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November 2021

Lab goals

Paper: PmrA / PriM
Re-do macrophage assay with ΔpmrA strains
Isolate gDNA from ΔpmrA experiment in (April?) and check by qPCR

Paper: ΔrpsU2
Re-do macrophage assay with ΔrpsU2 and complements

Sunday, November 7, 2021

To Do:

1. ~~Prep fresh DMEM-F~~
2. ~~Thaw macrophage (P5)~~

Results and Data:

Supplemented more DMEM with 10% FBS.
Thawed 1 vial of J774A.1 P5 cells, seeded into a 100 mm TC dish.

Monday, November 8, 2021

To Do:

1. Feed macrophage
2. Pour plates

Results and Data:

Replaced media (removed 10 mL, washed with 5 mL, added back 10 mL DMEM-F). Cells look happy under microscope.

Dan and Aisling helped me autoclave 4 flasks of CHA. I poured plates for the macrophage assay for Thursday

Wednesday, November 10, 2021

To Do:

1. ~~Streak cells for mac expt~~
2. ~~Seed macrophage~~

Results and Data:

Washed cells with 5 mL DMEMF, added back 8 mL and scraped up cells. Pelleted at 1,000xg for 5 mins in 15 mL conical tube- good pellet! Resuspended in 7mL DMEMF, plenty of cells (want at least 12 mL at 1.25e5).

1st measurement				
78	63	56	62	
Average				64.75
Undiluted				129.5
Density				1.30E+06

Dilute a bit high just in case (better to have more than less)- use 1.5 mL + 13.5 mL DMEMF. Dispense using multichannel into 2x 96 well plates and check concentration:

2nd measurement				
5	8	7	3	
8	5	6	8	
5	10	6	5	
Average				6.333333333
Undiluted				12.66666667
Density				1.27E+05

Perfect!

Use all remaining diluted cells and some of the resuspended cells (to make a volume of 10mL) and put into another TC dish as P6 cells. Put 96 well plates and dish into 37°C incubator with 5% CO2. Done around 5pm.

Final calculations in detail:

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)	15
Measured cells per ml	1.30E+06
Volume stock needed (mL)	1.5
Volume media for dilution	15.0
Measured cells per ml, seeded	1.27E+05
Measured cells per well	2.53E+04

Thursday, November 11, 2021

To Do:

1. ~~Infect macs~~
2. ~~Plate inoculum~~
3. ~~Wash macs~~
4. ~~T=2~~

Results and Data:

Plan for inoculum:

Bacterial Calculations	Actual
MOI	5
Macrophage cells per well	2.53E+04
Volume bacteria to add (mL)	0.05
Bacterial density needed (cells/mL)	2.53E+06
Cells/mL per OD600	5.81E+09
OD needed for given density	0.00044
Resuspend to	0.050
Final MOI 5, dilute 1:100	0.00050

Resuspend bacteria, check ODs, dilute and re-check, perform final dilution for a final volume of 4 mL in 3-chamber multi reservoirs.

			For final vol 1 mL at 0.05		For final vol 4 mL at 0.0005	
Number	Strain	Resuspend cells to (OD600)	Cells (uL)	OD600	Cells (uL)	Volume media (uL)
1	LVS pF	2.30	21.7	0.049	40.0	3960
2	ΔrpsU2 pF	1.23	40.7	0.049	40.0	3960
3	ΔrpsU2 pF-rpsU1	2.00	25.0	0.051	40.0	3960
4	ΔrpsU2 pF-rpsU2	2.48	20.2	0.050	40.0	3960
5	ΔrpsU2 pF-rpsU3	2.17	23.0	0.054	39.0	3961
6	ΔpigR	3.19	15.7	0.052	40.0	3960

Add 50 ul each inoculum to macrophage as below, 2x, put macrophage infection plates back in incubator. **Infected at 11:40am**.

	1	2	3	4	5	6	7	8	9	10	11	12
A	LVS pF	LVS pF	LVS pF		ΔrpsU pF	ΔrpsU pF	ΔrpsU pF		ΔrpsU pF-rpsU1	ΔrpsU pF-rpsU1	ΔrpsU pF-rpsU1	
B												
C												
D	ΔrpsU pF-rpsU2	ΔrpsU pF-rpsU2	ΔrpsU pF-rpsU2		ΔrpsU pF-rpsU3	ΔrpsU pF-rpsU3	ΔrpsU pF-rpsU3		ΔpigR	ΔpigR	ΔpigR	
E												
F												
G												
H												

Serially dilute inoculums 10x in PBS in 96-well plate, plate 10^0 – 10^{-4} on square CHA-Kan (#1-5) or square CHA (#6). Put plates at 37°C.

at 1:40pm, remove media from wells. Wash 2x with 1x PBS (280 uL) and replace media with 200 ul DMEMF with 10 ug/mL gentamycin.

10 ug/mL * 12,000 ul / 50,000 ug/mL = 2.4 ul

At 3:40, remove 1 plate from incubator. Remove media from wells. Wash 2x with 1x PBS (250 uL) and replace media with 200 ul 1% saponin in 1xPBS.

Friday, November 12, 2021

To Do:
1. T=24

Results and Data:

Words go here.

Saturday, November 13, 2021

To Do:
1. ~~Check / pull plates~~

Results and Data:

Can count plates with inoculums from 1, 3, 4, and 6 – pull, count, and leave on bench

Sunday, November 14, 2021

To Do:
1. ~~Check / pull plates~~

Results and Data:

Can count plates with inoculums from 2 and 5 – pull, count, and leave on bench
Pull and leave on bench the following T=2 plates: 1, 4, 6

Monday, November 115, 2021

To Do:
1. T=24

Results and Data:

Pull and count the following T=2 plates: 2, 3
Pull and count the following T=24 plates: 1, 2 (2-1 and 2-2; 2-3 needs another day), 3, 4

Future To-Do

Move 1° LVS pKR10-1 into strain box

Bibliography

Suh, Moo-Jin et al. “Extending ribosomal protein identifications to unsequenced bacterial strains using matrix-assisted laser desorption/ionization mass spectrometry.” Proteomics vol. 5,18 (2005): 4818-31. doi:10.1002/pmic.200402111