Big Idea 3

Genetics and Information Transfer

Baker's Dozen Lab 8: Bacterial Transformation

Objectives

Before doing this lab you should understand the principles of bacterial transformation, the conditions under which cells can be transformed, how a plasmid can be engineered to include a piece of foreign DNA, and how plasmid vectors are used to transfer genes.

After doing this lab you should be able to describe the biological process of transformation in bacteria, calculate transformation efficiency, and design a procedure to select positively for antibiotic-resistant transformed cells.

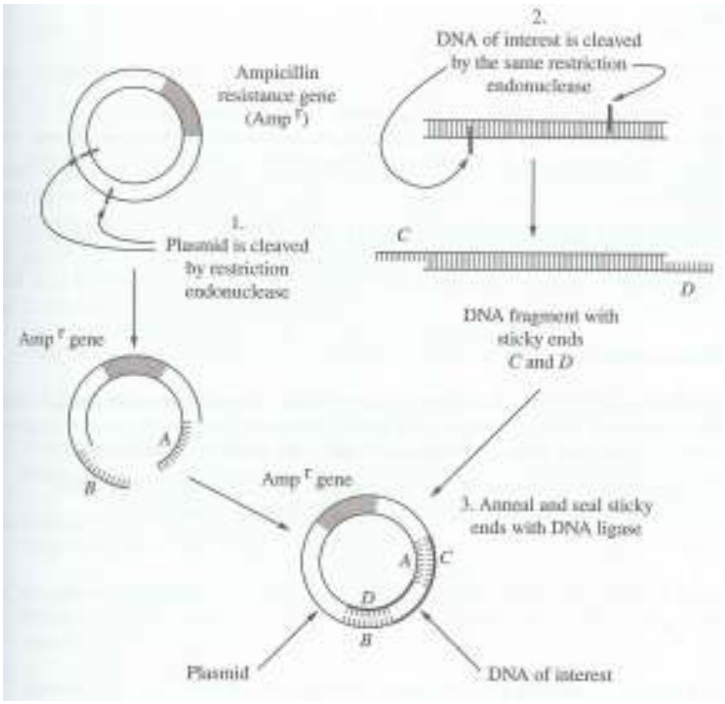
Introduction

The bacterium *Escherichia coli (E. coli)* is an ideal organism for molecular geneticists to manipulate. It has been used extensively in recombinant DNA research. It is a common inhabitant of the human colon and is easily grown in suspension culture in a nutrient medium such as Luria broth mixed with agar (LB agar) or nutrient agar.

The single circular chromosome of *E. coli* contains about five million DNA base pairs, only 1/600th the haploid amount of DNA in a human cell. *E. coli* cell may also contain small, circular DNA molecules (1,000 to 200,000 base pairs) called **plasmids**. The plasmids are extrachromosomal; they exist separately from the chromosome. Some plasmids replicate only when the bacterial chromosome replicates and exist only as single copies within the bacterial cell. Others replicate autonomously and occur in as many as 10 to 200 copies within a single bacterial cell. Certain plasmids, called R plasmids, carry genes for resistance to such antibiotics as ampicillin, kanamycin, or tetracycline.

In nature genes can be transferred between bacteria in three ways. **Conjugation** is a mating process during which genetic material is transferred from one bacterium to another of a different mating type. **Transduction** requires the presence of a virus to act as a **vector** (carrier) to transfer small pieces of DNA from one bacterium to another. **Bacterial transformation** involves transfer of genetic information into a cell by direct uptake of the DNA. During gene transfer, the uptake and expression of foreign DNA by a recipient bacterium can result in conferring a particular trait to a recipient lacking that trait. Transformation can occur naturally but the incidence is extremely low and is limited to relatively few bacterial strains. These bacteria can take up DNA only at the end of logarithmic growth when the cells are **competent**. Competence can be induced in *E. coli* with carefully controlled chemical growth conditions. Once competent, the cells are ready to accept DNA that is introduced from another source.

Plasmids can transfer genes that occur naturally, or they can act as vectors for introducing foreign DNA from other bacteria, plasmids, or even eukaryotes. In this lab, you will transform bacteria with two genes: ampicillin resistance gene (*bla*)and the **Green Fluorescent Protein** gene (*GFP*). The GFP gene comes from the bioluminescent jellyfish *Aequorea victoria*. These genes will be activated using the arabinose operon (*araC*). Restriction endonucleases areused to cut and insert pieces of foreign DNA into the plasmid vectors. These plasmid vectors can then be taken up by recipient bacteria, conferring the desired traits in those cells. This process requires four steps: 1) Create plasmid with gene of interest, 2) Transform recipient cells with plasmid DNA, 3) Plate recipients on ampicillin plates and select for resistant colonies, and 4) Isolate colonies carrying the plasmid.



Procedure:

1. Examine your starter plate and record your observations of the following characteristics:

   Number of colonies: _____

   Size of colonies: _____

   Distribution of colonies: _____

   Color of colonies: _____

   Visible appearance under a UV light: _____

2. Label the foam tube rack with the two labeled micro test tubes (+pGLO and –pGLO) with the names of your group members (or initials).
3. Open the two tubes and using a sterile transfer pipet, transfer 250 μl of transformation solution ($CaCl_2$).
4. Place the tubes back in the foam tube rack and place them on ice.
5. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the –pGLO tube.
6. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations here.

   _____

7. Immerse a new sterile loop into the plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring, similar to a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the +pGLO tube. Close the tube and return it to the rack on ice. Also close the –pGLO tube. Do NOT add plasmid DNA to the –pGLO tube.

   Why not? _____

8. Incubate the tubes on ices for 10 minutes. Make sure to push the tubes all the way down in the foam rack so the bottom of the tubes stick out and make contact with the ice.
9. While the tubes are sitting on ice, label your four agar plates on the bottom (NOT the lid) as follows: Label one LB/amp plate "+pGLO"; label the LB/amp/ara plate "+pGLO"; label the other LB/amp plate "-pGLO"; and label the LB plate "-pGLO."
10. Using the foam rack as a holder, transfer both the +pGLO and –pGLO tubes into the water bath (set at 42°C) for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the change from the ice (0°C) to 42°C and then back to the ice must be rapid. Incubate the tubes on ice for two minutes.
11. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250 μl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for ten minutes at room temperature.
12. Tap (or flick) the closed tubes with your finger to mix. Using a new sterile pipet for EACH tube, pipet 100 μl of the transformation and control suspensions onto the appropriate plates.

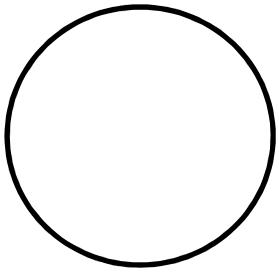
13. Using a NEW sterile loop for EACH plate, spread the suspensions evenly around the surface of the agar. Quickly skate the flat surface of the loop back and forth across the plate surface.
14. Stack up your plates and tape them together. Put your group name and class period on the bottom of the stack on the tape. Place the stack upside down in the 37°C incubator until the next day.

Data

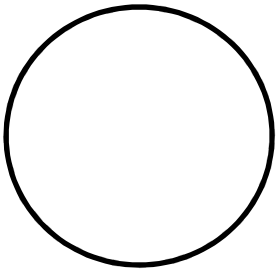
Observe the colonies through the bottom of the culture plate under normal room lighting. ***Do not open the plates!*** Then turn out the lights and hold the UV light over the plates. Draw what you see on each of the four plates in the correctly labeled circles below and on the next page. Record the following observations in the space provided to the right of each of your drawings: how much bacterial growth is present (individual colonies, a lawn, none at all), number of colonies (if present), and color of bacteria (under normal and UV light).

Transformation Plates Observations

+pGLO
LB/amp

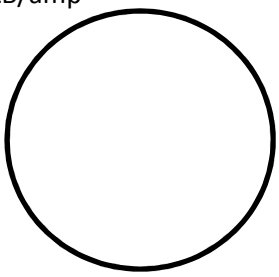


+pGLO
LB/amp/ara

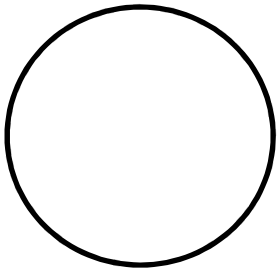


Control Plates Observations

-pGLO
LB/amp



-pGLO
LB



Analysis of Results

1. Identify two traits you originally observed for *E. coli* that were not altered. How do you know they stayed the same?

_____

_____

2. Identify two changes you observe in the new *E. coli* cells different from the original starter plate cells.

_____

_____

3. Based on your knowledge of DNA and proteins, what is the source of the fluorescence in the transformed bacteria?

_____

_____

4. What two factors must be present in the bacteria's environment for you to see the green color? (Hint: one factor is in the plate and the other is in how you look at them).

_____

_____

5. What advantage would there be for an organism to turn on or off particular genes in response to certain conditions?

_____

_____

_____

Transformation efficiency is expressed as the number of antibiotic-resistant colonies per microgram of pGLO. Because transformation is limited to only those cells that are competent, increasing the amount of plasmid used does not necessarily increase the probability that a cell will be transformed. A sample of competent cells is usually saturated with small amounts of plasmid, and excess DNA may actually interfere with the transformation process.

a. Determine the total mass of pGLO used. _____
(You used 10 μl of pGLO at a concentration of 0.005 μg/μl.) mass = volume x concentration

b. Calculate the total volume of cell suspension prepared. _____
(Volume of transformation solution + volume of pGLO + volume of LB-Broth)

c. Calculate the fraction of the total cell suspension that was spread on the plate. _____
(Number of μl spread/total volume)

d. Determine the mass of pGLO spread on plates. _____
(Total mass of pGLO x fraction spread)

e. Determine the number of colonies per μg of plasmid. Express in scientific notation. _____
(Number of colonies observed/mass pGLO spread = transformation efficiency)

6. Identify three factors that might influence transformation efficiency. Explain how each factor could affect the transformation efficiency of your experiment.

_____

_____

_____

_____

_____