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.

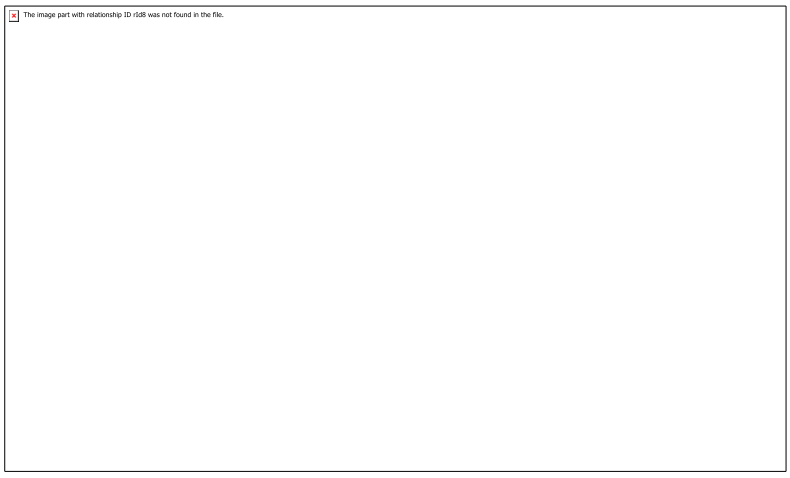


Figure 1

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).

January 2021

Thursday, January 14, 2021

To Do:

1. ~~Meet with Kathryn.~~
2. ~~Move things over into new lab space.~~
3. ~~Plan out schedule.~~

Results and Data:

Friday, January 15, 2021

To Do:

1. ~~Pour plates for next week.~~
2. ~~Make sure I have everything for next week.~~
3. ~~Dilute Strep and Hyg 1:2.~~

Results and Data:

Hygromycin was diluted to 27mg/ml and Strep to 35mg/ml.

Kan confirmation (duplicate x 4 strains x 1 antibiotic = 8 plates); Pilot (duplicate x 1 strain x 2 antibiotics = 4 plates); need 12 plates total.

For 600 mL of CHA

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!

Monday, January 18, 2021

To Do:

1. ~~Streak out cells.~~
2. ~~Make sure there are enough plates.~~

Results and Data:

Tuesday, January 19, 2021

To Do:

1. ~~Set up Kan experiment to confirm results.~~
2. ~~Set up pilot again with Strep and Hyg diluted 1:2.~~

Results and Data:

Plates seemed dry when spreading. Depending on growth of plates on Thursday, make plates on Monday and consider storing in box in 420. These plates were made on the Friday prior and left out on benchtop in 470.

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, January 20, 2021

To Do:

1. ~~Out of lab for retreat.~~

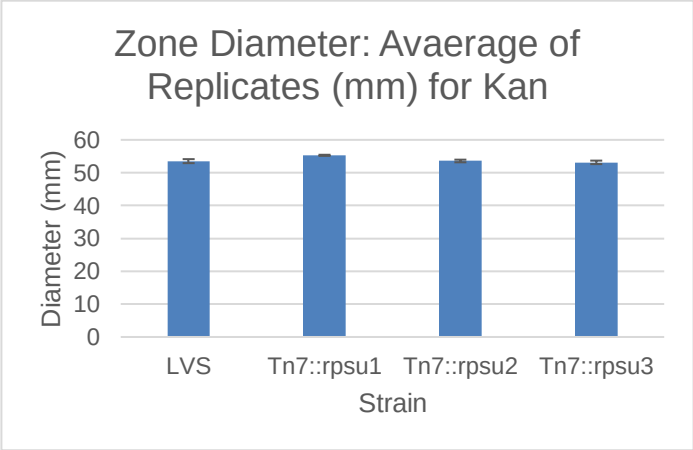
Results and Data:

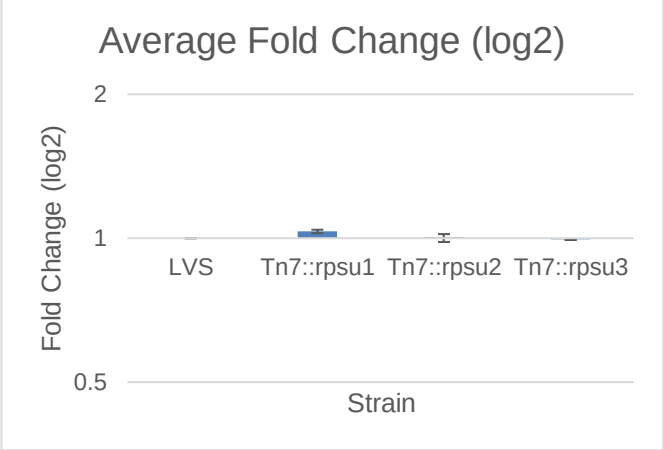
Thursday, January 21, 2021

To Do:

1. ~~Read Kan confirmation plates.~~
2. ~~Read pilot plates for Strep and Hyg.~~

Results and Data:





No difference between strains; matches previous Kan results.

Friday, January 22, 2021

To Do:

1. ~~Pour plates for next week?~~

Results and Data:

Monday, January 25, 2021

To Do:

1. ~~Streak out cells.~~
2. ~~Make plates.~~
3. ~~Check in with Dr. Ramsey about results and moving forward.~~

Results and Data:

For 600 mL of CHA

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)
    a. 150ml per beaker

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, January 26, 2021

To Do:

1. ~~Set up DDA for tetracycline.~~
2. ~~Streak out cells.~~

Results and Data:

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 (*methanol disk in the case of Tet or other methanol soluble antibiotic*) and a Kan disk on each CHAH plate.

Wednesday, January 27, 2021

To Do:

1. ~~Set up DDA for Hygromycin.~~

Results and Data:

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

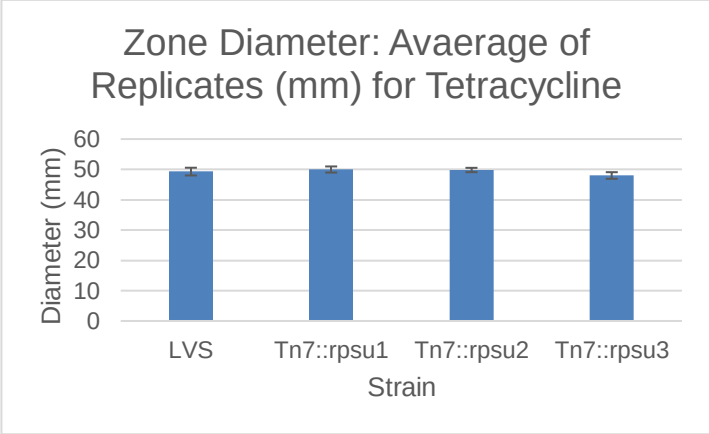
1. Resuspend cells for the strain being tested in about 400 µL of MHB. Measure the OD600.
2. Aim for an OD600 of 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2] Repeat steps 1 and 2 for a separate 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 (*methanol disk in the case of Tet or other methanol soluble antibiotic*) and a Kan disk on each CHAH plate.

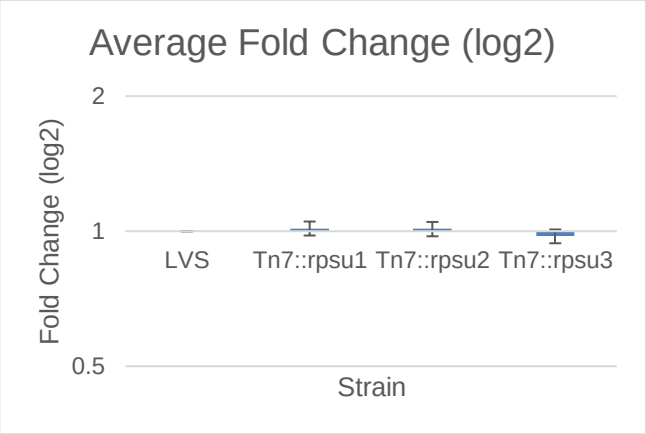
Thursday, January 28, 2021

To Do:

1. ~~Read Tetracycline DDA.~~

Results and Data:



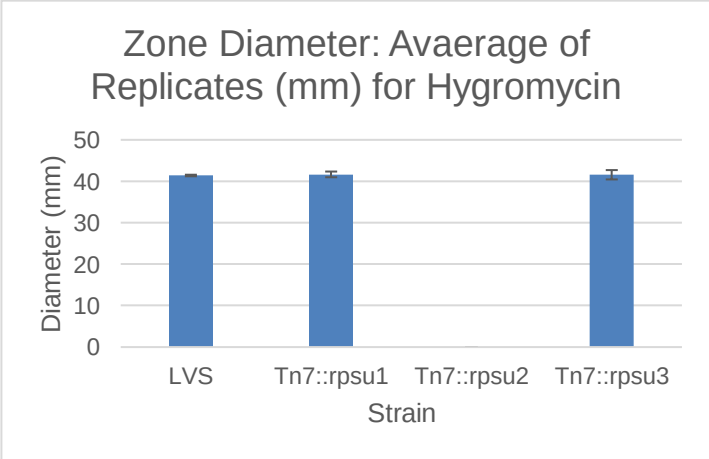


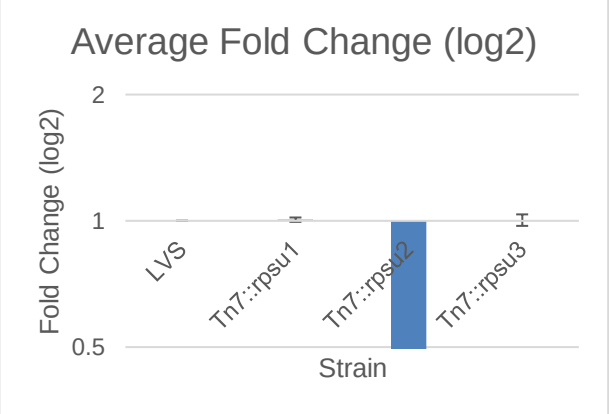
Friday, January 29, 2021

To Do:

1. ~~Read Hygromycin DDA.~~

Results and Data:





No halo on Tn7::rpsu2 plates.

February 2021

Tuesday, February 2, 2021

To Do:

1. ~~Pour plates.~~

2. ~~Streak out cells.~~

Results and Data:

Wednesday, February 3, 2021

To Do:

1. ~~Set up DDA for Strep.~~
2. ~~Keep cells plate for PCR.~~
3. ~~Reconstitute primers.~~

Results and Data:

Reconstitute by spinning down, adding 10*(nmol on side of primer tube) 0.1x EB to make 100uM solution, and diluting 1:10 (180ul EB and 20ul primer). Label tubes and place in freezer boxes.

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 (*methanol disk in the case of Tet or other methanol soluble antibiotic*) and a Kan disk on each CHAH plate.

Friday, February 5, 2021

To Do:

1. ~~PCR with LVS rpsu3 strains and KROL405+406.~~
2. ~~Read Strep plates.~~

Results and Data:

Total reaction volume	20
Total number of reactions	6

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	7
ddiH2O			12.4	86.8
PrimeSTAR GXL Buffer	5x	1x	4	28
dNTPs	2.5 mM	0.2 mM	1.6	11.2
oligo F	10 uM	0.3 uM	0.6	4.2
oligo R	10 uM	0.3 uM	0.6	4.2

template	100 ng/ul	2 ng/ul	0.4	2.8
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	0.4	2.8
		Total volume	20	140

-Take small amounts of each cross-out patch and resuspend in sterile water.

-Heat samples at 95°C for 10' to lyse and kill cells

-Dilute lysates 1:10. For controls, use LVS gDNA, the pEX-based allelic exchange vector, and water only.

Example colony PCR using KOD (use a master mix):

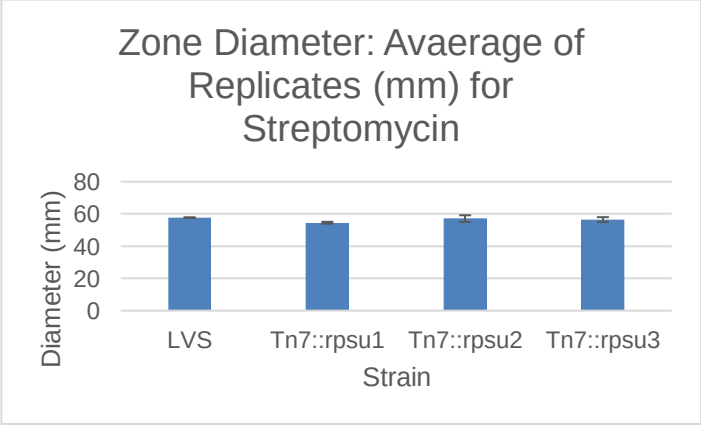
PCR Protocol (updated 7/1/19)

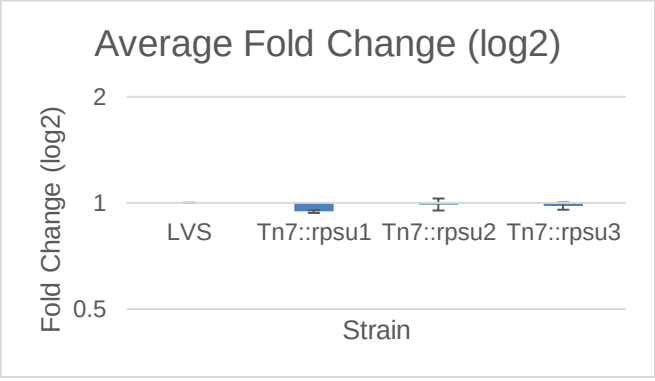
1. Acquire and label PCR tubes. Be sure to include at least 1 positive and 1 negative control for each PCR experiment.
2. Get a container of ice to keep the components on
3. Acquire the following components and put them on ice, labeling tubes if necessary:
    - Molecular grade H2O in 1.5 mL microfuge tube
    - KOD/primestar buffer
    - dNTPs
    - oligo F (10uM)
    - oligo R (10uM)
    - template (eg. LVS gDNA, plasmid, colony, etc.)
    - Note: KOD/primestar enzyme should be kept in the freezer until it is used as it is expensive and should be added last
4. Centrifuge the microfuge tubes to get any solution out of the microfuge tube cover
5. If any of the solutions are frozen, be sure to vortex the microfuge tube in order to dissolve it (tubes with frozen components may not be homogenized)
    - DO NOT vortex the enzyme itself or any solution with enzyme because vortexing will expose it to oxygen and degrade it
6. Use PCR_worksheet.xlsx to make establish the specifics of what will be added
    - The file is located in the Protocols folder
    - Also setup table below to specify which primers and source DNA will be used
7. Add appropriate volume (based on PCR worksheet) of each experiment specific primer (forward and reverse) to PCR tubes
8. Add ddi H2O to negative control tube
    - Template volume for 1 reaction
9. Prepare a master-mix in a 1.5 mL microfuge tube by adding the following according to the worksheet and using micropipettes:
    - Add ddi H2O
    - Add dNTPs
    - Add KOD buffer
    - Add KOD enzyme
10. Mix the master-mix solution by pipetting up and down

- Do not vortex to mix

11. Add appropriate volume of master-mix to negative control PCR tube
12. Add template to Master Mix
    - Factor template volume minus 1 template reaction volume
13. Add appropriate volume of master mix to each PCR tube (except negative control) and pipette up and down to mix (conserves tips)
14. Close PCR Tubes until the caps are tight
15. Place the PCR Tubes in the thermocycler on STN 1 – the following settings should be in place:
    - Heat at 94 degrees for 2 minutes,
    - 94 degrees C for 20 seconds
    - 50 degrees C for 30 seconds
    - 68 degrees C for 1 minute/kb (adjust based on expected size of product)
    - Go back to step 2
    - Repeat 32x
    - 68 degrees C for 5 minutes
    - 12 degrees C for infinity

Reaction Number	Plasmid/Region	Source DNA	Primers	Length (bps)
1	LVS	LVS gDNA	KROL405, KROL406	905
2	Tn7::rpsu1	Tn7::rpsu1 gDNA	KROL405, KROL406	905
3	Tn7::rpsu2	Tn7::rpsu2 gDNA	KROL405, KROL406	905
4	Tn7::rpsu3	Tn7::rpsu3 gDNA	KROL405, KROL406	905
5	pKR56	plasmid	KROL405, KROL406	905
6	Negative control	water	KROL405, KROL406	-





Monday, February 8, 2021

To Do:

1. ~~Run PCR on gel.~~
2. ~~Make more filter paper disks.~~
3. ~~Streak out cells.~~

Results and Data:

Agarose Gel Protocol – written by Joe Paquette

(Note: all ddiH2O is type I)

1. Mix 25x TAE buffer with ddiH_2O to obtain 1x TAE solution.
    - To make 1 L of 1x TAE, add 40 mL 25x TAE into 1 L graduated cylinder and fill to 1 L with ddiH2O.
2. Add 1 g agarose powder to 100 mL 1x TAE buffer in 250 mL.
3. Add stirbar to container.
4. Heat to dissolve the agarose while stirring (don't let over boil, should look like clear liquid, no solids).
5. Once the sugar has dissolved, make a 1x concentration of SYBR safe in the 1% agarose solution.
    - To make, add 10µL of 10,000x SYBR safe dye to the 1% agarose solution
6. Let the 1% agarose solution cool to approximately 50-55°C.
7. Apply autoclave tape to the edges of the gel cast (ensure the tape is tightly bound).
8. Pour 1% agarose - 1x SYBR safe solution into the cast and insert a comb to mold wells in the gel.
9. Let sit until the 1% agarose – 1x SYBR safe solution has cooled and solidified into a gel.
10. Carefully remove the comb.
11. Pour the 100 ml of 1x TAE buffer solution into the gel tank (add just enough to slightly submerge the gel itself).
12. Obtain 5 µL of each PCR sample.
13. Make 1x loading dye in 6 µL of solution.
    - To make 6 µL of 1x loading dye, combine 5 µL PCR sample with 1 µL of 6x loading dye
14. Load 10 µL of 2x log ladder into the first well.
15. Load 5 µL of the PCR-dye mixture into the wells in sequential order.
16. Insert the electrodes and run the gel at about 113 volts.

For 600 mL of CHA

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)
    a. 150ml per beaker

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, February 9, 2021

To Do:

1. ~~Set up DDA with Tetracycline.~~

Results and Data:

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

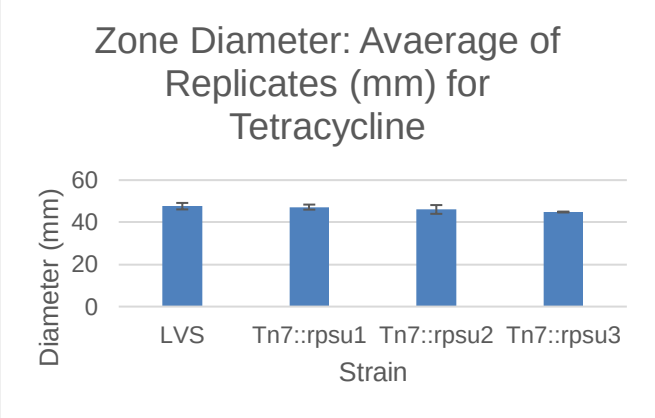
1. Resuspend cells for the strain being tested in about 400 µL of MHB. Measure the OD600.
2. Aim for an OD600 of 0.01. Dilute the appropriate amount of culture in a 15 mL conical that contains media to get required OD600, for a final volume of 10 mL. [Use C1V1=C2V2] Repeat steps 1 and 2 for a separate 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 (*methanol disk in the case of Tet or other methanol soluble antibiotic*) and a Kan disk on each CHAH plate.

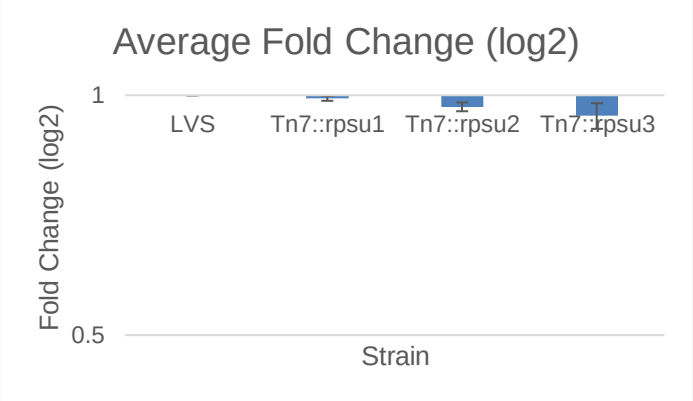
Thursday, February 11, 2021

To Do:

1. ~~Read Tet DDA.~~

Results and Data:





Friday, February 12, 2021

To Do:

1. ~~PCR with KOD enzyme and strains.~~

Results and Data:

-Take small amounts of each cross-out patch and resuspend in sterile water.

-Heat samples at 95°C for 10' to lyse and kill cells

-Dilute lysates 1:10. For controls, use LVS gDNA, the pEX-based allelic exchange vector, and water only.

Total reaction volume	20
Total number of reactions	6

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	7
ddiH2O			4	28
KOD buffer	2x	1x	10	70
dNTPs	2 mM	0.4 mM	4	28
oligo F	10 uM	0.3 uM	0.6	*4.2*
oligo R	10 uM	0.3 uM	0.6	*4.2*
template	100 ng/ul	2 ng/ul	0.4	2.8
KOD	1 U/ul	0.02 U/ul	0.4	2.8
		Total volume	20	131.6

PCR Protocol by John Church (New as of 11/14/18)

1. Acquire and label 4 PCR tubes with initials and designate as Tubes 1-4

- The tubes comes in strips of 8 and they can be split into 4 tube pieces so that the first 3 tubes are used and the fourth is unused

2. Get a container of ice to keep the components on
3. Acquire the following components and put them on ice, labeling tubes if necessary:
    - ddi H2O in 1.5 mL microfuge tube
    - uL KOD buffer
    - dNTPs
    - oligo F
    - oligo R
    - template
    - Note: KOD enzyme should be kept in the freezer until it is used as it is expensive and should be added last
4. Centrifuge the microfuge tubes to get any solution out of the microfuge tube cover
5. If any of the solutions are frozen, be sure to vortex the microfuge tube in order to dissolve it (tubes with frozen components may not be homogenized)
    - DO NOT vortex the KOD enzyme itself or any solution with KOD enzyme because vortexing will expose it to oxygen and degrade it
6. Use PCR_worksheet.xlsx to make establish the specifics of what will be added
    - The file is located in the Protocols folder
    - For this protocol, a “Total reaction volume” of 100 uL and 4 “Total number of reactions” were used – the following volumes are based on these specifications
7. Add 0.75 uL of each experiment specific primer (forward and reverse) to PCR Tubes 1 and 2 (oligos forward and reverse)
    - The amount added should be calculated by taking the total volume for 1 reaction (in worksheet) and subtracting the volumes for 1 reaction that have not yet been added to the master-mix
8. Add 0.75 uL of each control primer (oligos forward and reverse) to PCR Tubes 3 and 4
9. Add 0.5 uL ddi H2O to PCR Tube 4 so that all 4 PCR Tubes have an even amount of solution
    - Template volume for 1 reaction
10. Prepare a master-mix in a 1.5 mL microfuge tube by adding the following according to the worksheet and using micropipettes:
    - Add 27.5 uL ddi H2O
    - Add 27.5 uL dNTPs
    - Add 68.75 uL KOD buffer
    - Add 2.75 uL KOD enzyme
11. Mix the master-mix solution by pipetting up and down
    - Do not vortex to mix
12. Add 23 uL of master-mix to PCR Tube 4
13. Add 2.25 uL template to Master Mix
    - Factor template volume minus 1 template reaction volume.
14. Add 23.5 uL master mix to each PCR Tube 1-3 and pipette up and down to mix (conserves tips)

15. Close PCR Tubes 1-4 until the caps are tight (push until the caps do not squeak when you push on them)
16. Place the PCR Tubes in the thermocycler on STN 1 – the following settings should be in place:
    - Heat at 94 degrees for 2 minutes,
    - 94 degrees C for 20 seconds
    - 50 degrees C for 30 seconds
    - 68 degrees C for 1 minute
    - Go back to step 2
    - Repeat 32x
    - 68 degrees C for 5 minutes
    - 12 degrees C for infinity

Monday, February 15, 2021

To Do:

1. ~~Run PCR gel.~~
2. ~~Make plates (need 18).~~
3. ~~Streak out cells.~~

Results and Data:

Agarose Gel Protocol – written by Joe Paquette

(Note: all ddiH2O is type I)

1. Mix 25x TAE buffer with ddiH_2O to obtain 1x TAE solution.

    - To make 1 L of 1x TAE, add 40 mL 25x TAE into 1 L graduated cylinder and fill to 1 L with ddiH2O.

2. Add 1 g agarose powder to 100 mL 1x TAE buffer in 250 mL.
3. Add stirbar to container.
4. Heat to dissolve the agarose while stirring (don't let over boil, should look like clear liquid, no solids).
5. Once the sugar has dissolved, make a 1x concentration of SYBR safe in the 1% agarose solution.
    - To make, add 10µL of 10,000x SYBR safe dye to the 1% agarose solution
6. Let the 1% agarose solution cool to approximately 50-55°C.
7. Apply autoclave tape to the edges of the gel cast (ensure the tape is tightly bound).
8. Pour 1% agarose - 1x SYBR safe solution into the cast and insert a comb to mold wells in the gel.
9. Let sit until the 1% agarose – 1x SYBR safe solution has cooled and solidified into a gel.
10. Carefully remove the comb.
11. Pour the 100 ml of 1x TAE buffer solution into the gel tank (add just enough to slightly submerge the gel itself).
12. Obtain 5 µL of each PCR sample.
13. Make 1x loading dye in 6 µL of solution.

- To make 6 µL of 1x loading dye, combine 5 µL PCR sample with 1 µL of 6x loading dye. *Used 20ul sample with 4ul dye.*

14. Load 10 µL of 2x log ladder into the first well.
15. Load 5 µL of the PCR-dye mixture into the wells in sequential order.
16. Insert the electrodes and run the gel at about 113 volts.

For 600 mL of CHA

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)
    a. 150ml per beaker

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, February 16, 2021

To Do:

1. ~~Streak out cells.~~
2. ~~Set up DDA for Hygromycin confirmation.~~

Results and Data:

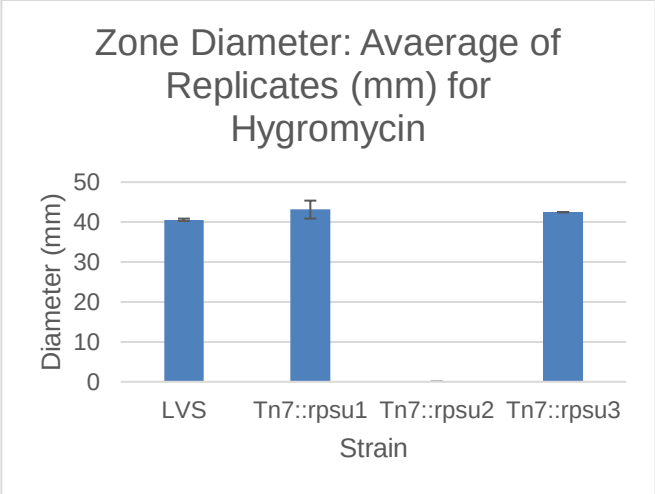
Thursday, February 18, 2021

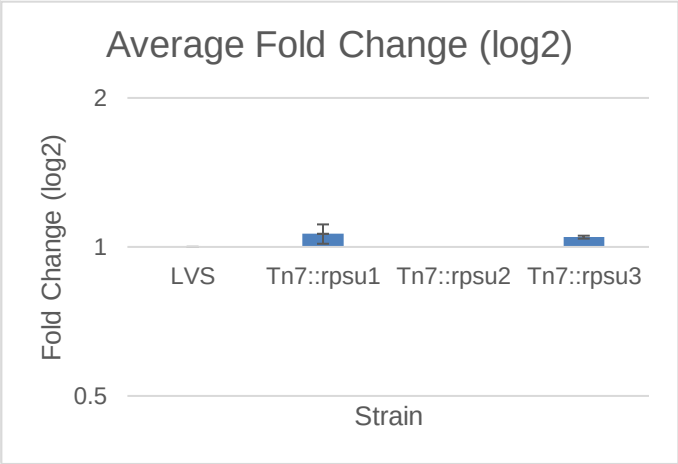
To Do:

1. ~~Read DDA for Hyg plates.~~

Results and Data:

Tn7::rpsu3 plates had single colonies inside halo, have not observed previously. Tn7::rpsu2 had no halo again.





Monday, February 22, 2021

To Do:

1. ~~Streak out cells.~~

Results and Data:

Tuesday, February 23, 2021

To Do:

1. ~~Set up Strep DDA for confirmation.~~

Results and Data:

Wednesday, February 24, 2021

To Do:

1. ~~Reconstitute primers.~~
2. ~~Set up PCR with KROL409 and 410.~~

Results and Data:

Mix media

For 600 mL of CHA

1. Weigh out 15.3g of cystine heart agar into 0.5L flask (non-baffled; 10.2g/100mL)
    a. 15.3g in 1 500ml flasks and use pressure cooker
2. Add 300mL of ddiH_2O (type I)
    a. 150ml per beaker

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 only 150ml hemoglobin into CHA using serological pipette

For CHAH-Hyg plates: full flask

Add 1.11mL of 54mg/mL hygromycin (stored at 4 ℃)

12. Using a 50mL pipet, aliquot 24mL of CHAH mixture into each 100mm plate (should make approximately 25 plates) Try to avoid bubbles!

Thursday, February 25, 2021

To Do:

1. ~~Read DDA for Strep confirmation.~~
2. ~~Check isolation plates.~~
3. ~~Design primers for 16S gene in *Francisella*.~~

Results and Data:

Friday, February 26, 2021

To Do:

1. ~~Set up PCR.~~

Results and Data:

Reconstitute by spinning down, adding 10*(nmol on side of primer tube) 0.1x EB to make 100uM solution, and diluting 1:10 (180ul EB and 20ul primer). Label tubes and place in freezer boxes.

Total reaction volume	20
Total number of reactions	6

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	7
ddiH2O			4	28
KOD buffer	2x	1x	10	70
dNTPs	2 mM	0.4 mM	4	28
oligo F	10 uM	0.3 uM	0.6	**4.2**
oligo R	10 uM	0.3 uM	0.6	**4.2**
template	100 ng/ul	2 ng/ul	0.4	2.8

KOD	1 U/ul	0.02 U/ul	0.4	2.8
		Total volume	20	131.6

PCR Protocol (updated 7/1/19)

1. Acquire and label PCR tubes. Be sure to include at least 1 positive and 1 negative control for each PCR experiment.
2. Get a container of ice to keep the components on
3. Acquire the following components and put them on ice, labeling tubes if necessary:
    - Molecular grade H2O in 1.5 mL microfuge tube
    - KOD/primestar buffer
    - dNTPs
    - oligo F (10uM)
    - oligo R (10uM)
    - template (eg. LVS gDNA, plasmid, colony, etc.)
    - Note: KOD/primestar enzyme should be kept in the freezer until it is used as it is expensive and should be added last
4. Centrifuge the microfuge tubes to get any solution out of the microfuge tube cover
5. If any of the solutions are frozen, be sure to vortex the microfuge tube in order to dissolve it (tubes with frozen components may not be homogenized)
    - DO NOT vortex the enzyme itself or any solution with enzyme because vortexing will expose it to oxygen and degrade it
6. Use PCR_worksheet.xlsx to make establish the specifics of what will be added
    - The file is located in the Protocols folder
    - Also setup table below to specify which primers and source DNA will be used
7. Add appropriate volume (based on PCR worksheet) of each experiment specific primer (forward and reverse) to PCR tubes
8. Add ddi H2O to negative control tube
    - Template volume for 1 reaction
9. Prepare a master-mix in a 1.5 mL microfuge tube by adding the following according to the worksheet and using micropipettes:
    - Add ddi H2O
    - Add dNTPs
    - Add KOD buffer
    - Add KOD enzyme
10. Mix the master-mix solution by pipetting up and down
    - Do not vortex to mix
11. Add appropriate volume of master-mix to negative control PCR tube
12. Add template to Master Mix
    - Factor template volume minus 1 template reaction volume
13. Add appropriate volume of master mix to each PCR tube (except negative control) and pipette up and down to mix (conserves tips)
14. Close PCR Tubes until the caps are tight
15. Place the PCR Tubes in the thermocycler on STN 1 – the following settings should be in place:

- Heat at 94 degrees for 2 minutes,
- 94 degrees C for 20 seconds
- 50 degrees C for 30 seconds
- 68 degrees C for 1 minute/kb (adjust based on expected size of product)
- Go back to step 2
- Repeat 32x
- 68 degrees C for 5 minutes
- 12 degrees C for infinity

Reaction Number	Plasmid/Region	Source DNA	Primers	Length (bps)
1	LVS	LVS gDNA	KROL409, KROL410	500
2	Tn7::rpsu1	Tn7::rpsu1 gDNA	KROL409, KROL410	500
3	Tn7::rpsu2	Tn7::rpsu2 gDNA	KROL409, KROL410	500
4	Tn7::rpsu3	Tn7::rpsu3 gDNA	KROL409, KROL410	500
5	pKR56	plasmid	KROL409, KROL410	500
6	Negative control	water	KROL409, KROL410	-

March 2021

Monday, March 1, 2021

To Do:

1. ~~Run agarose gel for PCR.~~
2. ~~Patch single colonies to CHA and CHA-Hyg.~~
3. ~~Pour plates.~~

Results and Data:

Talked with Kathryn and Hannah about PCR gel results. There appears to be matching bands in Tn7::rpsu2 and the + control pKR56 lanes, signifying Hygromycin resistance in the rpsu2 strain. Any new work on the rpsu2 strain and Hyg will be put on hold, but I will move forward with the rpsu3 strain and the single colonies. I am planning to run a colony PCR later to confirm that the strain is rpsu2 and no rpsu2 has been mixed in.

Agarose Gel Protocol – written by Joe Paquette

(Note: all ddiH2O is type I)

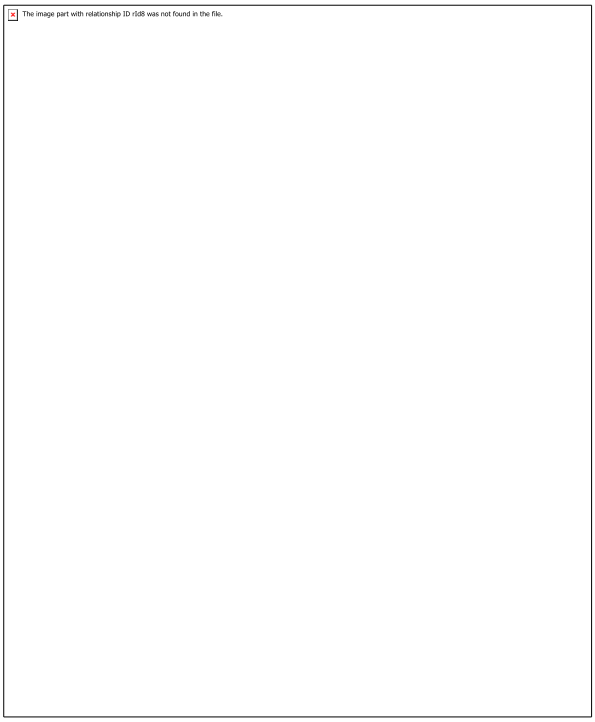
1. Mix 25x TAE buffer with ddiH_2O to obtain 1x TAE solution.

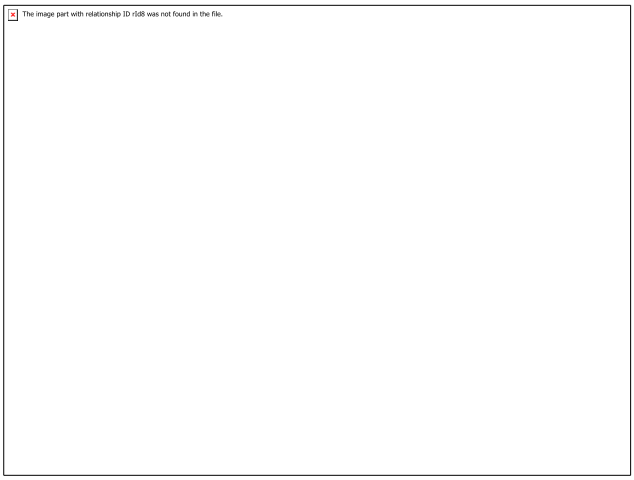
- To make 1 L of 1x TAE, add 40 mL 25x TAE into 1 L graduated cylinder and fill to 1 L with ddiH2O.

2. Add 1 g agarose powder to 100 mL 1x TAE buffer in 250 mL.
3. Add stirbar to container.
4. Heat to dissolve the agarose while stirring (don't let over boil, should look like clear liquid, no solids).
5. Once the sugar has dissolved, make a 1x concentration of SYBR safe in the 1% agarose solution.
   - To make, add 10µL of 10,000x SYBR safe dye to the 1% agarose solution
6. Let the 1% agarose solution cool to approximately 50-55°C.
7. Apply autoclave tape to the edges of the gel cast (ensure the tape is tightly bound).
8. Pour 1% agarose - 1x SYBR safe solution into the cast and insert a comb to mold wells in the gel.
9. Let sit until the 1% agarose – 1x SYBR safe solution has cooled and solidified into a gel.
10. Carefully remove the comb.
11. Pour the 100 ml of 1x TAE buffer solution into the gel tank (add just enough to slightly submerge the gel itself).
12. Obtain 5 µL of each PCR sample.
13. Make 1x loading dye in 6 µL of solution.

    - To make 6 µL of 1x loading dye, combine 5 µL PCR sample with 1 µL of 6x loading dye. *Used 20ul sample with 4ul dye.*

14. Load 10 µL of 2x log ladder into the first well.
15. Load 5 µL of the PCR-dye mixture into the wells in sequential order.
16. Insert the electrodes and run the gel at about 113 volts.





Wednesday, March 3, 2021

To Do:

1. ~~Scrape up patches from rpsu3 single colony plates.~~

Results and Data:

51/60 cross patches grew on Hyg media.

Making Glycerol Stocks Protocol

1. Make ~~3 cryovials~~ 1 cryovial for each ~~strain~~ patch (permanent stocks), label!
2. Prepare ~~2.4mL~~ 800ul of MHB in ~~a 50mL conical~~ cryovial (adjust if you are also making single use stocks)
3. Take ~~at least half of a thickly spread plate~~ most of patch and add cells to the MHB tube
4. Resuspend until there are no clumps in the MHB
5. Add ~~600ul~~ 200ul 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

1 – 1

2 – 2

3 – 4

4 – 5

5 – 6

6 – 8

7 – 9

8 – 49

9 – 57

10 – 58

Friday, March 5, 2021

To Do:

1. ~~Colony PCR with rpsu3 single colonies.~~
2. ~~Run gel.~~

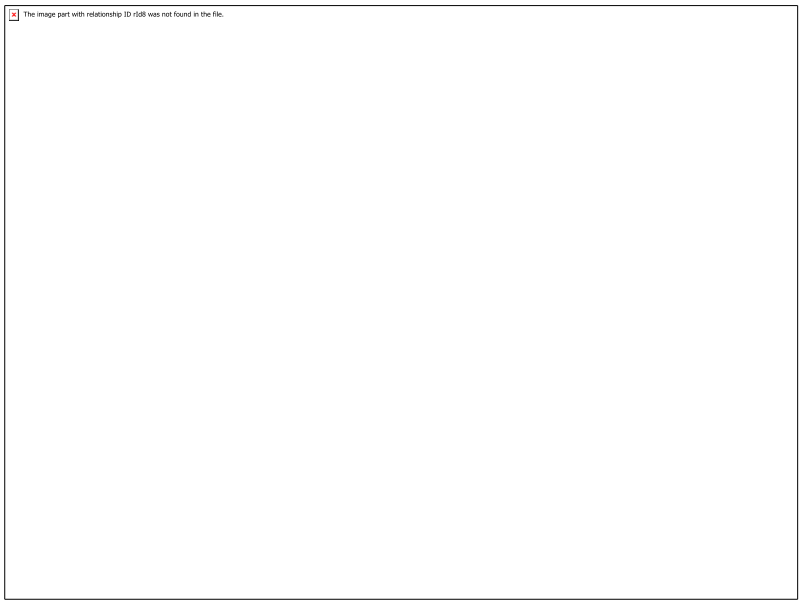
Results and Data:

Reaction Number	Plasmid/Region	Source DNA	Primers	Length (bps)
1	LVS gDNA		KROL409, KROL410	-
2	Patch 1		KROL409, KROL410	
3	Patch 2		KROL409, KROL410	
4	Patch 4		KROL409, KROL410	
5	Patch 5		KROL409, KROL410	
6	Patch 6		KROL409, KROL410	
7	Patch 8		KROL409, KROL410	
8	Patch 9		KROL409, KROL410	
9	Patch 49		KROL409, KROL410	
10	Patch 57		KROL409, KROL410	
11	Patch 58		KROL409, KROL410	
12	Tn7::rpsu2 gDNA		KROL409, KROL410	500
13	Tn7::rpsu3 gDNA		KROL409, KROL410	-
14	Water	- control	KROL409, KROL410	-

Total reaction volume	20
Total number of reactions	14

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	15
ddiH2O			4	60
KOD buffer	2x	1x	10	150
dNTPs	2 mM	0.4 mM	4	60
oligo F	10 uM	0.3 uM	0.6	9
oligo R	10 uM	0.3 uM	0.6	9
template	100 ng/ul	2 ng/ul	0.4	6
KOD	1 U/ul	0.02 U/ul	0.4	6
		Total volume	20	282

For gel: 6ul SYBR safe for small gel, 10ul for large gel.



Monday, March 8, 2021

To Do:

1. ~~Talk with Hannah about gel results.~~
2. ~~Prep for antibiotic pilot assays.~~
3. ~~Pour plates.~~
4. ~~Streak out LVS cells.~~

Results and Data:

I talked to Hannah about my gel results. The gel shows bands for all 10 patches, so it is likely that these patches were contaminated with Tn7::rpsu3 at some point. There also appears to be a band in the rpsu3 lane, though it is possible that this is spill over. Hannah is having WGS done on the Tn7 strains, so

anything wrong with the strains should be identified then. I will plan to move forward with more antibiotic assays this week.

Hannah found Kasugamycin and ciproflaxin in a box in the -20 named tetracycline. This box can be renamed. Ciproflaxin does not target ribosomes, so it can be used as a control (haloes should all be the same size).

For 600 mL of CHA

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)
    a. 150ml per beaker

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, March 9, 2021

To Do:

1. ~~Pilot assay for kasugamycin and ciproflaxin.~~

Results and Data:

Pilots are done in duplicate and I have 2 abx to test, so I will only need 4 plates for this assay.

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, March 10, 2021

To Do:

1. ~~PCR with 2 single colony patches and KROL252 and 253.~~
2. ~~Run gel.~~

Results and Data:

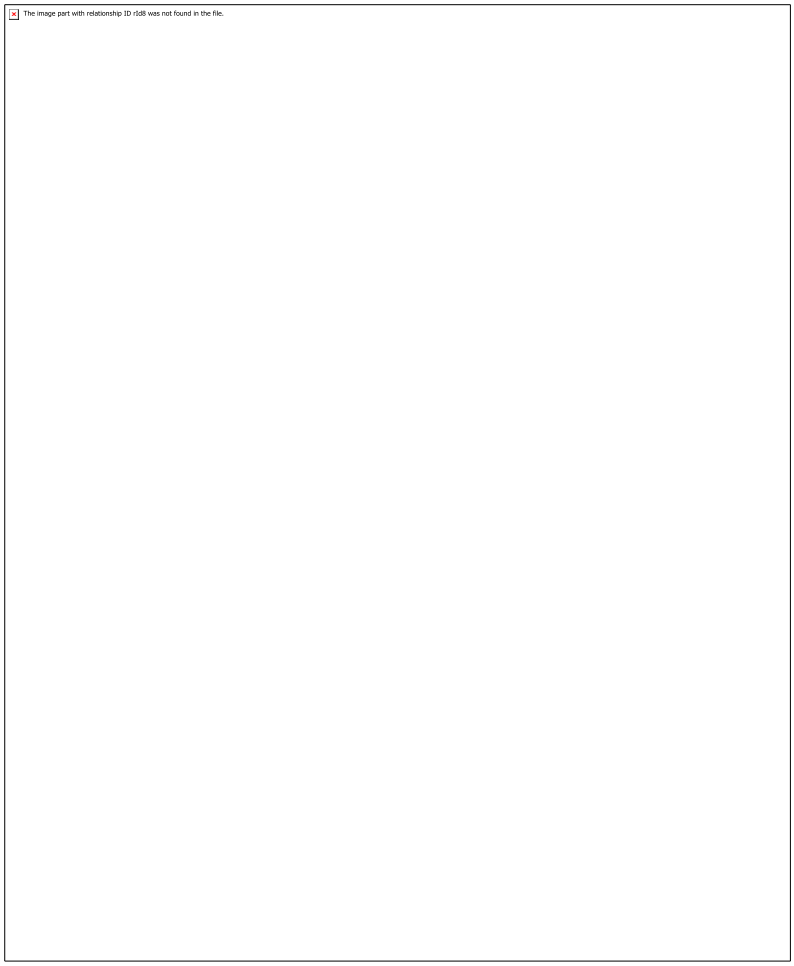
-Do a 50 uL PCR for any two of the 10 patches you made, as well as Tn7::rpsU2 gDNA, Tn7::rpsU3 gDNA and – control using KROL252 and 253 (total 5 samples)
- run 5 ul on a gel to make sure there is a band (should be ~2300 bps); if looks good, can throw out – control and proceed with only the 4 samples
- PCR purify with QIAgen kit, eluting in 35 uL of 0.1x EB
- Diagnostic digest with DraI (20 uL total volume, use Restriction Enzyme Digest Worksheet in the protocols folder); incubate for 1 hour - overnight
- run entire volume on a 1% agarose gel:
Expected that rpsU2 has 1 cut site so you should get two bands; but rpsU3 has 0 cut sites so you should get 1 band

Total reaction volume	50
Total number of reactions	5

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	6
ddiH2O			10	60
KOD buffer	2x	1x	25	150
dNTPs	2 mM	0.4 mM	10	60
oligo F	10 uM	0.3 uM	1.5	9
oligo R	10 uM	0.3 uM	1.5	9
template	100 ng/ul	2 ng/ul	1	6
KOD	1 U/ul	0.02 U/ul	1	6
		Total volume	50	282

Reaction Number	Plasmid/Region	Source DNA	Primers	Length (bps)
1	Tn7::rpsu2 gDNA		KROL252 and 253	2300
2	Tn7::rpsu3 gDNA		KROL252 and 253	2300

3	Patch 1	gDNA made from single colonies	KROL252 and 253	2300
4	Patch 2		KROL252 and 253	2300
5	- control	-	KROL252 and 253	-



Thursday, March 11, 2021

To Do:

1. ~~Image plates.~~

Results and Data:

The Kasugamycin pilots look good. The ciproflaxin pilots appear to have very little growth, so I will half the concentrations of antibiotic and run another pilot with the Kasugamycin DDA.

The PCR tubes were placed in John and Joe's colony PCR box at -20.

Monday, March 15, 2021

To Do:

1. ~~PCR purification and digest.~~
2. ~~Make plates.~~

Results and Data:

QIAquick PCR Purification Kit Protocol

1. Add 5 volumes Buffer PB to 1 volume of the PCR reaction and mix. (Have approximately 100 uL of PCR, so 500 uL Buffer PB will be used.) 225ul
2. Place a QIAquick column in the provided 2 mL tube.
3. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30-60s. Discard flow-through and place the QIAquick column back in the same tube.
4. To wash, add 750 uL Buffer PE to the QIAquick column and centrifuge for 30-60s. Discard flow-through and place the QIAquick column back in the same tube.
5. Centrifuge the QIAquick column once more in the provided 2 mL collection tube for 1 min to remove the residual wash buffer.
6. Place each QIAquick column in a clean 1.5 mL microcentrifuge tube.
7. To elute DNA, add 35 uL 0.1x Buffer EB (10mM Tris.Cl, pH 8.5) or water (pH 7.0-8.5) to the center of the QIAquick membrane and centrifuge the column for 1 min.

For diagnostic digest:

Number of samples	**4**
Master mix factor	**5**

Components	Volumes in 1 reaction (uL)	Volumes in Master Mix (uL)
H_2O	12	60
10x Buffer*	2	10
DNA	(5)	
Enzyme 1 (DraI)	0.5	2.5
Enzyme 2 (water)	0.5	2.5
Total	20.0 (15.0 actual b/c of DNA)	75

DNA digest

Protocol by John Church

1. Make a reaction table with desired digests:

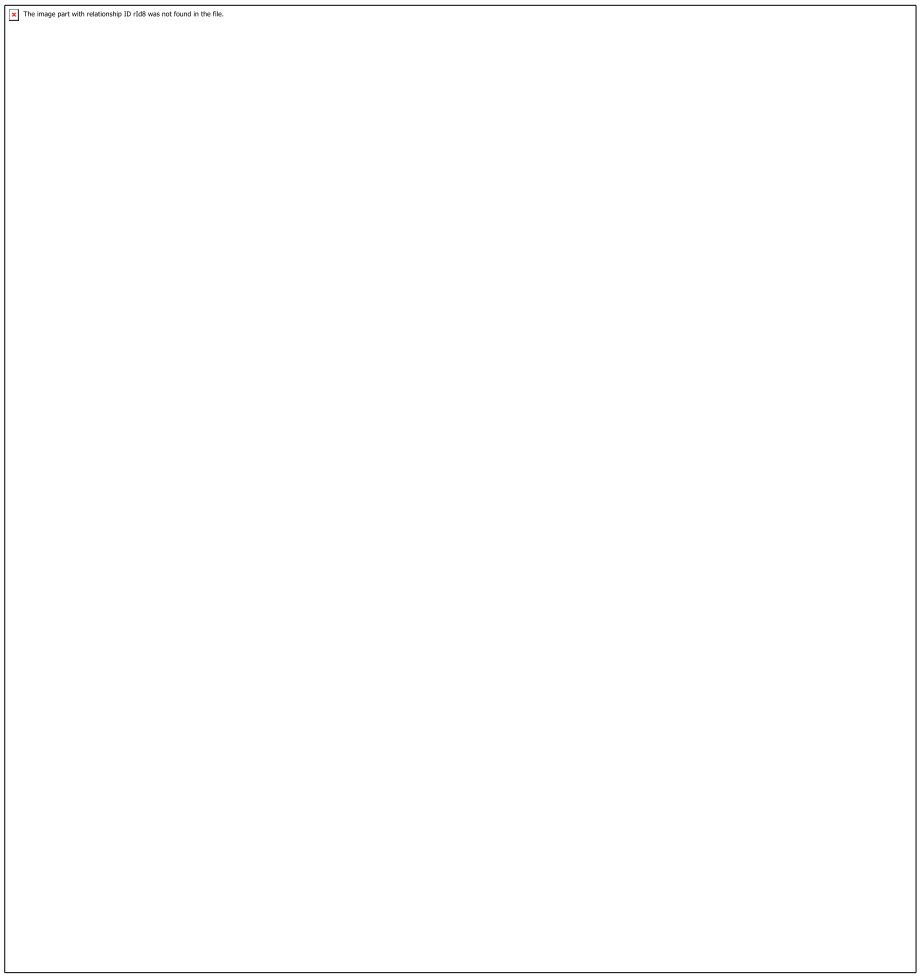
Tube	DNA	Enzyme(s)	DNA Volume (uL)	H_2O Volume (uL)
1	Tn7::rpsu2 gDNA	DraI, water	-	-
2	Tn7::rpsu3 gDNA	DraI, water	-	-

3	Patch 1	DraI, water		
4	Patch 2	DraI, water		

2. Set up master mix table:

*Cutsmart Buffer is used for all the “HF” enzymes. Check the NEB website for buffer compatibility with non-HF restriction enzymes.

3. Add indicated amounts of H_2O (___uL) and 10x buffer (_____uL) to master mix tube (MM).
4. Add indicated amount of DNA (___uL) to individual tubes for digest (1.5 mL microfuge tubes for digests in incubator, PCR strip tubes for reactions incubated in the thermocycler.
5. Add indicated amount of each enzyme (___uL) to the master mix tube (MM).
6. Mix the master mix by pipetting up and down.
7. Add master mix to individual tubes. Volume to add to each tube is the volume in 1 reaction minus the DNA volume (____ul).
8. Incubate at 37°C for 1 hour or up to overnight.
9. If using digest for plasmid construction then after incubation at 37°C, add 0.5ul of CIP enzyme to backbone tube, mix by pipetting and put back in37°C incubator for 10 minutes. (this step removes the phosphates from the ends of the plasmid to prevent re-ligation)



The colonies clearly appear to be Tn7::rpsu2 strains, indicating strain contamination and explaining hygromycin resistance.

Tuesday, March 16, 2021

To Do:

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

Results and Data:

Need 14 plates this week for assays. 12 for kasugamycin and 2 for another ciproflaxin pilot.

For 600 mL of CHA

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)
    a. 150ml per beaker

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!

Wednesday, March 17, 2021

To Do:

1. ~~DDA for kasugamycin.~~
2. ~~Pilot ciproflaxin for second time.~~

Results and Data:

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.

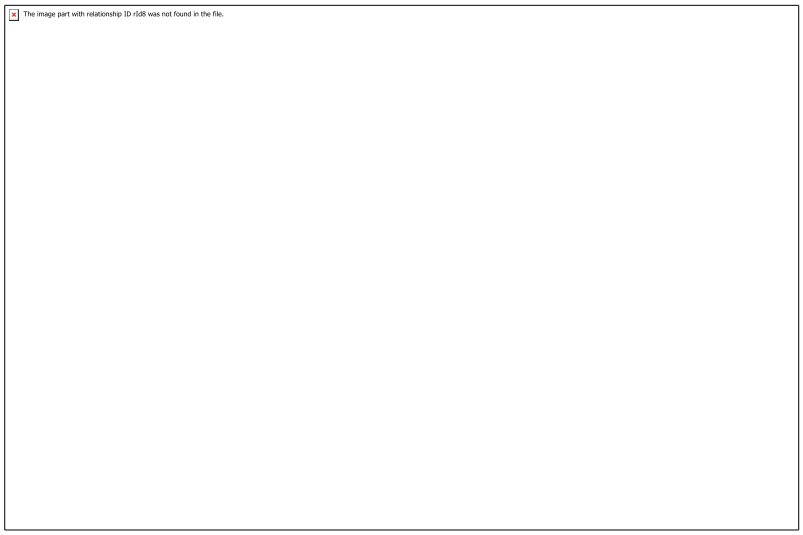
Friday, March 19, 2021

To Do:

1. ~~Read DDA plates.~~

Results and Data:

Note that there was still little to no growth on the ciprofloxacin plates. I will try using different dilutions to pilot in the future.



Monday, March 22, 2021

To Do:

1. ~~Work on paper presentation.~~
2. ~~Meet with Dan and Kathryn about presentation.~~

Results and Data:

Tuesday, March 23, 2021

To Do:

1. ~~Paper presentation at lab meeting.~~
2. ~~Make plates for assays this week.~~

Results and Data:

Wednesday, March 24, 2021

To Do:

1. ~~Repeat Kasugamycin assay~~
2. ~~Make more kasugamycin in Gregory lab.~~
3. ~~Pilot more ciprofloxacin concentrations.~~

Results and Data:

8 plates for kasugamycin,

6 plates for another ciprofloxacin pilot.

Need 14 plates total for assays.

Made more kasugamycin in the Gregory lab with Hannah. Used 0.050g or 50mg and 1ml MG H2O and then filter sterilized to produce 50mg/ml solution. I lost some of the solution in the syringe filter sterilizer, but this should be ok.

Diluted ciprofloxacin 1:4, 1:10, and 1:50.

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.

Thursday, March 25, 2021

To Do:

1. ~~Clean up work bench.~~
2. ~~Streak out cells to make more single use aliquots on Friday.~~

Results and Data:

Friday, March 26, 2021

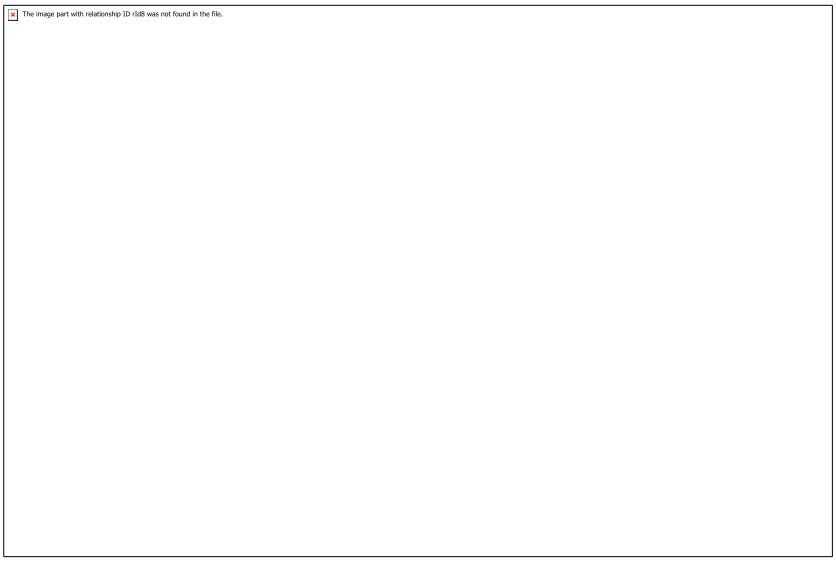
To Do:

1. ~~Make single use aliquots for Tn7::rpsu1, 2, and 3.~~
2. ~~Image plates from repeat kasugamycin assay and Ciprofloxacin pilot.~~

Results and Data:

Making Glycerol Stocks Protocol

1. ~~Make 3 cryovials for each strain patch (permanent stocks), label!~~
2. Prepare ~~2.4mL~~ 1ml of MHB in ~~a 50mL conical~~ cryovial (adjust if you are also making single use stocks)
3. Take ~~at least half of a thickly spread plate~~ most of patch and add cells to the MHB tube
4. Resuspend until there are no clumps in the MHB
5. Add ~~600ul~~ 250ul 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



Monday, March 29, 2021

To Do:

1. ~~Meet with Kathryn about recent results.~~
2. ~~Make CHA plates~~
3. ~~Streak out cells to test new single use aliquots.~~

Results and Data:

Met with Kathryn. Since there is a similar trend in the kasugamycin data but the numbers are different, I will repeat the assay this week in triplicate. Additionally, I am going to go back to my old data and see if the assays had similar diameters or if just the trends were the same. I will make a table of this data to indicate each strain's zone of inhibition for a given antibiotic. If the diameters are similar in the data, then I will average them for this table. If they are different, like in the kasugamycin assays, I will use a representative sample. Moving forward, Kathryn will check with the Gregory lab and see if they have any other antibiotics that I can run my assays with. If not, I may start running my assays with the double deletion strains that Tala was using and compare them to my data. Specifically, this may help uncover whether the differences between strains are due to the amount of bS21 or the identity of it. For my lab presentation, I will include the table of antibiotics and strains and a brief summary about the MICs that did not work out. I will also include a little bit about the mechanisms of the different antibiotics I am working with.

Tuesday, March 30, 2021

To Do:

1. ~~Streak out cells.~~
2. ~~Update lab notebook from meeting with Kathryn.~~
3. ~~Work on table for zone of inhibition assays.~~

Results and Data:

Wednesday, March 31, 2021

To Do:

1. ~~Set up DDA for kasugamycin again in triplicate.~~
2. ~~Repeat Ciprofloxacin pilot at 1:100 concentration..~~

Results and Data:

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.

April 2021

Friday, April 2, 2021

To Do:

1. ~~Meet with Kathryn.~~
2. ~~Read DDA plates for kasugamycin.~~

Results and Data:

I will add another table to the spreadsheet I made with standard deviations for reference. I will also add another table and highlight interesting differences (i.e. if a replicate differs from WT average by >3mm). It would also be good to do another kanamycin assay. Meeting with Kathryn again on Monday at 1pm.

Monday, April 5, 2021

To Do:

1. ~~Meet with Kathryn about presentation.~~
2. ~~Talk to Hannah about bS21 protein image for presentation.~~

Results and Data:

Made adjustments to presentation. Spoke to Kathryn about future directions with kasugamycin.

Tuesday, April 6, 2021

To Do:

1. ~~Present in lab.~~
2. ~~Make half flask of plates.~~
3. ~~Streak out cells.~~

Results and Data:

For 600 mL of CHA

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)
    a. 150ml per beaker

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!

Wednesday, April 7, 2021

To Do:

1. ~~Set up DDA for Kanamycin, tetracycline, and ciprofloxacin pilot.~~

Results and Data:

Kanamycin in triplicate, tetracycline in duplicate, and 2 plates for ciprofloxacin pilot. Need 22 plates total.

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, April 9, 2021

To Do:

1. ~~Read plates.~~

Results and Data:

Kanamycin

Strain	Rep 1 Zone (mm)	Rep 2 Zone (mm)	Rep 3 Zone (mm)	Average of Replicates	Std Dev
LVS	18.265	54.241	53.374	53.8075	0.613061579
Tn7::rpsu1	54.375	54.415	53.218	54.00266667	0.679835519
Tn7::rpsu2	53.187	53.704	54.269	53.72	0.54117742
Tn7::rpsu3	56.049	57.226	55.418	56.231	0.917637728

Tetracycline

Strain	Rep 1 Zone (mm)	Rep 2 Zone (mm)	Rep 3 Zone (mm)	Average of Replicates	Std Dev
LVS	45.364	46.69		46.027	0.937623592
Tn7::rpsu1	46.798	44.815		45.8065	1.402192747
Tn7::rpsu2	50.341	47.602		48.9715	1.936765474
Tn7::rpsu3	47.438	47.032		47.235	0.287085353

The tetracycline and kanamycin assays look more consistent with some of my previous data. All of the average zone of inhibition diameters were within 3mm of the average LVS diameter from previous assays. I will check in with Kathryn and Hannah and see if I should include all data I have recorded previously in the table of results I made or if I should remove data that is inconsistent in favor of the newer, more consistent data. The ciprofloxacin pilot plates still have relatively large haloes that just come into contact with the water control disks, but this may be ok.

Monday, April 12, 2021

To Do:

1. ~~Clean up in lab.~~
2. ~~Check in with Hannah and Kathryn about results from last weeks assays.~~

Results and Data:

Kathryn recommended that I add all data to the table I made so that all assays are present and that I move forward with ciprofloxacin at a 1:500 dilution.

Tuesday, April 13, 2021

To Do:

1. ~~Make CHA plates.~~
2. ~~Make more filter paper disks.~~
3. ~~Streak out cells.~~

Results and Data:

Wednesday, April 14, 2021

To Do:

1. ~~Set up DDA for ciprofloxacin at 1:500 dilution.~~

Results and Data:

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, April 16, 2021

To Do:

1. ~~Read DDA plates.~~

Results and Data:

Monday, April 19, 2021

To Do:

1. ~~Meet with Kathryn about Kasugamycin tube assay.~~

Results and Data:

Tuesday, April 27, 2021

To Do:

1. ~~Make CHA plates for DDA.~~

Results and Data:

Wednesday, April 28, 2021

To Do:

1. ~~Set up ciprofloaxacin assay in lab.~~

Results and Data:

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, April 30, 2021

To Do:

1. ~~Read ciprofloxacin assay results..~~

Results and Data:

May 2021

Monday, May 3, 2021

To Do:

1. ~~Talk to Kathryn and Hannah about setting up assay with double deletion strains in lab.~~

Results and Data:

I will set up a final disk diffusion assay in lab, rather than try to pilot a tube assay for kasugamycin. Hannah struck out the d1/d2 strain for me. Tomorrow I will streak out LVS, 2, and 3 strains for assay and make CHA plates.

Tuesday, May 4, 2021

To Do:

1. ~~Make CHA plates.~~
2. ~~Streak out cells for assay.~~

Results and Data:

For 600 mL of CHA

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)
    a. 150ml per beaker

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!

Wednesday, May 5, 2021

To Do:

1. ~~Set up DDA assay with kasugamycin.~~

Results and Data:

Friday, May 7, 2021

To Do:

1. ~~Check DDA plates.~~

Results and Data:

Plates did not grow well enough and will be given another day in the standing incubator. Pictures of d1/d3 were taken.

Saturday, May 8, 2021

To Do:

1. ~~Read DDA plates.~~

Results and Data:

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.