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

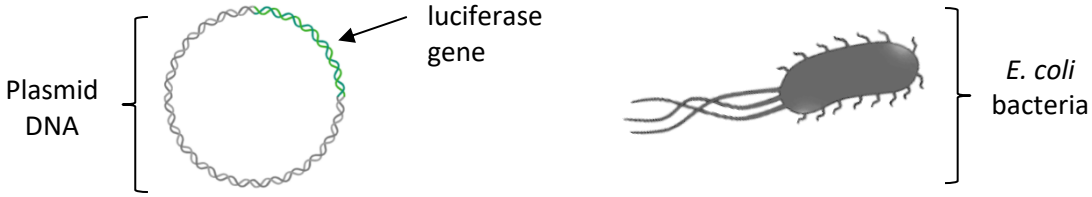
Bioluminescence & Genetic Transformation

Field Trip Background HS

Background Information

In the early 1970's, Stanley Cohen discovered that some bacteria contain small, circular pieces of DNA, which are copied and maintained in the bacteria and which can be transferred to different bacteria. These circular pieces of DNA are called **plasmids**. Since Dr. Cohen's original discovery of plasmids, molecular biologists have developed a variety of techniques to add useful genes to plasmids and to transfer the DNA into specific bacterial **hosts**. One common method of transferring DNA into bacteria is known as a **genetic transformation**. These relatively simple techniques of manipulating plasmid DNA and transferring it serve as a cornerstone for many modern advances in molecular biology.

In the bioluminescence field trip, the students simulate the genetic transformation of *E. coli* bacteria with a plasmid containing the click beetle **luciferase** gene. The luciferase gene is the DNA code that gives the click beetle the ability to make a protein that glows. **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**, which is not pathogenic to humans and is commonly used as a bacterial host in molecular biology labs. This transformation method uses *E. coli* cells that have been chemically treated to increase 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 process of transformation of competent *E. coli* cells has a low transformation efficiency; typically, 1 per 2-to-20 thousand. Because of the low efficiency of this technique and the relatively long 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 to allow 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 to encourage the bacteria to take the DNA into the cell. The transformed cells are returned to the ice and given growth media, **LB broth**, to help them recover and start growing. The cells are allowed to recover for only 5 minutes at room temperature in this step of our procedure.

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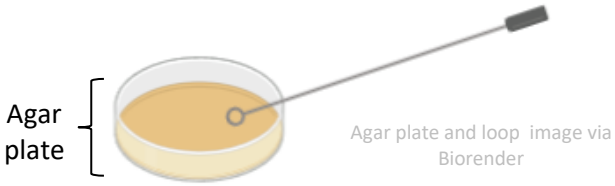
Antibiotic Selection

In addition to the luciferase gene and the necessary control elements, the plasmid that we use contains the **β-lactamase** gene. When expressed in bacteria, the β-lactamase protein allows the bacteria to counteract the normally lethal effects of **ampicillin**, which is a potent **antibiotic**. Students will plate the transformed bacteria on **agar plates** containing **LB media with ampicillin**. By doing this, they will select only the bacteria that have been transformed with the plasmid. Bacteria that do not contain the plasmid DNA will not grow on the ampicillin plates. In a typical transformation, the cells are allowed to grow for about 1 hour at 37°C to give the bacteria time to express the β-lactamase protein so that they will be resistant to the ampicillin in the agar plates.

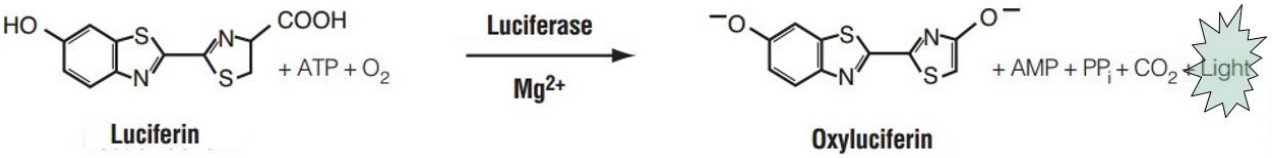


Bacterial Culture

We incubate the plated bacteria overnight at **30-31°C** or at room temperature for 2-3 days. The luciferase protein is less stable at 37°C (98°F), which is the optimal growth temperature for many laboratory bacterial strains. So that the students can see the results of the experiment the same day, they will scrape transformed bacteria from plates that were prepared by a previous field trip group. (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 **1mM luciferin**, which is the luciferase substrate, in **100mM sodium citrate, pH 5.5**. The luciferase reaction also requires the energy of **adenosine triphosphate** (ATP), which is provided by the living bacterial cells in this case, and **oxygen**, which is incorporated from the atmosphere by vigorously shaking the tube containing all of the other components.



In this lab the students will use three of the naturally-occurring **luciferase gene** variants, each of which produce 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!

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