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June 2019

Monday, June 24th, 2019

To Do:

1. PCR Purification
2. Pour plates
3. Observe Hannah streaking for isolation
4. Observe Jamie feeding microphage

Results and Data:

Protocol for PCR Purification:

1. Add 5 volumes of buffer PB to 1 volume of PCR and mix
 - a. I added 100 μ L of buffer to 20 μ L of PCR
2. Transfer the PCR/buffer solution to the QIAquick column
3. Centrifuge at 13,000 rpm for 1 min.
4. Discard flow-through.
5. Add 0.75 mL of Buffer PE to wash and centrifuge for 1 min
6. Discard flow-through and centrifuge again for 3 mins
7. Place the QIAquick column in a clean microcentrifuge
8. To elute, add 30 μ L of Buffer EB to the center and centrifuge for a min.
9. Discard the QIAquick column. (use the prepared diluted EB and not the one in the kit)

Make sure not to hit the second stop while pipetting

Pouring Plates (normal growth media)

1. We mix hemoglobin and CHA agar
2. Use an electronic pipette and pour about 24 mL per plate
3. Clean empty flask quickly before the agar solidifies (use citranox if needed)
4. Label the top plate on the stack

follow the aseptic technique and make sure to do it as close as possible to the flame

Streaking for Isolation

1. It's done in quadrants with tight zigzags
2. Streaking is done close to the corners and the loop can be dragged more than once since the bacteria is hard to grow.
3. Follow aseptic technique

Wednesday, June 26th, 2019**To Do:**

1. Preparing sucrose plates
2. Starting up culture

Methods and results:

Making sucrose plates

1. Add these ingredients to a 1 mL flask
 - a. 5 g of beef heart infusion
 - b. 5 g of protease peptone
 - c. 5 g of glucose
 - d. 0.5 g L-cystine
 - e. 7.5 g agar
2. Add 166.7 mL ddiH₂O
3. Stir on low heat (55) for 10 mins until fully dissolved
4. Autoclave on liquid 30, and add ice to the bin
 - a. Make sure not to shake the flask so that the agar does not get on the flask walls
5. Add hemoglobin and 60% sucrose to water bath at 56C
 - a. Hemoglobin can be found in the cold room
6. Add 83.3 mL 60% sucrose to CHA using aseptic technique and stir
7. Add 250 mL 2% hemoglobin to mixture and stir
8. Use the pipette to add 24 mL to each plate
9. Label plates and store at room temperature

** add the mixture at an angle to avoid bubbles **

Starting up culture

1. Next to the flame, add 400 µL MHB supplemented to a sterile tube
2. Obtain colonies from plate and aim to fill about 1/3 of the MHB volume
3. Mix well with pipette (avoid touching the clumps)
4. Use spectrophotometry cuvettes and add 1000 µL MHB to the blank
5. Add 950 µL MHB and 50 µL from the MHB/colony mixture to another cuvette
6. Cover cuvette with parafilm and shake well to mix
7. Use the spectrophotometer and measure absorbance at 600 nm
8. Use the equation $C1V1 = C2V2$

if the cuvettes need to be labeled make sure to write on the ribbed side

July 2019

Monday, July 1st, 2019

To Do:

1. Pour plating (sucrose, CHA, and Kanamycin)
2. Figure out MIC protocol with Hannah

Methods and results:

CHA plates protocol: For 600 mL of CHA

1. Weigh out 30.6g of cystine heart agar into 1L flask (non-baffled; 10.2g/100mL)
2. Add 300mL of ddiH₂O (type I)
3. Add stir bar to flask
4. Heat on low, stirring, for about 10 minutes (media should be totally dissolved)
5. Autoclave on 30' liquid cycle, filling the water bin up to the height of the media
6. Cool down (ideally to ~55°C)
7. Separately (before), prepare hemoglobin 2% solution
8. Add 6g freeze-dried hemoglobin to 300mL of ddiH₂O (type I)
9. Autoclave on 15' liquid cycle with water in the bin
10. Cool down (ideally to ~55°C)
11. Using sterile technique, pour hemoglobin into CHA
12. Using a 50mL pipet, aliquot 24mL of CHAH mixture into each 100mm plate (should make approximately 25 plates) Try to avoid bubbles!

Kanamycin plates protocol:

1. Mix 30.6 g cystine heart agar with 300 mL type I ddiH₂O
2. Autoclave 30', being very careful media does not boil over
3. While autoclaving, warm 300 mL of 2% hemoglobin (in 50°C oven or in water bath at 55°C)
4. Place CHA flask in 50°C oven or in water bath at 55°C, let temperature equilibrate
5. Wipe down flask and bottles with ethanol and using sterile technique, pour hemoglobin into CHA flask
6. FOR CHAH-Kan plates:
7. Add 60 µL of 50 mg/mL kanamycin
8. Mix media
9. Use sterile pipette, pour ~24 mL media per plate (25 plates for 600 mL media)

Monday, July 8th, 2019

To Do:

1. Work on MIC protocol and add hygromycin calculations
2. Prepare ethanol for Maria
3. Watch Hannah do sequence preparation
4. Prepare ONPG solution for Maria
5. Prepare 2.5% Iron Pyrophosphate

Methods and Results:

MIC protocol (not final):

1. For each strain to be tested, prepare 100 mL of media (supplemented MHB) in 2 sterile 50 mL conical.
2. Resuspend cells for strains being tested in about 400 uL of MHB (if necessary). Measure the OD600.
3. Aim for an OD600 of 0.05. Dilute the appropriate amount of culture in the 50 mL conical that contains media to get required OD600.
4. Add 1.1 mL of the diluted culture into the wells of a 96 well deep-well plate, such that three rows and all 12 columns are filled for each strain (see diagram below).
5. The next steps will vary based on the concentrations of antibiotic you want to use. An example for kanamycin, with the highest concentration in the wells of 50 ug/mL and 1:10 dilutions, is shown here. A starting concentration of 600 ug/mL in stock tube A is required, because it will be diluted 1:5 when added to the deep well plates. **An example for hygromycin, with the highest concentration in the wells of 200 ug/mL and 1:10 dilutions, is shown here. A starting concentration of 2400 ug/mL in stock tube A is required, because it will be dilute 1:10 when added to the deep well plates.**
6. In 12 sterile microcentrifuge tubes, prepare 360 uL of media.
7. Prepare a working stock of kanamycin that is a concentration of 6 mg/mL, by adding 6 uL of our stock 50 mg/mL solution to 44 uL of MHB. **Prepare a working stock of hygromycin that is at a concentration of 24 mg/mL, by adding 2.22 uL of our stock 54 mg/mL to 47.78 uL of MHB.**
8. Add 40 uL of this working stock to the first microcentrifuge tube and mix.
9. Serially dilute 1:10 by transferring 40 uL from the first microcentrifuge tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
10. Using the microchannel, pipet 100 uL from the microcentrifuge tubes to the corresponding wells on the plate with cells and mix (you can use dispense function and take up 300 uL, dispensing 100 uL into each of the three replicates).
11. Then take 200 uL from each well, and put into the smaller 96 well plate to compare different volumes.
12. Cover plate with film and put in 37C incubator overnight.
13. In the morning, pipet 200 uL from each well into a normal 96 well plate. Use the plate reader to measure OD600.

Ethanol

1. Ethanol is at 100% so we usually dilute it to 70% by adding 1.4 L ethanol to 600 mL of water to make 2 L.

Sequencing

Using the nanodrop (add 1 uL)

1. You wipe the tip first then you add water and read
2. Wipe the tip again and add the blank
3. Wipe the tip again and add what needs to be measured
4. Put the numbers in the table and modify it to a digit that you can pipette.

ONPG solution

1. Measure 160 mg of O-Nitrophenyl-beta-galactoside in 40 mL (4mg/mL) in Z buffer

** add 30-35 mL first then add the rest after it dissolves **

Preparing 2.5% iron pyrophosphate (found in MHB protocol)

1. Measured out .75g of iron pyrophosphate and add 30 mL of water
2. Dissolve over night

** add 20-25 mL first then add the rest after it dissolves **

Wednesday, July 10th, 2019**To Do:**

1. Prepare hemoglobin
2. Watch Hannah filter sterilize iron pyrophosphate

Methods and Results:

Hemoglobin preparation

1. Add 6g freeze-dried hemoglobin to 300mL of ddiH₂O (type I)
2. Autoclave on 20' liquid cycle with water in the bin (water should be at the liquid level)
3. Cool down (ideally to ~55C)

Monday, July 15th, 2019**To Do:**

1. MIC Protocol

Methods and Results:

1. In 12 sterile microcentrifuge tubes, prepare 405 uL of media (supplemented MHB)
 - a. Use more media next time (about 500 uL)
2. To prepare the working stock:
 - a. For kanamycin, in 44 uL of MHB add 6 uL
 - b. For hygromycin, in 47.8 uL of MHB add 2.2 uL
3. Add 45 uL of the working stock to the first microcentrifuge tube and mix
4. Serially dilute 1:10 by transferring 45 uL from the first microcentrifuge tube to the next, mix with a different pipette and transfer again. Repeat for all tubes except for the last one (no antibiotic)
5. Prepare 100 mLs of supplemented MHB in 2 sterile 50 mL conical
6. Resuspend cells for strains being tested in about 400 mL of MHB and measure OD₆₀₀
7. Aim for an OD₆₀₀ of 0.05. Dilute the appropriate amount of culture in the 50 mL MHB conical to get OD₆₀₀.
8. For 4th and 8th well with no cells add MHB without cells
9. Add 1.1 mL of diluted MHB into the rest of the wells of a 96 well deep.
10. Pipette 100 uL from the microcentrifuge tubes to the corresponding wells on the plate and mix
 - a. Use a different pipette when adding to 4th and 8th row
11. Take 200 uL from each well and put into a tissue 96 well plate to compare difference
12. Cover plates and incubate at 37 C overnight
 - a. We incubated at 1:30 pm

	Kan: 50 ug/mL	5 ug/mL	5x10 ⁻¹ ug/mL	5x10 ⁻² ug/mL	5x10 ⁻³ ug/mL	2x10 ⁻⁴ ug/mL	2x10 ⁻⁵ ug/mL	2x10 ⁻⁶ ug/mL	2x10 ⁻⁷ ug/mL	2x10 ⁻⁸ ug/mL	2x10 ⁻⁹ ug/mL	No antibiotic
Strain 1, replicate A												
Strain 1, replicate B												
Strain 1, replicate C												
No cells												
	Hygromycin: 200 ug/mL											
Strain 1, replicate A, antibiotic 1												
No cells												

Results:

Initial OD was .532 so we added 94 uL to MHB

Tuesday, July 16th, 2019

To Do:

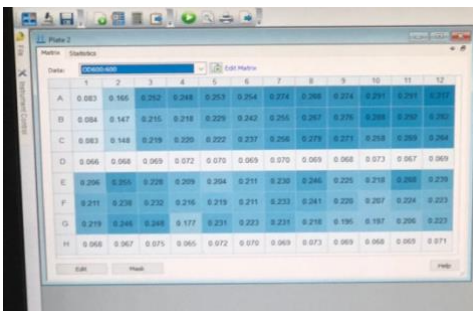
1. MIC Protocol Results
2. Spin Mini Prep

Methods and Results:

MIC Deep well plate results:



MIC tissue plate results:



Spin Mini Prep:

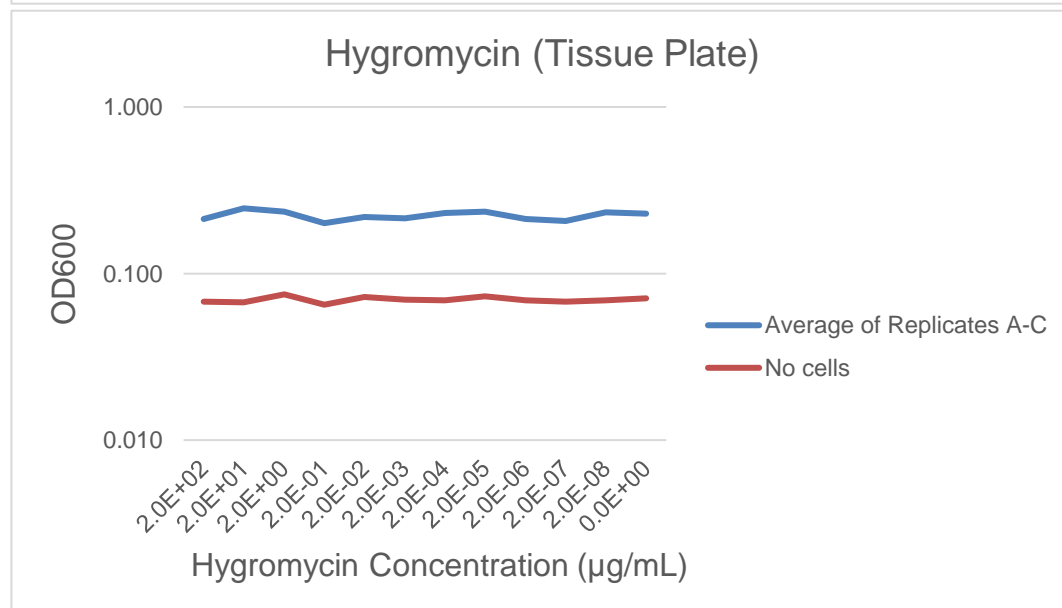
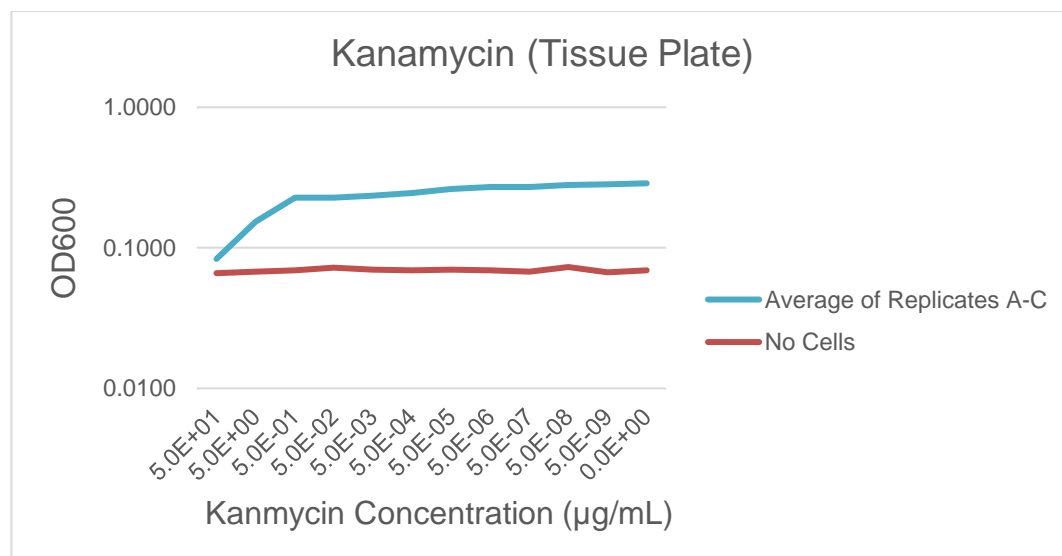
1. Pellet overnight bacteria by pouring into 1.5mL microcentrifuge tube and centrifuging it for 3 mins at highest speed
 - a. Repeat till all prepared bacteria is done
2. Resuspend pelleted bacterial cells in 250 μ L buffer P1
3. Add 250 μ L buffer P2 and invert tube 4 times
 - a. Don't let the lysis reaction proceed for more than 5 mins
4. Add 350 μ L buffer N3 and invert tube to mix
5. Centrifuge for 10 mins at 13,000 rpm
6. Add 800 μ L from step 5 to QIAprep spin column by pipetting.
7. Centrifuge for a minute and discard flow through
8. Wash by adding 500 μ L of buffer PB. Centrifuge for a minute and discard flow through
9. Wash THREE times by adding 750 μ L buffer PE. Centrifuge for a minute and discard flow through
10. Centrifuge for 3 mins to remove residual wash buffer
11. Place the spin column in a clean 1.5 mL micro centrifuge tube and add 50 μ L 0.1 X buffer EB to elute. Centrifuge for a minute.

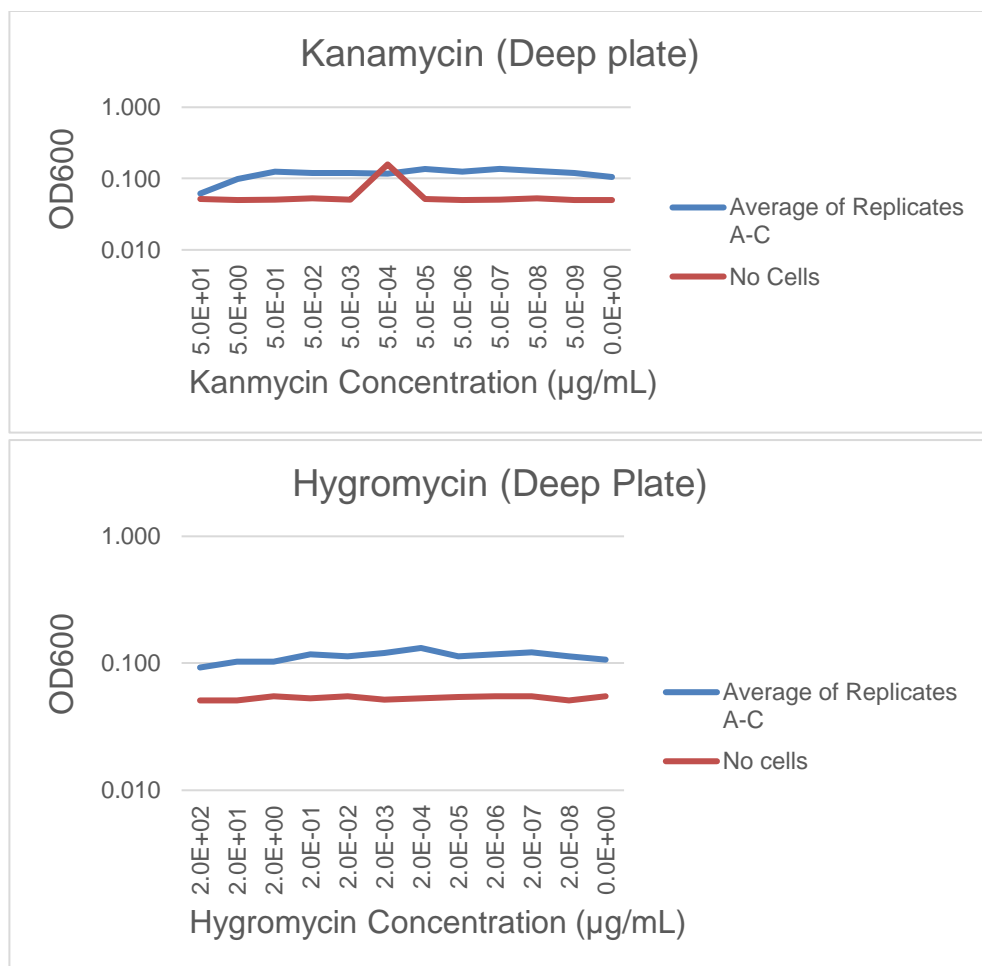
Wednesday, July 17th, 2019**To Do:**

1. Work on MIC graphs
2. Nanodrop plasmids for Hannah
3. Make LB agar

Results and Methods:

MIC graphs: [../MIC results /1VS/190716_TA_kan_hygro/190716_TA_MIC.xlsx](#)





Preparing LB-agar

1. For 500 mL of LB-agar, weigh out the following components and add to a 1 L (non-baffled) flask:
 - a. 6 g agar
 - b. 5 g NaCl
 - c. 5 g Tryptone
 - d. 2.5 g Yeast extract
2. Add stirbar to flask
3. Add 500 mL ddiH₂O
4. Mix on stirplate until components are dissolved
5. Cover top of flask with foil and add a small piece of autoclave tape
6. Autoclave on 30-minute liquid cycle

Streaking LVS:

1. Get LVS tube from -80C freezer in M.Ramsey lab
2. Get a regular plate
3. Open flame and pipette 50 μ L of LVS onto the plate
4. Using a stick, streak the bacteria to cover as much area as possible

Wednesday, July 24th, 2019**To Do:**

1. Perform MIC on LVS strain

Results and Methods:

Measured OD600 was 0.492 A

$$C1: .492 \times 50 = 24.6$$

$$C1V1 = C2V2$$

$$26.6 \times V1 = .05 \times 20,000$$

$$V1 = 40.65 \mu\text{L}$$

Measure OD600 (from 50 mL conical) was 0.047

Plate was incubated at 12:25 pm July 24th, 2019

To measure OD600:

1. Resuspend cells in 400 μ L of MHB supplemented in a centrifuge tube
2. Put 1 mL of MHB in blank cuvette
3. Put 980 μ L of MHB in another cuvette and add 20 μ L of cells
4. Invert tube to mix after covering it with film
5. Measure OD and calculate the volume needed using $c1v1 = c2v2$
6. Put the calculated amount of cells into the MHB conical and invert it
7. Put 500 μ L in a cuvette and measure the OD600

Thursday, July 25th, 2019**To Do:**

1. Read MIC results and work on data

Results and Methods:

Plate was removed from incubator **at 10:35 AM**

MIC graphs: [../MIC results /LVS/190724_TA_kan_hygro/190724_TA_MIC.xlsx](#)

Plate 1

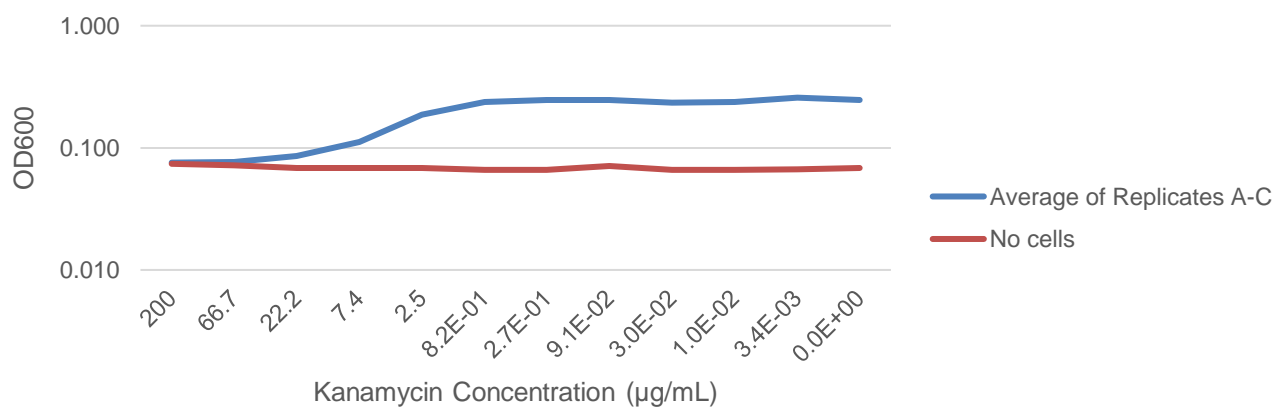
Matrix Statistics

Data: 600

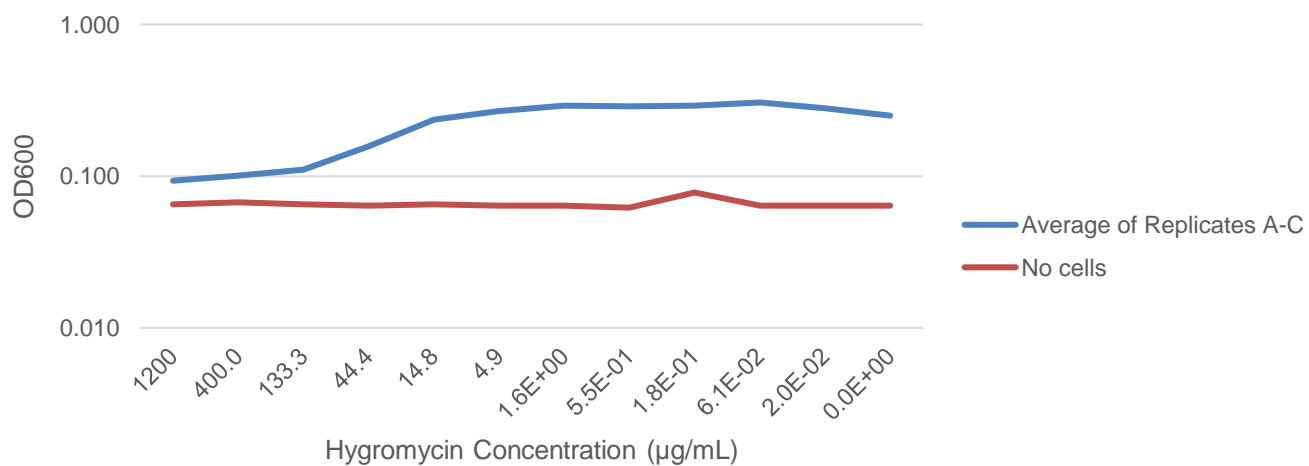
Edit Matrix

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.075	0.076	0.076	0.080	0.173	0.247	0.251	0.248	0.243	0.244	0.248	0.245
B	0.076	0.078	0.084	0.134	0.192	0.225	0.231	0.234	0.220	0.229	0.226	0.239
C	0.076	0.077	0.096	0.120	0.198	0.241	0.257	0.260	0.244	0.240	0.229	0.254
D	0.074	0.072	0.068	0.068	0.068	0.066	0.066	0.071	0.066	0.066	0.067	0.068
E	0.094	0.124	0.131	0.161	0.244	0.259	0.286	0.283	0.285	0.296	0.279	0.246
F	0.095	0.092	0.113	0.168	0.227	0.277	0.301	0.285	0.301	0.302	0.304	0.249
G	0.091	0.085	0.087	0.142	0.239	0.273	0.287	0.296	0.289	0.320	0.258	0.256
H	0.065	0.067	0.065	0.064	0.065	0.064	0.064	0.062	0.078	0.064	0.064	0.064

MIC of Kanamycin in LVS Strain



MIC of Hygromycin in LVS Strain



August 2019

Tuesday, August 6th, 2019

To Do:

1. Perform MIC on LVS strain

Results and Methods:

New MIC protocol:

1. For each strain to be tested, prepare 20 mLs of media (supplemented MHB) in 1 sterile 50 mL conical.
2. Resuspend cells for strains being tested in about 400 uL of MHB (if necessary). Measure the OD600.
3. Aim for an OD600 of 0.005. Dilute the appropriate amount of culture in the 50 mL conical that contains media to get required OD600.
4. Add 180 uL of the diluted culture into the wells of a non-treated 96 well plate, such that three rows and all 12 columns are filled for each strain (see diagram below).
5. In 4th row, add same volume of plain media
6. The next steps will vary based on the concentrations of antibiotic you want to use. An example for kanamycin, with the highest concentration in the wells of 200 ug/mL and 1:2 dilutions, is shown here. A starting concentration of 2,000 ug/mL in stock tube A is required, because it will be diluted 1:10 when added to the deep well plates. An example for hygromycin, with the highest concentration in the wells of 2700 ug/mL and 1:2 dilutions, is shown here. A starting concentration of 27,000 ug/mL in stock tube A is required, because it will be dilute 1:10 when added to the deep well plates.
7. In 12 sterile microcentrifuge tubes, prepare 100 uL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4 mg/mL, by adding 8.8 uL of our stock 50 mg/mL solution to 101.2 uL of MHB.
9. Add 100 uL of this working stock to the first microcentrifuge tube and mix.
10. Serially dilute 1:2 by transferring 100 uL from the first microcentrifuge tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
11. For Hygromycin, transfer 100 uL from the stock antibiotic to tube A.
12. Serially dilute 1:2 by transferring 100 uL from the first microcentrifuge tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
13. Using the multi-channel, pipet 20 uL from the microcentrifuge tubes to the corresponding wells on the plate with cells and mix (you can use dispense function and take up 80 uL, dispensing 20 uL into each of the four replicates).
14. Cover plate with film and put in 37C incubator overnight.
15. In the morning, use the plate reader to measure OD600.

We increased the hygromycin concentration so that only 5% of the 200 uL in the well is from the antibiotic. The highest concentration for hygromycin in the wells will be 2700 ug/mL.

The plate was incubated at 1:15 pm on August 6th, 2019

Wednesday, August 7th, 2019

To Do:

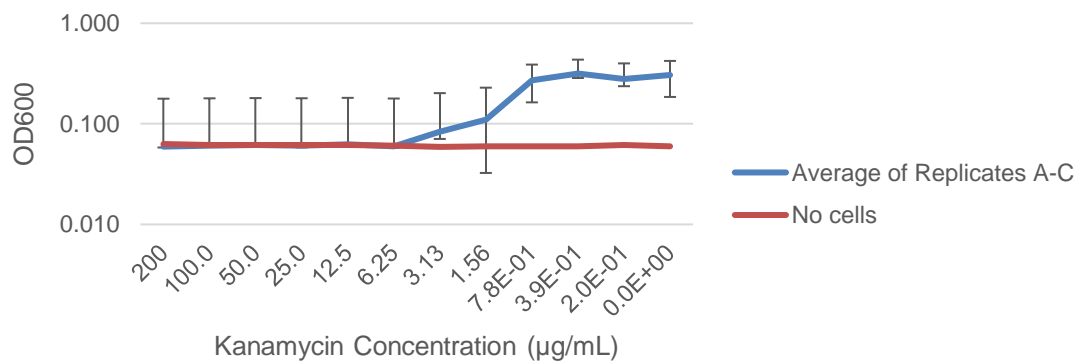
1. Read MIC results
2. Streak bacteria for new MIC

Results and Methods:

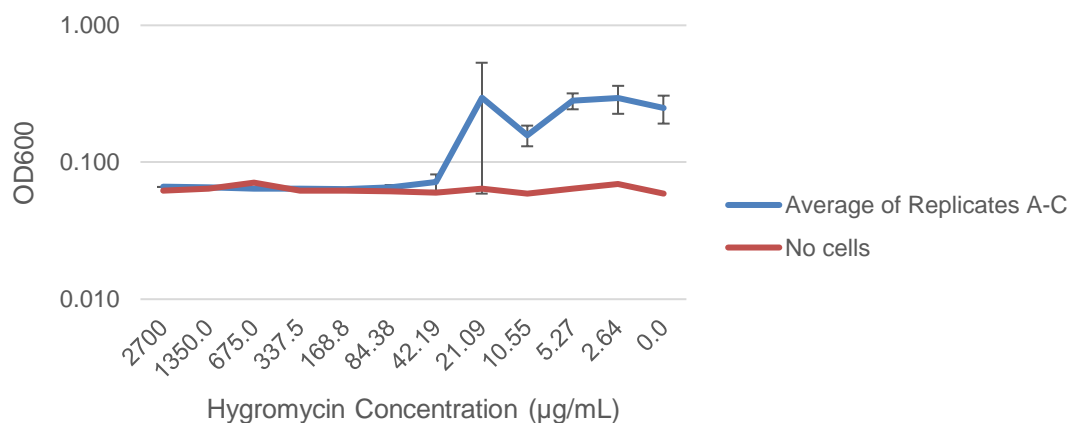
Plate was removed from incubator at 10:25 AM

MIC graphs: [../MIC_results/LVS/190807_TA_kan_hygro/190807_TA_MIC.xlsx](#)

MIC of Kanamycin in LVS Strain



MIC of Hygromycin in LVS Strain



Kanamycin Conc. (µg/mL)	Avg OD600 of LVS
200	0.059
100.0	0.061
50.0	0.062
25.0	0.061
12.5	0.063
6.25	0.060
3.13	0.083
1.56	0.110
7.8E-01	0.270
3.9E-01	0.316
2.0E-01	0.280
0	0.304

Hygromycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS
2700	0.066
1350.0	0.066
675.0	0.064
337.5	0.064
168.8	0.064
84.38	0.066
42.19	0.072
21.09	0.296
10.55	0.158
5.27	0.281
2.64	0.294
0.0	0.249

Streak LVS and $\Delta\text{rpsu } 1-\Delta\text{ rpsu } 3$

We will plate one antibiotic per plate and two different strains on each

Plates were incubated at 12:05 PM

Thursday, August 8th, 2019

To Do:

1. Perform MIC on LVS strain and $\Delta\text{rpsu } 1-\Delta\text{ rpsu } 3$

Results and Methods:

MIC protocol:

1. For each strain to be tested, prepare 20 mls of media (supplemented MHB) in 1 sterile 50 mL conical.
2. Resuspend cells for strains being tested in about 400 μL of MHB (if necessary). Measure the OD600.
3. Aim for an OD600 of 0.005. Dilute the appropriate amount of culture in the 50 mL conical that contains media to get required OD600.
4. Add 180 μL of the diluted culture into the wells of a non-treated 96 well plate, such that three rows and all 12 columns are filled for each strain (see diagram below).
5. In 4th row, add same volume of plain media
6. The next steps will vary based on the concentrations of antibiotic you want to use. An example for kanamycin, with the highest concentration in the wells of 200 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 2,000 $\mu\text{g/mL}$ in stock tube A is required, because it will be diluted 1:10 when added to the deep well plates. An example for hygromycin, with the highest concentration in the wells of 2700 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 27,000 $\mu\text{g/mL}$ in stock tube A is required, because it will be dilute 1:10 when added to the deep well plates.
7. In 12 sterile microcentrifuge tubes, prepare 100 μL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4 mg/mL , by adding 8.8 μL of our stock 50 mg/mL solution to 101.2 μL of MHB.
9. Add 100 μL of this working stock to the first microcentrifuge tube and mix.
10. Serially dilute 1:2 by transferring 100 μL from the first microcentrifuge tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
11. For Hygromycin, transfer 100 μL from the stock antibiotic to tube A.
12. Serially dilute 1:2 by transferring 100 μL from the first microcentrifuge tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
13. Using the multi-channel, pipet 20 μL from the microcentrifuge tubes to the corresponding wells on the plate with cells and mix (you can use dispense function and take up 80 μL , dispensing 20 μL into each of the four replicates).
14. Cover plate with film and put in 37C incubator overnight.

15. In the morning, use the plate reader to measure OD600.

Plates were incubated at 1:35 PM

OD600:

LVS: .225A Δ rpsu 1- Δ rpsu 3= .267. (Used X2 of MHB but then realized there was no need to X2 the amount)

$$C1V1=C2V2$$

$$(OD600 \times 50) \times V1 = .005 \times 40,000$$

Aim for an OD600 of .005

LVS V1= 17.78 μ L

Δ rpsu 1- Δ rpsu 3 V1= 14.98 μ L

Friday, August 9th, 2019

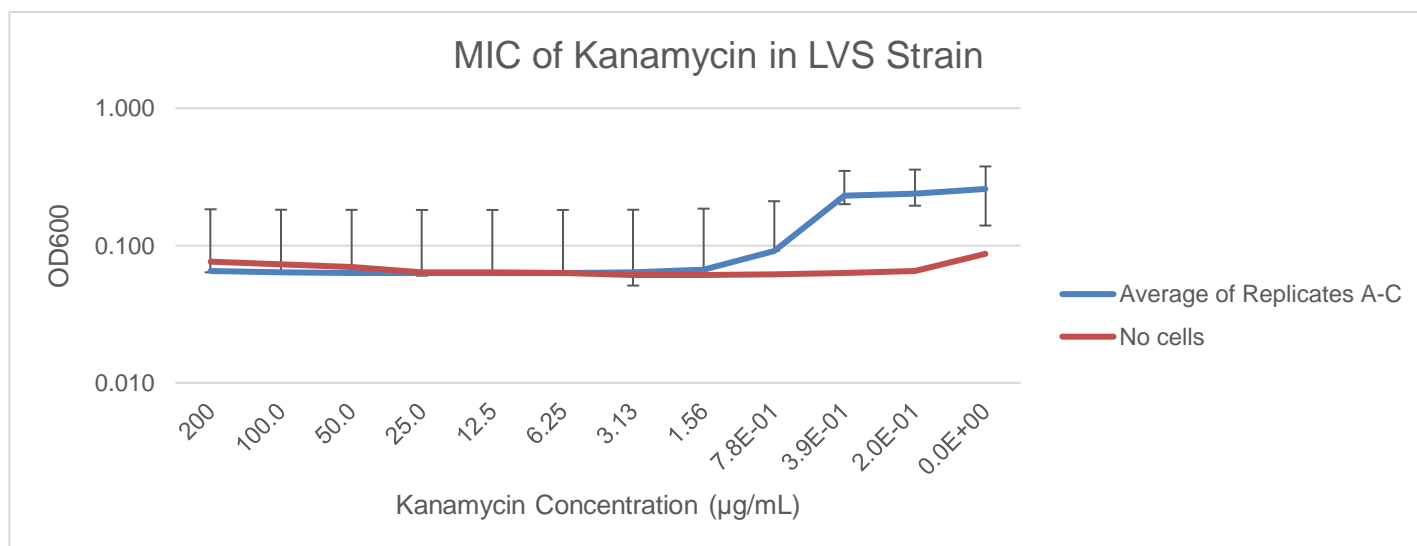
To Do:

1. Read MIC results

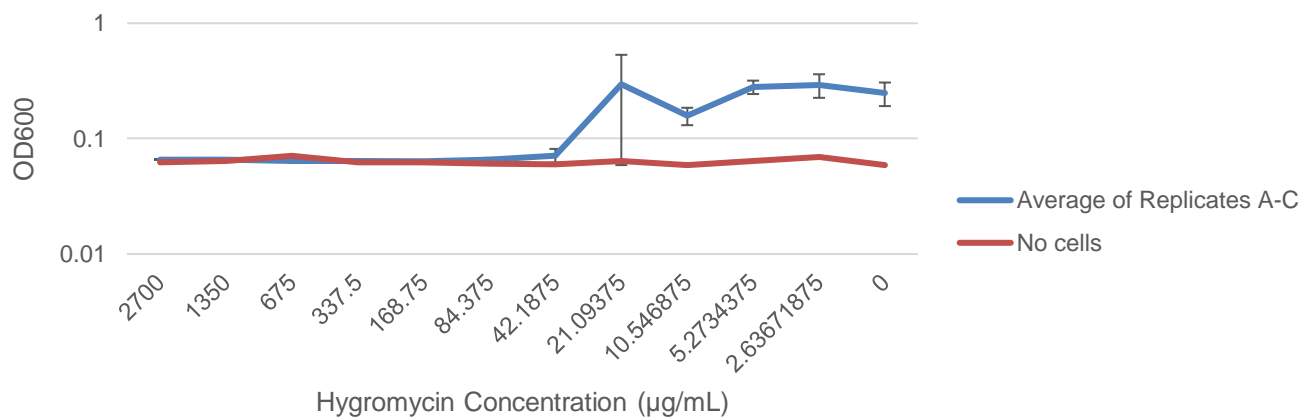
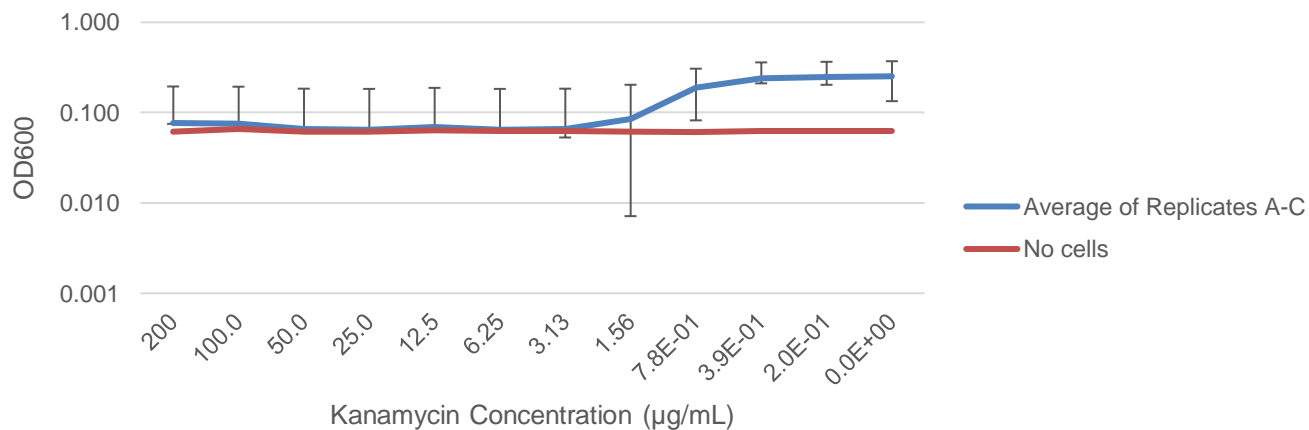
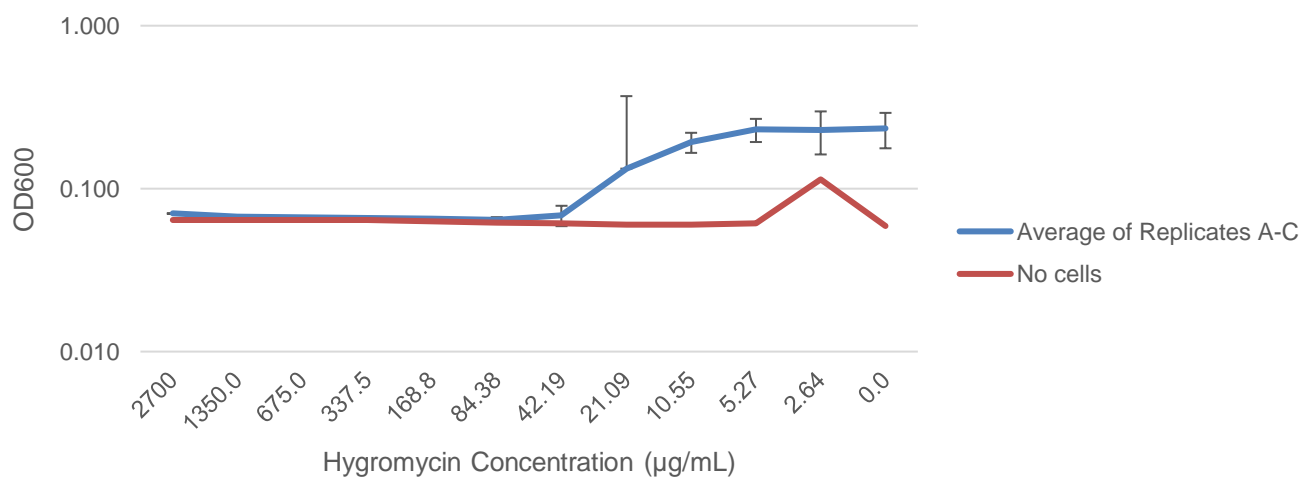
Results and Methods:

MIC graphs: [../MIC results /LVS and \$\Delta\$ rpsu1- \$\Delta\$ rpsu3/190809_TA_kan_hygro/190809_TA_MIC.xlsx](#)

Plates were removed from the incubator at 12:00PM



MIC of Hygromycin in LVS Strain

MIC of Kanamycin in $\Delta\text{rpsu1}-\Delta\text{rpsu3}$ StrainMIC of Hygromycin in $\Delta\text{rpsu1}-\Delta\text{rpsu3}$ 

Kanamycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu 3}$
200	0.065	0.076
100.0	0.064	0.075
50.0	0.063	0.066
25.0	0.063	0.065
12.5	0.063	0.070
6.25	0.063	0.065
3.13	0.064	0.066
1.56	0.067	0.085
7.8E-01	0.092	0.188
3.9E-01	0.231	0.242
2.0E-01	0.239	0.247
0	0.258	0.253

Hygromycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu 2}$
2700	0.069	0.070
1350.0	0.068	0.067
675.0	0.067	0.067
337.5	0.066	0.066
168.8	0.067	0.065
84.38	0.064	0.064
42.19	0.066	0.069
21.09	0.130	0.133
10.55	0.176	0.193
5.27	0.202	0.231
2.64	0.211	0.230
0.0	0.222	0.234

MIC of kan in LVS: 3.13 $\mu\text{g/mL}$.

MIC of kan in $\Delta\text{rpsu1-}\Delta\text{rpsu 3}$: 3.13 $\mu\text{g/mL}$

MIC of hygromycin in LVS: 42.19 $\mu\text{g/mL}$

MIC of hygromycin in $\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$: 42.19 $\mu\text{g/mL}$

Wednesday, August 14th, 2019

To Do:

1. Prepare CHA and Hemoglobin
2. Perform an MIC on LVS and $\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$
3. Make Kanamycin plates
4. Work on New MIC protocol

Results and Methods:

Plates were incubated at 12:45 PM

OD600 LVS: .171 A

OD600 $\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$: .156 A

LVS 8.55V1= .005*20,000 V1= 11.695

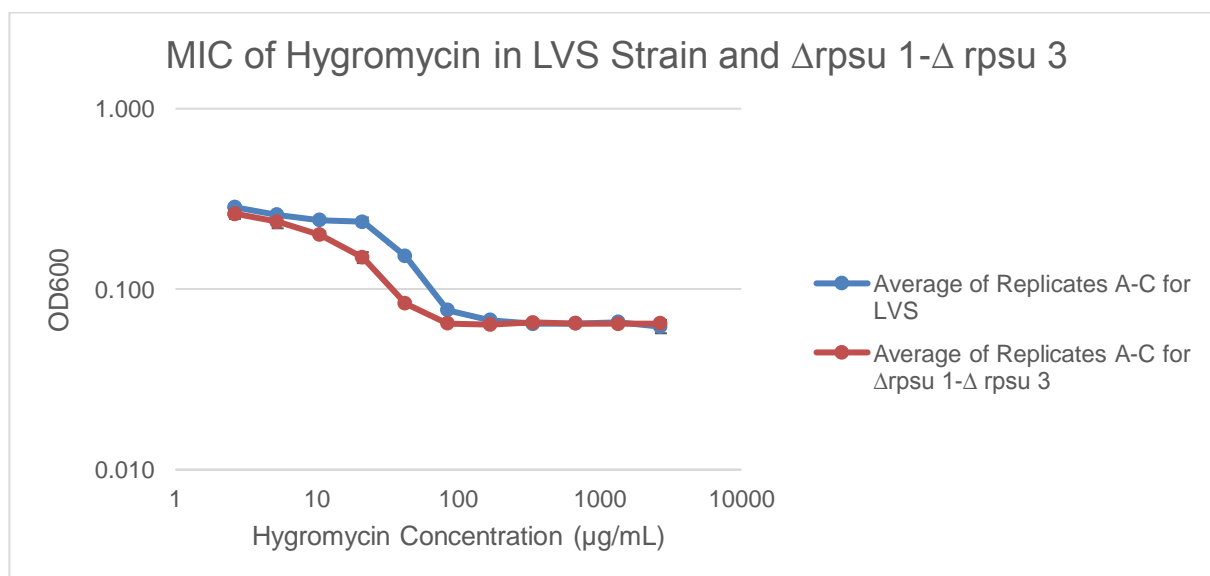
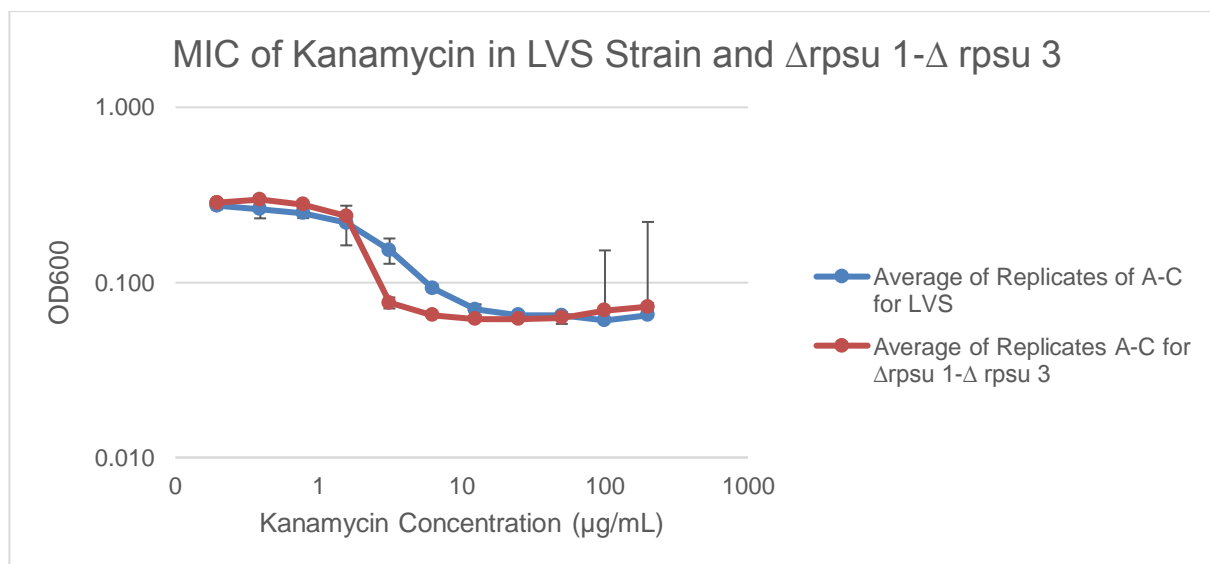
$\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$ 7.8 V1= .005*20,000 V1=12.82

Thursday, August 15th, 2019**To Do:**

1. Read MIC results
2. Prepare Hemoglobin

Results and Methods:

Plates were removed from the incubator at 11:10 PM

MIC graphs: [../MIC results /LVS and \$\Delta\$ rpsu1- \$\Delta\$ rpsu3/190815_TA_kan_hygro/190815_TA_MIC.xlsx](#)

Kanamycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu 2}$
200	0.065	0.073
100.0	0.061	0.069
50.0	0.065	0.063
25.0	0.065	0.062
12.5	0.070	0.062
6.25	0.093	0.065
3.13	0.153	0.077
1.56	0.219	0.239
7.8E-01	0.249	0.278
3.9E-01	0.262	0.297
2.0E-01	0.274	0.284
0	0.319	0.280

Hygromycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu 2}$
2700	0.062	0.065
1350.0	0.066	0.064
675.0	0.065	0.065
337.5	0.064	0.065
168.8	0.067	0.064
84.38	0.077	0.065
42.19	0.152	0.083
21.09	0.237	0.150
10.55	0.242	0.200
5.27	0.259	0.236
2.64	0.284	0.262
0.0	0.286	0.277

MIC of kan in LVS: 12.5 $\mu\text{g/mL}$.

MIC of kan in $\Delta\text{rpsu1-}\Delta\text{rpsu 3}$: 6.25 $\mu\text{g/mL}$

MIC of hygromycin in LVS: 168.8 $\mu\text{g/mL}$

MIC of hygromycin in $\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$: 84.38 $\mu\text{g/mL}$

We have decided to start putting the 96-well tubes in plastic containers and surround them with wet paper towel to provide a humid surrounding.

We will also start transferring 150 μL of our samples to new 96-well plate before reading the results.

Monday, August 19th, 2019

To Do:

1. Perform an MIC on LVS and $\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$
2. Make Regular Plates

Results and Methods:

New MIC Protocol:

1. For each strain to be tested, prepare 20 mL of media (supplemented MHB) in 1 sterile 50 mL conical.
2. Resuspend cells for strains being tested in about 400 μL of MHB (if necessary). Measure the OD600.
3. Aim for an OD600 of 0.005. Dilute the appropriate amount of culture in the 50 mL conical that contains media to get required OD600.

- Using the multichannel, add 190 μL of the diluted culture into the wells of a non-treated 96 well plate, such that three rows and all 12 columns are filled for each strain (see diagram below).
- In 4th row, add same volume of supplemented MHB using a new reservoir and multichannel.
- The next steps will vary based on the concentrations of antibiotic you want to use. An example for kanamycin, with the highest concentration in the wells of 200 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 4,000 $\mu\text{g/mL}$ in stock tube A is required, because it will be diluted 1:20 when added to the 96-well plate. **An example for hygromycin, with the highest concentration in the wells of 675 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 13,500 $\mu\text{g/mL}$ in stock tube A is required, because it will be dilute 1:20 when added to the 96-well plate**
- In 12 sterile strip tubes for each antibiotic, prepare 100 μL of media.
- Prepare a working stock of kanamycin that is a concentration of 8 mg/mL , by adding 17.6 μL of our stock 50 mg/mL solution to 92.4 μL of MHB.
- Prepare a working stock of hygromycin that is a concentration of 27 mg/mL , by adding 55 μL of our stock 54 mg/mL solution to 55 μL of MHB.**
- Add 100 μL of the working stocks to the first strip tube for each antibiotic and mix.
- Serially dilute 1:2 by transferring 100 μL from the first strip tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
- Using the multi-channel, pipet 10 μL from the strip tubes to the corresponding wells on the plate with media and mix (you can use pipet and mix function to take up 10 μL and mix with 155 μL 3x).
- Cover plate with lid and put in 37C incubator overnight.
- In the morning, mix and pipet 150 μL from each well to a new 96-well plate.
- Use the plate reader to measure OD600.

Plates were incubated at 12:10 PM

OD600 LVS: .184 A

OD600 $\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$: .194 A

$C1V1=C2V2$

LVS: $9.2V1=.005*20,000$ $V1= 10.87 \mu\text{L}$

$\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$: $9.7V1=.005*20,000$

$V1=. 10.31$

Tuesday, August 20th, 2019

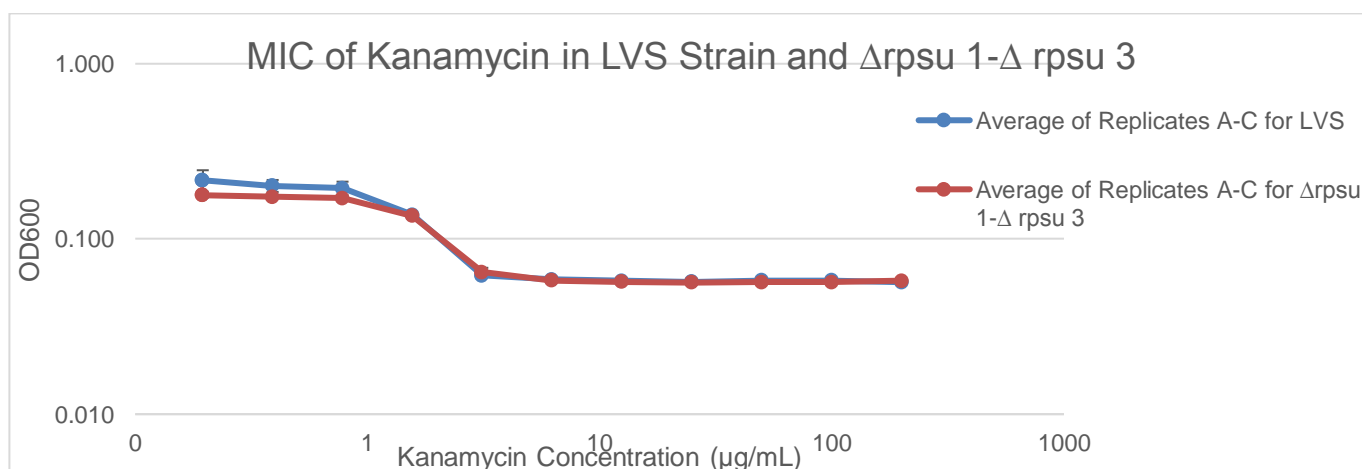
To Do:

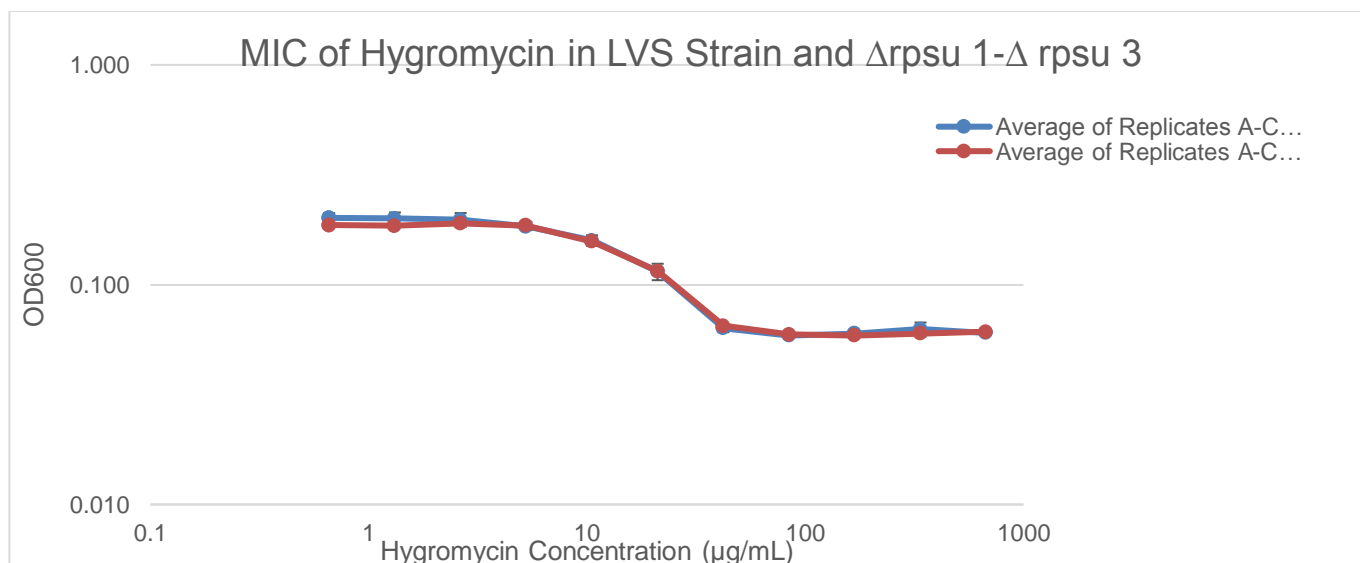
- Hannah will read the MIC Results

Results and Methods:

Plates were removed from the incubator at 10:35 PM

Mic graphs: [./MIC results /LVS and \$\Delta\text{rpsu1-}\Delta\text{rpsu3}\$ /190820_TA_kan_hygro/190820_TA_MIC.xlsx](#)





Kanamycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS	Avg OD600 of Δ rpsu1- Δ rpsu 2
200	0.057	0.058
100.0	0.058	0.057
50.0	0.058	0.057
25.0	0.057	0.056
12.5	0.058	0.057
6.25	0.059	0.058
3.13	0.062	0.065
1.56	0.137	0.135
7.8E-01	0.195	0.171
3.9E-01	0.201	0.174
2.0E-01	0.216	0.178
0	0.219	0.183

Hygromycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS	Avg OD600 of Δ rpsu1- Δ rpsu 2
675	0.060	0.061
337.5	0.063	0.060
168.8	0.060	0.059
84.38	0.059	0.059
42.19	0.063	0.065
21.09	0.115	0.115
10.55	0.159	0.157
5.27	0.184	0.186
2.64	0.197	0.190
1.32	0.200	0.185
0.66	0.201	0.186
0.00	0.211	0.199

MIC of kan in LVS: 6.25 $\mu\text{g/mL}$.

MIC of kan in Δ rpsu1- Δ rpsu 3: 6.25 $\mu\text{g/mL}$

MIC of hygromycin in LVS: 84.38 $\mu\text{g/mL}$

MIC of hygromycin in Δ rpsu 1- Δ rpsu 3: 84.38 $\mu\text{g/mL}$

Friday, August 23th, 2019**To Do:**

1. Perform an MIC on LVS and Δ rpsu 1- Δ rpsu 3
2. Make LB agar
3. Make MHB
4. Supplement MHB

Results and Methods:**Plates were incubated at 1:10 PM**

LB Agar protocol:

1. For 500 mL of LB-agar, weigh out the following components and add to a 1 L (non-baffled) flask:
 - a. 6 g agar
 - b. 5 g NaCl
 - c. 5 g Tryptone
 - d. 2.5g Yeast extract
2. Add stirbar to flask
3. Add 500 mL ddiH₂O
4. Mix on stirplate until components are dissolved
5. Cover top of flask with foil and add a small piece of autoclave tape
6. Autoclave on 30-minute liquid cycle

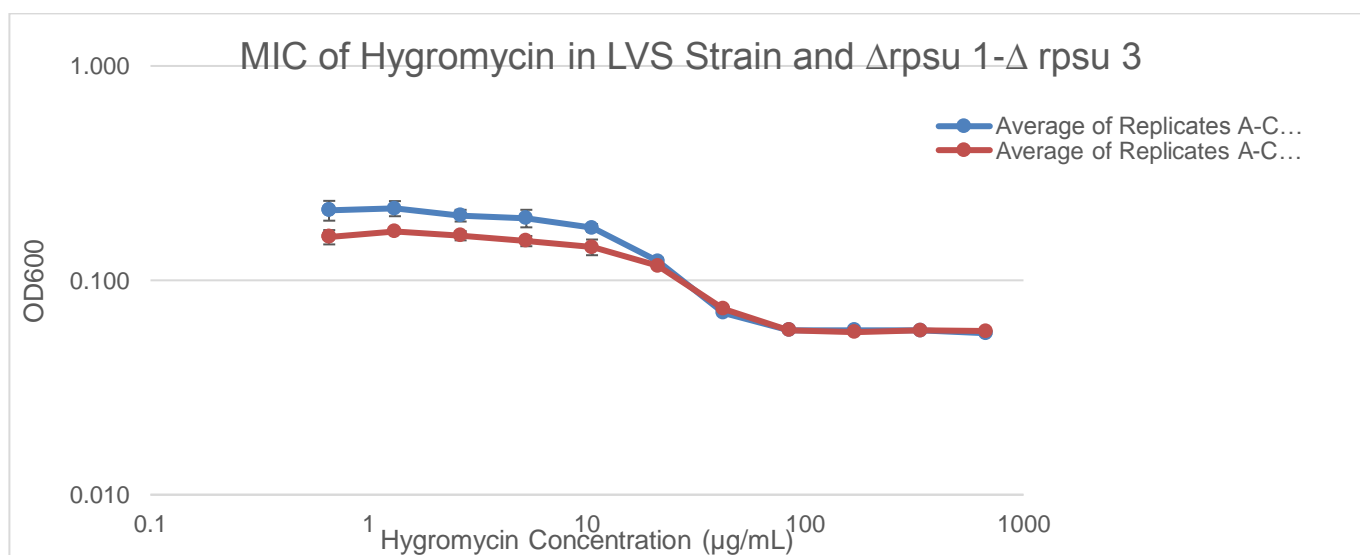
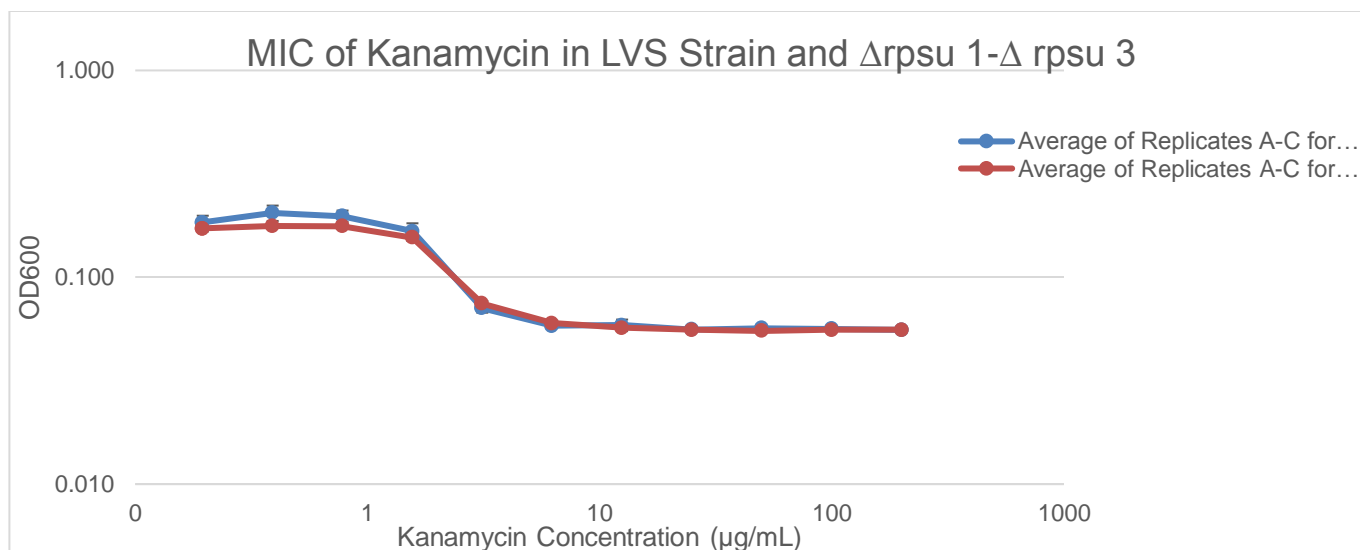
MHB protocol:

1. Weigh out 10.5g of Mueller-Hinton broth into 1L square-bottle
2. Add 500mL of ddiH₂O (type I)
3. Autoclave on 30' liquid cycle, filling the water bin up
4. Cool down to 37 °C or cooler
5. Can keep this sterile media indefinitely without supplements
6. Add 5mL of 10% glucose
7. Add 5mL of 2.5% iron pyrophosphate
8. Add isovitalax (combine 10mL from liquid vial with solids, add entire volume to broth)
9. Can keep this supplemented media for 2 weeks, storing at 4 °C

Saturday, August 24th, 2019**To Do:**

1. Read MIC results

Results and Methods:MIC graphs: [../MIC results /LVS and \$\Delta\$ rpsu1- \$\Delta\$ rpsu3/190824_TA_kan_hygro/190824_TA_MIC.xlsx](#)**Plates were removed from the incubator at 11:35 PM**



Kanamycin Conc. (μ g/mL)	Avg OD600 of LVS	Avg OD600 of Δ rpsu1- Δ rpsu 2
200	0.056	0.056
100.0	0.056	0.056
50.0	0.057	0.055
25.0	0.056	0.056
12.5	0.059	0.057
6.25	0.058	0.060
3.13	0.071	0.075
1.56	0.167	0.156
7.8E-01	0.198	0.176
3.9E-01	0.205	0.177
2.0E-01	0.184	0.172
0	0.192	0.171

Hygromycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu 2}$
675	0.057	0.058
337.5	0.058	0.058
168.8	0.059	0.057
84.38	0.059	0.059
42.19	0.071	0.074
21.09	0.123	0.117
10.55	0.176	0.143
5.27	0.195	0.153
2.64	0.201	0.162
1.32	0.217	0.169
0.66	0.212	0.159
0.00	0.223	0.173

MIC of kan in LVS: 6.25 $\mu\text{g/mL}$.

MIC of kan in $\Delta\text{rpsu1-}\Delta\text{rpsu 3}$: 6.25 $\mu\text{g/mL}$

MIC of hygromycin in LVS: 84.38 $\mu\text{g/mL}$

MIC of hygromycin in $\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$: 84.38 $\mu\text{g/mL}$

Tuesday, August 27th, 2019

To Do:

1. Prepare Tetracycline and Erythromycin stocks for MIC
2. Work on New MIC protocol
3. Streak LVS for MIC

Results and Methods:

Preparing Tetracycline:

1. Measure out .7g of tetracycline (it is stored in the -20°C)
2. Cover conical in foil
3. Add 20 mL of methanol
4. Vortex and store in the -20°C fridge

Preparing Erythromycin:

1. Measure out .2g of Erythromycin (it is stored in room temperature)
2. Add 10 mL of 100% Ethanol
3. Vortex and store in the -20°C fridge

New MIC Protocol for Tetracycline and Erythromycin

1. For each strain to be tested, prepare 20 mL of media (supplemented MHB) in 1 sterile 50 mL conical.
2. Resuspend cells for strains being tested in about 400 μL of MHB (if necessary). Measure the OD600.
3. Aim for an OD600 of 0.005. Dilute the appropriate amount of culture in the 50 mL conical that contains media to get required OD600.
4. Using the multichannel, add 190 μL of the diluted culture into the wells of a non-treated 96 well plate, such that three rows and all 12 columns are filled for each strain (see diagram below).
5. In 4th row, add same volume of supplemented MHB using a new reservoir and multichannel.
6. The next steps will vary based on the concentrations of antibiotic you want to use. An example for tetracycline, with the highest concentration in the wells of 64 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 1,280 $\mu\text{g/mL}$ in stock tube A is required, because it will be diluted 1:20 when added to the 96-well plate. An example for erythromycin, with the highest concentration in the wells of 1000 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 20,000 $\mu\text{g/mL}$ in stock tube A is required, because it will be dilute 1:20 when added to the 96-well plate

7. In 12 sterile strip tubes for each antibiotic, prepare 50 μL of media. **Don't add media to the tube A of erythromycin.**
8. Prepare a working stock of tetracycline that is a concentration of 2.56 mg/mL, by adding 8.0 μL of our stock 35 mg/mL solution to 102.0 μL of MHB.
9. Add 50 μL of the working stocks to the first strip tube for each antibiotic and mix.
10. **For Erythromycin, transfer 100 μL from the stock antibiotic to tube A.**
11. Serially dilute 1:2 by transferring 50 μL from the first strip tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
12. Using the multi-channel, pipet 10 μL from the strip tubes to the corresponding wells on the plate with media and mix (you can use pipet and mix function to take up 10 μL and mix with 155 μL 3x).
13. Cover plate with lid and put in 37°C incubator overnight.
14. In the morning, mix and pipet 150 μL from each well to a new 96-well plate.
15. Use the plate reader to measure OD600.

Wednesday, August 28th, 2019

To Do:

1. Perform MIC using tetracycline and erythromycin
2. Fill pipette boxes

Results and Methods:

Measuring OD600 for LVS:

OD600 LVS: .134 A

$C1V1 = C2V2$

$6.7 * V1 = .005 * 20,000$

$V1 = 14.93 \mu\text{L}$

Plate was incubated at 12:35 PM.

Thursday, August 29th, 2019

To Do:

1. Read MIC Results
2. Work on MIC Excel sheet
3. Work on MIC one strain and two strain protocol
4. Make LB-kanamycin plates
5. Make CHA kanamycin plates

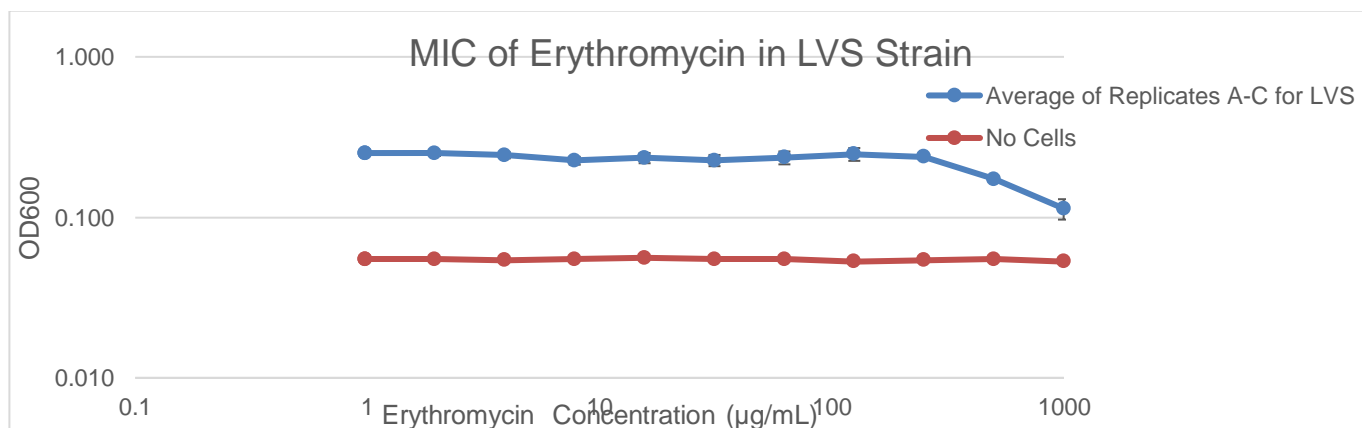
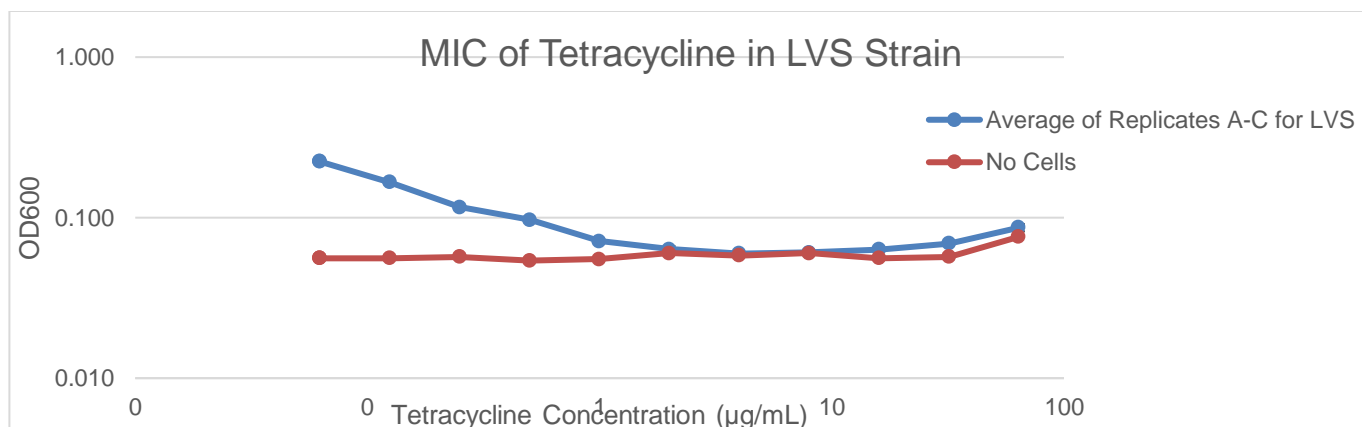
Results and Methods:

Plate was removed from incubator at 11:00 AM.

MIC Graph: [./MIC_results/LVS/190829_TA_tet_erythro/190829_TA_MIC.xlsx](#)

Tetracycline has a higher OD600 for the highest concentrations because of tetracycline color.

We will not test erythromycin again because the MIC seems to be higher than 1000 $\mu\text{g/mL}$ (5% of the 200 μL in the well is from the antibiotic and we shouldn't exceed that). Our strains might have developed resistance to erythromycin.



Tetracycline Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS
64	0.087
32.0	0.069
16.0	0.063
8.0	0.061
4.0	0.060
2.00	0.064
1.00	0.071
0.50	0.097
2.5E-01	0.116
1.3E-01	0.166
6.3E-02	0.223
0	0.282

Erythromycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS
1000	0.113
500.0	0.173
250.0	0.238
125.00	0.248
62.50	0.236
31.25	0.227
15.63	0.235
7.81	0.226
3.91	0.244
1.95	0.251
0.98	0.252
0.00	0.268

MIC of tet in LVS: 2.0 $\mu\text{g/mL}$.

MIC of eryth in LVS: >1000 $\mu\text{g/mL}$

LB-kanamycin plates protocol:

1. heat up LB-agar on hotplate, stirring, until entirely melted (**watch carefully** so it doesn't boil over! Don't walk away). Let agar cool down or place in warm over (50°C) until ready to pour plates.
2. add 500 μL of kanamycin to 500 mL of LB-agar media. Stir on stirplate to mix media.
3. Open a plate next to flame and pour ~20-25 mL LB-agar into plate; the bottom of the plate should be covered in media.
4. Immediately rinse out flask
5. Stack plates and mark side with blue marker for kanamycin

September 2019

Tuesday, September 3rd, 2019

To Do:

1. Steak LVS for MIC
2. Prepare antibiotic stocks for MIC
3. Work on streptomycin protocol

Results and Methods:

To make 50 mg/mL of streptomycin dilute 0.5g of powder in 10 mL of dH_2O , vortex and filter sterilize. Store in the -20C fridge.

MIC Protocol (One Strain):

1. Prepare 20 mL of media (supplemented MHB) in 1 sterile 50 mL conical.
2. Resuspend cells for the strain being tested in about 400 μL of MHB. Measure the OD600.
3. Aim for an OD600 of 0.005. Dilute the appropriate amount of culture in the 50 mL conical that contains media to get required OD600.
4. Using the multichannel and a reservoir, add 190 μL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 190 μL of the diluted culture into the empty wells of the plate, such that 6 rows and all 12 columns are filled with media.
6. The next steps will vary based on the concentrations of antibiotic you want to use. An example for tetracycline, with the highest concentration in the wells of 64 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 1,280 $\mu\text{g/mL}$ in stock tube A is required, because it will be diluted 1:20 when

added to the 96 well-plate. An example for streptomycin, with the highest concentration in the wells of 200 µg/mL and 1:2 dilutions, is shown here. A starting concentration of 4,000 µg/mL in stock tube A is required, because it will be diluted 1:20 when added to the 96-well plate.

7. In 12 strip tubes for each antibiotic, prepare 50 µL of media.
8. Prepare a working stock of tetracycline that is a concentration of 2.56 mg/mL, by adding 8.0 µL of our stock 35 mg/mL solution to 102.0 µL of MHB. Prepare a working stock of streptomycin that is a concentration of 8.00 mg/mL, by adding 17.6 µL of our stock 50 mg/mL solution to 92.4 µL of MHB.
9. Serially dilute 1:2 by transferring 50 µL from the first strip tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
10. Using the multichannel, pipet 10 µL from the strip tubes to the corresponding wells on the plate with media and mix (you can use pipet and mix function to take up 10 µL and mix with 100 µL 3x).
11. Cover plate with lid and put in 37°C incubator overnight.
12. In the morning, mix and pipet 150 µL from each well to a new 96-well plate.
13. Use the plate reader to measure OD600.

Wednesday, September 4th, 2019

To Do:

1. Make single use stocks for LVS and Δ rpsu 1- Δ rpsu 3
2. Perform an MIC on LVS using tetracycline and streptomycin

Results and Methods:

Plate was incubated at 12:15 PM.

Measuring OD600 for LVS:

OD600 LVS: .119 A

$$C1V1 = C2V2 \quad 5.95 * V1 = .005 * 20,000$$

$$V1 = 16.81 \mu\text{L}$$

Making single use aliquots:

1. Add 400 µL of MHB to a 1.5 mL microcentrifuge tube
2. Scrape all the cells on the plate and resuspend using the 200 µL pipette
3. Add 400 µL of more MHB (or whatever volume that makes an 800 µL solution)
4. Add 200 µL of 75% glycerol and mix by pipetting (don't vortex)
5. Pipette 50 µL of solution per microcentrifuge
6. Store in the -80°C freezer

Thursday, September 5th, 2019

To Do:

1. Read MIC results
2. Prepare 2.5% iron pyrophosphate

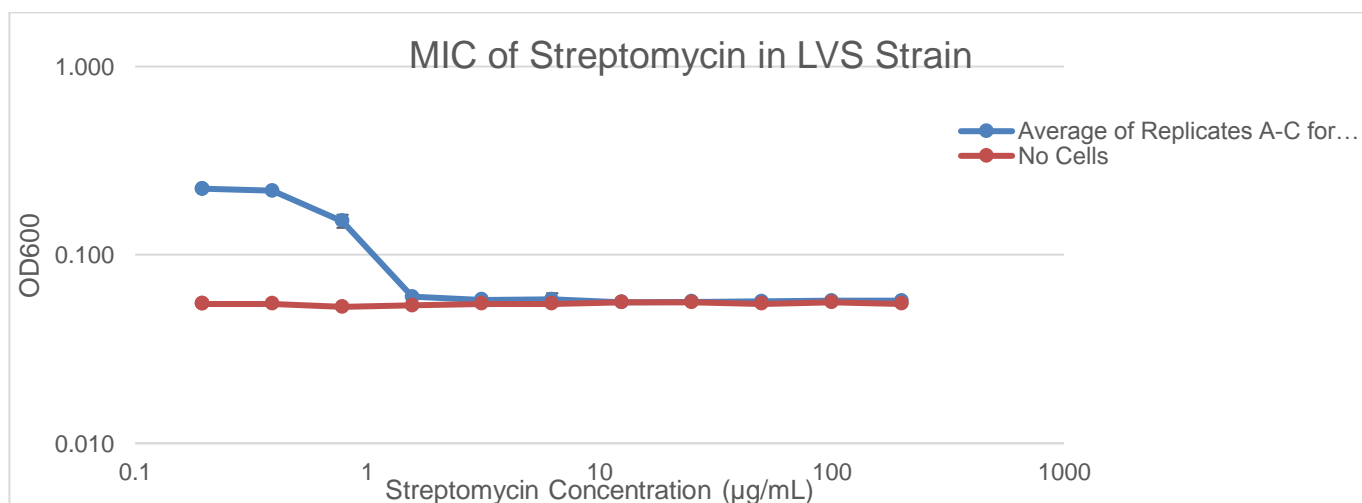
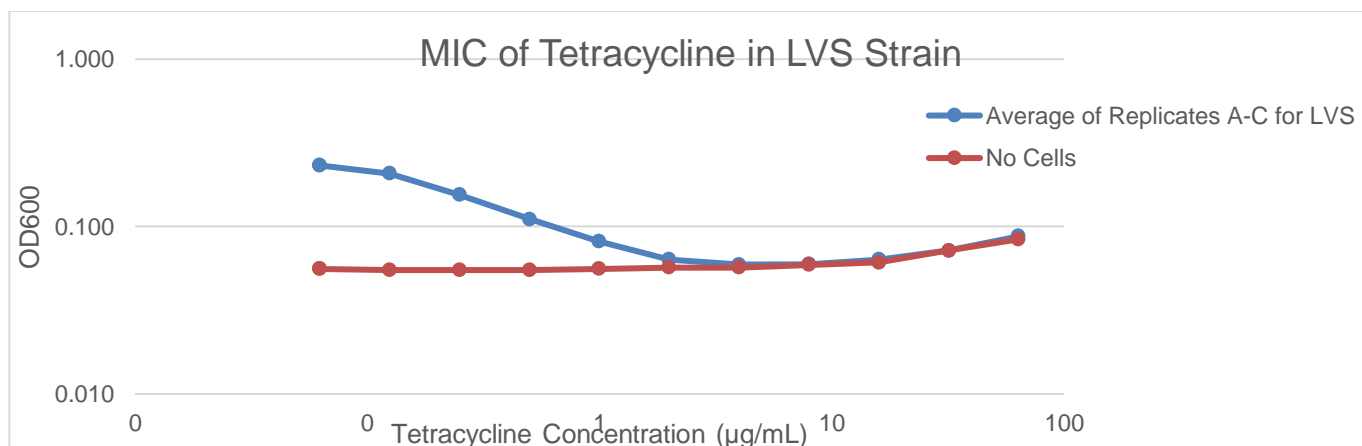
Results and Methods:

Plate was removed from the incubator at 10:40 AM.

MIC graphs: [../MIC results /LVS/190905_TA_tet_strep/190905_TA_MIC.xlsx](#)

MIC of tet in LVS: 4.0 µg/mL

MIC of strep in LVS: 3.13 µg/mL.



Tetracycline Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS
64	0.088
32.0	0.072
16.0	0.064
8.0	0.060
4.0	0.059
2.00	0.064
1.00	0.081
0.50	0.111
2.5E-01	0.155
1.3E-01	0.208
6.3E-02	0.232
0	0.253

Streptomycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS
200	0.057
100.0	0.057
50.0	0.057
25.00	0.056
12.50	0.056
6.25	0.058
3.13	0.058
1.56	0.060
0.78	0.151
0.39	0.219
0.20	0.225
0.00	0.238

Friday, September 6th, 2019

To Do:

1. Streak LVS for Monday's MIC
2. Filter sterilize iron pyrophosphate

Results and Methods:

Monday's MIC protocol:

1. Prepare 20 mL of media (supplemented MHB) in 1 sterile 50 mL conical.
2. Resuspend cells for the strain being tested in about 400 μL of MHB. Measure the OD600.
3. Aim for an OD600 of 0.005. Dilute the appropriate amount of culture in the 50 mL conical that contains media to get required OD600.
4. Using the multichannel and a reservoir, add 190 μL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 190 μL of the diluted culture into the empty wells of the plate, such that 6 rows and all 12 columns are filled with media.
6. The next steps will vary based on the concentrations of antibiotic you want to use. An example for tetracycline, with the highest concentration in the wells of 64 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 1,280 $\mu\text{g/mL}$ in stock tube A is required, because it will be diluted 1:20 when added to the 96 well-plate. **An example for streptomycin, with the highest concentration in the wells of 50 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 1,000 $\mu\text{g/mL}$ in stock tube A is required, because it will be diluted 1:20 when added to the 96-well plate.**
7. In 12 strip tubes for each antibiotic, prepare 50 μL of media.
8. Prepare a working stock of tetracycline that is a concentration of 2.56 mg/mL, by adding 8.0 μL of our stock 35 mg/mL solution to 102.0 μL of MHB. **Prepare a working stock of streptomycin that is a concentration of 2.00 mg/mL, by adding 4.4 μL of our stock 50 mg/mL solution to 105.6 μL of MHB.**
9. Serially dilute 1:2 by transferring 50 μL from the first strip tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
10. Using the multichannel, pipet 10 μL from the strip tubes to the corresponding wells on the plate with media and mix (you can use pipet and mix function to take up 10 μL and mix with 100 μL 3x).
11. Cover plate with lid and put in 37°C incubator overnight.
12. In the morning, mix and pipet 150 μL from each well to a new 96-well plate.
13. Use the plate reader to measure OD600.

Monday, September 9th, 2019

To Do:

1. Supplement MHB
2. Perform an MIC on LVS using tetracycline and Streptomycin

Results and Methods:

Plate was incubated at 12:05 PM

Tuesday, September 10th, 2019

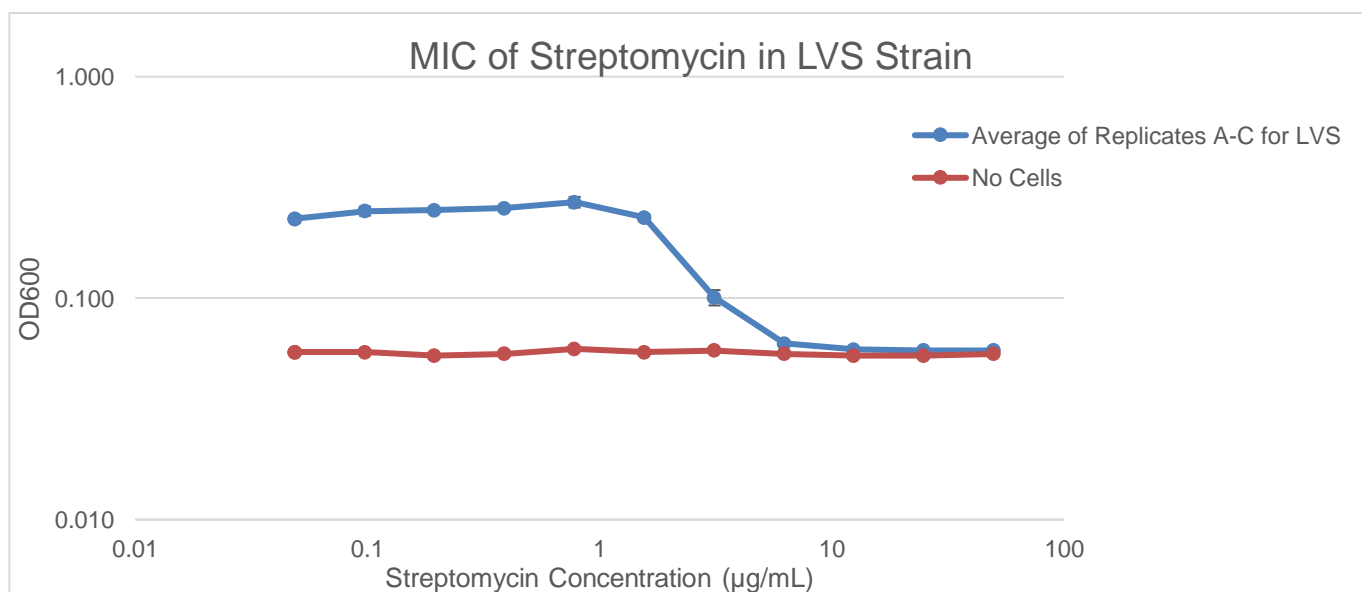
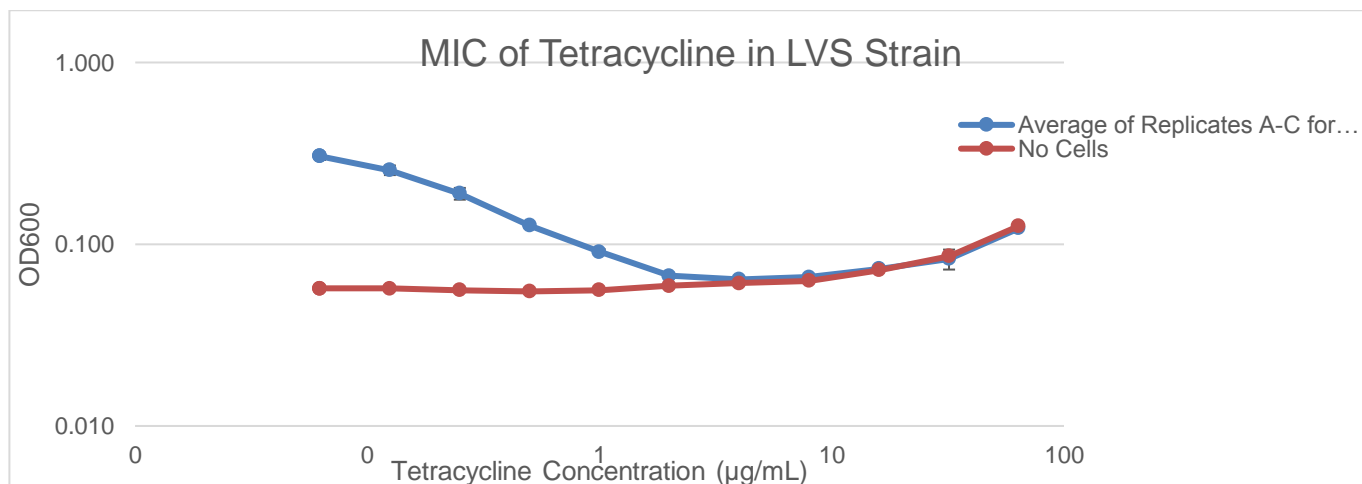
To Do:

1. Read MIC Results
2. Streak LVS for MIC
3. Streak $\Delta rpsu1-\Delta rpsu3$ for MIC
4. Prepare antibiotic dilutions for tetracycline and streptomycin
5. Refill autoclaved pipette tips

Results and Methods:

Plate was removed at 10:30 AM

MIC graphs: [../MIC_results/LVS/190910_TA_tet_strep/190910_TA_MIC.xlsx](#)



Tetracycline Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS
64	0.122
32.0	0.083
16.0	0.073
8.0	0.066
4.0	0.064
2.00	0.067
1.00	0.091
0.50	0.127
2.5E-01	0.190
1.3E-01	0.256
6.3E-02	0.305
0	0.350

Streptomycin Conc. ($\mu\text{g/mL}$)	Avg OD600 of LVS
50	0.058
25.0	0.058
12.5	0.059
6.25	0.062
3.13	0.101
1.56	0.231
0.78	0.271
0.39	0.255
0.20	0.249
0.10	0.247
0.05	0.228
0.00	0.280

MIC of tet in LVS: 4.0 $\mu\text{g/mL}$

MIC of strep in LVS: 12.5 $\mu\text{g/mL}$

MIC protocol

1. For each strain to be tested, prepare 20 mL of media (supplemented MHB) in 1 sterile 50 mL conical.
2. Resuspend cells for strains being tested in about 400 μL of MHB (if necessary). Measure the OD600.
3. Aim for an OD600 of 0.005. Dilute the appropriate amount of culture in the 50 mL conical that contains media to get required OD600.
4. Using the multichannel and a reservoir, add 190 μL of the diluted culture into the wells of a non-treated 96 well plate, such that three rows and all 12 columns are filled for each strain (see diagram below).
5. In 4th and 8th rows, add same volume of supplemented MHB using a new reservoir and multichannel.
6. The next steps will vary based on the concentrations of antibiotic you want to use. An example for tetracycline, with the highest concentration in the wells of 64 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 1,280 $\mu\text{g/mL}$ in stock tube A is required, because it will be diluted 1:20 when added to the 96-well plate. **An example for streptomycin, with the highest concentration in the wells of 50 $\mu\text{g/mL}$ and 1:2 dilutions, is shown here. A starting concentration of 1,000 $\mu\text{g/mL}$ in stock tube A is required, because it will be dilute 1:20 when added to the 96-well plate**
7. In 12 sterile strip tubes for each antibiotic, prepare 100 μL of media. Don't add media to tube A of erythromycin.
8. Prepare a working stock of tetracycline that is a concentration of 2.56 mg/mL, by adding 8.0 μL of our stock 35 mg/mL solution to 102.0 μL of MHB. **Prepare a working stock of streptomycin that is a concentration of 2.00 mg/mL, by adding 4.4 μL of our stock 50 mg/mL solution to 105.6 μL of MHB.**
9. Serially dilute 1:2 by transferring 100 μL from the first strip tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.

10. Using the multi-channel, pipet 10 μL from the strip tubes to the corresponding wells on the plate with media and mix (you can use pipet and mix function to take up 10 μL and mix with 100 μL 3x).
11. Cover plate with lid and put in 37°C incubator overnight.
12. In the morning, mix and pipet 150 μL from each well to a new 96-well plate.
13. Use the plate reader to measure OD600.

Antibiotic dilutions were prepared a day in advance. Streptomycin was done differently to avoid using such small volume (4.4 μL). A stock solution was made using 8.8 μL of 50 mg/mL streptomycin and then was diluted 1:2 into another 2mL microcentrifuge tube.

This MIC will have one plate to test tetracycline with LVS and double mutant and another to test streptomycin with LVS.

Wednesday, September 11th, 2019

To Do:

1. Perform an MIC on LVS strains and $\Delta\text{rpsu1-}\Delta\text{rpsu3}$ using tetracycline
2. Perform an MIC on LVS using streptomycin
3. Discuss allelic exchange protocol with Jamie

Results and Methods:

Plates were incubated at 11:35 AM.

Measuring OD600 for LVS:

OD600 LVS: .240 A

$$C1V1 = C2V2 \quad 12 * V1 = .005 * 20,000$$

$$V1 = 8.33 \mu\text{L}$$

Measuring OD600 for LVS:

OD600 $\Delta\text{rpsu1-}\Delta\text{rpsu3}$: .213 A

$$C1V1 = C2V2 \quad 10.65 * V1 = .005 * 20,000$$

$$V1 = 9.39 \mu\text{L}$$

Thursday, September 12th, 2019

To Do:

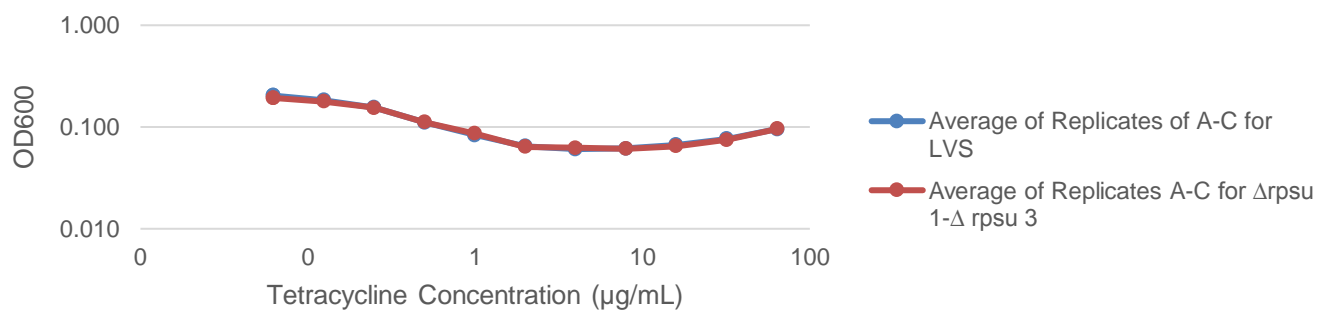
1. Read MIC Results

Results and Methods:

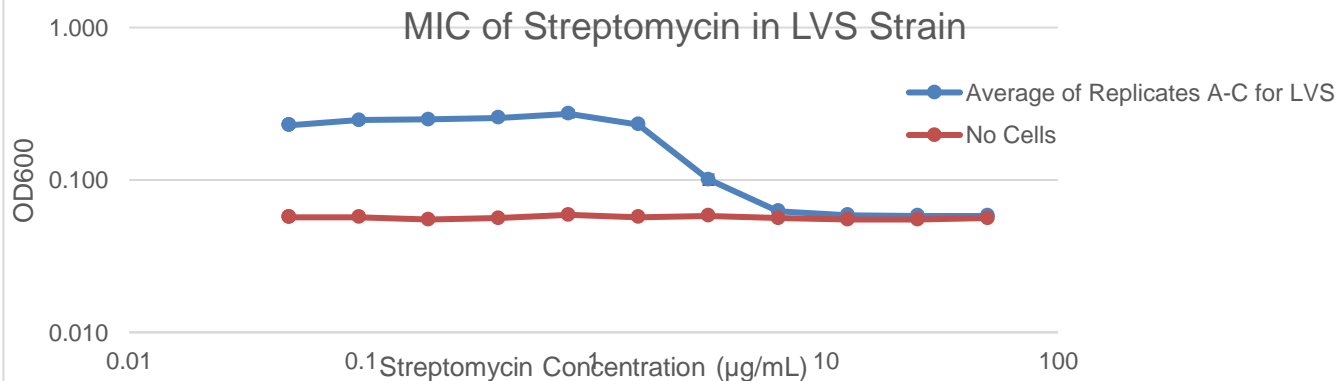
Plates were removed at 10:00 AM

MIC graphs: [../MIC results /LVS and \$\Delta\text{rpsu1-}\Delta\text{rpsu3}\$ /190912_TA_tet_strep/190912_TA_MIC.xlsx](#)

MIC of Tetracycline in LVS Strain and $\Delta rpsu 1-\Delta rpsu 3$



MIC of Streptomycin in LVS Strain



Tetracycline Conc. (μg/mL)	Avg OD600 of LVS	Avg OD600 of $\Delta rpsu1-\Delta rpsu 3$
64	0.095	0.096
32.0	0.076	0.075
16.0	0.067	0.065
8.0	0.062	0.061
4.0	0.061	0.063
2.00	0.065	0.064
1.00	0.083	0.087
0.50	0.111	0.112
2.5E-01	0.155	0.154
1.3E-01	0.184	0.178
6.3E-02	0.203	0.193
0	0.226	0.214

Streptomycin Conc. (μg/mL)	Avg OD600 of LVS
50	0.059
25.0	0.059
12.5	0.057
6.25	0.058
3.13	0.061
1.56	0.111
0.78	0.180
0.39	0.199
0.20	0.212

0.10	0.217
0.05	0.216
0.00	0.219

MIC of tet in LVS: 4.0 µg/mL

MIC of tet in Δ rpsu1- Δ rpsu 3: 4.0 µg/mL

MIC of strep in LVS: 3.13 µg/mL

Friday, September 13th, 2019

To Do:

1. Streak out LVS and Δ rpsu 1- Δ rpsu 3

Results and Methods:

Plates were allowed to grow in room temperature until Monday.

Monday, September 16th, 2019

To Do:

1. Perform an MIC on LVS and Δ rpsu 1- Δ rpsu 3 using tetracycline
2. Prepare hemoglobin
3. Watch Hannah run a PCR and gel

Results and Methods:

Plate was incubated at 11:35 AM

MIC Protocol

1. For each strain to be tested, prepare 20 mL of media (supplemented MHB) in 1 sterile 50 mL conical.
2. Resuspend cells for strains being tested in about 400 µL of MHB (if necessary). Measure the OD600.
3. Aim for an OD600 of 0.005. Dilute the appropriate amount of culture in the 50 mL conical that contains media to get required OD600.
4. Using the multichannel and a reservoir, add 190 µL of the diluted culture into the wells of a non-treated 96 well plate, such that three rows and all 12 columns are filled for each strain (see diagram below).
5. In 4th and 8th rows, add same volume of supplemented MHB using a new reservoir and multichannel.
6. The next steps will vary based on the concentrations of antibiotic you want to use. An example for tetracycline, with the highest concentration in the wells of 64 µg/mL and 1:2 dilutions, is shown here. A starting concentration of 1,280 µg/mL in stock tube A is required, because it will be diluted 1:20 when added to the 96-well plate.
7. In 12 sterile strip tubes for each antibiotic, prepare 100 µL of media. Don't add media to tube A of erythromycin.
8. Prepare a working stock of tetracycline that is a concentration of 2.56 mg/mL, by adding 8.0 µL of our stock 35 mg/mL solution to 102.0 µL of MHB.
9. Serially dilute 1:2 by transferring 100 µL from the first strip tube to the next, mixing, and repeating through the 11th tube. In the last tube, do not add any antibiotic. This will be the control.
10. Using the multi-channel, pipet 10 µL from the strip tubes to the corresponding wells on the plate with media and mix (you can use pipet and mix function to take up 10 µL and mix with 100 µL 3x).
11. Cover plate with lid and put in 37°C incubator overnight.
12. In the morning, mix and pipet 150 µL from each well to a new 96-well plate.
13. Use the plate reader to measure OD600.

Measuring OD600 for LVS:

OD600 LVS: .282 A

$C1V1 = C2V2$ $14.1 * V1 = .005 * 20,000$

$V1 = 7.09 \mu\text{L}$

Measuring OD600 for LVS:

OD600 $\Delta\text{rpsu1-}\Delta\text{rpsu3}$: .362 A

$C1V1 = C2V2$ $18.1 * V1 = .005 * 20,000$

$V1 = 5.52 \mu\text{L}$

Tuesday, September 17th, 2019

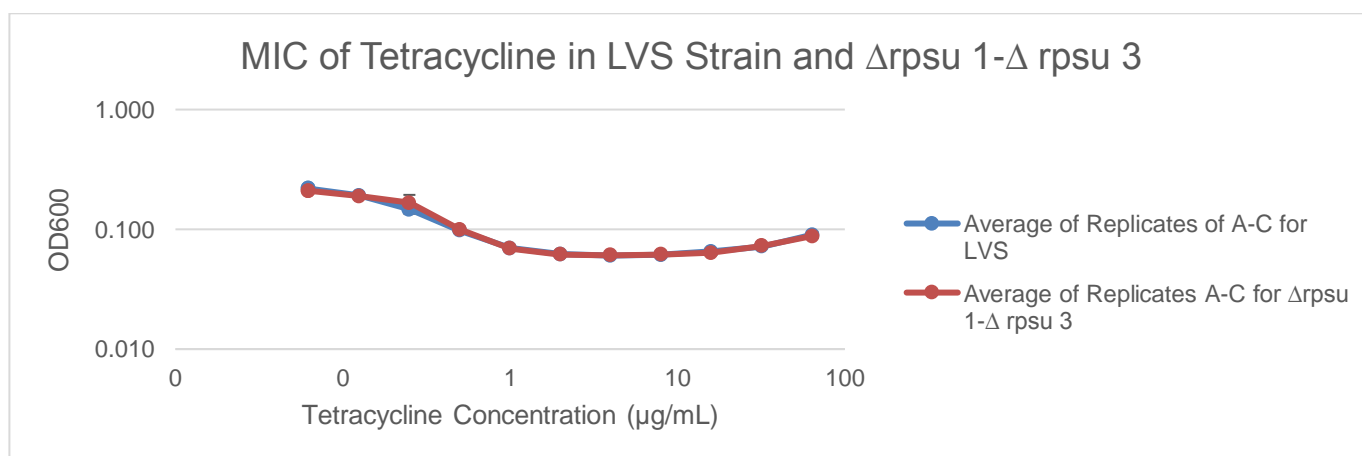
To Do:

1. Read MIC results
2. Make kan plates

Results and Methods:

Plate was removed from the incubator at 10:00 AM.

MIC graph: [../MIC results /LVS and Δrpsu1-Δrpsu3/190917_TA_tet/190917_TA_MIC.xlsx](#)



Tetracycline Conc. (μg/mL)	Avg OD600 of LVS	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu 3}$
64	0.090	0.088
32.0	0.072	0.073
16.0	0.065	0.064
8.0	0.061	0.062
4.0	0.060	0.061
2.00	0.062	0.062
1.00	0.070	0.069
0.50	0.098	0.100
2.5E-01	0.147	0.166
1.3E-01	0.192	0.190

6.3E-02	0.219	0.210
0	0.241	0.233

MIC of tet in LVS: 4.0 µg/mL

MIC of tet in $\Delta rpsu1$ - $\Delta rpsu$ 3: 4.0 µg/mL

Wednesday, September 18th, 2019

To Do:

1. Make hemoglobin
2. Make 50% sucrose

Friday, September 20th, 2019

To Do:

1. Refill tip boxes

Monday, September 23th, 2019

To Do:

1. make 2% hemoglobin
2. preform a mini prep for Hannah
3. streak LVS and $\Delta rpsu$ 1- $\Delta rpsu$ 3 for MIC

Tuesday, September 24th, 2019

To Do:

1. perform an MIC on streptomycin with LVS and $\Delta rpsu$ 1- $\Delta rpsu$ 3

Results and Methods:

Plate was incubated at 2:25 PM.

Measuring OD600 for LVS:

OD600 LVS: .215 A

$$C1V1 = C2V2 \quad 10.75 * V1 = .005 * 20,000$$

$$V1 = 9.30 \mu\text{L}$$

Measuring OD600 for LVS:

OD600 $\Delta rpsu1$ - $\Delta rpsu3$: .213 A

$$C1V1 = C2V2 \quad 10.65 * V1 = .005 * 20,000$$

$$V1 = 9.39 \mu\text{L}$$

Wednesday, September 25th, 2019

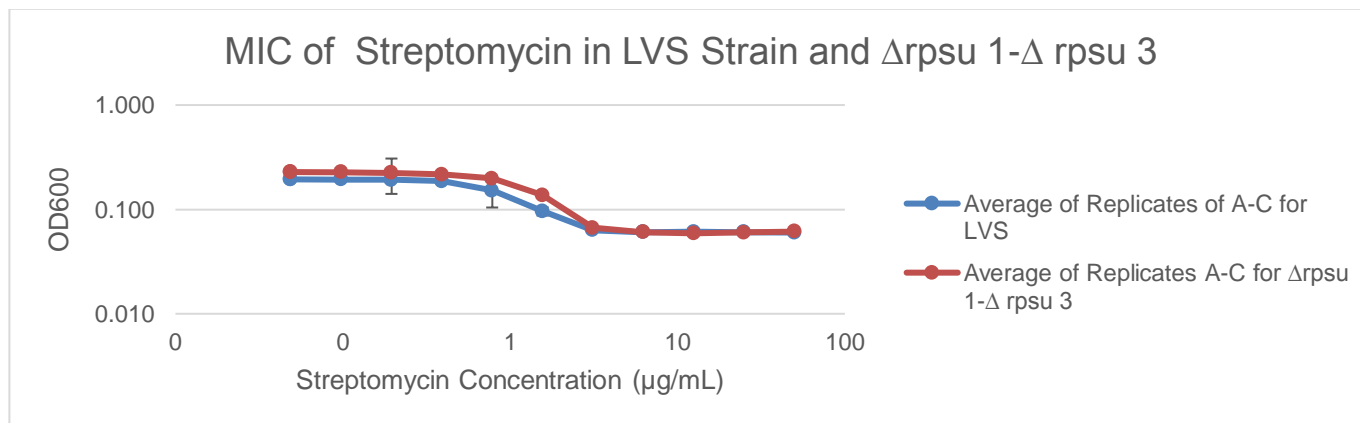
To Do:

1. read MIC results
2. streak LVS and $\Delta rpsu1$ - $\Delta rpsu$ 3
3. make streptomycin dilutions
4. refill tip boxes

Results and Methods:

Plate was removed from the incubator at 12:50 AM.

MIC graph: [./MIC results /LVS and Δrpsu1-Δrpsu3/190925_TA_strep/190925_TA_MIC.xlsx](#)



Streptomycin Conc. (μg/mL)	Avg OD600 of LVS	Avg OD600 of Δrpsu1-Δrpsu3
50	0.060	0.061
25.0	0.060	0.060
12.5	0.061	0.059
6.25	0.061	0.061
3.13	0.063	0.067
1.56	0.096	0.137
0.78	0.152	0.199
0.39	0.186	0.217
0.20	0.192	0.224
0.10	0.193	0.228
0.05	0.194	0.228
0.00	0.192	0.232

MIC of strep in LVS: 6.25 μg/mL

MIC of strep in Δrpsu1-Δrpsu 3: 6.25 μg/mL

Thursday, September 26th, 2019

To Do:

1. perform an MIC on LVS and Δrpsu1-Δrpsu 3 with streptomycin
2. make hemoglobin
3. autoclave trash

Results and Methods:

Plate was incubated at 4:27 PM

Measuring OD600 for LVS:

OD600 LVS: .261 A

C1V1=C2V2

13.05*V1=.005*20,000

$$V1 = 7.66 \mu\text{L}$$

Measuring OD600 for LVS:

$$\text{OD600 } \Delta\text{rpsu1-}\Delta\text{rpsu3: } .257 \text{ A}$$

$$C1V1 = C2V2 \quad 12.85 * V1 = .005 * 20,000$$

$$V1 = 7.78 \mu\text{L}$$

Friday, September 27th, 2019

To Do:

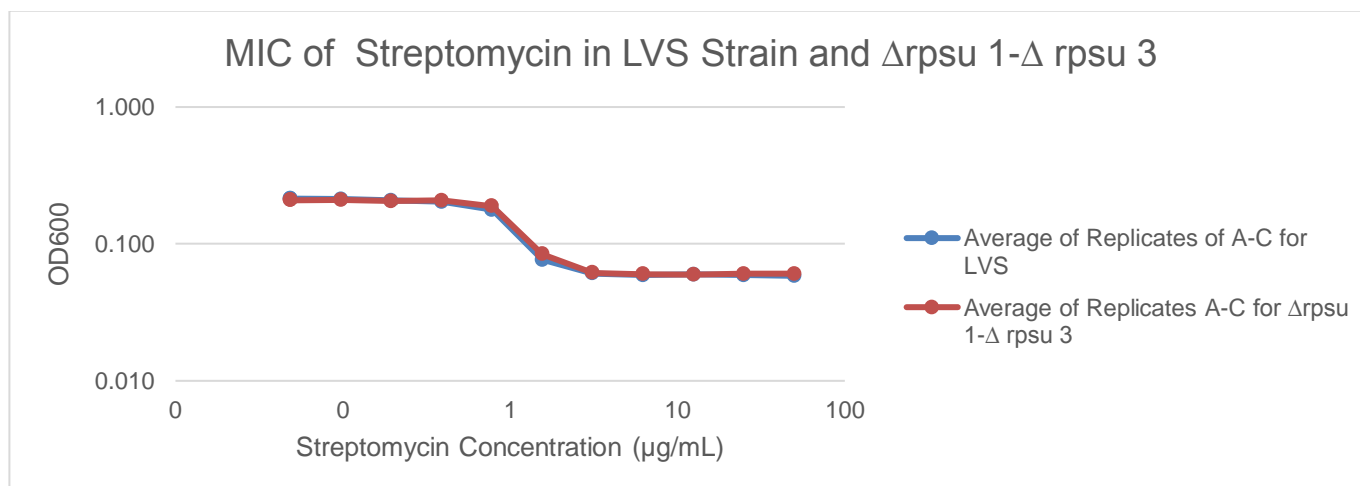
1. Read MIC Results
2. Make hemoglobin

Results and Methods:

Plate was removed from the incubator at 3:50 PM

**** Plate should have been removed at 2:50 PM but no change in results was detected****

MIC graph: [../MIC results /LVS and Δrpsu1-Δrpsu3/190927_TA_strep/190927_TA_MIC.xlsx](#)



Streptomycin Conc. (μg/mL)	Avg OD600 of LVS	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu3}$
50	0.059	0.060
25.0	0.059	0.061
12.5	0.060	0.060
6.25	0.059	0.060
3.13	0.061	0.062
1.56	0.077	0.084
0.78	0.178	0.189
0.39	0.203	0.208
0.20	0.208	0.205
0.10	0.213	0.210
0.05	0.214	0.208
0.00	0.226	0.212

MIC of strep in LVS: 3.13 $\mu\text{g/mL}$

MIC of strep in $\Delta\text{rpsu1-}\Delta\text{rpsu 3}$: 3.13 $\mu\text{g/mL}$

Monday, September 30th, 2019

To Do:

1. Make iron pyrophosphate
2. Streak LVS and $\Delta\text{rpsu 1-}\Delta\text{rpsu 3}$ for MIC with streptomycin

Results and Methods:

MHB was contaminated so couldn't do antibiotic dilutions

Bibliography

Ramsey, K. M. and Dove, S. L. (2016) ' A response regulator promotes *Francisella tularensis* intramacrophage growth by repressing an anti-virulence factor ', *Molecular Microbiology*. doi: 10.1111/mmi.13418.