2011 HKU iGEM

SUPER SILENCER

Development of a Novel Inducible Transcriptional Repressor mediates the formation of heterochromatin-like complex in *E.coli*

iGEM

Overview

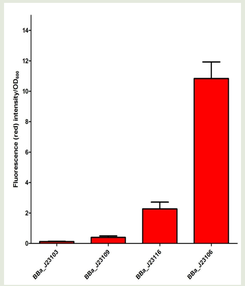
In eukaryotes, heterochromatin plays an important role in gene regulation. In the project, we use a synthetic biology approach to imitate heterochromatin in E.coli to achieve gene silencing. Specifically, fusion proteins comprising t etR and different parts of HNS (histone-like nucleoid structuring protein) were synthesized, they are 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.

Description

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 interaction between those proteins with DNA.

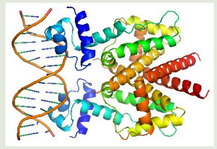
A. Promoter
By comparing the red fluorescence intensity among 4 different promoters, the result show that BBa_J23116 is the most suitable promoter to be used and further investigate.



B. Ribosome binding sites
The characterization of the ribosome binding sites is as important as the characterization of the promoters to fine tune our fusion protein expression. Our team finally decided to use BBa_B0034 in our project as its strength is one of the highest in the Registry collection.

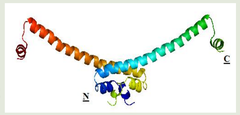
C. DNA-binding domain
DNA-binding domain (DBD) is a protein domain that contains at least one motif that recognizes double- or single-stranded DNA.
DBD can recognize and bind on a specific DNA sequence, which is called recognition site, with different affinity.

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.



TetR binds to tetO

D. Polymerization domain
H-NS 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 H-NS proteins in different bacteria species. Nonetheless, H-NS are involved in regulating transcription by repression, and the structuring of the nucleoid. The regulation of H-NS expression is complex. Its expression is like to be influenced by the external environment as well. However, it is also capable of autoregulating its own transcriptions.

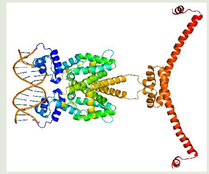


Positive/negative

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

Modelling

Gene regulation by HNS-tetR. The HNS-tetR fusion protein is arranged in and NC-NC manner, binding with its operator DNA. Through the subsequent oligomerization of HNS protein along the DNA, DNA is stretched and gene expression is suppressed. The figure shown above is the HNS-TetR binding with tetO.



Results

Our team have successfully inserted the tetO2-GFP sites ($tetO_2$-0 EGFP , tetO2-0 sfGFP and tetO2-1 EGFP) into the E.Coli MG1655 genome (attTn7 site) by recombination with our designed plasmid containing the tet-O2-GFP site.

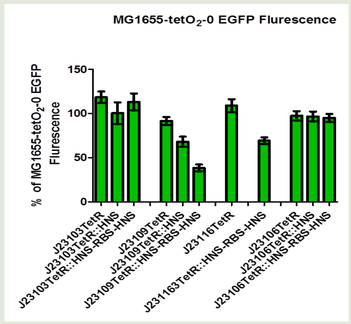
Subsequently, we transformed our silencer plasmid in to these tetO2-GP strains and the GFP intensity is measured.

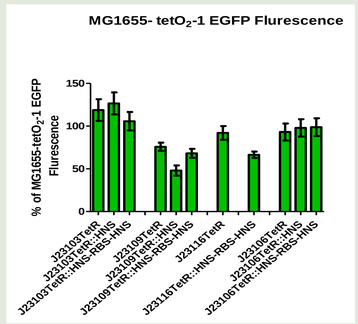
[EGFP: Enhanced Green Fluorescent Protein, sfGFP: Superfolder Green Fluorescent protein]

GFP intensity
The GFP intensity results are as follow:

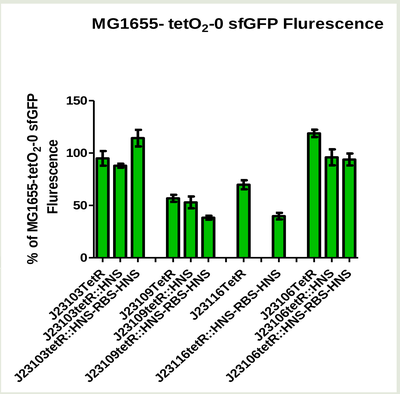
The untransformed E.Coli MG1655 ($tetO_2$-0 EGFP, tetO2-0 sfGFP or tetO2-1 EGFP incorporated into the genome) is set as control and the fluorescence intensity is calibrated as 100%.

As shown in below figures, when using promoters J23109, J23116, J23106, there is a subsequent lower fluorescence intensity detected. When only tetR is produced, there is already some repression. But the use of our fusion protein (tetR-HNS), the fluorescence is even lowered. This provides an insight for us that our designed repression system could be working. When we also expressed the native H-NS in addition to the bacteria own H-NS, the fluorescence intensity is the lowest. It can be reasoned that when our fusion protein tetR-HNS bind to the tet-O sites, the chance for oligomerization and hence repression is increased as there are more native H-NS available.





From the above result, in different promoter driven fusion tetR-HNS proteins system, their repressions are different. For example, when using J23103 promoter, all designed repressor and also the tetR have higher fluorescence intensity than the control. As for J23109, there is lower fluorescence intensity in tetR-HNS but higher intensity when native HNS is also expressed. In other two promoters, the results arealso unexpected. It seems that tetO-1 site is not suitable when the tetR recognition site is in too close proximity with the pLac promoter and the EGFP gene.



(All data collected are considered only when difference between values with the P-value <0.05)

BioBricks

Parts we have been constructed:
Tetracycline repressor selection --- (Constitutive Promoter Family + RBS + tetR)
K544001 – (Promoter: J23103)
K544002* - (Promoter: J23109)
K544003* - (Promoter: J23116)
K544012 - (Promoter: J23106)

Super silencer selection --- (Constitutive Promoter Family + RBS + tetR-$HNS_{(2\text{-}90)}$)
K544011 - (Promoter: J23103)
K544013* - (Promoter: J23109)
K544014 - (Promoter: J23106)

Dimeric Keima-Red Fluorescent Protein --- (dKeima)
K544021 - Dimeric version of the fluorescent protein "Keima-Red," as an alternative promoter

(*: Chosen as **favorite** parts because experimental results that their repression work better than others combination and should develop further on the designs.)

Human Practice

We hoped to convey the message that synthetic biology is not a pure academic subject: it is one that will find applications in daily life. We innovatively respond to the Human Practice by holding Open Day and School workshop. In addition to developing the interest of students, we reminded them to notice more about the topic since the prospective uses of synthetic biology applied to every corner of our life like environment aspect, medical aspect as well as food and safety, etc.

Although synthetic biology is still in infancy in Hong Kong, we see the potential development of it. We added a 'marketing' part in the workshop to provide a platform for the students to think about the future possibility of development. This successfully guided them to think different about synthetic biology.

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