

Genetic Material Uptake in E. Coli

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**Lab Section: 103
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Abstract:

This lab's purpose was to test E. coli's ability to take up and utilize DNA traits that did not originate within itself, but were taken up from the environment which explains its longevity on Earth. Our experiment can be broken down into two main sections: the control and the testable variants. The control allowed E. coli to grow at an exponential growth rate. This proved the E. coli was not harmed in the heat shock treatment used to take up the DNA. The second part of the control experiment was to prove that ampicillin is an effective drug used to kill any E. coli from growing/living. And so, our second plate containing no pGLO and ampicillin had no growth. This is good. It means our heat shock treatment went as planned and did not harm the microorganism being tested.

Secondly, we tested to make sure that pGLO was able to bring in bla which codes for the beta-lactase that provides ampicillin resistance to E. coli. The growth on this plate supports this idea. The last and final plate contained arabinose. The Arabinose is like an on/off switch for the GFP that is contained in pGLO. The araC complex then signals GFP production which allows the bacteria to fluorescently shine under UV. Our fourth plate of E. coli did shine like a sparkly diamond. This data supports the idea that non-self DNA can be taken up and used by a different source.

Introduction:

Genetic engineers specialize in their abilities to interpret and manipulate genetic materials in organisms such as bacteria. The genetic material of bacteria and most other organisms is DNA; the DNA is practically a recipe to synthesize proteins responsible for any type of traits an organism can contain. To change a trait an organism, scientists cause an organism to uptake a new gene into its genome by introducing it under optimal conditions (Unit 6, 2).

So, we all know it is currently impossible to design our babies with specific traits, but how do we manipulate bacteria such as E. coli to take in new DNA? E. coli is a single celled organism that contains not only one large chromosome, but also one or more plasmids. Plasmids are "small circular pieces of DNA" (2). The DNA in any given plasmid usually provides positive modifications for genes. E. coli and other bacteria have been able to share plasmids allowing for adaptation to various environments, hence the longevity of E. coli's presence on earth.

We will be testing the ability of E. coli to take on pGLO. Contained within pGLO are genes for the production of green fluorescent protein (GFP), bla, ori, and araC. The jellyfish *Aequorea Victoria* is the initial host containing the gene code of GFP (2). This gene irradiates light (glows) in the presence of ultraviolet light. The enzyme beta-lactase, whose synthesis instructions are contained in bla, allows E. coli to become ampicillin resistant. When scientists introduce arabinose to E. coli and it comes in contact with araC, the arabinose-araC complex functions as an on switch for the production of GFP (2-4). But is this possible?

Well, the cells have to be able to accept the new DNA without destroying it, much like a transplant patient must take immunosuppressant drugs to keep their body from rejecting the new organ. This is referred to as competency or a competent cell (4).

Competence has allowed bacteria to adapt to antibiotics, e.g. antibiotic resistant bacteria and superbugs such as MRSA, which is a new obstacle in the medicinal field.

To ensure E. coli will receive the non-self plasmid, we will use heat shock to induce competency. To heat shock E. coli, our E. coli sample which is suspended in CaCl_2 , is placed in a cold bath (0°C) then into a warm bath (42°C) (4). Why? If we slow down the movement of the entire cell to near death conditions without killing it and then slowly warm it up without denaturing any of the proteins, it will hinder the membrane's ability to effectively keep material which is usually non-permeable to the membrane due to a temperature gradient or convectional current that is created to heat the cell to more favored conditions (4). The desired result is to pass the plasmid into the cell before the membrane is functioning properly again.

How do we know if the E. coli actually took up our plasmid? This particular experiment has three testable factors to indicate if the experiment is successful. E. coli is normally killed by ampicillin; however, pGLO contains the gene that is responsible for the synthesis of beta-lactase. Why is this important? Ampicillin is broken down by beta-lactase. This means ampicillin is not an effective antibiotic when E. coli or other bacteria have the bla gene. This is a selectable marker because if you completed the experiment correctly, E. coli that is pGLO negative would not grow in the presence of ampicillin, but E. coli would grow in ampicillin if pGLO had been added to the E. coli before being streaked (or spread) onto an agar plate with ampicillin. You would also want an agar plate with E. coli and the broth. If you had no growth on the pGLO positive plate, when in theory you should, you would know by looking at agar plate sample with broth growth to know if there was live E. coli or if the E. coli had died or denatured at a step in the experiment.

Now that we know that the desired gene has been taken up by the E. coli, we need to know if the gene of interest is being expressed. GFP could not enter the E. coli's membrane even when being artificially induced. But with the facilitated transport provided by pGLO, GFP could signal if the gene actually was taken up by the E. coli. If this glows under the black light when arabinose is in the agar plate, then GFP would be a perfect example of a reporter gene. The glow signal not only allows the scientist to know that the DNA had been successfully transcribed, but also transcription had taken place. Transcription occurs when mRNA is produced, which in this case leads to the synthesis of araC protein which allows GFP to be switched on (5).

Materials and Methods:

Before beginning, the lab table was prepared with micropipettes and sterile tips, ice bucket with ice, sterile loops and cell spreaders, 4 agar plates (1 with agar only, two with ampicillin imbedded agar, and one with arabinose added to the agar), two sterile 1.5 ml tubes with rack for storage on ice, rubber bands, one 1.5 ml micro centrifuge tube of 600 μl transformation solution containing 50 mM calcium chloride with a pH of 6.1, and a pre-heated 37° and 42° water bath. Gloves and necessary health precautions should be taken, E. coli is disease causing.

Given a sample of E. coli to examine in growth and colonization, we first examine it. Without opening the lid (to prevent cross contamination), we observe and note the

number of colonies, size of largest, smallest, and average colony, the color and distribution. We also observed this specimen under UV light for comparison in the end.

Next, we micro pipetted, to the best of our abilities, 250 μ l of transformation solution into two micro centrifuge tubes one labeled +pGLO, the other -pGLO, both with group 7; then placed in the floating rack on ice in the bucket. Using separate sterile loops, Taylor and I each picked up a large colony of E. coli and spun the loop into the solutions labeled +/-pGLO. As this was occurring, Alex came around the room administering 10 μ l of pGLO in the correct micro centrifuge tubes. Then they were placed on ice for ten minutes monitored by a timer on Taylor's iphone. During this waiting period, we labeled four agar plates with - I (meaning -pGLO with LB broth), - II (-pGLO/LB/AMP), +II (+pGLO/LB/AMP), +III (+pGLO/AMP/ARA), and all four with our group #7.

Nearing the ten minute mark, we took our ice bucket with samples to the front of the room where the 42° C water bath was located; then submerged the samples labeled +/-pGLO for exactly 50 seconds, again timed by Taylor's iphone. We made sure that the test tubes were pushed down as far as possible in the floating rack to completely heat the whole sample. At the 50 second mark, the rack with samples was transferred back into the ice for exactly 2 minutes.

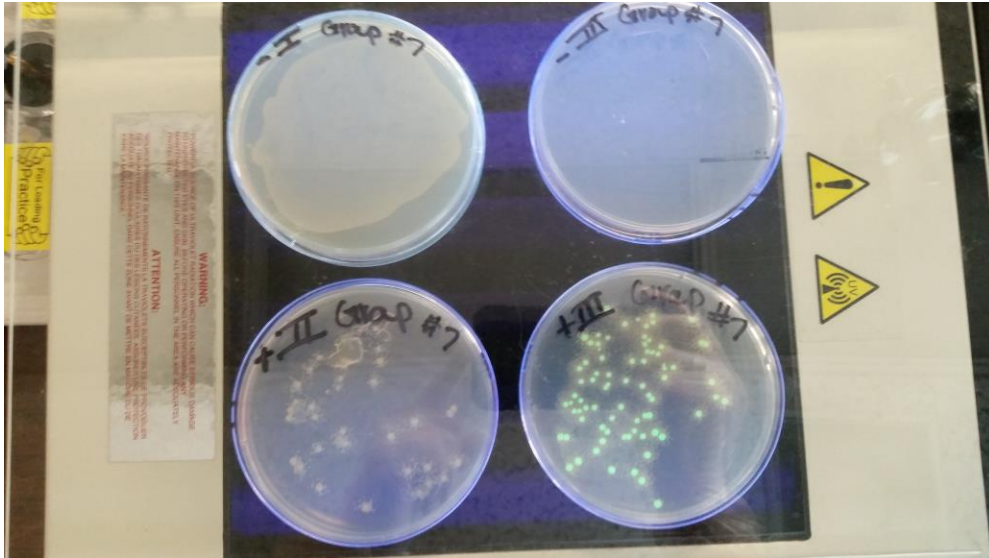
When the time was complete, we pipetted 250 μ l of LB nutrient broth into the + and - pGLO tubes using clean sterile tips for each procedure. Before placing them in the 37° C water bath for 15 minutes, we thumped them to ensure the liquids had interacted.

Upon rescuing the samples from the shock treatment and thumping to ensure the concentration is consistent throughout the tube, I pipetted 200 μ l of the +pGLO onto the +II and +III agar solutions without touching the agar gel. Simultaneously, Taylor pipetted 200 μ l of the -pGLO sample onto -I and -II agar plates with a separate micro pipette and sterile tip. We used sterile spreaders for each agar plate so that the pipetted E. coli could utilize as much surface area and nutrients of the agar gel as possible to grow. Faced down and rubber banded, the plates were taken to the front of the room to Alex, who incubated them at 37°C and stored the samples at 4°C for the next week's measurements and observations.

The next week, we then recorded the results. We measured and described the colonies. We tested to see if the GFP was taken up by shining UV light through the agar plates. Then, we calculated the transformation efficiency.

Results:

Figure 1: Escherich coli growth and UV Results



These are our E. coli growth results. As suspected, +III is the only one to glow, +II grew, -II did not grow due to the ampicillin and lack of antibiotic resistant gene from bla, and -I grew so exponentially it was basically one huge super molecule.

Table 1: Number o colonies counted under visible light and UV light

Plates	Growth Pattern	Relative number of colonies under Visible Light	Colony size before and after transformation	Color of colonies under visible light	Color of colonies under UV light
LB: -pGlo	Lawn	Numerous	Large	Whitish	Whitish
LB/AMP: -pGLO	None	None		Whitish	n/a
LB/AMP: +pGLO	Separate round colonies	Some	Small (with satellites)	Whitish	Whitish
LB/AMP/ARA: +pGLO	Separate round colonies	many		Whitish	Green

Table 1 explains the growth patterns observed from the results as well as size, number, and color.

Table 2/3: Numbers used to calculate transformation efficiency

	Experimental	Literature
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Number of Colonies on LB/AMP/ARA plate	77	277
Micrograms of pGLO DNA spread on the plates	0.314	.157
Transformation efficiency	2.452×10^2	1445.9

To find transformation efficiency of the experiment we divide the number of colonies on the LB/AMP/ARA plate by the micrograms of pGLO DNA spread on the plates.

Discussion:

So, in this experiment, we tested the ability of E. coli to take up foreign DNA with use of with the use of selective markers and reporter genes to support or disprove our hypothesis.

The first two plates which were –pGLO served as control group. As expected, the LB/-pGLO grew exponentially and killed the bacteria in the ampicillin containing agar solution. This is consistent with the literature and knowledge of scientists and doctors worldwide.

The third agar plate contained ampicillin and as previously noted we would be led to believe that E. coli would not grow; however, this E. coli had been positive for the pGLO gene which contains bla that makes E. coli ampicillin resistant. Based on the growth of E. coli in our +II plate, the data supports that E. coli can be forced to take up pGLO and become antibiotic resistant.

Taken it a step further to more thoroughly support the belief that E. coli can take up plasmid DNA for desirable characteristics, +III agar plate contains arabinose sugar. The sugar activates the on/off switch for the GFP gene. So, we took the plate and exposed it to UV light. Ta-Da! It glows! This is the optimal support needed to show that E. coli can not only take up new DNA, but it can also use the information and genetics provided by the transformation.

As noted in Table 2/3, our experimental data had a pretty low efficiency; nonetheless, our results still shows the anticipated trend that supports the belief that organisms, in particular E. coli, can take up foreign DNA and utilize it for sought after traits. These sought after traits have allowed E. coli to survive as long as it has on Earth.

Literature Cited:

Shin, S. (2015). General Biology Laboratory I Manual. Dept. Biol. Sci., University of Memphis, Memphis, TN.