Table of Contents

Introduction

Thursday, September 20, 2018

To Do:

1. ~~Explain lab notebook formatting~~
2. Media prep

Results and Data:

For each day, copy the previous entry headers and update the date. Save the same ongoing copy of the lab notebook to the lab google drive ONLY. Do not save separate physical copies. At the 1st of each new month, a read-only PDF file of the lab notebook must be saved and given to Dr. Ramsey electronically. **File contents converted to PDF MUST NOT BE EDITED after PDF conversion**. Continue to keep using the same word file until the end of the calendar year. New lab notebook files run in 6 month intervals.

For the To do list, update this each day with new tasks, as tasks are done, use the ~~strikethrough~~ font on the day they are completed and leave them out of the list on the next day.

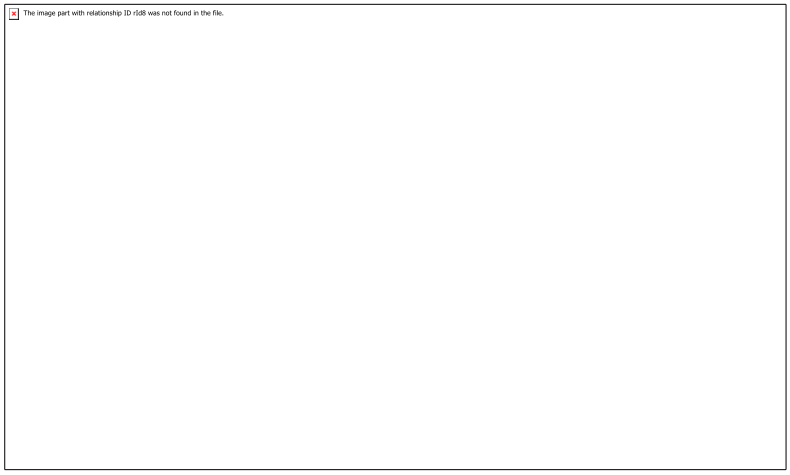


Figure 1

Figures are inserted as inline .png files when possible, .jpg, .gif, .tif otherwise are acceptable. Figure legends are always inserted in Word (right click – 'insert caption') and use "**Heading 2**" text to properly format in the TOC. Table legends are handled the same way. Figure images must be saved in a separate folder where the source data is saved.

Thoughts or questions. When you have a significant observation, question, confusing point or contradiction that you have identified in your data or protocol, use the "**Heading 3**" text heading on a descriptive brief title or single word heading so you can refer to it in your TOC later.

Protocol 1

1. This is the format for a protocol in your notebook.

2. The protocol title must be formatted in "**Heading 2.**"

3. Reagents which must be made for a specific protocol (buffers, solutions) should be listed in a "Reagents" Section, formatted in "**Heading 3**."

4. The protocol must be in numerical steps.

5. Use standard notation and carefully describe units for your protocol.

6. Use ½" indent for protocol text.

Reagents

Specific buffers

For initial lab notebooks, write as much detail as possible. As time goes by you will be able to refer to written protocols by their heading and/or page number within the document. If you make any modifications to a protocol you must state how and why.

File Formatting Protocol

1. Filenames begin with your initials, an underscore, and the date, formatted as the last 2 digits of the year, the month, then the day, ex: "KMR_180920_Sample_file1v1.xls"

2. This ensures all files will be sorted by their creator and by their date. You must use this file formatting system for all data files (including photos) that will be shared with the lab.

Bibliography data will be saved as (author/date) and using Mendeley at this time with the TOC inserted by order cited at the end of the lab notebook in the Bibliography section.

Example is a recent publication (Ramsey and Dove, 2016).

September 2020

Tuesday, September 15, 2020

To Do:

1. ~~Make glycerol stocks and single use aliquots of LVS and Tn7::rpsu1.~~

Results and Data:

Making Glycerol Stocks Protocol (edited for here)

1. Make 2 cryovials for each strain (permanent stocks), label! And 10 1.5 mL tubes
2. Prepare 2.4mL of MHB in a 50mL conical (adjust if you are also making single use stocks)
3. Take at least half of a thickly spread plate and add cells to the MHB tube
4. Resuspend until there are no clumps in the MHB
5. Add 600ul of 75% glycerol to the 2.4mL mix by pipetting
6. Aliquot 1mL per cryovial, freeze at -80.
7. For single use stocks follow the same protocol but pipet 50ul of solution to tubes

10 single use aliquots and 2 permanent stocks stored in -80 in Undergraduate Researchers box (frontmost) under E4.

Wednesday, September 16, 2020

To Do:

1. ~~Supplement MHB.~~
2. ~~Streak out plates for LVS and rpsu1.~~

Results and Data:

Thursday, September 17, 2020

To Do:

1. ~~Set up MIC assay.~~

Results and Data:

Started by making antibiotic before class and placing in fridge.

Updated by: Hannah Trautmann

2020/09/15

MIC Protocol (One Strain):

1. Prepare ~~20~~ 10 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 a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]

****

6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare 200 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of 200 uL, by adding 16.8 µL of our stock 50 mg/mL solution to 193.2 µL of MHB.
9. Serially dilute 1:2 by transferring 200 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube.
10. Transfer 200 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).

****

11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x).
12. Cover plate with lid and put in 37°C incubator overnight.
13. In the morning, mix and pipet 150 µL from each well to a new 96-well plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

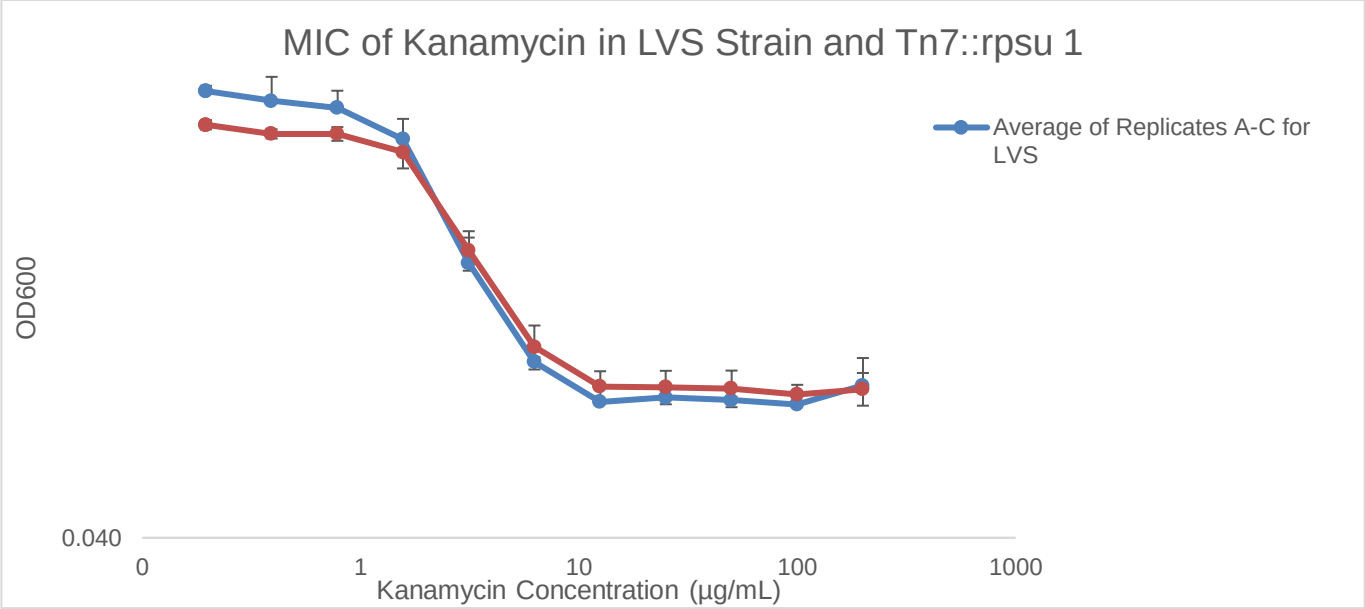
Friday, September 18, 2020

To Do:

1. ~~Transfer cell culture to a new plate~~
2. ~~Take OD readings in INBRE lab.~~

Results and Data:

Results were mixed, so I will start with an OD of 0.01 next time.



Monday, September 21, 2020

To Do:

1. ~~Make hemoglobin for lab.~~

Results and Data:

Tuesday, September 22, 2020

To Do:

1. ~~Set up MIC Assay.~~

Results and Data:

Updated by: Hannah Trautmann

2020/09/15

MIC Protocol (One Strain):

1. Prepare ~~20~~ 10 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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare 200 µL of media.

8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of 200 uL, by adding 16.8 µL of our stock 50 mg/mL solution to 193.2 µL of MHB.
9. Serially dilute 1:2 by transferring 200 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube.
10. Transfer 200 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x).
12. Cover plate with lid and put in 37°C incubator overnight.
13. In the morning, mix and pipet 150 µL from each well to a new 96-well plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

Wednesday, September 23, 2020

To Do:

1. ~~Mix and change plates to read results.~~
2. ~~Streak out LVS and Tn7::rpsu1.~~
3. ~~Check results with Dr. Ramsey.~~

Results and Data:

Thursday, September 24, 2020

To Do:

1. ~~Autoclave dry cycle.~~
2. ~~Check in with Hannah for LVS in MHB cultures.~~

Results and Data:

Friday, September 25, 2020

To Do:

1. ~~Make cultures of LVS in MHB and take 0, 2, and 4 hour timepoints.~~

Results and Data:

Measure 0.5 mL in spec, use 3 tubes, 6 mL in each tube, aim for a starting OD of 0.08.

Start ~12, have cultures incubating at 12:51:

1 – 0.078

2 – 0.080

3 – 0.133

2 hour timepoint at 2:51:

1 – 0.130

2 – 0.142

3 – 0.133

4 hour timepoint at 4:51:

1 – 0.210

2 – 0.219

3 – 0.197

Monday, September 28, 2020

To Do:

1. ~~Streak out cells for MIC.~~

Results and Data:

Tuesday, September 29, 2020

To Do:

1. ~~Repeat Kanamycin MIC assay with two plates to be measured at 24 and 48 hour timepoints.~~

Results and Data:

Updated by: Hannah Trautmann

2020/09/15

MIC Protocol (One Strain – 2 plates):

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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of ~~10~~ 20 mL. [Use C1V1=C2V2)

4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate and repeat with a second plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]. Repeat with second plate.
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare ~~200~~ 400 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of ~~200~~ 400 uL, by adding ~~16.8~~ 33.6 µL of our stock 50 mg/mL solution to ~~193.2~~ 386.4 µL of MHB.
9. Serially dilute 1:2 by transferring ~~200~~ 400 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube.
10. Transfer ~~200~~ 400 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x). Repeat with second plate.
12. Cover plate with lid and put in 37°C incubator overnight.
13. In the morning, mix and pipet 150 µL from each well to a new 96-well plate.
14. Use the plate reader to measure OD600.
15. Measure OD600 after 48 hours for second plate.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

Wednesday, September 30, 2020

To Do:

1. ~~Take 24 hour plate reading.~~

Results and Data:

October 2020

Thursday, October 1, 2020

To Do:

1. ~~Take 48 hour plate reading.~~
2. ~~Make more glycerol stocks.~~

Results and Data:

Friday, October 2, 2020

To Do:

1. ~~Make single use aliquots for LVS and Tn7::rpsu1.~~
2. ~~Streak out LVS and Tn7::rpsu1 cells for Monday.~~

Results and Data:

Making Glycerol Stocks Protocol (edited for here)

1. Make 2 cryovials for each strain (permanent stocks), label! And 10 1.5 mL tubes
2. Prepare 2.4mL of MHB in a 50mL conical (adjust if you are also making single use stocks)
3. Take at least half of a thickly spread plate and add cells to the MHB tube
4. Resuspend until there are no clumps in the MHB
5. Add 600ul of 75% glycerol to the 2.4mL mix by pipetting
6. Aliquot 1mL per cryovial, freeze at -80.
7. For single use stocks follow the same protocol but pipet 50ul of solution to tubes

15 single use aliquots for LVS and rpsu1 stored in -80 in Undergraduate Researchers box under E4. The older aliquots (from 9.15.2020) have “JTC” labeled on top and either have the older date listed or are missing a date.

Monday, October 5, 2020

To Do:

1. ~~Make permanent stocks and single use aliquots for Tn7::rpsu2 and Tn7::rpsu3.~~
2. ~~Set up MIC with Kanamycin, LVS, and rpsu1 (incubate 48 hours).~~
3. ~~Streak out LVS and Tn7::rpsu2 cells for Tuesday.~~

Results and Data:

Making Glycerol Stocks Protocol (edited for here)

1. Make 2 cryovials for each strain (permanent stocks), label! And 10 1.5 mL tubes
2. Prepare 2.4mL of MHB in a 50mL conical (adjust if you are also making single use stocks)
3. Take at least half of a thickly spread plate and add cells to the MHB tube
4. Resuspend until there are no clumps in the MHB

5. Add 600ul of 75% glycerol to the 2.4mL mix by pipetting
6. Aliquot 1mL per cryovial, freeze at -80.
7. For single use stocks follow the same protocol but pipet 50ul of solution to tubes

Updated by: Hannah Trautmann

2020/09/15

MIC Protocol (One Strain):

1. Prepare ~~20~~ 10 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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare 200 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of 200 uL, by adding 16.8 µL of our stock 50 mg/mL solution to 193.2 µL of MHB.
9. Serially dilute 1:2 by transferring 200 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube.
10. Transfer 200 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x).
12. Cover plate with lid and put in 37°C incubator overnight.
13. ~~In the morning~~ After 2 days~~,~~ mix and pipet 150 µL from each well to a new 96-well plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

Tuesday, October 6, 2020

To Do:

1. ~~Set up MIC with Kanamycin, LVS, and rpsu2 (incubate 48 hours).~~

Results and Data:

Updated by: Hannah Trautmann

2020/09/15

MIC Protocol (One Strain):

1. Prepare ~~20~~ 10 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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare 200 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of 200 uL, by adding 16.8 µL of our stock 50 mg/mL solution to 193.2 µL of MHB.
9. Serially dilute 1:2 by transferring 200 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube. SWITCH TIPS.
10. Transfer 200 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x).
12. Cover plate with lid and put in 37°C incubator overnight.
13. ~~In the morning~~ After 2 days~~,~~ mix and pipet 150 µL from each well to a new 96-well plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

Wednesday, October 7, 2020

To Do:

1. ~~Read first MIC.~~

Results and Data:

LVS and rpsu1 with Kan to confirm results at 48 hours.

Using MIC Protocol from 10/6/2020 moving forward unless otherwise specified.

Thursday, October 8, 2020

To Do:

1. ~~Read second MIC.~~

Results and Data:

LVS and rpsu2 with Kan (first round).

Friday, October 9, 2020

To Do:

1. ~~Lab tasks.~~
2. ~~Make 75% glycerol.~~
3. ~~Streak out cells.~~

Results and Data:

Monday, October 12, 2020

To Do:

1. ~~Set up MIC for LVS and rpsu2 with Kan.~~
2. ~~Streak out LVS and rpsu3 cells for MIC tomorrow.~~

Results and Data:

Tuesday, October 13, 2020

To Do:

1. ~~Set up MIC for LVS and rpsu3 with Kan.~~
2. ~~Streak out LVS and rpsu3 cells for MIC tomorrow.~~

Results and Data:

Wednesday, October 14, 2020

To Do:

1. ~~Set up MIC for LVS and rpsu3 with Kan.~~
2. ~~Read plate from Monday (rpsu2).~~

Results and Data:

Thursday, October 15, 2020

To Do:

1. ~~Read plate from Tuesday (rpsu3).~~
2. ~~Lab tasks?~~

Results and Data:

The plate of rpsu2 with Kanamycin read today had a different MIC for both LVS and rpsu2. This will be repeated next week. Note to switch tips when serially diluting in between dilutions.

Friday, October 16, 2020

To Do:

1. ~~Set up MIC for LVS and rpsu3 with Kan.~~
2. ~~Streak out LVS and rpsu3 cells for MIC tomorrow.~~

Results and Data:

Monday, October 19, 2020

To Do:

1. ~~Streak out LVS and rpsu1 cells for MIC comparison assay.~~
2. ~~Make single use stocks for LVS and rpsu2 from Friday's plate.~~
3. ~~Talk with Kathryn and Hannah about MICs and reproducibility.~~
4. ~~Work on lab presentation and review with Hannah.~~

Results and Data:

Making Glycerol Stocks Protocol (edited for here)

1. Make 2 cryovials for each strain (permanent stocks), label! And 10 1.5 mL tubes
2. Prepare 2.4mL of MHB in a 50mL conical (adjust if you are also making single use stocks)
3. Take at least half of a thickly spread plate and add cells to the MHB tube
4. Resuspend until there are no clumps in the MHB
5. Add 600ul of 75% glycerol to the 2.4mL mix by pipetting
6. Aliquot 1mL per cryovial, freeze at -80.
7. For single use stocks follow the same protocol but pipet 50ul of solution to tubes

Tuesday, October 20, 2020

To Do:

1. ~~Present in lab.~~

~~2. Set up 2 MICs of LVS and rpsu1 with Kanamycin, one in deep well plate (shaking incubator) and one in regular 96 well plate (standing incubator) and incubate for 48 hours to compare.~~

Results and Data:

MIC Protocol (One Strain – 2 plates):

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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of ~~10~~ 20 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate and repeat with a second plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]. Repeat with second plate.
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare ~~200~~ 400 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of ~~200~~ 400 uL, by adding ~~16.8~~ 33.6 µL of our stock 50 mg/mL solution to ~~193.2~~ 386.4 µL of MHB.
9. Serially dilute 1:2 by transferring ~~200~~ 400 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube.
10. Transfer ~~200~~ 400 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x). Repeat with second plate.
12. Cover plates with lids and put in 37°C incubator ~~overnight~~ for 48 hours.
13. ~~In the morning~~ After 48 hours, mix and pipet 150 µL from each well to a new 96-well plate. Repeat with second plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

MIC Protocol for hygromycin and two strains:

1. Prepare ~~20~~ 10 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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]
6. The next steps will vary based on the concentrations of antibiotic you want to use. For hygromycin: a starting concentration of 6750 ug/mL in stock tube A is required, because it will be diluted 1:10 when added to the 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare 200 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 13.5 mg/mL and a final volume of 210 uL, by adding 52.5 µL of our stock hyg 54 mg/mL solution to 157.5 µL of MHB.
9. Serially dilute 1:2 by transferring 200 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube. SWITCH TIPS.
10. Transfer 200 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x).
12. Cover plate with lid and put in 37°C incubator overnight.
13. ~~In the morning~~ After 2 days~~,~~ mix and pipet 150 µL from each well to a new 96-well plate.
14. Use the plate reader to measure OD600.

Thursday, October 22, 2020

To Do:

1. ~~Read both plates from each incubator.~~

Results and Data:

The shaking incubator plate grew well, but lost significant amounts of liquid from evaporation. Next week, try using cut ethanol container with many wet paper towels in the bottom and cover by placing saran wrap over top with an elastic band.

Friday, October 23, 2020

To Do:

1. ~~Streak out cells.~~
2. ~~Make hemoglobin.~~

Results and Data:

Monday, October 26, 2020

To Do:

1. ~~Comparison rpsu1 MICs again with new shaker setup.~~

Results and Data:

MIC Protocol (One Strain – 2 plates):

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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of ~~10~~ 20 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate and repeat with a second plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]. Repeat with second plate.
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare ~~200~~ 400 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of ~~200~~ 400 uL, by adding ~~16.8~~ 33.6 µL of our stock 50 mg/mL solution to ~~193.2~~ 386.4 µL of MHB.
9. Serially dilute 1:2 by transferring ~~200~~ 400 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube.
10. Transfer ~~200~~ 400 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x). Repeat with second plate.
12. Cover plates with lids and put in 37°C incubator ~~overnight~~ for 48 hours.
13. ~~In the morning~~ After 48 hours, mix and pipet 150 µL from each well to a new 96-well plate. Repeat with second plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

Tuesday, October 27, 2020

To Do:

1. ~~Streak out cells for Wednesday.~~
2. ~~Dry cycle?~~

Results and Data:

Wednesday, October 28, 2020

To Do:

1. ~~Read plates and compare.~~

Results and Data:

Consistent problem with 3.13 and 6.25ug/ul having very similar ODs.

Thursday, October 29, 2020

To Do:

1. ~~Set up 24 hours MIC with LVS and rpsu2 against Kan.~~

Results and Data:

First MIC assay done in shaking incubator for 24 hours. Start using results from here. (First plate read on 30th)

Tn7::rpsu2

MIC Protocol (One Strain – 2 plates):

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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of ~~10~~ 20 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate and repeat with a second plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]. Repeat with second plate.
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare ~~200~~ 400 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of ~~200~~ 400 uL, by adding ~~16.8~~ 33.6 µL of our stock 50 mg/mL solution to ~~193.2~~ 386.4 µL of MHB.
9. Serially dilute 1:2 by transferring ~~200~~ 400 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube.
10. Transfer ~~200~~ 400 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x). Repeat with second plate.
12. Cover plates with lids and put in 37°C incubator overnight.
13. In the morning, mix and pipet 150 µL from each well to a new 96-well plate. Repeat with second plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate												

A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

Friday, October 30, 2020

To Do:

1. ~~Read MIC plates.~~

Results and Data:

November 2020

Monday, November 2, 2020

To Do:

1. ~~Set up MIC with rpsu1 and Kanamycin.~~
2. ~~Take out glass waste.~~
3. ~~Streak out cells.~~

Results and Data:

Tn7::rpsu1

MIC Protocol (One Strain):

1. Prepare ~~20~~ 10 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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare 200 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of 200 uL, by adding 16.8 µL of our stock 50 mg/mL solution to 193.2 µL of MHB.

9. Serially dilute 1:2 by transferring 200 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube. SWITCH TIPS.
10. Transfer 200 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x).
12. Cover plate with lid and put in 37°C incubator overnight.
13. In the morning, mix and pipet 150 µL from each well to a new 96-well plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

Tuesday, November 3, 2020

To Do:

1. ~~Set up MIC with rpsu2 and Kan using 200ul tips.~~
2. ~~Read MIC from Monday.~~
3. ~~Streak out rpsu1 cells.~~

Results and Data:

Tn7::rpsu2

MIC Protocol (One Strain):

1. Prepare ~~20~~ 10 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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare 200 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of 200 uL, by adding 16.8 µL of our stock 50 mg/mL solution to 193.2 µL of MHB.

9. Serially dilute 1:2 by transferring 200 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube. SWITCH TIPS.
10. Transfer 200 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x).
12. Cover plate with lid and put in 37°C incubator overnight.
13. In the morning, mix and pipet 150 µL from each well to a new 96-well plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

Wednesday, November 4, 2020

To Do:

1. ~~Set up MIC with rpsu1 and Kan using 200ul tips.~~
2. ~~Read MIC from Tuesday.~~

Results and Data:

Tn7::rpsu1

MIC Protocol (One Strain):

1. Prepare ~~20~~ 10 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~~ 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2)
4. Using the multichannel and a reservoir, add 180 µL of supplemented MHB into the 4th and 8th row of a non-treated 96 well plate.
5. Add 180 µL of the diluted culture into the empty wells of the plate, such that entire plate is filled with media. [Shaded cells]
6. The next steps will vary based on the concentrations of antibiotic you want to use. For kanamycin: 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 96-well plate.
7. In eleven 1.5 mL tubes for each antibiotic, prepare 200 µL of media.
8. Prepare a working stock of kanamycin that is a concentration of 4.00 mg/mL and a final volume of 200 uL, by adding 16.8 µL of our stock 50 mg/mL solution to 193.2 µL of MHB.
9. Serially dilute 1:2 by transferring 200 µL from the working stock tube to the first of the 11 1.5 mL tubes, mixing, and repeating through the 11th tube. SWITCH TIPS.

10. Transfer 200 uL from each of the eleven 1.5 mL tubes to strip tubes. Include a twelfth strip tube at the end that has only 200 uL of media (no antibiotic).
11. Using the multichannel, pipet 20 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 20 µL and mix with 100 µL 3x).
12. Cover plate with lid and put in 37°C incubator overnight.
13. In the morning, mix and pipet 150 µL from each well to a new 96-well plate.
14. Use the plate reader to measure OD600.

	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate A												
Replicate B												
Replicate C												
No Cells												
Replicate A	Kanamycin 200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.125 µg/mL	1.56 µg/mL	0.78 µg/mL	0.39 µg/mL	0.195 µg/mL	No antibiotic
Replicate B												
Replicate C												
No Cells												

Thursday, November 5, 2020

To Do:

1. ~~Read plate from Wednesday.~~

Results and Data:

This plate appears to have an MIC of 3.13, differing from my other results. Because the results of 3.13 and 6.25ug/ml concentrations are always close or, in this case, virtually the same, I will talk to Dr. Ramsey and Hannah about what to do moving forward.

Friday, November 6, 2020

To Do:

1. ~~Streak out LVS and Tn7::rpsu1 cells.~~

Results and Data:

Monday, November 9, 2020

To Do:

1. ~~Talk with Hannah and Kathryn about MICs.~~
2. ~~Autoclave filter paper punchouts.~~
3. ~~Set up disk diffusion assay.~~

Results and Data:

After talking with Kathryn and Hannah, we decided to try and move forward with disk diffusion assays, specifically Kirby-Bauer Disk Diffusion Susceptibility tests (see: https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf). Source is also listed in bibliography. Tala had done some work with this in the past. See her protocol below and the modified protocol that I used to set up the disk diffusion assay.

Disk Diffusion Assay (written by Tala Allababidi):

1. Apply 20 µL of kanamycin and 200 proof ethanol to the filter disk
2. Allow to dry in the flow hood for at least 30 minutes
3. Plate 100 µL of LVS OD600 0.005 onto a regular plate
4. Use a plate spreader and wait for cells to dry before adding the disks.

Disk Diffusion Assay (written by Tala Allababidi) edited by John Church 11/9/2020

1. Resuspend cells for the strain being tested in about 400 µL of MHB. Measure the OD600.
2. Aim for an OD600 of 0.005. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2] Repeat steps 1 and 2 for a separate strain if necessary.
3. Add 100 µL of LVS cells at OD600 0.005 to a CHAH plate and spread with beads or a spreader. *For this protocol on 11/9, two plates were done with each type of spreading technique.* Repeat this a second time with a new plate if testing another strain. Allow plates to dry.
4. Using sterile tweezers, add sterile filter paper disks to a sterile square plate that has been separated by solvent and then add 20 µL of each solvent. *For protocol on 11/9, Kanamycin, H2O, and ethanol were used on each plate, with a fourth disk being kept sterile without solvent.* Allow these disks to dry for 15 minutes.
5. Using sterile tweezers, transfer disks from sterile plate to a CHAH plate that has been spread with *Francisella.*

1 – H2O

2 – Kanamycin

3 – Ethanol

4 – blank disk

Tuesday, November 10, 2020

To Do:

1. ~~Lab meeting.~~
2. ~~Check plates.~~

Results and Data:

Plates do not have enough growth yet. Maybe use a higher OD in the future to spread on plates. Protocol from ASM (Jan Hudzicki) uses a McFarland standard of 0.5, which is about an OD600 of 0.08-0.1. May be good to move up to an OD of at least 0.01.

Wednesday, November 11, 2020

To Do:

1. ~~Take pictures and measurements of plates.~~

Results and Data:

Very mixed results. Will try setting up another assay tomorrow with Kan and water as a control, so that there are only 2 discs per plate.

Spreader plate:

1 – H2O – 1.19in

2 – Kan – 1.85in (1.065in radius)

3 – ethanol – no halo

4 – blank disk – 0.072in (very small, may be residual from Kan)

Beads plate:

1 – H2O – 1.499in

2 – Kan – 2.153in

3 – ethanol – 1.028

4 – blank disk – 0.683in (may be residual from Kan)

Thursday, November 12, 2020

To Do:

1. ~~Talk to Kathryn and Hannah about DDA for Monday.~~

Results and Data:

4 plates: 2 LVS, 2 rpsu1. Each plate should have 2 discs, one with Kan and one with sterile water.

Friday, November 13, 2020

To Do:

1. ~~Make CHAH plates.~~
2. ~~Streak out cells for Monday.~~

Results and Data:

Monday, November 16, 2020

To Do:

1. ~~DDA with rpsu1 on 4 plates.~~

Results and Data:

Set up 4 plates: 2 of LVS, 2 of rpsu1. Each plate should have 2 disks: 1 Kan, 1 sterile water (control).

Some of the plates were made with high OD values, which will be noted when imaged (the correct ODs are noted in the image file name, the incorrect ODs are not).

Disk Diffusion Assay (written by Tala Allababidi) edited by John Church 11/16/2020

1. Resuspend cells for the strain being tested in about 400 µL of MHB. Measure the OD600.
2. Aim for an OD600 of 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2] Repeat steps 1 and 2 for a separate strain if necessary.
3. Add 100 µL each of LVS cells at OD600 0.01 to 2 CHAH plates and spread with a spreader. Repeat this a second time with new plates if testing another strain. Allow plates to dry.
4. Using sterile tweezers, add sterile filter paper disks to a sterile plate with dividers and then add 20 µL water and Kan. Allow these disks to dry for 15 minutes.
5. Using sterile tweezers, transfer disks from sterile plate to CHAH plates that have been spread with *Francisella* so that there is a water disk and a Kan disk on each CHAH plate.

Tuesday, November 17, 2020

To Do:

1. ~~Read plates that grew.~~

Results and Data:

These plates were incubated with high ODs of cells:

LVS plate 1: 1.291in

LVS plate 2: 1.305in

Rpsu1 plate: 1.666in

Wednesday, November 18, 2020

To Do:

1. ~~Read plates that grew.~~

Results and Data:

Note that plates with correct OD are noted with ”correctOD” in file name. These are listed below:

20201118_LVS.3correctOD: 2.132in

20201118_rpsu1.2: 2.085in

20201118_rpsu1.3correctOD: 2.150in

Friday, November 20, 2020

To Do:

1. ~~Ask Hannah to streak out strains for Monday.~~

Results and Data:

Monday, November 23, 2020

To Do:

1. ~~Set up DDA for all strains with Kanamycin.~~

Results and Data:

Note that rpsu1 cells that were struck out on Friday did not grow well over the weekend (still got enough to use for plates).

Disk Diffusion Assay (written by Tala Allababidi) edited by John Church 11/16/2020

1. Resuspend cells for the strain being tested in about 400 µL of MHB. Measure the OD600.
2. Aim for an OD600 of 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2] Repeat steps 1 and 2 for a separate strain if necessary.
3. Add 100 µL each of LVS cells at OD600 0.01 to 2 CHAH plates and spread with a spreader. Repeat this a second time with new plates if testing another strain. Allow plates to dry.
4. Using sterile tweezers, add sterile filter paper disks to a sterile plate with dividers and then add 20 µL water and Kan. Allow these disks to dry for 15 minutes.
5. Using sterile tweezers, transfer disks from sterile plate to CHAH plates that have been spread with *Francisella* so that there is a water disk and a Kan disk on each CHAH plate.

Tuesday, November 24, 2020

To Do:

1. ~~Check on DDA plates in 37 degree incubator.~~
2. ~~Check which boxes have my strains so I can notify Kathryn and she can streak out cells after break.~~

Results and Data:

Wednesday, November 25, 2020

To Do:

1. ~~Read DDA plates.~~
2. ~~Talk to Kathryn about DDA results if she has time.~~
3. ~~Autoclave plate waste.~~
4. ~~Make sure everything is all set for returning to lab after break (including asking Kathryn to streak out cells).~~

Results and Data:

These plates had some issues with bacterial growth and haloes around the water disks. Moving forward, streak out cells and incubate overnight at 37 degrees (rather than leaving out over weekend) and wash forceps with water to clean off antibiotic.

20201125_LVS.1: 2.046in

20201125_LVS.2: 2.359in

20201125_LVS.3: 2.036in

20201125_Tn7rpsu1.1: 2.364in

20201125_Tn7rpsu1.2: could not measure

20201125_Tn7rpsu1.3: 2.419in

20201125_Tn7rpsu2.1: 2.164in

20201125_Tn7rpsu2.2: 2.366in

20201125_Tn7rpsu2.3: 2.359in

20201125_Tn7rpsu3.1: CNM

20201125_Tn7rpsu3.2: 2.318

20201125_Tn7rpsu3.3: 2.365

December 2020

Monday, December 7, 2020

To Do:

1. ~~Streak out cells and incubate overnight at 37.~~

Results and Data:

Tuesday, December 8, 2020

To Do:

1. ~~Streak out cells for overnight incubation.~~

Results and Data:

Making Glycerol Stocks Protocol (edited for here)

1. Make 2 cryovials for each strain (permanent stocks), label! And 10 1.5 mL tubes
2. Prepare 2.4mL of MHB in a 50mL conical (adjust if you are also making single use stocks)
3. Take at least half of a thickly spread plate and add cells to the MHB tube

4. Resuspend until there are no clumps in the MHB
5. Add 600ul of 75% glycerol to the 2.4mL mix by pipetting
6. Aliquot 1mL per cryovial, freeze at -80.

For single use stocks follow the same protocol but pipet 50ul of solution to tubes

Single use aliquots made for Tn7::rpsu1 and Tn7::rpsu3 and stored at -80.

Wednesday, December 9, 2020

To Do:

1. ~~Make hemoglobin.~~
2. ~~Pour CHA plates.~~
3. ~~Set up DDA with LVS and rpsu1-3 using 12 CHA plates and Kanamycin disks.~~

Results and Data:

8. Add 6g freeze-dried hemoglobin to 300mL of ddiH_2O (type I)

9. Autoclave on 20' liquid cycle with water in the bin

10. Cool down (ideally to ~55C)

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!

Disk Diffusion Assay (written by Tala Allababidi) edited by John Church 11/16/2020

1. Resuspend cells for the strain being tested in about 400 µL of MHB. Measure the OD600.
2. Aim for an OD600 of 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2] Repeat steps 1 and 2 for a separate strain if necessary.
3. Add 100 µL each of LVS cells at OD600 0.01 to 2 CHAH plates and spread with a spreader. Repeat this a second time with new plates if testing another strain. Allow plates to dry.
4. Using sterile tweezers, add sterile filter paper disks to a sterile plate with dividers and then add 20 µL water and Kan. Allow these disks to dry for 15 minutes.
5. Using sterile tweezers, transfer disks from sterile plate to CHAH plates that have been spread with *Francisella* so that there is a water disk and a Kan disk on each CHAH plate.

Friday, December 11, 2020

To Do:

1. ~~Take pictures and measure plates.~~
2. ~~See about pouring more plates for next week.~~

Results and Data:

Monday, December 14, 2020

To Do:

1. ~~Make CHA.~~
2. ~~Pour plates.~~
3. ~~Streak out cells.~~

Results and Data:

1. Weigh out 30.6g of cystine heart agar into 1L flask (non-baffled; 10.2g/100mL)
    a. 15.3g in 2 500ml flasks and use pressure cooker

2. Add 300mL of ddiH_2O (type I)

3. Add stirbar 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 ~55C)

7. Separately (before), prepare hemoglobin 2% solution

10. Cool down (ideally to ~55C)

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!

Tuesday, December 15, 2020

To Do:

1. ~~Streak out cells?~~
2. ~~Set up 6 pilot plates for next set of antibiotics.~~

Results and Data:

Set up 6 plates in total to test hygromycin, streptomycin, and tetracycline in duplicate with controls. Tetracycline is in methanol, others are in water. Both streptomycin and tetracycline should be stored at -20.

Disk Diffusion Assay (written by Tala Allababidi) edited by John Church 11/16/2020

1. Resuspend cells for the strain being tested in about 400 µL of MHB. Measure the OD600.
2. Aim for an OD600 of 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2] Repeat steps 1 and 2 for a separate strain if necessary.
3. Add 100 µL each of LVS cells at OD600 0.01 to 2 CHAH plates and spread with a spreader. Repeat this a second time with new plates if testing another strain. Allow plates to dry.

4. Using sterile tweezers, add sterile filter paper disks to a sterile plate with dividers and then add 20 µL water and Kan. Allow these disks to dry for 15 minutes.
5. Using sterile tweezers, transfer disks from sterile plate to CHAH plates that have been spread with *Francisella* so that there is a water disk and a Kan disk on each CHAH plate.

Wednesday, December 16, 2020

To Do:

1. ~~Set up DDA to confirm Kan results in duplicate.~~
2. ~~Check antibiotic pilot.~~

Results and Data:

Note that LVS and rpsu1 plates may be mixed up.

Disk Diffusion Assay (written by Tala Allababidi) edited by John Church 11/16/2020

1. Resuspend cells for the strain being tested in about 400 µL of MHB. Measure the OD600.
2. Aim for an OD600 of 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2] Repeat steps 1 and 2 for a separate strain if necessary.
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4. Using sterile tweezers, add sterile filter paper disks to a sterile plate with dividers and then add 20 µL water and Kan. Allow these disks to dry for 15 minutes.
5. Using sterile tweezers, transfer disks from sterile plate to CHAH plates that have been spread with *Francisella* so that there is a water disk and a Kan disk on each CHAH plate.

Thursday, December 17, 2020

To Do:

1. ~~Read plates from antibiotics pilot.~~

Results and Data:

Note that pilotTET.1 was smudged by finger but still readable.

I also realized that filter paper with water and Kan were never placed on the plates to confirm Kan results (12.16.2020), but I put them back into the incubator to check growth on Friday because several of the pilot plates for new antibiotics had very poor growth. This may be due to the antibiotics, but I am unsure.

Friday, December 18, 2020

To Do:

1. ~~Read plates from DDA to confirm results on Kan.~~
2. ~~Toss out MHB tube in fridge before break.~~

Results and Data:

Talked to Hannah about plates from this week. The plates that were supposed to be for Kan that did not have filter paper (LVS and 3 rpsu strains) all grew pretty well. Hygromycin is 54mg/ml, the highest concentration of antibiotic, so I will try piloting again when I come back to lab with different concentrations. Hannah thinks it may also be because the plates with Hyg were older than the other plates or they were not spread as much as they should have been. In the future, set plates aside after making at the beginning of the week so that the assay only uses new plates and so there are enough to set up 2 assays during the week. Additionally, there appear to be some water halos again, so try getting a new water aliquot every day with a 1.5 ml tube and try setting up experiment again.

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.

Hudzicki, Jan (2009) "Kirby-Bauer Disk Diffusion Susceptibility Test Protocol," *American Society for Microbiology.* https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf