Summer 2022 Week 2

MONDAY, 6/6/2022

Plan For Tomorrow

- [x] Run GGA reaction to ligate AcrB to tdk/kan
- [ ] Set Up a transformation
- [ ] Help Jeffery make competent cells

AcrB Deletion

- Run Golden GGA reaction to ligate AcrB Homologies to tdk/kan cassette (pBTK622)
- Use GGA products to perform deletion of AcrB gene
- Determine the new strains susceptibility to ampicillin

ABR Gene Detector

- Edit and Order Primers by the END OF DAY
- Run PCR's to amplify the following sequences
  - ACAID 2049 Homologies
  - NPTII (pKD13)
  - Tem-1 (pBTK404)
- Determine if PCR products amplified the correct sequences

TUESDAY, 6/7/2022

Today

- [x] Run GGA reaction
- [x] Run PCR Amplifying ACAID2049 Homologies
- [x] Innoculate more pBTK622 E.coli
- [ ] Innoculate GGA Product in ADP1
- [x] Organize iGEM Boxes

Golden Gate Assembley Reaction

We want 20 uL of total reaction volume

- [x] 4.0 uL AcrB U
- [x] 4.1 uL AcrB D
- [x] 4.9 uL pBTK622
- [x] 3 uL Water
- [x] 2 uL 10x T4 dna Ligase Buffer
- [x] 1 uL Bsa1 HF
- [x] 1 uL T7 DNA Ligase

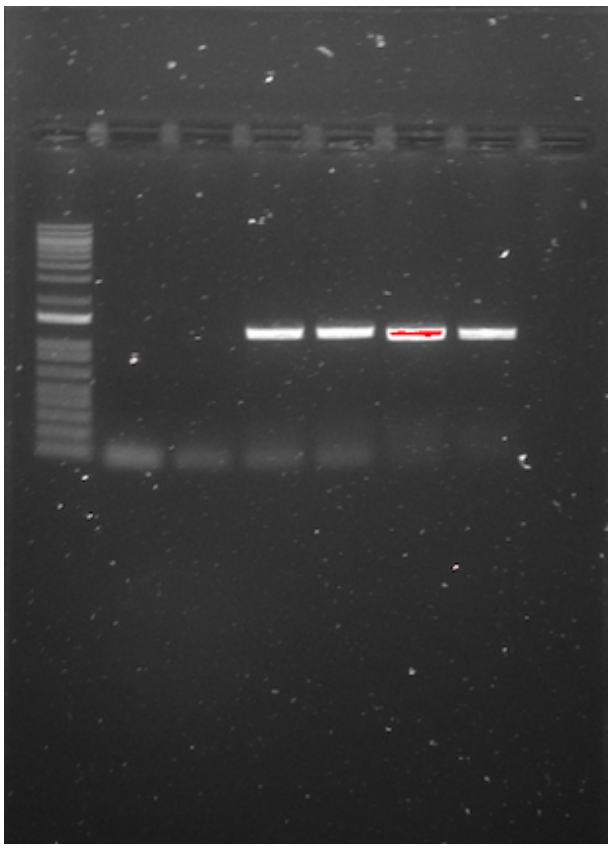
PCR of ACAID2049 Homologies

Run both up and downstream Homologies

- [x] 93 uL water
- [x] 30uL Phusion buffer
- [x] 7.5 uL Forward
- [x] 7.5 uL Reverse
- [x] 4.5 uL DMSO
- [x] 3 uL mM dNTP's
- [x] 1.5 uL Phusion polymerase

ADD 1 uL TEMPLATE DNA AFTER PUT INTO PCR Tubes
Gel Electrophoresis Order
Ladder-CU-CD-U1-U2-D1-D2
SUCCESSFUL PCR WITH AMAZING BANDS

2049 Gel.png



WEDNESDAY, 6/8/2022

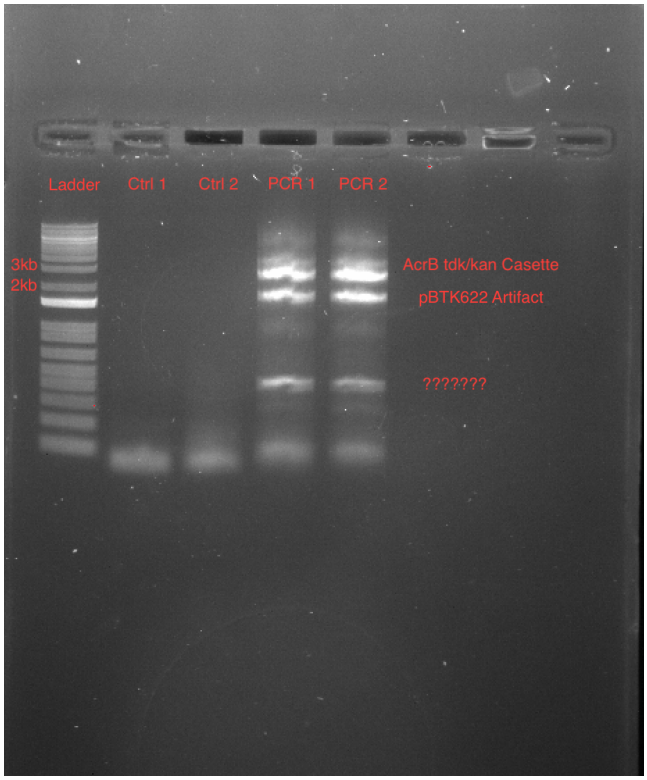
- [x] Perform Transformation
- [x] Clean and Concentrate PCR Product
- [x] Check to see if primers were delivered
- [x] Isolate pBTK622 Plasmid

Run PCR of GGA Product to amplify the AcrB Tdk/kan Casette

- [x] 62 uL water
- [x] 20uL Phusion buffer
- [x] 5 uL Up Forward
- [x] 5 uL Down Reverse
- [x] 3 uL DMSO
- [x] 2 uL mM dNTP's
- [x] 1 uL Phusion polymerase

ADD 1 uL TEMPLATE DNA AFTER PUT INTO PCR Tubes
Annealing temp- 61 degrees
Gel Electrophoresis Order
Ladder-C1-C2-1-2

AcrB tdk:kan Gel.png



Tdk/kan Casette= 1688bp AcrB Homologies= 2000 bp
Total Length of our Casette = roughly 3688 bp
Plasmid Total bp = 3340
TDK

I ran a PCR to amplify my GGA product but the results left me confused and unsure of how to proceed. I got the bands I expected at 3.7 kb (GGA product) and 1.7 kb (BSA1 Plasmid artifact) however I also got a band at around .4 kb. Does this band suggest some off-target annealing?
Also how do I purify only the 3.7 kb band and not the other two? Wouldn't the clean and concentrate kit purify ALL of the DNA present?

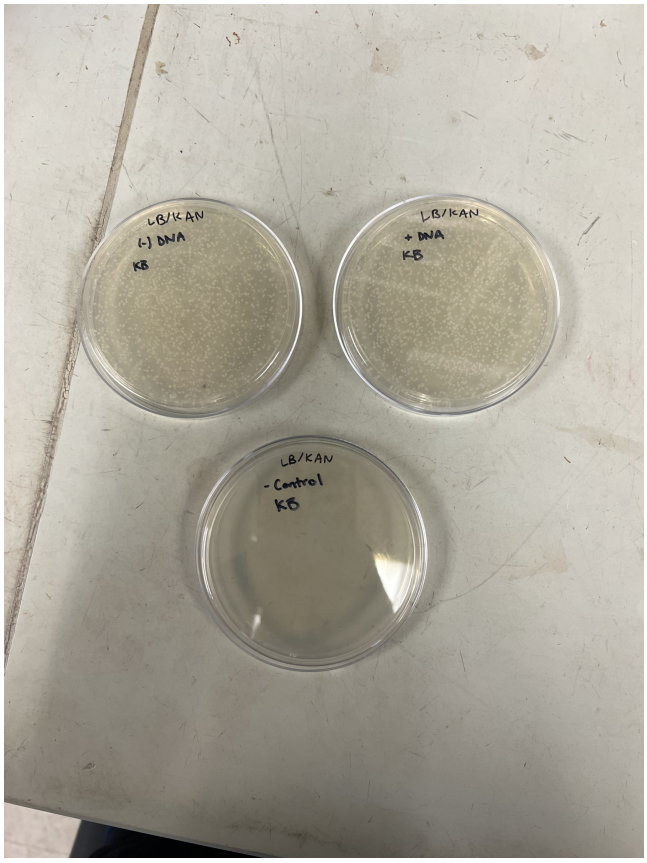
Conclusion-

- This PCR Product should still give me successful transformation because of two reasons
    a. The "plasmid artifact" has no homology to the ADP1 genome
    b. The offtarget products are too short for successful integration to occur
- As long as some of the correct Cassette was amplified then I will still get transformation

FRIDAY, 6/10/2022

Transformation Results

AcrB Deletion Transformation.JPG



Analysis-

Growth seen on both the negative and positive DNA plates

Potential Problems-

- **The plates did not have a high enough concentration of Kanamycin**
  - Today I will test on one of Dean's LB + Kan plates which he knows works.
- Some of the DNA got into the negative DNA
- The transformation was left to run for almost a day because of plating delays
- Contamination of the cells I used
- Does ADP1 have resistance to Kan? no right?

Goals For Today

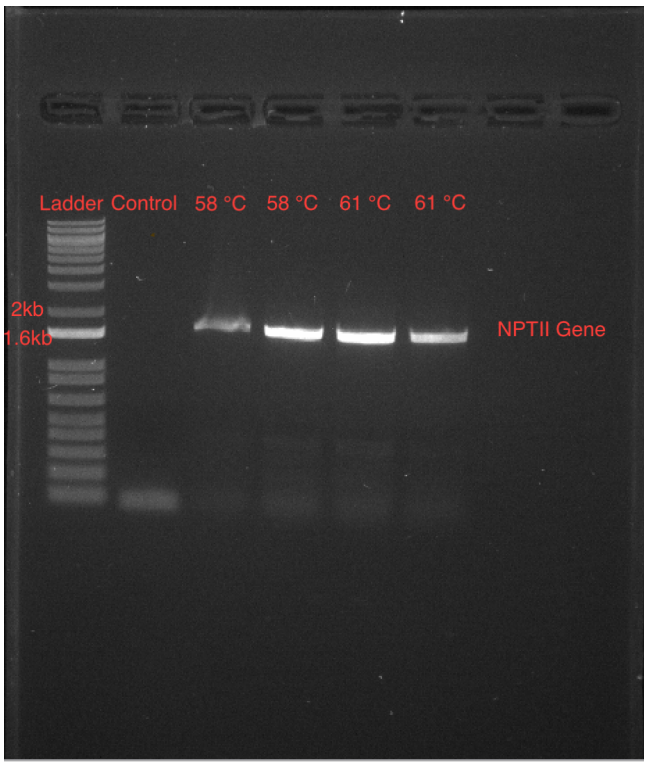
- [ ] Re-Innoculate the + and - DNA Cultures in LB to be run on Dean's LB + Kan Plates
- [x] Isolate the PBTK622 Plasmid
- [ ] Find pBTK404 plasmid
- [x] Clean and Concentrate the GGA PCR product
- [x] Run some PCR's of the new Primers

PCR of NPTII Gene on pKD13

- [x] 93 uL water
- [x] 30uL Phusion buffer
- [x] 7.5 uL Forward
- [x] 7.5 uL Reverse
- [x] 4.5 uL DMSO
- [x] 3 uL mM dNTP's
- [ ] 1.5 uL Phusion polymerase

ADD pKD13 as Template DNA
NEB Annealing Temp- 64°
Run at- 58° and 61°

NPTII Gel.png



Gel Results-
Ladder-Control-58-58-61-61
Looks Really Good! 58 and 61° C produced good bands!

In the Future, What will be needed?
ACIAD2049 Upstream Homology
ACAID2049 Downstream Homology
pYTK001 Entry Vector
May need to make more AcrB tdk/kan through GGA product for counterselection

Next 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