Cyanide Biosensor

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Goal

\[ \text{Cyanide} + \begin{bmatrix} :C\equiv N: \end{bmatrix}^- = \]

 Skull and Bones
Motivation

• Cyanide is toxic
• Traditional chemical tests are difficult

Titration  Distillation  Coloration
Past Cyanide Biosensors

- Measurement of inhibited respiration
  (Nakamura and Karube. 2003)

- Measurement of byproducts from *Pseudomonas fluorescens* (degrades cyanide)
  (Nakamura and Karube. 2003)
General Regulated System

TR Promoter     TR Gene       Promoter            Response

TR = Transcriptional Regulator
Implementation

- Modify genes from *Pseudomonas fluorescens*

Section of *Pseudomonas fluorescens* genome we dealt with (5.6 kb).

(Fernandez et al. 2004)
BioBricks

Promoter | Cyanide Nitrilase | Transcriptional | GFP
---|---|---|---
Gene | Regulator | |
Useful Combinations
Final Part for Our Project

TR Promoter     TR Gene     CN Promoter          GFP

TR = Transcriptional Regulator
CN = Cyanide Nitrilase
Procedure

• Transform cells with GFP
• Isolate *P.fluorescens* genes with PCR
• Cut genes with proper restriction enzymes
• Ligation
• Transform more cells
Results of PCR

Cyanide Nitrilease Promoter 0.27 kb
Transcriptional Regulator 1.3 kb
Negative control
Positive Control
Ladder

1kb DNA Ladder

bp
10,000
8,000
6,000
5,000
4,000
3,000
2,500
2,000
1,500
1,000
750
500
250
253
0.7% agarose
GFP BioBrick

GFP Amp
GFP Norm
NC Amp
NC Norm
PC Kan
PC Norm
YFP Amp
YFP Norm
Restriction Digests

Diagram modified from iGEM wiki
Limitations of Biosensor

- Cyanide could kill sensing bacteria
- Sensing bacteria can only detect free cyanide
Future Work

- Successfully build plasmid as planned
- Determine if cyanide nitrilase gene is regulated
- Determine correct transcriptional regulator
- Extend cyanide detecting system with complexed cyanide detecting capabilities
Conclusion:

Copy/paste in biology is harder than on computer.
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