

## Table of Contents

October 2019 .....	3
Tuesday, October 1st, 2019 .....	3
Wednesday, October 2nd, 2019 .....	3
Friday, October 4th, 2019 .....	5
Monday, October 7th, 2019 .....	7
Tuesday, October 8th, 2019 .....	7
Friday, October 11th, 2019 .....	8
Tuesday, October 15th, 2019 .....	10
Wednesday, October 16th, 2019 .....	10
MIC Protocol for Culture tubes (two strain): .....	10
Friday, October 18th, 2019 .....	11
Monday, October 21st, 2019 .....	11
Tuesday, October 22nd, 2019 .....	11
Wednesday, October 23rd, 2019 .....	11
Thursday, October 24th, 2019 .....	12
Friday, October 25th, 2019 .....	13
Monday, October 28th, 2019 .....	13
Tuesday, October 29th, 2019 .....	14
Wednesday, October 30th, 2019 .....	15
Thursday, October 31st, 2019 .....	15
November 2019 .....	16
Monday, November 4th, 2019 .....	16
Tuesday, November 5th, 2019 .....	16
Wednesday, November 6th, 2019 .....	17
Thursday, November 7th, 2019 .....	17
Tuesday, November 12th, 2019 .....	17
Wednesday, November 13th, 2019 .....	17
Thursday, November 14th, 2019 .....	18
Friday, November 15th, 2019 .....	19
Monday, November 18th, 2019 .....	19
Tuesday, November 19th, 2019 .....	19

Wednesday, November 20 <sup>th</sup> , 2019 .....	20
Thursday, November 21 <sup>th</sup> , 2019 .....	21
Friday, November 22 <sup>th</sup> , 2019 .....	21
December 2019 .....	22
Monday, December 2 <sup>nd</sup> , 2019 .....	22
Wednesday, December 4 <sup>th</sup> , 2019 .....	22
Thursday, December 5 <sup>th</sup> , 2019 .....	23
Friday, December 6 <sup>th</sup> , 2019 .....	24
Monday, December 9 <sup>th</sup> , 2019 .....	24
Tuesday, December 17 <sup>th</sup> , 2019 .....	25
Monday, December 23 <sup>rd</sup> , 2019 .....	25
Tuesday, December 24 <sup>th</sup> , 2019 .....	26
Wednesday, December 25 <sup>th</sup> , 2019 .....	26
Wednesday, December 26 <sup>th</sup> , 2019 .....	27
Sunday, December 29 <sup>th</sup> , 2019 .....	28
Monday, December 30 <sup>th</sup> , 2019 .....	28
Tuesday, December 31 <sup>st</sup> , 2019 .....	31
January 2020 .....	32
Wednesday, January 1 <sup>st</sup> , 2020 .....	32
Bibliography .....	35

## October 2019

### Tuesday, October 1st, 2019

**To Do:**

1. Perform an MIC on LVS and  $\Delta$ rpsu 1- $\Delta$ rpsu 3 with streptomycin
2. Streak LVS and  $\Delta$ rpsu 1- $\Delta$ rpsu2
3. Fill tip boxes

**Results and Methods:**

Plate was incubated at 9:05 AM.

Streptomycin:

Highest Conc. in the Wells ( $\mu\text{g/mL}$ )	Starting Conc. in Stock Tube A ( $\mu\text{g/mL}$ )			
50	1000			
Working Stock Conc. ( $\mu\text{g/mL}$ )	Antibiotic Source Conc. ( $\mu\text{g/mL}$ )	Total Volume of Working Stock ( $\mu\text{L}$ )	Volume of Antibiotic ( $\mu\text{L}$ )	Volume of MHB ( $\mu\text{L}$ )
2000	50,000	110	4.4	105.6

Measuring OD600 for LVS:

OD600 LVS: .220 A

$$C1V1 = C2V2 \quad 11 * V1 = .005 * 20,000$$

$$V1 = 9.09 \mu\text{L}$$

Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 3:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu3: .257 A

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

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

I streaked LVS and the new double mutant on regular plates. I will wait for Hannah to confirm with me which  $\Delta$ rpsu1- $\Delta$ rpsu 2 plate to use.

### Wednesday, October 2nd, 2019

**To Do:**

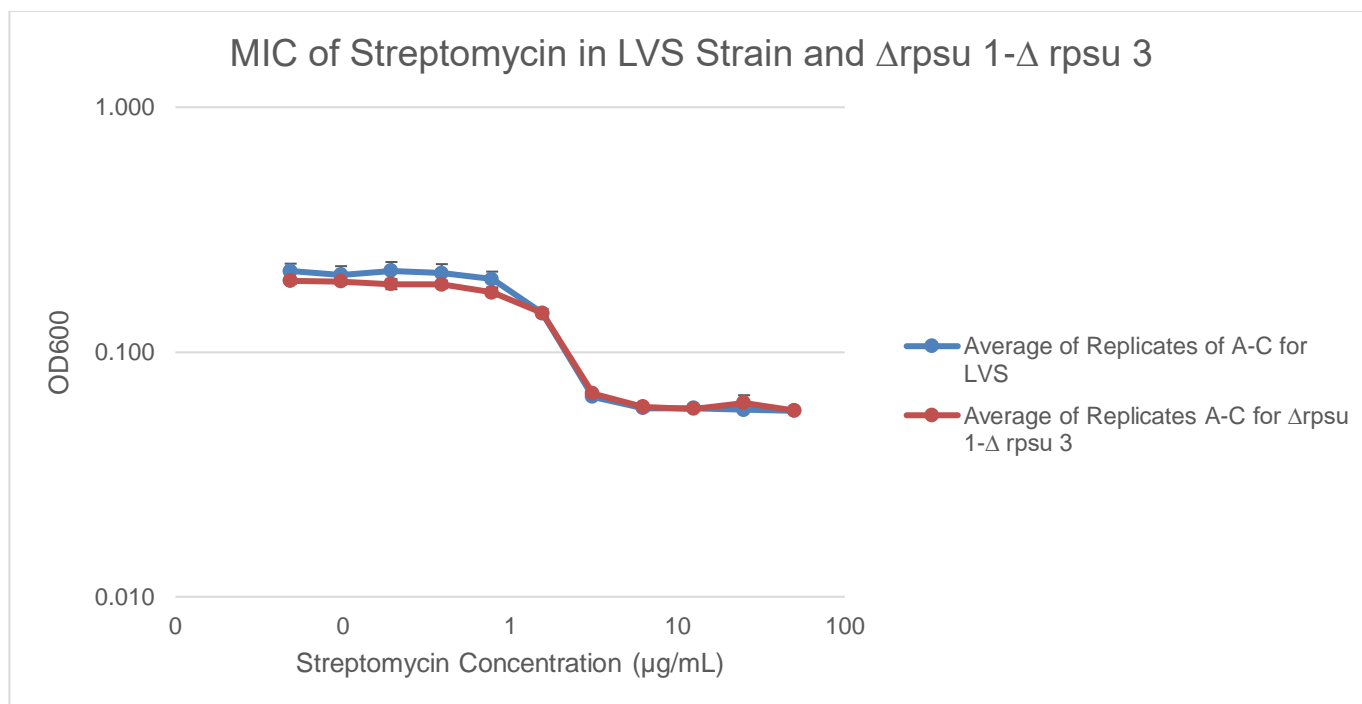
1. Perform an MIC on LVS and  $\Delta$ rpsu 1- $\Delta$ rpsu 2 with kanamycin and hygromycin
2. Read MIC results from yesterday
3. Make hemoglobin

**Results and Methods:**

Plate was removed from the incubator at 8:00 AM.

\*\* Plate should have been removed at 7:30 AM\*\*

MIC graph: [MIC results /LVS and Δrpsu1-Δrpsu3/191002\\_TA\\_Strep/191002\\_TA\\_MIC.xlsx](#)



Streptomycin Conc. ( $\mu\text{g/mL}$ )	Avg OD600 of LVS	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu 3
50	.058	.058
25	.058	.062
12.5	.059	.059
6.25	.059	.060
3.13	.066	.068
1.56	.144	.144
0.78	.198	.175
0.39	.210	.189
2.0E-01	.214	.190
9.8E-02	.207	.194
4.9E-02	.214	.195
0	.224	.205

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

MIC of strep in  $\Delta$ rpsu1- $\Delta$ rpsu3: 6.25  $\mu\text{g/mL}$

MIC of kan and hygromycin in LVS and  $\Delta$ rpsu1- $\Delta$ rpsu 2:

Plates were incubated at 12:25 PM

Kanamycin:

Highest Conc. in the Wells (µg/mL)	Starting Conc. in Stock Tube A (µg/mL)			
200	4000			
Working Stock Conc. (µg/mL)	Antibiotic Source Conc. (µg/mL)	Total Volume of Working Stock (µL)	Volume of Antibiotic (µL)	Volume of MHB (µL)
8000	50,000	110	17.6	92.4

Hygromycin:

Highest Conc. in the Wells (µg/mL)	Starting Conc. in Stock Tube A (µg/mL)			
675	13500			
Working Stock Conc. (µg/mL)	Antibiotic Source Conc. (µg/mL)	Total Volume of Working Stock (µL)	Volume of Antibiotic (µL)	Volume of MHB (µL)
27000	54,000	110	55.0	55.0

Measuring OD600 for LVS:

OD600 LVS: .217 A

$$C_1V_1 = C_2V_2 \quad 10.85 \cdot V_1 = .005 \cdot 20,000$$

$$V_1 = 9.22 \mu\text{L}$$

Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 2:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu2: .192 A

$$C_1V_1 = C_2V_2 \quad 9.6 \cdot V_1 = .005 \cdot 20,000$$

$$V_1 = 10.42 \mu\text{L}$$

**Friday, October 4<sup>th</sup>, 2019**

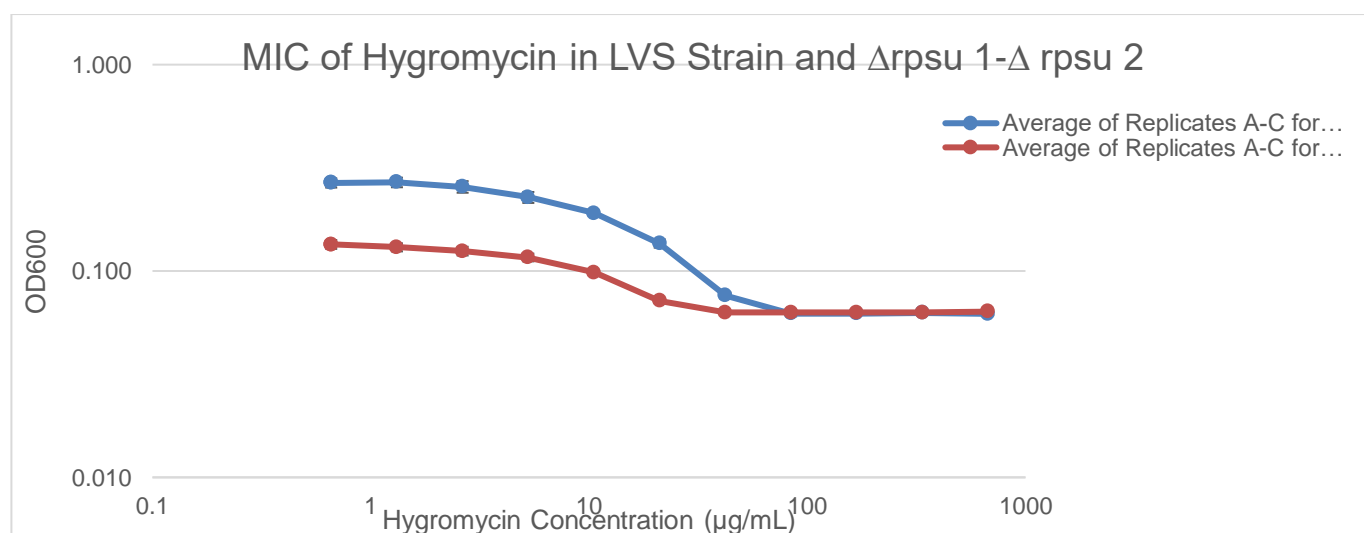
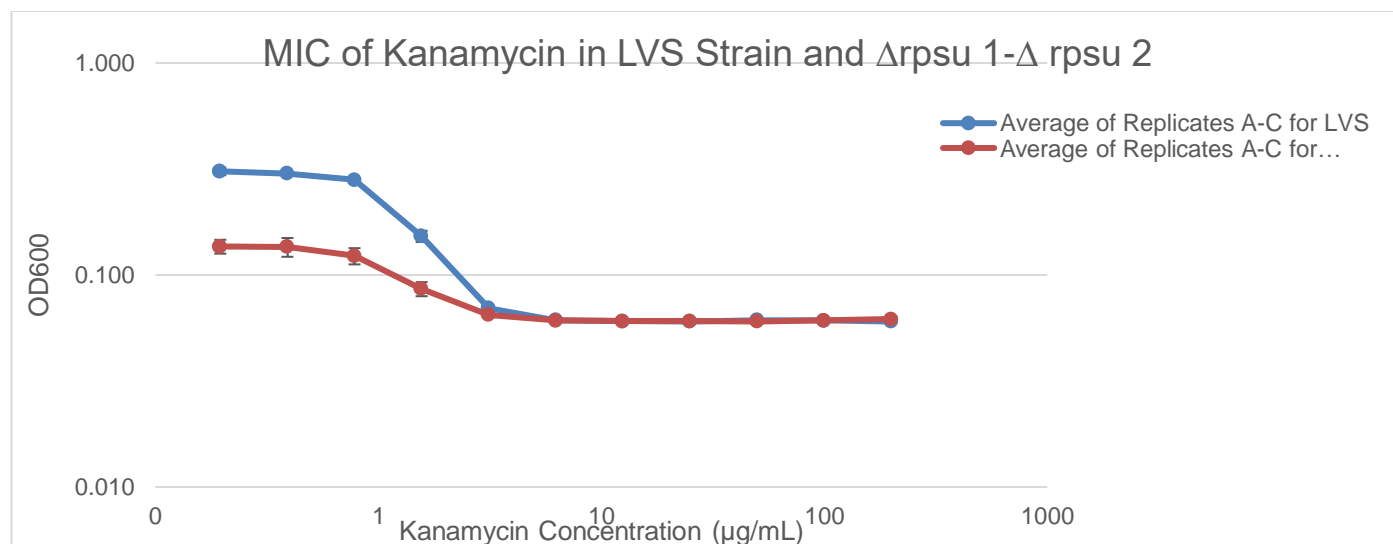
To Do:

1. Read MIC results for LVS and  $\Delta$ rpsu 1- $\Delta$ rpsu 2

**Results and Methods:**

Plates were removed from the incubator at 11AM

MIC graph: [MIC results /LVS and Δrpsu 1-Δrpsu2/191004\\_TA\\_MIC\\_Kan\\_hygro/191004\\_TA\\_MIC.xlsx](#)



Kanamycin Conc. ( $\mu$ g/mL)	Avg OD600 of LVS	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu 2
200	0.060	0.062
100.0	0.061	0.061
50.0	0.061	0.060
25.0	0.060	0.061
12.5	0.061	0.061
6.25	0.061	0.061
3.13	0.070	0.065
1.56	0.152	0.086
7.8E-01	0.281	0.123
3.9E-01	0.301	0.136
2.0E-01	0.308	0.136
0	0.314	0.146

Hygromycin Conc. ( $\mu$ g/mL)	Avg OD600 of LVS	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu 2
675	0.062	0.064

337.5	0.063	0.063
168.8	0.062	0.063
84.38	0.062	0.063
42.19	0.076	0.063
21.09	0.136	0.072
10.55	0.190	0.098
5.27	0.228	0.116
2.64	0.256	0.125
1.32	0.269	0.130
0.66	0.267	0.135
0.00	0.276	0.137

MIC of Kan in LVS: 6.25 µg/mL.

MIC of kan in  $\Delta$ rpsu1- $\Delta$ rpsu 2: 6.25 µg/mL

MIC of hygromycin in LVS: 84.38 µg/mL

MIC of hygromycin in  $\Delta$ rpsu 1- $\Delta$ rpsu 2: 42.19 µg/mL

### Monday, October 7<sup>th</sup>, 2019

#### To Do:

1. Streak LVS and  $\Delta$ rpsu1- $\Delta$ rpsu 2
2. Streak  $\Delta$ rpsu1- $\Delta$ rpsu 2 for single use aliquots
3. Make a 54 mg/mL hygromycin antibiotic stock
4. Prepare antibiotic dilutions for MIC

#### Results and Methods:

Making 54 mg/mL of hygromycin antibiotic stock with 1 g of supplied hygromycin: 1000 mg / 54mg/mL = 18.51 mL

Hygromycin is soluble in water so use molecular grade water then filter sterilize.

### Tuesday, October 8<sup>th</sup>, 2019

#### To Do:

1. Make Hemoglobin
2. Perform an MIC assay using kanamycin and hygromycin on LVS and  $\Delta$ rpsu1- $\Delta$ rpsu 2
3. Fill tip boxes

#### Results and Methods:

Plates were incubated at 1:12 PM. (incubate for three days)

$\Delta$ rpsu1- $\Delta$ rpsu 2 had some contamination in the agar on the bottom of the plate so won't be using to make single use aliquots.  
Kanamycin:

Highest Conc. in the Wells (µg/mL)	Starting Conc. in Stock Tube A (µg/mL)			
200	4000			
Working Stock Conc. (µg/mL)	Antibiotic Source Conc. (µg/mL)	Total Volume of Working Stock (µL)	Volume of Antibiotic (µL)	Volume of MHB (µL)
8000	50,000	110	17.6	92.4

Hygromycin:

Highest Conc. in the Wells (µg/mL)	Starting Conc. in Stock Tube A (µg/mL)			
675	13500			
Working Stock Conc. (µg/mL)	Antibiotic Source Conc. (µg/mL)	Total Volume of Working Stock (µL)	Volume of Antibiotic (µL)	Volume of MHB (µL)
27000	54,000	110	55.0	55.0

Measuring OD600 for LVS:

OD600 LVS: .244 A

$$C_1V_1 = C_2V_2 \quad 12.2 * V_1 = .005 * 20,000$$

$$V_1 = 8.19 \mu\text{L}$$

Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 2:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu2: .269 A

$$C_1V_1 = C_2V_2 \quad 13.45 * V_1 = .005 * 20,000$$

$$V_1 = 7.43 \mu\text{L}$$

**Friday, October 11<sup>th</sup>, 2019**

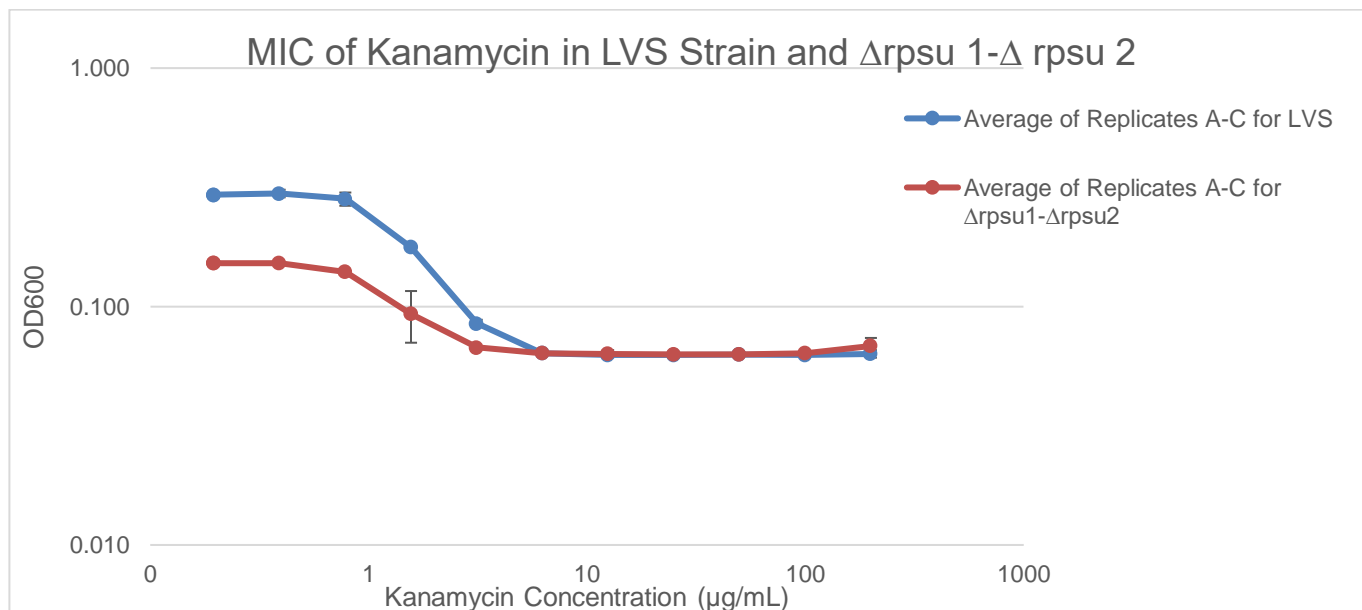
To Do:

1. Read MIC results

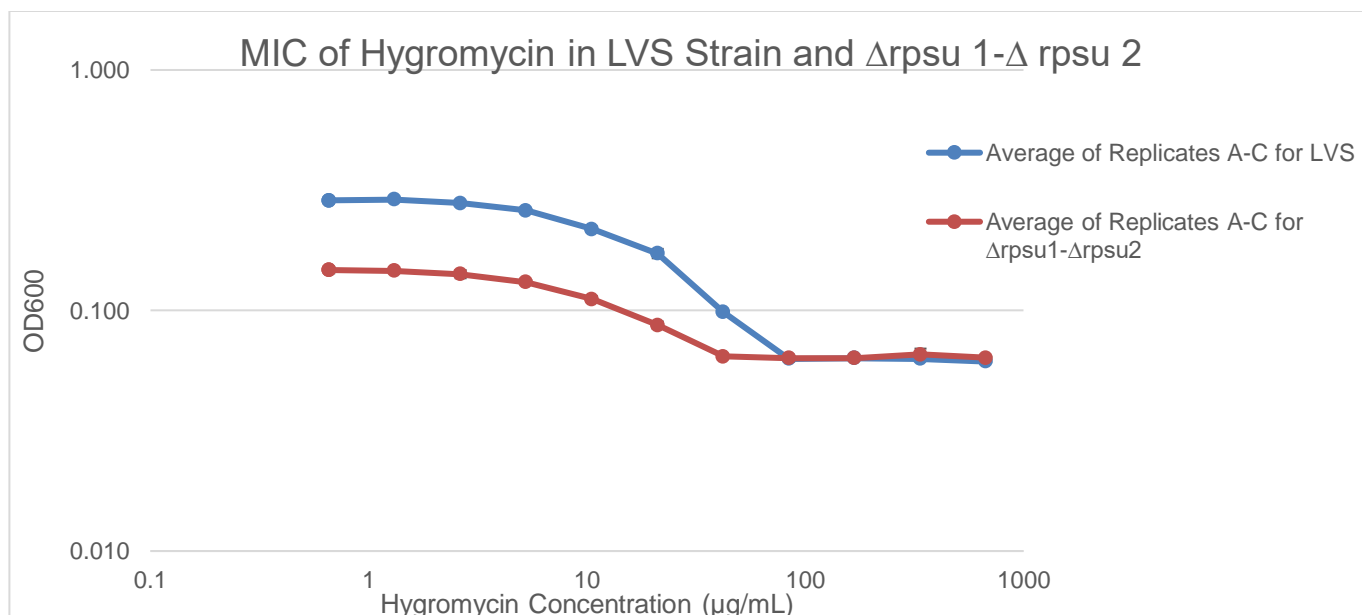
**Results and Methods:**

Plates were removed from the incubator at 11:30AM

MIC graphs: [MIC results /LVS and  \$\Delta\$ rpsu 1- \$\Delta\$ rpsu2/191011\\_TA\\_MIC\\_kan\\_hygro/191011\\_TA\\_MIC.xlsx](#)







Kanamycin Conc. ( $\mu$ g/mL)	Avg OD600 of LVS	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu 2
200	0.063	0.068
100.0	0.063	0.064
50.0	0.063	0.063
25.0	0.063	0.063
12.5	0.063	0.063
6.25	0.064	0.064
3.13	0.085	0.067
1.56	0.177	0.093
7.8E-01	0.283	0.140
3.9E-01	0.298	0.152
2.0E-01	0.294	0.152
0	0.279	0.153

Hygromycin Conc. ( $\mu$ g/mL)	Avg OD600 of LVS	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu 2
675	0.061	0.064
337.5	0.063	0.066
168.8	0.063	0.063
84.38	0.063	0.063
42.19	0.098	0.064
21.09	0.173	0.087
10.55	0.218	0.111
5.27	0.261	0.131
2.64	0.279	0.142
1.32	0.289	0.146
0.66	0.287	0.147
0.00	0.278	0.150

MIC of kan in LVS: 6.25 µg/mL.

MIC of kan in  $\Delta$ rpsu1- $\Delta$ rpsu 2: 6.25 µg/mL (seems to be between 6.25 and 3.13)

MIC of hygromycin in LVS: 84.38 µg/mL

MIC of hygromycin in  $\Delta$ rpsu 1- $\Delta$ rpsu 2: 42.19 µg/mL

## Tuesday, October 15<sup>th</sup>, 2019

### To Do:

1. Make hemoglobin
2. Streak LVS and  $\Delta$ rpsu1- $\Delta$ rpsu 2 for MIC
3. Fill tip boxes

## Wednesday, October 16<sup>th</sup>, 2019

### To Do:

1. work on new MIC protocol

### Results and Methods:

#### MIC Protocol for Culture tubes (two strain):

1. Create desired kanamycin concentration:
  - a. Make 200 µL of 5000 µg/mL of kanamycin by diluting 20 µL of kan in 180 µL of MHB.
  - b. Prepare antibiotic dilutions using the table below

Tube	Final Kanamycin concentration (µg/mL)	Final volume (µL)	Concentrated Kanamycin concentration	Final volume MHB in 2 mL tubes	MHB in tubes (- Kanamycin)	Stock Kanamycin concentration (ug/mL)	Volume stock Kanamycin to add (ul)
1	50	5000	1000	1250	1225.0	50000	25
2	12.5	5000	250	1250	1187.5	5000	62.5
3	6.25	5000	125	1250	1218.8	5000	31.25
4	3.13	5000	62.6	1250	1234.4	5000	15.65
5	1.56	5000	31.2	1250	1242.2	5000	7.8
6	0	5000	0			-	0

2. Preparing cell dilutions:
  - a. For each strain being tested:
    - i. Resuspend cells in 400 µL of MHB and measure OD600
    - ii. Prepare 100 mL of media (supplemented MHB) in a sterile flask
    - iii. Aim for an OD600 of 0.005. dilute the appropriate amount of culture in a 125 mL sterile flask that contains media to get required OD600.
3. Transfer 4.75 mL of LVS diluted culture into 11 test-tubes.
4. Transfer 4.75 mL of  $\Delta$ rpsu1- $\Delta$ rpsu2 diluted culture into 11 test-tubes.
5. Transfer 4.75 mL of each strain to two different test-tubes and add .25 mL of MHB. These will be the control.
6. Transfer 5 mL of MHB into 3 test-tubes. These will be the blank.
7. Transfer .25 mL from the antibiotic tubes to the corresponding culture tube and mix.
8. Place culture tubes in the shaking incubator overnight.
9. Using the aseptic technique, as you might have to incubate longer, transfer 150 µL from each culture tube to a 96-well plate. If no growth is detected incubate another night.

**Friday, October 18<sup>th</sup>, 2019****To Do:**

1. Make single use aliquots for  $\Delta$ rpsu1- $\Delta$ rpsu2

**Results and Methods:**

## Making aliquots:

1. Add 400  $\mu$ L of MHB to a 1.5 mL microcentrifuge tube
2. Scrape all the cells on the plate and resuspend
3. Add 400  $\mu$ L of more MHB (or amount needed to get to 800  $\mu$ L solution)
4. Add 200  $\mu$ L of 75% glycerol and mix by pipetting
5. Pipette 50  $\mu$ L of solution per microcentrifuge tube
6. Store in -80 freezer

\*\* Plate didn't have a good amount of growth, so I made the aliquots and tested one of the stocks by streaking a plate and leaving it at room temperature over the weekend\*\*

**Monday, October 21<sup>st</sup>, 2019****To Do:**

1. check the  $\Delta$ rpsu1- $\Delta$ rpsu2 plate for growth

**Results and Methods:**

plate had growth on it so I can use the single use stocks that were made.

**Tuesday, October 22<sup>nd</sup>, 2019****To Do:**

1. streak LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 for MIC with kanamycin following the culture tube protocol

**Wednesday, October 23<sup>rd</sup>, 2019****To Do:**

1. perform an MIC on LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 with kanamycin (culture tubes)
2. edit protocol
3. refill tip boxes

**Results and Methods:**

1. Create desired kanamycin concentration:
  - a. Make 200  $\mu$ L of 5000  $\mu$ g/mL of kanamycin by diluting 20  $\mu$ L of kan in 180  $\mu$ L of MHB.
  - b. Prepare antibiotic dilutions using the table below

Tube	Final Kanamycin concentration ( $\mu$ g/mL)	Final volume ( $\mu$ L)	Concentrated Kanamycin concentration	Final volume MHB in 2 mL tubes	MHB in tubes (- Kanamycin)	Stock Kanamycin concentration ( $\mu$ g/mL)	Volume stock Kanamycin to add ( $\mu$ L)
1	50	5000	1000	1250	1225.0	50000	25
2	12.5	5000	250	1250	1187.5	5000	62.5

3	6.25	5000	125	1250	1218.8	5000	31.25
4	3.13	5000	62.6	1250	1234.4	5000	15.65
5	1.56	5000	31.2	1250	1242.2	5000	7.8
6	0	5000	0			-	0

2. Preparing cell dilutions:
  - a. For each strain being tested:
    - i. Resuspend cells in 400  $\mu$ L of MHB and measure OD600
    - ii. Prepare 100 mL of media (supplemented MHB) in a sterile flask
    - iii. Aim for a final OD600 of 0.005. dilute the appropriate amount of culture in a 125 mL sterile flask that contains media to get required OD600.
3. Transfer 4.75 mL of LVS diluted culture into 12 test-tubes.
4. Transfer 4.75 mL of  $\Delta$ rpsu1- $\Delta$ rpsu2 diluted culture into 12 test-tubes.
5. Add .25 mL of MHB to the tubes with no antibiotic. These will be the control.
6. Transfer 5 mL of MHB into 3 test-tubes. These will be the blank.
7. Transfer .25 mL from the antibiotic tubes to the corresponding culture tube and mix.
8. Place culture tubes in the shaking incubator overnight.
9. Using the aseptic technique, as you might have to incubate longer, transfer 150  $\mu$ L from each culture tube to a 96-well plate. If no growth is detected incubate another night.

Measuring OD600 for LVS:

OD600 LVS: .449A

$$C1V1=C2V2 \quad 22.45 \cdot V1 = .0053 \cdot 100,000$$

$$V1 = 23.61 \mu\text{L}$$

Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 2:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu2: .306 A

$$C1V1=C2V2 \quad 15.30 \cdot V1 = .0053 \cdot 100,000$$

$$V1 = 34.64 \mu\text{L}$$

Culture tubes were incubated at 1:57 PM.

**Thursday, October 24<sup>th</sup>, 2019**

To Do:

1. Read OD600 of LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2

**Results and Methods:**

Reading was done at 4:10 PM

Kanamycin Conc. ( $\mu$ g/mL)	Avg OD600 of LVS	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu 2
50	0.062	0.063
12.5	0.066	0.064
6.25	0.067	0.064
3.13	0.081	0.065

1.56	0.125	0.066
0	0.236	0.067

MIC of Kan in LVS: 6.25 µg/mL.

MIC of kan in  $\Delta$ rpsu1- $\Delta$ rpsu 2: cannot be determined because no growth was observed

The culture tubes were placed back in the incubator

**Friday, October 25<sup>th</sup>, 2019**

To Do:

1. Read OD600 of LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 day 2

**Results and Methods:**

The culture tubes were removed from the incubator at 12:00 PM

Kanamycin Conc. (µg/mL)	Avg OD600 of LVS	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu 2
50	0.063	0.064
12.5	0.063	0.066
6.25	0.068	0.067
3.13	0.088	0.069
1.56	0.165	0.070
0	0.570	0.075

MIC of Kan in LVS: 12.5 µg/mL

MIC of kan in  $\Delta$ rpsu1- $\Delta$ rpsu 2: cannot be determined because no growth was observed

I will try doing this protocol with higher cell density.

**Monday, October 28<sup>th</sup>, 2019**

To Do:

1. Streak cells for an MIC
2. Prepare antibiotic dilutions
3. Make hemoglobin
4. Lab presentation

**Results and Methods:**

Antibiotic dilutions were made according to this table

Tube	Final Kanamycin concentration (µg/mL)	Final volume (µL)	Concentrated Kanamycin concentration	Final volume MHB in 2 mL tubes	MHB in tubes (- Kanamycin)	Stock Kanamycin concentration (ug/mL)	Volume stock Kanamycin to add (ul)
1	50	5000	1000	1250	1225.0	50000	25
2	12.5	5000	250	1250	1187.5	5000	62.5
3	6.25	5000	125	1250	1218.8	5000	31.25

4	3.13	5000	62.6	1250	1234.4	5000	15.65
5	1.56	5000	31.2	1250	1242.2	5000	7.8
6	0	5000	0	1250	1250	-	0

After talking to Dr. Ramsey, we have decided to wait on the MIC and conduct an assay with different cell concentrations to determine the best starting OD600 for  $\Delta rpsu1$ - $\Delta rpsu2$ . The antibiotic dilutions will remain in the fridge.

**Tuesday, October 29<sup>th</sup>, 2019**

**To Do:**

1. Inoculate cells with different OD600
2. Refill tip boxes
3. Make hemoglobin

**Results and Methods:**

Measuring OD600 for  $\Delta rpsu1$ - $\Delta rpsu2$ : (stock) 980  $\mu$ L MHB and 20  $\mu$ L of cells (1:50)

OD600  $\Delta rpsu1$ - $\Delta rpsu2$ : .136 A

Desired OD600 0.1:  $C1V1=C2V2$   $6.8 \times V1 = .1 \times 5000$

$V1 = 73.5 \mu$ L

Desired OD600 0.05:  $C1V1=C2V2$   $6.8 \times V1 = .05 \times 5000$

$V1 = 36.8 \mu$ L

Measuring OD600 for  $\Delta rpsu1$ - $\Delta rpsu2$ : (1:10 from stock) 400  $\mu$ L MHB and 100  $\mu$ L of cells (1:5)

OD600  $\Delta rpsu1$ - $\Delta rpsu2$ : .136 A

Desired OD600 .005:  $C1V1=C2V2$   $.68 \times V1 = .1 \times 5000$

$V1 = 36.8 \mu$ L

Desired OD600 .01:  $C1V1=C2V2$   $.68 \times V1 = .01 \times 5000$

$V1 = 73.5 \mu$ L

Measuring OD600 for LVS: (1:10 from stock) 400  $\mu$ L MHB and 100  $\mu$ L of cells (1:5)

OD600 LVS: .167 A

Desired OD600 .005:  $C1V1=C2V2$   $.835 \times V1 = .1 \times 5000$

$V1 = 29.9 \mu$ L

One of the culture tubes is blank with just 5 mL of MHB

**Culture tubes were incubated at 4:05 PM**

**Wednesday, October 30<sup>th</sup>, 2019****To Do:**

1. Read OD600 for LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 with different starting OD600 day 1

**Results and Methods:**

Strain	Initial OD600	Final OD600 tube 1	Final OD600 tube 2
LVS	.005	.264	.253
$\Delta$ rpsu1- $\Delta$ rpsu2	.005	.126	.088
$\Delta$ rpsu1- $\Delta$ rpsu2	.01	.111	.104
$\Delta$ rpsu1- $\Delta$ rpsu2	.05	.198	.197
$\Delta$ rpsu1- $\Delta$ rpsu2	.1	.252	.262

Control (MHB only) OD600: .055

I reread  $\Delta$ rpsu1- $\Delta$ rpsu2 OD600 .005 in order to plate the possibly contaminated tube but got different readings twice

	Tube 1	Tube 2
Reading 2	0.097	0.094
Reading 3	0.099	0.092

I also used the spectrophotometer and got 0.120 for tube 1 and .105 for tube 2

OD600 reading was done at 10:30 AM

**Thursday, October 31<sup>th</sup>, 2019****To Do:**

1. Read OD600 for LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 with different starting OD600 day 2

**Results and Methods:**

OD600 reading was done at 3:15 PM

**Reading done by plate reader**

Strain	Initial OD600	Final OD600 tube 1	Final OD600 tube 2
LVS	.005	.584	.578
$\Delta$ rpsu1- $\Delta$ rpsu2	.005	.175	.159
$\Delta$ rpsu1- $\Delta$ rpsu2	.01	.159	.173
$\Delta$ rpsu1- $\Delta$ rpsu2	.05	.228	.228
$\Delta$ rpsu1- $\Delta$ rpsu2	.1	.277	.264

Control (MHB only) OD600: .067

**Reading done by spectrophotometer**

Strain	Initial OD600	Final OD600 tube 1	Final OD600 tube 2
LVS	.005	1.154	1.185
$\Delta$ rpsu1- $\Delta$ rpsu2	.005	.314	.285
$\Delta$ rpsu1- $\Delta$ rpsu2	.01	.806	.784
$\Delta$ rpsu1- $\Delta$ rpsu2	.05	.466	.472
$\Delta$ rpsu1- $\Delta$ rpsu2	.1	.277	.310

## November 2019

Monday, November 4<sup>th</sup>, 2019

## To Do:

1. Streak cells
2. Try to figure out what went wrong with the readings on Thursday

## Results and Methods:

Hannah and I tried to figure out what went wrong. I scraped up some cells from a random plate in lab and resuspended cells in 400 mL of MHB. I first used the spec to read OD600 and got .238 (1:50 dilution). I read the same cuvette in Dr. Matthew Ramsey's lab and got .233. I added 500 mL of MHB to another cuvette with the same reading and got a .120 reading.

We used the 96 well plate reader to read the results and I transferred 150  $\mu$ L from the control and LVS cuvettes and got an OD600 reading of .051 and .134 respectively.

I have met with Dr. Ramsey and she informed me that the difference between the readings is due to the different light path between the two devices. We will aim for a starting OD600 of 0.005 for LVS and a starting OD600 of 0.01 for  $\Delta$ rpsu1- $\Delta$ rpsu2.

Tuesday, November 5<sup>th</sup>, 2019

## To Do:

1. Perform an MIC on LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 with kanamycin
2. Make hemoglobin

## Results and Methods:

Antibiotic dilutions were made according to this table

Tube	Final Kanamycin concentration ( $\mu$ g/mL)	Final volume ( $\mu$ L)	Concentrated Kanamycin concentration	Final volume MHB in 2 mL tubes	MHB in tubes (- Kanamycin)	Stock Kanamycin concentration ( $\mu$ g/mL)	Volume stock Kanamycin to add ( $\mu$ L)
1	50	5000	1000	1250	1225.0	50000	25
2	12.5	5000	250	1250	1187.5	5000	62.5
3	6.25	5000	125	1250	1218.8	5000	31.25
4	3.13	5000	62.6	1250	1234.4	5000	15.65
5	1.56	5000	31.2	1250	1242.2	5000	7.8
6	0	5000	0	1250	1250	-	0

Measuring OD600 for LVS:

OD600 LVS: .289A

$C_1V_1 = C_2V_2$

$1.445 \cdot V_1 = .0053 \cdot 100,000$

$V_1 = 366.8 \mu\text{L}$



Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 2:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu2: .281 A

$$C1V1=C2V2 \quad 1.405*V1=.0105*100,000$$

$$V1=747.3 \mu\text{L}$$

Culture tubes were incubated at 1:30 PM

**Wednesday, November 6<sup>th</sup>, 2019**

To Do:

1. Read MIC results day 1

Results and Methods:

Reading was done at 11:00 am

Kanamycin Conc. ( $\mu\text{g/mL}$ )	Avg OD600 of LVS (starting OD600 .005)	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu2 (starting OD600 0.01)
50	0.061	0.060
12.5	0.062	0.063
6.25	0.062	0.065
3.13	0.073	0.072
1.56	0.169	0.090
0	0.323	0.108

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

MIC of kan in  $\Delta$ rpsu1- $\Delta$ rpsu 2: 6.25  $\mu\text{g/mL}$

**Thursday, November 7<sup>th</sup>, 2019**

To Do:

1. Read MIC results day 2
2. Refill tip boxes

Results and Methods:

Culture tubes were removed from the incubator at 3:10 PM

Most of the culture tubes were contaminated including the two blank tubes so the OD600 was not measured.

This MIC will be repeated again next week.

**Tuesday, November 12<sup>th</sup>, 2019**

To Do:

1. Streak plates for MIC
2. Refill tip boxes

**Wednesday, November 13<sup>th</sup>, 2019**

To Do:

1. Perform an MIC on LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 with kanamycin

### Results and Methods:

Antibiotic dilutions were made according to this table

Tube	Final Kanamycin concentration (µg/mL)	Final volume (µL)	Concentrated Kanamycin concentration	Final volume MHB in 2 mL tubes	MHB in tubes (- Kanamycin)	Stock Kanamycin concentration (ug/mL)	Volume stock Kanamycin to add (ul)
1	50	5000	1000	1250	1225.0	50000	25
2	12.5	5000	250	1250	1187.5	5000	62.5
3	6.25	5000	125	1250	1218.8	5000	31.25
4	3.13	5000	62.6	1250	1234.4	5000	15.65
5	1.56	5000	31.2	1250	1242.2	5000	7.8
6	0	5000	0	1250	1250	-	0

Measuring OD600 for LVS:

OD600 LVS: .298A

$$C_1V_1 = C_2V_2 \quad 1.49 \cdot V_1 = .0053 \cdot 100,000$$

$$V_1 = 355.7 \mu\text{L}$$

Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 2:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu2: .352 A

$$C_1V_1 = C_2V_2 \quad 1.76 \cdot V_1 = .0105 \cdot 100,000$$

$$V_1 = 596.6 \mu\text{L}$$

Culture tubes were incubated at 11:55 AM

\*\*100 mL was way more than what I actually needed so I will start making only 70 mL\*\*

**Thursday, November 14th, 2019**

To Do:

1. Read MIC results day 1
2. Refill tip boxes

### Results and Methods:

At 7:45 AM, the culture tubes were contaminated and the two blank MHB tubes had a huge amount of growth. I will repeat this experiment again next week.

**Friday, November 15<sup>th</sup>, 2019****To Do:**

1. Make hemoglobin

**Monday, November 18<sup>th</sup>, 2019****To Do:**

1. Streak cells for MIC
2. Prepare kanamycin dilutions for MIC
3. Make hemoglobin

**Results and Methods:**

Antibiotic dilutions were made according to this table

Tube	Final Kanamycin concentration (µg/mL)	Final volume (µL)	Concentrated Kanamycin concentration	Final volume MHB in 2 mL tubes	MHB in tubes (- Kanamycin)	Stock Kanamycin concentration (ug/mL)	Volume stock Kanamycin to add (ul)
1	50	5000	1000	1250	1225.0	50000	25
2	12.5	5000	250	1250	1187.5	5000	62.5
3	6.25	5000	125	1250	1218.8	5000	31.25
4	3.13	5000	62.6	1250	1234.4	5000	15.65
5	1.56	5000	31.2	1250	1242.2	5000	7.8
6	0	5000	0	1250	1250	-	0

**Tuesday, November 19<sup>th</sup>, 2019****To Do:**

1. Perform an MIC on LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 using kanamycin
2. Refill tip boxes

**Results and Methods: Test tube codes:**

1	Kan 50 -1 LVS	2	Kan 50 -2 LVS
3	Kan 12.5 -1 LVS	4	Kan 12.5 -2 LVS
5	Kan 6.25 -1 LVS	6	Kan 6.25 -2 LVS
7	Kan 3.13 -1 LVS	8	Kan 3.13 -2 LVS
9	Kan 1.56 -1 LVS	10	Kan 1.56 -2 LVS
11	Kan 0 -1 LVS	12	Kan 0 -2 LVS
13	Kan 50 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	14	Kan 50 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
15	Kan 12.5 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	16	Kan 12.5 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
17	Kan 6.25 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	18	Kan 6.25 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
19	Kan 3.13 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	20	Kan 3.13 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
21	Kan 1.56 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	22	Kan 1.56 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
23	Kan 0 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	24	Kan 0 -2 $\Delta$ rpsu1- $\Delta$ rpsu2

Measuring OD600 for LVS:

OD600 LVS: .366A

$$C1V1=C2V2 \quad 1.83*V1=.0053*70,000$$

$$V1= 202.7 \mu\text{L}$$

Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 2:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu2: .344 A

$$C1V1=C2V2 \quad 1.72*V1=.0105*70,000$$

$$V1= 427.3 \mu\text{L}$$

Culture tubes were incubated at 1:53 PM

**Wednesday, November 20<sup>th</sup>, 2019**

To Do:

1. Make hemoglobin
2. Read MIC results day 1

Results and Methods:

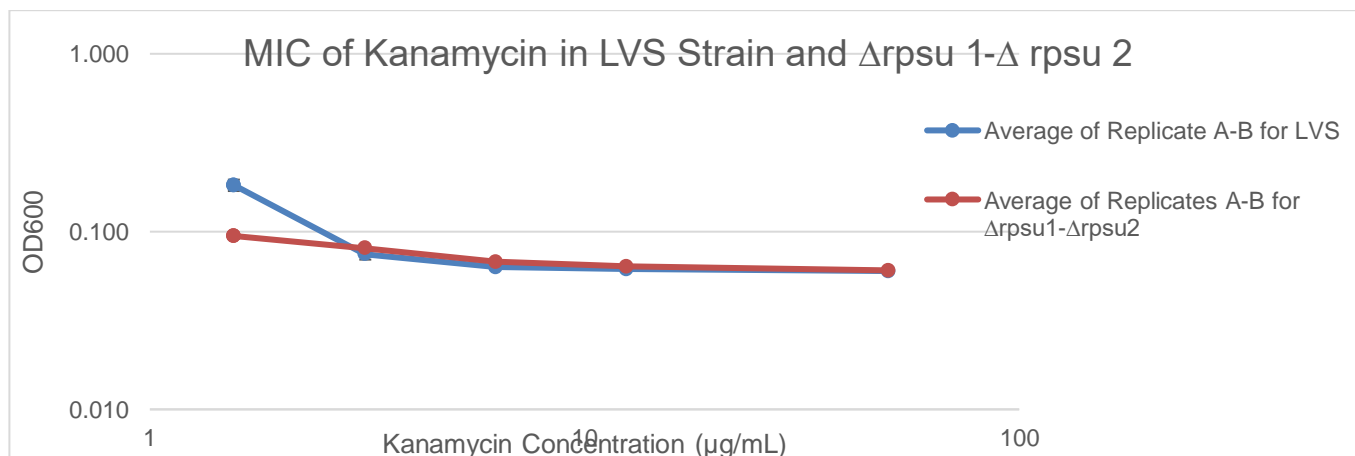
At 10:15 AM the  $\Delta$ rpsu1- $\Delta$ rpsu2 culture tubes had slight growth

Reading was done at 12:30PM.

Kanamycin Conc. ( $\mu\text{g/mL}$ )	Avg OD600 of LVS (starting OD600 .005)	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu2 (starting OD600 0.01)
50	0.060	0.061
12.5	0.062	0.064
6.25	0.063	0.068
3.13	0.075	0.081
1.56	0.183	0.095
0	0.377	0.109

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

MIC of kan in  $\Delta$ rpsu1- $\Delta$ rpsu 2: 6.25  $\mu\text{g/mL}$



**Thursday, November 21<sup>th</sup>, 2019**

To Do:

1. Read MIC results day 2

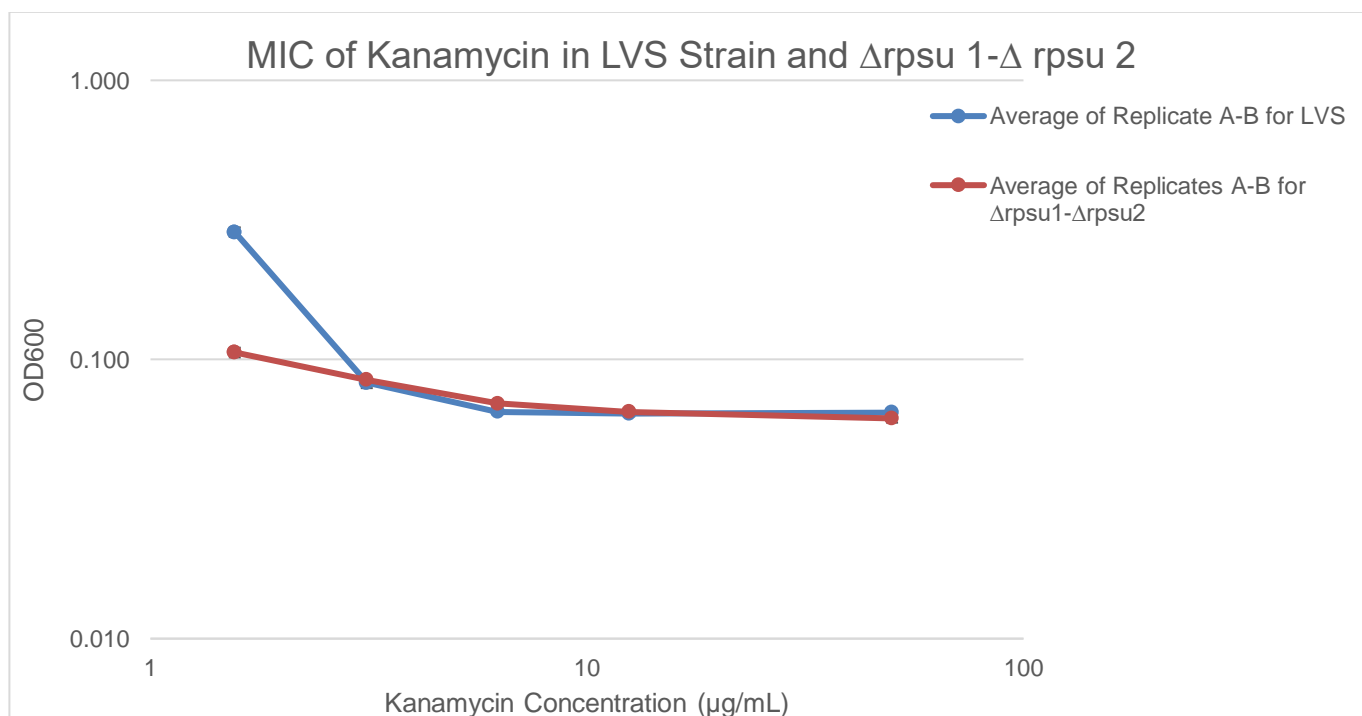
Results and Methods:

Reading was done at 2:20PM

Kanamycin Conc. ( $\mu\text{g/mL}$ )	Avg OD600 of LVS (starting OD600 .005)	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ (starting OD600 0.01)
50	0.065	0.062
12.5	0.064	0.065
6.25	0.065	0.070
3.13	0.083	0.085
1.56	0.286	0.106
0	0.590	0.129

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

MIC of kan in  $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ : 6.25  $\mu\text{g/mL}$



**Friday, November 22<sup>th</sup>, 2019**

To Do:

1. Bleach MIC tubes
2. Refill tip boxes

December 2019

**Monday, December 2<sup>nd</sup>, 2019****To Do:**

1. Streak cells for MIC (LVS and  $\Delta$ rpsu1-rpsu2)
2. Make iron pyrophosphate
3. Make hemoglobin
4. Make 50% sucrose
5. Prepare hygromycin antibiotic dilutions

**Results and Methods:**

Antibiotic dilutions will be made according to this table

Tube	Final hygromycin concentration ( $\mu$ g/mL)	Final volume ( $\mu$ L)	Concentrated hygromycin concentration	Final volume MHB in 2 mL tubes	MHB in tubes (- hygromycin)	Stock hygromycin concentration ( $\mu$ g/mL)	Volume stock hygromycin to add ( $\mu$ l)
1	337.5	5000	6750	1250	1093.8	54000	156.3
2	168.8	5000	3376	1250	1171.9	54000	78.1
3	84.38	5000	1687.6	1250	1210.9	54000	39.1
4	42.19	5000	843.8	1250	1230.5	54000	19.5
5	21.09	5000	421.8	1250	1240.2	54000	9.8
6	0	5000	0	1250	1250	-	0.0

I decided to wait on preparing the antibiotics so that I use the same MHB throughout the whole experiment.

**Wednesday, December 4<sup>th</sup>, 2019****To Do:**

1. Make hygromycin antibiotic dilutions
2. Supplement MHB
3. Perform an MIC on LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 using hygromycin
4. Refill tip boxes

**Results and Methods: Test tube codes:**

1	Hyg 337.5 -1 LVS	2	Hyg 337.5 -2 LVS
3	Hyg 168.8-1 LVS	4	Hyg 168.8-2 LVS
5	Hyg 84.38 -1 LVS	6	Hyg 84.38 -2 LVS
7	Hyg 42.19-1 LVS	8	Hyg 42.19 -2 LVS
9	Hyg 21.09-1 LVS	10	Hyg 21.09 -2 LVS
11	Hyg 0 -1 LVS	12	Hyg 0 -2 LVS
13	Hyg 337.5-1 $\Delta$ rpsu1- $\Delta$ rpsu2	14	Hyg 337.5-2 $\Delta$ rpsu1- $\Delta$ rpsu2
15	Hyg 168.8-1 $\Delta$ rpsu1- $\Delta$ rpsu2	16	Hyg 168.8 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
17	Hyg 84.38 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	18	Hyg 84.38 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
19	Hyg 42.19-1 $\Delta$ rpsu1- $\Delta$ rpsu2	20	Hyg 42.19 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
21	Hyg 21.09-1 $\Delta$ rpsu1- $\Delta$ rpsu2	22	Hyg 21.09-2 $\Delta$ rpsu1- $\Delta$ rpsu2
23	Hyg 0 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	24	Hyg 0 -2 $\Delta$ rpsu1- $\Delta$ rpsu2

Measuring OD600 for LVS:

OD600 LVS: .226 A

$$C1V1=C2V2 \quad 1.13*V1=.0053*70,000$$

$$V1= 328.3 \mu\text{L}$$

Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 2:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu2: .372 A

$$C1V1=C2V2 \quad 1.86*V1=.0105*70,000$$

$$V1= 395.2 \mu\text{L}$$

Culture tubes were incubated at 11:35 AM

Thursday, December 5<sup>th</sup>, 2019

To Do:

1. Make hemoglobin
2. Read MIC results day 1

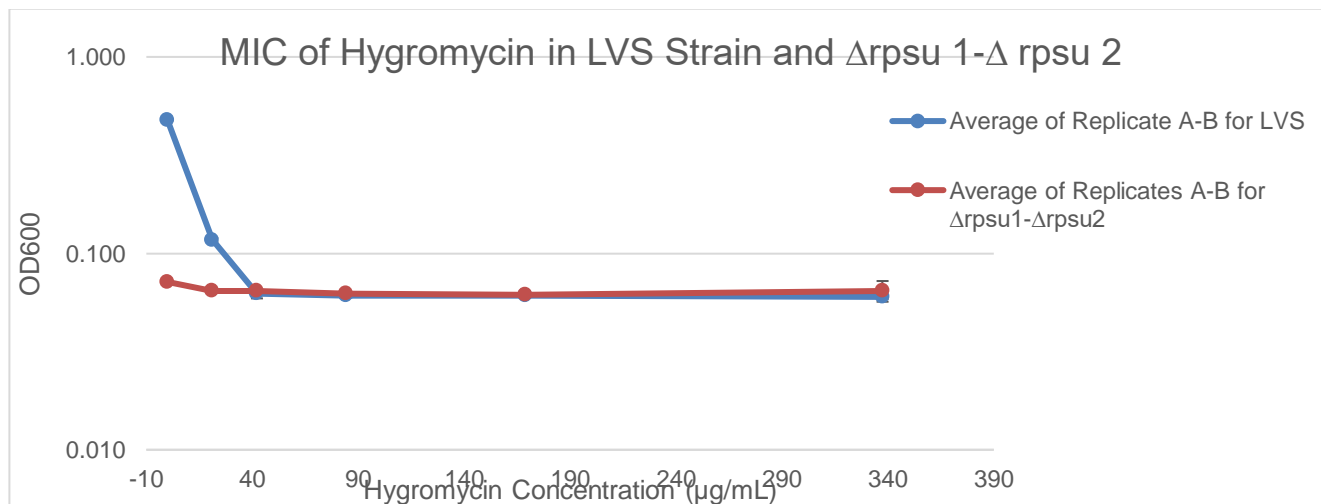
Results and Methods:

Reading was done at 2:30 PM

Hygromycin Conc. ( $\mu\text{g/mL}$ )	Avg OD600 of LVS (starting OD600 .005)	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu2 (starting OD600 0.01)
337.5	0.060	0.065
168.8	0.061	0.062
84.38	0.061	0.063
42.19	0.063	0.065
21.09	0.117	0.065
0	0.476	0.072

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

MIC of hyg in  $\Delta$ rpsu1- $\Delta$ rpsu 2: 21.09  $\mu\text{g/mL}$



The results obtained show difference in the MIC between the two strains but the MIC value is different from the one I got when I did the assay using a 96 well-plate.

**Friday, December 6<sup>th</sup>, 2019**

**To Do:**

1. Read MIC results day 2
2. Make hemoglobin for Jamie's mac assay and lab use

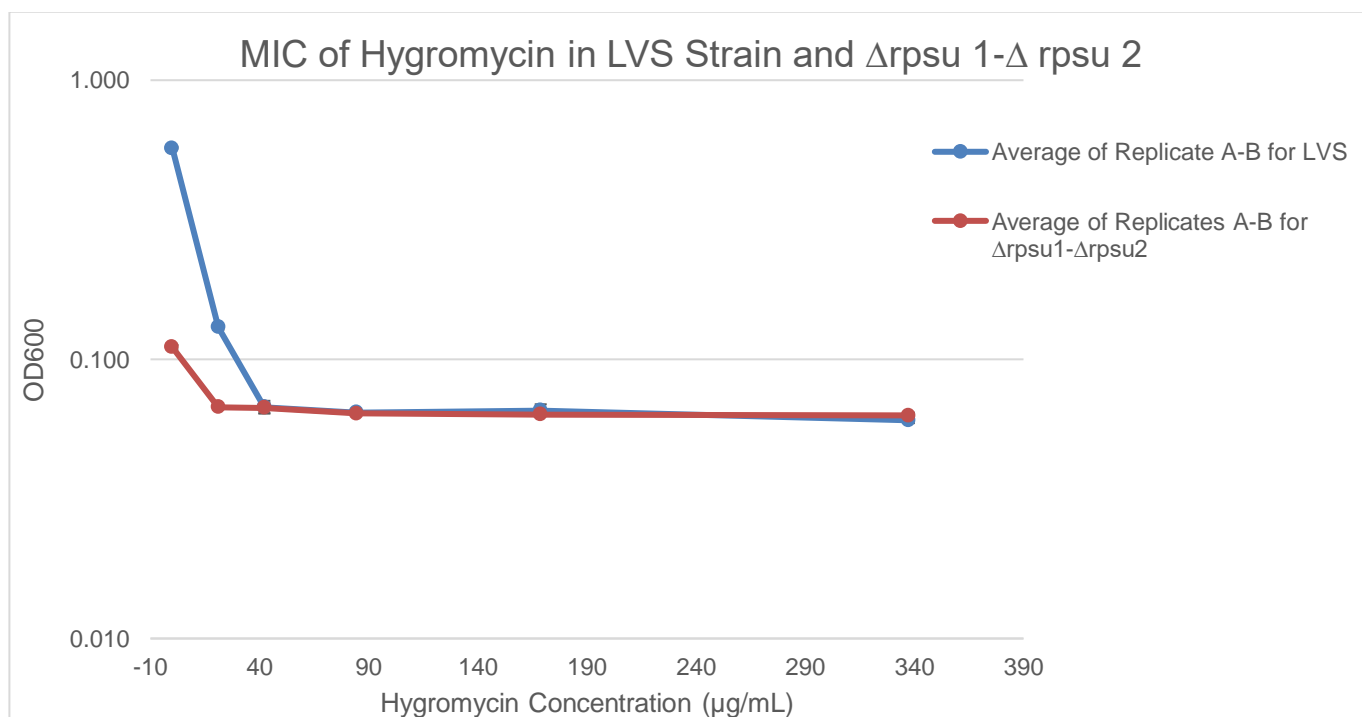
**Results and Methods:**

**Reading was done at 11:35 AM**

Hygromycin Conc. ( $\mu\text{g/mL}$ )	Avg OD600 of LVS (starting OD600 .005)	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ (starting OD600 0.01)
337.5	0.061	0.063
168.8	0.066	0.064
84.38	0.065	0.064
42.19	0.068	0.067
21.09	0.131	0.068
0	0.572	0.111

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

MIC of hyg in  $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ : 21.09  $\mu\text{g/mL}$



**I will repeat this assay after finals week.**

**Monday, December 9<sup>th</sup>, 2019**

**To Do:**

1. Meet with Dr. Ramsey



**Results and Methods:**

I will be starting the propolis assay after finals. To prep for this assay, I will conduct a disk diffusion assay using kanamycin.

I will first make disks from filter paper available in lab using a 3-whole puncher.

I will then sterilize these disks by placing them in a glass petri- dish in the autoclave.

We will be using kanamycin as a positive control.

**Preliminary Protocol:**

1. Prepare LVS dilution with starting OD600 of 0.05, 0.005, and 0.0005.
2. Pipette 100  $\mu$ L on regular plates evenly
3. Place kanamycin disks and grow cells
4. Measure inhibition zones

**Tuesday, December 17<sup>th</sup>, 2019****To Do:**

1. Refill tip boxes
2. Make hemoglobin for Dr. Ramsey

**Monday, December 23<sup>rd</sup>, 2019****To Do:**

1. Streak cells for MIC
2. Prepare hygromycin dilution
3. Cut out filter paper for disk diffusion

**Results and Methods:**

Antibiotic dilutions were made according to this table

Tube	Final hygromycin concentration ( $\mu$ g/mL)	Final volume ( $\mu$ L)	Concentrated hygromycin concentration	Final volume MHB in 2 mL tubes	MHB in tubes (- hygromycin)	Stock hygromycin concentration ( $\mu$ g/mL)	Volume stock hygromycin to add ( $\mu$ l)
1	168.8	5000	3376	1250	1171.9	54000	78.1
2	84.38	5000	1687.6	1250	1210.9	54000	39.1
3	42.19	5000	843.8	1250	1230.5	54000	19.5
4	21.09	5000	421.8	1250	1240.2	54000	9.8
5	10.55	5000	211	1250	1245.1	54000	4.9
6	0	5000	0	1250	1250	-	0.0

**Tuesday, December 24<sup>th</sup>, 2019**

To Do:

1. Make hemoglobin
2. Perform an MIC on LVS and  $\Delta$ rpsu1- $\Delta$ rpsu2 using hygromycin

**Results and Methods: Test tube codes:**

1	Hyg 168.8-1 LVS	2	Hyg 168.8-2 LVS
3	Hyg 84.38 -1 LVS	4	Hyg 84.38 -2 LVS
5	Hyg 42.19-1 LVS	6	Hyg 42.19 -2 LVS
7	Hyg 21.09-1 LVS	8	Hyg 21.09 -2 LVS
9	Hyg 10.55-1 LVS	10	Hyg 10.55-2 LVS
11	Hyg 0 -1 LVS	12	Hyg 0 -2 LVS
13	Hyg 168.8-1 $\Delta$ rpsu1- $\Delta$ rpsu2	14	Hyg 168.8 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
15	Hyg 84.38 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	16	Hyg 84.38 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
17	Hyg 42.19-1 $\Delta$ rpsu1- $\Delta$ rpsu2	18	Hyg 42.19 -2 $\Delta$ rpsu1- $\Delta$ rpsu2
19	Hyg 21.09-1 $\Delta$ rpsu1- $\Delta$ rpsu2	20	Hyg 21.09-2 $\Delta$ rpsu1- $\Delta$ rpsu2
21	Hyg 10.55-1 $\Delta$ rpsu1- $\Delta$ rpsu2	22	Hyg 10.55-2 $\Delta$ rpsu1- $\Delta$ rpsu2
23	Hyg 0 -1 $\Delta$ rpsu1- $\Delta$ rpsu2	24	Hyg 0 -2 $\Delta$ rpsu1- $\Delta$ rpsu2

Measuring OD600 for LVS:

OD600 LVS: .416 A

$$C1V1=C2V2 \quad 2.08*V1=.0053*70,000$$

$$V1= 178.4 \mu\text{L}$$

Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 2:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu2: .408 A

$$C1V1=C2V2 \quad 2.04*V1=.0105*70,000$$

$$V1= 360.3 \mu\text{L}$$

Culture tubes were incubated at 1:33 PM

**Wednesday, December 25<sup>th</sup>, 2019**

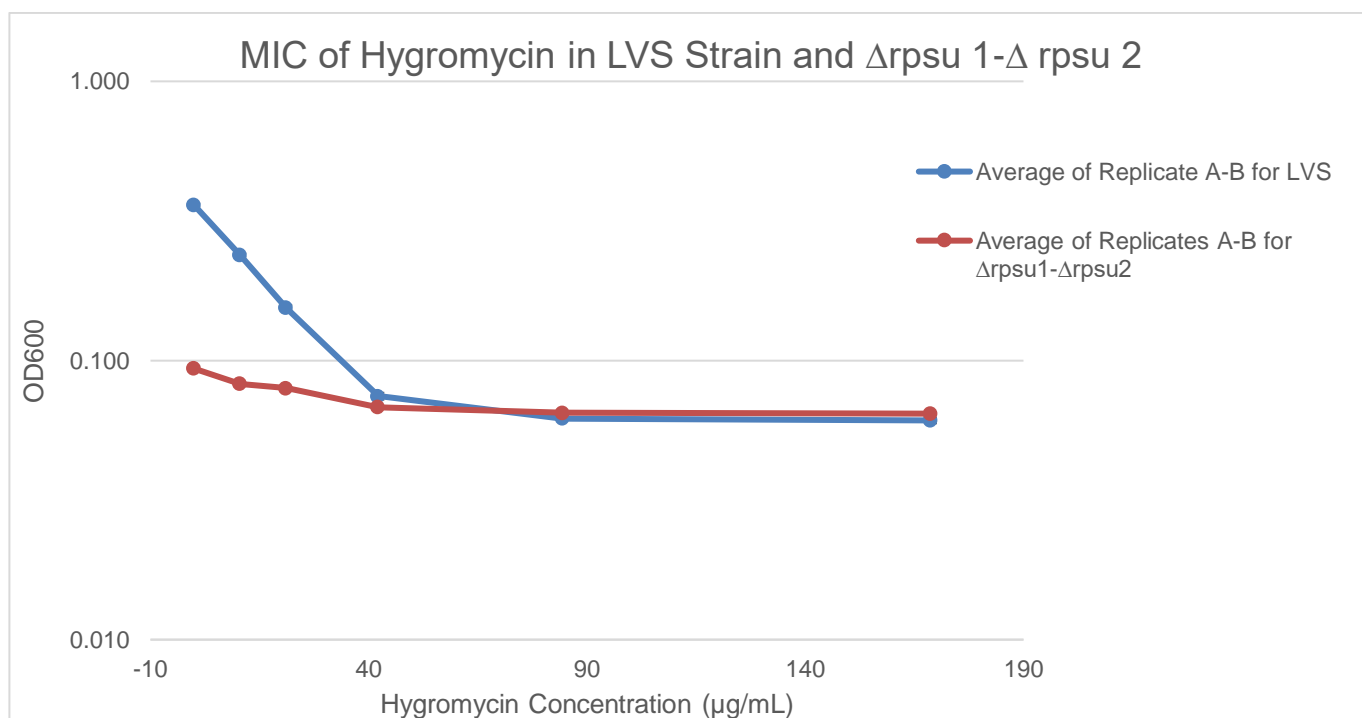
To Do:

1. Autoclave tip boxes
2. Read MIC results day 1

**Results and Methods:**

Reading was done at 1:00 PM

Hygromycin Conc. ( $\mu\text{g/mL}$ )	Avg OD600 of LVS (starting OD600 .005)	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ (starting OD600 0.01)
168.8	0.061	0.065
84.38	0.062	0.065
42.19	0.075	0.068
21.09	0.155	0.080
10.55	0.239	0.083
0	0.360	0.094

MIC of hyg in LVS: 84.38  $\mu\text{g/mL}$ MIC of hyg in  $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ : 42.19  $\mu\text{g/mL}$ 

These results match the ones I got from the 96 well plate MIC assay but don't match the results I got on December 5th.

**Wednesday, December 26th, 2019**

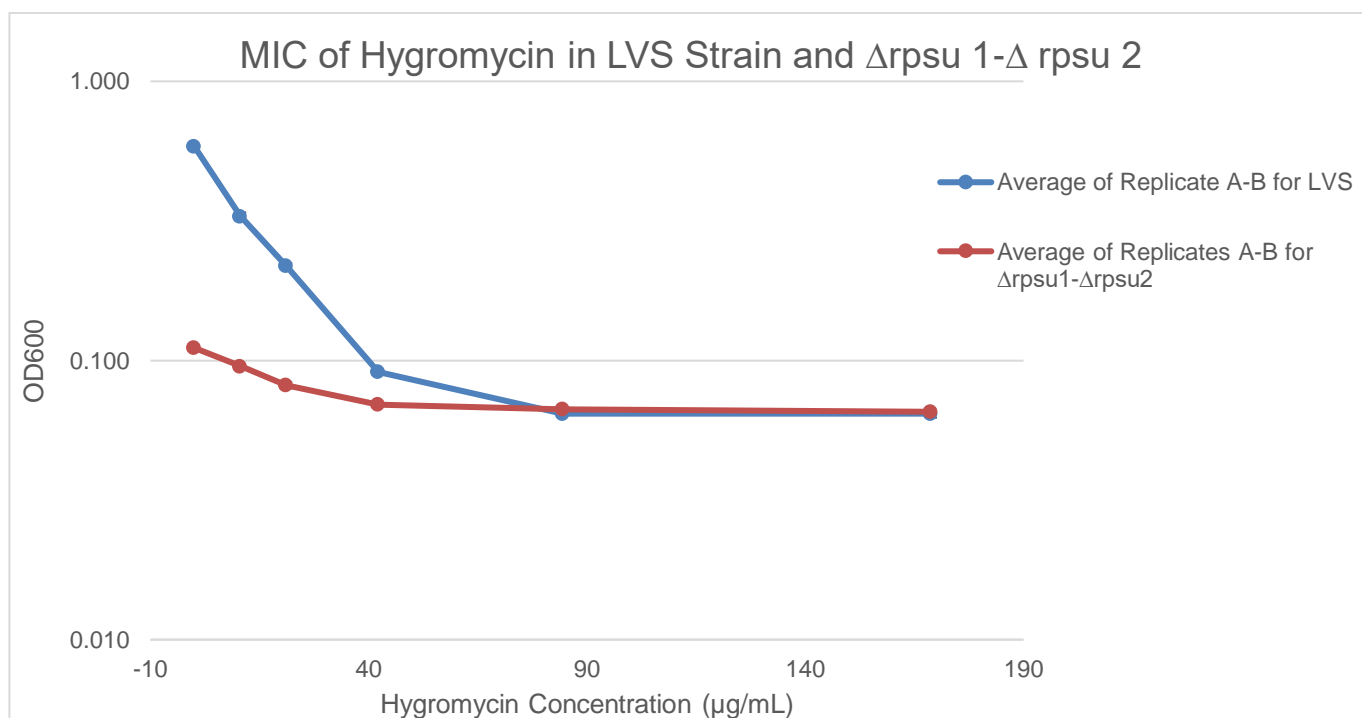
**To Do:**

1. Refill tip boxes
2. Read MIC results day 2

**Results and Methods:**

Reading was done at 10:40 AM

Hygromycin Conc. ( $\mu\text{g/mL}$ )	Avg OD600 of LVS (starting OD600 .005)	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ (starting OD600 0.01)
168.8	0.065	0.066
84.38	0.065	0.067
42.19	0.091	0.070
21.09	0.219	0.082
10.55	0.329	0.096
0	0.586	0.111

MIC of hyg in LVS: 84.38  $\mu\text{g/mL}$ MIC of hyg in  $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ : 42.19  $\mu\text{g/mL}$ **Sunday, December 29<sup>th</sup>, 2019****To Do:**

1. Streak cells for MIC assay

**Monday, December 30<sup>th</sup>, 2019****To Do:**

1. Perform an MIC on LVS and  $\Delta\text{rpsu1-}\Delta\text{rpsu2}$  using hygromycin
2. Perform an MIC on LVS and  $\Delta\text{rpsu1-}\Delta\text{rpsu2}$  using tetracycline and streptomycin (96-well plate assay)
3. Make filter disks and sterilize them

**Results and Methods:****Hygromycin assay:**

Antibiotic dilutions were made according to this table

Tube	Final hygromycin concentration (µg/mL)	Final volume (µL)	Concentrated hygromycin concentration	Final volume MHB in 2 mL tubes	MHB in tubes (- hygromycin)	Stock hygromycin concentration (µg/mL)	Volume stock hygromycin to add (µl)
1	168.8	5000	3376	1250	1171.9	54000	78.1
2	84.38	5000	1687.6	1250	1210.9	54000	39.1
3	42.19	5000	843.8	1250	1230.5	54000	19.5
4	21.09	5000	421.8	1250	1240.2	54000	9.8
5	10.55	5000	211	1250	1245.1	54000	4.9
6	0	5000	0	1250	1250	-	0.0

**Test tube codes:**

1	Hyg 168.8-1 LVS	2	Hyg 168.8-2 LVS
3	Hyg 84.38 -1 LVS	4	Hyg 84.38 -2 LVS
5	Hyg 42.19-1 LVS	6	Hyg 42.19 -2 LVS
7	Hyg 21.09-1 LVS	8	Hyg 21.09 -2 LVS
9	Hyg 10.55-1 LVS	10	Hyg 10.55-2 LVS
11	Hyg 0 -1 LVS	12	Hyg 0 -2 LVS
13	Hyg 168.8-1 Δrpsu1-Δrpsu2	14	Hyg 168.8 -2 Δrpsu1-Δrpsu2
15	Hyg 84.38 -1 Δrpsu1-Δrpsu2	16	Hyg 84.38 -2 Δrpsu1-Δrpsu2
17	Hyg 42.19-1 Δrpsu1-Δrpsu2	18	Hyg 42.19 -2 Δrpsu1-Δrpsu2
19	Hyg 21.09-1 Δrpsu1-Δrpsu2	20	Hyg 21.09-2 Δrpsu1-Δrpsu2
21	Hyg 10.55-1 Δrpsu1-Δrpsu2	22	Hyg 10.55-2 Δrpsu1-Δrpsu2
23	Hyg 0 -1 Δrpsu1-Δrpsu2	24	Hyg 0 -2 Δrpsu1-Δrpsu2

Measuring OD600 for LVS:

OD600 LVS: .279 A

$$C1V1=C2V2 \quad 1.395 \cdot V1 = .0053 \cdot 70,000$$

$$V1 = 265.9 \mu\text{L}$$

Measuring OD600 for Δrpsu 1-Δrpsu 2:

OD600 Δrpsu1-Δrpsu2: .395 A

$$C1V1=C2V2 \quad 1.975 \cdot V1 = .0105 \cdot 70,000$$

$$V1 = 372.2 \mu\text{L}$$

Culture tubes were incubated at 11:35 AM.

**Streptomycin assay:**

Highest Conc. in the Wells (µg/mL)	Starting Conc. in Stock Tube A (µg/mL)			
50	1000			
Working Stock Conc. (µg/mL)	Antibiotic Source Conc. (µg/mL)	Total Volume of Working Stock (µL)	Volume of Antibiotic (µL)	Volume of MHB (µL)
2000	50,000	110	4.4	105.6

Measuring OD600 for LVS:

OD600 LVS: .279 A

$$C_1V_1 = C_2V_2 \quad 1.395 * V_1 = .005 * 20,000$$

$$V_1 = 71.7 \mu\text{L}$$

Measuring OD600 for  $\Delta$ rpsu 1- $\Delta$ rpsu 3:

OD600  $\Delta$ rpsu1- $\Delta$ rpsu3: .395 A

$$C_1V_1 = C_2V_2 \quad 1.975 * V_1 = .005 * 20,000$$

$$V_1 = 50.6 \mu\text{L}$$

**Plate was incubated at 12:30 PM**

I have discussed with Dr. Ramsey the first step of the propolis experiment.

Next week, I will plate 3 plates with different starting OD600 of LVS (.05, .005, .0005) to see which one has the best growth for a disk diffusion assay.

**Tuesday, December 31<sup>st</sup>, 2019**

To Do:

1. Read hygromycin MIC results day 1

Results and Methods:

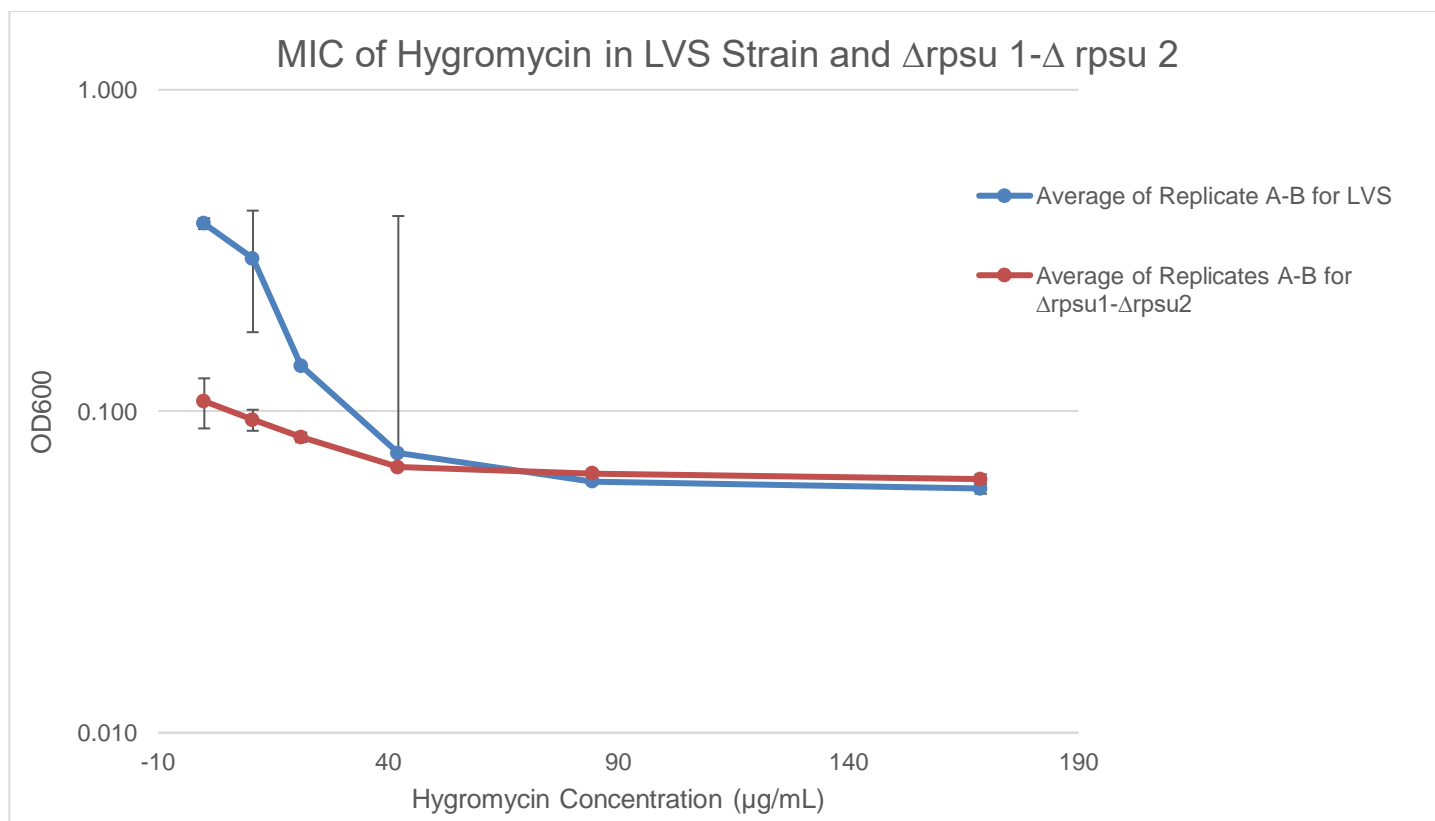
Reading was done at 11:30 AM

Hygromycin Conc. ( $\mu\text{g/mL}$ )	Avg OD600 of LVS (starting OD600 .005)	Avg OD600 of $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ (starting OD600 0.01)
168.8	0.058	0.062
84.38	0.061	0.064
42.19	0.308	0.067
21.09	0.139	0.083
10.55	0.299	0.094
0	0.384	0.108

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

MIC of hyg in  $\Delta\text{rpsu1-}\Delta\text{rpsu2}$ : 42.19  $\mu\text{g/mL}$

One of the LVS Hyg 42.19  $\mu\text{g/mL}$  showed growth and had a reading of 0.542. I excluded that one from the graph. I plated 50  $\mu\text{L}$  of that tube to see if there was possible contamination.



January 2020

Wednesday, January 1<sup>st</sup>, 2020

To Do:

1. Read hyg MIC day 2
2. Read streptomycin MIC results
3. Autoclave trash

Results and Methods:

Streptomycin MIC:

Plate was removed from the incubator at 10:35 AM

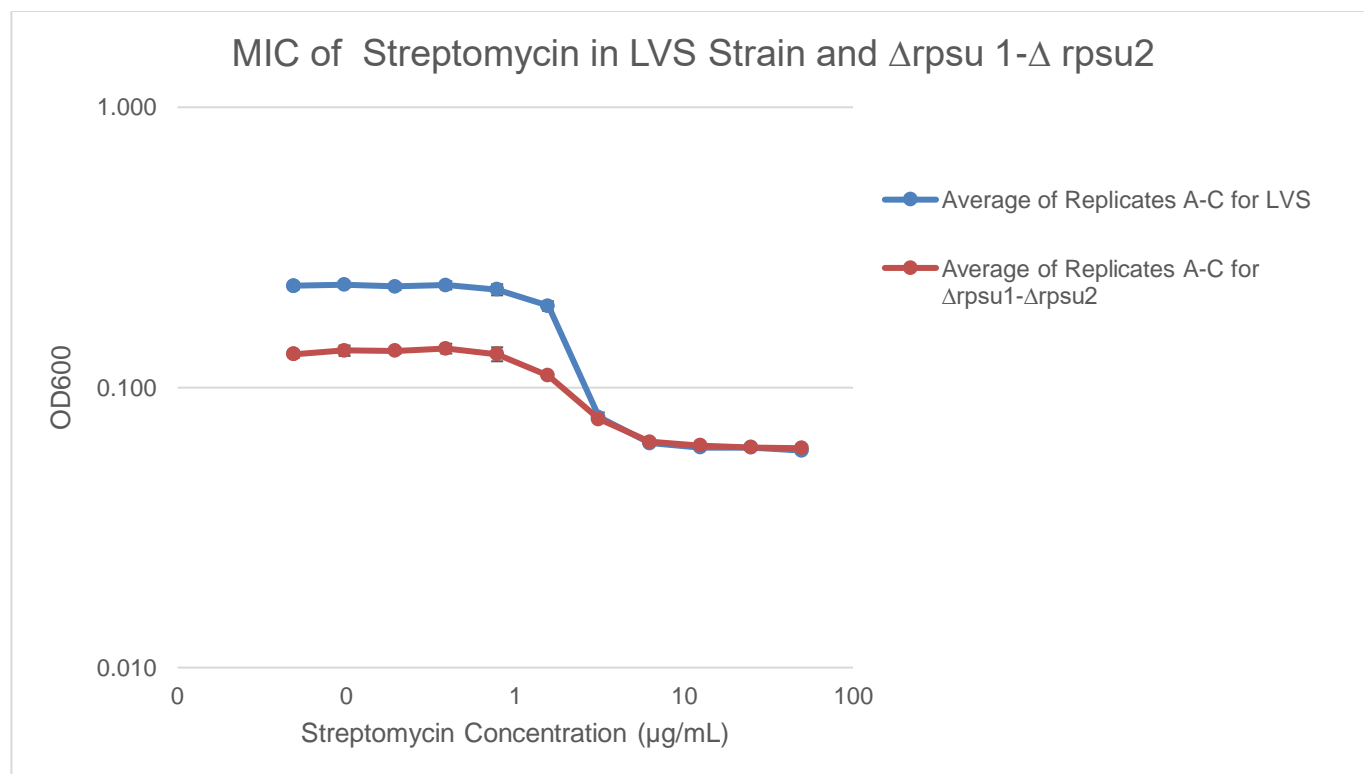
MIC graph: [MIC results /LVS and Δrpsu1-Δrpsu2/191230\\_TA\\_strep/191230\\_TA\\_MIC.xlsx](#)

Streptomycin Conc. (μg/mL)	Avg OD600 of LVS	Avg OD600 of Δrpsu1-Δrpsu2
50	0.060	0.061
25	0.061	0.061
12.5	0.061	0.062
6.25	0.063	0.064
3.13	0.078	0.077
1.56	0.195	0.110
0.78	0.223	0.132
0.39	0.232	0.138
2.0E-01	0.229	0.135
9.8E-02	0.233	0.135
4.9E-02	0.230	0.131
0	0.232	0.135

MIC of strep in LVS: 6.25 μg/mL

MIC of strep in Δrpsu1-Δrpsu2: 6.25 μg/mL





Hygromycin MIC:

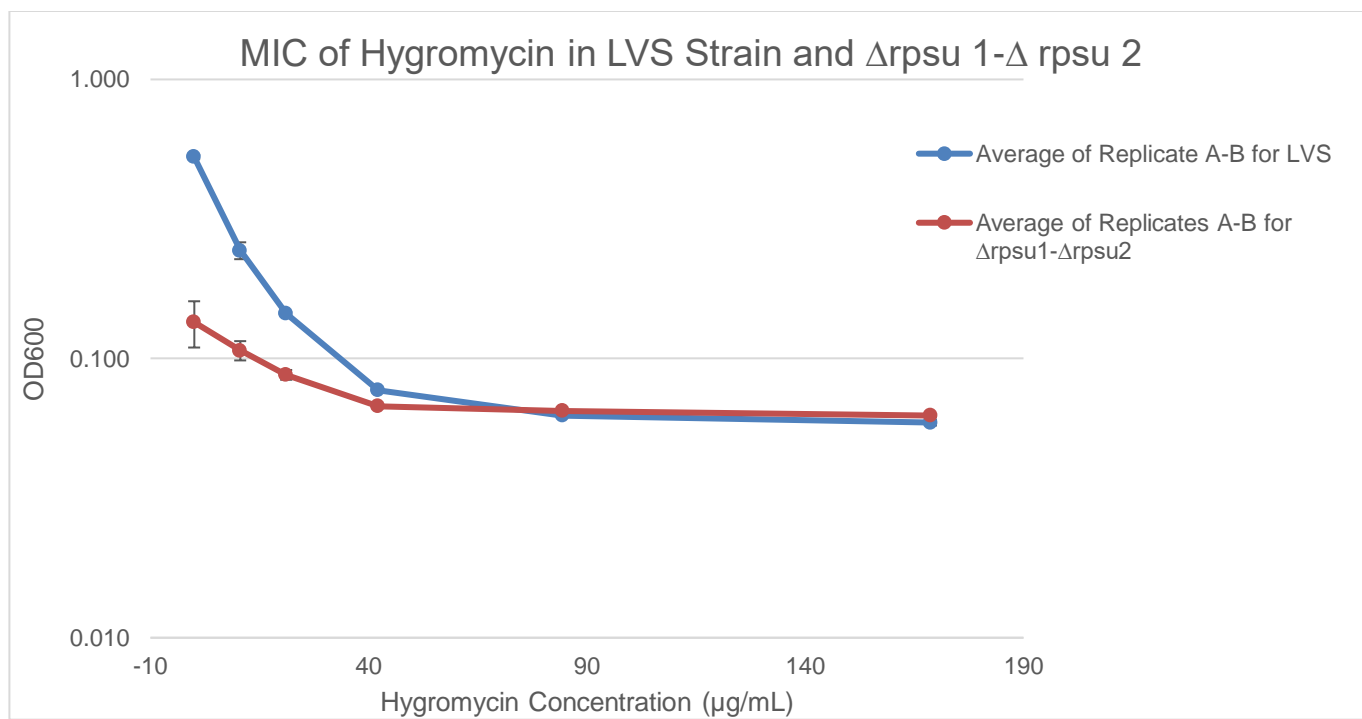
Reading was done at 11:15 AM

Hygromycin Conc. ( $\mu$ g/mL)	Avg OD600 of LVS (starting OD600 .005)	Avg OD600 of $\Delta$ rpsu1- $\Delta$ rpsu2 (starting OD600 0.01)
168.8	0.059	0.063
84.38	0.063	0.065
42.19	0.077	0.068
21.09	0.145	0.088
10.55	0.244	0.107
0	0.528	0.135

MIC of hyg in LVS: 84.38  $\mu$ g/mL

MIC of hyg in  $\Delta$ rpsu1- $\Delta$ rpsu 2: 42.19  $\mu$ g/mL

Tube 5 (Hyg 42.19  $\mu$ g/mL- LVS) was excluded from the results because the plating of that tube from the previous day showed obvious contamination. Plate had really gross growth and the contaminant formed bubbly looking colonies.



## 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.