

STUDENT ACTIVITY

Secrets of Sequencing

Project Details

GRADE RANGE

9–12

TIMING

2 45–60 minute classes

OVERVIEW

In this activity, students will learn how genomic sequencing is an important tool in the fields of microbiology and infectious diseases, and how it can be used to quickly determine what type of pathogen is affecting a person’s health. After learning about what next generation genomic sequencing is, students will form small groups and each group will be assigned a case where a patient has suddenly come down with a mysterious illness. They will build “reads” that are generated by next gen sequencing, and use the reads to assemble short, fragments of a genome. Each group will then use an online bioinformatics tool called BLAST (Basic Local Alignment Search Tool) to identify what type of pathogen their nucleotide sequence is from to diagnose their patients. They will research the pathogen and design a treatment plan for their patient.

OBJECTIVE

Students will explore Next Generation Sequencing and how it can be used for a variety of applications.

MATERIALS NEEDED

- Device with the ability to project
- Student devices (iPad, laptop)
- Student Sheet: **Secrets of Sequencing—NEXT GENERATION SEQUENCING BACKGROUND**
- Student Sheet: **ACTIVITY: MODELING NGS SEQUENCING and DATA ANALYSIS**
- Solid colored beads
- Pipe Cleaners
- Clear colored beads
- Student Sheet: **Patient Report: Diagnosis and Treatment Plan**

NEXT GENERATION SCIENCE STANDARDS

- HS-ETS1-3. Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics, as well as possible social, cultural, and environmental impacts.
- HS-LS1-1. 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.
- HS-LS3-2: Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

STANDARDS FOR TECHNOLOGICAL LITERACY

Standard 3: The Nature of Technology

K. The rate of technological development and diffusion is increasing rapidly.

L. Inventions and innovations are the results of specific, goal-oriented research.

Standard 14: Medical Technologies

K. Medical technologies include prevention and rehabilitation, vaccines and pharmaceuticals, medical and surgical procedures, genetic engineering, and the systems within which health is protected and maintained.

COMMON CORE STATE STANDARDS FOR ENGLISH LANGUAGE ARTS

Grades 9–10

- CCSS.ELA-LITERACY.RST.9-10.1 Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions.
- CCSS.ELA-LITERACY.SL.9-10.1.D Respond thoughtfully to diverse perspectives, summarize points of agreement and disagreement, and, when warranted, qualify or justify their own views and understanding and make new connections in light of the evidence and reasoning presented.

Grades 11–12

- CCSS.ELA-LITERACY.RST.11-12.2. Determine the central ideas or conclusions of a text; summarize complex concepts, processes, or information presented in a text by paraphrasing them in simpler but still accurate terms.
- CCSS.ELA-LITERACY.RST.11-12.6 Analyze the author's purpose in providing an explanation, describing a procedure, or discussing an experiment in a text, identifying important issues that remain unresolved.
- CCSS.ELA-LITERACY.SL.11-12.1.D. Respond thoughtfully to diverse perspectives; synthesize comments, claims, and evidence made on all sides of an issue; resolve contradictions when possible; and determine what additional information or research is required to deepen the investigation or complete the task.

PROCEDURE

1 Warm-Up Activity

Begin by asking students if they know what genomic sequencing is. Allow students to share their ideas with the class. Try breaking down the word genome into its word origin of “gene” and “chromosome” and adding on “sequencing” which may be a set of more familiar words for students to define and analyze. (If students have not been introduced to this, the instructor may show students the following short video: <https://www.youtube.com/watch?v=2JUu1WqidC4>)

Next, show the following video on the overhead screen for students that introduces a mysterious illness that threatens the life of a teenage boy. (Play the video to 4:45)

https://www.youtube.com/watch?time_continue=109&v=YOEq7mHsw4

Ask students to think about how metagenomic sequencing, which allows scientists to sample all of the genes in all organisms present in a sample, could help solve the mystery of Josh’s illness and other infectious diseases that are difficult to diagnose. Allow students to share their ideas with the class. Students should guess that because pathogens (bacteria, fungi, and bacteria) have genetic material, it may allow the type of pathogen to be identified through sequencing.

- 2 Provide a link to the following article for students to read on their devices, or display the article on the overhead screen: <https://www.hhmi.org/bulletin/spring-2014/one-test-rule-them-all>. Ask students to read through the article. Students should learn that metagenomic sequencing of Josh’s blood and spinal fluid revealed DNA sequences from *Leptospira* bacteria, which was causing his illness. Through a simple treatment of penicillin, Josh quickly recovered.
- 3 Next ask students what the advantage of next-generation sequencing (NGS) was over traditional methods of sequencing, and why it was so important in Josh’s case. Students should share their ideas, recognizing that NGS has advantages over older methods, including that it can be done faster, allowing for faster diagnosis and treatment for patients.
- 4 Give each student a copy of the Secrets of Sequencing Background sheet. Play the following video for the class that introduces the basics of Next-Generation Sequencing: https://www.youtube.com/watch?v=ToKUGz_YhC4
- 5 **Small Group Activity**
Tell students that in the next part of this activity, they will form groups of 3–4 and will review an article that looks at how Next Generation Sequencing and metagenomics is being used to help track and diagnose outbreaks in countries where technology and funding is lacking. They should obtain a copy of the article “A Simpler Way to Get to the Bottom of Mysterious Illnesses in Poor Countries” from the link provided (<https://www.theatlantic.com/science/archive/2018/10/dashboard-disease-detectives/573034/>).

- 6 Students should individually read the article at their seat. Ask them to highlight 1–2 sentences in the article that stood out for them in relation to the use of metagenomic sequencing. They should write each sentence on their student sheet in Part III.
- 7 Underneath each quote, they should write a few sentences explaining why they chose that quote: Why did they feel it was important in the article? Did it have a connection to their own lives and/or experiences? Did it cause them to have additional questions or thoughts about this topic?
- 8 Divide the students into groups of three, and then have them take A, B, and C roles within the group. Invite the A's to read one of their chosen quotations only (not the explanation). Ask students B and C to discuss the quote and why they think it's important or what it means. After a couple of minutes, have the A students read their explanation as to why they picked the quotation. Repeat the process with the Bs sharing, and then the Cs.
- 9 To complete the small group activity, ask students to share their thoughts and reflections on the article and other ways genomic sequencing might be used to help people with the whole class (if time allows).
- 10 **Culminating Activity:**
 Now that students have a basic understanding of what Next Generation Sequencing is and how it can be used for a variety of applications, explain to students that each group will play the role of a team of geneticists that are trying to determine the cause of a fictional patient's illness using NGS techniques. They will model the sequencing step and then enter their findings into an online bioinformatics tool called BLAST (Basic Local Alignment Search Tool) to search for the biological origin of their sequence, much as in Josh's case from the warm-up.
- 11 Each group should obtain the following to model the sequencing stage of NGS:
 - a. 8 pieces of pipe cleaner (cut to be roughly 6" long)
 - b. 64 solid beads—16 of each of 4 different colors, with each bead color representing one of the 4 nucleotide bases (A, G, C, or T)

***Make sure to specify to students which bead represents each nucleotide!**

 - c. 32 clear beads—8 of each of 4 different colors—THESE SHOULD BE THE SAME 4 COLORS AS THE SOLID BEADS and represent the same base
- 12 Once students have obtained all of their materials, assign each group a patient (A-F) as the focus of their activity.
- 13 Give each group a copy of the **ACTIVITY: MODELING NGS SEQUENCING and DATA ANALYSIS** sheet. Students should follow the instructions on the sheet to sequence their reads and use the BLAST website to discover what organism is the cause of their patient's illness.

- 14 The group will first take turns building the reads from their patient's sample. They should do this by adding beads to their pipe cleaner to create a nucleotide chain. These are the reads. Remind students to be careful that they have the exact sequence from the patient's sample and to be careful they do not mix up any beads! Once the reads are completed, they should complete the next steps to create the contig. This will give them the complete DNA nucleotide sequence that can then be analyzed using BLAST.
- 15 Once students have discovered the type of pathogen that their patient is infected with, they should work together to create a diagnosis and treatment plan for the patient. Give each group a copy of the **STUDENT SHEET: Patient Report: Diagnosis and Treatment Plan**.
- 16 If time allows, student groups can present their findings and treatment plans to the whole group for questions and feedback.

LINKS

<https://www.illumina.com/areas-of-interest/microbiology/microbial-sequencing-methods/shotgun-metagenomic-sequencing.html>

<https://www.genome.gov/genetics-glossary/Contig>

https://www.illumina.com/documents/products/illumina_sequencing_introduction.pdf

As you view the video: https://www.youtube.com/watch?v=ToKUGz_YhC4, take notes over the process of Next Generation Sequencing.

I. ADVANTAGES OF NGS OVER PREVIOUS METHODS:

II. 4 STEPS OF NEXT GENERATION SEQUENCING

Summarize each step with words and/or diagrams.

- 1 **Library Preparation**—What is PCR?
- 2 **Cluster Generation**
- 3 **Sequencing**—Why are the nucleotides fluorescently labeled in this step?
- 4 **Data Analysis**—What is a reference genome?

III. APPLICATIONS OF NGS: ARTICLE REVIEW—“A Simpler Way to Get to the Bottom of Mysterious Illnesses in Poor Countries”

Obtain a copy of the article and read it silently at your seat. After you have finished reading, choose 1–2 quotes from the article that you think are most important or stood out to you. Then, write the explanation or justification as to why you chose each quote below. Follow your teacher’s instructions to complete the small group activity.

- QUOTE 1:

- EXPLANATION:

- QUOTE 2:

- EXPLANATION:

ATTACHMENT II

STUDENT SHEET: ACTIVITY—MODELING NGS SEQUENCING and DATA ANALYSIS

Step 1: Create the Reads

Your patient’s sample has been sequenced to discover what is causing their illness. Your first task is to build reads from the genetic material.

What are reads?

read: NGS uses sophisticated instruments to determine the nucleotide sequence of a DNA or RNA sample. In general terms, a sequence "read" refers to the data string of A, T, C, and G bases corresponding to the sample DNA or RNA. With Illumina technology, millions of reads are generated in a single sequencing run.

Group members should take turns creating each of the 8 the reads by using the key below and adding beads to a piece of pipe cleaner in the correct order of the sequence given. Make sure to use the correct colored beads in the correct order! Mistakes are mutations and will affect your analysis!

PATIENT	READ SEQUENCE (5' → 3')							
	1	2	3	4	5	6	7	8
A	caggcca	aataact	agatgcc	ataactc	taactca	tgccagg	ccaggcc	atgccag
B	cgttctg	ttctgtt	tctttac	ctcgttc	tacatcc	ctgttct	tcgttct	ctttaca
C	ctgataa	caggcca	taaattc	ttctgat	ataaatt	aattcaa	gccaggc	aggataa
D	atcacag	tcatgtg	cagcagg	aatcac	caggatc	aatcaca	cacagca	gatcatg
E	tccggat	ggattcc	ttgtcaa	cggagga	gattgtc	attccgg	ggaggat	gaggatt
F	aaaagaa	aaataca	aagaaaa	caaccaa	tacaacc	aaccaat	acaaaag	aaaatac

Step 2: Attach Indexes

Next, you will attach indexes to your reads. In our model, this will be a sequence of 3 bases that will allow our fictional sequencing machine to distinguish your sample from others. Create an index of 3 bases (using clear beads of the correct colors) and attach the index to each of the 5" pipe cleaner end of each of your reads. Next create another index of 3 bases and attach these 3 beads to the 3" end of each read.

Step 3: Assembling the contig

What is a contig?



A contig—from the word "contiguous"—is a series of overlapping DNA sequences used to make a physical map that reconstructs the original DNA sequence of a chromosome or a region of a chromosome.

A contig can also refer to one of the DNA sequences used in making such a map.

Study your reads to determine where they overlap in sequence, using the colored beads to help you. Once you have assembled them correctly, you should have one continuous DNA sequence! Record this DNA sequence carefully in the box below. It should be 21 nucleotides long. (The indexes are NOT part of the contig and should be left out.)

CONTIG:

--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Step 4: Data Analysis using BLAST

On your student device, go to: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>

Click on “Nucleotide BLAST”



In the box that says: **Enter accession number(s), gi(s), or FASTA sequence(s)**, carefully type in the contig you determined in Step 3. Check to make sure that it is accurately typed in with no spaces between the bases, scroll down and click the button that says **BLAST**.

Step 5: Diagnosis of the Patient

Once your BLAST is completed (it may take up to a minute or two), scroll down until you see the “DESCRIPTIONS” section that says: **Sequences producing significant alignments:**

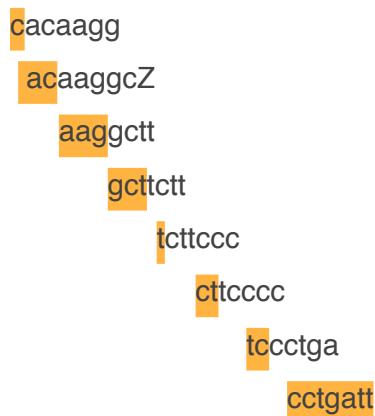
Find the first alignment listed, the scientific name of the organism will usually be the first two words of the listed alignment.

If you are not sure what this is, copy and paste it into your browser and discover what organism has a match with your DNA sequence! This is what has been afflicting your patient!

***TEACHER/STUDENT EXAMPLE, CREATING THE CONTIG FROM THE READS:**

PATIENT	READ SEQUENCE (5' → 3')							
	1	2	3	4	5	6	7	8
EXAMPLE READS	tcttccc	aaggctt	acaaggc	tccctga	cacaagg	cctgatt	gcttctt	cttcccc

Students should carefully assemble their **8 reads (seen in the chart above)** using the beads and pipe cleaners, then lay them out according to where they see overlap in the sequence as seen below. They should then be able to create the **contig**, as seen in the highlighted parts of the reads.



*COMPLETED CONTIG: **cacaaggcttcttccctgatt**

The **contig** will then be sequenced in BLAST, which should reveal the identity of the pathogen.

EX: SEQUENCE ALIGNMENT (from BLAST website): Human Immunodeficiency Virus (HIV)

Group A

*CONTIG: agatgccaggccaataactca

SEQUENCE ALIGNMENT: Plasmodium falciparum

Group B

CONTIG: ctcgttctgttctttacatcc

SEQUENCE ALIGNMENT: Vibrio cholerae

Group C

CONTIG: ttctgataaggataaattcaa

SEQUENCE ALIGNMENT: Marburg virus

Group D

CONTIG: aaatcacagcaggatcatgtg

SEQUENCE ALIGNMENT: Hantavirus

Group E

CONTIG: cggaggattccggattgtcaa

SEQUENCE ALIGNMENT: Zika virus

Group F

CONTIG: acaaaagaaaatacaaccaat

SEQUENCE ALIGNMENT: MRSA - Staphylococcus aureus

Patient Report: Diagnosis and Treatment Plan

Now that you have discovered the pathogen that is causing your patient’s illness, you need to do some research on the internet and create a treatment plan for your patient. Complete the chart below to create your plan!

Patient _____	Diagnosis:
Type of pathogen causing the illness (bacteria, fungi, virus)	
Method of infection	
Patient symptoms	
Prognosis (what are the chances of survival of the patient?)	
Can the patient infect others?	
Recommended treatments and medications	
Images related to this diagnosis	
Informational websites/support for the patient	