Jeffrey Chuong 9/3 - 9/10

FRIDAY, 9/2/2022

Primer Design for PCRs

SATURDAY, 9/3/2022

PCRs for 029 *P. destructans* Detector Strain

029 purified gDNA - purified from 3 different frozen strains (029A, 029B, 029C)
Tested in duplicates
Order: -control, A, A, B, B, C, C
Used 57C Annealing Temperature and 1:00 Extension Time for all reactions

Primer Pairs

077 and 078: 450 + 1072 + 110 = 1632 bp, 1:00 extension, annealing temp: 57C
079 and 080: 280 + 1675 + 175 = 2130 bp, 1:00 extension, annealing temp: 56C

- 079 binds to two places in genome (Target Sequence 3a, Target Sequence 3b)

081 and 082: 136 + 876 + 109 = 1121 bp, 0:30 extension, annealing temp: 57C
083 and 084: 71 + 1093 + 99 = 1250 bp, 0:30 extension, annealing temp: 56C

Results: https://docs.google.com/presentation/d/13NmDu4dU6AEPlgSL9_aBgP_vsylHMcMhP9M4xZQMVa4/edit?usp=sharing
Dr. Barrick - The 1st, 3rd, and 4th look reasonable to me. (Not sure why there are two bands in the 1st and the band in the control may mean there is some contamination of your stocks with gDNA or previous PCR products.)

If the upstream and downstream homologies have some internal repeats and match one another, then it may be impossible to get a good PCR product for anything that crosses both of them. Changing PCR conditions can't fix that.

So, I wouldn't redo anything. If it is Kan resistant and AZT sensitive, this seems like enough evidence that all of the parts are probably there. If it works in the mock transformation with the WNS fungus virus sequence that you make, then it's probably good to go, and then we can sequence the genome to give more definitive results than PCR will ever give.

MONDAY, 9/5/2022

Take 2 colonies of ADP1-WT, grow in LB, make glycerol stocks tomorrow
YFP Fluorescence Experiment
Day 0

- Streak out frozen stocks onto LB plates, LB-Kan plate
  - YFP only (021), YFP with cymR repressor (029) (LB-Kan), ISx, *E. coli* MG1655, *E. coli* with YFP plasmid (024)
- Prepare 3 culture tubes for each strain (biological triplicates, 15 total tubes)

TUESDAY, 9/6/2022

Make glycerol stocks of ADP1-WT (https://barricklab.org/twiki/bin/view/Lab/ProtocolsFreezingStrains)
#39 and 40 in iGEM -80 Rack

YFP Fluorescence Experiment
Day 1

- Add 5 mL of LB to each culture tube with appropiate antibiotic
- only plate, 3 colonies from YFP with cymR repressor plate, 3 colonies from *E. coli* with YFP
  ISx plate, 3 colonies from *E. coli*

- Dilute entire colony in 1 mL sterile saline
- Add 2 ul of saline mixture to appropiate culture tubes
- Grow at 30C for 16-24 hours (Start: 8:00 PM)

WEDNESDAY, 9/7/2022

YFP Fluorescence Experiment

Day 1

- Add 5 mL of LB to each culture tube with appropiate antibiotic
- Pick 3 colonies from YFP only plate, 3 colonies from YFP with cymR repressor plate, 3 colonies from *E. coli* with YFP plasmid, 3 colonies from ISx plate, 3 colonies from *E. coli*
- Dilute entire colony in 1 mL sterile saline
- Add 2 ul of saline mixture to appropiate culture tubes
- Grow at 30C O/N (Start: 8:00 PM)

Day 2

- Take pictures of culture tubes
- Add 200 ul of each overnight culture into a 96 well plate
- Measure YFP fluorescence of samples (End: 11 AM) on plate reader
    - results unclear...taking 200 ul to store in microcentrifuges O/N at 4C at 8 PM (after 24 hours of growth)
    - small colonies take longer to grow

MIC Experiments for ISx vs. ADP1-WT

Day 0

- Streak out frozen stocks onto LB plates
    - *E. coli* plate, ISx plate, ADP1-WT plate, Δ*pbpG* plate
- *E. coli* MG1655 will be used as a negative control'
- Δ*pbpG* negative control
- Prepare 9 culture tubes for each strain (8x 9, 72 total culture tubes)
    - -LB, 0, 2, 4, 8, 16, 32, 64, 128

THURSDAY, 9/8/2022

YFP Fluorescence Experiment

Day 2

- Take pictures of culture tubes
- Add 200 ul of each overnight culture into a 96 well plate
- Measure YFP fluorescence of samples (End: 11 AM) on plate reader
    - taking 200 ul to store in microcentrifuges O/N at 4C at 8 PM (after 24 hours of growth)
    - taking 200 ul to store in microcentrifuges for ≈5 hours at 4C at 10 AM (after 36 hours of growth)
    - small colonies take longer to grow
    - looks like our 029 repressor strain has a lot more YFP product vs. 021 YFP only (repressor does not work)

Plating 3 colonies on LB-AZT plates

- If 029 strain is correct with *tdk/kan*, we expect no growth on LB-AZT
- Hopefully this technique works, first time transferring a colony with a inoculating loop to another plate

Plating 1 colony of pAJM.712 on LB-AZT - should grow

MIC Experiments for ISx vs. ADP1-WT

Pause today, keep plates in 4C O/N, continue tomorrow

FRIDAY, 9/9/2022

MIC Experiments for ISx vs. ADP1-WT

Day 1

- Add 5 mL of LB to each culture tube with appropiate antibiotics
- Pick 3 colonies from ISx plate, 3 colonies from ADP1-WT plate, and 1 colony from *E. coli*, 1 colony from Δ*pbpG*
- Dilute entire colony in 1 mL sterile saline
- Add 2 ul of saline mixture to each culture tube
- Grow at 30C (Start: 10 PM)

Kanamycin Regulating *Acr* Operon Experiment

Hypothesis: Kanamycin is repressing acrR, which represses the promoter that activates the *acr* operon. Kanamycin is actually inducing YFP, which is in the place of *acrB*.

- Prepare 7 culture tubes (-LB ctrl, duplicates of LB only, LB-KAN, LB-intermediate KAN)
    - LB-KAN - 5 ul of 1000x 50 mg/mL KAN stock (final concentration: 50 ug/mL KAN)
    - LB-intermediate KAN - 2.5 ul of 1000x 50 mg/mL KAN stock (final concentration: 25 ug/mL KAN)
- Pick a colony from 029 LB-KAN plate
- Dilute entire colony in 1 mL sterile saline.
- Add 2 ul of saline mixture to each culture tube
- Grow at 30C (Start: 11 PM)

Confirmation of fluorescence levels in YFP only strain and detector strain

Streak 021A and 021B glycerol stocks onto ONE LB plate and onto ONE LB-KAN plate (half/half)

Streak 029A, 029B, and 029C glycerol stocks onto ONE LB plate and ONE LB-KAN plate (split into 3s)

SATURDAY, 9/10/2022

MIC Experiments for ISx vs. ADP1-WT

Day 2

- Take pictures of culture tubes
- Measure growth after 24 hours using plate reader

Results: https://docs.google.com/presentation/d/1-w30C0BjGEkx4gX4OVd-eMAX3nemJE6Df-utaTn5z_U/edit?usp=sharing

- Seems to be no difference in MIC between ISx and WT

Kanamycin Regulating *Acr* Operon Experiment

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YFP Experiments: https://docs.google.com/presentation/d/10s_i8qzLAoM-7sqrr5vgoXhlhq7VXxqBPyv1pMOxI_Q/edit?usp=sharing