Who is Wolbachia?
Wolbachia as an Emerging Pathogen

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Abstract:

I strongly believe that educating people is very important to fight emerging pathogens in our world. This module will help students to understand the nature of pathogens and how they evolve due to changes on our planet. This will also give us an opportunity to educate parents on emerging pathogens through pupil. By creating laboratory experience and hands-on activities, students will be equipped with the knowledge and skills required to understand the threats posed by the emerging pathogens. It is important to create human work force to fight future diseases and create a better world. This module will help students to acquire basic skills of biotechnology to understand and study pathogens.

Mission Statement

My goal is to introduce students to various biotechnology techniques used to study the nature of different pathogens which cause numerous diseases in our society. This will provide students with the opportunity to have a better understanding on emerging pathogens and how they cause diseases in human population; and also help students to understand how these pathogens evolve into more dangerous species in the future.

Description of Module

Students will do hands on activity to study how bacteria interact with other organisms in its environment including Humans. This module will help students learn the skills required to isolate and study the pathogens and understand their evolutionary patterns.

This module includes a series of labs and use of bioinformatics techniques like extraction of DNA, PCR, Gel electrophoresis, DNA sequencing, and constructing phylogenic tree.

Outcomes:

-Students will be able to learn the basic biotechnology skills and appreciate the way science understand the nature and understand the process of inquiry; and discovery based research.

-will isolate DNA and learn about DNA as a diagnostic tool to discover pathogens. Pupil will understand that DNA contains genes and how to use this information to identify the unknown DNA.

-will amplify the isolated DNA using Polymerase Chain Reaction technique and study how DNA samples separate based upon different sizes using gel electrophoresis.

-will sequence the DNA using bioinformatics techniques and study their phylogenic tree using INSDC.ORG.

Expertise of Principal Instructor:

PI has all the expertise and skills to guide students through this concept. PI has science background and 11 years of teaching experience in Biology in all levels. He has MS in Biology and a degree in teaching.
He is well trained in all Biotechnology modules including Immunotechniques, PCR and Protein purification techniques. He is IB and AP Biology trained. PI also has an Advanced Diploma in Bioinformatics.

Literature Cited:

1. Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32611, USA. ajey@gnv.ifas.ufl.edu

PMID: 10971717 [PubMed - indexed for MEDLINE]

2. Wolbachia, normally a symbiont of Drosophila, can be virulent, causing degeneration and early death
Kyung-Tai Min and Seymour Benzer
Proc Natl Acad Sci U S A. 1997 September 30; 94(20): 10792–10796.
PMCID: PMC23488

3. Ong PCR improves Wolbachia DNA amplification: wsp sequences found in 76% of sixty-three arthropod species.

Authors: Jeyaprakash, A.; Hoy, M. A.[1]

Source: Insect Molecular Biology, Volume 9, Number 4, August 2000, pp. 393-405(13)

Publisher: Blackwell Publishing

4. Xiao-Yue Hong, Tetsuo Gotoh and Hiroaki Noda: “Sensitivity comparison of PCR primers for detecting Wolbachia in spider mites” Applied Entomology and Zoology Vol. 37, pp.379-383 (2002).

5. No evidence of Wolbachia among Great Lakes area populations of Daphnia pulex (Crustacea: Cladocera**)**
Jay M. Fitzsimmons and David J. Innes*

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Budget:

DNA Isolation Kit	130.00
Primers	40.00
PCR reagents	20.00

Theme: Emerging Pathogens

Lesson Title	**Who is Wolbachia**
Grade Span	**11-12**
Content Emphasis (Mathematics or Science)	**Science**
Targeted Benchmark(s)	Standard 1: The student understands the competitive, interdependent, cyclic nature of living things in the environment. (SC.G.1.4) Benchmark SC.F.2.4.3: understands the mechanisms of change (e.g., mutation and natural selection) that lead to adaptations in a species and their ability to survive naturally in changing conditions and to increase species diversity. Benchmark SC.H.3.4.2: knows that technological problems often create a demand for new scientific knowledge and that new technologies make it possible for scientists to extend their research in a way that advances science
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Lesson Preparation

Learning goals: What will students be able to do as the result of this lesson?

Students will be able to :

1. learn the basic biotechnology skills and appreciate the way science understand the nature
2. understand the process of inquiry; and discovery based research.
3. will isolate DNA and learn about DNA as a diagnostic tool to discover pathogens. Pupil will understand that DNA contains genes and how to use this information to identify the unknown DNA.
4. will amplify the isolated DNA using Polymerase Chain Reaction technique and study how DNA samples separate based upon different sizes using gel electrophoresis.
5. will sequence the DNA using bioinformatics techniques and study their phylogenic tree using INSDC.ORG.

Estimated time: Please indicate whether this is a stand-alone lesson or a series of lessons.

This module includes a series of labs and use of bioinformatics techniques like extraction of DNA, PCR, Gel electrophoresis, DNA sequencing, and constructing phylogenic tree.

Periods: 4 (each 90 minutes) and One 60 minutes period

Note: 2.5 hour cycling period is needed outside of class time

Materials/Resources: Please list any materials or resources related to this lesson.

Thermal cycler	1	
Yellow tips	1 box	For teacher use with P200 (20-200ul pipet)
Large filter white tips	1 box	For teacher use with P1000 (200-1000ul pipet)
Medium filter white tips	10 boxes x 96 tips per box	For student use with P20 (2-20ul pipet)
P20 (2-20ul pipet)	9	8 student workstations plus teacher demo
P200 (20-200ul	1	Teacher use for aliquoting

pipet)		
P1000 (200-1000ul pipet)	1	Teacher use for aliquoting
Microcentrifuge	1	Teacher/shared workstation
Mini-sub cell GT electrophoresis box	4	Two student groups can utilize the same gel Casting tray and combs are included for teacher/student generated gels (ends of tray need to be taped prior to pouring gel)
PowerPac Basic	1	Four gel boxes can be run with one power supply
OR		
E-Gel Power Supply	8	8 student workstations
E-Gel Transilluminator	1	Teacher/shared workstation
Microcentrifuge tube rack	1	Teacher use for aliquoting
White light boxes	8	8 student workstations (*If staining with FastBlast)
UV lights	8	8 student workstations (*If using EtBr in agarose gels)

E-Gel 1.2% agarose with Sybr Safe	8	8 student workstations
ReadyAgarose Mini Gel	10	1% agarose/1XTAE, 8 well per gel with ethidium bromide
ReadyAgarose Mini Gel	10	1% agarose/1XTAE, 2 x 8 well per gel
50X TAE Buffer	100 ml	Dilute to 1X for use (1XTAE can be reused for multiple classes)
Fast Blast DNA Stain (500X)	100ml	Dilute prior to staining (Can be reused for multiple classes)
Agarose	5g	
Practice pipetting stations	8	20 agarose petri dishes (can be reused for multiple classes) Practice loading dye (4 colors, 10ml each color) Microfuge tubes for aliquoting dye
Microcentrifuge tube holders	16	Student stations: to hold DNA samples and/or practice loading dye

***Above material will be borrowed from CPET**

-Scissors
-Computers
-Markers
-Gloves
-Aprons
-Water bath

<u>Teacher Preparation:</u> What do you need to do to prepare for this lesson?

- Collecting samples from different locations.
-Designing Primers for PCR
-Gel casting
- Preparing the reagents
- Preparing PCR master mix
- Lab set up

Lesson Procedure and Evaluation

<u>Introduction:</u> Describe how you will make connections to prior knowledge and experiences

and how you will uncover misconceptions.

Introduce students to the PCR laboratory by going over the scenario for the experiment. Define PCR and explain how PCR can be used to detect organisms (e.g. PCR will be used to make many copies of a DNA sequence that is found in many organisms etc.).

Discuss the following questions with the students:

1. What components or "ingredients" are necessary to perform PCR?

2. What are "primers" and why are they needed for PCR?

3. How do primers "know" where to attach to DNA?

For: A great PCR tutorial and game for students….

Visit: http://nobelprize.org/educational_games/chemistry/pcr/

Exploration: **Describe in detail the activity or investigation the students will be engaged in and how you will facilitate the inquiry process to lead to student-developed conclusions**

Day One – construct a DNA template

- Have students construct a DNA template. They can decide how long they want the template to be (ex. 10 base pair or 15 base pair…) See DNA handout.

- Have each student complete a DNA review worksheet.

http://www.scienceteacherprogram.org/biology/RomeroReviewSheet06.html

- After completion of the worksheet students will view a PCR animation at http://www.dnai.org/b/index.html.

- Assign students to read an article on PCR as a homework assignment. Have students generate questions from what they read. You can select an article from the following web sites:

http://sunsite.berkeley.edu/PCR/whatisPCR.html
http://people.ku.edu/~jbrown/pcr.html
http://www.swbic.org/links/6.1.1.2.php
http://www.genome.gov/10000207
http://www.uq.edu.au/vdu/PDU_PCR.htm
http://lifesciences.asu.edu/resources/mamajis/pcr/pcr.html

Ref:
http://www.scienceteacherprogram.org/biology/Romero06.ht
Day Two – (90 minutes)
- Introduction and background material
. DNA ISOLATION LAB
(USE DNA ISOLATION KIT)
Day Three --- (60 minutes)
PCR Lab PART 1
Set up PCR reactions (50 min)
• Run PCR reactions (~ 3 hr)–typically overnight
Day Four ----- (90 minutes)
PCR Lab PART 2
Electrophoresis of DNA and staining of gels (50 min)
Analysis of results (40 min)

Day Five ---- (90 minutes)
. DNA Sequence Alignments and Phylogenetic Tree
Worksheets are attached
Ref: http://serc.carleton.edu/microbelife/k12/bioinformatics/index.html

Application: **Describe how students will be able to apply what they have learned to other situations.**

The intent of this lesson is to guide students through the thought process involved in a laboratory-based scientific

investigation. Students will learn about PCR, gel electrophoresis,DNA sequencing, and constructing phylogenetic tree while asking the question "How can a tiny amount of genetic material (DNA) be used to identify genotype of the organism.

The students' focus is not so much on the answer or result, but on how the result was obtained and substantiated by careful observation and analysis of their data. Students who engage in Biotechnology activities develop a positive sense of their ability to employ the scientific method to solve problems. Student involvement in this process results in an increased understanding of the value of approaching a scientific challenge in an organized and logical fashion.

Ref: Bio rad CrimScene investigation manual

Assessment: Describe how student knowledge is being assessed at the appropriate cognitive level for the targeted benchmarks.

Assessment worksheets are enclosed

Ref:

1. DNA Review worksheet

http://www.scienceteacherprogram.org/biology/RomeroReviewSheet06.html

2. Assign students to read an article on PCR as a homework assignment. Have students generate questions from what they read. You can select an article from the following web sites:

   http://sunsite.berkeley.edu/PCR/whatisPCR.html
   http://people.ku.edu/~jbrown/pcr.html
   http://www.swbic.org/links/6.1.1.2.php
   http://www.genome.gov/10000207
   http://www.uq.edu.au/vdu/PDU_PCR.htm
   http://lifesciences.asu.edu/resources/mamajis/pcr/pcr.html

3. Bio informatics assessment

Ref: http://serc.carleton.edu/microbelife/k12/bioinformatics/index.html

<u>Teacher Self-Reflection:</u> Record your thoughts on the lesson and describe any modifications you would recommend based on the outcomes.

***This could be filled after the actual lesson/lab.**

DNA Isolation Assessment worksheet

Please answer the following questions:

1) Briefly outline the steps required to isolate DNA, taking the cellular structures into consideration.

2) What are the cellular structures and elements that serve as a barrier to getting your DNA our of the cell and intact?

3) After completion of DNA extraction, what did the DNA look like?

4) Research scientists who are trying to determine the identification of a new flu virus would perform a DNA extraction from human tissues. Will the method used to isolate DNA be similar or different from the method you used to isolate insect and *Wolbachia* DNA?

5) What is the purpose of isolating the DNA? What is your next step in determining the frequency of *Wolbachia*?

BACKGROUND INFORMATION

What is *Wolbachia*?

Wolbachia is a genus of bacteria that infect arthropods. The symptoms of infection vary between host species, but include a skewing of the sex ratio of offspring from infected organisms toward females. Although the extent of the infection of arthropods by Wolbachia is unknown, it has been found infecting a wide variety of organisms and in a wide range of geographic areas.

During the lab , students will identify arthropod samples or bring samples from their local insect fauna. We will use molecular methods (PCR) to detect *Wolbachia* infections in the collected specimens. We will then sequence the infecting Wolbachia 16S rDNA amplified by PCR and conduct bioinformatic analyses. Using these equences, we will construct a phylogenic tree of the bacteria.

What is DNA and what does it do?

Deoxyribonucleic acid (DNA) is a molecule present in all living things, including bacteria, plants, and animals. DNA carries genetic information that is inherited, or passed down from parents to offspring. It is sometimes referred to as a biological "blueprint" because it determines all of an individual's physical features such as hair, eye, and skin color, height, shape of facial features, blood type, and countless others. Your DNA blueprint is a combination of your mother's DNA (from her egg) and your father's DNA (from his sperm) during conception.

DNA contains four chemical units, referred to by the first letters in their names: **A**(adenine), **G**(guanine), **T**(thymine), and **C**(cytosine). These four letters make up a code for genetic information. The letters of the DNA code function like letters of our alphabet. The 26 letters in the English alphabet spell words, which can be arranged in infinite ways to create messages and information. Similarly, the 4 chemical letters of DNA are organized to make messages that can be understood by cells, called **genes**. These genes contain the information to make **proteins**, which are the basis for almost all of a body's and cell's structures and functions.

Your DNA sequence is the particular arrangement or order of the chemical letters within your complete DNA collection, or **genome**. Scientists have determined that human DNA sequences are 99.9% identical. It is the <0.1% sequence variation from person to person that makes each of us unique.

Where is DNA found?

With only a few exceptions, DNA is found within practically every cell of an organism's body. In our cells, a compartment of the cell called the **nucleus** contains the DNA. Every time a cell divides (for growth, repair, or reproduction) the DNA within the cell's nucleus is copied and then coiled tightly into **chromosomes**. The human genetic blueprint is organized into 46 chromosomes, which contain approximately 40,000 genes that provide the instructions for constructing the human body.

What does DNA look like?

At the molecular level, DNA looks like a twisted ladder or a spiral staircase. The ladder actually

contains two strands of DNA, with pairs of the chemical letters **A**, **G**, **T**, and **C** forming the rungs. This structure is called a DNA **double helix** because of the spiral, or helical form made by the two DNA strands. Each strand of DNA is very long and thin and is coiled very tightly to make it fit into the cell's nucleus. If all 46 chromosomes from a human cell were uncoiled and placed end to end, the DNA would be 2 meters long — but only 2 nanometers (2 billionths of a meter) wide.

How can we make DNA visible?
We can see our DNA by collecting cells, breaking them open, and condensing the DNA from all of the cells together. Think of the long, thin DNA molecules as thin white threads. If the threads were stretched across a room they would be difficult to see, but piled all together on the floor they would be visible. This laboratory activity uses detergent and enzymes to break open cells collected from students' cheeks and release the DNA from within them. Salt and cold alcohol are then added to make the DNA come out of solution, or **precipitate**, into a mass that is big enough to see.

Extraction and Precipitation of DNA: How Does It Work?
Students will start this activity by gently chewing the insides of their cheeks to loosen cells from the inside of their mouth then rinsing their mouths with water to collect the cells. Lysis buffer is then added to the solution of cells. The lysis buffer contains a detergent that breaks apart the phospholipid cell membrane and nuclear membranes, allowing the DNA to be released. It also contains a buffering agent to maintain the pH of the solution so that the DNA stays stable.

Protease, an enzyme that digests proteins, is added to remove proteins bound to the DNA and to destroy cellular enzymes that would digest the DNA. This insures that you maximize the amount of intact DNA that is extracted. The cell extract containing protease is incubated at 50°C, the optimum temperature for protease activity.

DNA and other cellular components, such as fats, sugars, and proteins, dissolve in the lysis buffer. DNA has a negative electrical charge due to the phosphate groups on the DNA backbone, and the electrical charge makes it soluble. When salt is added to the sample, the positively charged sodium ions of the salt are attracted to the negative charges of the DNA, neutralizing the electrical charge of the DNA. This allows the DNA molecules to come together instead of repelling each other. The addition of the cold alcohol precipitates the DNA since it is insoluble in high salt and alcohol. The DNA precipitate starts to form visibly as fine white strands at the alcohol layer boundary, while the other cellular substances
remain in solution.

PCR LAB OVERVIEW
Most DNA analysis situations require fairly large amounts of DNA. Usually the amount in a few cells is not enough to fully analyze. A method called the polymerase chain reaction (PCR) has been developed to make many copies of DNA in a sample. PCR is essentially the microscope of the 21st century as it allows biologists to study the DNA of microorganisms that we cannot see by either eye or culture. It is revolutionizing research in microbial diversity, genetic disease diagnosis, forensic medicine, and evolution. In this portion of the lab series, you will use your samples from the DNA Extraction Lab to decipher if *Wolbachia* symbionts are present within your species. Your work could be new to science and potentially lead to new discoveries on the presence and absence of *Wolbachia* in Arthropods.
As in the previous lab, students should work in groups of two. Primers to specifically amplify a 438bp fragment of the 16S ribosomal RNA gene (ubiquitous in all *Wolbachia*) are WSPEC-F (5'-

CATACCTATTCGAAGGGATAG-3') and WSPEC-R (5'-AGCTTCGAGTGAAACCAATTC-3'). Primers to amplify a 658bp fragment of the CO1 cytochrome oxidase gene (ubiquitous in arthropod mitochondria) are LCO1490 (5'GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'TAAACTTCAGGGTGACCAAAAAATCA-3')

Introduction to Electrophoresis

Electrophoresis is a method of separating substances based on the rate of movement while under the influence of an electric field. Agarose is a polysaccharide purified from seaweed. An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. The result is a flexible gelatin-like slab. During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode. The DNA to be analyzed is pulled through the pores of the gel by the electrical current. Under an electrical field, DNA will move to the positive electrode (red) and away from the negative electrode (black). Several factors influence how fast the DNA moves, including; the strength of the electrical field, the concentration of agarose, and most importantly, the size of the DNA molecules. Smaller DNA molecules move through the agarose faster than larger molecules. DNA itself is not visible within an agarose gel. The DNA will be visualized by the use of a dye that binds to DNA.

Introduction to Bioinformatics

This exercise represents two interrelated modules designed to introduce the student to modern biological techniques in the area of Bioinformatics. Bioinformatics is the application of computer technology to the management of biological information. The need for Bioinformatics has arisen from the recent explosion of publicly available genomic information, such as that resulting from the Human Genome Project. To address this, the National Center for Biotechnology Information (NCBI) was established in 1988 as a national resource for molecular biology information. The NCBI creates public-access databases, develops software tools for analyzing genome data, and disseminates biomedical information - all for the better understanding of molecular processes affecting human health and disease. The NCBI is a virtual goldmine both in terms of available resources, and treasures yet to be discovered. We will investigate the GenBank DNA sequence database, which is responsible for organizing millions of nucleotide sequence records.

Online Resources: There are a number of online, educational resources devoted to learning bioinformatics. For details that summarize what we will cover in this exercise and more, see:

- BLAST for beginners (Helps the learner with a slide show; we will use this one!): http://www.geospiza.com/outreach/BLAST/index.html
- Similarity search (Summarizes the basic concepts and vocabulary of BLAST) http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/similarity.html
- NCBI Education (Provides educational tutorials, software, and mini-courses): http://www.ncbi.nlm.nih.gov/Education/index.html

Significance and Supplies Needed: By completing this lab, you will be exposed to the tools and databases currently used by researchers in molecular and evolutionary biology, and you will gain a better understanding of gene analysis, taxonomy, and evolution. While no computer programming skills are necessary to complete the modules in this work, prior exposure to personal computers and the Internet will be assumed. The main program that you will need is an Internet browser, such as Netscape Navigator or Internet Explorer.

Resources/References:

http://cndls.georgetown.edu/applications/posterTool/index.cfm?fuseaction=poster.display&posterID=3065

http://ctbiobus.org/download/electronicpcr.pdf

http://www.scienceteacherprogram.org/biology/Romero06.html

http://www.scienceteacherprogram.org/biology/Romero06.html

http://www.mbl.edu/education/courses/other_programs/pdf/dmw_application.pdf

http://discover.mbl.edu/labs.htm

http://www.bio-rad.com/cmc_upload/Literature/54133/4110034B.pdf