Tuesday 7/18

Samples were mini-preped. The concentration of the plasmids is as follows:

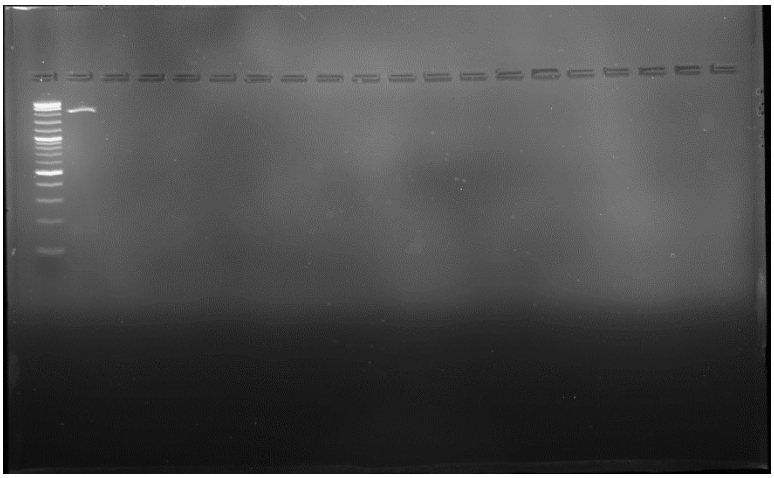
Plate	Well	Colony	DNA (ng/uL)
P3	14E	1	49.7
		2	23.8
		3	9.3
P4	17D	1	39.1
		2	22.5
		3	55.9

Thursday 7/20

The following digestion was carried out on P4 17D:

Reagent	1 Reaction
PstI Enzyme	1 uL
XbaI Enzyme	1 uL
10X Red Buffer	2.5 uL
Water	0
Subtotal	4.5 uL
DNA	20.5 uL
Total	25 uL
Number of Reactions	1

Gel Electrophoresis was used to analyze the digestion. The target band is at 200bps



Conclusion: The plasmid did not show the signature band at 200 bps. This could be due to a failed digestion. It is not due to negative supercoiling because a smudge is not observed.

Tuesday 7/25

PCR Conditions were as follows:

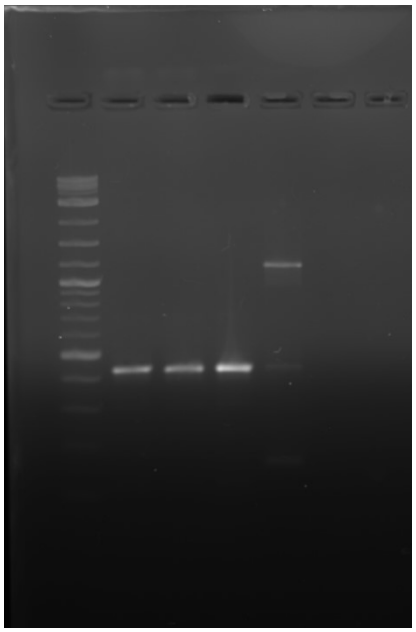
Template DNA was diluted 1:10 in water

Reagent	1 Reaction	Master Mix
Water	6 uL	24 uL
PCR Mastermix (5X)	2 uL	8 uL
Primer (10 uL)	1 uL	4 uL
Subtotal	9 uL	
Template DNA	1 uL	
Total	10 uL	
Number of Reactions	4	

Cycles:

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	95	15 min	1 X
Denature	95	30 sec	35-40 X
Anneal	60	30 sec	
Extend	72	1.5 min	
Final Extension	72	7 min	1 X
Hold	4	Hold	1 X

Gel Electrophoresis was used to analyze the results of PCR
Lane 1: Ladder
Lane 2-4: P4 17D
Lane 5: P3 14E



Conclusion: All lanes were successfully amplified. All transformations of P4 17D were successful. Therefore, the digestion on Thursday July 20 failed.

Thursday 7/27

PCR conditions were as follows:

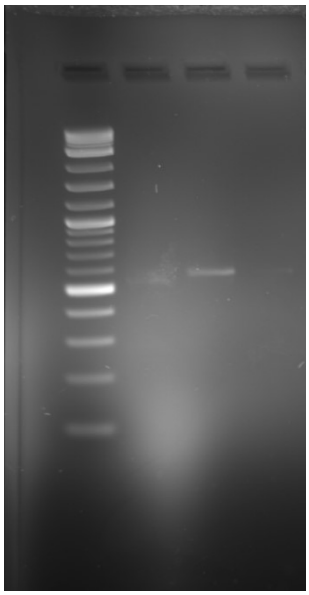
Template DNA diluted 1/10 in water

Reagent	1 Reaction	Mastermix
Water	6 uL	12 uL
PCR Mastermix (5X)	2 uL	4 uL
Primer (10uM)	1 uL	2 uL
Subtotal	9 uL	
Template DNA	1 uL	
Total	10 uL	
Number of Reactions	2	

Cycles:

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	95	5 min	1 X
Denature	95	30 sec	35-40 X
Anneal	60	30 sec	
Extend	72	45 sec	
Final Extension	72	7 min	1 X
Hold	4	Hold	1 X

Gel Electrophoresis was used to analyzes the results of PCR



Lane 1: DNA Ladder
Lane 2: lacI-Inducible Promoter BBa_R0010
Lane 3: pH Promoter BBa_K1231001

Conclusions:
BBa_K1231001 was successfully amplified. However, BBa_R0010 was not. Gel Extraction of BBa_K1231001 yielded a concentration of 1.25ng/uL.

Tuesday 8/1

The following plasmids were mini-prepped after transformation into competent *E. coli*: P4 2F, P4 2H, T7ptag, Hypoxia promoter.

The concentration of each mini-prepped was as follows:

Plate	Well	Colony	DNA (ng/uL)
P4	2F	1	255.85
		2	294.15
P4	2H	1	94.5
		2	95.85
T7ptag	Requested	1	137.75
		2	144.95
Hypoxia Promoter	Requested	1	50.15
		2	31.05

pH Promoter Cloning

The pH promoter was cloned into pSB1A10 using restriction enzyme double digest. Both the pH promoter and pSB1A10 were digested with *EcoRI* and *SpeI*.

EcoRI Digestion Conditions:

Reagent	pH Promoter PCR Product	RFP in pSB1A10
DNA to digest (500 ng)	30 uL	2 uL
10X EcoRI Buffer	5 uL	5 uL
EcoRI	1 uL	1 uL
Water	14 uL	42 uL
Total	50 uL	50 uL

The digestion was incubated at 37°C for 25 minutes and the products were column purified.

SpeI Digestion Conditions:

Reagent	pH Promoter PCR Product	RFP in pSB1A10
DNA to digest (500 ng)	30 uL	30 uL
10X CutSmart	5 uL	5 uL
SpeI	1 uL	1 uL
Water	14 uL	14 uL
Total	50 uL	50 uL

The digestion was incubated at 37°C for 20 minutes and the products were column purified.
Special Note if heat is inactivating:

37°C	**20 minutes**
80°C	20 minutes
Chill at 25°C	

Ligation of pH promoter into pSB1A10 Conditions:

Reagent	Ligation Reaction
Vector Backbone (pSB1A10)	20
Insert (pH promoter)	4.5
10X Ligase Buffer	3
Ligase	1
Water	1.5
Total	30 uL

The ligation was incubated overnight at 37°C.

Sunday 8/6

RBS+GFP Cloning

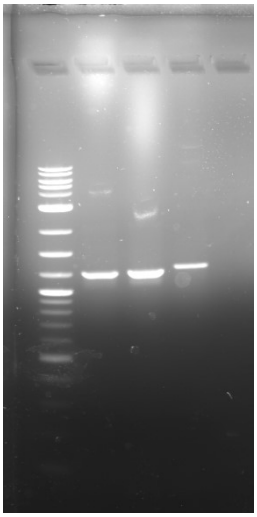
The plasmids in P2 24B, P1 4G, and P1 18A were amplified using PCR.
The PCR conditions are as follows:

Reagent	1 reaction	MasterMix
Water	6	18
PCR Mastermix (5X)	2	6
Primer (10uM)	1	3
Subtotal	9 uL	
Template DNA	1	
Total	10 uL	
Number of Reactions	3	

Monday 8/7

RBS+GFP Cloning

The PCR products from the day before were analyzed on a gel today.
Lane 1 - Ladder
Lane 2 - P2 24B
Lane 3 - P1 4G
Lane 4 - P1 18A



Conclusions: PCR was successful. All lanes show target band around 1.2 kb as expected. The bands were then extracted from the gel and the concentration of DNA was measured. The concentrations are as follows:

Plate	Well	DNA (ng/uL)
P2	24B	2.200
P1	4G	1.85
P1	18A	2.050

Digestion and Ligation of P2 24B, P1 4G, and P1 18A into the Backbone, pSB1A10

Six digestion reactions were setup. Both the inserts (P2 24B, P1 4G, P1 18A) and the backbones (pSB1A10) were cut with EcoRI and SpeI.

EcoRI Digestion Conditions:

Reagent	P2 24B	P1 4G	P1 18A	RFP in pSB1A10 (P4 2F)	RFP in pSB1A10 (P4 2F)	RFP in pSB1A10 (P4 2F)
DNA to digest (500 ng)	24	13	22	2	2	2
10X EcoRI Buffer	5	5	5	5	5	5
EcoRI	1	1	1	1	1	1
Water	20	31	22	42	42	42
Total	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL

The digestion was carried out at 37°C for 25 minutes and the products were column purified. The entire reaction was used for the following digestion.

SpeI Digestion Conditions:

Reagent	P2 24B	P1 4G	P1 18A	RFP in pSB1A10 (P4 2F)	RFP in pSB1A10 (P4 2F)	RFP in pSB1A10 (P4 2F)
DNA to digest (500 ng)	30	30	30	30	30	30
10X CutSmart	5	5	5	5	5	5
SpeI	1	1	1	1	1	1
Water	14	14	14	14	14	14
Total	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL

The digestion was carried out at 37°C overnight.

Tuesday 8/8

RBS+GFP Cloning

The previous digestions were cleaned up using a digestion cleanup kit.
The following table states the concentration of DNA and its purity for each digestion.

Reagent	DNA Concentration (ng/uL)	Purity
P4 2F	15.450	2.3
P4 2F	19.4	1.7
P4 2F	17.05	1.977
P2 24B	205	3.125
P1 4G	5.8	1.957
P1 18A	10.05	2.109

The digestions were then ligated into the appropriate backbones. In this case, the vector backbone was identical for all the inserts. The backbone was pSB1A10.

Ligation Conditions:

Reagents	**Inserts**		
	P2 24B	P1 4G	P1 18A
Vector Backbone (pSB1A10)	3	3	3
Insert DNA	20	9	6
10X Ligase Buffer	3	3	3
Ligase	1	1	1
Water	3	14	17
Total	30 uL	30 uL	30 uL

Distribution Kit Amplification

The following parts were amplified using PCR: P2 4B, P3 4G, P3 9N, P3 8M, P3 9P, P4 5L, and P5 12G.

PCR Conditions:

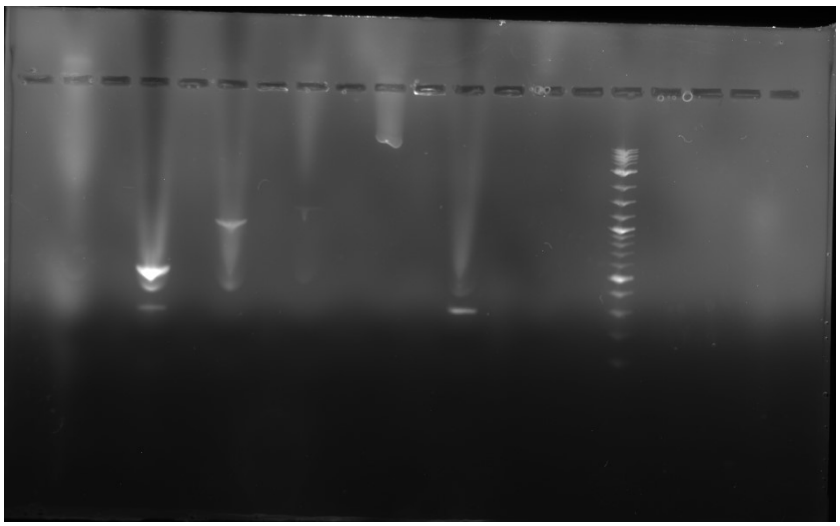
Template DNA was diluted 1/10 in water

Reagent	**1 Reaction**	**Mastermix**
Water	6	42
PCR Mastermix (5X)	2	14
Primer (10uM)	1	7
Subtotal	9 uL	
Template DNA	1	
Total	10 uL	
Number of Reactions	7	

Wednesday 8/9

The results of PCR were analyzed using gel electrophoresis.

Lane 2 – P2 4B
Lane 4 – P3 4G
Lane 6 – P3 9N
Lane 8 – P3 8M
Lane 10 – P3 9P
Lane 12 – P4 5L
Lane 14 – P5 12G
Lane 16 – Ladder



Conclusions: Only P3 4G, P3 9N, P3 9P and P4 5L were gel extracted and had their DNA concentrations measured. The concentrations are in the following table.

Parts	Concentration (ng/uL)
P3 4G	8.90
P3 9N	9.65
P4 5L	1.45
P3 9P	14.450

The DNA were then digested using EcoRI. The conditions were as follows:

Reagent	One Reaction
DNA	17
10X EcoRI Buffer	2
EcoRI	1
Water	0
Total	20 uL

The digestion products were column purified and digested using SpeI. The conditions were as follows:

Reagent	One Reaction
DNA	17
10X CutSmart	2
SpeI	1
Water	0
Total	20 uL

The digestion products were column purified and ligated. The DNA was eluted in 22 uL of water during the column purification. The ligation conditions were as follows:

Reagent	P1 4G	P3 9N	P4 5L	P 5 12G
Vector Backbone (pSB1A10)	2	2	2	2
Insert	20	20	20	20
10X Ligase Buffer	2.5	2.5	2.5	2.5
Ligase	1	1	1	1
Water	0	0	0	0
Total	25 uL	25 uL	25 uL	25 uL

The ligation products were transformed and plated.

Cloned Parts

The following parts were cloned into pSB1A10 and mini-prepped. The DNA concentration was as follows:

Part	Concentration (ng/uL)
P2 24B – 1	47.45
P2 24B – 2	53
P2 24B – 3	63.6
P2 24B – 4	47.20
P1 18A – 1	63.5
P1 18A – 2	61.15
P1 18A – 3	49.35
P1 18A – 4	49.3
P1 4G – 1	103.4
P1 4G – 2	71.65
P1 4G – 3	29.35

Monday 8/14

Distribution Kit Amplification

The ligations from last time were examined today. Only red colonies were found on P5 12G and P4 5L. This is evidence of a failed digestion/ligation. A single white colony was found on P3 9N and P3 4G. It was picked for colony PCR.

PCR amplification was done on the following samples under the given conditions:

Reagent	1 Reaction	Mastermix
Water	6	48
PCR Mastermix (5X)	2	16
Primer (10uM)	1	8
Subtotal	9 uL	
Template DNA	1	
Total	10 uL	
Number of Reactions	8	

For P2 4B, P3 8M, P4 3F, and P3 9N (colony), they were all diluted 1/10 and the following table represents their PCR cycles.

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	95	15 min	1X
Denature	95	30 sec	35-40X
Anneal	60	30 sec	
Extend	72	2 min	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

Tube1 – P2 4B, Tube2 – P3 8M, Tube3 – P3 9P, Tube4 – P4 3F, Tube5 – P3 9N (colony)

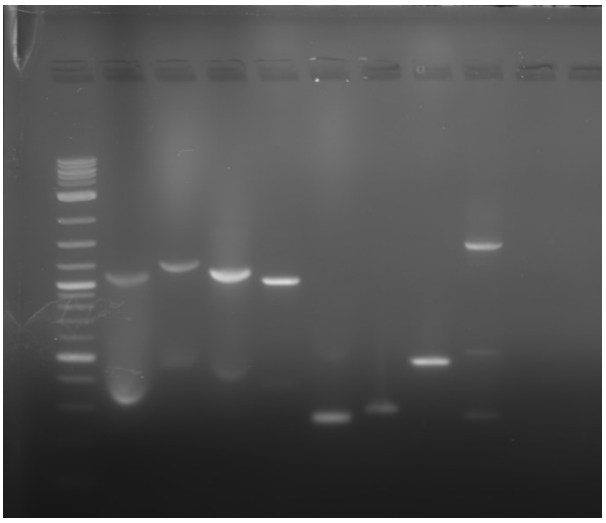
For P4 5L, P5 12G, and P3 4G (colony), they were all diluted 1/10 and the following table represents their PCR cycles.

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	95	15 min	1X
Denature	95	30 sec	35-40X
Anneal	60	30 sec	
Extend	72	1 min	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

Tube1 – P4 5L, Tube2 – P5 12G, Tube3 – P3 4G (colony)

Upon PCR, gel electrophoresis was conducted to analyze the results of PCR
DNA was loaded in the following order:

Lane 1 = Ladder
Lane 2 = P2 4B
Lane 3 = P3 8M
Lane 4 = P3 9P
Lane 5 = P4 3F
Lane 6 = P3 9N (Colony)
Lane 7 = P4 5L
Lane 8 = P5 12G
Lane 9 = P3 4G (Colony)



Conclusion:
Lanes 2, 3, 4, 5, 8, and 9 were gel extracted and the entire volumes were used for restriction enzyme digestion overnight.

The digestion conditions were as follows:

Reagent	1 Reaction
DNA	20
10X CutSmart Buffer	2.5
EcoRI	1
SpeI	1
Water	0.5
Total	25 uL

Digestion incubated at 37°C overnight.

Tuesday 8/29

Distribution Kit Amplification

All of the following parts were transformed today: P2 4B, P3 4G, P3 9N, P3 8M, P3 9P, P4 5L (2X, 4 uL and 1uL), P4 3F, P4 18C, P4 18E, P4 6B, P4 17O, P4 14O, P5 12G, CC10, CC50, CC100.

All transformations were done using 4 uL of DNA, heat shocked for 90 seconds, and chilled on ice for 2 minutes.
Competent cell test kit amplification was done using 1 uL of DNA, heat shocked for 90 seconds, and chilled on ice for 2 minutes.
Bacteria were incubated at 37°C for 1 hour in 600 uL of SOC prior to plating 200 uL.

The successful transformations were then picked and grown in an overnight culture of 7 mL with the appropriate antibiotic.

The cultures were then miniprepped and the final concentrations obtained were as follows:

Plate	Well	Colony	DNA (ng/uL)
P2	4B	1	79.850
P3	8M	1	74.500
		2	69.800
		3	126.75
P4	5L	1	39.300
		2	62.800
		3	95.500
CC 100		1	60.250
		2	62.150
		3	72.450`
P3	9N	1	71.450
		2	78.150
		3	96.000
P4	6B	1	63.200
		2	54.950
P4	3F	1	110.00
		2	139.05
CC 50		1	69.700

Thursday 8/31

3A Assembly

The following parts were digested using the following restriction enzymes:

pH – EcoRI, SpeI
GFP (P2 24B) – XbaI, PstI
J23100 (P4 17D) – EcoRI, SpeI
LasI (P3 9N) – XbaI, PstI
LasR (P1 18A) – XbaI, PstI
Chloramphenicol Backbone (CC 100) – EcoRI, PstI
Kanamycin Backbone (P4 6B) – EcoRI, PstI

EcoRI + SpeI Digestion Conditions:

Reagent	pH	J23100 (P4 17D)
DNA (500 ng)	10	10
10X CutSmart Buffer	5	5
EcoRI-HF	1	1
SpeI	1	1
Water	33	33
Total	50 uL	50 uL

XbaI + PstI Digestion Conditions:

Reagent	GFP (P2 24B)	LasI (P3 9N)	LasR (P1 18A)
DNA (500ng)	9	8	9
10X Buffer 3.1	5	5	5
XbaI	1	1	1
PstI	1	1	1
Water	34	35	34
Total	50 uL	50 uL	50 uL

EcoRI + PstI Digestion Conditions:

Reagent	Ch (CC 100)	Kan (P4 6B)
DNA (500 ng)	9	9
10X Buffer 2.1	5	5
EcoRI-HF	1	1
PstI	1	1
Water	34	34
Total	50 uL	50 uL

The digestions ran for 20 minutes at 37°C and they were then column purified

Using the digestions, the following plasmids were assembled by ligation:

Notation: Insert 1 + Insert 2 = Backbone
pH + P2 24B = CC100
P4 17D + P3 9N = CC100
P4 17D + P1 18A = CC100

The pH for 3A assembly was obtained from an earlier gel extraction.

Reagent	pH + P2 24B = CC100	P4 17D + P3 9N = CC100	P4 17D + P1 18A = CC100
Backbone	2	2	2
Insert 1	14	6	6
Insert 2	6	6	6
10X Ligase Buffer	3	2	2
Ligase	1	1	1
Water	4	3	3
Total	30 uL	20 uL	20 uL

Monday 9/4

3A Assembly
The ligation products from Thursday 8/31 were transformed (half volume of the reaction) into DH5α E. coli.

Tuesday 9/5

3A Assembly
P4 17D + P1 18A = CC 100 showed no colonies → failed transformation
The remaining ligation products: pH + P2 24B = CC100 and P4 17D + P3 9N = CC100 had colonies that were picked and grown overnight.

Thursday 9/7

3A Assembly
The liquid cultures form Tuesday were mini-prepped and amplified using PCR under the following conditions:

Reagent	1 reaction	Mastermix
Water	6	36
PCR Mastermix (5X)	2	12
Primer (10uM)	1	6
Subtotal	9 uL	
Template DNA	1	
Total	10 uL	
Number of Reactions	6	

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	95	15 min	1X
Denature	95	30 sec	50X
Anneal	60	30 sec	
Extend	72	2 min	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

Friday 9/8

3A Assembly
The following plasmids were 3A assembled:
P4 17D + P1 18A = CC100
pH + P2 24B = CC100
P4 17D + P3 9N = CC100

Reagent	Volume (uL)
Insert 1	3
Insert 2	3
Backbone	2
10X Ligase Buffer	1
Ligase	1
Water	0
Total	10 uL

The ligations were carried out for 45 minutes at 37°C and they were then transformed.

Saturday 9/9

3A Assembly
All 3 transformation plates from yesterday had colonies. Two colonies were picked from each plate and grown overnight in liquid cultures.

Monday 9/11

3A Assembly
The overnight cultures from Saturday were mini-prepped and the concentrations were as follows.

Part	Concentration (ng/uL)
P4 17D + P1 18A (1)	55.35
P4 17D + P1 18A (2)	30.65
pH + P2 24B (1)	73.35
pH + P2 24B (2)	68.75
P4 17D + P3 9N (1)	14.2
P4 17D + P3 9N (2)	23.9

Tuesday 9/12

3A Assembly
The following digestions were conducted under the following conditions:

EcoRI/SpeI Digestions:

Reagent	P1 4G	R0010 (P3 4G)	Hypoxia
DNA (500ng)	5	18	10
10X CutSmart Buffer	5	5	5
EcoRI-HF	1	2	1
SpeI	1	2	1
Water	38	23	33
Total	50 uL	50 uL	50 uL

XbaI/PstI Digestions:

Reagent	GFP (P2 24B)	K1497008 (P5 12G)	P2 4B
DNA (500ng)	18	16	7
10X Buffer 3.1	5	5	5
XbaI	2	1	1
PstI	2	1	1
Water	23	27	36
Total	50 uL	50 uL	50 uL

EcoRI/PstI Digestions:

Reagent	Ch (CC100)	Kan (P4 6B)	P4 2F
DNA (500ng)	14	18	4
10X Buffer 2.1	5	5	5
EcoRI-HF	2	2	2
PstI	2	2	2
Water	27	23	37
Total	50 uL	50 uL	50 uL

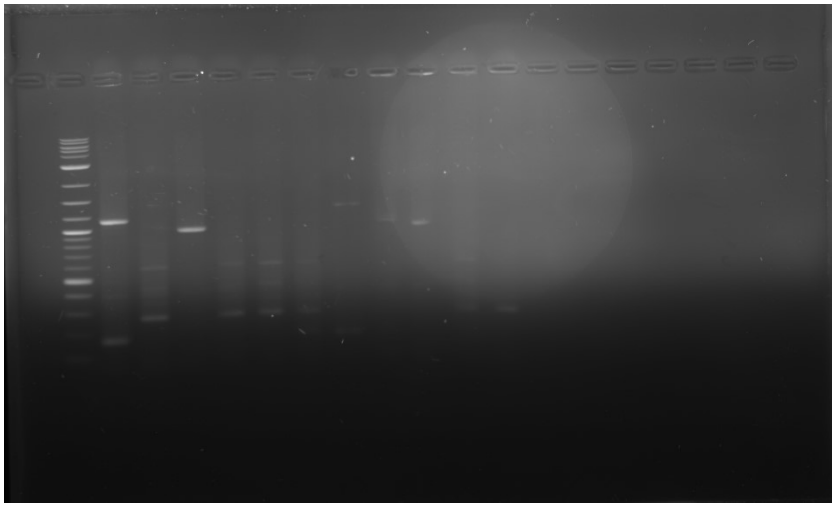
PCR:
DNA was diluted 1/100

Reagent	1 Reaction	Mastermix
Water	6	66
PCR Mastermix (5X)	2	22
Primer (10uM)	1	11
Subtotal	9 uL	
Template DNA	1	
Total	10 uL	
Number of Reactions	11	

Saturday 9/16

3A Assembly
The PCR samples from Tuesday were analyzed using gel electrophoresis.
Lanes:
1 – Blank
2 – Ladder
3 – P4 17D
4 – P1 18A
5 – P3 9N
6 – pH
7 – P2 24B

8 – P4 17D + P1 18A (1)
9 – P4 17D + P1 18A (2)
10 – P4 17D + P3 9N (1)
11 – P4 17D + P3 9N (2)
12 – pH + P2 24B (1)
13 – pH + P2 24B (2)



The overnight cultures from Tuesday were mini-prepped and their concentrations were as follows:

Plasmid	Concentration (ng/uL)
P3 4G + K1797008 (1)	28.15
P3 4G + K1797008 (2)	29.6
P1 4G + P2 24B (1)	8.15
P1 4G + P2 24B (2)	6.5
R0051 (1)	122.25
R0051 (2)	11.7

Tuesday 9/19

Distribution Kit Amplification

P4 1N (RBS) was amplified using PCR. The following table shows the PCR conditions:

Reagent	1 Reaction	Mastermix
Water	6	6
PCR Mastermix (5X)	2	2
Primer (10uM)	1	1
Subtotal	9 uL	
Template DNA	1	
Total	10 uL	
Number of Reactions	1	

Wednesday 9/20

Distribution Kit Amplification

P4 1N was gel extracted and its concentration was 4.60 ng/uL.

3A Assembly

The following digestions were conducted under the following conditions:

EcoRI/SpeI Digestions:

Reagent	B0034 (P4 1N)	R0051 (P4 5L)
DNA (500ng)	7	6
10X CutSmart Buffer	5	5
EcoRI-HF	1	1
SpeI	0.5	0.5
Water	36.5	37.5
Total	50 uL	50 uL

XbaI/PstI Digestions:

The P2 24B concentration here was 47.450 ng/uL

Reagent	K1497008 (P5 12G)	P2 4B	P3 9N	P1 18A	P2 24B	P2 24B	P2 24B
DNA (500ng)	16	7	7	8	11	11	11
10X Buffer 3.1	5	5	5	5	5	5	5
XbaI	1	1	1	1	1	1	1
PstI	1	1	1	1	1	1	1
Water	27	36	36	35	32	32	32
Total	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL

EcoRI/PstI Digestions:

The P2 24B concentration here was 53 ng/uL.

Reagent	P2 24B	P2 24B	P2 24B	CC100	CC100
DNA (500ng)	10	10	11	9	9
10X Buffer 2.1	5	5	5	5	5
EcoRI-HF	1	1	1	1	1
PstI	1	1	1	1	1
Water	33	33	32	34	34
Total	50 uL	50 uL	50 uL	50 uL	50 uL

The digestions were incubated at 37°C for 1 hour.

Ligations:

RBS (B0034) + HokD (P5 12G) = Amp P2 24B
RBS (B0034) + Cl Repressor (P2 4B) = Amp P2 24B
RBS (B0034) + lasR (P1 18A) = Chl CC100
RBS (B0034) + lasI (P3 9N) = Amp P2 24B
pCl + GFP = Chl CC100

Reagent	RBS + HokD = Amp P2 24B	RBS + Cl repressor = Amp P2 24B	RBS + lasR = Chl CC100	RBS + lasI = Amp P2 24B	pCl + GFP = Chl CC100
Backbone	2	2	2	2	2
Insert 1	3	3	3	3	3
Insert 2	3	3	3	3	3
10X Ligase Buffer	1	1	1	1	1
Ligase	1	1	1	1	1
Water	0	0	0	0	0
Total	10 uL	10 uL	10 uL	10 uL	10 uL

Ligations were incubated at 37°C overnight

Sunday 9/24

3A Assembly

Ligations from Wednesday were transformed and mini-prepped. The concentrations were as follows:

Ligation	Concentration (ng/uL)
B0034 + P2 4B (1)	32.950
pCl + GFP (1)	34.450
pCl + GFP (2)	15.1
B0034 + P3 9N (1)	45.500

The following digestions were conducted using the following conditions:

EcoRI/SpeI Digestions:

Reagent	P3 4G	pH	P1 4G	P3 4G
DNA (500ng)	8	12	7	8
10X CutSmart Buffer	5	5	5	5
EcoRI-HF	1	1	1	1
SpeI	0.5	0.5	0.5	0.5
Water	35.5	31.5	36.5	35.5
Total	50 uL	50 uL	50 uL	50 uL

EcoRI/PstI Digestions:

Reagent	P4 6B
DNA (500ng)	8
10X Buffer 2.1	5
EcoRI-HF	1
PstI	1
Water	35
Total	50 uL

The digestions were incubated at 37°C for 1 hour.

Ligations:
B0034 + P1 18A = CC100 (Ch)
P3 4G + P5 12G = P2 24B (Amp)
pH + P3 9N = P4 6B (Kan)
P3 4G + P2 4B = P2 24B (Amp)
P1 4G + P2 24B = CC100 (Ch)

Reagent	B0034 + P1 18A = CC100	P3 4G + P5 12G = P2 24B	pH + P3 9N = P4 6B	P3 4G + P2 4B = P2 24B	P1 4G + P2 24B = CC100
Backbone	2	2	2	2	2
Insert 1	3	3	3	3	3
Insert 2	3	3	3	3	3
10X Ligase Buffer	1	1	1	1	1
Ligase	1	1	1	1	1
Water	0	0	0	0	0
Total	10 uL	10 uL	10 uL	10 uL	10 uL

Ligations were incubated at 37°C for 30 minutes
The ligation products were then transformed into E. coli DH5α.

Distribution Kit Amplification
P4 1N, P4 13L, and P3 15D were transformed into E.coli DH5α.

Tuesday 9/26

3A Assembly
The concentrations of the successful ligations from Sunday 9/24 were as follows:

Ligation	Concentration (ng/uL)
pH + P3 9N	82.150
RBS + P1 18A	111.70

The following digestions were conducted under the following conditions:

EcoRI/SpeI Digestions:

Reagent	B0034	B0034	P3 4G	P3 4G
DNA (500ng)	7	7	8	8
10X CutSmart Buffer	5	5	5	5
EcoRI-HF	1	1	1	1
SpeI	0.5	0.5	0.5	0.5
Water	36.5	36.5	35.5	35.5
Total	50 uL	50 uL	50 uL	50 uL

XbaI/PstI Digestions:

Reagent	P5 12G	RBS + Cl	P1 18A	P2 24B
DNA (500ng)	16	16	8	8
10X Buffer 3.1	5	5	5	5
XbaI	1	1	1	1
PstI	1	1	1	1
Water	27	27	35	35
Total	50 uL	50 uL	50 uL	50 uL

EcoRI/PstI Digestions:

Reagent	P2 24B	P4 6B	P4 6B	CC100
DNA (500ng)	8	8	8	9
10X Buffer 2.1	5	5	5	5
EcoRI-HF	1	1	1	1
PstI	1	1	1	1
Water	35	35	35	34
Total	50 uL	50 uL	50 uL	50 uL

The digestions were incubated at 37°C for 1 hour.

Ligations:
The following ligations were created:
P4 1N + P1 18A = CC100 (Ch)
P4 1N + P5 12G = P2 24B (Amp)
P3 4G + [RBS + Cl] = P4 6B (Kan)
P3 4G + P2 24B = P4 6B (Kan)

Sunday 10/1

Distribution Kit Amplification
The following parts were mini-prepped and their concentrations were as follows:

Part	Colony	Concentration (ng/uL)
P3 15D	1	58.600
	2	57.250
	3	66.050
	4	79.850
P4 1N	1	56.450
	2	50.450
	3	61.300
	4	50.100

3A Assembly
The following digestions were conducted under the following conditions:

EcoRI/SpeI Digestions:

Reagent	pH	Hypoxia	Hypoxia	P4 17D	P4 1N	P4 1N
DNA (500ng)	12	10	10	13	9	9
10X CutSmart Buffer	5	5	5	5	5	5
EcoRI-HF	1	1	1	1	1	1
SpeI	0.5	0.5	0.5	0.5	0.5	0.5
Water	31.5	33.5	33.5	30.5	34.5	34.5
Total	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL

XbaI/PstI Digestions:

Reagent	P3 15D	P3 9N	RBS + P1 18A	RBS + P1 18A	P4 3F	P5 12G	P2 24B
DNA (500ng)	9	7	5	5	5	27	5
10X Buffer 3.1	5	5	5	5	5	5	5
XbaI	1	1	1	1	1	1	1
PstI	1	1	1	1	1	1	1
Water	34	36	38	38	38	16	38
Total	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL

EcoRI/PstI Digestions:

Reagent	P4 2F
DNA (500ng)	12
10X Buffer 2.1	5
EcoRI-HF	1
PstI	1
Water	41
Total	50 uL

The digestions were incubated at 37°C for 1 hour.

Ligations:
pH + P3 15D = P4 6B (Kan)
Hypoxia + [RBS + LasR] = P4 2F (Amp)
Hypoxia + P2 24B = P4 6B (Kan)
J23100 + [RBS + LasR] = P4 6B (Kan)
RBS + P5 12G = P4 6B (Kan)
RBS + P4 3F = CC100 (Chl)
RBS + P3 9N = P4 6B (Kan)

Reagent	pH + P3 15D = Kan	Hypoxia + [RBS + LasR] = Amp	Hypoxia + P2 24B = Kan	J23100 + [RBS + LasR] = Kan	RBS + P5 12G = Kan	RBS + P4 3F = Chl	RBS + P3 9N = Kan
Backbone	2	2	2	2	2	2	2
Insert 1	3	3	3	3	3	3	3
Insert 2	3	3	3	3	3	3	3
10X Ligase Buffer	1	1	1	1	1	1	1
Ligase	1	1	1	1	1	1	1
Water	0	0	0	0	0	0	0
Total	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL

Ligations were incubated at 37°C for 30 minutes and the products were transformed.

Wednesday 10/4

3A Assembly

The following parts were mini-prepped and their concentrations were as follows:

Part	Colony	Concentration (ng/uL)
Hypoxia + P2 24B	1	7.85
	2	9.1
P5 12G	1	9.5
	2	6.3
	3	65.800
P4 6B	1	6.4
Hypoxia + RBS + LasR	2	1.00
pH + P3 15D	1	16.2
P4 3F	1	5.85
	3	9.2
J23100 + RBS + LasR	1	4.1
	2	8.55
RBS + P3 9N	1	2.75

Sunday 10/8

Distribution Kit Amplification

The following parts were picked from glycerol stocks, grown overnight in cultures and mini-prepped. Their concentrations were as follows:

Sample	Concentration (ng/uL)
P4 3F	47.800
P4 3F	20.300
P5 12G	37.200
P5 12G	26.250
P5 12G	30.550

3A Assembly

The following ligations were mini-prepped after transformation and their concentrations were as follows:

Sample	Concentration (ng/uL)
Hypoxia + P2 24B	46.050
Hypoxia + P2 24B	42.950
Hypoxia + P2 24B	33.700
pH + P3 15D	20.600
pH + P3 15D	40.550
pH + P3 15D	36.050

The following digestions were conducted under the following conditions:

EcoRI/SpeI Digestions:

Reagent	P4 1N	P4 5L
DNA (500ng)	20	6
10X CutSmart Buffer	5	5
EcoRI-HF	2	1
SpeI	2	1
Water	21	37
Total	50 uL	50 uL

XbaI/SpeI Digestions:

Reagent	P1 18A (LasR)	P5 12G (HokD)	P4 3F (Cl)	P3 9N (LasI)	P2 24B (GFP)
DNA (500ng)	9	14	11	7	5
10X Buffer 3.1	5	5	5	5	5
XbaI	1	1	1	1	1
PstI	1	1	1	1	1
Water	34	29	32	36	38
Total	50 uL	50 uL	50 uL	50 uL	50 uL

EcoRI/PstI Digestions:

Reagent	P4 6B (Kan)
DNA (500ng)	20
10X Buffer 2.1	5
EcoRI-HF	2
PstI	2
Water	21
Total	50 uL

Digestions were incubated at 37°C for 30 minutes and column purified.

Ligations:
RBS + LasR = Kan
RBS + HokD = Kan
RBS + Cl = Kan
RBS + LasI = Kan
pCl + GFP = Kan

Reagent	RBS + LasR = Kan	RBS + HokD = Kan	RBS + Cl = Kan	RBS + LasI = Kan	pCl + GFP = Kan
Backbone	2	2	2	2	2
Insert 1	3	3	3	3	3
Insert 2	3	3	3	3	3
10X Ligase Buffer	1	1	1	1	1
Ligase	1	1	1	1	1
Water	0	0	0	0	0
Total	10 uL	10 uL	10 uL	10 uL	10 uL

All the ligation products were transformed and plated.
pH + P3 15D was also transformed into the expression strain, E. coli MG1655.

Tuesday 10/10

3A Assembly
Transformed colonies from Sunday 10/8 were picked and mini-prepped. The concentrations were as follows:

Part	Colony	Concentration (ng/uL)
RBS + Cl	1	17.5
	2	38.2
RBS + HokD	1	15.25
	2	50.65
RBS + pCl + GFP	1	305.85
	2	156.8
RBS + LasR	1	11.05
	2	130.3
RBS + LasI	1	51.35

The following digestions were conducted under the following conditions:
EcoRI/SpeI Digestions:

Reagent	P4 17D	P3 4G	Hypoxia	P4 5L	P3 4G	P4 17D
DNA (500ng)	13	4	10	6	4	13
10X CutSmart Buffer	5	5	5	5	5	5
EcoRI-HF	1	1	1	1	1	1
SpeI	0.5	0.5	0.5	0.5	0.5	0.5
Water	30.5	39.5	33.5	37.5	39.5	30.5
Total	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL

XbaI/PstI Digestions:

Reagent	RBS + LasR	RBS + HokD	RBS + LasR	RBS + HokD	RBS + Cl	P2 24B	RBS + LasI
DNA (500ng)	4	10	4	10	13	11	10
10X Buffer 3.1	5	5	5	5	5	5	5
XbaI	1	1	1	1	1	1	1
PstI	1	1	1	1	1	1	1
Water	39	33	39	33	30	32	33
Total	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL	50 uL

EcoRI/PstI Digestions:

Reagent	P4 2F	P4 2F	CC100	CC100
DNA (500ng)	2	2	8	8
10X Buffer 2.1	5	5	5	5
EcoRI-HF	1	1	1	1
PstI	1	1	1	1
Water	41	41	35	35
Total	50 uL	50 uL	50 uL	50 uL

Ligations:
Hypoxia + [RBS + LasR] = Amp P4 2F
J23100 + [RBS + LasR] = Chl CC100
pLac + [RBS + HokD] = Amp P4 2F
pCl + [RBS + HokD] = Chl CC100
pLac + [RBS + Cl] = Amp P4 2F
pLac + GFP = Kan P4 6B
J23100 + [RBS + LasI] = Chl CC100

Reagent	Hypoxia + [RBS + LasR] = Amp	J23100 + [RBS + LasR] = Chl	pLac + [RBS + HokD] = Amp	pCl + [RBS + HokD] = Chl	pLac + [RBS + Cl] = Amp	pLac + GFP = Kan	J23100 + [RBS + LasI] = Chl
Backbone	2	2	2	2	2	2	2
Insert 1	3	3	3	3	3	3	3
Insert 2	3	3	3	3	3	3	3
10X Ligase Buffer	1	1	1	1	1	1	1
Ligase	1	1	1	1	1	1	1
Water	0	0	0	0	0	0	0
Total	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL	10 uL

The ligations were incubated for 30 minutes at 37°C. The products were then transformed and plated.

Thursday 10/12

High Fidelity PCR (Phusion)

The following parts were amplified using PCR:

1 - P1 4G - pLasB - BBa_K575008 (1206)
2 - P1 18A - LasR/Ter/Ter - BBa_K574007 (1218)
3 - P2 4B - Cl Repressor - BBa_C0051 (1075)
4 - P2 24B - GFP - BBa_E0240 (1176)
5 -P3 4G - pLacI - BBa_R0010 (500)
6 - P3 9N - LasI - BBa_C0078 (967)
7 - P4 17F - J23101 - BBa_J23101 (335)
8 - P4 5L - pCl - BBa_R0051 (349)
9 - P4 13L - GFP - BBa_E0040 (1020)
10 - P4 1N - RBS - BBa_B0034 (312)
11 - P5 12G - HokD - BBa_K1497008 (456)
12 - pH Promoter + RBS - BBa_K123001 (1584)
13 - Hypoxia Promoter - BBa_K387003 (399)

The PCR reaction conditions were as follows:

Reagent	One Reaction	Mastermix
Water	19	285
PCR Mastermix (2X)	25	375
Primer (10uM)	5	75
Subtotal	49 uL	
Template DNA	1 uL	
Total	50 uL	
Number of Reactions	15	

The PCR reactions were separated into two categories based on length of DNA because they required different extension times.

PCR 1 – 30 second Extension Time:

5 -P3 4G - pLacI - BBa_R0010 (500)
7 - P4 17F - J23101 - BBa_J23101 (335)
8 - P4 5L - pCl - BBa_R0051 (349)
10 - P4 1N - RBS - BBa_B0034 (312)
11 - P5 12G - HokD - BBa_K1497008 (456)
13 - Hypoxia Promoter - BBa_K387003 (399)

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	98	5 min	1X
Denature	98	30 sec	35-40X
Anneal	64.6	30 sec	
Extend	72	30 sec	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

PCR 2 – 1 minute Extension Time:

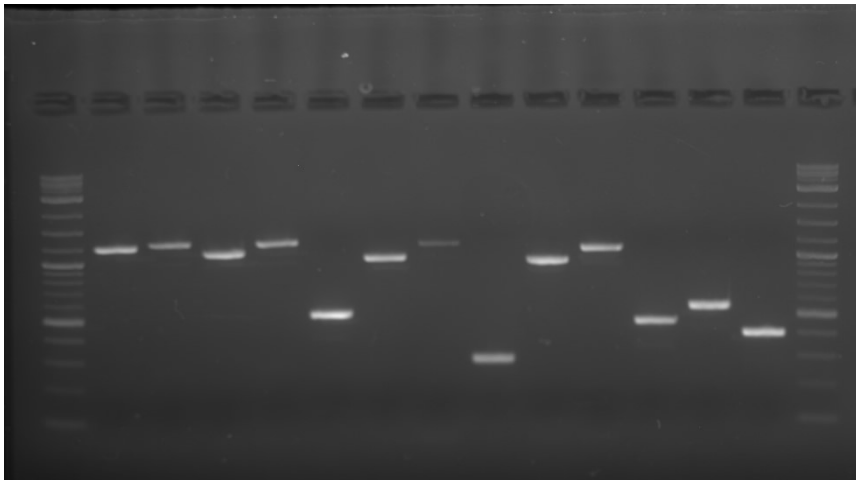
1 - P1 4G - pLasB - BBa_K575008 (1206)
2 - P1 18A - LasR/Ter/Ter - BBa_K574007 (1218)
3 - P2 4B - Cl Repressor - BBa_C0051 (1075)
4 - P2 24B - GFP - BBa_E0240 (1176)
6 - P3 9N - LasI - BBa_C0078 (967)
9 - P4 13L - GFP - BBa_E0040 (1020)
12 - pH Promoter + RBS - BBa_K123001 (1584)

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	98	5 min	1X
Denature	98	30 sec	35-40X
Anneal	64.6	30 sec	
Extend	72	1 min	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

Agarose Gel Electrophoresis

To confirm whether or not the PCR reactions worked, we ran them on an agarose gel. 3 uL of each PCR reaction was run on a 1.5% agarose gel. The lanes are as follows:

Lane 1 - DNA Ladder
Lane 2 - 1 - P1 4G - pLasB/RBS/GFP - BBa_K575008 (1206)
Lane 3 - 2 - P1 18A - LasR/Ter/Ter - BBa_K574007 (1218)
Lane 4 - 3 - P2 4B - Cl Repressor - BBa_C0051 (1075)
Lane 5 - 4 - P2 24B - GFP - BBa_E0240 (1176)
Lane 6 - 5 -P3 4G - pLacI - BBa_R0010 (500)
Lane 7 - 6 - P3 9N - LasI - BBa_C0078 (967)
Lane 8 - 7 - P4 17F - J23101 - BBa_J23101 (335)
Lane 9 - 8 - P4 5L - pCl - BBa_R0051 (349)
Lane 10 - 9 - P4 13L - GFP - BBa_E0040 (1020)
Lane 11 - 10 - P4 1N - RBS - BBa_B0034 (312)
Lane 12 - 11 - P5 12G - HokD - BBa_K1497008 (456)
Lane 13 - 12 - pH Promoter + RBS - BBa_K123001 (1584)
Lane 14 - 13 - Hypoxia Promoter - BBa_K387003 (399)



The gel showed that all of the PCR reactions were successful. Therefore, the PCR products were cleaned using the PCR clean up protocol.

Digestion

EcoRI/PstI Digestions:

Reagent	**CC100 = Chlor**	**P4 2F = Amp**	**P4 6B = Kan (500ng)**	**pLasB/RBS/GFP (P4 1G) (1)**
DNA (3ug)	45	11	10	20
10X Buffer 2.1	10	2.5	2.5	2.5
EcoRI-HF	2	2	1	1
PstI	2	2	1	1
Water	41	7.5	10.5	0.5
Total	100 uL	25 uL	25 uL	25 uL

EcoRI/SpeI Digestions:

Reagent	**pCI – P4 5L (8)**	**J23101 – P4 17F (7)**	**Hypoxia (13)**	**pLacI – P3 4G**	**[pH + RBS] (12)**	**RBS – P4 1N (10)**
DNA (PCR)	20	20	20	20	20	20
10X CutSmart	2.5	2.5	2.5	2.5	2.5	2.5
EcoRI-HF	1	1	1	1	1	1
SpeI-HF	1	1	1	1	1	1
Water	0.5	0.5	0.5	0.5	0.5	0.5
Total	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL

XbaI/PstI Digestions:

Reagent	[RBS + GFP] – P2 24B (4)	GFP – P4 13L (9)	LasI – P3 9N (6)	LasR – P118A (2)	HokD – P5 12G (11)	CI – P2 4B (3)
DNA (PCR)	20	20	20	20	20	20
10X Buffer 3.1	2.5	2.5	2.5	2.5	2.5	2.5
XbaI	1	1	1	1	1	1
PstI	1	1	1	1	1	1
Water	0.5	0.5	0.5	0.5	0.5	0.5
Total	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL

The digestion were cleaned using the PCR Cleanup Protocol.
CC100 (Chloro) was treated with CIP and the eluted 20 uL product was diluted to a total 100 uL
P4 2F (Amp) was eluted in 20 uL, which was then diluted to a total of 100 uL.

Ligations

All ligations were done into a CC100 E/P digested backbone.

Ligation conditions: 3uL Insert 1 (E/S) + 3uL Insert 2 (X/P) + 2uL Backbone (E/P) + 1uL 10X T4 Ligase Buffer + 1uL T4 Ligase

For pLasB/RBS/GFP: 6uL Insert (E/P) + 2uL Backbone (E/P) + 1uL 10X T4 Ligase Buffer + 1uL T4 Ligase

pCl [P4 5L = 8] + (RBS + GFP) [P2 24 B = 4]
J23101 [P4 17F = 7] + (RBS + GFP) [P2 24 B = 4]
Hypoxia [13] + (RBS + GFP) [P2 24B = 4]
pLacI [P3 4G = 5] + (RBS + GFP) [P2 24B = 4]
(pH + RBS) [12] + GFP [P4 13L = 9]
(pH + RBS) [12] + LasI [P3 9N = 6]
RBS [P4 1N = 10] + LasI [P3 9N = 6]
RBS [P4 1N = 10] + LasR [P1 18A = 2]
RBS [P4 1N = 10] + HokD [P5 12G = 11]
pLasB/RBS/GFP [P4 1G = 1]

Ligation were incubated at 25°C overnight.

Product Parts

(pH + RBS) + GFP
(pH + RBS) + LasI
RBS + LasR
RBS + LasI
RBS + HokD
RBS + Cl

Hypoxia + (RBS + GFP)
pCl + (RBS + GFP)
J23101 + (RBS + GFP)

High Fidelity PCR (Phusion)

The following parts were amplified using PCR:

1 - P2 4B - Cl Repressor - BBa_C0051 (1075)
2 - P3 5C - pLasB - BBa_R0079 (457)
3 - P4 1N - RBS - BBa_B0034 (312)
4 - P2 24B - GFP - BBa_E0240 (1176)

The PCR conditions were as follows:

Reagent	One Reaction	Mastermix
Water	19	76
PCR Mastermix (2X)	25	100
Primer (10uM)	5	20
Subtotal	49 uL	
Template DNA	1 uL	
Total	50 uL	
Number of Reactions	4	

The PCR reactions were separated into two categories based on length of DNA because they required different extension times.

PCR 1 – 30 second Extension Time:

2 - P3 5C - pLasB - BBa_R0079 (457)
3 - P4 1N - RBS - BBa_B0034 (312)

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	98	5 min	1X
Denature	98	30 sec	35-40X
Anneal	64.6	30 sec	
Extend	72	30 sec	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

PCR 2 – 2 minute Extension Time:

1 - P2 4B - Cl Repressor - BBa_C0051 (1075)
4 - P2 24B - GFP - BBa_E0240 (1176)

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	98	5 min	1X
Denature	98	30 sec	35-40X
Anneal	64.6	30 sec	
Extend	72	2 min	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

Friday 10/13

The PCR products from Thursday 10/12 were cleaned using the PCR clean up protocol:
P2 4B - Cl Repressor - BBa_C0051 (1075)
P2 24B - RBS + GFP - BBa_E0240 (1176)
P3 5C - pLasB - BBa_R0079 (457)
P4 1N - RBS - BBa_B0034 (312)

Digestions
Backbones and promoters were done on Thursday 10/12

EcoRI/SpeI Digestions:

Reagent	pLasB – P3 5C	RBS – P4 1N
DNA (PCR)	10	20
10X CutSmart	2.5	2.5
EcoRI-HF	1	1
SpeI-HF	1	1
Water	10.5	0.5
Total	25 uL	25 uL

XbaI/PstI Digestions:

Reagent	[RBS + GFP] – P2 24B	CI – P2 4B	pLasB – P3 5C
DNA (PCR)	20	20	10
10X Buffer 3.1	2.5	2.5	2.5
XbaI	1	1	1
PstI	1	1	1
Water	0.5	0.5	10.5
Total	25 uL	25 uL	25 uL

Digestions were incubated at 37°C for 45 minutes
Digestion products were cleaned up and eluted in 20uL water

Ligations
RBS [P4 1N] + Cl [P2 4B] = Chlor
pLasB [P3 5C] + (RBS + GFP) [P2 24B] = Chlor
pLac [P3 4G] + pLasB [P3 5C] = Chlor

Ligation Conditions were: 3uL Insert 1 (E/S) + 3uL Insert 2 (X/P) + 2uL Backbone (E/P) + 1uL 10X T4 Ligase Buffer + 1uL T4 Ligase

Ligations were run for 40 minutes at 37°C.

Transformations
Heat Shock for 90 seconds and Recovery in SOC media

The following ligations were transformed into *E. coli*:
1 - RBS + Cl
2 - RBS + LasR
3 - RBS + HokD
4 - RBS + LasI
5 - pH + RBS + LasI
6 - pH + RBS + GFP
7 - pLac + RBS + GFP
8 - pLac + pLasB
9 - J23101 + RBS + GFP
10 - Hypoxia + RBS + GFP
11 - pLasB + RBS + GFP
12 - pLasB + RBS + GFP (P1 4G)
13 - pCl + RBS + GFP

Sunday 10/15

Transformation Results:
Failed:
2 - RBS + LasR
3 - RBS + HokD
4 - RBS + LasI
5 - pH + RBS + LasI
6 - pH + RBS + GFP
7 - pLac + RBS + GFP
8 - pLac + pLasB
13 - pCl + RBS + GFP

Success:
1 - RBS + Cl = 2 colonies >1000bp
9 - J23101 + RBS + GFP = 2 colonies >1000bp
10 - Hypoxia + RBS + GFP = 6 colonies >1000bp
11 - pLasB + RBS + GFP = 7 colonies >1000 bp
12 - pLasB + RBS + GFP (P1 4G) = 3 colonies >1000bp

Colony PCR
Colonies were picked into 20 uL water.
The following colonies were amplified using Colony PCR:

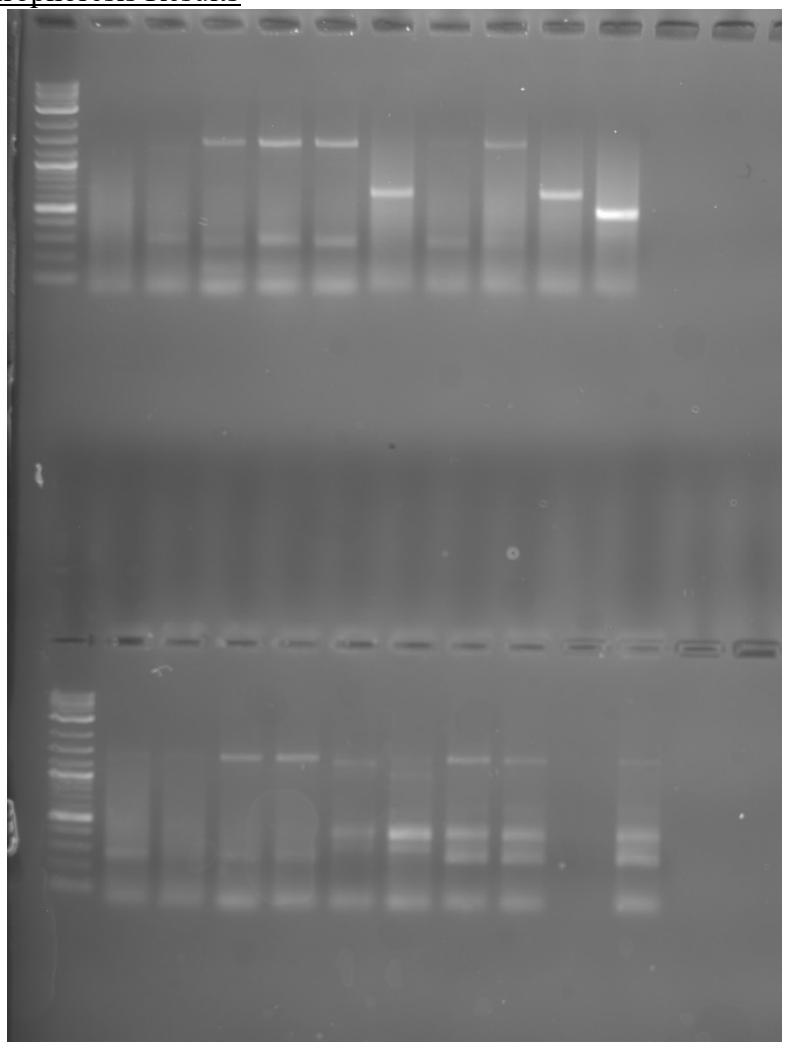
1 - [1 - RBS + Cl] 1 No	2 - [1 - RBS + Cl] 2 No
3 - [12 - pLasB + RBS + GFP (P1 4G)] 1	4 - [12 - pLasB + RBS + GFP (P1 4G)] 2
5 - [12 - pLasB + RBS + GFP (P1 4G)] 3	6 - [11 - pLasB + RBS + GFP] 1
7 - [11 - pLasB + RBS + GFP] 2	8 - [11 - pLasB + RBS + GFP] 3
9 - [11 - pLasB + RBS + GFP] 4	10 - [11 - pLasB + RBS + GFP] 5
11 - [11 - pLasB + RBS + GFP] 6	12 - [11 - pLasB + RBS + GFP] 7
13 - [9 - J23101 + RBS + GFP] 1	14 - [9 - J23101 + RBS + GFP] 2
15 - [10 - Hypoxia + RBS + GFP] 1	16 - [10 - Hypoxia + RBS + GFP] 2
17- [10 - Hypoxia + RBS + GFP] 3	18 - [10 - Hypoxia + RBS + GFP] 4

19 - [10 - Hypoxia + RBS + GFP] 5 20 - [10 - Hypoxia + RBS + GFP] 6

The PCR conditions were as follows:

Reagent	One Reaction	Mastermix
Water	6	132
PCR Mastermix (5X)	2	44
Primer (10uM)	1	22
Subtotal	9 uL	
Template DNA	1 uL	
Total	10 uL	
Number of Reactions	22	

Agarose Gel Electrophoresis Results



Through colony PCR, the following 3A assemblies did not work:

1 - [1 - RBS + Cl] 1
2 - [1 - RBS + Cl] 2
6 - [11 - pLasB + RBS + GFP] 1
7 - [11 - pLasB + RBS + GFP] 2
9 - [11 - pLasB + RBS + GFP] 4
10 - [11 - pLasB + RBS + GFP] 5
11 - [11 - pLasB + RBS + GFP] 6
12 - [11 - pLasB + RBS + GFP] 7
13 - [9 - J23101 + RBS + GFP] 1
14 - [9 - J23101 + RBS + GFP] 2
15 - [10 - Hypoxia + RBS + GFP] 1
16 - [10 - Hypoxia + RBS + GFP] 2
19 - [10 - Hypoxia + RBS + GFP] 5

Through colony PCR, the following 3A assemblies worked:

3 - [12 - pLasB + RBS + GFP (P1 4G)] 1
4 - [12 - pLasB + RBS + GFP (P1 4G)] 2
5 - [12 - pLasB + RBS + GFP (P1 4G)] 3
8 - [11 - pLasB + RBS + GFP] 3
17- [10 - Hypoxia + RBS + GFP] 3
18 - [10 - Hypoxia + RBS + GFP] 4
20 - [10 - Hypoxia + RBS + GFP] 6

The successful cPCR were cultured overnight at 37°C at 200 rpm.

Ligations

pCl [P4 5L = 8] + (RBS + GFP) [P2 24 B = 4] = Ch
pLacI [P3 4G = 5] + (RBS + GFP) [P2 24B = 4] = Ch
(pH + RBS) [12] + GFP [P4 13L = 9] = Ch
(pH + RBS) [12] + LasI [P3 9N = 6] = Ch
RBS [P4 1N = 10] + LasI [P3 9N = 6] = Ch
RBS [P4 1N = 10] + LasR [P1 18A = 2] = Ch
RBS [P4 1N = 10] + HokD [P5 12G = 11] = Ch
pLac [P3 4G] + pLasB [P3 5C] = Ch

(pH + RBS) [12] + GFP [P4 13L = 9] = Kan
(pH + RBS) [12] + LasI [P3 9N = 6] = Kan
RBS [P4 1N = 10] + LasI [P3 9N = 6] = Kan
RBS [P4 1N = 10] + LasR [P1 18A = 2] = Kan

Ligation Conditions were: 3uL Insert 1 (E/S) + 3uL Insert 2 (X/P) + 2uL Backbone (E/P) + 1uL 10X T4 Ligase Buffer + 1uL T4 Ligase

Ligations were run for 30 minutes at 37°C.

Transformations

All of the ligation products were transformed.
Heat shock for 90 seconds and incubated overnight at 37°C.

Monday 10/16

High Fidelity PCR (Phusion)

The following parts were amplified using PCR:

1 - P1 18A - LasR/Ter/Ter - BBa_K574007 (1218)
2 - P2 4B - Cl Repressor - BBa_C0051 (1075)
3 - P2 24B - GFP - BBa_E0240 (1176)
4 - P2 24B - GFP - BBa_E0240 (1176)
5 - P3 4G - pLacI - BBa_R0010 (500)
6 - P3 9N - LasI - BBa_C0078 (967)
7 - P4 1N - RBS - BBa_B0034 (312)
8 - P4 1N - RBS - BBa_B0034 (312)
9 - P5 12G - HokD - BBa_K1497008 (456)
10 - pH Promoter + RBS - BBa_K123001 (1584)
11 - P3 5C - pLasB - BBa_R0079 (457)

The PCR reaction conditions were as follows:

Reagent	One Reaction	Mastermix
Water	19	266
PCR Mastermix (2X)	25	350
Primer (10uM)	5	70
Subtotal	49 uL	
Template DNA	1 uL	
Total	50 uL	
Number of Reactions	14	

The PCR reactions were separated into two categories based on length of DNA because they required different extension times.

PCR 1 – 30 second Extension Time:

5 -P3 4G - pLacI - BBa_R0010 (500)
7 - P4 1N - RBS - BBa_B0034 (312)
8 - P4 1N - RBS - BBa_B0034 (312)
9 - P5 12G - HokD - BBa_K1497008 (456)
11 - P3 5C - pLasB - BBa_R0079 (457)

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	98	5 min	1X
Denature	98	30 sec	35-40X
Anneal	64.6	30 sec	
Extend	72	30 sec	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

PCR 2 – 1 minute Extension Time:

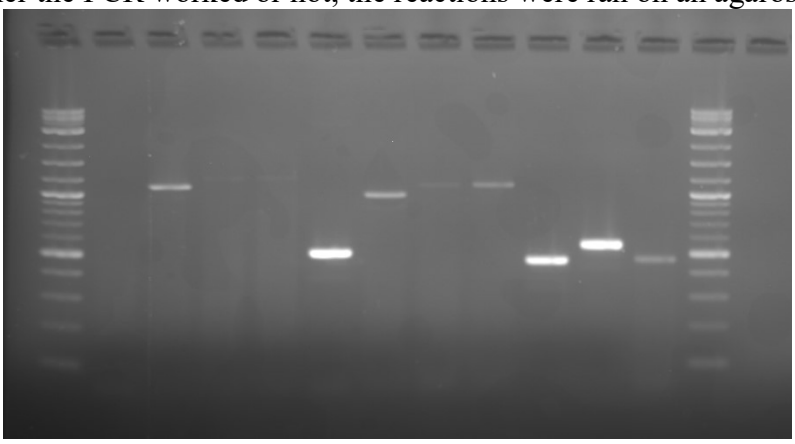
1 - P1 18A - LasR/Ter/Ter - BBa_K574007 (1218)

2 - P2 4B - Cl Repressor - BBa_C0051 (1075)
3 - P2 24B - GFP - BBa_E0240 (1176)
4 - P2 24B - GFP - BBa_E0240 (1176)
6 - P3 9N - LasI - BBa_C0078 (967)
10 - pH Promoter + RBS - BBa_K123001 (1584)

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	98	5 min	1X
Denature	98	30 sec	35-40X
Anneal	64.6	30 sec	
Extend	72	1 min	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

Agarose Gel Electrophoresis

To confirm whether the PCR worked or not, the reactions were ran on an agarose gel.



Succeeded PCR:

2 - P2 4B - Cl Repressor - BBa_C0051 (1075)
5 -P3 4G - pLacI - BBa_R0010 (500)
6 - P3 9N - LasI - BBa_C0078 (967)
8 - P4 1N - RBS - BBa_B0034 (312)
9 - P5 12G - HokD - BBa_K1497008 (456)
10 - pH Promoter + RBS - BBa_K123001 (1584)
11 - P3 5C - pLasB - BBa_R0079 (457)

Transformation Results from Sunday 10/15

Successful Transformations:

(pH + RBS) [12] + LasI [P3 9N = 6] = Ch
(pH + RBS) [12] + GFP [P4 13L = 9] = Kan
(pH + RBS) [12] + LasI [P3 9N = 6] = Kan
RBS [P4 1N = 10] + LasI [P3 9N = 6] = Kan
RBS [P4 1N = 10] + LasR [P1 18A = 2] = Kan

Failed Transformations:

pCl [P4 5L = 8] + (RBS + GFP) [P2 24 B = 4] = Ch
pLacI [P3 4G = 5] + (RBS + GFP) [P2 24B = 4] = Ch

(pH + RBS) [12] + GFP [P4 13L = 9] = Ch
RBS [P4 1N = 10] + LasI [P3 9N = 6] = Ch
RBS [P4 1N = 10] + LasR [P1 18A = 2] = Ch
RBS [P4 1N = 10] + HokD [P5 12G = 11] = Ch
pLac [P3 4G] + pLasB [P3 5C] = Ch

Colony PCR

The following parts were amplified using PCR:
Strip 1&2:
1-6 - pH + RBS + GFP = Kan (2604)
7-12 - pH + RBS + LasI = Kan (2551)
25-27 - pH + RBS + GFP = Ch (2604)
32 - pH + RBS + LasI = Ch (2551)

Strip 3&4:
13-18 - RBS + LasI = Kan (1279)
19-21 - RBS + LasR = Kan (1610)
22 - Hypoxia + RBS + GFP 1 = Ch (1575)
23 - P4 6B 2 = Kan (1369)
24 - P1 4G 3 = Ch (1206)
28 - pLacB + RBS + GFP = Ch (1633)
29 - RBS + HokD = Ch (768)
30 - pLac + pLasB = Ch (957)
31 - pLac + RBS + GFP = Ch (1676)

The PCR reaction conditions were as follows:

Reagent	**One Reaction**	**Mastermix**
Water	6	210
PCR Mastermix (5X)	2	70
Primer (10uM)	1	35
Subtotal	9 uL	
Template DNA	1 uL	
Total	10 uL	
Number of Reactions	35	

The PCR reactions were separated into two categories based on length of DNA because they required different extension times.

PCR 1 – 30 second Extension Time:

Strip 1 & 2

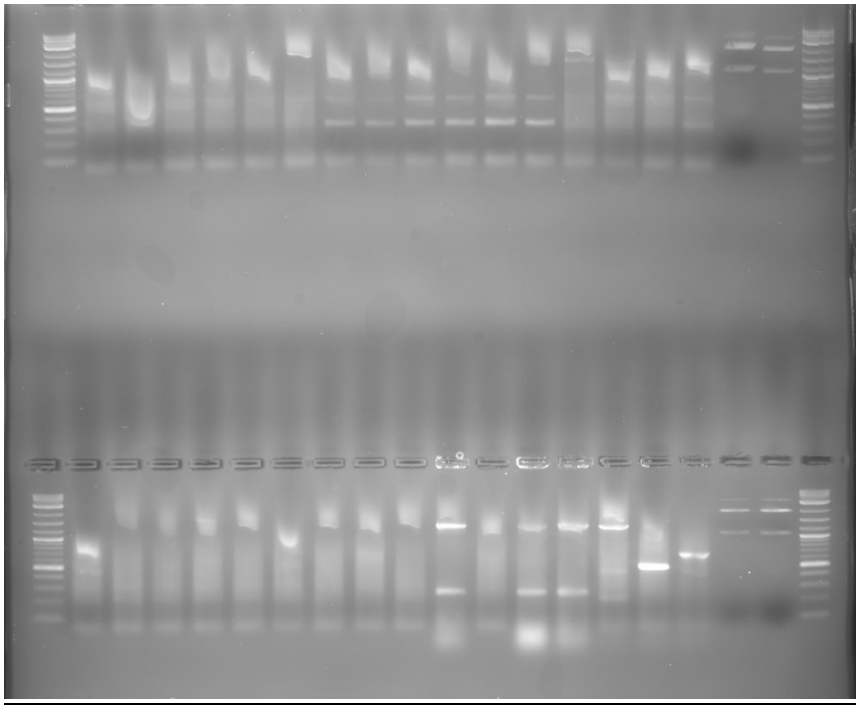
Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	98	5 min	1X
Denature	98	30 sec	35-40X
Anneal	64.6	30 sec	
Extend	72	30 sec	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

PCR 2 – 1 minute Extension Time:

Strip 3 & 4

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	98	5 min	1X
Denature	98	30 sec	35-40X
Anneal	64.6	30 sec	
Extend	72	1 min	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

Agarose Gel Results



Overnight High Fidelity PCR (Phusion)

The following parts were amplified using PCR:

1 - P1 18A - LasR/Ter/Ter - BBa_K574007 (1218)

2 - P2 24B - GFP - BBa_E0240 (1176)

3 - P2 24B - GFP - BBa_E0240 (1176)

4 - P4 1N - RBS - BBa_B0034 (1200)
5 - P4 1N - RBS - BBa_B0034 (1200)

The PCR reaction conditions were as follows:

Reagent	One Reaction	Mastermix
Water	19	133
PCR Mastermix (2X)	25	175
Primer (10uM)	5	35
Subtotal	49 uL	
Template DNA	1 uL	
Total	50 uL	
Number of Reactions	7	

The PCR reactions were separated into two categories based on length of DNA because they required different extension times.

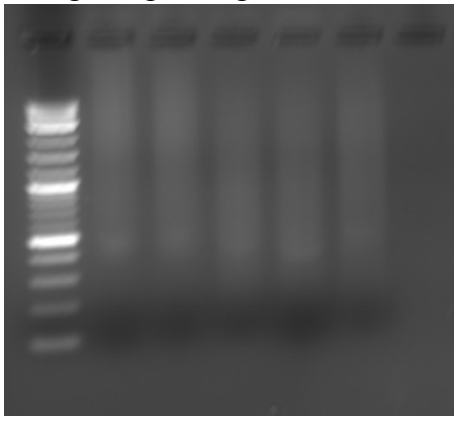
PCR 1 – 1 minute Extension Time:

1 - P1 18A - LasR/Ter/Ter - BBa_K574007 (1218)
2 - P2 24B - GFP - BBa_E0240 (1176)
3 - P2 24B - GFP - BBa_E0240 (1176)
4 - P4 1N - RBS - BBa_B0034 (1200)
5 - P4 1N - RBS - BBa_B0034 (1200)

Stage	Temperature (°C)	Time	Cycles
Enzyme Activation	98	5 min	1X
Denature	98	30 sec	35-40X
Anneal	64.6	30 sec	
Extend	72	1 min	
Final Extension	72	7 min	1X
Hold	4	Hold	1X

<u>Agarose Gel Electrophoresis</u>

The PCR results were analyzed using an agarose gel.



Based on the results of the gel, none of the overnight high-fidelity PCR reactions worked.

Wednesday 10/18

High Fidelity PCR (Phusion)

The following parts were amplified using PCR:

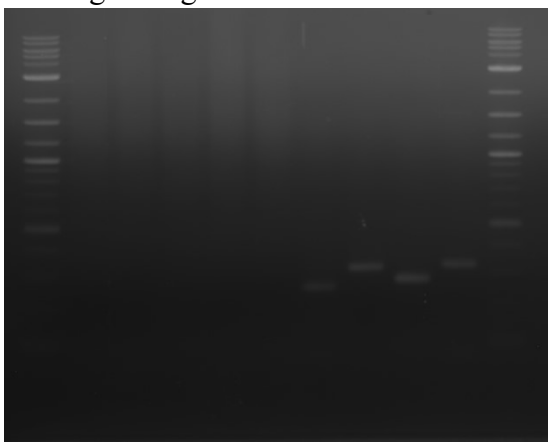
1 - P1 18A - LasR/Ter/Ter - BBa_K574007 (1218)
2 - P2 24B - GFP - BBa_E0240 (1176)
3 - P2 24B - GFP - BBa_E0240 (1176)
4 - P4 1N - RBS - BBa_B0034 (1200)
5 - P4 1N - RBS - BBa_B0034 (1200)
6 - Hok (257)
7 - Sok (225)
8 - RBS + Hok (257)
9 - pCl + RBS + Hok (329)

PCR reaction conditions:

Reagent	One Reaction	Mastermix
Water	19	209
PCR Mastermix (2X)	25	275
Primer (10uM)	5	55
Subtotal	49 uL	
Template DNA	1 uL	
Total	50 uL	
Number of Reactions	11	

Agarose Gel Result:

The PCR reaction was run on an agarose gel to confirm whether it worked.



From the gel, only lanes 6-9 were successful.

Colony PCR

The following parts were amplified using Colony PCR:

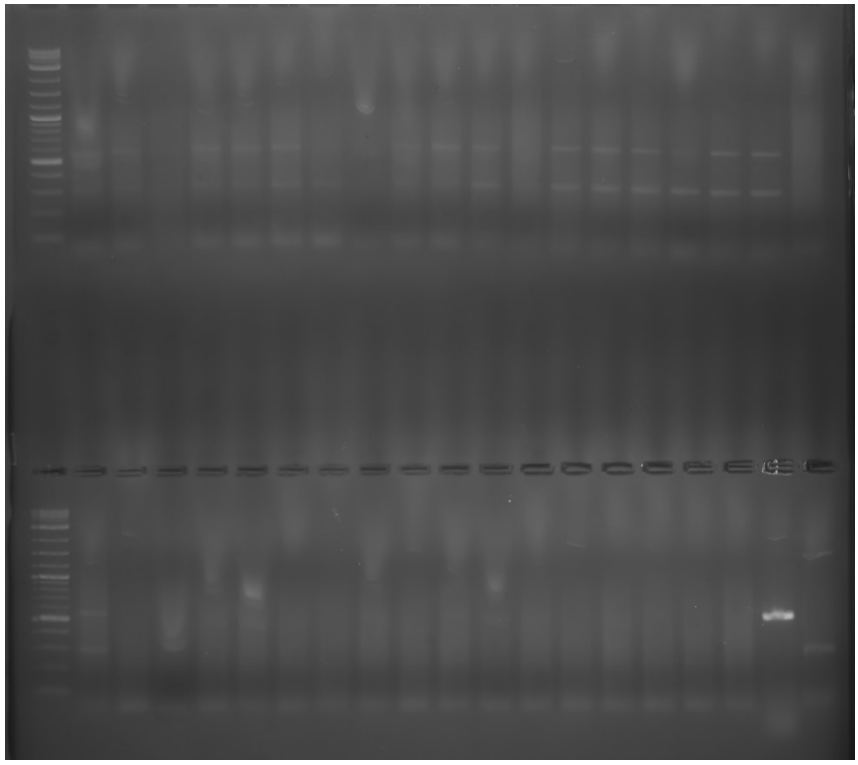
1-10 - pH + RBS + GFP = Kan (2604)
11-20 - pH + RBS + LasI = Kan (2551)
21-29 - RBS + LasR = Kan (1610)

30-38 - RBS + LasI = Kan (1279)
39 - pLac + pLasB = Ch (957)
40 - pH + RBS + GFP = Ch

PCR reaction conditions:

Reagent	One Reaction	Mastermix
Water	6	252
PCR Mastermix (5X)	2	84
Primer (10uM)	1	42
Subtotal	9 uL	
Template DNA	1 uL	
Total	10 uL	
Number of Reactions	42	

Agarose Gel Results



High Fidelity PCR (Phusion)

The following parts were amplified:

1 - P4 1N – 1265
2 - P4 1N – 1265
3 - P4 18G – 1076
4 - P4 18G – 1076
5 - P4 16C – 1180
6 - P4 16C – 1180

PCR Reaction Conditions:

Reagent	One Reaction	Mastermix
Water	19	152
PCR Mastermix (2X)	25	200
Primer (10uM)	5	40
Subtotal	49 uL	
Template DNA	1 uL	
Total	50 uL	
Number of Reactions	8	

Only P4 18G (RBS + GFP) was successful.

Thursday 10/19

Digestions

EcoRI/PstI Digestions:

Reagent	Chloro Linear	Hypoxia + RBS + LasR + Ter + Ter	Hypoxia + RBS + CFP + Ter + Ter	pH + RBS + LasI + Ter + Ter	pLac + pLasB + RBS + CI + Ter + Ter	Hok
DNA (500ng)	20	10	5	10	10	20
10X Buffer 2.1	2.5	2.5	2.5	2.5	2.5	2.5
EcoRI-HF	1	1	1	1	1	1
PstI	1	1	1	1	1	1
Water	0.5	10.5	15.5	10.5	10.5	0.5
Total	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL

Control Digestions:

Reagent	CC100 (Control)	CC100 (Control)
DNA (500ng)	10	10
10X Buffer 2.1	2.5	2.5
EcoRI-HF	1	0
PstI	0	1
Water	11.5	11.5
Total	25 uL	25 uL

XbaI/PstI Digestions:

Reagent	pLac + pLasB + RBS + CI
DNA (PCR)	5
10X Buffer 3.1	2.5
XbaI	1
PstI	1
Water	15.5
Total	25 uL

EcoRI/SpeI Digestions:

Reagent	pLac + pLasB + RBS + CI
DNA (PCR)	10
10X CutSmart	2.5
EcoRI-HF	1
SpeI-HF	1
Water	10.5
Total	25 uL

Transformations

The following parts were transformed into bacteria:

CC100 – Untouched
CC100 – Digested, but not ligated
CC100 – Digested and ligated

Friday 10/20

Predicted Parts

pLasB + RBS + GFP = BBa_K2493016
pLac + pLasB + RBS + GFP = BBa_K2493017
pLac + pLasB + RBS + Cl = BBa_K2493018
Sok = BBa_K2493019
Hok = BBa_K2493020
J23101 + Sok = BBa_K2493021
J23101 + RBS + Hok = BBa_K2493022
pH + Sok = BBa_K2493023
pCl + RBS + Hok = BBa_K2493024

Ligations

Hypoxia [13] + (RBS + LasR) [BBa_K2493002, Ch] = Kan
J23101 [P4 17F = 7] + (RBS + LasR) [BBa_K2493002, Ch] = Kan
pLacI [P3 4G = 5] + (RBS + HokD) [BBa_K2493004, Ch] = Kan
pCl [P4 5L = 8] + (RBS + HokD) [BBa_K2493004, Ch] = Kan
pLacI [P3 4G = 5]+ (RBS + Cl) [BBa_K2493005, Ch] = Kan
J23101 [P4 17F = 7] + (RBS + LasI) [BBa_K2493008, Ch] = Kan
RBS [P4 1N = 10] + Cl [P2 4B = 3]

Product Parts
pLacI + (RBS + GFP)
Hypoxia + RBS + LasR
J23101 + RBS + LasR
pLac + RBS + HokD
pCl + RBS + HokD
pLac + RBS + Cl
J23101 + RBS + LasI

Saturday 10/21

High Fidelity PCR (Phusion)
PCR Conditions:

Reagent	One Reaction	Mastermix
Water	19	209
PCR Mastermix (2X)	25	275
Primer (10uM)	5	55
Subtotal	49 uL	
Template DNA	1 uL	
Total	50 uL	
Number of Reactions	11	

Sunday 10/22

Colony PCR
PCR Conditions:

Reagent	One Reaction	Mastermix
Water	8	360
PCR Mastermix (2X)	10	450
Primer (10uM)	1	45
Subtotal	19 uL	
Template DNA	1 uL	
Total	20 uL	
Number of Reactions	45	

Wednesday 10/25

Colony PCR
PCR Conditions:

Reagent	One Reaction	Mastermix
Water	8	96
PCR Mastermix (2X)	10	120
Primer (10uM)	1	12
Subtotal	19 uL	
Template DNA	1 uL	
Total	20 uL	
Number of Reactions	12	

Friday 10/27

DNA Submission List
The following parts were submitted to the iGEM Parts Registry:
BBa_K2493001 – Hypoxia + RBS + CFP
BBa_K2493002 – RBS + Hok
BBa_K2493003 – pLac + pLasB + RBS + CI
BBa_K2493004 – Sok
BBa_K2493005 – Hok
BBa_K2493006 – pH + RBS + LasI
BBa_K2493007 – Hypoxia + RBS + LasR