iGEM 2016: Team Pittsburgh
Week 17 Lab Notebook

Monday, September 12

Mini-prep codon optimized BFP, GFP, RFP, YFP, and OFP (Maya)
Enzyme cleaned linearized PT3 and linearized PT7-RBS (Maya)
T3 Amplification from RBS-T3 (Maya)
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
50 uL reaction
10 uL 5X Phusion HF Buffer
1 uL 10mM dNTP
2.5uL 10 uM Xba-T3 Reverse 1
2.5uL 10 uM EcoRI-T3 Forward 1
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
Transform G switch
2 uL 42.1 ng/uL switch into 50 uL cells
500 LB (SOC contaminated)
Cell-free DNAzyme concentration (Claire)

Monday, Sep. 12.

Titration of DNAzyme amt (lead) cell-free

no trigger

0.52 nM
1 nM
3.74 nM ~~5 nM~~
10 nM
~~25 nM~~
50 nM
100 nM
1 μM

} DNAzyme and DNA

			no lead cleavage ~~cleavage~~ DNA	
I 11-12	no trigger	0.7 μL H_2O	~~0.26 μL 10nM hp~~	
I 13-18	0.52 nM	0.7 μL 3.74 nM	~~0.28 μL 66.9 nM hp~~ 0.417 μL 24 μM Pb 0.003 μL Pb buff.	0.7 μL 3.74 nM
	~~1 nM~~	~~0.5 μL 10 nM hp~~ ~~0.2 μL H_2O~~	~~0.25 μL 20 nM hp~~ ~~0.417 μL 24 μM Pb~~ 0.033 μL Pb buff.	~~0.5 μL 10 nM~~ ~~0.2 μL H_2O~~
I 19-24	3.74 nM	0.28 μL 66.9 nM hp 0.42 μL H_2O	0.28 μL 66.9 nM hp 0.417 μL 24 μM Pb 0.003 μL Pb buffer	~~0.374~~ ~~3.74~~ μL 50 nM 0.326 μL H_2O
J 11-16	10 nM	0.67 μL 74.8 nM hp 0.03 μL H_2O	0.25 μL 200 nM hp 0.417 μL 24 μM Pb 0.033 μL Pb buffer	0.5 μL 100 nM 0.2 μL H_2O
J 17-22	50 nM	0.25 μL 1 μM hp 0.45 μL H_2O	0.25 μL 1 μM hp 0.417 μL 24 μM Pb 0.033 μL Pb buffer	~~0.25~~ ~~0.25~~ μL 1 μM 0.45 μL H_2O
J 23-24	100 nM	0.25 μL 2 μM hp 0.45 μL H_2O	0.25 μL 2 μM hp 0.417 μL 24 μM Pb 0.033 μL Pb buffer	0.5 μL 1 μM 0.2 μL H_2O
K 11-14				

K15-20 1 μM 0.25 μL 20 μM hp 0.25 μL 20 μM hp 0.5 μL 10 μM
0.45 μL H_2O 0.417 μL 24 μM Pb 0.2 μL H_2O
0.033 μL Pb buffer

K21-22 5 μL leftover MM + 5 μL H_2O

~~20 nM hp~~: 1 part 74.8 nM : ~~2.74 parts water~~ ~~5 : 13.7~~

~~10 nM hp from ↑ (1:1)~~ → 2.5 74.8 nM : 13.7 H_2O

20 μM hp: ~~5 parts~~ 2 parts 74.8 μM : 5.48 H_2O

2 μM hp from ↑ (1:9)

1 μM hp from ↑ (1:1)

38 reactions (make 40):

Sol'n A:	2	80	ran out after 10 hp. Repeat.
B:	1.5	60	low on RNAse
RNAse:	0.25	× 40 = 10	
Sub:	0.25	10	
~~Fluor~~ Switch:	0.3	12	@ 72.1 ng/μL

2nd time: 10 μL @ 72.1
2 μL @ 61.9

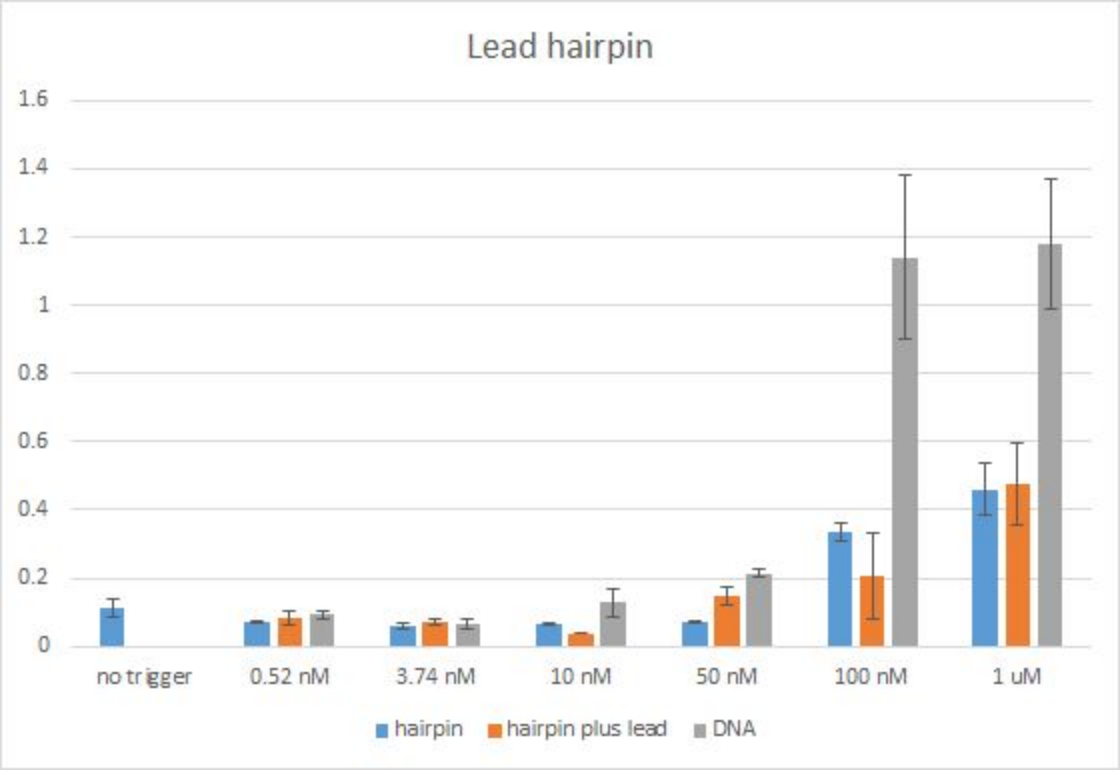
[NEED TO RE-RUN]

Heat hairpin: 5 μL of 74.8 μM.

95°	3 min
90°	2 min
85°	2 min
80°	2 min
70°	4 min
60°	4 min
40°	8 min
20°	8 min
4°	∞

re-run – 2 min. for every 5 deg.

Condition	hairpin				lead				DNA			
	1	2	average	st dev	1	2	average	st dev	1	2	average	st dev
no trigger	0.0936	0.1289	0.11125	0.024961								
0.52 nM	0.0699	0.0739	0.0719	0.002828	0.0689	0.0964	0.08265	0.019445	0.085	0.1003	0.09265	0.010819
3.74 nM	0.0512	0.065	0.0581	0.009758	0.0646	0.076	0.0703	0.008061	0.0565	0.0754	0.06595	0.013364
10 nM	0.0628	0.0668	0.0648	0.002828	0.0385	0.0381	0.0383	0.000283	0.1581	0.0969	0.1275	0.043275
50 nM	0.0687	0.0733	0.071	0.003253	0.1298	0.1662	0.148	0.025739	0.2234	0.2052	0.2143	0.012869
100 nM	0.3162	0.356	0.3361	0.028143	0.1149	0.293	0.20395	0.125936	0.9697	1.3128	1.14125	0.242608
1 uM	0.4054	0.5161	0.46075	0.078277	0.3923	0.5609	0.4766	0.119218	1.0448	1.316	1.1804	0.191767

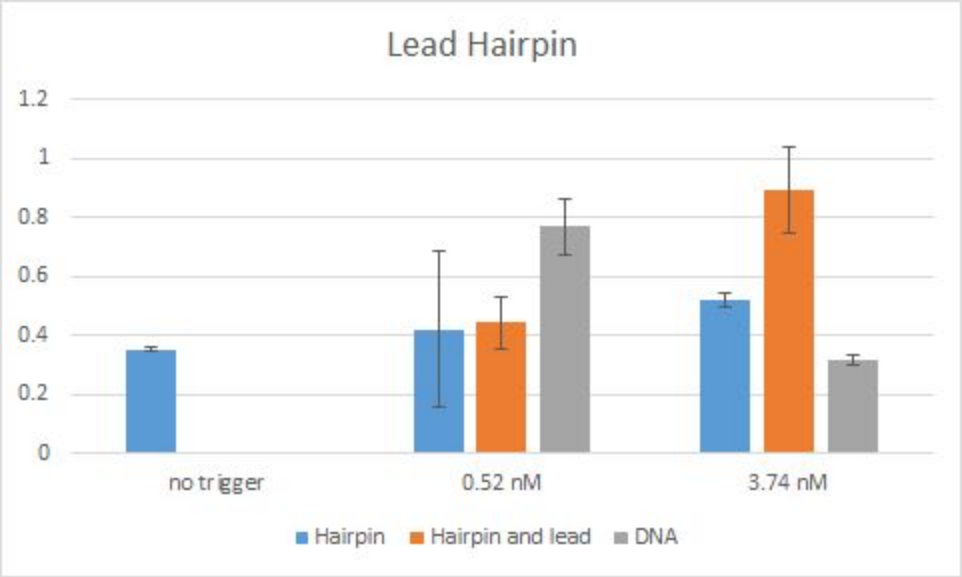


Tuesday, September 13

Cell-free concentrations (Praneeth)

No trigger
0.52 nM hairpin
0.52 nM hairpin + 2 uM lead + water
0.52 nM DNA
0.52 nM hairpin
0.52 nM hairpin + 2 uM lead + water
0.52 nM DNA

Condition	hairpin				lead				DNA			
	1	2	average	st dev	1	2	average	st dev	1	2	average	st dev
no trigger	0.3593	0.3474	0.35335	0.008415								
0.52 nM	0.6082	0.2351	0.42165	0.263822	0.5069	0.3827	0.4448	0.087823	0.7023	0.8368	0.76955	0.095106
3.74 nM	0.5345	0.5009	0.5177	0.023759	0.9972	0.7883	0.89275	0.147715	0.3262	0.3056	0.3159	0.014566



Wednesday, September 14

Cell-free lead hairpin reaction

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	0.40 uL at 61.9 ng/uL = 25 ng	4.84 uL
Trigger	0.60 uL	

Triggers:

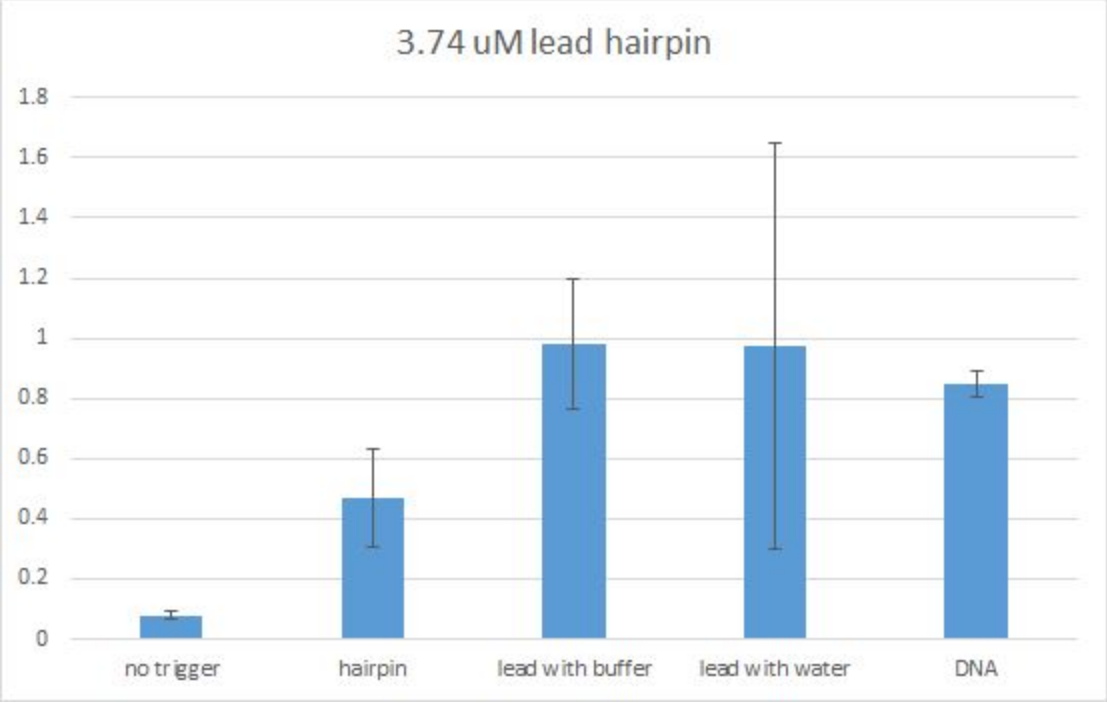
Plate	Condition	
C19-20	No trigger	0.60 uL water
C21-22	3.47 uM Pb Hairpin	0.25 uL 74.8 uM renatured hairpin 0.35 uL water
C23-24	3.47 uM Pb Hairpin + 2 uM lead + buffer	0.25 uL 74.8 uM renatured hairpin 0.25 uL Pb at 40 uM = 2 uM Pb 0.10 uL Pb buffer
D19-20	3.47 uM Pb Hairpin + 2 uM lead + buffer	0.25 uL 74.8 uM renatured hairpin 0.25 uL Pb at 40 uM = 2 uM Pb 0.10 uL water

D21-22	3.47 nM DNA trigger	0.374 uL 50 nM DNA trigger 0.226 uL water

PROCEDURE:

1. Make master mix
2. Add 8.8 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

Condition	1	2	average	st dev
no trigger	0.0868	0.0717	0.07925	0.010677
hairpin	0.3563	0.5855	0.4709	0.162069
lead with buffer	0.8271	1.134	0.98055	0.217011
lead with water	0.4966	1.4514	0.974	0.675146
DNA	0.8189	0.8812	0.85005	0.044053



Repeat with correct concentrations of trigger:

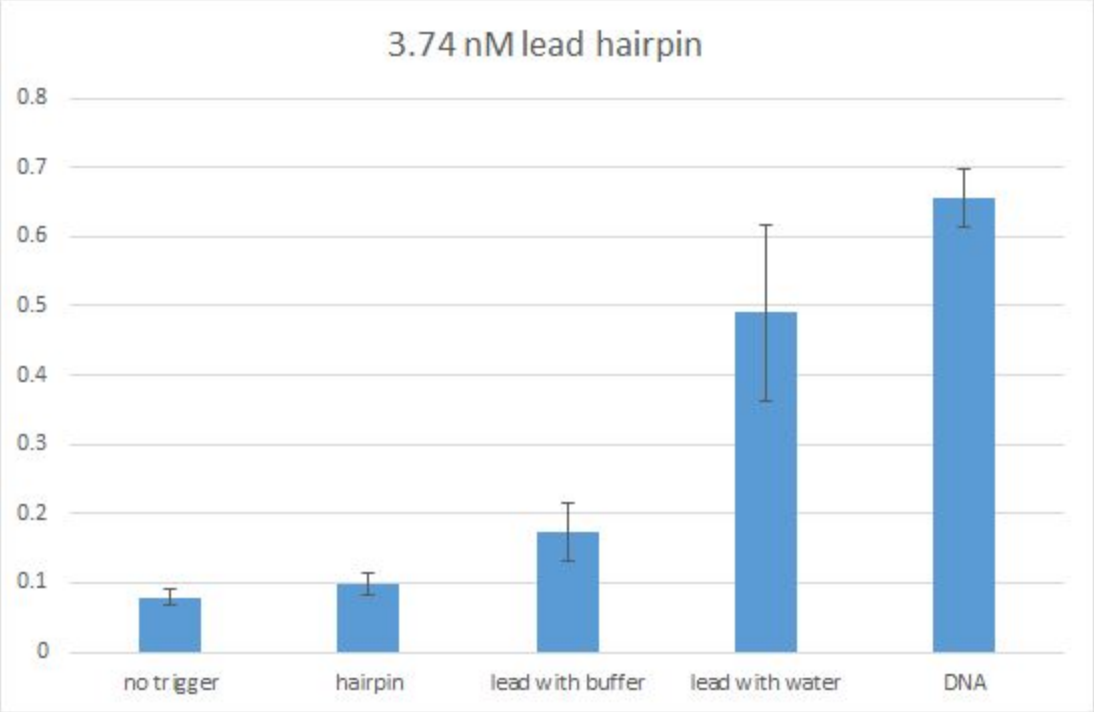
Triggers:

Plate	Condition	
D23-24	3.47 nM Pb Hairpin	0.25 uL 74.8 nM renatured hairpin 0.35 uL water
E19-20	3.47 nM Pb Hairpin + 2 uM	0.25 uL 74.8 nM renatured hairpin

	lead + buffer	0.25 uL Pb at 40 uM = 2 uM Pb 0.10 uL Pb buffer
E21-22	3.47 nM Pb Hairpin + 2 uM lead + buffer	0.25 uL 74.8 nM renatured hairpin 0.25 uL Pb at 40 uM = 2 uM Pb 0.10 uL water
E23-24	3.47 nM DNA trigger	0.374 uL 50 nM DNA trigger 0.226 uL water

Condition	1	2	average	st dev
no trigger	0.0868	0.0717	0.07925	0.010677
hairpin	0.1095	0.0881	0.0988	0.015132
lead with buffer	0.2032	0.1429	0.17305	0.042639
lead with water	0.5805	0.3999	0.4902	0.127703
DNA	0.6848	0.6271	0.65595	0.0408

*no trigger data from previous run



C

D

E

24 23 22 21 20 19

Agarose gel (Claire)

30 mL TAE buffer
0.3 g agarose
3 uL EtBr
Run at 100 V, 30 min
Lane 1: 1 kb ladder
Lane 2: lacZ 1
Lane 3: lacZ 2
Lane 4: T3 amp 1
Lane 5: T3 amp 2

Clear bands for T3 amp, but don't see anything for lacZ

Friday, September 16

Cell-free lead hairpin concentration variation

9/16

Friday, September 16

Redo concentration curve:

3.74 nM, 50 nM, 100 nM 20 rxns

25 ng switch @ ~~57.9~~ 50 ng/µL = ~~0.430~~ 0.5 µL/rxn

= 0.57 µL trigger/rxn

0 13-14	no trigger:	0.50 µL H_2O.
0 15-16	3.74 nM hp:	0.25 µL hp @ 74.8 nM ~~0.32~~ 0.25 µL H_2O
0 17-18	3.74 nM hp+: 2 µM Pb	0.25 µL hp @ 74.8 nM 0.25 µL Pb^{+2} @ 40 µM ~~0.07 µL H_2O~~
0 19-20	3.74 nM DNA:	0.5 µL DNA @ 34.7 nM
0 21-22	50 nM hp:	~~0.25~~ 0.25 µL hp @ 1 µM 0.25 µL H_2O
0 23-24	50 nM hp+: 2 µM Pb	0.25 µL hp @ 1 µM 0.25 µL Pb^{+2} @ 40 µM
P 17-18	50 nM DNA:	0.25 µL DNA @ 1 µM 0.25 H_2O
P 19-20	100 nM hp:	0.25 µL 2 µM hp 0.25 µL H_2O
P 21-22	100 nM hp+: 2 µM Pb	0.25 µL 2 µM hp 0.25 µL 40 µM Pb
P 23-24	100 nM DNA:	0.5 µL 1 µM DNA.

short on volume

0.25 µL → 0.3 µL

0.5 µL → 0.6 µL

Cell extract MM:

Sol'n A:	2 µL	2.4 µL	52.8 µL	14.4
B:	1.5 µL	1.8 µL	39.6	10.8
RNAse:	0.25 µL	0.3 µL	6.6	1.5
Sub:	0.25 µL	0.3 µL	6.6	1.5
Switch:	0.43 µL	0.52 µL	11.44	2.58
H_2O:	0.07 µL	0.08 µL	1.76	.42
trigger	1 µL	1.2 µL		
	5 µL	6 µL		

× 22 rxns =

10.8 µL/condition

Trigger MM:

no trigger: 0.6 µL × 2 = 1.2 µL H_2O

3.74 nM

hp: 0.3 µL hp @ 74.8 + 0.3 µL H_2O] 1 µL hp@74.8 + 1 µL H_2O ⇒ 1.2 µL

hp+Pb: 0.3 µL hp @ 74.8 + 0.3 µL Pb @ 40] 1 µL hp@74.8 + 1 µL Pb@40 ⇒ 1.2 µL

DNA: 1.2 µL @ ~~37.4~~ 37.4 nM

1 µL 50 + 0.34 µL H_2O = 3 µL 1.02

50 nM

hp: 0.3 µL hp @ 1 µM + 0.3 µL hp @ H_2O] 1 µL hp@1µM + 1 µL H_2O ⇒ 1.2 µL 500 nM hp

hp+Pb:] 1 µL hp@1µM + 1 µL Pb@40µM ⇒ 1.2 µL

DNA:] 1 µL DNA@1µM + 1 µL H_2O ⇒ 1.2 µL

pipetted seps

diff MM

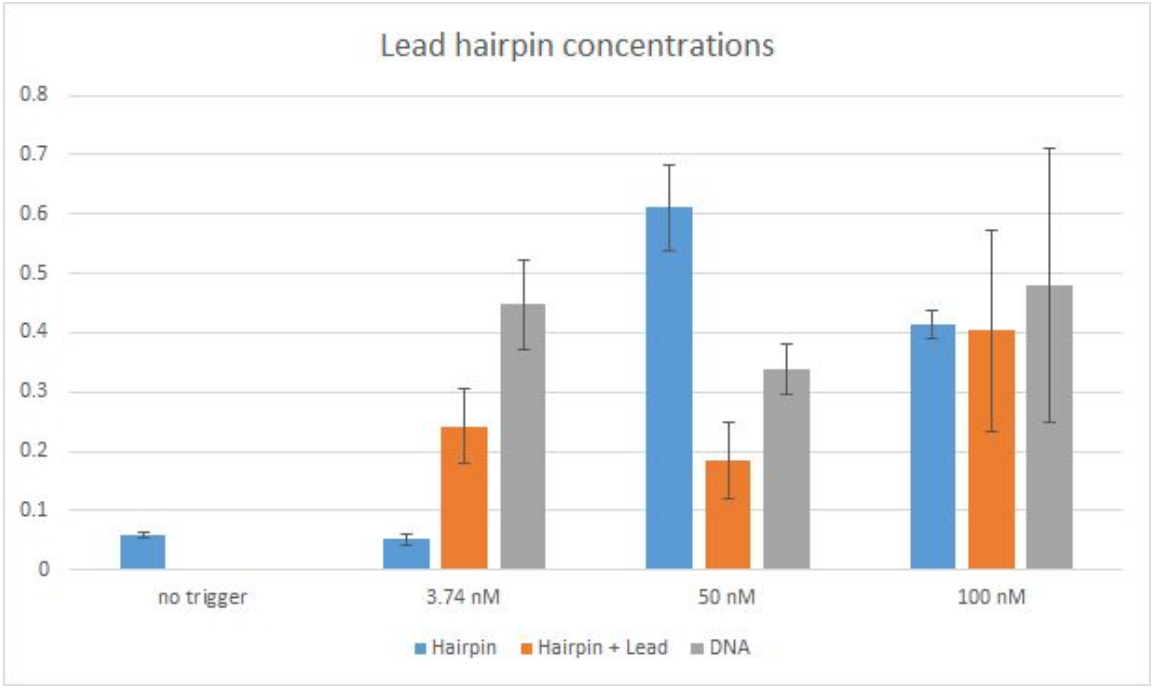
100 nM

hp:] 1 µL hp@2µM + 1 µL H_2O ⇒ 1.2 µL 1µM hp

hp+Pb] 1 µL hp@2µM + 1 µL Pb@40µM ⇒ 1.2 µL

DNA: 1.2 µL @ 1µM

Condition	hairpin				lead				DNA			
	1	2	average	st dev	1	2	average	st dev	1	2	average	st dev
no trigger	0.0557	0.0617	0.0587	0.004243								
3.74 nM	0.058	0.0454	0.0517	0.00891	0.1972	0.2863	0.24175	0.063003	0.4999	0.394	0.44695	0.074883
50 nM	0.6621	0.5589	0.6105	0.072973	0.2301	0.138	0.18405	0.065125	0.3075	0.3693	0.3384	0.043699
100 nM	0.3963	0.4312	0.41375	0.024678	0.2831	0.524	0.40355	0.170342	0.3158	0.6438	0.4798	0.231931



Saturday, September 17

Repeat above for 50 nM and 100 nM

9/17

Saturday, September 17.
Redo concentrations @ 50 nM & 100 nM

Cell extract MM

			×4
Sol'n A	2.4 µL	36 µL	9.6
B	1.8 µL	27 µL	7.2
RNAsei.	0.3 µL ×15 rxns =	4.5 µL	~~1.2~~
Sub.	0.3 µL	4.5 µL	1.2
Switch	0.45 @55.6 ng/µL =	6.75 µL	1.8 → ~~7.5 µL~~
~~H2O~~	~~0.05~~	~~0.75 µL~~ 2.25 µL	~~0.6~~
trig.	1.2 µL		0.6
	6 µL	10.8 /rxn	10.2/rxn

remainder + 1.8 µL H_2O → no trig.

~~9 µL / 1 µL / 100 µL~~

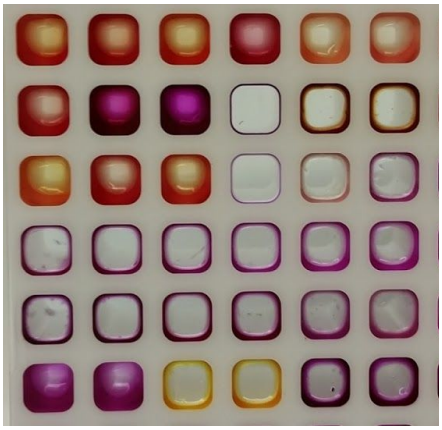
Triggers

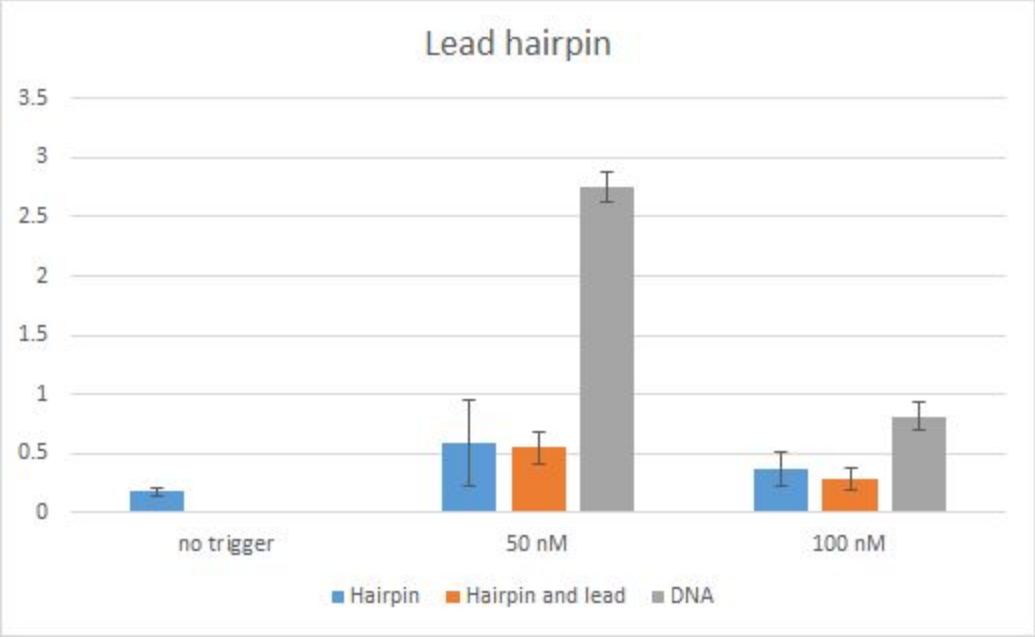
F21-22	50 nM hp	1.2 µL 500 nM hp
F23-24	50 nM hp+Pb 1 µL hp @ 1 µM + 1 µL Pb @ 40 µM ⇒	1.2 µL
G22-23	50 nM DNA	1.2 µL 500 nM DNA
G24, H21	100 nM hp	1.2 µL 1 µM hp
H22-23	100 nM hp+Pb 1 µL hp @ 2 µM + 1 µL Pb @ 40 µM ⇒	1.2 µL
diff: K23&24	100 nM DNA	1.2 µL 1 µM DNA

.25 µL RNAse + 0.35 µL H_2O added alone

no trig ~~A24~~ F19-20

Condition	hairpin				lead				DNA			
	1	2	average	st dev	1	2	average	st dev	1	2	average	st dev
no trigger	0.2048	0.1547	0.17975	0.035426								
50 nM	0.8517	0.3305	0.5911	0.368544	0.6468	0.4565	0.55165	0.134562	2.8397	2.6602	2.74995	0.126926
100 nM	0.4733	0.2644	0.36885	0.147715	0.3505	0.2162	0.28335	0.094964	0.7288	0.8967	0.81275	0.118723




Lead hairpin
3.5
3
2.5
2
1.5
1
0.5
0
no trigger
50 nM
100 nM
Hairpin
Hairpin and lead
DNA