Transforming E.Coli into Glowing Bacteria

Feb 19, 2024, Luke Yu

Bacteria are all around us, every day, everywhere. This experiment is about heat shocking E. coli so that it becomes glowing. I got the idea from a website called “Science Buddies” where many different kinds of science experiments are. This one intrigued me because it got me thinking about how you can transform a bacteria into something it normally wasn’t. Before the experiment, I started researching heat shock, and it started making sense. Heat shock is a technique used by many people to insert DNA into bacteria. It happens when the lipid layer of the bacteria is shocked because of the rapid temperature change.

My objective for this experiment is to determine how to transform E. coli with heat shock effectively. I decided to shock the bacteria for 30 seconds and 60 seconds, at 42 degrees Celsius, to see which is more efficient with transforming bacteria.

If I heat shock E. Coli for 60 seconds, at 42 degrees Celsius, it will have a higher transformation efficacy than if I had shocked it for 30 seconds because I think that the lipid bilayer will not be disrupted enough to allow plasmids to enter the bacteria. Since heat shock disrupts a bacteria membrane since it is hotter than the bacteria's ideal temperature, if I heat shock it for 60 seconds, it would be better than 30 seconds.

There were several variables involved in this experiment. My only manipulated variable was my heat shock time. For one tube of the bacteria infused with the plasmids, I will put it into the hot water for 30 seconds, for the other I will put it in for 60 seconds. I had many controlled variables, however. The incubation time, the incubation temperature the heat shocking temperature (42 degrees Celsius), and the amount of plasmids were all controlled variables. For controlled variables, I tried to limit as many sources of error. For example, during the heat-shocking temperature, I tried to maintain the temperature at 42 degrees Celsius for both tubes. I added hot water/room temperature water to fluctuate/maintain the temperature of the water. For the incubation temperature, I put both tubes in the same location in my house, to ensure an equality in temperature.

I needed various materials for this experiment, which I had to source from different websites online. Lab objects included plates, inoculation tubes, and microcentrifuge. The bacteria, the LB Agar Media, the transformation mix, and the plasmids I sourced from a website called The Odin. Objects like the bottle and the beaker I was able to source from my home.

The procedure is shown below:

Part 1
Preparing Agar Plates:

1. Add LB Agar media to a bottle.
2. Add 150mL of water to the bottle with LB Agar media.
3. Heat the agar in the microwave at 15-second intervals until almost boiling.
4. Once the LB Agar media looks clear, cool until warm to the touch.
5. Carefully remove the lid of the plates and pour agar to cover half the plate.
6. Cool agar for about 2 hours.
7. Use an inoculating loop to streak bacteria.
8. Store plate agar at room temperature for 48 hours.

Part 2
Transforming The Bacteria:

1. Pick a colony, and add it to the transformation mix.
2. Store in the fridge for 30 minutes
3. Add plasmids into the mix
4. Prepare the beaker with water at 42℃ using a thermometer. Temperatures too hot will cause damage to bacteria.
5. Split the mix into two microcentrifuge tubes
6. Heat the first tube for 30 seconds.
7. Heat the second tube for 60 seconds.
8. Leave at room temperature for more than 4 hours.
9. Prepare Kanamycin LB Agar plates (refer to Part 1)
10. Streak “transformed” bacteria and store plate at room temperature for 48 hours.

After experimenting, using my blue light, the 60-second one was the only one that glowed. To double-check, I used a fluorescent microscope and observed that 60

seconds transformed some bacteria. 30 seconds did not transform bacteria. This is very intriguing since I believed that 30 seconds would at least yield some glowing bacteria. Out of all 4 plates for 60 seconds, I only had one plate which glowed. This leads me to think that my transformation times were still not long enough. Perhaps a heat shocking time such as 90 seconds would perform better

Table of Transformation

30 seconds:	X	X	X	X
60 seconds:	✓	X	X	X

I conclude that 60 seconds is better than 30 seconds. I believe that a longer duration like 90 seconds would be more optimal because it will allow the membrane to be disrupted for longer, and more plasmids will be able to enter the bacteria. This was shown above in the table, where I had a transformation in 60 seconds, however, it was only one plate. If I were to repeat this experiment next time, I would try doing 60 seconds and 90 seconds, since my observations show that a longer time would be more effective.

I am grateful to my mom for supporting me in getting all the materials during my science fair journey. She provided aid with storage, materials, and ideas. I am grateful to my science tutor as well since she helped take microscopic pictures of my bacteria. This is an opportunity I normally would not have been able to have since it is a special type of microscope. Last but not least, this experiment would not have been possible without The Odin since it provided me with a transformation mix, and GFP plasmids for my bacteria, which are materials difficult to obtain in any typical store.

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