# Genetic Engineering: Transforming Bacteria

## Introduction

### *Lesson Introduction*

In this laboratory experiment, students will transform the phenotype of bacteria through the use of genetic engineering. A non-pathogenic strain of *E.coli* bacteria is mixed with a plasmid that contains the Lux gene and a plasmid containing the GFP gene. The Lux gene is a Luciferase gene that has been isolated and purified from a glowing bacteria, *Vibrio fischeri*. The GFP gene is a Green Fluorescent Protein gene that has been isolated and purified from the bioluminescent (fluorescent) jellyfish, Aequoria victoria.

***Grade Level:*** 10-12
***Time Needed:*** Two days (Plus pre-lab prep)

## Learning Objectives

After completing this lesson, students will be able to:

1. Define the terms plasmid, gene, phenotype, and bacteria
2. Transform bacterial cells with a plasmid and observe the growth of the bacteria and the genetically engineered phenotypic traists
3. Describe how genetic engineering can impact agricultural products

## Materials

- ☐ Items needed per six groups, per class
- ☐ Qiagen Maxi-Prep purified plasmid DNA (pLux and pGFP) – six aliquots at 100ng/5ul
- ☐ Water (six aliquots)
- ☐ Competent E.Coli cells (3 x 100 ul. Aliquots per group) on ice
- ☐ LB/AMP (Luria Broth with Ampicillin) plates (3 per group – 1 for pLux, 1 for pGFP, and 1 for Control) {No DNA}
- ☐ 37 degree Celcius Incubator
- ☐ Long wave UV lights
- ☐ Straws
- ☐ LB (Luria Broth) – 1 ml. aliquots per group
- ☐ 10 – 100 ul. Capacity micropipettors

## Next Generation Science Standards (NGSS)

As a result of activities in grades 9 - 12, all students should develop:

***Topic***

- **LS3:** Inheritance & Variation

***Performance Expectation***

- **HS-LS3-3:** Apply Concepts of statistics and probability to explain the variation and distribution of expressed traits in a population

***Dimension***

**Practices:**

- Asking Questions
- Carrying out Investigations
- Analyzing & Interpreting Data
- Constructing Explanations
- Engaging in Argument from Evidence

**Disciplinary Core Ideas:**

- LS3.B: Variation of Traits

**Cross-Cutting Concepts:**

- Patterns

## Background

The basic concept behind genetic engineering is the process of removing a functional DNA fragment from one organism and integrating it with the genetic material of another organism to make the desired protein for which the DNA codes. Within the field of agriculture, many plants are genetically modified so they acquire the genes necessary to increase their resistance to pests or diseases, improve their uptake of nitrogen, increase crop yield, or improve their taste.

For example, the soybean commonly produced by farmers has a strong grassy flavor. Although this flavor is not a problem when it is used as animal feed, people find it objectionable when soybeans are used in food products. The strong flavor of the soybean is caused by the action of an enzyme called lipoxygenase. Lipoxygenase is an enzyme is that is involved in the oxidation of lipids, or fat. This oxidation is what causes the grassy flavor.

By using a variety of genetic techniques, including hybridization, mutation, and selection, researchers are able to manipulate the genes that control the enzyme, thereby eliminating the strong grassy taste. Soybeans are now available that lack the lipoxygenase enzymes. These soybeans are being used to produce soymilk and other food products that no longer have the strong flavor of common soybeans.

In this laboratory experiment, students will transform the phenotype of bacteria through the use of genetic engineering. A non-pathogenic strain of *E.coli* bacteria is mixed with a plasmid that contains the Lux gene and a plasmid containing the GFP gene. The Lux gene is a Luciferase gene that has been isolated and purified from a glowing bacteria, *Vibrio fischeri*. The GFP gene is a Green Fluorescent Protein gene that has been isolated and purified from the bioluminescent (fluorescent) jellyfish, Aequoria victoria.

Transformation occurs when a cell incorporates and expresses DNA, which has been added. When viable *E.coli* cells are successfully transformed with these two different plasmids, they acquire an additional trait. The E.coli cells transformed with the pLux will glow in the dark, whereas the *E.coli* cells transformed with the pGFP will fluoresce under a long wave UV light.

## Procedure

**Prior to lab prep these items:**

- CCMB Buffer Solution
- Viable bacterial cells
- LB Agar Plates
- Plasmids

*CCMB Buffer Solution*

1. Make a solution with the following materials:
    - 11.8g $CaCl_2$
    - 4.0g $MnCl_2$
    - 2.0g $MgCl_2$
    - 0.7g KCl
    - 100 ml glycerol
2. Adjust pH to 6.4 with HCl
3. Bring final volume to 1 liter with water
4. Filter sterilize

*Creating Competent Bacterial Cells*

Bacterial cells normally will not take up plasmid DNA unless conditioned to do so. The following procedure will be used to prepare competent cells:

1. Grow an overnight culture of the bacteria (E.coli) in 2 ml of SOB media. SOB Media can be prepared as follows:

    Measure out the following and bring to 1 Liter total volume with water.
    - 20 g bactotryptone
    - 5 g bacto-yeast extract
    - 0.5 g NaCl
    - 0.2 g KCl

    Adjust pH to 7.0 with 5 N NaOH

    Autoclave solution for 20-30 minutes.

2. Transfer this 2 ml. culture to a flask and add 125 ml SOB
3. Incubate at 37$^0$C for two hours with shaking
4. Place culture on ice for 10 minutes.
5. Centrifuge the culture to pellet the cells.
6. Remove the supernatant and re-suspend the cell pellet in 40 ml. cold CCMB buffer.
7. Incubate on ice for 20 minutes
8. Centrifuge the culture to pellet the cells.
9. Remove the supernatant and re-suspend the cell pellet in 10 ml. cold CCMB buffer.
10. Aliquot the cells into 100 ul. volumes and quick freeze in liquid nitrogen or dry-ice ethanol bath.
11. Store the cells at -80$^0$C.
12. The day that cells are to be used, thaw the cells on ice (for a minimum of one hour

*LB Agar Plates*

1. Bring the following materials to volume with 1 Liter of water.
   - 10 g bactotryptone
   - 5gbacto-yeast extract
   - 10gNaCl
2. Add 15 g bacto-agar.
3. Autoclave 20 -30 minutes to sterilize solution.
4. Once cooled to around 55$^{0}$C (warm to touch flask), add 1 ml of 100 mg/ml ampicillin (filter-sterilize).
5. Mix and pour into petri dishes (approx. 15 ml/dish).
6. Let petri dishes sit overnight to allow agar to harden.

*Plasmids*

1. Use Qiagen Maxi-Preps for plasmid purification.
2. Take the plasmid stock and dilute to 100ng./5ul.
3. Store plasmids at -20$^{0}$C or -80$^{0}$.

## Student Activities

Students will conduct the bacterial transformation as follows: (divide the class into lab groups)

1. Take each plasmid DNA and mix it with 100 ul. competent bacterial cells.
   Tube 1 = 100 ul. bacteria + pLUX (10 ul.; 100ng)
   Tube 2 = 100 ul bacteria + pGFP (10 ul; 100 ng)
2. Incubate on ice for 15 minutes.
3. Incubate samples at 42$^{0}$C for two minutes.
4. Add 100 ul. LB broth to each tube and mix.
5. Incubate at 37$^{0}$C for 10 minutes (hold tubes in clenched fist).
6. Label LB agar plates containing ampicillin appropriately.
7. Pipet the contents of the tubes onto the agar plates.
8. Fold a straw into a triangle shape and use it as a spreader to spread the bacterial over the plates.

***USE A NEW STRAW FOR EACH PLATE.***

9. Incubate the plates overnight at 35$^{0}$C and the next day at room temperature for pLUX and 35$^{0}$C for pGFP.
   With pLUX, the luciferase gene product is not stable at 37$^{0}$C and therefore, will not glow unless grown for a day at room temperature.
10. Observe the plates for bacterial growth and phenotype.
    For pLUX, turn off the room light and look for "glowing".
    For pGFP, turn off room lights and observe plates using a blacklight.

At the conclusion of the laboratory:

1 Describe to students how the transformation occurred, explaining in detail how the plasmid integrates with the bacteria.
2 Ask students to describe the phenotypic differences in their transformed bacteria.
3 Facilitate a discussion with students about the potential of biotechnology to transform and improve agricultural products. Cite specific examples. (Specific examples can be found throughout the e-learning modules and presentations within *GetBiotechSmart.com*).