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# Construction of a thiF genetic disruption in Bacillus subtilis

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The goal of our research is to determine whether the level of transcription of a gene is correlated with the level of mutation in that gene. One factor involved in the mutability of a transcribed gene is the ability of the single stranded DNA to form secondary stem loop structures (SLS), in the wake of the transcription bubble, that contain unpaired mutable bases. We are interested in correlating the levels of mutation with transcription in the *thiF* gene, which is predicted by bioinformatic analysis to be highly mutable. To achieve this goal, Kathleen will first construct a non-polar *thiF* genetic knockout using a chloramphenicol cassette. Then, she will test the phenotype of the $ThiF^-$ strain. She will also build an IPTG-inducible construct containing *thiF* with a stop codon in the loop of a putative SLS. This will be introduced into $ThiF^-$ *Bacillus subtilis* and assayed for the accumulation of $Thy^+$ mutations under starvation conditions, in the presence and absence of IPTG.

# Construction of a *thiF* Genetic Disruption in *Bacillus subtilis*

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## Abstract

The goal of our research is to determine whether the level of transcription of a gene is correlated with the level of mutation in that gene. We are interested in observing whether increasing expression of the *thiF* gene, which is predicted by bioinformatic analysis to be highly mutable, will result in increased reversion of a point mutation in this gene. To achieve this goal a non-polar *thiF* genetic knockout using a chloramphenicol cassette was constructed and introduced into *Bacillus subtilis* strain YB955. The phenotype of the ThiF⁻ strain will be tested. An inducible construct containing the mutated *thiF* gene will then be introduced into the YB955 *thiF::cm* strain. This will allow us to assess the correlation between levels of *thiF* mutation with levels of transcription.

## Introduction

1.Stationary phase mutagenesis is the process by which non-dividing cells in a stressful environment may acquire adaptive mutations resulting in genetic diversity.

2.Previous experiments in *E. coli* and yeast have demonstrated that levels of transcription are correlated to levels of mutation of genes in growing cells (1,2).

3.Single-stranded DNA can form secondary loop structures in the transcription bubble (Fig. 1). The non-paired bases in these structures are predicted to be prone to higher mutability rates due to exposure to mutagenic substances (such as oxidative damage, lack of dinucleotide triphosphates, and conditions of low or no repair) in the cells' cytoplasm (3).

4. The *thiF* gene in *Bacillus subtilis* has been shown via bioinformatic analysis to be highly mutable.

5.We hypothesize that, in resting cells under non-lethal stress, increased transcription of genes induced under selective pressure results in a higher level of adaptive mutation of those genes.

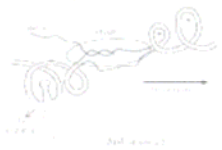

Figure 1. Trancription drives supercoiling of single-stranded DNA and the formation of stem-loop structures containing mutable unpaired bases.

**Objective: Is there a correlation between the level of transcription of *thiF* and the level of adaptive mutation of that gene?**

Goal One: Construct a *thiF* genetic disruption in *B. subtilis* strain YB955.

Goal Two: Integrate an expression vector containing a point-mutated *thiF* gene subcloned downstream of an IPTG-inducible promoter (Phyperspank) into the *amyE* locus.

Goal Three: Assay the accumulation of mutations in *Phs-thiF* during starvation conditions in stationary cells in the presence and absence of inducer.

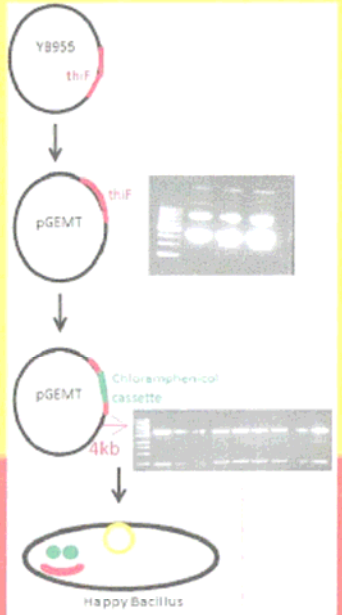


Figure 2. Experimental design.

## References

1.Wright, B.E. (2004).Stress-directed adaptive mutations and evolution. Molecular Microbiology, 52(3): 643-650.
2.Reimers, J.M., K.H. Schmidt, A. Longacre, D.K. Reschke, and B.E. Wright. (2004). Increased transcription rates correlate with increased reversion rates in leuB and argH Escherichia coli auxotrophs. Microbiology, 150: 1457-1466.
3.Datta, A. and S. Jinks-Robertson. (1995). Association of Increased Spontaneous Mutation Rates with High Levels of Transcription in Yeast. Science, 268 (5217): 1616-1619.

## Methods

1. Amplify the *thiF* gene from the genome of *B. subtilis* strain YB955 using PCR.
2. Subclone *thiF* into the pGEMT vector. Cut pGEMT-*thiF* with restriction enzymes ClaI and AfeI to remove a portion of *thiF*.
3. Amplify the chloramphenicol acetyltransferase cassette from the pMK4 vector by PCR.
4. Cut the cassette with ClaI and AfeI and ligate into cut pGEMT-*thiF*. This results in a genetic disruption of *thiF*.
5. Transform this construct into *B. subtilis* strain YB955 to disrupt chromosomal *thiF*. Assay this strain for the presence of *thiF::cam* by PCR and by testing the strain for thiamin (vitamin B1) auxotrophy.

## Future Directions

- A stop codon will be placed in a region of predicted high mutability in a putative stem loop structure of the *thiF* gene.
- This mutated gene will be subcloned downstream of an IPTG-inducible promoter.
- This construct will be introduced into strain YB955 and assayed for accumulation of Thy⁺ mutations under starvation conditions, in the presence and absence of IPTG.

## Acknowledgements

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Bob Marley for soothing my soul