FUTURELAB+

AG/ENVIRONMENTAL

Solution Seeking Microbes

Yogurt Fermentation

Laboratory Investigation

Developed in partnership with:

Bay Area Bioscience Education Community

In this Lesson Plan:

Print the **Teacher Section** →

Print the **Student Section** →

Teachers [T] *and* Student Resources [S] *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 or a range of pages:

Single Pages (use a comma): T3, T6	
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Cover Image
Lactobacillus casei is one of many friendly bacteria in your gut microbiome.

AG/ENVIRONMENTAL/ SOLUTION SEEKING MICROBES

Lab: Yogurt Fermentation

ACTIVITY DURATION

Five class sessions
(45 minutes each)

DRIVING QUESTION

How do microbes influence food and human health?

ESSENTIAL QUESTIONS

How are microbes utilized in the production of food?

What are connections between the microbiome and human health?

How are testable questions generated for a scientific investigation about the microbiome and probiotics?

OVERVIEW

Fermented foods are a regular part of diets worldwide. For example, fermented cabbage appears in both Korean cuisine as kimchi and German food as sauerkraut. Throughout history, fermentation was used primarily to preserve foods such as fresh vegetables and dairy. During fermentation, microorganisms convert the carbohydrates in the food into acid and ethanol, which in turn changes the environment so that it remains edible longer. Fermentation is also responsible for the tart, tangy flavor that makes fermented foods so delicious.

In this lab, students will observe the process of fermentation by making their own kefir yogurt. They will begin by observing milk and kefir samples under the microscope, and then predicting which can be used as a yogurt starter culture using their understanding of microorganisms. Students will then analyze data from previous studies showing how different variables influence yogurt production and choose one variable to change in their kefir yogurt fermentation experiment. They will then collect observational and pH data over a 48-hour period in order to determine how their changed variable affected the characteristics of the kefir yogurt. Students will also be given an opportunity to learn about connections between the microbiome and probiotics, as well as to generate their own testable question based on a real-world example from a scientist.

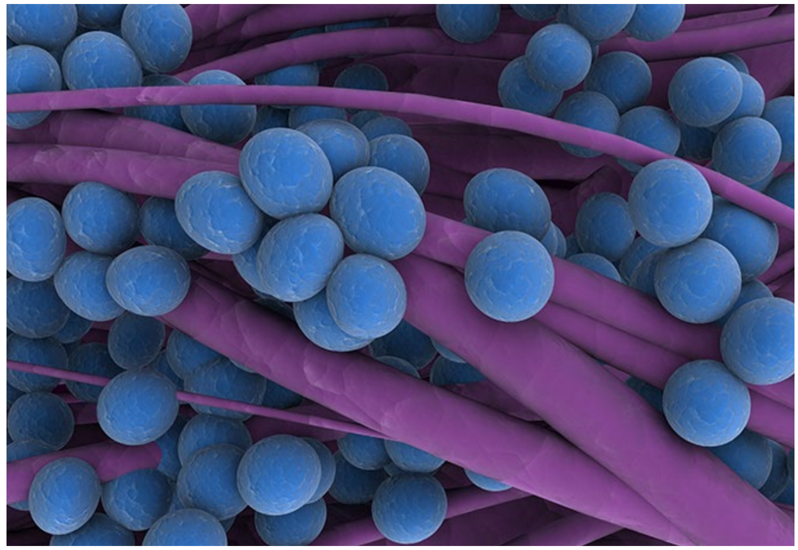
BACKGROUND INFORMATION

Students will begin this lesson by observing slides of milk and kefir under the microscope, a skill they practiced in the first lesson of the unit. Based on their observations and understanding of bacteria, students will determine which sample to use in the preparation of their own fermented product. Because they will be choosing a variable to manipulate in their experiment, it is helpful to have a foundational understanding of experimental design, i.e., control group, independent and dependent variables, controlled variables, data collection, etc. This lab introduces the concept of fermentation as it relates to food production and may connect to students' prior knowledge of metabolic pathways as an alternative to cellular respiration.

Have you ever wondered…

What does it mean for a food, such as yogurt to have "live active cultures?"

The phrase "live active cultures" in food refers to multiple species of microorganisms, especially bacteria, that are living inside the food. These microbes use a process called fermentation to break down sugars in the food and produce lactic acid and alcohol. In yogurt making, bacteria convert the lactose sugar in milk into lactic acid, which makes the milk more acidic and causes the proteins to coagulate and thicken. This gives yogurt its texture and tangy flavor.

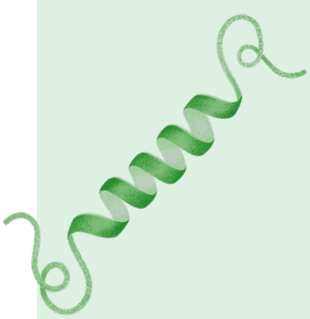


A magnified view of the bacteria staphylococcus (3D illustration).

MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

This unit connects how we can utilize microbes for human health and food production. The microbial relationship humans have with food production will be expanded on in an upcoming lesson.



How does this connect to careers?

Microbiologists study microscopic life forms, such as bacteria. They design and perform experiments to answer questions about the cellular processes and mechanisms governing microbe behavior.

Food scientists use lab and field studies to improve the safety, quality, and production of food. They use microbiology, engineering, and chemistry to meet the food safety requirements of the FDA and the needs of consumers and food manufacturers.

How does this relate to the product development life cycle?

In this lesson, students explain how bacteria are used in yogurt production as an example of how microorganisms can be used to develop other products. They also consider criteria for scaling up a product at a commercial level. These learnings connect to the Discovery and the Manufacturing parts of the product life cycle.

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 will communicate effectively with each other as they work in pairs and groups of three throughout the lesson. For example, they will design and perform their experiment as well as teach and learn from their classmates in two Jigsaw reading activities. This teamwork can help students develop positive relationships as they practice decision-making skills.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

This lesson focuses on how fermentation is used to make food products and introduces students to the beneficial types of relationships between humans and microorganisms. As many cultures around the world make some form of fermented food, students will likely have personal and cultural connections to this topic.

COMPUTATIONAL THINKING PRACTICES

Students will engage in logically organizing data, analyzing data, and finding patterns during the course of this lab. As students plan their experiment, they will consider how the independent variable they manipulate might influence dependent variables. Students will need to organize their data and observations such that they are able to analyze the results and support or refute their predictions. Students will also be asked to find trends in the data collected.

OBJECTIVES

Students will be able to:

Design and **conduct** an investigation to determine how a particular variable affects kefir yogurt fermentation using scientific data and text.

Make a claim about how the variable they changed affected the characteristics of their kefir yogurt and support it with evidence and reasoning using scientific text and experimental results.

Explain the role of micro-organisms in fermentation and the connection between the microbiome and probiotics using scientific text.

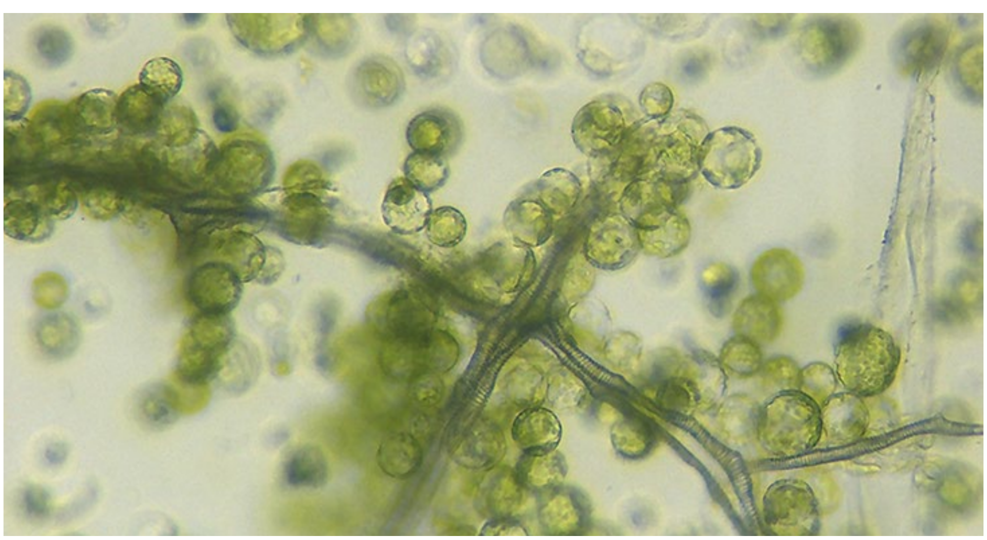
Ask a testable question relating to the microbiome using SMART goals.

Materials

Documents
Lab Preparation (for teacher)
Phenomenon: Food Chart (1 per pair)
Background Reading: Effects of Variables on Yogurt Production (Jigsaw) (1 per group of three)
Background Reading: Fermentation (1 per student)
Background Reading: The Microbiome (Jigsaw) (1 per student)
Background Reading: SMART Goals for Scientific Research (1 per student)
Career Profile: Celeste Allaban, DVM (1 per student)
Vocabulary Tool (1 per student)
Student Protocol (1 per pair)
Student Guide (1 per student)

Reagents
Kefir (15 mL per pair)
2% dairy milk and various plant-based "milks" and/or animal milk (150 mL of each milk per pair)

Equipment and Consumables
Clean containers with lids that can hold about 200 mL (2 per pair)
Transfer pipette (1 per pair) *P1000 micropipette and tips can be used in place of transfer pipette to help control for variation in the volume of kefir added.*
100-mL graduated cylinder (1 per pair)
Microwave (1 per class)
Incubator and/or fridge (1 per class, optional)
Permanent marker and lab tape (1 per pair)
Dry waste beaker (1 per group of four)
Sink or wet waste beaker (1 per group of four)



Chloroplasts in a plant cell viewed under a microscope.

Day 1

LEARNING OUTCOMES

Students will be able to:

Make observations and **ask** questions about fermented foods and describe a personal connection to a particular fermented food using images.

Identify differences between kefir and milk to determine which to use as a yogurt starter culture using microscopes and their knowledge of microorganisms.

Describe the process of bacterial fermentation to produce yogurt using scientific text.

Procedure

Teacher Note > *Before class, prepare slides with milk and kefir, and set up microscope stations around the room. (See Lab Preparation for more details.) If students are unfamiliar with microscopes, we suggest setting up each microscope with a slide and focusing the image before students make observations. Students can then adjust the fine focus, but don't need to do any additional prep.*

Whole Group (15 minutes)	
1	Give each student one copy of the *Student Guide* and one copy of *Phenomenon: Food Chart* (without the key) per pair of students. Ask students to observe the foods and answer Question #1 in *Student Guide, Part 1: Pre-Lab*.
2	Ask students to share what they think the foods have in common and, if a student knows what one of the foods is, to explain it to the class. This is a great opportunity to specifically bring in student voices who rarely share out (be sure to check in with them first to see if they are comfortable sharing their connection to that food and if they have identified it correctly).
3	Show students the key to the *Phenomenon: Food Chart* and ask them to share with the class what stands out to them and any questions they have.
4	Tell students that this lab focuses on fermented foods (a trait that all the foods in the chart share) and that they will be making yogurt as an example of a food made from bacterial fermentation. Share that the relationship between humans and microbes has a long history. From beer making in China starting around 7000 BC, bread making in Egypt around 1000 BC, and cheese making around 8000 BC, people have enjoyed the benefits of microbial fermentation. Ask students if they know what fermentation is.
5	Ask students to think of an example of a fermented food that is important to their culture and/or family and share with the class what it is, why it is important to them, and how it is made (if they know).This is a great opportunity to celebrate the diversity of cultures represented in the class and highlight the ubiquitousness of fermented foods around the world.

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Day 1

Continued

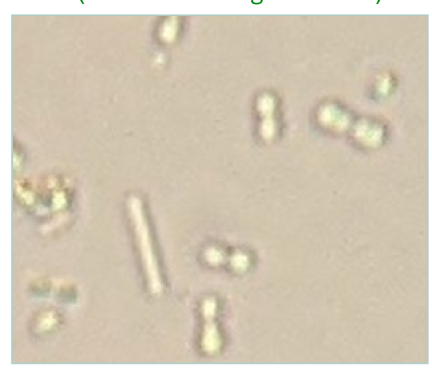
Procedure

Individual (10 minutes)	
1	Give each student a copy of *Background Reading: Fermentation*. Read the first few sentences as a class and model how to annotate to increase comprehension.
2	Ask students to complete the reading and answer the questions.

Small Group (15 minutes)	
1	Tell students that the first step to making yogurt is to choose a starter culture that contains bacteria. They will observe two solutions, one that does not contain bacteria (milk) and one that does (kefir), without knowing which is which (one is labeled “A” and the other is “B”). Based on previous experience in Lesson 1, where students observed various bacterial slides, they will have to use their knowledge to choose the right liquid culture. (Kefir will have smaller bacteria while milk will have large fat globules.)

a. Kefir (400X)

Kefir (400X with magnification)



b. 2% milk (400X)

2% milk (400X with magnification)

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Day 1
Continued

Procedure

2 If microscopes are already set up for students, point out the *fine focus* knob and ask them to turn it very gently and not adjust any other part of the microscope. If students are setting up the microscopes, remind them:

- **a.** Do not touch the glass part of the lenses with your fingers.
- **b.** Adjust the focus SLOWLY—be careful not to let the lens touch the slide.
- **c.** Use the lowest power objective to focus first.
- **d.** Use a drop of oil on the slide when using a 100X objective.
- **e.** Carry the microscope with BOTH hands and do not swing the microscope.

3 Break students into pairs for the lab and ask them to go to their lab stations, make observations of “A” and “B” slides, and complete Question #2 in *Student Guide, Part 1: Pre-Lab*.

4 As students work, ask them questions, such as “How do you know?” and “How does that observation connect to your claim?” to deepen their thinking and help them connect their observations of the size and particular shapes of bacteria to their claims.

Individual (5 minutes)

1 Hand out the *Vocabulary Tool* and ask students to write a sentence for each word for homework.

2 Exit Ticket: Think about one of the fermented foods you like to eat and describe how you think it is made.

Day 2

LEARNING OUTCOMES

Students will be able to:

Make claims about how different variables affect yogurt production using scientific data.

Plan a scientific investigation to determine how a particular variable affects kefir yogurt fermentation using their understanding of yogurt production.

Procedure

Whole Group (5 minutes)	
1	Warm-Up: Have you heard of probiotics? What do you think probiotics are? Are they safe? Are they beneficial? How?
2	Ask students to share with a partner and then randomly call on a few students to share with the class.
3	Tell students that today they are going to plan a scientific investigation to determine how a particular variable affects yogurt fermentation.

Small Group (20 minutes)	
1	Break students into groups of three and give each student one of the three variables in *Background Reading: Effects of Variables on Yogurt Production (Jigsaw)*. Tell students that they will review data from a scientific investigation in which a particular variable was changed in yogurt production. They will then describe their findings to the rest of their group. *Option:* Assign variables based on student reading level (the study in Variable 1 is the most straightforward and the study in Variable 3 is the most complex).
2	Before students read the study assigned to them, review the following definitions and write them on the board:

a. *Viscosity:* A property of a liquid that describes how thick it is. Viscosity an be measured with the unit (μ) where a high number means high viscosity, or very thick liquid.

b. *pH:* A scale of acidity from 0 (most acidic) to 14 (least acidic/most basic).

c. *Firmness:* A property of a solid that describes its texture. Firmnessan be measured with the unit (N) where a higher number means a firmer solid.

d. *Antioxidant activity:* The ability of a chemical to inhibit the production of free-radicals (molecules with an unpaired valence electron that can lead to cell damage). Having high antioxidant activity is considered beneficial for health.

e. *Proteolysis:* The process of breaking down proteins. Low proteolysis is considered desirable in yogurt.

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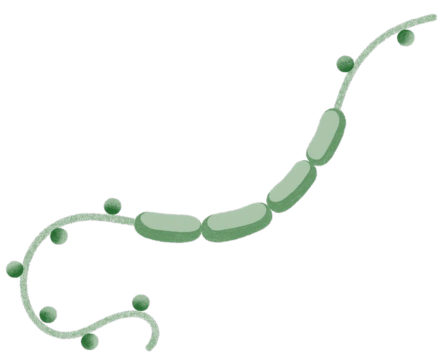
Day 2

Continued

Procedure

3 Ask students to read and answer the questions about their assigned variable. You may also want to have students form “expert groups” to work with other students on the same variable before returning to their “home group” to explain their variable to the rest of their group.

4 Ask students to take turns sharing a summary of the study they read to their group of three.



Small Group (15 minutes)

1 Ask students to work with their lab partners to plan their kefir yogurt fermentation experiments by choosing one variable to change and defining an experimental and a control group (question #3 in Student Guide, Part 1: Pre-Lab). Approve each pair’s plan before students can move on (make sure they have access to the necessary equipment and milk).

Suggested control groups:

- 2% dairy milk (if students are changing milk type or % fat).
- 21°C—room temperature (if changing incubation temperature).
- *37°C would be a good control if you have access to an incubator since this is the ideal temperature of the bacteria.*

2 Ask students to make a prediction about how the yogurt will be affected by their chosen variable (Question #4 in *Student Guide, Part 1: Pre-Lab*).

Individual (5 minutes)

Ask students to complete the first Lesson 2 question in their **Toolkit** using their understanding of fermentation.

Day 3

Procedure

LEARNING OUTCOMES

Students will be able to:

Design their scientific investigation to determine how a particular variable affects kefir yogurt fermentation and collect initial data.

Describe the role of the body's microbiome and probiotics and identify an area of research to explore further using scientific text.

Teacher Note > *Before class, set up student lab stations. (See Lab Preparation for more details.)*

Whole Group (10 minutes)	
1	Warm-up: Look at your answer to Question #2 in *Student Guide, Part 1: Pre-Lab*. What could you add to strengthen your reasoning for which slide showed milk and which showed kefir?
2	Ask students to vote for whether "A" or "B" should be the starter culture and call on a few volunteers to provide reasoning. Ask the class to come to a consensus on which to use. If they are struggling to correctly identify the kefir, search for a "kefir microscope slide" video to show students and point out the characteristics of kefir.
3	Tell students that today they will set up their kefir yogurt fermentation experiment and collect initial data for pH, smell, color, thickness, and texture. They will all use kefir as the starting culture regardless of their answer in the *Student Guide*. *For collecting data, you may wish to leave the observations completely open-ended, provide a scale for each of the qualitative parameters (examples below), or gather student input and come to a class consensus on the scales to use.* *Examples listed below:* • *Smell: very sour, sour, neutral, sweet, very sweet* • *Thickness and texture: all solid, mostly thick/solid, half solid/half liquid, mostly liquid, all liquid* • *Sample quantitative scale for these parameters*

Teacher Note > *Clarify that students should not taste their yogurts at any point during the experiment).*

Small Group (10 minutes)	
1	Ask students to go to their lab stations with their partners and follow their approved plan in the *Student Guide, Part 1: Pre-Lab* Question #3 to set up their experiments.
2	Ask students to collect initial data and record in the "0 hour" data table in *Student Guide, Part 2: Lab.*

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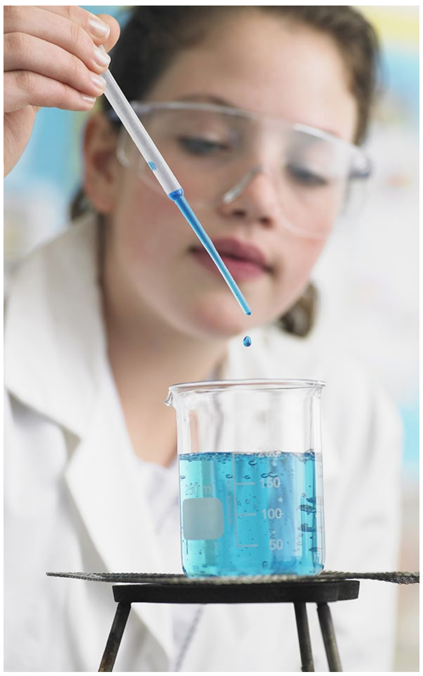
Day 3

Continued

Procedure



Whole Group (5 minutes)

1 Give each student a copy of *Background Reading: The Microbiome (Jigsaw)* and ask them to open the article *The Microbiome* from the Harvard School of Public Health on their computers or provide a printed copy.

2 Read "Section 1: What is the microbiome?" out loud and record three to four main ideas and one to three key words and their definitions in the table as a class.

Small Group (15 minutes)

1 Break students into groups of three and assign each student one of the next three sections (Sections 2–4) in *Background Reading: The Microbiome (Jigsaw)*. Tell students that they will read their section, record three to four main ideas and one to three key words with their definitions in the table, and share their findings with the rest of their group.

2 Ask students to take turns sharing a summary of the study they read to their group of three. As they listen to their peers, students should write down one main idea and one key word from each.

Individual (5 minutes)

Ask students to complete "Section 5: Future areas of research" in *Background Reading: The Microbiome (Jigsaw)*. They will return to this answer the next day when they learn how to write a testable SMART goal question for a scientific investigation.

Day 4

LEARNING OUTCOMES

Students will be able to:

Collect data to determine how a particular variable affects kefir yogurt fermentation.

Explain how scientists use SMART goals to drive their research and write a SMART goal question about microbiome and probiotics research using an interview transcript.

Procedure

Small Groups (5 minutes)

Ask students to collect data for their experimental and control groups, and record in the “24 hour” data table in *Student Guide, Part 2: Lab*. As they work, ask students to consider whether the data is supporting or refuting the prediction they made in *Student Guide, Part 1: Pre-Lab* Question #4.

Whole Group (15 minutes)

1	Have students share with their partners a goal they have had in the last year and how they achieved that goal.
2	Share that today they will read an interview with a scientist to find out how she uses a systematic way to set SMART goals, which lead to developing testable questions about the microbiome and probiotics.
3	Give students each a copy of *Background Reading: SMART Goals for Scientific Research*. Go over the example of a high school student setting a SMART goal for studying as a class.

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Day 4

Continued

Procedure

Small Group (15 minutes)	
1	Ask students to work with their lab partners to read the interview with Dr. Allaban and answer the embedded Questions #1–10. Explain that they will follow the steps she took to go from a broad question ("Do probiotics prevent cancer?") to a testable question that could be used in a scientific investigation using the SMART goals framework.

Teacher Note > *It may be helpful to briefly explain the process of qPCR (a technique for measuring the amount of DNA produced during PCR) as it is mentioned before Question #7.*

2	As an extension, invite students to read *Career Profile: Celeste Allaban, DVM*, the researcher showcased in the interview. Then, students can complete the final questions for Lesson 2 in their **Toolkit.**

Individual (10 minutes)	
1	Ask students to generate their own testable question (#11) about the microbiome and probiotics based on their answer to "Section 5: Future areas of research" in *Background Reading: The Microbiome (Jigsaw)*.
2	Ask students to share their questions with their partners and call on a few students to share with the class.

Day 5

LEARNING OUTCOMES

Students will be able to:

Collect final data to determine how a particular variable affects yogurt fermentation.

Make a claim about how the variable they changed affected the characteristics of their kefir yogurt and support it with evidence and reasoning using experimental results.

Procedure

Small Groups (5 minutes)

1 Ask students to collect final data for their experimental and control groups and record in the "48 hour" data table in *Student Guide, Part 2: Lab.*

2 Prompt students to complete Questions #1-2 on *Student Guide, Part 3: Data Analysis* with their lab partners.

Teacher Note > *Option to have students graph data, such as pH or other quantitative measurements, to use as part of their response to Student Guide, Part 3: Data Analysis Question #1 for data analysis.*

Small Group (20 minutes)

1 Have lab paira label their yogurts with numbers (e.g., 1–15).

2 Instruct students to walk around the room as inan art gallery to visit each lab pair's yogurts and record the variable each pair manipulated. Have them record their observations in Questions #3–4 on *Student Guide, Part 3: Data Analysis*.

Whole Group (10 minutes)

1 Remind students that an important part of the biotech Product Life Cycle is scaling up a new product for mass production and distribution. Discuss the following questions with the class using an instructional strategy such as Think-Pair-Share.

- **a.** Which of the yogurts would be worth scaling up? Which would not? Why?
- **b.** What resources are needed to scale up the production of yogurt?
- **c.** What potential issues may arise when scaling up a product? How could they be avoided?
- **d.** What are the financial considerations with scaling up a product?

Individual (10 minutes)

Ask students to write a complete CER paragraph in their own words and finish for homework (*Student Guide, Part 4: Answering the Driving Question*).

National Standards

Next Generation Science Standards

LS1.C
Organization for Matter and Energy Flow in Organisms
As matter and energy flow through different organizational levels of living systems, chemical elements are recombined in different ways to form different products.

Science and Engineering Practices
Conducting Investigations
Plan an investigation or test a design individually and collaboratively to produce data to serve as the basis for evidence as part of building and revising models, supporting explanations for phenomena, or testing solutions to problems.

Crosscutting Concepts
Cause and Effect
Using the concept of orders of magnitude.

Math

MP2 Reason abstractly and quantitatively
CStudents analyze qualitative and quantitative data collected from a scientific investigation to identify patterns and construct explanations.

Career and Technical Education (CTE)

A3.3
Employ standard techniques of DNA extraction, purification, restriction digests, bacterial cell culture, and agarose gel electrophoresis and document and evaluate results.

A4.2
Describe conditions that promote cell growth under aseptic conditions in the laboratory and workplace.

A4.3
Use various methods to monitor the growth of cell cultures.

A8.1
Follow written protocols and oral directions to perform a variety of laboratory and technical tasks.

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National Standards

CTE *Continued*	**A8.6** Properly and safely use and monitor a variety of scientific equipment, including pH meters, microscopes, spectrophotometers, pipettes, micropipettes, and balances.
	A8.7 Determine which equipment is appropriate to use for a given task and the units of measurement used.

Lab

Preparation

KEY

When the preparation task should take place in relationship to the lab

The amount of time necessary to complete the preparation task

Quick Tips	
1	Before continuing, check the *Materials List* to make sure you have all the necessary equipment and reagents for the lab.
2	We recommend having students complete this lab in **pairs**. • *Part 1*: The instructions below are designed for the teacher to set up stations with prepared slides so that students can simply take turns making observations. However, you have the option to provide these instructions and materials for each pair to create their own slides. • *Part 2*: Each pair is responsible for their own experimental and control yogurt groups.
3	This lab asks students to set up one control group and one experimental group, and monitor them for 48 hours. If you have additional supplies and/or time, *you may wish to have students set up more kefir yogurt samples and/or collect data over a longer time period.*
4	*Virtual Learning Options* substitutions for this lab, including digital-only resources, are provided.

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Lab

Continued

KEY

When the preparation task should take place in relationship to the lab

The amount of time necessary to complete the preparation task

Preparation

Lab, Part 1: Visualizing Culture on Microscope

1 Before the lab — 15 min

Prepare slides:

- [ ] Pipette 800 µL of water and 200 µL of Sample A (kefir) into a microtube. Flick tube to mix.
- [ ] Repeat for Sample B (2% dairy milk).
- [ ] Pipette 50 µL of each diluted sample onto two slides and place coverslip over each.

Note > *It would be best to prepare the slides right before class begins as they may dry out as the day goes on. This can be mitigated if kept in a humid or moist environment. For a demo of how to prep slides, search for a "kefir microscope slide" video.*

2 Before the lab — 15 min

Set up lab stations:

- [ ] If students are setting up microscopes and slides, each pair should have one microscope, one A slide (kefir) and one B slide (milk).
- [ ] If you are setting up microscopes and slides for students, each pair should have one microscope with either the A slide (kefir) OR the B slide (milk) and rotate to another microscope to view the other slide.

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Lab

Continued

Preparation

Lab, Part 2: Fermentation

1 Any time before the lab | 30 min

☐ Select type of milk for experiement.
Suggested milks include:

1	Dairy milk	— 0% — 2% — Whole
2	Other mammalian milks	— Sheep — Goat
3	Plant-based milks	— Coconut milk — Oat milk — Hemp milk — Soy milk — Any nut milk

☐ Aliquot reagents into appropriately sized beakers.
Example for a group of two students:

Reagent	Volume/Group
Kefir	15 mL
Milk(s)	150 mL

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Lab

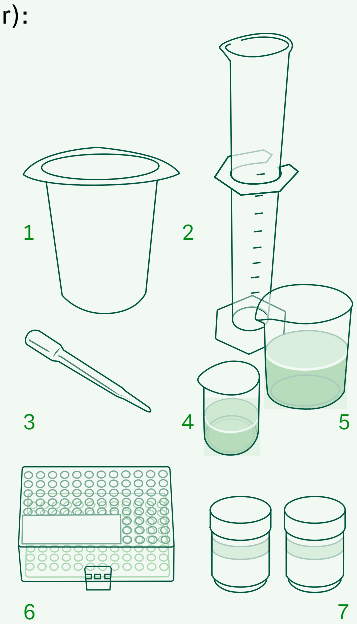
Continued

Preparation

2	Before the lab	30 min

☐ Set up lab stations (one set of supplies per pair):

1	Waste bucket
2	100 mL graduated cylinder
3	Transfer pipette or P1000
4	15 mL kefir
5	~150 mL of milk(s)
6	Optional: P1000 tips
7	2 clean jars with lids



Note > *Milk type and amount will depend on experimental design*

☐ Prepare milk choices and areas with different controlled temperatures. Suggested control groups:

- 2% dairy milk (if students are changing milk type or % fat)
- Room temperature, 21°C (if students are changing incubation temperature)
- 37°C would be a good control if you have access to an incubator because this is the ideal temperature of the bacteria.

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Lab

Continued

Virtual Learning Options

1	Anytime, Digital Only	30 min
☐	Microscope observations: Search for a “kefir microscope slide” video and ask students to record three characteristics of kefir.	
☐	Yogurt fermentation: Search for a “kefir fermentation time lapse” video and record five observations.	
☐	Ask students to choose one of the three readings from *Background Reading: Effects of Variables on Yogurt Production (Jigsaw)* and use the included data to complete Part 4 of the *Student Guide*.	
2	Anytime, At Home	2 hrs
☐	Students make yogurts at home. They will need: — Milk — Kefir — Small containers — pH test strips (optional)	

Effects of Variables on Yogurt Production (Jigsaw)

ANSWER KEY **Do not share with students**

Variable 1: Incubation Temperature

1. What variable did the scientists change?
   Name the experimental groups they analyzed.

Temperature at which the yogurt was incubated (°C) Groups: 30°C, 35°C, 40°C, 45°C

2. What variable(s) did the scientists measure?

pH and viscosity of the yogurt

3. Name two or three conclusions you can draw from the results.

— The pH of yogurt decreases (becomes more acidic) as the incubation temperature increases.

— The viscosity of yogurt increases (becomes thicker) as the incubation temperature increases.

4. On a scale of 1 to 5, how interested are you in seeing how this variable affects the yogurt you will make? Explain. (1 = not interested and 5 = very interested)

Answers vary: This question will help prepare students to discuss with their groups how to set up their experiments.

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Effects of Variables on Yogurt Production (Jigsaw)

ANSWER KEY **Do not share with students**

Continued

Variable 2: Milk Type

1. What variable did the scientists change?
   Name the experimental groups they analyzed.

Type of milk used to make yogurt
Groups: soy 1, soy 2, coconut, cashew, almond, hemp, dairy

2. What variable(s) did the scientists measure?

pH, viscosity, firmness, appearance, odor, flavor, texture, and acceptability of the yogurt

3. Name two or three conclusions you can draw from the results.

Examples:

— Soy and almond yogurt have the most similar viscosities to dairy yogurt.

— Hemp and coconut milk have the lowest pH (highest acidity).

— Hemp yogurt is more than twice as firm as the next firmest yogurt (soy 2).

— Almond yogurt had the lowest rating on “flavor” and dairy yogurt had the highest rating.

4. On a scale of 1 to 5, how interested are you in seeing how this variable affects the yogurt you will make? Explain. (1 = not interested and 5 = very interested)

Answers vary: This question will help prepare students to discuss with their groups how to set up their experiments.

Continues next page >

Effects of Variables on Yogurt Production (Jigsaw)

ANSWER KEY **Do not share with students**

Continued

Variable 3: Percent Milk Fat

1. What variable did the scientists change?
   Name the experimental groups they analyzed.

Percent of fat in milk used to make yogurt
Groups: No bacteria (control), maximum 0.5% fat (fat free), 2.1% fat (semi fat), 3.6% fat (full fat)

2. What variable(s) did the scientists measure?

pH, antioxidant activity, survival of bacteria over time

3. Name two or three conclusions you can draw
   from the results.

Examples:

— More bacteria survived in the fat free yogurt over time than in semi- or full fat.

— Semi-at yogurt became the most acidic (lowest pH) over 21 days of storage.

— Antioxidant activity peaked for all samples after 14 days and was the highest for fat free and semi-fat yogurt.

4. On a scale of 1 to 5, how interested are you in
   seeing how this variable affects the yogurt you will make?
   Explain. (1 = not interested and 5 = very interested)

Answers vary: This question will help prepare students to discuss with their groups how to set up their experiments.

Fermentation Questions

ANSWER KEY **Do not share with students**

1. How does the process of fermentation relate to microorganisms?

Microorganisms are what cause the process of fermentation. Through a natural metabolic process, these microorganisms convert a carbohydrate, such as starch or sugar, into either alcohol or acid.

2. What are the two strains of bacteria used in yogurt making?

Streptococcus thermophilus and *Lactobacillus bulgaricus*

3. Name two or three conclusions you can draw from the results.

Yogurt and kefir are both made through the bacterial fermentation of lactose (a milk sugar) into lactic acid. Both taste tangy as a result of this. Kefir is made from ‘grains’ that contain yeast, in addition to the bacteria. These additional microorganisms result in alcoholic fermentation and a thinner product.

Background Reading: The Microbiome (Jigsaw)

ANSWER KEY — **Do not share with students**

Directions

Open the article The Microbiome from the Harvard School of Public Health. Record main ideas and key words with their definitions for each section in the table below.

	Main Ideas (3–4)	Key Words and definitions (1–3)	How does this relate to fermented foods, such as kefir yogurt?
Section 1 *What is the microbiome?*	There are trillions of microorganisms inside our bodies. The microbiome plays many key roles in promoting smooth daily operations in the human body. A person is first exposed to microorganisms as an infant, during delivery in the birth canal and through the mother's breast milk. Each person has an entirely unique network of microbiota that is originally determined by one's DNA. The microbiome consists of microbes that are both helpful and potentially harmful.	Microbiome/microbiota/ microbes = the microorganisms inside our bodies Symbiotic = both the human body and microbiota benefit Pathogenic = promoting disease	Kefir yogurt, like breast milk, contains living microorganisms
Section 2 *How microbiota benefit the body*	Microbiota stimulate the immune system, break down potentially toxic food compounds, and synthesize certain vitamins and amino acids. Microbiota help to break down complex carbohydrates with their digestive enzymes. The microbiota of a healthy person will also provide protection from pathogenic organisms that enter the body, such as through drinking or eating contaminated water or food. Microbes are believed to prevent the overgrowth of harmful bacteria by competing for nutrients and attachment sites to the mucous membranes of the gut, a major site of immune activity and production of antimicrobial proteins.	Lactose = milk sugar Complex carbohydrates = starches and fibers	Kefir yogurt and other fermented foods contain living microorganisms.

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Background Reading: The Microbiome (Jigsaw)

ANSWER KEY **Do not share with students**

Continued

	Main Ideas (3–4)	Key Words and definitions (1–3)	How does this relate to fermented foods, such as kefir yogurt?
Section 3 *The role of probiotics*	Probiotics make up a multi-billion dollar industry that is evolving in tandem with quickly emerging research. Although published research is conflicting, there are specific situations in which probiotic supplements may be helpful, especially in the very young and very old. Probiotics may be helpful in situations of stress to the body: e.g., reducing severity of diarrhea after exposure to pathogens or replenishing normal bacteria in the intestine after a patient uses antibiotics. Probiotics are “supplements,” not food, so are not regulated by the FDA.	Probiotics = foods that naturally contain microbiota, or supplement pills that contain live active bacteria—advertised to promote digestive health	Like probiotic supplements, kefir yogurt and other fermented foods contain beneficial, live microorganisms. However, they are regulated by the FDA as “foods.”
Section 4 *Can diet affect one’s microbiota?*	Diet plays a large role in determining what kinds of microbiota live in the colon. A high-fiber diet in particular affects the type and amount of microbiota in the intestines. SCFA have wide-ranging effects on health, including stimulating immune cell activity and maintaining normal blood levels of glucose and cholesterol. Fruits, vegetables, beans, and grains are all good sources of prebiotic fibers. If one does not have food sensitivities, it is important to gradually implement a high-fiber diet because a low-fiber diet may not only reduce the amount of beneficial microbiota, but increase the growth of pathogenic bacteria that thrive in a lower acidic environment. Fermented foods are probiotic—their live microbiota may alter one’s microbiome.	Short chain fatty acids (SCFA) = molecules released after fermentation of fiber Prebiotics = fibers that feed beneficial microbes	Kefir yogurt is a fermented food so its live microbiota may alter one’s microbiome.

Continues next page >

Background Reading: The Microbiome (Jigsaw)

ANSWER KEY **Do not share with students**

Continued

	Which area of research would you be most interested in learning more about? Why? You will later write a testable SMART goal question about this area that could be used in a scientific investigation.
Section 5 *Future areas of research*	Answers will vary.

Background Reading: SMART Goals for Scientific Research

ANSWER KEY — Do not share with students

Directions
After reviewing the example SMART goal, read the Interview with a Scientist to see how SMART goals are used when planning experiments.

1. How did Dr. Allaband make the broad question more *achievable* here?

She went from using humans to using an animal model.

2. How was the experiment refined to be more *specific*?

She specified the type of cancer and identified the mouse model to use as well as the human microbiome of people with and without colon cancer.

3. After changing the test subject species, is the experiment still *relevant* to the original question? Explain.

The experiment is still relevant because there are several mouse models that have the same type of cancer found in humans, and each year many people are affected by cancer.

4. After checking the *CDC website for statistics*, do you think Dr. Allaband would use all male mice or include female mice? Why would this choice make the study more *relevant*?

Answers vary: Students might say that using male mice is more of a controlled experiment because it can be compared to what has been done in the past. However, they might also point out that females are also affected by this type of cancer and should be included in the study to make it more relevant.

5. How will the experiment be *measurable*? What will be measured to help answer the question?

Measure the size of the tumor before and after treatment. Additionally, the number and type of microbes would be measured.

6. How might you propose Dr. Allaband's team measures the changes in the microbiome of the colon over the course of the study? How often should a fecal sample be taken? What might the researchers be looking for? How long should the study be?

Answers vary: Students might say to culture the bacteria, quantify the bacteria, take samples weekly, daily, etc., over a period of months.

7. What is the *timeline* Dr. Allaband proposes for completing the experiment? Was what you proposed in #6 close to this? Do you think this can be obtained?

Answers vary: The experiment is planned to be completed over several months.

8. What important steps should be taken into account to make the data more *reliable*?

Mice need to be handled by the same person each day, which will reduce stress and maintain reliable data collection.

9. Why is communication among group members so important in making a goal achievable?

Each group member has strengths and if these are focused upon, discussed and executed in the experiment, then the goal is much more attainable.

10. After reading Dr. Allaband's interview, rewrite the original question, "Can probiotics be used to prevent cancer?" to fit each of the SMART goals.

Can mice be used as a model organism to study the effects of probiotics on cancer in humans?

(Too broad: Can probiotic bacteria be used in cancer prevention and treatment?)

Phenomenon: Food Chart

ANSWER KEY **Do not share with students**

Directions
Answer Question #1 in Student Guide, Part 1: Pre-Lab after carefully observing the foods.

1 Miso (fermented soybean, originated in Japan)

2 Kimchi (fermented cabbage/radish, originated in Korea)

3 Sauerkraut (fermented cabbage, common in German food)

4 Kombucha (fermented tea, originated in China)

Continues next page >

Phenomenon: Food Chart

ANSWER KEY **Do not share with students**

Continued

5 Yogurt (fermented milk, common in Greek and Indian food)

6 Curtido (fermented cabbage, common in Salvadorian food)

7 Shrimp Paste (fermented shrimp, common in Southeast Asia)

8 Injera (fermented flatbread, originated in Ethiopia/Eritrea)

Continues next page >

Phenomenon: Food Chart

ANSWER KEY **Do not share with students**

Continued

9 Tempeh (fermented soybean, originated in Indonesia)

10 Sourdough (fermented dough, common in Europe and USA)

Student Guide, Part 1: Pre-Lab

ANSWER KEY **Do not share with students**

Directions

In this lab, you will play the part of a clinical laboratory
In this lab, you will play the role of a food scientist exploring the effect of a particular variable on kefir yogurt production. To begin, review the images of different foods in the Phenomenon: Food Chart.

1. Phenomenon:

a. Which foods do you recognize? What do you think they are?

Answers will vary.

b. What do you think all the foods have in common?

Answers will vary.

c. What do you think all the foods have in common?

Answers will vary.

2. Choosing a yogurt starter culture:

Starter Culture A	**Starter Culture B**

2a. Observations

Kefir will have small oblong structures (bacteria).	Milk will look more uniform and globules (fat).

2b. Do you think it is milk or kefir? Provide evidence from your observations.

2c. Which solution do you think you should use as the starter culture for your kefir yogurt? Explain your reasoning.

Kefir should be used as a yogurt starter culture because it contains bacteria which will ferment the milk into yogurt.

It is fine if students do not have the correct answer here—this will be revisited before the students set up their experiments.

Continues next page >

Student Guide, Part 1: Pre-Lab

ANSWER KEY — **Do not share with students**

Continued

3. Kefir yogurt fermentation planning:

a. What variable will you *change* (independent variable)?

Milk type, % milk fat, or incubation temperature

b. What will be your c*ontrol group* (represents the "normal" or "baseline")?

Suggested control groups:

— 2% dairy milk (if students are changing milk type or % fat)
— 21°C: room temperature (if students are changing incubation temperature)
— 37°C would be a good control if you have access to an incubator because this is the ideal temperature of the bacteria.

c. What will be your *experimental group*?

Examples

— Whole dairy milk, skim dairy milk, soy milk, nut milk, help milk, coconut milk (if students are changing milk type or % fat)
— Fridge (4°C), Warm area, such as on top of a refrigerator (measure temp), incubator at any temp (e.g. 45°C) (if students are changing incubation temperature)

d. List at least three c*ontrolled variables* (these will be the same between the control group and experimental group):

Examples

— Incubation temperature of 21°C (if students are changing milk type or % fat)
— 2% milk (if students are changing incubation temperature)
— Volume of starter culture (kefir)
— Volume of milk
— Size and shape of container

e. You will *measure* the following characteristics for each group (dependent variables):

pH (lower = more acidic)

Smell

Color

Thickness and texture

f. Use your independent and dependent variables to write the driving question for your experiment.

(How does changing ______ affect the characteristics of the kefir yogurt?)

Examples:

— How does changing the type of milk used affect the characteristics of the kefir yogurt?
— How does changing the percent of milk fat used affect the characteristics of the kefir yogurt?
— How does changing the incubation temperature affect the characteristics of the kefir yogurt?

Continues next page >

Student Guide, Part 1: Pre-Lab

ANSWER KEY **Do not share with students**

Continued

4. Choose one of the variables you will measure and make a prediction about how your experimental group will compare to your control group. Explain.

   (The yogurt in the experimental group will __________ because ________ which causes/leads to _________.)

Example:

The yogurt in the experimental group (coconut milk) will have a higher pH than the control group (2% dairy milk) because coconut milk does not have lactose for the bacteria to break down, which leads to less fermentation and therefore less lactic acid produced.

5. CIf the controlled variables you listed in #3 are not kept constant, how might they impact your results?

   (If __________ is not kept constant between groups, then ________ because _________.)

Example:

The volume of kefir added to our control and experimental groups needs to be constant because a discrepancy might lead to more fermentation activity due to the amount of starter culture microbes added. More microbes would lead to faster fermentation.

Temperature needs to be constant because temperature affects the rate of fermentation. Higher temperature would lead to faster fermentation unless it is too hot for microbes to survive.

Volume of milk in both the control and experimental groups needs to be constant because this may also affect the rate of fermentation. Higher milk-to-kefir ratio would lead to a lower fermentation rate.

Student Guide, Part 2: Lab

ANSWER KEY **Do not share with students**

Directions
Make observations, measurements, and record your pictures as you complete the lab.

Data tables should be complete.
See complete set of tables in *Student Guide, Part 2: Lab*

Lab Notes

Con. = Control
Exp. = Experimental

	Time	pH	Smell	Color	Thickness and Texture	Picture
Con.	0 h					
Exp.	0 h					

	Time	pH	Smell	Color	Thickness and Texture	Picture
Con.	24 h					
Exp.	24 h					

Student Guide, Part 3: Data Analysis

ANSWER KEY — **Do not share with students**

Directions
Analyze your results from the lab by answering the questions below.

1. Review the data you collected over the 48-hour period and identify three to five trends and/or interesting observations:

Examples:

— The biggest difference between the yogurts was in smell.
— The experimental group became sweeter-smelling whereas the control group became more sour.
— Both kefir yogurts became more acidic.
— Both kefir yogurts became thicker.
— There was no significant difference in color between the yogurts.

2. How do your results compare to your predictions? What does this mean? *(My results supported / did not support my prediction because ….This means that…)*

Example:
My results did not support my prediction because the experimental group (coconut milk) had almost the same pH as the control group (2% dairy milk) throughout the 48 hours. This means that the bacteria and other microorganisms in the kefir starter were able to use other sugars besides lactose in the coconut milk during fermentation resulting in yogurt still being produced.

3. Perform a Gallery Walk of the other kefir yogurt samples produced by each lab pair in your class. Use the table on the next page to capture what variable they manipulated and any noteworthy results. Put a star next to any yogurts you think are best for scaling up.

Answers will vary.

4. Consider the observations you made and discuss the following questions:

a. Which of the yogurts would be worth scaling up? Which would not? Why?

Answers will vary.

b. What resources are needed to scale up the production of yogurt?

Answers will vary.

c. What potential issues may arise when scaling up a product? How could they be avoided?

Answers will vary.

d. What are the financial considerations with scaling up a product?

Answers will vary.

Student Guide, Part 4: Answer the Driving Question

ANSWER KEY — **Do not share with students**

Directions

Use the organizer below to write a complete Claim, Evidence, Reasoning paragraph or two that answers the question: How did changing your independent variable (the temperature or milk type) affect the characteristics of the kefir yogurt?

CER Organizer

Optional Sentence Frames/Guiding Questions	
Claim	Statement that answers the driving question
Evidence: data collected from the lab	What were the trends you identified in Part 3 #1?
	Were some characteristics more affected than others?
	Cite specific evidence from your experiment.
Reasoning: scientific principles that explain the data	________ indicates that ________ because ________ .
	____________ explains why ____________ .
	____________ occurs as a result of ____________ .

Key Vocabulary
Experimental group
Control group
Fermentation
Lactose
Lactic acid
Microorganism/ microbes
Culture/starter

CER Paragraph Example:

Changing the milk type most greatly impacted the smell. In our control group, the smell of the kefir yogurt became more sour at 24 hours, and increasingly more so at 48 hours, whereas our experimental group smelled increasingly sweeter as time went on. In terms of thickness and texture, both the control and experimental groups became thicker. Our measurements at the end showed that there was 60 mL of liquid that could be extracted from the control group and 50 mL of liquid from the experimental group. The pH of our control group went from 6.6 to 4.5, and the pH of our control group went from 6.8 to 4.9. This means that both became more acidic, with a greater change in pH in the control group (2.1) versus experimental (1.9). Lastly, the color of both groups went from white to an off-white, with no remarkable differences between the two.

Over the 48 hours, the texture and consistency of both milks gradually changed from a liquid state into one more coagulated and gelatinous. This is a result of the microbes found in dairy products (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) fermenting the sugars in milk and producing lactic acid within the culture. The production of the lactic acid caused the sample to become thicker, more acidic in pH, and tart or sour smelling. Since we started with a kefir culture, there were likely other microorganisms, such as yeast, also fermenting the milks into alcohol, which likely is why the samples still were mostly liquid and had a slightly yeast-like smell.

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Background Reading: Effects of Variables on Yogurt Production (Jigsaw)

Directions

Read an article about one of the three variables and answer the associated questions.

Variable 1: Incubation Temperature

Article Overview

Title	Effects of Incubation Temperature on the Physical and Chemical Properties of Yoghurt
Scientists	Temitayo. E. Oladimeji, Iyi-Eweka. E., Oyinlola. R. Obanla, Joseph. O. Odigure
Institutions	Covenant University and Federal University of Technology, Nigeria
Year	2016

Oladimeji, Temitayo. E. et al. "Effects of Incubation Temperature on the Physical and Chemical Properties of Yoghurt" 3rd International Conference on African Development Issues, 2016.

Methods

- Milk to make yogurt was prepared with skim milk powder (Cowbell brand).
- Milk was inoculated with plain yogurt as the starter culture.
- 24-hour incubation at various temperatures

Results

Acidity and viscosity of yogurts incubated at different temperatures:

Incubation Temp (°C)	pH	Viscosity (μ)
30	4.51	56.5
35	4.32	60
40	4.20	68
45	3.80	76

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Background Reading: Effects of Variables on Yogurt Production (Jigsaw)

Continued

Variable 1: Incubation Temperature

1. What variable did the scientists change? Name the experimental groups they analyzed.

2. What variable(s) did the scientists measure?

3. Name two or three conclusions you can draw from the results.

4. On a scale of 1 to 5, how interested are you in seeing how this variable affects the yogurt you will make? Explain. (1 = not interested and 5 = very interested)

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Background Reading:
Effects of Variables on Yogurt Production (Jigsaw)

Continued

Variable 2: Milk Type

Article Overview

Title	Composition, Physicochemical, and Sensorial Properties of Commercial Plant-Based Yogurts
Scientists	Nadia Grasso, Loreto Alonso-Miravalles, and James A. O'Mahony
Institutions	University College Cork, Ireland
Year	2020

Grasso, Nadia et al. "Composition, Physicochemical and Sensorial Properties of Commercial Plant-Based Yogurts." Foods (Basel, Switzerland) vol. 9,3 252. 26 Feb. 2020, doi:10.3390/foods9030252

Methods

Six commercial plant-based yogurts and one dairy yogurt were analysed, all "plain type" (not flavored).

Products were purchased from Irish supermarkets (Quay Co-op, Tesco and Lidl).

For sensory evaluation: Consumer panel (25 panelists, two sessions) consisting of students and staff recruited within University College Cork, Ireland. Samples (10 g) were served at 4°C in a randomized order in transparent plastic cups.

Results

Firmness, acidity, and viscosity of yogurts:

Yogurt	pH	Viscosity (μ)	Firmness (N)
Soy 1	4.38	0.29	0.46
Soy 2	4.56	0.23	0.73
Coconut	4.00	0.75	0.44
Cashew	4.16	0.42	0.51
Almond	4.28	0.31	0.72
Hemp	3.99	0.55	1.78
Dairy	4.15	0.24	0.36

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Background Reading:
Effects of Variables on Yogurt Production (Jigsaw)

Continued

Variable 2: Milk Type

Results

Sensory evaluation of yogurts

	Soy 1	Soy 2	Coconut	Cashew	Almond	Dairy
Appearance	6.82	4.81	6.93	5.46	6.21	7.17
Odor	6.29	4.03	6.43	4.61	5.09	6.33
Flavor	5.75	2.54	4.79	2.60	2.88	5.67
Texture	6.49	4.05	6.37	5.17	4.83	6.33
Acceptability	5.95	2.80	5.19	3.61	3.54	5.95

0 = extremely disliked

10 = extremely liked

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Background Reading: Effects of Variables on Yogurt Production (Jigsaw)

Continued

Variable 2: Milk Type

1. What variable did the scientists change? Name the experimental groups they analyzed.

2. What variable(s) did the scientists measure?

3. Name two or three conclusions you can draw from the results.

4. On a scale of 1 to 5, how interested are you in seeing how this variable affects the yogurt you will make? Explain. (1 = not interested and 5 = very interested)

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Background Reading:
Effects of Variables on Yogurt Production (Jigsaw)

Continued

Variable 3: Percent Milk Fat

Article Overview

Title	Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt
Scientists	Maryam Tavakoli, Mohammad B. Habibi Najafi, and Mohebbat Mohebbi
Institutions	Ferdowsi University of Mashhad, Iran
Year	2019

Tavakoli, Maryam et al. "Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt." Heliyon vol. 5,2 e01204. 3 Feb. 2019, doi:10.1016/j.heliyon.2019.e01204

Methods

Milks to make yogurt were prepared with skim milk powder (0.05% fat) and whole milk powder (35% fat) that were purchased from Golshad Dairy Product Company in Mashhad, Iran.

Starter culture previously isolated from Iranian traditional yogurt was provided by the microbial collection of Ferdowsi University of Mashhad.

Control sample = yogurt without probiotic bacteria.

Fat free yogurt = yogurt with probiotic bacteria and maximum 0.5% fat.

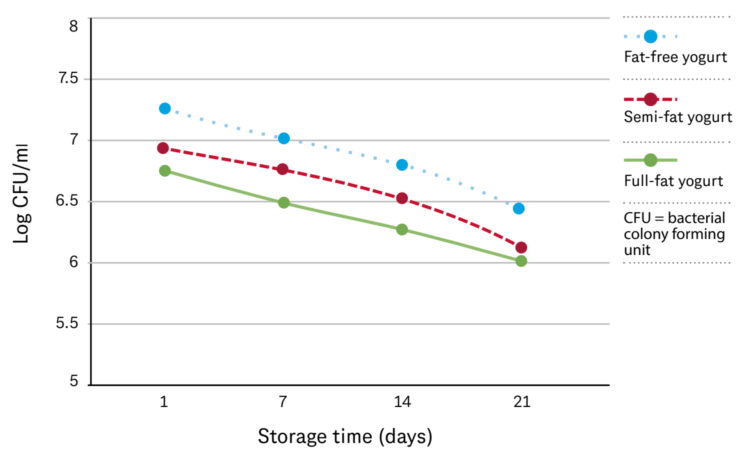
Semi fat yogurt = yogurt with probiotic bacteria and 2.1% fat.

Full fat yogurt = yogurt with probiotic bacteria and 3.6% fat.

After inoculation, all samples were incubated at 37 °C until the pH reached 4.65. At the end of the fermentation process, all containers were immediately cooled down and stored in a refrigerator at 4 °C and analyzed for 21 days.

Results

Survival of *Lactobacillus acidophilus* bacteria in yogurt



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Background Reading:
Effects of Variables on Yogurt Production (Jigsaw)

Continued

Variable 3: Percent Milk Fat

Results

Acidity of yogurts

	pH			
	Day 1	Day 7	Day 14	Day 21
Control	4.21	4.18	4.08	3.94
Fat Free	4.3	4.25	4.11	3.87
Semi Fat	4.22	4.09	4.03	3.84
Full Fat	4.34	4.27	4.15	4.1

Antioxidant activity of yogurts

	Antioxidant activity (%)			
	Day 1	Day 7	Day 14	Day 21
Control	41.18	43.52	45.35	44.08
Fat Free	49.52	51.23	54.82	52.31
Semi Fat	49.38	52.12	55.28	53.11
Full Fat	44.93	46.25	48.01	47.14

Antioxidant activity is measured in percentage of inhibition of DPPH (a chemical that is a free-radical and traps other free-radicals). Free-radicals are molecules with an unpaired valence electron that can lead to cell damage. Antioxidants inhibit production of free radicals.

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Background Reading:
Effects of Variables on Yogurt Production (Jigsaw)

Continued

Variable 3: Percent Milk Fat

1. What variable did the scientists change? Name the experimental groups they analyzed.

2. What variable(s) did the scientists measure?

3. Name two or three conclusions you can draw from the results.

4. On a scale of 1 to 5, how interested are you in seeing how this variable affects the yogurt you will make? Explain. (1 = not interested and 5 = very interested)

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Background Reading: Fermentation

Directions
Answer the questions below after closely reading the background material.

Have you ever seen a food with a label that says "probiotics" or "live active cultures?" This means that it contains one or more species of living microorganisms, usually bacteria. Microorganisms play an important role in human health, and you naturally have an estimated 500–1,000 different species living inside your body (Eisenstein, 2020). The collection of all the microorganisms (and all their genetic material) in your body is called your microbiome. In food making, microorganisms have been exploited for their ability to transform raw ingredients into delicious new dishes through the process of fermentation.

Fermented foods are a regular part of diets worldwide. For example, fermented cabbage appears in both Korean cuisine as kimchi and German food as sauerkraut. Throughout history, fermentation was used primarily to preserve foods such as fresh vegetables and dairy. During fermentation, microorganisms convert the carbohydrates in the food into acid and ethanol, which in turn changes the environment so that it remains edible longer. Fermentation is also responsible for the tart, tangy flavor that makes fermented foods so delicious.

Yogurt and kefir are both examples of foods made from fermented milk. Kefir is a sour-tasting drink that is fermented using kefir grains—pale yellow globules that contain multiple species of bacteria and yeast (Prado et al., 2015). Yogurt is thicker than kefir and always contains the two bacterial species *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Charles, 2015). To make yogurt, these bacteria (and sometimes additional species) are added to milk that is then heated. The warm temperature allows the bacteria to reproduce and break down lactose (sugar) in the milk to produce lactic acid. This increases the acidity of the environment, causing the milk proteins to form clumps and increasing the thickness (viscosity) of the solution. As a result, the yogurt develops its characteristically tart flavor and creamy texture. Since kefir contains the necessary bacteria, it can be used as a starter culture to make yogurt.

Sources:

The History and Health Benefits of Fermented Food

Hey Yogurt-Maker, Where'd You Get Those Microbes?

Is Fermentation in Chemistry?

The Hunt for a Healthy Microbiome

Milk Kefir

Fermented Foods

Kefir (fermented milk)

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Fermentation Questions

1. How does the process of fermentation relate to microorganisms?

2. What are the two strains of bacteria used in yogurt making?

3. How are yogurt and kefir similar? How are they different?

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Background Reading: The Microbiome (Jigsaw)

Directions
Open the article The Microbiome from the Harvard School of Public Health. Record main ideas and key words with their definitions for each section in the table below.

	Main Ideas (3–4)	Key Words and definitions (1–3)	How does this relate to fermented foods, such as kefir yogurt?
Section 1 *What is the microbiome?*			
Section 2 *How microbiota benefit the body*			

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Background Reading: The Microbiome (Jigsaw)

Continued

	Main Ideas (3–4)	Key Words and definitions (1–3)	How does this relate to fermented foods, such as kefir yogurt?
Section 3 *The role of probiotics*			
Section 4 *Can diet affect one's microbiota?*			

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Background Reading: The Microbiome (Jigsaw)

Continued

	Which area of research would you be most interested in learning more about? Why? You will later write a testable SMART goal question about this area that could be used in a scientific investigation.
Section 5 *Future areas of research*	

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Background Reading: SMART Goals for Scientific Research

Directions

After reviewing the example SMART goal, read the Interview with a Scientist to see how SMART goals are used when planning experiments.

SMART Goal Example

SMART goals are used by many different industries and are helpful in both professional and personal life. SMART is an acronym for Specific, Measurable, Achievable, Realistic, and Timed. It provides a framework for writing goals that both help you grow and are realistic. Below is an example of a SMART goal that a high school student wrote to help them improve their test scores.

Source:
How to write SMART Goals

SMART Goal Steps

SMART Goal Question: Can I improve my chemistry test scores by taking notes?

	Specific	**Measureable**	**Acheivable**	**Relevant**	**Timed**
1	*What will you do?*	*What data will you track?*	*Is this possible?*	*Why is this important?*	*What is the timeline?*
2	*Which specific actions will you take?*	*How will you know when you have achieved it?*	*Do you have the necessary resources?*	*Does this fit with your other goals?*	*When will it be complete?*
	I will take at least one page of Cornell notes after every chemistry class about the lesson from that day.	I will calculate my test score average. I will meet my goal when the average increases from 70% to 80%.	I will set aside 30 minutes after school on each day I have chemistry. I have time after soccer practice and before I help make dinner. I will ask a friend from class or the teacher if I need help reviewing the lesson.	I need to get a good grade in chemistry because it will help prepare me for college and the more advanced science classes that I want to take.	We have four chemistry tests left in the semester. I will recalculate my test score average after each test.

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Interview With A Scientist

Celeste Allaband, DVM, *is a Small Animal Veterinarian and a PhD Candidate in the Rob Knight microbiome lab at the University of California, San Diego. She has thought a lot about experimental design in both humans and animals. Read the interview with Dr. Allaband below and answer the questions to see how she uses SMART goals in her research strategy in studying the connection between probiotics and cancer.*

Dr. Allaband

Hi there! I'm glad you asked about SMART goals as they are so important in helping us plan. Working with animals and studying the microbiome is fascinating, and sometimes we have really big questions that need scaffolding to help us design an experiment around them. Let's say a question we have is "*Can probiotics be used to prevent cancer?*" Is that testable and can we use the SMART goals to answer it?

The best questions are sometimes the hardest to answer. For instance, does eating extra fiber or does eating a probiotic help prevent cancer? Those kinds of questions are just really hard because it's so hard to do long-term experiments. And those aren't as measurable as we would like. SMART goals are discussed in a lot of different fields and using them helps guide experimentation.

I would really love to know if a probiotic could help prevent cancer, but I can't look at that in people because we don't do primary/exploratory experiments on people as it's considered unethical. When we have proven it works in the lab (in vitro) and in an animal model (in vivo), and we think it seems like it will work we can then do clinical trials. There are a lot of different factors we have to account for like how different genetic backgrounds could influence their risk of cancer, or how differences in diets influence the microbiome among people. So that makes it really difficult to study the microbiome in people, soI can't look at that question in people. Usually the next best thing in most laboratory settings is to start looking at that in a mouse model.

1. How did Dr. Allaband make the broad question more *achievable* here?

Continues next page >

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Interview With A Scientist

Continued

Dr. Allaband

Now we need to think about another few questions. Does the mouse model relatively accurately replicate that cancer that I'm interested in? And what specific cancer are we questioning that the microbiome might have an effect on? Colon cancer which has the strongest links to the microbiome? Gastrointestinal lymphoma? Breast cancer? According to the *CDC*, every year, an estimated 147,950 adults in the United States will be diagnosed with colorectal cancer. These numbers include 104,610 new cases of colon cancer (52,340 men and 52,270 women) and 43,340 new cases of rectal cancer (25,960 men and 17,380 women). Therefore, this might be a reasonable cancer to focus on in this study, and we would obtain a *mouse model of colon cancer*, of which there are several species. Rather than a probiotic, it might be more reasonable to see the effect of the existing microbiome on colon cancer. We could use the human colon microbiome from people with and without colon cancer in our experiment with this mouse model.

2. How was the experiment refined to be more *specific*?

3. After changing the test subject species, is the experiment still *relevant* to the original question? Explain.

Continues next page >

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Interview With A Scientist

Continued

Dr. Allaband

So, what exactly are we wanting to do with these mice? What's the treatment and what's the control and how will we know it's working? It's reasonable to measure the size of the tumor as our indicator that our treatment is working. So, once we have our model, specifically FabplCre;Apc(15lox/+) mouse, which has few tumor numbers but are larger in size, making them easy to measure, we need to think about the equipment we need and the things to control in the experiment. For equipment, if we aren't able to use ultrasound, or MRI to look at tumors while the mice are alive, we are only going to be able to measure the lesions once-after death.

Now, what are we going to control? We can control every aspect of their diet, circadian rhythms (amount of light/dark in the room), socializing the animals, etc. There are all of these different factors that I need to attempt to control for, so that the results, and when they get written up, someone else could do that in a laboratory 3000 miles away and get those same results. Traditionally, all studies similar to what we're planning were run in male mice only. Would we still choose all male mice or would we include females?

4. After checking the *CDC website for statistics*, do you think Dr. Allaband would use all male mice or include female mice? Why would this choice make the study more ***relevant***?

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Interview With A Scientist

Continued

Dr. Allaband

Besides tumor size, what else are we measuring in this study?

Remember, our question is focused on the microbiome and how it affects cancer. At the start of the experiment, we could give the mice antibiotics to kill off any bacteria in their gut to give us a baseline for the type and number of bacteria populating their colon. We could then perform a fecal transplant from human patients known to have colon cancer in some of the mice and from healthy people into some of the mice as our control. At the end of the study, we could see if the transplant from people with cancer makes the tumors bigger than the healthy controls. Then, with the microbiome data, we could try to figure out if there is a unique microbe or an overabundance of a particular microbe that may have caused that to happen.

5. How will the experiment be ***measurable?*** What will be measured to help answer the question?

6. How might you propose Dr. Allaband's team measures the changes in the microbiome of the colon over the course of the study? How often should a fecal sample be taken? What might the researchers be looking for? How long should the study be?

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Interview With A Scientist

Continued

Dr. Allaband

Using DNA sequencing and qPCR, we could measure the quantities and types of microbes in the mice every week. This data would be compared with tumor protein biomarkers in the mouse models over a period of several months.

Something that should be noted is that the problem with the microbiome is that it is very changeable, even when we're trying to control all of the factors in the experiment (preventing contamination, making sure our equipment is working properly, knowing everything about the animal's conditions, etc) there's a lot of things that can change. Mice get stressed out when different people handle them. It's been found that mice and rats that are in laboratories have favorite people who take care of them and are more calm with some people than with others. And there's a whole range of factors to account for.

7. What is the *timeline* Dr. Allaband proposes for completing the experiment? Was what you proposed in #6 close to this? Do you think this can be obtained?

8. What important steps should be taken into account to make the data more reliable?

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Interview With A Scientist

Continued

Dr. Allaband

And finally, you have to know your group and their strengths and what everyone will contribute to the study. For instance, who will handle the animals? Who will look through the data and make sense of it? If you like crunching numbers, but aren't as into handling mice on a daily basis, this should be discussed with your group. This makes the goal much more attainable in the end, as everyone is contributing in a realistic way.

9. Why is communication among group members so important in making a goal *achievable*?

10. After reading Dr. Allaband's interview, rewrite the original question, "Can probiotics be used to prevent cancer?" to fit each of the SMART goals.

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Interview With A Scientist

Continued

11. Look back at your response to "Section 5: Future areas of research" in *Background Reading: The Microbiome (Jigsaw)*. Using the area of research in which you are most interested, generate your own testable SMART Goal question. Explain how the question fits each of the SMART goal characteristics in the table below. Feel free to search the internet for more information and examples of past scientific studies to help you.

SMART Goal Question:

	Specific	**Measureable**	**Acheivable**	**Relevant**	**Timed**
1	*What will you do?*	*What data will you track?*	*Is this possible?*	*Why is this important?*	*What is the timeline?*
2	*Which specific actions will you take?*	*How will you know when you have achieved it?*	*Do you have the necessary resources?*	*Does this fit with your other goals?*	*When will it be complete?*

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Career Profile

Directions

Read the profile below to learn more about a scientist who studies the microbiome.

Celeste Allaban, DVM

University of California, San Diego,
Small Animal Veterinarian and PhD Candidate

What do you do and how did you get here?

I am currently a PhD candidate at UCSD. I collect and analyze data to examine the fundamental properties of the microbiome and how it applies to both human and animal health. After working as a small animal veterinarian, I was able to find this position, where I am able to combine my undergraduate interest in medical microbiology with my understanding of animal health.

What was your favorite subject in high school, and why did you love it?

My favorite subject in high school was physics. I had a really great teacher who was enthusiastic about the subject and we got to do a bunch of fun in-class experiments with springs, paper airplanes, etc.

If you could give a piece of advice to your younger self, what would it be?

My advice for my younger self would be to worry less. I was always measuring myself against others and how far "behind" I was compared to some people. Now, I love all of the detours my life has taken.

What skills do you use on a daily basis?

One of my core skills that I use every day is writing—whether that's writing a formal scientific paper or grant, an email to a colleague, or a friendly text message. Knowing who your audience is and how to make sure both parties are effectively talking about likes, dislikes, expectations, problems, etc is really important. Remember to be kind as much as possible—assume positive intent from every message. Maybe they are just having a bad day. Give them the benefit of the doubt that you would want. And, yes, people sometimes do want to hurt you with their words, but they get really frustrated when you don't smile.

What's most fulfilling about your job? What's most challenging?

The most fulfilling part of my career is having great conversations with other scientists who are passionate about their specialties. I learn so much all the time! I also like knowing that I am helping to make the medicine of the future. The most challenging thing is when you are trying to make sense of all of the data you collect. There is no one there to tell you how to do it right or better, you have to figure that out yourself. Although, being the first person on the planet to know something is so cool!

If you could instantly learn any language, which would you choose and why?

If I could instantly learn any language, I would probably pick either Mandarin or Cantonese. There are so many people in the world who speak the language, including some close friends of mine. The written characters are gorgeous and have such a rich history, too. Plus, I am absolutely terrible at it. I cannot hear the different tones at all to even get started. Sigh.

What is your most used phone app?

My most used phone app is Webtoon, which has online comics. I love exploring new stories, genres, and art styles!

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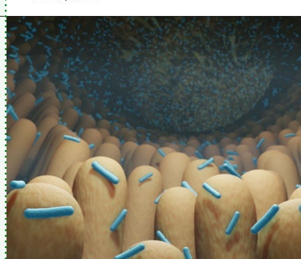
Vocabulary Tool

Directions
For each vocabulary word, write a new sentence that helps you practice using it.

Word	Image	Definition	Example Sentence	My Sentence
Fermentation		A process in which microorganisms break down carbohydrates into acid and alcohol. This happens in the absence of oxygen.	There are so many delicious and unique foods that are created through the process of *fermentation*.	
Yogurt Starter or Culture		A sample containing one or more species of microorganisms that begins the process of fermentation when added to food.	In order to make my own yogurt at home, I had to obtain milk and a reliable *yogurt starter (or culture).*	
Probiotic		A microorganism that is consumed by people to improve their health.	*Probiotic* supplements are commonly sold in the vitamin section of stores.	
Microbiome		The genomes of all the microorganism populations that live together inside a human body.	Your *microbiome* is sometimes commonly referred to as your "gut bacteria" though it includes other organisms such as fungi.	
Kefir		A sour-tasting drink made by fermenting milk.	Though still fairly uncommon, *kefir* is increasing in popularity in the United States.	

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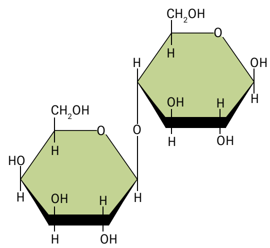
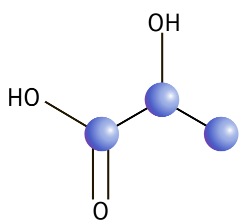
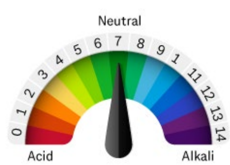
Vocabulary Tool

Continued

Word	Image	Definition	Example Sentence	My Sentence
Lactose		A sugar that is naturally present in milk.	If you are *lactose* intolerant, your body cannot process the sugar in dairy products.	
Lactic Acid		A product of fermentation that decreases the pH of the environment, leading to the sour taste of fermented foods.	As *lactic acid* levels increase, clots begin to form in milk converting it to yogurt.	
pH		A scale of acidity from 0 (most acidic) to 14 (least acidic or most basic).	Fermented foods have a low *pH* because of their high levels of lactic acid.	
Viscosity		A property of a liquid that describes how thick it is. High viscosity = very thick.	Yogurt has a higher *viscosity* (is more viscous) than milk because of the clots that form as a result of fermentation.	

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Phenomenon: Food Chart

Directions

Answer Question #1 in Student Guide, Part 1: Pre-Lab after carefully observing the foods.

1

2

3

4

Continues next page >

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Phenomenon: Food Chart

Continued

5

6

7

8

Continues next page >

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Phenomenon: Food Chart

Continued

9

10

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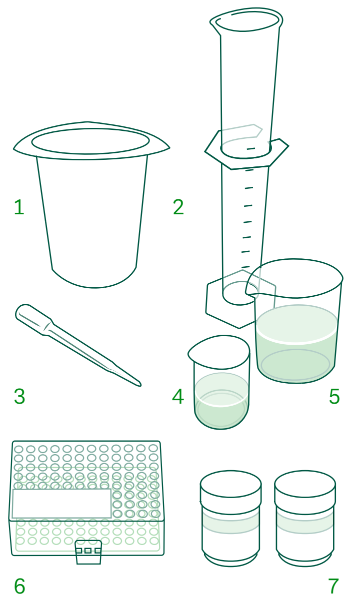
Student Protocol

1 Clean the lab surface and gather all the materials needed to complete the lab.

☐ Materials needed per student:

1	Waste bucket
2	100 mL graduated cylinder
3	Transfer pipette or P1000
4	15 mL kefir
5	~150 mL of milk(s)
6	Optional: P1000 tips
7	2 clean jars with lids

Note > *Milk type and amount will depend on experimental design*



2 Add chosen milk to clean container.

☐ Add 100 mL of 2% dairy milk or other chosen milk to a clean container that can be microwaved.

3 Microwave for ~30 seconds.

☐ If a microwave is not available, a beaker and hot plate set to medium heat may also be used to warm the milk.

4 Swirl milk and touch the container sides with your fingers to gauge the temperature.

☐ If it is comfortably warm to the touch, go to the next step.

☐ If it is too hot, cool until comfortable to touch.

☐ If it is too cool, microwave for 10 more seconds until it is comfortably warm to the touch.

5 Add 5 mL (5,000 µL) kefir to the warm milk and swirl to mix.

6 Incubate for 48 hours at room temperature or other chosen temperature.

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Student Guide, Part 1: Pre-Lab

Directions

In this lab, you will play the part of a clinical laboratory
In this lab, you will play the role of a food scientist exploring the effect of a particular variable on kefir yogurt production. To begin, review the images of different foods in the Phenomenon: Food Chart.

1. Phenomenon:

a. Which foods do you recognize? What do you think they are?

b. What do you think all the foods have in common?

c. What do you think all the foods have in common?

Continues next page >

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Student Guide, Part 1: Pre-Lab

Continued

2. Choosing a yogurt starter culture:

Starter Culture A	**Starter Culture B**

2a. Observations

2b. Do you think it is milk or kefir? Provide evidence from your observations.

2c. Which solution do you think you should use as the starter culture for your kefir yogurt? Explain your reasoning.

Continues next page >

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Student Guide, Part 1: Pre-Lab

Continued

3. Kefir yogurt fermentation planning:

a. What variable will you *change* (independent variable)?

b. What will be your *control group* (represents the "normal" or "baseline")?

c. What will be your *experimental group*?

d. List at least three *controlled variables* (these will be the same between the control group and experimental group):

e. You will *measure* the following characteristics for each group (dependent variables):

- pH (lower = more acidic)
- Smell
- Color
- Thickness and texture

f. Use your independent and dependent variables to write the driving question for your experiment.

(How does changing ______ affect the characteristics of the kefir yogurt?)

Continues next page >

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Student Guide, Part 1: Pre-Lab

Continued

4. Choose one of the variables you will measure and make a prediction about how your experimental group will compare to your control group. Explain.

   (The yogurt in the experimental group will ____________ because _________ which causes/leads to ___________.)

5. If the controlled variables you listed in #3 are not kept constant, how might they impact your results?

   (If ______________ is not kept constant between groups, then _________ because ___________.)

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Student Guide, Part 2: Lab

Directions
Make observations, measurements, and record your pictures as you complete the lab.

Lab Notes

Con. = Control
Exp. = Experimental

	Time	pH	Smell	Color	Thickness and Texture	Picture
Con.	0 h					
Exp.	0 h					

	Time	pH	Smell	Color	Thickness and Texture	Picture
Con.	24 h					
Exp.	24 h					

Continues next page >

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Student Guide, Part 2: Lab

Continued

	Time	pH	Smell	Color	Thickness and Texture	Picture
Con.	48 h					
Exp.	48 h					

Final Liquid Content

Liquid = whey
Solids = curds

	Time	Liquid leftover after straining (mL)
Con.	48 h	
Exp.	48 h	

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Student Guide, Part 3: Data Analysis

Directions
Analyze your results from the lab by answering the questions below.

1. Review the data you collected over the 48-hour period and identify three to five trends and/or interesting observations:

2. How do your results compare to your predictions? What does this mean? *(My results supported / did not support my prediction because ….This means that…)*

3. Perform a Gallery Walk of the other kefir yogurt samples produced by each lab pair in your class. Use the table on the next page to capture what variable they manipulated and any noteworthy results. Put a star next to any yogurts you think are best for scaling up.

Continues on next page >

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Student Guide, Part 3: Data Analysis

Continued

Gallery Walk Table

Group #	Variable Manipulated	Results / Notes
1		
2		
3		
4		
5		
6		
7		
8		

Continues next page >

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Student Guide, Part 3: Data Analysis

Continued

Gallery Walk Table

Group #	Variable Manipulated	Results / Notes
9		
10		
11		
12		
13		
14		
15		

Continues next page >

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Student Guide, Part 3: Data Analysis

Continued

4. Consider the observations you made and discuss the following questions:

a. Which of the yogurts would be worth scaling up? Which would not? Why?

b. What resources are needed to scale up the production of yogurt?

c. What potential issues may arise when scaling up a product? How could they be avoided?

d. What are the financial considerations with scaling up a product?

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Student Guide, Part 4: Answer the Driving Question

Directions

Use the organizer below to write a complete Claim, Evidence, Reasoning paragraph or two that answers the question: How did changing your independent variable (the temperature or milk type) affect the characteristics of the kefir yogurt?

CER Organizer

Optional Sentence Frames/Guiding Questions	
Claim	Statement that answers the driving question
Evidence: data collected from the lab	What were the trends you identified in Part 3 #1?
	Were some characteristics more affected than others?
	Cite specific evidence from your experiment.
Reasoning: scientific principles that explain the data	________ indicates that ________ because ________ .
	________________ explains why ________________ .
	_____________ occurs as a result of _____________ .

Key Vocabulary
Experimental group
Control group
Fermentation
Lactose
Lactic acid
Microorganism/ microbes
Culture/starter

CER Paragraph:

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CER Rubric

Score	4	3	2	1
Claim	In the first sentence, a claim is made which clearly and specifically answers the guiding question.	In the first sentence, a claim is made which clearly answers the guiding question.	A claim is made which answers the guiding question. This claim is unclear OR not in the first sentence.	A claim is made but does not answer the guiding question.
Evidence	At least three pieces of evidence are provided that strongly support the claim. The evidence is very clear (including data analysis and comparison), logical, and relevant to the claim.	At least three pieces of evidence are provided that support the claim. The evidence is mostly clear, logical, and relevant to the claim.	Some evidence (at least two pieces) is provided that supports the claim.	Very little evidence (one piece) is provided that supports the claim. Evidence is irrelevant, unclear, or illogical.
Reasoning	The reasoning uses the evidence to communicate the claim in a convincing way with significant use of scientific principles. Language is clear, explicit, and thorough.	The reasoning clearly and accurately relates the evidence to the claim with some use of scientific principles or real world connections.	The reasoning begins to relate the evidence to the claim. There is some relevant reasoning, but not enough.	The reasoning attempts to relate the evidence and the claim.
Final Score				