CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

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Project Number

S1410

Project Title

Multi-Factor Optimization of Gene Expression in Saccharomyces cerevisiae

Abstract

Objectives/Goals

The goal was to determine optimal conditions for the induction of native soluble Ste7 and Kss1, proteins involved in the Saccharomyces cerevisiae pheromone pathway.

Methods/Materials

Factors identified as likely to improve solubility were IPTG induction level, induction temperature, ethanol, and NaCl. Orthogonal arrays were used to design the experiments so that all four factors could be varied simultaneously. A stastical method was used to find a robust, high-output combination of levels of factors. The initial conditions were 1mM IPTG at 30^C, without ethanol or NaCl. We predicted that decreasing the induction temperature and IPTG level, anad adding NaCl would increase the expression of soluble protein. This hypothesis was tested by inducing E.coli under the sets of conditions dictated by the orthogonal array. The amount of protein produced was quantified via spectrophotometry of SDS-PAGE gels and Western blotting membranes.

Results

The best conditions for Ste7 were 0.1 to 0.5mM IPTG at 25^C, without ethanol or NaCl. Analysis of variance showed that 70.5% of the increase in soluble protein resulted from changing the IPTG level and 9.6% from induction temperature. Ethanol and NaCl were ineffective at increasing solubility. Bradford protein assays showed that purification of a 100-mL culture increased by 240% when conducted at 0.1 mM and 30^C. Kss1 was difficult to express, but after changing to ER2256 strain, a small amount of Kss1 was detected.

Conclusions/Discussion

Additional testing should be done to refine levels of factors to better understand signal transmission and amplification by cells.

Summary Statement

The goal was to determine optimal conditions for the induction of native soluble Ste7 and Kss1, proteins involved in the Saccharomyces cerevisiae pheromone pathway.

Help Received

The team used lab equipment at Molecular Sciences Institute under the supervision of Dr. Myron Williams.