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Constructing an ArgF- strain of 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 *argF* gene, which is predicted by bioinformatic analysis to be highly mutable. To achieve this goal, Allison will first construct a non-polar *argF* genetic knockout using a kanamycin cassette. Then, she will test the phenotype of the $ArgF^-$ strain. If a biochemical suppressor is present, she will disrupt the next possible genetic candidate. She will also build an IPTG-inducible construct containing *argF* with a stop codon in the loop of a putative SLS. This will be introduced into $ArgF^-$ *Bacillus subtilis* and assayed for the accumulation of mutations under starvation conditions, in the presence and absence of IPTG.

Constructing an ArgF- strain of *Bacillus subtilis*

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Abstract

The goal of our research is to determine whether and increase in the level of transcription of a gene results in an increased rate of mutation in that gene. The ability of the single stranded DNA to form secondary stem-loop structures (SLS) in the wake of transcription is one factor mediating mutations. A stable SLS has been predicted by bioinformatic analysis to be highly mutable. I am interested in testing whether there is a correlation between levels of transcription and accumulation of mutations in the *argF* gene. To achieve this goal, I constructed a non-polar *argF* genetic knockout using a kanamycin cassette. I will assay the phenotype of the ArgF- strain by plating on selective media, to determine appropriate growth conditions for all future work. Also, an IPTG-inducible *argF* construct will be mutated by site directed mutagenesis to contain a stop codon produced by a single base mutation in the *argF* gene. These constructs once introduced into *B. subtilis* will be assayed for the accumulation of mutations under conditions of arginine deprivation and in the presence and absence of IPTG.

Hypothesis

Increased transcription of genes containing mutable DNA sequences during non-lethal stress in arrested cells results in an increased mutation frequency.

Goal one: Introduce a genetic disruption of *argF* into *B. subtilis* strain YB955.
Goal two: Subclone *argF* containing a stop codon into an IPTG-inducible vector. (see figure 1)
Goal three: Test the *B. subtilis* YB955 strain containing the *pHS-argF* in an *argF::kan* background for the accumulation of Arg+ revertants under stressful conditions in the presence and absence of inducer.

Background

•A significant fraction of mutations arise via growth dependant processes.

•Interestingly there are mutagenic processes occurring in non-dividing cells. These mutagenic processes have been implicated in the generation of mutations that ultimately result in cancer.

•Some factors involved in the generation of mutations in non-dividing cells include error-prone polymerases, differentiation of cell subpopulations and transcription factors.

•Davis (1989) proposed transcription as a process mediating the formation of mutation in non-dividing cells.

•Wright et al., (2000) postulated that the process of transcription generates DNA structures that are prone to damage. (see figure 1)

•In this work, we generate an allele to test the concept of transcription associated mutagenesis.

Figure 1
Transcription induced stem loop structure
Red 'T' is a potential point mutation resulting in a stop codon in future studies

Methods

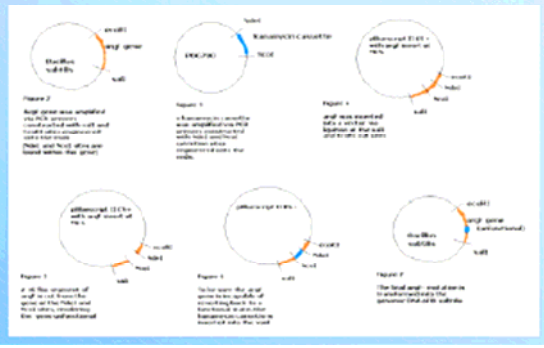
•The *argF* gene was amplified from *Bacillus subtilis* using primers designed with *SalI* and *EcoRI* sites on the ends. (Figure 2)

•A kanamycin cassette was amplified from PDG780 with primers designed to add restriction sites for *NcoI* and *NdeI* to the ends of the cassette. This cassette was later used to knock out the *argF* gene (Figure 3)

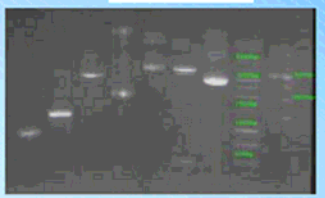
•Once the plasmid had been dephosphorylated, *argF* was then inserted into pBluescript KS+ II (pBSK). Successful ligation of the *argF* gene into the pBSK vector was identified by white colonies of *E. coli* into which, the plasmid was transformed. (Figure 4)

•pBSK with the *argF* insert was cut with the restriction enzymes *NcoI* and *NdeI*, providing a ligation site for the kanamycin cassette, which disrupts the *argF* gene. (Figure 5 and 6)

•The plasmid was cut with *ScaI* to linearize the vector, and was transformed into *B. subtilis YB955*. The phenotype of the *argF::kan* mutant will be determined.



Results



The image above of a gel electrophoresis identifies the products at each step of the process of constructing the *argF*- plasmid. Each lane is described below:

1. The kanamycin cassette amplified from PDG780
2. The *argF* gene amplified from *B. subtilis*
3. pBSK linearized via a restriction diigest with *EcoRI*
4. pBSK purified from *E.coli* cells (supercoiled)
5. pBSK containing *argF* (pBSK: *argF*)
6. pBSK: *argF* w/ 0.4kb cut from gene sequence
7. pBSK: *argF*- with kanamycin cassette insert into *argF*
8. 1kb ladder (as labeled)
9. supercoiled ladder (as labeled)

Discussion

•The first goal of this experiment was to produce an *argF*- allele in *Bacillus subtilis*. The *argF* - strain of *B. subtilis* is available for continuation of this project.

•From this experience I have learned numerous methods of restriction digesting as well as ligating and understand that different methods are successful under different circumstances.

Acknowledgement

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