



MARINE BIOTECHNOLOGY & BIOINFORMATICS FOR TEACHERS
MOSS LANDING MARINE LABS NSF ITEST GRANT
TEACHER LESSON PLAN FOR CLASSROOM USE
WHICH MUSSELS LIVES IN THE
SALT MARSH ESTUARY OF JAMAICA BAY?

Title of Lesson: Which Mussels Live in the Salt Marsh Estuary of Jamaica Bay?

Designed by

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Background

Students will be engaged in a nature study field trip involving collection and identification of local mussel species. Dissection and extraction of DNA from each species will follow. Students will then perform PCR, gel electrophoresis, photography and band analysis of different species. This is a multi-day lesson that can be extended into a several week unit on biotechnology and bioinformatics.

Description of Audience: This biotechnology/bioinformatics activity is designed for use by Grades10-12 and AP Biology.

State Standards: This biotechnology/bioinformatics activity fulfills the following State of New York Science Standards:

- Standard 1: Students will use mathematical analysis, scientific inquiry, and engineering design, as appropriate, to pose questions, seek answers, and develop solutions.
- Standard 2: Students will access, generate, process, and transfer information using appropriate technologies.
- Standard 4: Students will understand and apply scientific concepts, principles, and theories pertaining to the physical setting and living environment.
- Standard 6: Students will understand the relationships and common themes that connect mathematics, science, and technology and apply the themes to these and other areas of learning.
- Standard 7: Students will apply the knowledge and thinking skills of mathematics, science, and technology to address real-life problems and make informed decisions.

National Standards: This biotechnology/bioinformatics activity fulfills the following National Science Standards:

- Structure the time available so that students are able to engage in extended investigations.
- Create a setting for student work that is flexible and supportive of science inquiry.
- Ensure a safe working environment.
- Make the available science tools, materials, media, and technological resources accessible to students.
- Identify and use resources outside the school.

STEM Connection. Science careers: Forensics, Biotechnology and Bioinformatics, Naturalist, Biologist

Technology Integration. Gel Electrophoresis, Computer Bioinformatics.

Goals(s):

The goal of this lesson is to:

- Familiarize students with DNA technology and Gel electrophoresis
- Introduce students to Bioinformatics activities

Learning Objective(s)

Upon completion of this lesson, students will be able to:

- Extract and prep DNA for Gel Electrophoresis, learn the use of pipetters, learn how to make agarose gels, learn how to load gels, learn how to run and photograph gels and understand and interpret the results.
- Use Blast to search for sequences of *Mytilus* species.

Purpose/Rationale

To demonstrate the most scientific way of learning about living things and their genomes. The significance, relevance, and reason for teaching and learning this lesson is to make available science tools, materials, media, and technological resources which would otherwise be inaccessible to students. Students will become familiar with a plethora of lab procedures and activities.

Standards

To provide opportunities for both high school students to build the skills and knowledge needed to advance their study, and to function and contribute in a technologically rich society.

Materials/Resources

In order to complete this lesson, the following materials are needed for 40 samples:

| ☐ 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 µl 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

| ☐ Pipetters (1 set of 2-20 µl 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

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)

Prior Teacher Preparation

Purchases of necessary supplies, lab set up and organization of room for easy access, worksheet preparation and copying, and set of up LCD projector and computer.

Day1 Lab: Obtain and set up Qiagen Easy Clean kit at each station. Copy a modified instruction sheet.

Day 2 Lab: Set up for the addition of restriction enzymes and PCR. Copy a modified instruction sheet. Ask for help from an ITEST Center for doing the PCR and possibly the sequencing of the DNA samples later on.

Day 3 Lab: Set up for Gel Electrophoresis.

1. Purchase or prepare agarose gel. (Instructions below)

- Decide on amount of agarose needed. The concentration is .8%.
- Measure 1X TAE buffer using a graduated cylinder; add to flask.
- Weigh out agarose and add to 1X TAE buffer in flask.
- Add magnetic stir bar to flask and put on hot plate; set to medium heat with stirrer turned on. Heat until solution is completely melted (no visible particles).
 - Agarose solution can also be heated in a microwave. Heat for two-minute intervals, checking in between. Make sure flask is uncovered and sitting in an outer container in case of bubbling over.
- While agarose is melting, place casting tray in gel box so that there are four solid sides. Add combs with enough wells for samples plus markers.
- Using an insulated glove, carefully take flask off hot plate and let cool for about 3 minutes. If agarose begins to solidify, return to heat plate until dissolved, and cool again.
- Add Ethidium Bromide to cooled solution (1 μ l per 100 ml agarose solution).

- Pour agarose into casting tray so that comb teeth are partially covered. Remove any bubbles with a pipet tip.
- Let cool for about an hour (until milky) at room temperature. For faster cooling, move to 4 °C for 10 minutes after solid (about ½ hour) or immediately onto a level shelf at 4 °C for 20 minutes.

3-Step Procedure

#1 Introduction

- Prior knowledge of DNA extraction is preferable. Review the protocols.
- Go over prior learning on restriction enzymes and their functions.
- Vocabulary necessary for each day's lesson will vary each day. Some new words are: AGAROSE, ELECTROPHORESIS, RESTRICTION ENZYME, BUFFER, BASE PAIR, TAC POLYMERASE, POLYMERASE CHAIN REACTION, ANODE, CATHODE
- Use video clips on Restriction enzymes, gel electrophoresis, PPT on Gel Electrophoresis, handouts.
- Students form groups of 4 or 5 for lab activities, engage class discussion and procedures, and begin lab report.
- Students put on gloves and safety goggles.

#2 Exploration

- The path of inquiry involves the procedure and rationale for Collection, Dissection, Extraction, Cleaning, PCR, and Gel Electrophoresis.

Questions:

1. What safety precautions do we need for these Labs?
2. What is the best method for collecting mussels?
3. What are the parts of a mussel?
4. Why do we extract gill tissue?
5. Why do we need to "clean" the DNA?
6. What is PCR?
7. Why do we add restriction enzymes to the DNA before PCR?
8. How does the Electrophoresis apparatus function?
9. What charge does a DNA molecule have?
10. Which direction will the DNA go in the gel?
11. What does a "marker" do?
12. How can we record the results of running a gel?
13. Why do we use a UV light source to photograph the gels?
14. What do the resulting bands show?
15. Why do we see differences in the samples of DNA we have ran?
16. How can we use this information to track invasive species?
17. What is BLAST?
18. How can we find possible Mytilus species in NCBI?
19. How can we compare the species in CLustal?
20. What does a "tree" tell us about several species?

Day 1 Lab:

1. Using scientific collection techniques, collect different species of mussels.
2. Bring the mussels back to the classroom and dissect. Remove the gill tissue and place in plastic vials.
3. Use a commercially prepared "DNA Easy Clean" kit to purify the DNA.

Day 2 Lab:

4. Mix DNA with restriction enzymes.
5. Do the PCR process on the DNA.

Day 3 Lab:

1. Put on gloves and cover bare skin. *Ethidium Bromide is toxic and should not touch skin. It may be present on gel box and UV light box.*
2. Pipette 2 μl of loading dye ("dye dot") for every 10 μl of sample onto parafilm, taking care that they do not run into each other. Some samples (such as PCR products) may already have loading dye in them. If so, skip to step 5.
3. Carry your parafilm and DNA samples to the gel box.
4. Pipette 10 μl of your first DNA sample onto the first dye dot. Pipette up and down to mix.
5. Place a piece of colored paper under the gel box to make the wells in the gel easier to see. Set pipet to 10 μl volume, press plunger down to first stop and slowly aspirate your sample. This will leave behind some of your sample on the parafilm but will insure that no bubbles are in the pipet tip. With a very steady hand, insert pipet tip into well, taking care not to break through the bottom. Slowly dispense the 10 μl of DNA-dye mix into the gel well, allowing the sample to fill up the well. Dispensing the sample too quickly can cause the sample to spill out of the well. Avoid trapping air bubbles in the wells.

Take care not to touch gel box with bare skin, as residual Ethidium Bromide may be present.

6. Mark the gel-loading sheet with the sample name.
7. Repeat steps 4 through 6 until all DNA samples are loaded into gel. Return your original DNA sample tubes to the instructor for cold storage.
8. Once all DNA is loaded, the instructor will load the molecular weight marker onto the gel (1 to 2 lanes per row), put the top on the gel box (make sure that red top is with red bottom, and black is with black) and plug into power source. Set the power source and run for 1 hour at 80 volts.

LAB SHEET for students to record data, answer questions.

- Students answer questions about procedure, explain the process of Electrophoresis, predict results of the experiment, explore the results using the PPT created from the photographs. We compare with other gels used during the summer workshop. We compare species of mussels on the west coast and the east coast. We then tie in what we have learned with a discussion about invasive species. How can invasive species disrupt an ecosystem?
- Conclude by having students write a list of skills, ideas and future projects they would like to do with this technology. Evaluation takes place through grading of the Lab Reports and a test or quiz.

Day 4 and Day 5 Computer Classroom

1. Look up the sequences of the mussel species in the NCBI website. (Google NCBI and it will bring up the homepage).
2. Have them copy and paste 3 to 5 species sequences of similar DNA onto notepad.
3. Open the Clustal program and upload the sequences into the program.
4. Do an alignment and then draw a N_ J long branch tree.
5. Discuss the relatedness of the species.

#3 Application

- Learning to do Gel Electrophoresis is an essential skill found in many biotechnology careers.
- The value of this lesson was made relevant by discussing crime scene investigations, invasive species problems, and endangered and exploited species (Black sturgeon poaching, Chilean sea bass).

- Extension: Do ITS digest and CO3 DNA sequencing as part of the unit on Biotechnology and Bioinformatics.
- A good follow-up activity would be "How Many CATS" , a paper forensics activity showing band analysis .
- Provide relevant homework, class work, parent-involvement activity, research assignment...**TBD**

- **Career Connection:**

Have students go to the website: <http://science.education.nih.gov/LifeWorks.nsf/feature/indexhtm>

Have them report on one of the following careers

Biologist

Research or study basic principles of plant and animal life, such as origin, relationship, development, anatomy, and functions.

Medical Scientist

Conduct research dealing with the understanding of human diseases and the improvement of human health. Engage in clinical investigation or other research, production, technical writing, and related activities.

Biochemist

Research or study chemical composition and processes of living organisms that affect vital processes -- such as growth and aging -- to determine chemical actions and effects on **Microbiologist**

Investigate the growth, structure, development, and other characteristics of microscopic organisms, such as bacteria, algae, or fungi. Includes medical microbiologists who study the relationship between organisms and disease or the effects of antibiotics on microorganisms.

Industrial Production Manager (Drug Manufacturing)

Plan, direct, or coordinate the work activities and resources necessary for manufacturing medicinal and other health-related products.

Biological Technician

Assist biological and medical scientists in laboratories. Set up, operate, and maintain laboratory instruments and equipment, monitor experiments, make observations, and calculate and record results. May analyze organic substances, such as blood, food, and drugs.

Chemical Engineer

Apply the principles of chemistry and engineering to solve problems involving the production or use of chemicals; builds a bridge between science and manufacturing.

Immunologist

"an immunologist is a research scientist who investigates the immune system of vertebrates (including the human immune system

Genetic Counselor

Genetic counselors often work in clinical practice with prenatal, pediatric, adult, and/or cancer genetics patients.

Chemist

Conduct qualitative and quantitative chemical analyses or chemical experiments in laboratories for quality or process control or to develop new products or knowledge.

Pharmacist

Compound and dispense medications following prescriptions issued by physicians, dentists, or other authorized medical practitioners.

Assessment

- Completed Lab Worksheets, follow up quizzes (paper and on line QUIZ STAR)
- Design a worksheet, journal recording, test, quiz, or performance-based activity for students to demonstrate what they have learned. [To be done later](#)
- Have your Goals and Learning Objectives been met? [To be done later](#)
- How will you do to assist those who do not "get it"? Provide an alternative activity for a student with a special need. [To be done later](#)
- How might you extend the lesson, dig deeper, go beyond? [To be done later](#)

Please include several copies of students' work, ideas, journals, and completed lab sheets. Include copies of any text pages you used as well as any handouts, lab sheets, and workbook pages.

Teachers' Self Evaluation

Reflect on strengths and weaknesses of the lesson as taught.

- Describe individual student responses to techniques used. How did they react?
- Discuss student "thinking" and ideas.
- Include samples of students answers on lab sheet or journal entry (photocopy is fine).
- Ask students for a brief evaluation of lesson. Include their responses.
- Discuss fulfilled and unfulfilled expectations. Any surprises?
- In retrospect, how would you modify this lesson?