Cyanide Biosensor

Southern Utah University

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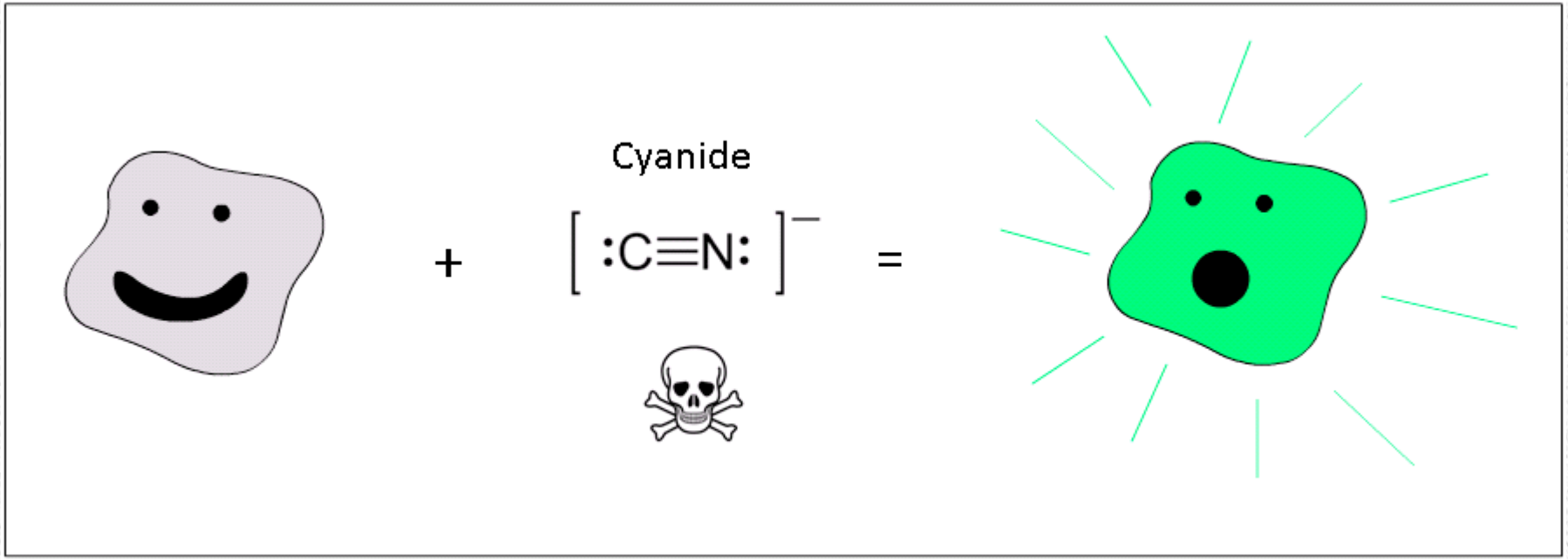
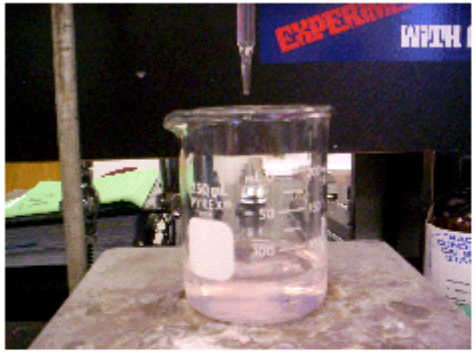
Goal



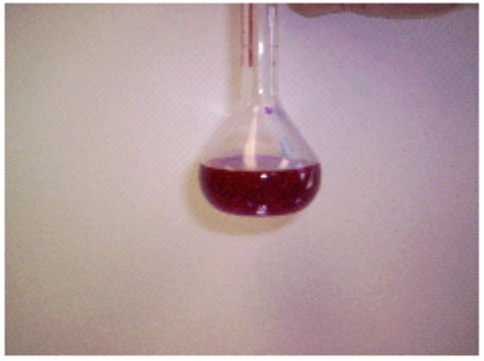
Motivation

- 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)

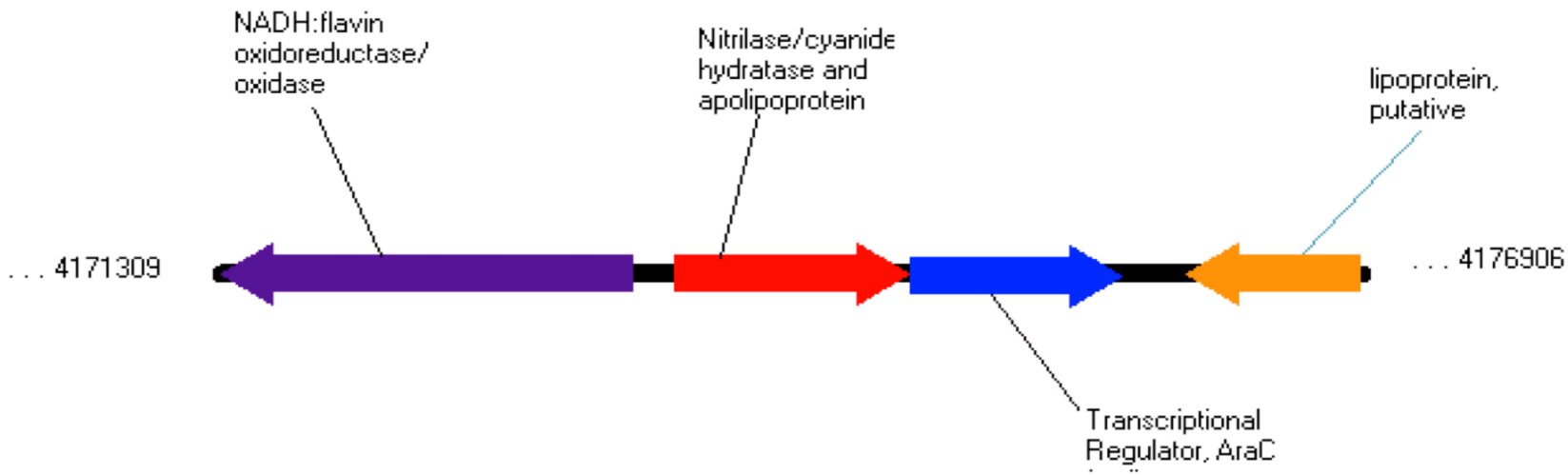
General Regulated System

TR Promoter TR Gene Promoter Response

TR = Transcriptional Regulator

Implementation

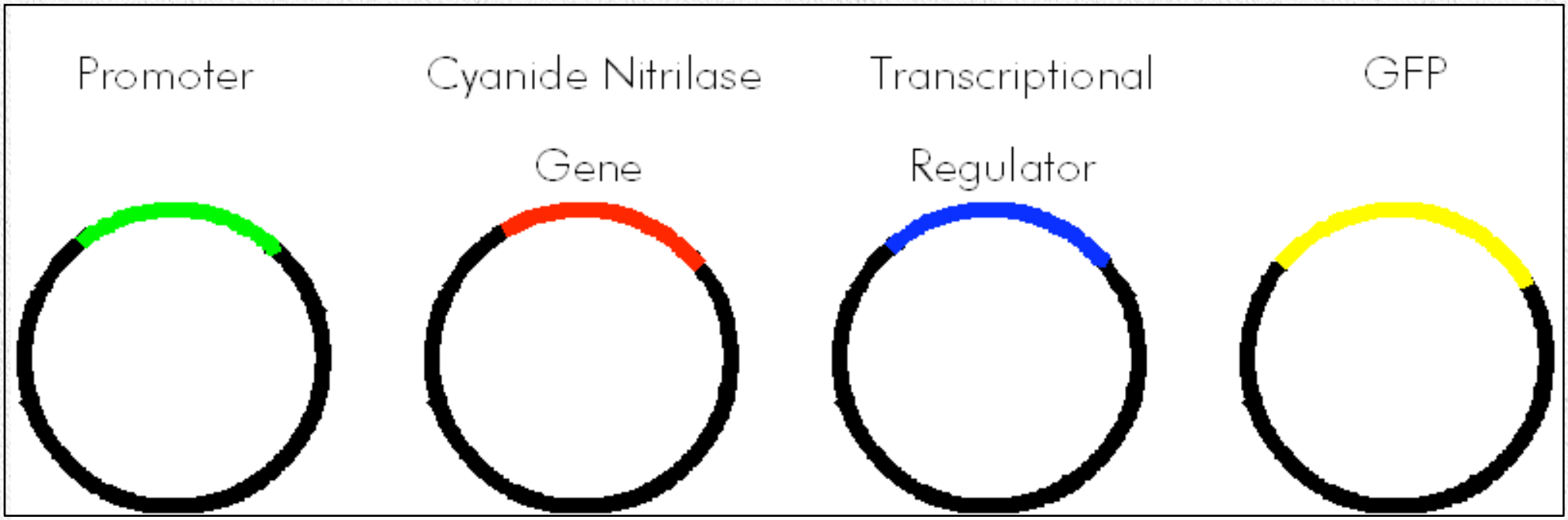
- Modify genes from *Pseudomonas fluorescens*



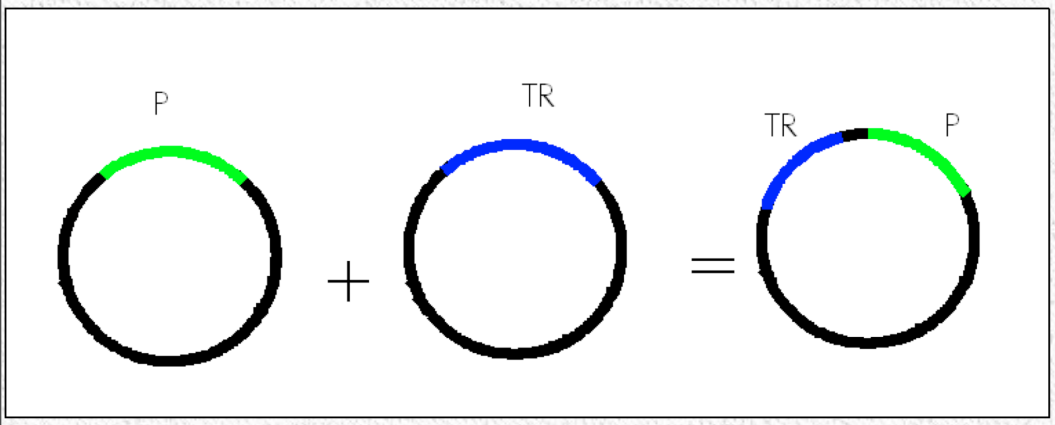
Section of *Pseudomponas fluorescens* genome we dealt with (5.6 kb).

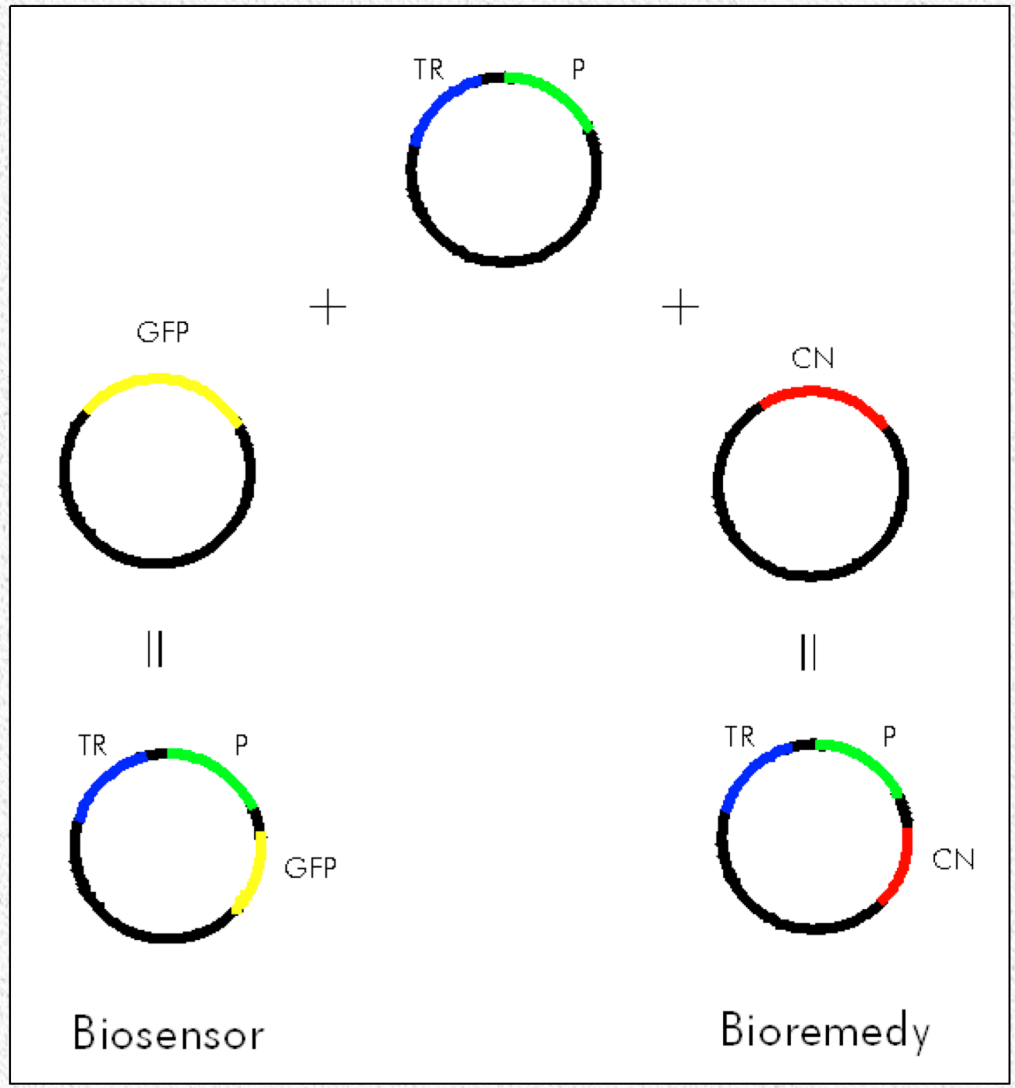
(Fernandez *et al.* 2004)

BioBricks

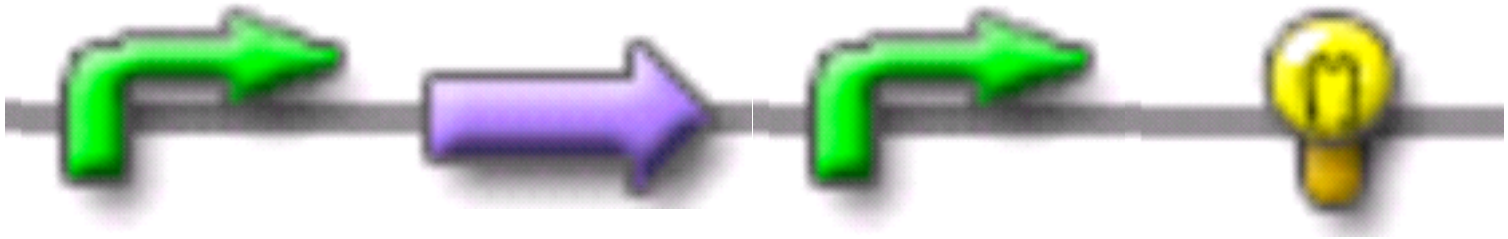

Promoter
Cyanide Nitrilase Gene
Transcriptional Regulator
GFP

Useful Combinations





Final Part for Our Project

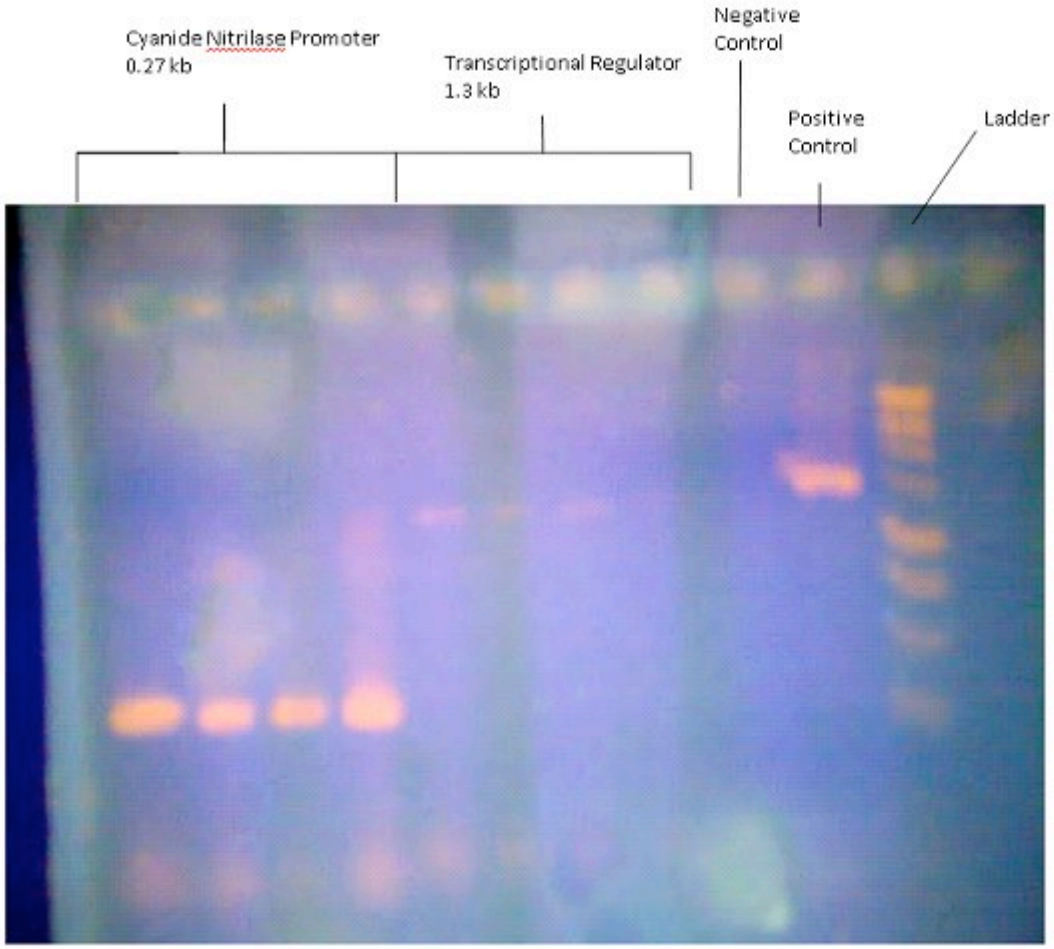


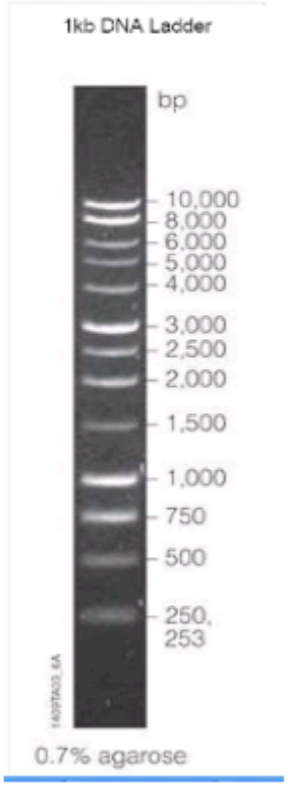
TR = Transcriptional Regulator
CN = Cyanide Nitrilase

Procedure

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

Results of PCR



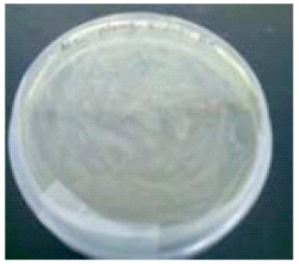


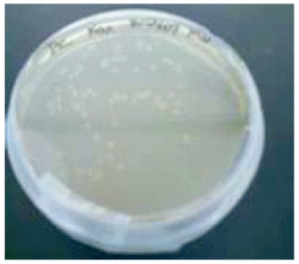
GFP BioBrick


GFP Amp

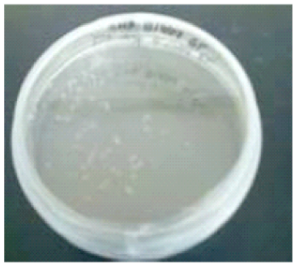

GFP Norm


NC Amp


NC Norm

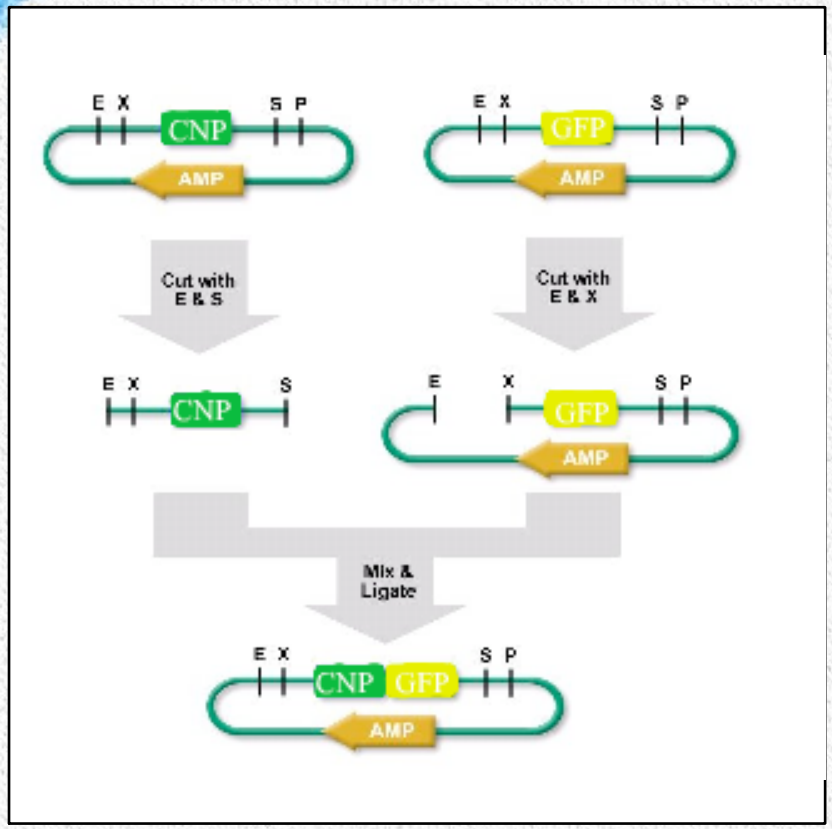

PC Kan


PC Norm


YFP Amp


YFP Norm

Restriction Digests



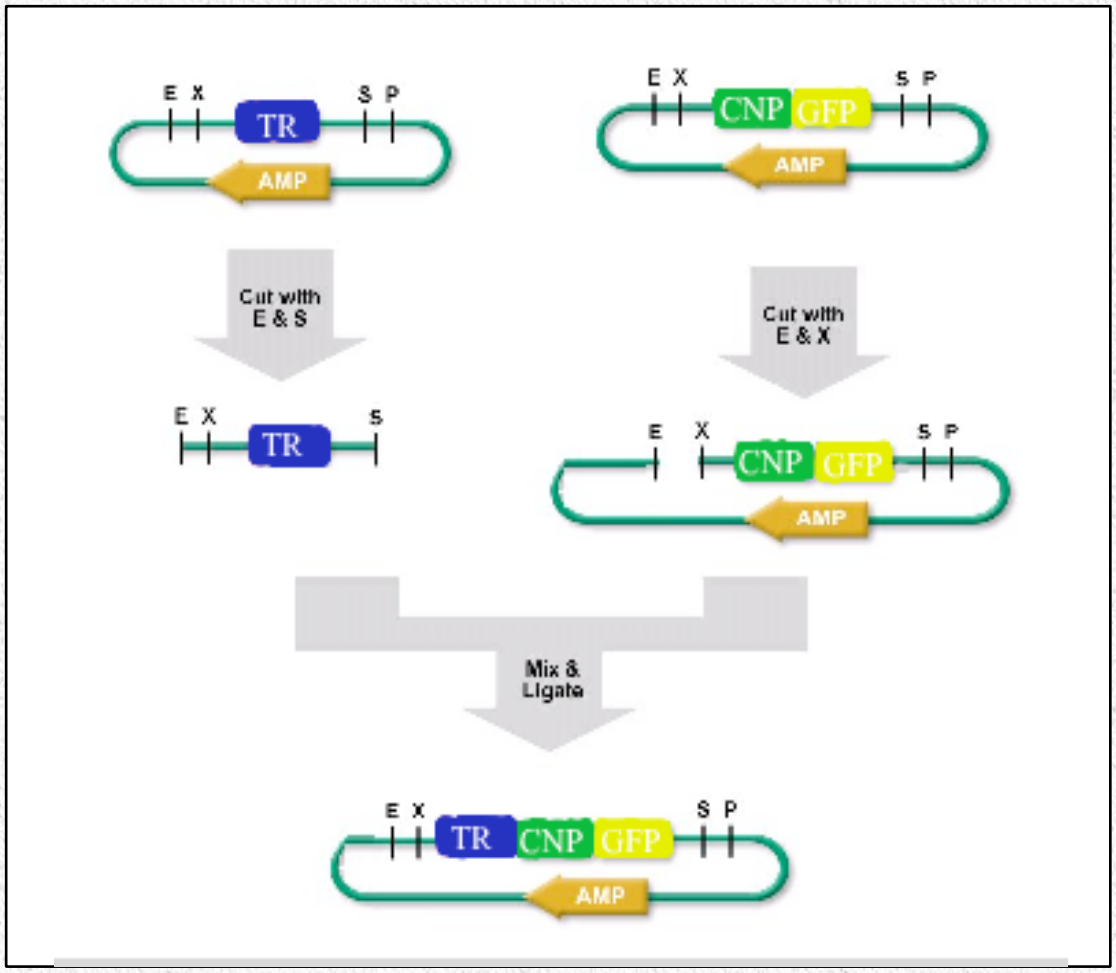


Diagram modified from iGEM wiki

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

Acknowledgments

- Chief Advisor: Charlotte Rosendahl Pedersen
- Advisors: Bruce Howard, Colette Calmelet
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- Southern Utah University

Citations

1. Nakamura, H., Karube, I. (2003). Current research activity in biosensors. Anal Bioanal Chem, 377, 446-468.

2. Fernandez, R.F., Dolghih, E., Kunz, D.A. (2004). Enzymatic Assimilation of Cyanide via Pterin-Dependent Oxygenolytic Cleavage to Ammonia and Formate in *Pseudomonas fluorescens* NCIMB 11764. Applied and Environmental Microbiology, 70, 121-128.