Method

Introduction

Measuring the Entire Genome
One common use of microarrays is to determine which genes are induced and which genes are repressed when two populations of cells grown under different conditions are compared. Unlike some methods—such as a northern blot, where only a handful of genes are analyzed in a single experiment—a microarray allows investigators to simultaneously measure the expression of the entire genome in one experiment.

Demonstration
The following section demonstrates how a DNA microarray experiment is performed. By proceeding through these animations, we will learn how microarrays are used to compare gene expression in yeast cells grown under two different experimental conditions. We'll also see how yeast cells provide a model system that illustrates the principles of the microarray method. After completing this section, we will have the tools to discuss other applications of microarrays, such as studying the changes in gene expression that occur in human disease or during development.

Experimental Design

Yeast Experiment
Yeast cells are capable of growing both aerobically—in the presence of oxygen—or anaerobically, in the absence of oxygen. To grow under these different conditions, yeast cells synthesize specialized proteins that facilitate their adaptation to a particular environment. In other words, yeast cells express different genes to survive under different growth conditions.

We will perform a DNA microarray experiment to examine the difference in gene expression between yeast cells grown under control, or aerobic, conditions, and experimental, or anaerobic conditions. The microarray will allow us to measure the response of the entire yeast genome to an experimental environment.

Experimental cells vs. Control Cells
At the beginning of the experiment, cells are added to a growth medium in two culture tubes. A stopper is inserted into one tube to create an anaerobic environment. The cells in both tubes start to divide.

As experimental cells grow in the absence of oxygen, certain genes are repressed, others are induced, and some do not change their expression at all when compared to the same genes in the control cells.
Generating Microarray Probes

Isolate Yeast cells
To analyze the changes in gene expression using a microarray, microarray probes must be generated. The first step in generating probes is to isolate mRNA from both control and experimental cells.

To isolate mRNA, use a centrifuge to spin the culture tubes in which the cells were grown. The yeast cells form a pellet at the bottom of the tube.

Use a pipette to remove the growth medium, taking care to leave the pellet at the bottom of the tube.

Isolate mRNA
Add buffer to the pelleted cells to extract the mRNA, which in a real experiment would involve several steps. At the end of the mRNA extraction protocol, mRNA is located in the extraction buffer.

Finally, remove the mRNA and place it in a fresh microfuge tube. The mRNA has now been isolated.

Differential Gene Expression
There are millions of mRNA molecules in the microfuge tubes. To keep things simple, we will look at just three molecules from each tube. These mRNA molecules are shown in the inset. Remember that gene expression is different under aerobic and anaerobic conditions, so the population of mRNA molecules in each tube is different.

cDNA Synthesis (reverse transcription)
An enzyme called reverse transcriptase allows the synthesis of cDNAs from mRNA templates. As cDNAs are synthesized from control mRNAs, green fluorescent dye is incorporated into their sequences. The green fluorescent cDNAs represent the total transcriptional output, or the transcriptome, of cells grown under aerobic conditions. Likewise, red fluorescent dye is used to label the cDNAs representing the genes transcribed under anaerobic conditions.

RNase Degrades RNA
An enzyme called RNase is used to degrade the mRNAs. The cDNAs that remain are complementary to the mRNA molecules from which they were made. The cDNAs are also complementary to the antisense strand of the gene.

Mix Control and Experimental cDNA
Mix the red and green cDNAs together in one tube. These combined cDNAs will be used to probe the microarray.

Hybridization

Yeast Genome on Microarray
This is a slide containing a DNA microarray. This microarray was designed so that each spot contains a portion of the coding sequence from a different yeast gene. Taken together, all the spots on the microarray represent the yeast genome. In a real experiment, there would be about 6,200 spots on this microarray, one for each gene in the yeast genome. In this animation,
we will focus on only three spots. Each spot contains many identical copies of a unique DNA molecule. This could be single or double-stranded DNA depending on the technique used. The cDNA for each gene will bind to its complementary antisense DNA sequence. On a microarray with 6,200 spots, 6,200 genes are represented.

**Incubate cDNA with Microarray**

Let's incubate the mixed cDNA with the DNA chip. This process is called hybridization.

During the hybridization process, both green cDNAs and red cDNAs bind to the DNA spotted on the microarray. The binding of labeled cDNAs to a spot tells us whether the expression of the gene in this spot was changed by the conditions under which the cells were grown.

For example, if a gene were expressed only under aerobic conditions, only green cDNAs would bind to the spot on the microarray that contains this gene.

Notice that some of the labeled cDNA has bound to our three spots. Mouse over each spot to see its base pairing.

**Wash unbound cDNA**

Use buffer to wash off the unbound cDNAs to prepare the microarray for detection.

Now it is time to detect the bound cDNAs so they can be visualized.

**Data Analysis**

**Scan Microarray Slide**

For simplicity, we will follow only three spots through the detection process. The microscope slide containing the microarray is placed inside a microarray scanner, where the slide is scanned with two lasers to detect the bound green and red cDNAs.

First the green cDNAs are detected. **(Pause for sound effect)** Note the location of our three spots. This image is stored on the computer for later analysis.

Now the red cDNAs are detected. **(Pause for sound effect)** Again, this image is stored on the computer for later analysis.

**Analyze Results**

Eject the microscope slide and store it. Use the computer to analyze your results to reveal which genes, if any, altered their transcription when cells were grown under anaerobic conditions, and whether the alterations resulted in induction, repression, or no change in expression of a given gene when compared to control cells.

**Merge Red and Green Images**

First retrieve the stored green image…

Then retrieve the stored red image…

Using the computer, we can create a merged image. Notice that only green cDNAs bound to gene #127, indicating that the gene was only expressed in the cells grown under aerobic conditions. Gene #2619 was expressed in both conditions, resulting in the appearance of a
yellow spot in the merged image. Gene #5854 was expressed only in cells grown under anaerobic conditions.

**Merged Images**
Merged images would be obtained for all 6200 spots. A representative microarray is shown here.

Notice that this microarray includes some black spots, in addition to the green, yellow, and red ones we have already discussed. Black spots indicate that no cDNAs bound to the spot, which means that the gene was not transcribed under either of the two conditions tested.

Also, note that there are variations in intensity of red and green spots, with some spots appearing orange or lime green. These variations indicate levels of induction or repression of a particular gene.

**Summary**

In the animation you just completed, the genome of one population of cells had to respond to the presence of oxygen, while the genome of the other population of cells had to respond to the absence of oxygen. Although the two populations were genetically identical, their genomes responded differently.

A DNA microarray allowed us to simultaneously measure the response of every gene in the genome to the presence or absence of oxygen. With this technology, we can compare any two cell types to measure how their genomes respond to changing environments.

Imagine the power of this technology when it is used to compare gene expression in human cells under different conditions. Microarrays could be used to diagnose genetic diseases, create customized medical treatments, and predict who will suffer toxic side effects from a particular treatment regimen. Microarrays have the potential to make a revolutionary impact on healthcare and quality of life issues because of the valuable genetic information they provide.