CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

Megan Li; Michael Lin

Project Number

S0410

Project Title

Novel Method for Genetic Screening for Elements of Transduction Pathways

Abstract

Objectives/Goals

Vital to our understanding of cellular responses is the identification of regulatory proteins in the transduction pathways responsible for the expression of genes. To increase the efficiency of such genetic screens, we have tested a assay using a cell sorter and a Saccharomyces cerevisiae (yeast) deletion collection.

Methods/Materials

Rapamycin and the Mep2 yeast gene are used to test this technology. The controls in this procedure are Ho1, a wildtype strain; Fpr1, a rapamycin receptor; and Gln3 and Gat1, known regulators of Mep2 pathway.

The gene expression reporter in this experiment is a plasmid made up of the Mep2 promoter and the green fluorescent protein (GFP) gene. A collection of yeast deletion strains, each with one specific gene removed and identified by two unique DNA barcodes, is transformed with the plasmid and treated with rapamycin. The cells are fed into a Fluorescence-Activated Cell Sorter (FACS), which scans and sorts individual cells by fluorescence. Polymerase chain reactions (PCR) amplify the DNA barcodes in both pools, which are hybridized to yeast genome microarray chips. The chips are scanned to measure the knockout strain concentrations. The strains that show the highest GFP- to GFP+ ratios are individually examined with rapamycin under a fluorescent microscope to confirm that they encode components of the signal pathway.

Results

Strains that do not transcribe of the vector (GFP) in the presence of rapamycin lack a gene that codes for an element of the promoter's pathway. The assay was successful in identifying the controls Gln3 and Gat1 as regulatory elements of Mep2 transcription. The individually analyzed experimental strains had partial defects in GFP induction; they were less fluorescent than the Ho1 strain, suggesting reduced transcription.

Conclusions/Discussion

This assay using the FACS and deletion strains was successful with the entire pool. It sorted out the control strains as likely candidates and also selected strains with reduced induction, indicating that the assay can effectively pinpoint potential regulators. Classical screens involve mutagenesis to few select strains; this technological development can simultaneously monitor the transcription of a gene in all knockout strains, greatly restricting the number of strains to be individually examined in genetic screens. This will make the construction of models of signal transduction pathways for genes more efficient.

Summary Statement

Our project tested the accuracy of novel method using a yeast deletion collection to efficiently identify potential pathway regulators.

Help Received

Used lab space and equipment at Stanford University under supervision of Taavi Neklesa.