CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY

Name(s)
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Project Number
J1718

Project Title
Optimizing Bacterial Transformation Efficiency: A Study of Heat and Cold Shock Parameters and DNA Plasmid Concentration

Abstract

Objectives/Goals
Bacterial transformation is widely used in commercial applications and research laboratories as a way to introduce or transfer one or more new genes into a cell. My project aimed to investigate how different heat and cold shock parameters and DNA plasmid concentration affect the transformation efficiency of bacteria in a two-step chemical transformation protocol. I hypothesized that longer the heat shock and cold shock duration at the recommended temperature with the recommended DNA concentration should result in the highest transformation efficiency.

Methods/Materials
My control was the experiment at the recommended (nominal) condition (42C heat shock temperature, 50 second heat shock duration, 2 minute cold shock duration, 1X DNA plasmid concentration). I varied the following parameters: heat shock temperature, heat shock duration, cold shock duration, and DNA plasmid concentration. Experiments were conducted such that only one parameter varied at a time, while keeping other parameters at nominal values. I used Bio-RadOs pGLO plasmid, which consists of three genes: a Green Fluorescent Protein (GFP), ampicillin resistance, and an ara operon gene. I used Escherichia coli HB 101 K-12, a non-pathogenic, gram-negative bacterium.

Results
Amongst the cold shock durations tested, 30 minutes produced the best results, 975 transformants/µg. For the heat shock temperatures tested, 37C was optimal, 294 transformants/µg. Amongst the heat shock durations tested, both 25 and 50 seconds yielded the highest transformation efficiency, 84 transformants/µg. For the DNA plasmid concentration, both 0.1X and 0.01X concentration yielded 625 transformants/µg.

Conclusions/Discussion
The 25 or 50 second heat shock duration was optimal because it allowed enough time for the cells to become susceptible to the plasmid DNA, and not damaged by the heat. The 30 minute cold shock duration was the best because it gave the cells sufficient time to repair after the heat shock. I think that 37C produced the highest transformation efficiency because there was a good probability of cells undergoing transformation as these cells were not damaged by an elevated temperature. For the DNA plasmid concentrations, I concluded that the saturation point of the DNA plasmid lied within 0.1X and 1X, since the transformation efficiency remained constant for concentrations below 0.1X and decreased for concentrations above 0.1X.

Summary Statement
This project aimed to investigate how different key parameters in a two-step chemical transformation protocol affected the transformation efficiency of bacteria.

Help Received
I used lab equipment at Stanford University under the supervision of Prof. Allan Campbell; Prof. C. Ouverney (SJSU) guided me on Epi-fluorescent microscope; Prof. I. Gabashvili (SJSU) introduced me to Blastx; Prof. C. Hackworth (West Valley College) advised me on experimental parameters.