

Break-It-Down:

Programming Cell Death

***Sarah Wright, Madeline O'Grady, Hao Xing,
Ishwar Kohale, Shruthi Narayanan, Jiaqi Xie***

*Technical Specification Review
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Motivation

Goal of our project:

- To allow for better bioremediation - specifically of marine oil spills

Current problem;

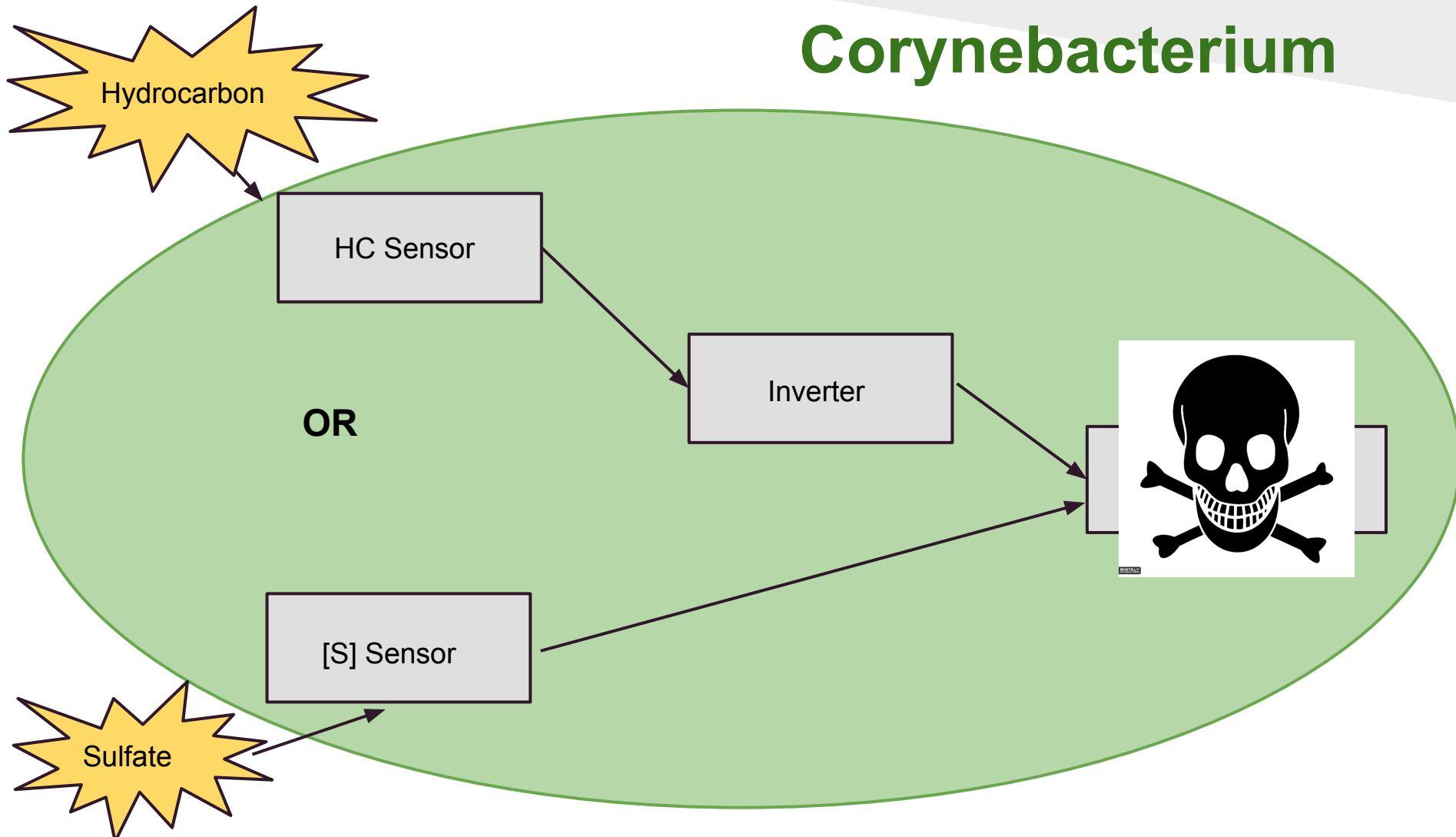
- Bioremediation takes time
- Research is confined to labs

Our solution;

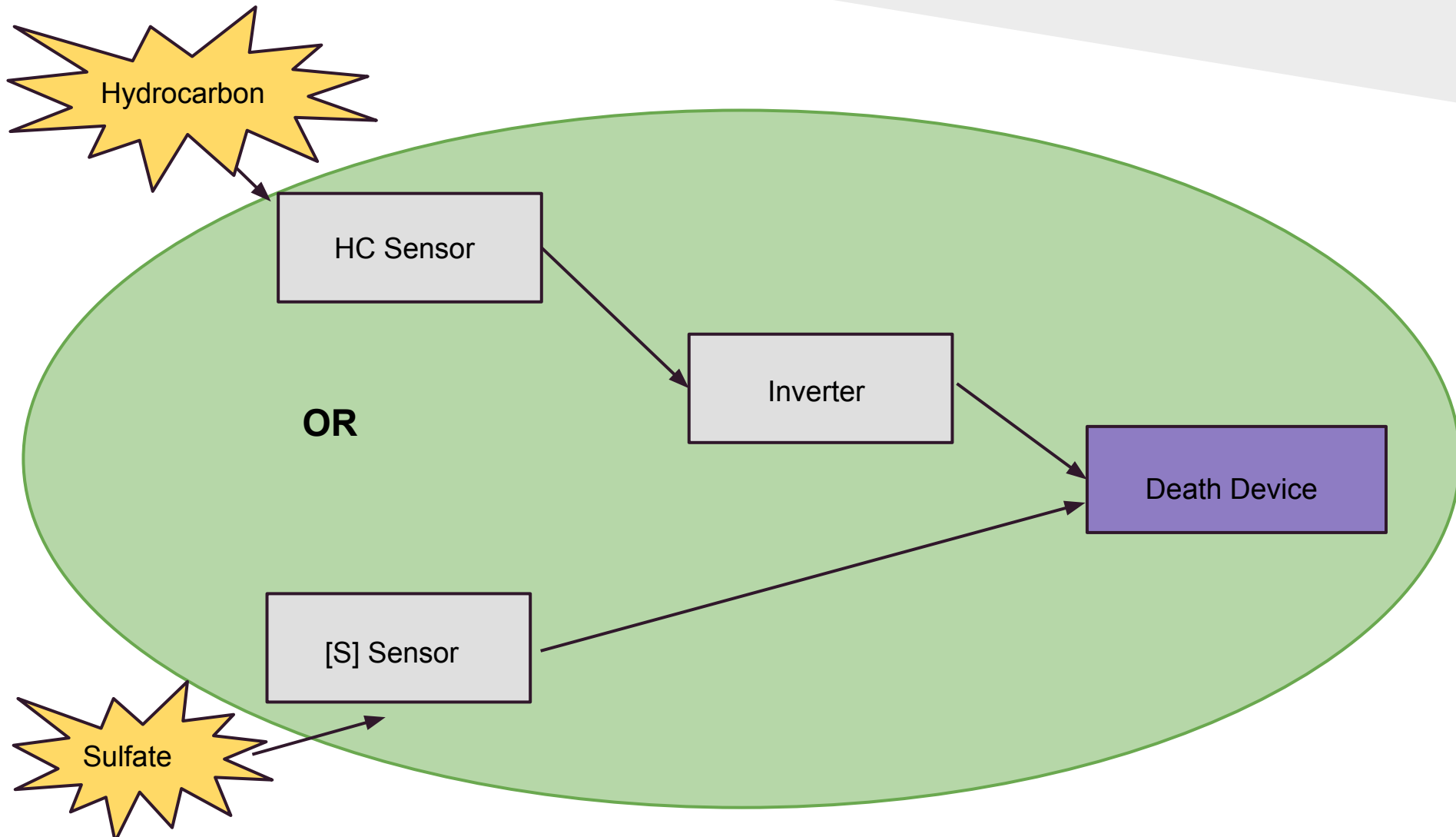
- To engineer the cells to die after bioremediation has occurred.

Device-level model

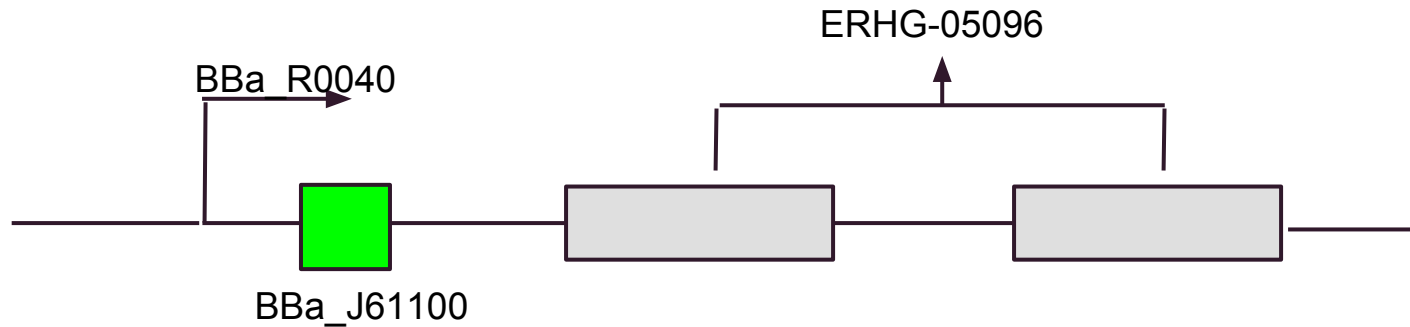
Corynebacterium



Death Device



Death Device

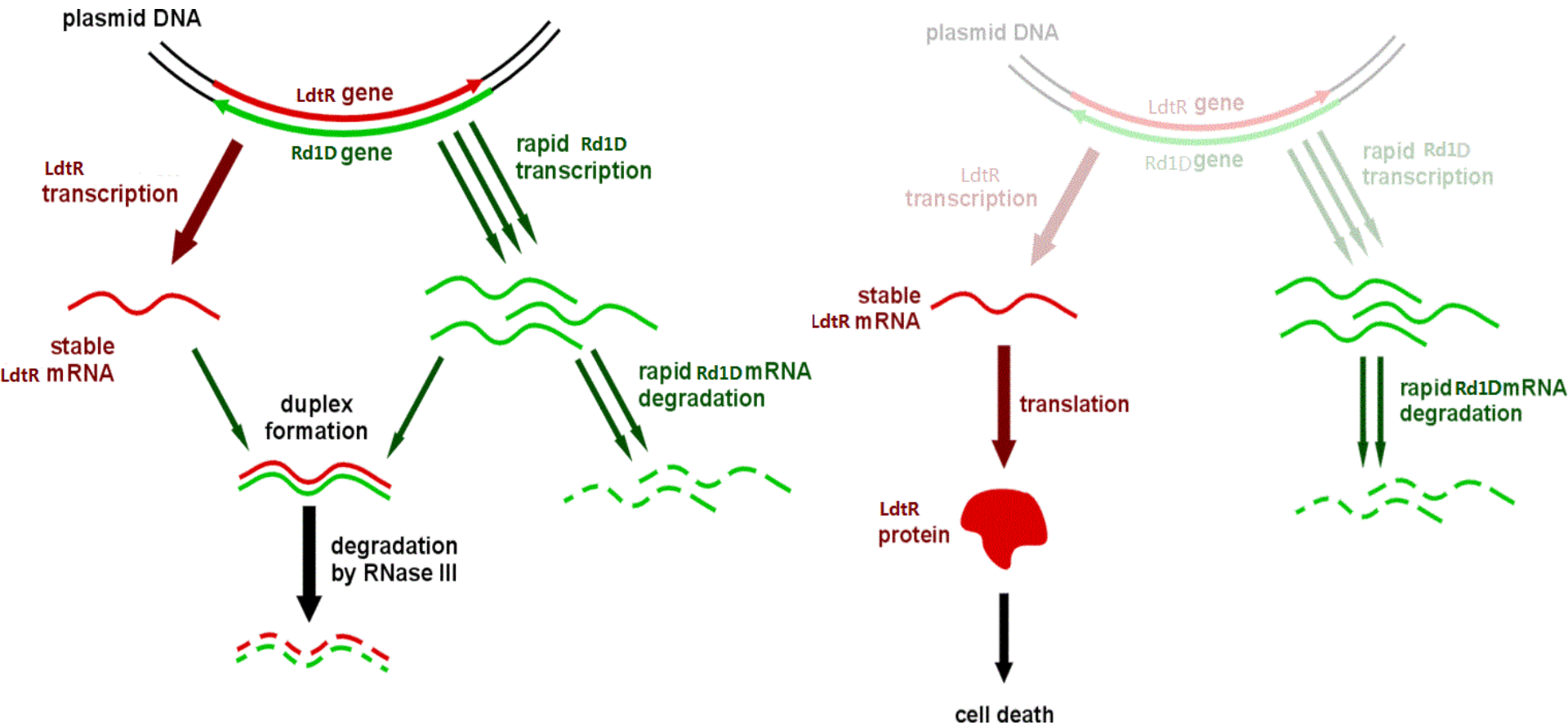


BBa_R0040: TetR repressible promoter

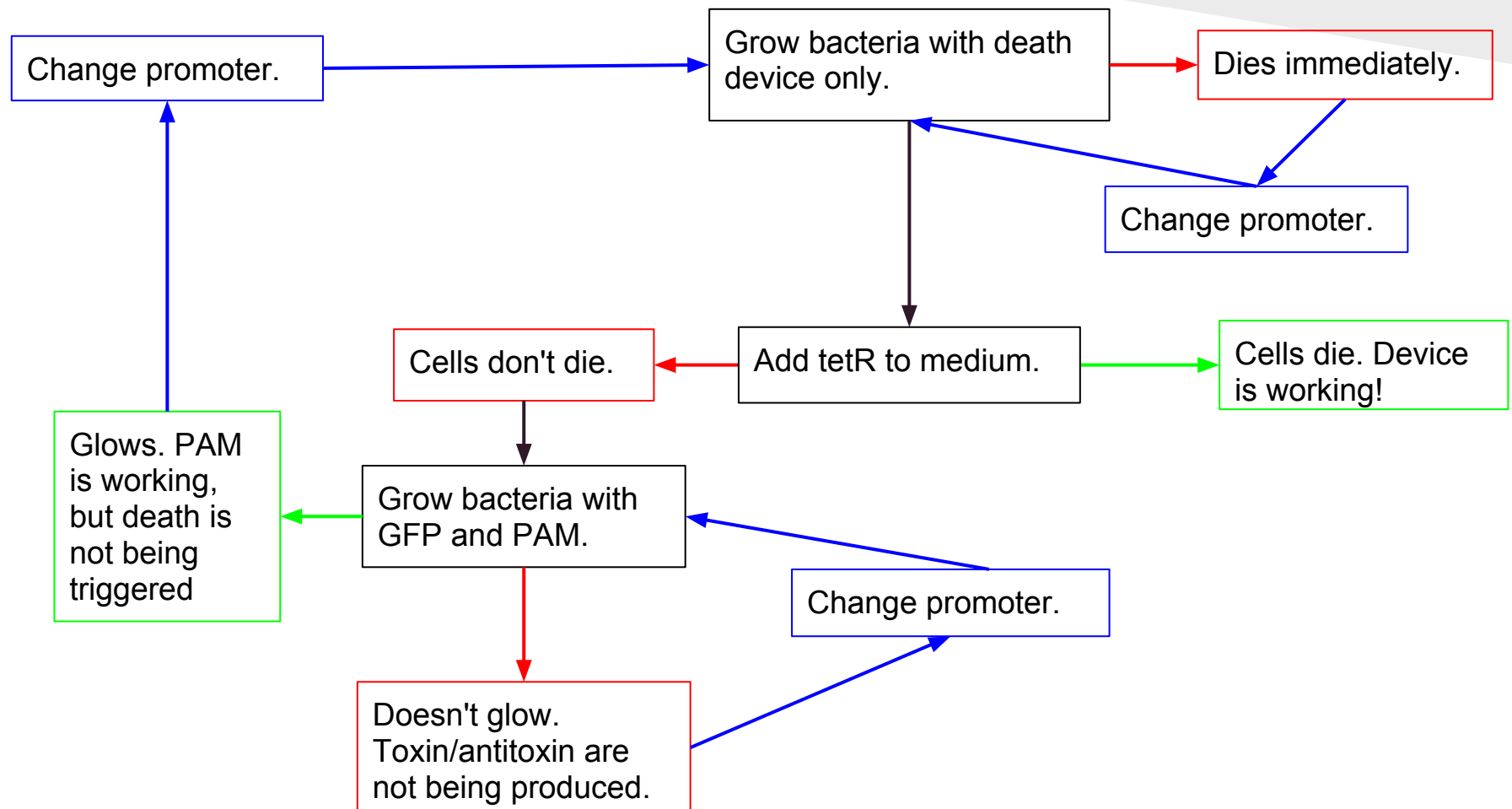
BBa_J61100: Ribosome binding site

ERHG-05096: LdtR-Rd1D sequence

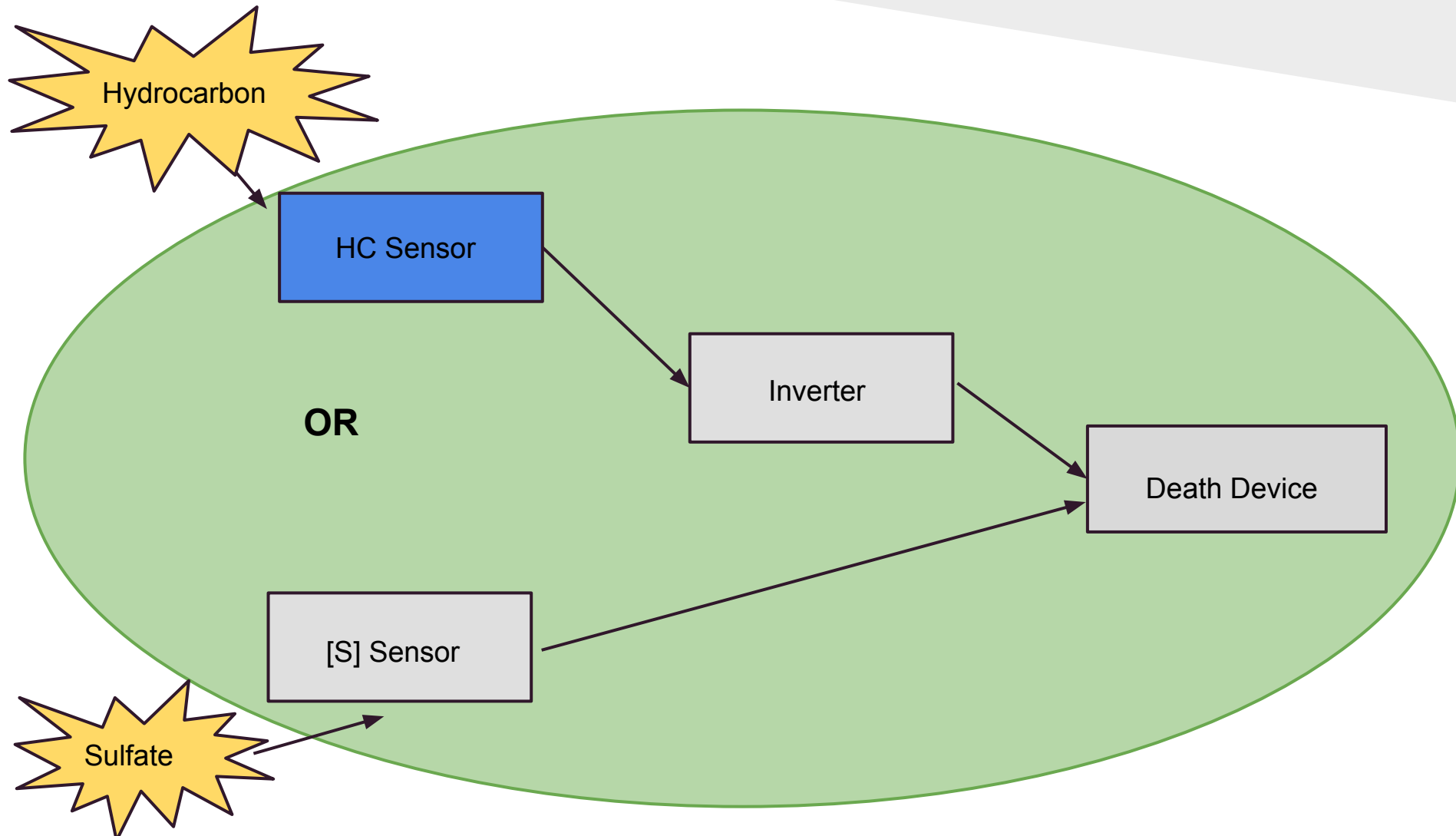
Addiction System



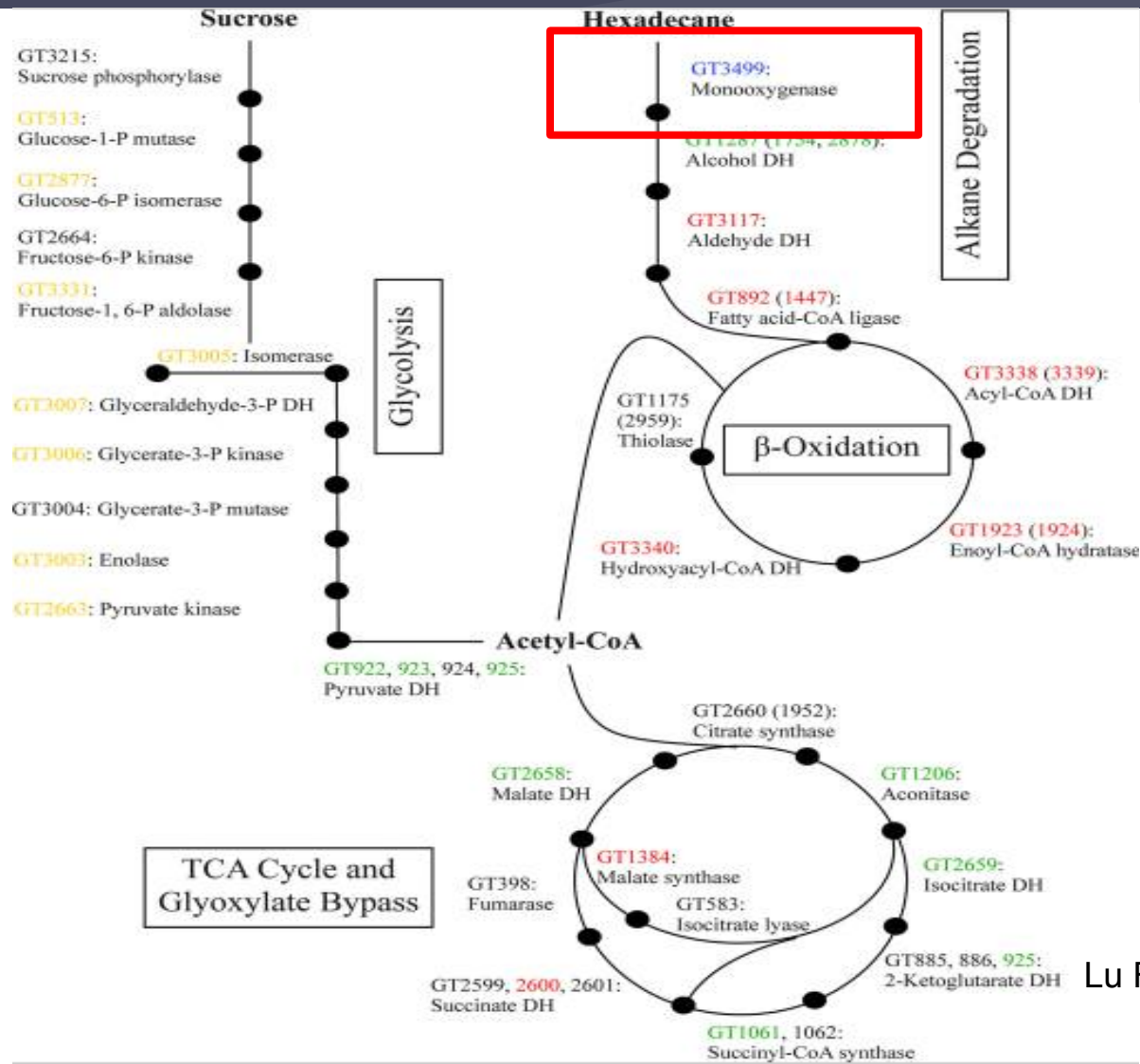
Testing/Debugging: Death Device



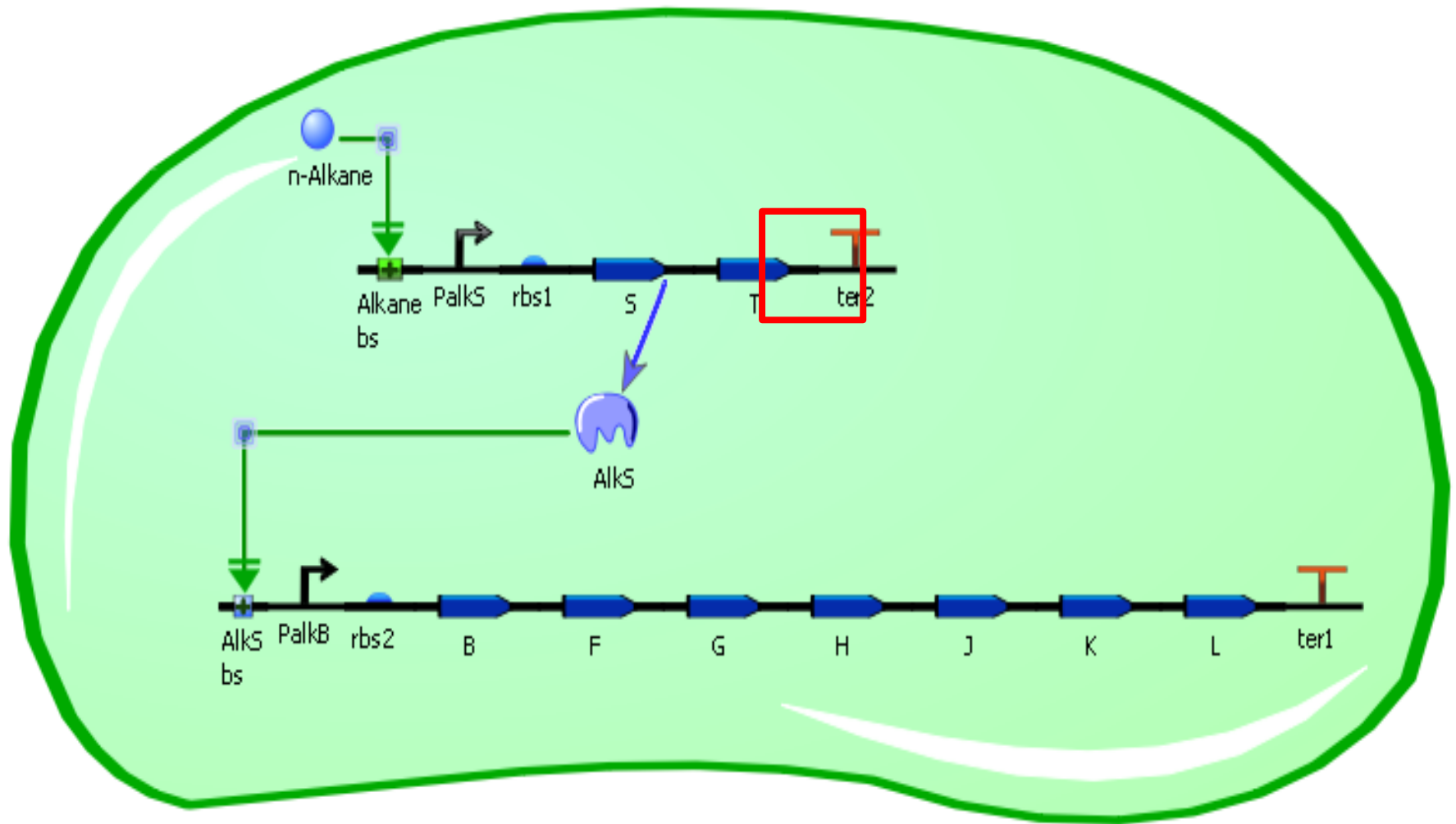
Hydrocarbon Sensor



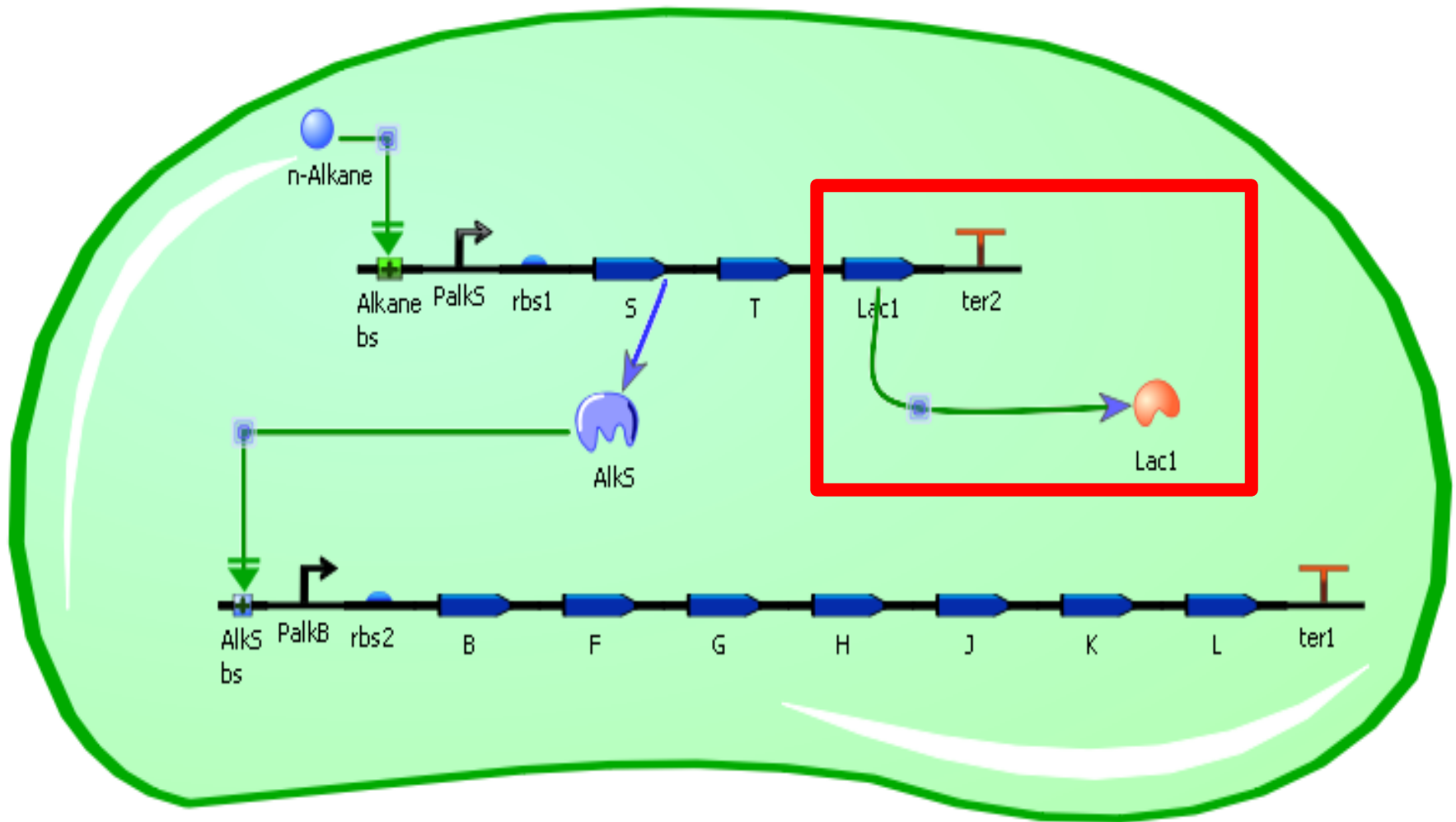
Hydrocarbon Degradation System



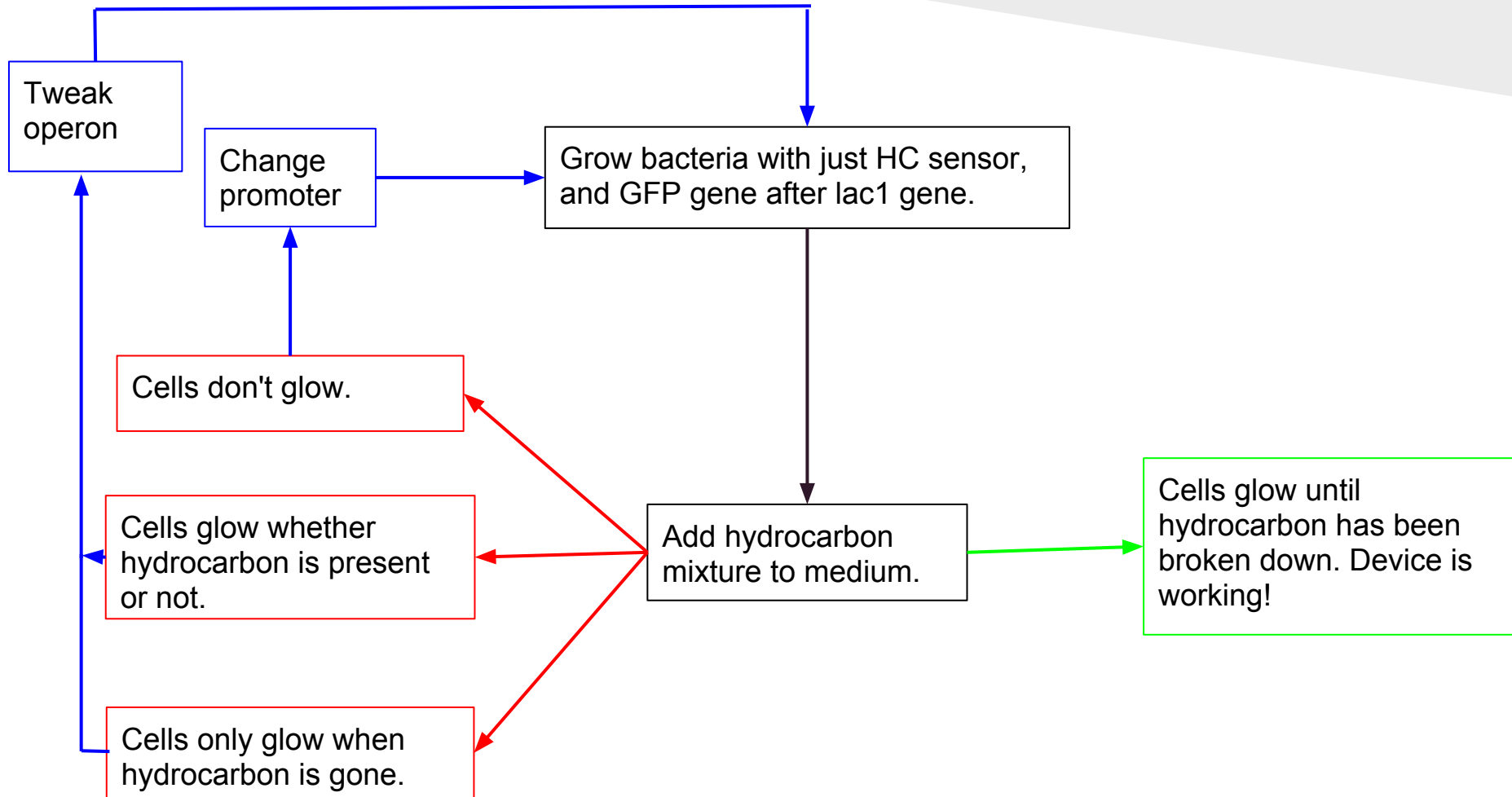
Hydrocarbon Sensor



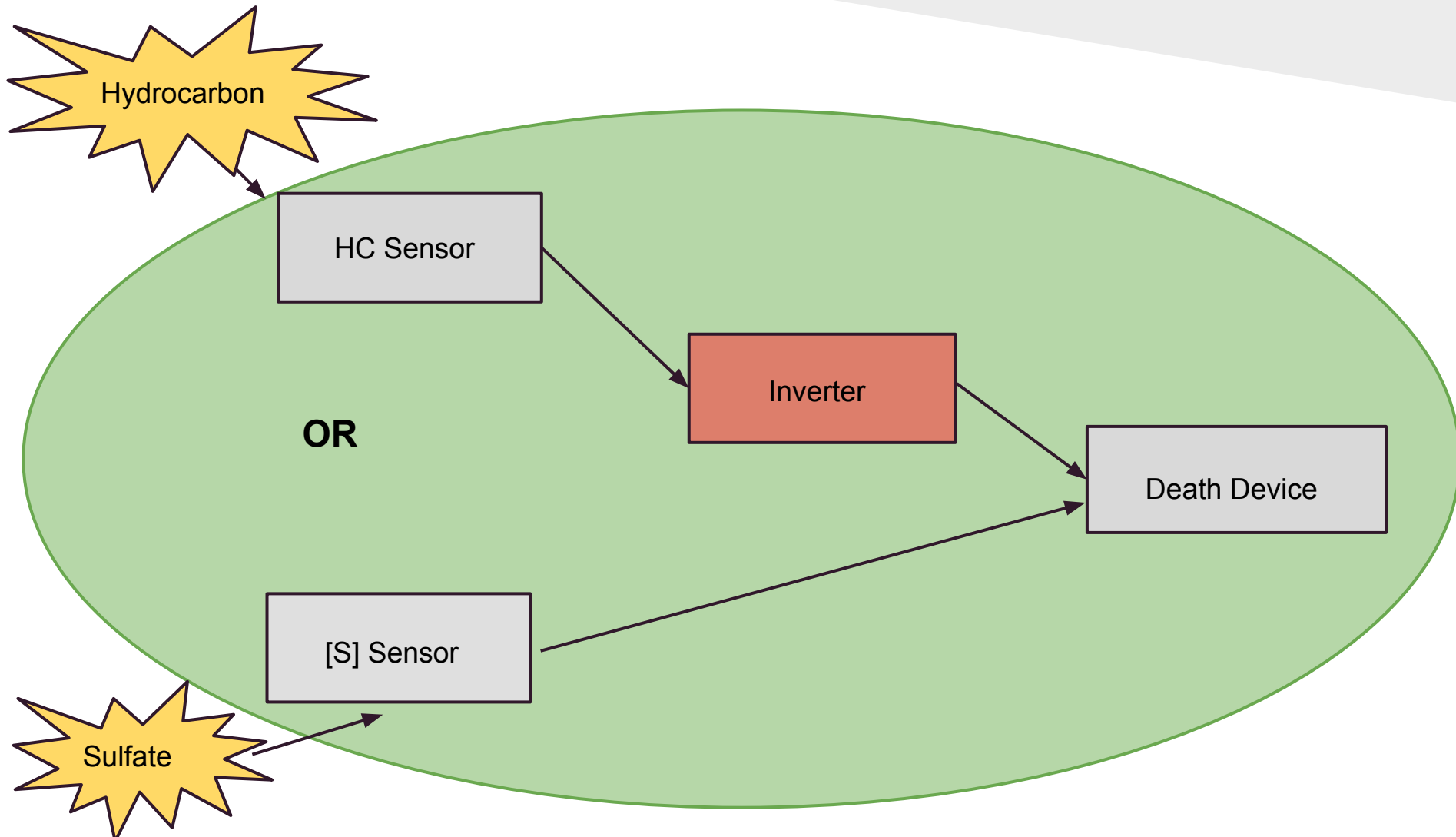
Hydrocarbon Sensor



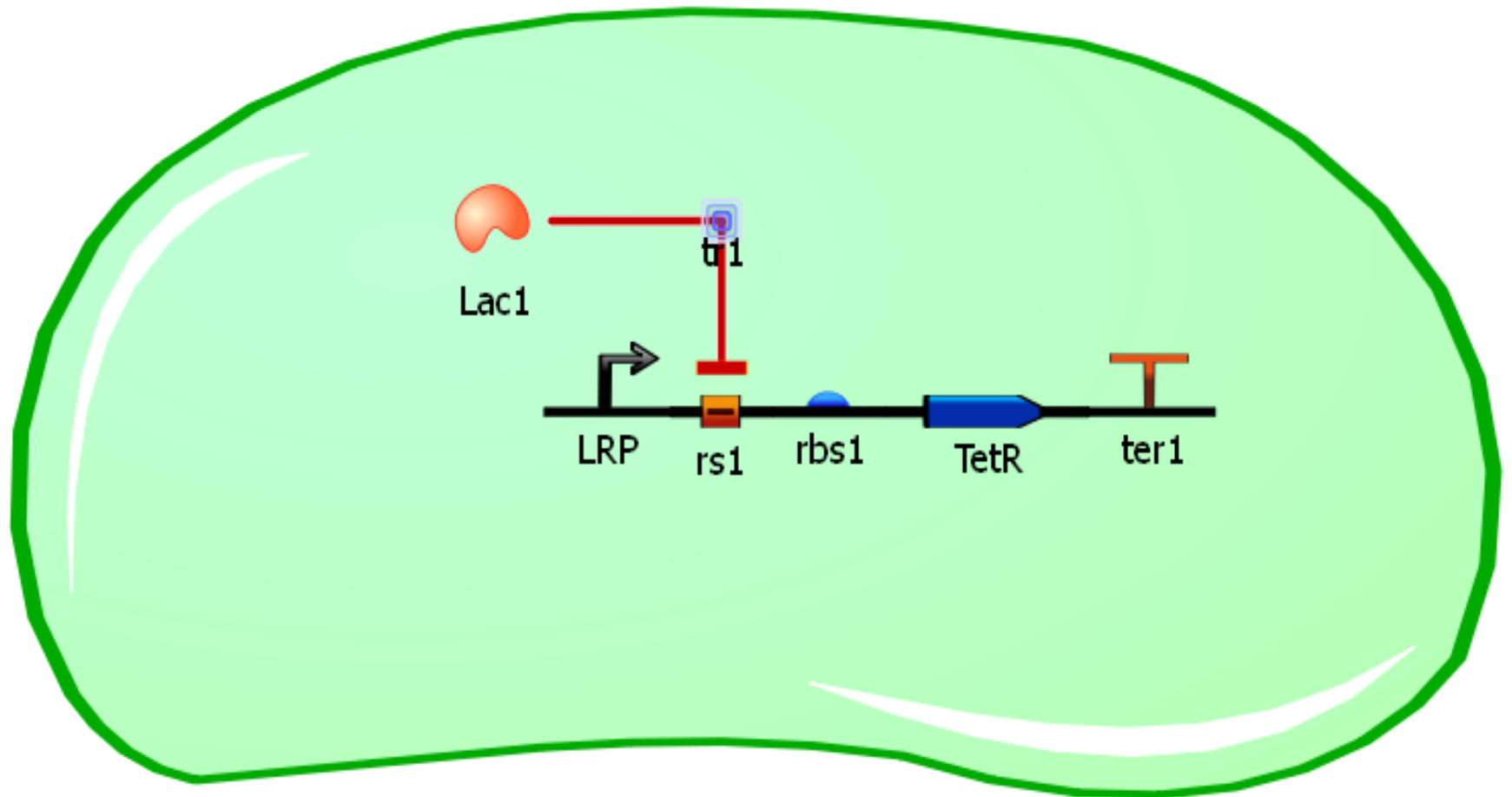
Testing/Debugging: HC Sensor



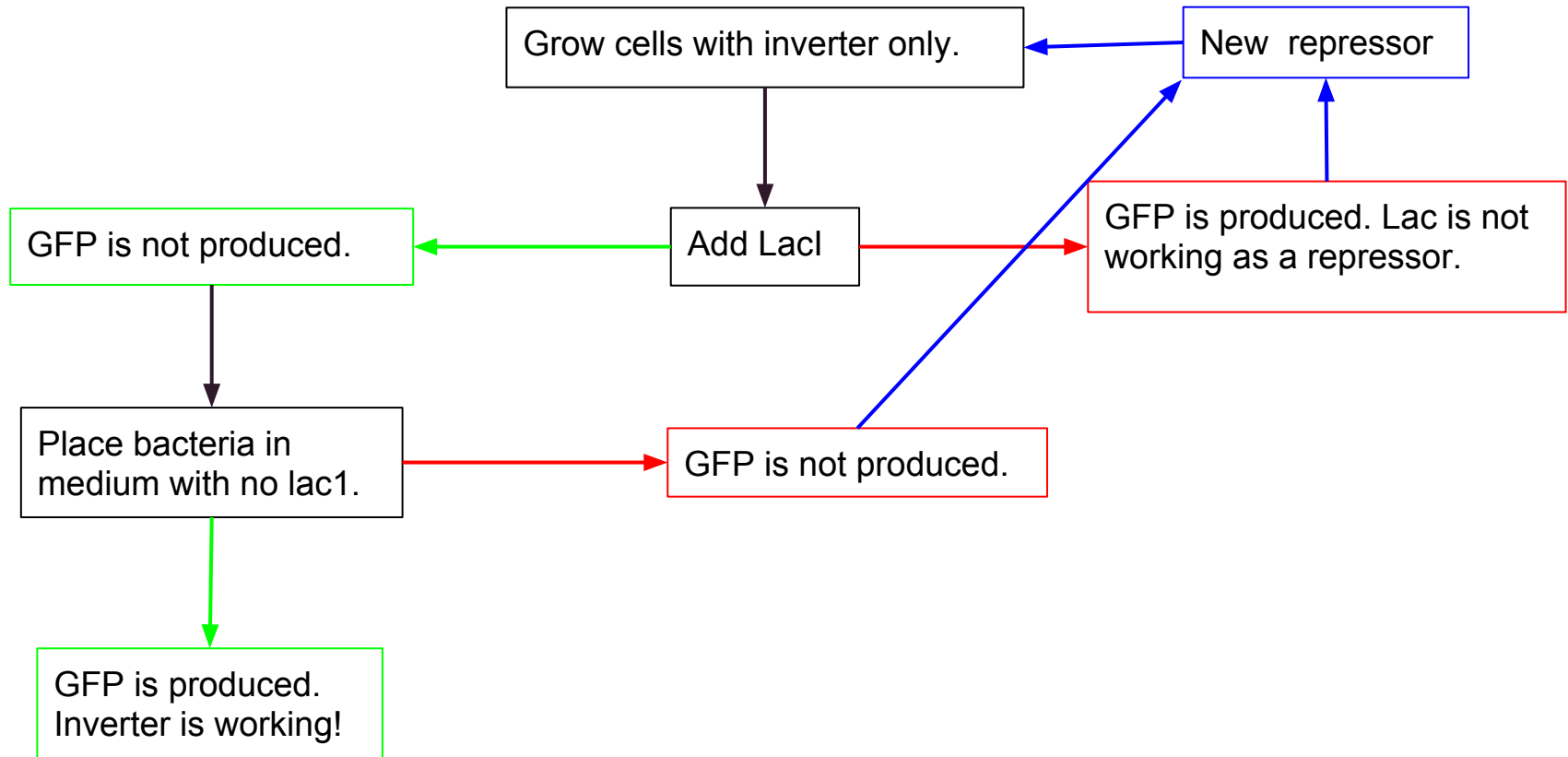
Inverter



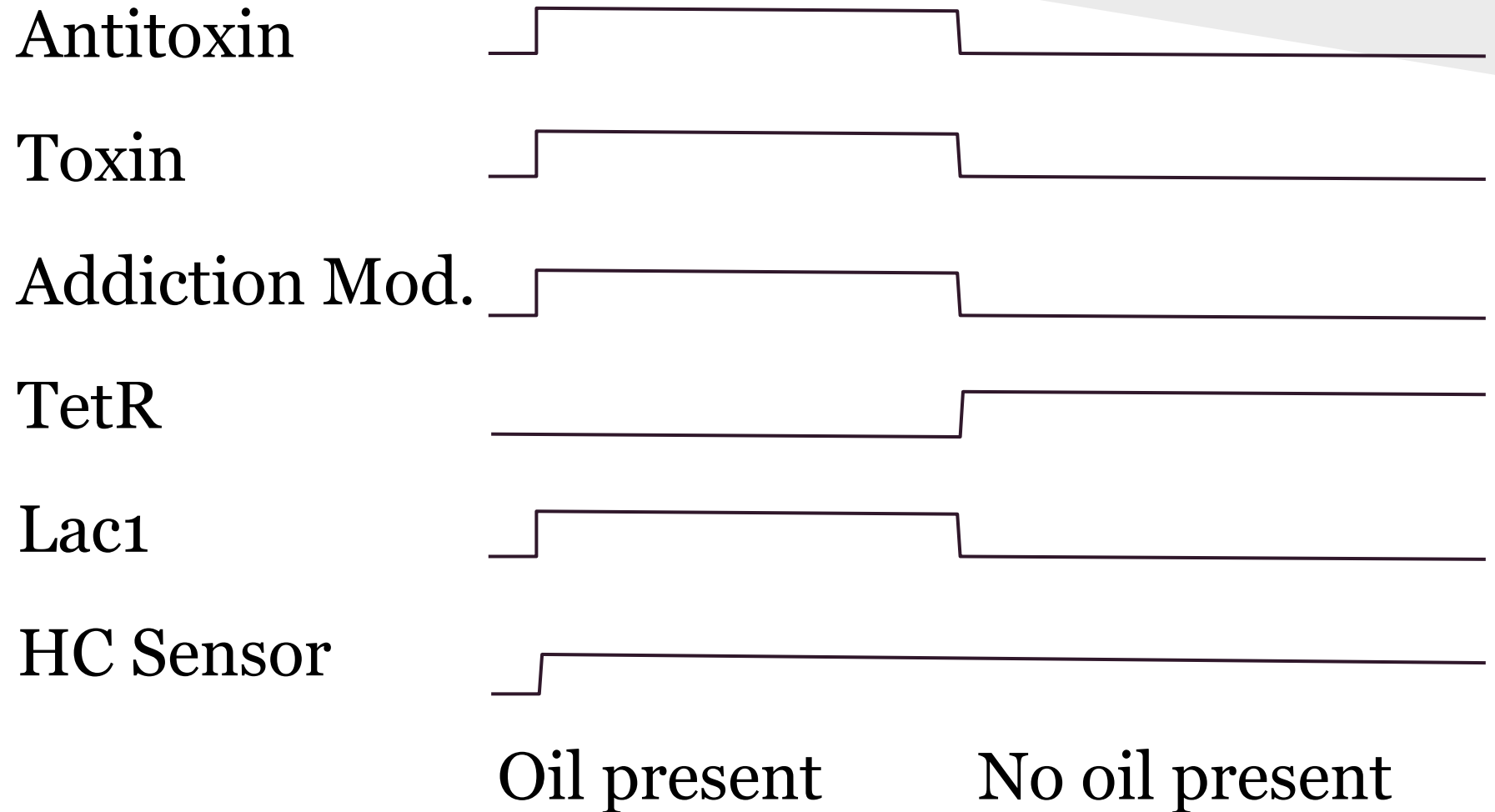
Inverter



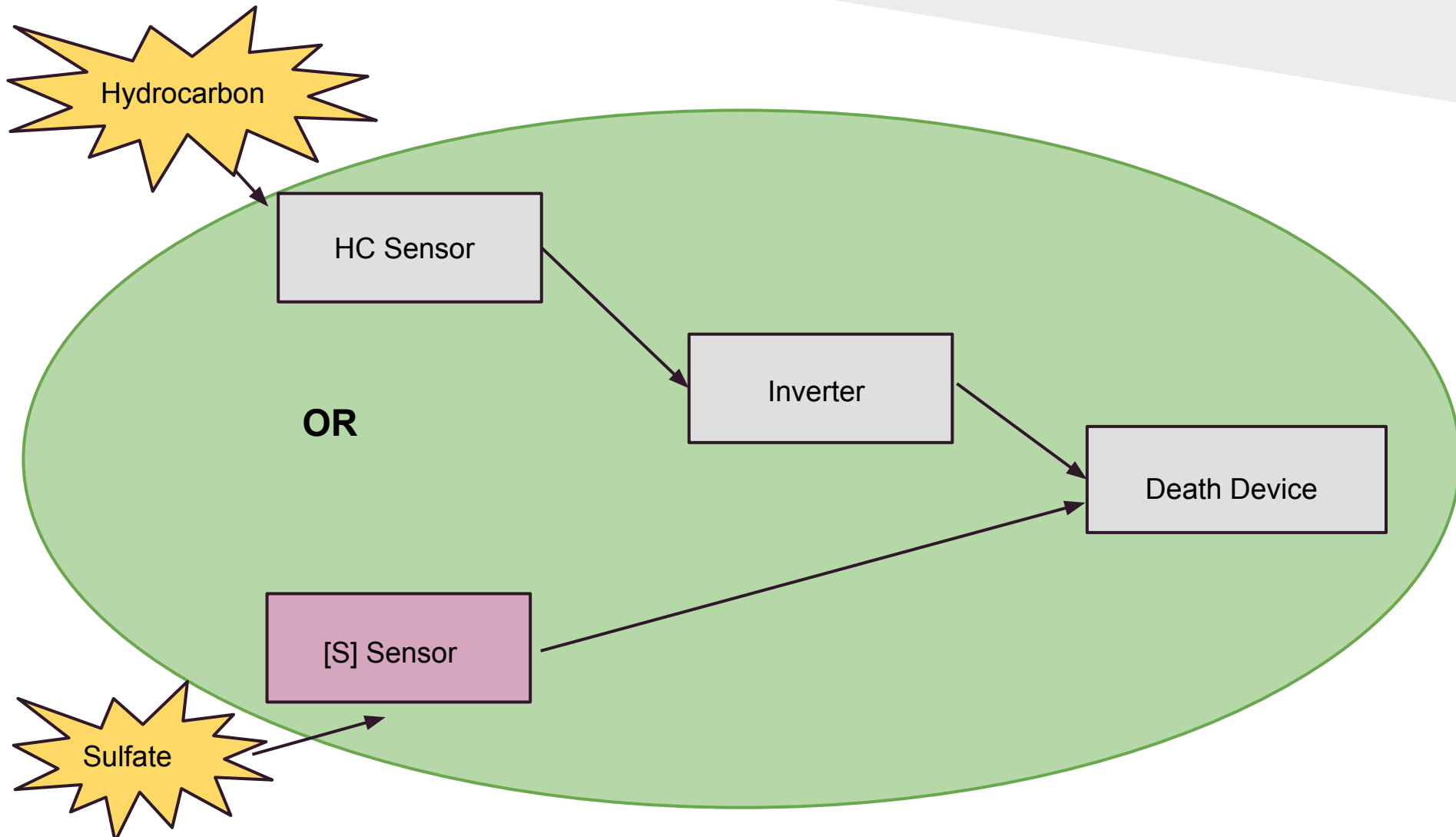
Testing/Debugging: Inverter



Timing Diagram (HC sensor model)



Sulfate Sensor



Sulfate Sensor

Cysteine is as a
result produced in
larger numbers

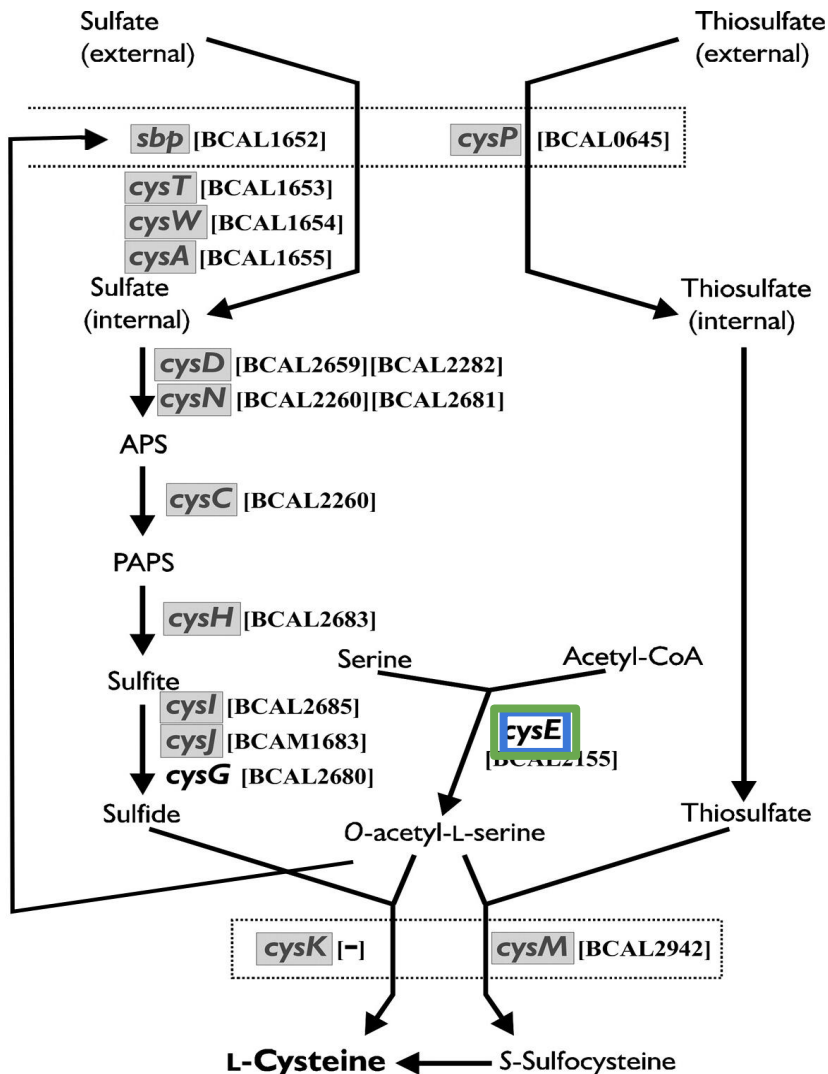
Tet-R, which
triggers death, is
produced.



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

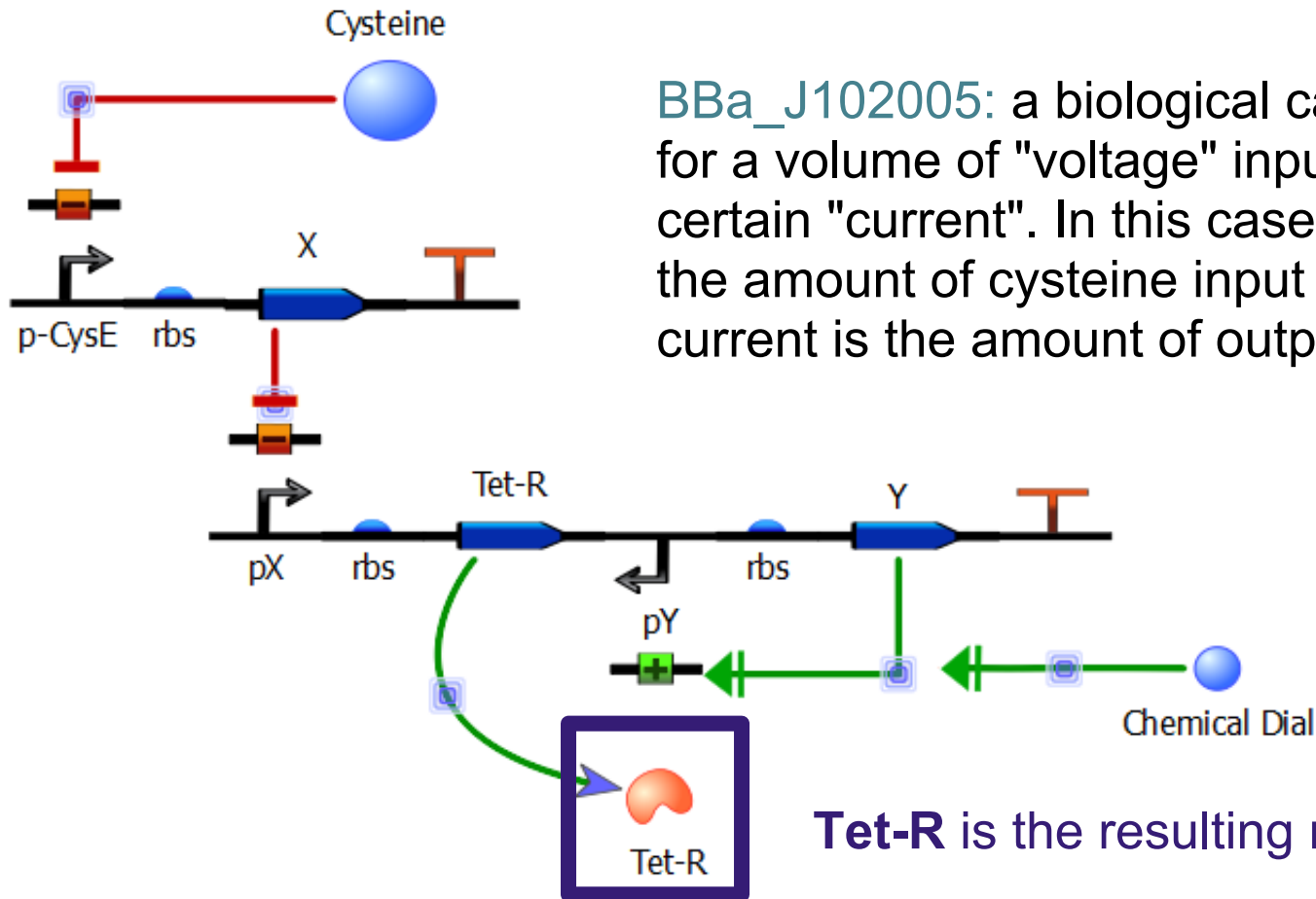
Sulfate and Cysteine



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.

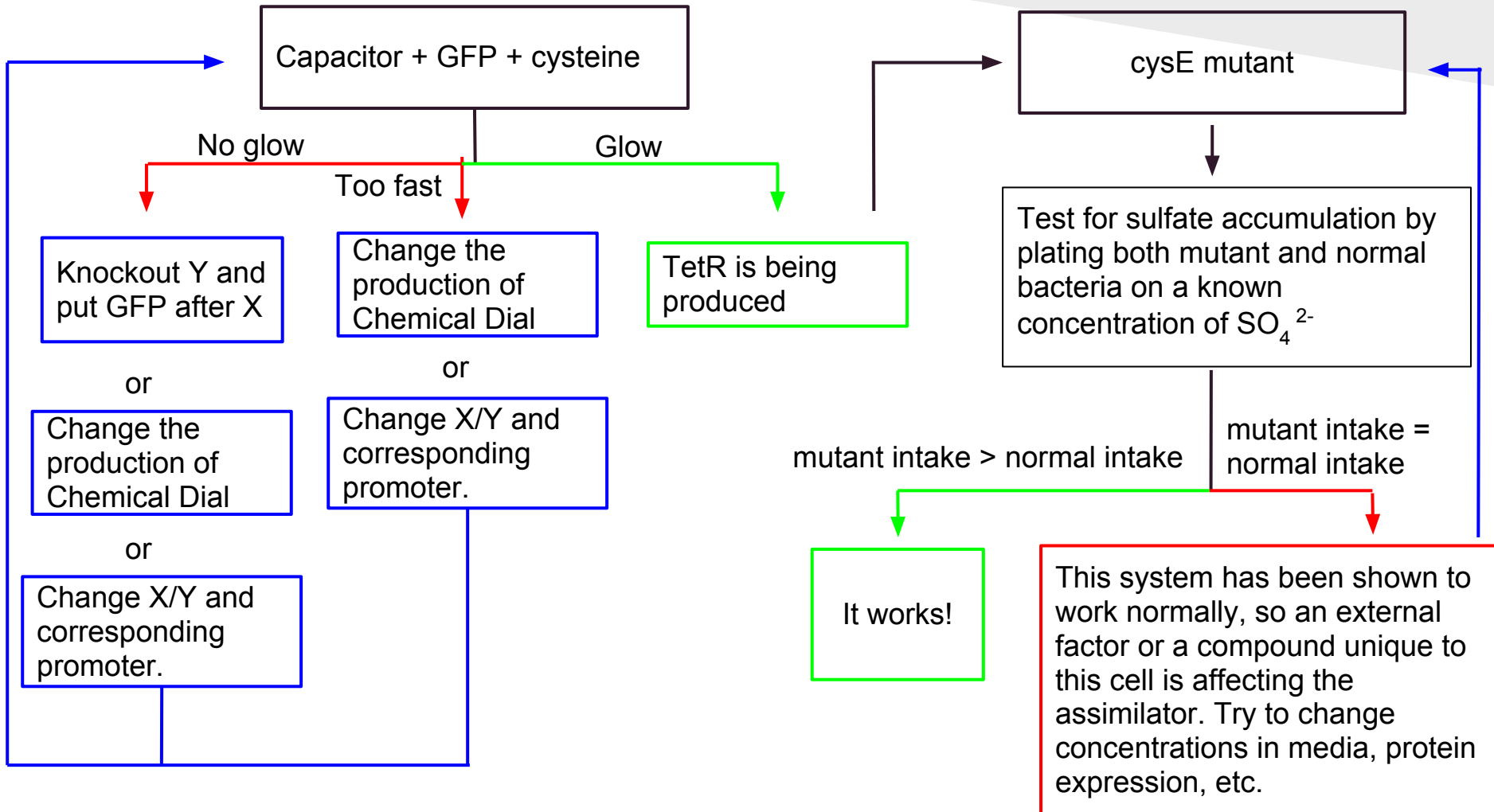
Capacitor To Produce Tet-R



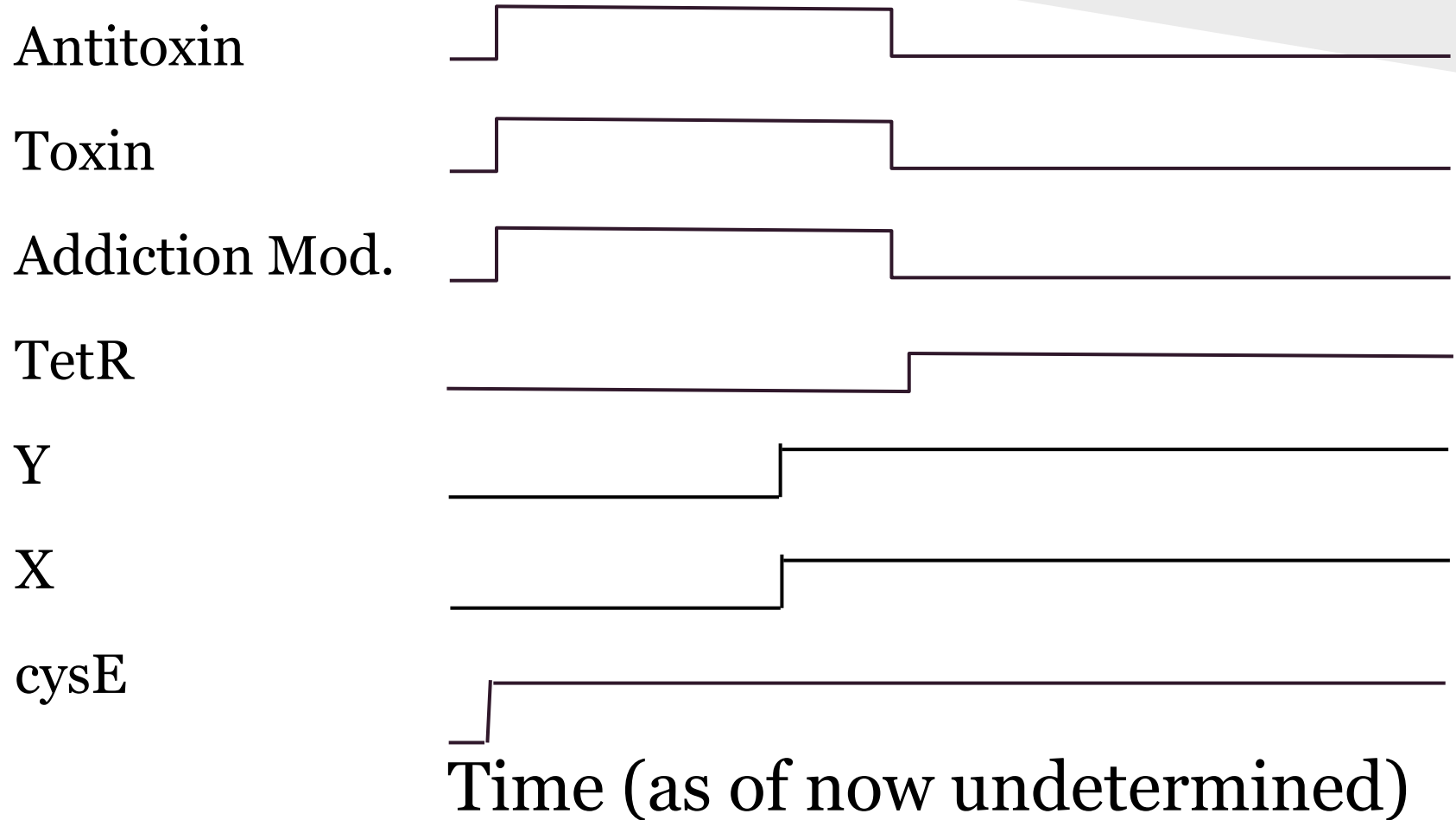
BBa_J102005: a biological capacitor that for a volume of "voltage" input produces a certain "current". In this case, the voltage is the amount of cysteine input and the current is the amount of output.

Tet-R is the resulting molecule.

Testing/Debugging



Timing Diagram (Sulfate sensor model)



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?



References

Death device:

Engelberg-Kulka, Hanna; Gad Glaser (October 1999). "Addiction modules and programmed cell death and antideath in bacterial cultures". *Annual Review of Microbiology* (Annual Reviews) **53**: 43–70. doi:10.1146/annurev.micro.53.1.43. PMID 10547685. Retrieved 2007-06-20.

Conceptualizing “suicidal genetically engineered microorganisms” for bioremediation applications. Gunjan Pandey et. al. <http://www.sciencedirect.com/science/article/pii/S0006291X04028335>

Parts: Registry of Biological Parts

ERHG-05096: LdtR-Rd1D sequence

http://partsregistry.org/wiki/index.php?title=Part:BBa_R0040

http://partsregistry.org/wiki/index.php?title=Part:BBa_R1051

http://partsregistry.org/wiki/index.php/Part:BBa_J61100

http://partsregistry.org/wiki/index.php?title=Part:BBa_B0010

Bioremediation:

Genetically engineered oil-eating microbes for bioremediation: Prospects and regulatory challenges. Obidimma C. Ezezika and Peter A. Singer. <http://www.sciencedirect.com/science/article/pii/S0160791X10000771>

References

HC Sensor

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<http://www.ncbi.nlm.nih.gov/pubmed/10971768>

<http://jb.asm.org/content/194/24/6972.full>

<http://chemistry.berea.edu/~biochemistry/2011/it/>

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC107560/>

<http://www.nature.com/nbt/journal/v24/n8/abs/nbt1232.html>

<http://www.nature.com/srep/2012/120423/srep00377/full/srep00377.html>

http://partsregistry.org/Part:BBa_K398014

References

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http://partsregistry.org/wiki/index.php/Part:BBa_K731010

http://partsregistry.org/Part:BBa_K731030

http://partsregistry.org/wiki/index.php/Part:BBa_J102005

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC94713/>

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC94713/figure/F6/>