Jeffrey Chuong 10/4 - 10/12

TUESDAY, 10/4/2022

Experimental Design

How well do iGEM_013 and iGEM_019 sensors parse through foreign *E. coli* MG1655 foreign DNA to detect the *nptII* and *TEM-1* WT genes?

1. Streak out a culture of 013 and 019. PCR *nptII* and *TEM-1* WT fragments and purify (if necessary).
2. Inoculate three colonies from each plate into LB for triplicates.
3. Add 35 uL of O/N culture to 500 uL of LB. Add 0.545 ng TOTAL of WT gene. Add DNA amounts ranging from 0, 0.545, 5.45, 54.5, 545 ng TOTAL of foreign DNA.
    a. 0.545 ng TOTAL of WT gene was chosen since it had 10^-4 Transformation Frequency (countable colonies? I'm not too sure about the logic behind this number)
    b. Run appropriate +DNA and -DNA transformation controls.
4. Dilute transformation 0x, 10x, 100x, 1000x, 10000x. Plate 5 uL of diluted transformation (spot plating) on a selective plate and non-selective plate.
    a. Plate appropriate controls.
5. Count CFUs on selective and non-selective plates. Back-calculate to get the total cell count using the non-selective plates. Calculate transformation frequency (with total cell count) with selective plates.

FRIDAY, 10/7/2022

Streaked out plates of 013 and 019 on LB Plates O/N

Inoculated 35 uL of O/N culture (iGEM_034 *P. destructans* detector strain) + 20 uL GGA rescue

- +DNA, -DNA, off-target DNA, -LB ctrl

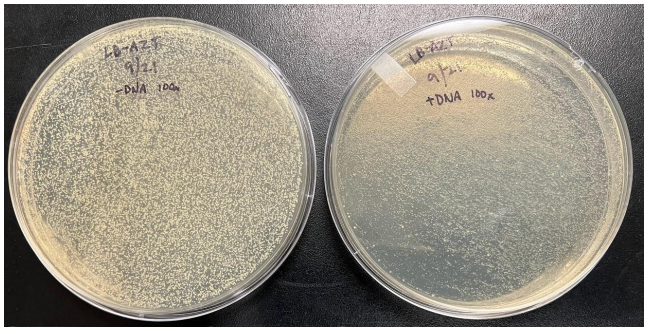
SATURDAY, 10/8/2022

Inoculated triplicates from 013 and 019 into LB O/N

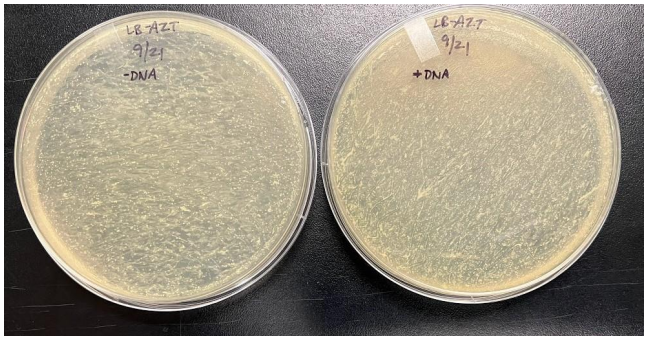
Plated *P. destructans* rescue transformations on LB-AZT

- +DNA, -DNA, +DNA 100x, -DNA 100x

10-9-22 P. destructans rescue 100x.jpg



10-9-22 P. destructans rescue 0x.jpg



SUNDAY, 10/9/2022

triplicates of 013 *nptII* and 019 *TEM-1*
500 ul LB + *nptII* or *TEM-1* DNA + *E. coli* MG1655 gDNA

0: -DNA
1-5: contain 10 ul of 0.0545 ng DNA of WT gene (TOTAL 0.545 ng DNA), 5 ul of gDNA
TOTAL AMOUNTS of *E. coli* gDNA

- 1 - 0 gDNA
- 2 - 545 ng gDNA
- 3 - 54.5 ng gDNA
- 4 - 5.45 ng gDNA
- 5 - 0.545 ng gDNA

MONDAY, 10/10/2022

Spot Plating - https://barricklab.org/twiki/bin/view/Lab/ProtocolsCFUCounts
3 replicates each for *nptII* and *TEM-1* detectors on selective and non-selective plates, 12 total plates

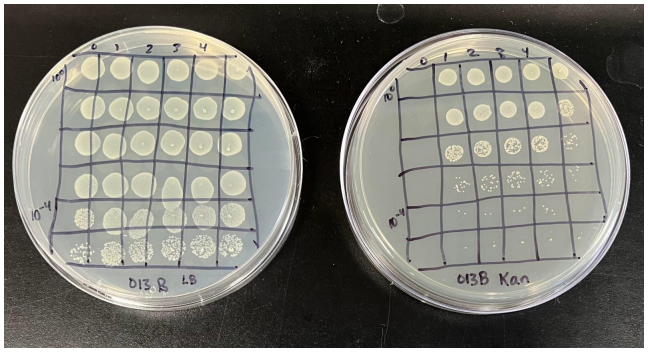
On each plate
0: -DNA
1: 0 gDNA
2: 0.545 ng gDNA
3: 5.45 ng gDNA
4: 54.5 gDNA
5: 545 gDNA

Table1

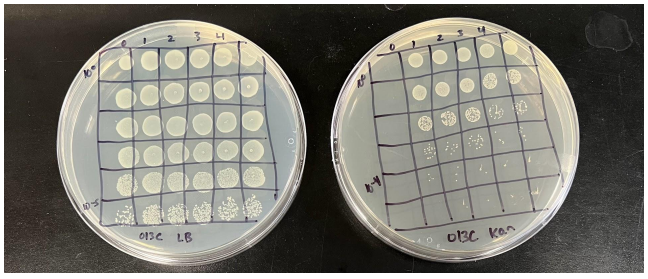
	Dilutions	0: -DNA	1: 0 gDNA	2: 0.545 ng gDNA	3: 5.45 ng gDNA	4: 54.5 ng gDNA	5: 545 ng gDNA
1	10^0						
2	10^-1						
3	10^-2						
4	10^-3						
5	10^-4						
6	10^-5						

TUESDAY, 10/11/2022

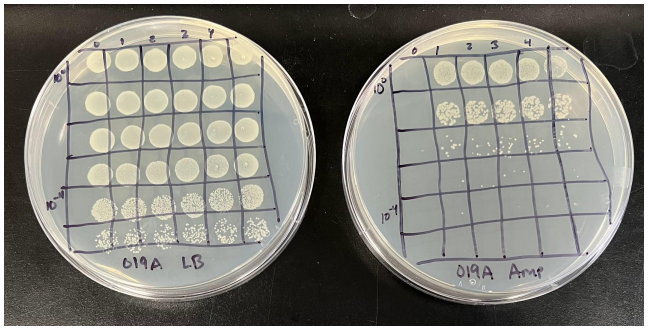
013B.jpg



013C.jpg



019A.jpg



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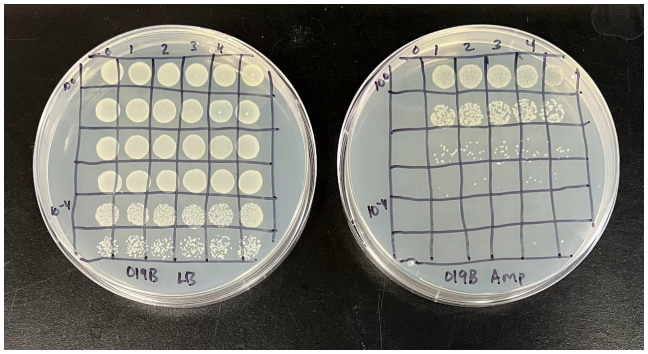

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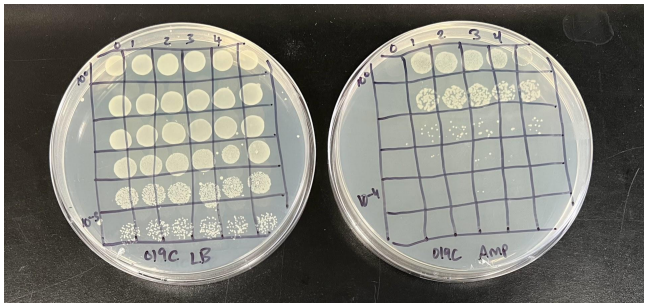

013A.jpg