Characterization of Quorum Sensing by Synthetic Circuit Controllers in Cyanobacteria

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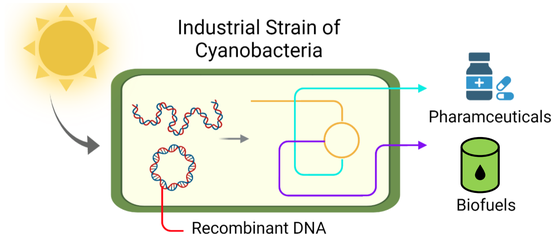
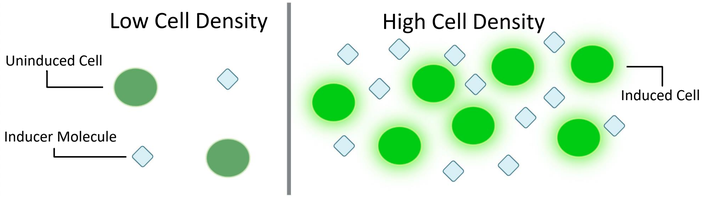
Sustainability of Cyanobacteria



***Synechococcus* sp. PCC 7002** was the strain selected for this study. Its industrial chassis makes PCC 7002 an ideal candidate for sustainable production of specialty chemicals due to its:

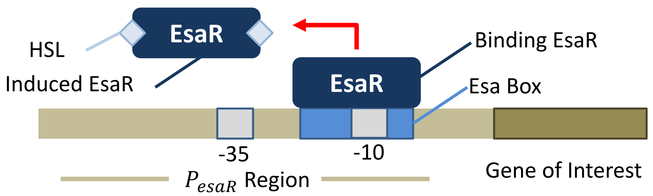
- **Fast doubling time (~2.6 h)**
- **High salt tolerance (open raceway production)**
- **Independence of freshwater resources**

Quorum Sensing



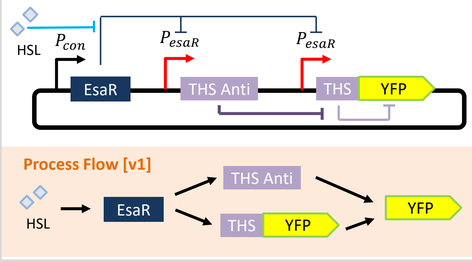
Quorum Sensing is a form of bacterial communication in which a signaling molecule – produced by the cell – induces one or more genes. Induction occurs when a **critical concentration** of signaling molecules is reached.

In this study, promoter P_{esaR}, was induced by signaling molecule **HSL**. Molecule **EsaR** – also produced endogenously – represses P_{esaR} by binding to esa boxes when uninduced. At the critical concentration of HSL, the repressing molecule EsaR is cleared by HSL driving expression.



Vectors & Integrated Controllers

Feed Forward Control Plasmid [v1]



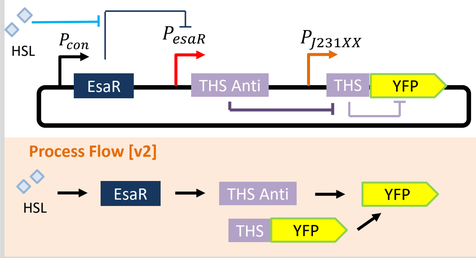
Feed Forward [v1]

A feed forward control circuit was designed and implemented to create first prototype vector.

THS (Toehold switch) was implemented before the gene of interest (YFP) to act as a secondary controller with P_{esaR}. The THS sequence spontaneously forms a hairpin upon transcription, preventing all further expression.

This hairpin collapses upon interaction with its antisense complement, also under control of P_{esaR}. This two factor control acts as a feed-forward loop.

Series Control Plasmid [v2]



In Series [v2]

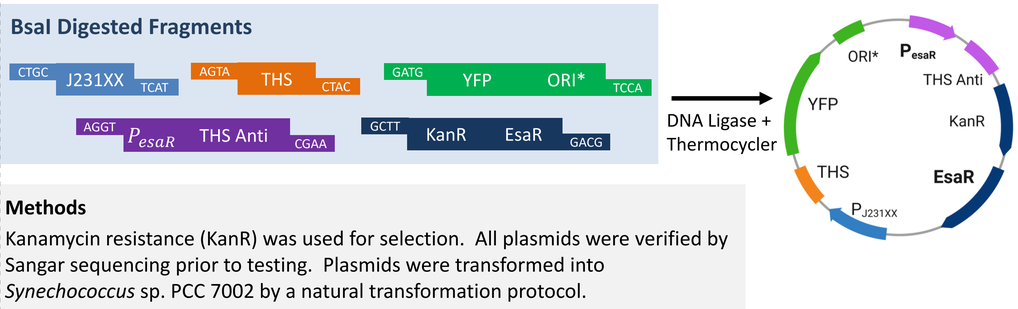
In reaction to an excess of regulation in the first prototype, a second vector was designed to employ a series configuration.

The QS circuit in this vector only modulates the antisense complement of THS, while the GOI is expressed constitutively. Two different Anderson Promoters (J23119, J23114) were used for the constitutive expression. To ensure optimal tunability, promoters were selected by unique relative expression levels.

Assembly and Transformation

Golden Gate Assembly

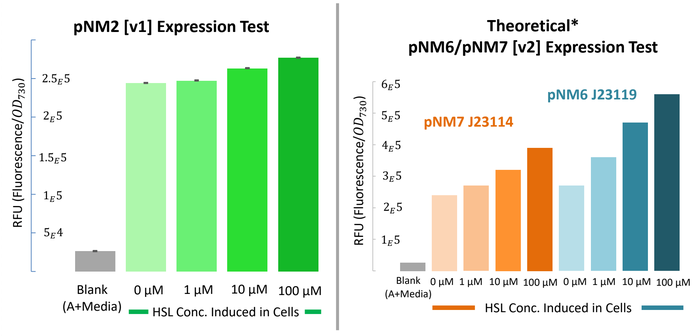
The assembly of each plasmid was accomplished using the Golden Gate method. This assembly technique requires that fragments contain type IIs endonuclease sites (which can also be introduced via primer design) and were digested prior to the reaction. Golden Gate was chosen due to the ease in which reassembly is possible.



Methods

Kanamycin resistance (KanR) was used for selection. All plasmids were verified by Sangar sequencing prior to testing. Plasmids were transformed into *Synechococcus* sp. PCC 7002 by a natural transformation protocol.

Fluorometry Results



Figures display fluorometric data measured in RFU (relative fluorescence units) to quantify expression of the gene of interest, YFP. Plasmid pNM2 employed the feed-forward control [v1] which resulted in the over suppression of YFP, as is evident by the small dynamic range of **~13.4%**.

Although data has not been collected from the series [v2] vectors pNM6 and pNM7, a theoretical chart can be created for the expected data. The expected RFU values and dynamic ranges of the [v2] plasmids were estimated by previously charted normalized expression values for Anderson Promoters.

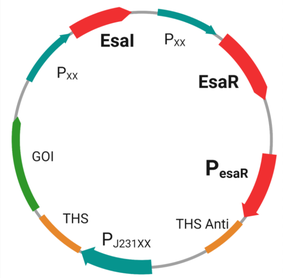
Further Research

Kinetic Analysis

Implementing of a full QS system would require a kinetics analysis of chemical composition. Reaction rate data would theoretically resemble a batch reactor where HSL would be considered a reactant and YFP a product. By incrementally taking samples for HPLC after induction, a nonlinear regression model would be used to find reaction rate constants.

EsaI (HSL Synthase)

EsaI is a gene that encodes for the HSL synthase molecule. The last step prior to achieving a full circuit QS system would be implementing this gene into the expression plasmid. Using the rate data collected in kinetics, the EsaI and EsaR genes would be tuned using a promoter library. With a gradient of inducer/repressor expression, GOI expression response would be dependent on selectable cell density.



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