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The Gulf of Mexico Deepwater Habitats Expedition

Gellin

Focus
DNA analysis

Grade Level
9-12 (Life Science/Chemistry

Focus Question
What are some techniques used to study DNA?

Learning Objectives
Students will be able to explain and carry out a simple process for separating DNA from tissue samples.

Students will be able to explain and carry out a simple process for separating complex mixtures.

Students will be able to explain the process of restriction enzyme analysis.

Materials
- ❒ Electrophoresis chambers and power supplies (see "Learning Procedure")
- ❒ 1% agarose gel (Carolina Biological Supply No. WW-21-7075)
- ❒ TRIS/Borate/EDTA buffer (Carolina Biological Supply No. WW-21-9025)
- ❒ Gel casting trays (Carolina Biological Supply No. WW-21-3655)
- ❒ Four test tubes, each containing a pure food color, plus a fifth test tube containing a mixture of food colors; one set for each student group
- ❒ Five micropipets (Carolina Biological Supply No. WW-21-1022)
- ❒ Liquid dish detergent, approximately 100 ml
- ❒ Sodium chloride, approximately 100 g
- ❒ Fresh meat tenderizer, approximately 50 g
- ❒ Distilled water, approximately 2 l
- ❒ 95% ethanol
- ❒ Ice, crushed, approximately 3 kg
- ❒ Paper towels
- ❒ Plastic sandwich bag, one for each student group
- ❒ Student instruction handout for each student (see "Learning Procedure")
- ❒ OPTIONAL: additional supplies for electrophoresis of student-prepared DNA extracts (see Learning Procedure, Steps 5 and 6)

Audio/Visual Materials
- ❒ Marker board, blackboard, or overhead projector with transparencies for group discussions

Teaching Time
Three or four 45-minute class periods, depending upon the number of activities selected

Seating Arrangement
Laboratory groups of 2 – 3 students

Maximum Number of Students
30

Key Words
DNA
Electrophoresis
Restriction enzyme
Lophelia pertusa

Background Information

Deep-water coral reefs were discovered in the Gulf of Mexico nearly 50 years ago, but very little is known about the ecology of these communities or the basic biology of the corals that produce them. In contrast, deep-water coral reefs near the coasts of Europe have been intensively studied, and scientists have found a great abundance and variety of species associated with these communities. *Lophelia pertusa* is the dominant coral species in these communities. Technically, *Lophelia* is ahermatypic (non-reef-building), but branches of living coral grow on mounds of dead coral branches that can be several meters deep and hundreds of meters long. Unlike hermatypic corals that produce reefs in shallower waters, *Lophelia* does not have symbiotic algae and receives nutrition from plankton and particulate material captured by its polyps from the surrounding water. *Lophelia* mounds alter the flow of currents and provide habitats for a variety of filter feeders. Several commercially-important species are associated with *Lophelia* reefs in European waters, and scientists suspect that the same may be true for deep-water reefs in the Gulf of Mexico. But they don't know for sure, because most of these communities are almost entirely unexplored.

Most reports of *Lophelia* reefs in the Gulf of Mexico were the result of investigations directed toward hydrocarbon seepage and/or chemosynthetic communities. Scientists studying deep-water reefs on the Norwegian continental shelf have found that many large *Lophelia* banks occur at sites where there were relatively high levels of light hydrocarbons present in the sediments. The reason for this correlation is not known, nor is it known whether a similar correlation exists in the hydrocarbon-rich Gulf of Mexico.

As scientists have begun to learn more about *Lophelia* reefs, there is increasing concern that these reefs and their associated resources may be in serious danger. Many investigations have reported large-scale damage due to commercial fishing trawlers, and there is also concern about damage that might result from exploration and extraction of fossil fuels. The primary objectives of the Gulf of Mexico Deepwater Habitats Expedition are:

- to locate deep-water coral reefs in the Gulf of Mexico;
- to describe biological communities and geological features associated with these reefs; and
- to improve our understanding of the ecology of *Lophelia* and deep-water reef communities.

In addition to documenting the visible organisms associated with *Lophelia* reefs, the Gulf of Mexico Deepwater Habitats Expedition will also investigate microbial components of these communities. This research will involve examination of bacterial DNA. This lesson is intended to introduce students to several common DNA analysis techniques that may be used for studies ranging from deep sea biology to crime scene investigations.

Learning Procedure

[NOTE: This lesson is based upon activities designed by Ellen Averill, Karen Kyker, Sandy Collins, and Theresa Knapp while participating in the 1993 Woodrow Wilson Biology Institute. These activities are used with permission from the Woodrow Wilson National Fellowship Foundation. Visit http://woodrow.org for information on other activities and current programs. The restriction enzyme activity is obtained from the Access Excellence Classic Collection (http://www.accessexcellence.org)]

1. Download the following activities:
   - "Isolation of DNA from Onion" (http://www.woodrow.org/teachers/bi/1993/isolation2.html)
   - "Electrophoresis Analogy" (http://www.woodrow.org/teachers/bi/1993/electrophoresis.html)
   - "Rainbow Electrophoresis" (http://www.woodrow.org/teachers/bi/1993/rainbow.html)
   - "Desktop Electrophoresis Lab - Moving Molecules" http://www.woodrow.org/teachers/bi/1993/moving.html
   - "How Restriction Enzyme, Probes and RFLP's Work" (http://www.accessexcellence.org/AE/AEC/CC/word_activity.html)

There are two options for obtaining the equipment needed for the electrophoresis activity. The first is to buy electrophoresis chambers and power supplies from a laboratory supply company (cost approximately $400 and up). The second is to build your own chambers and power supplies as directed in the "Desktop Electrophoresis Lab" activity (cost approximately $20 per system).

2. Prepare detergent/salt solution, meat tenderizer solution, sodium chloride solution, electrophoresis gels, TRIS/Borate/EDTA buffer, and student instruction sheets (from the downloaded activities) prior to the lab. If you want to use the (lower cost) "Desktop Electrophoresis" apparatus, prepare the chambers and power supplies as well, unless you plan to have students do this.

3. Briefly review Background Information on the Gulf of Mexico Deepwater Habitats Expedition, and deep-water reefs. Be sure students understand that these reefs have a high diversity of species and large number of individual organisms like coral reefs in shallower water, but are virtually unexplored in the Gulf of Mexico. Compare and contrast deep-water reef corals (e.g., *Lophelia pertusa*) with reef-building corals in shallow water. Visit http://oceanexplorer.noaa.gov/explorations/islands01/background/islands/sup10_lophelia.html for more background on *Lophelia* reefs.

4. Tell students that bacteria are present in every biological community, and often are essential to fundamental community processes. You may want to briefly discuss chemotrophic communities and the role of bacterial symbionts as an example (see http://www.pmel.noaa.gov/vents/home.html for more information). Point out that despite their importance, bacteria are ignored in many ecological studies because of the difficulty of observing and identifying these organisms. Recent advances in techniques for analyzing DNA provide ways to overcome these difficulties, and are a powerful tool for many kinds of scientific investigations. Tell students that the purpose of these activities is to introduce three widely used techniques for studying DNA.

5. Have students complete the "Isolation of DNA from Onion" lab activity as directed in the student instructions.

   If you want students to use their DNA isolates for the following electrophoresis activity, have them transfer the DNA to a clean test tube, rinse with 70% ethanol to remove excess salts, then decant (pour off) the ethanol from the test tube. Add 0.5 ml distilled water to the test tube, cover, and refrigerate until the next lab period. Each student or student group should record their procedures and results in a lab notebook or written report.

6. Introduce the technique of electrophoresis using the "Electrophoresis Analogy." Have students complete the "Rainbow Electrophoresis" lab activity as directed in the student instructions. Each group should prepare written answers to the questions included in the activity, either in a lab notebook or separate written report.

   If you are having students use their DNA extracts for this activity, have them put 85 µl of their extract into a clean test tube, add 15 µl TRIS/Borate/EDTA buffer, and load the electrophoresis gels as directed in the student instruction sheets. Run the gels at 81 volts (use nine batteries in the power supply) for about one hour. Stain the gels by soaking overnight in a 0.02% solution of methylene blue in distilled water. Procedures and results should be recorded in a lab notebook or written report.

7. Introduce the technique of restriction enzyme cleavage using the word analogy activity described in "How Restriction Enzyme, Probes and RFLP's Work." If you want to do an actual restriction enzyme cleavage procedure, visit the University of Arizona's Biotech Project website (http://biotech.biology.arizona.edu/default.html) for detailed instructions.

The Bridge Connection

http://www.vims.edu/bridge/reef.html;
http://www.vims.edu/bridge/lesson.html

The "Me" Connection

Have students write a short essay describing how DNA analysis could be useful or important to their own lives.

Connections to Other Subjects

English/Language Arts, Chemistry

Evaluation

Written reports prepared in Step 6 provide an opportunity for assessment.

Extensions

Log on to http://oceanexplorer.noaa.gov to keep up to date with the latest Gulf of Mexico Deepwater Habitats Expedition discoveries, and to find out what explorers are learning about deep-water coral communities.

Visit http://www.woodrow.org/teachers/bi/1993/ for more activities related to biotechnology from the 1993 Woodrow Wilson Biology Institute.

Biological supply companies have a variety of materials and kits suitable for other DNA research techniques.

Resources

http://oceanica.cofc.edu/activities.htm – Project Oceanica website, with a variety of resources on ocean exploration topics

http://biotech.biology.arizona.edu/default.html – Website for the University of Arizona's Biotech Project

http://www.dnaftb.org/dnaftb/ – An animated primer on the basics of DNA, genes, and heredity from the DNA Learning Center at the Cold Spring Harbor Laboratory

http://www.woodrow.org/teachers/bi/1993/ – Background and activities from the 1993 Woodrow Wilson Biology Institute on biotechnology

Roberts, S. and M. Hirshfield. Deep Sea Corals: Out of sight but no longer out of mind. http://www.oceana.org/uploads/oceana_coral_report.pdf — Background on deep-water coral reefs

National Science Education Standards

Content Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard B: Physical Science

- Chemical reactions

Content Standard C: Life Science

- The cell
- Molecular basis of heredity
- Interdependence of organisms
- Matter, energy, and organization in living systems

Content Standard E: Science and Technology

- Understandings about science and technology

Content Standard F: Science in Personal and Social Perspectives

- Natural resources
- Science and technology in local, national, and global challenges

For More Information

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