Transformation Efficiency Calculation

DNA UNIT PRESENTATIONS

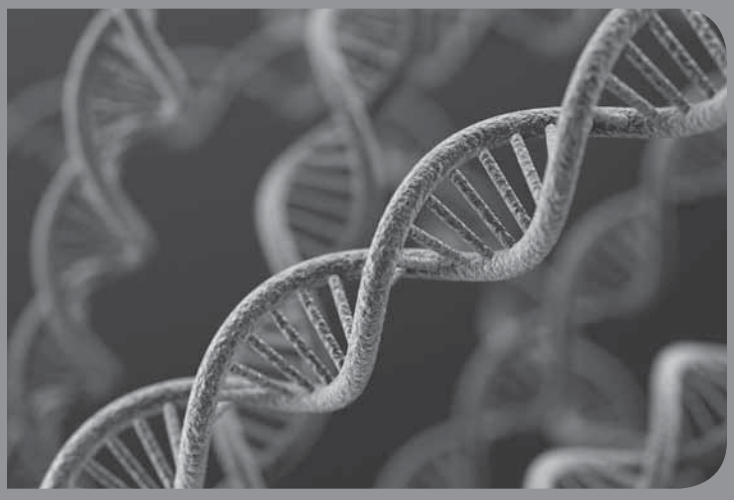
WORK ON BEAN BEETLE PRESENTATIONS FOR NEXT WEEK

LEARNING OBJECTIVES

1. Calculate transformation efficiency from pGLO transformation experiment.
2. Communicate central dogma/gene transformation information to various target audiences.
3. Develop an oral and written presentation of a semester-long experiment of your design.

INTRODUCTION

In the previous lab, you learned how to transform bacteria with the pGLO gene (using pGLO plasmid), which encodes the green fluorescent protein (GFP) of the bioluminescent jellyfish *Aequorea victoria*. In this lab, you will begin investigations with the bacteria you genetically transformed with the plasmid, pGLO. You will learn to multiply bacteria (containing the pGLO plasmid) for producing large quantities of GFP.



Lab 3

One of the basic tools of modern biotechnology is DNA splicing: cutting DNA and linking it to other DNA molecules. The basic concept behind DNA splicing is to remove a functional DNA fragment—let's say a gene—from one organism and combine it with the DNA of another organism in order to make the protein that gene codes for. The desired result of gene splicing is for the recipient organism to carry out the genetic instructions provided by its newly acquired gene. For example, certain plants can be given the genes for resistance to pests or disease, and in a few cases to date, functional genes can be given to people with nonfunctional or mutated genes, such as in a genetic disease like cystic fibrosis.

Genes can be cut out of human, animal, or plant DNA and placed inside bacteria (using plasmids). 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. When allowed to multiply in gigantic vats (fermenters) these bacteria can be used to mass produce the human insulin protein. This genetically engineered insulin is purified using protein chromatography and used to treat patients with the genetic disease diabetes, whose insulin genes do not function normally.

A common problem in purifying genetically engineered "designer" proteins from transformed bacteria is contamination by endogenous bacterial proteins. Chromatography is a powerful method used in biotechnology industry for separating and purifying proteins of interest from other proteins (e.g., bacterial proteins). Proteins purified by chromatography can be used as medicine to treat human disease, or for household agents such as natural enzymes to make better laundry detergents. The cloning and expressing of the GFP gene, followed by the purification of its protein, is completely analogous to the processes used in the biotechnology industry to produce and purify proteins with commercial value.

The pGLO plasmid that contains the GFP gene also contains the gene for beta-lactamase, a protein that provides bacteria with resistance to the antibiotic ampicillin. The beta-lactamase protein is produced and secreted by bacteria that contain the plasmid. The secreted beta-lactamase inactivates ampicillin, allowing only pGLO cells to grow in its presence.

The bacteria that were transformed with the pGLO plasmid were plated onto LB/amp and LB/amp/ara agar plates. Expression of the GFP gene is under the regulatory control of the arabinose promoter. Thus, when the bacteria were grown on LB agar containing arabinose (LB/amp/ara), GFP was expressed and the colonies appeared bright green. Conversely, when the bacteria were grown on LB agar that did not contain arabinose (LB/amp), the gene was turned off and the colonies appeared white.

ACTIVITY 1: DATA COLLECTION FROM TRANSFORMATION PLATES

Observe the results you obtained from the previous week's transformation lab under normal room lighting. Then turn out the lights and hold the ultraviolet light over the plates. **NOTE: To prevent damage to your skin or eyes, avoid exposure to the UV light. Never look directly into the UV lamp. Wear safety glasses for prolonged viewing.**

1. Observe carefully and sketch what you see on each of the four plates. 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. Under UV light, use a Sharpie marker to place a dot on each transformed (glowing) colony.

Table 1. Observed results.

E. coli Transformation with pGLO	–DNA LB	–DNA LB/amp	+DNA LB/amp	+DNA LB/amp/ara
# of colonies				
Amp resistance?				
White or green under normal light?				
White or green under UV light?				
GFP expressed?				

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

3. If you picked a colony from the fourth column of the table above (+DNA, LB/amp) and plated it on a new dish containing LB/amp/ara, what would happen? Would it grow? Would it glow? Justify your answer.

4. If the genetically transformed cells have acquired the ability to live in the presence of the antibiotic ampicillin, then what might be inferred about the other genes on the plasmid that you used in your transformation procedure?

5. From the results you obtained, how would you know these changes were due to the procedure that you performed?

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

ACTIVITY 2: CALCULATING 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 (each colony grew from a single cell). 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.

Transformation efficiency 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:

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the LB/amp/ara + DNA plate}}{\text{Amount of DNA spread on the plate}}$$

Transformation efficiency = _____ # of transformants/µg

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)

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.

Start the calculations to determine your group transformation efficiency below.

DETERMINING THE AMOUNT OF DNA (pGLO) IN THE BACTERIAL CELLS SPREAD ON THE LB/amp/ara PLATE

Be sure to include the units as you set up each calculation and in each of your answers below.

$$\textbf{DNA(µg)} = (\text{concentration of DNA}) \times (\text{volume of DNA µL})$$

1. In this experiment you used 10 µL of pGLO at a 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.

   Show the calculation:

   Enter that total here:

2. 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:

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

   You spread 100 µL of cells containing DNA from a test tube containing a total volume of 510 µL of solution. (Look in the laboratory procedure and locate all the steps where you added liquid to the reaction tube.)

   What is in this 510 µL of solution? List each component and corresponding volume.

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

   Show the calculation:

   Enter that total here:

3. Determine how many micrograms of DNA 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) × fraction of DNA

Show the calculation:

Enter that total here:

4. Using these calculations from above:

Transformation efficiency =

$$\frac{\text{Total number of colonies growing on the LB/amp/ara + DNA plate}}{\text{Amount of DNA spread on the plate}}$$

Show the calculation:

Enter that total here:

Transformation efficiency = _____ transformants/µg

Transformation efficiency written in scientific notation: _____ transformants/µg

Record your data in scientific notation along with the class data in Table 2 and answer the questions at the end of this unit.

Table 2. Class results.

	# of Transformed Colonies on the LB/amp/ara Plate	Calculated Transformation Efficiency
Group 1		
Group 2		
Group 3		
Group 4		
Group 5		
Group 6		

DISCUSSION QUESTIONS

TRANSFORMATION EFFICIENCY CALCULATION

Name

Date *Section*

1. How does your transformation efficiency compare with the above ideal range of efficiency?

2. How does your transformation efficiency compare with other groups in your class?

3. What might account for these differences?

Before you leave lab, complete this lab notebook and turn it in to your TA.