# pVIB Bacterial Transformation- Pre-Lab

Introduction:
In this lab you will perform a procedure known as a genetic transformation. Remember that a gene is a piece of DNA which provides the instructions for making (coding for) a protein which gives an organism a particular trait. Genetic transformation literally means change caused by genes and it involves the insertion of a gene(s) into an organism in order to change the organism's trait(s). Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be genetically transformed into plants. In bio-remediation, bacteria can be genetically transformed with genes enabling them to digest oil spills. In medicine, diseases caused by defective genes are beginning to be treated by gene therapy; that is, by genetically transforming a sick person's cells with healthy copies of the gene involved in their disease.

The genetic system required for luminescence in the bacterium *Vibiro fischeri* is the lux operon. This operon contains a gene for luciferase (the enzyme that catalyzes the light-emitting reaction) and genes for enzymes which produce the luciferins (which are the substrates for the light-emitting reaction.). More specifically there are five genes within the lux operon (*LuxCDABE*) that have been identified as active in the emission of visible light, and two genes (*LuxR* and *LuxI*) that are involved in regulating the operon.
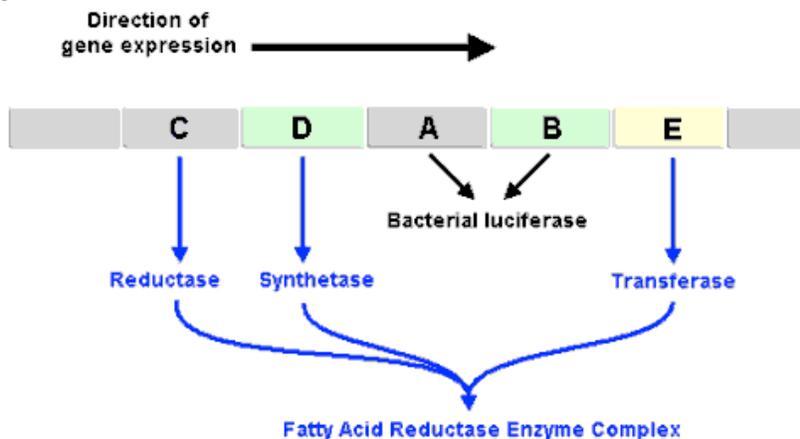

Luciferase
There are many types of luciferases depending upon the organism (ie bacteria, fireflies, fungi etc.). Bacterial luciferase is a heterodimer, composed of two different polypeptides, designated α and ß (of molecular mass 40 kDa and 37 kDa, respectively) and encoded by the *lux*A and *lux*B genes, respectively. The active site is located within the α subunit.
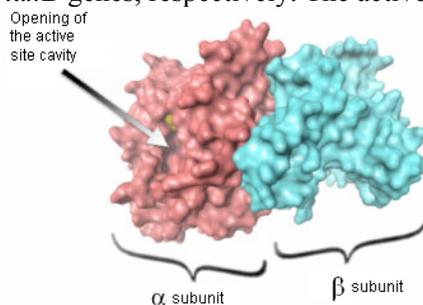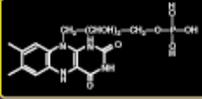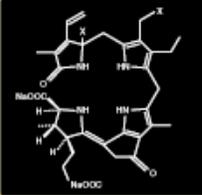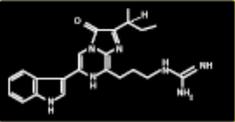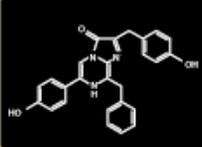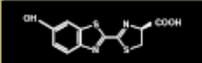

In the absence of the ß subunit, the α subunit alone functions inefficiently with a poor light yield. Evidence suggests extensive interactions and complex binding patterns between several side chains and backbone amides of the α and ß subunits, the function of the ß subunit has been assumed to act as a supporting scaffold assisting in the conformational change of the α subunit during the catalysis.
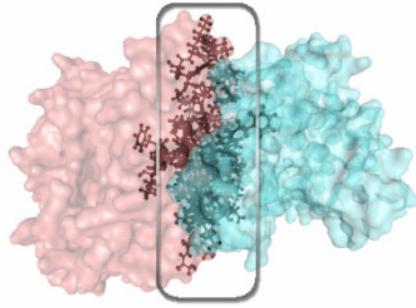
Luciferins

Luciferins (from the Latin *lucifer*, "light-bringer") are a class of light-emitting biological pigments found in organisms capable of bioluminescence. Luciferins are a class of small-molecule substrate each for their corresponding protein enzyme luciferase. Luciferins are oxidized in the presence of the enzyme luciferase to produce oxyluciferin and energy in the form of light. There are five general types of luciferins. Below is a list of the major luciferin types.

Objectives

In this activity, you will learn about the process of moving genes from one organism to another with the aid of a plasmid. In addition to one large chromosome, bacteria naturally contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for one or more traits that may be beneficial to bacterial survival. In nature, bacteria can transfer plasmids back and forth allowing them to share these beneficial genes. This natural mechanism allows bacteria to adapt to new environments. The recent occurrence of bacterial resistance to antibiotics is due to the transmission of plasmids. Below is the plasmid map for pVIB, the plasmid containing the *Lux* operon. Note that the plasmid also contains an ampicillin resistance gene as well as regulatory portions of the *Lux* operon.
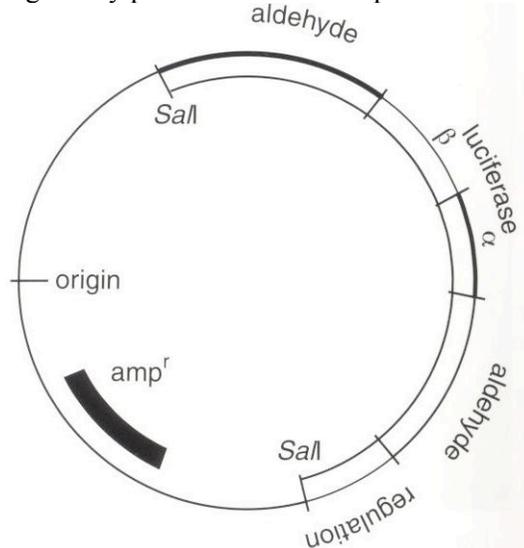
Note- LuxA and LuxB are labeled as Luxα and Luxβ

Transformed cells will glow in the dark as seen below.
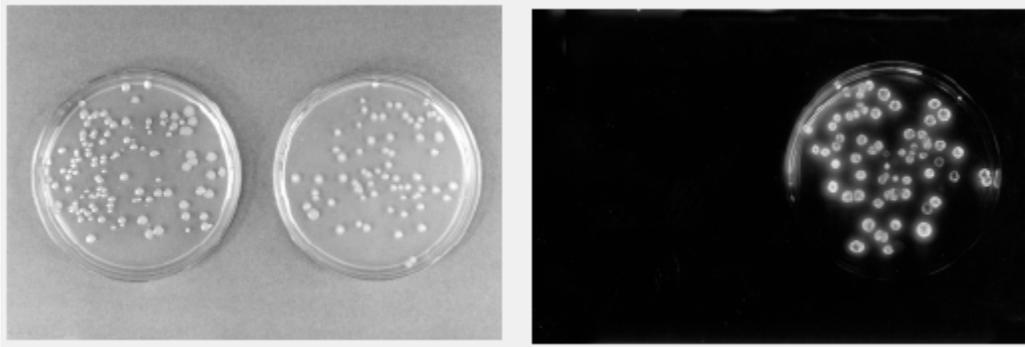
In both panels, control plasmid plate is to the left and pVIB plate is to the right. The right panel shows the plates photographed in light conditions. The photograph in the right panel was taken in total darkness using only the light emitted from the bacteria that were transformed with plasmid pVIB.

You will be provided with the tools and a protocol for performing genetic transformation.
Your task will be:
1. To do the genetic transformation.
2. To determine the degree of success in your efforts to genetically alter an organism.

Pre-Lab Considerations:

There are many considerations that need to be thought through in the process of planning a scientific laboratory investigation. Below are a few for you to ponder and answer as you take on the challenge of doing a genetic transformation. Since scientific laboratory investigations are designed to get information about a question, our first step might be to formulate a question for this investigation.

Consideration 1: Can I genetically transform an organism? Which organism?
1. To genetically transform an entire organism, you must insert the new gene(s) into every cell in the organism. Which organism is better suited for total genetic transformation-one composed of many cells, or one composed of a single cell? Why?

2. Scientists often want to know if the genetically transformed organism can pass its new traits on to its offspring and future generations. To get this information, which would be a better candidate for your investigation, an organism in which each new generation develops and reproduces quickly, or one which does this more slowly? Why?

3. Safety is another important consideration in choosing an experimental organism. What traits or characteristics should the organism have (or not have) to be sure it won't harm you or the environment? Why?

4. Based on the above considerations, which would be the best choice for a genetic transformation:
bacteria, earthworm, fish, or mouse? Describe your reasoning.

Consideration 2: How can I tell if cells have been genetically transformed?
Recall that the goal of genetic transformation is to change an organism's traits (phenotype). Before any change in the phenotype of an organism can be detected, a thorough examination of its natural (pre-transformation) phenotype must be made. Look at the colonies of *E. coli* on your starter plates. List all observable traits or characteristics that can be described:

The following pre-transformation observations of *E. coli* might provide base-line data to make reference to when attempting to determine if any genetic transformation has occurred.
a) Number of colonies
b) Size of:
        1) the largest colony
        2) the smallest colony
        3) the majority of colonies
c) Color of the colonies
d) Distribution of the colonies on the plate
e) Visible appearance when viewed with ultraviolet (UV) light
f) The ability of the cells to live and reproduce in the presence of an antibiotic such as ampicillin

1. Describe how you could use two LB/agar plates, some *E. coli* and some ampicillin to determine how *E. coli* cells are affected by ampicillin (an antibiotic).

2. What would you expect your experimental results to indicate about the effect ampicillin has on the *E. coli* cells?


Consideration 3: The Genes
Genetic transformation involves the insertion of some new DNA into the *E. coli* cells. In addition to one large chromosome, bacteria often contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for more than one trait. Scientists can use a process called genetic engineering to insert genes coding for new traits into a plasmid. In this case, the pVIB plasmid carries the operon (*Lux*) which produces the bioluminescent and a gene *(amp^r)* that codes for a protein that gives the bacteria resistance to an antibiotic.

1) Why is it important to have the ampicillin resistance gene in the same plasmid as the *Lux* operon? Explain your reasoning.

Consideration 4: The Act of Transformation

This transformation procedure involves three main steps. These steps are intended to introduce the plasmid DNA into the *E. coli* cells and provide an environment for the cells to express their newly acquired genes.
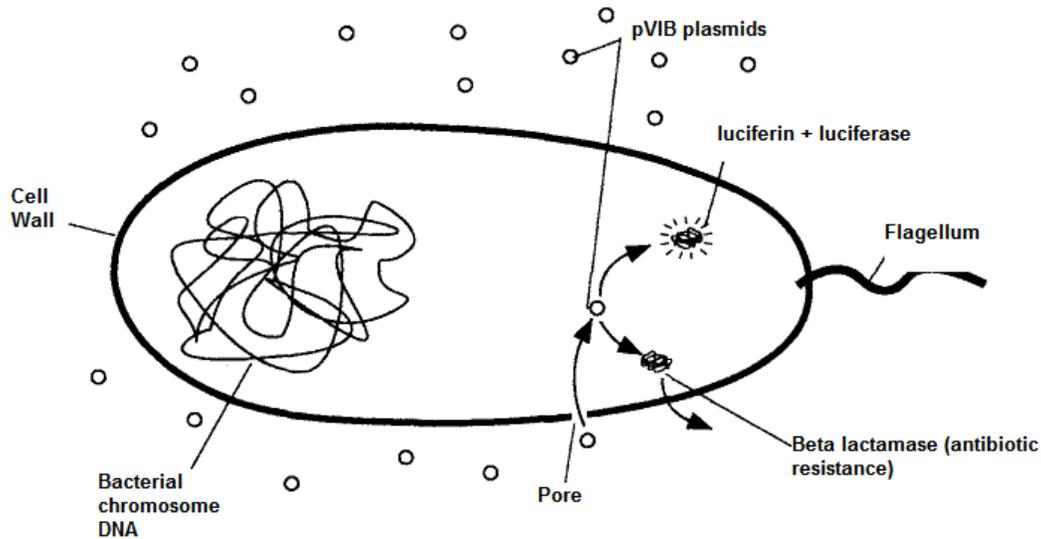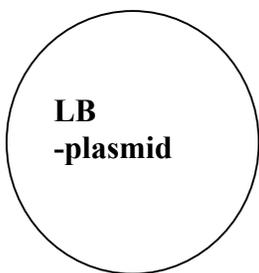

To move the plasmid DNA - pVIB through the cell membrane you will:

1. Use a transformation solution of CaCl2 (calcium chloride). Explain why?

2. Carry out a procedure referred to as heat shock. Explain what this does?

For transformed cells to grow in the presence of ampicillin you must:

3. Provide them with nutrients and a short incubation period to begin expressing their newly acquired genes. You will have four culture plates per group: one with just LB agar (the nutrient media) and non-transformed bacteria; one with LB agar and transformed bacteria; one with LB agar, ampicillin and non-transformed bacteria; and one with LB agar, ampicillin and transformed bacteria. Predict what you will see on each plate, give a reason for your prediction, designate if that plate is part of the control group or experimental group, and if it part of the control group identify if it is either the positive or negative control.
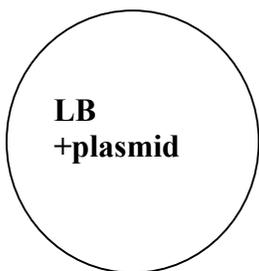
LB -plasmid

Prediction of Results Why?:

Reason:

Control or Experimental Group

If Control: Positive or Negative Control

LB +plasmid

Prediction of Results Why?:

Reason:

Control or Experimental Group

If Control: Positive or Negative Control

LB/amp -plasmid

Prediction of Results Why?:

Reason:

Control or Experimental Group

If Control: Positive or Negative Control

LB/amp +plasmid

Prediction of Results Why?:

Reason:

Control or Experimental Group

If Control: Positive or Negative Control