Creating a cost effective and faster way to synthesize DNA by using temperature variants

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Abstract

This experiment focuses on the effects of temperature on the growth of synthesized e.coli DNA and how the e.coli is affected if it is exposed to four different temperatures. Synthetic DNA is the remaking of existing biological entities. It was hypothesized that if synthetic DNA and proteins are grown at hot temperatures then the absorption rates would be higher because the enzymes speed up metabolism and cells increase rapidly in size when it is grown in any type of bacteria in warmer temperatures.

For the experiment, there is a growth media consisting of Luria Broth, ampicillin, and isoamyl alcohol and this is the Synthetic DNA part because this media is what changes the DNA. Then, there are 4 different temperature groups (120℉, 100℉,70℉,50℉). Put media into every tube of the 4 groups, put the e.coli in each of the tubes. Then they were put into their temperature zones.

In the results, most absorbance rates were in the hotter temperature zones of 120℉ and 100℉ and in the spectrophotometer, it came out to be 0.8 which means higher absorbance and less light passing through it, the 70℉ had lower absorbance rates and lower results, in the spectrophotometer, it came out to be 0.3, and in the 50℉, it had the lowest absorbance with 0.2 in the spectrophotometer. So the hypothesis was proven correct.

Introduction

Synthetic DNA is the reengineering or remaking of existing biological entities or systems in a gene. Synthetic DNA is still an emerging platform, but it can still be very useful and important to as it can be helpful in curing diseases that have no cure and can improve the medical industry generously. Synthetic DNA is a process of genetic engineering which transforms a living organism's building blocks, genes and DNA which can change the organism into something completely different. Synthetic DNA is really flexible making it easy to work with, this genetic modification process can also be edited on computers, and it provides so much information about the organism that modifying the genetic code is possible. Synthetic DNA can lead to many cures to incurable diseases such as cancer, diabetes, the normal cold, and many other diseases out there. Synthetic DNA is just starting to be used but the innovation and great progression it is making can be seen already, for example, the Moderna vaccine is a Synthetic DNA based vaccine and it was a very successful vaccine, and these vaccines are developed much faster than the normal vaccines and they have more accuracy. This is the same case for the cures or advances made with Synthetic DNA.

The problem that this project aims to solve is to make this process much simpler. Another purpose is to make it available to everyone but still make it possible that it actually changes something about the organism but it is a simple process, and show the benefits of this technology if used at a bigger scale. Simplifying this process can be useful in many ways, there can be courses for synthetic DNA since it the future of science and it is a must to to teach others about it, so it can use a simpler process to learn, this can also lower costs of synthetic DNA to make it accessible to everyone and making this process simpler allows for new innovations quicker and

faster and can pave the way for more scientific discoveries and answers to the problems that scientist try to find the answers to.

The purpose of this project is that synthetic DNA is expensive right now, and this project aims to bring this technology and make it available to everyone. The price for synthetic DNA can range from $100-$2000 and gene therapies cost $373,000 per dose. So the main purpose of this project is to make this technology affordable and show people how effective Synthetic DNA actually is.

The hypothesis for this project is, if synthetic DNA and proteins can be grown at cold or hot temperatures then what are the effects of those different temperatures on the Synthetic DNA? Protein can be processed at a very cold temperature, but on the other side, the bacteria cannot be grown in a very cold environment because then my experiment will go wrong because of the bacteria getting very cold or even frozen. So it is not possible to decrease the temperature so much that the bacteria gets frozen. The bacteria this project experiments on is E. coli can survive up to 39 and 113* Fahrenheit. So in order to complete this project, there would have to be a system in place to track the temperature that these e.coli are going to grow in.

Methodology

This project is going to experiment on different, safe, e.coli strains and see how synthetic DNA changes the e.coli. So basically what synthetic DNA is doing is that it creates something which is synthesized and makes the organism think that, that is their DNA and “tricks” them into using that DNA which causes the changes in the organism. There is a growth media that serves as the Synthetic DNA because this part will “trick” the e.coli into thinking that its growth media has its DNA so it should develop with it causing it to change something about itself. The prediction of

the result of this experiment will mainly affect how it looks and how it smells because there is an added material that acts as a substrate that interacts with the enzyme causing it to mainly change the smell. There are 4 established data groups (each of these groups would be in different temperatures, for example, group 1 could be in 70 degrees Fahrenheit and so on) Here are the steps to the science fair experiment:

1. Make growth media for the e.coli to grow in and change in by adding 600 ml of Luria broth (Luria broth provides food for the bacteria), 3 ml of ampicillin, and 500 ml of isoamyl alcohol (this is the substance that acts as a substrate for the enzyme and specializes in mainly changing the smell of an organism).
2. Then make 4 groups of 4 tubes, each group will be in a separate temperature zone and add the growth media, and then proceed to add the different strains of e.coli in each tube (add the 4 types of different e.coli strains so that each tube has a different e.coli)
3. The names of the 4 groups depending on what temperature or setting they are grown in. The first group is the lowest group (this is grown in 50 degrees Fahrenheit), the second group is moderate cold (70 degrees Fahrenheit), the third group is called mid-zone(100 degrees Fahrenheit), and lastly, the fourth group is moderately hot 120 degrees Fahrenheit).
4. Then record how many days each group takes to fully develop and grow, take pictures of results, and analyze how smell, appearance, size, etc grew with the Synthesized DNA growth media given to the e.coli how different temperatures affected the growth.
5. If possible, test this in a spectrophotometer which allows measuring the cell density.

Data and Results

This graph shows the relationship between the number of days it took each e.coli to grow in its given temperature phase.

By the way:

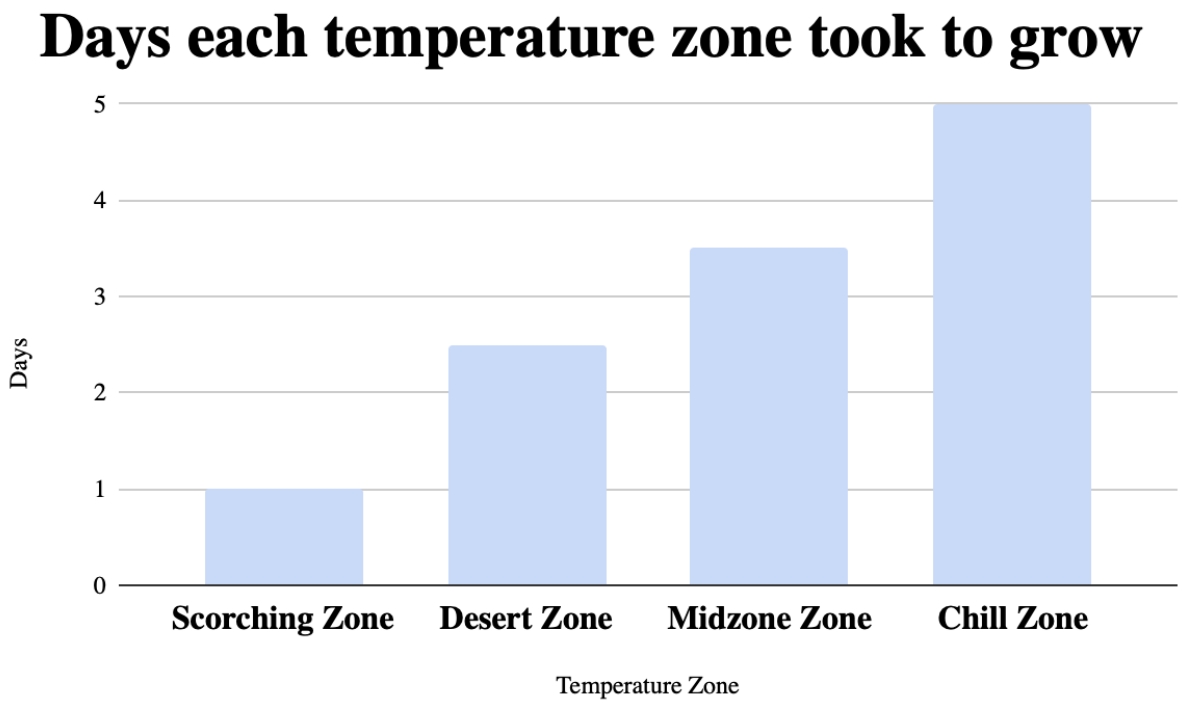
Chill Zone: 50 degrees Fahrenheit

midzone: 70 degrees Fahrenheit

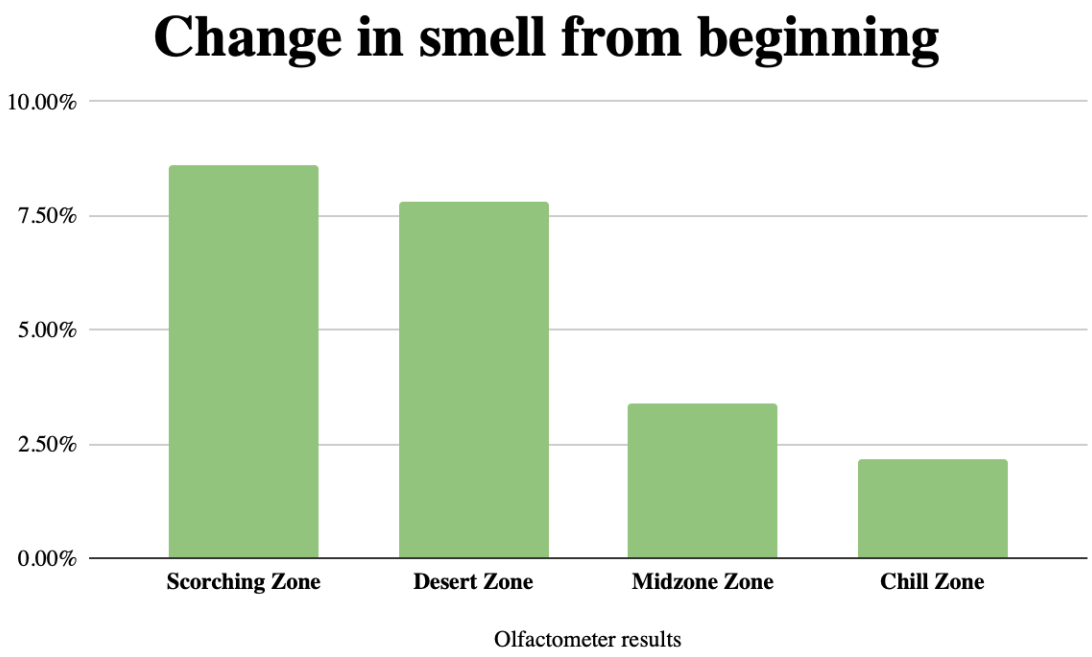
Desert zone : 100 degrees Fahrenheit

Scorching zone: 75 degrees Fahrenheit

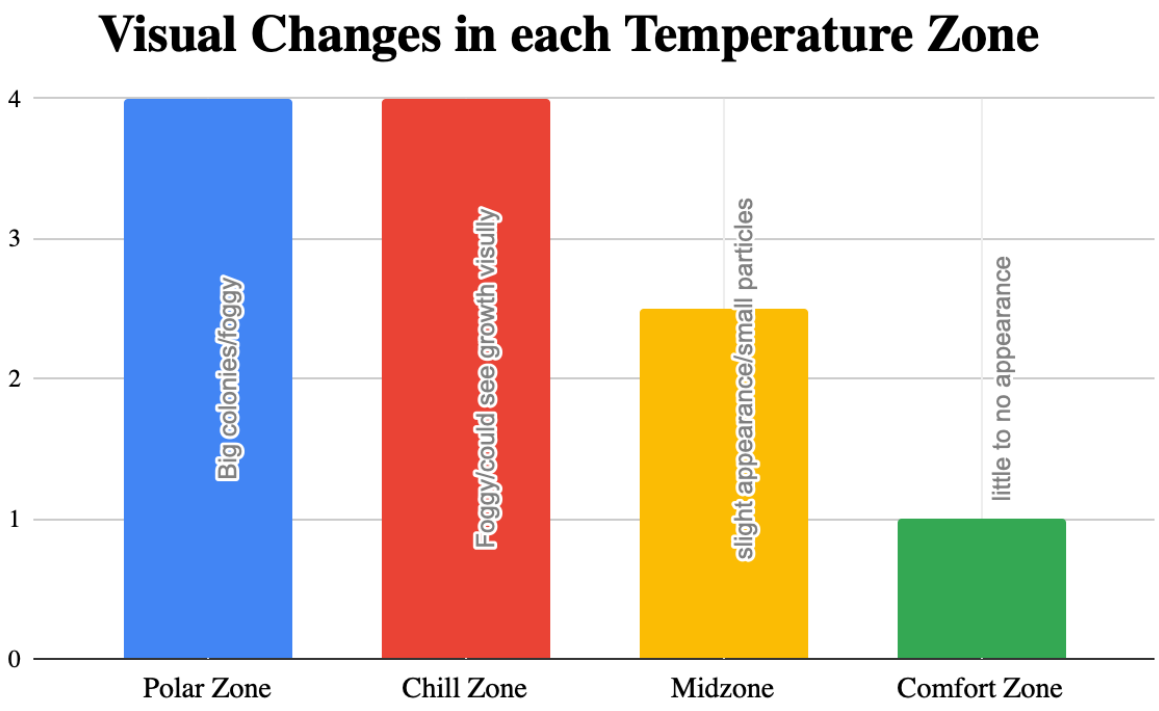
From this graph, it is inferred that it took the least amount of time for the colder temperature's ecolis' to grow compared to the e.coli growing in high temperatures.



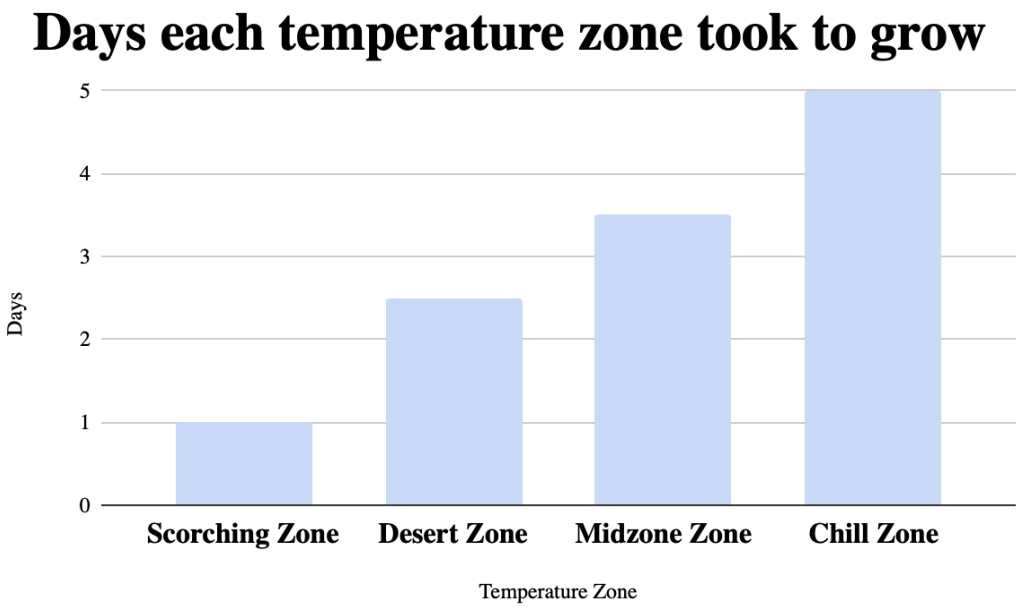
This graph shows the smell changes that happened to synthesized e.coli in each temperature zone, as it is shown, the colder temperature had a higher change in smell (the isoamyl alcohol added to this will change the smell into a sweeter scent which was a banana scent). The higher temperature ones again had the lowest amount of smell change too.



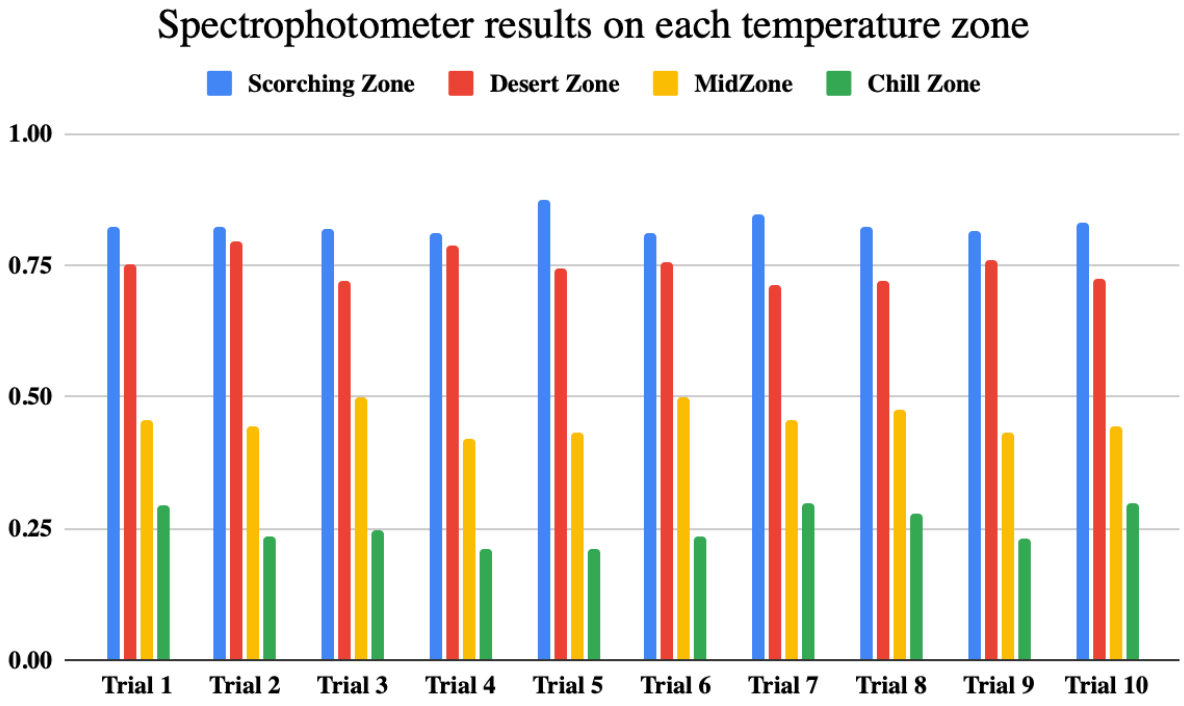
In this next graph, the visual changes each synthesized e.coli had are graphed, in the scorching zone, the e.coli had a lot of changes with big colonies, most amount of smell and visual changes,, in the desert zone, there were smaller colonies but they were still visible and they also had many smell and visual changes, in the Midzone, the e.coli growth was in “dust particles” not in big groups but rather in a particle form and it was difficult to see what was going on, and in the last one, the Polar zone, there was little particles/colonies and it was the hardest to see what was going on.



This next graph shows how many days each zone took to grow. It is shown that the scorching zone of 110 degrees had only one day while the chill zone of 50 degrees fahrenheit had five days of growth and it is shown that the chill zone had the least amount of results with the most time but on the other hand, the scorching zone of 110 degrees had the most amount of results with less growth period.

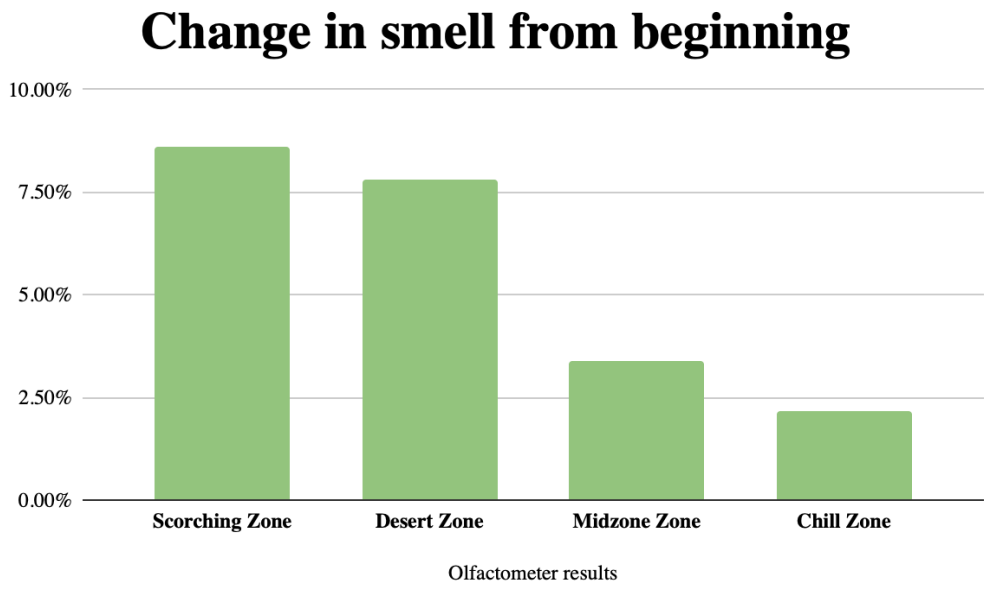


After the growth period of each zone, they were put into a spectrophotometer to see which temperature gave the most absorption rates. As shown, averagely for all ten trials, the scorching zone had the most amount of absorption rates with 0.8 absorption units and chill zone of 50 degrees only had 0.2 absorption units.

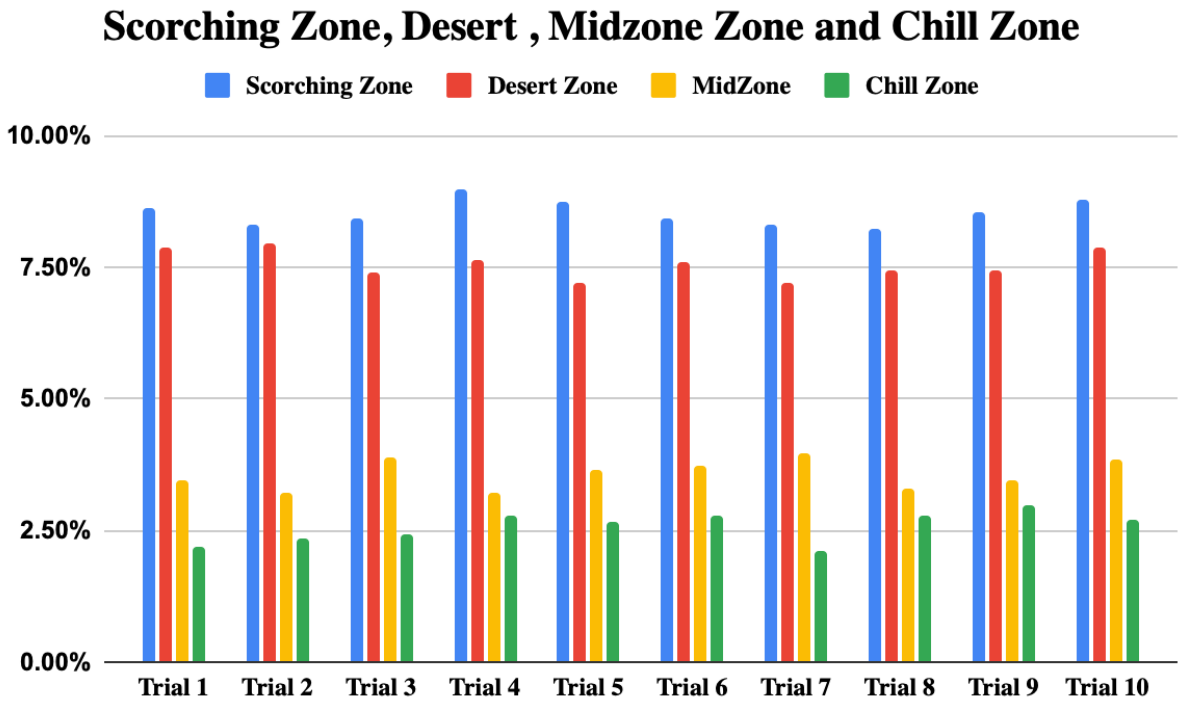


In this project, it is mentioned that there would be a smell change because of the isoamyl acetate produced by the isoamyl alcohol. Each zone was put into an olfactometer, a machine that measures the amount of smell change in a substance. In the scorching zone of 110 degrees, there

was a 8.5% change in smell while the chill zone of 50 degrees only had 2% smell change.



The following graph shows the average of all ten trials of each temperature zone in the olfactometer, and on average the scorching one still has the most amount of smell change while chill zone of 50 degrees only has 2% average.



Conclusion

This project is about how temperature affects the growth of synthesized e.coli DNA. The project's purpose is to make an efficient process of synthetic DNA so it is less expensive, more successful and is available to all because right now per unit it can cost anywhere from $100-$2000 and a treatment with synthetic DNA can cost from $72,000 to even millions of dollars. The reason why this project is focused on temperature is because finding the optimal temperature at which synthetic DNA is best grown would make the process easier, simpler, faster which would bring the cost of the process down. In addition the idea of changing an organism however it is wanted without any limits sparks creativity and this topic as a whole is an interesting field of science.

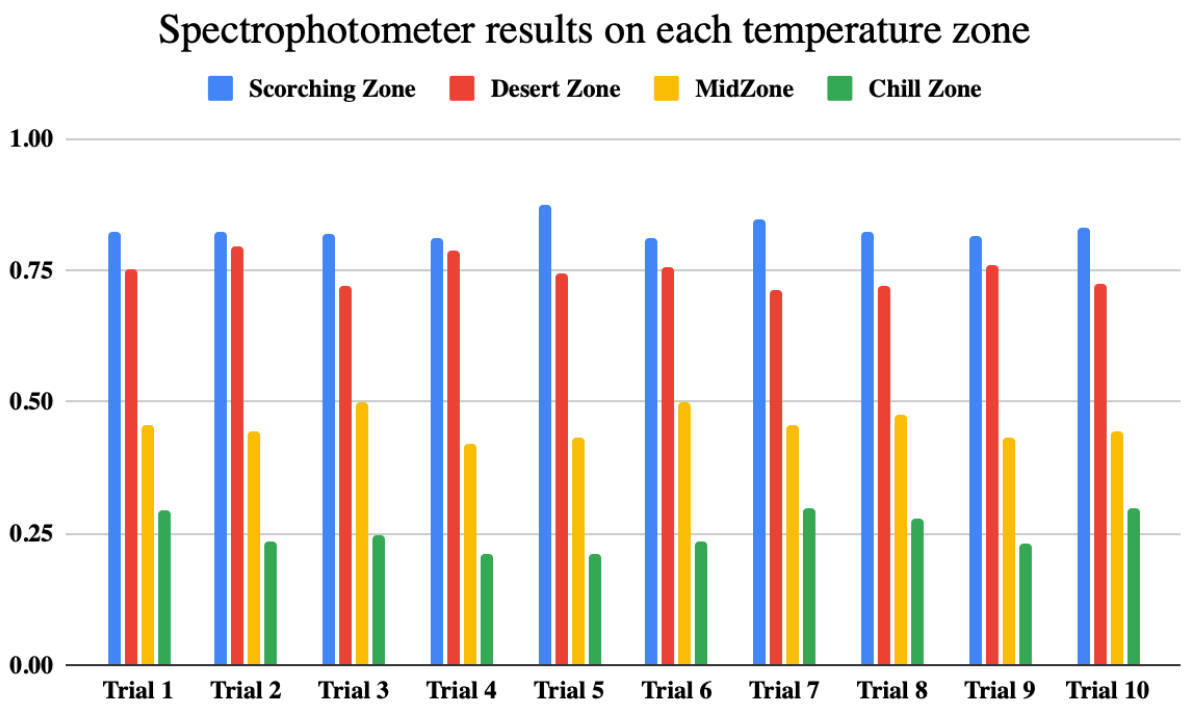
The hypothesis for this project was, if synthetic DNA and proteins are grown at hot temperatures then the absorption rates would be higher because the enzymes speed up metabolism and cells increase rapidly in size when any type of bacteria in warmer temperatures was grown. The reason why hotter temperatures will have more successful results is because as the temperature of the environment goes up it makes the molecules move faster, the enzymes speed up metabolism and it results in the cells rapidly increasing in size.

The way this project was conducted is by making a growth bacteria in which the e.coli would grow in. The media consisted of Luria broth (provides food for the e.coli), ampicillin and isoamyl alcohol; isoamyl alcohol is a substance that changes the substrate of an enzyme and isoamyl alcohol specializes in changing smell, so the expected outcome was that the e.coli

should have a smell change after its growth period. There were 4 temperature zones, 120°F, 100℉,70℉, and 50℉. The temperature zones had names according to what temperature zone they were in, the 120℉ was “scorching zone”, the 100℉ was “desert zone”, te 70℉ was “midzone" and the 50℉ was “chill Zone”. After making the growth media, they were placed into 4 groups of tubes and placed each tube to its temperature zone and repeated this 10 times. After the growth, each group was measured in a spectrophotometer which would tell the absorbance rates of each zone, and the higher the absorbance is the more successful the project was. To measure change in smell, an olfactometer was used, which measures the change in smell of a substance. So these both were ways that this project had quantified results.

In conclusion it was found that the 120℉ and 100℉ both had the highest results out of them, in the spectrophotometer, the tubes that grew in 120℉ had absorbance rate of 0.8 and the 100℉ tubes had an absorbance rate of 0.6. The 70℉ tubes had a lower absorbance rate of 0.4 and the 50℉ tubes had the lowest absorbance rate of all of them at 0.2. Here is the graph and table on

this data:

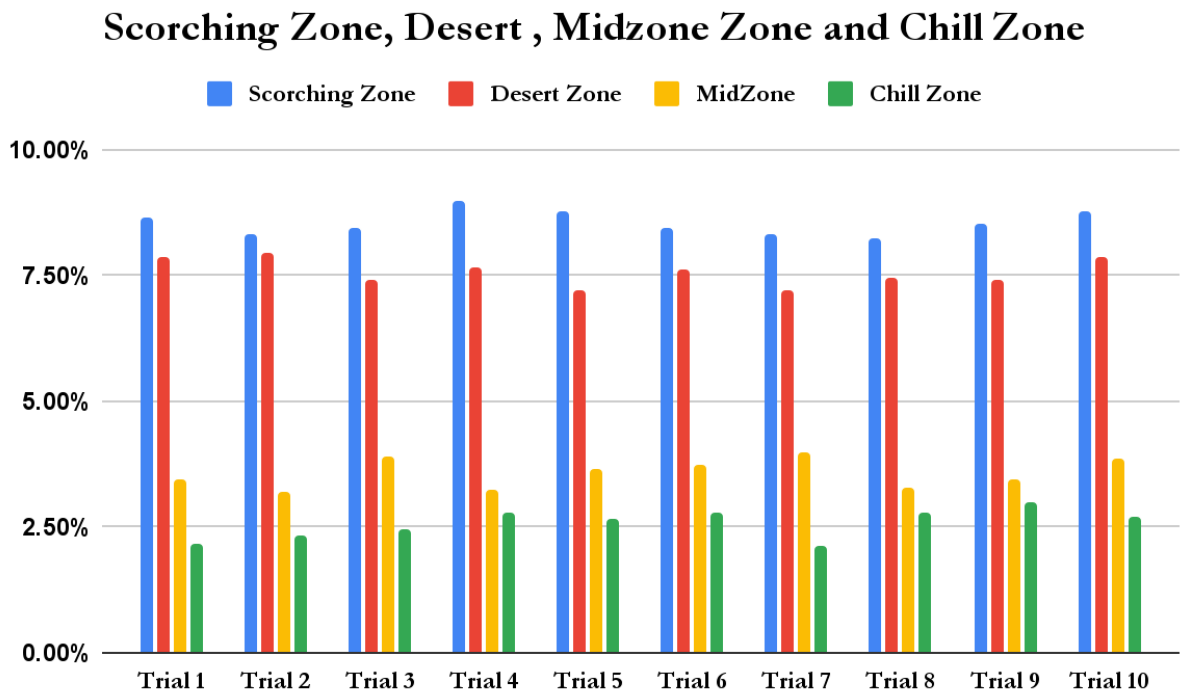


Spectrophotometer Results

	Scorching Zone	Desert Zone	MidZone	Chill Zone
Trial 1	0.8235	0.7542	0.4546	0.2932
Trial 2	0.8245	0.7961	0.4432	0.23332
Trial 3	0.8212	0.7223	0.4987	0.2456
Trial 4	0.8101	0.7865	0.4218	0.2112
Trial 5	0.8732	0.7432	0.4323	0.2098
Trial 6	0.813	0.7554	0.4998	0.2347
Trial 7	0.8474	0.712	0.4569	0.2996
Trial 8	0.8221	0.7189	0.4754	0.2768
Trial 9	0.8173	0.7604	0.4329	0.2309
Trial 10	0.8324	0.7234	0.4449	0.2991

In the spectrophotometer, it is shown that the most amount of absorbance was by the highest temperature zones (comfort zone and Midzone) with 0.8 and 0.6 absorbance rates with the lowest ones being 0.4 and 0.2.

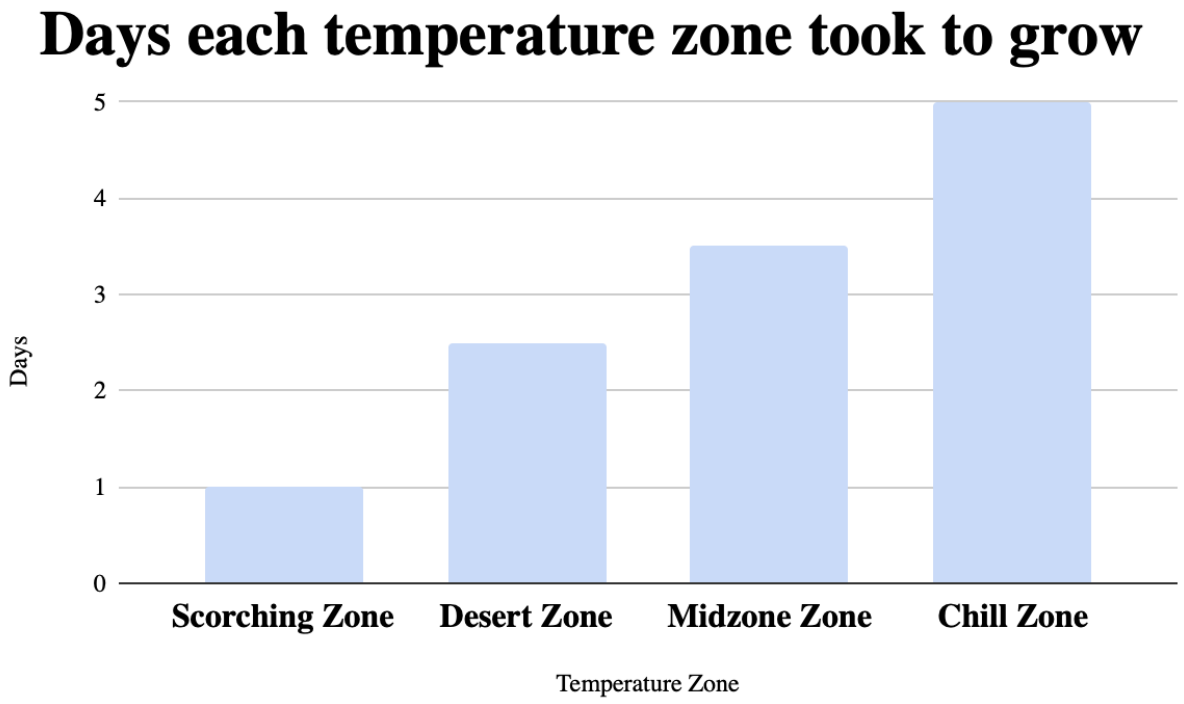
After the spectrophotometer, it was observed that there was a distinct difference in the smell, the higher absorbance tubes had a banana like smell and the tubes with the lowest had some smell change so this was measured as well. A machine called an olfactometer was used, which basically measures the smell change in percentage. These were the results:



	Scorching Zone	Desert Zone	MidZone	Chill Zone
Trial 1	8.65%	7.88%	3.44%	2.18%
Trial 2	8.32%	7.96%	3.21%	2.34%
Trial 3	8.43%	7.40%	3.89%	2.44%
Trial 4	8.97%	7.65%	3.22%	2.78%
Trial 5	8.76%	7.21%	3.67%	2.65%
Trial 6	8.43%	7.62%	3.74%	2.77%
Trial 7	8.32%	7.22%	3.99%	2.13%
Trial 8	8.23%	7.44%	3.29%	2.78%
Trial 9	8.54%	7.43%	3.45%	2.99%
Trial 10	8.78%	7.88%	3.86%	2.69%

As shown in the graph and the table,the zones with higher temperature zones (comfort zone and Midzone) had the highest amount of smell change compared to all of the others, the highest temperature zone had a change of 8.6% and the lowest temperature zone had a percent change of 2.18%.

The project purpose was to also make a process that makes the synthetic DNA process faster so the cost goes lower so a measurement of how many days it took for each group to grow was taken and here are the results on a bar graph:



In this graph the Comfort zone took the least amount of time to grow with only 2 days of growth time and the Polar Zone took the longest to grow with 5 days in total.

To show these results, it has been quantified in a t-test. These were compared all of the temperatures to the control of room temperature and compared the spectrophotometer results by each. Here is what the t-test table looks like:

Control is room temperature (80 degrees Fahrenheit)				
	Sample Mean	Sample Standard Deviation	t-value	p-value
110°F vs Control	0.8	0.10606	17.889	.05 > .00001
100°F vs. Contro	0.754	0.13859	22.817	.05>.00001
70°F vs Control	0.4	0.3889	5.65	0.5>.000023
50°F vs control	0.219	0.51689	7.17	0.5>.000411

In this table the p-value of .05 is greater than all of the temperature zones meaning that the null hypothesis can be rejected and accept the hypothesis of if synthetic DNA and proteins are grown at hot temperatures then the absorption rates would be higher.

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