Developing the CRISPR Interference System to Understand Bacterial Gene Function

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THE PROBLEM

- With an increasing amount of DNA sequences available, there is a demand for new tools for *functional genomics* to reveal the function of genes identified by genome sequencing projects

THE SOLUTION

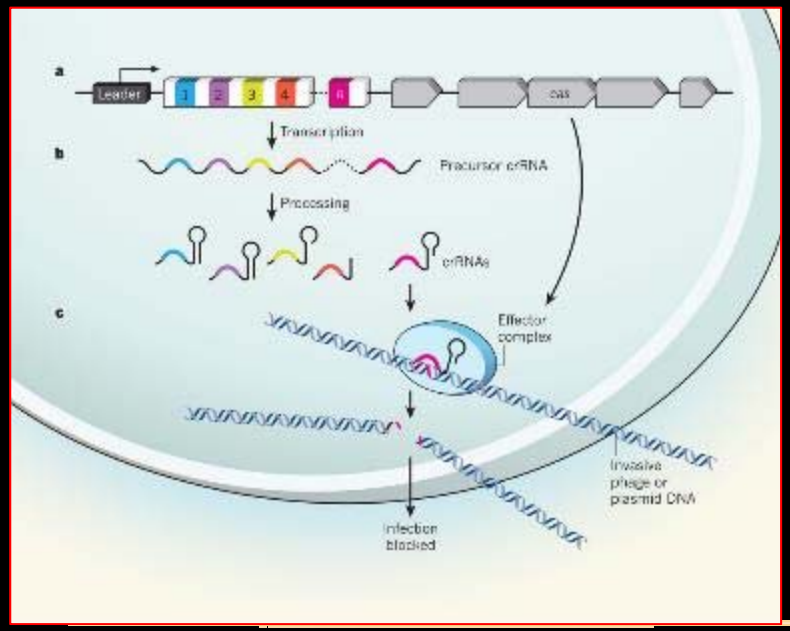
- Development of a genetic system to repress expression of any targeted gene by use of CRISPRi technology
  - Useful to study gene function in multiple bacterial species
  - Can target multiple genes for simultaneous inactivation

Devaki B, M Davison, R Barrangou. CRISPR-Cas Systems in Bacteria and Archaea: Versatile Small RNAs for Adaptive Defense and Regulation. 2011. *Annu. Rev. Gen.* Vol. **45**:273-297.

Tao X, L Yongchao, JD Van Nostrand, Z He, J Zhou. Cas9-Based Tools for Targeted Genome Editing and Transcriptional Control. 2014. *Appl. Environ. Microbiol.* **80**(5):1544.

INTRODUCTION

- Bacterial CRISPR (clustered regularly interspaced short palindromic repeats) System - a newly understood bacterial defense mechanism against horizontal transfer of bacteriophage and plasmid DNA
- Cas9 protein - degrades foreign DNA when bound to guide sequences



Sontheimer EJ, LA Marraffini. Microbiology: Slicer for DNA. 2010. *Nature*. **468**:45-46.

CRISPR INTERFERENCE (CRISPRi)

- Repressor proteins are effective to shut off gene expression (e.g., LacI, cI, Cro, GalR)
- Cas9 modified by inactivation of nuclease activity (Cas9*)
- Cas9* can be targeted to specific genes by co-expression of RNA guide sequences
- Cas9* functions as a repressor of transcription of targeted genes

Qi L, M Larson, L Gilbert, J Doudna, J Weissman, A Arkin, W Lim. 2013. Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression. Cell. Vol. **152**(5): 1173-1183.

CRISPRi SYSTEM

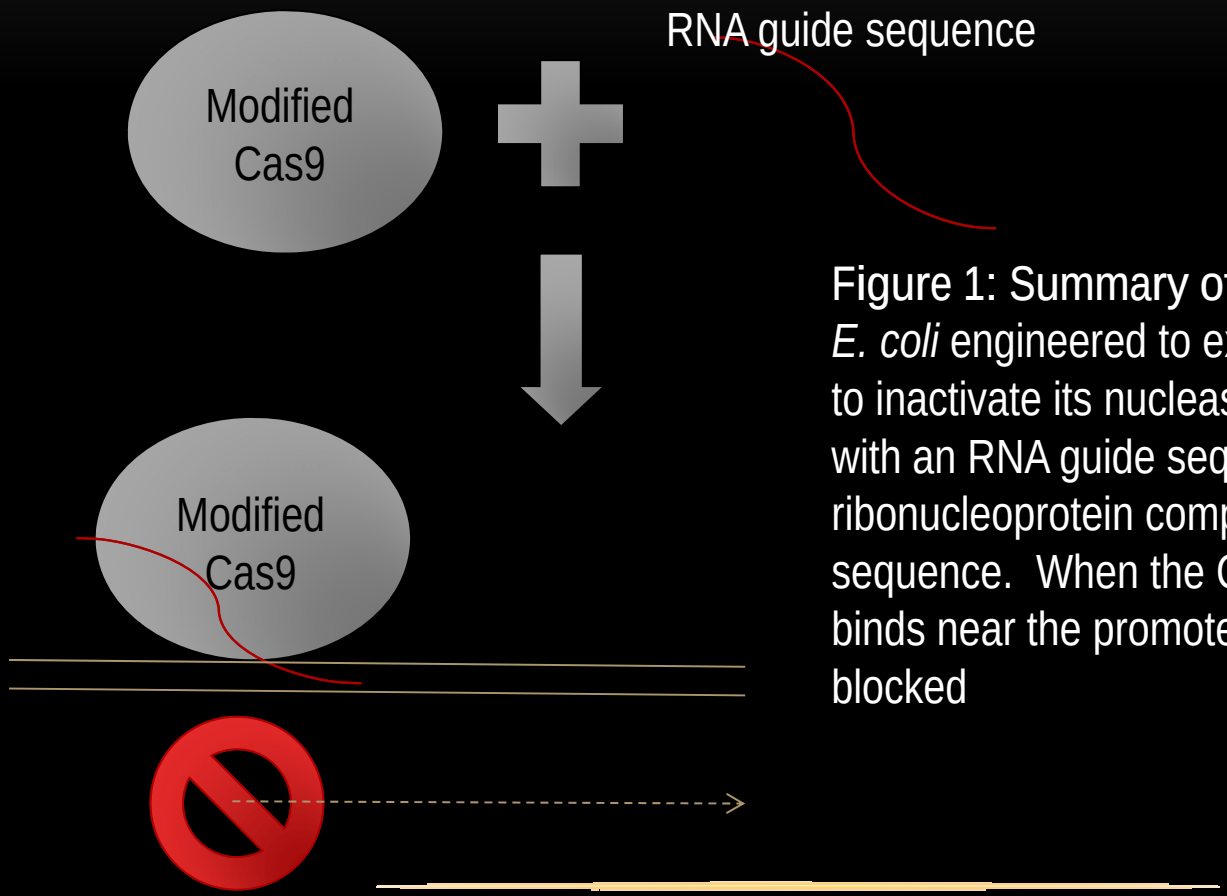


Figure 1: Summary of CRISPRi system
E. coli engineered to express Cas9 modified to inactivate its nuclease activity complexes with an RNA guide sequence to target the ribonucleoprotein complex to specific DNA sequence. When the Cas9-RNA complex binds near the promoter region transcription is blocked

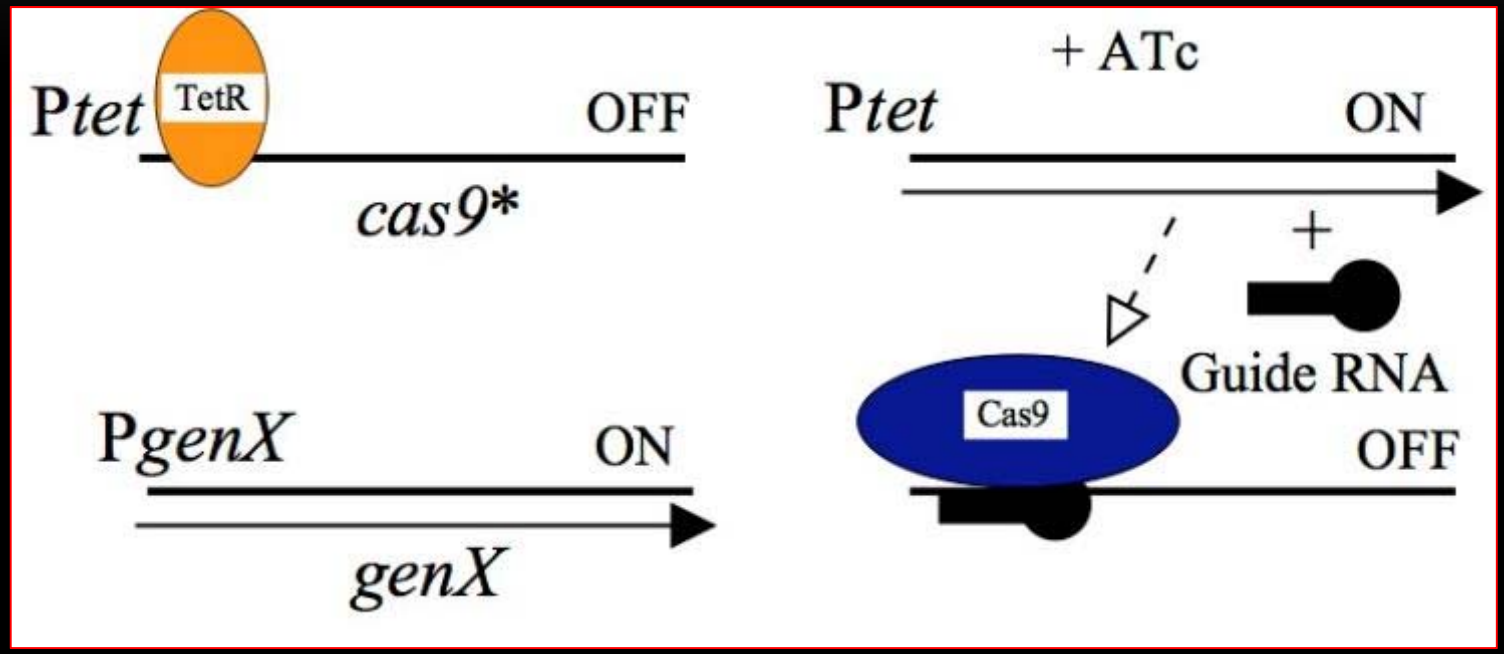
METHODS

- To adapt this system as an effective means to silence bacterial genes, we:
  - (1) Placed Cas9* expression under control of the *tet* promoter, and
  - (2) Integrated the P*tet-cas9** into the *E. coli* chromosome using CRIM Integration
  - (3) Constructed RNA guide sequences via PCR amplification
  - (4) Tested the system to repress expression of genes for:
    - Carbohydrate metabolism (*lac*)
    - Essential genes

Haldimann A, BL Wanner. Conditional-Replication, Integration, Excision, and Retrieval Plasmid-Host Systems for Gene Structure-Function Studies of Bacteria. 2001. *J. Bacteriol.* Vol. **183**(21): 6384-6393.

Qi L, M Larson, L Gilbert, J Doudna, J Weissman, A Arkin, W Lim. 2013. Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression. Cell. Vol. **152**(5): 1173-1183.

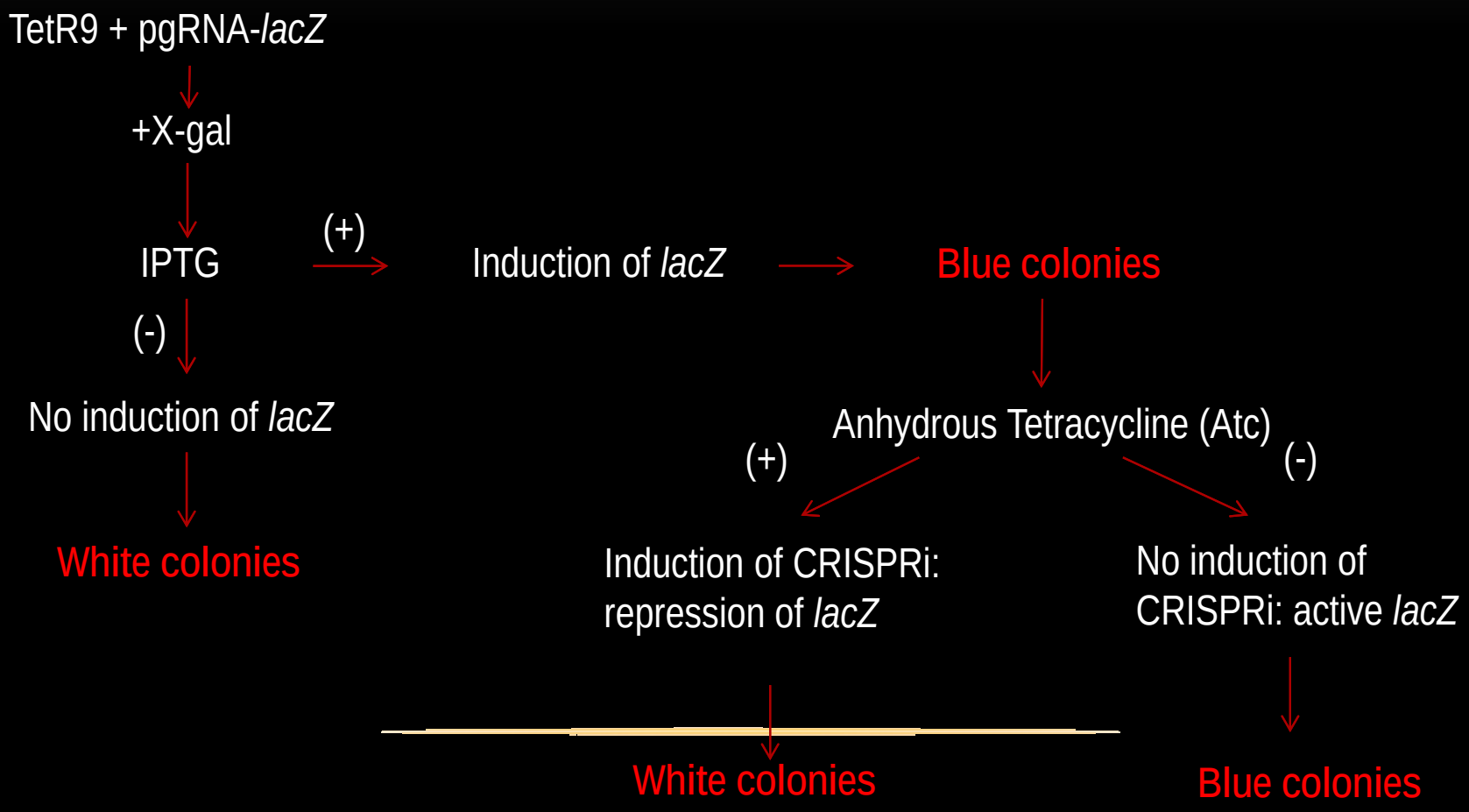
FIGURE 2: SUMMARY OF CAS9* EXPRESSION SYSTEM



genX = gene of interest
TetR = Tetracycline Represser

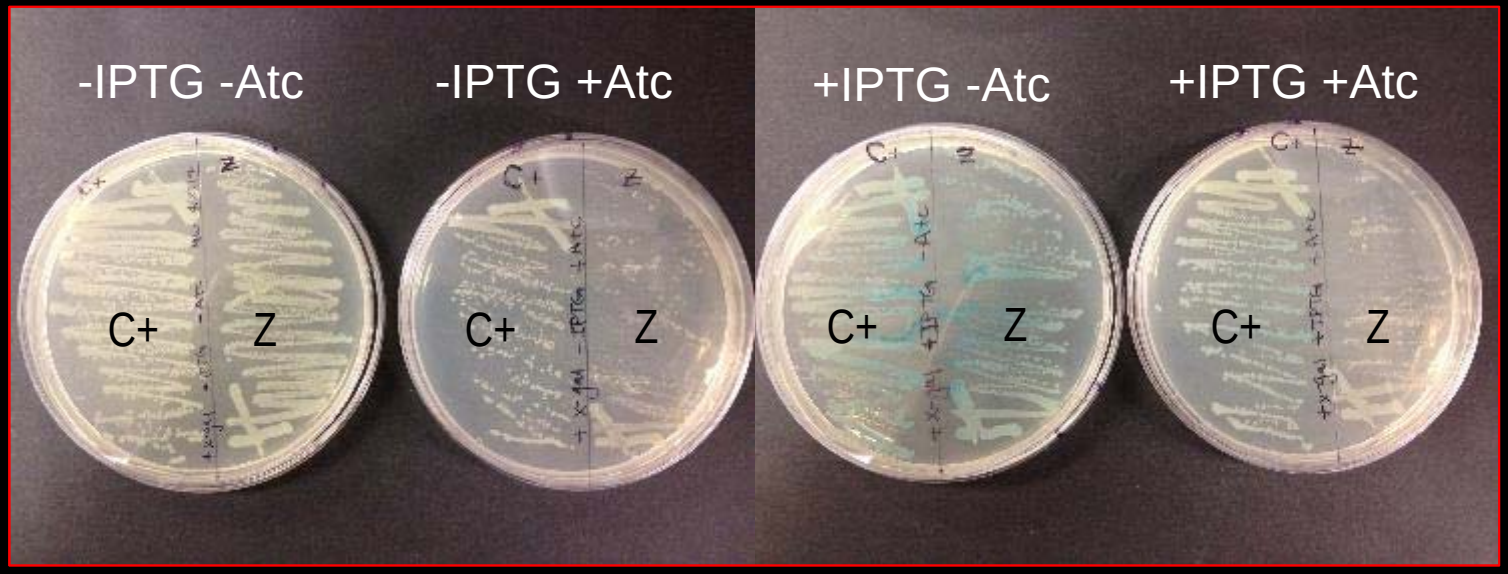
Atc = Inducer
P*tet* = *tet* promoter

FIGURE 3:
ANHYDROUS TETRACYCLINE CONTROL



RESULTS

Figure 4: Repression of *lacZ* by CRISPRi

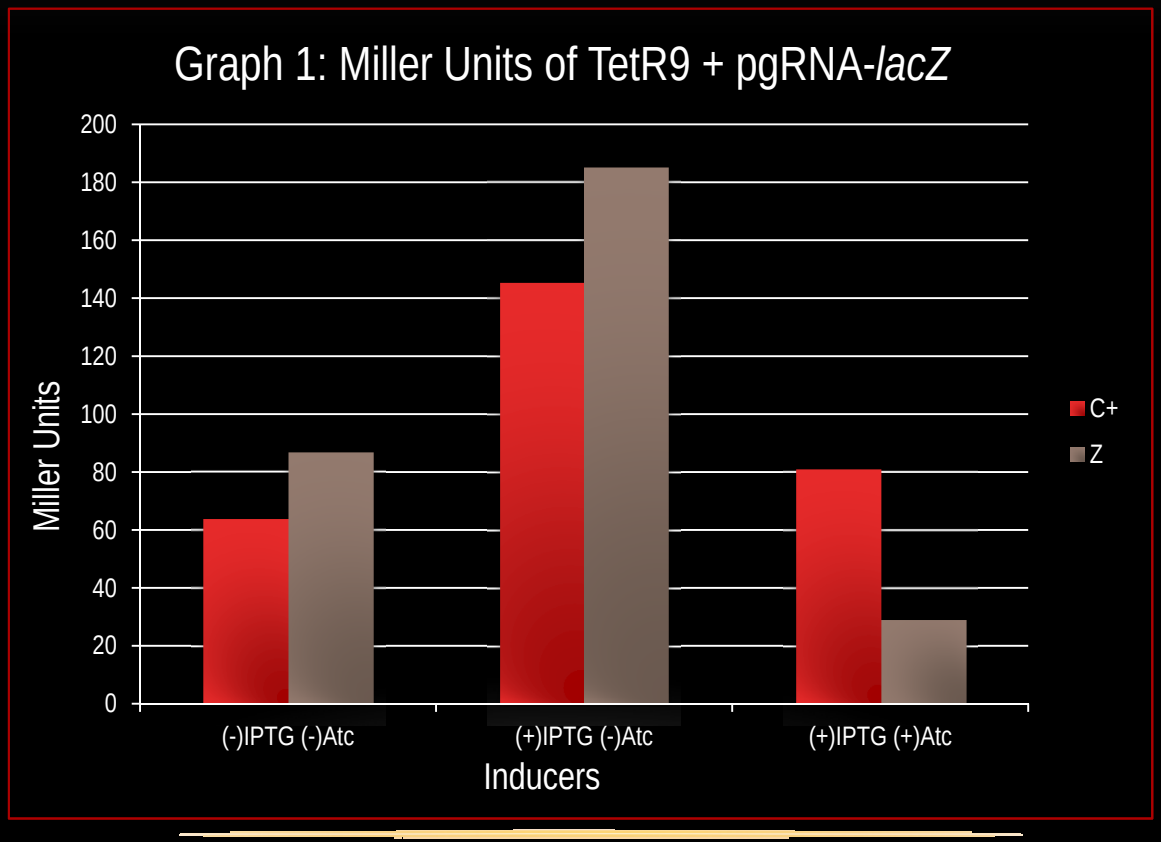


IPTG = Inducer of *lac* operon Atc = Inducer of CRISPRi

C+ = CRISPRi + control Z = CRISPRi + pgRNA-*lacZ*

RESULTS

Figure 5: Beta-Galactosidase assay using TetR-9



C+ - pgRNA control

Z - pgRNA-*lacZ*

RESULTS

Figure 6: Repression of essential genes using TetR9 CRISPRi system

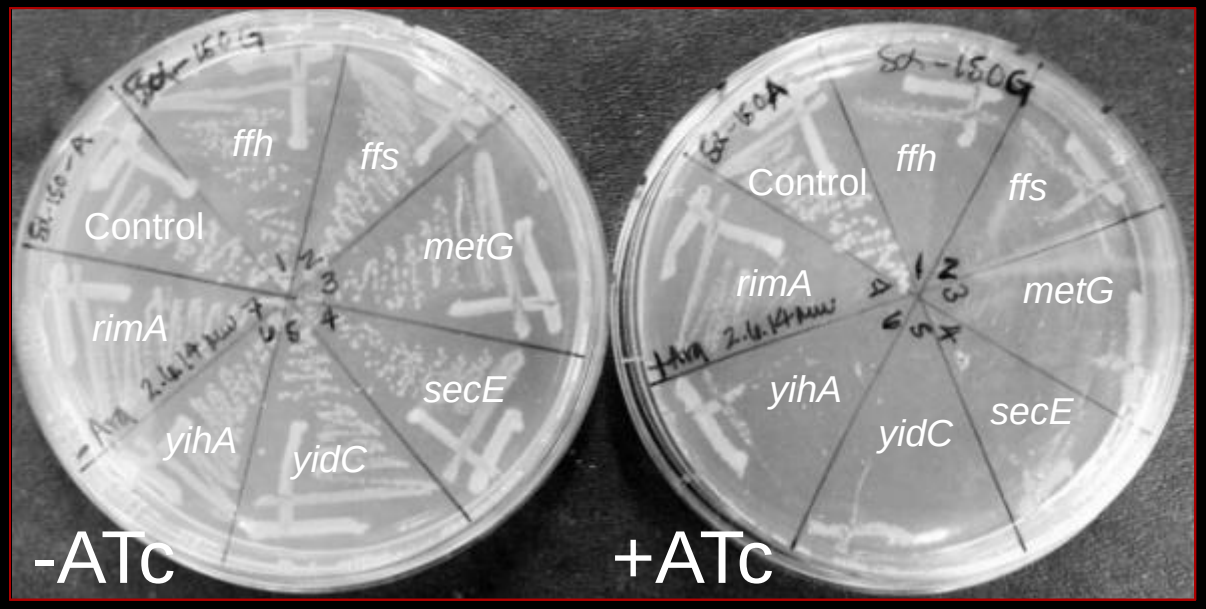


Table 1: Genes targeted for repression

Gene	Function
metG	Protein synthesis
ffs	Signal recognition particle
ffh	Signal recognition particle
secE	Protein translocation
yidC	Membrane protein insertion
yihA	GTP binding protein
rimA	Protein translation

CONCLUSIONS

- Successful development of CRISPRi for conditional expression of *E. coli* genes
- Cas9* under P_{tet} control & integrated into chromosome
- Multiple genes targeted by construction of plasmids expressing guide RNAs
  - Essential genes
  - *lacZ* (beta-galactosidase expression)

FUTURE STUDIES

- Silencing of multiple genes with redundant function to better understand antibiotic persistence
- Use of CRISPRi in other bacterial species, including pathogenic bacteria as a new means to identify virulence factors
- Direct visualization of chromosome dynamics (Cas9*-GFP)