HOT METAL SWITCH

2016 iGEM Jamboree

Hynes Convention Center, Boston, MA

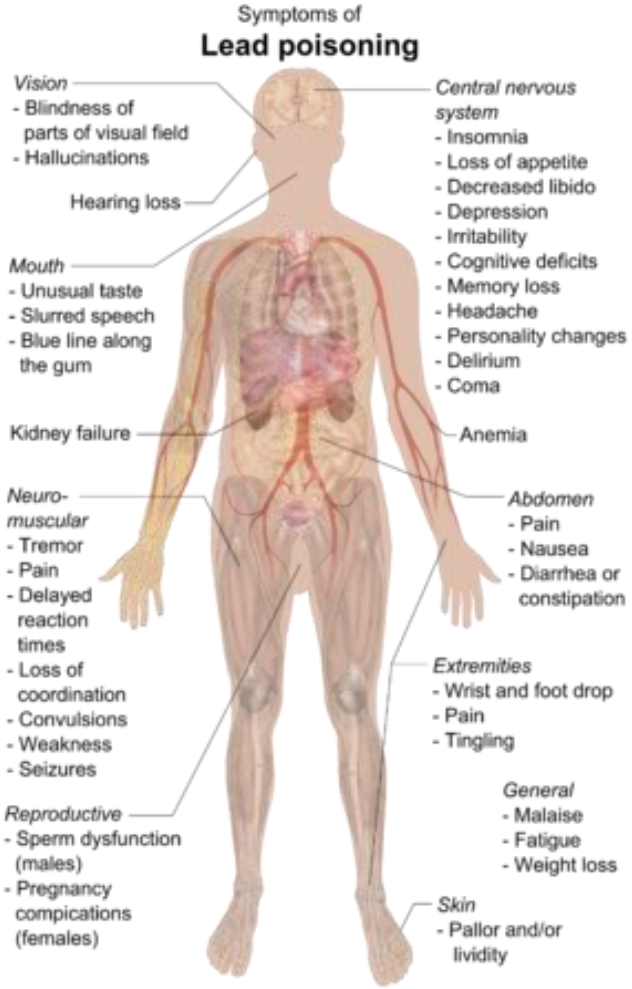
October 29, 2016


HOT METAL SWITCH

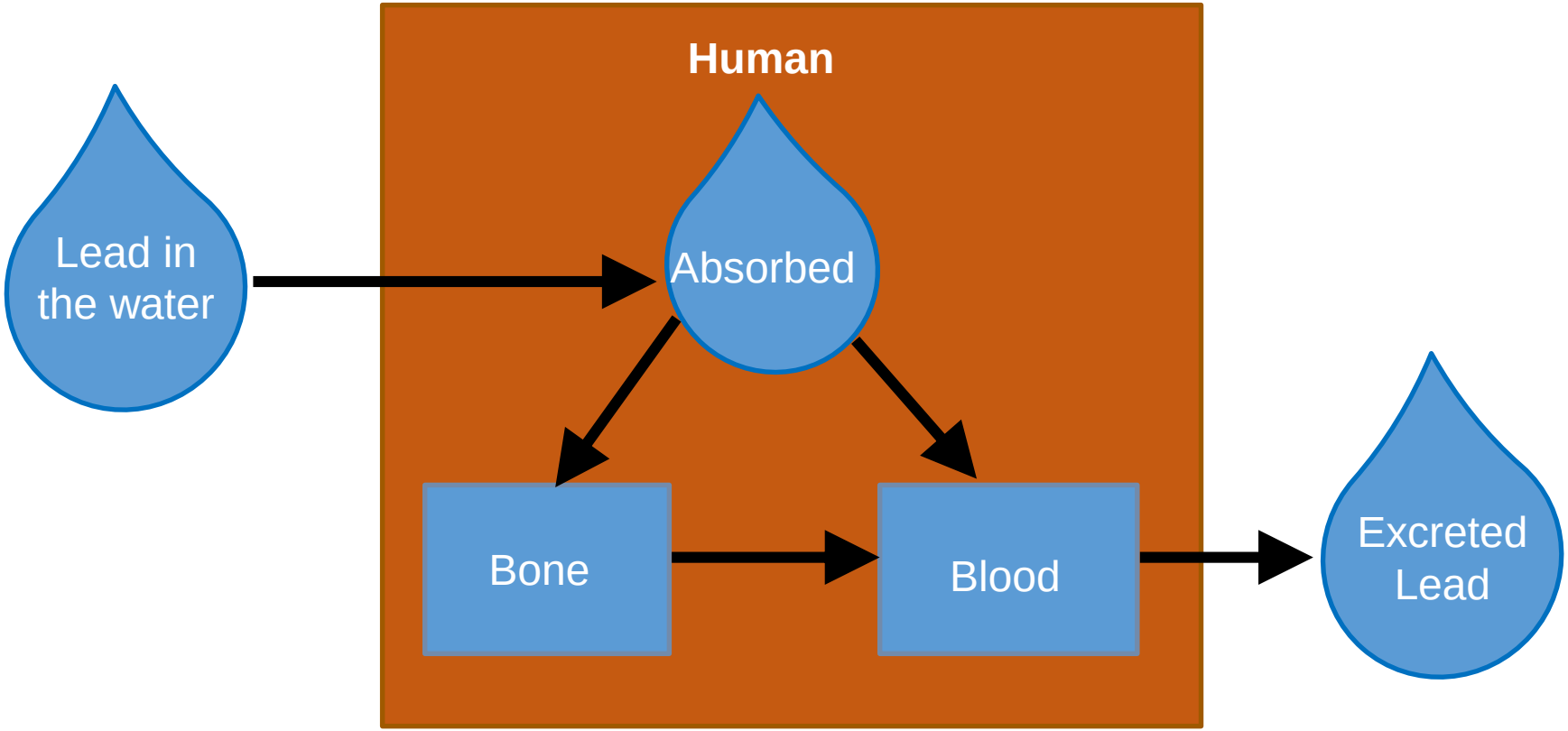

Flint, MI
Pittsburgh, PA
Washington, D.C.

Global Issue

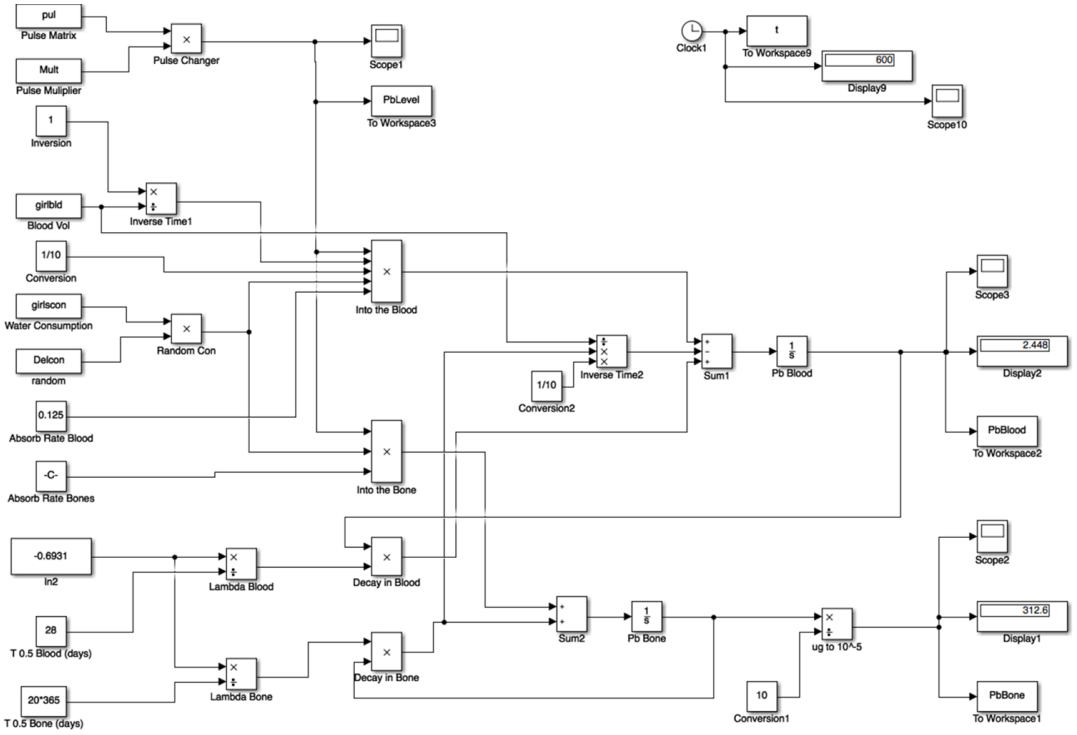
- WHO Lead Poisoning Prevention Week
  - October 23-29th
  - Toxic
    - Children: Behavioral, Neurological
    - Adults: Liver and kidney damage



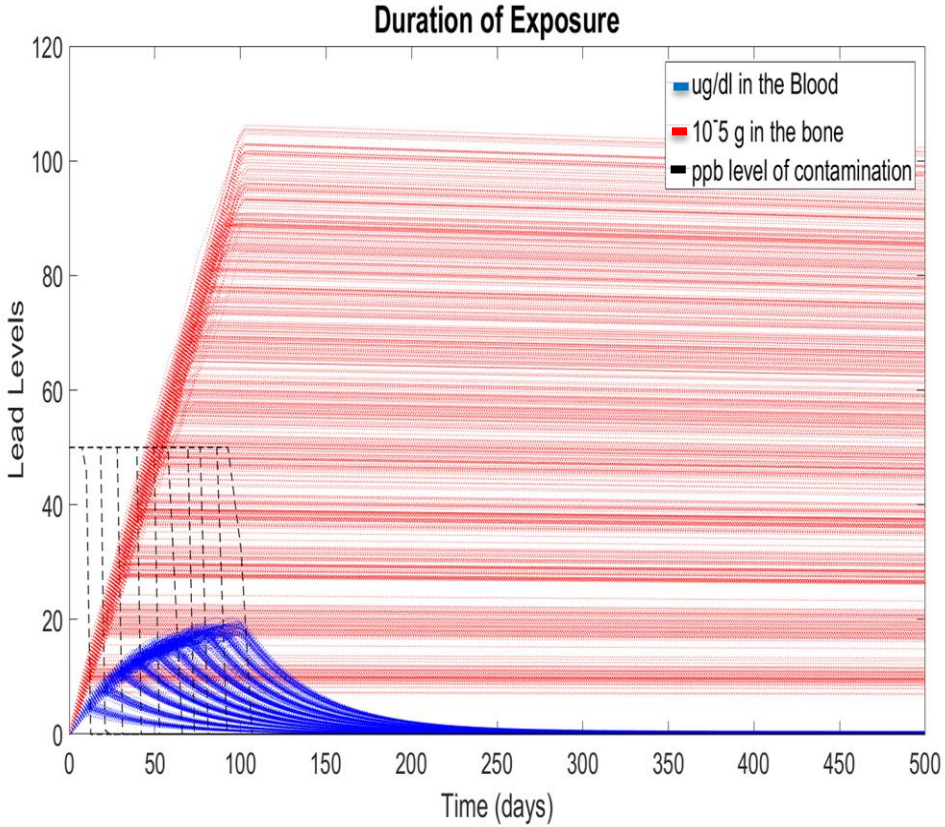
Model

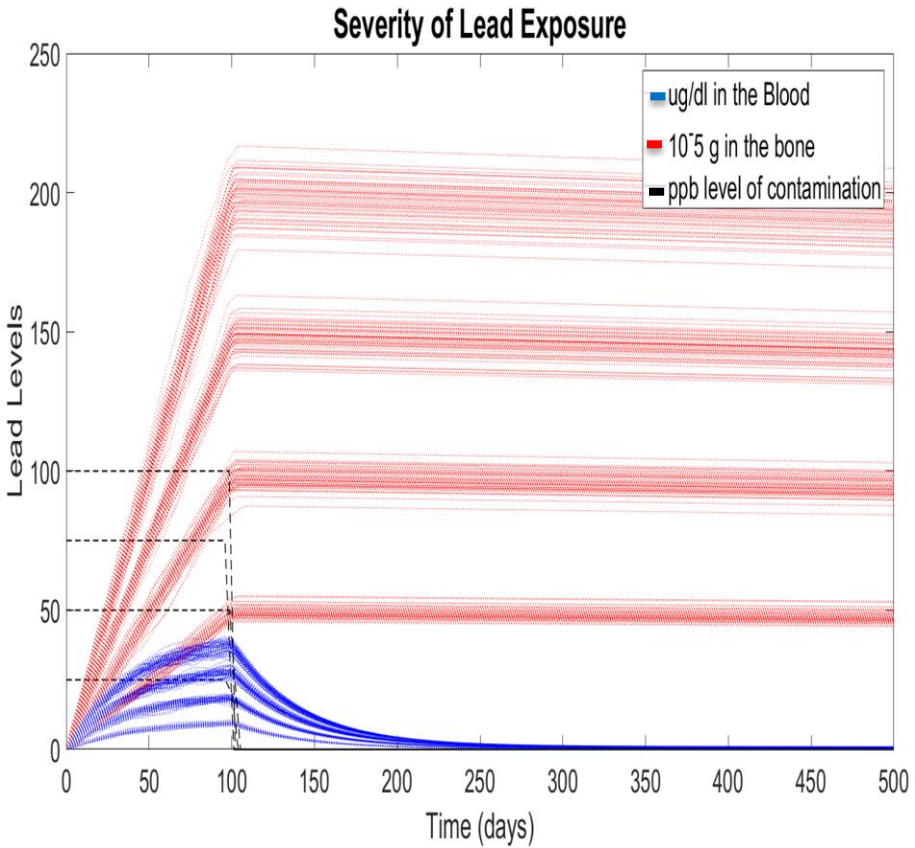


Simulink

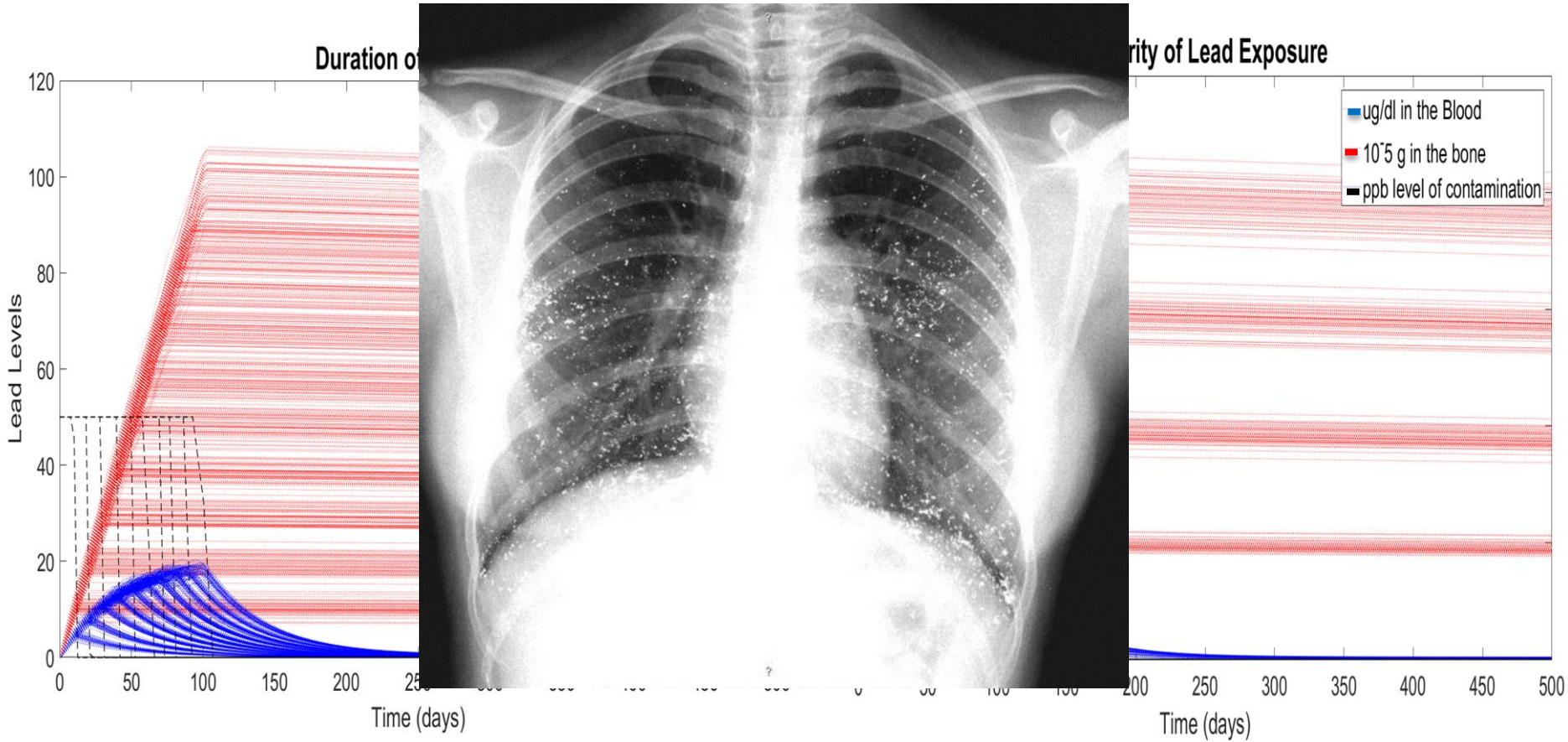

pul
Pulse Matrix
Mult
Pulse Muliplier
Pulse Changer
Scope1
PbLevel
To Workspace3
1
Inversion
girlbld
Blood Vol
Inverse Time1
1/10
Conversion
girlscon
Water Consumption
Random Con
Delcon
random
0.125
Absorb Rate Blood
-C-
Absorb Rate Bones
Into the Blood
Into the Bone
Clock1
t
To Workspace9
600
Display9
Scope10
Inverse Time2
1/10
Conversion2
Sum1
Pb Blood
Scope3
2.448
Display2
PbBlood
To Workspace2
-0.6931
in2
Lambda Blood
Decay in Blood
28
T 0.5 Blood (days)
20*365
T 0.5 Bone (days)
Lambda Bone
Decay in Bone
Sum2
Pb Bone
ug to 10^-5
10
Conversion1
Scope2
312.6
Display1
PbBone
To Workspace1

Model Results: Retention of Lead in Bone and Blood

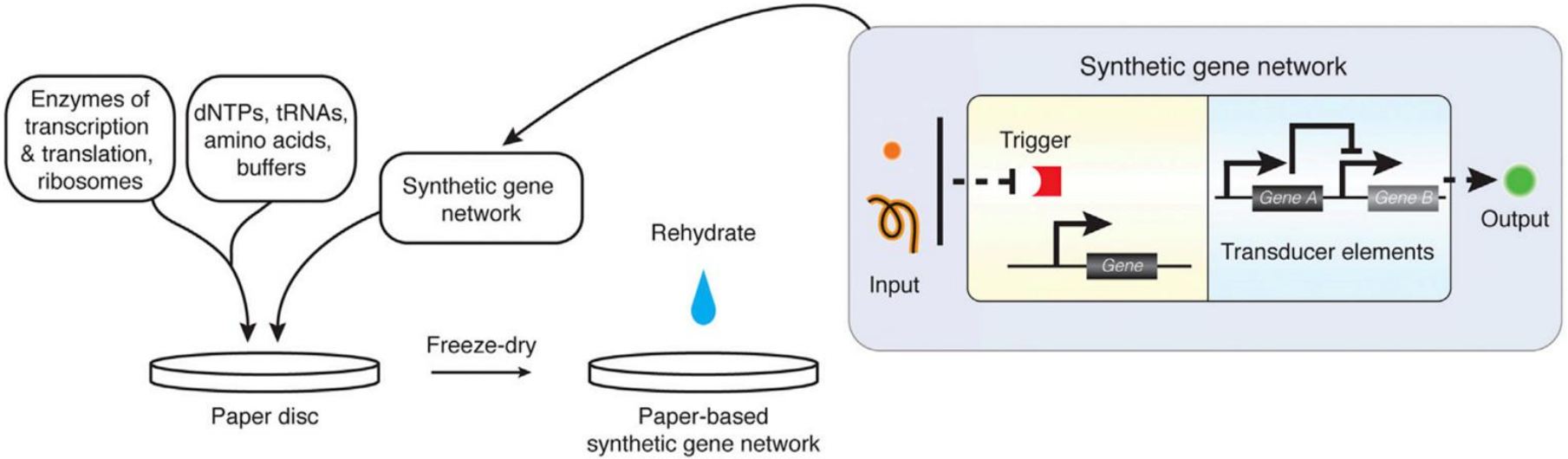




Model Results: Retention of Lead in Bone and Blood

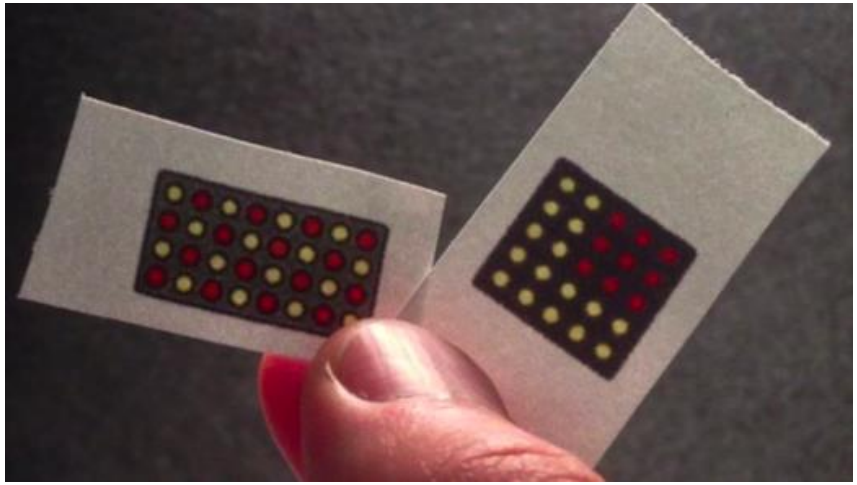


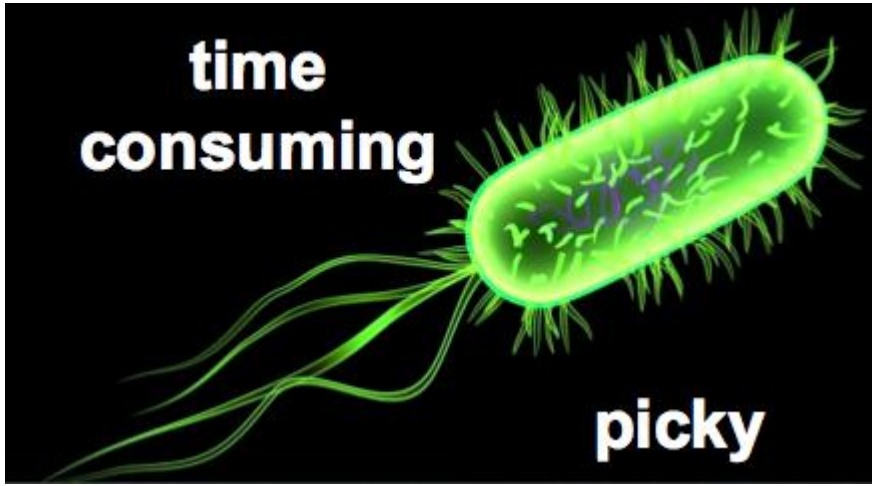
Background



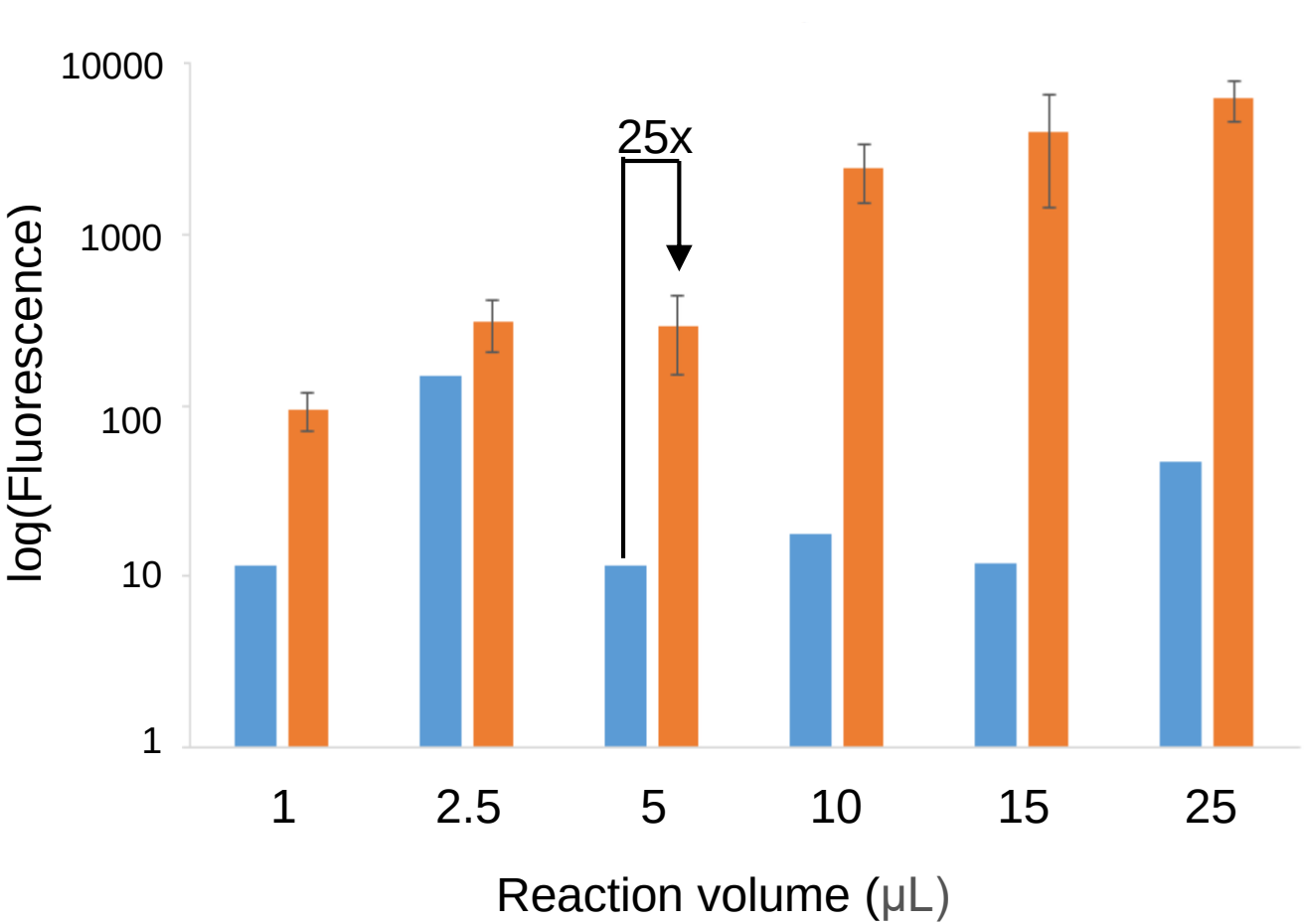
Pardee, Green, Ferrante, Cameron, DaleyKeyser, Yin, Collins. *Cell*.2014.

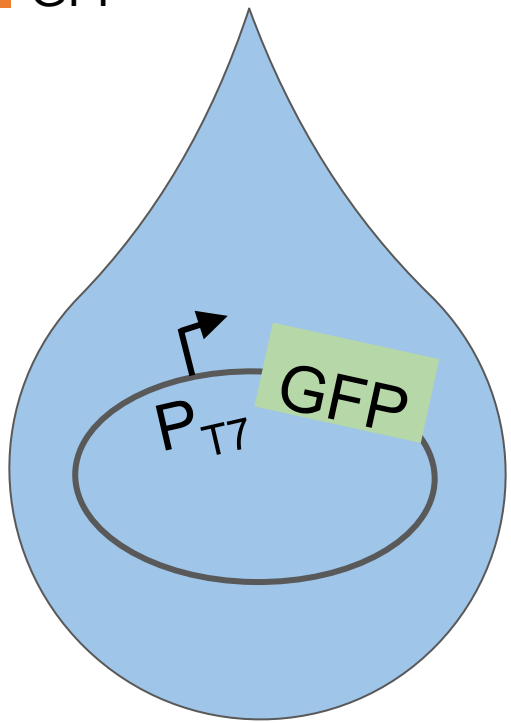
Why?



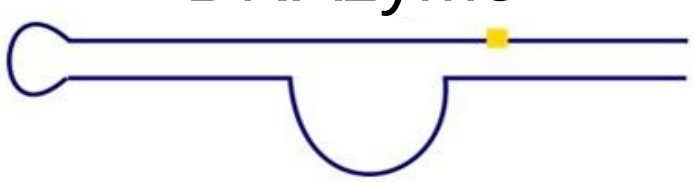


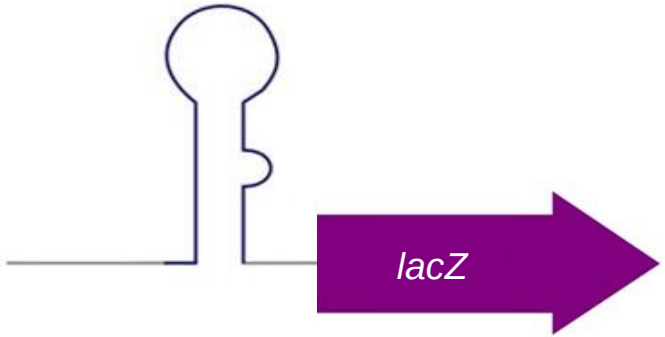
Reaction Volume Minimization





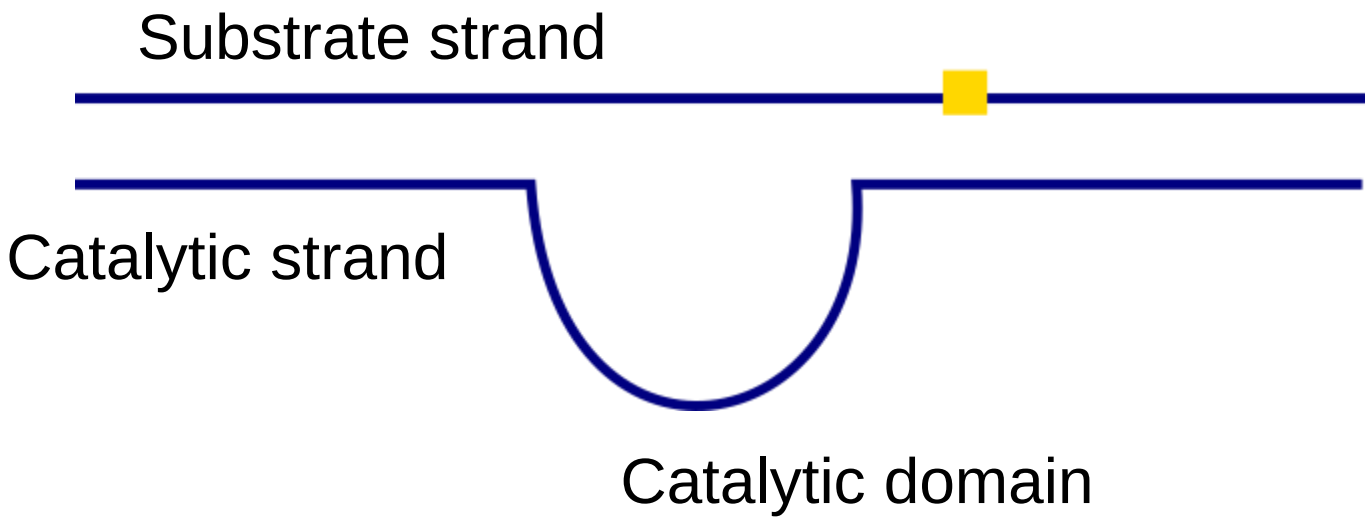
DNAzyme



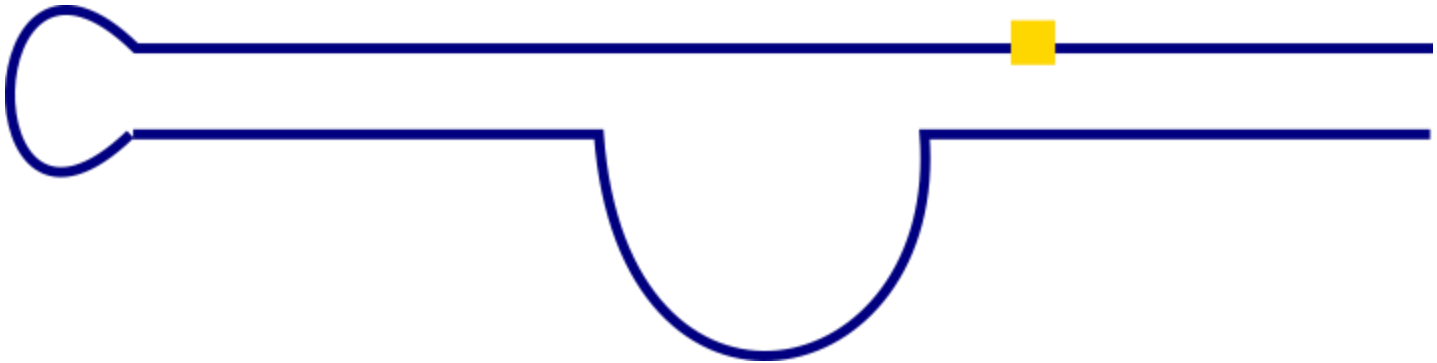


Reporter

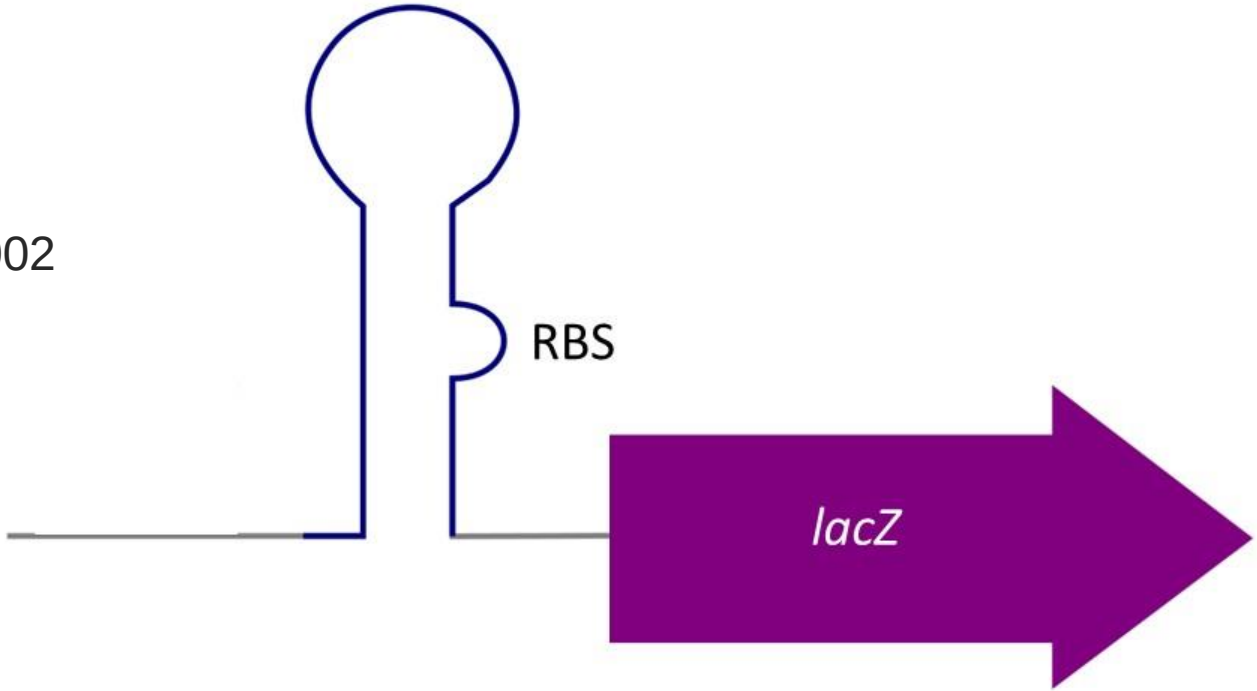
Toehold Switch



Lan, Furuya, & Lu. *Chem. Commun.* 2010.



BBa_K2084002

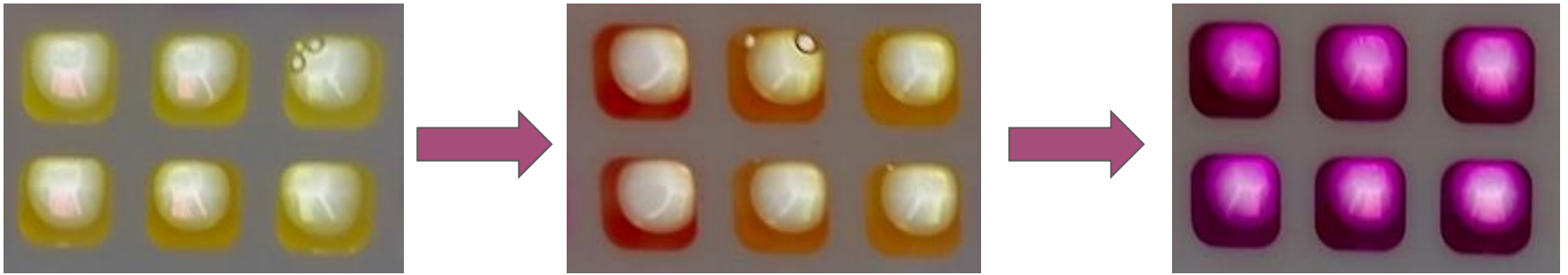


Pardee, Green, Ferrante, Cameron, DaleyKeyser, Yin, Collins. *Cell*. 2014.

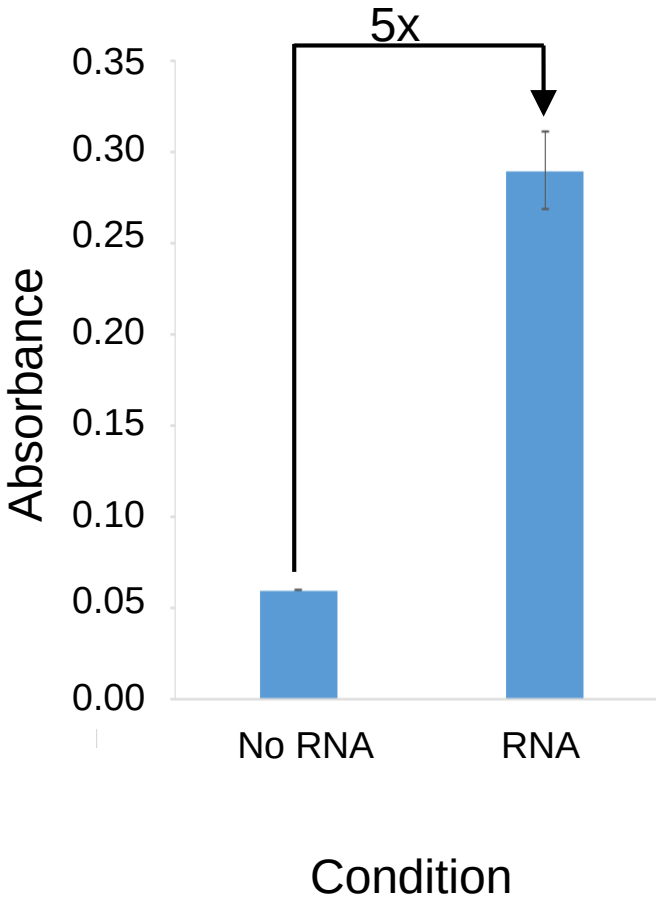
Reporter Substrate

CPRG (Chlorophenol Red-β-D-galactopyranoside)

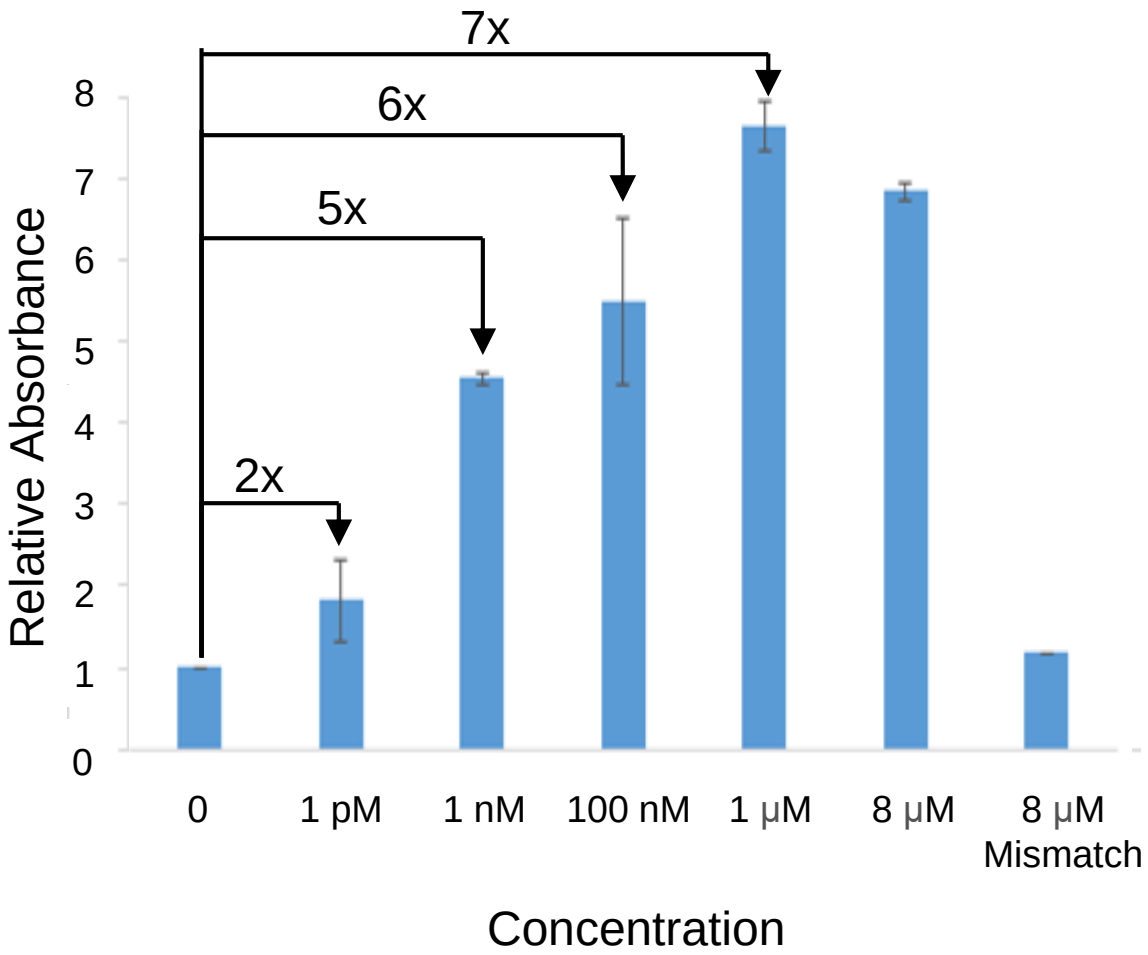
Undergoes color change when lacZ is expressed



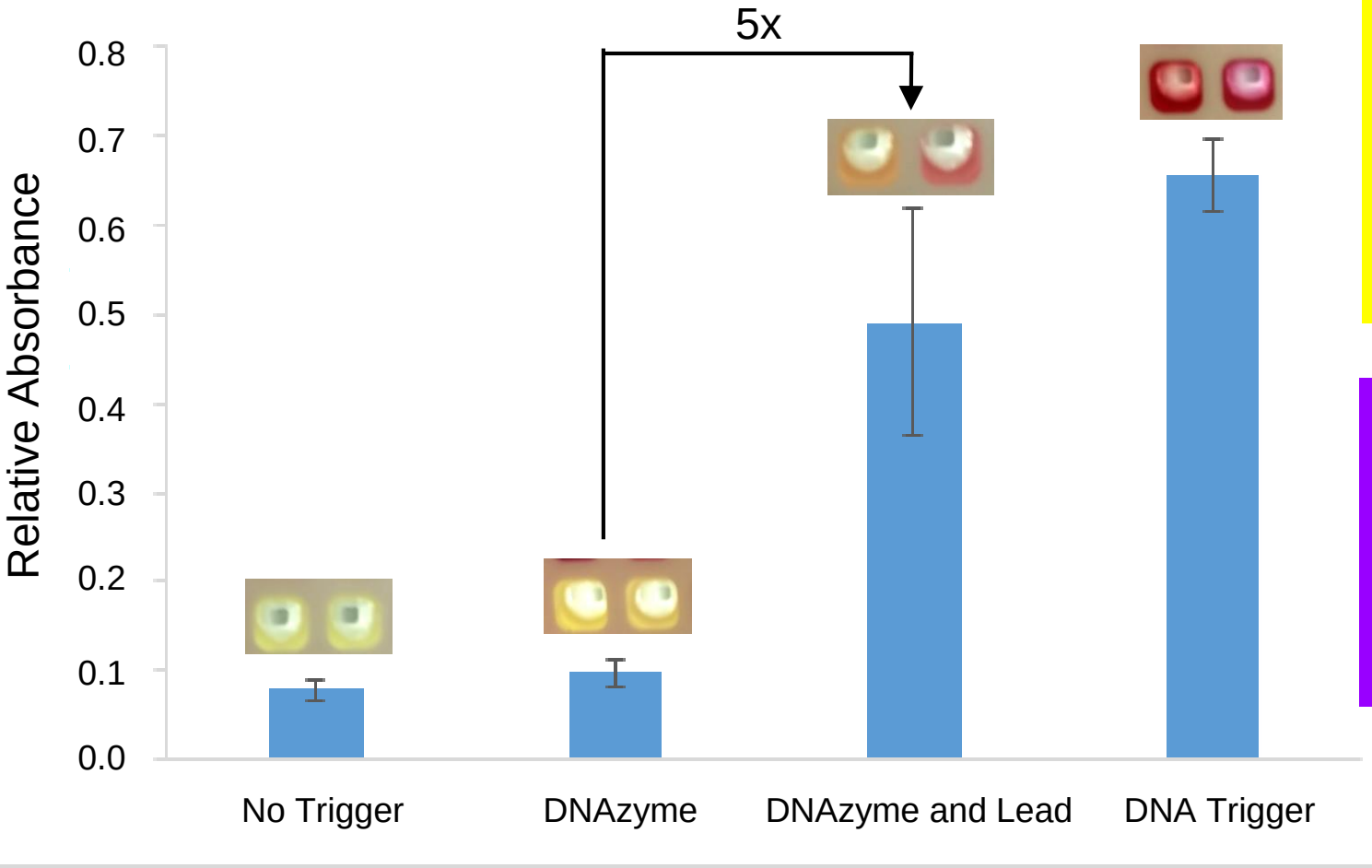
Activation with RNA

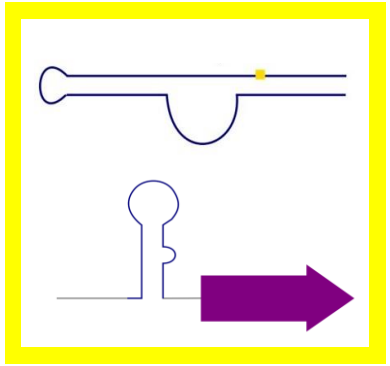


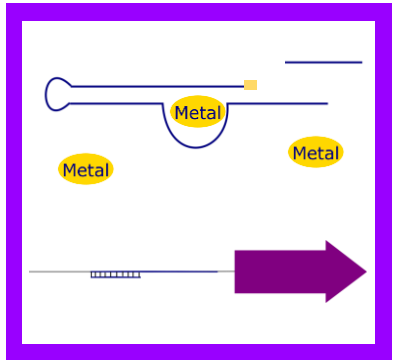
Activation with DNA



lacZ Expression with 2 μM Lead



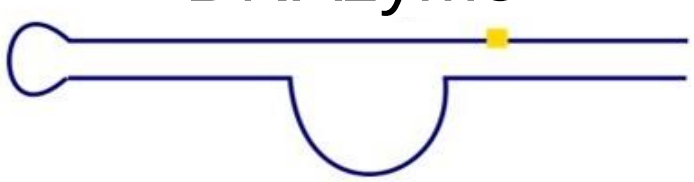


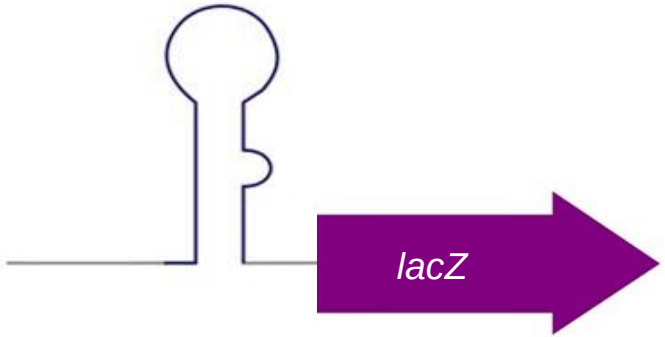


Comparison of Lead Concentrations

Source	Concentration (ppb)	Concentration (nM)
EPA MCL	15	72.4
Hot Metal Switch	429	2,000
Flint, MI	13,200	63,712

DNAzyme





Reporter

Toehold Switch

Current Project: Forward Feedback Amplification

DNAzyme

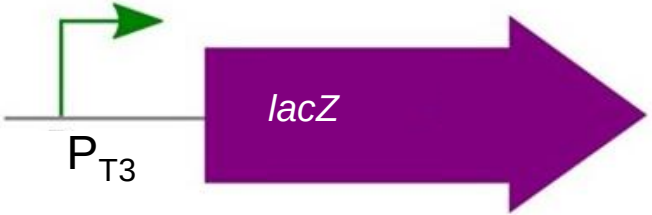
Toehold Switch

BBa_K2084007

Amplifier

BBa_K2084004

BBa_K2084001



Reporter BBa_K2084009

Flexibility

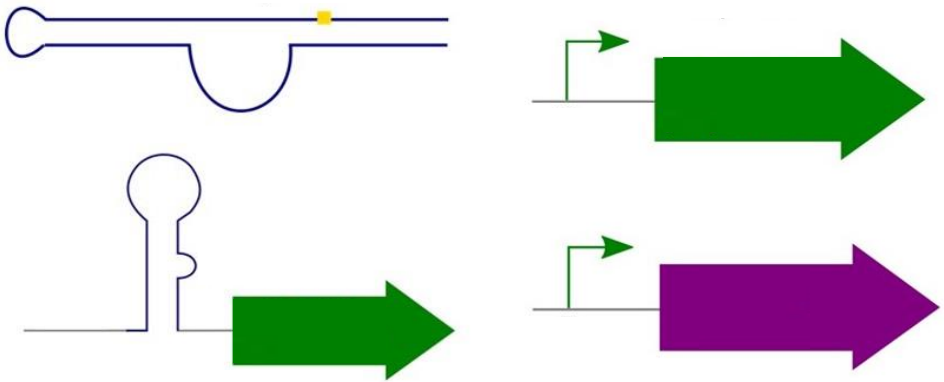
Thallium

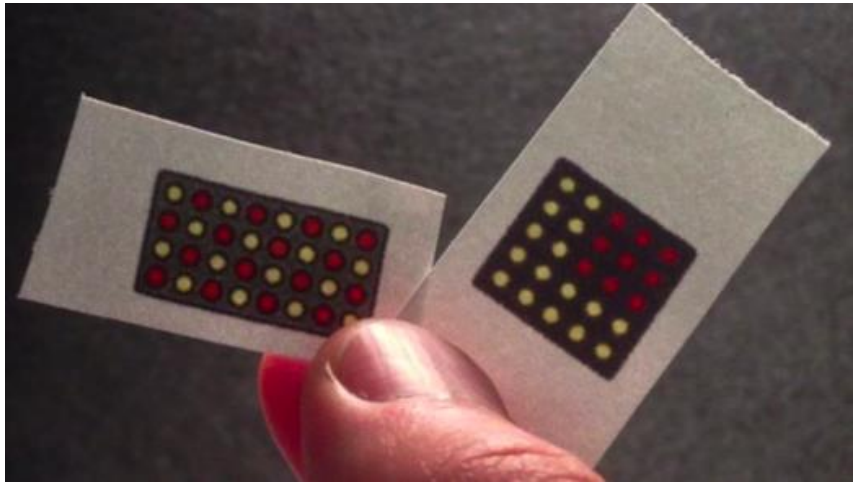
Lead

Mercury

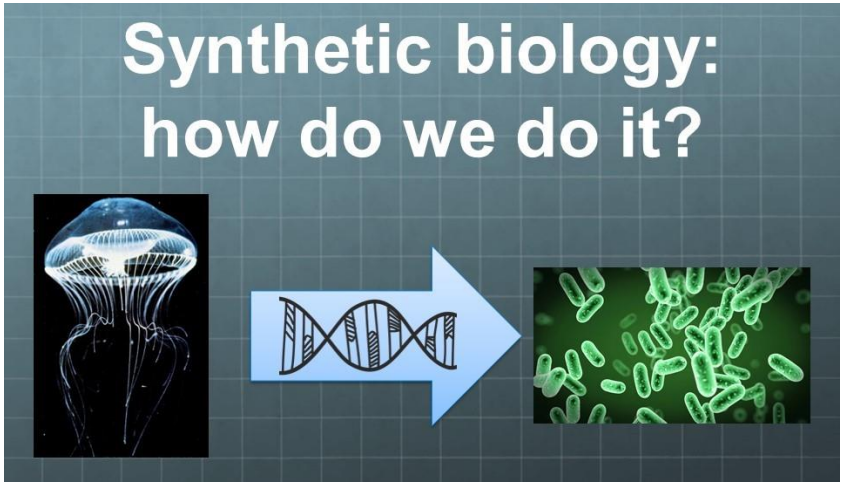
Arsenic

Future work





Human Practices



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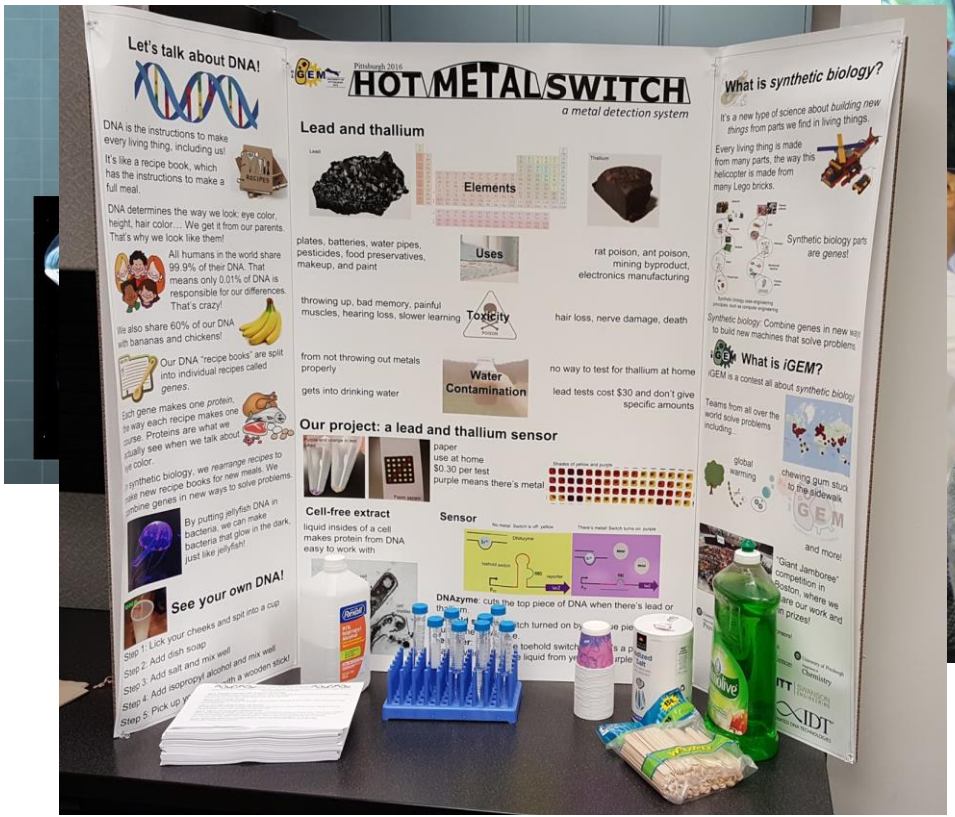

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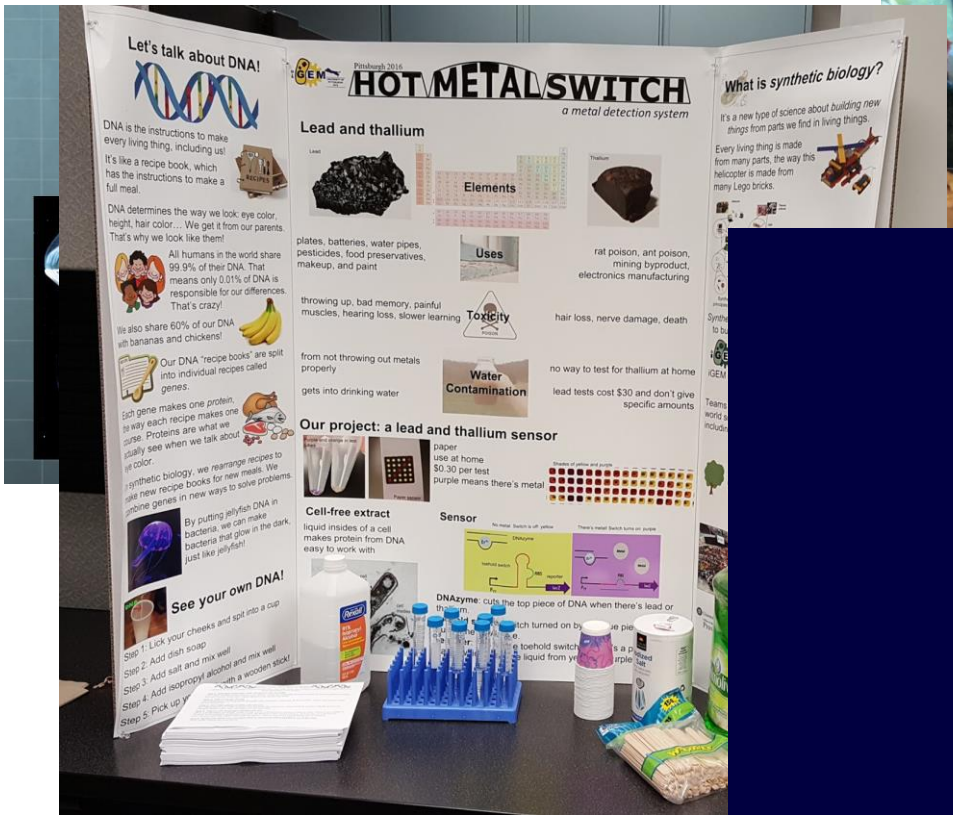
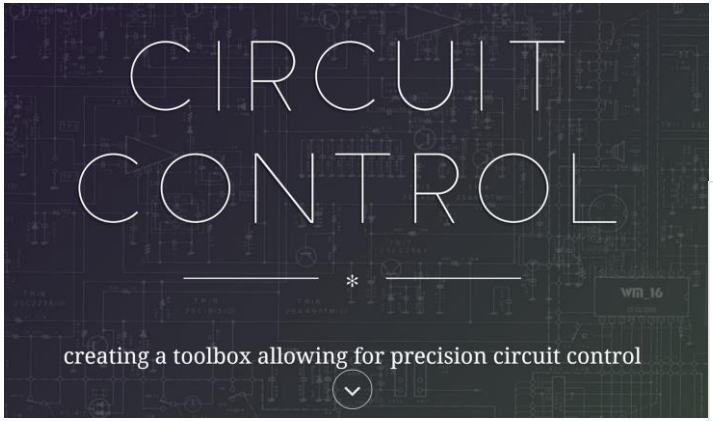
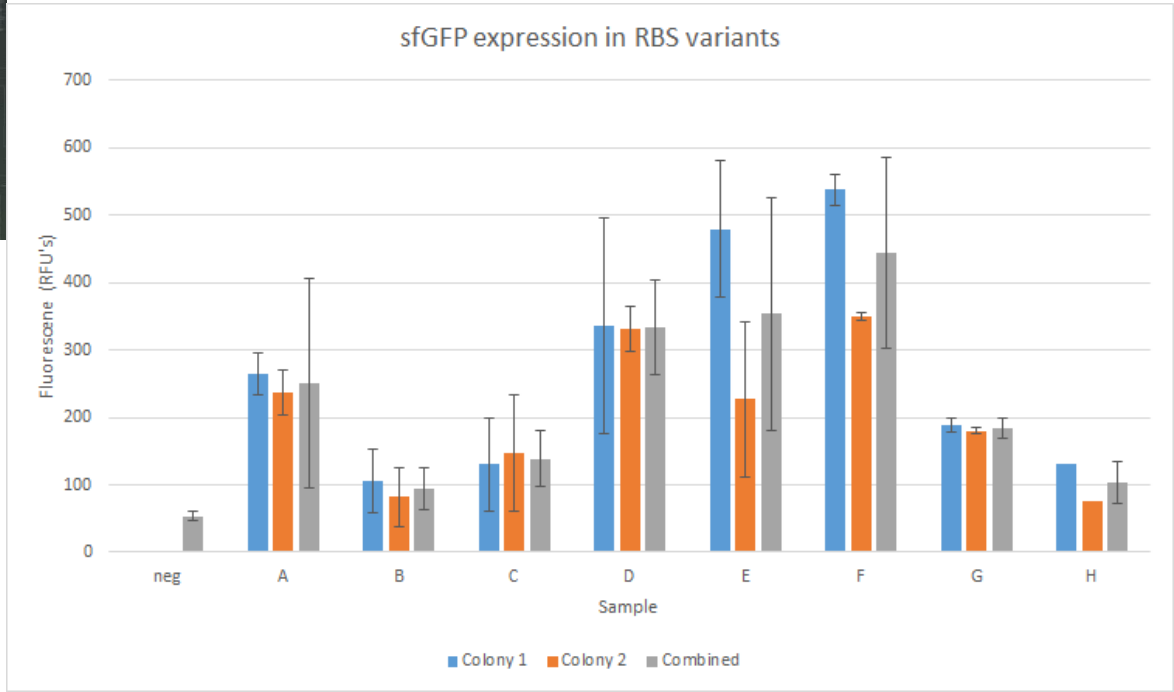


Lead

Pitt iGEM 2016

Collaborations





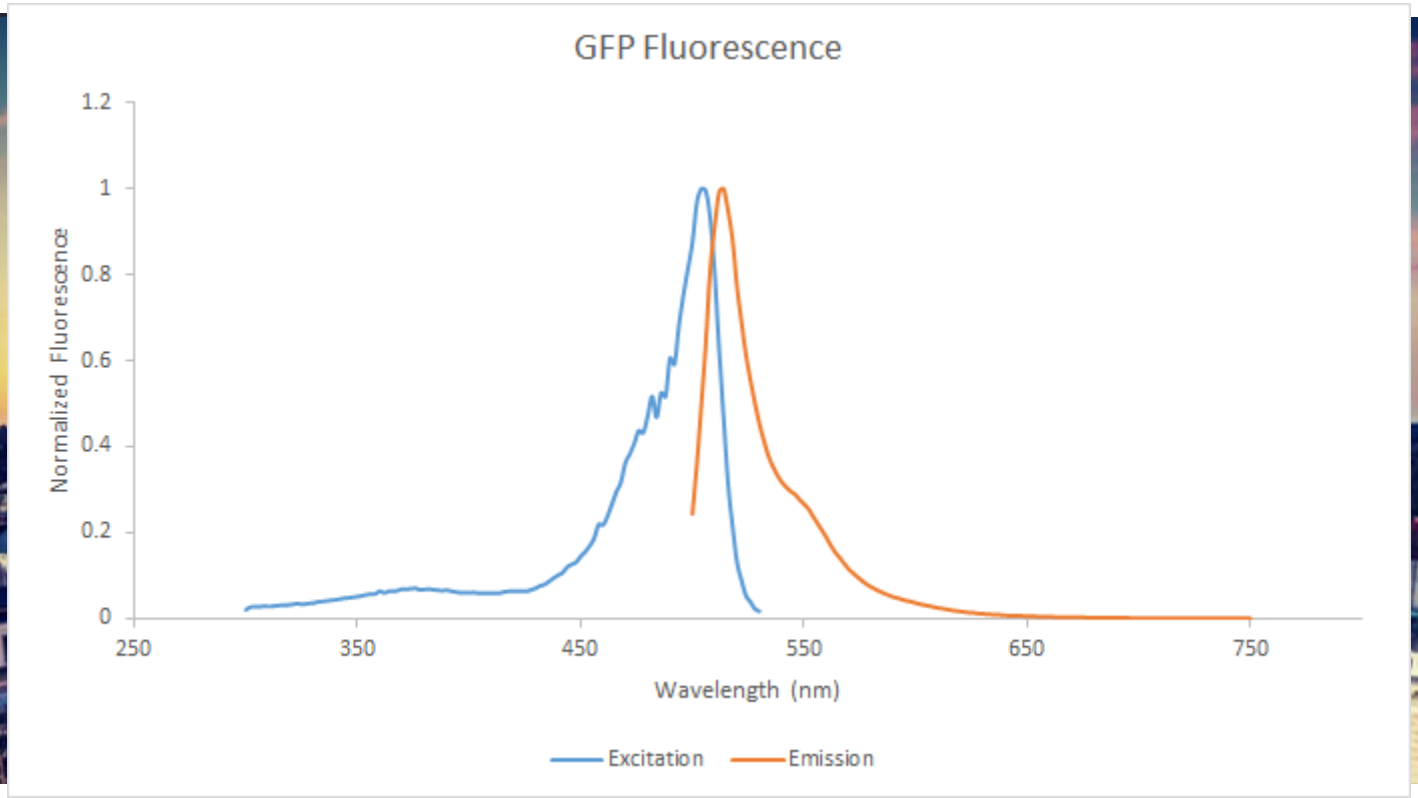
Collaborations

Sample

Colony 1 Colony 2 Combined

Collaborations

Collaborations



Conclusion

- Modeled the consequences of lead exposure
- Demonstrated DNA trigger of toehold switch
- Developed promising circuit with regulatory RBS
- Taught community about synthetic biology

Thank You!

Team

Aife Ni Chochlain

Sponsors

University of Pittsburgh Office of the Provost

PITT | SWANSON ENGINEERING

PITT | SWANSON ENGINEERING BIOENGINEERING

University of Pittsburgh Department of Biological Sciences

University of Pittsburgh Department of Chemistry

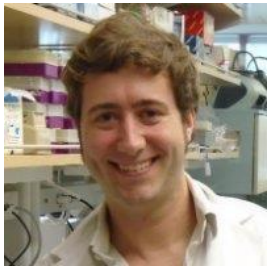
NEW ENGLAND BioLabs Inc.

experiment

IDT INTEGRATED DNA TECHNOLOGIES

Advisors and Mentors

Dr. Alexander Deiters



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