Bacterial Transformation

XXXXXXXXX XXXXX

Bio Lab Thursday 3-5:45

21 November 2013

Abstract

The major objective of this experiment was to determine whether E. Coli cells would take up the pGLO plasmid and glow in the presence of three manipulated environmental factors; arabinose sugar, the antibiotic ampicillin, and LB nutrient broth. This was tested through the use of five plates, each of which contained E. Coli cells and different combinations of the three environmental factors. It was found that E. Coli will only fluoresce when all three factors are present. These results went against the hypothesis stating that, in the presence of arabinose, many E. Coli cells exposed to the pGLO plasmid would all take up the plasmid and glow.

Introduction

In this experiment, E. Coli bacteria are transformed with the pGLO plasmid. This plasmid contains three genes: bl, coding for beta lactamase, araC, coding for araC protein, and GFP, coding for Green Fluorescent Protein. Beta Lactamase is an enzyme that provides antibiotic resistance, specifically to the antibiotic ampicillin. Green Fluorescent Protein is a protein found in the jellyfish *Aequorea Victoria* which causes the jellyfish to fluoresce around its rim. In this investigation, the desired result is bacteria that fluoresces in the same way under ultraviolet light (Lab Manual).

The pGLO plasmid has been used to transform E.Coli bacteria in other experiments prior to this one. One such example is the mutagenesis experiment of Bassiri (2011). The investigation entailed first transforming E.Coli bacteria with the pGLO plasmid, then using the transformed bacteria to conduct further research, such as the effects of Kanamycin. Bassiri found that the GFP gene only transcribed in the presence of the sugar arabinose, due to the araC repressor present in the pGLO plasmid.

The experimenters in this investigation hypothesized that, in the presence of the sugar arabinose, the E.Coli cells would pick up the pGLO plasmid, transcribe the GFP gene and fluoresce. They predicted that the beta lactamase in the pGLO plasmid would protect the cells who picked it up from the harmful effects of ampicillin. The experimenters also predicted that the LB growth medium would cause exponential growth in plates that did not contain ampicillin, and smaller growth in only cells that picked up the pGLO plasmid in the presence of ampicillin.

Materials and Methods

The independent variables in this experiment were the predetermined presence or absence of arabinose and ampicillin on each of the experimental plates, as well as the addition or omittance of the pGLO plasmid. The dependent variables included whether the E. Coli cells showed growth, whether they took up the pGLO plasmid and whether those that did take up the plasmid fluoresced.

The first step was to label one capped micro test tube as +pGLO and another as –pGLO and place the tubes in a foam tube rack. Next, each tube was opened and 250 ul of transformation solution was added to each. The transformation solution, CaCI2, was added to the tubes using a sterile transfer pipet. The tubes were then left open and placed on ice. While the tubes were on ice, a sterile loop was used to pick up a single colony of E. Coli bacteria from the starter plate and immerse the colony into the transformation solution at the bottom of the tube labeled +pGLO. Once the colony was completely dispersed in the solution and no floating chunks were visible, the same process was repeated for the –pGLO tube using a new sterile loop.

Next, another new sterile loop was used to transfer a loopful of pGLO plasmid DNA from the stock tube into the cell suspension of the +pGLO tube. No plasmid was added to the –pGLO tube, but both tubes were closed. They were then both incubated on ice for 10 minutes. After ice incubation, the tubes were transferred into a water bath set at 42° C for exactly 50 seconds, then placed rapidly back on ice for another two minutes.

After the final two minutes of incubation on ice, the rack holding the tubes was removed from ice and placed on a flat surface. 250 ul of LB nutrient broth was then added to both tubes, using a new sterile pipet for each tube, and the tubes were incubated for 20 minutes at room temperature. Next, again using a new sterile pipet for each tube, 100 ul of the transformation and

control suspensions (+pGLO tube and –pGLO tube respectively) were transferred onto the appropriate nutrient agar plates.

5 nutrient agar plates were used. Plates 1 and 2 received 100 ul from the –pGLO tube, while Plates 3, 4 and 5 received 100 ul from the +pGLO tube. Before the transformation and control suspensions were added, Plate 1 contained only LB nutrient broth, Plates 2 and 3 contained both LB and ampicillin, Plate 4 contained LB and arabinose and Plate 5 contained LB, ampicillin and arabinose.

The suspensions were then spread evenly around each plate's agar by lightly gliding the flat surface of a sterile loop back and forth across the plate surface. The plates were then stacked upside down to prevent contamination of the samples by the condensation that forms on the underside of the lid during overnight incubation. Lastly, the stack of plates was placed in the incubator at 37° C until the next day, at which time they were each observed under an ultraviolet (UV) light in order to detect fluorescence. Visible colonies indicated that the LB nutrient broth worked as intended, causing colonies to grow. Colonies that were both visible and glowing indicated that the E. Coli cells took up the pGLO plasmid while the absence of glowing colonies indicated the opposite.

Results

When observed under the UV light, only one plate possessed any glowing colonies. This was Plate 5, containing E. Coli, LB nutrient broth, ampicillin, arabinose and the pGLO plasmid DNA. This plate had three visible bacteria colonies, all of which fluoresced. Plates 1 and 4 showed exponential growth with a large number of colonies that were impossible to count, but none of these colonies glowed. Plate 2 showed neither growth nor fluorescence. Plate 3

contained 2 visible colonies, neither of which glowed. This information is displayed below in Figure 1.

	Plate 1 -pGLO, LB	Plate 2 -pGLO, LB+Amp	Plate 3 +pGLO, LB+Amp	Plate 4 +pGLO, LB+Ara	Plate 5 +pGLO, LB+Amp+Ara
Relative Growth (+++, ++, + or -)	+++	-	+	+++	+
Fluorescent under UV	No	No	No	No	Yes

In Figure 1, "+++" means that the plate contained too many visible colonies to count, "+" stands for 1-4 visible colonies, and "–" stands for zero visible colonies. In the same table, "ampicillin" has been shortened to Amp and "arabinose" has been shortened to Ara. The LB nutrient broth is represented simply as LB.

Discussion

We originally hypothesized that, in the presence of arabinose, E. Coli cells would take up the pGLO plasmid and fluoresce. While the plate that showed fluorescing colonies did contain arabinose, Plate 4 contained arabinose as well and showed no glowing colonies. This result disagreed with our hypothesis. The colonies on Plate 4, which contained LB nutrient broth and Arabinose, did not grow due to fact that the cells did not take up the pGLO plasmid, which can be explained by the lack of ampicillin on the plate. The presence of ampicillin is a motivator for the E. Coli cells to pick up pGLO, which contains a gene coding for beta lactamase. Beta

lactamase acts against ampicillin, protecting the cell from the antibiotic's harmful effects. Without the threat of ampicillin, the cells have no reason to take up the pGLO plasmid, and therefore the presence of arabinose has no effect on whether the cells fluoresce or not.

Ampicillin has been used to drive cells to pick up plasmids in multiple experiments. In mutagenesis experiment by Lewis and Thompson (1990), investigators attempted to mutate DNA with a novel plasmid. The plasmid contained, among other things, a correction primer that corrects a defect in the ampicillin resistance gene on the DNA and makes it resistant to the antibiotic. The average yield of mutated DNA in the absence of ampicillin was only a few percent. When ampicillin was added as a motivator for the DNA to pick up the plasmid, Lewis and Thompson (1990) observed 60-90% yield in mutated DNA.

Schleif (2000) found that araC protein, whose coding gene is found in the pGLO plasmid, regulated the expression of other genes in the arabinose operon. Schleif discovered that, without the presence of Arabinose, araC acted as a repressor and would not allow the transcription of the other genes in the operon. This explains why, even in the presence of ampicillin, no E.Coli cells fluoresced unless arabinose was also present.

All of the other plates were in line with predictions made at the start of the experiment. Plate 1 showed exponential growth due to the presence of the LB nutrient broth and did not glow since pGLO was absent. Plate 2 showed no growth even in the presence of LB because the ampicillin counteracted its effects. Again, there was no glow due to the absence of pGLO. Plate 3 showed growth by only the E. Coli cells that took up the pGLO plasmid since the gene coding for beta lactamase counteracted the effects of the ampicillin, while those that did not take up the plasmid were affected by the antibiotic. Lastly, Plate 5 possessed colonies that showed both

growth and fluorescence because the cells that took up pGLO were able to transcribe the GFP (green fluorescent protein) gene in the presence of arabinose as well as counteract the ampicillin.

While we conducted this experiment carefully, there were still many sources of possible error. For instance, if the lid of the agar plate was lifted for too long, bacteria from the air may have entered the plate and contaminated the sample. Also, if any condensation had dripped from the lid onto the plate prior to the plate being flipped, it would have been another source of plate contamination.

References

Bassiri, E. A. (2011). PGLO Mutagenesis. *Biochemistry and Molecular Biology Education, 39*(6), 432-39. Print.

Biology 281 Conceptual Approach Bio Majors 1. Laboratory Exercises for General Biology 1. Plymouth: Hayden-McNeil Publishing, 2013. Print.

Lewis, M. K., and D. V. Thompson (1990). Efficient Site Directed in Vitro Mutagenesis Using Ampicillin Selection. *Nucleic Acids Res.* 3439-443. Print.

Schleif, R. (2000). Regulation of the L-arabinose Operon of Escherichia Coli. *Trends in Genetics 16*(12), 559-65. Retrieved from Web of Science.