Jeffrey Chuong 9/19 - 9/25

SUNDAY, 9/18/2022

MONDAY, 9/19/2022

recJ Deletion in Antibiotic Detector Strains 013 (broken *nptII*) and 019 (broken *TEM-1*)

Day 4

Inoculated two transformed colonies into LB-Kan. Incubate O/N. Set up rescue Golden Gate Ligation of Upstream and Downstream fragments O/N reaction.

- Only 013 transformed on LB-Kan, wondering if that has anything to do with the broken *nptII* potentially being fixed somehow? hopefully tdk/kan was actually inserted (can test with a LB-AZT plate)
    - Same GGA rxn (40 ul) added to both, same growth conditions
    - 9/20 BLASTED together, no hits

NGS Genome Sequencing

Take 1 colony each from 013a, 019a, and 029a and grow O/N in liquid LB or LB-Kan.

MIC Final Experiments

E. coli MG1655, BD413, ADP1-WT, ISx, Δ*pbpG*, Δ*acrB*

Conditions: 30°C O/N

Day 2

Measure OD600 on Plate reader (End Time:)

DID NOT MEASURE because cultures are not turbid yet (except for LB without any antibiotics)

- Inoculated 2 ul from resuspended colony in 1 mL, colonies were pretty tiny

TUESDAY, 9/20/2022

NGS Genome Sequencing

Coordinate with Dan for culture handoff.

MIC Final Experiments

E. coli MG1655, BD413, ADP1-WT, ISx, Δ*pbpG*, Δ*acrB*

Conditions: 30°C O/N

Day 3

Measure OD600 on Plate reader (End Time: 4 PM)

- computer won't turn on, loaded samples into 96 well plate, put lid and stored in 4C O/N

recJ Deletion in Antibiotic Detector Strain 13 (broken *nptII*)

Day 5

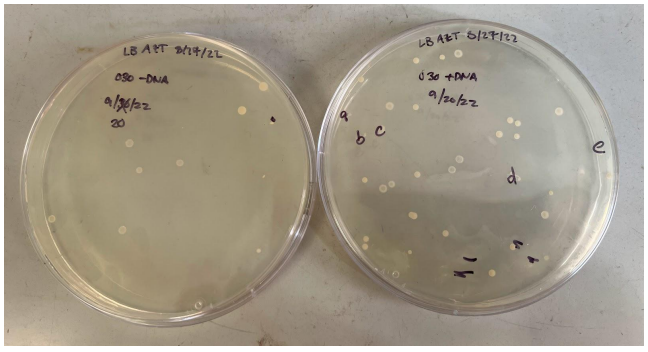
Freeze stocks of *tdk/kan* insert 013 strains. Set up transformation of *tdk/kan* insert strain with rescue GGA ligation and -DNA ctrl. Incubate at least 6 hours. (Start Time: 11:30 AM)

- New strain: 030 is the 013 strain with a *tdk/kan* inserted in place of *recJ*

Spread transformations on LB-AZT plates O/N.

- Dilute each transformation 100x in sterile saline.(+DNA and -DNA)
- Plate 50 ul of diluted transformation mix on LB-AZT
    - AZT plates are a little sus

9-20-22 Transformation of iGEM_030 with ligated re cJ rescue homology.jpg



recJ Deletion in Antibiotic Detector Strain 019 (broken *TEM-1*)

Day 1

Golden Gate Ligation of Upstream/Downstream fragments to *tdk/kan* O/N reaction. Inoculate 019 in LB O/N.

WEDNESDAY, 9/21/2022

Make LB-AZT plates

MIC Experiments 30C vs 37C

- Test BD413 as a pilot for growth conditions

recJ Deletion in Antibiotic Detector Strain 13 (broken *nptII*)

Day 6

030 (013 strain with *tdk/kan* inserted in place of *recJ*) rescue (*recJ* deletion) transformations did not grow on LB-AZT

recJ Deletion in Antibiotic Detector Strain 019 (broken *TEM-1*)

Day 2

Transform 019 with GGA product and -DNA ctrl. Incubate at least 6 hours (Start Time 11:30, End time 5:30)
Spread transformations on LB-Kan plates O/N.

MIC Final Experiments

Order of 96 well plate: *E. coli* MG1655, BD413, ADP1-WT, ISx, Δ*acrB*, Δ*pbpG*
Conditions: 30°C, 48 hours

Day 3

Measure OD600 on Plate reader

- samples were stored O/N in 4C, in 96 well plate

FRIDAY, 9/23/2022

MIC Ampicillin Experiments 30C vs 37C

- Test BD413 as a pilot for growth conditions

5 uL of O/N BD413 culture (grown in 37C) diluted 1000x in 5 mL of LB with appropiate amounts of antibiotics (Growth conditions: 30C and 37C)
2 uL of pUC19 *E. coli* strain in 5 mL of LB with appropiate amounts of antibiotics (Growth conditions: 30C and 37C, acts as a positive control)

recJ Deletion in Antibiotic Detector Strain 13 (broken *nptII*)

Day 6

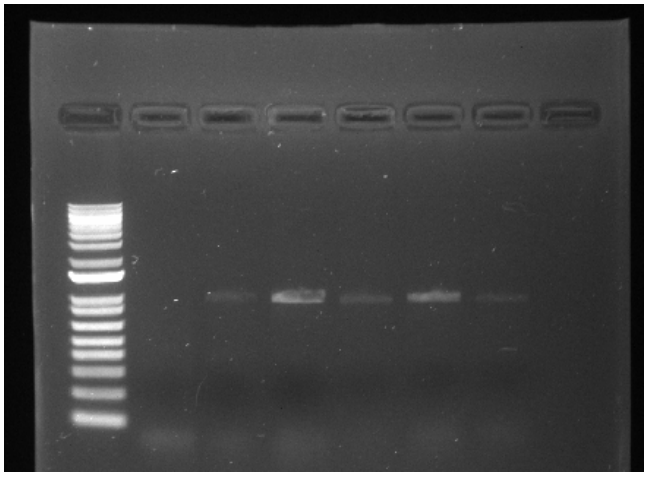
+DNA transformations saturated on LB-AZT because I left the plates in 37C incubator for 3 days, -control also had growth
BUT plates I stored from 9/20 had some +DNA colonies, potentially transformations that worked -> going to test colony PCR on these

- picked 5 colonies to inoculate O/N and make glycerol stocks tomorrow
- new strain is named iGEM_031a-e, five replicates stored in Kaitlin's box for now
- gel looks like a 1 kb band
- my conclusion: primers may have been labeled wrong, incorrect primer stocks? I tried to use *recJ* upstream forward and *recJ* downstream reverse, but perhaps the primers used just amplified a homology flank?

Colony PCR Gel

Order: Ladder, -ctrl, a, b, c, d, e

9-24-22 recJ deletion in 013 nptII detector via colony PCR.png



recJ Deletion in Antibiotic Detector Strain 019 (broken *TEM-1*)

Day 2

LB-Kan Plates did not grow, transformation failed

Starting Over

Day 1

Golden Gate Ligation of Upstream/Downstream fragments to *tdk/kan* O/N reaction. Inoculate 019 in LB O/N.

SATURDAY, 9/24/2022

MIC Ampicillin Experiments 30C vs 37C

- Test BD413 as a pilot for growth conditions

Day 2

use plate reader to measure OD600 (24 hours of growth)

Conclusions:

- Got pretty much the same MIC results, BD413 is susceptible to ampicillin, with an MIC of 2-4 ug/mL, no matter the temperature. Positive *E. coli* control with AmpR plasmid grew at all concentrations
- Got some sort of cell aggregation, cell death in BD413 cultures that grew in Ampicillin (only at 2 and 4 ug/mL)

recJ Deletion in Antibiotic Detector Strain 13 (broken *nptII*)

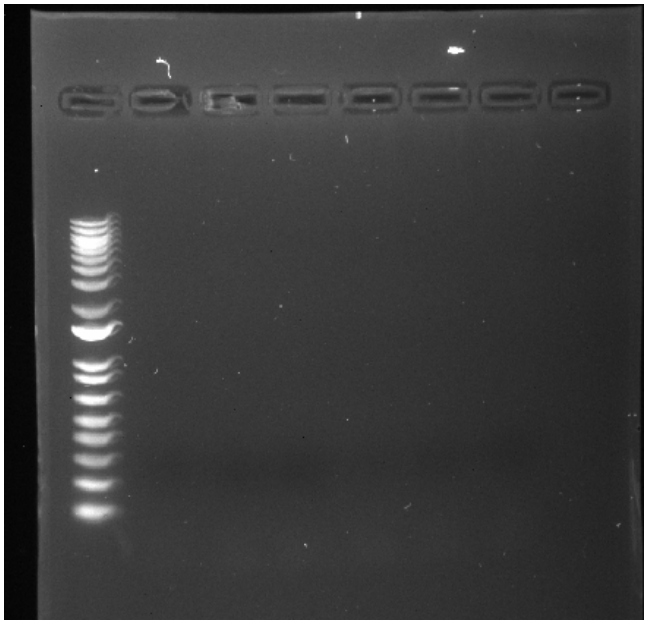
- Made glycerol stocks for colonies a, b, c, d, e
- Colony PCR Gel is not the correct size (should be 2 kb)
- Conducting a Whole-Cell PCR (re-made primer stocks to see if I potentially used the incorrect primers that resulted in only a 1 kb band)

Whole-Cell PCR Gel

- 94C 10 min to lyse cells, 56 annealing temp for *recJ* primers, did not work

Order: Ladder, a, b, c, d, e

9-25-22 recJ deletion in 013 nptII detector via WC PCR.png



recJ Deletion in Antibiotic Detector Strain 019 (broken *TEM-1*)

Day 2

Transformed GGA ligation (*recJ* homology flanks and *tdk/kan* cassette) with 019 O/N culture

SUNDAY, 9/25/2022

Make LB-Kan plates

Take pics of MIC BD413 30C vs 37C

To Confirm *recJ* deletion in 013 strain

- Streak out 013 on LB, 030 on LB-Kan, and 031a, 031b, 031c on LB
- Perform colony PCRs
- Expected band sizes: 013 should be 2 kb flanks + 1.7 kb *recJ* CDS, 030 should be 2 kb flanks + 1.7 kb tdk/kan, 031a,b,c should be 2 kb flanks only
    - watch out for extension times, 4 kb = 2:00, 2 kb = 1:00

Gel Order: -ctrl, 013 (3.7 kb), 030 (3.7 kb), 031a, b, c (2 kb)

recJ Deletion in Antibiotic Detector Strain 019 (broken *TEM-1*)

Day 3

Plated Transformation

9-25-22 Transformation of iGEM_019 with GGA ligation of pBTK622 tdk kan and recJ homology.jpg