DNA Microarray Model Activity
Participant Guide

Description and Estimated Time to Complete

In this activity you study how DNA (Deoxyribonucleic acid) microarrays are fabricated using a photolithography process developed by the semiconductor manufacturing industry. You then apply this knowledge to building a macro-size DNA microarray model. A second activity allows you to simulate the process of matching probes to targets and interpreting the results. These activities should improve your understanding of how DNA microarrays are made and how they identify complementary single-stranded DNA (ssDNA) in a sample.

A bonus activity takes the DNA microarray fabrication process one step further and asks you to design a 4 x 4 microarray, identifying the DNA sequence for each feature, and then designing the masks for microarray fabrication.

If you have not reviewed the unit <u>DNA Microarrays</u>, you should do so before completing this activity. This <u>DNA Microarray</u> unit provides the background information needed to best understand the concepts presented in this activity.

<u>Estimated Time to Complete</u>
Allow at least two hours to complete this activity.

Introduction

DNA microarrays are helping researchers learn more about human diseases, what causes them, how to identify them, and how to treat them. We now know more about complex diseases such as diabetes, multiple sclerosis, heart disease, and cancer than we have ever known before. For some diseases, such as multiple sclerosis, researchers have been able to identify specific genes that influence the risk of getting the disease.[Stimson, 2007] They have found that most genetic disorders are influenced by many, many genes and not just one or two. Such discoveries may eventually lead to the development of the therapeutics needed to diagnose and prevent a disease or to possibly cure it.

DNA microarrays use gene sequencing and DNA transcription and hybridization to analyze and identify thousands of genes simultaneously. Each microarray consists of hundreds or thousands of gene sequences (or ssDNA molecules) which are mounted on a chip and used as "probes". These probes detect complementary DNA fragments or cDNA copied from messenger RNA (mRNA) in a sample. The cDNA are the target molecules *(as shown in the graphic)*.

The DNA microarray relies on hybridization of ssDNA fragments to an oligonucleotide (oligo) DNA sequence (a specific A,C, G, T combination). In DNA microarrays synthetic DNA oligos are fabricated and used as the capture molecules or probes. These synthetic DNA oligos are fabricated onto a solid surface (substrate) before the hybridization step. In the graphic to the right, the probes are the oligos.

This graphic illustrates six identical probes on a substrate and several possible targets. Three of the probes have identified cDNA (targets) and have reannealed or are in the process of reannealing into a double-stranded DNA or dsDNA (i.e., hybridization). Those cDNA that do not match this specific probe's sequence continue to move through the microarray looking for a matching sequence at another address or feature within the array.
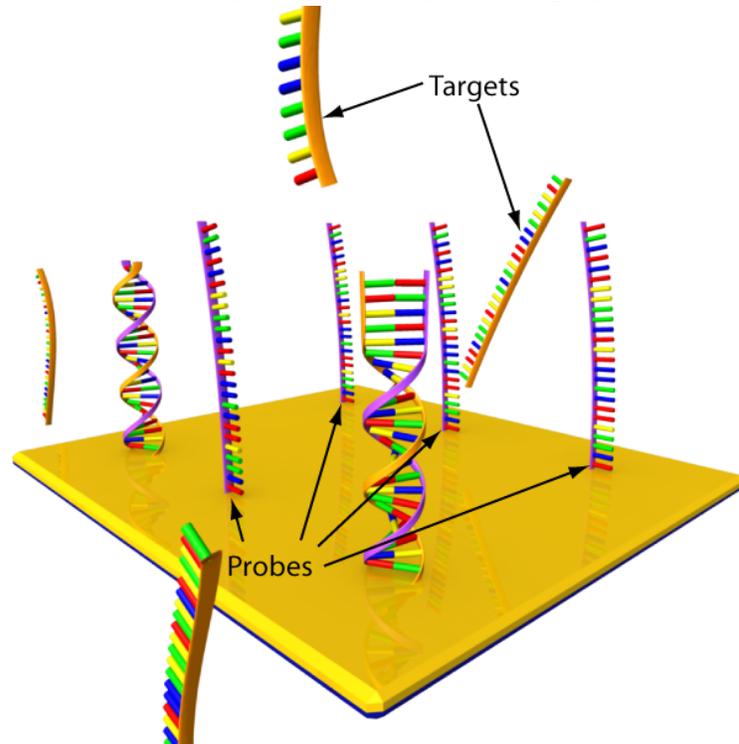
Activity Objectives and Outcomes

Activity Objectives

- Using the components provided in a SCME DNA Microarray kit, build a macro-size DNA microarray with at least a 3 nucleotide sequence using the photolithography process developed by Affymetrix for the GeneChip^{TM}.
- Outline and explain the fabrication steps for an oligonucleotide array.

Activity Outcomes
At the end of this activity, you will be able to answer the following questions:
- How are oligonucleotides used in a DNA microarray?
- How are synthetic oligonucleotides fabricated on a DNA microarray?
- How does a DNA microarray identify different target molecules simultaneously?

**Resources (Can be downloaded from scme-nm.org – Educational Materials)**

SCME DNA Microarray Primary Knowledge unit
SCME DNA Microarray Model Kit

DNA Microarray Fabrication

Many of the fabrication methods for microarrays come from semiconductor fabrication technology as well as microtechnology. For example, one type of microarray fabrication technique uses a printer similar to an ink-jet printhead (a macro-size device with micro-size elements called nozzles) to print the addresses onto the microarray slide. However, the "ink" is an oligonucleotide solution. Another method of microarray fabrication uses the photolithography process used in micro-fabrication. Let's take a closer look at both processes.

The DNA microarrayer (below left) "prints" oligonucleotide (oligo) probes to specific addresses on a microscope slide using the microtechnology of an ink-jet printer (below right). The inkjet "printhead" consists of an ink reservoir for each color and piezoelectric actuators that allow the fluid ink to flow from the reservoir to the nozzles. Because the diameter of the nozzles is in the micrometer-scale (between 1 to 100 micrometers), capillary action moves the molecules of ink through the tiny nozzles. This same technology is used to achieve the nano-size probes when printing microarrays. Because the size of a probe is in the nano-scale, the printhead nozzles are smaller allowing a single microarray to have as many as a million to two million addresses printed onto one slide. As the printhead moves across the array, actuation of the piezoelectric crystal causes the prepared oligos to be delivered through the nano-size nozzles to the slide surface without physical contact.
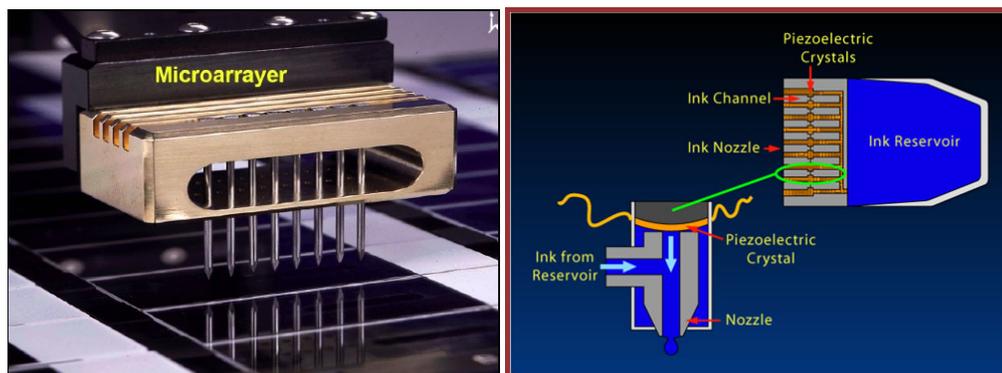
Take a break and watch a video showing the robotic printing of DNA microarrays:
"Diagnostic Microarrays" - **http://www.youtube.com/watch?v=8hB0hy6_oKg&feature=related**

The printing of a DNA microarray is very laborious due to all of the oligonucleotide "inks" that must be prepared for each of the addresses on the microarray. These oligos may be synthesized by chemical methods, one at a time, or they can be prepared enzymatically by a reverse transcription of mRNA isolated from cells to copy DNA or cDNA. Both of these methods of preparing oligo probes from each address of a DNA microarray are long procedures and require a long time to prepare before you have all the sequences needed to print onto a microarray.

Alternatively, the photolithography method uses the photolithography process borrowed from the semiconductor fabrication industry in combination with chemical reactions to synthesize oligonucleotides probes on a silicon surface. The Affymetrix GeneChip® *(image right)* is a DNA microarray, but more specifically an **oligonucleotide array** that has thousands of synthetic oligonucleotide (oligo) probes fabricated on a silicon chip. The oligos on these arrays are generally 20 nucleotides long and the array itself, is smaller than other types of DNA microarrays. Each address on a GeneChip® may be as small as 50 nanometers (nm) square – almost 2000 times smaller than the width of a strand of hair! Even smaller are the oligos themselves. Each address may contain 11 to 16 DNA copies of the same gene meaning 11 to 16 oligos per 50 nm square address! [(Zaccheo, 2005)]

DNA microarray (2008) allows for assay of approximately 500,000 polymorphisms in a single genome.
[Image courtesy of Affymetrix]

In this activity you will simulate the GeneChip® fabrication process for a Oligonucleotide Array which is patented by Affymetrix. So let's take a more in-depth look into that process.

Fabrication of a Oligonucleotide Array

As previously mentioned, oligonucleotide microarray fabrication uses the photolithography process developed from the semiconductor industry and chemical reactions to construct synthetic oligonucleotides onto a silicon substrate. In this approach oligos of different sequences are built from the bottom up in each address of the GeneChip^{TM} by repeating a three step process –Protect , Deprotect, Addition. Each cycle of the three step process adds a new nucleotide base (i.e., A (adenine), T (thymine), G (guanine), or C (cytosine)) to the sequence, allowing the oligos to be built up one nucleotide at a time. Let's take a look at this process.

Initially, a light-sensitive blocking chemical or agent is washed over the entire silicon wafer (one wafer contains hundreds of microchips or microarrays). The blocking agent protects the "surface" of the wafer. In subsequent steps, the blocking agent attaches to the nucleotides in the *Protect* step of the process.

Deprotect (Photolithography)
Deprotect uses photolithography which requires a patterned mask and ultraviolet (UV) light to "deprotect" select features of the microarray. Each exposed feature is a location for the addition of the next nucleotide base in a specific sequence. The graphic illustrates the UV light, mask and substrate. Each square in the mask is a select feature or address in the array. The light that travels through the mask degrades the chemical blocking agent, "deprotecting" the areas exposed to UV.

Addition- After "deprotect" the wafer is washed with a solution of the specific nucleotide base being added at that step. The nucleotide bases in the solution attach through a chemical reaction to the deprotected areas on the wafer. The blocking agent protects all of the features that were not exposed to the UV light, preventing the addition of a nucleotide base in those features.
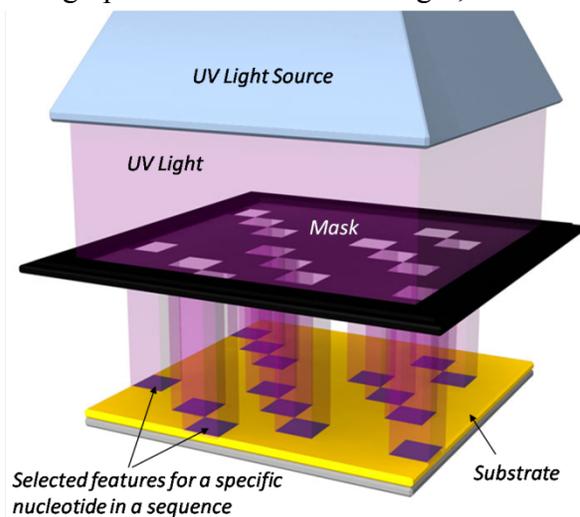
Protect
Once the exposed areas have increased in size by one nucleotide base, a photosensitive blocking or "protective" agent is added to "protect" the new nucleotide bases on the array. The microarray is now ready for another mask or "deprotect" step.

Take a few minutes and watch the animation "GeneChip" on YouTube. This animation illustrates the three step process described above. (*NOTE: The animation uses "filter" in place of "mask".)* http://www.youtube.com/watch?v=V8uNJCO7Qqo

As seen in the animation, this three step cycle is repeated until the desired oligonucleotides are fabricated throughout the array. For each cycle of the process a mask with a unique pattern is used. An array with 25 nucleotide probes may require as many as 100 masks and thus 100 cycles of the deprotect, addition, and protect process. Therefore, since a new mask is required for each deprotect step of a cycle, the component that ultimately controls the building of ssDNA probes (oligos) on selected positions in the array is the mask.

With the first few masks, most of the deprotected areas "link" a nucleotide directly to the substrate. The other deprotected areas attach the nucleotide to a deprotected nucleotide already linked to the substrate. In order to construct an array with specific nucleotide sequences in each feature, each mask is unique and each mask deals with one and only one nucleotide at a time.

For example, the following four graphics illustrates the first two masks of a process and the outcome at the end of a three step cycle. Mask 1 (a) identifies 16 features (addresses) that start with the A nucleotide base as the first nucleotide in the sequence. The (b) graphic is the result. The yellow dots represent the A nucleotide that has linked to the substrate. Mask 2 (c) identifies 20 features for the C nucleotide *(16 attached to the base and 4 attached to an existing A nucleotide)*. The (d) graphic is the result. The red dots represent the C nucleotide.

Mask 1 – Identifies location of A bases   **Mask 2 – Identifies location of C bases**

(a)   (b)   (c)   (d)
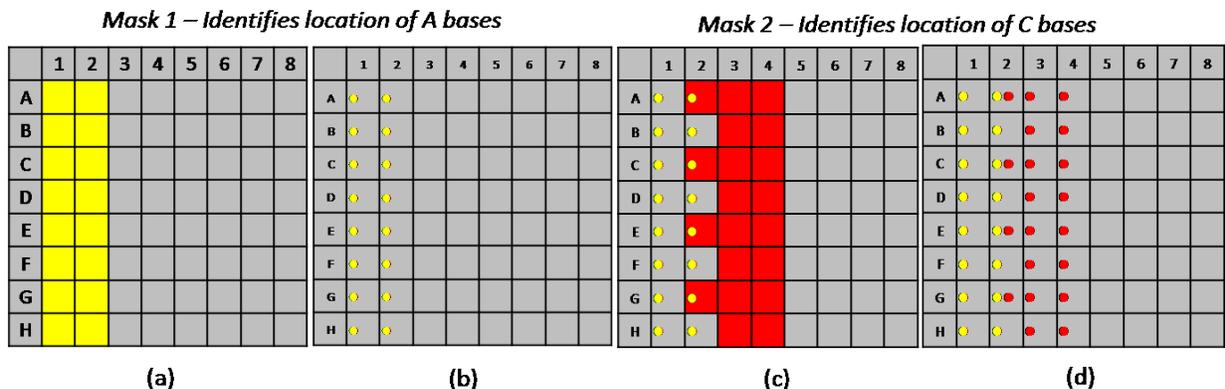
Mask 1 indicates "A" as the first nucleotide in the sequence (*in yellow)* in columns 1 and 2. Mask 2 indicates "C" as the first nucleotide in the sequence *(in red)* in columns 3 and 4. However, as you can see in figures (c) and (d), column 2, rows A, C, E, and G, a sequence of A-C is indicated. Mask 2 allowed "C" to synthesize onto the deprotected base "A". *(Note: C actually is "on top of" A, not side by side as shown.)* This indicates that in these four addresses the first two bases of the oligo sequence is A-C. Each subsequent mask controls the rapid synthesis of the oligo probes by allowing each probe's sequence to pick up one more link in the chain (i.e., a sequence of A-C-T could become A-C-T-C). "Computer algorithms calculate the optimal design of the masks that will minimize the number of reactions needed." [(Antler, 2004)]

So putting things in perspective – in the previous example, after two masks, we've only just begun with half of the array. As previously mentioned, if we want an array of 100 oligonucleotides, then we'll need at least 100 masks!

Here's another presentation by Affymetrix, the developer of this process.

- DNA Chips and microarrays – A YouTube video by Affymetrix on the DNA microarray. http://www.youtube.com/watch?v=ui4BOtwJEXs&feature=related

Are you ready? Let build a model of a DNA microarray simulating the process used to fabricate Oligonucleotide Microarrays.

Team

This activity would be more beneficial by working with 1 to 2 other participants. Working as a team will promote discussion and further exploration into DNA microarray fabrication, operation and applications.

Workspace

A workspace with a flat table and plenty of elbow room is all you need to complete this activity.

Supplies / Equipment

SCME Kit – DNA Microarray Model
Optional: A flashlight to simulate the UV light

The kit contains
- a "substrate" (below left) representing an 8 x 8 microarray (64 addresses / features),
- many nucleotide bases (beads indicating A, C, G, or T),
- a set of masks needed to fabricate a three nucleotide chain in each address, and
- two sample bags – Sample 1 and Sample 2. (not shown below)
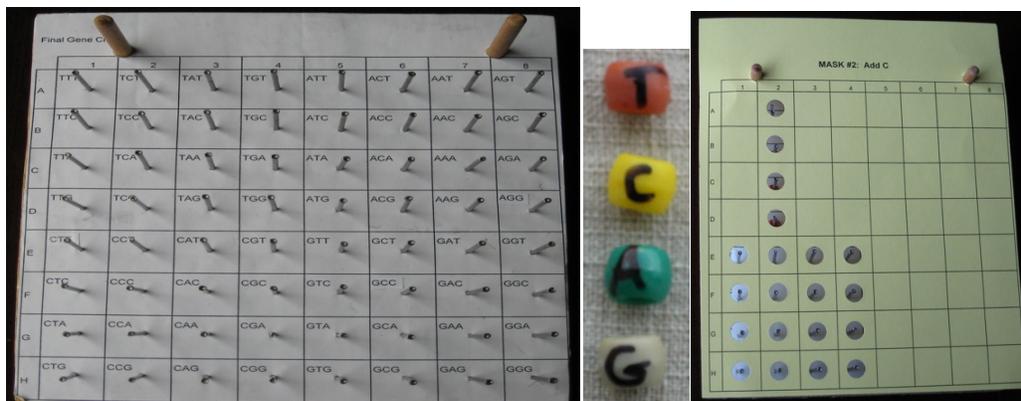
(Pictures may not match the new kit parts)

Documentation

1. A table indicating the specific oligo for each feature in the area,
2. The cDNA sequence from the control or test samples that have attached to an oligo (indicate on the table).
3. Answers to the Post-Activity Questions.

NOTE: Be sure to include ALL sources and references to data and graphics when applicable.

Activity: Building a DNA Microarray – Part I

Procedure:

Building the synthetic oligos

1. Check your supplies. You should have an 8 x 8 array, 4 different colors of beads, 12 masks, and a small bag of "fluorescent labeled nucleotide sequences" (3 beads and a sequin on a paperclip).

2. Note the legend at the top of the array. There are four nucleotide bases; therefore, there are four colors of beads, each color representing one specific nucleotide. Indicate the color for each nucleotide base. (*Reminder: A nitrogenous base with a sugar and at least one phosphate is a nucleotide or nucleotide base.*)

      a. Adenine - _____

      b. Thymine - _____

      c. Guanine - _____

      d. Cytosine -   _____

3. Assuming the substrate (8 x 8 array) has been fully protected with a blocking agent, use Mask 1 to identify the features that will be deprotected with UV light. (Align Mask 1 to the array using the two pegs at the top of the board.)

4. At this time in a real fabrication process, UV light would be used to expose or deprotect the open features in the mask. If you have a flashlight, you can simulate this by holding the flashlight directly over the mask, turning the light ON for 2 seconds, then OFF.*This step is deprotect.*

5. "Add" the first nucleotide to the deprotected areas by dropping the correct color of bead through the holes. (The nucleotide base for each mask is indicated by both the color of the mask and the text at the top of the mask.) This step is *addition.*

6. Remove Mask 1. In a real fabrication process, the new nucleotides are protected with a blocking agent in preparation for the next mask. This step is *protect.*

7. Align Mask 2 to the array. *Deprotect* the select features.

8. "Add" the second nucleotide to the deprotected areas by dropping the correct color bead through the holes.

9. Continue to build your oligos using remaining masks. After Mask 12 you should have a 3 nucleotide oligo on each feature of your array.

10. Using the 8 x 8 array template (GeneChip® Model Template) on the next page, identify the oligo in each feature of the array. (e.g., TTT, TCA, CAT, AGG)

11. Complete the Part I: Post-Activity Questions.

DNA Microarray Model Template

	1	2	3	4	5	6	7	8
A								
B								
C								
D								
E								
F								
G								
H								

Part I: Post-Activity Questions

1. Explain the function of the synthetic oligonucleotides on a DNA microarray.

2. How does a DNA microarray identify thousands of different genes (target ssDNA) simultaneously?

3. What is the purpose of the blocking agent in this fabrication process?

4. What is the purpose of the mask in each cycle of the fabrication process?

5. What happens during the "addition" step of the fabrication process?

6. Approximately how many masks would be required to fabricate a 30 base oligonucleotide in each feature of an 8 x 8 array?

7. Graphically, outline the fabrication process that utilizes photolithography and chemical reactions to build synthetic oligos. For each step, write a short description of the process.

An Online Review of DNA Microarray and Genechip® Fabrication Methods.

Go to http://www.dnai.org/d/index.html
Go go to "Genes and Medicine"
then "Genetic Profiling",
then "Techniques".

Watch the sections for DNA Microarrays, GeneChips and then "Making GeneChips"

Interpreting Your Results

Each feature (also called address) of a DNA microarray has a different set of synthetic oligo probes attached to it. Each feature may contain hundreds or thousands of identical probes, while each array may contain tens of thousands of features. Each feature is looking for a specific gene sequence (allele) found in a particular organism. Because an array contains thousands of features, it can simultaneously search and "find" thousands of specific genes.

One way to help to ensure the accuracy of any DNA microarray test as well as to allow easier interpretation of the results, a control sample is used along with the test sample. Each gene in the control sample is tagged with a green fluorescent dye molecule. Each gene or cDNA in the target sample is tagged with a red fluorescent dye molecule. After hybridization, a laser scans the microarray and the presence of an attached target DNA is detected by the fluorescence of the label on the microarray. The image to the right is a scanned DNA microarray showing the hybridization results at each feature in the array.

- Yellow indicates hybridization with cDNA from both control and test samples
- Red indicates hybridization with cDNA from mostly or only the test sample
- Green indicates hybridization with cDNA from mostly or only the control sample
- Black indicates no hybridization with either sample
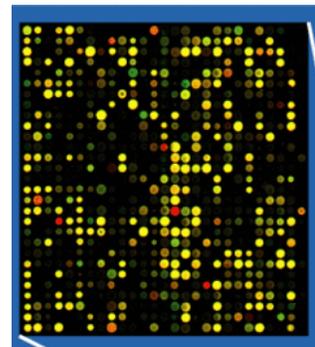
Another way to ensure accurate test results is to build controls into the microarray during fabrication. Such control includes positive and negative control as well as direct comparison controls.

Positive and Negative controls
Positive and negative controls exist in all biological assays (tests that analyze biomolecules in specific events or conditions). The function of positive and negative controls is even more important for DNA microarrays because of the complexity of microarray fabrication. The purpose of positive and negative controls is to verify the overall performance of the microarray (Is it accurate and can it be trusted?) and the analytical technique (Were the samples prepared properly and the procedures executed correctly?).

A negative control is an array feature that is designed to have NO binding or hybridization. Its purpose is to avoid getting "false-positives" – positive results that should have been negative. In the design of a DNA microarray, bogus features that have no oligos attached are interspersed throughout the array. If something does bind to one of these features, then the results of the test should be questioned and the test repeated. Prior to repeating the test, adjustments should be made to the preparation to better ensure accurate results.

A positive control is one that you expect to show a positive result. An example is a feature that contains a gene sequence that is ALWAYS present; therefore, you should see hybridization with

both the control and test DNA samples. If this positive control does not show hybrization with both samples, then one would have to assume that there may be "false negatives" elsewhere in the array. Therefore, the assay results should not be trusted. In the case of a "negative" positive control, one should examine the procedure used to prepare the DNA samples to ensure that it was correct. If the procedure was correct, then one should suspect that the microarray is defective. In this case, the test should be discarded.

Direct Comparison Controls
In this type of control, each feature of the array is a comparison of the test sample DNA to the control sample DNA. Such controls are for the direct comparison of each address (or each gene) between two different cells. The control DNA sample provides a comparison for evaluating the test DNA sample and thereby rules out other factors that might play a role in a low signal strength (low amount of test DNA binding or hybridization) or a high signal strength (high amount of test DNA hybridization). For example, if during fabrication of the microarray a more abundant density of capture oligonucleotide (probe) is made in one of the features, this could result in a higher amount of test DNA bound to that address in the array. Conversely, an address with a lower density of oligo synthesized will result in fewer test DNA bound there. By comparing a *ratio of test DNA to control DNA* bound at all of the arrays, it is possible to rule out these types of problems that can arise during fabrication.

Activity: Testing and Interpretation – Part II

Mapping your array and identifying specific genes in the control and test samples

NOTE: The purpose of Part II of this activity is to provide you with a little exposure into the world of DNA microarray interpretation. There is a tremendous amount of information supplied in a completed DNA microarray test. Therefore, one must have a strong foundation in biology and genetics to be able to understand and interpret the information that is available from such tests. **This is only an exercise, not an actual test result**.

Procedure:

1. Ask your instructor for a bag of targets. Each bag of targets contains cDNA from both a control cell (sample) and a test cell. (Reminder: The cDNA in the control and test samples are specific gene sequences or alleles to be identified by the DNA microarray.)

2. Separate the target DNA sequences into control sample (green tags) and test sample (red tags).

3. Match each gene sequence (cDNA) to the complementary oligo on the array. Remember that the tag is at the TOP of the cDNA.

4. To your array template, indicate the features with a cDNA in the control sample and in the test sample by writing in the cDNA sequence and the specific sample. (For example -the feature for GGA would require a cDNA of CCT; therefore, if that match is made on your array with a green tagged sequence, your template would read "GGA-CCT (Gr)".) [*Review of DNA base pairs: A-T, T-A, G-C, C-G*]
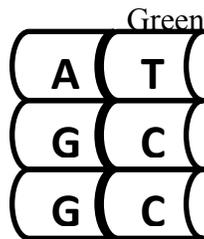
5. Answer the Post-Activity questions for Part II.

Part II: Post-Activity Questions

1. The array features H5 and H7 are positive controls.
    a. What is the purpose of a positive control feature?

    b. What can one infer when both positive control features have hybrids from both the control and test samples?

    c. What would one see in the case of a "negative-positive" control?

2. The array features H6 and H8 are negative controls.
    a. What can one infer if either H6 or H8 or both indicate binding with a cDNA from either the control or test sample?

    b. What can one infer if neither H6 nor H8 indicates binding with any of the cDNA from the samples?

3. What process is performed prior to testing to help distinguish between the targets in the control and test samples?

Following are tables that will assist you in the interpretation of this specific Genechip® test.
    a. The first table (List of the Gene Sequences in the Samples and the related Array Addresses) shows all of cDNA that are available in the control and test samples. The table briefly describes what gene sequence or allele is being "identified" in a specific array addresses.
    b. The second table (Notes for Interpretation of DNA Microarray) provides information on what a hybrid in a specific address may indicate.

Using the tables below, answer the following. Keep in mind that this is only an exercise to give you a taste of DNA microarray interpretation. There is A LOT more to it than is indicated here.

4. Is you test valid? _____
   a. If YES, explain.

   b. If NO, explain.

5. Based on the results of your test, make assumptions as to the patient's health and prognosis. Support your assumptions with specific results from the microarray. (If you have an invalid test, go ahead and complete an analysis of your results, assuming a valid test.)

List of the Gene in the Samples and the related Array Addresses

Array Address	Gene Name	Abbreviated Explanation
A5	BRCA1	Tumor suppressor gene that protects cells from cancer (normal allele)
A6	BRACA1-11	Mutation of BRCA1 tumor suppressor gene. This mutation is responsible for more than 80% of inherited breast and ovarian cancers
A7	BRCA2	Tumor suppressor gene that protects cells from cancer (normal allele)
A8	BRCA2-26	Mutation of BRCA2 tumor suppressor gene. This mutation is responsible for more than 80% of inherited breast and ovarian cancers
B1	DAB	Tumor suppressor gene (normal allele)
B2	DAB2	Mutation of DAB that enhances cell spreading
B7	ESR	Estrogen receptor cell signaling protein: proto-oncogene
B8	ESR1	Oncogene associated with breast and endothelial cancer.
C3	DCC	Tumor suppressor gene (normal allele)
C4	DCC-02	One of 3 mutations of the DCC gene, associated with advanced colon cancer
C8	IL6-01	Oncogene associated with numerous cancers
D3	ID4	Tumor suppressor gene (normal allele)
F7	TP53	Tumor suppressor (normal allele)

F8	TP53-D4	Mutation associated with smoking and confers an aggressive cancer with poor prognosis
H5	CP450	Positive Control (All cells have this gene)
H6		Negative Control
H7	TUB	Positive Control (All cells have this gene)
H8	No oligo	Negative Control

Notes for Interpretation of DNA Microarray

Array Address	Meaning of Green and/or Red
H5, H7	A green and red on H5 and H7, AND *H6 and H8 blank*, validates the results. (It implies a properly fabricated array and the use of proper experimental procedures for making the oligos from the sample tissue.)
H6 and H8	Negative control. Both should be blank. A red or green marker in either of these addresses indicates an invalid test.
B7	A proto-oncogene (normal allele)
B8, C8	Oncogene – Biologically, it only takes one oncogene allele to make cancerous tissue. This is because an oncogene is a dominant gene. Therefore, a red on this feature could indicate bad news for the patient.
A5, A7, B1, C3, D3, F7	Tumor suppressors – Protect the cell from becoming cancerous. If one of the pairs of tumor suppressor alleles (e.g., BRCA1 and BRCA2) is mutated and no longer functions, the remaining tumor suppressor can compensate (i.e., BRCA1 can protect against a mutation of BRCA2). However, if both tumor suppressor genes are mutated (e.g., BRACA1-11 and BRCA2-26) and there is no normal allele left (e.g., BRCA1 or BRCA2), this is bad news for the patient.
A6, A8, B2, C4, F8	Gene mutations that aid in the development of cancer and may be used to determine the patient's prognosis.
	If a feature for a normal allele is GREEN only, this means that only the mutant allele is found in the test sample DNA.
	If a feature for a mutant allele is BLACK, this means that neither the control nor the test sample has that mutation.
	If a feature for a mutant allele is RED, this means that the test sample has this mutation.

Abbreviated Glossary

Allele – One member of a pair or variation of a gene that occupy a specific position on a specific chromosome.

Gene–The basic biological unit of heredity. A length of DNA sequence or segment of DNA that "contributes to phenotype/function".(Guidelines for Human Gene Nomenclature)This region is usually associated with regulatory regions of DNA sequence.

Genome – An organism's genetic material which is made up of molecules of DNA and found in the nucleus of eukaryotic cells and in the cytoplasm of prokaryotic cells.

Hybridization–A process of combining two complementary single-stranded DNA into a single double-stranded molecule through base pairing.

Nucleotides – Nitrogenous bases with a sugar and at least one phosphate, that when joined together, make up the structural unit of RNA and DNA.

Oligonucleotide – A short fragment of single-stranded DNA typically 5 to 50 nucleotides long.

Oncogene – A mutated gene that causes the transformation of normal healthy cells, into cancer cells.

Proto-oncogene – A normal gene which, when altered by mutation, becomes an oncogene that can contribute to caner.

Tumor Suppressor gene - A gene that protects a cell from cancer. If both pairs of tumor suppressor genes are mutated, the cell loses its protective effect and can become cancerous.

BONUS Activity: Designing a DNA Microarray

Team Member Names: _____ _____

Objective: Design and simulate the fabrication of a DNA microarray by determining the DNA sequences for each of the features in a 4 x 4 array based a specific scenario. Once the sequences are determined, design the process masks that will yield those sequences.

Activity Part I: Design a 4 x 4 Direct Detection DNA microarray. Your array should meet the following criteria. (Use the notes and *Activity Gene Table* provided at the end of this activity to gather the information for the criteria.)

1. Design feature oligos to detect specific genes (3 nucleotides sequences) that could indicate a

   possible disease or trait (e.g., breast cancer, mild or aggressive) in a test sample. Create a scenario

   for your test. Write your scenario below. (e.g., This DNA test is to determine if the subject has the

   genes for breast cancer and if genes are present that indicate that the cancer may be of an

   aggressive or non-aggressive form.)

   _____

   _____

   _____

2. Each oligo sequence should be 3 nucleotides long and each feature should have one sequence.

   (Use the sequences and table at the end of this worksheet for a guide.)

3. Develop at least 12 unique sequences. (Identify sequences that would be found in both the test and

   control samples and at least two sequences found only in the test sample.)

4. Use controls to ensure test validity.

5. Complete the table on the next page to describe the content of each feature (the sequence, gene

   name, and whether the feature is a control, control gene, test gene or both).

DNA Microarray Layout

In each of the array features, indicate…
1. the 3 sequence oligo (ATT) for that particular feature (first base at substrate)
2. the Gene Name from table at the end of this worksheet (e.g., BRCA1)
3. whether it is a control, control gene, test gene or both

	1	2	3	4
A				
B				
C				
D				

Activity Part II:

Design the masks for your array.

1. Determine the sequence of nucleotide application for your microarray. (i.e., which nucleotide is applied first, second, third, etc.)

2. For your masks, you can either physically make the masks using paper or create a graphic or series of graphics that show the layout of each mask. Design the layout of each mask for each step.

3. Based on your sequences, how many masks did you need? _____

4. To test your masks, give them to another team and have it create an array from your masks

Post Activity Questions

1. What are the TARGET sequences that would hybridize with select features in your array yielding a "valid" test and showing the presence of at least one gene from the test sample only.

2. A DNA microarray can have as many as 30 bases in one oligo. What is the maximum number of masks that could be needed for an array with 30 base oligos?

3. Be prepared to state the purpose of each feature in your array.

Activity Gene Table (The DNA sequences in the features for the following gene are hypothetical and strictly for the purpose of this activity.)

Array Address	Gene Name	Cellular Effects
A1	CDKN2	Tumor suppressor gene that encodes a protein called p16 that protects cells from cancer by stabilizing the tumor suppressor protein p23.
A2	CDKN2A-032	One of 27 different mutations to CDKN2
A3	CDKN1	Tumor suppressor encodes a protein called p21 that protects against cancer by inhibiting the action of cell signaling proteins and triggering cell death when DNA has been damaged beyond repair. Vitamin D activates the activity of p21, and this protein works with p53.
A4	CDKN1A-02	One of 5 different mutations to CDKN1
A5	BRCA1	Tumor suppressor gene that encodes a protein that plays a role in DNA repair and protects cells from cancer.
A6	BRCA1-11	One of 20 mutations to BRCA1. Mutations of the BRCA tumor suppressor gene are responsible for more than 80% of inherited breast and ovarian cancers.
A7	BRCA2	Tumor suppressor gene that encodes a protein that plays a role in DNA repair and protects cells from cancer.
A8	BRCA2-26	One of 20 mutations to BRCA1. Mutations of the BRCA tumor suppressor gene are responsible for more than 80% of inherited breast and ovarian cancers.
B1	DAB	Tumor suppressor
B2	DAB2	Mutation of DAB that enhances cell spreading.
B3	DRD	Proto-oncogene, dopamine receptor.
B4	DRD2	Oncogene that is associated with metastasis and confers a poor prognosis in cancer patients.
B5	END	Endothelin signaling protein: proto-oncogene.
B6	END1	Oncogene that is associated with aggressive cancer and confers a poor prognosis in cancer patients.
B7	ESR	Estrogen receptor cell signaling protein: proto-oncogene.
B8	ESR1	Oncogene that is associated with breast and endothelial cancer.
C1	ERBB2	The first proto-oncogene that was found, it stabilizes a family of growth factor cell receptors that are involved in triggering normal cell division.
C2	ERBB2-01	Mutation of ERBB2 that forms an oncogene that is linked to a poor prognosis in breast and ovarian cancer. This is one of 2 mutations of ERBB2
C3	DCC	Tumor suppressor
C4	DCC-02	One of 3 mutations that inactivate the tumor suppressor activity of the DCC gene, associated with advanced colon cancer.
C5	FHIT	Tumor suppressor gene that plays an important role in protecting the cell from carcinogens and in inducing cell death in response to DNA damage beyond repair.
C6	FHIT-01	Mutations in this tumor suppressor gene have been found in about half of all esophageal, stomach, and colon carcinomas, and is a component of aggressive pancreatic cancer.
C7	IL6	This proto-oncogene is also known as: Interferon Beta-2 (IFNB-2), B-Cell differentiation factor, B-Cell stimulatory facto 2 (BSF2), Hepatocyte stimulatory factor (HSF) and Hybridoma

		growth factor (HGF).
C8	IL6-01	Oncogene associated with numerous cancers, especially lymphomas.
D1	IFR1 (upstream sequence)	Interferon regulatory factor. Deletions of this tumor suppressor are found in a number of leukemia's.
D2	IFR1 (downstream sequence)	Interferon regulatory factor. Deletions of this tumor suppressor are found in a number of leukemia's.
D3	ID4	Tumor suppressor gene that encodes Inhibitor of DNA Binding protein.
D4	ID4-01	Loss of the ID4 function results in aberrant methylation of DNA and is associated with leukemias and other cancers.
D5	INHA	Proto-oncogene that inhibits expression of certain growth factors.
D6	INHA	Oncogene often associated with prostate cancer.
D7	MYC	Proto-oncogene that encodes a protein that acts in the signaling pathway for normal cell proliferation.
D8	MYC-25	Leukemias and lymphomas are associated with 15 different mutations that create oncogenes of MYC.
E1	RB1	Tumor suppressor gene that encodes a protein that is a negative regulator of the cell cycle and was the first tumor suppressor gene found.
E2	RB1-01	This mutational defect of the RB1 tumor suppressor gene is a cause of childhood cancer retinoblastomas, bladder cancer, and osteogenic sarcoma.
E3	RNASEL	This tumor suppressor gene encodes a protein that helps to control cell division.
E4	RNASEL-12	One of 12 different mutations that have been linked to prostate cancer.
E5	SMAD4	Tumor suppressor gene.
E6	SMAD4-04	One of 3 mutations that inactive SMAD4, associated with advanced colon cancer.
E7	STAT3	Proto-oncogene.
E8	STAT3-03	One of 6 mutations of STAT3 that create an oncogene associated with ovarian, pancreatic, lung, renal, esophageal, cervical, colon, and gastrointestinal tumors.
F1	SS18	X-linked tumor suppressor
F2	SS18-01	Chromosomal translocation-associated oncogene associated with synovial sarcoma.
F3	ERG	Proto-oncogene
F4	TRPSS2	Chromosomal translocation of ERG and ETV1 creates an oncogene associated prostate, pancreatic, liver, lung and small intestinal cancers.
F5	TGFBR2	Tumor suppressor associated with RB1 function.
F6	TGFBR2-01	Inactivation of TGFBR2 associated with esophageal carcinoma.
F7	TP53	Tumor suppressor encodes a protein called p53 that protects against cancer by allowing DNA repair or triggers apoptosis when DNA damage is extensive.
F8	TP53-04	Inactivation of TP53 is associated with over 60% of all types of cancers. Over 19 mutations of TP53 have been characterized. This particular mutation is associated with smoking and confers an aggressive cancer and very poor prognosis.
G1	PALB2	Tumor suppressor gene that promotes stabilized associations of the BRCA2 protein with DNA

		to allow for DNA repair.
G2	FANCN	Mutation of PALB2 that is associated with cancers of the esophagus, breast, prostate, and stomach.
G3	XPA	Tumor suppressor gene that encodes a DNA excision enzyme that repairs DNA damaged by ultraviolet light (UV).
G4	XPA-01	Mutation of XPA is associated with skin cancer. Loss of function of XPA leads to aggressive cancer, with a poor prognosis.
G5	VHL	Tumor suppressor gene encodes a protein involved in the ubiquitination and degradation of a transcription factor that plays a central role in the regulation of gene expression by oxygen.
G6	VHL-01	Loss of function of VHL leads to cancers of the cerebellum, spinal cord, kidney and eye.
G7	WRN	Tumor suppressor gene that encodes a helicase involved in DNA repair.
G8	WRN-01	Loss of function of WRN leads to defective DNA repair and aggressive forms of cancer.
H1	ACD	A guardian gene. This gene encodes telomerase. In differentiated cells, this enzyme is not expressed, limiting the number of cell divisions that they can undergo.
H2	ACD-01	A telomerase that is inappropriately expressed in cancer cells allow them to divide indefinitely.
H3	GAPC	Glyceraldehyde phosphate dehydrogenase, a glycolytic enzyme essential to all living organisms. A "housekeeping" gene.
H4	H1	All cells have histone genes that provide the structural basis of nucleosomes in the chromatin. A "housekeeping" gene.
H5	CP450	All cells must have functional cytochrome p450 genes. A "housekeeping" gene.
H6	No oligo	(negative control reaction)
H7	TUB	All cells have functional tubulin genes that provide cytoskeletal microtubules. A "housekeeping" gene.
H8	No oligo	(negative control reaction)

Summary

DNA microarray fabrication requires the construction of thousands or millions of oligonucleotides within the features of an array. One fabrication process uses the technology of the inkjet printer while another process uses photolithography. The photolithography process results in synthetic oligos built one nucleotide at a time using specially designed masks and UV light.   Each of these processes has the ability to construct large microarrays capable of identifying thousands of different genes simultaneously.

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