Environmental Science Institute
THE UNIVERSITY OF TEXAS AT AUSTIN

1 University Station C9000
Austin, TX 78712
(512) 471-5847
www.esi.utexas.edu

DNA Mass Production

Lesson plan for grades 6-12
Length of lesson: 60-85 minutes (1.5 class periods)
Authored by: Brotee Rahman, Environmental Science Institute, 5/17/2013

SOURCES AND RESOURCES:

- Andrew Ellington – Diagnosing Ourselves
  http://www.esi.utexas.edu/k-12-a-the-community/hot-science-cool-talks/diagnosing-ourselves-biotechnology-in-your-back-pocket
- National Center for Biotechnology Information (NCBI) Polymerase Chain Reaction (PCR) Overview:
  http://www.ncbi.nlm.nih.gov/projects/genome/probe/doc/TechPCR.shtml
- *Device Helps Find DNA 'Needles' In Genetic 'Haystack'
  http://www.the-scientist.com/?articles.view/articleNo/9912/title/Device-Helps-Find-DNA--Needles--In-Genetic--Haystack-/

POTENTIAL CONCEPTS TEKS ADDRESSED THROUGH THIS LESSON:

§112.34. Scientific Processes, Grades 9-12 c2A
§112.34. Scientific Skills, Grades 9-12 c6A

PERFORMANCE OBJECTIVES (in order of increasing difficulty to permit tailoring to various age groups):

Students will be able to:

- Make a basic list or flowchart of the components needed in a PCR
- Explain why amplification of DNA is important, and how it can be used in real-world applications

MATERIALS (per group of two-four students):

- Computer with Microsoft Word and an overhead
- Long strip of paper (long enough to wrap around the classroom's walls made of several smaller pieces of 8.5 x 11 paper) to represent the genome
- Notecards given to students with A, T, C, G in a combination of 2 letters (AT, CG, GA, TC, etc)
- Blank notecards for the students (10-20 for each group of students) as future DNA templates
- Color markers for illustrating DNA sequences on the paper used to construct the genome
- Masking tape

Environmental Science Institute
THE UNIVERSITY OF TEXAS AT AUSTIN

1 University Station C9000
Austin, TX 78712
(512) 471-5847
www.esi.utexas.edu

CONCEPTS:

Genome: the entire DNA sequence of an organism

DNA: the initial template of an organism's genetic blueprint, which gets transcripted to RNA. DNA is made of nucleotides T, G, C, A (Thymine, Guanine, Cytosine, and Adenine)

RNA: the template made from DNA (which uses U, Uracil, instead of T, Thymine) which is processed by ribosomes into proteins

Nucleotide: building blocks of DNA and RNA. Triplets of nucleotides from RNA will make up amino acids that make up

Gene of resistance: A small DNA sequence, which gets transcribed (in other words, translated) to RNA, which is then used to manufacture a protein (specifically, antibodies). This protein is what the organism uses to defend against an illness or disease.

Assay: method of getting quantitative information about the presence of a microorganisms, cells, or viruses in a sample

PCR (Polymerase Chain Reaction) is a method that can to copy a segment of DNA by over 1,000,000 fold. The purpose of copying a DNA segment is to "amplify" the segments presence, thereby making it easier to detect by laboratory instruments or tests.

There are two key components in a PCR vital to copying a specific segment within the huge DNA strand (the part of the genome targeted for copying): 2 primers, which are strands of a nucleic acid that serve to mark the beginning and end of the segment we wish to copy, and DNA polymerase as the enzyme; a molecule that copies the segment using free nucleotides (C, G, T, A).

Once the primers locate the small sequence (maybe 200-300 nucleotides long) out of 1,000,000 nucleotides (about 1/4000 of the genome we need), the polymerase will make many copies so instead of struggling to see a single copy of the sequence, we have a gigantic pile of DNA segments with which we can scan the sequence and detect it easier. The best analogy for this is "finding a needle in a haystack, then making a stack of needles from the one needle"* (See the third bullet in Sources and Resources).

Environmental Science Institute
THE UNIVERSITY OF TEXAS AT AUSTIN

1 University Station C9000
Austin, TX 78712
(512) 471-5847
www.esi.utexas.edu

BACKGROUND:

Molecules – special structures made of atoms - contain a code of instructions guiding how all living things are constructed and function. Those molecules are made of DeoxyriboNucleic Acids (DNA) and the scientific field of genetics focuses on DNA and all of its components and processes. Almost all living things, including microscopic organisms and that cause sickness and disease - have cells with the genetic code DNA. When dealing with diseases, we sometimes have to look to different organisms aside from humans in order to develop vaccines. Out of a certain organism's entire DNA sequence, we only need the "gene of resistance". Compared to the entire sequence, the gene of resistance is a very small set of genetic codes. This small DNA sequence gets transcribed to RNA, which is then translated to protein (specifically, antibodies), which is what the organism needs to resist illness or disease.

This specific combination of C, G, T, and A nucleotides in the DNA strand is so small, we need a special way to detect its presence (there exists no magnifying glass for DNA sequences with only a few strands). The gene must be reliably detected in order to report its presence as part of a diagnosis or other medical assessment! In order to detect the gene, scientists have devised a way to make a tremendous number of copies of the sequence, thereby "amplifying" its presence. The best way to achieve this amplification in a normal lab setting is via a Polymerase Chain Reaction (PCR), which is the focal concept of this lesson. An effective PCR sets the stage for a detection test called an Assay. Based on the analogy previously discussed, the PCR has made "a stack of needles", for which the Assay can detect "within a haystack"!

Dr. Andrew Ellington talks about amplification in his April 2013 *Hot Science - Cool Talks* lecture (minute mark 14:29). His lab is working to detect a multi-drug resistant tuberculosis gene. Dr. Ellington and his lab members often encounter challenges a problem that even with amplification. Assays are often still not sensitive enough to detect the tuberculosis gene (minute mark 18:34), or even when amplified over 1,000,000-fold, there are too many impurities or it can take as long as 20 hours (minute mark 20:40) to register a detection. Even in PCR, impurities can be amplified as well, showing how challenging this area of medical science is.

PREPARATION: (10 minutes)

First, the teacher (with student help) can construct a long strip of paper made by attaching several sheets of 8.5 x 11 paper together (using either tape or staples to attach the sheets together). Students will fill in random combinations of C, A, T, and G before the actual activity onto the sheet wrapped around the room.

1 University Station C9000
Austin, TX 78712
(512) 471-5847
www.esi.utexas.edu

Have a computer than can shown on the overhead with the web page (below) on a news article. This will be to show the students how the Find, Copy, and Paste functions are essentially what a PCR does.

http://www.dailytexanonline.com/person/andrew-ellington

Make notecards with the 2-letter combinations as described in materials to act as primers for the students primers (see Concepts section)

ENGAGE: (15 minutes)

Teacher Asks: "Do you think it easy to see one single bacteria with the naked eye or a microscope?" Allow the class to respond.

"The answer is 'No' – it is impossible in many cases to do that. Do you think it might be easier to see a whole pile of bacteria? Like when a colony grows over a petri dish?"

"In this case, it's easier to see the bacteria. The same can be said with DNA. Does anyone know what DNA is? If so please provide a description to the class"

Make sure the class is familiar with these terms:

1) DNA – organic blueprint of an organisms made up of nucleotides C, T, G, A
2) Genome – entire DNA sequence of an organism
3) Nucleotide – C, T, A, G which make up amino acids for proteins. A DNA sequence is displayed as many nucleotides (Ex: CTAGCTGATCTTAGCTAGTCG)
4) DNA Polymerase – the main enzyme used to copy DNA

1 University Station C9000
Austin, TX 78712
(512) 471-5847
www.esi.utexas.edu

5) Primer – needed for DNA Polymerase to attach to the DNA template and make copies. Without a primer, DNA Polymerase cannot start replication

However, the molecules that make up DNA are even smaller than bacteria! So how might we try to see more of it so it's easier to study?" Allow the class to try answering the question.

Teacher Answers: "We make more DNA! In almost all cases of research and medicine, we are not interested in making copies of the entire DNA strand – we are just interested in segment of it that is responsible for causing something like an illness. Making copies of a DNA segment is the essence of something called a Polymerase Chain Reaction (PCR) – this is an essential part of modern medical science. "

Teacher's PCR Analogy Demonstration:

Teacher Says: "I'd like to show you an Analogy for how a PCR reaction functions"

On the computer, have an article ready to view so it is visible on the overhead. Now out of this whole article, all we want is the author's name. Copy the whole article text, and paste it onto the Notepad application on your computer (available on almost any machine under "Accessories", so the text no longer has spacing or formatting). It should be emphasized to students that an entire DNA strand has no format aside from being a giant sequence of letters. Now we can read through the whole article, or use something to locate the name instantly. The search function run by pressing "Ctrl+F" on the keyboard at the same time in Notepad allows us to instantly find the author's name without reading through the whole article. Then, the command "Ctrl+C"(copy) and "Ctrl +V" (paste) allows us to make as many copies of the name as we want onto another document. This is how PCR works in a very simplified way.

EXPLORE: (20 minutes)

Class PCR Targeting Analogy Demonstration

1. Teacher says: "Now we will perform class simulation of a PCR on a larger scale, ***with a focus on finding specific parts of a DNA sequence***: Here, the different components of a DNA strand will be labeled and visualized.

2. On the premade strip of paper wrapped around the room, students will fill in C, T, A, and G in random orders all over the paper. Have 8 characters per sheet, with each student doing multiple sheets until the whole strip is filled. The size of the paper might (and should) seem overwhelming to the students.

1 University Station C9000
Austin, TX 78712
(512) 471-5847
www.esi.utexas.edu

3. Group the students into groups of 2-4 students each. Each group will get 2 notecards with the 2-letter combos. These will be primers that the students have to locate on the giant strip and find DNA segments whose beginning and ending consist of these primers.

4. Students will find segments using their pair of primers as the beginning and ending of the segments (they can choose which primer can be the beginning or ending). They will copy the specific C-A-T-G sequences down onto their empty notecards and mark where it was on the original giant strip using initials for their group members.

**********while this is going on, the teacher should not only make sure the students are doing the activity properly, but also scan the sheet to look for short DNA segments (preferably seen more than once on the sheet, so about 4-5 nucleotides, or even shorter if repetitions cannot be found) to give to the students to look for themselves in the next activity*********

5. After the students have gotten used to using primers to locate random sequences along the DNA strand, the teacher will give each group a certain sequence (a "target") to find in the giant strip (these target sequences should consist mostly of the repeated segments the class used when making the giant strip).

6. When the students find the "target" DNA sequence, they need to use the right primers to mark the beginning and end of the sequence before copying the sequence onto their notecards. The students can access the notecards for all primers. Students should use masking tape to attach the correct primers to their "target" DNA sequence.

EXPLAIN : (15 minutes)

The teacher should ask the following questions to the class and have them explain in their own words.

Teacher says "I'd like to ask you some questions about what you observed while finding different parts of the DNA sequence – these are the "targets" that might be pieces of DNA that would be copied many times in order to be detected by a chemical test.

- What do the giant strip of paper (genome), notecards (Primer), and the students (DNA Polymerase) represent in terms of a PCR?
- When you had two primers, were there multiple DNA segments with similar beginnings and endings but differed in the main sequence? There probably were. Example sequences like **AG**CTAG**TG** and **AG**GGAT**TG** (same primers for beginning and ending, but different sequences in the middle)

1 University Station C9000
Austin, TX 78712
(512) 471-5847
www.esi.utexas.edu

- In a PCR, when using 2 primers, only one segment is found instead of multiple different segments. What changes in the experiment can be done to pinpoint only one segment? Make a longer primer that can only recognize the sequence of interest.

ELABORATE: (15 minutes)

- Now, from the sequence the students had to find given by the teacher (the last activity), the students will make several copies of that sequence onto sheets of paper, and tape those onto the genome wrapped around the room. This will reinforce that students are acting as the DNA Polymerase.
- Now ask students to find some of the replicated sequences. Ask if the multiplication of those sequences made it easier to find among the whole genome than if there was no multiplication.
- Since the multiplication made it easier to find sequences, detection for the "target" strand is much easier, and the detection is called the "assay."
- To make the amplification seem more useful, say that we want to know if a certain strand on our genome wrapped around the room actually exists. If only one copy exists, then while we do know it exists, it can be easy to not see it. The PCR activity makes it much easier to detect the strand and not miss it.

EVALUATE: (10 minutes)

- How is the PCR similar to the "needle in a haystack" analogy? In terms of the PCR, what is the needle and what is the haystack? (needle is target sequence of interest and haystack is genome)
- Trying to connect the article example with the activity and an actual PCR: how was a target amplified in each? (Copy and Past/Ctrl + C and Ctrl + V, the students, and DNA Polymerase respectively)