PCR Reaction of Hsp60 Promoter June 25, 2014
Purpose: Amplification of Hsp60 Promoter for insertion into biobrick

Reaction Reagents:
1. mCherry Fushion hsp60 promoter template
2. Primers
    a. Hsp60BBfwd
    b. Hsp60rev comps
    c. RBS_BBrev comps
3. PCR Buffer
4. Double distilled water
5. DNA Polymerase
6. 1kb DNA Ladder

Primer Numbering and Concentration:
1. Hsp60BBfwd: 29.1 nmol/100ul
2. Hsp60rev comps: 26.2 nmol/100ul
3. RBS_BBrev comps 27.7 nmol/100ul

Final Concentration 100 uM of DNA

Reagent Table 1:

Reagent	3x (ul)
Clear Color Buffer	12
dNTP's	1.2
Primers: 1 + 3	6
Template DNA	3
DNA Polymerase	0.6
ddH_2O	37.2
Total Volume	60

Reagent Table 2:

Reagent	3x (ul)
Clear Color Buffer	12
dNTP's	1.2
Primers: 1 + 2	6
Template DNA	3
DNA Polymerase	0.6
ddH_2O	37.2
Total Volume	60

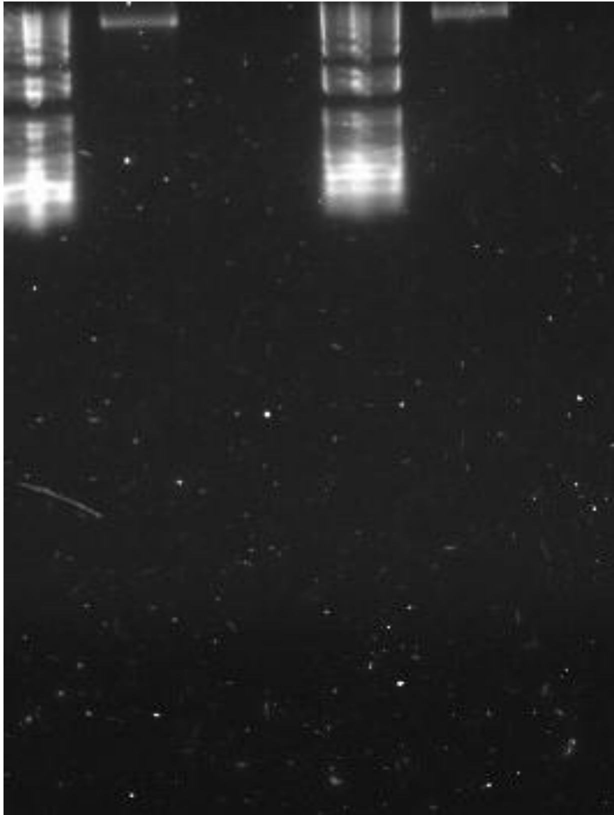
Loading Buffer Concentration Table:
x= total loading volume

$$x = total\ loading\ volume$$

$$x - \frac{1}{5}x = 5$$

Reaction loaded (ul)	Loading Buffer (ul)
4	1
5	1.25
8	2
10	2.5

Result of PCR with Hsp60 June 25th, 2014
Friday, June 27, 2014
Result of PCR with Hsp60



Gel Analysis:
Loading Table

Lane	Sample	Amount Loaded (ul)
1	1kb DNA Ladder	2
2	(1) Hsp60BBfwd (3) RBS_BBrev	6.25
3	Control, no primers	6.25
4	Ladder	6.25
5	(1) Hsp60BBfwd (2) Hsp60BBrev	6.25
6	Control, no primers	6.25

It seems that too much template DNA was used hence the bands did not travel far

Liquid Culture with DAM- E. coli in LB
Thursday, June 26, 2014

1. Set up 3 liquid cultures with 4 ml of LB each and 4 ul of Ampicillin (1:1000 Concentration)
2. Incubated in 37˚C shaker overnight

Mini-Prep DAM- Negative Liquid Culture
Friday, June 27, 2014

Buffer Preparation:
Resuspension Solution: add provided RNAse Al store at 4˚C for up to 6 months
Wash Solution: Add 35 ml of 96% EtOH to 20 ml wash solution. Store at room temperature (RT); we have 100% EtOH

$$C_1V_1 = C_2\ V_2$$
$$(1)V_1 = 0.96(35ml)$$
$$V_1 = 33.6\ ml\ of\ 100\%\ EtOH + 1.4\ ml\ ddH_2O$$

1. Pipet 1.5 mL of culture into microcentrifuge tube and spin for 2 minutes
2. Resuspend pellet in 250 mL of Resuspension solution
3. Add 250 mL of Lysis solution and mix by inverting tube
4. Add 360 mL of neutralization and mix immediately by inversion 4-6 times
5. Centrifuge for 5 minutes to pellet cell debris
6. Transfer supernatant to spin column
7. Centrifuge for 1 minute and discard flow through
8. Add 500 mL of wash solution and centrifuge for 30-60 seconds and discard flow through
9. Repeat wash using 500 mL of wash solution
10. Transfer spin column to fresh 1.5 mL tubes and add 50 mL of elution buffer to center of column
11. Incubate at RT for 2 minutes centrifuge for 2 minutes
12. Store flow through (purified plasmid DNA) at -20 degree Celsius.

Result:

Tube	Concentration (ng/uL)
1	29.1
2	29.5
3	18.4
4	25.9
5	18.5
6	16.8
7	23.9
8	15.6

PCR of Hsp60 with and without RBS
Friday, June 27, 2014

Making a 1:13 dilution of our template DNA which is 140 ng/uL

Components	Amount (ul)
deionized water	12
Template DNA	1

Reagent Table 1 (w/o RBS):

Reagent	3x (ul)
Clear Color Buffer	12
dNTP's	1.2
Primers: 1 + 3	3
Template DNA	3
DNA Polymerase	0.6
ddH_2O	37.2
Total Volume	57

Reagent Table 2 (w/ RBS):

Reagent	3x (ul)
Clear Color Buffer	12
dNTP's	1.2
Primers: 1 + 2	3
Template DNA	3
DNA Polymerase	0.6
ddH_2O	37.2
Total Volume	57

Primer Numbering and Concentration:
1. Hsp60BBfwd: 29.1 nmol/100ul
2. Hsp60rev comps: 26.2 nmol/100ul
3. RBS_BBrev comps 27.7 nmol/100ul

Final Concentration 100 uM of DNA

1.25 uL of loading dye + 5 uL PCR reaction
2ul 1 kb DNA ladder

Started gel at 4:26 pm
Stopped gel at 4:55 pm

Results:
There were no PCR Products

PCR with Hsp60
Monday, June 30, 2014

Reagent Table 1 (w/o RBS):

Reagent	2.5x (ul)
Clear Color Buffer	25
dNTP's	2.5
Primers: 1 + 3	12 (6 each)
Template DNA	6
Phusion	1.25
ddH_2O	77.5
Total Volume	125

Reagent Table 2 (w/ RBS):

Reagent	2.5x (ul)
Clear Color Buffer	12
dNTP's	1.2
Primers: 1 + 2	12 (6 each)
Template DNA	6
Phusion	1.25
ddH_2O	77.5
Total Volume	125

PCR Tube Labeling:
1 = fwd primer + rev primer RBS
2 = fwd primer + rev primer Hsp60 (without RBS)

New PCR Condition:

Temperature (℃)	Time
98	30 secs
98	10 secs
64	15 secs
72	15 secs
72	5 mins
4	hold

Some housekeeping tasks
Monday, June 30, 2014
0.8% Agarose Gel:
25 mL 1x TBE buffer
0.2 g Agarose
1uL EtBr
100 mL LB and autoclaved:
1g tryptone
0.5g yeast extract
1g NaCl

Loaded 1 kb of volume 1 ul and then 6.25 ul of samples (w/RBS in lane 2 and w/o RBS lane 3)

Gel started at 3:30 pm
Gel stopped at 4:40 pm

Made 10, 5 mL LB+AMP, E. coli dam- pBRES36a culture at 6 pm

Mini-Prep
Tuesday, July 1, 2014

Mini-prepped 50 mL of E. coli dam- pBRES36a in LB+AMP

Obtained:
450 ul of 17.6 ng/ul of DNA
50 ul of 14.6 ng/ul of DNA

Primer Walking and PCR Purification
Wednesday, July 2, 2014
Primer Walking:
Belle submitted the plasmid (pBRES36a) to GeneWiz for Primer Walking

PCR Purification:
Purified 45 mL of both RBS and no RBS PCR products using new SV Wizard Kit from Promega
Added 75 mL of 95% EtOH to membrane wash solution

Final Concentration:

Tube Labeling	PCR Product	Concentration (ng/uL)
1	RBS	17.0
2	No RBS	20.9

Restriction Digest of PCR Product with XbaI and PstI
Thursday, July 3, 2014
Result

1. 8.6 ug/mL for RBS PCR product
2. 11.5 ug/mL for no RBS PCR product

Mini-Prep
Tuesday, July 8, 2014
Mini-prepped 5x5 mL of E. coli dam- pBRES36a in LB+amp
    Ran out of lysis solution ,unable to mini prep the remaining 25 mL

Yield:
5x50 mL = 250 mL
Concentration: 42 ug/mL, equivalent to10.5 ng plasmid

Testing old Tubes and Preparing LB Agar (100mL)
Wednesday, July 9, 2014

Testing Old Mini-prep Columns and Tubes:
Use 20 uL of 3.0 ug/mL DNA
Result
New tubes: 52 ug/mL
Old Tubes: 20 ug/mL

Conclusion: old tubes can no longer be used purchased new tubes

100 mL LB Agar and autoclaved:
1g tryptone
0.5g yeast extract
1g NaCl
1.5 g Agar (1.5%)
100 mL Water (ddH2O)

50 mL aliquots

Ligating Hsp60 Inserts Into Vector Backbone
Monday, July 14, 2014

1. Digestion

Materials:

Reagent	Amount (ul)
DNA	40
Buffer	5
XbaI restriction enzyme	1.5
PstI restriction enzyme	1.5
ddH2O	2
Total	50

Procedures:
1. Mixed materials together in microcentrifuge tubes
2. Incubate 30 minutes at 37 degrees Celsius

2. PCR Purification (using Promega Wizard SV gel clean up kit)
3. Ligations
   a. Ligation 1: RBS and Vector
   b. Ligation 2: no RBS and Vector

Materials for Ligation 1:

Reagent	Amount (ul)
Ligase	1
Buffer T4	2
30 ng of RBS insert	4
60 ng of vector plasmid	3.5
ddH2O (Volume to 20)	9.5

Materials for Ligation 2

Reagent	Amount

	(ul)
Ligase	1
Buffer T4	2
30 ng of no RBS insert	3
60 ng of vector plasmid	3.5
ddH2O (Volume to 20)	10.5

Procedures:
1. 15 minutes at room temperature
2. Put on ice until transformation

4. Transformation (see transformation protocol)

Housekeeping
Monday, July 14, 2014

30 mL LB Agar and autoclaved:
0.3 g tryptone
0.18 g yeast extract
0.3 g NaCl
0.45 g Agar (1.5%)
30 mL Water (ddH2O)

Chloramphenicol (CAM)
10 mg in 1 mL of 95% EtOH
5ug/mL workign concentration is needed

$$C_1V_1 = C_2\ V_2$$
$$\left(10\frac{mg}{mL}\right)V_1 = 5.0\frac{ug}{mL}(30\ mL)$$
$$V_1 = 15\ mL$$

Used 35 ug/mL final

Prep 50 mL LB agar +CAM:

0.5 g tryptone
0.25 g yeast extract
0.5 g NaCl
0.75 g Agar (1.5%)
48 mL Water (ddH2O)
25 uL of 10 mg/mL CAM

Competent cells growing

Housekeeping and Ligation
Wednesday, July 16, 2014

Prep 75 m
0.75 g tryptone
0.375 g yeast extract
0.5 g NaCl
1.125 g Agar (1.5%)
50 mL Water (ddH2O)

Ligations

Ligation 1: RBS and Vector
Ligation 2: no RBS and Vector

Materials for Ligation 1:

Reagent	Amount (ul)
Ligase	1
Buffer T4	2
30 ng of RBS insert	4
60 ng of vector plasmid	3.5
ddH2O (Volume to 20)	9.5

Materials for Ligation 2

Reagent	Amount (ul)
Ligase	1
Buffer T4	2
30 ng of no RBS insert	3
60 ng of vector plasmid	3.5
ddH2O (Volume to 20)	10.5

Transformation:
50 mL comp cells + 5 uL plasmid (shipment plasmid + RBS/no RBS)

On ice for 2 minutes
Heat shock 42 degree Celsius for 45 seconds
Ice 2 minutes
550 mL SOC media into mixture
Shake at 37 degree Celsius for 1 hour (recovery)
Plate onto CAM plate + incubate at 4 pm

Plan + CAM plate

Friday, July 18, 2014

Plan of Attack with pBRES36a

1. Digest with individual restriction enzymes and a negative control
   a. No enzyme
   b. XbaI
   c. PstI
   d. SpeI
   e. EcoRI
2. Visualize on Gel
3. Double Digest with Pair from Step 1
4. Visualize on gel
5. Map the sequence

CAM plates made with 35 ug/mL

Transformation with:

1. Ligation product (2 of them)
2. Plasmid that's known to express CAM (for control)

7/20/14

2nd Double Digestion: For the purpose of confirming that there was no mix up in the previous digestion.

Components	Amount (uL)
10x Fast Digest Buffer	2
pBRES36a (42ng/uL)	16
SpeI	1
PstI	1
Total	20

Lane # (from left)	Sample
1	Ladder (not visible)
2	No enzyme
3	PstI and SpeI
4	No enzyme
5	PstI and SpeI

Housekeeping

Wednesday, July 23, 2014

Cam plates: 35 ug/mL, dilute 1:1000

    Used 75 mL, used 75 ul
    For 4 plates

Transformation with RBS:

    50 mL E. coli comp cells, 5 uL RBS ligation
    50 mL E. coli comp cells, 5 uL no RBS ligation

    Transformed and recovered for 1 hour

    Plated 50 uL of transformed cells onto cam plates

Restriction Digest of pBRES36a Plasmid
Monday, July 28, 2014

1. Digestion

Purpose: cut with restriction enzymes to provide a rough map for the pBRES36a plasmid and confirm the plasmid's identity

Materials:

Reagents	Amount (ul)
pBRES36a plasmid	17
Buffer	2
Restriction Enzyme	1
Total Volume	20

Procedures:

1. Mix reagents together and spin down gently.
2. Incubated for 10 minutes
3. Pipet up and down gently to mix samples
4. Load each sample onto the agarose gel
5. Run electrophoresis according to the conditions specified.

Enzyme Used:

1. XbaI
2. PstI
3. SpeI
4. EcoRI

2. Gel Electrophoresis

Run 1: Gel Set Up (7/18/14)

lane	Reaction/Reagent	Amount loaded (ul)
1	1 kb Marker	1
2	Plasmid + XbaI	5
3	Plasmid + PstI	5
4	Empty Lane	0
5	Plasmid + SpeI	5
6	Plasmid +EcoRI	5
7	Plasmid only	5

Run 1 Condition:

1. 2.0 hours
2. 80 Volts
3. 0.8% Agarose Gel
4. 1x TBE Buffer

Run 2: Gel Set Up (7/28/14)

lane	Reaction/Reagent	Amount loaded (ul)
1	1 kb Marker	1
2	Plasmid + XbaI	5
3	Plasmid + PstI	5
4	Plasmid + SpeI	5
5	Plasmid +EcoRI	5
6	Plasmid +SpeI and EcoRI	5
7	Hsp60 + XbaI and PstI	5
8	mCherry + XbaI and PstI	5

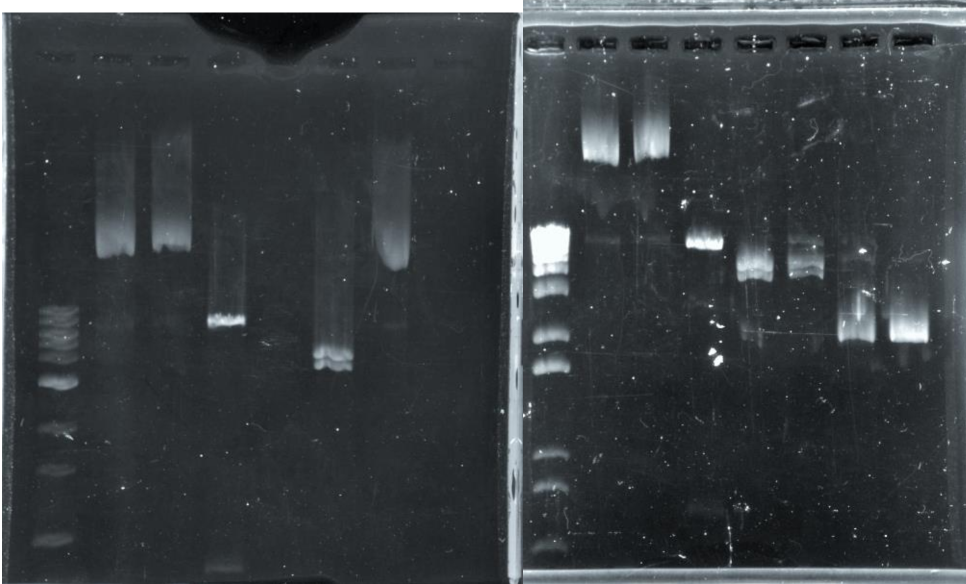
Run 2 Condition:

1. 1.5 hours
2. 80 Volts
3. 0.8% Agarose Gel
4. 1x TBE Buffer

July 28, 2014 con't:

Results

Run 1 (7/18/14) Left and Run 2 (7/28/14) Right



Ligation, Transformation, Selection
Thursday, July 24, 2014
Ligations

Ligation 1: RBS and Vector
Ligation 2: no RBS and Vector

Materials for Ligation 1:

Reagent	Amount (ul)
Ligase	1
Buffer T4	2
30 ng of RBS insert	4
60 ng of vector plasmid	3.5
ddH2O (Volume to 20)	9.5

Materials for Ligation 2

Reagent	Amount
	(ul)
Ligase	1
Buffer T4	2
30 ng of no RBS insert	3
60 ng of vector plasmid	3.5
ddH2O (Volume to 20)	10.5

Incubate at RT for 15 minutes

Transformation

1. 50 mL E. coli comp cells
2. 6 uL plasmid
3. 2 min on ice
4. 45s heat shock at 42 degrees Celsius
5. 2 min on ice
6. 950 mL SOC growth media
7. 1 hour recovery at 37 degrees Celsius

Selection:
plate 60 mL of transformed cells onto selection plate (LB + CAM plates)

8/1/14

1. Take out overnight liquid culture around 11:00 am
2. Mini prep iGEM plasmid parts

Part	Amount (ng/uL)
Desaturase	42.6
Cathelicidin	12.7
mCherry	12.7

8/4/14 Lab

Make 1% Gel (30 mL):
0.3 g Agarose
30 mL 1x TBE
1 uL EtBr

Restriction Digests:

hsp60 PCR products:

RBS:	no RBS:
6 uL ddH_2O	6 uL ddH_2O
2 uL Buffer	2 uL Buffer
1 uL XbaI	1 uL XbaI
1 uL PstI	1 uL PstI
10 uL PCR product	10 uL PCR product

iGEM Parts:

mCherry:	Cathelicidin:	deSaturase:
6 uL ddH_2O	6 uL ddH_2O	6 uL ddH_2O
2 uL FD Green Buffer	2 uL FD Green Buffer	2 uL FD Green Buffer
1 uL XbaI	1 uL XbaI	1 uL XbaI
1 uL PstI	1 uL PstI	1 uL PstI
10 uL mCherry	10 uL Cathelicidin	10 uL deSaturase

PCR Purification:
Run PCR purification of hsp60 digests (RBS and no RBS)
1: RBS concentration-
2: no RBS concentration –

Gel Electrophoresis:

Ladder – mcherry – cath – desat - empty - cath – mcherry – desat

Failed. Will upload pic later

Ligation:
Ligate hsp60 RBS and no RBS into pSB1C3 backbones

RBS:	no RBS:
9.5 uL ddH_2O	10.5 uL ddH_2O
3.5 uL backbone	3.5 uL backbone
4 uL insert	3 uL insert
2 uL buffer	2 uL buffer

1 uL ligase 1 uL ligase

Transformation:
Transform RBS and no RBS ligations into *E. coli* competent cells

5 uL plasmid
100 uL competent cells

Ice 2 minutes

Heat shock (42C) 45s

Ice 2 minutes

Add 950 uL outgrowth media

Recover with shaking (37C) for 1 hour

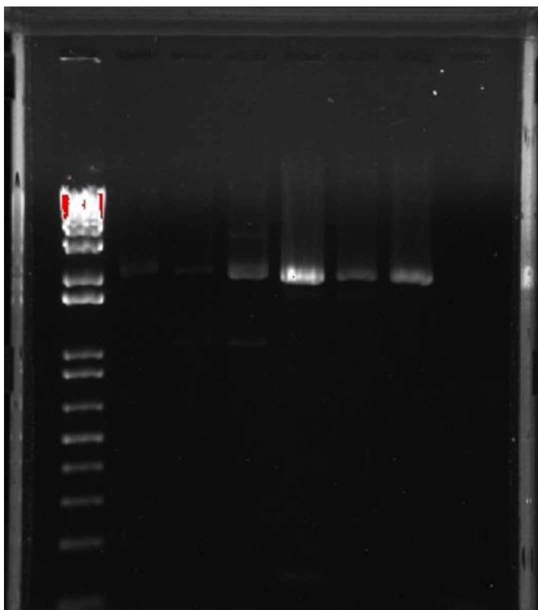
Plate onto selection plate (chloramphenicol)

Restriction Digest:
Cut mCherry prep, Cath. Prep., deSat. Prep, 1 RBS, 1 no RBS, 1 mCherry with XbaI and PstI

6 uL ddH_2O
2 uL FD Green Buffer
1 uL XbaI
1 uL PstI
10 uL plasmid DNA

Gel Electrophoresis:

1 kb+ ladder – mCherry prep – Cath. Prep – deSat prep – 1 RBS – 1 no RBS – 1 mCherry -



8/5/15 Lab

Make 1% Gel (35 mL)
0.35 g Agarose
35 mL 1x TBE
1 uL EtBR

Restriction Digests:

Master Mix (for 6 reactions):	x6.5 =
6 uL ddH_2O	39 uL
2 uL Green Buffer	13 uL
1 uL XbaI	6.5 uL
1 uL PstI	6.5 uL

Combine 10 uL of Master Mix with 10 uL of each plasmid:

mCherry Prep, Cath. Prep, deSat prep., 1 RBS, 1 no RBS, 1 mCherry

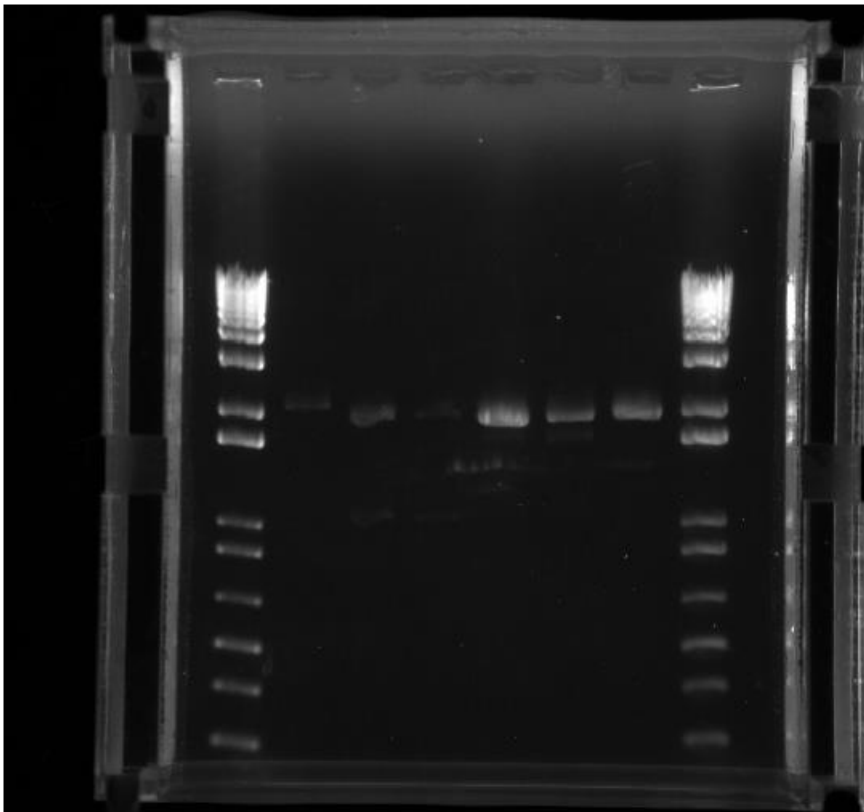
Incubate 45 mins at 37C

Gel Electrophoresis:

Run 1 hour 30 mins

Lanes:

1 kb+ ladder – mCherry mini – Desat – Cath – 1 RBS – 1 no RBS – 1 mCherry – 1 kb+ ladder

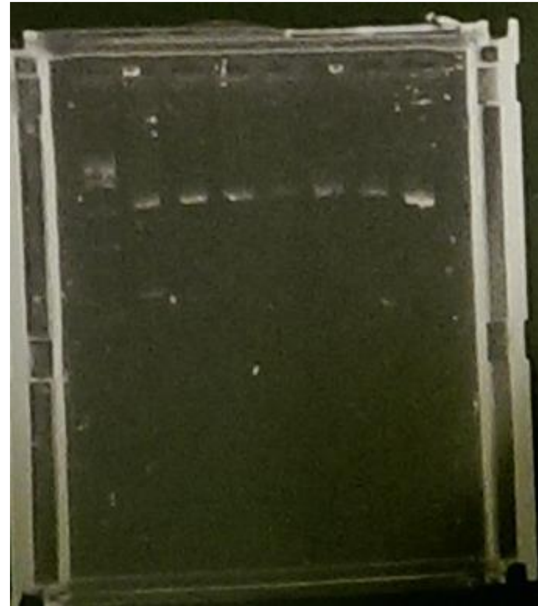


08/08/14

Used the culture collected from 8/1/14 mini-prep for the subsequent restriction digest

1. Restriction digest (45 minutes)
2. Run agarose gel

	Plasmid	Expected Band (kb)
1	Desaturase	~1
2		
3	Cathelicidin	~0.1
4		
5	RBS	~0.3
6		
7	No RBS	~0.3
8		



Digestion was incomplete and need to repeat experiment

Experiment was repeated on 8/9/14 and digestion was successful. The desire bands were excised and purified. Unfortunately the image file was lost due to a technical problem prior to routine file backup. However, further experiments continued to use parts isolated on this date and bands of correct size were present through all of these steps.

Lab Work 8/12/14:

Gel Purification:

Gel Fragment	Gel + Tube Mass (g)	Tube Mass (g)	Gel Mass (g)	Membrane Binding Solution Added (uL)	DNA Concentration (ng/uL)
1 Cath. Part	1.133	1.050	0.083	83	2.9
2 Cath. Part	1.146	1.050	0.096	96	3.9
3 Desat. Part	1.173	1.050	0.123	123	3.1
4 Desat. Part	1.192	1.050	0.142	142	3.1
1 Backbone	1.160	1.050	0.110	110	3.2
2 Backbone	1.152	1.050	0.102	102	3.2
3 Backbone	1.151	1.050	0.101	101	3.2
4 Backbone	1.200	1.050	0.150	150	4.3
5 Backbone	1.201	1.050	0.151	151	6.6
~~6 Backbone~~	~~1.058~~	~~1.050~~	~~0.008~~	~~8~~	N/A
7 Backbone	1.243	1.050	0.193	193	5.7
8 Backbone	1.159	1.050	0.109	109	5.7
Backbone	1.300	1.050	0.250	250	4.4

*Add 10 uL of Membrane Binding Solution per 10 mg of Gel slice

-Vortex Gel and solution
-Incubate at 55C for 10 mins to melt gel
-Spin Down
-Gel Purify

Miniprep:

Miniprepped mRFP1 and MelA liquid cultures
-used same elution buffer for two of the same sample to obtain double DNA concentration

Concentrations:
1 mRFP: 516 ng/uL
2 mRFP: 462.5 ng/uL
3 mRFP: 243.5 ng/uL

1 MelA: 117.9 ng/uL
2 MelA: 158.3 ng/uL
3 MelA: 146.1 ng/uL

Lab Work 8/13/14:

Restriction Digest:

Digesting 1 mRFP, 2 mRFP, 3 mRFP, 1 MelA, 2 MelA, 3 MelA with XbaI and PstI.

20 uL Reactions:	Master Mix:
5 uL Plasmid DNA	13 uL FD Green Buffer
2 uL FD Green Buffer	6.5 uL XbaI
1 uL XbaI	6.5 uL PstI
1 uL PstI	78 uL ddH_2O
12 uL ddH_2O	104 uL Total

*Reactions are 5 uL Plasmid + 15 uL Master Mix
*There are 6 reactions. Master Mix is 6.5x
*Incubate for 45 minutes at 37C

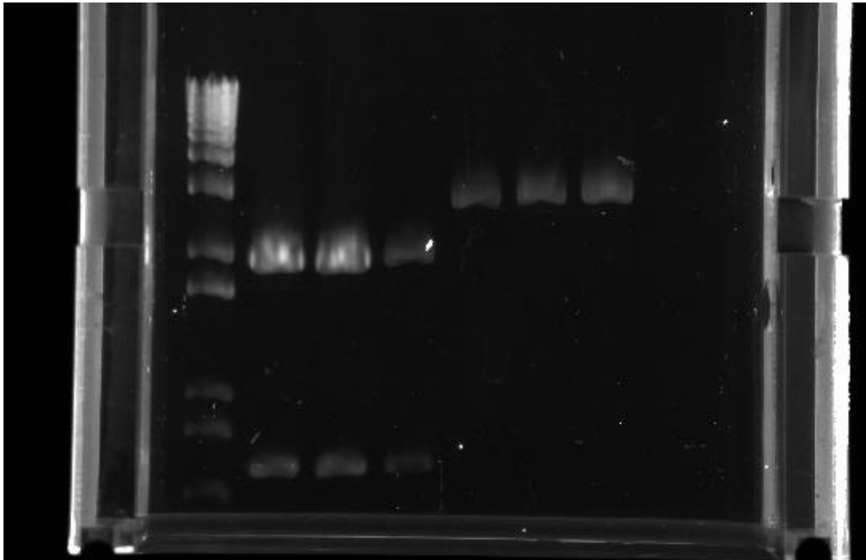
Gel Electrophoresis:

Running a gel to check the validity of the mRFP1 and MelA mini-prepped DNA.

Expect to see mRFP1 band at 706 bp and MelA band at 1844 bp.

Lanes:
1 kb+ --- 1 mRFP --- 2 mRFP --- 3 mRFP --- 1 MelA --- 2 MelA --- 3 MelA --- EMPTY

Gel:



Gel Purification:

Gel Fragment	Gel + Tube Mass (g)	Tube Mass (g)	Gel Mass (g)	Membrane Binding Solution Added (uL)	DNA Concentration (ng/uL)
1 mRFP	1.142	1.017	0.125		
2 mRFP	1.143	1.009	0.134		
3 mRFP	1.149	1.012	0.137		

*Did not complete Gel Purification

Plan for Tomorrow:

1) Finish Gel Purification (Possibly Re-Run. I believe I cut wrong fragment)
2) Make chloramphenicol plates
3) Resuspend YF1 & FixJ, Blue Light Sensor (Plate 1, Well 10N; 1:10N)
4) Resuspend FixK2 Promoter (Plate 1, Well 19G; 1:19G)
5) Transform and Plate (YF1 & FixJ) and FixK2 Promoter

If able to obtain Kanamycin:

1) Make Kanamycin plates (2-3)
2) Transform and Plate mCherry Bomb (3.4 uL of plasmid left)

Lab Work 8/14/14:

Plan:

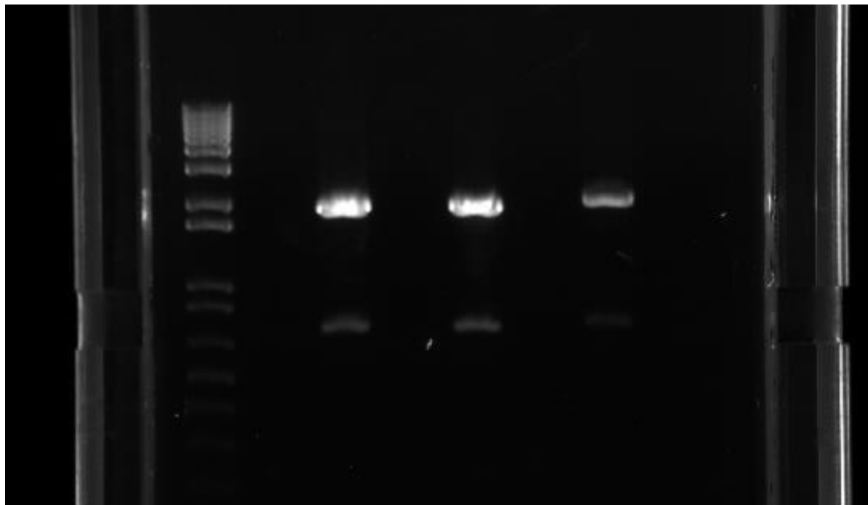
1) Re-Run mRFP1 on Gel and then Gel Purify
2) Make Chloramphenicol Plates
3) Dilute mCherry Bomb and nanodrop
    ~~3a) If enough DNA is present, run PCR~~ (1.4 ng/uL only)
    3b) If not enough DNA is present, make Kanamycin plates
4) Resuspend YF1 & FixJ, Blue Light Sensor (Plate 1, Well 10N; 1:10N)
5) Resuspend FixK2 Promoter (Plate 1, Well 19G; 1:19G)
6) Transform and Plate (YF1 & FixJ) and FixK2 Promoter (Chloramphenicol)
7) Transform and Plate mCherry Bomb plasmid (Kanamycin)

mRFP1 Gel Electrophoresis:

-Create 25 mL or 1% agarose gel and let solidify
-Load digested mRFP1 DNA from 8/13/14

Lanes:

1 kb+ Ladder --- Empty --- 1 mRFP --- empty --- 2 mRFP --- empty --- 3 mRFP --- empty



Dilute mCherry Bomb and nanodrop:

-1 uL mCherry Bomb into 9 uL ddH_2O
-Concentration of 1.4 ng/uL

Make Plates:

*1 Kanamycin; 3 Chloramphenicol
-Kanamycin working concentration of 50 ng/mL
-CAM working concentration of 25 ng/mL

Resuspend DNA:

*Added 10 uL ddH$_2$O to each part to resuspend

Transform:

Transformed Blue Light Sensor, Blue Light Promoter, and mCherry Bomb
*Plates put in 37C incubator at 4:16 PM

Gel Purification:

Gel Fragment	Gel + Tube Mass (g)	Tube Mass (g)	Gel Mass (g)	Membrane Binding Solution Added (uL)	DNA Concentration (ng/uL)
1 mRFP	1.195	1.014	0.181	181	3.6
2 mRFP	1.217	1.002	0.215	215	2.0
3 mRFP	1.158	1.012	0.146	146	4.6

Lab Work 8/15/15:

Morning:

Took out agar plates of Blue Light Sensor, Blue Light Promoter, and mCherry Bomb
*Colonies were spotted on each Plate

Evening:

Made liquid cultures for Blue Light Sensor, Blue Light Promoter, and mCherry Bomb
*5 mL per liquid culture
*2 Liquid cultures per plasmid
*Added CAM to Blue Light Sensor and Blue Light Promoter cultures at working concentration of 25 ng/mL
*Added Kan to mCherry Bomb culture at working concentration of 50 ng/mL

Lab Work 8/16/14:

Miniprep:
-Miniprepped liquid cultures of Blue Light Promoter, Blue Light Sensor, and mCherry Bomb

Concentrations:
Blue Light Promoter (1:19G): 44.5 ng/uL
Blue Light Sensor (1:10N): 36.6 ng/uL
mCherry Bomb: 35 ng/uL

Lab Work 8/18/14:

PCR:

-PCRing hsp60 out of plasmid mCherry Bomb

Master Mix			Primers and DNA			PCR Reaction	
Reagent	1x Rxn (uL)	2.5x Rxn (uL)	Primer	RBS (uL)	no RBS (uL)	Reagent/DNA	Volume (uL)
Buffer	10	25	Fwd Primer	2.5	2.5	Master Mix	11.5
dNTP	1	2.5	Rev Primer	2.5	2.5	Primers and DNA	7.5
Phusion	0.5	1.25	mCherry Bomb	2.5	2.5	ddH2O	31
Total	11.5	28.75	Total	7.5	7.5	Total	50

*Two 20 uL PCR reactions of each (RBS and no RBS) were carried out

PCR conditions:

98C for 30s
98C for 10s
66C for 30s x30 cycles
72C for 30s
72C for 5 min
4C holding

Gel Electrophoresis:

Ran two PCR products with a ladder on a gel. There were no bands shown. PCR was unsuccessful

Lab Work 8/19/14:

Restriction Digest:

Digesting Blue Light Sensor and Blue Light Promoter

20 uL Reactions:
5 uL Plasmid DNA
2 uL FD Green Buffer
1 uL XbaI
1 uL PstI
12 uL ddH_2O

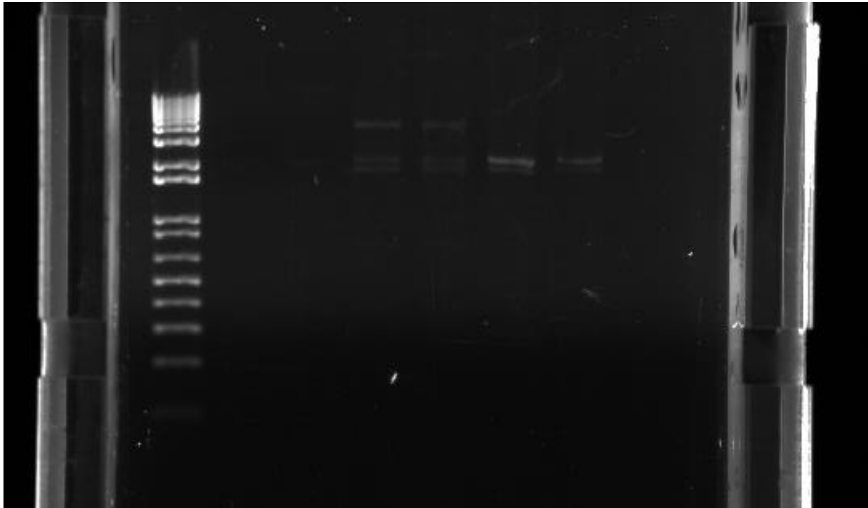
*Incubate for 45 minutes at 37C

Gel Electrophoresis:

Running a gel to check the validity of the Blue Light Sensor (1796 bp), Blue Light Promoter (250 bp), and PCR products (~380 bp).

Lanes:

1 kb+ ladder --- RBS PCR --- no RBS PCR --- Blue Light Sensor --- Blue Light Sensor --- Blue Light Promoter --- Blue Light Promoter --- empty



9/12/14
Goal:

1. PCR purify Hsp60 with and without RBS
2. Digestion of purified product with XbaI and PstI
3. Gel purification of digestion products
    a. Cut out slices and store at 4 ℃

Experiments:
Tubes Labeled by MJ

Tube label	Content
1, PCR purification product, no RBS	1st elution: PCR purification product, no RBS
2, PCR purification product, no RBS	2nd elution: PCR purification product, no RBS
1, PCR purification product, RBS	1st elution: PCR purification product, RBS
2, PCR purification product, RBS	2nd elution: PCR purification product, RBS

1. PCR purification

Step	Hsp60 with RBS (uL)	Components	Hsp60 without RBS (uL)
1	34.5	Membrane binding solution	34.5
2	700	Membrane Wash solution	700
3	500	Membrane Wash solution	500
4	50	1st elution with Nuclease free ddH_2O	50
5	30	2nd elution with Nuclease free ddH_2O	30

2. Digestion of purified product

Components	Amount for Hsp60 with RBS (uL)	Amount for Hsp60 without RBS (uL)
10x Fast Digest Green Buffer	2	2
PCR Product of Hsp60	7	7
XbaI	1	1
PstI	1	1
ddH_2O	13	13
Total	24	24

Incubated at 37 ℃ for 30 minutes
2 reactions each were digested for 1st elution of both with and without RBS; 1 reaction each for the 2nd elution for both with and without RBS

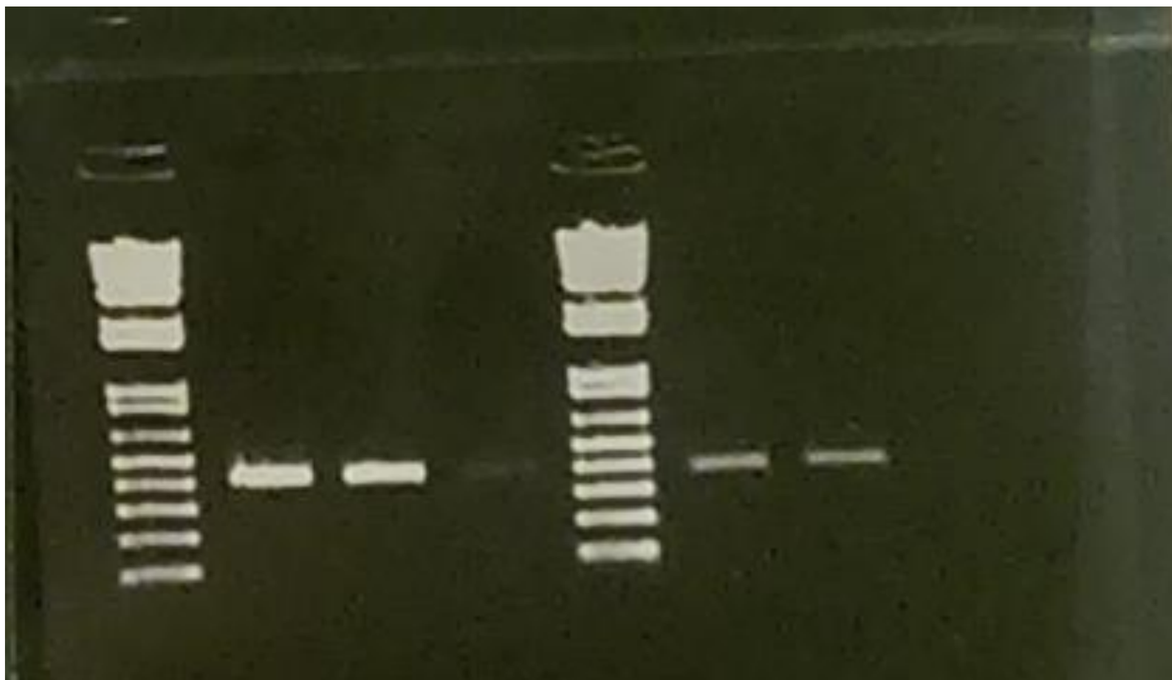
9/12/14 continued

3. Gel Set Up:

Lane	Sample	Amount (uL)	Expected band size (bp)
1	1kb DNA Ladder	2	None
2	Elution 1, no RBS digested	5	~ 300 bp
3	Elution 1, no RBS digested	5	
4	Elution 2, no RBS digested	5	
5	1kb DNA Ladder	2	None
6	Elution 1, RBS digested	5	~300 bp
7	Elution 1, RBS digested	5	
8	Elution 2, RBS digested	5	

4. Mass of tube and tube plus sample

Tube label	Sample (lane #)	Mass of tube (g)	Mass of Tube plus sample (g)
1	2	1.012	1.1
2	3	1.005	1.098
3	4	1.018	1.121
4	6	1.01	1.105
5	7	1.02	1.113
6	8	1.003	1.094



Lab Work 9/17/14:

Goals:

1) Digestion of Blue Light Parts w/ X+P
2) Run Parts on Gel
3) Gel Purify Blue Light Parts, hsp60 parts, vector backbone
4) Ligate hsp60 + backbone

Digestion:

9 uL ddH$_2$O
2 uL Fast Digest Green Buffer
1 uL XbaI
1 uL PstI
7 uL Plasmid (Blue Light Sensor and Blue Light Promoter)
20 uL rxn

Incubate at 37 C for 30 min

Gel Electrophoresis:

Lanes:

1 kb+ ladder – Blue Sensor – Blue Sensor – Blue Promoter – Blue Promoter – empty

Image at 30 mins:

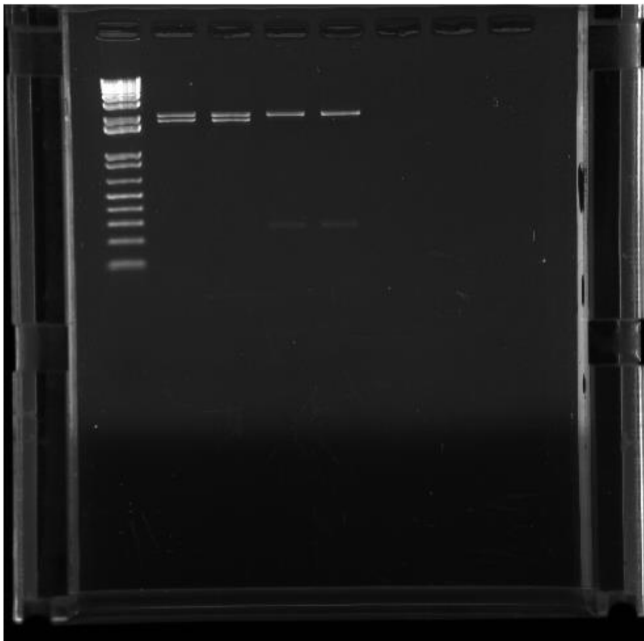


Image at 1 hour, 15 mins:

*Lanes 4/5 extracted at 30 mins

Purification:

	Tube Weight (g)	Tube + Gel Weight (g)	Gel Fragment Weight (g)	Membrane Binding Solution (uL)
Backbone	1.021	1.183	0.162	162
Blue Promoter	1.016	1.198	0.182	182
Backbone	1.016	1.187	0.171	171
Blue Sensor	1.004	1.126	0.122	122
no RBS			0.088	88
no RBS			0.093	93
RBS			0.095	95
RBS			0.093	93

Ligation:

RBS	no RBS
1 uL ligase	1 uL ligase
2 uL Buffer	2 uL Buffer
4 uL Insert	3 uL Insert
3.5 uL Backbone	3.5 uL Backbone
9.5 uL ddH_2O	10.5 uL ddH_2O
20 uL	20 uL

9/22/14

Goals:

1. Digestion of desaturase, and mRFP-1
2. Purification of desaturase and mRFP-1
3. Ligation into the vector
4. Digestion of 7 uL of plasmid DNA
5. Purification

Experiment:

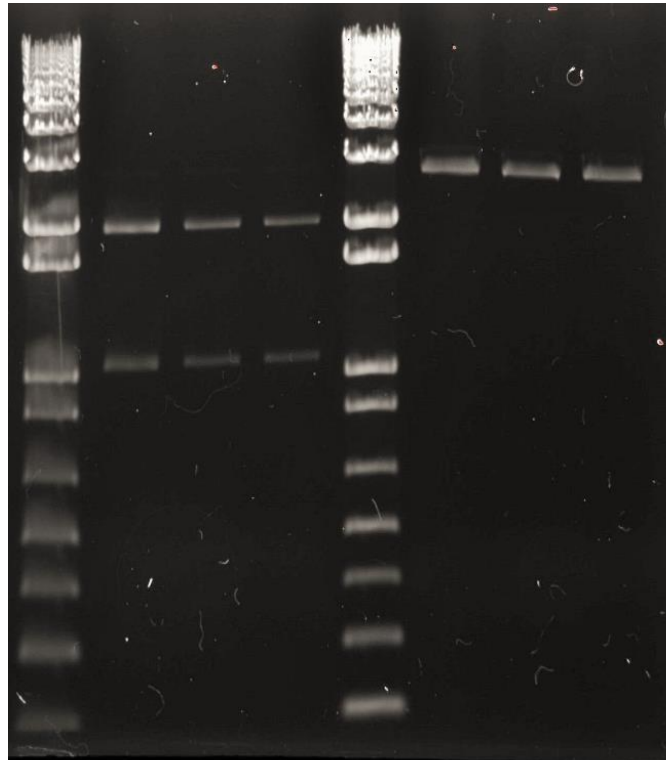
Cut mRPF-1 with EcoRI and XbaI

Desaturase with EcoRI and SpeI

Components mRFP-1	Amount (uL)	Components desaturase
10x Fast Digest Buffer	2	10x Fast Digest Buffer
plasmid	7	plasmid
EcoRI	1	EcoRI
XbaI	1	SpeI
ddH$_2$O	9	ddH$_2$O
Total	19	Total

Gel:

Lane	1	2	3	4
Components	1kb DNA Ladder	Desaturase (EcoRI+SpeI)		
Amount (uL)	2	5	5	5
Lane	5	6	7	8
Components	1kb DNA Ladder	mRFP-1 (EcoRI+XbaI)		
Amount (uL)	2	5	5	5



9/24/14

mRFP-1 and desaturase plasmids were previously collected and this is the resulting digestion (9/22/14) followed by gel purification of the previous experiments.

1. Gel purification: and resulting concentrations:

Tube label	Part excised	Mass (g)	Concentration (ng/uL)
1	Desaturase Vector Backbone	Not used since this is the backbone	
2			
3			
4	Desaturase Part	0.124	3.9
5		0.123	4.7
6		0.095	4.2
7	mRFP-1 linearized plasmid	0.095	4.4
8		0.103	4.4
9		0.133	4.8

9/26/14

1. Ligation reaction BH

Components	Amount (uL)
Insert (desaturase)	9
Vector Backbone (single cut mRFP-1)	3.5
Ligase	1
Buffer	1.5
ddH_2O	5
Total	20

   Negative control: just VB (labeled A)
   Ligation 1 (labeled B)
   Ligation 2 (labeled C)

2. Transformation into E. coli competent cells BH

   Incubation at 37 ℃ on LB + CAM from 2 pm

      Note: the plate was examined once at 6 hours and another time at 8 hours—yielded no usable colonies

3. Mini-Prep (BH) of colonies culture grown by MJ

   Labeling maintained the same (1-6)
   Resuspended in 50 uL of elution buffer
   Undetermined concentration

   Ligation and Transformation were repeated 2 times yet the reaction never yielded colonies that survived in LB+CAM culture.

Lab Work 9/29/14:

Diagnostic Digest:

Hsp60(no RBS) – mRFP1 (~1090 bp) 1) X+P

Blue Promoter – mRFP1 (~1050 bp) 2) X+P, 3) S+P

Hsp60 (no RBS) – Blue Sensor (~2160 bp) 4) X+P

MelA (~1896 bp) 5) X+P

20 uL Rxns
9 uL ddH_20
2 uL FD Green
1 uL XbaI/SpeI

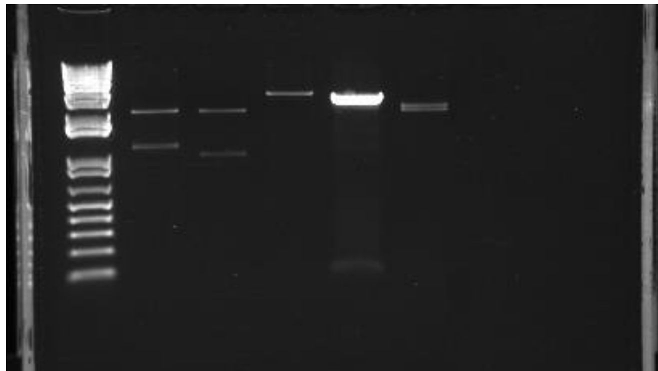
1 uL PstI
7 uL Plasmid

*Incubate for 30 min @ 37C

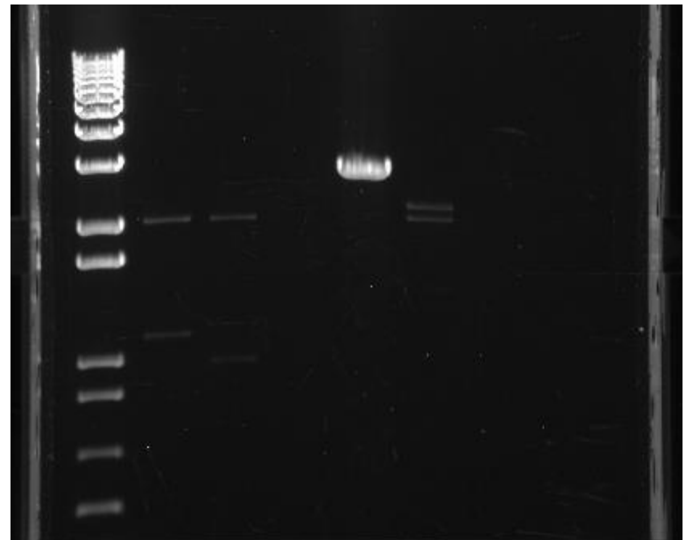
Gel Electrophoresis:

1 kb+ ladder – 1 – 2 – 3 – 5 – 4 – empty

Gel @ 30 mins



Gel @ 1 hr, 30 mins



*Note: Band 3 was extracted after 30 minutes. The lower of band 5 was extracted at 1 hr, 30 min

Gel Extraction:

Blue Promoter – mRFP1 S+P gel weight: 0.086 g

Hsp60 (no RBS) – Blue Sensor X+P gel weight: 0.062 g

Made 6 CAM plates & 2 AMP plates

Transformation:

Transformed RBS (4:1N) and Terminator (4:16G).
Plate onto AMP plates

Lab Work 10/1/14:

Mini Prep:

Mini Prep RBS and Terminator Liquid Cultures

Gel Purify:

Add 86 uL Membrane Binding Solution to Blue Promoter – mRFP1 (S+P) part

Add 62 uL Membrane Binding Solution to hsp60 (no RBS) – Blue Sensor (X+P) part

Restriction Digests:

Terminator (E+X) (~2150 bp)

RBS (E+X) (~2082 bp)

Cathelicidin (E+S) (~123 bp)

Blue Promoter (E+S) (~250 bp)

20 uL rxns:
9 uL ddH_2O
2 uL FD Green
1 uL EcoRI
1 uL XbaI/SpeI
7 uL Plasmid

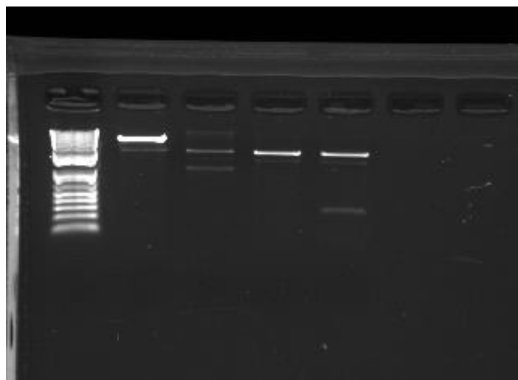
Incubate 30 mins @ 37C

Gel Electrophoresis:

Lanes:

1 kb+ ladder – Terminator (E+X) – Cathelicidin (E+S) – RBS (E+X) – B. Promoter (E+S) – empty

*Run Gel 15 minutes



Gel Purifiy:

RBS (E+X)
Blue Promoter (E+S)

Gel Fragment	Gel + Tube Mass (g)	Tube mass (g)	Gel Mass (g)	Membrane Binding Solution (uL)	DNA Concentration (ng/uL)
~~Terminator (E+X)~~	-	~~1.106~~	-	-	-
~~Cathelicidin (E+S)~~	-	~~1.015~~	-	-	-
RBS (E+X)	1.033	0.985	0.048	48	1.7
Blue Promoter (E+S)	1.082	1.005	0.077	77	1.8

Ligation:

Blue Promoter – RBS

<u>15 uL Rxn:</u>
3.5 uL Insert (Blue Promoter)
9 uL VB (RBS)
1.5 uL Buffer
1 uL Ligase
0 uL ddH_2O

Transformation:

Transform Blue Promoter – RBS onto AMP plate

Lab Work 10/4/14:

Morning:

Made Liquid Cultures of Blue Promoter – RBS part in Turbo E.coli Comp Cells

Afternoon:

Mini Prep:

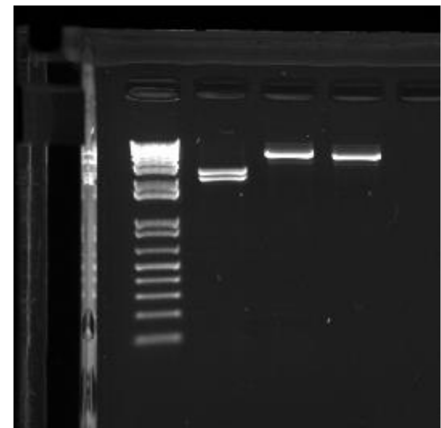
Mini Prepped Blue Promoter – RBS : ??? ng/uL

Restriction Digest:

Blue Promoter – RBS (X/P)
Blue Promoter – RBS (S/P)
Terminator (S/P)

20 uL rxns:
9 uL ddH_2O
2 uL FD Green
1 uL XbaI/SpeI
1 uL PstI
7 uL Plasmid

Gel Electrophoresis:



Lanes:

1) 1 kb+ ladder
2) Blue Promoter – RBS (X+P) (~262 bp)
3) Blue Promoter – RBS (S+P) (~2350 bp)
4) Terminator (S+P) (~2150 bp)

Gel Purify:

Gel Fragment	Tube (g)	Gel + Tube (g)	Gel (g)	Membrane Binding Solution (uL)	Concentration (ng/uL)
Blue Promoter-RBS (S/P)	1.002	1.067	0.065	65	5.0
Terminator (S/P)	1.020	1.093	0.073	73	2.1

Ligation:

1) Blue Promoter – mRFP1 (S/P) (~3000 bp) + Cathelicidin (X/P) (~120 bp)
2) Blue Promoter – RBS (S/P) (~2350 bp) + Cathelicidin (X/P) (~120 bp)
3) Terminator (S/P) (~2150 bp) + hsp60 (no RBS) – Blue Sensor (X/P) (~2160 bp)
4) Blue Promoter – mRFP1 (S/P) (~3000 bp) + hsp60 (no RBS) – Blue Sensor (X/P) (~2160 bp)

Ligation	VB bp	VB concentration (ng/uL)	Insert bp	Insert concentration (ng/uL)	VB (uL)	Insert (uL)
1	3000	2.9	120	3.9	4.00	8.50
2	2350	5.0	120	3.9	3.50	9.00
3	2150	2.1	2160	4.9	3.00	9.50
4	3000	2.9	2160	4.9	3.50	9.00

1.5 uL Buffer
1 uL Ligase

Transformation:

Blue Promoter – mRFP1 – Cathelicidin (CAM)
Blue Promoter – RBS – Cathelicidin (AMP)
Terminator – hsp60 (no RBS) – Blue Sensor (AMP)
Blue Promoter – mRFP1 – hsp60 (no RBS) – Blue Sensor (CAM)

Lab Work 10/5/14:

Ligation:

Ligation 3: Terminator (S/P) + hsp60 (no RBS) – Blue Sensor (X/P)

1.5 uL Buffer
1 uL Ligase
6 uL VB (Terminator)
6.5 uL Insert (hsp60-Blue Sensor)

Transformation:

Transform 3: Terminator – hsp60 (no RBS) – Blue Sensor
AMP Plate

MiniPrep:

Mini 1: Blue Promoter – mRFP1 – Cathelicidin 61.5 ng/uL
Mini 2: Blue Promoter – RBS – Cathelicidin 85.9 ng/uL
Mini 4: Blue Promoter – mRFP1 – hsp60 (no RBS) – Blue Sensor 81.0 ng/uL

Restriction Digest:

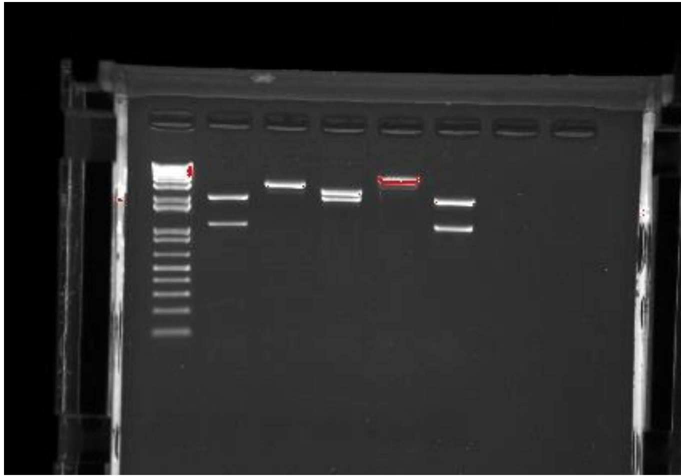
1) Mini 1: Blue Promoter – mRFP1 – Cathelicidin (X/P)
2) Mini 1: Blue Promoter – mRFP1 – Cathelicidin (S/P)
3) Mini 2: Blue Promoter – RBS – Cathelicidin (X/P)
4) Mini 2: Blue Promoter – RBS – Cathelicidin (S/P)
5) Mini 4: Blue Promoter – mRFP1 – hsp60 (no RBS) – Blue Sensor (X/P)

20 uL rxns:
9 uL ddH_2O
2 uL FD Green
1 uL XbaI/SpeI
1 uL PstI
7 uL Plasmid

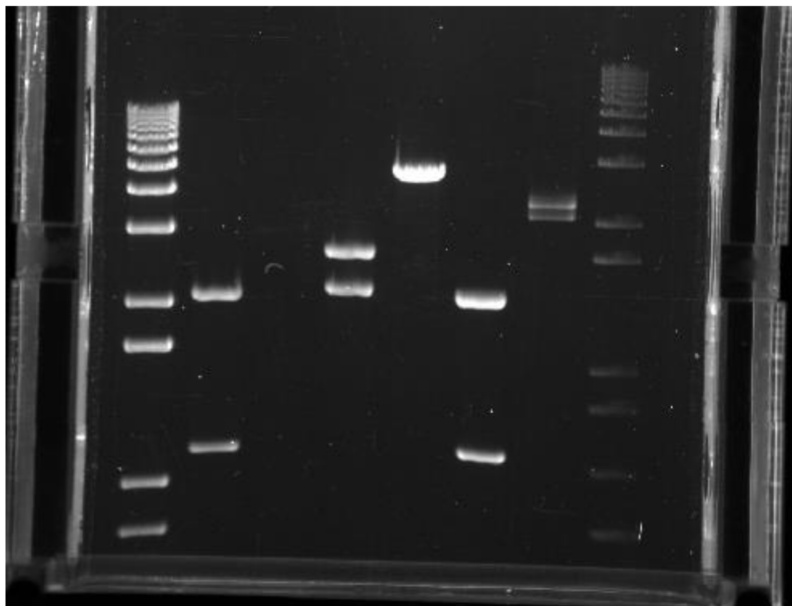
Gel Electrophoresis:

Lanes:

1) 1 kb+ ladder
2) Mini 1: Blue Promoter – mRFP1 – Cathelicidin (X/P) (~1000 bp) (Part)
3) Mini 1: Blue Promoter – mRFP1 – Cathelicidin (S/P) (~3100 bp) (VB)
4) Mini 2: Blue Promoter – RBS – Cathelicidin (X/P) (~370 bp) (Part)
5) Mini 2: Blue Promoter – RBS – Cathelicidin (S/P) (~2470 bp) (VB)
6) Mini 4: Blue Promoter – mRFP1 – hsp60 (no RBS) – Blue Sensor (X/P)(~3160 bp) (Part)



*After 30 mins, hsp60 (no RBS) – Blue Sensor (X/P) (~2160 bp) added to lane 7. Ladder added lane 8.



Gel Purify:

Gel Fragment	Tube (g)	Gel + Tube (g)	Gel (g)	Membrane Binding Solution (uL)	Concentration (
Mini 1: B.P. - mRFP1 - Cath (S/P)	1.013	1.072	0.059	59	
no RBS - Blue Sensor (X/P)	1.016	1.065	0.049	49	

Ligation:

1.5 uL Ligation Buffer
1 uL Ligase

Lig 1:
9 uL Blue Promoter (Insert) (~200 bp)
3.5 uL RBS (VB) (~2100 bp)

Lig 2:
6 uL Terminator (VB) (~2100 bp)
6.5 uL hsp60 (no RBS) – B. Sensor (Insert) (~2160)

Lig 3:
3.5 uL B. Prom – mRFP1 (VB) (~3100)
9 uL hsp60 (no RBS) – B. Sensor (Insert) (~2160 bp)

Transformation:

Transform 1: Blue Promoter – RBS onto AMP plate
Transform 2: Terminator – hsp60 (no RBS) – Blue Sensor onto AMP plate
Transform 3: Blue Promoter – mRFP1 – hsp60 (no RBS) – Blue Sensor onto CAM plate

Make Liquid Cultures:

Made 2 5mL Liquid cultures of LB + AMP, Blue Promoter – RBS

Lab Work 10/6/14:

Make Liquid Cultures:

(AMP) Transform 1: Blue Promoter – RBS
(AMP) Transform 2: Terminator – hsp60 (no RBS) – Blue Sensor
(CAM) Transform 3: Blue Promoter – mRFP1 – hsp60 (no RBS) – Blue Sensor

Mini prep:

Mini 1: Blue Promoter – RBS
Mini 2: Terminator – hsp60 (no RBS) – Blue Sensor
Mini 3: Blue Promoter – mRFP1 – hsp60 (no RBS) – Blue Sensor

Restriction Digest:

1) Blue Promoter – RBS (X/P)
2) Blue Promoter – RBS (S/P)
3) Terminator – hsp60 (no RBS) – Blue Sensor (X/P)
4) Blue Promoter – mRFP1 – hsp60 (no RBS) – Blue Sensor (X/P)

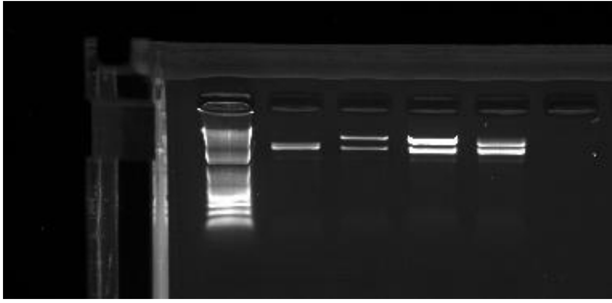
20 uL Rxns
9 uL ddH_2O
2 uL FD Green
1 uL XbaI/SpeI
1 uL PstI
7 uL Plasmid

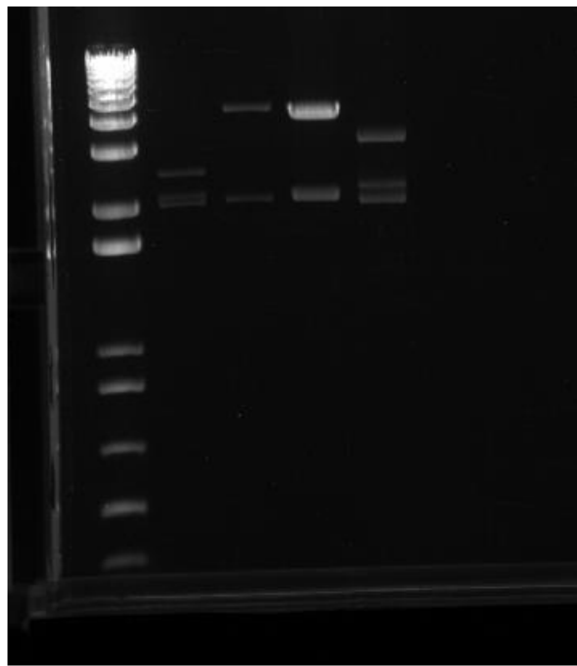
Gel Electrophoresis:

Lanes:

1) 1 kb+ ladder
2) Blue Promoter – RBS (X/P) (~262 bp)
3) Blue Promoter – RBS (S/P) (~2341)
4) Terminator – hsp60 (no RBS) – Blue Sensor (X/P) (~2236)
5) Blue Promoter – mRFP1 – hsp60 (no RBS) – Blue Sensor (X/P) (~3100)

*Note: In lane four, the VB is ~2100, will need long separation to see. Part will be bigger than backbone
*Stop at 15 mins and check lane 2, if correct band is present, extract lane 3 before continuing.
*Run for another hour and fifteen before stopping a second time and extracting lane 4





Gel Purify:

Ligation:

~~Ligation 1: Blue Promoter – RBS (X/P) + Cathelicidin (S/P)~~
Ligation 2: Blue Promoter – mRFP1 – Cathelicidin (S/P) + Terminator – hsp60 (no RBS) – Blue Sensor (X/P)

Ligation	VB bp	VB Concentration (ng/uL)	Insert bp	Insert Concentration (ng/uL)	VB (uL)	Insert (uL)
~~1~~	~~2341~~	-	~~123~~	-	-	-
2	2500		2236			