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AG/ENVIRONMENTAL

Community Science

Tech 2: Sanger Sequencing

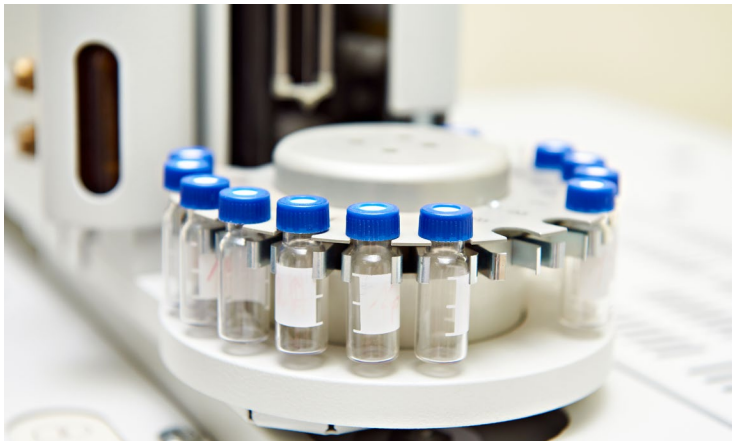
Developed in partnership with:

Discovery Education and Ignited

In this Lesson Plan:

Print the **Teacher Section** →

Print the **Student Section** →



The image shows a biochemical analyzer in a science laboratory.

—

Cover Image
Bacteria in a water sample is a potential source of environmental DNA (eDNA).

This document is separated into two sections, For Teachers [T] *and* Student Resources [S]*, which can be printed independently.*

Select the appropriate printer icon above to print either section in its entirety.

Follow the tips below in the Range field of your Print panel to print single pages or page ranges:

Single Pages (use a comma): T3, T6

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AG/ENVIRONMENTAL / COMMUNITY SCIENCE

Technical (Tech) Lesson 2: Sanger Sequencing

DRIVING QUESTION

How does Sanger sequencing work as a DNA identification technique?

OVERVIEW

The most cutting-edge and advanced biotechnology techniques are the product of years of work and are only possible due to the technologies that came before them. As the techniques become more complicated, faster, and more readily available, the scientific discoveries that support them tend to become more complicated. In order to understand how DNA identification works, it is necessary to start with the foundational technology that was developed by Frederick Sanger.

In this lesson, students are presented chromatograms that are the result of automated Sanger sequencing, and must infer their purpose. They will then model what happens in Sanger sequencing. After the modeling activity, they will read about Sanger sequencing with a partner and each will be responsible for identifying the pros and cons of the technique. The final portion of the lesson will be to add what they learned about the process of Sanger sequencing to their DNA Identification Technologies table that is part of the Decision Tree Assessment.

ACTIVITY DURATION

Two class sessions
(45 minutes each)

ESSENTIAL QUESTIONS

How can Sanger sequencing be used to answer questions related to DNA identification?

How does Sanger sequencing connect to other DNA tools we have learned about (BLAST, PCR)?

OBJECTIVES

Students will be able to:

Explain the purpose of Sanger sequencing.

List types of information that can be gathered using Sanger sequencing.

Evaluate the pros and cons of Sanger sequencing as a DNA identification technique.

Materials

- **Four Color Chromatogram Capture Sheet**
- **Modeling Sanger Sequencing Capture Sheet**
- **Baggies of beads (four colors with two different-sized or -shaped bead for each color)**
- **Background Reading: Sanger Sequencing**
- **Technology Overview Capture Sheet, Part 1: DNA Identification**

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.

SOCIAL-EMOTIONAL LEARNING

Students must use self-discipline and self-motivation to stay on task during the lab work. Students need to communicate clearly with their groups and with other groups to produce the decision tree and provide feedback. This lesson also asks students to listen actively, cooperate, and work collaboratively to problem-solve, negotiate conflict constructively, and seek or offer help when needed. Students will work on making a reasoned judgment on which technique to use after analyzing information, data, and facts.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

This lesson connects to real-world issues identified by students in their own communities, as Sanger sequencing will be an option to include in their final proposal. In making their decision tree, students will need to be aware of possible biases and combat the influences of those biases in having a negative or inequitable impact on community groups.

ADVANCING INCLUSIVE RESEARCH

Because of our wide geographic dispersal, various genes have evolved in different ways. In order for scientists to create solutions that help all people, they must first map the DNA sequence for that gene, and collect representative samples from as diverse a participant pool as possible. In this lesson, students will discuss the limitations of technological advances that are created when certain communities are excluded or left out of the research process.

COMPUTATIONAL THINKING PRACTICES

Students will understand how automation works in Sanger sequencing technology. Students will extract key information about Sanger sequencing to complete the lesson. Depending on the lab chosen, students will also collect data, use digital tools to analyze them, and represent data in various ways to facilitate problem-solving and decision making.

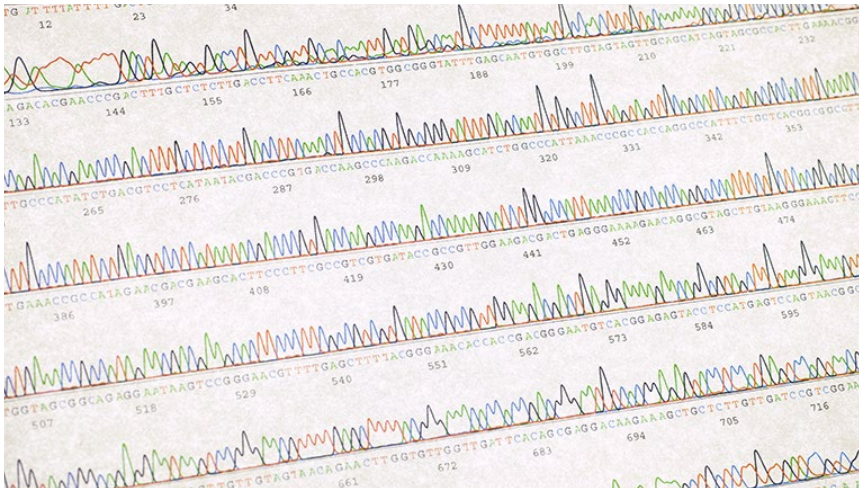
CONNECTION TO THE PRODUCT LIFE CYCLE

In this lesson, students learn about Sanger sequencing and compare it to other DNA sequencing methods. This dive into the science and technology principles behind GE products connects to the **discover** aspect of the product life cycle, as students learn about biotechnology lab procedures.

Have you ever wondered…

How do scientists figure out the sequence of DNA?

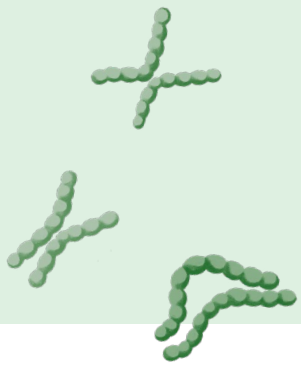
The order of nucleotides (A, T, C, and G) that a strand of DNA can provide is one of the first steps to figuring out the source of the DNA. Determining the sequence of DNA is a technology that has been advancing rapidly to become less expensive and quicker. In order to understand the newest sequencing technologies it is essential to understand the beginning of sequencing. The first widely used sequencing technique is Sanger sequencing.



MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

This lesson is the second lesson in a series on major technologies used in DNA identification. Students will be selecting among these technologies as they prepare their final artifact proposals.



How does this connect to careers?

Laboratory technicians and ***biotechnologists*** are responsible for carrying out the laboratory procedures used to collect and analyze DNA. They are responsible for following quality assurance protocols, keeping up with evolving laboratory protocols, and collecting raw data. Sanger sequencing is a common tool used by many laboratory technicians.

How does this connect to our world?

Sanger sequencing is the foundation for how scientists determine the order of DNA nucleotides. It is becoming more obsolete, but it is necessary to fully understand the sequencing work that is done in most labs.

Sanger sequencing will be one of the options students can select when designing their final artifact for the unit.

Day 1

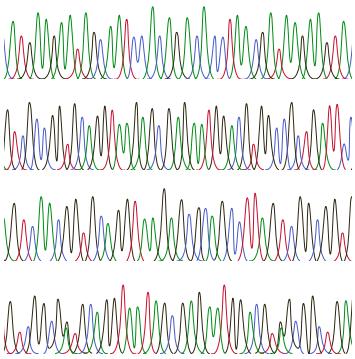
Procedure

LEARNING OUTCOMES

Students will be able to:

Explain the purpose of Sanger sequencing.

List types of information that can be gathered using Sanger sequencing.



Whole Group (10 minutes)

1	Start the class by activating prior knowledge about DNA and reminding students of the work done in DNA Technologies (Tech) Lesson 1: DNA Recap.
2	Hand each student one of the chromatograms from the *Four Color Chromatogram Capture Sheet*. These should be printed in color. Ask students what they think this is.

Teacher Note > *The chromatograms do not have the associated nucleotide labeled, just the colored peaks.*

3	After students guess, write the following on the board: Black-G, Red-T, Blue-C, Green-A. Once again, ask students what they think they are holding.
4	Now ask students to decode their "picture". Students can do this on the bottom of their slips of paper, or they can do it on another piece of paper to save the copies for future use.
5	Finally, lead into the modeling lab by telling students that these are called four color chromatograms and are what automated DNA sequencers make to show the results of a sequencing run. Explain that these automated sequencers use a technique called Sanger sequencing and that the students will act as the computers for today's modeling activity.

Small Group (35 minutes)

In small groups, students will complete a Sanger sequencing modeling activity by using the *Modeling Sanger Sequencing Capture Sheet* and baggies of beads.

Teacher Note > *There are a variety of Sanger sequencing "dry labs" widely available, using everything from purchased kit materials to pop beads, paper models, and case studies. The one in the Modeling Sanger Sequencing Modeling Capture Sheet is a simple example of modeling that can be used, or others can be found with an Internet search.*

INDUSTRY AND CAREER CONNECTION

Remind students that modeling lab techniques before using them can help us understand the results, and that this predictive stage is an essential part of the work of lab technicians and scientists in creating protocols.

Day 2

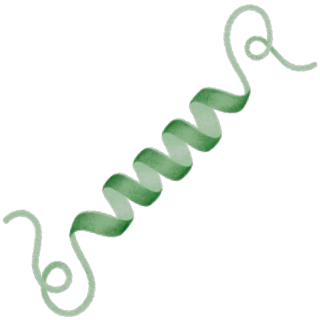
Procedure

LEARNING OUTCOMES

Students will be able to:

Evaluate the pros and cons of Sanger sequencing as a DNA identification technique.

Small Group or Individual Work (30 minutes)	
1	Students will read this short article from the Nobel Prize organization on the biochemist *Fredrick Sanger*, who won two Nobel Prizes for his work on proteins and DNA sequencing.
2	Next, students will evaluate the effectiveness of Sanger sequencing using the *Background Reading: Sanger Sequencing*. While reading the article, one student in the pair should write out the pros for Sanger sequencing and while the other partner identifies the cons.
Individual Work (15 minutes)	
1	Introduce the *Technology Overview Capture Sheet, Part 1: DNA Identification*. This table will also be used in DNA Technology (Tech) Lessons 3 and 4 to summarize student learning about two additional DNA identification techniques. The first time students fill out the table for Sanger sequencing, it will be used as a formative assessment of their learning.
2	After students fill out the tables, collect them and give individual feedback or go over the Sanger sequencing section as a class. In subsequent lessons, this capture sheet will be used as a summative assessment so students will need to understand how to evaluate the pros and cons of the technique and provide a summary of how it works.



National Standards

Next Generation Science Standards	**LS-1-1 From Molecules to Organisms: Structures and Processes** Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.
	ETS1-2 Engineering Design Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.
	Science and Engineering Practices Analyzing and interpreting data Analyze data to identify design features or characteristics of the components of a proposed process or system to optimize it relative to criteria for success.
Career and Technical Education (CTE)	**A3.1** Use data to explain how biotechnology fields such as pharmaceuticals, agriculture, diagnostics, industrial products, instrumentation, and research and development are impacting human life.
	A1.6 Explore and outline the various science and non-science fields and careers associated with biotechnology.
	A5.2 Use a variety of methods, including literature searches in libraries, computer databases, and online for gathering background information, making observations, and collecting and organizing data.

Modeling Sanger Sequencing Capture Sheet

Part 1: Modeling the Sequencing-PCR Product

ANSWER KEY **Do not share with students**

1. Use the small beads from the bag to make the template strand of DNA by putting the nucleotides (beads) in order. Fill in the key below for what base each color represents.

DNA Sequence										
Template Strand	C	G	A	A	T	A	T	G	C	G
Nontemplate Strand	G	C	T	T	A	T	A	C	G	C

Adenine	Responses will vary
Thymine	Responses will vary
Cytosine	Responses will vary
Guanine	Responses will vary

2. The template strand is the DNA you will use to copy with Sanger sequencing (selected by the primer used). The process is a modified PCR. In the PCR tube you have your sequence of interest, dNTPs, ddNTPs for one of the four bases, the primer, and the rest of the reagents needed for PCR. As the thermocycler heats and cools, the dNTPs are added to the template strand to copy. Sometimes, however, instead of adding a dNTP, a ddNTP is added which stops the production of that fragment. The end result is a mixture of many different-sized fragments. Fill in Segment 4 with the correct sequence using the formulas below.

Segment (n)	Primer + (n-1) dNTP bases + 1 ddNTP base

Segment 1	Primer + 1 ddNTP base
Segment 2	Primer + 1 dNTP base + 1 ddNTP base
Segment 3	Primer + 2 dNTP bases + 1 ddNTP base
Segment 4	Primer + 3 dNTP bases + 1 ddNTP base

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Modeling Sanger Sequencing Capture Sheet

Part 1: Modeling the Sequencing-PCR Product

ANSWER KEY **Do not share with students**

Continued

3. Putting the segments in order by size and repeating the process for all the bases (using the other three ddNTPs, one at a time) reveals the sequence of DNA. Let's model this process.

   In your baggie you will notice a larger bead for each color. That is the ddNTP which will terminate the copying of the DNA.

   Continuing from the primer below, make the shortest possible fragment of DNA that would be made with the complete Sanger sequencing process (with all possible ddNTPs). *Hint: The ddNTP for this first, shortest one will be T.*

   Fill in this fragment below, or take a picture of the beads that represent it.

Shaded boxes indicate the primer bases. Note, this is a shortened version of the primer that would actually be used. Typically, primers are ~20 nucleotides long.

Template Strand	C	G	A	A	T	A	T	G	C	G
Shortest fragment of DNA	G	C	T	T						

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Modeling Sanger Sequencing Capture Sheet
Part 1: Modeling the Sequencing-PCR Product

ANSWER KEY **Do not share with students**

Continued

4. Continue the process below, using the smaller beads for the dNTPs and the larger beads for the ddNTPs. When the DNA segment is fully sequenced, you will have a total of seven different-sized fragments, and they will all start with the mini primer above. Fill in the segments below, or take a picture of the beads that represent them.

Template Strand	C	G	A	A	T	A	T	G	C	G
Fragment 1 *Shortest fragment of DNA*	G	C	T	T						
Fragment 2	G	C	T	T	A					
Fragment 3	G	C	T	T	A	T				
Fragment 4	G	C	T	T	A	T	A			
Fragment 5	G	C	T	T	A	T	A	C		
Fragment 6	G	C	T	T	A	T	A	C	G	
Fragment 7	G	C	T	T	A	T	A	C	G	C

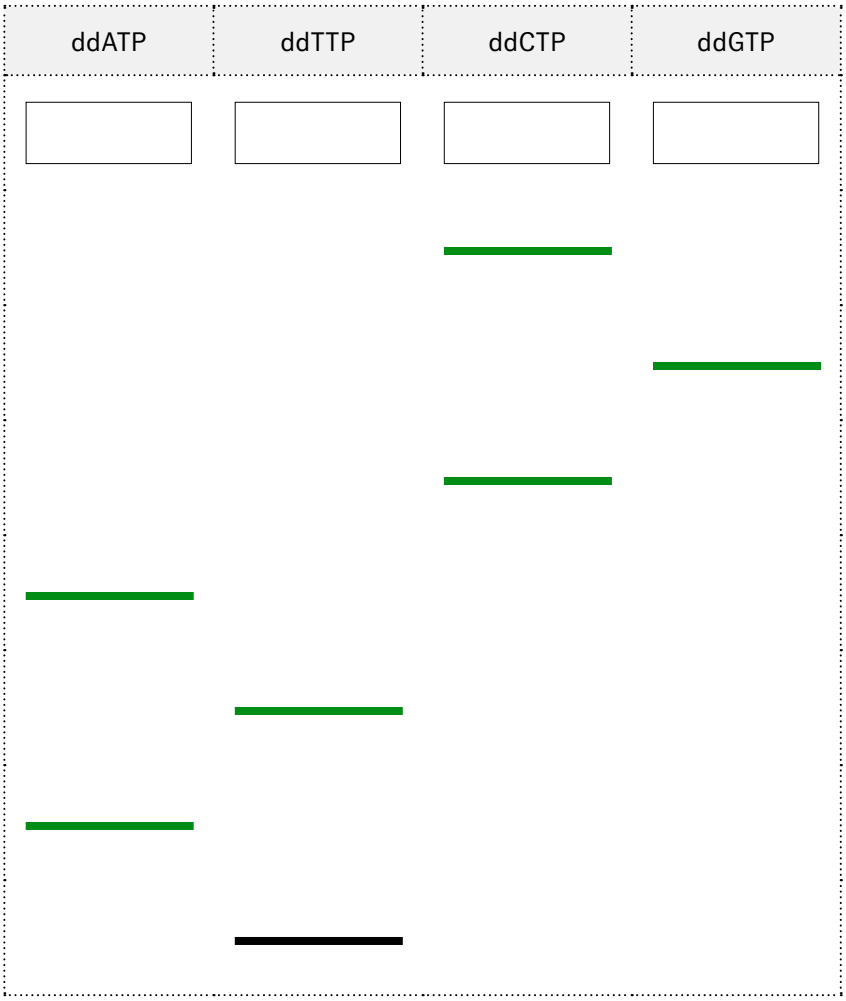
Modeling Sanger Sequencing Capture Sheet

Part 2: Hypothesizing the Gel from the PCR Product

ANSWER KEY **Do not share with students**

1. In order to see the different-sized segments, technicians or scientists used to use gel electrophoresis. Each lane in the gel represents the outcome of the Sanger sequencing PCR process using a different ddNTP (A, T, C, G). The size of the fragments on the gel and the lane they are in reveal the sequence.

   Use the simplified diagram of the gel below to hypothesize where each of the bands from the seven segments on the previous page would be located.



Example: The first band is four base pairs long and ends with ddTTP.

Positive end of gel chamber

Technology Overview Capture Sheet

Part 1: DNA Identification

ANSWER KEY **Do not share with students**

Directions

After each technology lesson, use the corresponding table to summarize what you learned about that DNA identification technique. Save this page for comparison in Lesson 9.

Tech Lesson 2		**Sanger Sequencing**	
Describe	Summarize how this technique works.	This process determines the sequence of DNA nucleotides through PCR. As the PCR reaction copies the fluorescent chain, terminator nucleotides mark the end of different segment lengths. The segment lengths are put in order and a program reads the end nucleotide fluorescence to determine sequence.	
Discuss	List the pros and cons you identify for the technique.	Pros	Cons
		Easily accessible	Slow
		Most established technology	Expensive per region you want to sequence
		Good at cloning individual genes	Can only sequence the target region because you have to design a primer
		Easily manipulated for initial plasmid research	
		Good for checking that insertion of a gene in a plasmid worked	
Support	Provide examples.	Forensics—animals or humans Genotyping—Determining presence of different alleles (sequences) Determining viral variants Other examples not mentioned in the lesson do exist.	

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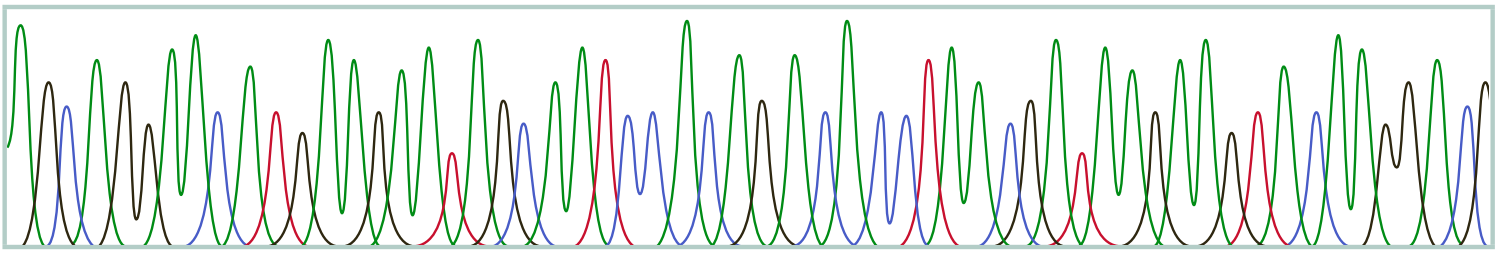
Four Color Chromatogram Capture Sheet

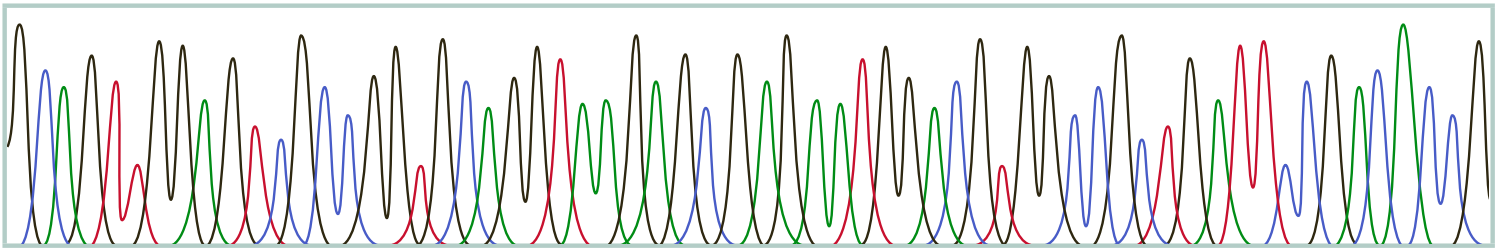
Directions

Print this page with a color printer, if possible, so that you see the black, red, blue, and green peaks in each of the four examples. Select one of the four examples to analyze.

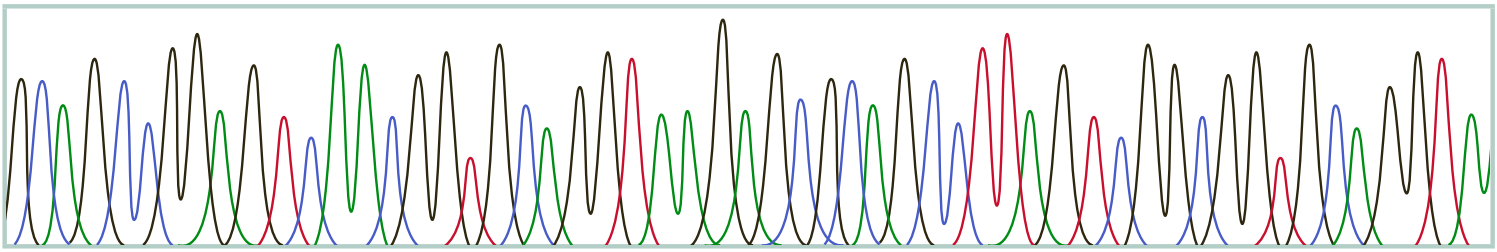
Example 1



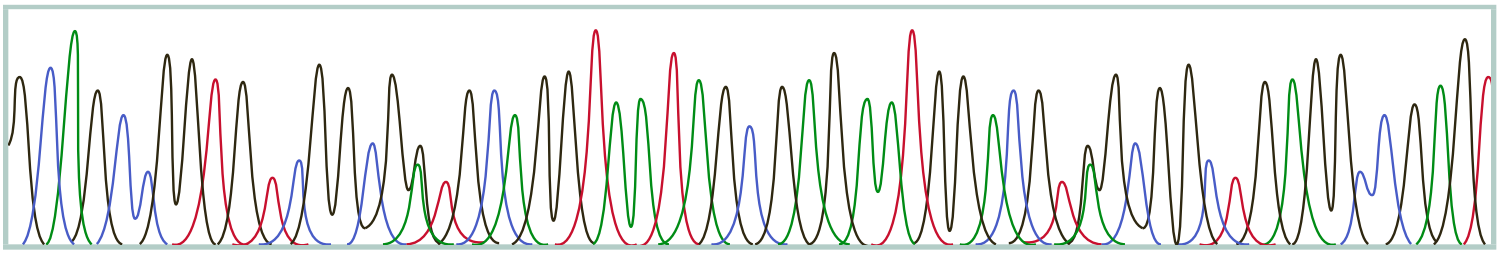
Example 2



Example 3



Example 4



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Modeling Sanger Sequencing Capture Sheet

Directions

The goal of this modeling activity is to determine the sequence of a template strand of DNA using a method that models Sanger sequencing.

Background Information

For this activity, you are going to do things a little backwards. Normally you would have a segment of DNA and then you would try to figure out the sequence using the Sanger method. In this case, however, you will be given an extra short sequence of DNA and will need to determine the polymerase chain reaction (PCR) product that would be the result of Sanger sequencing (Part 1). Then you will hypothesize what those products would look like on a gel (Part 2).

As you complete the following steps it is important for you to remember what you have learned in previous units about the process of PCR and gel electrophoresis. Please refer back to your notes or resources if you need a reminder. In addition, use the important vocabulary listed here as needed.

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Important Vocabulary

dNTP	*(deoxyribonucleotide triphosphate)* These are the nucleotides with three phosphate groups that are the building blocks of DNA, and are used in PCR reactions. dATP, dCTP, dGTP, and dTTP are needed to copy DNA. Because Sanger sequencing is a modified PCR reaction, these are necessary reagents.
ddNTP	*(dideoxynucleotides triphosphates)* For Sanger sequencing, these are used as a substance to stop the copying of DNA. This is possible because ddNTP lacks the hydroxyl group needed to build on the next DNA base. Similarly to dNTPs, they are named after the nucleotides (ddATP, ddCTP).
Template strand	This is the sequence of DNA that is used as the template or the directions that will be copied to determine the sequence.
Primer	This is a short, single-stranded DNA sequence that provides a starting point for DNA synthesis in PCR. When using the PCR technique, a pair of primers is used to hybridize with the sample DNA and flank the target region of the DNA that will be amplified. In Sanger sequencing, only a single primer is used.

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Modeling Sanger Sequencing Capture Sheet

Part 1: Modeling the Sequencing-PCR Product

1. Use the small beads from the bag to make the template strand of DNA by putting the nucleotides (beads) in order. Fill in the key below for what base each color represents.

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Template Strand	C	G	A	A	T	A	T	G	C	G
Nontemplate Strand	G	C	T	T	A	T	A	C	G	C

Adenine	
Thymine	
Cytosine	
Guanine	

2. The template strand is the DNA you will use to copy with Sanger sequencing (selected by the primer used). The process is a modified PCR. In the PCR tube you have your sequence of interest, dNTPs, ddNTPs for one of the four bases, the primer, and the rest of the reagents needed for PCR. As the thermocycler heats and cools, the dNTPs are added to the template strand to copy. Sometimes, however, instead of adding a dNTP, a ddNTP is added which stops the production of that fragment. The end result is a mixture of many different-sized fragments. Fill in Segment 4 with the correct sequence using the formulas below.

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Segment 4	

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Modeling Sanger Sequencing Capture Sheet

Part 1: Modeling the Sequencing-PCR Product

Continued

3. Putting the segments in order by size and repeating the process for all the bases (using the other three ddNTPs, one at a time) reveals the sequence of DNA. Let's model this process.

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   Continuing from the primer below, make the shortest possible fragment of DNA that would be made with the complete Sanger sequencing process (with all possible ddNTPs). *Hint: The ddNTP for this first, shortest one will be T.*

   Fill in this fragment below, or take a picture of the beads that represent it.

Shaded boxes indicate the primer bases. Note, this is a shortened version of the primer that would actually be used. Typically, primers are ~20 nucleotides long.

Template Strand	C	G	A	A	T	A	T	G	C	G
Shortest fragment of DNA	G	C	T							

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Modeling Sanger Sequencing Capture Sheet

Part 1: Modeling the Sequencing-PCR Product

Continued

4. Continue the process below, using the smaller beads for the dNTPs and the larger beads for the ddNTPs. When the DNA segment is fully sequenced, you will have a total of seven different-sized fragments, and they will all start with the mini primer above. Fill in the segments below, or take a picture of the beads that represent them.

Template Strand	C	G	A	A	T	A	T	G	C	G
Fragment 1 *Shortest fragment of DNA*	G	C	T							
Fragment 2	G	C	T							
Fragment 3	G	C	T							
Fragment 4	G	C	T							
Fragment 5	G	C	T							
Fragment 6	G	C	T							
Fragment 7	G	C	T							

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Modeling Sanger Sequencing Capture Sheet

Part 2: Hypothesizing the Gel from the PCR Product

1. In order to see the different-sized segments, technicians or scientists used to use gel electrophoresis. Each lane in the gel represents the outcome of the Sanger sequencing PCR process using a different ddNTP (A, T, C, G). The size of the fragments on the gel and the lane they are in reveal the sequence.

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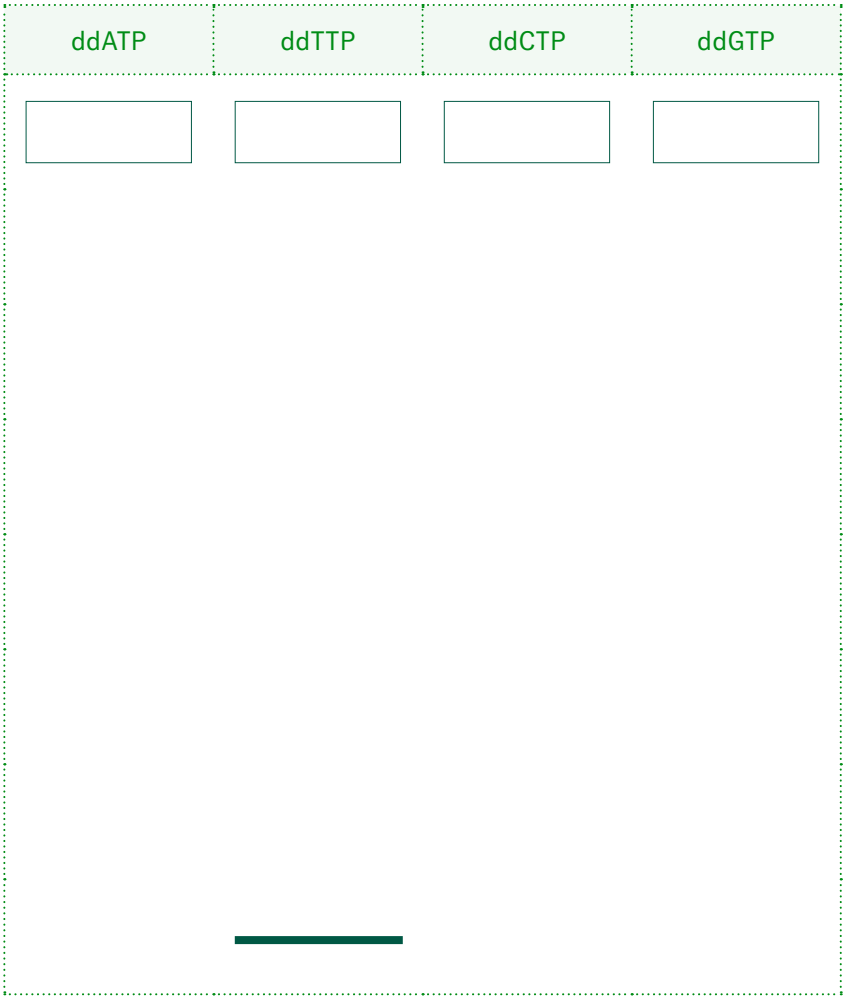

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Background Reading: Sanger Sequencing

Sanger sequencing has been the standard for DNA sequencing for the last 30 years. Over the past few years, this method of sequencing has been largely replaced by newer sequencing methods, which allow for the rapid generation of large amounts of sequence data. Sanger sequencing is still widely used in small-scale experiments. It can also be used for segments of DNA that aren't easily sequenced with newer sequencing methods, such as highly repetitive DNA regions.

All sequencing platforms determine a DNA sequence by finding the order of base pairs in small segments of the DNA called "reads". The reads are then put together to form the full sequence. Sanger sequencing reads are usually 800–1000 base pairs long, while newer sequencing methods have shorter reads. Read length is crucial to full sequence assembly, because those are pieces of the sequence puzzle. When a genome is complex and repetitive (same sequences over and over again) it is hard to assemble from short reads or small puzzle pieces. This is the case for the human genome, which is complicated because it is repetitive.

Sanger sequencing can navigate around these shortcomings. Sanger sequencing platforms can allow up to 50,000 reads to be generated at once, while also being low cost. It is also important to note since Sanger sequencing was one of the first effective methods of sequencing there are more facilities with that technology. Sanger sequencing technology is currently easier to access than newer more expensive methods.

Sources: *Sanger sequencing is not dead?—Wired*

Frederick Sanger, a biochemist who won a second Nobel Prize in Chemistry for a method to sequence DNA, referred to as Sanger sequencing.

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Technology Overview Capture Sheet

Part 1: DNA Identification

Directions

After each technology lesson, use the corresponding table to summarize what you learned about that DNA identification technique. Save this page for comparison in Lesson 9.

Tech Lesson 2		**Sanger Sequencing**	
Describe	Summarize how this technique works.		
Discuss	List the pros and cons you identify for the technique.	Pros	Cons
Support	Provide examples.		