

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 antibiotic plates. Transformed cells will appear white (wild-type phenotype) on plates not containing arabinose, and fluorescent green 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:

1. To do the genetic transformation.
2. To determine the degree of success in your efforts to genetically alter an organism.

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Calif Palo Alto



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Table of Contents Teacher Manual Pglo Transformation Answers

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

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