Student Manual

pGLO Transformation

Lesson 1 Introduction to Transformation

In this lab you will perform a procedure known as a genetic transformation. Remember that a gene is a piece of DNA which provides the instructions for making (coding for) a protein which gives an organism a particular trait. Genetic transformation literally means change caused by genes and it involves the insertion of a gene(s) into an organism in order to change the organism's trait(s). Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be genetically transformed into plants. In bio-remediation, bacteria can be genetically transformed with genes enabling 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 gene involved in their disease.

You will use a procedure to transform bacteria with a gene that codes for a Green Fluorescent Protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*. The gene codes for a Green Fluorescent Protein which causes the jellyfish to fluoresce and 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 ultraviolet 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 allowing them to share these beneficial genes. This natural mechanism allows bacteria to adapt to new environments. The recent occurrence of bacterial resistance to antibiotics is due to the transmission of plasmids.

Bio-Rad's unique pGLO plasmid encodes the gene for the Green Fluorescent Protein (GFP) and a gene for resistance to the antibiotic, ampicillin. pGLO also incorporates a special gene regulation system which can be used to control expression of the fluorescent protein in transformed cells. The gene for the Green Fluorescent Protein can be switched on in transformed cells by adding the sugar, arabinose, to the cells nutrient medium. Selection for cells that have been transformed with 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.

You will be provided with the tools and a protocol for performing genetic transformation.

Your task will be:

1. To do the genetic transformation.
2. To determine the degree of success in your efforts to genetically alter an organism.

Lesson 1 Focus Questions

There are many considerations that need to be thought through in the process of planning a scientific laboratory investigation. Below are a few for you to ponder as you take on the challenge of doing a genetic transformation.

Since scientific laboratory investigations are designed to get information about a question, our first step might be to formulate a question for this investigation.

Consideration 1 Can I genetically transform an organism? Which organism?

1. To genetically transform an entire organism, you must insert the new gene(s) into every cell in the organism. Which organism is better suited 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 in which each new generation develops and reproduces quickly, or one which does this 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 won't harm you or the environment?

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

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

Recall that the goal of genetic transformation is to change an organism's traits (phenotype). Before any change in the phenotype of an organism can be detected, a thorough examination of its natural (pre-transformation) phenotype must be made. Look at the colonies of *E. coli* on your starter plates. List all observable traits or characteristics that can be described:

The following pre-transformation observations of *E. coli* might provide base-line data to make reference to when attempting to determine if any genetic transformation has occurred.

a) Number of colonies

b) Size of : 1) the largest colony
2) the smallest colony
3) the majority of colonies

c) Color of the colonies

d) Distribution of the colonies on the plate

e) Visible appearance when viewed with ultraviolet (UV) light

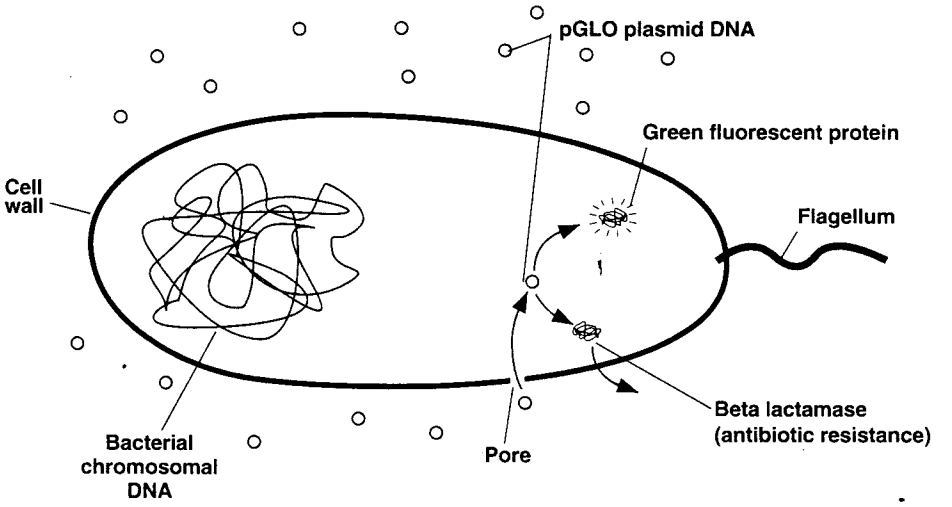
f) The ability of the cells to live and reproduce in the presence of an antibiotic such as ampicillin

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 ampicillin has on the *E. coli* cells?

Consideration 3 The Genes

Genetic transformation involves the insertion of some new DNA into the *E. coli* cells. In addition to one large chromosome, bacteria often contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for more than one trait. Scientists can use a process called genetic engineering to insert genes coding for new traits into a plasmid. In this case, the pGLO plasmid carries the gene (GFP) which produces the green fluorescent protein and a gene (*bla*) that codes for a protein that gives the bacteria resistance to an antibiotic. The genetically engineered plasmid can then be used to genetically transform bacteria to give them this new trait(s).



Consideration 4 The Act of Transformation

This transformation procedure involves three main steps. These steps are intended to introduce the plasmid DNA into the *E. coli* cells and provide an environment for the cells to express their newly acquired genes.

To move the plasmid DNA - pGLO through the cell membrane you will:

1. Use a transformation solution of $CaCl_2$ (calcium chloride)
2. Carry out a procedure referred to as heat shock

For transformed cells to grow in the presence of ampicillin you must:

3. Provide them with nutrients and a short incubation period to begin expressing their newly acquired genes

Lesson 2 Transformation Lab

Workstation Check (✔) List

Your workstation: Materials and supplies that should be present at your workstation prior to beginning this lab are listed below.

Student workstations	Number required	(✔)
E. coli starter plate	1	❑
Poured agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)	4	❑
Transformation Solution	1	❑
LB broth	1	❑
Inoculation loops	7 (1 pk of 10)	❑
Pipettes	5	❑
Foam microtube holder/float	1	❑
Container full of crushed ice (Styrofoam cup)	1	❑
Marking pen	1	❑
Copy of Quick Guide	1	❑

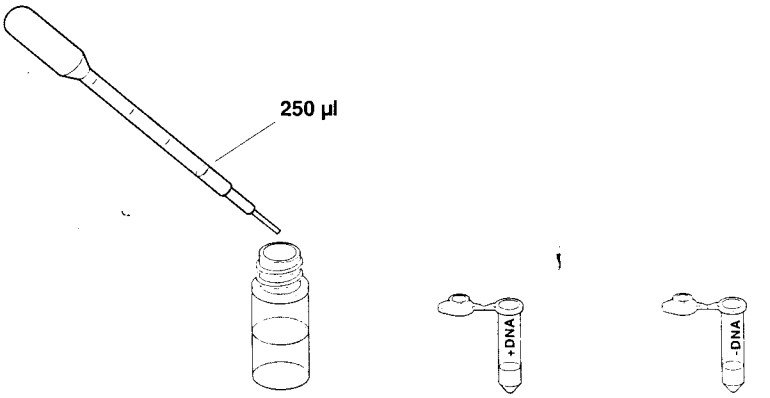
Instructors (common) Workstation. A list of materials, supplies and equipment that should be present at a common location to be accessed by your team is also listed below.

Hydrated pGLO plasmid	1 vial	❑
42 °C water bath and thermometer	1	❑
37 °C incubator (optional, see General lab Skills-Incubation)	1	❑

Transformation Procedure

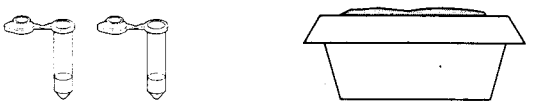
1. Label one closed micro test tube **+DNA** and another **-DNA**. Label both tubes with your group's name. Place them in the foam tube rack.

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



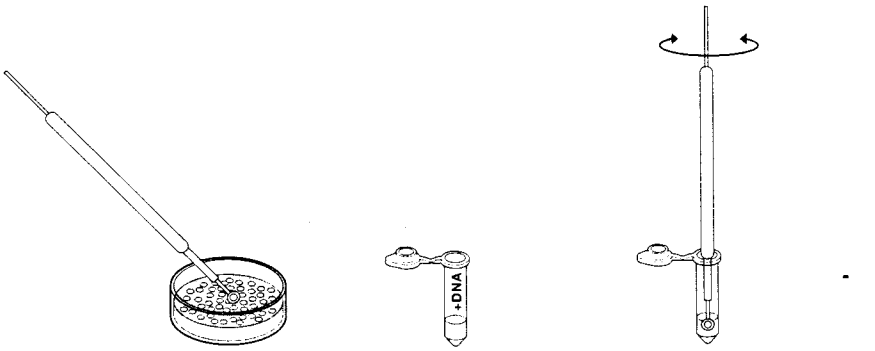
Transformation solution

3. Place the tubes on ice.



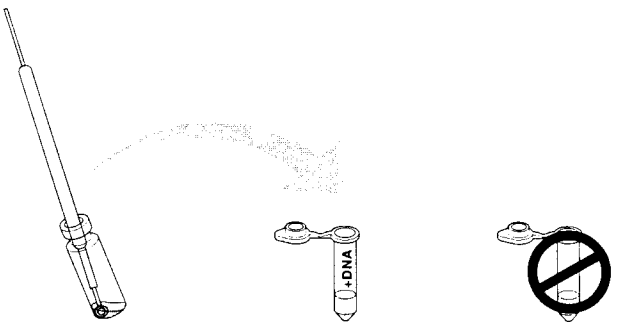
Ice

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.



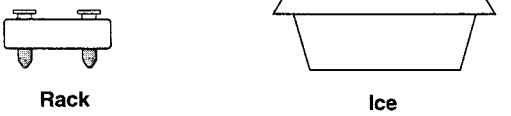
(+DNA) (-DNA)

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?



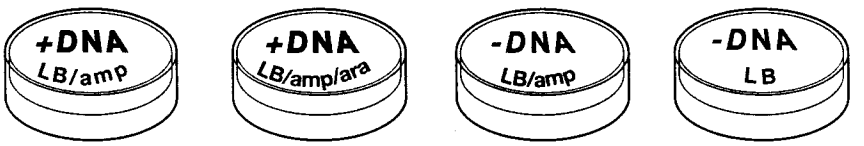
Plasmid DNA (+DNA) (-DNA)

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.



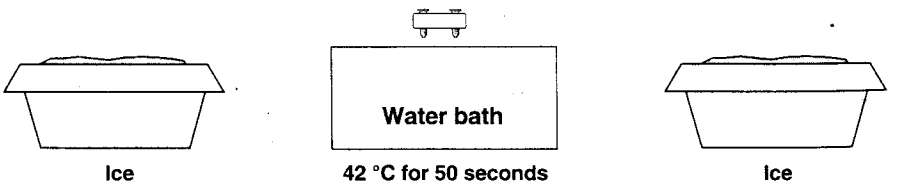
7. While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as follows:

- 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**

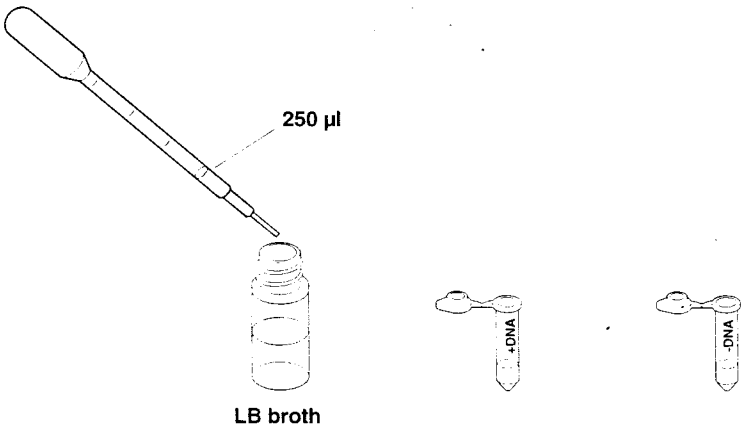


8. **Heat shock.** Using the foam rack as a holder, transfer both the (+) and (-) tubes into the water bath set at 42 °C for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water.

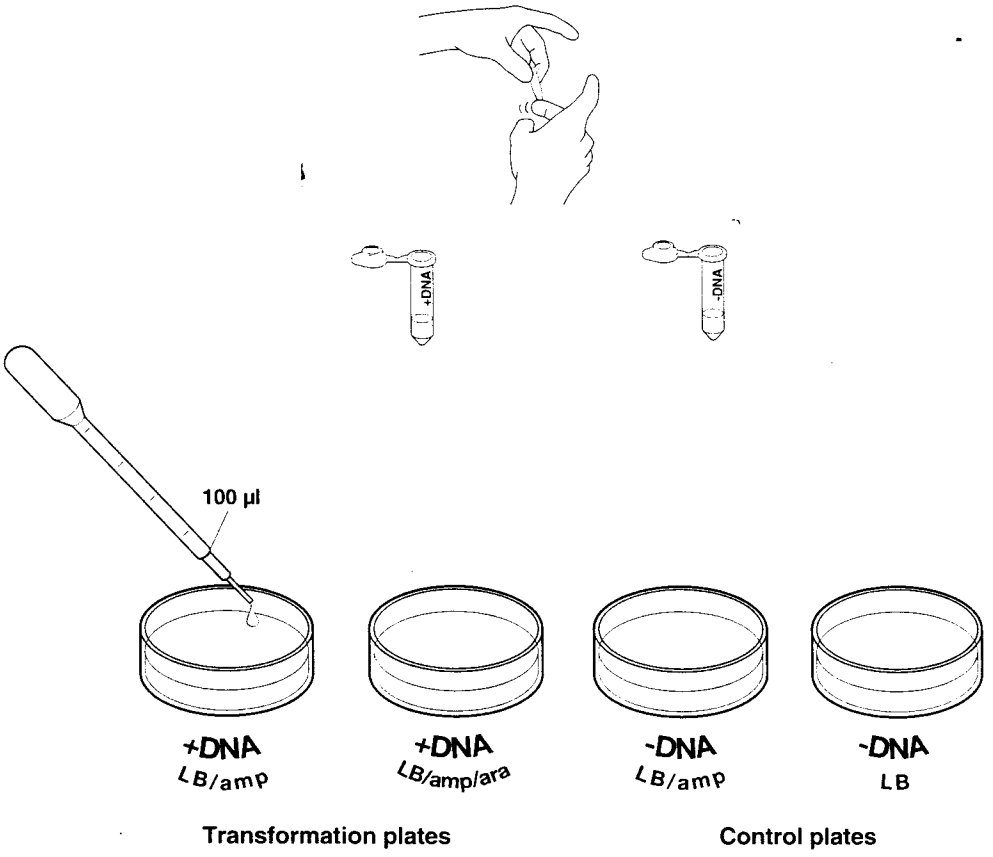
   When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the change from the ice (0 °C) to 42 °C and then back to the ice must be rapid. Incubate tubes on ice for 2 minutes.



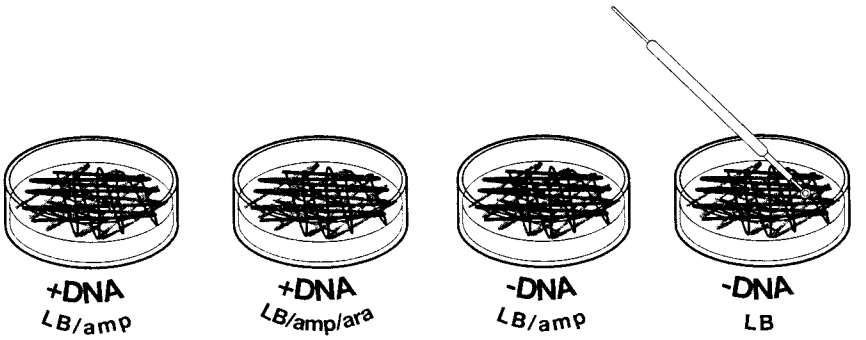
9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipette, add 250 µl of LB broth to the tube and re-close it. Repeat with a new sterile pipette for the other tube. Incubate the tubes for 10 minutes at room temperature.



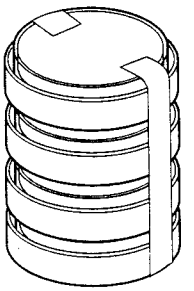
10. Tap the closed tubes with your finger to mix. Using a new sterile pipette for each tube, pipette 100 µl of the transformation and control suspensions onto the appropriate plates.



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.



Lesson 2 Review Questions Name _____

Before collecting data and analyzing your results answer the following questions.

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

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

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

4. What is meant by control plate? What purpose does a control serve?

Lesson 3 Data Collection and Analysis

A. 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).

Transformation plates

	Observations
+DNA LB/amp	
+DNA LB/amp/ara	

Control plates

	Observations
-DNA LB/amp	
-DNA LB	

Lesson 4 Extension Activity

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 ⇨

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

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

In this experiment you used 10 µl of pGLO at concentration of 0.03 µg/µl. 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 ⇨

Total amount of DNA (µg) used in this experiment = _____

How will you use this piece of information?

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

$$\text{Fraction of DNA used} = \frac{\text{Volume spread on LB/AMP plate}}{\text{Total sample volume in test tube}}$$

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 ⇨ Fraction of DNA = _____

- How will you use this piece of information?

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.

pGLO DNA spread (µg) = Total 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 =	
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 ⇨ Transformation efficiency = _____ transformants/µg

Analysis

Transformation efficiency calculations result in very large numbers. Scientists often use a mathematical shorthand referred to as scientific notation. 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)

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

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

5 x 10^3 transformants/µg (1,000 times 5)

- 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 (1,000 times 2.6)

Similarly:

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

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

- Report your calculated transformation efficiency in scientific notation.

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

Biotechnologists are in general agreement that the transformation protocol that you have just completed generally has a transformation efficiency of between 8.0×10^2 and 7.0×10^3 transformants per microgram of DNA.

- How does your transformation efficiency compare with the above?

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

Team	Efficiency

- How does your transformation efficiency compare with theirs?

- Calculate the transformation efficiency of the following experiment using the information and the results listed below.

DNA plasmid concentration—0.03 µg/µl

250 µl $CaCl_2$ transformation buffer

10 µl plasmid solution

250 µl LB broth

100 µl cells spread on agar

227 colonies of transformants counted

Fill in the following chart and show your calculations to your teacher:

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

- Extra Credit Challenge:

If a particular experiment was known to have a transformation efficiency of 3×10^3 bacteria/µg of DNA, how many transformant colonies would be expected to grow on the LB/amp/ara plate? You can assume that the concentration of DNA and fraction of cells spread on the LB agar are the same for the pGLO lab.