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Edvo-Kit #
935

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Battling Bacteria:

Ecosystem Dynamics in a Petri Dish

Experiment Objective:

By using bacteria with short generation times, students can observe, test, and collect their own data on key ecological phenomena in a week! In this experiment, students will create their own microbial ecosystems and investigate the dynamics of competition and change on community structure.

See page 3 for storage instructions.

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Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

Experiment Components

Components		Storage	Check (✓)
•	BactoBeads™ transformed with purple plasmid	4 °C	❑
•	BactoBeads™ *E. coli* chromogenic host	4 °C	❑
A	IPTG	-20 °C	❑
B	Ampicillin	-20 °C	❑
C	Nutrient powder	4 °C	❑
D	Salt	4 °C	❑

This experiment is designed for 10 groups of students.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

REAGENTS & SUPPLIES

Store all components below at room temperature.

- ReadyPour™ agar ❑
- Recovery broth ❑
- Petri dishes ❑
- Sterile loops ❑
- Microcentrifuge tubes ❑
- 50 mL conical tubes ❑
- 10 mL wrapped pipet (sterile) ❑

Requirements *(not included in this experiment)*

- Micropipettes
- Incubator
- Marking pens
- Bunsen burner, hot plate, or microwave
- Hot gloves
- Pipet pump or bulb

Background Information

Ecology - Life in Context

Often when considering a conversation or statement, we hear about the importance of context. That's because interpreting a comment or a sentence without the surrounding words or circumstances can be difficult or confusing. Something similar happens in biology. When studying living organisms, it is extremely important to consider the environment that they live in. Ecology is the branch of biology that examines the relations of organisms to one another (the living or biotic context) and to their physical surroundings (the non-living or abiotic context). This can be done on many different scales ranging from a single individual to the entire planet (Figure 1)!

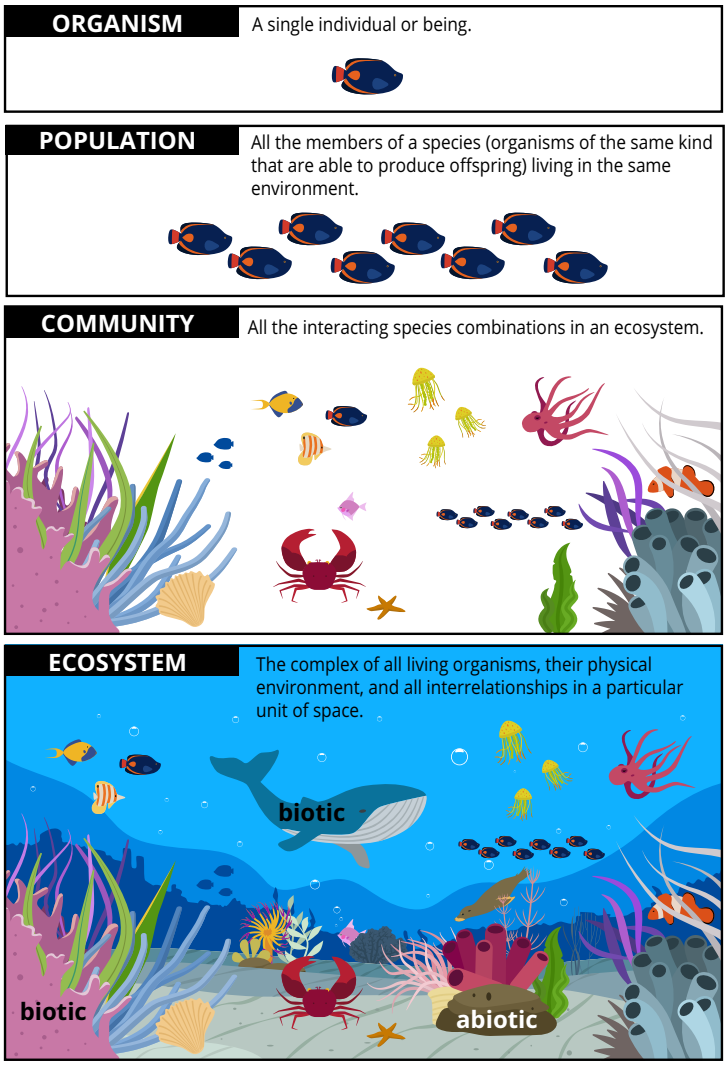


Figure 1: Key Levels of Organization in Ecology.

Organisms are influenced by the presence and activities of other organisms. These are the biotic factors of an ecosystem. Interactions between individuals can be both intraspecific - between members of the same species - and interspecific - between members of different species. They can also be both direct, such as when a prairie dog warns other members of its colony that a snake is nearby, and indirect, such as when a growing maple tree creates increased shade for other nearby plants. Ecologists study how individuals, populations, and species respond to the presence of other individuals, populations, and species. This research helps scientists better understand, explain, and even predict global patterns of survival and biodiversity. Table 1 lists some of the most commonly observed and well-studied biotic interactions as well as the effect they have on both parties involved.

TABLE 1: Common Biotic Interactions

Interaction	Effect
Mutualism	+/+
Competition	-/-
Predation	+/-
Parasitism	+/-
Commensalism	+/0

Anywhere an individual lives also has a physical environment that influences how they live and how successful they are. These non-living aspects of a location are called abiotic factors. Key abiotic factors include temperature, water availability, sunlight, wind, and soil composition. Abiotic factors are in constant flux and can change over time and vary by latitude, elevation, water depth, and climate. Such changes have a direct effect on the growth and survival of

individual organisms, the size of local populations, and, consequently, the size and composition of a community. When the scarcity of a specific abiotic factor reduces any of these it's called a limiting resource.

A species ability to live and thrive in a particular area is determined by both biotic and abiotic conditions. In turn, a species can influence and change the biotic and abiotic makeup of its surroundings. A niche is the role a species plays within an ecosystem. This includes all the factors a member of that species needs to survive and reproduce, how they use these resources, and how they alter the environment. For example, the niche of a seaside bird would include: its geographic range; when, where, and what it ate; what ate it (prey and parasites); all it's reproduction and nesting behavior; and what other species it competed against - to name a few. Needless to say, "niche" is a useful shorthand for the many complex aspects of a species!

Competition

When niches overlap, the result is competition. Competition is the struggle between individuals for a limited environmental resource. This struggle negatively affects both parties. Most competition between individuals or groups can be classified as interference, exploitative, or apparent (Figure 2). Interference competition occurs when one individual or species directly inhibits the foraging, survival or reproduction of other individuals or species. Exploitative competition occurs when one individual or species denies others access to a resource by consuming or claiming it first. The third type of competition is apparent competition. This occurs when two species are both preyed upon by the same predator. In this case, a population increase in one prey species causes an increase in the population size of the predatory species, which in turn, causes a population decline in the other prey species.

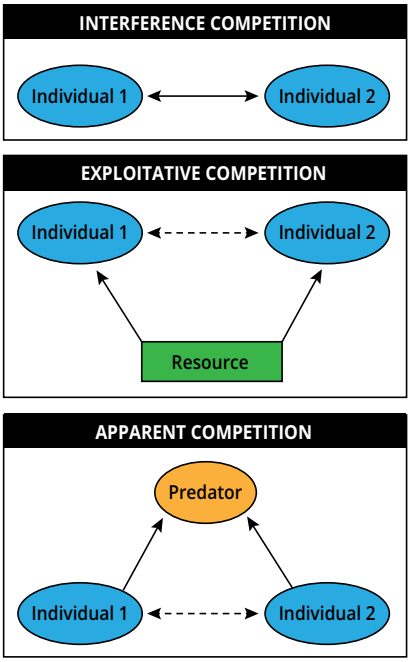


Figure 2: Types of Competition

In competition, individuals adversely affect others in their quest for food, living spaces, mates, and other common needs. However, the cost of competition is rarely equal. Rather one party is usually slightly better at obtaining the resources than the other. In such cases, the inferior competitor will - over time - experience a more pronounced reduction in growth and/or decreases in population size while the superior competitor will experience faster growth and/or increases in population size. The compounding effect of different competitive abilities leads to one of the key laws of ecology - that two species with identical niches cannot coexist together indefinitely. Rather one species will have a slight (or significant) advantage that will allow it to eventually dominate. This idea is known as the Competitive Exclusion Principle.

The Competitive Exclusion Principle was explored and illustrated by the Russian scientist G.F. Gauss (and is sometimes called Gauss's Law). Gauss conducted a series of test-tube experiments using two species of single-celled freshwater animals called paramecium (Figure 3). First, he grew both species alone in a test tube while adding food daily. Alone both species would grow until their populations hit carrying capacity - i.e. until their population size was limited by the size of the tube and the amount of food added. Next, he grew both species together. In the same tube, one

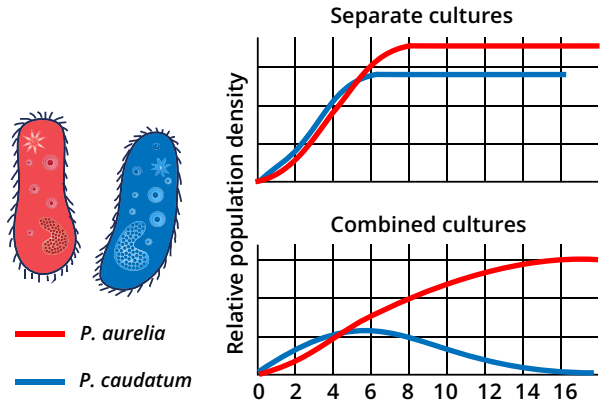


Figure 3: Gauss' Paramecium Experiment

species grew rapidly for the first few days but then began to decline while the second species grew steadily until it became the only species in the tube. Gauss observed a similar pattern when he grew two yeast species separately and then together. In addition, mathematical models of competitive interactions such as the Lotka-Volterra competition equations also predict the frequent extinction of one species when two similar species try to coexist.

While the Competitive Exclusion Principle has been demonstrated in experiments and is predicted in most species interaction models it is rarely observed in natural communities. For example, many species of plankton (microscopic and motionless plants and animals that drift in water currents) coexist in oceans and lakes even though their overlapping ecological niches would suggest that one species should dominate. A major goal in biology, and specifically in community ecology, is to resolve this disconnect and answer the question: if similar species cannot coexist—as is predicted by the Competitive Exclusion Principle—then why is so much diversity observed in natural ecosystems?

Getting Along and Creating Diversity

In nature, organisms can and often do increase their chance of survival by behaving in ways that minimize competition. This can be done by using subtypes of a limited resource, using shared resources at different times, or using the same resources but in different locations. While this resource partitioning can be behavioral, natural selection often reinforces such behavioral changes with morphological adaptations. For example, birds with overlapping niches can survive by specializing in a particular type of food (termites in a tree, seeds protected by a hard shell, fruit from a tree etc.) Over time this has led to the evolution of a huge diversity of bird beaks!

Limited resources can also be partitioned spatially. If you've ever slept in a small room with bunk beds then you've experienced how competition (in this case for sleeping space) can be reduced by looking up! *Anolis* lizards of the Caribbean island of Bimini use a similar strategy. These lizards all eat a similar diet of insects but coexist because each species forages and lives in different types of vegetation and/or at different heights (Figure 4).

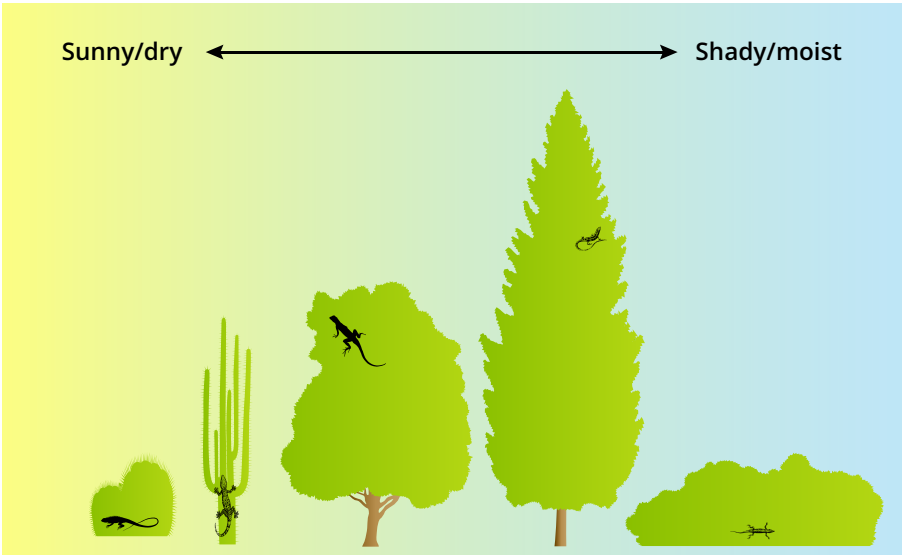


Figure 4: Resource Partitioning in *Anolis* Lizards

While resource partitioning reduces the pressure of competition it does have a cost. The specializing organism now has a smaller set of abiotic and biotic factors that it can effectively use. This narrower niche is referred to as it's "realized niche", while the wider set of possible conditions that the individual could originally thrive in is called its "fundamental niche". However, such reductions are not set in stone. In fact, one way to identify resource partitioning in the wild is to observe a character trait that is accentuated in places where a species overlaps with other similar species but is absent in places where the species exists alone.

The environment that plants and animals live in is also constantly changing, providing another way for individuals and species to diversify. These changes can range from the daily fluctuations in water temperature, salinity, and oxygen experienced in tidal pools to the gradual shift of an abandoned field from grassland to forest. In some cases, the source of the change is an abiotic (and often dramatic) event like a forest fire, hurricane, or volcanic eruption. In other cases, the sources of change are the individuals living in that environment. Two examples of biotic drivers of ecosystem change are a tree falling and creating a new light and nutrient rich patch of forest and beavers building a dam that in turn creates a new lake. Humans too are a major source of environmental change! We introduce pollutants into the ecosystem, cause climate change, and repurpose land for roads, agriculture, and homes. We also restore areas to their original state in a process known as ecological restoration.

Organisms respond to environmental changes in a variety of ways. Some organisms thrive on change. These "pioneering species" are plants and animals who have adopted a life history strategy of quickly colonizing new areas where there are few competitors rather than competing in well-established communities. Similarly, generalists - plants and animals with broad or flexible habitat requirements - are also adept at handling changes. For example, fox populations have been increasing in urban and suburban settings where humans are creating large and frequent environmental shifts.

In this experiment, you will be examining competition between two similar bacteria species. You will test how the initial population size of these two species affects their ability to compete, survive and reproduce in a controlled environment. Next, you will investigate how this community of two bacteria species responds to different types of environmental change.

Experiment Overview

EXPERIMENT OBJECTIVE

By using bacteria with short generation times, students can observe, test, and collect their own data on key ecological phenomena in a week! In this experiment, students will create their own microbial ecosystems and investigate the dynamics of competition and change on community structure.

LABORATORY SAFETY

1. Wear gloves and goggles while working in the laboratory.
2. The *E. coli* bacteria used in this experiment are not considered pathogenic. Regardless, it is important to follow simple safety guidelines in handling and disposing of materials contaminated with bacteria. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant. All materials, including Petri plates, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage.
3. Disinfect materials as soon as possible after use in one of the following ways:
   - Autoclave at 121 °C for 20 minutes. Tape several Petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.
   - Soak in 10% bleach solution. Immerse Petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR.

Wear gloves and safety goggles

LABORATORY NOTEBOOK

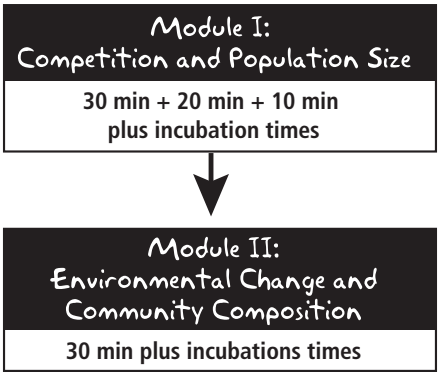
Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:
- Record your observations in your lab notebook.

After the Experiment:
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

MODULE OVERVIEW



Experiment Overview, continued

PRELAB QUESTIONS

1. Sort the following words into abiotic or biotic factors: grass, sunlight, earthworm, pollinator, pH, parasite, tree, rain, wind, mushroom, mountain, plankton, water current, fire. Brainstorm 1 or 2 additional factors to add to both lists.

2. Label the interactions below as either mutualism (+/+), competition (-,-), predation (+,-), parasitism (+,-), or commensalism (+,0).
   - A lion defending its hunting territory from a pack of hyenas.
   - Moose bulls sparring with their antlers.
   - A squirrel stealing seeds from a bird feeder.
   - A bear catching salmon in the river.
   - A rabbit eating grass.
   - A tree frog using a plant leaf to avoid drying out in the sun.
   - Clownfish and anemone living together and protecting each other from predators.
   - Flowers feeding bees and bees spreading the flower's pollen.
   - A tick living on a field mouse.

3. Define niche.

4. What does the Competitive Exclusion Principle predict will happen if two species live in the same area and have the same niche?

5. Describe at least two ways that species avoid competition.

Module I: Competition and Population Size

In this module, you will compare the competitive dynamics between two classroom safe E. coli strains (Box 1). Using the common microbiology techniques of bacteria plating and colony counting, you will compare the population size of both strains at the start of the experiment, immediately after they are first mixed together, after several generations of living together, and after several generations of growing in isolation.

BOX 1: THE COMPETITORS

Wild-type: A popular model organism used in many microbiology experiments due to it's fast generation time (about 15 min.) and its ability to survive in a range of conditions. Colonies of this strain will appear white on the provided plates.

Purple-type: A specialized strain that can create its own antibiotic defense and that produces a purple colored pigment when grown in the presence of IPTG. Producing these require additional energy. However, they also allow the strain to survive and to look beautiful.

MODULE I, DAY 1

1. **COLLECT** a purple-type bacteria tube, a wild-type bacteria tube, ten empty 1.5 mL tubes, a 50 mL tube of "recovery broth", three LB plates, and three sterile loops. ***NOTE: Label the tube of recovery broth with your group ID. Do not throw this tube away. You will use it tomorrow and in Module II.***
2. **DETERMINE** a starting ratio for the two strains that your group would like to test. For example, a 9:1 ratio would have 9 times more purple-type bacteria than wild-type bacteria, while a 1:1 ratio would have an equal volume of both strains. **RECORD** your starting ratio here: ____:____ (purple:wild) and **LABEL** a 1.5 mL tube with your group number and this ratio.
3. **PREPARE** your mixed sample with a final volume of 500 µL. To do this:
   a. **ADD** the two numbers you recorded in Step 2.

      _____
   b. **DIVIDE** 500 µL by this number. _____
   c. **MULTIPLY** the answer from Step 3b by the first number of your ratio. This is the µL volume of purple-type bacteria to add to the mixed tube.

      _____
   d. **ADD** this volume of purple-type bacteria to the tube.
   e. **MULTIPLY** the answer from Step 3b by the second number in your ratio. This is the µL volume of wild-type bacteria to add to the mixed tube.

      _____
   f. **ADD** this volume of wild-type bacteria to the tube.
   g. Invert the tube several times to **MIX**.

Example for Step 3 Using a 9:1 Ratio

a. 9:1 would be 9+1=10
b. 500 µL ÷ 10 = 50 µL
c. 50 µL x 9 = 450 µL purple-type bacteria
d. Add 450 µL purple-type bacteria to tube.
e. 50 µL x 1 = 50 µL wild-type bacteria
f. Add 50 µL wild-type bacteria to tube.
g. Invert the tube several times to mix.

Module I: Competition and Population Size, continued

4. **PREPARE** a bacteria sample for plating. (The bacteria grows so well that they need to be diluted to 1:100,000 before plating in order to see individual colonies!)
   a. **LABEL** three 1.5 mL tubes as M1, M2, and M3 for the three dilutions.
   b. **ADD** 900 µL of recovery broth to each tube.
   c. **TRANSFER** 100 µL of your sample from Step 3 to the tube labelled "M1". Invert or vortex to **MIX**.
   d. **TRANSFER** 100 µL of your sample from tube M1 to the M2 tube. Invert or vortex to **MIX**.
   e. **TRANSFER** 100 µL of your sample from tube M2 to the M3 tube. Invert or vortex to **MIX**.
5. **LABEL** a Petri plate "Mixed, Time 0". **TRANSFER** 50 µL of the bacteria mixture in the M3 tube into the middle of the plate. Using a sterile loop, **SPREAD** the bacteria all around the plate to cover the entire area. **INCUBATE** the plate for 5 minutes at room temperature and then **INVERT** the plate. **DISCARD** your M1, M2, and M3 tubes.
6. **REPEAT** Steps 4 and 5 for the purple-type and wild-type bacteria. **LABEL** the dilutions P1, P2, and P3 for the purple-type bacteria dilutions and W1, W2, and W3 for the wild-type bacteria dilutions. **LABEL** the plates "Purple, Time 0" and "Wild, Time 0". **USE** a new sterile loop each time.
7. **INCUBATE** the plates at 37 °C for 3 days.
8. **INCUBATE** the remaining mixed solution from Step 3 and the remaining wild-type and purple-type solutions from Step 1 overnight at room temperature. You will need them for Module I, Day 2.

MODULE I, DAY 2

9. **COLLECT** twelve empty tubes, your tube of recovery broth, three LB plates, and three sterile loops.
10. **RETRIEVE** your tubes of mixed, wild-type, and purple-type bacteria solutions from day 1. Overnight, the bacteria may have become concentrated in the bottom of each tube so **MIX** by inverting or vortexing briefly.
11. **PREPARE** the mixed bacteria sample for plating. (The bacteria need to be diluted even more after growing overnight.)
    a. **LABEL** four 1.5 mL tubes as M1, M2, M3, and M4.
    b. **ADD** 900 µL of recovery broth to each tube.
    c. **TRANSFER** 100 µL of your mixed sample from Step 4 to the tube labelled "M1". Invert or vortex to **MIX**.
    d. **TRANSFER** 100 µL of your sample from tube M1 to the M2 tube. Invert or vortex to **MIX**.
    e. **TRANSFER** 100 µL of your sample from tube M2 to the M3 tube. Invert or vortex to **MIX**.
    f. **TRANSFER** 100 µL of your sample from tube M3 to the M4 tube. Invert or vortex to **MIX**.
12. **LABEL** a Petri plate "Mixed, Time 1". **TRANSFER** 50 µL of the bacteria solution from your M4 tube to the middle of the plate. Using a sterile loop, **SPREAD** the bacteria all around the plate to cover the entire area. **INCUBATE** the plate for 5 minutes at room temperature and then **INVERT** the plate. **DISCARD** your M1, M2, M3, and M4 tubes.
13. **REPEAT** Steps 11 and 12 for the purple-type and wild-type bacteria. **LABEL** the dilutions P1, P2, P3, P4 and W1, W2, W3, W4. **LABEL** the plates "Purple, Time 1" and "Wild, Time 1". **USE** a new sterile loop each time.
14. **INCUBATE** the plates at 37 °C for 2 days.

NOTE:
White bacteria growth can be seen after 24 hrs. However, vibrant color differences will not show until ~48 hrs.

Module I: Competition and Population Size, continued

DAY 3

15. Continue to **INCUBATE** the Petri plates.

DAY 4

16. **RETRIEVE** all six plates (mixed, purple, and wild at times zero and one). **OBSERVE** or count* the number of white and purple colonies on each plate.

Plate	# of White Colonies	# of Purple Colonies
Mixed, Time 0		
Mixed, Time 1		
Wild, Time 0		
Wild, Time 1		
Purple, Time 0		
Purple, Time 1		

** If there are many colonies, divide each plate into four equal sections, count the colonies in one section, and multiply by four. If there are still too many colonies to count, label as ">500".*

17. (OPTIONAL) **GRAPH** your results.

Module II: Environmental Change and Community Composition

In this experiment, you will observe the response of both species to a changing environment. A mixture of both species will be grown in test tube solutions identical to Module I but will then be plated on Petri plates with different conditions such as added antibiotics, extra food, high salt, or a condition of your choosing.

DAY 1

1. **DECIDE** on an environmental change that you would like to test. Depending on your class, you may be choosing between an environment with an antibiotic stressor, a high nutrient environment, and a high salt environment OR you may be creating your own conditions.
2. **OBTAIN** an agar plate with your chosen environmental modification, three empty 1.5 mL tubes, one sterile loop, and your "recovery broth" from Module I.
3. **LABEL** the plate with your group ID.
4. **COLLECT** a tube of "mix" bacteria sample from your instructor. (This is a 1:1 solution of purple-type and wild-type.)
5. **PREPARE** the bacteria sample for plating.
   a. **LABEL** three 1.5 mL tubes as B1, B2, and B3.
   b. **ADD** 900 µL of "recovery broth" to tubes B1, B2, and B3.
   c. **TRANSFER** 100 µL of bacteria from the "mix" tube to the B1 tube. Invert or vortex to **MIX**.
   d. **TRANSFER** 100 µL of bacteria solution from the B1 tube to the B2 tube. Invert or vortex to **MIX**.
   e. **TRANSFER** 100 µL of bacteria solution from the B2 tube to the B3 tube. Invert or vortex to **MIX**.
6. **TRANSFER** 50 µL of bacteria solution from the B3 tube to the middle of the agar plate. Using a sterile loop, **SPREAD** the bacteria all around the plate to cover the entire area. **COVER** and **INCUBATE** the plate for 5 minutes at room temperature and then **INVERT** the plate.
7. **INCUBATE** the plates at 37 °C for 2 days.

DAY 2

8. Continue to **INCUBATE** the Petri plates.

DAY 3

9. **OBSERVE** or **COUNT** the number of white and purple colonies on the plate.
10. **COMPARE** your results with other groups.

Experiment Questions

1. In Module I, which species was most abundant on the Time 1 mixed plate? What does this tell you about the relative competitive abilities of both bacteria?

2. Compare your Module I results with other groups that used a different purple-type and wild-type ratio. Did this make a difference in the final mixed plate result?

3. In Module I why do you think you prepared a mixed plate immediately after mixing the bacteria? Why did you prepare pure purple-type and wild-type plates?

4. BONUS: Colorful animals are frequently observed in nature. Yet (similar to the bacteria in this experiment) these colors do not always help an organism eat or even survive. Why might they still be beneficial to an individual?

Instructor's Guide

EXPERIMENT TIMELINE

This lab is designed for a week long ecology section. A timeline is provided. However, tube incubation times can be shortened to as little as 1 hour, plate incubation times can be extended to 4 days at room temperature, and Module I and II can be performed on the same days. Consequently, many alternative schedules are possible.

The Week Before
- Prelab: Prepare LB agar plates for Modules I and II.

Monday
- Prelab: Prepare bacteria solutions (purple-type & wild-type) as close to class time as possible. Set incubator to 37 °C.
- Module I: Students create bacteria mix plus control samples and incubate overnight. Students create time zero plates and incubate for 3 days.

Tuesday
- Module I: Student create remaining 3 plates and incubate for 2 days.

Wednesday
- Prelab: Prepare a 1:1 BactoBead™ mix. Set incubator to 37 °C.
- Module II: Students plate bacteria in new environment and incubate for 2 days.

Thursday
- Module I: Students view and analyze Module I results.

Friday
- Module II: Students view and analyze Module II results.

OVERVIEW OF PRELAB PREPARATIONS

Preparation For:	What to do:	When:	Time Required:
Module I: Competition and Population Size	Prepare plates for Module I (and plates for Module II).	2-7 days before Module I	30 min.
	Prepare wild-type and purple-type bacteria solutions. Aliquot recovery broth.	Day of Module I	10 min.
	Bring plates to room temperature and set incubator temperature.	Day of Module I	5 min.
Module II: Environmental Change and Community Composition	Prepare mix bacteria solution.	Day of Module II	10 min.
	Bring plates to room temperature and set incubator temperature.	Day of Module II	5 min.

OPENINGS FOR INQUIRY

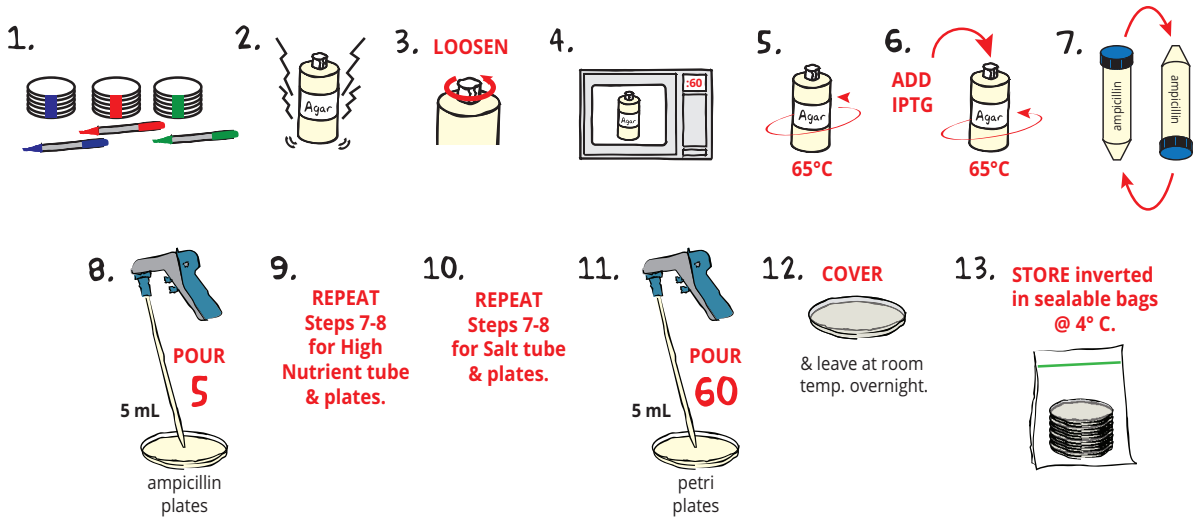
Module II is a great opportunity for students to create their own experiment! The prelab instructions below allow student groups to choose between three preset conditions (an environment with an antibiotic stressor, a high nutrient environment, or a high salt environment). However, students can brainstorm and then test all types of environmental conditions.

For Example:

- Students can select any type of powder or liquid prior to making the Module II Petri plates and incorporate these into the plates instead of—or in addition to—ampicillin, nutrient powder, and salt. Spices or powdered vitamins are two example.
- Students can add solutions that you have in your classroom—dish soap, food dye, orange juice, coffee, etc.—directly to the prepared plates and then allow the liquid to be absorbed overnight. For example adding 2 mL of vinegar creates a new high acidity environment.
- Students can vary the incubation conditions of their plates in ways that change the temperature, moisture, or light levels of the environment. For example, placing Petri plates in a sealed sandwich bag with a wet towel will create a high humidity environment.
- Students can introduce additional species. For example, *C. elegans* can be cultivated on LB plates and love to eat bacteria. Students can also leave a Petri plate at room temperature for an hour or two before the experiment to allow local bacteria to colonize the area before introducing the wild-type and purple-type mix. (Make sure that students wear gloves!)

Creating an open-ended experiment will require additional classroom time and modified prelab steps.

Preparing LB Agar Plates for Modules I and II



If your class is creating their own environmental conditions (Openings for Inquiry, page 16), Step 1 and Steps 7-10 may need to be modified.

1. **LABEL** 5 Petri plates "Ampicillin" either on the bottom plate or with a stripe using a colored permanent marker. **LABEL** 5 Petri plates "High Nutrient" either on the bottom plate or with a stripe using a different colored permanent marker. **LABEL** 5 Petri plates "Salt" either on the side of the bottom plate or with a stripe using a different colored permanent marker.
2. **BREAK** the bottle of solid ReadyPour™ LB Agar into small chunks by vigorously squeezing and shaking the plastic bottle.
3. **LOOSEN**, but DO NOT REMOVE, the cap on the ReadyPour™ Agar bottle. This allows the steam to vent during heating. ***CAUTION: Failure to loosen the cap prior to heating may cause the bottle to break or explode.***
4. **MICROWAVE** the ReadyPour™ Agar on high for 60 seconds to melt the agar. Carefully **REMOVE** the bottle from the microwave and **MIX** by swirling the bottle. Continue to **HEAT** the solution in 30-second intervals until the agar is completely dissolved (the amber-colored solution should be clear and free of small particles).
5. **COOL** the ReadyPour™ Agar to 65 °C with careful swirling to promote even dissipation of heat.
6. **ADD** the IPTG to the cooled ReadyPour™ Agar. **RECAP** the bottle and swirl to **MIX** the reagents. ***NOTE: The agar will quickly cool and solidify so work quickly during the next steps.***
7. **POUR** 30 mL of the IPTG ReadyPour™ Agar directly into the Ampicillin tube (Component B)—use the volume markings on the side to measure. **RECAP** and **MIX** by inverting the tube.
8. Using a fresh 10 mL pipet, **POUR** 5 mL of the Ampicillin-Agar mix into the 5 Ampicillin Petri plates.
9. **REPEAT** Steps 7 and 8 for the High Nutrient tube (Component C) and Petri plates.
10. **REPEAT** Steps 7 and 8 for the Salt tube (Component D) and Petri plates.
11. Using a fresh 10 mL pipet, **POUR** 5 mL of the remaining IPTG ReadyPour™ Agar into 60 unlabeled Petri plates.
12. **COVER** and **WAIT** for all the agar plates to solidify. For optimal results, leave plates at room temp. overnight.
13. **STORE** plates at 4 °C for up to two weeks. Plates should be inverted and placed in a sealable bag to ensure they do not dry out.

NOTE for Step 4:
Use extra care and make sure the agar does not boil out of the bottle. Pay close attention and stop the heating if it starts to bubble up.

Prelab Preparations

MODULE I

1. If plates have been refrigerated, **TAKE** them out and let them return to room temperature.
2. **LABEL** two 1.5 mL conical tubes "purple" and "wild" and **ADD** 1 mL recovery broth to each.
3. **TRANSFER** three wild-type BactoBeads™ (BactoBeads™ *E. coli* chromogenic host) to the "wild" 1.5 mL tube. **VORTEX** or invert to **MIX**. (The remaining BactoBeads™ should be left in the vial and placed in the refrigerator to be used for Module II preparations.) **REPEAT** for the purple-type BactoBeads™ (BactoBeads™ transformed with purple plasmid).
4. **LABEL** two 50 mL conical tubes "purple" and "wild" and **ADD** 40 mL recovery broth to each.
5. **TRANSFER** 40 µL of the solution from Step 3 to the "wild" 50 mL tube. Vortex or invert to **MIX**. **REPEAT** for the purple-type BactoBead™ solution.
6. **LABEL** ten 1.5 mL tubes "wild-type" and **TRANSFER** 1 mL of the "wild" solution from Step 5 into each tube.
7. **LABEL** ten 1.5 mL tubes "purple-type" and **TRANSFER** 1 mL of the "purple" solution from Step 5 into each tube.
8. **ALIQUOT** 23 mL of recovery broth into ten 50 mL tubes and **LABEL** as "recovery broth". These tubes will be used in *BOTH* Modules I and II so make sure that groups label them, keep them sealed whenever possible (to reduce contamination), and DO NOT throw them away before the end of Module II day 1.
9. **SET** an incubator to 37 °C.
10. *FOR DAY ONE*, **DISTRIBUTE** to each group: one 1.5 mL tube of "purple-type" bacteria, one 1.5 mL tube of "wild-type" bacteria, ten empty 1.5 mL tubes, one 50 µL tube of "recovery broth", three LB plates, and three sterile loops.
11. *FOR DAY TWO*, **DISTRIBUTE** to each group: twelve empty 1.5 mL tubes, three LB plates, and three sterile loops. Also, make sure each group has their tube of recovery broth and their incubated purple, wild, and mixed tubes from Module I, day 1.

FOR MODULE I - DAY 1
Each group will need:

- One "purple-type" bacteria tube
- One "wild-type" bacteria tube
- Ten empty 1.5 mL tubes
- One 50 mL tube "recovery broth"
- Three LB plates
- Three sterile loops

FOR MODULE I - DAY 2
Each group will need:

- Twelve empty 1.5 mL tubes
- Three LB plates
- Three sterile loops
- Their incubated purple, wild, and mixed tubes from day 1
- Their tube of "recovery broth"

MODULE II

1. If plates have been refrigerated, **TAKE** them out and let them return to room temperature.
2. **PREPARE** a 50 mL tube with 30 mL recovery broth and 2 purple-type BactoBeads™ and 2 wild-type BactoBeads™.
3. **LABEL** ten 1.5 mL tubes "mix" and **TRANSFER** 120 µL of the 1:1 BactoBead™ solution (from Step 2) into each tube.
4. **DISTRIBUTE** to each group: one 1.5 mL tube of "mix" bacteria, three empty 1.5 mL tubes, one environmental plate, and one sterile loop. Also, make sure each group still has their tube of recovery broth from Module I.

FOR MODULE 2
Each group will need:

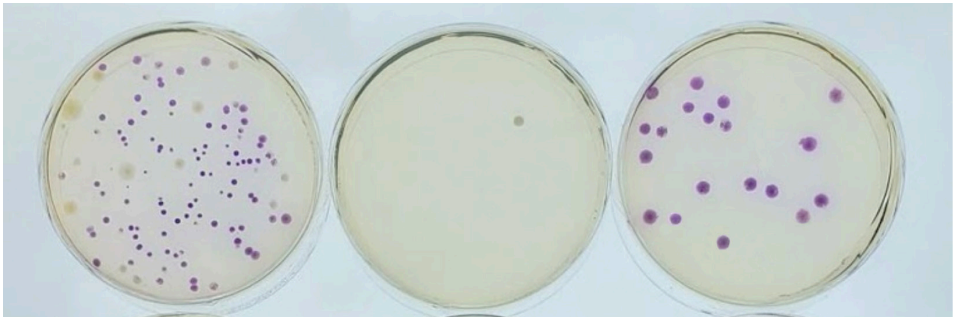
- One "mix" bacteria tube (1:1 mix)
- Three empty 1.5 mL tubes
- One environmental plate
- One sterile loop
- Their tube of "recovery broth"

Experiment Results and Analysis

MODULE I

Below are sample results using a starting 9:1 ratio of purple-type to wild-type.

Mixed, Time 0 Wild-type, Time 0 Purple-type, Time 0



Mixed, Time 1 Wild-type, Time 1 Purple-type, Time 1

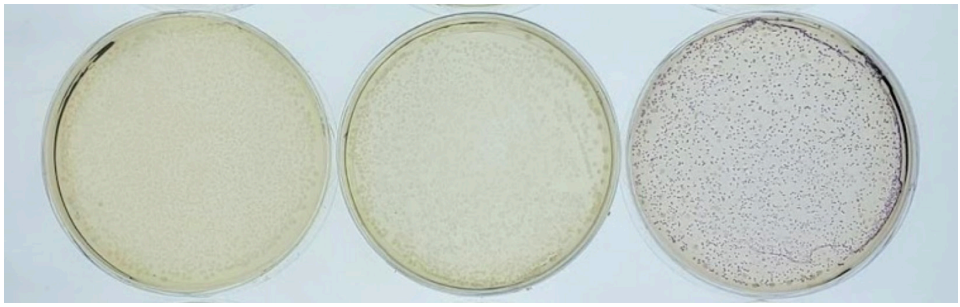
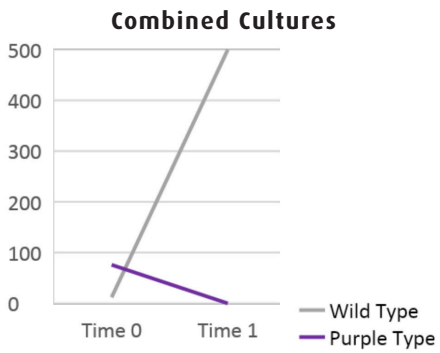


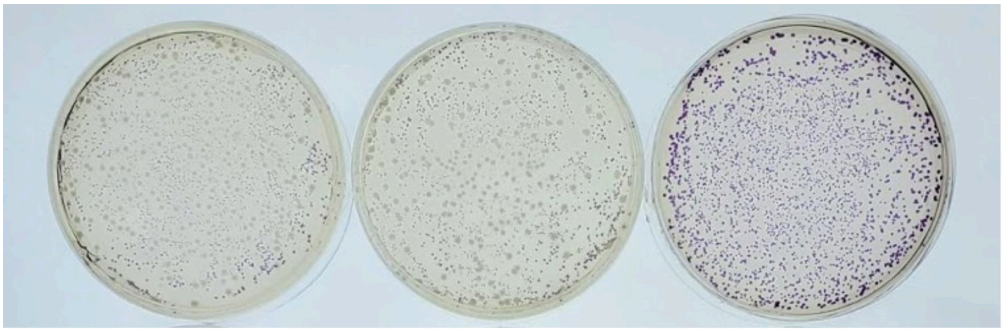
Plate	# of White Colonies	# of Purple Colonies
Mixed, Time 0	12	76
Mixed, Time 1	>500	0
Wild, Time 0	1	0
Wild, Time 1	>500	0
Purple, Time 0	0	20
Purple, Time 1	0	>500



Experiment Results and Analysis, continued

MODULE II

High Salt	High Nutrition	Ampicillin



Wild type: >500 Purple type: >500	Wild type: >500 Purple type: >500	Wild type: 0 Purple type: >500

Please refer to the kit insert for the Answers to Study Questions

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