Title: Dengue: Using Breakbone Fever to Introduce Proteomics

Name: Robb Bartenslager
Palm Beach Central High School
Wellington, FL

Abstract:

High school students often have little knowledge about the workings of biotechnology and are intimidated to try and understand molecular biology. Dengue fever, while sounding exotic, is an emerging pathogen that could affect Florida students today. The study of this disease will be used as a hook to get students to utilize biotechnology tools and protocols to understand proteomics. Mosquito egg clusters will be reared in the lab, with specimens being removed at the larval, pupal and adult stages. After DNA is extracted from each stage, it will be run through PCR and sent to an outside source for sequencing to confirm identical DNA at all stages. Protein will be extracted from each stage as well, and gels will be compared to find differences in protein expression. Samples will be run through the MALDI mass spectrometer at the University of Florida with the results being sent back to students at a later date.

Rationale:

Understanding biology at the molecular level is critical in any of the life science fields students may pursue at the secondary level. Biology teachers at my school do not conduct lab activities associated with biotechnology, leaving students with a high level of ignorance and intimidation concerning molecular biology. This is reflected by the number of students taking Advanced Placement Biology and Genetics Honors. With a student population of about 3,000 students, Palm Beach Central currently has ten sections of Advanced Placement Environmental Science which shows an interest in higher level science. However, there are only two sections of Advanced Placement Biology and one section of Genetics Honors which indicates a significant drop in students advancing to the more difficult science courses. This unit will expose students to many of the concepts of these courses, and an increase in enrollment of these courses is expected. In addition, many Research Honors students need to incorporate biotechnology into their individual projects but lack the knowledge of how to do this. The practical experience of this unit will assist them in following protocols for several common biotechnology tasks.

Description of Teaching Unit:

The following unit will take approximately five weeks, which will be followed by extension activities for the following semester. Since students are in grades 9-12, with some not having taken biology yet and others having completed AP Biology, the Viral Quest curriculum will be utilized to bring all students up to a baseline level of molecular biology.

Day One:	Pre Test on emerging pathogens and biotechnology
Day Two:	Viral Quest
Day Three:	Viral Quest
Day Four:	Viral Quest
Day Five:	Viral Quest
Day Six:	Viral Quest
Day Seven:	Viral Quest
Day Eight:	Viral Quest
Day Nine:	Viral Quest
Day Ten:	Viral Quest
Day Eleven:	Viral Quest
Day Twelve:	Lecture on insect taxonomy and anatomy
	Assessment: Mosquito life cycle diagram
Day Thirteen:	Grasshopper dissection
	Assessment: Lab Packet
Day Fourteen:	Mosquito IPM DVD
	Assessment: Video worksheet
Day Fifteen:	Dengue Fever WebQuest
	Assessment: WebQuest packet
Day Sixteen:	HHMI PCR Virtual Lab
	Assessment: Lab worksheet
Day Seventeen:	Micropipette activity
Day Eighteen:	DNA extraction and PCR
	Assessment: Lab report
Day Nineteen:	Run PCR product on gels, send out for sequencing
	Assessment: Lab report
Day Twenty:	Protein extraction
	Assessment: Lab report
Day Twenty One:	Protein gels and digestion
	Assessment: Lab report
Day Twenty Two:	Lecture on mass spectrometry
Day Twenty Three:	Post Test on emerging pathogens and biotechnology
	Samples sent to Dr. Chen's lab for mass spec.

Extensions:

Classroom visit by person who had Dengue
- the human side of disease

Classroom visit by emergency room physician (former student)
- how doctors detect uncommon pathogens
- academic pathways to a medical career

Classroom visit by Scripps research scientist
- how researchers up/down regulate protein expression

Classroom visit by UF entomologist

Student insect collection

Group presentation on an emerging pathogen

Confirmation of mass spec by using BLAST

Data Collection:

In addition to completing a pre test and post test, nine assignments will be collected and graded along with the Viral Quest materials. The number of students who incorporated biotechnology in their individual projects will be recorded this year and compared to the number incorporating them last year. Enrollment numbers for AP Biology and Genetics Honors will be monitored to determine any increase.

Equipment Usage:

The equipment locker will be needed during the week of DNA and protein extraction to use the micropipettes and gel chambers. Samples will be run on the MALDI in Dr. Chen's lab at UF.

ICORE Connections:

Knowledge of Dengue was obtained from a lecture by Dr. Connelly during ICORE. The labs for DNA extraction, PCR, protein extraction and mass spec were conducted during ICORE.

Budget:

Mosquito Breeding Chamber		$20
Culex egg cluster		$20
Primer		$10
DNA sequencing	($8x9)	$72

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Key Questions: What is Dengue Fever and why should I care about it?

Science Subject: Research Honors, Advanced Placement Environmental Science

Grade and Ability Level: Honors (9-12), Advanced Placement (10-12)

Science Concepts: How are vector borne diseases transmitted?

Overall Time Estimate: Unit will take 25 days, lesson will take 50 minutes.

Learning Styles: Students will be viewing video clips and reading text from various web sites.

Vocabulary: symptom, vector, endemic, pandemic, epidemic, serotype, antigen, antibody, vaccine

Lesson Summary: Students will review several web sites to understand what Dengue Fever is, where it is found, how it is transmitted along with current research.

Student Learning Objectives with Standards:

1. Student will describe the symptoms of Dengue Fever.
2. Student will describe the transmission of Dengue Fever.
3. Student will construct a graph of Dengue Fever cases and calculate percent increase over time.
4. Student will describe current research relating to the development of a Dengue Fever vaccine.

Florida Standard: SC.912.2.14.6 Explain the significance of genetic factors, environmental factors, and pathogen agents to health from the perspective of both individual and public health.

Materials: Dengue Fever packet, computer with internet access

Background Information: Dengue Fever is a viral disease most commonly found in tropical climates. However, it has been increasing in both number of cases worldwide and in the number of geographic areas affected. Although Dengue Fever is not endemic to Florida, it has been found occasionally in travelers returning from an overseas trip and has been transmitted locally. The virus that causes Dengue Fever is transmitted by mosquitoes in the *Aedes* genus which limits the virus to areas where the mosquito lives. A mosquito bites an infected person and picks up the virus, and 8-10 days later it can transmit the virus through its salivary secretions when it bites another human. Typical symptoms of Dengue Fever include headache, fever, rash, and severe joint pain which last about a week. There are four different strains of viruses that cause Dengue Fever, so the body does not build up an immunity to the other strains after being infected with one variety. Some patients develop a more serious form of Dengue called Dengue Hemorrhagic Fever which causes a loss of blood and can be fatal. To prevent Dengue Fever, communities need to monitor mosquito populations and remove breeding areas that *Aedes*

prefers, like small containers that collect water. Scientists are currently working on creating a vaccine for Dengue Fever, but have had difficulty because there are four serotypes.

Symptom – sign of abnormality of function in a patient
Vector – organism that transmits a pathogen
Endemic – disease is normally found in a population
Pandemic – when a disease spreads across continents
Epidemic – new cases of a disease increase rapidly
Serotype – variety of virus based on its antigens
Antigen – molecule that causes the body to produce antibodies
Antibody – protein produced by the body to identify foreign particles
Vaccine – weakened/dead pathogen introduced so the body produces antibodies

Advance Preparation: It is best to run through the Web Quest to make sure all links are still operating.

Procedure and Discussion Questions with Time Estimates: To introduce the lesson, I will ask if anyone has heard of Dengue Fever before, and if so, what they think they know about the disease. It is expected that no student will have background knowledge about Dengue, and this should only take 2 minutes. Students will then move quickly to complete the Dengue web quest, which will take 40 minutes. If it takes longer for the students to complete the web quest, the follow-up discussion will take place the following class period. Discussion questions will include:

What are the symptoms of Dengue Fever?
Fever, headache, joint pain.

Where is Dengue usually found? (endemic)
Tropical Asia, Central America, South America, Puerto Rico.

How could someone in Florida get Dengue?
By traveling to an area that has Dengue. There is a lag time of several days from being bitten to showing symptoms.

What limits the spread of Dengue?
Only Aedes mosquitoes transmit Dengue. There needs to be mosquitoes and the virus present to start an epidemic.

So what actually causes Dengue?
Dengue virus.

Why are the female mosquitoes important?
They are the vectors, transmitting the virus from one infected person to another.

What government agencies track diseases like Dengue?
CDC in the U.S. and WHO worldwide

What do we see worldwide as far as the number of Dengue cases?
Large increase (200%) over the last two decades.

What is the most serious form of Dengue?
Dengue Hemorrhagic Fever, which can be fatal and more often acquired after having Dengue multiple times.

How many different types of Dengue virus are there?
There are four serotypes.

Why is creating a vaccine so difficult for Dengue?
The difficulty has been getting one vaccine that is good for all four serotypes. Trials are currently underway in Thailand.

Assessment Suggestions: In addition to the web quest packet, questions will be on the pre/post test.

1. The agent that causes Dengue Fever is a
    a. Virus
    b. Bacterium
    c. Protozoan
    d. Mosquito
    e. None of the above
2. Dengue gets introduced into the U.S. by
    a. Infected food products
    b. Migratory birds
    c. International travelers
    d. Tainted medical supplies
    e. All of the above
3. The vector for Dengue is
    a. Ticks
    b. Mosquitoes
    c. Lice
    d. Bed bugs
    e. All of the above
4. How many varieties of DEN are there?
    a. One
    b. Two
    c. Three
    d. Four
    e. Five

5. In the U.S. diseases like Dengue are tracked by the
    a. USDA
    b. USFWS
    c. USPS
    d. CDC
    e. EPA
6. The most severe form of Dengue is
    a. Dengue Hemorrhagic Fever
    b. Dengue Encephalitis

c. Dengue Dysentery
d. Dengue Sleeping Syndrome
e. There is only one form of Dengue.

7. Why has the development of a Dengue vaccine taken so long?
    a. There are so few cases of Dengue worldwide, it isn't cost effective to produce.
    b. The vaccine must work for all Dengue varieties.
    c. Birds are resistant to most antibiotics.
    d. Dengue cases have been declining steadily worldwide.
    e. A different vaccine must be produced for each human ethnic group.
8. In which stage of the vector life cycle is Dengue transmitted?
    a. Egg
    b. Larva
    c. Pupa
    d. Adult
    e. All stages
9. Symptoms of Dengue include
    a. Fever
    b. Rash
    c. Headache
    d. Joint pain
    e. All of the above

Extensions: This is part of a unit, which will include using ViralQuest, HHMI virtual PCR lab, UF IPM for Mosquito Control DVD and print resources, rearing of mosquitoes, DNA extraction, and protein extraction.

Articles:

Linda Marsa, *Discover*, The Hot Zone, December 2010

Peter Arensburger *et al*, *Science* 330, Sequencing of *Culex quinquefasciatus* Establishes a Platform for Mosquito Comparative Genomics, October 2010

Resources: Several web sites are utilized in this activity.

BBC News http://news.bbc.co.uk/2/hi/health/8237529

ABC News www.abcnews.go.com/Health/video/dengue-fever-outbreak-risk-mosquito-10730699

U.S. Centers for Disease Control www.cdc.gov/dengue

Wikipedia www.wikipedia.org

Thai News www.dailymotion.com/video/xhqq06_dengue-fever-vaccine-developed-in-thailand_news