iGEM 2016: Team Pittsburgh

Week 16 Lab Notebook

Tuesday, September 6

Checking G Switch (Maya)

Switches Used: G2 (61.9 ng/uL) , G3 (42.9 ng/uL), G4 (84.1 ng/uL)

Master mix of (4.5 uL each):

6 uL Solution A

4.5 uL Solution B

0.75 uL RNAse Inhibitor

0.75 uL Substrate

1.5 uL 50 nM Trigger

G2: 0.404 uL DNA

0.96 uL H2O

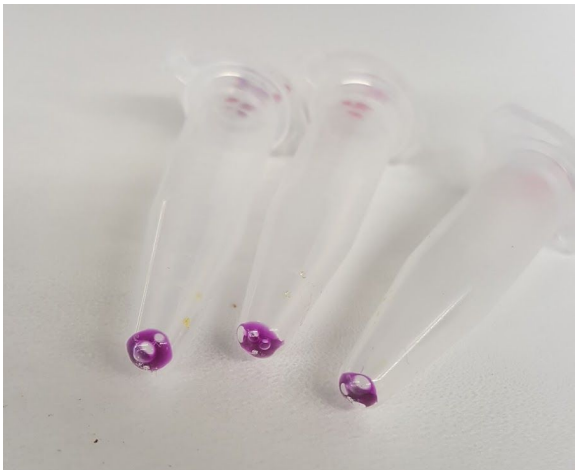
G3: 0.583 uL DNA

0 uL H2O

G4: 0.297 uL DNA

0.203 uL H2O

Incubate at 37: Check at:



Wednesday, September 7

Cell-free lead and thallium test (Claire)

Solution A	2 uL	36 uL
Solution B	1.5 uL	27 uL
RNAse inhibitor	0.25 uL x18 reactions =	4.5 uL
Substrate	0.25 uL	4.5 uL
Switch	0.30 uL at 84.1 ng/uL = 19.8 ng	5.4 uL (1 uL 84.1, 4.4 uL 61.9)

Trigger	0.70 uL	

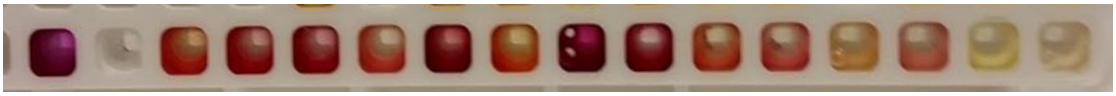
Triggers:

Condition	
No trigger	0.70 uL water
1.42 nM Pb Hairpin	0.70 uL 5 nM original hairpin
1.42 nM Pb Hairpin + 2 uM lead	0.35 uL 10 nM original hairpin 0.25 uL Pb at 40 uM = 2 uM Pb 0.10 uL Pb buffer
Tl Hairpin	0.35 uL at 10 uM = 0.7 uM hairpin 0.35 uL water
Hairpin + Er	0.35 uL at 10 uM = 0.7 uM hairpin 0.25 uL at 200 uM = 10 uM Er 0.10 uL water
Hairpin, Er, 10 uM Tl	0.35 uL at 10 uM = 0.7 uM hairpin 0.25 uL at 200 uM = 10 uM Er 0.2 uL 100 uM Tl --no buffer--
1.42 nM DNA trigger Pb	0.7 uL 4 nM DNA oligo
0.7 uM DNA trigger Tl	0.35 uL at 10 uM DNA oligo 0.35 uL water

PROCEDURE:

1. Make master mix
2. Add 8.6 uL master mix to 8 tubes
3. Add 2x what is listed in “Triggers” to each tube
4. Separate each tube into 2 tubes of 5 uL each
5. Incubate 2 hours at 37 degrees

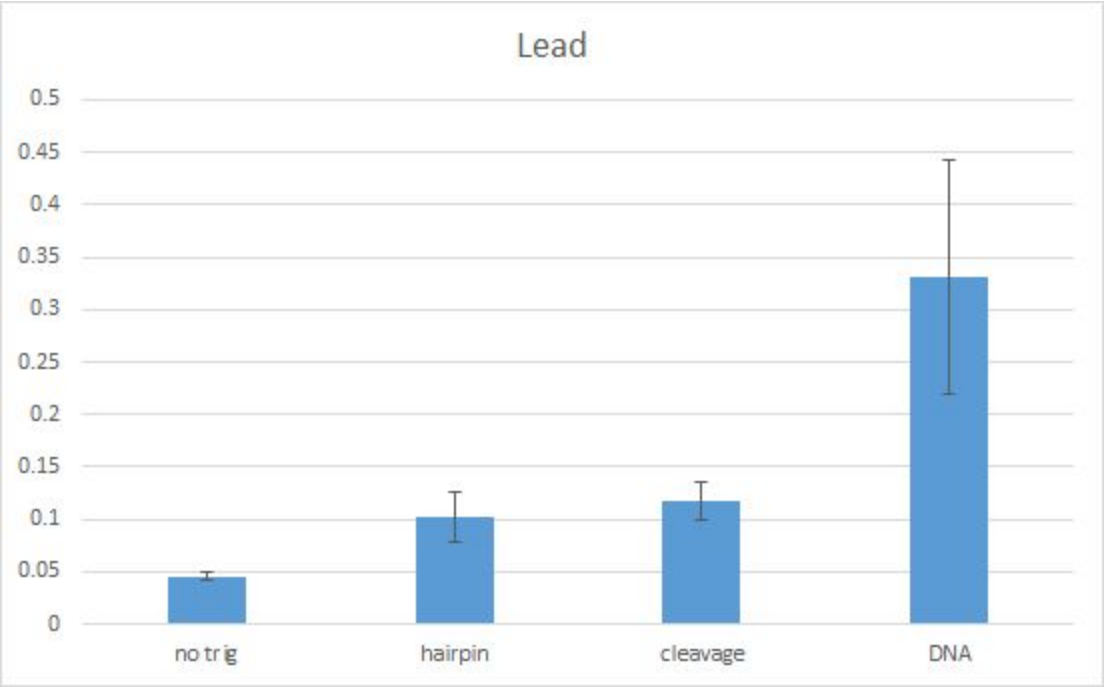
Needed to add to master mix for last 3 reactions. Ran out again so pipetted components of DNA Tl individually. Not enough to make duplicates for DNA Tl



Lead

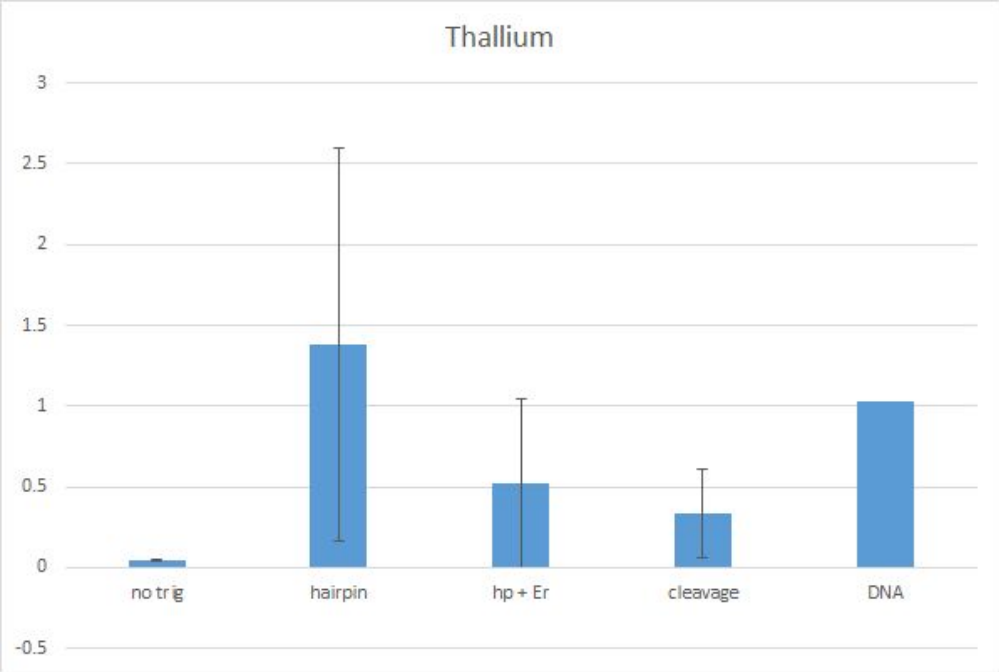
Condition	1	2	average	st dev
no trig	0.0479	0.0431	0.0455	0.003394

hairpin	0.1181	0.0844	0.10125	0.02383
cleavage	0.1041	0.1307	0.1174	0.018809
DNA	0.4094	0.2523	0.33085	0.111086



Thallium

Condition	1	2	average	st dev
no trig	0.0479	0.0431	0.0455	0.003394
hairpin	0.5196	2.2396	1.3796	1.216224
hp + Er	0.1516	0.8927	0.52215	0.524037
cleavage	0.1435	0.5284	0.33595	0.272165
DNA	1.0293		1.0293	



Cell-free Collins 15 switch test (Claire)

Solution A	2 uL
Solution B	1.5 uL
RNAse inhibitor	0.25 uL
Substrate	0.25 uL
Switch	0.50 uL at about 270 ng/uL
Trigger	0.50 uL of 5 nM

Pipette reactions separately. Test switches “Collins 15,” “Collins 1-3”

Incubate. Collins 1-3 yellow; toss plasmids

Digest T3 (Claire)

2 uL XbaI

2 uL SpeI

1 uL DpnI

4 uL buffer

5 uL plasmid at 38.3 ng/uL

30 uL water

TAE Buffer (Maddie)

500 mL

121 g Tris base

9.305 g EDTA

28.55 mL glacial acetic acid

Obtained 4 tubes (200uL) competent cells from Cheryl

Obtained plate of codon-optimized OFP, GFP, BFP, YFP, and RFP

Thursday, September 8th

Liquid cultures of codon-optimized OFP, GFP, BFP, YFP, and RFP

T3 Amplification from RBS-T3

1 50 uL reaction

10 uL 5X Phusion HF Buffer
1 uL 10mM dNTP
2.5uL 10 uM PstI-T3 Reverse 2
2.5uL 10 uM XbaI-T3 Forward 2
0.4 uL DNA (26.3 ng/uL) RBS-T3
0.5 uL Phusion
28.6 uL Water

PCR: 98C for 30sec
[98C for 10 sec
52C for 30 sec
72C for 2:30] 30X
72C 10 min
4C hold

PST-T3 Reverse 2 and Xba T3-Forward 2

Friday, September 9th

Lab meeting

Cell-free test of lead (replicate 8/19, Week 13 experiment) (Claire)

Solution A	2 uL	24 uL
Solution B	1.5 uL	18 uL
RNAse inhibitor	0.25 uL x12 reactions =	3 uL
Substrate	0.25 uL	3 uL
Switch	0.30 uL at 84.8 ng/uL = 25.44 ng	3.6
Trigger	0.70 uL	Final concentration 0.52 nM

Triggers:

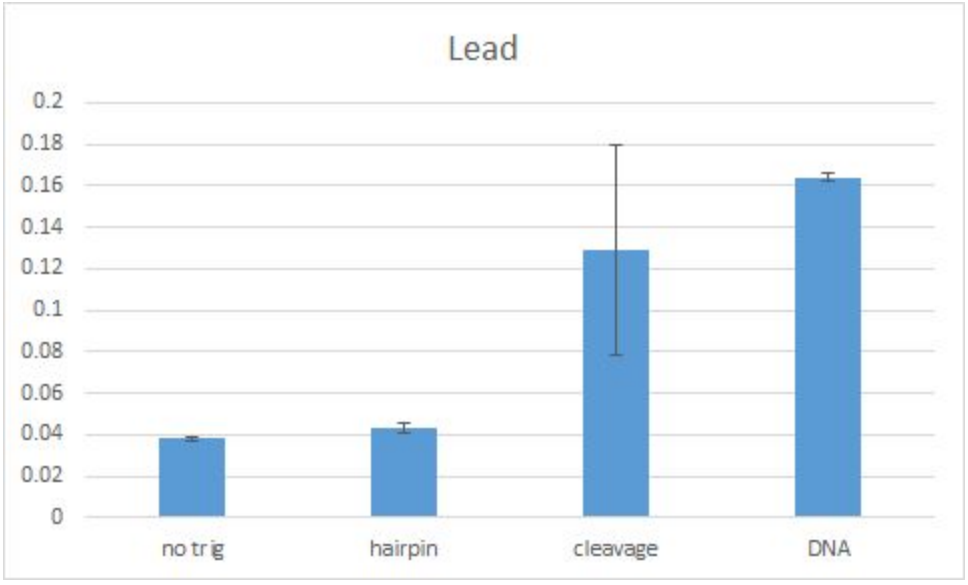
Condition	

No trigger	0.70 uL water
0.52 nM Pb Hairpin	0.70 uL 3.74 nM original hairpin
0.52 nM Pb Hairpin + 2 uM lead	0.28 uL 66.9 nM original hairpin 0.417 uL Pb at 24 uM = 2 uM Pb 0.003 uL Pb buffer (just dabbed droplet onto side of tube)
0.52 nM DNA trigger Pb	0.7 uL 3.74 nM DNA oligo

PROCEDURE:

1. Make master mix
2. Add 8.6 uL master mix to 4 tubes
3. Add 2x what is listed in “Triggers” to each tube
4. Separate each tube into 2 tubes of 5 uL each
5. Incubate 2 hours at 37 degrees

Condition	1	2	average	st dev
no trig	0.0372	0.0388	0.038	0.001131
hairpin	0.0415	0.0449	0.0432	0.002404
cleavage	0.1643	0.0929	0.1286	0.050487
DNA	0.1651	0.1625	0.1638	0.001838



Run 2 T3 amp on 1% agarose gel (Claire)

No bands visible; may have run off gel?

Digest of PT3 and T7