

GeneChip® Microarrays



Teacher's Guide

This guide will help you implement the entire module of five activities in *GeneChip Microarrays*. It is put together to help you make decisions on which activities to teach, how to adjust the sections to fit your classroom style and how to best set up your lessons. Many of the lessons can be easily adjusted to fit the schedule of your class. You may decide to use this module and the activities in it to supplement a unit on DNA, genetics, or even one on the Human Genome Project.

The entire module is organized to go from a basic introduction of the GeneChip microarray and then build from there. After the basic introduction, the module moves onto learning about the structure and function of the GeneChip microarrays and how they are manufactured, and then onto a challenging group data analysis activity. Finally, it ends with a thought provoking discussion on the ethical implications of using genetic tools such as GeneChip microarrays. This allows the students to relate the technology to their own lives and the world around them.



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I. Implementing the Module – Introduction

As mentioned above, one of the goals of this module is to be flexible to fit your needs as a teacher. In each section below entitled “Implementing the Module: Suggestions & Hints”, there are suggested ways to change around the primary directions and different ways to use the module in your classroom. Unlike some module teaching guides, however, the general directions found in the student module are not repeated here. Rather than repeat all the student handouts with teacher directions added to it, the teacher directions provided here are completely separate. You should have a copy of the student directions and handouts handy while looking this over.

One important note you should keep in mind: there are a number of different types of DNA microarrays that are used in research in today’s genetics labs. The different names you may hear are DNA chips, biochips, DNA arrays, gene array, or Genome Chip. Many of these work in similar fashions and are based on similar biochemical concepts. It all depends on who manufactures them. The DNA microarray that is discussed here in this module is the GeneChip® microarray. There are three basic types of these arrays – the Gene Expression Array, the Genotyping Array, and the Resequencing Array. These chips are produced by the company Affymetrix who have trademarked the “GeneChip” terminology.

II. Implementing the Module: Hints & Suggestions – Activity # 1

This first activity is meant to be a very general introduction to GeneChip microarrays. It should be done with little introduction by the teacher to GeneChip microarrays. The idea is to let the students begin thinking about this technology and begin to ask questions. The students, thus, are doing their own introduction and building their own framework for the rest of the module. Because it is an introduction, the language is kept simple by using newspaper articles and other articles found on the web.

* **The Primary Goals** of Activity #1 for the students are:

- (1) to connect with the material at whatever level they are coming from at the start of the module
- (2) to become aware of the multiple uses of these arrays and how powerful of a research tool they are
- (3) to spark their interest and get them excited for the module



- (4) to begin getting used to working within a small group (which will be a common occurrence throughout the module)

* **Procedure** for Activity #1 for the teacher:

- (1) do a very brief introduction to what GeneChip microarrays are and introduce the schedule for the module in your class
- (2) organize students into groups of 5
- (3) pass out the Student Handout for Activity #1 as well as a different article to each person in each group
- (4) give the students 35-40 minutes to read their article, take down the main points, share out to the group about their article while others write down some of the main points given, discuss the articles as a group, and do the “Final Questions” on the Student Handout
- (5) make your own final comments, take questions, and go over the “Final Questions” (possibly introduce Activity #2)

* **Hints & Suggestions** for Activity #1:

There are 5 articles provided that cover several exciting areas of research that involve the use of microarrays. However, you could very easily use other articles you find on the internet or in a newspaper. There are new ones coming out every week. In the directions of the activity, the students are to work in groups of about 5. You could make these groups smaller and remove some of the articles or make the groups bigger by adding more articles. It all depends on what group sizes you feel comfortable having in your classroom.

Encourage the students to actively discuss the articles, share their ideas about their specific article, and give their opinions. It is highly suggested you walk around during their discussions to hear where they are coming from, get an idea of their interest, and listen to some of the common questions that come up.

The student handout provided is mostly a place to write down notes and/or get the main points of the article down in one area. You could use this as an informal assessment or simply skip this part, using only the “Final



Questions” as an assessment and proof of participation in the activity.

After the students are done reading their article and sharing the main points with the rest of the group, it is suggested that you do a wrap up by holding a short class discussion on what they learned, go over the “Final Questions” on the student handout, and maybe give some of your own comments before the module takes a big step forward in the next activity.

If you are limited in time, you may want to skip this activity all together or maybe pass out one article to the whole class, have each person in the class read it, and then discuss the single article as a class as the introduction before you move onto how the chips function (Activity 2).

III. Implementing the Module: Hints & Suggestions – Activity # 2

This is not really an activity, but rather a lesson that requires the students to read and answer questions about that material. The students will read information about the structure of GeneChip microarrays, how the different types of arrays work, and how they can be used in the field of research and medicine. While doing the reading, the students answer questions from the Student Handout for Activity #2 to help them check their understanding and allow for assessment by you.

* **The Primary Goals** of Activity #2 for the students are:

- a. to learn about the basic structure of a GeneChip microarray
- b. to learn the importance of the probes to the function of microarrays
- c. to learn how a Gene Expression microarray works and how they can be used in research into drug discovery and effectiveness
- d. to learn how a Genotyping microarray works and how the results can be used in medicine
- e. to learn how a Resequencing array works and how they can be used research and DNA analysis
- f. to review basic concepts of DNA structure, DNA function, and basic Mendelian genetics



* **Hints & Suggestions** for Activity #2:

Before you do any planning for this activity, you should read the information about the structure and function of GeneChip microarrays found in the Activity #2 in the Student Manual. This reading has all the information you will need. If you are looking for further information and more background knowledge, go to the “Additional Resource Links” found on the main page. Links to websites that provide further information are included. For example: “GeneChip Essentials” found on Affymetrix.com may be useful.

As it is shown in the “Suggested Calendar”, the reading for the students could be assigned as homework over a one or two day time period. There are a lot of questions, though most of them are basic feedback type of questions. You may decide to cut out some of the questions to lessen the workload. Or, you could have the students write their own questions for the material and then bring them to class the next day to switch with other students. Then, in class they could answer each others questions as the warm up for the day.

Depending on the level of your students and your timetable for the module, you may not want to assign the entire reading. If you are lecturing, you may not want to assign the reading at all, though it is suggested you do so. The reading is split into three parts. Each part gets progressively more detailed and harder to understand as it goes from Gene Expression arrays to Genotyping arrays to Resequencing arrays.

Also, which parts you assign may affect how you approach Activity 4, where the students work in groups to analyze different research scenarios involving the microarrays. Analyzing the arrays will require some knowledge of how the specific array works. For example, if you decide to skip the reading (and do no teaching of it whatsoever) on the on resequencing arrays, you most likely will want to then skip the Activity 4 scenarios that use resequencing arrays. Though reading the results of resequencing arrays is very simple – it is the understanding of how these microarrays work to get the results which is the hard part.

Because this material is quite high level, it is strongly suggested that you supplement the reading of the material with a lecture or at least a review of the questions on the Student Handout for Activity #2. Masters that can be used to produce overhead transparencies are provided for you to download and use as part of the lecture. These are found towards the end of the Teacher’s Guide.

If you are not a teacher that uses lectures as a teaching tool, there are a few other, more student-driven and active options that can be used. One way to cover this material is to do what is known as a jigsaw. You could break students into groups of three or more and have them each cover a certain section of the reading. Then, they



can each teach their section to their specific group. Once each student is done, the group could then answer the questions together. Be careful with this approach. When you cover reading this way, a student that is assigned a section from the middle or end of the reading may have a hard time understanding their portion since they had not read the material directly before it. To alleviate this, you may want to assign the reading the night before the students come to class to do the jigsaw.

One of the benefits of teaching about GeneChip microarrays is that they help to review and reinforce the basic concepts of Mendelian genetics, DNA structure, the Central Dogma, and Protein Synthesis. In fact, this entire module could be used to supplement your DNA or Genetics unit in a general Biology class. If you feel your students are weak in this area or need to revisit these topics, you may want to assign the “DNA Basics” reading found in Appendix A in the Student Manual. This should be done before the start of the unit and will help them review the important concepts about DNA structure and function that they need to know to understand how GeneChip microarrays work (but does not cover Mendelian genetics).

As you can see, there are many approaches to covering this material. However, covering this material is important, especially when you move to Activity 3 and 4. How you cover it is really up to you and should take into account the level of your students as well as how you prefer to organize your class.



* **Question Set Answers** for Activity#2:

Below are the answers to the student questions for activity #2. Some answers are straight from the reading, but many will demand extra thought and the ability to synthesize concepts from previous topics in this course. These answers are in no way complete, but do make sure to get to the major points of the question.

Part I – Intro, and Gene Expression Microarrays

(1) What is gene expression? What can affect gene expression?

- **When a gene is used to build an mRNA copy (transcription) which is then used to guide the synthesis of a protein (translation), the gene has been “expressed”. Thus the term gene expression refers to anytime a gene is “turned on”, leading to the synthesis of the protein it codes for.**
- **Gene expression can be affected by mutations or environmental factors. Thus the expression of genes is not always consistent within an organism.**



- (2) Are all genes expressed in all cells? Explain why or why not. How do scientists study gene expression?
- **No, not all genes are expressed in every single cell of an organism. Genes that code for vital functions needed by all cells (like getting energy from food) maybe expressed in all cells, while those needed by only specific cells will be found expressed in those cells only (such as pigments that protect skin cells).**
 - **By measuring the amount of RNA copies a gene produces, scientists can study that gene's level of expression. A highly expressed gene will produce a lot more RNA than a gene that is expressed in small amounts. A gene expression microarray can be used to detect both the presence and amount of RNA present in a cell.**
- (3) What would researchers have to do in order to make a hypothesis about gene expression in the past, before the use of the microarrays? What can they now do with the use of microarrays?
- **Scientists would have to refer to previous research on similar diseases or topics and extrapolate from that information. They would then have to form a hypothesis based on any links they find in this old research.**
 - **With the use of microarrays, scientists are able to look at the entire genome at once, measuring the expression of every gene in the organism. They can then make comparisons of which genes are expressed in the cells of different organisms – for example: diseased versus normal cells.**
- (4) How many genes are found in the human genome according to the latest studies?
- **The latest scientific research points to there being approximately 30,000 genes in the human genome. However, this number is not definite.**
- (5) Write the complementary (opposite match) DNA strand to AGGCTAGAC.
- **TCCGATCTG**
- (6) What is the term for the short piece of DNA bound to the glass chip? How many base pairs long is this piece of DNA? What does it represent?
- **This piece of DNA is a probe and it is 25 base pairs long. It represents a small, unique section of the entire gene.**



- (7) Why does the probe not have to represent the entire gene? What does the binding of the RNA to the probe show?
- **This 25 base pair segment is unique to that specific area of the gene. It is so unique that it cannot be found anywhere else in the entire genome.**
 - **So, when the RNA binds to the probe, you know that this gene was expressed since no other gene has this specific sequence. This way, you do not need to worry about building a probe that covers the entire gene. This allows the probes to be much smaller, more specific and easier to build.**
- (8) What is hybridization? How is the process of hybridization used by microarrays?
- **Hybridization is the basic attraction between two pieces of nucleic acids – either DNA to DNA, RNA to DNA, or RNA to RNA. It occurs due to the basic attraction between A and T as well as C and G.**
 - **This process is used to determine which RNA sequences are present in a sample by “fishing” out the specific sequences from a large sample of thousands of different RNAs. Thus, if you have a piece of DNA that is ATCATG, and another piece hybridizes to it, you know the other piece (if it is RNA) must have the sequence of UAGUAC.**
- (9) How specific and accurate is the detection of microarrays?
- **They can detect one single specific RNA molecule in a sample of a mixture of over 100,000 different RNAs.**
- (10) Describe the surface of the microarray chip. Be sure to include the dimensions of the entire array and a single feature. Also discuss the probes in each feature.
- **The array is a 1.25 cm by 1.25 cm checkerboard, with each square (or section) known as a feature. There can be up to 6.5 million features on one chip.**
 - **Each square feature is about 11 micrometers by 11 micrometers and built on it is one specific probe. Each feature has about a million copies of a specific probe sequence. In the latest chips the features are actually as small as 5 micrometers by 5 micrometers and have up to 6.5 million features on them.**
- (11) What are the first few steps done when using a Gene Expression microarray?



- **The first step is to isolate all of the RNA from the cell and make copies of the RNA through the process of PCR. These RNA pieces are then fragmented and labeled with biotin.**

(12) What is the purpose of the biotin and the fluorescent molecules? What happens when the RNA sample is washed over the microarray?

- **The biotin attaches to each RNA fragment and acts like a molecular glue for fluorescent molecules that will be washed over the array. The fluorescent molecules will then glow and show specifically which feature the RNA has hybridized to.**
- **When the sample is washed over the array, there are millions upon millions of tagged RNA pieces floating around and coming in contact with the million of probes on each feature. Most will not find a match, but somewhere a match may be made and that specific RNA will stick to the probes on the feature.**

(13) How can you tell if the sample matches a probe? What if it doesn't?

- **To look for a match, the excess sample is washed off the array, which leaves only the attached RNA (stuck to the probe it matches). To visualize which feature the RNA stuck to and in what amount, a fluorescent molecule is washed over the array and will stick to the biotin on the RNA fragments. The feature that the RNA has combined with will then fluoresce or glow when shined on with a laser from the scanner.**
- **If there is no match between the RNA and the feature probes, all the RNA will wash away and there will be no biotin for the fluorescent dye to stick to. Thus, when it is hit with a laser, it will not fluoresce or glow.**

(14) How can you tell if the gene was highly expressed?

- **A gene that is highly expressed will create more RNA copies. If this occurs, then many RNA molecules will stick to the probes and the feature will shine brightly when scanned. Those genes expressed at a low level will create a small amount of RNA which will stick to the probes on the feature but will shine with less intensity.**

(15) How can a Gene Expression microarray be used to determine which genes are taking part in the disease or trait being studied? What can scientists do once they have identified the specific genes responsible?



- **Scientists can look at which genes are expressed in people or organisms with a specific trait or disease. For example, they could identify which genes are expressed specifically in people that are loud speakers and not expressed in those that are not loud speakers.**
- **They can then do further studies to look at what is the function of the proteins created by these genes and find out how they result in the disease or trait. Scientists could do comparison studies using multiple chips to give them even more data.**

(16) Using heat map results, how could a scientist classify a disease based on genetics? What advantage does that give the researchers?

- **Scientists compare gene expression patterns (heat maps) from people with the disease they are studying to patterns from people with similar diseases or no diseases at all. They do this to look for expression patterns for each situation. This way, they are specifically classifying each disease by the genetics behind it.**
- **This has an advantage because it allows researchers to develop therapies or drugs targeted at the specific genetics that cause the disease and not a general target for a group of similar diseases.**

(17) In the black and white gene expression image, what colors represent a strong intensity? What does that tell you about the gene expression level of the gene the feature represents? In a colors display image, what gene expression level does each color indicate?

- **Strong intensity is indicated by white and grey features.**
- **These tell you that the gene represented by that feature is expressed at a high level, resulting in a lot of RNA from that gene.**
- **In reality, the computer reads the image in black and white with shades of grey in between. However, the images are converted to color for aesthetic purposes. In a color image display, from highest expression level to lowest level to no expression level, it is white, red, yellow, green, light blue, dark blue, and black.**

(18) Before developing a treatment, what must a scientist who has identified a disease pathway do? How can Gene Expression microarrays be used for this?

- **They need to know if disrupting the specific gene pathway will actually disrupt the disease. To do this in the lab, they can block the function of**



the gene in a cell and see how it responds. They can then look at how the cell responds to disrupting the function of other genes in the pathway.

- **Micorarrays can then be used to find out which gene or combination of genes should be blocked to treat the disease.**

(19) How could Gene Expression microarrays be used to find a successful drug? What else can microarrays tell scientists about the affects of the drug?

- **Using gene expression microarrays, scientists can screen a large number of chemical compounds to see how each compound affects the expression of all the genes in the genome of the organism. This is done by looking at the effect the drug has on the gene expression of the organism. This allows them to look specifically at the gene(s) identified earlier.**
- **It also allows them to look at the effect the compound has on other genes in the genome, giving them clues as to possible side affects from the compound. There may even be changes in gene expression that might be helpful in fighting other diseases.**

(20) What is personalized medicine? How can these microarrays be used for this?

- **Personalized medicine is choosing the best treatment for a specific patient by identifying which drugs would work best on them based on their specific genetic pattern.**
- **Microarrays can be used to determine how people with each type of genetic pattern respond to a specific drug. They can identify particular genetic differences and predict which type of patients will respond best to the drug and which will respond poorly or not at all.**

Part II – The GeneChip Genotype Microarray for SNPs

(21) Define genotype. Explain what a person's genotype is and give an example.

- **Every person has two of each chromosome – one from each parent. These may or may not be exact duplicates of each other, and thus, genotype is the actual combination the person has for a section of DNA or genes on the two chromosomes.**



- **If allele A represents one version of a gene or section of DNA and allele B represents another version of the gene or section of the DNA, there are three possible genotypes. They could be the A/A, B/B, or A/B genotypes.**

(22) What is a SNP?

- **A SNP is a single nucleotide polymorphism. In the simplest terms, it is a single base pair difference between two people. At one specific area of the human DNA, one person has a T, while someone else might have a C.**

(23) How can genotyping SNPs be used to find a disease gene?

- **Researchers can determine which SNPs are found with people of the disease. For example, studies may show that 500 people with the disease share the same dozen or so SNPs. This helps the scientists to pinpoint the areas of the genome to focus their studies and look for the disease gene(s).**

(24) Why won't a DNA with the sequence ATCATG bind to DNA with the sequence TATGAC?

- **The second T in the DNA sequence will not match up to the C in the DNA. These strands are not truly “complementary”.**

(25) How does knowing the sequence of one DNA strand help you to determine the SNP genotype the person has? How are probes built to find this out?

- **The probes of a SNP genotyping microarray are designed to detect the SNP by having the middle base on the probe be variable. For example, the first probe may read ATTCATG while the second probe may read ATTTTATG. These two probes are the exact same except for the middle base of the 7. The middle is used because that is the exact spot where the SNP has been identified. All people have the exact same DNA in the area this probe represents except for the middle base.**
- **If the person's DNA sticks to first probe, you know that they must have G in their DNA at that spot (and C on their opposite strand, thus the person has the C/G SNP genotype). If the DNA sticks to the second probe, you know they must have an A at that exact spot (and T on their opposite strand, thus the person has the A/T SNP genotype).**

(26) How many SNPs are on the newest Genotyping arrays?

- **The newest arrays can look for up to 500,000 SNPs or more on a single array.**



(27) Where is the SNP found on the 25 base long probe?

- **The middle position, or base pair number 13 out of the 25 bases of the probe, represents the place of the SNP.**

(28) Once the probes and the microarray are made, what is the first step to genotyping a sample with a genotyping array? How is this different from the use of gene expression arrays?

- **Extract the DNA from the saliva or blood from the subject.**
- **The gene expression arrays need RNA from the sample to be tested rather than DNA. The specific type and amount of this RNA in a cell is different for each type of cell (because not all genes are expressed in every cell in the body nor are all genes expressed in the same amount in every cell) However, the DNA of an organism is the same for each and every cell in their body.**

(29) What are the rest of the steps to get a DNA sample ready for genotype analysis?

- **The rest of the steps are very similar to the steps when using the gene expression array:**
 1. **After extracting the DNA, it must be amplified into large amounts by PCR, then labeled with Biotin.**
 2. **The labeled DNA is then randomly fragmented into pieces.**
 3. **The labeled fragments are washed over the array and those DNA fragments that are complementary to a probe will stick to the array.**
 4. **The array is then washed to remove those fragments not bound and then washed with a fluorescent dye which sticks only to the Biotin-labeled DNA fragments.**
 5. **The array is then scanned to look for which features show matches, indicating which type of SNP.**

(30) Why does a sample that binds to an ATTCATG probe have the C/G genotype?

- **Since the middle base is a C, when DNA binds to this probe, it must have a G at that exact spot. Thus, the person has a C on the opposite strand of DNA at that spot. So, they have the C/G genotype.**

(31) Explain the difference between someone heterozygous for a genotype versus someone who is homozygous for a genotype. Which is implicated in causing more diseases? Why?



- Since everyone has two copies of their DNA (one from each parent) they technically have two copies of each segment of DNA. However, they are not *exact* copies since everyone's DNA is slightly different. So, the DNA that someone receives from their mother will have differences from the DNA inherited from the father.
- For example, if a person inherited the C/G genotype for a SNP from both parents, they have the exact same sequence at this point of both chromosomes and would be considered homozygous for this SNP. If they received the C/G genotype for this SNP from one parent and the A/T genotype from another, they are heterozygous for this SNP.
- Diseases sometimes develop when a person is homozygous for a SNP genotype. For example, let's say a specific SNP (A/T) has been linked to the disease gene. If the person has the A/T SNP on both his/her chromosomes, then they most likely also have two copies of the disease gene. They would be homozygous for the SNP and the disease gene. Because most diseases are recessive, it takes two copies of the disease gene to actually get the disease. If a person is heterozygous for this SNP (having the A/T SNP on one chromosome and C/G SNP on the other), they would most likely also have one disease version of the gene and one normal version. The normal version is dominant, so the person does not end up with the disease.

Part III – GeneChip Resequencing Microarrays

(32) Why is this array known as a “resequencing” array? Give an example.

- The array is not used to sequence an unknown piece of DNA for the first time. The probes are built based on the already determined sequence of the organism(s) being detected.
- For example, if a chip was designed to test a blood sample to see which strain of malaria a person is infected with, the sequences of the different strains of malaria would be need to known ahead of time to build the probes representing each strain type. Thus, these chips are used for identification purposes and not to perform new sequencing studies.

(33) What are some of the uses of the GeneChip Resequencing microarray?

- Resequencing arrays can tell you the exact sequence of DNA in a given region.



- They can be used to determine the origin of DNA in a sample. For example, the array could examine a virus to determine which strain is present. This could be used in the case of an outbreak.
- They could also be used to monitor pathogens in the water or food supply or to see if a sample of food is contaminated (for example, is the tuna you are buying really tuna or is there some salmon mixed in?).

(34) How many probes are used to determine each base in a DNA sequence?

- A set of four probes is used to determine each specific base of the DNA.

(35) How many bases make up each probe? Which one is the variable base that is used to determine the base at the specific spot in the sequence? Draw a simple diagram that illustrates this and explains how a probe set can detect the base at a specific spot.

- 25 bases make up each probe. The middle base (#13) is the variable base used to determine the base at a specific spot. The probes below are exactly the same except for the middle base.

Probe #1	-----A-----
Probe #2	-----T-----
Probe #3	-----C-----
Probe #4	-----G-----

- If the DNA fragment hybridizes up to probe #1, it must have T at that spot, while if it hybridizes with probe #2, it must have an A at that spot, etc.

(36) If a hybridization occurs at a probe with C as its variable base, why is the actual DNA base read as a G?

- GeneChip microarrays work because of the complementary base pairing of DNA or RNA in a sample to the DNA of the array probes. Because only G hybridizes with C, if the probe has a C at the middle spot, then the actual sequence of the DNA sample at that spot must have a G.

(37) Draw a simplified GeneChip Resequencing microarray readout for the following sequence: ATGCCTAAGTCT



A			■						■			
C				■	■						■	
G	■						■	■				
T												

IV. Implementing the Module: Hints & Suggestions – Activity #3

This section contains one of the more interesting and fun activities in the module that focuses on the application of microarrays. Therefore, if you are pressed for time, this may be an activity to skip and move onto Activity #4. This activity is focused on teaching how these microarrays are actually manufactured, which is a truly amazing process. It shows how one industry (manufacturing of computer processors) can influence a completely different industry (genetic testing and research). It also provides an activity that gives the students a creative way to illustrate their understanding of the material by building a model of the manufacturing process out of household and office supply items and presenting these models to the class.

* **The Primary Goals** of Activity #3 for the students are:

- (1) to learn about the process of photolithography and how it uses masks to manufacture tiny microchips
- (2) to understand the steps used to build the small DNA probes on a microarray
- (3) to learn the basic idea of how the manufacturing plant builds these arrays
- (4) to use models to show their understanding of a complex scientific process

* **Procedure** of Activity #3 for the teacher:

- (1) Have the students read through the reading in Activity #3 and answer the questions in the Student Handout for Activity #3 for homework
- (2) The next day, do a brief lecture on the manufacturing process, paying close attention to the photolithography steps and the use of the mask
- (3) Go over the questions in the Student Handout with the class
- (4) Organize the students into groups of 2 to 4



- (5) Provide each group with the materials in “suggested materials for model building” in the student manual
- (6) Give the students 25 – 30 minutes to work with their group to brainstorm how they can use the materials to build a model or models of the manufacturing process they have just learned about and to build their model(s)
- (7) Each group then presents their model(s) to the class, explaining what each item represents and how their model(s) illustrate the process

* **Hints & Suggestions** for Activity #3:

Before planning the unit, you should read over the information in Activity #3 of the Student Manual. It has three sections that cover all the aspects of the manufacturing of GeneChip microarrays that you will need to know to put together a lecture for the students.

This activity is actually a two part lesson. The first part has the students learning about the manufacturing process and the second part allows them to show their understanding by building model or models. Like Activity #2, a main portion of this activity is actually set up as reading followed by a lecture. Refer to Activity #2 for a few suggestions on the lecture and alternatives to doing the lecture. There are a good number of diagrams and pictures provided in the “Downloadable Masters” section below that can be used during a lecture. Most of these diagrams are the same ones that are shown in the student reading, but some of the other ones cover what the actual manufacturing machinery looks like as well as the actual fabrication room. It is suggested that you use these pictures at the end of the lecture to give the students a feel for what really goes on during the manufacturing process and how the workers fit into the process.

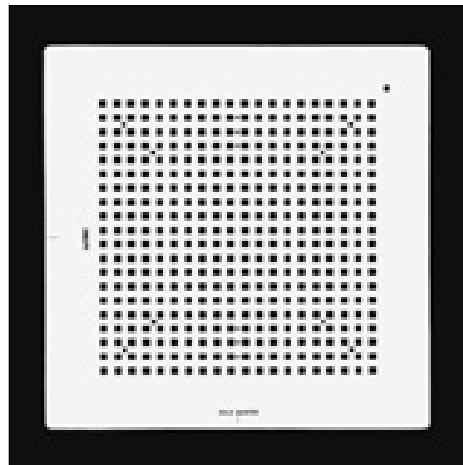
Once again, like in Activity #2, you could just have the students read and skip the in class lecture, moving directly to the activity. Or you could skip the reading of the material by the students and only lecture on it. You may want to at least go over the questions in the Student Handout for Activity #3 before doing the model building portion. The process is not overly complex, but building the models may be pretty difficult to do without a good understanding of the process.

You could run into a lot of problems and questions if you do not check their understanding of the process before moving on to the model building. Exactly how you approach this is up to you. You may not want to repeat the exact same set up as you did in Activity #2 (reading – questions – lecture) and may want to mix it up simply for variety for the students.



The building of the models is one of the more creative and active portions of the entire module. It provides the students with a different means to show their understanding besides the answering of questions. In fact, to assess their understanding of the process, you may want to skip the questions all together. However, this is a group activity and may make it difficult to assess each student's understanding on an individual basis

The idea behind the model building is to let the students loose to come up with any creative way of illustrating that process that they can think of. However, you may need to be careful with what your goals to the activity are. If you just turn them loose to build a model of the process, you may get groups that simply build a model of a final microarray, which really doesn't show the entire process. You may want to stress that they need to illustrate the ENTIRE process and not just the final product. This may require them to build a series of arrays at different steps of the process.



The suggested material list can be found in the student directions for Activity #3. You are not limited to these materials, nor do you need to use all of them. Here are a few ideas on how each item could be used. Some of the items are red herrings, although you never know what the students will come up with). Once again, these are just suggestions!

- Aluminum Foil – cut into squares and punch holes in it to produce a “mask”
- 3X5 cards – cut into squares to become the entire wafer or a single feature
- flashlight – shine above the mask act as the light source
- pens and pencils – more to throw off the students, but could be used to mark the wafer into individual features or something
- paper clips – could be used as single DNA bases or blocking agents
- candy – used as DNA bases (different colors could be used for each base)
- glue –used to stick DNA bases together as probes and stick probes to wafer
- tape – used just like the glue
- paper – could possibly used to build a mask or act as the wafer
- scissors – to cut the paper and cards, not really used as part of the real model
- plastic wrap – mainly there to confuse students, but could be used as a mask
- small beads – like the candy, could be used as DNA bases
- string – mainly just there to make the students think about it
- wine bottle corks – another item that could be used as DNA bases or as the blocking agent
- pushpins – used as blocking agents or DNA bases (or could be the silicane or



linker molecule?)

- rubber stoppers – used just like the corks
- popsicle sticks – could be used to build the probes by gluing the candy or other DNA bases to the sticks instead of stacking them
- pipe cleaners – could be used to string the beads the beads that are being used to illustrate the DNA bases

When the students are done, have each group give a brief explanation to the class on how their model shows the manufacturing process in action. Make sure that they discuss what each item represents and that they go through the how their model shows the process. This can be a time for you assess whether they really understand the process and in what detail. Also, you could give out some sort of incentive (extra credit, etc.) to the most creative models or the ones that best illustrate the process. How you grade each group is up to you, though it is suggested that it is kept somewhat informal as Activity #4 is much more formal and probably will be more stressful for the students. Keep it relaxing and fun and let the students really use their creative side!

* **Questions Set Answers** for Activity #3

Below are the answers to the student questions for activity #2. Some answers are straight from the reading, but many require extra thinking and work. They are in no way complete, but do make sure to get to the major points of the question.

Part 1- Introduction / Photolithography

- (1) GeneChip microarrays are built based on manufacturing techniques of from which industry?

They are made based on techniques from the semiconductor (computer chip) industry.

- (2) What tool, used in photolithography, ultimately controls the building of the DNA probes? How does this tool work to control it?

The tool is a “mask”. The mask is used to control the synthesis of the probes by directing UV light onto specific sections (features) of the chip. The mask has tiny openings designed to let light through only at specific spots. Light is then used to allow the growth of the nucleotide chain through a process of deprotection and addition of new bases onto specific chains / probes.

- (3) What is the name for the tiny sections (squares) that make up the DNA chip?



Each tiny section or square is called a feature.

- (4) What is the difference between the deprotected and protected part of the chip?

The features that have deprotected nucleotide chains (which become the future probes) will grow in the next step when free nucleotides are added. Because the top nucleotide in the chain is deprotected, the free nucleotide will bind to the top of the chain. The chain is now one base longer than before and has essentially “grown”. The features that have protected (light did not touch them) nucleotide chains will not grow. This is because the top nucleotide in their chain has a molecule on it that does not allow the free nucleotide to bind to it. So, when the free nucleotides are added, they do not bind to the chain.

Part 2 – The Manufacturing Process

- (5) What is the wafer made of?

The wafer is made of a matrix of glass.

- (6) What is the purpose of the silane coating of the wafer?

The silane molecules combine with the glass and provide a starting point for the growth of the nucleotide chains (which form the probes of the chip). Each spot along the glass wafer that has a silane molecule on it will allow the binding of the first molecule of the chain known as the “linker” molecule in the next step of the process.

- (7) What is the purpose of the linker – photosensitive molecule conjugate?

The linker molecule joins with the silane molecules and serves as the initial attachment point for the nucleotide chain. They are the starting points that will combine with the first free nucleotide base only if the photosensitive molecule is removed through deprotection. The silane / linker molecule combination starts each growing nucleotide chain. Everywhere there is one on the wafer, a nucleotide chain probe will be built.

- (8) What occurs when the UV light gets through the mask and hits the wafer?

The masks are designed to allow the UV light to hit only specific features at a time. Once they do, the nucleotide chain (or the linker molecule if this is the first step) loses its’ protection. The photosensitive molecules are essentially removed and this allows the addition of a new nucleotide to all the chains in the deprotected feature.

- (9) How do the different probes in the same feature compare (once finished)?



Each probe in the same feature should be made of the exact same sequence of nucleotides.

- (10) When a nucleotide is washed over the wafer after the UV light exposure, what happens? How does this “build” the DNA probe?

Those features that were exposed to the UV light have chains are no longer protected. Thus, when the nucleotides are washed over the wafer, they will combine with these unprotected chains. They will not combine with the protected chains that the UV light never reached. With the addition of a new nucleotide, the chains (which are really the probes) have grown. The subsequent addition of new nucleotides in later steps will build the probes vertically away from the wafer.

- (11) From the point after the addition of the linker molecule, summarize the three steps in the process of building the nucleotide chains which will become the probes on the features.

The process is basically a repeating of three basic steps. A mask is placed over the chip, and UV light is shined on the chip from above, deprotecting specific features that the light hits. The next step will only occur at these features and not the features that were not touched by the light. The next step is the washing of the free nucleotide over the entire chip. These nucleotides will combine with any chain that is deprotected and will not combine with the protected chains. The steps are then repeated – place a new mask over the chip, shine the UV light through the mask and onto the chip and then wash with the next nucleotide. This continues until each chain is of a specific 25 base sequence.

- (12) What is the purpose of the protection side group (or “P”)?

These side protection groups prevent the small possibility of free nucleotides combining to the wrong part of the nucleotides chain. To build probes, the addition of each new nucleotide must occur at the top of the chain. However, the chemistry of nucleotides does allow the possibility of the free nucleotides adding to the side of the nucleotides on the chain. This is the normal hydrogen bonding / attraction of base pairs in DNA (C with G and A with T). This is prevented by the addition of a side protection group to each free nucleotides before they hook up to the chain. Once added, they will prevent the hydrogen bonding between the nucleotides in further steps.

- (13) About how many steps does it take to build the millions of DNA probes? How is this possible?

It normally takes about 100 steps to build all of the 25 base pair probes



in each array. This is possible because you are not adding one base at a time to one feature at a time. Everything is precisely calculated and each mask pre-made precisely to add one nucleotide at a time to multiple features at a time. There are over 6.5 million features on a chip. So, an A may be added to only 500,000 specific features in one step, and then a T may be added to 750,000 different features in the next step. The probes in different features are built unevenly, but eventually they will all be 25 base pairs in length.

- (14) What happens when a nucleotide does not add when it should? How is this problem overcome?

When a nucleotide does not combine when it should, the entire probe will be of the incorrect sequence and will not function properly. If this occurred at each probe of an entire feature, then it is a big deal. The whole feature would be useless and could give false results. If it is just one probe out of a million or so in a feature, this is not that big of a problem. Because of this, the incorrect probe can essentially be “sacrificed”. A capping agent is added after each nucleotide wash. This cap will only combine to any chain where a nucleotide did not combine for some untold reason. Remember that after the addition of free nucleotide to the chain, the chain is protected since each free nucleotide has a photosensitive molecule on it. Any chain that did not have a nucleotide added to it will not be protected and the cap will combine with it. Doing this makes it so you do not have to go back and check each individual probe and redo each mistake. It keeps the process moving as quick as possible.

- (15) What happens once the final nucleotide wash is added and the synthesis completed?

The protection side groups and capping agents are removed.

Part 3 – Building In Bulk

- (16) What are the two main parts to manufacturing GeneChip microarrays? In general, what happens in each part?

The two parts of the manufacturing are the chemistry and photolithography. In the chemistry portion, liquids are washed over the wafer in perfect conditions at precise points in time. Anything that does not combine is washed away and the next step is preformed. In the photolithography step, the wafer is moved to a different chamber and a specific mask is placed precisely over the wafer and locked into place. the UV light is shined from above and chains are deprotected. The wafer is then removed from the chamber and moved to the chemistry chamber



for the addition of the next nucleotide through the chemical wash step. The steps are repeated, moving the wafer from chamber to chamber.

(17) Why must the mask be precisely placed on top of the wafer during photolithography?

Because you want only specific features to be hit with the UV light. And because each feature is so tiny, each opening in the mask is also tiny. Any small mistakes where the mask is slightly shifted in any direction can ruin the entire process.

(18) What happens once the wafer is complete?

Once the wafer is completed, the wafer is removed and cut into smaller chips using glass blades. Each chip is carefully placed into a cartridge and labeled correctly. For quality control purposes, a few chips in each batch are tested to make sure they work correctly.

V. Implementing the Module: Hints & Suggestions – Activity #4

This activity is definitely one of the more involved portions of *GeneChip Microarrays*. However, it is also one of the more interesting and challenging section that really gets the students to sharpen their ability to analyze data, work in groups and present the scientific information to a larger group. The six different scenarios provided in this activity include a wide range of applications of GeneChip microarrays and also are of different difficulty levels. Scenarios range from using GeneChip microarrays to research contamination of sushi to studying the genetics of cancer to looking at the gene expression in wine grapes.

Once the groups have finished analyzing and interpreting the data, they put together a short presentation to inform the class about their scenario, the results, and their interpretation of what the results mean.

* **The Primary Goals** of Activity #4 for students are:

- (1) to apply their knowledge of the three types of GeneChip microarrays to the analysis of results of a simulated experiment using these microarrays to collect genetic data
- (2) to work as a group to analyze and interpret data
- (3) to work as a group to organize and present the results of an experiment to a class in a formal presentation
- (4) to work on communication of scientific information within a group and a class

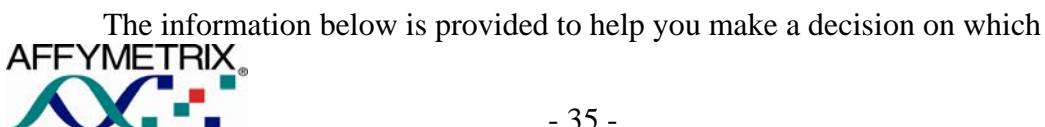


* **Procedure** of Activity #4 for the teacher:

- (1) Preview all the activities you will use as part of this lesson to get a good idea on the results of each situation and what the results mean (use the explanations below to help you out if needed).
- (2) Organize students into 6 groups (or less, depending on how many scenarios you will cover) and assign each group a scenario.
- (3) Give the students about 20 minutes to read their scenario and interpret the results with minimal help from you.
- (4) After about 20 minutes, walk around the class and help out each group and have them tell you what they think the results indicate.
- (5) Give the students about 20 more minutes to organize a short, 5-7 minute, presentation about their scenario and the results.
- (6) Since these presentations should include visuals of the data and other important information, provide the students with overhead transparencies or posters along with appropriate pens.
- (7) On the next day, pass out the Student Handout for Activity #4. This is basically a summary sheet for each student to write down key points and what they learned from the other presentations.
- (8) Have each group present their results, using the rubric provided to grade their performance – be sure to allow time for questions from the rest of the class and any further clarification from you.
- (9) It is strongly suggested that you take a few minutes after each presentation to take questions and make sure everyone understands the data involved in the scenario and how it was interpreted.

* **Hints & Suggestions** for Activity #4:

A big part of this activity is deciding on which scenarios you will use in the classroom. The scenarios are not all of the same difficulty and do not all use the same type of GeneChip microarray. So, which scenarios you choose to assign will depend on how much you covered in Activity #2 (all three arrays? only gene expression?), the level of your students, and the amount of time you have for this section.



scenarios to cover in the activity. For each scenario, there is an estimated difficulty level, an explanation of the results and what is happening, and some hints you can give the students. The levels go from basic (A) to average (C and F) to difficult (B and E) to advanced (E).

It should be noted that the one key thing about this activity is that you do not have to assist on perfect interpretation of the results. Some of the data actually does not have one exact answer, having multiple possibilities. It is designed to allow student come up with their own interpretation. The most important thing is that the groups make sure to explain the results and back up their interpretations with facts from the data. In the end, it is the “journey” that is more important than the final answer. Working together and stretching their brains to come up with an interpretation is just as important as coming up with the exact answer.



* Scenario A – Fish CSI?

Difficulty level: Basic

GeneChip Microarray used: Genotyping (?)

This is by far the most simple of all the scenarios. The data is the most basic and the set up of the experiment is the least complex. The experiment does not really use a “true” genotyping microarray, though. There is only one type of array used and it is simplified so that if a specific feature with probes from a specific fish fluoresces, than DNA from that fish is present in the sample. It does not really try to identify the actual genotype of certain SNPs of the fish. However, it does blend part of a gene expression array in that this array can measure the level of DNA found in the sample (i.e., if there was a lot of DNA, meaning a lot of that particular fish in the sample).

Is the sample contaminated or not? That is what the students must determine from the results. Students are provided with an explanation of what fish each feature of the array represents. There are only four samples that are looked at. If you would like to make this scenario a bit more challenging, you can come up with some more samples and results for them to analyze.

Here is the basic idea of what happens with each sample:

#1 – Some of the test showed pure salmon, which is what the restaurant was claiming the sushi was. However a few tests indicated the salmon was actually tuna! And most of the results indicated the presence of tuna in the salmon sushi. This implies that the restaurant is mixing their fish to save money.

#2 – Nothing is wrong here. The restaurant says it is serving Japanese eel and all of the 20 tests run indicated only Japanese eel in the sample

#3 – Just like #2, there is nothing wrong with this sample. The restaurant says it is serving Atlantic mackerel and all 20 tests run show only the presence of Atlantic mackerel. (This is a pretty cheap fish.)

#4 – Here, there was not Blue-finned Tuna in the samples from what the restaurant was claiming was truly Blue-finned Tuna. The tests either indicated the tuna was really Skipjack tuna (much cheaper), Spotted tunny (also cheaper), or mainly a mixture of the two varieties.

Hints: Most of this is pretty straight forward. Make sure they read the “directions” on the handout of the scenario as it has some questions to help them. Make sure they are looking back at the diagram of what fish each feature represents. Also, they should ask themselves why they might switch the particular fish they did. Maybe you could have them research the different types of fish and how expensive they are.



Hints: There is a lot of data to keep track of. Encourage the students to make a chart of all the results to help look for patterns. Ask them to see what common alleles are found with each type of metabolizer and to look at the differences between the different phenotype. You may need to help them with the UMs situation. Make sure they read the “Directions” at the end of their handout which has some key questions for them to help on.

*** Scenario C – HIV Genotyping**

Difficulty Level: Average

GeneChip Microarray used: Resequencing

This scenario has the students look at changes in the genome of HIV that occur while an infected patient takes two different anti-HIV drug cocktails. It is a real life scenario that is occurring all the time as scientists try to determine the best use of drugs to fight off the AIDs epidemic. The background of this situation is similar to how bacteria become resistant to antibiotics. HIV is very prone to mutations. Most of those mutations do nothing, but some make the virus weaker or unable to replicate, and some even make the virus stronger and even more deadly. As the patient takes an HIV drug or a combination of HIV drugs (known as a cocktail) the weaker strains with less resistance will be eliminated by the body, but those with the increased resistance will survive and replicate until only the stronger strains are left. This is a pretty horrible situation to be in, since the drugs the patient is taking no longer work.

This scenario has the students look for mutations in the gene for reverse transcriptase enzyme and connect those mutations with changes in resistance level of the HIV virus (with the mutation) to anti-reverse transcriptase drugs. The study is kept simple by looking at only two different drug combinations and trying to eliminate all possible variable factors. The study has two parts. First, they must look over data showing the change in resistance of a patient’s HIV while they are administrated the drug cocktail over a year period. Secondly, they must analyze mutations shown in two different exons (74 & 184) of the reverse transcriptase enzyme. To keep it simple, they only look at two of the areas that commonly show mutations (called “hot spots”).

The data is kept very simple as there really is only one type of mutation with exon 74 and one type with exon 184. Resequencing results are only shown if a mutation occurred, which does kind of give it a way. That is, if the sequence is shown, you know there was a mutation. The analysis is simplified because it is stated that none of the patients show any mutations in these exons originally. The data is organized into the starting or pre-experiment test results (known as a baseline) and the Final Results (taken after the patients take the drug cocktail for one year). Reading resequencing outputs is very simple, even if the students do not know how it works.

You can add a small level of complexity by asking them to identify exactly where the



mutation occurred (there are only two mutation types and they are both single base pair changes – known as point mutations).

Here is a simple chart that explains the results of the two groups:

<u>Patient #</u>	<u>Resistance Level Change</u>	<u>Mutation? (Which exon?)</u>
1	1 → 2 (x2)	yes, exon 74
2	1 → 1	no
3	1 → 2 (x2)	yes, exon 74
4	1 → 1	no
5	1 → 1	no
6	2 → 2	no
7	2 → 4 (x2)	yes, exon 74
8	1 → 1	no
9	3 → 15 (x5)	yes, exon 184
10	3 → 3	no

*Patients #1-10 took all the same drug cocktail: AZT + sustiva + videx. Notice that only 4 out of 10 (40%) patients had any change and three of those had a 2x resistance change. The patients that had a resistance increases of double all had exon 74 mutated while the one patient with a five times increase had exon 184 mutated. Also, anytime there was not mutation, there was no resistance change.

<u>Patient #</u>	<u>Resistance Level Change</u>	<u>Mutation? (Which exon?)</u>
11	2 → 4	yes, exon 74
12	1 → 1	no
13	1 → 10 (x10)	yes, exon 74 & 184
14	1 → 10 (x10)	yes, exon 74 & 184
15	1 → 2 (x2)	yes, exon 74
16	1 → 1	no
17	2 → 20 (x10)	yes, exon 74 & 184
18	2 → 10 (x5)	yes, exon 184
19	1 → 1	no
20	2 → 2	no

*Patients #11-20 all took the same drug cocktail: epivir + sustiva + videx. This is only different from the other group by one drug (epivir instead of AZT), but the difference is obvious. 6 out of 10 patients (60%) had mutations and a resulting change in the resistance of the HIV they were infected with. The results are consistent with the first group. Any time someone had a mutation in exon 74, the resistance increased two times (patient #11 and 15). Anytime a patient had HIV that had mutation in exon 18, the resistance increased five times. The new situation is that anytime the HIV mutated in BOTH exons 74 and 184, the



resistance increased 10 times (which is basically the multiplication of the other two individual resistance levels, so it makes sense). So, taking this drug combination tends to lead to more mutations (60% versus 40%) and it leads to more mutations in the exon that seems to lead to a more resistance increase – exon 184. Thus substituting the AZT with epivir leads to many more resistance level changes in the HIV of infected patients.

Hints: The data is pretty straight forward (and never would be this clean in a real life experiment!), but the initial analysis may confuse the students. Suggest they make a simple chart like the one shown above and stress that they look at the changes and pattern. You may want to have them identify the actually base pair change in each exon as well (exon 74 showed an A to C change in base pair #7 while exon 184 showed a G to A change in base pair #13 shown). Once again, tell them to make sure to read all the background information and look at all the data. One thing to point out is that it is only 20 patients, so it is not a rock solid study, though this is how initial human drug studies usually start and that this is made up data. In no way has epivir (and actual drug) been shown to cause these mutations in real life.

*** Scenario D – Grape Gene Expression Study**

Difficulty Level: Advanced

GeneChip Microarray used: Gene Expression

This study has the students look at gene expression of grapes under specific conditions. It is the type of study that scientists working for wine makers are performing every day, looking for ways to maximize their crop production by determining the best conditions the grapes need for growth. In wine making it is also vital that the wine maker has grapes with the perfect sugar and acidity content. Not only do they want perfect growth rate, but also a consistent result with these conditions. All of this is meant to maximize profit while decreasing the effort needed in growing of the grapes.

The difficulty level of this scenario is considered advanced because, other than a few instances, the data does not fit one perfect pattern. Patterns do exist and if analyzed correctly, they will make themselves clear to the students. Also, there is a lot of data to makes sense of. The array output is easy to read, but connecting that to all the different conditions that were tested, it can get tricky.

Some key information and hints to think about:

- (1) The best rating for the grape is a 5, so take a look at the gene expression for the 5 grapes (and 4 rated grapes would be good to look at as well)



- (2) To find out which genes are important for the best grapes, you should compare all of the genes and how they change for each type of grape rating
- (3) The temperature is increased from low to medium to high, so do a comparison on what is needed at each level to get a 4 (or 5)
- (4) The actual levels for the temperature, water, and pH are not that vital, so just keep them in the “low, medium, and high” category to keep things a bit more simplified.

Here is a chart to help you compare the results of the gene expression and to identify which genes lead to which type of grape ratings (5 being the best).

+++ = high expression (black feature) ++ = medium expression (dark grey feature)
 + = low expression (light grey) 0 = no expression (white feature)

Ratings → Gene	1	2	3	4	5
1	+++	++	+	0	0
2	0	0	+	++	+++
3	0	0	0	0	0
4	0	0	0	+	++
5	0	+	+	++	+++
6	++	+	0	0	0

These patterns show which type of gene lead to which type of grape rating. This is one of the easiest analyses to do with this data. The results say the following about each gene:

- Gene 1 – As the grape quality goes down from 3 to 1, gene 1 is expressed more (this must be a gene that leads to poorer quality grapes)
- Gene 2 – As the grape quality increases from 3 to 5, this gene increases in its’ expression level, so it must be a important gene for high quality grapes
- Gene 3 – This gene is not expressed in any of the grapes, so it must not be important for the growth or quality of the grape.
- Gene 4 – This gene is only expressed in the best quality grapes (4 and 5), so it is really key in production of high quality grapes.
- Gene 5 – As the quality of the grape goes down (from 5 to 2), so does the expression of this gene, so this must be an important gene for good



quality grapes.

Gene 6 – This gene is only expressed in the poor quality grapes, so its code must lead to unwanted qualities in the grape.

**Gene 2, 4, and 5 are important for the best quality grapes. As their expression goes down, so does the quality of the grape. Gene 1 and 6 seem to be expressed only in the poorer quality grapes, so they must code for proteins that do not lead to the best quality of grapes with the perfect sugar and acid amounts.

As mentioned above, there is a lot of data to make sense of, and much of it does not fit just one pattern. The results above show the gene expression patterns producing a specific quality grape. However, the experiment was also about finding out what conditions produce the best quality grapes (by changing the gene expression, of course). There are many patterns to look at, so we will only list a few of them. First of all, let's look at only those grapes given a 5 rating – conditions D, N and Z.

condition D → medium pH, low H₂O, and low temperature

condition N → medium pH, medium H₂O, and medium temperature

condition Z → high pH, medium H₂O, and high temperature

*It is clear that a 5 rating can be arrived at any temperature. It just depends on changing the water and pH levels to get it. Water level must be increased as the temperature increases, though never to high levels. The pH must always be at least medium (close to neutral) but at high temperatures, it must be at high.

This is just one example that the students can look at. The tricky part comes if they try to arrive at patterns of the 4 rating grapes. There is no one set pattern here. Remember, the goal is to try to get all the grapes at a 5, but understanding the 4 quality grapes would also be important. There is so much to look at, but patterns do exist. For example, the students may look at all the 4 quality grapes from the low temperature settings and find that as the pH is increased, the water must also be increased, but never to a high level. They may decide to look at the 2 quality grapes to compare to the 5 quality ones. They may find that you can not get a 2 quality grape grown at medium temperature or that high temperature and low pH usually lead to 2 quality grapes.

Hints: A lot of hints are given above. Those could be given to the students. Tell them not to worry about looking at all the data and just concentrate at a few specific points. If you want 5 quality grapes, how do you get them? Tell them to look at the expression patterns for each quality grape and make a chart for comparing the different types. They could write just next to the outputs under each temperature to help with the comparisons or maybe make some other charts. The main thing is that they do not try to understand all the data.



*** Scenario E – Monitoring Gene Expression of Cancer**

Difficulty: Difficult

GeneChip Microarray used: Gene Expression

This scenario has the students analyze gene expression results from a cancer study. This is the easier of the two gene expression scenarios. They look at the gene expression pattern of 10 genes as the cells go from normal to cancerous. There is only one array output and not nearly as much background as some of the other scenarios, but the reason it is given a difficult rating is that many students may not really have a strong background on gene expression. Also, there is no one perfect answer here, though the patterns of gene expression are pretty clear as the cells go from normal through the 4 stages of cancer.

The stages of cancer given are actually summaries of the real stages of Small Cell Lung Cancer. Each stage also has sub-stages which are not really explained, but later in the study it is mentioned that the students will look at 8 samples take from 7 stages of cancer - early and middle stage 1, early and middle stage 2, early and middle stage 3, and stage 4 or full blown cancer – as well as a cell in its' normal state (the control) This is why there are 8 columns in the simplified array given (one for each sample take). There are also 10 genes that that are already identified as possible important genes to study. To simplify things, these genes are already given to the students and each gene is given a number. The actual gene symbols are real gene symbols of genes known to be involved in regulation of cell division.

So, overall, the array used has 80 features or squares. Each one represents the activity of the gene at the specific stage. The activity is “color” coded in that a white feature means the gene is inactive at that stage. A light grey color means the gene is on, but not too active while a dark grey feature means the gene is fairly active and probably important for the cell at that stage. Lastly, a black feature means the gene is highly active and most likely vital for the cell at that stage.

Looking at the data, the genes can be broken down into two categories: those that are important for the normal cell and those important for the cancerous cell. Here is a summary of each gene.

Genes that are active when a cell is Normal: 3, 4, 5, 7 and 9:

Genes 3 & 5 – active when a cell is normal and only early stages (1 and 2)

Genes 4 & 7 – only active when a cell is normal

Gene 9 – mainly on when cell is normal and very early stage 1 only

Genes that are active when a cell is Cancerous: 1, 2, 6, 8, and 10:

Gene 1 – active during all cancer stages and seems to become more active



with each progressive stage
Gene 10 – very similar to gene 1 but suddenly turns off just as the cell reaches stage 4
Gene 8 – similar to gene 10, but takes a little longer to start being active
Genes 2 & 6 – active in later stages of cancer and fully active by stage 4

*There are a lot of ways to interpret the results and come up with ways to attack the cancer. Students may choose to try to turn on those genes that turn off as the cell becomes cancerous or turn off genes that become active as the cell become cancerous. There are multiple possibilities. For example, they may say to try to keep gene 4 and 9 active to keep the cell from becoming cancerous. Or, they may say to turn off genes 1 and 2 as they seem important for the full development of cancer.

Hints: There really is no one answer here and it is mainly important that they back up what they conclude with evidence from the results. Note that the function of the genes is not discussed. All the students need to do is figure out which genes are important where and identify which one they think should be looked at further as possible targets for drug treatments of Small Cell Lung Cancer. Suggest that they look at which genes are on during the normal and cancerous stages and that they should compare the two. Also, remind them too look at the level of activity of each gene at each stage. The most important thing is that they back up there conclusions and suggestions with data from the results. Remind them that there is no one perfect answer here.

* **Scenario F – An E. coli Outbreak**

Difficulty: Average

GeneChip Microarray Used: Resequencing

This study has the students use their basic knowledge of microarrays to solve a mystery. The mystery is centered on a small outbreak of E. coli that has plagued a small town. The students must organize all the data, interpret what is happening, determine what strain of E. coli is making the people sick, and figure out where it is coming from. The situation is quite basic, though looking through all the background information and sifting through all the data will take the students some time. The interpretation, however, is pretty straight forward. And, actually, the students really do not need a lot of understanding of resequencing arrays. All they really need to do is be able to understand how to read the outputs (which is pretty simple). So, if they have little knowledge about these types of arrays, you can still assign this scenario and maybe just give the group a short explanation on how to read the outputs.

The students are given some facts about the town and surrounding area as well as a map of the area. They are then given a list of 17 samples taken from the area to



analyze as possible sources of contamination. Finally, the situation is complicated as they are given a description of six different E. coli strains. The test results will show that more than one type of E. coli can be found in the area. However, some of the E. coli is natural and does not harm to humans. The students must determine which strain is causing the sickness and where it is coming from. The introduction to the students hints that it may be the deadly O157 strain, but as you will find out, the actual problem strain is something else. You should read the student handout before moving on.

As far as the data given, here is a simple breakdown with some explanations to help you out.

- Sample #1 (Apple Juice from store) – no contamination
- Sample #2 (milk from store) – contaminated with strain JM101; similar contamination found at dairy plant in cow feces; coming from the cows, but this strain is harmless to people (NOT THE SOURCE OF THE PROBLEM)
- Sample #3 (meat from store) – no contamination
- Sample #4 (water from well A) – no contamination
- Sample #5 (water from lake) – contamination from strains Y1088 and RR1; RR1 is waterborne but harmless to humans; strain Y1088 is also found in the soil of the apple farm just north of the lake; this strain does not kill but causes some severe sickness; Y1088 mostly is found in moist soil, but can become waterborne; most likely came from the apple farm and was washed down into the lake during the most recent storm (THIS IS THE MAIN PROBLEM STRAIN BUT NOT THE SOURCE)
- Sample #6, 7, and 8 (water from river at three spots)– none of these show any contamination, though the river becomes contaminated once it gets below the connection from the lake
- Sample #9 (feces from cow farm) – contaminated with strain O111; this strain comes from intestines of cows and does cause some sickness in humans, but nothing too severe; there is no way to trace this to the human sickness as the meat from the cows on the farm is found NOT to be contaminated and all other areas contaminated with O111 would have no way to get to the humans, unless they all work at the farm or sewage plant, which they do not (THIS IS NOT THE SOURCE OF THE PROBLEM)
- Sample #10 (cow feces from dairy plant) – contaminated with JM101; this strain is finding its way into the milk in the store as it also tested positive for



it; but, this E. coli strain is harmless to humans so this could not be it (THIS IS NOT THE SOURCE OF THE PROBLEM)

- Sample #11 (water from river after lake tributary) – contaminated with strain Y1088 and RR1; this is no surprise as these two strains are also found in the lake which flows into this river; notice that these two strains are not found to contaminate the river before the lake; see Sample #5 for more information; this is another possible place where the people are getting sick from Y1088, but not the source as this strain is normally found in moist soil (THIS IS THE MAIN PROBLEM STRAIN BUT NOT THE SOURCE)
- Sample #12 (raw sewage from treatment plant) – contaminated with Q358 and O111; not surprising, as Q358 is also found in the drainage pipe from the town that brings the sewage to the plant; Q358 most likely coming from human waste, but is harmless to humans; O111 can cause some sickness and seems to be coming from the cow farm via the drainage pipe; however, there is no real way for this source to get to the others of the town unless it is the workers of the plant, which it is not; the sewage after treatment is clean of E. coli (THIS IS NOT THE SOURCE OF THE PROBLEM)
- Sample #13 (water from well B) – contaminated with strain Q358; however, this strain is harmless to humans; found normally in the human gut so this does indicate some source of human contamination; finding this in the well most likely coincides with finding it in the pipe just north of the well that takes waste from the town – with human wastes most likely – and brings it to the sewage plant for treatment; maybe the drain is leaking (THIS IS NOT THE SOURCE OF THE PROBLEM)
- Sample #14 (from treated sewage) – no contamination
- Sample #15 (water from town sewage drain pipe to treatment plant) – contaminated with Q358; this is discussed in sample #13 above; this strain is coming from human waste, but is not harmful to humans (THIS IS NOT THE SOURCE OF THE PROBLEM)
- Sample #16 (water from cow farm drainage pipe to treatment plant) – contaminated with O111; discussed in sample #9 above; can cause sickness in humans, but no evidence that they are getting sick from the sewage in the drainage pipe (THIS IS NOT THE SOURCE OF THE PROBLEM)
- Sample #17 (samples from soil taken at the apple farm) – contaminated with strain Y1088; discussed in sample #5 above; most likely became an issue after the most recent storm as this strain becomes active in moist soil; seems to



have been washed into the lake during the storm and then getting to the people when they swim in the lake (**THIS IS THE SOURCE OF THE PROBLEM**).

Looking at all the data, there seems to be three problems:

- (1) the drainage pipe from the town to the sewage treatment plant is leaking and some of it is getting into to well B; but, this Q358 is a harmless strain, so it can't be the main problem
- (2) some of the JM101 bacteria from the cow farm is getting into some of the milk that is processed there and is being sold at the store; but, this strain is harmless to humans
- (3) the strain Y1088 causes sickness in humans and is found in two common swimming areas – the lake and the river (below the lake); since this is also found in the apple farm above the lake and there was a recent storm, it seems to be the bacteria (which can be waterborne) is take from the farm to the lake where people swimming in it are becoming sick

Hints: Unlike some of the other scenarios, this one is designed to have a single definite answer. Have the students organize all the data somehow. They could write on the map which strain is found at what spot. They should also make sure to read the description of each strain as some strains are harmless. Even though they are found in various areas, they are not meant to be the problem. They could write on the descriptions where they are found in the town, as well. As far as reading the outputs, they do not have to look at the entire sequence, but only the variable areas. Also, they could just write down each strain would look like and then simply compare the diagrams for identification.

VI. Implementing the Module: Hints & Suggestions – Activity #5

This culminating activity is set up to have the students think about the various ethical issues that involve the use of GeneChip microarrays (and in reality, any other advanced genetic analysis tool) in our society. It provides them with a forum to share their point of view with others, hear other points of views, and learn about some ideas and issues they may not have thought already. Once again, they will work in groups to do some initial discussion and brainstorming before bringing their thoughts to the rest of the class. A small class discussion around the issues will then take place. The activity finishes with the students acting as an ethical committee for a “fake” GeneChip microarray company. Their job is to put together an ethical statement about their company’s point of view on the ethical issues raised by the use of their technology.



* **The Primary Goals** of Activity #5 for the students are:

- (1) to become informed about the many different ethical issues that our society is presented with the use of advanced genetic analysis tools like GeneChip microarrays
- (2) to formulate some of their own ideas and opinions on the issues raised
- (3) to think about how these ethical issues apply to a biotechnology company that is in the business of analyzing genetic information
- (4) to learn to look at both the positive and negative sides of the issues raised
- (5) to strengthen their abilities to formulate and present an opinion in a clear manner

* **Procedure** of the activity for the teacher

- (1) Introduce the activity with the students by covering the goals, the importance of looking at the ethical implications of technology in society, and to go over the rules and expectations for the discussion being held in this activity
- (2) Organize the students into at least nine groups and handout the Student Handout for Activity #5
- (3) Assign each group a scenarios and explain to them that they need to think about and brainstorm all the possible pros or cons about the situation (or cover both sides).
- (4) Give the student 15 to 20 minutes to brainstorm and discuss in their groups
- (5) Once time is up, have one person from each group read out load their issue and give the top 4 or 5 points they came up with. Allow time for a small discussion and comments from the rest of the class (keeping in mind you have nine issued to discuss)
- (6) After each group has presented, have the students return to their group for the next portion
- (7) Tell them they are now role playing the part of an ethical committee for a local GeneChip microarray company. They need to come up with a paragraph or two that acts as a “ethical policy statement” from the company. In other words, they need to come up with a statement that clearly explains their



companies position on the type of ethical issues brought up in the earlier discussion

- (8) Give them 10 to 15 minutes to come up with their statement and have them write it down on the Student Handout for Activity #5
- (9) If time permits that day or during the next day, have a different member from each group read their policy to the class and have a class discussion around some of the statements made. Identify common areas and those that seemed unique. If you would like, you could come up with one final statement from all of the ethical “committees”.

* **Hints & Suggestions** for Activity#5 in the classroom

It is strongly suggested that you cover the rules and expectations you have for the discussions and debates that this activity involves. A list of 5 rules can be found in the student directions for the activity. Everyone should participate in a courteous manner, and no one should try to prove others wrong or be vindictive in any way. Stress that it is a time for a discussion around the issues as well as time to become more aware of the issues.

The procedure above pretty much sums up most of the plans of the activity, though, you may want to change the groups around by adding more scenarios or removing some of them. This would require you to make the groups smaller or larger as needed. Alternatively, you could assign the same scenario to each set of two groups and have one group take the pro point of view and the other take the con point of view. This would require you to cover fewer scenarios, but may allow time for a deeper discussion and even a formal debate around a few of the issues. The way you approach this activity is very flexible and depends on the class you have. However, you may want to find a way to cover as many of the scenarios as possible so that the students are exposed to the wide range of ethical issues this technology brings up.

In terms of the final “ethical committee” part of the activity, the idea is to have the students think about how these issues might affect a public company and how that company might need to deal with the possible future problems. Most of the high level companies in the fields of biotechnology and genetics research have these committees and tackle the hard questions that are brought about by their own technology. Most come up with statements that give their company’s stance on the issues. It is good public relations for the company that does not want to be seen as group of mad scientists out to tinker with the human race.

As an example, here is the ethical policy statement from Affymetrix, the leading manufacturer of GeneChip microarrays in the world. You may or may not want to read this to your students. Giving it to them early may influence their statements and



therefore you may want to only give the students a basic idea of what a policy like this entails. You could read it to them after they come up with their own statements.

Example Ethical Policy Statement

"Affymetrix products enable scientists to turn genetic information into specialized detailed knowledge. We believe this enhanced knowledge will transform the quality of our lives, and help us more fully understand ourselves.

For society to benefit from these extraordinary biological advances, Affymetrix believes that informed public discussion, meaningful application of ethical principles, and thoughtful public policy must foster the constructive uses of genetic information. Education of the public and health care providers will help us become genetically literate, and enable us to make informed choices."

Whether you do this part of the activity is up to you. This part of the activity adds a twist to the common debate and discussion around scientific ethical issues that usually occurs in science classes. It is something different that actually has a real life application as most genetic and biotechnology companies have committees similar to this situation.

VII. Example Planning Calendars and Flow Charts

Below are some example flow charts of the activities to help you plan out how you will implement the module. There are three charts to show the different ways you could approach the module – Basic, Middle of the Road, or Full Module. The charts suggest what to teach in each day, but do not include homework assignments. Here is how the charts are set up:

- (1) "Minimum" or "Basic" chart to help you determine what to do if you are limited in the time you can spend on the module and/ or you are teaching a regular Biology class (and worried about the high level nature of the material)
- (2) "Average" or "Middle of the Road" chart to help you organize what to do if you have only about a week to spend on the module and/ or you are teaching maybe a Biotechnology class that is a step below an Advanced Placement level class
- (3) "Maximum" or "Full Module" chart to help you organize what to do if you have time for the full module (7-8 days) and/ or you are teaching an advanced Biotechnology or Advanced Placement Biology class or a college level class



After the charts there is a one and a half week Calendar. This calendar illustrates the suggested “Maximum” level coverage of the module if you do all the activities in your class, and provide time to do each portion of each activity. It takes 8 days, not including any formal assessment at the end such as a test or quiz. However, you may not have this amount of time, so suggested alternative charts are provided.

**“Minimum / Basic” Activity Flow Chart
(for In-Class)**

Day 1

Introduce the Unit / Goals

Activity #1 - use only 1 article / class discussion only

Day 2

Activity #2 – teacher covers only the material from Section 1 (Gene Expression Arrays) through an in-class lecture

Day 3 & 4

Activity #4 – organize 3 groups and cover only Scenario A, E, and F (analyze one day and present the next)

**“Middle of the Road” Activity Flow Chart
(for In-Class)**

Day 1

Introduce the Unit / Goals

Activity # 1 – all articles covered & discussed

Day 2

Activity #2 - teacher covers only material from Sections 1 & 2 (Gene Expression and Genotyping Arrays) through an in-class lecture

Day 3 & 4

Activity #4 – organize 4 groups and cover only scenarios A, C, E & F (analyze one day and present the next)

Day 5

Activity #5 – only cover all 9 issues given and skip the ethical policy statement



**“Maximum / Advanced” Activity Flow Chart
(for In-Class)**

Day 1

**Introduce the Unit / Goals
Activity # 1 – all articles covered**

Day 2

Activity #2 - teacher covers all of the material from Sections 1-3 (all three Arrays) through an in-class lecture

Day 3 & 4

Activity #3 – all the material from Section 1 -3 is covered by doing an in-class jigsaw on the material followed by the building of the models of the manufacturing process

Day 5 & 6

Activity #4 – organize 6 groups and cover all scenarios A - F

Day 7 & 8

Activity #5 –cover all 9 issues given and include the writing of the ethical policy statement



Week 1	Monday	Tuesday	Wednesday	Thursday	Friday
In Class	<p>* Intro. to unit – go over Unit Outline and Goals</p> <p>* Activity #1 (Article Reading and Group Share)</p> <p>* General class discussion of what was learned about the application of GeneChip microarrays</p>	<p>* Activity #2 Lecture or Jigsaw – cover all material from Activity #2 (structure and function of GeneChip microarrays)</p> <p>* Review the answers and take questions on #1-44 from Activity #2</p>	<p>* Review main points from Activity #2</p> <p>* Activity #3 Lecture or Jigsaw – cover all material from Activity #3 (the manufacturing of GeneChip microarrays)</p>	<p>* Review the answers and take questions on #1-18 from Activity #3</p> <p>* Do 2nd part of Activity #3 on GeneChip microarray manufacturing (model building and brief class presentation)</p>	<p>* Introduce and start Activity #4 (GeneChip microarray research scenario analysis and presentation)</p> <p>* Finish analysis of scenario data and put together presentation for class</p>
Home-work	<p>* Read sections #2-3 from Activity #2 and do Questions #26-44 from Student Handout</p>	<p>* Read Sections #1-3 from Activity #3</p> <p>* Review Lecture Notes from today</p>	<p>* Review Lecture Notes from today</p> <p>* Do questions #1-18 from Activity #3</p>	<p>* Read over Activity #4 as preparation for tomorrow</p> <p>* Review all information from Activity #2 and 3</p>	<p>* Work on Presentations for next Monday</p>



Week 2	Monday	Tuesday	Wednesday	Thursday
In Class	<p>*Activity #4 Continued - group presentations on scenario data analysis from last Friday</p>	<p>*Activity #5 – Ethical Scenario Discussion, Debate, and simulation of the formation of a company’s ethical policy statement</p>	<p>*Activity #5 Continued – Groups present their ethical policy statement from yesterday (further class discussion) *Wrap - Up</p>	<p>*Formal Assessment (Test of Quiz?)</p>
Homework	*Preview Activity #5 & read through ethical scenarios		*Study for Test or Quiz the next day?	



VIII. Power Point Slide Shows and “Masters” for Lecture Visuals

Available to help you out are two power point slide shows to be used with Activity #2 and #3. The slideshow for Activity #2 focuses mainly on the structure, function, and application of the gene expression microarray. The slide show for Activity #3 helps the explanation of the manufacturing process of GeneChip® microarrays. To download them, go to the following website and look for the additional resources sections:

http://www.affymetrix.com/corporate/outreach/lesson_plan/index.affx

Also available as part of this curriculum, is a set of visual masters. You could print them out and use them as masters for making overhead transparencies or passing out to students. Most of these visuals are found in the Student Manual in Activity #2 and #3. Each page is organized to help you determine how to use it.

Masters for Showing Basic DNA concepts:

“DNA Basics”

“Transcription Translation”

Masters of Basic Microarray Pictures / Diagrams (Activity #2):

“An Actual Array Image”

“Chip Probes Output”

“Food Expert-ID Array”

Masters Illustrating how Gene Expression Arrays Work (Activity #2):

“Good Match Bad Match”

“Detail of a Single Feature”

“Hyb Wash Step”

“No Match Visualization”

“Gene Expression Assay”

Masters Showing Different Array Outputs (Activity #2):

“Comparison of Three Outputs”

“Sequencing Output”

“Yeast Gene Expression Study Results”

Masters of Experimentation with Arrays (Activity #2):

“Assay Pictures”

Masters Illustrating the Manufacturing Steps of Arrays (Activity #3):

“Photolithography”

“Photolithography Basics:

“Wafer Manufacturing #1”

“Wafer Manufacturing #2”

“Wafer Manufacturing #3”

“Wafer Manufacturing #4”

“Wafer Manufacturing #5”

“Wafer Manufacturing #6”

“Wafer Manufacturing #7”

“Packaging Clips”

“Wafer Coating Washing”

“Washing Wafer and Aligning”



*To access these master files in pdf format or download the power point slide show with each of the files, please go to the website mentioned at the top of the page.

IX. Assessment

There are many possible areas of assessment during this module. What you decide to assess is ultimately up to you. There are a few means of assessment that are included in this module. Activity 1, 2, 3, and 5 all have student handouts that can be used to grade the students on their work during these units. Student handouts for Activity 1 and 5 are mainly brainstorm or list worksheets where there isn't much formal questioning. Student handouts for Activity 2 and 3 are question sets to go with the reading. You may decide to use all of the questions or cut some of them out.

Since a lot of this activity involves working in groups and discussions in class, you should come up with a way to assess them in these areas. Maybe have some sort of simple participation guide for grading their participation in the discussions and some guidelines for working in groups. Also, there is no formal test or quiz questions provided, but these can easily be made to fit your means of testing. Questions could range from drawing and diagramming how a GeneChip microarray functions, to analyzing microarray outputs, to discussing the differences between the three arrays and their various uses.

Lastly, Activity 5 ends with group presentations around the analysis of the scenario assigned to each group. The grading of these presentations is up to you, but it is suggested that you use some sort of rubric or scoring guide as an assessment tool. One page 58 is an example rubric that you may use to help with the grading of these presentations. How you equate this into to points may vary. The standard equation is a 4 equals 100%, a 3 equals an 85%, a 2 equals a 70%, and a 1 equals a 50%.

Below are a set of multiple choice and open ended questions to help you with some formal assessment at the end of the unit or with any quizzes you may want to do along the way.

Multiple Choice Questions

Topic: Gene Chip Structure

- (1) The small strands of DNA / RND that stick out from the microarray surface are known as:
- | | |
|-------------------------|------------|
| (A) complementary bases | (B) genes |
| (C) features | (D) probes |
- (2) How long is a typical probe?



- (A) 8 base pairs
(C) 100 base pairs
- (B) 25 base pairs**
(D) there is no set length to the probes
- (3) Why are the probes of gene chips made 25 bases long rather than a shorter length (such as 5 bases long)?
(A) At a 25 base pair length, one can be sure that this sequence is not found anywhere else in the genome of study
(B) DNA fragments will not stick to 5 base pair long probes
(C) most genes are about 25 base pairs long
(D) 25 is the “magic” number, anything shorter or longer does not work
- (4) Each tiny square on the microarray is known as a:
(A) **feature** (B) chip (C) probe (D) SNP
- (5) Which of the following is the smallest in size?
(A) wafer (B) microarray (C) **feature** (D) gene chip cartridge
- (6) In each feature, how many different types of probes are there?
(A) **1** (B) 10 (C) 25 (D) 10,000
- (7) What is the first step of an experiment using a gene expression microarray?
(A) apply the sample to the chip (B) **extract the RNA from the cell**
(C) label the RNA with biotin (D) multiply the RNA through PCR
- (8) In an experiment using a GeneChip® Microarray, what is the purpose of the biotin used to label the RNA?
(A) to act as a molecular “glue” to the fluorescent molecules added later on
(B) to aid in the visualization of which features the RNA sticks to
(C) to help the RNA to stick to the probes in the features
(D) **both A and B explain the purpose of the biotin labeling**
- (9) In an experiment using a GeneChip® Microarray, after the RNA sample from a cell is labeled with biotin and fragmented, what is the next step?
(A) scan the chip with a laser
(B) label the chip with fluorescent molecules
(C) **add the sample to the chip so that it is washed over the entire surface**
(D) make more copies of the RNA through PCR
- (10) In an experiment using a GeneChip® Microarray, if a gene in a cell is highly expressed:
(A) the feature representing the gene will shine with very little intensity
(B) **the feature representing the gene will shine with a lot of intensity**
(C) the feature representing the gene will be a black color
(D) there is no way to detect expression levels, only if the gene is expressed or not



- (11) In an experiment using a GeneChip® Microarray, the molecules are very small and can not be seen with the human eye. Therefore, how does the scientist know which features the RNA has hybridized to?
- (A) **The chip is washed with a fluorescent molecule that sticks to the biotin on the RNA fragments. It is then scanned with a laser to find out where it happened.**
- (B) The scientists use electron microscopes to scan in and see where the matches occurred.
- (C) A computer is used to analyze where the matches occurred.
- (D) The RNA is radioactively labeled. So, after the addition of the RNA to the chip, a detection counter is used to look for matches.
- (12) When using a gene expression microarray, you know that a gene is not expressed because the feature representing that gene:
- (A) **does not glow** (B) glows
- (C) glows brightly (D) there is no way to tell
- (13) In the color microarray image, which colors represent that a lot of the RNA has hybridized with the probes in the feature (and thus the gene is highly expressed)?
- (A) black and blue
- (B) light blue and green
- (C) **red and white**
- (D) the color does not indicate how much has hybridized

Topic: Gene Chip Function (Gene Expression chip)

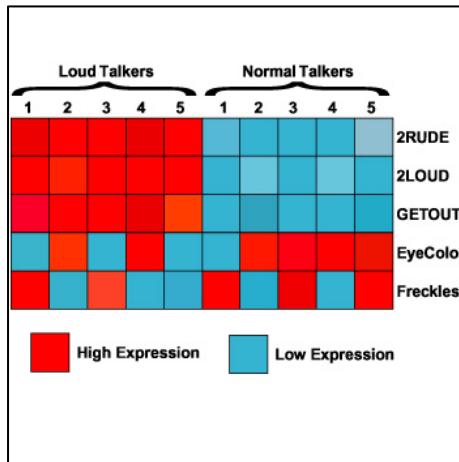
- (14) In order for a scientist to measure gene expression using a GeneChip® Microarray, what molecule are they technically measuring?
- (A) the DNA (B) **the mRNA** (C) the proteins (D) the lipids
- (15) How can GeneChip® Microarrays help a scientist that is studying a disease?
- (A) They could help them determine which genes are active in the diseased cell
- (B) They could help them determine an SNP pattern associated with the disease
- (C) They could help them determine the unknown sequence of the gene
- (D) **both A and B are correct**
- (16) The great thing about a gene expression chip is:
- (A) It allows the scientist to find out the exact function of the gene
- (B) **It allows the scientist to measure the expression of all the genes in a cell**
- (C) It allows the scientist to determine what mutations cause the disease
- (D) It allows the scientist to determine which cell will become diseased



- (17) Which of the following is NOT an advantage for researchers using gene chips when doing a study on the gene expression of a diseased cell compared to the older methods of the genetic research?
- (A) in the past, scientists would have to look at older research to find genetic links to similar diseases and make a hypothesis based on this research - with gene chips, this does not need to be done
 - (B) the gene chip allows scientists to study every single gene in the human genome
 - (C) in the past scientists would have to test hundreds of different hypotheses, but with the gene chip, they can go into the experiment without an hypothesis and see what happens
 - (D) the gene chip allows them to easily determine the specific function of each gene much quicker than in the past**
- (18) When two pieces of complementary DNA pieces come together, it is known as:
- (A) Hybridization**
 - (B) Transcription
 - (C) Translation
 - (D) Replication
- (19) This basic principle is the main basis for how GeneChip® Microarrays are able to identify which RNA or DNA is in the sample being tested.
- (A) Replication
 - (B) Translation
 - (C) Hybridization**
 - (D) Transcription
- (20) How can a probe represent only one gene out of the entire genome?
- (A) The probe is long enough to be complementary to the entire gene
 - (B) The probe is made of different sections from different sections of the gene
 - (C) The probe is unique, so that it is found nowhere else in the genome**
 - (D) none of the above explain how a probe can represent a gene
- (21) A powerful aspect to the gene expression microarray is:
- (A) it allows a scientist to look at many genes at once and see what genes are on
 - (B) it helps a scientist to determine which genes may contribute to a disease
 - (C) it allows a scientists to compare the gene expression of two different cells
 - (D) all of the above are made possible by gene expression microarrays**
- (22) When using gene expression gene chips, how can scientists tell the level of expression of the gene (i.e., how can they tell whether the gene was expressed a lot or a little)?
- (A) the level of fluorescence tells them how much the gene was on – blue for high expression and white for low expression
 - (B) the level of fluorescence tells them how much the gene was on – white for high expression and blue for low expression**



- (C) the scanner used looks for the amount DNA stuck to the probes and the computer prints the level out
- (D) this can not be done with gene chips – the chip is not really quantitative
- (23) Which of the following is NOT explained by the following heat map??



- (A) 2RUDE, 2LOUD, and GETOUT are all involved in how people talk
- (B) High expression of the 2RUDE, 2LOUD, and GETOUT genes leads to loud talking
- (C) **High expression of the 2RUDE, 2LOUD, and GETOUT genes leads to normal talking**
- (D) Eye Color and Freckles genes seem to be closely connected to how someone talks

- (24) How can a gene expression microarray help with testing a medical drug?
- (A) It can show exactly how the drug interacts with the gene
- (B) It can show which genes are affected by the drug and which genes are not**
- (C) It can show how genes combine to cause the disease
- (D) none of the above explain it can help with testing a medical drug
- (25) How can a gene expression microarray help increase the success rate of a medical drug?
- (A) It can be used to perform experiments to find out which gene expression pattern in people leads results in the best affect from the drug.
- (B) It can help doctors determine whether the drug will work on a person or not depending on their gene expression pattern
- (C) It can show scientists whether the drug turns off the genes they need to affect
- (D) all of the above**

Topic: GeneChip Microarray Function (Genotyping & Resequencing chips)

- (26) Which of the following is the best definition of genotype?
- (A) The number of genes someone has in their genome.
- (B) The combination of the versions of a gene or section of DNA a person has.**
- (C) The type of genes a person has in their genome.
- (D) It tells which genes combinations cause a disease.

- (27) SNP stands for
- (A) Single Nucleotide Placements
- (B) Several New Polymorphisms
- (C) Single Nucleotide Polymorphisms**
- (D) Somewhat Normal Person



- (28) What is an SNP?
 (A) Difference between two people's DNA due to a deletion of a section of DNA
 (B) Difference between two people's DNA due to an insertion of a section of DNA
 (C) **A difference between two people's DNA at a specific single base pair**
 (D) A difference between two people's DNA within a single gene
- (29) How can SNPs be used to help a scientist find the cause of a disease?
 (A) **They could tell you where the disease gene is because certain patterns of SNPs are usually associated with specific genes**
 (B) SNPs are normally the cause of defective genes and thus lead to diseases
 (C) Certain types of SNPs usually lead to diseases
 (D) none of the above
- (30) In the SNP genotyping microarrays, which base among the 25 base pairs of the probe is used to determine the specific SNP?
 (A) base #1 (B) base #7 (C) **base #13** (D) base #25
- (31) If the DNA from a sample matches with a probe on a SNP Genotyping Microarray that has a C as its middle base, what SNP genotype would the person have for that specific SNP?
 (A) A/T (B) **C/G** (C) C/T (D) A/G
- (32) When using a genotype microarray, what must you first isolate from the cell of the organisms you are genotyping?
 (A) **DNA** (B) mRNA (C) protein (D) lipids
- (33) If a person has the C/G genotype for an SNP on one chromosome, and the A/T genotype for the SNP on the other chromosome, then they are considered for this SNP.
 (A) homozygous (B) **heterozygous** (C) a mixture (D) a combination
- (34) If a person has the A/T genotype for an SNP on one chromosome, and the A/T genotype for the SNP on the other chromosome, then they are considered for this SNP
 (A) **homozygous** (B) heterozygous
 (C) a mixture (D) a combination
- (35) Which is NOT something a resequencing chip could be used for?
 (A) identify the origin of DNA in a sample
 (B) determine the strain of a virus that someone is sick from
 (C) **determine the sequence of an unknown DNA in a sample**
 (D) test a sample of food for DNA from another organism



probes?

(A) about 10 (B) about 50 (C) **about 100** (D) about 250

(49) When manufacturing gene chips, if you wanted to add an adenine nucleotide to 500,000 specific features on the chip, how many openings must there be in the mask used just before the addition of the base?

(A) 1,000,000 (B) 500 (C) **500,000** (D) 100,000

Free Response / Open Questions

(50) Diagram the basic structure of a GeneChip® Microarray. Use the diagram to illustrate and explain the size and layout of the whole chip as well as a feature.

(51) Describe the steps performed in an experiment using a gene expression microarray. Explain each step going starting from the cell all the way to scanning the chip.

(52) Explain what a probe on a GeneChip® Microarray is and how it is used to detect the presence of a specific RNA or DNA in a sample. Use diagrams to help you out and be sure to explain the importance of hybridization.

(53) Explain how the use of gene expression microarrays can help with studying a disease, discovering medicines used to fight the disease, and the prescription of the medicine by doctors. Include a discussion on personalized medicine.

(54) Use simple diagrams to explain how probes are designed and set up to sequence DNA in a resequencing microarray.

(55) Describe the steps performed in an experiment using a genotyping microarray. Explain each step going starting from the cell all the way to scanning the chip.

(56) Explain how probes in an SNP Genotyping Microarray are used to determine what version of an SNP a person has in their DNA.

(57) Explain how a gene expression GeneChip® microarray is able to detect if a gene is expressed or not in a cell. How is the whole thing visualized?

(58) Describe and explain how the two step process of shining a light through a mask followed by the addition of free nucleotides is used to build probes on GeneChip® Microarray. Be sure to explain the importance of the photosensitive molecule. Use diagrams to help in your explanation.

(59) Explain how the process of photolithography is used to build DNA probes onto a GeneChip® microarray





Score →	1	2	3	4
Analysis	<p>* presentation shows very little thought or analysis of the data / analysis does not make sense</p> <p>*most of the data is left out or not used in the analysis by the group</p>	<p>* presentation shows a minimally thought out analysis of the data / analysis is somewhat confusing</p> <p>*some of the data is left out and not accounted for in the analysis by the group</p>	<p>* presentation shows a well thought out analysis of the data / analysis is clear</p> <p>* most of the data is looked at and accounted for in the analysis by the group</p>	<p>*presentation shows an extremely well thought out and strong analysis of the data / analysis is very clear</p> <p>*all data is looked at and accounted for in the analysis by the group</p>
Conceptual Understanding	<p>*presentation shows a little or no understanding of the concepts of microarrays and they are used and of the background of the scenario the group analyzed</p>	<p>*presentation shows a basic understanding of the concepts of microarrays and how they are used and of the background of the scenario the group analyzed</p>	<p>*presentation shows a good understanding of the concepts of microarrays and how they are used and of the background of the scenario the group analyzed</p>	<p>*presentation shows a very strong understanding of the concepts of microarrays and how they are used and of the background of the scenario the group analyzed</p>
Visual	<p>*very poor use of visuals or no use of visuals at all</p> <p>*visuals very ineffective and messy and unclear</p>	<p>*a good, basic use of a few visuals</p> <p>*visuals somewhat effective and clear</p>	<p>*a good, strong use of visuals that help out the explanation</p> <p>*visuals effective and neat and clear</p>	<p>*very strong use of multiple visuals that enhanced the explanation</p> <p>*visuals very effective and neat and clear</p>
Organization	<p>*presentation very unorganized and not well thought out or organized with little or no teamwork</p>	<p>*presentation show some basic organization though it seems somewhat rushed with the input of only a few members of the group</p>	<p>*presentation well organized and thought out and organized with good teamwork</p>	<p>*presentation very well organized and thought out and organized with great teamwork from all</p>
Communication	<p>*poor communication of ideas and confusing at times / speech is hard to hear and mumbled at times</p>	<p>*presenters communicated their ideas clear at times and unclear at others / speech could be louder and clearer</p>	<p>*presenters communicated their ideas clearly / speech clear & somewhat loud</p>	<p>*presenters communicated their ideas very clearly / speech nice & loud & clear</p>



X. Feedback: Teacher & Student Surveys

The GeneChip® Microarray curriculum is in its' second year of development. It is still being piloted by various teachers. To get your help in making improvement to the curriculum for future use, we ask that you please fill out the teacher feedback survey. The survey can be found by going to the Affymetrix.com website, going to resources for educators, and clicking on the GeneChip Microarray curriculum. Also, you can go to the following web address and click on “feedback” towards the top.

http://www.affymetrix.com/corporate/outreach/lesson_plan/index.affx

If you can, please fill out the form electronically and send as an attachment to the following e-mail address below. Feel free to add further comments in the e-mail.

outreach@affymetrix.com

If there is a problem with doing this, please send an e-mail to the address above and we can come up with another way for you to get it to us.

Along with your feedback, we would also like to get an idea of the impact of the curriculum on the student and get their ideas for improvements. If time permits, we would be very appreciative if you would have them fill the student questionnaire and survey out. It is a bit long as there is room for feedback on all five activities as well as some places for rating their knowledge on the main topics. Please give them enough time to be thorough and complete with their answers. You may want to even give it to them as a homework assignment after you have completed the curriculum. Please have them only give feedback on the activities you did in your classroom and encourage them to be as thorough and detailed as possible. The questionnaire / survey can be found by going to the following web address:

http://www.affymetrix.com/corporate/outreach/lesson_plan/index.affx

Please click on “Student Manual”, and then click on “Student Survey” to download it. Please collect them and organize them into a single folder. To get them to us, you can either send them to the same address as the teacher survey given above, or you may send an e-mail to outreach@affymetrix.com and we will organize someone to pick them up from you.

