# 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 oppositionity 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 Shinura, Marty Chalfie and Roger Tsien in 2006 of 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 splits 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**

William T. Smith, Jesse Hermon Wood

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#### **Table of Contents Pglo Transformation Lab Instructor Manual**

- 1. Understanding the eBook Pglo Transformation Lab Instructor Manual
  - The Rise of Digital Reading Pglo Transformation Lab Instructor Manual
  - Advantages of eBooks Over Traditional Books
- 2. Identifying Pglo Transformation Lab Instructor Manual
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
- 3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Pglo Transformation Lab Instructor Manual
  - User-Friendly Interface
- 4. Exploring eBook Recommendations from Pglo Transformation Lab Instructor Manual
  - Personalized Recommendations
  - Pglo Transformation Lab Instructor Manual User Reviews and Ratings
  - Pglo Transformation Lab Instructor Manual and Bestseller Lists
- 5. Accessing Pglo Transformation Lab Instructor Manual Free and Paid eBooks

- Pglo Transformation Lab Instructor Manual Public Domain eBooks
- Pglo Transformation Lab Instructor Manual eBook Subscription Services
- Pglo Transformation Lab Instructor Manual Budget-Friendly Options
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  - o ePub, PDF, MOBI, and More
  - Pglo Transformation Lab Instructor Manual Compatibility with Devices
  - Pglo Transformation Lab Instructor Manual Enhanced eBook Features
- 7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Pglo Transformation Lab Instructor Manual
  - Highlighting and Note-Taking Pglo Transformation Lab Instructor Manual
  - Interactive Elements Pglo Transformation Lab Instructor Manual
- 8. Staying Engaged with Pglo Transformation Lab Instructor Manual
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Pglo Transformation Lab Instructor Manual
- 9. Balancing eBooks and Physical Books Pglo Transformation Lab Instructor Manual
  - Benefits of a Digital Library
  - o Creating a Diverse Reading Collection Pglo Transformation Lab Instructor Manual
- 10. Overcoming Reading Challenges
  - Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
- 11. Cultivating a Reading Routine Pglo Transformation Lab Instructor Manual
  - Setting Reading Goals Pglo Transformation Lab Instructor Manual
  - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Pglo Transformation Lab Instructor Manual
  - Fact-Checking eBook Content of Pglo Transformation Lab Instructor Manual
  - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning
  - Utilizing eBooks for Skill Development

- Exploring Educational eBooks
- 14. Embracing eBook Trends
  - Integration of Multimedia Elements
  - Interactive and Gamified eBooks

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