

Week 9 Notebook

July 27, 2015 - July 31, 2015

July 27, 2015

Kayla/Julie

- Quantified biofilm plates stained on 7/25/15 and 7/26/15
- Prepared liquid cultures of EMG2:Kλ, 26C, GaAFP, RiAFP, TiAFP and ZeAFP for biofilm assay

Chloe/Charlotte

On Friday, it was discovered that we never had the 28C vector in the first place, so it was decided that we will no longer try to clone GFP+AFP parts. Instead, we will focus on cloning the remaining AFPs into the 27C vector.

Another colony PCR was prepared using plates from the previous two colony PCRs in an attempt to find colonies with correct BclA+AFP inserts.

Cultures of confirmed AFPs were grown up overnight for use in a freeze survival assay tomorrow.

Dave/Eddie

- Picked colonies and ran PCR from the plates from 7/22 and 7/24:
 - 15-35C [5]
 - 15-38C [5]
 - 15-40C [5]
 - 15-41C [5]
 - 15-42C [5]
 - 15-44C [5]
 - 15-46C [5]
 - Positive Control
 - Negative Control
- Miniprepmed the samples from Friday that were spun down on Saturday.
- Held a baby llama.

July 28, 2015

Kayla/Julie

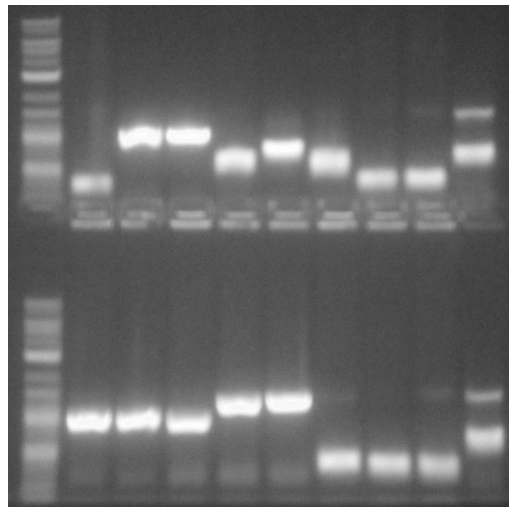
- Set up a polyacrylamide gel to determine if AFPs are being expressed by EMG2:Kλ
 - 18% with 4% stacking gel

- ran samples prepared from EMG2:Kλ, 26C, GaAFP, LpAFP, MaAFP, RiAFP, TiAFP and ZeAFP liquid cultures at 120V for 2 hours
- stained gel with Coomassie Brilliant Blue, rinsed with water, and left on shaker overnight
- Set up a new biofilm plate with EMG2:Kλ, 26C, GaAFP, RiAFP, TiAFP and ZeAFP
- Transformed 11 antifreeze proteins into EMG2:Kλ

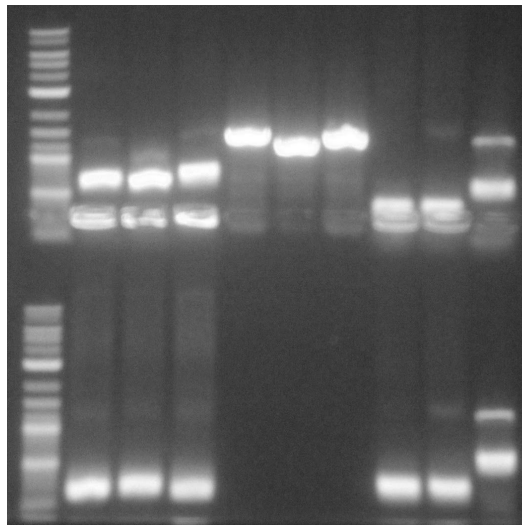
Chloe/Charlotte

The colony PCR from yesterday was run on gels to determine which (if any) colonies had a correct-looking insert, pictured below. Based on this gel, cultures of X, X, X, and X were grown up overnight to be miniprep and sent for sequencing.

Gel1:



Gel2:



A new freeze survival assay was conducted using all of the confirmed AFPs so far (19C, 20C, 21C, 22C, 33C, 34C, and 43C).

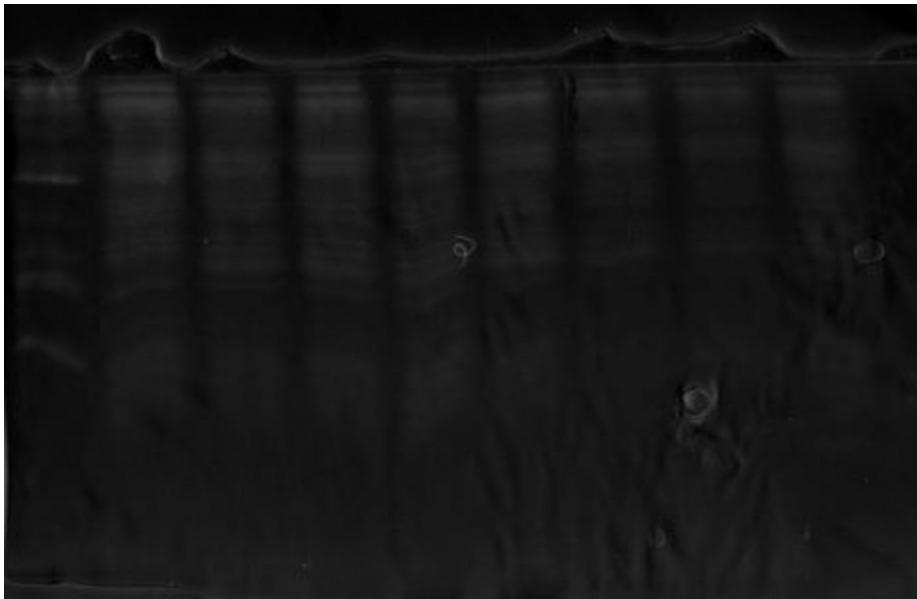
Dave/Eddie

- Sequencing came back, mostly looked bad
- We got PpAFP!
- Mo had weird sequencing, IA, CF, and Ap all had bad sequencing, all others came back as empty vectors.
- We transformed PpAFP in order to make a glycerol stock. 2 ul miniprep used, transformed and made directly into a liquid culture.
- Picked five colonies from every AFP we don't currently have sequence confirmed for growth in liquid medium overnight, to be miniprep tomorrow. 60 colonies total.

July 29, 2015

Kayla/Julie

- Checked transformation plates
 - colonies grew on all 11 plates
- Checked polyacrylamide gel
 - bands were present in all lanes
 - proteins ran about halfway rather than running completely off as we had thought



- Poured new polyacrylamide gels
- Prepared 19 liquid cultures for biofilm assay (EMG2:Kλ, 26C,Cf27, Ch27, Dc27, Ep27, Ga, Ga27, IAFGP27, Lp, Ma, Ma27, Mp27, Pp, Ri, Ri27, Ti, Ti27, and Ze)

Chloe/Charlotte

Minipreps were prepared of the promising PCR 3 cultures grown up overnight, which were all sent for sequencing.

The second half of the freeze survival assay was conducted.

MTS Freeze Assay Results (OD ₆₀₀) 7/29 Assay A					average	avg - blank
17C Pre-freeze	0.445	0.609	0.48	0.511	0.51125	0.17425
17C After -20 freeze	0.293	0.339	0.326	0.322	0.32	0.0225
17C After -80 freeze	0.294	0.338	0.307	0.311	0.3125	0.0145
19C Pre-freeze	0.387	0.525	0.501	0.535	0.487	0.15
19C After -20 freeze	0.309	0.335	0.324		0.322666667	0.025166667
19C After -80 freeze	0.327	0.341	0.373	0.386	0.35675	0.05875
20C Pre-freeze	0.591	0.628	0.613	0.661	0.62325	0.28625
20C After -20 freeze	0.304	0.296	0.351	0.333	0.321	0.0235
20C After -80 freeze	0.341	0.309	0.331	0.334	0.32875	0.03075
21C Pre-freeze	0.529	0.584	0.588	0.493	0.5485	0.2115
21C After -20 freeze	0.303	0.295	0.298	0.294	0.2975	0
21C After -80 freeze	0.341	0.345	0.339	0.371	0.349	0.051
22C Pre-freeze	0.559	0.596	0.633	0.641	0.60725	0.27025
22C After -20 freeze	0.288	0.302	0.297	0.295	0.2955	-0.002
22C After -80 freeze	0.311	0.324	0.322	0.324	0.32025	0.02225
33C Pre-freeze	0.865	0.933	0.931	0.75	0.86975	0.53275
33C After -20 freeze	0.343	0.35	0.316	0.257	0.3165	0.019
33C After -80 freeze	0.309	0.343	0.328	0.345	0.33125	0.03325

34C Pre-freeze	0.757	0.729	0.771	0.848	0.77625	0.43925
34C After -20 freeze	0.338	0.277	0.305	0.336	0.314	0.0165
34C After -80 freeze	0.31	0.318	0.36	0.338	0.3315	0.0335
43C Pre-freeze	0.416	0.45	0.439	0.387	0.423	0.086
43C After -20 freeze	0.307	0.318	0.324	0.327	0.319	0.0215
43C After -80 freeze	0.283	0.327	0.316	0.313	0.30975	0.01175
Blank Pre-freeze	0.29	0.384			0.337	
Blank After -20 freeze	0.296	0.299			0.2975	
Blank After -80 freeze	0.3	0.296			0.298	

Dave/Eddie

- Miniprepmed the 60 colonies from yesterday using the new miniprep kit (because it's awesome!).
- Digested all 60 with E and P for a test digest, 20 ul total with 5 ul of DNA, 1 ul of each enzyme and 2 ul of buffer. Ran on four gels, starting in numerical order until the last well of the first gel, which is 15-39C #5. The rest are again numerical, without 15-39C #5.
 - Gel 1:
 - 15-23C #1-5
 - 15-32C #1-5
 - 15-35C #1-5
 - 15-36C #1-2
 - 15-39C #5
 - Gel 2:
 - 15-36C #3-5
 - 15-37C #1-5
 - 15-38C #1-5
 - 15-39C #1-4
 - 15-40C #1
 - Gel 3:
 - 15-40C #2-5
 - 15-41C #1-5
 - 15-42C #1-5
 - 15-44C #1-4

- Gel 4:
 - 15-44C #5
 - 15-46C #1-5
- Made a glycerol stock of PpAFP.

July 30, 2015

Kayla/Julie

- Prepared 1:100 dilutions for all 19 liquid cultures in LB and M9
 - plated 100 μ L of each dilution in sets of 4 wells on 96 well plate
- Prepared SDS PAGE samples from all 19 liquid cultures
 - diluted cultures to OD 0.3
 - Pelleted cells and resuspended in 50 μ L 2X SDS Sample Buffer +DTT
 - Heated samples on heat block at 100°C for 10 minutes
 - Spun samples down for 10 seconds at 13,000 rpm
- Ran 15 μ L of the 26C, GaAFP, LpAFP, MaAFP, PpAFP, RiAFP, TiAFP, and ZeAFP samples on an 18% polyacrylamide gel at 120V for 1.5 hours
 - rinsed gel with water
 - stained with Coomassie Brilliant Blue for 20 minutes
 - Rinsed with water overnight
- Prepared 5mL liquid cultures of EMG2:K λ , 26C, Ga, Ga27, Ma, Ma27, Ri, Ri27, Ti, and Ti27 for biofilm assay

Chloe/Charlotte

A new freeze survival assay was conducted using all of the following cultures: PpAFP, Ch27, Ti27, Ga27, Dc27, Cf27, and Mp27.

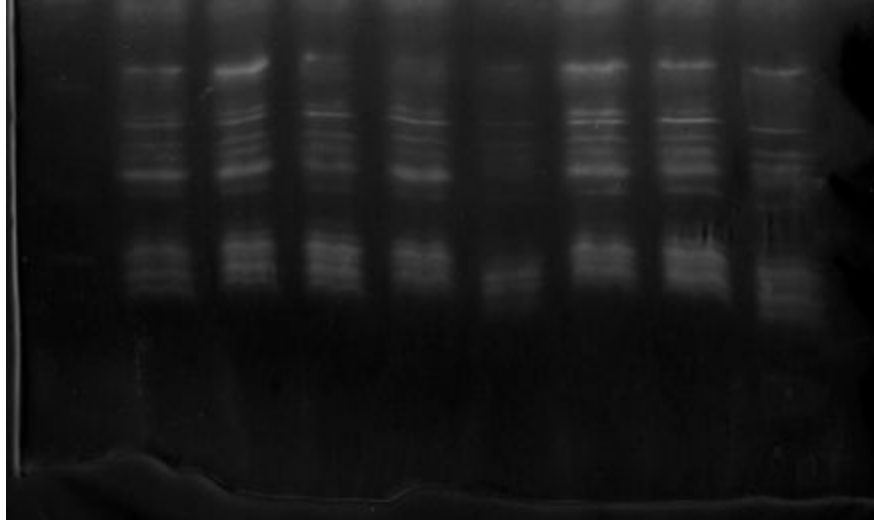
Dave/Eddie

- Sent away 15-23C #3, 15-32C #2 and #4, 15-36C #3, 15-37C #2, 15-38C #2, 15-39C #1, 15-40C #2 and #3, and 15-41C #4.
- Picked 36 colonies for growth overnight and miniprep tomorrow, assuming those sequences come back bad.

July 31, 2015

Kayla/Julie

- Set up a new biofilm assay with 26C, GaAFP, GaAFP 27, MaAFP, MaAFP 27, RiAFP, RiAFP 27, TiAFP, and TiAFP 27
 - no positive control because EMG2:K λ culture did not grow
- Checked polyacrylamide gel



Chloe/Charlotte

The second half of the freeze survival assay was conducted.

MTS Freeze Assay Results (OD_{600}) 7/31 Assay B					average	avg - blank
17C Pre-freeze	0.365	0.402	0.359	0.349	0.369	0.097
17C After -20 freeze	0.313	0.319	0.403	0.336	0.343	0.077
17C After -80 freeze	0.275	0.226	0.270	0.305	0.269	0.001
Pp Pre-freeze	0.381	0.472	0.459		0.437	0.165
Pp After -20 freeze	0.334	0.321	0.358	0.362	0.344	0.078
Pp After -80 freeze	0.281	0.281	0.254	0.320	0.284	0.016
Ch27 Pre-freeze	0.399	0.409	0.387		0.398	0.126
Ch27 After -20 freeze	0.321	0.329	0.378	0.333	0.340	0.074
Ch27 After -80 freeze	0.296	0.298	0.304	0.273	0.293	0.025
Ti27 Pre-freeze	0.411	0.396	0.404	0.418	0.407	0.135
Ti27 After -20 freeze	0.317	0.323	0.251	0.297	0.297	0.031
Ti27 After -80 freeze	0.288	0.291	0.287	0.286	0.273	0.005
Ga27 Pre-freeze	0.440	0.415	0.443	0.424	0.431	0.159
Ga27 After -20 freeze	0.322	0.337	0.316	0.313	0.322	0.056
Ga27 After -80 freeze	0.294	0.298	0.293	0.293	0.295	0.027
Dc27 Pre-freeze	0.446	0.458	0.460	0.448	0.453	0.181

Dc27 After -20 freeze	0.363	0.347	0.350	0.316	0.344	0.078
Dc27 After -80 freeze	0.305	0.297	0.267	0.305	0.294	0.026
Cf27 Pre-freeze	0.419	0.415	0.402	0.419	0.414	0.142
Cf27 After -20 freeze	0.312	0.333	0.312	0.330	0.322	0.056
Cf27 After -80 freeze	0.290	0.302	0.301	0.310	0.301	0.033
Mp27 Pre-freeze	0.380	0.376	0.341	0.376	0.361	0.089
Mp27 After -20 freeze	0.288	0.311	0.299	0.300	0.300	0.034
Mp27 After -80 freeze	0.296	0.290	0.313	0.274	0.293	0.025
Blank Pre-freeze	0.249	0.295			0.272	
Blank After -20 freeze	0.245	0.287			0.266	
Blank After -80 freeze	0.260	0.276			0.268	

Dave/Eddie

- We got: Tm, Ch (4, not 2), Mo, and Ap
 - There was a problem with the confirmation sequence, at some point the sequence above, Mo, got dragged down and replaced the sequence for Ap, so the trace looks good, but we can't align the sequences.
- Something weird happened with IA, it's missing a section in the middle of the sequence
- Dc looked good, but it's so long that we don't have enough sequencing to confirm it the whole way. The ends both look good though, so we've really probably got it.
- Conducted minipreps of the liquid cultures for 15-35C, 15-38C, 15-40C, and 15-42C and ran a test digest. The first gel was 15-35C, 15-38C and 15-42C in order, and the second gel was entirely 15-40C.
- Sent away 15-40C #2, #4, and #11; 15-38C #2; and 15-42C #1.

August 1, 2015

Kayla/Julie

- Stained biofilm plate from Thursday, 7/30

August 2, 2015

Kayla/Julie

- Stained biofilm plate from Friday, 7/31
 - biofilm formation in LB wells most likely due to contaminated LB
 - No visible difference between M9 wells. All had robust biofilm formation.