Collective Lab Notebook

WEDNESDAY, 5/31/2017

PCR Amplification of LysrR+pmmsa (promoter) and pmmsa from 1μL from the G-block

THURSDAY, 6/1/2017

XbaI & SpeI restriction enzymes were used to digest the Lux vector (part k823025)

FRIDAY, 6/2/2017

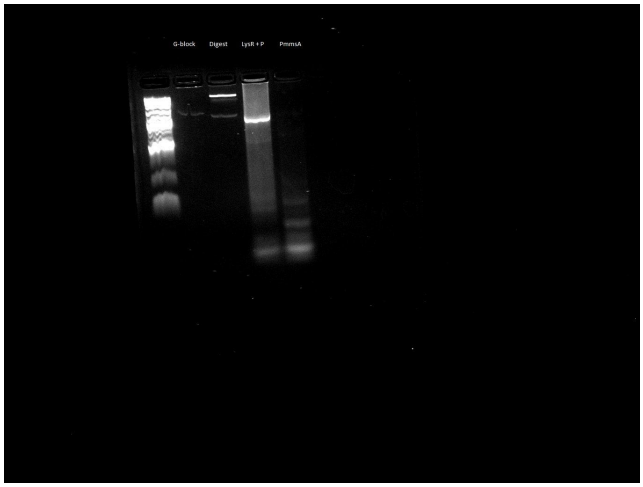
2 2% agarose gel (*Agarose gels and Agarose Gel Electrophoresis* protocol linked) were created. One of the gels was designated a "diagnostic" gel and 4 µL of the restriction digests, LysR+pmmsa, and pmmsa (with 6x dye) were loaded in wells 3, 4, and 5 respectively (labeled in *Figure 1)* with 5µl of a log2 ladder on well 1 in addition 1µl of the g-block in well 2. Gel electrophoresis at 75V for 1 hour was done until the dna separated effectively. **THIS WAS A TEST RUN TO PRACTICE THE SKILLS.**

Agarose gels and Agarose Gel Electrophoresis

The bright bands were cut out (~1kb for the LysR+pmmsa, ~200bp pmmsa) and the *Extraction of DNA fragments from an Agrose Gel* protocol (linked below) was run to achieve pure DNA. We only purified the Lux vector. The dna was then discarded.

Extraction of DNA fragments from an Agarose Gel

Figure 1



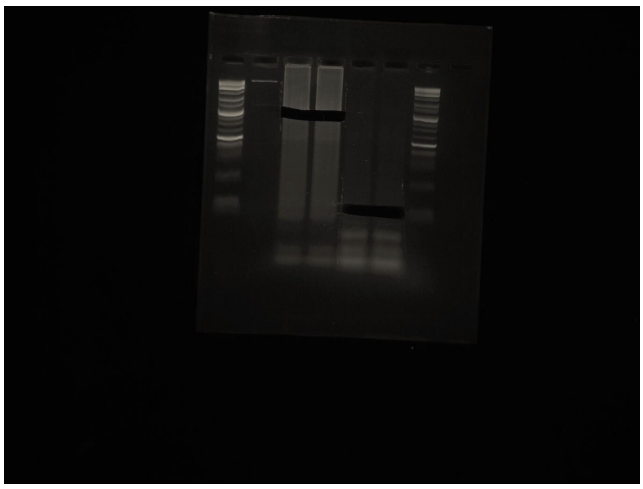
The same procedure as above was run with a well setup as follows. This part was for real.

Table1

	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7
1	2-Log Ladder	Restriction Enzyme (removed)	22.5 µl (LysR & pmmsa) + 7.5 µl 6x loading dye	22.5 µl (LysR & pmmsa) + 7.5 µl 6x loading dye	22.5 µl (pmmsa) + 7.5 µl 6x loading dye	22.5 µl (pmmsa) + 7.5 µl 6x loading dye	2-Log Ladder

The resulting gel with the LysR&pmmsa and pmmsa cut out is pictured in *Figure 2*.

Figure 2



The following protocol was used to extract the DNA.

Extraction of DNA fragments from an Agarose Gel

THE RESTRICTION DIGEST of lux WAS NOT GEL PURIFIED HOWEVER. Instead 3 volumes of QG buffer to 1 volume of the restriction digest was added and the sample was incubated at 50˚C. The rest of the procedure was then followed starting from **"Bind the DNA to a QIAquick 1column"**

The following data was collected from the nano spectrometer:

Table2

	A	Concentration (ng/microliter)	Absorbance 260/280	Absorbance 260/230
1	LysR-PmmsA	17.2	1.26	0.08
2	PmmsA	0.5	1.26	0.00
3	Restriction Digest of Lux Vector	4.3	2.58	0.02

Gibson Assembly:

A gibson assembly of the LysR-PmmsA sequence and restriction digested Lux vector was then done.

6 µl of the lux vector + 1 µl of the lysR-PmmsA insert were combined and spun down (Call this the dna mix). The numbers were calculated accounting for the homologous ends.

Then 2.5 µl of the dna mix was added to 2.5 µl of Gibson Assembly master mix. The solution was placed in the green pcr tubes and placed in the thermocycler at 50˚C for 30 minute.

The resulting 5µl of plasmid was used to transform our stock E. Coli cells using the following procedure using the outgrowth (1hr) method.

Fast and Slow Transformation of Z-Competent E. coli

After an hour the cells were plated in a labeled AMP plate and allowed to grow overnight at 37˚C.

Let's pray for white culture tomorrow morning.

SATURDAY, 6/3/2017

Previous experiment did not work. Restriction digest was reperformed with 750 ng of K823025 in 60 mL of solution and eluted in 25 microliters of EB giving a yield of 13.2 ng/uL. Gibson was reperformed using the Ellermeier calculator on google drive. LysR-PmmsA was re-nanospeced due to disbelief in the 17.2 ng/ul concenctration and 3 readings gave an average of 8 ng/ul which was used in the calculator. .9 micro of lysR PmmsA was added to 5 microliter of vector k823025 and mixed thoroughly. 2.5 microliter of the dna mix was added to 2.5 microliter of master mix and placed in thermocycler at 50 for 30 minutes and transformed after outgrowth.- ML

SUNDAY, 6/4/2017

Previous experiment did not work. As per the idea of Dr. Ellermeier, 1.5 microliter of K823025 lux digested vector (XbaI+SpeI) was combined with 1.5 microliter of the LysR-PmmsA PCR product. 2.5 microliter of this DNA mix was added to 2.5 microliter of Gibson Master Mix and put in the thermocycler following the programed gibson thermocycler protocol. The product transformed into competent E. coli cells, sat on ice for 10 minutes, and incubated with 4 volumes of SOC media for 1 hr at 37 C. The entire volume was plated onto an amp plate and incubated overnight at 37 C.- ML

MONDAY, 6/5/2017

Previous experiment did not work. Dr. Ellermeier suspects it's the competence of the cells.

TUESDAY, 6/6/2017

As per the idea of Dr. Ellermeier, 1.5 microliter of K823025 lux digested vector (XbaI+SpeI) was combined with 1.5 microliter of the LysR-PmmsA PCR product. 2.5 microliter of this DNA mix was added to 2.5 microliter of Gibson Master Mix and put in the thermocycler following the programed gibson thermocycler protocol. The Gibson products were brought to Craig's lab and heat shocked with his competent at 42 C for 45 seconds. The cells were outgrown in 1 mL of SOC and incubated for 1.25 hours at 37 C. Cells were spun down and resuspended in 150 microliters. Cells were then plated on a warmed AMP plate and incubated overnight.- ML

WEDNESDAY, 6/7/2017

Cells were successfully grown and isolated from the 6/5 experiment. Today, cells will be miniprepped and prepared for storage coming from 4 unique colonies. Prepared plasmids will be quanitfied and sequenced to verify plasmid construct is valid. Restriction digest was performed with XbaI SpeI to confirm construct.- ML

6.7.17_resdigestLuxLysR.tif

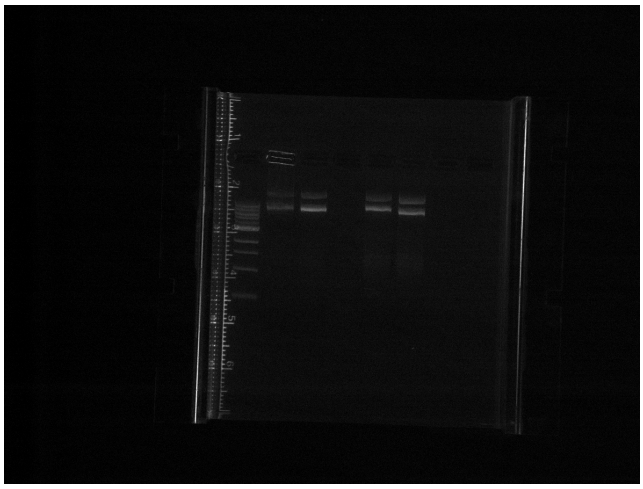


Table3

	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6
1	**1kB ladder**	**22 (I)**	**23 (II)**	**Blank**	**21 (IV)**	**24 (V)**

^Image of diagnostic agarose gel electrophoresis for XbaI SpeI restriction digest to confirm construct.
In each well, 12uL restriction digest was combined with 2.5uL loading dye (to create a 1X loading dye concentration). Gel was 1% agarose and samples ran at 75V for ~50 minutes. No bands are obseved at 1,000BP or 100BP as we hoped for. Therefore, we conclude that either the restriction digest of construct failed. Likely, we will need to attempt the restriction digest again before proceeding with the sequencing. -PN

THURSDAY, 6/8/2017

- Continuing the growth curve for our DH5a
- Finished growth curve and created competent cells --> tested for competence and aliquotted in the -80
- Re-doing the restriction digest with the plasmids obtained from the 4 unique colonies of the transformed Ecoli from 6/5. Test with 2 colonies at 250 ng DNA, .5 microliter of each restriction enzyme, and running incubation for 60 minutes.

Table 4: Completed Growth Curve from Wednesday 6/7/2017

OD600

	A	B	C
1	9:00 am 06/07	0	
2	10:30	0.008	
3	12:00 pm	0.009	
4	1:30	0.016	
5	3:00		
6	4:30	not turbid .01	
7	6:00	-0.004	<--we suspect blank is contaminated?
8	7:30	0.03	<--measured with fresh blank
9	9:00	0.110	
10	10:30	0.042	
11	12:00 am	0.06	<--measured with fresh blank
12	1:30 am 06/08	0.082	
13	3:00	0.099	
14	4:30	--	
15	6:00		
16	7:30		

6-7-17 res Digest Xbal Spel LysR-Lux.jpg

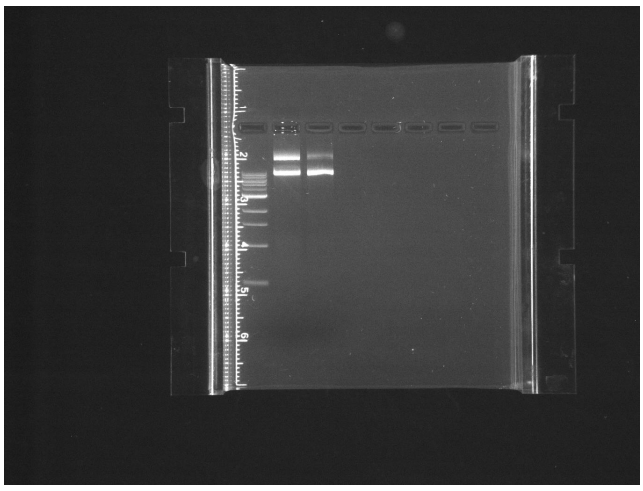


Table4

	A	B	C	D	E
1	1 kb ladder	II	I		

SATURDAY, 6/10/2017

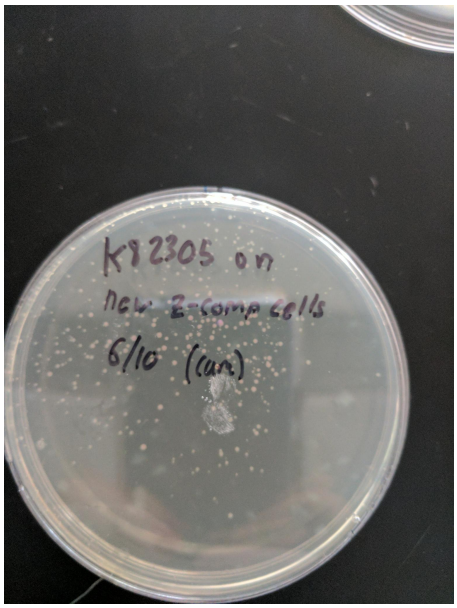
PCR failed on 6/9 being rerun today. K823025 Lux transformed to purify tomorrow for use in gibson assembly. We will digest this vector with both XbaI/SpeI and EcoRI/PstI pairs. Previous Gibson transformations did not pass Restriction Digest control indicating our insert was likely not present in the culture. We are starting from scratch on Gibson in order to remove poor quality DNA parts as a source of error.- ML

MONDAY, 6/12/2017

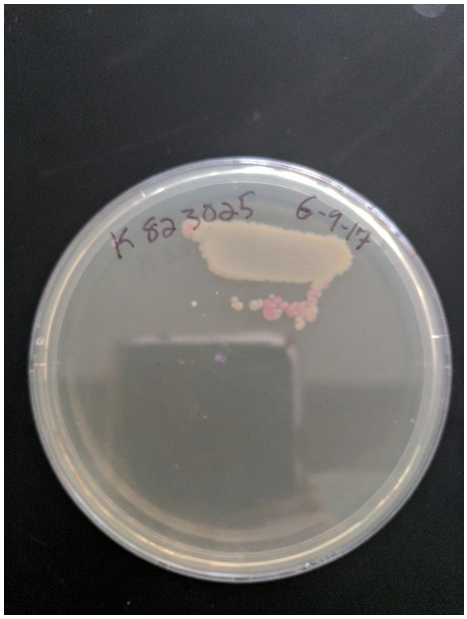
K82305 tranformation, new comp cells, CAM and AMP plates

IMG_20170612_092904.jpg

IMG_20170612_092859.jpg



IMG_20170612_092850.jpg



Doing PCR of LysR-PmmsA following standard protocol outlined in images on the PCR for Gibson protocol.
PCR for PmmsA alone will be done by changing the extension temp to 70 C and lowering annealing time to 45 sec.
LysR-PmmsA extracted from gel:

NanodropOne

	260/280	260/230	ng/uL	D
1	2.48	0.02	4.1	
2	2.3	0.04	6.5	

TUESDAY, 6/13/2017

Cleaning K823025 digest of Xba/Spe and EcoRI/Pst. Nested PCR of LysR-PmmsA
D:98-10s; A:53-20s; E: 68-45s
Nested PCR of PmmsA
D:98-10s; A: 55-20s; E 68-30s
Used 6/10 LysRPmmsA and 6/12 LysRPmmsA as templates following PCR for Gibson protocol

image.png

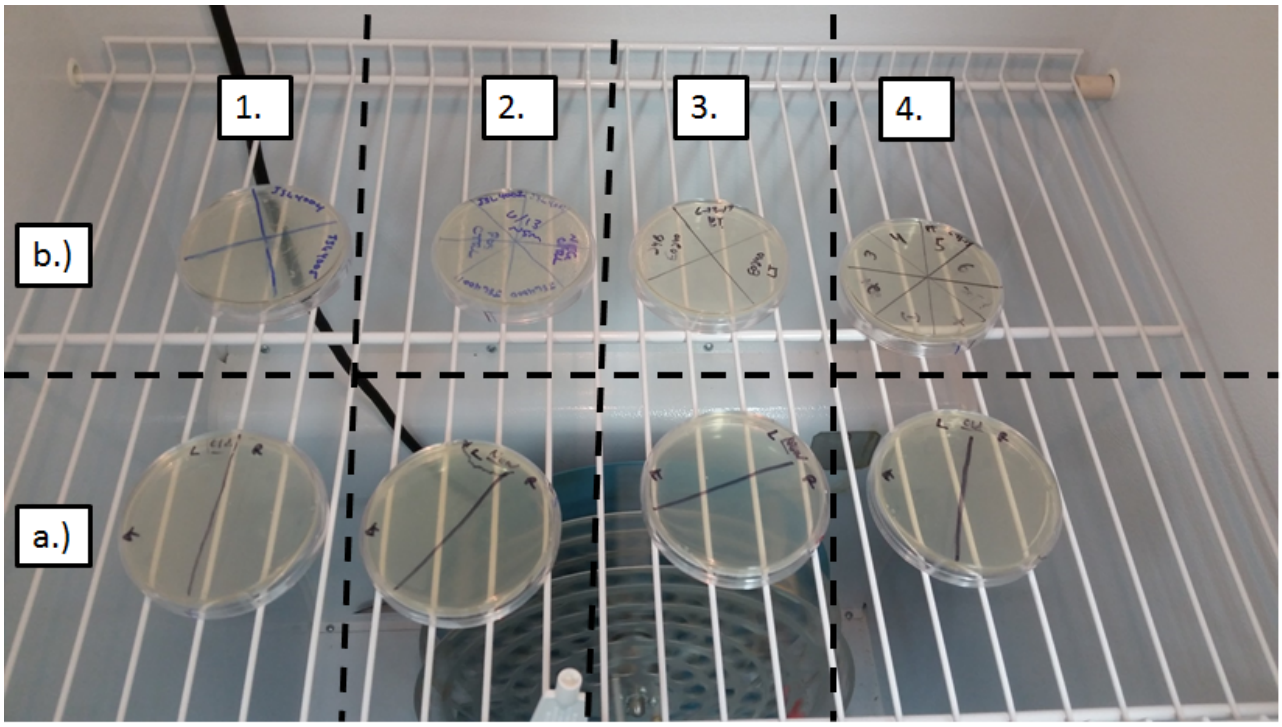


Figure : (a) Paul Retransform old/new part k823025 onto old/new AMP/CAM plates. (b) Paul/Niko restreak Interlab transformed cells onto old/new CAM plates.

Table 5: Identification of the plates in the incubator 6/13/17. I note first which plates where used (antibiotic and if it was the old/new batch). Then I note what part specifically is in the plates.

Table5

	A	B	C	D	E	F	G	H
1		1	2	3	4			
2	b.)	CAM(old)/Misc. Interlab Parts	CAM(old)/Main Interlab Parts	CAM(new)/Misc. Interlab Parts	CAM(new)/Main Interlab Parts			
3	a.)	CAM(old)/K823025	CAM(new)/K823025	AMP(new)/K823025	AMP(old)/K823025	R = Fresh Plasmid Obtained 6/12	L = Old Plasmid Obtained 5/25	Used second batch of DH5a cells

Further explanation:

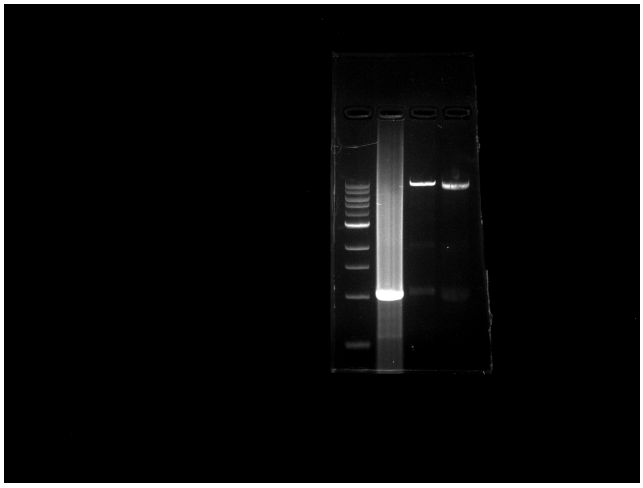
For row a.)

I redid the transformation of our standard part K823025. Nina mentioned something weird about the a260/280/230 values on the part that was collected on 6/13, so I decided to go transform an older stock of the K823025 part from 5/25. Thus, on each plate, there are two transformations: The 6/13 plasmid into DH5a (these sides are labeled "R" on the petri dish) and the 5/25 plasmid into DH5a (these sides are labeled "L" on the petri dish). Additionally, we were unsure as to whether our old plates were good, so I performed the transformation on both old & new CAM & AMP plates. I hope it works.

For row b.)

In preparation to perform the interlab study measurements, we restreaked the stock parts from the -80 onto old/new CAM plates. The plan is to select singles tomorrow and grow up overnight cultures in liquid media. God Speed.

6-12 Res Digest and LysRPmmsA.tif

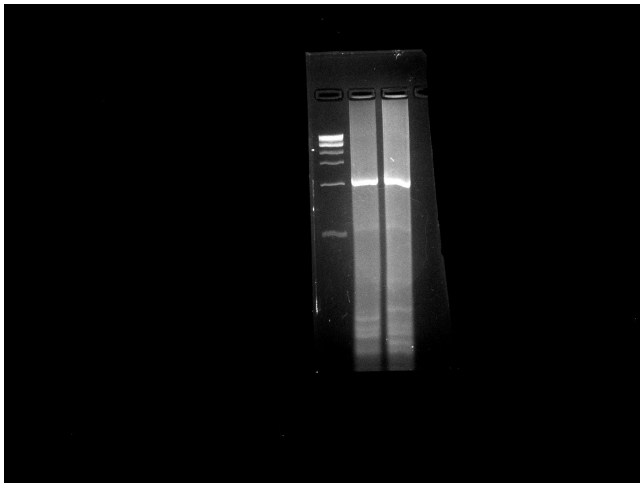


1kb ladder, LysR-Pmmsa, Xba/Spe RD of k823025, EcoRI/Pst RD of k823025

6-13 PCR PmmsA.jpg

2 log ladder, PmmsA fomr 6/12 template, PmmsA from 6/10 template

6-13 PCR LysR-PmmsA.jpg

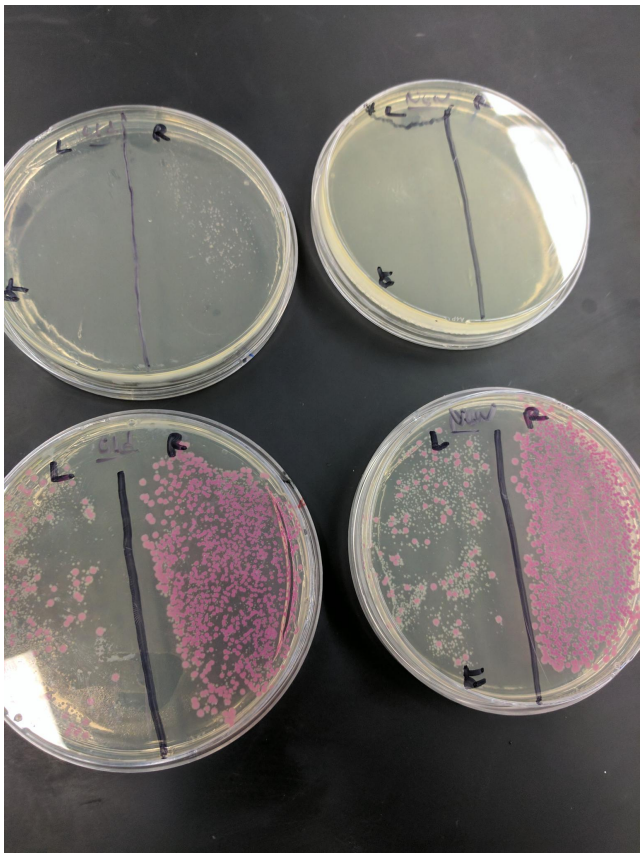


1 kb, LysR-PmmsA 6/12 template, PmmsA from 6/10 template

WEDNESDAY, 6/14/2017

The redid transformation of K82305:

IMG_20170614_103022.jpg



We observe the same results as last time where cells grow at a profoundly lower efficiency on CAM. In addition, our old stock (5/25) of K82305 has many colonies not exhibiting RFP and also has a lower efficiency. The newer (6/13) stock also has colonies (not satellite) the do not exhibit RFP but exhibit antibiotic resistance.

THURSDAY, 6/15/2017

K823025 transformation is over-grown
Picked 2 colonies for overnight culture (grown in 5ml LB + amp)
into 37C at 10am

IMG_2118.JPG

Gibson assembly sequence overview:

- [x] - Use calculator to test concentration (06/15)
  2.5 uL DNA + 2.5 uL Gibson = 5 uL to transform
- [x] - Gibson protocol on the leftmost thermocycler (06/15)
- [x] - Transform (06/15)
  ligation protocol w outgrowth onto AMP plates (5 plates -4 gibson 1 k823025 + control)
- [x] - Incubate 10-14 hr- TRY NOT TO INCUBATE MUCH LONGER
  in at 3 --> out between 1-5 am
- [x] - Pick 8 "sick" white colonies
  each colony: streak for singles, overnight in LB-AMP
- [ ] - Miniprep + restriction digest

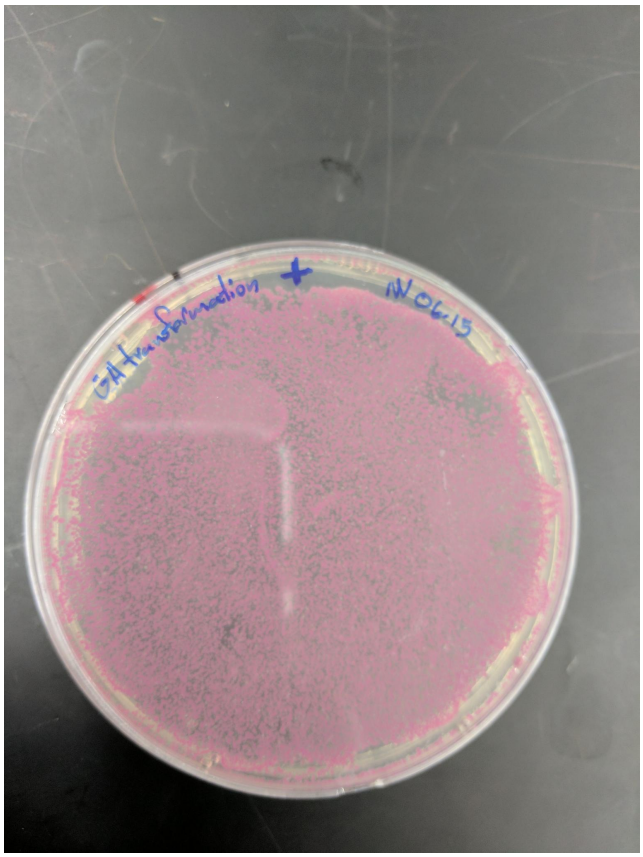
Gibson Assembly 06/15

	A	B	C
1		no insert	LysR-PmmsA, K823025
2	Xba/SpeI	#23	#20
3	EcoR/PstI	#24	#22

FRIDAY, 6/16/2017

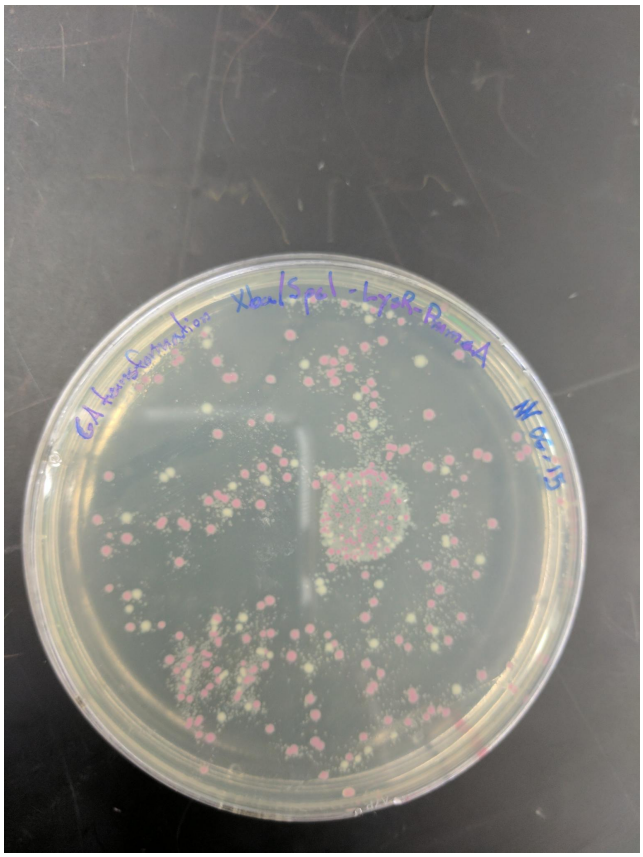
GA transformation plates taken out at 10am:

IMG_20170616_101812.jpg



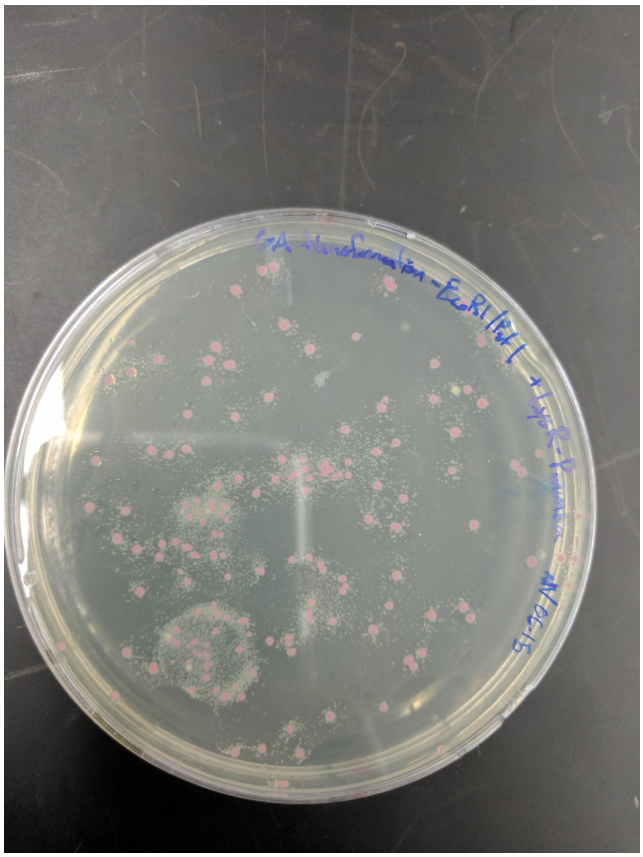
Positive control

IMG_20170616_101819.jpg



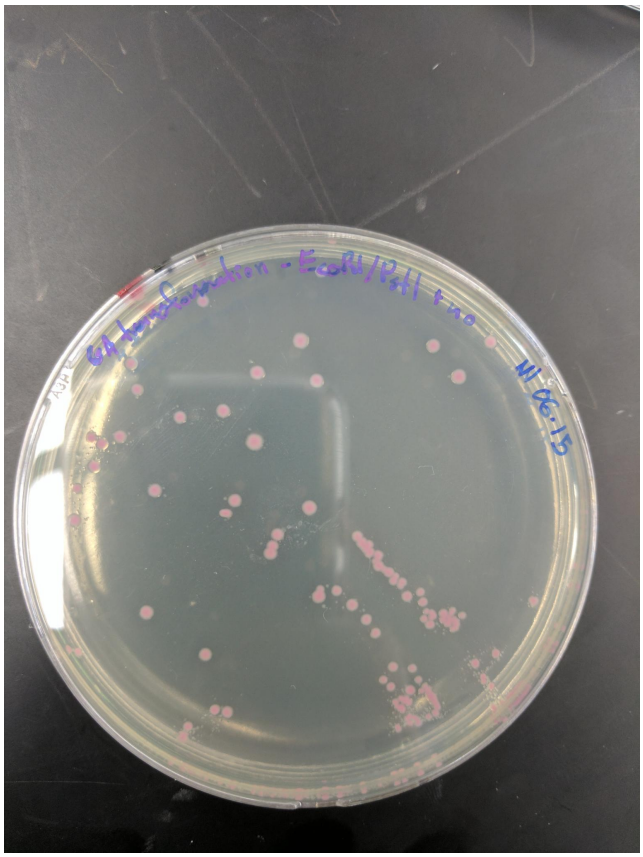
Xba/SpeI, LysR-PmmsA (1)

IMG_20170616_101825.jpg



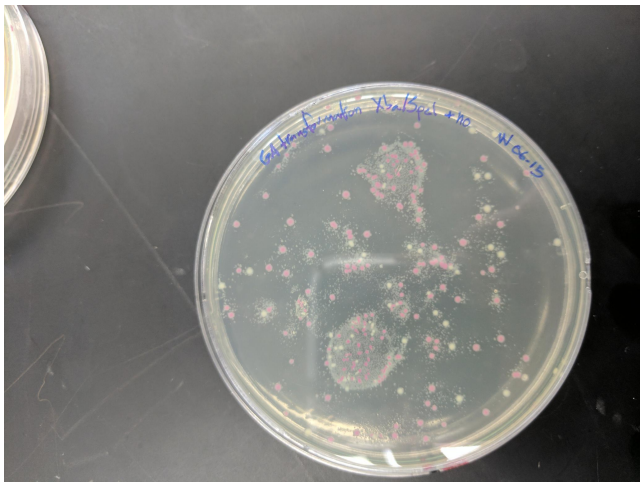
EcoRI/PstI, LysR-PmmsA (2)

IMG_20170616_101834.jpg



EcoRI/PstI, no insert (3)

IMG_20170616_101844.jpg



Xba/SpeI, no insert (4)

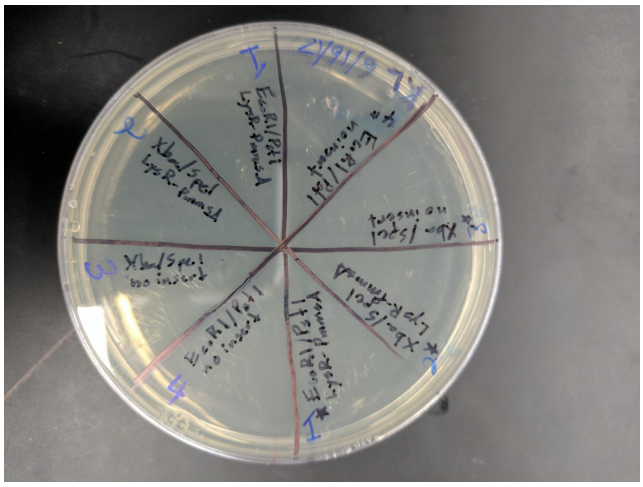
(^ These plates are currently parafilmed and stored in the fridge in case people want to see them in person after the weekend 06/19)

Streaking for single colonies: 2 segments per plate

- Dot or star corresponds to which colony was used on original plate
- Note: NO white colonies on {EcoRI/PstI, no insert}

New labeling:

IMG_20170616_124302.jpg



Liquid overnights: 2 5 mL cultures per plate

- Dot or star corresponds to which colony was used on original plate

- Miniprep K823025 overnights from 06/15am (also recorded on the plasmid list page)

Nanospec 06/16

	A	B	C	D	E
1		label	ng/uL	260/280	260/230
2	K823025	1A	33.4	2.10	1.82
3	K823025	1B	38.6	2.21	1.28
4	K823025	1C	49.4	2.06	1.70
5	K823025	2A	54.5	2.12	1.02
6	K823025	2B	34.1	2.34	1.41
7	K823025	2C	58.0	2.13	1.20

Met with Dr. Fassler: bad plates (unexpected RFP) likely the result of reuptake of undigested plasmids
--> Repeat restriction digest with EcoRI/PstI on K823025

SATURDAY, 6/17/2017

Gel for restriction digest showed no difference in band pattern between digested and undigested plasmid (06/17)
--> Repeat digest of K823025 with EcoRI/PstI?

Colony PCR: streak plates from 06/15 GA

- thermocycler protocol called "colony"
  - D: 5 min, 95°C
  - A: 35 cycles: 1 min, 95°C; 1 min, 53.8°C; 1 min, 72°C
  - E: 5 min, 72°C

PCR loading pattern

	A	B	C	D	E	F
1	1					
2		2	3	4	5	6
3	7	8	9	10	11	12
4	13	14	15	16		

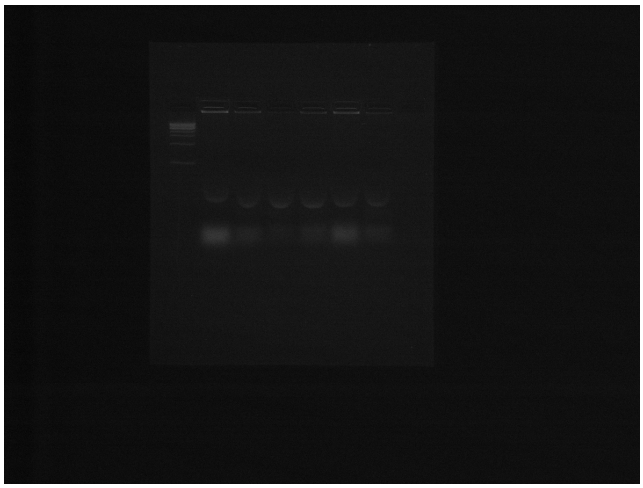
Innoculate liquid LB+AMP w streaked cultures undergoing colony PCR

Colony PCR Diagnostic Agarose gel from 6/15 GA
-10uL PCR product + 2 uL sample dye into each well
-2.5uL 1KB ladder loaded

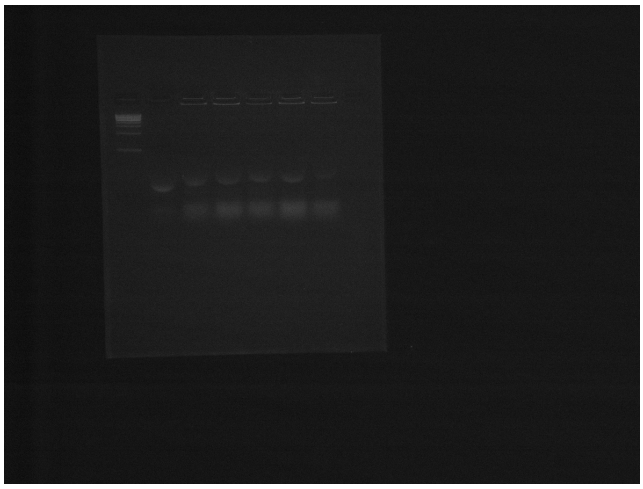
Table6

	A	B	C	D	E	F	G	H
1		Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
2	Gel 1	1KB Ladder	k8	1	2	3	4	5
3	Gel 2	1KB Ladder	6	7	8	9	10	11
4	Gel 3	1KB Ladder	(+)	13	14	12	15	16

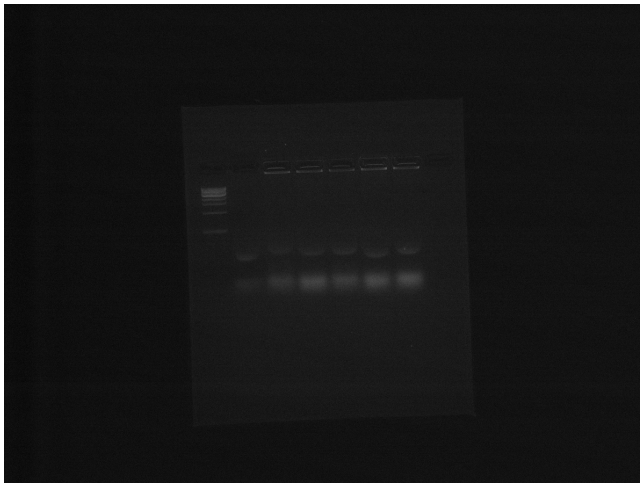
6.17.17_colonyPCR_gel1.tif



6.17.17_colonyPCR_gel2.tif



6.17.17_colonyPCR_gel3.tif



All lanes in all gels appear similar. Ladder is weak, but controls look similar to the gel lanes so we believe we have obtained the expected ~1.1kB product or primers aren't binding properly resulting in all negatives. May need to run another gel with more ladder to confirm. Gels are stored in 4' on the side door, PCR tubes with remaining PCR product are in the -20. ~PN + TH

Please use jpeg not tif files!!

MONDAY, 6/19/2017

Interlab experiment --> expand

TUESDAY, 6/20/2017

Minipreps:

LysR-Lux

	A	B	C	D
1	Tube name	260/280	260/230	ng/uL
2	9	1.88	2.25	142.6
3	15	1.89	2.32	158.1
4	12	1.88	2.30	226.4
5	10	1.89	2.25	149.7
6	16	1.89	2.18	131.6

Restriction digest:

Tube order

	A	B	C	D	E	F
1	12, XbaI/SpeI					
2		9, EcoRI/PstI	15, EcoRI/PstI	16, EcoRI/PstI	10, EcoRI/PstI	12, EcoRI/PstI

Gel: 1%

6-20-17 RD of Gibson Assembly.jpg

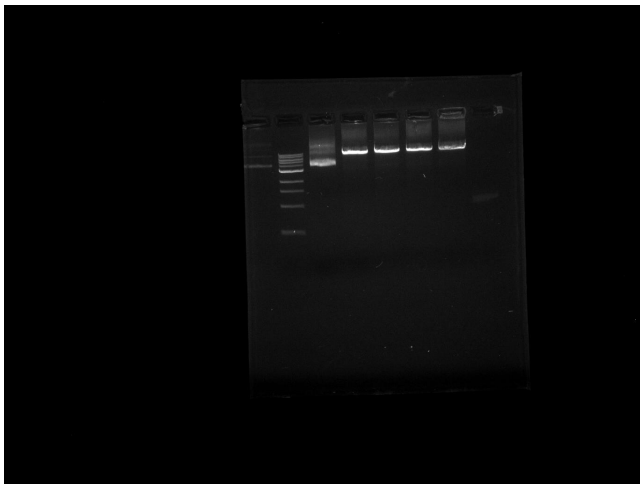


Table7

	A	B	C	D	E	F	G	H
1	12 Xba	1 kb	9	15	16	10	12 Eco	LysRPmmsa

WEDNESDAY, 6/21/2017

Faculty meeting -->

THURSDAY, 6/22/2017

MONDAY, 6/26/2017

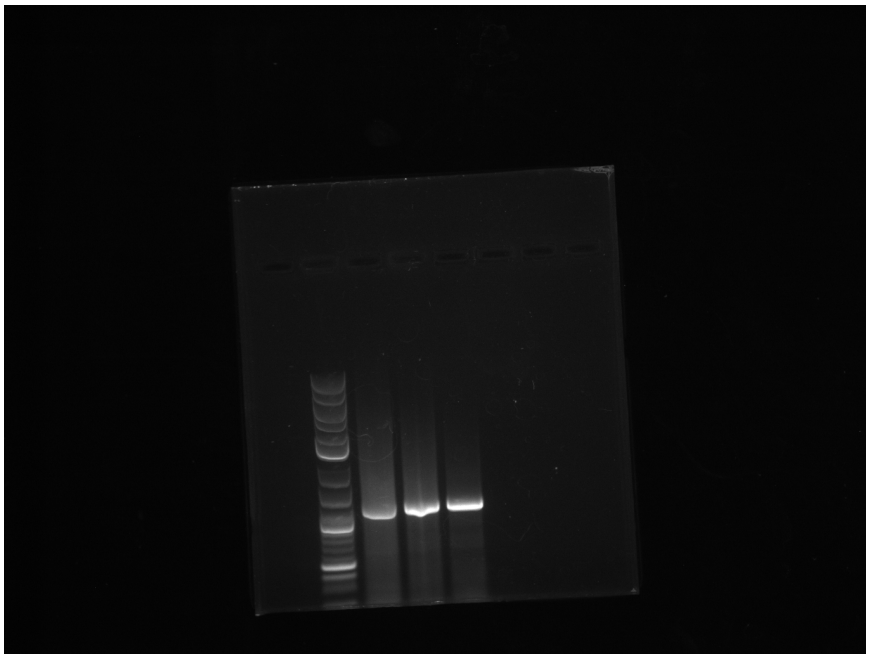
Fragment preparation -VDZ

1. PCR amplify the PmmsA LysR combination with primers 80, 81
    #20: 1 X [] of template (1 ul)
    #21: 0.1 X
    #22: 0.01 X
2. Run the LysR/PmmsA PCR products on a gel to evaluate size, quality/quantity.
    2.5uL purple 2 log ladder, 5 uL PCR product + 1 uL 6X purple loading dye (expect 1.1 kB)
    Run @ 75 V --> start 2:30

Gel 06/26

	Well 1	Well 2	Well 3	Well 4
1	2 log ladder	1 uL template	0.1 uL template	0.01 uL template

6.27.17_GblockPCRproducts.tif

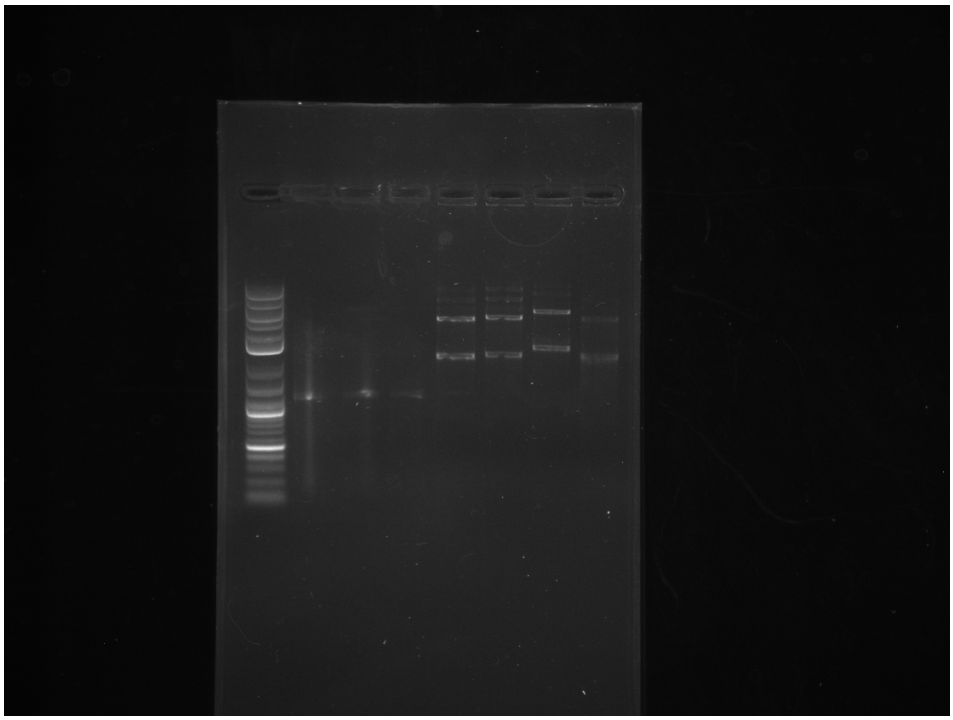


3. Do a PCR clean up reaction (Qiagen kit)
4. Digest a portion of the purified PCR products with EcoRI and PstI (Save some PCR product in the undigested form)

Vector preparation (from email)
1. Resuspend a linearized backbone (provided in the kit) in 50 microl ddH20 => 25 ng/microl
2. Digest 4 microl backbone with EcoRI-HF and PstI-HF + DpnI. (37C 30m -3h followed by heat kill 80C for 20m). See http://parts.igem.org/Help:Protocols/Linearized_Plasmid_Backbones.

Step 3. A vector only ligation (done as a control alongside the vector+fragment ligations) can be analyzed on a gel. An efficient ligation will yield more double length bands than single. If the control ligation is transformed (use 1 microl), there should be very few transformants because the two cuts (RI and PstI) do not leave complementary ends. If there are (too many) transformants, this may be due to the presence of circular DNA (template) from the PCR reaction used to generate the linear backbone in the kit. The inclusion of DpnI is meant to eliminate any circular template (the plasmid will be methylated but the PCR product will not be, and Dpn1 only cuts methylated DNA).
Other notes on vectors: Vectors provided in liberalized form are based on the pMB1 replicon. They are high copy number plasmids (COPY NUMBER IS 100—300). They are each approximately 2 kb in size.
Seehttp://parts.igem.org/Plasmid_backbones/Assembly for details. They were originally provided with the ccdB gene, but I believe they now have the RFP gene instead. The RFP fragment is ~1 kb but it is not included as part of the linearized PCR product provided in the kit.
All Biobrick parts have to be submitted in pSB1C3 so we should consider using that backbone.

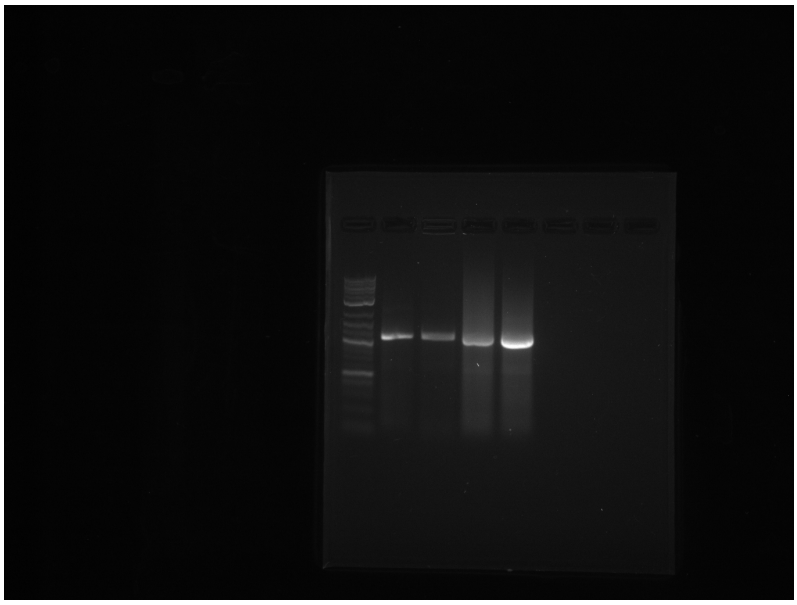
6.27.17_gblock&4vectors.tif



TUESDAY, 6/27/2017

Rerun Yesterday's Gel with digested fragment

6.17.17GblockPCR&digestion.tif



Finish fragment preparation-

5. Gel purify the products using Qiagen gel purification kit.
6. Check PCR product recovery from the gel; simultaneously run a small aliquot of vector.

Ligation

Step 1. Combine the vector and fragment at 1:3 molar ratio
Step 2. Set up a ligation reaction and allow it to incubate for an hour or more at 16 degrees
Step 3 (option) Set up ligations into Topo T/A plasmid using uncut PCR product

Fragment preparation

- [x] Repeat PCR amplification on Gblock with 80,81 primers, Q5 Master Mix -VDZ
  - 0.01 X [.08 ng/uL] of template (1 uL)
  - 0.001 X [.008 ng/uL]
  - 0.0001 X[.0008 ng/uL]
  - (left machine: td-1, "colony 2")
- [x] Run the Gblock PCR products on gel -VDZ
  - 2.5uL purple 2 log ladder, 5 uL PCR product + 1 uL 6X purple loading dye
  - Run @ 75 V

PCR products 06/27

	Well 1	Well 3	Well 4	D	Well 5
1	2.5 uL 2 log ladder	5 uL 0.01X	5 uL 0.01X (excess dye)	5 uL 0.001X	5 uL 0.0001X

- [ ] Gel purify

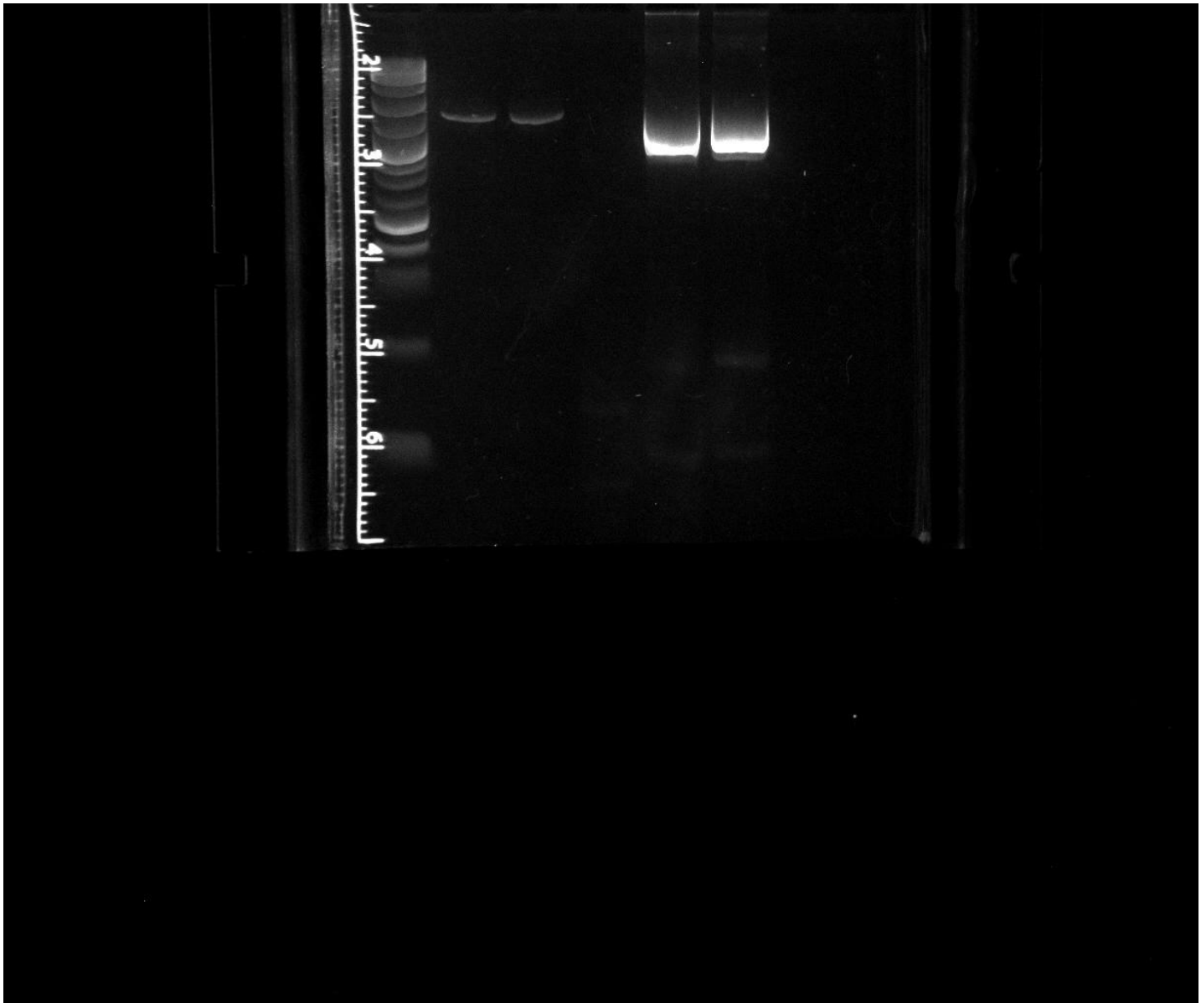
WEDNESDAY, 6/28/2017

Transformation Results:

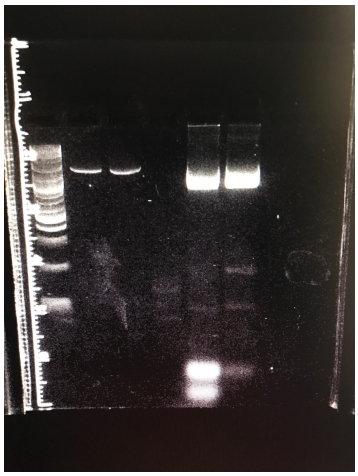
(psb1c3 vector)
+ Vector-alone --> a little growth
+ Vector+Frag --> much more growth
+ additional vectors (a3, k3, t3) --> no growth on CAM plates
--> Streaked for singles and started 4 overnight liquid cultures
---> Colony PCR Tomorrow

Well 1: 2 log
Well 2+3: LysRPmmsA
Well 4:PmmsA
Well 5+6: Digest of 2 red colonies with Xba/SpeI

6-22 RFP PCR.jpg



IMG_0123.JPG



Same as above, different contrast

THURSDAY, 6/29/2017

New CAM Plates
Miniprepped GA colonies and digested with Xba/SpeI (Figure below)
Gel 1: 1 kb ladder, no insert, LysR, Gblock, LysR,no insert, 1 kb ladder

20170629_Gel1_5wells.jpg

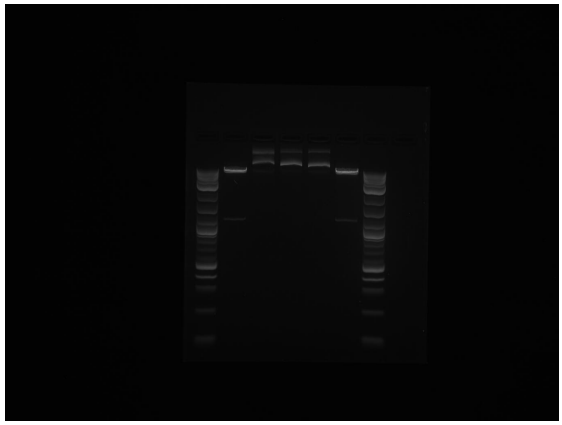


Figure : Gibson Assembly Products

Colony PCR Results (Displayed below): No fragment detected.
- Used K823025 as a colony PCR control (RFP lane)
- Used PCR product of fragment as gel control
- "+ fragment" indicates ligation with vector and fragment
- "- fragment" indicates ligation with vector alone

6.29.17ColonyPCRGel1.jpg

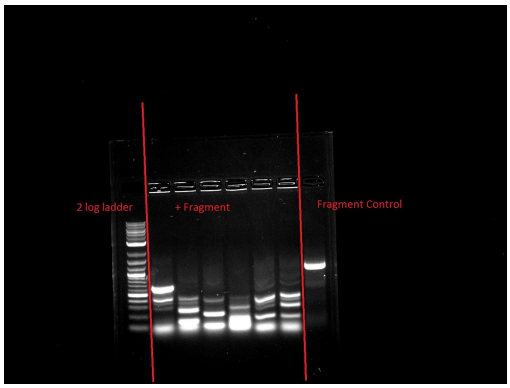


Figure: Overnight colonies from vector + fragment, and fragment control

6.29.17ColonyPCRGel2.jpg

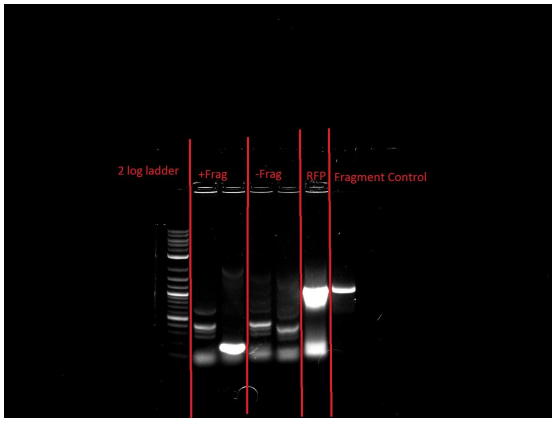


Figure: Overnight colonies from vector +/- fragment

FRIDAY, 6/30/2017

Colony PCR on remaining colonies from 6/27/17 Transformation (pSB1C3 + Fragment, pSB1C3 alone)

- RFP contains band @ about 1 kb
- Frag control contains a faint band @ about 1 kb
- None of the transformants contain a band @ about 1 kb --> Matches previous results from 6/29/17

6.30.17ColonyPCRResults.png

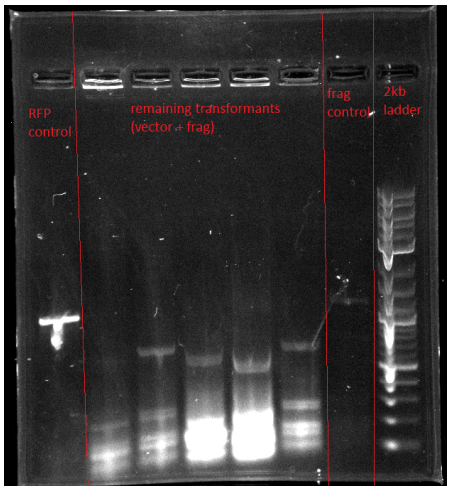


Figure : Colony PCR results from remaining transformants, an RFP control, and a lysR/pmmsA PCR control.

Transform remaining ligation mixtures from 6/27/17 (pSB1C3 + Frag, pSB1C3 alone, pSB1A3 + Fragment)

The following experiments utilized the linear backbones provided by iGEM (Located in the -20)
Restriction Digest:
- pSB1C3 --> EcorR1 + Pst1 + Dpn1
- pSB1A3 --> EcorR1 + Pst1 + Dpn1

6.30.17Digested VectorFrag.png

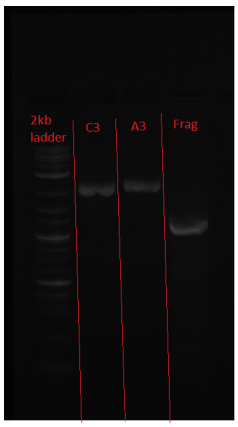


Figure : Gel with 2kb ladder, Vector pSB1C3 digested in lane 1, Vector pSB1A3 digested in lane 2, and digested Fragment in lane 3.

Gel Purification:
- pSB1C3 (digested)

- pSB1A3 (digested)
- Fragment [LysR + pmmSA] (digested)

6.30.17.png

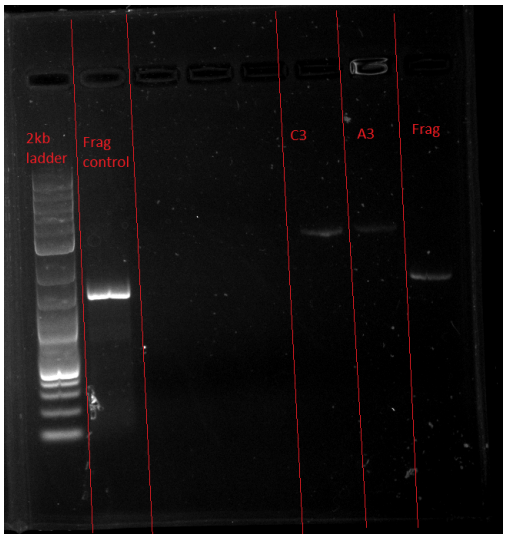


Figure : Diagnostic gel to check relative concentration of vector to fragment (last 3 wells are vectors, 2 well was a PCR fragment control)

note: for next gel purication, resuspend in 25 ul of EB instead of 50 ul EB (higher concentration)

Ligation:
1.) pSB1C3 + Fragment + Ligase
2.) pSB1C3 + Ligase
3.) pSB1C3 - Ligase
4.) pSB1A3 + Fragment + Ligase
-->Ligation placed in the fridge to incubate overnight. Start time: 3:30 PM. Will be taken out tomorrow morning ~ 10 AM to be transformed

SATURDAY, 7/1/2017

Transform overnight Ligation from 6/30/17
1.) pSB1C3 + Fragment + Ligase
2.) pSB1C3 + Ligase
3.) pSB1C3 - Ligase
4.) pSB1A3 + Fragment + Ligase
5.) control plasmid
6.) 0 DNA

MONDAY, 7/3/2017

Linear vector digest (EcoR1/PstI) repeat:

07/03

	digest	260/280	260/230	ng/uL
1	pSB1C3	7.48	0.03	23
2	pSB1K3	194.93	0.01	14
3	pSB1A3	203.68	0.01	12.5
4	pSB1T3	7.08	0.02	18.7
5	fragment	12.63	0.01	15.2

Ligation:
--> incubating in fridge ON

Table8

	A
1	pSB1C3+fragment+ligase
2	pSB1K3pSB1C3+fragment+ligase
3	pSB1A3pSB1C3+fragment+ligase
4	pSB1T3pSB1C3+fragment+ligase
5	pSB1C3+ligase
6	pSB1K3+ligase
7	pSB1A3+ligase
8	pSB1T3+ligase

Transformation Results:
1.) pSB1C3 + Fragment + Ligase --> Few Cells
2.) pSB1C3 + Ligase --> Few Cells
3.) pSB1C3 - Ligase --> No Cells
4.) pSB1A3 + Fragment + Ligase --> Few Cells
--> Performed Colony PCR on 1, 2, 4
--> Restreaked 1, 2, 4

Colony PCR Results:

7.2.17ColonyPCRgel1(2).tif

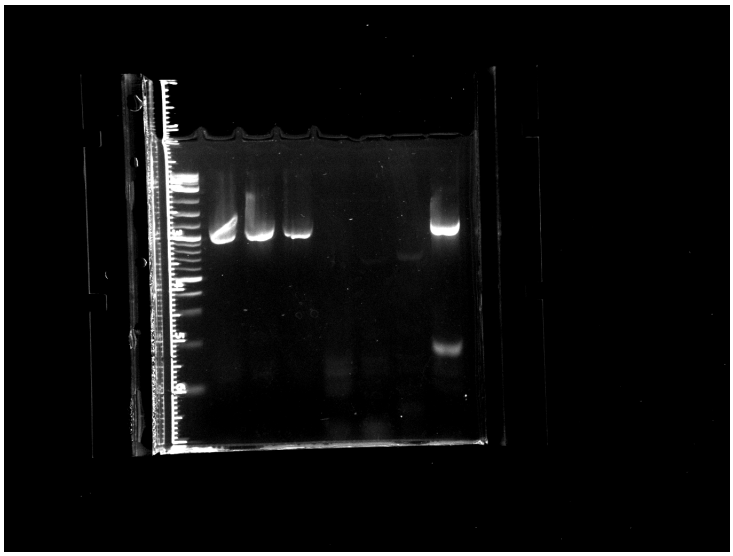


Figure : Colony PCR on transformants 1, 4, and a control colony (K823025).

7.2.17ColonyPCRgel2(1).tif

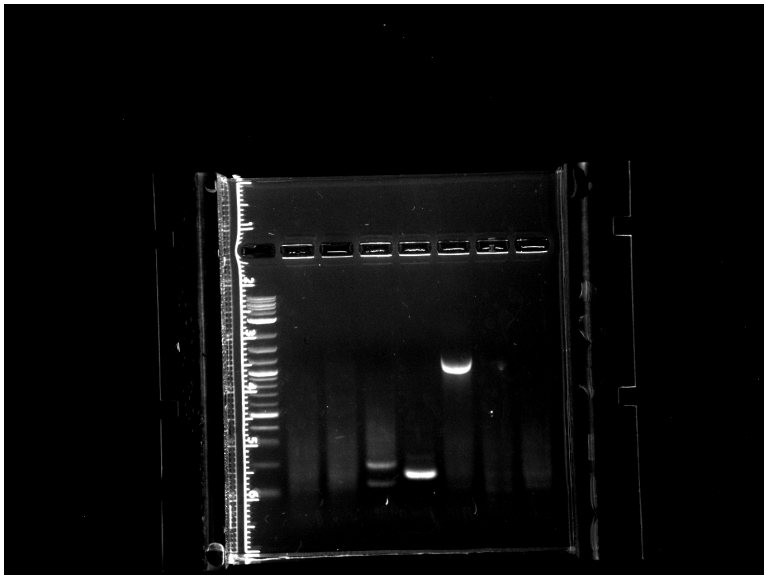


Figure : Colony PCR on ML transformants (Lux Vector + Fragment)

Table 9: Loading order for Colony PCR gel 1 & 2

Table9

	A	B	C	D	E	F	G	H	I
1		Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 8
2	gel 1	2kb Ladder	1	1	1	4	4	4	Control
3	gel 2	2kb Ladder	ML	ML	ML	ML	ML	ML	ML

Running new gels Wednesday.

TUESDAY, 7/4/2017

Transformation of ligation reactions from 07/03
--> Only pSB1C3 and pSB1A3, no tetracyclin or kanamycin plates
Plates into 37C at 12pm

WEDNESDAY, 7/5/2017

Cleaned up Colony PCR gel:

7.5.17CleanColonyPCRgel(1).tif

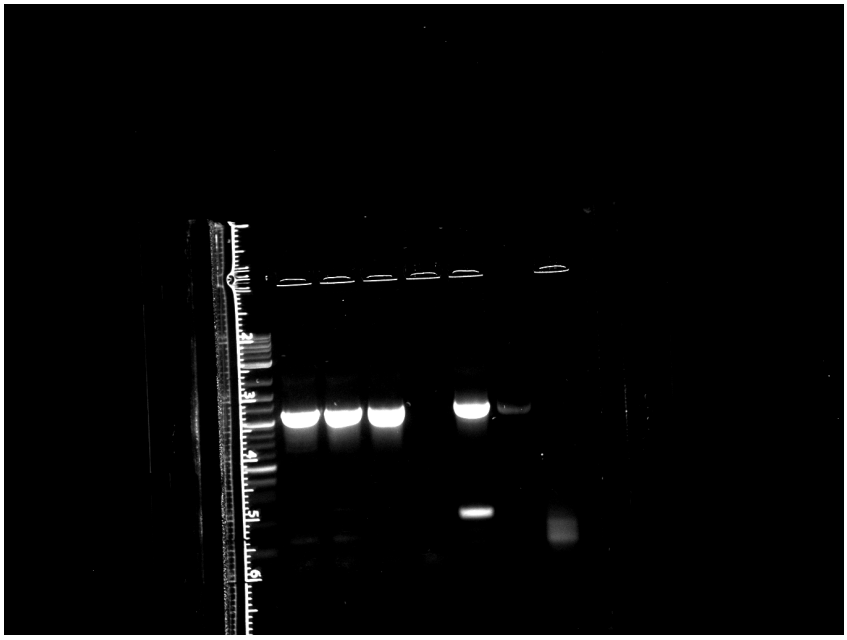


Figure : Colony PCR results with proper controls

Table 10: Loading order of Cleaned up Colony PCR gel.

Table10

	A	B	C	D	E	F	G	H
1	2kb Ladder	Vector/Frag	Vector/Frag	Vector/Frag	Vector Alone	RFP Control	Frag PCR	ML colony

THURSDAY, 7/6/2017

Colony PCR --> Can't find Mason's sacred colony
Isolate Vector DNA from pSB1C3 + Frag colonies
Design primers for sequencing pSB1C3 + Frag (our first biobrick)

FRIDAY, 7/7/2017

Started overnight on new Lux-LysR colonies. If unsuccessful, will work w Paul to do clean ligation. Recovered 8 sketchy colonies which were streaked to confirm identity.

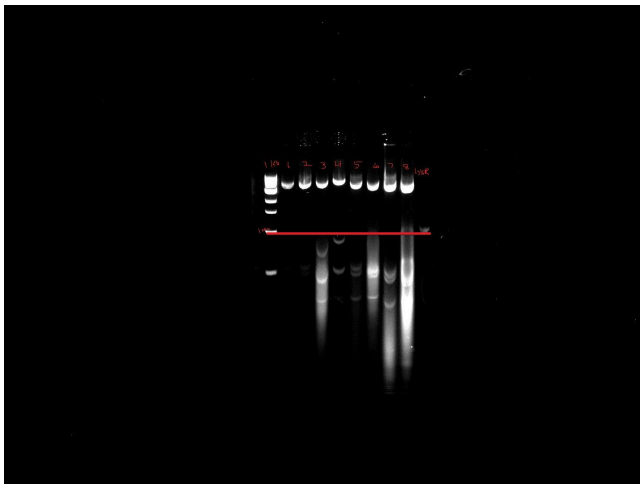
MONDAY, 7/10/2017

PCR and digestion products run on 2% AG gel.

TUESDAY, 7/11/2017

Lux-LysR: Ligation with clean DNA... what could we improve/change

7-11-ResDigest LysRLux.jpg



WEDNESDAY, 7/12/2017

Beginning a very precise LysR and PmmsA journey... I will be taking this slow and methodically.

Gel:
2kb ladder (2.5 uL) - J20200 (10uL+2dye) - E2022 (10uL+2dye)

MONDAY, 7/24/2017

- Racked pipette tips, ready to be autoclaved
- Continuing 3A assembly with GFP reporter (colony PCR)
- more info: LysR w/ Other Reporters (Lux suxs)

WellOrientation

	A	B	C	D	E	F
1	plate 1, dot colony	1, star	2, dot	2, star	3, dot	3, star

-Bands very smeared, not readable (saved on back table in saran wrap) - repeat tommorrow with saved product

WEDNESDAY, 8/2/2017

Started LysR-RFP construct with E1010, LuxLysR, and pSBK3. 3A Tfx today. If doesn't work, will PCR backbone, purify each part, and religate.
PmmsA-Lux- Rerunning digests/gel today to confirm presence. If not real, will repeat and retry with 500 insert to 1 backbone.

SATURDAY, 8/5/2017

LysR-E1010-Kan backbone ligation worked and the gel is below. Next step is to put into psbC3 backbone and send for sequencing to verify.

LysRRfp8_4.pdf