# Summer 2022 Week 11

MONDAY, 8/8/2022

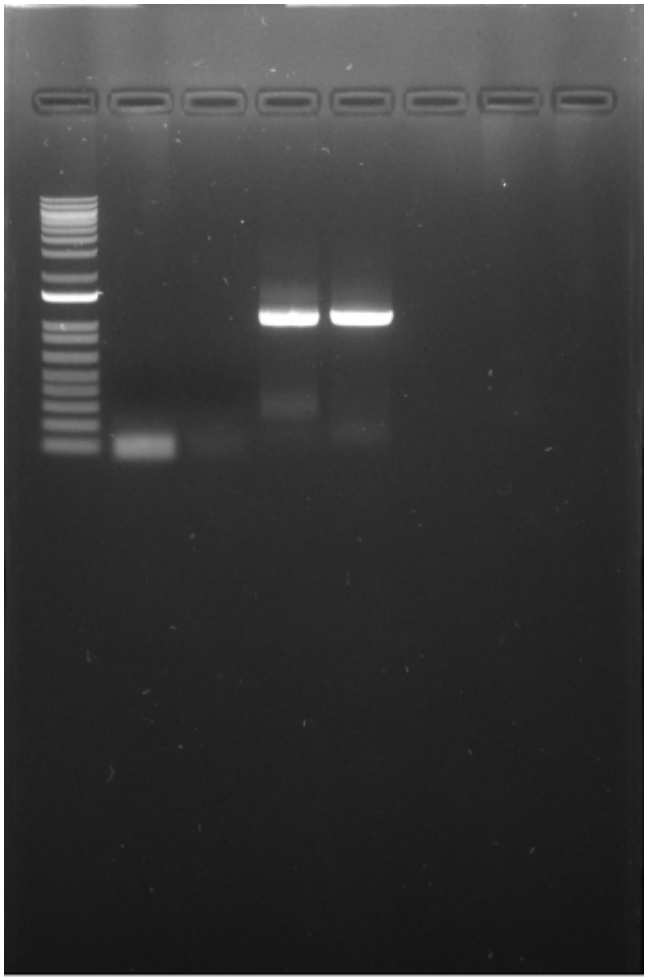
## Detector Experiments

- 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

P.Destructans_target_gel.png



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

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

TUESDAY, 8/9/2022

## Pathogen Detector-

- Run a PCR of the GGA rxn 1 w/ 064 and 001
- We cannot run a PCR of the other GGA

GGA 1 PCR

- 064 AND 001 primers

Tm = 59 °

GG2 PCR

- 065 and 004 primers

Tm = 60°

## YFP Insertion-

- Run a PCR w/ 061 and 062 to amplify the Repressor genes for insertion into the YFP strains
  - They should be ligated to the 2049 homologies
  - Tm = 61 °
- Run a GGA reaction w/ homologies and repressor sequences to ligate them
  - Inoculate a culture of the CymR insertion for a transformation tomorrow

## Detector Experiments-

- Performed Transformations with decreasing the decreasing concentrations for both 019 and 013 strains
  - ~~Follow Up -~~
    - ~~Spot plate the transformations with decreasing concentrations~~
- Performed a transformation with 019 strain to insert the Tdk/kan into the 2049 gene
  - This will be used for the co-culture experiment
  - Follow Up-
    - Plate onto kanamycin to check for growth
    - If succssful, Innoculate two cultures
      - One for 30°c growth
      - One for 37° C growth (for co culture experiment)

Thank you, here are the sequences!
iGEM22_071:
GCATCGTCTCATCGGTCTCAGCTGagctgctttggcagtttattcttgacatgtag

Igem22_072:
ATGCCGTCTCAGGTCTCAACTCcagataaaatatttgctcatgagcccg

iGEM22_073:
atgccgtctcaggtctcatcggACAAACTTAACGGGGTTCCCC

iGEM22_074:
gcatcgtctcatcggtctcaccctGACTGAGCGGAACGAGCTATGA

WEDNESDAY, 8/10/2022

## Pathogen Detector

- PCR was run for GGA1- 064 and 001 (2049 UP F)
- PCR was run for GGA2- 065 and 004 (2049 Down R)
  - Follow Up-
    - Run a gel to determine the success

## YFP Insertion

- PCR ran for CymR- 061 and 062
  - Follow up-
    - Run a gel to determine the success
    - If successful, run a GGA reaction

## Detector Experiments-

- Spot plated the transformatins for 013 and 019
    - ~~Follow Up-~~
        - ~~Analyze the results and take pictures of the plates~~
        - Results look really good, Pictures were taken
- Plated the 019 2049 transformation
    - Follow Up-
        - If successful, freeze a glycerol stock
        - Glycerol stocks were grown the next day because the colonies were fairly small

FRIDAY, 8/12/2022

## Detector Experiments-

- Glycerol stocks of 019 tdk/kan strain were made

## Pathogen Detector

- Run a PCR of GGA 1, GGA 2
- Gel Order- Ladder-CG-CC-GGA1-GGA1-CymR1-CymR1

## YFP Insertion

- RAn a PCR of CymR
- Gel Order- Ladder-CG-CC-GGA1-GGA1-CymR1-CymR1