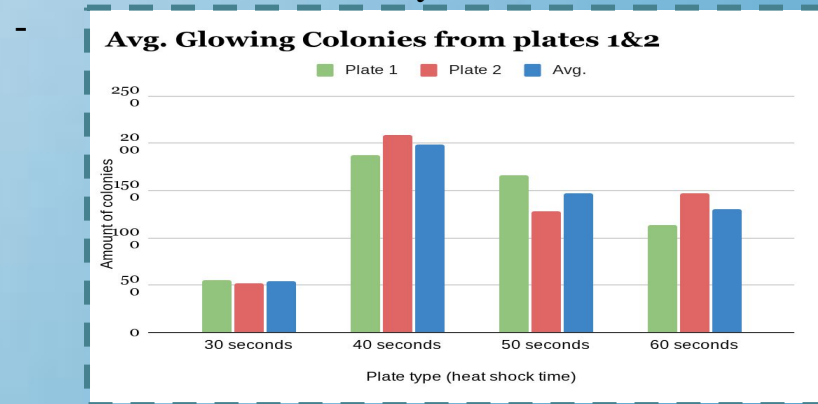


Glowing *E.coli*

Research Question

How do different heat shock times affect transformation efficiency?

Data Analysis and results



Methodology

- Put *E.coli* and GFP in the tube
- Placed it in ice then in heat block for allotted time then back to ice
- Put LB broth in tube, hand warmed for 10 minutes
- Made two bacterial lawn plates with bacteria
- Repeated for rest of trials then incubated
- The next day, tested plates under uv light for glow and counted the colonies and did calculations for avg.

Conclusions

- I did the project on transforming bacteria really wanted to work with that, so one of the ways was to manipulate the methods for transforming *E.coli* into glowing bacteria with GFP. As the heat shock times increase, last longer, the transformation efficiency would increase along with it; the transformation efficiency would increase long with the heat shock times. Although from the results I have garther theses predictions were mostly right.

Introduction

Research done before, specifically for heat shocking for transformation, was by Irma M. Van Die. They studied at what temperature the peak for transforming *E.coli* was incubated at different temperatures. Their results show that the most efficient transformation happens when the heat is 30 °C - 42°C. It also showed that not cold shocking the cells after heat shocking didn't change much in how many transformed *E.coli*, but with the cold shock, there was a bit less transformed *E.coli*. Research by M. Rahimzadeh showed that between the chemical treatment in the buffer, compared to the standard heat shock methods, brought little to no higher efficiency in transforming *E.coli*.



Question

Question

How do different heat shock times affect transformation efficiency?

Hypothesis 1

The heat shock times would make no difference for the transformation efficiency, all outcomes of colonies in the plates would be around the same amount.

Hypothesis 2

As the heat shock times increase, last longer, the transformation efficiency would in increase along with it.

Hypothesis 3

The transformation efficiency would increase along with the heat shock times, but would hit a peak at 40 seconds then the transformation efficiency would start to decrease.

Methods

Day 1:

1. Get 20 microliters of GFP DNA solution from a GFP DNA tube into your micropipette
2. Pipette the 20 microliters of the GFP DNA into *E.coli* cells tube
3. Label tube of *E.coli* cells: 30 (for number of seconds on the heat block)
4. Put *E.coli* tube on ice for 5 minutes
5. During that time, get 2 LB agar + ampicillin & arabinose plates. Write initial and 30 small on the bottom of the plate.
6. Take the *E.coli* tube from the ice and put it into a 42°C heat block for 30 seconds.
7. Put *E.coli* tube from heat block to ice for 2 minutes
8. Get an LB broth tube, add all of the LB broth into the *E.coli* tube by tipping the LB tube upside down onto the tube and flicking the LB tube.
9. Incubate *E.coli* tube in your hand for 10 minutes.
10. Use a micropipette to add all of the mixture from the *E.coli* tube to the LB agar + ampicillin & arabinose plate from the *E.coli* tube.
11. Use a sterile Q-tip to spread the mixture on the plate. Use different tips for each plate.
12. Repeat steps 1-11 for the corresponding *E.coli* tubes to LB agar + ampicillin & arabinose plate with labels : 40, 50, 60 (for each different seconds on the heat block)
13. Let plates sit up right for a few minutes to dry and put them upside down in the 37° C incubator for 24 hours

Methods

Day 2:

1. Take plates out of the incubator.
2. Count the amount of glowing colonies for both plates.
3. Calculate the Average. #of colonies on plate 1 + #of colonies on plate 2 divided by the number of plates (2)

Independent variable:
Heat Shock time

Dependent variable: Number of *E.coli* colonies produced

Control: Standard 30 seconds heat shock time

Materials:

Heat block Sterile Q-tips
Ice Tube of LB broth
Waste bag micropipette
UV light

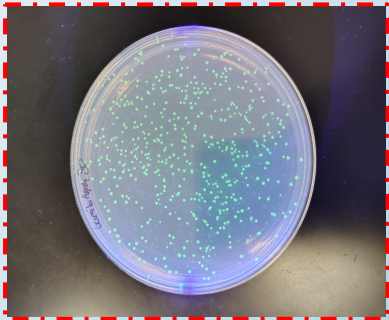
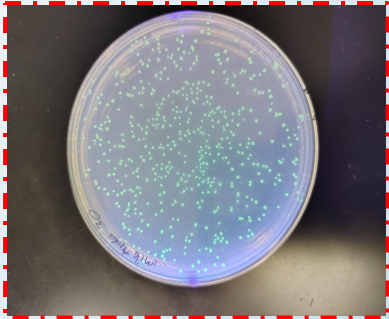
Results

- The 40 second plate produced the most amount of bacterial colonies over all.
- The 30 second plates produced the the least amount of bacterial colonies
- After the 40 second plates the amount of bacterial colonies produce decreases, which each plate after producing less than the ones before but still higher than the 30 second plates.

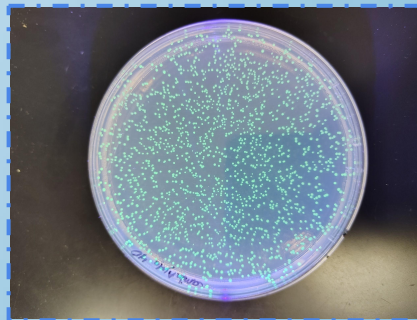
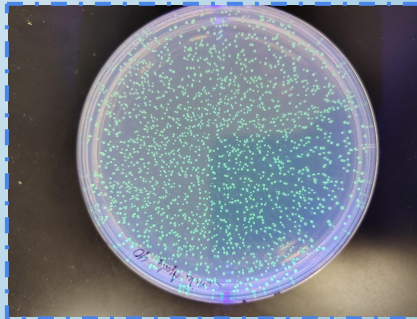
Plate type (heat shock time)	Plate 1	Plate 2	Avg.
30 seconds	559	523	541
40 seconds	1876	2089	1982.5
50 seconds	1664	1282	1,473
60 seconds	1139	1468	1303.5

Results Photos

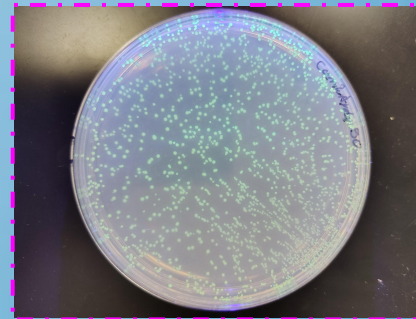
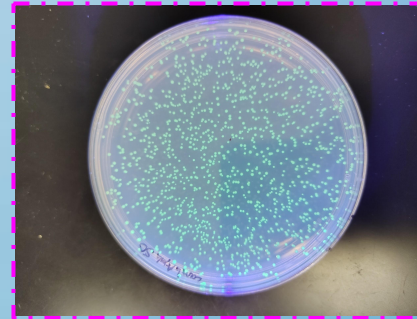
30 sec. plate



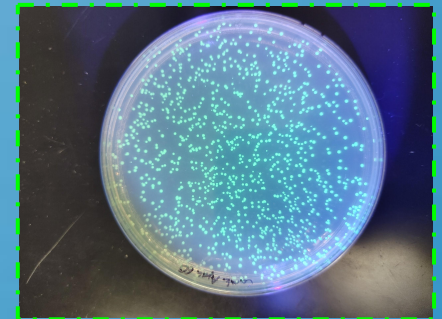
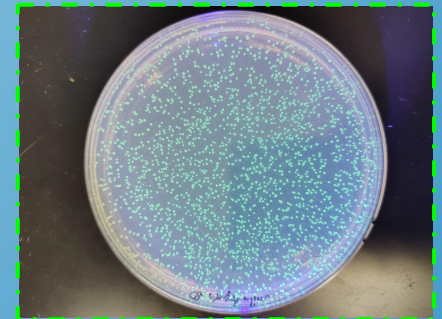
40 sec. plate



50 sec. plate

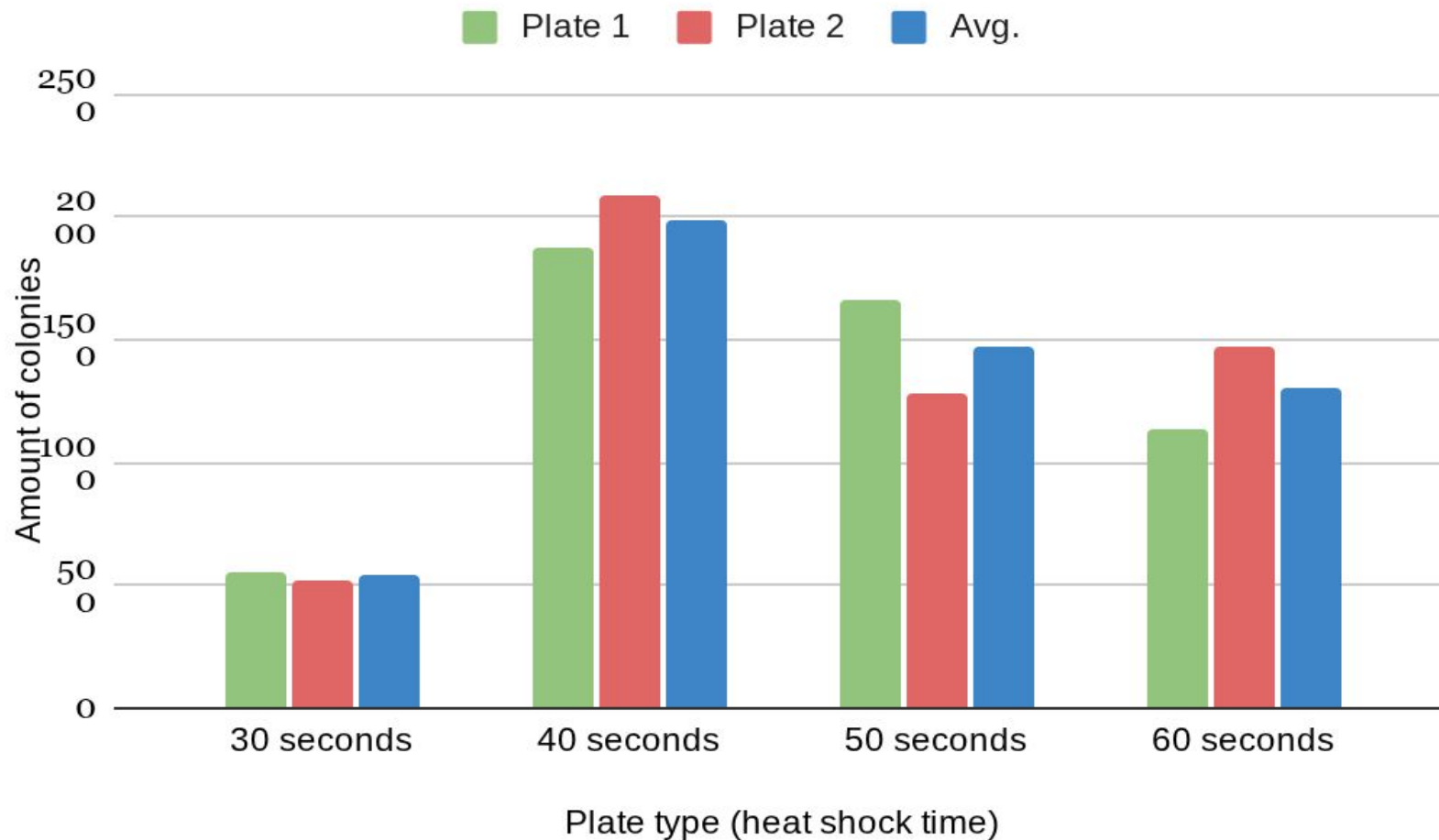


60 sec. plate



Results: Graph

Avg. Glowing Colonies from plates 1&2



Discussion

How do different heat shock times affect transformation efficiency? I think it is so interesting that we can change the DNA of an organism by adding proteins by plasmids, and I really wanted to work with that, so one of the ways was to manipulate the methods for transforming *E.coli* into glowing bacteria with GFP. As the heat shock times increase, they last longer, the transformation efficiency would increase along with it; the transformation efficiency would increase along with the heat shock times, but would hit a peak at 40 seconds, then the transformation efficiency would start to decrease, where my two claims. The transformation efficiency was measured by the average number of colonies for each time shock second trials, the averages. I thought 60 seconds was so long that the heat would kill most of the bacteria instead of just breaking the membrane open enough for the plasmids to go in. For the results from trials in 30s the the production of colonies was so little because during the time of heat shock, it could have resulted from there not being enough cells opened in that time frame, resulting in fewer transformed bacteria. For the rest, the membrane of the bacteria during the heat shock timing was enough to be open and also too long as after the 40s trials the colonies decreased, which means that the bacteria were dying from being in the heat for too long. These results supported my alt. hypothesis because there was a peak at 40s before it started to decrease, and the data partially supported my first claim that the heat shock times increase, so do the colonies, but not fully supported as the transformation efficiency decreased at the end. A challenge while doing the experiment was making sure that all of the trials were done under the same conditions, each having the same amount of time in ice, and warming up in room temperature, so that the only difference was the heat shocking time that would affect the bacteria. Something that could have influenced the experiment was the way the plating/spreading of the bacteria was done, as there is one that looks far different from the others, as what the bacteria pattern looks like. A limit to the experiment was the sample size because it would be more accurate and would show more effect if I did it other times, but the whole experiment takes a long time to do, and that just isn't enough time to have all of those trials done.

Implications for Future Research

It is important for other uses of the same heat shock method, as it was established that there is little to no difference between the heat method to the chemical method, so it is better to improve on the heat shock method. The information is useful to scientists because they can use it for producing more plasmids transforming into organisms. Like using it to study things they can't see, creating a vaccine, they can have a better transformation efficiency. New questions that come up were; do different types of bacteria and plasmid size affect the transformation efficiency significantly? Next experiment could be using different types of plasmid proteins and seeing how they differ in transformation efficiency. Could use the same experiment be change different variables and have more than one thing changing to see the transformation efficiency.

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