Summer 2022 Week 3

MONDAY, 6/13/2022

This Week-

- AcrB Deletion
  - Perform a successful Transformation
  - Counterselect on AZT plates
  - Test to see if the cells grow on Carb
- NPTII
  - Insert NPTII PCR Product into Entry Vector
  - Transform NPTII Entry vector into NEB-5-α cells
  - Screen and sequence colonies to determine correct insertion
  - Order Primers for NcoI deletion
- Tem-1
  - Find the pBTK404 plasmid
  - Run PCR of Tem-1 gene on pBTK404
  - Insert PCR product into Entry Vector
  - Transform NPTII Entry Vector into NEB-5-α cells
  - Screen and sequence colonies to determine correct insertion
  - Run PCR for deletion product
  - Order more deletion primers

Experiment 1 (AcrB Deletion

- I innoculated 2 Cultures of ADP1 ISX for transformation tomorrow
  - Follow Up- Add 35 uL of culture into a + DNA and a -DNA tube. Add GGA product into +DNA tube
- Ran GGA reaction with AcrB homologies and pBTK622
  - Run a Gel With the following
    - Pre GGA reaction (i.e no enzymes added to the reaction)
    - No template DNA added
    - Homology flank's by themselves
    - Plasmid by itself
    - Cut plasmid by itself?
- GGA Checklist
  - 20 uL of total reaction volume
    - [x] 4.0 uL AcrB U
    - [x] 4.1 uL AcrB D
    - [x] 3.7 uL pBTK622
    - [x] 4.2 uL Water
    - [x] 2 uL 10x T4 dna Ligase Buffer
    - [x] 1 uL Bsa1 HF
    - [x] 1 uL T7 DNA Ligase
- **3 GGA reactions were set up**
  a. Typical GGA reaction
     Expected Gel- AcrB tdk/kan casette at 3.7 pBTK622 backbone at 1.6
  b. No Enzymes Reaction
     Expected Gel- Homologies at 1kb and Plasmid at 3.3 kb
  c. No Homologies Reaction
     Expected Gel- tdk/kan cassette at 1.6 kb and Plasmid backbone at 1.6
  - Follow Up- Run the Gel in the manner discussed later in this page

- New Kanamycin plates were made
    - Follow Up- Check for contamination

Experiment 2 (NPTII Gene Detector)

- Ran a PCR of NPTII gene on pKD13
    - Unsuccessful PCR

Experiment 3 (Tem-1 Gene)

- Beth Innoculated a culture of pBTK404 strain for me

Plan for Tomorrow's GGA Gel

Up Homology

4.0 uL of UpH = 150 ng -----> Load- 4.0 uL UpH + 1.0 uL Water + 1.0 uL Dye

Down Homology

4.1 uL Down H = 150 ng -----> Load 4.1 uL Downh + 0.9 Ul Water + 1.0 uL Dye

Plasmid

3.7 uL Plasmid = 250 ng -----> load 3.7 uL pBTK622 + 1.3 uL water + 1.0 uL Dye

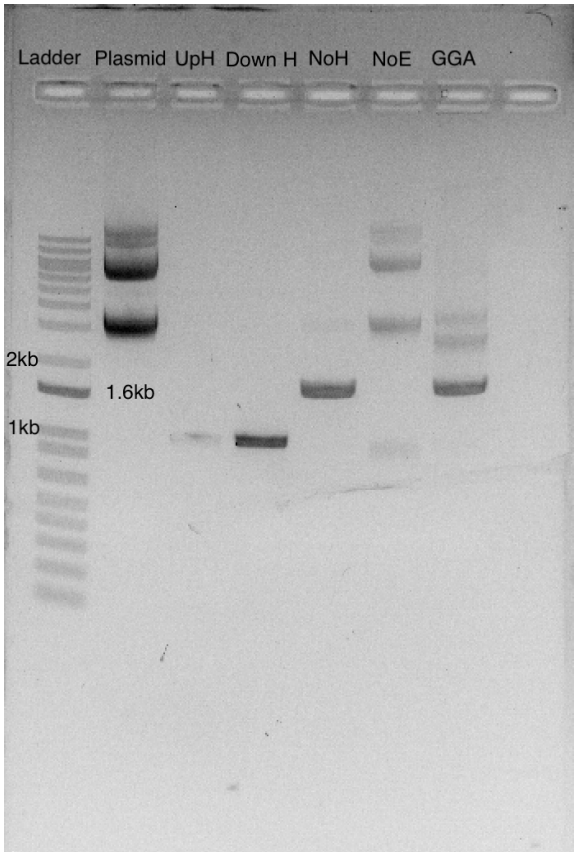
Ladder-Plasmid-UpH-DownH-NoH-NoE-GGA

TUESDAY, 6/14/2022

Experiment 1 (AcrB Deletion)

- No Contamination was seen On the Kanamycin Plates
- Plated ADP1 ISX on kan plates
    - No growth should be seen
    - Follow Up- check back at 5:30 to see if we got growth
- Innoculated Three overnight Cultures w/ +DNA -DNA and No cell's
    - Follow up- @ 5:30 to plate the cell's
- Ran the proposed Gel
    - Ladder-Plasmid-UpH-DownH-NoH-NoE-GGA

KB 5.png



- Ran a successful PCR of AcrB Homologies
  - Up Tm- 58° or 60°
  - Down Tm- 60°
- Cleaned AcrB Homology PCR Product
  - Follow Up- Quibit for [] and use for GGA w/ pBTK622
- Plated -DNA and +DNA on LB-Kan plates
  - Follow Up- Check to see if +DNA grew and -DNA didn't.
    - WE GOT EXPECTED RESULTS
      - Growth on +DNA no Growth on -DNA
  - Follow Up- Innoculate a couple of colonies in LB+Kan Tubes
    - One for Whole Cell PCR
    - One to be frozen
    - One for counter selection

Experiment 2 (NPTII Gene Detector)

- Ran an unsuccessful PCR of NPTII Gene
  - Tm- 58°
  - Follow Up- Run PCR at 61° next time
- Located pBTK1001 plasmid
  - Strain-JEB1435
    - Grows in LB+Cam
  - Box- Barrick 14
  - Rack- Barrick 2
  - Follow Up- Innoculate a Cuture of JEB1435

Experiment 3 (Tem-1 Gene Detector)

- Isolated the pBTK404 Plasmid from an overnight culture
    - 10.4 ng/uL
- Froze a glycerol stock of pBTK404 E. coli strain
- Ran a successful PCR of Tem-1 Gene
    - Tm- 56
- Cleaned the Tem-1 PCR Product
    - Follow Up- Quibit for [] and Insert into the pBTK1001 Entry vECTOR

Mega Gel Electrophoresis

Order: L-C1-CU-CD-N1-N2-T1-T1-U1-U2-D1-D2

Tm's

AcrB Up= 58 or 60

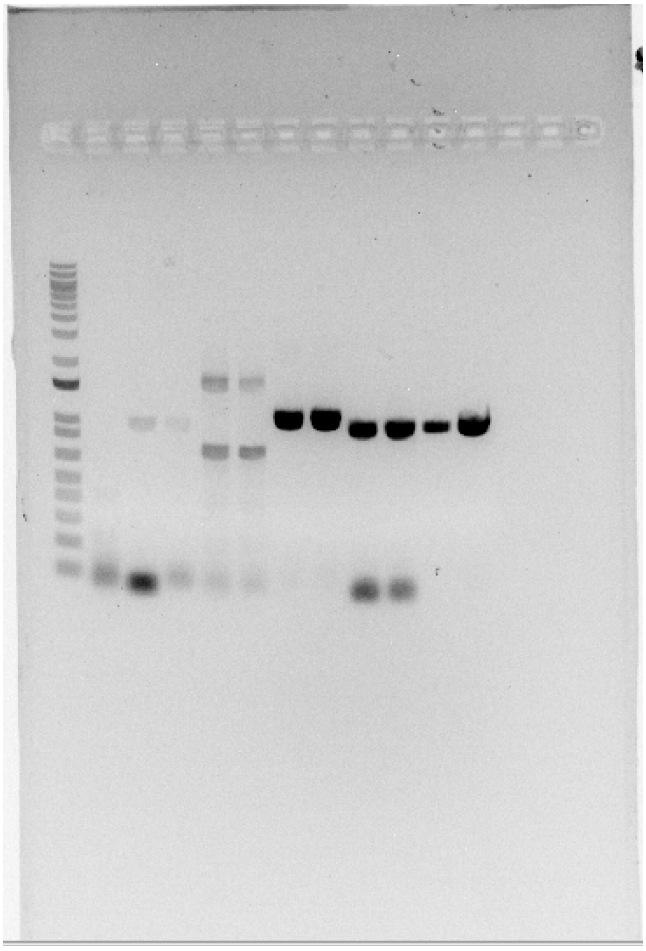
AcrB Down = 60

NPTII = 58

Tem-1 =56

Results:

kb6.png

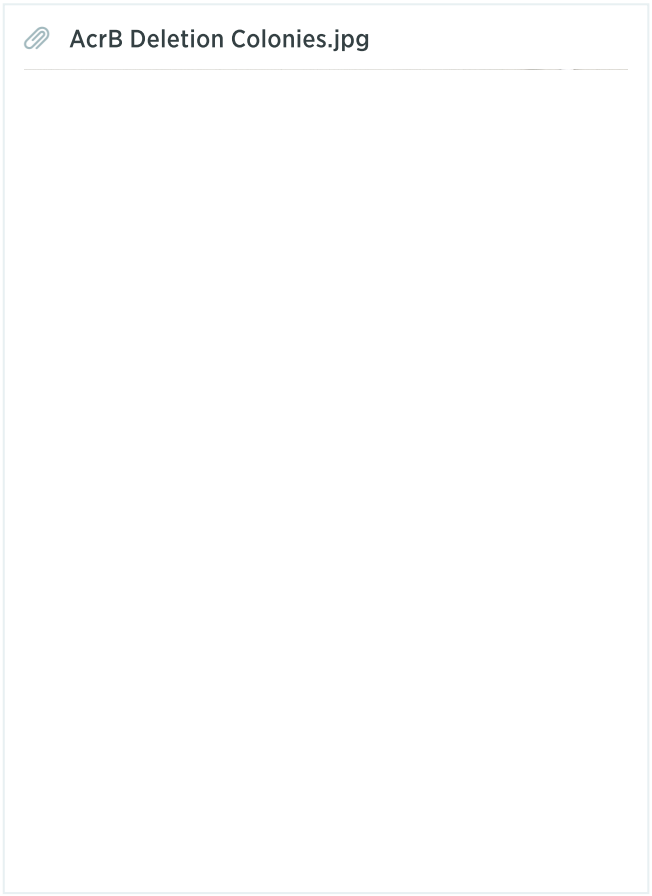


WEDNESDAY, 6/15/2022

Experiment 1 (AcrB Deletion)

- Performed a Successful transformation
  - Saw growth on +DNA and no growth on -DNA
- Innoculated Colonies
  a. LB+Kan+Colony = Whole Cell PCR
  b. LB+Kan+Colony = Glycerol Stock
  c. LB+Kan+Colony = Counter Selection w/ AZT
  d. LB + Colony = + Control
  e. LB + Kan = - Control
  - Follow Up-
    - Make glycerol stock of one culture
    - Whole Cell PCR of another culture
    - Proceed w/ counterselection of final culture
- 344 CFU's were seen

AcrB Deletion Colonies.jpg



- Determine [] of AcrB Homolgy PCR's
- Ran BsmbI GGA reaction w/ AcrB homologies for counter selection
  - [x] 2.9 uL Acrb Up
  - [x] 2.7 uL Acrb Down
  - [x] 2 uL T4 ligase buffer
  - [x] 1 uL T7 DNA ligase
  - [x] 1 uL BsmbI
  - [x] 10.4 uL Water
  - Follow Up- Run Gel to confirm and perform AZT transformation

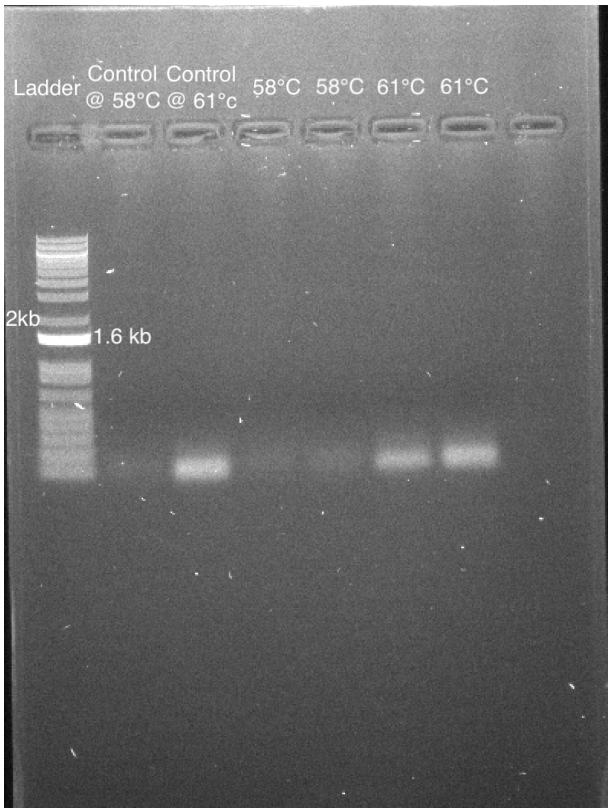
- One w/ no enzyme and one normal GGA rxn

- Made some AZT and LB plates
  - Follow Up- Check for contamination on plates, label and move to fridge

Experiment 2 (NPTII Gene Detector)

- Ran a PCR of NPTII
  - Tm's @ 58°C, and 61°C
- NPTII PCR Gel Electrophoresis
  - Order: Ladder-C58-C60-58-58-61-61

NPTII PCR.png



Experiment 3 (Tem-1 Gene Detector)

- Innoculated Culture of JEB1435
  - Box- Barrick 14
  - Rack- Barrick 2
  - Grow on LB + Cam
  - 3 tubes
    - LB + Cam + pBTK1001
      - Growth Expected
    - LB + Cam + MG1655
      - No growth
    - LB + Cam + No cell's
      - No growth
    - LB + pBTK1001
      - Growth

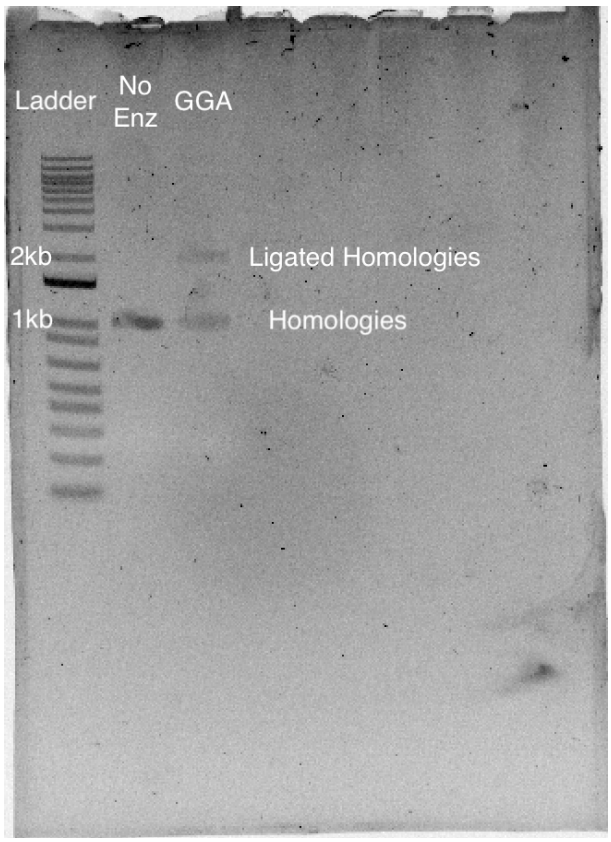
        - LB + MG1655
            - Growth
        - LB + No Cell's
            - No Growth
    - Follow Up Tomorrow-
        - Freeze Glycerol Stock of LB +CAM +pBTK1001
        - Run a plasmid mini prep
- Determine [] of yesterday's PCR

THURSDAY, 6/16/2022

Experiment 1 (AcrB Deletion)

- Ran a gel to confirm ligation of GGA reaction
    - Order: Ladder - No Enzymes - GGA

AcrB Counterselection Gel.png



- Innoculated a +DNA and -DNA culture
    - Follow Up-
        - Plate +DNA and -DNA on AZT plates
        - Plate +DNA and -DNA on LB only
- Made two Glycerol Sotock's of AcrB tdk/kan strain
    - Follow Up- Make an iGEM Strain database
- 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

15-150= 135

- Innoculate a culture of ADP1-ISX and the AcrB transformants
  - LB+Kan+Transformant = Growth
  - LB + Transformant = Growth
  - LB + Kan +ADP1-ISX = No Growth
  - LB + ADP1-ISX = Growth
  - LB + KAN = No Growth
  - LB = No Growth
  - Run another Whole Cell PCR w/ the tranformant and a culture of regular ADP1-ISX

Experiment 2 (NPTII Gene Detector)

Experiment 3 (Tem-1 Gene Detector)

- Froze a glycerol stock of pBTK1001
- Isolated the pBTK1001 Plasmid
- Run GGA rxn w/ entry vector and TEM-1

- [x] 2.9 uL Acrb Up
- [x] 2.7 uL Acrb Down
- [x] 2 uL T4 ligase buffer
- [x] 1 uL T7 DNA ligase
- [x] 1 uL BsmbI
- [x] 10.4 uL Water

FRIDAY, 6/17/2022

Experiment 1 (AcrB Deletion)

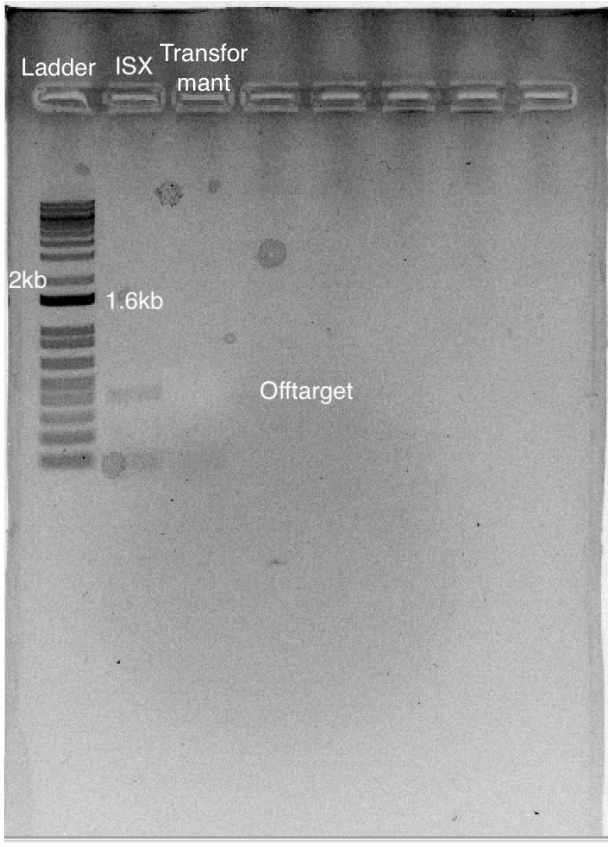
- Plated +DNA on an AZT plate and an LB plate
  - Follow Up-
    - +DNA on AZT = Growth
    - +DNA on LB = Growth
- Plated -DNA on an AZT plate and an LB PLate
  - Follow Up-
    - -DNA on AZT = No growth
    - -DNA on LB = Growth
- Follow Up- Choose three colonies to innoculate
- Set up a Whole Cel PCR

- [x] 27 µL water
- [x] 10 µL of Phusion HF buffer
- [x] 2.5 µL Forward Primer
- [x] 2.5 µl Rverse Primer
- [x] 1.5 uL DMSO
- [x] 1 µL of mM dNTPs

- [x] .5 µL Phusion DNA polymerase

- Run at 58° C
- Follow up- Run Gel to determine success of the Whole Cell PCR

Whole Cell PCR AcrB.png



UNSUCCESSFUL!!

Experiment 2 (NPTII Gene Detector)

- Follow Up- Run a PCR to amplify NPTII gene

Experiment 3 (Tem-1 Gene Detector)

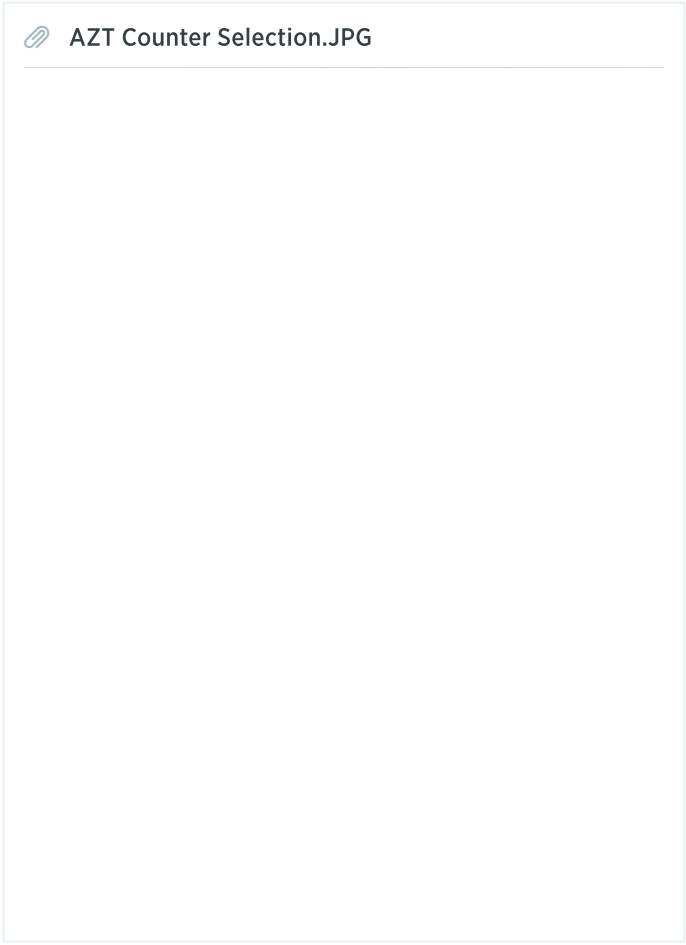
- Follow Up- Run a GGA Reaction to ligate Tem-1 gene to Entry Vector

SATURDAY, 6/18/2022

Experiment 1 AcrB Deletion

- Saw growth on LB plates, no growth on AZT -DNA and growth on AZT+ DNA

AZT Counter Selection.JPG



The bottom right dish shows the AZT counterselection

- Innoculate Cultures of Counter Selection
    - LB No Cells
    - LB + Colony for Glycerol Stock
    - LB + Colony + Kan = No growth should be observed
    - LB + Kan No cells
    - Follow Up- Innoculate a overnight culture of glycerol stock for WCPCR

Question 10
Question 11
Question 12
Question 20 - Show figure of circuit or workflow?