Genetically Engineering a Plasmid to Develop a Live-Dead Reporter System in *Mycolicibacterium smegmatis*

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Background on Tuberculosis

Mycobacterium tuberculosis (Mtb) is an infectious bacteria that causes damage to the lungs, and is often lethal in humans. It is successful due to the ability to resist and tolerate antibiotics. Tuberculosis (TB) progresses through the formation of the granuloma. The conditions inside of the granuloma are very stressful for the bacteria yet it is still able to survive.

Current methods of determining if cells are alive after antibiotic treatment are cumbersome and may not be accurate. **The goal of our research is to develop a live-dead fluorescent reporter system to detect cell viability after treatment with antibiotics**

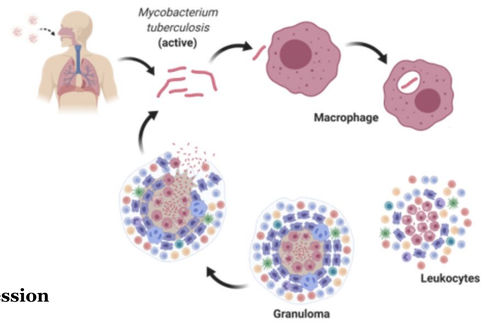


Figure 1. Granuloma formation and progression of TB

UN Sustainable Development Goals

The UN Sustainable Development Goal that our research project took inspiration from is: Good Health & Well Being. This relates to our research because TB effects people globally and disproportionately affects impoverished countries.

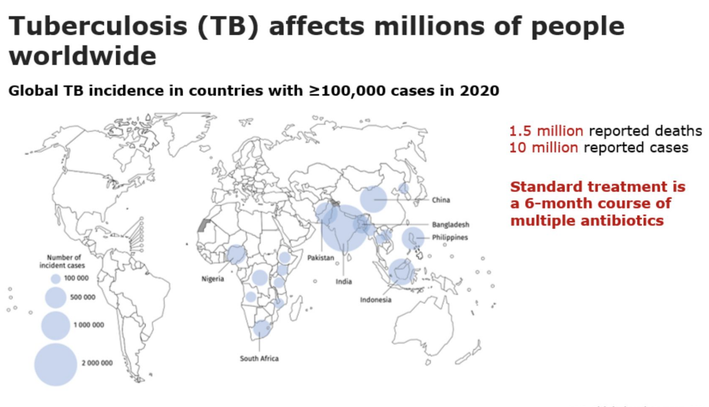
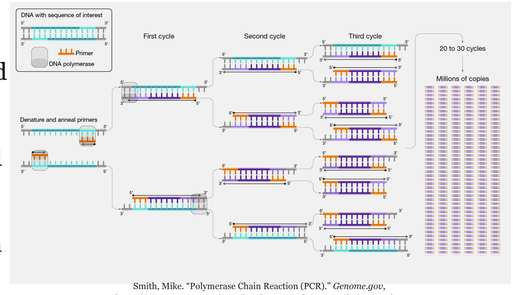


Figure 2. Worldwide effects of TB

Methods and Results

1. Design primers to create DNA fragments, which are segments of DNA sequenced from existing plasmids. The plasmids we used were a backbone which is a plasmid already containing GFP, a promoter region, mScarlet fluorescent protein. We then amplified fragments using PCR (Polymerase Chain Reaction).



Smith, Mike. "Polymerase Chain Reaction (PCR)." *Genome.gov*, https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction

Figure 3. Polymerase Chain Reaction Steps and Process

2. Treat PCR products with the DpnI enzyme, and run gel electrophoresis to check the size of the products. Extract and purify the DNA bands.

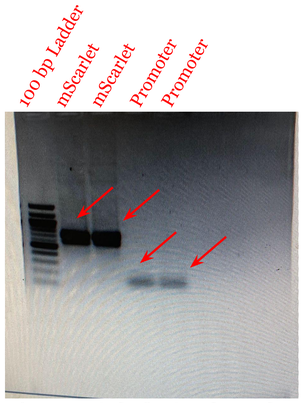


Figure 4. Gel of mScarlet and promoter PCR products, arrows indicate bands

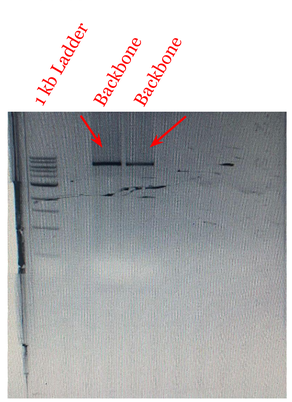


Figure 5. Gel of backbone PCR product, arrows indicate bands

3. Perform HiFi assembly and transform the plasmid into *E. coli*.

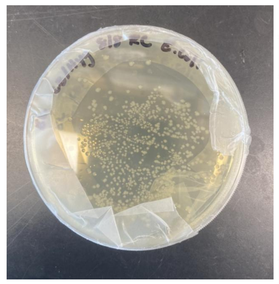
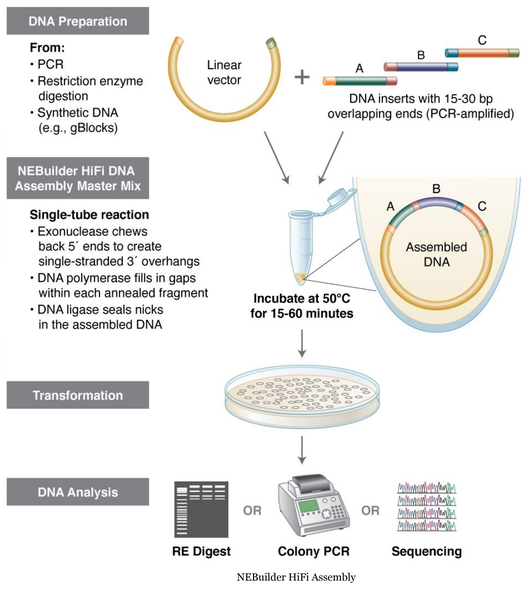


Figure 6. HiFi Assembly Steps, Process, and Results

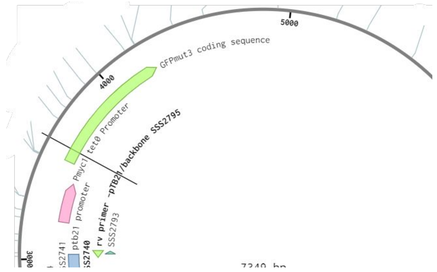


NEBuilder HiFi Assembly

Conclusions

Our primers were successful in generating the correct DNA fragments needed for creation of the plasmid shown. This plasmid will be next transformed into *M. smegmatis* and used in future works.

Figure 7. Engineered Plasmid



Classroom Connection

Middle School: ***Case Study on Tuberculosis and Antibiotic Resistance.***

- Allow students to select a country to research and represent at a mock UN conference centered on the topics of antibiotic resistance, and TB treatment.

High School: ***Develop a New Antibiotic***

- Students act as medical researchers by creating a novel treatment that inhibits expression of antibiotic resistance genes.

Future Work

- Transform the plasmid into *M. smegmatis* and check the fluorescence to see if mScarlet works in combination with the pTB21 promoter

- Treat with antibiotics and use flow cytometry to measure cells viability using red and green fluorescent proteins to distinguish alive vs. dead cells.

Acknowledgements

This material is based upon work supported by the National Science Foundation Grant No. EEC-2055507. We would also like to thank Scarlet Shell and the members of the Shell Lab!

https://wp.wpi.edu/ret-stem/

References

Smith, Mike. "Polymerase Chain Reaction (PCR)." *Genome.gov*, https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction.

NEBuilder HiFi Assembly

Shell Lab Research Slides