Break-It-Down:
Programming Cell Death

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Technical Specification Review
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Motivation

Goal of our project:
- To allow for better bioremediation - specifically of marine oil spills

Current problem;
- Bioremediation takes time
- Research is confined to labs

Our solution;
- To engineer the cells to die after bioremediation has occurred.
Device-level model

Corynebacterium

- Hydrocarbon
- [S] Sensor
- Inverter
- OR
- Sulfate
Death Device

Hydrocarbon

HC Sensor

Inverter

OR

[S] Sensor

Death Device

Sulfate
Death Device

BBa_R0040: TetR repressible promoter
BBa_J61100: Ribosome binding site
ERHG-05096: LdtR-Rd1D sequence
Addiction System

plasmid DNA

LdtR transcription

Rd1D gene

rapid Rd1D transcription

stable LdtR mRNA

duplex formation

degradation by RNase III

LdtR protein

cell death

translation

rapid Rd1DmRNA degradation
Testing/Debugging: Death Device

- **Change promoter.**
- **Grow bacteria with death device only.**
  - Dies immediately.
  - **Change promoter.**
- **Cells don't die.**
  - Add tetR to medium.
  - **Cells die. Device is working!**
- **Grow bacteria with GFP and PAM.**
  - Glows. PAM is working, but death is not being triggered
  - **Change promoter.**
- **Doesn't glow. Toxin/antitoxin are not being produced.**
Hydrocarbon Degradation System

Hydrocarbon Sensor
Hydrocarbon Sensor
Testing/Debugging: HC Sensor

- Grow bacteria with just HC sensor, and GFP gene after lac1 gene.
- Add hydrocarbon mixture to medium.
- Cells don't glow.
- Cells only glow when hydrocarbon is gone.
- Cells glow whether hydrocarbon is present or not.
- Cells glow until hydrocarbon has been broken down. Device is working!

Tweak operon
Change promoter
Inverter
Testing/Debugging: Inverter

Grow cells with inverter only.
- GFP is produced.
- Lac is not working as a repressor.

Add LacI
- GFP is not produced.

Place bacteria in medium with no lac1.
- GFP is not produced.

GFP is produced. Lac is not working as a repressor.

New repressor
- GFP is produced. Inverter is working!
Sulfate Sensor

Cysteine is as a result produced in larger numbers

Tet-R, which triggers death, is produced.

Encourages the assimilation of the salt sulfate from the ocean water

Input of cysteine is tuned in a "capacitor" fashion to produce an output of a certain volume
Sulfate and Cysteine

cysE: important in assimilating sulfate and making cysteine. Mutant version of this gene (BBa_K1010) will constitutively assimilate sulfate.

p-cysE: is normally sensitive to cysteine concentration. The mutant version of this is not, but the original promoter is useful because cysteine can repress it.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1855706/figure/f1/
Capacitor To Produce Tet-R

**BBa_J102005:** a biological capacitor that for a volume of "voltage" input produces a certain "current". In this case, the voltage is the amount of cysteine input and the current is the amount of output. Tet-R is the resulting molecule.
Testing/Debugging

Capacitor + GFP + cysteine

- No glow
  - Knockout Y and put GFP after X
  - Change the production of Chemical Dial
  - Change X/Y and corresponding promoter.

- Too fast
  - Change the production of Chemical Dial
  - Change X/Y and corresponding promoter.

Glow

- TetR is being produced
  - Change the production of Chemical Dial

- cysE mutant
  - Test for sulfate accumulation by plating both mutant and normal bacteria on a known concentration of $\text{SO}_4^{2-}$
  - mutant intake > normal intake
  - mutant intake = normal intake
  - It works!

This system has been shown to work normally, so an external factor or a compound unique to this cell is affecting the assimilator. Try to change concentrations in media, protein expression, etc.
Timing Diagram (Sulfate sensor model)

Antitoxin
Toxin
Addiction Mod.
TetR
Y
X
cysE

Time (as of now undetermined)
Impact Description

- Allows for use of engineered bacteria
- Implementation in a range of bacteria
- Potential use in other situations
- Faster bioremediation = fewer side effects
Open Issues

- Which sensor is safer/more effective?
- Time delay for sulfate system
- Fail-safe system?
- How to block horizontal gene transfer?
- Will toxin be released into the environment after death and affect other bacteria?
Go/No Go?

GO.
Any Questions?
Death device:


**Parts:** Registry of Biological Parts

ERHG-05096: LdtR-Rd1D sequence


Bioremediation:

References

HC Sensor

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