BioPharmaceutical Technology Center
I N S T I T U T E

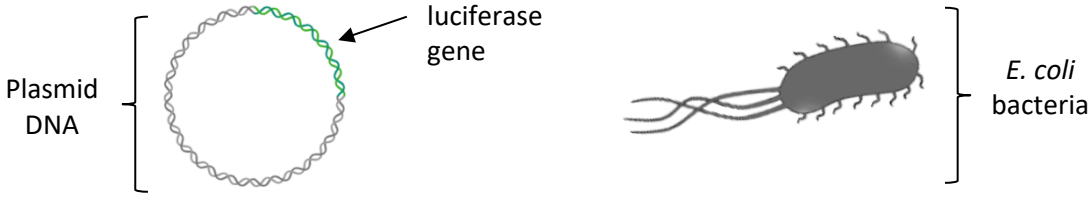
# Bioluminescence & Genetic Transformation

*Field Trip Background MS*

## Background Information

In the early 1970's Stanley Cohen made a discovery. He found that some bacteria contain small, circular pieces of DNA. He called these circular pieces of DNA "**plasmids**." Plasmids are copied in the bacteria and can be moved to different bacteria. Since the discovery of plasmids, molecular biologists have developed a variety of techniques to use plasmids. For example, they can add useful pieces of DNA called "**genes**" to plasmids. Then, they can transfer the plasmid into specific bacterial **hosts**. One method of transferring DNA into bacteria is known as a **genetic transformation**. This technique is a cornerstone for many modern advances in molecular biology.

In the bioluminescence field trip, the students simulate a genetic transformation. They will transform *E. coli* bacteria with a plasmid containing the click beetle **luciferase** gene. The luciferase gene is the DNA code which is used to produce, or express, the luciferase protein. This protein gives the click beetle the ability to glow. **Sterile techniques** and equipment will be discussed and used in this lab.




Plasmid DNA image via Biorender

E. coli image free for commercial use by Jomar Junior from Pixabay

## Genetic Transformation of Bacteria in the Laboratory

We use ***E. coli* strain JM109**. It does not make humans sick and is commonly used in molecular biology labs. The *E. coli* cells we use have been chemically treated. The treatment increases their ability to take up the plasmid DNA. After this chemical treatment, we refer to the bacterial cells as **competent cells**. A typical transformation follows the same steps that we use in this lab but requires longer incubation times (about 20 minutes on ice and 60 minutes at 37°C). The transformation of competent *E. coli* cells has a low transformation efficiency; typically, 1 transformation per 2-to-20 thousand *E. coli* cells. Because of the low efficiency of this technique and the longer incubation times, we give the students pre-transformed cells to ensure success.

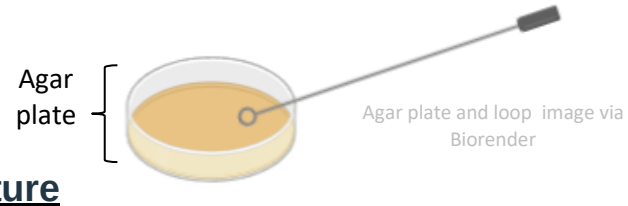
After combining the DNA and the bacterial cells, the mixture is incubated on ice. This allows the DNA to bind to the outer wall of the bacteria. In the lab, we allow about 5 minutes for this step, which usually takes 15-30 minutes. The bacterial cells are then heat shocked at 42°C for 45 seconds. This encourages the bacteria to take the DNA into the cell. The transformed cells are returned to the ice and given growth media, **LB broth**. This helps them recover and start growing. The cells recover for only 5 minutes at room temperature in our procedure.

*Supported in part by NSF/ATE grants:*
*DUE #9454555; 9752027*

*Revised January 2020*

## Antibiotic Selection

The plasmid we use also contains the **β-lactamase** gene. When the β-lactamase protein is expressed, the bacteria can counteract the lethal effects of **ampicillin**. Ampicillin is a potent **antibiotic**. Students will use a loop to put the transformed bacteria on **agar plates** containing **LB growth media with ampicillin**. By doing this, only the bacteria that have been transformed with the plasmid will grow. These bacteria are resistant to the antibiotic. Bacteria that do not contain the plasmid DNA will not grow on the ampicillin plates. In a typical transformation, the cells grow for about 1 hour at 37°C. This gives the bacteria time to make the β-lactamase protein so that they will be resistant to the ampicillin in the agar plates.

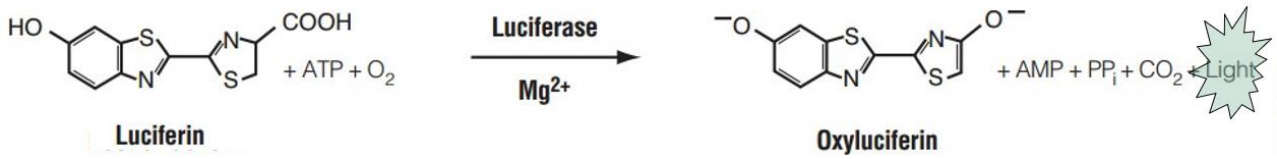



## Bacterial Culture

We incubate the plated bacteria overnight at **30-31°C** or at room temperature for 2-3 days. 30-31°C is the optimal temperature for the bacteria to make the luciferase protein. Students will plate with transformed bacteria that were prepared by a previous field trip group so they can see the results of the experiment the same day. (Your students' plates will provide the bacteria for a future field trip group.)

## Luciferase as an Enzyme

Bacterial cells expressing the luciferase protein will be added to a tube containing the **luciferase assay reagent** (LAR). LAR is a solution of **luciferin**, which is the luciferase substrate. The luciferase reaction also requires the energy of **adenosine triphosphate** (ATP). ATP is provided by the living bacterial cells. In addition, the reaction requires **oxygen**. This comes from the atmosphere by shaking the tube containing all of the other components.




In this lab the students will use three of the naturally-occurring **luciferase genes.** Each gene produces a different color of bioluminescent light: **green**, **yellow** and **orange**. The students will view the tubes in a dark room and should be able to easily distinguish the three colors.

If you have any questions or would like more information before you bring your students to the BTC Institute for the bioluminescence/genetic transformation field trip, please contact us. Alternatively, bring your questions along and we can discuss them during the lab. We look forward to seeing you and your group on your scheduled field trip day. Thank you for your interest in the BTC Institute's Biotechnology Field Trips program!

5445 East Cheryl Parkway
Madison, WI 53711
(608) 273-9737
Fax: (608) 273-6992
www.btci.org

*Supported in part by NSF/ATE grants: DUE #9454555; 9752027*

*Revised January 2020*