Edvo-Kit # 235

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DNA/RNA Microarrays

Experiment Objective:

In this experiment, students will master the experimental concepts and methodology behind genomic microarrays. This simulated two-color microarray experiment examines differences in gene expression across four cancer patients.

See page 3 for storage instructions.

Version 235.180419

SAMPLE LITERATURE
Please refer to included weblink for correct version

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Experiment Components

Component		Check (✓)
A	Equilibration buffer	❑
B	Control cDNA solution	❑
C	Hybridization buffer	❑
•	Patient sample Microarray QuickStrip™ Plates	❑
•	Snap top tubes	❑
•	Microarray cards	❑

Storage:
Store all components and Microarray QuickStrips™ at 4°C.

This experiment is designed for 10 groups.

Requirements

(Not included with this kit)

- Automatic micropipets (0-50 µL) or 5 µL single volume pipets and tips
- Hand-held long wave UV light (Cat. #969 recommended) or UV transilluminator
- Incubation oven (37°C, optional but highly recommended)
- UV Safety goggles
- Disposable lab gloves

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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Background Information

UNLOCKING THE HUMAN GENOME

An organism's genome contains the genetic information necessary for its growth, development, and survival. In humans, this information is contained within 23 pairs of chromosomes contained within a cell's nucleus. In the early 1990s, researchers resolved to sequence the entire human genome (six billion base pairs of DNA). This international undertaking, called the Human Genome Project, launched the field of "genomics" (the study of the sequence and structure of the genome). As a result of the Human Genome Project, a vast amount of information about the DNA sequence has been made publicly available.

After the complete consensus sequence of the human genome was published in April 2003, scientists began investigating the information hidden within the DNA sequences. Using the sequence information, specific genes can be mapped to their chromosomal location, and novel genes are still being identified today. However, sequence analysis has determined that there are only 21,000 protein-producing genes in the human genome, a number much lower than estimates made before the Human Genome Project.

Data from the Human Genome Project has shown that DNA sequences only differ approximately 0.2% between individuals (roughly one base in every 500 is changed). Specific variations in an individual's genome can be used as markers to predict predisposition for particular diseases. Scientists can analyze these genetic differences to explore human diversity and evolution at the DNA sequence level. In addition to DNA sequences that code for proteins, the genome includes DNA sequences that influence protein production via other mechanisms. For example, sequences known as promoters control transcription of a specific mRNA. Other DNA sequences code for ribosomal RNA, transfer RNA, and microRNA, which work together to regulate translation of proteins. For these reasons, a fundamental understanding of the entire human genome sequence is critical, even if a majority of the genome does not code for proteins.

ANALYZING GENE EXPRESSION USING MICROARRAYS

Precise regulation of gene expression is essential for the normal function of cells and tissues. Depending on the characteristics of its promoter, the expression of a particular mRNA may vary from no expression to hundreds of copies per cell. Traditional techniques such as Northern blotting analyze the expression of only a few genes at a time, making genomics research very time consuming. The emergence of DNA Microarray technology has made it possible to produce and analyze data measuring the levels of mRNA from thousands of genes in a single experiment.

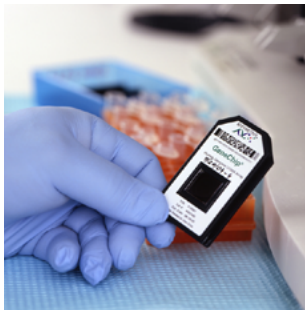

Figure 1: An example of a Gene Chip.

Microarrays (or "gene chips") have made it possible to identify, classify, and assign functions to many uncharacterized genes, simply by determining when the genes are expressed or repressed. Their small size and ability to analyze expression from large number of genes simultaneously have made microarrays an important tool for genomics research in diverse fields such as drug discovery, toxicology, and medical diagnostics.

Each chip consists of short, single-stranded pieces of DNA called oligonucleotides (or oligos) affixed to a glass slide (Figure 1). The chip contains a grid comprising thousands of oligos, each with a known sequence that corresponds to a particular gene to be analyzed. Because a single chip contains thousands of spots, each experiment can accurately analyze the expression levels of thousands of genes.

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Two-color microarray technology allows a comparison of the expression profiles between two different samples (for example, normal skin cells versus skin cancer cells). There are four basic steps involved in a two-color microarray (Figure 2):

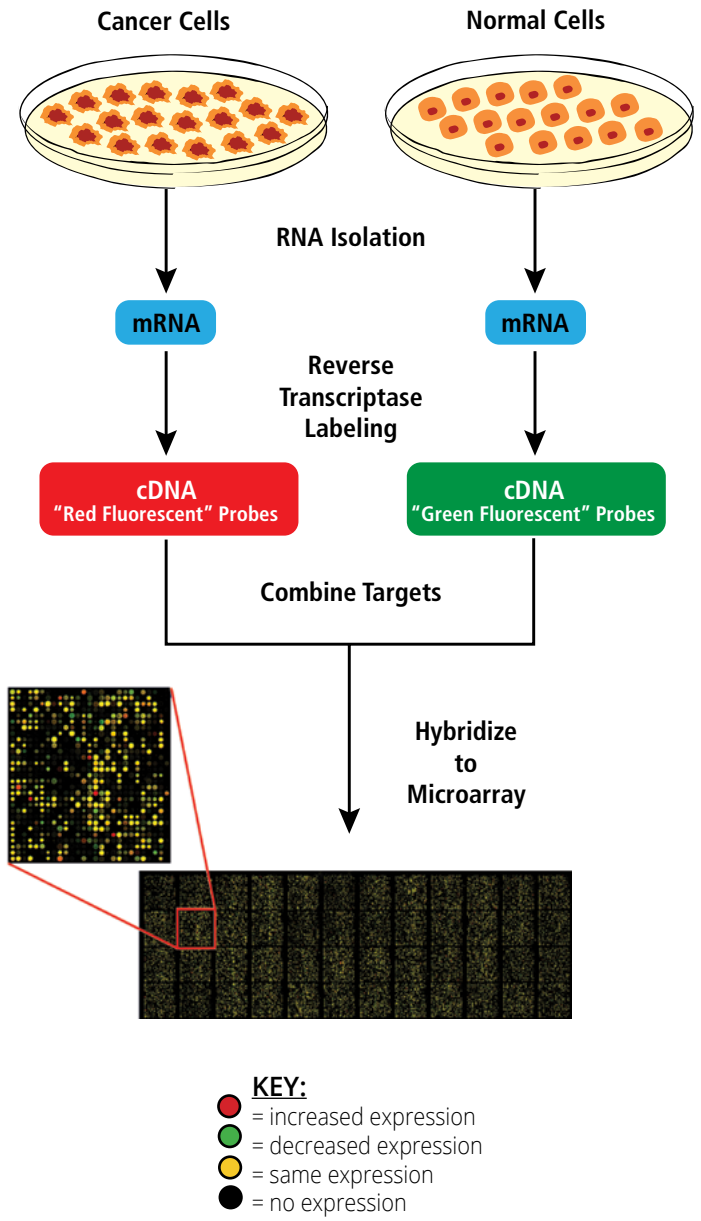
1. **Sample Preparation**
   Total mRNA is extracted from control and experimental samples.

2. **cDNA Synthesis**
   Complementary DNA (cDNA) libraries are generated from the RNA samples using reverse transcriptase (RT), an enzyme that uses RNA as a template to produce DNA. The cDNA libraries are labeled with fluorescent probes. The cDNA isolated from the control sample is typically labeled with a green fluorescent tag, whereas cDNA isolated from the experimental sample is labeled with a red fluorescent tag.

3. **Hybridization**
   The labeled cDNA from both control and experimental samples is placed on the microarray chip, where it binds (or hybridizes) with the spot containing the oligo with a complementary sequence. This hybridization results in a stable, double-stranded DNA helix. Following hybridization, the chip is washed several times to remove any cDNA that has not bound to an oligo on the chip.

4. **Scanning and Data Analysis**
   The chip is scanned with a laser that excites the fluorescent tags. The fluorescence information at each spot is collected and processed using a specialized program that creates a color image of the microarray. The image is then analyzed using a program that interprets the fluorescence at each spot.

Figure 2: Principles of Two-color Microarray Analysis



The color and intensity of fluorescence at each particular spot allows researchers to identify key genetic differences, such as those between normal cells and cancer cells. The control cDNA library represents the normal level of gene expression in the cells. If we only hybridized the control library to our microarray, different spots would appear as various intensities of green. Bright green spots indicate spots that have captured high levels of cDNA (suggesting a high expression level), and pale green or black spots suggest low/no mRNA expression. Conversely, if the same chip were hybridized exclusively with the cancer cDNA library, varying intensities of red would be seen. When both libraries are hybridized simultaneously to the same chip, four colors are expected in the analysis—black (no expression), green, red, or yellow. Yellow spots result when both the control and experimental samples hybridize in equivalent amounts. A spot that appears green indicates the presence of more

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cDNA from the control (healthy) sample than from the experimental (diseased cell) sample. Thus, a green spot reveals that less mRNA is present in the cancer cell than normal, and the gene is said to be "down-regulated". Conversely, a red spot would mean that the gene is "up-regulated" in the cancer sample.

CURRENT CHALLENGES AND FUTURE PROMISES

As microarray experiments became more common, researchers recognized the need for information management systems to organize and analyze the vast amounts of data being generated. As a result, scientists began to employ computer technologies for storing and processing biological data. Consequently, the interdisciplinary field of bioinformatics—which integrates computer science, biology, and information technology—evolved to develop extensive databases of biological data. Some examples of microarray databases are Gene Expression Omnibus (GEO) by the National Center for Biotechnology Information (NCBI), Array Express by the European Bioinformatics Institute (EBI), the Stanford Microarray Database, and others. These databases allow scientists around the world to access and share large amounts of data during their genomic studies.

Microarrays and their resulting analyses have already contributed significantly to scientific discovery. Expression analysis is presently used in drug development, drug response studies, and therapeutic development. Expression data also holds tremendous promise for personalized medicine, in which treatments are tailored to an individual's specific genetic profile to treat a particular disease more effectively. As scientists develop more effective ways to analyze the expression data generated by microarrays, the pace of discovery is likely to accelerate.

In this experiment, you will explore DNA microarrays. Cancer biopsies were collected from four patients and have been converted to cDNAs in the presence of a red fluorescent tag. Students will mix the patient cDNA with green-labeled cDNA obtained from normal control cells. The mixed samples are then spotted onto prepared microarray cards and a hybridization buffer is used to bind the cDNA samples to the microarray oligos. Each row of the microarray card contains oligos that correspond to four control samples (1-4), followed by four genes of interest (5-8). Once dry, the microarrays are analyzed under UV light to determine the expression of each gene.

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Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will master the experimental concepts and methodology behind genomic microarrays. This simulated two-color microarray experiment examines differences in gene expression across four cancer patients.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Wear gloves and goggles while working in the laboratory. Wear UV goggles when working with black/UV light.
2. Always exercise extreme caution when working in the laboratory.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR!

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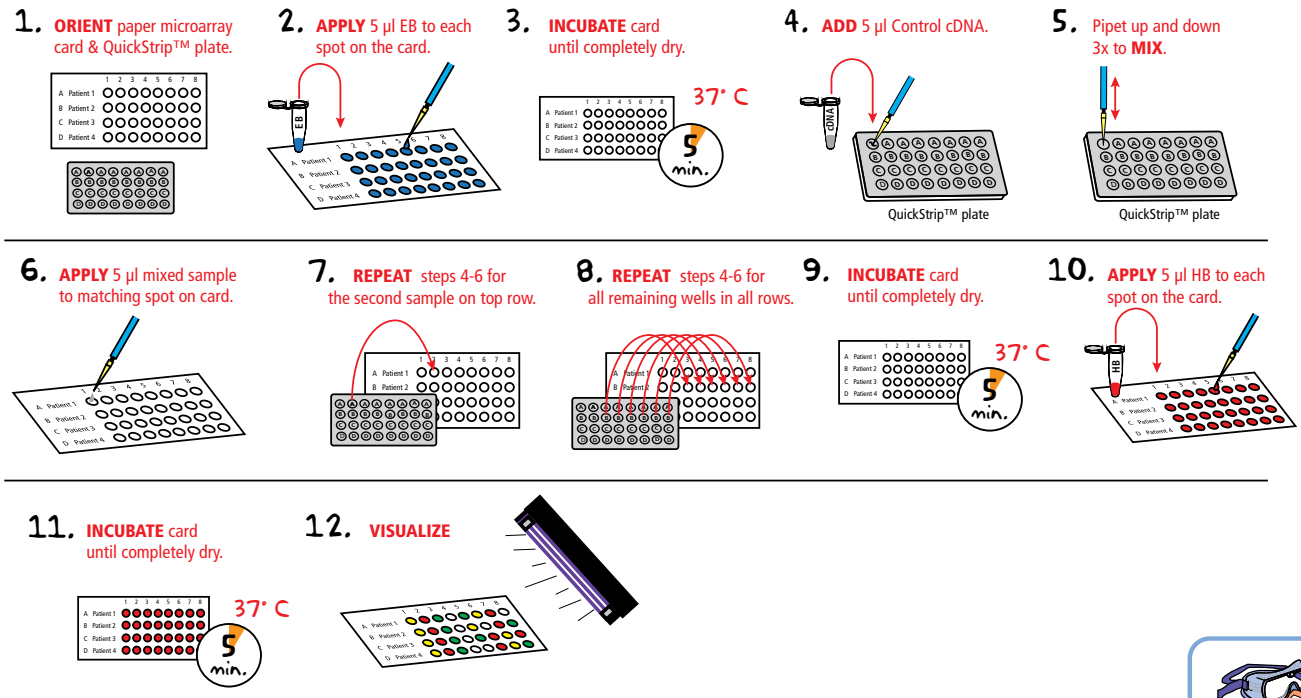
Performing the Microarray



Before starting the experiment, gather one paper microarray card, one QuickStrip™ plate of patient cDNA samples, and tubes of Equilibration Buffer (EB), Hybridization Buffer (HB), and Control cDNA for your group.

1. **ORIENT** the paper microarray card and QuickStrip™ plate so that Patient 1 (row "A" or "E") is in the top left corner of each. **LABEL** the microarray card with your initials or group number.
2. Using a micropipet, **APPLY** 5 µL Equilibration Buffer (EB) to each spot on the microarray card.
3. **INCUBATE** the microarray card in a 37°C incubator for 5 minutes, or at room temperature for 10 minutes, to allow samples to completely dry.
4. Using a fresh micropipet tip, **ADD** 5 µL of Control cDNA (cDNA) to the top left well of the QuickStrip™ plate by punching through the foil.
5. With the same micropipet tip, pipet up and down 3 times to **MIX**.
6. Still using the same micropipet tip, **APPLY** 5 µL of the mixed sample to the top left spot of the microarray card.
7. Using a fresh micropipet tip, **REPEAT** Steps 4-6 for the second sample on the top row.
8. Continue to **REPEAT** Steps 4-6 for each additional well on the top row, then continue with the remaining 3 rows. Once all 32 samples have been mixed with control cDNA and added to the paper microarray card, continue with Step 9.
9. **INCUBATE** the microarray card in a 37°C incubator for 5 minutes, or at room temperature for 10 minutes, to allow samples to completely dry.
10. **APPLY** 5 µL Hybridization Buffer (HB) to each spot on the microarray card.
11. **INCUBATE** the microarray card in a 37°C incubator for 5 minutes, or at room temperature for 10 minutes, to allow samples to completely dry.
12. **VISUALIZE** the microarray using a long-wave handheld UV light or mid-range transilluminator. Microarray cards can be stored and analyzed for up to a week if protected from light.

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Study Questions

Answer the following study questions in your laboratory notebook.

1. What new information has become available as a consequence of the human genome project?
2. Explain the core technology behind microarrays and why it is important for biotechnology and medicine.
3. How are cDNAs libraries made?
4. What information have DNA microarrays made possible?
5. How are the individual spots on a microarray chip identified and analyzed?

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Instructor's Guide

Prior to starting this experiment, carefully check the list of Components and Requirements (Page 3) to ensure that you have all the necessary components and equipment. The guidelines prepared in the manual are based on 10 laboratory groups.

Approximate Time Requirements:

Preparation for:	What to do:	When:	Time:
Module I: DNA Microarray	Aliquot reagents	Anytime before performing the experiment	20 min.
	Gather Materials for Students	Anytime before performing the experiment	10 min.
	Prepare 37°C incubation oven	Anytime before performing the experiment	5 min.

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PreLab Preparations

The following instructions are meant to assist lab instructors in preparing for their microarray simulation lab. Please read and perform the necessary steps below in preparation for the lab.

Preparing the Microarray QuickStrip™ Plates and Paper Microarray Cards

1. Using a pair of scissors, **CUT** the microarray QuickStrip™ plates horizontally between rows D and E, then **CUT** vertically between columns 8 and 9. ***NOTE: Columns 9-12 are not used in this experiments, have been intentionally left empty, and can be discarded.***
2. **PAIR** each 4-row microarray QuickStrip™ plate with a matching paper microarray card. Plates containing rows A-D should be paired with a card for rows A-D, while plates with rows E-H will be paired with a card for rows E-H.
3. Each group will receive one of the microarray QuickStrip™ plates and matching microarray card. Samples are identical across the plates; samples for patient 1 are contained in rows A and E, patient 2 in rows B and F, etc.
4. Remind students to gently tap the microarray QuickStrip™ plates on the lab bench to ensure that all of the samples are at the bottom of the wells.

Preparation of Buffers and Control cDNA Samples:

1. Dispense 200 µL of Equilibration buffer (Component A) into 10 microcentrifuge tubes. Label the tubes as "EB".
2. Dispense 200 µL of Control cDNA (Component B) into 10 microcentrifuge tubes. Label the tubes as "cDNA".
3. Dispense 200 µL of Hybridization buffer (Component C) into 10 microcentrifuge tubes. Label the tubes as "HB".

Gather Materials for Students:

In order to perform the microarray experiment each group will require one adjustable volume micropipet or 5 µL fixed volume pipet and disposable tips. Analysis of the microarray cards requires a long wave handheld UV light source or transilluminator, which can be shared between groups. The experiment can be paused after any of the incubation steps and resumed at a later point. Store the fully dried microarray cards at room temperature and protected from light.

Preheat Incubation Oven:

If using an incubation oven set the temperature to 37°C. Alternatively, the microarray cards can be dried at room temperature. Microarray cards must be allowed to fully dry between each step.

Each Student Group Should Receive:

- QuickStrip™ plate containing patient samples (Rows A-D or Rows E-H)
- Matching paper microarray card
- Microcentrifuge tube containing 200 µL of Equilibration buffer
- Microcentrifuge tube containing 200 µL of Hybridization buffer
- Microcentrifuge tube containing 200 µL of Control cDNA

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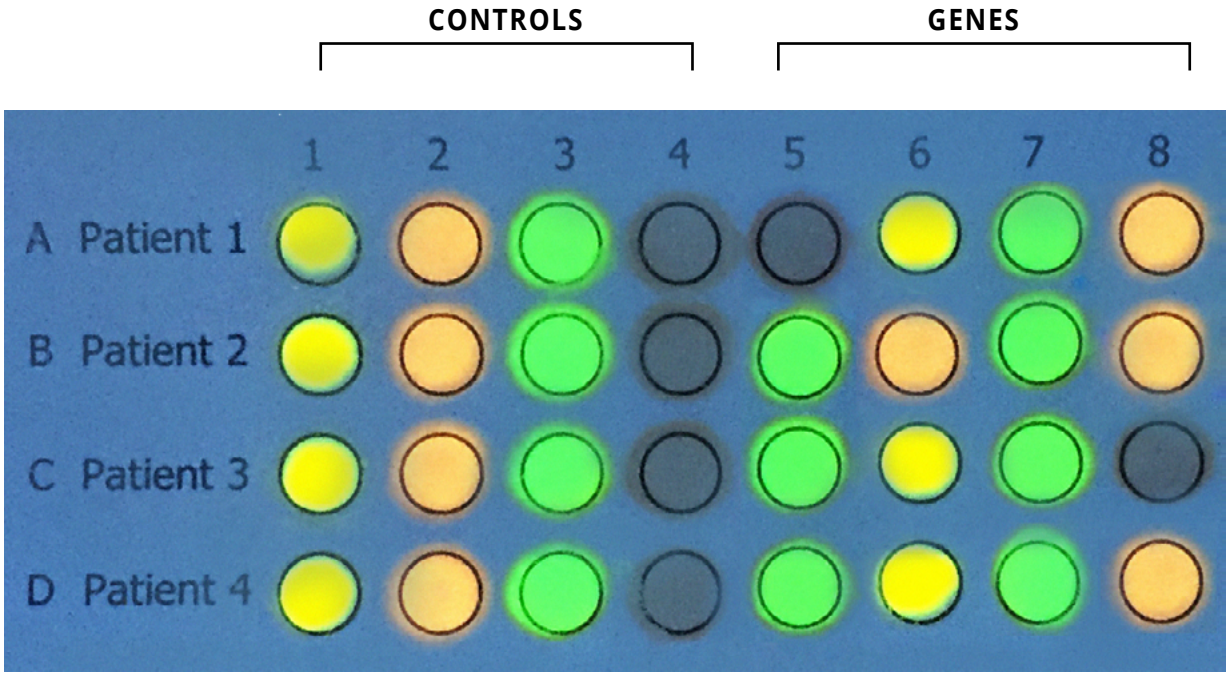
Experiment Results and Analysis

The microarray results indicate changes in expression between the four patient samples and control cDNA. The first four columns represent the control samples and should be checked first to ensure that the experiment worked as expected. Once the controls have been verified the four genes of interest can be analyzed in each of the patients to determine up- or down-regulation of the genes.

Results from an example experiment can be seen below:



	1	2	3	4	5	6	7	8
	Normal	Up	Down	Blank	Gene 1	Gene 2	Gene 3	Gene 4
Patient 1	N	↑	↓	-	-	N	↓	↑
Patient 2	N	↑	↓	-	↓	↑	↓	↑
Patient 3	N	↑	↓	-	↓	N	↓	-
Patient 4	N	↑	↓	-	↓	N	↓	↑

Gene samples that fluoresce yellow show similar expression as the Control cDNA, while red fluorescence indicates that the gene has increased expression and green fluorescence indicates that the gene expression is decreased. No fluorescence indicates that there is no expression. For example, Patient 1 has increased expression of gene 4, decreased expression of gene 3, similar expression of gene 2, and no expression of gene 1.

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Please refer to the kit insert for the Answers to Study Questions