Expression and characterization of MtrCAB in *Synechocystis sp. PCC 6803*

Student: Devansh Kumar; Supervisor: Prof Louise Horsfall; School of Biological Sciences, University of Edinburgh.

Introduction:

The urgent need to tackle the effect of climate change has resulted in the growing need for renewable energy sources to shift us into a low carbon energy economy. In recent years there has been an emergence in the development of biological systems for energy production. To this effect, cyanobacteria, photosynthetic organisms, are potential chassis for the conversion of light energy into electrical energy through repurposing the photosynthetic electron transport chain [1]. The MtrCAB complex is a key component in *Shewanella Oneidensis MR-1's* extracellular electron transfer capabilities by facilitating the free flow of electrons across the bacterial outer membrane, where an external electron acceptor can be reduced, or in the case of an anode, directly feed a current into an electrical circuit (Figure 1) [2]. The project has focused on plasmid design and assembly of the MtrCAB complex and CymA into a CyanoGate level T plasmid, to enable the recombinant expression of the MtrCAB complex in a cyanobacteria species, *Synechocysteis sp. PCC 6803* using the CyanoGate assembly method.

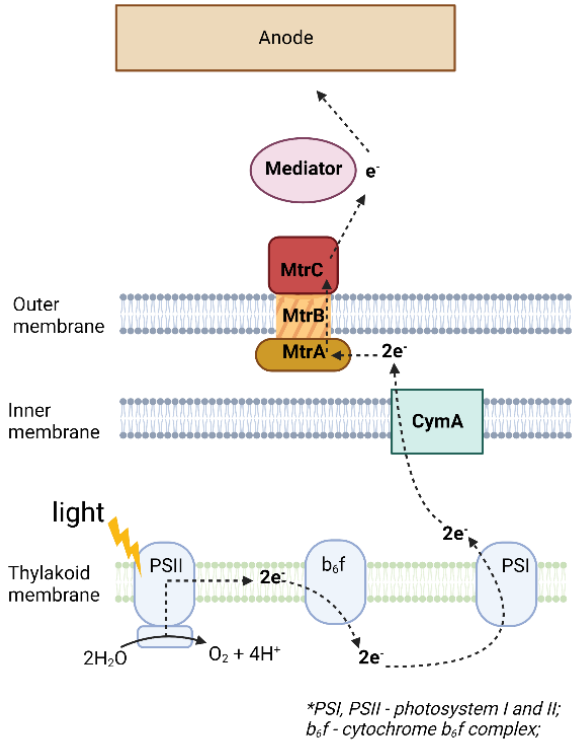

Figure 1: Illustration of electron transfer facilitated by Mtr complex and CymA protein. Created with BioRender.com

Aims of the project:

- Design vectors encoding Mtr operons and assemble using the CyanoGate kit
- Transform level T expression vectors into *Synechocystis sp. PCC 6803* and *E. coli*
- Characterising MtrCAB function with the ferrocyanide assay

Summary of the work undertaken:

MtrCAB, MtrCA and MtrAB genes were extracted from *Shewanella Oneidensis MR-1* via PCR. The PCR primers were designed to introduce CyanoGate (similar to golden gate but for cyanobacteria [3]) overhangs in the extracted operons allowing their integration into plasmid vectors. The operons with overhangs (now called g-blocks) were integrated into level 0 plasmid (Figure 2). Ptrc10 Promotor lv0 (*Synechocysteis* and *E. coli promotor*), g-block lv0 and TrrnB terminator lv0 plasmids were assembled into a level 1 plasmid as a CyanoGate cassette. Finally, the level 1 cassette was assembled into a level T vector that can be transformed into both *Synechocystis sp. PCC 6803* and *E. coli*.

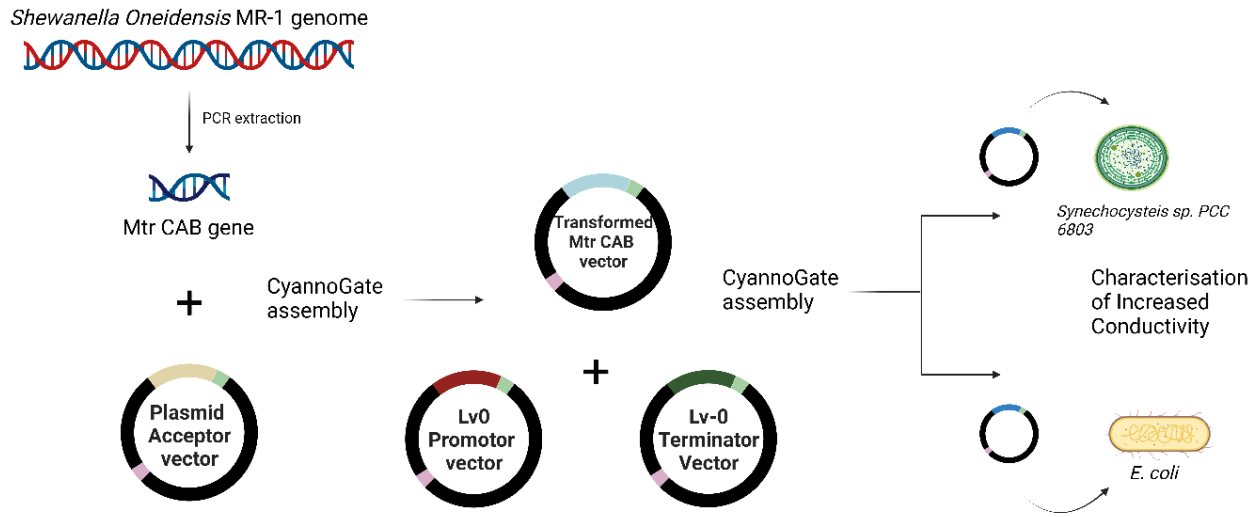

Figure 2: Illustration showing the workflow of introducing MtrCAB gene from Shewanella Oneidensis MR-1 genomine into Synecosystis 6803 via a replicative plasmid. Created with BioRender.com

The extracted operons' size were confirmed via DNA gel electrophoresis. Moreover, the correct assembly was determined by colony PCR and restriction digestion (refer results). While these methods suggested correct extraction and assembly, the final confirmation was achieved by whole plasmid sequencing done by the Edinburgh genome foundry.

Notably, domestication was required for the g-blocks; MtrCAB and MtrCA because MtrC gene contained an additional restriction enzyme recognition site (BsaI site). This step was conducted when these g-blocks were in lv0 vector. Domestication was done by PCR followed by restriction digestion and ligation. Herein, primers were designed allowing elimination of the restriction site by changing a single base pair.

Unfortunately, due to time constraints and complexity of transformation in *Synechocystis sp. PCC 6803,* I was unable to characterize the effects of MtrCAB in *Synechocystis sp. PCC 6803*. Nevertheless, the ferrocyanide assay was performed on wild type *Synechocystis sp. PCC 6803* providing base readings.

Assessment of results and outcomes of studentship:

The PCR extraction was successful after several rounds of troubleshooting. The troubleshooting involved; use of different enzymes such as One-Taq, and Q5; different annealing temperatures; and different extension times. Figure 3 shows positive result of PCR extraction for all 3 operons. The correct sizes strongly indicate that the gene extraction was successful.

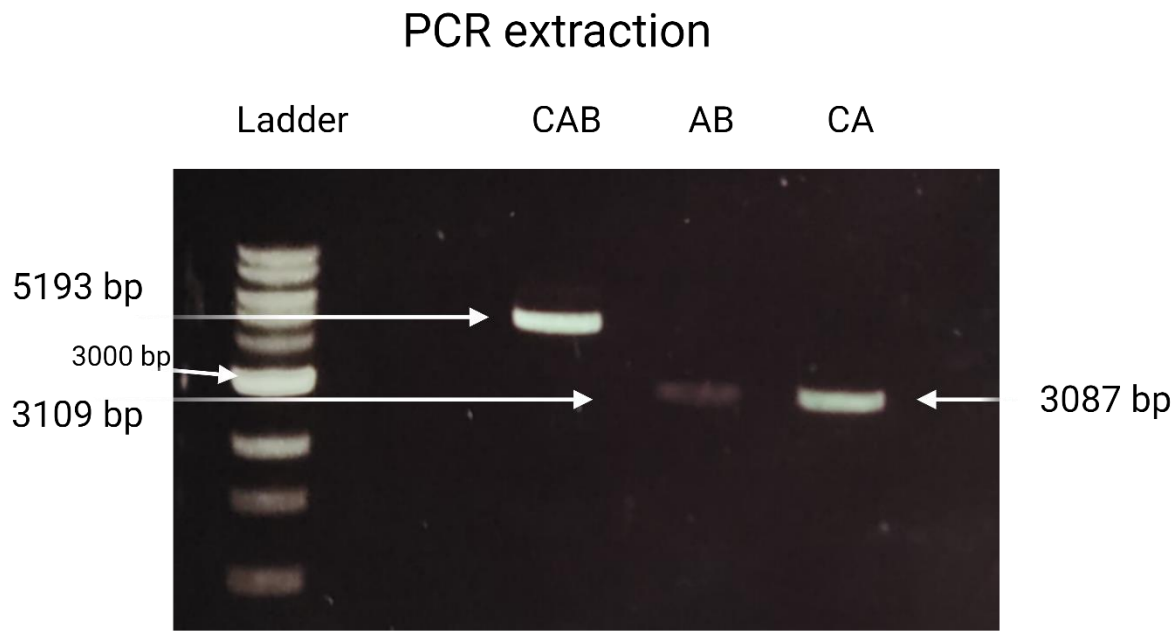

Figure 3: Annotated gel showing bands corresponding to PCR extracted genes from Shewanella Oneidensis MR-1.

Assembly into Level 0 vectors was sucessful for all 3 operons on the first attempt. Initially colony PCR allowed identification of colonies with correct inserts which were then confirmed by diagnositc digestions shown in figure 4. While digestion with BsaI cuts out the gene from the vector via two cuts, digestion with PvuI leads to linerisation allowing checking of the construct's size. Notably, The 242bp faint bands visible for MtrCAB and MtrCA demonstrate the presence of an extra BsaI site and thus the requirement for domestication. Moreover, as MtrAB did not require the additional step of domestication, it sucessfully was taken from lv0 to lv1 vector on the 2nd attempt and then from lv1 to lvT vector after 3 attemps. While domestication for MtrCAB and MtrCA was sucessful, due to time constraints, MtrCAB and MtrCA were not assembled into lvT vectors.

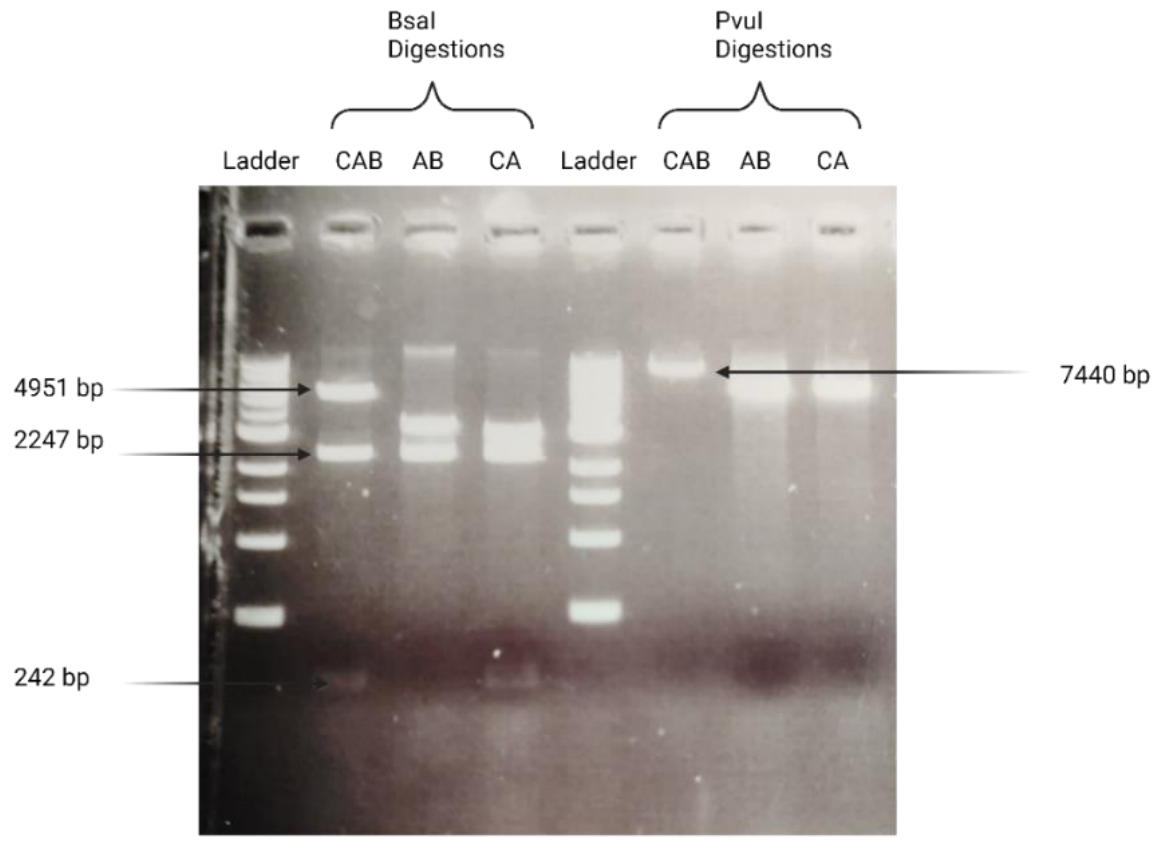

Figure 4: Annotated Gel showing diagnostic digestions of MtrCAB, CA, AB in Lv0 Vectors.

Future directions:

In the future, MtrCAB expression cassette can be characterized by measuring reduction of extracellular electron mediators (like Ferrocyanide) via Optical density measurements. Moreover, due to the design of Cyanogate, complex constructs can be created, for example MtrCAB and CymA in the same LvT vector reducing metabolic burden created by expressing the system via two separate plasmids. Lastly, this biological cassette and system can be further tested not only via ferrocyanide but in Bio photovoltaic prototypes [4] [5].

Impact of the work/results:

The move away from non-renewable energy has led to an increasing focus on newer sustainable solutions. Cyanobacteria as biophotovoltacis establishes great promise to harness the sun's energy. Moreover, genetically modifying them to become more efficient is essential to develop this technology further as done in this project. Furthermore, the work and results show that the newly developed CyanoGate system is user friendly and can be a good option for any scientist interested in introducing new genes/operons in cyanobacteria. This project helps the biochemistry society achieve its strategy by researching areas important for the betterment of the society, keeping SDGs in mind, and by fostering a collaborative spirit wherein Cyanogate parts were borrowed from a McCormick lab.

Value of studentship:

The summer studentship was an enriching experience that helped me gain and improve many skills essential for someone interested in pursuing research. The studentship provided hands-on exposure to numerous fundamental laboratory techniques, such as PCR, vector assembly and cloning, transformations, and cell culture preparation. Moreover, planning the project from scratch, designing and executing experiments, analysing data, and troubleshooting issues that inevitably strengthened my intellectual autonomy and self-reliance. Additionally, the collaborative nature of lab work, learning from experienced scientists, and sharing results during weekly lab meetings fostered my communication and presentation skills. The sense of accomplishment from contributing to ongoing research project was very rewarding. Lastly, the project helped establishing CyanoGate assembly as a robust DNA construction tool that was not routinely used in the lab before.

References:

1. Lea-Smith, D.J., et al., *Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria.* Biochim Biophys Acta, 2016. **1857**(3): p. 247-55.
2. Jensen, H.M., et al., *CymA and Exogenous Flavins Improve Extracellular Electron Transfer and Couple It to Cell Growth in Mtr-Expressing Escherichia coli.* ACS Synthetic Biology, 2016. **5**(7): p. 679-688.
3. Vasudevan, R., et al., *CyanoGate: A Modular Cloning Suite for Engineering Cyanobacteria Based on the Plant MoClo Syntax.* Plant Physiology, 2019. **180**(1): p. 39-55.
4. Melania, R., et al., *Living Photovoltaics based on Recombinant Expression of MtrA Decaheme in Photosynthetic Bacteria.* bioRxiv, 2023: p. 2023.02.28.530417.
5. Schuergers, N., et al., *A synthetic biology approach to engineering living photovoltaics.* Energy & Environmental Science, 2017. **10**(5): p. 1102-1115.

Photo of Devansh in the Lab: