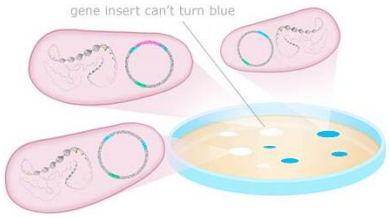
BACTERIAL TRANSFORMATION - AP LAB



☐ BACKGROUND INFORMATION

Are genetically modified foods safe? There is ongoing debate about whether it is safe to eat fruit and vegetables that are genetically modified to contain toxins that ward off pests. For instance, biotechnologists have succeeded in inserting a gene (Bt) from the bacterium Bacillus thuringiensis into the corn genome. When expressed, the Bt toxin kills caterpillars and controls earworms that damage corn, but is the corn safe for human consumption?

Genetic information passed from parent to offspring via DNA provides for continuity of life. In order for information in DNA to direct cellular activities, it must be transcribed into RNA. Some of the RNAs are used immediately for ribosomes or to control other cellular processes. Other RNAs are translated into proteins that have important roles in determining metabolism and development, i.e., cellular activities and phenotypes (traits). When the DNA of a cell changes, the RNAs and proteins they produce often change, which in turn changes how that cell functions.

DNA inside a cell can change several ways. It can be mutated, either spontaneously or after the DNA replication machinery makes an error. Biotechnologists may cause an intentional mutation in a cell's own DNA as a way to change how that cell behaves. The most powerful tool biotechnologists have, though, is the ability to transfer DNA from one organism to another and make it function there. With this tool, they can make cells produce novel protein products the cells did not make previously.

Examples of this powerful tool are all around us. Insulin that people take to control their blood sugar levels is often made from engineered bacteria. Some vaccines, as well as enzymes used for manufacturing denim jeans, are also made using engineered cells. In the near future, engineered bacteria and other cells being developed could help clean up spilled oil or chemicals, produce fuel for cars and trucks, and even store excess carbon dioxide to help slow global climate change. Can you think of other possible applications of genetic engineering? However, biotechnology and human manipulation of DNA raise several ethical, social, and medical issues, such as the safety of genetically modified foods. Can you think of other issues to consider?

This biotechnology depends on plasmids, small circles of DNA that were found first in bacteria. Plasmids allow molecular biologists to manipulate genetic information in a laboratory setting to understand more fully how DNA operates. Plasmids also let us move DNA from one bacterium to another easily.

In this investigation, you will learn how to transform Escherichia coli (E. coli) bacteria with DNA it has not possessed before so that it expresses new genetic information. Bacterial cells that are able to take up exogenous (external) genetic material are said to be "competent" and are capable of being transformed. You also will calculate transformation efficiency to find out how well the E. coli took up the "foreign" DNA. Using these techniques, you will have the opportunity to explore the field of biotechnology further.

☐ GETTING STARTED

DNA provides the instructions necessary for the survival, growth, and reproduction of an organism. When genetic information changes, either through natural processes or genetic engineering, the results may be observable in the organism. These changes may be advantageous for the long-term survival and evolution of a species, but it also may be disadvantageous to the individuals who possess the different genetic information.

In bacteria, genetic variation does not happen by mutation alone. It also can be introduced through the lateral (horizontal) transfer of genetic material between cells. Some bacteria undergo conjugation, which is direct cell-to-cell transfer. Other bacteria acquire DNA by transduction (viral transmission of genetic information). The third route is transformation, which is uptake of "naked" DNA from the environment outside the cell.

The concept of cell transformation raises the following questions, among others:

- To transform an organism to express new genetic information, do you need to insert the new gene into every cell in a multicellular organism or just one? Which organism is best suited for total genetic transformation — one composed of many cells or one composed of a single cell?
- Can a genetically transformed organism pass its new traits on to its offspring? To get this information, which would be a better candidate for your investigation — an organism in which each new generation develops and reproduces quickly or one that does this more slowly?
- Based on how you answered the first two sets of questions, what organism would be a good choice for investigating genetic transformation — a bacterium, earthworm, fish, or mouse?

If your answer to the last question is "bacterium," you are on the right track. Genetic transformation of bacteria most often occurs when bacteria take up plasmids from their environment. Plasmids are not part of the main DNA of a bacterium. They are small, circular pieces of DNA that usually contain genes for one or more traits that may be beneficial to survival. Many plasmids contain genes that code for resistance to antibiotics like ampicillin and tetracycline. [Antibiotic-resistant bacteria are responsible for a number of human health concerns, such as methicillin-resistant Staphylococcus aureas (MRSA) infections.] Other plasmids code for an enzyme, toxin, or other protein that gives bacteria with that plasmid some survival advantage. In nature, bacteria may swap these beneficial plasmids from time to time. This process increases the variation between bacteria — variation that natural selection can act on. In the laboratory, scientists use plasmids to insert "genes of interest" into an organism to change the organism's phenotype, thus "transforming" the recipient cell. Using restriction enzymes, genes can be cut out of human, animal, or plant DNA and, using plasmids as vectors (carriers of genetic information), inserted into bacteria. If transformation is successful, the recipient bacteria will express the newly acquired genetic information in its phenotype (Figure 1).

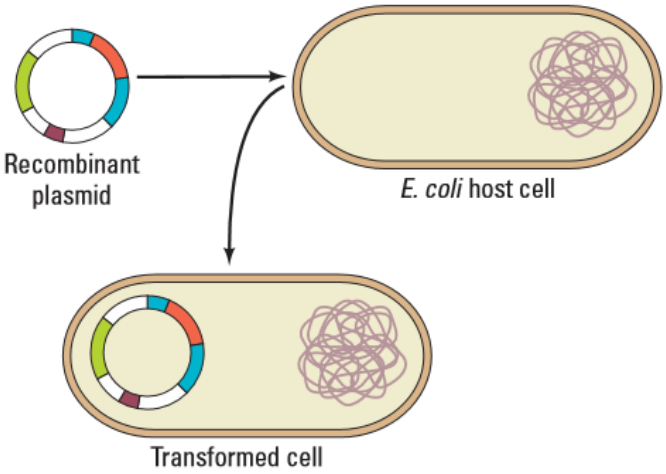


Figure 1. Transformation of Bacteria

In nature, the efficiency of transformation is low and limited to relatively few bacterial strains. Also, bacteria can take up DNA only at the end of logarithmic growth; at this time, the cells are said to be "competent." In the lab, you have discovered several ways to increase the rate of transformation. Now, rather than just a few bacteria taking up a plasmid you want them to use, millions of bacteria can be transformed. The number of bacteria that take up a plasmid successfully is called the "transformation efficiency." This is one of the values you will calculate in this lab unit.

In this investigation, you will use a predefined procedure to transform E. coli bacteria with a plasmid carrying a foreign gene. There are several different plasmids your instructor can choose from; you will be instructed about which one to work with for this unit. E. coli is an ideal organism for the molecular geneticist to manipulate because it naturally inhabits the human colon and easily can be grown in a nutrient medium such as LB broth.

☐ PRE-LAB QUESTIONS

1. How could you use two LB/agar plates, some E. coli, and some ampicillin (an antibiotic) to determine how E. coli cells are affected by ampicillin?

2. What would you expect your experimental results to indicate about the effect of ampicillin on the E. coli cells?

3. Do you think that exposure to ampicillin will cause the E. coli cells to evolve resistance to ampicillin? Why or why not?

4. How will you be able to tell if host E. coli cells have been genetically transformed? *(Hint: the gene of interest is an ampicillin resistance gene)*

☐ PROCEDURE

1. Please visit the following website:
   http://www.classzone.com/books/hs/ca/sc/bio_07/virtual_labs/virtualLabs.html

2. Click on Bacterial Transformation.

3. Once in the laboratory, familiarize yourself with the equipment.

4. Click on the procedure tab on the top left-hand corner and follow steps 1-11 outlined in the program.

5. Complete your predictions and observations in the table below.

Table 1. E.coli-amp vs. E.coli + amp

	PETRI DISH			
CONDITIONS	*1.Control Agar with E.coli +amp*	*2. Agar + Ampicillin with E.coli + amp*	*3. Control Agar with E.coli – amp*	*4. Agar + Ampicillin with E.coli - amp*
GROWTH PREDICTIONS				
GROWTH OBSERVATIONS				

☐ ANALYSIS OF DATA

1. What does the genome of the transformed E.coli cell contain that a normal E.coli cell does not contain? Based on your observations, how can you tell which, if any, of the bacterial cells were transformed.

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2. Which petri dishes showed the most growth and which showed the least?

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3. What does the culture with partial growth tell you about whether all of the bacterial cells incorporated the amp^R gene?

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4. Look at the two petri dishes that had the most growth. What do they have in common? Explain why the ampR gene offers or does not offer an advantage when grown in the environment of these petri dishes.

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5. How does the petri dish with no growth support the conclusion that some bacterial cells did not take in the amp^R gene?

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6. How can the procedures described above (addition of CI_2 and "heat shocking") help facilitate the introduction of plasmids into the E. coli cells?

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