Week 3 (Sol 9) 7/9/19
To do today:

1. Make Formaldehyde LB Agar plates
2. Plate test bacteria

Procedure:

Item 1: Make Formaldehyde LB Agar plates
Note do all mixing in the chemical hood

1. (Made 800 mL) Mix 20 g LB broth, 12 g of Agar, and 800 mL of dH20 in a 1 mL bottle
2. Send to autoclave
3. Make 6 100mL bottles with the following concentrations of HCHO
    1. 0.1%
    2. 0.03%
    3. 0.01%
    4. 0.003%
    5. 0.001%
    6. 0%
        1. *We do this so we can get a range of HCHO concentration where E.coli can grow
4. Do this by adding 100 mL of LB from autoclave + X uL of HCHO as follows.
    1. Bottle 1: 100 mL LB + 625 uL 16% (Stock) HCHO
    2. Bottle 2: 100 mL LB + 187.5 uL 16% (Stock) HCHO
    3. Bottle 3: 100 mL LB+ 62.5 uL 16% (Stock) HCHO
    4. Bottle 4: 100 mL LB + 18.75 uL 16% (Stock) HCHO
    5. Bottle 5: 100 mL LB + 6.25 uL 16% (Stock) HCHO
    6. Bottle 6: 100 mL LB
5. Pour each bottle into appropriately labeled Petri dishes (we were able to make 5 of each concentration plus little extra of the plain LB) and leave the plates — closed — in the hood fo ~30 mins.
    1. Note on labeling- write date, initials, and “LB X% HCHO"
6. Set up the Bunsen burner, perform the rest of the experiment here
7. Prepare the BL21 competent cells
    1. Dilute the bacteria as follows
        1. Tube 1: 10^5
        2. Tube 2: 10^7
        3. Tube 3: 10^9
            1. (So essentially there will be 18 plates)
    2. How?

1. Take 10 uL from the original BL21 sample and put it in 990 uL of LB (100x dilution)
2. Resuspend
3. Take 10 uL from this diluted sample and transfer to new tube with 990 of uL LB (100x)
4. Resuspend
5. Take 100 uL from this diluted sample and transfer to a new tube with 900 uL of LB (10x) Now this is a 10^5 dilution
6. Take 10 uL from this 10^5 sample into a new tube with 990 uL of LB
7. Resuspend. This is the 10^7 sample
8. Take 10 uL from the 10^7 sample and into a new tube with 990 uL of LB.
9. Resuspend. This is the 10^9 sample.

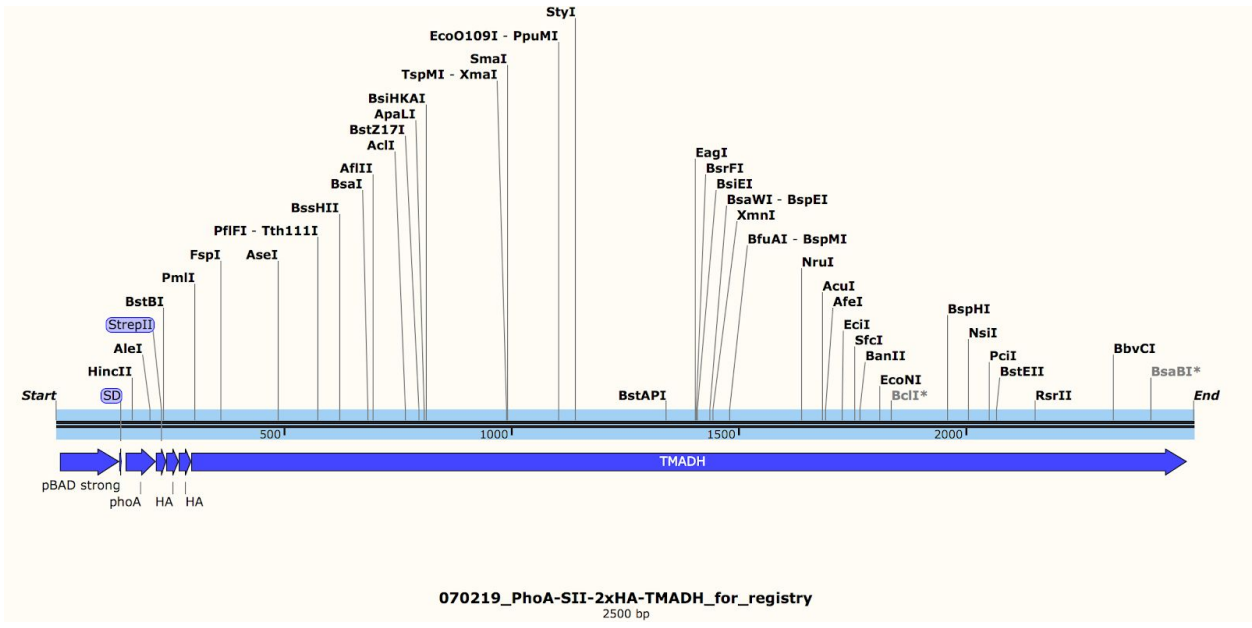
8. Get the plates and the diluted bacterial samples
    1. Label each plate with concentration of bacteria that is there too
9. Plate the bacterial samples (can use the same spreader for 10^5 samples, use the same spreader for the 10^7 samples, etc.)
    1. Plate 50 uL each plate
10. Flip over and incubate in 37C overnight

Data/Results:

No results from this experiment yet, but…

Construct organization and sequences below

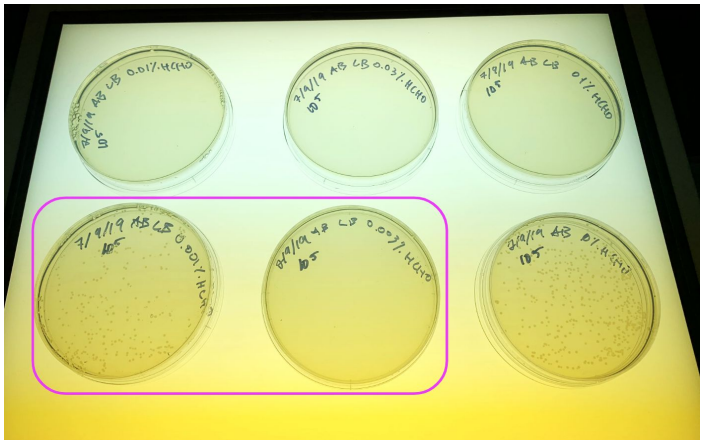
Recombinant 1: (minus pBAD vector)

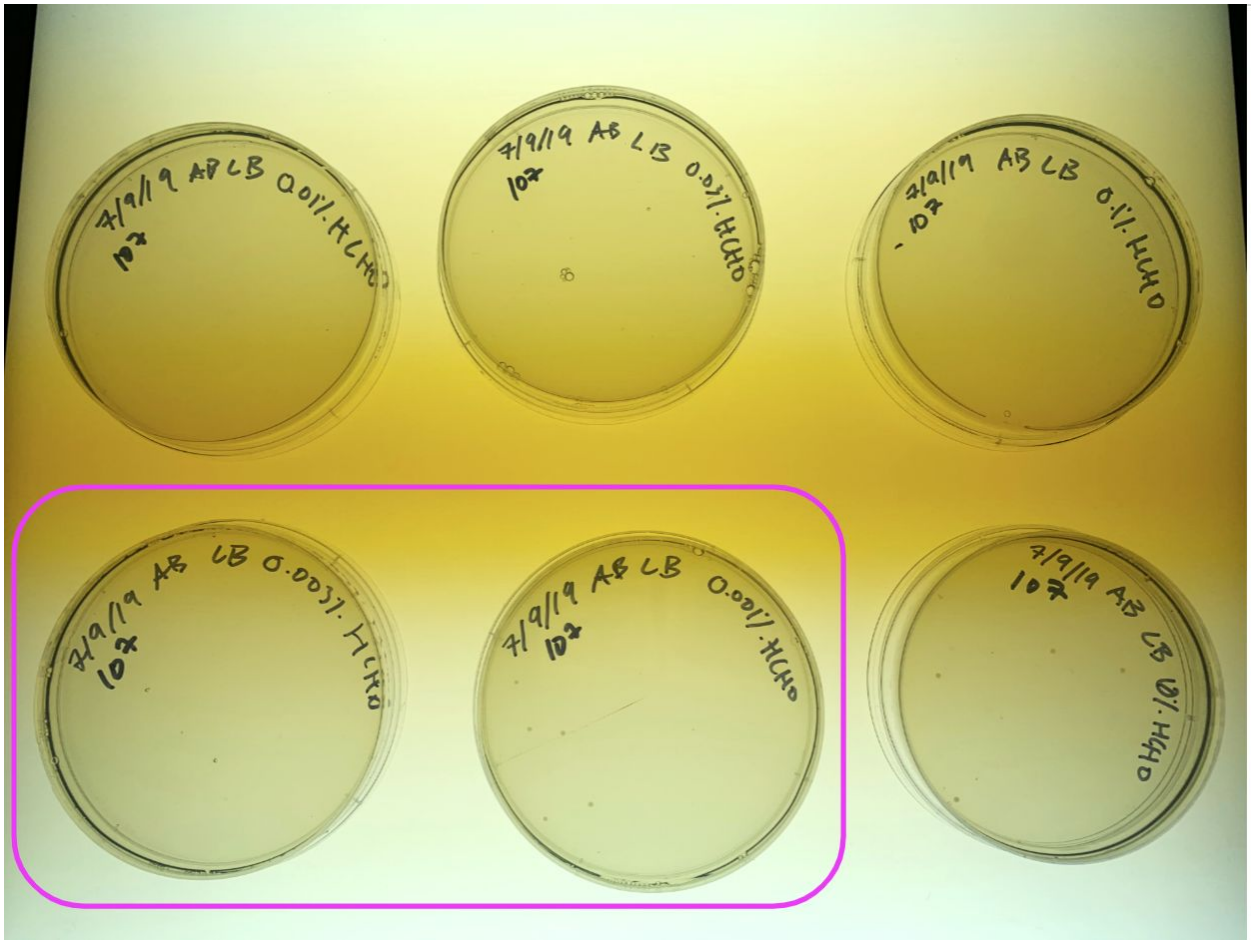


(Sequence:

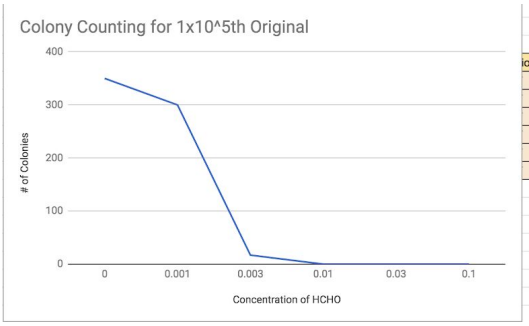
Week 3 (Sol 10) 7/10/19
To do today:
Analyze results of bacterial cultures from Sol 9

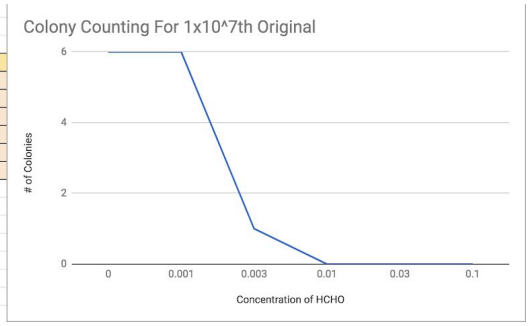
Data/Results (LAB):





Dilution	% Concentration HCHO	Approx. # of colonies
10^5	0.1	0
10^5	0.03	0
10^5	0.01	0
10^5	0.003	17
10^5	0.001	300
10^5	0	350
10^7	0.1	0
10^7	0.03	0
10^7	0.01	0
10^7	0.003	1
10^7	0.001	6
10^7	0	6





Observations:

For the samples on top (10,000th of the original cell sample) it is clear that an increase in HCHO concentration increasingly inhibits colony formation. From this, we can conclude that the ideal range for HCHO concentration to test out FDH+ bacteria is within 0.001%-0.003% HCHO. We can determine his be observing that There are still colonies at 0.003% concentrate but no significant growth after. Additionally, at 0.001% there is significant colony growth but is not to the excessive extent observed at the 0% negative control.

A similar relationship is observed in the bottom samples (10,000,000th of the original cell sample).

Conclusion: FDH plate assays will contain 0.001%-0.003% HCHO

Week 3 (Sol 11) 7/11/19
To do today:

1. Test kill curve with DH5a cells

Procedures:

See Sol 10

Week 3 (Sol 12) 7/12/19
To do today:

1. Record and graph results of Kill curve from Sol 11
2. Make DH5a competent cells

Procedures:

Item 2: Making DH5a competent cells

Materials:

RF1 Solution

Purpose: It treats the DH5a cells so that we can use them in experiments later

- 40 mL of 100 mM RbCl
- 20 mL of 50 mM MnCl2x 4H20
- 24 mL of 30 mM Potassium Acetate (pH 7.5)
    - Adjust pH to 5.4 with acetate
- 20 mL of 10 mM CaCl2x2H20
- 66 mL of 15% glycerol (diluted with H20)

RF2

- 2 mL of 1mM MOPS Buffer
- 4 mL of 10 mM RbCl
- 15 mL of 75 mM CaCl2x2H20
- 47 mL of 15% glycerol (diluted with H20)

Protocol:

1. Culture DH5a cells in 4 mL LB medium overnight (use a 15 mL round bottom culture tube)
2. Subculture the DH5a cells into three flasks
    1. Flask 1: take 300 uL of original culture into 30 mL of LB
    2. Flask 2: take 100 uL of original cell culture into 30 mL of LB
    3. Flask 3: take 50 uL of original cell culture into 3o mL of LB
3. Incubate on shaker at 37C for ~2-2.5 hrs
4. Measure OD600 for Flask 1 (600 nm)
    1. Absorbance should be within 0.3-0.35 if it is over wait 15 mins and repeat with flask 2. (If over again do it with flask 1)
5. Once OD600 measures within 0.3-0.35 transfer the culture into a centrifuge tube and spin at 4000 rpm for 15 mins at 4C
6. Aspirate supernatant

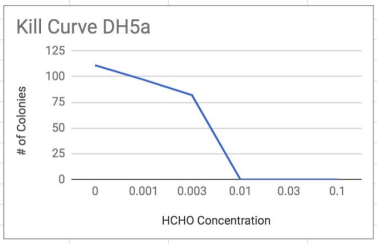
7. Re-suspend in 10 mL RF1 and incubate on ice for 15 mins
8. Spin cells down at 4000 amp for 15 mins at 4C
9. Aspirate Supernatant
10. Re-suspend cells in2.4 mL RF2 and incubate on ice for 15 mins
11. Make and aliquot of 100 uL each in eppendorf tubes and store in -80C

Data/Results:

Item 1: Observe kill curve for DH5a cells

Purpose: we wanted to test the range of HCHO concentration in which DH5a cells could survive (without FDH) in order to see what range we need for when we express our recombinants in DH5a cells

Dilution	Concentration of HCHO	Approx. # of colonies
10^6	0	111
10^6	0.001	97
10^6	0.003	82
10^6	0.01	0
10^6	0.03	0
10^6	0.1	0



Item 1: Plated cultures for kill curve test

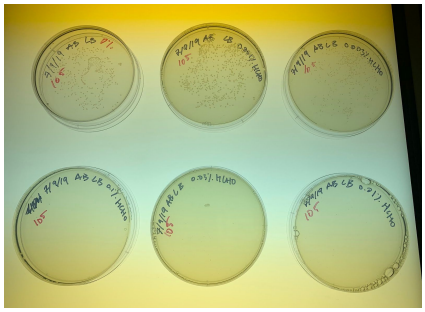


Fig.1 Plates after diluting original bacteria 1:100,000

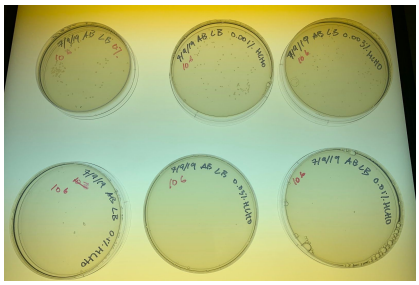


Fig. 2 Plates after diluting original bacteria 1:1,000,000

Week 4 (sol 13) 7/15/19
To do today:

1. Prepare a O/N culture just in case competent cells don't work
2. Test DH5a competent cells
3. Make Endo Agar plates?

Procedures:
Item 1: Prepare O/N culture

1. Aliquot 4 mL of LB media into a round bottom culture tube
2. Take 1 uL of DH5a cells (not competent)
3. Leave in Shaker at 37C O/N

Item 2: Test DH5a competent cells

1. Thaw Top10 and DH5a on ice
2. Dilute cells as follows

1:10 20 uL of concentrated bacteria into 180 uL of LB
1:100 20 uL from 1:10 dilution into 180 uL of LB

3. Add 5 ml DNA plasmid on ice for 30 mins
4. Heat shock at 42C waterbathe for exactly 45 seconds
5. Immediately transfer to ice for 2 mins
6. Add 200 ml SOC media and incubate cells at 37C for 1 hour
7. Warm LB amp plates for 1 hour
8. Plate 50 uL overnight inverted at 32C

Data/Results:
None

Week 4 (Sol 14-15) 7/16-17/19
To do today(s):

1. Resuspend gBlock from IDT
2. Performing restriction digest

Procedures:

All procedures for Phase 1 Part 1 can be found here: Protocol: Phase 1 Part 1

Item 1: Resuspend gBlock from IDT

1. Spin for ~5-10 seconds
2. Add 100 uL of MilliQ H_2O so that the final concentration is 10ng/uL
3. Vortex briefly
4. Incubate at 50C for 20 mins
5. Briefly vortex and do a quick spin
6. Transfer to 1.5 mL eppendorf tube
7. Store in -20C

Item 2: Performing Restriction Digest

Vector: pBAD-D4

1. Cut 5ug in a final volume of 100uL with BamHI-HF and EcoRI-HF
   a. DNA (123.7 ng/uL) 40.4 uL
   b. 10x CUT smart buffer 10uL
   c. EcoRI-HF 4uL
   d. BamHI-HF 4uL
2. Digest for at least 6 hrs in 37C bacterial incubator

Insert: FDH gBlock (~2.7 kb)

3. Mix all of the following in a PCR tube
   a. gBlock 80uL
   b. H2O 3uL
   c. 10x Buffer2.1 10uL
   d. EcoRI-HF 3uL

Insert: TMADH gBlock (~2.7 kb)

4. Mix all of the following in a PCR tube
   a. gBlock 80uL
   b. H2O 3uL
   c. 10x Buffer2.1 10uL
   d. EcoRI-HF 3uL
5. Incubate both samples at 37 °C for 2 hrs

Vector: pBAD-D4

6. Mix all of the following into the same PCR tube used in step 1
   a. 5M NaCl 1 µL
   b. BglII 2.5 µL

Insert: FDH gBlock (~2.7 kb)

7. Mix all of the following into the same PCR tube used in step 2
   a. 5M NaCl 1 µL
   b. BglII 2 µL

Insert: FDH gBlock (~2.7 kb)

8. Mix all of the following into the same PCR tube used in step 2
   a. 5M NaCl 1 µL
   b. BglII 2 µL
9. Incubate at 37 °C 2 hrs
10. Store in -20C O/N

Data/Results:

None

Week 4 (Sol 16) 7/18/19
To do today:
1. PCR purification for inserts
2. Gel purification for vector
3. Make competent cells
Procedures:

Item 1: PCR purification for inserts
1. Add 5 volumes of PB buffer per 1 volume of PCR reaction
2. Place column in tube and add sample to column
3. Spin at max speed for 1 minute, discard flow through
4. Repeat until all of sample has been binded to column
5. Add 750 uL Buffer PE and centrifuge at max speed for 1 minute
6. Discard flow through and spin at max speed for 1 minute
   1. Removes any residual buffer
7. Place column in 15 mL eppendorf
8. Add 15 uL Buffer EB to column, let stand for 1 minute, and spin for 1 minute
9. Repeat step 9, keep flow through
10. Quantify with QubitMix
   1. 4.5 µL HS dye with 900 µL HS buffer (for 4 samples+a little extra)
   2. Put 190 µL of solution + 10 µL of HS Standard 1 into one tube and 190 µL solution+ 10 µL of HS Standard 2 into another tube
   3. Add 199 µL of solution + 1 µL of FDH to one tube
   4. Add 199 µL of solution + 1 µL TMADH to one tube
   5. Use Qubit DNA HS machine to analyze concentration

Item 2: Gel Purification for vector
Part 3b: Gel Purification (For Vector Only)
We expect to see three bands for the pBAD samples: 3.9kb, 2.0kb, 0.2kb. After running the sample on a gel we will perform gel purification using the Qiagen gel purification kit. It is important to note that this process of purification is for the pBAD vector only. The inserts will be processed via PCR purification described above.
1. Make a 0.8% agarose gel
   1. Put 125 mL TAE 1x buffer in a flask
   2. Add 0.84 g agarose
   3. Heat up in microwave
   4. Add 12 µL ethidium bromide (*carcinogen! Be extra careful)
   5. Cool until you can touch it
   6. Pour into frame and wait until solid (don't forget to add the comb)
2. Add DNA Ladder (10 µL) and pBAD DNA sample (add all of the sample but mix with 20 µL 6x purple loading dye first)
3. Run gel at 170 V for about 1 hr
4. Take a picture of the gel and cut out at ~3.9 kb

1. Add 3 volumes of Buffer QG to 1 volume of gel (weigh the gel, 1g = 1mL)
2. Incubate at 50 ℃ for ~10 minutes, vortex occasionally during this time
3. Add 1 volume of 100% isopropanol
4. Transfer to QIAquick column and spin at 8000 rpm for 1 minute (repeat until all of solution has been spun down) discard flow through
5. Add 500 µL QG buffer and spin again @ 8000 rpm for 1 min
6. Add 700 µL PE buffer and spin @ 13,000 rpm for 1 minute (2x) and discard flow through each time
7. Centrifuge at 13,000 rpm for 2 minutes (to dry)
8. Transfer column to clean 1.5 eppendorf
9. Add 15 µL water/EB buffer, let stand for 1 minute, and spin @ max speed for 1 minute (2x)
10. Keep the flow-through

5. Quantify with Qubit

Item 3: Make Competent DH5a cells

RF1 Solution

Purpose: It treats the DH5a cells so that we can use them in experiments later

* 40 mL of 100 mM RbCl
* 20 mL of 50 mM MnCl2x 4H20
* 24 mL of 30 mM Potassium Acetate (pH 7.5)
  * Adjust pH to 5.4 with acetate
* 20 mL of 10 mM CaCl2x2H20
* 66 mL of 15% glycerol (diluted with H20)

RF2

* 2 mL of 1mM MOPS Buffer
* 4 mL of 10 mM RbCl
* 15 mL of 75 mM CaCl2x2H20
* 47 mL of 15% glycerol (diluted with H20)

Protocol:

KEEP EVERYTHING COLD

1. Culture DH5a cells in 4 mL LB medium overnight (use a 15 mL round bottom culture tube)
2. Subculture the DH5a cells into three flasks
   1. Flask 1: take 300 uL of original culture into 30 mL of LB
   2. Flask 2: take 100 uL of original cell culture into 30 mL of LB
   3. Flask 3: take 50 uL of original cell culture into 3o mL of LB
3. Incubate on shaker at 37C for ~2-2.5 hrs
4. Measure OD600 for Flask 1 (600 nm)
   1. Absorbance should be within 0.3-0.35 if it is over wait 15 mins and repeat with flask 2. (If over again do it with flask 1)
5. Once OD600 measures within 0.3-0.35 transfer the culture into a centrifuge tube and spin at 4000 rpm for 15 mins at 4C
6. Aspirate supernatant
7. Re-suspend in 10 mL RF1 and incubate on ice for 15 mins

8. Spin cells down at 4000 amp for 15 mins at 4C
9. Aspirate Supernatant
10. Re-suspend cells in2.4 mL RF2 and incubate on ice for 15 mins
11. Make and aliquot of 100 uL each in eppendorf tubes and store in -80C

Data/Results:

Item 1:
FDH Concentration: 9.80 ng/uL
TMADH Concentration: 4.5 ng/uL

Item 2:
pBAD concentration: 19.6 ng/L

Week 4 (Sol 17) 7/19/19
To do today:

1. Perform Ligation
2. Perform transformation of pBAD+Insert(s) and pBAD only
3. Make and test endo agar plate
4. Test competent cells

Procedures:

Item 1: Ligation

There must always be a vector only ligation reaction in order so that there is something to compare to. There will be 3 reactions in 3 different tubes.

Tube 1- Vector only

1. Mix the following in a PCR tube
    1. Vector (want 50 ng) X uL
    2. 2x ligation buffer 10 uL
    3. Water X uL (to 20 uL)
    4. T4 DNA ligase 0.5 uL
    5. Total volume 20 uL

Tube 2- Vector + TMADH

2. Mix the following in a PCR tube
    1. Vector (want 50 ng) X uL
    2. 2x ligation buffer 10 uL
    3. Insert (want 75 ng) X uL
    4. T4 DNA ligase 0.5 uL
    5. Total volume 20 uL

Tube 3- Vector + FDH

3. Mix the following in a PCR tube
    1. Vector (want 50 ng) X uL
    2. 2x ligation buffer 10 uL
    3. Insert (want 75 ng) X uL
    4. T4 DNA ligase 0.5 uL
    5. Total volume 20 uL
4. Incubate at RT for 10 mins

Item 2: Transform bacteria

1. Add 3 uL ligation to 30 uL of competent cells
2. Proceed with normal transformation
    1. Leave on ice for 30 mins
    2. Heat shock in 42C water bath for 45 seconds (EXACTLY!)
    3. Immediately put on ice for 2 mins
    4. Add 200 uL of SOC recovery media
    5. Incubate at 37C for 1 hr to recover
3. Plate 100 uL of cells on LB Amp agar plates
4. Incubate at RT over the weekend

<u>Item 3: Make and test Endo agar plates</u>

We will be making two kinds of endo agar plates to test to see if the absence or presence of lactose will have any effect on the efficiency of the plates and to see how our bacteria will respond on the plates themselves in both situations

1. Endo agar plates +lactose
    1. Peptone 10 g/L
    2. Lactose 10 g/L
    3. Potassium Phosphate di basic 3.5 g/L
    4. Sodium Sulfite 2.5 g/L
    5. Agar 10 g/L
    6. Basic Fuchsin 6 mL
        1. Diluted to 10% (50:50 ethanol:water)
    7. MilliQ Water 1 L
2. Endo agar plates -lactose
    1. Lennox Broth 20g
    2. Agar 12g
    3. Sodium Sulfite 2.5 g/L
    4. Basic Fuchsin 4.8 mL
        1. Diluted to 10% (50:50 ethanol:water)
    5. MilliQ Water 800 mL
3. Mix in a 1 L flask and send to autoclave for 1 hr
4. Pour 500 mL into plates and let cool for 1-2 hrs

Note these plate tests are not testing with our genes of interest but testing the effectiveness of the plates themselves

1. Test 1: HCHO drop test
    1. Drop 50 uL of 15% HCHO onto both of the endo agar plates
    2. Observe and record results
2. Test 2: Lactose +/- test
    1. Plate 100 uL of TOP10 bacteria on Endo agar plates with lactose

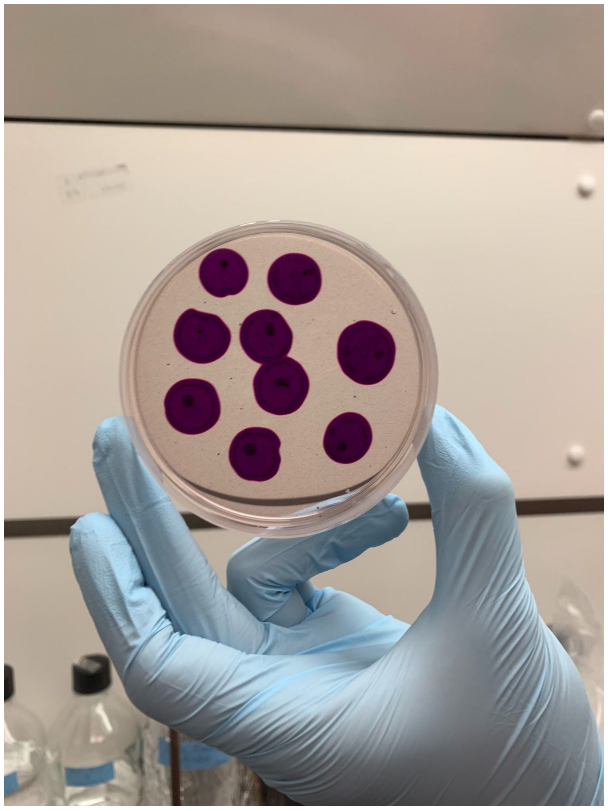
2. Plate 100 uL of DH5a bacteria on endo agar plates without lactose
3. Incubate inverted at 37C O/N

Item 3: Test competent cells

1. Transform homemade DH5a cells with the pSU6 DNA
2. Transform lab made DH5a cells with the pSU6 DNA
3. Plate the samples on LB ampicillin plates O/N at 37C

Data/Results:

Item 3: Endo agar plate drop test



Observation:

The plates react to formaldehyde

Week 5 (Sol 18) 7/22/19
To do today:
Prepare culture for mini prep tomorrow

Procedures:

Item 1: prepare culture/samples for mini prep

1. Count colonies
2. Pick 5 colonies from the Vector+TMADH plate and 5 colonies from the Vector+FDH plate
    1. Take a no filter p200 tip and poke the colony
    2. Put the tip into 4 mL of LB+Amp
        1. Made 50 mL of LB + 50 uL of Amp (100ug/uL)
3. Leave in shaker at 37C O/N

Data/Results:

Item 1: Samples cultures (counting colonies)
Vector only:
3 colonies

Vector+TMADH
11 colonies

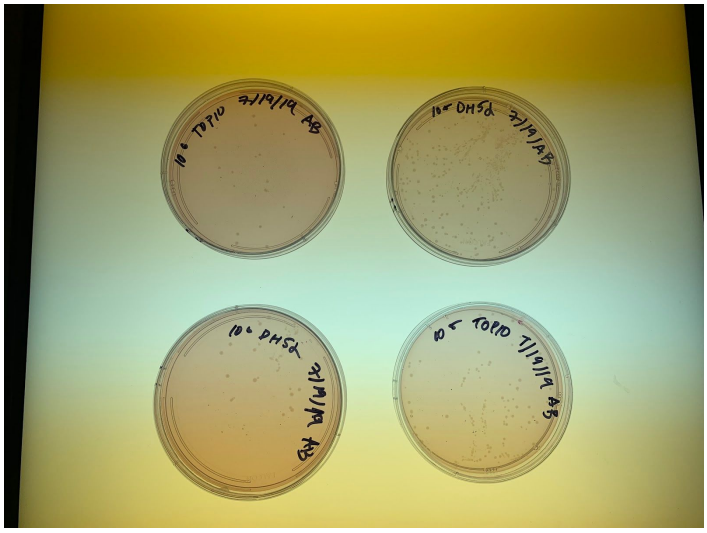
Vector+FDH
5 colonies
the vector only colony is the negative control and we should always have more colonies in the vector+insert transformations otherwise that means that either the ligation, transformation, or the sequence is not correct. In theory the vector only shouldn't be able to survive at all but there is always that possibility.

Cell counting for competent cell test
Top10 (1:10 dilution)
8 colonies

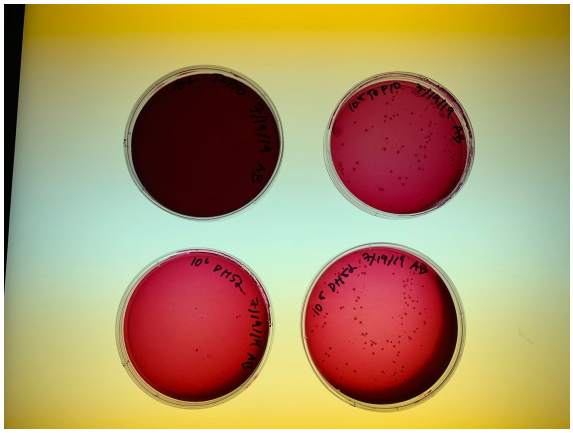
DH5a Homemade (1:10 dilution)
70 colonies
Thus the efficiency of our homemade DH5a cells are 9x10^7 cfu/ug which is within the range of what we want
(How to calculate? (# of colonies/amount of DNA plated)x300 (final volume)= Xcfu/amount of DNA then convert it into cfu/ug

Testing homemade Endo agar plates

Endo agar plates with lactose



Endo agar plates without lactose



Observation:

The plates without the lactose turned red very fast. However, as time went on the plates without lactose slowly turned more pink over time. The reason that the plates without lactose changed color so fast may be due to the fact that the Lennox broth was added to the agar. However, upon completing this experiment we realized that the plates are light sensitive and the fact that the plates were incubated at room temperature may have resulted in the lack of aldehyde production. Further, tests with plates incubated at 37C and in the dark will be performed.

Week 5 (sol 19) 7/23/19
To do today:

1. Miniprep DNA

Procedure:
Item 1: Miniprep DNA
Tube 1: Vector+TMADH

1. Add the following into a 1.5 mL micro centrifuge tube

2. Centifugation at 5000 x g for 10 mins. at room temperature

3. Decant the medium and discard so that the palette is left in the micro centrifuge tube.

4. Add 500 uL Solution 1/RNase A. Pipet up and down to mix thoroughly. Resuspension of cell pallet is necessary to obtain good yields.

5. Transfer suspension into new 2 mL microcentrifuge tube.

6. Add 500 uL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

7. Add 250 uL ice cold N3 Buffer. Invert several times until white precipitate forms.

8. Centrifuge at maximum speed (13000 x g) for 10 mins. A compact white pellet will form. Promptly precede to the next step.

9. Transfer the clear lysate to a new 1.5 mL microcentrifuge tube. Measure the volume of the clear lysate transferred.

10. Add 1 volume ETR Binding Buffer. Invert the tube 10 times to mix thoroughly.

NOTE: If you transferred 500 uL cleared lysate, then add 500 uL ETR Binding Buffer.

use of vacuum volume 1,150 uL -amount of lysate and ETR buffer that we used

NOTE: tubes 1-4 are vector + FDH; tube 5-9 vector + TMADH

11. Insert a HiBind DNA Mini Column II into a 2mL Collection Tube

Optional Protocol For Column Equilibration (WE DID IT):

1. Add 100uL 3M NaOH to the HiBind DNA Mini Column
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

12. Transfer 700uL lysate from Step 10 to the HiBind DNA Mini Column II.

13. Centrifuge at maximum speed for 1 minute.

14. Discard the filtrate and reuse the collection tube.

15. Repeat steps 12-14 until all of the lysate has been transferred to the column.

16. Add 500 uL ETR Wash Buffer.

17. Centrifuge at maximum speed foe 1 minute.

18. Discard the filtrate and reuse the collection tube.

19. Add 500 uL HBC Buffer.

NOTE: HBC Buffer must be diluted with 100% isopropanol before use.

20. Centrifuge at maximum speed for 1 minute.

21. Discard the filtrate and reuse the collection tube.

22. Add 700 uL DNA Wash Buffer.

NOTE: DNA Wash Buffer must be diluted with 100% ethanol prior to use.

23. Centrifuge at maximum speed for 1 minute

24. Discard the filtrate and reuse the collection tube.

25. Repeat Steps 22-24 for a second DNA Wash Buffer wash step.

26. Centrifuge the empty HiBind DNA Mini Column II matrix for 2 minutes at maximum speed to dry the column matrix.

27. Transfer the HiBind DNA Mini Column II to a clean 1.5 mL micro centrifuge tube.

28. Add 15 uL Elution Buffer directly to the center of the column membrane.

29. Spin it in the centrifuge.

30. Add 15 more uL Elution Buffer directly to the center of the column membrane.

31. Centrifuge at maximum speed for 1 minute.

32. Store DNA at -20 degrees celsius.

Data/results:

Endo agar plate test in the dark at 37C (with lactose only)

Observations:

So, it is clear that the plates change color with increased exposure to light and that incubation at 37C allows for TOP10 bacteria to take up lactose and secrete aldehydes.

Week 5 (Sol 20) 7/24/19
To do today:

1. Quantify purified DNA sample
2. Perform restriction digest on the purified DNA

Procedures:

Item 2: Restriction Digest
Cut 500ng plasmids with BglI and BglII (remember to include pBAD-D4)

1. Make a 2x enzyme mix (x12) x12

10x buffer 3.1	2.0uL	24
BglI	0.5uL	6
BglII	0.5uL	6
DNA+H2O	to 17 uL	

2. Incubate for 45mins ~ 2 hrs at 37C bacterial incubator

Data/Results:
DNA concentration

1-4= Vector+FDH
5-9= Vector+TMADH

1. 62.7 ng/uL
1. 61.5 ng/uL
2. 100.6 ng/uL
3. 45.4 ng/uL
4. 93.3 ng/uL
5. 35.2 ng/uL
6. 39.3 ng/uL
7. 51.5 ng/uL
8. 33.3 ng/uL

pBAD-D4= 123.7 ng/uL

Week 5 (Sol 21) 7/25/19
To do today:

1. Run gel with purified DNA
2. Send to sequencing company

Procedures:

Item 1: Run gel with purified DNA

1. Prepare a 1% gel
2. Add 20uL of 2x DNA loading dye to each tube, mix ~5 times and load 20uL to the gel
    1. Make 50 uL of 6x loading dye in 100 uL of H2O
3. 50 uL of 6x Loading dye in 100 uL of H2O
4. Run at 150V for 45 mins to resolve the gel
5. Take a picture of the gel using bio-rad

Data/Results:

Week 5 (Sol 22) 7/26/19
To do today:

1. Rerun digest

Procedure:

Item 1: Rerun digest

1. Make “Master Mix"
    1. Water. 11.5 uL
    2. CutSmart buffer 33 uL
    3. EcoRV. 3.5 uL

Total volume: 15 uL/per tube (explanation: there is 15 uL for each tube but will make enough for 11 samples so that we don’t have to pipette such a small amount for each tube)

2. Mix DNA + H2O to 15 uL
    1. 62.7 ng/uL
    2. 61.5 ng/uL
    3. 100.6 ng/uL
    4. 45.4 ng/uL
    5. 93.3 ng/uL
    6. 35.2 ng/uL
    7. 39.3 ng/uL
    8. 51.5 ng/uL
    9. 33.3 ng/uL
3. Incubate at 37C for 1 hr
    1. While incubating make 1% gel
4. Add 30 uL of 2x loading dye
5. Load 10 uL of DNA ladder
6. Load samples as follows
    1. Wells 2-5 pBAD-FDH
    2. Wells 6-10 pBAD-TMADH
    3. Well 11 pBAD-D4
7. Run at 150 V for 45 min
8. Take a photo of the gel

Data/Results:

Week 6 (Sol 23) 7/29/19
To do today:

1. Perform PCR on gBlock

Procedures:

Item 1: Perform PCR amplification on gBlock

1. Prepare Primers
    1. After receiving the primers from IDT, spin the tube at 13,000 rpm for 1 min to collect all material at the tube bottom
    2. Use filter tips, add 1xTE to the tube for a final concentration of 100uM
        1. For 100uM, the volume (in uL) is 25 nmol times 10
        2. Therefore add 250 uL of 1x TE
    3. After adding the buffer, cap the tube and invert 5 times to mix
    4. Flick the tube to get the most liquid to the bottom and vortex for 10”
    5. Spin at 13,000 rpm for 10 mins
    6. Use a round sticker (green/white) to label the top of the tube with the primer name
    7. Stock the primer at -20C
2. Prepare PCR reaction mix
    1. Forward primer (100uM) 5uL
    2. Reverse primer (100uM) 5uL
    3. H2O 40uL
    4. Label the tube as XXX F+R 10uM and date on the side of the tube
3. Setup the following on a metal rack on ice (!) in PCR tubes
        1. Pipet larger volume first to the PCR tubes for easier transfer of smaller volume
        2. If setting up multiple reactions, consider setting up a master mix (remember to include ~10% extra to account for pipetting loss)
    2. Template (10ng) 1 uL
    3. Primer mix (10uM) 2.5uL
    4. 2x Phusion mix 25.0uL
    5. H2O to 50.0uL
4. Quick Spin to collect all the liquid at the bottom
5. Set up PCR reaction conditions on PCR machine

98C 30”

98C 5” \
60C 30” 20 cycles (will get 10ug in theory)
72C 75” (30” / kb) /

72C 150"

16C Forever

Data/Results:

None

Week 6 (Sol 24) 7/30/19
To do today:

1. PCR gel check
2. Qiagen gel purification/PCR clean up
3. Set up digest

Procedures:

Item 1: PCR gel check

1. Make a 1% agarose gel
    1. 100 mL of 1x TAE+ 1g of agarose
2. Load as follows
    1. Ladder (8 uL)
    2. FDH (2 uL + 8 uL **1x** loading dye)
    3. FDH (2 uL + 8 uL **1x** loading dye)
    4. Blank
    5. TMADH (2 uL + 8 uL **1x** loading dye)
    6. TMADH (2 uL + 8 uL **1x** loading dye)
3. Run the gel at 150 V for 1 hr

negative, no result. Did PCR again.

Item 1 Trial 2: PCR + PCR gel check

Use aliquoted DNase- and RNase-free water. Always use filtered tips when handling PCR. Always spin the tube before opening. Stock primer concentration should always be 100uM (blue cap). Keep primers and DNA on ice when working. Work in PCR tubes

1. Prepare Primers
    1. After receiving the primers from IDT, spin the tube at 13,000 rpm for 1 min to collect all material at the tube bottom
    2. Use filter tips, add 1xTE to the tube for a final concentration of 100uM
        1. For 100uM, the volume (in uL) is 25 nmol times 10
        2. Therefore add 250 uL of 1x TE
    3. After adding the buffer, cap the tube and invert 5 times to mix
    4. Flick the tube to get the most liquid to the bottom and vortex for 10”
    5. Spin at 13,000 rpm for 10 mins
    6. Use a round sticker (green/white) to label the top of the tube with the primer name
    7. Stock the primer at -20C
2. Prepare PCR reaction mix
    1. Forward primer (100uM) 5uL

2. Reverse primer (100uM) 5uL
3. H2O 40uL
4. Label the tube as XXX F+R 10uM and date on the side of the tube

3. Setup the following on a metal rack on ice (!) in PCR tubes
    1. Pipet larger volume first to the PCR tubes for easier transfer of smaller volume
    2. If setting up multiple reactions, consider setting up a master mix (remember to include ~10% extra to account for pipetting loss)
    2. Template (10ng) **x** uL
    3. Primer mix (10uM) 2.5uL
    4. 2x Phusion mix 25.0uL
    5. H2O to 50.0uL
4. Quick Spin to collect all the liquid at the bottom
5. Set up PCR reaction conditions on PCR machine

98C 30”

98C 5” \
58C 30” 20 cycles (will get 10ug in theory)
72C 75” (30” / kb) /

72C X * 2 “
16C Forever

6. Save DNA in -20C
7. Check DNA
    1. 10 uL DNA + 10uL 2x Loading dye
    2. Load into 100 mL 1% agarose gel at 130 V for 1 hr

Item 2: Qiagen PCR clean up

1. Add 5 volumes of PB buffer per 1 volume of PCR reaction
2. Place column in tube and add sample to column
3. Spin at max speed for 1 minute, discard flow through
4. Repeat until all of sample has been binded to column
5. Add 750 uL Buffer PE and centrifuge at max speed for 1 minute
6. Discard flow through and spin at max speed for 1 minute
    1. Removes any residual buffer
7. Place column in 15 mL eppendorf
8. Add 15 uL Buffer EB to column, let stand for 1 minute, and spin for 1 minute
9. Repeat step 9, keep flow through
10. Quantify with QubitMix
    1. 4.5 µL HS dye with 900 µL HS buffer (for 4 samples+a little extra)

2. Put 190 µL of solution + 10 µL of HS Standard 1 into one tube and 190 µL solution+ 10 µL of HS Standard 2 into another tube
3. Add 199 µL of solution + 1 µL of FDH to one tube
4. Add 199 µL of solution + 1 µL TMADH to one tube
5. Use Qubit DNA HS machine to analyze concentration

Item 3: Set up digest

Insert: FDH gBlock (~2.7 kb)

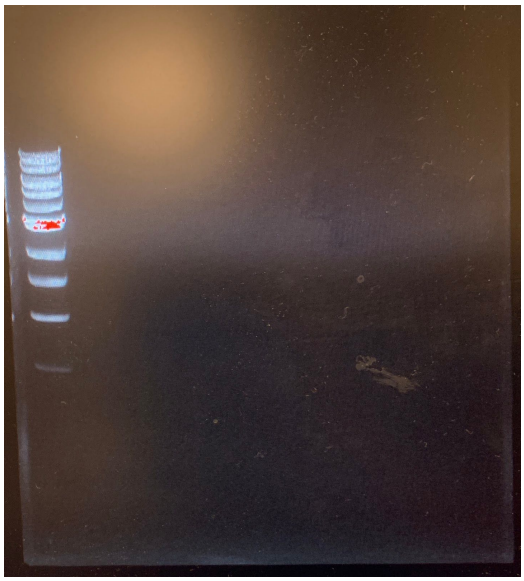
1. Mix all of the following in a PCR tube
    1. gBlock 80uL
    2. H2O 3uL
    3. 10x Buffer2.1 10uL
    4. EcoRI-HF 3uL

Insert: TMADH gBlock (~2.7 kb)

2. Mix all of the following in a PCR tube
    1. gBlock 80uL
    2. H2O 3uL
    3. 10x Buffer2.1 10uL
    4. EcoRI-HF 3uL
3. Incubate both samples at 37 ℃ for O/N

Data/results:

Item 1:



Item 1 Trial 2:

Observations:

The cause of the lack of bands in the first trial could be due partially because of pipetting error. Also the adjusted PCR conditions may also be more optimal for the primers we are using.

Week 6 (Sol 25) 7/31/19

To do today:

1. Finish Digest
2. Run gel and purify gBlock bands

Procedures:

Item 1: Finish Digest

Insert: FDH gBlock (~2.7 kb)

1. Mix all of the following into the same PCR tube used in step 2
    1. 5M NaCl1 µL
    2. BglII2 µL

Insert: FDH gBlock (~2.7 kb)

2. Mix all of the following into the same PCR tube used in step 2
    1. 5M NaCl1 µL
    2. BglII2 µL
3. Incubate at 37 ℃ 2 hrs
4. Store in -20C O/N

Item 2: Run gel and purify gBlock bands

1. Make a 1% gel with 100 mL 1x TAE
    1. Prepare each sample with 20 uL of 6x loading dye
2. Load all of the sample
    1. Marker
    2. 30 uL FDH
    3. 30 uL FDH
    4. 30 uL FDH
    5. 30 uL FDH
    6. Blank
    7. 30 uL TMADH
    8. 30 uL TMADH

9. 30 uL TMADH
10. 30 uL TMADH

3. Run gel at 170 V for 1 hr
4. Take a picture of the gel and cut out...
    1. FDH- 1 kb
    2. TMADH- 2 kb
5. Add 3 volumes of Buffer QG to 1 volume of gel (weigh the gel, 1g = 1mL)
6. Incubate at 50 ℃ for ~10 minutes, vortex occasionally during this time
7. Add 1 volume of 100% isopropanol
8. Transfer to QIAquick column and spin at 8000 rpm for 1 minute (repeat until all of solution has been spun down) discard flow through
9. Add 500 µL QG buffer and spin again @ 8000 rpm for 1 min
10. Add 700 µL PE buffer and spin @ 13,000 rpm for 1 minute (2x) and discard flow through each time
11. Centrifuge at 13,000 rpm for 2 minutes (to dry)
12. Transfer column to clean 1.5 eppendorf
13. Add 15 µL water/EB buffer, let stand for 1 minute, and spin @ max speed for 1 minute (2x)
14. Keep the flow-through
15. Quantify with Qubit

Item 3: Ligation

Tube 1- Vector only

1. Mix the following in a PCR tube
    1. Vector (want 50 ng)X uL
    2. 2x ligation buffer10 uL
    3. WaterX uL (to 20 uL)
    4. T4 DNA ligase0.5 uL
    5. Total volume20 uL

Tube 2- Vector + TMADH

2. Mix the following in a PCR tube
    1. Vector (want 50 ng)X uL
    2. 2x ligation buffer10 uL
    3. Insert (want 75 ng)X uL
    4. T4 DNA ligase0.5 uL
    5. Total volume20 uL

Tube 3- Vector + FDH

3. Mix the following in a PCR tube
    1. Vector (want 50 ng)X uL
    2. 2x ligation buffer10 uL
    3. Insert (want 75 ng)X uL
    4. T4 DNA ligase0.5 uL
    5. Total volume20 uL
4. Incubate at RT for 10 mins

Item 4: Transformation

3. Add 3 uL ligation to 30 uL of competent cells
4. Proceed with normal transformation
    1. Leave on ice for 30 mins
    2. Heat shock in 42C water bath for 45 seconds (EXACTLY!)
    3. Immediately put on ice for 2 mins
    4. Add 200 uL of SOC recovery media
    5. Incubate at 37C for 1 hr to recover
5. Plate 100 uL of cells on LB Amp agar plates
6. Incubate at RT over the weekend

Data/Results:

Item 2: Gel

Item 2 (Cont): DNA concentration

TMADH- 16.3 ng/uL

FDH- 11.8 ng/uL

Observations:

Allison is dummy and forgot to digest the vector. Will do that tomorrow.

Week 6 (Sol 26) 8/1/19

To do today:

1. Restriction digest pBAD and gel purify

Procedures:
Restriction digest pBAD and gel purify: see sol 20 & 24 respectively

Week 6 (Sol 27) 8/2/19

To do today:

1. Quantify all DNA
2. Ligation
3. Transformation

Procedures:

Item 1: Quantify all DNA

1. 4.5 µL HS dye with 900 µL HS buffer (for 4 samples+a little extra)
2. Put 190 µL of solution + 10 µL of HS Standard 1 into one tube and 190 µL solution+ 10 µL of HS Standard 2 into another tube
3. Add 199 µL of solution + 1 µL of FDH to one tube
4. Add 199 µL of solution + 1 µL TMADH to one tube
5. Use Qubit DNA HS machine to analyze concentration

Item 2: Ligation
*Tip: make a big 'master mix' that adds up all the

1. 50 uL of 2x Quick ligation buffer
2. 2.5 uL of T4 DNA ligase

1. Tube 1- **Vector Only**
    1. Vector (25 g) 6.22 uL
    2. H2O (to 20 uL). 3.28 uL
    3. “Master Mix”. 10.5 uL
2. Tube 2- **Vector+TMADH**
    1. Vector (25 ng). 6.22 uL
    2. TMADH (16 ng). 0.98 uL
    3. H2O (to 20 uL). 2.3 uL
    4. “Master Mix”. 10.5 uL
3. Tube 3- **Vector+FDH (Short)**
    1. Vector (25 ng). 6.22 uL
    2. FDH (Short) (6 ng). 0.508 uL
    3. H2O (to 20 uL). 2.7 uL
    4. “Master Mix”. 10.5 uL
4. Tube 4- **Vector+FDH (long)**
    1. Vector (25 ng) 6.22 uL
    2. FDH (long) (10 ng). 2.34 uL
    3. H2O (to 20 uL). 0.94 uL
    4. “Master Mix”. 10.5 uL
5. Incubate at RT for 10 mins

<u>Item 2: Transformation into DH5a cells</u>

1. While ligation is incubating thaw DH5a competent cells on ice
2. Add 5 uL of ligation to 50 uL of cells (store ligation in -20C for later use… we used all of the vector only)
3. Incubate on ice for 30 mins
4. Heat shock in 42C water bath for 45 seconds exactly!
5. Put on ice for 2 mins
6. Add 300 uL of SOC recovery to cells
7. Recover at 37C shaking for 1 hr
    1. While this is happening dry LB amp plates at 37C
8. Spin cells down at 8000 rpm for 5 mins
    1. This is so that we can resuspend the pellet in a lower volume of SOC and thereby increase the concentration of bacteria we plate
9. Remove 250 uL of supernatant
10. Resuspend bacterial pellet in remaining 100 uL of SOC
11. Plate 100 uL of bacteria
12. Incubate at 37C inverted O/N

Data/Results:

Item 1: Quantify all DNA
pBAD- 4.02 ng/uL
TMADH- 16.3 ng/uL
FDH (Short)- 11.8 ng/uL
FDH (long)- 4.28 ng/uL

Observations:

- If we had eluted the DNA from yesterday in 30 uL of buffer EB instead of 50 uL our concentration of DNA for pBAD and FDH (long) would probably have been larger.
- The original protocol called for 50 ng of Vector in 75 ng of insert; however, since we had such a low concentration of DNA we determined the amount of insert necessary for 25 ng of vector (instead of 50 ng) using the following equation
    - (X ng Vector)*([Insert]/[Vector])= Xng of Insert
- The reason that there are two FDH samples is because after doing digest there were two fragments (~1 kb and ~1.5 kb) so we cut and purified both of them I order to see which one is the “real” FDH

Week 6 (Sol 28) 8/5/19

To do today:

1. Try transformation again (it didn’t work)

Week 6 (Sol 29) 8/6/19

To do today:

1. Make TMA plates
2. Test TMA kill curve

Procedures:

Item 1: Make TMA plates

Endo agar plate recipe

Note: endo agar plates are light sensitive. Meaning that if the plates are left in open bench light (versus in a dark incubator) the plate will start to turn pink.

Ingredients		**g/L**
Peptone		10
Lactose		10
Potassium Phosphate (dibasic)		3.5
Sodium Sulfite		2.5
Agar		10
Basic Fuchsin*		6 mL**
pH 7.5 +/- 0.2 at 25 C		

*Basic Fuchsin is a potential carcinogenic. Avoid inhalation of powder or contact with skin.
**To prepare, suspend 36 g of Basic Fuchsin powder in 1 L Distilled H2O. Make a 10% w/v solution by diluting it into a 50:50 ratio of ethanol:distilled water.
Mixing instructions:

1. Measure peptone, lactose, potassium phosphate dibasic, sodium sulfite in a large 2 L flask.
2. Separate into 6 100 mL bottles and add 2 g of agar to
3. Send to autoclave

4. Add 600 uL basic Fuchsin to the mix
5. Add Arabinose so it’s 0.2% final
    1. 10% stock
6. Add TMA in a serial dilution with final concentrations as follows (stock is 25%)
    1. 8%
    2. 4%
    3. 2%
    4. 1%
    5. 0.5%
    6. 0%
7. Mix thoroughly
8. Pour plates

Week 6 (Sol 30) 8/7/19

To do today:

1. PCR TMADH to amplify the gBlock
2. Send FDH samples for sequencing

Procedures:

Item 1: PCR TMADH to amplify the gBlock

1. Setup the following on a metal rack on ice (!) in PCR tubes
    1. Pipet larger volume first to the PCR tubes for easier transfer of smaller volume
    2. If setting up multiple reactions, consider setting up a master mix (remember to include ~10% extra to account for pipetting loss)
    2. Template (10ng) **x**uL
    3. Primer mix (10uM) 2.5uL
    4. 2x Phusion mix 25.0uL
    5. H2Oto 50.0uL
2. Quick Spin to collect all the liquid at the bottom
3. Set up PCR reaction conditions on PCR machine

98C30”

98C5” \
59C30” 20 cycles (will get 10ug in theory)
72C75” (30” / kb) /

72C150”
16CForever

4. Save DNA in -20C
5. Check DNA
    1. 10 uL DNA + 10uL 2x Loading dye
    2. Load into 100 mL 1% agarose gel at 130 V for 1 hr
6. Purification if PCR is successful (Qiagen PCR clean up)
7. Once done purifying quantify 1 uL of PCR clean up with Qubit

Item 2: Send FDH samples for sequencing

1. Measure the concentrations of DNA
    1. 150 ng/uL
    2. 128.6 ng/uL
    3. 139.1 ng/uL
2. Add 500 ng of DNA to each PCR tube (load as follows)
    1. 3.33 uL
    2. 3.89 uL
    3. 3.59 uL
3. Add H2O to each tube for a final volume of 10 uL
    1. 6.67 uL
    2. 6.11 uL
    3. 6.41 uL
4. Print out sequencing information sheet and send to Genewiz

---

Data/Results:

TMA kill curve

Week 6 (Sol 31) 8/8/19

To do today:

1. Do digest on pBAD (modified) + TMADH
2. Mini prep Vector+TMADH colony
3. Gel purify pBAD and TMADH
4. Quantify both mini prep and gel purification

Procedures:

Item 1: Digest in pBAD and TMADH (modified)

1. Tube 1: pBAD D4 (1)
    1. DNA (5 ug) 40.4 uL
    2. 10x Cutsmart buffer. 10 uL
    3. EcoRI-HF. 4 uL
    4. BamHI-HF. 4 uL
2. Tube 2: pBAD D4 (2)
    1. DNA (5 ug). 40.4uL
    2. 10x Cut smart buffer. 10 uL
    3. EcoRI-HF. 4uL
    4. MluI-HF. 4 uL
3. Tube 3: TMADH (already cut with EcoRI O/N)
    1. Digest
    2. 10x Cut smart buffer. 10 uL
    3. BglII. 4 uL
4. Incubate at 37C for at least 2 hrs
5. Run samples on a gel
    1. Add 1 gram of agar and 100 mL of TAE buffer into a flask
    2. Microwave for 30 sec - 1 minute until it is clear
    3. Cool the bottom of the flask with water
    4. Put in 10 uL of ethidium bromide (or a 1:10,000 ratio)
    5. Mix and put in the mold
    6. Cold room for 15 minutes
    7. Before loading, mix each sample with 20 uL of 6x purple loading dye
    8. Load the gel. For each well…
        1. 8 uL 1kb DNA ladder

2. 40 uL PBAD-D4 (1) - already has 6x loading dye
3. 40 uL PBAD-D4 (1) - already has 6x loading dye
4. 40 uL pBAD-D4 (2) - already has 6x loading dye
5. 40 uL pBAD-D4 (2) - already has 6x loading dye
6. 40 uL TMADH - already has 6x loading dye
7. 40 uL TMADH - already has 6x loading dye

9. Run gel at 150 V for 1 hour

Item 2: Miniprep Vector+TMADH
Item 3: Gel Purify gel from item 1w

Week 6 (Sol 32) 8/9/19

To do today:

1. Pick colonies from TMADH transformation
2. Perform PCR on TMADH insert (modified)
3. Do gel check and PCR purification on PCR product
4. Start Digest on TMADH insert

Procedures:

Item 2: Perform PCR on TMADH insert (modified)

1. Prepare MluI primer
    1. After receiving the primers from IDT, spin the tube at 13,000 rpm for 1 min to collect all material at the tube bottom
    2. Use filter tips, add 1xTE to the tube for a final concentration of 100uM
        1. For 100uM, the volume (in uL) is25 nmoltimes 10
        2. Therefore add 250 uL of 1x TE
    3. After adding the buffer, cap the tube and invert 5 times to mix
    4. Flick the tube to get the most liquid to the bottom and vortex for 10”
    5. Spin at 13,000 rpm for 10 mins
    6. Use a round sticker (green/white) to label the top of the tube with the primer name
    7. Stock the primer at -20C
2. Prepare PCR reaction mix
    1. Tube 1- TMADH (BglII Primer)
        1. Template (10ng) 1 uL
        2. Primer mix from 7/29/19(10uM) 2.5uL
        3. 2x Phusion mix 25.0uL
        4. H2O to 50.0uL. 21.5 uL
    2. Tube 2- TMADH (BglII Primer) Replicate
        1. Template (10ng) 1 uL
        2. Primer mix from 7/29/19(10uM) 2.5uL
        3. 2x Phusion mix 25.0uL
        4. H2O to 50.0uL. 21.5 uL
    3. Tube 3- TMADH (MluI Primer)
        1. Template (10ng) 1 uL

2. Primer mix from 8/9/19 (10uM) 2.5uL
3. 2x Phusion mix 25.0uL
4. H2O to 50.0uL. 21.5 uL

4. Tube 4- TMADH (MluI Primer) Replicate
    1. Template (10ng) 1 uL
    2. Primer mix from 8/9/19 (10uM) 2.5uL
    3. 2x Phusion mix 25.0uL
    4. H2O to 50.0uL. 21.5 uL

3. Set up PCR conditions

98C30”
98C5”\
59C30”x20
72C75”/
72C150”
12 **

<u>Item 4: Restriction Digest on PCR product</u>

1. Tube 1- TMADH (BglII)
    1. DNA from PCR clean up 30 uL
    2. 10x CutSmart Buffer. 5 uL
    3. EcoRI-HF 2 uL
    4. H2O (to 50 uL). 13 uL
    5. Incubate at 37C O/N
2. Tube 2- TMADH (MluI)
    1. DNA from PCR clean up 30 uL
    2. 10x CutSmart Buffer. 5 uL
    3. EcoRI-HF 2 uL
    4. MluI-HF 2 uL
    5. H2O (to 50 uL). 13 uL
    6. Incubate at 37C for 2 hrs
3. Tube 1- TMADH (BglII) Part 2
    1. BglII 2 uL
    2. 5M NaCl 2 uL
    3. Incubate at 37C for 2 hrs
4. Store digest at -20C

Week 7 (Sol 33) 8/12/19

To do today:

1. Miniprep samples
2. Redo ligation and transformation for TMADH plan B

Procedures:

Item 2: Ligation and Transformation

1. Tube 1- Vector only (BamHI+EcoRI)
    1. pBAD (25 ng) 1.131 uL
    2. 2x Ligation Buffer 10 uL
    3. T4 DNA Ligase. 0.5 uL
    4. H2O (to 20uL) 8.369 uL
2. Tube 2- Vector Only (MluI+EcoRI)
    1. pBAD (25 ng) 1.412 uL
    2. 2x Ligation Buffer 10 uL
    3. T4 DNA Ligase. 0.5 uL
    4. H2O (to 20uL) 8.088 uL
3. Tube 3- Vector+TMADH (MluI)
    1. TMADH (16 ng) 4.372 uL
    2. pBAD (25 ng) 1.412 uL
    3. 2x Ligation Buffer 10 uL
    4. T4 DNA Ligase. 0.5 uL
    5. H2O (to 20 uL) 3.716 uL
4. Tube 4- Vector+TMADH (BglII)
    1. TMADH (16 ng) 4 uL
    2. pBAD (25 ng) 1.131 uL
    3. 2x Ligation Buffer 10 uL
    4. T4 DNA Ligase. 0.5 uL
    5. H2O (to 20uL) 4.369 uL
5. Incubate at RT for 10 mins
6. Add 5 uL of ligation to 50 uL of DH5a
7. Incubate on ice for 30 mins
8. Heat shock in 42C water bath for 45 seconds
9. Immediately transfer to ice for 2 mins

10. Add 300 uL of SOC media
11. Recover at 37C shaking for 1 hr
12. Spin bacteria down at 8000 rpm for 5 mins
13. Remove 200 uL of supernatant
14. Resuspend cells in remaining SOC
15. Plate everything
16. Incubate at 37C O/N

Data/results:

Item 1: DNA concentration (Nanodrop result)

1. 156.8 ng/uL
2. 132.6 ng/uL
3. 205.1 ng/uL
4. 173.6 ng/uL
5. 160.4 ng/uL
6. 170.9 ng/uL
7. 193.5 ng/uL
8. 177.7 ng/uL

Week 7 (Sol 34) 8/13/19

To do today:

1. Make TMA Plates

Procedures:

Item 2: Make TMA plates

Ingredients		**g/L**
Peptone		10
Lactose		10
Potassium Phosphate (dibasic)		3.5
Sodium Sulfite		2.5
Agar		10
Basic Fuchsin*		6 mL**
pH 7.5 +/- 0.2 at 25 C		

1. While at the autoclave perform a serial dilution of TMA so the concentrations are as follows
    1. 2%
    2. 1%
    3. 0.5%
    4. 0.25%
    5. 0.125%
    6. 0%

2. After sending to the autoclave and adding the basic fuchsin add the TMA to the solution so that there are 6 different kinds of endo agar mixtures (all varying in TMA concentration)
3. Next pour the plates and let them set for ~30 mins at RT
4. Perform two dilutions on DH5a cells
    1. 10^5 and 10^6
5. Incubate at 37C O/N

Week 7 (Sol 35) 8/14/19

To do today:

1. Do digest on TMADH
    1. PCR purify and quantify
2. Do digest on FDH
    1. PCR purify and quantify

Procedures:

Item 1+2: Digest

Did a sequential digest of both inserts of EcoRI-HF and MluI

Week 7 (Sol 36) 8/15/19

To do today:

1. PCR TMADH
    1. Why? When quantifying the insert that we digested there was no DNA so we are doing PCR again
2. Start testing PCR conditions for mutagenesis
3. Did digest on TMADH to prepare for ligation

Procedures:

Item 2: Test PCR conditions for mutagenesis

1. Make 2x Buffer+dNTP (1mL)-- one with MnCl2one without.

10x Buffer (contain final 1.5mM MgCl2)200uL→1x

2M MgCl23.5uL→5mM

1M MnCl22uL→0.5mM

100mM dATP4uL→0.4mM

100mM dGTP4uL→0.4mM

100mM dCTP20uL→2.0mM

100mM dTTP20uL→2.0mM

Final 1x buffer also contain: 10mM TrisHCl pH8.3; 50mM KCl

(We already have both of these reagents so that we don't)

2. Setup PCR reaction

Template 1ng1 uL

Primer mix (10uM)10uL

2x buffer+dNTP25uL

Taq 1uL

H2O to 50uL

Total will have 4 conditions: 2 primer mixes; +/- MnCl2

3. PCR reaction cycle (~1.5 hrs)

94C2’

94C15”\

46C30”x20

68C2’30’’/

68C5’

12C**

4. Load 2 uL of PCR product in 8 uL of 1x purple loading dye in a 100 mL 1% agarose gel
5. Run at 150V for 1 h

Data/Results:

None

Week 7 (Sol 37) 8/18/19

To do today:

1. Gel purification of the digested things
2. Quantify

Data/Results:

There was nothing lol

Week 8 (Sol 38) 8/19/19

To do today:

1. Redo PCR on the inserts
2. Run test gel for PCR product of inserts and error prone
3. Perform digest on inserts
4. Ligation and transformation

Procedures:

Item 1: Redo PCR on the inserts

Do as always

Item 2: Run test gel on PCR products of inserts and error prone

2 uL of PCR product into 8 uL of 1x purple loading dye

Run gel at 150 V for 1 hr

Do PCR clean up on inserts once confirmed PCR products

Item 3: Perform digest on inserts

Each tube should be as follows

1. DNA (All)
2. 6 uL of 10x CutSmart Buffer
3. 2 uL of EcoRI-HF
4. 2 uL of MluI-HF (FINALLYY)
5. Incubate at 37C for 1-3 hrs

Item 4: Ligation and transformation

Do as normal

Data/results:

Item 2: Quantify after PCR clean up

TMADH- 72.1 ng/uL

FDH- 60.6 ng/uL

Week 9 (Sol 39) 9/4/19

WE GOT CLONES MY DUDES

didn't update in a while because was just repeating the same process until got successful clones

To do today:

1. Send plasmids for sequencing with pBAD Reverse primer
2. Transform TMADH, FDH, and pBAD-D4 into DH5a
3. Did PCR for mutagenesis

Procedures:

Item 3: PCR for mutagenesis

Note: we set up two reactions, each with different Tm's. We are testing two PCR conditions at the same time

Template 5ng

Primer mix (10 uM) 10 uL

2x buffer +dNTP 25 uL

Taq 1 uL

Water to 50 uL

PCR conditions

94 C 2’

94 C 30'

46 C/50C 1’ \

72 C 4’ x20

72 C 8’ /

12C **

Observations:

Week 9 (Sol 40) 9/5/19

To do today:

1. Pick colonies for arabinose induction, TMA kill curve (scratch), and TMA plate assay
2. Make TMA plates
3. Test kill curve of TMA
4. Set up PCR again

Procedures:

Item 2: Make TMA plates

Start with 50 mL of Endo agar

Add 300 uL of 10% Fuchsin

Add 100 uL of 10% Arabinose

Add TMA as follows…

1. Add 1000 uL from 25% stock concentration into a micro centrifuge tube
    1. This will become 0.1% final concentration in 50 mL of Endo agar
2. Take 333 uL from tube 1 into 666 uL of H2O
    1. This will become 0.03% final concentration in 50 mL of Endo agar
3. Take 333 uL from tube 2 into 666 uL of H2O
    1. This will become 0.01% final concentration in 50 mL of Endo agar
4. Take 333 uL from tube 3 into 666 uL of H2O
    1. This will become 0.003% final concentration in 50 mL of Endo agar
5. Take 333 uL from tube 4 into 666 uL of H2O
    1. This will become 0.001% final concentration in 50 mL of Endo agar
6. Don't add any TMA to tube/bottle 6 as this is the 0% TMA negative control

Item 4: Mutagenesis PCR

Note we are testing two different PCR conditions in this procedures

Also note to dilute the PCR product being mutated in this experiment before use

PCR Reaction:

Template. 5 ng

Primer mix (10 uM) 10 uL

2x buffer+ dNTP 25 uL

Taq 2 uL

H2O to 50 uL

PCR conditions:

94C 2’

94C 15” \

46C/50C 30” x20

68C 4’ /

68C 8’

12C **

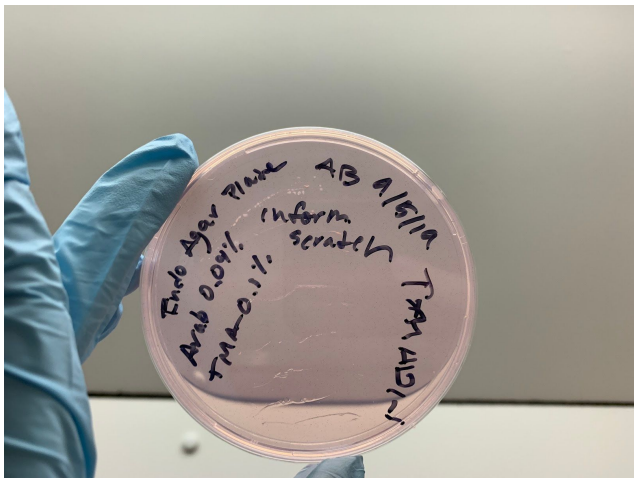
Week 9 (Sol 41) 9/6/19

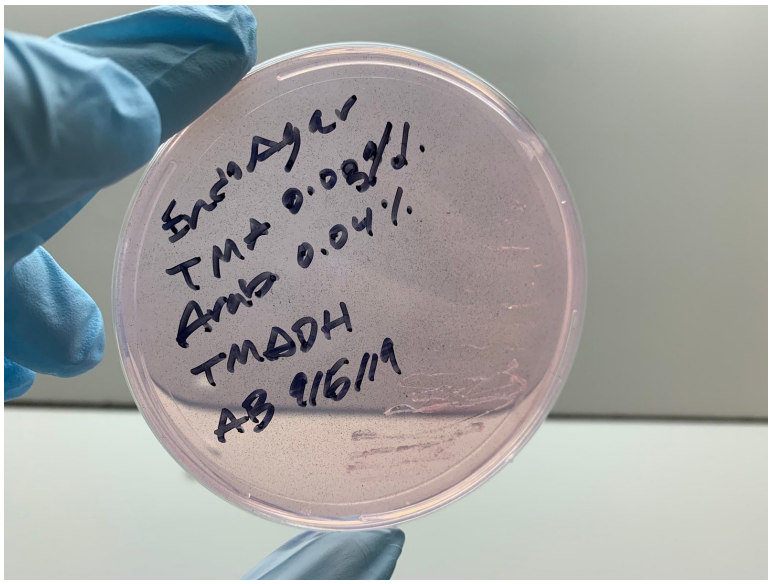
To do today:

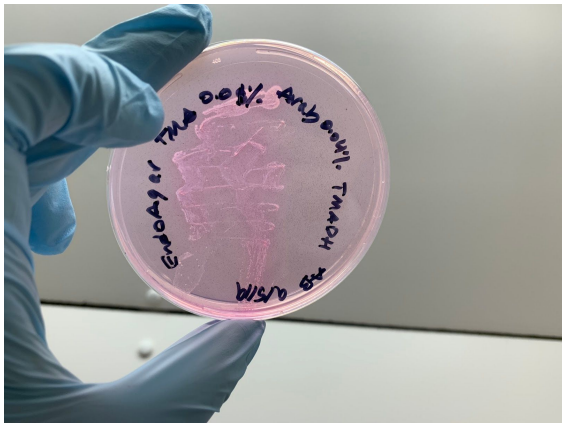
1. Make TMA plates fresh
2. Take colonies selected from Sol 40 onto TMA plates for testing
3. Gel check for PCR mutagenesis (load everything)

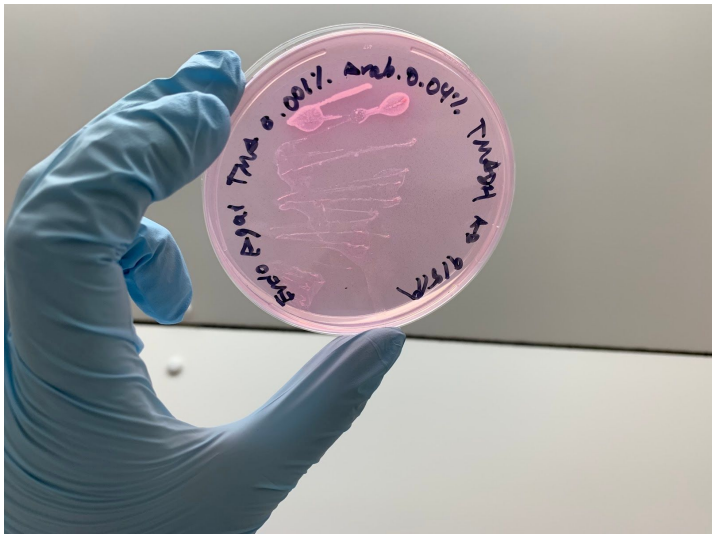
Data/Results:u

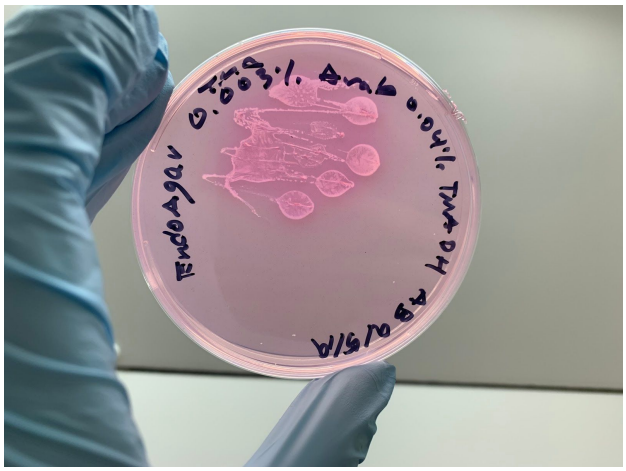
Result 1: Informal plate test.

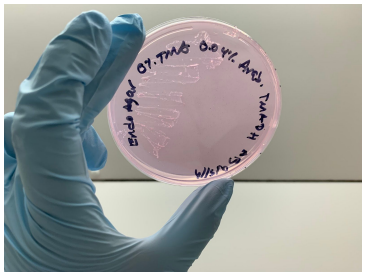



TMA 0.08%
Amb 0.04%
TMADH
AB 9/16/19

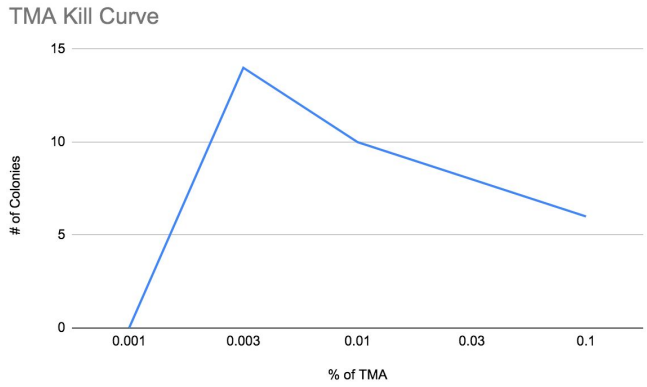

TMA 0.08%
TMADH







Result 2: TMA Kill curve



Concentration (% of TMA)	0	0.001	0.003	0.01	0.03	0.1
# of colonies	0	14	10	8	6	0

Week 10 (Sol 42) 9/9/19

To do today:

1. Make TMA and HCHO plates
2. Plate constructs on the bacteria
3. Arabinose Induction

Procedures:

Item 1: Make TMA and HCHO plates

a. TMA plates

1. Melt the prepared 50 mL endo agar already prepared in the microwave
2. Add 300 uL of 10% Basic Fuchsin
3. Add 10 uL of 10% arabinose (final concentration of 0.02%)
4. Perform a serial dilution of TMA as follows
    1. 1 mL of 25% (Final is 0.1%)
        1. Take 333 uL from this
    2. Add 666 uL of H2O (final is 0.03%)
        1. Take 333 uL from this
    3. Add 666 uL of H2O (final is 0.01%)
        1. Take 333 uL from this
    4. Add 666 uL of H2O (final is 0.003%)
        1. Take 333 uL from this
    5. Add 666 uL of H2O (final is 0.001%)
5. Add 200 uL of the serial dilution to six bottles of the endo agar with fuchsin and arabinose
    1. For the sixth bottle add 200 uL of water
6. Mix thoroughly

b. HCHO plates

1. Melt the prepared 50 mL endo agar already prepared in the microwave
2. Add 300 uL of 10% Basic Fuchsin
3. Add 10 uL of 10% arabinose (final concentration of 0.02%)
4. Add HCHO to the mixes as follows

1. 93.75 uL of 16% HCHO (final is 0.03% HCHO)
2. 31.25 uL of 16% HCHO (final is 0.01% HCHO)
3. 9.375 uL of 16% HCHO (final is 0.003% HCHO)

Item 2: Plate constructs with bacteria

1. Take 1 mL of LB and put it into a FACS tube
2. Pick 10 colonies from the TMADH plate with the same no filter p200 filter tip and put it into the tube in step 1
3. Vortex for ~5 seconds
4. Remove the tip
5. Repeat step 2-4
6. Repeat step 1-5 for FDH and pBAD-D4
7. Measure the OD600 of the sample
    1. Can return it back to the FACS tube after being put in the cuvette
8. Calculate the concentration of cells
    1. Since OD600 of 1.0= 8x10^8 cells/mL
    2. OD of samples were as follows
        1. TMADH- 0.09
        2. FDH- 0.101
        3. pBAD- 0.088
    3. Therefore the concentration of the cells were as follows
        1. [TMADH]= 7.36x10^7 cells/mL
        2. [FDH]= 8.08x10^7 cells/mL
        3. [pBAD]= 7.04x10^7 cells/mL
9. Then dilute your sample
    1. We are aiming for a concentration of 2000 cells/mL
        1. Since we will be plating 50 uL and we want around 100 colonies/cells
10. To do this first dilute the sample so that it is 1x10^6 cells/mL then do a 1:50 dilution and then a 1:10 dilution

Item 3: Arabinose induction

Note we are only adding arabinose to the bacteria and will do western blot tomorrow

1. Select colonies from FDH and TMADH and leave in 4 mL of LB-Amp O/N
2. Label two sets of 4 tubes with 5 mL of bacteria culture and shake at 37C for 4 hrs

3. Perform a serial dilution of arabinose as follows

Tube	Stock Concentration (after serial dilution)	How much to add to each tube	Final concentration
1	10%	50 uL	0.2%
2	1%	50 uL	0.02%
3	0.1%	50 uL	0.002%
4	0%	50 uL	0%

1. Add arabinose and leave in shaker at 37C for 4 hrs
2. Take 1 mL of the bacteria culture and spin at max speed for 1 min, aspirate supernatant, (freeze pellet at -20C for long term storage)

Observations:

- When plating the bacteria, make sure to invert them so that the condensation doesn't drip onto the bacteria
- Write the labeling on the bottom of the plate so it is easier to see
- OD600 of 1.0= 8x10^8 cells/mL

Week 11 (Sol 43) 9/10/19

To do today:

1. Try doing a streak test (bc the plates from yesterday didn't have anything)
2. Make more TMA plates
3. Western blot arabinose induction product form yesterday

Procedures:

Item 1: Streak test

note we just streaked on top of the plates that we used yesterday

Item 3: Western blot

1. SDS-PAGE
    1. Dilute 4x Sample buffer to 1x as follows
        1. 225 uL of Sample Buffer
        2. 675 uL of H2O
        3. 22.5 uL of Protein 2ME
    2. Resuspend pellet in 1x Sample buffer
    3. Heat at 95C for 10 mins
    4. Put on ice for 5 mins
    5. Load gel
        1. We used two gels (two replicates) each gel as the wells as follows:
            1. 10 uL of protein marker
            2. 20 uL of sample buffer (blank)
            3. 20 uL of 10% arab TMADH
            4. 20 uL of 1% arab TMADH
            5. 20 uL of 0.1% arab TMADH

6. 20 uL of 0% arab TMADH
7. 20 uL of 10% arab FDH
8. 20 uL of 1% arab FDH
9. 20 uL of 0.1% arab FDH
10. 20 uL of 0% arab FDH

6. Run the gel at 150 V for 1 hr

2. Transfer
    1. Prepare the transfer box and transfer buffer
    2. Fill the transfer box with transfer buffer and soak the membranes (bc we have two) while preparing the gel
    3. Get another smaller box and put the frame in it with transfer buffer
        1. Put the black side down and put foam and then cardboard on each side
    4. Break the gel cast and carefully detach the SDS-PAGE gel from it's cast
    5. Put the membrane from the transfer box on top of the cardboard
    6. Slide the detached gel on top of the membrane
    7. Stack the other cardboard and foam on top for the gel and close the transfer Fram
    8. Repeat for both membranes/gels
    9. Slide the transfer frames into the transfer box, fill the box with transfer buffer
    10. Run transfer at 50V at 4C for 1 hr
3. Blotting

Data/Results:

Need to do western blot again, did initial staining with Ponceau S red, didn't see any protein

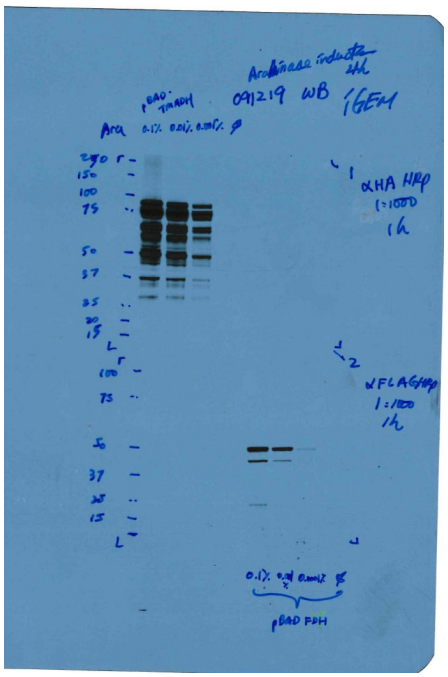
Week 12 (Sol 44) 9/16/19

To do today:

1. Sub culture for streak test tomorrow
2. Sub culture for mini prep (so we can extra DNA)
3. Make TMA plates

Data/Results:

Arabinose induction WB results:



Explanation:

The wells in both images are as follows

1. Protein ladder (marked)
2. 0.1% Arabinose TMADH
3. 0.01% Arabinose TMADH
4. 0.001% arabinose TMADH
5. 0% arabinose TMADH
6. 0.1% arabinose FDH
7. 0.01% arabinose FDH
8. 0.001% arabinose FDH
9. 0% arabinose FDH

The top image is the FDH and TMADH proteins on a membrane with anti-HA antibody. The bottom image is the FDH and TMADH membrane with anti-FLAG antibody.

Observation based on this result:

The top membrane shows many bands most likely because there was protein degradation. Ignoring this, the first three lanes show protein bands and if we measure the size of the top band it is correct to the predicted size of pBAD-TMADH protein. The bottom membrane just has two bands which is expected and is also the correct size for pBAD-FDH

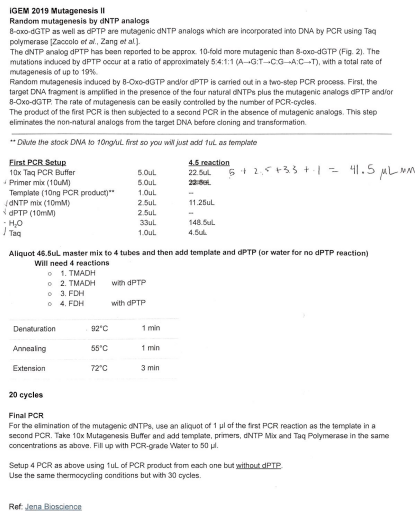
Week 12 (Sol 45) 9/17/19

To do today:

1. Miniprep sub culture from yesterday
2. Do error prone PCR with dPTP
3. Do streak test

Procedures:

Item 2: Error prone PCR with dPTP



iGEM 2019 Mutagenesis II
Random mutagenesis by dNTP analogs
8-oxo-dGTP as well as dPTP are mutagenic dNTP analogs which are incorporated into DNA by PCR using Taq polymerase [Zaccolo *et al.*, Zang *et al.*].
The dNTP analog dPTP has been reported to be approx. 10-fold more mutagenic than 8-oxo-dGTP (Fig. 2). The mutations induced by dPTP occur at a ratio of approximately 5:4:1:1 (A→G:T→C:G→A:C→T), with a total rate of mutagenesis of up to 19%.
Random mutagenesis induced by 8-Oxo-dGTP and/or dPTP is carried out in a two-step PCR process. First, the target DNA fragment is amplified in the presence of the four natural dNTPs plus the mutagenic analogs dPTP and/or 8-Oxo-dGTP. The rate of mutagenesis can be easily controlled by the number of PCR-cycles.
The product of the first PCR is then subjected to a second PCR in the absence of mutagenic analogs. This step eliminates the non-natural analogs from the target DNA before cloning and transformation.

*** Dilute the stock DNA to 10ng/uL first so you will just add 1uL as template*

First PCR Setup		4.5 reaction
10x Taq PCR Buffer	5.0uL	22.5uL
Primer mix (10uM)	5.0uL	~~22.5uL~~
Template (10ng PCR product)**	1.0uL	--
dNTP mix (10mM)	2.5uL	11.25uL
dPTP (10mM)	2.5uL	--
H_2O	33uL	148.5uL
Taq	1.0uL	4.5uL

5 + 2.5 + 33 + 1 = 41.5 μL rxn

Aliquot 46.5uL master mix to 4 tubes and then add template and dPTP (or water for no dPTP reaction)
Will need 4 reactions
- 1. TMADH
- 2. TMADH with dPTP
- 3. FDH
- 4. FDH with dPTP

Denaturation	92°C	1 min
Annealing	55°C	1 min
Extension	72°C	3 min

20 cycles

Final PCR
For the elimination of the mutagenic dNTPs, use an aliquot of 1 µl of the first PCR reaction as the template in a second PCR. Take 10x Mutagenesis Buffer and add template, primers, dNTP Mix and Taq Polymerase in the same concentrations as above. Fill up with PCR-grade Water to 50 µl.

Setup 4 PCR as above using 1uL of PCR product from each one but without dPTP.
Use the same thermocycling conditions but with 30 cycles.

Ref: Jena Bioscience

Observe that we are using dPTP instead of Mn2+ as it causes mutations at a higher level in DNA polymerase. Also it is important to note that we are using the full plasmid as the template instead of the usual insert only. It should speed up the process of everything.

(Original source: https://www.jenabioscience.com/molecular-biology/cloning-and-mutagenesis/random-mutagenesis-kits/pp-101-jbs-dntp-mutagenesis-kit)

Item 3: Do streak test

Week 12 (Sol 46) 9/18/19

To do today:

1. Gel purify Mutagenesis from yesterday
2. Send Miniprep for sequencing
3. Do PCR mutagenesis again

Procedures:

Item 2: Send for sequencing

Send 800 ng in a total volume of 10 uL

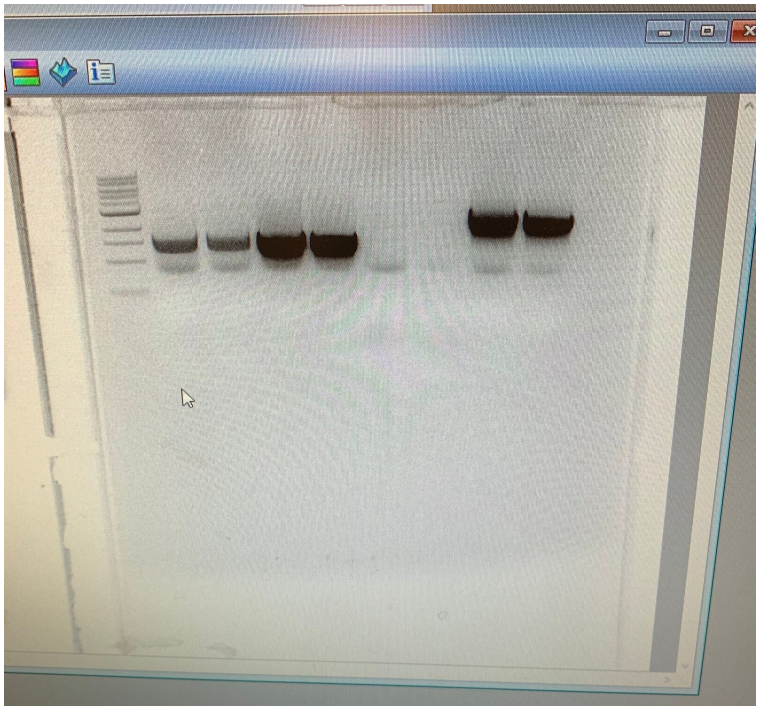
Item 3: Do PCR again

Explanation: so the results from item 1 show that PCR worked for the dPTP+ for FDH but not for TMADH. I hypothesize that the reason may be that the concentration of dTPT inhibits the PCR reaction somehow because even the FDH sample is fainter for the +dPTP sample. So for this one we did a serial dilution of dPTP so that the tubes are as follows:

1. 2.5 uL of dPTP
2. 2.0 uL of dPTP
3. 1.5 uL of dPTP
4. 1.0 uL of dPTP
5. 0.5 uL of dPTP
6. 0 uL of of dPTP

The volume of water added to each sample was adjusted from the original protocol so that the final volume in all tubes is still 50 uL.

Data/Results:



Explanation: the first lane is the DNA ladder, lanes 2-3 are FDH +, 4-5 FDH -, 6-7 TMADH+, 8-9 TMADH-. We ran the gel at 150 for 30 mins. As explained in the procedures, it is pretty clear that the +dPTP samples are fainter than the -dPTP samples

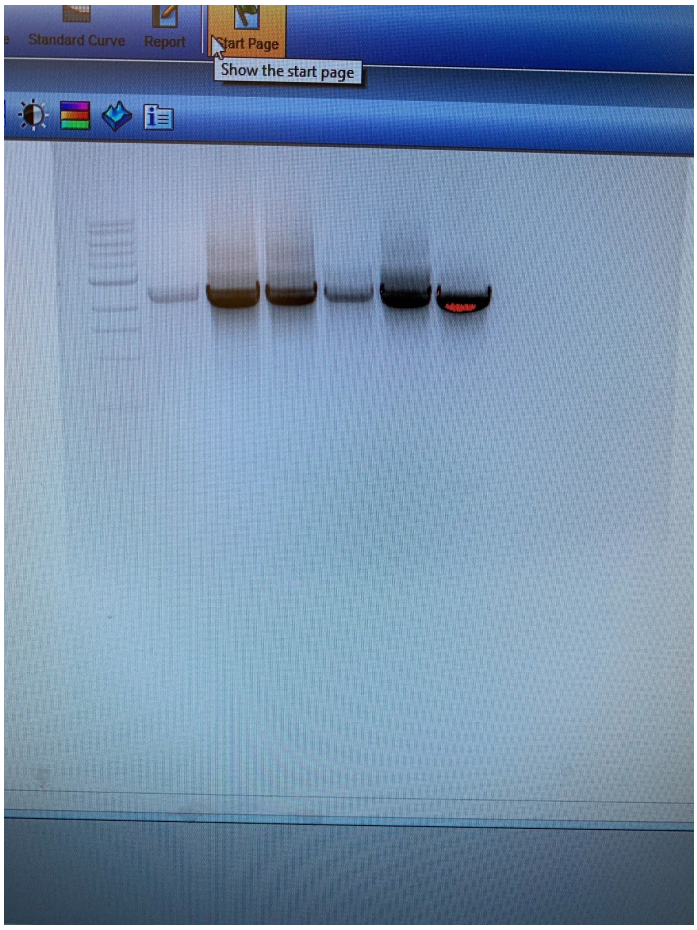
Week 12 (Sol 47) 9/19/19

To do today:

1. Run test gel of TMADH 1st PCR (testing dPTP concentration)
2. Quantify everything

Data/Results:

Item 1:



Week 12 (Sol 48) 9/20/19

To do today:

1. PCR Purify PCR product from yesterday
2. Run test gel
3. Send Mutagenesis PCR products for sequencing
4. Do 2nd PCR for FDH Mutagenesis

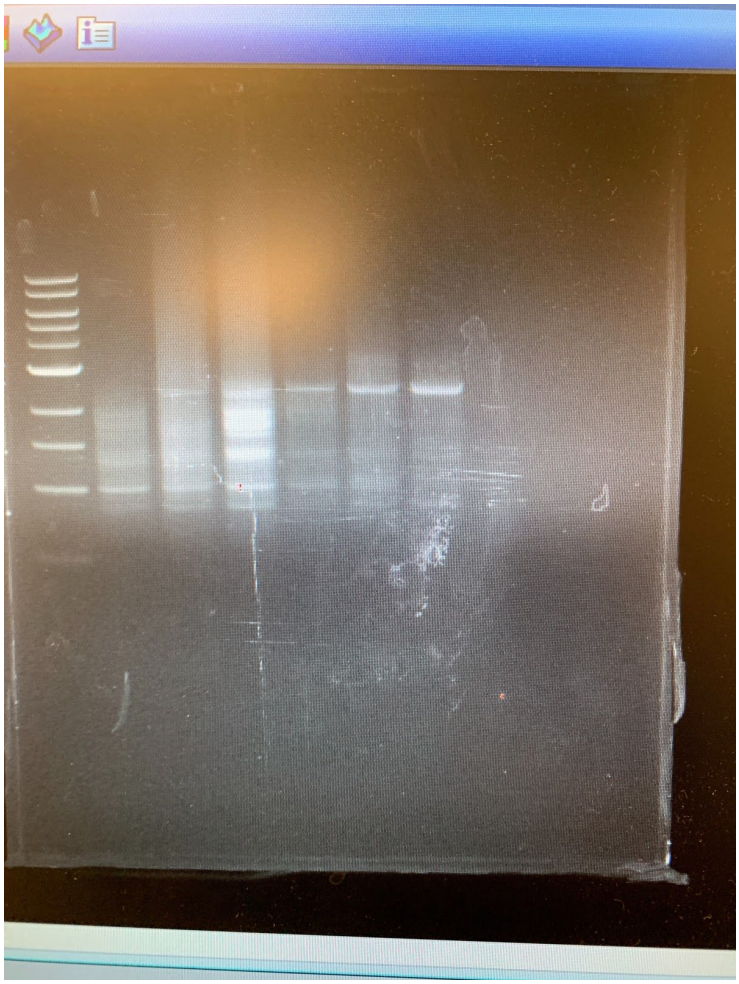
Procedures:

Item 1: PCR purification

REMEMBER TO ELUTE IN 30 uL NEXT TIME

Data/Results:

Item 2: Run test gel for the 2nd TMADH PCR product



Analysis:

This is the gel after PCR purification and after the 2nd PCR for TMADH. There are a lot of bands (obviously) and this could be because between the 2nd and 1st PCR we didn't do gel purification (only PCR purification) this could mean that there were non specific/trash DNA that was in the PCR product after the 1st PCR. We will do a test gel after we do the 2nd PCR for FDH

Week 12 (Sol 49) 9/21/19

To do today:

1. Run test gel for FDH 2nd PCR
2. PCR Purify 2nd FDH PCR product
3. Made TMA and HCHO Plates

Procedures:

Item 1: Run test gel
10 uL of 2x loading dye and 10 uL of PCR product. 150 V for 1 hr

Item 2: 2nd PCR for FDH PCR product
Note: don't add dPTP to ANY of the samples in the 2nd PCR, everything else can be the same (including the PCR conditions)

Item 3: TMA and HCHO plates
Make endo agar (without fuchsin, TMA, or anything) and send to autoclave
For TMA mix as follows into the melted agar:

1. 10 % Basic Fuchsin 300 uL
2. 10% Arabinose. 100 uL
3. TMA perform serial dilution and put 200 uL of each dilution
    1. 0.1% (Final)
        1. Take 333 uL from this
    2. Into 666 uL of H2O = 0.03% (final)
    3. Repeat until 0.001% and then have a 0% one

For HCHO plates mix as follows into the melted agar

1. 10% basic fuchsin 300 uL
2. 10% arabinose. 100 uL
3. 16% HCHO
    1. 31.25 uL (final= 0.03%)

2. 9.275 uL (final= 0.01%)
3. 3.125 uL. (Final= 0.003%)
4. 0 uL

Note: do not make more than two bottles at a time (otherwise the agar will solidify). Store the remaining bottles in the 50C waterbath until use and for long term storage leaving them on the bench is fine

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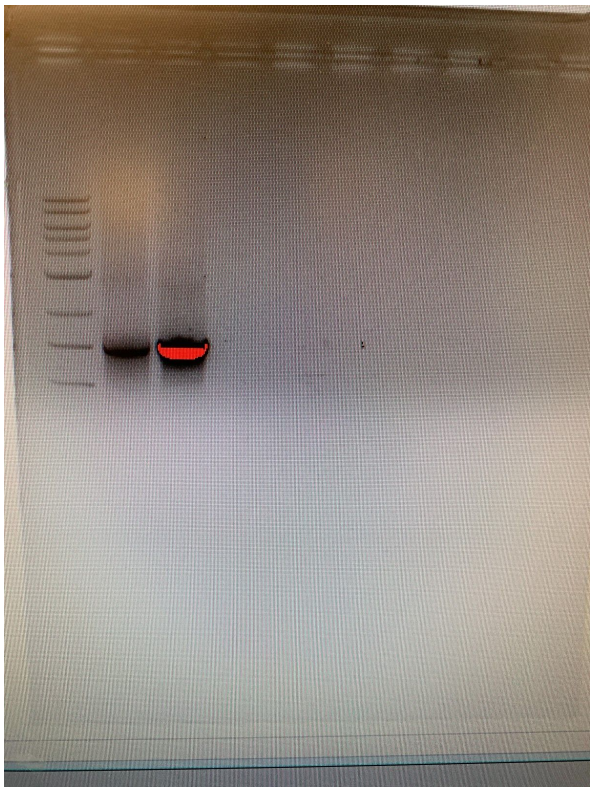
Data/Results:

Item 1: FDH 2nd PCR product test gel



Analysis: This is the gel of the FDH 2nd PCR product, which had a gel purification step between the 1st and the 2nd PCR. It is noteworthy to observe that this gel only has one band per lane, and each band is the approximately the size that we predict it to be. When compared to the results of the gel from the TMADH 2nd PCR product, we can conclude that performing gel extraction between each PCR will significantly decrease the amount of background we get.

Week 12 (Sol 50) 9/22/19

To do today:

1. Do PCR Mutagenesis on TMADH again
2. Run gel and gel extract from item 1
3. Start 2nd PCR for TMADH
4. Plate bacteria on TMA and HCHO plates (PLATE ASSAY)
5. Do liquid HCHO culture assay

Procedures:

Item 4: liquid HCHO culture

1. Make 50 mL of LB with 50 uL of Ampicillin and 1000 uL of arabinose
2. Aliquot 4 mL of this mix into one culture tube and 2 mL in six other culture tubes
3. Add 50 uL of 16% HCHO into tube 1 (4 mL)
    1. Final concentration of HCHO= 0.2%
4. Mix thoroughly by vortexing
5. Take 2 mL from this tube into the next tube
    1. Final concentration of HCHO= 0.1%
6. Repeat this process for tube 3-6
7. Don't add anything to tube 7 (0% HCHO control)
8. Add 5 uL of pBAD-FDH bacteria to each tube
9. Repeat the entire process and then add 5 uL of pBAD-D4 (negative control) to each tube
10. Incubate at 37C shaking for 16 hours

Data/Results:

Week 13 (Sol 51) 9/23/19

To do today:

1. Gel check TMADH
2. Redo PCR for TMADH (bc it didn't work)
3. Measure absorbance of FDH subculture
    1. Need to redo and change conditions

Procedures:

Item 2: PCR for TMADH

Set up two reactions one with TM of 46C and one with TM of 48C. Changed the extension time to 3 minutes for both of them

Item 3.1: Need to change FDH subculture conditions

1. Change the HCHO starting concentration to 0.1% and do a 10 fold serial dilution (there are four samples in total, three dilution and a 0% negative control)
2. Still add the same amount of ampicillin and LB to each tube
3. Changed the arabinose concentration to 0.05%

Week 13 (Sol 52) 9/24/19

To do today:

1. Measure OD600 of FDH liquid culture
2. Gel check for PCR retry from Sol 51
3. Make TMA and HCHO plates

Procedures and Observations:

Item 1: Measure OD600 for FDH liquid culture

This is the results of the overnight culture from Sol 51. We changed the conditions as the results of the first trial did not show much bacteria growth.

Item 3: Make TMA and HCHO plates

It is important to note that the concentration for arabinose has changed to 0.05%, this is based on the results of the Arabinose induction which showed that TMADH had slight over expression at 0.1% arabinose but normal expression at 0.01% while FDH had optimal expression at 0.1% it also had acceptable expression levels at 0.01%. Thus, we compromised the concentration to be 0.05% so that the same concentration of arabinose could be applied to both samples during testing. It is also important to note that the qualifiers that distinguished “normal,” “optimal,” and “over” expression is based on qualitative observation of the brightness of the bands on the western blot membrane. Store them in -4C O/N

Data/Results:

Item 1: Measuring OD600

% of HCHO	0.2	0.1	0.05	0.025	0.0125	0
OD600 FDH	0.082	0.077	0.08	0.079	0.081	1.443
OD600 pBAD	0.079	0.077	0.079	0.079	0.077	1.257

Analysis: Based on these results, both FDH (the test group) and pBAD (the negative control) bacteria have a hard time surviving in the presence of formaldehyde at any concentration. Some possible reasons could be that the concentration of bacteria is too low in comparison to the concentration of formaldehyde or the concentration of arabinose is too high causing the bacteria to react negatively. We will retry with a lower concentration of arabinose.

Item 2: Gel check for PCR

No bands yet again…

Although, it is most likely due to human error, we will retry again tomorrow.

Week 13 (Sol 53) 9/25/19

To do today:

1. Measure OD600 of FDH liquid culture Trial 2
2. Set up PCR again
3. Plate bacteria

Procedures:

Item 1: New conditions used for FDH liquid culture (set up was done on Sol 52)

1. Mix 50 mL of LB, 50 uL of Ampicillin, and 250 uL of 10% arabinose (final concentration= 0.05%)
2. Aliquot this mix into four tubes as follows
    1. 2.2 mL
    2. 1.8 mL
    3. 1.8 mL
    4. 1.8 mL
3. Add 25 uL of 16% HCHO to the first tube (final [HCHO]= 0.1%)
4. Mix by vortexing
5. Take 200 uL from Tube 1 and add to tube 2 (final [HCHO]= 0.01%)
6. Mix by vortexing
7. Take 200 uL from Tube 2 and add to tube 3 (final [HCHO]= 0.001%)
8. Mix by vortexing
9. Add 200 uL of LB to tube 4 (final [HCHO]= 0%)
10. Add 5 uL of FDH culture to each tube
11. Repeat steps 2-10 for pBAD sub culture
12. Incubate at 37C shaking O/N

Item 3: Plate bacteria

Plate 50 uL of bacteria from subculture of confirmed clones. Be sure to keep hand with cell spreader stationary and rotate the plate itself to spread the bacteria instead of using the cell spreader hand alone to spread the bacteria.

Data/Results:

Item 1: Measure OD600 of FDH liquid culture Trial 2

% HCHO	0.1	0.01	0.001	0
FDH	0.081	0.08	1.499	1.38
pBAD	0.08	0.079	1.497	1.503

Analysis: We see the same results here even after adjusting the concentration of arabinose and widening the serial dilution range to allow for more room for the bacteria to grow. Potential explanations for this could be that when the HCHO is distributed in the solution, especially at such a small volume, it can reach a higher concentration of bacteria thus making it harder for the bacteria to survive. This explanation is kind of shaky though, so the results of these tests can confidently be rules as inconclusive.

Week 13 (Sol 54) 9/26/19

To do today:

1. Set up 2nd PCR for TMADH
2. Collect results for plates from Sol 52

Procedures:

Item 1: Set up 2nd PCR

For the first PCR we tested two conditions (two TMs) 46C and 48C, the results of the gel check (shown in results) showed that both conditions worked but the band for 48C was slightly brighter so we gel purified the samples form 48C and proceeded to do the 2nd PCR with this new conditions. All other set up is the same.

Data/Results:

Item 1: Gel from 1st PCR

Well 1: NEB 1kb marker
Well 2-3: 46C TMADH +dPTP
Well 4-5: 46C TMADH -dPTP
Well 6-7: 48C TMADH +dPTP
Well 8: 48C TMADH -dPTP

Item 2: Plate Results
See below for all plate assay results:

https://docs.google.com/presentation/d/1l_rBsvSjkIVJHjWFIqzF08ySdI4rpZMSan4D_nVfcGY/edit?usp=sharing

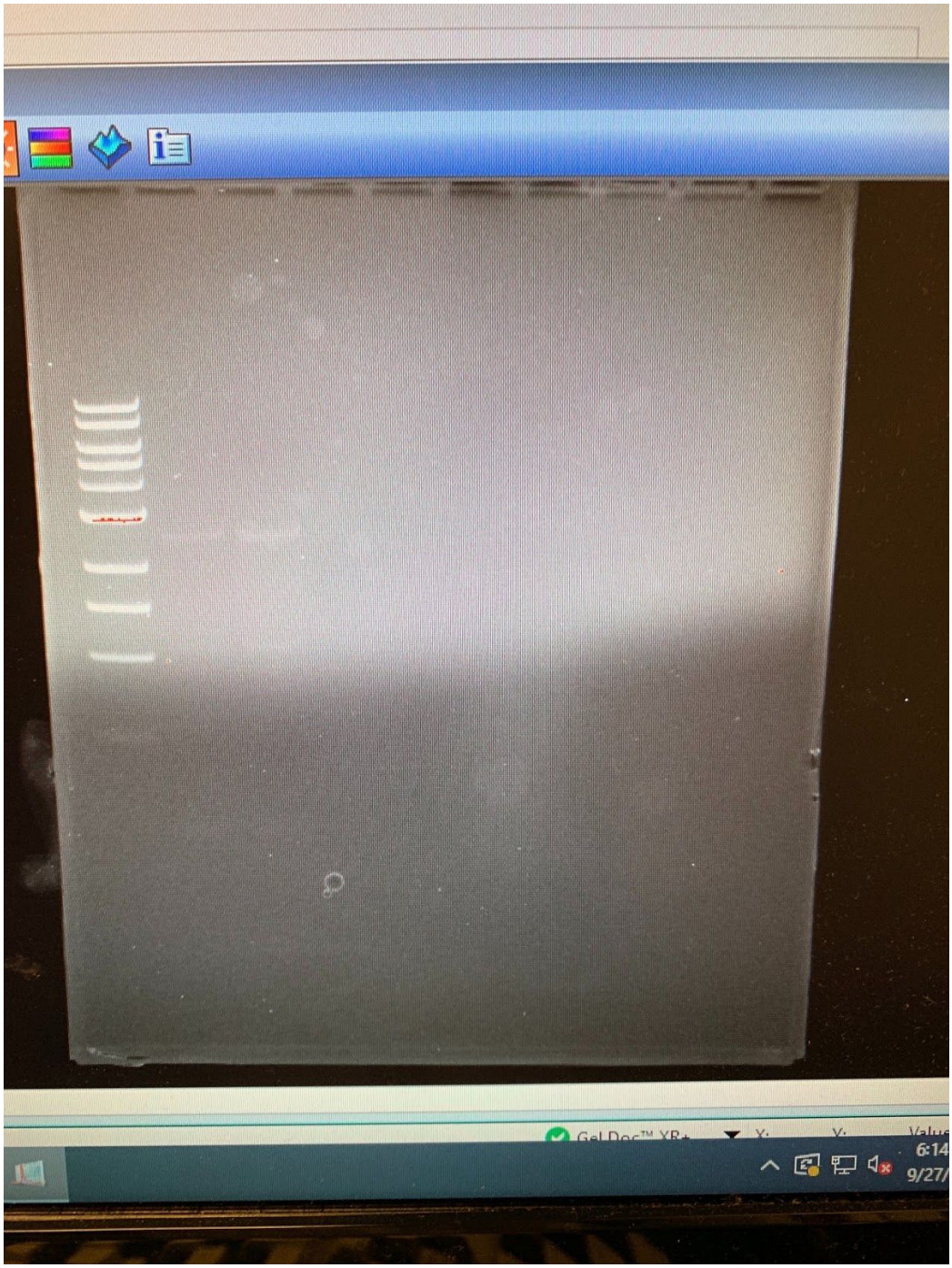
Week 13 (Sol 55) 9/27/19

To do today:

1. Re made plates (making sure to perfect everything)
2. Gel for second PCR

Data/Results:

Item 2: Gel for 2nd PCR



Well 1: 1kb DNA ladder
Well 2: TMADH +dPTP
Well 3: TMADH -dPTP
Analysis: it is really hard to see but there are faint bands at the correct size at both wells 2 and 3 showing that the PCR was successful

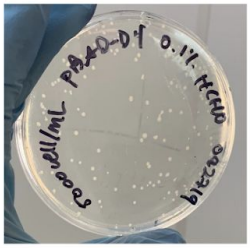
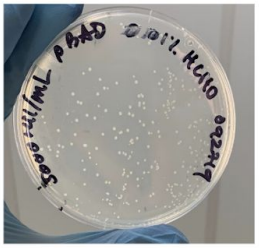
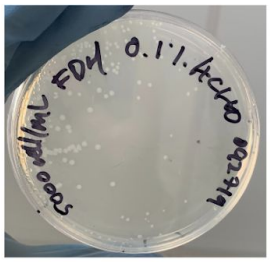
Week 13 (Sol 57) 9/29/19

To do today:

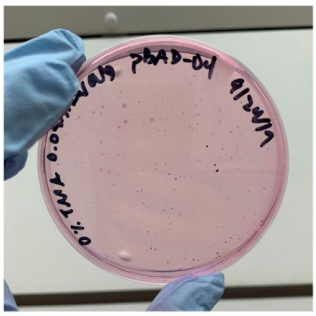
1. Collect results for TMADH plate assay

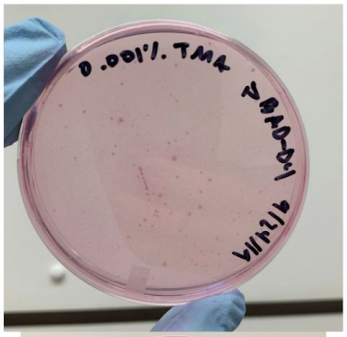
Data/results:

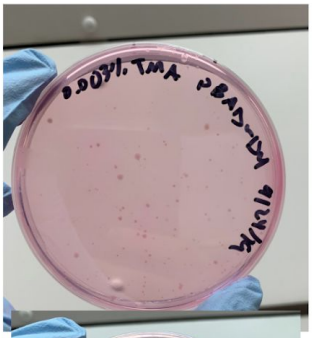
Bio Assay: FA Culture Plate

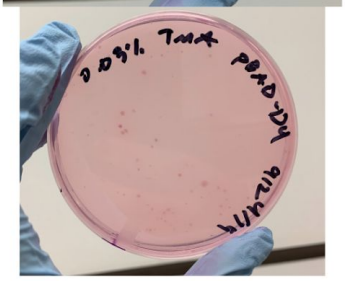
	No FA	0.1% FA	0.01% FA
-FDH			
+FDH			

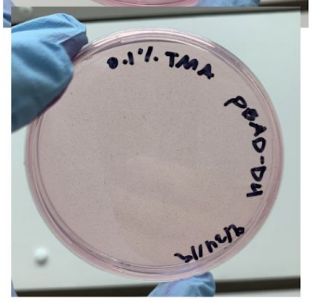
pBAD-D4











pBAD-TMADH