

Introduction to Genetic Engineering

Bacterial Transformation with Green Fluorescent Protein (pGLO)

Genetic engineering is an umbrella term that encompasses many different techniques for moving DNA between different organisms. **Transformation** the process by which an organism acquires and expresses a whole new gene. In this activity, you will have the opportunity to genetically transform bacteria cells; altering them so that they can make an entirely new protein. This procedure is used widely in biotechnology laboratories all over the world, enabling scientists to manipulate and study genes and proteins in exciting new ways.

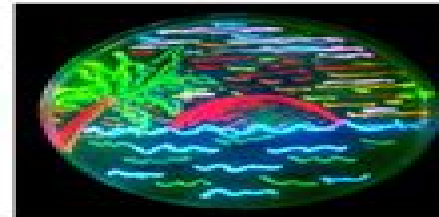
Adding a new gene to bacteria cells is a very simple procedure. You will add a gene that codes for Green Fluorescent Protein (GFP). This protein was discovered in the bioluminescent jellyfish called *Aequorea victoria*, a jellyfish that fluoresces and glows in the dark (Figure 1).

The gene for GFP was isolated in 1994 and was quickly used in laboratories as a way to brightly label proteins in a living cell. This "tagging" of proteins allowed researchers to visualize specific proteins to learn more about their biological functions in exciting new ways. The discovery of GFP proved to be so important that the Nobel Prize in Chemistry in was awarded to Osamu Shimomura, Marty Chalfie and Roger Tsien in 2008 for their work. Since then, Roger Tsien's laboratory at UCSD has altered the GFP gene to make a full rainbow of proteins. Figure 2 shows how bacterial expressing many different colored fluorescent proteins can be grown together on one plate.

Figure 1
Aequorea victoria
glowing under UV light



Figure 2
A rainbow of fluorescent
growing on an agar plate



Bacteria are commonly used for genetic transformation experiments because they are simple, single-celled organisms that grow and reproduce very quickly. Bacteria cells store their DNA on one large, circular chromosome. But they may also contain one or more small circular pieces of DNA called plasmids. Because bacteria reproduce asexually, plasmid DNA allows for the addition of new traits into a cell. Plasmids are able to replicate independently of the large bacterial chromosome, and can transfer easily between cells. Figure 3 shows the circular DNA chromosome and plasmid DNA inside of a cell.

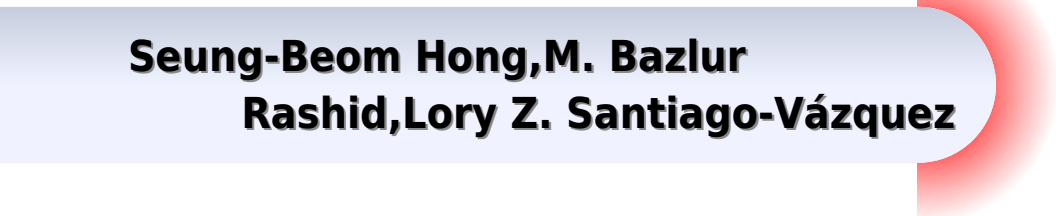
Figure 3
Genetic material in bacteria
takes 2 forms.



Bacterial evolution and adaptation in the wild often occur via plasmid transfers from one bacterium to another. An example of bacterial adaptation is resistance to antibiotics via the transmission of plasmids. This natural process can be modified by humans to increase our quality of life. In agriculture, genes are added to help plants survive difficult climatic conditions or damage from insects, and to increase their absorption of nutrients. Toxic chemical spills can often be bioremediated (cleaned-up) by transformed bacteria specifically engineered to do the task. Currently, many people with diabetes rely on insulin made from bacteria transformed with the human insulin gene. Scientists use transformation as a tool to study and manipulate genes all the time.

Pglo Transformation Lab Instructor Manual

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Rashid, Lory Z. Santiago-Vázquez**



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