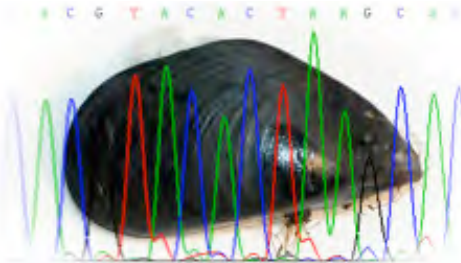


Marine Biotechnology and Bioinformatics



A program of ITEST (Information Technology Experiences for Students and Teachers) funded by the National Science Foundation

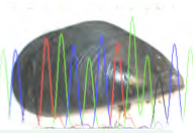


Invasive Species Investigation



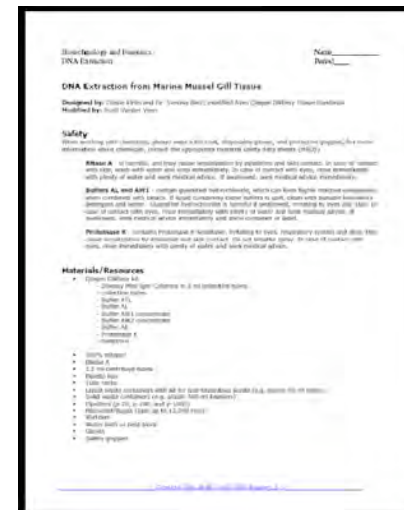
Mussel Collection, Mussel Dissection, DNA Extraction, PCR Purification, and ITS Digest

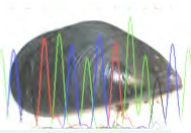
Biotechnology 10th - 12th
Scott Vander Veen
Valley Christian High School
San Jose, CA



Background Context

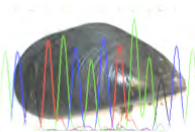
- ▶ First year Biotechnology class of 10th – 12th grade students. (four sections)
- ▶ Needed a real scientific study as application of techniques that they had been learning during the semester
- ▶ Responsibilities
 - ▶▶ Developing procedures
 - ▶▶ Implementing labs
 - ▶▶ Analyzing results





Instructional Goal

- ▶ The goals of this lesson were:
 - ▶▶ to allow students to apply techniques and skills that they developed during the semester
 - ▶▶ to allow application into the scientific research process
 - ▶▶ to allow students to work outside the “normal” classroom environment in order to improve comprehension



State Standards

▶ Mussel Dissection

- Biology
 - Grade 7: 5a, b
 - Grades 9-12: 9a, b, c, d, e, f, g, h, i

▶ DNA Extraction

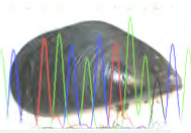
- Chemistry
 - Grade 8: 6c
 - Grades 9-12: 6, 7, 8
- Biology
 - Grade 7: 2e, 3a
 - Grades 9-12: 2, 7
- Investigation and Experimentation
 - Grade 7: a
 - Grade 8: a
 - Grades 9-12: b

▶ PCR Purification

- Chemistry
 - Grade 8: 5a, 6c
 - Grades 9-12: 2a-c, 6a-f, 8a-d
- Biology
 - Grade 7: 2e, 3a
 - Grades 9-12: 1b, 1d, 1h, 2a-g, 4a-f, 5a, 5b, 5d
- Investigation and Experimentation
 - Grades 6-7: 7b
 - Grade 8: 9a
 - Grades 9-12: 1 a/b, 1.1

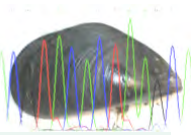
▶ ITS Digest

- Chemistry
 - Grade 8: 6c
 - Grades 9-12: 6, 7, 8
- Biology
 - Grade 7: 2e, 3a
 - Grades 9-12: 5
- Investigation and Experimentation
 - Grade 7: a
 - Grade 8: a
 - Grades 9-12: a, b, c, d, i, k



Instructional Objectives

- ▶ Random Sampling and collection
 - ▶▶ Students will use accepted methods to randomly sample mussel species
 - ▶▶ Student will incorporate proper collection technique and safety
- ▶ Dissection
 - ▶▶ Students will be able to properly dissect two mussel samples and avoid contamination



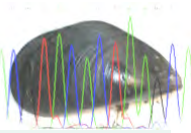
Instructional Objectives

▶ DNA extraction

- ▶▶ Students will use Qiagen DNEasy kits to extract DNA from marine mussel gill tissue

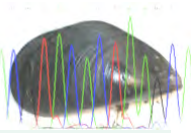
▶ ITS PCR

- ▶▶ Students will apply polymerase chain reaction in order to amplify ITS product



Instructional Objectives

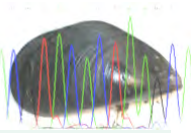
- ▶ ITS Restriction Digest
 - ▶▶ Student will use enzymes to cut DNA in order to prepare it for gel electrophoresis
- ▶ Gel Electrophoresis
 - ▶▶ Using ITS PCR products in gel electrophoresis to determine *mytilus* species



Materials and Resources 1/9

Mussel Collection

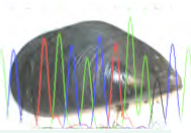
- ▶ Snack size resealable plastic bag
 - ▶▶ (1 per group)
- ▶ Wet wipes or paper towels and soap
 - ▶▶ (to clean hands in the field)
- ▶ Ice or coldpacks
 - ▶▶ (enough to cover bottom of bucket)
- ▶ Permanent markers



Materials and Resources 2/9

Mussel Collection

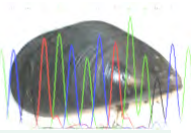
- ▶ 5 m transect line—either a meter tape or a pre-measured string with increments marked every 10 cm
 - ▶▶ (if using a string, label marks with flags of colored tape for ease of use)
- ▶ List of randomly generated numbers
- ▶ Bucket or cooler
 - ▶▶ (approx. size 2 gallon, with handle to transport mussels)
- ▶ Fish & Game fishing license



Materials and Resources 3/9

DNA extraction

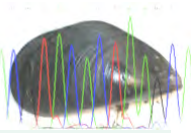
- ▶ Qiagen DNEasy kit
 - ▶▶ DNeasy Mini Spin Columns in 2 ml collection tubes
 - ▶▶ collection tubes
 - ▶▶ Buffer ATL
 - ▶▶ Buffer AL
 - ▶▶ Buffer AW1 concentrate
 - ▶▶ Buffer AW2 concentrate
 - ▶▶ Buffer AE
 - ▶▶ Proteinase K
 - ▶▶ handbook
- ▶ 100% ethanol
- ▶ RNase A
- ▶ 1.5 ml centrifuge tubes
- ▶ Pipette tips
- ▶ Tube racks
- ▶ Liquid waste containers with lid for non-hazardous waste (e.g. plastic 50 ml tubes)
- ▶ Solid waste containers (e.g. plastic 500 ml beakers)
- ▶ Pipettors (p-20, p-200, and p-1000)



Materials and Resources 4/9

DNA extraction

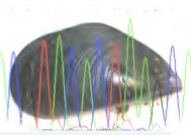
- ▶ Microcentrifuges
 - ▶▶ (Spin up to 13,000 rpm)
- ▶ Vortexer
- ▶ Water bath or heat block
- ▶ Gloves
- ▶ Safety goggles



Materials and Resources 5/9

ITS PCR

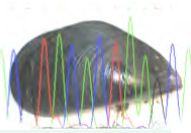
- ▶ GoTaq Green 2X PCR Mix (contains GoTaq® DNA Polymerase, 2X Green GoTaq® Reaction Buffer, 400 μ M dATP, 400 μ M dGTP, 400 μ M dCTP, 400 μ M dTTP, 3 mM MgCl₂ and loading dye).
- ▶ PCR grade water
- ▶ ITS forward primer
- ▶ ITS reverse primer
- ▶ PCR tubes
- ▶ 1.5 ml centrifuge tubes
- ▶ Tube racks
- ▶ Pipette tips
- ▶ Solid waste containers



Materials and Resources 6/9

ITS PCR

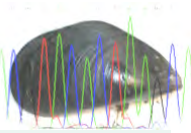
- ▶ Containers with ice
- ▶ Pipettors (2-20 μ l and 20-200)
- ▶ Microcentrifuge
- ▶ PCR machine
- ▶ Working DNA stock



Materials and Resources 7/9

ITS Digest

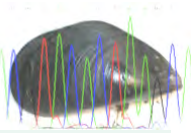
- ▶ FastDigest™ Hha I restriction enzyme (store at -20°C)
- ▶ FastDigest™ 10X buffer (store at -20°C)
- ▶ Nuclease-free H₂O
 - ▶ (store at room temp)
- ▶ 1.5 ml centrifuge tubes
- ▶ Tube racks
- ▶ Pipette tips
- ▶ Pipettors (p-20 µl and p-200)
- ▶ Solid waste containers for non-hazardous waste
- ▶ Microcentrifuge
- ▶ Vortexer
- ▶ Water bath
- ▶ Gloves



Materials and Resources 8/9

Gel Electrophoresis

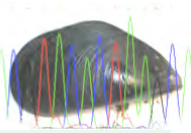
- ▶ 6X Loading dye
- ▶ Molecular weight markers. For large samples (e.g. genomic DNA), use lambda-HindIII; for small samples (e.g. PCR products) use 100bp ladder.
- ▶ Parafilm or wax paper (cut into 2 inch squares, one per student)
- ▶ Pipette tips: (1 box of 200 μ l capacity per group and 1 box at each gel loading station)
- ▶ Tube racks: 1 per group
- ▶ Solid waste containers (e.g. plastic 500 ml beakers): 1 per group and 1 at each gel loading station
- ▶ Pipettors (1 set of 2-20 μ l capacity)
- ▶ 1X Tris Acetate-EDTA (TAE) Buffer
- ▶ Molecular-grade agarose (i.e. Fisher catalog # BP160-100)
- ▶ 10mg/ml Ethidium Bromide (EtBr)
- ▶ Weigh boat or weigh paper
- ▶ Spatula



Materials and Resources 9/9

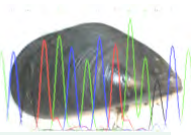
Gel Electrophoresis

- ▶ 1.5ml microcentrifuge tubes (for marker and loading dye preparation; reagent distribution)
- ▶ 500 ml flask for melting agarose
- ▶ Graduated cylinder
- ▶ Magnetic stir bar
- ▶ 2 Gel rigs (gel box with lid, casting tray and combs); 12-well combs are preferable
- ▶ Power source
- ▶ Insulated glove for handling hot glassware
- ▶ Hot plate or microwave
- ▶ Balance
- ▶ UV light box
- ▶ Camera with appropriate filters for photographing ethidium bromide-stained gels
- ▶ Gloves (one pair per student)
- ▶ Goggles (one pair per student)



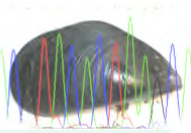
Instructional Strategies

- ▶ Lab skills used:
 1. Mussel Dissection
 2. DNA extraction / purification
 3. PCR
 4. PCR Purification
 5. Gel Electrophoresis
- ▶ Direct presentation of background information and procedure



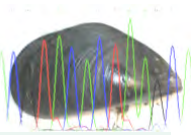
Assessment

- ▶ Student lab notebooks
- ▶ Informal questioning and observation



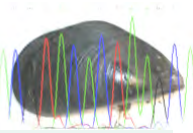
Learning Outcomes

- ▶ The students gained a bigger and better understanding of the scientific process.
- ▶ The students were able to take basic lab skills and apply them to detailed and involved investigations.
- ▶ The students gained a positive outlook on the process of scientific investigation.



Lessons Learned

- ▶ As with all labs, time management is KEY and planning is CRUCIAL!
- ▶ Working with new resources and materials is complex and rewarding.
- ▶ Perfect results are not the only way to experience science.
- ▶ Students will respond and rise to the challenge!



Contact

- ▶ For more information about this lesson, contact:
 - ▶▶ Scott Vander Veen
 - ▶▶ AP Biology and Anatomy and Physiology
 - ▶▶ Valley Christian High School, San Jose, CA
 - ▶▶ svanderveen@vcs.net