

Lab - Transformation of *E. coli* Bacteria with the pGLO plasmid

I. INTRODUCTION:

Green Fluorescent Protein is used extensively in biology research and work. It is one among several fluorescent proteins that allow DNA to be sequenced automatically by lasers. It is used quite frequently to label cells for studying how organisms develop as embryos and it is a key part of ongoing stem cell research. The protein itself does not glow but it can be made to fluoresce with a green light when exposed to ultraviolet (UV) light.

Bacteria grow well with the food source Luria Broth (LB). You can grow them either in liquid LB or on the surface of a petri dish with a mixture of LB and agar that solidifies into a thick gel. Antibiotics are sometimes also added to the agar to help control the growth of bacteria that are unwanted at the end of the experiment. Most antibiotics either kill the bacteria or keep them from reproducing. We will be using an antibiotic called ampicillin (amp).

Lab groups will prepare bacterial cells using calcium chloride to help the small plasmid circles of DNA get through the membrane of the cell. Then, you will get a plasmid into half of these cells by weakening the membrane further with heat shocks (cold, hot cold). A small percentage of the millions of bacteria in your sample should successfully take in plasmids.

Each plasmid contains a gene coding for green fluorescent protein (GFP), a gene coding for an enzyme (beta-lactamase) that breaks down the antibiotic ampicillin and a region that is like a lock or on/off switch for the green fluorescent protein gene. The on/off switch or lock is called an "inducible operon" that is usually in the "off" or locked position. The sugar arabinose (ara) is the key that opens the lock and turns on the GFP region of the plasmid.

You will put your transformation mixes (and your control sample) onto petri dishes to compare what happens with ones that have the plasmid to ones that have not received the plasmid. After the bacteria have had a chance to grow we will look at them under normal and ultraviolet light. It will take at least a day to show results.



Pre-lab Questions

1. What is a plasmid?
2. What is a gene?
3. What is luria broth (LB)?

Wards Transformation Of E Coli Lab Answers

F Rizvi



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Table of Contents Wards Transformation Of E Coli Lab Answers

1. Understanding the eBook Wards Transformation Of E Coli Lab Answers
 - The Rise of Digital Reading Wards Transformation Of E Coli Lab Answers
 - Advantages of eBooks Over Traditional Books
2. Identifying Wards Transformation Of E Coli Lab 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 Wards Transformation Of E Coli Lab Answers
 - User-Friendly Interface
4. Exploring eBook Recommendations from Wards Transformation Of E Coli Lab Answers
 - Personalized Recommendations
 - Wards Transformation Of E Coli Lab Answers User Reviews and Ratings
 - Wards Transformation Of E Coli Lab Answers and Bestseller Lists
5. Accessing Wards Transformation Of E Coli Lab Answers Free and Paid eBooks
 - Wards Transformation Of E Coli Lab Answers Public Domain eBooks
 - Wards Transformation Of E Coli Lab Answers eBook Subscription Services
 - Wards Transformation Of E Coli Lab Answers Budget-Friendly Options
6. Navigating Wards Transformation Of E Coli Lab Answers eBook Formats

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