Jeffrey Chuong 8/22 - 8/30

MONDAY, 8/22/2022

MIC Experiments for *acrB* and *pbpG* knockouts

Make stocks of Carbenicillin at concentrations (mg/mL): 0, 2, 4, 8, 16, 32, 64, 128

- 3.2g of Carb powder in 25 mL 50% ethanol (50 mL conical), push syringe through 0.2 uM filter into 50 mL conical
- 2x Serial Dilutions starting from 128 mg/mL fo 2 mg/mL in 1.7 microcentrifuge tubes, 1000x concentrations
- store at -20C

Expected MIC for *acrB*: 8 ug/mL

Expected MIC for *pbpG*: 8 ug/mL

TUESDAY, 8/23/2022

MIC Experiments for *acrB* and *pbpG* knockouts

9 culture tubes for each Carb concentration (8x)

- *acrB* strain, *pbpG* strain in technical triplicates, *E. coli* negative control, ADP1-ISx positive control in duplicates
- Expected growth in ISx at all concentrations
- Expected growth in *E. coli* in low concentrations?
- Expected growth in *acrB*, *pbpG* strains at 0, 2, 4, 8 ug/mL, Expected no growth at 16, 32, 64, 128
- need clarification on triplicates

Confirm *P. destructans* detector genome via PCR

2049 Up + Pd 3a Up (1300+500) (1:00 extension time)

- Primers 001 and 064, need new primer pairs because these have too big of a difference in melting temperature

Repressor + Pd 3b + 2049 Down (1000 + 500 + 1200) (1:30 extension time)

- Primers 004 and 071, need new primer pairs because these have too big of a difference in melting temperature

Ran a PCR to confirm Repressor Integration

- Primers 071 and 072, 61 temperature, 1kb (0:30 extension)

SATURDAY, 8/27/2022

MIC Experiments for *acrB* and *pbpG* knockouts

Day 0

- Streak out frozen stocks onto LB plates
  - *acrB* plate, *pbpG* plate, ISx plate
- *E. coli* MG1655 will be used as a negative control - directly from frozen stock
- Prepare 9 culture tubes for each Carb concentration (8x, 72 tubes total)

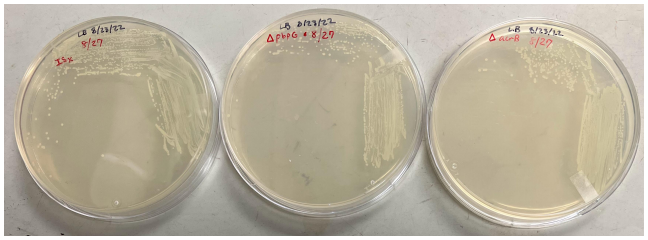
SUNDAY, 8/28/2022

MIC Experiments for *acrB* and *pbpG* knockouts

Day 1

- Add 5 mL of LB to each culture tube with appropiate antibiotic
- Pick 3 colonies from *acrB* plate, 3 colonies from *pbpG* plate, and 2 colonies from ISx plate
- Dilute entire colony in 1 mL sterile saline
- Add 2 ul of saline mixture to appropiate culture tubes
- Inoculate frozen *E. coli* MG1655 stocks into appropiate culture tubes

8-28-22 ISx, pbpG KO, acrB KO streaks.jpg



- Grow at 30C for 16 hours (Start: 6 PM)

MONDAY, 8/29/2022

MIC Experiments for *acrB* and *pbpG* knockouts

Day 1

- Add 5 mL of LB to each culture tube with appropiate antibiotic
- Pick 3 colonies from *acrB* plate, 3 colonies from *pbpG* plate, and 2 colonies from ISx plate
- Dilute entire colony in 1 mL sterile saline
- Add 2 ul of saline mixture to appropiate culture tubes
- Inoculate frozen *E. coli* MG1655 stocks into appropiate culture tubes
- Grow at 30C for 16 hours (Start: 6 PM)

Day 2

- Take pictures of culture tubes
- Measure growth after 16 hours (End: 10 AM) using plate reader

10 AM

- Concerns: No growth in ISx starting from Carb 16 ug/mL to 128 ug/mL, *acrB* KO has no growth in all Carb concentrations (2-128 ug/mL)
- Expected: Growth in ISx up to 64 ug/mL or 128 ug/mL, *acrB* KO growth in 2 and 4 ug/mL
- *pbpG* and *E. coli* look great
- Identical results across duplicates, triplicates

7 PM

- Stored 200 ul in microcentrifuge tubes because Cameron was using plate reader, kept all tubes in 4C
- Will use plate reader on these samples tomorrow

Order: -LBcontrol, 128, 64, 32, 16, 8, 4, 2, LB
1-9: E. coli
10-18: ISx A
19-27: ISx B
28-36: pbpG A
37-45: pbpG B
46-54: pbpG C
55-63: acrB A
64-72: acrB B
73-81: acrB C

https://docs.google.com/presentation/d/1DMf4JC8ewZmKfi7BRXG0J0NyYOIq2JyercZi5IrdfOs/edit?usp=sharing

TUESDAY, 8/30/2022

MIC Experiments for *acrB* and *pbpG* knockouts

Day 0

- Streak out frozen stocks onto LB plates
    - *acrB* plate, *pbpG* plate, ISx plate
- *E. coli* MG1655 will be used as a negative control - directly from frozen stock
- Prepare 9 culture tubes for each Carb concentration (8x, 72 tubes total)

Day 1

- Add 5 mL of LB to each culture tube with appropiate antibiotic
- Pick 3 colonies from *acrB* plate, 3 colonies from *pbpG* plate, and 2 colonies from ISx plate
- Dilute entire colony in 1 mL sterile saline
- Add 2 ul of saline mixture to appropiate culture tubes
- Inoculate frozen *E. coli* MG1655 stocks into appropiate culture tubes
- Grow at 30C for 16 hours (Start: 6 PM)

Day 2

- Take pictures of culture tubes
- Measure growth after 16 hours (End: 10 AM) using plate reader

Day 3

- Measure samples (taken after 24h, kept in 4C O/N) on Plate Reader

Future direction: Test WT ADP1 vs. ISx with Carb stocks and maybe *E. coli*