pGLO TRANSFORMATION EFFICIENCY

The transformation efficiency is a quantitative number that shows the extent to which you genetically transformed *E. coli* cells in this experiment. 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.

Transformation efficiency is a number that 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:

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

So…you need two pieces of information:

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

1. 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.____________________

2. The total amount of DNA we began with is equal to the product of the concentration and the total volume used:

DNA (ug) = (concentration of DNA) x (volume of DNA ul)

In this experiment you used 10 ug of pGLO at concentration 0.03 ug/ul. This means that each microliter of solution contained 0.03 ug of pGLO DNA. So now you can calculate the total amount of DNA used in this experiment.

3. Since not all the DNA you added to the bacterial cells will be transformed to the agar plate, you need to find out what fraction of the DNA was actually spread onto the LB/amp/ara plate. Divide the volume of DNA you spread on this plate by the total volume of liquid in the test tube containing the DNA.

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

You spread 100ul of cells containing DNA from a test tube containing a total volume of 510ul of solution. Calculate the Fraction of DNA you spread on the LB/amp/ara plate.

4. So now you can calculate how many micrograms of DNA you spread on the LB/amp/ara plate. You need to multiply the total amount of DNA used in the experiment by the fraction of DNA you spread on the LB/amp/ara plate.

pGLO DNA spread (ug) = Total amount of DNA used (ug) x fraction of DNA

5. Fill in the following table. Decide which numbers you calculated belong in the 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 from the formula given at the beginning of this lesson.

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 1000 bacteria/ug of DNA they often report this number as
10^3 transformants/ug. Suppose that an efficiency is calculated as 5000 bacteria/ug of DNA. This would be reported as 5×10^3 transformants/ug. If 2600 transformants/ug were calculated, then the scientific notation for this number would be 2.6×10^3 transformants/ug.

1. Write the following transformation efficiencies in scientific notation:
   a. 10,000 transformants/ug

   b. 40,000 transformants/ug

   c. 960,000 transformants/ug

   d. Write your own transformation efficiency in scientific notation.

2. 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 predicted amount? How can you account for any differences?

3. List the transformation efficiency for other groups in the class. How does your transformation efficiency compare with theirs?

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

DNA plasmid concentration = 0.03 ug/ul

250 ul $CaCl_2$ transformation buffer

10 ul plasmid solution

250 ul LB broth

100 ul cells spread on agar

227 colonies of transformants counted