iGEM 2016: Pittsburgh
Week 5 Lab Notebook

Monday, June 20

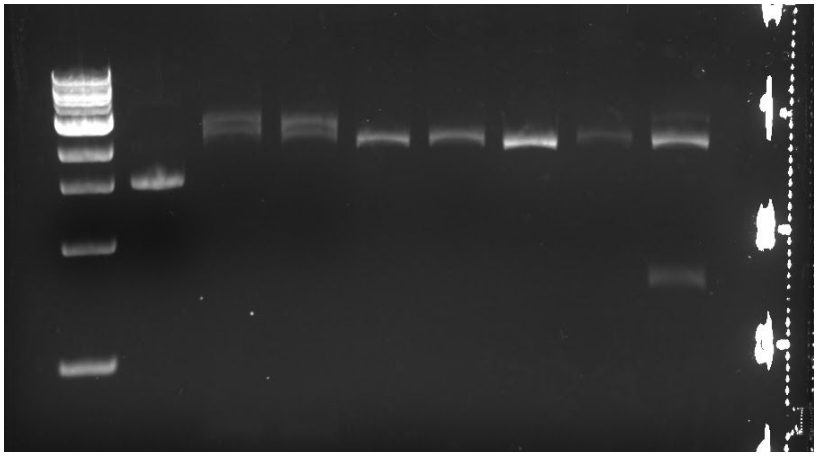
Lab meeting

Double digest of T7-RBS -- lacZ/eGFP to check for insert (Praneeth)

- 0.5 uL plasmid
- 0.5 uL EcoRI
- 0.5 uL SpeI
- 1 uL 10x buffer
- 7.5 uL H2O

Gel electrophoresis check

Lane		
	1	ladder
	2	control
	3	T7 LacZ 1
	4	T7 LacZ 2
	5	T7 eGFP 1
	6	T7 eGFP 2
	7	T7 eGFP 3
	8	T7 eGFP 4
	9	T7 eGFP 5



Transformation of Collins plasmids (Claire)

- D_LacZ (13), kan resistance
- pAG_TS2AT05 (14), amp resistance
- G_LacZ (15), kan resistance
- pAG_TS2AT03 (16), amp resistance

Sequence of T7-RBS -- amilCP not convincing for amilCP insertion direction

- Correct sequence from wrong primer--mislabeled primers?
- Double digest of 3-2 and 3-5 T7-RBS -- amilCP with EcoRI and SpeI

Sign up for InterLab study

Email Ronald Joseph (Pittsburgh Public Schools water testing) rj1@pghboe.net

Tuesday, June 21

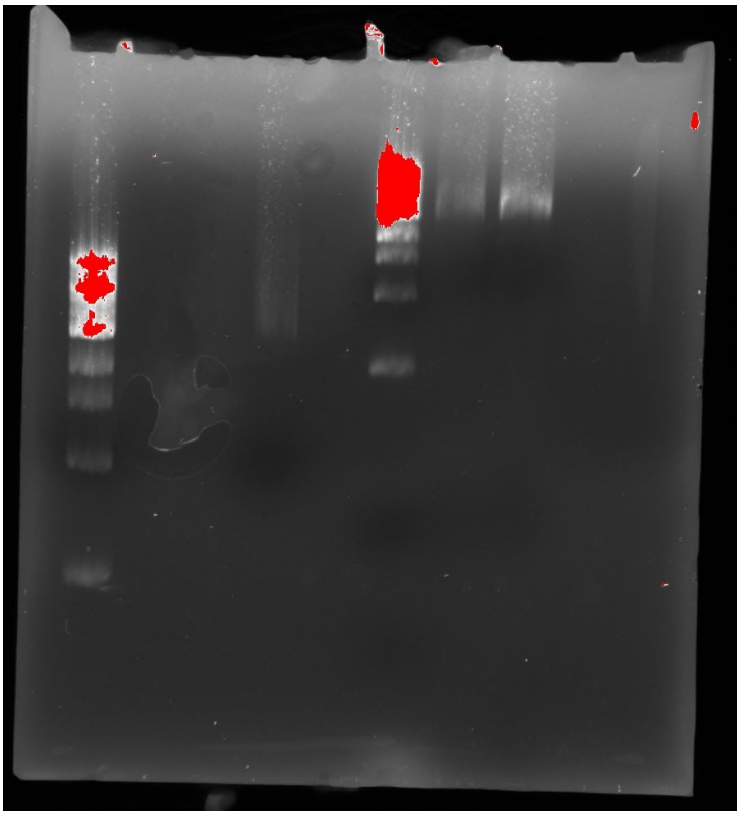
Gel electrophoresis of double digest 3-2 and 3-5 T7-RBS -- amilCP (Claire)

1% agarose gel

Lane 1 ladder
2 3-2 (1 uL)
3 3-5 (1 uL)
4 uncut 3-5 control

Couldn't see lanes 2 and 3, run again

Lane 6 ladder
7 3-2 (remaining rxn)
8 3-5 (remaining rxn)



Did not confirm amilCP direction

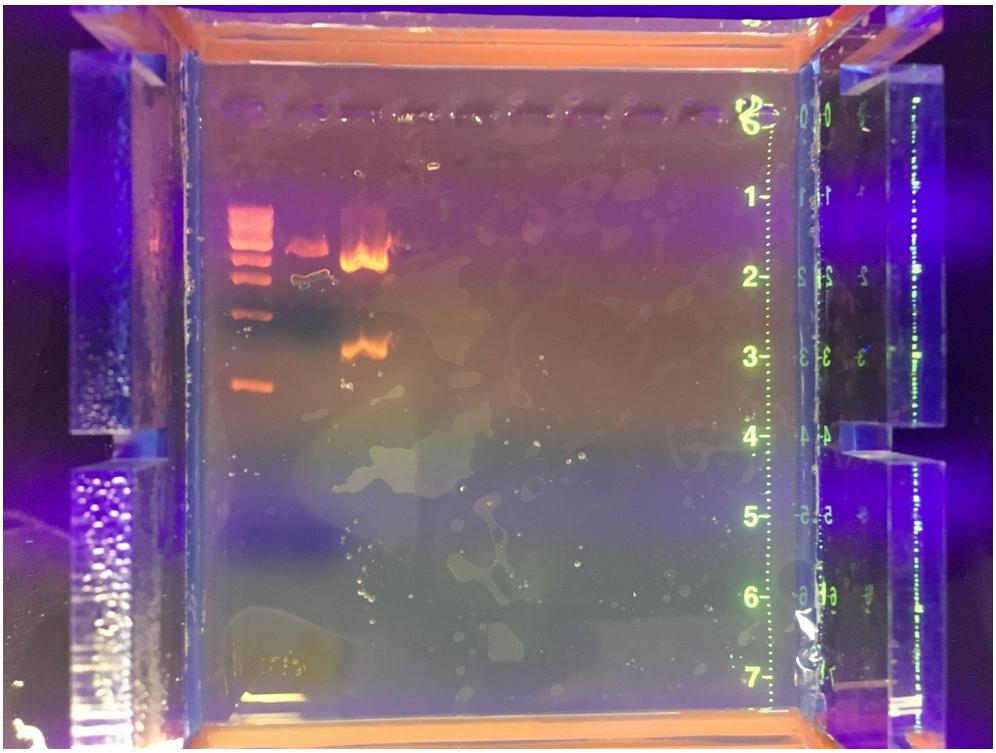
Double digest of 3-2 T7-RBS -- amilCP (Maddie)

~1 uL SpeI (left over)
1 uL EcoRI
2 ug DNA = 15.34 uL DNA
2 uL 10x buffer
.66 uL H2O
No heat inactivation

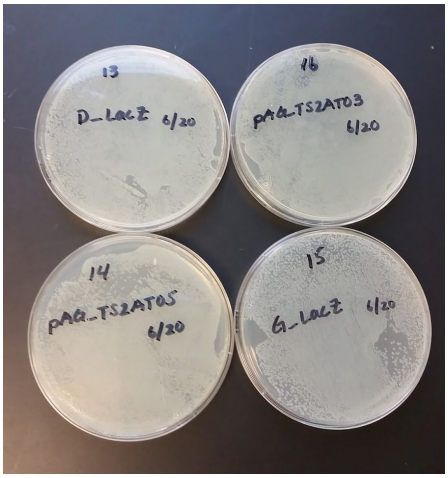
Gel electrophoresis of digested 3-2 T7-RBS -- amilCP (Maddie)

Lane 1 ladder

2 undigested plasmid
3 digest reaction



Liquid cultures of Collins plasmids (2 of each plasmid), into shaker at 2 pm (Claire)



Wednesday, June 22

Ligation of T7-RBS -- amilCP to terminator (Claire, Maddie)

Control	1 uL	terminator
	0 uL	T7-RBS -- amilCP
	2 uL	buffer
	16 uL	H2O
	1 uL	T4 ligase
3:1	1 uL	terminator
	50 ng= 4.76 uL	T7-RBS -- amilCP
	2 uL	buffer

11.24 uL H2O
1 uL T4 ligase
x:1 1 uL terminator
5.76 uL T7-RBS -- amilCP
2 uL buffer
10.76 uL H2O
1 uL T4 ligase

Transformation of ligation reaction (Claire)

5 uL of each reaction into 66.6 uL of Cheryl's competent cells
Ice 10 min
42 degC 2 min
Ice 5 min
200 uL SOC media
Shaker at 37 degC for 1 hr. 45 min.
Into incubator at 12:45 pm

Mini-prep Collins plasmids liquid cultures (Maddie, Praneeth)

Concentrations (ng/uL)

	13	**14**	**15**	**16**
1	81.3	162.5	95.4	141.9
2	83.6	129.1	71.6	148.6

Cell-free extract volume optimization (Maddie, Praneeth)

T7--GFP (concentration = 120.8 ng/uL)
DNA amount = 5 ng/uL

	25 µL	**25 Control**	**20 µL**	**20 C**	**10 µL**	**10 C**	**5 µL**	**5 C**
Solution A	10	10	8	8	4 µL	4 µL	2 µL	2 µL
Solution B	7.5	7.5	6	6	3 µL	3 µL	1.5 µL	1.5 µL
RNAse	0.125	0.125	*0.2*	*0.2*	*0.1 µL*	*0.1 µL*	*0.05*	*0.05*
H2O	6.345	7.355	4.972	5.8	2.486	2.9	1.243	1.45
DNA	1.03	0	0.828	0	0.414	0	0.207	0

New RNAse at twice the concentration of old RNAse

* all 25 uL and 20 uL reactions, including controls, were made from Solutions A and B that were mixed on June 17, then frozen at -80 degC because RNAse was low

Incubate 2 hours at 37 degC
Top off all reactions with water to 25 uL (min volume of plate reader)

	1	2	3	4	5	6	7	8	9	10	11	12
A					5 C	5-1	5-2	10 C	10-1	10-2	20 C	20-1
B	20-2	25 C	25-1	25-2								

Results (rfu) 485 emission, 585 excitation

Read multiple times because wasn't sure if plate reader settings were correct

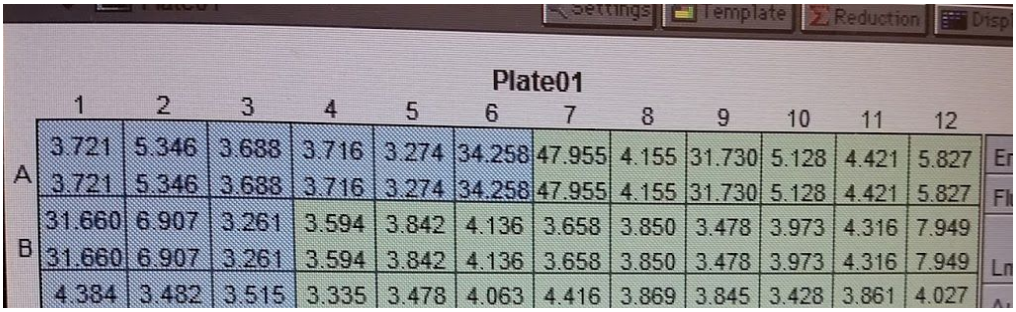


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.721	5.346	3.688	3.716	3.274	34.258	47.955	4.155	31.730	5.128	4.421	5.827
A	3.721	5.346	3.688	3.716	3.274	34.258	47.955	4.155	31.730	5.128	4.421	5.827
B	31.660	6.907	3.261	3.594	3.842	4.136	3.658	3.850	3.478	3.973	4.316	7.949
B	31.660	6.907	3.261	3.594	3.842	4.136	3.658	3.850	3.478	3.973	4.316	7.949
	4.384	3.482	3.515	3.335	3.478	4.063	4.416	3.869	3.845	3.428	3.861	4.027

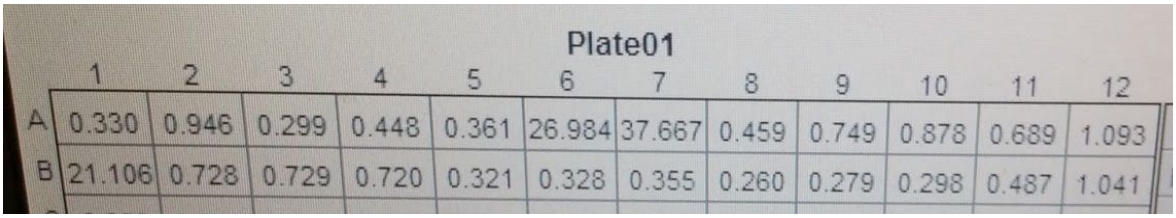


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.330	0.946	0.299	0.448	0.361	26.984	37.667	0.459	0.749	0.878	0.689	1.093
B	21.106	0.728	0.729	0.720	0.321	0.328	0.355	0.260	0.279	0.298	0.487	1.041

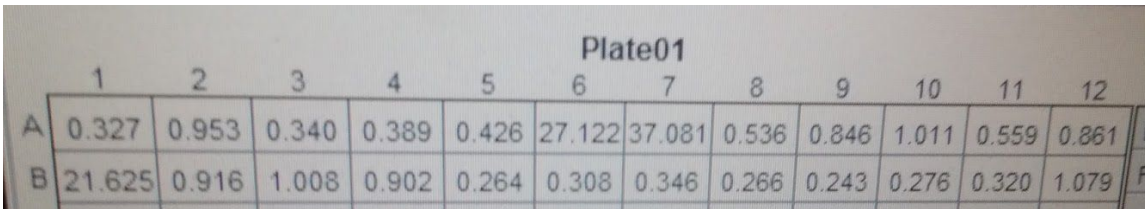


Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.327	0.953	0.340	0.389	0.426	27.122	37.081	0.536	0.846	1.011	0.559	0.861
B	21.625	0.916	1.008	0.902	0.264	0.308	0.346	0.266	0.243	0.276	0.320	1.079

Only 5-mL worked. Forgot to add DNA? Refrozen cell extract?

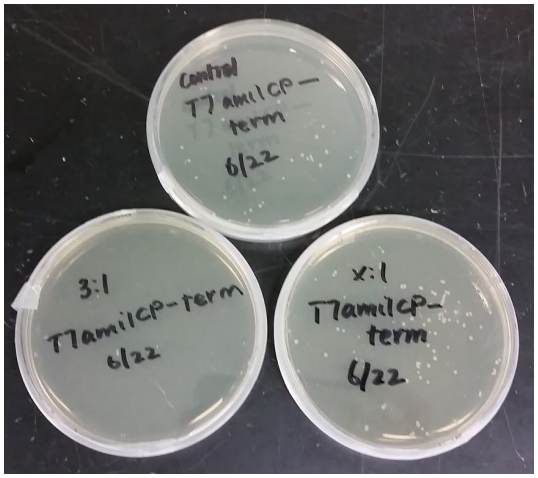
Linear T7-RBS -- amilCP in cell extract (Aife)

T7-RBS -- amilCP digested with EcoRI, concentration = 52.16 ng/uL

	5 uL	**5 uL control**	**1 uL**	**1 uL control**
Sol'n A	2	2	0.4	0.4
Sol'n B	1.5	1.5	0.3	0.3
RNAse	0.25	0.25	0.05	0.05
DNA	0.96	0	0.19	0
water	0.29	1.25	0.06	0.25

Thursday, June 23

Colonies grew on x:1 and control plates (but more on x:1) for T7amilCP--term



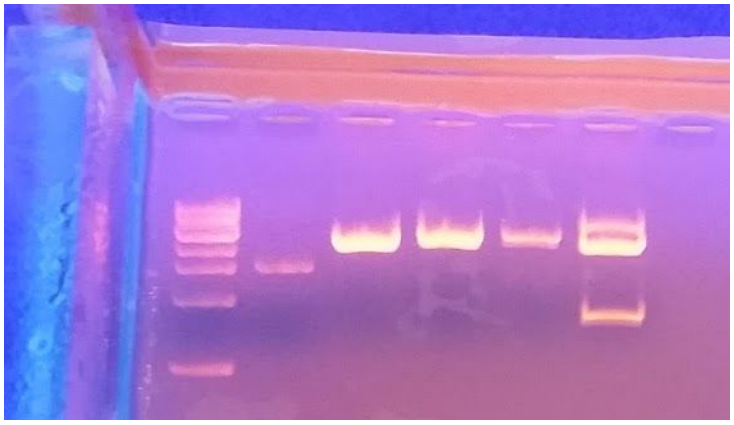
Liquid cultures of 5 colonies from x:1 T7amilCP--term plate (Praneeth)

Digest T7-eGFP 2, 3, 4, 5 with EcoRI and SpeI (Claire)

1 uL SpeI		
1 uL EcoRI		
2 ug DNA =	13.24 uL	T7-eGFP 2
	10.07 uL	T7-eGFP 3
	10.94 uL	T7-eGFP 4
	9.58 uL	T7-eGFP 5
2 uL buffer		
Water =	2.76 uL	2
	5.93 uL	3
	5.06 uL	4
	6.42 uL	5

Gel electrophoresis T7-eGFP (Claire)

Lane	1	ladder
	2	uncut T7-eGFP 2
	3	T7-eGFP 2
	4	T7-eGFP 3
	5	T7-eGFP 4
	6	T7-eGFP 5 (extract smallest band)



Gel extraction of lane 6 (T7-eGFP 5) (Claire)

Concentration = 23.1 ng/uL

Ligation T7-eGFP -- terminator (Claire)

Control
- 1 uL terminator (double digested)
- 0 uL T7-eGFP
- 2 uL buffer
- 16 uL H2O
- 1 uL ligase

3:1
- 1 uL terminator (double digested)

50 ng = 2.16 uL T7-eGFP
- 2 uL buffer
- 13.84 uL H2O
- 1 uL ligase

7:1
- 1 uL terminator (double digested)

115 ng = 4.98 uL T7-eGFP
- 2 uL buffer
- 11.02 uL H2O
- 1 uL ligase

Transformation: 5 uL of each reaction into 66.6 uL Cheryl's cells (Claire)

Collins plasmids in cell-free extract (Praneeth)

Substrate: Chlorophenol Red-β-D-galactopyranoside at 12 mg/mL (20x)

working concentration = 0.6 mg/mL

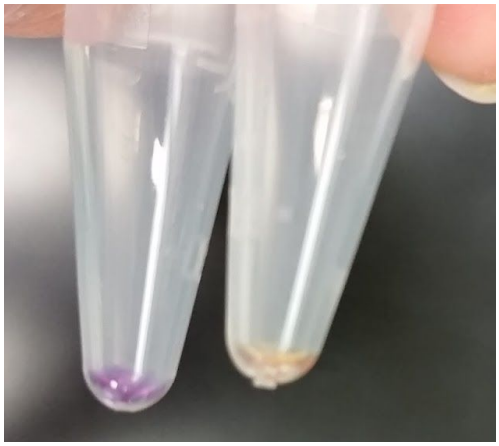
Switch: D_LacZ (13) Trigger: pAG_Ts2AT05 (14)

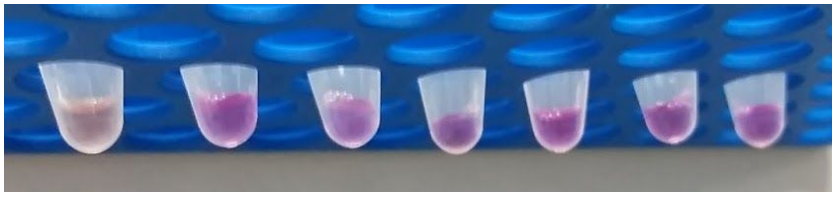
Amt trigger	**50 ng**	**25 ng**	**10 ng**	**0 ng**
Solution A	2	2	2	2
Solution B	1.5	1.5	1.5	1.5
RNAse	0.25	0.25	0.25	0.25
Switch (13-2) 50 ng	0.60	0.60	0.60	0.60
Trigger (14-1)	0.31	0.15	0.06	0.00
Substrate	0.25	0.25	0.25	0.25
Water	0.09	0.25	0.34	0.40

Switch: G_LacZ (15) Trigger: pAG_Ts2AT03 (15)

Amt trigger	**50 ng**	**25 ng**	**10 ng**	**0 ng**
Solution A	2	2	2	2
Solution B	1.5	1.5	1.5	1.5
RNAse	0.25	0.25	0.25	0.25
Switch (15-1) 50 ng	0.52	0.52	0.52	0.52
Trigger (16-2)	0.34	0.17	0.07	0.00
Substrate	0.25	0.25	0.25	0.25
Water	0.14	0.31	0.41	0.48

Incubate 2 hours at 37 degC; color change visible around 1 hour





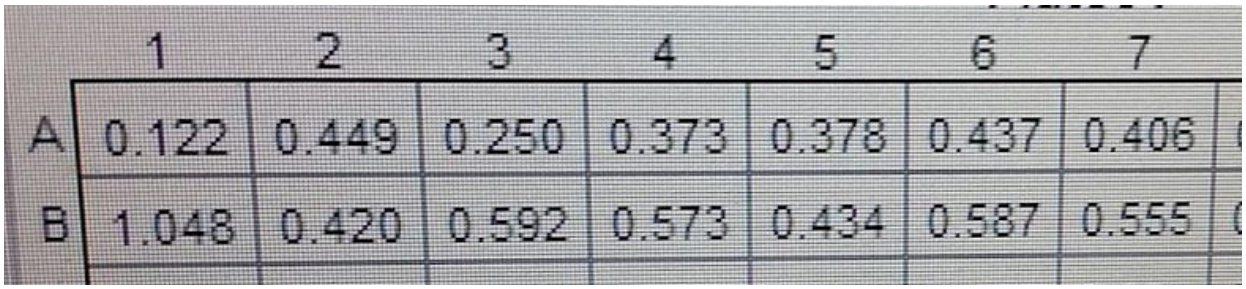
D plasmids (13 and 15), increasing concentration from left to right

G plasmids (14 and 16), increasing concentration from left to right

Clear plate:

	1	2	3	4	5	6	7
A	13-0	13-10 1	13-10 2	13-25 1	13-25 2	13-50 1	13-50 2
B	15-0	15-10 1	15-10 2	15-25 1	15-25 2	15-50 1	15-50 2

Add 20 uL water to each well to achieve plate reader's min concentration
Wavelength = 570 nm



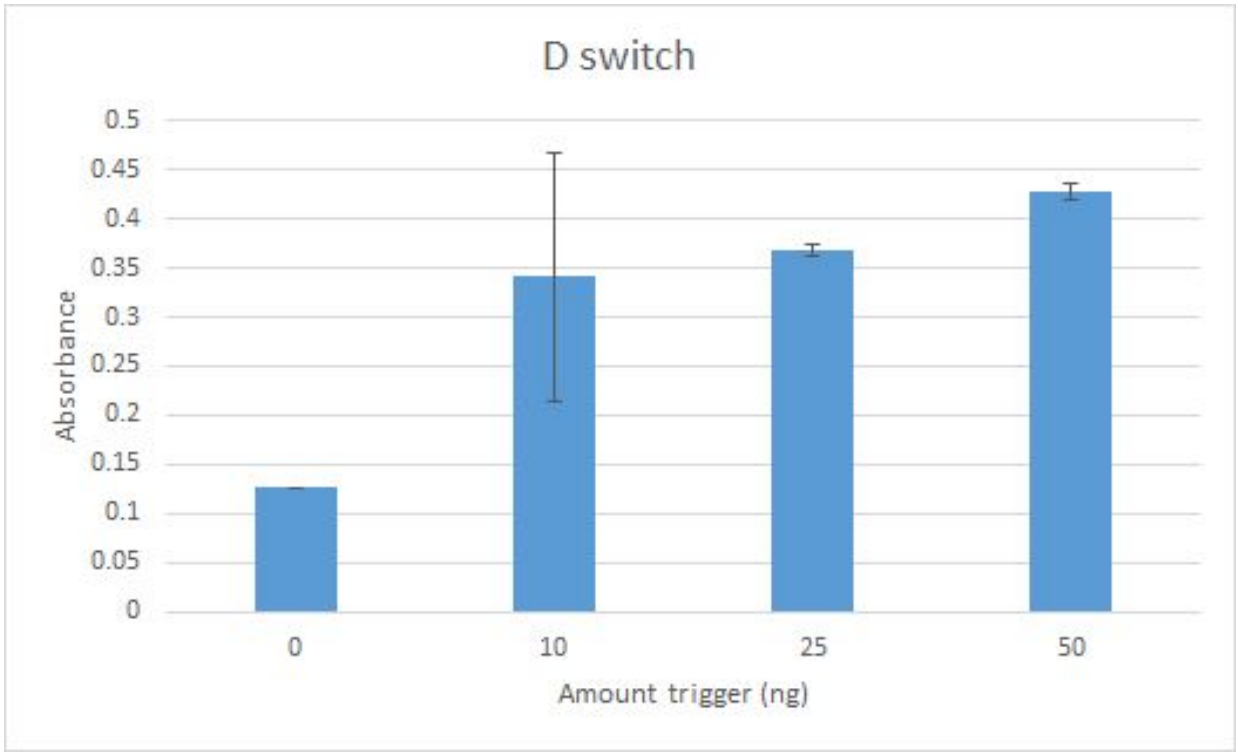
	1	2	3	4	5	6	7
A	0.122	0.449	0.250	0.373	0.378	0.437	0.406
B	1.048	0.420	0.592	0.573	0.434	0.587	0.555

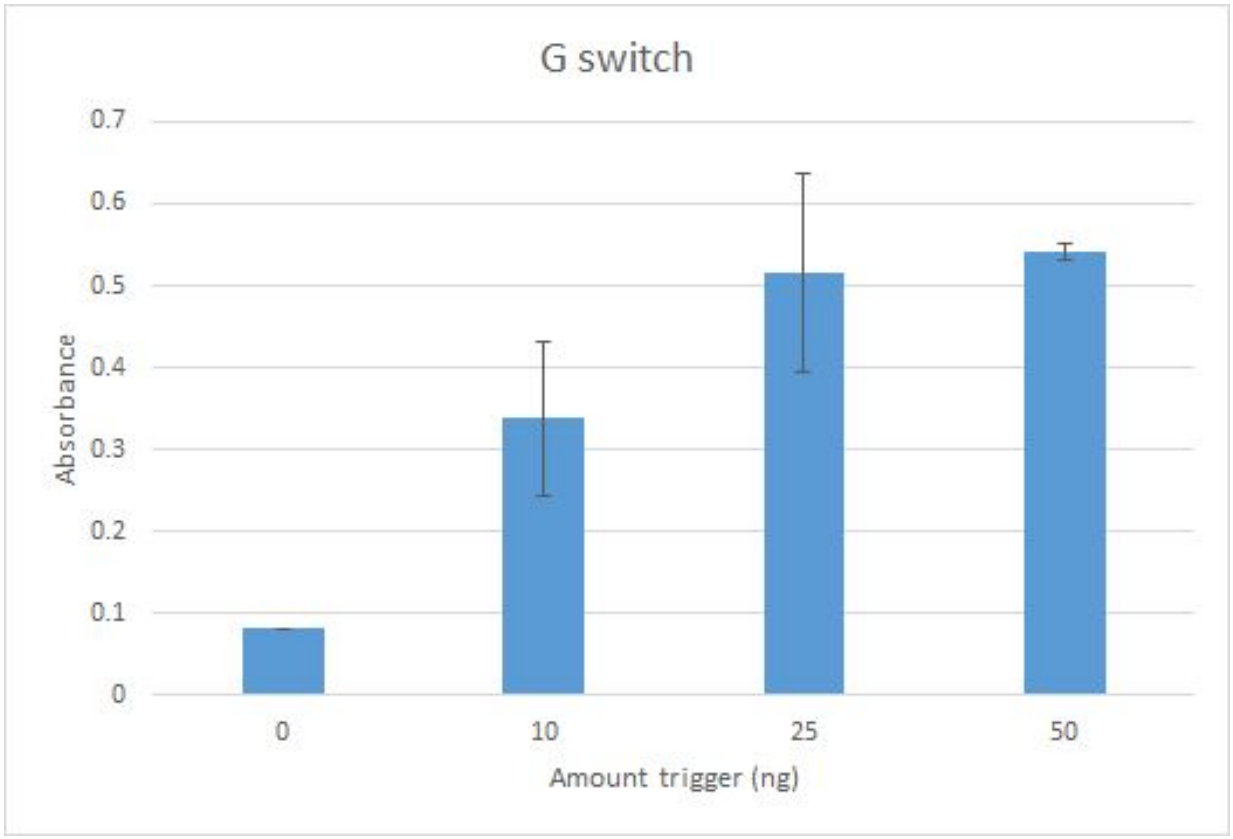
Wells not evenly covered, so add 25 uL more water (45 uL total)
Results from Roy lab plate reader similar, so Roy lab reader good for LacZ

	1	2	3	4	5	6	7
A	0.127	0.440	0.255	0.369	0.379	0.435	0.422
B	0.565	0.409	0.578	0.316	0.430	0.543	0.551

Pipette out bubbles from B1 and B3, read at Roy lab plate reader

	1	2	3	4	5	6	7
A	0.126	0.431	0.252	0.364	0.372	0.434	0.421
B	0.082	0.404	0.272	0.600	0.429	0.534	0.548





Error bars represent standard deviation

Inequivalence of samples of same concentration may be due to pipetting error--very small volumes of DNA (e.g., 0.06 uL) are difficult to achieve precision with. Next time, dilute DNA.

Friday, June 24

T7-eGFP -- terminator plates (growth on 3:1, none on 7:1, none on control!)

Mini-prep T7amilCP--terminator liquid cultures

Concentrations (ng/uL):	1	109.7
	2	167.9
	3	130.6
	4	175.5
	5	170.3

Digest mini-prepped DNA to check for ligations with EcoRI and PstI

0.5 uL EcoRI

0.5 uL PstI

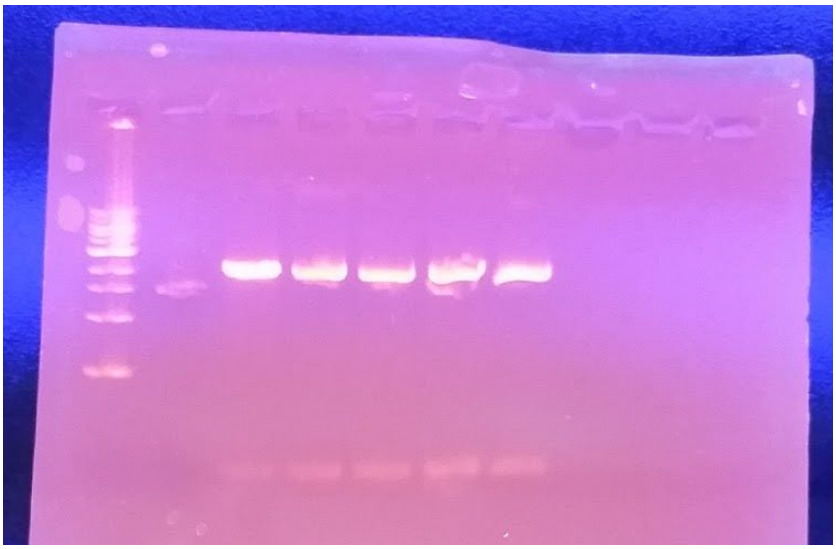
1 ug DNA =	9.12 uL	1
	5.96 uL	2
	7.66 uL	3
	5.70 uL	4
	5.87 uL	5

1 uL buffer

H2O =	0 uL	1
	2.04 uL	2
	0.34 uL	3
	2.30 uL	4
	2.13 uL	5

Gel electrophoresis of digests

Lane	1	2 uL ladder + 0.5 uL loading dye	
	2	2 uL MLK terminator (uncut) + 0.5 uL loading dye	
	3	1	
	4	2	
	5	3	
	6	4	
	7	5	No successful ligations?



LB + CM plates

Sunday, June 26

Liquid cultures (Maddie)

x:1 T7 amilCP -- terminator (x5)

3:1 T7 eGFP -- terminator (x5)