International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Algal Biofuel: Modified Tag Production

Joseph Rop[1]*, Shawn Kraus[2]

[1]Academy for Science and Design, Nashua, New Hampshire, USA

*Corresponding Author: josephrop649[at]gmail.com

[2]Advisor, Research and Design Instructor, Academy for Science and Design, shawn.kraus[at]asdnh.org

Abstract: ***Algal biofuel today presents some major limitations which keep it from being used as a primary energy source globally. The two major methods of production are: open biofuel systems and closed bio photoreactors. These present two extremes of algae biofuel limitations in the present day. While external factors are what limit these forms of production, internal factors must not be ignored. This research project focuses on the development of a method for altering the genetic component found in the algae species chlorella vulgaris to increase its biofuel productivity rates. The process will comprise 2 important processes: the selection of an appropriate genome which, if changed, could increase productivity, as well as the use of the genome editing tool, CRISPR/Cas9 to replace this genome. This design portfolio will include the following: costs of development, process of documentation, and constraints/limitations. To help with refining the biofuel reactor model it should be assessed in industry to determine its commercial application/viability.***

Keywords: Biofuel, tag production, CRISPR, Cas9, triacylglycerol (TAG), G3DPH, plasmid

1.Introduction

Modern energy is dominated by fossil fuels which are extracted from leftover organic materials and processed into coal, oil, or natural gases. Our dominance on this single energy source does not necessarily positively correlate with its overall benefit to human use and the environment. Firstly, fossil fuels are extremely harmful to the environment on a multitude of layers. Fossil fuel can be extracted from mining as well as fracking, both of which involve the disruption of rock structures and often cause the oil to be extracted to mix with underground water reservoirs[1]. Additionally, fossil fuels are known producers of GHGs (Greenhouse Gases) which are responsible for depleting the ozone layer protective layer in the atmosphere responsible for blocking potentially harmful Ultraviolet sun rays. As this layer is destroyed, global temperatures increase, causing the loss of diverse ecosystems in the process. Secondly, our extreme reliance on fossil fuels as our major source of energy is quite a large concern due to its depletion rates. With a collective depletion rate of 1.3%, the IEA (International Energy Association) predicts that biofuels could run out in the next 50-52 years.

This marks the beginning of algal biofuel production: a new alternative to fossil fuels. First cultivated as a biofuel in the early 1970s, algae made headway as a key player in the replacement of fossil fuels as a sustainable, clean source of energy. Since this breakthrough, algal biofuel advancements seemed to plateau except for 2 major classifications: open biofuel systems and closed photobioreactors.

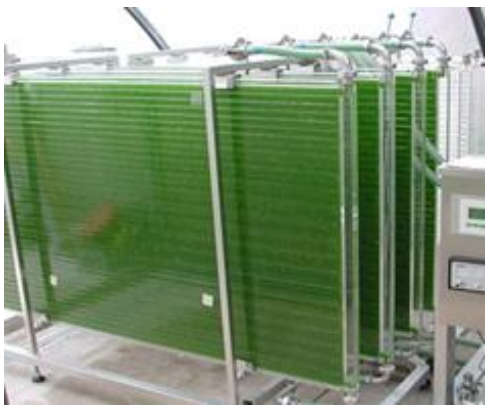
1.1 Modern Algal Biofuel: The Problem

Open and closed algal biofuel systems currently comprise most (if not all) of the total biofuel using algae.

1.11 Open Biofuel Systems

Open biofuel reactors present the first set of pros and cons: open pond systems are cheap and do not require significant quality control measures to keep production levels at their regular levels (Cavelius et al., 2023). Additionally, lighting is not an issue which allows photosynthesis within algal cells to occur. This photosynthetic process allows algae cells to grow and thus produce the necessary lipids used as biofuels (Cavelius et al., 2023).

1.12 Closed Bio Photoreactors



[1] Important to note that although this is the case, the mixture is not significantly toxic enough to be considered a major concern.

Closed biofuel systems, bio photoreactors, are often desired over open pond systems due to their high levels of biofuel output. Being closed, these systems can generate higher levels of lipids due to the level of control of pH, CO_2 concentration, and temperature researchers have (Cavelius et al., 2023). However, these closed systems are also much more expensive to operate, costing as much as $9.29 per square footage to run compared to open ponds which cost about $0.87 to operate. On top of this, closed systems have significantly lower levels of light penetration when compared to open pond systems, which can be attributed directly to the glass thickness in which algae compounds are stored during cultivation (Cavelius et al., 2023). This lowers the amount of photosynthesis that can occur, thus lowering the level of growth and therefore production of lipids (Cavelius et al., 2023).

Between these two systems, there is an apparent gap in the advancement and progression of algal biofuel as fossil fuels replacement. The benefits and faults of each production type mirror each other in a way that makes biofuel unnecessary and unprofitable to large energy production companies.

1.2 CRISPR Technology

CRISPR, also known as Clustered Regularly Interspaced Short Palindromic Repeats, is a "recently" developed piece of technology used for gene editing. The 2012-developed system is also called CRISPR/Cas 9 due to its pairing with the enzyme, Cas 9. By itself, the misinterpreted "CRISPR" system is a grouping of organized repeats, which is often used by bacteria and other prokaryotic organisms such as archaea to better identify the external DNA or foreign DNA of other organisms. However, CRISPR functions using two main components: guide RNA and the Cas 9 enzyme family (Redman et al., 2016).

1.21 Guide RNA (sgRNA or gRNA)

In the CRISPR system, the guide RNA plays an important role in the identification of the target area for gene editing to take place. Also known as sgRNA, this RNA segment binds to the target area by matching to the specific base nucleotide pairs (complementary base pair matching). The sgRNA marks these sites as "cleavage sites" and almost alerts the Cas 9 enzymes that these areas should be cut out as they are "foreign" (Schneider, 2020).

1.22 Cas 9 Enzyme (Endonuclease)

Cas9, also known as CRISPR-associated protein 9, is a crucial enzyme used in CRISPR technology. The enzyme is a specific type of enzyme known as endonuclease which allows for the erosion of double strands through its use of restriction enzymes—similarly functioning "cutting" enzymes used in recombinant DNA. In the CRISPR system process, the enzyme "cuts" the hydrogen bonds between the nitrogenous bases of the target DNA segment identified by the guide RNA (Redman et al., 2016).

CRISPR technology has many different applications in biotechnology. This system can be used to make an entire plant species resistant to a brand of pesticides or even change the growth capabilities and skin color of one's future offspring (Thomas, 2023; Urry et al., 2017).

1.3 Recombinant DNA and Plasmid Integration

A plasmid is a circular piece of DNA originating exclusively from prokaryotic cells (single celled organisms such as bacteria or archaea). Plasmids contain much less genetic information than the DNA found in eukaryotic cells; this makes sense because prokaryotic cells are much less complex than eukaryotic cells as eukaryotic cells need to be managed in a way where they can express genetic information in extremely complex ways. An example of this complexity is the difference between human body parts: the eye and foot of humans contain the same cells; however, they look and function differently. This is made possible by the gene expression that occurs to the DNA within the nucleus of the cell (Urry et al., 2017).

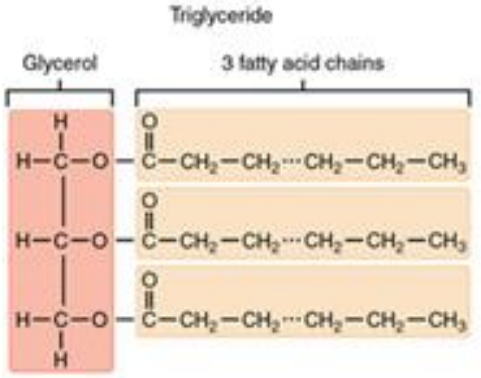
Recombinant DNA is the resulting DNA formed after DNA from two different sites is joined. These sites can be from as close as from within the same species to different species entirely. Recombinant DNA exists as a plasmid usually as DNA is most easily transferable for simpler prokaryotic genomes. To create recombinant DNA, one must obtain an original or template plasmid and a donor plasmid. Next, the restriction site—the area that is cut during the recombination process—is identified and targeted by restriction enzymes. These enzymes act like helicases during DNA replication and break the hydrogen bonds holding the nitrogenous bases together. This process occurs for both the donor plasmid and the template plasmid; however, in the template plasmid, the smaller DNA segment is removed whereas in the donor plasmid, the smaller segment is kept and combined with the edited template plasmid. The two plasmids are joined together using an essential known as DNA ligase. DNA ligase is an important enzyme used to join okazaki and DNA primer fragments during DNA replication.

Once this recombinant process is complete the resultant DNA can be used for various purposes, namely DNA cloning. This is when the formed recombinant bacterium is inserted back into the original bacteria cell and produced to make identical copies. These copies can either be used to make additional copies or produce proteins after gene expression (Urry et al., 2017).

1.4 Triacylglycerol and G3DPH

1.41 Triacylglycerol (TAG)

Triacylglycerol (TAG) is a fatty acid produced by microalgae. This fatty acid is an essential component in biodiesel production. As a fatty acid, TAG is primarily produced in the smooth endoplasmic reticulum which uses numerous proteins to convert the fat from its earlier form to its final complex form.



Triacylglycerol is essential to biodiesel production due to its structure. This structure features a repeating methylene group (CH_2) and a glycerol group. Methylene is comprised of a Carbon triple bonded to two Hydrogen molecules. This triple bonded structure allows the TAG molecule to store extremely high amounts of energy which are broken down into simpler forms and used as energy, nonetheless.

1.42 G3DPH

Short for glycerol-3-phosphate dehydrogenase, G3DPH is an essential enzyme responsible for the creation of glycerol-3-phosphate (G3P). G3P is equally important to the production of TAG. G3P is a simpler form of G6P, Glucose-6-phosphate, and is produced because of the Calvin Cycle in the chloroplast. Following the cycle, this molecule is used in the formation of a pyruvate molecule, which in turn is used to create Acetyl-coenzyme A (Acetyl-CoA). Once Acetyl-CoA is created, this molecule undergoes a series of reactions with enzymes embedded in the smooth endoplasmic reticulum and assumes its final form: Triacylglycerol.

1.5 Project Aim: CRISPR-Induced Algae TAG Production & Modified G3DPH Plasmid

The inefficiencies created by current methods cause their underuse by large energy production companies. Open and closed systems simply have too many gaps in production to compete and replace fossil fuel alternatives which dominate current global energy outputs.

This design portfolio attempts to solve this issue and make algae-based biofuels the most viable option. This overarching goal will be achieved in two procedures: an organized CRISPR implementation process involving CRISPR usage, plasmid introduction, and growth, and an original G3DPH plasmid design. These two combined aspects of the portfolio seek to achieve higher TAG fatty acid outputs which will enable higher biodiesel capabilities for the algae species Chlorella vulgaris.

2.Literature Review: Sustainable Energy - Biofuel

Ambaye et al. (2021) writes that in modern times the increase of society and industrial affairs increase the need for energy. Ambaye et al. elaborate on this, stating society currently relies on fossil fuels for energy which could be an issue due to the worrying depletion rates of fossil fuel reserves (1.3%). Ambaye et al. suggest this decrease in fossil fuels and their negative impacts on the environment via their production of greenhouse gases create a need for an alternative energy source. Ambaye et al. indicates biofuels could fulfill this need and define biofuels as a fuel source made from any organic material or biomass (such as plants, plant residue, and crops). The author proposes that society replaces biofuels with fossil fuels because of economic reliability as well as accessibility, leading to our current dilemma regarding energy. Ambaye et al. note biofuels, despite existing two main forms (biodiesel and bioethanol), are more commonly seen as bioethanol which comprises about 80% of the current biofuel production industry. They suggest biofuels are more favorable than fossil fuels as they produce about 10% to 45% oxygen and produce significantly less sulfur and nitrogen levels. In addition, the author states that since 2020, it was found that fossil fuels—in the form of petroleum, oil, and natural gas—had the possibility of being exhausted according to the International Energy Association. Ambaye et al. propose biofuels as a substitute for fossil fuels and state biofuels are a renewable and sustainable energy source as their production uses CO_2, a harmful greenhouse gas, and are sustainable as a large portion of biofuels are made from crops which can be continuously grown.

A similar article by Mahapatra et al. (2021) finds fossil fuels such as coal, natural gas, and petroleum account for almost 80% of the world's global energy production and have produced 54 gigatons of carbon dioxide. Mahapatra et al. estimate that this metric will increase to 87 gigatons by about 2050 which will have significant, negative impacts on the environment.

2.1 Biofuel Generations

2.11 First Generation biofuels

Cavelius et al. (2023) affirm that first generation biofuels are designated as biofuels made from edible food sources such as crops. Cavelius et al. state this type of biofuel can be further categorized into 2 end products: bioethanol and biodiesel. The study explains biodiesel is produced from edible seed oils from various continents including South America, Asia, and Europe. The author contrasts biodiesel from bioethanol, stating the biodiesel production process involves enzymatic catalysis and only partial biosynthesis unlike bioethanol which is produced through biosynthesis. Following this, Cavelius et al. state first generation biofuels have a major downside: their use of edible food as this creates a conflict between food's usage for biofuel vs its use for human and animal consumption. The study also notes first generation biofuels require a large amount of land as well as water which has led to deforestation. The author writes that the available land required to cultivate oil-based, first-generation biofuels would need to double to meet the global energy demand.

2.12 Second Generation Biofuels

Cavelius et al. (2023) mention second generation biofuels are currently produced from lignocellulosic materials as

well as organic waste as a solution to the issues presented by first generation biofuels. Adding to this, Cavelius et al. write to convert second generation biofuels such as lignocellulosic materials and non-edible plant parts into bioethanol, microorganisms break down cellulose from plant stems and lignin found in wood into sugar monosaccharides. They continue, explaining that these microorganisms use anaerobic fermentation to convert sugars into ethanol afterwards. Cavelius et al. state microorganisms have been found to produce even higher yields from carbon sources found in waste such as glucose, decreasing waste from the environment in the process. The study highlights another second-generation biofuel source known as syngas which is composed of carbon monoxide, carbon dioxide, and hydrogen gas. Cavelius et al. write syngas have numerous benefits as a fuel source compared to other second-generation biofuels including higher availability and lower competition with other industry needs, higher energy output, and higher carbon dioxide use. Building on these benefits, the author claims second generation biofuels occupy significantly less land and do not create conflicts for food usage unlike first generation biofuels. However, Cavelius et al. note, despite the benefits of second-generation biofuels, additional steps such as pretreatment are needed to produce more of the biofuels. The author provides lignin as an example of a polysaccharide molecule that needs to be treated during biofuel production. According to the study, this pretreatment increases both production times and costs. The study names gasification as an alternative to pretreatment-dependent biofuels that use complex polysaccharide molecules in their larger states, eliminating the extensive pretreatment task entirely.

2.13 Third Generation Biofuels

Cavelius et al highlight third generation biofuels are obtained from microalgae as well as cyanobacteria biomass. They claim algae are advantageous for the purpose of fuel production as they are two to four times as efficient at photosynthesis than most terrestrial plants and do not need land space or fresh water. They also emphasize that algae production utilizes carbon dioxide to make algal oils (about 70% of the carbon dioxide provided), making algae a viable carbon-negative option. Cavelius et al. illustrates the benefits and setbacks of algae bioreactors. They state algae bioreactor options such as open ponds are cheap but produce less bioethanol than enclosed, artificial ponds due to a lack of temperature control and water loss (from evaporation). Regarding artificial algae bioreactors, the article implies these reactors include enclosed ponds called closed photobioreactors which can be controlled to a higher degree than their cheaper counterparts which increase production concentrations. The author highlights the advantages of the enclosed bioreactors come at the cost of a significantly higher maintenance cost of the photobioreactors. Cavelius et al. point out third generation biofuels in general do not share a need for land usage with first- or second-generation biofuels. Additionally, they claim the potential for biofuel output is much higher in algae compared to terrestrial plants as in algae lipids can build up in each individual cell in high concentrations. The article then highlights the setbacks of third generation biofuels including microalgae's low resistance to pH changes—which results in difficulties during biofuel production—and increased energy need for downstream processing.

2.14 Fourth Generation Biofuels

Cavelius et al. define fourth generation biofuels as biofuels derived from the genetic engineering of organisms to boost certain aspects of the organism that can be used in biofuel production. Their study categorizes these traits as the use of various sugars, increased lipid creation, photosynthesis, and carbon fixation rates. The author notes that for certain microorganisms such as Escherichia coli and Saccharomyces cerevisiae, many genetic engineering techniques can be implemented to achieve greater biofuel outputs. However, Cavelius et al. also emphasize for other natural biofuel producers, these genetical engineering techniques are less available. The author states that across all fourth-generation biofuel production, 2 main techniques have been utilized in the biofuel industry. These techniques are native biofuel producer pathways and readvanced engineered pathways for ideal microorganisms (like Escherichia coli). The article highlights examples of heterologous hosting for engineering pathways, saying two bacterium E. coli, Pseudomonas putida and Bacillus subtilis have been introduced to butanol pathway genes from the bacteria Clostridium. The author implies the introduction of these genes into microorganisms can be used to produce biofuels. The article also notes that processes such as these can lead to membranous errors as the introduction of foreign genes can cause a cell's membrane to become unable to regulate the entry and exit of substances. The article claims algae is being modified using CRISPR to produce higher product concentrations than natural algae by enhancing their photosynthetic capabilities. Cavelius et al. shift to cyanobacteria, writing that fourth generation procedures aim to produce ethanol as well as butanol, isobutanol, and some fatty acids. According to the article, around 500 mg/L of 1-butanol and 5.5 g/L of bioethanol have been produced through fourth generation efforts. Following this, the author describes an alternative to the standard genetic modification to organisms, naming random mutagenesis as the alternative. Random mutagenesis is described as the purposeful increased evolution of microorganisms through UV lighting, chemicals, or neutron irradiation. The author observed that this process resulted in yeast C. oleaginosus successfully producing increased levels of oil to be used in biodiesel production.

2.2 The Impacts of Biofuels In the Energy-Production Industry

2.21 Current Impacts

Cavelius et al. (2023) writes that recent progress in the collection of native producer genome sequences has been made, allowing for faster genome sequence collection. They suggest the collection of these genomes is crucial as their collection allows for the recreation of metabolic pathways which can be used to further advance biofuel production.

A similar article, by Shurin et al. (2016), claims that the overall understanding of algal genome sequencing has significantly increased. The author suggests this understanding has led to a greater development of algae with desired traits which has the potential to create higher product-yielding algae.

2.22 Future Biofuel Potential and Setbacks

Ambaye et al. (2021) estimate biofuels, if used to their full potential, could produce 10% to as high as 50% of the world's global energy consumption. Ambaye et al. indicate the research in the biofuel industry is concentrated on advancing the output of biofuels from crops and other forms of biomass to make biofuels more efficient. The author observed the increase in global biofuel production from 2007 to 2017 (11.4%), crediting the European Union as the highest biofuel producer globally (38.5 Giga Watts) and China falling just shy (36.27 GW). However, Ambaye et al. note, despite the obvious growth in the level of biofuel production with each coming year, this growth rate is minimal when considering global energy needs. The author links this claim to ethanol's inability to be made in high enough quantities, resulting in its mixture with gasoline. Additionally, the author also names economic uncertainty, limitations in technology, and political uncertainty as factors in the slow growth of biofuels in the fuel industry. Ambaye et al. claim that regardless of these limitations, biofuel production is still increasing along with the advancement of biofuels in multiple generations but fourth generation biofuels (micro-organisms).

A similar article, by Suhara et al. (2024), highlights that if environmental, economic, and social aspects are seriously considered in biofuel production, biofuels can become more successful and sustainable as a standard fuel source. The author elaborates on this, stating the success of biofuels is dependent on sustainability. The article claims measures such as eliminating competition of food usage between the biofuel and agricultural industries as well as protecting land rights, ensuring civic participation, and wildlife conservation should all be considered to establish biofuels as viable, sustainable options in the future.

In summary, this literature review aims to present the current state and potentials of biofuels and their use as a replacement for fossil fuels. Many of the articles share similar viewpoints and support each other in their respective areas such as biofuels as a replacement for fossil fuels, what is currently being advanced in biofuel production, and the potential of biofuels in the future. Despite these considerations, a major area yet to be explored is the creation of new biofuel options. This area regards the creation of biofuels that do not have the climates that support algal growth for example. Additionally, most literature sources on the biofuel industry fail to mention the details of biofuel use in certain countries that are not leading biofuel producers. Therefore, this research paper aims to contribute to literature on biofuel systems that use algae as a source of energy. For my paper, I plan to create a cheaper and efficient method for transforming algae into biofuel using an enclosed system or transforming food waste into crude biofuel with a higher yield. I will use my literature sources to identify the current methods of biomass transformation for these biofuel types and improve these methods so they can be used in more environments at cheaper costs and higher yields.

3.Materials and Methods

3.1 Methodology

A review of current literature (Cavelius et al., 2023) on biofuel production revealed that most manufacturers used open and closed biofuel fuel systems as a means of producing the majority of algae-based biofuel. A similar article by Lee et al., 2023 discusses the use of CRISPR technology to increase the efficiency of carbon-fixation in microalgae; however, this article does not discuss using CRISPR to directly alter the genes involved in producing the TAG lipid body in algae. An opportunity exists for the use of CRISPR technology to increase TAG production in microalgae in the hopes of also increasing the algae's lipid output.

3.2 Pre-Phase 1: Growth Phase

This is the first "pre-phase" in the design portfolio's pathway. In this phase, growth is the key focus. For optimal efficiency, 10 agar plates will be arranged per set of trials, or batch. These agar plates will contain a standard of 5 Chlorella vulgaris colonies (1 cm diameter, placed by pipette). Each batch will be grown for a minimum of 1 month to a maximum of 4 months to ensure proper growth has been achieved.

3.3 Phase 1: CRISPR

This phase involves the physical use of CRISPR to edit each algae colony's G3DPH genome. After growth has occurred, the colonies will be transferred to each of their own centrifuge tubes. Each of the tubes will be spun on the highest setting for 2 minutes. This step is crucial to ensure the algae is uniform. The algae colonies will then be transferred to each of their own trays and placed into an electroporation system known as a Nucleofector. This machine will create tiny pores within each cell and its membrane using high voltage electricity suspension. The pores created by this machine will make the membrane of each cell permeable and thus allow our plasmids to take effect once inserted.

As mentioned, at this point the **modified G3DPH plasmids** will be inserted directly into each cell culture using a micropipette. It is crucial that this step occurs during the repair mechanisms employed by both the cell and artificially. The artificial repair mechanisms used in this pathway are known as HDR, or Homology Directed Repair. This slower-acting process allows for higher precision during the insertion of the plasmid following the cut to the plasmid made by the Cas9 enzyme that is injected into the cell culture. Following the injection of the plasmids, the trays containing the cultured cells will be placed into an incubator for 18 hours to allow for optimal conditions for the RNA transfer to occur.

3.4 Phase 2: FACS Sorting

During this step, the algae is sorted based on its GFP activity, or reflection of light to indicate successful gene transformation. The steps are simple: place your tray containing the cell culture into your cell sorter and record the results. The cells will be scanned using a laser from inside the machine and the optics hardware inside the machine will display which wavelengths are being reflected. The reflected wavelengths are the fluorescent GFP indicating activity and successful transformation. Cells with 395 nm to 470 nm are indicative of successful gene transformation.

3.5 Phase 3: Separation

Phase 3 marks the separation and physical sorting stage. Successfully transformed colonies will now be organized and placed onto new agar plates. Organization of the colonies will not be based on output (as we cannot determine this yet; at this point we only know which colonies have the potential to yield high TAG concentrations). Instead, organization will be as follows: for 1 to 4 months algae will spend time growing in agar plates then eventually to outdoor settings as needed.

3.6 Phase 4: Growth

The final phase of the TAG production pathway is the final growth stage. During this phase, the modified algae will permanently be transferred to an outdoor biofuel system where it will receive the standard CO_2, light, and pH measures to ensure sustained growth. Like most algae systems, the final product will be biodiesel made from the high concentration triacylglycerol fatty acid produced from the pathway.

3.7 Modified G3DPH Plasmid

During Phase 1 of the pathway, the following plasmid will be inserted directly into each cell colony to ensure identical replication and results are met. This plasmid is designed to act along with the standard CRISPR/Cas9 plasmid following the electroporation of cell cultures.

This plasmid will work as follows: once injected into the cell, Cas9 cuts out the original G3DPH gene (as identified by the gRNA which has complementary codons). The modified plasmid will then copy the genetic code for the G3DPH gene and insert into the gap created by the Cas9 cut. cells the RNA polymerase reads the gene; the cell will facilitate the production of G3DPH which produces the G3P molecule. This molecule is then naturally used by the algae cell to create TAG after processing in the smooth ER.

The plasmid is presented below:

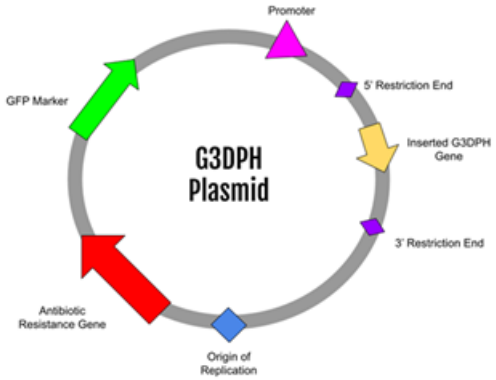


Figure 1: Concept G3DPH plasmid inserted into algae cells following the CRISPR/Cas9 process. This plasmid features a promoter, 5' and 3' restriction end, the inserted G3DPH gene, an antibiotic resistance gene, and a GFP marker.

3.8 Cost Estimates

This design was created to benefit the use of biofuels. However, due to various drawbacks of the design process, this may not be foolproof. The first concern is cost. To use CRISPR technology, fund research, and create an entirely new plasmid the cost comes out to $24.5 million (Lassoued et al., 2019). This estimate is after taking every detail into account; however, this number is still a concern as more realistic estimates are not extremely far off. This begs the question: does the model really benefit biofuels in terms of commercial use? The answer to this question is uncertain. With all technology, the older and more widespread it becomes, the less expensive it will become. This is especially true for concepts like CRISPR as it is being heavily researched using government spending. This will drive down costs by a significant margin, causing the methodology presented in this design to become a more viable option.

4.Discussion

There are multiple concerns surrounding the design and overall project viability not involving the costs. Safety is a large issue involving CRISPR usage. When altering genetic codes at such a precise level, errors are bound to happen, and this could have larger-than-expected effects on the environment. If, let us say, that a genetically modified strain of algae was to contact other natural algae, entire gene pools of algae within the Chlorella vulgaris species could be affected.

Additionally, this project is a best-case scenario project. For this design to yield significant results, every step would need to occur perfectly. This is because improving algae at such a specific degree is already limiting itself so every alteration would need to be operating at the most optimal level for beneficial results to be seen.

5.Conclusion

In conclusion, this project seeks to advance the progress of algal biofuel production on a commercial scale. This action is being done to combat the obvious and dangerous reliance the world has on fossil fuels. To combat these harmful and non-sustainable fuels. This design portfolio suggests an alternative using modified algae. This modification occurs in the G3DPH gene and is transmitted by plasmid to the cell of the algae species Chlorella vulgaris after CRISPR. The G3DPH gene is transmitted into the plant via plasmid and causes an increase in the production of TAG, a fatty acid, which is a major component of the by-product, bioethanol.

Despite economic setbacks of the current design presented in this portfolio, there is potential for the improvement of this design. As research for CRISPR continues to happen at its currently increasing rate, this design could be more attainable to more companies in the future, creating a world less reliant on harmful fossil fuels and more able to sustain itself and those in still developing areas.

References

[1] Ambaye, T. G., Vaccari, M., Bonilla-Petriciolet, A., Prasad, S., van Hullebusch, E. D., & Rtimi, S. (2021). Emerging technologies for biofuel production: A critical review on recent progress, challenges, and perspectives. Journal of Environmental Management, 290(112627). https://doi.org/10.1016/j.jenvman.2021.112627

[2] Cavelius, P., Engelhart-Straub, S., Mehlmer, N., Lercher, J., Awad, D., & Brück, T. (2023). The potential of biofuels from first to fourth generation. PLOS Biology, 21(3), e3002063. https://doi.org/10.1371/journal.pbio.3002063

[3] Lassoued, R., Phillips, P. W. B., Smyth, S. J., & Hesseln, H. (2019). Estimating the cost of regulating genome edited crops: expert judgment and overconfidence. GM Crops & Food, 10(1), 1–19. https://doi.org/10.1080/21645698.2019.1612689

[4] Mahapatra, S., Kumar, D., Singh, B., & Sachan, P. K. (2021). Biofuels and their sources of production: A review on cleaner sustainable alternative against conventional fuel, in the framework of the food and energy nexus. Energy Nexus, 4, 100036. https://doi.org/10.1016/j.nexus.2021.100036

[5] Morgan, K. (2014). Plasmids 101: The Promoter Region – Let's Go! Addgene.org. https://blog.addgene.org/plasmids-101-the-promoter-region

[6] Radakovits, R., Jinkerson, R. E., Darzins, A., & Posewitz, M. C. (2010). Genetic Engineering of Algae for Enhanced Biofuel Production. Eukaryotic Cell, 9(4), 486–501. https://doi.org/10.1128/ec.00364-09

[7] Redman, M., King, A., Watson, C., & King, D. (2016). What Is CRISPR/Cas9? Archives of Disease in Childhood - Education & Practice Edition, 101(4), 213–215. https://doi.org/10.1136/archdischild-2016-310459

[8] Schneider, A. (2020). A short history of guide RNAs. EMBO Reports, 21(12). https://doi.org/10.15252/embr.202051918

[9] Shurin, J. B., Burkart, M. D., Mayfield, S. P., & Smith, V. H. (2016). Recent progress and future challenges in algal biofuel production. F1000Research, 5(2), 2434. https://doi.org/10.12688/f1000research.9217.1

[10] Suhara, A., Karyadi, Herawan, S. G., Tirta, A., Idris, M., Roslan, M. F., Putra, N. R., Hananto, A. L., & Veza, I. (2024). Biodiesel Sustainability: Review of Progress and Challenges of Biodiesel as Sustainable Biofuel. Clean Technologies, 6(3), 886–906. https://doi.org/10.3390/cleantechnol6030045

[11] Thomas, U. (2023, August 10). CRISPR Probes Links between Genes and Skin Color Regulation. GEN - Genetic Engineering and Biotechnology News. https://www.genengnews.com/topics/genome-editing/stanford-study-uses-crispr-to-probe-links-between-genes-and-skin-color-regulation/

[12] Urry, L. A., Taylor, M. R., Pollock, M., & Campbell, N. A. (2017). Campbell Biology (11th ed.). New York Pearson.

[13] Yang, H., Ren, S., Yu, S., Pan, H., Li, T., Ge, S., Zhang, J., & Xia, N. (2020). Methods Favoring Homology-Directed Repair Choice in Response to CRISPR/Cas9 Induced-Double Strand Breaks. International Journal of Molecular Sciences, 21(18), 6461. https://doi.org/10.3390/ijms21186461