Summer 2022 Week 4

MONDAY, 6/20/2022

10-15 min presentation

- Introduce detection of environmental DNA
- Show how adp1 is good for this
- Introduce the two examples of sntibiotic resistance and WNS
- Have an idea of how you want them to contact us

Experiment 1 (AcrB Deletion)

- Test the AcrB deletion's growth on Carb
  - Innoculate AcrB deletion Cultures
  - Innoculate a culture of AcrB on Carb
    - No growth in Carb means AcrB deletion does cause Carb sensitivity
  - Innoculate a culture of ISX on Carb
- Follow Up-
  - Check Carb Cultures To determine susceptability to Carb
    - ISX should gow and AcrB deletion should not
  - Run Whole Cell PCR of all three AcrB deletions w/ UF and DR primers

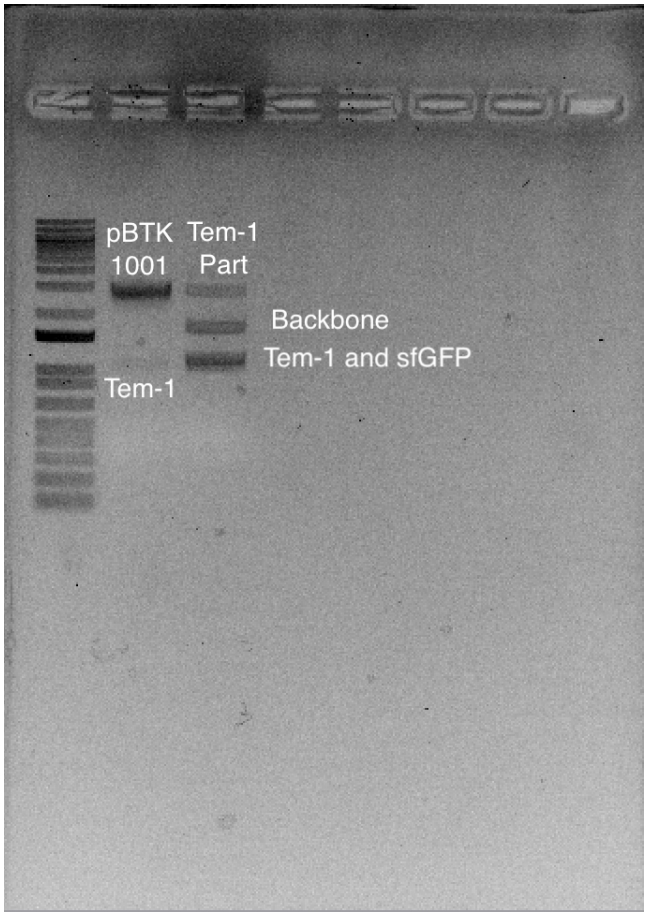
Experiment 2 (NPTII Gene Detector)

- Ran a PCR for NPTII
  - Tm of 58 and 61
- Follow Up- Run gel for PCR of NPTII Gene Detector

Experiment 3 (Tem-1 Gene Detector)

- Inserted Tem-1 PCR Product into pBTK1001 entry vector
- Reagants
  - [x] .80 uL of Tem-1?
  - [x] 2.58 uL ng pBTK1001
  - [x] 2uL T4 DNA Ligase buffer
  - [x] 1 uL BsmbI mix
  - [x] 13.6 uL
- Thermalcycler Conditions
  - 42°C for 5 min
  - 60°C for 5 min
- Ran a gel to confirm the product

Tem-1 Part Gel.png



- Follow Up- Transform the entry vector into NEB-5-α cells

TUESDAY, 6/21/2022

Experiment 1 (AcrB Deletion)

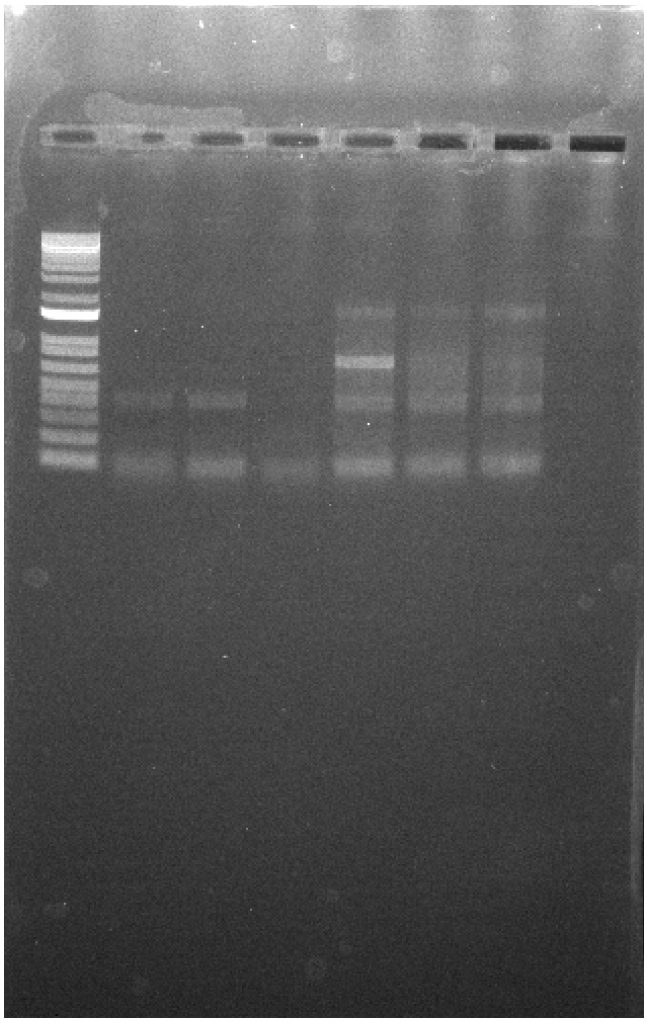
- Saw no growth in Carb for both ISX and AcrB deletion strains
- Whole Cel PCR
  - [x] 108 µL water
  - [x] 40 µL of Phusion HF buffer
  - [x] 10 µL Forward Primer
  - [x] 10 µl Rverse Primer
  - [x] 6 uL DMSO
  - [x] 4 µL of mM dNTPs
  - [x] 2 µL Phusion DNA polymerase
- Ran at 62°C
- Follow Up- Run a gel to see if it worked
  - Gel has been prepared for tomorrow
  - DID NOT WORK
- Innoculate Cultures for WCPCR tomorrow
  - Control
  - AcrB Deletion 1
  - Acrb Deletion 2
  - AcrB Deletion 3

    - ISX
    - tdk/kan insertion on Kan
- Follow Up- Purify the genome of all of these

Experiment 2 (NPTII Gene Detector)

- Ran a gel to determine success of the PCR
- Gel Order: Ladder-C58-C61-58-58-61-61

nptII Sai gel.png

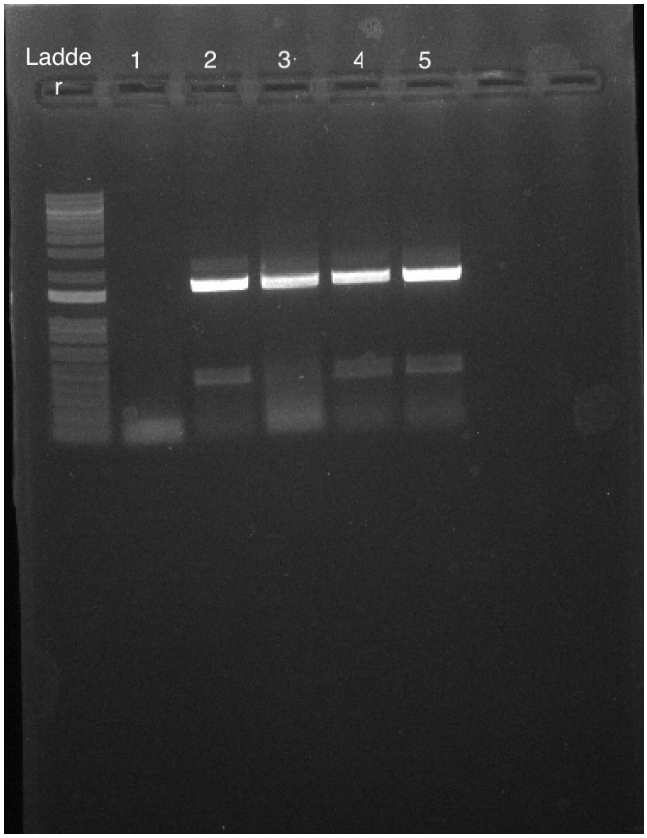


- Large amount of off target annealing so annealing temp seems to be too low.
    - At 61° I got the target but a lot of offtarget as well
    - Run a Temperature gradient from 61°-66°
- Ran a second PCR of NPTII
    - 63, 65°
        - [x] 155 uL water
        - [x] 50 uL Phusion buffer
        - [x] 12.5 uL Forward
        - [x] 12.5 uL Reverse
        - [x] 7.5 uL DMSO
        - [x] 5 uL mM dNTP's
        - [x] 2.5 uL Phusion polymerase

      ADD 1 uL TEMPLATE DNA AFTER PUT INTO PCR Tubes

- Ran a gel to determine the success of the PCR
- Gel Order: Ladder-C-63-63-65-65

Screen Shot 2022-06-21 at 5.54.03 PM.png



- Follow Up- Clean and Concentrate PCR products
- Ver Dramatic offtarget annealing

Experiment 3 Tem-1 Gene Detector

- Performed a transformation of Tem-1 Entry vector
- Plated 3 Tem-1 Entry Vector Cultures
  - 10 uL
  - 50 uL
  - Pellet
- Plated 3 BB190 Cultures
  - 10uL
  - 50uL
  - Pellet
- Follow Up- look at colonies under the light to find NON Fluorescent colonies
  - Select non fluorescent colonies to...
    - Run a miniprep
    - PCR
    - Glycerol Stock

WEDNESDAY, 6/22/2022

Experiment 1 (AcrB Deletion)

- Purified genome of AcrB Deletion, and tdk/kan insertion
- Run PCR of purified tdk/kan, Acrb Deletion and ISX
    - [x] 186 uL water
    - [x] 60 uL Phusion buffer
    - [x] 15 uL Forward
    - [x] 15 uL Reverse
    - [x] 9 uL DMSO
    - [x] 6 uL mM dNTP's
    - [x] 3 uL Phusion polymerase

  ADD 1 uL TEMPLATE DNA AFTER PUT INTO PCR Tubes
- Control, ISx, Tdk and AcrB 1 run at 60°
- AcrB 2 and AcrB 3 run at 62°C
- Follow Up- Run gel to determine correct bands

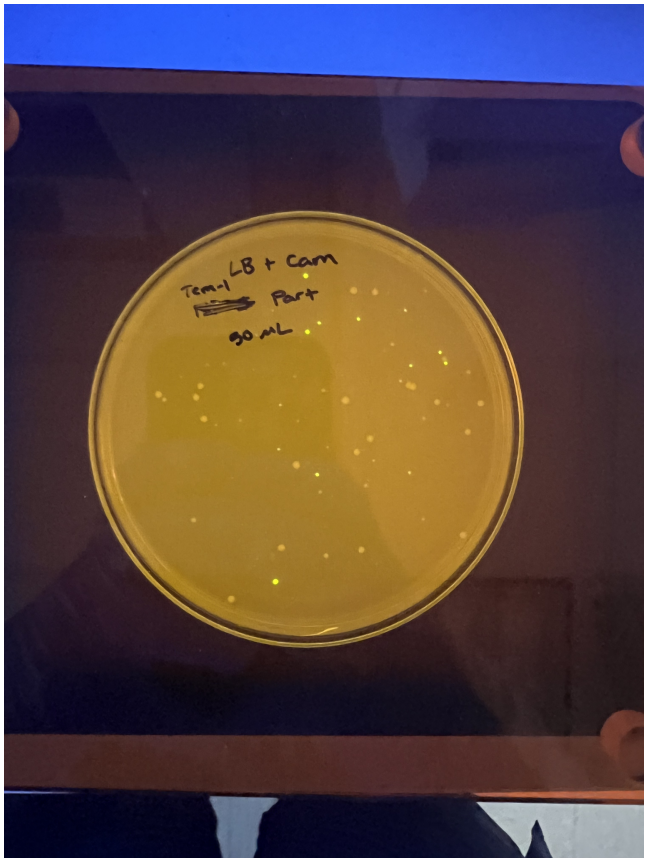
Experiment 2 NPTII Gene Detector

- Ran a GGA to make NPTII Part
- Reagants
    - [x] .53 uL of NPTII
    - [x] 1 uL ng pBTK1001
    - [x] 2uL T4 DNA Ligase buffer
    - [x] 1 uL BsmbI mix
    - [x] 15.5 uL water
- Reaction Conditions
    - 42°C 5 min
    - 60°C 5 min
- Follow Up- Run Gel to confirm correct Ligation
    - Transform part plasmid into NEB-5-α Cells

Experiment 3 Tem-1 Detector

- Growth was very minimal and I will check back tomorrow
- Follow Up- Check growth against Blue light

Tem-1 Part Culture.JPG



- Select non fluorescent colonies to Innoculate for...
  - A miniprep
  - PCR
  - Glycerol Stock

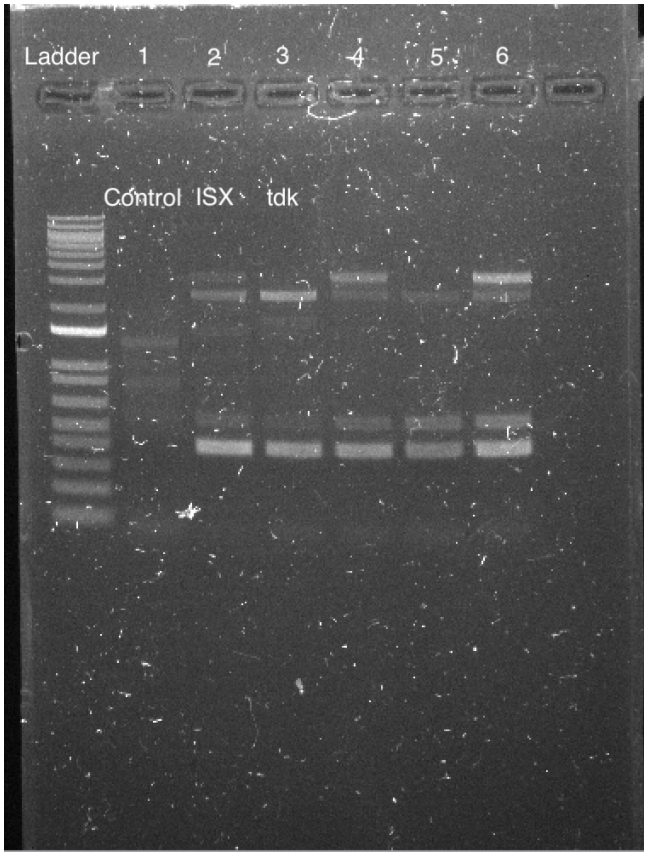
THURSDAY, 6/23/2022

Experiment 1 AcrB Deletion

- Run a Gel to determine if the correct sequences were inserted
- Order: Ladder-Control-ISX-TDK-AcrBD1-AcrBD2-AcrBD3
  - Expected Bands
    - ISX- 5191 bp
    - TDK- 1000+1011+1675 = 3686 bp
    - AcrB Deletions- 1000+1011 = 2011 bp

  30 SECONDS/KB

Deletion Confirmations.png



- Results:
  - Quite a few unwanted bands and offtarget annealing
  - Extension time was far too short, will run it again with a much longer extension time
  - Extension time should be 30 seconds/ kb so I will run it with a 3:00 extension time
- Ran a second PCR w/ 3:00 extension time
  Run PCR of purified tdk/kan, Acrb Deletion and ISX
  - [x] 186 uL water
  - [x] 60 uL Phusion buffer
  - [x] 15 uL Forward
  - [x] 15 uL Reverse
  - [x] 9 uL DMSO
  - [x] 6 uL mM dNTP's
  - [x] 3 uL Phusion polymerase

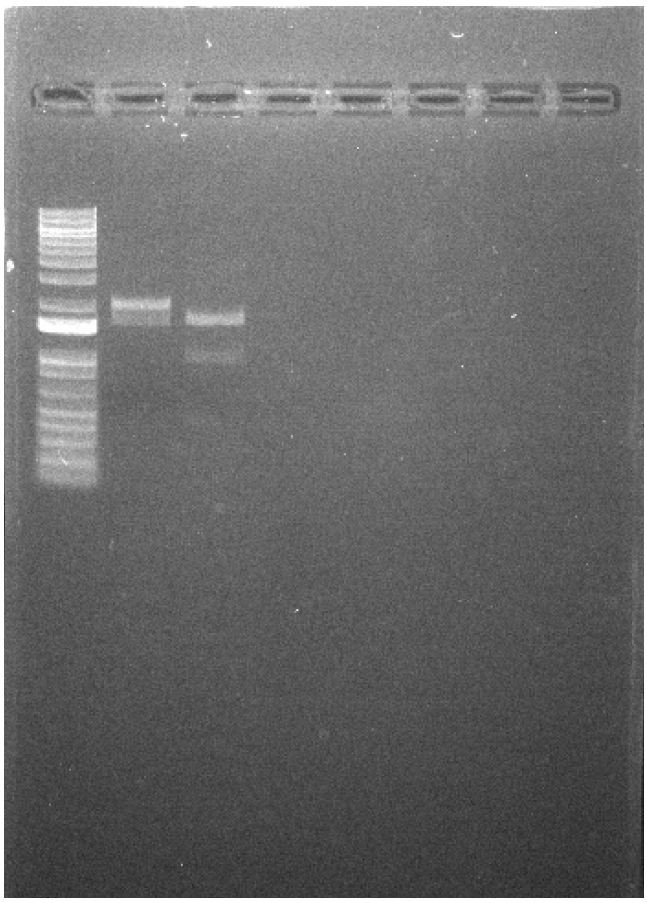
  ADD 1 uL TEMPLATE DNA AFTER PUT INTO PCR Tubes
  - Follow Up- Run a gel

Minimum Inhibitory [Amp] Experiment
Concentration Gradient for ISX Cultures
Concentration Gradient for AcrB deletion Cultures

Experiment 2 NPTII Gene Detector

- Ran a gel to determine if part creation was successful

NPTII Part Gel.png



- It doesn't look like my part creation worked because I dont see a band at my expected of 3kb
  - I can BARELY see something.
  - Follow Up-Run another gel tomorrow and see if you can make out whether or not that is a band at 3kb
  - Follow Up- Run another part creation reaction
- Perform a transformation w/ neb-5-α Cells
  - Follow Up- Look at tranformations and see if ANY of them worked successfully

Experiment 3 Tem-1 Gene Detector

- Innoculated 3 Colonies,
  - one for Miniprep
  - Two for glycerol stock
- Follow Up-
  - Perform a miniprep
  - PCR the miniprep
  - Sequence the plasmid
  - Freeze Glycerol Stock
- Run PCR of purified tdk/kan, Acrb Deletion and ISX
  - [x] 186 uL water
  - [x] 60 uL Phusion buffer
  - [x] 15 uL Forward
  - [x] 15 uL Reverse
  - [x] 9 uL DMSO
  - [x] 6 uL mM dNTP's

- [x] 3 uL Phusion polymerase

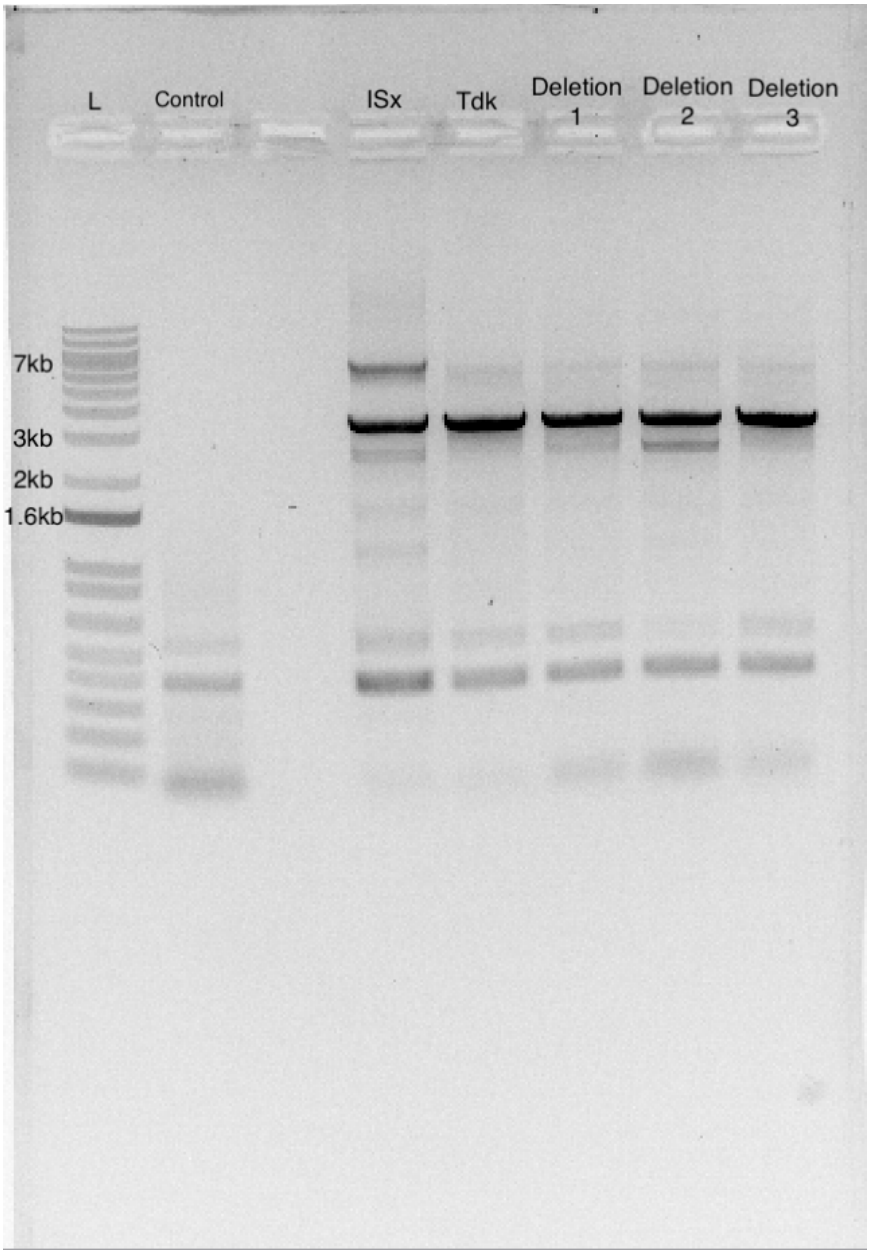
ADD 1 uL TEMPLATE DNA AFTER PUT INTO PCR Tubes

FRIDAY, 6/24/2022

Experiment 1 AcrB Deletion

- Run a gel to determine if the PCR of the AcrB deletion was successful

Deletion gel 2.png



- Trying the PCR one more time at 66° and 67°
  - Control, ISX, TDK, AcrB1,
    - [x] 124 uL water
    - [x] 40 uL Phusion buffer
    - [x] 10 uL Forward

- [x] 10 uL Reverse
- [x] 6 uL DMSO
- [x] 4 uL mM dNTP's
- [x] 2 uL Phusion polymerase

ADD 1 uL TEMPLATE DNA AFTER PUT INTO PCR Tubes

Experiment 2 NPTII Gene Detector

- Re-run the Ligation reaction w/ NPTII and pBTK1001
- Reagants
    - [x] .87 uL of NPTII
    - [x] 2.58 uL ng pBTK1001
    - [x] 2uL T4 DNA Ligase buffer
    - [x] 1 uL BsmbI mix
    - [x] 13.55 uL water
    - Reaction Conditions
        - 42°C 5 min
        - 60°C 5 min
- Negative Control- No gene, just pBTK1001

RAN A GEL TO CONFIRM CORRECT LIGATION

- Order: Laddder-NoGene-NoPlasmid-GGA

Experiment 3 Tem-1 Gene Detector

- After one night, the tubes didn't become turbid
    - Why?
        - The initial plates took two nights to produce visible colonies so more time may be required for growth in the Overnight culture
            - Follow Up- Check in tomorrow to see if the tubes become turbid
        - Our Chloromphenicol plates could be faulty and thus we got the growth of any and all cells no matter the transformation status
            - Follow Up- Run another transformation to see if the same results occur. If so, make new CAM plates
- Miniprep the Tem-1 Part Plasmid
- PCR for the part plasmid
- Freeze glycerol stock
- Prepare plasmid for sequencing

Type 2-4 parts of each Repressor and Reporter
Type 6 reverse promoter RBS (Coding sequence +Terminator) (
type 7 reverse other gene