Workshop, Department of Molecular Biology, Princeton University

[3] http://www.tsienlab.ucsd.edu/Images/General/IMAGE-%20Composite.jpg

[4] http://www.tsienlab.ucsd.edu/HTML/Images/IMAGE%20-%20PLATE%20-%20Beach.jpg

Procedure:

1. Label the bottom of an LB/amp plate with your class ID number.

2. Label a microtube tube with your class ID number.

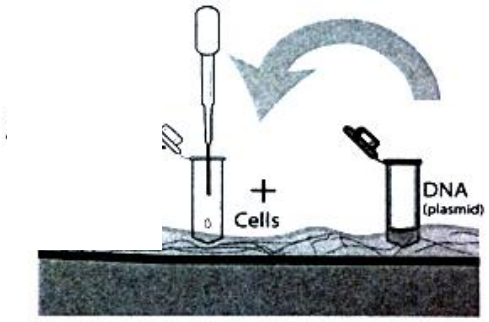
3. Add 500 μL of 50 mM $CaCl_2$, into this tube.

4. Set up an ice bath in a foam container. Place the tube in ice for at least 2 minutes.

5. Using a sterile loop/tip, gently scrape up 1 large (or 3 – 5 small colonies if there aren't single large colonies available) of MM294 from one of the LB agar **starter plates**. Transfer the bacteria to your labeled tube containing $CaCl_2$ solution.

6. Mix the contents of the tube by inverting the tube. The solution should look cloudy with no chunks. If the solution does not appear cloudy, add more colonies (check with Goldberg first).

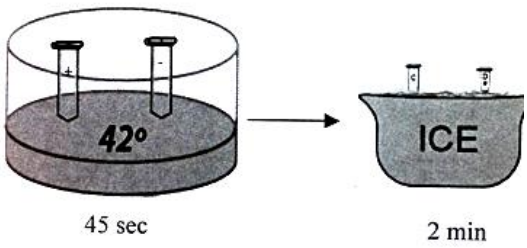
7. Using a sterile tip, add 10 μL of the plasmid solution you are using (PM1 or PM2) into your tube of $CaCl_2$. Discard the used tip, and tap the tube gently to mix.



8. Incubate the tube on ice for 10 minutes. Make sure the tube is immersed in the ice. Tap your tube gently to mix once or twice during this incubation.

9. While you are waiting, clean your work area: dispose of items that came in contact with bacteria in the red-striped waste containers.

10. Take your foam ice container with your tube still in the ice to the 42 °C water bath. Make sure the water bath is at 42 °C. Transfer the tube to the hot water for **<u>exactly</u> 45 seconds**. Make sure the tube are in the contact with the hot water. <u>Immediately after</u> return the tubes to the ice.



11. Incubate the tubes on ice for an additional 2 minutes.

12. Tap your tube gently to mix. Using a new sterile tip, transfer 150 μL of the cell mixture in your tube to your LB/amp plate. Discard the tip.

13. Using a sterile spreader or sterile glass beads (proper technique is required!) spread the cells on the plates.

14. Incubate your plates at 37 °C for 24 – 30 hours and then observe under long wave UV light.

15. The next day, label a new microtube with your class ID number.

16. Add 100 μL of fresh LB broth to this tube.

17. Using a sterile loop, pick up a 2 – 4 large fluorescent colonies **that have no signs of satellite colonies** and resuspend them in your new tube with the LB broth.

18. Spread this suspension (all 100 μL) onto a new LB/amp plate using a properly flamed and cooled spreader.

19. Incubate this plate at 37 °C for 24 – 30 hours and then use the new lawn as your bacterial paint!

Questions and Analysis:

1. What is fluorescence?

2. How does fluorescence differ from bioluminescence?

3. What is the purpose of using ampicillin on the plates?

4. What would you expect to observe if you did not include ampicillin in the plates?

5. Are any colonies more than one color? If so, why might this be?