"Biotechnological Applications to Emerging Pathogens"

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Abstract:
The growing awareness of emerging pathogens, coupled with the advancements in biotechnology research has created a demand for qualified and motivated students and future scientists. In order to bridge the gap from a high school student sitting in an 11th grade biology class to an adult scientist gathering scientific data or developing a cure for a disease requires exposure of those students to the latest information and techniques in biotechnology. By creating rigorous and relevant laboratory experiences, students will become equipped with the tools necessary to explore their desire to advance in the sciences and be aware of the real world applications of biotechnology. Such relevant skills to be practiced include micropipette use, gel electrophoresis, Southern blotting, clean room technique, transformation of bacteria, ELISA, and crystallization of proteins. In addition, field trips to research and industrial biotechnology sites will provide students the opportunity to see these various techniques in practice.

Mission Statement:
It is one thing to learn a concept or technique in theory, but another thing altogether to actually perform a task for oneself. Oftentimes, students memorize what occurs during gel electrophoresis or PCR, but if you put a gel box in front of them, they would not know where to begin. With the field of biotechnology having application in numerous disciplines, becoming proficient in basic biotechnology techniques will be a valuable tool for all students who plan on pursuing any career in the sciences. By designing a progressive unit, students will not only learn about the history and theory of biotechnology, but will be engaged in valuable hands on experiences that will make this exciting field more relevant. The umbrella of "emerging pathogens" provides an avenue for dialogue and specific applications of such techniques. Since the subject of emerging pathogens is ever developing and changing, this will provide a dynamic foundation that will capture the attention of students and bring relevance to the techniques that are explored.

Description of Teaching Unit:
This teaching unit will be implemented in Advanced Placement Biology. This course currently has a unit on DNA/genetics and DNA technology. However, the specific techniques associated with biotechnology have only been discussed in theory rather than in practice. The primary purpose of this unit will be to supplement the aforementioned theory with hands-on biotechnology techniques, using the umbrella of emerging pathogens as the arena for this exploration. To accomplish this goal, the unit of *"Biotechnological Applications to Emerging Pathogens"* will have the following components:

- *1. Lecture notes on related chapters from the textbook.*
    - Will provide a foundation of biotechnology theory.
    - Expected Outcomes: Students will be familiar with biotechnology history, terminology, techniques and applications.
- *2. Micropipette Use.*

    - Nearly every biotechnology technique utilizes micropipettes. Being familiar, comfortable and proficient with this tool is an invaluable skill in becoming successful in the activities which will be performed. Specific skills to be practiced:
        - Attaching/detaching disposable tips.
        - Adjusting volume.
        - Using micropipette to transfer solutions.
    - In addition to acquiring the manual skill of using the micropipette, this is also an excellent opportunity to review over the metric system, making conversions, precision and accuracy.
    - Expected Outcome: Students will be able to efficiently use micropipettes.
- *3. Gel Electrophoresis and Southern Blotting*
    - Test will use Dr. Lawrence's protocol: "The High Rise Killer – DNA Fingerprint" to introduce students to the technique of gel electrophoresis and Southern Blotting.
    - Expected Outcomes: Students will be able to perform gel electrophoresis and Southern Blotting and understand their applications.
- 4. *Field trip to UF. (To be arranged with Erin Kelso).*
    - Anticipated activities:
        - Clean room technique.
        - Transformation of bacteria (mechanism for adaptation and possible emergence as a pathogen).
        - Campus tour.
        - Tour of researchers' lab(s).
    - Expected Outcomes: Students will perform bacterial transformation. Students will be exposed to the research setting of the University and the campus.

The previously discussed activities will specifically be associated with the chapters from the AP Biology textbook devoted to DNA technology. However, to make biotechnology more relevant, additional techniques and activities will be implemented in other locations throughout the curriculum in order to illustrate the application of biotechnology in a variety of different instances. For example:

- 1. ELISA Allergy Test (Protocol by Dr. Lawrence).
    - To be conducted during unit devoted to immune system.
- 2. Possible field trip to Progress Corporate Park for lab activities and tour of industrial biotech facilities.
    - To be conducted as examples of careers in science.

Expertise of the Principal Instructor:

- AP Biology and AP Environmental Science instructor for the past 4 years
- Science Department Chair at Santa Fe High School
- Selected "Teacher of the Year" at Santa Fe High School, 2007-2008
- Attended 2008 ICORE Emerging Pathogens Workshop hosted by UF's CPET

Literature Cited:

Campbell, N., Reece, J., 2005. *Biology, 7th Edition*. San Francisco, Benjamin Cummings.

Woolhouse, M., Gowtage-Sequeria, S., "Host Range and Emerging and Reemerging Pathogens". *Emerging Infectious Diseases*, December, 2005.

Protocol for “The High Rise Killer – DNA Fingerprint”. From Dr. Charles Lawrence.

Protocol for “ELISA Allergy Test”. From Dr. Charles Lawrence.

Protocol for “Crystallization of Proteins”. From Doctors Robert and Mavis McKenna.

Budget and Budget Justification:

The grant will be allocated for purchasing materials needed for the lab activities conducted in the classroom (specifics to follow with lesson plans). In order to successfully implement the stated goals, the students must have the required materials. Since our school already has micropipettes and all equipment needed for gel electrophoresis, the money would be best spent on consumable materials that can be utilized for hands-on lab activities. Also, field trip transportation cost of visiting the Progress Corporate Park.

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Lesson Plan Title: "Developing Micropipet Technique"
Subject: 9th Grade Biology Honors

Developing Micropipet Technique

Oftentimes, procedures in biotechnology use very small volumes of DNA and reagents. Dispensing these volumes require the use of adjustable micropipets. Micropipets come in many different models and volume ranges…as little as one microliter (ul) -- a millionth of a liter!

Metric Conversions Involving Small Volumes:

Familiarize yourself with metric units of measurements and their conversions. We will use the volume measurement (base unit: liter) but the prefixes we learn would also apply to mass (base unit: gram) or linear measurement (base unit: meter). The two most prevalent units of liquid measurement in molecular biology are the milliliter (mL) and the microliter (ul).

1 mL = 0.001 liter or 1/1,000 liter 1,000 mL = 1 liter

1 ul = 0.000001 liter or 1/1,000,000 liter 1,000,000 ul = 1 liter

1 ul = 0.001 mL or 1/1,000mL 1,000 ul = 1 ml

Proper Uses: How to Use a Micropipette

1. Select the correct size micropipet and tips.

2. Dial the volume adjustment knob to set the proper volume.

3. Place the tip securely on the micropipet.

4. Hold the micropipet vertically over the solution and push the plunger down to the first stop.

5. Insert the tip into the solution.

6. Slowly release the plunger and note that the solution is drawn into the tip (DO NOT ALLOW PLUNGER TO "SNAP BACK" UP).

7. Look at the tip to be sure that you do not have bubbles in the tip. (If bubbles exist, expel the solution and try again.)

8. Dispense the solution touching the tip to the side of the target container. Slowly depress the plunger to the second stop. Before releasing the plunger, remove tip from target container.

9. Be sure the tip is empty, then use the tip ejector to dispose of the tip into an appropriate disposal container.

Note: You must use a fresh tip for every transfer or you will contaminate your solutions!

Lab Details

Student Objectives

1. Become familiar with the function of a micropipet.
2. Practice using the micropipette as a tool for dispensing minute volumes.
3. Develop their sense of metric scale as they deal with very small volumes.
4. Develop confidence in using the tool, so that later explorations in biotechnology will be simpler.

Materials Needed:

a. 100mL beaker filled with water and red food coloring (to make the solution easier to see).
b. 2 microcentrifuge tubes per student (label them “1” and “2”).
c. 20-200uL micropipet
d. Disposable micropipet tips

Procedure:

a. Using the instructions on the previous page, students will be transferring colored water from the solution in the beaker into the “1” microcentrifuge tube. The following volumes should be added to microcentrifuge tube “1”:
    a. 25ul
    b. 150ul
    c. 75ul
    d. 200ul
    e. 50ul

b. After the previous volumes have been added to microcentrifuge tube “1”, students will be transferring the solution from tube “1” to microcentrifuge tube “2”. The following volumes should be removed from tube “1” and placed into tube “2”:
    a. 125ul
    b. 35ul
    c. 70ul
    d. 220ul
    e. 50 ul

*By conducting the above procedure, students should have transferred a total of 500ul into microcentrifuge tube “1”. Next, they should have removed a total of 500ul from tube “1” and transferred it to microcentrifuge tube “2”. If they followed the instructions and measured accurately/precisely, then there should be no solution remaining in tube “1”.

*This activity should take around 10-15 minutes per student (depending on their proficiency of using the micropipettes).

*A suggested assessment would be to have students complete a similar exercise for time. Items assessed would include use of micropipette (dispose of olds tips, correctly adjust volume, etc.) as well as length of time required to complete the task.

Standards Addressed:

SC.912.N.1.1	
Benchmark Description:	Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following: ***1. use tools to gather, analyze, and interpret data (this includes the use of measurement in metric and other systems, and also the generation and interpretation of graphical representations of data, including data tables and graphs),***

References:

Daugherty, Ellyn. Biotechnology – Science for the New Millenium, Saint Paul, MN. Paradigm Publishing. 2007.