I. Introduction to Bacterial Transformation

Background

In this lab, you will perform a genetic transformation, which literally means "change caused by genes." A gene is a piece of DNA that provides the instructions for making (it codes for, or encodes) a protein. This protein usually serves some cellular function and gives an organism a particular trait, also known as a phenotype. Genetic transformation involves inserting a gene into an organism in order to change a trait of that organism.

Genetic transformation is used in many areas of biotechnology. In the lab, bacterial transformation is a common method for producing more copies of genes (cloning) for insertion of that gene into a variety of model organisms. Expressing genes in model organisms can help determine the function of those genes in more complex organisms. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be genetically transformed into plants. In bioremediation, bacteria can be transformed with genes that enable them to digest oil spills. In medicine, diseases caused by defective genes are beginning to be treated by gene therapy; that is, by genetically transforming a sick person's cells with healthy copies of the defective gene that causes the disease.

Genes can be obtained from human, animal, or plant DNA and placed inside bacteria for a variety of purposes. One purpose is to use bacterial cells as microscopic factories, either to clone more copies of DNA or to produce a gene product. For example, the healthy human gene for the hormone insulin can be put into bacteria. Under the right conditions, these bacteria can make authentic human insulin, which can then be used to treat patients with diabetes, in whom insulin-producing cells do not function normally.

You will transform bacteria with a gene that codes for green fluorescent protein (GFP). The source of this gene is the bioluminescent jellyfish *Aequorea victoria*. When exposed to ultraviolet (UV) light, the GFP protein causes the jellyfish to fluoresce and glow green. Following the transformation procedure, your bacteria will express their newly acquired jellyfish gene and produce the fluorescent protein, which will cause them to glow a brilliant green under UV light.

In this activity, you will learn about the process of moving genes from one organism to another with the aid of a **plasmid**. In addition to one large chromosome, bacteria naturally contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for one or more traits that may be beneficial to bacterial survival. In nature, bacteria can transfer plasmids back and forth among themselves, allowing them to share genes, which may permit them to adapt to new environments. The development of bacterial resistance to antibiotics is often due to such transmission of plasmids.

Bio-Rad's unique pGLO plasmid contains several elements critical to its function:

- *Aequorea victoria* GFP gene, which confers green fluorescence in the presence of UV light when the gene is expressed
- *bla* gene, whose gene product confers ampicillin resistance to bacteria when it is expressed
- *araC* gene, a component of the arabinose operon whose encoded protein stimulates GFP gene expression from the P_{BAD} promoter when **arabinose** is present

Following bacterial transformation, selection for cells that have been transformed with pGLO DNA is accomplished by growth on **ampicillin** containing plates. When pGLO transformed bacterial cells are grown in nutrient medium containing **arabinose** as well, GFP expression is stimulated and the bacteria will glow brilliant green upon exposure to UV light. When **arabinose** is absent from the nutrient medium, the GFP gene remains turned off and the colonies appear white.

This is a an excellent example of the central dogma of biology in action; that is, **DNA > RNA > Protein > Trait.** For more detailed information on the **arabinose** operon and how it is used in the pGLO plasmid to regulate GFP expression, see Appendix B.

ThINQ! Exercises

What are other ways to use genetic transformation? Search the Internet and explain other real-world applications of genetic transformation that are used in biotechnology.

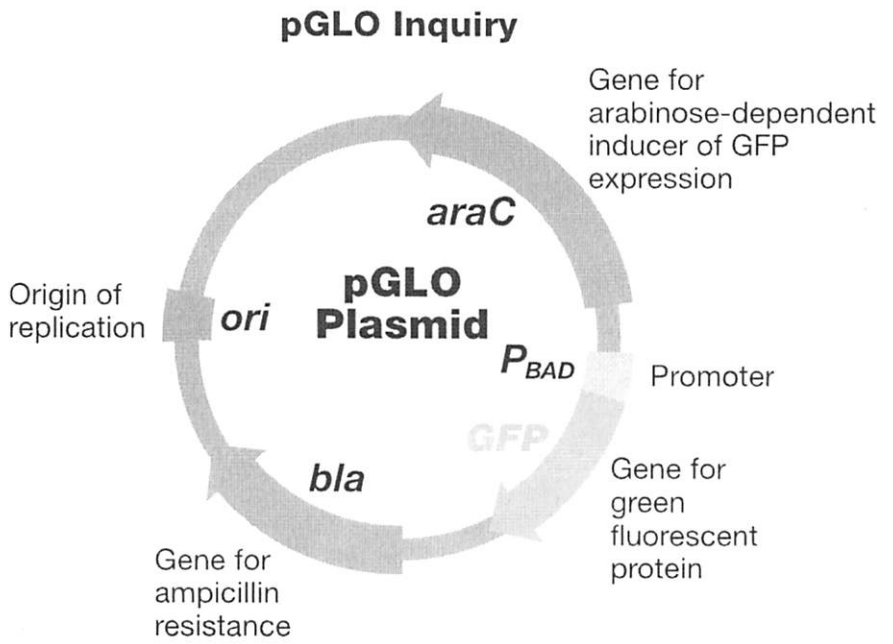
Define the following:

Plasmid:

LB nutrient medium:

Ampicillin:

Arabinose:



The pGLO plasmid. *GFP, green fluorescent protein; araC, gene for arabinose-dependent inducer of GFP expression; bla, gene for ampicillin resistance; ori, origin of replication.*

You will be provided with tools and a protocol for performing genetic transformation. Your task will be to:

1. **Perform the genetic transformation**. The procedure involves three main steps: two to move the pGLO plasmid DNA through the *E. coli* cell membrane and one to provide an environment for the cells to express the newly acquired genes.
   a) Incubate actively growing bacteria with plasmid DNA in $CaCl_2$ solution.
   b) Rapidly heat and cool the bacteria in a process known as heat shock during which the plasmid DNA enters the bacteria.
   c) Transfer the bacteria to growth media to recover and express their newly acquired genes.
2. **Assess the degree of success**. You will determine transformation efficiency.

Along the way, you will learn about the reasons for each step and how and to what extent each step influences gene transfer.

ThINQ! Exercises

Steps for designing a scientific investigation:

- Observe the natural world
- Ask questions about your observations
- Formulate a reasonable hypothesis to explain the observations
- Create and execute experiments testing the hypothesis and generating data
- Analyze the data, compare to the hypothesis, and communicate your findings

Define the following:

Phenotype:

Genotype:

Focus Questions

Scientific investigations begin with an observation about the natural world and the formulation of questions about that observation. Below are a few questions for you to ponder as you take on the challenge of performing a genetic transformation lab.

Question 1: Which organism should I choose, and why?

1. To genetically transform an entire organism, the new gene must be in every cell in the organism. Considering this, which organism would be the simplest to work with for total genetic transformation: one composed of many cells, or one composed of a single cell?

2. Scientists often want to know if the genetically transformed organism can pass its new traits on to its offspring and future generations. To get this information, which would be a better candidate for your investigation: an organism that develops each new generation quickly, or one that reproduces more slowly?

3. Safety is another important consideration in choosing an experimental organism. What traits or characteristics should the organism have (or not have) to be sure it will not harm you or the environment?

4. Based on the above considerations, which would be the best choice for a genetic transformation: bacterium, earthworm, fish, or mouse? Describe your reasoning.

Question 2: How can I tell if cells have been genetically transformed?

The goal of genetic transformation is to change an organism's traits, also known as its **phenotype**. Before a change in the phenotype can be detected, however, a thorough examination of its natural (pretransformation) phenotype must be made.

1. Describe how you could use two LB/agar plates, some *E. coli*, and some ampicillin 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?

II. Investigation #1: pGLO Bacterial Transformation Laboratory (Structured Inquiry)

In this activity, you will perform a bacterial transformation, transforming a stock *E. coli* culture with the pGLO plasmid.

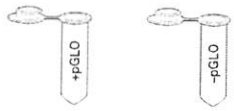
Student workstations	***Quantity***
☐ *E. coli* starter plate	1
☐ Poured nutrient agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)	4
☐ Transformation solution (1 ml)	1
☐ LB nutrient broth (1 ml)	1
☐ Inoculation loops (1 pk of 10)	7
☐ Disposable plastic transfer pipets (DPTPs)	4
☐ Foam microcentrifuge tube holder/float	1
☐ Container full of crushed ice (foam/paper cups)	1
☐ Microcentrifuge tubes	2
☐ Marking pen	1

Common workstation	***Quantity***
☐ Rehydrated pGLO plasmid, vial	1
☐ 42°C water bath and thermometer	1
☐ UV pen light	1
☐ 37°C incubator	1
☐ Clock or timer for counting seconds	1

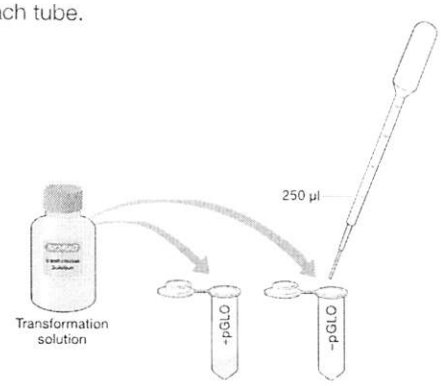
Optional	***Quantity***
☐ 2–20 µl adjustable volume micropipet	1
☐ 2–20 µl micropipet tips, box	1

Protocol

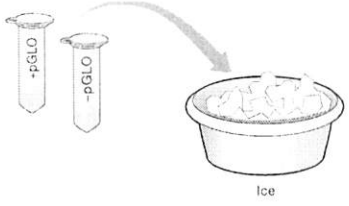
1. Label one microcentrifuge tube **+pGLO** and another **–pGLO**. Label both tubes with your group's name. Place them in the foam tube rack.



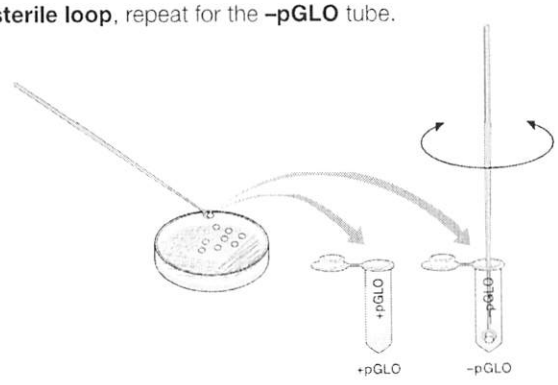
2. Open the tubes and use a sterile DPTP to transfer 250 ul of transformation solution (50 mM $CaCl_2$) into each tube.



3. Place the tubes on ice.



4. Use a sterile loop to pick **2–4 large colonies of bacteria** from the starter plate. Select colonies that are "fat" (1–2 mm in diameter). It is important to take individual colonies (not a swab of bacteria from the dense portion of the plate), since the bacteria must be actively growing to achieve high transformation efficiency. Pick up the **+pGLO** tube and immerse the loop into the transformation solution in the tube. Spin the loop between your index finger and thumb until the colonies are dispersed in the transformation solution (there are no floating chunks). Place the tube back in the tube rack in the ice. Using a **new sterile loop**, repeat for the **–pGLO** tube.



ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

Examine the bottle of pGLO plasmid DNA solution with the UV lamp. What do you see? Note your observations:

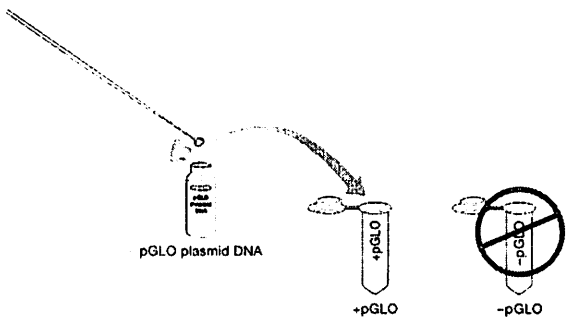
Look at the individual colonies of *E.coli* on your starter plates. On a separate piece of paper list all observable traits or characteristics that can be described. For example:

- Color of colonies
- Size of: 1) the largest colony; 2) the smallest colony; 3) the majority of colonies
- Shape of colonies (both 2-D and 3-D)
- Appearance of the colonies under regular and UV light

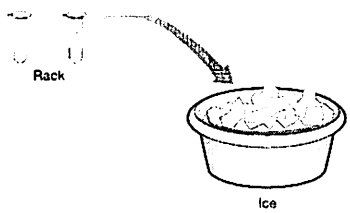
Why do you add the $CaCl_2$ transformation solution?

Why do you place the tubes on ice?

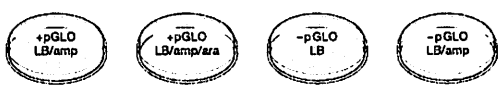
5. Immerse a **new sterile loop** into the tube of 0.8 x 10^{-1} µg/µl solution of pGLO plasmid DNA stock tube. Withdraw a loopful (10 µl). You should see a film of plasmid solution across the ring, similar to the soapy film across a ring for blowing soap bubbles. Mix the loop into the cell suspension of the **+pGLO** tube. **Do not add plasmid DNA to the –pGLO tube**. Close both the **+pGLO** and **–pGLO** tubes and return them to the rack on ice.



6. Incubate the tubes on ice for 10 min. Push the tubes all the way down in the rack so the bottoms of the tubes make contact with the ice.



7. While the tubes are on ice, label the four LB nutrient agar plates on the bottom (not the lid):
   - Label one **LB/amp** plate: **+pGLO**
   - Label the **LB/amp/ara** plate: **+pGLO**
   - Label the **LB** plate: **–pGLO**
   - Label the other **LB/amp** plate: **–pGLO**



ThINQ! Exercises

Collaborate and use outside resources to answer the following:

Approximately how much volume is picked up by the loop if the solution is 0.8 x 10^{-1} µg/µl and a loopful of solution contains 0.8 µg of pGLO plasmid?

Why do you incubate the tubes on ice for 10 minutes?

Why do you use only four LB nutrient agar plates, as opposed to 6 plates (2 LB, 2 LB/amp, and 2 LB/amp/ara)?

What would you expect to grow on the following LB nutrient agar plates?

+pGLO on LB:

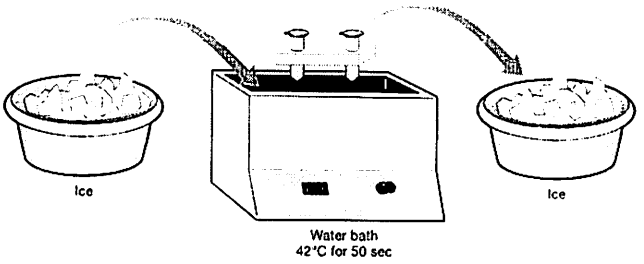
–pGLO on LB/amp/ara:

Why do you heat shock the cells?

Why do you incubate on ice for 2 minutes?

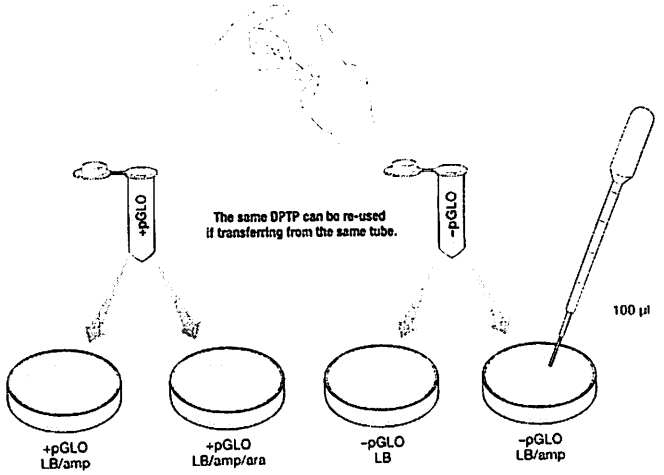
8. **Heat shock.** Using the foam holder as a rack, transfer both the **+pGLO** and **–pGLO** tubes into the **42°C** water bath for **exactly 50 sec.** Push the tubes all the way down in the rack so the bottoms of the tubes make contact with the warm water.

   When the 50 sec has passed, place both tubes back on ice. For best results, the transfer from the ice **(0°C)** to the **42°C** water and back to the ice must be rapid. Incubate tubes on ice for **2 min.**



9. Remove the rack of tubes from the ice and place it on the benchtop. Open a tube and use a new sterile DPTP to add 250 µl of LB nutrient broth. Close the tube. Use the same DPTP for the other tube. Incubate the tubes for 10 min at room temperature.

10. Gently flick the closed tubes with your finger to mix and resuspend the bacteria. Using a new sterile DPTP for each tube, pipet 100 µl of the transformation and control suspensions onto the appropriate agar plates.

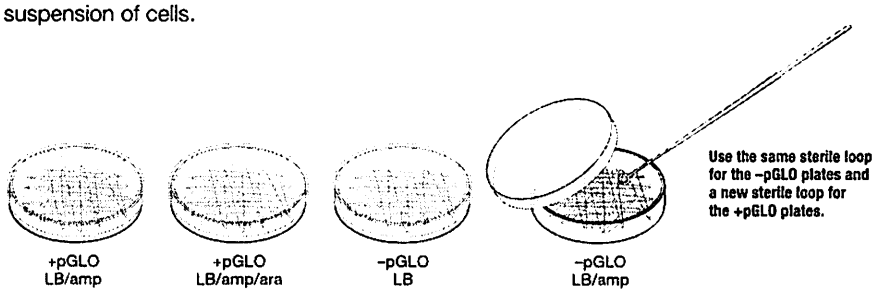


ThINQ! Exercises

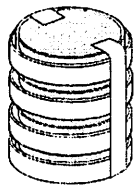
Collaborate and use outside resources to answer the following.

Which is your control plate? Why?

11. **Spread the suspensions evenly using one new sterile loop for the +pGLO plates and one new sterile loop for the –pGLO plates.** Spread the +pGLO LB/amp plate first, then the +pGLO LB/amp/ara plate. Then, using a new sterile loop for the –pGLO plates, spread the –pGLO LB plate first, then the –pGLO LB/amp plate. On the surface of each LB nutrient agar plate quickly skate the flat surface of the sterile loop back and forth across the plate surface. Do not press into the agar. Minimize contamination by uncovering one plate at a time and re-covering it immediately after spreading the suspension of cells.



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



IMPORTANT: After analyzing the results, **save transformed pGLO plates** for the inquiry labs that follow.

ThINQ! Exercises

Collaborate and use outside resources to answer the following questions:

Why do you need to place the stack of plates upside down?

Alternatively, you could incubate your plates at room temperature. What difference would you expect if plates were incubated at 37°C vs. room temperature (22°C)?

Review Questions:

On which of the plates would you expect to find bacteria most like the original untransformed *E. coli* colonies you initially observed? Explain your prediction.

If there are any transformed bacterial cells, on which plate(s) would they most likely be located? Explain your prediction.

Which plates should be compared to determine if any genetic transformation has occurred? Why?

Data Collection

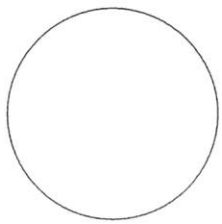
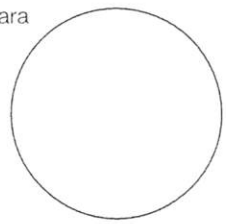
Observe the results you obtained from the transformation lab under normal room lighting. Then turn out the lights and hold the ultraviolet (UV) light over the plates.

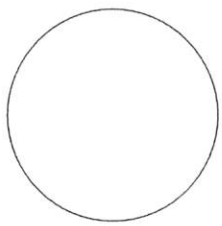
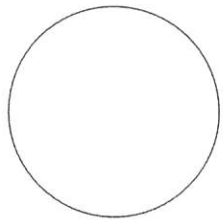
Carefully observe and draw what you see on each of the four plates. Put your drawings in the data table below. Record your data to allow you to compare your observations of the **+pGLO** cells with your observations of the non-transformed *E. coli*. Write down the following observations for each plate.

1. How much bacterial growth do you see on each plate, relatively speaking?

2. What color are the bacteria under normal light and UV light conditions?

3. How many bacterial colonies are on each plate (count the colonies you see).

	Observations
Transformation plates	+pGLO LB/amp 
	+pGLO LB/amp/ara 

	Observations
Control plates	–pGLO LB/amp 
	–pGLO LB 

Analysis of Results

The goal of this analysis is to determine whether genetic transformation has occurred.

1. How did the traits you originally observed for *E. coli* alter?

2. If the transformed cells have acquired the ability to grow in the presence of ampicillin, then what might be inferred about their ability to glow bright green under UV light?

3. From the results that you obtained, how could you provide evidence to support your hypothesis/argument that the changes that occurred were due to the procedure that you performed?

The Interaction between Genes and Environment

Look again at the four plates. Do you observe some *E. coli* growing on the LB plate that does not contain ampicillin or arabinose?

1. From your results, can you tell if these bacteria are ampicillin resistant by looking at them on the LB plate? Explain your answer.

2. What might happen to these bacteria if you moved them to plates containing ampicillin?

3. Often an organism's traits are caused by a combination of its genes and its environment. Think about the green color you saw in the genetically transformed bacteria as you consider these questions:

   a) What two factors must be present in the bacteria's environment for you to see the green color?

   b) Provide another example of a change in the environment causing expression of a different trait.

   c) What advantage would there be for an organism to be able to turn on or off particular genes in response to certain conditions?

Calculation of Transformation Efficiency

The next task in this investigation will be to determine how many of the *E. coli* cells were transformed. This quantitative measurement is referred to as transformation efficiency.

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

The Task

You are about to calculate the transformation efficiency for this experiment, which indicates how effective you were in getting new DNA molecules into bacterial cells.

Transformation efficiency is a number: the total number of colonies growing on the plate divided by the amount of DNA spread on the plate. It represents the total number of bacterial cells transformed using one microgram of DNA. Each colony on the plate can be assumed to derive from a single cell. As individual cells reproduce, more and more cells accumulate, developing into a colony. The most direct way to determine the total number of bacteria that were transformed with the pGLO plasmid is to count the number of colonies on the plate. Transformation efficiency is calculated using the following formula:

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the agar plate}}{\text{Amount of DNA spread on the agar plate (in µg)}}$$

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 pGLO plasmid DNA used for bacterial transformation that was spread on the LB/amp/ara plate.

1. Determining the Total Number of Transformed Green Fluorescent Colonies

Place the LB/amp/ara plate near a UV light source. Count the number of green fluorescent colonies that glow under UV light on the plate.

Enter that number here → Total number of colonies = _____

2. Determining the Amount of pGLO DNA in the Cells Spread on the LB/Amp/Ara Plate

You need two pieces of information to determine the amount of pGLO DNA in the bacterial cells spread on the LB/amp/ara plate in this experiment: (a) the total amount of DNA we began the experiment with, and (b) the fraction of the DNA (in the bacteria) that was spread onto the LB/amp/ara plates.

Once you calculate these data, you multiply the total amount of pGLO DNA used in this experiment by the fraction of DNA you spread on the LB/amp/ara plate. This will tell you the amount of pGLO DNA in the bacterial cells that were spread on the LB/amp/ara plate.

A. Determining the total amount of pGLO plasmid DNA

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

(DNA in µg) = (concentration of DNA in µg/µl) x (volume of DNA in µl)

In this experiment you used 10 µl of pGLO at a concentration of 0.08 µg/µl. This means that each microliter of solution contained 0.08 µg of pGLO DNA. Calculate the **total amount of DNA** used in this experiment

Enter that number here → Total amount of pGLO DNA, µg used in this experiment = _____

How will you use this piece of information?

B. Determining the fraction of pGLO plasmid DNA (in the bacteria)

Not all the DNA you added to the bacterial cells was transferred to the agar plate; therefore, you must determine the fraction of the DNA 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:

$$\text{Fraction of DNA used} = \frac{\text{Sample volume spread on LB/amp/ara plate, µl}}{\text{Total sample volume in microcentrifuge tube, µl}}$$

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 pGLO plasmid DNA** you spread on the LB/amp/ara plate.

Enter that number here ➔ | Fraction of DNA = _____ |

How will you use this piece of information?

C. So how many micrograms of pGLO DNA did you spread on the LB/amp/ara plate?

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

pGLO DNA spread (µg) = amount of DNA used (µg) x fraction of DNA

Enter that number here ➔ | pGLO DNA spread, µg = _____ |

What will this number tell you?

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 = _____
pGLO DNA spread on the plate, µg = _____

Now use the data in the table to calculate the efficiency of the pGLO transformation:

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the agar plate}}{\text{Amount of DNA spread on the agar plate (in µg)}}$$

Enter that number here → Transformation efficiency = _____ transformants/µg

The Analysis

Transformation efficiency calculations result in very large numbers. Scientists often use a mathematical shorthand, referred to as scientific notation, to express large numbers. For example, if the calculated transformation efficiency is 1,000 bacteria/µg of DNA, they often report this number as:

10^3 transformants/µg (10^3 is another way of saying 10 x 10 x 10 or 1,000)

1. How would scientists report 10,000 transformants/µg in scientific notation?

Carrying this idea a little further, suppose scientists calculated an efficiency of 5,000 bacteria/µg of DNA. This would be reported as:

5×10^3 transformants/µg (5 x 1,000)

2. How would scientists report 40,000 transformants/µg in scientific notation?

One final example: If 2,600 transformants/µg were calculated, then the scientific notation for this number would be:

2.6×10^3 transformants/µg (2.6 x 1,000)

Similarly:

$5{,}600 = 5.6 \times 10^3$ $271{,}000 = 2.71 \times 10^5$ $2{,}420{,}000 = 2.42 \times 10^6$

3. How would scientists report 960,000 transformants/µg in scientific notation?

4. Report your calculated transformation efficiency in scientific notation.

5. Use a sentence or two to explain what your calculation of transformation efficiency means.

Biotechnologists generally agree that the transformation protocol you just completed usually has a transformation efficiency of between 8.0×10^2 and 7.0×10^3 transformants per microgram of DNA.

6. How does your transformation efficiency compare with the above?

7. In the table below, report the transformation efficiency of several of the teams in the class.

Team	*Efficiency*

How does your transformation efficiency compare to theirs?

IMPORTANT: Save the **transformed pGLO plates** for the inquiry labs that follow.