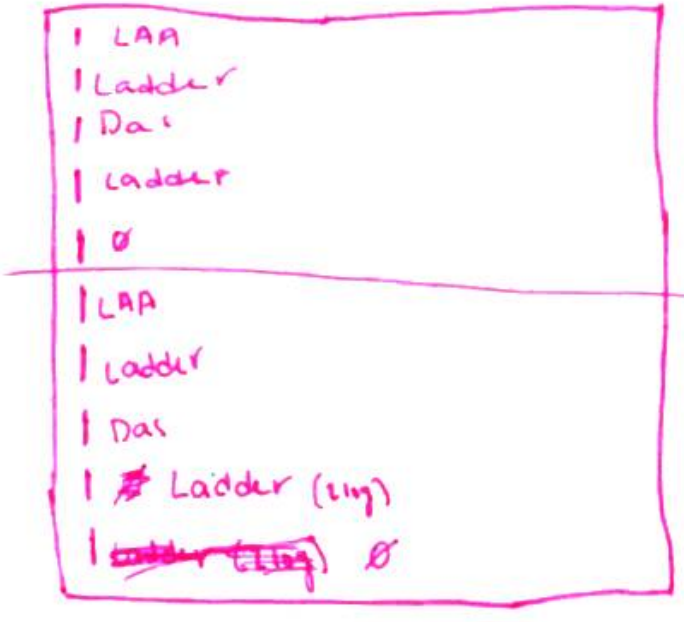
10 ng/µl $\frac{1000\ ng}{100\ µl} = 1\ µg$ DNA

Digest 31/7/2017

- 150 ml solution in microcentrifuge tube + alloquat into 3 PCR tubes.
- running on a gel

Gel 1/8/2017



15 µl of DNA, 3 loading dye

1st time
- no bands

2nd time
- purple spots
- no bands

ask stands

purple bands are just dye. The gel had no DNA.
↳ ask to test other tubes

order more TSPurple

Transformation 2/6/2017

pAR LacI GFP LAA AK3 on Kan plate:
↳ colonies grew, no fluorescence

pLG DAS ClpXP CI 1:100 on Plain plate
↳ colonies grew, no fluorescence

pLG ClpXP CI 1:100 on Plain plate
↳ colonies grew, no fluorescence

R11 ClpXP CI 1K3 on Kan plate
↳ colonies grew, SUCCESSFUL

Next steps:

- miniprep R11 ClpXP CI 1K3
- transform pAR LacI, GFP DAS, and GFP LAA to miniprep and obtain more DNA

written on 2/22/17

Transformation 2/9/17

pλR LacI in 3T5
↳ colonies grew → fluorescence (not expected)
↳ liquid cultures (x3)

GFP DAS in 1C3
↳ colonies grew → no fluorescence (was expected: GFP)
↳ streak to singles

GFP LAA in 1C3
↳ no growth

next: colony PCR + gel pλR LacI + GFP DAS
order GFP LAA
send pλR LacI + GFP DAS

2/23/17

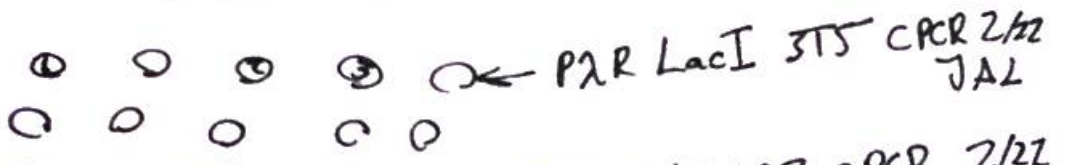
Colony PCR of transformations

Completed in triplicates
→ colonies pulled from plates

→ PλR LacI in 3T5

→ GFP DAS in 1C3

In Thermocycler



Next: run a gel

into gel
- S → 10 µL of DNA
- Z → 2 µL of Loading Die

10	
9	10 µL of 2 log ladder into ~~loading~~ gel
8	PλR LacI 1
7	PλR LacI 2 → not clear
6	PλR LacI 3
5	2 Log Ladder
4	GFP DAS 1 → blah
3	GFP DAS 2 → blah
2	GFP DAS 3

Notes – From Google Call 2/24 3:45

- HSV or HSL values
- Pellet From liquid Culture

- Make sure to account For # of cells + time of cells + Multiple measures oF same sample

- What would you expect graphs to look like?
  Bar Graph

pLAC – synthetic Leakiness has been eliminated

(T7 promoter) interesting but not on topic

- Varying Clp ClpX aminoacid - Probably NOT Good idea - difficult
- Varying tags to other known working tags
- Use the sequences in Registry
- Feedback times

Slide 10 – Don't mutate tags
Keio strain Question – Not essential to address
#9 = Absolutely
#8 - Yes!! + also background motivation or why

3/23/2017 Transformation * used NEB protocol *
↳ includes heat block

Parts:

① RII ClpXP 3T5
3/23 transf

② RII ClpXP 3T5
33 ClpP
3/23 transf

③ 33 ClpX AK3
33 ClpP
3/23 transf

④ pλR 33 LACI 3T5
3/23 transf

⑤ B33 CI 1C3
3/23 transf

⑥ pλR LACI RII RFP 3K5
3/23 transf

50uL cells
10uL plasmid (diluted)

green microcentrifuge tubes

3 Tet plates
2 Kan plates
1 Cam plate

plated 250uL onto each plate

Results:

① RII ClpXP 3T5 - growth → liquid culture in ~~tet~~ resistance

② RII ClpXP 3T5 - no growth → replating on tet
33 ClpP

③ 33 ClpX AK3 - growth → liquid culture in kan resistance
33 ClpP

④ pλR 33 LacI / 3T5 - no growth → replating on tet

⑤ B33 CI 1C3 - growth → liquid culture in cam resistance

⑥ pλR lacI RII / RFP 3K5 - growth → liquid culture in kan resistance

3/29/2017 - Transformation *used NEB Protocol*
↳ includes heat block

Parts/Results:

① PAR LacI [3T5] - growth on plain
- no growth on tet

3/30/2017 Colony PCR

Parts:

PAR LacI [3T5]
① ② ③

Results:

4/14/17: colony PCR
8:00: made gel
· made liquid cultures
- R0011 ClPXP (3T5)
- CI (IC3)

3rd period: colony PCR JL & AF
· R0011 ClPXP (3T5)
· CI (IC3)

4/14/17 - Part Lengths

PART	LENGTH	
ClpP	624bp	} = 2804
ClpX	1275bp	
pR11	~~2029~~	
CI	775bp	

CL and GA

4/17/17: miniprep of LCa from 4/14/17 JL & AF
· R0011 ClPXP (3T5) A260: .821, A260/A280: 1.35, 41.2 ng/mL
· CI (IC3) A260: 1.059, A260/A280: 1.42, 52.9 ng/mL
re-miniprep ↑ for better results

JLE AT

4/18/17 miniprep (redo)

- R011 CIPXP(3T5)
- CI (IK3)

R011 CIPXP
A260 : .479 / 1.49
A260/A280 : 1.55 / 1.57
24 ng/µL / 24.5

CI
A260 : .422
A260/A280 : 1.81
21.1 ng/µL

4/28/2017 Digest of R11 CIpXP CI IK3

92.8 ng/uL

$$\frac{92.8\ ng / 1\ ug}{1\ uL \mid 1000\ ng} = \frac{0.0928\ ug}{1\ uL} = \frac{1\ ug}{x\ uL} \quad x = 10.78\ uL$$

2 uL X (10X Re-MIX)
1 uL P (standard enzyme)
10.8 uL DNA
6.2 uL dH_2O

GEL

used 100 bp (only access) so could not tell

5/02/2017 Re-Digest

4 R11 CIpXP CI IK3
4 92.8 ng/uL

$$\frac{92.8\ ng / 1\ ug}{1\ uL \mid 1000\ ng} = \frac{0.0928\ ug}{1\ uL} = \frac{1\ ug}{x\ uL} \quad x = 10.78\ uL$$

2uL X (10x Remix)
1uL P (Standard enzyme)
10.8 uL DNA
6.2 uL dH_2O

5/07/2017 miniprep

R0011 ClpXP CI in 1K3

A260 (10 mm): 0.687

A260/A280 : 1.83

34.4 ng/µl

as this is the 2nd or 3rd miniprep with low concentration we think that the generation of R11 ClpXP CI is straining for the cells to generate, resulting in low cell growth

Colony PCR

12.5 µL Q5

1.25 µL VF2

1.25 µL VR

6 µL DNA

4 µL H_2O

↓

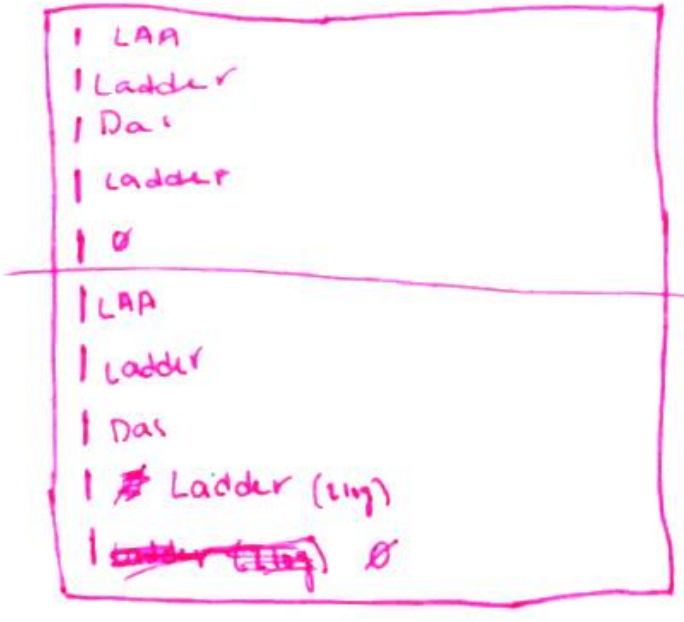
using junction's in a 1K3 also

10 ng/µl $\frac{1000\ ng}{100\ µl} = 1\ µg$ DNA

Digest 31/7/2017

- 150 ml solution in microcentrifuge tube + alloquat into 3 PCR tubes.
- running on a gel

Gel 1/8/2017



15 µl of DNA, 3 loading dye

1st time
- no bands

2nd time
- purple spots
- no bands

ask stands

purple bands are just dye. The gel had no DNA.
↳ ask to test other tubes

order more TSPurple

Ligation Calculation 1/8/2017 (waiting on pAr LacI backbo

vector:

Part 1: TS Purple

Backbone Digest 1/8/2017 → should we run on gel? ask Stanley

1C3

1A3

1T3

1K3

(all with EP)

can't ↳ trust

Transformation 8/1/2017

might redo w/ heat [illegible]

(x2) PλR LacI 1K3 (plating on all 4 resistant plates) → out of Kan
miniprep 3/22

ClpXP Carb Miniprep AK3
miniprep 4/28

ClpXP CI 3K5 → cell growth on plain
↓
streak onto Kan
→ all grew on Kan

PλR LacI 1K3
- plain - ✓ growth → streak onto kan
- cam - X no growth
- carb - X no growth
- tet - X no growth
- kan - ✓ growth → from streaks

Miniprep

PλR LacI (1K3)

liquid culture made 6/4/2017

failed

August 4th

Gel Electro.

- [ ] LAA
- [ ] Ladder
- [ ] DAs
- [ ] ~~Ladder~~
- [ ] ~~Ladder~~
- [ ] ~~Ø~~

- [ ] LAA
- [ ] Ladder
- [ ] DAs
- [ ] Ladder 2log
- [ ] ~~Ø~~

results: ladders were clear and visible, however no DNA ~~was~~ in the wells w/ our constructs.
↳ reordered parts 8/4/17 → IDPURPLE
- JL & EG

8/8/17 - replacing PXR lalT with weaker plac
IL and LH transformed from DNA in freezer
· wear plac - transformed
· ROOII dpXPC1 - ☺
· tzpuppies - ordered
estimated shipping 8/11
estimated arrival 8/17

reminder: you are all wonderful & lovely people. we got this!

Wednesday, August 9, 2017

* Result: Growth of weak pLac AK3 on Kan plate from transformation on 8/8/2017
* Lab: Liquid cultures (3 5mL LC tubes) ⇒ inoculation

Thursday, August 10, 2017

* Liquid cultures ⇒ did not grow (used AF Kan LB 10-14-16)
  - ↳ new liquid cultures: 3 new Kan LB, 1 plain LB, 1 from 8/9
* Digest of tsPurple, tsPurple LAA, tsPurple DAS after hydration
  - ↳ Protocol for Hydration: Pipette 10μL milliQ H_2O to make 100 ng/μL
  - ↳ 15.5μL H_2O + 2.5μL DNA + 2μL E + 1μL P

Friday, August 11, 2017

* Ligate tsPurple into iC3 (in box ⇒ ligase box MIA)

8/11 minprep (pLac) JL, LH, EG

1:	18.1 ng/μL	A260: 0.362	A260/A280: 1.98
2:	11.7 ng/μL	A260: 0.235	A260/A280: 1.99
3:	19.5 ng/mL	A260: 0.391	A260/A280: 1.57
4:	11.5 ng/μL	A260: 0.231	A260/A280: 2.07

Next minprep: open new kit

R0011 ClpXP CI backbone: 1K3 (8/9/2017)

↳ miniprep → stored at 0

↳ digest with E + P ✓ ark standby

↳ 21 - 25 of august

Friday, August 10

Transformation of TS Purple (Ø, DAS, LAA)

Tuesday, August 15

Ligation of TS Purple and AK3 (Ø, DAS, LAA)
plated on K (all 3 grew + control → 10 Betta, grew)

Wednesday, August 16

Made Amp & Kan LB
Liquid culture of TS Purp (Ø, DAS, LAA) AK3
Liquid culture of pAR LacI Tet LB

Friday, August 18 (miniprep, AF)
nanodrop (ng/μL)

Ø ⇒	0.6 ng/uL, A260/A280 = 4.31	1.5 ng/uL, A260/A280 = 1.61
DAS ⇒	1.3 ng/uL, A260/A280 = 2.47	2.9 ng/uL, A260/A280 = 1.56
LAA ⇒	1.1 ng/uL, A260/A280 = 1.37	1.1 ng/uL, A260/A280 = 1.26

pλR LacI 3T5 from 3/22 : 2.3 ng/uL A260/A280 : 1.86

pλR LacI 1T3 from 3/22 : 7.0 ng/uL A260/A280 : 1.77

pλR LacI 3T5 from 3/22 : 4.0 ng/uL A260/A280 : 1.71

pλRLacI 3T5 from 8/17/17 : 4.3 ng/uL A260/A280 : 2.00

pλRLacI 3T5 from 8/17/17 : 16.8 ng/uL A260/A280 : 1.54 (18.5 ng/uL original)

ROSA1ClpXPC1 from 5/15/17 41.2 ng/uL A260/A280 : 1.89

8/22 re minipreps from 8/16

tripple	5.4
Dan	11.8
Lan	6.3

Wednesday 8/23

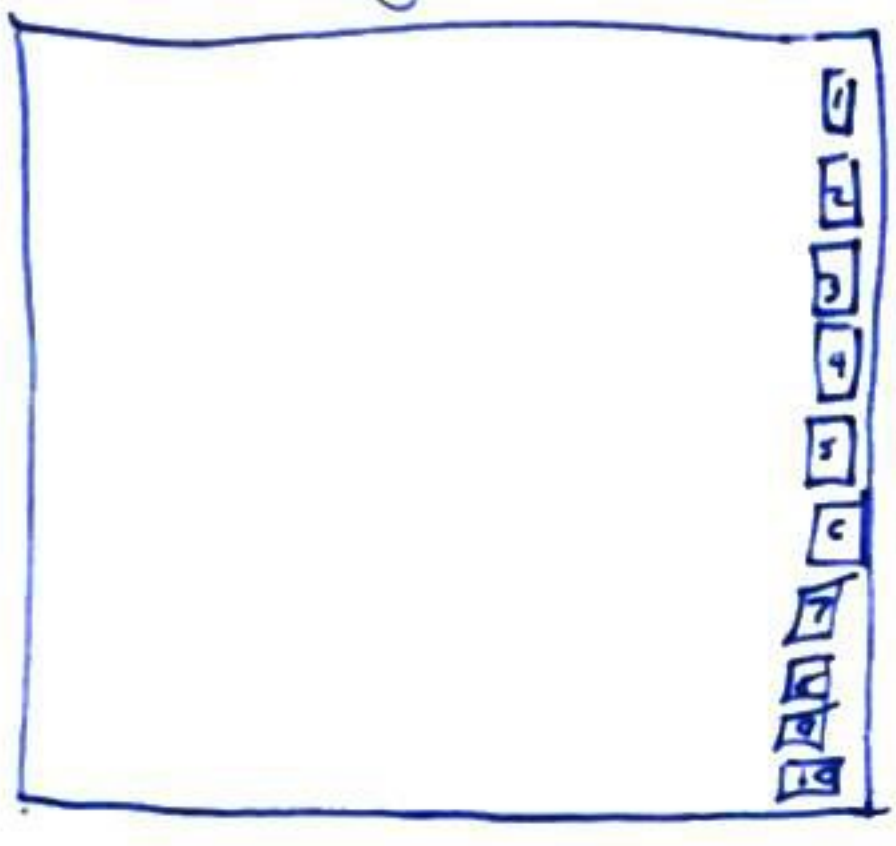
PCR:

① P2R LacI 3T5 (6.0 ng/uL)

② P2R LacI 3T5 (18.5 ng/uL)

③ R0011 ClpXP CI

Thursday 8/24



3 - P2R LacI 3T5 (6.0 ng/uL)
4 - P2R LacI 3T5 (18.5 ng/uL)
5 - 2-Log-Ladder
6 - R0011 ClpXP CI

8/29/17

sent for sequencing:

AHA 772 → R0011 ClpXP(1 with VF_2 (5 nL DNA, 0.8 nL primer VF_2, 6.2 nL millipure H_2O)

ANV 224 → pARLacI 3T5 1 with VF_2 (2.5 nL DNA, 0.8 nL VF_2 primer, 8.7 nL H_2O)

ANV 226 → pARLacI 3T5 2 with VR (2.5 nL DNA, 0.8 nL VR primer, 8.7 nL H_2O)

Digest from IDT:

2.5 uL	DNA
2 uL	E or X
1 uL	S or P
14.5 uL	H_2O

Ligation from IDT:

1.4 uL	DNA
2 uL	ligase buffer
1 uL	ligase
14.6 uL	H_2O
1 uL	1C3

Hydrating constructs from IDT:

* 10uL H_2O for 100ng/uL concentration
* Digest
* Ligate

Send for sequencing:

800 ng DNA for sequencing

0.8 uL primer VF_2 & VR

Bring to 12uL w/ H_2O

TsPurple (TsPurple DAS, + TsPurple LAA) 8/30/17

20 NL ~~Digest~~ Digest starting off
- 1.4 NL for ligation
- 6 NL for gel

12.6 NL left

Gel 8/30/2017

1 L —
12. —
13. —
14. —
15. +sPurple LAA
16. 2 Log ladder
17. +sPurple
18. +sPurple DAS
19. —
110—

expected lengths: 788 bp DAS
750 bp LAA
∅

Results: lengths look good! successful digest

100 V, 60 min

8/31/2017 Ligation of +sPurple, +sPurple DAS, +sPurple LAA

Program: 002 on thermocycler

4.5 uL	DNA	plate on both	other tubes
1 uL	~~IC3~~ AT3 or AK3	the same (+s Purp or +s Purp DAS, +s Purp LAA)	14.6 uL H_2O
+ 5.5 uL	sticky-end ligase MM		1.4 uL DNA
11.0 uL	↳ 2X, refer to protocol		

stored in freezer w/ DNA

* Ran out of IC3, used digested backbones from one year ago
  ↳ Digest more backbone

9/1/2017 – Transformation of tsPurple, tsPurple DAS, tsPurple LAA

- 2uL DNA
- 50uL DH10 beta cells
- Heat shock for 30 sec @42°C, ice for 2 min
- plated on Tet & Kan plates

9/5/2017

Results from Transformation:

↳ Tet plates: no growth on control nor tsPurple (+ DT) plates

↳ Kan plates: growth on all four plates (control, tsPurple, tsPurple DAS, tsPurple LAA

9/6/17 ☺

Transformations:

PKR Lacl (18.5ng/uL), ROCK1C/p XPC1, GFP (from DNA miniprep Box on Control), RFP

(Following NEB 10 Beta Transformation Protocol)

9/6/17 - lunch period - julia

- colony PCR

PCR setup

C	DAS
0	LAA

- control (C) labels
- ts purple 0 (0)
- tspurple DAS (DAS)
- tspurple LAA (LAA

DNA dilutions in ~~blue rack~~ (freezer) julia/emily/lauren's box

Sequencing - 9/6/2017

RUO11 C1PXP C1 (63.2 ng/uL): VF_2 – AHA782

VR – AHA774

9/8/17 gel of colony PCR a
- trp purple

1. control
2. tz purple 8
3. tz purple 9
4. tz purple 10
5. ladder (2-log)
6. pXR 2a 1
7. RO0H C1PXPC1

12 µL reactions
10µL DNA
2µL loading dye

4µL 2-log ladder

expected lengths
J ~ 760
Kan ~ 790
Laa ~ 790
pXR 2a 1 ~ 1115
RO011 C1PXPC1 ~ [redacted] 2012

* tubes in weigh boat in top left

RFP Transformation 9/8/17 NS

RFP → 5ul, 10ul ∑ PXRLacI R011RFP → DH10 Beta

Puc19 → 5ul ∑ (+) control → DH10 Beta

↘ → DH10 ~~Beta~~ (-) control

* put in incubator for 1 hr at 2:28 p.m

X Spread on Kan and plain plates

- ~~P~~XRLacI R011RFP 5ul → ~~kan~~ plain plate
- PXRLacI R011 RFP 10uL → kan plate
- PUC19 5uL → plain plate
- plain DH10 Beta → ~~plain plate~~ Carb plate
  ↳ expect to see nothing b/c on a Carb plate

9/13/17 RFP Streak to Singles

* Results from Friday → lawns on both RFP plate
  → controls: colonies on Carb plate of DH10
  lawn / colonies of Puc 19
  - colonies (look visually different from lawn-colonies) most likely contamination

- pXR LacI R011 RFP 5uL → plated on kan / streak to singles
- PXR LacI R011 RFP 10uL → plated on Kan / streak to Singles

9/13
- inoculated liquid cultures of pXRLacI and R011ClpXP1 need to retransform triplex constructs

9/13 lunch
transformations of triplex 6, DAS, CA1 ∑ puc 19 ↓ control

↳ did not work

9/14/17 - gone back to 8/18 minipreps to transform, cPCR, and inoculate

ligations
11
-2
-2
7ml left

9/14/17 transformations of tryptophan from 8/22 JL

Transformations: JL tsPurple AK3, tsPurple DAS AK3, tsPurple LAT AK3

↳ plated 100uL on amp + kan plates (+ puc 19 DNA control)

* Note: heat shock occurred a little later than 30 min.

Transformations: LH RIICIpXP 3T9, B33 CJ IC3

↳ plated 100uL on tet and cam plates (+ puc19 DNA control)

* Note: heat shock occurred a little later than 30 min

* Next step: colony PCR

9/15/17

transformation results did not work

↳ re-digesting from hydrated (tryptophan) digested tryptophan

(transfer into purple microcentrifuge tubes

9/15/17 - Gaurav B.

Miniprep of RIICIpXPCI and PKRlacI

Results -

RIICIpXPCI - A260 (10mm): 3.859

A260/A280: 1.86

Concentration: 193.0 ng/uL

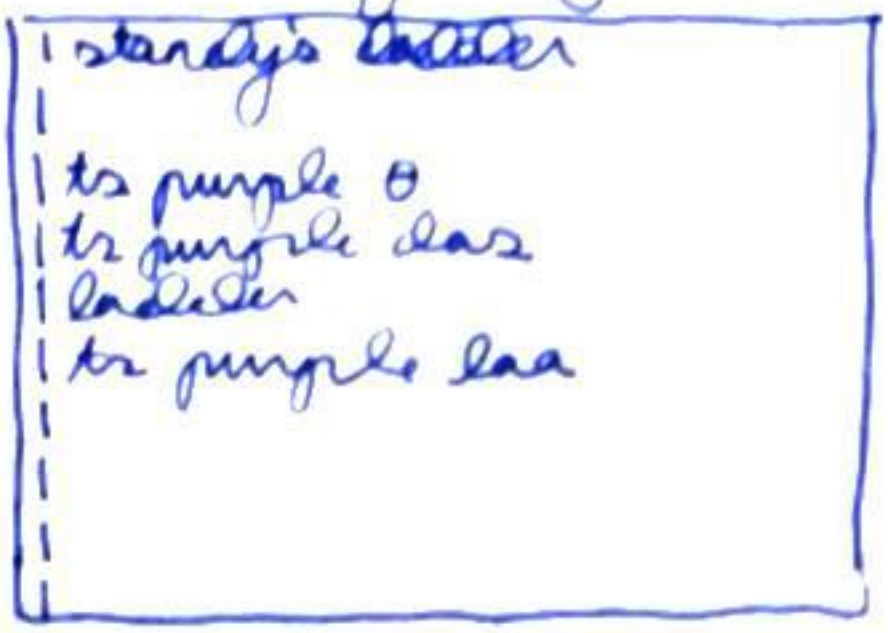
PKRlacI - A260(10mm): 0.429

A260/A280: 1.91

Concentration: 21.5 ng/uL

9/15/17 Julia

5th-run gel of tripurple digests w/ ladder



5ul DNA, 1ul ~~loading dye~~

expected lengths

0 - 750

5ul DNA - 780

9/16/2017

Digests ⇒ stored in pink PCR-tube box in junior drawer!

(stored in pink trays in junior drawer)

pAR LacI 1K3 E2s
tsPurple / tsPurple DAS / tsPurple LAA X&P
} 25uL rxns

Gel

pAR LacI
2-log ladder
tsPurple
tsPurple DAS
tsPurple LAA

5uL DNA, 1 uL loading dye

6 uL 2-log ladder

RESULT: pAR LacI did not show up

tsPurples look good ✓

[ ] too low

Ligations ⇒ stored in pink PCR-tube box in junior drawer!

Parts	Length of Insert	Length of Vector	Total	1/conc	uL	
1C3	1000	2200	3200	0.31	1	2uL ligation buffer
pAR LacI	1115	2204	3324	0.30	3.1	
tsPurple	690	0	690	1.45	0.64	+ 1uL ligase
tsPurple DAS	920	0	920	1.087	0.86	
tsPurple LAA	920	0	920	1.087	0.86	

→ + 12.24uL mg H_2O → 12.04uL mg H_2O

* Part length of tsPurple should be 756 bp, 788 bp w/ DAS & LAA *

9/19

- calculations were off → wrong tsPurple part lengths
- pAR conc. too low → re-inoculating from 9/7/17 GB pAR lacI transformation

Laa: no colonies — 0 colonies, purple (RFP :( )

das: colonies, white — qucl9 - no growth :)

~~re~~ inoculating 0 3 cols to run digest gel diagnostics

10/2 Hydrated amilcp, amajLime, + cjBlue
- stored in freezer (big) in box that says Standy's

10/3/2017

Parts	l of insert	l of vector	Total	[DNA]	µL
IC3	1982	2070	4052	0.248	2
pAR LacI	1227	3189	4416	0.226	2.20
sPurple	755	0	755	1.325	0.38
IC3	2015	2070	4085	0.245	2
pAR LacI	1227	3189	4416	0.226	2.16
sPurple DAS/LAA	788	0	788	1.269	0.38

1uL ligase + 2uL lig buffer 12.92uL dH2O

1uL ligase + 2uL lig buffer 12.46uL dH2O

10/4/17
transformation results (puc19 [illegible]
pAR LacI triangle 0, 0.45, LAA [illegible]
+ control: :)
everything else: :(cry :(

10/4/17 (Gam) -
Digest
PAR - 16.5uL DNA in 25uL reaction
RBS - ~~12.2~~ 12.2 uL DNA in 50uL reaction

16.5 DNA
2.5 CutSmart
.5 EcoRI-HF
.5 SpeI-HF
5 mgH2O

12.2 DNA
5.0 CutSmart
1.0 XbaI
1.0 PstI
30.9 mgH2O

10/14/17 GB
ligation of pAR, [illegible], and RBS
* almost out of T4 ligase

Parts	L of Part	Lot Vector	Total	1/kb	uL
IA3	60	2155	2355	0.443	1
PAR	49	2079	2129	0.152	2.94
RBS	11	2070	2081	0.481	2.76

1 uL IA3
2.94 uL PAR Dig
2.76 uL RBS Dig
2 uL Ligase Buffer

1 uL Ligase
11.3 uL mqH_2O

10/9/17 - GB

Restriction Digest

	pAR +RBS	LacI
DNA	11.99 uL	4.40 uL
CutSmart	2.5 uL	2.5 uL
EcoRI-HF	0.5 uL	0.5 uL
SpeI-HF	0.5 uL	0.5 uL
mqH_2O	9.51 uL	17.1 uL

Program 003

10/17/2017

Miniprep: 260.9 ng/uL , A260/A280 : 1.93 #2

161.8 ng/uL , A260/A280 : 1.92 #1

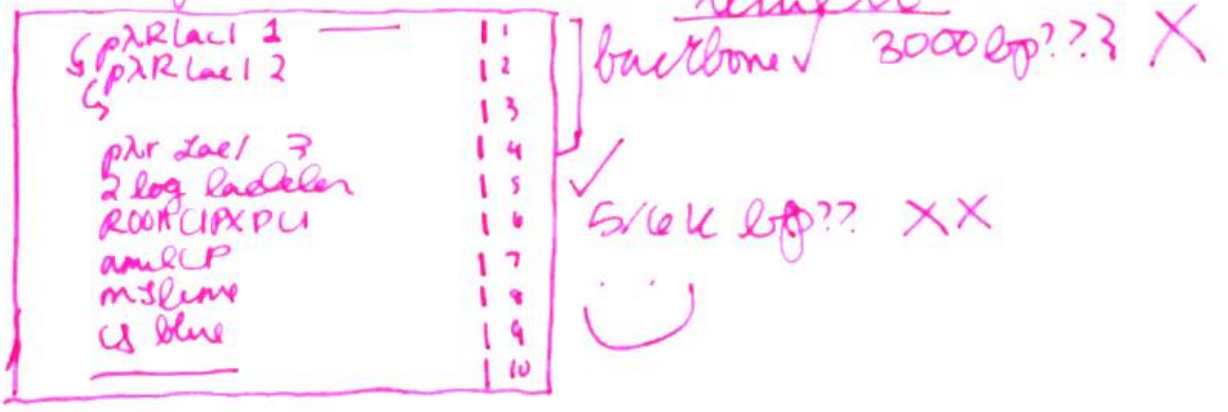
3 106.8 ng/uL , A260/A280 : 1.89 #3

10/18/17

digest of ~~pXR~~ GIB

- pXR Lac1 1
- pXR Lac1 2
- pXR Lac1 3
- R00 II clpXP1 2
- amilCP
- mSline
- CS blue

~ran gel (JC) 6pl rxns



10/18 - Ligation Calculations

Part	L of 1	L of Vector	Total	1/kb	uL
IT3	1942 / 1975	2461	4403 / 4436	0.227 / 0.225	1
PARLacI	1187	2204	3391	0.295	0.769 / 0.763 x3 : 2.307 / 2.289
TsPurple	755	0	755	1.325	0.171 x3 : 0.513
TsPurple DAS	788	0	788	1.269	0.177 x3 : 0.531
TsPurple LAA	788	0	788	1.269	0.177 x3 : 0.531

PARLacI-TsPurple

1uL IT3

2.307 uL PARLacI

0.513 uL TsPurple

2uL Ligase Buffer

1uL Ligase

13.19 uL mgH_2O

PARLacI - TsPurple DAS/LAA

1uL IT3

2.289 uL PARLacI

0.531 uL TsPurple DAS/LAA

2uL Ligase Buffer

1uL Ligase

13.18uL mgH_2O

10/18

- forward/reverse ROCKCPAPCI middle primers annealed
  - hydrated
    - ↳ 5uL in sequencing box
    - ↳ 5uL in Brandy's DNT box (closet)

sent for sequencing.

- ROCKCPAPCI (middle area) (two tubes, F & R)

FORWARD	REVERSE
ANV213	ANV214

- 4uL dna (193 ng/uL)
- 7.2uL water (milli-Q)
- 0.8uL primer

10/18 Sequencing (continued): Alyssa ↲

19 CJB VF2   22 ACP VF2
20 CJB VR   23 ACP VR

10/25/17 JL
liquid cultures of:
- J23101 (2) cam
- GFP (2) cam
- R0011 LepXPCI reg (2) amp
- R0011 LepXPCI (2) amp
- PAR LacI bjpurple (2) tet

10/25/17 - (Graham) continuation of Ligation of TsPurple into 1C3

Part	L of I	L of V	Total	1/kb	uL	(Digestion by Lauren)
1C3	755	2070	2825	0.354	1	15.198 ul mq H_2O
TsPurple ⌀	755	0	785	1.325	0.802	
1C3	788	2070	2858	0.350	1	
TsPurple DAS/LAA	788	0	788	1.269	0.827	15.173 ul mq H_2O

2 ul Buffer
1 ul Ligase

Gel -

10/26/2017 – Virginia Violet T5/Amp/pUC/HC

1) Centrifuge tube briefly before opening to ensure DNA is not in the lid.

2) Add 20uL sterile water or 1X TE and incubate 10 min at room temp.

3) Dissolve DNA by pipetting up and down a few times.

4) Long-term storage at -20°C.

* 1 ng/uL stock ⇒ 1uL solution, 99 uL H_2O

20 ul 2 ug dna

2000 ng / 20 ul = 100 ng/ul

1 ul + 99 ul H_2O = 1 ng/ul

10/26 LH

transformation

- Virginia violet
- media
- transLAA
- transRAS

>10/26/17 -

Gaurav / Lauren Miniprops

Part	Conc	A260/A280	A240
1) J23101 #1	76.9 ng/uL	1.85	1.538
2) J23101 #2	95.9 ng/uL	1.82	1.919
3) RIICIpXPCI #1	312.3 ng/uL	1.84	6.246
4) pARLacI TsPurple	30.1 ng/uL	1.47	0.602
5) RIICIpXPCI Seq #1	243.7 ng/uL	1.85	4.874
~~6) RIICIpXPCI Seq #2~~			
7) GFP #1	106.8 ng/uL	1.89	2.137
8) RIICIpXPCI #2	159.0 ng/uL	1.82	3.180
9) RFP Unknown #1	248.6 ng/uL	1.83	4.973
90) RFP Unknown #2	204.8 ng/uL	1.74	4.097
11) GFP #2	173.5 ng/uL	1.61	3.470

Parts

*TSp= TS Purple

49	PλR	Lamda Promoter
11	B33	weak RBS
34	B34	Strong RBS
1064	LacI	Protein generator
717	eGFP	Green Flourescent Protein
129	B15	double terminator
816	CI	Lamda Repressor
753	GFP-DAS	GFP w/ deg tag
753	GFP-LAA	GFP w/ deg tag
624	ClpP	Part 1 of Protease
1317	ClpX	Part 2 of Protease
55	R0011	pLac Promoter
→ 755	Tspurple	Purple Chromoprotein
→ 788	TSP DAS*	↓ w/ deg tag
→ 788	TSP LAA*	↓ w/ deg tag
33	LAA+DAS	deg tags (each seperate 33)
1113	PλR-LacI	
1875	PλR-B33-LacI-B34-GFP	
2147	B15-R0011-B33-clpx-B33-ClpP	
1963	B33-ClpX-B33-ClpP	
1253	B15-PλR-B33-LacI	
827	B33-CI	
→ 787	B34-GFP-DAS	
→ 787	B34-GFP-LAA	
→ 1848	PλR-B33-LacI-B34-TSPurple	
→ 2079	PλR-B33-LacI-B34-TSpurpleDAS	
→ 2078	PλR-B33-LacI-B34-TS Purple LAA	
2974	B15-R0011-1333-ClpX-1333-ClpP-B33-CI	
→ 724	B34-TS Purple	
→ 1911	PλR-B33-LacI-B34-GFPLAA	

Backbones

2204	iK3
2070	IC3

tspurp 755

tspurp·das 788

tspurp·laa 788

nanodrop

ACP 3 = A260 (10mm) 0.625
A260/A280 2.00
31.3 ng/ul

ACP 1 = A260 (10mm) : 1.697
A260/A280 : 2.01
84.9 ng/ul

ACP 2 = ~~A260 (10mm) : 25.378~~
~~A260/A280 : 1.05~~
1268.9 ng/ul

mL 1 = A260 (10mm) 1.513
A260/A280 1.84
75.7 ng/ul

mL 3 = A260 (10mm) 0.773
A260/A280 : 2.12
38.6 ng/ul

CJB 3 = A260 (10mm) 3.574
A260/A28 · 1.49
178.7 ng/mL

CJB 1 = A260 (10mm) : 1.236
A260/A280 · 1.69
61.8 ng/ul

CJB 2 = A260 (10mm) : 1.836
A260/A280 : 1.93
91.8 ng/ul

mL 2 = A260 (10mm) : 0.943
A260/280 : 1.67
47.2 ng/ul

Digest 9/20/2017

CJB3: 178.7 ng / 1 uL = 1 ug / 5 uL 37.4 uL H2O 1 uL X, 1 uL P 5 uL cutsmart

mL 1: 75.7 ng / 1 uL = 1 ug / 13.21 uL 29.79 uL H2O 1 uL X, 1 uL P 5 uL cutsmart

ACP 1: 54.9 ng / uL = 1 ug / 11.15 uL 31.22 uL H2O 1 uL X, 1 uL P 5 uL cutsmart

Gel 9/21 (of digests)

CJD 3
2 Log Ladder
MJL 1
2 Log Ladder
ACP1

pause

CJB 3
2-LL
MJL 1
2-LL
ACP1

Gel 9/15

CJB 1 M1
CJB2 1 2
2Log3 1 3
MJL1 1 4
MJL2 1 5
MJL3 1 6
2Log 1 7
ACP1 1 8
ACP2 1 9
ACP3 1 10

← failed w/ smears

← looked decent, restreak

... maybe PCR materials

4 liquid cultures → draw from new colonies on plates but use MJL 1 + 2 dilutions instead of plates

Ordered parts from IDT 8/c time

W/ orbeed parts

Hydration:

1000 ng / 40 mL = 25 ng/mL

MJL
CJB
ACP

10/2/2017: Hydrated amicp, amaj lime, cjb lne
- stored in big freezer in Sandy's box

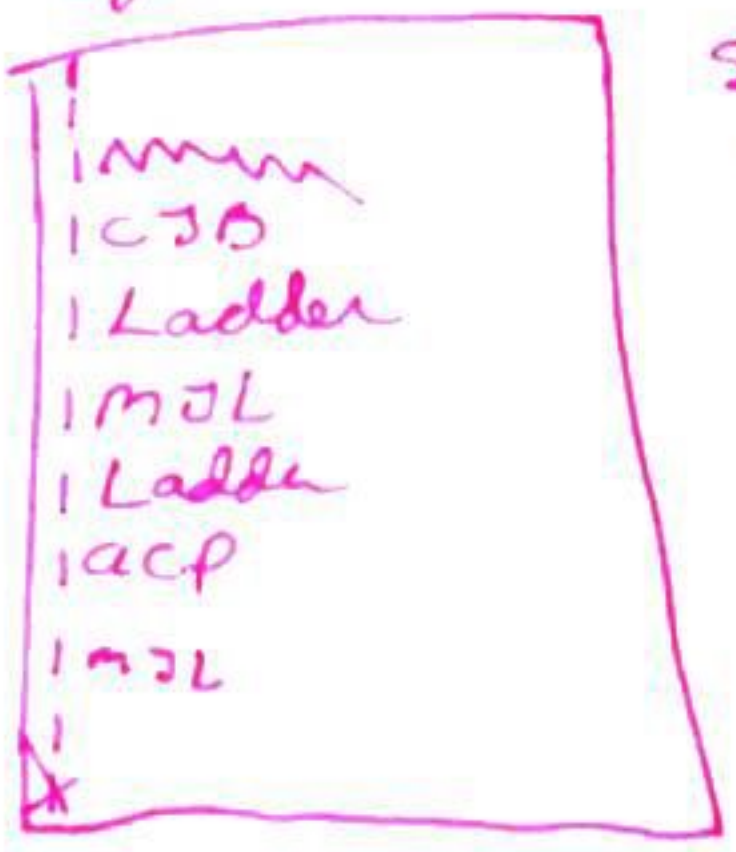
Digestion: 10/3/17

10 mL DNA of 25 ng/mL
2 mL ~~X~~ E
1 mL P
7 mL H_2O

gel 10/4

25 mL 10/10/17

10 DNA
2.5 2.1 Buffer
.5 EcoRI
.5 PstI
11.5 H2O



Success & correct (the acp didn't show well)

Ligation: AF 10/5 → failed → due to wrong digestion

Parts	L pack	L vector	total	1/Kb	mL	
IC3	822	2070	2.892	.346	1 / 2	1 mL Ligase
CJB	822	0	.822	1.21	.857	2 mL Li. Buff. 15.143 mL H_2O
IC3	822	2070	2.859	.3498	1 / 2	1 mL Ligase
ACP	789	0	.789	1.26	.833	2 mL Li. Buff 15.167 mL H_2O
IC3	822	2070	2.883	.347	1 / 2	1 mL Ligase
MJL	813	0	.813	1.23	.846	2 mL Li. Buff. 15.154 mL H_2O

Transformation 10/6/17

CJB IC3 - growth, no color
ACP IC3 - growth, no color
MJL IC3 - growth, No color
control - No growth

ligation failed, IC3 closed on self.

↓

digest → phosphotase IC3
retransform & religate

Digest 10/9/17

linearized IC3
↳ iGEM protocol

Digest 10/10/17

colors: MJL, CJB, ACP

25 mL sdn
10 mL DNA
2.5 mL 2.1 Buffer
.5 mL Eco-1
.5 mL Pst 1
+ 11.5 mL H_2O

Ligation 10/10/17

using 10/5 calculations

failed

positive control worked

check digestion on gel

Gel 10/11/17

all are correct, ligation is which failed (+ transformation)

Ligation 10/10/17

5 mL overnight LB → miniprep → sequencing

10/18/2017 - 10/19/2017

- Digest of Amil CP, Amaj Lime, CJ Blue

  10 uL DNA

  5uL NEBuffer 2.1

  1 uL EcoRI-HF

  1 uL PstI

  33uL dH2O

- Gel ⟹ Part lengths look correct
- Ligate to 1A3

Part	Part Length	Vector Length	Total Length	fmol	uL	
1A3	822	2155	2977	0.336	1	+ 2uL lig buffer + 1uL ligase + 15.172uL H_2O
CJ Blue	822	0	822	1.217	0.828	
1A3	789	2155	2944	0.340	1	+ 2uL lig buffer + 1uL ligase + 15.195uL H_2O
Amil CP	789	0	789	1.267	0.805	
1A3	813	2155	2968	0.337	1	+ 2uL lig buffer + 1uL ligase + 15.178 H_2O
Amaj Lime	813	0	813	1.230	0.822	

23°C for 10 min, 65°C for 10 min

10/25/17 Liquid Culture (amp resistance)

Tinsel Purple → 1mM ✓ IPTG
Scrooge orange → 100 uM ✓
10 uM →
Ø → mal growth ✓

in incubator
Shaking
↳ YES, WORK
room temp
Shaker
↳ NO, WORK, drop

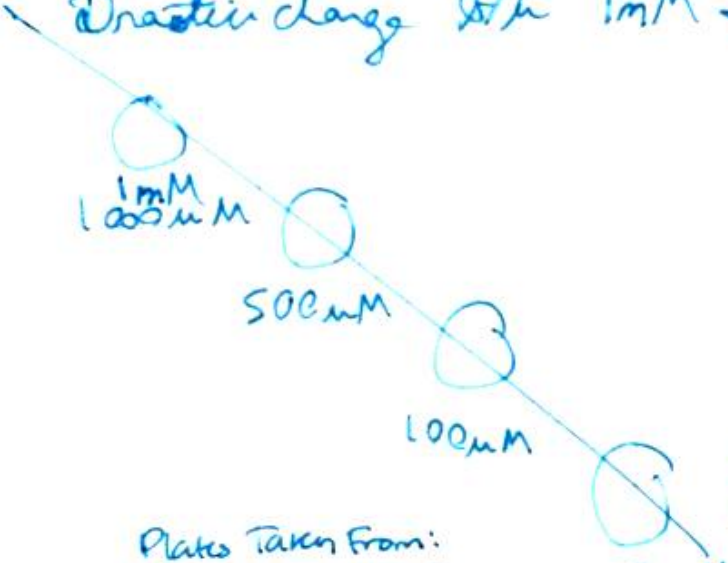
cells from TP: 1ng/uL SO: 10ng/uL

10/26/18

Dramatic change b/w 1mM & 100 uM so adding 500 uM

(amp resistance)

triplicates



TP from: 1mM IPTG
500 uM IPTG
100 uM IPTG
___ ng/uL 10 uM IPTG
Ø IPTG
resistance

SO from: 1mM IPTG
500 uM IPTG
100 uM IPTG
___ ng/uL 10 uM IPTG
Ø IPTG
resistance

Plates Taken From:
* Scrooge Orange 10ng/uL (10/24 Trans JAS)
* Tinsel Purple 1ng/uL (10/24 Trans JAS)
* added (-) control to test sterility LB