Summer 2022 Week 10

MONDAY, 8/1/2022

YFP Insertions

- Run PCR of 2049 TDK/kan
- Innoculated the following cultures...
  - ISX
  - E. Coli
  - 020
  - 021
  - 022
  - 023
  - 024
  - 025
- ~~Follow Up -~~
  - ~~Put into a plate to measure fluorescence~~

Detector Experiments

- Innocualte a culture of...
  - 013A @ 59
  - 019A @ 66°
  - ~~Follow Up -~~
    - ~~Perform transformation w/ NPTII for 013~~
    - ~~Perform transformation w/ Tem-1 for 019~~
- Streaked a culture of ISx on LB and LB + Kan plates to test if the kan plates work
  - ~~Follow Up -~~
    - ~~No growth on the kan plate= the plates are successful~~
- Run a PCR of 019A and 013A

013A (NPTII) @ 59°C (2049 gene)
Expected Length- 1266 + 1223 + 1593 = 4082

- [x] 93 Water
- [x] 30 HF Buffer
- [x] 7.5 2049 Up F
- [x] 7.5 2049 Down R
- [x] 4.5 DMSO
- [x] 3 dNTP's
- [x] 1.5 Polymerase

2049 tdk/kan @ 59° C 1226 + 1223 + 1675 =4124

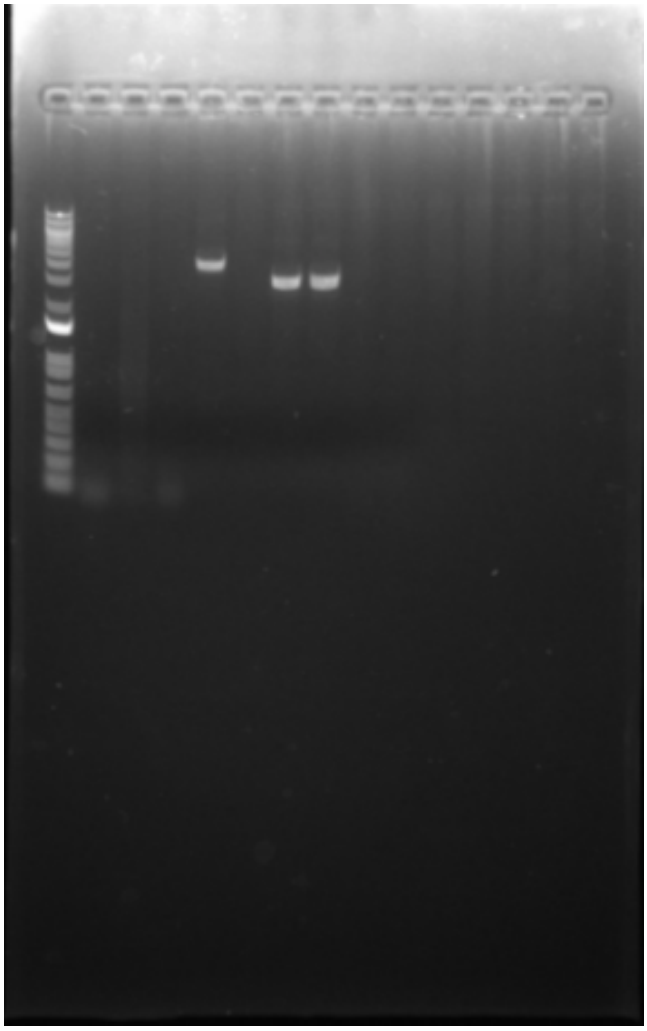
- [x] 93 Water
- [x] 30 HF Buffer
- [x] 7.5 2049 Up F
- [x] 7.5 2049 Down R
- [x] 4.5 DMSO
- [x] 3 dNTP's
- [x] 1.5 Polymerase

019A (Tem-1) @ 66°C 1000 + 1011 + 1087 = 3098

- [x] 93 Water
- [x] 30 HF Buffer
- [x] 7.5 028
- [x] 7.5 030
- [x] 4.5 DMSO
- [x] 3 dNTP's
- [x] 1.5 Polymerase

Gel Order: Ladder-C2-C3-C9-2049-2049-013-013-019-019

Screen Shot 2022-08-01 at 3.12.32 PM.png



Interlab-

- Perform transformations of the BBa plasmids
- Performing Transformations of the following plasmids
    - pKD13 (Negative Control for plates)
    - pBTK622 (Poisitive Control for plates)
    - BBa_J428100 (negative Control)
    - BBa_I20270 (Positive Control)
    - BBa_J428112 (Test Device 1)
    - BBa_J428110 (Test Device 2)

- BBa_J428111 (Test Device 3)
- BBa_J428101 (Test Device 4)
- BBa_J428108 (Test Device 5)
- BBa_J428106 (Test Device 6)

TUESDAY, 8/2/2022

YFP Insertion-

- Excited at 488 and meaured emission at 530
- Isolated DNA from 020 and 021 for PCR reactions
- Perform Golden Transforation of 023
  - Add 50 ng of 2049 tdk/kan to 500 uL of LB + 35 uL of Overnight culture
- ~~Follow Up-~~
  - ~~Plate the transformation on kanamycin~~
- Run a PCR of 020 and 021

- [x] 155 Water
- [x] 50 HF
- [x] 12.5 028
- [x] 12.5 030
- [x] 7.5 DMSO
- [x] 5 dNTP's
- [x] 2.5 Phusion

- ~~Follow Up-~~
  - ~~Run gel to determine if the PCR was successful~~

RSF1010 Plasmid

Cam or Spec

Check paper where they tested a bunch of promoters

025 = E. Coli w/ YFP controlled by BetI promoter
024 = E. Coli w/ YFP controlled by CymR promoter
023 = ADP1 w/ YFP controlled by LacI promoter
022 = ADP1 w/ YFP controlled by VanR
021 = ADP1 w/ YFP controlled by CymR
020 = ADP1 w/ YFP controlled by BetI
E. Coli
ISX

Detector Experiments-

019A (Tem-1) @ 66°C 1000 + 1011 + 1087 = 3098

- [x] 93 Water
- [x] 30 HF Buffer
- [x] 7.5 028
- [x] 7.5 030
- [x] 4.5 DMSO
- [x] 3 dNTP's
- [x] 1.5 Polymerase

013A (NPTII) @ 63°°C (2049 gene)

Expected Length- 1266 + 1223 + 1593 = 4082

- [x] 93 Water
- [x] 30 HF Buffer
- [x] 7.5 2049 2049 Up F
- [x] 7.5 2049 2049 Down R
- [x] 4.5 DMSO
- [x] 3 dNTP's
- [x] 1.5 Polymerase

Gel Order: Ladder-C3-013-013-C9-019-019

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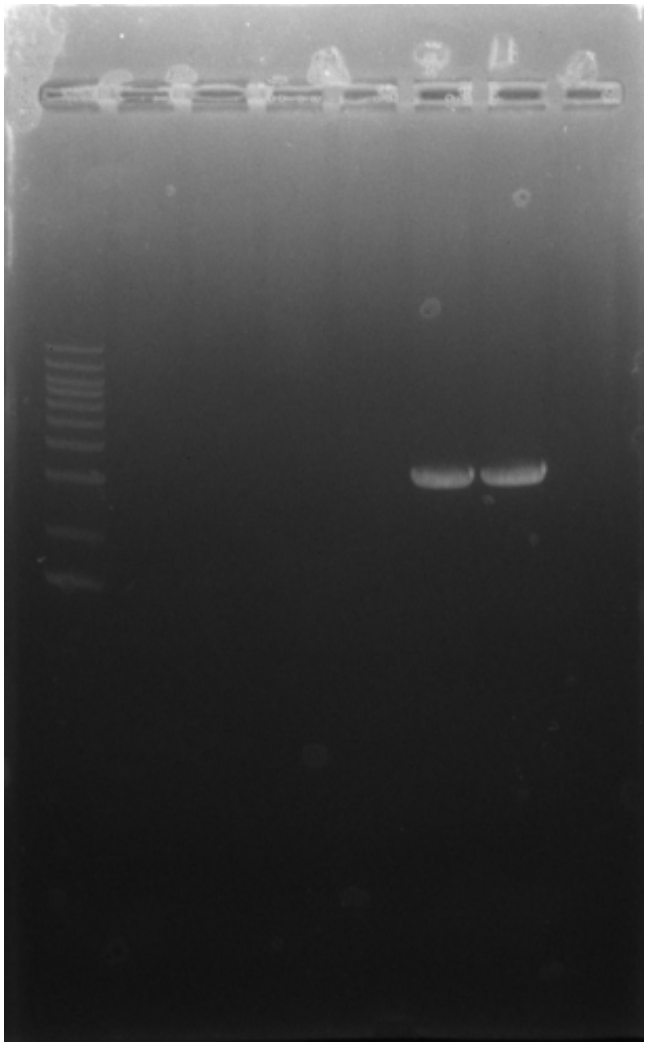


- The gel looks good, I will clean and concentrate and have prepped for sequencing
- Innoculated cultures of 019A and 013A
    - ~~Follow Up-~~
        - ~~Perform transformation w/ the correct genes to figure out the best dilutions~~

~~R~~

Interlab-

RSF1010 Plasmid
    Cam or Spec
    Check paper where they tested a bunch of promoters

025 = E. Coli w/ YFP controlled by BetI promoter
024 = E. Coli w/ YFP controlled by CymR promoter
023 = ADP1 w/ YFP controlled by LacI promoter
022 = ADP1 w/ YFP controlled by VanR
021 = ADP1 w/ YFP controlled by CymR
020 = ADP1 w/ YFP controlled by BetI
E. Coli
ISX

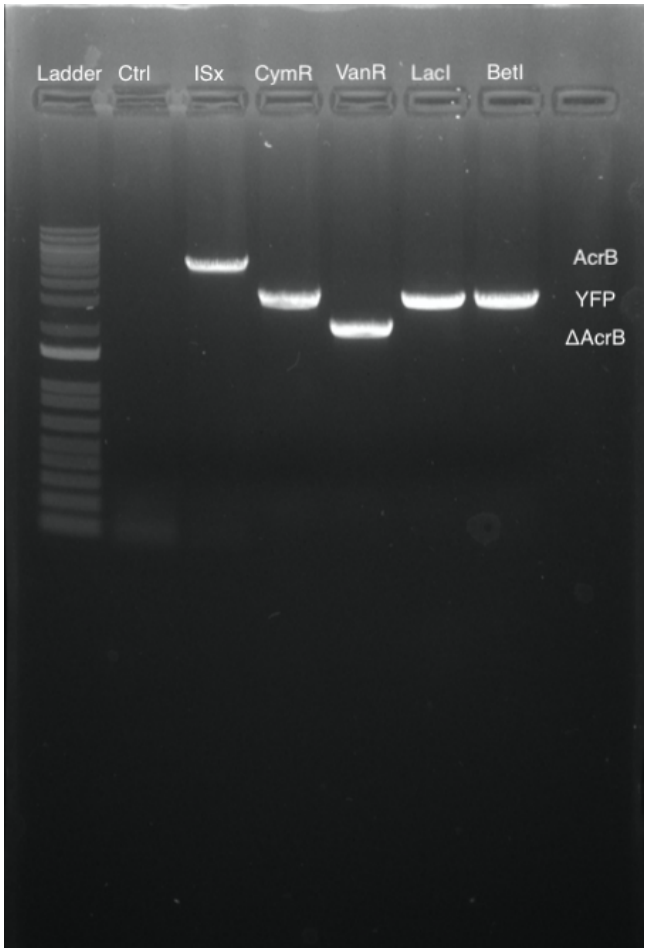
WEDNESDAY, 8/3/2022

YFP Insertion-

- Ran a gel to determine if 020 and 021 have the correct sized insert
- The transformation was contaminated so I will have to try again tomorrow
    - Innoculated a culture of 020, 021, and 023
- Follow Up-
    - Perform a transformation using the 2049 tdk/kan PCR product

- Run a PCR of 020-021-022-023- and ISX to show what strains have the inserts
    - [x] 186 Water
    - [x] 60 HF
    - [x] 15 028
    - [x] 15 030
    - [x] 9 DMSO
    - [x] 6 dNTP's
    - [x] 3.0 Phusion

YFP_insertion_Gel.png



- 020, 021, and 023 seem to have the correct insert

Detector Experiments-

- The transformation was contaminated so I will have to try again tomorrow
- Innoculated the following cultures
  - ~~Three cultures of 019A~~
    - ~~Follow Up- Perform transformations w/ Tem-1 PCR Product, No DNA, and the blank~~
  - ~~Three Cultures of 013A~~
    - ~~Follow Up- Perform transformations w/ NPTII PCR product, No DNA, and the blank~~

- Ran a PCR of NPTII
  - 005 and 006
- Ran a PCR of Tem-1
  - 007 and 008

FRIDAY, 8/5/2022

Detector Experiments-

- Plate all 3 transformations of both 013 and 019
  - Dilute + DNA, -DNA and Control

        - What should I dilute into?
    - Plate on LB and a selective plate

YFP Insertion-

- Plate the transformation onto LB-Kan
    - 020 +DNA and -DNA on LB and LB kan
    - 021 -DNA and +DNA on LB and LB Kan
    - 023 +DNA and -DNA on LB and LB Kan

Pathogen Detector-

- Run a PCR of the 2049 homologies w/ new primers
- If successful, clean and concentrate
- Run the following GGA reactions
    - 2049 UP + Target Up (Run 4 of these reactions)
    - Target Down + Repressor + 2049 Down (Run 1 reaction/repressor)

P. destructans Detector Workflow

- PCR 2049 homologies
    - 001 and 0069 for up
    - 004 and 007 for down
- C&C PCR product
- Run the following BsaI GGA reactions
    - 2049 Up homolgy + PD seq 3A
    - 2049 Down Homology + BetI + PD Sequence 3B

SATURDAY, 8/6/2022

Detector Experiments

- Innoculated a culture of 013
- Innoculated a culture of 019
    - Follow Up-
        - Perform a transformation with the following DNA concentrations
            - 100 ng/mL
            - 10 ng/mL
            - 1 ng/mL
            - .1 ng/mL
            - .01 ng/mL
            - .001 ng/mL
    - Follow Up-
        - Perform a transformation of 019 w/ the 2049 tdk/kan product

Pathogen Detector

- Ran a PCR of PD seq 3A and PD seq 3B at 62°C

        - Run a gel to determine the success of the PCR
            - Expected sizes
                - 3a = 1050
                - 3b = 1026

- Ran a GGA reaction of 2049 Up + Pd seq 3a
- Ran a GGA reaction of CymR + PD seq 3B + 2049

Order the right primers tomorrow...

PD workflow day one-

1.) Clean & Concentrate 2049 PCR product

2.) Resuspend G-Blocks in nuclease free water

3.) Run a GGA rxn w/ 2049 Up Homology and PD seq 3A