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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 assay 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 gene 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.

Background

- A significant fraction of mutations arise via growth dependent 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.

Methods

- The argF gene was amplified from *Bacillus subtilis* using primers designed with *SacI* 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 *SacI* to linearize the vector, and was transformed into B. subtilis YB955. The phenotype of the argF-kan mutant will be determined.

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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