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July 2020

Tuesday, July 28, 2020

To Do:

1. Streak out plates for RNA

Results and Data:

Streak out:

Number	Description	Strain number
1	LVS	0
2	LVS FTL_0146(F315L)	KRLVS69
3	LVS ΔpmrA	KRLVS40
4	LVS ΔpmrA FTL_0146(F213L)	KRLVS81
5	LVS ΔpmrA(sup)	KMLFT37

Note that KMLFT37 was struck from Jamie's stock (label ΔpmrA suppressor)
LVS ΔpmrA was a single-use aliquot from Jamie
Both KRLVS69 and KRLVS81 need to be moved from Jamie's boxes to the lab strain boxes

Thursday, July 30, 2020

To Do:

1. Make glycerol stocks for strains
2. Make single-use aliquots for strains
3. Streak out to single colony again, just in case.

Results and Data:

Made a single 1.2 mL glycerol stock per strain and 6x100uL single use stocks. (1500uL cells in MH+ and 380 uL 75% glycerol)

August 2020

Saturday, August 1, 2020

To Do:

1. Patch out single colonies

Results and Data:

Will need to validate the identity of the strains I'm using. Confirm:

Presence/absence of *pmrA*
Presence/absence of *priM*
Presence/absence of FTL_0146(F315L) mutation

Sunday, August 2, 2020

To Do:

1. Grow cells, isolate RNA

Results and Data:

Setup:

Number	Description	Strain number
1	LVS	0
2	LVS FTL_0146(F315L)	KRLVS69
3	LVS ΔpmrA	KRLVS40
4	LVS ΔpmrA FTL_0146(F213L)	KRLVS81
5	LVS ΔpmrA(sup)	KMLFT37

Note that LVS ΔpmrA(sup) didn't grow very well- 6 patches, 5 with little to no growth and the last with pretty robust growth (which makes me concerned!). The robust growth patch is replicate "C". I could use a single patch for replicate "B", and I had to scrape up some extra, non-single colony cells for replicate "A."

		Diluted OD600	Dilution Factor	Actual OD600	Desired OD600	Total volume (mL)	Volume cells (uL)	Volume MHB (ul, + 7 mL)
1A	LVS	0.347	20	6.94	0.08	8	92.2	907.8
1B		0.424	20	8.48	0.08	8	75.5	924.5
1C		0.406	20	8.12	0.08	8	78.8	921.2
2A	LVS FTL_0146(F315L)	0.34	20	6.8	0.08	8	94.1	905.9
2B		0.302	20	6.04	0.08	8	106.0	894.0
2C		0.332	20	6.64	0.08	8	96.4	903.6
3A	LVS ΔpmrA	0.29	20	5.8	0.08	8	110.3	889.7
3B		0.162	20	3.24	0.08	8	197.5	802.5
3C		0.175	20	3.5	0.08	8	182.9	817.1
4A	LVS ΔpmrA FTL_0146(F213L)	0.268	20	5.36	0.08	8	119.4	880.6
4B		0.215	20	4.3	0.08	8	148.8	851.2
4C		0.106	20	2.12	0.08	8	301.9	698.1
5A	LVS ΔpmrA(sup)	0.436	10	4.36	0.08	8	146.8	853.2
5B		0.22	10	2.2	0.08	8	290.9	709.1
5C		0.349	20	6.98	0.08	8	91.7	908.3

Tuesday, August 4, 2020

To Do:

1. Final RNA purification
2. Run gel
3. Make cDNA

Results and Data:

Generate cDNA (half protocol)

REACTION SIZE CUT IN HALF from Lory lab microarray protocol
Combine the first components for primer annealing:

Component	Volume or Amount	Final Concentration
RNA	3 ug	267 - 333 ng/ ul
$(NS)_5$ Primer (250 ng/ul)	1.5 ul	25 ng/ul
RNase-free water	up to 15 ul	

	Gel		cDNA	
Sample ID	RNA (1.5 ug)	Water	RNA (3 ug)	Water
1A	3.53	6.47	7.06	6.44
1B	3.57	6.43	7.15	6.35
1C	3.33	6.67	6.67	6.83
2A	2.96	7.04	5.91	7.59
2B	2.72	7.28	5.44	8.06
2C	2.87	7.13	5.74	7.76
3A	3.55	6.45	7.10	6.40
3B	5.12	4.88	10.24	3.26
3C	4.75	5.25	9.51	3.99
4A	3.17	6.83	6.35	7.15
4B	2.93	7.07	5.87	7.63
4C	2.92	7.08	5.84	7.66
5A	4.22	5.78	8.44	5.06
5B	3.55	6.45	7.11	6.39
5C	2.79	7.21	5.58	7.92

Incubate using program JSScDNA1:

Step	Temp	Time
1	70°C	10'
2	25°C	10'
3	4°C	hold

While waiting, prepare the cDNA synthesis reaction in master mix format:

Component	Final Concentration	Volume or Amount	x 16.5
5X 1st strand buffer	1x	6 ul	8.25
RNase-free water		2.88 ul	47.52
100 mM DTT	10 mM	3.0 ul	49.5
10 mM dNTPs	0.5 mM	1.5 ul	24.75
Superscript III (200 U/ul)	10.8 U/ul	1.63	26.9

Aliquot 15 ul per reaction
Screwed up- check out that buffer volume indicated above- waaay off! Will need to re-do another time. ☹

Wednesday, August 26, 2020

To Do:
1. Make cDNA

Results and Data:

Generate cDNA (half protocol)

REACTION SIZE CUT IN HALF from Lory lab microarray protocol
Combine the first components for primer annealing:

Component	Volume or Amount	Final Concentration
RNA	3 ug	267 - 333 ng/ ul
$(NS)_5$ Primer (250 ng/ul)	1.5 ul	25 ng/ul
RNase-free water	up to 15 ul	

					cDNA		
Number	Sample ID		Date isolated	RNA Conc (ng/ul)	RNA	H2O	Total RNA
1	1A	LVS	8/4/20	424.9	7.06	6.44	3000.0
2	1B			419.7	7.15	6.35	3000.0
3	1C			449.9	6.67	6.83	3000.0
4	2A	LVS FTL_0146(F315L)		507.3	5.91	7.59	3000.0
5	2B			551.9	5.44	8.06	3000.0
6	2C			522.7	5.74	7.76	3000.0
7	3A	LVS ΔpmrA		422.7	7.10	6.40	3000.0
8	3B			292.9	10.24	3.26	3000.0
9	3C			315.6	9.51	3.99	3000.0
10	4A	LVS ΔpmrA FTL_0146(F213L)		472.8	6.35	7.15	3000.0
11	4B			511.5	5.87	7.63	3000.0
12	4C			513.8	5.84	7.66	3000.0
13	5A	LVS ΔpmrA(sup)		355.6	8.44	5.06	3000.0
14	5B			422.2	7.11	6.39	3000.0
15	5C			537.3	5.58	7.92	3000.0
16	1A	LVS ΔpmrA (old)	11/14/19	150.96	13.50	0.00	2038.0
17	1B			196.01	13.50	0.00	2646.1
18	1C			185.08	13.50	0.00	2498.6
19	2A	LVS ΔpmrA (new)		383.44	7.82	5.68	3000.0
20	2B			353.3	8.49	5.01	3000.0
21	2C			388.73	7.72	5.78	3000.0

Incubate using program JSScDNA1:

Step	Temp	Time
1	70°C	10'
2	25°C	10'
3	4°C	hold

While waiting, prepare the cDNA synthesis reaction in master mix format:

Component	Final Concentration	Volume	x 22.5
5X 1st strand buffer	1x	6	135
RNase-free water		2.88	64.8
100 mM DTT	10 mM	3	67.5
10 mM dNTPs	0.5 mM	1.5	33.75
Superscript III (200 U/ul)	10.8 U/ul	1.63	36.68

Aliquot 15 ul per reaction

Incubate using program JSScDNA2

Step	Temp	Time
1	25°C	10'
2	37°C	60'
4	42°C	60'
5	70°C	10'
6	4°C	hold

Move samples to -80°C until I can purify them.

Monday, August 31, 2020

To Do:

1. ~~Purify cDNA~~
2. Set up RT-PCR reactions

Results and Data:

Add 10 ul of 1N NaOH
Incubate 65°C for 30'
Neutralize with 10 ul of 1N HCl
Final volume is 50 ul
Purify cDNA using Qiagen PCR clean-up column
Elute in 60 ul of 0.1x EB

Check concentration by Nanodrop:

Number	Sample ID	Strain	Nucleic Acid Conc (ng/µl)	A260	A280	260/280	260/230
1	1A	LVS	19.4	0.587	0.321	1.83	1.29
2	1B		54.4	1.649	0.915	1.8	1.76
3	1C		21.1	0.639	0.349	1.83	1.57
4	2A	LVS FTL_0146(F315L)	59	1.788	1.003	1.78	1.9
5	2B		53	1.607	0.902	1.78	1.75
6	2C		44.7	1.355	0.758	1.79	1.87
7	3A	LVS ΔpmrA	46.5	1.41	0.791	1.78	2.08
8	3B		47.7	1.447	0.81	1.79	1.7
9	3C		38.6	1.171	0.653	1.79	1.78
10	4A	LVS ΔpmrA FTL_0146(F213L)	55.3	1.674	0.936	1.79	1.68
11	4B		39.8	1.206	0.669	1.8	1.29
12	4C		50.7	1.536	0.865	1.78	1.66
13	5A	LVS ΔpmrA(sup)	26.3	0.796	0.441	1.81	1.56
14	5B		48	1.453	0.831	1.75	2.02
15	5C		40.6	1.229	0.68	1.81	1.58
16	1A	LVS ΔpmrA (old)	26.8	0.813	0.448	1.82	1.49
17	1B		36.9	1.118	0.624	1.79	0.97
18	1C		34.4	1.042	0.572	1.82	1.25
19	2A	LVS ΔpmrA (new)	41.1	1.246	0.689	1.81	2.07
20	2B		34.1	1.034	0.563	1.84	2.03
21	2C		38.8	1.174	0.657	1.79	1.9
1	1A	LVS	19.2	0.582	0.304	1.91	1.24

Multiple samples have poor results (high 260/280 ratios). ☹

September 2020

Saturday, September 12, 2020

To Do:

1. Set up RT-PCR reactions
2. Streak out cells for gDNA isolation
    a. ΔpmrA ΔpriM KMLFT49
    b. ΔpmrA KMLFT37
    c. ΔpmrA priM(S) KMLFT67
    d. ΔpmrA mreA(S) KMLFT69
    e. LVS

Results and Data:

Testing *priM* and *tul4* transcript abundances. Can only fit 16 samples per plate with 2 primer pairs.

Dilute cDNA to 1.5 ng/uL

		Volumes to dilute for 1.5 ng/uL	
Sample	**[cDNA]**	**cDNA**	**H2O**
1	19.4	2	23.9
2	54.4	2	70.5
3	21.1	2	26.1
4	59	2	76.7
5	53	2	68.7
6	44.7	2	57.6
7	46.5	2	60.0
8	47.7	2	61.6
9	38.6	2	49.5
10	55.3	2	71.7
11	39.8	2	51.1
12	50.7	2	65.6
13	26.3	2	33.1
14	48	2	62.0
15	40.6	2	52.1
16	26.8	2	33.7

Can fit 16 samples per plate, analyzing *priM* and *tul4*

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1			A9			B1			B9		
B	A2			A10			B2			B10		
C	A3			A11			B3			B11		
D	A4			A12			B4			B12		
E	A5			A13			B5			B13		
F	A6			A14			B6			B14		
G	A7			A15			B7			B15		
H	A8			A16			B8			B16		

16 samples * 3.5 + 3.5 = 59.5 reactions per primer pair

Reactions		
A	*priM*	P437 P438
B	*tul4*	KROL63 KROL64

	1x	Master Mix
2x Power Up	10	595
5 uM primers	1	59.5
H2O	8	476

-Aliquot 3.5 uL cDNA per tube
-Add 66.5 uL master mix
-Dispense 20uL per well

Place in real-time machine and run rxn.
Parameters for old step-one program:
1. 95°C 10'
2. 95°C 15"
3. 60°C 60"
4. Go to step 2, 39x (total 40 cycles)
5. 95°C 10"
6. 65°C 60"
7. 97°C 60"

Sunday, September 13, 2020

To Do:
1. Analyze RT-PCR data
2. Set up second set of RT-PCR reactions
3. Isolate gDNA

Results and Data:

Set up second set of RT-PCR reactions
Dilute cDNA to 1.5 ng/uL

		Volumes to dilute for 1.5 ng/uL	
Sample	**[cDNA]**	**cDNA**	**H2O**
17	36.9	2	47.2
18	34.4	2	43.9
19	41.1	2	52.8
20	34.1	2	43.5
21	38.8	2	49.7

	1	2	3	4	5	6
A	A17			B17		
B	A18			B18		
C	A19			B19		
D	A20			B20		
E	A21			B21		
F						
G						
H						

5 samples * 3.5 + 3.5 = 21 rxns per primer pair

Reactions		
A	*priM*	P437 P438
B	*tul4*	KROL63 KROL64

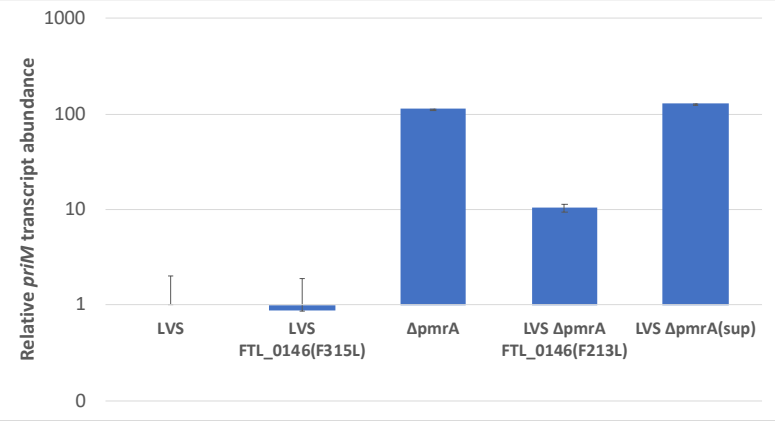
	1x	Master Mix
2x Power Up	10	210
5 uM primers	1	21
H2O	8	168

-Aliquot 3.5 uL cDNA per tube
-Add 66.5 uL master mix
-Dispense 20uL per well

Place in real-time machine and run rxn.
Parameters for old step-one program:
1. 95°C 10'
2. 95°C 15"
3. 60°C 60"
4. Go to step 2, 39x (total 40 cycles)
5. 95°C 10"
6. 65°C 60"
7. 97°C 60"

Get data from last plate and perform analysis on first set of samples:

qRT-PCR results from 9/12/20



Thoughts

Looking over the data, the Δ*pmrA* A sample is quite a bit of an outlier. Most of the samples with *pmrA* (WT background) have very little *priM*, so high ΔCt values for *priM*. The *pmrA* replicates B and C are consistent with previous findings- much lower ΔCt values for *priM*. If I include replicate A in the analysis, there is only 44x more *priM* in Δ*pmrA* than in wild-type. But if I exclude replicate A, there is a 112x difference in *priM* abundance. The second scenario is more similar to what we've seen before (although lower than the 300x both Jamie and I have found previously). Going back to the growth curves, I was actually quite suspicious of the Δ*pmrA* replicate A culture- it grew faster and reached a higher final density than replicates B and C. For these reasons, I suspect it was contaminated with a different strain- don't use that replicate in the analysis.

Looking back at the growth curves, there was also significant differences in the Δ*pmrA*(sup) strain; replicate C grew faster and achieved a higher final density than replicates A and B. If I include that replicate, the fold change is 127x. If I exclude it, the fold change increases to 167. This is all strange because Jamie replicated that the Δ*pmrA*(sup) strain had about 30-40x increase in *priM* over wild-type, compared to 200-300x increase of *priM* in Δ*pmrA*. I'm a bit concerned about the genotypes of these cells! For now, keep all the replicates.

Data Analysis

priM	average	stdev	*tul4*	average	stdev	DCt
LVS A	28.14	0.056	LVS A	19.66	0.031	8.483
LVS B	26.70	0.038	LVS B	17.72	0.010	8.983
LVS C	28.79	0.000	LVS C	20.00	0.015	8.787
LVS FTL_0146(F315L) A	26.53	0.074	LVS FTL_0146(F315L) A	17.63	0.021	8.900
LVS FTL_0146(F315L) B	26.78	0.095	LVS FTL_0146(F315L) B	17.72	0.015	9.063
LVS FTL_0146(F315L) C	26.88	0.006	LVS FTL_0146(F315L) C	17.91	0.030	8.967
LVS ΔpmrA A	22.82	0.042	LVS ΔpmrA A	17.39	0.040	5.423
LVS ΔpmrA B	18.02	0.015	LVS ΔpmrA B	17.06	0.012	0.967
LVS ΔpmrA C	17.92	0.035	LVS ΔpmrA C	17.45	0.006	0.468
LVS ΔpmrA FTL_0146(F213L) A	22.25	0.096	LVS ΔpmrA FTL_0146(F213L) A	17.27	0.035	4.973
LVS ΔpmrA FTL_0146(F213L) B	23.38	0.135	LVS ΔpmrA FTL_0146(F213L) B	19.02	0.015	4.363
LVS ΔpmrA FTL_0146(F213L) C	23.00	0.047	LVS ΔpmrA FTL_0146(F213L) C	18.02	0.021	4.980
LVS ΔpmrA(sup) A	20.21	0.015	LVS ΔpmrA(sup) A	19.84	0.015	0.370
LVS ΔpmrA(sup) B	17.77	0.031	LVS ΔpmrA(sup) B	18.06	0.006	-0.290
LVS ΔpmrA(sup) C	20.05	0.114	LVS ΔpmrA(sup) C	18.63	0.006	1.420
LVS ΔpmrA (old) A	18.04	0.036	LVS ΔpmrA (old) A	18.78	0.026	-0.740

	average DCt	stdev	DDCT vs control	s	1.8^-averDDCT	DDCT +/- stdev	1.8^-DDCT+/- stdev		error bars
LVS	8.751	0.252	0.000	0.356	1.000	0.356	0.81	+	0.189
						-0.356	1.23	-	0.233
LVS FTL_0146(F315L)	8.977	0.082	0.226	0.265	0.876	0.490	0.75	+	0.126
						-0.039	1.02	-	0.148
ΔpmrA	0.717	0.352	-8.034	0.433	112.398	-7.600	87.13	+	25.264
						-8.467	144.99	-	32.589
LVS ΔpmrA FTL_0146(F213L)	4.772	0.354	-3.979	0.435	10.368	-3.544	8.03	+	2.337
						-4.413	13.39	-	3.017
LVS ΔpmrA(sup)	0.500	0.862	-8.251	0.898	127.727	-7.353	75.33	+	52.401
						-9.150	216.58	-	88.855

Started DNA purification but accidentally put tubes at 95°C instead of 65°C! ☹
Re-streak cells and do tomorrow.

Monday, September 14, 2020

To Do:

1. Analyze RT-PCR data
2. Isolate gDNA

Results and Data:

Purify gDNA
Samples:

1	KMLFT49	ΔpmrA ΔpriM
2	KMLFT37	ΔpmrA
3	KMLFT67	ΔpmrA priM(S)
4	KMLFT69	ΔpmrA mreA(S)
5		LVS

DNA Purification Protocol

1. Dilute 1 µl of Proteinase K into 300 µl of Tissue and Cell Lysis Solution for each sample (can use 310 uL to account for pipetting error). **5x310ul = 1550 uL + 5.17 uL Proteinase K**
2. Scrape up cells from plate, resuspend in 1X PBS, pellet, and discard the supernatant, leaving approximately 25 µl of liquid. **Put at -20°C for ~30' until ready for next step.**
3. Vortex for 10 seconds to resuspend the cell pellet.
4. Add 300 ul of Tissue and Cell Lysis Solution containing the Proteinase K and mix thoroughly.
5. Incubate at 65°C for 15 minutes; vortex every 5 minutes.
6. Cool the samples to 37°C and add 2 µl of 5 mg/ml RNase A to the sample; mix thoroughly. **Added 1uL RNase A (oops).**
7. Incubate at 37°C for 30 minutes.
8. Place the samples on ice for 3-5 minutes and then proceed with total DNA precipitation. **Put samples at -80°C for 3'**.

1. Add 150 µl of MPC Protein Precipitation Reagent to 300 µl of lysed sample and vortex vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4°C for 10 minutes at ≥10,000 x g in a microcentrifuge. If the resultant pellet is clear, small, or loose, add an additional 25 µl of MPC Protein Precipitation Reagent, mix, and pellet the debris again.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 500 µl of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
5. Pellet the DNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.
6. Carefully pour off the isopropanol without dislodging the DNA pellet.
7. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet and let dry completely under hood.
8. Resuspend the DNA in 35 µl of 0.1x EB Buffer. Put on ice to help dissolve, and add 50 µl of additional buffer if DNA is very goopy. Added 35 ul to all but #1 (70 uL). Put at -20°C until have time to move forward.

Wednesday, September 16, 2020

To Do:

- ~~1. Quantify gDNA~~
- ~~2. Set up PCR reactions~~
- ~~3. Purify PCR reactions~~
- ~~4. Send to sequence~~

Results and Data:

gDNA quantity and quality look good

#	Sample ID	[DNA] (ng/ul)	A260	A280	260/280	260/230	Volume (ul) for dilutions (100 n/ul) gDNA	0.1x EB
1	KMLFT49 ΔpmrA ΔpriM	1557.9	31.159	15.053	2.07	1.68	5	72.9
2	KMLFT37 ΔpmrA	3087.8	61.756	29.726	2.08	1.96	5	149.4
3	KMLFT67 ΔpmrA priM(S)	1694.1	33.882	16.88	2.01	1.88	5	79.7
4	KMLFT69 ΔpmrA mreA(S)	1949.1	38.983	20.223	1.93	1.73	5	92.5
5	LVS	1161.9	23.239	11.428	2.03	1.64	5	53.1

Make indicated dilutions and set up 3 PCR reactions:

Reaction	Locus	Primers	Expected size (WT)	Expected size (Δ)	Sequencing primer
A	*pmrA*	KROL61, KROL62	1204	535	-
B	*priM*	KROL155, KROL156	1792	352	-
C	FTL_0146	KROL270, KROL271	1320	-	KROL270

Sample Number	Strain number	Strain name
1	KMLFT49	ΔpmrA ΔpriM
2	KMLFT37	ΔpmrA
3	KMLFT67	ΔpmrA priM(S)
4	KMLFT69	ΔpmrA mreA(S)
5		LVS
6		-DNA

Total reaction volume	30
Total number of reactions	6
Total number of master mixes	3

Component	Stock concentration	Final concentration	1 rxn volume	3 master mixes 22	Individual master mix 7
ddiH2O			18.6	409.2	
PrimeSTAR GXL Buffer	5x	1x	6	132	
dNTPs	2.5 mM	0.2 mM	2.4	52.8	193.2
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.6	13.2	
Oligo 1	10 uM	0.3 uM	0.9	-	6.3
Oligo 2	10 uM	0.3 uM	0.9	-	6.3
template	100 ng/ul	2 ng/ul	0.6		
		Total volume	30	607.2	205.8

Use STN1 with a 2' extension time.

PCR-purify reactions C1-C5

#	Sample ID	[DNA] (ng/ul)	A260	A280	260/280	260/230
C1	FTL_0146_ΔpmrAΔpriM	68.9	1.377	0.767	1.8	2.39
C2	FTL_0146_ΔpmrA	67.7	1.353	0.754	1.8	2.35
C3	FTL_0146_ΔpmrApriM(S)	76	1.52	0.848	1.79	2.4
C4	FTL_0146_ΔpmrAmreA(S)	75.1	1.501	0.842	1.78	2.36
C5	FTL_0146_LVS	74.3	1.487	0.841	1.77	2.37

Dilute all gDNA 1:10.

Set up and submit sequencing reactions with KROL270

Sample number	Template Name	Primer Name	A.	B.	C.	D.	F.
			Template Size (bases)	Template Stock Conc. (ng/µl)	**PCR** template:	**PCR** template:	Volume **H_2O**
					ng needed =	Volume =	
					(A ÷ 100) × 2.5	**(C ÷ B)µl**	**(12 less D or E - 2.56)µl**
KMR_C1	FTL_0146_ΔpmrAΔpriM	KROL270	1320	6.8	33.00	4.85	4.59
KMR_C2	FTL_0146_ΔpmrA	KROL270	1320	6.8	33.00	4.85	4.59
KMR_C3	FTL_0146_ΔpmrApriM(S)	KROL270	1320	6.8	33.00	4.85	4.59
KMR_C4	FTL_0146_ΔpmrAmreA(S)	KROL270	1320	6.8	33.00	4.85	4.59
KMR_C5	FTL_0146_LVS	KROL270	1320	6.8	33.00	4.85	4.59

Thursday, September 17, 2020

To Do:

1. Finish analyzing and reviewing qRT-PCR data

Results and Data:

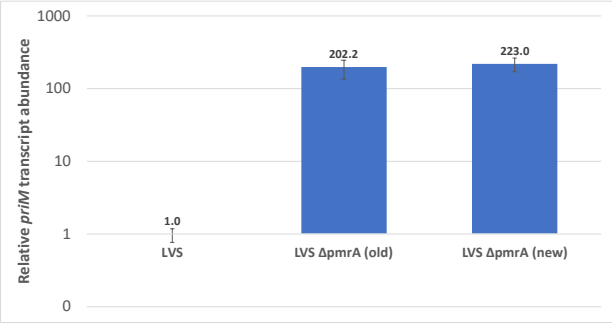
Have data from 9/13/20 that I never put into the spreadsheet. Analyze now:

priM	**average**	**stdev**	***tul4***	**average**	**stdev**	**DCt**
LVS ΔpmrA (old) A	18.04	0.036	LVS ΔpmrA (old) A	18.78	0.026	-0.740
LVS ΔpmrA (old) B	17.81	0.059	LVS ΔpmrA (old) B	17.86	0.035	-0.047
LVS ΔpmrA (old) C	17.86	0.021	LVS ΔpmrA (old) C	17.91	0.025	-0.057
LVS ΔpmrA (new) A	17.81	0.04	LVS ΔpmrA (new) A	17.97	0.025	-0.153
LVS ΔpmrA (new) B	18.23	0.015	LVS ΔpmrA (new) B	18.87	0.045	-0.633
LVS ΔpmrA (new) C	18.19	0.026	LVS ΔpmrA (new) C	18.75	0.006	-0.557

This RNA was isolated on a different day that the LVS control (for the RNA-Seq experiment), but make the comparison for this particular experiment:

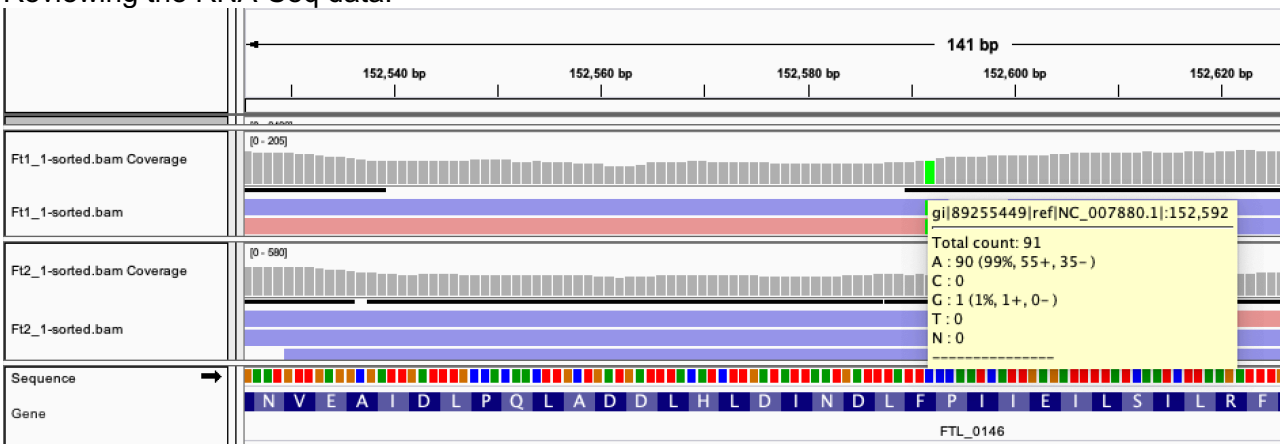
	average DCt	**stdev**	**DDCT vs control**	**s**	**1.8^-averDDCT**	**DDCT +/- stdev**	**1.8^-DDCT+/-stdev**		**error bars**
LVS	8.751	0.252	0.000	0.356	1.000	0.356	0.81	+	0.189
						-0.356	1.23	-	0.233
LVS ΔpmrA (old)	-0.281	0.397	-9.032	0.471	202.152	-8.562	153.31	+	48.845
						-9.503	266.56	-	64.407
LVS ΔpmrA (new)	-0.448	0.258	-9.199	0.360	222.958	-8.838	180.39	+	42.571
						-9.559	275.58	-	52.618

Both of these samples look like they are expressing quite a bit of *priM*!



This is notable because we didn't see a difference in the RNA-Seq data comparing the ΔpmrA and Δ*pmrA*(sup) strain; this experiment validates that finding but also highlights that both strains are producing high levels of *priM*!

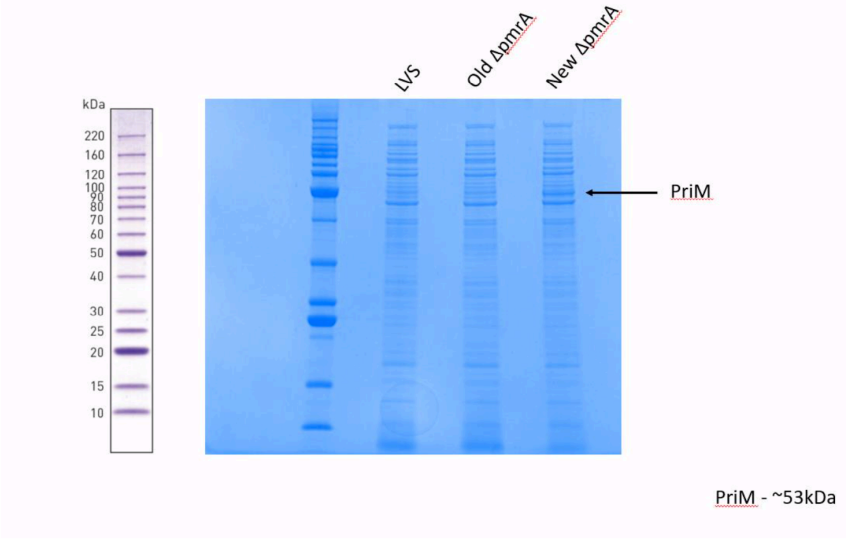
Reviewing the RNA-Seq data:



The FTL_0146 mutation is absolutely present in the Δ*pmrA*(old) sample.

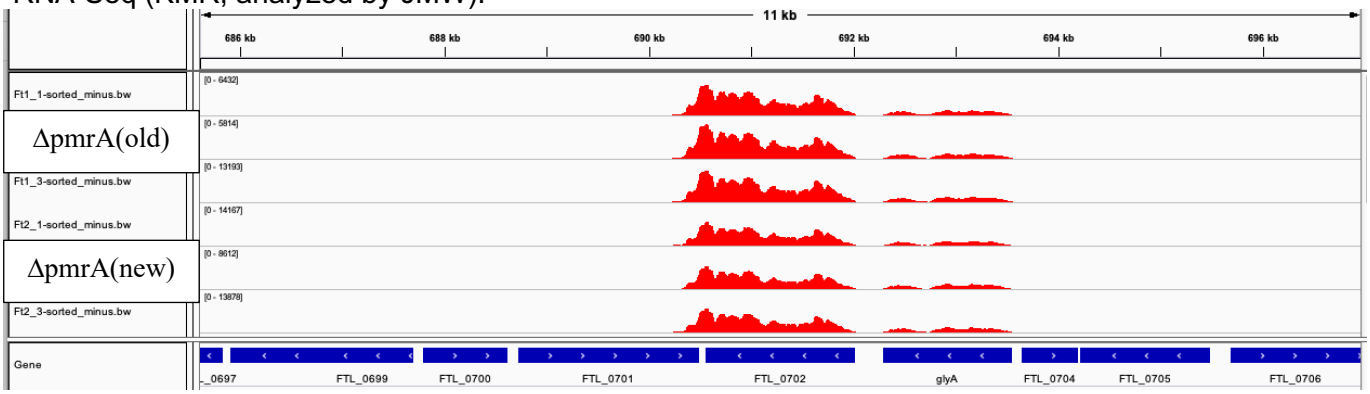
The conflicting results:
Old ΔpmrA = ΔpmrA(sup); New ΔpmrA = ΔpmrA

-Coomassie gel (JMW, 8/20/19):



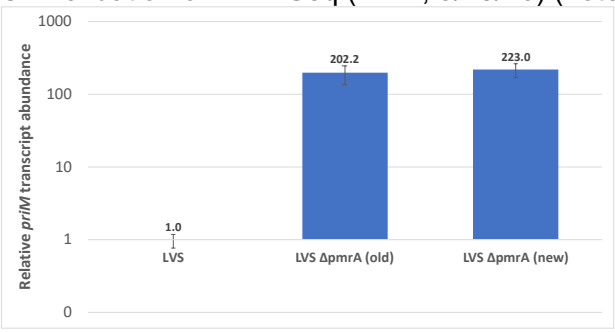
There is clearly more PriM produced in the new ΔpmrA strain

-RNA-Seq (KMR, analyzed by JMW):



ΔpmrA(new) and ΔpmrA(old) have similar amounts of priM transcript, and it is quite abundant (no other transcript of similar abundance nearby)

-qRT-PCR validation of RNA-Seq (KMR, 9/13/20) (note that LVS sample isn't the perfect comparison).

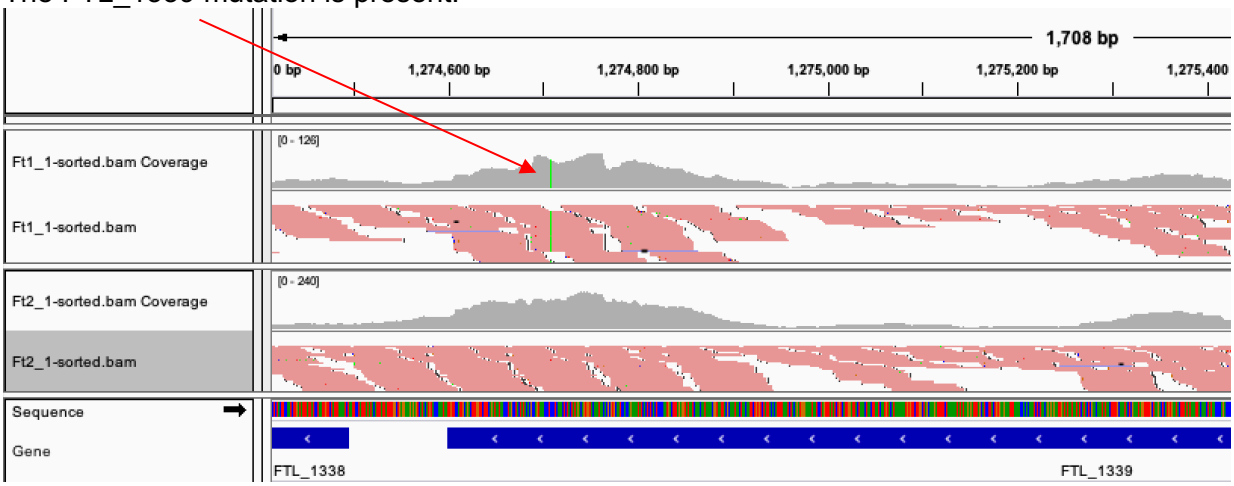


*****Conflict between transcript abundance detected and protein levels**
Jamie struck out these cells; maybe picked the wrong "old" ΔpmrA?

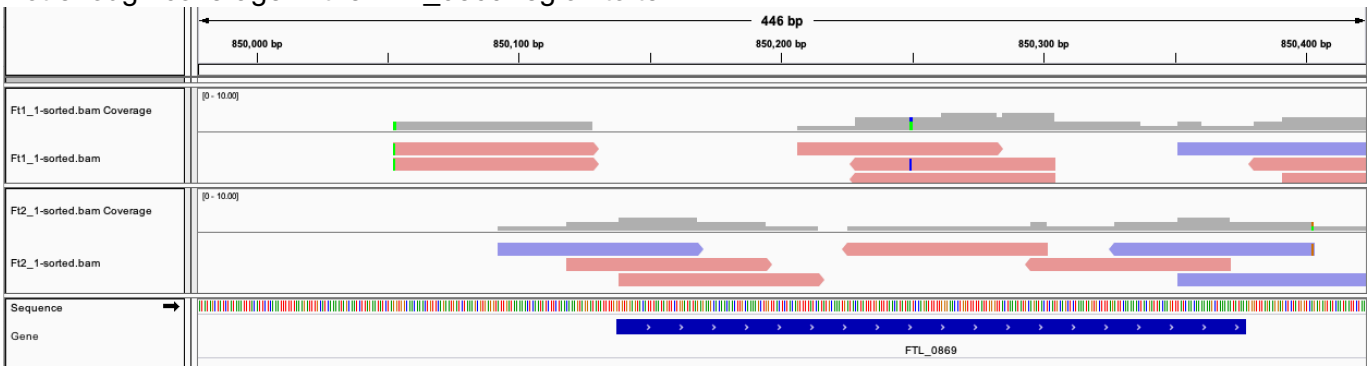
-Are these actually different strains? I double-checked that the RNA-Seq samples have the FTL_0146 mutation, but do they have the other two mutations Jamie found in the ΔpmrA(sup) strain?

Gene or Region	Locus Number	Nucleotide Change	Mutations
ATP Binding Protein	FTL_0146	C152592A	F315L
Proton-dependent oligopeptide transport	FTL_1339	C1274708A	G421G
Upstream of hypothetical protein	Upstream of FTL_0869	G849877T	N/A

The FTL_1339 mutation is present:



Not enough coverage in the FTL_0869 region to tell.



If the discrepancies between the RNA-Seq data and Jamie's qPCR data are due to accidentally using different strains, maybe they would grow differently?

When I isolated RNA, the old grew better than the new.

Friday, September 18, 2020

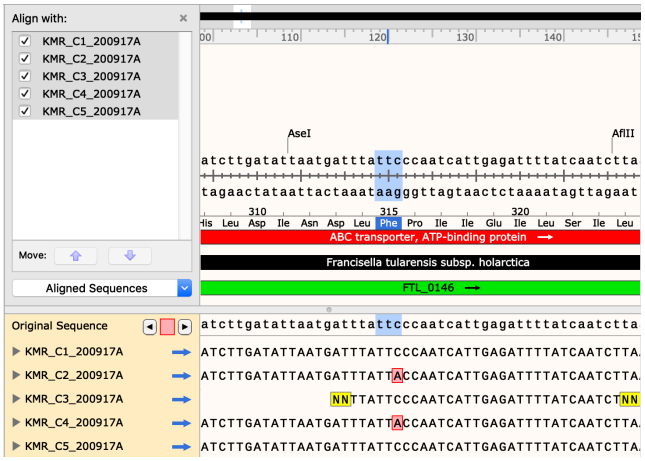
To Do:

1. Review sequencing data
2. Run gel of PCR products
3. Determine genotype of strains struck out from glycerol stocks

Results and Data:

Results of sequencing the FTL_0146 mutation region:

Sample name	Strain	Results
KMR_C1	KMLFT49 ΔpmrA ΔpriM	No SNP
KMR_C2	KMLFT37 ΔpmrA	SNP
KMR_C3	KMLFT67 ΔpmrA priM(S)	No SNP
KMR_C4	KMLFT69 ΔpmrA mreA(S)	SNP
KMR_C5	LVS	No SNP

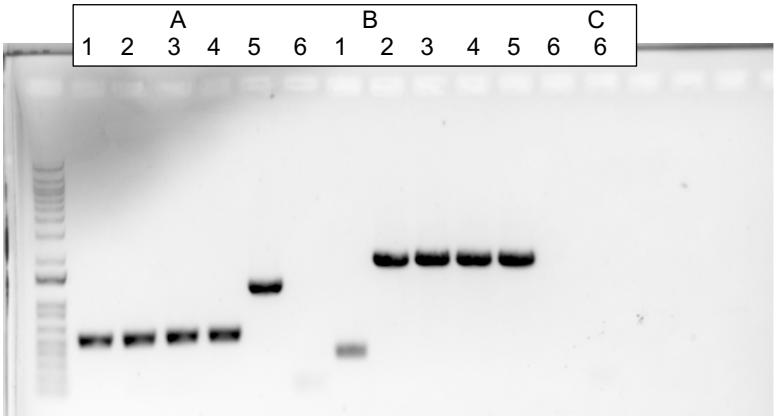


The Δ*pmrA mreA*(S) strain has the FTL_0146 F315L mutation, but I found that it could not replicate in macrophage.

I don't think the FTL_0146 SNP is causing the Δ*pmrA*(sup) intramacrophage replication phenotype.

Reaction	Locus	Primers	Expected size (WT)	Expected size (Δ)	Sequencing primer
A	*pmrA*	KROL61, KROL62	1204	535	-
B	*priM*	KROL155, KROL156	1792	352	-
C	FTL_0146	KROL270, KROL271	1320	-	KROL270

Sample Number	Strain number	Strain name	Result Rxn A	Result Rxn B
1	KMLFT49	ΔpmrA ΔpriM	~535	~352
2	KMLFT37	ΔpmrA	~535	~1792
3	KMLFT67	ΔpmrA priM(S)	~535	~1792
4	KMLFT69	ΔpmrA mreA(S)	~535	~1792
5		LVS	~1204	~1792
6		-DNA	-	



All *pmrA* and *priM* genotypes as expected.

Consider re-doing the WGRS all these strains:

1	LVS	
2	ΔpmrA(sup)	KMLFT37
3	ΔpmrA (new)	KRLVS40
4	ΔpmrA ΔpriM	KMLFT49
5	ΔpmrA priM(S)	KMLFT67
6	ΔpmrA mreA(S)	KMLFT69
7	LVS FTL_0146 F315L	KRLVS69
8	ΔpmrA FTL_0146 F315L	KRLVS81

But for now, review the WGRS data (did we miss anything in the variant calling??) and check the SDS-sensitivity of Δ*pmrA* cells.

Monday, September 21, 2020

To Do:

Results and Data:

Reviewing old NGS data. Folks from MiGS (sequencing facility) have no idea why the coverage is so variable.

Looking back to see if there is another undetected SNP. In the new ΔpmrA strain, there is a mutation in pepN (C 1883383 A which changes the codon for methionine 819 from ATG to ATT, so isoleucine (M819I). This mutation looks like it's present in our LVS cells (about 50/50 WT : mutant) but not completely fixed and became fixed in the new ΔpmrA strain.

Future To-Do

Move 1° LVS pKR10-1 into strain box