Table of Contents

June 2019 2
August 2019 6
Bibliography 22

June 2019

Tuesday, June 11, 2019

To Do:

1. ~~Thaw macrophage~~

Wednesday, June 12, 2019

To Do:

1. ~~Patch out LVS, ΔchaC, ΔchaC Tn7:chaC~~
2. ~~Seed macrophage~~
3. ~~Prep media +/- cysteine~~

Results and Data:

Patched out strains for macrophage assay (LVS, Δ*chaC*, Δ*chaC* Tn7:P*bfr-chaC*) plus the other strains from the Mougous lab to make single-use stocks for later use.

Received the following strains from the Mougous lab:
wild-type *F. novicida* U112
TOP10 E. coli with plasmid pMP720
TOP10 E. coli with plasmid pMP749
TOP10 E. coli with plasmid pMP672

Plasmids in *E. coli* are for Tn7 integration system (LoVullo et al., 2009).

Prepare to seed macrophage for experiment -/+ added cysteine.
Need 5mM cysteine (or equiv added PBS) in each well.

Make 100x cysteine stock in PBS: 500 mM, 25 mL

```
?g? = |500mmol | 0.025 L |   1 mol    | 175.63 g| = 2.195 g = 2.2 g
      | 1 L.   |    1    | 1000 mmol  | 1 mol   |
```

Plans:
If I add this into individual wells, I'd only be adding 2 uL/well. Not ideal.
When seeding cells, plate at slightly higher density so I can add 150 uL of cells and 50 uL of media with either PBS or cysteine. The media plus cysteine would need 20 mM cysteine.

For each media addition, would need 12 wells worth = 600uL, round up to 1 mL.
Media supplementation:
40 uL either 500 mM cysteine in 1x PBS or just 1x PBS
960 uL DMEMF

Use 50 uL per well.

The change to my seeding plans:

Normal:

Macrophage Calculations	Actual
Cells per well	2.00E+04
Volume to plate (mL)	0.2
Density needed (cells/mL)	1.00E+05
Total volume needed (mL)	7
Measured cells per ml	-
Volume stock needed (mL)	-
Volume media for dilution	-
Measured cells per ml, seeded	7.67E+04
Measured cells per well	1.53E+04

Altered:

Macrophage Calculations	Actual
Cells per well	2.00E+04
Volume to plate (mL)	0.15
Density needed (cells/mL)	1.33E+05
Total volume needed (mL)	5.25

Note that I based the total volume needed off the normal estimate of 7mL, which is theoretically enough to seed 35 wells (only need 26 wells).

When I add the bacteria, made two different inoculums per strain- one in cysteine and one in media plus PBS. Will just need to deal with extra inoculum plates!

Seeding macrophage

Plate setup:

	1	2	3	4	5	6	7	8	9	10	11	12
A	LVS	LVS		LVS Δ*chaC*	LVS Δ*chaC*		LVS Δ*chaC* Tn7:*chaC*	LVS Δ*chaC* Tn7:*chaC*		LVS only		
B												
C												
D	LVS	LVS		LVS Δ*chaC*	LVS Δ*chaC*		LVS Δ*chaC* Tn7:*chaC*	LVS Δ*chaC* Tn7:*chaC*		mac only		
E												

Scrape up all cells (thawed yesterday), spin down (5' at 900 rpm), and resuspend in 5.5 mL DMEMF.

Resuspension

Set	Counts			
1	7	8	2	0
2	14	16	18	12
3	21	2	3	10
4	6	12	16	3
Average	9.38			
Cells per mL	1.88E+05			

Great, more cells than needed! Note that they are pretty clumpy, though. Dilute 3.8 mL with 1.5 mL DMEMF, seed 150 uL per well as indicated. For row A, add 50 uL DMEM+1xPBS. For row D, add 50 uL DMEM + 20 uM cysteine in 1x PBS (except mac control, added DMEM+PBS)
Two plates total for T=2 and T=24.

Check final cell density in dilution:

Final Dilution

Set	Counts			
1	4	3	0	6
2	6	1	0	0
3	2	3	3	3
4	2	3	2	7
5	3	13	1	9
6	5	3	0	5
Average	3.50			
Cells per mL	7.00E+04			

Darn- I think the clumpiness really threw the calculations off! Definitely half the concentration I was aiming for: wanted $1.3x10^5$, have $7x10^4$. ☹ Move forward anyway.

Macrophage Calculations	**Actual**
Cells per well	2.00E+04
Volume to plate (mL)	0.15
Density needed (cells/mL)	1.33E+05
Total volume needed (mL)	5.25
Measured cells per ml	1.88E+05
Volume stock needed (mL)	3.8
Volume media for dilution	1.5
Measured cells per ml, seeded	7.00E+04
Measured cells per well	1.05E+04

Put remaining macrophage in TC dish for growth. Now P7 J774As.

Thursday, June 13, 2019

To Do:

1. ~~Infect macrophage~~
2. ~~Wash macrophage~~
3. ~~Take T=2 mac timepoint~~
4. ~~Western blot on cells grown in macrophage~~
5. ~~Check Jamie's plates- inoculum~~

Results and Data:

Western blot:
Incubate in 10 mL blocking buffer + 4.5 uL anti-VSVG for ~1.5 hrs
Wash 4x 10' in TBS+NP40
Re-block in 10 mL blocking buffer for ~45'.

Incubate in 10 mL blocking buffer + 4 uL anti-rabbit for 1 hour
Wash 4x 10' in TBS+NP40
Leave in wash until ready to develop
Develop with Pico kit ~4', bring to dark room

<u>Macrophage expt: *chaC* deletion</u>
Resuspend bacteria (Δ*chaC* is really, really mucoid! The complement is not) in 400 uL DMEMF
1: LVS
2: Δ*chaC*
3: Δ*chaC* Tn7:*chaC*
Check OD600, dilute to calculated OD of 0.025 in 1.3 mL
Check OD600, all around 0.02, also need to add either PBS or cysteine
Calculations for a final OD of about 0.00025:
  12.5 uL bacteria
  10 uL either 500 mM cysteine or 1X PBS
977.5 uL DMEMF
1 – 3 = 1x PBS supplementation
4 – 6 = 5 mM cysteine supplementation

			For final vol 1.3 mL at 0.028			
Number	Strain	Resuspend cells to (OD600)	Cells (uL)	Media (x2, uL)	OD600	Volume to dilute in 1 mL (usually 1:100)
1	LVS	0.9	36.1	1263.9	0.019	12.5
2	Δ*chaC*	1.1	29.5	1270.5	0.02	12.5
3	Δ*chaC* Tn7:*chaC*	1.27	25.6	1274.4	0.021	12.5

10:20: Infect each well as indicated with 50 uL (use PBS supp media in control wells)
Plate for CFU for all 6 inoculumns

After washing, will need to replace media with either cysteine-supplemented media or PBS.
Prep 4 mL each supplementation type:
4 mL DMEMF
40 uL either 500 mM cysteine or 1X PBS
0.8 uL gentamycin (50 mg/mL)

Wash ~12:20

Lyse cells from one plate at ~2:20 and plate **75uL** each well 2x.

Check on Jamie's plates:

Friday, June 14, 2019

To Do:

1. ~~Take T=24 mac timepoint~~
2. ~~Check Jamie's plates- 2, 5, 24 hours~~
3. ~~Feed P7 macrophage~~

Results and Data:
Notes here.

August 2019

Friday, August 9, 2019

To Do:

1. ~~Electroporate for ΔchaC complementation~~

Results and Data:

Performed 4 electroporations:

Electroporation	Strain	EC cells	Plasmid	Notes
52	LVS	JW 7.16.19	pF	Arc
53	LVS	JW 7.16.19	pKL138 pF-chaC	
54	LVS ΔchaC	KMR unknown date	pF	Arc
55	LVS ΔchaC	KMR unknown date	pKL138 pF-chaC	Arc

Recovering at ~11am

Jamie plated 20 and 200 ul for me on CHA-Kan plates at about 2pm.

Monday, August 12, 2019

To Do:

1. ~~Patch colonies from Friday's EP~~

Results and Data:
All plates from 8/9 electroporation have colonies.
Patch out 2 plates (duplicate), each with quadrants corresponding to:
LVS pF
LVS pF-chaC
ΔchaC pF
ΔchaC pF-chaC

Tuesday, August 13, 2019

To Do:

1. ~~Look at patches~~
2. ~~Plate 1° PchaC-lacZ onto sucrose plates~~

Results and Data:

Resuspended 1° pKR10 integrant in PBS, performed 10x serial dilutions to 10^{-7}, plated 100 ul of 10^{-2} -> 10^{-7} on sucrose plates, plated 100 ul of 10^{-7} on CHA

chaC complementation:
Not clear just from the patches if the pF-*chaC* plasmid complemented the mucoid phenotype (nothing looks mucoid?).

Test the number of viable cells per OD600:
Resuspend each strain in DMEMF, dilute to an OD600 of 0.03, wait about 10', serially dilute in PBS and plate on square CHA plates.

Number	Strain	OD after resuspension (1:10)	Volume for 1.5 mL at 0.03 (uL)	Final OD600
1	LVS pF	0.301	15	0.036
2	LVS pKL138 pF-chaC	0.374	12	0.037
3	ΔchaC pF	0.485	9	0.037
4	ΔchaC pKL138 pF-chaC	0.561	8	0.041

Plated 10 uL of undiluted -> 10^{-5} (6 spots total)

Wednesday, August 14, 2019

To Do:

1. ~~Look at patches~~
2. ~~Check for primers for PchaC-lacZ~~

Results and Data:

Ordered new primers to check for integration of *chaC* in Tn7 site (Jamie attempted to amplify the region and did not see any differences between LVS and the presumed complements).

Looked at Δ*chaC* pF vs pKL138 pF-chaC patches- can't really see mucoid phenotype.

To confirm lacZ integration into *chaC* locus (pKR10 integration) after sucrose selection, use P795 and KROL182 (integration = 900bp)

Thursday, August 15, 2019

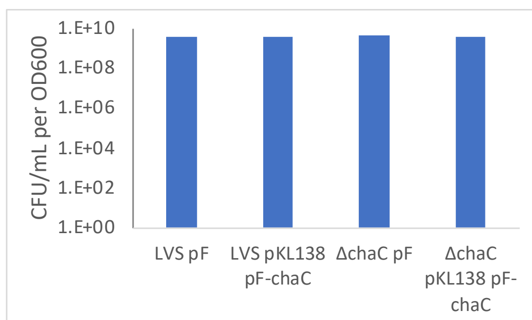
To Do:

1. ~~Count colonies for ΔchaC complementation expt~~
2. ~~PCR to confirm ΔchaC~~
3. ~~Streak out strains to make EC cells tomorrow~~
4. ~~Check for enough plasmid~~
5. Mail Hannah L plasmid to delete chaC

Results and Data:

Counted colonies for Δ*chaC* complementation expt. No difference between LVS pF and Δ*chaC* pF!

	Track Plate 1						Track Plate 2										
	1	2	3	4	5	6	1	2	3	4	5	6	**Dilution factor counted**	**Average Cells**	**CFU per mL**	**OD600**	**CFU per mL per OD600**
LVS pF	TMTC	TMTC	TMTC	TMTC	TMTC	**11**	TMTC	TMTC	TMTC	TMTC	TMTC	**19**	0.00001	15	1.50E+08	0.036	4.17E+09
LVS pKL138 pF-chaC	TMTC	TMTC	TMTC	TMTC	TMTC	**20**	TMTC	TMTC	TMTC	TMTC	TMTC	**10**	0.00001	15	1.50E+08	0.037	4.05E+09
ΔchaC pF	TMTC	TMTC	TMTC	TMTC	TMTC	**20**	TMTC	TMTC	TMTC	TMTC	TMTC	**12**	0.00001	16	1.60E+08	0.037	4.32E+09
ΔchaC pKL138 pF-chaC	TMTC	TMTC	TMTC	TMTC	TMTC	**23**	TMTC	TMTC	TMTC	TMTC	TMTC	**12**	0.00001	18	1.75E+08	0.041	4.27E+09
Dilution Factor	1	0.1	0.01	0.001	1E-04	1E-05	1	0.1	0.01	0.001	1E-04	1E-05					



Did I really use Δ*chaC* cells for my electroporation? Check by PCR.

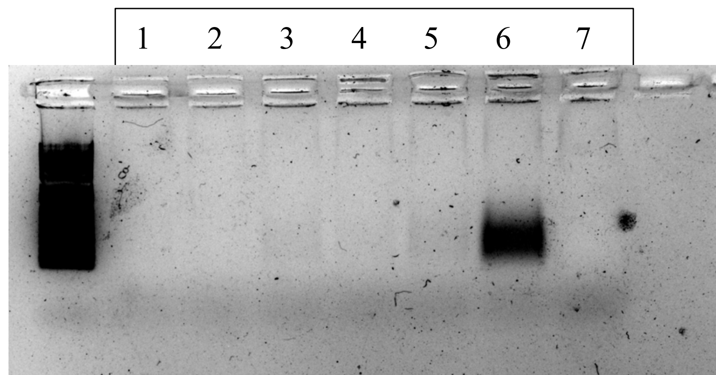
PCR to confirm Δ*chaC* using PrimeStarGXL polymerase:
Use primer set P794 and P795:

Reaction numbers	Sample	Expected size
1	Colony LVS pF	2243
2	Colony LVS pF-chaC	2243
3	Colony ΔchaC pF	1460
4	Colony ΔchaC pF-chaC	1460
5	Colony ΔchaC	1460
6	LVS gDNA	2243
7	-DNA	no product

Total reaction volume	20
Total number of reactions	7

Component	Stock concentration	Final concentration	1 rxn volume	Factor 8
ddiH2O			6	48
PrimeSTAR GXL Buffer	5x	1x	10	80
dNTPs	2.5 mM	0.2 mM	2	16
P794	10 uM	0.3 uM	0.6	4.8
P795	10 uM	0.3 uM	0.6	4.8
template	100 ng/ul	2 ng/ul	0.4	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	3.2
		Total volume	20	156.8

Run standard program STN1 with 2:30 extension.



Yikes! Looks like the PrimeStar polymerase didn't work out super well… Oh, wait! I didn't adjust the buffer concentration and there is WAY too much! Try again tomorrow.

Struck out LVS and ∆*chaC* to make EC cells tomorrow.

Tomorrow, would potentially like to do the following:
Electroporate for re-created ∆*chaC* strain (pKL133)
Electroporate for pF-*chaC* and pF in LVS and ∆*chaC* strains (test complementation): pKL138
Electroporate pSD26 into *rpsU1-lacZ* strain? (would need Kan-X-gal plates for this)

Friday, August 16, 2019

To Do:

1. ~~Electroporate for new ∆chaC~~
2. ~~Re-do chaC PCR~~
3. Re-do pF-chaC electroporations? NO.
4. ~~Patch potential PchaC-lacZ colonies from sucrose plates~~
5. Mail Hannah L plasmid to delete chaC

Results and Data:

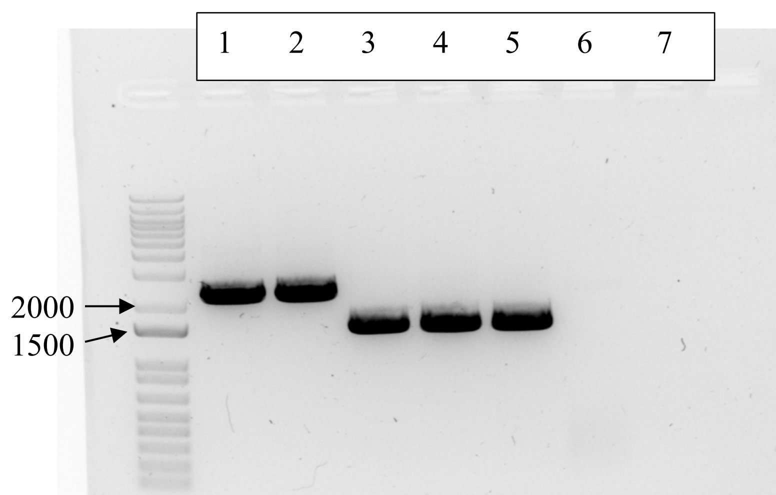
Check for ∆*chaC* using primers P794 and P795:

Reaction numbers	Sample	Expected size
1	Colony LVS pF	2243
2	Colony LVS pF-chaC	2243
3	Colony ΔchaC pF	1460
4	Colony ΔchaC pF-chaC	1460
5	Colony ΔchaC	1460
6	LVS gDNA	2243
7	-DNA	no product

Total reaction volume	20
Total number of reactions	7

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	8
ddiH2O			12.4	99.2
PrimeSTAR GXL Buffer	5x	1x	4	32
dNTPs	2.5 mM	0.2 mM	1.6	12.8
P794	10 uM	0.3 uM	0.6	4.8
P795	10 uM	0.3 uM	0.6	4.8
template	100 ng/ul	2 ng/ul	0.4	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	3.2
		Total volume	20	160

Run standard program STN1 with 2:30 extension in MRamsey lab thermocycler.



Hannah loaded gel and took picture. Looks like I probably forgot to add gDNA to 6th tube.
Results indicate that I did use the correct cells for my complementation experiment. DO NOT need to re-electroporate.
Addition of a replicating plasmid and selection on kanamycin prevents the mucoid phenotype and viability defect of the Δ*chaC* mutant? Might actually be worth testing in a macrophage assay.

Made new aliquots of EC LVS cells (from LVS not from single-use vial, but from original stock tube).

Re-making Δ*chaC*: Performed 2 electroporations:

Electroporation	Strain	EC cells	Plasmid	Notes
56	LVS	KMR 8.16.19	pKL133 pEXΔFTL_1548	
57	LVS	KMR 8.16.19	pKL133 pEXΔFTL_1548	

Recovering at ~12:45PM – 6:45PM. Plated each EP on 2 CHA-Kan plates.

P_{chaC}-*lacZ* strain construction:
Patched 18 colonies (mix of small and large) from pKR10-1 sucrose plates to CHA and CHA-Kan, put at 37°C.

Saturday, August 17, 2019

To Do:

1. ~~Start PCR to check potential PchaC-lacZ colonies~~

Results and Data:

To check patches for PchaC-lacZ, use primers P794 and P795.
WT 2243 bp
PchaC-lacZ 4577 bp

(chaC = 783 bp, lacZ insert = 3117 bp)

Reaction numbers	Sample	Expected size
1	Colony 10-1-1	2243 or 4577
2	Colony 10-1-2	2243 or 4577
3	Colony 10-1-3	2243 or 4577
4	Colony 10-1-4	2243 or 4577
5	Colony 10-1-5	2243 or 4577

6	Colony 10-1-6	2243 or 4577
7	Colony 10-1-7	2243 or 4577
8	Colony 10-1-8	2243 or 4577
9	Colony 10-1-9	2243 or 4577
10	Colony 10-1-10	2243 or 4577
11	Colony 10-1-11	2243 or 4577
12	Colony 10-1-12	2243 or 4577
13	Colony 10-1-13	2243 or 4577
14	Colony 10-1-14	2243 or 4577
15	Colony 10-1-15	2243 or 4577
16	Colony 10-1-16	2243 or 4577
17	Colony 10-1-17	2243 or 4577
18	Colony 10-1-18	2243 or 4577
19	LVS colony	2243
20	LVS gDNA	2243
21	pKR10 (1:10)	no product
22	-DNA	no product

Total reaction volume	20
Total number of reactions	22

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	23
ddiH2O			12.4	285.2
PrimeSTAR GXL Buffer	5x	1x	4	92
dNTPs	2.5 mM	0.2 mM	1.6	36.8
P794	10 uM	0.3 uM	0.6	13.8
P795	10 uM	0.3 uM	0.6	13.8
template	100 ng/ul	2 ng/ul	0.4	9.2
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	9.2
		Total volume	20	460

Use STN1 with 4:30 extension- leave O/N.
Note that a number of patches looked goopy/mucoid and did not grown on the Kan plate, including #s 1, 3, and 4. Streak 1, 3, and 4 to single colony on CHA plate.

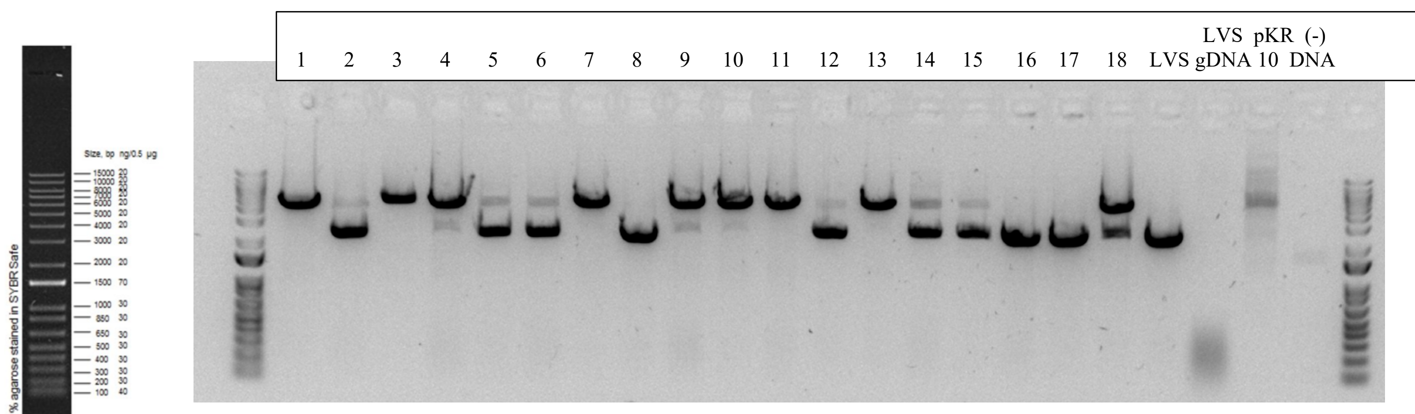
Monday, August 19, 2019

To Do:

1. ~~Run gel of PchaC-lacZ PCR~~
2. ~~Patch out 1° ΔchaC colonies from Friday, 8/16~~
3. ~~Patch out 3 colonies each from PchaC-lacZ strains struck out on Saturday.~~
4. ~~Mail Hannah L plasmid to delete chaC~~

Results and Data:

Run gel of PCR from 8/17/19:



Not entirely sure why LVS gDNA didn't work (didn't have concentration on that tube), but it looks like my hypothesis was correct. I picked 1, 3, and 4 as mucoid strains that probably lacked chaC, and they do!

Patch single colonies originating from those patches and cross-patch to CHA and CHA-Kan.

Tuesday, August 20, 2019

To Do:

1. ~~PCR to check PchaC-lacZ colonies~~
2. ~~Freeze down ΔchaC 1° integrants~~
3. ~~Freeze down PchaC-lacZ strain~~
4. ~~Transform E. coli with pF and pF-chaC~~

Results and Data:

To check purified patches for PchaC-lacZ, use primers P794 and P795.
WT 2243 bp
PchaC-lacZ 4577 bp

(chaC = 783 bp, lacZ insert = 3117 bp)

Reaction numbers	Sample	Expected size
1	10-1-1A	2243 or 4577
2	10-1-1B	2243 or 4577
3	10-1-1C	2243 or 4577
4	10-1-3A	2243 or 4577
5	10-1-3B	2243 or 4577
6	10-1-3C	2243 or 4577
7	10-1-4A	2243 or 4577
8	10-1-4B	2243 or 4577
9	10-1-4C	2243 or 4577
10	LVS colony	2243
11	LVS gDNA	2243
12	pKR10 (1:10)	no product
13	-DNA	no product

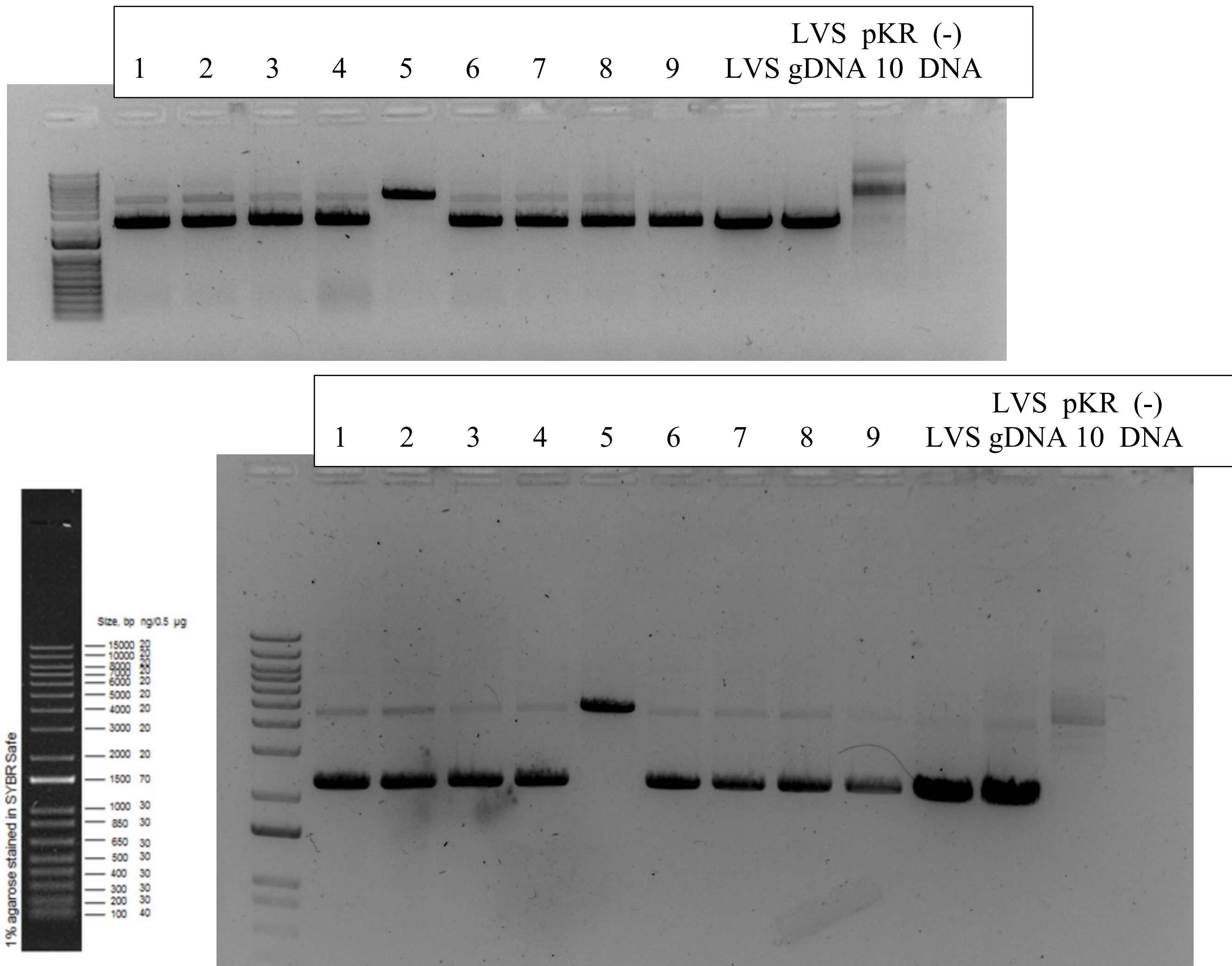
Total reaction volume	20
Total number of reactions	13

Component	Stock concentration	Final concentration	1 rxn volume	Factor 14.5
ddiH2O			12.4	179.8
PrimeSTAR GXL Buffer	5x	1x	4	58
dNTPs	2.5 mM	0.2 mM	1.6	23.2
P794	10 uM	0.3 uM	0.6	8.7
P795	10 uM	0.3 uM	0.6	8.7
template	100 ng/ul	2 ng/ul	0.4	-
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	5.8
		Total volume	20	290

Use STN1 with 4:30 extension. Takes about 3.5 hours.

Froze down all 6 1° integrants of pKL133, 1 vial each.

Transformed chemically competent XL1 Blue cells with pF, pKL138 (pF-*chaC*), and pKL75 (for JMW). Put transformations of pF and pKL138 at 37°C O/N, put transformation of pKL75 at 30°C O/N (has growth issues at 37°C).



Clearly only KR10-1-3B is correct. Freeze down as KRLVS39.1 and (when Jamie is back) move the 1° integrant pKR10-1 into the strain box.

Wednesday, August 21, 2019

To Do:

1. ~~Streak out ΔchaC 1° integrants~~
2. ~~Freeze down JMW's 1° integrants.~~
3. ~~Streak out ΔchaC~~
4. ~~Streak out rpsU1-lacZ~~
5. ~~Make single use aliquots of PchaC-lacZ, streak out one aliquot~~
6. ~~Start O/N cultures of E. coli with pF and pF-chaC~~

Thursday, August 22, 2019

To Do:

1. ~~Plate ΔchaC 1° integrants on sucrose~~
2. Make EC ΔchaC cells
3. Make EC PchaC-lacZ cells

4. ~~Make EC rpsU-lacZ cells~~
5. Miniprep pF and pF-chaC
6. ~~Make square plates, Kan-Xgal plates~~

Results and Data:

Friday, August 23, 2019

To Do:
1. ~~Miniprep pF and pF-chaC~~
2. ~~Make EC ΔchaC cells~~
3. ~~Make EC PchaC-lacZ cells~~
4. ~~Electroporate pF, pF-chaC into LVS, ΔchaC~~
5. ~~Electroporate pSD26 into PchaC-lacZ and rpsU-lacZ~~
6. Streak PchaC-lacZ and PrpsU1-lacZ to single colonies on X-gal plates

Results and Data:

Dilute pSD26 so I have 1 ug DNA per 3 uL (333 ng/uL):
(333.3 ng/ul)(30 uL)/ 1231 ng/uL = 8.12 uL into

Check Δ*chaC*: viability

Electroporation	Strain	EC cells	Plasmid	Volume	Notes
58	LVS	KMR 8.16.19	pF (Kan)	3 uL	
59	LVS	KMR 8.16.19	pKR30 pF (Nat)	3 uL	Arced
60	LVS	KMR 8.16.19	pKL138 pF-chaC	3 uL	
61	LVS	KMR 8.16.19	pKL139 pF2-chaC	3 uL	
62	ΔchaC	KMR 8.23.19	pF (Kan)	3 uL	
63	ΔchaC	KMR 8.23.19	pKR30 pF (Nat)	3 uL	
64	ΔchaC	KMR 8.23.19	pKL138 pF-chaC	3 uL	
65	ΔchaC	KMR 8.23.19	pKL139 pF2-chaC	3 uL	arced

Recovering at ~1:30pm – 4:40pm. Plated 20 and 200 ul of each EP on CHA-Kan (for #60 and 63, on CHA-nat).

Transposon mutagenesis of reporter strains:

Electroporation	Strain	EC cells	Plasmid	Volume	Notes
66	PchaC-lacZ	KMR 8.23.19	pSD26	3 uL	
67	PchaC-lacZ	KMR 8.23.19	pSD26	3 uL	Arced
68	PrpsU1-lacZ	KMR 8.22.19	pSD26	3 uL	Arced
69	PrpsU1-lacZ	KMR 8.22.19	pSD26	3 uL	

Recovering at ~1:30 – 5pm. Plated 1x 100 ul, 1x 300 ul, 2x 500 ul on CHA-Kan-X-gal

Monday, August 26, 2019

To Do:
1. ~~Pick potential ΔchaC colonies, cross-patch to CHA and CHA-Kan~~
2. ~~Patch single colonies from pF EPs to new plates~~
3. ~~Check reporter strain plates~~

4. ~~Streak PchaC-lacZ to make EC cells again~~
5. ~~Streak out ∆chaC for viable cells expt~~

Results and Data:

Plates from PchaC-lacZ electroporations (EP 66 and 67) are contaminated. Will need to re-do.

On PrpsU1-lacZ plates, there are white colonies that are larger, but most of blue colonies remain small. Leave at 37°C for another night and check tomorrow.

Tuesday, August 27, 2019

To Do:

1. ~~Check ∆chaC by PCR~~
2. ~~Streak potential ∆chaC to single colony~~
3. ~~[Make EC PchaC-lacZ] Re-streak PchaC-lacZ for EC cells~~
4. ~~Resuspend strains with pF plasmids and ∆chaC, plate for CFU~~

Results and Data:

Patch of PchaC-lacZ seems to have some contamination. Re-streak from the glycerol stock to make EC cells tomorrow.

PCR to check for ∆chaC

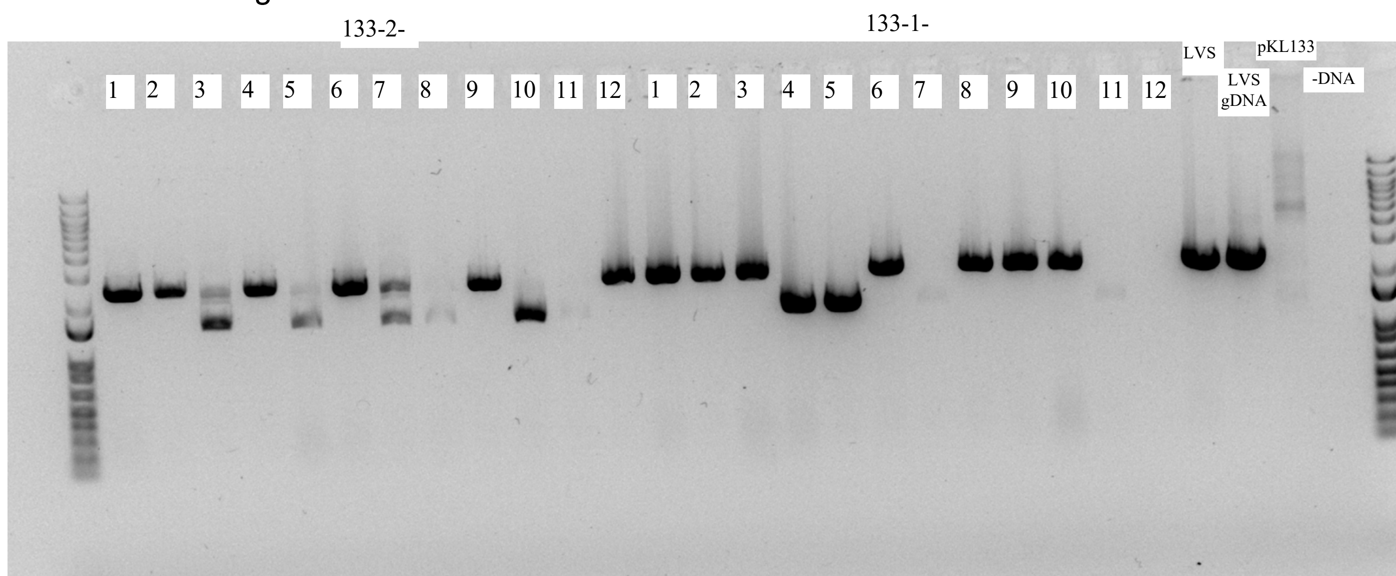
Reaction numbers	Sample	Expected size
1	Colony 2-1	2243 or 1460
2	Colony 2-2	2244 or 1460
3	Colony 2-3	2245 or 1460
4	Colony 2-4	2246 or 1460
5	Colony 2-5	2247 or 1460
6	Colony 2-6	2248 or 1460
7	Colony 2-7	2249 or 1460
8	Colony 2-8	2250 or 1460
9	Colony 2-9	2251 or 1460
10	Colony 2-10	2252 or 1460
11	Colony 2-11	2253 or 1460
12	Colony 2-12	2254 or 1460
13	Colony 1-1	2255 or 1460
14	Colony 1-2	2256 or 1460
15	Colony 1-3	2257 or 1460
16	Colony 1-4	2258 or 1460
17	Colony 1-5	2259 or 1460
18	Colony 1-6	2260 or 1460
19	Colony 1-7	2261 or 1460
20	Colony 1-8	2262 or 1460
21	Colony 1-9	2263 or 1460
22	Colony 1-10	2264 or 1460
23	Colony 1-11	2265 or 1460
24	Colony 1-12	2266 or 1460
25	LVS colony	2243
26	LVS gDNA	2243
27	pKL133 (1:10)	no product
28	-DNA	no product

Total reaction volume	20
Total number of reactions	28

Component	Stock concentration	Final concentration	1 rxn volume	Factor 30
ddiH2O			12.4	372
PrimeSTAR GXL Buffer	5x	1x	4	120
dNTPs	2.5 mM	0.2 mM	1.6	48
P794	10 uM	0.3 uM	0.6	18
P795	10 uM	0.3 uM	0.6	18
template	100 ng/ul	2 ng/ul	0.4	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	12
		Total volume	20	588

Run PCR with STN1, 2.5' extension time.

Ran ~12.5 uL on gel:



Streak to single colony: 2-10, 2-11, 1-4, 1-5 (all mucoid!)

Check viable CFU per OD600:

Number	Strain	Measured OD600	Actual OD600	Volume for 0.03 in 1 mL (uL)	Final OD600
1	LVS pF	0.145	1.45	20.69	0.038
2	LVS pF-chaC	0.348	3.48	8.62	0.036
3	LVS pF2-chaC	0.194	1.94	15.46	0.035
4	ΔchaC pF	0.29	2.9	10.34	0.035
5	ΔchaC pF2-chaC	0.208	2.08	14.42	0.034
6	LVS pF (nat)	0.249	2.49	12.05	0.034
7	ΔchaC pF (nat)	0.347	3.47	8.65	0.036
8	ΔchaC	0.276	2.76	10.87	0.035

(Note that ΔchaC pF-chaC plate must have been contaminated- nothing grew on the patch).
All cells resuspended in DMEM, diluted serially to 10^{-5} in 1X PBS, plated 10 uL undiluted plus all 5 dilutions on track plates, put at 37°C.

Wednesday, August 28, 2019

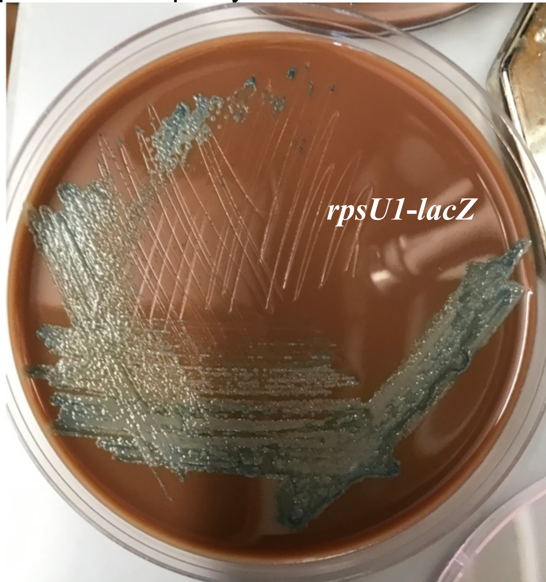
To Do:

1. Pour CHA X-gal Kan plates
2. ~~Check patches of Tn insertions in rpsU1-lacZ~~
3. ~~Make EC PchaC-lacZ~~
4. ~~Move plates for CFU/OD600 to room temp until Friday~~

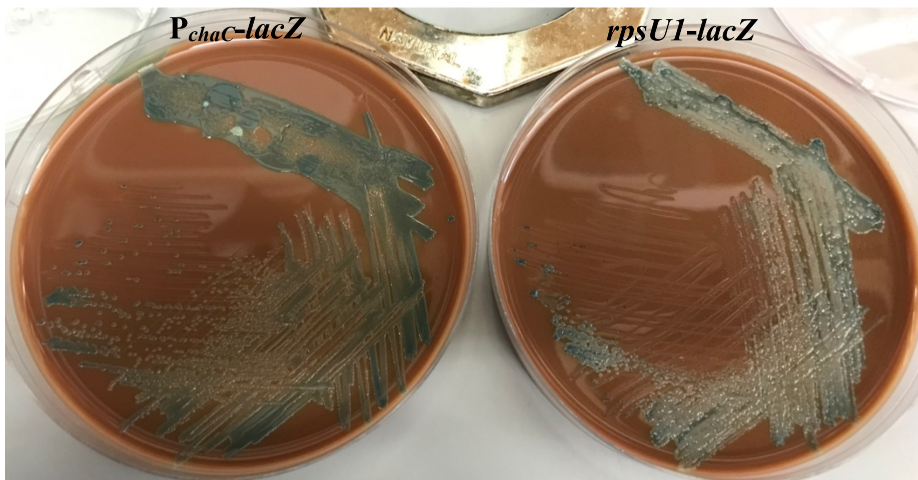
Results and Data:

rpsU1-lacZ transposon mutagenesis

When the *rpsU1-lacZ* strain (KRLVS28.1) is struck out on a plate containing X-gal, the blue color / lacZ production is pretty variable.



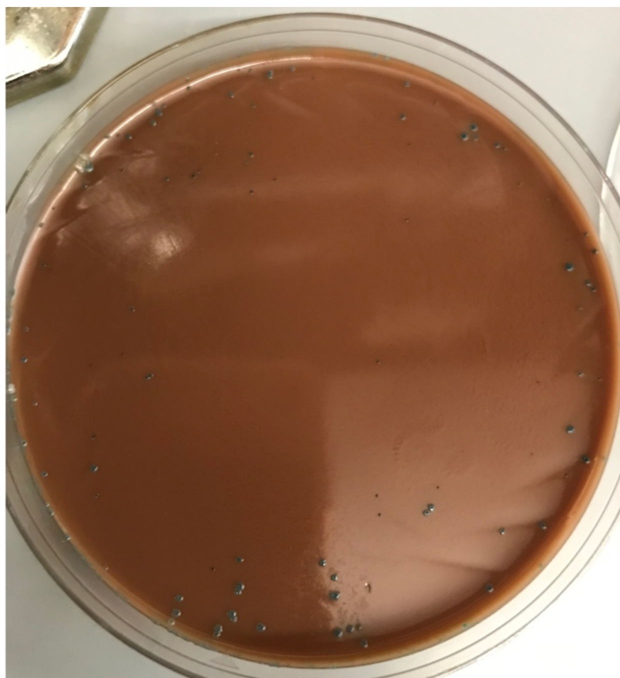
More blue at edges, extremely dense areas, and single colonies.

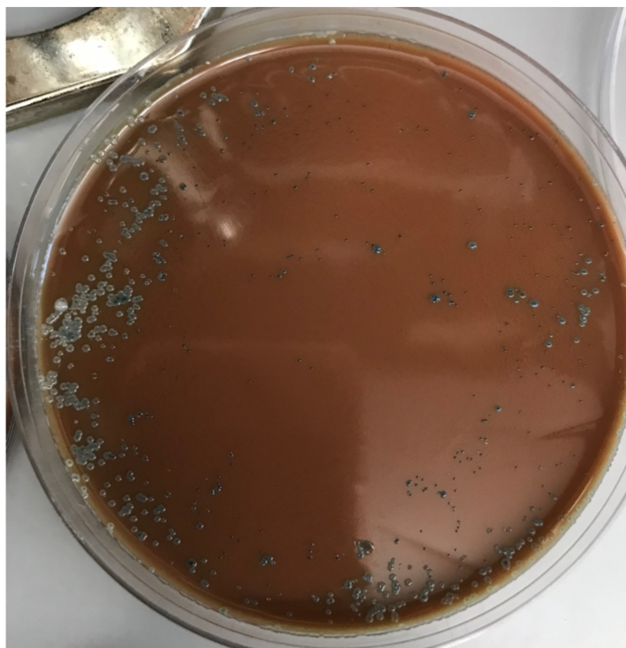


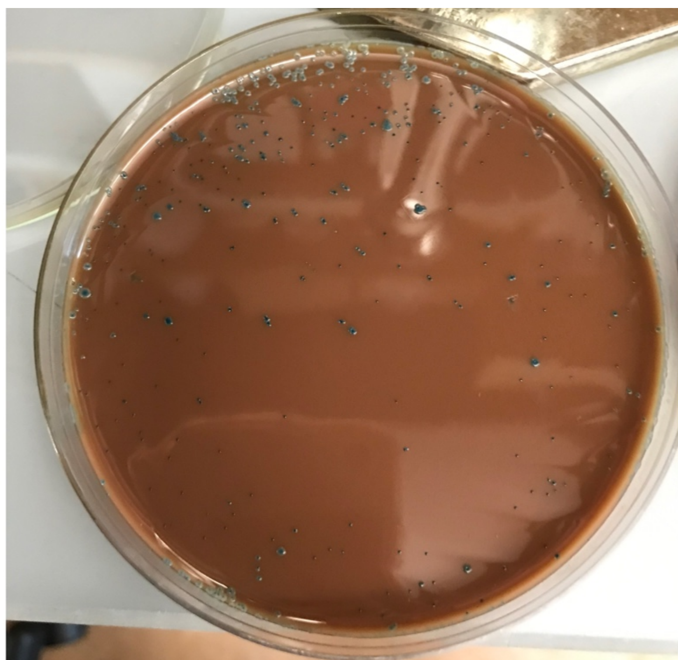
This is in contrast to other reporter strains like PchaC-lacZ (KRLVS39.1), which is more uniform in lacZ production.

This makes evaluating single colonies after transposon mutagenesis difficult; the strain itself would typically have color variation!

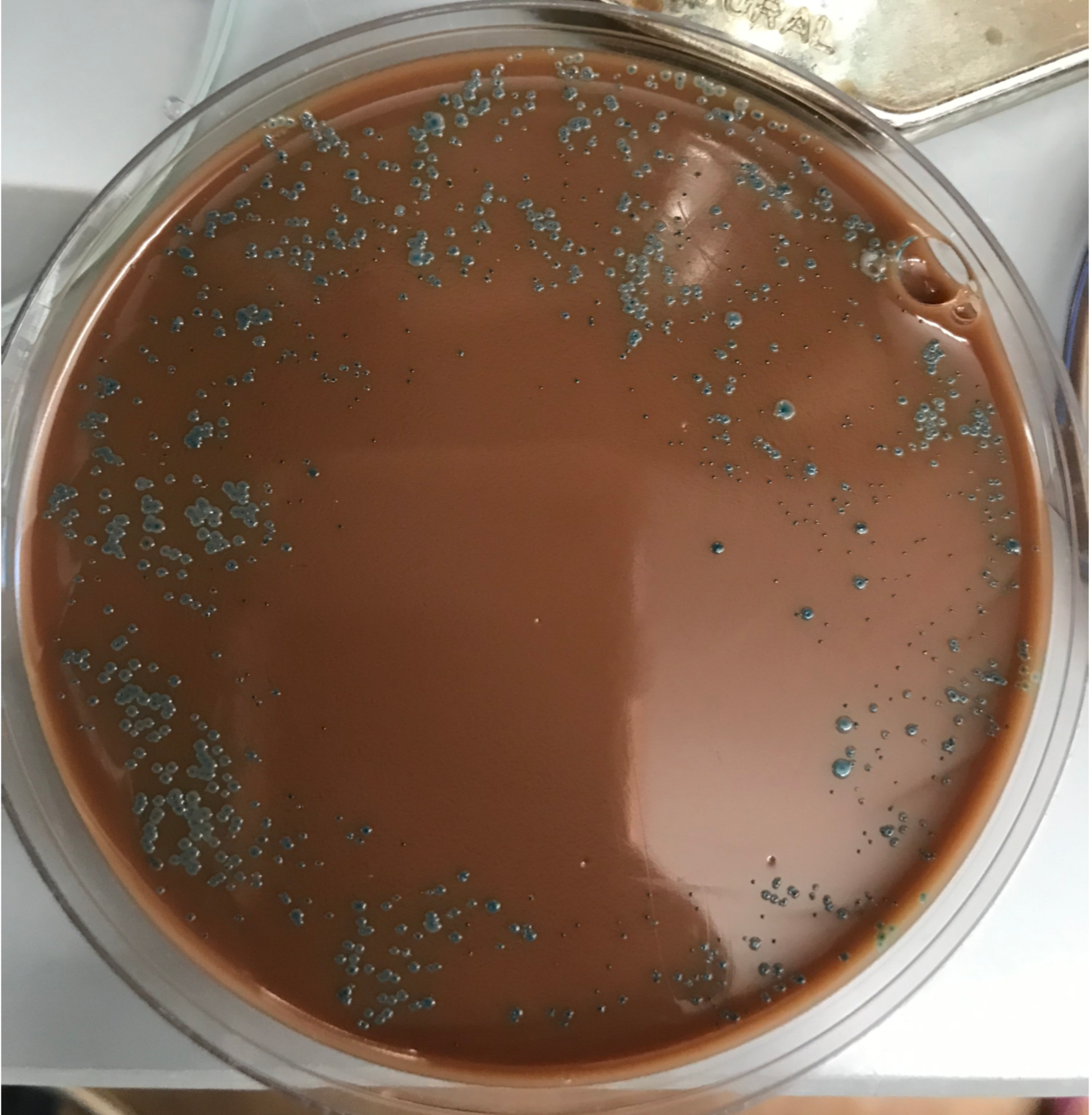
Some examples of plates:







KIMURA



There is frequently more pigment in the center of the colonies and there are many tiny blue colonies. I tried picking some of them yesterday and patching to a new X-gal plate but they did not grow. The colonies I did pick looked variable upon patching (similarly to the un-mutagenized strain), making them difficult to evaluate, not worth pursuing. I attempted to patch more tiny blue colonies to a Kan plate (no X-gal) and left at room temp to see if they are simply slow-growing strains.

I also picked white colonies yesterday and patched them out to X-gal plates. They all appeared white, with one having some small amount of blue. I'm hoping that means that in that strain, lacZ might be intact and there may just be lower levels of rpsU1 (it is likely that all the white colonies are the result of the transposon hopping into lacZ itself).

I froze down single tubes of glycerol stocks for each of the 4 white colonies (1 -> 4, EP68-1, EP68-2, EP69-1, EP69-2) and took samples of the patch for colony PCR.

Reaction numbers	Sample	Expected size
1	rpsU1-lacZ pSD26-1 (68-1)	3841 or 4282
2	rpsU1-lacZ pSD26-2 (68-2)	3841 or 4282
3	rpsU1-lacZ pSD26-3 (69-1)	3841 or 4282
4	rpsU1-lacZ pSD26-4 (69-2)	3841 or 4282
5	rpsU1-lacZ	3841
6	LVS gDNA	723
7	-DNA	no product

Total reaction volume	20
Total number of reactions	7

Component	Stock concentration	Final concentration	1 rxn volume	Factor 8.5
ddiH2O			12.4	105.4
PrimeSTAR GXL Buffer	5x	1x	4	34
dNTPs	2.5 mM	0.2 mM	1.6	13.6
KROL233 / P744	10 uM	0.3 uM	0.6	5.1
P742	10 uM	0.3 uM	0.6	5.1
template	100 ng/ul	2 ng/ul	0.4	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	3.4
		Total volume	20	166.6

STN1 with 4:30 extension (O/N).

Friday, August 30, 2019

To Do:

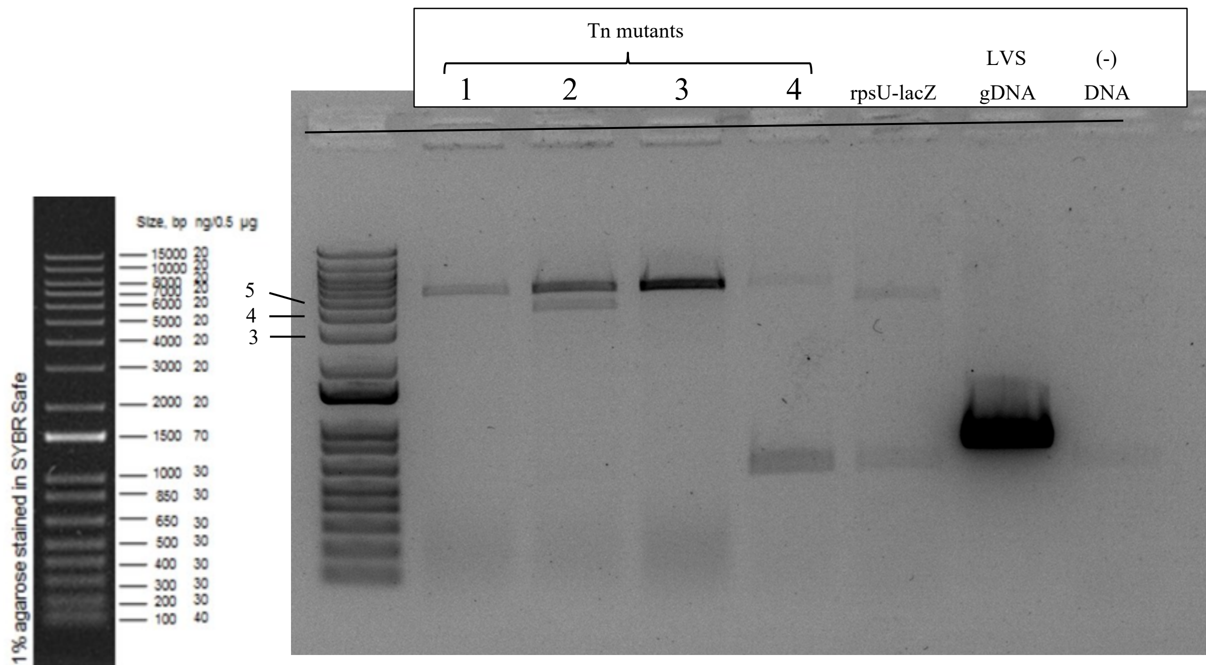
1. ~~Electroporate PchaC-lacZ with pSD26~~
2. ~~Plate electroporations~~
3. ~~Check PCR on gel~~
4. Count colonies from viable cells expt

Results and Data:

Transposon mutagenesis of reporter strains:

Electroporation	Strain	EC cells	Plasmid	Volume	Notes
70	PchaC-lacZ	KMR 8.23.19	pSD26	3 uL	
71	PchaC-lacZ	KMR 8.23.19	pSD26	3 uL	

pSD26 was at 333 ng/uL so about 1 ug per electroporation.
Recovering at ~8:30am – 11:30. Plated 3x 100 ul, 1x 300 ul, 2x 500 ul on CHA-Kan-X-gal (HT and JW poured yesterday)



These results aren't entirely clear.

Reaction numbers	Sample	Expected size	Apparent size
1	rpsU1-lacZ pSD26-1 (68-1)	3841 or 4282	>6 kb
2	rpsU1-lacZ pSD26-2 (68-2)	3841 or 4282	>6 kb
3	rpsU1-lacZ pSD26-3 (69-1)	3841 or 4282	>6 kb, 4.2kb?
4	rpsU1-lacZ pSD26-4 (69-2)	3841 or 4282	>6 kb?, ~650bp
5	rpsU1-lacZ	3841	4.2kb?, ^50bp?
6	LVS gDNA	723	~850bb
7	-DNA	no product	Faint band

There is a faint band in the – control- I should re-do the reaction. Everything looks a bit off in terms of size. However, there is some consistency in a high band being present in all the white colonies (1->4) with some interesting lower bands in 3 and 4. Re-doing the PCR and running it out further should help.

Bibliography