

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 measurement 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 laboratory, and then put back into the patient. The more cells that are transformed to produce the needed protein, the more likely that the therapy will work. The transformation efficiency is calculated to help scientists determine how well the transformation is working.

The Task

You are about to calculate the transformation efficiency, which gives you an indication of how effective you were in getting DNA molecules into 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.) The transformation efficiency is calculated using the following formula:

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

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 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. 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 bacteria that were transformed with the pGLO plasmid** is to count the colonies on the plate.

Enter that number here →

Total number of colonies =

2. Determining the Amount of pGLO DNA in the Bacterial Cells Spread on the LB/amp/ara Plate

We need two pieces of information to find out the amount of pGLO DNA 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 the DNA (in the bacteria) actually got spread onto the LB/amp/ara plates.

Once you calculate this data, you will 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

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

In this experiment you used 10 μl of pGLO at concentration of 0.08 $\mu\text{g}/\mu\text{l}$. 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) 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

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

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?

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

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.

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

Enter that number here →

pGLO DNA spread (μg) = _____
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- 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

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

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 $10 \times 10 \times 10$ 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:

5×10^3 transformants/ μg ($5 \times 1,000$)

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

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

DNA plasmid concentration: 0.08 $\mu\text{g}/\mu\text{l}$

250 μl CaCl_2 transformation solution

10 μl pGLO plasmid solution

250 μl LB broth

100 μl cells spread on agar

227 colonies of transformants

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 were 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 as that of the pGLO laboratory.