ONLINE INQUIRY & INVESTIGATION

Microarrays Made Simple: "DNA Chips" Paper Activity

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Microarray technology is revolutionizing biological science. DNA microarrays (also called DNA chips) allow simultaneous screening of many genes for changes in expression between different cells. Now researchers can obtain information about genes in days or weeks that used to take months or years. This paper activity allows students to visualize how cells grown under different conditions would be analyzed using a DNA microarray. This activity also reinforces concepts related to DNA structure, transcription, and experimental design.

DNA chip technology stems from the basic chemistry of DNA. Most biology students can recite that "A pairs with T" and "G pairs with C." This base complementarity is what allows DNA from cells to bind specifically to known DNA sequences on a chip. Each sequence is unique to a specific gene. A microarray can have tens of thousands of genes in an area smaller than the size of a postage stamp. Since a cell expresses hundreds or even thousands of genes at any given time, a "snapshot" of gene activity can be inferred using microarray technology.

People in many fields use microarray technology. Scientists use this tool to help them understand basic cellular functions such as cell division or photosynthesis. People in the pharmaceutical industry are using microarrays to predict mechanisms of toxicity in drugs, and to develop drugs that promote healthy living and actually enhance lifespan. Cancer researchers use microarrays to identify tumor-related genes. Winemakers have created a DNA chip containing the grape genome to help them develop grapes that are better suited to certain climates.

Microarray experiments involve sophisticated equipment and a high level of technical skill. Because a single microaarray experiment can provide researchers with huge amounts of information, this technology has created a demand for people with a broad range of expertise. People in fields such as computer science and statistics work with researchers in the physical and life sciences to understand how to interpret the information from microarray experiments. Students who enter these fields will be well served by educational practices that include experiences with microarray technology.

Microarray technology is an exciting topic in the study of DNA. After studying DNA structure, base pairing, and transcription/translation, students can be introduced to reverse transcription and to some of the uses of microarray technology before they do this paper activity. It is recommended that students complete the "Student Reading" before the activity. This activity highlights the use of a biological molecule, DNA, for technological advancement—a perfect example of "bio-technology."

Description

Grade level: high school

Class time required: approximately 30 minutes

Student prior knowledge: DNA base complementarity, transcription, reverse transcription

Vocabulary: DNA, transcription, reverse transcriptase, messenger RNA (mRNA), complementary DNA (cDNA), antiparallel, target DNA, probe DNA, hybridization, gene expression

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Materials for Each Student Group (2-4 Students)

- 2-4 pairs of scissors
- clear tape
- small colored stickers (red and green, or any two different colors; approximately 1 cm diameter, eight of each) or markers in two different colors
- 2-4 copies of "Student Reading" (one for each person)
- 2-4 copies of "Student Instructions" (one for each person)
- 1 copy of RNA from light tissue (Figure 1)
- 1 copy of RNA from dark tissue (Figure 2)
- 1 copy of DNA chip (Figure 3)

Microarray Paper Activity: Student Instructions

This simple microarray experiment involves looking for differences in gene expression of seedlings grown under two conditions: One set of seedlings receives full light and the other set germinates completely in the dark. Light-grown seedlings are green with open cotyledons (first leaves) and short hypocotyls (the stem between the root and the cotyledons). Dark-grown seedlings are yellowish with closed cotyledons and long hypocotyls.

1. Divide your group into two: seedlings grown in light and seedlings grown in dark.
2. Cut out each rectangle of mRNA from light-grown and from dark-grown cells.
3. Each person can take one or two of the mRNA transcripts from the organism grown in one of the two conditions. Each transcript represents a gene that was expressed in that organism at the time the seedlings were harvested. Note: some transcripts are *rare*, some are *abundant* (abundant means many copies are present). Transcripts may also vary in *length*, since DNA coding regions vary in length. (In reality, mRNAs are typically much longer than those used here.)

Figure 1. Light-grown mRNA. Cut these rectangles apart at solid lines.

(light conditions) mRNA: 5′ U U G G C A 3′ reverse transcriptase cDNA: 5′

(light conditions) mRNA: 5′ A A G G C C 3′ reverse transcriptase cDNA: 5′

(light conditions) mRNA: 5′ A A G G C C 3′ reverse transcriptase cDNA: 5′

(light conditions) mRNA: 5′ A A G G C C 3′ reverse transcriptase cDNA: 5′

(light conditions) mRNA: 5′ C G G G A U 3′ reverse transcriptase cDNA: 5′

(light conditions) mRNA: 5′ A A C C G U 3′ reverse transcriptase cDNA: 5′

(light conditions) mRNA: 5′ A A A U U U 3′ reverse transcriptase cDNA: 5′

(light conditions) mRNA: 5′ G A C C U 3′ reverse transcriptase cDNA: 5′

Figure 2. Dark-grown mRNA. Cut these rectangles apart at the solid lines.

(dark conditions) mRNA: 5′ U U G G C A 3′ reverse transcriptase cDNA: 5′

(dark conditions) mRNA: 5′ C G G G A U 3′ reverse transcriptase cDNA: 5′

(dark conditions) mRNA: 5′ A U G G G C 3′ reverse transcriptase cDNA: 5′

(dark conditions) mRNA: 5′ G A C C U 3′ reverse transcriptase cDNA: 5′

(dark conditions) mRNA: 5′ C U C C U A 3′ reverse transcriptase cDNA: 5′

(dark conditions) mRNA: 5′ C U C A A 3′ reverse transcriptase cDNA: 5′

(dark conditions) mRNA: 5′ A U U A G C 3′ reverse transcriptase cDNA: 5′

(dark conditions) mRNA: 5′ A G U A C 3′ reverse transcriptase cDNA: 5′

4. You are the enzyme *reverse transcriptase*. Write the complementary DNA sequence (cDNA).
5. On the word "cDNA," place a red sticker on the light cDNA and a green sticker on the dark cDNA. (Alternatively, use a colored pen or pencil.)
6. Degrade the mRNA by cutting it off. Now you have a single strand of cDNA (**target** DNA).
7. Hybridize your labeled cDNA to the "spots" on the microarray slide using tape. Be sure to obey the rules of *base complementarity*, and remember that double stranded DNA is *antiparallel*. For this activity you may assume the temperature is high enough so that there must be at least four bases in a row that match, otherwise the cDNA won't bind to the probe DNA. (In reality, microarrays use DNA probes that are 20-70 bases long and cDNA that is much longer than 6 bases. Researchers use sophisticated computational methods for determining how many bases must match for binding to be specific to a single cDNA sequence.)
8. Wash away any cDNAs that don't match.
9. Note which probe molecules have been hybridized with light cDNAs, dark cDNAs, both, or neither.

Figure 3. These are the probe DNA sequences that are "spotted" onto the glass in an ordered array. This is the DNA chip. Do not cut these apart.

5′TCAC3′	5′ACCC3′
5′ATTA	5′CGGG3′
5′AACC3′	5′ATGG3′
5′ATTT3′	5′ATAT3′
5′CTCC3′	5′GTCA3′
5′GACC3′	5′AAGG3′
5′CATA3′	5′TCGG3′
5′AGTA3′	5′TTGG3′

Do not cut these apart

Questions

1. Identify which genes are expressed by looking at the Grid Layout (Figure 5).
2. What was the outcome of each control spot, and what does this indicate?
3. Were any gene transcripts *abundant*? *Rare*? Which ones? What does this tell you about the expression of these genes and why might there be differences in expression levels?
4. Which steps in the flowchart (Figure 4) correspond to the steps you did?
5. Are there any cDNA target molecules (from mRNA transcripts) that did *not* match the probe spots on the DNA chip? What does this indicate?
6. What are some applications of microarray technology? Use your favorite search engine.

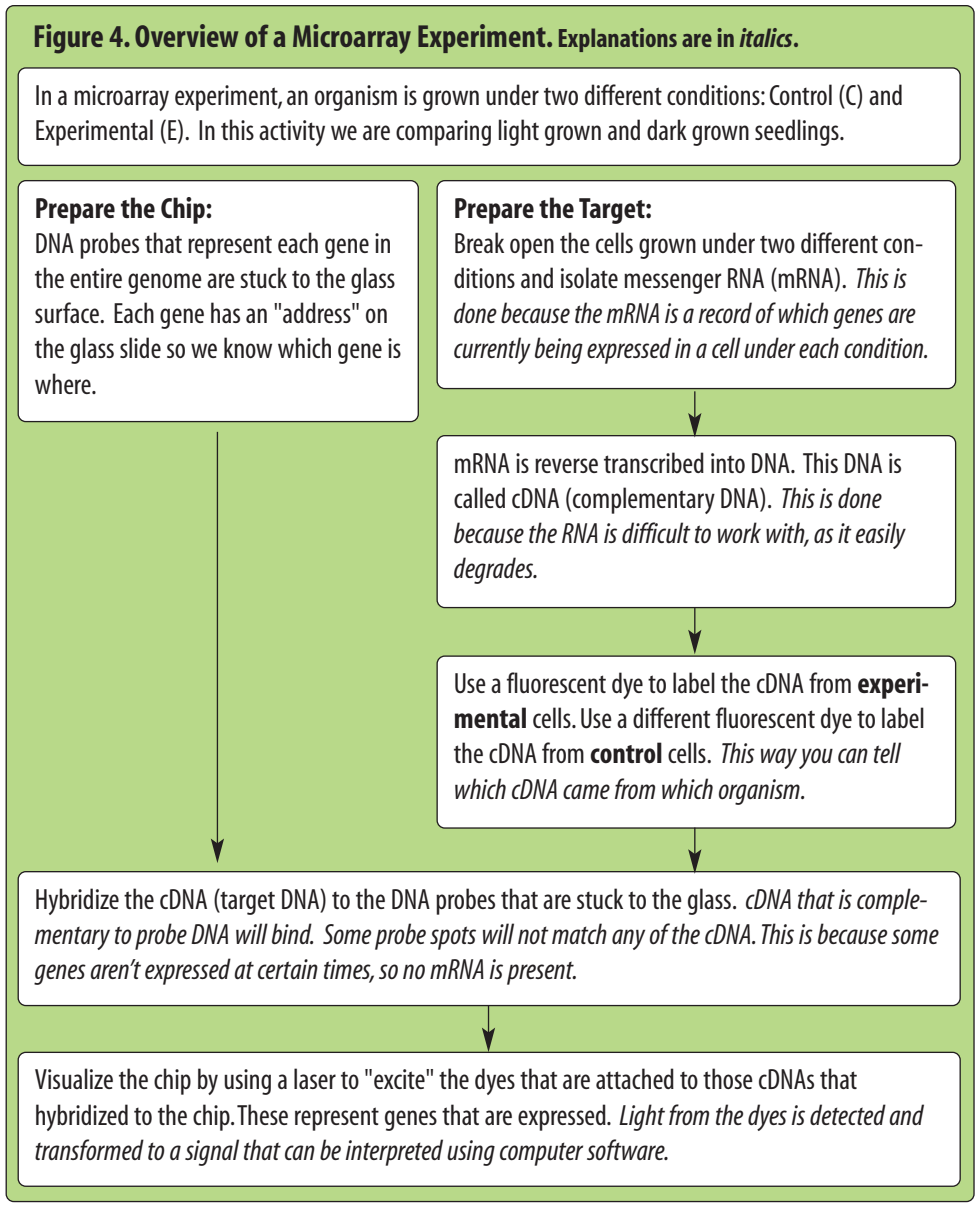
Discussion

In this activity, the results indicate that some genes are expressed in each condition, some in both conditions, and some in neither condition. Light is a signal that can start a cascade of events that "turn on" many genes in a seedling, some of which are identified using this simple chip. If a gene is highly expressed, that mRNA transcript is abundant whereas some genes are expressed at low levels so those mRNA transcripts are rare. Some genes in an organism could be expressed but not detected since microarrays don't usually have every possible gene spotted onto them—this exemplifies a limitation of microarray technology. Some spots don't have anything attached, so that particular gene was probably not expressed in that organism at that time under either condition.

In order to visualize which cDNAs were attached to the probe DNA on the chip, all you had to do was use your eyes to distinguish which cDNA was attached at each location. In real microarray experiments, the cDNA has a fluorescent dye attached to it. A real DNA chip is scanned using one or more lasers. When the laser energizes the dye, the dye gives off light and a machine detects which spot that light came from. Researchers use computer software and sophisticated statistical methods to interpret the spots from a chip that might have as many as 30,000 genes on it. There are many complicated steps involved in analyzing and interpreting microarray data. This activity is an oversimplification to the extent that playing *Monopoly* compares to actually investing in real estate.

The controls in this activity validate that the chip is indeed testing what it is intended to test. For example, the negative control spot has no cDNA bound to it. This spot could be DNA from an unrelated organism, which shows that non-specific binding isn't taking place under the

hybridization conditions. In a real experiment, if the negative control spot had cDNA bound to it, this might indicate that the sample was contaminated, or perhaps the hybridization temperature was too low, allowing for non-complementary base pairing between a few bases. The positive control spot indicates that the materials and method are working properly, i.e. the mRNA was isolated intact, the reverse transcription worked, the chip printing worked, and the hybridization and scanning worked. This positive control spot could be the DNA of a gene that is known to be expressed in both conditions (also called "constituitively expressed"). DNA chips usually have replicate spots in different places to give the experimenter information about the reliability of the information from a single spot.



Student Reading

Microarrays Made Simple: "DNA Chips" Paper Activity

A DNA microarray (DNA chip) is an ordered array of different *known* sequences of DNA (~20-70 bases long). These DNA sequences represent many of the genes in an organism. Many copies of each different sequence are stuck to one "spot" on a solid surface (glass). A DNA chip can have thousands of different spots, representing thousands of different genes. A microarray can have tens of thousands of genes in an area smaller than the size of a postage stamp. In this activity, each DNA sequence stuck to the chip is called a **probe**.

DNA chip technology is based on the basic chemistry of DNA. Adenine pairs with thymine and guanine pairs with cytosine. This base complementarity is what allows DNA from cells to bind specifically to known DNA sequences (probes) on a chip. Since a cell expresses hundreds or even thousands of genes at any given time, a "snapshot" of gene activity can be inferred using microarray technology. Without this technology, it could take years to analyze each gene one at a time.

In this activity you will use only a few probes, rather than thousands, to detect differences in gene expression between seedlings that have been sprouted under two different conditions: light and dark (See Figure 4).

A real microarray experiment requires a high level of skill to prepare the samples and sophisticated equipment and software to collect and analyze the data. Because this technology has only been commonly available since the 1990s, it is still being developed, refined, and perfected. One thing is certain: This technology is not going away—in fact, it is being adapted for studying other types of molecules in cells such as proteins and carbohydrates.

Acknowledgments

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Figure 5. Grid Layout and Gene Description.

GRID LAYOUT

Gene 1	Gene 9
Gene 2	Gene 10
Gene 3	Gene 11
Gene 4	Gene 12
Gene 5	Gene 13
Gene 6	Gene 14
Gene 7	Negative control
Gene 8	Positive control

GENE NUMBER	DESCRIPTION
1	Abscission (losing leaves)
2	Cell elongation 1
3	Light signal processor I
4	Light signal processor II
5	Stress response
6	Cell division (Mitosis)
7	Temperature response
8	Cell elongation II
9	Reproduction (makes pollen)
10	Cellular metabolism
11	Yellow pigment
12	Reproduction (makes ovaries)
13	Salt response
14	Chlorophyll
15	Animal gene
16	Water transport

References

Campbell, M.A. & Heyer, L. J. (2002). *Genomics, Proteomics & Bioinformatics*. San Francisco: Cold Spring Harbor Press & Benjamin Cummings.

Dhahbi, J.M., Kim, H.J., Mote, P.L, Beaver, R.J. & Spindler, S.R. (2004). Temporal linkage between the phenotypic and genomic responses to caloric restriction. *The Way Ahead*. Available online at http://www.affymetrix.com/community/wayahead/index.affx.

Karpinets, T.V., Foy, B.D. & Frazier, J.M. (2004) Tailored gene array databases: applications in mechanistic toxicology. *Bioinformatics, 20,* 507-517.

Schena, M., Shalon, D., Davis, R.W. & Brown, P.O. (1995). Quantitative monitoring of gene expression patterns with a complimentary DNA microarray. *Science, 270,*467-470.