NAME PERIOD DATE

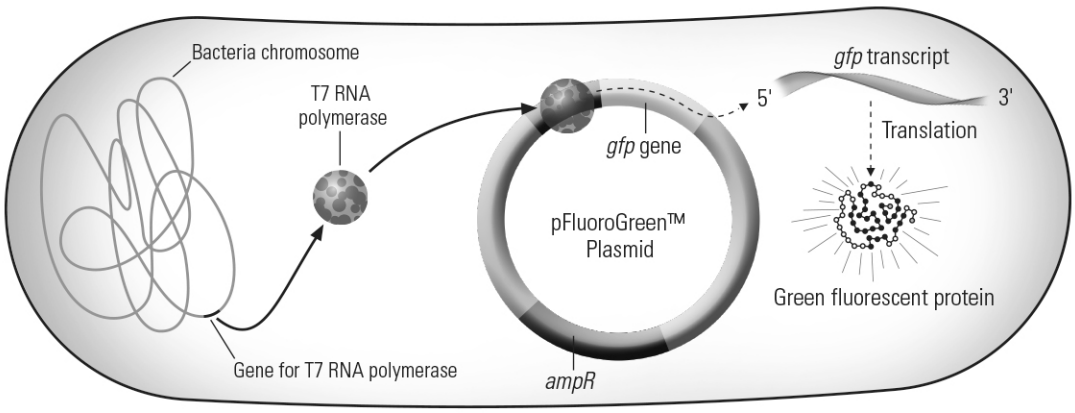
TRANSFORMATION

Background

Early in the 20th century there was great debate and uncertainty as to the identity of the molecule responsible for heredity. Some thought it was DNA, others thought it was protein. Experiments performed by Fred Griffith and Oswald Avery demonstrated that mixing cellular material, specifically DNA, from heat-killed pathogenic bacteria with nonpathogenic bacteria could transform the nonpathogenic type into the disease-causing type. This identified DNA as being the molecule responsible for heredity.

In nature, some bacteria can "transform" by taking in pieces of DNA from their environment. This is an important mechanism for maintaining genetic diversity in bacteria populations. Biologists have taken advantage of this characteristic of bacteria and use transformation in numerous biotechnology applications. For example, transformation is used to create bacteria that produce a protein of interest such as insulin. Transformation is also used to clone genes and make gene libraries (the library is "housed" in the genetically engineered organism).

This investigation provides you an opportunity to transform bacteria with the *gfp* gene for green fluorescent protein (GFP). This gene comes from the bioluminescent jellyfish, *Aquorea victoria*. If exposed to long-wave UV light, GFP emits a bright green light, a characteristic known as *fluorescence*.



Genetically engineered *E. coli* (elements not to scale proportionally)

The *gfp* gene is introduced into *E. coli* via a genetically engineered plasmid, pFluoroGreen. If the *gfp* gene is transcribed, the cell will produce GFP. As is commonly done in the biotechnology industry, this plasmid was engineered with a promoter sequence that helps ensure transcription of the gene. A special RNA polymerase, T7 RNA polymerase, recognizes this promoter and initiates transcription of *gfp*. The *E. coli* used in this investigation have been engineered to have the gene that codes for this polymerase in their chromosome.

Also on the plasmid is a *selectable marker*, an ampicillin resistance gene. This *ampR* gene codes for the enzyme β-lactamase, which destroys ampicillin. Culturing bacteria on agar that contains an antibiotic is a technique often used in industry to screen for bacteria that have been successfully transformed.

While research has led to the development of specific protocols for effective transformation, transformation is never 100% efficient. Not all bacteria cells exposed to plasmids will take in a plasmid. A transformation efficiency of 1×10^5 to 1×10^8 cells per microgram of plasmid DNA is expected in industrial applications.

Driving Question

How can foreign genes be added to bacteria? What is the efficiency of transformation, and do bacteria always express foreign genes?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- LB (Luria Broth) Petri plate
- LB/Amp Petri plate (2)
- LB/Amp/IPTG Petri plate
- Inoculating loops (2), sterile
- Transfer pipets (4), 1-mL, sterile
- Micropipet with a sterile tip
- Microcentrifuge tubes (2)
- Small cup or beaker, 100-mL, for ice
- Tube with 0.5 M Calcium chloride ($CaCl_2$), 1 mL[1]
- Tube with Recovery Broth, 1.5 mL
- Tube with pFluoroGreen™ (pGFP) plasmid, 12 µL[1]
- Toothpick, sterile
- Ice
- Permanent marker, fine
- Masking tape

[1]Keep these materials on ice.

For Class Use

- *E. coli* host cells (on 5 large Petri plates)
- Warm water baths (2), 37 °C and 42 °C
- Incubator (37 °C)
- Long wave UV light source
- Disinfectant

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

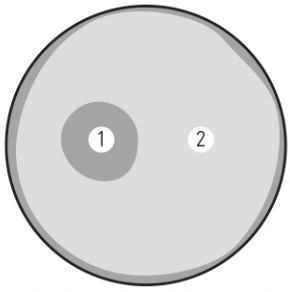
- Wear safety goggles at all times.
- Students with an allergy to antibiotics such as penicillin, ampicillin, kanamycin, or tetracycline should not participate in this experiment.
- Follow proper sterile technique while carrying out all procedures.
- Always wash hands thoroughly with soap and water after working with bacteria and use a 10% bleach solution to disinfect lab surfaces before and after the experiment.

  NOTE: wear gloves and goggles when working with bleach.
- Disinfect used materials (Petri plates, loops, pipets, and tubes) by placing them in an autoclavable, disposable bag and autoclaving at 121 °C for 20 minutes, or by soaking the materials in a 10% bleach solution overnight.
- Never look directly at a UV light. If available, wear UV safety goggles when using a long-wave UV light source.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

Before the transformation experiment



1 = Ampicillin / 2 = Distilled water

1. *E. coli* was grown on Luria Broth (LB) agar containing two paper discs. One disc was soaked in ampicillin and the other was soaked in distilled water before being placed on the surface of the agar. The dark area on the Petri plate indicates bacteria growth. What does the diagram suggest about the effect of ampicillin on *E. coli*? Explain your answer.

2. Some bacteria are resistant to antibiotics such as ampicillin. Bacteria that are sensitive to an antibiotic can become resistant if they acquire a resistance gene. The ability to survive in the presence of the antibiotic results from the gene coding for a beneficial protein, for example, for an enzyme that degrades the antibiotic. In nature, how might bacteria acquire new genes? Identify two or more possibilities and give a description of each.

3. For successful transformation, you will need to work efficiently. You will not have time to figure out the steps along the way. To prepare for a successful experiment, read the procedures of the investigation carefully and create a flow chart to guide you through the steps of the transformation procedure.

4. By introducing a plasmid into the cells you are altering the genotype of the bacteria. How will this affect the phenotype of the bacteria? In other words, how will you know if the transformation is successful?

5. Identify the purpose of each of the following:

   a. The labels "+" and "–" on the microcentrifuge tubes.

   b. Suspending cells in $CaCl_2$.

   c. Incubation of cells in 42 °C water, followed by incubation on ice.

   d. Culturing cells on an agar plate containing ampicillin ("LB/Amp").

Transformation experiment

6. Put on your safety goggles.

7. Add ice to half-fill the small beaker or cup and put the plasmid and calcium chloride tubes into it.

8. Use a sterile 1-mL pipet to transfer 0.5 mL (500 µL) of cold $CaCl_2$ solution to one of the microcentrifuge tubes. Place that tube into the ice.

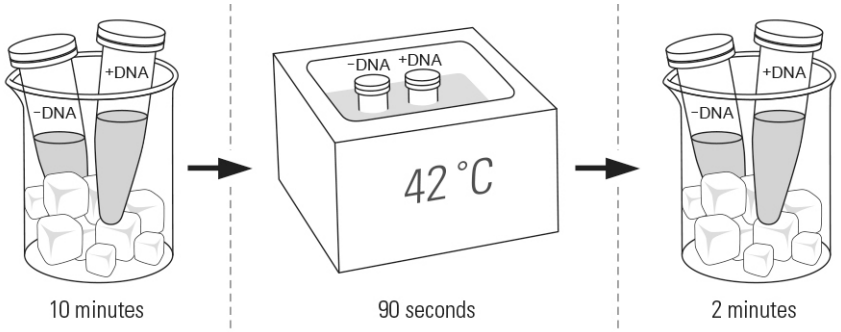
9. With a sterile toothpick, transfer the cells of 15 colonies from an *E. coli* source plate to the tube you just filled with the $CaCl_2$. Swirl the toothpick vigorously in the solution to dislodge the cells and flick the tube to fully suspend them. The suspension should look homogenous, without clumps.

10. Using the same pipet, transfer 0.25 mL (250 µL) of the cell suspension into a second microcentrifuge tube.

11. Use a permanent marker to mark one of the microcentrifuge tubes with the cell suspension with a "+" and the second one with a "–".

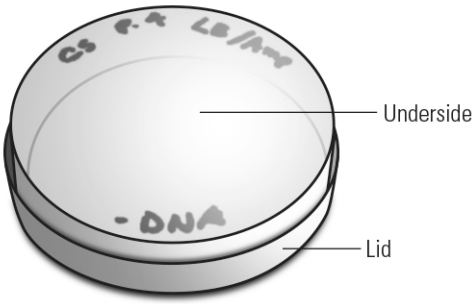
12. Place the "–" tube back in the ice. Use a micropipet with a sterile tip to add 10 µL of plasmid (pGFP) solution to the "+" tube and place it in ice. Leave both tubes in the ice for 10 minutes.

13. After the 10 minutes on ice, move the tubes to a 42 °C water bath for 90 seconds. Then return the tubes to ice for 2 minutes.



14. Use a fresh sterile pipet to add 250 µL of Recovery Broth to each of the tubes and mix them. Place the tubes in a 37 °C water bath for a 30-minute recovery period.

15. While you wait, label the four Petri plates with a permanent marker. Make all labels small and near the edge of the underside of the bottom of the plate (not on the removable lid) so as not to obscure the view of bacteria colonies that will form.



    a. Write your group's initials, class period, and the date on each plate.

    b. On the LB plate, write "–DNA".

    c. On one LB/Amp plate write "–DNA". Write "+DNA" on the second LB/Amp plate.

    d. On the LB/Amp/IPTG plate write "+DNA."

16. When the recovery period is over, bring the tubes back to your lab station. Use a sterile pipet to transfer 0.25 mL (250 µL) of cell suspension from the "–" tube to each of the two plates labeled "–DNA." Use a fresh sterile inoculating loop to spread the cell suspension over the entire plate (for both plates).

17. Use a fresh pipet to add 0.25 mL of cell suspension from the "+" tube to the plates labeled "+DNA" and use a fresh sterile inoculating loop to spread the cell suspension over the plates.

18. Leave the plates undisturbed for at least five minutes to allow the liquid to be absorbed into the agar in each plate.

? 19. On which plate(s) do you expect to observe growth of the transformed bacteria cells? Explain the reasoning for your prediction.

20. After the liquid has been absorbed, stack the set of plates and tape them together. Write your group's initials and class period on the tape. Invert the plates and place them in a 37 °C incubator for 16–20 hours.

21. Thoroughly disinfect your work space, place the used materials in the location designated by your teacher for disinfection and disposal, and wash your hands with soap.

Results of the transformation experiment

NOTE: Do NOT remove the lid of the Petri plates. Colonies are easily observed by keeping the plates inverted and viewing them through the bottom of the plate.

22. After the incubation period, obtain your stack of Petri plates. Record detailed observations and sketch the appearance of each of the plates.

23. Darken the room and use a long wave UV light to determine if any transformed colonies fluoresce, due to production of GFP. Record your observations.

? 24. Did you get any surprising or unexpected results? Explain your answer.

Data Analysis

1. Which plate has the greatest bacteria growth? Explain why this is the case.

2. Which plate has no bacteria growth? Explain why this is the case.

3. Control groups are an important part of good experimental design.

   a. Which plate or plates represent a control group for the transformation experiment? Explain your answer.

   b. Why are control groups essential?

Transformation efficiency

Transformation efficiency is an indicator of the success of the experiment and is obtained by determining the number of cells transformed per 1 µg of plasmid DNA.

4. To calculate the transformation efficiency:

   a. Begin by counting the number of colonies on the LB/Amp/IPTG plate. It may be helpful to mark counted colonies with a dry erase marker on the outside of the plate. Record the total number of colonies in your lab notebook.

   b. Use the colony count to calculate the transformation efficiency using the formula below and the accompanying information:

   Total DNA used: 0.050 µg

   Recovery volume: 0.50 mL

   Volume plated: 0.25 mL

$$\text{Transformation Efficiency} = \frac{\text{Number of transformants/plate}}{\mu\text{g DNA/plate}}$$

$$\text{where the number of } \mu\text{g DNA/plate} = \text{Total DNA } (\mu\text{g}) \times \frac{\text{Volume plated}}{\text{Recovery volume}}$$

5. In research laboratories, transformation efficiency ranges from 1×10^5 to 1×10^8 cells per microgram of plasmid DNA. How does the transformation efficiency of this investigation compare to that of a research laboratory?

6. How do the transformation efficiencies acquired by different student groups compare? What might account for differences in efficiency?

7. Count the colonies on the plate labeled LB/Amp (+DNA) and calculate the transformation efficiency. Record the data in your notebook. Is the efficiency similar between the two experimental group plates (LB/Amp and LB/Amp/IPTG)?

8. Identify any new questions that have arisen as a result of your research.

Synthesis Questions

The *E. coli* used in this experiment have been genetically engineered to be able to produce a special RNA polymerase, called *T7 RNA Polymerase*. The gene for this polymerase is under the control of the inducible promoter of the *lac* operon. IPTG is a substance that binds to the *lac* repressor, thereby inducing transcription of the T7 RNA polymerase gene. The pGFP plasmid used in the investigation was engineered to have a T7-specific promoter just upstream of the *gfp* gene. You may find it helpful to refer to the image in the Background section.

1. Think about how gene expression is regulated by an inducible promoter.

   a. What is the role of a repressor protein in gene regulation?

   b. What effect does IPTG in the growth medium have on the expression of the T7 RNA polymerase gene within the *E. coli* bacteria cells?

2. IPTG affects the expression of the *gfp* gene, and therefore affects whether *E. coli* colonies fluoresce. Why is the expression of the *gfp* gene dependent upon IPTG?

3. Explain how the transformation experiment demonstrates:

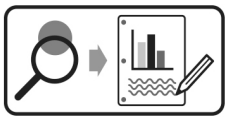
   a. the relationship between genotype and phenotype.

   b. the relationship between phenotype and the environment.

4. The *ampR* gene codes for a digestive enzyme that degrades ampicillin, thereby allowing *E. coli* cells to grow and reproduce in the presence of the antibiotic. Describe in detail the molecular processes involved in gene expression. That is, how does the DNA sequence of a gene result in the synthesis of a particular protein within cells?

Design and Conduct an Experiment

If your teacher determines there is sufficient time and materials for you to carry out an experiment of your own design, explore other aspects of transformation or antibiotic resistance.



Design and carry out your experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan.

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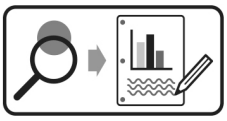
Design and Conduct an Experiment Worksheet

Explore other aspects of transformation or antibiotic resistance.



Develop and conduct your experiment using the following guide.

1. Create a driving question: develop a testable question for your experiment.

2. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

3. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

4. Write a testable hypothesis (If…then…).

5. What conditions will need to be held constant in the experiment? Quantify these values where possible.

6. How many trials will be run for each experimental group? Justify your choice.

7. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

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8. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

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9. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

10. Have your teacher approve your answers to these questions and your plan before beginning the experiment.