DNA/RNA MICROARRAYS

This protocol is based on the EDVOTEK® protocol "DNA/RNA Microarrays".

10 groups of students

NOTE: USE THIS KIT WITHIN 6 MONTHS OF RECEIPT.

1. EXPERIMENT OBJECTIVE

The objective of this experiment is to understand the basics of microarrays as applied to functional genomics. This simulation is designed to provide students with the opportunity to analyze differences in gene expression using a two-color microarray.

2. EXPERIMENT COMPONENTS for 10 groups of students

COMPONENTS	STORE
Four different Microarray sample QuickStrips™	
Patient 1	Refrigerator
Patient 2	Refrigerator
Patient 3	Refrigerator
Patient 4	Refrigerator
Microarray Card	Refrigerator

NOTE: NO HUMAN MATERIAL IS USED IN THIS EXPERIMENT. ALL 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.

NOTE: STORE ENTIRE EXPERIMENT IN THE REFRIGERATOR UPON RECEIPT.

2.1 Requirements

- Fixed-volume (5 µl) or variable volume micropipettes.
- Pipette tips.
- Long wave U.V. light source.

3. 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.

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.

Figure 1: An example of a Gene Chip.

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 micro- array. The image is then analyzed using a program that interprets the fluorescence at each spot.

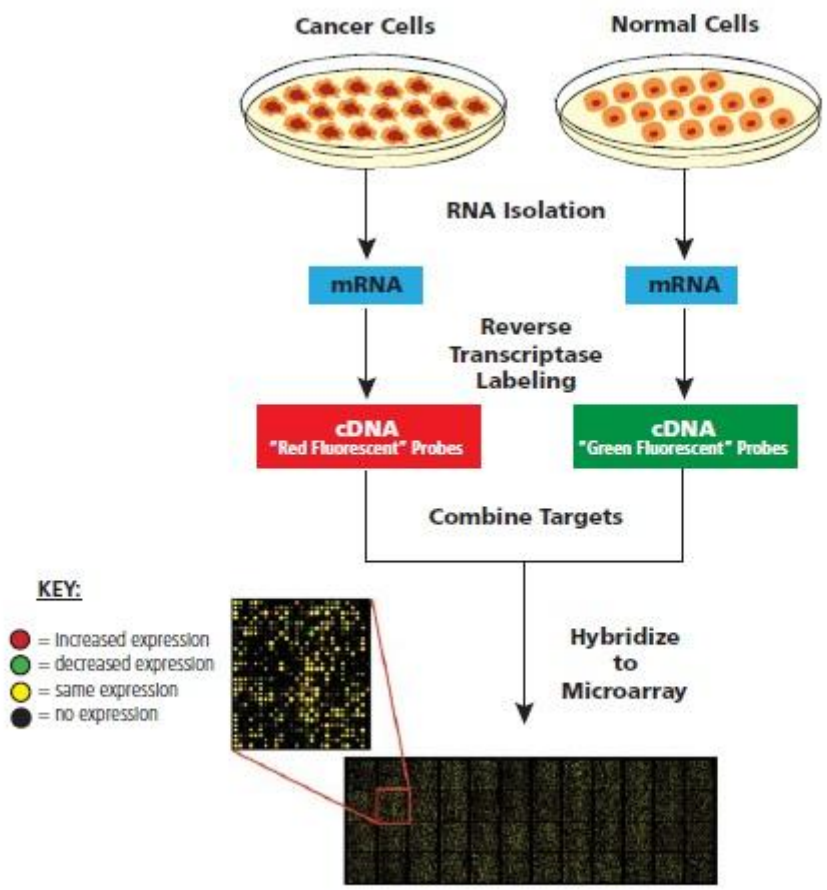


Figure 2: Principles of two-color microarrays analysis.

The color and intensity of fluorescence at each particular spot allows researchers to identify key genetic differences, such as those between normal skin cells and skin cancer cells. The control cDNA library represents the normal level of gene expression in skin 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 skin 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 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

In the early era of genomics, researchers recognized the need for information management systems to organize the data generated by microarrays. Additionally, scientists needed to figure out a way to recognize trends and correlations that would otherwise be missed in the vast amount of data. 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 genomicists develop more effective ways to analyze the expression data generated by microarrays, the pace of discovery is likely to accelerate.

4. EXPERIMENTAL PROCEDURES

The objective of this experiment is to understand the basics of microarrays as applied to functional genomics. This simulation is designed to provide students with the opportunity to analyze differences in gene expression using a two-color microarray.

BRIEF DESCRIPTION OF THE EXPERIMENT
For this experiment, 16 different samples (four per patient) have been pre-converted to cDNAs in the presence of a fluorescent tag incorporated during synthesis. The patient cDNA libraries are pre-mixed with the labeled cDNA obtained from normal control cells. Students will spot the samples on microarray cards in the order provided for each patient. The sample spots on the cards contain synthesized cDNA fragments (from PCR) for each patient mRNAs. The first four samples in each microarray QuickStrips™ are control samples (A to D), followed by four patient samples (E to H). The microarray samples are labeled with stripe(s), for example, Patient 1 (one stripe), Patient 2 (two stripes), and so on. To obtain correct results, accuracy in pipetting and keeping individual patient samples in the correct order is essential.

Microarrays detect up and down regulation of genes based on mRNA expression of cells. Patient mRNA for specific genes is converted to cDNA and is labeled with a red fluorescent tag, while the normal (control) sample is labeled with a green fluorescent tag. The two samples (patient and control cDNAs) are mixed and hybridized to a microarray gene chip. After hybridization, the microarray gene chip is read by a laser-induced fluorescence scanner. Information obtained from the chip is stored in a computer and analyzed using a program that specifically interprets microarray data.

4.1 Laboratory safety
No human material is used in this experiment.

1. Gloves and safety goggles should be worn at all times as good laboratory practice.

2. NOT PIPETTE WITH THE MOUTH, use appropriate devices.

3. Exercise caution when working with equipment using together heat and mix of reagents.

4. Wash hands with soap and water after working in the laboratory or after using biological reagents and materials.

4.2 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.

PRE-ALIQUOTED MICROARRAY SAMPLE QUICKSTRIPS™

The simulated cDNA samples have been pre-aliquoted and packaged in Microarray QuickStrips™. Pre-lab preparation only requires the separation of the sample sets into strips.

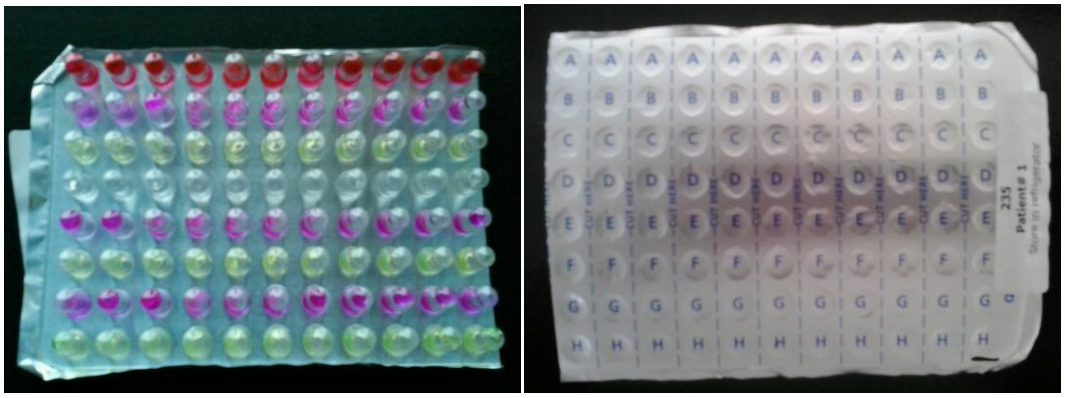


Figure 3: Microarrays QuickStrip™

1. Use a marker to stripe the end of each Microarray QuickStrips™ as shown below:

Plate	# of Stripes
Patient # 1	1 / I
Patient # 2	2 / II
Patient # 3	3 / III
Patient # 4	4/ IIII

2. Carefully separate the QuickStrips™ as indicated in the packaging with a pair of scissors.

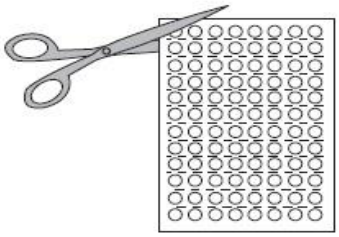


Figure 4: Each microarray QuickStrip™ plate is for one of the four patients. Before plates are cut, as shown, stripe along the end according to the patient #.

NOTE: Be sure not to puncture the foil covering samples!

3. Each student will receive a set of four QuickStrips™ coded with black stripes for each patient.

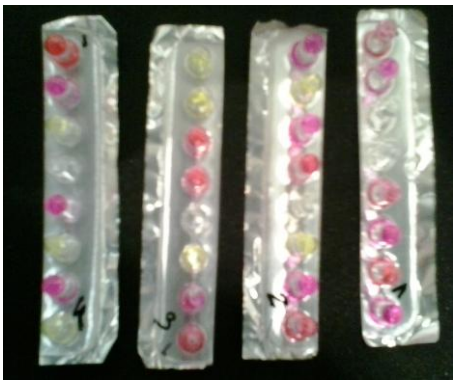


Figure 5: Microarrays QuickStrip™ of the four patients.

4. Remind students to gently tap the QuickStrips™ on the lab bench to ensure that all of the samples are at the bottom of the tube.

5. A microarray card will be given to each group. On the card, spots A-D represent control samples and spots E-H represent experimental samples.

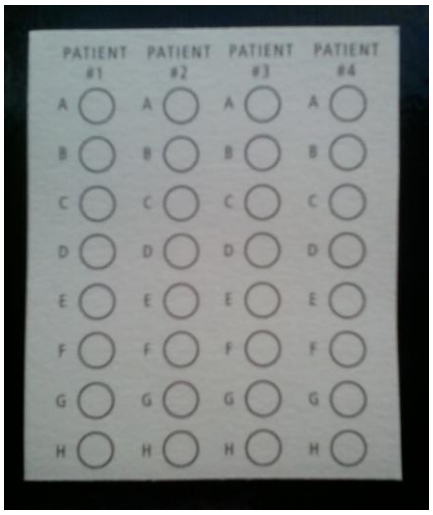


Figure 6: Microarrays card.

4.3 Material that should receive each group

Assemble the following materials for each of the student groups:

- Four different patient microarray QuickStrips™ (Striped 1, 2, 3 and 4):
  - Patient one microarray sample strip (#1)
  - Patient two microarray sample strip (#2)
  - Patient three microarray sample strip (#3)
  - Patient four microarray sample strip (#4)
- Microarray Card
- Pipets (5 µl) fixed or variable pipets
- Pipet tips
- Long wave U.V. light source (black light) to be shared by all student groups

5. STUDENT EXPERIMENTAL PROCEDURES

PERFORMING THE MICROARRAY
Wear gloves and safety goggles.

For this microarray simulation, there are no hybridization and washing steps. A long wave UV light source will be used to detect the fluorescence.

1. OBTAIN a microarray card.
2. OBTAIN four individual microarray QuickStrips™ of cDNA samples coded for patients 1, 2, 3, and 4, (as shown below):

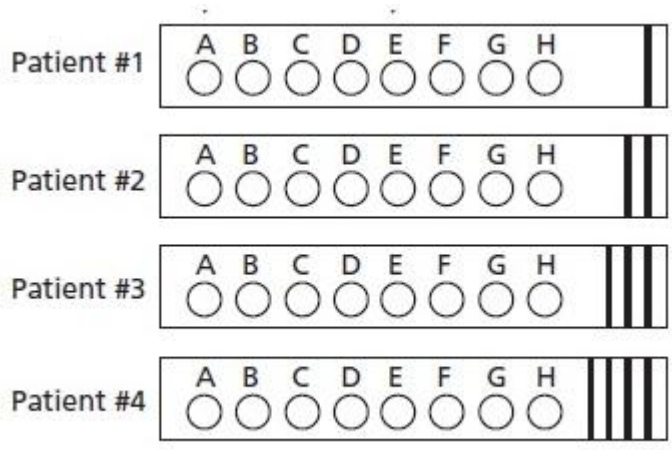


Figure 7: Example of student microarray QuickStrips™. Each row of samples (strip) constitutes a complete sample for each patient to be used by each student group.

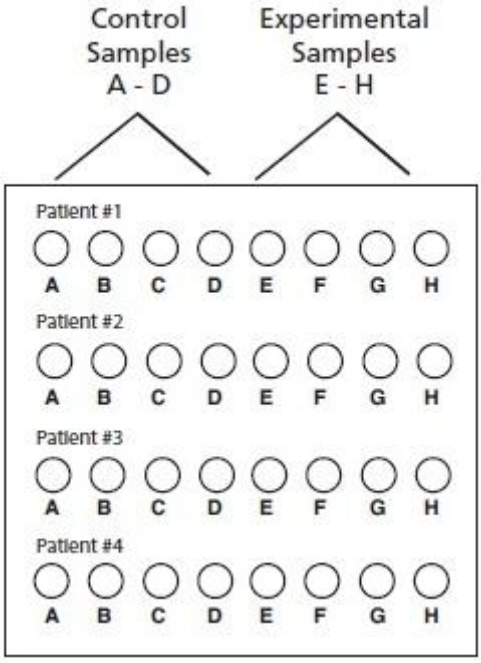
3. APPLY 5µl of each cDNA sample to the appropriately labeled spots for each patient as shown below.

Figure 8
Control Samples A-D For all Four Patients:

A = Normal
B = Up regulation
C = Down regulation
D = Blank

Experimental Samples E-H for all four patients:

Spots E - H on the micro- array card represent individual genes.



<u>NOTE:</u> To puncture the foil covering of microarray QuickStrips™ can be used the same pipette tip, taking care not to lose the sample is inside the well.

<u>NOTE:</u> If the tips consumption is too high for the budget of the center, you can re-use the same tip for all patients, explaining students that it is not the right way to work in a laboratory. In this case, the tip should be cleaned with distilled water between each sample/well and making sure the tip is not water that can dilute the sample before applying it on the card.

4. PLACE the membrane in a 37°C incubator for five minutes to allow the samples to dry.

5. VISUALIZE the microarray using a long-wave handheld UV transilluminator.

6. (Optional) RECORD your results by photographing the microarray card using either a Digital or a Polaroid camera.

- Recommended camera settings are aperture f 5.6 for 2 seconds.
- If the photograph is too light, change the aperture to f 8 and ex- pose for 2 seconds.
- If too dark, keep the aperture at f 5.6 and reduce the shutter speed to 1 second.

Specific settings will vary depending upon the photodocumentation system you are using. For additional information, refer to the instructions included with your photodocumentation system.

6. EXPERIMENTAL RESULTS

6.1 Experimental Results and Analysis

Using the appropriate symbols, record your results in the sample microarray card (see key below right).

(N)	normal (yellow).
(–)	no expression (black).
(↑)	up regulated (red).
(↓)	down regulated (green).

Figure 9: Key.

The results obtained are as follows (figure 10):

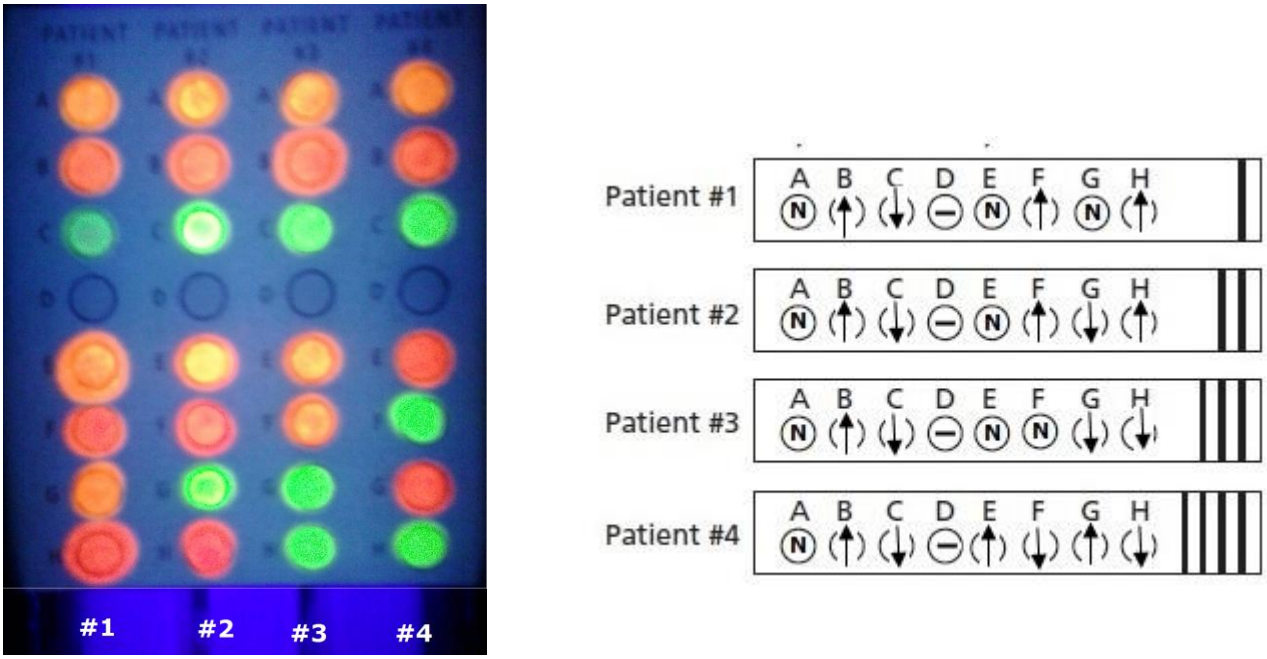


Figure 10: Results obtained.

6.2 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 is it important for biotechnology and medicine.
3. How are cDNAs libraries made?
4. What information has DNA microarrays made possible?
5. How are the individual spots on a microarray chip identified and analyzed?