Summer 2022 Week 8

WEDNESDAY, 7/20/2022

NPTII Gene Detctor

- Design an experiment to quantify the detectors ability to detect the gene

Tem-1 Gene Detector

- Run preliminary test to determine if the detector works

AcrB MIC experiment

Detector Test Experimental Design

- What type of information do I want to gather?
  - Ability to sift through noise
    - Can we detect the gene whenever it is in an E. Coli Genome
  - Sensistivity/transformation efficiency
    - Determine how much of the DNA was used to fix the gene?
    - Determine how much DNA was detected?
    - Perhaps determine a ratio of amount of DNA added to amount of DNA detected
- What are we trying to answer?
  - Can you detect in the presence of a lot of noise?
  - How efficient is the sensor?
  - What is the concentration threshold?

Do everything in triplicates
How much DNA went in and how many CFU's were observed
How much background CFU's were observed

- Negative Control

Next experiment would be what the lowest quantity

- similar to the MIC stuff

Extract E.COLI GENOME AND MIX WITH NPTII

- Match mixture target gene to the detector threshold results

When doing the quantity gradient

- Precision goes away at low uL so add the same volume of each dilution into the same

What dilutions do I want to plate with each colony

- 30-300

Determine what a good dilution will be and then move on to next steps
Test concentrations first and

9.6x10^6 cfu's/mL = 300 CFU's/mL

Detection Threshold-

1. Grow an Overnight culture of 013 strain
2. Dilute the overnight culture to get 30-300 CFU's

3. Plate on LB
4. Grow an overnight culture of 3 different colonies
5. Quantity gradient

Assign one YFP gene of the FRI students

- we have yfp PCR product

Samer and Adam will be here in the no Lease period

- Make more AcrB up and down flanks
- Make more 2049 flanks

Pathogen detector Cassette

- 3 seperate GGA reactions to create the cassette

Marionette

1. Create YFP strains
2. Create YFP-tdk/kan strains
3. Create YFP-Repressor strains

Pathogen Detector-

1. Run GGA w/ 2049 up + Pd Up
2. Run GGA w/ Repressor + Pd down + 2049 down
3. Run GGA w/ GGA1 + GGA2 + tdk
4. Insert into a YFP + tdk strain

THURSDAY, 7/21/2022

YFP Insertions-

- Ligate AcrB homologies to YFP gene via Bsa1 digestion
    - For initial YFP strain
- Ligate 2049 homologies to tdk-kan via Bsa1 Digestion
    - For YFP TDK-KAN strain
- Ran a GGA reaction to ligate VanR, BetI, Cym and LacI with homoogies

  CymR
  - [x] 9.6 water
  - [x] 2.7 of Up Homology
  - [x] 1.8 uL CymR CDS
  - [x] 1.9 uL of Down Homology
  - [x] 2 uL T4 DNA ligase buffer
  - [x] 1 uL of T7 DNA Ligase
  - [x] 1 uL of Bsa1

  LacI
  - [x] 10 uLwater
  - [x] 2.7 of Up Homology
  - [x] 1.4 uL LacI CDS
  - [x] 1.9 uL of Down Homology
  - [x] 2 uL T4 DNA ligase buffer
  - [x] 1 uL of T7 DNA Ligase
  - [x] 1 uL of Bsa1

  BetI
  - [x] 6.5 water

- [x] 2.7 of Up Homology
- [x] 4.9 uL BetI CDS
- [x] 1.9 uL of Down Homology
- [x] 2 uL T4 DNA ligase buffer
- [x] 1 uL of T7 DNA Ligase
- [x] 1 uL of Bsa1

- Innoculated an overnight culture of AcrB tdk/kan strain
  - ~~Follow Up-~~
    - ~~Add 35 uL of culture to 500uL of LB + GGA product~~
      - This will knockout the tdk/kan and insert the YFP
- Innoculated a culture of the

Tem-1 Detector Preliminary Test

- Innoculated an overnight culture of 019A, 019B and 019C
  - Add 35 uL of culture into 500 uL of LB + DNA

NPTII Detector Preliminary Test

- Innoculated an overnight culture culture of 013A
  - Add 35 uL of culture into 500 uL of LB + DNA
    - We want to use this to test different dilutions determine the

Broadening our Human Practices-

Perhaps reach out to people whose papers we have read about sensing
- *B. subtillis* people
- Cancer detection guy
- Biodiversity monitoring with our system
  - "Metagenomics" studies

Perhaps talk to people using ADP1 for biotechnology
- Interested in designing ADP1 to detect certain sequences, curious to get feedback from ADP1 experts on our design
- How do they see ADP1 fitting into synthetic biology and do they see any other avenues for which ADP1 can be applied?

FRIDAY, 7/22/2022

What to do today?
- Perform transformation w/ GGA product
- Run a GGA reaction with VanR product from yesterday
- Perform a transformation of 013 strain w/ target DNA
- Make Amp plates

YFP Insertions-

- Innoculated + and - DNA cultures for transformation
  - CymR
  - BetI
  - LacI
- Follow Up-
  - Dilute each transformation
  - Plate on AZT

Tem-1 Detector Preliminary Test

- Made Ampicillan plates
- Innoculated cultures of 019
    - 019A
    - 019B
    - 019C
- Follow Up-
    - Put 35 uL of culture into 500 uL LB w/ DNA
        - Add 54.5 ng of NPTII (random DNA)
        - Add 54.5 ng of Tem-1 (Target DNA)
        - No DNA in final tube

NPTII Detector Experiment

- Innoculate 35 uL of 013 Overnight culture in 500 uL of LB
    - Add 54.5 ng NPTII (target DNA) to one tube
        - 54.5 ng gives a [DNA] of 100 ng/mL
    - Add 54.5 ng Tem-1 (random DNA) to another tube
        - 54.5 ng gives a [DNA] of 100 ng/mL
    - No DNA in the final tube
- This is an initial test to determine what dilution I should make for the next part of the experiment
        - What dilutions do I want to try?

How to Dilute the Tem-1 PCR product?

- We want 10 uL of the solution to have 54.5 ng

Step 1- Take 1 uL of PCR product and put into 16 uL of elution buffer

- This gives a [DNA] of 5.45 ng/uL

Step 2- Take 10 uL of the dilution in step 1 and add it to the tube

How to Dilute the NPTII PCR product?

- We want 10 uL of the solution to have 54.5 ng

Step 1- Take 1 uL of PCR product and put into 21 uL of elution buffer

- This gives a [DNA] of 5.55 ng/uL

Step 2- Take 10 uL of the dilution in step 1 and add it to the tube

- Follow Up
    - make three different dilutions to get the following expected CFU's
        - 48 CFU's from 50 uL
        - 96 CFU's from 50 uL
        - 192 CFU's from 50 uL
    - Plate the cultures on Kanamycin
    - We ran out of kan plates so this experiment was not carried out

SUNDAY, 7/24/2022

YFP Insertions-

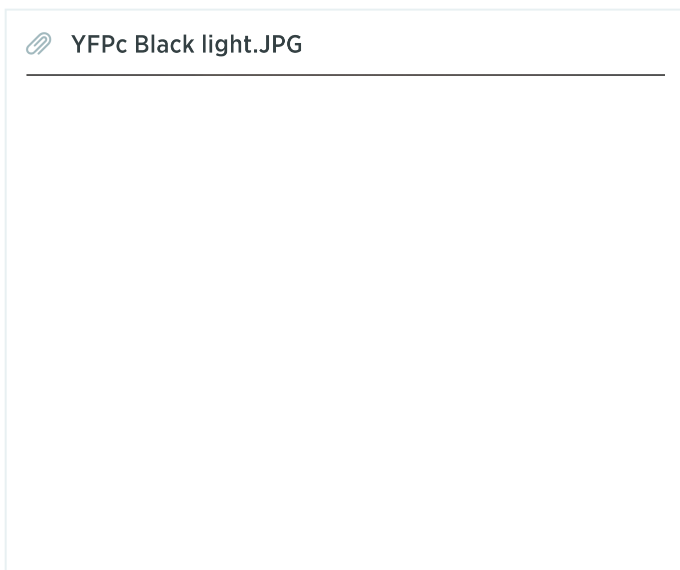
- Plated the transformations on AZT
    - Saw growth on CymR and BetI
        - Saw no fluorescence though so I'm skeptikal about whether or not the correct gene was inserted

YFPb Insertion.JPG

YFPb Black light.JPG

YFPc Insertion.JPG

YFPc Black light.JPG



- LacI did not work

YFPI insertion.JPG