Table of Contents

January 2023

Tuesday, January 24th, 2023

To Do:

1. ~~Make LB agar x2~~
2. ~~Make MHB x2~~

Results and Data:

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 stir bar to flask
3. Add 500 mL ddiH20
4. Mix on stir plate until components are dissolved
5. Cover top of flask with foil and add a small piece of autoclave tape
6. Autoclave on liquid30 cycle

Preparing MHB

1. For every 500mL of MHB, weigh 10.5g of MHB dehydrated powder into a 1L orange capped bottle
2. Add 500mL of ddiH2O and shake
3. Autoclave on liquid 30 cycle

Prepared two bottles each of LB-agar and MHB.

Wednesday, January 25th, 2023

To Do:

1. ~~Laurenobiolide calculation~~

Results and Data:

Received 2.6mg of laurenobiolide from Dr. Bertin's lab. 8mg/ml CH_3OH concentration needed, 325ul of methanol calculated to be added to reach desired concentration.

Friday, January 27th, 2023

To Do:

1. ~~Meeting with Kathryn~~
2. ~~Sign independent study contract~~

Results and Data:

Completed the independent study contract with Kathryn to obtain permission number for BPS498. Set meeting schedule of biweekly meetings with Kathryn (every other Monday at 10:30-11 (for now)).

Retrieved data from last semester and recreated excel sheet with measurements from the 11.18.22 experiment. 3 of the measurements had no zone of inhibition; these three discs were impregnated with the laurenobiolide of the remaining stock that I had, as there was not enough in the new aliquot that I had received, results show it must have been lower concentration or purity compared to the new one.

Monday, January 30th, 2023

To Do:

1. ~~Meeting with Kathryn~~
2. ~~Streak out cells~~
3. ~~Pour LB plates~~
4. ~~UV sterilize EP cuvettes~~

Results and Data:

Met with Kathryn to touch base on some things; revisited information about my last experiment (11.18) and how moving forward how important it is to take from the same aliquot of laurenobiolide.

Prepped for DDA tomorrow. Struck out SA113, 4A3M1-M4 onto LB agar. Poured non abx LB plates onto round plates. Some of the plates are wobbly so I laid out most of the plates on the bench. Will need to dry the plates by the flame before I plate any bacteria onto them.

Restocked 50ml serological pipets above the lab bench.

Sterilized EP cuvettes in the UV sterilizer.

Tuesday, January 31st, 2023

To Do:

1. ~~DDA~~
2. ~~Sterilize cuvettes~~

Results and Data:

Made sure to set up everything I needed before I took the bacteria out of the incubator (labeled tubes, cuvettes, plates. Added beads to plates, added LB in proper amounts according to labeled tube or cuvette)

400ul in the initial resuspension, 900ul in the 1:10 dilution tubes, 950ul in the cuvettes, and 1000ul in the final volume tubes.

I also made sure to pipet methanol and laurenobiolide onto the discs and let them dry in advance. I noticed that there wasn't a full amount of 20ul on one of the laurenobiolide discs, so I made sure to put it onto SA113-1 just so it wouldn't interfere with any of the potential mutants' measurements.

Measurements of ODs;

SA .262 – 95.4ul

LR4A3M1 OD .192 diluted 1:10, requires 130.2ul cells into 1ml LB

LR4A3M2 OD .313 diluted 1:10, requires 79.9ul cells into 1ml LB

LR4A3M3 OD .135 diluted 1:10, requires 185.2ul cells into 1ml LB

LR4A3M4 OD .291 diluted 1:10, requires 85.9ul cells into 1ml LB

Upon determining these values, I would first take that amount of the final volume tube of LB and then put the volume of resuspended cells from the 1:10 dilution into the final tube.

I pipetted 100ul of each strain on the corresponding labeled plates and shook them with the beads. Some plates took longer to dry than others so I would take turns shaking some of them and letting them rest before shaking them again. It started to take more than 10 minutes to get the plates to dry so I ended up leaving about 4 plates, which were SA113, 4A3M2, and 4A3M4 with their lids slightly open around the flame to dry them out a little bit before closing them and shaking the plates and spreading the beads around.

When placing some of the discs, some of them fell while I was placing them and may have had some of the compound get onto part of the plate where I did not end up putting the disc, as it may have fallen close to the edge or near the control disc which would make my results harder to discern. I don't think this will be the case since it wasn't pressed into the plate yet and there was no prolonged exposure, I should be able to see what the results are tomorrow.

I also had sterilized the EP cuvettes in the UV sterilizer, capped them and placed them into the box in the cabinet under Hannah's bench.

Wednesday, February 1st, 2023

To Do:

1. Analysis and Measurements
2. Moving forward?

Results and Data:

Insert measurements of diameters and excel sheet, representative images of the mutants and WT.

Moving forward: need to isolate gDNA!

Wrapped the DDA plates into parafilm and stored them in the 4C refrigerator in the meantime.

Tomorrow's plan is to go ahead and pellet at least half a plate of cells per tube: SA, M1 ,M3, M4. HT suggested that overnight cultures will be needed at 5mL to grow more bacteria, and that the lysostaphin will be the limiting reagent. May not yield enough gDNA, potentially may need more cells?

Reference January 26, 2022, in Hannah's notebook for information about the lysostaphin protocol. There is also a modified protocol for isolating S. aureus gDNA which is in the protocols folder.

Steps from KMR:

Label microfuge tubes well

Add ~300 ul 1xPBS to each tube

Scrape up a bunch of cells (ask HT how much), get them off the loop and into the PBS

Spin down cells

Remove excess PBS

Store at -20C until ready to isolate gDNA

Thursday, February 2nd, 2023

To Do:

1. Pellet bacteria from SA113, M1,3,4
2. Lysostaphin Master Mix

Results and Data:

Made PBS aliquot, took 1ml from 10x PBS using aseptic technique with a serological pipet and then added 9ml of DI water to the aliquot – 10ml of 1x PBS.

Took parafilm wrapped plates out of the 4C fridge. I took 4 microfuge tubes and labeled them SA pl (pellet), M1 pl, M3 pl, and M4 pl. Scraped up about half a plate per tube, inoculated into 300ul 1x PBS. Bacteria was taken from DDA plates, avoiding contact near the laurenobiolide discs. SA113-1, LR4A3M1-1, LR4A3M3-3, LR4A3M4-1. Centrifuged at max speed for 3 minutes.

gDNA Materials Needed:

most stuff in DNA RNA box

Ice

150 ul Lysostaphin - -20C antibiotics box

1x Tissue and Cell Lysis Solution – box above water bath

Lysozyme crystals - in full plastic Tupperware dark round container

Proteinase K – furthest left enzyme box in front row (red)

RNAse A– furthest left enzyme box in front row (red)

MPC Protein Precipitation Reagent – box above water bath

Isopropanol

70% ethanol

.1X EB

Lysostaphin Preparation (10 mg/mL)

1. Make up ~30 ml of 20 mM sodium acetate in MQ water.

2. Adjust pH to 4.5 with acetic acid or NaOH as needed, adding dropwise.

3. Filter sterilize solution.

4. Dissolve whole vial of lysostaphin (5 mg) in 0.5 ml buffer.

5. Aliquot solution out into 65 ul aliquots in sterile Eppendorf tubes.

6. Label and store at -20 (in antibiotics box). Avoid repeated freeze-thawing of aliquots.

DNA Purification Protocol – *S. aureus*

Cell Samples

1. Dilute lysostaphin in Tissue and Cell Lysis Solution solution to 1 mg/mL (i.e. 30 ul of 10 mg/ml stick in 270 ul TCL)

2. Add lysozyme crystals to 1 mg/mL and dissolve by pipetting up and down. Used 1.8aimed for 1.2

3. Pellet cells by centrifugation (0.5-1 x 106 mammalian cells; 0.1-0.5 ml of an overnight culture of E. coli) and discard the supernatant, leaving approximately 25 µl of liquid.

4. Vortex for 10 seconds to resuspend the cell pellet.

5. Add 300 ul of Tissue and Cell Lysis Solution containing the lysozyme and lysostaphin and mix thoroughly.

6. Incubate at 37°C for 30 minutes

7. Add 1 ul of Proteinase K to each sample and mix thoroughly.

8. Incubate at 65°C for 15 minutes; vortex every 5 minutes.

9. Cool the samples to 37°C and add 1 µl of 20 mg/ml RNase A to the sample; mix thoroughly.

10. Incubate at 37°C for 30 minutes. – start centrifuge cool down

11. Place the samples on ice for 3-5 minutes and then proceed with total DNA precipitation (below).

Precipitation of Total DNA (for all biological samples)

1. Add 150 µl of MPC Protein Precipitation Reagent to 300 µl of lysed sample and vortex vigorously for 10 seconds.

2. Pellet the debris by centrifugation at 4°C for 10 minutes at ≥10,000 x g in a microcentrifuge. If the resultant pellet is clear, small, or loose, add an additional 25 µl of MPC Protein Precipitation Reagent, mix, and pellet the debris again.

3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.

4. Add 500 µl of isopropanol to the recovered supernatant. Invert the tube 30-40 times.

5. Pellet the DNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.

6. Carefully pour off the isopropanol without dislodging the DNA pellet.

7. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet and let dry completely under hood.

8. Resuspend the DNA in 35 µl of 0.1x EB Buffer. Put on ice to help dissolve, and add 50 µl of additional buffer if DNA is very goopy.

9. Check concentration and purity by nanodrop.

Friday, February 3rd, 2023

To Do:

1. Work on proposal and updating Notebook

Results and Data:

Monday, February 6th, 2023

To Do:

1. Work on proposal and updating Notebook

Results and Data:

I initially was planning to do the Lysostaphin extraction experiment today, but I did not have enough time since Hannah suggested that I budget at least 2 to 2 ½ hours to completing this experiment.

Tuesday, February 7th, 2023

To Do:

1. Lysostaphin gDNA extraction

Results and Data:

In only 30ul of .1xEB

Wednesday, February 8th, 2023

To Do:

1. gDNA nanodrop and dilution for samples
2. create shipping order to send out samples to SeqCenter through Fedex

Results and Data:

Friday, February 10th, 2023

To Do

1. Make MHB
2. Make LB agar

Results and Data:

Since I don't have any bench work to do since neither I nor Dr. Bertin have laurenobiolide, I decided to do lab tasks and some house keeping things while I wait for the sequencing results and for more laurenobiolide to be accessible.

I made Mueller Hinton Broth in two 1L autoclave bottles with orange tops which contained 500ml DI water and 10.5g of the MHB dried powder solution and mixed by shaking

Monday - Wednesday, February 13th-15th, 2023

To Do:

1. Work on completing the ASP grant proposal

Results and Data:

Since Kathryn was still traveling on Monday during our normal meeting time, we met on Tuesday morning at 10am to discuss the proposal and any changes that I needed to make before we submitted the final draft. I wasn't in the lab this week as I was focused on completing the proposal by Wednesday

Friday, February 17th, 2023

Wednesday, February 22nd, 2023

To Do:

1. Organize the enzyme boxes

Results and Data:

Organizing the enzyme boxes, filter out the refills to go into the left most box. Potentially organize enzymes alphabetically.

I organized the boxes and filed everything in an excel spreadsheet in the Inventories folder. There is a refills box on the far left, then box #2 is containing rna/restriction, protein, and gDNA. Box #3 contains only restriction enzymes. The box on the far right (box #4) contains Inseq and Cloning/PCR.

Wednesday, March 1st, 2023

To Do:

1. Creating spreadsheet to find mutations
2. Mutation sorting

Results and Data:

Thursday, March 2nd, 2023

To Do:

3. Looking at mutations at same base pair being different from SA113

Results and Data:

Friday, March 3rd, 2023

To Do:

4. Meeting with Kathryn
5. Joint Lab Meeting

Results and Data:

Monday, March 6th, 2023

To Do:

6. Create rplU protein in SnapGene

Results and Data:

Tuesday, March 7th, 2023

To Do:

1. Streak out cells
2. Pour fresh LB agar plates

Results and Data:

Wednesday, March 8th, 2023

To Do:

1. Literature search on SAM methyltransferase and rplU
2. ~~***E. coli* pEPSA5 strain from MRamsey lab~~
3. Reconstitute laurenobiolide
4. Update lab notebook!

Results and Data:

Notes from Kathryn's email:

Friday, March 10th, 2023

To Do:

1. Literature search on SAM methyltransferase and rplU
2. Lab meeting

Results and Data:

Lab meeting today; Aisling and Julia present.

Monday, March 13th – Friday, March 17th, 2023 -SPRING BREAK

Monday, March 20th, 2023

To Do:

1. ~~Make LB agar~~
2. ~~Make MHB~~
3. Update Lab Notebook
4. Kathryn's notes?? – next steps for the plasmid
5. Respond to Dr. Gregory/Kathryn emails

Results and Data:

Made 2 autoclave bottles of MHB and 2 flasks of 500ml LB agar. Placed in the autoclave 11:35 – need to take out in between my classes.

I was able to take the MHB and LB agar out of the autoclave, but my class ran overtime and I only had a couple minutes to label the media, I had asked Sierra to help out and if she could just label the ones I didn't get to which were the LB agar flasks.

Wednesday, March 22nd, 2023

To Do:

1. ~~Meeting with Kathryn and Kira~~
2. ~~Meeting with Kira~~

Results and Data:

Notes from meetings with KMR and KB:

Now using Microsoft web-based planner to help keep track of overall themes and next steps for experiments in the project. There is the option to put notes and comments in each card if need be, Kathryn has already written some notes for me and has invited Kira to the planner as well. V

Prioritize finding resistant mutants over validation experiments.

Cloning plasmids

1. Introduce WT and mutant version into cell and see what happens.
2. Needs two plasmids for each gene assessed.

Purify pEPSA5 from E. coli by miniprepping and then it will need to be sent for sequencing at Plasmidsaurus.

Design plasmid -> clone -> validate

SAOUHSC_02245 gene will have pKR185, pKR186 as plasmids. Has a xylR inducible system (t5X promoter) where the gene won't be expressed unless grown on xylose. Information on this is in other_plasmids folder.

pKR185 – Wild Type

pKR186 – From mutant strain (LR4A3M1)

Primers to be used are KROL620, KROL621 for both plasmids.

Amplify the DNA from WT and mutant and design another plasmid with Kira.

When cloning, carbenicillin plates should be used.

In the excel sheet, need to indicate when made and when Sanger Sequencing has been completed.

If any questions for Kathryn to be brought up at the next meeting, add to agenda in the Microsoft planner.

Next week – clone, protocols in KMR lab files

Ligation protocol in folder, worksheet for master mix, and ligation multipiece calculator. Nanodrop after miniprep and record concentration

The inserts for the ligations are the two primers

Once fragment amplified, PCR to quick check.

Next week, longer PCR needed to get more product. Then PCR clean up/purification kit (Qiagen).

Can use Gel Purification or PCR purification, if gel – need to do same day, if PCR, can wait by storing in the freezer.

Thursday, March 23rd, 2023

To Do:

1. Pick Colonies
2. Reconstitute primers
3. gDNA from SA113 purification

Results and Data:

Reconstitute primers onto SA113 gDNA (miniprep TODAY) check in box to see if I have any, if not, KB had struck out SA113 for me yesterday in order for me to miniprep today if I had no gDNA.

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).
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.
   b. If a sequencing primer, make a 2.5 uM stock by diluting 5 uL of the 100 uM stock into 195 uL of 0.1x EB. Label with blue sticker and put in appropriate freezer box.
7. Put Certificate of Analysis sheet in "Oligos Spec Sheet" binder and shipping sheet in Invoices box.

DNA Purification Protocol – *S. aureus*

Cell Samples

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3. Pellet cells by centrifugation (0.5-1 x 106 mammalian cells; 0.1-0.5 ml of an overnight culture of E. coli) and discard the supernatant, leaving approximately 25 µl of liquid.

4. Vortex for 10 seconds to resuspend the cell pellet.

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6. Incubate at 37°C for 30 minutes

7. Add 1 ul of Proteinase K to each sample and mix thoroughly.

8. Incubate at 65°C for 15 minutes; vortex every 5 minutes.

9. Cool the samples to 37°C and add 1 µl of 20 mg/ml RNase A to the sample; mix thoroughly.

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11. Place the samples on ice for 3-5 minutes and then proceed with total DNA precipitation (below).

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3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.

4. Add 500 µl of isopropanol to the recovered supernatant. Invert the tube 30-40 times.

5. Pellet the DNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.

6. Carefully pour off the isopropanol without dislodging the DNA pellet.

7. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet and let dry completely under hood.

8. Resuspend the DNA in 35 µl of 0.1x EB Buffer. Put on ice to help dissolve, and add 50 µl of additional buffer if DNA is very goopy.

9. Check concentration and purity by nanodrop.

I ended up stopping midway through the gDNA purification protocol because I started feeling unwell, my plan was to redo it the next day instead.

I also had patched out 4 of the colonies that I had picked from the pEPSA5 plate on an LB-carb plate.

Friday, March 24th, 2023

To Do:

1. Miniprep (pEPSA5)
2. Restart gDNA

Results and Data:

Well, unfortunately my plan to miniprep could not get done today. I should not have patched out the colonies but instead have made overnight cultures because it slipped my mind that you would

Freeze down strain (S. aureus)

Use aseptic technique

Per strain, label 2 cryotubes with strain number. Include SA, strain number, genotype, date.

Add 200 uL sterile 75% glycerol to each tube (2 per strain).

In a sterile 2 mL tube (1 per strain), add 400 uL of LB

Resuspend patch (all of what you have) in LBto homogeneity

Correct volume to 1700 uL (add 2x 650 uL LB)

Transfer 800 uL to each cryotube (final volume should be 1 mL)

Vortex cryotube

Quickly spin (mini-fuge) to get liquid to the bottom of the tube

Freeze at -80°C in appropriate strain box

Since I made a mistake and patched out the cells instead of making overnight cultures, I decided to freeze down the colonies just in case, and to have one to streak out to single colony again. They are stored in my #2 box in the -80. They are labeled: ECpEPSA5 COL (COLONY) 1-4

Monday, March 27th, 2023

To Do:

1. Meeting with Kathryn
2. Streak out LVS, SA113, pEPSA5

Results and Data:

Tuesday, March 28th, 2023

To Do:

1. DDA for LVS and SA113
2. Reconstitute LB (with instructions from planner/slack)

Results and Data:

Wednesday, March 29th, 2023

To Do:

1. PCR
2. Image SA113 DDA plates

Results and Data:

Amplified region 868

55 seconds

Aisling showed me how to use the snapgene file to figure out how many base pairs I need to look for to amplify

Thursday, March 30th, 2023

To Do:

1. Image LVS plates

Results and Data:

Friday, March 31st, 2023

To Do:

1. Hannah's Defense

Results and Data:

No lab work today, was not able to attend the Ramsey-Gregory joint lab meeting as I needed to do some last minute studying for my exam; I wanted to make sure that I could be there for Hannah's defense as it took place within the hour right before my exam.

Tuesday, April 4th, 2023

To Do:

1. Overnight cultures for pEPSA5

Results and Data:

I was able to come into the lab later on Tuesday rather than in the morning in order to start the overnight cultures in order to do the miniprep on Wednesday. I knew I wouldn't be in the lab on that Thursday as I had a pharmacy meeting so this was the best time for me to do it this week.

Overnight Cultures Protocol for pEPSA5

1. For a new plasmid, use 3 culture tubes (one tube per colony) with 5mL of LB in each (E. coli loves LB for standard reference, NEVER use MHB)
2. 5ul for 5mL of carbenicillin into each culture tube.
3. Inoculate one colony into each culture tube by using a sterile stick
4. Place in the shaking incubator and allow for 16-22 hrs incubation overnight
5. ODs do not need to be checked unless you are doing a growth experiment

the 100ug/ml carbenicillin aliquots are located in the -20C antibiotics box.

Wednesday, April 5th, 2023

To Do:

1. Miniprep pEPSA5
2. Check LVS/SA plates for colonies

Results and Data:

I looked at the LVS and SA113 plates to check if they had any colonies. I did see that there were background colonies popping up within the SA113 plates but nothing close to the laurenobiolide disc itself. I did not see any colonies on the LVS plates, and although their zones of inhibition were larger than when I did previous disc diffusions with 8mg/ml (I had made sure to do 16mg/ml for the concentration for 2 LVS plates and 8mg/ml for 3 SA113 plates) we still haven't seen any changes– hopefully in the future days or weeks we can start to see ones pop up really close to the discs containing laurenobiolide.

Miniprepping does not need to be done aseptically. When taking the culture tubes out, no need for pipetting– just carefully pour the culture from the tube into a 2ml microcentrifuge tube.

Spin Miniprep

1. Pellet 5ml from bacterial overnight culture by centrifugation at >/=10000 rpm for 3 minutes at room temperature in a 2ml tube and discard supernatant
2. Resuspend pelleted bacterial cells in 250ul of Buffer P1 from fridge and transfer to a 1.5ml microcentrifuge tube
3. Add 250ul Buffer P2 and mix thoroughly by inverting the tube 4-6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 minutes
4. Add 350ul of Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times.
5. Centrifuge for 10 min at 13,000 rpm in a table top microcentrifuge
6. Apply 800ul supernatant from step 5 to the 2.0 spin column by pipetting. For centrifuge processing, centrifuge 60 seconds and discard the flowthrough in a liquid waste container
7. Wash the spin column by adding 500ul Buffer PB. Centrifuge for 60 seconds and discard the flowthrough in a liquid waste container.
8. Wash the spin column by adding 750ull Buffer PE. Centrifuge for 60 seconds and discard the flow through. Do this wash **3 times.**
9. Centrifuge for 3 minutes to remove residual wash buffer
10. Place the column in a clean 1.5 microcentrifuge tube. To elute DNA, add 50 ul Buffer .1x EB to the center of the spin column, let stand for 1 min, and centrifuge for 1 minute.
11. If the extracted DNA is to be analyzed on a gel, add 1 volume of loading dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

I had three culture tubes (one for each colony that I had picked from the single colony plate that i had patched out awhile ago and had stored in the fridge for the next time I would have enough time to miniprep) that I then turned into 3 miniprep microfuge tubes. They are in my 2nd box in the -20C freezer. I will still need to check the concentrations of the minipreps with the nanodrop (send one out to plasmidsaurus?)

Thursday, April 5th, 2023 - not in lab due to meeting with my faculty advisor

Friday, April 7th, 2023

To Do: Reconstitute laurenobiolide?

Results and Data:

I didn't have much time to be in the lab today and wasn't able to attend the lab meeting. I wanted to ask some questions and waited around in the lab after the lab meeting but I didn't see anyone come back up. I decided to organize my folders instead.

I received ~2.3 mg of Laurenobiolide from the Bertin lab. I need to reconstitute it when I am back in the lab on Tuesday. Need to check in with Kathryn before I reconstitute it to make sure that I have the right concentration for the right experiment: I'm not sure what I'm doing next, I know that I need to prioritize making resistant mutants especially for LVS as we are the most interested in that one.

Monday, April 10th, 2023

To Do:

1. **Make a list/plan for the week ahead**
2. **Get notes in order for discussion with Kathryn on Microsoft Planner**

Results and Data:

Not in the lab today as I will be traveling back to Rhode Island, however Kathryn asked me to make a list of questions in the Microsoft Planner to discuss during our next meeting (this Thursday 4/13 at 3pm).

Added "laurenobiolide update" on the meeting agenda for 4/13. To discuss: I received another vial of dried down laurenobiolide from the Bertin lab: I think it was 2.3mg but I need to check when I go back into lab and go back and verify to make sure that number is correct before the discussion.

- What concentration will we be aiming for when I reconstitute it?
- What is the next experiment that I will be using the LB for?
    - More LVS DDA?

I have been holding off on running my PCR products on a gel as I know I need to find a time where I can fully run the gel. Would I need to gel purify the PCR products of the SA113 or is the gel purification for when I run the plasmid on a gel? Need some clarification because I have been confused with what step goes to what since there are multiple experiments going on with the SA113 wildtype and LR4A3M1 mutant compared to this plasmid pEPSA5 backbone that I am trying to get out of E. coli and insert it into SA113- it has been a bit confusing so I will be asking to clear some of this up at the next meeting.

- I recall asking Kathryn whether I would need to PCR purify or gel purify; either could be possible.
- Could either save PCR product and only put some of it on the gel and verify that the primers are working
- Or put all product on gel then gel purify

Need to obtain concentrations for my pEPSA5 plasmid minipreps on the nanodropper. I recall something about plasmidsaurus? What are the next steps now that I have completed a miniprep? (Restriction digest? I don't remember which step comes next)

Also would not be a bad idea to make more LB liquid media, revisit the protocol for that. Check to see if there is any other media to be made (MHB, unsupplemented and LB agar?)

I also remember conversations about sequencing, when would I need to do that and for what product? I feel like I would need to make a table or list for all the upcoming steps so that I can be more organized in knowing what is going on.

Inform Kathryn about the abstract I will be writing for the ASP Travel Grant through the PCog sponsorship application.

Tuesday, April 11th, 2023

To Do:

1. **Work on ASP Travel Grant**
2. **Update lab notebook**

Results and Data:

Not i

Wednesday, April 12th, 2023

To Do:

1. ~~**Make MHB**~~
2. ~~**Make LB media**~~
3. **Continue working on ASP Travel Grant**
4. ~~**Prepare gel for tomorrow**~~

5. **Continue updating lab notebook**
6. **Poster session 4/27/23**
7. **Update agenda in Planner**

Results and Data:

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 ddiH20
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

I made MHB (10.5g in 500ml ddiH2O) and LB media and placed it in the autoclave.

Took Aisling's media out of the instantpot and put it in the waterbath at 56C.

Kathryn and I talked about getting the gel ready to run it for tomorrow, because I don't have to make the gel and run it on the same day. She suggested to heat up the gel, make it (add 10ul sybrsafe) and let it solidify (pro tip: place it level in the fridge so it can be opaque and I can take the comb out). Then, to put it in a plastic container with enough buffer to cover the gel (add 30ul sybrsafe to the buffer), place tin foil on top of the container and place it in the large 4C fridge. That way, tomorrow when I come in, I just have to take the gel and load it into the rig, load the ladder and positive (SA113) and negative controls (water), and then use FRESH (used) 1x TAE and save the buffer that I had stored the gel in for later.

Thursday, April 13th, 2023

To Do:

1. **Work on ASP Travel Grant**
2. **Run Gel**
3. **Meeting w Kathryn**

Results and Data:

Notes from meeting:

Miniprep: Check concentration on nanodropper, pool 3 minipreps into one tube. Send to plasmidsaurus (coordinate with Ben- he may be sending plasmids to plasmidsaurus soon)

Design plasmid to clone both genes (rplU, hypothetical) – hypothetical might already have been designed? - work with kira to create

pEPSA5 is an empty vector

restriction digest cuts the empty vector, PCR purification then digest PCR product from mutant and wild type then gel purify , then ligation, then transform into E. coli to grow up and propagate the plasmid – get single colonies, then miniprep those single colonies and send those for sequencing

then put into wild type and mutant s aureus by electroporation then do 2 ddas (one for each gene)

only want the stuff that's digested, separate by size (run on a huge gel rig) want to put the other pieces into that plasmid, want to make sure any thing that was already in there is gone away so theres no crossing(?) kick the enzyme outta there, separates protein from dna, making sure we have right size dna

design plasmid with kira asap! Get primers ordered asap

Monday, April 24th, 2023

To Do:

1. **Meeting w Kathryn**

Results and Data:

Notes from meeting:

Miniprep

Tuesday, April 25th, 2023

To Do:

1. **Resuspend Primers**
2. **Large Volume PCR for SAM**

Results and Data:

Large Volume PCR with all 3 mutants, WT, and H2O

Total reaction volume	100
Total number of reactions	5

				Factor
Component	**Stock concentration**	**Final concentration**	**1 rxn volume**	6
ddiH2O			54.0	324
PrimeSTAR GXL Buffer	5x	1x	20.0	120
dNTPs	2.5 mM	0.2 mM	8.0	48
oligo F	10 uM	0.3 uM	3.0	18
oligo R	10 uM	0.3 uM	3.0	18
template	100 ng/ul	2 ng/ul	10.0	
PrimeSTAR GXL DNA Polymerase	1.25 U/ul	0.025 U/ul	2.0	12
		Total volume	100	540

Receiving Primers Protocol

Wednesday, April 26th, 2023

To Do:

1. **Resuspend Primers**
2. **Large Volume PCR GEL and PURIFICATION for SAM**

Results and Data:

Large Volume

DIGEST TMRW

PCR for check if primers work tmrw

- Maybe do in morning? Run gel later then do another PCR and run overnight

Thursday, April 27th, 2023

To Do:

1. **Small volume pcr check**
2. **Gel for rplu**
3. **Digest**
4. **Large volume pcr for rplu**

Results and Data:

small Volume

Rplu – 308 bp

Friday, April 28th, 2023

To Do:

1. **Check on a gel**

Results and Data:

small Volume

Monday, May 1st, 2023

To Do:

1. **PCR Purification**
2. **Digest**

Take pictures of our boxes

Results and Data:

475ul pb

35ul eb

Do travel authorization

Make more LB agar and LB media

Check to see if we have enough carb plates – need 14 carb plates, melt gel (for digest) and agar for plates

Monday/Today: pcr purification and digest, gel and gel purify, 10 minute ligation and the transformation, NEED LB plates

Tuesday: pick colonies, grow overnight

Wednesday: miniprep colonies, set up sequencing reactions, send to sequence

Thursday: sequence

Friday: seq results, prep plates for abx plates SA (chloramphenicol?)

Monday: prep Electrocompetent Cells SA, electroporate SA

Tuesday: colonies, patch out, pour plates

Wednesday: DDA

Large gel rig – 12ul of sybrsafe

Gel Extraction Kit

Tuesday, May 2nd, 2023

To Do:

1. **Transformation**

Results and Data:

475ul pb

Wednesday, May 3rd, 2023

To Do:

1. **Redo ligation**
2. **Redo Transformation**
3. **take pictures of boxes**
4. **pour carb plates**
5. **make more lb agar**

Results and Data:

#	Sample ID	User name	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	260/280	260/230	Sample T
1	SA113_rplU-wt	Science	18.2	ng/µl	0.365	0.146	2.5	0.03	DNA
2	SA113_rplU-mut	Science	18.5	ng/µl	0.37	0.221	1.67	0.04	DNA
3	SA113_SAM-wt	Science	22.2	ng/µl	0.445	0.252	1.77	0.05	DNA
4	SA113_SAM-mut	Science	16.7	ng/µl	0.333	0.178	1.87	0.03	DNA
5	pEPSA5	Science	6.9	ng/µl	0.138	0.057	2.4	0.01	DNA

Backbone: 1152 bp

rplU: 309 bp

SAM: 845/846 bp

WT rplU:

WT rplU	3X	5X	BB only
Water	8.04	6.57	10.25
Ligation Buffer	2	2	2
Backbone	7.25	7.25	7.25
Insert	2.21	3.68	-
Ligase	0.5	0.5	0.5

MUT rplU

MUT rplU	3X	5X
Water	8.08	6.63
Ligation Buffer	2	2
Backbone	7.25	7.25
Insert	2.17	3.62
Ligase	0.5	0.5

WT SAM	3X	5X
Water	5.27	1.94
Ligation Buffer	2	2
Backbone	7.25	7.25
Insert	4.99	8.31
Ligase	0.5	0.5

MUT SAM	3X	5X	
Water	3.63	-0.78	don't add water
Ligation Buffer	2	2	
Backbone	7.25	7.25	
Insert	6.62	11.03	
Ligase	0.5	0.5	

MASTER MIX: 5ul LIGASE

20ul LIGATION BUFFER

72.5ul BACKBONE

Ended up doing the one molar volume ligation with normal calculations as opposed to the 3x 5x, did not have enough left over from the gel extraction. Ligation was left overnight

Thursday, May 4th, 2023

To Do:

1. **Redo Transformation**
2. **take pictures of boxes**
3. **pour carb plates**
4. **make more lb agar**
5. **need to insert protocols**

Results and Data:

Today I poured carb plates (100ug/ml) and redid the transformation. My plates were really wet however and it took a long time for them to dry. While my cells were recovering I made more LB agar (x2) and LB media (x2).

Friday was not in lab

Saturday, May 6th, 2023

To Do:

1. **Redo Transformation**
1. **Miniprep transformed plates**
1. **need to insert protocols!!**

Results and Data:

#	Sample ID	User name	Date and Time	Nucleic Acid	Unit
1	2-1	Science	5/6/2023 2:42:37 PM	38.1	ng/µl
2	2-2	Science	5/6/2023 2:44:09 PM	113.3	ng/µl
3	2-4	Science	5/6/2023 2:45:50 PM	59.8	ng/µl
4	2-3	Science	5/6/2023 2:46:25 PM	77.1	ng/µl
5	2-1-retry	Science	5/6/2023 2:47:03 PM	44.1	ng/µl
6	4-1	Science	5/6/2023 2:47:55 PM	89.9	ng/µl
7	4-3	Science	5/6/2023 2:49:04 PM	82.7	ng/µl
8	4-3-retry	Science	5/6/2023 2:49:34 PM	82.8	ng/µl
9	4-2	Science	5/6/2023 2:50:41 PM	97.7	ng/µl
10	pEPSA5	Science	5/6/2023 2:53:33 PM	78.3	ng/µl

I was having issues with the nanodrop and getting the concentrations- I had to redo it a couple times.

Monday May 8th 2023

Ligation 2 Colony 1 was not included in the sequencing because of the concentration being too low. Everything is now off by one: L2C2-4 OH1-3, L4C1-3 OH4-6, pEPSA5 OH7

Tuesday – overnight cultures

Wednesday – minipreps, nanodrop, check sequencing, lb agar

Sample ID	User name	Date and Time	Nucleic Acid
MT4	Science	5/10/2023 5:39:32 PM	2.7
MT3	Science	5/10/2023 5:40:36 PM	3.2
MT2	Science	5/10/2023 5:41:05 PM	6
MT1	Science	5/10/2023 5:41:32 PM	5.4
RPLU1	Science	5/10/2023 5:42:08 PM	10.5

Miniprep names in box?

Thursday – make agar