NAME _____

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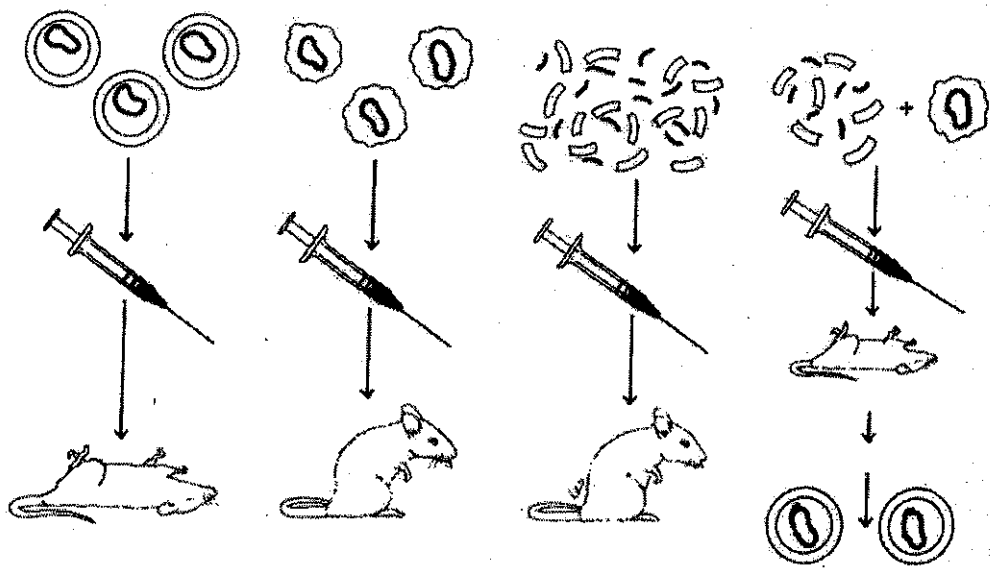
Carolina™ Transformation for AP Biology

Background

Transformation and Its Role in Discovering the Function of DNA

The discovery of transformation in bacteria was crucial in understanding that DNA is the material of inheritance. Transformation is the uptake of exogenous, naked DNA by a cell. The newly adopted DNA can become a heritable part of the cell's genetic material. Transformation was discovered through work done by several scientists, or groups of scientists, each of them building on the work of the others. In 1928, the English scientist Frederick Griffith was studying the bacterium *Streptococcus pneunomiae*. This organism causes pneumonia, which was a leading cause of death. Griffith worked with two strains of *S. pneumoniae*: one that caused disease and whose colonies were smooth in appearance, and another that did not cause disease and whose colonies were rough in appearance. The smooth appearance of the pathogenic form of *S. pneunomiae* resulted from a specific type of external polysaccharide coating, which is now known to be the mechanism by which the strain evades a host's immune system.

Griffith's experiments involved injecting the mice with the two different strains of *S. pneumoniae*. When he injected the smooth strain, the mice became ill and died. When he injected the rough strain, the mice stayed healthy. However, when Griffith mixed heat-killed smooth cells (which caused no disease when injected into mice) with living rough cells (which caused no disease when injected into mice) and injected that combination into mice, surprisingly, the injected mice became ill as if they had been injected with live smooth cells. When Griffith isolated *S. pneunomiae* cells from the dead mice, the bacteria formed smooth colonies. Griffith concluded that the living rough cells had been transformed into smooth cells as the result of being mixed with the dead smooth cells. Sixteen years later Avery, McCarty, and MacLeod showed that the "transforming principle," the substance from the heat-killed smooth strain that caused the transformation, was DNA. They did so by demonstrating that purified DNA from the killed smooth cells was able to transform the live rough cells and that enzymes which destroyed DNA destroyed the "transforming principle."



Griffith's transformation experiment with smooth and rough strains of *Streptococcus pneumoniae* and mice

Natural Transformation—an Example of Lateral (Horizontal) Transfer of Genetic Material

Some bacteria, including *Streptococcus pneumoniae*, undergo transformation naturally. Bacteria that transform naturally have mechanisms for transporting the DNA into the bacterial cell. Once inside the cell, the base sequence of the new DNA is compared to the bacterium's DNA. If enough similarity in sequence exists between the new DNA and part of the bacterium's existing DNA, the new DNA can be substituted for the homologous (like) region of the bacterium's DNA. If the new DNA is not similar to the bacterium's DNA, it is not incorporated into the genome and is broken down by intracellular enzymes.

Why would bacteria have mechanisms for taking up free DNA from the environment? Taking up free DNA from the environment increases the organism's access to diverse genetic material and thus increases the ability of the organism to adapt to the environment. For example, *Neisseria gonorrhea*, the causative agent of gonorrhea, takes up DNA from members of its own species. In doing so, it often takes up DNA that allows it to alter the character of its surface proteins. Since our immune system makes antibodies to these surface proteins in order to fight infection by *N. gonorrhea*, if the organism changes the nature of these surface proteins, it is more likely to evade our immune system.

From this example it is easy to understand how lateral (also called horizontal) gene transfer, the transfer of genetic material between organisms that do not have a parent/offspring relationship, plays a role in how microorganisms adapt to their environment. (In contrast, transfer of genetic material from parent to offspring is referred to as vertical gene transfer.) Naturally occurring transformation is one example of horizontal gene transfer. See the section on plasmids for information regarding conjugation, another mechanism of horizontal gene transfer.

Artificial Transformation

Scientists took natural transformation and developed it into something they could use as a powerful tool in the lab. It is relatively rare for most bacteria to take up DNA naturally from the environment. However, by subjecting bacteria to specific artificial conditions, scientists are able to cause bacteria to consistently take up DNA. Bacteria that are able to take up DNA are referred to as competent. Making cells in the lab competent usually involves changing the ionic strength of their medium and subjecting them to several temperature changes, one of which involves heating the cells in the presence of positive ions (usually calcium.) Another method for making cells take up DNA at an artificially high rate involves exposing them to high voltage. This method is called electroporation.

Once DNA is taken into a host bacterial cell, the genes coded for by the DNA need to be expressed (i.e., transcribed and translated). In addition, the DNA needs to persist in the cell. However, as discussed above, if transformed DNA is to persist in the cell, it must be integrated into the genome, and in order to integrate into the genome it must be similar in sequence to at least some part of the organism's DNA. Often, when researchers want to introduce DNA into a bacterial cell, they want to introduce DNA that is quite different from that of the existing bacterial genome. To ensure that the introduced DNA will persist in the cell, scientists insert the DNA of interest into something called a plasmid and then transform the bacterial cells with the plasmid DNA.

Plasmids

Plasmids are small, usually circular pieces of extra-chromosomal DNA that exist in nature in bacteria and yeasts and are sometimes transferred between individuals. Bacteria transfer them in a process called "conjugation," in which there is physical contact between the two cells. Plasmids have been harnessed and modified to serve a pivotal role in molecular biology. Each plasmid has an origin of replication (a sequence of bases at which DNA replication begins). Because of this origin of replication, plasmid DNA (unlike

fragments of transformed DNA that are not integrated into the organism's genome) can replicate in the host bacterial cell. In addition, each daughter cell receives copies of the plasmid upon cell division. Thus, DNA inserted into a plasmid introduced into a bacterial cell can persist in the cell and in the cell's offspring. Because of this, plasmids provide an ideal mechanism for introducing foreign DNA into bacterial cells and were critical in the development of genetic engineering.

Selective Markers

There is one more key step involved in making transformation work in the lab. In the preceding paragraph, it was stated that DNA introduced into a cell via a plasmid will persist in the cell and its offspring. This is true, but there is a catch. Bacteria, in nature and in a crowded culture flask, are in intense competition for survival. Plasmids do not always contain genes that are beneficial for survival, and they do take extra energy to maintain. The tendency is for bacteria to lose their plasmids unless there is an advantage to retaining them. One way to force a bacterium to keep a plasmid is to maintain the bacterium in the presence of an antibiotic that will kill it unless it develops resistance to the antibiotic. Consequently, researchers grow transformed bacteria in the presence of antibiotic and include the appropriate antibiotic-resistance gene along with the other desired DNA on the plasmid that they want the bacteria to retain. The gene for antibiotic resistance is called a selective marker; not only does it force the bacteria to keep the plasmid, it allows the researcher to select for those bacteria that have successfully taken up the plasmid of interest. Only those bacteria that have taken up the desired plasmid will survive.

The plasmids that you will work with in this lab activity use either ampicillin or kanamycin as a selective marker. You will use either ampicillin or kanamycin to select for bacteria that have actually taken up the plasmid.

Antibiotics, Antibiotic Resistance, and Evolution

This lab demonstrates one way in which bacteria can acquire antibiotic resistance—through the acquisition of a plasmid with a gene for antibiotic resistance. In this lab, the bacteria were artificially induced to take up a plasmid with an antibiotic resistance gene. However, remember that in nature plasmids are passed back and forth between bacteria, and that many naturally occurring plasmids contain antibiotic-resistance genes as well.

While antibiotic resistance is a helpful tool in the lab, the development of antibiotic resistance in nature is a serious problem. The discovery of antibiotics is one of the most important medical breakthroughs; it has allowed many people to survive what would have once been lethal bacterial infections. However the effectiveness of many antibiotics has decreased over the years as bacteria have developed resistance to them.

A bacterial population's development of antibiotic resistance is an example of evolution at work. Through random mutation (e.g., some bacteria have developed resistance through mutations in the gene coding for the 30S ribosome) or possession of a plasmid with an antibiotic-resistance gene, bacterial populations include a few individuals with genes providing resistance to one or more antibiotics. When the population is exposed to an antibiotic, there is selective pressure in favor of the few individual bacteria that are resistant to the antibiotic. When these antibiotic-resistant bacteria divide, they pass the resistance trait on to their offspring. If the antibiotic persists in their environment, these offspring then come to dominate and eventually to constitute most, if not all, of the population.

The more prevalent the use of an antibiotic, the greater is the selective pressure in favor of antibiotic resistance, and the more likely that bacterial populations evolve to become resistant. Some practices in animal husbandry provide a good example of this dynamic. On many large farms, farmers add antibiotics to the animals' feed to minimize infection among the animals, especially if they are grown in crowded

conditions. This sustained use of antibiotics contributes to the evolution of antibiotic-resistant bacteria. Another factor involved in increasing drug resistance in bacteria is doctors prescribing antibiotics to patients who do not really need them. Some doctors tend to prescribe or are pressured into prescribing antibiotics for patients who have viral infections, even though viruses are not killed by antibiotics.

To combat the problem of antibiotic resistance, researchers continually try to develop new antibiotics to kill pathogenic bacteria in novel ways. To do this the researchers study bacteria to gain a thorough understanding of how they function.

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Pre-Laboratory Inquiry

You work on a team in a research lab. You have three separate plasmids in your lab, but the labels have come off of the tubes and you no longer know which plasmid is which. Come up with an experiment that will give you a definitive answer as to which plasmid is which. For the time being, you will refer to the plasmids as plasmids 1, 2, and 3. You know the following:

- One plasmid has a kanamycin-resistance gene.
- Two plasmids have an ampicillin-resistance gene.
- In addition to having an antibiotic-resistance marker, one plasmid also codes for the gene for green fluorescent protein (GFP), a protein from the bioluminescent jellyfish *Aequorea victoria*.

Bacteria that produce green fluorescent protein look green under white light and fluoresce under ultraviolet light.

Assume that each research team has access to the following materials and is responsible for identifying one of the three plasmids. (**Note:** You will not actually use any materials during this pre-lab inquiry.) Before planning your experiment, answer the questions below to ensure that you understand some of the basic concepts involved in the lab. Some of these questions are specifically designed to help you think about setting up your experiment.

starter plates (plates containing *E. coli* in the rapid, or exponential, stage of growth)
kanamycin plates
ampicillin plates
LB plates
plasmids 1, 2, and 3
transformation reagents, tubes, and equipment (inoculating loops, calcium chloride, LB broth, ice, 42°C water bath)
glass beads for spreading bacteria

1. What is a plasmid?

2. What is transformation?

3. Why is naturally occurring transformation beneficial to bacteria?

4. Why is transformation useful to research scientists?

Note: Questions 5–7 relate directly to the experiment you are planning.

5. Should you plate some of your transformed bacteria onto plates with antibiotics? Why or why not?

6. What would you expect to see if you plated some of your transformed bacteria onto a plate without antibiotic? Would there be an advantage to doing this (in terms of understanding your results)? Explain.

7. To transform bacteria with plasmids, technicians first make the bacteria competent (capable of taking up DNA) by placing them in calcium chloride and chilling them. Plasmid is then added to the competent bacteria and the plasmid/bacteria combination is taken through a few more steps to make the bacteria take up the DNA. In your experiment, should you treat a tube of bacteria that you do not add plasmid to exactly as you do the tube of bacteria that you will transform? Why or why not?

Other things to consider in planning

1. What controls will you include in your experiment?
   a. How will you differentiate between bacteria that have taken up the plasmid and those that have not?
   b. Are there any controls you can include which would help you determine why the transformation did not work if it does not?
   c. What controls could you include to definitively demonstrate that any phenotypic changes you see are a direct result of the introduction of the plasmid into the bacteria?
2. What do you expect to observe, given how you have set up the experiment?
3. Draw any tables or any other method for organizing the data you will collect. Remember that if you collect data from other groups, you will have to have a way of recording their data as well.

Transformation Laboratory Procedure

After discussing your experimental design with the class, it is time to conduct the actual transformation to determine the identity of the plasmids. Each group will be assigned one plasmid to work with.

Remember the following:

- One plasmid has a kanamycin-resistance gene.
- Two plasmids have an ampicillin-resistance gene.
- In addition to having an antibiotic-resistance marker, one plasmid also codes for the gene for green fluorescent protein (GFP), a protein from the bioluminescent jellyfish *Aequorea victoria*.

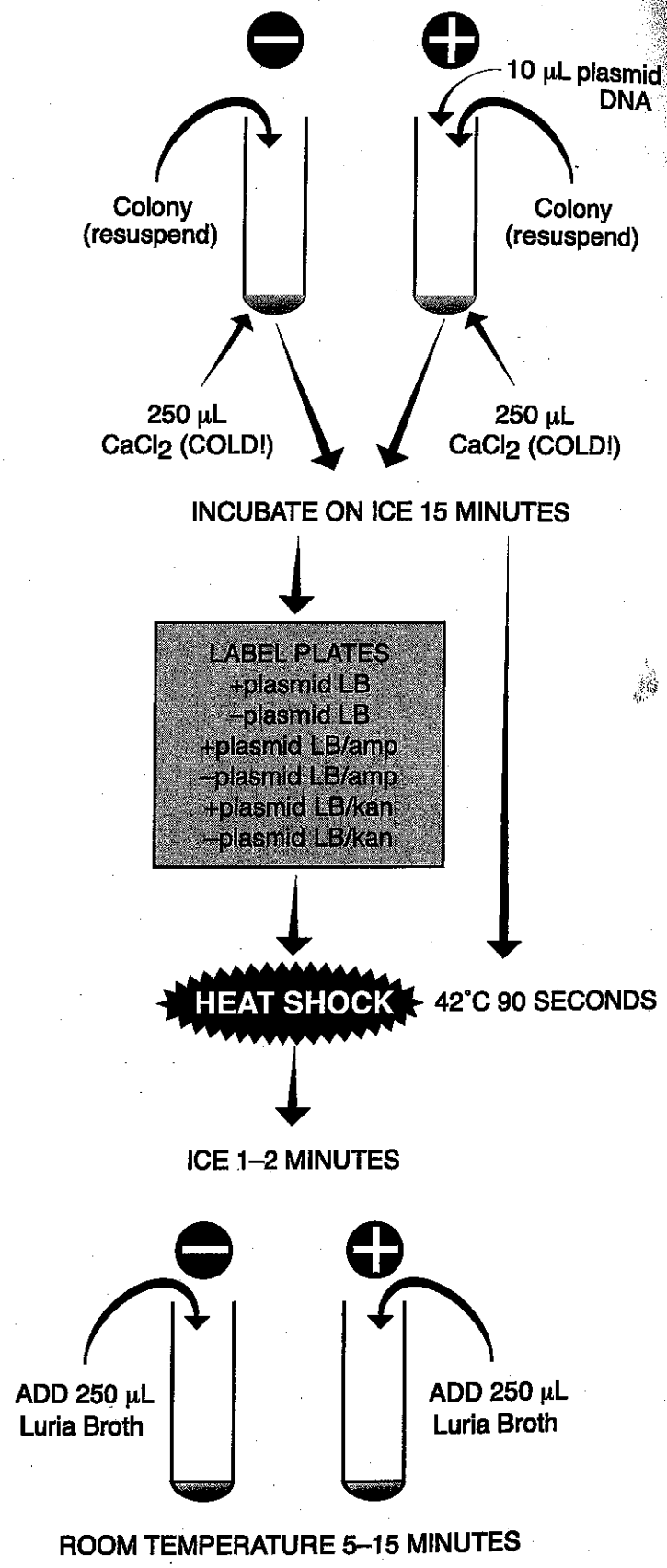
Materials

For your group:

2 LB plates
2 kanamycin plates
2 ampicillin plates
2 sterile transformation tubes
container with crushed ice
rack for holding transformation tubes
3 sterile inoculating loops
6 sterile transfer pipets
waste container
3-mL vial LB
3-mL vial $CaCl_2$ (on ice)

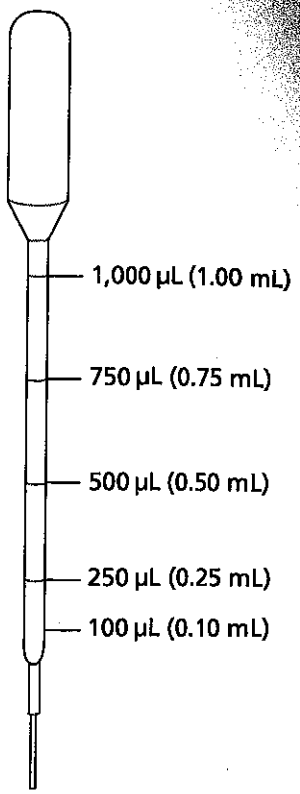
To share:

glass beads for spreading
water bath (42°C)
incubator (if used)
starter plate (shared between two groups)
plasmids (will be marked "Plasmid 1," "Plasmid 2," or "Plasmid 3")



Procedure

When performing all of the following steps use sterile technique.

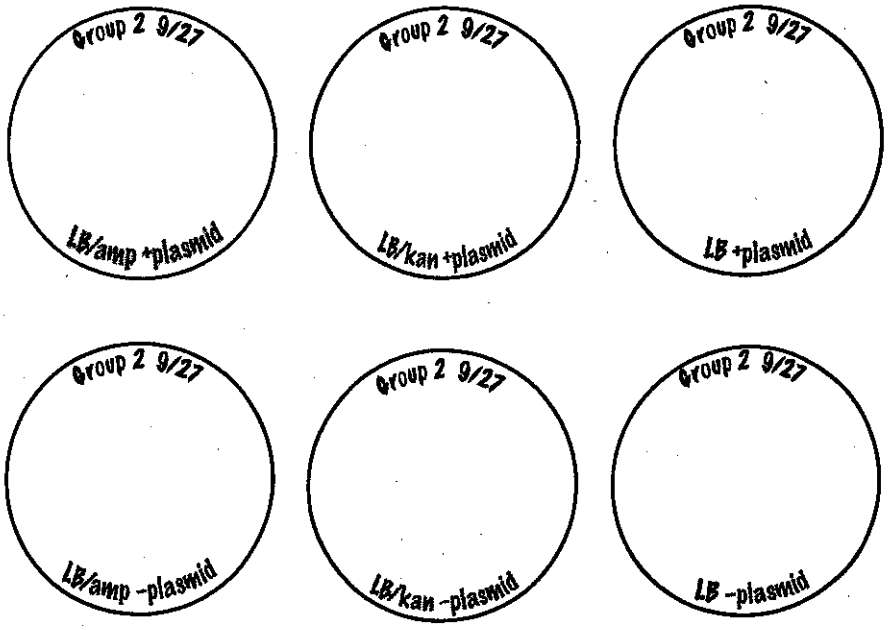


1. Mark one sterile 15-mL tube "+plasmid." Mark another "–plasmid." Plasmid DNA will be added only to the +plasmid tube.
2. Use a sterile transfer pipet to add 250 µL of ice-cold calcium chloride to each tube. See the figure at the right for reference. **Note:** Pressing the conical area between the stem and bulb of the pipet provides better control of the amount of liquid being aspirated.
3. Place both tubes on ice.
4. Use a sterile disposable inoculating loop to transfer isolated colonies of *E. coli* from the starter plate to the +plasmid tube. The total area of the colonies picked should be about half the size of the top of a pencil eraser (the ball of cells on the loop should be approximately 1–2 mm in diameter.)

a. Do not transfer any agar from the plate along with the cell mass.

b. Immerse the cells on the loop in the calcium chloride solution in the +plasmid tube and vigorously spin the loop in the solution to dislodge the cell mass. Hold the tube up to the light to verify that the cell mass has fallen off the loop.

5. **Immediately** suspend the cells by repeatedly pipetting in and out with a sterile transfer pipet. It is not necessary to draw the suspension all the way up into the tube; keep the solution in the stem of the pipet. Examine the tube against the light to confirm that no visible clumps of cells remain in the tube or are lost in the bulb of the transfer pipet. The suspension should appear milky white.
6. Return the +plasmid tube to ice.
7. Transfer a mass of cells to the –plasmid tube and resuspend as described in steps 4 and 5 above.
8. Return the –plasmid tube to ice. Both tubes should now be on ice.
9. Use a new sterile disposable inoculating loop to add one loopful of plasmid DNA to the +plasmid tube **only**. When the DNA solution forms a film across the loop opening, its volume is 10 µL. If you do not see this film, you do not have enough DNA on the loop. Immerse the loopful of plasmid DNA *directly into* the cell suspension and spin the loop to mix the DNA with the cells.
10. Return the +plasmid tube to ice and incubate both tubes on ice for **15 minutes**.
11. While the tubes are incubating, label your media plates as indicated in the diagram and with your lab group name and date. Think about why you are labeling these plates as you are.



12. Following the 15-minute incubation on ice, heat shock the cells as follows. Remove both tubes directly from ice and immediately immerse them in the 42°C water bath for 90 seconds. Gently agitate the tubes while they are in the water bath. Return both tubes directly to ice for 1 minute or more. The abrupt transfer of the tubes from ice to 42°C and back again is critical.
13. Use a sterile transfer pipet to add 250 µL Luria broth (LB) to each tube. Gently agitate the tubes with your finger to mix the LB with the cell suspension. Allow the tubes to sit at room temperature for a 5–15 minute recovery. During this incubation your instructor may have you add the glass beads for spreading your bacteria to your plates. See 14a. for instructions on how to do this.
14. Using the procedure below, spread the cells in the +plasmid tube onto the +plasmid plates and the cells in the –plasmid tube onto the –plasmid plates.
    a. Place the plates lid side down. Slightly open the plate on one side and carefully pour 4–6 glass beads onto each plate. Flip the plate over so that the beads are now resting on the agar.
    b. Using a sterile transfer pipet add 100 µL of cells from the –plasmid transformation tube to each of the appropriate plates.
    c. Using a new sterile transfer pipet add 100 µL of cells from the +plasmid transformation tube to each of the appropriate plates.
    d. Spread the bacteria evenly across the plate by using a back-and-forth motion (not swirling round and round) to move the glass beads across the entire surface of each plate. Shake each plate for several minutes.
    e. When you finish spreading, let the plates sit for several minutes to allow the agar to absorb the cell suspension.
    f. To remove the glass beads, hold each plate vertically over a waste container, slightly open the lower edge, and tap out the beads into the container.
15. Stack your plates together and incubate them as instructed by your instructor.

Laboratory Questions

1. Record your results and conclusions using the organizational method you devised in the Pre-laboratory Inquiry Activity.

2. Did you observe what you expected to? If not, how would you explain your observations?