



MAD SCIENCE TRANSFORMATIONS

INTRODUCTION

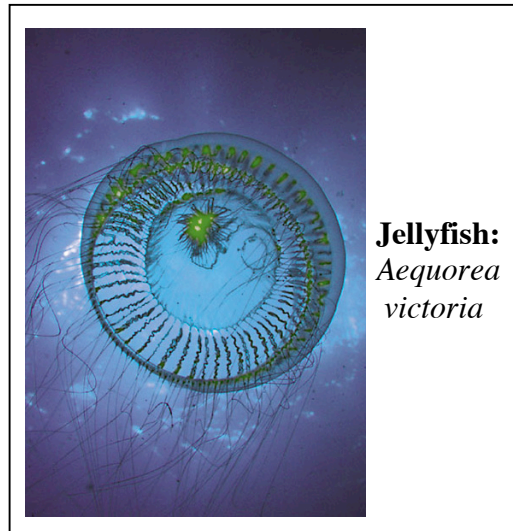
In 1928, Fredrick Griffith witnessed a miraculous event, a transformation in the literal sense of the word. During the course of an experiment, a living organism had changed in physical form. The virulent bacteria, isolated from a mouse that had died from a pneumonic infection, appeared different from the ones injected into a healthy animal two days prior. At the start of the experiment, Griffith had injected the mouse with a mixture of a heat killed smooth (S) strain of pneumococcus bacteria and a living but nonvirulent rough (R) strain. The smooth polysaccharide capsule of the S strain is essential for infection; the R strain, which appears rough, lacks the polysaccharide capsule and thus is incapable of infection. When injected alone, neither the heat-killed S strain nor the living R strain caused infection in the mouse, but co-injection of the two strains killed it. Griffith had isolated the S strain from the dead mouse.

Griffith hypothesized that some transforming principle was transferred from the heat-killed S bacteria to the R bacteria that converted it to a virulent state. Transformation appeared to be a genetic phenomenon. This association was strengthened by the one gene-one enzyme hypothesis proposed by George Beadle and Edward Tatum in 1940; according to this hypothesis, the transforming principle involved one or more genes that produced enzymes needed to synthesize the polysaccharide coat. In 1944 a team of researchers at the Rockefeller Institute, headed by Oswald Avery, purified the transforming principle from pneumococcus. Biochemical tests revealed it to be deoxyribonucleic acid (DNA). Taken together, all this evidence pointed to DNA as the components of genes.

The phenomenon of transformation, which provided a key clue to understanding the molecular basis of the gene, also provided a tool for manipulating the genetic makeup of living organisms. To a large extent, genetic engineering relies on adding relatively short segments of DNA containing a foreign or modified gene to living cells. Transformation- the uptake and expression of DNA by a living cell - is the limiting factor in the genetic engineering of any species.

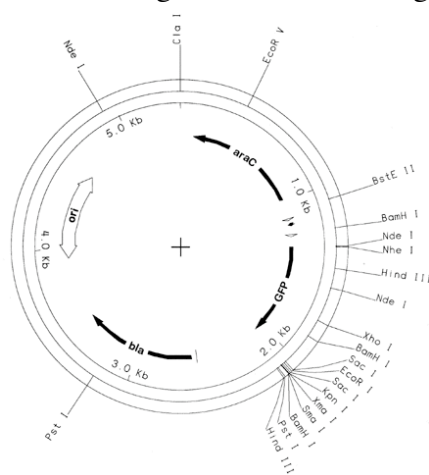
Genes can be cut from human, animal, or plant DNA and placed inside bacteria. For example, a healthy human gene for the hormone insulin can be put into bacteria. Under the right conditions, these bacteria can make authentic human insulin. This insulin can then be used to treat patients with the genetic disease, diabetes, whose insulin genes do not function normally.

In this lab you will be transforming the bacteria *Escherichia coli* with a gene that codes for Green Fluorescent Protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*. This gene codes for Green Fluorescent Protein, which causes the jellyfish to glow in the dark. Following the transformation procedure, the bacteria express their newly acquired jellyfish gene and produce the fluorescent protein, which causes them to glow a brilliant green color under UV light.



Jellyfish:
Aequorea victoria

You will be moving the gene for GFP into the *E. coli* with the plasmid pGLO. This plasmid encodes the gene for GFP and a gene for resistance to the antibiotic ampicillin.



pGlo Plasmid

The gene for GFP can be turned on in transformed cells by adding the sugar arabinose to the cells' nutrient medium. Selection for cells that have been transformed with the pGLO DNA is accomplished by growth on antibiotic plates. Transformed cells will appear white (wild type phenotype) on plates not containing arabinose, and fluorescent green when arabinose is included in the nutrient agar.

GENE REGULATION, ONE GENE, ONE PROTEIN

Our bodies contain thousands of different proteins which perform many different jobs. Digestive enzymes are proteins; some of the hormone signals that run through our bodies and the antibodies protecting us from disease are proteins. The information for assembling a protein is carried in our DNA. The section of DNA which contains the code for making a protein is called a gene. There are over 100,000 genes in the human genome. Each gene codes for a unique protein: one gene-one protein. The gene which makes a digestive enzyme in your mouth is different from one which makes an antibody or the pigments that color your eyes.

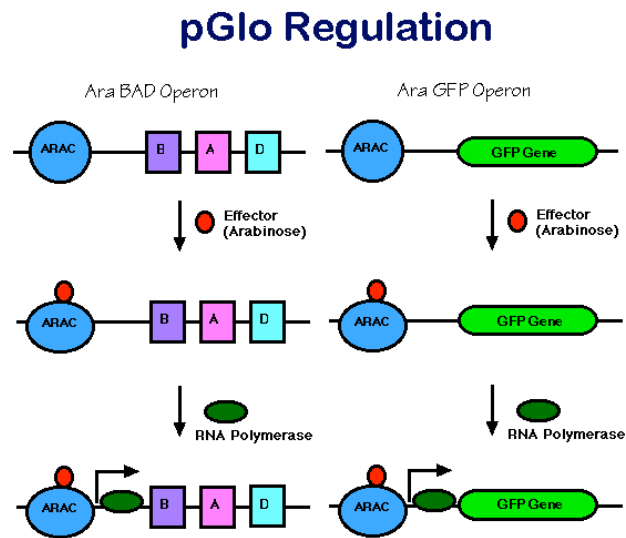
Organisms regulate expression of their genes and ultimately the amounts and kinds of proteins present within their cells for a myriad of reasons including developmental, cellular specialization and adaptation to the environment. Gene regulation not only allows for adaptation to differing conditions, but also prevents wasteful overproduction of unneeded proteins which would put the organism at a competitive disadvantage. The genes involved in the transport and breakdown (catabolism) of food are good examples of highly regulated genes. For example, the sugar arabinose is both a source of energy and a

source of carbon. *E. coli* bacteria produce three enzymes (proteins) needed to digest arabinose as a food source. The genes which code for these enzymes are not expressed when arabinose is absent, but they are expressed when arabinose is present in their environment. How is this so?

Regulation of the expression of proteins often occurs at the level of transcription from DNA into RNA. This regulation takes place at a very specific location on the DNA template, called a promoter, where RNA polymerase sits down on the DNA and begins transcription of the gene. In bacteria, groups of related genes are often clustered together and transcribed into RNA from one promoter. These clusters of genes controlled by a single promoter are called operons.

The three genes (*araB*, *araA* and *araD*) that code for three digestive enzymes involved in the breakdown of arabinose are clustered together in what is known as the arabinose operon.³ These three proteins are dependent on initiation of transcription from a single promoter, (P_{BAD}). Transcription of these three genes requires the simultaneous presence of the DNA template (promoter and operon), RNA polymerase, a DNA binding protein called *araC* and arabinose. *araC* binds to the DNA at the binding site for the RNA polymerase (the beginning of the arabinose operon). When arabinose is present in the environment, bacteria take it up. Once inside, the arabinose interacts directly with *araC* which is bound to the DNA. The interaction causes *araC* to change its shape which in turn promotes (actually helps) the binding of RNA polymerase and the three genes B, A and D, are transcribed. Three enzymes are produced, they do their job, and eventually the arabinose runs out. In the absence of arabinose the *araC* returns to its original shape and transcription is shut off.

The DNA code of the pGLO plasmid has been engineered to incorporate aspects of the arabinose operon. Both the promoter (P_{BAD}) and the *araC* gene are present. However, the genes which code for arabinose catabolism, *araB*, A and D, have been replaced by the single gene which codes for the Green Fluorescent Protein (GFP). Therefore, in the presence of arabinose, *araC* protein promotes the binding of RNA polymerase and GFP is produced. Cells fluoresce a brilliant green color as they produce more and more protein. In the absence of arabinose, *araC* no longer facilitates the binding of RNA polymerase and the GFP gene is not transcribed. When the GFP protein is not made, bacteria colonies will appear to have a wild type (natural) phenotype - of white colonies with no fluorescence.



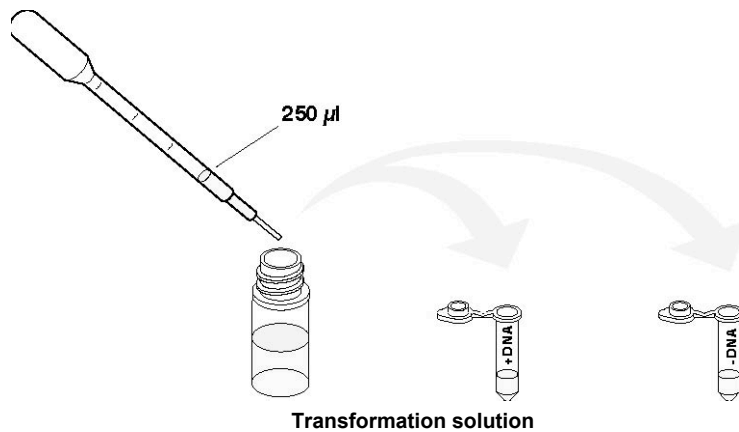
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TRANSFORMATION PROCEDURE

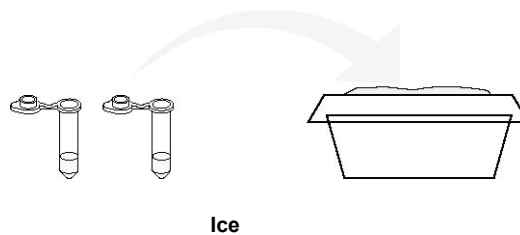
1. Label one closed micro test tube **+DNA** and another **-DNA**. Label both tubes with your group's name. Place them in the foam tube rack.



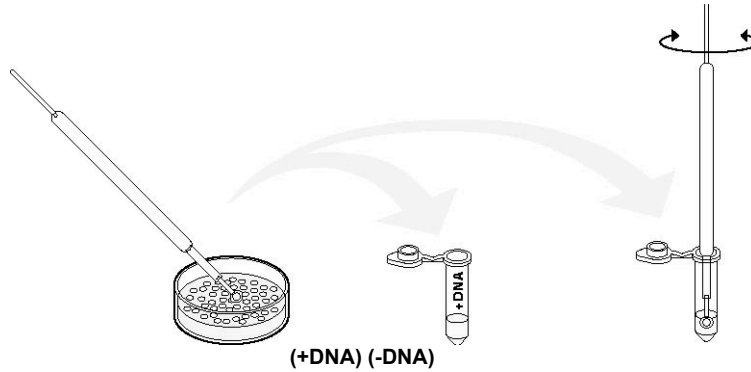
2. Open the tubes and, using a sterile transfer pipette, transfer 250 μ l of Transformation Solution (CaCl_2) into each tube.



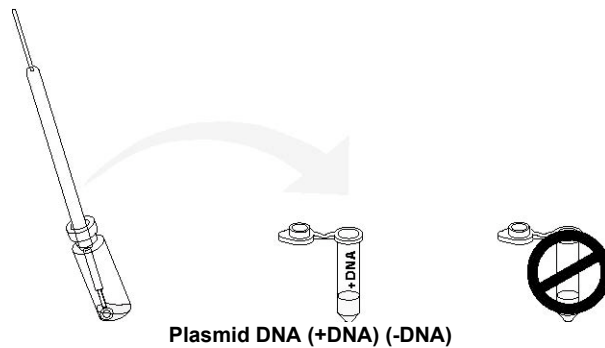
3. Place the tubes on ice.



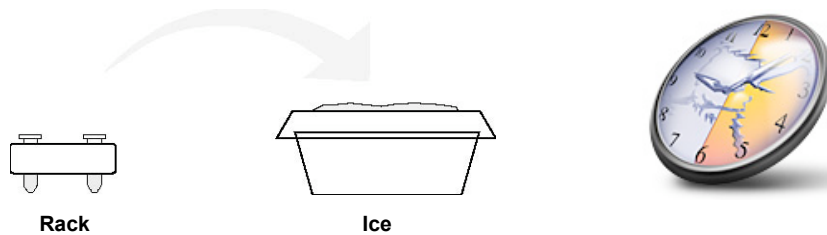
4. Use a sterile loop to pick up **one single colony of bacteria** from your starter plate. Pick up the **+DNA** tube and immerse the loop into the Transformation Solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the Transformation Solution (no floating chunks). Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the **-DNA** tube.



5. Examine the pGLO DNA solution with the UV lamp. Note your observations. Immerse a **new sterile loop** into the plasmid DNA stock tube. Withdraw a loop full. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loop full into the cell suspension of the **+DNA** tube. Close the tube and return it to the rack on ice. Also close the **-DNA** tube. **Do not** add plasmid DNA to the **-DNA** tube. Why not?

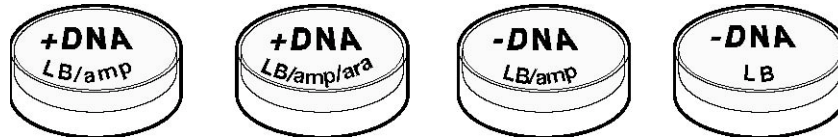


6. Incubate the tubes on ice for 10 minutes. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.



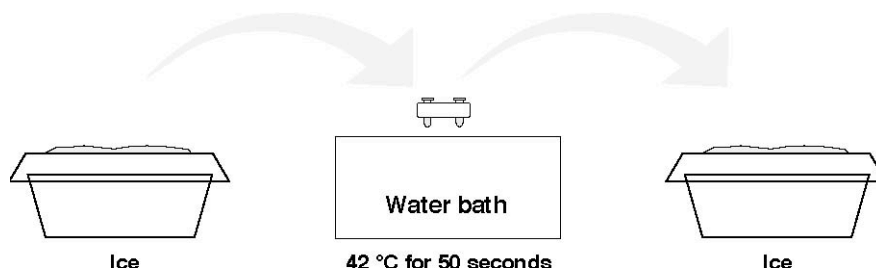
7. While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as follows:

- Label one **LB/amp** plate: **+ DNA**
- Label the **LB/amp/ara** plate: **+ DNA**
- Label the other **LB/amp** plate: **- DNA**
- Label the **LB** plate: **- DNA**

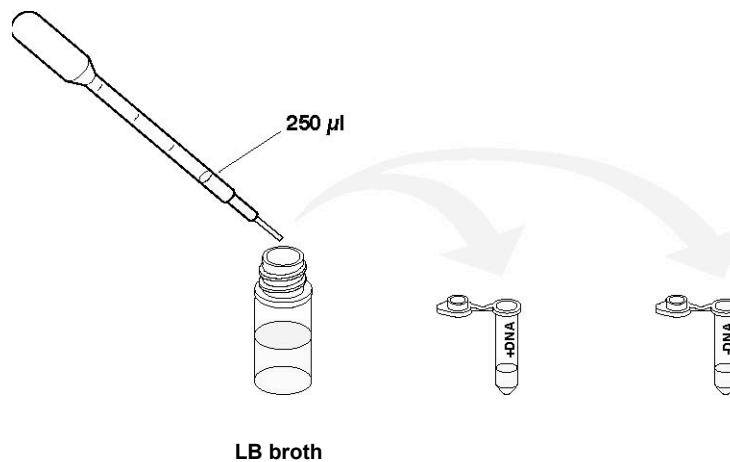


8. **Heat shock.** Using the foam rack as a holder, transfer both the (+) and (-) tubes into the water bath set at 42 °C for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water.

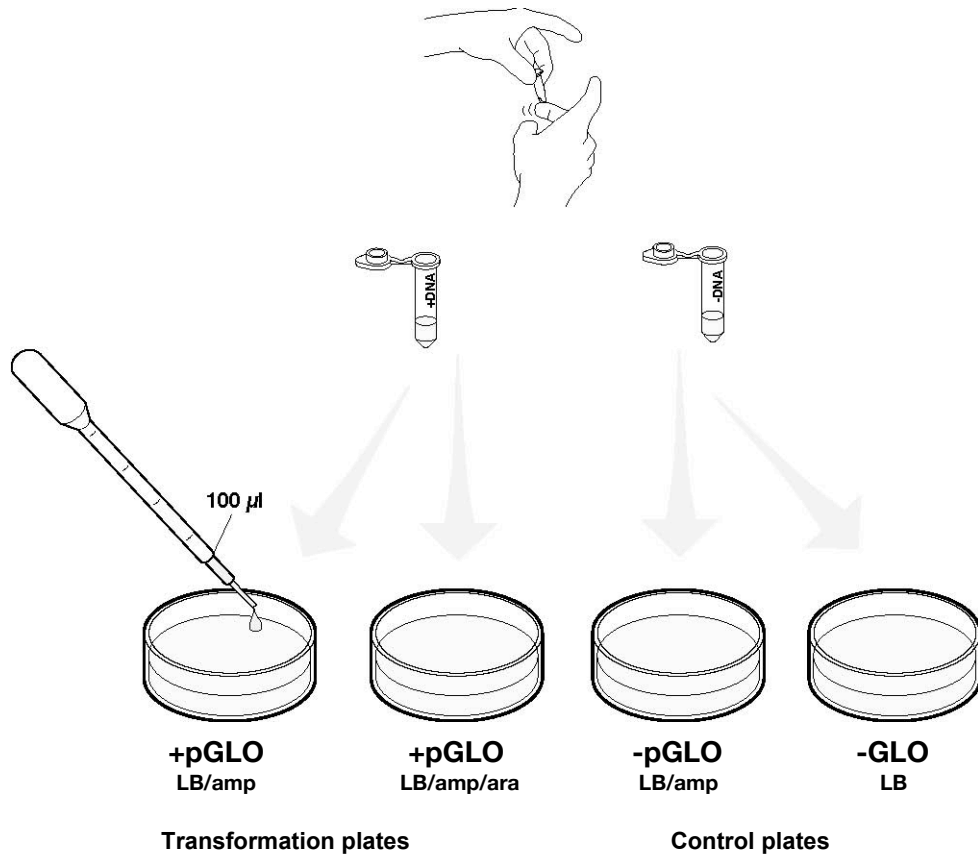
When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the change from the ice (0 °C) to 42 °C and then back to the ice must be rapid. Incubate tubes on ice for 2 minutes.



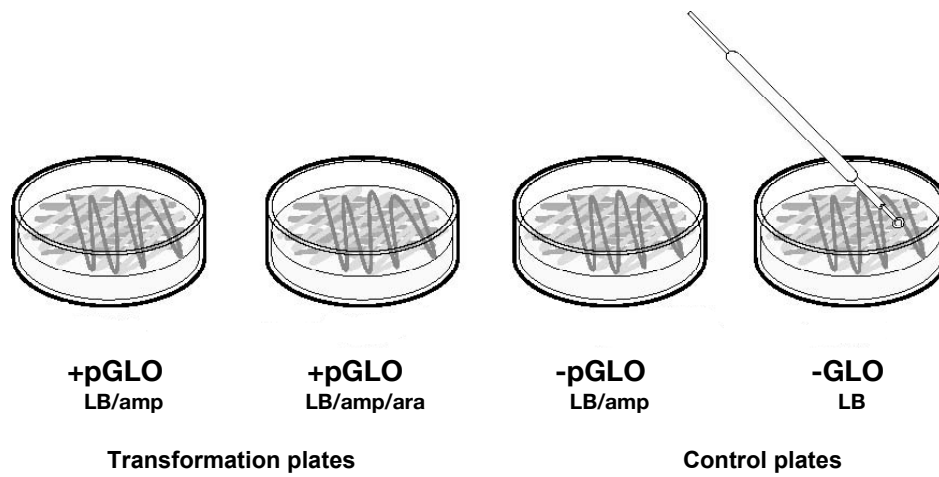
9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipette, add 250 μ l of LB broth to the tube and re-close it. Repeat with a new sterile pipette for the other tube. Incubate the tubes for 10 minutes at room temperature.



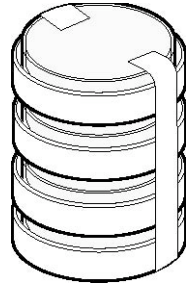
10. Tap the closed tubes with your finger to mix. Using a new sterile pipette for each tube, pipette 100 μ l of the transformation and control suspensions onto the appropriate plates.



11. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.



12. Stack up your plates and tape them together. Put your group name and class period on the bottom of the stack and place it **upside down** in the 37 °C incubator until the next day.



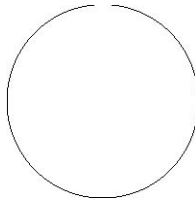
DATA COLLECTION

Observe the results you obtained from the transformation lab under normal room lighting. Then turn out the lights and hold the ultraviolet light over the plates.

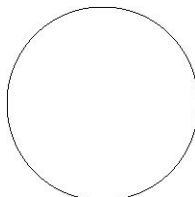
1. Observe and draw what you see on each of the four plates carefully. Put your drawings in the data table in the column on the right. Record your data to allow you to compare observations of the “+ DNA” cells with those you record for the non-transformed *E. coli*. Write down the following observations for each plate.
2. How much bacterial growth do you see on each, relatively speaking?
3. What color are the bacteria?
4. Count how many bacterial colonies there are on each plate (the spots you see).

Observations

+DNA LB/amp

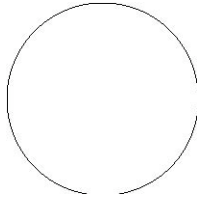


+DNA LB/amp/ara

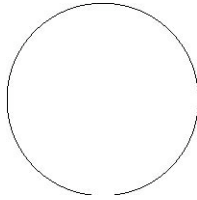


Observations

-DNA LB/amp



-DNA LB



ANALYSIS

Calculate Transformation Efficiency

Your next task in this investigation will be to learn how to determine the extent to which you genetically transformed *E. coli* cells. This quantitative number is referred to as the transformation efficiency.

In many experiments, it is important to genetically transform as many cells as possible. For example, in some types of gene therapy, cells are collected from the patient, transformed in the lab, and then put back into the patient. The more cells that are transformed to produce the needed protein, the more likely it is that the therapy will work. A number called transformation efficiency is calculated to help scientists determine how well the transformation is working.

The Task

You are about to engage in calculating the transformation efficiency from the information you collected in the laboratory procedure. Transformation efficiency gives you an indication of how effective you were in getting DNA molecules into a colony of bacterial cells. Transformation efficiency is a number. It represents the total number of bacterial cells that express the green protein divided by the amount of DNA used in the experiment. (It tells us the total number of bacterial cells transformed by one microgram of DNA.) In formula terms this can be symbolized as:

$$\text{Transformation efficiency} = \frac{\text{Total number of cells growing on the agar plate}}{\text{Amount of DNA spread on the agar plate}}$$

Therefore, before you can calculate the efficiency of your transformation, you will need two pieces of information:

- (1) The total number of green fluorescent colonies growing on your LB/amp/ara plate.
- (2) The total amount of DNA (pGLO) in the bacterial cells spread on the LB/amp/ara plate.

1. DETERMINING THE TOTAL NUMBER OF GREEN FLUORESCENT CELLS

Place your LB/amp/ara plate near a UV light source. Each colony on the plate can be assumed to be derived from a single cell. As individual cells reproduce, more and more cells are formed and develop into what is

termed a colony. The most direct way to determine the **total number of green fluorescent cells** is to count the colonies on the plate.

Enter that number here \Rightarrow

Total number of cells = _____

2. DETERMINING THE AMOUNT OF DNA (pGLO) IN THE BACTERIAL CELLS SPREAD ON THE LB/AMP/ARA PLATE

We need two pieces of information to find out the amount of DNA (pGLO) in the bacterial cells spread on the LB/amp/ara plate in this experiment. (a) What was the total amount of DNA we began the experiment with, and (b) What fraction of DNA (in the bacteria) actually got spread onto the LB/amp/ara plates.

Once you calculate this data, you will need to multiply the **total amount of DNA** Used in this experiment by the **fraction of DNA** you spread on the LB/amp/ara plate. The answer to this multiplication will tell you the amount of DNA (pGLO) in the bacterial cells that were spread on the LB/amp/ara plate.

a. Determining the Total Amount of DNA

The total amount of DNA we began with is equal to the product of the concentration and the total volume used, or

$$\text{DNA}(\mu\text{g}) = (\text{concentration of DNA}) \times (\text{volume of DNA } \mu\text{l})$$

In this experiment you used 10 μl of pGLO at concentration of 0.03 $\mu\text{g}/\mu\text{l}$. This means that each microliter of solution contained 0.03 μg of pGLO DNA. Calculate the **total amount of DNA** used in this experiment.

Enter that number here \Rightarrow

Total amount of DNA (μg) used in this experiment = _____
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b. Determining the fraction of DNA (in the bacteria) that actually got spread onto the LB/amp/ara plate

Since not all the DNA you added to the bacterial cells will be transferred to the agar plate, you need to find out what fraction of the DNA was actually spread onto the LB/amp/ara plate. To do this, divide the volume of DNA you spread on the LB/amp/ara plate by the total volume of liquid in the test tube containing the DNA. A formula for this statement is

$$\frac{\text{Volume spread on LB/AMP plate}}{\text{Total sample volume in test tube}}$$

Fraction of DNA used =

You spread 100 μl of cells containing DNA from a test tube containing a total volume of 510 μl of solution. Do you remember why there is 510 μl total solution? Look in the laboratory procedure and locate all the steps where you added liquid to the reaction tube. Add the volumes.

Use the above formula to calculate the **fraction of DNA** you spread on the LB/amp/ara plate.

Enter that number here \Rightarrow

Fraction of DNA = _____

So, how many micrograms of DNA did you spread on the LB/amp/ara plates?

To answer this question, you will need to multiply the **total amount of DNA used** in this experiment by the **fraction of DNA** you spread on the LB/amp/ara plate.

$$\text{pGLO DNA spread } (\mu\text{g}) = \text{Total amount of DNA used } (\mu\text{g}) \times \text{fraction of DNA}$$

Enter that number here \Rightarrow

pGLO DNA spread (μg) = _____
--

Look at all your calculations above. Decide which of the numbers you calculated belong in the table below. Fill in the following table.

Number of colonies on LB/amp/ara plate =	
Micrograms of pGLO DNA spread on the plates	

Now use the data in the table to calculate the efficiency of the pGLO Transformation

$$\text{Transformation efficiency} = \frac{\text{Total number of cells growing on the agar plate}}{\text{Amount of DNA spread on the agar plate}}$$

Enter that number here \Rightarrow

Transformation efficiency = _____ transformants/ μg
