

Bacterial transformation efficiency: e.coli with pglo



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Bacterial Transformation Efficiency in E. Coli with pGLO Plasmids

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Introduction

“ The conversion of one genotype into another by the introduction of exogenous DNA (that is, bits of DNA from an external source) is termed transformation. The transformation was discovered in *Streptococcus pneumoniae* in 1928 by Frederick Griffith; in 1944, Oswald T. Avery, Colin M. MacLeod, and Maclyn McCarty demonstrated that the “ transforming principle” was DNA. Both results are milestones in the elucidation of the molecular nature of genes.” ¹

Bacteria transformation is the process of a bacteria absorbing and expressing foreign genetic information using plasmids. Plasmids are small circular molecules of DNA that holds a small number of genes. The plasmids used in the experiment have the ampicillin resistance gene. Ampicillin (amp) is an antibiotic used to kill bacteria such as E. coli, the bacteria used in the experiment. E. coli (*Escherichia coli*) is a simple bacterium commonly found in our body's and in everyday life but most commonly found in mammal's intestines. *Glowing Fluorescent Proteins* (GFP's) is the gene found in jellyfish that holds bioluminescent properties and “ glow” under UV light. By knowing the location of the gene, scientists can “ cut out” the GFP gene from the jellyfish DNA. They do this using restriction enzymes to which recognize and cut DNA in a specific region of nucleotides to acquire a specific gene. Once the gene is isolated, it can be used in the experiment and “ glued” into a plasmid that contains the AMP gene. This is done by sticky ends as the

Jellyfish DNA binds to the amp resistance plasmid using hydrogen bonds which are then sealed by DNA ligase. This creates pGLO a plasmid which is used in the experiment in the transformation of the bacteria. Before it can be part of the transformation the bacteria must be made competent to accept the pGLO. This is done by "heat shocking" the bacteria which makes it easier for the pGLO to be incorporated into the bacteria. For the bacteria to fluoresce under UV light it must be in presence of arabinose sugars, which "turns on" the gene for the production of Glowing Fluorescent Proteins.² The amp Resistance gene enables bacteria to survive in the presence of the antibiotic ampicillin. When a plasmid containing both the GFP gene and AMP gene (pGLO) is transferred into an E. coli bacterium, the transformed cells can be grown in a culture dish that contains ampicillin. Only a small number of bacteria cells will be transformed and grow on the LB (lysogeny broth) and amp plates and glow.³

The experiment demonstrates how Bacteria is modified to express a specific gene through the process of bacterial transformation. The purpose of this experiment is to find the efficiency of bacterial transformation in E. Coli bacteria by observing their expression of the plasmids. This is calculated by determining the frequency of the bacterium with GFP's and arabinose sugars by counting the glowing colonies.

It was the results for each plate was hypothesized before the experiment. The LB plate with only the bacteria and no pGLO administered will grow a lawn of bacteria and have no glowing properties. The LB with ampicillin but bacteria without pGLO will not survive at all and there will be no bacteria

growth. The LB plate with amp and bacteria with the pGLO will have bioluminescent properties but only a very small percentage of the bacteria will survive the amp and bacterial transformation will occur. Finally, the LB with no amp but the bacteria with the pGLO will form a lawn of bacteria and the bacteria that is transformed will glow like the previous plate. The efficiency of the bacterial transformation is hypothesized using in class discussion and background knowledge, to be about $8 \times 10^{-4} \%$.⁴

Materials and Methods

- *E. coli* bacteria cultures
- 100-1000 μl micropipette
- 0. 5-10 μl micropipette
- sterile tips
- 2 sterile 15-ml test tubes
- 500 $\hat{1}\frac{1}{4}\text{L}$ of ice cold 0. 05 *M* CaCl_2 (ph. 6. 1)
- 500 $\hat{1}\frac{1}{4}\text{L}$ of lysogeny broth/agar
- a spreading rod
- Bunsen burner
- 4 agar plates: 2 ampicillins+ and 2 ampicillin -
- an incubator
- a sterile inoculating loop
- 10 $\hat{1}\frac{1}{4}\text{L}$ of pAMP solution
- a timer
- ice
- tape
- sterile glass beads

- a water bath

1. Use a permanent marker to label one sterile 15-ml tube "+", and another "-".

2. Use a 100-1000 μl micropipette and sterile tip to add 250 μl of CaCl_2 (calcium chloride) solution to each tube.

3. Place both tubes on ice.

4. Use a sterile inoculating loop to transfer a visible mass of *E. coli* from a starter plate to the + tube:

a. Sterilize loop in Bunsen burner flame until it glows red hot.

b. Carefully, stab loop into agar to cool.

c. Scrape up a visible mass of *E. coli*, but be careful not to transfer any agar. (Impurities in agar can inhibit transformation.)

d. Immerse loop tip in CaCl_2 solution and vigorously tap against the wall of the tube to dislodge bacteria. Hold tube up to light to observe the bacteria drop off into the calcium chloride solution. Make sure cell mass is not left on a loop or on side of tube.

e. Sterilize loop before setting it on the lab bench.

5. Immediately suspend cells in the + tube by repeatedly pipetting in and out, using a 100-1000 μl micropipette with a fresh sterile tip. a. Pipet carefully to avoid making bubbles in suspension or splashing suspension far up sides of the tube.

b. Hold tube up to light to check that suspension is homogeneous. No visible clumps of cells should remain.

6. Return + tube to ice.

7. Transfer the second mass of cells to - tube as described in Step 4, and resuspend cells as described in Step 5.

8. Return - tube to ice. Both tubes should be on the ice.

9. Use a 0.5-10 μl micropipette to add 10 μl of 0.005 $\mu\text{g}/\mu\text{l}$ pGFP solution directly into cell suspension in the + tube. Tap tube with a finger to mix. Avoid making bubbles in suspension or splashing suspension up to the sides of the tube. [DO NOT ADD pGFP TO THE "-" TUBE.]

10. Return + tube to ice. Incubate both tubes on ice for 15 minutes.

11. While cells are incubating, use a permanent marker to label two LB plates and two LB/amp plates with name and the date.

Label one LB/amp plate "+ GFP". This is the experimental plate.

Label the other LB/amp plate "- GFP". This is a negative control.

Label one LB plate "+ GFP". This is a positive control.

Label the other LB plate "- GFP". This is a negative control.

12. Following the 15-minute incubation on ice, heat shock the cells in both the + and - tubes. It is critical that cells receive a sharp and distinct shock:

a. Carry ice beaker to the water bath. Remove tubes from ice, and immediately immerse in 42°C water bath for 90 seconds.

b. Immediately return both tubes to ice, and let stand on ice for at least 1 additional minute.

13. Place + and - tubes in test tube rack at room temperature.

14. Use a 100-1000 µl micropipette with a fresh sterile tip to add 250 µl of sterile LB medium to each tube. Gently tap tubes to mix. This will allow the cells to recover from the heat shock.

15. Use the matrix below as a checklist as + and - cells are spread on each plate:

16. Use a 100-1000 µl micropipette with a fresh sterile tip to add 100 µl of cell suspension from the - tube onto the - LB plate and another 100 µl onto the - LB/amp plate.

17. Use a 100-1000 µl micropipette with a fresh sterile tip to add 100 µl of cell suspension from the + tube onto + LB plate and another 100 µl of cell suspension onto + LB/amp plate. [Do not let suspensions sit on plates too long before proceeding to Step 18.]

18. Use sterile glass beads to spread cells over the surface of each - plate:

a. Obtain four 1.5 ml tubes containing at least five sterilized glass beads.

b. Lift lid of one - plate, only enough to allow pouring of the beads from one of the 1.5 ml tubes onto the surface of the agar. Replace plate lid; do not set the lid down on the lab bench. Repeat for all plates.

c. Use beads to spread bacteria evenly on plates by moving plates side to side several times. Do not move plates in a circular motion.

d. Rotate plates $\frac{1}{4}$ turn, and repeat spreading motion. Repeat two more times. The object is to separate cells on agar so that each gives rise to a distinct colony of clones.

19. Let plates set for several minutes to allowing the suspension to become absorbed into the agar. Then wrap together with tape.

20. Place plates upside down in 37°C incubator, and incubate for 12-24 hours, or store at room temperature for approximately 48 hours. ⁵

Results

<u>Transformed cells</u>	<u>Non-transformed cells</u>	
<u>LB/amp</u>	Bacterial Growth in form of green colonies	No growth on plate
<u>LB</u>	Growth spread across	Growth spread

	across
	entire
entire plate	plate
(bacteria	(bacter
lawn)	ia
	lawn)

Table 1. the E. coli bacterial plates after incubation.

Discussion

Before the experiment was conducted the results of each plate was hypothesized. It was believed that the plate with only the LB and no plasmids added would grow a lawn of bacteria, this was proven correct by the experiment. The plate with LB and ampicillin but no pGLO was predicted to have no growth, which was also proven correct by the experiment. The plate with LB and ampicillin but the bacteria was administered with the pGLO was predicted to survive the amp but not in very large quantities. Finally, for the plate with only LB but with the pGLO administered to the bacteria it was hypothesized that it would glow, not necessarily in large quantities but at least a little. This was different from the results of the experiment in which the bacteria did not show bioluminescent properties. This can occur for numerous reasons, the lack of bacteria that was transformed, unsterile equipment, improper heat shocking to make the bacteria competent. While all these are the possible reasoning for the experiment results the most probable cause for the plates to not grow is the lack of arabinose sugar which is an important part in the expression of the GFP'S (see introduction). If the plates lack the arabinose sugar the GFP proteins may not be

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expressed. This explains why the LB only plate with the pGLO did not produce transformed bacteria. This also draws questions to why the plate with LB and ampicillin and the transformed bacteria. Why would it glow if it didn't have any arabinose sugar? This most likely is explained by the fact that it must have been administered in the LB but not in the others. ³

The transformation Efficiency was determined by counting the number of colonies on the LB/amp plate pGFP. Any bacteria that shows light under the UV light must have accepted the plasmids and successfully transformed the desired genes to survive the lb/amp plate and express the GFP gene. Each colony represents one bacteria that has been transformed. Using this the efficiency can be determined. Transformation efficiency is expressed as the number of antibiotic resistant colonies per μg of pGFP DNA. To find this the mass of the pGFP used must first be determined by the formula

Concentration X Volume = Mass. This is shown in *figure 1* and was calculated using the formula $0.005 \mu\text{g} / \mu\text{l} \times 10 \mu\text{l} = 0.05 \mu\text{g}$. Then using the formula to determine the total number of cells per plate the fraction of cells suspended onto the +LB/Amp plate. This is shown in *figure 1* and was calculated using the formula $.005 \mu\text{g} / 510 \mu\text{l} = 9.8 \times 10^{-5} \mu\text{g} / \mu\text{l}$ this number must then be multiplied by 100 because there are approximately 100 cells in use. This is calculated in *figure 1* and is solved to be 9.8×10^{-3} . To determine the transformants per microgram the formula *(total transformed cells/cells per plate)/10000* to find the efficiency in transformants per microgram. This is solved in *figure 1* to be 8.673 transformants per microgram. Then the Transformation Efficiency can be found. This is shown in *figure 1* which uses the formula *(Total cells to start / total microliters) x 100 microliters* to find

the total number of cells on the plate. Then the formula $(\text{Transformants} / \text{Total cells}) \times 100 = \text{percent of efficiency}$. This is calculated as $(8.673 \text{ transformants} / 1,960,784,314) \times 100$ to calculate a transformation efficiency of .000004335% or in scientific notation $10 \times 4.3355 \times 10^{-6}$

Before conducting the experiment, it was hypothesized that the transformation efficiency would be about $8 \times 10^{-4}\%$. After doing the experiment the transformation efficiency was found to be $4.335 \times 10^{-6}\%$ or 8.673 transformants per microgram. This proves the percentage of efficiency to be significantly lower than hypothesized. The transformation efficiency being lower than expected shows the rarity of this specific form of genetic modification. The experiment tests how rare it is for the genetic modification to occur and demonstrates the results of the modification and its effect on an organism.

Citations

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