MOLEBIO LAB #23: Transformation of *E. coli* with the Rainbow of BioBridge Fluorescent Plasmids

**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. Shimomura 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 photoorgans 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:


**Figure 1: Molecular Structure of GFP**

![Molecular Structure of GFP](image)

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.

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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**

![Fluorescent Proteins Image](http://www.tsienlab.ucsd.edu/Images/General/IMAGE-%20Composite.jpg)

![Fluorescent Proteins Image](http://www.tsienlab.ucsd.edu/HTML/Images/IMAGE-%20PLATE%20-%20Beach.jpg)
**Procedure:**
1. Label your two prepared agar plates (mark the bottom of the plate with your lab group number along with the description of the plate (LB/amp + and LB/amp -).

2. Label one 1.7 mL microfuge tube with a + and your lab group number.

3. Obtain a tube containing 500 µL of 50 mM CaCl₂, label it with a (−) and your lab group number.

4. Set up an ice bath in a foam container. Place both tubes 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 (or JM109 – or whatever E. coli type we are using) from the top of the LB agar class starter plate. Transfer the colonies to the tube of CaCl₂ 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. Place the tube on ice.

7. Transfer 250 µL from the (−) tube into the (+) tube.

8. Using a sterile tip, add 10 µL of the plasmid solution you are using (PM1 through PM6, depending on what was available) into the positive (+) tube with CaCl₂. BE SURE THAT YOU ONLY TRANSFER THE PLASMID TO THE (+) TUBE ONLY! Discard the used tip, and tap the tube gently to mix.

9. Incubate both tubes on ice for 10 minutes. Make sure the tubes are immersed in the ice. Tap your tubes gently to mix once or twice during this incubation.

10. While you are waiting, clean your work area: dispose of items that came in contact with bacteria in the red waste containers (bleach bins); dispose of 'clean' items that in the normal trash.
11. Take your foam ice container with your tubes still in the ice to the 42°C water bath. Make sure the water bath is at 42°C. Transfer the tubes to the hot water for **exactly 45 seconds**. Make sure the tubes are in the contact with the hot water. Immediately return the tubes to the ice.

12. Incubate the tubes on ice for 2 minutes.

13. Tap your tubes gently to mix. Using a new sterile tip, transfer 150 µL of the cell mixture from the negative (-) tube to the **LB/amp (-)** dish. Discard the tip.

14. With a sterile tip, transfer 150 µL from the positive (+) tube of the cell mixture to ONLY the **LB/amp (+)** plate. Discard the tip.

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

16. Incubate your plates at 37°C for 24-30 hours and observe under long wave UV light.
Questions and Analysis:
1. What is fluorescence?

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2. How does fluorescence differ from bioluminescence?

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

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

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5. Do you observe fluorescent colonies on both plates?

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

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Page 5 of 6
7. Transformation efficiency is expressed as the number of transformed bacteria per microgram of DNA used. Using the steps below (which are similar to the steps that we used in the previous lab), calculate the transformation efficiency of each of the different color plasmids that we used to transform *E. coli*. Show all of your calculations...

   a. The most direct way to determine the total number of transformed cells is to count the total number of green fluorescent colonies. (Each colony developed from one cell—so the number of colonies equal the number of cells)

   b. Determine the total mass of plasmid used. (You used 10 μL of PMx at a concentration of the 0.05 μg/μL.)

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   \text{Total Mass of PMx Used} = \text{volume} \times \text{concentration}
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   c. Calculate the total volume of the cell suspension prepared. (Hint: go back through the procedure and add up the total volume of all materials added with the cells before spreading them onto the agar plates.

   d. Now calculate the fraction of the total cell suspension that was actually spread on the plate.

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   \text{Fraction of Suspension} = \frac{\text{number of } \mu\text{L spread}}{\text{total volume prepared}}
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   e. Determine the mass of PMx actually spread on the agar plate.

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   \text{Total Mass of PMx Spread} = (\text{total mass of PMx used}) \times (\text{fraction of suspension})
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   f. Determine the number of colonies per microgram of plasmid. Express in scientific notation. This is the transformation efficiency.

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   \text{Transformation Efficiency} = \frac{\text{number of colonies observed}}{(\text{mass of PMx spread})}
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