UNIVERSITY OF MARYLAND
HONORS COLLEGE

Spread Prevention and Eradication of Resistant Bacterial Growth

Team SUPERBUG

Neha Sripathi, Joshua Kim, PhucLam Dinh, Amber Rayford, Nicholas Breymaier, Cristina Zhang, Mackenzie Hillman, Dr. Daniel Stein

GEMSTONE
Honors College
University of Maryland

Introduction

- Diseases caused by drug resistant bacteria are a pressing public health threat
- This is due to a lack of new antibiotics and the evolution of multidrug resistance
- Drug resistance is caused by mutant or novel genes known as resistance genes
- CRISPR-Cas9 gene editing has been shown to edit resistance genes and increase susceptibility to antibiotics
  - We aim to improve upon the efficiency of previous studies.

Research Problem

- Drug resistance disproportionately affects minorities and people of lower socioeconomic status (1).
  - Antibiotic resistant infections are more expensive to treat, further burdening disadvantaged populations (2).
- We are studying a common nalidixic acid resistance mutation in the gyrase A gene (*gyrA*) in *E. coli*.
  - Single nucleotide substitution at codon 87 in the gyrase A gene
  - Ideal for targeting with CRISPR-Cas9 and homology directed repair (3)
- Urinary Tract Infections (UTIs) caused by *E. coli* are the most common type of bacterial infection in females (4).
  - UTIs are rapidly becoming nalidixic acid resistant.
  - Colistin, a harsher antibiotic, is the only current alternative treatment.

Research Question

Can we efficiently deliver a CRISPR-Cas9 gene editing system into nalidixic acid resistant *E. coli* in order to edit a single nucleotide substitution in *gyrA* and resensitize it to nalidixic acid?

Methodology Overview

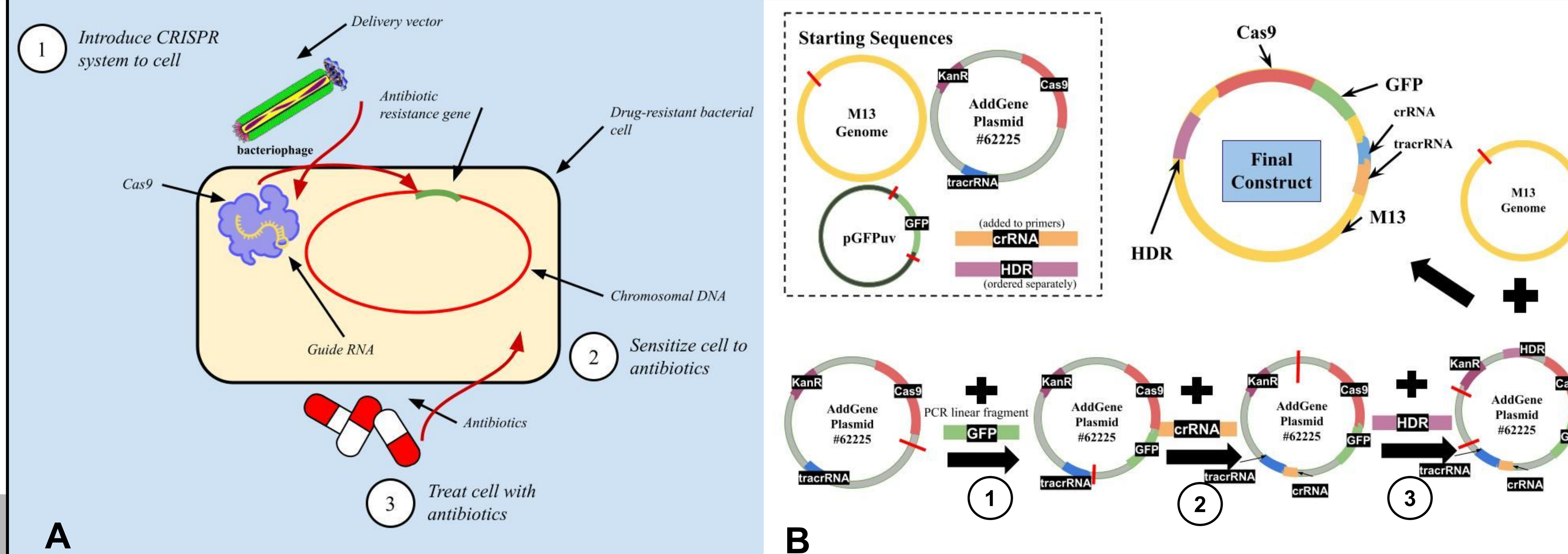

Figure 1. A) Graphical overview of methodology, outlined below. B) Bioinformatic design of the final CRISPR-Cas9 construct.

1. Create JM101 *E. coli* mutants and culture JM101 and JM109 strains
   a. Verify strain resistance
   b. Verify sequences
2. Design gene sequence of the bacteriophage M13 insert which will be used to deliver the CRISPR-Cas9 system to *E. coli cells*
3. **Conduct PCR and restriction digest experiments to create the final construct**
4. Insert construct into M13 vector, isolate transductants, and verify codon 87 editing & re-sensitivity

Results

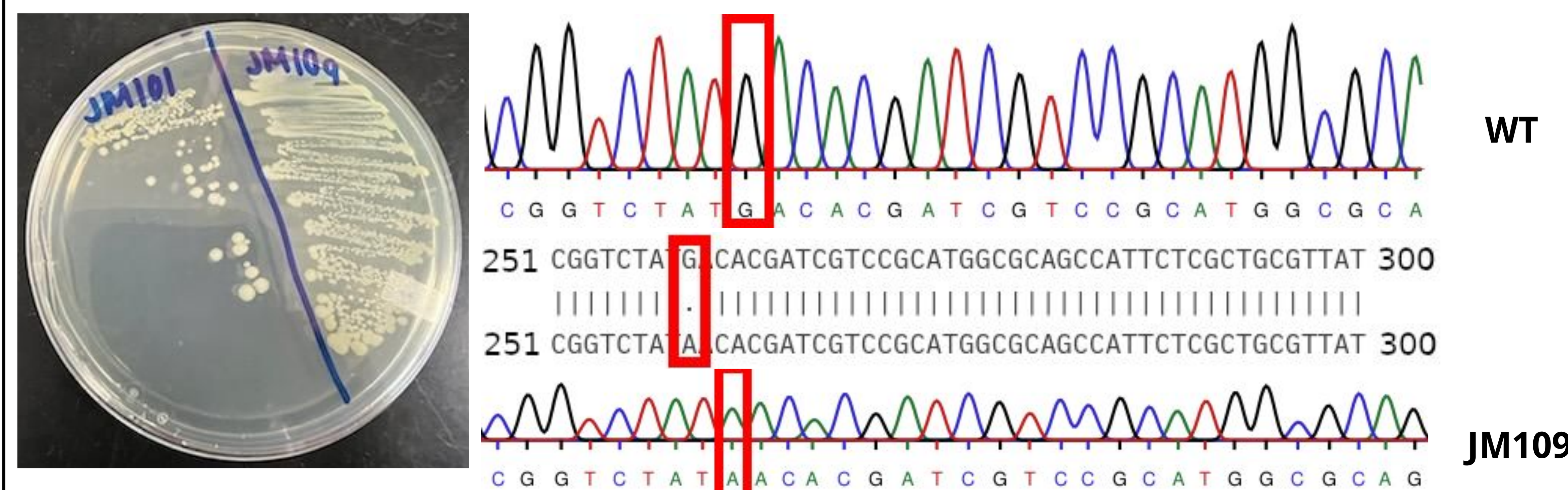

Figure 2. Generation of JM101 nalidixic acid resistant mutants & validation of JM109 resistance and *gyrA* codon 87 mutation.

Table 1. Primer Design

Primer Function	Forward Sequence	Reverse Sequence
gyrA sequencing	5'-CAGATGTCCGAGATGGCCTG-3'	5'-CGGCCATCAGTTCATGGGCA-3'
Adding Cas9 plasmid FseI restriction site	5'-attGGCCGGCCttttagatgaagattatttct-3'	5'-actGGCCGGCCtatacttcagtcacctccta-3'
Adding FseI restriction sites to GFP sequence	5'-cagtcaGGCCGGCCaacaatttcacacaggaaacagctatga-3'	5'-ctgacaGGCCGGCCggaattcattatttgtagag-3'
Inserting crRNA	5'-ctggtaacaggattagcaga-3'	5'-CTATAACACGATCGTCCGCAgttttagagctagaaatagc-3'

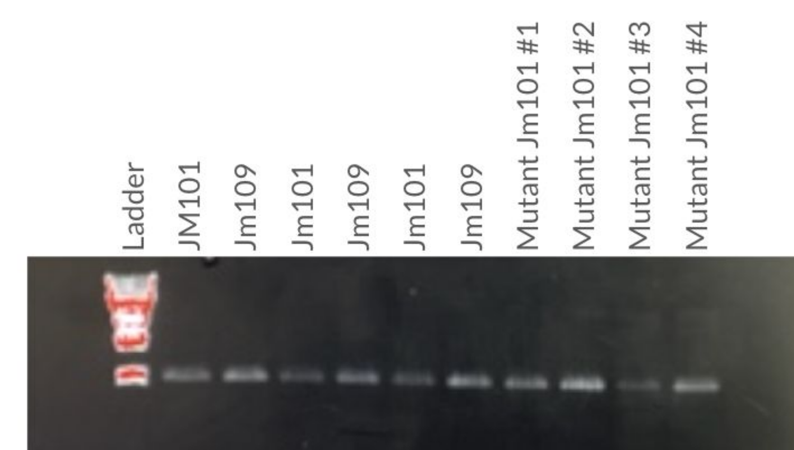

Figure 3. PCR of *gyrA* across JM101 and JM109 strains; primer validation.

Current Work

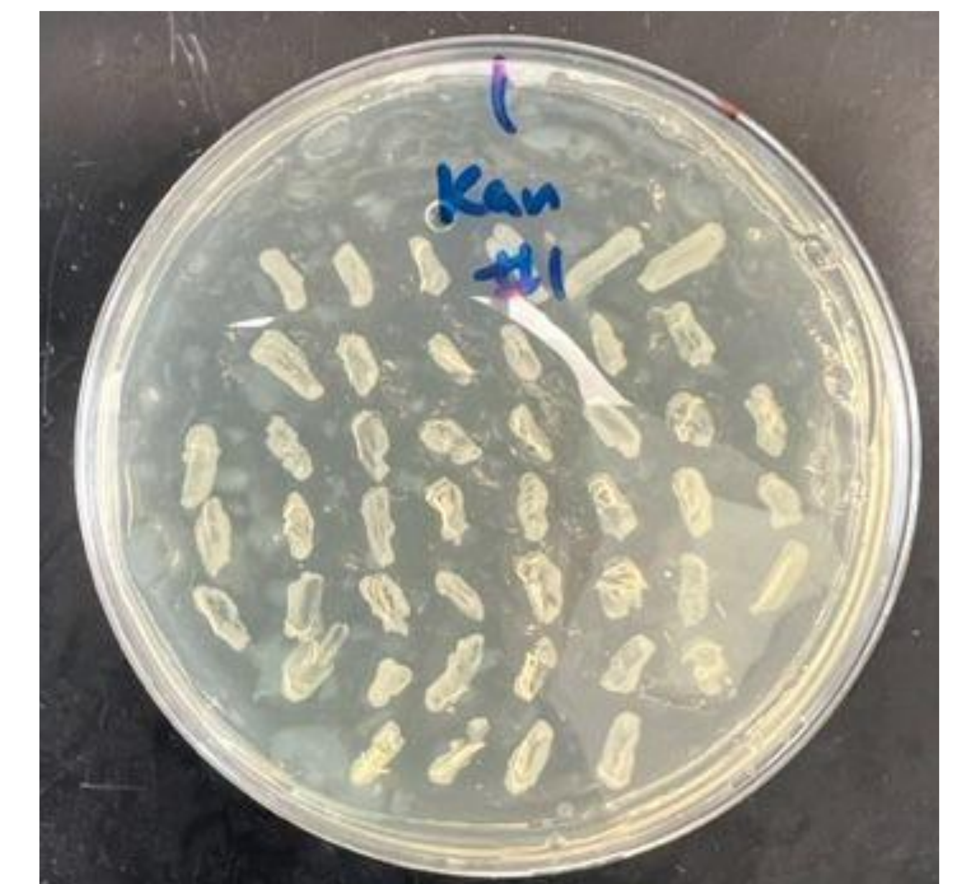

Figure 4. Cas9-GFP transformants

- Validating plasmid modification and bacterial transformation using GFP expression
- Working on adding additional CRISPR and HDR components to plasmid

Remaining Work

1. Transform modified CRISPR plasmid into JM109 colonies
2. Screen for Cas9-GFP expression and nalidixic acid sensitivity
3. Sequence transformants to verify successful editing of codon 87
4. Insert modified CRISPR plasmid into M13 genome and produce modified virions
5. Infect JM109 with modified M13 viruses
6. Screen for effective delivery and expression of CRISPR and reversion of codon 87 mutation

Acknowledgements and References

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