Northeast High School AP Biology: Creating Relevance in the Biotechnology Lab

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Abstract:
During my time at ICORE it became apparent that the PCR and real time PCR are an integral part of every academic, industrial and hospital lab. My goal is to incorporate PCR into my AP electrophoreses biotech lab. I have chosen to use the Tomato Spotted Wilt Viruses and peanut plant as the basis for this lab.

Students will learn through this lab how to perform PCR and electrophoresis and that plants are affected by viral pathogens as well as humans. The students Northeast serves are from an urban area and have little knowledge of the agriculture that produces the food they eat. A farmer seeking help with diseased crops will help them understand a relevant application of biotechnology and research science and that it is an important component of agriculture and vital for the worlds food supply.

Mission Statement:
To develop a purposeful Biotechnology lab that not only meets but exceeds the required electrophoresis component of the College Board AP Biology Lab #6.

Description of Teaching Unit:
Lecture on TSWV to include summation of history of its emergence and effect on Florida tomato and peanut crops. Overview of the virus, thrips its insect vector and research efforts to create a genetically engineered variety of peanut plants that will be resistant to the pathogens.

Field trip to UF Agricultural Ext Facility in Davie, FL

- Students will visit the UF facility to learn the impact of TSWV on Florida crops and see what measures can be taken to minimize its impact.

Visual and Quick testing of TWWV in peanut plants.

- Student will perform ICORE Immunostrip Assay Lab #1 following the Student Manual to identify TSWV in a farmer's peanut field. Testing will allow students to understand the testing for viral proteins within plant material is a more definitive process than visual identification.

Students will then follow ICORE scenario to help a farmer identify genetically engineered peanut seeds sent for research in his field from his commercial peanut seeds.

- The purpose of this lab is for student to perform DNA extraction from peanut seeds Lesson #2, use PCR to amplify the extracted DNA Lesson #3 in order to obtain enough to DNA to perform Lesson #4 which is gel electrophoresis to identify which seed has had the viral DNA inserted in hopes of giving the plant grown for this seed resistance to the effects of TSWV.

Post lab discussion will include presentation for and against the allowing of genetically engineered plants for commercial crop production.

Extension activity: students will meet with Oakland Park Horticulturist Charles Levo to create an informational brochure that will hopefully be shared with community gardeners at one of the cities monthly lecture series.

Resources required for this lab are:

- The ICORE Student lab manual by Julie Bokor
- Agdia ImmunoStrip Test kit from www.agdia.com
- Peanut plants and seeds supplied by Dr. Gallo, UF
- ICORE PCR lab locker

Indication of Expertise of PI:

BS in Biology from Springfield College
3yrs industrial experience as lab tech
1yr pharmacy tech experience
12 yrs Biology, H Biology & H Earth Space teaching experience
3yrs AP Biology Teaching experience
ICORE Biotech and AP Biology Collage Board training

Literature References:

ICORE Investigating TSWV: Can we stop it?
Biology Lab Manuel The College Board Advanced Placement Program
aspnet.org Plant Disease Lesson
plantpath.ifas.ufl.edu/takextpub/factsheets/ciro914.pdf

Budget:

Agdia ImmunoStrip Tests $105. 00
County School Buss for Field Trip $80
UF PCR locker $0
Knowledge & experience for students PRICELESS!

ICORE Lesson Plan
Marianne Snow

Title: Biotechnology at Work – Testing for presence of pathogens and genetically altered food.

Subject: Biology, Biotechnology, And Environmental Science

Grade: 10-12 Honors/AP Students

Overview: AP Biology Lab #6 Molecular Biology - alt.

Time Estimate: 3-part lab activity will require 3 days of 90 min block schedule

Key Questions: Why would we want to change a plant?

How are pathogens transmitted from one infected organism to another?

How are we able to genetically alter a one species by inserting DNA from another organism?

Student Learning Objectives:

Students will be able to:

1. Recognize that plants as well as animals are subject to disease?
2. Understand that viruses as well as other microorganisms can be pathogenic.
3. Use standard laboratory techniques to:
   - test for the presence of viral proteins
   - understand how restriction enzymes are used to cut and amplification DNA - demonstrate how gel electrophoresis separates DNA fragments by length

Benchmarks:
SC.912.L.16.6 Discuss the mechanisms for regulation of gene expression in prokaryotes and eukaryotes at transcription and translation level.

SC.912.L.16.7 Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.

SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.

SC.912.L.16.12 Describe how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).

Vocabulary:
Immunoassay- biochemical test that detects the presence of a protein in a biological substance by using the reaction of an antigen to its antibody

Gel electrophoreses- method of separating DNA fragments according to length

Pathogen – agent that infects an organ and causes disease

PCR- polymerase chain reaction. A method that rapidly generates many copies of a specific DNA fragments.

Restriction Enzyme – type of enzyme that cuts specific base sequences in DNA

Thrips – small insect vector of Tospoviruses that feeds on plant flowers and vegetation

TSWV- tomato spotted wilt virus, negative, single stranded RNA plant pathogen virus in the genus Tospovius

Vector- an insect or other animal that carries a pathogen between hosts.
- a plasmid used in genetic engineering to insert a fragment of DNA into a new genome

virus- noncellular infectious particle consists of DNA or RNA, a protein coat and, in some an outer lipid envelope: it can be replicated only after its genetic material enters a hast cell and subverts the host's metabolic machinery.

Lesson Summary:
This lesson will consist of a three-part lab conducted in two parts.
First students will be prepared with lecture and discussion on disease caused by viral pathogens and their effect on agriculture and food crops. Students will then observe peanut plant root, stems and leaves for the presence of a viral infection. The students will then perform an immunoassay test on the plant material for the presence of TSWV. Secondly, students will be given peanut seeds from a farmer who needs to identify which of his mixed-up seed bags contain a genetically engineered Varity he was to test in his fields. Lecture and discussion of genetically angering procedures will be followed by PCR and gel electrophoreses to determine a match to a know sample of engineered peanuts.

Background Information:
Viruses are very small organisms that consist of a nucleic acid that contains their genetic information surrounded by a protein capsid. Viruses are incapable of reproducing without a hosts cell and are therefore considered obligate parasites. Once a virus is able to enter a specific cell it will disable the host DNA's direction of activities and use host ribosome's to reproduce viral nucleic acids and protein coats.
Since viral infections disrupt the normal function of its host cells they are **pathogenic** and cause diseases that can range from minor irritation to death. Although we are most familiar with human viral pathogens such as those that cause influenza, AIDS, polio, encephalitis, small pox and yellow fever there are also viruses that attack plants.
The modern methods of agriculture that rely on production of large amounts of a single crop at one time have become vulnerable to the adverse effects of a single **pathogen.** Plant viruses can enter the host plant through wounds, during pollen transmitting or by vectors. The most common vectors for plants pathogens are insects. **TSWV** is transmitted from an infected plant to a healthy plant when insects called **thrips** feed on the infected plant and then a healthy plant.

The **TSWV pathogen** though named for the tomato plant it was originally found in infects over 800 different species of plants including the economically important peanut crop. Plants infected by the virus may have stunted growth and marred fruits and can cause low yields of food crops. **Thrips** are not effectively controlled by insecticides so it is important to educate farmers on effective planting and harvesting of crops to minimize the economic losses to **TSWV** and the development of genetically breed plants with resistance to the disease caused by the virus. Early detection of the presence of **TSWV** virus also becomes important in the field in order to prevent its spread by **thrips.** Since visual detection of the virus is difficult, as it can range from asymptomatic to spotting on leaves and fruits to severe stunting of growth a biochemical test for the viruses presence is important. An **immunoassay** strip that detects the presence of **TSWV** proteins can easily be use in the lab or even in the field to determine the presence of this pathogen in plant material.

The development of genetically engineered crop plants has met with opposition from some groups preventing its wide acceptance of food production. It is therefore necessary for agricultural to carefully test these crops before they will become widely accepted of human consumption. When tested genetically engineered crops must be separated from crops farmers grow for market. **PCR** and **gel electrophoresis** lab techniques can be utilized to identify if seeds such as peanuts have been genetically altered by adding a resistant gene into its genome with a plasmid **vector.** The DNA of the seed can be cut with a **restriction enzyme** into fragments. The amount of DNA can then be increased by amplification with **PCR.** These fragments can then be separated on an agrose gel with **electrophoresis** by running a charge through the gel from one side to the other. DNA fragments will travel through the gel because of the charged areas within their backbone that contains ionic phosphate groups on one end and separate according to size. Smaller pieces can travel farther that larger ones. Comparison of gels created with genetically engineered seeds will be different than non-altered DNA and can then be identified by comparison to other engineered DNA.

Advanced Preparations and materials:
Agdia ImmunoStripsTM Test Strips must be ordered from www.Agdia.com
Peanut plants and seeds will be provided by Dr Gallo at UF
PCR and electrophoresis equipment are required along with agrose and buffer.

Procedures:
The procedures from ICORE's Investigating Tomato Spotted Wilt Virus: Can We Stop It?
Lesson 1ImmunoStrip Assay Lab
Lesson 2 Genetic Engineering- DNA extractions
Lesson 3 Genetic Engineering-DNA amplification
Lesson 4 Genetic Engineering—Gel Electrophoresis

Assessments:
Students will write a report for Farmer John summarizing the steps taken to determine if TSWV is present in his crops and identify which bag of unlabeled seeds contain the genetically engineered peanut seeds.

Extension Activities:
Students can research the TSWV and the work of George Washington Carver on peanuts crops.

Discuss the advantages and disadvantages of modern large monoculture farming after a field trip to visit the local agricultural extension agency.

Recourses:

ICORE TSWV Lab by Julie Bokor at CPET,UF
Power point on TSWV Dr Gallo UF
AP Biology Campbell and Reece
The College Board Biology Lab Manual
Cpet ICORE PCR/Electrophori's lab Equipment locker