# MARY FISH  ACTION PROPOSAL - ICORE 2010

## Spanish River High School

## Biotechnology Academy

## *MODULE UNIT TITLE: Modeling DNA Microarray Technology To Screen For And Compare Resistance Genes Amongst Antimicrobial Resistant Bacteria*

**Background:** Antibiotics or antimicrobial compounds have been used for years to fight infections by bacteria. But some bacterial pathogens, like Salmonella, E. coli, Campylobacter, Staphylococcus, and Enterococcus, are becoming resistant to the antibiotics that are used to fight them off. Many bacterial strains are not just resistant to one antibiotic but many. They are called MDR's or multiple drug resistance strains. The result has been the re-emergence of such diseases as tuberculosis, and the development of such recent scourges as MRSA. Natural selection, the rate of evolution in bacteria and the overuse of antibiotics have been the driving forces in this development. Bacteria are exchanging genetic information for antibiotic resistance to their progeny and the creation of mutations that fine tune the advantage to survival. There is evidence that completely different strains are exchanging genetic information with each other in every possible place that you find multiple strains of bacteria: in soils, waterways, plants, and in animals including us. This new development has been the subject of intense investigation because they can be acquired horizontally via conjugation, transformation or transduction, and they may be transmitted in groups of genes on plasmids, transposons or integrons.

Scientists need to know which bacteria are resistant to which antibiotics and how the bacteria continue to develop resistance to other ones. Many methods have been used to identify and study antibiotic resistance in bacteria. Traditionally, but still widely used, is the technique to grow bacterial strains on antibiotic-rich medium to check for resistance or to use the zone of inhibition technique that employs antibiotic disks placed on plates to see which antibiotics bacterial strains are resistant. The advancement of molecular biology and biotechnological techniques, the mapping of the human genome and DNA sequencing techniques have made it possible to identify and compare the actual resistance genes that are responsible for the production of enzymes and/or other mechanisms the bacteria employ to disable, pump out or keep out the antibiotic. DNA/plasmid isolation, primers and PCR, and gel electrophoresis/southern blotting identify genes but now that there are so many of them in many strains these methods can be too time consuming. Real time PCR and DNA Microarray technology is being used because these techniques can identify and compare many resistance genes in many bacterial strains at the same time.

**Abstract**: This proposal will implement a simulation activity for identifying resistance genes in antimicrobial resistant bacteria using DNA Microarray technology. Microarray technology is a biotechnological technique that is taught in our biotechnology program at our school. Although

performing an actual microarray is cost prohibitive in our lab, this will give our students a virtual way to understand this important tool for screening and diagnostics in laboratory research and in industry. The DNA Microarray model is modified from the Viral Microarray Simulation Kit developed by Dr. Chuck Lawrence in the ICORE program at UF. The data for the simulation comes from an actual published experiment done using this technology found in the literature.

**Mission**: Students will be given a scenario. They will be told that they are scientists at the CDC research laboratories who need to identify and compare the resistance genes in 7 bacterial strains in order to find out the products or mechanisms that the bacteria employ to resist the antibiotics that are now used on them. Let them know that it may lead to creating a new line of antibiotics that will quench some of this resistance and reduce the re-emergence of such diseases as tuberculosis. Once the results are in, students can use graphing software to analyze the genes that were identified in multiple strains. Discussion of how these genes found themselves in distantly related strains follows. Assessment will be a post test about the unit on antibiotic resistance, a bacterial disease report, and a lab protocol on DNA microarray technique.

**Implementation**: This simulation activity will be the last but focal part of a three week module unit on antibiotic resistance. The target audience will be our second year biotechnology students ($10^{th}$ graders). During the first part of the school year, the second year biotech students are taught about sterile technique and the biology of microorganisms. They would have already prepared media, grown cultures of bacteria, and analyzed cultures using staining techniques. Also, they would have completed DNA extraction from bacteria, running a gel to study the DNA fragments after restriction enzyme digestion, and some experiments involving protein isolation and analysis. So it looks like the best spot for the antibiotic resistant growth experiments and the DNA microarray lesson will be in the second year.

## Outline and Expected Outcomes:

**MODULE UNIT TITLE**: Modeling DNA Microarray Technology To Identify And Compare Resistance Genes Amongst Antimicrobial Resistant Bacteria

Time Period: Three to Four Weeks

First Week:

1. Introduction to Antimicrobial Resistance (AR)
    a. Students will complete a pre-test about bacteria and growth information, and a lab quiz about media preparation and sterile techniques based on a previous unit in the school year.
    b. Activity: Student Quiz about the impact of infectious diseases and a classification exercise distinguishing emerging, re-emerging and endemic diseases. (1)

c. Video: Secrets of the Sequence: Superbugs: Bacterial Drug Resistance and activity (2)

d. Direct Instruction about Antimicrobial Resistance (PPT)- Assign Bacterial Disease Report – Good for the JSEHS.

e. Zone of Inhibition Lab/Kirby-Bauer Test (3)-Start

Second Week:

2. Antibiotic Resistance and Evolution

   a. Pseudomonas fluorescens Growth Experiment (4)- Example of Vertical Gene Transfer

   b. Instruction: The Evolution of Antibacterial Resistance

      - PBS Video/Questions: Tuberculosis (5)

   c. Optional Lab: Horizontal Gene Transfer Between Two Bacterial Organisms (6)

   d. Assessment- Quiz , Lab Reports

Third Week:

3. Techniques for Screening and Diagnosing Antibiotic Resistance

   a. Review of techniques that have been used so far (DNA isolation and purification, PCR, gel electrophoresis).

   b. Introduce the use of DNA Microarrays to Screen Antimicrobial Resistance Genes

      - DNA Microarray Animation (7)

      - DNA Microarray Fact Sheets/Articles and optional modeling activity (8)

   **c. DNA Microarray to Screen Resistance Genes in Bacterial Strains Simulation (9)**

   d. REVIEW; lab practical; Presentations of Bacterial Disease Reports; Culminating Solution Activity; TEST

**Expected Outcomes:**

1. Students become proficient in aseptic technique.

2. Students become proficient in media preparation, growing a plate culture, and analyzing results accurately.

3. Students can identify the reasons why certain bacterial strains have become multi-drug resistant.

4. Students can see the evolutionary basis of antimicrobial resistance.
5. **Students will be able to identify the importance for controls in experiments.**
6. **Students can evaluate the need for DNA Microarrays to accurately screen for resistance genes in bacterial stains and see the importance in that.**

**National Science Standards:**

**Content Standard A**

**As a result of activities in grades 9-12, all students should develop:**

- **Abilities necessary to do scientific inquiry**
- **Understandings about scientific inquiry**

## Content Standard C

**As a result of their activities in grades 9-12, all students should develop understanding of**

- The cell
- Molecular basis of heredity
- Biological evolution
- Interdependence of organisms
- Matter, energy, and organization in living systems
- Behavior of organisms

Proposed List of Materials Needed:

1. For Zone of Inhibition Lab: *S. epidermidis* and *E. coli** , *TSA, antibiotic disks*
2. For Pseudomonas lab: Pseudomonas fluorescens
3. Viral Microarray Kit from ICORE (Loan out)

The list of materials to order with the $200 stipend has not been determined yet. The search for cultures is still being done. As a biotech facility we have plenty of the materials in order to grow bacteria.

References:

1. NIH Curriculum Packet: Emerging and Re-Emerging Diseases – Activity 1
2. http://www.pubinfo.vcu.edu/secretsofthesequence/playlist.frame.asp
3. Biological Science Initiative: Kirby-Bauer Test- http://www.hhmi.org/biointeractive/disease/pdf/antibiotic_resistance/antibiotics_activities.pdf
4. NIH Curriculum Packet: Activity 3
5. PBS Evolution Site: Video 6- Why Does Evolution Matter Now?
6. http://www.eurovolvox.org/Protocols/PDFs/Antibiotic1.3_UK_eng.pdf
7. Learn.Genetics- Microarray
8. **Fishing for genes: DNA microarrays in the classroom-Science In Schools**

9. DNA Microarray Simulation Information – **DNA microarray detection of antimicrobial resistance genes in diverse bacteria Jonathan G. Frye[a], Troy Jesse[a], Fred Long[b], Gaelle Rondeau[b], Steffen Porwollik[b], Michael McClelland[b], Charlene R. Jackson[a], Mark Englen[a] and Paula J. Fedorka-Cray[a]** Bacterial Epidemiology and Antimicrobial Resistance Research Unit, U.S. Department of Agriculture, Agriculture Research Service, Richard B. Russell Research Center, 950 College Station Road, Athens, GA 30605, USA /Sidney Kimmel Cancer Center, 10835 Road to the Cure, San Diego, CA 92121, USA

**DNA MICROARRAY DATA SHEET**

Objective: To determine the type of resistance genes found in two DNA samples of two bacterial strains that have shown to be antibiotic resistant phenotypically.

Directions: Fill in the spots that fluoresce blue. Use the DNA Microarray Key to determine the resistance gene being identified at each spot on the microarray that fluoresced and fill in the name of the resistance gene on the right side of the page. Then draw a line from that fluorescent spot to the name of the resistance gene from the strain sample.

Strain Name:_____

| | | | | |
|---|---|---|---|---|
| 1 | x | o | x | o |
| 2 | o | x | o | o |
| 3 | o | o | o | x |
| 4 | o | x | x | o |
| 5 | o | o | x | o |
| | A | B | C | D |

Resistance Gene #1:_____

Resistance Gene #2:_____

Resistance Gene #3:_____

Resistance Gene #4:_____

Resistance Gene #5:_____

Resistance Gene #6:_____

Resistance Gene #7:_____

Resistance Gene #8:_____

Resistance Gene #9:_____

Resistance Gene #10:_____

DNA Microarray Key

| | | | | |
|---|---|---|---|---|
| 1 | 1 | 2 | 3 | 4 |
| 2 | 5 | 6 | 7 | 8 |
| 3 | 9 | 10 | 11 | 12 |
| 4 | 13 | 14 | 15 | 16 |
| 5 | 17 | 18 | 19 | 20 |
| | A | B | C | D |

1 – 5aph6 (strB) (Aminoglycosides)

2- vanC (Vancomycins)

3- 9dfrA1 (Trimethroprims)

4- tetO (Tetracyclines)

5- mecA (Beta-Lactams)

6-29adA1- (Aminoglycosides)

7-catP (Chloromphenicol)

8-aadA7 (Aminoglycosides)

9-bla oxa -27 (Beta-Lactams)

10-erm(B) (Erythromycins)

11-vanA (Vancomycins)

12-21bla (Beta-Lactams)

13-ermC (Erythromycins)

14-28aph3(strA) (Aminoglycosides)

15-42cat4 (Chloramphenicol)

16-sat4 (Streptothricins)

17-dfr1 (Trimethoprims)

18- vanB2 (Vancomycins)

19- aph3(strA) (Aminoglycosides)

20-vanX (Vancomycins)