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July 2021

Lab goals for July and future

Paper: PmrA / PriM

Re-do macrophage assay with Δ pmrA strains

Isolate gDNA from Δ pmrA experiment in (April?) and check by qPCR

Paper: Δ rpsU2

Re-do macrophage assay with Δ rpsU2 and complements

Plan out future grants. New Investigator? Beckman? R21?

Friday, July 16, 2021

To Do:

1. ~~Plan next week's macrophage experiments~~
2. ~~Pour plates for macrophage experiments~~
3. ~~Thaw J774A.1 cells~~
4. Check on items received

Results and Data:

For 300 mL CHA:

- 6 g Beef Heart Infusion
- 6 g Protease Peptone
- 6 g Glucose
- 0.6 g L-Cystine
- 3 g salt
- 9 g Agar

For 900 mL CHA:

- 18 g Beef Heart Infusion
- 18 g Protease Peptone
- 18 g Glucose
- 1.8 g L-Cystine
- 9 g salt
- 27 g Agar

Poured plates:

	CHA		CHA-Kan		Total
	Round	Square	Round	Square	
Total plates plus extra	30	50	40	45	Round plates: 24 mL, 25 per flask
Flasks of 600 mL CHA	1.2	2.5	1.6	2.25	Square plates: 30 mL, 20 per flask
Total number of CHA flasks	3.7		3.85		7.55

Rounded up to 8 flasks, 4 CHA and 4 CHA-Kan (1L flask with 300 mL and 2 L flask with 900 mL each)

Thawed vial of P4 J774A.1 cells, plated in 100mm tissue culture dish with fresh DMEM + 10% FBS.

Sunday, July 18, 2021

To Do:

1. ~~Feed macrophage~~

Results and Data:

Fed macrophage. Pretty dense, but wait to split until I seed tomorrow.

Monday, July 19, 2021

To Do:

1. ~~Streak out strains for mac expt~~
2. ~~Seed macrophage~~
3. Move 1° LVS pKR10-1 into strain box
4. start gDNA isolations
5. Check on items received

Results and Data:

Scraped up all macrophage (quite a bit!) in 8 mL. Pellet (900xg 5'), remove sup, replace with ~5 mL, add another ~6 mL. Cells quite dense. Only need 540 uL for 6 mL of cells (only one 96-well plate bc only 24 hr timepoint)

1st measurement			
55	60	86	75
Average			69
Undiluted			138
Density			1.38E+06

2nd measurement			
6	4	1	4
4	5	7	10
5	7	8	8
Average			5.75
Undiluted			11.5
Density			1.15E+05

Dilution a tiny bit low, but good.

Macrophage Calculations

Cells per well	2.50E+04
Volume to plate (mL)	0.2
Density needed (cells/mL)	1.25E+05
Total volume needed (mL)	6
Measured cells per ml	1.38E+06
Volume stock needed (mL)	0.54
Volume media for dilution	5.5
Measured cells per ml, seeded	1.15E+05
Measured cells per well	1.73E+04

Seeded macrophage ~5:15pm. Took remainder of cells (~10 mL), and distributed into 5x 100 mm tissue-culture plates (+8 mL DMEMF). Will use some tomorrow, freeze down remainder.

Tuesday, July 20, 2021**To Do:**

1. ~~Infect macrophage~~
2. Wash macrophage
3. ~~Streak out strains for mac expt #2~~
4. ~~Seed macrophage for mac expt #2~~
5. Move 1° LVS pKR10-1 into strain box
6. start gDNA isolations
7. Check on items received

Results and Data:

Infected macrophage at 12:25pm.

Prepare for wash. Make and sterilize more 1x PBS from 10x PBS (250 mL). Prepare DMEM-F with gentamycin. Make 6 mL DMEMF+gent at 10 ug/mL, so add 1.2 uL of 50 mg/mL gent.

Wash at 2:30 pm. Remove media, replace with 200 mL 1xPBS, repeat for total of 2 washes, replace with media containing 10 ug/mL gentamycin.

Wednesday, July 21, 2021**To Do:**

1. ~~Infect macrophage~~
2. Wash macrophage
3. ~~Streak out strains for mac expt #2~~
4. Seed macrophage for mac expt #2
5. Move 1° LVS pKR10-1 into strain box
6. start gDNA isolations
7. Check on items received

Results and Data:

Infected macrophage at 10:15

Wash at 12:15

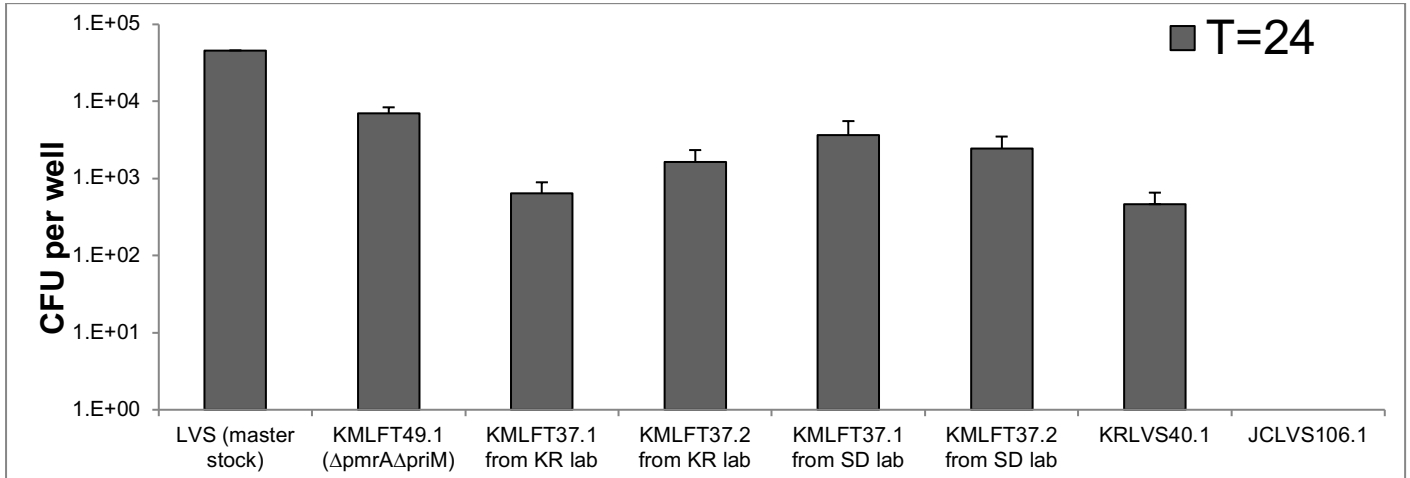
Saturday, July 24, 2021**To Do:**

1. Count plates
2. Analyze data

Results and Data:

	Average CFU per well	St Dev	Original MOI	T-test (vs LVS)	T-test (vs Δ pmrA Δ pri M)	T-test (vs KRLVS40)	Fold change from LVS	Fold change from Δ pmrA Δ priM
LVS (master stock)	4.60E+04	0.00E+00	5.8		0.0006	0.0000	1.00	6.52
KMLFT49.1 (Δ pmrA Δ priM)	7.05E+03	1.34E+03	11.2	0.0006	1.0000	0.0206	-6.52	1.00

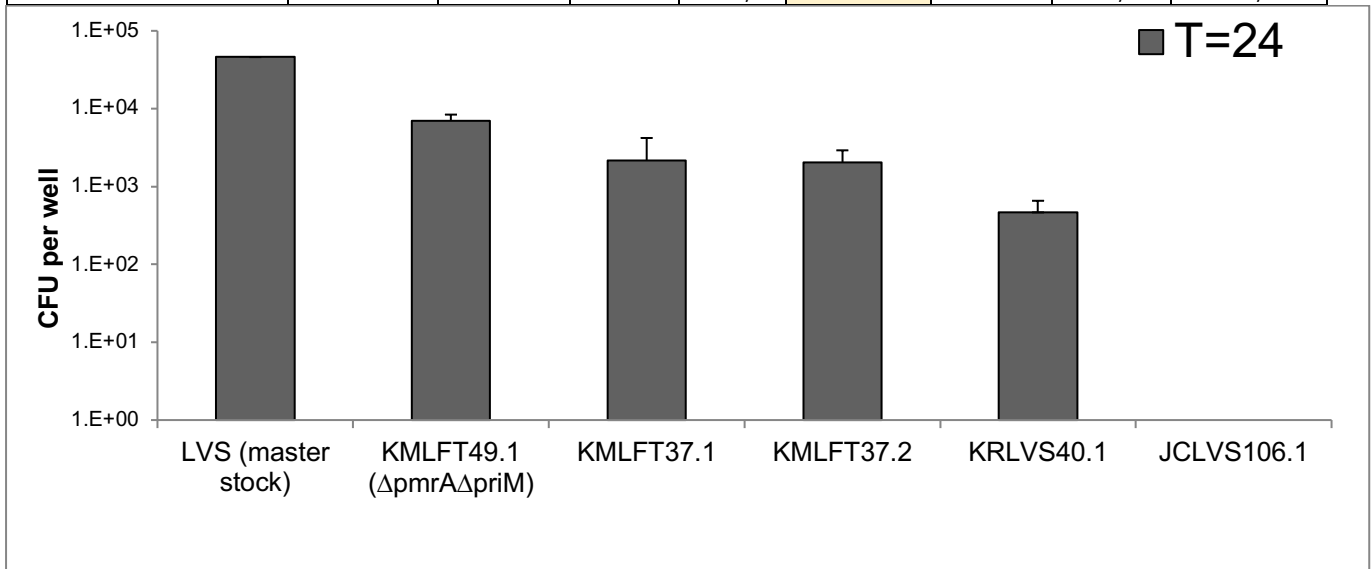
KMLFT37.1 from KR lab	6.43E+02	2.51E+02	3.5	0.0000	0.0220	0.5095	-71.60	-10.97
KMLFT37.2 from KR lab	1.66E+03	6.86E+02	3.2	0.0001	0.0369	0.1419	-27.79	-4.26
KMLFT37.1 from SD lab	3.64E+03	1.92E+03	2.9	0.0010	0.1762	0.1458	-12.64	-1.94
KMLFT37.2 from SD lab	2.47E+03	1.04E+03	3.0	0.0003	0.0623	0.1158	-18.66	-2.86
KRLVS40.1	4.65E+02	1.91E+02	3.9	0.0000	0.0206	1.0000	-98.92	-15.16
JCLVS106.1	0.00E+00	0.00E+00	4.9	#DIV/0!	0.0177	0.0749	#DIV/0!	#DIV/0!



The newly-made KRLVS40 is the most attenuated, although it's only about 2 logs lower than WT cells. And it's still very close to KRLVS37.1 (KR labs).

If I combine all the KMLFT37.1 replicate data together and all the KMLFT37.2 replicate data together (ignoring source labs):

	Average CFU per well	St Dev	Original MOI	T-test (vs LVS)	T-test (vs ΔpmrAΔpriM)	T-test (vs KRLVS40)	Fold change from LVS	Fold change from ΔpmrAΔpriM
LVS (master stock)	4.60E+04	0.00E+00	5.8		0.0006	0.0000	1.00	6.52
KMLFT49.1 (ΔpmrAΔpriM)	7.05E+03	1.34E+03	11.2	0.0006	1.0000	0.0206	-6.52	1.00
KMLFT37.1	2.14E+03	2.06E+03	3.5	0.0000	0.0411	0.3398	-21.48	-3.29
KMLFT37.2	2.06E+03	8.58E+02	3.2	0.0000	0.0045	0.0697	-22.33	-3.42
KRLVS40.1	4.65E+02	1.91E+02	3.9	0.0000	0.0206	1.0000	-98.92	-15.16
JCLVS106.1	0.00E+00	0.00E+00	4.9	#DIV/0!	0.0177	0.0749	#DIV/0!	#DIV/0!



Seems like I should double-check the phenotypes of:

LVS

KMLFT37.1 (KR lab fine)

KRLVS40.1

Δ prmA Δ priM

The question is:

Is the Δ prmA originally from Simon's lab still attenuated or has it acquired a suppression mutation?

Note that **on plates, KRLVS40 grows slower.**

Froze down P5 J774A.1 cells:

Future To-Do

Move 1° LVS pKR10-1 into strain box

Bibliography

Chalabaev, S., Anderson, C., Onderdonk, A., Kasper, D. (2011). **Sensitivity of *Francisella tularensis* to ultrapure water and deoxycholate: implications for bacterial intracellular growth assay in macrophages.** *Journal of microbiological methods* 85(3), 230 - 232.
<https://dx.doi.org/10.1016/j.mimet.2011.03.006>

Hoang, K., Fitch, J., White, P., Mohapatra, N., Gunn, J. (2020). **The sensor kinase QseC regulates the unlinked PmrA response regulator and downstream gene expression in *Francisella*** *Journal of Bacteriology* <https://dx.doi.org/10.1128/jb.00321-20>