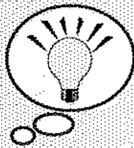

DID YOU KNOW?
According to the U.S. Environmental Protection Agency, the presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination.

OBJECTIVES

- Investigate transformation as a mechanism of genetic exchange
- Create competent cells by chemically and thermally treating *E. coli* cells
- Insert a plasmid containing antibiotic-resistance genes into competent *E. coli* cells
- Screen the transformed cells to determine which have been genetically altered
- Calculate the efficiency of the transformation reaction

MATERIALS

MATERIALS NEEDED PER GROUP

2 Luria agar plates
2 Luria agar plates with ampicillin
2 Microcentrifuge tubes
1 Inoculating loop
2 Bacti-spreader
4 Sterile graduated pipets
1 Rubber pipet bulb
1 Capillary micropipet

SHARED MATERIALS

Calcium chloride
Luria broth
Plasmid pUC8
Waterbath
Starter plate of *E. coli*

Figure 1

PROCEDURE

Prior to conducting the experiment, make sure all materials are present and ready to use. A 42°C waterbath should be available and the calcium chloride should be in an ice bath and kept cold throughout the experiment.

1. Obtain two microcentrifuge tubes and mark one tube "+", the other "–". The "+" tube will have the plasmid added to it.

2. Using a sterile pipet, add 0.25 ml (250 µl) ice cold calcium chloride to each tube (Figure 1).

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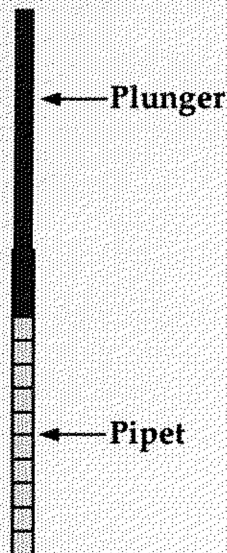
3. Obtain a starter plate. Use a sterile inoculating loop to transfer a large colony of bacteria from the starter plate to each tube of cold calcium chloride. Be sure not to transfer any agar to the tube.

Figure 2



4. To remove the bacteria from the transfer loop, place the loop into the calcium chloride and twirl rapidly. Dispose of the loop according to your instructor.

Gently tapping the loop against the side of the tube may help dislodge the bacteria.

5. Using the provided capillary micropipets and plungers, add 10 µl (Figure 2) of the plasmid pUC8 solution, which carries the antibiotic resistance gene, to the "+" tube.

6. Gently tap the tube with your finger to mix the plasmid into the solution.

7. Incubate both tubes on ice for 15 minutes.

8. While the tubes are incubating, obtain two Luria agar plates and two Luria agar plates with ampicillin. Label one Luria agar plate "+", the other "–". Do the same for the Luria agar plates with ampicillin. Be sure to label all four plates with your group name.

Both time and temperature are critical in the following heat-shock protocol. Be sure your waterbath is at 42°C and do not exceed 90 seconds in the waterbath.

Figure 3

9. The bacterial cells must be heat shocked to allow the plasmid to enter the cell. Remove the tubes from ice and immediately place in a 42°C hot waterbath for 60 to 90 seconds.

10. Remove the tubes from the 42°C waterbath and immediately place on ice for two minutes.

11. Remove the tubes from the ice bath and add 0.25 ml (250 µl) of room temperature Luria broth to each tube with a sterile disposable pipet. Gently tap the tube with your finger to mix the solution. The tubes may now be kept at room temperature.

12. Add 0.1 ml (100 µl) (Figure 3) of the "+" solution to the two "+" plates with another sterile disposable pipet. Add 0.1 ml (100 µl) of the "–" solution to the two "–" plates with a different sterile disposable pipet.

13. Using a sterile Bacti-spreader, spread the cells over the entire surface of the Luria agar "-" plate. Then, using the same Bacti-spreader, spread the liquid on the Luria agar w/ampicillin "-" plate.

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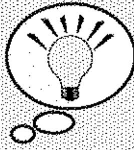

DID YOU KNOW?

Streptococcus pneumoniae infections cause 3,000 cases of meningitis, 50,000 blood infections, and 100,000 - 150,000 hospitalizations for pneumonia each year.

14. Using a new Bacti-spreader, repeat the procedure for both of the "+" plates. Spread the liquid on the Luria agar "+" plate first followed by the Luria agar w/ampicillin "+" plate. Dispose of the Bacti-spreaders according to your instructor.

15. Let the plates sit for five minutes to absorb the liquid. Place the plates in a 37°C incubator, inverted, overnight.

16. The next day, remove the plates from the incubator. Count and record the number of colonies on each plate. If the bacteria has grown over the entire surface so that individual colonies cannot be distinguished, write "lawn". Record your results in the Analysis section.

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Name: ____________________
Group: ____________________
Date: ____________________

ANALYSIS

Luria agar + ____________

Luria agar – ____________

Luria agar with ampicillin + ____________

Luria agar with ampicillin – ____________

A cell must be competent for transformation to occur. Not all cells in the solution become competent and therefore never receive the gene for antibiotic resistance. Transformation efficiency is the number of resistant colonies per microgram of plasmid. Using the directions below, calculate transformation efficiency.

Total mass of plasmid used
(total mass = volume x concentration) ____________________

Total volume of suspension ____________________

Fraction of cell suspension put on plate
(µl on plate/total volume) ____________________

Total mass of plasmid in fraction
(mass of plasmid x fraction on plate) ____________________

Number of colonies per µg of plasmid
(# of colonies counted/mass of plasmid put on plate) ____________________

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Name: ______________________
Group: ______________________
Date: ______________________

ASSESSMENT

1. Based on your experimental results, did transformation occur? Why or why not?

2. What other methods can be used to verify that transformation occurred? Explain.

3. Transformation is one type of genetic exchange among bacteria. Research another type of genetic exchange that occurs in bacteria and briefly describe or draw the mechanism by which exchange occurs.

4. Your expected transformation results for each of the four plates are listed below. Briefly explain next to each one why the listed results occurred.

   Luria "-": bacterial lawn

   Luria "+": bacterial lawn

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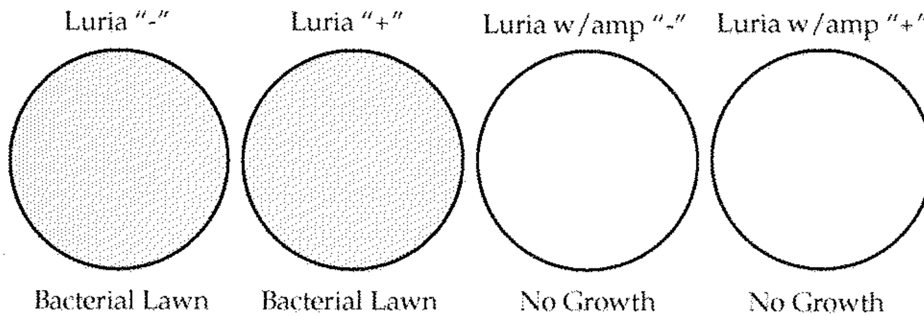
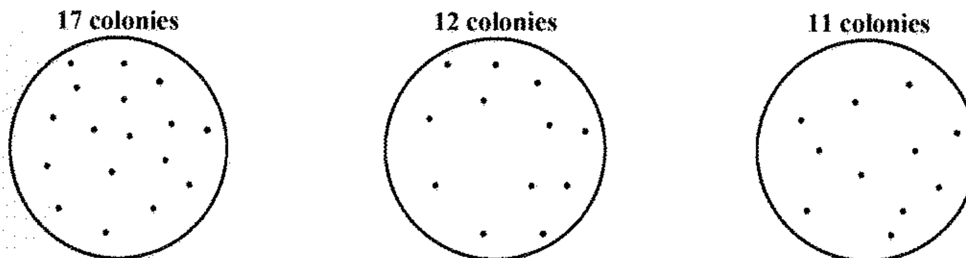
Luria w/amp "-": no growth

Luria w/amp "+": several colonies

5. You repeated the experiment and examined the results the next day, below is what your plates looked like when you checked them. Explain what may or may not have occurred.



6. You performed a transformation and got the results on the three plates shown below. You added 10 µg of plasmid at a concentration of 0.005 µg/µl to cells suspended in 300 µl of $CaCl_2$. After your heat-shock step, 200 µl of Luria broth was added to each reaction. After the experiment was complete, you placed 200 µl of the final solution on each plate and incubated. Calculate the transformation efficiency of each reaction and then calculate the average transformation efficiency for all of your trials.



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Calculate the results using the formula for transformation efficiency for each plate:

Total mass of plasmid used:

Total volume of suspension:

Fraction of suspension put on plate:

Total mass of plasmid in fraction:

Number of colonies per μg of plasmid:

Plate 1 Plate 2 Plate 3

Avg:

7. Which of the following statements is/are not true about bacterial transformation?

a) Cells can only be transformed when they are in a competent state

b) Transformation may only be performed using plasmid DNA containing antibiotic-resistance genes (R factors)

c) Transformed cells are capable of passing their newly acquired traits onto succeeding generations

d) Transformation was first discovered in the bacterium *Streptococcus pneumoniae*

e) Cells must first be treated artificially in the laboratory before they are capable of undergoing transformation.

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8. Using an external source, such as the internet or your school library, research the history of transformation and fill in the missing information in the following paragraphs. A list of terms to be used is included (not all of the terms will be used and some may be used more than once).

List of terms:

Alexander Fleming	living non-pathogenic protein	transforming principle
conjugation	Oswald Avery	transduction
heat-killed pathogenic	living pathogenic	1928
RNA	1895	rabbits
transformation	heat-killed non-pathogenic	Watson & Crick
T.H. Morgan	*Streptococcus pneumoniae*	*Bacillus subtilis*
mice	DNA	
1953	guinea pigs	
Eschericia coli	Frederick Griffith	

The phenomenon of ______________ was first discovered in ______________ by ______________. In his now-famous experiment, he injected ______________ with ______________ cells of the bacterium ______________ and with ______________ cells of the same bacterium. The results displayed the properties of ______________ cells. This led him to conclude that the ______________ cells must have been altered in some way by the material from the ______________ cells. Though the exact substance causing the change in the cells was unknown at the time, he proposed that some sort of ______________ caused the genetic exchange when the two types of cells were combined.

Years later, in the early 1940's, the work of ______________, along with several colleagues, demonstrated exactly what the substance was that transformed the cells. By isolating specific components from the ______________ cells and exposing each component individually to the ______________ cells, it was demonstrated the only material that could cause transformation of the cells was ______________. Their work was met with much skepticism initially because until that point it had been assumed that ______________ was the source of genetic material. However, repetition of the experiment and the work of several others proved conclusively that ______________ is indeed the transforming substance, as well as the source of genetic material.

9. Divide the class into two parts. One half of the class will represent a team of doctors from a local hospital. The other half of the class will represent a team of genetic engineers from a local biotechnology company. Explain to each other why you as a group believe antibiotic-resistant organisms are helpful or harmful.

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