



FRALIN LIFE SCIENCES INSTITUTE
VIRGINIA TECH™

Caging the Blob: Studying Slime Mold Behavior

INFORMATION MANUAL

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Fralin Life Sciences Institute

Virginia Tech

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Calendar of Activities Week 1: Slime Mold Guided Inquiry

Class schedule	Day 1	Day 2	Day 3	Day 4	Day 5
45–50 minute periods with no blocks	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Introduce lab (15 min) • Students design and construct mazes (20 min) 	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Pour agar into mazes and allow to dry (20 min) • Inoculate mazes with cultures on oats and wrap plates (20 min) 	<p><i>Only part of the block will be needed</i></p> <ul style="list-style-type: none"> • Remove inoculated oats from plates (10 min) • Place fresh oats at two ends of maze (5 min) 	<p><i>Only part of the block will be needed</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) 	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) • Answer lab questions (20 min)
45–50 minute periods plus one block (90–120 minutes)	<p><i>Block day</i></p> <ul style="list-style-type: none"> • Introduce lab (15 min) • Students design and construct mazes (20 min) • Pour agar into mazes and allow to dry (20 min) • Inoculate mazes with cultures on oats and wrap plates (20 min) 	<p><i>Regular day</i></p> <ul style="list-style-type: none"> • Remove inoculated oats from plates (10 min) • Place fresh oats at two ends of maze (5 min) 	<p><i>Regular day</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) 	<p><i>Regular day</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) • Answer lab questions (20 min) 	<p><i>Regular day</i></p> <ul style="list-style-type: none"> • Class discussion of questions (20–30 min) • Design of open inquiry experiment (20 min)
All blocks (90–120 minutes)	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Introduce lab (15 min) • Students design and construct mazes (20 min) • Pour agar into mazes and allow to dry (20 min) • Inoculate mazes with cultures on oats and wrap plates (20 min) 	<p><i>Only part of the block will be needed</i></p> <ul style="list-style-type: none"> • Remove inoculated oats from plates (10 min) • Place fresh oats at two ends of maze (5 min) 	<p><i>Only part of the block will be needed</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) 	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) • Answer lab questions (20 min) • Class discussion of questions (20–30 min) • Design of open inquiry experiment (20 min) 	

Calendar of Activities Week 2: Slime Mold Open Inquiry

Class schedule	Day 1	Day 2	Day 3	Day 4	Day 5
45–50 minute periods with no blocks	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Class discussion of questions (20 min) • Design of open inquiry experiment (20 min) 	<p><i>Some of the block will be needed</i></p> <ul style="list-style-type: none"> • Teacher approval and discussion with groups about experimental design (20 min) • Students gather and organize materials for experiment (15–30 min) 	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Students set-up and inoculate experimental and control plates (40 min) 	<p><i>Only part of the block will be needed</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) 	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) • Group discussion of results and conclusions (30 min)
45–50 minute periods plus one block (90–120 minutes)	<p><i>Block day</i></p> <ul style="list-style-type: none"> • Teacher approval and discussion with groups about experimental design (20 min) • Students set-up and inoculate experimental and control plates (45–60 min) 	<p><i>Regular day</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) 	<p><i>Regular day</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) • Group discussion of results and conclusions (30 min) 	<p><i>Optional: Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Group presentations of experiments 	
All blocks (90–120 minutes)	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Teacher approval and discussion with groups about experimental design (20 min) • Students set-up and inoculate experimental and control plates (45–60 min) 	<p><i>Only part of the block will be needed</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) 	<p><i>Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Observe plates, take pictures, make measurements (15 min) • Group discussion of results and conclusions (30 min) 	<p><i>Optional: Most of the block will be needed</i></p> <ul style="list-style-type: none"> • Group presentations of experiments 	

Introduction To The Slime Mold Kit

The Slime Mold Kit from the Fralin Life Science Institute contains the materials need to test the ability of *Physarum polycephalum* to navigate a simple maze made of Lego® blocks. Teachers will need to provide alcohol and materials for the open inquiry part of the investigation. The kit is available from the Center for a 3-week loan period.

Few laboratory exercises are designed to teach biology students about barriers that may constrain the movement of organisms. Here, we describe a unique inquiry-based exercise involving Lego® mazes (the barrier) and the plasmodial slime mold, *Physarum polycephalum* (the organism). During guided inquiry, students construct mazes using Lego® blocks and the slime mold is allowed to ‘navigate’ through the maze and ‘respond’ to the barrier. Students then generate and test hypotheses about the movement of the slime mold in response to different barriers in the open inquiry phase of the investigation.

This manual is divided into three sections. The first section includes a calendar of activity, a list of kit contents, and a section introducing the concept of barriers and the basics of *P. polycephalum*. The instructor can decide the complexity of the material to be presented to the students.

The second section is the experimental section, containing an overview of each part of the experiment, teacher notes, and the student experimental procedure.

The final section is the Appendix, which contains everything else, including student questions (and some answers), pictures of student mazes, an example of an open-inquiry experiment, correlations to Virginia SOLs, and references.

If you come up with any other ideas or study questions, please send us a copy and we'll incorporate them in the manual as well.

Feel free to call Dr. Kristi DeCourcy at Fralin or Cindy Bohland with any questions about the material. Corrections and suggestions will be gratefully received. Kristi's phone is (540) 231-7959 (email is decourcy@vt.edu). Cindy's e-mail is cbohland@rvgs.k12.va.us.

Slime Mold Kit Contents

Description	Quantity
Slime mold sclerotia (foil packet)	1
Oat flakes (Ziploc bag)	1
Agar [#]	20 g
Parafilm [®]	1 box
Aluminum foil	1 roll
Forceps	8
Gloves	1 box
Pipette pump (green, 10-ml)	2
10-ml serological pipettes, sterile	10
Thermal grippers	1
Mister bottles	2
Lab markers	4
Lego [®] set*	1
Sieve (for clean-up)	1
Petri dishes, 100 mm, 25/sleeve	2
Prepoured 100 mm Petris [§]	2
Digital camera	1
Extra cable ties	2

[#] This agar is granulated and coarse, not like higher grade agar or agarose. It is brownish in color, not white.

* Lego[®] set includes 176 blocks in six sizes, 48 corner plates, and 96 plates in three sizes. See page 31 in the manual for more details.

[§] store in refrigerator at 4 °C until needed.

Materials/equipment to be supplied by the instructor

Alcohol (70% isopropyl or ethanol will work)

Distilled water

Microwave or autoclave for agar prep

Water bath (preferred) or hot plate stirrer for holding molten agar until needed

Flask or bottle for agar prep

Bleach (10% solution, for cleanup)

Materials for open inquiry experiments

Safety Notes

Slime Mold Cultures

Physarum polycephalum is non-pathogenic and safe for use in the biology classroom. While working with slime molds, students should practice sterile technique to prevent contamination and growth of unknown microorganisms that could possibly be harmful. Students should not be allowed to open any contaminated plates. Students should also adhere to general good laboratory practices. All students should wash their hands at the conclusion of the lab, and food and drink should not be permitted in the laboratory. At the conclusion of the experiment, the slime mold can be disposed of by soaking Petri dishes in a solution of 10% bleach or by autoclaving. The dishes can then be placed in the general trash.

Basics tips for using sterile technique

1. Wipe lab benches down with 70% isopropyl alcohol before and after working with slime mold.
2. Use sterile forceps when transferring slime mold or food. Forceps can be wrapped in foil and sterilized in an autoclave or can be sprayed with 70% isopropyl alcohol. Never touch a surface with forceps or allow a sterilized instrument to be exposed to air longer than necessary. Sterilize forceps after use.
3. Never remove the lid of a Petri dish and place that lid on a lab bench surface. Always open plates by using one hand to crack and hold the lid, giving just enough space to work (with the other hand), while allowing as little air as possible into the dish.

Molten Agar

Molten agar for maze experiments may be kept slowly stirring on a hot plate at 40–50°C or held in a 50–60°C water bath. Students should take care in pipetting agar from this flask.

If you chose to prepare agar ahead of time and then melt it down before lab (not recommended), use extreme caution in melting agar in the microwave. Use 50% power and melt in short bursts. Agar heated in the microwave may become superheated and bubble and spill over when you swirl the container, so allow the agar time to cool before handling flasks from the microwave.

Chemicals

Due to the open-ended nature of this activity, there are many different kinds of chemicals with which the students may choose to work. Some are obviously safe (such as sodium chloride), but other may not be. Teachers should have students find and print the Material Safety Data Sheets (MSDS) for the chemical chosen for the experiment. Information from the MSDS can be used in deciding whether or not to use a chemical and can be used as a guide for proper disposal.

Introduction

In this two-part inquiry-based lab, students use slime molds to understand the effects of barriers on the movement of organisms. During guided inquiry, students learn to work with the plasmodial slime mold (*Physarum polycephalum*) and construct mazes using Lego® blocks as a barrier. During open-inquiry, students design their own experiments to test barriers they predict will be effective at controlling the movement of slime mold. This investigation is a fun way to introduce scientific method or can be integrated through content areas such as cell biology, ecology, and behavior of organisms.

Movement of Organisms: What's the big deal?

Organisms move from one place to another through land, water, and/or air. Understanding the ways in which organisms utilize land, water, and air to move from place to place is essential knowledge in studying ecosystems. Sometimes, the movement of a population of organisms into a new space causes diseases to other organisms or damage to the ecosystem. For example, the zebra mussels that moved through ballast water into the Great Lakes in the 1980s have caused extensive ecological and economic damage (Miller & Spoolman 2009). The creation of barriers to prevent the movement of exotic or disease-causing species seems a reasonable solution to this problem. For example, ships are advised to take measures to avoid transporting exotic species in ballast water, such as exchanging water only in designated zones (Grodowitz 2002). This creates a barrier to the movement of aquatic organisms, but only if every ship always complies with these voluntary recommendations.

The creation of barriers to prevent the movement of exotic or disease causing organisms becomes an even more complex issue when organisms can move through the air. Microorganisms that can travel great distances through the atmosphere are responsible for many plant diseases. The Irish Potato Famine, a devastating episode in history that resulted in the deaths of over one million people, was caused by an airborne plant pathogen known as *Phytophthora infestans* (Schumann et al. 2000). This fungus-like organism produces small lemon-shaped spores that may be transported through the air to healthy potato fields (Aylor et al. 2011). If scientists can understand how plant pathogens travel through the air, then they can find ways to control the movement and minimize the damage of these organisms to the crops on which we depend. Part of understanding this movement involves understanding barriers to the movement (Isard et al. 2001). For example, a mountain range might create a barrier, effectively isolating a population of microorganisms. Or other factors, such as severe weather (e.g., hurricanes), might diminish barriers and accelerate the spread of a potentially devastating disease to crop plants across the country (Isard et al. 2005). Subtler atmospheric phenomena, perhaps due to the long-term effects of global climate change, can also lead to a dynamic landscape of “invisible” atmospheric barriers (Lekien & Ross 2010), leading to changes in cyclic patterns of large-scale movements of microorganisms.

Slime molds

Many of us are familiar with slime molds through the seemingly overnight appearance of yellow or orange “slime” on a mulch pile or in a garden bed. Slime molds are not actually molds or even fungi; rather, they are classified as amoeboid protozoans. Slime molds exist in two forms:

- plasmodium (pl. plasmodia) – a streaming packet of cytoplasm containing many nuclei
- sclerotium (pl. sclerotia) – a dry structure. In this form, the slime mold can survive environmental extremes until conditions are favorable for growth.

This exercise makes use of a plasmodial slime mold known as *Physarum polycephalum*. In the plasmodial stage, *P. polycephalum* exhibits an amoeboid type movement and is known to respond to a variety of environmental stimuli, including light, physical barriers, food sources, and chemical repellants (Adamatzky 2010; Latty & Beekman 2010; Nakagaki et al. 2007; Nakagaki et al. 2000). This response can be easily observed with the naked eye as the plasmodium grows, creating new pseudopodia and thickening tubes in some areas and shrinking back pseudopodia and allowing tubes to die in other areas (tubes that have died appear white rather than yellow). As more materials are exchanged between two parts of a tube, the tube thickens and is reinforced. As fewer materials are exchanged, the tube thins and eventually dies (Nakagaki et al. 2007).

Slime molds have been used in diverse fields of scientific research. Ecologists have used slime mold to study optimal foraging strategies (Latty & Beekman 2010) and computer scientists have used slime mold to build a robot controlled by the movement of the slime mold (Knight 2006). The student activities described in this manual were inspired by two clever experiments that demonstrate the “primitive intelligence” of *P. polycephalum*. Nakagaki et al. (2000) placed *P. polycephalum* in a maze created by cutting out a pattern of plastic film. They showed that after filling the maze, *P. polycephalum* shrinks its plasmodium so that it connects two food sources placed at two ends through the shortest distance of the maze (a time-lapse video of the experiment can be viewed at http://www.youtube.com/watch?v=F3z_mdaQ5ac). Tero et al. (2010) used *P. polycephalum* to model the Tokyo rail system. By placing food in areas of concentrated human populations and by shining light to restrict growth to model areas of natural barriers (mountains, lakes, and oceans), they showed that *P. polycephalum* forms an efficient network of rail-lines. The design of both of these experiments is contingent upon the ability of *P. polycephalum* to sense and respond to barriers in its environment.

Barriers

There are three general types of barriers to the movement of organisms: static, invisible, and dynamic. Static barriers are solid, physical entities. In the guided inquiry part of this experiment, Lego[®] blocks are used as static barriers to impede movement. The maze experiment described by Nakagaki et al. (2000) used plastic film as a static barrier. The rail system experiment described by Tero et al. (2010) used light as an invisible barrier (slime molds grow away from light). The term “dynamic” describes barriers that are changing; for example, solid barriers can either move slowly and smoothly or they can be added or removed abruptly. Barriers also can be both dynamic and invisible. For instance, an area can be lighted at one time, then dark at another time. Of course, in nature, all three of these types of barriers exist at once and organisms that move must have mechanisms that allow them to sense and respond in a way that maximizes survival and reproduction. In the open inquiry part of this experiment, students choose, design, and create a barrier to impose on *P. polycephalum*.

Guided Inquiry: Slime Molds in Mazes

Instructor's Preparation

These are the things that the instructor needs to prepare for the experiments. Notes and detailed directions are below.

1. **Start slime mold cultures.** Start the slime mold cultures from the provided sclerotia. Do this as soon as possible after the kit arrives, so that you will have plenty of oat flakes well colonized with slime mold by the time the students need them. Two agar plates are provided in the kit, so you can start the growth immediately.
2. **Prepare water agar.** The experiment uses agar in water, with no additional nutrients added. The water agar will be used to culture the slime mold and to prepare the student mazes.
3. **Fill mist bottles.** The mist bottles need to be filled with 70% isopropyl alcohol (provided by instructor) for sterilization of forceps and mazes.

Instructor's Notes

1. The slime mold used in this experiment (*Physarum polycephalum*) grows readily over uncooked oat flakes. *P. polycephalum* covered oats are yellow in appearance and can be used as an easy way to transfer the slime mold from one place to another. A covered oat flake can be picked up with forceps and placed slime mold side (yellow-side) down on the new surface. Unlike yeast or bacteria, you cannot simply scrape *P. polycephalum* from one plate and spread it on another plate.
2. The agar in this kit is a coarse, granulated agar. It is light brown in color, so it does not look anything like agarose or Bacto Agar. The container of agar included in the kit may be labeled "Slime Agar" for us to distinguish it from agarose.
3. Sterilize forceps with alcohol. Since the forceps provided in the kit are plastic, please do not flame them.
4. Be certain that your students know how to seal their plates with Parafilm[®]. It may help to give them pre-cut strips the right size for sealing plates.
5. Since slime molds eat bacteria, students need not have perfected sterile technique for this experiment. However, it is still a good idea to have them think about and practice sterile technique during this experiment.
6. Slime molds will grow well at room temperature or in a 25°C incubator.
7. It is important to keep *P. polycephalum* in the dark (wrap plates with aluminum foil) to avoid inducing spore-formation. (It is okay to use light as a variable in the open-inquiry part of the experiment.)
8. For the guided inquiry part of this experiment, it is helpful to have some non-sterile Petri dishes that students can use to test the dimensions of their mazes.
9. Lego[®] blocks: For instructors planning to use this kit with more than one classes, sets of Lego[®] blocks in different colors have been provided. Please keep the sets separate, if at all possible. It would make it very difficult for us if we had to separate the sets of blocks between the loan periods.

10. Lego® maze height: For the 100-mm Petri dishes included in this kit, the maximum maze height is one Lego® block plus one Lego® plate. With that height, the lid will still fit on the Petri dish. If the student mazes are taller than that, plastic wrap (not included in kit) could be used to cover the Petri dish.

Other dishes are available from supply houses, such as deep dishes, but they cost considerably more than standard depth Petri dishes. Square dishes are also available, and can be used to construct larger mazes.

Documentation

There are several ways that students can document their progress and results of this experiment.

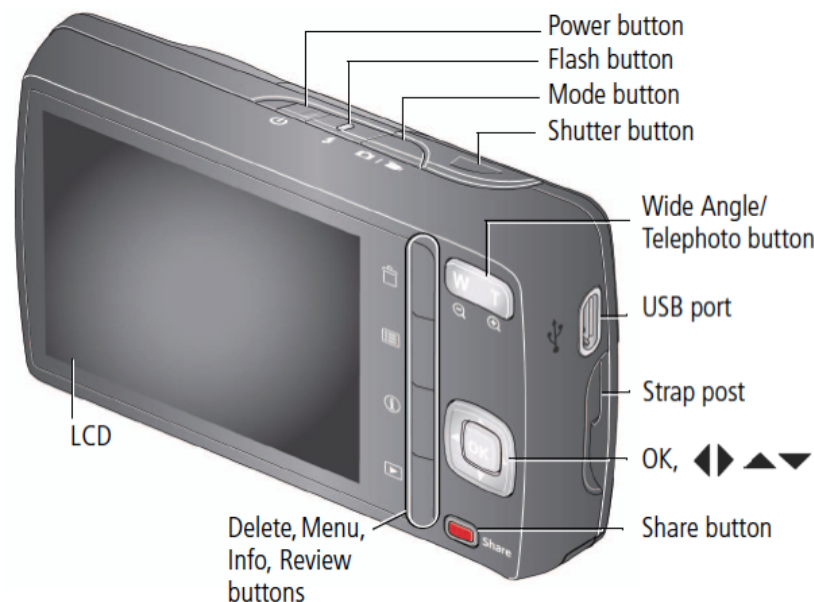
Growth of the plasmodia can be documented with photographs. A digital camera is provided in the kit, and most teachers and students have cell phone cameras. It is often easier to photograph the growth from the bottom of the plate.

Time-lapse photos of changes in the plasmodia can be made into movies. See Extension Activities on page 28 for more information. See Cynthia Bohland's time lapse videos at <http://www.schooltube.com/user/slimemold>.

Camera Directions

The camera included in the kit is a Kodak EasyShare M530, with a 4 GB memory card, USB cable, and wall plug. It should be pretty easy to use, but the link to the manual is at resources.kodak.com/support/pdf/en/manuals/.../M530_xUG_GLB_en.pdf

If you have some tech-savvy students, they may know how to use the Share button to upload to Facebook, etc. Otherwise, pictures can be uploaded to a computer using the USB cable (connect with camera off, then power up camera). If it is the first time you've connected the camera, you will be prompted to install the software.



Instructor's preparation: Water Agar

Water agar at 2% works best for this experiment. (There is less movement of the slime mold on 1% agar.)

- For each 100 ml of 2% water agar, add 2 grams of agar to 100 ml of distilled water. Use a container that will tolerate heat, a bottle or flask. The agar has to be heated to dissolve, so do not swirl or stir the flask at this point.
- Heat to dissolve the agar.
 - If you have an autoclave available, autoclave agar solution in an unsealed container on a liquid cycle.
 - To prepare in a microwave, heat water and agar in a flask or uncapped bottle until agar is dissolved. See suggested procedure in box below. Note: Be very careful handling superheated agar solution. (See Safety Notes on page 7.)
- Hold the molten water agar in a water bath at 40–60°C until needed. Alternatively, use a stirring hot plate to maintain the agar at 40–50°C.

Volumes needed:

- For culturing slime mold in Petri dishes, allow 25 ml per each 100 mm Petri dish and 10 ml for each 60 mm Petri dish.
- For agar for mazes in 100 mm Petri dishes, a generous estimate is 10 ml of agar per maze.
- For larger dishes, estimate volume needed by filling first with water.

KD suggestion for dissolving agar in the microwave:

- When I prepared 100 ml of 2% agar, I started with 2 g of agar in 100 ml of distilled water in a 500 ml flask. I used a lab marker to mark the volume on the flask, then added another 50 ml of distilled water. Do not swirl the flask, as it won't help and will put agar on the sides of the flask.
- I heated the flask in the microwave on high for about a minute (long enough to heat the water to near boiling, but not to boiling).
- Then I microwaved on medium power for 1-minute intervals, watching the flask carefully to be sure it didn't boil over. (My microwave is older — you may have to go lower than medium power on newer microwaves.)
- After each minute, I removed the flask using thermal grippers and swirled lightly, looking for undissolved agar particles (watch out for superheating!).
- In my microwave, it took about 5 minutes to get it all dissolved. With the evaporation during heating, the final volume was close to the mark I had made for 100 ml. It can be a little off without a problem.

Instructor's preparation: *Physarum polycephalum*

P. polycephalum has been provided for you in its sclerotial stage. Slime molds in this state will keep almost indefinitely. The slime mold sclerotium is on the filter paper in the foil wrapped packet.

To start the sclerotia growing, place a small piece of filter paper containing the sclerotium on a water agar plate:

- Pick up the filter paper with sterile forceps and snip off a piece with scissors.
- Place it in the center of the water agar plate (see Figure 1a). (It doesn't matter which side of the filter paper is up. The slime mold will grow either way.)
- Wrap the plate with foil and leave at room temperature.
- By the next day, the slime mold will be growing out from the filter paper across the agar (see Figure 1b). Feed it by placing oat flakes at the margins of the growth (see Figure 1c).
- Within 24 h, the oats should be covered with yellow plasmodia (see Figure 1d), but ideally let them grow for another 24 h. The more slime mold on the oats, the better the student mazes will be colonized after an overnight incubation.

Each student group will need 4–6 oats colonized with *P. polycephalum* to start the growth in their mazes (more for larger mazes). Prepare several colonized 100 ml Petri dishes for the class. Note: Two prepared 100-mm agar plates have been included in the kit. They may be used to jumpstart the slime mold growth — you can start the growth immediately, before you prepare your own agar dishes.

The figures below show the same three Petri dishes (60-mm size) at different time points.

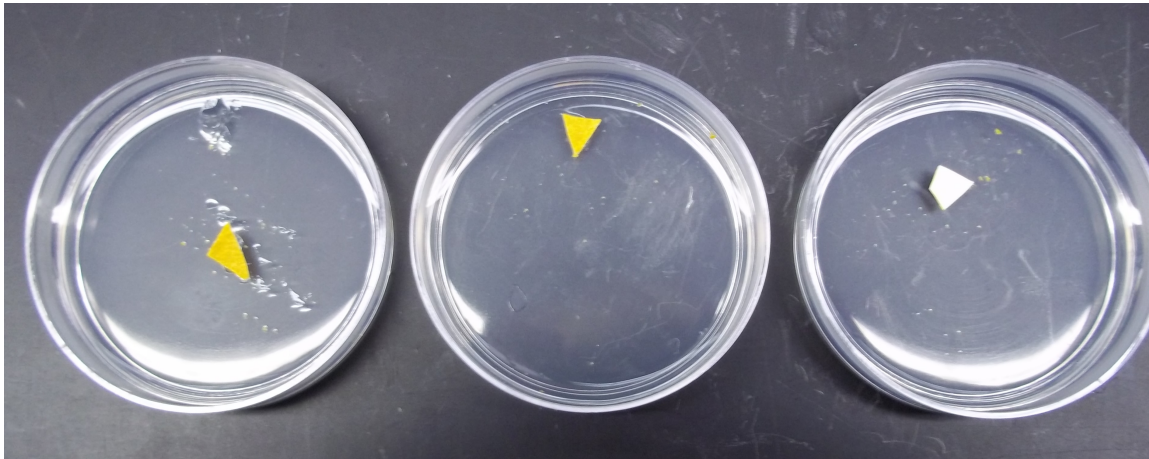


Figure 1a. Sclerotia on filter paper placed on 2% water agar in 60 mm Petri dishes. Dishes were wrapped in foil and incubated overnight at room temperature.

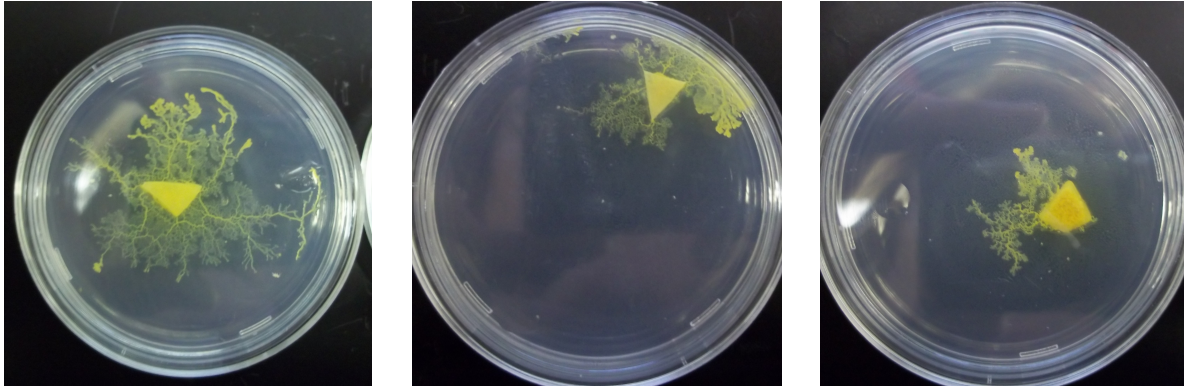


Figure 1b. Growth of plasmodia on 2% water agar after overnight incubation at room temperature.

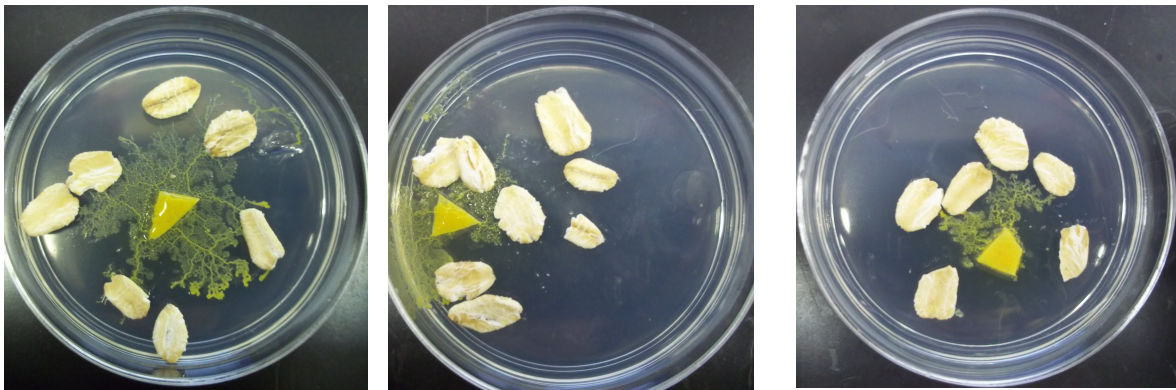


Figure 1c. Feeding of plasmodia. Oats were placed at the margins of the growth. Dishes were wrapped in foil and incubated overnight at room temperature.



Figure 1d. Overnight growth of plasmodia after feeding.

Student Experiment

Materials

- Assorted Lego® blocks
 - long, thin pieces (1x6 and 1x8 blocks) to construct the outside of the maze
 - shorter, thin pieces to construct the interior of the maze (1x1, 1x2, 1x3, 1x4 blocks)
 - corner plates to connect the outside of the maze (1x2x2)
 - flat plates to connect the interior pieces and help hold the maze together
- Molten 2% water agar
- Petri dishes
- Sterile pipettes
- Pipette pumps
- Forceps
- Lab markers
- 70% isopropyl alcohol in spray bottles
- Parafilm®
- Aluminum foil
- Oat flakes, uncooked
- Culture of *P. polycephalum* growing on oats

Objectives:

Through this lab experience, the student will:

1. Describe the importance of barriers in constraining the movement of organisms, particularly in the context of pathogens and invasive species.
2. Evaluate the effect of static barriers on the movement of *P. polycephalum* and relate these movements to the concept that living things sense and respond to their environment.
3. Discuss the question of when 'sense and respond to the environment' becomes 'primitive intelligence' and create a definition of intelligence.
4. Design a controlled experiment that collects quantitative data to test the effects of a new static, invisible, or dynamic barrier on the movement of *P. polycephalum*.
5. Analyze data and make appropriate conclusions based on the data from each experiment.
6. Evaluate the experimental design of each lab group and make suggestions to improve control groups, constants, and collection of data.
7. Generate a list of new questions that arise after each experiment and consider new experiments to test these new questions.

Experimental Procedure

1. Construct a maze that will fit in the Petri dish. Disinfect the maze by spraying it down with alcohol and wiping it with a paper towel.
2. Pipette 2% water agar into the maze to cover the bottom of the dish. (Do not overfill the dish; 10 ml should be more than enough for a 100 mm Petri dish.)
3. Allow agar to solidify.
4. Use sterile forceps to place 4–6 oats colonized with *P. polycephalum* (culture side down) throughout maze. Note: forceps tips can be sterilized with alcohol.
5. Seal the sides of the plate with Parafilm[®] and cover in foil.
6. Incubate at 22–25°C for about 24 hours.
7. After *P. polycephalum* has filled in all parts of the maze, remove the inoculated oats with sterile forceps.
8. Place fresh oats at the two ends of the maze.
9. Incubate at 22–25°C in the dark for another 24 hours.
10. Observe the growth of the *P. polycephalum* for the next few days.
 - Has the plasmodium shrunk back from the dead ends?
 - Is the plasmodium connecting the two food sources?
 - If there was more than one route from one oat to the other, did the plasmodium connect the two oats through the shortest distance?
 - What color is the plasmodium?

Open Inquiry: Students Design Barriers

Instructor's preparation:

- Prepare another batch of *P. polycephalum* from sclerotia, or keep feeding any unused cultures from the previous experiments. Growing *P. polycephalum* needs to be fed every 2–3 days. Just keep sprinkling oats over the plates.
- Prepare plates of 2% water agar for maze experiments.

Instructor Notes And Materials

During this part of the experiment, it is important to allow the students to be creative in designing their experiments. A good rule of thumb is to allow the students to try whatever they wish *as long as they do not intentionally kill the slime mold*. You may provide chemicals from your classroom or the chemistry stockroom, or students may wish to bring materials from home.

Experimental Procedure

Each experiment will be unique. Students should base their own experiments on observations from the maze experiments and information they can find about slime molds. After a quick internet search, students will find that slime molds prefer dark and moist areas. More sophisticated students might do a literature search and find examples of research in which slime molds are routed using light or chemical repellants (such as salt). Students should be encouraged to try something totally new and different. For example, students might try the effects of essential oils or spices. Since many groups will choose a chemical barrier or a lack of moisture barrier, this is a good time to introduce or review diffusion and osmosis.

The relative ease in setting up these experiments and collecting data allows students to set up multiple controls and revise their experiments as needed. For this reason, the open inquiry phase of this investigation is particularly powerful in teaching students the logic behind experimental design and the necessity of controls. (For an example of the use of controls in an experiment, see the appendix.)

Students will also need help in making sense of their results. Allow student groups time to talk through their observations and come to conclusions and then ask them to give quantitative evidence to support their conclusions.

Data Analysis

Was the student-imposed barrier effective? Students should be able to provide quantitative data and use both their control and experimental plates to support their claim.

Plan future experiments. Did your results produce more questions than answers? Have your students suggest other experiments that might provide additional information.

Student presentations. Since each group will have completed a different experiment, it may be fun to have them present their experiments to the class. These could be done as Powerpoint presentations, or could be presented in video format.

Clean-Up

1. Legos[®]

- If you had more than one Lego[®] set in your kit (for multiple classes), please keep the sets separate!
- Soak Legos[®] in alcohol or 10% bleach solution.
- Wipe off any remaining slime mold with a paper towel or a small brush.
- Wash with soap and water.
- Note: Do not autoclave or otherwise heat Legos[®]! The Legos[®] are sensitive to high temperatures and will melt and change shape.

Tip: Be careful not to lose the many small pieces. A kitchen sieve is included in the kit that you may use for clean-up.

As I moved the Lego[®] blocks from the bleach, I used a small brush (a toothbrush would work here) to clean off any remaining traces of mold, then dropped the pieces into soapy water. To rinse, I poured them into the sieve, rinsed them in the sieve, then poured the Lego[®] blocks onto paper towels to dry.

2. Soak cultures and Petri dishes in a 10% bleach solution or autoclave. Discard.
3. Empty and rinse the alcohol mister bottles.

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Guided Inquiry Questions

1. After two days of growth, did *P. polycephalum* connect the oats through the shortest distance?
 - a. Can you provide evidence through quantitative data?
 - b. Does this mean the Lego[®] blocks acted as an effective barrier for the slime mold?
2. What other observations did you make after two or three days of growth? Provide pictures or quantitative data.
3. Why is it important that *P. polycephalum* be able to sense and respond to barriers?
 - a. How would the behavior of limiting growth to the shortest distance between the oats be the best behavior for survival?
 - b. In what situations would finding ways around the barrier be beneficial to survival?
4. Find and describe an example in which a human-imposed barrier has limited the spread of a plant, animal, or human pathogen or an invasive species.
5. Find and describe an example in which barriers have not been effective in controlling a plant, animal, or human pathogen or an invasive species.
6. Do you think *P. polycephalum* exhibits intelligence? Define intelligence and defend your answer.

Possible Answers for Guided Inquiry Questions

1. After two days of growth, did *P. polycephalum* connect the oats through the shortest distance?

a. Can you provide evidence through quantitative data?

Students should be able to replicate Nakagaki's results (Nakagaki et al. 2000), demonstrating that slime mold will connect the shortest distance between two oats (for examples of maze results, see the next section of the appendix). Although quantifying this claim may seem obvious, some students will need to be prompted to take measurements. Asking the student groups "How could you convince somebody else that the slime mold did find the shortest distance?" is usually enough to guide them to the point of measuring with a ruler the distances of all the possible routes. At this point, the concept of dynamic barriers could be introduced by having students ask additional questions regarding adaptability of the slime mold. For instance, if the shortest maze route gets "shut down" by rearrangement or addition of a new Lego[®] barrier, then the previously longer route now becomes the shortest and students can consider if the plasmodium responds accordingly and reinforce the new shortest route. This could be related to larger themes of unpredictability and serendipity in the movement and development of organisms to new habitats.

b. Does this mean that the Lego[®] blocks acted as an effective barrier for the slime mold?

a. Student answers will vary here. Although the slime mold may connect the shortest distance through the maze, the slime mold will grow over and out of the maze occasionally. You can have a class discussion debating the effectiveness of the Lego[®] blocks as barriers. This discussion might naturally lead to students thinking about other types of barriers (the open inquiry part of the experiment).

2. What other observations did you make after two or three days of growth? Provide pictures or quantitative data.

3. Why is it important that *P. polycephalum* be able to sense and respond to barriers?

a. How would the behavior of limiting growth to the shortest distance between the oats be the best behavior for survival?

Slime molds that are able to "determine" the shortest distance would obtain more net energy from the food sources, since energy would not be wasted feeding plasmodia going to "dead ends." From an evolutionary perspective, slime molds that were most efficient in utilizing food sources would have been most likely to survive and reproduce, leading to the evolution of this behavior.

b. In what situations would finding ways around the barrier be beneficial to survival?

Finding ways around barriers is a matter of life and death. Motile organisms must constantly find ways around barriers to obtain food, water, and other necessary resources.

4. Find and describe an example in which a human-imposed barrier has limited the spread of a plant, animal, or human pathogen or an invasive species.

Students can find and cite specific examples. Here are some general acceptable answers, all of which are physical barriers:

- Use of gloves to prevent spread of bacteria in hospitals and food service
- Use of nets to prevent the spread of mosquito borne illnesses such as malaria in developing countries
- Use of a quarantine to create a physical barrier to prevent spread of a disease. For examples, rabies has been nearly eradicated from England for over 100 years due to two physical barriers: water, as England is separated from other land masses by water, and lengthy quarantine periods for all pets entering the country.
- Use of condoms to prevent the spread of some STDs including HIV. (Many of the students who alpha-tested this kit had the idea of vaccines creating an immunological barrier to the spread of many diseases.)

5. Find and describe an example in which barriers have not been effective in controlling a plant, animal, or human pathogen or an invasive species.

Students can find and cite specific examples. Some acceptable answers might include:

- Difficulty in containing flu viruses and other emergent diseases in humans
- The appearance of Tropical Race Four, a fungus that infects bananas, that has spread from Asia to Australia despite efforts to stop the spread (Peed 2011)
- The spread of *Hydrilla* (an invasive species) in waterways from Texas to Connecticut (including Virginia)
- The spread of certain STDs such as HPV that are not stopped by condom barriers

6. Do you think *P. polycephalum* exhibits intelligence? Define intelligence and defend your answer.

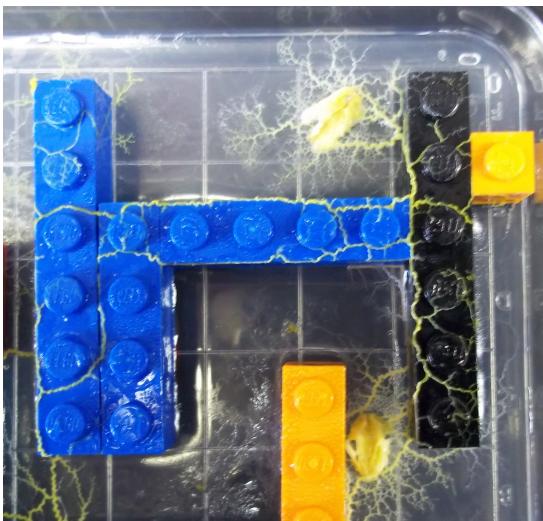
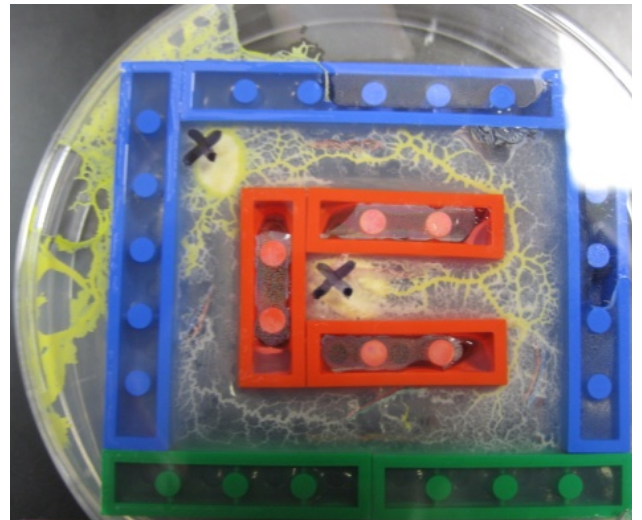
This question provides an opportunity for students to have a class discussion about the nature of intelligence. It may be useful for the teacher to review Nakagaki's article (Nakagaki 2001) in which the author argues that *P. polycephalum* does exhibit primitive intelligence. Thomas' article (Thomas 1980) describing a hierarchy of learning abilities may also be helpful. This element of the lab is one that may appeal more to the humanities and social-science oriented learners in the classroom.

Sample Results from Maze Experiment



(left) *P. polycephalum* is connecting the food sources through the shortest distance of the maze. Areas where the plasmodia have shrunk back are white.

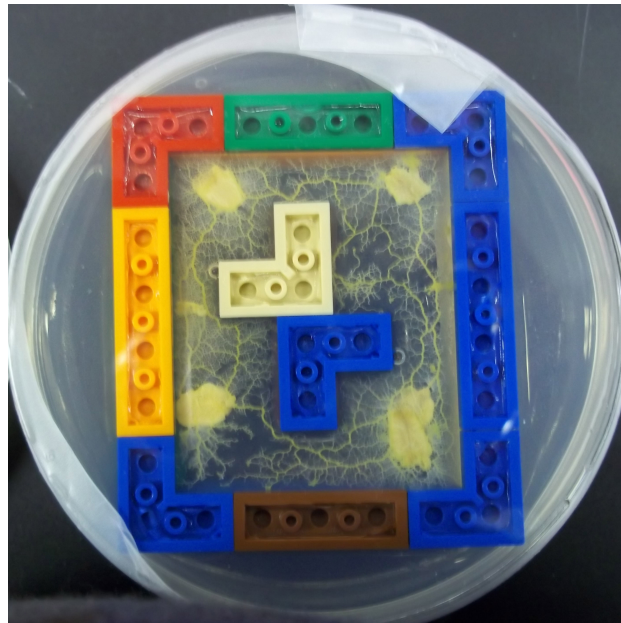
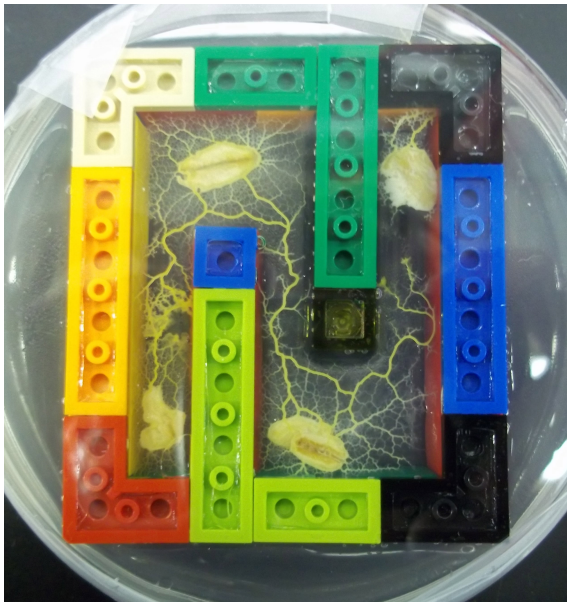
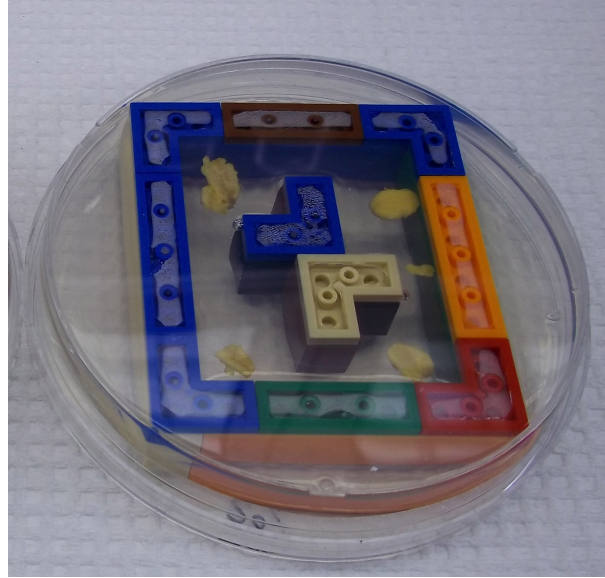
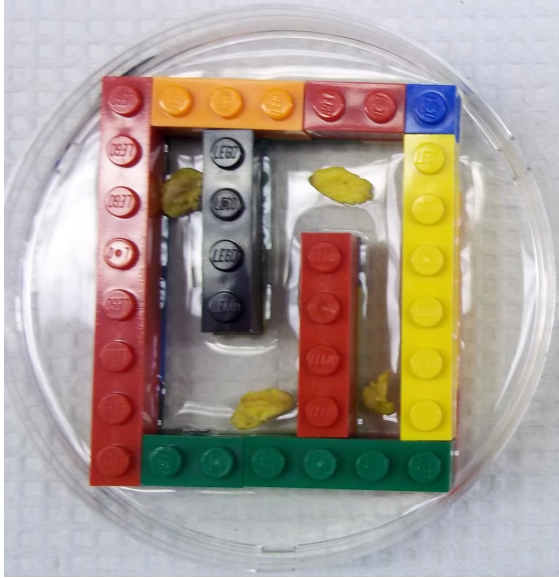
(right) View from the bottom of a Petri dish showing results of a maze experiment two days after fresh oats were placed (at Xs). Living plasmodia connect the two oat flakes in the maze through the shortest distance, with white dead plasmodia in other regions; however, in this case, the Lego® blocks were not an effective barrier. Some *P. polycephalum* “escaped” on the left hand side.



(left) In this experiment, the *P. polycephalum* found the shortest route between the oat flakes — right over the Lego® blocks!

Appendix: Sample Results of Maze Experiment

Here are a few more maze photos. The top two were taken right after the mazes were constructed and inoculated with oat flakes covered with *P. polycephalum*. The lower two photos were taken after overnight incubation in the dark at room temperature. Note that frequently it is easier to photograph the plates from the bottom.



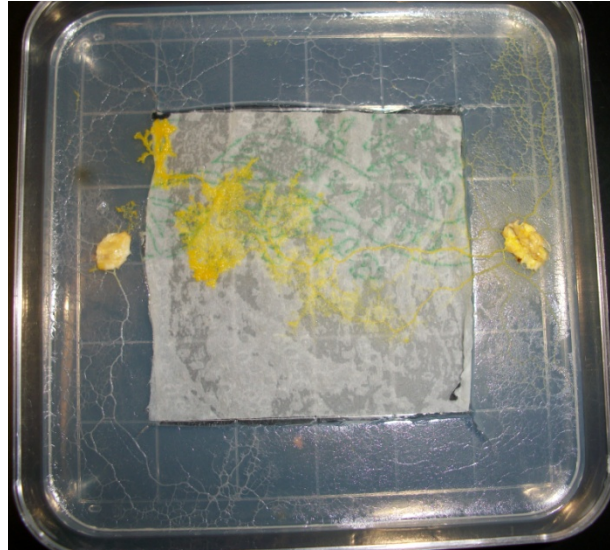
Importance of Controls in Open Inquiry Experiment

During the open inquiry phase of this investigation, it is important that the teacher help guide each student group in thinking about control groups and constants. The table below shows the design of a group experiment to examine the lack of moisture as a barrier to the movement of the slime mold, *P. polycephalum*. For this experiment, students were provided with square Petri dishes. Barriers were created either by cutting a square piece out of the center (exterior agar) or by cutting a strip of agar spanning the width of the Petri dish (divided agar).

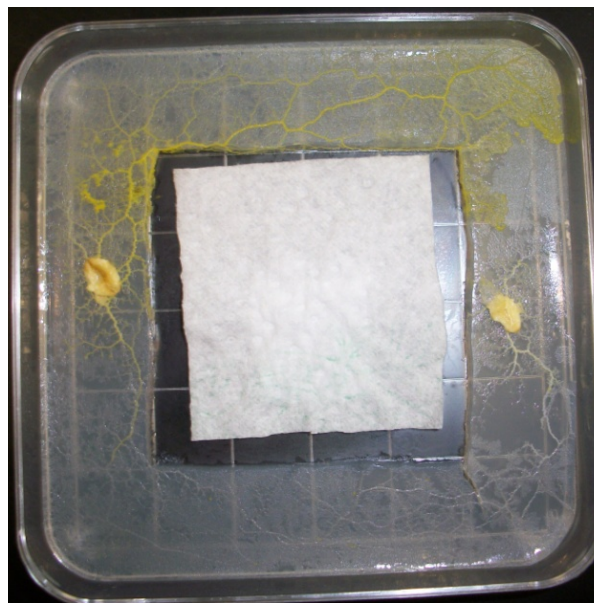
Experimental Design	Purpose	Explanation
Exterior Agar/ Dry Center	Experimental Plate	Will the slime mold cross a dry paper towel or grow around it?
Divided Agar/ Dry Center	Experimental Plate	Will the slime mold cross a dry paper towel or grow around it?
All Agar	Control Plate	Demonstrates the pattern of growth from one oat to another under optimal conditions.
All Wet Paper Towel	Control Plate	Demonstrates that moisture, not something else about the paper towel, is the factor preventing growth. Growth is expected on this plate.
All Dry Paper Towel	Control Plate	Demonstrates that the slime mold cannot grow without moisture. No growth is expected on this plate.
Exterior Agar/ Wet Center	Control Plate	Demonstrates that moisture is the barrier, not the fact that there is a ledge of agar or something else. Growth is expected across the center.
Divided Agar/ Wet Center	Control Plate	Demonstrates that moisture is the barrier, not the fact that there is a ledge of agar or something else. Growth is expected across the center.

Sample Results from an Open Inquiry Experiment

These images show growth of the slime mold three days after inoculation on plates with a wet (top) or dry (bottom) center. Students used a piece of paper towel slightly smaller than the cut area so that the paper towel would not absorb moisture from the agar.



On the wet plate, the slime mold eventually connected the two oats through the shortest distance across the wet paper towel.



On the dry plate (above), the slime molds grew around the barrier of the dry paper towel.

On both plates, areas of dead plasmodia (and places where the slime mold once grew) can be seen as white tubes.

Extension Activities

Here are a few ideas for extension activities.

Examine slime mold microscopically. If there is a dissecting microscope available, look at the slime mold plasmodia on the agar plate. If you look at the edges of the growth area, you can see the protoplasm streaming in the veins. This does not require high magnification to observe. Remember that plasmodial form of slime mold is a syncytium (a multinucleate supercell filled with cytoplasm).

Prepare sclerotia from slime mold cultures. Paul Davison at the University of North Alabama has graciously shared his method for preparing sclerotia:

Producing Sclerotia

Sclerotium formation is most successful in 3–4 day old cultures of vigorous plasmodia growing on moistened filter paper in a Petri dish. To initiate the formation of sclerotia, simply prop open the lid to the Petri dish such that there is an opening a few millimeters wide at one end of the lid, and place the dish in a dark place (e.g., a cabinet or drawer). The paper containing the plasmodium will dry over a period of 2–3 days. During this drying period, the plasmodium may migrate and attempt to get out of the container, but usually a nice sclerotium will form on the filter paper. Once formed and dry, cut out the filter paper that has the sclerotium and place in a sealed plastic bag and refrigerate. A Petri dish stored in a plastic bag can be used to hold the sclerotia. Stored under refrigeration, sclerotia should remain viable for up to a year (or longer?).

His website with additional information can be found at:

<http://www.una.edu/faculty/pgdavison/PHYSARUM%20culture%20for%20web.html>

Prepare time-lapse videos of movement of *P. polycephalum*.

Cynthia Bohland used a document camera to take her videos. The time lapse was set to 1 frame every 10 minutes, and the plate was left in the light for the entire filming period. Since the camera took the pictures in video mode, no conversion was necessary. Her videos can be found here:

<http://www.schooltube.com/user/slimemold>

If a document camera is not available, try taking conventional digital images at regular intervals. To make this easier, make sure that the Petri dishes are always in the same orientation (perhaps a mark on the side of the dish lined up with a mark on whatever surface you're setting the dish on for photographs) and make sure that the camera is always the same distance from the plate.

A series of images can be made into a movie using programs such as iMovie or Windows Movie Maker.

Virginia Standards of Learning: Where do slime molds and barriers fit?

To determine where this lab fits into the Virginia State Standards, the applicable standards are listed below (adapted from the website). To see a complete list of standards, please refer to the website: <http://www.pen.k12.va.us/VDOE/Superintendent/Sols/home.shtml>

Organizing Topics	Related Standards of Learning
Scientific Method	BIO. 1 a, b, c, e, f, h, l, j, k, l, m
Cell Structure and Function	BIO. 4 b, c, d
Life Functions and Processes	BIO. 5 a, c
Ecology	BIO. 9 a, d
Evolution	BIO. 2 b

Organizing Topic	Essential Knowledge, Understandings, and Skills	SOL
Scientific Method	<p>Active participation in scientific investigations is necessary to develop an understanding of biology as an experimental science.</p> <p>The continual use and development of cognitive and manipulative skills associated with the formulation of the scientific explanations is important.</p> <p>The design of sound scientific experiments relies on systematic preliminary observations and data collected in the laboratory. Prior establishment of an adequate knowledge base is essential before hypotheses can be developed and tested.</p>	BIO. 1 a, b, c, l, j, m
	<p>Apply the following principles of scientific investigation:</p> <ol style="list-style-type: none"> 1. Collect preliminary observations. 2. Formulate hypotheses based on cause-and-effect relationships. 3. Justify hypotheses based on both preliminary observations and scientific literature. 4. Identify variables that must be held constant. 5. Establish controls as appropriate. 6. Identify the independent variable in an experiment. 7. Select dependent variables that allow collection of quantitative data. 8. Collect preliminary observations. 9. Make clear distinctions among observations, inferences, and predictions. 	Bio. 1 a, b, c, i

Appendix: Alignment with Virginia Standards of Learning

Scientific Method	A hypothesis can be supported, modified, or rejected based on collected data. A <i>hypothesis</i> is a tentative explanation that accounts for a set of facts and that can be tested by further investigation. A <i>theory</i> is an explanation of a large body of information, experimental and inferential, and serves as an overarching framework for numerous concepts. It is subject to change as new evidence becomes available.	BIO. 1 h, k, l
	<ol style="list-style-type: none"> 1. Use evidence, apply logic, and construct an argument for conclusions based on reported data. 2. Determine the extent to which data supports/does not support a hypothesis, and propose further hypotheses and directions for continued research. 	BIO. 1 h, k, l
Cell Structure and Function	<ol style="list-style-type: none"> 1. Cellular activities necessary for life include chemical reactions that facilitate acquiring energy, reproduction, and adaptation/maintaining homeostasis. 	BIO. 4 c, d
	<ol style="list-style-type: none"> 1. Diffusion occurs in cells when substances that are dissolved in water move from an area of higher concentration to an area of lower concentration. 2. Osmosis refers to the movement of water molecules through a semi-permeable membrane from an area of higher water concentration to an area of lower water concentration. 	BIO. 4 b, c, d
Life Forms and Functions	<p>Differentiate and give examples of the following:</p> <ol style="list-style-type: none"> 1. Multicellular and unicellular organisms 2. Motile and non-motile organisms 3. Behavioral responses to the environment 	BIO. 5 a, c
Evolution	Natural selection is a process by which organisms with traits well suited to an environment survive and reproduce at a greater rate than organisms less suited to that environment.	BIO. 2 b
Ecology	As any population grows, it is held in check by interactions among a variety of biotic and abiotic factors.	BIO. 9 a
	<p>Describe an ecosystem in terms of the following:</p> <ol style="list-style-type: none"> 1. Effects of biotic and abiotic components 2. Examples of interdependence 3. Evidence of human influences 	BIO. 9 d

Sources For Materials Used In This Kit

Lego® blocks

Customized Lego® sets can be ordered from the company in the Pick a Brick section of the Lego® shop at <http://shop.lego.com/pab/?warning=false>. Select Bricks or Plates in the Brick Search pull down menu on the left side of the page.

The following table shows a sample order for 8 student groups. Prices are from 2011.

Lego® Piece	Number per group	Total for 8 groups	Unit cost	Total cost
Bricks				
1x8	4	32	0.25	\$ 8.00
1x6	4	32	0.25	8.00
1x4	2	16	0.20	3.20
1x3	2	16	0.20	3.20
1x2	5	40	0.15	6.00
1x1	5	40	0.10	4.00
Corner plate				
1x2x2	6	48	0.10	4.80
Plates				
1x2	4	32	0.10	3.20
1x3	4	32	0.10	3.20
1x4	4	32	0.10	3.20
Total				\$ 46.80

Other materials

Item	Source	Catalog #	Quantity	Price
Slime mold sclerotia	Carolina Biological Supply Company	156190	box	\$11.85
Agar, granulated*	Fisher Scientific	BP1423-500	500 g	\$143.08
Agar	Carolina Biological Supply Company	796200	100 g	\$62.80
Forceps	Nasco	SB18875B	ea	\$ 0.81

* This does not have to be high grade (e.g., Bacto Agar)

Other materials are widely available from biological supply houses, such as Petri dishes, sterile serological pipettes, pipette pumps, gloves, etc.

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DIRECTIONS FOR RETURN SHIPPING

Please return the checklist with the kit.

- Repackage the materials in the shipping container. Please be sure that heavier items are on the bottom of the box.
- **Please** check off items as they are repacked. This kit has a lot of small parts that are easy to lose track of if you are not careful. Any items that are not shaded on the form are expected to be returned!
- Please return *unused* expendables. Do not return used items. Particularly, do **not** mix used items with unused items.
- If you need additional packing material, please use wadded paper. Please **do not** use Styrofoam peanuts!
- Shipping:
 - Remove the original FedEx shipping label from the shipping container.
 - Seal the box with cable ties.
Please make sure that the cable ties are secure. Note that there is a right way and a wrong way to insert the tab in the cable tie. Look at the end you put the tab through. The tab should be put in from the side that is smooth with the tie, not the end that sticks out. If it is done the wrong way, the cable tie will open when you pull on it. Please test the cable tie by pulling to be sure that you've done it correctly.
 - Remove the backing paper from the plastic airbill sleeve and place the pre-printed airbill on top of the box.

Call FedEx for pickup. They require at least a 3-hour notice on pickups.