

Introduction to the Microbiome Guide for Teachers

*Using Models and Model Organisms to
Understand Biological Systems*

Developed by the Gene You Project
Center for Educational Outreach
Baylor College of Medicine
Houston, Texas



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BioEdSM

**TEACHER RESOURCES FROM THE CENTER FOR EDUCATIONAL OUTREACH
AT BAYLOR COLLEGE OF MEDICINE**

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CONTENTS

1. Comparing Microbiomes	1
Does the composition of bacterial populations differ among locations on the human body and among individuals?	
2. How Many Cells Are in Your Body?	8
How many human and microbial cells make up your body?	
3. Bacterial Quorum Sensing	13
Do bacteria communicate? Does population density affect bacterial signaling or communication?	
4. Using Bacteria to Treat Disease	18
Can the microbiome be manipulated to treat disease?	
5. Feeding Your Microbiome	24
Can the balance of different microbes in the gut be altered?	
6. Got Fiber?	30
Why is fiber in food important to the health of the microbiome of the gut and to the general health of humans? Where can we find good sources of fiber?	

Unit Materials

Follow all school district recommended safety practices for working with live organisms in the classroom.

INVESTIGATION 1

Per student

- Clear tape
- Pair of disposable latex or vinyl gloves
- Permanent marker
- Petri dish (pre-poured with agar and allowed to set)
- Science notebook for observations and written conclusions
- Sterile swabs
- Sticky notes for concluding discussion and class graph

INVESTIGATION 2

- Smart board or computer and projector with speakers

Per Student Group

- Copy of the “3 - 2 - 1” (p. 12)

Per Student

- “Gut bacteria gene complement dwarfs human genome” article (see “Before You Start”)

INVESTIGATION 3

- 50 ml of isopropyl alcohol, 70%, in a small beaker (approximate amount)
- 10 pairs of disposable gloves
- Bioluminescent Bacterium Kit - Item 154750 (See “Before You Start,” p. 13)
- Plastic bag for disposal of cultures at the end of the investigation
- Smart board or computer and projector with speakers
- Test tube racks to hold 10 tubes

Per Student Group

- Paper towels
- Permanent markers and tape to label test tubes

INVESTIGATION 4

- Smart board or computer and projector with speakers

Per student

- Copy of “About the Microbiome Article” (p. 22–23)
- Copy of “NewsFlash: Fecal transplant pill may bring new balance to microbiome” (p. 21)

INVESTIGATION 5

Per Student

- Copy of the student sheets (p. 27–29)

INVESTIGATION 6

- 10 packaged non-perishable food items—or empty packages (see “Before You Start” below, for suggested items and information)

Per Student

- Copy of “Fiber Content Challenge,” (p. 33)

INVESTIGATION 1

Comparing Microbiomes

OVERVIEW

Students will examine differences in the bacterial populations on different skin areas by cultivating bacteria collected from at least 3 areas of the body. Sites recommended include the crease behind the knee, crease behind the ear and the side of the nose. Students will compare their cultures from the 3 sites and then the cultures of other students to investigate similarities and differences among microbial populations. They will discover that microbial ecosystems exist on the skin, differ from person to person and even among locations on the body.

TIME

2 sessions, with 2–3 days in between (if you are teaching the unit continuously, consider conducting Activity 2, while you are waiting for results from Activity 1).

MATERIALS

Per Student

- Clear tape
- Pair of disposable latex or vinyl gloves
- Permanent marker
- Petri dish (pre-poured with agar and allowed to set)
- Sterile swabs
- Science notebook for observations and written conclusions
- Sticky notes for concluding discussion and class graph

BEFORE YOU START

Each student will need a prepared petri dish. If you start with empty petri dishes, it will be necessary to fill each with agar nutrient to a depth of about 0.5 cm. Microwave the agar in its bottle until liquid. Open each petri dish just enough to carefully pour the agar into the bottom half. Place the lid so that it covers the agar, but leaves some gaps to avoid water condensation forming on the lid (as shown, right).



Be ready to use a smart board or computer and projector to show the following video to students.

Exploring the invisible universe that lives on us —and in us

<http://www.npr.org/sections/health-shots/2013/11/01/242361826/exploring-the-invisible-universe-that-lives-on-us-and-in-us>

SAFETY

Follow all school district recommended safety practices for working with live organisms in the classroom.

Once students have inoculated the petri dishes, the lids should be sealed closed with tape and kept sealed until the dishes are disposed of. The cultures could contain potentially harmful microbes. To dispose of petri dishes with microbe colonies, open the dishes slightly and immerse them in a 10% chlorine bleach solution (1 part bleach to 9 parts water). Then, seal the dishes in a plastic bag for disposal.

BACKGROUND

It was once thought that humans were independent beings—capable of carrying out all life functions, except for nutrition, without assistance from other organisms. Biologists assumed that the human genome (all of a person’s DNA) contained a complete set of instructions for life, encoded as 20,000 to 25,000 genes.

That assumption of independence turned out to be untrue. In addition to our human genes, there are more than 300 times more genes in the microbes that live on and within our bodies. If just counting genes, we are more microbes than human.

The genome contains the genetic information (complete set of DNA) for all the inheritable traits of an organism. This amounts to all the genetic information in the chromosomes including its genes and DNA sequence. In humans, a copy of the entire genome, more than 3 billion DNA base pairs, is found within all cells that have a nucleus. Cells are the working units in all living things. The DNA in each cell contains about 20,000 to 25,000 genes, organized as sequences of base pairs that provide the instructions for building proteins. Other regions of each DNA molecule help regulate which genes are activated or have structural functions.

Mitochondria and chloroplasts, which are organelles, have their own DNA.

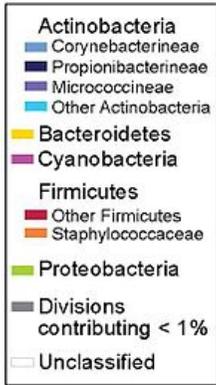
The collection of microbes on and in your body is your microbiome. In essence, it is your second genome. In fact, since many of the bacteria inside the body have not been cultured or studied, scientists only can identify them through their DNA.

The entire human body is a host for a variety of microbes. More than 40 trillion microbes live in our gut, on our skin, and on and in mucosal surfaces of our bodies. Microbes are found in every nook and cranny of our bodies, but the majority of the microorganisms are located in the gut. These unseen inhabitants are mostly bacteria, but viruses, fungi and protozoans also are present. The microbiome can be thought of as the “ecological community of commensal, symbiotic and pathogenic microorganisms that share our body space”¹ or as “the set of microbial genes that are found in and on the body.”

The skin microbiome is colonized during the first few days after birth. Most skin bacteria are categorized into four different phyla: Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. Some skin diseases, such as psoriasis, are associated with changes in the populations of bacteria present.

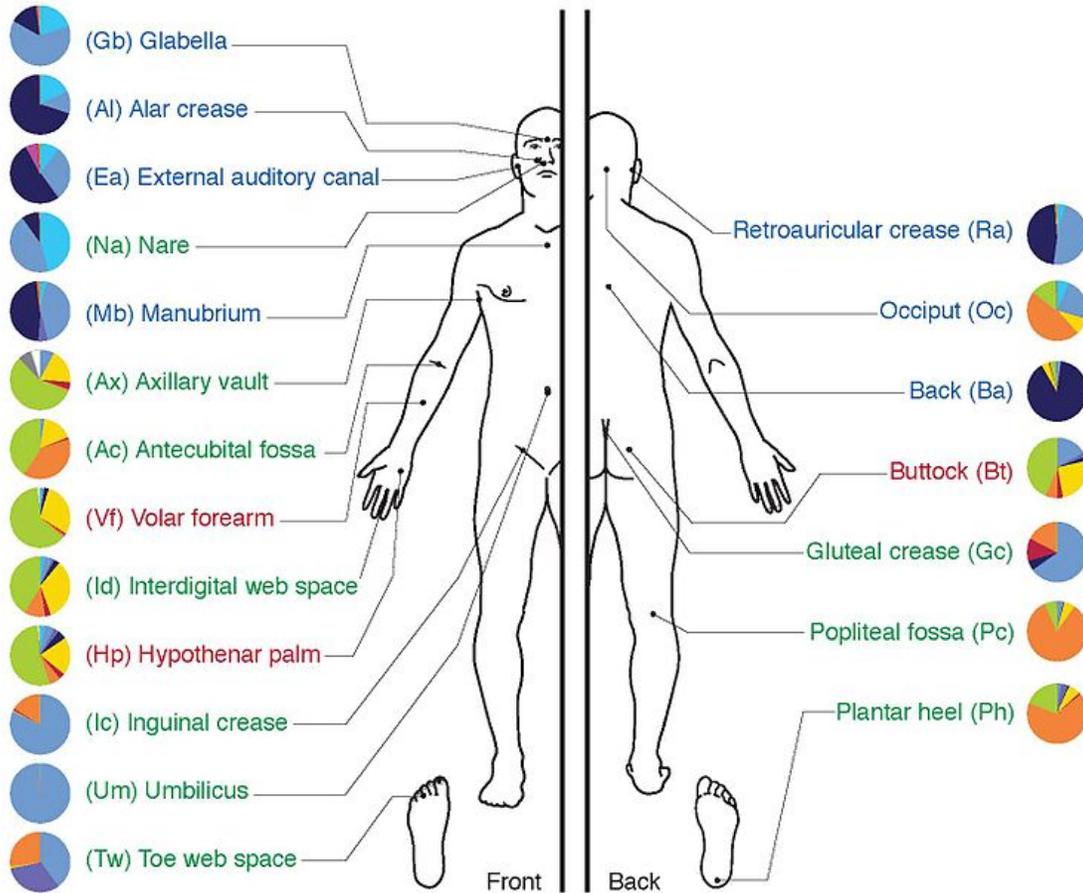
¹ A framework for human microbiome research. Nature 2012; 486:215-21.

Normal Human Microbiome on Skin



Actinobacteria – phylum of Gram-positive bacteria with guanine and cytosine content in their DNA.
 Firmicutes – phylum of mostly Gram-positive bacteria, also present in large numbers in the gut.
 Proteobacteria – phylum of Gram-negative bacteria, including *E. coli*, *Vibrio* and *Helicobacter*.
 Bacteroidetes – phylum of Gram-negative bacteria found widely in the environment.

This diagram may be accessed online at <http://www.genome.gov/dmd/img.cfm?node=Photos/Graphics&id=85320>



Skin is populated predominantly in skin folds. Bacteria also are found in moist areas of the nose and respiratory tract. The oral cavity (tongue, teeth and gums) contains high number of bacteria (10^{12}). The largest portion of our microbiome, however, lives in the large intestine, which contains about 10^{11} bacterial cells per gram of intestinal content.² Surprisingly, about 70% of these bacterial species have not been cultivated successfully outside of the human body.

The microbiome has a major influence our health. It is responsible for many relevant functions including breakdown of complex food molecules, prevention of disease-causing pathogens from entering the body, and the synthesis for essential nutrients and vitamins. In the gut, microbes play important roles in the digestion of food. (Technically, gut microbes metabolize food.) But, the kinds of microbes contained in our guts vary from person to person. In other words, each individual's gut microbiome works a bit differently. Some studies even have shown that obese people have a somewhat different composition of gut bacteria compared to thin people—which may explain why some people gain weight more easily than others.³

Not only are the gut microbes unique to each individual, the microbes living on the surfaces of the human body also are specific to each individual, just like fingerprints. Skin, our largest organ, plays an important protective role against infection and invasion. The microbes living on skin vary in both species and abundance due to a huge variety of factors including environment, gender, diet, medications, climate, age, occupation, hygiene and even genes. And even more specifically, the microbes in and on each individual vary from location to location on the body. “Knee” microbes are different species from “toe” microbes, and so on!

WHAT'S THE QUESTION?

Does the composition of bacterial populations differ among locations on the human body and among individuals?

PROCEDURE

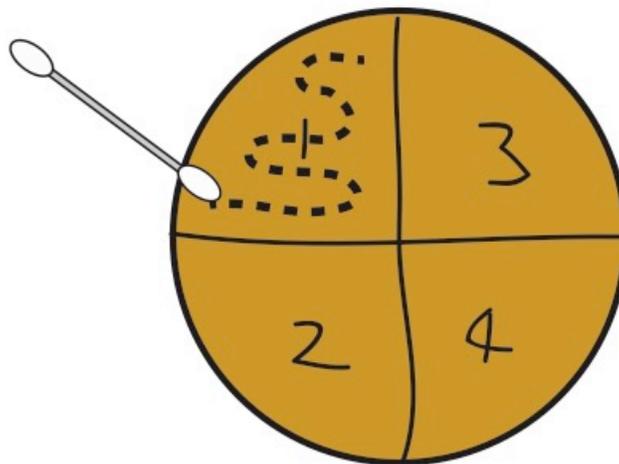
Part 1: Introduction and initiate experiments

1. Have students sit in groups of two or four. Ask students to record a list of all the “things” on their skin. This list will most likely include dead skin, lotion, sweat, perfume—but not microbes. After each group has compiled a list, ask each group in turn to share one item from their list—until no one has additional ideas to share.
2. Tell students that there is something else on each student's skin: invisible bacteria, viruses and fungi. In other words, each person's skin contains large numbers of microorganisms or microbes.
3. Show students the video, Exploring the invisible universe that lives on us — and in us, (For video URL, see “Before You Start,” p. 1)

² Tlaskalová-Hogenová H, et al. 2011. The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. *Cellular & Molecular Human Immunology* 8: 110-120.

³ European Food Information Council. 2013. The role of gut microorganisms in human health. Accessed online, http://www.eufic.org/article/en/artid/The_role_of_gut_microorganisms_in_human_health/

4. Discuss some of the major concepts introduced in the video. Ask students, *what is your microbiome?* Explain that microbes or microscopic organisms, both beneficial and harmful, live on every square centimeter of our skin, as well as inside our bodies.
5. Ask, *do you think all the microbes are similar or might there be a number of varieties? Why or why not? If so, on what might these differences depend?* Explain to students that they will be culturing microbes from specific areas—behind the ear, behind the knee and the crease on the side of the nose—to look for the variety in the microbes on their own skin and then compare it with their team members’ results.
6. Give each student a petri dish with agar. Instruct them to keep it closed to avoid contamination until ready to use. Students will divide the plate into four equal parts. Tell students to turn the dish upside down—*keeping the lid in place*—and use a permanent marker on the outside of the dish to divide it into numbered quadrants, as shown below.



Divided petri dish showing swabbing technique.

7. Create a key to identify each quadrant with a different skin area that will be sampled. These areas should be the same for each student. (For example, 1 – Crease behind knee, 2 – Crease behind the ear, 3 – Side of the nose, 4 – Control - no inoculation.)
8. Demonstrate how to take the sample and explain that the samples will need to be comparable among students. Explain that students should hold only the “stick” part of the swab to avoid contamination. To collect a sample, they should rub the swab against the region of skin back and forth *only three times*. This will assure that all students use comparable methods to take their samples.
9. Each student will sample him or herself. Have the students swab one of the three specified areas. Then, lift the lid on the dish and gently rub in a zig-zag motion across the surface of the agar. Instruct them to repeat this with each sample using a clean end

of a swab. Again, have all students inoculate in the same manner that you have demonstrated.

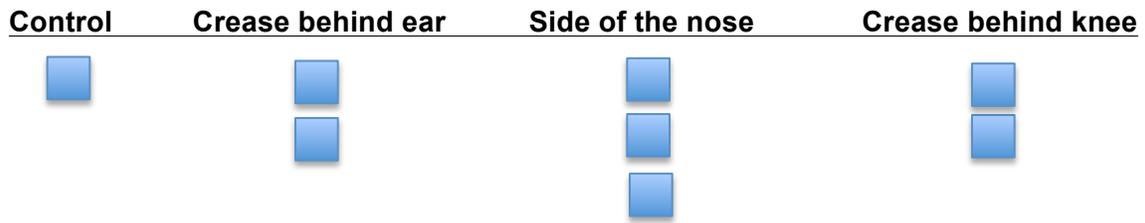
10. Since harmful bacteria can be present, make sure the dishes are sealed once students have collected all three samples. Use clear tape to hold the lid over the dish. Also, use a long strip of tape to seal the gap around the bottom to avoid contamination.
11. Place all the dishes, upside down, in a warm (37° C), dark place and examine daily or in 3–4 days.
12. After students have completed the inoculation of the dishes, have them describe the areas they sampled in their notebooks. They should describe the environment of each of the sampled locations: oily, damp or dry; warm or cool; frequency with which the area is washed (for example, face might be washed several times during the day, while area behind knee might be washed only once per day).

In addition, have students make predictions in response to the following questions.

- Which sampled location do you predict will have more kinds of bacteria (as judged by the appearance of the colonies)? Which location will have the fewest different kinds of bacteria? Why do you think so?
- Which location will produce the most bacteria (area of the plate that is covered by bacteria)? Which location will have the least? Why?
- Will the types and numbers of bacterial colonies be consistent for sample locations as compared across different individuals? Why do you think so?

Part 2: Observations, discussion and assessment

1. Have students sit with their groups again. First, each student should observe and draw the bacterial colonies (present as clumps, mounds or stripes) on his or her petri dish. Have them use colored pencils or markers to match the colony colors. Then, each student should describe the colonies in his or her notebook. Ask, *Were the four sections similar or different? What happened in the control section? If the control section is not free of bacterial colonies, what might have happened?*
2. Next, have students compare their microbe growth with the results obtained by other students in their group. Have students discuss the results as compared to their predictions.
3. Ask each group to report their findings, and conduct a class discussion of the results. Or, create a class histogram of the findings by sample area and have each students place a sticky note in the sample area with the most microbes. Create a second histogram on which students use a sticky note to identify the area with the greatest diversity of microbes. (Diversity = different kinds of microbes)



WRAP IT UP

1. Ask, *What conclusions can be drawn from your observations and comparisons?* There are many varieties of microbes and no two individual's microbes are exactly the same. However, some parts of the body host a greater diversity of microbes than other parts. In addition, the population of microbes varies based on the skin environment.

Explain that all the microbes on us and inside us are our microbiome. The microorganisms inhabit microscopic ecosystems on and within the body. We have more microbes than human cells in our body! Project or make copies of the attached diagram that shows the major kinds of bacteria that inhabit human skin and the types of bacteria typically found in different skin environments.

2. Have each group prepare a written summary of their findings.

INVESTIGATION 2

How Many Cells Are In Your Body?

OVERVIEW

Students will calculate the number of cells in human body and compare that number to the number of microorganism inside and on the body.

TIME

1 session

MATERIALS

- Smart board or computer and projector with speakers

Per Student Group

- Copies of the “3 – 2 - 1” (p. 12)

Per Student

- “Gut bacteria gene complement dwarfs human genome” article (see “Before You Start”)

BEFORE YOU START

Download or access the online article below.

Gut bacteria gene complement dwarfs human genome

<http://www.bioedonline.org/news/nature-news/gut-bacteria-gene-complement-dwarfs-human-genome/>

Review the articles in the “Resources” section (p. 11)

BACKGROUND

Counting cells in the human body is beset with many problems. Different organs have cells of different sizes. Some cells are easy to count but others, like tangled neurons are difficult to distinguish.

There are so many variables in counting cells in large organisms that it is only practical to estimate the number. One way is to estimate the number of cells is by their mass. Many references estimate a typical human body cell to have a mass of 1 nanogram. Therefore, a typical human with a mass of 70 kilograms would have about 70 trillion cells. The problem with this estimate is that cells for the different human organs have different masses and the 1 nanogram is a “ballpark number.”

Another problematic strategy is to estimate the number of cells by volume. A typical cell has a volume of 0.000000004 cubic centimeters (again, an approximate number).

Therefore, a typical human would have about 15 trillion cells. Both strategies are fraught with problems. There isn’t such a thing as a typical cell with a typical mass and volume.

In a paper done for the *Annals of Human Biology*, the authors estimated the number of cells found in a typical human. Their typical human weighs 70 kg, has 1.85 square meters of

skin, and is 1.7 meters tall). The authors of the paper made their estimates of the number of cells in the typical human the old fashioned way. They estimated the cell types in each organ of the body. They arrived at the number 37.2 trillion cells!

This makes one wonder about the wisdom of a Star Trek transporter. Disassembling 37.2 trillion cells, converting them to an energy beam, and reassembling them on a distant planetary surface (with no mistakes!) seems more than far-fetched. Then again, having 37.2 trillion cells all cooperating together to make a functioning human instead of a soupy mess of competing cells, sounds pretty far-fetched too. But humans are real.

Even more amazing than the human cell count is the population of microorganisms that humans have residing in their guts, and in and on all other parts of their bodies. Some studies indicate that there are ten microbes for every human cell. If correct, that comes out to over 300 trillion microbes! On a cell count alone, humans are more microbe than human. Just this year, a new estimate challenged the conventional wisdom about the number of bacterial cells that we host. The new estimate based on updated calculations about the numbers of bacteria in the colon yields a ratio of 1.3 bacterial cells to each human cell.

Read more about the new calculation at the web page below.

Scientists bust myth that our bodies have more bacteria than human cells
<http://www.bioedonline.org/news/nature-news/scientists-bust-myth-that-our-bodies-have-more-bacteria-than-human-cells/>

Obviously, these numbers are only estimates but it is fun and amazing to think about them.

WHAT'S THE QUESTION?

How many human and microbial cells make up your body?

PROCEDURE

- 1 Review what students know about cells. *What are cells? What are they made up of? What are the major kinds of cells in the human body?* Then ask students, *How many cells do you have in your body?* Write down the numbers students propose on the board.
- 2 Tell your students that they will be checking their ideas about the total number of cells in a human body by estimating the number of cells for a typical human. Write the following equation on the board.

$$N = g \times 5.314 \times 10^8$$

N Approximate number of cells in subject's body

g Subject's total body mass in grams

Subject: Typical 30-year-old male, 170 cm tall, 70 kg mass

Note: The 5.314×10^8 factor is a derived number based on the results of the scientific study. It is approximately equal to the estimate number of cells per gram of body mass of the typical human referenced in the study.

- 3 Have students calculate the total number of cells in the subject.

Answer

$N = 70,000 \times 5.314 \times 10^8 = 3.7198 \times 10^{13}$ or 37,198,000,000,000 or rounded off to 37.2 trillion cells.

- 4 Have students compare their calculations to the estimates on the board. *How close were the initial student estimates?*
- 5 Challenge students to privately calculate the estimated number of cells in their own bodies. Follow with a discussion about variables that could affect their estimates, such as body type, height, age or muscle mass.
- 6 Announce that their calculation only accounts for the number of human cells in the body. It doesn't account for the number of microbes in and on humans. Tell them that by some estimates, there are about 1.3 microbes for every human cell. Have students calculate number of microbes they personally host in and on their bodies.

To arrive at the calculation, student should multiple the total number of cells for their own body, by 1.3.

- 7 Now ask students, *how many genes are represented in each of your cells?* (approximately 21,000-23,000)? Remind them that genes code for all of the molecules and functions needed for life. Follow by asking, *do you think the bacteria in your body have fewer or greater numbers of genes than your own genome?* Record students' ideas on the board.
- 8 Distribute copies of the article, "Gut bacteria gene complement dwarfs human genome" (see article URL, p. 8).

Encourage students to highlight and look up any words in the article that are unfamiliar to them. Some examples of new vocabulary or different uses of words include: "catalogue" as a verb (create a complete listing); microbiome (all of the genes represented by the microbes in and on an organism); faecal (alternate spelling of "fecal"); transiently (for a short period of time); inflammatory bowel disease (a disease characterized by pain and diarrhea, caused by ongoing irritation of the intestinal tract); therapeutic potential (possibility of developing a useful treatment for a condition).

WRAP IT UP

Have students work in groups to complete a "3 - 2 - 1" sheet based their calculations and the article they read.

RESOURCES

The original research article estimating the number of human cells.

Bianconi E1, et al, 2013, An estimation of the number of cells in the human body, *Ann Hum Biol.* 2013 Nov-Dec;40(6):463-71 An estimation of the number of cells in the human body. The article is available at the following website.

Taylor Francis Online

<http://www.tandfonline.com/doi/full/10.3109/03014460.2013.807878>

The article below updates the number of bacterial cells calculated for the body.

Scientists bust myth that our bodies have more bacteria than human cells
<http://www.bioedonline.org/news/nature-news/scientists-bust-myth-that-our-bodies-have-more-bacteria-than-human-cells/>

Additional online resources on this topic are listed below.

How many cells are in your body?

<http://phenomena.nationalgeographic.com/2013/10/23/how-many-cells-are-in-your-body/>

37.2 Trillion: Galaxies or human cells?

http://www.nytimes.com/2015/06/23/science/37-2-trillion-galaxies-or-human-cells.html?_r=0

Microbiome: Your body houses 10x more bacteria than cells

<http://discovermagazine.com/galleries/zen-photo/m/microbiome>

3 – 2 – 1

Answer each question in the space provided.

3 List three things that you learned about cells in the human body and the bacteria that are found in and on the body.

1. _____

2. _____

3. _____

2 List two things that you found interesting and would like to learn more about.

1. _____

2. _____

1 Write one question that you have about the bacteria and the human body.

1. _____

INVESTIGATION 3

Bacterial Quorum Sensing

OVERVIEW

Students will learn about bacterial communication and investigate the phenomenon of quorum sensing in the fluorescent bacterium, *Vibrio fischeri*.

TIME

2 sessions

MATERIALS

- 50 ml of isopropyl alcohol, 70%, in a small beaker (approximate amount)
- 10 pairs of disposable gloves
- Bioluminescent Bacterium Kit - Item 154750 (See “Before You Start,” p. 13)
- Plastic bag for disposal of cultures at the end of the investigation
- Smart board or computer and projector with speakers
- Test tube racks to hold 10 tubes

Per Student Group

- Paper towels
- Permanent markers and tape to label test tubes

BEFORE YOU START

Obtain a Bioluminescent Bacterium Kit from Carolina Biological Supply Company. Plan to receive the kit the day before you plan to use it. See “Materials” for contact information. Download the *Vibrio fischeri* care sheet to familiarize yourself with the procedures for working with the bacteria prior to its arrival.

<http://www.carolina.com/teacher-resources/Document/vibrio-care-handling-instructions/tr23902.tr>

The actual inoculation of the agar tubes should begin as soon as possible after the arrival of the kit. Depending upon transport and storage conditions, the kit may still be viable for several days. It is best not to wait.

Download or bookmark the following “talks” from TED to show to students.

The weird, wonderful world of bioluminescence

https://www.ted.com/talks/edith_widder_the_weird_and_wonderful_world_of_bioluminescence?language=en

How bacteria “talk”

https://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate?language=en

BACKGROUND

Bacteria are not isolated cells. They exist in communities and sometimes orchestrate their group responses by releasing signaling molecules, a chemical language, into their environment. In one mechanism, called “quorum sensing,” bacteria are able to detect the amount of signaling molecules that are present and use the signals to coordinate their behavior.

With quorum sensing, bacteria communicate and coordinate their activities to achieve the greatest response. Each individual bacterium produces a chemical, called an autoinducer, which is released into the immediate environment. When enough of the autoinducer is released and detected—all of the bacteria respond together. In other words, when the right number of bacteria, a quorum, is present and producing the autoinducer, the quorum is sensed by all the bacteria. When this stage is reached, an event is triggered. The type of event depends upon the kind of bacteria.

Quorum sensing makes it possible for bacterial populations to respond rapidly to changes in their environment. Some disease causing (pathogenic) bacteria, for example, coordinate their attack on the host by waiting until they have reached a certain population level. This enables the bacteria to escape elimination by the host’s immune system until they are sufficiently numerous to establish a successful infection. With *Vibrio cholerae*, which causes cholera—approximately 100 million of the bacteria must be present in a person before the quorum is achieved. *Vibrio cholerae* begins wreaking havoc on the afflicted individual only when the population of bacterial cells reaches a critical level in the intestines.

In the case of the *Vibrio fischeri*, the bacterial species that students investigate in this activity, the quorum causes all the individual bacteria to emit light that can be seen in a dark room. This is referred to as bioluminescence.



Quorum sensing was discovered by Dr. Bonnie Bassler of Princeton University. Dr. Bassler spent years studying the harmless bioluminescent bacterium, *Vibrio harveyi*. In small numbers, the bacteria do not produce light—but as the bacteria divide and multiply, a

quorum is reached and all the bacteria begin producing light at the same time. They trigger the luminescent response by releasing a hormone-like “signaling molecule,” which she called “autoinducer.” As Dr. Bassler describes it, the bacteria have little detectors on their membranes, kind of like a lock and key, where the autoinducer molecules fit perfectly. When the numbers of autoinducer reach a threshold level, gene expression is altered within each cell leading to a coordinated response.

Dr. Bassler’s discoveries led to the realization that all bacteria communicate in a similar fashion. The *Vibrio fischeri* bacterium is especially interesting because it is very easy to work with and results in bioluminescence when a quorum is reached. *Vibrio fischeri* have a symbiotic relationship with a very small squid called the Hawaiian bobtail squid. The bacteria reside inside and grow within two light-emitting organs on the squid’s underside. The squid provides nutrients for the bacteria to grow and the bacteria emit light to help the squid in its nocturnal hunting in shallow waters.

This amazing relationship involves light detectors on the backs of the squid that sense moonlight and starlight. The squid then replicates the same amount of light with the bacteria as it swims near the bottom. The light released exactly matches the overhead moon and starlight so that the squid does not cast a shadow on the bottom. This helps the squid avoid predators that would dine on it.

At first, Dr. Bassler’s discoveries were not thought to be very important but as her research confirmed that quorum sensing is a property of all bacteria, it was realized there is a great opportunity to benefit humans. Dr. Bassler’s research team among others, is looking for ways of using the chemicals in quorum sensing to neutralize harmful bacteria. It is hoped that drugs may be found that will shut off quorum sensing in the harmful bacteria, such *Vibrio cholerae*, so that they become harmless.

For More Information

The following Internet videos tell the story of quorum sensing and its universal potential through the eyes of its discoverer, Dr. Bonnie Bassler.

Quorum sensing in bacteria

<https://www.youtube.com/watch?v=LebqwdQSFHE>

Profile: Bonnie Bassler

<http://www.pbs.org/wgbh/nova/body/bonnie-bassler.html>

WHAT’S THE QUESTION

Do bacteria communicate? Does population density affect bacterial signaling or communication?

SPECIAL PROCEDURES

The actual inoculation of the agar tubes should begin as soon as possible after the arrival of the kit, *preferably early in the school week*. Depending upon transport and storage conditions, the kit may still be viable for several days. It is best not to wait.

PROCEDURE

Part 1: Setting up *Vibrio fischeri* cultures

1. Show students the video “The weird, wonderful world of bioluminescence (see URL, p. 13)
2. After watching the video, discuss bioluminescence with students. Ask, *What needs do organisms fulfill by producing their own light (bioluminescence)?* Have students share their ideas.
3. Tell students that they will be investigating bioluminescent bacteria. Prepare your students for the arrival of the Bioluminescent Bacterium Kit by reviewing the procedures for inoculating the nutrient agar with the *Vibrio fischeri* bacteria.
4. Select an area of the classroom and create a station where students will be able to inoculate their bacterial cultures. Have students work in groups of 2-4 and assign one or two tubes to each group, depending on your number of students. Each team should inoculate their culture as described below.

The kit will contain ten prepared capped test tubes with agar nutrient. One additional tube will contain the bacteria culture.

First, each group of students should label their tubes with their group name and date and time of inoculation.

Before opening any tube, the area where inoculation takes place should be sterilized by wiping with isopropyl alcohol.

Students doing the inoculation should wash their hands with soap and water and wear disposable gloves.

The kit comes with a nichrome wire inoculating loop. This loop must be shared with all students involved in the inoculation. Students should not touch the loop. If they do, they should dip the loop in a container with alcohol and let the loop dry before proceeding.

To inoculate a fresh tube of agar—a small piece of the culture, about the size of the loop should be cut from the culture and inserted into the middle of the nutrient agar. The tube should be loosely capped and placed in a test tube rack.

5. When all ten tubes have been inoculated, place them in a very dark place, such as a cabinet or closet. Keep the tubes in the dark for at least 24 hours.

6. Have students observe the tubes at least two times during the next 24 hours. For each observation, the students should take their culture tube into a room without any windows. They should turn out the lights and wait for their eyes to adjust to the darkness. This should take only a minute or two. Students should notice whether there are any changes in their culture, and write their observations in their science notebooks.

Part 2: Summary of results, discussion and extension

1. The next day, take the tubes and your students into a room without any windows. Turn out the lights and wait for eyes to adjust to the darkness. By this time, the bacterial cultures will have reached a quorum and begun glowing. (Eventually, the individual cultures will become exhausted and stop glowing. They should last for more than a day.)
2. Conduct a class discussion about their observations. Ask, *When did the cultures begin glowing? Did every tube begin glowing at about the same time? Was there a gradual increase in the amount of luminescence or did it appear at a particular time?*

WRAP IT UP

Hold a class discussion on quorum sensing. Point out that bacteria can communicate with each other. Show students the TED Talk video of Dr. Bonnie Bassler, in which she explains quorum sensing in the bioluminescent bacterium, *Vibrio fischeri*. (See video URL, p. 13.)

Have each student write a short paper on the issues, practices, and promise of quorum sensing research, in which they address one of the following questions.

- What did they observe with the *Vibrio fischeri* cultures.
- How does bacterial communication work?
- What is the role of communication in the life of a bacterial colony?
- Why is it important to understand quorum sensing?
- How will this knowledge about bacterial communication help humans?

INVESTIGATION 4

Using Microbes to Treat Disease

OVERVIEW

Students will learn about a new treatment for *Clostridium difficile* infection that replaces an unhealthy gut microbiome with the microbial flora from a healthy person.

TIME

1 to 2 sessions

MATERIALS

- Smart board or computer and projector with speakers

Per student

- Copy of “About the Microbiome Article” (p. 22–23)
- Copy of “NewsFlash: Fecal transplant pill may bring new balance to microbiome (p. 21)

BEFORE YOU START

Make copies of the articles listed above (one per student).

Load the following National Geographic story about microbiomes on your computer for playback to the class or link to the site and project the video on a smartboard.

Why would anyone get a fecal transplant? Watch a brother and sister explain <http://phenomena.nationalgeographic.com/2015/06/22/fmt-film/>

BACKGROUND

Bacteria exist as communities of different species that reside in the same area and communicate together. These mixed communities of bacteria can be found in multiple niches in the environment—near deep sea ocean vents, in thermal hot springs, on the surfaces of plant leaves and roots, on our skin, intestines and on other body surfaces. With the development of new techniques to quickly sequence DNA, scientists can easily collect environmental samples and identify different species of bacteria within these communities.

The human microbiome can be thought of as the “ecological community of commensal, symbiotic and pathogenic microorganisms that share our body space”⁴ or as “the set of microbial genes that are found in and on the body.” Other organisms also host their own microbiomes, which also are found as communities in other environments.

Identification of the many different kinds of bacteria within our microbiome is important, and scientists are constantly discovering new species of bacteria that have never been identified before! Since many species of bacteria cannot be cultured in the lab, new bacterial sequencing techniques have enabled a better appreciation for the diversity of

⁴ A framework for human microbiome research. Nature 2012; 486:215-21.

microbes and the genes they contain. However, identification of bacterial species yields only superficial data. Scientists are much more interested in understanding how these bacteria communicate with each other, what they're saying, and how they can communicate with larger organisms (like plants and us!).

Bacterial communication occurs when bacteria secrete both waste and non waste products. Other organisms, such as other species of bacteria, fungi, worms, plants and humans, can detect these small molecules. Once detected, these communication molecules influence health and development in many ways. For instance, mice that are grown in a completely sterile environment develop bowel and immune system dis-functions due to the lack of bacteria in their gut. Furthermore, certain species of bacteria can enhance plant development by recycling and modifying nutrients and defending against pathogenic bacteria. A similar form of this bacterial recycling and nutrient supplementation occurs within our gut, too. In fact, disruption of the human gut microbiota can influence the development of obesity, inflammatory bowel disease and type-2 diabetes. These data indicate that the behavior of larger organisms (like plants and us!) could be controlled by both our genes AND the genes of the bacteria that live inside and outside of us. In fact, humans and our bacterial partners together sometimes are considered to be part of a larger "super-organism."

Since emerging evidence suggests that the human microbiome has a large influence on the development of human diseases, the National Institutes of Health recently started The Human Microbiome Project (HMP, <https://commonfund.nih.gov/hmp/index>.) The goal of this project is to identify and characterize the species and communications that occur within human microbiomes and analyze the role of microbes in human health and disease. Since the start of the project in 2009, over 340 papers associated with the HMP have been published and research is still ongoing!

In this activity, students will analyze an article discussing whether microbiome transplants can be effective in disease prevention or treatment in humans. They will then complete a worksheet assessing their comprehension of the article.

WHAT'S THE QUESTION

Can the microbiome be manipulated to treat disease?

PROCEDURE

1. Ask students to think about the investigation they just conducted about how bacteria send signals to one another. Ask, *Do you think the bacteria inside our bodies also can communicate with our own cells?* Help students understand that the bacteria that comprise our microbiome produce molecules that affect the immune system, promote or suppress inflammation and even protect from other harmful microbes. This communication has a large influence on the development of human diseases.
2. Tell students that when their gut microflora becomes dominated or overpopulated by a harmful bacterium, serious illness can result. This is what happens when someone

becomes sick as a result of infection with the bacterium, *Clostridium difficile* (called, “difficile,” because it is difficult to grow in the lab). *C. difficile* infections usually occur after a lengthy course of antibiotics that destroy the normal ecosystem with the intestines. One promising new treatment involves transplanting gut bacteria from a health person back into the sick person in a process called, fecal transplantation.

3. Show students the video, “Why would anyone get a fecal transplant? Watch a brother and sister explain” (see video URL, p. 18)
4. As a follow-up to the video, have students read the article from BioEd Online (see p. 21).

Please note: The article uses some out-of-date data regarding the estimated number of gut microbes. Be sure to point this out to students before they begin reading.

Distribute and have students complete the student page, “About the Microbiome Article.”

5. Organize students into small study groups to discuss what they learned and their reactions to the new information contained in the article and video.

Wrap It Up

Hold a class discussion using the worksheet as a guide to questions. Have the small groups discuss their conclusions and ideas about new understandings of the roles of bacteria in health and disease.

Students also can listen to the following NPR broadcast.

Staying healthy may mean learning to love our microbes
<http://www.npr.org/blogs/health/2013/07/22/203659797/staying-healthy-may-mean-learning-to-love-our-microbiomes>

Additional information about FMT (fecal microbial transplants) can be found at the site below.

Fecal transplantation (Bacteriotherapy)
http://www.hopkinsmedicine.org/gastroenterology_hepatology/clinical_services/advanced_endoscopy/fecal_transplantation.html

Fecal Transplant Pill May Bring Balance to Microbiomes

Our gut flora, 100 trillion microorganisms in our intestines also known as the gut microbiome, does much more than help us to digest food. It plays an important role in promoting overall health by regulating inflammation and metabolism, and by controlling infection.



One such infection is caused by the bacterium, *C. difficile*. Each year, about 500,000 people in the US become infected by *C. difficile*, and about 14,000 die. *C. difficile* is naturally present (and non-harmful) in a small percentage of the population, but it can be spread to anyone through resilient spores sometimes found in hospitals and nursing homes. Infection can disrupt the normal gut microbiome. No surprise, then, that people with *C. difficile* infection can experience fever and serious intestinal problems, including nausea, cramping and severe diarrhea. In extreme cases or particularly vulnerable patients, infection can lead even to death.

Initial treatment consists of certain antibiotics, but the infection sometimes proves resistant to these medications. Fortunately, alternative treatments using fecal transplants have shown success in clearing persistent *C. difficile* infections. However, these treatments require the placement of tubes down a patient's nose or the use of enemas to introduce the transplanted material, so they are uncomfortable, and sometimes impractical.

An even newer development comes in the form of a pill. Donor stool, usually from a relative, is processed to extract the resident microflora, encapsulated, and then triple-coated in gelatin to ensure the pills reach the intestines before dissolving. The full dose (ranging from 24 to 34 capsules) appears to promote the resurgence of diverse and health intestinal flora. A notable drawback to this treatment is that the pills must be custom processed for each patient just before treatment, with microflora collected from a very specific donor.

Given the large number of people who suffer from *C. difficile* and other intestinal disruptions (such as ulcerative colitis and Crohn's Disease), there actually had been a significant "do-it-yourself" market for fecal microbiota therapy (fecal transplants), despite the potential "gross-out" factor. Recently, however, the US Food and Drug Administration identified the pills as an investigational drug. So future access to these treatments are likely to be regulated by federal processes and oversight. As research into the human microbiome progresses, doctors and scientists hope to produce a pill that encompasses the full diversity of healthy intestinal flora, and/or identify characteristics a "universal donor" to provide safe and effective treatment for *C. difficile* and other intestinal disruptions.

Name: _____

About the Microbiome Article

Read the article and complete the questions below.

1. Making connections: How does the topic of this article relate to you? Have you ever had food poisoning or the stomach flu? How did you treat it?

2. Summarizing: Why are doctors performing fecal transplants?

3. Critical Thinking: Are fecal transplants similar to other kinds of human transplants? Compare and contrast the fecal transplant with a heart transplant.

4. Inferring from the data: How can we determine whether a potential fecal transplant donor is "good"? Could we use this form of treatment for all kinds of diseases, or only certain ones?

6. Synthesizing: Why do doctors transplant patients with a fecal microbiome, and not with a specific bacterium? How does a microbiome differ from a single, specific strain of bacteria?

7. Questions: What additional questions do you have after finishing this video and article?

8. Research the Next Steps: Pick a couple of key words out of your questions above. Use these key words to research and find answers to your questions. Summarize your results below.

INVESTIGATION 5

Feeding Your Microbiome

OVERVIEW

Students will compare and discuss data generated by a recent experiment in which the gut bacteria of subjects was studied with and without the addition of dietary fiber.

TIME NEEDED

1 to 2 sessions

MATERIALS

Per Student

- Copy of “Bacterial Populations”(p. 27)
- Copy of “About Bacterial Populations” (p. 28-29)

BEFORE YOU START

Make one set of copies of the student sheets for each student.

BACKGROUND

Bacteria exist as communities of different species within and on our bodies. As students discovered in the first activity of this unit, the diversity of microbes varies by location. In this activity, students will examine recent experimental data that demonstrates that food sources also affect the bacterial species that are present in a bacterial ecosystem.

The large intestine contains the largest bacterial ecosystem in the body, and may contain more than 35,000 bacterial species. However, the composition of the bacterial flora in the gut changes based on food sources. Recent research suggests that the ratios of different major groups of bacteria can shift within 2–3 days in response to a change in diet. This adaptability in gut flora might have important consequences for the host when food sources change. Relatively rapid changes in populations of intestinal bacteria will make it possible for the host to take advantage of a range of food sources as they become available.

Obesity researchers are discovering that individuals who eat diets comprised mainly of highly refined sugars and starches (such as white bread, processed cookies and candies, potato chips, etc.) have different amounts of certain groups of bacteria in their intestines. Some of the bacteria associated with a refined food diet, such as members of the Phylum Firmicutes, are suspected to play roles in obesity and even inflammation throughout the body. Members of other bacterial groups, such as the Bacteroidetes, are associated with diets higher in fiber and also tend to be more prevalent in lean individuals.⁵

In this activity, students will examine real data from an experiment that examined the relative composition of Firmicutes and Bacteroidetes in the intestines of subjects with and without fiber supplementation. Fiber in the large intestine serves as food primarily for

⁵ <http://www.scientificamerican.com/article/how-gut-bacteria-help-make-us-fat-and-thin/>

Bacteroidetes. These bacteria break fiber down to obtain energy through fermentation. These bacteria use a variety of fibers as food, including resistant starch (found in bananas, oats, beans); soluble fiber (in onions and other root vegetables, nuts); and insoluble fiber (in whole grains, especially bran, and avocados). The less a food is processed, the more fiber gets through the intestinal track and becomes available as an energy source for bacteria. Foods, such as these, are referred to as “prebiotics,” because they promote the growth of helpful bacteria in the large intestine. Probiotics, on the other hand, are helpful bacteria that are found in fermented food sources, such as yogurt, sauerkraut and kimchi.

In the experiment, subjects were given snack bars without supplementary for three weeks, and bars with two different sources of soluble fiber (polydextrose and corn fiber) for three weeks each. Both of these fibers are partially fermented by gut bacteria. The laboratory used a randomized, double-blind, cross-over design for the experiment. This means that the persons providing the snack bars and the participants were unaware of the fiber content of the bars at all times. DNA from fecal samples was used to estimate the composition of the gut microbiome after each treatment.

WHAT'S THE QUESTION?

Can the balance of different microbes in the gut be altered?

PROCEDURE

1. Have students sit in groups of four and provide each student with a copy of the sheet, “Bacterial Populations.” Students should read the paragraph describing the experiment, and then complete “About Bacterial Populations” page that require them to interpret the results as depicted on the graph.
2. After each group has completed their analysis of the results, conduct a class discussion about what they learned. Make certain that students understand that the addition of either kind of fiber led to similar changes in the gut flora—in both cases, a shift to a greater proportion of Bacteroidetes as compared to Firmicutes.
3. Finally, discuss the additional experiments with mice that demonstrate how the ratio of Bacteroidetes to Firmicutes may play a role in obesity.

WRAP IT UP

Have students share their conclusions regarding the possible role of diet and body composition. Ask if this new information provides a motivation to eat fewer processed foods. You may want to list several examples of processed foods vs. healthier options: white bread vs. whole grain bread, fruit roll-ups vs. fresh fruit or cookies vs. whole grain snack or breakfast bars.

EXTENSION

Researchers believe that treatment with antibiotics causes weight gains in live stock (such as beef cattle), because the antibiotics change the composition of their gut microbiome.

Have students investigate the relationship between antibiotics and the microbiome and report their findings. Or show the following interview from CBC News in class.

How the gut's "microbiome" affects weight gain

<http://www.cbsnews.com/news/how-the-guts-microbiome-affects-weight-gain/>

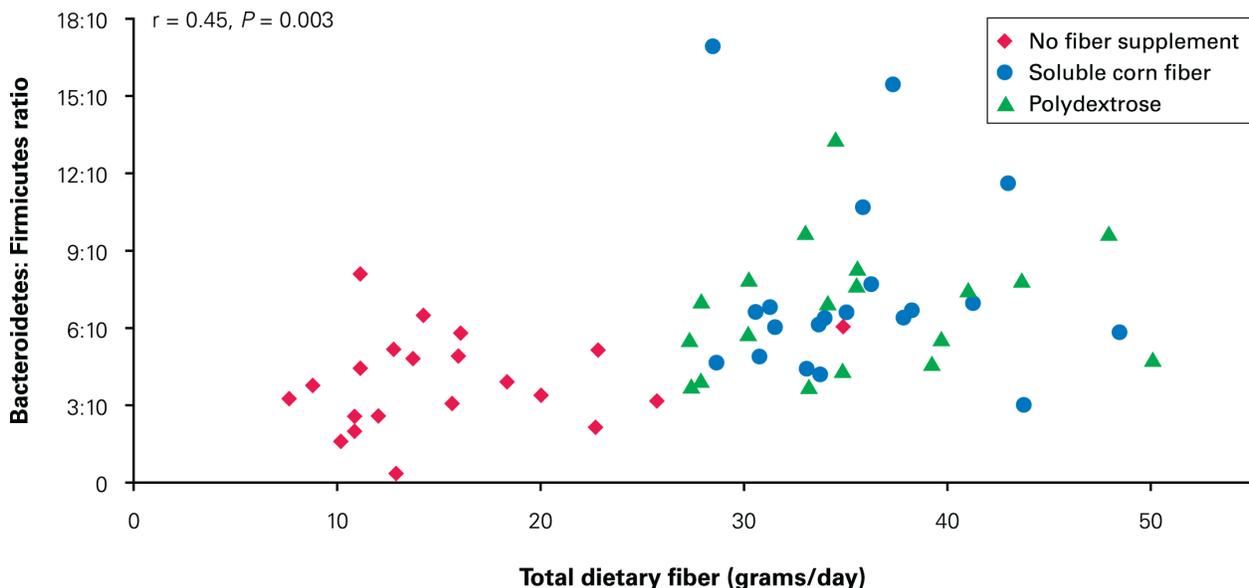
Bacterial Populations

Recently, scientists at the University of Illinois conducted a double-blind experiment to see if adding fiber to a person's diet would shift change the balance between two different kinds of bacteria in the large intestine. In a double-blind experiment, neither the experimenters nor the participants know who is receiving which treatment at any time.

21 health adult men participated in the experiment. They were divided randomly into three treatment groups. Each person was given three "rice crisp" bars per day to eat in addition to his regular diet. There were three kinds of bars: bars without fiber, bars with 7 grams of supplemental fiber in the form of polydextrose, and bars with 7 grams of fiber in the form of soluble corn fiber. All the bars looked and tasted the same.

Each person ate one kind of bar for three weeks, before switching to the next kind of bar. They also reported all other food sources, so that their total daily amount of dietary fiber could be estimated. The total length of the experiment was nine weeks (each person randomly was assigned a different kind of bar for each three-week period).

Fecal samples were collected from each person during the final five days of each three-week period. Bacterial DNA was extracted from the samples and used to identify the kinds and amounts of two different families of bacteria: Firmicutes and Bacteroidetes. The results are shown below. The colored points represent individuals who participated in each three-week block of the experiment.



Name: _____

About Bacterial Populations

Discuss and answer the following questions related to the fiber-bar experiment.

1 What question was being investigated?

2. Why was it important for all the bars to look and taste the same?

3. What happened to the relative amount of Bacteroidetes bacteria in the gut when the amount of fiber in the diet was increased.

(continued on the following page)

Name: _____

About Bacterial Populations (*continued*)

Now, consider the following additional information. Investigators also have looked at the ratio of Firmicutes and Bacteroidetes in the intestinal microbiomes of lean and obese mice. They found that obese mice have a higher proportion of Firmicutes to Bacteroidetes than lean mice. The same results have been observed in obese humans compared to lean subjects.

The effects of Firmicutes on weight gain are believed to be related to the abilities of Firmicutes to make more calories from food available to the host—as compared to the amount of calories made available by Bacteroidetes.

4. What insight does this new information provide related to the effects of diet on body weight?

INVESTIGATION 6

Got Fiber?

OVERVIEW

Students will estimate and rank the amount of fiber that they expect will be found in common food items, compare their estimates to the real ranking and create a menu for a fiber-rich meal.

TIME

1 session

MATERIALS

- 10 packaged non-perishable food items—or empty packages (see “Before You Start” below, for suggested items and information)
- Student copies of “Fiber Content Challenge,” (p. 33)

BEFORE YOU START

Collect 10 packaged food items (or empty packages) that do not require refrigeration with different fiber contents per serving as expressed on nutrition labels. Specific brands of food items are not important. Suggested items should include foods such as canned beans, rice, vegetable juice, canned meat product, breakfast cereal, spaghetti noodles, energy bar or raisins.

Check the nutrition labels for fiber content for each item to be sure that there is a wide range of fiber content among the products. Create a chart of the actual fiber content of each food. Do not immediately reveal this information to students.

Tape a paper cover over each food label.

You also may substitute fresh foods and look up the amount of fiber at the following website.

Fiber chart

<https://www.wehealny.org/healthinfo/dietaryfiber/fibercontentchart.html>

BACKGROUND

Previously, students learned one benefit of fiber in the diet. Fiber serves as an energy source for healthy bacteria in the large intestine. Fiber does other things as well. Soluble fiber, found in beans, peas, lentils, oatmeal, nuts, seeds, strawberries, and blueberries helps lower LDL or bad cholesterol. It also helps to regulate blood sugar and may lower the risk of heart disease and type 2 diabetes. Insoluble fiber, found in whole grains, brown rice, wheat bran, cucumbers, celery, green beans, raisins, and tomatoes, helps to prevent constipation. It also aids in reducing the chance of getting diverticular disease.

The U.S. Department of Agriculture recommends 14 grams of fiber for every 1,000 calories of food consumed. For a typical man under the age of 50, the diet should include a total of 38 grams assuming a daily intake of 2,700 calories. Over 50, the recommended fiber intake is 30 grams. Women under 50 should take in 25 grams of fiber and 21 grams if over 50. While the benefits of fiber are many, many of our popular foods contain little or no fiber. Foods that are highly processed usually lose their fiber during the processing. A piece of fresh fruit, for example, will have more fiber than 100% juice made from that fruit. Brown rice has more fiber than white rice, which has been processed to remove the bran (much of its fiber) and much of the germ (plant embryo contained within the seed).

Shown below is an example chart of real food items and their relative fiber content

Food Item*	Grams of Fiber	Daily Percentage Estimate
Hormel Chili Vegetarian with Beans	10g	40%
Kroger Green Split Peas	9g	36%
Kroger Raisin Bran	7g	28%
Kroger Extra Fancy Pork and Beans	6g	24%
Fiber One Streusel Bar	5g	20%
Kroger Movie Theater Butter Popcorn	4g	18%
V8 Original 100% Vegetable Juice	3g	12%
Uncle Ben's Brown Rice, Whole Grain	2g	10%
Homestyle Chicken Noodle Soup	1g	4%
Hormel Chili Vegetarian with Beans	0g	0 %

One of the best ways to increase fiber intake is to read food labels. Labels identify the number of fiber grams per serving and the percentage of daily requirements. The daily requirement is generalized for all populations. Every gram of fiber equals about 4% of recommended intake. Food labels on different foods round off numbers. One package may say 3 grams and 10% and another may say 3 grams at 12%.

WHAT'S THE QUESTION

Why is fiber in food important to the health of the microbiome of the gut and to the general health of humans? Where can we find good sources of fiber?

PROCEDURE

1. Display the food items on a table in no particular order (tape a paper cover over the food labels before class). Distribute copies of the "Fiber Content Challenge." Have students complete the chart content without looking at the nutrition labels.
2. When students have completed their lists, reveal the actual fiber content list. Have students put check marks on their lists for every position on the chart. Check the following example. In this example, the student made six correct order choices.

Sample of a ranking chart with a student order column inserted before the score

Fiber Rank	Name of Food Item	Sample Student Order	Score
10 (most)	Hormel Chili Vegetarian with Beans	Vegetarian chili	✓
9	Kroger Green Split Peas	Fiber One Streusel Bar	
8	Kroger Raisin Bran	Raisin bran	✓
7	Kroger Extra Fancy Pork and Beans	Pork and brans	✓
6	Fiber One Streusel Bar	Peas	
5	Kroger Movie Theater Butter Popcorn	Popcorn	✓
4	V8 Original 100% Vegetable Juice	V8 juice	✓
3	Uncle Ben's Brown Rice, Whole Grain	Brown Rice	✓
2	Homestyle Chicken Noodle Soup	Tuna	
1 (least)	Starkist Chunk Light Tuna	Chicken noodle soup	

3. Compare student choices and determine which student is most fiber content conscious. If desired, present the student with a fiber-rich snack as a reward. Review with the class why a fiber-rich diet is important to their microbiome and their overall health.

WRAP IT UP

Have students create a menu for a fiber-rich meal. The menu should contain 10–12 grams of fiber and be no more than 800 calories. Have students identify the items on the menu based on nutrition labels from the Internet. They should search by food type and product name. (product name, nutrition label)

Specific brands of food items above are not important. These items were available locally. Choose any local or national brands that are convenient. Check the nutrition labels for fiber content and create a new table showing ten items with different fiber content.

Fiber Content Challenge

Ten food items are displayed. Without checking the food label, estimate the relative amount of fiber contained in a serving of each of the foods.

In the table below, rank the food items from most to least amount of fiber per serving. Write the names of food items in the appropriate column starting with the highest fiber per serving and descending to the lowest at the bottom.

Check off each correct ranking when the answers are provided. How many correct estimates did you make?

Ranking of Fiber per Serving	Name of Food Item	Score (Place a check mark for each item that you ranked correctly.)
Most fiber 10		
9		
8		
7		
6		
5		
4		
3		
2		
1 Least fiber		