Viral Transmission within Mulberry Leaves – A Pathogenic Simulation using Silkworms

Stace Alcala
Mulberry Senior High School – Honors Biology

Abstract:
This honors biology study will involve students “chasing” a scenario that involves both application of a wide variety of biotechnology practices as well as determining the validity of pathogenic transmission of simulated virus via silkworm larva.

This simulation will include a hypothetical introduction of a viral pathogen presented to non-infected mulberry leaves via silkworm larva purchased from China. The students will undergo several hands-on activities that utilize biotechnology in their quest to a greater understanding of pathogenic emergence, viral/vector relationships, pathogenic lifecycles, and the molecular/genetic physiology of the silkworm (as a means of comparison toward other metamorphic specimens).

Rationale:
This simulation will allow the student to become familiar with a number of high-tech science practices that include: micropipette use, PCR, gel electrophoresis, ELISA antigen/antibody determination, protein sequencing and evaluation using GenBank data banks. As well, the student will become familiar with the biological basics of: cytology, biochemistry of cells and organisms, genetics, and some amounts of botany and zoology. The incorporation of the “mystery” involved within the scenario involving an unknown disease, new and engaging biotech labs, and the husbandry involved with rearing the silkworms should motivate the students toward a desire to learn at a greater than usual expectation. In addition, this unit should increase awareness of post high school requirements as well as future career opportunities.

Module 1: Introduction and Review
An overview of scientific methods including data collection, scientific measurement and statistics, biotech instruments, data analysis, and lab safety protocols will equip the students with lab tools necessary for successful interaction with activities presented.

Assessment:
Pre and Post Test assessment will provide a reasonable basis for success via knowledge base and application.

Activities – Group I: Introduction of specimens and scenario
Students will read and discuss provided scenario producing individual hypothesis (giving reason for local leaf deterioration).

- lab safety protocols – district required permission/signature
- discussion of complete metamorphosis and life cycle of Chinese silkworm
- introduction of viruses and viral pathogens
- brief discussion of plant leaf biology
- time allowance for growth of larva (−four-five weeks)

Silkworm Simulation – p2
Alcala – Honors Biology
Action Proposal

Activities – Group II: Fruit Loop Lab
Students utilize measuring techniques to become adept at data collection and analysis using common scientific measuring devices and graphical representation of outcomes.

- lab handout, students work in lab groups of 2’s
- class data collection (record on front board)
- class discussion of results
Module 2: Cytology, DNA and the Power of Proteins

Becoming familiar with cell structure, transport mechanisms, DNA structure/analysis and protein synthesis as the central dogma for understanding much of biology. Students will use several approaches to gain a working knowledge of cellular and molecular biology including: observation of cellular specimens, producing cellular/tissue slide specimens, DNA and protein simulations, biotechnology techniques that include PCR, Gel-Electrophoresis, and ELISA simulations. If possible, either simulation or actual protein analysis (-omics) utilizing Dr. Chen’s laboratory and mass spectrometry will give students a hands-on approach to cutting edge technology. Mass spec. analysis will then be programmed into GenBank’s data reservoir for potential protein probabilities. Reared larva and moths will be used for DNA and protein analysis, as well as hypothetically infected and non-infected mulberry leaves.

Assessment:
Pre and Post Test assessment, teacher checklist rubric demonstrating student specimen preparation, gel banding – ELISA success assessment, worksheets and lab write-ups

Activities Group I : Cytology and fundamental tissue Histology
➢ Graphic Organizers (GO’s) and outlines in guided lecture pertaining to cell structure/function
➢ non-mandatory 3D cell model project (criteria/rubric provided in handout to student)
➢ membrane transport mechanisms using membrane models and the “Potato Lab”
➢ microscopy with preserved cellular and tissue samples, in addition, students practice the production of “wet mounts” using such specimens as onion tissue layers
➢ basic elements of biochemistry will be learned through the “Breakfast Lab” entertaining the concept of macromolecules (primarily hitting on proteins and nucleic acids)

Activities Group II: Biotechnology Applications
➢ micropipette by coordinates discovery
➢ Dactylography “Whorls, Swirls and Arches” fingerprinting activity (introduction to DNA fingerprinting and Gel Electrophoresis)
➢ restriction enzymes and PCR activity (handout - cutting DNA, producing segmental copies) the use of a thermal cycler
➢ PCR and Gel Electrophoresis – a comparison of larval and adult SW (silkworm) DNA
➢ PCR and Gel Electrophoresis – a comparison of local mulberry leaves and hypothetically infected Chinese mulberry leaves
➢ Introduction of ELISA antigen/antibody analysis (a simulation)
➢ student analysis through group/individual discussion/reporting (both guided and independent)
➢ class discussion – interpretation of results

Activities Group III: Protein Analysis (per time and perceived level of understanding)
➢ Sequencing differential banding of gels for protein sequence and mass spectrometry
➢ GenBank protein BLAST program for protein probabilities

Silkworm Simulation – p3
Alcala – Honors Biology
Action Proposal

Module 3: Review of Techniques and Summary of Unit

Students will review all techniques through independent, paired, group, and class activities using gained knowledge and skills toward problem solving introductory scenario among other hypothetical scenarios.

Assessment:
A Final Unit Test to evaluate success of learned material and skills
A survey of student interest, self-evaluation and constructive criticisms
Activities Group I: Review and Wrap-Up
- JigSaw – students identified and grouped as special interest, government officials, health officials, various scientists etc... to identify a potential pathogenic threat (similar to RVF with Dr. Gibbs)
- Group presentations of various biotech instruments and techniques
- Review worksheets and games

Activities Group II: Intro to historical/known worldwide pathogens and Career Opportunities
- Class surfing of CDC website and various websites concerning pathogens (i.e. WHO)
- guest speakers involved in careers involving in biotechnology, science and health, and research

Budget Considerations:

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplier</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silkworm eggs</td>
<td>Carolina Biological Co.</td>
<td>$60</td>
</tr>
<tr>
<td>Silkworm larva food</td>
<td>Carolina Biological Co.</td>
<td>$45/lb</td>
</tr>
<tr>
<td>micropipette tips</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 well microplates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Expected Locker Usage:

- Simulated ELISA kit (Dr. Lawrence)
- Gel Electrophoresis Kit (including all PCR (thermal cycler) needs, gel plates and chambers)
- Micropipette Kit (if separate from above)
- microcentrifuge

- all digester enzymes, buffers, indicators etc...

References:

ICORE and affiliates

Lesson Plan

5 weeks of Feasting ends with 5 days of Fasting! (and serious reproduction!)
“The Industrious Silkworm” – Life Cycle, Husbandry, and a Genetic Close-Up

Mulberry Senior High School
Honors Biology (10th grade)

Intention: Students will gain a greater understanding of biotechnology techniques and strategies through a 2-month study of silkworm life cycles. Several broad areas of biology will be addressed such as: Cytology, Genetics, Plant biology, Molecular and Chemical biology, as well as viral and bacterial simulations.

Learning Goals: Students will be able to identify, answer, perform, teach, and demonstrate the following:
data collection techniques (various measurement tools – qualitative/quantitative methods)
cell structure, membrane transport, cell division, DNA replication, protein synthesis
complete/incomplete insect metamorphosis
PCR and Gel-Electrophoresis
micropipette use
lytic and lysogenic viral “life” cycles
ELISA simulations of plant pathogens

**Estimated Time:** The silkworm life cycle is accomplished in approximately 2 months. While worms are going through in-star stages students will be involved in cellular, viral, and measurement lessons. Practice of micropipette use, gel-electrophoresis simulations, DNA digestion and segmental replication simulations, as well as cellular studies involving membrane transport, mitotic division and viral/bacterial reproduction. Pre and Post-Test assessments will help determine student learning curves and lesson success.

**Materials/Resources:** lab groups of 4-5 students (5 stations)

**Lab Equipment:** microscopes, metric rulers, digital scales, micropipettes and tips, microplates, thermal cycler, vertical gel chambers, magnifying glasses, fingerprint stain

**ICORE Locker Needs:** thermal cycler, microcentrifuge, class set of micropipettes, tips, plates, 5 vertical electrophoresis chambers, gel slabs (pre-made), power sources, ELISA simulation pack (Dr. Lawrence), all buffers, digesters

**Lab supplementary Items:** tissue samples, onion root-tip mitosis slides, fruit loops, silkworms, mulberry leaves, mulberry leave gelatin, macromolecules for breakfast lab (toast, butter, jelly, bacon, eggs, juice)

**Teacher Preparation:**
- prepare pre and post test questions
- lab write-ups for Breakfast Lab, Fruit Loop Lab, Mitosis Lab, Silkworm DNA Comparison Lab (larval and adult silkworms), Mulberry Leaf Comparison Lab (normal and simulated pathogenic)
- prepare review activities and discussion questions for student collaboration

**Lesson Plan (continued) p2**

**Targeted Benchmarks:**
- cellular biology
- molecular biology
- plant/botany biology
- measurements/graphing
- taxonomy/zoology

**Assessments:**
- pre-test on cell components, metric conversions, plant leaf anatomy, viral/bacterial reproduction
- demonstrate “wet-mount” slide preparation
- demonstrate proper micropipette use
➢ demonstrate proper gel “well loading”
➢ various worksheets (homework) involving vocabulary, cycles, anatomy etc...
➢ lab write-ups for Fruit Loop, Mitosis, Breakfast, DNA and Leaf gel comparisons, 3-D cell project, and Fingerprints Labs
➢ post-test over all materials
Silkworm DNA Extraction and PCR

DNA Extraction

1. Label a **screwtop** microcentrifuge tube with the sample type and an identifying mark for your group. Repeat for your remaining samples. You will repeat each of the following steps for each sample that you run.
2. Pipette 100 mL of Extraction Solution (ES) into a **screwtop** microcentrifuge tube.
3. Add 25 mL of Tissue Preparation Solution (TPS) to the tube and pipette up and down to mix.
4. Rinse the scalpel and forceps in ethanol prior to use and between different samples. Place a 2–10 mg piece of tissue into the solution and close the tube. Mix thoroughly by flicking the tube. Ensure the tissue is in the solution.
5. Incubate sample at room temperature for 10 minutes.
6. Incubate sample at 95°C in the heat block for 3 minutes. Be careful, the heat block is VERY hot!
7. Add 100 mL of Neutralization Solution B (NSB) to sample and mix by flicking the tube.
8. Use DNA sample immediately for PCR.

PCR

1. Label a PCR tube with the sample type and an identifying mark for your group. Repeat for your remaining samples.
2. Half of the groups will use primer sets 1, and the other half will use primer sets 2. Wait for your instructor to indicate which set your group will run, and label your tubes with a 1 or 2, accordingly.
3. Add the following reagents to your PCR tube:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Master Mix (reaction mix, primers, water)</td>
<td>16 µl</td>
</tr>
<tr>
<td>DNA sample</td>
<td>4 µl</td>
</tr>
</tbody>
</table>

4. Take your PCR tubes over to the thermocycler to run through the temperature cycles. Once the cycles have run, the amplified DNA will be kept cold, at 4°C until ready for gel electrophoresis.
DNA Gel Electrophoresis

1. Plug red E-Gel PowerBase™ into an electrical outlet.
2. Remove gel cassette from package.
3. Insert the gel (with comb in place) into the base right edge first. The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the gel cassette in the E-Gel PowerBase™. A steady, red light will illuminate if the gel cassette is correctly inserted.
4. Remove and discard comb from the E-Gel® cassette.
5. Add 10μl sterile distilled H₂O to wells 4-8.
6. Add 20μl sterile distilled H₂O to wells 1-3, 9-12.
7. Add 8μl DNA samples to wells 5-8.
8. Add 8μl marker to well 4.

<table>
<thead>
<tr>
<th>Well #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>What to add to the well</td>
<td>20μl water</td>
<td>20μl water</td>
<td>20μl water</td>
<td>10μl water + marker</td>
<td>10μl water + 8μl DNA</td>
<td>10μl water + 8μl DNA</td>
<td>10μl water + 8μl DNA</td>
<td>10μl water + 8μl DNA</td>
<td>20μl water</td>
<td>20μl water</td>
<td>20μl water</td>
<td>20μl water</td>
</tr>
</tbody>
</table>

9. Press and release the 30 minute button on the E-Gel® PowerBase™ to begin electrophoresis.
10. At the end of the run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping, and unplug the E-Gel® PowerBase™.
11. Remove the gel cassette and analyze your results by viewing on one of the transilluminators.
Silkworm PCR & Gel Electrophoresis
Introduction to PCR

• 1983 Kary Mullis (Cetus Corp) developed the molecular biology technique that has revolutionized genetic research: the Polymerase Chain Reaction

• PCR quickly transformed molecular biology into a multidisciplinary research field
Introduction to PCR

• Object of PCR: produce a large amount of DNA in a test tube (in vitro) starting with only a trace amount

• Controlled enzymatic amplification of a DNA sequence, or gene, of interest

• Tiny amounts of genomic DNA from a drop of blood, single hair, or cheek cell can generate enough DNA to study
Introduction to PCR

• PCR impacted several areas of genetic research:

  – used as a medical diagnostic tool to detect specific mutations that may cause genetic disease
  – used in criminal investigations and courts of law to identify suspects
  – used in the sequencing of the human genome
Introduction to PCR

• Prior to PCR, the use of molecular biology techniques for therapeutic, forensic, pharmaceutical, agricultural, or medical diagnostic purposes was neither practical nor cost-effective

• The development of PCR transformed molecular biology from a difficult science to one of the most accessible and widely used disciplines of biotechnology
PCR amplification

• With PCR, you can target and make millions of copies (amplify) a specific piece of DNA (or gene) out of a complete genome

• In our experiment, you will amplify a region within your own chromosome 16
PCR amplification

- PCR makes use of the same basic processes that cells use to duplicate their DNA (replication)
  - Complementary DNA strand hybridization
  - DNA strand synthesis via DNA polymerase
PCR amplification

Recipe for a PCR amplification of DNA:

- DNA template: Your DNA, containing the intact sequence of DNA to be amplified
- Deoxynucleotides: raw material of DNA
- DNA polymerase: enzyme that assembles the nucleotides into a new DNA chain
- Magnesium ion (Mg$^{2+}$): cofactor (catalyst) required by DNA polymerase to create the DNA chain
- Primers: pieces of DNA complementary to the template that tell DNA polymerase exactly where to start
- Salt buffer: provides the optimum ionic environment and pH for the PCR reaction
PCR amplification

• PCR: three main steps
  – Denaturation (94 degrees, 1min)
  – Annealing (60 degrees, 1min)
  – Extension (72 degrees, 2min)
    • 40 cycles
    • Amplified exponentially
    • Results in $1.1 \times 10^{12}$ sets of precise-length DNA
DNA Amplification Using Polymerase Chain Reaction

Reaction mixture contains target DNA sequence to be amplified, two primers (P1, P2) and heat-stable Taq polymerase.

Reaction mixture is heated to 95°C to denature target DNA. Subsequent cooling to 37°C allows primers to hybridize to complementary sequences in target DNA.

When heated to 72°C, Taq polymerase extends complementary strands from primers.

First synthesis cycle results in two copies of target DNA sequence.

Second synthesis cycle results in four copies of target DNA sequence.

Source: DNA Science, see Fig. 13.
Gel loading tips

• Always use a clean pipet tip prior to drawing sample
• Push the plunger to the **first stop** to push air or sample out of the pipet
• Place your tip into the well and expel the sample without jabbing the tip through the bottom of the gel (this will cause a hole to form at the bottom of the well, allowing your DNA to leak out)
Gel electrophoresis

- Migration of charged particles under the influence of an electric field
  - DNA is ____________ charged
  - Moves toward _________ electrode
Gel electrophoresis

- Horizontal gel made of agarose
  - Agarose is a substance extracted from seaweed, similar to gelatin
  - Different size fragments of DNA move through the agarose gel at different speeds due to the sieving action of agarose
DNA exposed

- Fragments of DNA are separated on the gel revealing bands or lines
- Use dye and UV light to see the bands
- See bands of different sizes which represent fragments of DNA
- This will not tell us the DNA sequence
Silkworm Protein Extraction and Electrophoresis

Extraction
1. Label one 1.5 ml **fliptop** micro tube for each of the silkworm life stage samples. Also label one **screwcap** microtube for each sample.
2. Add 250 μl of Laemml sample buffer (blue liquid, labeled LB) to each labeled **fliptop** microtube.
3. Cut a piece of each silkworm life stage sample about 0.25 x 0.25x 0.25 cm³ and transfer each piece into a labeled **fliptop** micro test tube. Close the lids.
4. Flick the microtubes 15 times to agitate the tissue in the sample buffer.
5. Incubate for 5 minutes at room temperature.
6. Carefully transfer the buffer by pouring from each **fliptop** microtube into a labeled **screwcap** microtube. Do not transfer the silkworm!
7. Heat the samples in screwcap microtubes for 5 minutes at 95°C in the heat block. Be careful – heat block is very hot!

Gel Electrophoresis
1. Your instructor has set up the gel boxes with 15% polyacrylamide gels, with a 1% TGS buffer as the electrophoresis running buffer.
2. Load your gel using the p20 pipette and the special Protein Gel Pipette tips (available from your instructor) as follows:

<table>
<thead>
<tr>
<th>Lane</th>
<th>Volume</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Empty</td>
<td>Empty</td>
</tr>
<tr>
<td>2</td>
<td>Empty</td>
<td>Empty</td>
</tr>
<tr>
<td>3</td>
<td>Empty</td>
<td>Empty</td>
</tr>
<tr>
<td>4</td>
<td>5 μl</td>
<td>Protein Standards</td>
</tr>
<tr>
<td>5</td>
<td>10 μl</td>
<td>Silkworm sample 1</td>
</tr>
<tr>
<td>6</td>
<td>10 μl</td>
<td>Silkworm sample 2</td>
</tr>
<tr>
<td>7</td>
<td>10 μl</td>
<td>Silkworm sample 3</td>
</tr>
<tr>
<td>8</td>
<td>10 μl</td>
<td>Silkworm sample 4 (or empty)</td>
</tr>
<tr>
<td>9</td>
<td>Empty</td>
<td>Empty</td>
</tr>
<tr>
<td>10</td>
<td>Empty</td>
<td>Empty</td>
</tr>
</tbody>
</table>
3. Electrophorese for 30 minutes at 200 V in 1x TGS electrophoresis buffer.
4. Remove gel from gel cassette by removing the cover sticker and slowly and gently sliding the gel out in a gel tray with purified water. Gently swish your gel in the water.
5. Pour out water and rinse once more with water. Pour out water.
6. Add 25 ml of Coomassie Blue safe stain to your gel tray. Let sit for at least 1 hour.
7. Pour out gel stain and rinse with water. Let sit for at least 1 hour.
8. View gels.
Viral Transmission within Mulberry Leaves – A Pathogenic Simulation using Silkworms

Stace Alcala
Mulberry Senior High School – Honors Biology

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This simulation will include a hypothetical introduction of a viral pathogen presented to non-infected mulberry leaves via silkworm larva purchased from China. The students will undergo several hands-on activities that utilize biotechnology in their quest to a greater understanding of pathogenic emergence, viral/vector relationships, pathogenic lifecycles, and the molecular/genetic physiology of the silkworm (as a means of comparison toward other metamorphic specimens).

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- micropipette use
- lytic and lysogenic viral “life” cycles
- ELISA simulations of plant pathogens

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Total Time:  Approximately 60 days - High School Biology

Silkworm lab activities

pre-silkworm preparation – measurement strategies, foundational biology recaps
Fruit Loop measurement lab – 1 class period (45 minutes)
Potato Lab (transport mechanisms – diffusion/osmosis) 1 lab period (90 minutes (ideal))
Breakfast Lab (macromolecule study – lipids, proteins, nucleic acids, carbohydrates) 1 lab period (90min)

biotechnology practices – introduction/review of biotech instrumentation
Viral comparison (lytic vs lysogenic) (portion of class period)
DNA extraction of strawberries 1 class period (45 minutes)
Micropipette use (micro-well pictures) 1 class period (45 minutes)
ELISA simulation (antigen recognition) 1 class period (45 minutes)
Real-time PCR vs Traditional (simulations) 1 class period (45 minutes)

silkworm “rearing” phase (followed by these labs)
Silkworm Husbandry – feeding and care of larval silkworm moths, cocoons/pupae, adult moths
DNA gel-electrophoresis of simulated pathogens (Missouri, Georgia, Pennsylvania viral outbreaks) 1 lab period
DNA extraction of silkworm (SW) metamorphic stages (egg, larva, pupa, adult) 1 lab period
Protein gel-electrophoresis 1 lab period (or more)
  PCR and amplification of DNA segments (using specific primers for membrane and silk protein production)
  Gel-electrophoresis segregating membrane and silk proteins

Targeted Benchmarks:

- cellular biology
- molecular biology
- taxonomy/zoology
- plant/botany biology
- measurements/graphing

Assessments – Students Will Be Able To Demonstrate

- prior knowledge of - cell components, metric conversions, graphs/data collection/illustration,
  leaf anatomy, viral/bacterial reproduction, biotech vocab in a pre-test assessment
- “wet-mount” slide preparation
- proper micropipette use
- proper gel “well loading”
- knowledge of various vocabularies, cycles, anatomy, equipment use (through worksheets (in/out of class))
- lab write-ups for Fruit Loop, Mitosis, Breakfast, DNA gel comparisons, 3-D cell project, Fingerprints Labs
- post knowledge of overall project benchmarks through post-test assessment
Action Proposal Implementation

Students were introduced to the action proposal “Viral Transmission within Mulberry Leaves” as a general concept at the inception of the school year. Throughout the fall term we employed several labs that would teach and review scientific processes. These labs included: data collection through measurement, visual display of data through graphic representation, and microscope use (within the “Fruit Loop Lab”). In addition, understanding of: cellular components, membrane transport mechanisms, and molecular organization of cellular constructs were solidified with the “Potato and Breakfast Labs”. Each of these labs involved hands-on practices that reinforced concepts that would be useful in the upcoming action proposal (see attached lab exercises).

This study involved two Honors Biology (tenth grade) audiences of approximately fifty students. The instructor was compelled to complete instruction on molecular and genetic processes in a unit prior to the implementation of the action proposal. As such, this moved the time line into the second half of the school year. As well, the availability of fresh food sources (for the developing silkworm larva) was unavailable until new mulberry leave emergence occurred during the “spring”. As we waited for this time, the students began a ten-day unit on viral transmission and pathogenic emergence in a hypothetical simulation called “Viral Quest”. This simulation (developed by a team at the University of Florida for high school students) brought into “play” many biotech applications that were completely unfamiliar to the biology students. This was a great springboard for our action proposal, as many of the techniques would be practiced and subsequently used with our silkworm study. During this simulation, students became familiar and even well-versed in both the vernacular and practice of biotechnology instrumentation. In addition, the “life-cycles”, transport, and response to pathogenic infection were highlighted throughout the simulation. During this “quest”, students gained practice with micropipettes, gel-electrophoresis chambers, DNA extraction techniques, graphic simulation of real-time and conventional PCR, and simulated ELISA antigen identification. All of these actions would become a vital and integral part of the understanding needed in the silkworm lab. The primary purpose of this lab is to demonstrate accurate methodology of biotechnology practices and instrumentation use. Silkworms are used in this study as they show significant morphological differences at each metamorphic stage of development, indicating alternative gene expression, though DNA remains unchanged.

Finally, after much waiting, the time for hatching silkworm larva from their dormancy within the “frozen” eggs was upon us. Larva (approximately a millimeter in length) emerged and began heartily eating the available mulberry leaves (their only food source). Students were able to view growth rates as the larva progressed through several “in-star” stages. After approximately one month, the larva (now some 7.5 centimeters in length) began the task of spinning silk for cocooning themselves to continue their metamorphosis (approximately three days). Again, the wait as the metamorphosis into adult moths requires three weeks. During this time we prepared for the lab’s molecular aspects. Members of the University of Florida acquired necessary “primers” for two DNA locations. One location for PCR amplification involved the gene sequence for silk, while the other involved a gene for cellular membrane construct. These primers are to be used for gene amplification in a PCR reaction for the subsequent DNA gels.

At last, the long awaited lab began on a Thursday morning (approximately three and one half hours.) During this time, all fifty students (grouped into teams of six) performed several of the biotech practices previously learned, only this time on the silkworms. DNA extractions of all metamorphic stages were employed for banding segregation using gel-electrophoresis chambers. In addition, protein extractions were performed and segregated with gel-electrophoresis. The extracted DNA was then placed within a thermal cycler along with the necessary primers to amplify the two gene sequences on all metamorphic stages (egg, larva, pupa, adult).
These amplifications were then used within gel chambers to indicate segregation of the two band lengths. Oddly, the banding was most evident within the egg phase of the life cycle. Although visible, the banding within the other wells (larva, pupa, and adult) was scant and almost “ghost-like”. However, consistency was seen within the egg well for all student lab groups (see gel photos).

In summary, students became familiar with all desired biotechnology practices indicated at onset. The pre-training instruction, practice, and hands-on labs provided necessary motivation for students to become engaged and proactive in their knowledge acquisition. Although the title of the proposal indicates the involvement of mulberry leaves and viral transmission, the students were never introduced to this aspect. The instructor deemed the other components involved were sufficient to adequately represent the desired biotech implementation. If the viral transmission aspects were desired, a simulation involving mulberry leaves and any other plant species could be used to demonstrate gel segregations and “pretend” vector transfer of virus components occurred.
Viral Quest Log  -  Mulberry Senior High  
Stace Alcala

Day One: Outbreak

Students began Viral Quest study with Outbreak reading and Mandy Gomez. Discussions involved possible causes, identification (hypothesis), and impacts on society (individuals). In addition, overview of course and pre-testing.

Day Two: Viruses

Powerpoint presentation on virus structure, “life” cycle, and comparisons between lytic and lysogenic viruses. As well, students overviewed both DNA and RNA (as we have already studied both molecular and cellular concepts).

Day Three: Media and Viral News

Students performed jigsaw activity to gain data from various media sources, breaking into discussion groups that allowed for the sharing of the characteristics of both HIV and HPV viruses.

Day Four: Viral WhoDunit (Crime Scene)

Students read crime scene scenario. Class discussion involved crime scene details and a comparison of data from crime scene samples and how those could be used similarly to viral identification. Specific biotech procedures were discussed to amplify DNA through PCR.

Day Five: DNA Extraction (Strawberry DNA)

After setting up several lab stations, students used materials to extract DNA from fresh strawberries. Discussion involved the whys and hows of the extraction. As well, a summary of the week prepared students for the first Quiz.

Day Six: PCR

After watching both BioRad’s PCR and GTCA youtube videos (as well as the first version of “We are the World”, discussion involved PCR protocols for both traditional and real-time methodologies. The powerpoint presentation was utilized to illustrate and explain both processes as the students completed worksheet outlines.

Day Seven: Reverse Trascription

We viewed the powerpoint presentation of RNA – DNA PCR amplification and reverse transcription. Discussion of Mandy and the HIV or HPV virus identification for the unknown outbreak ensued. Students completed worksheets associated with this lesson.

Day Eight: Real-Time PCR Analysis

After viewing the PCR powerpoint (highlighting the “threshold” cycle). Students identified various results from the examples provided on the powerpoint. A hardcopy “lab” was distributed and students broke into small groups to discuss and complete the handouts. Class discussion followed as we summarized the findings.
Day Nine:  Clean Up Info day

We reviewed and summarized the various techniques. Took the second quiz and completed all worksheets in the student viral quest notebook.

Day Ten:  Biotechnology careers

Although we did not research careers in our media dept., we discussed potential job opportunities and viewed career “spotlights” from teacher resources. Review of the program an summarization also took place

Day Eleven:  Assessment

Post Test of content. As well, students completed Viral Quest Student Notebooks.

Each day included Bell Ringer beginning and Journal Entry completion. Some days had interruptions that forced the completion of each of these the following day. As well, students completed the majority of worksheets associated with each lesson.