BIO-RAD Explorer

BacTalk Cell Signaling Kit

Catalog #12024250EDU

Student Guide

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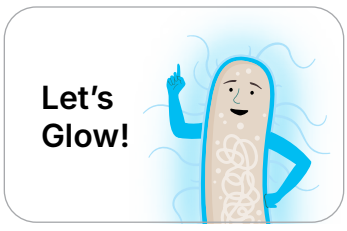


Activity 1. Modeling the Lux Signal Transduction Pathway

Part 1: Introduction to Bioluminescence and Quorum Sensing

Bioluminescence: An Enlightening Phenomenon

What do milky seas, anglerfish, and the Hawaiian bobtail squid (Figure 1) all have in common?

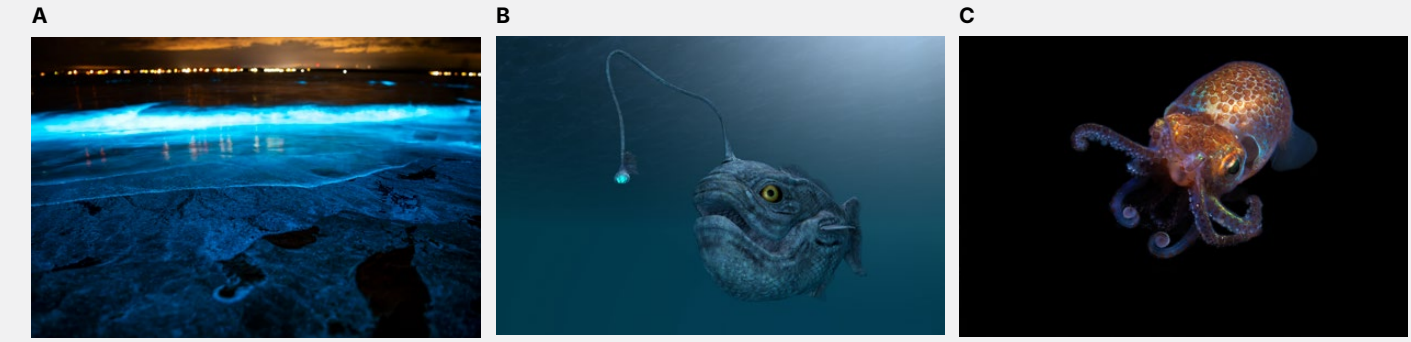


Fig. 1. Bioluminescence in nature. (**A**) Ocean waves glowing with bioluminescent plankton, (**B**) an anglerfish using its bioluminescent esca to attract prey, and (**C**) a Hawaiian bobtail squid emitting bioluminescent light to camouflage itself from predators. Bioluminescence is a natural phenomenon in which living organisms produce light through chemical reactions.

For centuries, people have observed that ocean waters can sometimes glow ghostly blue or white at night. This phenomenon occurs over vast areas of tropical and subtropical oceans — up to 16,000 square kilometers (about the size of the state of Connecticut). Charles Darwin recorded one of the earliest scientific mentions of milky seas during his voyage aboard the HMS Beagle in the 1830s, and it has also been mentioned in classic works of literature, such as Jules Verne's 20,000 Leagues Under the Sea and Herman Melville's Moby-Dick. More recent satellite images have confirmed the scale and duration of these milky seas, which have been attributed to marine bacteria. This hauntingly beautiful glow can persist continuously for several nights and is similar to, but distinct from, the glowing blue light produced in crashing waves by marine plankton (Figure 1A).

Deep-sea anglerfish typically live at ocean depths of 200–2,000 meters (660–6,560 feet). To attract prey in these pitch-black waters, an anglerfish lies motionless, bobbing a glowing appendage called an esca that dangles from the end of a dorsal spine extending out of its head (Figure 1B). The light produced by bacteria living in the esca mimics smaller organisms such as worms or shrimp, and it draws the attention of curious would-be predators. When an unsuspecting organism gets close enough to try to eat the glowing "prey", the anglerfish snaps its jaws shut with incredible speed and force. The would-be predator becomes prey for the anglerfish.

The nocturnal Hawaiian bobtail squid lives off the coast of Hawai'i in shallow, knee-deep water. During the day, it buries itself in the sand to avoid predators and to sleep. At night, it hunts for small crustaceans, such as shrimp, small fish, and annelids (worms). On starry or moonlit nights, the bobtail squid has enough light to hunt by. Too much light, however, casts a shadow that the squid's predators can use to calculate the squid's trajectory to track and eat it.

Luckily for the bobtail squid (Figure 1C), it uses light produced by bacteria living in two specialized organs inside its ventral mantle cavity for counter-illumination camouflage. The light produced is just enough to allow the squid to hunt without casting a shadow. The light-producing organs have a shutter that can open and close to regulate the amount of light released into the environment. The squid also has light detectors on its back to detect the amount of ambient light hitting its back. The detectors signal the shutters to release the same amount of light hitting the squid's back, so it does not produce a shadow.

The bacteria that live in the light organs of the bobtail squid are *Aliivibrio fischeri* (formerly *Vibrio fischeri*), and they have a short lifespan. They grow and divide over the course of a night in the squid's light organs. Soon after the squid buries itself in the sand, a pump in each light organ pumps out nearly all the bacteria. Over the course of the day, the remaining bacteria continue to grow, and by nighttime, there are enough bacteria to produce the light the squid needs.

The milky seas, anglerfish, and Hawaiian bobtail squid all produce light through chemical reactions. This type of light is called **bioluminescence**. The chemical reaction that produces bioluminescence requires a molecule called luciferin, which emits light when it reacts with oxygen. The specific details of this reaction depend on the species producing it: there are distinct types of luciferins in nature, and many other organisms also require an enzyme called **luciferase** to produce light.

In some organisms, like *A. fischeri*, the production of bioluminescence is tightly regulated by a type of cell signaling called **quorum sensing**[1]. In this series of lab activities, you will explore the process of cell signaling that leads to and regulates bioluminescence in the bacterium *Vibrio campbellii*. Like its cousin *A. fischeri*, *V. campbellii* bacteria coordinate production of bioluminescence through quorum sensing.

Cell Signaling and the Lux Pathway

Cell signaling is the process by which cells interact with the environment, themselves, and other cells. In multicellular organisms such as yourself, each cell is programmed to respond to specific extracellular signals; this is the basis of growth, development, and **homeostasis**. Errors in signaling can cause diseases such as cancer, autoimmunity, and even diabetes.

Intriguingly, even single-celled bacteria use cell signaling to regulate their growth, division, and responses to environmental changes and even to coordinate activities with other bacteria.

How do they do this?

[1] While quorum sensing regulates bioluminescence in certain bacteria, it is not the universal mechanism for all bioluminescent organisms. Different species have evolved various biochemical pathways to produce light.

1. **Watch the video by Dr. Bonnie Bassler, which is titled, "How Bacteria "Talk"" (bio-rad.com/qs-talk) or read Appendix A.** As you watch or read, record key things you notice about bacteria, *Vibrio*, and quorum sensing in the table below. Then record what these observations make you wonder about. Be prepared to share what you noticed and wonder.

What I **noticed** as I watched/read:	What this makes me **wonder**:

To coordinate their production of bioluminescence, *Vibrio* bacteria talk to each other. The conversation begins when they "speak" using chemical "words" they produce and release into their environment (Figure 2).

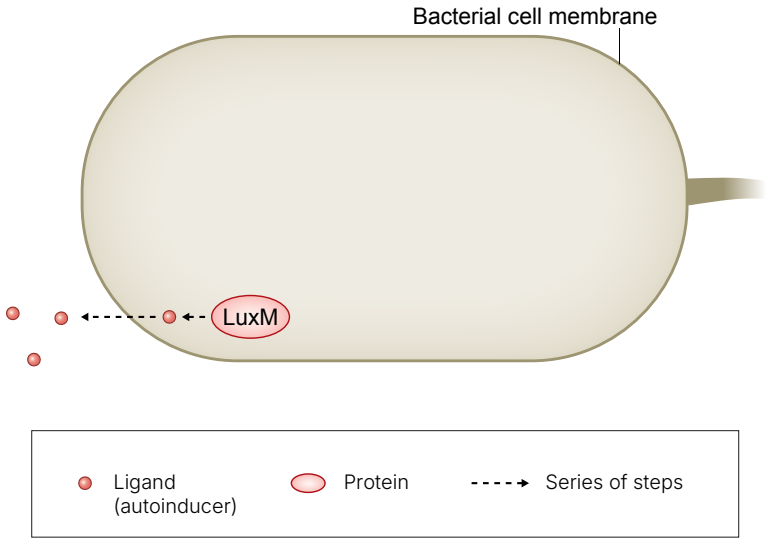


Fig. 2. How *V. campbellii* bacteria "speak": the "words" they use. (Note the details of quorum sensing in *V. campbellii* are different from those in *A. fischeri*).

2. Refer to the video or Appendix A, as well as to Figure 2, to answer the following questions:

a. What are the chemical "words" that *Vibrio* "speak" with?

b. In *V. campbellii*, which molecule produces the chemical words (Figure 2)?

Many species of bacteria "hear" and "respond" to chemical "words" using a signal transduction pathway. **Signal transduction** involves a series of molecular reactions that receive, relay, and convert a signal ("words") into a response. Simply put, signal transduction is the process by which a cell responds to an external signal and converts that information (transduction) into a response.

The pathway that produces bioluminescence in *V. campbellii* is called the **Lux pathway** (Figure 3). Bioluminescence occurs through a chemical reaction catalyzed by the enzyme luciferase, and in this system, luciferase is made up of the LuxA and LuxB proteins. Another protein, LuxR, is required by the cell to make LuxA and LuxB.

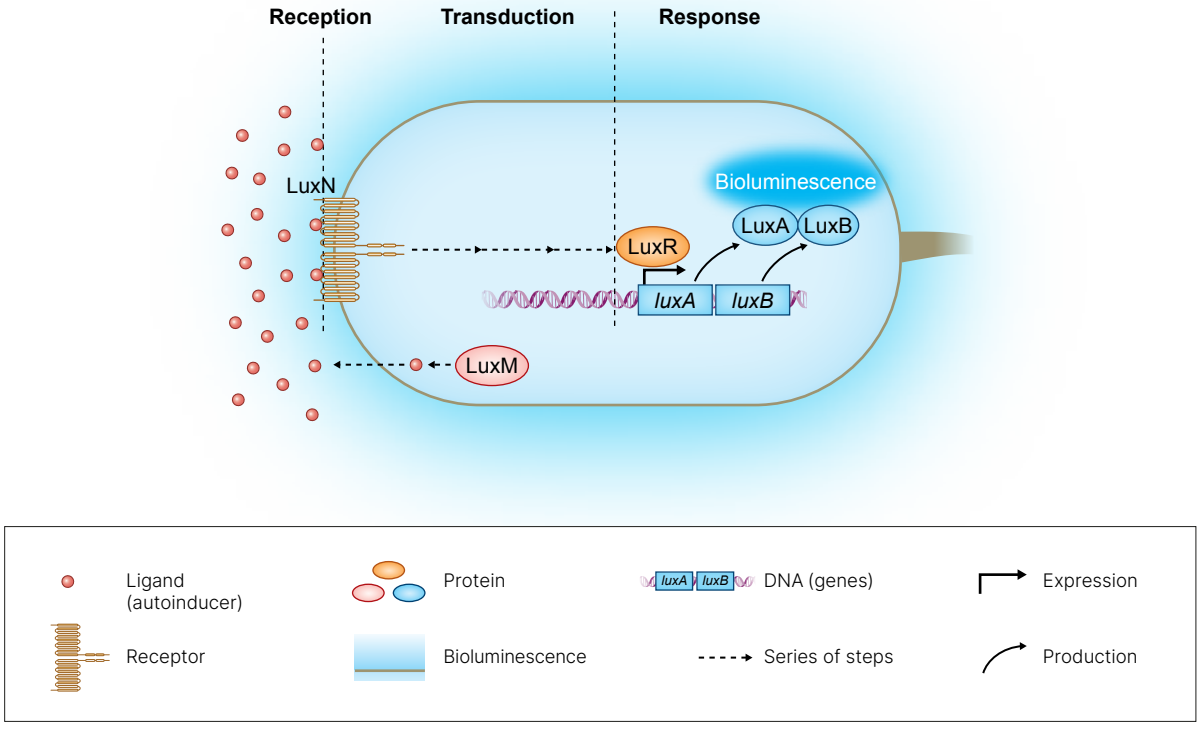


Fig. 3. How *V. campbellii* bacteria "speak", "hear", and "respond" in the Lux pathway. There are many steps and molecules involved in this pathway. The molecules that are relevant to this set of labs are shown here.

3. Use the model shown in Figure 3 to describe the three main phases of signal transduction and the Lux pathway.

Quorum Sensing

The chemical reaction that produces bioluminescence in *V. campbellii* uses luciferin, luciferase, oxygen, and a lot of energy, specifically in the form of ATP. Because of this, bacteria only glow (produce bioluminescence) when there are enough other bacteria nearby to make the light noticeable (Figure 4). This coordination is called **quorum**[2] sensing.

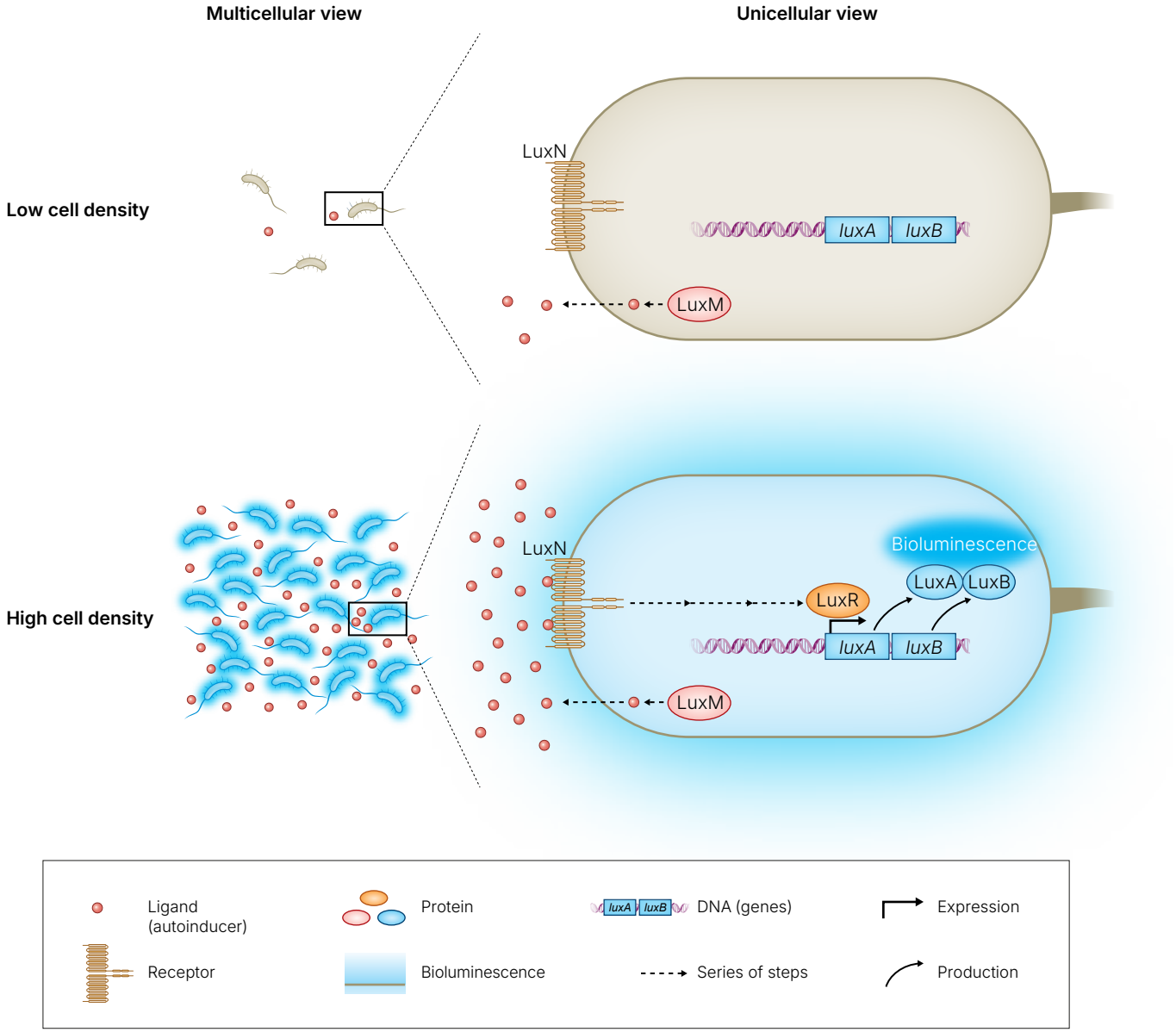


Fig. 4. Quorum sensing and the Lux signal transduction pathway in *V. campbellii*. Bioluminescence requires the combined activity of both the LuxA and LuxB proteins. For more details about how signal reception leads to the production of LuxA and LuxB, refer to Appendix B. Quorum sensing allows bacteria like *Vibrio* to work together to save energy by controlling when they produce light. Quorum sensing also helps bacteria manage other processes and responses to their environment, such as causing disease (pathogenesis), forming beneficial relationships (symbiosis), and reacting to environmental stress.

[2] Quorum = the minimum number of members of a group required to be present at a given meeting to transact business.

4. **Use the models of bacterial communication shown in Figure 4 to explain how a high cell density is required for quorum sensing by *V. campbellii* to produce bioluminescence.**

To learn more about the details behind how signal reception leads to the production of LuxA and LuxB, refer to Appendix B.

Part 2: Simulate the Molecular Mechanism of Quorum Sensing in *Vibrio*

Analogy Map

1. Follow your teacher's instructions to construct an analogy map of the Lux pathway using the table below.

This part of the model...	...is like...	this aspect of human communication.	They are alike because...
LuxM producing ligand		*speaking* to someone	
Ligand binding to LuxN			
LuxA and LuxB producing bioluminescence			

Paper Model: The Lux Signal Transduction Pathway and Quorum Sensing

2. Use the paper model parts A–I and Figures 3 and 4 to model the Lux pathway:

a. At low bacterial cell densities (no quorum).

b. At high bacterial cell densities (at quorum).

Be prepared to explain how and why you constructed each of your models the way you did.

Paper Model: Effects of Disrupting the Lux Signal Transduction Pathway

Changes in the Lux pathway could prevent bioluminescence, even when the density of *Vibrio* bacterial cells is high enough for quorum sensing to occur. The lab activities you will perform investigate the three disruptions shown in Figure 5.

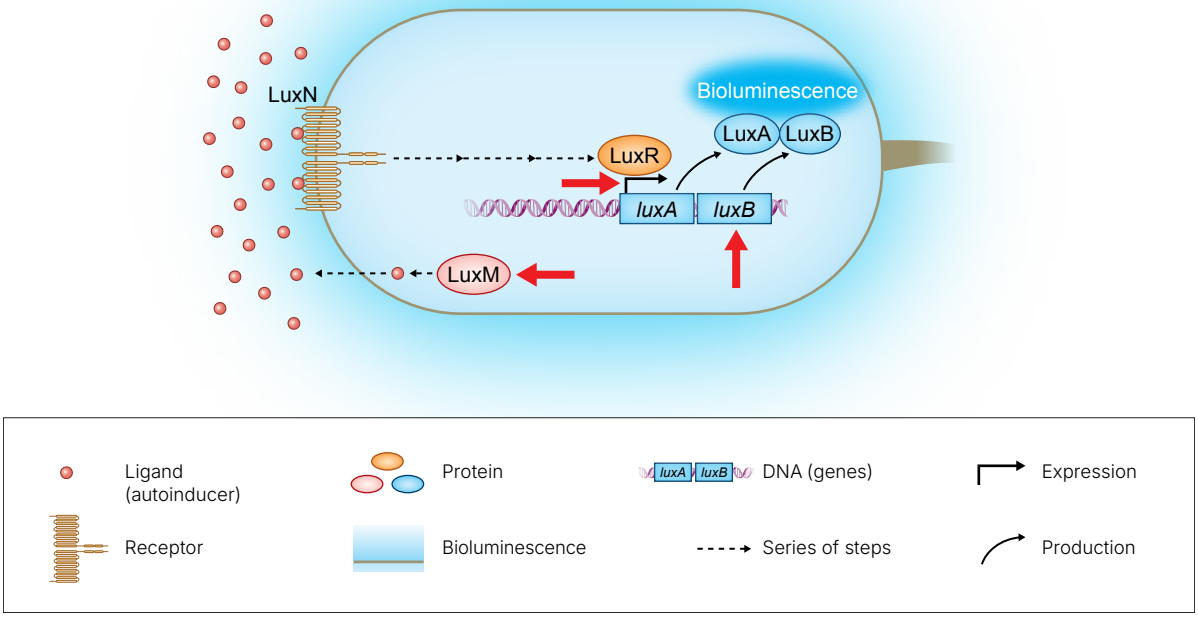


Fig. 5. Lux signal transduction pathway. Arrows point to three components of the *V. campbellii* Lux pathway that will be disrupted in the lab activities.

Use the paper model parts A–I and Figures 4 and 5 to provide answers for questions 3–6:

3. **A *V. campbellii* strain that does not produce LuxM is called a *luxM-* strain. When *luxM-* bacteria are at quorum (high concentrations), will they produce bioluminescence? Provide evidence to support your answer.**

4. **Could *luxM*- bacteria produce bioluminescence in the presence of wild-type (WT) bacteria? Remember that in WT bacteria, all the components of the Lux pathway are present and functional, including LuxM. Provide evidence to support your answer.**

5. **Another strain of *V. campbellii* (*luxB*-) cannot produce the LuxB protein. When *luxB*- bacteria are at quorum, will they produce bioluminescence? Provide evidence to support your answer.**

6. Could *luxB*- bacteria produce bioluminescence in the presence of wild-type (WT) bacteria? Provide evidence to support your answer.

7. Summarize what you have figured out about the three strains of *V. campbellii* (WT, *luxM-*, and *luxB-*) in the table below. Enter a plus (+) for the processes each strain can carry out. You may use your analogy map and paper models to decide whether each strain can "speak," "hear," and/or "respond."

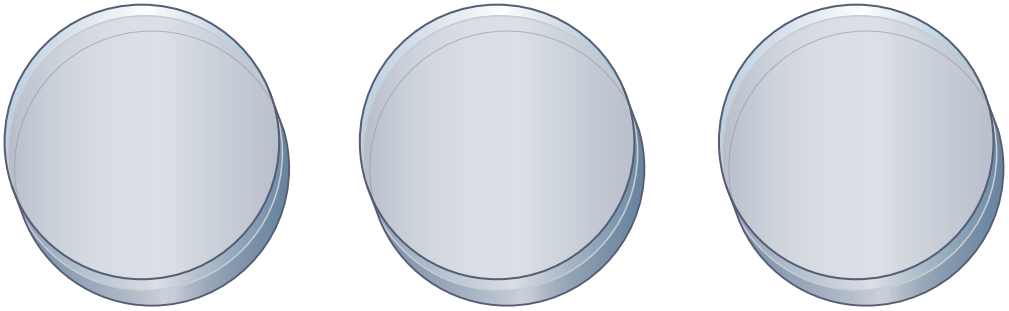
Strain	Signal Can "speak" (produce ligand)	Reception & Transduction Can "hear" (perceive ligand)	Response Can "respond" (produce bioluminescence if ligand is made or provided)	Reasoning
Wild type (WT) All pathway components function				
luxM- LuxM protein is not produced				
luxB- LuxB protein is not produced				

Part 3: Observe Demonstration Plates

Your teacher has provided a set of three agar plates. Each plate contains a culture of a single strain: WT, *luxM*-, or *luxB*-. The strains are labeled with symbols to hide their identity.

1. **Observe the demonstration plates in the light (to document growth) and in the dark (to document bioluminescence). (Alternatively, observe the example plate images provided.)**

   a. Photograph the plates and/or sketch your observations below. Where did you see growth? Bioluminescence?



   b. Indicate in the table below which strain(s) produced bioluminescence.

Plate	Strain	Bioluminescence?
1	□	
2	Δ	
3	○	

2. Can you determine which strain is which from your observations? Record your ideas below.

Plate	Strain	Can you identify the strain?	Reasoning
1	□		
2	Δ		
3	○		

Activity 2. Co-Plating of Wild-Type, *luxM-*, and *luxB- Vibrio* Strains

In this activity, you will determine the identities of the three *Vibrio* strains you examined in Activity 1. You will inoculate a set of three agar plates with different combinations of the unknown strains. This type of experiment is known as co-culturing or **co-plating**.

Part 1: Understand Co-Plating

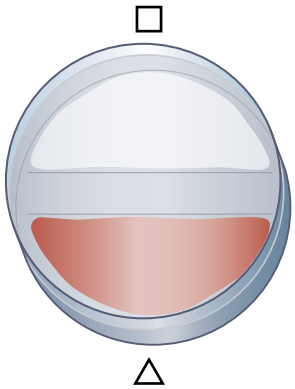
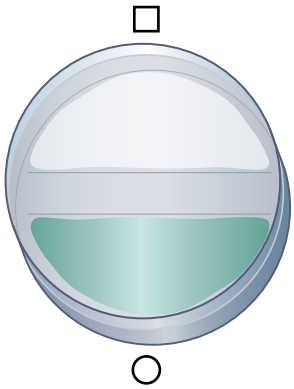
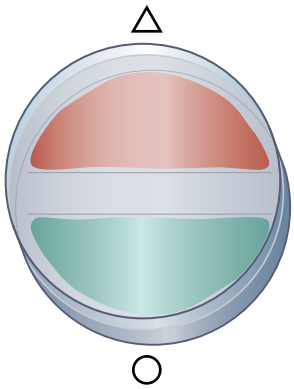
One way we can identify the strains of bacteria is through a process called co-culturing, or co-plating. This involves inoculating one strain on one side of a plate and then inoculating the other side with the second strain.

Use what you have learned about the strains and quorum sensing in Activity 1 and the paper model to predict where bioluminescence will be observed on each plate below.

1. On the diagrams below:

a. Write "yes" to indicate areas on each half of the plate where you predict you would observe bioluminescence.

b. Add your reasoning in the space below.

Combination 1	Combination 2	Combination 3
		
Reasoning	**Reasoning**	**Reasoning**

Part 2: Perform Co-Plating

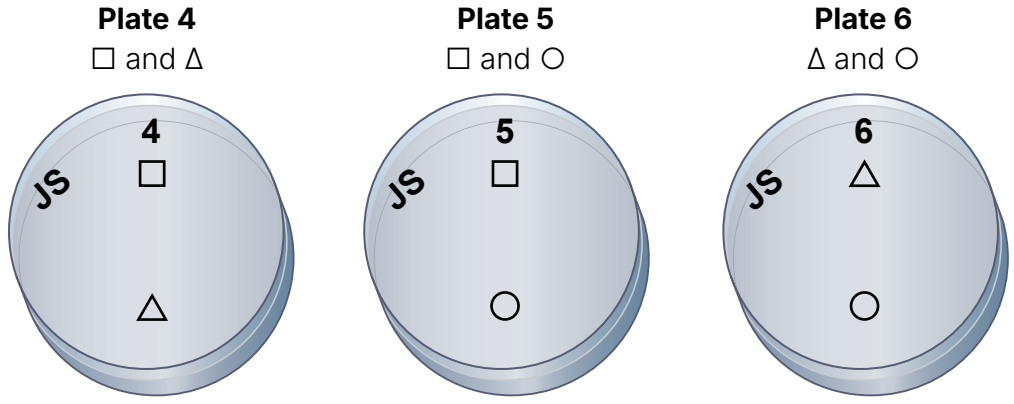
Student Workstation	
Material	**Quantity**
Student Guide	1
Paper model	1
Viewing box (or access to a dark space)	1
Plate templates	1
VA plates	3
Set of *V. campbellii* strains in microcentrifuge tubes (☐, Δ, and O)	1
Inoculation loops	6
20 µl adjustable-volume micropipet (or 20 µl fixed-volume micropipet)	1
Micropipet tips	1 box
Permanent marking pen	1
Biohazard waste collection	1

Also needed
Camera capable of dark imaging (for example, a cell phone camera)

Protocol

For this activity, you will inoculate two strains on each plate, with a space left between them. Use the guide template provided or use a ruler to draw guidelines on the plates being streaked.

1. Label the bottoms of three agar plates "4", "5", and "6" writing at the edge of each plate. Add each group designation or person's initials near the plate number, then add each strain designation:



2. Inoculate the plates with *Vibrio* strains. For each plate (see Figure 6):

   a. Use the plate templates to guide your labels and plating of bacteria. The template is important for maintaining the 0.5 cm distance between strains. To use the template, either:

      Place the plate agar-side-up on the template and mark the area on the bottom of each plate before plating (preferred).
      -or-
      Place the plate agar-side-down (lid-side up) on the template while you plate.

   b. Gently flick the bacterial cultures ~10 times to resuspend the bacteria prior to use.

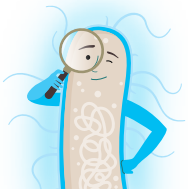
   c. Use a micropipet and a clean tip to add 20 µl of the specified strain to the plate. Dispose of the tip in the biohazard waste receptacle.

   d. Use a fresh inoculation loop to spread the bacteria within its designated region. Use a "painting on gelatin technique" to gently spread the culture evenly across the designated plating area for that strain.

   e. Dispose of the loop in the biohazard waste receptacle.

   f. Repeat steps 2b–e with the second strain, taking care to **use a clean pipet tip and inoculation loop each time**.

Focus Questions



Why is it important to maintain a gap between the different strains?

How might mixing or cross-contaminating the strains affect your interpretation of the results?

Important notes about plating the bacteria:

- Spread the bacteria evenly across the designated part of each plate. The resulting culture should look like a lawn, **covered completely with bacteria** (you are NOT using a streak plate method to generate single colonies)
- To avoid cross-contamination of strains:
  - Maintain ~0.5 cm distance between the two strains on each plate. The strains should not touch or mix
  - Use clean pipet tips and inoculation loops for each strain. For environmental safety, dispose of these in the special waste container provided

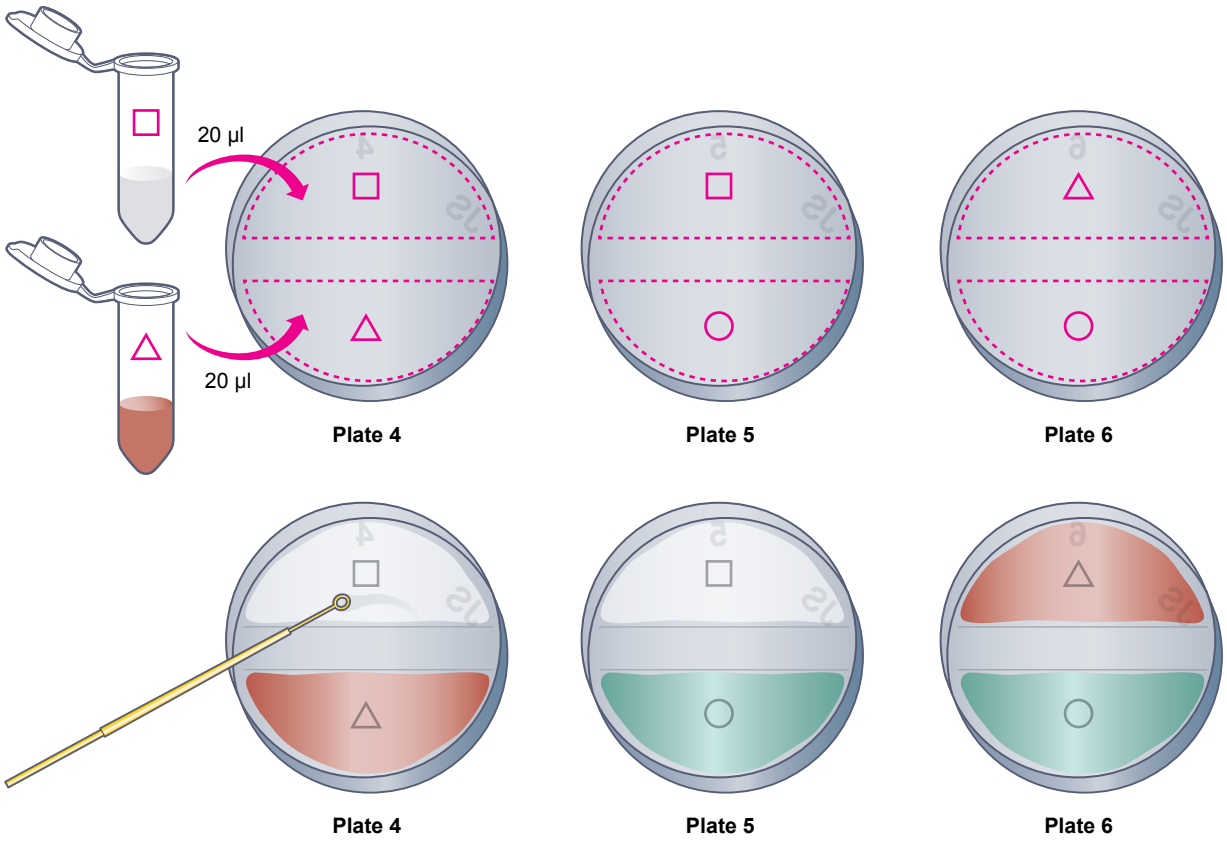


Fig. 6. Inoculation patterns for plates 4–6.

3. Allow the plates to dry until no liquid is observed (~1–15 minutes).

4. Stack the plates upside-down (agar-side-up) and incubate them as instructed (for example, for 22–24 hours at 30°C).

STOP **Stop**. Ask your teacher whether to stop now or proceed to Activity 3.

Part 3: Record and Analyze Results

1. Observe the plates both in the light (for growth) and in a dark environment (for bioluminescence).

a. It is helpful to take a picture of the plates both in the light and the dark for the purpose of comparison.

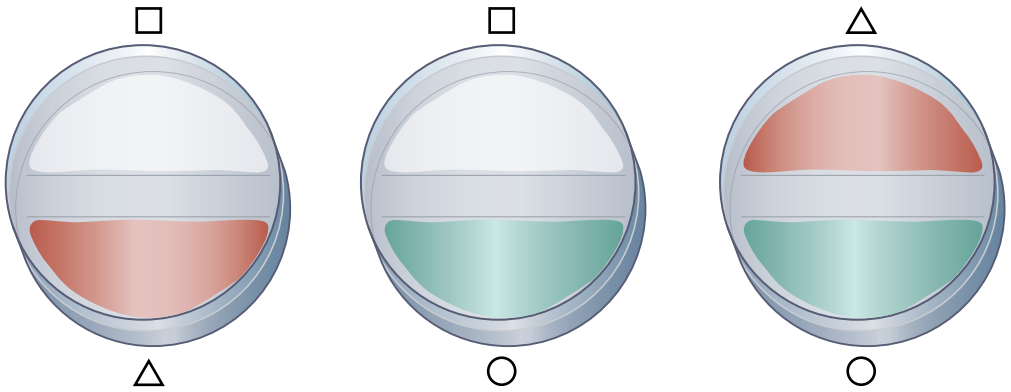
a. To observe bioluminescence, use a viewing box, a dark cover, or a dark room, as directed by your teacher.

b. It may be difficult to observe bioluminescence with the naked eye.

- If viewing in a darkened room, it can take up to 5 minutes for your eyes to adjust and for bioluminescence to be observable.
- If you can, capture a photograph of the plates with a camera. If using a cell phone camera, use "Night Mode" or "Night Sight" settings, if available.

Note: If there is condensation on the lids of your plates, observe them with the lids down (agar-side up) to ensure the condensation does not fall onto the plates.

2. In the diagram below, record where you observed growth and where you observed bioluminescence. You may also take photos of the plates using a cell phone camera.



3. Do you notice any interesting patterns in bioluminescence or growth?

4. Summarize your observations in the table below.

Plate	Strain	Does the strain produce bioluminescence?
4	□	
	Δ	
5	□	
	○	
6	Δ	
	○	

5. Make a claim about the identity of each of the strains. Use evidence from the analogy map, paper model, and plate observations to support your claim.

Strain	Identity	I think this because...
□		
Δ		
○		

Activity 3. Co-Plating with Inhibitor

To increase your confidence in the claims you made in Activity 2 about the identities of each *Vibrio* strain, repeat the co-plating experiment but this time, apply a chemical inhibitor of the Lux signal transduction pathway.

Part 1: Make Predictions

QStatin is a chemical inhibitor that blocks LuxR from activating production of LuxA and LuxB (Figure 7).

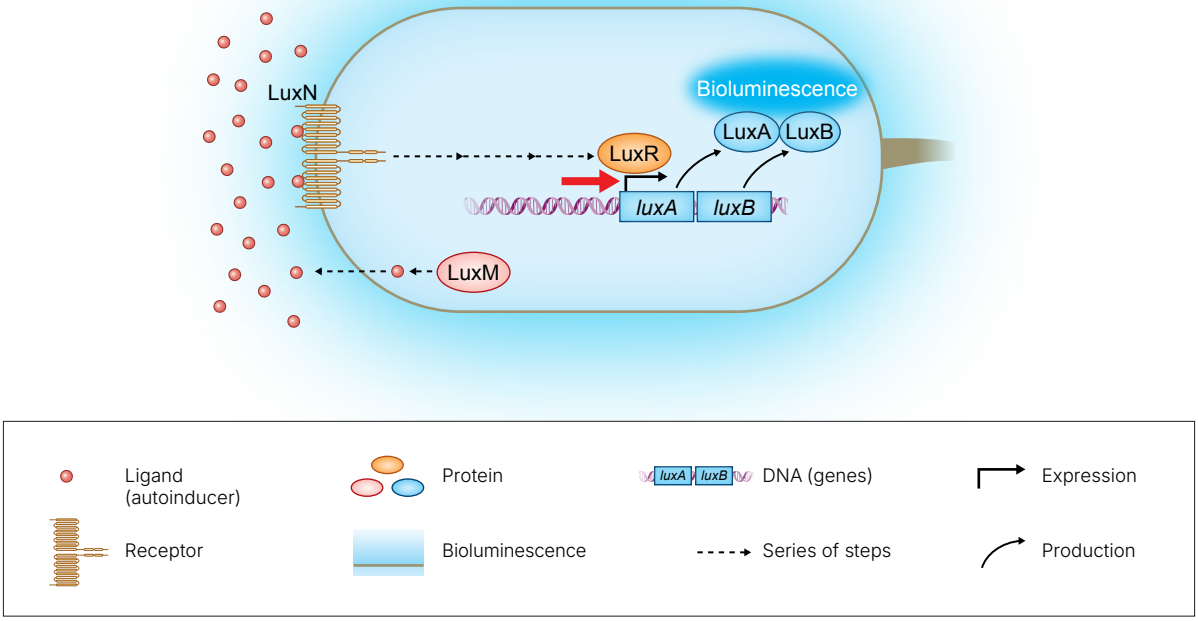
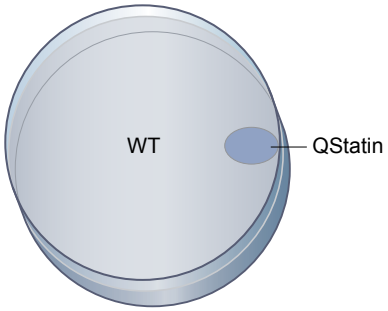


Fig. 7. Location of action of QStatin inhibitor. QStatin blocks the LuxR protein from initiating expression of the *lux* genes.

In the presence of QStatin, would you expect any of the strains (WT, *luxM*-, or *luxB*-) to produce bioluminescence?

1. **Use the paper model to predict the outcome of adding QStatin to the area around each strain. Be prepared to share your ideas.**

2. What would you observe if you added a small amount of QStatin to the edge of a plate containing the WT strain? Would you see growth? Bioluminescence? Draw your predictions below and explain your ideas.



3. In these investigations, QStatin is dissolved in a solvent (alcohol) before it can be added to the agar plate. The solvent is lethal if it is applied directly to the bacteria. With this additional information:

a. How might you design a control for the investigation described above?

b. What information would the control provide?

Part 2: Perform Co-Plating with Inhibitor

Student Workstation	
Material	**Quantity**
Student Guide	1
Paper model	1
Viewing box (or access to a dark space)	1
Plate templates	1
VA plates	3
Set of *V. campbellii* strains in microcentrifuge tubes (☐, Δ, and O)	1
QStatin (Q)	1
Solvent control (S)	1
Inoculation loops	6
20 µl adjustable-volume micropipet (or 10- and 20 µl fixed-volume micropipets)	1
Micropipet tips	1 box
Permanent marking pen	1
Biohazard waste collection	1

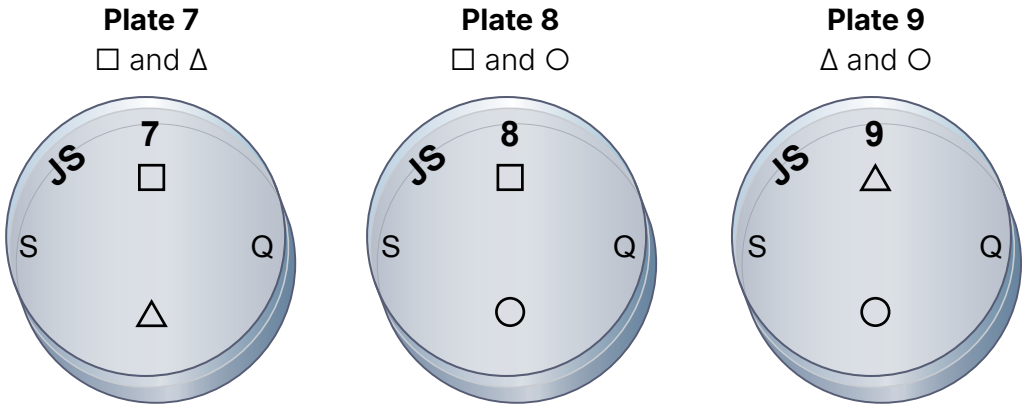
Also needed
Camera capable of dark imaging (for example, a cell phone camera)

Protocol

For this activity, you will use the same plating technique you used in Activity 2. Before you plate the bacteria, however, you will apply QStatin and a control to each plate, in the space between the two strains. Maintain ~4.0 cm distance between the solvent control and QStatin addition points (refer to the plate templates).

1. Label your plates "7", "8", and "9". On each plate, add your group designation or initials.

2. Using the plate templates, label the far-left side of the center of the plate "S" (for solvent control) and the far right with a "Q" (for QStatin). Then label where each strain will be added:



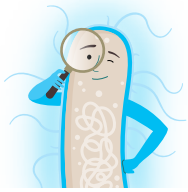
3. To plate 7, add 10 µl Solvent (S) on the indicated position. Repeat with plates 8 and 9.

4. Using a new tip, add 10 µl QStatin on the indicated position on plate 7. Repeat with plates 8 and 9.

5. Allow the spots to dry until you no longer see liquid (~10–15 minutes).

6. Once the solutions have dried, inoculate the plates as shown in Figure 8. Use 20 µl of the *Vibrio* strains and the same techniques you used in Activity 2. Try not to cross through the Q and S spots with the inoculation loops.

Focus Questions



Why is it important to:

- Apply the QStatin and control before inoculating the plates with *Vibrio*?
- Allow the QStatin and control to dry before inoculating the plates?

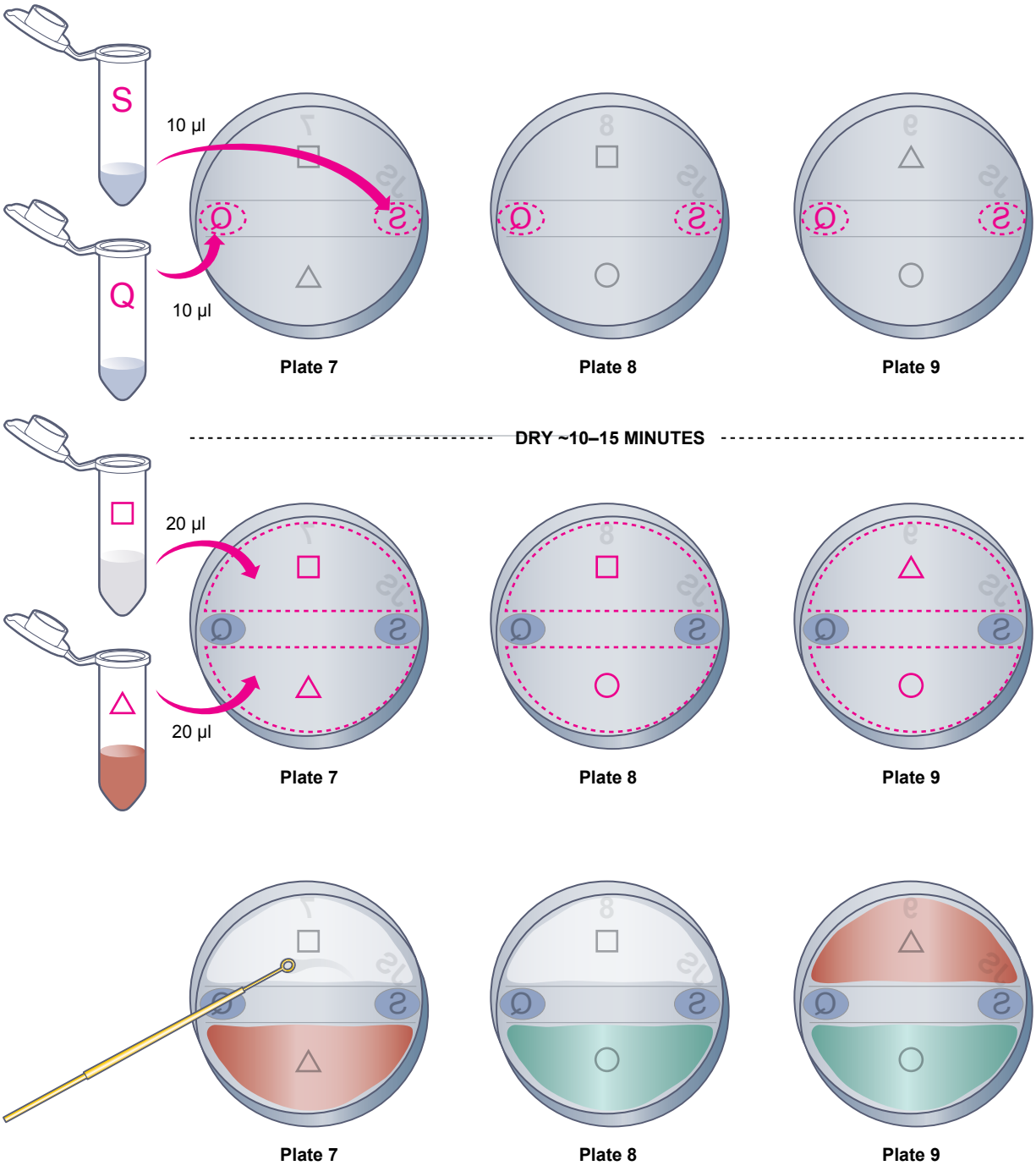


Fig. 8. Inoculation patterns for plates 7–9.

7. Allow the plates to dry (1–15 minutes), then place them upside-down and incubate them as instructed.

8. On the diagram below, predict where bioluminescence will be observed on each plate.

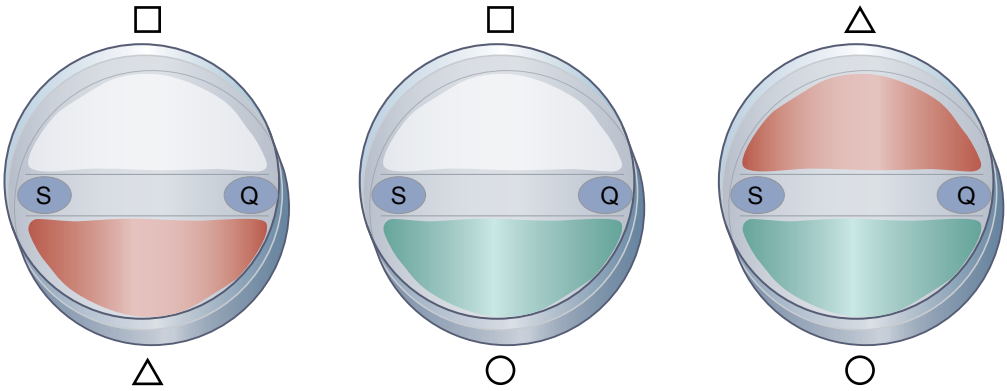


Plate 7 Plate 8 Plate 9

STOP **Stop**. Ask your teacher whether to stop now or proceed to Activity 4.

Part 3: Record and Analyze Results

1. **Record where you observe growth and where you observe bioluminescence. Use the techniques you used in Activity 2.**

   Record the results in the diagram below or take photos using a cell phone camera.

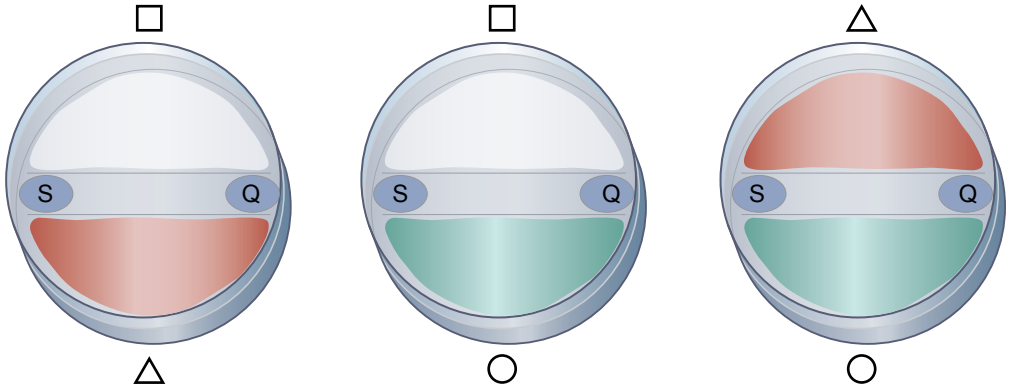


Plate 7 Plate 8 Plate 9

Note: If you examine the plates agar-side up (lids facing downward), the location of QStatin and solvent will be on the opposite sides of the plate than they were for plating.

2. Describe your observations in the table below.

Plate	Strain	Description of bioluminescence and growth
7	□	
	Δ	
8	□	
	○	
9	Δ	
	○	

3. Do your observations support your predictions and previous identification of the strains? Provide reasons why these observations support your identification (or not).

Activity 4. Student-Designed Co-Plating Investigation

In this activity, you will investigate a question of interest using one agar plate. You will have access to all three *Vibrio* strains, QStatin, and the solvent.

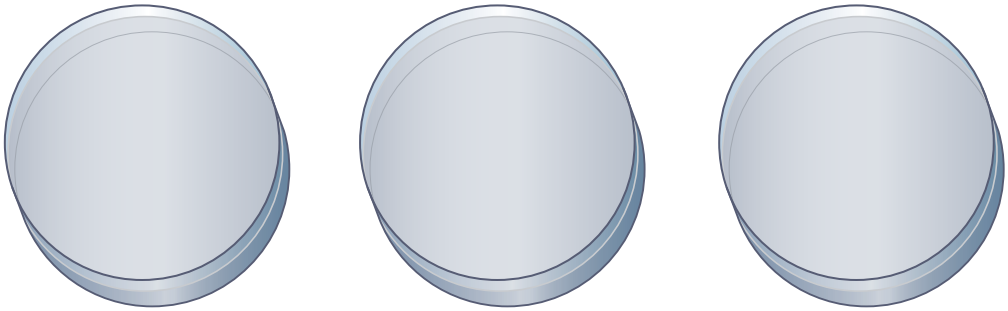
Part 1: Plan Your Experiment

Student Workstation	
Material	**Quantity**
Student Guide	1
Paper model	1
Viewing box (or access to a dark space)	1
Plate templates	1
VA plate	1
Set of *V. campbellii* strains in microcentrifuge tubes (☐, Δ, and O)	1
QStatin (Q)	1
Solvent control (S)	1
Inoculation loops	6
20 µl adjustable-volume micropipet (or 10- and 20 µl fixed-volume micropipets)	1
Micropipet tips	1 box
Permanent marking pen	1
Biohazard waste collection	1

Also needed
Camera capable of dark imaging (for example, a cell phone camera)

1. **With a partner or group, brainstorm several questions you could investigate with some or all the materials available to you. Record your questions below.**

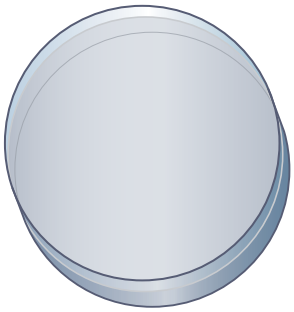
2. **For each question, draw a labeled sketch of how you would add materials to investigate your question. Be prepared to share your question and investigation design.**



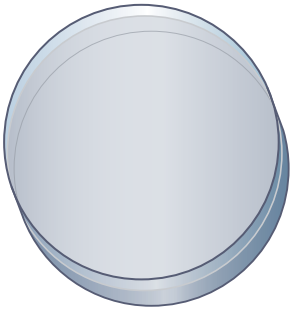
3. **Select and record the question you will investigate. Draw the investigation design below.**

Investigation question

Labeled sketch of investigation design



4. **Use your understanding of the Lux signal transduction pathway, quorum sensing, and the abilities and limitations of each *Vibrio* strain to predict the outcome of your investigation. Include a sketch of your prediction and your reasoning.**



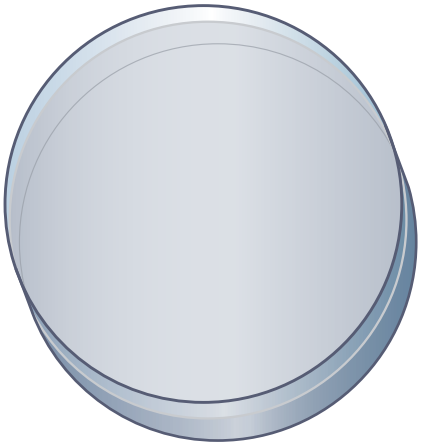
Part 2: Conduct Your Investigation

1. **Label the plate with your initials and the number "10" at the top of the plate.**

2. **Using the techniques you used in Activities 2 and 3, inoculate your plate and add the chemicals according to your design from Part 1. Record the volumes of each strain and/or chemical that you added.**

3. **Incubate your plate upside-down as instructed.**

4. **Retrieve your plates and record your results. Use the figure below to indicate where you observe bioluminescence.**



5. **Demonstrate your understanding of quorum sensing in *Vibrio* and the Lux signal transduction pathway by explaining your observations.**

Appendix A

Quorum Sensing: A Form of Bacterial Communication

This appendix provides background information from the TED Talk "How Bacteria "Talk"" by Dr. Bonnie Bassler (bio-rad.com/qs-talk).

Bacteria as Social Creatures

For a long time, biologists thought bacteria were microscopic, independent creatures that did not interact much with each other. But recent research has shown that bacteria can do some amazing things. They can sense changes around them, communicate with each other, and even work together to launch coordinated responses.

While bacteria can act alone, they can also detect other bacteria nearby and change their behaviors accordingly. These changes can be beneficial or harmful to other organisms. For example, bacteria can make toxin when they sense there are enough of them to be effective. They can also use group behavior to produce helpful chemicals and form beneficial relationships. Sometimes, they even collaborate to produce light, known as bioluminescence, which can lead to symbiotic or parasitic relationships with animals.

So, how do bacteria know when they are alone or in a group? How do they work together to coordinate the making of toxins or even the production of light? And why is this coordination beneficial?

These questions highlight the complex and social nature of bacteria, showing that they are far more interactive and cooperative than previously thought.

Bacteria and Bioluminescence

Researchers, including Bonnie Bassler at Princeton University and Julia van Kessel at Indiana University, have studied the phenomenon of bioluminescence in a marine bacterium called *Vibrio*. They have learned *Vibrio* can "speak" to each other using chemical "words" called ligands (or autoinducers). *Vibrio* make and secrete these small molecules, and when the bacteria are in low cell densities, the ligand molecules are also at low concentrations and just float away. However, when the bacteria are at high cell densities, the concentration of ligand molecules increases, and when they reach a threshold level, the bacteria all produce light — bioluminescence — at the same time.

If bacteria "speak" to each other using small molecules they release into their environment, how do they "hear"? The answer depends on the *Vibrio* species.

Some *Vibrio* species, like *V. campbellii*, which you will be using in these lab activities, have protein receptors on their cell surface. The shapes of the protein receptor and ligand fit together like a lock and key. Once the ligand binds to the receptor, a chain of chemical reactions ensues within the bacterial cell. The series of chemical reactions and the molecules involved in the reactions are collectively called a signal transduction pathway, and in this example, the result of these reactions is light. The pathway that produces bioluminescence in *Vibrio* is called the Lux signal transduction pathway.

Other species of *Vibrio* may produce bioluminescence through variations of this pathway. Regardless of the pathway, producing bioluminescent light is always an energy-intensive process. By working together to produce light together, *Vibrio* ensure that each bacterium invests energy to produce light only when there are enough individuals nearby to create a noticeable effect.

Quorum Sensing

Cell-to cell-communication is not limited to the Lux pathway and bioluminescence in *Vibrio*. Many other bacterial species can communicate with each other to turn on group behaviors that are only successful when a critical concentration of cells participates in unison. The scientific term for this coordinated response is quorum sensing.

In the case of *V. campbellii*, each bacterium "votes" by secreting ligand. When there are enough ligand "votes" (a quorum), all the bacteria receiving that signal respond. This allows a population of bacteria to have collective behaviors and conduct tasks that they could never accomplish as individuals alone.

In addition to bioluminescence, bacteria can use quorum sensing to become pathogenic (disease causing). One way that pathogenic bacteria make you sick is by secreting toxins, enzymes, or other molecules that damage your tissues or inhibit your immune responses. Whereas a few bacteria in your body could not secrete enough of these molecules to cause symptoms of infection, many bacteria all producing them at the same time may quickly overwhelm your body's ability to respond. This is what happens in the disease known as cholera, which is caused by *Vibrio cholerae*. Quorum sensing can also stimulate bacteria to produce enzymes that degrade antibiotics and to upregulate efflux pumps that rid the bacterial cell of antibiotics.

Quorum sensing can enable bacterial cells of the same species to communicate and coordinate activities with each other. In some species, the ligand and receptor molecules have a unique three-dimensional shape. These shapes confer languages unique to them, enabling "private conversations" understood only by others of the same species.

But bacterial species are rarely found by themselves; they live in diverse mixtures. So, can one species of bacteria communicate with another species? Can they "spy" on the conversations of other bacteria? They can!

Research has shown that some bacteria are, in fact, multilingual. Each species has a specific language as well as an interspecies language. Many bacteria can produce the same universal ligand and a corresponding universal receptor. In this way, each species can "count" how many bacterial cells are of their species and how many are of other species. With this information, they can coordinate and perform different processes, depending on which species are in the majority, and which are in the minority.

Implications of Understanding Quorum Sensing

Quorum sensing is a common feature in pathogenic bacteria that we currently treat with antibiotics. Antibiotics inhibit bacterial growth by disrupting their cell membrane, preventing replication of their DNA, or affecting protein synthesis or general metabolism. Antibiotic resistance is a growing health concern. Many species of bacteria are multidrug resistant. According to the Centers for Disease Control's 2019 Antibiotic Resistance Threats Report[3], every year, about 2.8 million people get infections that our current antibiotics can no longer treat; more than 35,000 of them die.

Could our understanding of signal transduction pathways and quorum sensing lead to new kinds of antibiotics[4]? How might we prevent bacteria from "speaking" or "hearing"?

In one example, researchers have developed species-specific or disease-specific molecules that have similar shapes to ligands. When these molecules bind with the bacterial receptor, they do not initiate a signal transduction pathway. Instead, the molecules attach permanently to the receptor, blocking the actual ligand from binding. In this way, individual bacterial cells act as if they are in low concentrations and do not produce the toxins that make us sick.

Molecules can be designed to block virtually any step along a signal transduction pathway. In addition, work being done to block universal ligands and receptors has raised the hope that these antiquorum sensing molecules could be used as broad-spectrum drugs that could work against all species of pathogenic bacteria.

Experiments in mice have supported the idea that antiquorum sensing molecules can be used as therapeutics. Mice injected with a pathogenic bacterium do not become sick until the bacterial population is large enough for quorum sensing to occur. Once quorum sensing is initiated, the mice become sick and die. When mice are injected with a multidrug-resistant pathogenic bacterium and antiquorum sensing molecules, more mice survive.

Researchers are also exploring proquorum sensing molecules that can speed up quorum sensing in bacterial populations to support our immune systems and keep us healthy. The study of quorum sensing also has applications for medical instrument safety, dentistry, agriculture, and even the safety of water and petroleum pipelines.

3 www.cdc.gov/antimicrobial-resistance/data-research/facts-stats/index.html

4 Rodríguez-Urretavizcaya B et al.(2024) Strategies for quorum sensing inhibition as a tool for controlling *Pseudomonas aeruginosa* infections, Int. J. Antimicrobial Agents 64.

Evolutionarily, quorum sensing by single-celled bacteria could be a precursor to multicellularity. By using quorum sensing, bacteria behave in many ways like a multicellular organism. Quorum sensing allows bacteria to distinguish their own species (self) from other species (other). A similar process allows differentiated cells, tissues, and organs in our body to communicate and function separately and in conjunction with other cells, tissues, and organs. Your heart and kidneys function as separate organs yet work in conjunction with each other to maintain homeostasis.

By studying bacteria and quorum sensing, we may gain insights about multicellularity in humans and other organisms.

Appendix B

The Lux Pathway in *Vibrio campbellii*

Many bacteria exhibit cooperative behaviors, coordinating their activities and working together. These behaviors are often regulated through a cell density-dependent, signal-mediated communication system known as quorum sensing and can include changes in pathogenicity or even the production of beautiful, blue bioluminescence.

In these lab activities, you investigated the Lux pathway of *V. campbellii*, which leads to bioluminescence when the bacteria are at high cell density (Figure 4). Here, we dive deeper into this response to describe how the signal (high concentration of ligand) is transmitted and turns the bacteria to glowing blue.

These details apply to the Lux pathway in *V. campbellii*. Other quorum-sensing pathways in other bacteria and even in other *Vibrio* species have pathways and regulatory mechanisms of their own.

The Basics

Using quorum sensing, *V. campbellii* only triggers the Lux pathway when there is a threshold amount of ligand available in the external environment (Figure 4).

Recall from Activity 1 and Figures 2–4 that signaling begins with the reception of a signal; that signal is transmitted throughout the cell, and the pathway ends in the production of bioluminescence. Bioluminescence is produced by an enzyme called luciferase.

Signaling Requires LuxM and LuxN

V. campbellii produces signaling molecules called ligands (autoinducers are another term used when speaking of quorum sensing), which are synthesized by the enzyme LuxM (Figure 2). As the bacterial population grows, the concentration of ligand increases in the environment. When the concentration reaches a critical threshold, it binds to the LuxN receptor on the bacterial cell membrane. This initiates a signaling pathway that ends with the bacteria producing bioluminescence (Activity 2 and Figures 3 and 4).

Bioluminescence Requires Both LuxA and LuxB (Luciferase)

You observed bioluminescence as the response in your experiments. Bioluminescence occurred when the luciferase enzyme was present in cells. Luciferase is made up of two subunits, the LuxA and LuxB proteins. Both subunits are required for luciferase activity (Activity 2).

LuxR — a Key Regulator

Production of LuxA and LuxB is tightly regulated by the LuxR protein. LuxR is a transcription factor, a protein that can bind to a piece of DNA and signal the cell's gene expression machinery to begin transcription. When LuxR is present, LuxA and LuxB proteins are produced, and the cell can produce bioluminescence (Figure 4). QStatin is a compound that inhibits the ability of LuxR to initiate production of LuxA and LuxB (Activity 3).

More Details

A more detailed view of the Lux pathway and quorum sensing is shown in Figure 9. It involves a sensor **kinase** (the LuxN receptor), which senses environmental changes, and a **response regulator** (LuxO), which modulates expression of other proteins, including LuxR. LuxN transmits signaling information to the response regulator LuxO via a **phosphorelay** mechanism. When LuxN is not bound to ligand, the LuxN kinase activity leads to phosphorylation of LuxO, and in this phosphorylated state, LuxO prevents the production of the LuxR protein. When LuxN is bound to ligand, the LuxN kinase domain switches to an inactive state and LuxO loses its phosphorylation. LuxR is produced and activates production of LuxA and LuxB.

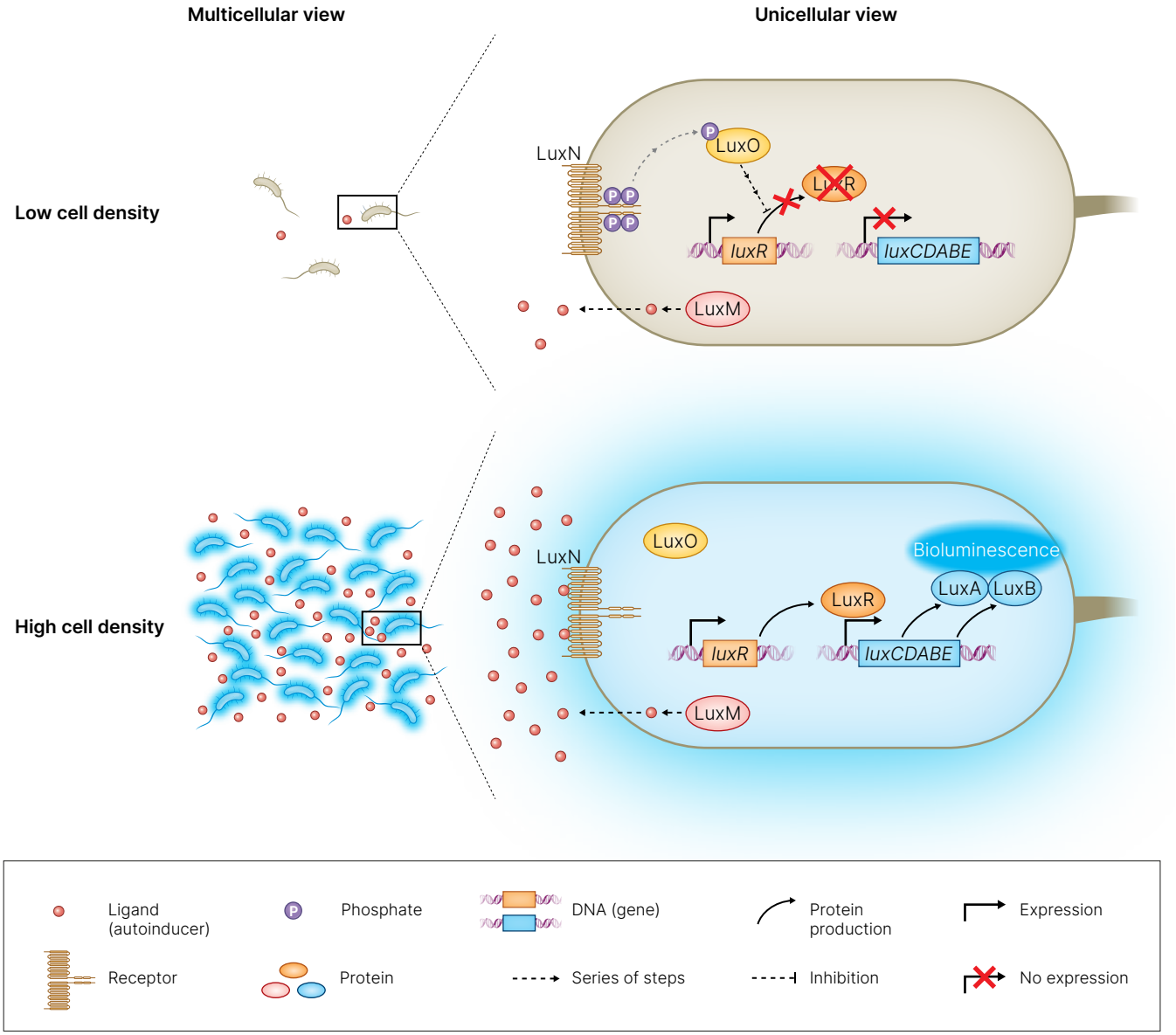


Fig. 9. Quorum sensing in *V. campbellii*. Note the involvement of LuxN as a receptor kinase that initiates a phosphorelay system that results in the phosphorylation of LuxO. Phosphorylated LuxO inhibits production of the LuxR protein. At high cell density, LuxN binds ligand molecules and shuts off the kinase activity, leading to dephosphorylation of LuxO, production of LuxR, and production of LuxA and LuxB proteins, which produce bioluminescence.

Lux Operon — the Bioluminescence Genes

In prokaryotes, an operon is a functional unit of DNA in which a cluster of genes codes for a set of functionally related proteins, and these genes are transcribed together from a single promoter into a single mRNA molecule. In *V. campbellii*, the lux operon includes the *luxA* and *luxB* genes that encode LuxA and LuxB, as well as other genes involved in the synthesis of the substrates required for the luciferase reaction.

In *Vibrio campbellii*, when LuxR is present, it binds to the lux operon promoter to activate expression of the lux genes, including those that encode LuxA and LuxB (Figure 9).

LuxO — the Regulator in Between

LuxO is a negative regulator of bioluminescence in the Lux pathway. LuxO can exist in a phosphorylated or nonphosphorylated state. When it is phosphorylated, LuxO acts with other cellular components to inhibit production of LuxR protein. When it is not phosphorylated, LuxO no longer inhibits LuxR production (Figure 9).

So, what determines the phosphorylation state of LuxO?

LuxN — a Receptor Kinase

The LuxN receptor in *V. campbellii* is a receptor kinase, meaning it can transfer phosphate molecules from ATP to other proteins.

- When there is no quorum and LuxN is not bound to the ligand, it acts as a kinase — it transfers phosphates to a phosphorelay system that phosphorylates LuxO. Phosphorylated LuxO inhibits production of LuxR (Figure 9)
- When there is quorum and LuxN binds ligand, its kinase activity stops — LuxO eventually becomes dephosphorylated (Figure 9)

Summary

In summary, the Lux pathway involves the binding of a ligand with the LuxN receptor. This binding triggers a stop to the phosphorelay system and the eventual dephosphorylation of LuxO. Once dephosphorylated, LuxO no longer inhibits LuxR production. LuxR is produced, and promotes the expression of genes responsible for bioluminescence, including the luciferase proteins LuxA and LuxB. The enzymatic reaction between LuxA and LuxB produces light, resulting in the beautiful bioluminescence you observed in *Vibrio* (Figure 9).

Appendix C

Glossary

Bioluminescence – light produced by a living organism through a chemical reaction, most commonly involving luciferin and luciferase, where energy is released as light

Cell signaling – process by which cells communicate with each other and sense their environment, transmitting information to coordinate cellular activities; also referred to as cell-to-cell communication

Co-plating – simultaneous cultivation of different bacterial strains or species on a solid culture medium, often an agar plate; technique used to study the interactions between or combined effects of different strains or species; also referred to as co-culturing or co-streaking

Homeostasis – process by which living organisms maintain a stable internal environment despite changes in external conditions

Kinase – enzyme that catalyzes phosphorylation, the transfer of phosphate groups from high-energy, phosphate-donating molecules like ATP to other proteins and substrates

Ligand – in cell signaling, refers to a molecule that binds to a specific receptor to initiate signal transduction

Luciferase – enzyme that catalyzes the production of bioluminescence in many organisms; in *Vibrio campbellii*, luciferase is made up of two component proteins: LuxA and LuxB

Lux pathway – the quorum-sensing pathway in *Vibrio* bacteria that results in the production of bioluminescence

LuxA – protein subunit of the luciferase enzyme in *Vibrio campbellii*; encoded by the *luxA* gene and works with LuxB to create a functional luciferase enzyme

LuxB – protein subunit of the luciferase enzyme in *V. campbellii*; encoded by the *luxB* gene and works with LuxA to create a functional luciferase enzyme

LuxM – protein in *V. campbellii* that is required for production of ligand molecules

LuxN – receptor protein in *V. campbellii*

LuxO – response regulator protein in the Lux pathway; in its phosphorylated state, prevents production of LuxR; binding of ligand to the LuxN receptor leads to dephosphorylation of LuxO and subsequent signal transduction and bioluminescence

LuxR – master regulator protein; in the Lux pathway, a transcription factor that regulates expression of lux operon genes

Master regulator – protein that controls the expression of other genes to orchestrate a specific cellular process or developmental pathway; in the Lux pathway, LuxR acts as the master regulator

Operon – in prokaryotes, a cluster of genes that is transcribed into a single mRNA molecule to enable coordinated regulation of related functions

Phosphorelay – signal transduction mechanism particularly common in bacteria, involving multiple steps of phosphoryl transfer to relay a signal from a sensor protein to a response regulator; a more complex variation of the simpler two-component system

QStatin – chemical inhibitor of quorum sensing in *Vibrio* bacteria that blocks LuxR from activating production of LuxA and LuxB

Quorum – minimum number of members of a group required to be present at a given meeting to transact business

Quorum sensing – cell-to-cell communication process used by bacteria to monitor their population density and coordinate their behavior

Reception – first phase of the triphasic cell signaling process; refers to the binding of a ligand to a receptor

Receptor – in cell signaling, refers to the protein that interacts with the signaling molecule (ligand) to initiate signal transduction; receptor proteins often occur on the cell membrane but can also be soluble, intracellular proteins

Response – third and final phase of the triphasic cell signaling process; refers to the cellular change that occurs

Response regulator – protein component of a two-component system that acts as a signal receiver and output device in prokaryotes; activated by phosphorylation from a kinase and then triggers downstream cellular responses, often by regulating gene transcription; in the Lux pathway, the LuxO protein is the response regulator

Signal transduction – second phase of the triphasic cell signaling process; refers to the series of molecular changes by which cells convert an extracellular signal into a specific cellular response

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