Genetically Engineering a Plasmid to Develop a Live-Dead Reporter System in *Mycobacterium 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.

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).

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. 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.

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

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.

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References

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