Summer 2022 Week 6

WEDNESDAY, 7/6/2022

AcrB Amp MIC

- Plated E.Coli, ISx and AcrB deletion
- Follow Up-
    - Innoculate 3 ISx colonies
    - Innoculate 3 AcrB Colonies
    - Innoculate 1 E. Coli Colony

LB dilutions

Tem-1 Gene Detector

- Run a PCR of the AcrB Homologies
- Upstream
        - [x] 93 uL water
        - [x] 30 uL Phusion buffer
        - [x] 7.5 uL 027
        - [x] 7.5 uL AcrB Up Forward
        - [x] 4.5 uL DMSO
        - [x] 3 uL mM dNTP's
        - [x] 1.5 uL Phusion polymerase
    - NEB = 65°C
    - Run at 62°C
- Downstream
        - [x] 93 uL water
        - [x] 30 uL Phusion buffer
        - [x] 7.5 uL 029
        - [x] 7.5 uL AcrB Down Revers
        - [x] 4.5 uL DMSO
        - [x] 3 uL mM dNTP's
        - [x] 1.5 uL Phusion polymerase
    - NEB = 66°C
    - Run at 62°C

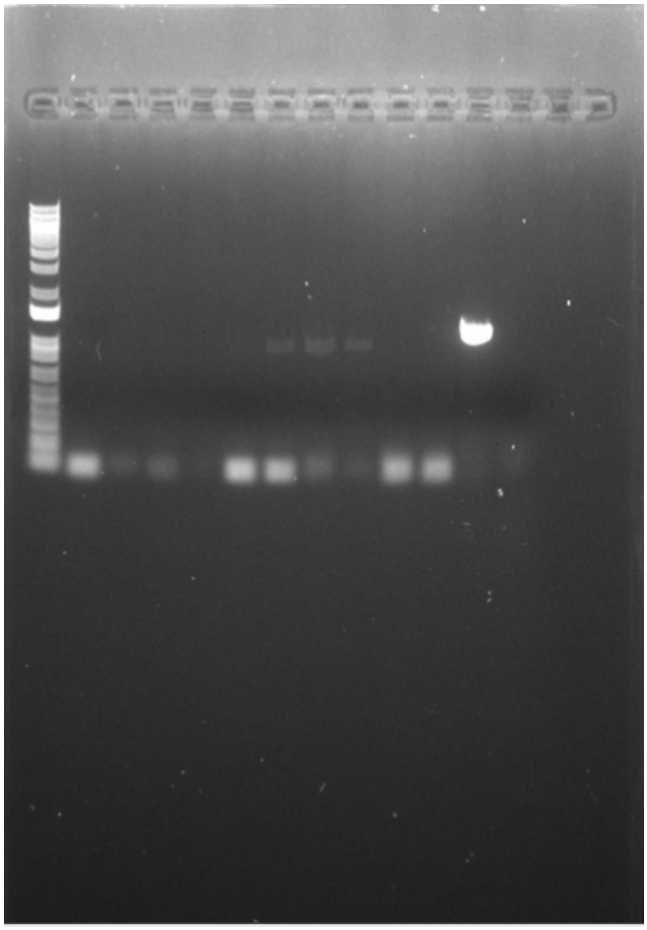
NPTII Gene Detector

- Run a PCR of the 2049 Homologies
- Upstream
        - [x] 93 uL water
        - [x] 30 uL Phusion buffer
        - [x] 7.5 uL 001
        - [x] 7.5 uL 025
        - [x] 4.5 uL DMSO
        - [x] 3 uL mM dNTP's
        - [x] 1.5 uL Phusion polymerase
    - NEB= 59°C
    - Run at 59
- Downstream
        - [x] 93 uL water
        - [x] 30 uL Phusion buffer
        - [x] 7.5 uL 026

- [x] 7.5 uL 004
- [x] 4.5 uL DMSO
- [x] 3 uL mM dNTP's
- [x] 1.5 uL Phusion polymerase

    - NEB = 60°C
    - Run at 60°C
- Run a gel to determine success of all PCR's
- Gel Order: Ladder-CAU-CAD-C2U-C2D-AcrU-AcrU-AcrD-AcrD-2049U-2049U-2049D-2049D

Screen Shot 2022-07-07 at 3.03.29 PM.png



- AcrB
    - Annealing temps were too high
        - Perhaps try a temperature gradient
- 2049
    - Annealing Temps were too high
        - Try running them at 56°C ?

THURSDAY, 7/7/2022

AcrB deletion-

- Innoculated 3 colonies of ADP1-Isx
- Innoculated 3 colonies of ADP1 AcrB deletion
- Innoculated 1 colony of E. Coli

- Follow Up-
  - Add 2 uL of each stock to the following dilutions
    - 128 ug/mL
    - 64 ug/mL
    - 32 ug/mL
    - 16 ug/mL
    - 8 ug/mL
    - 4 ug/mL
    - 2 ug/mL
    - 0 ug/mL
- Set Up

Tem-1 Gene Detector

- Follow Up- Run a PCR of the AcrB homologies against a temperature gradient?
  - Run at 60° and 64° ?

NPTII Gene Detector

- Run a PCR of the 2049 Homologies
- Upstream
  - [x] 93 uL water
  - [x] 30 uL Phusion buffer
  - [x] 7.5 uL 001
  - [x] 7.5 uL 025
  - [x] 4.5 uL DMSO
  - [x] 3 uL mM dNTP's
  - [x] 1.5 uL Phusion polymerase
  - NEB= 59°C
  - Run at 56°C
- Downstream
  - [x] 93 uL water
  - [x] 30 uL Phusion buffer
  - [x] 7.5 uL 026
  - [x] 7.5 uL 004
  - [x] 4.5 uL DMSO
  - [x] 3 uL mM dNTP's
  - [x] 1.5 uL Phusion polymerase
  - NEB = 60°C
  - Run at 56°C
- ~~Follow Up- Run a gel to determine success of 2049 PCR~~

Expected-

FRIDAY, 7/8/2022

AcrB deletion-

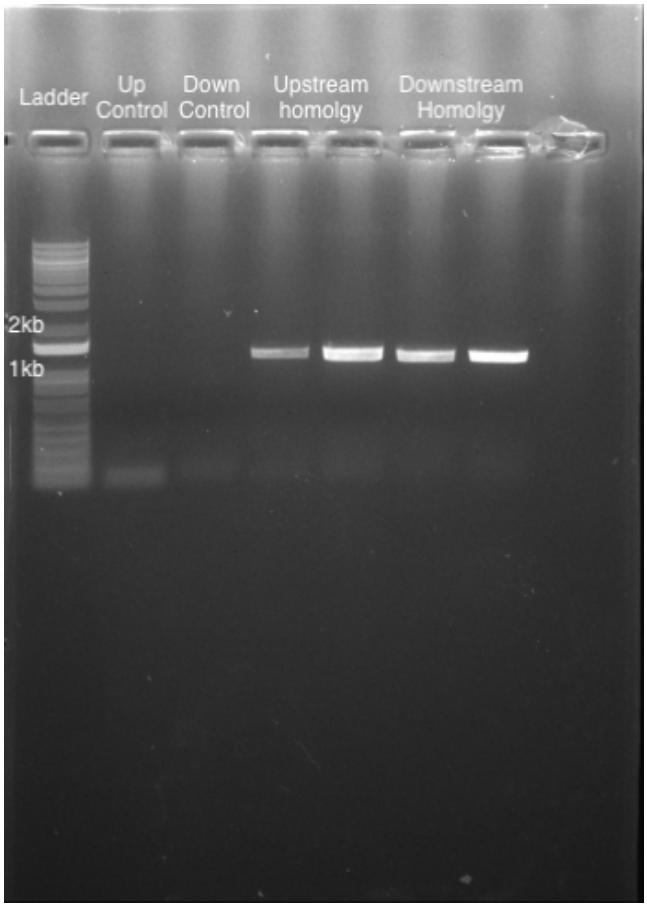
- ~~Follow Up-~~
  - ~~Put 200 uL of each dilution into the 96 well plate~~
  - ~~Run it in the plate reader-~~
    - OD Measures light scattering
      - High OD measurement = High scattering = Large amount of particles = turbidity
      - Low OD = Low Scattering = Low amt of particles = no turbidity

Tem-1 Gene Detector

NPTII Gene Detector

- Ran a gel to determine the success of the PCR
  - Expected Results
    - Upstream = 1266
    - Downstream 1213
  - Gel Order: Ladder-CU-CD-U-U-D-D

NPTII Detector Homologies.png



- The Gel looks really good!
- Clean and Concentrate the Product and start a GGA reaction
- GGA Reaction Conditions
  - [x] 10.8 uL water
  - [x] 2 uL T4 ligase Buffer
  - [x] 2 uL BsmbI mix
  - [x] 2 uL Upstream
  - [x] 1.3 uL ng Downstream
  - [x] 1.1 uL PCR 1
  - [x] .8 uL PCR 2
- Grew an overnight culture of 2049 deletion strain
- ~~Follow Up~~
  - ~~Run gel to determine if the expected product is seen~~
  - ~~Put 15 uL into culture tube w/ 500 uL LB and 35 uL overnight Culture~~

~~Follow Up- Cameron made a plate of the marionette strains, tomorrow I need to innoculate cultures of those~~

- Follow Up-
    - Plate +DNA and -DNA cultures onto AZT plates
        - 10^{-2} dilution of +DNA
        - Serial dilution of - DNA

Design More Mutations

NPTII (pKD13)

- 10 bp deletion @ site 1440-1449
- Proposed Deletions-
    - Mutate the catalytic site
    - 10 bp deletion at the catalytic site
    - premature stop codon at the catalytic site

Tem-1 (pBTK404)

- 10 bp deletion @ site 1312-1321
- Mutate Catalytic Serine from AGC -> CTC @ site 1112-1114
- Proposed Deletions-
    - Mutate one of the Leucines to a polar AA at site 1312-1321
    - Premature stop codon at site 1312-1321
    - 10 bp deletion of te catalytic site @1112-1114