Sequencing

Description of activity

Sequencing provides students with an opportunity to use web-based resources and in-class activities to understand modern methods of DNA sequencing. Students will work together to determine the sequence of a hypothetical segment of DNA. Students can also make a bookmark that illustrates two methods of visualizing DNA sequence.

Learning Outcomes

Students will:

1. observe how cycle sequencing works using the animations on DNAi.
2. understand one way scientists solve problems
3. work together to model the molecular processing involved in the most commonly used method of DNA sequencing

Assumptions of Prior Knowledge

This activity is best done after students are familiar with some other techniques used in molecular biology research such as gel electrophoresis and PCR.

Both of these techniques lend themselves nicely to teaching the principles of cycle sequencing.

Misconceptions

Students sometimes have trouble understanding that each peak on the electropherogram represents many DNA molecules of the same size, and not just a single DNA molecule.

Students may also not understand that DNA polymerase forms phosphodiester bonds on the sugar-phosphate backbone and *NOT* the hydrogen bonds between the bases. The bases (A, C, T, and G) represented in this activity really consist of a base, a sugar, and a phosphate.

Implementing the Lesson

Become familiar with the *DNA Interactive (DNAi)* web site (www.dnai.org) and how to navigate through it. Provide students with information about navigating the site, and how to play animations and video clips.

Before class:

Photocopy the *Sequencing* worksheet and a *DNA sequence* bookmark template for each student. There are two versions of the bookmark. If you are not able to print and photocopy in color, use the "B&W bookmark" template. Make five copies of the student worksheets and five copies of both template sheets.

Arrange for students to have access to a computer connected to the Internet for at least part of the class (ideally a computer each, or one for each team).

Gather the following materials:

- scissors (for each student);
- clear cellophane tape (five rolls);
- 20 cups;
- five sets of four colored pencils/markers: red, blue, green and yellow (for sequencing and the sequencing bookmark);
- card stock (for Part II, the bookmark); and
- colored yarn (for Part II, the bookmark).
- glue (for Part II, the bookmark)

During class:

Sequencing

Give each student a copy of the *Sequencing* worksheet. For Part I of the sequencing simulation, divide the students into five groups. Give each group a copy of both handouts.

Direct students to follow the instructions on their worksheets. The instructions with the computer symbol on the left indicate that students need access to animations or materials from the DNAi web site to complete the step.

Run Part II of the *Sequencing* activity with students working together as a single group. Use a blackboard or any empty wall space as the model gel.

DNA sequence bookmark

Give each student a copy of the bookmark template. NOTE: The sequences are different on the left and right sides of the bookmark.

Looking at the "bar code" version of the sequence on the right side of the bookmark, explain to the students that the sequence is read from the bottom up (from the smallest to longest fragments).

Have students work in teams of two. One student can read the sequence and the second can record it along the bottom of the bookmark in the spaces provided. The recorder will also be the timer and record the amount of time it takes to finish the sequence. The two students should then switch so that each one has the chance to read and record their sequence. Then have the students determine their reading rate in nucleotides per minute.

Discuss with the class how the use of automated sequencing speeded up the process. Use clips from www.dnai.org > **Genome** > **The Project** > **players** > **technology** to show the workings of an automated sequencer. Discuss the advantages of cycle sequencing e.g. the use of fluorescent dyes to identifying each base, and the use of computers to compile sequence data, and the speed that is now possible with current sequencing machines.

If students are using the black and white copy of the bookmark have them use their four colored markers to color the electropherogram next to the sequence on the gel. The color key is located at the top of each bookmark. Direct students to glue their paper template to card stock (if provided) or just to fold the bookmark in half and glue it together. The holes at the top of the bookmark are for punching and threading a piece of colored yarn to form a tassel.

Further Explorations

Sanger Method Discovery Activity

Ask the students to design an experimental method for determining the sequence. This time they will not have access to an automated sequencing machine (represented by the drawing of the electropherogram). Inform students that they can still use electrophoresis, but the only way they will be able to see the different bands is through radioactive labeling which doesn't distinguish between fragments tagged with different types of dideoxynucleotides All the bands will look the same. This activity should lead students to design a sequencing method similar to the one originally used by Fred Sanger. A different cycling reaction was set up for each nucleotide and four adjacent lanes on a single polyacrylamide gel were run simultaneously. Each lane represents a different letter in the sequence.

Writing

Have students pretend to be a scientist in the 1980s asking the Federal Government for a grant to fund the sequencing of the entire human genome (Human Genome Project). Write a letter that provides convincing arguments for why the government should approve the use of public money for this project.

Investigating the Sequencing Methods in the Human Genome Project (HGP)

Write a report that explains how automated cycle sequencing affected the way scientists in both the public and private sectors finished the Human Genome Project. Students should refer to **www.dnai.org > Genome > The Project.**

Glossary

Dideoxynucleotide

dNTP (Dexoynucleotide Triphosphate)

Electropherogram

Polyacrylamide

Thermocycler

DNA polymerase

Phosphodiester bond

Denature

Anneal

Resources

Web

Cold Spring Harbor Laboratory (2002). *DNA From the Beginning: an animated primer on the basics of DNA, genes, and heredity*, www.dnaftb.org

Howard Hughes Medical Institute *Biointeractive* http://www.biointeractive.org > *Virtual Bacterial Identification Lab*

Human Genome Management Information System (2003). *DOEgenomes.org: genome programs of the U.S. Department of Energy Office of Science*, doegenomes.org

Video/DVD

DNA Interactive (2003). NTSC version produced by Cold Spring Harbor Laboratory and Red Green & Blue Company; funded by Howard Hughes Medical Institute. Available at www.dnai.org

Article

Sanger, F. (1981). Determination of nucleotide sequences in DNA. *Science*. Dec 11;214(4526): 1205-10.

Books

Micklos, David A., Freyer, Greg A., and Crotty, David A. (2003). *DNA Science: A First Course, (2nd Edition)*, Cold Spring Harbor Laboratory Press, New York.

Watson, James D., with Berry, Andrew (2003). *DNA: The Secret of Life*, Alfred A. Knopf, New York.

Richard Robinson (2003). *Genetics*, Macmillan Reference USA, New York.

Activity pages include:

Student worksheets: nucleotide template; primer and sequence template page; question sheets.

Bookmark templates: black and white (B&W); and color.

Answer sheets.