dnaE temperature sensitive conditional mutation in order to synchronize a population of cells at G1

Renée Nicolas and Professor Katherine Gibson
University of Massachusetts Boston, Boston MA 02125

Methods and Results:

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Ligation of dnaE and pK18mobsacB:

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Transformation of plasmids into E.coli:

1. Competent Cell Negative Control - 0 colonies
2. Ligation Negative Control - 0 colonies
3. Ligation of dnaE and pK18mobsacB - 21 colonies

Transformation of ligation products into E.coil:

This procedure and the transformation of the plasmid, pK18mobsacB, into E.coli are identical.

Conclusion:

I worked to create a temperature-sensitive conditional allele of dnaE. I found that the ideal annealing temperature for the dnaE primers is 59°C with 5.5 amplification cycles. An intermediate PCR cloning step into pCR-Blunt II-TOPO was attempted, however this proved to be impossible due to the size of dnaE. Using the restriction enzymes EcoRI and XbaI, a double digest was performed with dnaE and pK18mobsacB. A ligation was set up with the dnaE insert and the plasmid. The ligations were transformed into DH5α to select for recombinant plasmids, however the presence of colonies on the negative control plate provided evidence that the double digest was unsuccessful since digested plasmid was able to reanneal during the ligation reaction.

Future Direction:

The next step of this project is to successfully clone dnaE into pK18mobsacB and then perform site-directed mutagenesis in order to create the dnaE::tau allele. From there, this dnaE::tau allele will be recombined into the S. meliloti genome and tested for a growth phenotype by assayable viability of cells expressing the altered dnaE allele grown at 30°C versus 37°C. Flow cytometry will be utilized to determine the amount of DNA in the population of cells at both 30°C and 37°C in order to test whether this allele synchronizes cells in G1. If successful, our lab will then be able to utilize this dnaE strain to study cell cycle progression using a population of cells all at the G1 phase.

Works Cited:


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