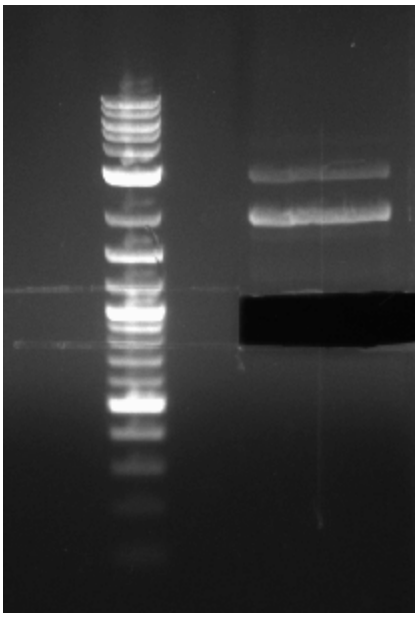
Day 35 - Monday- 07/21/14

- Alex and Chloe gel purified the CIP-treated 25K insert and ligated it into a Chlor vector. The resulting ligation was plated at two different concentrations

	25.1	25.2
pSB1C3 Volume (uL)	1	1
Insert Volume (uL)	3	6
dH2O Volume (uL)	16	10
Ligase Buffer Volume (uL)	2	2
Ligase Volume (uL)	1	1
Total Volume (uL)	20	20

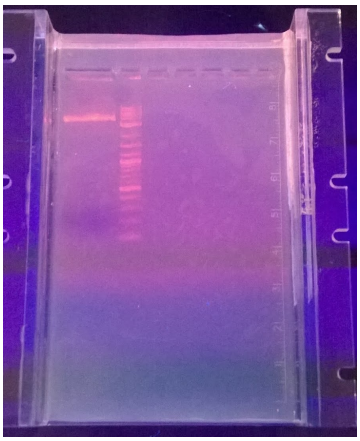
Gel Purification of 25K 7/21/14



- Various buffers for performing a Western Blot were also made.
- Kayla and Mike looked at the sequencing results for 26C minipreps 4 and 5. Miniprep 4 did not contain any parts of the banana odor construct while miniprep 5 contained the promoter with a prefix and a suffix. Thus, the promoter did not ligate to the RBS/ATF1/DT and create a scar site. To fix this problem, part 23C, the RBS/ATF1/DT, was digested again with EcoRI and XbaI. The annealed constitutive promoter oligos were also digested, but with EcoRI and SpeI. Both digests were left in the 37ºC water bath for one hour. Following the hour, 1 uL of CIP was added to the 23C digest and it was placed back

in the 37ºC water bath for an additional 20 minutes. The constitutive promoter digest was moved to the 80ºC water bath for 20 minutes. Once 23C and the constitutive promoter were digested, 5 uL of 6x LB were added to the 23C digest eppie and all 30 uL were loaded into a gel for gel purification. The gel was run at 72V for 45 minutes and the 4 kb band was cut out for gel purification. The gel can be seen below:

7/21/14 Gel Purification



After the DNA was purified from the gel, four ligations were set up. The ligation products were then used in 5 separate transformations. The contents of the ligation tubes can be seen in the table below.

7/21/14 Ligations

Tube	23C DNA (uL)	Arsenic Promoter DNA (uL)	Constitutive Promoter DNA (uL)
1	2	2	0
2	2	1	0
3	2	0	2
4	2	0	1
Control	2	0	0

- Shawna and Corbyn checked the sequence of 13C (BCLA+CAEV) and 27C (BCLA+CAEV) and made sure the reading frame was correct. Then the forms for the IRB as well as a solidified survey draft were completed and sent to Professor Farny. The miniprep tubes for both BCLA+CAEV and BCLA+YFP from the previous week were relabeled to 13C and 27 C, respectively and moved to the sequence confirmed box. The draft of the rainbow gel curriculum was updated and nearly completed, aside from the appendices. The survey questions were uploaded onto qualtrics as well. At the end of the

day,we liquid cultures 6 colonies from plate 3 of RBS+ Promoter and then we left them in the shaker overnight.

Day 36 - Tuesday - 07/22/14

- Kayla and Mike checked the constitutive promoter/RBS/ATF1/DT and arsenic promoter/RBS/ATF1/DT transformation plates. No colonies grew on the control plate that was transformed with the open plasmid, so all colony growth on other plates did not result from the plasmid ligating to itself. There were over 100 colonies on the two plates for the arsenic promoter/RBS/ATF1/DT. However, no colonies grew on the two plates for the constitutive promoter/RBS/ATF1/DT. Thus, 7 new constitutive promoter/RBS/ATF1/DT ligations were prepared according to the table below. Following the one hour bench top ligations, 7 transformations were performed with the ligated DNA. 6 colonies from the arsenic promoter/RBS/ATF1/DT plates were selected for liquid culture. Additionally, another trial of the agglutination quantification was started. The four eppies containing 600 uL of liquid culture, 600 uL of 5% BSA in PBS, and the varying amounts of antibody were prepared and initial ODs were measured before placing them on the rotator to rotate overnight.

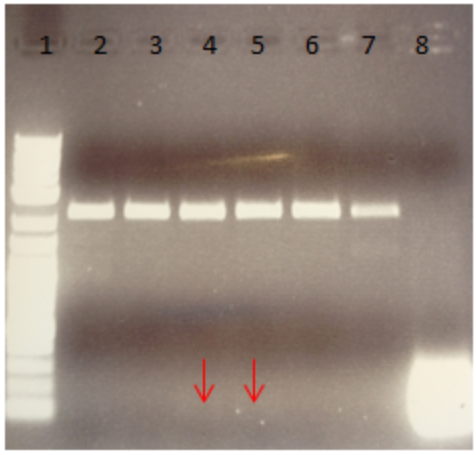
7/22/14 Constitutive Promoter/RBS/ATF1/DT Ligations

Tube	Constitutive Promoter Digest (uL)	RBS/ATF1/DT Digest (uL)
1	2	2
2	1	2
3	2	1
4	1	1
5	1	5
6	5	1
Control	0	2

- Alex and Chloe ran another set of samples on the plate reader, this time not making any dilutions and running 3 sets of 4 for each sample. This should be the final experiment for the interlab study, as it includes the rest of the data that we

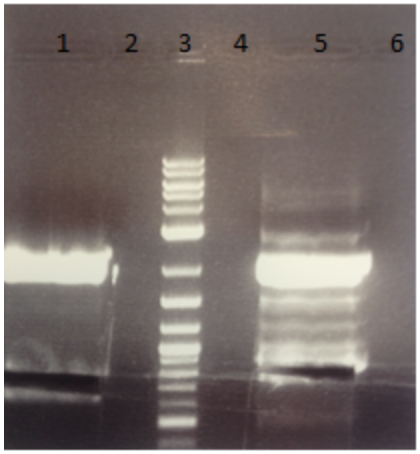
need to finish the documentation. Official documents for the interlab study were filled out. The immunofluorescence staining protocol (located in the protocol folder) was followed for the microscopy of 25K construct along with the 2.3B low promoter construct (used as a control because it was left over). There was too much cell death and the experiment was altogether a failure. The protocol will be updated after more research is done on more ideal conditions for the immunofluorescence staining of *E. coli*.

- Corbyn and Shawna started the day by collecting the 6 liquid cultures of what should be RBS+Promoter. We then miniprepped all 6 samples and then used the nanodrop to find their concentrations. We subsequently relabeled the tubes as 28A (1-6) and test digested with E and P for 1hr 20min. We ran the digested samples on a 1% gel at ~70V for 40 minutes, along with a promoter digest with E and S for a control. A picture of the gel with labeled lanes can be seen below, and you can see very faintly that it appears as there is a band the correct size (~100) in lanes 3 and 4. The last lane (promoter E-S) might be so bright because that was an older sample that was stored in the freezer for some time. We sent both 28A3 and 28A4 for sequencing.



Lane	Contents
1	Ladder
2	E-P digest 28A sample 1
3	E-P digest 28A sample 2
4	E-P digest 28A sample 3
5	E-P digest 28A sample 4
6	E-P digest 28A sample 5
7	E-P digest 28A sample 6
8	E-S digest Promoter

We also made a 50uL digest of both 13C4 and 27C4 with X and P with the intention of gel purification.After allowing digestion, we loaded the samples into the larger wells on a 1% agarose gel that ran at ~ 70 V for a little over an hour. We successfully cut out the bands and gel purified the samples, and then checked their concentration using the nanodrop. A picture of the gel after the bands had been cut out can be seen below.

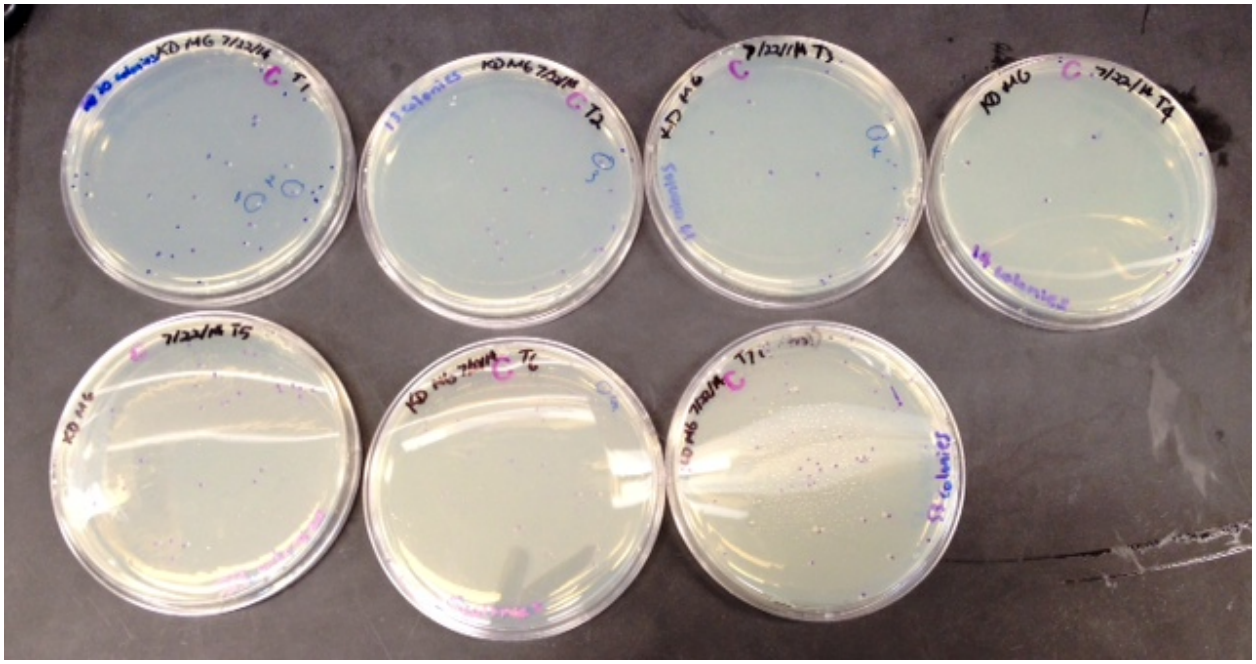


Lane	Contents
1	50uL 13C4 X-P
2	empty
3	ladder
4	empty
5	50uL 27C4 X-P
6	empty

At the end of the day, we prepared a 25uL digest of the two promising Promoter+RBS samples (28A3 & 28A4) with S-P and allowed them to sit in the 37 degree water bath overnight.

Day 37-Wednesday-7/23/14

- Kayla and Mike checked the plates for the constitutive promoter/RBS/ATF1/DT transformation. Unfortunately, the colonies that grew were small and barely visible, so all the plates were placed back in the warm room to allow for more growth. After an extended growth period, the colonies were counted. A photo of the plates and a table with colony counts can be seen below. Five liquid cultures were prepared from the larger colonies.
  7/23/14 Transformation Plates



7/23/14 Colony Counts

Plate	Colonies
1	30
2	13
3	17
4	14
5	28
6	25
7 (control)	53

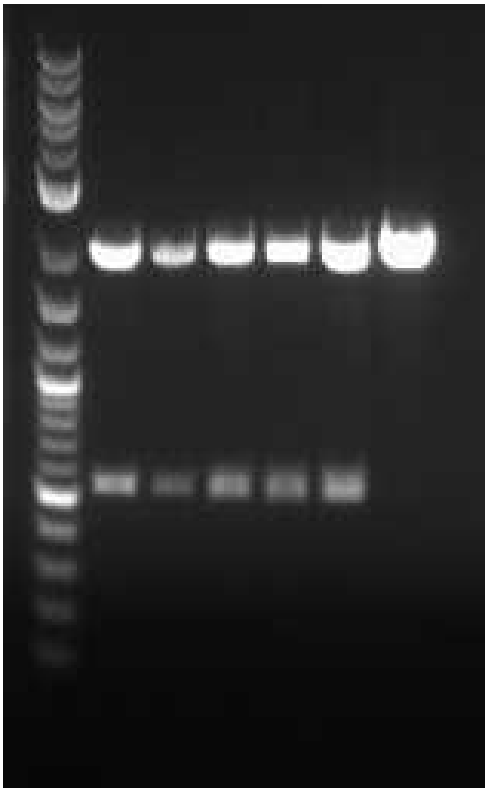
While the colonies were allowed to grow, six minipreps were prepared from the six 26C liquid cultures. The concentrations of the minipreps can be seen in the table below.

7/23/14 26C Miniprep Concentrations

Miniprep #	**Concentration (ng/ul)**
1	279.5
2	261.7
3	334.6
4	280.4
5	248.9
6	489.4

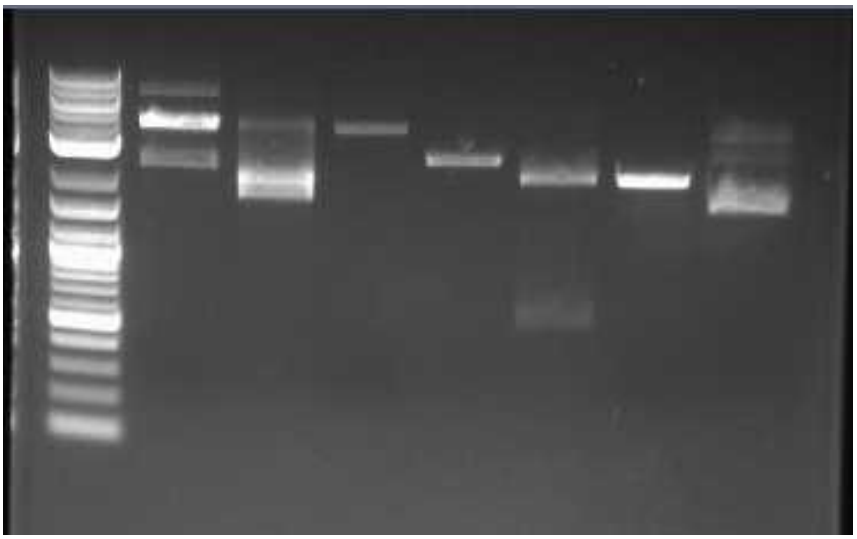
After the minipreps were made, six digests were set up. All six minipreps were digested with EcoRI and PstI for an hour and then run on a gel, which can also be seen below. Lane one contained the 2 log ladder and lanes 2-7 contained 26C minipreps 1-6 in order. Each lane on the gel had a band at about 2000 bp and 500 bp, so none of the minipreps had the correct insert.

7/23/14 26C Minprep Digests



In order to figure out what happened, the minipreps for 23C and 7C were each digested with EcoRI and XbaI and EcoRI and PstI. These digests along with the 23C and 7C gel purified DNA were run on another gel, which can be seen below.

7/23/14 7C and 23C Test Digests



Gel Lanes

Lane	Contains
1	Marker
2	23C (mini) Digested with E+X
3	23C(mini) Digested with E+P
4	Gel Purified 23C
5	7C (mini) Digested with E+X
6	7C (mini) Digested with E+P
7	Gel Purified 7C
8	7C Digested with E+S (Old Digest)

Based on the size of the gel purified 7C band, the wrong band was cut out of the gel during gel purification. The gel purified DNA should have been 500 bp in length rather than about 2000 bp in length. Unfortunately, this result does not explain why only the pARS sequence was found on the latest DNA sampling sent to Eton.Because the original gel purification product was incorrect, a new 40 uL digest of 7C was prepared and placed in the 37^{o}C water bath for the night.
Finally, Kayla passed the samples for the agglutination quantification experiment through 30 micron columns and measured the final OD. There was no difference between the final ODs of the 3 samples, so the protocol will have to be amended again or abandoned altogether.

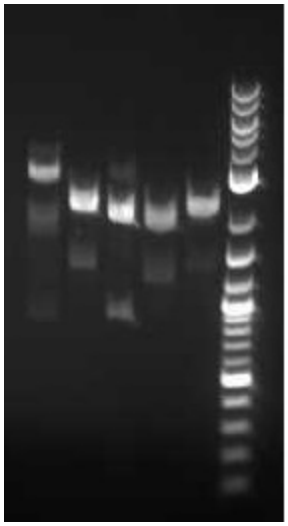
- Shawna and Corbyn started the day by checking the sequence of their promoter to RBS ligation (28A3 and 28A4). Both 28A3 and 28A4 were confirmed as the correct sequence. They then heat killed the digestion of those two constructs from the previous day in 80degrees for 20 minutes. 28A4 was used in the following ligations.

Tube	Promoter/RBS digest (uL)	BCLA YFP Digest (uL)	BCLA/CAEV Digest (uL)
C1	2	0	2
C2	1	0	2
C3	2	0	1
Y1	2	2	0

Y2	1	2	0
Y3	2	1	0

Once these ligations had run for just over an hour, they were transformed into competent *E. coli*. We also transformed the S-P digest of 28A4 as a control. The expected constructs are : Promoter-RBS-BCLA-CAEV in pSB1A3 and Promoter-RBS-BCLA-YFP in pSB1A3 .The transformed *E. coli* were plated on ampicillin in the afternoon and stored in the warm room overnight. During the day Shawna and Corbyn completed the edits to the Qualtrics survey, and prepared a word document with the questions to send to the IRB.

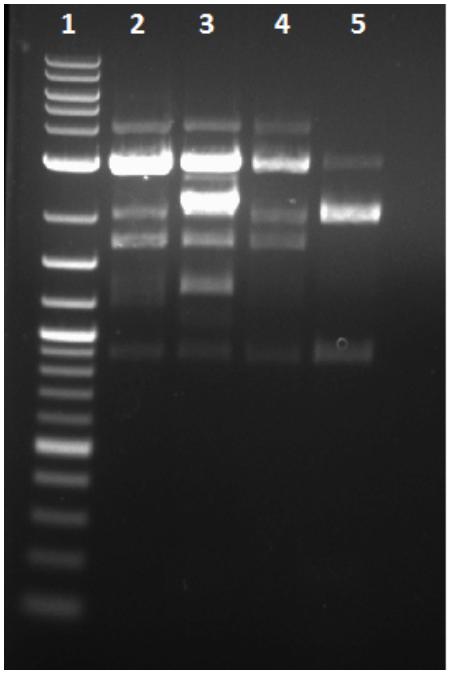
- Alex and Chloe prepared a miniprep of the 5 liquid cultures from the ligation of 25K into the Chlor vector. Then, a test digest was run to check if the insert was present. The resulting gel was very strange, so 3 of the samples were sent for sequencing to double check for correctness. As a precaution, 5 new colonies were picked and liquid cultured from the opposite ligation plate.



Lane	Contents
1	25C (colony 1)
2	25C (colony 2)
3	25C (colony 3)
4	25C (colony 4)
5	25C (colony 5)
6	Ladder

Day 38-Thursday-7/24/14

- Alex and Chloe checked the sequences for colonies 1, 2, and 3 of 25C and confirmed that the correct insert was present in colony 1 and 3, though 3's sequence was not as accurate as 1's sequence. A new test digest was prepared of both colony 1 and 3 and ran on a gel next to the old 1 and 3 digests. Both digests of colony 1 looked the same on the gel, and the sequence had already been confirmed as correct. Based on this, it was determined that there is a problem with one of the enzymes, not the clone itself.



Lane	Contents
1	Ladder
2	New Digest of 25C Colony 1
3	Old Digest of 25C Colony 1
4	New Digest of 25C Colony 3
5	Old Digest of 25C Colony 3

- Alex and Chloe also conducted a test on antibiotics to see if antibiotic stocks left out overnight were actually less effective than stocks kept frozen. 4 Liquid cultures were prepared of 25C (because they would be needed tomorrow anyway) and either fresh chloramphenicol kept frozen or chloramphenicol, ampicillin or kanamycin left out overnight was mixed in to each culture. Because we have been getting strange results with digests lately, a large series of test digests was prepared to check if all of the enzymes are working correctly. Using 25C and 23C, two sets of six digests were set up. The tubes all contained 500 ng DNA, 5 uL NEB Buffer, and 1 uL BSA, like a standard restriction digest, but tube 1 was EcoRI only, tube 2 was XbaI only, tube 3 was SpeI only, tube 4 was PstI only, tube 5 was both X and S, and tube 6 was also both X and S, but contained suspicious BSA from a new aliquot we had used for the first time with the strange-looking digests. These were left in 37 degrees overnight.
- Shawna and Corbyn collected their plates from the warm room. Each of the plates had over 100 colonies except for the Y1 plate which had ~30 colonies. They began to work on the parts information for the biobrick that will be submitted. They compiled a list of emails for contacting farmers in the New England area. At the end of the day 6 colonies were chosen from both the C2 and the Y2 plate and liquid cultured overnight in the shaker. Shawna and Corbyn also prepared overnight digests of 28A (E-S) and the BCLA biobrick

(X-P) to begin construction of a Promoter-RBS-BCLA biobrick.

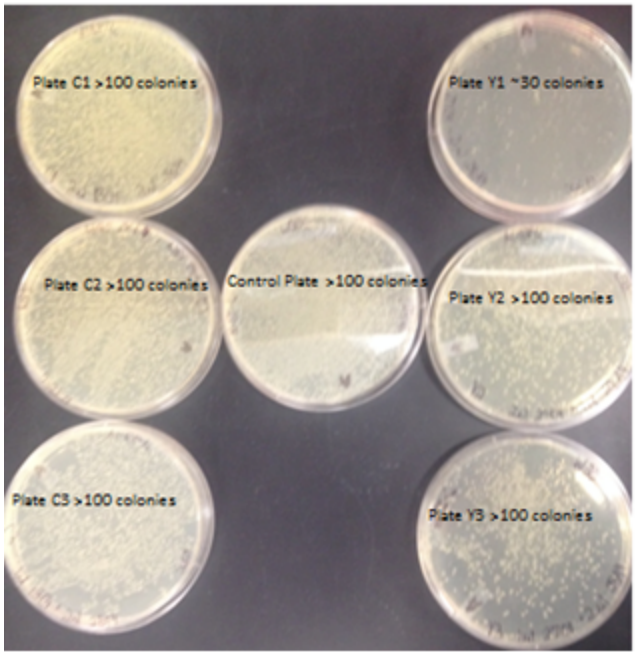


Plate	# of Colonies
C1	>100
C2	>100
C3	>100
Y1	~30
Y2	>100
Y3	>100

- Kayla and Mike made a gel to gel purify the new 7C digest. The gel was run at 72V for one hour, and the band at approximately 500 bp was cut out and placed in a conical tube for purification. The newly gel purified DNA was used in 4 ligations, which were prepared according to the table below. 4 transformations were performed with the ligation DNA.

7/24/14 Ligations

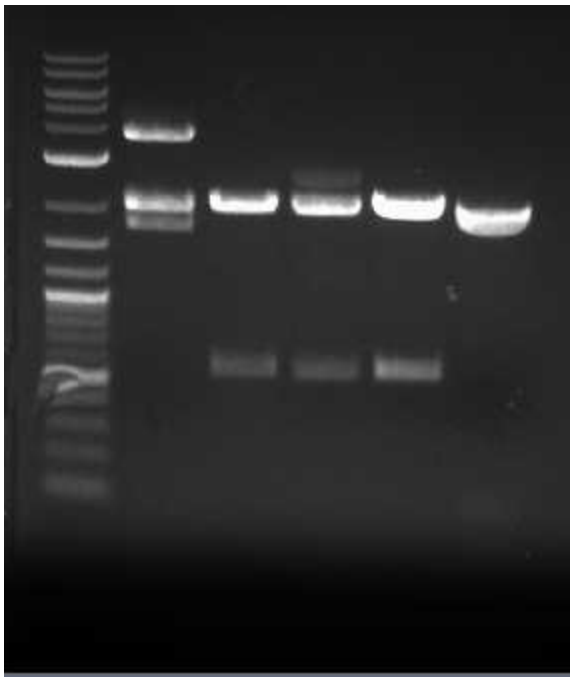
Ligation	7C DNA (uL)	23C DNA (uL)
1	2	2
2	1	2
3	2	1
4 (Control)	0	2

Additionally, 5 minipreps were made from the 29C liquid cultures (constitutive promoter/RBS/ATF1/DT). The concentrations of the five minipreps can be seen in the table below. Once the minipreps were made, five 25 uL digests with EcoRI and PstI were prepared. These digests were then run on a gel at 72V for 1 hour. The first lane contained the 2 log ladder while lanes 2-6 contained minipreps 1-5. The picture of the gel can be seen below. Because the miniprep 1 digest had 2 bands around 2 kb, it was sent off for sequencing.

7/24/14 29C Miniprep Concentrations

Miniprep	Concentration (ng/uL)
1	351.3
2	200.1
3	276.2
4	303.3
5	209.8

7/24/14 Test Digest



Day 38-Friday-7/25/14

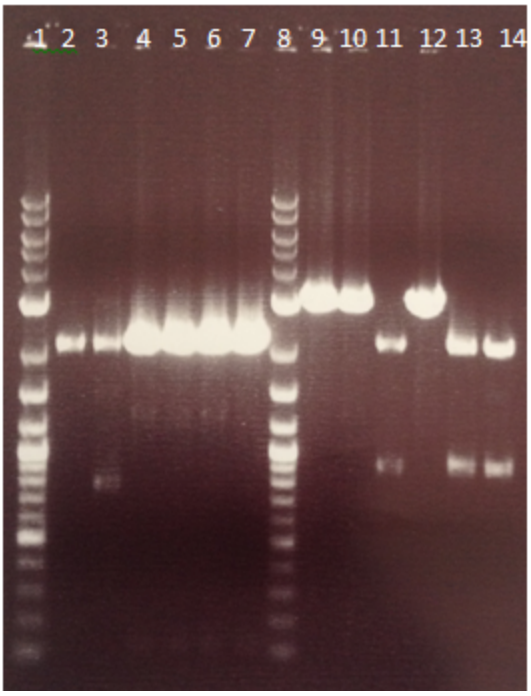
- Alex and Chloe ran a gel on the restriction digests that had been left overnight, determining that the problems we'd encountered with restriction digests had been because our EcoRI is not working properly. A new protocol for fixation was developed and a quick test was run on 17K. The process seemed to work much better than our previous protocol, though the 17K was very dim under the microscope. This was probably the fault of the stock itself, not the fixation process. Another attempt at immunofluorescence staining 25C was made.

- Corbyn and Shawna collected the liquid cultures of P+RBS+BCLA+CAEV and P+RBS+BCLA+YFP and then miniprepped them. We checked the concentrations with the nanodrop and then relabeled these tubes as P+RBS+BCLA+CAEV (29A1-6) and P+RBS+BCLA+YFP (30A1-6). We also heat killed the 28A3 (E-S) digest and BCLA BB3 (X-P) digestions that had been digesting overnight. Then, we performed ligations of the

Promoter-RBS complex into the BCLA BB. The table below shows the ratios of ligations we performed, transformed, and then plated. We also transformed and plated BCLA BB3 X-P digest as a control. All 4 plates were left on the benchtop to grow over the weekend.

Tube	BCLA BB (uL)	P-R (28A3) [uL]
1	2	2
2	1	2
3	2	1

We also performed an E-P test digest of all 6 29A and 30A samples. The digests were run on a 1% gel at 71 V for about 1.5 hours. A picture of the gel can be seen below, and based on its results we sent samples 29A2 as well as 30A3,5,and 6 for sequencing. I also sent 29A1 for sequencing just in case.



Lane	Contents
1	Ladder
2	$29A_1$
3	$29A_2$
4	$29A_3$
5	$29A_4$
6	$29A_5$
7	$29A_6$
8	Ladder
9	$30A_1$
10	$30A_2$
11	$30A_3$
12	$30A_4$
13	$30A_5$
14	$30A_6$