Name _____ Class _____ Date_____

A Glowing Transformation

Inserting Useful Genes Into Bacteria

Question How can bacterial cells be genetically transformed with plasmid DNA containing a jellyfish gene?

Lab Overview In this investigation you will mix plasmid DNA containing the gene for green fluorescent protein (GFP) with *E. coli* bacteria. You will culture the bacteria and then check for "glowing" bacteria that have the GFP gene and produce the GFP protein.

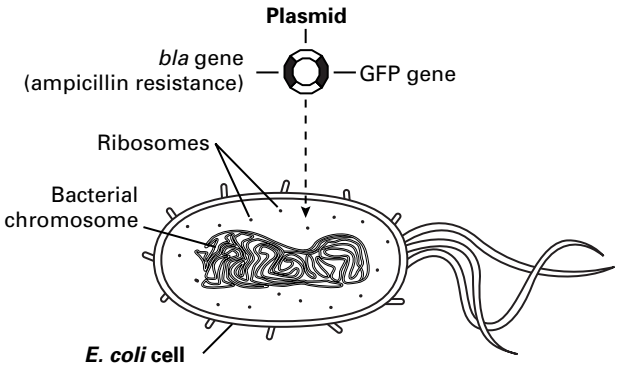
Introduction As you may recall from earlier chapters, bacteria are prokaryotes. Although prokaryotes do not undergo meiosis, they can undergo other processes that result in genetic mixing. For example, in transformation, bacteria pick up plasmids containing different genes from the environment. To start your investigation, you will explore what happens in a bacterial cell when it is genetically transformed. You will learn about the pGLO plasmid that you will use in the laboratory, which contains recombinant DNA. Using materials from the pGLO™ Bacterial Transformation Kit developed by Bio-Rad Laboratories, you will discover how this plasmid can be used to move jellyfish genes into bacterial cells, and find out how to select for transformed bacteria that express these jellyfish genes.

Background Small circular DNA molecules called plasmids occur naturally in many bacteria. Although plasmids come in different sizes, they are much smaller than the bacterial chromosome, and generally contain only a few genes. Plasmid DNA is replicated and expressed inside bacterial cells. Copies of a plasmid can also move from one bacterial cell to another.

Using restriction enzymes, biologists can "cut and paste" desired genes into a plasmid. The recombinant plasmid you will use, called pGLO, has been engineered with several different genes, including one from a bioluminescent (glowing) jellyfish. This gene codes for green fluorescent protein (GFP), a protein that glows a brilliant green color when exposed to ultraviolet (UV) light. The GFP gene is "switched on" in the presence of the sugar arabinose. When grown on agar containing arabinose, transformed bacteria that contain the pGLO plasmid make the GFP and appear bright green in UV light. When no arabinose is present, these bacteria appear white under UV light because GFP is not produced. The pGLO plasmid also carries a gene for producing beta-lactamase (*bla*), a protein that provides resistance to the antibiotic ampicillin. Bacterial cells that contain the pGLO plasmid produce beta-lactamase and can grow into colonies on agar plates containing ampicillin, whereas other bacterial cells would die.

You will move copies of the pGLO plasmid into bacterial cells through the process of genetic transformation. To begin, you will mix plasmid DNA with bacterial cells in a solution of calcium chloride ($CaCl_2$). You will then "shock" the bacteria by exposing them to heat. This treatment makes the cell walls of some of the bacteria permeable enough for the plasmid to enter the cells. After these steps, you will culture (grow) the bacteria on agar plates containing ampicillin and arabinose to select for transformed bacteria that contain the pGLO plasmid.

Prelab Activity Study the drawing that shows what happens in a bacterial cell during transformation. Afterward, answer the Prelab Questions.



Once the plasmid is within the bacterium, protein synthesis begins. Beta-lactamase is produced and provides resistance to ampicillin. If arabinose is present, then GFP is also produced.

Prelab Questions

1. Many different structures play a role in the bacterial transformation technique that you will carry out. Order the following items from smallest to largest: bacterial chromosome, plasmid, ribosome, bacterial colony, bacterial cell.

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2. The GFP gene comes from the jellyfish *Aequorea victoria*. Why do you think an *E. coli* bacterial cell can produce a protein from genetic information in jellyfish DNA?

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3. What is the significance of the *bla* gene? How does it allow you to select for transformed bacteria cells containing the pGLO plasmid?

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4. Will the colonies that grow on agar plates containing ampicillin also glow in the presence of arabinose? Explain your prediction.

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Materials

- hand-held UV lamp (one per class)
- two microcentrifuge tubes
- foam tube rack
- marker
- calcium chloride ($CaCl_2$) solution
- 5 sterile transfer pipettes
- plastic cup for biohazard waste containing 10% bleach solution
- crushed ice
- 2 foam cups
- starter agar plate with colonies of *E. coli*
- 6 sterile inoculating loops
- pGLO plasmid DNA
- hot and cold tap water
- thermometer
- LB nutrient broth
- 3 agar plates (2 with ampicillin, 1 with ampicillin and arabinose)
- clock or watch with second hand

IMPORTANT: You will be working with a non-disease-causing laboratory strain of bacteria called *E. coli* K-12. When working with these bacteria, however, it is important to use sterile techniques to avoid contaminating your culture with other bacteria. In general, anything that will come into direct contact with the *E. coli* bacteria, such as the tips of the inoculating loops and the transfer pipettes, should not touch any laboratory surfaces or your skin. Carefully follow all instructions regarding proper handling and disposal of materials.

Procedure

Part A: Transforming *E. coli* Cells With Plasmid DNA

1. Before starting the transformation procedure, observe the starter plate (*do not remove the petri dish lid*) with colonies of *E. coli*. Each bacterial colony appears as a small rounded growth on the agar surface. Shine the UV lamp on the colonies and observe them. **CAUTION:** *Avoid looking directly into the UV lamp or at reflected UV light for an extended time.* Then, shine the UV lamp on the tube of pGLO plasmid DNA to determine whether the DNA glows. Record your observations below.

   Bacteria: _____

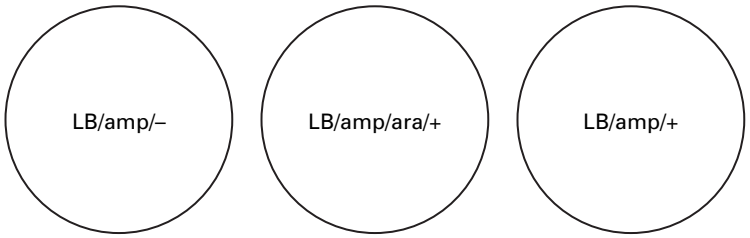
   pGLO plasmid DNA: _____

2. Label one of the closed microcentrifuge tubes with a plus (+) sign (for "with plasmid") and the other with a minus (−) sign (for "without plasmid"). Place both tubes in the foam tube rack.
3. Open the microcentrifuge tubes. Using a sterile transfer pipette, add two drops of calcium chloride ($CaCl_2$) solution to each tube.
4. Fill one foam cup with crushed ice. Then, place the rack with both tubes on ice.
5. Remove the lid from the *E. coli* starter plate. Using a new inoculating loop, gently scoop up one colony of bacteria and place it into the microcentrifuge tube labeled "+." Swirl the loop gently so that all the bacteria become suspended in the solution. Discard the loop in the plastic cup for biohazard waste. Using a new loop, scoop up a different bacterial colony and swirl it into the microcentrifuge tube labeled "−." Discard the second loop in the biohazard waste cup.
6. Use a new inoculating loop to obtain a small amount of pGLO plasmid DNA from its tube. To do this, dip the loop in the plasmid solution so that the loop fills with a thin film. Check to make sure that liquid is present inside the loop. Place the loop with the plasmid solution into the microcentrifuge tube labeled "+" and carefully mix the loopful of DNA into the suspension of *E. coli* cells. Do not add anything to the tube labeled with the minus sign (−). Close both tubes tightly. Place the used loop into the biohazard waste cup.
7. Keep the rack with both tubes on ice for 10 min.
8. Prepare a warm water bath for the heat shock step. Mix hot and cold tap water in the second foam cup until the water temperature is 42°C.

9. After the tubes have been on ice for 10 min, move the rack with both tubes into the 42°C water bath for 50 sec. Make sure the tubes are pushed all the way down into the rack so that they come in contact with the water. After 50 sec, put the rack and tubes back on the ice for 2 min.

10. Remove the rack from the ice. Using a new sterile transfer pipette, add three drops of LB nutrient broth to each tube. Then, allow the tubes to sit at room temperature for 10 min.

11. You will receive three agar plates labeled as in the figure below. Write the date and your initials on each plate. The label "LB" means that the agar was mixed with LB nutrient broth. The label "amp" means that the agar was mixed with ampicillin. The label "ara" means the agar was also mixed with arabinose. The "+" and "−" labels indicate which type of bacteria you will add to the plates—the bacteria with plasmids or the bacteria without plasmids.



12. Open the plate labeled "LB/amp/−." With a new transfer pipette, place two drops from the tube of untransformed cells (labeled "−") on the agar surface. Use a new loop to spread the liquid on the agar surface. (**NOTE:** *Do not dig the loop into the agar*.) Quickly cover the plate with its lid. Place the used pipette and loop into the biohazard waste cup.

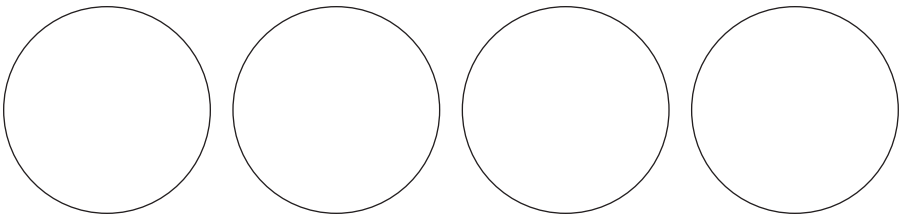
13. Open the plate labeled "LB/amp/ara/+." With a new transfer pipette, place two drops from the tube containing transformed cells (labeled "+") on the agar surface. Use a new inoculating loop to spread the liquid, then cover the plate. Place the used pipette and loop into the biohazard waste cup.

14. Open the plate labeled "LB/amp/+." With a new transfer pipette, place two drops from the tube of transformed cells (labeled "+") on the agar surface. Use a new inoculating loop to spread the liquid, then cover the plate. Place the used pipette and loop into the biohazard waste cup.

15. Incubate the plates upside down over the weekend at room temperature, or overnight at 37°C, as directed by your teacher. The *E. coli* starter plate should be left out at room temperature.

Part B: Observing Colonies of Transformed and Untransformed *E. Coli* Cells

1. Observe the colonies of *E. coli* cells on the three plates you prepared in steps 12–14 of Part A and those on your *E. coli* starter plate. In the space below, draw a sketch of each plate. Label each plate, and add notes describing what you observe.



2. Use the hand-held UV light to look for the presence of the fluorescent protein. In the space below, describe the appearance of the colonies on each plate under UV light. Record your observations in Data Table 1.

Data Table 1

Plate	Appearance Under UV Light

Analysis and Conclusions

1. What did you see when you observed *E. coli* bacteria under UV light in Step 1 of Part A? Was it what you expected? Explain.

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2. What did you see when you observed pGLO plasmid DNA under UV light in Step 1 of Part A? Was it what you expected? Explain.

3. Did bacteria that were not mixed with pGLO plasmid DNA grow on the agar plate containing ampicillin? Explain.

4. Did bacteria that were mixed with pGLO plasmid DNA grow on the plates containing ampicillin? Explain.

5. Do the colonies on both the + plasmid plates glow under UV light? Are the results what you expected? Why or why not?

Extension

Take on the role of a biotechnologist who wants to find commercial uses for green fluorescent protein. What useful applications might there be for a protein that glows? Think about ways that GFP might be used in industry, medicine, research, or in consumer goods. Then create a chart listing your ideas for possible uses and potential products.