Lab Journal

June 16, 2009

- meeting between Bogdan, Bryant, James and I
- finished the sequence for the surface expression construct
- figured out the to-do list
- mini-prep the following:
  - linker K157013 *Plate 3, well 3G, plasmid Bba_K157000 (A)*
  - TEV protease
    - I712078 (C-terminus) *Plate 2, well 14M, plasmid J70003 (A)*
    - I712077 (N-terminus) *Plate 2, well 14K, plasmid J70003 (A)*
  - pLux
    - R0062 (not leaky) *plate 1, well 6O, pSB1A2 (A)*
    - R1062 (median strength in the absence of luxR/HSL) *plate 1, 8G, pSB1A2 (A)*
  - HO-pcyA
    - K098010 *plate 3, well 11N, plasmid pSB3K3 (K)*
  - terminator
    - B0015 *plate 1, well 23L, plasmid pSB1AK3 (AK)*
  - RFP
    - E1010 *plate 1, well 18F, plasmid pSB2K3 (K)*

2009-06-17

- transformed the following parts into Top10

1.	K157013	*Plate 3*	*well 3G*	*K157000*	*Resis:A*
2.	I712078	*Plate 2*	*well 14M*	*J70003*	*Resis:A*
3.	I712077	*Plate 2*	*well 14K*	*J70003*	*Resis:A*
4.	R0062	*Plate 1*	*well 6O*	*pSB1A2*	*Resis:A*
5.	R1062	*Plate 1*	*well 8G*	*pSB1A2*	*Resis:A*
6.	K098910	*Plate 3*	*well 11N*	*pSB3K3*	*Resis:K*
7.	B0015	*Plate 1*	*well 23L*	*pSB1AK3*	*Resis:AK*
8.	E1010	*Plate 2*	*well 18F*	*pSB2K3*	*Resis:K*

- left in 37C for four hours and left on the lab bench in room temperature overnight

2009-06-18

- no colonies were observed on the plates
- left the plates in 37C for four hours
- Picked one colony from each plate and made overnight broth culture for glycerol stock.
- K098010 plate did not grow, so it was left in the 37C overnight.

2009-06-19
- made the glycerol stocks from the overnight broth cultures
- started broth culture for K098010
- re-transformed the E1010 in Top10

2009-06-22
- re-started the broth culture for K098010 because it was overgrown (already in stationary phase)
- Observed no colonies on the E1010 plate. We found out that E101 should be on Kanamycin resistance plate.

Things to do
- get out the standard plasmid backbones
- High copy number assembly plasmid backbone
  - pSB1A3 *Plate 1, well 1K,*

2009-6-23
- submitted the VLA construct to Mr. GENE for synthesis
- placed orders for PCR primers

2009-6-24
- transformed the following parts into Top10

9.	RBS-luxR	*J37033*	*Plate 3*	*4O*	*pSB1A2*	*Resis: A*
10.	RBS-LuxI-ter	*F1610*	*Plate 2*	*24G*	*pSB1AK3*	*Resis: AK*
11.	RBS-LuxI	*K081008*	*Plate 2*	*10L*	*pSB1A2*	*Resis: A*
12.	RBS-LuxR-ter	*I0462*	*Plate 1*	*8O*	*pSB1A2*	*Resis: A*
13.	Pconst	*J23119*	*Plate1*	*18A*	*pSB1A2*	*Resis: A*
14.	LuxR	*C0062*	*Plate1*	*4O*	*pSB1A2*	*Resis: A*

2009-6-25
- purchased a QIAminiprep kit from BioBar
- picked colonies from plates and re-cultured in broth; left overnight
- ordered ITGA4 cDNA plasmid from OpenBioSystem.

2009-6-26
- made glycerol stocks for the parts transformed on 6-23
- designed primer for PCR out VLA cDNA plasmid and primer for pLux-RBS-HindIII
- Transformed the following parts into Top10 and left the plates in 37C overnight.

15.	RBS	*B0034*	*Plate1*	*2M*	*pSB1A2*	*Resis: A*
16.	HemeC	*I716154*	*Plate1*	*17B*	*pSB1A2*	*Resis: A*
17.	HemeD	*I716155*	*Plate1*	*17D*	*pSB1A2*	*Resis; A*

2009-6-27

- picked colonies from plates and made glycerol stocks

2009-6-28

- transformed glycerol stocks into broth culture

2009-6-29

- purified plasmids containing parts 1-17 from Top10 using Qiagen Spin Miniprep Kit
- performed Agarose Gel Electrophoresis to check the size of the parts. Bends did not migrate very far, possibly due to the fact that circular plasmids migrate very slowly. We decided to digest the plasmids with a BioBrick restriction enzyme and then run gel again.

2009-7-2

- We rerun the Agarose Gel Electrophoresis to check the size of the parts. It worked.

2009-07-06

- We transformed the following parts into Top10

18.	GFP	*E0040*	*Plate1*	*14K*	*pSB1A2*	*A*
19.	GFP constr.	*E0840*	*Plate1*	*12O*	*pSB1A2*	*A*
20.	pTet+GFP	*I13522*	*Plate2*	*8A*	*pSB1A2*	*A*
21.	LuxR construct	*K091204*	*Plate2*	*8J*	*pSB1A2*	*A*
22.	luxI + GFP	*J37034*	*Plate2*	*7I*	*pSB1A2*	*AK*
23.	pSB1AC3		*Plate1*	*11A*	*pSB1AC3*	*AC*
24.	pSB1AK3		*Plate1*	*13A*		*AK*
25.	pSB1AT3		*Plate1*	*15A*		*AT*

2009-07-07

- We picked colonies from the plates and made broth culture, which was left to grow overnight.

2009-07-08

- We purified the plasmids of part 18 to 25 using QIA spin miniprep kit.

2009-07-09

- We ran a 1% Agarose gel electrophoresis to confirm the plasmid lengths of part 18 to 25.
- Gel loading and concentrations:

I.	E0040	120ug/uL	loaded 12ul
II.	pSB1AC3	120ug/uL	loaded 12ul
III.	R0062	120ug/uL	loaded 12ul
IV.	pSB1AT3	120ug/uL	loaded 12ul
V.	J23119	120ug/uL	loaded 12ul

VI.	J37034	120ug/uL	loaded 12ul
VII.	K091204	120ug/uL	loaded 12ul
VIII.	E0840	120ug/uL	loaded 10ul
IX.	B0034	50ug/uL	loaded 10ul
X.	F1610	50ug/uL	loaded 10ul
XI.	I13522	120ug/uL	loaded 10ul

- We figured out the relative concentrations of the parts by comparing the bands with the ladder (The 5,000bp ladder is about 120ng/ul)

- Parts digested

I.	R0062	P_{lux}	prefix	EcoR1+Spe1
II.	pSB1AT3		backbone	EcoR1+Pst1
III.	J23119	P_{const}	prefix	EcoR1+Spe1
IV.	B0034	RBS	suffix	Xba1+Pst1
V.	F1610	RBS-LuxI-STOP	suffix	Xba1+Pst1
VI.	B0015	Terminator	prefix	EcoR1+Spe1

- restriction digestion mix recipe:
  - 600ng of DNA
  - 4ul restriction buffer
  - 0.5ul EcoR1 and 0.5ul Spe1, or
  - 0.5ul Xba1 and 0.5ul Pst1
  - Up to 35ul of ddH_2O
- We digested the parts using corresponding BioBrick restriction enzymes, and then purified the parts using QIAquick PCR Purification Kit.
- Stored the purified DNA in -20C overnight.

2009-07-10

- We ran a 1% Agarose gel electrophoresis to confirm the products of restriction digestions.
- The expected bands of the parts did not show up. This is probably due to the fact that most of the parts have lengths within 100bp and the QIA quick PCR Purification Kit removes DNA below 100bp length.
- The miniprep plasmids of the parts are digested again with corresponding restriction enzymes for 2 hours.
- Enzymes are deactivated (denatured) by heating at 65C

2009-07-13

- ligated the parts into the following constructs using the *T4 ligase protocol*.
  - P_{const} – RBS – LuxI – 2xSTOP
  - P_{lux} – RBS
  - P_{const} – RBS (Heme Oxygenase)
  - Terminator – RBS
- Transformed the ligated constructs into Top10

- made broth culture from the ITGA4 cDNA glycerol stock
- Transformed ANP construct and kill switch (I716462) into Top10.
  I716462 *plate 2* *well 3C* *Amp*
-

2009-07-14

- purified the plasmid for ITGA4 using QIAminiprep spin kit.
- Recultured ITGA4 containing cells in broth.
- Picked colonies and made broth culture for the ligated constructs in triplicates.
- Picked colonies and made broth culture for ANP construct and the kill switch (I716462)
  - ANP construct plate was overgrown

2009-07-15

- PCR the colonies to check ligations
- Broth culture for ANP construct did not contain cells. Therefore transformed Top10 cells were cultured in broth overnight again. Broth culture was also made for I716462.
- Run gel to confirm ligations and ITGA4
- working constructs from ligation
  - C1 (P_{const} – RBS)
  - PL3 (P_{lux} – RBS)
  - T3 (Ter- RBS)

  Note: BioBrick primers worked for pSB1AT3 backbone.

Wed, Sept 2

SUMMARY →

① All P_{tet}-RBS-Lpp OmpA broth cultures were red upon harvest gain
② All P_{lacUV5}-RBS-Lpp OmpA also red
③ P_{tet}-RBS-SAA plates gridded, some white clones

ACTIONS TAKEN →

① Regrew broth cultures from P_{tet}-Lpp and P_{lacUV5}-Lpp plates; hope to miniprep tomorrow
② Cast a gel (in fridge)
③ Serial PCR started ~~from~~, using VF2 and R2, ~~3~~ and the products from PCR round A from last time
→ tubes labelled LOB^*_1 and LOB^*_2
④ PCR purify LOB^*_1 + LOB^*_2
⑤ Start PCR for LOC^*_1 and LOC^*_2 (1:30 - 3:20)
⑥ Start gel on LOB samples (2:10 - 3:10)

→ Loading order:

1	2	3	4	5
Ladder	LOB^*_1	LOB^*_1	LOB^*_2	-VC

expected length: ~>500 bp

[redacted]

2:11 PM

Scans

Actions
- PCR Purified run 2 → labelled loc*1, 2, -'ve and "~~run~~ end run 3" (actually ran 3!)
- Set up new PCR w/ VF2 and R3
- Imaged gel from run 2 → weak bands ~ 600 and < 200 bp. Will see how next run turns out.

- Phone call w/ Bryant:
  - ↳ he has already done band 3.
  - ↳ throw out current PCR and start again w/ VF2 and R1.
  - ↳ actually left in PCR machine and just restarted program w/ new samples

(band 4)
- New samples called loc*1, loc*2, -'ve
- Imaging band 3 on gel

ladder	1	2	-'ve
↓	↓	↓	↓

Observations
- 1st set bands from previous gel ~ 600 vanished. 2nd set ~ 100-200 nonsense.
- Will see if repeat samples (above mistake) work different.

To do

- PCR purdby round 4 samples
- Run gel for round 3 and round 4 samples.
- Redo (round 2 (overnight) if round 3 and 4 do not work.

Gel of rum 3 and 4

ladder | lab1 | lab2 | -ve | lab1 | lab2 | -ve | lab1 | lab2

* 1st lab1 and lab2 loaded poorly. Repeated at end

Evening Update

- Gel 4 showed bands ~ 200 :., repeat PCR from step 2.
- Tubes labelled lab 1 rep 2, lab 2 rep 2 and -ve rep 2
- PCR running w/ DEPC H_2O (now in rack w/ primers).
- Will use communal PCR machine in Botterell

Thurs Sept 3

- PCR purified Lo81 +2, rep 2. (and gel)
- Miniprep: P_{tet} - RBS - SAA ②
  P_{tet} - RBS - Lpp-OmpA → (SAA1 truncated red)

LOADING ORDER:
ladder, Lo81, Lo82, -ve, Lo82

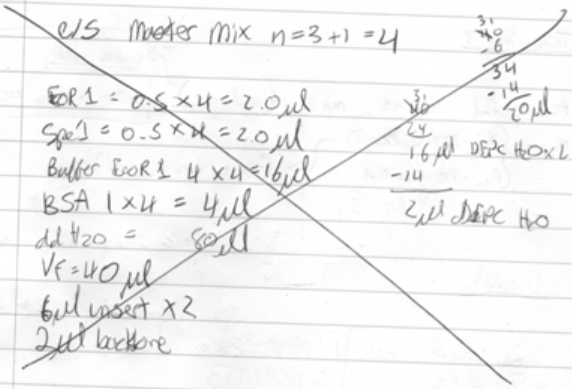
Digest

s2 e/s
s8 e/s
B0015 X/P
(plasmid - RBS - SAA) in pSB1AT3

#9 ptet - RBS - SAA e/s
~~#10 ptet - RBS - SAA~~
B0015 X/P
in pSB1AT3

Above digests are to put terminators onto SAA construct.

s2D one labels for digests
s8D
B0015D
#9D



Sept 03. 2009 (Hamy)

- Run PCR # 4 on = $\frac{\angle ODC1}{rep2}$ + $\frac{\angle ODC2}{rep2}$, and on RBS
- run gel for $\frac{\angle ODC1}{rep2}$ + $\frac{\angle ODC2}{rep}$ + -CTL
- Ligated S2 - BB015
  S8 - BB015
  #9 - BB015 x2 (duplicates)
- test digest with EcoR1 + Pst1
- run gel to check lengths. (loading next page)
- transformed the ligations and plated on pSB1AT3 pl
-

Gel loading

ladder, 20ul (rep2), 20C2, (rep2), S2-B0015, S8-B0015, S-B0015(1), S-B0015(2), -CTL(PCR).

Conversation w/ Ian

- Lower annealing temp to 3.0°C below Tm of R3.
- Use 2:10 use 2ul/rxn of purified R2 for R3.
- Try to re-amplify R2 and if ok use as R3 template
- Remove aliquots @ 25 cycles

Protocol IGEM -°C

50.°C	5min	
95°C	5min	
95°C	30s	↑
52°C	30s	x39
72°C	30s	
72°C	10min	
10°C	15min	
4°C	6h	

PCR R2 and R3

A * Dilute R2 → for R3
B Regular R2 → for R3
Purified band

✓ C * Dilute R2 → reamplify
D Regular R2 → reamplify

E * Dilute R2 → for R3
F Regular R2 → for R3
PCR Purifying Kit

G * Dilute R2 → reamplify
H Regular R2 → reamplify

n = 8 + 1 = 9

	n = 1	n = 9
10x PCR Buffer	6.0 x 9	= 54
dNTPs	0.4 x 9	= 3.6
DEPC H_2O	37.1 x 9	= 333.9

SPLIT

195.75 µl

n = 4.5

R2 = 36 µl
VF2 = 36 µl

R3 = 36 µl
VF2 = 36 µl

For R2
n = 5 (4+1)

	n=1	n=5
10x PCR Buffer	6 x 5	= 30 µl
dNTPs	0.4 x 5	= 2.0 µl
DEPC H_2O	37.1 x 5	= 185.5 µl
R2	6.0 x 5	= 30 µl
VF2	6.0 x 5	= 30 µl
Taq	0.5 x 5	= 2.5 µl

+ template

For R3
n = 5 (4+1)
* same as above (w/ R3 instead of R2)

Update

~~PCR~~ Fast digest may not have worked - will retry w/ regular digest reagent.
- Re-doing PCR 3 w/ updates as per above.
- checked plates: will leave bacteria to grow longer.

* Removed 7µl of p50 PCR samples @ end of cycle 28 and deactivated @ 72°C 10min. *

Gels Run

Cycle 28 aliquot loaded ladder A → H, space, ladder.

Full Run PCR loaded ladder A → H, poor loading of wells B, D, G.

Gel: 28 cycle Observations
- All bands per 1 showing.
- Excise all R3 bands.
* - Ian suggests hot start and separate mm for +ve ctl (ie: reamplification) *

To DO

- Run PCR R4 with purified (gel) and kit (sample) PCR 3 products.
- Use protocol "iGEM - °C".
- Pick colonies for later miniprep.

Note: PCR products in blue strip of tape, iGEM Rack -20°C.

Sept. 5 (Hany)

- Run PCR#4 on A, B, E, F (see page 50)
- picked colonies from pTac-RBS-SAA-Ter 1+2
  and pConst-RBS-SAA-Ter (S8-Boo1)
- plates for S8-Boo1S and S2-Boo1S were put back into 37°C due to the lack of colonies.

* - miniprepped pSB1AC3 & pSB1AT3 ~~both cultures were white → need to remake both from glycerol stock~~
  (pellet for pSB1AT3 was pink)

PCR purification samples: (on green rack)

LOD A	LOD B	LOD E	LOD F

Sept 6 (Hany)

- pick colonies from S8-Boo1S place
  (tube 2+3)
  pick colonies from S2-Boo1S
  (tube 1)
- made glycerol stocks for 9

* - miniprepped Ptac-RBS-SAA-Ter
  - placed in the green rack

Gel from Henry RH.

Ladder A B E F B

lost

No bands > 500 bp. RH did not amplify. Check dT and return on Tuesday

↳ Wrong protocol was used!!! Repeat w/ Protocol "iGEM PCR - °C."

6d

Tomorrow (Tues 8th Sept)

- PCR → if OK digest, ligate, transform
- Pick colonies
- Buy new miniprep kit
- Get order (buffers) Jenet.

Jml

- digest Pcon-RBS-SAA-Ter with EcoRI + PstI
- run gel to confirm.
- Return Myc to Peter.

Gel of PCR products:
order = ladder, A, B, B, B, E, F

- Boogdan miniprep of SAA culture
  Ptet-RBS-SAA-Ter called "SAA Full"
  in freezer.

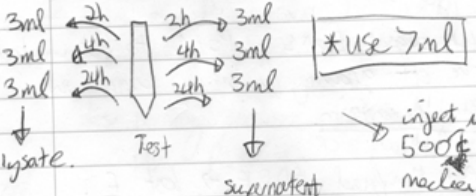
✓ • PCR VLA digest EcoR1 + Pst1 to test in backbone
✓ • SAA +-ive ctl growing for set up tomorrow.
✓ • Digest SAA to confirm.
- ~~Re-run~~ RH w/ PCR

(Note: PCR purified ADEF → lost "E".
- Re-run RH on P. 57 gel. w/ PCR purified samples

SAA experiment planning.

Collect 2hrs, 4hrs, overnight

- 3 ml for each timepoint
- 2x 50 ml Falcon Tube. (1:100 overnight seed)



→ repeat w/ PSB1AT3 negative + media control

-'ve

- Run pancreum to normalize
  - ↳ re-run gel if req'd w/ normalizations
- Probe for SAA
- Lyse w/ 800 ul sample buffer (western) w/ 200 ul 1M DTT. in 1:1 ratio. Boil 7mins.
- Freeze @ -20°C remaining sample
- Measure OD upon collection.

Gel RH combination

ladder A B F -ve SAA

* Have digested and ligated previous RH for test purposes although gel did not show bands.
* Re-run RH on above gel after new RH PCR run.

SUCCESS! ALL WORKED!
Excised band.

To do

Reamplify + digest + ligate PCR RH.

Gel purified samples labelled RH A, RH B, RH F

overnight in Rotterell. (GA = gel purified A
B
F
PA = PCR kit purified.
B
F

Gel of PCR samples:
ladder blank GA GB GF PA PB PF

SAA Experiment Started @ 11:30
- T2 = 1:30
- T4 = 3:30
overnight = 11:30

OD Instructions (OD 600)
- Cuvette w/ 1ml media (blank)
- Δ on cuvette sideways
- Press neb to blank
- OD > 1 means dilute sample or "- - -"

11:30	T0	OD 600
1.5ml	pSBIAT3 sup	0.058
1.5ml	pSBIAT3 wcl	0.063
1.5ml	SAA sup	0.040
1.5ml	SAA wcl	0.036

1:30	T2	OD 600	
3ml	pSBIAT3 sup *	0.560	→ lost -lve
3ml	pSBIAT3 wcl	0.600	10.8mL
3ml	SAA sup	~~0.190~~	0.330 → 3ml, 19.6mL
3ml	SAA wcl	0.324	20mL

3:30
3ml
3ml
3ml
3ml

1:30
3ml
3ml
3ml
3ml

5:30 — T4

			OD600	
3ml	psB1AT3	sup	1.601	4.05 µL
3ml	psB1AT3	wcl	1.582	4.10 µL
3ml	SAA	sup	1.306	4.96 µL
3ml	SAA	wcl	1.321	4.91 µL

1:30 — T24

			OD600	
3ml	psB1AT3	sup	2.716	2.39 µL
3ml	psB1AT3	wcl	2.827	2.30 µL
3ml	SAA	sup	1.358	4.77 µL
3ml	SAA	wcl	2.841	2.28 µL

1:10

*Note: You must control for the volume of sample removed when resuspending whole cell lysate to lyse. You need to keep the same [ ] of cells! Ie: If 3ml removed resuspend relative to sample if 1.5ml removed.

Also all listed volumes are X-1 ml as 1ml reg'd for OD600.

14 September 2009

- Western normalized against OD 0.324
- Boiled ~~up~~ all w/ buffer 7mins.

loaded as follows

T0 + T2 on gel 1
T14 + T24 on gel 2

left.	media ctl	
	psB1AT3 sup	repeats for
T0	SAA sup	T14 / T24.
	psB1AT3 wcl	
	SAA wcl	
	ladder	
	psB1AT3 sup (missing for T2)	
	SAA sup	
T2	psB1AT3 wcl	
right	SAA wcl	

- transfered to nitrocellulose blot
- blocked at 4°C overnight
-

15 Sept. 2009

- made broth culture for ~~RBS~~ - LAp-OmpA - TEV x 2 - linker imp SBIAT3
- Washed the blots 3X 15 min in TBS-T
- incubated with 1° Ab 1:5000 2% skim milk
- Washed 3x 15 min in TBST
- incubated with 2° Ab 1:40000 2% skim milk overnight at 4°C

16 Sept. 2009

- broth cultures did not grow → need to remake broth cultures
- To ligate RBS and Myc
  1. Digest RBS + Myc with Dra I
  2. PCR purify the digestion
  3. ligate

17 Sept. 2009

- Tee plates do not work

- Digestion

1) ~~SAA Protein~~

PA, PB, PF, pSBIAT3	EcoRI + PstI
2) RBS + Myc	Dra I

Loading scheme (suggested by Peer)

Gel #1

+ CTL (10)	+ CTL (1)	- CTL (To WCL 10)	- CTL (To WCL 1)	Ladder	To SAA sup 10	To
To SAA WCl 10	To SAA WCl 1	Ladder				

Gel #2

First 6 wells are the same + To SAA sup 10 | To SAA sup 1 | To SAA WCl 10 | To

Ladder

Sept 28, 2017

- received SAA + CTL sample (1 mg/mL)
- SAA antibody (1:1000 dilution) → 1 mL in 1 mL solution

Sept 29, 2009

Ran SDS-PAGE on SAA samples

Sample preparation:

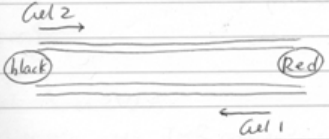
SAA + CTL c (ug/mL): ~~added~~

- conc. 10 : added 55 ul and 11 ul of 6x SDS buffer
- conc. 1 : added 5.5 ul sample into 49.5 ul dd H_2O, and mixed with 11 ul of 6x SDS buffer

This method was used to prepare all samples.

- made 120 ul of SAA To WCl and T4 WCl c SDS buffer added ; boiled)

Loading



Running 40mA constant Amp. for 90min

- Blocked in milk for 1hr
- incubated with 1° Ab overnight at 4°C
-

~~Regina~~

Oct. 1. 2009

Run SDS-PAGE

Gel #1	+CTL 10	+CTL 1	-CTL 10	-CTL 1	Ladder	SAA T24 10 Sup	SAA T24 1 Sup
	~~Ladder~~	SAA T24 WCL 10	SAA T24 WCL 1	Ladder			
Gel #2	+CTL 10	+CTL 1	-CTL 10	-CTL 1	Ladder	SAA T24 10 WCL	SAA T24 1 WCL

- transfered to nitrocellulose blot
- blocked for 1 hr
- 1° Ab overnight

I

II

III

IV

X

pare together - 3:30)

Planning

Oct 2, 2009

I PCR reactions

① PCR Ptet-RBS using BioBrick primers
② PCR construct for stretching Ptet-RBS (using primer: Ptet stitch FWD + PCR Whole REV)
③ PCR VLA4 using primer: VLA4 Partial FWD + REV
④ PCR Whole construct to put into backbone
⑤ ~~PCR~~

II PCR purify; run gel to confirm band
III PCR stitch Ptet-RBS to construct using outside primers (7:45 - 6:00)
IV PCR purify

Oct ~~3~~ 5, 2009

- PCR stitch III to VLA
- 3 Way ligation with TetR construct (K081018)

PCR round 1

tubes
#1. Ptet-RBS amplified with BB primers
#2 Mr.Gene PCR construct amplified with Ptet-stitch FWD + VLA stitch REV
X #3 VLA4 amplified with VLA partial FWD + VLA Partial REV
#4. Whole construct amplified with PCR Whole FWD + REV

none #3 evaporated, need to redo PCR

GAWA6ADE

PCR round 2

#5 Stitch Ptet-RBS to construct using primers:
BB FWD V2F + VLA stitch REV
- Note: this rxn contains 2 templates

#3 VLA4 amplified with VLA Rad61 FWD + REV

Ran gel of 1st PCR purified products

order = marker/ladder | Ptet-RBS stitch (#1) | copy of Construct stitch (#2) | Amplified Construct (#4)

5 lanes

- when the gel was loaded some of the samples floated to the top

- gel file called: PCR Gel #1 - Oct 2 Bogdan

Checking gel

Construct stitch → 492 bp. → faint band
amplified construct ~ 750 bp → worked

Oct. 5. 2009

Things to do:

1. PCR stitch VLA to construct
   template: "VLA stitch frag Oct.2"
   ~~"Construct Stitch Oct.2"~~
   "Ptet-construct Oct.2"
   Primers, - V2F BB FWD
   - VLA Partial REV.

2. Run Gel to confirm.
   samples: "VLA stitch frag Oct.2"
   "Ptet-construct Oct.2"
   + product from the new PCR

3. PCR ANP out of construct
   template: "Amplified Construct Oct.2"
   primers: ANP FWD + PCR Whole REV.

Dogchin

Mastermix. 6.0 PCR buffer x3
0.4dNTP x3
37.1 ddH2O x3
0.5 taq x3

Agarose Gel Loading
Ladder, Binding Construct, ANP, Blank, Binding Construct, ANP, -Ctl
Ptet-RBS-construct, VLAq
↑ verified ↑ worked fairly well

Plan

- amplify construct stitch with Pcec-stitch FWD + VLA stitch REV.
- amplify binding construct with V2F and VLA Partial REV
- amplify Pcec-construct with V2F and VLA stitch REV.
- amplify ANP with ANP FWD + PCR Whole REV.
- amplify V2A using (Partial VLA 1 + VLA stitch 2)
  primer VLA partial FWD + VLA Partial REV

master mix × 8

Loading

Gel: PCR Oct. 6

ladder, VLA stitch 1 (loading error), VLA stitch 2, Pcec construct, VLA stitch 3

ANP construct stitch, Binding Construct

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- Parts that can be ligated into backbone:
  "VLA stitch frag-2 Oct.6"
  "ANP2 Oct.6"
  "AmpHfiedConstruct. Oct. 2"

Rerun PCR #2

1) template. "Ptet-RBS stitch Oct 2"
   "Construct stitch Oct 2"
   primers. V2F + VLA stitch REV

- Sample labelled "Ptet-construct Oct. 7"

Gel
Ladder - CTL unpurified PCR sample purified PCR sample

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PCR #3

1) template "Ptet-RBS- Construct Oct. 7" add 4ul
   "VLA stitch frag 2 Oct6" add 2ul
   primers: V2F + VLA Pst61 REV

Samples: - CTL
Binding Construct Oct 7. - use purified sample
Binding 2. - use unpurified sample