Week of Sep 2

TUESDAY, 9/4/2018

Made Liquid Cultures

- piG 207, 209, 224 (2), 225 (2)

Ran Digest and Gel Analysis

- 37C for 1 hr
- Hold at 4C

Left Chamber

	A	B	C	D	E
1	Well 1	Well 2	Well 3	Well 4	Well 5
2	Ladder	231	235	223	

Right Chamber

	A	B	C	D	E
1	Well 1	Well 2	Well 3	Well 4	Well 5
2	Ladder	227	236	230	

Origin Tests: C. glut and S. mel

1. Transformed C. glut and S. mel with 100 ng of plasmid (should this be more?) containing RSF1010, pBBR1, and pWV01 origins (one plasmid per transformation)
    a. DNA used was unmethylated
    b. electroporated C. glut, 2.5 kV, electroporated S. mel, 2.1 kV
    c. Let rest in LB in shaking incubator; C. glut rested 1 hr; S. mel rested 2 hrs (should have been 3, but it was really late)
2. Plated 25 uL ea. of C. glut transformants on plain m17 agar and Kan50 LB agar plates
    a. also plated untransformed C. glut on Kan plate as neg. control
3. Plated 25 uL ea. of S. mel on plain m17 agar and Neo100 LB agar plates
    a. also plated untransformed S. mel on Kan plate as neg. control
4. In appropriate incubator at 11:50-ish pm

WEDNESDAY, 9/5/2018

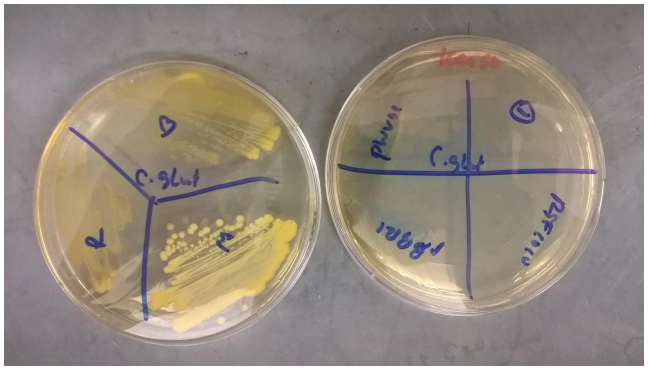
Minipreps

THURSDAY, 9/6/2018

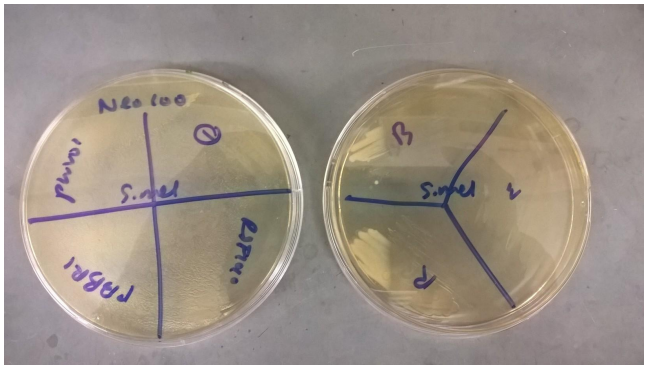
Origin Test Results: C. glut and S. mel

1. No growth for any origin on the antibiotic plates; growth for all origins on plain LB plate

Origin testing C. glut 2.jpg



Origin testing S. mel 2.jpg



FRIDAY, 9/7/2018

Cloning piG230, 231, 232, 233

SATURDAY, 9/8/2018

Making electrocompetent S. oneidensis

1. Picked a colony of S. onei into 5 mL plain LB
2. In 30C shaking incubator at 5:00 pm

Week of Sept 9

SUNDAY, 9/9/2018

Cloning of cassettes for orthogonal translation

RSF1010 cassettes only had green colonies. p15A cassettes has white colinies.

Colony PCR piG231, piG233

Untitled.png

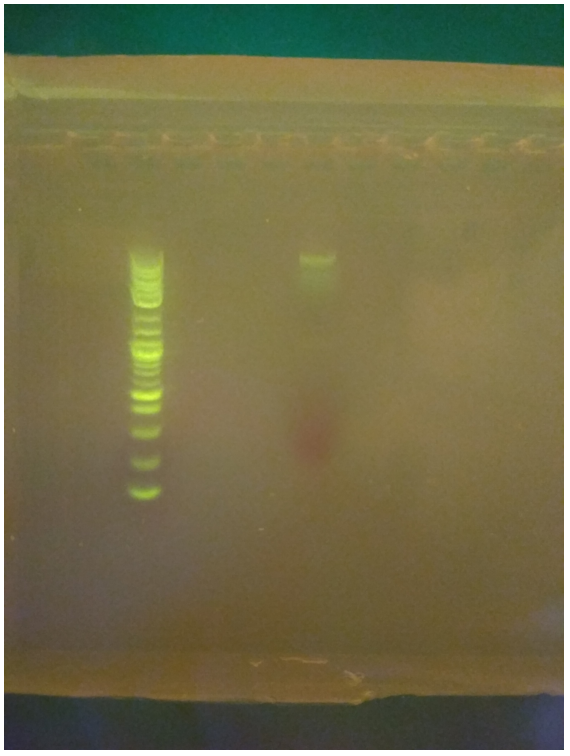
Optimal Q5/Phusion Pol PCR						Optimal Q5/Phusion Pol PCR, copy 1					
Reactions	8	*GC Enhancer?*	no			*Reactions*	4	*GC Enhancer?*	no		
Final [DMSO]	0%	*Stock [Primer]*	100	µM		*Final [DMSO]*	0%	*Stock [Primer]*	100	µM	
Excess	5%	*0%*	–			*Excess*	5%	*0%*	–		
Reactions	8.4	8.	*1 ref*		*Target:*	*Reactions*	4.2	4.	*1 ref*		*Tarç*
diH_2O	310.8	296.	37.	µL		diH_2O	155.4	148.	37.	µL	
5× Q5 reaction buffer	84.	80.	10.	µL	*1×*	5× Q5 reaction buffer	42.	40.	10.	µL	*1×*
10 mM dNTPs	8.4	8.	1.	µL	*0.2 mM*	10 mM dNTPs	4.2	4.	1.	µL	*0.2*
DMSO	0		0	µL	*0–5%*	DMSO	0		0	µL	*0–5*
5× GC Enhancer	0		0	µL	*1×*	5× GC Enhancer	0		0	µL	*1×*
2 U/µL DNA polymerase	4.2	4.	0.5	µL	*0.5 µL/50 µL rxn;0.*	2 U/µL Q5 DNA polymerase	2.1	2.	0.5	µL	*0.5*
Primer F	2.1	2.	0.25	µL	*0.5 µM*	Primer F	1.05	1.	0.25	µL	*0.5*
Primer R	2.1	2.	0.25	µL	*0.5 µM*	Primer R	1.05	1.	0.25	µL	*0.5*
1 pg – 1 µg template	8.4	8.	1.	µL	*1 pg–1 µg*	1 pg – 1 µg template	4.2	4.	1.	µL	*1 pg*
Total:	420.	400.	50.	µL		*Total:*	210.	200.	50.	µL	
Total–Template:	411.6	392.	49.	µL		*Total–Template:*	205.8	196.	49.	µL	
Total–Primers:	415.8	396.	49.5	µL		*Total–Primers:*	207.9	198.	49.5	µL	
Total–Template & Primers:	407.4	388.	48.5	µL		*Total–Template & Primers:*	203.7	194.	48.5	µL	

Digest of piG223

Digested with BsaI to check

Result: Looks as expected. (there's a faint band at 1 kb)

image.png



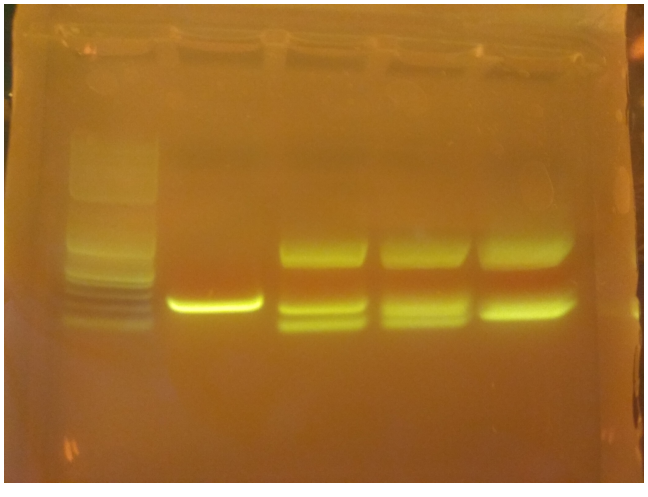
Transformations

Transformed piG241 into P. outida and S. oneidensis

PCR on the assembled cassettes

Did PCR with B17, B18 primers . Worked!

image.png



mKate overnight fluorescence measurements for piG241 for DH10B, P. putida, BL21, and Nissle

Set up the assay in LB to measure fluorescence over time of piG241 construct in the strains indicated.

Fluorescence Measurements for HW elements in P. putida

Making Competent S. oneidensis

1. Streaked out S. onei onto plain M17 agar plate
2. Left in 30C

MONDAY, 9/10/2018

Transformations

Completed a total of 9 transformations via electroporation

9/10 Transformations

	A	B	C	D	E	F	G	H	I	J
1	**DNA**	piG202	piG204	piG207	piG208	piG209	dcm- gfp	piG241	piG241	gfp
2	**Cells**	S. onedensis	S. onedensis	S. onedensis	S. onedensis	S. onedensis	S. onedensis	BL21	P. putida	P. putida

Procedure:

- Used 1 uL of DNA for all samples
- Note: Aliquots of comp cells varied in volume. 204 and 207 even had more than 100 uL (of S. onedensis); noticed some liquid lying above the space between the plates. I also apparently cannot count nor read, and I initially thought there were 7 Shewanella transformations when we had only 6 aliquots left in the freezer. I went through a complicated process of dividing one of the aliquots in two, only to kick myself when I realized I needed 6 to begin with. **Important part of this story: I put the now extra aliquot of S. onedensis comp cells back in the -80 freezer for later use.** I don't know if this was a good idea and how it will affect the competency of the cells, so note for future reference.
- Shocked cells. The readings for these ranged from 3-5.7 ms
- Saved cells with 1 mL SOB
- Let cells shake and recover for 1 hour
- After 1 hour, plated on Kan
    - Plated 500 uL for piG202, then reeled it back to 300 uL for the rest of the plates because there was a LOT of excess culture that would drip on to the lid
    - Used streaking pattern
    - Stored plates around 11:10 PM in top incubator in Keck

Liquid culture

Made overnight liquid culture from 225-m plate

Procedure:

- 4 mL LB
- 4 uL Kan50
- Toothpick to pick circled colony
- Placed in incubator around 10:00 pm

Prepping Gels

Notes: Made two 1% gels to use tomorrow and left them on the gel casting table. Used the 1.5 combs; I hope the wells are big/small enough for whatever you plan to do. One of the gels is a little shallow because I ran out of pre-made gel solution.

Restocked all the bottles of pre-made gel:

1x TAE

- 16 mL 50x TAE
- 800 mL H2O

0.8%

- 1.98 g Agarose
- 250 mL TAE

1%

- 2.50 g Agarose
- 250 mL TAE

1.5%

- 3.75 g Agarose
- 250 mL TAE

2%

- 5.00 g Agarose
- 250 mL TAE

Making Competent S. oneidensis

1. PIcked one colony of S. oneidensis into 5 mL LB
2. Left in 30C shaking incubator overnight

Cloning piG226, 231, 233

1. Transformed piG226, 231, and 233 into NEB Turbo
    a. heat shock w/ 4 uL of DNA
        I. thawed Turbo on ice, added 4 uL DNA, incubated for 30 min on ice
        II. put aliquots in 42C heat block for 30 sec
        III. immediately put back on ice for 1 min
        IV. added 150 uL SOB to aliquots and let rest in 37C shaking incubator for 1 hr
2. Plated 10 uL of ea. transformant on Amp100 plate

TUESDAY, 9/11/2018

Golden Gate Assemblies

Making Competent S. oneidensis

1. Inoculated 50 mL LB with 500 uL of S. oneidensis culture from yesterday
2. In 30C shaking incubator at 9:30 am
3. Took out at 8:30 pm; culture was very turbid
4. Pelleted by centrifugation (4000xg, 5 min, 20C)
5. Discarded supernatant
6. Resuspended pellet w/ 6 mL D sorbitol
7. Pelleted by centrifugation (3 min, highest rpm of microcentrifuge, room temp)
8. Discarded supernatant
9. repeat steps 6-8 three more times

10. Resuspended final pellet in 1 mL total D sorbitol
11. portioned into 50 uL aliquots

Cloning piG226, 231, 233

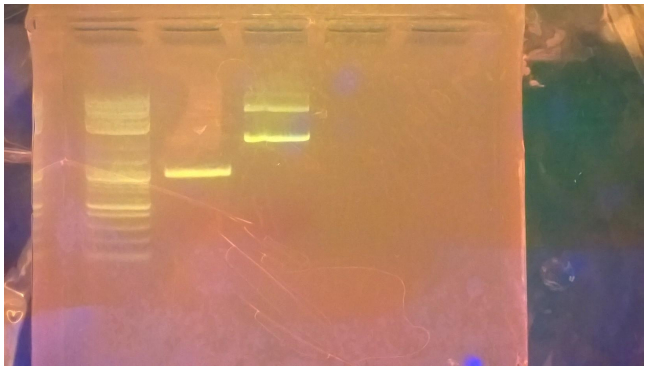
1. Took out 226, 231, 233 plates; only 226 and 233 had a non-green colony
2. Picked non green colony of 226 and 233 ea. into 4 mL LB + Amp
3. Incubated culture in 37C shaking for about 11 hrs
4. Miniprepped 226 and 233
    a. [226] = 138 ng/uL
    b. [233] = 500 ng/uL

WEDNESDAY, 9/12/2018

Cloning piG226, 231, 233

1. Digested 226 and 233 w/ Esp3I and NotI, respectively (37C for 1 hr)
2. Ran 226 and 233 digest on 1% agarose gel, 100V
    a. from left to right, 1 kb ladder, piG226, piG233
    b. 226 expected band sizes: ~2.0 and 1.0 kb
    c. 233 expected band sizes: ~2.0 and 1.0 kb
    d. 226 is missing a band; both 233 bands are too big

20180912 Digest of piG226 and 233 Gel.jpg



1. Plated 3 Amp100 plates w/ 20 uL ea. of piG231

Table1

	A	B	C	D
1	piG226	3.5	piG233	1
2	NEB 3.1	1.5	NEB 3.1	1.5
3	Esp3I	0.5	NotI	0.5
4	H2O	9.5	H2O	12
5	Total	15 uL	Total	15 uL

Growth curves and antibiotic titration/making EC DH10B and MG1655

1. Streaked out all strains of bacteria onto m17 agar plates; put plates in appropriate incubator around 8:00 pm

a. in 37C: MG1655, DH10B, BL21, Nissle1917, B. sub, C. glut, L. lactis
b. in 30C: P. putida, S. mel, S. onei

THURSDAY, 9/13/2018

Cloning piG231

1. Took out plates
    a. all green colonies; 2 of the plates looked really contaminated

Growth curves and antibiotic titration

1. Took out all plates; everything grew except S. mel and L. lactis

Making EC DH10B and MG1655

1. Picked one colony of DH10B and MG1655 into 5 mL LB
2. in 37C shaking at 4:00pm

FRIDAY, 9/14/2018

CLONING EVERYTHING

piG230
piG230 with 439
piG231
piG 231 with 439
Control (same cassette with regular RBS)
piG232
piG233
piG226 (with 439)
Control (same cassette with Pcon)
Negative control cassette with RSF1010: RSF1010, gBlock (2-part assembly)
piG239

image.png

Golden Gate Assembly					
Reactions	9		BSA?	yes	
# Parts/Cassettes	7			1	
# Common Parts/Vector	0			1	
# PCR Pdt/Oligo Anneals	0			1	
Excess	4%	0%	0%	-	
Reactions	9.36	9	1	1 ref	
diH_2O	70.2	67.5	7.5	7.5	µL
10× Ligase Buffer	14.04	13.5	1.5	1.5	µL
10× BSA	14.04	13.5	1.5	1.5	µL
T_4 DNA Ligase	4.68	4.5	0.5	0.5	µL
Bsm BI/*Bsa* I/*Bbs* I	4.68	4.5	0.5	0.5	µL
		Total:		Each:	
Parts/Cassettes	32.76	31.5	3.5	0.5	µL
Common Parts/Vector	0	0	0	0.5	µL
PCRs/Oligo Anneal(s)	0	0	0	0.5	µL
Total:	140.4	135	15	15	µL
Total – DNA:	107.64	103.5	11.5	11.5	µL
Total – PCR pdt & Oligo Anl:	140.4	135	15	-	µL

Transformations

- Transformed the assemblies from yesterday into E. coli Turbo (heat shock)
- co-transform UBER + mKate into DH10B, Nissle, BL21

Making EC DH10B and MG1655

1. Inoculated 500 mL of plain LB w/ 5 mL of DH10B; did same for MG1655
2. Let grow in 37C shaker for several hours until turbid
3. Pelleted by centrifugation; 4000xg, 10 min, 25C
4. Discarded supernatant
5. Resuspended in 250 mL glycerol
6. repeated steps 3-5
7. pelleted by centrifugation; same setting as before
8. discarded supernatant
9. resuspended in 100 mL glycerol
10. pelleted, discarded supernatant
11. resuspended in 10 mL glycerol
12. pelleted, discarded supernatant
13. resuspended in 1 mL glycerol
14. divided into 50 uL aliquots and flash froze
15. stored in -80C freezer

Growth curves and antibiotic titration

1. Restreaked L. lactis and S. mel onto both plain LB and m17 agar plates

SUNDAY, 9/16/2018

Growth curves and antibiotic titration

1. Took out L. lactis and S. mel plates

Got plates out of the incubator. around 50/50 white/green colonies

Colony PCR

Did colony PCR on 232, 230, 231, 233, 226. Used red colony from HW-mKate cassette and piG204 plasmid as a control

Lengths:

232 - 3 kb
230 - 3 kb
231 - 1 kb
233 - 1 kb
226 - 1kb
controls - 1kb

Tm = 66 C

Week of Sept 16

MONDAY, 9/17/2018

Growth curves and antibiotic titration

1. Picked one colony of each strain into 4 mL LB
    a. for C. glut and L. lac, added glucose and ascorbate
2. In appropriate incubator at 8:30 pm

UT collaboration

1. Transformed 4 uL ea. of contents from tube 1 and 2 into NEB Turbo
    a. used heat shock
2. Let rest for 45 min. in 150 uL SOB
3. plated 33.3 uL of 1/10 dilution onto plates
    a. kan plate for tube 2
    b. kan, chl, and amp for tube 1 (was supposed to plate on tet plate as well, but we didn't have any)
4. In 37C shaking incubator at 8:30 pm

PCR of piG225 to fix the mutation, mKate gBlock

piG225
Primers Tm = 67 C
Length = 11 kb
iG083 and iG084
Tm = 68

mKate gBlock
iG013 and oG014
Tm = 64

PCR

	A	B	C	D	E	F	G	H	I
1		**Phusion HF buffer**	**dNTPs**	**Fwd Primer (45)**	**Rev Primer (48)**	**Phusion DNA polymerase**	**Template DNA**	**H2O**	
2		10	1	2.5	2.5	0.5	0.4	33.1	

PCR on GG assembly products

image.png

Optimal Q5/Phusion Pol PCR, copy 1				
Reactions	7	*GC Enhancer?*	no	
Final [DMSO]	0%	*Stock [Primer]*	10	µM
Excess	5%	*0%*	–	
Reactions	7.35	7.	*1 ref*	
diH_2O	242.55	231.	33.	µL
5× Q5 reaction buffer	73.5	70.	10.	µL
10 mM dNTPs	7.35	7.	1.	µL
DMSO	0	0	0	µL
5× GC Enhancer	0	0	0	µL
Q5 DNA polymerase	3.68	3.5	0.5	µL
Primer F	18.38	17.5	2.5	µL
Primer R	18.38	17.5	2.5	µL
1 pg – 1 µg template	3.68	3.5	0.5	µL
Total:	367.5	350.	50.	µL
Total–Template:	363.83	346.5	49.5	µL
Total–Primers:	330.75	315.	45.	µL
Total–Template & Primers:	327.08	311.5	44.5	µL

Constructs Digest with NotI

image.png

DNA Restriction Digest					
Notes	**Reactions**	9	**BSA?**	no	
	Excess	4%	0%		
	Reactions	9.36	9	*1 ref*	
	diH_2O	290.16	279	31	µL
CutSmart	**10× rxn buffer**	37.44	36	4	µL
	10× BSA	0	0	0	µL
EcoRI	**Enzyme 1**	9.36	9	1	µL
SpeI	**Enzyme 2**	0	0	0	µL
	Enzyme 3	0	0	0	µL
	DNA	37.44	36	4	µL
	Total:	374.4	360	40	µL
	Total–DNA:	336.96	324	36	µL
	Total–Enz:	365.04	351	39	µL
	Total–DNA/Enz:	327.6	315	35	µL

TUESDAY, 9/18/2018

Biobrick Assembly

Parts Digest

Backbone Digest

	A	B	C	D	E	F	G
1	*Volumes = uL*	**EcoRI-HF**	**PstI**	**DNA (200ng)**	**Cut Smart Buffer**	**NF Water**	**Total**
2	Backbone	1	1	8	5	35	50
3	Backbone 2	1	1	8	5	35	50

Part Plasmid Digest

	A	B	C	D	E	F	G
1	*Volumes = uL*	**EcoRI-HF**	**PstI**	**DNA (1000ng)**	**Cut Smart Buffer**	**NF Water**	**Total**
2	piG 002	1	1	4.4	5	38.6	50
3	piG 003	1	1	3.6	5	39.4	50
4	piG 004	1	1	4.4	5	38.6	50
5	piG 006	1	1	3.3	5	39.7	50
6	piG 007	1	1	1.3	5	41.7	50
7	piG 008	1	1	1.8	5	41.2	50
8	piG 009	1	1	2.9	5	40.1	50
9	piG 010	1	1	2.7	5	40.3	50
10	piG 011	1	1	2.9	5	40.1	50

- Digest at 37C for 1 hour, 80C for 20 minutes, 12C hold
- Made 2 2% gels with 6uL gel green
- Added 8.3uL loading dye to samples

Table1

	A	B	C	D	E
1	Well 1	Well 2	Well 3	Well 4	Well 5
2	Ladder	2	3	4	6

Table2

	A	B	C	D	E
1	Well 1	Well 2	Well 3	Well 4	Well 5
2	7	8	9	10	11

WEDNESDAY, 9/19/2018

Making competent S. meliloti

1. Prepared 500 mL of TY medium (5 g/L tryptone, 3 g/L yeast extract, 0.87 g/L CaCl2 dihydrate); autoclaved and cooled
2. Inoculated TY medium with 4 mL of S. mel culture
3. put on shaking incubator, room temp
4. let grow overnight

Making competent L. lactis

1. Into 100 mL of "universal medium," added sterile ascorbate (to 0.5 g/L), glycine (to 1%) and glucose (to 0.5%)
2. Inoculated media w/ 1 mL of L. lactis culture
3. put in shaking incubator, 30C
4. let grow overnight

Kan antibiotic titration repeat w/ finalized strains

1. Spotted 10 uL of every strain onto 0, 12.5 ug/mL, 25 ug/mL, 50 ug/mL, 100 ug/mL, and 200 ug/mL Kan plates
    a. DH10B, MG1655, Nissle1917, BL21, C. glut, B. subtilis and L. lactis were put in 37C incubator
    b. P. put, S. mel, S. onei, and L. lactis (L. lactis was spotted twice) were put in 30C incubator

Neomycin antibiotic titration

1. Prepped plain plates by adding neomycin to concentration of 0, 12.5 ug/mL, 25 ug/mL, 50 ug/mL, 100 ug/mL, and 200 ug/mL (assuming plates were 20 mL)
2. Let dry overnight

THURSDAY, 9/20/2018

Making competent S. meliloti

1. Pelleted S. mel; 4000g, 10 min, 4C
2. Washed twice w/ 500 mL water, washed once with 500 mL 10% glycerol
3. Resuspended in 1 mL 10% glycerol; divided into 100 uL aliquots
4. Stored in -80C freezer

Making competent L. lactis

1. Pelleted L. lactis culture; 5000g, 15 min, 20C
2. Washed twice w/ 0.5M sucrose + 10% glycerol
3. Resuspended in 1 mL 0.5 M sucrose + 10% glycerol; divided into 50 uL aliquots
4. Stored in -80C freezer

Neomycin antibiotic titration

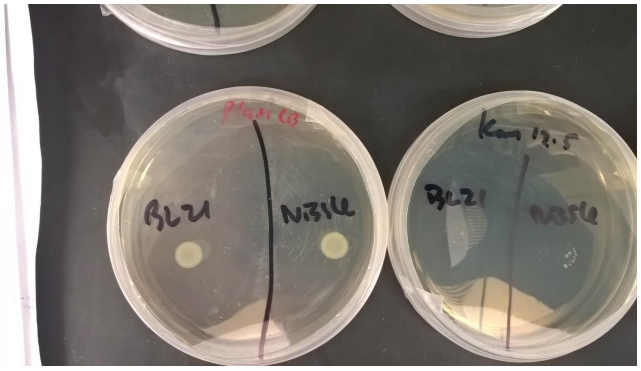
1. Spotted 10 uL of each strain onto plain, 12.5, 25, 100, 200 ug/mL neomycin plates

FRIDAY, 9/21/2018

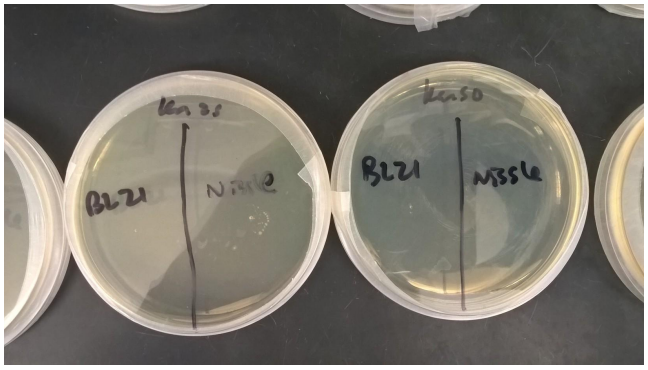
Kanamycin and neomycin antibiotic titration results

1. Kanamycin:

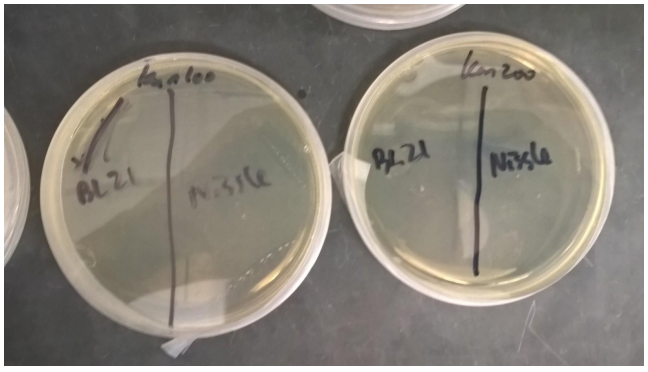
BL21 Nissle Kan 0 12.5.jpg



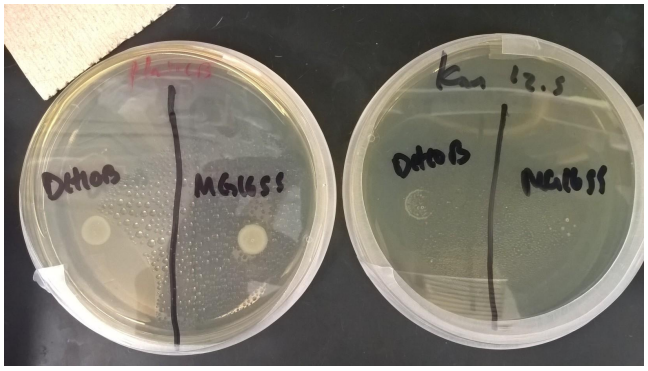
BL21 Nissle Kan 25 50.jpg



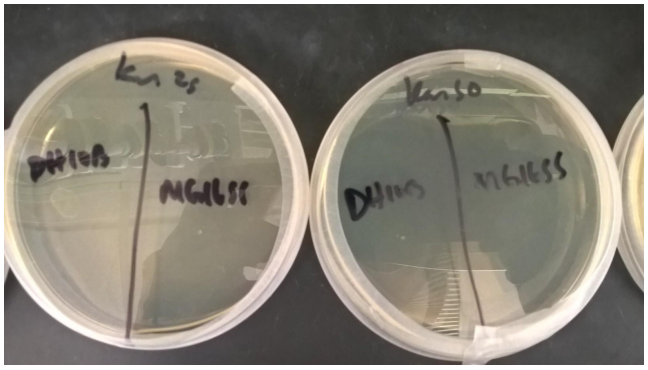
BL21 Nissle Kan 50 100.jpg



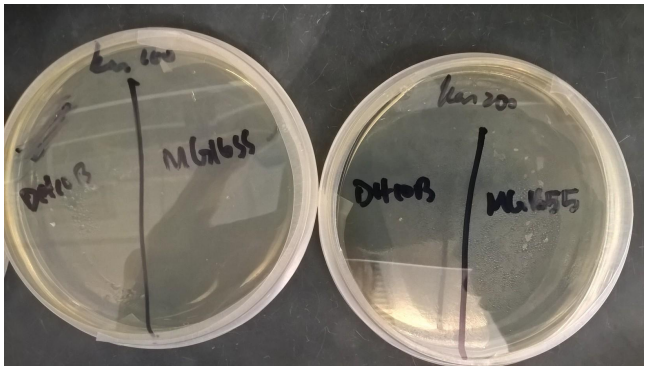
DH10 MG1655 Kan 0 12.5.jpg



DH10 MG1655 Kan 25 50.jpg

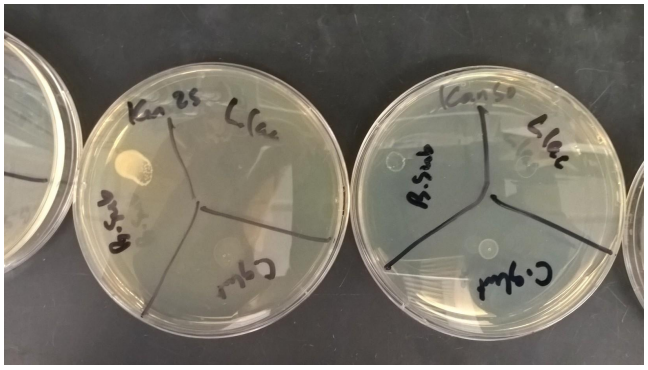


DH10 MG1655 Kan 100 200.jpg

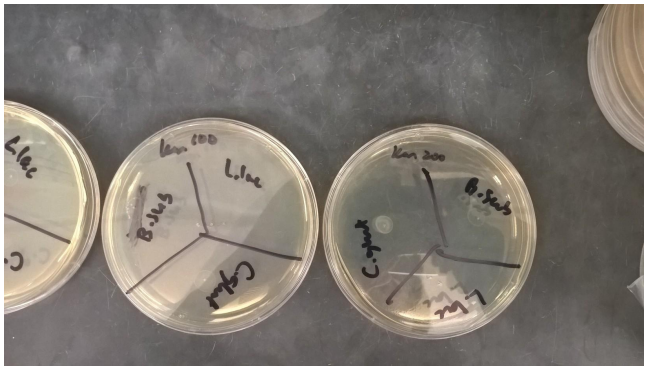


L lac C glut B sub Kan 0 12.5.jpg

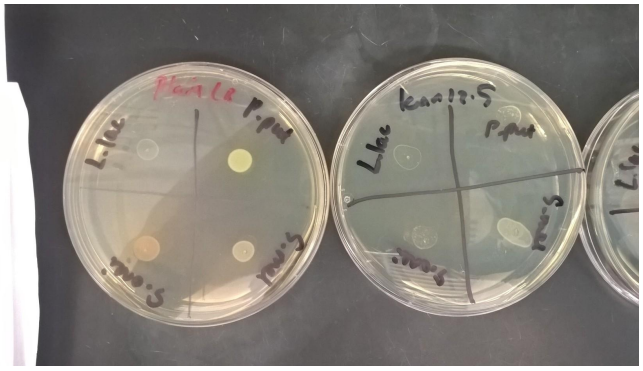
L lac C glut B sub Kan 25 50.jpg



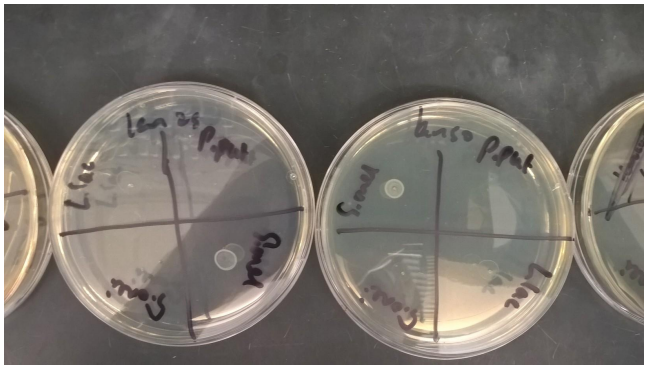
L lac C glut B sub Kan 100 200.jpg



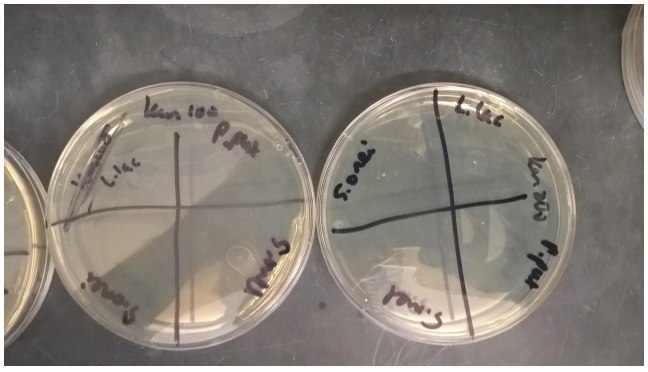
L lac P put S mel S onei Kan 0 12.5.jpg



L lac P put S mel S onei Kan 25 50.jpg

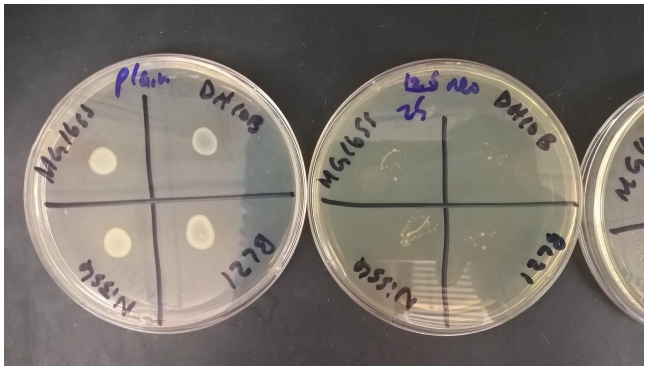


L lac P put S mel S onei Kan 100 200.jpg

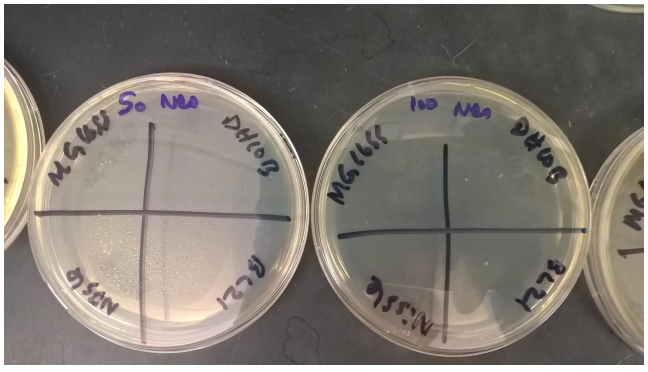


Neomycin:

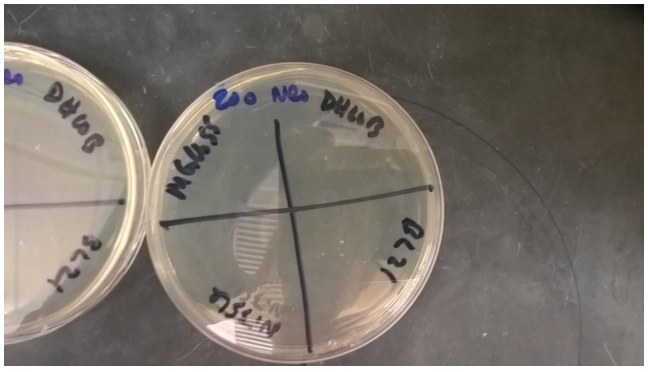
MG1655 DH10B BL2 Nissle Neo 0 25.jpg



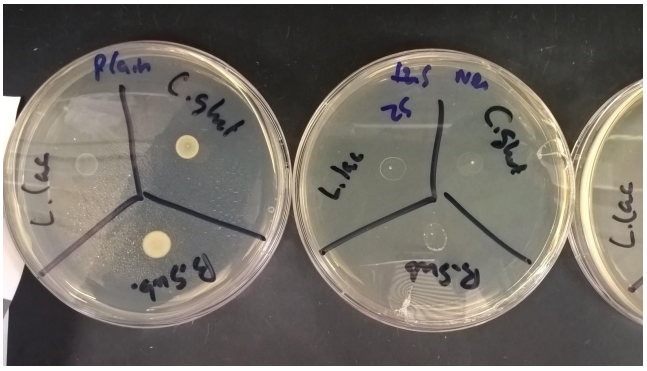
MG1655 DH10B BL2 Nissle Neo 50 100.jpg



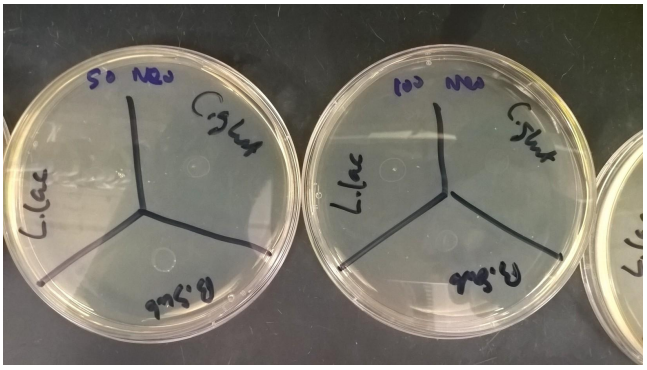
MG1655 DH10B BL2 Nissle Neo 200.jpg



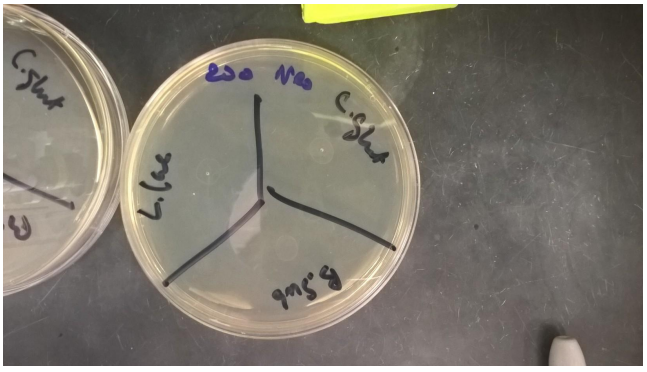
L lac C glut B sub Neo 0 25.jpg



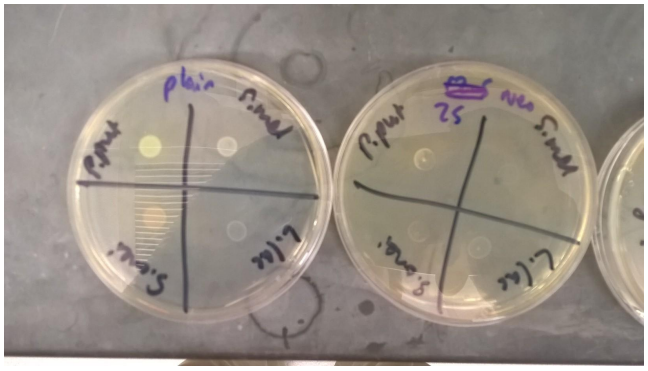
L lac C glut B sub Neo 50 100.jpg



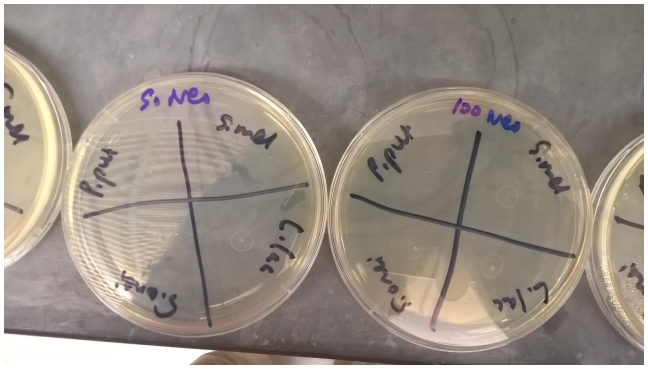
L lac C glut B sub Neo 200.jpg



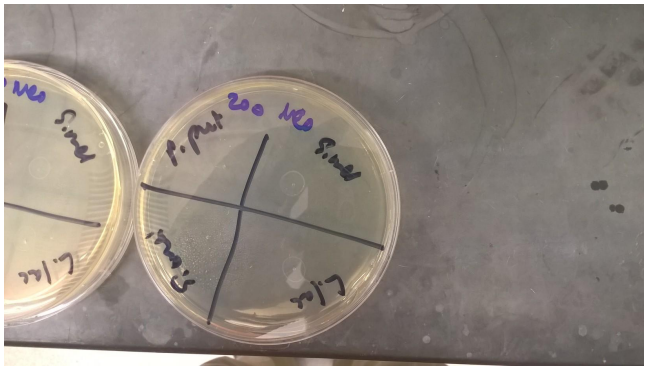
P put S mel L lac S onei Neo 0 25.jpg



P put S mel L lac S onei Neo 50 100.jpg



P put S mel L lac S onei Neo 200.jpg



Week of Sept 23

SUNDAY, 9/23/2018

230, 231, 232, 233, 223 cassettes & 225 multigene digest Digest

Digested the cassettes for orthogonal translation with NotI (left table). DIgested piG223 with BsaI and piG225 with Esp3I.

image.png

DNA Restriction Digest

Notes	Reactions	10	BSA?	no	
	Excess	4%	0%		
	Reactions	10.4	10	*1 ref*	
	diH_2O	150.8	145	14.5	µL
CutSmart	10× rxn buffer	20.8	20	2	µL
	10× BSA				µL
EcoRI	Enzyme 1	5.2	5	0.5	µL
SpeI	Enzyme 2	0	0	0	µL
	Enzyme 3	0	0	0	µL
	DNA	31.2	30	3	µL
	Total:	208	200	20	µL
	Total–DNA:	176.8	170	17	µL
	Total–Enz:	202.8	195	19.5	µL
	Total–DNA/Enz:	171.6	165	16.5	µL

< 2 µL total

Consider copy#

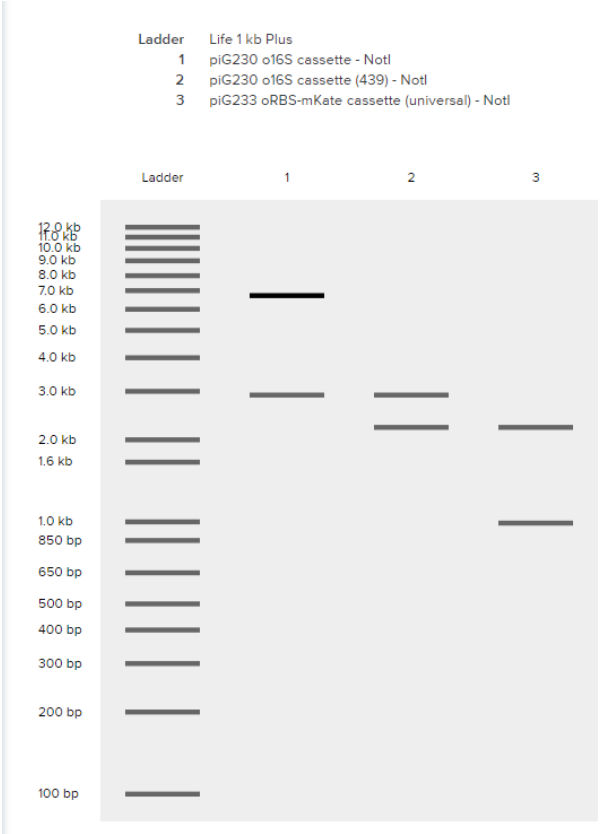
DNA Restriction Digest (copy)

Notes	Reactions	2	BSA?	no	
	Excess	4%	0%		
	Reactions	2.08	2	*1 ref*	
	diH_2O	29.12	28	14	µL
	10× rxn buffer	4.16	4	2	µL
	10× BSA				µL
	Enzyme 1	2.08	2	1	µL
	Enzyme 2	0	0	0	µL
	Enzyme 3	0	0	0	µL
	DNA	6.24	6	3	µL
	Total:	41.6	40	20	µL
	Total–DNA:	35.36	34	17	µL
	Total–Enz:	39.52	38	19	µL
	Total–DNA/Enz:	33.28	32	16	µL

< 2 µ tota

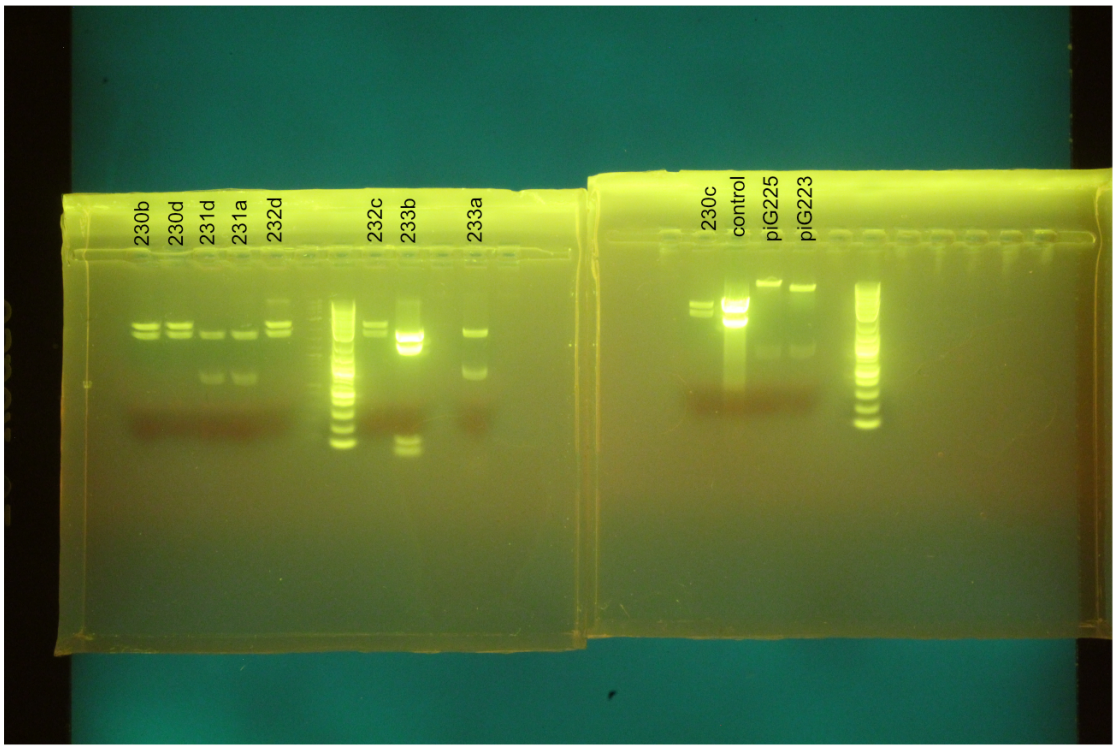
Expected results:

image.png



Observed results:

18-09-23 0002.png



MONDAY, 9/24/2018

Transformations

Transformed assembilies of ER1, OR1 016S part plasmids and 439 + gBlock assembly into DH10B (electroporation)

Samples for sequencing

Table1

	A	B	C	D	E
1	sample	conc	DNA	Primers	H2O
2	225	78	6.4102564103	2.5	6.0897435897
3	piG012 (U pp2)	300	1.6666666667	2.5	10.8333333333
4	piG012 (U pp2)	300	1.6666666667	2.5	10.8333333333
5	230b	70	7.1428571429	2.5	5.3571428571
6	231a	96	5.2083333333	2.5	7.2916666667
7	231a	96	5.2083333333	2.5	7.2916666667
8	233a	121	4.132231405	2.5	8.367768595
9	233a	121	4.132231405	2.5	8.367768595

TUESDAY, 9/25/2018

Gathering growth over time data for all strains

1. Previously made liquid cultures of ea. strain
2. Diluted each culture to OD = 0.1
3. Added 20 uL of culture to 180 uL of liquid media in 96-well plate; arrangement of strains and identity of liquid media in table:

Table3

	A	B	C	D	E	F	G	H	I	J	K	L
1	1	1	1	2	2	2	3	3	3	4	4	4
2	1	1	1	2	2	2	3	3	3	4	4	4
3	1	1	1	2	2	2	3	3	3	4	4	4
4	5	5	5	6	6	6	7	7	7	8	8	8
5	5	5	5	6	6	6	7	7	7	8	8	8
6	5	5	5	6	6	6	7	7	7	8	8	8
7	9	9	9	9	9	9	10	10	10	10	10	10
8	9	9	9				10	10	10			

white = LB + 0.5% glucose
light grey = universal medium + 0.5% glucose
darker grey = universal medium + 0.5% glucose + ascorbate

1 = BL21
2 = MG1655
3 = DH10B
4 = Nissle
5 = B. sub
6 = C. glut
7 = L. lac
8 = P. put
9 = S. onei
10 = S. mel

Making electrocompetent DH5a

1. Streaked out DH5a onto plain LB plate

Making EC B. subtilis

1. Streaked out B. sub onto plain LB plate

WEDNESDAY, 9/26/2018

Samples for sequencing

Table2

	A	B	C	D	E
1	sample	conc	DNA	Primers	H2O
2	225 (E11)	78	6.4102564103	2.5	6.0897435897
3	241 (E11)	96.4	5.1867219917	2.5	7.3132780083
4	230b (B17)	70	7.1428571429	2.5	5.3571428571
5	232c (B17)	75.2	6.6489361702	2.5	5.8510638298
6	232c (B18)	75.2	6.6489361702	2.5	5.8510638298
7					
8					

Nanodrop

	A	B	C
1		**[DNA]**	**DNA (250ng)**
2	piG 002	286.0	1.144
3	piG 003	176.9	0.7076
4	piG 004	177.2	0.7088
5	piG 006	305.3	1.2212
6	piG 007	193.8	0.7752
7	piG 009	141.4	0.5656
8	piG 010	149.6	0.5984
9	piG 011	189.1	0.7564

Part Plasmid Digest

	A	B	C	D	E	F	G
1	*Volumes = uL*	**EcoRI-HF**	**PstI**	**DNA (250ng)**	**Cut Smart Buffer**	**NF Water**	**Total**
2	piG 002	0.5	0.5	2	2.5	19.5	25
3	piG 003	0.5	0.5	2	2.5	19.5	25
4	piG 004	0.5	0.5	2	2.5	19.5	25
5	piG 006	0.5	0.5	2	2.5	19.5	25
6	piG 007	0.5	0.5	2	2.5	19.5	25
7	piG 009	0.5	0.5	2	2.5	19.5	25
8	piG 010	0.5	0.5	2	2.5	19.5	25
9	piG 011	0.5	0.5	2	2.5	19.5	25
10	**MM**	**4**	**4**	**16**	**20**	**156**	**200**

Used Wetlab Calculator and added 8% excess of volumes listed above for MM

Origin Testing L. lactis

1. Transformed RSF1010, pBBR1, pWV01 into L. lactis
    a. electroporated 200 ng of DNA into L. lactis; 2.0 kV
2. Let rest in LB for 2 hrs in 30C shaking incubator
3. Plated 25 uL, streaked

Making electrocompetent DH5a

1. Picked colony of DH5a into 5 mL LB

Making EC B. subtilis

1. Picked colony of DH5a into 5 mL LB

FRIDAY, 9/28/2018

Samples for sequencing

Table4

	A
1	226
2	232
3	piG015
4	piG031

SATURDAY, 9/29/2018

BioBricks Assembly Part 2

Table5

	A	B	C	D	E	F	G	H
1	*Volumes = uL*	**EcoRI-HF**	**PstI**	**DNA (1000ng)**		**Cut Smart Buffer**	**NF Water**	**Total**
2	piG 001	1	1	18		5	25	50
3	piG 005	1	1	18		5	25	50
4	piG 016	1	1	18		5	25	50
5	piG 018	1	1	18		5	25	50
6	piG 026	1	1	18		5	25	50
7	piG 027	1	1	18		5	25	50
8	piG 029	1	1	18		5	25	50
9	piG 030	1	1	18		5	25	50
10	pSPB 639	1	1	18		5	25	50
11	**MM**	**9.3**	**9.3**			**46.5**	**232.5**	

- Digest at 37C for 1 hour, 80C for 20 minutes, 12C hold

Week of Sept 30

MONDAY, 10/1/2018

BioBricks

- Made three 2% gels with 6uL gel green
- Added 8.3uL loading dye to samples

Sample Arrangment

	A	B	C	D	E	F
1	Gel	Well 1	Well 2	Well 3	Well 4	Well 5
2	Left	Ladder	18	26	1	
3	Middle	Ladder	5	16	30	
4	Right	Ladder	639	27	29	

TUESDAY, 10/2/2018

BioBricks

- Cut out DNA slices
- Gel purification
- Ligation

Table1

	A	B	C	D	E	F
1	**Part**	**Backbone (50ng)**	**DNA (50ng)**	**T4 DNA ligase buffer**	**T4 DNA ligase**	**Water**
2	**1**	2	8	1.5	0.5	3
3	**5**	2	8	1.5	0.5	3
4	**16**	2	8	1.5	0.5	3
5	**26**	2	8	1.5	0.5	3
6	**27**	2	8	1.5	0.5	3
7	**29**	2	8	1.5	0.5	3
8	**30**	2	8	1.5	0.5	3

WEDNESDAY, 10/3/2018

Thessaloniki collab

Before dilution:

Abs600 of Diluted Cultures ...

	A	B
1	**Sample**	**OD**
2	TD1 1	0.3908
3	TD1 2	0.3736
4	TD2 1	0.3754
5	TD2 2	0.3741
6	TD3 1	0.3897
7	TD3 2	0.3901
8	TD4 1	0.3625
9	TD4 2	0.3925

SATURDAY, 10/6/2018

Backbone Digest

	A	B	C	D	E	F	G
1	*Volumes = uL*	**EcoRI-HF**	**PstI**	**DNA (200ng)**	**Cut Smart Buffer**	**NF Water**	**Total**
2	Backbone	1	1	8	2.5	12.5	25

Table5

	A	B	C	D	E	F	G	H
1	*Volumes = uL*	**EcoRI-HF**	**PstI**	**DNA (1000ng)**		**Cut Smart Buffer**	**NF Water**	**Total**
2	piG 001	1	1	10		5	33	50
3		8.5	8.5			42.5	280.5	340
4								0
5								0
6								0
7								0
8								0
9								0
10								0
11								

Table2

	A	B	C	D	E	F
1	**Part**	**Backbone (50ng)**	**DNA (50ng)**	**T4 DNA ligase buffer**	**T4 DNA ligase**	**Water**
2	**1**	2	5	1.5	0.5	6
3		14.6		10.95	3.65	43.8