Title: Viral Transmission within Mulberry Leaves –A Pathogenic Simulation using Silkworms

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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)

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

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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:

Silkworm eggs	Carolina Biological Co.	$60
Silkworm larva food	Carolina Biological Co.	$45/lb
micropipette tips		
96 well microplates		

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 with 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