

Student Manual

pGLO Transformation

Lesson 1 Introduction to Transformation

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.

Bio-Rad's unique pGLO plasmid encodes the gene for GFP and a gene for resistance to the antibiotic ampicillin. pGLO also incorporates a special gene regulation system, which can be used to control expression of the fluorescent protein in transformed cells. The gene for GFP can be switched on in transformed cells by adding the sugar arabinose to the cells' nutrient medium. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on ampicillin plates. Transformed cells will appear white (wild-type phenotype) on plates not containing arabinose, and fluorescent green under UV light when arabinose is included in the nutrient agar medium.

You will be provided with the tools and a protocol for performing genetic transformation. Your task will be to:

1. Do the genetic transformation.
2. Determine the degree of success in your efforts to genetically alter an organism.

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Table of Contents Pglo Transformation Lab Student Manual Answers

1. Understanding the eBook Pglo Transformation Lab Student Manual Answers
 - The Rise of Digital Reading Pglo Transformation Lab Student Manual Answers
 - Advantages of eBooks Over Traditional Books
2. Identifying Pglo Transformation Lab Student Manual Answers
 - 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 Student Manual Answers
 - User-Friendly Interface
4. Exploring eBook Recommendations from Pglo Transformation Lab Student Manual Answers
 - Personalized Recommendations
 - Pglo Transformation Lab Student Manual Answers User Reviews and Ratings
 - Pglo Transformation Lab Student Manual Answers and Bestseller Lists
5. Accessing Pglo Transformation Lab Student Manual Answers Free and Paid eBooks

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