Jeffrey Chuong 9/11 - 9/18

SATURDAY, 9/10/2022

SUNDAY, 9/11/2022

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

Day 1

PCR flanking upstream and downstream homologies of *recJ*. Run gel to confirm. Purify PCR products.
Golden Gate Ligation of Upstream/Downstream fragments to *tdk/kan* O/N reaction. Inoculate 013 and 019 in LB O/N.

Day 2

Transform 013 and 019 with GGA product and -DNA ctrl. Incubate at least 6 hours or O/N

Day 3

Spread transformations on LB-Kan plates O/N.

Day 4

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

Day 5

Freeze stocks of *tdk/kan* insert 013 and 019 strains. Set up transformation of *tdk/kan* insert strain with rescue GGA ligation and -DNA ctrl. Incubate at least 6 hours.
Spread transformations on LB-AZT plates O/N.

Day 6

Inoculate two transformed colonies into LB O/N.

Day 7

Freeze stocks of deletion rescue strains. Confirm deletion with Whole-Cell PCR. *recJ* is now deleted from 013 and 019 strains.

THURSDAY, 9/15/2022

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

Day 1 - Thursday 9/15

PCR flanking upstream and downstream homologies of *recJ*. Run gel to confirm. Purify PCR products.
Golden Gate Ligation of Upstream/Downstream fragments to *tdk/kan* O/N reaction. Inoculate 013 and 019 in LB O/N.
Inoculated from 013A and 019A

ADP1 BD413

Inoculate two colonies of BD413 to make glycerol stocks tomorrow

- scraped entire colony into culture tube, 30C O/N

FRIDAY, 9/16/2022

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

Day 2 - Friday 9/16

Transform 013 and 019 with GGA product and -DNA ctrl. Incubate O/N
35 ul of O/N culture + 20 ul GGA product (flanks + *tdk/kan*) in 500 ul LB

MIC Final Experiments

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

Conditons: 30°C O/N

Just do 1 each and make ampicillin stocks

SATURDAY, 9/17/2022

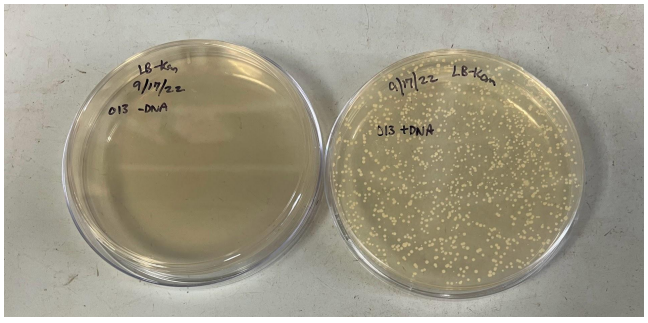
Making LB and LB-Kan plates, 40 min power level 3 in B lab microwave, 30 min in 55C water bath

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

Day 3

Dilute each transformation 100x in saline. Plate 50 ul of diluted transformation on LB-Kan plates. Incubate O/N.

9-17-22 Transformation of iGEM_013 with GGA ligati on of pBTK622 tdk kan and recJ homology.jpg



MIC Final Experiments

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

Conditions: 30°C O/N

Day 0

Make Ampicillin stocks: 3.2g of Ampicillin sodium salt powder in 25 mL of ddH2O. Filter through 20 mL syringe fitted with 0.22 µm filter

Streak out plates of *E. coli* MG1655, BD413, ADP1-WT, ISx, Δ*pbpG*, Δ*acrB* on LB plates

Set up culture tubes.

SUNDAY, 9/18/2022

Made more LB plates

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

Day 4

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

Plates not ready, no growth on 013 +DNA

NGS Genome Sequencing

Streak out 013a and 019a on LB plates and 029a on LB-Kan plate

MIC Final Experiments

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

Conditions: 30°C O/N

Day 1

Take pictures of streaked plates

5 mL and 5 ul of appropiate antibiotics in each culture tube.

Grow O/N (Start Time: 5 PM)