

pGLO Bacterial Transformation

Objectives

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.

Introduction

In this lab you will perform a procedure known as genetic transformation. Remember that a gene is a piece of DNA which provides the instructions for making (codes for) a protein. This protein gives an organism a particular trait. Genetic transformation literally means change caused by genes, and involves the insertion of a gene into an organism in order to change the organism's trait. 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 bioremediation, 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 defective gene that causes the disease.

You will use a procedure to transform bacteria with a gene that codes for Green Fluorescent Protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*. Green Fluorescent Protein causes the jellyfish to fluoresce and glow in the dark. Following the transformation procedure, the bacteria express their newly acquired jellyfish gene and produce the fluorescent protein, which causes them to glow a brilliant green color under ultraviolet light.

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.

The pGLO plasmid encodes the gene for GFP and a gene for resistance to the antibiotic ampicillin. The pGLO plasmid has been constructed by modifying an existing plasmid that contains the arabinose operon. This operon allows bacteria to break down the sugar arabinose. The operon's structural genes have been replaced with the pGLO gene, but the regulatory regions have been left intact (see the diagrams below).



DNA Sequences in pGLO Plasmid

- | | |
|---------|--|
| • ori: | origin of replication |
| • araC: | gene for regulatory protein |
| • GFP: | gene for green fluorescent protein |
| • bla: | gene for beta-lactamase (breaks down ampicillin) |

Pglo Transformation Lab Instructor Manual

IM Harris



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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
- 6. Navigating Pglo Transformation Lab Instructor Manual eBook Formats
 - 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
 - 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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