Day 16

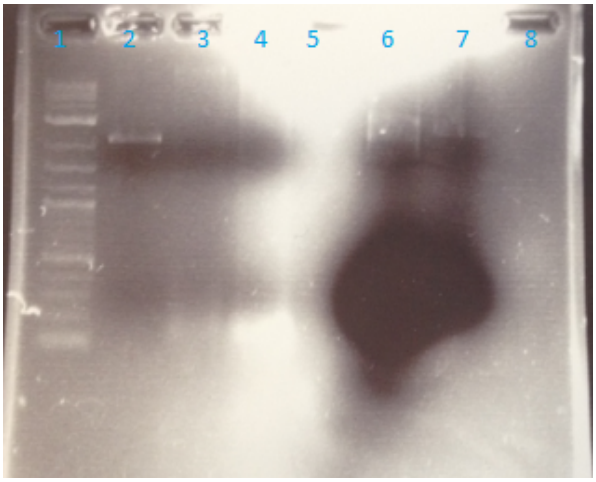
Team meeting

- Kayla and Mike started off by preparing digests of part 16K and the linearized plasmid pSB1C3. The amended digest protocol, which can be found in the protocol folder, was followed. The second 16K miniprep, which had a concentration of 64.3 ng/uL, was used. Therefore, the volume containing 500 ng of DNA was 7.88 uL. Following the digest, 4 ligations were set up. The volumes added to each ligation tube can be seen in the table below. Following the ligations, 4 transformations were set up according to the iGEM transformation protocol.

  Ligation Volumes

	Tube 1	Tube 2	Tube 3	Tube 4
pSB1C3 Volume (uL)	2	2	2	2
16K Volume (uL)	2	2	2	2
RBS Volume (uL)	0.5	1	2	5
dH2O Volume (uL)	12.5	12	11	8
Ligase Buffer Volume (uL)	2	2	2	2
Ligase Volume (uL)	1	1	1	1
Total Volume (uL)	20	20	20	20

- Alex and Chloe began preparing the cells for the Interlab Measurement Study. Parts 17K, 18C, 19C, and 20C were transformed into competent cells and plated. A liquid culture of 14K was prepared to be used with 5M salicylic acid dissolved in DMSO tomorrow. A liquid culture of 5A was prepared to be used in an agglutination assay tomorrow.
- Shawna and Corbyn digested the PSB1C3 backbone with E and P as well as BCLA and CAEV in an attempt to create biobricks once again, because the first attempt had failed (as confirmed by sequencing). The results of this digest were run on a gel along with a digest of BCLA with E and S. The gel was inconclusive, and can be seen below with labeled lanes. The blur may be due to the fact that the wells 6 and 7 exploded when being loaded, or had too much dye. In order to solve the theorized issue of extra restriction sites possibly being in the BCLA stock, Corbyn and Shawna performed a PCR using the prefix and suffix primers on the BCLA stock. This should take the BCLA out of the backbone provided by IDT, allowing the correct digestions in the future. The samples from the digest and the PCR will be run on a gel in the morning. The primer labels for the prefix and suffix primers are iGEM1 and iGEM7.



Lane	Contents
1	Ladder
2	BCLA E-S
3	BCLA E-P
4	CAEV E-P
5	skip
6	ATF1
7	ATF1 DT

- Professor Farny and Michael discussed ways to quantify the results of our agglutination assays. We found some suitable paper centrifuge filters at 10 microns, and another set of polyethylene filters with 30 micron pores, these were ordered and will be tested later in the week.

Day 17

- Alex and Chloe prepared a 1:10 dilution of the liquid culture of 5A for an agglutination assay test. The 14AK culture was diluted 1:5 and incubated for 3 hours to an OD of 0.6. Various solvents containing Salicylic Acid were combined with the culture and grown for 2 hours. The dilutions are shown in the following table.

	Control	DMSO	75% EtOH	95% EtOH
Concentration of Salicylic Acid	None	5M	1M	1M
Smell	No	Medium	Strong	Stronger

- The 5A culture was incubated for 5 hours to an OD of 1.3. The results of the agglutination assay test are shown in the following table:

	1:100 Antibody	1:1000 Antibody	1:10000 Antibody
1:1 Overnight E.coli Control	Pink Dot	Pink Dot	Pink Dot
1:1 Overnight E. coli Ab	Wide Pink Dot	Pink Dot	Pink Dot
2:1 Overnight E. coli Control	Faint Pink Dot	Faint Pink Dot	Faint Pink Dot
2:1 Overnight E. coli Ab	Wide Faint Pink Dot	Faint Pink Dot	Faint Pink Dot
1:1 Back Dilution E. coli Control	Faint Pink Dot	Faint Pink Dot	Faint Pink Dot
1:1 Back Dilution E. coli Ab	Wide Faint Pink Ring/Dot	Wide Faint Pink Dot	Faint Pink Dot
2:1 Back Dilution E. coli Control	Faint Pink Dot	Faint Pink Dot	Faint Pink Dot
2:1 Back Dilution E. coli Ab	Wide Faint Pink Ring	Wide Faint Pink Ring	Faint Pink Ring

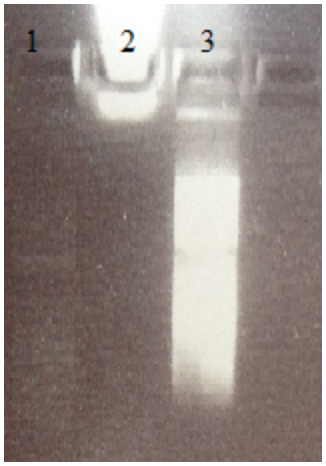
- Finally, liquid cultures for the four Interlab study cultures were created.
- Kayla and Mike counted colonies on the four transformation plates. Colonies grew on three out of the four plates, so the transformation of *E. coli* with the RBS/ATF1/Double Terminator construct (part 21C) was successful. Five liquid cultures were prepared in the afternoon.

Kayla and Mike's Transformation Results

	Plate 1	Plate 2	Plate 3	Plate 4
RBS Ligation Volume (uL)	0.5	1	2	5
16K Ligation Volume (uL)	2	2	2	2
pSB1C3 Ligation Volume (uL)	2	2	2	2
Transformation Volume Plated (uL)	250	250	250	250
Number of Colonies	~140	6	12	0

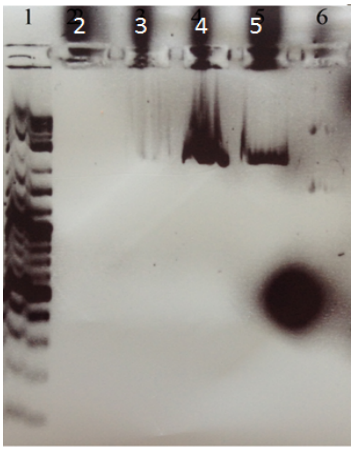
- Corbyn and Shawna started the day by running a gel of BCLA digested with E-S, BCLA digested with E-P, the PCR of BCLA, and the E-P digestion of CAEV. This gel had very inconclusive results due to the improper loading of some adjacent wells, so no picture was taken. The BCLA PCR was run on another gel and the result showed that the PCR was unsuccessful, the PCR of BCLA did not even create a band on the gel . A picture of the resulting gel can be seen below with labeled lanes.

Lane	Contents
1	Ladder w/o ETBR
2	BCLA from PCR
3	Ladder w/ ETBR



Single digests of the BCLA miniprep were prepared with each of the enzymes(E,X,S,P) and a larger double digest of the BCLA (50ul) was prepared with E-P (this larger double digest was left in the 37 water bath over night).The single digests were run on a gel along with the uncut BCLA. A picture of the resulting gel can be seen below, with labeled lanes. It revealed that most

of the restriction sites were working, although the lane E did not show any bands.Also, it showed two bands for the BCLA uncut, both circular and supercoiled.



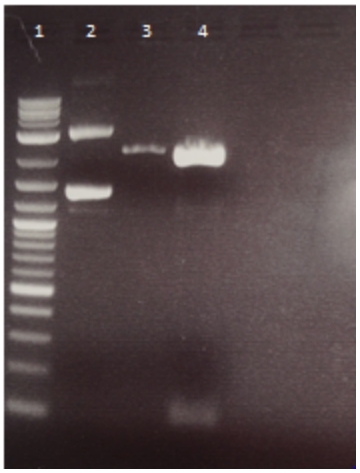
Lane	Contents
1	Ladders
2	BCLA digest w/E
3	BCLA digest w/X
4	BCLA digest w/S
5	BCLA digest w/P
6	uncut BCLA

The CAEV piece was digested with E-P and then ligated and transformed into competent pSB1C3 cell room to grow overnight.

Day 18

- Corbyn and Shawna ran a 1.5% agarose gel at 71volts containing: Ladder, BCLA miniprep uncut, BCLA single digest with E , and BCLA double digest with E-P. The picture of the gel with labeled lanes can be seen below. Using this gel they determined that the BCLA had successfully been cut out of the vector and now they can move on with that BCLA E-P digest to create the BCLA biobrick. The digested BCLA (E-P) was ligated into pSB1C3 backbone and transformed in competent E. coli. These cells were plated (5 different concentrations) and left in the warm room overnight.They collected their CAEV transformation plate from the overnight incubation in the warm room. They counted 9 colonies on the plate. (Picture can be found in the plates picture folder from

this week). At the end of the day, we performed liquid cultures on the CAEV transformation plate (using 3 colonies) and put them in the shaker overnight.



Lane	Content
1	Ladder
2	Miniprep of BCLA
3	E digest BCLA
4	E+P digest BCLA

- Kayla and Mike prepared minipreps of the five liquid cultures of *E. coli* transformed with part 21C (RBS+ATF1+DT). The DNA concentrations, in ng/uL, of the five minipreps can be seen below. Miniprep 3, which had the highest DNA concentration, was then used to make a digest. 3.44 uL of miniprep 3 were added to the digest tube to get the required 500 ng of DNA. It was then digested with XbaI and PstI. After the new plasmid was digested, a ligation reaction with the new digest, the pSB1A3 digest, and the arsenic promoter (part 7C) was performed. Following the ligation, a transformation was performed with the newly ligated plasmid.

  Miniprep DNA Concentration

	DNA Concentration (ng/uL)	500 ng Volume (uL)
Miniprep 1	95.5	5.24
Miniprep 2	81.6	6.13
Miniprep 3	145.3	3.44

Miniprep 4	109.4	4.57
Miniprep 5	108.9	4.59

Ligation Volumes

	Tube 1	Tube 2	Tube 3	Tube 4
pSB1A3 Volume (uL)	2	2	2	2
21C Volume (uL)	2	2	2	2
7C Volume (uL)	0.5	1	2	5
dH2O Volume (uL)	12.5	12	11	8
Ligase Buffer Volume (uL)	2	2	2	2
Ligase Volume (uL)	1	1	1	1
Total Volume (uL)	20	20	20	20

- Alex and Chloe Miniprepped and Digested the 18C, 19C, and 20C cultures for the Interlab study. Ligations were performed with the following volumes:

	Promoter 1.1	Promoter 1.2	Promoter 2.1	Promoter 2.2
pSB1C3 Volume (uL)	2	2	2	2
18C Volume (uL)	1	2		
19C Volume (uL)	2	2	2	2
20C Volume (uL)			1	2
dH2O Volume (uL)	12	11	12	11
Ligase Buffer Volume (uL)	2	2	2	2
Ligase Volume (uL)	1	1	1	1
Total Volume (uL)	20	20	20	20

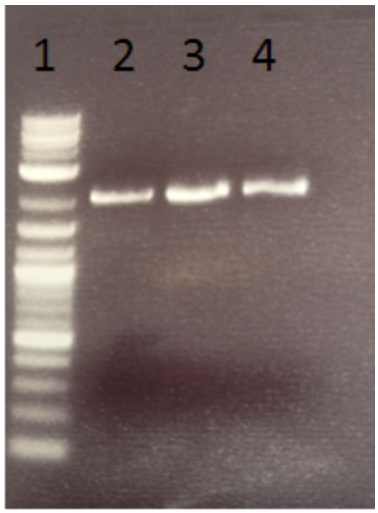
The ligation results were then transformed into competent cells and plated.

Day 19

- Shawna and Corbyn collected their BCLA plates and counted the colonies.

Plate 1	5 colonies
Plate 2	5 colonies
Plate 3	2 colonies
Plate 4	137 colonies
Plate 5	145 colonies

In the afternoon 5 liquid cultures of BCLA were prepared from plate 5. Minipreps were prepared of the CAEV liquid cultures from Wednesday. The minipreps were digested with E and P. They were then run on a 1.3% agarose gel at 71V for an hour and photographed using the gel doc system. (Picture below). As you can see, all three lanes produced bands one band, indicating that the CAEV insert did not insert into the backbone.



Lane	Contents
1	Ladder
2	CAEV E-P 1
3	CAEV E-P 2
4	CAEV E-P 3

In the afternoon digests of the pSBC13 backbone (E-P), pSB1K3 backbone (E-P), CAEV (E-P), RBS(X-P), and promoter (E-S) were prepared. These were left at 37 degrees overnight to digest.

- Alex and Chloe checked the transformation plates containing the Interlab Study ligations. All plates had 100+ colonies, but the plates with the ligations that had lower volume of the promoter had noticeably higher growth.

- Kayla and Mike checked the 22A transformation plates from the day before. There was no growth on plates 1 and 4, but plates 2 and 3 showed colony growth. The table below shows colony counts for each plate. Liquid cultures were also made from five different colonies on plate number 3.

  6/26/14 22A Transformation Colony Counts

	Colonies
Plate 1	0
Plate 2	1
Plate 3	41
Plate 4	0

- In exploring the O/D filter based agglutination test, Kayla and Mike ran a few spin tests on some unused RFP E.Coli. The table below details the trials. It was discovered that 1 minute at 2000 rpm is optimal for the column with 30 micron pores and 1 minute at 1000 rpm is optimal for the column with 10 micron pores.

Time (min/sec)	RPM	Filter(s) Used	Notes
:30	100	10micron, 30micron	no flow through
:30	500	10micron, 30micron	minimal flow through in10micron filter
:30	1000	10micron, 30micron	Minimal flow through in both
:30	1250	10micron, 30micron	Small pellet in 10micron, large pellet in 30micron
1:00	1000	10micron, 30micron	minimal flow through in 30micron, half flowed through in 10micron
+:30(1:30)	1000	10micron, 30micron	same
+30(2:00)	1000	10micron, 30micron	small pellets in both

+5:00(7:00)	500	10micron, 30micron	same
+5:00(12:00)	750	10micron, 30micron	same
5:00	1000	30micron	minimal flow through with small pelet
+5:00	2000	30micron	complete flow
5:00	2000	30micron	Pellet and half flow through
1:00	2000	30 micron w/ 400 uL	complete flow through, no pellet
1:00	1000	10 micron w/ 400 uL	complete flow through, no pellet

Day 20:

- Shawna and Corbyn started the day by heat killing the digestions of PSB1C3, PSBIK3, CAEV, RBS, and promoter from the day before. These were then ligated into the appropriate backbones (CAEV into the PSBIC3 backbone and RBS&promoter into the PSB1K3 backbone)and transformed into competent cells. The transformed cells were plated and then left to incubate on the bench over the weekend. Minipreps of the five liquid cultures of BCLA from the day before were prepared, and each sample was also put on the nanodrop to determine the DNA concentration.
- Kayla spent the day testing culture retention with the spin columns. 2.5 mL of the overnight liquid *E. coli* culture were transferred to a new tube containing 22.5 mL of liquid LB to make a 1:10 dilution. Then, 800 uL of the original liquid culture were added to six cuvettes. The OD at 595 nm of the liquid in each cuvette was measured. The liquid in the cuvettes were then divided into 12 spin columns. Samples were split up into two 400 uL volumes and added to two of the same type of spin column. Thus, there were 6 30 micron columns and 6 10 micron columns, each containing 400 uL of liquid culture. The 30 micron columns were then spun at 2000 rpm for 1 minute while the 10 micron columns were spun at 1000 rpm for 1 minute. Once the liquid had passed completely through the columns, the liquid was poured back into its corresponding cuvette. The optical density of each sample was measured again and recorded so percent retention could be calculated. This procedure was repeated with the 1:10 dilution prepared at 9:30, and then again at 12:30 and 2:00. The results of this experiment can be seen in the Excel spreadsheet titled Column Testing in the week four folder.
- Alex and Chloe Miniprepped and ran a gel with the two Interlab Study constructs. The cultures were then read from a 96-well plate using the plate reader.
- Michael took the 5 liquid stocks(Ars-RBS-Ban-DT) from the previous day, and created 5 minipreps. The end concentrations are in the table below:

Miniprep #	Concentration: micrograms/microliter
1	.12
2	.08
3	.10
4	.15
5	.08

- Michael Created a restriction digest of the materials in tube 4 and injected it into the gel that Alex and Chloe ran @71 volts for ~30 minutes. The results appear to show a plasmid, but in only one piece. Another restriction digest will be created on Monday.