WARD'S

Transformation of *E. coli* with pUC8 Lab Activity

Student Study Guide

DID YOU KNOW?

Transformation was discovered in the late 1920s by Fred Griffith, an English medical officer, while he was studying the bacteria responsible for a pneumonia epidemic in London.

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.

BACKGROUND

The ability to exchange genes within a population is a nearly universal attribute of all living things. Among prokaryotes, there is no known case where genetic exchange is an obligatory step (as it often is in eukaryotes) in the completion of an organism's life cycle. Rather, genetic exchange in prokaryotes seems to be an occasional process that occurs through three different mechanisms in various prokaryotes. These three mechanisms are transformation, transduction, and conjugation.

In transformation, DNA is released from cells into the surrounding medium, and recipient cells are then able to incorporate it into themselves from the medium. Transduction occurs when a phage viron attaches to a bacterial cell and transfers some or all of its DNA into the bacteria. Conjugation is controlled by plasmid-borne genes and occurs between cells that are in direct contact with one another. Usually only the plasmid itself is transferred, although sometimes chromosomal genes can be transferred as well.

Although the existence of plasmids was inferred from genetic studies in the 1950s, it has only been during recent decades, with the advent of more accurate means of detection, that the impact of plasmids on the biology and ecology of prokaryotes has been fully appreciated. Plasmids are circular molecules of double-stranded DNA and generally function as small chromosomes. They are self-replicating and can encode for a variety of cellular functions. However, unlike chromosomes, they are dispensable. The types of functions they encode only benefit the cell in a limited set of environments, and none are known to encode for essential cellular functions. Many, but not all, bacteria may contain anywhere from one to several dozen plasmids and although they are capable of autonomous replication, the number of plasmids remains fairly constant from one generation to the next. These bits of DNA vary in size from a few to several hundred Kb (kilobase) pairs in length.

Many plasmids, called R factors, carry genes that confer resistance to antibiotics on the host cell. First discovered in 1955, R factors have spread rapidly among pathogenic bacteria in recent years, profoundly affecting medical science by causing many strains of pathogenic bacteria to be highly resistant to antibiotics. The number of in-

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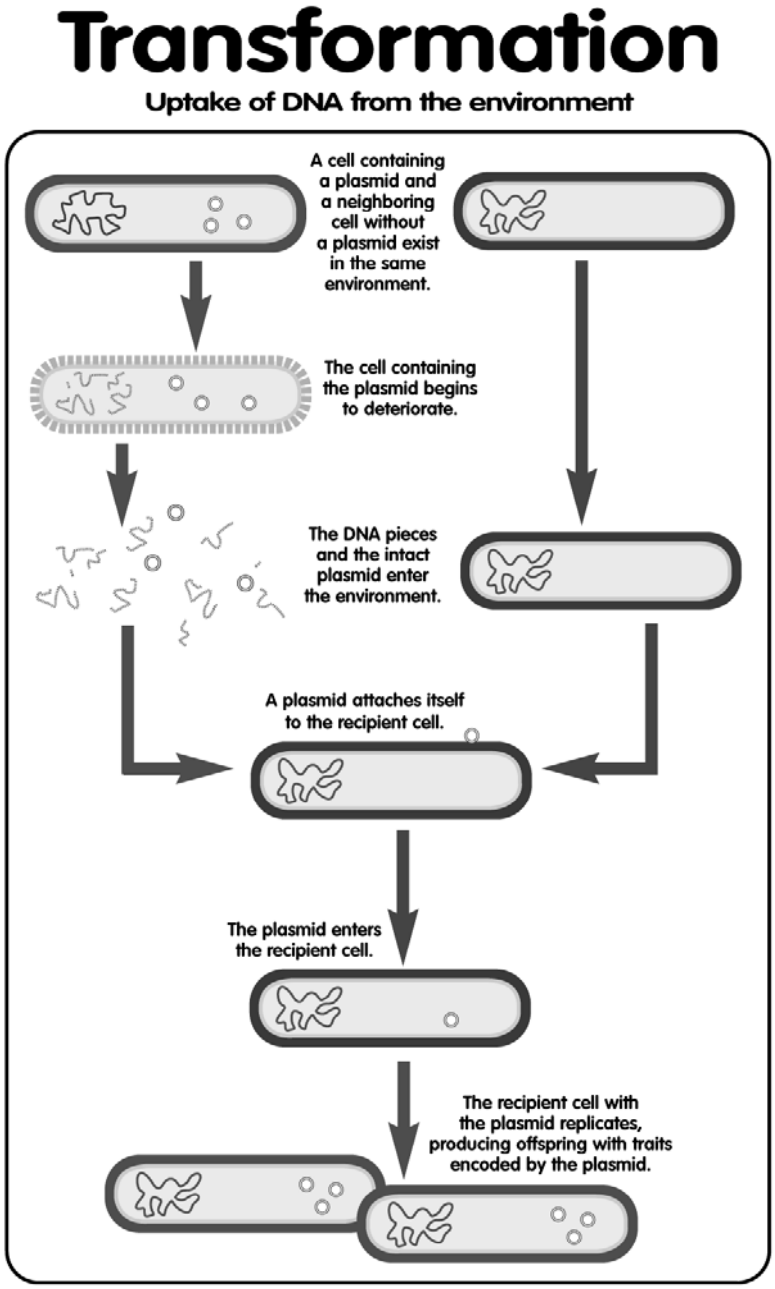
DID YOU KNOW?

Although *E. coli* has often been in the news as a food-borne pathogen, the vast majority of *E. coli* strains are harmless, including those commonly used by scientists in genetic laboratories.

hibitory substances for R-mediated resistance has grown to almost all antibiotics, many other chemotherapeutic agents, and a variety of heavy metals. The mechanisms of resistance conferred by these genes tend to be different from those that are chromosomally determined. Plasmid genes often encode enzymes that chemically inactivate the drug or eliminate it from the cell by active transport. In contrast, chromosomal mutations usually modify the cellular target of the drug, rendering the cell resistant to the drug's action.



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DID YOU KNOW?

According to the CDC, U.S. doctors prescribe approximately 100 million courses of antibiotics each year. More than 50 million of these are unnecessary, because they are given mostly for colds and other viral infections for which antibiotics offer no benefit.

The first mechanism of bacterial genetic exchange to be discovered was transformation. In 1928, a now-famous experiment demonstrated that injecting mice with an non-pathogenic (not capable of causing disease) strain of *Streptococcus pneumoniae*, together with heat-killed cells of a pathogenic (disease-causing) strain killed mice, while injecting these strains separately did not. This and subsequent experiments established that the surviving cells were recombinant, meaning that they exhibited certain properties (including the ability to cause disease) that were typical of the killed cells and others that were typical of the non-pathogenic culture. A genetic exchange of the DNA dissolved in the external medium had occurred between the dead cells and the live ones. At the time, it was thought that a particular substance, a "transforming principle", caused the exchange to take place. Thus the word "transformation" came to be used to describe genetic exchange among prokaryotes.

When cells are able to be transformed by DNA in their environments they are called "competent". In a significant number of bacteria, entry into a competent state is encoded by chromosomal genes and signaled by certain environmental conditions. Such bacteria are said to be capable of undergoing natural transformation. Many other bacteria do not become competent under ordinary conditions but can be made competent by exposing them to a variety of artificial treatments, such as exposure to high concentrations of divalent cations.

E. coli cells, which do not possess a natural system for transformation, are capable of being artificially transformed. They become competent only after the cultured cells are exposed to calcium chloride solution. These newly-competent cells are now receptive to an insertion of foreign DNA contained in a plasmid.

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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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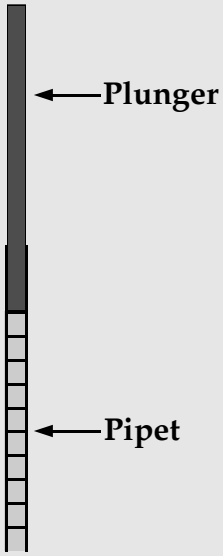
Figure 2



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.

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?

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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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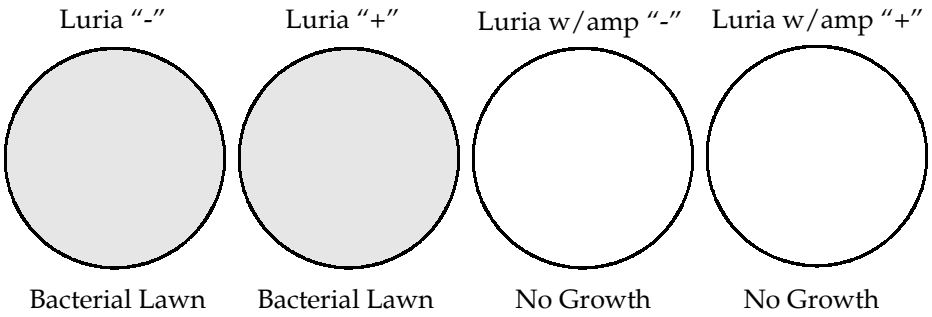
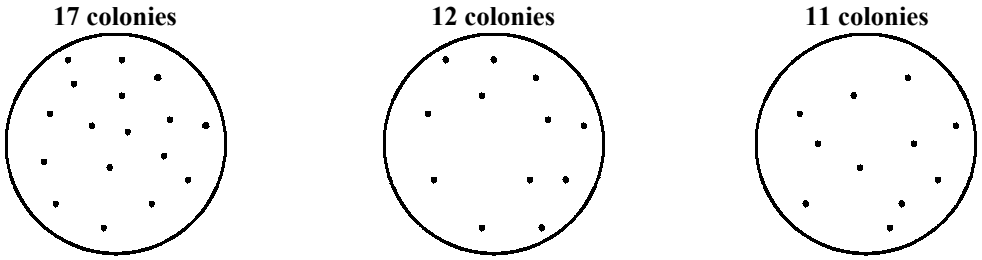
Luria w/amp "-": no growth

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 <u>not</u> 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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