Teacher Notes: Deadly Letters: The Anthrax Mystery

In this activity, your students will investigate a fictional anthrax case using paper to model the **Sanger method of DNA sequencing**. Modeling #4 of the DNA sequencing technique outlined below, each student group will receive an envelope containing strips of paper that represent the incomplete copies of anthrax DNA from a suspected lab. Then, modeling #5 of the DNA sequencing technique, students will sort these strips to determine the DNA sequence from that lab. Finally, as a class, students will compare their sequences to the one found in the fictional anthrax-laced letter to determine which lab is guilty.

Background Information

To solve the mystery of the anthrax-laced letters, as presented in the *Anthrax* video, investigators turned to technology to identify the research lab where the harmful bacteria originated. First, they determined which strain of *Bacillus anthracis*, the bacterium that causes anthrax in humans, was in the letters. Yet even after pinpointing the specific type, they realized that many research laboratories around the world have that particular strain. So investigators needed to analyze the anthrax in a more detailed way.

A. How mutations occur during cellular replication

The investigators suspected that the bacteria's DNA might be slightly different in some laboratories. Why? As the *Bacillus anthracis* was distributed to different labs over the years, the cells grew and reproduced, replicating their own DNA with each division. During normal DNA replication, the DNA's double helix unzips into two strands. At that point, each strand can act as a template. Nucleotides arrange themselves one by one, in complementary pairs corresponding to the sequence on the templates, with adenine (abbreviated A) matching thymine (T), and cytosine (C) matching guanine (G). Sometimes, however, a small error occurs, and the wrong nucleotide inserts itself in the sequence. The result is called a mutation. While some mutations can change how a cell's DNA functions, most have no effect. Cells with such benign mutations can function just as well as cells without the mutation. However, if the mistake is not reversed by the cell's repair mechanisms, the resulting copy of DNA (and all subsequent copies) will be different from the original. Mutations are useful because they can help scientists identify differences within a single strain of anthrax.

B. The science behind identifying the suspected lab

In the anthrax case, the scientists used DNA sequencing technology to determine the exact sequence of the DNA obtained from each suspected lab. The result was a string of approximately five million nucleotides, represented by As, Ts, Gs, and Cs, from each lab. After comparing these sequences, scientists found a handful of differences among the suspected labs. And when they compared the labs' results to the sequence contained in the letters, they struck gold—the *Bacillus anthracis* DNA in the letters matched the DNA sequence from a lab at the US Army Medical Research Institute for Infectious Disease (USAMRIID) in Fort Detrick, Maryland.

C. Summary of a common DNA sequencing technique

The Sanger method is a well-known DNA sequencing technique whose main procedures are:

1: Mix the components—In a test tube, scientists combine template DNA, nucleotides (A, T, G, and C), helper proteins, and special fluorescent versions of A, T, G, and C.

2: Build DNA strands—The helper proteins bind to the template DNA and pull nucleotides out of the solution to build a new DNA molecule, nucleotide by nucleotide.

3: Incorporate a fluorescent nucleotide—Eventually a fluorescent A, T, G, or C will attach to the end of the strand. The fluorescent versions interrupt and stop the copying process. Because the process is interrupted at random places, before long the test tube contains many incomplete copies of the DNA strand, each one of a varying length and each one ending with a fluorescent A, T, G, or C.

4: Sort the strands—A sequencing machine sorts the copies by length and "reads" which fluorescent nucleotide is at the end of the strand.

5: Determine the sequence—By knowing the length of the strand and which nucleotide is at the end of the DNA strand, scientists can determine the sequence. For example, if a strand is five nucleotides long and the one at the end is a "T", then the fifth position must be a "T." Similarly, if a DNA strand has 20 nucleotides, and the one on the end is a "G," then position 20 must be "G."

Glossary

Consider providing these terms to students.

- **Anthrax:** a very rare but deadly disease caused by the bacterium *Bacillus anthracis*
- ***Bacillus anthracis*:** the scientific name of the bacterium that causes anthrax
- **DNA:** a molecule, known as deoxyribonucleic acid, that contains the genetic instructions for the development and functioning of organisms
- **Nucleotides:** the four building blocks of DNA: adenine, guanine, cytosine, and thymine
- **Mutation:** a change to the DNA sequence of an organism
- **DNA sequencing:** a process used to determine the order of nucleotides in DNA

Answer Key

Step 2: The DNA differences from lab to lab would have been caused by mutations.

Step 4: Sample description of the main steps of the Sanger method of DNA sequencing. Student answers will vary, but they should include:

- Scientists combine template DNA, nucleotides (A, T, G, and C), and fluorescent nucleotides.
- Nucleotides line up to complement the DNA template.
- Eventually, a fluorescent nucleotide gets added, stopping replication and creating many incomplete copies of DNA.
- A machine sorts fragments by size and reads the fluorescent nucleotides.

Step 5: Depending on the sequence students received, they will have one of the following:

- TGACAATCGG (the "innocent" sequence, determined by all the groups but one)
- TGACAATCAG (the "guilty" sequence, determined by only one pair of students)

Step 6: As you review students' answers, check that every sequence has ten nucleotides. At this point, do not tell students whether or not they have the "guilty" sequence; simply check for accuracy. Answers should read either **TGACAATCGG** (innocent) or **TGACAATCAG** (guilty).

Step 9: The match identifies the source of the fictional DNA found with the letter.

Step 10: One difference is that the investigators compared five million nucleotides, while the class only compared ten.

Step 11: (a)

"Innocent" DNA:	TGA-CAA-TCG-G
Transcribed to mRNA codons:	ACU-GUU-AGG-C
Translated to amino acids:	threonine-valine-arginine-
"Guilty" DNA:	TGA-CAA-TCA-G
Transcribed to mRNA codons:	ACU-GUU-AGU-C
Translated to amino acids:	threonine-valine-serine-

Check for Understanding:

Use the Teacher Notes information to determine how well your students are able to explain how they solved the anthrax mystery, particularly as it relates to the Sanger method for DNA sequencing.