Sea Turtle CSI

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Grade Level: High School 9-12
Lesson Groupings: Individual & Small Groups

State Standards
ALCOS (Alabama Course of Study): Genetics
7. Describe the structure and function of DNA, including replication, translation, and transcription.

- Applying the genetic code to predict amino acid sequence
- Describing methods cells use to regulate gene expression
- Defining the role of RNA in protein synthesis.

9**.** Differentiate among major areas in modern biotechnology, including plant, animal, microbial, forensic and marine.

- Examples: hybridization, cloning, insulin production, DNA profiling, bioremediation
- Describing techniques used with recombinant DNA
- Examples: DNA sequencing, isolation of DNA segments, polymerase chain reaction, gel electrophoresis

National Standards

NS.9-12.3 LIFE SCIENCE
As a result of their activities in grades 9-12, all students should develop an understanding of

- The cell
- Molecular basis of heredity
- Biological evolution

NS.9-12.5 SCIENCE AND TECHNOLOGY
As a result of activities in grades 9-12, all students should develop

- Abilities of technological design
- Understandings about science and technology

NS.9-12.6 PERSONAL AND SOCIAL PERSPECTIVES
As a result of activities in grades 9-12, all students should develop an understanding of

- Personal and community health
- Population growth
- Natural resources

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- Environmental quality
- Natural and human-induced hazards
- Science and technology in local, national, and global challenges

Ocean Literacy the Essential Principles of Ocean Sciences

- 5. The ocean supports a great diversity of life and ecosystems.
- 6. The ocean and humans are inextricably interconnected.

http://www.coexploration.org/oceanliteracy/documents/OceanLitChart.pdf

http://www.education-world.com/standards/national/science/9_12.shtml#ns.9-12.1

Topic

Deoxyribonucleic acid, or DNA, consists of four bases, a sugar and a phosphate. In the 1950s, DNA became known as the source for heredity. The structure of the DNA molecule was unknown until James Watson, Francis Crick, Maurice Wilkins and Rosalind Franklin discovered the molecular structure. "The DNA molecule consists of two sugar-phosphate strands, with one nucleotide base connected to each sugar. The strands are connected to each other by hydrogen bonds that form between the bases. The base adenine (A) always pairs with thymine (T), and guanine (G) with cytosine (C). Paired strands of bases are twisted around each other to obtain the shape of a double helix."

"In 1977, two methods for sequencing DNA were introduced." The Sanger method is the most popular technique for sequencing DNA, because it is easier. "Sequencing with the Sanger method was carried out using radioactivity for detection, a process which took at least a day and a half to obtain 12 sequences, each sequence being 300 base pairs long."

Individual sequences are difficult to obtain unless they are amplified to make many copies. In the early 1980s there was still no method to amplify specific units of DNA in an easy and fast way. The gene or DNA sequences under study had to be "cloned" into bacteria. The bacteria were cultivated, and later the bacterial DNA with the amplified gene was extracted. However, this method took several days to complete for every sequence of interest.

DNA is located in the nucleus of all cells. To analyze DNA, it has to be removed from the cell and separated from the rest of the particles. This is the process for DNA isolation.

In the first step in DNA isolation tissues have to be collected and ground up. "Then an enzyme called proteinase K is added to digest the proteins of the cell. Proteinase K breaks down the proteins and releases the DNA into solution." The next step is to add chemical reagents such as phenol and chloroform and centrifuge the sample. The DNA is dissolved in water and can be removed. "Alcohol and salts are added to force the DNA out of the solution. Sometimes, if there is a huge amount of DNA, the DNA can even be seen by the naked eye. It looks a little bit like white slime." The mixture is put in a centrifuge to concentrate it into a pellet of pure DNA. The pellet is diluted to a desired concentration.

"Researchers usually do not want to look at all the DNA sequences; instead they are concerned with the study of specific gene sequences. Even in the case of concentrated DNA, there are only a small number of copies of any one gene present, which is not enough for effective sequencing. This problem was solved with the invention of PCR."

"The basic principle of PCR is to amplify a target sequence millions of times" so there is more DNA to analyze. The first step in PCR is to separate the DNA double stranded molecule by heating it. Then the solution is cooled to allow primers, two small pieces of synthetic DNA, each complementing a specific sequence at one end of the target sequence, to attach to its complementary sequence. Polymerases start at each primer and copy the sequence of that strand. Within a short time, exact replicas of the target sequence have been produced. In new cycles, double-stranded molecules of both the original DNA and the copies are separated; primers bind again to complementary sequences and the

polymerase replicates them. At the end of many cycles, an exponential number of pieces of DNA that have the target sequence are available for analysis.

The DNA is then run through gel electrophoresis where DNA of different sizes are separated using electricity and a gel matrix that separates according to size, allowing the smaller pieces to move through the gel matrix faster than longer pieces. There are two different types of gels used. “To detect PCR fragments, agarose gels are used (which look like white-grayish Jell-O). For the detection of the products of sequencing reactions, very high-resolution gels are needed that are made out of polyacrylamide. The gel is different, but the principles of analysis are the same. A comb-like device is used to poke holes or slots in the gels in which different DNA samples are loaded.”

“The entire gel is then placed in a chamber filled with a buffer solution that contains salts. Electrodes are on both the bottom and the top of the chamber. One electrode is connected to a negative charge and the other to a positive charge in order to create an electrical current. (DNA) Deoxyribonucleic acid is negatively charged. When an electric field is applied, the DNA will migrate toward the electrode with the positive charge. However, large DNA fragments will take longer to pass physically through the gel matrix than small ones. The latter are able to go through the pores in the matrix more easily. Thus, if one does a PCR of a 500-base pair (bp) DNA fragment, it will run more slowly than a PCR of a 100-bp DNA fragment. “

Researchers run a DNA standard that consists of DNA fragments of known sizes at the same time, in the same gel, to see and compare the size of the fragment that has been amplified.
The simplest and shortest sequencing technique is “direct” sequencing of the PCR product.
Only a fraction of the whole PCR product is used to identify the order of bases in the DNA strand being studied. Scientists are looking for the amino acids that are coded by a triplet code. “Modern DNA sequencing is a chemical procedure that allows one to determine the order of bases of DNA.” It follows the Sanger method, but it is an automated procedure that allows the generation of enormous amounts of data.

“The procedure of sequencing is similar to the PCR reaction; the only major differences are that only one primer is used (and therefore the reaction is not exponential), and a fraction of the nucleotides added are modified in a way that stops the elongation of the DNA strand randomly. These nucleotides are also labeled with a fluorescent dye, which is needed for detection.”

Once the sequence is determined, it is compared to other known sequences. “For example, if a researcher were sequencing a gene that might play a role in cancer, he or she would want to know if the sequence in a tumor cell is different from the sequence in a normal cell. In many evolutionary biology studies, the researcher wants to determine how organisms are related to each other.” Over time, DNA can change. This is called a mutation. “Closely related organisms will have DNA sequences that are more similar than those of distantly related organisms. This information allows researchers to compare the differences between alleles, individuals, populations and finally, species, by looking at how similar or how different their DNA sequences are.”

Source:
History of Molecular Lab Techniques by Dr. Claudia Englbrecht
http://sunsite.berkeley.edu/biotech/pcr/whatisPCR.html

Curriculum Links

Mendelian Genetics
DNA Extraction/Isolation
Gel Electrophoresis

Objectives

Understand realistic applications for the techniques introduced.
Describe the structure of DNA.
Explain DNA replication, Transcription, and Translation.
Apply PCR technique to model strands of DNA.

Materials:

Computer
Sea Turtle CSI handout
LCD projector/screen
Twizzlers – pull 'n' peels
Spice Drops - 4 colors to represent A, T, C, G
*Laminated colored pieces to represent A, T, C, G
*Laminated white strips
Haplotype key
Unknown Template Cards
BioEdit Program
PowerPoint Presentation
Lecture materials
PCR Signs
Scissors
Tooth picks
Tape dispensers
Paper plates

(*If candy is not allowed, use laminated colored pieces and white strips to create your DNA molecules.)

Time
90 minutes

PCR Simulator

Scenario: You are Investigators with The US Fish and Wildlife Service. Your department has seized meat believed to be from Loggerhead Sea Turtles (*Caretta caretta*). The population distribution is in the temperate and subtropical waters around the world. Sea turtles travel long distances from their natal beach but return once fully mature to breed and lay eggs. Therefore, the Loggerhead Sea Turtles have a variety of haplotypes, or genetic identifications, in their mitochondrial DNA according to their home beaches. In other words, you can ID where the turtle was born.

Procedure:

Students should wash their hands before starting this exercise.

First we will create a beginning DNA strand.

1. Print and cut out the Unknown Template Cards.
2. Label the back of each card with Unknown #1 - Unknown #6. (you may want to laminate cards for future use)
3. Pass one Unknown Template Card to each student.
4. Place the Twizzlers, toothpicks and 4 colors of Spice Drops on paper plates.
5. Name the spice drops to represent the nucleotide bases (one color for each base; adenine, cytosine, guanine and thymine)
6. Spread plates out on different tables around the room.
7. Have students build their DNA molecule to match their Unknown Template Cards.
8. Connect two different colored spice drops together using the toothpicks.
9. Be sure to match A (adenine) with T (thymine) and G (guanine) with C (cytosine).
10. Connect the toothpicks with the spice drops (nucleotide bases to the Twizzlers) so that the candy pieces are between the two strands.
11. Holding the ends of the Twizzlers, twist the structure slightly.
12. You now have your beginning DNA strand.

Next we will design Primers for the PCR Simulation

13. Students should recognize the 3' and 5' ends of their DNA strands.
14. They will then design Primers for their DNA.
15. Cut a Twizzler in half and connect the 3 complementary nucleotides (spice drops). (OR use laminated white strips and colored pieces to build your primer)
16. Several Primers should be made. These will then be placed on a paper plate, and later on the table for the annealing step of the simulated PCR.
17. Set up the room as if it were a Macro PCR.
18. Spread the PCR signs/steps around the room on separate tables.
19. Place candy and toothpicks on the *Extension* table. (OR laminated white strips and colored pieces).
20. Place the primers on the *Annealing* table.
21. Have the students begin the process of running through the PCR with their Unknown DNA Strand.
22. After the students are able to recognize the exponential product growth, stop their "PCR".
23. Have them do the math to see how many strands they should have after __ cycles.
24. Lay out the Known Haplotypes and get the students to figure out which one matches their DNA.

BioEdit

In this exercise, DNA Extraction and Isolation would occur first. Next, we perform a PCR. Then, with the PCR product, Gel Electrophoresis is completed. Next, we send our Sea Turtle DNA gel off to be sequenced. Once we get it back, we will use BIO EDIT to help figure out the haplotype.

1. On your computer open the BIO EDIT program.
2. Click FILE, scroll to OPEN, Select the file CORD Loggerhead June 06 then your unknown file# (Each group has their own#)
3. Next Click on FILE, Scroll to IMPORT, SEQUENCE ALIGNMENT FILES. You are looking for ALL FILES in file type. Click on HAP A, then while holding the shift key. Click on HAP J this should choose/highlight all sequences. And OPEN.
4. Click on VIEW, VIEW MODE, NORMAL
5. Click on ALIGNMENT, PLOT IDENTITIES TO FIRST SEQUENCE WITH A DOT

The first sequence listed is your unknown. All the others are compared to the unknown. Letters indicate a difference in sequence and a dot means a match. If a line is all dots, then this matches your unknown.

Haplotypes

Hap A	North Carolina South Carolina Florida Georgia
Hap B	Georgia Florida Mexico Greece
Hap C	Gulf of Mexico side Florida Mexico
Hap D	Brazil
Hap E	SE Florida
Hap F	Greece
Hap G	Gulf of Mexico side of Florida
Hap H	Mexico
Hap I	Mexico
Hap J	Mexico

Source:
UAB -CORD Summer science Institute Laboratory, Sea turtle Genetics, An experiment in Molecular Biology, By Thane Wibbels, PhD and Jenny Estes M.S.