Ap bio pglo transformation formal lab report essay sample

Science, Genetics

Abstract:

The topic of this research involved the occurrence of genetic transformation in bacteria (E. Coli). More specifically, a previously prepared pGLO plasmid–which consisted of the gene to be cloned–was used to transform non-pathogenic bacteria. The pGLO plasmid contained a gene for the Green Fluorescent Protein (GFP) from a bioluminescent jellyfish and a gene for resistance to ampicillin, an antibiotic. Essentially, we wanted to determine the conditions of the bacteria that would glow. Our hypothesis was that the transformed solution with no plasmid DNA and ampicillin would produce no bacteria colonies, as it wouldn’t be able to grow without the gene for ampicillin resistance. Also, the transformed solution with just LB and ampicillin would produce bacteria colonies but the transformed solution with LB/ampicillin/Arabinose would produce glowing bacteria colonies (as Arabinose allows the GFP gene to be expressed, but in both cases bacteria colonies would be present because of the gene of resistance to the antibiotic, ampicillin).

We essentially made the required transformed solutions–and the controls–swiped them on the agar plate, and then observed to see whether or not bacteria colonies grew and whether or not they glowed. Our data fully supported our hypothesis. We can thus conclude that bacteria can take in foreign DNA through the process of transformation and that this foreign DNA can fundamentally change the bacteria (ex: making it glow). Future research can involve inserting other pieces of DNA into bacteria from different organisms, making the bacteria take on various phenotypic characteristics.

Background Information:

Genetic transformation is one of the most important processes in biotechnology. Essentially, genetic transformation involves the process where a cell (in this lab, a bacterial cell) takes up foreign DNA from its surroundings and incorporates it into its own DNA. This gene transfer is accomplished with the aid of a plasmid, a naturally-occuring small piece of circular DNA in bacteria. Bacteria can efficiently adapt to new environments by transferring plasmids with beneficial genes aiding survival to other bacterial cells.

In biotechnology laboratories, plasmids are transformed. Essentially, restriction enzymes are used to make cuts in the plasmid–the ‘ vector’–and in the DNA sequence containing the gene to be transferred to the bacteria. Then, this gene to be transferred is forged into the plasmid (in this lab we used previously made plasmids called pGLO, containing a bioluminescent gene and a gene for ampicillin resistance). Plasmids are then ‘ forced’ into bacterial cells using a process called “ heat shock,” where small pores in the bacteria open up. In the end–in order to find out which bacteria received the new gene–the transformed solution can be incubated on agar gel. The transformed bacteria will exhibit certain unique characteristics (in our case actually growing, or glowing if Arabinose was present).

Materials and Methods:

For this experiment, the following materials were used: two micro test tubes, sterile transfer pipettes, 500 µl of Transformation Solution (CaCl2), an ice

bath, a foam rack for the tubes to keep them afloat in the ice-bath, non-pathogenic E. Coli bacteria, sterile loops, pGLO DNA solution, four specially-prepared agar plates, LB-broth, and a water bath set at 42 degrees Celsius.

First we labeled one micro test tube '+DNA' and the other '-DNA.' Then we transferred 250 µl of Transformation Solution (CaCl2)–using a sterile pipette–into each micro test tube, and placed the tubes in a foam rack, which floated on an icy water bath. Next, we used two sterile loops to transfer E. Coli. Bacteria from the starter plate to each of the tubes ('+DNA' and '-DNA' tubes). Now that we had added the required bacteria, we proceeded in our experiment by adding the pGLO DNA solution–using a new sterile loop–to the micro test tube labeled '+DNA.'

The next part of the experiment involved the heat shock process, where plasmids were fused into bacteria. We incubated both micro test tubes on ice for 10 minutes. Then, we transferred both of them into the water bath set at 42 ºC for exactly 50 seconds, making sure to place them back on ice after the 50 seconds were ' up.' After letting the tubes incubate on ice for 2 minutes, we used a sterile pipette to add 250 µl of LB broth to the '+DNA' tube. We repeated this process with the '-DNA' tube. We let these tubes sit for ten minutes at room temperature.

Finally, each transformation solution was prepared. Using new sterile loops for each plate, we spread the suspensions evenly around the surface of the agar of each of their four respective plates (plates were labeled +DNA LB/amp ; +DNA LB/amp/ara ; -DNA LB/amp ; -DNA LB for what they contained

or didn’t contain [Arabinose, ampicillin, LB]). Lastly, we put our plates in an incubator so bacteria could grow, and observed them the next day under UV light.

Results:

The nature of this research involved fusing pGLO plasmids containing the GFP glowing gene and a gene for ampicillin resistance into E. Coli bacteria, only some of which would take in this plasmid. The solutions having transformed bacteria DNA from the plasmid (+DNA) and the solutions where its bacteria did not receive plasmid DNA (-DNA) were spread on agar plates; the plates were then observed in the hopes of drawing important conclusions about gene transfer and transformation in bacteria. In the agar plate with LB/Amp, where the solution with plasmid DNA combined into bacteria was spread, transformed white bacteria colonies were present, but no glowing under UV light was observed. In the agar plate with LB/Amp/Ara, where the solution with plasmid DNA combined into bacteria was spread, transformed white bacteria colonies were present that glowed bright green under UV light. In the agar plate with LB/Amp and -DNA, no bacteria were present. In the agar plate with just LB and -DNA, a bacteria lawn was present (bacteria were everywhere, but they did not glow).

Discussion:

Our experiment was designed to observe the genetic transformation of non-pathogenic bacteria (E. Coli) via pGLO plasmids fusing into the bacteria. From careful analysis of our results, it becomes clear that our hypothesis was

supported. Originally, we hypothesized that the only bacteria that would glow would be the bacteria that had received the plasmid DNA–that had been transformed–but that had also been cultivated in Arabinose. Also, we predicted the solutions with just the bacteria–not the plasmid DNA in addition to the bacteria–would NOT grow if ampicillin was present in the growth medium, but would grow if ampicillin was absent from the growth medium (agar). Our results confirmed our hypothesis.

Clearly, when analyzing our experiment, our hypothesis was supported by our results because it makes sense scientifically. First of all, any bacteria growing on the '+DNA' plates must have been transformed, since the plates have ampicillin, an antibiotic. In being transformed, bacteria received the gene for ampicillin resistance from the pGLO plasmid. Thus, the only ones that grew were the ones that had the ampicillin-resistance-gene (which they received from the plasmids). Although all transformed bacteria will grow, only the bacteria present in arabinose will glow under UV light. This Arabinose is a sugar which turns on the GFP glowing gene. Furthermore, even bacteria which had not received the plasmid genes ('-DNA'), and thus could not glow, obviously would grow on a medium with LB, favoring the growth of bacteria. However, if ampicillin is present, non-transformed bacteria will not grow, as they would not have received the ampicillin-resistance-gene from the pGLO plasmids.

From this lab, we can conclude that bacteria–through the process of transformation–are able to take up foreign DNA (such as in the form of a pGLO plasmid) and express it in phenotypic characteristics (such as

glowing). We also learned that transformation is never fully effective; only cells that are competent enough are able to take up foreign DNA. This can be seen by the fact that the control (-DNA/LB) plate had a lawn of bacteria, whereas the ampicillin plates had small bacteria colonies (only transformed bacteria could grow).

Our lab results are subject to imperfections, such as the fact that the heat shock timing might not have been perfect. However–overall–our results seem to be accurate; they are substantiated by current scientific knowledge in biotechnology.