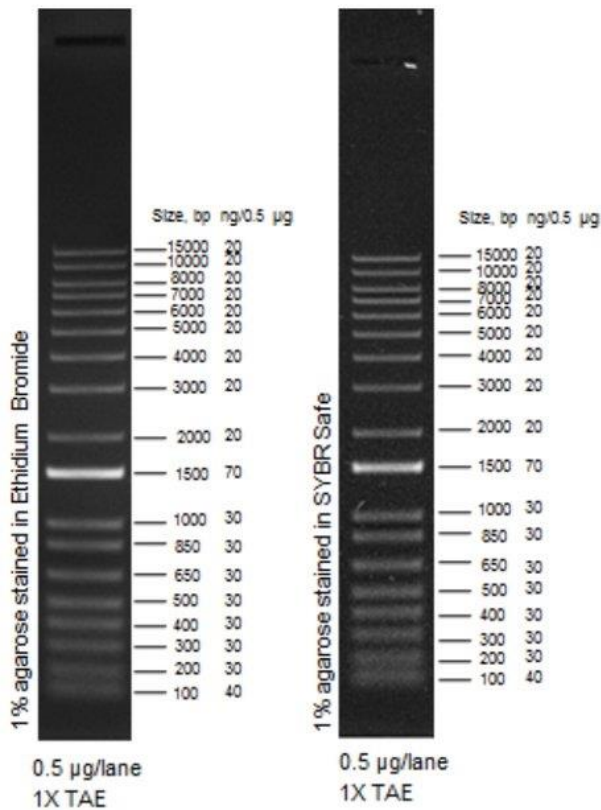


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DNA ladder used throughout this notebook.

January 2022

QIAquick Gel Extraction Kit

1. Weigh the gel slice in a 2ml tube. Add 3 volumes Buffer QG to 1 volume gel.
 - a. Tube 1 = 0.27g so 810ul of QG
 - b. Tube 2 = 0.25g so 750ul of QG
2. Incubate at 53°C for 5 minutes or until fully dissolved. Vortex every 2-3 minutes to help dissolve. After dissolved make sure that the mixture is yellow.
 - a. Had to wait for the heat block to cool down for 20 min so that's why only 5 min. It was already mostly dissolved
3. Add 1 gel volume isopropanol to the sample and mix.

- a. Tube 1 = 270 ul
- b. Tube 2 = 250ul
4. Add 750 ul at first then spin down and then add remaining.
5. Place a column in collection tube and centrifuge for 1 minute at 13000 rpm. Discard flow through and place the column back into the same tube.
6. Add 500 ul of Buffer QG to the column and centrifuge for 1 minute. Discard flow through and place the column back into the same tube.

Hannah did the rest of these steps. Thanks Hannah!

7. To wash, add 750ul of Buffer PE to column and centrifuge for 1 minute. Discard flow through and place the column back into the same tube. Centrifuge for 3 minutes to remove residual wash buffer.
8. Place column into a clean 1.5ml microcentrifuge tube.
9. To elute DNA, add 35ul Buffer 0.1xEB to the center of the membrane, and let stand for 4 minutes, and then centrifuge for 1 minute.

Friday, February 4, 2022

To Do:

- ~~1. Streak out~~
2. Count plates from Wednesday

Streak for Isolation:

1. Pull out single use aliquots of 111, 112, 148, and 149 from the -80*
2. Pipette 20 ul onto a CHAN-KAN plate
3. Streak for isolation
 - a. Tightly packed together the streaks for *F. tularensis*

Plated my LVS glycerol stock to see if there is contamination. My plates were contaminated so I couldn't count.

Monday, February 7, 2022

To Do:

- ~~1. Move plates to room temperature~~
2. CHAN plates

Messed up 😞

Tuesday, February 8, 2022

To Do:

- ~~1. Patch out cells for beta-gal~~
- ~~2. Make CHA Plates~~
- ~~3. Make single use aliquots and glycerol stocks~~
4. Talk to Kim about scheduling training
5. Prep for beta-gal

Monday, January 31, 2022

To Do:

- ~~1. Make 10 square CHAN plates~~
2. Make LVS glycerol stocks

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

For 300 mL of CHA

1. Take out 15.3g of cystine heart agar into 1L flask (non-baffled; 10.2g/100mL)
2. Add 150mL of ddiH₂O (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
8. Add 3g freeze-dried hemoglobin to 150mL of ddiH₂O (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 30mL of CHAH mixture into each 100mm plate (should make approximately 20 plates) Try to avoid bubbles!

February 2022

Tuesday, February 1, 2022

To Do:

- ~~1. Make LVS glycerol stocks~~
- ~~2. Make LB-agar plates~~
- ~~3. Make MHB~~
- ~~4. Make 10 x PBS~~

Making Glycerol Stocks Protocol

1. Make 2 cryovials for each strain (permanent stocks), label!
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

2 x 1 mL glycerol stocks in screwcap cryo vials AND 9 x 50 mL single use aliquots in flip top microfuge tubes

Preparing LB-agar

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

Mueller-Hinton broth protocol- by Jamie Wandzilak

For 500 mL of MHB

1. Weigh out 10.5g of Mueller-Hinton broth into 1L square-bottle

2. Add 500mL of ddiH₂O (type I)
3. Autoclave on 30' liquid cycle, filling the water bin up
4. Cool down to 37 °C or cooler
5. Can keep this sterile media indefinitely without supplements

10X PBS recipe

Start with 800 ml of distilled water:

Add 80 g of NaCl.

Add 2 g of KCl.

Add 14.4 g of Na₂HPO₄.

Add 2.4 g of KH₂PO₄.

Adjust the pH to 7.4 with HCl or NaOH.

Bring 20ul and 200ul pipettes, pipettes tips, water, waste beaker, 5 M sodium hydroxide to INBRE
Add distilled water to a total volume of 1 liter.

Amount of PBS needed for dilutions for viable cell plate counting:

$$6 \times 3 = 18 \text{ wells}$$

$$18 \times 180 \text{ ul} = 3240 \text{ ul} = 3.24 \text{ mL}$$

Prepared lawn of *F. tularensis* for practice run of using the multichannel tomorrow. Used a 50ul single use aliquot that I made earlier and pipetted it onto a non-antibiotic CHAN plate. Used a wooden stick to spread.

Wednesday, February 2, 2022

To Do:

- ~~1. Complete viable cell count plates practice~~
- ~~2. Ask how many days to wait until count (4?)~~

Cell Count Plates Protocol

Plating LVS in PBS, 6 plates total

Check OD = 0.03 in 1 mL and use $C_1V_1 = C_2V_2$

1. Get 96 well plate, cuvette, MHB, 2sterile 1.5 mL, and cells prepared. Label all plates prior to starting.
2. Test plate to see if dry enough with 10uL of PBS

3. Get a sterile 1.5 ml tube and pipette 300ul of MHB
4. 1 loop of cells that were prepared the day before into the tube of MHB
5. 900 ul of MHB and 100 ul of resuspended cells into cuvette
6. Place parafilm on top and invert 3 to 5 times
7. Use spectrometer to measure OD
8. $V1 = (0.03 \times 650) / (0.480 \times 10)$
 - a. V1 needs to be greater than 20ul
 - b. V1 ul of cells minus 650 ul = amount of MHB needed
 - c. Diluted 1:10
 - d. $V1 = 40.625$ ul
 - e. MHB needed = 609.375 ul
9. Pipette 180 ul 1 x PBS into wells B1/2/3 – G1/2/3
10. Pipette 200ul of the OD = 0.03 cells into wells A1, A2, A3
11. Using the multichannel pipette, pipette 20 ul from A into B
12. Program to mix 150 ul of the volume three times
13. Repeat steps 11 and 12 for B to C, C to D, ect. for each row
 - a. CHANGE THE TIP EACH TIME!
14. Using a different multichannel pipette set to 10 ul and change the orientation of the plate to vertical
15. Pipette 10 ul from A1-G1 onto one plate letting each drip down the plate
16. Hold and watch to see drip down to $\sim 2/3^{\text{rd}}$ of plate. Set between two pipette boxes.

6 plates, 1A/B, 2A/B. 3A/B

*if drips run into each other, redo!

*need dry plates

Went well for the first time, a few bubbles, on two of the plates. On 3a the last two columns may have run into each other.

Thursday, February 3, 2022

To Do:

- ~~1. Continue working on making pKR122~~
 - ~~a. DNA Digest~~
2. Read literature on viable cell plate counting
 - a. Temperatures
 - b. OD

DNA digest

1. Make a reaction table with desired digests:

Tube	DNA	Enzyme(s)	DNA Volume (uL)	H ₂ O Volume (uL)
1	Purified PCR (rps2 promoter, tul4 5' UTR)	NotI-HF/KpnI-HF	15	-
2	Backbone (pKR89)	NotI-HF/KpnI-HF	5	10

2. Set up master mix table:

Components	Volumes in 1 reaction (uL)	Volumes in Master Mix 3x (uL)
H ₂ O	10.8	32.4
10x Buffer*	3.0	9.0
DNA	(15.0)	-
Enzyme 1 – NotI-HF	0.6	1.8
Enzyme 2 – KpnI-HF	0.6	1.8
Total	30.0 (15.0 actual b/c of DNA)	

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

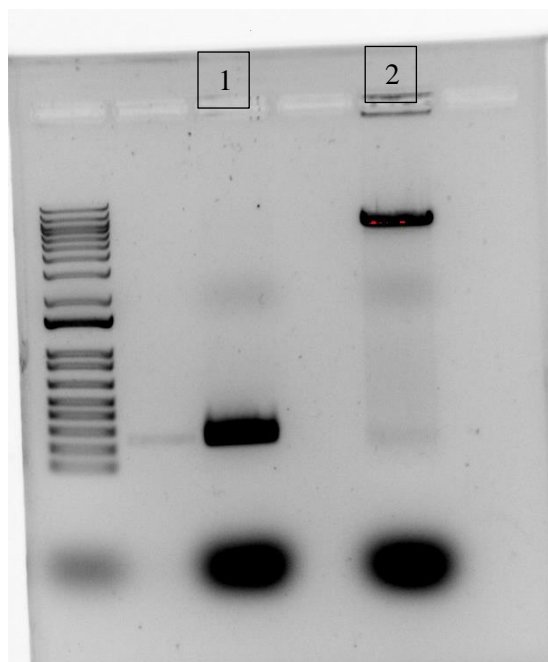
3. Add indicated amounts of H₂O and 10x buffer to master mix tube (MM).
4. Add indicated amount of DNA or DNA+water (for backbone tubes) to individual tubes for digest (1.5 mL microfuge tubes for digests in incubator, PCR strip tubes for reactions incubated in the thermocycler).
5. Add indicated amount of each enzyme (_1.8_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 (_15_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 in 37°C incubator for 10 minutes. (this step removes the phosphates from the ends of the plasmid to prevent re-ligation). **If using the new Quick CIP – add 1 uL, mix, incubate for 10 mins, then put at 80C for 2 minutes to inactivate the CIP.**

Made a mistake and used the 20ul PCR of the insert for pKR122 that dan made on 12/3 instead of the 100ul PCR that I made on 12/13 and then someone purified on 12/14. So I had to re-do the insert part. The backbone stayed in the incubator for 2 hours and the PCR stayed in for 1 hour.

Transform into pure 1 cells because pKR89 is maxi prep

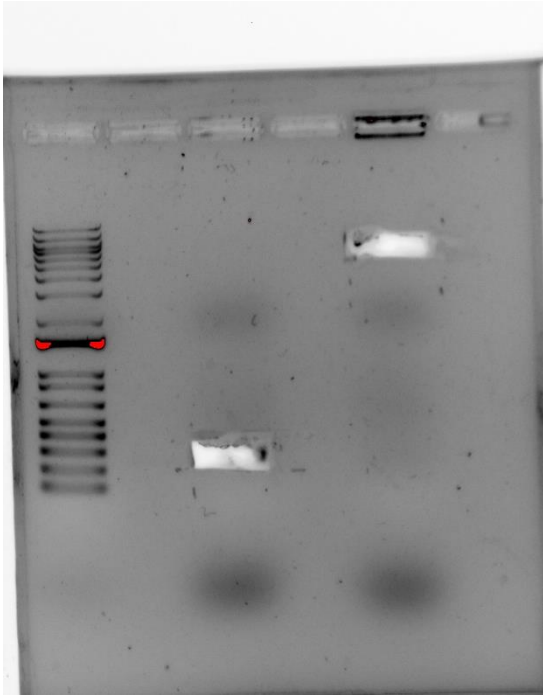
Used 5ul of cybersafe. 10 ul of ladder, all 36 ul (30ul of digest + 6ul of dye) for each digest when loading.
SKIP A LANE!! And when imaging, use the blue screen!

Before cutting it out



Lane	Tube	expected	y/n
3	1 - PCR	250-300 bps	y
5	2- Backbone	should yield 6250 bp fragment and 207 bp fragment; keep larger fragment	y

After cutting it out:



Patching out cells

1. Take one single colony with wooden stick and make a small patch (like a lawn)
2. Three patches per strain minimally
3. Put in incubator

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

For 300 mL of CHA

1. Take out 15.3g of cystine heart agar into 1L flask (non-baffled; 10.2g/100mL)
2. Add 150mL of ddiH₂O (type I)
3. Add stirbar to flask
4. Heat on low, stirring, for about 10 minutes (media should be totally dissolved)
5. Pressure cook for 30 minutes and then let depressurize for 20 minutes. Then put in water bath to cool down for ~15 minutes or more.
6. Cool down (ideally to ~55C)
7. Separately (before), prepare hemoglobin 2% solution
8. Add ½ flask of hemoglobin into water bath
10. Cool down (ideally to ~55C)

11. Using sterile technique, pour hemoglobin into CHA
12. Using a 50mL pipet, aliquot 30mL of CHAH mixture into each 100mm plate (should make approximately 10 plates) Try to avoid bubbles!

Making Glycerol Stocks Protocol

1. Make 2 cryovials for each strain (permanent stocks), label!
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

2 x 1 mL glycerol stocks in screwcap cryo vials AND 9 x 50 mL single use aliquots in flip top microfuge tubes

Beta-Galactosidase Assay Prep

- Mix final volume of MHB with kanamycin in sterile
 - C1 Kanamycin = 50 mg/mL
 - C2 Kanamycin = 0.005 mg/mL
 - V1 = 9.5 uL of kanamycin added
 - V2 = 95 mL
- Cuvettes with 950 ul of MHB
 - 13 cuvettes x 950 ul = 12,350 ul = 12.35 mL
- Cultures tubes with 6 mL of MHB
 - 12 tubes x 6 mL = 72 mL
- Sterile 1.5 mL with 500ul of MHB
 - 12 tubes x 500 ul = 6 mL
- Total MHB needed = 90.35 mL = 95 mL
- Look up estimate times based on previous data if possible for step 17

Wednesday, February 9, 2022

To Do:

1. Beta-gal for 149.1, 148.1, 112, 111

2. Move culture tubes to 37°C shaker
3. Use loop to scrape patch into microtube with MHB (3 per strain)
4. Pipette up and down until no clumps
5. Repeat for all 18 patches
6. 50 uL from cell suspension and add to cuvette with 950 uL MHB
 - a. Invert to mix with parafilm
7. Read OD600 in spec: MUST BE BELOW 0.05-0.6
8. Calculate volume to add with $C1V1=C2V2$
 - a. $V2=6000$ uL
 - b. $C2$ for LVS = 0.1 uL
 - c. $C2$ for rpsu2 = 0.135 uL
 - d. $C1 = OD600 \times 20$
 - e. $V1 = ((6000)(0.1 \text{ or } 0.135)) / (OD600 \times 20)$
9. Add appropriate volume to culture tubes
10. Put in shaking incubator at 37°C
11. Start timer counting up

Tubes for culturing:

Tube #	Strain	
1-3	149 (LVS)	Prpsu2-rpsU2UTR
4-6	148 ($\Delta 2$)	Δ rpsU2 PrpsU2_rpsU2UTR
7-9	112 (LVS)	Ptul4-tul4 5'UTR
10-12	111 ($\Delta 2$)	Δ rpsU2 Ptul4-tul4-5'UTR

MUST BE BETWEEN 0.05-0.6

OD600 for initial:

Tube	OD	V1 (uL)	Tube	OD	V1 (uL)
1	0.385	77.9	7	0.334	89.8
2	0.28	107.1	8	0.36	83.3
3	0.214	140.2	9	0.321	93.5
4	0.277	140.8	10	0.333	117.1
5	0.252	154.8	11	0.234	166.7
6	0.373	104.6	12	0.271	143.9

Added to culture tubes and then took 500 uL from culture tube and added to cuvette to get the time zero OD600.

The range of OD600 could be from 0.07 to 0.14.

T=0 OD600

Tube	OD	Tube	OD
1		7	
2		8	
3		9	
4		10	
5		11	
6		12	

Assay

1. Grow 6ml cultures until OD600 = 0.3
2. Turn on 28°C water bath and get ice
3. Check ONPG (in 20* on bottom left, covered in foil) and thaw in water bath
4. Determine amount of Z-buffer needed (0.8ml x 2 x # of cultures plus 1, the 2 is for running duplicates, the 1 is for a blank replicate). Add BME to Z-buffer (2.72 x Xml Z-buffer = μ l of BME).
 - a. $0.8\text{ml} \times 2 \times 12 = 19.2\text{ mL} + 2\text{mL} = 21.2\text{ mL} = 23\text{mL Z-buffer}$
 - b. $2.72 \times 23\text{mL Z-buffer} = 62.56\text{ ul} = 62.6\text{ ul of BME}$
 - c. $24\text{ tubes} + 2\text{ blank tubes} = 26\text{ tubes}$
 - d. Z-buffer is sterile so use the flame to aliquot into a 50mL conical
 - e. BME = 2-mercaptoethanol which is under the fume hood, add into the conical under the fume hood
 - f. Blank is two because we know the two groups of strains will finish at the same time
 - g. End blank at the same as the last sample
5. Set up reaction tubes with 800 μ l Z-buffer, put on lids
 - a. Use the repeat pipettor to do this step
 - b. The tips are behind the dishwashing to-do
 - c. To release the tip, push both the top and bottom buttons down
6. Turn on spec and gather cuvettes
7. After ~3.5 hours of the cultures being in the shaker, check the two expected highest and two expected lowest and measure OD₆₀₀
 - a. Looking to be between 0.3-0.4, they all are move all onto ice!
 - b. Time on incubator: 3:55:09
8. Once cultures reach OD₆₀₀ = 0.3, place on ice 30 min and put ONPG in water bath
 - a. While waiting on ice:
 - i. Add media to blanks
 - ii. Prep cuvettes
 - iii. Get repeat pipettor stop tip (on very top of shelves above bench)
9. After cells have incubated on ice, measure OD₆₀₀ of bacterial cultures
 - a. Put back on ice after measuring out 500 ul for OD₆₀₀

10. Add 200µl culture to each reaction tube (add 200µl culture media to blank tube)
 - a. No need to use the flame
11. Add 30µl 0.1% SDS to each reaction tube
 - a. $30 \text{ ul} \times 26 = 780 \text{ ul}$ SDS
 - b. In 50 mL conical personal
12. Add 60µl CHCl₃ (chloroform) to each reaction tube
 - a. $60 \text{ ul} \times 26 \text{ tubes} = 1.56 \text{ mL}$
 - b. Under fume hood in 1L jar
13. Vortex reaction pairs on high for 6 secs (time precisely with timer)
14. Put in water bath for 10 min
15. Prepare repeater pipette with 1M Na₂CO₃ (stop)
 - a. 11.5 mL
16. Add 200µl ONPG in 5 sec intervals (use timer with hours)
 - a. $200\text{ul} \times 26 = 5400 \text{ ul} = 5.2 \text{ mL}$
17. Shake gently and watch for yellow (goal OD₄₂₀ is 0.6-0.9)
18. Stop with 500µl 1M Na₂CO₃, record time, vortex at 4 for 10 sec
 - a. $500 \times 26 = 11.5 \text{ mL}$
19. Give all reaction at least 2 hours
20. Remove 1 mL from reaction (avoid chloroform at bottom), measure OD₄₂₀ and OD₅₅₀, using blank reaction as blank in spectrophotometer.

Clean-up

- ✓ Beta-gal tubes put in hazardous waste and then rinse them out with water
- ✓ Pour all culture tubes into the liquid waste and then bleach with 100% in waste container and small amounts of bleach into the tubes
- ✓ Throw away all cuvettes
- ✓ Turn off water bath!!

Calculations

$$\text{Miller Units} = 1000 \times [(\text{OD}_{420} - 1.75 \times \text{OD}_{550}) / (t \times v \times \text{OD}_{600})]$$

Note that “t” is the time of the reaction in minutes, “v” is the volume of the culture used in the assay in mL (i.e., 0.2) and the OD₆₀₀ is that determined for the culture used in each assay. Use spreadsheet.

Thursday, February 10, 2022

To Do:

- ~~1. Plate cells~~
2. Z-buffer
- ~~3. colony per~~
4. put Rh123 in -20* bottom right hand of fridge

Colony PCR from patches

-Take small amounts of each patch and resuspend in 50 μ L molecular water using a sterile toothpick.

Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8	Tube 9	Tube 10
Patch 1	Patch 2	Patch 3	Patch 4	Patch 5	Patch 6	Patch 10	Patch 7	LVS gDNA	water

- Heat samples at 95°C for 10' to lyse and kill cells
 - only for tubes 1 through 8 NOT LVS gDNA!
- Dilute lysates 1:10
 - 18 μ L of molecular grade water to 2 μ L of cells

-Use 1-2 μ L of heat-killed cells as template in colony PCR to check for desired mutation. As of August 2019, the KRamsey lab is primarily using PrimeStar GXL polymerase (Takara Bio). For controls, use LVS cells, LVS gDNA, the Tn7 plasmid, and water only.

	Number of samples	10
	Master mix factor	11
Components	Volumes in 1 reaction (uL)	Volumes in Master Mix (uL)
H ₂ O	11.8	129.8
5X PrimeStar GXL buffer	4	44
2.5 mM dNTPS	1.6	17.6
Forward primer (10 uM)	0.6	6.6
Reverse primer (10 uM)	0.6	6.6
PrimeStar DNA polymerase	0.4	4.4
Cell lysate	1	
Total	20	209

Primers used KROL147 and KROL148.

-Use PCR program:

1. 94°C 2'
2. 94°C 20"
3. 50°C 30"
4. 68°C (1' per kb)
5. Go to step 2, rep 32x
6. 68°C 5'
7. Hold 12°C

- This is the PCR program SNT1 which is already programmed. The extension time is 1.5 minutes because the DNA should be ~1300 bp. The amount in the tubes is 20 uL.
- LVS gDNA was 0.5 uL so 19.5 ul of Master mix was added
- 1 ul of 1-8 and 10 used 1ul so 19 ul of Master mix was added

Didn't save the image by accident, but there were no bands. Possible sources of error: I forgot to add something into the mastermix, the enzyme didn't work, or something else wasn't working.

Count Plates Protocol

Plating LVS in PBS, 6 plates total

Check OD = 0.03 in 1 mL and use $C1V1=C2V2$

1. Get 96 well plate, cuvette, MHB, and cells prepared
2. Test plate
3. Get a sterile 1.5 ml tube and pipette 300ul of MHB
4. 1 loop of cells that were prepared the day before into the tube of MHB
5. 900 ul of MHB and 100 ul of resuspended cells into cuvette
6. Place parafilm on top and invert 3 to 5 times
7. Use spectrometer to measure OD
8. $V1 = (0.03 \times 650) / (0.095 \times 10)$
 - a. V1 needs to be greater than 20ul
 - b. V1 ul of cells minus 650 ul = amount of MHB needed
 - c. $V1 = 20.53 \text{ ul} = 20.6 \text{ ul}$ used
 - d. MHB needed = 629.47 ul
 - i. Put 650 ul of MHB into 1.5 tube and then removed 20.6 ul before adding the cells
9. Pipette 180 ul 1 x PBS into wells B1/2/3 – G1/2/3
10. Pipette 200ul of the OD = 0.03 cells into wells A1, A2, A3
11. Using the multichannel pipette, pipette 20 ul from A into B
12. Program to mix 150 ul of the volume three times
13. Repeat steps 11 and 12 for B to C, C to D, ect. for each row
 - a. CHANGE THE TIP EACH TIME!
14. Using a different multichannel pipette set to 10 ul and change the orientation of the plate to vertical
15. Pipette 10 ul from A1-G1 onto one plate letting each drip down the plate
16. Pipette at 45° and let dry in between two tip boxes
17. Two plates in each column

6 plates, 1A/B, 2A/B. 3A/B

*if drips run into each other, redo!

*need dry plates

The first two plates didn't drip as much as anticipated. Dried out plates 2A/B and 3A/B in the bls hood for 15 minutes. The rest were fine, but had to put at vertical to get enough dripping. It was hard to see on the funky plates, but it did grow overnight, so we will see what happens on Monday.

Friday, February 11, 2022

To Do:

1. ~~Re-do of the Colony PCR~~
2. ~~1 M Na₂CO₃~~

Colony PCR from patches

Used the dilutions from yesterday.

-Use 1-2 µL of heat-killed cells as template in colony PCR to check for desired mutation. As of August 2019, the KRamsey lab is primarily using PrimeStar GXL polymerase (Takara Bio). For controls, use LVS cells, LVS gDNA, the Tn7 plasmid, and water only.

	Number of samples	10
	Master mix factor	11
Components	Volumes in 1 reaction (uL)	Volumes in Master Mix (uL)
H ₂ O	11.8	129.8
5X Primestar GXL buffer	4	44
2.5 mM dNTPS	1.6	17.6
Forward primer (10 uM)	0.6	6.6
Reverse primer (10 uM)	0.6	6.6
Primestar DNA polymerase	0.4	4.4
Cell lysate	1	
Total	20	209

Primers used KROL147 and KROL148.

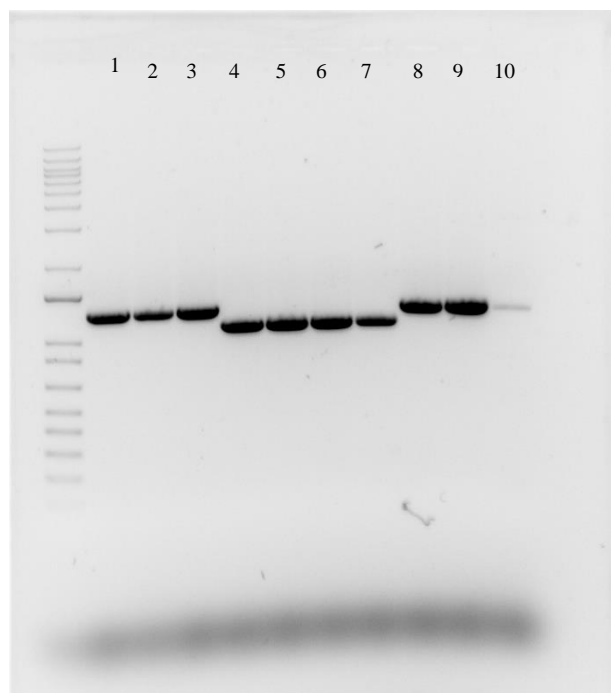
-Use PCR program:

1. 94°C 2'
2. 94°C 20"
3. 50°C 30"
4. 68°C (1' per kb)
5. Go to step 2, rep 32x
6. 68°C 5'

7. Hold 12°C

- This is the PCR program SNT1 which is already programed. The extension time is 1.5 minutes because the DNA should be ~1300 bp. The amount in the tubes is 20 uL.
- LVS gDNA was 0.5 uL so 19.5 ul of Master mix was added
- 1 ul of 1-8 and 10 used 1ul so 19 ul of Master mix was added

Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8	Tube 9	Tube 10
Patch 1	Patch 2	Patch 3	Patch 4	Patch 5	Patch 6	Patch 10	Patch 7	LVS gDNA	water
149 (LVS)	149 (LVS)	149 (LVS)	148 Δ2	148 Δ2	148 Δ2	111 Δ2	112 (LVS)		



Lane	Expected	Y/N
1	1300	Y
2	1300	Y
3	1300	Y
4	1100	Y
5	1100	Y
6	1100	Y
7	1100	Y
8	1300	Y
9	1300	Y
10	0	Y

1 M Na₂CO₃

- Mix 53 g of sodium carbonate and 500 mL of diH₂O in a 600 mL beaker
- Stir on plate with stir bar
 - Needs to be fully dissolved (nothing granular seen)
 - Add heat if needed
- Vacuum Filter sterilize
 - 500 mL filter sterilize equipment (above sierras bench on top shelf)
 - Attach the vacuum and turn on BEFORE pouring
 - Wipe down bench and turn on flame

- Bring the green cap and filter piece over to the flame
- Undo the top part and place the green cap on
- Don't touch the inside of the green cap!
- Belongs on the right middle of shelving unit above the water bath

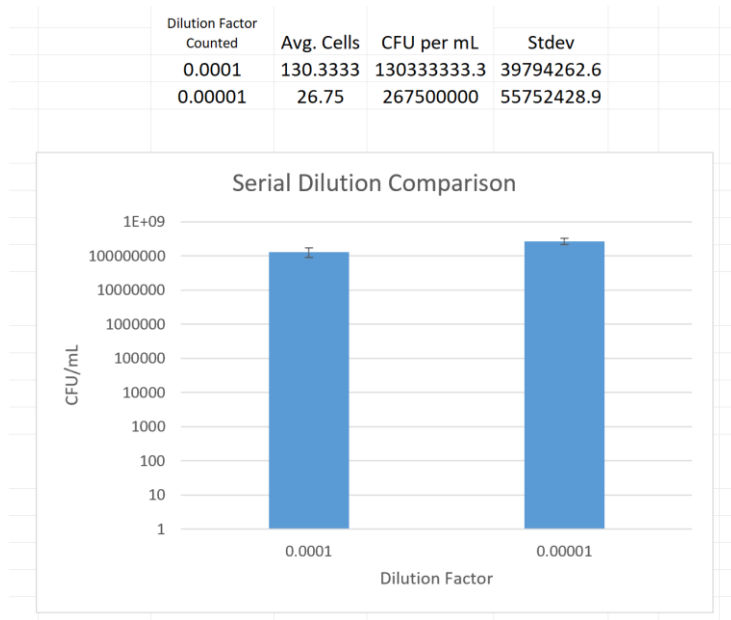
Monday, February 14, 2022

To Do:

1. Count Plates

There was snow this morning, so only one hour in lab. I counted my plates from 2/10/22 for the 0.0001 and 0.00001 dilutions. All the results are in the spreadsheet.

Track Plate 1								Dilution Factor Counted	Avg. Cells	CFU per mL
1	2	3	4	5	6	7				
A				96	13			0.0001	89.5	89500000
B				83	10			0.00001	11.5	1.15E+08
Dilution Factor	1	0.1	0.01	0.001	0.0001	0.00001	0.000001			
Track Plate 2								Dilution Factor Counted	Avg. Cells	CFU per mL
1	2	3	4	5	6	7				
A				103	13			0.0001	132.5	1.33E+08
B				162	31			0.00001	22	2.2E+08
Dilution Factor	1	0.1	0.01	0.001	0.0001	0.00001	0.000001			
Track Plate 3								Dilution Factor Counted	Avg. Cells	CFU per mL
1	2	3	4	5	6	7				
A				175	20			0.0001	169	1.69E+08
B				163	20			0.00001	20	2E+08
Dilution Factor	1	0.1	0.01	0.001	0.0001	0.00001	0.000001			



Tuesday, February 15, 2022

1. ~~Z buffer~~
2. ~~Filter Water~~
3. ~~Make CHAH plates~~
4. ~~Streak for isolation LVS~~

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

For 600 mL of CHA

1. Take out 15.3g of cystine heart agar into two 0.5L flasks (non-baffled; 10.2g/100mL)
2. Add 150mL of ddiH₂O (type I) into both flasks
3. Add stirbar to flask
4. Heat on low, stirring, for about 10 minutes (media will not be fully dissolved because of glucose)

Heat to 50°C

5. Pressure cook for 30 minutes and then let depressurize for 20 minutes. Then put in water bath to cool down for ~15 minutes or more.
6. Cool down (ideally to ~55°C)

7. Separately (before), prepare hemoglobin 2% solution
8. Add ½ flask of hemoglobin into water bath
10. Cool down (ideally to ~55C)
11. Using sterile technique, pour hemoglobin into CHA

Do it all quickly otherwise agar starts to solidify

12. Using a 50mL pipet, aliquot 30mL of CHAH mixture into each 100mm plate (should make approximately 20 plates) Try to avoid bubbles!

Z-Buffer (1L)

16.1g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$

5.5g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$

0.75g KCl

0.246g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

997.3ml ddH₂O

pH 7

Added about 17*20 ul of NaOH to get the pH to 7.00 , started at a pH of 6.95.

Wednesday, February 16, 2022

- ~~1. Making plate (20)~~
- ~~2. Make a lawn of LVS (two plates)~~
- ~~3. Prepare for tomorrow~~

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

Making plates went a lot smoother today. Took the CHA out of the pressure cooker at exactly 20 minutes.

At first, I misunderstood and thought that I was streaking for isolation. Then I pulled out the plates and from the densest region, spread as much as I could so I could create a lawn for getting cells tomorrow.

Prepared the 100mL of sterile freshwater in a 250 mL flask for the OD, and a 50 mL of sterile freshwater. Labeled all the flasks and the plates for tomorrow and Friday.

Thursday, February 17, 2022

- ~~1. Day 0 of plating~~
- ~~2. Scrape cells from plate~~

3. ~~OD first~~
4. ~~Original 100ml do 4 plates~~
5. ~~Ask sierra if she is coming in on Saturday~~

Inoculation

- Prepare 96 well plate, cuvette, parafilm, freshwater samples, square CHAH plate, and 1xPBS
 - Test plate to see if it is dry enough by pipetting 10 uL of freshwater and watching it drip.
- Get a sterile 1.5 mL tube and pipette 500 uL of freshwater into it.
- Scrap up all of cells that were prepared the previous day (which I messed up) into the 1.5mL tube with freshwater. Resuspend the cells.
- Add 500 mL more freshwater to the 1.5 tube
- Pipette 980 uL of freshwater and 20 uL of resuspended cells into the cuvette. Place parafilm on top and invert 3 to 5 times, slowly.
- Use spectrometer to measure OD. Obtain an OD₆₀₀ of 0.03. Don't forget the blank!
 - Needs to be between 0.05-0.60 (dynamic range of the spec), if more than 0.6 need to dilute
 - $C1V1=C2V2$
 - $V1 = 280 \text{ uL}$
 - Amount of resuspended cells to add
 - $C1 = \text{the OD measured} \times 50 \text{ (amount diluted)}$
 - 0.214
 - $C2 = 0.03$
 - $V2 = 100 \text{ mL}$
- Take prepared 100 mL freshwater in 250mL baffled flask and add V1 of resuspended cells to it.
- Put the cells into three technical replicates. 10 mL in 3 different flasks for the three different conditions. 9 flasks total.
- Put flasks in respective locations.
 - 4°C – cold room
 - 16°C – Jenkins Lab
 - 25°C – cold room

Flask 1	Flask 2	Flask 3	Flask 4	Flask 5	Flask 6	Flask 7	Flask 8	Flask 9
4°C	4°C	4°C	16°C	16°C	16°C	25°C	25°C	25°C

Plating Protocol

- Prepare 96 well plate, cuvette, parafilm, freshwater samples, square CHAH plate, and 1xPBS
- Test plate to see if it is dry enough by pipetting 10 uL of freshwater and watching it drip.
- 1. Pipette 180 uL of 1 x PBS into wells row B1/2-G1/2.
 - a. Use multichannel and a reservoir.
- 2. Pipette 200 uL from 100mL (which is now 10mL) of resuspended cells and freshwater into A1 and A2
 - a. Use a 200 uL pipette.

3. Using the multichannel pipette, pipette 20 uL from A into B. Mix 150 uL of the volume three times.
4. Repeat step 3 for B to C, C to D.
 - a. hint = say it out loud so you don't lose your place.
 - b. Change the tip each time!
5. Use the less volume multichannel pipette set to 10 uL and change the orientation of the plate to vertical before starting the dripping process.
6. Pipette 10 ul from A1-G1 onto the plate, letting each drip down the plate.
 - a. Remember to stay within the 6 inches of the flame, don't put the handle of the multichannel pipette to the flame, have the plates at roughly a 45-degree angle, and place to dry in between two pipette boxes at the same angle.
 - b. Two plates for each column. So should be 18 total plates
 - c. Place in incubator for 2 days and then pull out and

Plating is done in order to get the starting point for all of the samples.

Put labeled plates that I made yesterday (2/16) into container with wet paper towels to try and keep moist for Monday.

Friday, February 18, 2022

- ~~1. Day 1~~
- ~~2. Need to pull out on Sunday~~

Plating Protocol

- Prepare 96 well plate, freshwater samples, square CHAH plate, and 1xPBS
 - Test plate to see if it is dry enough by pipetting 10 uL of freshwater and watching it drip.
1. Pipette 180 uL of 1 x PBS into wells row B1/2-G1/2.
 - a. Use multichannel and a reservoir.
 2. Pipette 200 uL from one flask into row A1/2.
 - a. Use a 200 uL pipette.
 - b. Swirled before pipetting for about ~10 seconds each.
 - c. Went over to CBLS first and pipetted in Dr. MRamsey's lab? For flasks 4, 5,6
 - d. Mixed all before diluting by using the 200ul pipette and resuspending ~5 times. I changed the tip each time.
 3. Using the multichannel pipette, pipette 20 uL from A into B. Mix 150 uL of the volume three times.
 4. Repeat step 9 for B to C, C to D. hint = say it out loud so you don't lose your place.
 - a. Change the tip each time!
 5. Use the less volume multichannel pipette set to 10 uL and change the orientation of the plate to vertical before starting the dripping process.
 6. Pipette 10 ul from A1-G1 onto the plate, letting each drip down the plate.

- Remember to stay within the 6 inches of the flame, don't put the handle of the multichannel pipette to the flame, have the plates at roughly a 45-degree angle, and place to dry in between two pipette boxes at the same angle.
- Two plates for each column. 18 total plates, 6 for each of the three temperatures.
- I had issues with Plate 2A and 2B but I did a third one... still had issues with the dry spots. So they all converged so I don't think I can use that data?
- I also had issues with 8A, I must have been holding the multichannel weird because it wasn't sucking up anything from 8G until I tried like 15 times. I think it went well after, but only time will tell.
- Overall, the plates were kinda hit or miss. Some of them were dry, some were perfect, and others were still kind of wet so they dripped really fast.

Sunday, February 20, 2022

- ~~Pull out day 1 plates~~
- Check out day 0 plates? Should already be pulled out

Unfortunately, the plates were not taken out 😞 Also I pulled out the day one plates, but I should have kept them in the incubator because they were too small.

Monday, February 21, 2022

- ~~Day 4 Plate~~
- ~~Count Day 0~~

Put the day 1 plates back into the incubator in the morning. Hannah said she would check them in the afternoon.

	Track Plate 1							Factor Counted	Avg. Cells	CFU per mL
	1	2	3	4	5	6	7			
A				126	13			0.0001	11.5	11500000
B				98	10			0.001	112	11200000
Dilution Factor	1	0.1	0.01	0.001	0.0001	0.00001	0.000001			
	Track Plate 2							Factor Counted	Avg. Cells	CFU per mL
	1	2	3	4	5	6	7			
A				12	65			0.0001	68	68000000
B					71			0.001	12	1200000
Dilution Factor	1	0.1	0.01	0.001	0.0001	0.00001	0.000001			

There were some smaller (pinpoint) colonies on the Day 0 plates as well. Not sure what to think about that, so I asked Dr. Ramsey.

Plating Protocol

- Prepare 96 well plate, freshwater samples, square CHAH plate, and 1xPBS
 - Test plate to see if it is dry enough by pipetting 10 uL of freshwater and watching it drip.
- Pipette 180 uL of 1 x PBS into wells row B1/2-G1/2.
 - Use multichannel and a reservoir.
 - Pipette 200 uL from one flask into row A1/2.

- a. Use a 200 uL pipette.
 - b. Swirled before pipetting for about ~10 seconds each.
 - c. Went over to CBLS first and pipetted in Dr. MRamsey's lab? For flasks 4, 5,6
 - d. Mixed all before diluting by using the 200ul pipette and resuspending ~5 times. I changed the tip each time.
9. Using the multichannel pipette, pipette 20 uL from A into B. Mix 150 uL of the volume three times.
 10. Repeat step 9 for B to C, C to D. hint = say it out loud so you don't lose your place.
 - a. Change the tip each time!
 11. Use the less volume multichannel pipette set to 10 uL and change the orientation of the plate to vertical before starting the dripping process.
 12. Pipette 10 ul from A1-G1 onto the plate, letting each drip down the plate.
 - a. Remember to stay within the 6 inches of the flame, don't put the handle of the multichannel pipette to the flame, have the plates at roughly a 45-degree angle, and place to dry in between two pipette boxes at the same angle.
 - b. Two plates for each column.

Plating day 4 went well. I think about 2 or 3 plates had pretty bad dry spots so the drips converged. The water in the warmer temperature flasks looked a little cloudy.

Tuesday, February 22, 2022

- ~~1. Make plates~~
 - ~~a. Use a big flask for CHAH because all of its going to the autoclave~~
- ~~2. Meet with Kim and Kathryn at 1pm~~
- ~~3. Make MHB~~
- ~~4. Make LB agar~~
- ~~5. Add 1xPBS to 50 conical~~
- ~~6. Count Day 1~~

Pulled out Day 1 plates after they were in the incubator for 3 days and 1 day at room temperature.

Propidium Iodine - the dye has excitation/emission maxima of 493 / 636 nm – thermofisher

Rh123 - excitation/emission spectra 507/529 – thermofisher

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

For 600 mL of CHA

1. Take out 15.3g of cystine heart agar into two 0.5L flasks (non-baffled; 10.2g/100mL)
2. Add 150mL of ddiH₂O (type I) into both flasks
3. Add stirbar to flask

4. Heat on low, stirring, for about 10 minutes (media will not be fully dissolved because of glucose)

Heat to 50°C

5. autoclave
6. Cool down (ideally to ~55°C)
7. Separately (before), prepare hemoglobin 2% solution
8. Add ½ flask of hemoglobin into water bath
10. Cool down (ideally to ~55°C)
11. Using sterile technique, pour hemoglobin into CHA

Do it all quickly otherwise agar starts to solidify

12. Using a 50mL pipet, aliquot 30mL of CHAH mixture into each 100mm plate (should make approximately 20 plates) Try to avoid bubbles!

Mueller-Hinton broth protocol- by Jamie Wandzilak

For 500 mL of MHB

1. Weigh out 10.5g of Mueller-Hinton broth into 1L square-bottle
2. Add 500mL of ddiH₂O (type I)
3. Autoclave on 30' liquid cycle, filling the water bin up
4. Cool down to 37 °C or cooler
5. Can keep this sterile media indefinitely without supplements

Preparing LB-agar

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

Counting Day 1 plates:

Counted both the big and small colonies (total) and just the big colonies. It's a lot so its in the spreadsheet = "22_Plate Counts"

Meeting with Kim:

- FACS is gone in ~6 weeks
- Click the origin to change the scatter
- Rh123 – live cells (live then kill)
- Propidium iodine – all cells
- Hit tube then properties
 - 10,000 counts up to 2.5 million
- Extra buffer

Wednesday, February 23, 2022

- ~~1. Pull out Day 4 – look to see if colonies are big enough~~
- ~~2. Take plate single use aliquot as a lawn onto CHA plate~~
 - ~~1. Messed up again and started to streak for isolation, but then did my best to move it across the entire of the plate.~~

Looked at the Day 4 colonies. The 4C looks like there is some growth, but it will definitely need at least 1 or 2 more days in the incubator before I can count them. Unfortunately, I think that the 16C and the 24C are no longer viable. I will plate them again on day 7 to confirm, but I will ask Dr. Ramsey if this is the right move.

Thursday, February 24, 2022

- ~~1. Day 7 – Plate~~
 - ~~1. Plate the small amount from the single use aliquot~~
- ~~2. Work on abstract~~
- ~~3. Look at day 4 plates~~
- ~~4. Cover rh123!~~

Checking to see if the small colonies appear:

- Take a small amount from lawn and do another 0.03 in 1xPBS

Plating Protocol

1. Get a sterile 1.5 ml tube and pipette 300ul of PBS
2. 1 loop of cells that were prepared the day before into the tube of PBS
3. 900 ul of PBS and 100 ul of resuspended cells into cuvette
4. Place parafilm on top and invert 3 to 5 times
5. Use spectrometer to measure OD
6. $V1 = (0.03 \times 650) / (0.280 \times 10)$
 - a. V1 needs to be greater than 20ul
 - b. V1 ul of cells minus 650 ul = amount of PBS needed

- c. Diluted by adding 900 ul of PBS into another sterile tube and adding 100 ul of resuspended cells.
- d. $V_1 = 6.96$
- e. PBS needed =
 - i. Put 650 ul of PBS into 1.5 tube and then removed 69.6 ul before adding the cells

Plating went smoothly today. The only plate that gave me any issues was plate 9B. There was more condensation than I originally realized, and then the first column diverged with the second.

Looked at the day four plates. There was enough growth to count the 4C, and there is growth on the 16C that will hopefully be countable on Monday. The 25C has some growth in the undiluted column that I will count on Monday as well. I counted day four, it was a bit tricky as the colonies were pretty small, but I did my best. For the 25C flasks (7,8,9) Dr. Ramsey had me take 6 circular plates and did the following to see the amount of viable cell colonies.

- Pipette 300ul from one flask onto the circular plate
- Add ~20 beads. And shake vigorously.
- Wait for all of the liquid to absorb.
- Take off beads and put into the 50 mL conical.
- Place in the incubator. Ask sierra to take them out on Sunday.

Friday, February 25, 2022

1. Count Day 4

Snow day.

Sunday, February 27, 2022

- ~~1. Streak out a lawn~~
- ~~2. Look at day 7~~

Sierra did this for me ! Much thanks.

Monday, February 28, 2022

3. Stain
- ~~4. Count day 4~~

Reagents and supplies

Amber-colored 1.5 mL microfuge tubes (do not need to be sterile)

1X PBS (use filter-sterilized to minimize extracellular debris but do not need to keep sterile)

1.040 uM Rhodamine 123 (MW: 381 g/mol) dissolved in ethanol (protect from light)

- Resuspend 5 mg rhodamine 123 in 2 mL ethanol to make a 2.5 mg/mL (6561.7 uM) stock
- Aliquot 10 tubes with 15 ul of 2.5 mg/mL stock, label and store at -20°C. Label tube with remainder properly and store at -20°C also
- Make 65.6 uM stock: mix 10 uL with 990 uL ethanol
- Make 1.04 uM stock: mix 10 uL of 65.6 uM stock with 621 uL ethanol

formaldehyde solution (37% stock solution)

3 ug/mL Propidium iodide dissolved in water (protect from light)

- Resuspend 5 mg propidium iodide in 5 mL water to make a 1 mg/mL stock. Store unused 1 mg/mL stock at -20°C
- Make 3 mg/mL stock: mix 4.5 uL with 1495.5 uL water
- Make 3 ug/mL stock: mix 4.5 uL with 1495.5 uL water

The powder for both was sticking to the weigh paper, and I was making a general mess. So didn't go to well, and threw it away. I will try again soon hopefully.

I counted my day 4 plates for 16C and 25C and the day 7 plates for 4C. In an ideal world I would have pulled the 4C day 7 plates yesterday as the colonies were on the larger size. The serial dilutions from the stock were only medium and glistening colonies. No pinpoint colonies. This suggests that a sub population is not growing well in the water conditions and not contamination.

March 2022

Tuesday, March 1, 2022

- ~~1. Make CHA plates~~
- ~~2. Count day 7 for 16C and 25C~~
3. Try #2 of making the propidium iodide
4. Streak out KRLVS 148 on KAN-CHA

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

For 600 mL of CHA

1. Take out 30.6g of cystine heart agar into a 1L flasks (non-baffled; 10.2g/100mL)
2. Add 300mL of ddiH₂O (type I) into flask
3. Add stirbar to flask
4. Heat on low, stirring, for about 10 minutes (media will not be fully dissolved because of glucose)

Heat to 50°C

5. autoclave at liquid 20

6. Cool down (ideally to ~55C)
7. Separately (before), prepare hemoglobin 2% solution
8. Add ½ flask of hemoglobin into water bath
10. Cool down (ideally to ~55C)
11. Using sterile technique, pour hemoglobin into CHA

Do it all quickly otherwise agar starts to solidify

12. Using a 50mL pipet, aliquot 30mL of CHAH mixture into each 100mm plate (should make approximately 20 plates) Try to avoid bubbles!

Counted Day 7 for 16C and 25C. Here's the summary:

Track	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
Plates 1,2,3	0.01	122.6667	1226667	69342.15
	0.001	19.83333	1983333	301385.7
Track	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
Plates 4,5,6	1	30.5	3050	1760.918
	0.1	4	4000	2309.401
Track	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
Plates 7,8,9	1	4.25	425	256.5801
	0.00001	0	0	#DIV/0!

Try #2

3 ug/mL Propidium iodide dissolved in water (protect from light)

- Resuspend 14.1 mg propidium iodide in 14.1 mL water to make a 1 mg/mL stock. Store unused 1 mg/mL stock at -20°C
- Make 3 ug/mL stock: mix 4.5 uL with 1495.5 uL water

Took me two tries but I did it!

Wednesday, March 2, 2022

- ~~1. Label everything for tomorrow~~
2. Talk about protocol

Thursday, March 3, 2022

- ~~1. Plate day 14~~
 - ~~1. Round plates for 4-9~~
- ~~2. Streak to single colony LVS 149~~

Plating: Did the beads with 300 ul for flasks 4 – 9 in duplicate. Will check on them on Sunday. Did the normal plating procedure for flasks 1-3. Plate 3B converged (or almost) in the first two columns so I re-did it since I had extra plates. Will also check on those on Sunday.

Streaking to single colony: Took a single use aliquot from the -80C of LVS149.1. Pipetted it onto a CHA-KAN plate. Used wooden sticks to streak into four quadrants.

Friday, March 4, 2022

- ~~1. Prepped for Monday~~
- ~~2. Send Dr. R abstract~~

Sunday, March 6, 2022

- ~~1. Patch out cells for LVS 149 and LVS 148~~
- ~~2. Streak out lawn for staining of LVS~~

Monday, March 7, 2022

- ~~3. RNA seq day~~
 - ~~1. 8 cultures~~
4. Staining
- ~~5. Check 4C cell counts~~

No staining today, there is an error with the FACS machine. No growth on the 5-9 today, left at room temperature just in case to check in the next couple of days.

Staining for FC Protocol

- Prepare 4 amber-colored tubes with LVS.
- Scrape up small amount of LVS cells into 400 mL 1x PBS
- Resuspend cells completely by pipetting
- Check OD600 by diluting 100 uL in 900 uL 1X PBS in a cuvette (blank with 1X PBS)
- Calculate how much bacterial suspension is required to obtain a final OD600 of 0.03 in 1 mL
- $(0.03)(1000\text{uL}) / (\text{measured OD600} \times 10) = \text{uL}$
- If smaller than 30 uL, dilute bacterial suspension and repeat check OD and calculations

Prepare 4 amber-colored 1.5 mL microfuge tubes with 1 mL of LVS at 0.03

- Follow protocols below for each step, adhering to plan in table above. (i.e., tubes 1-3 fixed with formaldehyde first, tube 4 stained with rhodamine first, etc)

sample #	form	rh123	formaldehyde	propidium idonine	expectations
1	yes	no	no	no	dead cells- no fluorescene
2	yes	no	no	yes	stains DNA - stains everything (all cells)
3	yes	yes	no	yes	should only be dead - no rh123

4	no	yes	yes	yes	should see both rh123 and propidium (some cells that are only p
5					no cells

Stain with Rhodamine

- Add 1 uL of 1.04 uM stock Rhodamine 123 to 1 mL sample
- Incubate at room temp for 15 minutes in the dark (in amber tube or covered is sufficient)
- Pellet cells by spinning at 10,000xg for 7 minutes
- Remove and discard supernant
- Add 1 mL 1x PBS, resuspend the cells by pipetting (first wash)
- Pellet cells by spinning at 10,000xg for 7 minutes
- Remove and discard supernant
- Add 1 mL 1x PBS, resuspend the cells by pipetting (second wash)
- Pellet cells by spinning at 10,000xg for 7 minutes
- Resuspend cells in 1 mL 1x PBS

Fix with formaldehyde

- Add 28 uL formaldehyde to 1 mL sample (37% stock; final concentration will be 1%)
- Incubate (with gentle rocking) for 15 minutes
- Wash twice with 1x PBS as above

Supernant goes into hazardous waste

- Resuspend cells in 1 mL 1x PBS

Propidium iodide

- Add 6 uL of 3 ug/mL PI to 1 mL sample
- store cells at 4°C until ready for flow cytometry

Culture growth for RNA isolation

Day -3

Streak strains to single colony

Day 0

Patch out single colonies in triplicate

Day 1 (RNA isolation day)

For each sample, scrape up one patch of cells and resuspend in ~300 uL MHB in sterile 1.5 mL tube

Add 700 uL more MHB, be sure cells are well-resuspended

Check OD600:

Dilute resuspended cells 1:10 in MHB (100 uL cells, 900 uL MHB)

Check OD600 using 1 mL MHB as blank

Calculate actual OD600 (measured OD600 multiplied by dilution factor [10])*

Calculate what volume of cells you need to obtain an OD600 of 0.08 in 10 mL*

Swirl tube to distribute cells – didn't do

Remove 1 mL and use to measure OD600

Put tubes at 37°C shaking

Check OD600 after ~ 2 hours to be sure cells have come close to doubling (document OD600!*)

Cool down the centrifuge

When OD600 reaches between 0.3 – 0.4 (4-6 hours, document OD600!*):

Transfer 1.8 mL into 2 mL tube

Centrifuge max speed at 4°C for 3 minutes

Transfer 1.8 mL into 2 mL tube

Centrifuge max speed at 4°C for 3 minutes

Resuspend cell pellet in 1 mL TRI-Reagent (step 1 of RNA isolation protocol)

RNA isolation – Dan and Dr. Ramsey did

Purify nucleic acids

1. Resuspend cell pellet (1.8 mL - 10 mL) in 1 mL TRI-Reagent (phenol – inactivates)
2. Incubate at 60°C for 10 min
Heat block – lyse open the cells
3. Spin at 4°C for 10 min at max speed
RNase free!!
4. Transfer supernatant to new 2mL tube (~950 uL).
Don't care about the pellet (nothing of interest)
5. Add equal volume 100% ethanol add to the supernatant tube
6. Pass sample over Directzol column, 600 uL per spin, 30 sec per spin at max speed, discarding flow-through in phenol and methanol waste
7. Place spin column in new collection tube
8. Wash twice with 400 uL RNA PreWash buffer, discarding flow-through in phenol and methanol waste
9. Add 700 ul of Wash buffer, let sit on column for 3 min
10. Spin max speed for 2 min
11. Wash again with 700 ul Wash buffer
12. Spin max speed for 2 min
13. Place column in new collection tube
14. Spin max speed for 3 min
15. Place column in clean 1.5 mL tube
16. Add 90 uL RNase-free water, let sit on column 2 min
17. Spin max speed 1 min
18. Place flow-through on column again, spin 1 min

Check RNA concentration and quality by Nanodrop. The 260/280 ratio should be close to 2.0 and the 260/230 ratio should be greater than 1.9. Run on a gel.

Store nucleic acids at -80°C if not moving directly to the next step

Tuesday, March 8, 2022

1. ~~Check counts for day 14 and see if circular plates are better~~
2. ~~Make CHA plates – 6 square and 12 circular~~
3. ~~LB Agar~~
4. ~~LB Media~~
5. ~~MHB broth~~

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

Made 13 circular plates and 10 square plates

Mueller-Hinton broth protocol- by Jamie Wandzilak

Preparing LB-agar

Preparing LB media

1. For 250 mL of LB, weigh out the following components and add to a 500 mL bottle:
 - a. 2.5 g NaCl
 - b. 2.5 g Tryptone
 - c. 1.25 g Yeast extract
2. Add 250 mL type I ddiH₂O
3. Close tightly and shake to mix
4. Loosen cap and add a small piece of autoclave tape with the date
5. Autoclave on 30 minute liquid cycle
6. Sterile media can be stored indefinitely

Completed the DNase treatment with Dr. Ramsey. Took around 2.5 hours total (?). No changes in the protocol.

Nanodropped before starting:

#	Sample ID	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	260/280	260/230	Sample Type	Factor
1	Rna1	448	ng/μl	11.201	5.275	2.12	2.42	RNA	40
2	Rna2	492.3	ng/μl	12.307	5.86	2.1	2.41	RNA	40
3	Rna3	542.3	ng/μl	13.557	6.357	2.13	2.42	RNA	40
4	Rna4	438.6	ng/μl	10.964	5.223	2.1	2.43	RNA	40
5	Rna5	322.6	ng/μl	8.065	3.799	2.12	2.25	RNA	40
6	Rna6	370.8	ng/μl	9.27	4.387	2.11	2.48	RNA	40

DNase treatment

~100 ul of sample

1. Add 10 uL RNase-free DNase buffer and 10 uL RNase-free DNase (Promega, RQ1)
Add buffer to everything first

2. Incubate at 37°C for 1 hour
3. Add 300 uL TRI-Reagent
4. Add 400 uL 100% ethanol
5. Go to step 6 in "Purify nucleic acids" protocol above and follow protocol, elute purified RNA in 100 uL RNase-free water.

Nanodropped:

RNA isolation – Dan and Dr. Ramsey did

Purify nucleic acids

6. Pass sample over Directzol column, 600 uL per spin, 30 sec per spin at max speed, discarding flow-through in phenol and methanol waste
7. Place spin column in new collection tube
8. Wash twice with 400 uL RNA PreWash buffer, discarding flow-through in phenol and methanol waste
9. Add 700 ul of Wash buffer, let sit on column for 3 min
10. Spin max speed for 2 min
11. Wash again with 700 ul Wash buffer
12. Spin max speed for 2 min
13. Place column in new collection tube
14. Spin max speed for 3 min
15. Place column in clean 1.5 mL tube
16. Add 90 uL RNase-free water, let sit on column 2 min
17. Spin max speed 1 min
18. Place flow-through on column again, spin 1 min

Nanodropped again:

#	Sample ID	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	260/280	260/230	Sample Type	Factor
1	RNA1	300	ng/μl	7.501	3.496	2.15	2.45	RNA	40
2	RNA2	415.1	ng/μl	10.376	4.813	2.16	2.43	RNA	40
3	RNA3	361.4	ng/μl	9.035	4.204	2.15	2.36	RNA	40
4	RNA4	273.8	ng/μl	6.845	3.205	2.14	2.45	RNA	40
5	RNA5	220.4	ng/μl	5.51	2.588	2.13	2.38	RNA	40
6	RNA6	240.4	ng/μl	6.01	2.805	2.14	2.39	RNA	40

Put in the -80C.

Wednesday, March 9, 2022

1. ~~Label things for tomorrow~~
2. ~~Work on poster~~

Thursday, March 10, 2022

1. ~~Plating Day 21~~
 1. ~~1-3 do serial dilutions~~
 2. ~~4-9 300 ul circular plates~~
2. ~~Take out phenol from under other hood~~

Plating Protocol

Plating: Did the beads with 300 ul for flasks 4 – 9 in duplicate. Will check on them on Sunday. Did the normal plating procedure for flasks 1-3.

Friday, March 11, 2022

1. ~~Get hype for spring break!!~~
2. ~~Make plates for Hannah to use on 3/17~~
3. ~~Send Hannah the protocol for 3/17~~

Monday, March 21, 2022

1. ~~Check plates~~
2. ~~Submit abstract at 12pm~~
3. ~~Count day 21 and 28~~

Today I pulled out the plates that Hannah plated for me on 3/17. I counted them.

Track	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
Plates	1	4.833333	483.3333	577.3503
1,2,3	0.1	0	0	0

I also counted the plates from 3/10 from the pictures that Hannah sent me via slack.

Total:

Track	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
Plates	1	74	7400	5463.744
1,2,3	0.1	8.666667	8666.667	11718.93

Big:

Track	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
Plates	1	53.66667	5366.667	4271.222
1,2,3	0.1	5.666667	5666.667	7371.115

Did the math for the cDNA

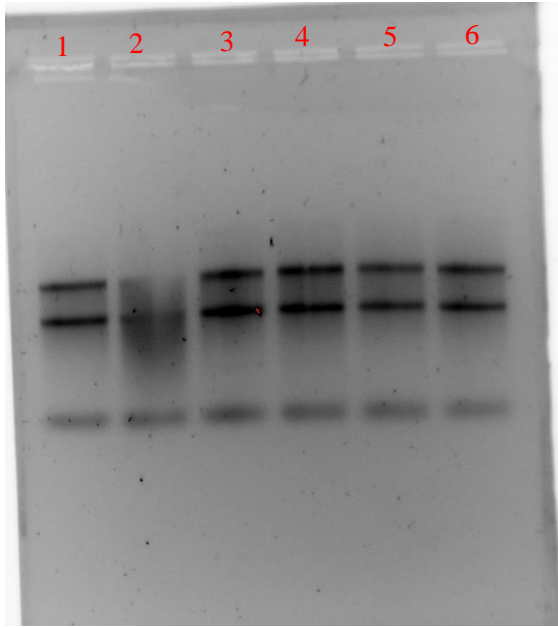
Tuesday, March 22, 2022

1. Make plates
2. cDNA half protocol
3. run gel

Gel for RNA

- rinse comb and gel box with DI water
 - used small gel box and the 6 lane comb
- ethanol both the comb and gel box
- Make fresh agarose (1 g agarose and 100ml of fresh 1 x TAE)
- Add stir bar and heat until boiling
- Cool down to ~60C
- Add 5 ul of cybersafe before pouring
- Pour gel and cover with tin foil
- Load 2 ul of purple dye that can be found in the misc. buffers in -20C
- Load 12ul of each sample into each lane.

Sample	Nanodrop (ng/ul)	RNA Volume (ul)	Water Volume (ul)
RNA1	300	5.00	5.00
RNA2	415.1	3.61	6.39
RNA3	361.4	4.15	5.85
RNA4	273.8	5.48	4.52
RNA5	220.4	6.81	3.19
RNA6	240.4	6.24	3.76



The top band is ____ and the middle band is _____. The bottom band is the 5s. Every sample looks as expected except for 2.

Generate cDNA (half protocol)*

***REACTION SIZE CUT IN HALF** from Lory lab microarray protocol

Combine the first components for primer annealing:

Component	Volume or Amount	Final Concentration
RNA	3 ug	267 - 333 ng/ ul
(NS) ₅ Primer (250 ng/ul)	1.5 ul	25 ng/ul
RNase-free water	up to 15 ul	

To normalize all the cDNA samples to the same DNA mass, dilute with RNase-free water in PCR strip tubes.
Table with volumes of cDNA and water

Number		Date isolated	RNA Conc (ng/ul)	RNA	cDNA	
					H2O	Total RNA
1	148.1 LVS ΔrpsU2	3/8/2022	300	10.00	3.50	3000
2	Tn7_PrpsU2_rpsU2U		415.1	7.23	6.27	3000
3	TR_lacZ aphA E-1		361.4	8.30	5.20	3000
4	149.1 LVS		273.8	10.96	2.54	3000
5	Tn7_PrpsU2_rpsU2U		220.4	13.50	0.00	2975.4
6	TR_lacZ aphA E-1		240.4	12.48	1.02	3000

Note the total volume of cDNA and water is 13.5 uL. Also note the change in total RNA for #5.

Add the 1.5 uL (NS)₅ oligo (stored in small aliquots at -80°C; take your own aliquot to use and move it to your own stock box; minimize freeze/thaw cycles) to the tubes.

- Vortexed and spun down before putting in thermocycler

Incubate using program JSScDNA1 in the thermocycler:

Step	Temp	Time
1	70°C	10'

2 25°C 10'
3 4°C hold

Didn't change that the volume was 15ul not 30ul.

While waiting, prepare the cDNA synthesis reaction in master mix format. The number of reactions you have plus 1.5 should be the factor you multiply by.

Example master mix calculations- this master mix would be enough for 21 cDNA reactions:

Component	Final Concentration	Volume	x 7.5
5X 1st strand buffer	1x	6	45
RNase-free water		2.88	21.6
100 mM DTT	10 mM	3	22.5
10 mM dNTPs	0.5 mM	1.5	11.25
Superscript III (200 U/ul)	10.8 U/ul	1.63	12.225

*Buffer, DTT, and dNTPs should be from the cDNA reagents box (they are RNase free!)

- mixed the master without the enzyme by vortex and spinning down
- pipetted up and down 8 times after enzyme with 100 ul

Aliquot 15 ul of master mix per reaction into each PCR tube from the first reaction (total volume now 30ul)

Incubate using program JSScDNA2

Step	Temp	Time
1	25°C	10'
2	37°C	60'
4	42°C	60'
5	70°C	10'
6	4°C	hold

*potential stopping point- samples can be stored at -80°C if necessary

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

Made 12 plates. 24 ml onto each plate. 15.3 g of CHA and 150 ml of di water.

Wednesday, March 23, 2022

- ~~1. label~~
2. ask about cleaning up flasks 4-9
 1. bleach
- 3.

Remove RNA from sample by degrading with sodium hydroxide:

- ☐ Add 10 ul of 1N NaOH
 - o Pipetted up and down 5 times
- ☐ Incubate 65°C for 30'
 - o Dan did the steps after this
- ☐ Neutralize with 10 ul of 1N HCl
- ☐ Final volume is 50 ul
- ☐ Purify cDNA using Qiagen PCR clean-up column (be very sure there is no ethanol carry-over!)
 - o Added 250 ul of PB to the 50 ul of the PCR reactions
 - o
- ☐ Elute in 60 ul of 0.1x EB

Check concentration by Nanodrop

Store cDNA at -80°C

Thursday, March 24, 2022

1. ~~Plate day 35~~
 1. ~~Only plating 1-3 on circular plates~~
2. ~~Update the graphs~~
3. Nanodrop 5



total								
Sample	Day 0	Day 1	Day 4	Day 7	Day 14	Day 21	Day 28	Day 35
1	8850000	5750000	1505000	1150000	59500	2350	150	
2	8850000	5100000	1275000	1285000	157000	13200	1150	
3	8850000	3950000	1060000	1245000	92000	6650	150	
4	8850000	1145000	400000	3050	466.6667	0	0	
5	8850000	3700000	540000	0	0	0	0	0
6	8850000	1130000	0	0	0	0	0	0
7	8850000	1600000	3750	0	0	0	0	0
8	8850000	1435000	16900	265	0	0	0	0
9	8850000	1525000	14450	218.3333	0	0	0	0

Big only								
Sample	Day 0	Day 1	Day 4	Day 7	Day 14	Day 21	Day 28	Day 35
1	8850000	3950000	1065000	820000	38500	1800	150	
2	8850000	3400000	1065000	1070000	93000	10100	1150	
3	8850000	2350000	830000	920000	61000	4200	150	
4	8850000	825000	325000	2350	451.6667	0	0	
5	8850000	2200000	360000	0	0	0	0	0
6	8850000	1130000	0	0	0	0	0	0
7	8850000	1040000	3250	0	0	0	0	0
8	8850000	960000	13450	121.6667	0	0	0	0
9	8850000	370000	11950	108.3333	0	0	0	0

Dan changed the factor so it is measuring the ssDNA:

dsDNA calculations	Sample ID	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	260/280	260/230
	1	26.4	ng/μl	0.528	0.278	1.89	1.49
	2	30.5	ng/μl	0.61	0.296	2.06	1.92
	3	32.3	ng/μl	0.647	0.329	1.97	1.74
	4	27.5	ng/μl	0.55	0.259	2.13	1.94
	5	30	ng/μl	0.6	0.335	1.79	1.14
	6	23.1	ng/μl	0.462	0.298	1.55	1.65
	7	24.5	ng/μl	0.491	0.317	1.55	1.42
	8 #5 redo	16.1	ng/μl	0.486	0.234	2.08	1.44
ssDNA calculations	Sample ID	Nucleic Acid	Unit	A260 (Abs)	Factor		
	1 cDNA sample 1	17.5	ng/μl	0.528	33.3		
	2 cDNA sample 2	20.3	ng/μl	0.61	33.3		
	3 cDNA sample 3	21.5	ng/μl	0.647	33.3		
	4 cDNA sample 4	18.3	ng/μl	0.55	33.3		
	5 cDNA sample 5 (the redo)	16.1	ng/μl	0.486	33.3		
	6 cDNA sample 6	15.3	ng/μl	0.462	33.3		

Friday, March 25, 2022**1. Clean flasks**

Put bleach so 1/2 sample, 1/2 bleach and let sit for 10 minutes. Went down the drain with a lot of water.

Monday, March 28, 2022**1. Prep DNA samples****2. Check plates**

I checked the plates, there was minimal growth. The colonies were tiny, barely visible. I put them back into the incubator for further growth.

Determine concentration of cDNA

- Sample 3 = 21.5 ng/ul

Make a dilution that's final concentration is 1.5ng/ul

- $C_1V_1 = C_2V_2$
- $C_1 = 21.5$
- $V_1 = 5.93$ from RNA tube 3
- $C_2 = 1.5$ ng/ul
- $V_2 = 85$ ul
- $V_1 = (1.5 \times 20)/(C_1)$

Make 1:10 serial dilutions so you have 4 different DNA concentrations (1.5ng/ul, 0.15ng/ul, 0.015ng/ul, 0.0015ng/ul)

0.1 x EB to dilute

Final concentration	0.1 x EB (ul)	DNA (ul)	Source
DNA A: 1.5 ng/ul	79.07	5.93	RNA tube 3
DNA B: 0.15 ng/ul	76.5	8.5 ul	Tube A
DNA C: 0.015 ng/ul	76.5	8.5	Tube B
DNA D: 0.0015 ng/ul	76.5	8.5	Tube C

Switch tips!!

Tuesday, March 29, 2022

1. make plates
 1. check plates to see growth first
2. Real time PCR Primer Efficiency
3. Take out trash

Real-Time Primer Efficiency Test

Note: Efficiencies should be run on same "type" of DNA as you plan to do the experiment on; i.e. ChIP= sonicated DNA, RNA expression = cDNA or RNA w/ 2-step kit

- Only doing one sample to test the efficiency

Put 17.5ul of each DNA sample into separate tubes (enough for 3.5rxns)

Set up primer master mixes in separate eppendorfs (make one for each primer pair)

PRIMER SET 1 = KROL395,396

PRIMER SET 2 = KROL397,398

PRIMER SET 3 = KROL399,400

PRIMER SET 4 = (tul 4 control)

For 4 DNA samples, multiply the volume of each master mix component by 17.5 to account for pipetting error

example:

10 ul of SYBR green x 17.5 = 175

1 ul of 5uM primer set x 17.5 = 17.5

4 ul ddH₂O x 17.5 = 70

TOTAL = 262.5

Add enough master mix so that there are 3.5 rxns worth in each tube. Since 1 rxn=20ul, 3.5 rxn x 20ul rxn volume = 70ul. Tubes already have 17.5ul of DNA, so add 52.5ul (70-17.5) of primer master mix to each DNA tube

Pipette 20ul of each rxn into 3 separate wells on the 96 well plate

1A	1A	1A	1B	1B	1B	1C	1C	1C	1D	1D	1D
2A	2A	2A	2B	2B	2B	2C	2C	2C	2D	2D	2D
3A	3A	3A	3B	3B	3B	3C	3C	3C	3D	3D	3D
4A	4A	4A	4B	4B	4B	4C	4C	4C	4D	4D	4D

Spin plate down – 2 min at 500 rcf

Place in real-time machine and run rxn

Old StepOne Plus (2-step amplification)

95°C 10'

95°C 15"

60°C 60"

Go to step 2, 39x (total 40 cycles)

95°C 10"

65°C 60"

97°C 60"

Melt curve (95°C 10", 65°C 60", 97°C 60" with continuous ramp)

Analyze on RT machine

Calculations

ChIP enrichment

Primer Efficiency ^ (Ct_diff.)

Ct_diff. = (control_ChIP_Ct – test_ChIP_Ct) - (control_Input_Ct – test_Input_Ct)

Primer Efficiency = (10^{^(-1/slope)})

Where the slope is the slope of the standard curve plot

GSC computer login:

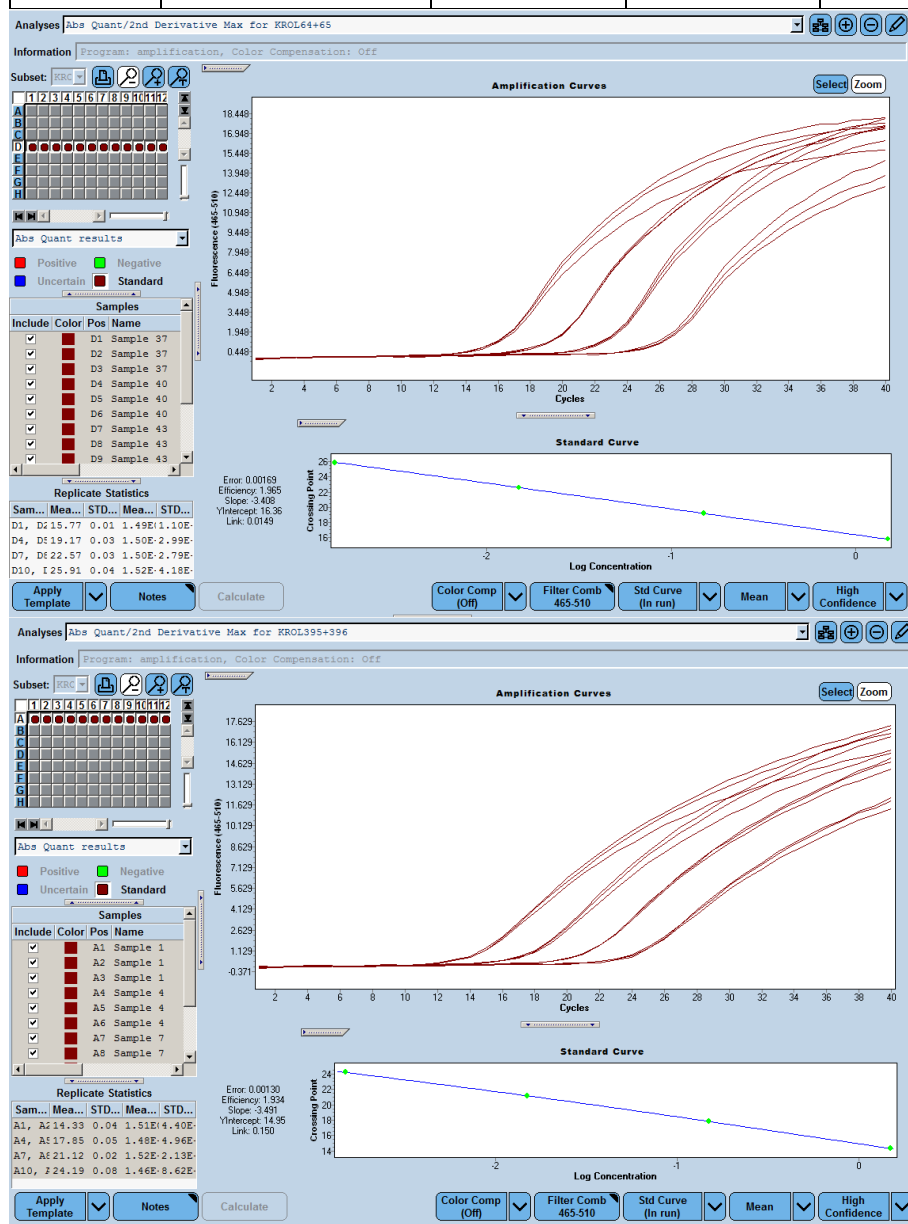
K_Ramsey Lab

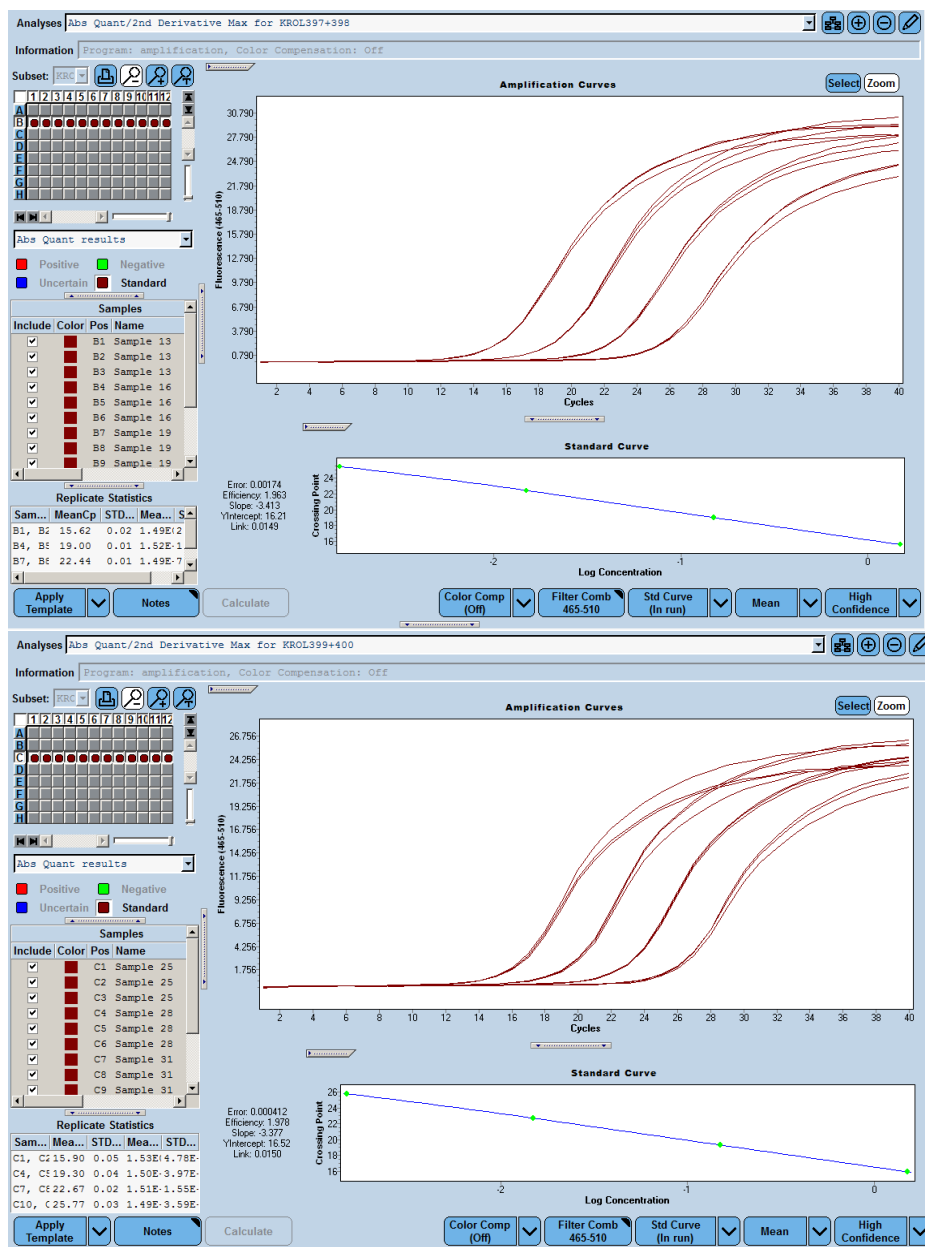
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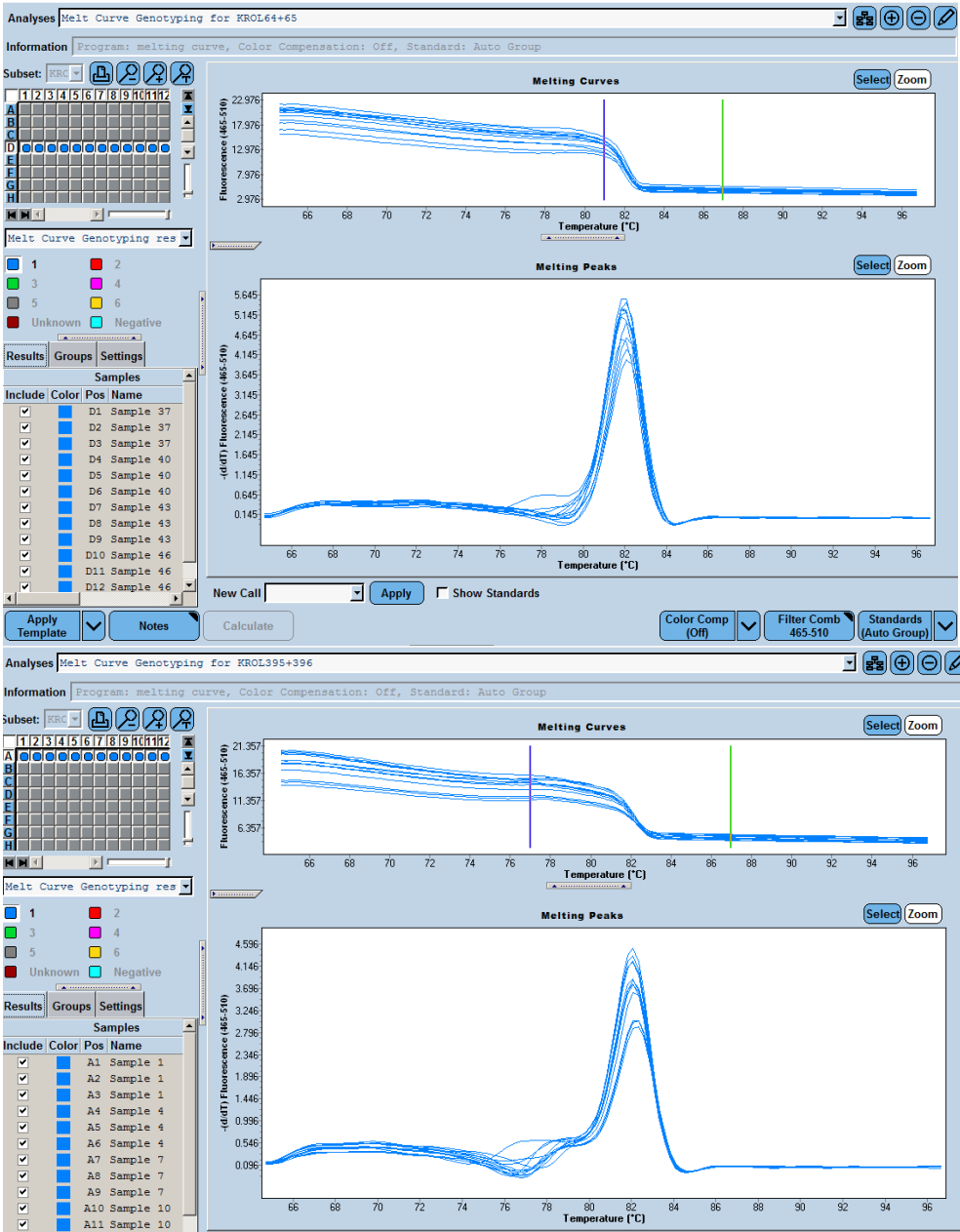
Roche480

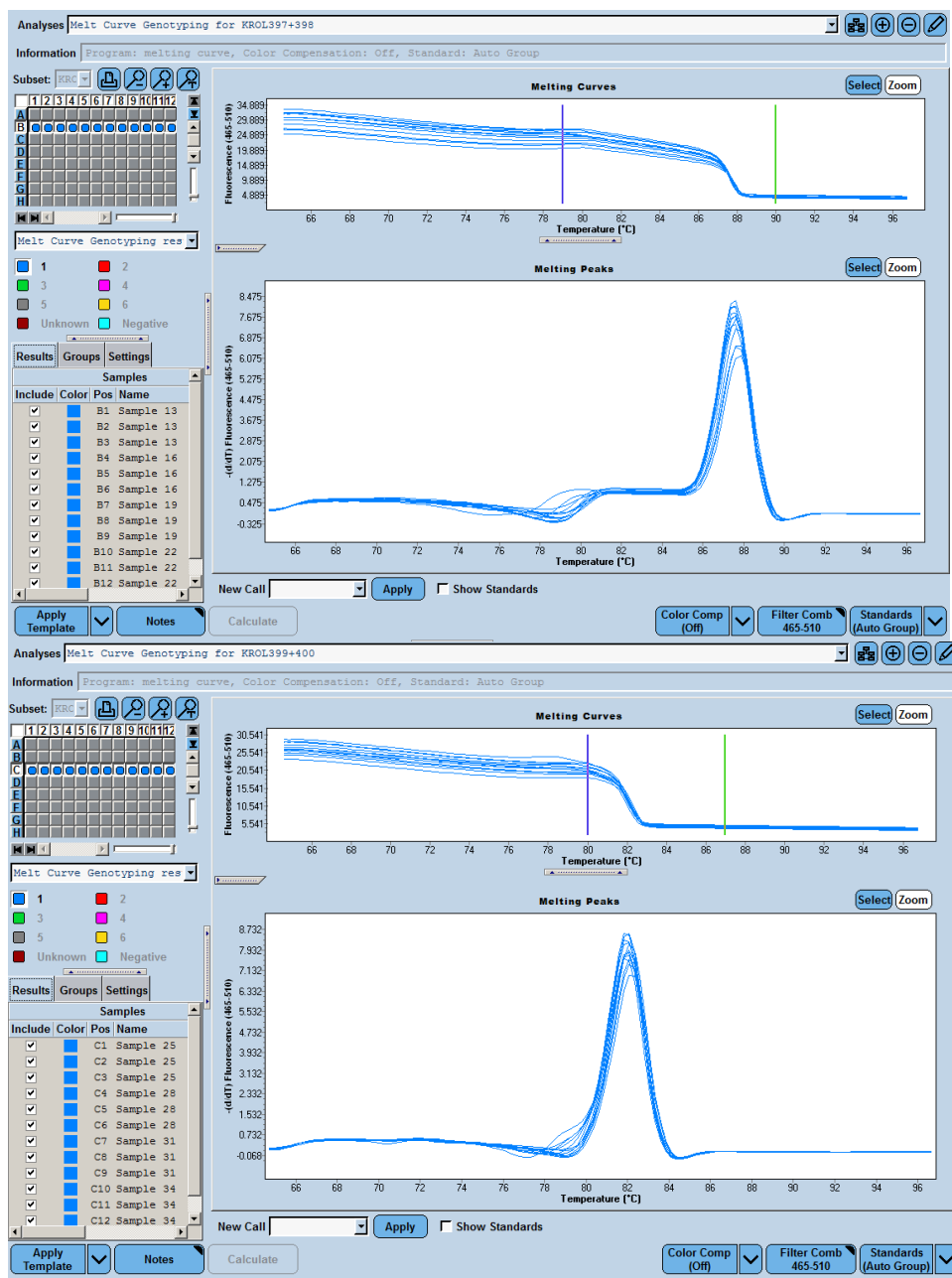
Results

Primer Set ID	Primers	Efficiency	CP value 1.5	CP value 0.15	CP value 0.015	MP
PS1	KROL 395/396	1.934	14.33	17.85	21.12	~82C with 1 small shoulder
PS2	KROL 397/398	1.963	15.62	19.00	22.44	~87.5C with 2 small shoulders
PS3	KROL 399/400	1.978	15.90	19.30	22.67	~82C clean
PS4	KROL63/64	1.965	15.77	19.17	22.57	~82C with 1 small shoulder









Primer Set ID	Primers	Efficiency	CP value 1.5	CP value 0.15	CP value 0.015	MP
PS1	KROL 395/396	1.934	14.33	17.85	21.12	~82C with 1 small shoulder
PS2	KROL 397/398	1.963	15.62	19.00	22.44	~87.5C with 2 small shoulders
PS3	KROL 399/400	1.978	15.90	19.30	22.67	~82C clean
PS4	KROL63/64	1.965	15.77	19.17	22.57	~82C with 1 small shoulder

Wednesday, March 30, 2022

- ~~1. Label~~
- ~~2. Check amount of KROL 343/344~~
- ~~3. Make dilutions for the rt-per~~
- ~~4. Take out trash and clean non-hazardous tubes~~

Make more KROL 343/344

- Take 15 ul of each (KROL 343 and 344) and combine in 1.5 ul tube
- Add 270 ul of 0.1 x EB
- 300 ul of 5 mM PS 343/344

Dilution Math:

Sample ID	Nucleic Acid (ng/ul) C1	C2	V1	V2	EB
cDNA sample 1	17.5	0.5	1.43	50	48.57
cDNA sample 2	20.3	0.5	1.23	50	48.77
cDNA sample 3	21.5	0.5	1.16	50	48.84
cDNA sample 4	18.3	0.5	1.37	50	48.63
cDNA sample 5 (the redo)	16.1	0.5	1.55	50	48.45
cDNA sample 6	15.3	0.5	1.63	50	48.37

Thursday, March 31, 2022

- ~~1. plate day 42~~
- ~~2. more LB media and MHB~~
- ~~3. RT-PCR~~

Plated day 42 onto circular plates with 300 ul of sample from flasks 1-3. Used beads. Went well.

Real-Time PCR on cDNA samples

Original protocol by Heather McManus, edited by Kathryn Ramsey

Each experiment will need at least one test primer and one control primer for each sample

Example: 6 biological replicates = 6 DNA samples

Using three primer pairs = 18 different reactions

PS1: KROL399/400 - lacZ

PS2: KROL63/64 – tul 4

PS3: KROL343/344 – 5' UTR

Each different reaction type must be run in triplicate on the real-time plate

Example: 18 reactions = 54 wells

Each Individual reaction = 20 µL

10 µL PowerUp SYBR Green Master Mix

1 µL 5uM combined forward and reverse primer

1 µL cDNA (~1.5 ng/ul, can base off efficiency tests)

8 µL ddiH₂O

The use of master mixes increases the consistency between samples; therefore follow the method below for setting up the plate

For each reaction type (primer/DNA combination) set up a master mix equal to 3.5 reactions:
Put 3.5 μ L of DNA type into strip tubes

To determine the amount of each primer master mix to create, identify the number of reactions for each primer pair (i.e. #DNA samples x 3.5) and add 3.5 to this number to account for additional pipetting error

Example: 6 DNA samples per primer pair = $6 \times 3.5 + 3.5 = 24.5$

Set up primer master mixes in separate 1.5 mL tubes (make one for each primer pair)

Example:

10 μ L PowerUp SYBR Green Master Mix $\times 24.5 = 245.0 \mu\text{L}$

1 μ L 5 μ M combined F and R primer $\times 24.5 = 24.5 \mu\text{L}$

8 μ L ddiH₂O $\times 24.5 = 171.5 \mu\text{L}$

TOTAL = 465.5 μ L

Add primer master mix to tubes containing DNA.

3.5 reactions $\times 20 \mu\text{L}$ volume = 70 μ L. DNA tubes already have 3.5 μ L of DNA. Add 66.5 μ L of primer master mix to each tube

Pipette 20 μ L of each reaction into 3 separate wells on 96 well plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1A	1A	1A	1B	1B	1B	1C	1C	1C	1D	1D	1D
B	1E	1E	1E	1F	1F	1F						
C	2A	2A	2A	2B	2B	2B	2C	2C	2C	2D	2D	2D
D	2E	2E	2E	2F	2F	2F						
E	3A	3A	3A	3B	3B	3B	3C	3C	3C	3D	3D	3D
F	3E	3E	3E	3F	3F	3F						

Spin plate down for 2 minutes at 500 rcf and pray it opens

Place in real-time machine and run using the same program used to determine that the primers are appropriately efficient.

GSC computer login:

K_Ramsey Lab

Password:

Roche480

Mueller-Hinton broth protocol- by Jamie Wandzilak

For 500 mL of MHB

1. Weigh out 10.5g of Mueller-Hinton broth into 1L square-bottle
2. Add 500mL of ddiH₂O (type I)
3. Autoclave on 30' liquid cycle, filling the water bin up

4. Cool down to 37 °C or cooler
5. Can keep this sterile media indefinitely without supplements

Preparing LB media

1. For 250 mL of LB, weigh out the following components and add to a 500 mL bottle:
 - a. 2.5 g NaCl
 - b. 2.5 g Tryptone
 - c. 1.25 g Yeast extract
2. Add 250 mL type I ddiH₂O
3. Close tightly and shake to mix
4. Loosen cap and add a small piece of autoclave tape with the date
5. Autoclave on 30 minute liquid cycle
6. Sterile media can be stored indefinitely

Preparing LB-agar

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

April 2022

Friday, April 1, 2022

1. Look at results from yesterdays rt-pcr

deltaCt = comparing gene of interest to control gene = subtract gene of interest – control gene averages

average delta Ct = average the delta Ct value for the biological replicates

stdev of the delta ct

delta delta ct vs control = normalize WT = 1 , easier to look at multiple genes at once

$$= \text{delta CT} - \text{WT delta CT}$$

\$ = dragging down the column, the number stays the same

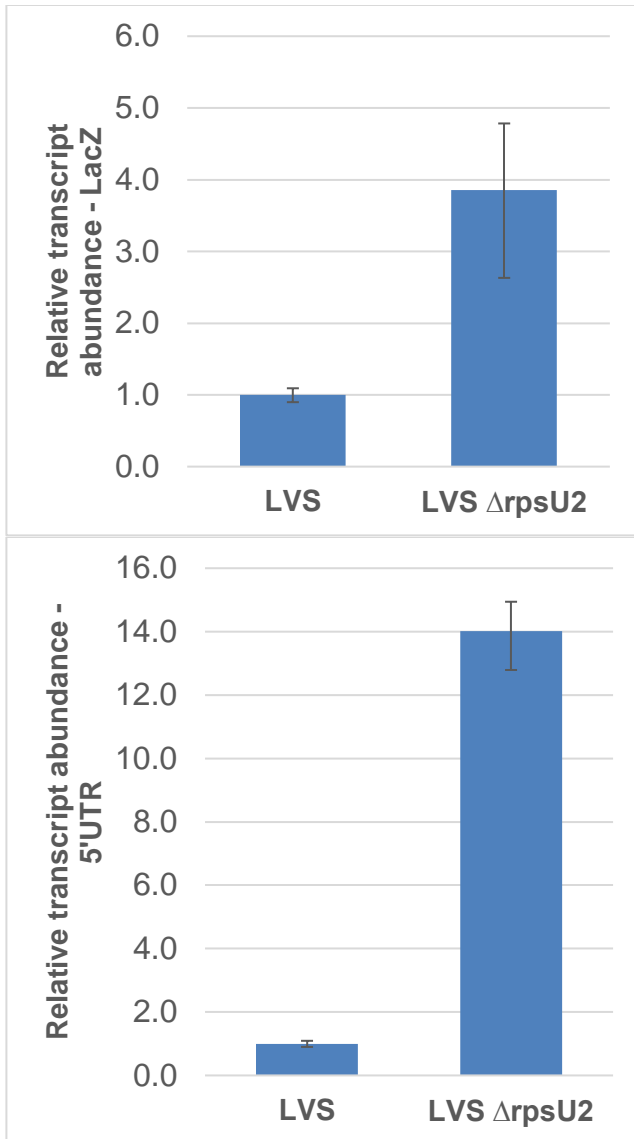
S = standard error

$$= \sqrt{WT^2 + \text{delta}^2}$$

1.8 (doubling DNA every time) primers not efficient, 1.8 is lower limit for acceptable efficiencies

Separate plus and minus bars for stdev

Column O = height of error bars



Monday, April 4, 2022

1. ~~Prep primers~~
2. ~~Prep for rt per experiment~~

Looked at plates for samples 1-3. No growth. Leaving at room temp and will check tomorrow.

Receiving Primers Protocol

By: Hannah Trautmann

1. Spin primers at maximum speed in tabletop centrifuge for 3 minutes so dessicated primers go to the bottom of the tube.
2. Add 0.1x EB to a final concentration of 100 uM. Calculate this by multiplying the reported nm by 10 and adding that volume in ul (i.e. 12.7 nmoles = add 127 uL of 0.1xEB).
 - a. KROL504 = 29.57 nm x 10 = 295.7 ul

- b. $\text{KROL505} = 26.59 \text{ nm} \times 10 = 265.9 \text{ ul}$
- c. $\text{KROL506} = 29.77 \text{ nm} \times 10 = 297.7 \text{ ul}$
- d. $\text{KROL507} = 38.28 \text{ nm} \times 10 = 382.8 \text{ ul}$
3. Put on 42°C heat block for 5 minutes to help primers dissolve
4. Vortex and brief spin.
5. Label tubes with KROL numbers on the top and put in the appropriate 100 uM stock box in the -20°C freezer.
6. Optional: Make dilution for intended purpose of primer.
 - a. If a PCR primer, make a 10 uM stock by diluting 20 uL of the 100 uM stock into 180 uL of 0.1xEB. Label with purple sticker and put in appropriate freezer box.
7. Put Certificate of Analysis sheet in “Oligos Spec Sheet” binder and shipping sheet in Invoices box.

Determine concentration of cDNA

- Sample 2 = 20.3 ng/ul

Make a dilution that's final concentration is 1.5ng/ul

- $C1V1 = C2V2$
- $C1 = 20.3$
- $V1 = 6.28$ from RNA tube 2
- $C2 = 1.5 \text{ ng/ul}$
- $V2 = 85 \text{ ul}$
- $V1 = (1.5 \times 85)/(C1)$

Make 1:10 serial dilutions so you have 4 different DNA concentrations (1.5ng/ul, 0.15ng/ul, 0.015ng/ul, 0.0015ng/ul)

0.1 x EB to dilute

Final concentration	0.1 x EB (ul)	DNA (ul)	Source
DNA A: 1.5 ng/ul	78.71	6.28	RNA tube 2
DNA B: 0.15 ng/ul	76.5	8.5	Tube A
DNA C: 0.015 ng/ul	76.5	8.5	Tube B
DNA D: 0.0015 ng/ul	76.5	8.5	Tube C

Switch tips!! Vortexed and spun before pulling 8.5 ul from the previous tube.

Tuesday, April 5, 2022

1. ~~Check plates~~
2. ~~Make plates depending on growth~~
3. Rt-pcr

Make KROL504/505

- Take 15 ul of each (KROL 504 and 505) and combine in 1.5 ul tube
- Add 270 ul of 0.1 x EB
- 300 ul of 5 mM PS 504/505

Real-Time Primer Efficiency Test

Note: Efficiencies should be run on same “type” of DNA as you plan to do the experiment on; i.e. ChIP= sonicated DNA, RNA expression = cDNA or RNA w/ 2-step kit

- Only doing one sample to test the efficiency

Put 17.5ul of each DNA sample into separate tubes (enough for 3.5rxns)

Set up primer master mixes in separate eppendorfs (make one for each primer pair)

PRIMER SET 1 = KROL399,400 - LacZ

PRIMER SET 2 = KROL504,505 – downstream rpsu2

PRIMER SET 3 = KROL506,507 – downstream rpsu2

PRIMER SET 4 = (tul 4 control) 63,64

For 4 DNA samples, multiply the volume of each master mix component by 17.5 to account for pipetting error

example:

10 ul of SYBR green x 17.5 = 175

1 ul of 5uM primer set x 17.5 = 17.5

4 ul ddH2O x 17.5 = 70

TOTAL = 262.5

Pipetted up and down ~10 times when adding SYBR green to mix.

Add enough master mix so that there are 3.5 rxns worth in each tube. Since 1 rxn=20ul, 3.5 rxn x 20ul rxn volume = 70ul. Tubes already have 17.5ul of DNA, so add 52.5ul (70-17.5) of primer master mix to each DNA tube

Pipette 20ul of each rxn into 3 separate wells on the 96 well plate

1A	1A	1A	1B	1B	1B	1C	1C	1C	1D	1D	1D
2A	2A	2A	2B	2B	2B	2C	2C	2C	2D	2D	2D
3A	3A	3A	3B	3B	3B	3C	3C	3C	3D	3D	3D
4A	4A	4A	4B	4B	4B	4C	4C	4C	4D	4D	4D

Spin plate down – 2 min at 500 rcf

Place in real-time machine and run rxn

Old StepOne Plus (2-step amplification)

95°C 10'

95°C 15"

60°C 60"

Go to step 2, 39x (total 40 cycles)

95°C 10"

65°C 60"

97°C 60"

Melt curve (95°C 10", 65°C 60", 97°C 60" with continuous ramp)

Analyze on RT machine

Calculations

ChIP enrichment

Primer Efficiency \wedge (Ct_diff.)

Ct_diff. = (control_ChIP_Ct – test_ChIP_Ct) - (control_Input_Ct – test_Input_Ct)

Primer Efficiency = $(10^{(-1/\text{slope})})$

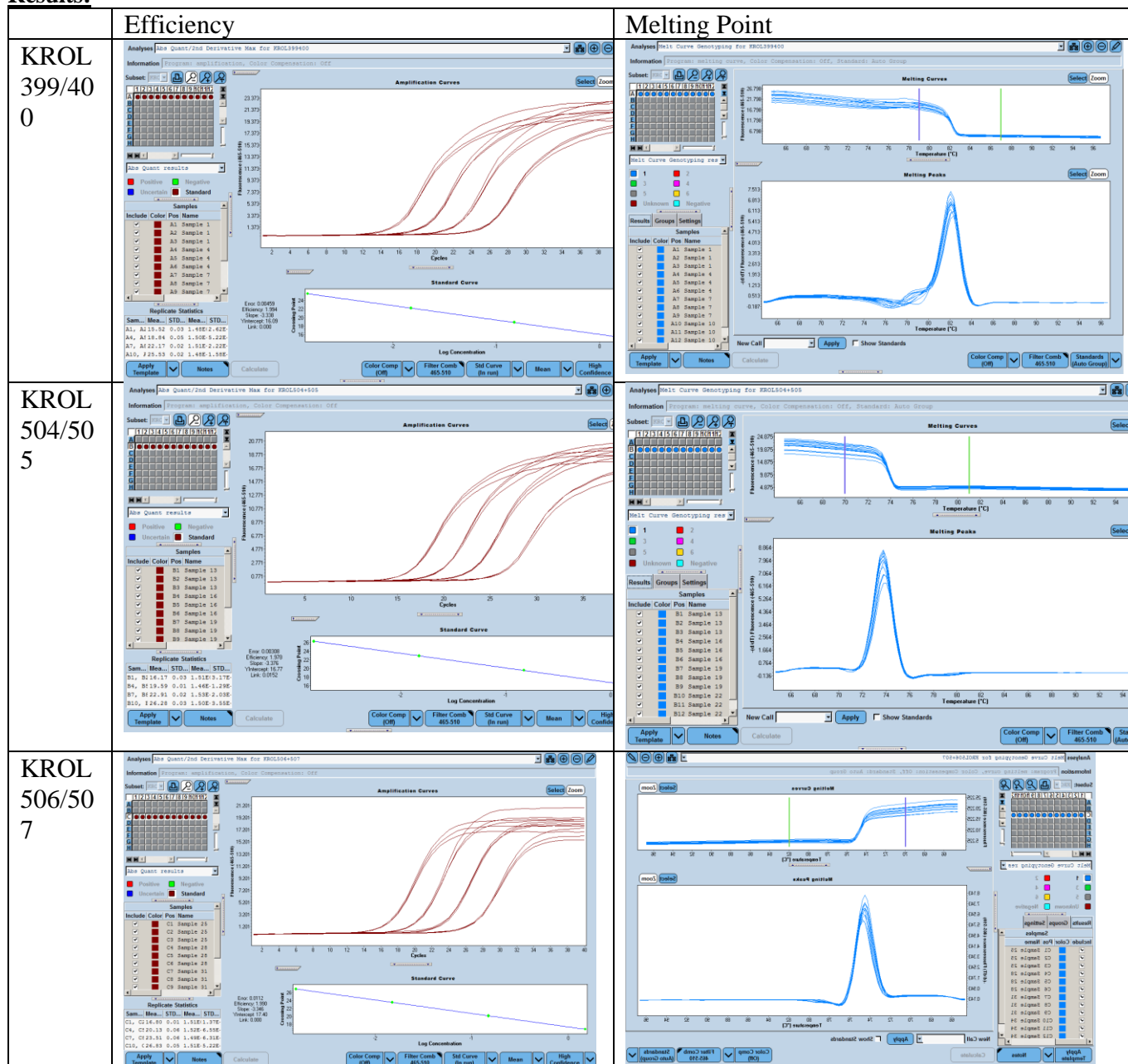
Where the slope is the slope of the standard curve plot

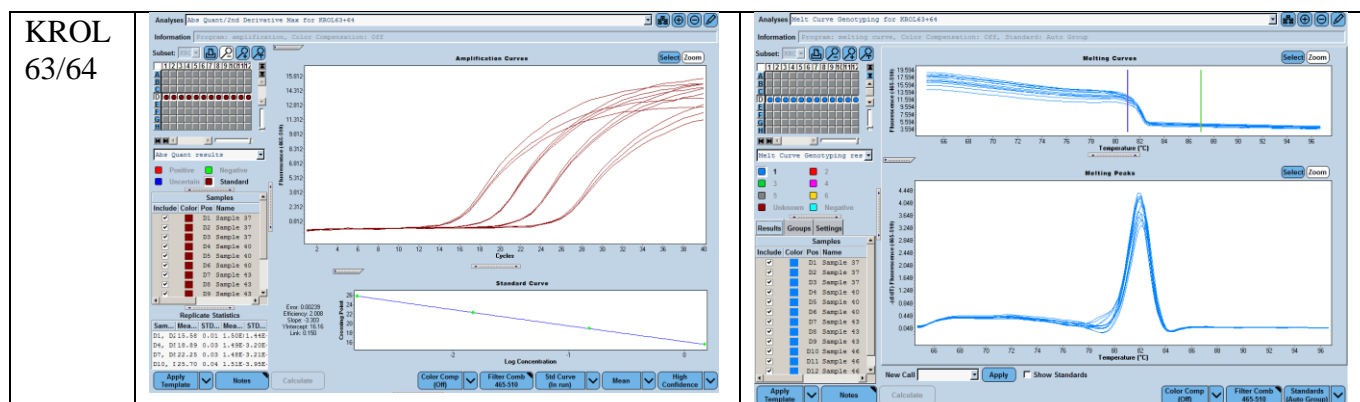
GSC computer login:

K_Ramsey Lab

Password:

Roche480

Results:



PS ID	Primers	Efficiency	CP 1.5	CP 0.15	CP 0.015	Melting Point
PS1	KROL 399+400	1.994	15.52	18.84	22.17	~82 small shoulder
PS2	KROL504/5	1.978	16.17	19.59	22.91	~74
PS3	KROL506/7	1.99	16.80	20.13	23.51	~75
PS4	KROL63/4	2.008	15.58	18.89	22.25	~82

Wednesday, April 6, 2022

1. Prep for plating

Labeled, and in these are notes from lab meeting:

Next RT-PCR

PS 504/5, PS63/64 and all of our samples (6 cDNA) and old samples (cDNA march 4th 2020, samples 1-6 and 10-12)

1-3 – LVS pF

4-6 delta2 pF

10-12 is delta2 pF-rpsu2V

Thursday, April 7, 2022

1. Finish up presentation
2. Finish up poster
3. Plate day 49
4. Check old cDNA concentrations
5. Get correct graph for KROL504/505

Nanodrop of cDNA samples from 3/4/2020. Going to use them for the RT-PCR next Thursday (4/14).

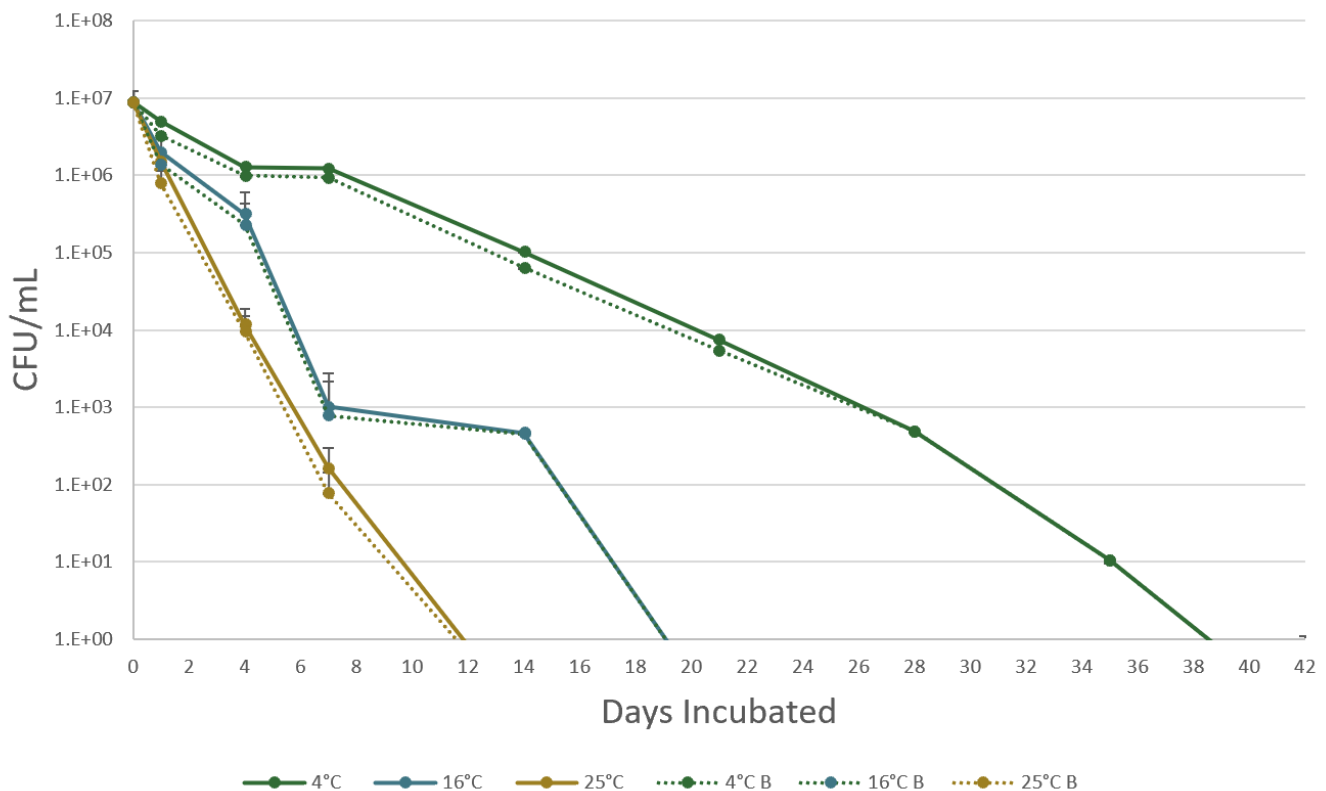
#	Sample ID	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	260/280	260/230	Sample Type	Factor
1	3	29.8	ng/μl	0.903	0.462	1.95	2.7	ssDNA	33
2	1	33.3	ng/μl	1.008	0.533	1.89	2.36	ssDNA	33
3	2	34.5	ng/μl	1.045	0.576	1.81	2.29	ssDNA	33
4	4	23.2	ng/μl	0.702	0.367	1.91	3.41	ssDNA	33

5	5	31.4	ng/μl	0.951	0.511	1.86	2.7	ssDNA	33
7	6b	26.6	ng/μl	0.805	0.436	1.85	2.47	ssDNA	33
8	7	22.8	ng/μl	0.692	0.358	1.93	2.77	ssDNA	33
9	8	24.6	ng/μl	0.744	0.383	1.94	3.01	ssDNA	33
11	9	29.7	ng/μl	0.9	0.518	1.74	1.96	ssDNA	33
12	10b	28.5	ng/μl	0.864	0.479	1.81	2.55	ssDNA	33
13	11	37.1	ng/μl	1.124	0.624	1.8	2.5	ssDNA	33
15	12b	37.7	ng/μl	1.144	0.596	1.92	2.51	ssDNA	33

Friday, April 8, 2022

- ~~1. Presentation day!~~
- 2. Final graph for counts**

Final graph for First run of viable cell plates at 4C, 16C, 25C in freshwater



lines are for total and dots are for big only, big is arbitrary and non-pinpoint colonies.

Monday, April 11, 2022

- ~~1. Figure out protocol for rt-per~~
- ~~2. Check day 49~~

No growth on any plates. Contamination on 1A.

Tuesday, April 12, 2022

- ~~1. Check day 49~~

~~2. LB Agar~~**Preparing LB-agar**

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

No growth on any of my plates.

Wednesday, April 13, 2022

- ~~Prep cDNA and primers and labels for tubes tomorrow~~
- ~~Measure everything needed for tomorrow~~

Sample ID	new ID	Nucleic Acid (ng/ul) C1	C2	V1	V2	EB
sample 1 - Δ2 lac	A	17.5	0.5	1.43	50	48.57
sample 2 - Δ2 lac	B	20.3	0.5	1.23	50	48.77
sample 3 - Δ2 lac	C	21.5	0.5	1.16	50	48.84
sample 4 - LVS lac	D	18.3	0.5	1.37	50	48.63
sample 5 - LVS lac	E	16.1	0.5	1.55	50	48.45
sample 6 - LVS lac	F	15.3	0.5	1.63	50	48.37
1 - LVS pf	G	33.3	0.5	0.75	50	49.25
2 - LVS pf	H	34.5	0.5	0.72	50	49.28
3 - LVS pf	I	29.8	0.5	0.84	50	49.16
4 - Δ2 Pf	J	23.2	0.5	1.08	50	48.92
5 - Δ2 Pf	K	31.4	0.5	0.80	50	49.20
6 - Δ2 Pf	L	26.6	0.5	0.94	50	49.06
10 - Δ2 Pf-rpsu2	M	28.5	0.5	0.88	50	49.12
11 - Δ2 Pf-rpsu2	N	37.1	0.5	0.67	50	49.33
12 - Δ2 Pf-rpsu2	O	37.7	0.5	0.66	50	49.34

Went well. I would have done it differently if I thought ahead. I put the cDNA first then the EB, so I used the 200ul pipette and it wasn't super exact. If I were to do it again, I would put 50 ul of the EB then take out the amount of cDNA and then put the cDNA in to be more exact. I don't think it will effect the results drastically, but something to remember for next time.

Thursday, April 14, 2022

- Next RT-PCR to compare our strains (with *lacZ*) to the previous strains

Notes from lab meeting:

PS 504/5, PS63/64 and all of our samples (6 cDNA) and old samples (cDNA march 4th 2020, samples 1-6 and 10-12)

1-3 – LVS pF

4-6 delta2 pF

10-12 is delta2 pF-rpsu2V

Real-Time PCR on cDNA samples

Original protocol by Heather McManus, edited by Kathryn Ramsey

Each experiment will need at least one test primer and one control primer for each sample

Example: 15 biological replicates = 15 DNA samples

Using 2 primer pairs = 30 different reactions

PS1: KROL504/5 - *lacZ*

PS2: KROL63/64 – *tul 4*

Each different reaction type must be run in triplicate on the real-time plate

Example: 30 reactions = 90 wells

Each Individual reaction = 20 μ L

10 μ L PowerUp SYBR Green Master Mix

1 μ L 5uM combined forward and reverse primer

1 μ L cDNA (~1.5 ng/ μ L, can base off efficiency tests)

8 μ L ddiH₂O

The use of master mixes increases the consistency between samples; therefore follow the method below for setting up the plate

For each reaction type (primer/DNA combination) set up a master mix equal to 3.5 reactions:

Put 3.5 μ L of DNA type into strip tubes

To determine the amount of each primer master mix to create, identify the number of reactions for each primer pair (i.e. #DNA samples x 3.5) and add 3.5 to this number to account for additional pipetting error

Example: 6 DNA samples per primer pair = 6 x 3.5 + 3.5 = 24.5

15 DNA samples per primer pair = 15 x 3.5 + 3.5 = 56

Set up primer master mixes in separate 1.5 mL tubes (make one for each primer pair)

Example:

10 μ L PowerUp SYBR Green Master Mix x 56 = 560 μ L

1 μ L 5 μ M combined F and R primer x 56 = 56 μ L

8 μ L ddiH₂O x 56 = 448 μ L

TOTAL = 1064 μ L

Add primer master mix to tubes containing DNA. Pipetted up and down 3-5 times to mix before adding to wells.

3.5 reactions x 20 μ L volume = 70 μ L. DNA tubes already have 3.5 μ L of DNA. Add 66.5 μ L of primer master mix to each tube

Pipette 20 μ L of each reaction into 3 separate wells. Pipetted up and down 3-5 times to mix before adding to wells.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1A	1A	1A	1B	1B	1B	1C	1C	1C	1D	1D	1D
B	1E	1E	1E	1F	1F	1F	1G	1G	1G	1H	1H	1H

C	1I	1I	1I	1J	1J	1J	1K	1K	1K	1L	1L	1L
D	1M	1M	1M	1N	1N	1N	1O	1O	1O			
E	2A	2A	2A	2B	2B	2B	2C	2C	2C	2D	2D	2D
F	2E	2E	2E	2F	2F	2F	2G	2G	2G	2H	2H	2H
G	2I	2I	2I	2I	2J	2J	2K	2K	2K	2L	2L	2L
H	2M	2M	2M	2N	2N	2N	2O	2O	2O			

Spin plate down for 2 minutes at 500 rcf and pray it opens

Place in real-time machine and run using the same program used to determine that the primers are appropriately efficient.

GSC computer login:

K_Ramsey Lab

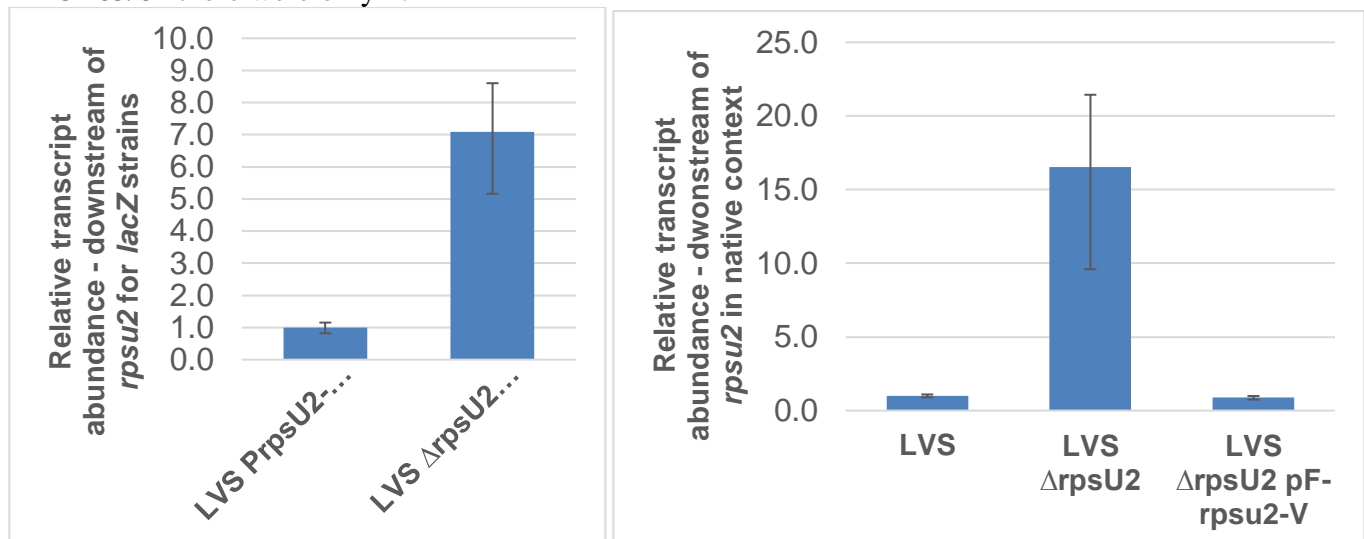
Password:

Roche480

Results

No need to group by primers. Go to analysis, do the abs. quant and save to batch and ensure that it goes to the right folder on the usb stick. Should be a text file.

Well, the results don't look super-hot. In the KROL504/5 there were 11 changes while with the KROL63/64 there were only 2.



There is a lot of error with both, not super ideal. The native contexts were within reason to assume that they validate the previous data I think, even though it is only about 16-fold instead of 25-fold increase of transcript abundance. Our strains have about a 7-fold increase which is a little less than half of what was found with the native context. The primers are measuring the downstream region of *rpsu2* on the chromosome. One reason for this difference could be that a transcription factor or bS21-2 could be pulled in 2 directions in our engineered strains in comparison to the native context? Not really sure.

Friday, April 15, 2022

- ~~1. Figure out what to do next week~~
- ~~2. Pull out dans plates, wrap in parafilm and foil, put in fridge~~

Monday, April 18, 2022

1. Work on draft for final report
- ~~2. Make 10xPBS~~

10X PBS recipe

Start with a beaker to then transfer into a 1 L bottle. Easier to pH.

Start with 800 ml of distilled water:

Add 80 g of NaCl. (61g + 19 g)

Add 2 g of KCl.

Add 14.4 g of Na₂HPO₄. - dibasic

Add 2.4 g of KH₂PO₄.

Adjust the pH to 7.4 with HCl or NaOH.

Bring 20ul and 200ul pipettes, pipettes tips, water, waste beaker, 5 M sodium hydroxide to INBRE.

Used a LOT of 5 N sodium hydroxide to bring pH up to 7.4.

Add distilled water to a total volume of 1 liter. Added like 50 mL too much water.

Split into two bottles, label, add autoclave tape, and autoclave on liquid 20.

Tuesday, April 19, 2022

- ~~1. Plan next experiment for freshwater viability~~
- ~~2. De ice -80C~~

Wednesday, April 20, 2022

- ~~1. Check to see if there are hemoglobin~~
- ~~2. De ice -80C~~

Thursday, April 21, 2022

- ~~1. Prep spreadsheet~~
- ~~2. De ice -80C~~
3. Work on final report

Friday, April 22, 2022

- ~~1. Make CHAH plates~~
- ~~2. Check multichannel and pipette tips~~

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

For 900 mL of CHA

1. Take out 15.3g of cystine heart agar into three 0.5L flasks (non-baffled; 10.2g/100mL)
2. Add 150mL of ddiH₂O (type I) into all flasks
3. Add stirbar to flasks
4. Heat on low, stirring, for about 10 minutes (media will not be fully dissolved because of glucose)

Heat to 50°C

5. Pressure cook for 30 minutes and then let depressurize for 20 minutes. Then put in water bath to cool down for ~15 minutes or more.
6. Cool down (ideally to ~55°C)
7. Separately (before), prepare hemoglobin 2% solution
8. Add ½ flask of hemoglobin into water bath
10. Cool down (ideally to ~55°C)
11. Using sterile technique, pour hemoglobin into CHA

Do it all quickly otherwise agar starts to solidify

12. Using a 50mL pipet, aliquot 30mL of CHAH mixture into each 100mm plate (should make approximately 30 plates) Try to avoid bubbles!

Monday, April 25, 2022

- ~~1. Make a lawn of LVS on two CHAH plates (square plates)~~
- ~~2. Prepare 100 mL of sterile freshwater into a 250 mL flask for OD~~
- ~~3. Prepare 50 mL of sterile freshwater into a conical~~
- ~~4. Get 9 small flasks and label~~
- ~~5. Have 4 square plates ready and labeled for day 0~~
- ~~6. Have 18 square plates for day 1~~
- ~~7. Put small water bath in cold room~~
- ~~8. Prepare poster script~~

Poster Script

For my project I worked with *Francisella tularensis*, which is a pathogenic bacterium. It is the causative agent of tularemia. There is a relationship between aquatic reservoirs and this bacterium, and my project

aimed to determine the laboratory conditions that lead to the longest survival to investigate the genetics in the future. Using filter sterilized water from the Beaver River, three samples with LVS *F. tularensis* were incubated. I kept the samples at 4C, 16C, and 25C. On days 0,1,2,4,7 and weekly afterwards I serially diluted from 1 to 10^{-6} , and incubated at 37C or room temperature for 4-7 days. The variation in incubation was due to the colonies needing to be a countable size. After counting the cells, I added to a spreadsheet where I kept track and analyzed the data.

The cells remained viable from 35 days at 4C. Another significant piece of information is at Day 7, there was significant death of cells at 16C and 25C. Additionally, there were variation in colony sizes, a medium, glistening, round edge colony, and a pinpoint colony. I decided to keep track of the total colonies and just the “big” colonies, the line and dotted line, respectively. There were no significant differences found between temperature condition and colony size. The difference in size could be due to some cells responding the stressors of these conditions. These results connect back to literature as a large amount of tularemia cases can be found in central and norther Norway. There river water is warmest between at 7.7C – 20.3C in summer months, and cases can be found throughout the year. I am currently replicating this experiment to validate results, and hopefully over the summer, I will create a mutant library to find the gene or genes that are responsible for this response or use flow cytometry to enumerate cells.

Tuesday, April 26, 2022

~~1. Take OD~~

~~2. Plate Day 0~~

- **Take OD first!**
- Get a sterile 1.5 mL tube and pipette 500 uL of freshwater into it.
- Scrap up all of cells that were prepared the previous day into the 1.5mL tube with freshwater. Resuspend the cells.
- Add 500 mL more freshwater to the 1.5 tube
- Pipette 980 uL of freshwater and 20 uL of resuspended cells into the cuvette. Place parafilm on top and invert 3 to 5 times, slowly.
- Use spectrometer to measure OD. Obtain an OD₆₀₀ of 0.03. Don't forget the blank!
 - o Needs to be between 0.05-0.60 (dynamic range of the spec), if more than 0.6 need to dilute
 - o $C1V1=C2V2$
 - o $V1 = 0.229 \text{ mL} = 229 \text{ uL}$
 - Amount of resuspended cells to add
 - o $C1 = 0.262$
 - the OD measured x 50 (amount diluted)
 - o $C2 = 0.03$
 - o $V2 = 100 \text{ mL}$
- Take prepared 100 mL freshwater in 250mL baffled flask and add V1 of resuspended cells to it.
- Put the cells into three technical replicates. 10 mL in 3 different flasks for the three different conditions. 9 flasks total.
- Put flasks in respective locations.
 - o 4°C – cold room
 - o 16°C – cold room in water bath

- 25°C – warm room

Flask 1	Flask 2	Flask 3	Flask 4	Flask 5	Flask 6	Flask 7	Flask 8	Flask 9
4°C	4°C	4°C	16°C	16°C	16°C	25°C	25°C	25°C

Plating Protocol

- Prepare 96 well plate, cuvette, parafilm, freshwater samples, square CHAH plate, and 1xPBS
 - Test plate to see if it is dry enough by pipetting 10 uL of freshwater and watching it drip.
- Pipette 180 uL of 1 x PBS into wells row B1/2-G1/2.
 - Use multichannel and a reservoir.
 - Pipette 200 uL from 100mL (which is now 10ml) of resuspended cells and freshwater into A1 and A2
 - Use a 200 uL pipette.
 - Using the multichannel pipette, pipette 20 uL from A into B. Mix 150 uL of the volume three times.
 - Repeat step 3 for B to C, C to D.
 - hint = say it out loud so you don't lose your place.
 - Change the tip each time!
 - Use the less volume multichannel pipette set to 10 uL and change the orientation of the plate to vertical before starting the dripping process.
 - Pipette 10 ul from A1-G1 onto the plate, letting each drip down the plate.
 - Remember to stay within the 6 inches of the flame, don't put the handle of the multichannel pipette to the flame, have the plates at roughly a 45-degree angle, and place to dry in between two pipette boxes at the same angle.
 - Two plates for each column. So should be 4 total plates
 - Place in incubator for 2 days and then pull out and count

Plating went well for the most part. Plate 1A and 1B converged, so I did an extra plate for that. Plates for 2 went well.

Plating is done to get the starting point for all of the samples.

Wednesday, April 27, 2022

- ~~1. Plate Day 1~~
- ~~2. Make more plates for Day 3~~

Plating Protocol

- Prepare 96 well plate, freshwater samples, square CHAH plate, and 1xPBS
 - Test plate to see if it is dry enough by pipetting 10 uL of freshwater and watching it drip.
- Pipette 180 uL of 1 x PBS into wells row B1/2-G1/2.
 - Use multichannel and a reservoir.
 - Pipette 200 uL from one flask into row A1-9
 - Use a 200 uL pipette.

- b. Swirled before pipetting for about ~10 seconds each.
3. Using the multichannel pipette, pipette 20 uL from A into B. Mix 150 uL of the volume three times.
4. Repeat step 3 for B to C, C to D. hint = say it out loud so you don't lose your place.
 - a. Change the tip each time!
5. Use the less volume multichannel pipette set to 10 uL and change the orientation of the plate to vertical before starting the dripping process.
6. Pipette 10 ul from A1-G1 onto the plate, letting each drip down the plate.
 - a. Remember to stay within the 6 inches of the flame, don't put the handle of the multichannel pipette to the flame, have the plates at roughly a 45-degree angle, and place to dry in between two pipette boxes at the same angle.
 - c. Two plates for each column. So 18 plates total.

Plating went ok. I forgot to skip a column between the 4C and the 16C but I did remember to skip between 16C and 25C. Some of my plates were too dry, so they converged. Hopefully, I still count them on Friday!

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak

Poster Script

For my project I worked with *Francisella tularensis*, which is a pathogenic bacterium. It is the causative agent of tularemia. (Describe the two pictures at the top) There is a relationship between aquatic reservoirs and this bacterium, and my project aimed to determine the laboratory conditions that lead to the longest survival to investigate the genetics in the future. For this project we were interested changing the temperatures. Using filter sterilized water from the Beaver River, three samples with LVS *F. tularensis* were incubated. I kept the samples at 4C, 16C, and 25C. On days 0,1,2,4,7 and weekly afterwards I serially diluted from 1 to 10^{-6} , and incubated at 37C or room temperature for 4-7 days. The variation in incubation was due to the colonies needing to be a countable size. After counting the cells, I added to a spreadsheet where I kept track and analyzed the data.

The cells remained viable from 35 days at 4C. Another significant piece of information is at Day 7, there was significant death of cells at 16C and 25C. Additionally, there were variation in colony sizes, a medium, glistening, round edge colony, and a pinpoint colony. I decided to keep track of the total colonies and just the "big" colonies, the line and dotted line, respectively. There were no significant differences found between temperature condition and colony size. The difference in size could be due to some cells responding the stressors of these conditions. These results connect back to literature as a large amount of tularemia cases can be found in central and norther Norway. There river water is warmest between at 7.7C – 20.3C in summer months, and cases can be found throughout the year. I am currently replicating this experiment to validate results, and hopefully over the summer, I will create a mutant library to find the gene or genes that are responsible for this response or use flow cytometry to enumerate cells.

Thursday, April 28, 2022

- ~~1. Check Day 0~~
- ~~2. Make MHB~~
- ~~3. Make LB agar~~
- ~~4. Label plates~~

I checked the day 0 plates. They were not super pretty (plates 1A and 1B) but there is growth on all, but not large enough to count. I took them out of the incubator and will leave them at room temperature today to count tomorrow.

Mueller-Hinton broth protocol- by Jamie Wandzilak**Preparing LB-agar**

- Made two flasks

Friday, April 29, 2022

1. Check Day 0 and Day 1 plates
 1. Count if possible
- ~~2. Plate Day 3 (instead of day 4)~~
- ~~3. Make plates for day 7~~
 - ~~1. Ask dan to put into a box with wet paper towels in the afternoon~~

Looked at the Day 0, and still too small to count. So, I put it back in the incubator for the morning.

Plated day 3, 5A and 5B converged so I redid it on 5C. Other than that the plates were borderline perfect, not to brag.

Cystine heart agar with hemoglobin plates protocol- by Jamie Wandzilak**May 2022****Monday, May 2, 2022**

- ~~1. Prepare plates for day 7~~
- ~~2. Check day 0,1 and 3 and count~~

DAY 0 DATA:

Dilution Factor Counted	Avg. Cells	CFU per mL	
			Stdev
0.0001	57.75	57750000	6010408
0.001	#DIV/0!	#DIV/0!	#DIV/0!

DAY 1 DATA

Track Plates 1,2,3	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
	0.001	0	0	0
	0.0001	57.5	57500000	1802776
Track Plates 4,5,6	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
	0.01	#DIV/0!	#DIV/0!	#DIV/0!
	0.0001	46.66667	46666667	5484828
Track Plates 7,8,9	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
	0.01	0	0	0
	0.0001	22	22000000	3122499

Day 3 was not countable. Took out of the incubator and then leaving at room temperature to count tomorrow.

Tuesday, May 3, 2022

1. Plate day 7
2. ~~LB Media~~
3. ~~LB Agar~~

Sort of a mess today. The four-degree room broke yesterday, so Hannah kindly moved my samples to the fridge in the lab. She also moved the water bath to four-degree room on the third floor. When I got to lab today the 25C room was 28C and the 3rd floor 4C room was 14C. The waterbath was still 16C though so I didn't move it. I did move the 25C samples to our lab under the big waterbath. I plated as normal and some of the plates were wicked wet, while others were perfect.

I also counted Day 3 plates. Results:

Track Plates 1,2,3	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
	0.001	0	0	0
	0.0001	53.33333	53333333	5299371
	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev

Track Plates 4,5,6	0.001	103.5	10350000	16723075
	0.0001	27.33333	27333333	16072751
Track Plates 7,8,9	Dilution Factor Counted	Avg. Cells	CFU per mL	
	0.01	90.5	905000	160000
	0.0001	#DIV/0!	#DIV/0!	#DIV/0!

Made LB Media and Agar.

Preparing LB-agar

Preparing LB media

- For 250 mL of LB, weigh out the following components and add to a 500 mL bottle:
 - 2.5 g NaCl
 - 2.5 g Tryptone
 - 1.25 g Yeast extract
- Add 250 mL type I ddiH₂O
- Close tightly and shake to mix
- Loosen cap and add a small piece of autoclave tape with the date
- Autoclave on 30 minute liquid cycle
- Sterile media can be stored indefinitely

Wednesday, May 4, 2022

- Presentation day!!

Thursday, May 5, 2022

- Nothing

Friday, May 6, 2022

- ~~Make plates for day 14~~
- ~~Count day 7 plates~~

Making circular plates for samples 4-9 and square plates for 1-6.

I pulled out the day 7 plates and they are super small still and I will come back on Sunday or Monday to check on them.

Got covid, went home early. Hannah plated for my day 7 and day 14 on 5/10 and 5/17 respectively. Thank you Hannah!!

Day 7 Results:

Track Plates 1,2,3	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
	0.001	0	0	0
	0.0001	27.75	27750000	353553.4
Track Plates 4,5,6	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
	0.01	108.5	1085000	445477.3
	0.0001	#DIV/0!	#DIV/0!	#DIV/0!
Track Plates 7,8,9	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
	0.01	0	0	0
	0.0001	0	0	0

Day 14 Results:

*track plates 4,5,6 were not track plates, but the circular plates with 300 ul of sample. Same with track plates 7,8,9, they were circular plates.

Track Plates 1,2,3	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
	0.001	55.83333	5583333	1451149
	0.0001	0	0	0
Track Plates 4,5,6	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
		27.66667	92.22222	142.5008
Track Plates 7,8,9	Dilution Factor Counted	Avg. Cells	CFU per mL	Stdev
	0.01	0	0	0
	0.0001	0	0	0

Bibliography

