

Expanding Gene Expression Tools in Cyanobacteria

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Introduction

Synechococcus sp. PCC 7002, a marine strain of cyanobacteria is a promising candidate as a cell factory for production of value-added chemicals due to its rapid growth rate. Quorum sensing (QS) systems provide bacteria the ability to coordinate their behavior based on population density. This system is less expensive than more widely used inducible promoter systems since cells are responsible for self-induction without need to add additional chemicals. Integrating a non-native QS system in cyanobacteria will allow for better understanding of how these systems work in heterologous systems, and will lead to cheaper, more efficient methods of producing this next generation of sustainable biofuels.

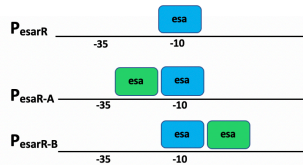


Figure 1: PesarR Promoter System box arrangements

Previously, we attempted to introduce LasI, Auhl, and LuxI activating QS systems into *Synechococcus* sp. PCC 7002, however when tested, did not express fluorescence when induced. That said, the EsaR promoter system is a promising contender to have expression due to the fact that it is a repressor rather than an activator. In this research, the EsaR promoter system with two separate *esa* boxes in different configurations were introduced into *Synechococcus* sp. PCC 7002. The designs replicate the PesarR-A and PesarR-B box arrangements in Figure 1, and another a control.

Future

Presently, it is known that EsaR can be successfully integrated into *Synechococcus* sp. PCC 7002. The next step entails performing a fluorescence test on the developed promoters to determine their relative and absolute activities, as well as determine how leaky they are. Further tests can be done during the transformation step of the procedure to quantify the number of multimers created, to see how efficient this method of transformation is for these plasmids.

Methods

Plasmids, paes1, with P_{Esar-A} integrated, paes2, with P_{Esar-B} integrated, and paes3, the control were designed in Benchling shown in Figure 2, then constructed and introduced into *Synechococcus* sp. PCC 7002 through multimer transformation. Oligo fragments were ordered then were amplified through standard PCR methods. The products were then verified by gel electrophoresis. Products that displayed single bands first had their excess template removed by digestion from the *dpn1* enzyme then purified through Zymo Research's DNA Clean and Concentrator-25 kit, while products that displayed multiple bands were purified through QIAGEN's QIAquick® Gel Extraction Kit. Since PCR is not meant for large fragments of DNA and Paes1, Paes2, and Paes3 were all ~6000 -7000 bp in length, PCR and purification was performed three times in order to obtain enough DNA to assemble the plasmids. Golden Gate Assembly was then performed to obtain the plasmids from the DNA product. Afterwards, the plasmids were integrated into *Escherichia coli* via natural transformation. Then colony PCR was performed on multiple colonies for each plasmid to confirm correctness. Once the plasmids were verified, they were further tested by sequencing. The correct plasmids were then transformed into *Synechococcus* sp. PCC 7002.

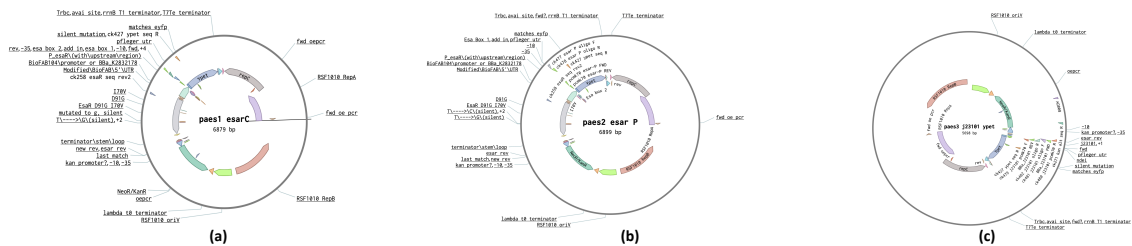


Figure 2: Plasmids paes1 (a), paes2(b), paes3 (c)

Results

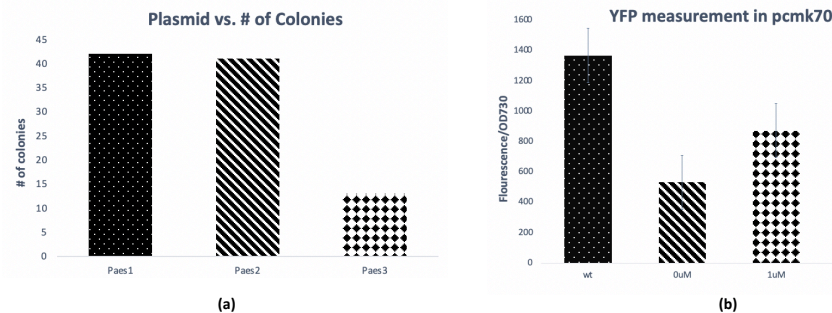


Figure 3a: Plasmid type and the number of colonies produced. The colonies with quorum sensing integrated depicts significantly more growth than the control. Figure 3b: Fluorescence measured of YFP protein in *pcmk70*, a plasmid with a single EsaR box of wild type control, 0uM HSL, 10uM HSL, and 100uM HSL induced. HSL induces quorum sensing in bacteria. After induction, YFP (yellow fluorescent protein) is transcribed by the promoter. Therefore, a measurement of fluorescence is a measurement of how well a promoter transcribes a protein. This plasmid depicts high leakiness due to the amount of fluorescence measured without induction.