(RT2-1) Investigating a Plasmid and Recombination-Based System for Genome Editing in *Enterobacter ludwigii*

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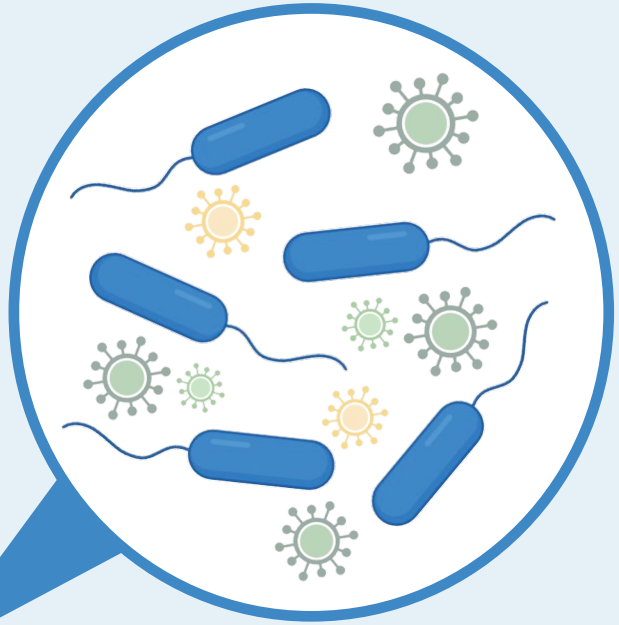
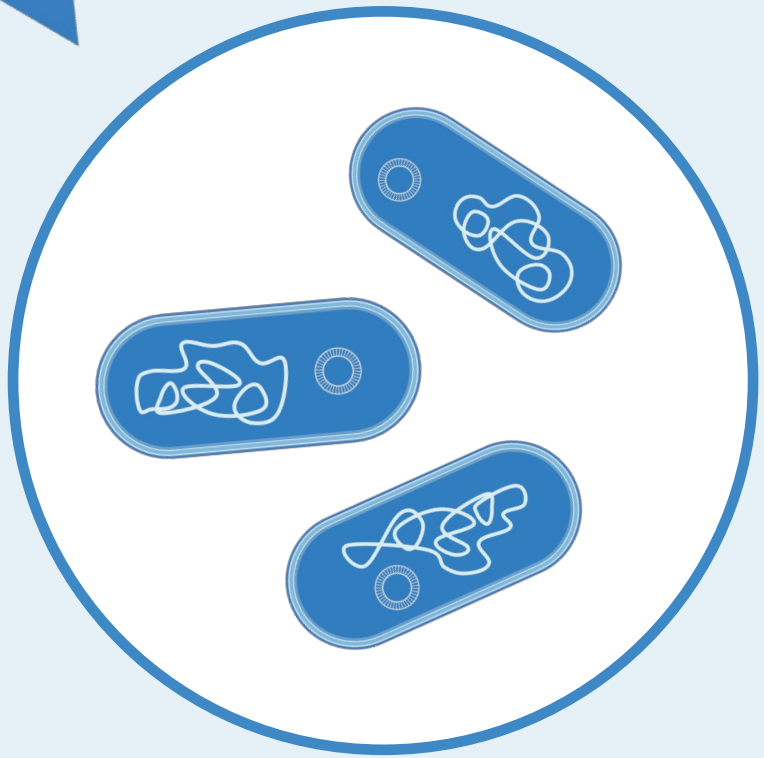
Overview

Context & Overarching Project Goals

Use of chemicals is prevalent and problematic



1. Develop better biological understanding of sink-adapted *Enterobacter ludwigii*
2. Genetically engineer *E. ludwigii* to be more probiotic and/or antimicrobial



Computational

1. Identify possible locations for insertion
2. Return information about potential downstream effects of insertion

Wet Lab

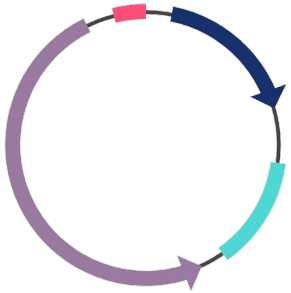
Conduct foundational genome editing experiments, attempting recombination at chosen locations

Methods

1

Identify positions for insertion in genome *(Prokka-annotated E1o genome and chose ampE, poxB, and hcp1)*

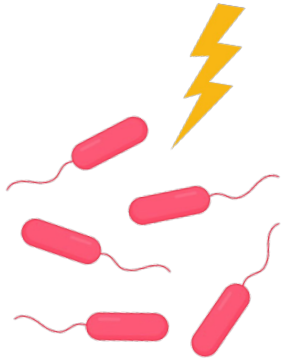
2



Plasmid construction *(Design, PCR, and Gibson assembly)*

(x3)

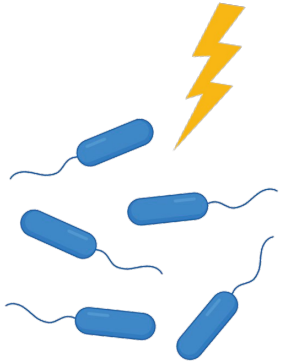
3



Delivery into PIR1 *E. coli* cells *(Electroporation)*

(x3)

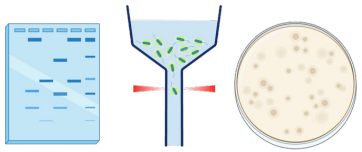
4



Delivery to E1o *E. ludwigii* cells *(Electroporation)*

(x3)

5

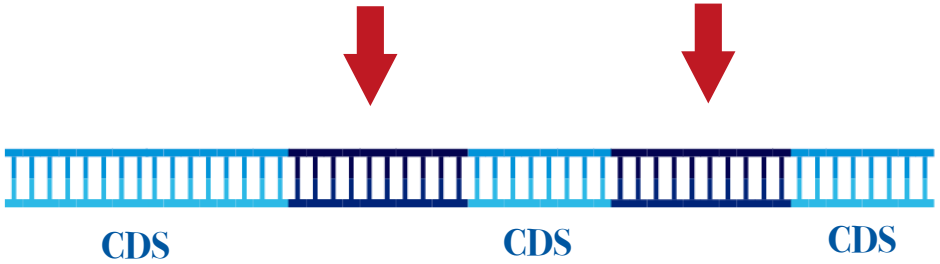


Analysis of recombination success *(4 colonies per knockout type)*

(x3)

Results

a Locating specific, known gene(s) to be knocked out in place of a desired insert



b Locating intervals that fall between coding sequences (CDSs), insertion will *not* directly disrupt a gene

Results Pt. 1

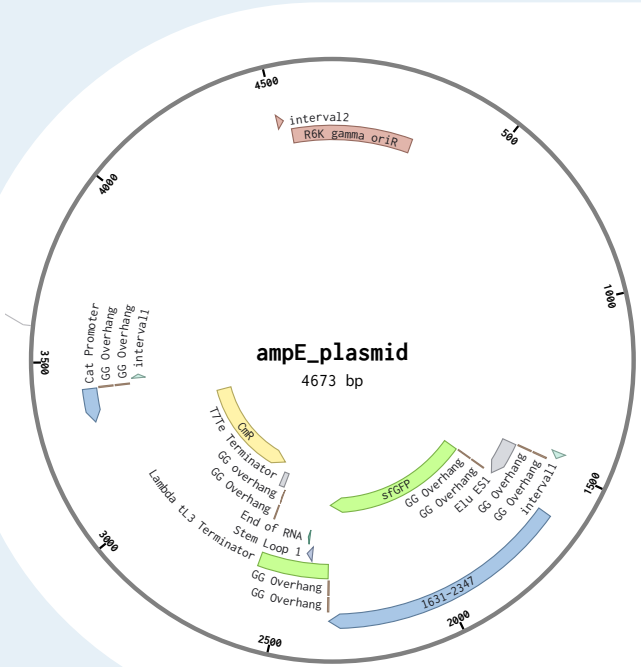
Successes in Computational Outputs

- Program to locate sites for insertion
- Program to identify genes that may be lost or gained due to an insertion
- Shared and documented on GitHub

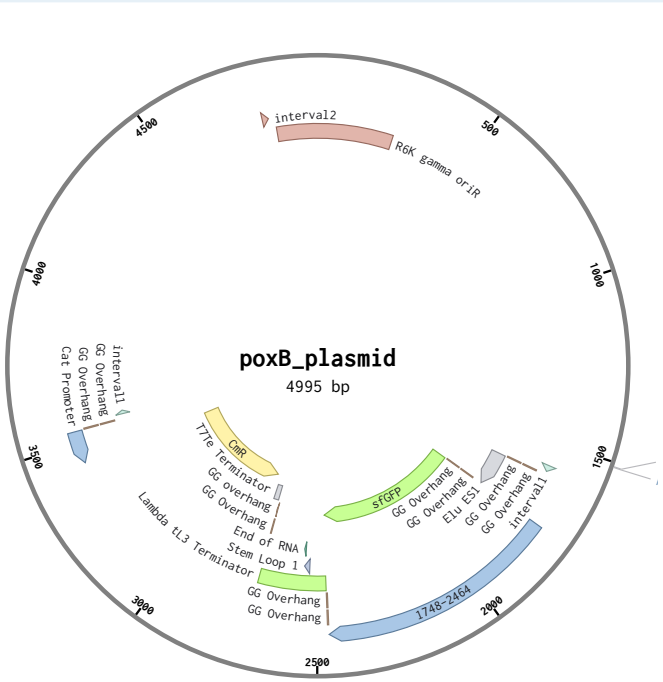
Designed Plasmids

Specific origin, GFP, CmR, and homologous arms

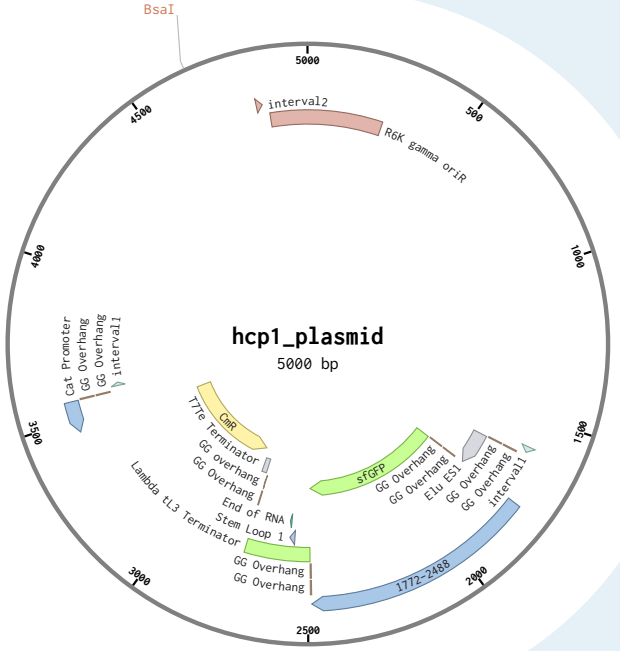
ampE_plasmid (4673 bp)



poxB_plasmid (4995 bp)



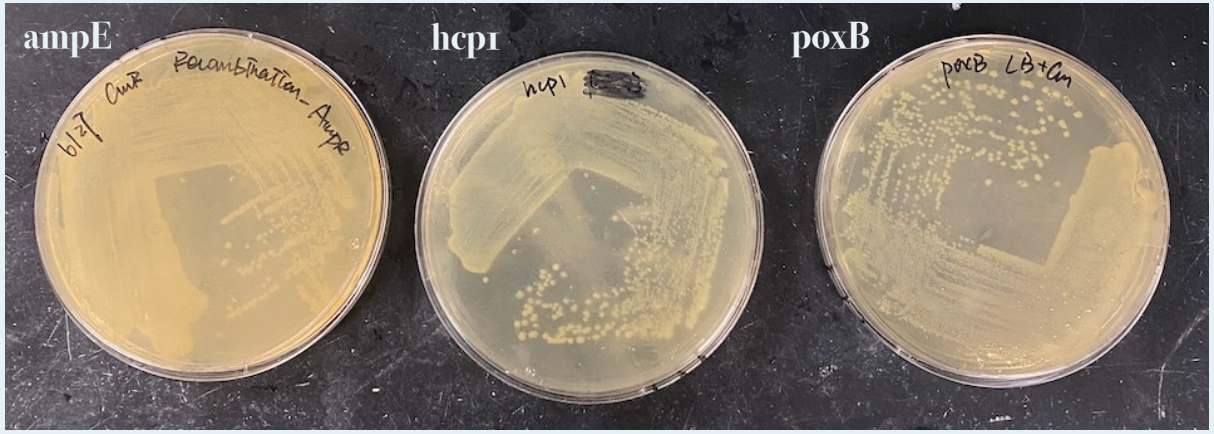
hcp1_plasmid (5000 bp)



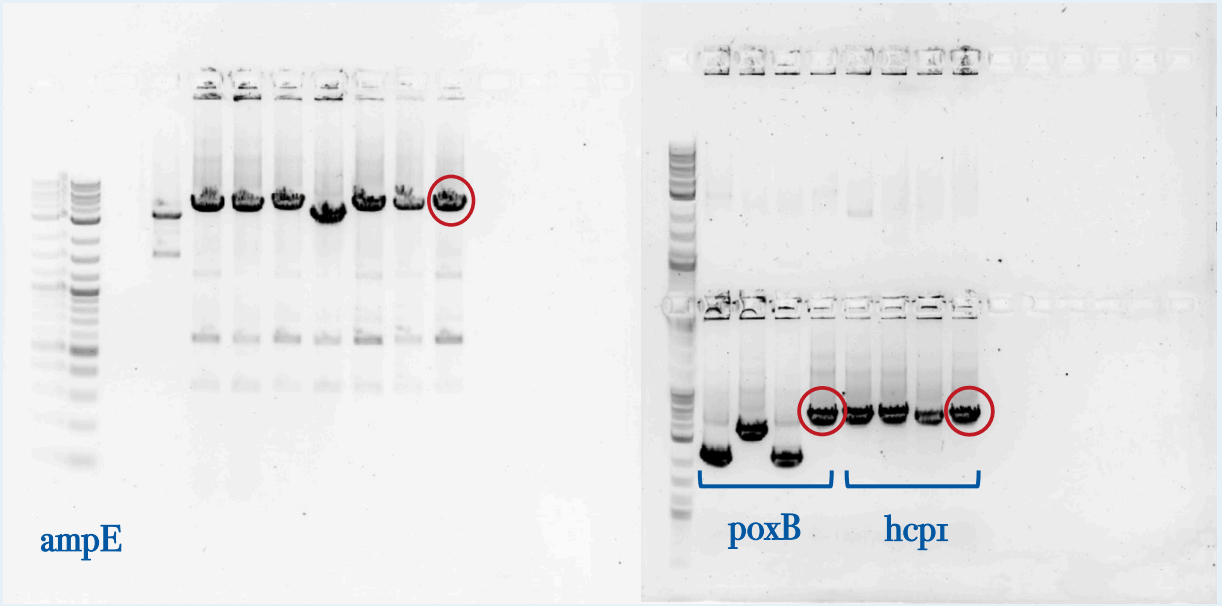
Results Pt. 2

Successes in Plasmid Delivery

- Successful plasmid design and creation
  - Illumina sequenced
- Transformation into PIR1 (*E. coli*)
- Likely successful delivery of plasmids into E10
  - Reasonable voltage and time of electroporation
  - Chloramphenicol resistance



Survival of transformed PIR1 (*E. coli*) on selective media

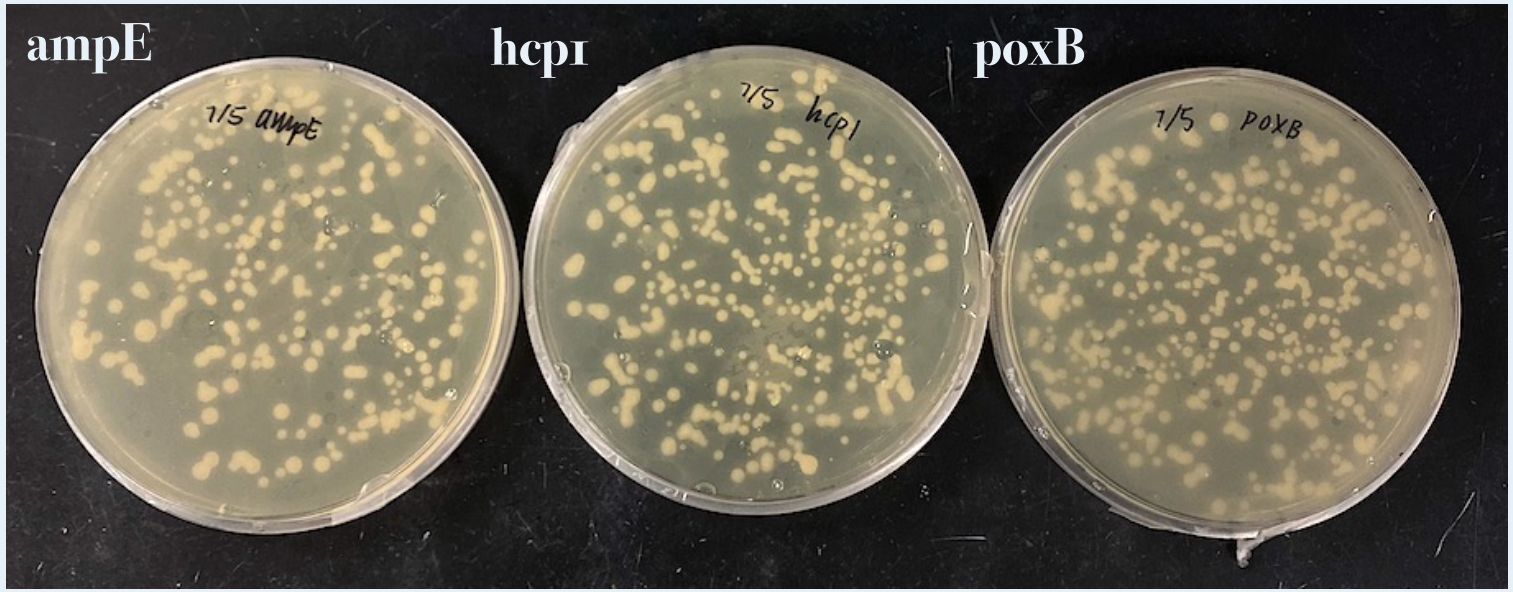


Validation of plasmids' presence in sample PIR1 colonies

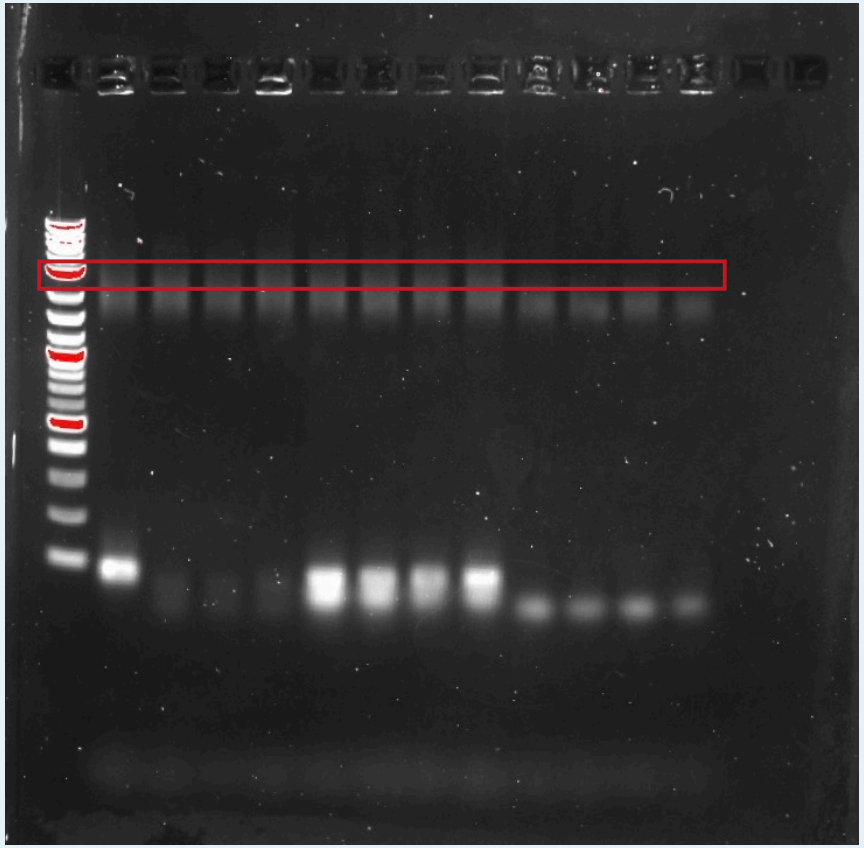
Results Pt. 3

Recombination Outcomes

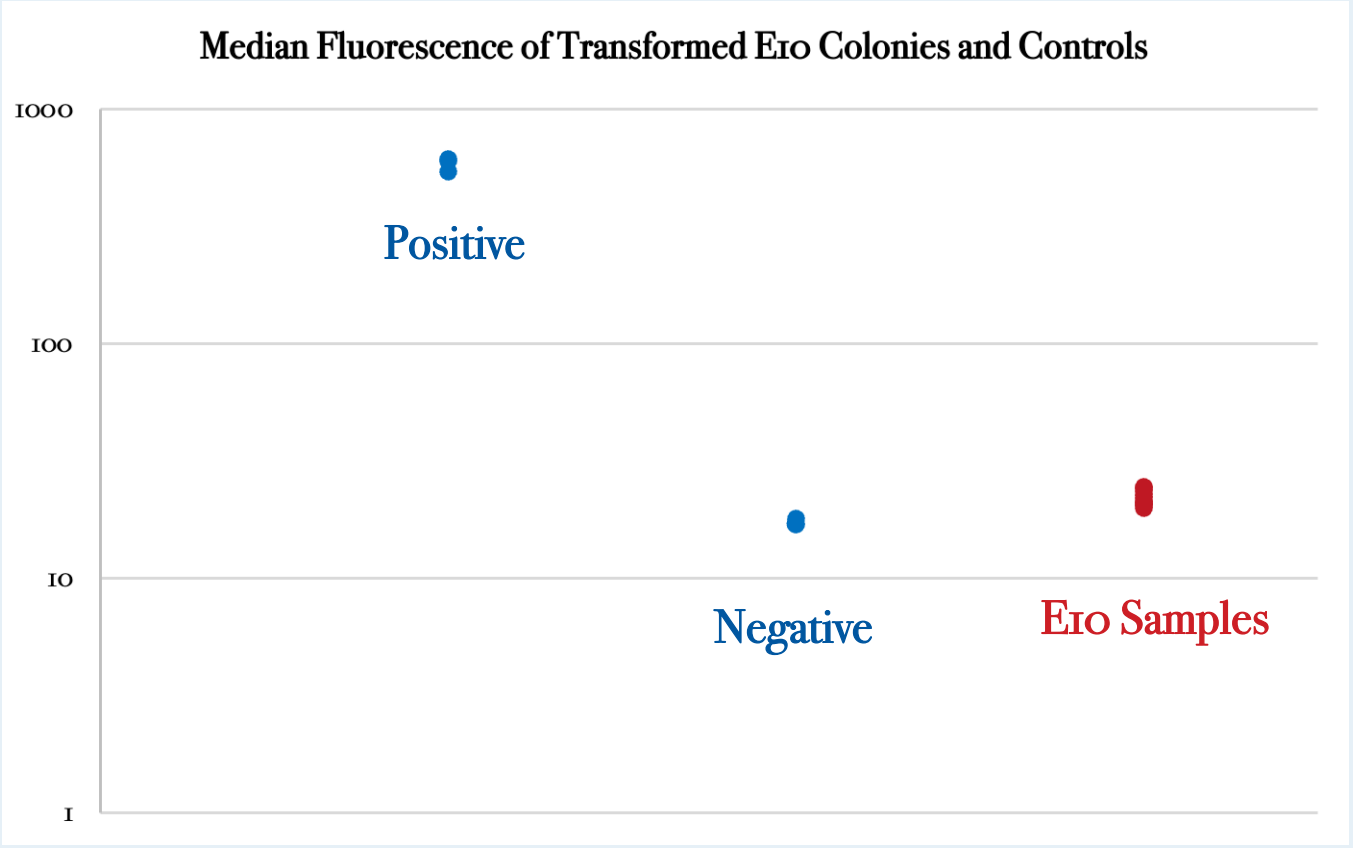
- 4 colonies isolated per transformation (12 total)
- Detection of false positives
- Inserts were *not* integrated into our samples' genomes



Survival of transformed E10 on selective media



No apparent amplification of target sequence (roughly 3 kb)

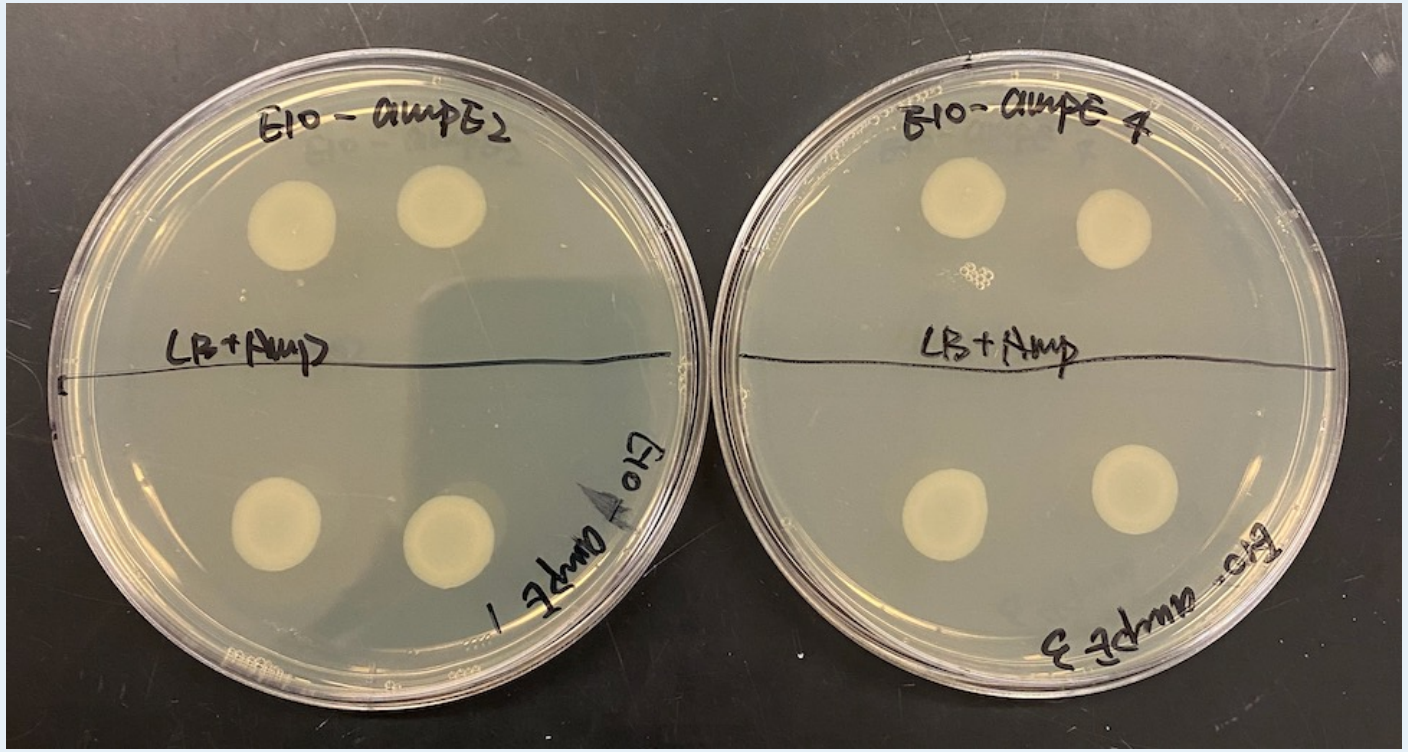


Median fluorescence levels of E10 samples resembled those of negative controls

Results Pt. 3 (Continued)

All Attempted Knockouts

- Following colony PCR of transformed E10 samples, no bands of appropriate length on gel
- No fluorescence detected via Flow cytometry



Results Pt. 3 (Continued)

ampE Attempted Knockout

- Unintended growth on selective media (ampicillin)

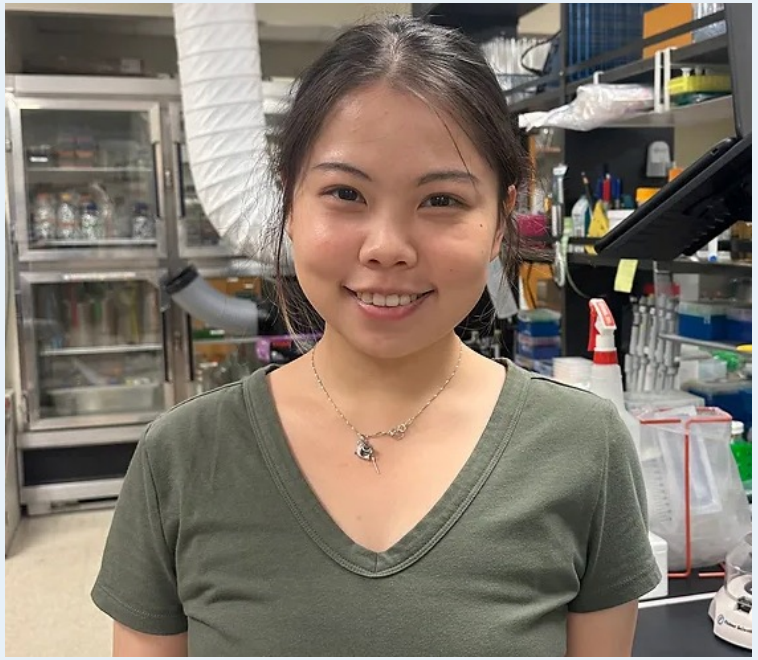
Future Directions

Immediate

- Continue utilizing program to find locations for insertion and analyze desirability
- Repeat experiment, tweaking certain aspects of protocol
  - Ex: increase length of homologous arms in designed plasmids, sample more *E. ludwigii* colonies following transformation, etc.
- Explore other possible methods of genetic engineering
  - Ex: CRISPR technology

Long Term

- Determine useful mechanisms for *E. ludwigii* to employ within sink microbiome
- Identify and harness specific functional genes to increase *E. ludwigii* robustness and probiotic/antimicrobial characteristics



Thanks to Dr. Crook and Qiaochu Li!

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