Method Exploration

Introduction

Introduction to Asthma
In the Method section, you explored the microarray experiment, measuring gene expression in 2 populations of yeast. In the same way that you compared gene expression in yeast under aerobic and anaerobic conditions, this experiment will challenge you to examine the differences in gene expression between asthmatic and non-asthmatic individuals. In this example, we will look at mice as a model organism for studying the human condition of asthma.

Some mice, which we'll call “asthmatic mice,” respond to bronchoconstrictor agents with an exaggerated narrowing of their airways, just as humans with asthma do. The experiment that you will perform will address the question, “What is the difference in gene expression in an “asthmatic” mouse compared to a “non-asthmatic mouse” 30 minutes after exposure to a bronchoconstrictor?”

Experimental Design

Control and Experimental Groups
The first component of a successfully designed experiment is to define the control and experimental test groups. Data collected from the experimental condition is compared against a control to draw conclusions about the experiment.

Using this design, assign mice to the experimental and control groups in such a way that you can determine which genes are repressed and which are induced in “asthmatic mice” as compared to “non-asthmatic mice”. You can distinguish between Asthmatic and Non-Asthmatic individuals by exposing the mice to a bronchoconstrictor agent and tracking each mouse's respiratory response. Select mice that will give you the greatest difference in gene expression between the experimental and control populations.

Remember that mice with the greatest physiological difference will yield the greatest differences in gene expression. Sample from individuals at the extremes of the spectrum to identify mice with distinct Asthmatic and Non-asthmatic symptoms.

Next, assign a cDNA probe color to each condition. In this experiment, the colors you choose are significant only in that they distinguish one group from the other.

A Merged Image
At the end of this experiment, the experimental and control probes bound to the microarray will be scanned to generate a merged data image. As a result of your experimental design, the colored spots on the merged image will represent gene induction and repression in the asthmatic state relative to the non-asthmatic condition.
Refer to your experimental design to answer the following questions about what red and green spots will represent on your merged image:

**Generating Microarray Probes**

*Template Extraction*
In order to quantify the differences in the genes that respond to an asthma-inducing stimulus, gene expression will need to be measured locally in the lung tissue of the mice. The two populations of mice are euthanized and their lung tissue is removed. To continue the experiment, extract a molecule from the cells that will serve as an indicator of gene expression for the two physiological conditions. Use the ‘zoom lens’ to explore a cell and identify this molecule. Remember that some molecules are found exclusively within certain organelles.

*Probe Components*
mRNA is extracted from the cellular material, and placed into 2 microfuge tubes.

Now use the mRNAs as a template to synthesize a fluorescent-labeled molecule that will hybridize to the DNA microarray.

First, you'll need an enzyme that will synthesize the microarray probes from an mRNA template.

You'll also need free monomers to polymerize into the chain of molecules in the new probes.

**Hybridization**

*Probe Hybridized to Slide*
The cDNAs are hybridized to the microarray overnight.

**Data Analysis**

*Merged Image Produced*
The bound red and green cDNAs are scanned and a merged image is produced on the computer.

Select three different spots on the microarray corresponding to genes that were induced, *to some degree*, in the lungs of the Asthmatic mice.

**Relevance of Asthma**

*Asthma and Model Organisms*
Click on a spot that represents a gene which was expressed equally in the lung tissue of asthmatic and non-asthmatic mice.