Written Project CYSF:

Genetic Modification on Cyanobacteria to Increase Carbon Capture

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Contents

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Introduction and Research

This science fair project is about increasing carbon capture in cyanobacteria. Cyanobacteria is found in freshwater and salt water environments around the world. Cyanobacteria are photosynthetic, crucial for the production of the oxygen that we breathe, while also absorbing carbon dioxide, the primary greenhouse gas.

Cyanobacteria is a division of microorganisms that are related to the bacteria but are capable of photosynthesis. They are prokaryotic and represent the earliest known form of life on the earth (XX dictionary, 2024). Prokaryotic cells do not contain a nucleus and membrane bound organelles. A key feature in Prokaryotic cells is the presence of plasmids. Plasmids are a genetic structure in a cell that can replicate independently of the chromosomes, typically a small circular DNA strand in the cytoplasm of a bacterium or protozoan. Plasmids are used in the laboratory in manipulation of genes (found in an online dictionary for science). Cyanobacteria evolution developed from bacteria. It was one of the first organisms able to complete photosynthesis. This bacteria contains both plasmids like prokaryotic cells but also contains membrane bound organelles such as the chloroplast. Cyanobacteria are photosynthetic prokaryotes and are one of the main contributors to biogeochemical cycles. The organism has been around for billions of years. Not only this organism is found in almost all regions of the Earth making it a good candidate for this study. The type of Cyanobacteria being studied is unicellular. Recent studies have been focusing on genetic engineering through the use of plasmids that contain a Gene of Interest (GOI) that codes for the enzyme Carbonic anhydrase (CA). This enzyme catalyzes the reaction of converting gaseous CO2 to dissolved bicarbonate, which can easily be taken up by microalgae in the culture media (XX dictionary, 2024). In Genetic Engineering, GOIs give instructions that are needed in life for living organisms coding for specific traits. These provide the organism with a key feature, which can lead to unique solutions, such as optimized carbon (CO2) capture. Through the use of meticulously designed gene regulation, plasmids can be created for sustainable environmental solutions. Gene regulation is what controls in a cell to determine what portions of DNA are used. Some of the research that was conducted is as follows.

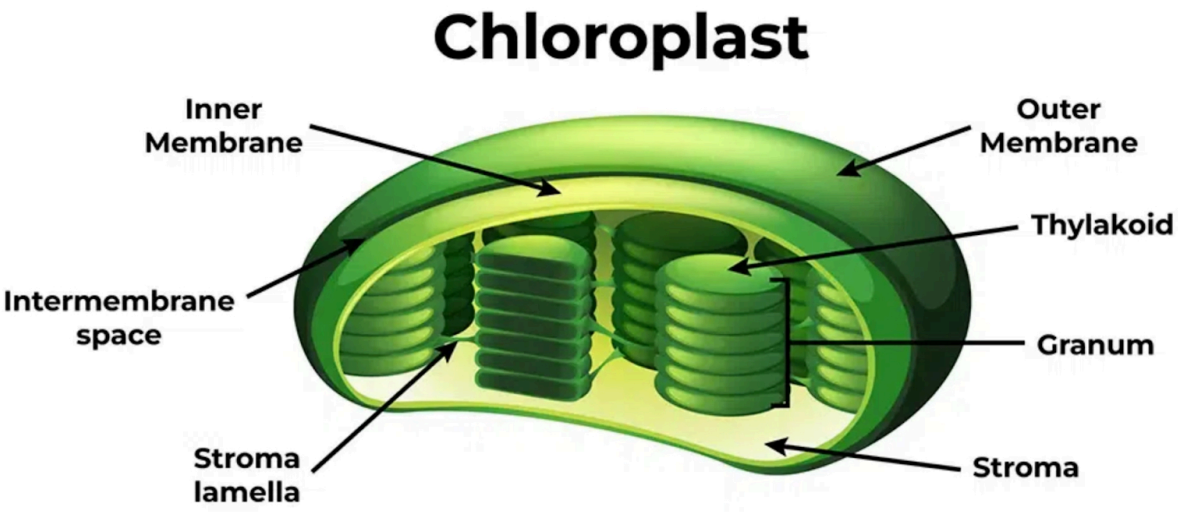
The studies include in depth research of all topics; photosynthesis, DNA, gene regulation, synthetic biology, plasmid design and function was conducted.

Photosynthesis

Photosynthesis studies included learning about chloroplast, light-dependent reactions, light-independent reactions, and electron transport chain. Photosynthesis is a complex process that occurs in the chloroplast of plants and Cyanobacteria cells, where carbon dioxide and water can be converted into glucose. Chloroplasts are specialized membrane bound organelles that contain a unique structure that allows for photosynthesis to occur. Some of those structures include a stack of thylakoid membranes, which is called a granum which is a biology term for stack. The space around the granum is a liquid called the stroma. The stroma is where the guard cells are. The guard cells allow carbon dioxide in and oxygen out of the plant or bacteria.

Figure 1

The Inside of a Chloroplast



Note: Chloroplasts: Diagram, Structure and Functions. (2023). *The Inside of a Chloroplast* [image] Retrieved March 8, 2024, from https://www.geeksforgeeks.org/chloroplasts-diagram-structure-and-functions/

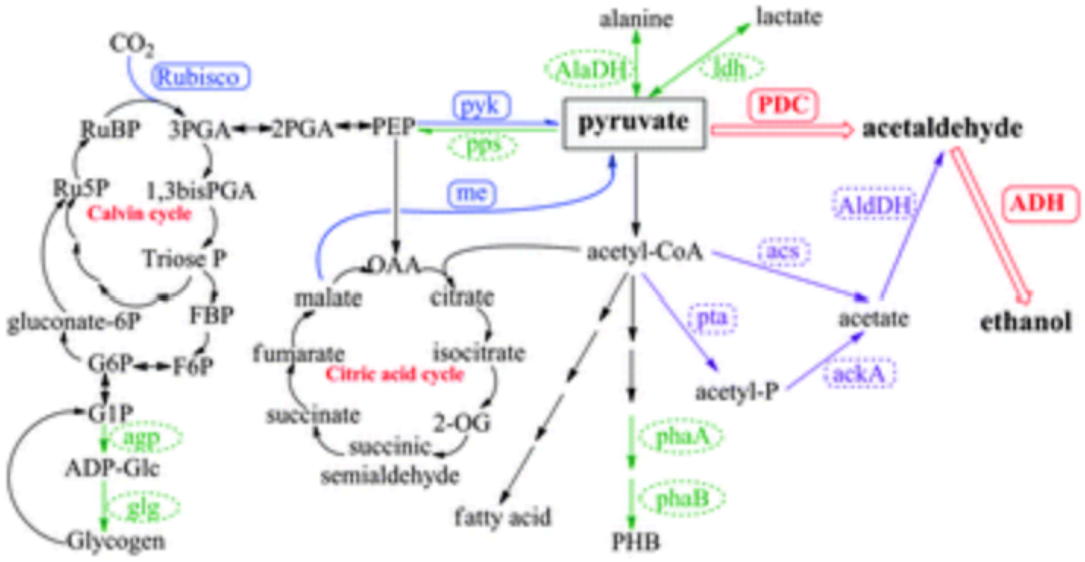
Photosynthesis can be divided into two main steps: Light dependent-reactions and Light Independent reactions. First, the light dependent reaction occurs in the thylakoid membrane of the chloroplast. The light dependent reaction produces two things, adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH). These are used later in the second step, the Calvin cycle. Water is used as a reactant for the photosynthesis equation. The water enters the cell and is split into protons, electrons and oxygen. When this process is taking place hydrogen is a waste product. After creating the necessary chemicals ATP and NADPH, the Calvin cycle reaction takes place. This takes place in the stroma which is the fluid in the chloroplast. Carbon dioxide

enters through the guard cells and is fixed by an enzyme which then can be used later on. NADPH will supply reduced power, which means it will add high energy electrons to the process. The ATP will act as an energy currency for the Calvin cycle. Using all of this NADPH, ATP and fixed carbon will lead to the creation of glucose and oxygen completing the two steps of the photosynthesis reaction.

In order to see this process scientists use chromatography, which allows people to see chlorophyll. Two different types of chlorophyll were studied. Chlorophyll A which predominantly absorbs the light wavelengths violet, blue and red. Chlorophyll B predominantly absorbs blue orange and red., The result is the reflected green pigment of the plant

Figure 2

Photosynthesis Cycle



Note:✍. (2022). *Photosynthesis Cycle* [image] Retrieved January 3, 2024, from https://pubs.rsc.org/en/content/articlelanding/2012/ee/c2ee22675h/unauth

Note: On the left it shows the chemical reactions in the Calvin Cycle. On the right, the chemical reactions from cellular respiration. This figure is illustrating how carbon dioxide from cellular respiration is used in photosynthesis and oxygen released from photosynthesis is used in cellular respiration.

DNA Structure and Function

DNA is found in all living organisms and is a self-replicating material that carries genetic information. The acronym stands for Deoxyribonucleic Nucleic Acid. A plasmid is a circular piece of DNA found in prokaryotic cells, in this case, Cyanobacteria. It is used to control the cell's functions and give instructions to different parts of the cell. In this project it is studied to find that this genetic material is used to give instruction for protein synthesis which is part of the overall topic of gene regulation. The structure of DNA includes two chains of nucleotides and genes. Nucleotides are the building blocks of nucleic acids, which include two families RNA and DNA. They also code for a structure of proteins synthesized. Several nucleotides are coenzymes which speed up the process in this case CA. Genes are made up of DNA. Their main function is to control the synthesis of proteins. This is where gene regulation comes into play.

Gene Regulation

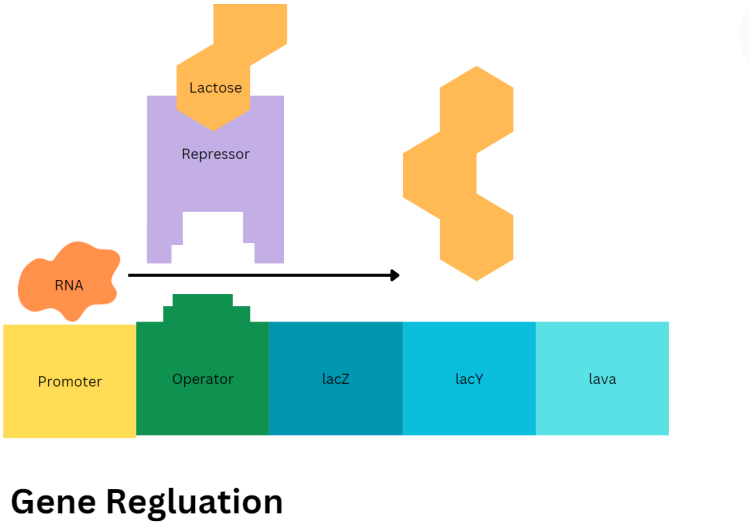
Gene regulation is controlled by switching genes on or off. (this is a good source for a general description) A gene is a specific strand of DNA.

An operon is a cluster of genes that are transcribed together to give a single messenger RNA (mRNA) molecule, which therefore encodes multiple proteins (Fig. 16.11). Such polycistronic mRNA is typically found in prokaryotes. (Clark et al., 2019) . Gene regulation includes protein synthesis. The has two main steps: transcription and translation. These two steps play a key role in gene regulation. Transcription and translation are the processes by which a protein is made. Transcription is the first step that occurs when RNA will connect to the bases of the DNA to form a strand of mRNA (m stands for messenger). Before the next step there is a lot of editing done within the mRNA before it completes its job. Ribosomes build proteins following the MRNA and tRNA steps. Furthermore, inside the cytoplasm of cyanobacteria there is a tRNA (t stands for transfer) amino acids are found on the ends of the tRNA which is the monomer for a protein. The mRNA directs the tRNA. They direct the tRNA to look for complementary bases on the to transfer the amino acids on the mRNA. The tRNA reads the “letters' ' which are read in threes called a codon. The acids are then left and held together by a peptide bond. Over many of the acids being left behind there is a stop codon which signals to the ribosome that the protein creation is finished. The DNA is in control over the entire process. In addition, to the RNA polymerase they need a promoter to bind to. In other words in order to create the enzyme to break down lactose (sugar) the RNA needs the promoter to build the enzyme. Beside the promoter on the DNA strand the operator is included which can bind with the repressor to prevent the enzyme from breaking down

lactose. The reason the repressor is used is to save energy. If there is no lactose then the repressor blocks the RNA. If there is lactose present then the lacoste will bind to the repressor allowing the RNA to pass through which then breaks down the lactose which is used for energy.

Figure 3

Gene Regulation Diagram



Note: Alyssa.B (2024). *Gene Regulation Diagram* [image]

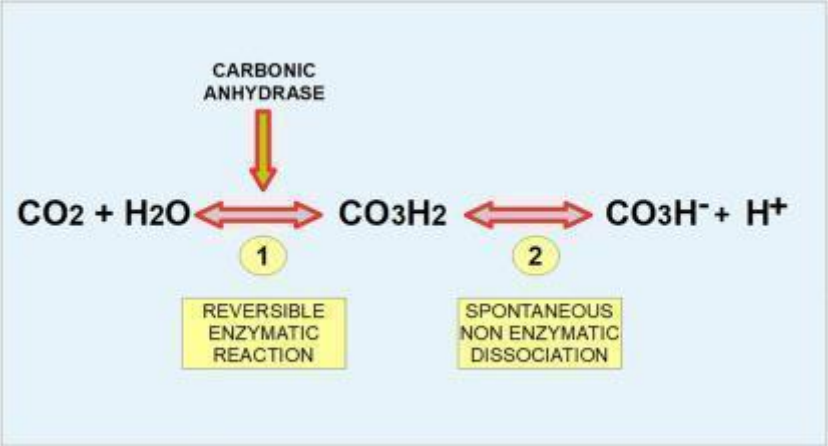
Synthetic Biology

Next main topic that was studied was Synthetic Biology. What is Synthetic Biology? It is a field of research in which the main objective is to create fully operational biological systems from the smallest constituent parts possible, including DNA, proteins, and other organic molecules (Rugnetta, 2024). Some research was done on a specific enzyme called Carbonic Anhydrase also known as CA. CA is an enzyme that catalyzes the reaction of converting gaseous CO2 to dissolved bicarbonate, which can easily be taken up by microalgae in the culture media. CA is an enzyme that catalyzes the interconversion of dissolved bicarbonates and carbon dioxide. (Found from iGem team). This enzyme was chosen because it can dissolve carbon dioxide at a faster rate which increases carbon dioxide capture. There is a chemical formula which occurs in Cyanobacteria plasmid and is shown in Figure 4..

Formula: CO 2 + H 2 O ↔ H + + HCO 3

Figure 4

Carbonic Anhydrase Formula in Cyanobacteria



Note: Team:Mingdao. (2019 a).*Chemical Formula of the iGem Team Biobrick.* [image] iGEM 2019. Retrieved January 21, 2024, from https://2019.igem.org/Team:Mingdao

HCO 3 stands for bicarbonate. The CA, C02, and H20 create carbonic acid (C03H2). Carbonic anhydrase dramatically increases the speed of this reaction and is one of the fastest-known enzymatically catalyzed biological processes (found on ScienceDirect) In addition, biological processes determining cell behavior are defined by the complex interactions among all molecules which is part of three main processes. H+ is an acidic solution that has a high concentration of hydrogen ions (found in the formula).

To conclude, the enzyme that will be used in this project is Carbonic Anhydrase which carries out the function of dissolving carbon dioxide at a faster rate.

Plasmid Design

A plasmid is a circular piece of DNA that is found within Cyanobacteria. The group that was researched is called the AHUT China team. The team developed four different plasmid designs, with the goal of speeding up the process of photosynthesis. In order to construct the protein they must purify the proteins, which are used through the process of gene regulation. The team had to go through a number of steps including maintaining a 3D structure of the enzyme and shortening the distance between the protein

transfer. Their main goal was to purify the protein so it could be inserted into a plasmid design. The enzyme that was used is Carbonic Anhydrase (CA). It was found to speed up the process of photosynthesis.

Adding the CA is a 10 step process. The ten steps process includes; selecting a CA gene, designing a plasmid vector, polymerase chain reaction, amplification, restriction digestion, ligation, transformation, screening and verification, expression, protein purification and characterization. The team developed a genetically modified version of Carbonic Anhydrase, called CA2, which is the purified form of the enzyme. This took in depth research on gene regulation to be able to generate it on a computer.

A lego set to build a tower is a metaphor that can be used to explain the plasmid design and function. The set contains an instruction manual and the bricks to create the lego tower. The bricks represent RNA, mRNA, tRNA, lactose, etc., while the instruction manual represents the plasmid and the finished tower represents the protein. The plasmid instructs the bricks how to build the tower. The main function of plasmids is to give instructions to 'the bricks' creating a protein and to build the organism. Additionally enzyme CA is an optional add on to accelerate the build. Think of it as a short cut in the instruction manual to build the lego tower faster. In order for it to be added correctly there must be instructions for this attachment. For example, if you try to do the short cut in the instruction manual at the wrong time, the lego tower will not be built properly. The CA, when inserted correctly, will speed up the process of photosynthesis allowing the protein to be created faster. The iGEM teams have created plasmids to inform the CA what to compete.

Problem and Potential Solution

Figure 5 below shows the greenhouse gas emissions for several countries and compares them for the years 2005 and 2020. In 2020 China, the United States and India are the largest emitters and account for nearly 47% of global emissions. Canada by comparison emits 1.5%. Globally emissions continue to rise contributing to overall warming and changes to climates around the world. These changes are causing many negative consequences including sea level rise, extreme weather events, drought and flooding to name a few. Attempts are being made to reduce carbon emissions that come primarily from fossil fuels, however such reduction is complex depending on geography.

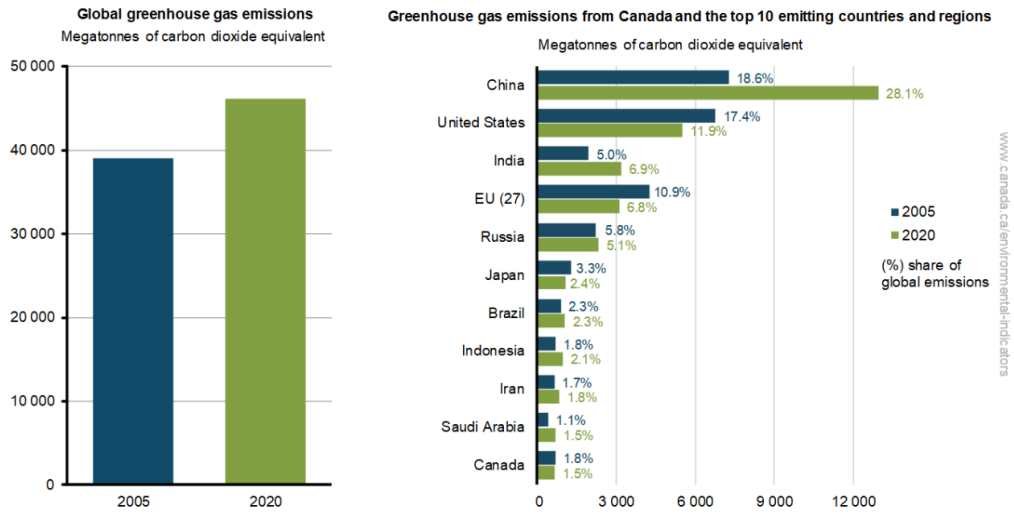
Sequestration of carbon is a way to reduce greenhouse gas from the atmosphere. This project focusing on photosynthesis could be a potential solution among others being studied.

For example, there are groups that are working towards a similar goal that this project is conducting, such as the Mingdao iGem Team, who have done a similar type of

project in 2019. The team has a potential carbon solution. The research that this iGem Team has developed is changing the plasmid design of algae. Furthermore, the reason the iGem Team developed this project is because they wanted to improve air quality within their hometown in Taiwan. The team researched CO2 and VOCs to create a Biobrick. The team used the enzyme CA to increase the dissolution rate of the CO2 in the algal culture medium. Within this study there was a second team looking into AHUT China, their goal was similar to the other Team. They wanted to speed up the process of photosynthesis. It uses an already existing Biobrick to create four different plasmid designs (included at the bottom of the paper). The team used the enzyme CA2 instead of CA, in addition. The parts that were used in the Biobricks include Team Mindago: BBa_K2932000 and the AHUT China Team using part BBa_K2232000. Team Mindago developed the Biobrick which is the part above. The AHUT China team used an already existing Biobrick to include in their research. The team's final design was the third plasmid listed below.

Figure 5

Greenhouse Gas Emissions

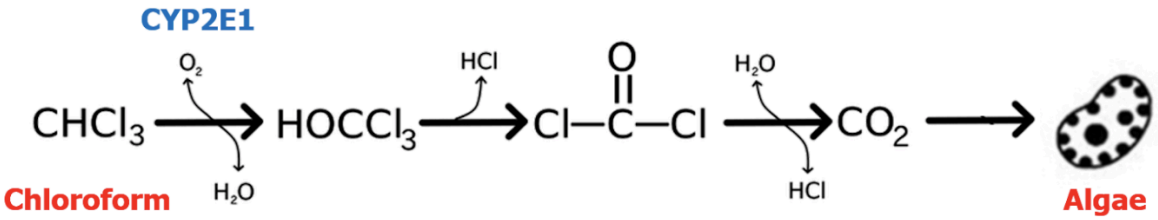


Note: Is an information bar chart showing the levels in 2005 and 2020. This shows the increase in some of the major countries contributing to carbon dioxide increase.

Statistics Canada. (2023, September 22). *Greenhouse gas emissions*. [Image]. Canada.ca. Retrieved January 20, 2024, from https://www.canada.ca/en/environment-climate-change/services/environmental-indicators/global-greenhouse-gas-emissions.html

Figure 6

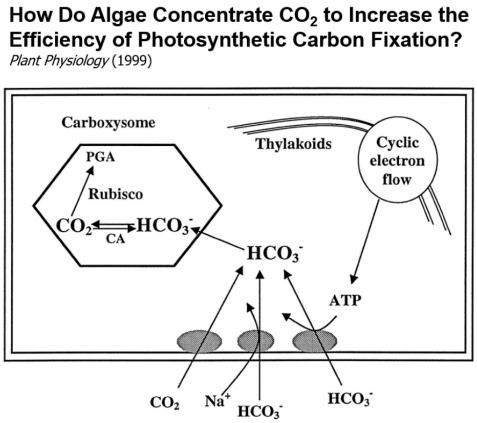
Chemical Formula of the iGem Team Biobrick



Note. Team:Mingdao. (2019 b).*Chemical Formula of the iGem Team Biobrick.* [image] iGEM 2019. Retrieved January 21, 2024, from https://2019.igem.org/Team:Mingdao

Figure 7

Algae Concentrate to Increase Carbon Fixation



Note: Team:Mingdao. (2019 c).*Chemical Formula of the iGem Team Biobrick.* [image] iGEM 2019. Retrieved January 21, 2024, from https://2019.igem.org/Team:Mingdao

Method

Advanced study has been done on Cells and Systems, photosynthesis, DNA, gene regulation with an in depth understanding of transcription and translation. As well as synthetic biology. In addition, intensify study and research on plasmid design, structure and function. I used the iGem team from 2019 as one of my main sources because the research that they had done was valuable for the project. It gave an overview of all the topics that were looked into. A spent a long time looking into this source. The CCDS was

the other main source of this project, it allowed plasmids and gene sequencing to be generated. I used this source near the end of my research project so I could understand the figure. The amoeba sisters were a source that helped explain what was happening as a visual. The process of this project included notes on all the topics which I then converted into this paper. I created a mock up of the plasmid and two trifolds. The total amount of time I spent on this project started in October up until March. Lots of work was done over the breaks that I had including the Christmas break. The process started with research and understanding, which then developed into finding a possible solution.

Data

The data base for this project is comparing the two iGEM teams to understand, how are they different, what was their approach, what genes did they use, and how did it work for each group. Both groups used CA in their design. Team Mindago developed a Biobrick, which is one of the first steps into developing the plasmid they were hoping to design to increase carbon dioxide capture.

A Possible Solution

A possible solution that could be explored is combining the successes of both teams to develop a plasmid design further. This includes four main things to combine, photosynthesis, gene regulation, Carbonic Anhydrase 2 and a Biobrick. Team Mindago biobrick was more developed then the other team, and explored photosynthesis at a deeper level. For the purpose of the best outcome, I would use this team’s biobrick. On the other hand, using the CA2 would add a whole new level to the speed of photosynthesis in the Cyanobacteria. Reinforcing the plasmid design that the AHUT China team developed may also be beneficial for the best outcome. Their team has a solid plasmid design but adding the Biobrick that team Mindago developed would bring a whole new level to the design. Using both teams ideas of gene editing allowed them both to have the success they came too. The main difference of what I am proposing is taking both teams' incredible research on plasmids, and instead of starting over, combining them to create the ultimate plasmid design for Cyanobacteria to increase carbon dioxide capture.

Plasmid Design

Gene Sequence

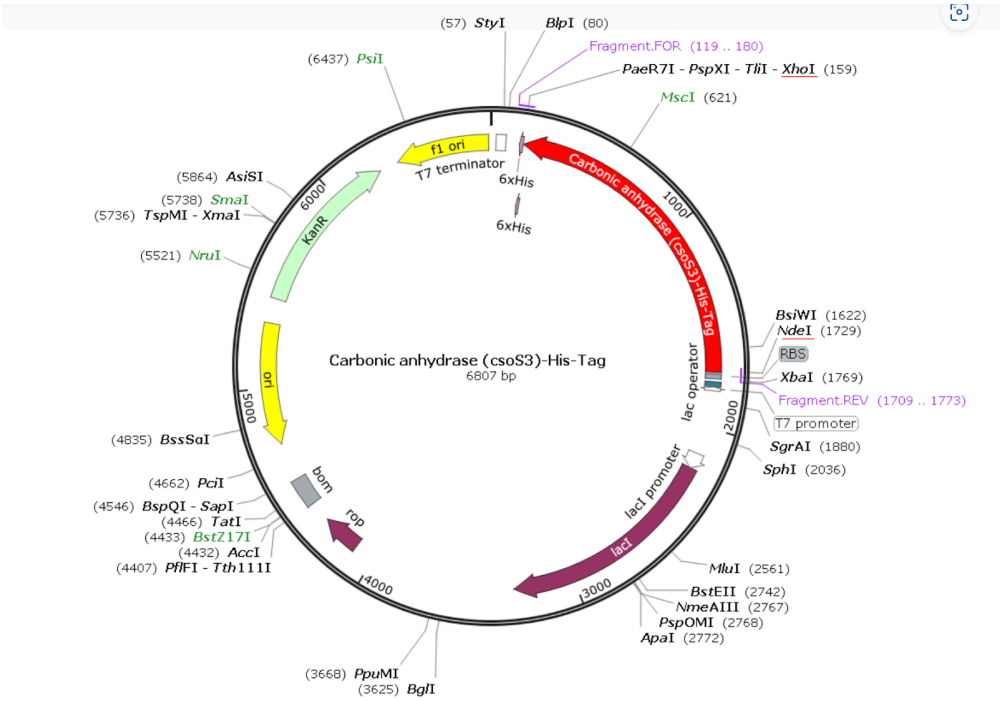
Note: This is the gene sequence that is used to increase carbon capture in cyanobacteria of a plasmid.

Nucleotide Sequence (783 nt):

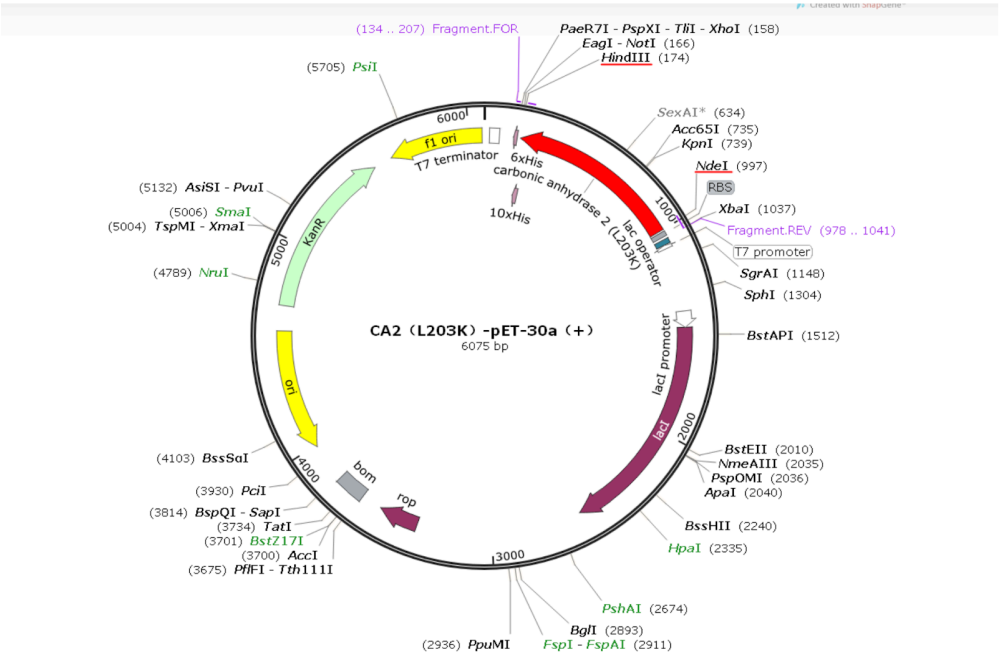
ATGTCCCATCACTGGGGGTACGGCAAACACAACGGACCTGAGCACTGGC
ATAAGGACTTCCCCATTGCCA
AGGGAGAGCGCCAGTCCCCTGTTGACATCGACACTCATACAGCCAAGTAT
GACCCTTCCCTGAAGCCCCT
GTCTGTTTCCTATGATCAAGCAACTTCCCTGAGGATCCTCAACAATGGTCA
TGCTTTCAACGTGGAGTTT
GATGACTCTCAGGACAAAGCAGTGCTCAAGGGAGGACCCCTGGATGGCA
CTTACAGATTGATTCAGTTTC
ACTTTCACTGGGGTTCACTTGATGGACAAGGTTCAGAGCATACTGTGGAT
AAAAAGAAATATGCTGCAGA
ACTTCACTTGGTTCACTGGAACACCAAATATGGGGATTTTGGGAAAGCTG
TGCAGCAACCTGATGGACTG
GCCGTTCTAGGTATTTTTTTGAAGGTTGGCAGCGCTAAACCGGGCCTTCA
GAAAGTTGTTGATGTGCTGG
ATTCCATTAAAACAAAGGGCAAGAGTGCTGACTTCACTAACTTCGATCCT
CGTGGCCTCCTTCCTGAATC
CTTGGATTACTGGACCTACCCAGGCTCACTGACCACCCCTCCTCTTCTGG
AATGTGTGACCTGGATTGTG
CTCAAGGAACCCATCAGCGTCAGCAGCGAGCAGGTGTTGAAATTCCGTA
AACTTAACTTCAATGGGGAGG
GTGAACCCGAAGAACTGATGGTGGACAACTGGCGCCCAGCTCAGCCACT
GAAGAACAGGCAAATCAAAGC
TTCCTTCAAATAA (National Library of Medicine, n.d.)

Translation (260 aa):

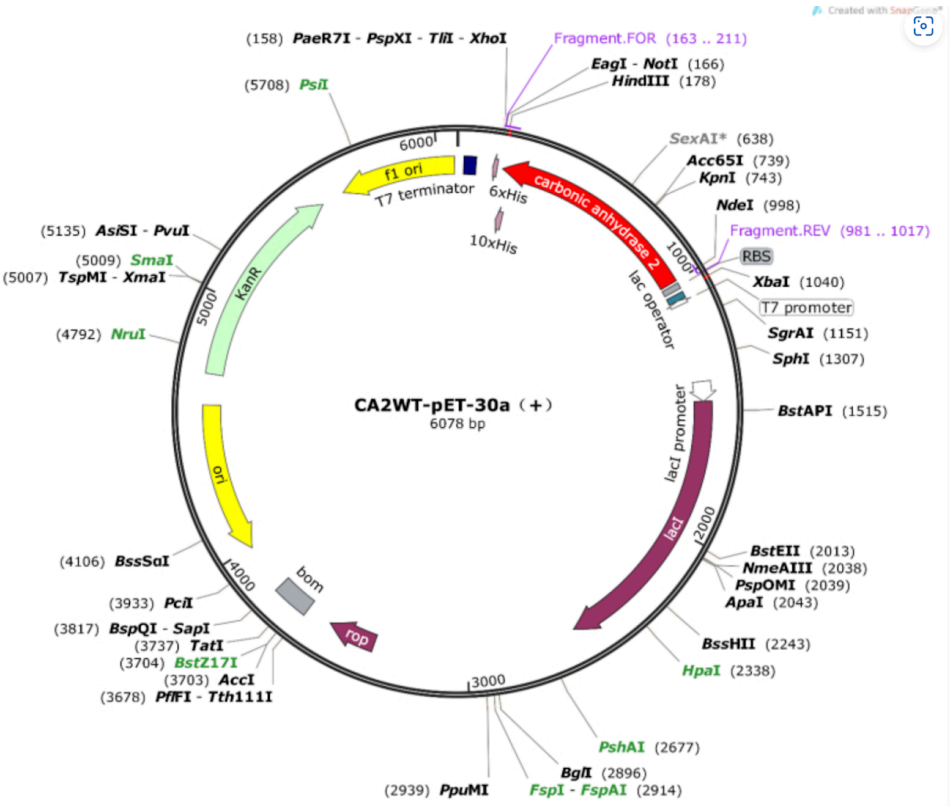
MSHHWGYGKHNGPEHWHKDFPIAKGERQSPVDIDTHTAKYDPSLKPLSVS
YDQATSLRILNNGHAFNVEF
DDSQDKAVLKGGPLDGTYRLIQFHFHWGSLDGQGSEHTVDKKKYAAELHL
VHWNTKYGDFGKAVQQPDGL
AVLGIFLKVGSAKPGLQKVVDVLDSIKTKGKSADFTNFDPRGLLPESLDYW
TYPGSLTTPPLLECVTWIV
LKEPISVSSEQVLKFRKLNFNGEGEPEELMVDNWRPAQPLKNRQIKASFK
(National Library of Medicine, n.d.)

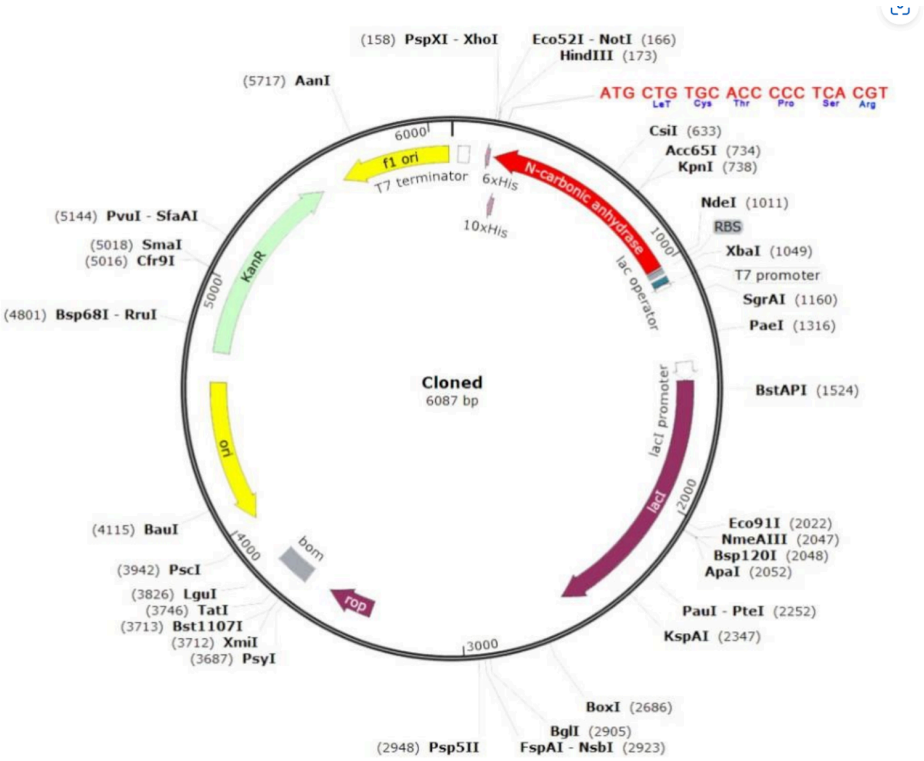


Specifically, the coding sequence of Carbonic anhydrase csoS3 was codon-optimized, and His-tag was added to the end, so that Carbonic anhydrase csoS3 could be expressed in E. coli BL21 (DE3) and had good carbonic anhydrase activity.(Team:AHUT China/New Parts, n.d.)



Because wild-type CA2 has the fastest reaction rate at 37 °C and loses its activity at 50 °C, it may not be suitable for using wide type CA2 to capture CO2 under industrial operating conditions. Therefore, we use molecular simulation to design new high-efficiency and stable carbonic anhydrases by improving their catalytic properties and stability. (Team:AHUT China/New Parts, n.d.)


(158) PaeR7I - PspXI - TliI - XhoI
Fragment.FOR (163 .. 211)
EagI - NotI (166)
HindIII (178)
(5708) PsiI
SexAI* (638)
Acc65I (739)
KpnI (743)
NdeI (998)
Fragment.REV (981 .. 1017)
RBS
XbaI (1040)
T7 promoter
SgrAI (1151)
SphI (1307)
BstAPI (1515)
(5135) AsiSI - PvuI
(5009) SmaI
(5007) TspMI - XmaI
(4792) NruI
(4106) BssSαI
(3933) PciI
(3817) BspQI - SapI
(3737) TatI
(3704) BstZ17I
(3703) AccI
(3678) PflFI - Tth111I
BstEII (2013)
NmeAIII (2038)
PspOMI (2039)
ApaI (2043)
BssHII (2243)
HpaI (2338)
PshAI (2677)
BglI (2896)
(2939) PpuMI
FspI - FspAI (2914)
f1 ori
T7 terminator
6xHis
10xHis
carbonic anhydrase 2
lac operator
lacI promoter
lacI
KanR
ori
bom
rop
CA2WT-pET-30a (+)
6078 bp


(158) PspXI - XhoI
Eco52I - NotI (166)
HindIII (173)
(5717) AanI
ATG CTG TGC ACC CCC TCA CGT
CsiI (633)
Acc65I (734)
KpnI (738)
NdeI (1011)
RBS
XbaI (1049)
T7 promoter
SgrAI (1160)
PaeI (1316)
BstAPI (1524)
(5144) PvuI - SfaAI
(5018) SmaI
(5016) Cfr9I
(4801) Bsp68I - RruI
(4115) BauI
(3942) PscI
(3826) LguI
(3746) TatI
(3713) Bst1107I
(3712) XmiI
(3687) PsyI
Eco91I (2022)
NmeAIII (2047)
Bsp120I (2048)
ApaI (2052)
PauI - PteI (2252)
KspAI (2347)
BoxI (2686)
BglI (2905)
(2948) Psp5II
FspAI - NsbI (2923)
f1 ori
T7 terminator
6xHis
10xHis
N-carbonic anhydrase
lac operator
lacI promoter
lacI
KanR
ori
bom
rop
Cloned
6087 bp

Conclusion

In conclusion, the problem of reducing carbon dioxide in the atmosphere is a complex issue. A potential solution explored in this research project is to edit the plasmid design and function of Cyanobacteria, a common form of bacteria that is found in many ecosystems. The two iGEM teams researched, have created a starting point using biobricks, and a genetically altered form of Carbonic Anhydrase (CA2) to speed up the process of photosynthesis. Theoretically, this would cause the chemical reaction in the Calvin cycle to occur faster which would speed up the absorption of Carbon Dioxide and ultimately release Oxygen at a quicker rate.

Future steps that are possible with this research include taking the proposed solution and designing a plasmid that combines both teams exceptional research. In addition, a biobrick could be designed that uses both models and then creates a plasmid design that could be tested in a lab. Just think, if this was successful, the practical applications in the world could involve releasing the Cyanobacteria in local storm ponds, wetlands, lakes, etc of large communities or cities and therefore decreasing the carbon emissions as a result.

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Acknowledgements

There are many people that I would like to thank for being supportive of this project and inspiring me to do my best. Thank you Dr. Soares for guiding me through this process. Thank you both mom and dad for supporting the project as well. Thank you grandad for helping me edit this paper and being very supportive of this project. Inspiration played a key role in the development of this project. Being passionate about helping the environment was also a key motivation. Overtime, I have realized the growing concern about carbon emissions and wanted to help. At first, it was simple tasks, like recycling but it always felt like we could do more. After learning about cells and systems in science I thought that this could develop into a project. With the help and guidance of Dr. Soares, she led me into this topic of gene modification and the project grew from there. I learned the process of developing a plasmid for Cyanobacteria that is capable of increasing carbon capture. This research on the topic of synthetic biology has inspired me to take this new passion further and explore the amazing possibilities that hold in the future. I hope to take this project forward and learn more about this science.