Abstract

The aim of this project is to engineer a genetic bistable switch that produces two mutually exclusive outputs when given two different inputs. The switch is based on the repressor/anti-repressor system of the Salmonella phages GI4-1 and GI4-2 and the λ-phage anti-terminator system. The latest induced output will remain stable through generations, even after the input changes, due to the phage regulatory systems. We present the framework for this development and characterize the regulatory mechanisms by using fluorescent proteins as the reporters (outputs). We have also submitted the promoters, repressors and anti-repressors from the Salmonella phages, as well as the anti-terminator protein from the λ-phage, as BioBricks.

There are multiple potential applications for a biological “switch” like ours in the world of biotechnology e.g. within the medical and environmental field.

The Switch

In order to create a robust bistable switch, regulatory systems are needed [2]. Here, the three systems applied in the switch design are outlined: Repressible promoters, anti-repressors and anti-terminators.

Promoter-Repressor System

The GI4 phages with their respective promoters are important regulatory elements in the switch and this is why they have been characterized and submitted as BioBricks. The two plasmids shown below have been constructed for each GI4 phage and tested in the microfermentation system Biolister.

Anti-terminator System

In order to characterize the regulatory function of the terminator – anti-terminator subpart of our system, the BioBrick devices shown in fig. 8 were constructed. Results from the fluorescence microscopy are shown in fig. 9. Due to the high toxicity from the FP’s and thus low stability and high mutation rate of the constructs, further characterization and verification was not achieved.

DTU SPL Standard

Usage of a Synthetic Promoter Library is an alternative method for gene regulation. When wanting to fine-tune gene expression, it is essential to be able to work with small increments in expression strengths, which is achievable when working with an SPL.

As a proof of concept, the SPL was ligated with BBa_K374008, which contains an RFP gene, namely BBa_K374008. This was done in strains X3L-Blue and DH5α. RFP expression was measured from 25 random colonies for both strains using the microfermentation system Biolister. Relative Promoter Units (RPUs) were calculated using BBa_J23100 as the reference standard promoter as per Kelly et al. (2009) - BBF RFC 13 [4]. The DTU SPL standard was then created – BBF RFC 63.

Conclusion

In this project, we have outlined a design for a bistable biological switch, successfully characterized two regulatory systems contained in the switch, and submitted them as BioBricks. The regulatory systems are from the GI4 and lambda phages, namely the two sets of represors, promoters and the anti-terminator reporter. The characterizations were carried out using fluorescence and also bioluminescence. We also developed a new tool for creating a BioBrick compatible Synthetic Promoter Library that can be used for fine-tuning the expression of BioBrick parts and devices.

Background

Biological signal processing and switches are required in order to engineer more complex biological circuits. It is an advantage if the system is able to stay stable in one state even if induction is removed [1]. There is a need to further improve biological switches as they have many applications, such as:

- Cheap proof reading or control mechanism in production plants. On exposure to undesired treatments or conditions, a reporter is expressed.
- Elucidation of biofilm formation. By inducing the biological matrix with different inducers at different time points, layers in the matrix will develop depending on the last inducer. In this way movements and development can be tracked.

References: