Abstract

Streptococcus pneumoniae causes health complications such as pneumonia and meningitis, killing around 1.6 million people a year. Although there are existing tools to detect S. pneumoniae, they are not readily accessible in developing countries. So, we designed a new, more accessible diagnostic tool, Pneumosensor. Pneumosensor has a rewired ComCDE signal transduction pathway native to S. pneumoniae that detects autoinducer molecules released specifically by S. pneumoniae. It is also equipped with a new promoter for specific and tight regulation of target gene expression in E. coli. It is important that biosensor has minimum leakage to prevent false alarm. Hence we worked on characterizing riboregulators to increase specificity of expression and minimize leakage that can be applied to our own and other teams’ project. We also took time to characterize PBad promoter because it is a critical part for the riboregulator system.

Pneumosensor

Background

Pneumosensor adopts the quorum sensing pathway in S. pneumoniae to detect its populations. The main advantage of this system is its detection specificity to eliminate possible cross-talk of autoinducer molecules, competence-stimulating peptide (CSP) with native E. coli molecules. This is then incorporated with a highly specific reporting system, whereby σX (ComX) associated with RNAP binds to promoters PMar and Pconf containing 8 bp Com-Box sequence specific to σX for activation.

Characterization

1. We managed to build and characterize σX CDS (BBa_K1379004), PMar (BBa_K1379000) and Pconf (BBa_K1379001).
2. GFP intensities were measured over time to obtain GFP synthesis rates, which then compared to BBa_J23101 as reference promoter to obtain Relative Promoter Units.

Riboregulator

Pneumosensor needs to have minimum leakage to prevent false alarm. Riboregulators can fulfill this requirement. However, a lot of riboregulators in Part Registry are lacking characterization data. Therefore, we characterized those parts so that our team and other teams could use the parts confidently.

Riboregulator consists of two components:
1. crRNA is mRNA containing a cis-repressing sequence to sequester the RBS.
2. taRNA contains a sequence complementary to cis-repressive sequence to expose the RBS.

**Results**

Since PBad Promoter was an essential part of the riboregulator system, we took some time characterizing the promoter. We calculated Relative Promoter Units (RPU) of the promoter in DH10B, BW25113 and DH5α strains to understand how cell strain variability affect the promoter function. All-or-none response was observed for all three cell strains. The levels of RPU, however, were different in the three cell strains.

**PBad Characterization**

Since PBad Promoter was an essential part of the riboregulator system, we took some time characterizing the promoter. We calculated Relative Promoter Units (RPU) of the promoter in DH10B, BW25113 and DH5α strains to understand how cell strain variability affect the promoter function. All-or-none response was observed for all three cell strains. The levels of RPU, however, were different in the three cell strains.

**Human Practice - Start-up Kit**

Start-up Kit is the first comprehensive and handy tool that aims to help iGEM teams, particularly the new iGEMers, in initiating new human practice projects. It includes several items such as:
1. Search Engine
2. Handbook
3. Interview with past iGEM Human Practice judges
4. Report

****Acknowledgements****

We would like to thank these parties for helping and supporting our teams in completing our projects:
1. Office of the Provost, HKUST
2. School of Science, HKUST
3. Office of the Dean of Science, HKUST
4. Office of the Provost, HKUST
5. Prof. Lam Hon Ming
6. Prof. Tom Richard
7. Ms. Christine Chiu
8. Office of the Dean of Science, HKUST
9. Past iGEM Teams (South University of Science and Technology of China 2014, Tec Monterrey 2013 iGEM, Manchester 2013, Cornell 2013)
10. Wellcome Trust Sanger Institute, Technology of China 2014, Tec Monterrey 2013 iGEM, Manchester 2013, Cornell 2013