Notebook: (Camille)

5/30:

bacteria=growth/expression factory for plasmid

bacteria will copy small bits of DNA

cut space in bacteria and insert plasmid

whatever is in plasmid will be expressed and replicated in bacteria

plate and select from bacteria containing plasmid

in plasmid:

ORI- place where plasmid replication begins

Selectable marker- gene expressed from plasmid not in bacteria (antibiotic resistant gene) shows transformation worked, CAM, AMP, KAN

GFP (except in neg control)

Promoter- tells gene expression where to start, 20-60 nucleotides long, different sequences=different power of promoter

Neg control=no GFP, not making anything

Pos control=strong promoter, high GFP expression

Competent cells-> holes in the membrane to insert plasmids, fragile

5/31:

Check plates from Interlab and count colonies (~220)

Interlab serial dilution protocol 3

6/1:

Dilution from cultures (Interlab colonies)

1. 0.174- 0.174/0.02=8.7 (8.7x more concentrated than we want), 12mL/8.7=**1.38mL** (of culture), 12-1.38=**10.92mL** (of media)
2. 0.399- 0.399/0.02=19.95, 12/19.95=**0.602mL** (culture), 12-0.602=**11.398mL** (media)

target=0.02

final volume=12mLs

+12 μL CAM

500 μL into tube

neg/pos dilution (Interlab)

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
neg 1	0.36	0.1	3.6	5	1.388888889	3.611111111	0.103
neg 2	0.352	0.1	3.52	5	1.420454545	3.579545455	0.1
pos 1	0.389	0.1	3.89	5	1.285347044	3.714652956	0.105
pos 2	0.16	0.1	1.6	5	3.125	1.875	0.1

dilution factor target= 0.1

final volume= 5mL

100 μL into 1.9mL LB (1:20 dilution)

*well 4 and 5 100 μL into 0.90mL LB (1:10 dilution) – change in protocol

100 μL on each plate and incubate

6/4:

Made LB plates for bacteria art for Touch Tomorrow

*Recipe in protocols

Week 2 goals:

Finish Interlab and submit data- excel sheets, surveys

Pouring plates and bacteria art- transformation for RFP

Strawberry DNA extraction

Transform bacteria for banana smell

Project Planning- bacteria that cause biofilms, model organisms, safe salmonella, learn how to make/quantify biofilms, figure out which plant/pathogen combination is most harmful/common, understand risks of GMOs, organic vs. non-organic foods on bacterial growth, add curcumin to grown biofilms (crop plants to produce it, bacteria to produce it and apply to plants)

Streak AFPs we have- Ia4, Ia5, Ze1, Ze4

RFP transformation

*Procedures used under Protocols

6/5:

To do:

Let colonies from incubator sit out to make more proteins but not divide anymore (RFP)

AFP streaking

LB plates with no antibiotics in autoclave (for biofilms)

Banana smell transformation for Touch Tomorrow

Bacterial art for Touch Tomorrow

ZeAFP- from fish, AMP plasmid

IAFGP- from tick (+ control), AMP plasmid

Plasmid- pET21a

- ORI (E. coli)
- AMP res gene (AMP plates)
- LACI repressor gene (always made from this plasmid)
- LACO
- GFP or AFP

Purify AFP, take plasmid and put into other strain that makes or does not make biofilms

- E. coli EMG2Kλ- yes
- E. coli NCTC9001- yes
- E. coli DH5a- no

Streak out E. coli strains on plain LB plates

Liquid culture of EMG E. coli over night

20% glucose for M9

40g into 200mL water

6/6:

Banana transformation

- 10mL LB + 10 μL CAM + 1 colony and shake all day, at the end of the day add arsenic

Filter glucose for M9 liquid

Finish M9 liquid procedure from MQP

*M9 recipe and procedure found in Protocols

Biofilm Assay

Blank=M9+LB OD=0.1 Volume=10mL

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
EMG	0.33	0.1	3.3	10	3.03030303	6.96969697	0.103
NCTC	0.198	0.1	1.98	10	5.050505051	4.949494949	0.099

Put into wells

Colonies from streak plates (AFPs) into shaker over night

6/7:

New banana transformation

- 10mL LB

200 μL culture

10 μL CAM

20 μL arsenic

Crystal violet dilution

1:100 dilution of crystal violet 0.1% becomes 0.001%

2-fold serial dilution- start with 200 μL and transfer 100 μL to each plate

100 μL water in wells 2-12, 200 μL in well 1

prepare 4 different dilutions

495 μL water + 5 μL crystal violet, vortex

*Do another CV dilution plate for standard curve using a dilution of 1:10 (450 μL water + 50 μL UV)

For new biofilms

10mL LB + 1 colony from each plate (DH5a, EMG, NCTC, S. epidermis and S. aureus)

6/8:

Standard curve CV assay

New biofilms for DH5a, EMG, NCTC, S. epidermis, S. aureus, M9)

Bacteria	Absorbances	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
NCTC	0.053	0.1	0.53	10	18.86792453	-8.867924528	0.099
DH5a	0.159	0.1	1.59	10	6.289308176	3.710691824	0.098
EMG	0.179	0.1	1.79	10	5.586592179	4.413407821	0.099
S. epi	0.205	0.1	2.05	10	4.87804878	5.12195122	0.102
S. aureus	0.281	0.1	2.81	10	3.558718861	6.441281139	0.1

*Note for NCTC the amount of culture added was 9.52mL and the amount of M9 media was 0.48mL

6/11:

IPTG turns on gene for AFP expression

Made CCMB80 buffer

*recipe in Protocols, originally from parts.iGEM

Over night culture for competent EMG and NCTC cells

5mL LB + 1 colony

Making CV solution (0.1%)- 0.1g in 100mL water

Autoclave LB in 500mL flask (broth)

25g LB powder + 1 L water

Cultures for mini preps

5mL LB + 5 μL AMP, colonies from Ia, Ze, GFP streak plates

6/12:

competent cell protocol OD (0.01-0.1), shaker until OD (0.3-0.3) about 2-3 hours

test transformation empty pET21a

mini prep plasmids

1mL culture into 200mL LB- check OD

adjust to 0.1

shake 200mL flasks for 2-3 hours to let cultures grow

check OD- should be between 0.3-0.4

mini prep of plasmids from cultures Ia, Ze, GFP

spin cultures for 10 min (3000)

dump out liquid

250 μL A1 buffer, pipette up and down, move everything to microfuge tube

250 μL A2 buffer to each tube, mix, sit for 5 min

300 μL A3 buffer

spin for 5 min, add to columns using pipette and avoiding the pellet at the bottom

spin 30 sec

dump out liquid

600 μL A4 buffer, spin 30 sec

dump liquid, spin 2 min, throw out liquid

put column in another microfuge tube and add 50 μL AE buffer

sit for 1 min, spin for 1 min

measuring DNA from plasmids

Blank= AE buffer 2 μL blank twice, 2 μL of each plasmid

Check OD from flasks

EMG: 0.38- on ice

NCTC: 0.152- back in shaker

Competent cells

Put EMG, NCTC liquid on ice

*everything should stay cold

spin for 10 min, dump out liquid

re-suspend in 80mL CCMB80, ice for 20 min

spin 10 min, dump out liquid

re-suspend cell pellet in 10mL CCMB80 buffer

combine cells, 20 min on ice

400 μL in each tube, store -80°C

6/13:

Transformation of competent cells and plasmids from mini prep

GFP and RFP mini prep and GFP, RFP, DH5a over night cultures for high school

6/14:

Checked transformation of competent cells

EMG plates grew colonies but NCTC did not

Read about procedures for competent NCTC cells and redo experiment

SOC plain LB plate + NCTC competent cells to test cells

Ca2+ competent cell new protocol found

Over night culture of AFP in EMG

5mL LB + 5 μL AMP + 1 colony

6/15:

NCTC Ca2+ media OD=0.2

Start with 1 L media + 30mL over night culture NCTC

Absorbance= 0.032

Shake for 1 hour

Absorbance= 0.066

Shake another hour

OD=0.205

Split culture into 8 parts in 8 50mL tubes (400mL which is 40% of the total)

Centrifuge for 15 min at 4°C 100mL (0.4) = 40mL 40mL/8=5mL per tube

Decant and re-suspend each tube in cold $MgCl_2$, centrifuge for 15 min

Decant and re-suspend pellet in 200mL $CaCl_2$, ice for 20 min

1.5mL microfuge tubes on ice at this time

Centrifuge for 15 min

Decant and re-suspend pellet in 2.5mL 80mM $CaCl_2$, 15% glycerol

Freeze -80°C

Over night culture of EMG + plasmids

Measure OD, adjust 0.1 in 5mL in 50mL tube

+ 5 μL AMP

+50 μL IPTG

grow 4 hours in shaker

Absorbance:

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
GFP	0.129	0.1	1.29	10	7.751937984	2.248062016	0.098
Ia4	0.569	0.1	5.69	10	1.757469244	8.242530756	0.12
Ze1	0.206	0.1	2.06	10	4.854368932	5.145631068	0.093
Ze4	0.184	0.1	1.84	10	5.434782609	4.565217391	0.102
Ia5	0.33	0.1	3.3	10	3.03030303	6.96969697	0.111

Set up biofilm plate with M9 should contain AMP (1:1000)

Absorbance:

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
GFP	0.448	0.1	4.48	5	1.116071429	3.883928571	0.098
Ia4	0.985	0.1	9.85	5	0.507614213	4.492385787	0.097
Ze1	0.383	0.1	3.83	5	1.305483029	3.694516971	0.96
Ze4	0.45	0.1	4.5	5	1.111111111	3.888888889	0.101
Ia5	0.504	0.1	5.04	5	0.992063492	4.007936508	0.097
pET21a	0.108	0.1	1.08	5	4.62962963	0.37037037	0.119

Grow plate for 72 hours

6/18:

37°C and RT 72 hour plate wash, stain, dry, re-suspend, plate reader

New NCTC cells- transform with AFP, GFP, pET21a, plate

New stuff:

- Sonicator biofilm assay
- Curcumin assay with 5 biofilm forming strains
- Replicates of 5 strains and AFP biofilms- 3 per experiment
- Cloning stuff- 2 plant AFPs that were not tested last year
  - tcAFP from carrots- design new primers
  - zpAFP from grass- design new primers
  - New protein from research

Over night cultures to make plates for EMG (from competent cells)

- Repeat 2x
- 10mL LB + 1 colony

6/19:

EMG over night culture transformations

Absorbance:

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
GFP	1.55	0.1	15.5	10	0.64516129	9.35483871	0.101
Ia4	1.518	0.1	15.18	10	0.658761528	9.341238472	0.103
Ze1	1.583	0.1	15.83	10	0.631711939	9.368288061	0.103
Ze4	1.413	0.1	14.13	10	0.707714084	9.292285916	0.103
Ia5	1.779	0.1	17.79	10	0.562113547	9.437886453	0.099
pET21a	1.583	0.1	15.83	10	0.631711939	9.368288061	0.106

+ 5 μL AMP

+50 μL IPTG

Grow 4 hours

Absorbance:

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
GFP	1.55	0.1	15.5	5	0.322580645	4.677419355	0.093
Ia4	0.689	0.1	6.89	5	0.725689405	4.274310595	0.104
Ze1	0.459	0.1	4.59	5	1.089324619	3.910675381	0.092
Ze4	0.18	0.1	1.8	5	2.777777778	2.222222222	0.078
Ia5	0.785	0.1	7.85	5	0.636942675	4.363057325	0.103
pET21a	1.009	0.1	10.09	5	0.495540139	4.504459861	0.126

*diluted with M9 + AMP

plate and let grow for 72 hours

Read NCTC plates

Electroporation procedure because plates did not grow

NCTC transformed liquid into plates

Make 5 over night cultures 10mL for electro-competent cells

EMG plates

Process and read

30% acetic acid, 100 μL into each well, pipette up and down

Sit for 10 minutes, transfer to flat bottom wells, read and save data for analysis

6/20:

E. coli electro-competent cells

Over night cultures from NCTC plate- need 50mL of culture (5 @ 10mL)

50mL over night culture + 500mL

Check OD- 0.095

Shake until OD = 0.4 (0.394)

Centrifuge for 10 min @ 4°C, dump out liquid and re-suspend in 500mL cold DI water (rinse and spin 3xs)

Re-suspend in 1mL cold 10% glycerol

Keep in -80°C freezer

6/21:

Electroporation

Cuvette size 1 (100 μL)

50 μL NCTC cells + 1mL DNA (Ze, Ia, GFP, empty pET21a) + 50 μL water into microfuge tube

transfer to electroporation cuvette, 2500 volts, 900 μL SOC media to cuvette, transfer back to tube

*want time constant to be between 4-5

Ze1- 5 accidently put too much DNA

Ze4- 4.8

Ia5- 4.6

Ia4- 4.4

GFP- 4.8

pET21a- 4.6

sit for 1 hour at 37°C in shaker, spin for 1 min, take out 900 μL, re-suspend with remaining 100 μL

plate

6/22:

No colonies on the NCTC electroporation plates

Mini preps

Absorbance:

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
GFP	1.749	0.1	17.49	10	0.571755289	9.428244711	0.168
Ia4	1.693	0.1	16.93	10	0.590667454	9.409332546	0.16
Ze1	1.764	0.1	17.64	10	0.566893424	9.433106576	0.163
Ze4	1.804	0.1	18.04	10	0.554323725	9.445676275	0.178
Ia5	1.731	0.1	17.31	10	0.577700751	9.422299249	0.163

+ 10 μL AMP

+ 10 μL IPTG

shake for 4 hours

New Absorbance

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
Ze1	0.419	0.1	4.19	5	1.193317422	3.806682578	0.097
Ze4	0.169	0.1	1.69	5	2.958579882	2.041420118	0.103
Ia4	0.192	0.1	1.92	5	2.604166667	2.395833333	0.09
Ia5	0.227	0.1	2.27	5	2.202643172	2.797356828	0.092
GFP	0.649	0.1	6.49	5	0.770416025	4.229583975	0.103

One plate 37°C one plate RT both for 72 hours

***Note all EMG plates made up to this point will not be used in the final data analysis because they either did not contain enough IPTG or AMP

6/25:

2 replicates of pET21a- Kylie

Read third EMG plate- even though it will not be used in the final data analysis

Over night cultures to be split for PAGE gel- 6 plasmids 10mL + AMP

Over night cultures from 2 different colonies of pET21a +AMP

*procedures found under Protocol

6/26:

Plate 1 Absorbance

*Blank LB

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
pET21a	1.295	0.1	12.95	5	0.386100386	4.613899614	0.146
Ia5	0.563	0.1	5.63	5	0.888099467	4.111900533	0.099
Ia4	0.388	0.1	3.88	5	1.288659794	3.711340206	0.106
Ze4	0.308	0.1	3.08	5	1.623376623	3.376623377	0.102
Ze1	1.453	0.1	14.53	5	0.344115623	4.655884377	0.148
GFP	0.682	0.1	6.82	5	0.73313783	4.26686217	0.113

+5 μL AMP

+50 μL APTG

4 hours in the shaker

New Absorbance

*Blank 1:20 of LB:M9

Bacteria	Absorbance	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
pET21a	0.198	0.1	1.98	5	2.525252525	2.474747475	0.112
Ia5	0.414	0.1	4.14	5	1.207729469	3.792270531	0.096
Ia4	0.24	0.1	2.4	5	2.083333333	2.916666667	0.104
Ze4	0.256	0.1	2.56	5	1.953125	3.046875	0.106
Ze1	0.66	0.1	6.6	5	0.757575758	4.242424242	0.113
GFP	0.374	0.1	3.74	5	1.336898396	3.663101604	0.104

Plate, 1 in RT, 1 in 37°C for 72 hours

Plate orientation:

Ze1->6 pET21a1->12

Ze4->6 pET21a2->12

Ia4->6

Ia5->6

GFP->6

pET21a->6

M9->6

Make 250mL M9 + AMP

175mL water

50mL M9 salts

0.5mL 1M $MgSO_4$

5mL 20% glucose

20 μL 1M $CaCl_2$

Filter and then add 250 μL AMP

Make new EMG plates from old cultures- streak from 4 different cultures

Over night cultures from 6 EMG plates for 3rd well plate reading

Over night cultures from yesterday split in half with half getting IPTG and half not getting any

Kept in -80°C freezer for PAGE gel test

6/27:

Bacteria	Absorbances	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
Ze1	0.001	0.1	0.01	5	500	-495	
Ze4	0.016	0.1	0.16	5	31.25	-26.25	
Ia4	0.976	0.1	9.76	5	0.512295082	4.487704918	0.106
Ia5	0.261	0.1	2.61	5	1.915708812	3.084291188	0.111
GFP	0.905	0.1	9.05	5	0.552486188	4.447513812	0.115
pET21a	0.408	0.1	4.08	5	1.225490196	3.774509804	0.11

*the absorbance for Ze1 and Ze4 were too low and show that the bacteria did not grow in the overnight colony so new overnight cultures were made and Ze1 and Ze4 will not be included on this plate (plate 3)

+ 5μL AMP
+ 50μL 1M IPTG

In the shaker for 4 hours

New Absorbance

Bacteria	Absorbances	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
Ia4	0.519	0.1	5.19	5	0.963391137	4.036608863	0.116
Ia5	0.516	0.1	5.16	5	0.968992	4.031007	0.116

					248	752	
GFP	0.371	0.1	3.71	5	1.347708895	3.652291105	0.115
pET21a	0.28	0.1	2.8	5	1.785714286	3.214285714	0.108

Plate 3 setup:
A Ia4->6
B Ia5->6
C GFP->6
D pET21a->6
E M9->6
F Ia5 (missing from plate 2 because it did not grow well overnight)->6
G pET21a (missing from plate 2 because it did not grow well overnight)->6
Concentration of pET21a = 485.7 ng/μL-> 2.0589μL
Final volume = 20μL
*keep all enzymes on ice, do not vortex, spin 10 sec before use
1μL ecoRI enzyme + 1μL Hendi3 enzyme + 2.1μL pET21a plasmid +2μL buffer (10x) + 13.9μL filtered water
37°C incubator for 1 hour
80℃ water bath for 20 min
Store in 20℃ freezer- waiting on primers

6/28:

Absorbances for Ze1 and Ze4 from plate 3 that did not grow well yesterday

Bacteria	Absorbances	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
Ze1	1.142	0.1	11.42	5	0.437828371	4.562171629	0.124
Ze4	0.55	0.1	5.5	5	0.909090909	4.090909091	0.109

+5μL AMP
+50μL IPTG
Shaker for 4 hours
New absorbance:
*blank 1:20 LB:M9

Bacteria	Absorbances	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD

Ze1	1.113	0.1	11.13	5	0.449236298	4.550763702	0.121
Ze4	1.088	0.1	10.88	5	0.459558824	4.540441176	0.125

Plate setup:
Ze1 (plate 3)->6
Ze4 (plate 3)->6
M9+AMP->6
Grow for 72 hours
PAGE gel
Clean parts with diluted soap and ethanol
*Lower % of gel the further the protein can move through
*higher % gel means smaller holes to migrate through and a better resolution of small proteins
Using 15% gel
Make first gel recipe
3.75μL 1M Tris pH 8.8
100μL 10% SDS
5 mL 30% Acrylamide
1.05mL water
When ready to pour add
100μL 10% Ammonium persulfate
4μL TEMED
Pour gel, level with isopropanol and let sit for 45 min
Pour out isopropanol and make top layer of gel
0.63mL 1M Tris pH 6.8
50μL 10% SDS
1mL 30% Acrylamide
3.7mL water
When ready to pour add
50μL 10% Ammonium persulfate
5μL TEMED
Pour into gel mould and insert the combs, let sit for 45 min
Store in damp paper towel until ready to use
Primers
Spin down solid form for 5 min
Used lpAFP ~500-600bp
Want a final concentration of 100mM
23.8nmol + 238 AE buffer = 100mM vortex
Make 1:10 primer dilution 9μL di water + 1μL primer (do it for both tubes)
PCR reaction
*add water first

1μL template DNA
1.25 (1.3)μL each diluted primer (2)
21.5μL water
25μL 2x master mix (green)- this is the enzyme that will make the copies of DNA (polymerase)

PCR machine and store in freezer

6/29:

Rinse and stain EMG plate 1 and 2
Agarose gel- 1% agarose, DNA, something to visualize results (sybr green) PCR from yesterday
Pour gel, insert comb and wait for gel to harden- 20 min
2μL dying gel + 5μL PCR mix and add to gel
Order:
Ladder
Lp
Cf
Dc
PCR reaction gel:
+ 5μL denaturing agent to each sample (Ia, Ze, GFP and pET21 with and without IPTG)
Boil @80℃ for 5 min, cool and load into gel
Take combs out of gel and fill cracks with buffer
Map of gel:

Gel 1:
Ladder (5μL)
pET21a - (15μL)
pET21a +
GFP -
GFP +
Ia4 -
Ia4 +
Ia5 -
Ia5 +
Gel 2:
Ladder (5μL)
Ladder (5μL)
pET21a - (15μL)
pET21a +
GFP -
GFP +
Ze1 -
Ze1 +
Ze4-
Ze4+

Pour into sink

Peel apart glass holding gel
Put gel into boxes and rinse with water for 5 min 3x
Pour blue dye and shake for 1 hour
Rinse with water and keep in water over the weekend

7/2:

Purifying PCR

- 45μL PCR rnx (Lp, Cf and Dc) + 90μL NTI buffer
- Mix for 1 min
- 700μL Silica membrane wash
- Mix 1 min
- Dry mix 2 min
- 40μL AE buffer
- RT for 1 min and spin for 1 min
- Nanodrop

Lp 105ng/μL
Cf 275ng/μL
Dc 64.1ng/μL
PCR machine for 1 hour
2x assembly- 10μL

- 50ng vector- 1μL in each
- Lp 1μL + 8μL water
- Cf 0.5μL + 8.5μL water
- Dc 2μL + 7μL water
- (-) vector only 1μL vector + 1μL + 9μL water
- (+) 10μL NE positive control
- +10μL G2x
- 60 min @ 50℃

Transformation

- 2μL assembly rxn- keep the rest @-20℃
- 50μL cells (NED5 alpha)
- Ice 30 min
- 42℃ heat shock for 30 sec
- Ice 5 min
- 950μL SOC medium
- 37℃ 2 hours
- Plate on AMP plates- 10 total (one with 100μL and the other with 900V for each sample)

Plate EMG plates 1, 2 and 3 MAP
A Ze1 (37℃)->6 Ze1(RT)->6
B Ze4 (37℃)->6 Ze4 (RT)->6
C Ia4 (37℃)->6 Ia4 (RT)->6
D Ia5 (37℃)->6 Ia5 (RT)->6
E GFP (37℃)->6 GFP (RT)->6
F pET21a (37℃)-> pET21a (RT)->6

G M9+AMP (37℃)->6 M9+AMP (RT)->6
H Standard curve->12

7/3:

Counted colonies from cloned plates:

*All colonies were very small and there were colonies on our negative control plates

Lp

100μL ~104 colonies

900μL ~60-70 colonies

Cf

100μL ~164 colonies

900μL ~ over 200 colonies

Dc

100μL ~128 colonies

900μL ~488 colonies

Positive control

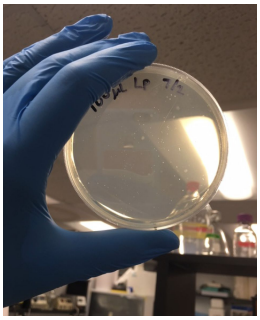
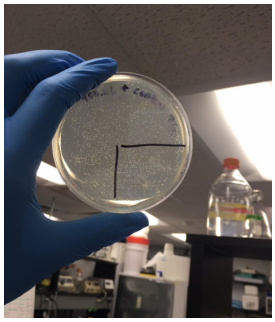
100μL ~80 colonies

900μL~ a lot

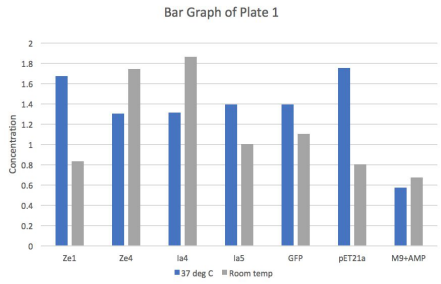
Negative control

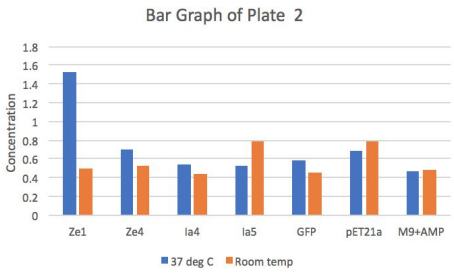
100μL ~100 colonies

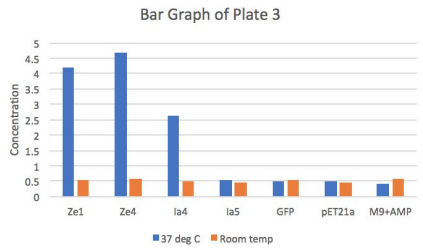
900μL ~over 200 colonies

Data from EMG plates:







7/10:

Over nights for another PAGE gel were made the day before

Bacteria	Absorbances	Target OD	OD/Target OD	Target Volume	Culture (mL)	Media (mL)	New OD
Ze1	0.575	0.1	5.75	10	1.739130435	8.260869565	0.108
Ze4	0.799	0.1	7.99	10	1.251564456	8.748435544	0.117
Ia4	1.032	0.1	10.32	10	0.968992248	9.031007752	0.118
Ia5	0.423	0.1	4.23	10	2.364066194	7.635933806	0.105
GFP	1.552	0.1	15.52	10	0.644329897	9.355670103	0.113
pET21a	0.84	0.1	8.4	10	1.19047619	8.80952381	0.117

The cultures were split 5mL and 5mL, 5μL IPTG was added to ½ the cultures, 5μL of AMP was added to all of them

In the shaker for 4 hours, spun down, liquid dumped and all put in in the -80°C freezer

7/12:

2mL cold/filtered water + 2mm square from NCTC plate made using beads into tube, mix until there are no chunks left

*keep on ice

Spin 10 min 3X total, transform with GFP to see results

Used CAM plates

PAGE gel:

100μL buffer per sample + denaturing agent

Use syringe to pipette up and down to prevent the samples from being too thick to pipette into the wells

Add 100μL buffer to samples to re-suspend and transfer to small tube, heat to 80°C for 10 min

Spin down 10 sec, load into gel

3μL ladder, 15μL samples

Fixing solution for gels:

100mL ethanol

20mL acetic acid

200mL ultra pure water

Gel 1:

Ladder
Ze1
Ze4
Ia4
Ia5
GFP
pET21a

Gel 2:

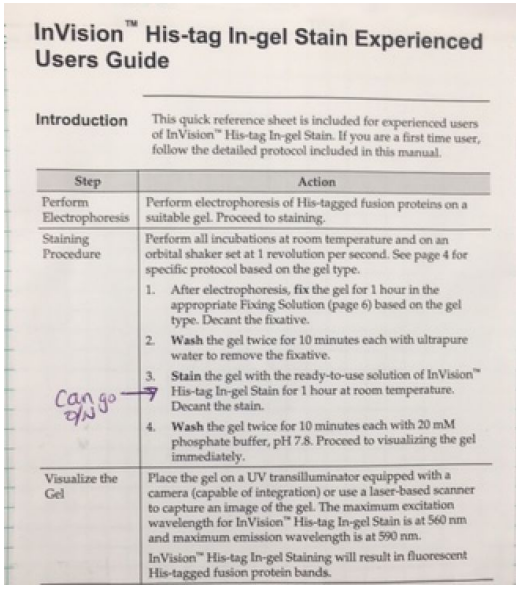
Ladder
Ladder
Ze1
Ze4
Ia4
Ia5
GFP
pET21a

Gels from 7/12 did not work because lid disrupts the electrical circuit

Use premade gel:

3μL ladder, 12μL samples (same samples from other gel)

Followed this procedure for the gel:



InVision™ His-tag In-gel Stain Experienced Users Guide

Introduction This quick reference sheet is included for experienced users of InVision™ His-tag In-gel Stain. If you are a first time user, follow the detailed protocol included in this manual.

Step	Action
Perform Electrophoresis	Perform electrophoresis of His-tagged fusion proteins on a suitable gel. Proceed to staining.
Staining Procedure	Perform all incubations at room temperature and on an orbital shaker set at 1 revolution per second. See page 4 for specific protocol based on the gel type. 1. After electrophoresis, **fix** the gel for 1 hour in the appropriate Fixing Solution (page 6) based on the gel type. Decant the fixative. 2. **Wash** the gel twice for 10 minutes each with ultrapure water to remove the fixative. 3. **Stain** the gel with the ready-to-use solution of InVision™ His-tag In-gel Stain for 1 hour at room temperature. Decant the stain. 4. **Wash** the gel twice for 10 minutes each with 20 mM phosphate buffer, pH 7.8. Proceed to visualizing the gel immediately.
Visualize the Gel	Place the gel on a UV transilluminator equipped with a camera (capable of integration) or use a laser-based scanner to capture an image of the gel. The maximum excitation wavelength for InVision™ His-tag In-gel Stain is at 560 nm and maximum emission wavelength is at 590 nm. InVision™ His-tag In-gel Staining will result in fluorescent His-tagged fusion protein bands.

Also viewed gel

7/16:

Analysis of results from PAGE gel

Cloning:

New vector prep

1. Digest- 5μg/0.4857= 10.3μL DNA + 30.7μL water + 2μL of each enzyme + 5μL cutsmart buffer (50μL total volume) 2.5 hours in 37℃
2. Add 1μL SAP 1 hour in 37℃

7/17:

DNA gel 1% agarose

60mL 1X TAE buffer + 0.6g DNA agarose microwave for 2 minutes

Pour gel and let sit for 45 minutes, pour buffer and add samples and run the gel fpr 45 minutes- 10μL 6X dye added to vector samples 20μL into each of the 3 wells 5μL buffer into first well

Gel did not work…

10μL ladder in new well

3μL mini prep + 3μL dye

Nanodrop of pET21a 474.8 ng/μL and 432 ng/μL

Will need to redo vector and miniprep

Transforming +/- control from interlab into EMG (50μL) competent cells + 1μL DNA (with red dye) 100μL to CAM plate

*Follow Transformation protocol

***for tomorrow- make overnight cultures of pET21a + GFP to look under the microscope and determine if proteins are being expressed- no evidence in gel- make 4 overnights total from 2 colonies from each plate

7/18:

Over nights of:

Inducible GFP

+/- GFP

pET21a from old plate that did not work yesterday

Streaked plates for Ze1, Ze4, Ia4, Ia5, GFP

Streaked plates from pET21a freezer stock

7/19:

+/- GFP worked! It glowed under the fluorescent lights

pET21a from old plate spun down for 10 minutes

Inducible GFP in EMG- only one worked

NCTC plates from 7/12- 4 and 2 colonies no color (yet)

Back dilute inducible GFP with IPTG

1mL cultures in 4 tubes, add 1μL AMP to each tube, 0μL, 1μL, 10μL, and 100μL IPTG to 4 tubes, grow 4 hours in shaker in 37℃, 100μL in black clear bottom plates and read OD:

1.000- 5/10=0.5mL-5= 4.5mL New OD: 0.127- 0.109

*NCTC slow growing and slow expressing

NCTC overnight, over night iptg instead of 4 hours, 30℃, store for Histag gel stain

Rest of GFP samples back in 37℃ overnight, read samples Friday note time***start of IPTG

Nanodrop, digest, SAP, gel analysis friday

NCTC overnights + AMP to test and see if they are actually NCTC

7/20:

NCTC overnights

plates - take out monday

OD 0.354 and 0.358

Absorbance with plate reader

Put 1μL of culture in spinner at 37℃ + 1μL AMP

Gel

.6g agarose

60mL TAE buffer

Pour and let sit 45 min

50μL digest + 10μL dye = 60μL total / 3 wells

10μL ladder- skip well in between ladder and digest, run gel, imaged **Digest was bad**

7/23:

Mini prep on agarose gel (Natalie's)

Vector again (2) from overnight made from frozen stock streak plates

20mL 50x TAE buffer + 980mL water

Don't digest mini preps yet… waiting to see results 5mL into 1 well (of each)

10μL ladder, 5μL mini preps + 1μL loading dye

Nanodrop: 109.4 and 150.5

Plate setup:

Well 1	**Well 2**	**Well 3**	**Well 4**	**Well 5**	**Well 6**
Ladder	Natalie's mini prep	Old mini prep 1	Old mini prep 2	New mini prep 1	New mini prep 2

Results from IPTG experiment plate reading inconclusive… IPTG possibly not working need

to redo experiment, also try something new: AraC

AraC from MQP, run gel

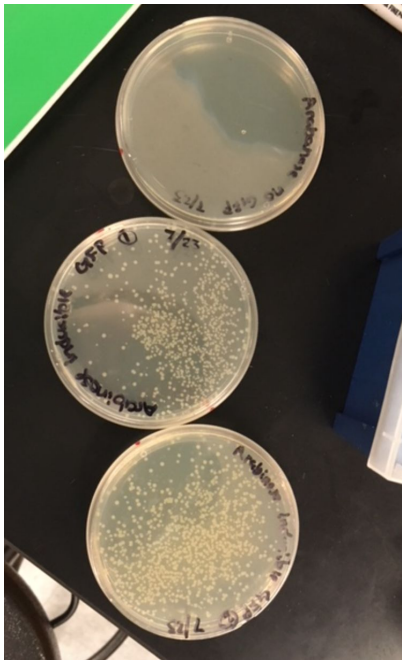
Gel setup:

Well 1	**Well 2**	**Well 3**	**Well 4**
Ladder	pRpos/AraC (no GFP)	AraC/GFP 1	AracC/GFP 2

Vector nanodrop: 150.5 -> .1505 5/.1505 = 33.2μL vector, 2μL each of the enzymes, 5μL cutsmart, 7.8μL water
Incubate for 1 hour, 1μL SAP
EMG inducible GFP overnights made to redo IPTG experiments
0.6mg gel 200μL/100mg -> 1.6mL buffer to digest gel
+250μL SOC medium, shake for 1 hour
Nanodrop: 19.8 ng/μL
Put in freezer, Gibson assembly tomororw

7/24:

OD of GFP overnights
1: 0.699 2: 1.201 3: 1.084 4: 0.602
12.01 5/12.01= 0.416mL 4.583mL LB OD: 0.136- 0.111
6.12 ⅝.12= 0.817mL 4.183mL LB OD: 0.105
+5μL AMP to each, split and add 0μL,1μL,10μL and 100μL to each tube (8 total, 1 from each of the 2 overnights)
Nanodrop: 17.9
50/17.9= 2.79μL of vector from Gibson assembly
1 hour at 50℃ + 750μL SOC medium, incubate 1 hour, plate on AMP plate and grow overnight
Overnights made from AraC plates



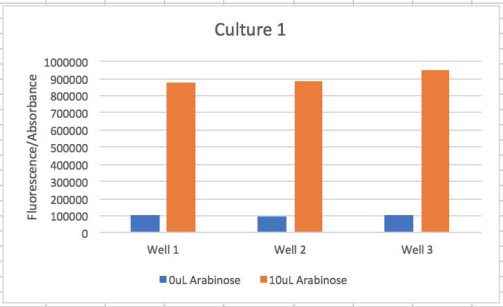
1st culture OD: 1.807 18.07 5/18.07=0.277mL culture 4.723mL LB OD: 0.247->0.118
2nd culture OD: 1.913 19.13 5/19.13=0.262mL culture 4.738mL LB OD: 0.323->0.111
+5μL Cam split into 1mL samples, 2 from each culture, to 1 of each sample add 0μL 10% arabinose and to the others add 10μL 10% arabinose

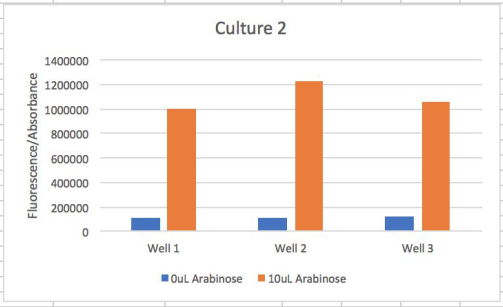
Arabinose experiment worked after 4 hours of incubation! The samples with no arabinose did not glow while the samples with arabinose did! Put 100uL in 3 wells for each sample and read on plate reader

Culture 1		
Absorbance		
0.208	0.230	0.228
0.206	0.214	0.222
Fluorescence		
21526	23005	22873
180552	189178	210861

Culture 2		
0.182	0.202	0.196
0.207	0.210	0.219
20856	23279	23461
206866	257066	232019

Culture 1		
Well 1	Well 2	Well 3
103303.7375	99906.72256	100467.4132
878482.5336	884790.3941	948314.549
Culture 2		
Well 1	Well 2	Well 3
114314.1459	115353.7872	119414.752
998374.1391	1225443.535	1057500.17





Will now need to reclone AFPs

7/26:

Transformed AraC into DH5a cells on plate from yesterday had colonies on negative control

Pour agarose gels 0.6g agarose + 60mL TAE buffer

Gel Set up

Well 1	Well 2	Well 3	Well 4	Well 5
10uL ladder	5uL purified vector + 1uL dye	5uL pET21a mini prep + 1uL dye	5uL AraC with GFP + 1uL dye	5uL AracC with no GFP + 1uL dye

Ran gel- AraC with and without GFP appropriate lengths, mini prep normal and purified vector appeared normal

Transformation into DH5a

1uL AraC 1 GFP, AraC 2 GFP, and AraC no GFP +25uL DH5a *regular transformation procedure

+250uL SOC medium

***¼ of total volume of sample 65% gylcerol

AraC on Cam plates

Plate 100uL on Cam plate rest used for glycerol stocks

Total concentration of glycerol stocks should be 15% glycerol

Volume 175uL x 15% = 26.25% of 100% glycerol x 2 = 52.5uL of 50% glycerol

Actually added 17% (59.5uL) to account for change in volume when glycerol is added to stocks

7/25:

Overnights of AraC without GFP, and AraC 1 and 2 with GFP (3x 5mL + 5uL Cam)

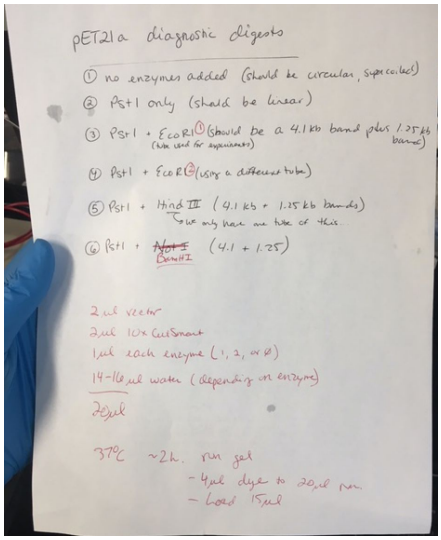
Overnight of plain EMG with no antibiotics

7/26:

Plates:

Strain	Original OD	Calculation	Amount of culture	Amount of LB	Final OD
AraC no GFP	1.667	(5/16.67)-(5)	0.299mL	4.725mL	0.115
AraC GFP 1	1.665	(5/16.65)-(5)	0.300mL	4.94mL	0.109
AraC GFP 2	1.816	(5.18.16)-(5)	0.275mL	4.970mL	0.122
EMG	1.091	(5/10.91)-(5)	0.458mL	4.566mL	0.109

+5uL Cam, split cultures in half and add 5uL Arabinose to half
No GFP (2.75uL each) + 2.8uL Arabinose
GFP 1 (3.5uLuL each) + 3.5uL Arabinose
GFP 2 (3.5uL each) + 3.5uL Arabinose
Incubate 4 hours @ 37℃
Freezer Stocks:
500uL cells straight from over nights + 500uL 50% glycerol (25% total concentration) and freeze in -80℃
Enzyme troubleshooting:



Run gel for 45 min
Mini Prep:
*follow protocol
Nanodrop
GFP2: 6.8
GFP1: 5.4
No GFP: 14.8

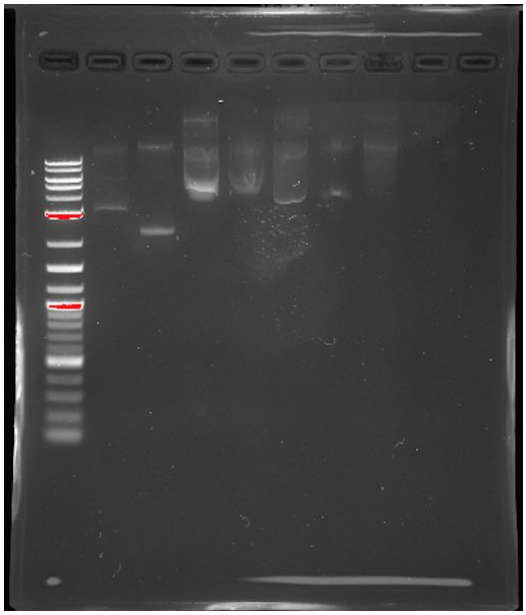
Plate Setup:

	1	2	3	4	5	6	7	8	9	10	11	12
A	AraC +	AraC +	AraC +	AraC +	AraC +	AraC +						
B	AraC -	AraC -	AraC -	AraC -	AraC -	AraC -						
C	GFP 1+	GFP 1+	GFP 1+	GFP 1+	GFP 1+	GFP 1+						
D	GFP 1-	GFP 1-	GFP 1-	GFP 1-	GFP 1-	GFP 1-						
E	GFP 2+	GFP 2+	GFP 2+	GFP 2+	GFP 2+	GFP 2+						
F	GFP 2-	GFP 2-	GFP 2-	GFP 2-	GFP 2-	GFP 2-						
G	emg	emg	emg	emg	emg	emg						
H	M9+	M9+	M9+	M9+	M9+	M9+	M9-	M9-	M9-	M9-	M9-	M9-

7/31:
2uL vector pET21
2uL 10X cutsmart
(+/-) 1uL EcoRI (2) and BamHI
14-16uL water
(20uL total volume)
Digest 37℃ 2 hours
Heat @ 80℃ for 20 minutes
Spin down quick
Transform 2uL into 50uL DH5a cells
Ice 30 minutes, heat shock 30 sec @ 42℃, ice for 5 minutes, 950uL SOC medium, 37℃ for 2 hours, plate 100uL AMP plates
Overnights of plain EMG (no plasmid), AraC GFP 1, 2 and no GFP from original and streaked plates
8/1:
Minipreps of overnights from yesterday
Test samples in agarose gel

Gel setup:

Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8
Ladder	No GFP original plate	No GFP streaked plate	GFP1 original plate	GFP1 streaked plate	GFP2 original plate	GFP2 streaked plate	Plain EMG



Wash 48 hour plate

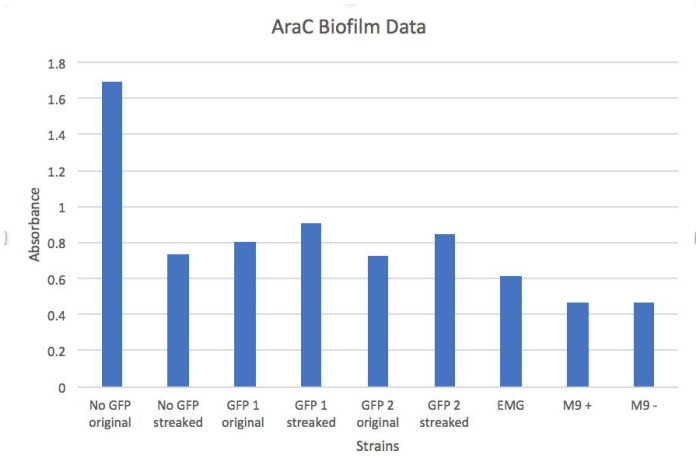
Nanodrop if sample:

- No GFP original plate- 6.1
- No GFP streaked plate- 46.0
- EMG- 17.6
- GFP1 original plate- 38.3
- GFP1 streaked plate- 7.7
- GFP2 original plate- 41.8
- GFP2 streaked plate- 33.4

Rinse 48 hour plate with water, 125uL crystal violet dye for 10 minutes, rinse 3-4 time with water and let dry overnight

8/2:

Read AraC biofilm plate



Make AMP and CAM plates- could be why we have been having contamination problems
*used standard procedures under protocols

9/6:

Vector/gel

1uL Prpos AraC vector (~150-180 mg/uL) -> 1/0.142= 7.03uL

1uL Pst1 + 1uL Spe1 + 2uL cutsmart buffer

Degest 2 hours at 37 C, add 1uL SAP and incubate 20 min at 37 C

Heat block 80 C for 20 minutes

DATE??

22 mini preps of IA, ZE and 2 Neg controls

9/16

168uL water

+24uL cutsmart buffer

+12uL pst1

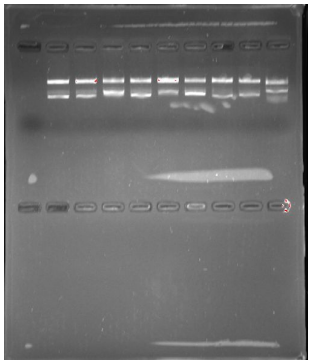
+12uL ecor1

Mix, 9uL per tube and to each tube add 1uL plasmid (8 of IA, 8 of Ze and 2 neg controls) and 2uL loading dye

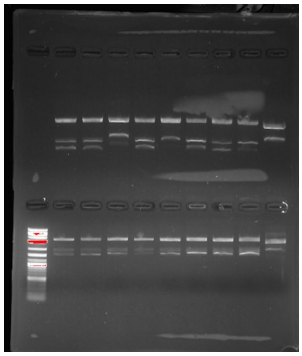
Run gel

Gel picture: Ladder did not contain dye

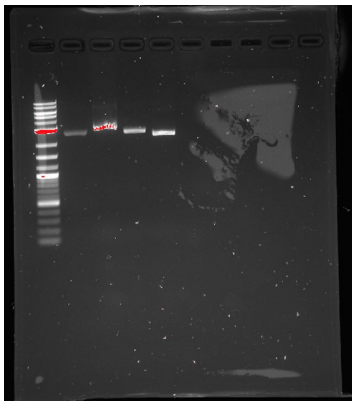
Well 1= ladder, Wells 2-11= ZE, Well 12= neg control 1



Gel 2: Well 1=ladder, Wells 2-11=IA, Well 12= neg control 2



9/21:
Ran another gel
3.5uL EcoR1
+3.5uL Pst1
+7uL cutsmart buffer
+42uL water
Mix and split into 5 tubes, add 2 uL plasmid (IA1, Ze2, Ze3, neg control and ArC GFP) + 2uL dye
Gel: Well 1=ladder, Well 2=IA1, Well 3= Ze2, Well 4= Ze3, Well 5= neg control, Well 6= GFP



10/3
85% agarose gel- 0.5mL agarose in 60mL
7 uL ecor1
14 uL cutsmart buffer
49 uL water
+2uL dye

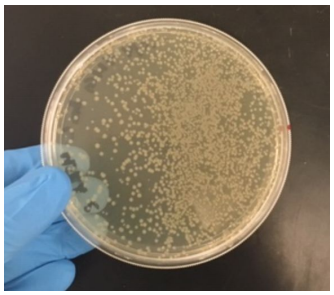
+1uL Cf, Ap or Mrs1

10/4

Cloning of Ia, Ze, Cf, Ap, Msr1, GFP, empty

Used cloning protocol

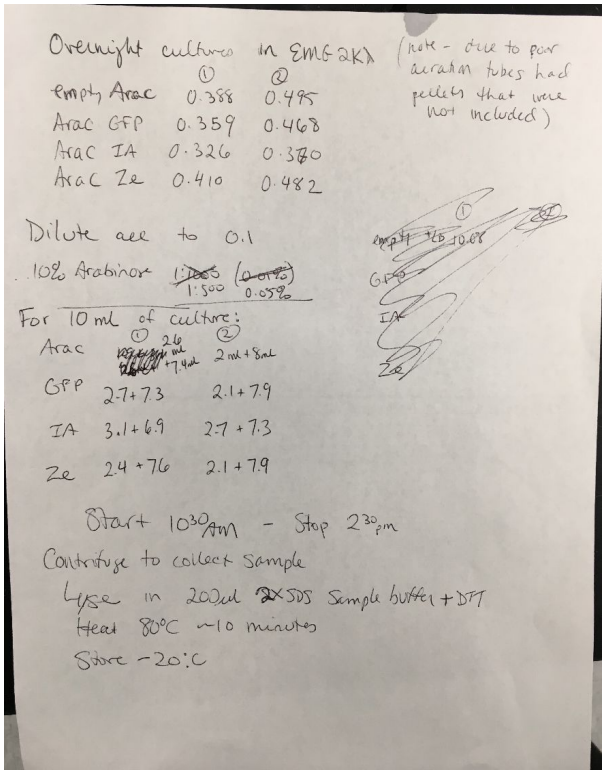
Plated



10/5

Over nights made with CAM

10/6



Crystal Violet assay plate with +/- arabinose and CAM
Followed protocol
OD

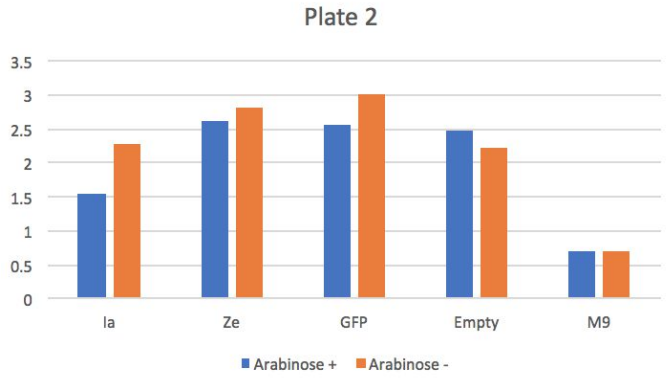
Ia -	Ia +	Ze -	Ze +	GFP -	GFP +	Empty -	Empty +
1.374	1.189	1.254	1.48	1.387	1.392	1.384	1.472
0.15	0.14	0.18	0.21	0.15	0.17	0.17	0.18

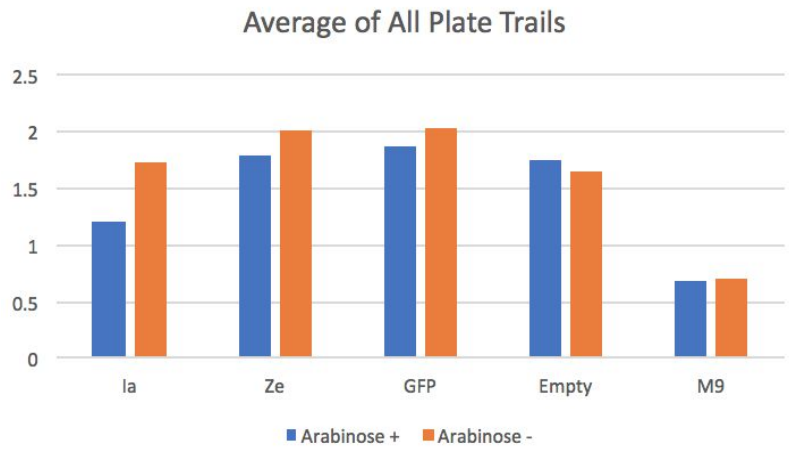
IA	Ze	Cf	apbd	msr1	gfp	empty
0.257	0.307	1.434	0.528	0.428	0.771	0.276
0.624	0.297	0.564	0.317	0.345	0.433	0.393
0.306	0.457	0.315	0.956	0.29	0.406	0.318

IA	Ze	Cf	apbd	msr1	gfp	empty							
0.257	0.307	1.434	0.528	0.428	0.771	0.276							
0.624	0.297	0.564	0.317	0.345	0.433	0.393							
0.306	0.457	0.315	0.956	0.29	0.406	0.318							
Ia +	Ia -	Ze +	Ze -	Cf +	Cf -	apbd +	apbd -	msr1 +	msr1 -	gfp +	gfp -	empty +	empty -
0.725	1.356	1.416	1.251	1.579	1.482	1.457	1.468	1.463	1.381	1.411	1.37	1.455	1.31
7.25	13.56	14.16	12.51	15.79	14.82	14.57	14.68	14.63	13.81	14.11	13.7	14.55	13.1
1.3793103	0.7374631	0.7062147	0.7993605	0.6333122	0.6747638	0.6863418	0.6811989	0.683527	0.724113	0.7087172	0.729927	0.6872852	0.7633588
8.6206897	9.2625369	9.2937853	9.2006395	9.3666878	9.3252362	9.3136582	9.3188011	9.316473	9.275887	9.2912828	9.270073	9.3127148	9.2366412
1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1.326	1.527	1.945	1.337	1.536	1.559	1.455	1.511	0.689	1.411	1.475	1.439	1.471	1.41	
13.26	15.27	19.45	13.37	15.36	15.59	14.55	15.11	6.89	14.11	14.75	14.39	14.71	14.1	
0.3770739	0.3274394	0.2570694	0.3739716	0.3255208	0.3207184	0.3436426	0.3309067	0.7256894	0.3543586	0.3389831	0.3474635	0.3399048	0.3546099	bacteria
4.6229261	4.6725606	4.7429306	4.6260284	4.6744792	4.6792816	4.6563574	4.6690933	4.2743106	4.6456414	4.6610169	4.6525365	4.6600952	4.6453901	M9

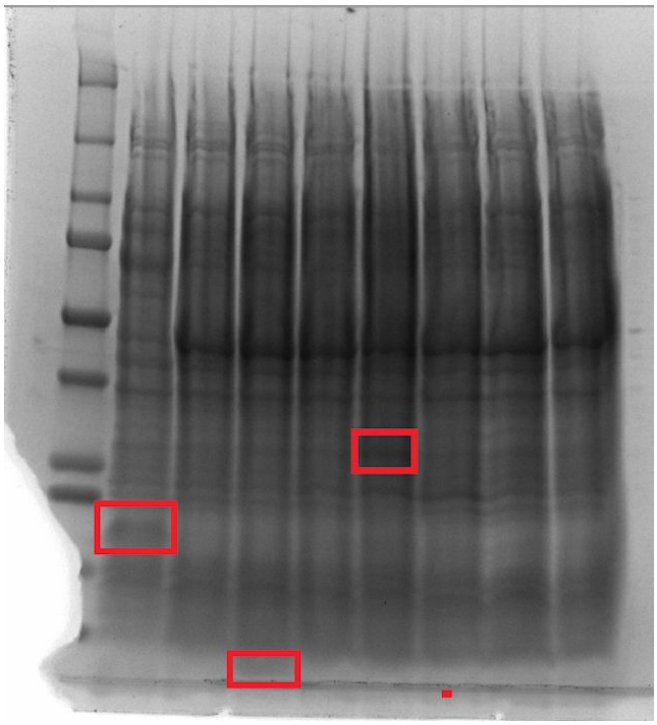
10/10

Results from crystal violet assay plate





Protein gel that confirms expression of AFPs. The red boxes outline the IaAFP with arabinose, ZeAFP with arabinose and GFP with arabinose. The samples of IaAFP, ZeAFP and GFP without arabinose do not show the band that shows expression of the proteins. The last two wells contained samples of empty psB1C3 plasmid and therefore do not have the bands of protein.



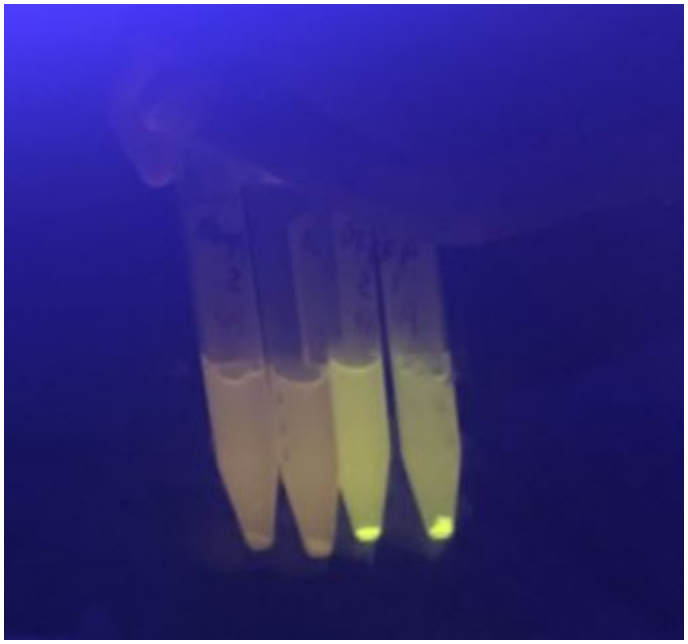


Image of GFP cultures. The two tubes on the right that contain arabinose and the two tubes on the left do not. It can be seen that the tubes containing arabinose are glowing and therefore confirm the expression of the GFPs.