Day 11

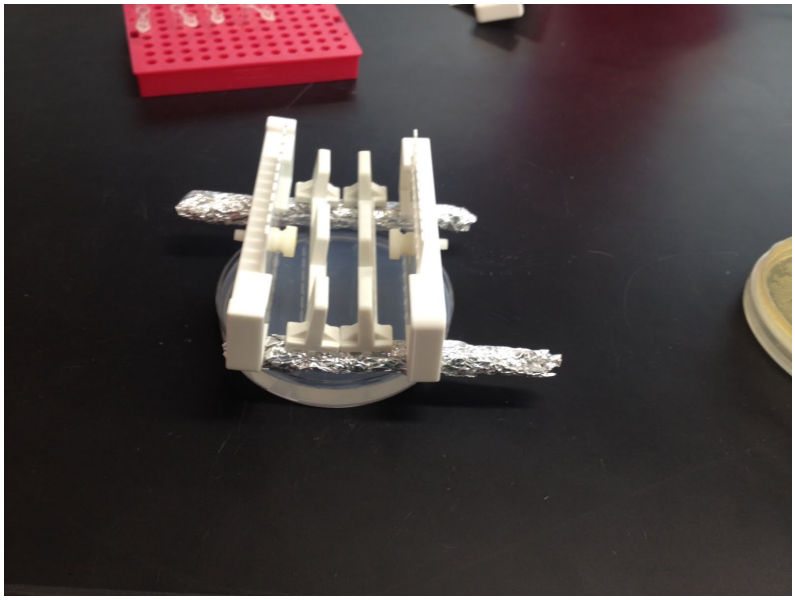
- Kayla and Mike worked on a survey for local farmers and the PowerPoint presentation for Friday's NEGEM meeting at Boston University on Friday. Three liquid cultures of the *E. coli* transformed with the ATF1/Double terminator ligation were made. Lists of local goat farmers was and potential survey questions were also made.
- Shawna and Corbyn ligated RBS to the BCLA+CAEV fragment into an ampicillin resistant backbone (15A). Then, they transformed the ligation into competent E.coli and plated them and let them grow overnight in 37°C.
- Alex and Chloe prepared a liquid culture with the 5A (Red High Expression Constitutive Promoter) and with the 13/14AK (salicylic acid carboxyl methyltransferase). A third liquid culture that was identical to the first 13/14AK culture was prepared and 30 mg of acetylsalicylic acid was added. A test agglutination assay was also performed and is shown in the following table: (All paraformaldehyde wells contained indiscernible undyed clumps)

	1:100	1:200	1:400	1:800	1:1600	1:3200
E (Heat Fixed Monoclonal Control)	Small Clump	Med Clump	Med Clump	Med Clump		
F (Heat Fixed Monoclonal)	Large Clump	Large Clump	Large Clump	Large Clump	Large Clump	Large Clump
E (Heat Fixed Polyclonal Control)	Small Clump	Small Clump	Med Clump	Med Clump		
F (Heat Fixed Polyclonal)	Med Clump	Med Clump	Med Clump	Med Clump	Med Clump	

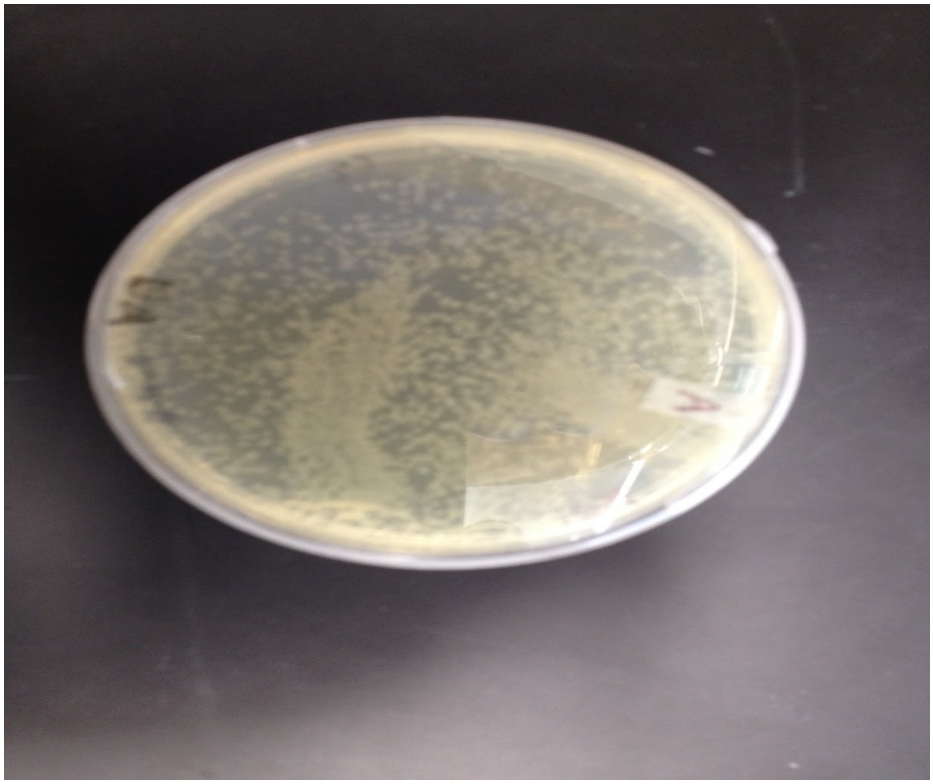
Day 12

- The team met with Professor Duffy and discussed where they had accomplished thus far and what they were each working on.

- Shawna and Corbyn made and tested a practice gel for the "women in science" camp. (See picture below) The protocol was subsequently altered slightly. The 15A plate was collected from the warm room and checked for colonies. There were many colonies on the plate. (see picture below) Three colonies were selected for liquid culture in the afternoon and placed in the 37°C shaker to incubate overnight. The BCLA and CAEV biobrick minipreps as well as the BCLA-CAEV ligation miniprep were used to prepare samples for DNA sequencing and sent to Eton Biotech. The protocol for DNA sequencing was added to the protocol folders of the google drive.



Practice Rainbow Gel



15 A Plate after ligation

- Alex and Chloe prepared a 1:10 back dilution of both the 5A and the 13/14AK cultures. The third culture with the acetylsalicylic acid did not grow. A glycerol stock of the 14AK was prepared. A miniprep for the 14AK culture was performed, but the yield was very low; at 29 ng/ml. A test agglutination assay was set up using 5A bacteria and an E. coli antibody.

- Kayla and Mike prepared three minipreps of the plasmid containing the ATF1 gene and the double terminator from the three liquid cultures. The DNA concentration of each miniprep was measured. All of the DNA concentrations were well below the expected concentration of 100 ng/uL or greater, so a digest could not be performed. Instead, three new liquid cultures were prepared. A tube library for the freezer boxes was also created.

Day 13

- Kayla and Mike repeated the miniprep procedure with the overnight liquid cultures. Once again, the DNA concentrations of the miniprep were all about 20 ng/uL, so digests could not be prepared. Fortunately, we discovered that ethanol had not yet been added to the PE buffer in the new miniprep kit. Three more liquid cultures were prepared so proper minipreps could be made. Additionally, the powerpoint presentation for the NEGEM meeting and the survey questions were edited.
- Shawna and Corbyn completed minipreps of the 15A liquid culture that had been prepared the day before. The resulting DNA concentrations of the miniprep were all very low due to an issue with the miniprep kit, so digests could not be prepared. Thus, we once again selected three colonies for liquid culture in the afternoon and placed in the 37^{o}C shaker to incubate overnight. We also received the sequence of our possible BCLA biobrick, CAEV biobrick, and the combination of BCLA and CAEV in the biobrick. With help from our advisor Professor Duffy we began to analyze the sequence to check if we have correctly ligated the parts into the backbone. However, we were missing the sequences of both the BCLA and CAEV genes, so we will need to get those and then perform further analysis to learn if our ligations were successful. Today we were also introduced to GCK, which will help with our analysis of the sequenced DNA. Corbyn created a plasmid of the psb1cs vector highlighting its restriction sites.
- Chloe and Alex repeated the miniprep for the 14AK culture and met success upon using a different stock of buffer PE, yielding a concentration of 190.3 ng/mL, proving that the trouble with the previous attempts had been with the kit's buffer PE, which had not had ethanol added to it. A 1M stock of salicylic acid was also prepared. The acid crystals would not dissolve into water at all, even with heat added, but it did finally mix into 95% ethanol. A 1:200 dilution of this was added to a new culture of 14AK, but because the high concentration of ethanol posed the threat of killing off the culture, a second culture was prepared with nothing added to it so that if the culture did die overnight, tests may be done tomorrow as well.

Day 14:

As a team, we all worked on the powerpoint and presentation for NEGEM 3.1 at Boston University tomorrow, the 20th. We also began to look into the survey and brainstorm questions and think of ways to distribute it.

- Corbyn and Shawna prepared minipreps of 15A that were very successful, with high concentrations of DNA. We also once again viewed the sequence to check if our digestions and ligations were correct, (for BCLA, CAEV, and BCLA+CAEV), but we need

some assistance from our advisors to definitively decide if our experiments were successful.

- Kayla and Mike prepared three minipreps for part 16K. The DNA concentrations for the three minipreps were 64.3, 50.3, and 43.3 ng/uL.