Dye Electrophoresis Lab

Microbe Hunters

miniPCR Learning Lab™
Dye Electrophoresis Lab:
Microbe Hunters

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Student's Guide

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Background information

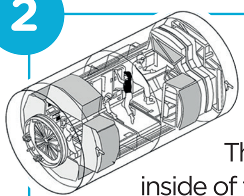
The International Space Station (ISS)

Image credit: NASA

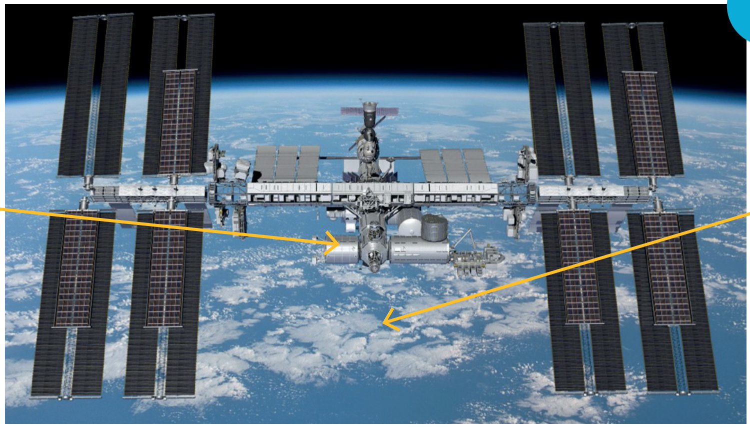
1

The **International Space Station (ISS)** is a spaceship that has been in orbit around the Earth for more than 20 years. The ISS was built as a place for humanity to learn how to live in space. The ISS contains laboratories where astronauts can do science experiments that help us understand the challenges space travelers face and invent tools that will help them survive on long missions.

2



The inside of the ISS is about as big as a five-bedroom house. It hosts a small crew of only six or seven astronauts at a time.



3

A much larger team on Earth supports the ISS crew as they complete their missions.

4

Since its construction began in 1998, more than 200 people have lived and worked on the ISS. Living in such an extreme environment is incredibly dangerous. Along with the obvious hazards of traveling in outer space, there is the danger that if you get sick in space there is no hospital to go to. **Today, it's your job to make sure that the ISS is free of harmful microbes that might infect and endanger the ISS crew.**

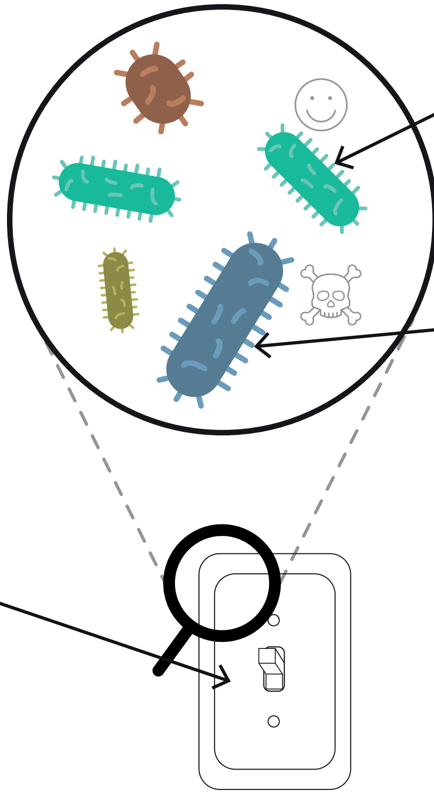
What are bacteria?

1

<u>Bacteria</u> are tiny, single-celled microorganisms (or microbes) that live virtually everywhere on Earth.

2

Many microorganisms live in and on the bodies of larger organisms, including humans. Because they are all over our bodies, we leave bacteria behind everywhere we go: on the surfaces in our homes, in our cars, and even on the ISS.



3

Most bacteria are harmless to people. Many are even helpful, like the ones that live in our guts and help us digest food.

4

Some bacteria can make us sick. If you've ever had strep throat, for example, it was caused by a harmful type of bacteria—also known as a germ.

5

Disease can spread from person to person when we pass on the disease-causing bacteria. This can happen by touching an infected person or even by touching surfaces where infectious bacteria have been left behind.

Background: Stop and think

The following two statements are false. Rewrite them so that they are correct:

1. The International Space Station (ISS) is a spaceship that orbits the moon.

2. All bacteria are harmful to humans.

Answer the following questions:

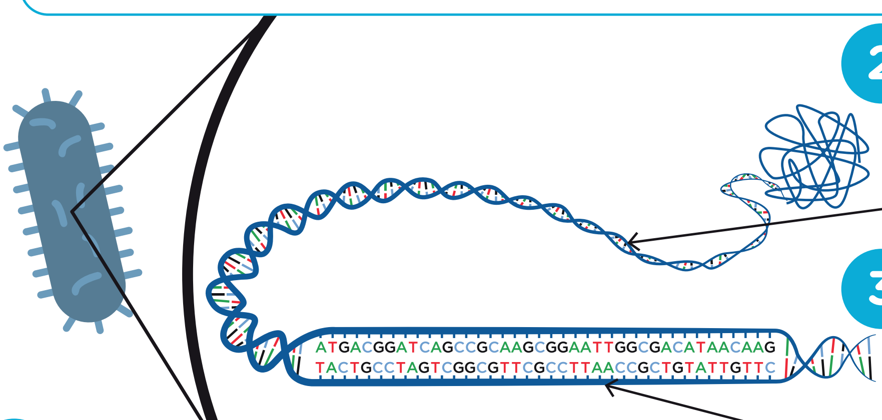
3. Would it be a good idea to try to eliminate all bacteria from astronauts traveling to space? Why or why not?

4. Why do you think it would be so important to keep astronauts from getting sick during long-distance space travel?

What is DNA?

1

Bacteria are too small to see with the naked eye. But **we can find out if harmful bacteria are present aboard the ISS by looking for their DNA.** **DNA** is a molecule that contains all the instructions that cells need to carry out vital functions like growing and dividing, disposing of waste, and more.



2

DNA can be found inside the cells of every living thing. DNA is made of two long, parallel chain-like structures.

3

In the middle of the two chains are pairs of molecules called nitrogen bases (bases for short) that hold the two strands together. There are four possible bases. They are named adenine (shortened to A), thymine (T), cytosine (C), and guanine (G).

4

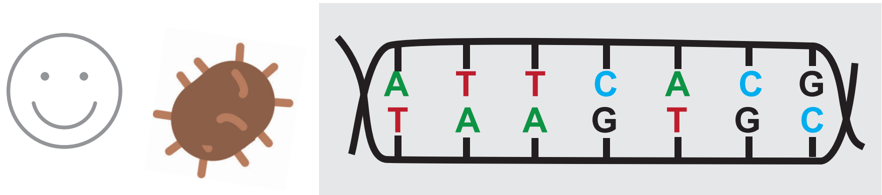
The sequences of A's, T's, C's, and G's are read by the cell almost like letters in a word. The sequence of the bases works like a molecular instruction book for the cell. But scientists can also read the bases in a cell's DNA, using special lab equipment.

Using DNA to identify bacteria

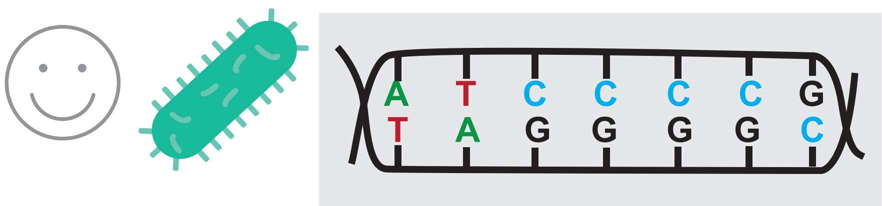
1

All organisms have unique DNA sequences. These sequences are so unique that scientists can use them to identify bacteria in an environment. Let's look at these three types of bacteria for example.

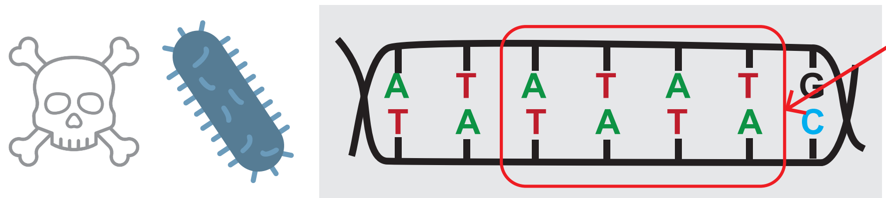
Staphylococcus hominis



Staphylococcus epidermidis



Salmonella enterica



2

Beside each type of bacteria, you can see a short segment of its DNA. Scientists usually study much longer DNA sequences, but today we'll look at these short sequences as examples.

3

The DNA sequences of these three bacteria are similar, but not identical. The "ATAT" sequence outlined in the red box is unique to the harmful *Salmonella* bacteria.

4

In this example, if you found the "ATAT" DNA sequence on your kitchen table, you would have good reason to believe that *Salmonella* bacteria are growing there. Eating *Salmonella* can cause food poisoning. You would know that you should clean your table carefully before eating off of it.

But how would you find the "ATAT" sequence on your table to begin with? Scientists can study bacterial DNA using some of the same tools you will use in the lab today.

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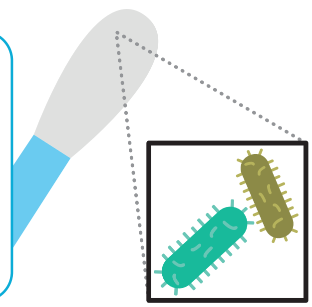
How can we study bacterial DNA?

1

As you learned on the previous page, different types of bacteria have unique DNA sequences. But to tell bacteria apart, scientists don't actually need to read their DNA sequences. Instead, they can use tools called PCR and gel electrophoresis to tell bacterial DNA segments apart based on their length. Here is how that works:

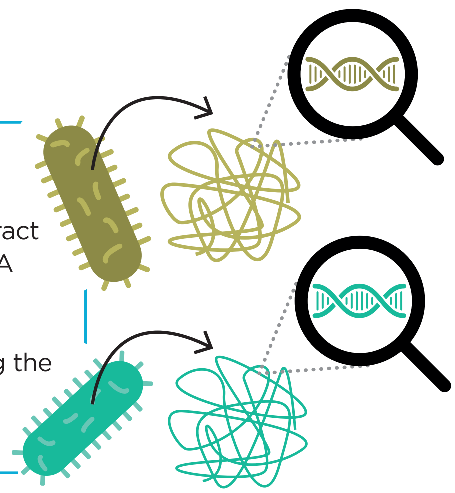
2

Sample collection: A scientist will first collect bacteria by swabbing the surface they wish to test.



3

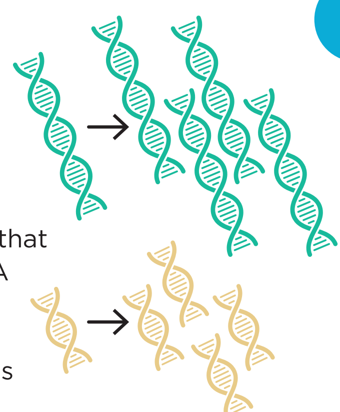
DNA extraction: Then, they use chemicals to extract (or take out) DNA from any cells present in the sample, including the bacteria they are looking for.



4

PCR: Next, they use a process called **<u>PCR</u>** (which stands for polymerase chain reaction) to attempt to make billions of copies of a DNA sequence specific to the kinds of bacteria they are looking for.

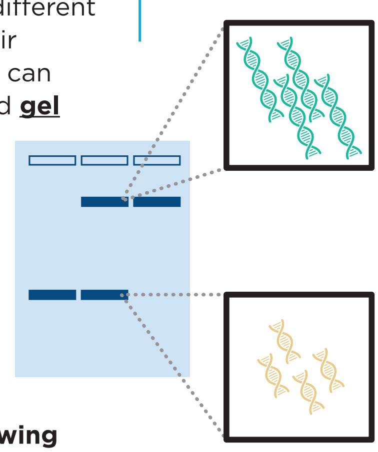
If DNA from the kinds of bacteria that the scientist is looking for is present, then the PCR will make billions of copies of that small piece of DNA. If DNA from the bacteria is <u>not</u> present, the PCR won't be able to make any copies of the DNA.



Importantly, scientists can design the experiment so that the size of the copy from one type of bacteria will be different from other types. This way they can test for more than one type of bacteria at the same time.

5

Gel electrophoresis: Finally, to see how long the different DNA segments in their sample are, scientists can use a technique called **<u>gel electrophoresis</u>.** This is what allows them to identify the bacteria that contributed DNA to their samples.



Today, you will use this technique to identify bacteria growing aboard the ISS.

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Background: Stop and think

Statements 5-8 are false. Rewrite them so that they are correct:

5. All bacteria share the same DNA sequence.

6. We can identify the bacteria growing on a surface by studying their fingerprints.

7. Gel electrophoresis is a technique that can be used to make copies of a DNA segment.

8. In today's lab, we will identify the bacteria growing aboard the ISS by reading their DNA sequences.

Answer the following question:

9. Why is it important to specifically look for dangerous bacteria and not just see if any bacteria are present?

Glossary

International Space Station (ISS): A laboratory in orbit around the Earth. The ISS is a spacecraft where astronauts live and work. The ISS contains lab modules where astronauts conduct experiments to better understand the challenges faced by space travelers.

Bacteria: Tiny, single-celled microorganisms. Bacteria live virtually everywhere on Earth, including on the bodies of larger organisms like humans. Most types of bacteria are harmless, some are helpful, and a few can be harmful to humans. We sometimes refer to harmful bacteria as germs.

DNA: A molecule that contains the instructions that cells need to carry out the basic processes of life. DNA is made of two long, chain-like structures. In the middle of the two chains are pairs of molecules called nitrogen bases (bases, for short) that hold the two chains together. The sequence of bases in a DNA strand can be read by a cell or a scientist like letters in a word.

Polymerase chain reaction (PCR): A method used to make many copies of a DNA segment you are interested in studying.

Gel electrophoresis: A method used to separate molecules by size and charge.

Module: A building block of the ISS inside which astronauts live and work. Modules function like rooms in a house.

Control: Samples that help you ensure that your experiment is working properly and help you make sense of your results.

Agarose gel: A type of gel commonly used for gel electrophoresis. Agarose is a sugar from seaweed. At the microscopic level, the inside of an agarose gel looks like a web or a sponge. Small molecules can move through the holes with ease, but larger molecules get slowed down. This allows scientists to separate molecules of different sizes.

Well: A pocket in a gel where samples are placed at the start of a gel electrophoresis experiment.

Band: A visible group of molecules that traveled together through an electrophoresis gel.

Lane: The straight line through which molecules travel through an electrophoresis gel.

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Today's mission

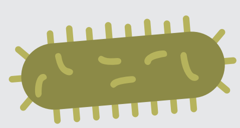
The ISS crew is tested for illness before they launch to space, and astronauts work with the NASA Microbiology team at Johnson Space Center in Houston, Texas to keep the ISS clean and free from dangerous germs. Members of the NASA Microbiology team have special knowledge about the different types of microbes that astronauts might encounter and give the astronauts advice on how to protect themselves from infection. But even so, disease can sometimes break out.

Weeks ago, an astronaut aboard the ISS developed a skin infection caused by a type of bacteria called *Klebsiella aerogenes.* It started with a routine injury: the astronaut accidentally cut her hand with a screwdriver while repairing a cargo hold. The wound was minor, so the astronaut bandaged it and continued with her work. But a few days later, the cut began oozing pus, and the area around it became red and swollen. The astronaut contacted her doctors on Earth, who were concerned that, if left untreated, the infection could spread to the astronaut's bloodstream and cause serious illness. They advised her to treat the cut with antibiotic ointment. Fortunately, the infection cleared up, and the cut has now healed.

Now, a new crew is on its way to the ISS, and you need to make sure there are no *Klebsiella* living on ISS surfaces where they might infect the new arrivals. Astronauts collected samples on the ISS and sent them back to Earth for testing. **You are a member of the team at Johnson Space Center and will analyze samples from the ISS to see whether they contain disease-causing *Klebsiella* bacteria.**

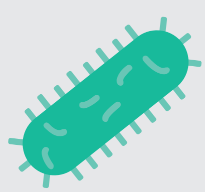
The bacteria

Remember that there are many, many different types of bacteria, and while a few are harmful to people, most are harmless. The team at Johnson Space Center regularly monitors the ISS for dozens of different types of microbes, including the potentially dangerous *Klebsiella* that caused the skin infection described above.
Today, you will just be looking for two types of bacteria:



Klebsiella aerogenes
(pronunciation:
kleb-see-ELL-uh
AIR-oh-genes)

Klebsiella typically lives in the human digestive tract, where it does not cause harm. If *Klebsiella* bacteria get into other parts of the body—like the lungs or a wound on the skin, for example—they can cause dangerous infections. Surfaces contaminated with *Klebsiella aerogenes* <u>must</u> be disinfected to prevent disease.



Staphylococcus epidermidis
(pronunciation:
sta-fuh-luh-KAA-cus
epp-ee-DERM-id-iss)

Staphylococcus epidermidis lives on human skin, and is easily left behind on surfaces that people touch.
This type of *Staphylococcus* is <u>not</u> dangerous to humans. Surfaces contaminated with *Staphylococcus epidermidis* do <u>not</u> require special cleaning.

The samples

The astronauts aboard the ISS have swabbed areas in four different **modules** aboard the ISS. You can think of modules as rooms; they are the building blocks of the space station inside which astronauts live and work. Harmful bacteria may be growing in any of these areas. Your job is to tell the astronauts where it is safe and where they need to clean to prevent future disease.

Image credit: NASA

Sample H (for Harmony):
Wall outside sleep quarters in Harmony module

Sample D (for Destiny):
Laboratory counter top in Destiny module

Sample U (for Unity):
Dining table in Unity module

Sample T (for Tranquility):
Handrail near toilet in Tranquility module

The experiment

You will complete this step

Step 1: DNA collection

Astronauts wiped cotton swabs on different ISS surfaces. If bacteria have been growing on those surfaces, they will be found on the corresponding cotton swabs.

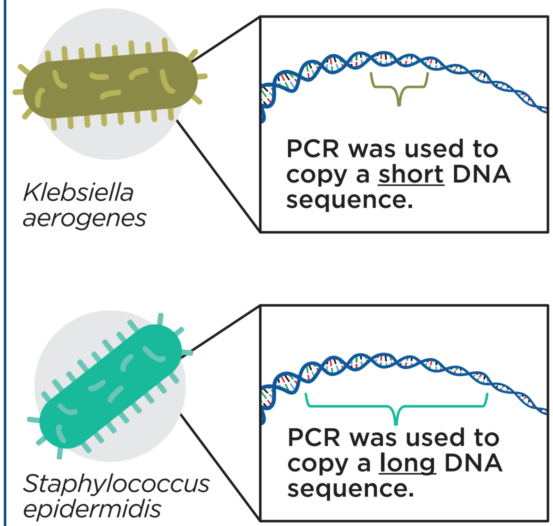
The swabs were sent back to Johnson Space Center for testing. Other members of your team have already sampled DNA from each of those cotton swabs.

Step 2: PCR

One of your teammates has used PCR to copy segments of the bacterial DNA present in those samples.

Step 3: Electrophoresis

You will use gel electrophoresis to see the DNA that was copied from the two types of bacteria. The DNA segments copied by PCR are unique to the bacteria you are looking for, and their lengths can be used to tell them apart:



The electrophoresis gel

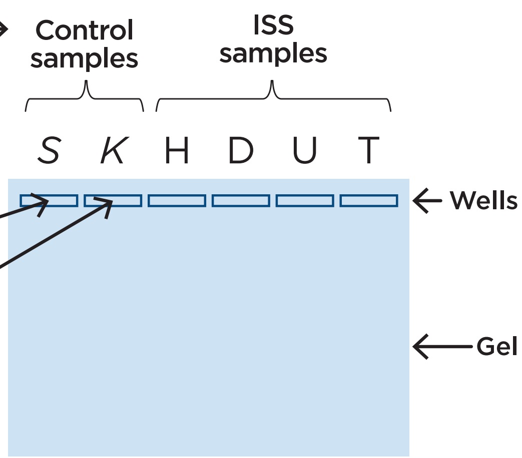
1 You will load DNA from each ISS sample into its own **well** in an **agarose gel.** Wells are like pockets that hold your DNA samples.

2 You will also load two **control** samples. Controls help you ensure that your experiment is working properly, and help you make sense of your results.

3 One control sample contains DNA segments from *Staphylococcus epidermidis* bacteria grown in a lab.

The second sample contains DNA segments from *Klebsiella aerogenes* bacteria grown in a lab.

These controls will tell you what it looks like when *Klebsiella* or *Staphylococcus* DNA is present in a sample.



S: Staphylococcus control
K: Klebsiella control
H: Harmony module sample
D: Destiny module sample
U: Unity module sample
T: Tranquility module sample

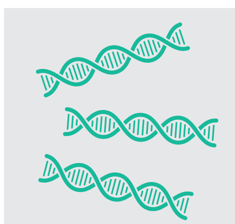
The results

1

When your experiment is complete, you will see **bands** in your electrophoresis gel. Each band contains billions of DNA molecules. As they move through the gel, bands travel in a straight line that is the same width as the well above. This area is referred to as a **lane.**

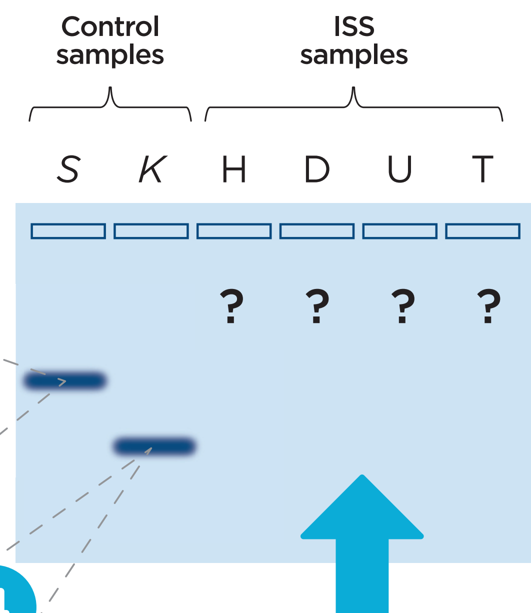
2

The band in the *Staphylococcus* lane should be close to the well, since it is made of long *Staphylococcus* DNA segments that move slowly through the gel.



3

The band in the *Klebsiella* lane should be farther from the well, since it is made of shorter *Klebsiella* DNA segments that move quickly through the gel.



4

Your job will be to match the bands you see in the ISS sample lanes H, D, U, and T to the *Klebsiella* and *Staphylococcus* bands you see in the control lanes. **If you see a *Klebsiella* DNA band, you will need to notify the crew so they can disinfect the surfaces. Work carefully today—our astronauts' safety is at stake!**

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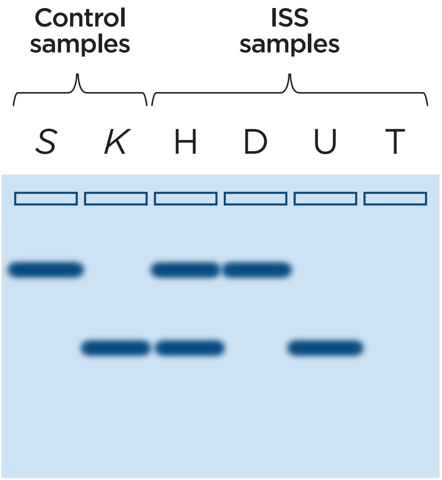
Background: Stop and think



The gel on the right shows you one way your results might look when you are done with this experiment. Refer to the gel as you answer the questions below.

10. Which band contains DNA molecules that are larger?
    A. Sample D
    B. Sample U

    Explain how you can tell:

11. Which sample contains only *Klebsiella* DNA?
    A. Sample H
    B. Sample D
    C. Sample U

    Explain how you can tell:

12. Which sample contains both *Staphylococcus* and *Klebsiella* DNA?
    A. Sample H
    B. Sample D
    C. Sample U

    Explain how you can tell:

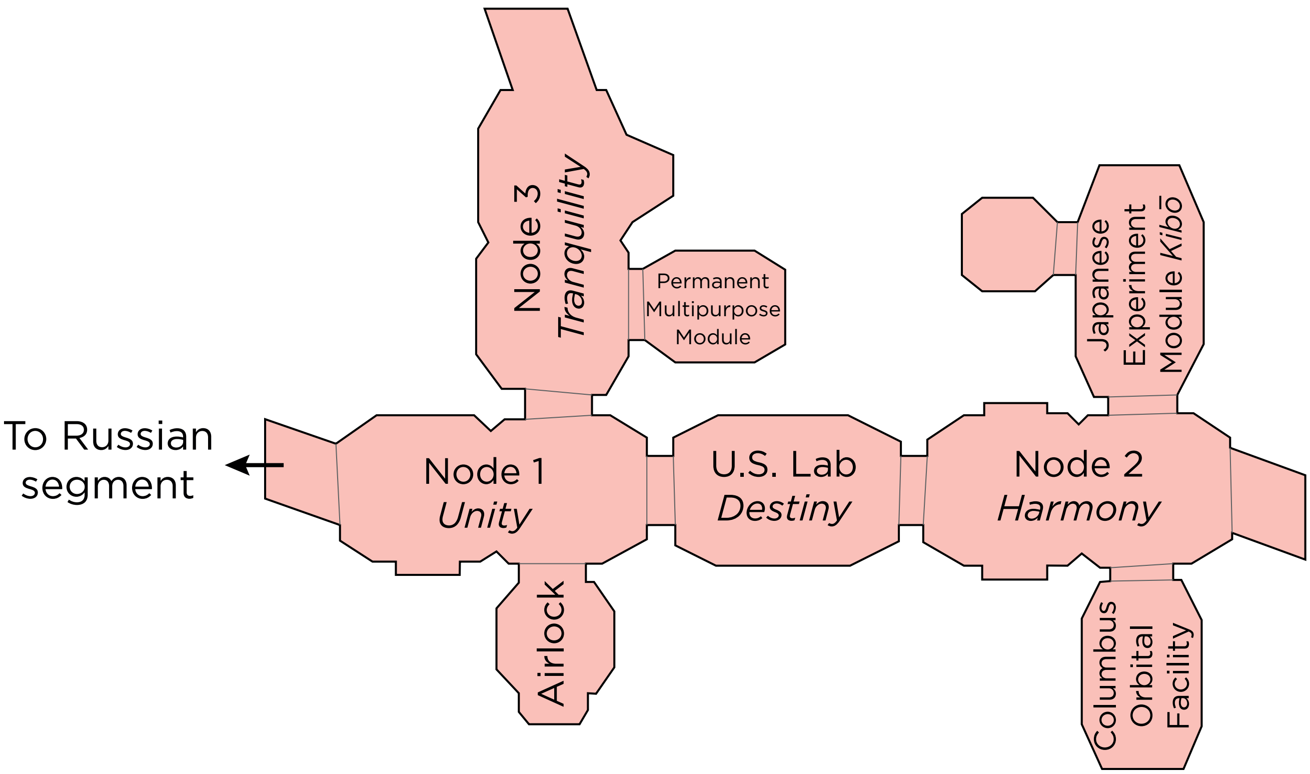
13. In the Sample T lane, draw the band(s) you would expect to see if that sample contained only *Staphylococcus* DNA. Explain your reasoning.

Map of International Space Station

Below is a floor plan of the International Space Station.

Remember that you will be working with the following samples:

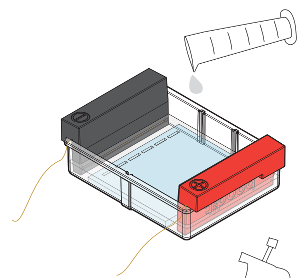
- Sample H: Wall outside sleep quarters in Harmony module
- Sample D: Laboratory countertop in Destiny module
- Sample U: Dining table in Unity module
- Sample T: Handrail outside toilet in Tranquility module



Laboratory guide

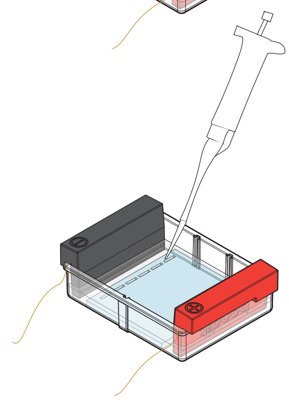
Protective gloves and eyewear should be worn for the entirety of this experiment.

See detailed assembly and gel pouring instructions for the Bandit™ STEM Electrophoresis Kit
https://www.minipcr.com/bandit-assembly/



1. Submerge your gel in enough TBE buffer to just cover the gel and fill the wells

- If using a Bandit™ or blueGel™ electrophoresis system you will need approximately 30 ml of TBE buffer.



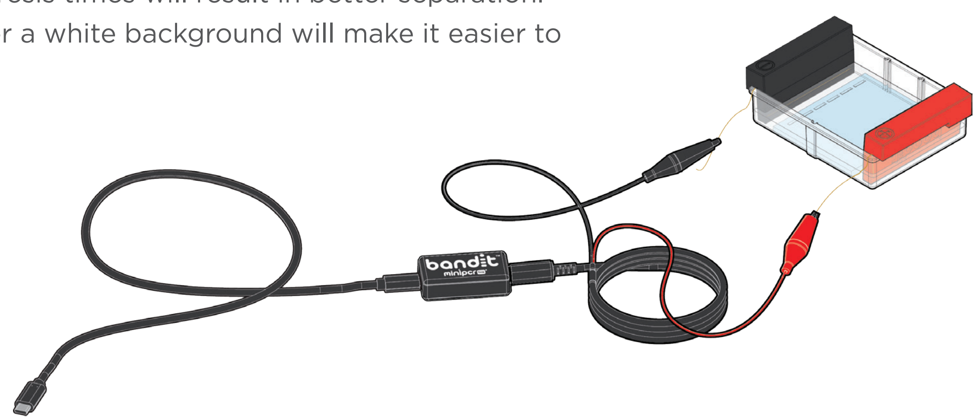
2. Use a micropipette to load samples onto the gel from the corresponding tubes in your Load Ready™ Strip

- **Lane 1:** 10 µl from tube 1, *Staphylococcus* control
- **Lane 2:** 10 µl from tube 2, *Klebsiella* control
- **Lane 3:** 10 µl from tube 3, ISS sample H
- **Lane 4:**10 µl from tube 4, ISS sample D
- **Lane 5:** 10 µl from tube 5, ISS sample U
- **Lane 6:** 10 µl from tube 6, ISS sample T

3. Connect the electrodes and turn on your gel electrophoresis system

4. Conduct electrophoresis for 15-25 minutes

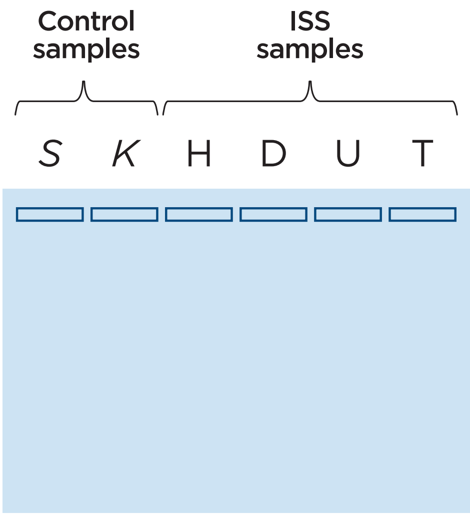
- Longer electrophoresis times will result in better separation.
- Placing the gel over a white background will make it easier to see your results.



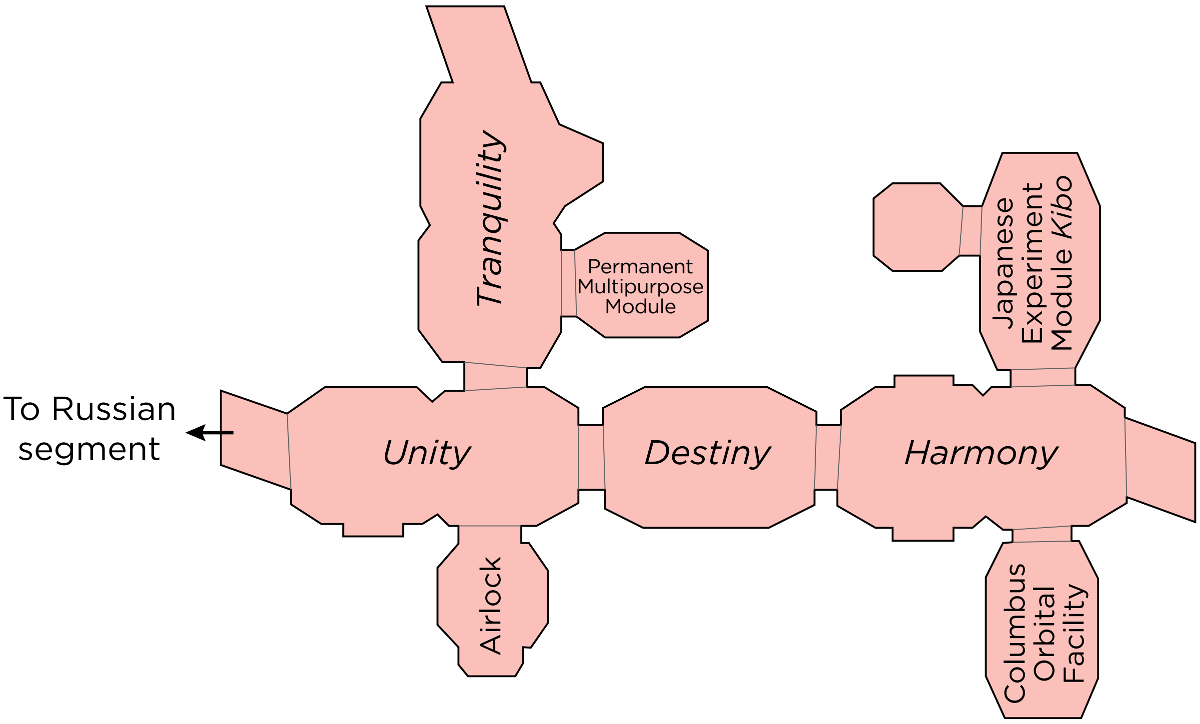
Post-lab study questions

Interpreting results

1. Use the image of a gel on the right to draw what your gel looks like. For each sample, draw the bands that you see on your actual gel.



2. Below is the ISS floor plan. Mark each module where *Klebsiella* were found with an "X." Mark each module where *Staphylococcus* were found with an "O."



3. Based on what you found, what surfaces do you recommend the astronauts clean to prevent the spread of disease?

Critical thinking

1. Name the surfaces where you found *Staphylococcus epidermidis.*

2. Remember that *Staphylococcus epidermidis* bacteria are usually found on human skin. How do you think this type of bacteria ended up on the surfaces where you discovered it?

3. Name the surfaces where you found *Klebsiella aerogenes.*

4. Remember that *Klebsiella aerogenes* bacteria are usually found in the human digestive tract. How do you think this type of bacteria ended up on the surfaces where you discovered it?

5. *Klebsiella aerogenes* can cause dangerous infections, so it's important to keep it from growing throughout the ISS. Return to the ISS floor plan on page 26. Based on where you found *Klebsiella,* where else might you want to test for *Klebsiella* to ensure the ISS remains safe?

CER table

Fill in the table based on your results from the lab. Use the rubric on the next page to help your answers.

Question:

On which surfaces aboard the ISS are there harmful Klebsiella bacteria?

Claim Make a clear statement that answers the above question.	
Evidence Provide data from the lab that supports your claim.	
Reasoning Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.	

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Score	4	3	2	1
CLAIM A statement that answers the original question/problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is hightly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric Score	3	4	5	6	7	8	9	10	11	12
Equivalent	55	60	65	70	75	80	85	90	95	100

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