Overview

In eukaryotes, heterochromatin plays an important role in gene regulation. In the project, we use a synthetic biology approach to imitate heterochromatin in E.coli to achieve gene silencing. Specifically, fusion proteins comprising of TetR and different parts of HNS (histone-like nucleoid structuring protein) were expressed, they were expected to bind DNA specifically and carry out polymerization among the fusion HNS and the native HNS to create a densely packed DNA form, which may block the transcription.

We have two main objectives; the first is to create a total of eight BioBricks and the second is to show the possibility of silencing specific genes using fusion protein constructs and engineered DNA sequences.

Results

We produced constructs with tetO sites upstream or downstream of lac promoter and EGFP gene. Then we used standard constitutive promoters with different activity to drive our fusion proteins to find the optimum expression level. Moreover, tetR, HNS and fusion proteins were purified and gel shift assay would be utilized to detect the interactions between those proteins with DNA.

A. Promoter

By comparing the red fluorescence intensity among 4 different promoters, the result show that B ministers J23116 in the most suitable promoter to be used and further investigate.

B. Silosome binding sites

The characterization of the silosome binding sites is an important as the characterization of the promoters to fine tune our fusion protein expression. Our team finally decided to use B ministers B0034 in our project as its strength is one of the highest in the Registry collection.

C. DNA binding domain

DNA-binding domain (DBD) is a protein domain that contains at least one motif that recognizes double- or single-stranded DNA. DBD can recognize and bind to a specific DNA sequence, which is called recognition site, with different affinity.

The Tet repressor (tetR) we used in the experiment is a transcription factor for regulating gene expression. It binds to the operator site (tetO) by using a multi-helical DBD of the N-terminal of the proteins, while the C-terminal is for regulation of DNA binding which depends on the co-factors.

D. Polymerization domain

HNS proteins are a type of histone-like proteins found in abundance in Gram-negative bacteria (such as E.coli). Phylogenetic studies have shown there are some varieties of HNS proteins in different bacteria species. Nonetheless, HNS are involved in regulating transcription repression, and the structuring of the regulation. The HNS expression is complex. Its expression is like to be influenced by the external environment as well. However, it is also capable of autoregulaiona in cell transcription.

E. Achieving Repression

By expressing the tetR-HNS fusion proteins, the tetR part of the proteins would recognize and specifically bind to the tetO binding region (tetO2). There are two tet-R binding sites as tetR to form a dimer as in the tetR-driven promoter system. As the fusion proteins bound to tetO, it is expected that the HNS part of the fusion protein would attract and oligomerize with other native HNS proteins inside the cell. With the oligomerization of the fusion proteins and native HNS, the DNA covered by the oligomers is expected to trap the RNA polymerase during transcription, thus gene repression is achieved.

Reference

[1] Our institutions: The University of Hong Kong, The Chinese University of Hong Kong, and The Hong Kong Polytechnic University.

[2] Our sponsors: The Research Grant Council (RGC), the Health and Medical Research Fund (HMRF), the Research Committee of the University Grants Committee (UGC), the Science and Technology Committee, the Innovation and Technology Committee, the Innovation and Technology Commission, and the Innovation and Technology Fund (ITF).