August

Made with Benchling

Project: Imperial iGEM 2016 Shared Project

Authors: Akash

Dates: 2016-08-02 to 2016-10-17

TUESDAY, 8/2

Abbreviations:

c: colony
r: replica
e: EcoR1
s: SpeI
p: PstI
x: XbaI

WEDNESDAY, 8/3

Resuspended plasmids from the distribution kit in 10 µl distilled water:

THURSDAY, 8/4

Transformed NEB 5a competent cells with 2 µl of resuspension
Streaked ampicillin agar plates with 20 µl and 500 µl of transformation reaction

Quorum

Resuspended plasmids
from the distribution kit (see above)Transformed NEB 5a competant cells and plated on CAM plates
>luxR S03119
>GFP I13504
>pSB1C3

Table2

	A	B	C	D
1	**DNA**	**Plate**	**Well**	**Antibiotic**
2	LuxR (BBa_503119)	3	5A	CAM
3	GFP (BBa_I13504)	3	17C	CAM
4	araC (BBa_K1321333)	5	12N	CAM
5	Reverse Terminator (B0025)	4	1F	AMP
6	pSB1C3 +RFP	6	12P	CAM

STAR

Transformation
PSB1C3 with RFP in NEB 5a cells

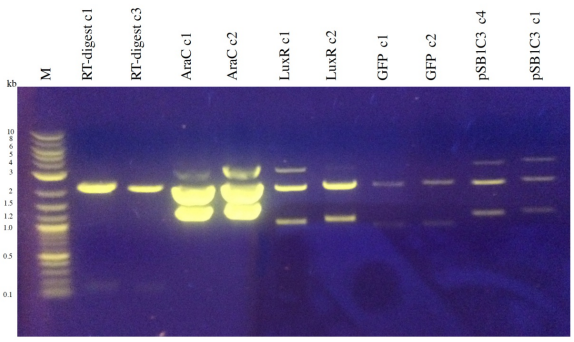
FRIDAY, 8/5

Quorum

overnight cultures
(4x for each transformation)
>LuxR
>GFP
>pSB1C3

Gel of plasmids:

3rd August.png



Growth

overnight cultures
4 overnight cultures from 20ul plates (4x replicates of each transformation)
>psB1A2 + pBAD c1-4
> reverse terminator c1-4

SATURDAY, 8/6

Growth

Miniprep
psB1A2 + araC + pBAD (c1-2) and reverse terminator plasmids (c1 and c3R), only 2 clones of each trnasformation
Remaining 2 clones were centrifuged under 250 µl of P1 buffer had already been added. Excess liquid was discarded and pellets were stored in the cold room.
Quantified DNA samples

Table1

	A	B
1	**Sample**	**Concentration (ng/µl)**
2	araC c1	140
3	araC c2	200
4	b0025 c1	50
5	b0025 c3	82.5

Test digest

Table3

	A	B	C
1	DNA	Insert size (bp)	Plasmid size (bp)
2	LuxR	998	
3	GFP	875	
4	araC	1210	
5	Reverse terminator	129	
6	pSB1C3		2070

MONDAY, 8/8

STAR

Transformation
PSB3K3 and F2620

Overnight culture
S03119, I13504, K1321333, B0025, pSB1C3
Reverse Terminator (c1)
F2620 (luxR) (c1)
araC (c3)
pSB1C3 (c1)

Growth

overnight cultures:

- Reverse terminator (b0025) c1
- araC+pBAD (K1321333) c3
- pSB1C3 c1

TUESDAY, 8/9

STAR

Overnight cultures
for pSB1C3, pSB3K3, F2620, k1321333, pSB1A2

WEDNESDAY, 8/10

Quorum

PCR

Table4

	A	B	C
1	DNA	Primers	Function
2	F2620	VF2 and VR	Amplify insert
3	F2620	AA001 and AA002	Remove pTET, add J23101 promoter
4			

Overnight culture for GFP (BBa_I13504)

STAR

Miniprep
pSB3K3, pSB1C3, F2026, k231333, B0025

Table11

	A	B
1	Sample	**Concentration (ng/µl)**
2	pSB3K3 c1	93.5
3	pSB3K3 c2	116.5
4	K1321333 R1	419.1
5	K1321333 R2	453.1
6	F12620 R1	100.7
7	F12620 R2	11.3
8	B0025 R1	149.8
9	pSB1C3 R1	184.3
10	pSB1C3 R2	204.6

EcoR1 + Spe1 restriction digest

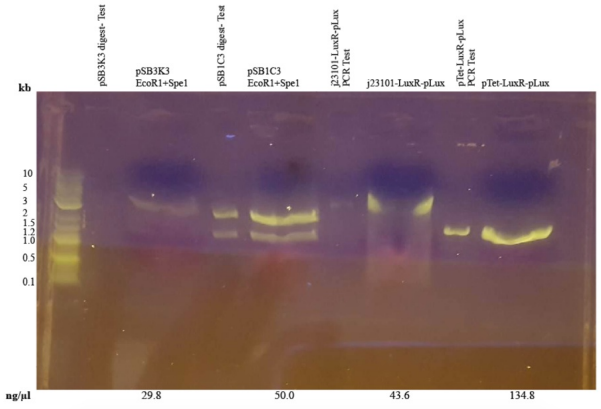
THURSDAY, 8/11

Quorum

Gel

F2620 PCR products (with primers AA and VF2/VR), and pSB1C3, psb3K3.

11th august gel photo.png



Gel extraction and nanodropping:

Table5

	A	B	C
1	Sample	Primers	Concentration (ng/ul)
2	F2620	AA01, AA02	43.6
3	F2620	VR, VF2	134.8
4	psB1C3		50
5	psB3K3		29.8
6	AraC		35.5
7	B0025		68.5
8	GFP - I13504 (from miniprep)		108.9
9			

Circularization

Phosphorylation of F2620 (amplified by AA01 and AA02)(pAA001)

-100ng DNA

-2 ul buffer T4 ligase

1 uL PnK

up to 20 ul with ddH2O

Ligation of pAA001

FRIDAY, 8/12

Miniprep, gel extraction and purification

pSB1C3, pSB3K3

MONDAY, 8/15

Quorum

RhlR biobrick assembly:

Transferred RhlR from psB1A2 to psB1C3
up to 50 ul H2O
5 ul NEB buffer 2.1
2000 ng DNA
1 ul Spe1 and EcoR1
Leave at 31C overnight
Heat to 80C for 20 minutes

STAR

Overnight culture

E.Coli BL21, DHIOB and Yeast 4741

Transformation

STAR response (SFGFP) into NEB 5a

Growth

Restriction digest of:

- Reverse terminator c1 with SpeI and PstI
- araC+pBAD c3 with XbaI and PstI

Gel electrophoresis run of digestion product
Lanes:

Table15

	A	B	C	D	E	F	G
1	ladder	x	cat	x	AraC, X/p	x	RT, S/P

Miniprep

Table16

	A	B
1	Sample	Concentration (ng/ul)
2	cat	153.8
3	AraC	58.5
4	b0025	68.8

Ligation

Ligated digested b0025 with araC insert, transformed into cells

TUESDAY, 8/16

Quorum

RhlR dirty ligation:

Digested psB1C3 (50ng/ul) 1ul (E/S digest)
100ng digested insert (E/S) digest
1ul ligase
dH2O up to 20 ul

Incubated at room temperature for 20 minutes

Transformation

Transformed Neb10 with circularised pAA001

Sequencing

Used the mix2seq kit

Table6

	A	B	C
1	Sample	Primter	Sequence ID
2	araC	VF2	FRI2893530
3	RT	VF2	FRI2893538
4	F2620	VF2	FRI2893522
5	I13504	VF2	FRI2893514

Transformations

Table7

	A	B
1	Sample	Cells/ul
2	RhlR-1C3	50
3	RhlI	33.3
4	CinI	33.3
5	LuxI	33.3
6	pBAD AraC	50

Overnight Cultures

pAA001 4 x
4 colonies of LuxR (F2620AA)

STAR

Overnight Cultures

4 colonies from 10ul plate of SFGFP

Growth

Transormations od synthesised and ligated CinI, RhlI, LuxI (10, 100, 900 ul) and RhlR (100, 900 ul)

WEDNESDAY, 8/17

Quorum

Miniprep

pAA001, 2 cultures miniprepped

Overnight cultures (4x)

LuxI
CinI
RhlI
B0025+ pBAD (Growth)

Sequencing

pAA001 c2-
pAA001 c4-
RT+pBAD c1
RT+pBAD c3
Rhlr-1C3 c3- CORRECT
Rhlr-1C3 c4- CORRECT

STAR

Miniprep

SFGFP 3 and 4 (pellets of 1 and 2 stored in freezer)

Transformation

STAR and AntiSTAR (10, 100, 900(100) ul) plates

THURSDAY, 8/18

Quorum

Miniprep, Nanodrop, Test Digest

Table8

	A	B	C
1	Name	DNA ng/ul	Lane in test dig
2	sfGFP 4	408.5	1
3	sfGFP 3	444.2	2
4	pAA001 2	169.9	3
5	pAA001 4	380	4
6	pBAD 1	407.1	5
7	pBAD 3	478.5	6
8	pBAD 4	445.6	
9	luxI 1	589.3	7
10	luxI 2	590.7	8
11	luxI 3	538.1	
12	luxI 4	153.4	
13	CinI 1	203.8	9
14	CinI 4	217.1	10
15	RhlI 1	580.7	11
16	RhlI 3	221.2	12
17	RhlR-1C3 1	407.5	
18	RhlR-1C3 3	297.3	13
19	RhlR-1C3 4	199.2	14

STAR

Overnight culture
STAR and antiSTAR construct

Nanodrop
SFGF

Table12

	A	B
1	Sample	DNA ng/ul
2	SFGFP c3	444.2
3	SFGFP c4	408.5

FRIDAY, 8/19

STAR

Ligation
SFGFP (c4) - digested with EcoR1-HF and Spe1 - and PSB3K3 + PSB1C3

Miniprep
STAR and antiSTAR
- nanodrop and test digest
- restriction digest
- ligate over the weekend

Nanodrop:

Table13

	A	B
1	**Sample**	**DNA ng/ul**
2	STAR 1	349.9
3	STAR 2	390.6
4	A.STAR 1	167.1
5	A.STAR 2	213.6

Gel extraction
SFGFP (4) : 43.7 ng/ul

Ligation
SFGFP ligation with PSB3K3 and PSB1C3

Growth

Restriction digest
>SFGRP c3, X/P
>pBAD+RT c3, S/P
>cut amplicon with X/P

Gel electrophoresis

Table17

	A	B	C	D	E	F	G
1	ladder	x	pBAD+RT	x	sfGFP	x	cat

MONDAY, 8/22

Quorum

Assembly
RhlR digest+ pSB3K3 digested
Transformed into Turbo cells

Sequencing

pAA001 with VR primer, as already sequenced with VF- C1 CORRECT

Overnight culture
RhlR+pSB1C3 c3, c4

STAR

Transformation
ligated constructs into turbo cells and grown on plates overnight
- AntiSTAR+pSB1C3
- STAR+pSB1C3
- SFGFP+pSB1C3
- SFGFP+pSB3K3

Growth

Nanodrop of digests

Table18

	A	B
1	Sample	Concentration ng/ul
2	pBAD+RT	98.7
3	cat	76.1
4	sfGFP	26.3

Ligation
pBAD+RT ligated with cat and sfGFP

TUESDAY, 8/23

Quorum

Glycerol Stock
20% glycerol stalk of RhlR+pSB1C3 c2, c4, and pAA001 c2

Day Culture
RhlR+pSB3K3 ligation

AHL Experiment: C4
- 100 mM C4 AHL stock prepared
- Activation range of RhlR: 100uM C4, so must dilute 1,000x
- Plate reader used to record OD and fluorescence over 720 minutes
- Recorded fluoresnce of the RhlR+pRhl+GFP construct in pSB1C3 backbone, with pAA001 as control

Samples preparation
-dilute overnight cultures 100x, incubate at 37 degrees for 2 hours
- Induced: 50 uL cells + 2uL AHL + 148uL LB + .2 uL Cam
- Non-Induced: 50 uL cells + 150uL LB + .2 uL Cam

Organization of plate: I before construct name= induced by AHL

Table9

	A	B	C	D	E	F	G	H	I
1	x	x	x	pAA001	pAA001	pAA001	lpAA001	lpAA001	lpAA001
2	x	x	x	RhlR3	RhlR3	RhlR3	IRhlR3	IRhlR3	IRhlR3
3	x	x	x	RhlR4	RhlR4	RhlR4	IRhlR4	IRhlR4	IRhlR4
4	x	x	x	LB	LB	LB	ILB	ILB	ILB

Nanodrop

Table10

	A	B
1	Construct	ng/uL
2	RhlR+3k3 c1	97
3	RhlR+3k3 c2	84
4	sfGFP+1C3 1	118.3
5	sfGFP+1C3 1	195.5
6	sfGFP+1C3 2	110.5
7	STAR+1C3 2	90.8
8	STAR+1C3 3	123.2
9	A.STAR+1C3 3	86
10	A.STAR+1C3 4	75.7

All sent for sequencing

STAR

Overnight cultures

of colonies picked from previous days transformations

Transformations

of empty vectors (pSB1C3 and pSB3K3) into Turbo cells

Growth

Transformation and overnight cultures

pBAD+RT ligated with cat and sfGFP

WEDNESDAY, 8/24

Quorum

Assembly

pAA001+I13504=pAA002

-pAA001 digested with S and P

-I13504 digested with X and P

-ligated

Growth

Miniprep and Nanodrop

pBAD+RT ligated with cat and sfGFP overnight cultures

Table19

	A	B
1	Sample	Concentration ng/ul
2	c1	121.2
3	c2	132.9
4	c3	137.6
5	c4	101.4

Sequencing

Catc1 and sfGFPc3

Restriction digest

pBAD+RT+sfGFP c3,c1 digested by E/S

I13504, X/P

Gel electrophoresis

Table20

	A	B	C	D	E	F
1	ladder	x	pBAD+RT+cat c1	x	I13504 X/P	

THURSDAY, 8/25

Quorum

Transformation

pAA001+I13504=pAA002

-pAA002 transformed into Turbo cells

STAR

Restriction Digests

- pSB1C3 (4)
- P3K3 (1)
- P1C3 (3)
- P3K3 (3)

Growth

Miniprep and nanodrop

Table21

	A	B
1	Sample	Concentration ng/ul
2	GFP c1	121.9
3	GFP c2	190.6
4	pBAD+RT+cat c2	207.1
5	pBAD+RT+cat c3	56.2

Sequencing

GFP c2 and pBAD+RT+CATc2

Restriction Digest

pBAD+RT+CATc1, E/S
pBAD+RT+CATc2, E/S
pBAD+RT+GFP c4, E/S
pBAD+RT+CATc4, E/S
I13504, S/P
I13504, X/P

Ligation

Ligated pBAD+RT digest with I13504

Ligated I13504 c2(X/P) and cat (X/P) with pBAD+araC+RT (S/P)

Sequencing results suggest cat did not insert into pBAD+araC+RT, repeat ligation

PCR

Repeat PCR of cat from pSB1C3

Gel electrophoresis:

Table22

	A	B	C	D	E
1	ladder	x	GFP X/P digest	x	cat PCR product

FRIDAY, 8/26

Quorum

Day cultures

pAA002 put in 37 for day culture
spun down for future miniprep

Sequencing

pSB3K3+RhlR C1 and C2 sent for sequencing with primers VF2 and VR

C1: WRONG

C2: Correct

STAR

Miniprep and test digest

no STAR/a.STAR seen

Nanodrop

Table14

	A	B
1	**Sample**	**DNA ng/ul**
2	STAR c3	353.7
3	STAR c4	479.4
4	SFGFP c1	506.2
5	SFGFP c2	385.7
6	A.STAR c1	353.5
7	A.STAR c4	222.5
8	SFGFP-pSB3K3 c1	180.9
9	SFGFP-pSB3K3 c2	375.9
10	SFGFP-pSB3K3 c3	623.4
11	SFGFP-pSB1C3 c1	146.5
12	A.STAR-pSB1C3 c1	231.3
13	A.STAR-pSB1C3 c2	239
14	A.STAR-pSB1C3 c3	346.7
15	A.STAR-pSB1C3 c4	279.1
16	STAR-pSB1C3 c1	248.7
17	STAR-pSB1C3 c2	180.1
18	STAR-pSB1C3 c3	350.9
19	STAR-pSB1C3 c4	153.3
20		

pSB1C3 amd pSB3K3 (empty vectors) - major digest (deactivated)

Growth

LIgation

- Sequencing results suggest cat did not insert into pBAD+araC+RT vector. Therefore repeated ligation and PCR
- I13504 c2 X/P digest and cat X/P digest with pBAD+araC+RT S/P digest

TUESDAY, 8/30

Quorum

Day Cultures

Day cultures of RhlR-3K3 prepared

Miniprep

pAA002 c1
pAA002 c2
RhlR-3k3 c2 r1
RhlR-3k3 c2 r2

Table24

	A	B
1	Sample	Concentration ng/ul
2	pAA002 c1	108.1
3	pAA002 c2	234.2
4	RhlR-3K3 c2 r1	172.4
5	RhlR-3K3 c2 r2	180.3

Sequencing

pAA002 (colonies 1 and 2) sent for sequencing with VF2 and VR primers

Glycerol stock

RhlR-3K3 c2 r1, c2 r2

STAR

Digestion and gel extraction

- A.STAR 1
- A.STAR 4
- pSB1C3

Nanodrop

Table29

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	A.STAR 1	17.9
3	A.STAR 4	10.5
4	p1C3	51.5
5	p3K3	76.9

Growth

Nanodrop

Table23

	A	B
1	Sample	Concentration ng/ul
2	pBAD+GFP 1	402
3	pBAD+ GFP 1a	828.4
4	pBAD+CAT 2A	591.2
5	CAT amplicon	71
6	GFP (X/P)	84.7

Ligation

Transformations from last Friday were contaminated/unsuccessful, repeating transformations with ligations from August 25th

Sequencing

pBAD+GFP 1
pBAD+GFP 1a
pBAD +CAT 2a

Each sample sent with VF2 and VR primers

Colony PCR

Performed colony PCR on *E. coli* (Turbo)
25ul RodTaq
1 uL DNA (1 colony in 50 uL, boiled at 95 for a minute)
2 ul Sho1 and Sho2 primers
22ul H20

WEDNESDAY, 8/31

Quorum

Test digest

pAA002 colonies 1 and 2 digested by e/s

Gel electrophoresis

Table25

	A	B	C	D	E
1	ladder	x	c1	x	c2

Transformation
Transformed RhlR-3K3 and RhlR-1C3 into MG16 cells

Overnight Cultures
pAA001 from glycerol stock, 4 replica c2

STAR

Ligation
sfGFP-pSB3K3
STAR-psB1C3

THURSDAY, 9/1

Quorum

Miniprep
pAA001 r1 and r2 miniprepped, r3 and r4 spun down

Table26

	A	B
1	Samples	Concentration ng/ul
2	pAA001 c1r1	159.5
3	pAA001 c1r2	131.9

Day cultures
3x colonies from pSB1C3 RhlR 900 uL plate (Mg-R cells)

Assembly
I13504 (x/p) gel extracted and digested

Ligation
digestes pAA001 and I13504, transformed into Turbo cells

Transformation
transformation of empty vectors (pSB1C3 and pSB3K3) into MG16 cells (transformation failed!!)

Experiments
RhlR - pSB1C3 colony 2 (glycerol stocks made and stored in -80 freezer)
tested with AHLs (concs: 0, 1nM, 10nM, 100nM, 1uM, 10uM, 100uM, 1mM)

overnight cultures of RhlR-pSB3K3 for tomorrows experiments
data analysed using Prism

STAR

Daytime cultures
x12 (4 for each construct)

Miniprep
x11 (sfGFP-p3K3(2)) had no colonies on replica plate

Growth

Miniprep

Table33

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	pBAD + GFP c1	336.7
3	pBAD + GFP c2	373.8
4	GP2 c1	321.8
5	GP2 c3	312.9

Sequencing
- pBAD+GFP c2 - CORRECT

FRIDAY, 9/2

Quorum

Nanodrop
Diluted LasR

Table27

	A	B
1	**Sample**	**Conc (ng/ul)**
2	LasR	1459.8

Grew cells
from RhlR-pSB3K3 overnight cultures

Digestion
LasR-pSB1A2

Ligation
LasR into pSB1C3 and pSB3K3

Experiments
RhlR-pSB3K3 colony 3 (glycerol stocks made and stored in -80 freezer)
tested with AHLs (concs: 0, 1nM, 10nM, 100nM, 1uM, 10uM, 100uM, 1mM)

Miniprep
Spun down pAA002 day cultures in preparation for mini-prep on monday

STAR

Nanodrop

Table30

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	sfGFP p3k3 1	282.1
3	sfGFP p3k3 3	69.5
4	sfGFP p3k3 4	150.5
5	STAR p1c3 1	218
6	STAR p1c3 2	120.9
7	STAR p1c3 3	165.4
8	STAR p1c3 4	169
9	A.STAR p1c3 1	138
10	A.STAR p1c3 2	238.7
11	A.STAR p1c3 3	174.3
12	A.STAR p1c3 4	142.6

Sequencing
- sfGFP 3 +4
- STAR 3 + 4
- A.STAR 3 + 4

Growth

Dirty ligation and gel extraction
GP2 with pSB1C3 (X/P)

Pelleted
overnight cultures from previous day and stored in freezer

Test digest
GP2 c3 (after c1 failed digest)

MONDAY, 9/5

Quorum

Miniprep

Miniprepped pAA002 c1 and c2
Nanodropped: c1: 91.2 ng/ul, c2: 42.4 ng/ul

Test digest
pAA002 c1 and c2, analyzed by gel electrophoresis
Both failed, indicating failed ligation

Transformation
lasR-3K3
lasR-1C3

STAR

Digest A.STAR with EcoR1 and XBal
Ligate to STAR (E + S)
Transform into Turbo
Co-transform STAR-p1C3 and sfGFP-p3K3

Growth

Performed arabinose induction experiments in plate reader

Miniprep
pBAD c1-c4

Nanodrop

Table35

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	pBAD + b0025 c1	42.7
3	pBAD + b0025 c2	146.8
4	pBAD + b0025 c3	103.4
5	pBAD + b0025 c4	140.4

TUESDAY, 9/6

Quorum

Day cultures
lasR-3K3
lasR-1C3

Test digest
pAA001 r1 from stock solution

Transformed
pAA002 ligation, pAA002 colony 2 DNA

Miniprep and nanodrop

Table28

	A	B
1	**Samples**	**Concentration (ng/ul)**
2	lasR/pSB3K3 c3	
3	lasR/pSB3K3 c4	
4	lasR/pSB31C3 c3	
5	lasR/pSB31C3 c4	

STAR

Day cultures

- STAR-p1C3 + sfGFP-p3K3
- Ligated STAR + A.STAR

Experiment

Investigate RNA logic activation by STAR

- Sample 1: ts-sfGFP/pSB3K3 + STAR/pSB1C3 [Top10]
- Sample 2: ts-sfGFP/pSB3K3 + J23119/pSB1C3 [Top10]
- Control cell autofluoresence J23119/pSB1C3 + pSB3K3 (empty) [Top10]

Growth

PCR

of cat from pSB1C3 template with Fprbs + cat and RPcat primers (cat PCR failed, repeating with gel extracted cat PCR amplicon from 30/8)

Gel

Run gel for PCRs of cat, GP0.4 and LeuB (got band in gel but too much template DNA used, repeat with more dilute DNA)

Performed araBAD characterisation experiments

pSH001 (Neb5a) [araBAD-GFP/pSB1A2]

Nanodrop

Table36

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	pSB1C3 X/P	74.9
3	LeuB	90.6
4	gp0.4	157.4

WEDNESDAY, 9/7

Quorum

Test Digest

LasR-pSB1C3 colonies 3 and 4, LasR-pSB3K3 colonies 3 and 4
Gel visualisation was faint, repeat tomorrow

Transformation

In OneShot TOP10 cells:
- RhlR/pSB3k3
- RhlR/pSB1C3

STAR

Test digest

- STAR and A.STAR construct (mistakenly put dye into miniprep DNA)
- miniprep colony 1 and 4 and test digest again
- got plasmid backbone band but no insert (testing again with company backbone)

Growth

Transformation

In OneShot TOP10 cells:
- pBAD+GFP

PCR

- Repeated PCR round two for cat with diluted template and for LeuB

Restriction Digest

Heat inactivation, ligation and transformation for cat(X/P) digest
Restriction digest of gp0.4 with X/P

Nanodrop

Table37

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	CAT 2A	99
3	CAT 2B	101.5
4	LeuB	127.5
5	Gp2	75.9

Gel

(all correct)

THURSDAY, 9/8

Quorum

Test Digest

LasR-pSB1C3 colonies 3 and 4, LasR-pSB3K3 colonies 3 and 4 using E and S
Gel visualisation successful
Lane 1: ladder
Lane 2: LasR-1C3 colony 3
Lane 3: LasR-1C3 colony 4
Lane 4: LasR-3K3 colony 3
Lane 5: LasR-3K3 colony 4

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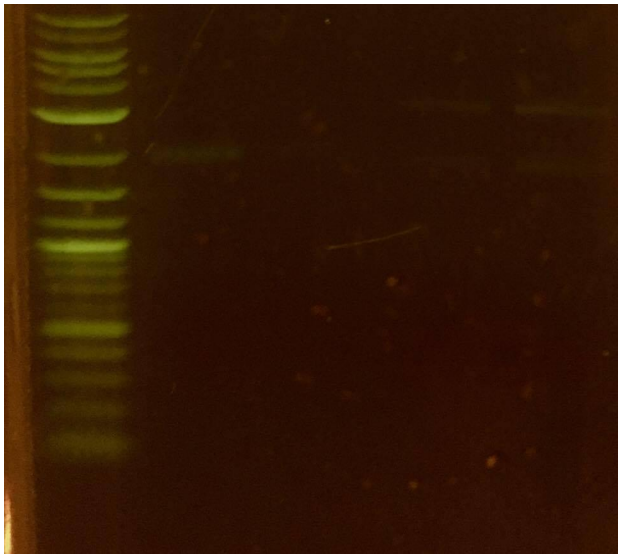

Sequencing:
Sent LasR3K3 colony 3 and LasR1C3 colony 3 CORRECT

Miniprep
pAA001 and pAA002 transformations

Table31

	A	B
1	pAA001 c2	157.6
2	pAA001 c3	153.2
3	pAA001 c4	142.7
4	pAA001 c1	124.6
5	pAA001 c2	202.4
6	pAA001 c3	105.4
7	pAA001 c4	138.4

Test Digest pAA001 and pAA002
Single digest with EcoRI, double with E/S
Gel visualisation FAILED
lane 1: ladder
lane 2:pAA001 c2 -Eco
lane 3: pAA001 c3-Eco

lane 4:pAA001 c2-Eco/Spe
lane 5:pAA001 c3-Eco/Spe
lane 6: pAA002 c1 Eco/Spe
lane 7: pAA002 c2 Eco/Spe

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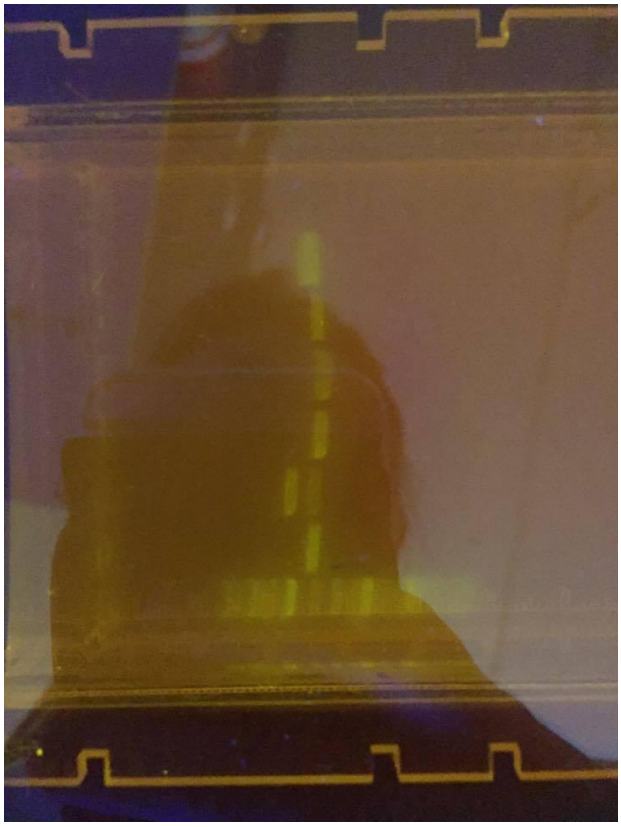

Overnight cultures
2x colonies from RhlR/pSB3K3 + RhlR/pSB1C3 (in TOP10 cells)

STAR

Digestion
- A.STAR-pSB1C3 [EcoR1 + Xba1]
- STAR-company vector [EcoR1 + Spe1]

Ligation
Vector: a.STAR-p1C3
Insert: STAR

Gel extraction
- STAR and a.STAR and stored in freezer

Transformation
into Turbo cells with a.STAR-STAR ligated construct

Growth

PCR purification and gel extraction
pBAD + b0025

Nanodrop

Table38

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	Cat 2A (X/P)	118.2
3	Cat 2B (X/P)	114.3
4	Leu B (X/P)	148.8
5	GP 0.4 (X/P)	98.4
6	GP2 (X/P)	74.3

Transformation and day cultures

Into TOP10 cells:
- Cat2A
- Cat2B
- LeuB
- gp0.4
- gp2

Performed arabinose GFP induction in TOP10 characterisation experiments

FRIDAY, 9/9

Quorum

Transformation
In OneShot TOP10 cells:
- LasR/pSB3k3
- LasR/pSB1c3
(left to grow on bench over weekend)

Glycerol Stocks
- RhlR/pSB3K3 + RhlR/pSB1C3

Daytime culture and miniprep
of STAR-a.STAR constructs
test digest (both single and double) with EcoR1 and Pst1

STAR

Day cultures and miniprep
of STAR/A.STAR ligated constructs

Test digest
with both single and double digest (EcoR1 + PstI)

Gel
revealed that ligation did not work (single digest band too high up - too large)

Growth Experiments

Glycerol stocks
TOP10, DH10, MG65, Turbo, S.Cerevisiae, Pichia, B.Subtilis

SUNDAY, 9/11

Quorum

Daytime Cultures
2x colonies from LasR/pSB3K3 + LasR/pSB1C3 (in TOP10 cells)
THROWN AWAY
Repeated as overnight cultures!! (N.B. no CAM plates left, so 1c3 replicas on stefans plate!!)
All four colonies from ORIGINAL pAA001 replica plate (labelled F2620AA)

MONDAY, 9/12

Quorum

Miniprep
C1: 78.2 ng/ul
C2: 74.8
C3: 47.0
C4: 58.5

Test digests
Eco+Pst and Eco for each colony C1-C4

Gel visualisation: ALL APPEAR CORRECT EXCEPT C2
lane 1: ladder
lane 2:pAA001 c1 -Eco
lane 3: pAA001 c2-Eco
lane 4:pAA001 c3-Eco
lane 5:pAA001 c4-Eco
lane 6: pAA001 c1 Eco/Pst
lane 7: pAA001 c2 Eco/Pst
lane 8: pAA001 c3 Eco/Pst
lane 9: pAA001 c4 Eco/Pst

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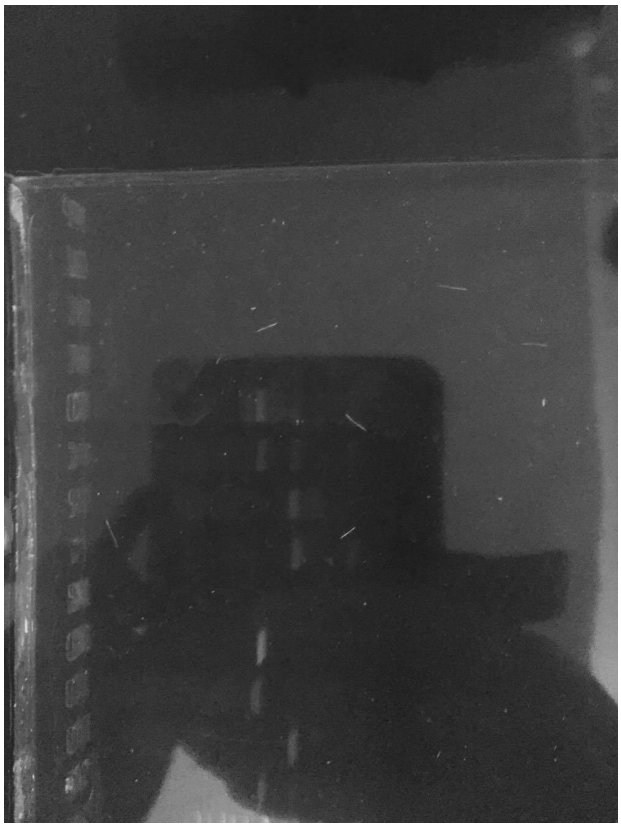


STAR

Overweekend plates

of SFGFP+J23119 and PSB3K3+J23119 (fungal infections)

Overnight cultures

of above, and SFGFP-STAR

Growth

Miniprep

CAT 2A c1 + c2

CAT 2B c1 + c2

LeuB c2 + c3

gp0.4 c2 + c3

Restriction digest

pSB1C3 with X/P

Nanodrop

Table39

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	Cat2B c1	87.1
3	Cat2B c2	95.5
4	Cat2A c1	100.3
5	Cat2A c2	85.5
6	gp0.4 c2	68.2
7	gp0.4 c3	41.4
8	leuB c2	40.5
9	leuB c3	70.6

Sequencing
pBAD+b0025

Glycerol stocks
pBAD+b0025

Growth Experiments

Overnight cultures
B. Subtilis (168 and WB 800N)

TUESDAY, 9/13

STAR

Overnight culture
of pSB3K3 + J23119 (did not grow)

Growth

Miniprep
of overnight pSB1C3 cultures

Nanodrop

Table40

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	pSB1C3 c1	183.9
3	pSB1C3 c2	178.7
4	pSB1C3 c3	172.5
5	pSB1C3 c4	185.8

Restriction digest
of pSB1C3 c4 with EcoR1, XbaI and PstI

Growth Experiment

Overnight culture
Pichia (X33)

WEDNESDAY, 9/14

Quorum

Sequencing
pAA001 C1 and C4 sent for sequencing with primer VF2
Both Correct

Growth

Restriction digest
of pBAD+b0025 with S/P

Ligation
repeated from 14/9 of pBAD+b0025 with CAT, LeuB, gp0.4, gp2

Glycerol stocks
made of empty pBAD+araC+B0025 vector (c3) and araC vector with GFP (c2)

Overnight culture
empty pBAD vector (c3)

Miniprep
pBAD vector

Nanodrop

Table41

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	pBAD+araC+B0025 c3	322.6

Digestion
of pBAD+B0025 with S/P

Gel extraction and nanodrop
of pBAD+araC+B0025

Table42

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	pBAD+araC+B0025	41.5

Ligation
pBAD vector and target genes

Miniprep
of day cultures (3x gp0.4 cultures did not work)

THURSDAY, 9/15

Quorum

Ligation
digested PAA001 colony 1 with S/P
ligated with I13504 (X/P)

FRIDAY, 9/16

Quorum

Transformation
of pAA002 ligation

MONDAY, 9/19

Quorum

Overnight cultures for characterisation experiments
lasR/pSB1C3 (colony 1)
lasR/pSB3K3 (colony 1)
RhlR/pSB1C3 (colony 2)

Day Culture
pAA002

Growth

Nanodrop

Table43

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	CAT2A c2	165.9
3	CAT2A c4	147.6
4	LeuB c2	125.6
5	LeuB c4	159.7
6	Gp2 c1	76.8
7	Gp2 c2	117.1
8	Gp0.4 c2	106.5

Test digest
of Gp0.4 c2

Sequencing
- CAT2A c2
- LeuB c2
- LeuB c4
- Gp2 c1
- Gp2 c2
- Gp0.4 c2

PCR
Cat, amplified from pSB1C3

Restriction digest
- Of cat amplicon and pSB1C3 c1 with X/P
- Gel run of CAT 2A amplicon

TUESDAY, 9/20

Quorum

Miniprep
pAA002

Characterisation experiments
Performed cross talk experiments on RhlR/pSB1C3

Gel
pAA002: c1, c2, c3, c4

Digestion
pAA001 (S/P)

STAR

Overnight culture
of STAR-pSB1C3 and A.STAR-pSB1C3

WEDNESDAY, 9/21

Quorum

Characterisation experiments
Performed cross talk experiments on lasR/pSB3K3 and lasR/pSB1C3

Growth

Gel extraction
of cat2 X/P and pSB1C3 X/P

Ligation
- B0025 + pBAD S/P
    - gp0.4 (x/p)
    - cat 2A (x/p)
    - cat 2B (x/p)
- pSB1C3 X/P
    - cat 2A (x/p)
    - cat 2B (x/p)
    - leuB (x/p)
    - gp0.4 x/p
    - gp2 x/p

Transformation
In Turbo:
- Cat 2B
- GP0.4
- CAT 2B
- LeuB
- GP0.4
- GP2

In Top10:
- GP2 + araBAD

STAR

Miniprep
of STAR-pSB1C3 and A.STAR-pSB1C3

Restriction digest, Gel, Gel extraction + Nanodrop

Table45

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	STAR/pSB1C3 (E/S)	21.4
3	A.STAR/pSB1C3 (E/X)	80.2

Ligation
of above using T4 DNA ligase buffer

Transformation
of above into Turbo cells

THURSDAY, 9/22

Quorum

Transformation
pAA002 and cinR/pSB1C3 into Turbo cells

Day culture + PCR
LasR/pSB1c3 and RhlR/pSB1C3

STAR

Nanodrop

Table46

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	STAR 1	73.9
3	A.STAR 1	74.1

Restriction digest
STAR (E+S)
A.STAR (E+S)
A.STAR (E+X)

Growth

Nanodrop

Table44

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	pBAD + cat 2B c1	105.6
3	pBAD + cat 2B c2	145.9
4	pBAD + cat 2B c3	71.5
5	pBAD + cat 2B c4	94.6
6	pBAD + gp0.4 c1	130.6
7	pBAD + gp0.4 c2	108.6
8	pBAD + gp0.4 c3	28.4
9	pBAD + gp0.4 c4	10.5
10	pSB1C3 + cat c1	121.5
11	pSB1C3 + cat c2	166.1
12	pSB1C3 + cat c3	165.9
13	pSB1C3 + LeuB c1	103
14	pSB1C3 + LeuB c2	120.6
15	pSB1C3 + LeuB c4	48.3
16	pSB1C3 + gp0.4 c1	102.2
17	pSB1C3 + gp0.4 c2	84.4
18	pSB1C3 + gp0.4 c3	73.9
19	pSB1C3 + gp0.4 c4	79.6
20	pSB1C3 + gp2 c2	16.3

MONDAY, 9/26

Quorum

Miniprep

cinR/pSB1C3.pAA002 (colony 1-4)

Test Digest

cinR (colony 3 + 4)

pAA002 (colony 1 + 3)

STAR

PCR, run on gel, gel extraction + nanodrop

Table47

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	STAR	37.9
3	A.STAR	35.2

SpeI digestion
PCR purification
PNK phosphorylation

Growth

Repeat of test digests from 22/9

TUESDAY, 9/27

Quorum

Sequencing
cinR colony 1

Gel
Of Las and Rhl PCR products

Test Digest
- CinI/X-P
- LuxI/X-P (J23111)
- RhlI/X-P (J23105)
- RhlI/X-P (J23100)
- RhlI/X-S (J23100)
- RhlI/X-S (J23111)

(All loaded on gel)

Nanodrop

Table32

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	LuxI J23100 c1	249.4
3	LuxI J23100 c2	269.9
4	LuxI J23100 c3	209.2
5	LuxI J23111 c1	228.5
6	LuxI J23111 c3	169.9
7	LuxI J23105 c2	166.4
8	RhlI J23100 c1	280.5
9	RhlI J23100 c2	273
10	RhlI J23100 c3	233.9
11	RhlI J23111 c1	374
12	RhlI J23111 c2	210
13	RhlI J23105 c1	274.6
14	RhlI J23105 c2	228.6
15	CinI J23100 c1	274.9
16	CinI J23100 c2	255.3
17	CinI J23111 c3	253.1
18	CinI J23105 c2	256.4

STAR

overnight cultures

SFGFP/pSB1C3
SFGFP/pSB3K3
STAR/pSB1C3
A.STAR/pSB1C3

WEDNESDAY, 9/28

Quorum

Sequencing

CinI J23111
CinI J23100
RhlI J23111
RhlI J23100
RhlI J23105
LuxI J23105
LuxI J23100
LuxI J23111

Overnight Culture

CinI J23105

Digest
pAA001 c1r1 (S/P)

STAR

Transformation
of original synthesised STAR and A.STAR (both from company)

THURSDAY, 9/29

Quorum

Day culture
Las + Rhl (no GFP)

Miniprep
Cin c1

Test Digest
Cin c1 (S/P)

STAR

Miniprep
of original synthesised STAR and A.STAR (bothf from company)

Day cultures
of original synthesised STAR and A.STAR (bothf from company)

Digest
of original synthesised STAR and A.STAR (bothf from company)

Ran above on gel
concentration too low to see anything

Overnight culture
2x STAR and 2x A.STAR (both from company)

FRIDAY, 9/30

Quorum

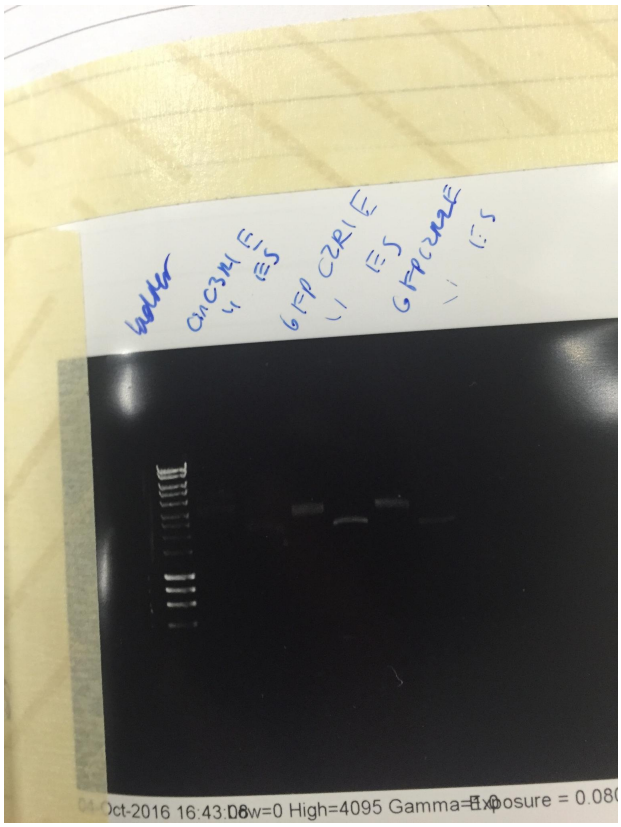
Day culture
pAA002

Sequencing
c1 (Las no GFP)
c2 (Rhl no GFP)

Miniprep
pAA002 (c1 and 3)
Cin + GFP (c2 and 4)

Gel
Cin-GFP C3 looks good

IMG_7341.jpg



Nanodrop

Table34

	A	B
1	**Sample**	**Concentration (ng/ul)**
2	CinI J23105 c1	235.6
3	CinI J23105 c2	149.9
4	CinI J23105 c3	191.3
5	pAA002 c1	44.8
6	pAA002 c3	46.3
7	Cin c2	37.3
8	Cin c4	15.6

Test digest (Eco, EcoHF, Spe1)
pAA002 (c1 + c3)
Cin (c2)

STAR

Miniprep

of original synthesised STAR and A.STAR (both from company)

Assembly digest
STAR (E/S), A.STAR (E/X), A.STAR (E/X)

Overnight ligations
in 2:1, 3:1 and 5:1, insert:vector ratios

SATURDAY, 10/1

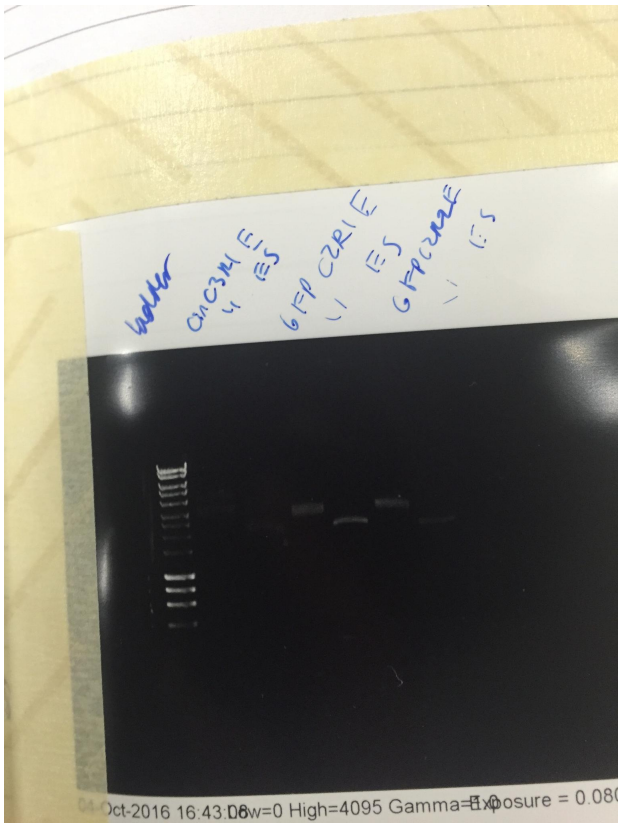
Transformation
of 2:1, 3:1, 5:1 ligations (STAR-A.STAR/ampicillin ligations)

SUNDAY, 10/2

Quorum

Miniprep
cinR+GFP colony 3
Ran on a gel - appears correct

IMG_7341 (1).jpg



STAR

Daytime cultures and miniprep
STAR-A.STAR

Digest
STAR-A.STAR/ampicillin with E/P

Growth

miniprep

AraC
GFP+pBAD
Gp2+pBAD
pSB1C3

Growth Experiments

Overnight cultures

- E. Coli: MG65, BL21, Top10, Turbo
- B. Subtilis: 168, WB800N
- Yeast: BY1471, Pichia(X33)

MONDAY, 10/3

Quorum

Sequencing

Sent CinR+GFP C3 R1

Transformation

CinR+GFP C3 R1 into top10 cells

STAR

Ran STAR-A.STAR on gel

seems no ligations were successful (all bands less than 200bp)
sent for sequencing

Overnight culture

of glycerol stocks of STAR/pSB1C3 (c1-4) and A.STAR/pSB1C3 (c1-4)
(cells grew on -ve control plates, perhaps enzymes not working properly?)

Ligation

of A.STAR E+X and transformation

Growth

Ligation

Gp0.4

Digestion

araC, GFP + Gp2 / pSB1C3

Sequencing

Gp0.4

TUESDAY, 10/4

Quorum

Miniprep

I13504 c2r1 + c2r2

Test digest

Cin c3 r1 (E + E/S)

Sequencing

Cin3 (c1)

STAR

Miniprep
of glycerol stocks of STAR/pSB1C3 (c1-4) and A.STAR/pSB1C3 (c1-4) - (very low concentrations)

Sequencing
results for STAR-A.STAR came back incorrect

Inverse PCR
of STAR/pSB1C3 and A.STAR/pSB1C3. Ran on gel, Dpn1 digest

Overnight cultures
of glycerol stocks of STAR/pSB1C3 and A.STAR/pSB1C3 (c1-4)

WEDNESDAY, 10/5

Quorum

Miniprep
CinR + GFP (c1, c2, c3 - TOP10)

Test digest
CinR + GFP c1 + c2

Overnight culture
CinR + GFP (x4)
pAA001 (x4)

STAR

Miniprep
STAR/pSB1C3 (1-4) and Anti-STAR/pSB1C3 (1-4)

PCR
Inverse PCR of STAR/pSB1C3 and Anti-STAR/pSB1C3 to remove the j23119 promoter
Dpn1 digest

PCR Purification
Run the STAR NP/pSB1C3 and Anti-STAR NP/pSB1C3
Gel extraction

Ligation
PNK Phosphorylation
Blunt-ended ligation of the STAR NP and Anti-STAR NP

Transformation
No promoter constructs into the Turbo cells

THURSDAY, 10/6

Quorum

Observe CinR + GFP in blue box, colony 7 looks promising

Digest

Cin R (no GFP)
Cin R (+ GFP)

STAR

Overnight Cultures
STAR NP/pSB1C3 (1-4) and Anti-STAR NP/pSB1C3 (1-4)

FRIDAY, 10/7

Quorum

Miniprep
CinR(+GFP)
c2r1
c2r2

Test Digest
CinR(+GFP) c2r1 (E + E/S)

Sequencing
CinR(+GFP) c2r1 and c2r2 (CORRECT)

STAR

Miniprep
STAR NP/pSB1C3 (1-4) and Anti-STAR NP/pSB1C3 (1-4)
- Send for sequencing

Assembly Digest
Anti-STAR 1 with E+X
Anti-STAR 2 with E+S
Anti-STAR 3 with X+P
Anti-STAR 4 with S+P
STAR 1 with E+S
STAR 2 with E+X
STAR 3 with S+P
STAR 4 with X+P

Ligation
PNK phosphorylate: Anti-STAR 1, Anti-STAR 4, STAR 2, STAR 3
Ligate in 3:1 and 5:1:
Anti-STAR 1 and STAR 1
Anti-STAR 2 and STAR 2
Anti-STAR 3 and STAR 3
Anti-STAR 4 and STAR 4

SATURDAY, 10/8

Quorum

Transformation
CinR(+GFP) c2r2 into TOP10 cells

STAR

Sequencing results
STAR NP/pSB1C3 (1-4): All of these are wrong, have a couple of nucleotides missing
Anti-STAR NP/pSB1C3 (1-4): All of these have the first nucleotide at 5'end of Anti-STAR missing

Transform

Turbo cells

- Anti-STAR 1 and STAR 1 3:1 and 5:1
- Anti-STAR 2 and STAR 2 3:1 and 5:1
- Anti-STAR 3 and STAR 3 3:1 and 5:1
- Anti-STAR 4 and STAR 4 3:1 and 5:1

Assembly Digest

Rhl and Las with E+S

STAR/pSB1C3 and Anti-STAR/pSB1C3 with E+X

Gel purification

Ran on gel --> DNA was lost (leads connected the wrong way)

SUNDAY, 10/9

Growth

Gel extraction and ligation

Gp2 + SFGFP

Sequencing

GP2+SFGFP c1 - INCORRECT

GP2+SFGFP c2 - INCORRECT

Miniprep

SFGFP+GP2

STAR

Assembly Digest

anti.STAR no promoter 3 E+X (6/10) –done on 9/10/16

CIP

Overnight Cultures

Anti-STAR 1 and STAR 1 3:1 and 5:1

Anti-STAR 2 and STAR 2 3:1 and 5:1

Anti-STAR 3 and STAR 3 3:1 and 5:1

MONDAY, 10/10

STAR

Assembly Digest

1. Digest Las colony 1 (1) with EcoR1-HF and Spe1-HF (E+S)
2. Digest Rhl colony 2 (1) with EcoR1-HF and Spe1-HF (E+S)

Gel purification

Ran on gel:

- anti.STAR no promoter 3 E+X (6/10) –done on 9/10/16
- anti.STAR no promoter 1 E+X – done on 9/10/16
- anti.STAR no promoter 2 E+X – done on 9/10/16
- anti.STAR no promoter 3 E+X – done on 9/10/16
- anti.STAR no promoter 4 E+X – done on 9/10/16
- STAR no promoter 1 E+X – done on 9/10/16
- STAR no promoter 2 E+X – done on 9/10/16
- STAR no promoter 3 E+X – done on 9/10/16

- STAR no promoter 4 E+X – done on 9/10/16
- Las c1 (1) E+S – done on 10/10/16
- Rhl c2 (1) E+S - done on 10/10/16

Gel extraction

- anti.STAR no promoter 3 E+X (6/10) –done on 9/10/16
- Las c1 (1) E+S – done on 10/10/16
- Rhl c2 (1) E+S - done on 10/10/16

Ligation

Treat STAR/pSB1C3 and Anti-STAR/pSB1C3 with E+X with CIP

Ligate STAR/pSB1C3 and Anti-STAR/pSB1C3 to Las (E+S) and Rhl (E+S)

Transformation

STAR/pSB1C3 and Anti-STAR/pSB1C3 to Las (E+S) and Rhl (E+S)

Miniprep

- Anti.STAR **E+X** & STAR **E+S**
    - 3:1 and 5:1 ligations *i.e. insert to vector ratios*
- Anti.STAR **E+S** & STAR **E+X**
    - 3:1 and 5:1 ligations
- Anti.STAR **X+P** & STAR **S+P**

    3:1 and 5:1 ligations

Test Digests

All Anti-STAR and STAR ligations on pSB1C3

Ran on gel--> all less than 200 bp bands, no 360 bp bands, i.e. ligation didn't happen

TUESDAY, 10/11

Growth

Dirty Ligation

Gp4 into pSB1C3

WEDNESDAY, 10/12

Quorum

Overnight culture

CinR(TOP10) - x6

Growth

Test digest

SFGFP + GP2 (c1+c2)

Miniprep

SFGFP + GP2 (c1+c2)

FRIDAY, 10/14

Quorum

Miniprep and test digest

CinR(TOP10) - INCORRECT

SATURDAY, 10/15

Quorum

Test digest and gel

CinR(no GFP) - (c2r2) - CORRECT

MONDAY, 10/17

Quorum

Transformation

CinR-GFP ligation into TOP10