miniPCR bio Learning Lab™

BioBits®: Antibiotic Resistance

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Antibiotic Resistance

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Student's Guide Contents

Background information

In your lifetime, you have likely taken antibiotics many times to combat bacterial infections. The use of antibiotics is estimated to save millions of lives across the globe every year and is considered one of the great medical advances of the 20th century.

But a new threat has emerged: bacteria are increasingly becoming resistant to antibiotic drugs. It is estimated that well over one million people die worldwide every year as a direct result of infections caused by antibiotic resistant bacteria.[1]

In this lab, you will investigate how bacteria can survive in the presence of antibiotics. But before we get started, let's go over some basics about antibiotics and antibiotic resistance.

Antibiotics

An antibiotic is a small chemical molecule that kills bacteria by disrupting an essential cellular function. Antibiotics can kill bacteria in many different ways, but one thing they all have in common is that they specifically target bacterial molecules. For this reason, antibiotics kill bacteria without harming animal cells, such as your own cells.

In this lab, we will explore two different antibiotics that work by a similar mechanism: they both disrupt the cellular process known as *translation*. Translation is a key step in protein synthesis, and when cells can no longer make proteins, they die.

Resistance to antibiotics

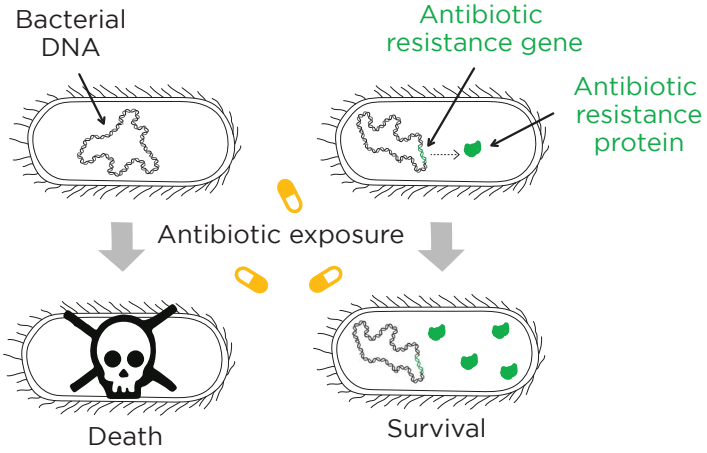
We say bacteria are *resistant* when they can survive in the presence of an antibiotic that would otherwise kill them. Luckily for us, this protection is not universal—most resistant bacteria are able to evade just one specific antibiotic, or a class of chemically similar antibiotics. There are many different ways in which bacteria can resist antibiotic action; for example, by chemically modifying the antibiotic to render it non-toxic, or by actively pumping antibiotic molecules out of the cell.

The common feature of all mechanisms of antibiotic resistance is that the bacteria produce specific proteins that protect them against the action of an antibiotic. Because the instructions for making

[1] Murray, Christopher JL, Kevin Shunji Ikuta, Fablina Sharara, Lucien Swetschinski, Gisela Robles Aguilar, Authia Gray, Chieh Han, *et al.*, "Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis." The Lancet 399, no. 10325 (February 12, 2022): 629–55. https://doi.org/10.1016/S0140-6736(21)02724-0.

proteins are encoded in DNA, it is genetic information that makes bacteria immune to antibiotics. A stretch of DNA that can make bacteria resistant to antibiotics is called an *antibiotic resistance gene*.

In today's lab, you will work directly with a bacterial antibiotic resistance gene. This particular gene does not pose a direct threat to human health, but it will help you test a mechanism that can make bacteria immune to antibiotics. Equipped with a mechanistic understanding, scientists can develop new approaches to combat the spread of antibiotic resistance.



Today's lab

Today, your class will characterize an antibiotic resistance gene and two antibiotics. You will confirm that both antibiotics work by blocking translation and you will identify which of the two antibiotics is vulnerable to the resistance gene.

Studying antibiotics, the cell-free way

Scientists often use live bacteria to study antibiotics and antibiotic resistance. In the presence of antibiotics, susceptible bacteria will stop dividing and die, while resistant bacteria will continue to thrive. But culturing bacteria is time-consuming and also requires a special laboratory setup where you can safely grow microorganisms.

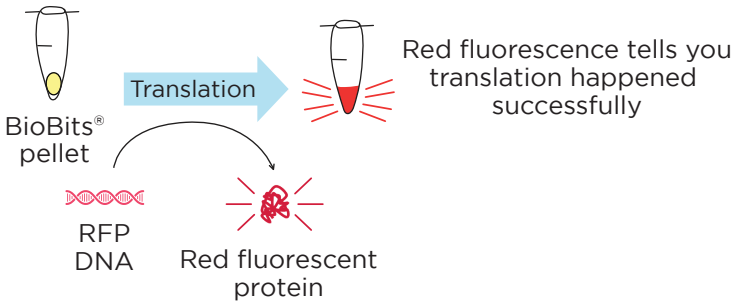
Borrowing tools from synthetic biology, today you will investigate antibiotic resistance in a simpler way. You will use a cell-free system to directly observe the effects of antibiotics that block translation.

You will use a cell-free protein synthesis system called BioBits®

- BioBits® takes the protein synthesis machinery from bacteria and puts it inside a test tube.
- All you need to do is provide instructions to make a protein, in the form of DNA.
- The molecular machinery in BioBits® will decode the DNA to make a protein, following the same molecular steps that bacterial cells would perform.

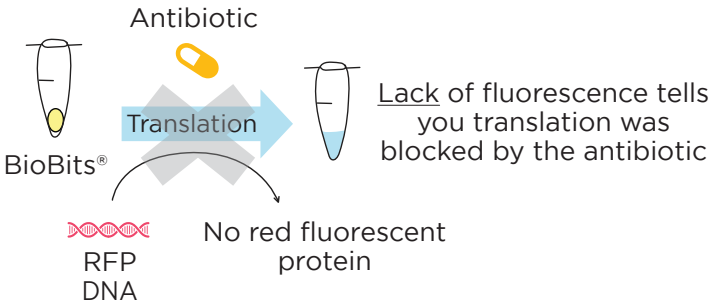
Red fluorescence will signal that protein has been made

- BioBits® can make essentially any bacterial protein; today we will be making a red fluorescent protein (RFP).
- This protein is encoded in **RFP DNA** and it glows red once translated.
- When BioBits® glows red under a blue light, you will know that translation worked and protein synthesis has occurred!



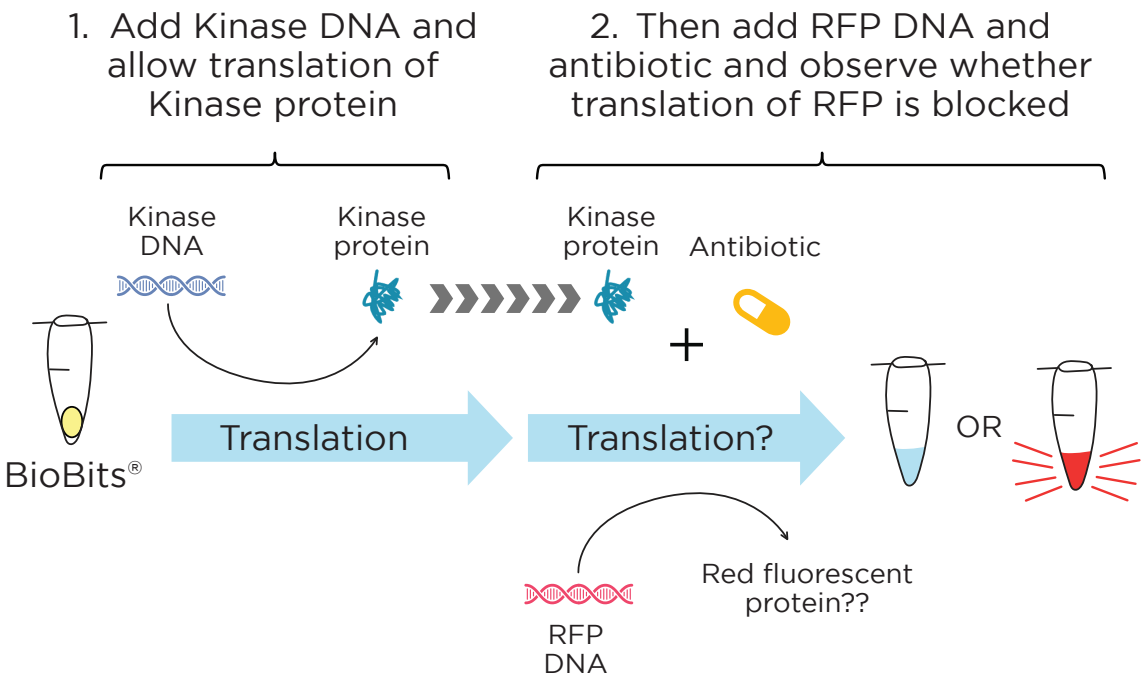
Antibiotics that block translation in bacteria will block translation in BioBits®

- **Kanamycin** and **streptomycin** are antibiotics that kill bacteria by blocking translation.
- They will also block translation in BioBits®, which are made of bacterial components.
- If translation is blocked, you will not see red fluorescence after adding RFP DNA.



Your goal: to test whether an antibiotic resistance gene can restore translation

- **Kinase DNA** encodes a protein known as *aminoglycoside kinase* that confers resistance to some antibiotics.
- Your job will be to test whether this antibiotic resistance gene confers resistance to **kanamycin or streptomycin**.



Note that the antibiotics you are testing inhibit all translation—including that of the kinase!

- If we were to add the Kinase DNA and the antibiotic at the same time, the antibiotic would block translation of the kinase protein; then the kinase gene would not have a chance to confer resistance against the antibiotic.
- Instead, you will add the Kinase DNA first, so the system has time to make the kinase protein before antibiotics are added.

Pre-lab activities

You will be provided 4 tubes to use in your experiment, each containing a BioBits® cell-free pellet. BioBits® are desiccated and need water and DNA to start making proteins. So you will also be provided:

Component	Function
Kinase DNA	Provides resistance to some antibiotics
Antibiotic (kanamycin or streptomycin)	Inhibits protein synthesis by interfering with ribosomes
RFP DNA	Encodes red fluorescent protein
Water	Added to equalize volumes as needed

The reagents that will be used in each tube are listed in the table below.

Predict what you expect to see in each of the following reactions. Use the information from the background to fill out the following table:

	Tube 1	Tube 2	Tube 3	Tube 4
BioBits® cell-free pellet	✔	✔	✔	✔
Kinase DNA	No	No	No	✔
Antibiotic (Kanamycin or Streptomycin)	No	No	✔	✔
RFP DNA	No	✔	✔	✔
Water	✔	✔	✔	✔
Do you expect to see red fluorescent protein at the end of the experiment?	Yes / No / Unknown (circle one)	Yes / No / Unknown (circle one)	Yes / No / Unknown (circle one)	Yes / No / Unknown (circle one)
Justify your prediction				

Laboratory guide

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

You will use the BioBits® cell-free system to make fluorescent proteins and see how adding antibiotics and a specific antibiotic resistance gene will impact protein production. You will have a total of four samples, including a negative control where you only add water.

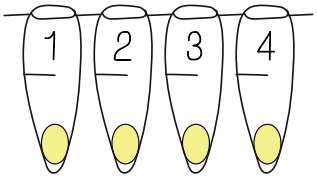
Selecting the antibiotic for your group

Your instructor will assign your group an antibiotic to test. Circle the antibiotic that you will be testing:

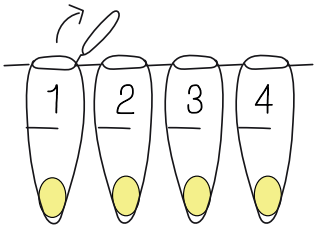
Kanamycin **Streptomycin**

Setting up BioBits® reactions

1. **Number each tube in your strip of four BioBits® pellets, 1-4.**
   - Label the numbers on the sides, not cap, of the tube.

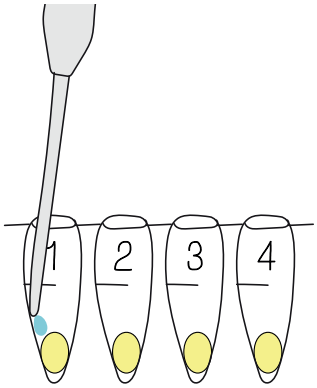


2. **Uncap the BioBits® strip tubes.**
   - BEFORE UNCAPPING, gently tap tubes on the table to collect pellets at the bottom.
   - To open tubes, CAREFULLY remove each cap in the strip one at a time, taking care not to dislodge BioBits® pellets.



How to pipette when working with BioBits®:

- Touch your pipette tip to the side of the tube and dispense the liquid, then tap the tube on the table top to collect the liquid in the bottom of the tube.
- Do not touch your pipette tip to the pellet.
- Do not pipette up and down to mix.



3. Add reagents according to the chart below, using a new tip for each sample.

	Tube 1	Tube 2	Tube 3	Tube 4
Reagent	3 µl water	3 µl water	3 µl water	3 µl Kinase DNA

Wait 10 minutes before proceeding to the next step.

- Cap tubes and tap them on your table to make sure liquid is collected at the bottom and pellets are dissolved.

4. After 10 minutes have elapsed, add reagents according to the chart below.

- In the table below, for tubes 3 and 4, circle the antibiotic that you will be using (either kanamycin or streptomycin) and then use the corresponding RFP DNA + antibiotic mix.
- Other groups will test the function of the other antibiotic.
- Add the additional reagents directly to the liquid already in the tube.
- To avoid introducing bubbles, do not pipette up and down to mix.
- Use a new tip for each sample.

	Tube 1	Tube 2	Tube 3	Tube 4
Reagent added	3 µl water	3 µl RFP DNA	3 µl pre-mix of RFP DNA + [kanamycin OR streptomycin]	3 µl pre-mix of RFP DNA + [kanamycin OR streptomycin]

5. **Close the caps on the tubes.**
    - You should feel the caps "click" into place when they are closed correctly.
    - Tap tubes on your table to make sure all liquids collect at the bottom.

6. **Observe your tubes under blue light in the P51™ viewer through an orange filter.**
    - Dim the ambient lights so it's easier to observe any fluorescence.
    - Make sure the blue light in the P51™ is ON.
    - Observe the tubes through the front window of the P51™.
    - You may use a different blue light illuminator with an orange filter if a P51™ is not available.
    - Record your observations in Table 1 on the next page. In the row labeled "0 hours", record whether the tubes appear to be fluorescing and if so, what color.

7. **Let the reactions proceed for 8 to 72 hours at room temperature.**
    - You may leave the tubes lying flat on the lab bench or table.
    - Although indoor ambient light is fine, avoid storing your tubes in direct sunlight.

Observations (to be taken 8 to 72 hours later)

1. **Observe your tubes under blue light in the P51™ viewer through an orange filter.**
    - Tap tubes on your table to make sure all liquids collect at the bottom.
    - Dim the ambient lights so it's easier to observe any fluorescence.
    - Make sure the blue light in the P51™ is ON.
    - Observe the tubes through the front window of the P51™ using the orange filter.
    - Record your observations in Table 1 below. Record whether the tubes appear to be fluorescing and if so, what color.

Table 1: Record the presence or absence of fluorescence, and its color.

Time	Tube 1	Tube 2	Tube 3	Tube 4
0 hours	[YES / NO] Color:	[YES / NO] Color:	[YES / NO] Color:	[YES / NO] Color:
Final observation (8-72 h later)	[YES / NO] Color:	[YES / NO] Color:	[YES / NO] Color:	[YES / NO] Color:

Pre-lab study questions

Review

1. **What is an antibiotic?**

2. **Why is the problem of antibiotic resistance of such a concern?**

3. **Aminoglycosides, like the kanamycin and streptomycin you will use in this lab, are antibiotics that work by binding to the bacterial ribosome and preventing translation from proceeding. Explain why this would be toxic to a cell.**

4. **Antibiotic resistance genes allow bacteria to survive in the presence of an antibiotic. But the gene doesn't directly interact with the antibiotic. Explain how genes provide resistance to antibiotics.**

5. **Would it be likely to find an antibiotic resistant gene that confers resistance to all antibiotics? Explain your answer.**

Critical thinking

6. **One common target of antibiotics is cell wall formation. Why would interfering with cell wall formation be an especially good target for an antibiotic? Consider in your answer how important it is for antibiotics to be nontoxic to patients.**

7. **Many antibiotics, such as the ones used in this lab, block protein synthesis by binding to the bacterial ribosome. Hypothesize how these antibiotics can specifically inhibit bacterial ribosomes, but not animal ribosomes.**

8. **The aminoglycoside kinase encoded by the Kinase DNA is a protein expected to provide resistance to at least one of the two antibiotics you are testing. Antibiotic resistance functions by one of four general mechanisms: (i) by removing antibiotics from the cell, (ii) by chemically modifying antibiotics making them inactive, (iii) by interfering with an antibiotic's target, or (iv) by creating new biochemical pathways that bypass the antibiotic's action. Based on the fact that today's kinase must protect from antibiotics in a cell-free system, which resistance mechanism(s) could potentially describe how aminoglycoside kinase functions? Explain why some resistance mechanisms cannot be tested in a cell-free system.**

9. **Both streptomycin and kanamycin are classified as aminoglycosides, which means they share some structural and functional similarities. Based on this information and the information in the introduction, do you consider it likely that a specific antibiotic resistance gene could confer resistance to both streptomycin and kanamycin? Justify your answer.**

10. **Many plasmids that spread resistance genes actually contain several different resistance genes on a single plasmid. Antibiotic resistance has been described as one of the great examples of natural selection in action. Why would having multiple antibiotic resistance genes allow a plasmid to be more successful in terms of natural selection than a plasmid that contains only one antibiotic resistance gene?**

Post-lab study questions:

Analyzing your data

Which antibiotic did you test? (circle one) **Kanamycin** **Streptomycin**

1. **A positive control is one that confirms that a test works as expected. Looking back at your results from today's experiment, which tube behaved as a positive control for the ability of BioBits® to produce a protein?**

2. **In your experiment, which tube(s) demonstrated that the antibiotic you were testing was able to disrupt protein production? Use evidence from the lab to support your answer.**

3. **In your experiment, which tube(s) demonstrated whether the antibiotic resistance gene rescued protein production in the presence of the antibiotic? Explain your answer.**

4. **Did the antibiotic resistance gene that your group tested rescue protein production in the presence of an antibiotic? Refer to your experimental results to support your claim.**

5. **Compare your results with those of other groups.**
   a. **Did groups that tested the same antibiotic obtain the same results as yours? If not, what might explain the difference(s)?**

   b. **Did groups that tested the other antibiotic see the same results as yours? Describe any differences observed.**

6. **Some antibiotic resistance mechanisms are specific to certain antibiotics, while other resistance mechanisms protect broadly against multiple antibiotics. Based on today's experimental outcomes, do you think Kinase DNA confers broad resistance to antibiotics, or specific resistance to some antibiotics? Cite experimental evidence from at least two different groups in your answer.**

Critical thinking:

7. **You were instructed to add the Kinase DNA 10 minutes before adding the antibiotics and RFP DNA. What would have happened if you had added the Kinase DNA at the same time as the antibiotics and RFP DNA?**

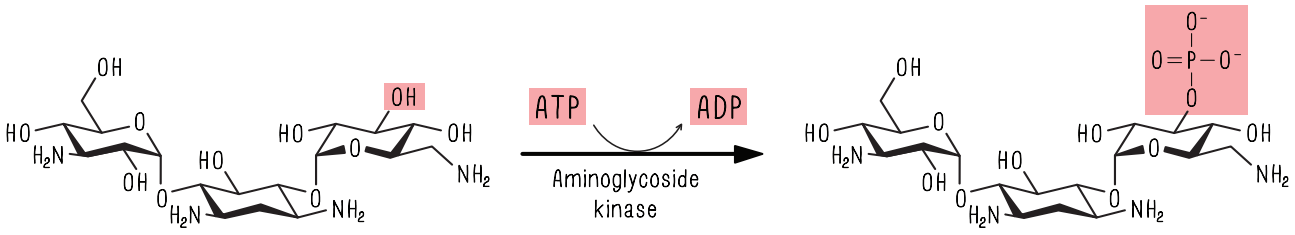
8. **What two steps took place in the BioBits® system after you added the Kinase DNA? Explain how these steps were important for the antibiotic resistance to manifest itself.**

9. **After adding Kinase DNA, you had to incubate the reaction for just 10 minutes prior to adding the antibiotics and RFP DNA. Then, after adding RFP DNA, you waited at least 8 hours before observing the results. Based on your answer to question 8 above, in your opinion, what was different between Kinase DNA and RFP DNA that the waiting times were so different? Explain why you think that.**

Advanced questions:

10. **Lateral gene transfer is when genes move between different organisms that are not related. This is different from how genes are normally passed down from parents to their offspring. When thinking of the BioBits® reaction as a model for a cell, how does adding the Kinase DNA model how bacteria are able to acquire resistance in nature? Is it more similar to a DNA mutation conferring resistance or to a cell acquiring resistance through lateral gene transfer? Explain your answer.**

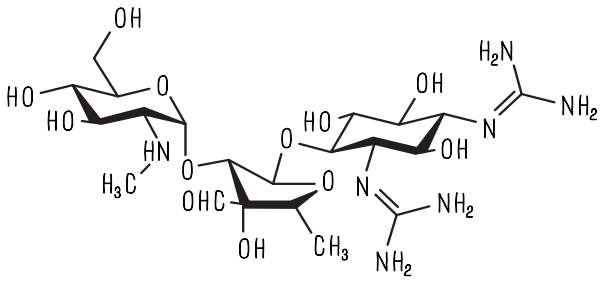
11. **The reaction you observed in this lab is illustrated below. The chemicals or functional groups that are directly changed by the reaction are highlighted in red. Can you use information from this reaction to say whether using aminoglycoside kinase to survive in the presence of high levels of kanamycin would be energetically costly to a cell? Justify your answer.**



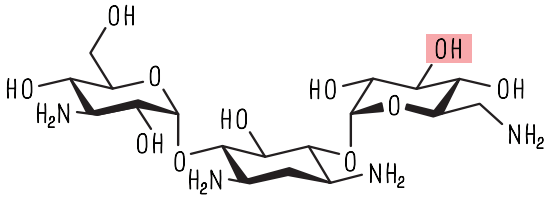
Kanamycin

Kanamycin (3')-phosphate

12. **Below are the chemical structures of both streptomycin and kanamycin. Again the critical hydroxyl group that is modified by aminoglycoside kinase is highlighted in red in the kanamycin molecule. Compare the structures of the two molecules. Can you propose a simple hypothesis as to why aminoglycoside kinase is not effective at conferring resistance to streptomycin?**



Streptomycin



Kanamycin

CER Table

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

Question:

Against which antibiotic(s), kanamycin and/or streptomycin, does the aminoglycoside kinase confer resistance?

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.

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 highly 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 that 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 Grade	55	60	65	70	75	80	85	90	95	100