Macromodel of Microarray

Abstract

A teacher-led demonstration of microarray technology using a model created from a pizza box and ping-pong balls. This "macroarray" model demonstrates how single-stranded DNA segments affixed to a solid support are used to separate and identify DNA segments in a solution.

Learning Objectives

- Students will learn that DNA molecules in a solution can be separated and identified using microarray technology.
- Students will observe how microarray analysis allows scientists to test which genes are on or off in different tissue samples (optional).

Estimated time

- Class time 30 minutes
- Prep time 2 hours

Materials

- ping-pong balls (12 or more in at least 4 different colors)
- cardboard box (copy paper box lid or clean pizza box)
- Velcro® hook-and-loop fastener (2 cm x 30 cm)
- ceramic magnets
- packaging tape
- exacto knife
- scissors
- markers
- a large clear plastic container (optional)
- puzzle pieces (optional)
- small box (optional)

Background Information

What is a microarray?

A microarray is a solid support, roughly the size of a microscope slide, that has single-stranded DNA fragments of known sequences bound to it in an ordered and known arrangement. A single microarray will have thousands of DNA sequences (fragments of genes) bound to it. Microarrays can be used to analyze targeted sequences of DNA and to measure relative levels of gene expression. Microarray analysis also plays an important role in diagnosis of disease, detection of gene variations, and drug development. Microarray technology is revolutionizing the study of genomics.

How is a microarray made?

Using robotic technology, numerous small fragments of known DNA sequences are deposited in small spots of 50-500 μm in diameter on a silicon chip, nylon membrane or glass slide. The DNA fragments come from clones or libraries of commercially synthesized DNA. The array is then boiled to make the DNA single stranded so that sample DNA applied to the array can bind to the deposited DNA. The array can be either custom-designed or mass-produced.

How are microarrays used?

1. Depending on the purpose of the experiment, copy or complimentary DNA (cDNA) made from the mRNA of active genes is amplified and labeled with a fluorescent probe. DNA or cDNA from a control sample is amplified and labeled with a different fluorescent probe.

2. The DNA or cDNA is then fragmented into smaller sizes and made single-stranded by boiling or applying an enzyme treatment.

3. The DNA or cDNA from both the experimental and control samples is applied to the microarray where it will bind to complementary spots on the microarray.

4. To detect and record the spots on the microarray to which DNA or cDNA has bound, the microarray is scanned with laser light. The light will detect and create an image of the fluorescent probes added to the DNA or cDNA in the beginning steps.

5. The fluorescent images are displayed on a computer for comparison and analysis. Spots to which DNA from the experimental sample are affixed are displayed as a different color (red) than the spots to which DNA from the control sample have affixed (green). Spots on the microarray to which DNA from both the experimental and control samples have affixed are displayed as a third color (yellow).

Scientists usually purchase their microarrays from biotechnology companies where they are manufactured in large quantities.

Invitation to Learn

1. Place puzzle pieces in a box, shake the box, and ask your students: "Is it possible to assemble two pieces of a puzzle by placing the pieces in a box and then shaking the box?"

2. Invite a student to try it then pose the following question to the class (assuming the student was unsuccessful): "What could we do to increase the chances that the two pieces of puzzle will be assembled inside the box?"

3. Record student responses on the white or chalk board. You may want to try responses that are reasonable. Ultimately, you'll want the class to arrive at the following suggestions:

    - Fix one piece of the puzzle to the bottom of the box. It is more likely the puzzle pieces will fit together if only one piece is free to move around rather than two.
    - Make multiple copies of the free moving piece of the puzzle and add the copies to the box. With multiple pieces, the chance that one free moving piece will match up with the attached puzzle piece will increase.

- Create a form of attraction at the point where the two complementary puzzle pieces fit together. If a randomly moving puzzle piece falls close to the binding site, the attractive force might draw the two pieces together, thus increasing the chance that they will assemble.

4. Inform your students that geneticists are using a similar strategy called microarray analysis to study genes. This strategy is considered to be groud-breaking in that it is far more efficient and a faster way to study genes than other techniques.

Instructions

1. Share the following background information with students:

- Geneticists have created an efficient method for analyzing DNA or gene activity. It is called microarray analysis and has revolutionized genetic research.
- Microarray takes advantage of the following properties of DNA:
  - DNA always follows the base-pairing rules (A-T, C-G).
  - When heated, DNA separates into single strands.
  - When cooled, single-stranded DNA reforms into a double-stranded molecule.
- Microarrays are similar to microscope slides and have DNA fragments of known sequence affixed to them.

2. Hold up the macroarray model and use it to explain that microarray technology works in the following way:

The locations on the bottom of the box labeled 1, 2, and 3 represent spots containing single-stranded DNA segments of a known sequence. A microarray contains thousands of these spots affixed to a piece of glass, silicon, or nylon that is roughly the size of a microscope slide.

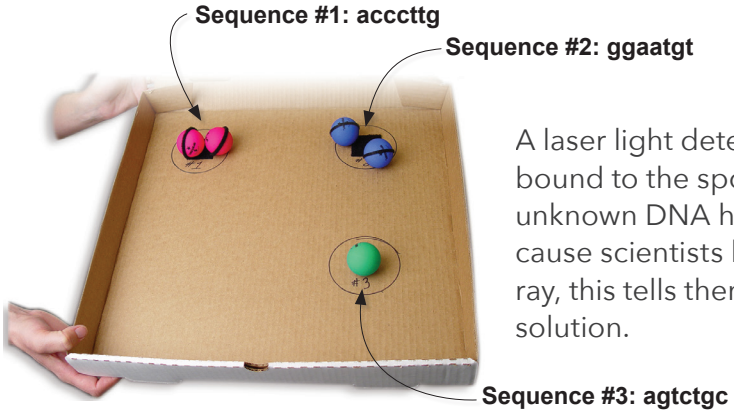
A solution containing DNA segments of unknown sequence with a fluorescent tag added to them is washed over the microarray.

- Add the ping-pong balls, then close and shake the box in a slightly circular motion.

The DNA segments (with fluorescent tags) in the solution will bind to complementary sequences on the microarray.

The microarray is then washed with another solution to rinse the unbound DNA segments away.

- Remove the unbound ping-pong balls from the box.



A laser light detects the fluorescent tags on the DNA that have bound to the spots on the microarray. The spots to which the unknown DNA has affixed are recorded on a computer. Because scientists know the sequence of the DNA on the microarray, this tells them the sequence of the DNA segments from the solution.

3. Using the macroarray model, demonstrate how microarray technology can be used to determine the differences in gene activity or expression between two tissue types.

Explain that if a gene is active, mRNA copies of it will be present in a tissue sample. Scientists are often interested in knowing which genes are active in a certain tissue type relative to a control sample. Performing a microarray analysis allows them to answer this question.

- Place the ping-pong balls, minus those with hook Velcro® affixed to them, in a clear container labeled Sample 1.

A microarray is obtained to which segments of the genes of interest are affixed.

mRNA is isolated from a tissue sample.

DNA copies (cDNA's) of the mRNA are synthesized and labeled with fluorescent tags.

This solution (containing the labeled DNA copies) is washed over the microarray.

- Close and shake the box in a circular motion, then remove the unbound ping-pong balls from the box.

The DNA segments (with fluorescent tags) in the solution will bind to complementary sequences on the microarray. The microarray is then washed with another solution to rinse away unbound DNA segments.

A laser light detects the fluorescent tags on the DNA sequences that have bound to the spots on the

microarray. The spots, representing genes, to which the DNA has affixed are recorded on a computer. The spots (genes) to which no DNA has bound will appear dark.

In order for this data to be meaningful, scientists must compare it against a control sample. DNA from a control sample with a fluorescent tag of a different color is washed over an identical microarray. The experimental sample is typically labeled with a red fluorescent tag, whereas the control sample is typically labeled with a green fluorescent tag.

- Create a control sample by placing the ping-pong balls, minus those with pile Velcro® affixed to them, in a clear container labeled Sample 2. Close and shake the box, then remove the unbound balls.

The data obtained from both the experimental and control samples is then compared by a computer. Green spots indicate a gene is active only in the control tissue, red spots indicate a gene is only active in the experimental tissue, and yellow spots indicate gene activity is similar in both tissue samples.

Identifying the differences in gene expression in a healthy tissue sample (control) versus a diseased one (experimental) clues scientists in on which genes to study further.

Extension

Visit DNA Microarray in the Biotechniques Laboratory on the Genetic Science Learning Center website to perform a virtual microarray experiment and determine which genes are active in healthy versus cancerous tissue. This activity contains an in-depth explanation of the process demonstrated above.

Adaptations

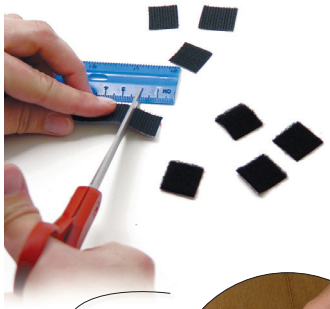
You may want to label the "spots" on the macroarray model with DNA base-pair sequences. Once you have completed the demonstration, have students write out the sequence of each complimentary DNA segment (ping-pong ball) attached to the model.

Funding for this module was provided by a Science Education Partnership Award (No. 1 R25 RR16291) from the National Center for Research Resources, a component of the National Institutes of Health.

Detailed Preparation Guide

Materials

Creating the Box



Cut four 2.0 cm x 2.0 cm pieces of both the pile and hook Velcro®.

In one corner of the box, paste the squares of hook Velcro® in a cluster to form a 4.0 cm x 4.0 cm square. Do the same with the pile Velcro® in the opposite corner. Draw a circle around each cluster to resent spots on a microarray.

rep-

These will simulate the DNA segments of known sequence that are affixed to a microarray. Label them Sequence #1 and Sequence #2 (or 1 and 2 for short).

Tape 1-2 ceramic magnets to the underside of the box lid or pizza box in a location that is a comfortable distance from the position of Sequences 1 and 2.

Draw a circle on the right side of the box directly above the magnet.

Label this Sequence #3 (or 3).

Creating the Balls

Cut a 13 cm long pieces of both pile and hook Velcro®. Carefully cut off three 0.2 cm wide strips.

Glue one thin strip of hook Velcro® to each of 3 ping-pong balls of the same color. (These will represent DNA segments of unknown sequence in solution.)

Glue one thin strip of the pile Velcro® to each of 3 ping-pong balls of a different color. (These will represent DNA segments of unknown sequence in solution.)

Using the exacto knife, slice a very small hole into the ping-pong ball of the third color. Insert the rare earth magnet inside.

NOTE If you are having trouble getting the ping-pong balls to stick to the box, you may wish to place an additional 0.2 cm strip of Velcro® on the balls.