Summary

Goal: design biosensor capable of detecting Pseudomonas Aeruginosa quickly, inexpensively, and effectively

- Pseudomonas Aeruginosa is known to cause a wide variety of severe nosocomial infections, especially in immunocompromised patients
- We achieved our goal by engineering a biosensor system in E. coli, using components from the P. Aeruginosa quorum sensing system
- Our biosensor constructs detect and report the presence of autoinducer molecules unique to the P. Aeruginosa quorum sensing system
- Our biosensor constructs reached steady state in approximately two hours, which is much faster than other existing detection methods

Biobrick Assemblies:
- Las/PAI-1 constructs provide an excellent binary test for presence of P. Aeruginosa autoinducers
- RhlR/PAI-2 constructs yield output fluorescence readings proportional to autoinducer concentration

Novel Parts:
- Newly isolated genomic promoters provide novel mechanisms for detecting P. Aeruginosa

System Overview

1. Constructs constitutively express Las or Rhl receptor proteins
2. When appropriate autoinducer is present, autoinducer dimerizes with receptor protein
3. Dimer activates inducible promoter on plasmid
4. Promoter drives transcription of GFP

Testing Methods

- Grew cultures overnight at 37° while shaking
- Used clear NB media to supplement cells
- The next morning: took OD readings at 600nm and diluted cultures to OD values around 0.05
- Grew cultures until all OD values fell within range of 0.5-0.7
- Chopped samples and controls into 96/384 well plates
- Added corresponding autoinducer molecules to each well and immediately began making measurements
- Measured both OD and fluorescence over course of 4 hours

Mathematical Model

- Performed sensitivity analysis and optimized model parameters to qualitatively recapitulate biosensor performance
- Sensitivity analysis showed that LasR concentration was sensitive to both transcription of mRNA and translation of LasR from mRNA
- Analysis further showed that autoinducer concentrations was most sensitive to autoinducer degradation rates

Results

- Basal fluorescence insignificant, easily discernable from induced samples
- Well suited for binary test

- Superior discrimination between different autoinducer concentrations
- Discriminates between initial and steady state autoinducer concentrations

Conclusions

- Biosensor constructs exhibited significant fluorescence in the presence of autoinducer molecules
- Steady state fluorescence was reached in approximately two hours
- Characterization of existing and newly isolated promoters identified excellent candidates for both binary and concentration dependent testing

References


Human Practices

We created a video discussing economic and ethical implications of using synthetic biology in a clinical setting. The video features conversations with:
- Dr. Dale Mortensen, Nobel Laureate in Economics
- Dr. Keith Tyyn, Synthetic Biologist
- Dr. Laurie Zoloth, Bioethicist

We showed our video in the Northwestern University Technological Institute lobby to get students thinking about synthetic biology.

Application

Photodiode Well
Orange filter
Blue LED

Schematic overview of photodiode-based clinical detector

Our system’s success means My N.U. P.A.L. will have many advantages:
- Easily portable
- No expensive or complicated equipment
- Fast (1-2 hr) detection of Pseudomonas Aeruginosa

The device could be used in several ways to reduce the impact of infections:
- Checking hospital equipment for contamination
- Determining identity of blood or tissue sample
- Checking hospital rooms before a patient enters

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