Bacterial transformation plasmid isolation

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Transformation is the process by which bacteria take up genes from its surrounding environment. Scientists take advantage of this as a way to introduce foreign genes into a bacterial cell in order to amplify that specific gene. They isolate those bacteria (or select them) by selectively destroying those bacteria that did not take up the plasmid. They begin this process by incorporating a desired gene (in this case the gene is pGlo) into a vector (a piece of DNA that is used to transfer a gene into a host cell), such as an ampicillin-resistant gene for example, and then inserting that gene and extra DNA (called a plasmid) into the bacteria via transformation. To aid in transferring the plasmid into the bacterial DNA, the bacteria is placed in a solution of $CaCl_2$ and is heat shocked (removed from a cold bath and quickly inserted into a hot bath of ~43° C). Then, the entire bacterial culture is placed into an environment that contains ampicillin, thus killing those bacteria that did not take in the plasmid

Arabinose is an inducer, so in its presence, the RNA polymerase will be able to transcribe certain genes that are usually “ shut off” when arabinose is not present. By replacing those certain genes with a gene to make GFP (Green Fluorescent Protein, from a bioluminescent jellyfish), the E. coli cells will transcribe and translate the genes for GFP instead of its original genes.

There are 4 important components of the pGlo plasmid: the araC, GFP, bla, and ori-gene. The araC (arabinose C gene) is a “ promoter region that regulates the expression of GFP,” (Miyada, et. al). The GFP (green fluorescent protein gene) is the gene obtained from a bioluminescent jellyfish that causes it to glow green under UV light. The bla gene (Beta-lactamase enzyme) is what gives the bacteria resistance to beta-lactam

antibiotics like penicillins (Antibiotics). The ori is the origin of the plasmid where replication will begin (ori-gene).

The purpose for having a negative control is to prove that the E. coli will not grow in a medium containing ampicillin unless an ampicillin-resistant gene has been taken in by that bacteria (labeled number #3 control in this experiment). The purpose for a positive control is to prove that the lauria broth is a medium that will allow the growth of E. coli and to prove that it is not the reason that the bacteria do not grow (labeled as #4 control in this experiment). Therefore, the following is what I would expect for the tests performed:

#1 pGlo" plate contains transcribed cells, LB, and ampicillin; I would expect bacterial growth but I would not expect it to glow green under UV light. The bacteria have taken in the ampicillin-resistant gene so it will grow in an environment that has ampicillin, however, since there is no arabinose present, the pGlo gene will not be activated and the bacteria will not shine green under UV light.

#2 pGlo" plate contains the transcribed cells and the LB/AMP/Arabinose; I would expect growth in this plate and I would expect for the cells to glow green under UV light. Since the bacteria contains the ampicillin-resistant gene, it can grow in an environment that contains ampicillin, and since there is arabinose in the environment as well, then the cells will transcribe the genes for GFP and glow green under UV light.

#3 control" plate contains the non-transformed cells LB, and ampicillin; I would expect no growth because these bacteria do not have an ampicillin-

resistant gene and will therefore not grow in an environment that has ampicillin.

#4 control” plate with the non-transformed bacteria and LB, I would expect growth but it would not glow green under UV light. The cells are not transformed, so they do not contain genes for GFP, however there is also no ampicillin present in its environment, therefore the bacteria is able to survive but it does not transcribe any GFP.

The plasmid DNA will be isolated from cells and its components by the combined methods of Alkaline Lysis and Ion Exchange Chromatography. The alkaline lysing process will break apart the cell’s membrane. This is accomplished by first resuspending the cells in a buffer of EDTA (which will bind to the divalent cations in the cell membrane) and RNase (which will digest any RNA to avoid contamination). This is then treated with sodium dodecyl sulfate (SDS, a detergent that dissolves the plasma membrane and denatures cell proteins causing cell lysis) and sodium hydroxide (denatures plasmid DNA and chromosomal DNA into single strands). Adding potassium acetate and acetic acid next will form a precipitate of chromosomal DNA/cell proteins/lipids. Taking advantage of the ability of DNA plasmid to reanneal itself completely, centrifugation is used to separate the plasmid DNA (in the supernatant) from the rest of the cell parts (in the pellet). Next, Ion Exchange Chromatography will be used to purify the plasmid. DNA is negatively charged (due to the negatively charged phosphate group in its backbone), so it is easily attracted to the positively charged beads in the chromatography column in a high salt condition. After this column is washed

several times with various buffers to remove contaminants, the DNA is eluted from the column with a low salt buffer.

A restriction enzyme is a DNA cutting enzyme that will cut open a double or a single-stranded DNA at a certain nucleotide sequence, called a DNA recognition sequence (sequences vary with each restriction enzyme used). The sizes of the sequence will also vary with each restriction enzyme; three different restriction enzymes are used in this lab: the EcoR1, the EcoRV, and the Sca1. The enzyme EcoR1 will recognize and cut DNA at the phosphodiester bond between the G and the A nucleotides of the specific sequence GAATTC in an offset fashion that will create sticky ends, as shown in figure 1. EcoR5 restriction enzyme will cut DNA at GATATC in a blunt cut, as shown in figure 2. Sca1 is a protease that “ cleaves sterol regulatory element-binding protein-1 (SREBP-1) and SREBP-2 in vitro. These factors are basic helix-loop-helix-leucine zipper proteins involved in the activation of the transcription of the genes for the low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl CoA synthase,” (Sca-1).

All of the results in this experiment are not accurate; non-transcribed bacteria grown in an environment containing ampicillin should not have grown, but it was explained that this was purposely done due to lack of non-transcribed E. coli. In a real experiment, the results would have been retested, and the odd results would have been due to a mutation in the E. coli, contamination of other bacteria in the air, a mix up in bacteria used (like what happened in this lab) and other such possibilities. Due to lack of time, however, another test will not be performed, and the false positive will be excluded since it is known what went wrong.

A control was set up in which non-transcribed E. coli bacteria should have been unable to grow in an environment containing ampicillin (Test 3-control, figure 5), so it will be treated as if growth did not occur. This test was supposed to have been the negative control. A positive control was also set up (4-control, figure 6) where non-transcribed E. coli was grown in an environment that contained only lauria broth, which showed growth, but it did not glow green under UV light. Two other experiments were set up that included transcribed E. coli, in one experiment (figure-3, 1-pGlo) transcribed bacteria was grown in an environment that contained lauria broth and ampicillin, bacteria was grown but it did not glow green under UV light since no arabinose was present. Therefore, in the second experiment involving transcribed E. coli (Figure-4, 2-pGlo), the bacteria were allowed to grow in an environment that contained lauria broth, ampicillin, and arabinose. The results of this lab show that bacteria is able to grow in an environment that contains ampicillin and it is also able to glow green under UV light when arabinose is present.

Discussion:

I predicted that non-transcribed bacteria would grow in an environment that contained only lauria broth, but that they would not grow in an environment that contained ampicillin, and neither of them should glow green under UV lighting since they do not contain the pGlo gene, no is there arabinose present in the environment. Based on the results, non-transcribed E. coli bacteria were able to grow in an environment that contained lauria broth and did not glow green under UV lightin, and they should not have been able to grow in an environment that had ampicillin, however in this lab a false

positive was obtained for the control that did have non-transcribed E. coli growing in an environment with ampicillin. This could have been due to contamination of bacteria in the air, the ampicillin may not have been ampicillin, or the wrong type of bacteria may have been used. We were told that the E. coli that were used were actually transformed bacteria, they had the ampicillin-resistant gene, so the entire class obtained false-positives for this test. In an actual experiment, this entire experiment would have been redone, however due to lack of time, instead of redoing the experiment, we will ignore the results obtained from this false positive. The false-positive also did not glow green under UV lighting.

I predicted that for the transcribed bacteria grown in an environment with lauria broth and ampicillin would show growth, and that when arabinose was added to the environment that they would also glow green under UV lighting. My predictions were correct as seen in figures 4 and figure 3. Transformed E. coli bacteria are able to grow in an environment that contains ampicillin, and they are able to glow green under UV lighting when grown in an environment that contains arabinose. These results indicate that the bacteria were successfully transformed.

Conclusion:

What I learned from the results of the experiment (excluding the results from the false-positive from experiment 3-control), non-transformed E. coli grown in medium containing only lauria broth should be able to show growth, nontransformed E. coli grown on medium that contains ampicillin should not grow, E. coli transformed with the pGlo plasmid that is grown in an environment with ampicillin should show growth, and the same transformed

bacteria grown in the presence of arabinose should glow green under UV lighting.

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