Week 4 Notebook

June 22, 2015 - June 26, 2015

June 22, 2015

Kayla/Julie

        - 1 µL 15-5A
        - 25 µL 2x PCR Master Mix
        - 22.5 µL dH2O
        - 1.25 µL 5' or VF2
        - 1.25 µL 3' or VR
    - Cycle=
    - Ran 10 µL PCR product on 1.0% agarose gel at 95 V for 45 minutes
        - bands almost ran off gel
        - ladder was not visible
        - did not run enough PCR product to gel purify insert
- Transformations of J23101, J23106, and J23117 in NEB DH5α and RFP (20 pg/ µL from transformation efficiency test kit), 14-34C, and 14-35C in EMG2:Kλ
    - plated all 250 µL

Chloe/Charlotte

Cut PCR products with X and P. Intended to ligate them into the pSB1C3 vector, but realized that the vector had to be cut with E and P before ligating and did not contain an X site. Redid PCR of 14-34C and 14-17K with the 5' and 3' Biobrick primers. Ran a gel of the finished PCR and placed products in the freezer.

Worked on graphics and wiki design.

Potential antifreeze protein from: *Solanum dulcamera*

Dave/Eddie

- We resuspended the AFPs that were delivered, the GaAFP, RiAFP, BrAFP, and TmAFP. They were centrifuged at max speed 5 min, then 50 ul H2O was added. 10 ul of that was used in a 20 ul digest with X and P, 1 ul each, with 2 ul buffer and 6 ul H2O. Incubation at 37C for 1 hr and heat kill at 80C for 20 min. Ligations followed, left to incubate for 1 hr.

Ligation table

	J23119 Vector (ul)	Insert (ul)	Buffer (ul)	H2O (ul)	Total (ul)

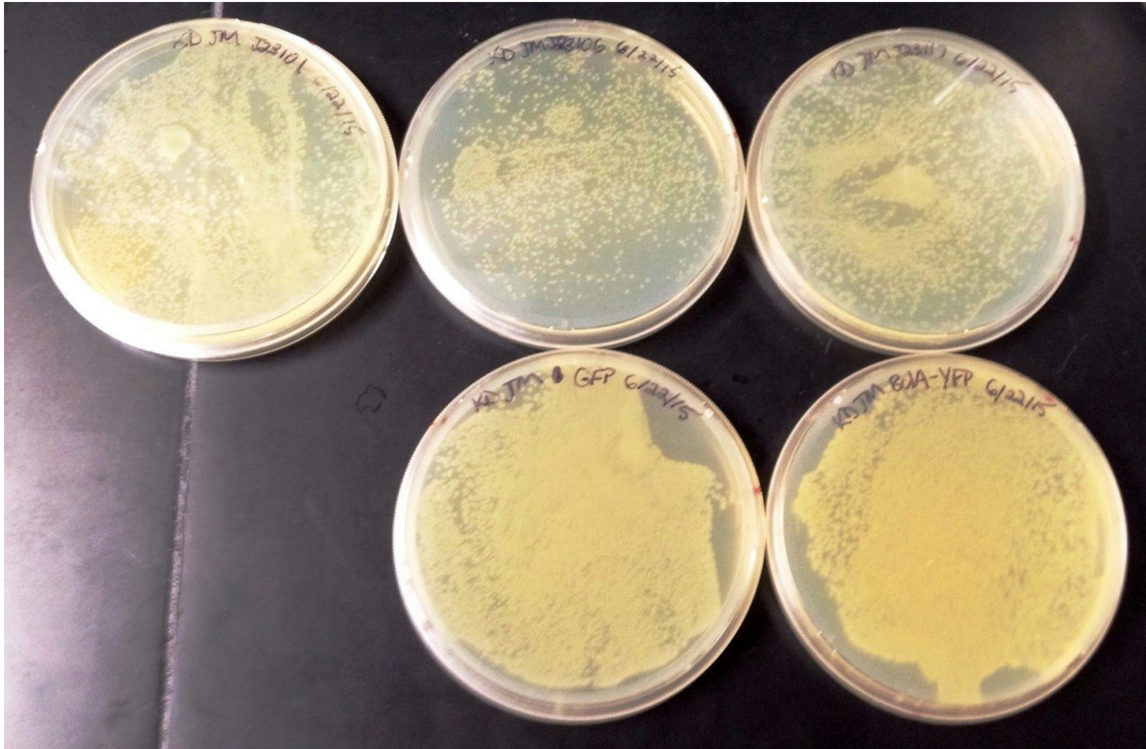
1	2	0	Control	2	15	20
2	2	2	ZeAFP	2	13	20
3	2	5	ZeAFP	2	10	20
4	1	5	ZeAFP	2	11	20
5	2	2	GaAFP	2	13	20
6	2	5	GaAFP	2	10	20
7	2	2	RiAFP	2	13	20
8	2	5	RiAFp	2	10	20
9	2	2	TmAFP	2	13	20
10	2	5	TmAFP	2	10	20
11	2	2	BrAFP	2	13	20
12	2	5	BrAFP	2	10	20

- We ran out of the vector and thus 1-6 were ligated for an hour and transformed today. 7-12 were left to ligate overnight as by the time the vector was digested and purified it was too late in the day to transform.
- We anticipated running out of vector, so we used the miniprepped supply that we had and made more purified backbone. 20 ul of vector, 282 ug/nl was used in a 50 ul reaction. It was digested with S and P. It was CIP treated with 1 ul CIP for 20 min at 37C after the digestion for 1 hr at 37C. It was gel purified using two lanes.
- No growth was seen on last weeks transformations.

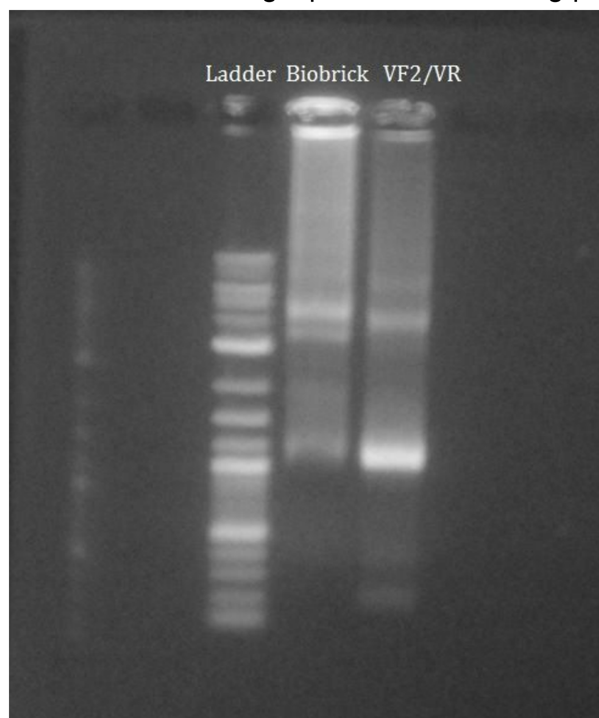
June 23, 2015

Kayla/Julie

- Checked transformation plates
    - colonies grew on all plates except for the EMG2:Kλ RFP plate



- Ran remaining PCR product (~20µL) on a 1% agarose gel at 80V for 45 minutes
    - the band for VF2/VR primers was brighter than the band for the Biobrick primers, so the VF2/VR band was cut and gel purified
        - DNA concentration of gel purified GFP=35 ng/µL



- Digested gel purified GFP with XbaI and PstI

    - 31µL H2O, 10 µL GFP, 5µL buffer, 2 µL XbaI, 2µL PstI
    - 2 hours at 37°C
- Prepared 5mL liquid cultures of BclA-YFP and GFP (EMG2:Kλ) and J23101, J23106, and J23117 in LB

Chloe/Charlotte
Realized that the wrong primers were used on yesterday's PCR and repeated the PCR of 14-17K and 14-34C with the VF2 and iGEM 10 (BclA) or iGEM11 (GFP) primers. Ran a gel on the PCR products and found everything to be the right size. Performed a restriction digest of the PCR products and the pSB1C3 vector with E and P. Ligated the digested 14-34C and 14-17K into the digested vector overnight.

Ligation:
- 3 ul vector
- 3 ul insert
- 2 ul buffer
- 1 ul ligase
- q.s. to 20 with water

Worked on graphics and wiki design.

Dave/Eddie
- Two of the transformed plates from last night appeared to have a colony. The plates were returned to the incubator for the day to allow for more growth.
    - later in the day 2 colonies from the GaAFP 2:5 and one from ZeAFP 1:5 were seen and used to make liquid cultures so that minpreps and glycerol stocks could be made tomorrow.
- The remaining six ligations were transformed and plated and left in the incubator overnight.
- Started liquid cultures of two GaAFP colonies and one ZeAFP colony.

June 24, 2015

Kayla/Julie
- Minipreps of J23101 (15-24C), J23106 (15-25C), and J23117 (15-26C)
- PCR on GFP using 1:10 dilutions of VR and VF2
    - 50 µL reactions
        - 1 µL 15-5A
        - 25 µL 2x PCR Master Mix
        - 22.5 µL dH2O
        - 1.25 µL 5' VF2
        - 1.25 µL 3' VR

    - Cycle=
- Purified GFP plasmid with PCR purification kit
- Prepared 5 mL liquid cultures of EMG2:Kλ expressing nothing, GFP, and BclA-YFP
- Digested 15-24C2, 15-25C2, and 15-26C1 with SpeI and PstI
    - 50 μL reaction
        - 5 μg miniprep
        - 2 μL SpeI
        - 2 μL PstI
        - 5 μL NEB buffer
        - H_2O to 50 μL
    - 2 hours at 37°C

Chloe/Charlotte

The freeze assay was repeated (again with 14-34C, 14-35C, and RFP from the transformation kit), this time plating using only the 10^{-6} dilution, but done in triplicate. The pre-freeze plates were placed in the incubator at 11 am.

The ligations of 15-27C and 15-28C were transformed, and the transformations were plated and left to incubate overnight at 37℃.

A miniprep of 14-34C and 14-35C was prepared.

A test digest of the new minipreps of 14-34C and 14-35C was prepared using only S to test whether or not there is a problem with the inserts.

14-34C and 14-35C were sent in with forward primer to be sequenced.

Dave/Eddie

- Conducted a miniprep of the ZeAFP colony and the two GaAFP colonies and made glycerol stocks. Spectrophotometer Table

	@ 260nm	@280nm	Concentration (ug/ul)
15-19C	0.0141	0.0094	233
15-20C 1	0.0160	0.0113	264
15-20C 2	0.0169	0.0118	279

Spec used with 9 ul DNA in 291 ul H2O

- Ran a test digest with E and P.
    - test digest used 5 ul of DNA in 20 ul total solution
    - Gel ran:
        - 1 Ladder
        - 2 15-19C

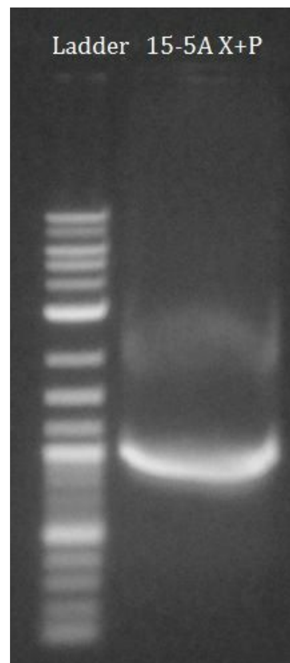
    - 3 15-20C 1
    - 4 15-20C 2
- Colonies from the transformed ligations after two nights (with the exception of 2:5 GaAFP and 1:5 ZeAFP which were only left for one night):

	2:0::Vector:Insert	1:5::Vector:Insert	2:2::Vector:Insert	2:5::Vector:Insert
ZeAFP (15-19C)	1	1	98	18
GaAFP (15-20C)	--	--	0	2
RiAFP (15-21C)	--	--	3	0
BrAFP (15-22C)	--	--	0	1
TmAFP (15-23C)	--	--	1	0

June 25, 2015

Kayla/Julie

- EMG2:Kλ liquid culture did not grow because chloramphenicol was added to it
    - could not proceed with the biofilm assay as planned
- Ran GFP digest on a 1.0% agarose gel at 80V for 1 hour
    - cut and gel purified insert band



- Ligated 15-5A digest into 15-24C, 15-25C, and 15-26C vectors
    - 20 µL reactions
        - 3µL 15-5A digest (0 µL for control)
        - 3 µL digested vector
        - 2 µL buffer
        - 1 µL ligase
        - 11 µL H_2O (14µL for control)
- Transformed DH5α with 2 µL of each ligation
- Prepared 5 mL liquid cultures of EMG2:Kλ expressing nothing, GFP, and BclA-YFP

Chloe/Charlotte
No colonies were found on the plates from the transformation of 15-27C and 15-28C. The plates were left to incubate at 37℃ for another night.

At 11 am, colonies were counted on the pre-freeze plates for the freeze survival assay. At 1 pm, three serial dilutions each were made of the slow-freeze and fast-freeze cultures stored at both -20℃ and -80℃ (18 dilutions total). The 6th serial dilution of each series was plated and left to incubate overnight at 37℃.

The four antifreeze protein sequences ordered from IDT were PCR'ed with primers containing the correct ribosome binding sites to repair the parts.

Colonies of Stationary Phase Growth *E. coli* Plates (Before Freeze)					
Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies
34C 10-6 (1)	1311	35C 10-6 (1)	(650x4) = 2600	RFP 10-6 (1)	(878x4) = 3512
34C 10-6 (2)	(1526x2) = 3052	35C 10-6 (2)	1604	RFP 10-6 (2)	(975x4) = 3900
34C 10-6 (3)	(869x4) = 3476	35C 10-6 (3)	495	RFP 10-6 (3)	(924x4) = 3696
Average:	2613	**Average:**	1566.33	**Average:**	3702.67

Dave/Eddie
- Picked a colony from the TmAFP plate to grow up a liquid culture.
- Made minipreps and glycerol stocks for RiAFP and BrAFP.
    - Spec'd 9 ul in 291 ul water

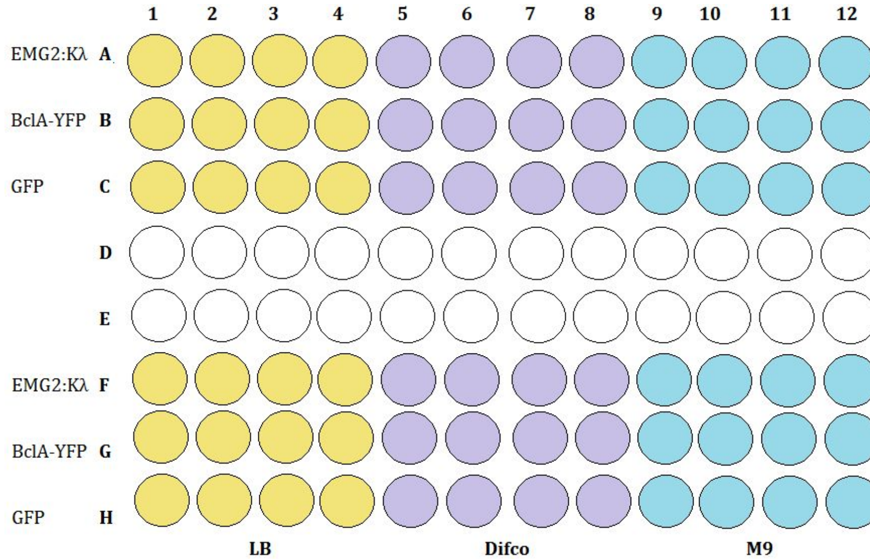
	260nm	280nm	Concentration (ug/ul)
15-21C	0.0309	0.0226	509
15-22C	0.0183	0.0119	301

- Set up a test digest with with E and P with 5 ul of DNA in 20 ul total solution
- Gel ran:
  - 1 Ladder
  - 2 15-21C
  - 3 15-22C
- The gel didn't look good, so we will re-ligate later.
- Set up a freeze assay with 15-21C and 15-22C at noon:
  - Plated three plates of 10^{-6} dilutions of 15-21C and 15-22C and put them in the incubator
  - Put 200 ul of each AFP of the original culture in both the -20°C and -80°C freezers
- Sent away 15-19C and 15-20C (2nd colony) for sequencing.

June 26, 2015

Kayla/Julie

- Checked interlab transformation plates
  - Colonies grew on all plates, including controls, but there were more colonies on the ligation plates than the control plates.
  - Some colonies glowed. This will help in the selection process when making liquid cultures.
- Set up biofilm assay
  - 1:100 dilutions of EMG2:Kλ, BclA-YFP, and GFP in LB, Difco Minimal, and M9 Minimal media
  - Plated 100μL of the dilution according to the schematic below



 - Incubate at 37°C for 48 hours (check plates Sunday afternoon)
- Spoke to Lindsay Lozeau about other biofilm forming *E. coli* strains

Chloe/Charlotte

The plates from the transformation of 15-27C and 15-28C were checked again; no colonies were found. The ligations were repeated, with the following modifications:

- Vector/insert ratio and amount modified (four ways):
  - 2 ul : 2 ul
  - 2 ul : 5 ul
  - 5 ul : 2 ul
  - 5 ul : 5 ul
- Two controls with 5 and 2 ul of vector, respectively, were used.
- After vector, insert, and water were added, tubes were placed in the PCR machine to run for 5 minutes at 65°C.

Colonies of Post-Freeze *E. coli* Plates							
Strain	Temp (°C)	Freeze speed	Plate 1	Plate 2	Plate 3	Average number of colonies	% survival (average)
34C	-20	slow	1434	1416	1667	1506.67	0.5766
34C	-20	fast	1830	1860	783	1491	0.5706
RFP	-20	slow	518	463	871	617.33	0.3941
RFP	-20	fast	479	580	790	616.33	0.3935
34C	-80	slow	62	38	66	55.33	0.0212

34C	-80	fast	0	0	0	0	0
RFP	-80	slow	55	10	90	51.67	0.0330
RFP	-80	fast	0	0	0	0	0

Dave/Eddie
- Sequencing for GaAFP looks good, the ZeAFP we sent in was in the original plasmid..
- Conducted a miniprep and made a glycerol stock of 15-23C.
- Found the Concentration:

	Absorbance @260	Absorbance @280	Concentration (ug/ul)
15-23C	0.0106	0.0084	175

- Abandoned freeze assay for our samples, the sequence needs to be fixed via PCR anyway.
- Conducted transformations and ligations according to the following guidelines:
  - Heat kill PCR product 80C 10min
  - column purify - elute 30ul H2O
  - Ligate
    - 2ul vector, 2ul insert
    - control only 2ul vector
    - `Add DNA and water only, - PCR tubes heat 65C 5 min, cool to RT, add 2ul buffer and 1 ul ligase, incubate 30 min RT
  - Transform
    - 30ul competent cells
    - 5ul ligation, 120ul SOC
    - plate on one plate per rxn

June 27, 2015

Chloe/Charlotte

Checked transformation plates for 15-27C and 15-28C that we made, as well as the AFP transformation plates that Dave and Eddie made. Only one colony was found, on the RiAFP plate. All plates were placed back into their incubators.

June 28, 2015

Kayla/Julie
- Prepared liquid cultures from colonies on the 29C, 30C, and 31C transformation plates
  - tried to pick colonies that glowed

    - picked 5 colonies from each plate
- Stained biofilm plate with crystal violet to visualize biofilm formation
    - all three EMG2:Kλ strains were able to form biofilms in M9 minimal media

Dave/Eddie

- Picked a colony from the RiAFP plate (15-21C) and made a liquid culture.