TITLE: Biotechnology in High School Chemistry and Biology

NAME AND CORRESPONDENCE:
David Frantz
Bayshore High School / Manatee County

ABSTRACT:
High School Chemistry curriculum typically includes the Unit: “Organic Chemistry and Nuclear Chemistry” at the end of the year (second semester), if at all. I propose to disturb this traditional order by placing a small “Biotech Unit” at the beginning of the first semester of the Chemistry and Biology classes I’m teaching. The “Biotech Unit” will initially review the basic structure of Sugars, Carbohydrates, Proteins and Nucleic Acids and their functionality. This unit is primarily defined by conducting a series of standard Biotechnology lab procedures from “Biotech In The Classroom (BTC).” “BTC” labs are based on diagnosing the presence of the Tomato Spotted Wilt Virus (TSWV) and include: The ImmunoStrip Assay test for TSWV, DNA extraction from plant cells, PCR of two plant samples and finally Gel Electrophoresis of amplified DNA.

RATIONAL:
Biotech has the potential to become one of Florida’s largest growing industries in the near future and it needs a trained workforce. Thus Biotech is extremely relevant to today’s chemistry student, yet it is not covered in the latest textbooks. Biotech is incomprehensible without a basic understanding of molecular structures and the forces that affect them. This Biotech Unit will revisit and review these molecular structures and the associated charges that are manipulated in Biotech to understand the sorting and molecular movement.

This lab intensive unit is placed at the beginning of the year to heighten the student awareness of the practical application of Biochemistry. In addition, this early exposure to the organic molecules can serve as a linking review of the prior year’s Biology course content.

DESCRIPTION OF THE TEACHING UNIT:
The primary component of the teaching unit is the “Biotech in The Classroom,” (BTC, Bokor, 2010) which is a series of biotech labs. The first lab in BTC is based on measuring the existence of TSWV in plant cell extract. The TSWV can infect many different cash crops including Tomato and Peanut. In Peanut, TSWV displays a wide array of symptoms that range from minor spotting on leaves to severe plant stunting and death due to severe root crown damage. However, peanuts can be asymptomatic, and still contain TSWV. Immunoassays of the peanut leaf and root can be tested for the presence of TSWV. The common type of immunoassay is an enzyme-linked immunosorbant assay (ELISA). This test assay turns a color when the enzyme and antibody bind to the antigen expressed on the virus. When the enzyme-antibody is placed on a test strip and this test

strip is placed in an assay from an infected leaf or root, a colored line appears. These immunosorbant test strips are widely used for diagnostics in other infectious diseases such as HIV and rapid strep test as well.

In the first lab, the student will collect immunoassays from the peanut plant leaves and simply test to see if the STWV is present in the plant tissues. The student must complete Pre-Lab focus questions concerning the theory and procedure as well as Post-Lab data and questions and a final written report of the labs findings to a fictitious farmer concerned about the crop.

The second lab is called, "Getting the DNA Out- DNA extraction. Extracted DNA can be used to create DNA fingerprints to help diagnose genetic diseases, solve criminal cases, identify victims of disaster and war and establish paternity or maternity.

In this lab the DNA is extracted from the plant cell to analyze the DNA. The cell wall, the cell membrane and the nuclear membrane must be broken to release the DNA. A physical micropestle is used to break down the stiff cell wall but the inner membranes must use heat (55*C) for 10 minutes to soften the membranes to release the DNA and then (95*C) for 3 minutes to destroy/denature the DNA chewing enzymes, DNase. Two seed extracts are obtained from two different samples and prepared as described and placed in a thermal control device called a thermocycle. After these two samples are heat-treated they will be used in the next experiment. The students have both Pre and Post – Lab questions.

The third lab is called Making Lots of Copies – DNA amplification. The treated sample extracts from experiment #2 are multiplied in this third experiment. The amplification process is called Polymerase Chain Reaction, (PCR). The process begins with heat (94*C) that splits the DNA into single strands exposing the nucleotide bases. The next step lowers the heat to (60*C) in order for the annealing process to start building new strands of DNA by first adding primers to either end of a small known sequence of DNA like book ends.

The fourth step is called extension, where Taq polymerase will extend the primer by bringing in complementary nucleotides as it moves along the template strand. All four nucleotides are free in solution and are added as Taq adds them to the template strand.

Inserting a specific gene sequence of DNA into a plant genome gives increased resistance to the TSWV. One method of determining whether this plant's genome contains the added gene is to perform this PCR and then separate the DNA through Gel electrophoresis. The students have Pre-Lab and Post –Lab questions to complete.

The fourth Lab is visualizing the DNA – Gel Electrophoresis. This final lab separates the multiplied DNA as it is moved by electrical charge through an agarose matrix using gel electrophoresis. DNA is negatively charged due to the negative charge associated with the phosphate groups on the DNA backbone and therefore, when placed in a matrix and electric field, travels toward the positive charge. As the two samples of DNA are pulled through the gel matrix , the shorter lengths travel farther and faster while the longer sections travel slower. The result is a ladder like separation of the different lengths of DNA. Three samples are drawn out side by side, so these ladder marks can be compared. One sample is the "marker" which has a series of base lengths, a gradation of increasing base lengths cut to which the plant DNA samples can be compared, including the resistance DNA sequence. The size of the inserted gene sequence is known, and thus the two plant samples can be compared to the marker lane and measured to see if either sample contains this resistance gene length of DNA.

DATA COLLECTION:
At the outset and the finish of the Biotech Unit the students will be given a pretest and posttest respectively, which cover the Biotech basics. The pre-tests will be graded but this assessment will only be used for comparison to the posttest that will count toward their semester grade.

LEARNING OBJECTIVES:
1) Lean to operate lab equipment:
- Master the use of the micropipette*
- Microcentrifuge
- Thermal cycler
- Mini-sub cell GT electrophoresis box
- E-Gel Power Supply
- E-Gel transilluminator
- Bio-Rad PAGE Gel Boxes
- White Light Boxes
- UV lights
- Hot blocks

2) The students performing the labs will touch on all of the following State / National Science Standards

Next Generation Science Standards Correlated to Experiments

Benchmark	Exp 1	Exp 2	Exp 3	Exp 4
SC.912.N.1.6: Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied	✓	✓		
SC.912.L.14.2: Relate structure to function for the components of plant and animal cells. Explain the role of cell membranes as a highly selective barrier.		✓		
SC.912.L.14.3: Compare and contrast the general structures of plant and animal cells.		✓		
SC.912.L.14.52: Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics	✓			
SC.912.L.16.3: Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.			✓	
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.16.9: Explain how and why the genetic code is universal and is common to almost all organisms.			✓	
SC.912.L.16.12: Describe the basic DNA technology (restriction digestion, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules.			✓	✓
SC.912.L.17.6: Compare and contrast the relationships among organisms, including predation, parasitism, competition, commensalism, and mutualism.	✓			
SC.912.L.18.1: Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.			✓	✓
SC.912.L.18.3: Describe the structures of fatty acids, triglycerides, phospholipids, and steroids. Explain the functions of lipids in living		✓		
SC.912.L.18.11: Explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions. Identify factors, such as pH and temperature, and their effect on enzyme activity.			✓	

ICORE SUMMER INSTITUTE CONNECTIONS:

The primary source of curriculum in this action proposal is “Biotech in the Classroom: Laboratory Manual Tomato Spotted Wilt Virus” was obtained at HHMI – UF CPET / 2010 summer institute.

LITERATURE CITED:

1. Bokor, Julie, Biotech in the Classroom: Laboratory Manual Tomato Spotted Wilt Virus, April 2010, http://biotechintheclassroom.webs.com
2. Gallo, Maria, Dr., **History and Impact of TSWV** biotechintheclassroom.webs.com/TSWV-Overview.ppt

3. University of Florida Center for Precollegiate Education and Training, Peanut DNA extraction and amplification Power Point, biotechintheclassroom.webs.com

BUDGET:

1. The travel expenses of (2) UF CPET coordinators/ staff from Gainesville FL, to Bradenton FL / two days
2. (200) Biotech in the Classroom: Laboratory Manual Tomato Spotted Wilt Virus

Source	**Student workstation**	**Number required per group**	**Experiment**
Peanut plants showing signs of TSWV infection – local grower, UF collaborator, class-grown plants	Farmer John's plant samples	Variable 2-4	1
Agdia http://www.agdia.com/ Agdia ImmunoStrip™ TSWV Tests Item ISK 39300/0025 25 sample bags and strips=$105	Sample extract bags	2-4	1
	Test strips	2-4	1
Classroom laboratory	Sharpie	1	1, 2, 3, 4
Classroom laboratory	Scissors	1	1
Classroom laboratory	Colored pencils/crayons	Assortment	1
Sigma Aldrich http://www.sigmaaldrich.com REDExtract-N-Amp Seed™ PCR Kit 10 extractions/ 10 PCR reactions : XNASS-1KT, $26.50 100 extractions/ 100 PCR reactions : XNAS-1KT, $213.00	Seed extraction buffer, 100µl	1 tube	2, 3
	Seed preparation buffer, 15µl	1 tube	2, 3
	Neutralization buffer, 120µl	1 tube	2, 3
Fisher Scientific http://www.fishersci.com/ Cat. No. K749520-0090, $132.23 PK of 100 individually wrapped microtubes and micropestles	1.5ml microcentrifuge tubes	2	2
	Sterile blue micropestle	2	2
Fisher Scientific http://www.fishersci.com/ Cat. No. 14-230-225, $125.00 Case of 1000	0.2ml PCR tubes	2	2, 3
Classroom laboratory	Sterile distilled H_2O, 200µl	1 tube	2, 3, 4
Bio-Rad http://www.bio-rad.com Classroom 20–200 µl Digital Micropipet 166-0551EDU, $111.00	P20 pipette	1	2, 3, 4

Bio-Rad http://www.bio-rad.com Classroom 20–200 µl Digital Micropipet 166-0552EDU, $111.00	P200 pipette	1	2, 3
Fisher Scientific http://www.fishersci.com/ Cat. No. 50863814 Pack of 960 for $120.99 (10 boxes of tips with 96 tips per box)	P20 pipette tips	1 box	2, 3, 4
Fisher Scientific http://www.fishersci.com/ Cat. No. 50863815 Pack of 960 for $120.89 (10 boxes of tips with 96 tips per box)	P200 pipette tips	1 box	2, 3
UF Collaborator or UF CPET	Genetically engineered peanut seeds	1	2
Local grocery store, unsalted peanuts	Wild-type peanut seeds	1	2
Bio-Rad http://www.bio-rad.com MJ Mini 48-Well Personal Thermal Cycler PTC-1148EDU, $3,196.00	Thermal cycler	1	2, 3
Classroom laboratory	70% ethanol	1 squeeze bottle	2
Classroom laboratory	Analytical scale	1	2
Classroom laboratory	Scalpel	1	2
Classroom laboratory	Kimwipes	1 box	2
Bio-Rad http://www.bio-rad.com 100–1000 µl Digital Micropipet Cat. No. 166-0508EDU, $221.00	P1000 pipette	1	2, 3, 4
Fisher Scientific http://www.fishersci.com/ Cat. No. 50863812 Pack of 560 for $66.98	P1000 pipette tips	1 box	2, 3, 4
Fisher Scientific http://www.fishersci.com/ Cat. No. 02-681-5, $17.67 Pack of 250	1.5ml microcentrifuge tubes	Variable; need at least four per group for reagent aliquots	2, 3, 4
Seed A, Seed B from Experiment 2 Positive control from UF collaborator	DNA samples, 5µl each (Seed A, Seed B, Positive Control)	3	3
Sigma Aldrich REDExtract-N-Amp Seed™ PCR Kit http://www.sigmaaldrich.com 10 extractions/ 10 PCR reactions: Cat. No. XNASS-1KT, $26.50 **OR**	PCR reaction mix, 60µl (Contains reaction buffer,Mg2+, dNTPs, Taq polymerase)	1 tube	2, 3

100 extractions/ 100 PCR reactions: Cat. No. XNAS-1KT, $213.00			
Integrated DNA Technologies (IDT) http://www.idtdna.com/ , $8.05 257: GCCAAGACAACACTGATCATCTC	Primer 1, 5µl	1 tube	3
Integrated DNA Technologies (IDT) http://www.idtdna.com/ , $8.05 259: TGACCTTCAGAAGGCTTGATAGC	Primer 2, 5µl	1 tube	3
Classroom laboratory **OR** Invitrogen http://www.invitrogen.com/ UltraPure™ DNase/RNase-Free Distilled Water Cat. No. 10977-015, $24.72	Sterile distilled H_2O, 50µl	1 tube	2, 3, 4

1 x 500ml			
Bio-Rad http://www.bio-rad.com BR-2000 Vortexer 166-0610EDU, $275.00	Vortex (optional)	1	3
Bio-Rad http://www.bio-rad.com Cat. No. 166-0603EDU, $299.00	Mini centrifuge (0.2ml tubes) (optional)	1	3, 4
Invitrogen http://www.invitrogen.com/ E-Gel® 50 bp DNA Ladder Cat. No. 10488-099, $117.00	Molecular weight marker, 25µl aliquot	1 tube	4
Invitrogen http://www.invitrogen.com/ E-Gel® 1.2% with SYBR Safe™ Starter Kit Cat. No. G6206-01, $109.00 (includes PowerBase, adaptor plug, and six 1.2% E-Gels)	E-Gel® PowerBase™ and adaptor plug	1	4
Invitrogen http://www.invitrogen.com/ E-Gel® 1.2% with SYBR Safe™18-Pak Cat. No. G5218-01, $170.00 (Gels only)	E-Gel® 1.2% with SYBR Safe™	1	4
Fisher Scientific http://www.fishersci.com/ Fotodyne* FOTO/Phoresis* UV Transilluminator – minigel model Cat. No. FD11430, $856.06	UV Transilluminator	1	4
OR			
Invitrogen http://www.invitrogen.com/ E-Gel® iBase™ and E-Gel® Safe Imager™ Combo Kit Cat. No. G6465, $824.00	E-Gel Safe Imager Transilluminator and iBase	1-4	4

TITLE: Biotech in High school Chemistry and Biology / "Testing for the dreaded Tomato spotted wilt virus (TSWV). "

NAME AND CORRESPONDENCE:

David Frantz
Bayshore High School / Manatee County
5401 34th Street West
Bradenton Florida 34209
Frantz1d@fc.manatee.k12.fl.us
941-751-7004 ext. 2345

ABSTRACT:

This lab/ lesson is an introduction to Biotech lab procedures. The students will be extracting plant cell effluent in hopes of detecting the TSWV. The student will use the Agdia ImmunoStrip Test to ascertain if the plant has viral contamination or not.

PROPOSED OUTCOMES:

After performing this lab/lesson, the students will be familiar with the name of the pathogen responsible for TSWV as well as one biotech / laboratory procedure utilized in testing for the presence of TSWV. The students will also be informed of TSWV's present threat and risks facing the peanut and tomato crops/ industry not only locally, but also nationally and internationally. Finally the students will see a practical and valuable reason to learn and understand basic biochemistry as it applies to managing and growing healthy basic food crops.

STUDENT LEARNING OBJECTIVES:

1. Describe the physical characteristics of a plant suspected of viral infection
2. Use technology to test for the presence of TSWV.
3. Explain antigen/antibody interactions and how their properties are used in diagnostics

NEXT GENERATION FLORIDA SCIENCE STANDARDS:

Benchmark (SC.912.N.1.6): Describe how scientific inferences are drawn from scientific observations
and provide examples from the content being studied
Benchmark (SC.912.L.14.52): Explain the basic functions of the human immune system, including specific
and nonspecific immune response, vaccines, and antibiotics
Benchmark (SC.912.L.16.7): Describe how viruses and bacteria transfer genetic material between cells
and the role of this process in biotechnology
Benchmark (SC.912.L.17.6): Compare and contrast the relationships among organisms, including
predation, parasitism, competition, commensalism, and mutualism.

LESSON OUTLINE:

1) The teacher will present a “***SHORT PPT***” presentation of TSWV background information and pictures of the pathogen and the lethal damage it causes to the plant.

2) Students receive the “Biotech in the Classroom Laboratory Manuals” and we review / read together “The Background Information” and “The Setting the Stage” printed in the Student Pages in pre lab experiment 1.

3) The students will receive the actual plant material to be tested in this lab, and note the appearance of the plant samples and record their observations in the pre lab experiment 1 section.

4) The teacher will explain / demonstrate the Laboratory Method for Agdia ImmunoStrip Test and the location of all the necessary and additional testing materials.

5) The students will perform the laboratory method and collect the necessary data and then analyze this data by answering the questions in “Biotech in the Classroom Lab Manual” – post lab experiment 1.

MATERIALS:

For Each Student Lab Pair Workstation:

2-4 leaves /Peanut plants showing signs of TSWV infection – local grower or UF collaborator
2-4 Test strips
2-4 Sample extract bags
(Agdia ImmunoStrip™ TSWV Tests/ Item ISK 39300/0025 / Agdia / http://www.agdia.com/
25 sample bags and strips=$105)
1 Sharpie
1 Scissors
Colored pencils/Markers/ crayons Assortment

Vocabulary:

Immunoassay- biochemical test that measures the concentration of a substance in a biological substance, using the reaction of an antibody to its antigen.
Tomato spotted wilt virus (TSWV)- a negative RNA virus vectored by thrips affecting ~800 species of agricultural and ornamental plants. It causes spotting, stunted growth, loss of production, and eventual plant death.

CONCLUSION:
All student lab pairs will collectively report / record their data and results on an overhead or the white board. The teacher will involve the class in a discussion and analysis of the lab results, the successes or failures and finally the merits of the lab/lesson.

ASSESSMENT/ HOMEWORK:
The student is required to write a report to send to Mr. Bailey detailing the lab procedure's findings. The student is to include the methods used to reach the conclusion. The student is to discuss the use of the ImmunoStrip, how it works, and the results obtained from Mr. Bailey's samples. The students will be graded on a rubric, provided in the teachers Biotech in the Classroom Laboratory Manual.

REFERENCE:

1. Bokor, Julie, Biotech in the Classroom: Laboratory Manual Tomato Spotted Wilt Virus, April 2010, http://biotechintheclassroom.webs.com
2. Gallo, Maria, Dr., **History and Impact of TSWV** biotechintheclassroom.webs.com/TSWV-Overview.ppt
3. Koroly, Mary Jo, Ph.D. Director of UF CPET, ICORE.
4. UF HHMI ICORE Interdisciplinary Center for Ongoing Research/Education Summer Institute: July 11-23, 2010.

TEACHER BACKGROUND:
B.S. Biology/ Secondary Ed. U. Pittsburgh
M.Div. Pittsburgh Theological Seminary
M.S. Medical Science U.S.Florida
Teaching High school Florida 11 yrs. Biology, Genetics, Chemistry, Physics
AP Biology, AP Chemistry, AP Physics workshops