BILD 4 Introductory Biology Lab

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| LB0 | Laboratory Basics and Safety |

Before beginning in the laboratory each week

1. Check that everyone has appropriate laboratory attire, which includes closed-toed shoes and long pants (or equivalent). No skin can be showing below the waist.

2. Check that everyone has appropriate personal protective equipment (PPE). PPE includes a laboratory coat, safety glasses or goggles, and gloves.

3. Wipe the bench with 70% ethanol. Be sure to have appropriate PPE on before you start.

4. Read through the entire protocol as a group. Divide up responsibilities, including writing in the research notebook and entering data onto the shared Google spreadsheet. Be sure to rotate responsibilities regularly so that everyone gets a chance to do everything.

5. If there is wait time in the protocols, decide how you would like to be productive, such as staggering the experiments, writing in the research notebook, entering data onto the shared Google spreadsheet, and/or working on assignments.

Before leaving the laboratory each week

1. Discard all waste in appropriate containers. If you are unsure, check with the instructional assistants for appropriate waste disposal.

2. Wipe the bench with 70% ethanol.

3. Remove and store your PPE. Thoroughly wash your hands with soap and water.

4. Finish writing in the research notebook and entering data into any shared Google spreadsheets.

| LB1 | Research Notebook Guidelines |

The research notebook will be a shared Google document. It is a record of your group's work for your own use and will be graded for thoughtful completion every week as an item to your contribution grade. It is not a laboratory report.

The first page of the research notebook should include a table of contents. Fill in the table of contents throughout the quarter to create a list of experiments and activities and their corresponding dates. Each week should start with a new page that is clearly labeled and dated. In some weeks, there may be multiple experiments.

Create a new entry in your research notebook for each day's activities. Answer the reflection questions below. During some weeks there will be additional prompts given in laboratory.

1. Purpose (no more than a few sentences): What question(s) did we address today?

2. Make sure to record all the necessary data and items from the laboratory protocol for today.

3. Conclusion (about 100 words): In a paragraph, write your group's conclusion(s) to the question(s) above. **If appropriate:** Include a table summarizing important information, statistics, and/or Excel formulas; create figure(s) that accurately display the appropriate comparisons of the data; include appropriate caption(s) for your table(s) and/or figure(s).

4. Reflection (about 200 words): Write a paragraph summarizing and reflecting on what you learned in the laboratory today. What was particularly challenging and why? Was everything as you expected, or were you surprised about anything? Why or why not?

5. Collaboration and author contributions (about 100 words): Write a paragraph describing how each team member contributed to the activities and how the team collaborated together. How might you as a team improve the collaborative process?

6. Please feel free to add your own notes and any other details that you would like to record and remember as a team for future reference.

PROGRESS CHECK: Once your group has completed all the laboratory activities and research notebook items, check in with the instructional assistant(s) before leaving the laboratory.

| LB2 | Community Building |

1. Please begin by briefly introducing yourselves to one another, e.g. your name, major, and college. **Record this information in the research notebook.**

2. Pick two questions from the list below and share your individual answers. As a team, you can either decide to use the same questions for everyone, or you can individually choose the questions that you would like to share about yourself.

   - What is one song that reminds you of a time when you were younger?
   - What is something you hesitated to do but ended up liking?
   - What is an experience that has taken you out of your comfort zone?
   - What are some strategies for communicating with people who are different from you?
   - What do you believe is the most important factor for a successful team?
   - When working in a team, how do you deal with conflicts?

3. Identify one thing that everyone has in common. This should be something more substantive than "everyone is a UCSD student" or "biology majors!" – try and think hard about everyone's interests and experiences! **Record this information in the research notebook.**

4. Take a blank sheet of paper. Create a group logo, using the information discussed so far and any additional ideas that you have together. All the group logos will be displayed in the laboratory. **Record this information in the research notebook.**

5. Discuss the following community guidelines built from campus Principles of Community. Consider how we – as a team and as individuals – might act to promote and uphold these norms. **Record this information in the research notebook.**

   Campus principles of community: https://ucsd.edu/about/principles.html

UC San Diego is dedicated to learning, teaching, and serving society through education, research, and public service. Our international reputation for excellence is due in large part to the cooperative and entrepreneurial nature of the UC San Diego community. UC San Diego faculty, staff, and students are encouraged to be creative and are rewarded for individual as well as collaborative achievements.

In this course, we hope to foster open, respectful, and productive dialogue and collaborative teamwork. To do so, we agree to:

- Participate to the fullest of our ability and listen actively
- Share responsibility for including all voices in the conversation
- Ensure equitable contribution from ourselves and everyone else
- Speak from our own experience instead of generalizing
- Differentiate between opinion and informed knowledge
- Be willing to grapple with challenging ideas

6. Please share contact information with one another. **Consider recording this in the research notebook, so you know where to find the contact information in the future.**

| SL0 | Introduction to Scientific Literature |

Throughout the course, we will be using one or more databases to find peer-reviewed scientific articles. These articles will provide background and support for scientific arguments in both writing assignments and group final research proposals. Please consult the general biology and course-specific library guides: https://ucsd.libguides.com/biology

Peer-review process

When scientist send their work to a scientific journal for possible publication, an editor at the journal will decide if they think the results are worth publishing. If not, the manuscript is rejected outright. If the editor does think that the manuscript is potentially worthy of publication, they will send the manuscript to several other scientists in the same field, scientific peers, who would be in a strong position to evaluate the work. This is called the peer-review process. If, after peer-review, the editor concludes that the work is scientifically sound and worth publishing, the article will appear in a scientific journal. Typically, a manuscript will be revised one or more time for additional peer review before it is accepted for publication.

Primary original research articles

These involves the collection of original research data. Scientists carry out experiments in a laboratory or in the environment. Once they have sufficient results to tell a story, the scientists will send the results to a scientific journal for possible publication. The manuscript describing the results will be peer reviewed. When published after the peer-review process, this is a primary research article. Such articles typically include an abstract that summarizes the work, introduction that situates the current work in the existing research literature, methods that allow for others to replicates the experiments or studies, results that present the data collected, and discussion that explains the results in the context of what is known in the field.

Secondary scientific review articles

This other type of articles provides an overview of the research in a given field. A secondary review article is a summary of results from multiple primary studies in a single peer-reviewed article. Review articles are considered secondary as they do not involve the collection of original research data.

However, they are a very good place to start to get a broad overview of a topic, and they provide citations and references to many primary research articles that contributed to the field. We will use both primary and secondary peer-reviewed articles in the course when seeking background information and support for scientific arguments.

Tertiary popular science articles

Unlike primary research and secondary review articles, popular science articles are summaries of scientific results intended for a broad general audience. Popular science articles are generally written by science journalists or scientists, but they are not peer reviewed in the same way as the other two types of articles. A popular science article will sometimes provide links to primary research or secondary review articles, but they should not be cited in assignments.

| SL1 | Identifying Features of Scientific Literature |

1. Open the articles or papers posted on Canvas. There are three sets of articles numbered 1A-1C, 2A-2C, and 3A-3C. Each group is assigned one set of articles below. The articles are also labeled as popular science (tertiary), scientific review (secondary), or original research (primary).

    - Group A: articles 1A-1C
    - Group B: articles 2A-2C
    - Group C: articles 3A-3C
    - Group D: articles 1A-1C
    - Group E: articles 2A-2C
    - Group F: articles 3A-3C

2. Look at – look but do not read! – your assigned articles. Comment on their differences and similarities in diagrams, figures, sections, and overall structure.

    PROGRESS CHECK: Write down the differences and similarities that your group has identified and agreed upon across the different types of articles.

3. Comment on the audiences that you think each type of article is trying to reach.

    PROGRESS CHECK: Write down the different potential audiences that your group has identified and agreed upon across the different types of articles.

| SL2 | Literature Search |

1. Use Google Scholar to find the journal article: “Does pizza protect against cancer?”. Click on one of the links below the article information on Google Scholar to find articles that have cited this article. Record the Vancouver citation format of the article in research notebook. This should be authored by Levi et al. in 2004.

2. Find a primary original research article with the following characteristics: topic = microbiome, co-author = Dutton, publication year = 2017. Please write a one-sentence summary of the article abstract. Record this information in research notebook.

3. Find a secondary scientific review article about microbiomes. Record reference information of the article in research notebook using any format of your choice. Include the DOI for this article.

4. Find one interesting primary original research article and one secondary scientific review article on related topics using the tools above. The goal is to find papers to inspire the group in finding a question to study for the research proposal poster presentation! Some points to consider:

    - Choose topics that everyone in the group finds interesting.
    - Choose papers published within the last 5-10 years.
    - Choose papers that are not so technical that it would be very difficult to do enough background reading in a few weeks to create a workable research proposal plan.

    5. Record reference information of the two articles in research notebook using any format of your choice. Include the DOI for these articles.

    6. In research notebook, list at least 3 biological topics that the group is interested in learning more about and using for the research proposal poster presentation, even if these topics have nothing to do with the papers that the group has found or may eventually choose later on.

| FW0 | Introduction to Field Work |

The Scripps Coastal Reserve (SCR) is part of the larger University of California (UC) Natural Reserve System (http://www.ucnrs.org), which manages 39 wild lands across the state of California. Each campus manages their share of the reserves. UC San Diego manages the SCR, Elliot Chaparral Reserve (near Marine Corps Air Station Miramar), Dawson Los Manos Reserve (Vista), and Kendall Frost Mission Bay Reserve (Pacific Beach) (http://nrs.ucsd.edu). These reserves are different from National or State parks in that the UC reserves are managed for education and research, consistent with the mission of the university. It is the largest university-run reserve system in the world and covers most California habitats, including alpine, desert, chaparral, estuarine, ocean, and more. Some reserves are small like the SCR; others are large and include laboratories and dormitories.

Southern California is a biodiversity hotspot. We have a large number of species overall, as well as a large number of threatened and endangered species. This is particularly true for plants; San Diego County has more plant species and more threatened / endangered plant species than any other county in the United States. And, many of our species are endemic (only occur here, which makes them more vulnerable to extinction) because our Mediterranean climate (hot dry summers and cool wet winters) is quite unusual. The SCR alone has over 200 plant species.

The plant community we will see at the SCR is coastal sage scrub. The plants are mostly low growing, aromatic, and have adaptations to survive San Diego's hot dry summers. The namesake plant is California sage (Artemisia californica), a gray-green bush with feathery foliage. This plant handles the dry season by being drought-deciduous and loses its leaves in summer. A different strategy is used by lemonade berry (Rhus integrifolia). This plant has thick, tough (sclerophyllous) leaves that stay on the plant all year, and water loss is reduced by the waxy covering. Although this plant retains its leaves all year and thus is able to photosynthesize all year, the rate of photosynthesis is lower because the waxy coating reduces both water loss and carbon dioxide (CO^2) intake. We can see both strategies (drought deciduous vs. sclerophyllous leaves) involve trade-offs.

The SCR land was used by the La Jolla Indians more than 8,000 years ago and more recently by the Kumeyaay, an Indigenous people of the Americas who live at the northern border of Baja California in

Mexico and the southern border of California in the United States. The SCR land is rich in archeological artifacts, including middens, or old trash heaps. It was farmed by Europeans around 1900, and oil tycoon William F. Black ranched here (namesake of Black's beach). The land was also a look out during World War II, and a bunker can still be seen.

Given the history of use, this land is far from pristine native vegetation. There are many plants here that are from elsewhere, and many of these exotic species are invasive; they outcompete native species, reduce biodiversity, and sometimes even create monocultures. Most scientists and non-scientists alike agree that biodiversity is important for various reasons. We hypothesize that biodiversity may increase ecosystem productivity and resilience although that has been hard to prove. Easier to see is the importance of biodiversity for ecosystem services, or the ways that nature helps humans, e.g. pollination, erosion control, pest suppression, CO_2 reduction, pollution control, etc.

Some of the common invasive plants found at SCR are:

- Erodeum circutarium (heron's bill)
- Mesembryanthemum crystallinum (crystalline ice plant)
- Brassica nigra and others, (various mustards)
- Avena spp. (wild oats)
- Hordeum spp. (barley)

The most common birds we will see at SCR include the black phoebe, brown towhee, California thrasher, and Anna's hummingbird. All are year-round residents.

Extensive research has been done at the SCR. The remains of one project can be seen at the very west end of the property, where there is a vague grid of square meter plots. This project explores why only a small fraction of plants that are introduced to new places, both intentionally and accidentally, become invasive. One hypothesis is that these species are better able to take advantage of the extra resources that occur in disturbed areas, such as extra water from irrigation run-off, extra nitrogen from fertilizer run-off or nitrogen dioxide from air pollution, etc. One way to test this hypothesis is to reduce resources and see if the native plants regain a competitive advantage. Professor Elsa Cleland and her plant ecology students planted a California sage seedling in the middle of each plot amidst

the crystalline ice plant and reduced nitrogen levels. The method they used to reduce nitrogen levels was to add carbon. This works because microbes in the soil are heterotrophs, and they eat other organisms. The main energy source for autotrophs is carbon, but they use nitrogen for building cell structure too, although in smaller quantities. If we increase carbon in the soil, the microbial community or microbiome will grow and use up all the nitrogen – gardeners call this “robbing” the soil of nitrogen. The Cleland Laboratory tested how effective different sources of carbon (e.g. saw dust, rice straw, etc.) were at reducing the competitive advantage that invasive plants usually have.

PROGRESS CHECK: Using the library resources in the Scientific Literature section of the laboratory manual, try and find the primary research article by Professor Elsa Cleland and her colleagues that report the results of the research study at the SCR described above.

Additional resources

- California Academy of Sciences. What is a diversity hotspot? https://www.calacademy.org/explore-science/what-is-a-biodiversity-hotspot
- California Academy of Sciences. Biodiversity hotspot case study: California. https://www.calacademy.org/explore-science/biodiversity-hotspot-case-study-california

| FW1 | Collecting Soil Samples

Each lab group will work as a team for the soil collection. Every quarter, we will identify two designated plants to study their associated microbiomes as a course. Half of the student groups will collect soil from underneath one designed plant, and the other half will collect soil from beneath another designed plant. Each individual student from the group will practice using the soil corer, but only 10 ml of soil are to be collected in total from beneath the plant assigned to you.

1. Download the free PlantNet app for Android or iOS onto your phone. If you have run into any issues and are not able to do this, please do not worry.

2. Take a picture of your plant and try to confirm it using the PlantNet app. Note the plant's common name and scientific name including genus and species.

   PROGRESS CHECK: Include a picture of your plant.

   PROGRESS CHECK: Write down the common name and scientific name of your plant.

3. Put on a pair of gloves and find a spot under the plant where you plan to take a soil sample. Clear away any sticks and leaves.

4. Uncap an empty sterile 50-mL canonical tube.

5. Make sure the central plunger of the corer is sitting securely in the handle, in a straight line with the rest of the handle.

6. Push the entire T-shaped plastic soil corer into the soil and rotate back and forth until the bottom of the plunger is filled with soil. Pull the soil corer out of the soil.


7. Turn the central plunger at a 90 degrees angle and slowly push the plunger down, transferring the soil into the empty 50-mL conical tube.



8. All members of the group should collect a soil core and add it to the same 50-mL conical tube.

9. Take the tube of soil back to the laboratory where you will conduct subsequent experiments. **PROGRESS CHECK: Do not throw away your soil sample. Make sure to store it in the laboratory according to instructions provided in class.**

| FW2 | Sample Storage for Other Experiments

Objectives

- Aliquot and store soil samples for taxonomic, functional, and genetic biodiversity experiments.

Overview

We will aliquot and store soil samples for subsequent experiments. This is important, so we are prepared for the other parts of our project and also do not lose track of our soil samples.

Protocol

Throughout the laboratory manual, the “completed” column of boxes on the left in all the protocols are for the group to check off the steps as they are performed. This will help the group keep track of where everyone is together in the experiments. The “progress check” column on the right in all the protocols are questions and notes to consider as the experiments are being performed. Please make sure to include all the relevant information from this column and elsewhere in the research notebooks.

Graphic summary

Researchers and scientists often use schematic diagrams to represent information. Such a summary provides a visual representation to help the reader to quickly gain an overview on the protocol, as well as some salient details. Producing such a summary can also help us develop a deeper understanding of the protocol. In some cases, we will create graphic summaries to help us prepare for laboratory work, and we may refer to these summaries in the laboratory as references.

Sample storage

Completed	Step	Protocol	Progress check
	1	For taxonomic biodiversity (TB) experiments, add 0.5 g of soil* to a 50-mL conical tube. Be as accurate as possible and record mass. Store at room temperature. *Weigh paper should always be used when weighing soil.	
	2	For functional biodiversity (FB) experiments, add 0.5 g of soil to a different 50-mL conical tube. This is a second conical tube and is different from the one for TB experiments. Be as accurate as possible and record mass. Store at room temperature.	
	3	For genetic biodiversity (GB) experiments, label the lids of 2 sterile PowerBead Pro tubes from the Qiagen DNeasy PowerSoil Pro Kit. Each group will process 2 aliquots for the same soil samples. Add 0.25 g of soil to each PowerBead Pro tube. Be as accurate as possible and record mass. Store at -20°C. A typical household freezer is also -20°C, but laboratory freezers do not have an auto-defrost feature, which subjects the freezer's contents to multiple freeze-thaw cycles, which can damage laboratory samples.	

| BB0 | Beginning Basics |

In this course, we will develop an understanding of research in the biological sciences through discovery-based laboratory experiments. We will work in teams to collect, analyze, and present original research data while learning foundational biological concepts and laboratory skills. Data collected in this course will contribute to an ongoing research project on soil microbiomes at the Scripps Coastal Reserve.

In this first part of the course, Beginning Basics, we will develop skills that are essential for collecting reliable data and for drawing appropriate conclusions. This will serve as a foundation for subsequent work in the course, where we will conduct multiple experiments related to the overall research project. Specifically, these experiments investigate soil properties such as pH and moisture content, as well as the taxonomic, functional, and genetic biodiversity of microbiomes within our soil samples. Some experiments may span multiple weeks.

Make sure to split the work up equitably within the group, so everyone has the opportunity to engage and learn! For example, if someone is already proficient with pipetting, then help others to figure things out, or if someone is not experienced with pipetting, then make sure they have the focus on learning to pipet. Similarly, if someone is especially proficient in Excel, then help others to figure out the formulas, or if someone is not experienced in Excel, then make sure that they really engage in data analysis. We will be using both micropipettes to collect data and Excel to analyze data for all our future experiments as well. So, this is our first opportunity to learn, and we will continue to develop these skills and other skills together throughout the course!

| BB1 | Using Micropipettes

Learning objectives

- Practice proper pipetting technique
- Become familiarized with common pipetting errors

Overview

The success of our research project and future laboratory experiments depends, in part, on our ability to pipette. Micropipettes are used to accurately and precisely transfer liquid volumes in the range of 1-1000 µL, where 1 µL or one microliter is one millionth of a liter, which is a very small quantity! A typical set of micropipettes includes one for small volumes (e.g. 1-10 µL) called a P10, one for intermediate volumes (e.g. 10-100 µL) called a P100, and one for large volumes (e.g. 100-1000 µL) called a P1000. If a desired volume can be transferred with more than one micropipette, select the micropipette with the smaller volume range because it will be more accurate.

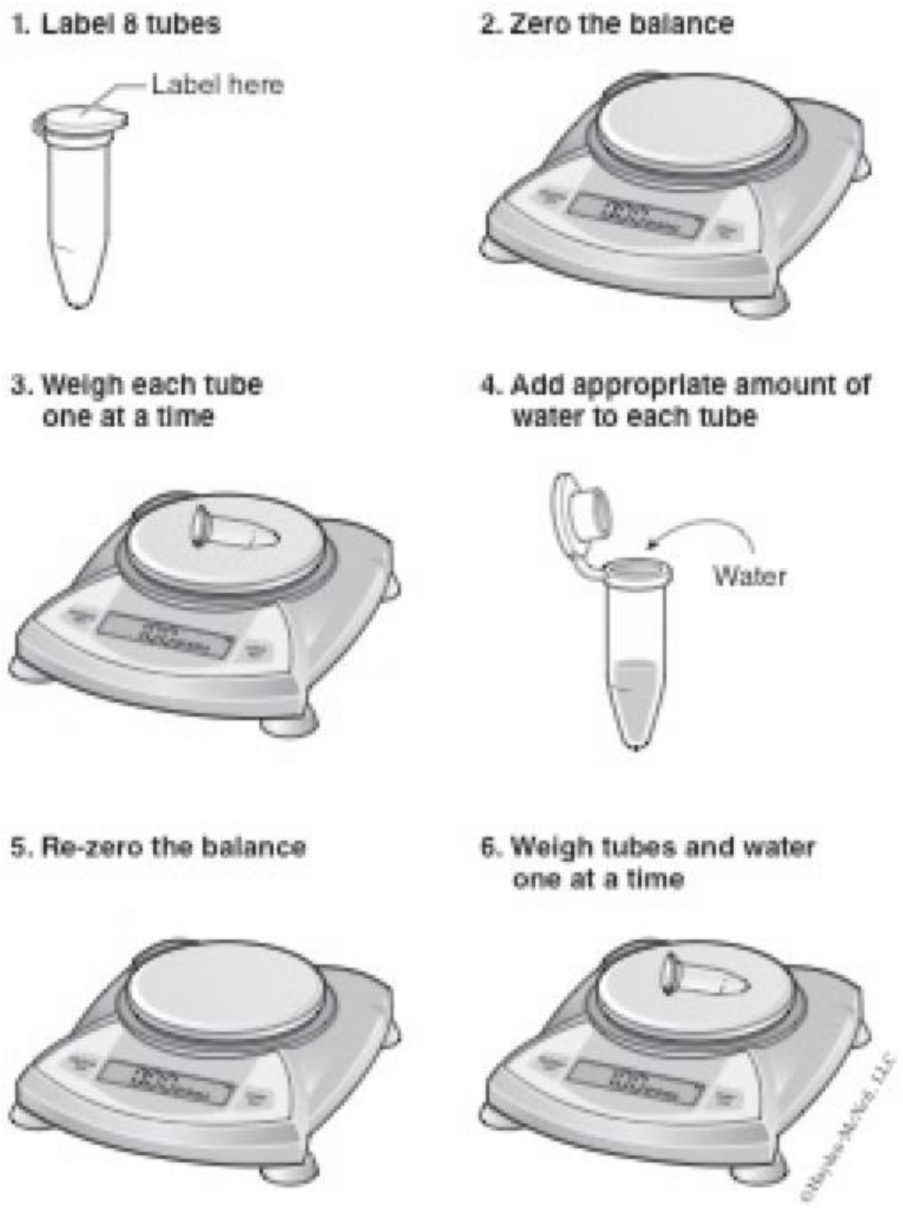
In this experiment, we will learn to pipette correctly and examine a common way that micropipettes are used incorrectly. We will transfer water into pre-weighed microtubes using both correct and incorrect techniques. Next, we will determine the mass of water within each tube and then compare the measured mass with what is expected based on the density of water. Replicates of each sample will be prepared so that precision (i.e. variability among measurements) can be estimated from the standard deviation of the measurements. A smaller standard deviation indicates less variability.

Protocol

Throughout the laboratory manual, the “completed” column of boxes on the left in all the protocols are for the group to check off the steps as they are performed. This will help the group keep track of where everyone is together in the experiments. The “progress check” column on the right in all the protocols are questions and notes to consider as the experiments are being performed. Please make sure to include all the relevant information from this column and elsewhere in the research notebooks.

Graphic summary

Researchers and scientists often use schematic diagrams to represent information. A graphic summary of the protocol is provided here. Such a summary provides a visual representation to help the reader to quickly gain an overview on the protocol, as well as some salient details. Producing such a summary can also help us develop a deeper understanding of the protocol.



Data table

Mass of each tube before and after adding water

Tube	Tube (g)	Tube + Water (g)
1		
2		
3		
4		
5		
6		
7		
8		

Make sure to write down all the relevant data collected in this protocol. Afterwards, this information needs to be transferred to the shared data sheet. Measurements (numbers) must be entered into computer spreadsheets without units (letters) to perform calculations. **Record this information in the research notebook.**

Excel functions and operations

- Subtract (-): A minus sign is used to subtract numbers or expressions, e.g. =A2-A4 or =12-2
- Divide (/): A forward slash is used to divide numbers or expressions, e.g. =A2/A4 or =(A1+2)/(8-6)
- Multiply (*): An asterisk is used to multiply numbers or expressions, e.g. =A2*A4 or =(5+2)*(B3-4)
- Autofill data: https://support.microsoft.com/en-us/office/fill-data-automatically-in-worksheet-cells-74e31bdd-d993-45da-aa82-35a236c5b5db

Additional resources

- JoVE Science Education Database. General laboratory techniques: An introduction to the micropipettor. Journal of Visual Experimentation, Cambridge, MA (2024). https://app.jove.com/v/5033/an-introduction-to-the-micropipettor

Part A: Determining the mass of microtubes

Completed	Step	Protocol	Progress check
	1	Obtain 8 non-sterile microtubes from an instructional assistant.	
	2	Label the microtube lids with section, group, “wat” for water, and 1-8, e.g. B02-C-wat5. Consistent labeling is important throughout the course, as there are many samples being collected and analyzed.	
	3	Before measuring the tubes, zero the balance (i.e. tare it with nothing on it). Make sure it is displaying mass in grams (g).	How many digits are displayed by the balance?
	4	Weigh tube 1. Enter the mass in Data Table using the appropriate number of significant figures as displayed on the balance.	
	5	Repeat for tubes 2-8.	

Part B: Using micropipettes

Completed	Step	Protocol	Progress check
	1	Pick up any micropipette Familiarize with the micropipette by identifying and examining the features of the micropipettes listed below.	
	2	**Plunger:** Depress the plunger. There are two stops when pressing down. Importance of these stops is examined in our experiment.	
	3	**Dial:** Use the dial to set the desired volume to be delivered. Do not turn the dial past the minimum or maximum volume!	What do you think will happen if the dial is turned past the minimum or maximum volume?
	4	**Display:** The display indicates how many µL of liquid the micropipette is set to measure. Adjust this amount by turning the dial. The numbers are read from the top (closest to the plunger) to the bottom.	
	5	**Shaft:** Disposable tips are attached to the bottom of the shaft by firmly pushing down onto a tip. Tips come in various sizes. We will use the same small diameter tips with the P10 and P100 and large diameter tips with the P1000. Tips must be used to transfer liquids or solutions.	Why do you think we must use pipette tips when transferring solutions with micropipettes?
	6	**Eject button:** Press the eject button to release a used tip directly into the appropriate waste container. Do not remove used tips with hands.	Why do you think we should not remove used pipette tips with our hands?

Part C: Pipetting water into microtubes

Completed	Step	Protocol	Progress check
	1	Fill a small beaker with water from the sink.	
	2	Determine which micropipette is appropriate to use to pipette 60 µL.	Which micropipette is the most appropriate for 60 µL?
	3	Using the correct method here, pipette 60 µL of water each into microtubes 1 and 2: a. Attach the appropriately sized tip. b. Depress the plunger to the first stop and gently hold your thumb in position. Do not go past the first stop! c. Keeping your thumb in position, insert the end of the tip just below the water. d. Slowly release the plunger to draw the water into the tip. e. Visually inspect the fluid level in the tip and check for air bubbles. f. Move the micropipette to an empty microtube. g. Slowly press the plunger to the second stop to deliver all of the liquid within the tip. There should be no residual liquid. h. Slowly release the plunger. i. Eject the tip into the appropriate waste container (non-hazardous sharps). j. Close the tube.	What do you think will happen if the plunger is depressed past the first stop?
	4	Using the incorrect method here, pipette 60 µL of water each into microtubes 3 and 4: a. Attach the appropriately sized tip.	

		b. Depress the plunger to the second stop and gently hold your thumb in position. Make sure to go past the first stop! c. Keeping your thumb in position, insert the end of the tip just below the water. d. Slowly release the plunger to draw the water into the tip. e. Visually inspect the fluid level in the tip and check for air bubbles. You should notice that, compared to the correct method, there is more fluid in the tip. f. Move the micropipette to an empty microtube. g. Slowly press the plunger to the second stop to deliver the liquid. Some residual liquid may be left in the tip. h. Slowly release the plunger. i. Eject the tip into the appropriate waste container (non-hazardous sharps). j. Close the tubes.	What do you think will happen when the plunger is depressed to the second stop? Why do you think there is now more liquid in the tip compared to the correct method?
	5	Determine which micropipette is appropriate to use to pipette 120 µL.	Which micropipette is the most appropriate for 120 µL?
	6	Using the correct method, pipette 120 µL of water each into microtubes 5 and 6.	
	7	Using the incorrect method, pipette 120 µL of water each into microtubes 7 and 8.	

Part D: Determine the mass of water in each tube

Completed	Step	Protocol	Progress check
	1	Zero the balance.	
	2	Determine the mass of each microtube containing water. Record these data in the data table.	
	3	Open a new Excel file and create the data table in the spreadsheet.	
	4	Calculate the mass of water in the first microtube in Excel using the minus "-" function (e.g. =A3-A2).	
	5	Convert the mass of water in the first microtube from grams (g) to milligrams (mg) using the multiple "*" and/or divide "/" function as appropriate.	Why do you think it is better to do calculations in Excel instead of just entering the results of our calculations?
	6	Calculate the mass of water and perform the same conversion from g into mg by filling data automatically in the spreadsheet. Filling data automatically is a very important skill to learn. Ask an instructional assistant for help!	What do you think are some advantages of filling data automatically instead of performing the multiple calculations separately?
	7	Adjust all values so that they display an appropriate number of significant figures. Use the "→.0" or "←.0" button in the Ribbon (along the top of the spreadsheet) or the Format pull-down menu to change the number of decimal places displayed.	How many digits or decimal places do you think would be appropriate in this case? Why?
	8	Enter the data into the appropriate columns in shared Google spreadsheet.	

| BB2 | Asking Scientific Questions and Testing Hypotheses |

Asking questions and scientifically testing them while also minimizing bias is both simple and takes lots of intentional practice. It is a challenging skill to master despite it often feeling intuitive. Here is a short list of things to consider as we consider asking questions and testing hypotheses.

STEP 1: What is our question? The key is to be specific. Who, what, where, when, how, and why are all things to think about when asking a question. Assessing context and assumptions can help minimize bias. Example question: How effective is the Pfizer vaccine?

- **Who?** Are we talking about a specific age group, sex, health history? Be specific about the group you want to learn about.
- **What?** What do we mean by “efficacy”? Are we talking about preventing any infection, any symptoms, or preventing hospitalization or death?
- **Where?** Where will we be finding our test subjects? A study in South Africa may not be easily generalizable to a US population.
- **When?** When will the data be collected and how often will individuals be tested? Are we collecting short or long-term health data? Might data be collected at a time when a particularly nasty COVID variant is prevalent?
- **How?** How will we collect data? Will you use questionnaires, or will you test subjects for COVID? Each method has its own errors and biases.
- **Why?** What is our reason for conducting your experiment? Are lives at stake? The answer to this may affect the amount of error you allow when making conclusions or recommendations.

STEP 2: Yay! We have a question you’re interested in. Now it is time to construct a hypothesis and design an experiment. Before we do, there is one huge point to remember. The scope of our question will likely be broader than the sample we are actually able to test in your experiment.

Example: Let's say our question asks about the efficacy of the Pfizer vaccine in preventing COVID-related hospitalization in all adults over 16 years old. Will we be able to test all adults over the age of 16? No, we might be able to test as many as 50,000, but that is just a subset of the group.

Our question addresses an entire group of interest. Our experiment measures only a sample of it.

This distinction means there are two answers to our research question:

- The effect that exists in reality for the entire group of interest.
- The effect observed in our data collected from our sample.

We hope that both answers are the same. Since our experiment is a subset of all potential data, there is a chance that our experimental results will not match the true answer – and it's not even our fault!

Question: Is the Pfizer vaccine more effective than placebo?

		Effect observed in your data	
		YES my data show the vaccine and placebo are different	**NO,** my data does not show the vaccine and placebo are different
Effect that exists in reality	**YES** vaccine and placebo are truly different	True positive	False negative
	NO vaccine and placebo are not truly different	False positive	True negative

As a researcher, we are hoping our results match reality (true positive or negative). Through good design and analysis, we can minimize and measure the probability our results are different from reality (false positive or negative).

STEP 3: How can we begin to assess the probability that our results are wrong?

Part A: Understanding the false positive and the magical mysterious p-value
Take our two types of hypotheses:

- Alternate hypotheses: There is an effect! Vaccine is more effective than placebo.
- Null hypothesis: There is no effect. Vaccines are equal in efficacy with placebo.

Assuming the null hypothesis is true, the p-value is the probability that we could have gotten our results or something more extremely different by chance. The lower this probability is, the more likely our results represent a true positive, i.e. significantly different experimental groups.

For example, let's say we have a perfectly fair coin, such that the null hypothesis is true, and the probability of flipping heads or tails is the same. We flip the coin 10 times and get 2 heads. Doesn't sound like a fair coin, but what is the probability of flipping 2 heads by chance with a fair coin? About 4.4%. The p-value would be the probability of flipping 2 heads or something more extreme. This would be the sum of the probabilities of flipping 0, 1, 2, 8, 9, and 10 heads, which happens to be about 11% or a p-value = 0.11.

Yet another way to think about p-values: Let's say we run our experiment and have a p-value of 0.05. Yay! It is a significant difference, and we won science, and all our grants will get funded! Well, maybe. Once again, let's assume that the null is actually true. And imagine we ran our exact same experiment 100 different times. In 5 of those experiments, by random chance, we would think there is a difference, but there really is not. How do we know whether our results are part of that rare 5%, and our positive results are a random fluke? We do not. Only replication and higher sample size might help us better figure that out.

So why is 0.05 the magical p-value number for significance? No particularly specific reason. Historically it was recommended by Fisher in 1925 out of convenience. Everything had to be calculated by hand at that time anyway. This threshold is called the alpha value, and the researcher should set it based on the amount of risk they are willing to assume if the results are a false positive.

That is why the significance threshold should be as low as 0.01 or 0.001 or even smaller if we are testing the safety of a medicine that could possibly result in death.

Part B: What about false negatives? Are they not powerfully important too?
p-values do not directly tell us anything about the power of an analysis, which is the ability of our experiment to see an effect that is actually present. Statistical power = 1 i.e. the probability of a false negative. Good power is often thought to be around 0.80. This means that if an effect is present, our data analysis will see it 80% of the time. Unfortunately, much of the research published today has much lower power, and it is often not reported in articles. Is this not awful? Tell me about it! Change the system and speak truth to power.

STEP 4: How do we put all of this together?
Does a p-value tell us anything about effect size, i.e. how different two groups are? No, not directly. This is often measured by other statistics such as Cohen's D or Glass' Delta. It is also not reported as often as it should be. Well, that does not sound right. What should we do? It is good to report all relevant statistics: p-value, power, sample size, and effect size. In fact, these are things we should incorporate as early as the design phase of our experiment.

- Determine the effect size we would like to be able to see in our experiment. Sometimes we do not need to be able to tell if the difference between groups is 0.001%.
- Determine power and p-value thresholds we are comfortable with before we collect all our data.
- Decide on the appropriate statistical test(s) we will use to test our hypothesis.
- Use a power analysis to estimate the sample size needed to be able to see a given effect at our proposed power.
- Run our experiment with that sample size and calculate the actual (i.e. post-hoc) measures of power, effect size, and p-value. Report our results.
- Do not selectively change our analysis, experimental design, or sample size without sufficient reason. Doing so is p-hacking and N-hacking and leads to biased results and conclusions.

In this course, we are not doing a power analysis and mostly making figures and reporting p-values. Is that okay? Yes! Nothing we are doing is wrong. It is just that we will continue to learn more about some of these additional considerations in other courses.

Additional resources

- JoVE Laboratory Manual Database. Biology: Scientific method. Journal of Visual Experimentation, Cambridge, MA (2024). https://app.jove.com/science-education/v/10552/concepts/scientific-method
- Nuzzo, R. Scientific method: Statistical errors. Nature 506, 150-152 (2014). https://doi.org/10.1038/506150a

| BB3 | Performing Statistical Analysis |

Learning objectives

- Increase proficiency with Microsoft Excel
- Compare correct and incorrect pipetting techniques using statistics

Overview

Recall that we pipetted correctly (depressing the plunger to the first stop before filling the tip) and incorrectly (depressing the plunger to the second stop before filling the tip). We will use statistics to determine whether the volume pipetted using the correct method is different from the volume pipetted using the incorrect method.

A t-test will be performed to determine if the volume pipetted using the correct technique is the same as the volume pipetted using the incorrect technique. A t-test is a common statistical test to compare two sets of continuous data sampled from populations that are normally distributed. There are 1-tailed and 2-tailed versions of the t-test. We will use the 2-tailed version because we are not making any assumptions regarding which pipetting method (correct or incorrect) leads to larger measurements.

The result of the t-test will be a p-value, which quantifies the probability that the two samples come from the same population. The lower the p-value, the less likely the samples are to have come from the same population. A common (but relatively arbitrary) threshold is $p=0.05$. If $p<0.05$, there is less than a 5% chance that pipetting correctly gave the same volume as pipetting incorrectly. Therefore, we can conclude that the samples pipetted using the correct technique have a different volume than the samples pipetted using the incorrect technique. This result would validate the importance of using the correct technique.

Protocol

Throughout the laboratory manual, the “completed” column of boxes on the left in all the protocols are for the group to check off the steps as they are performed. This will help the group keep track of where everyone is together in the experiments. The “progress check” column on the right in all the protocols are questions and notes to consider as the experiments are being performed. Please make sure to include all the relevant information from this column and elsewhere in the research notebooks.

Data table

Statistical analysis for correct versus incorrect method of pipetting

Volume (µL)	Method	Average (mg)	St. Dev. (mg)	p-value
60	Correct			
60	Incorrect			
120	Correct			
120	Incorrect			

Make sure to write down all the relevant data collected in this protocol. Afterwards, this information needs to be transferred to the shared data sheet. Measurements (numbers) must be entered into computer spreadsheets without units (letters) to perform calculations. **Record this information in the research notebook.**

Excel functions and operations

- **=AVERAGE()**: A function to calculate the arithmetic mean. The syntax is: =AVERAGE(argument_#1,argument_#2,….). Example: =AVERAGE(22,67,F12), where 22 and 67 are values, and F12 references the value within cell F12.
- **=STDEV()**: A function to calculate the standard deviation. The syntax is: =STDEV(argument_#1,argument_#2,….). Example: =STDEV(22,67,F12), where 22 and 67 are values, and F12 references the value within cell F12.
- **Sort**: A feature used to arrange data in alphabetical, numerical, or other defined orders. Sort can be found under the Data menu.
- **=TTEST()**: A function to calculate p-value associated with a t-test. The syntax to perform a 2-tailed t-test to compare samples drawn from populations with unequal variance is: =TTEST(array1,array2,2,3), where the two arrays are the cell ranges containing the two data sets.

Performing t-tests and calculating p-values

Completed	Step	Protocol	Progress check
	1	When all the groups in the lab section (one lab room) have entered their data onto the shared Google spreadsheet, download it as an Excel file to work on it independently.	
	2	Using the “sort” function under the “data” tab, sort the data so that all the related data are close to one another. For each volume, we will compare mass of water pipetted using correct versus incorrect method. Determine which column(s) need to be sorted in which order.	
	3	Check with an instructional assistant to make sure that the data are sorted in the most efficient manner for the analysis.	
	4	Use the =AVERAGE() and =STDEV() functions to calculate the averages and standard deviations for each of the data sets (i.e. 60 µL correct, 60 µL incorrect, 120 µL correct, and 120 µL incorrect). Record this information in the data table.	
	5	When using Excel for calculations, it is good practice to do a reality check by comparing the calculated values with the raw data.	What should we be comparing here? What might indicate that we could have a problem?
	6	For the 60 µL data, use the =TTEST() function to determine whether the volume pipetted using the correct versus incorrect method is different.	Do you think the data would be different based on the t-test result? Why or why not?

	7	Perform another t-test using the =TTEST() function to compare the 120 µL data using the correct versus incorrect method.	Do you think the results would be the same as the 60 µL data? Why or why not?
	8	Based on the p-values, determine if pipetting correctly versus incorrectly matters in terms of the volume being transferred.	Did you reach the same conclusion for the 60 µL data as for the 120 µL data?
	9	Excel has a help feature that provides additional information about the t-test and other Excel functions. Search for information online at: https://support.office.com	What is one additional thing that you learned about Excel functions by doing this search?

Additional thought questions

1. If we obtained a p-value of 0.04 from one of the pipetting experiments, does that mean that pipetting to the first and second stops are definitively different? Why?
2. If we obtained a p-value of 0.06 from one of pipetting experiments, does that mean that pipetting to the first and second stops are definitively the same? Why?
3. If we obtained a p-value of 0.06 from the dataset based on our lab section, would we expect the p-value from the entire dataset based on all four lab sections to be different? Why?
4. Let's say that there was one additional 60-µL first stop data point with a mass of 82 mg. How would that affect the p-value and your conclusions? Why? What might have happened?
5. We find out for some of our data points that one of our group members accidentally forgot to subtract the mass of the tube. How might we handle this as the research group? Why?
6. Compare the p-values for the 60-µL and 120-µL experiments. Which p-value is smaller? Why might this be the case?

| SP0 | Soil Properties |

To characterize the environments from which the soil samples were collected, we will measure pH and moisture content. These data will be analyzed to determine if, on the day the samples were collected, pH and/or moisture content of the soil samples were different. Similar data are collected each quarter and pooled, so that in the future, we can investigate trends over time.

Resources

- The roles of microbes in plant nutrition
- What is pH?
- Using a pH meter
- Determination of moisture content in soil

| SP1 | Soil pH

Objectives

- Test if pH is different for between our soil samples

Overview

To begin to characterize our soil samples, we will first re-suspend the soil in water and measure the pH of the soil and water mixture using a pH meter.

Protocol

Throughout the laboratory manual, the “completed” column of boxes on the left in all the protocols are for the group to check off the steps as they are performed. This will help the group keep track of where everyone is together in the experiments. The “progress check” column on the right in all the protocols are questions and notes to consider as the experiments are being performed. Please make sure to include all the relevant information from this column and elsewhere in the research notebooks.

Graphic summary

Researchers and scientists often use schematic diagrams to represent information. Such a summary provides a visual representation to help the reader to quickly gain an overview on the protocol, as well as some salient details. Producing such a summary can also help us develop a deeper understanding of the protocol. In some cases, we will create graphic summaries to help us prepare for laboratory work, and we may refer back to these summaries in the laboratory as references.

Data table

Plant soil	Invasive or native	Average	St. Dev.	p-value

Make sure to write down all the relevant data collected in this protocol. Afterwards, this information needs to be transferred to the shared data sheet. Measurements (numbers) must be entered into

computer spreadsheets without units (letters) to perform calculations. **Record this information in the research notebook.**

Excel functions and operations

- **=AVERAGE()**: A function to calculate the arithmetic mean. The syntax is: =AVERAGE(argument_#1,argument_#2,….). Example: =AVERAGE(22,67,F12), where 22 and 67 are values, and F12 references the value within cell F12.
- **=STDEV()**: A function to calculate the standard deviation. The syntax is: =STDEV(argument_#1,argument_#2,….). Example: =STDEV(22,67,F12), where 22 and 67 are values, and F12 references the value within cell F12.
- **Sort**: A feature used to arrange data in alphabetical, numerical, or other defined orders. Sort can be found under the Data menu.
- **=TTEST()**: A function to calculate p-value associated with a t-test. The syntax to perform a 2-tailed t-test to compare samples drawn from populations with unequal variance is: =TTEST(array1,array2,2,3), where the two arrays are the cell ranges containing the two data sets.

Part A: Data collection

Completed	Step	Protocol	Progress check
	1	Obtain the soil aliquot and a pre-labeled vial. An aliquot is a portion of a sample.	
	2	Tare the balance on the empty vial.	
	3	Add approximately 3 g of soil to vial*. *Weigh paper should be used whenever weighing soil.	
	4	Measure and record mass of soil using number of significant figures that represents precision of the balance.	How many digits are displayed by the balance?
	5	Add 12 mL of sterile deionized (DI) water to soil and tightly cap the vial.	
	6	With the vortex mixer set to “Auto” and the speed set at maximum, push the vial gently down onto the platform to initiate mixing. Vortex at maximum speed for 1 min to thoroughly mix soil and water. Use an online timer on lab computers to avoid touching your cell phone to prevent contamination.	
	7	Remove the cap and incubate the mixture at room temperature for 30 min while swirling gently every 5 min. This time allows the soil and water to equilibrate.	Are there other parts of the project that can be completed during this incubation?
	8	Measure pH using the pH meter, which is an instrument used to measure concentration of hydrogen ions [H^+] in a solution. The pH meter was calibrated before laboratory sections. pH meters require periodic calibration to maintain accuracy. Check with	

		an instructional assistant if you think the pH meter requires re-calibration. a. Remove the pH probe from the storage solution and rinse it with water. b. Place the probe in the sample and wait for the reading to stabilize. c. Rinse the pH probe with water and return it to the storage solution.	
	9	Record the pH in research notebook and shared data spreadsheet.	

Part B: Data analysis

Completed	Step	Protocol	Progress check
	1	When all groups' teams have entered their data into the shared data spreadsheet, download it as an Excel file to work on the data independently.	
	2	Use the "Sort" function under the "Data" tab to sort the data so that all of the related data are close to one another. The Sort function arranges data in alphabetical, numerical, or other defined orders.	
	3	Calculate averages and standard deviations for each data sets. **Record this information** in the data table and in research notebook.	What Excel functions would you use for these calculations?
	4	Determine if pH is statistically different for the two soil samples. **Record this information** in the data table and in research notebook.	What Excel functions would you use for these calculations?

| SP2 | Moisture Content Sample Preparation

Objectives

- Initiate an experiment to test if moisture content is different for across soil samples

Overview

We will measure the soil moisture by determining the mass of approximately 1 gram of soil before and after drying, which evaporates water from the soil.

Protocol

Throughout the laboratory manual, the “completed” column of boxes on the left in all the protocols are for the group to check off the steps as they are performed. This will help the group keep track of where everyone is together in the experiments. The “progress check” column on the right in all the protocols are questions and notes to consider as the experiments are being performed. Please make sure to include all the relevant information from this column and elsewhere in the research notebooks.

Graphic summary

Researchers and scientists often use schematic diagrams to represent information. Such a summary provides a visual representation to help the reader to quickly gain an overview on the protocol, as well as some salient details. Producing such a summary can also help us develop a deeper understanding of the protocol. In some cases, we will create graphic summaries to help us prepare for laboratory work, and we may refer back to these summaries in the laboratory as references.

Data table

Moisture content of soil sample

Measurement or calculation	Mass (g)
Measured mass of vial and cap	
Measured mass of vial, cap, and soil	
Calculated mass of soil	
Measured mass of vial, cap, and dried soil	Experiment SP3
Calculated mass of dried soil	Experiment SP3

Make sure to write down all the relevant data collected in this protocol. Afterwards, this information needs to be transferred to the shared data sheet. Measurements (numbers) must be entered into computer spreadsheets without units (letters) to perform calculations. **Record this information in the research notebook.**

Sample preparation

Completed	Step	Protocol	Progress check
	1	Obtain a pre-labeled vial.	
	2	Measure and record mass of vial and cap. Record this information in the data table and in research notebook. Use the ←.0 or →.0 button to change the number of decimal places displayed.	Why is it necessary to weigh the vial and cap rather than tare the balance on the vial as we did for measuring pH?
	3	Add approximately 1 g of soil to vial*. Measure and record mass of the vial, cap, and soil. Record this information in the data table and in research notebook using the appropriate number of significant figures. *Weigh paper should be used whenever weighing soil.	
	4	Calculate and record mass of soil using the number of significant figures that represents precision of the balance.	Describe in your own words how you would perform this calculation. Write an equation for how you would perform this calculation. When you are writing your explanation and equation, think about why they make sense.
	5	Remove the cap and store it for use in the next experiment.	Why do you think the cap must be removed before placing the vial in the oven?
	6	Place the soil sample in a laboratory oven set at 105–110°C and dry for 1 week.	

| SP3 | Moisture Content Analysis |

Objectives

- Test if moisture content is different for the soil samples.

Overview

The soil samples were dried for one week in a laboratory oven set at 105-110°C. The instructional assistants removed each vial of soil from the drying oven and immediately sealed the vial using the same cap that was measured previously.

Protocol

Throughout the laboratory manual, the “completed” column of boxes on the left in all the protocols are for the group to check off the steps as they are performed. This will help the group keep track of where everyone is together in the experiments. The “progress check” column on the right in all the protocols are questions and notes to consider as the experiments are being performed. Please make sure to include all the relevant information from this column and elsewhere in the research notebooks.

Graphic summary

Researchers and scientists often use schematic diagrams to represent information. Such a summary provides a visual representation to help the reader to quickly gain an overview on the protocol, as well as some salient details. Producing such a summary can also help us develop a deeper understanding of the protocol. In some cases, we will create graphic summaries to help us prepare for laboratory work, and we may refer back to these summaries in the laboratory as references.

Data table

Moisture content of soil sample

Measurement or calculation	Mass (g)
Measured mass of vial and cap	Transfer from Experiment SP2
Measured mass of vial, cap, and soil	Transfer from Experiment SP2
Calculated mass of soil	Transfer from Experiment SP2
Measured mass of vial, cap, and dried soil	
Calculated mass of dried soil	

Statistical analysis for moisture content of soil samples

Plant soil	Invasive or native	Average	St. Dev.	p-value

Make sure to write down all the relevant data collected in this protocol. Afterwards, this information needs to be transferred to the shared data sheet. Measurements (numbers) must be entered into computer spreadsheets without units (letters) to perform calculations. **Record this information in the research notebook.**

Excel functions and operations

- **=AVERAGE()**: A function to calculate the arithmetic mean. The syntax is: =AVERAGE(argument_#1,argument_#2,….). Example: =AVERAGE(22,67,F12), where 22 and 67 are values, and F12 references the value within cell F12.
- **=STDEV()**: A function to calculate the standard deviation. The syntax is: =STDEV(argument_#1,argument_#2,….). Example: =STDEV(22,67,F12), where 22 and 67 are values, and F12 references the value within cell F12.
- **=TTEST()**: A function to calculate p-value associated with a t-test. The syntax to perform a 2-tailed t-test to compare samples drawn from populations with unequal variance is: =TTEST(array1,array2,2,3), where the two arrays are the cell ranges containing the two data sets.

- **Sort**: A feature used to arrange data in alphabetical, numerical, or other defined orders. Sort can be found under the Data menu. More details: https://support.microsoft.com/en-us/office/sort-data-in-a-range-or-table-62d0b95d-2a90-4610-a6ae-2e545c4a4654
- **Autofill**: A feature used to fill cells with data that follows a pattern (of values and/or formulas) or are based on data in other cells. More details: https://support.microsoft.com/en-us/office/fill-data-automatically-in-worksheet-cells-74e31bdd-d993-45da-aa82-35a236c5b5db

Part A: Data Collection

Completed	Step	Protocol	Progress check
	1	Measure and record mass of vial, cap, and dry soil. Record this information in the data table and in research notebook. Use the ←.0 or →.0 button to change the number of decimal places displayed.	What would a reasonable number of significant figures to use in this calculation? Why?
	2	Calculate and record mass of soil using the number of significant figures that represents precision of the balance.	Describe in your own words how you would perform this calculation. Write an equation for how you would perform this calculation. When you are writing your explanation and equation, think about why they make sense.
	3	Calculate and record percent moisture in the original soil sample. Record this information in the data table and in research notebook.	

Part B: Data Analysis

Completed	Step	Protocol	Progress check
	1	When all groups' teams have entered their data into the shared data spreadsheet, download it as an Excel file to work on the data independently.	

	2	Use the “Sort” function under the “Data” tab to sort the data so that all of the related data are close to one another. The Sort function arranges data in alphabetical, numerical, or other defined orders.	
	3	Calculate moisture content for the first row of data. Make sure to use dry soil mass in the denominator as we learned in our reading.	Why is it more useful to report moisture content rather than mass of moisture?
	4	Calculate moisture content for all the soil samples by filling data automatically in the spreadsheet. If you have questions, ask an instructional assistant!	Why do you think it is important to fill data automatically versus manually repeating the calculations for different cells?
	5	Calculate averages and standard deviations for each data sets. Record this information in the data table and in research notebook.	What Excel functions would you use for these calculations?
	6	Determine if moisture content is statistically different for the two soil samples. Record this information in the data table and in research notebook.	What Excel functions would you use for these calculations?

| TB0 | Taxonomic Biodiversity |

The soil samples are home to a large and diverse group of microorganisms, making up the soil microbiome. These microorganisms need specific nutrients and growth conditions to survive. While it is not possible to create the conditions in the laboratory that will support the growth of all of the microorganisms in our soil samples, a subset of these microorganisms will grow on plates containing culture media with different sets of nutrients.

In this project, we will add our soil samples to plates containing three different types of standard laboratory media: trypticase soy agar (TSA), lysogeny broth (LB), and MacConkey agar (MA). We will then observe what grows on these plates. We will be able to compare this taxonomic biodiversity of our samples with the functional and genetic biodiversity results that we will collect and analyze.

Resources

- Microbial and fungal diversity
- Culturing and enumerating bacteria from soil samples
- Enrichment cultures: Culturing aerobic and anaerobic microbes on selective and differential media

| TB1 | Plating Soil Samples on Different Culture Media |

Part A: Practice Plate

Completed	Step	Protocol	Progress check
	1	Obtain a microtube filled with dye.	
	2	Pipette 100 µL of dye into the center of the practice plate using the “clam-shell” technique to open the plate. Share a plate for practice with your lab partner, and the instructional team will demonstrate how to do this “clam-shell” technique.	Why do you think such a technique is important to avoid contamination?
	3	Open the package of sterile plastic cell spreaders being careful to touch only the handles to avoid contamination.	
	4	With the sterile plastic cell spreader, spread the dye evenly over the surface of the plate medium using the “clam-shell” technique to open the plate.	
	5	Discard the used cell spreader and all waste appropriately.	

Part B: Culture Media Plates

Completed	Step	Protocol	Progress check
	1	Label six plates as described: TSA Dilution #1, TSA Dilution #2, LB Dilution #1, LB Dilution #2, MA Dilution #1, and MA Dilution #2. Be sure to label the bottom edge of the plates and not the lids. Also include section, group, and soil sample in addition culture medium on each plate.	Why do you think it is important to label the bottom of the plates and not the lids?
	2	Add 14.5 mL of sterile water to the 50-ml tube with 0.5 g of previously weighed soil. Be careful not to touch the pipet to the soil when adding water. Vortex for 5 seconds. Label this tube “Soil Solution”.	
	3	Using the same pipette, add 14.5 mL of sterile water to a new 50-mL conical tube. Label this tube “Soil Dilution #1”.	Why do you think it is okay to use the same pipet here?
	4	Transfer 500 µL of soil solution to the new tube above. Vortex for 5 seconds.	
	5	Using proper technique to open the plate, pipette 100 µL of Soil Dilution #1 into the center of TSA Dilution #1. Using a sterile cell spreader, spread the solution evenly over the surface of the plate. Discard the spreader appropriately.	
	6	Repeat the above step for the LB Dilution #1 and MA Dilution #1 plates using a new sterile cell spreader each time.	Why do you think it is important to use a new sterile spreader each time?
	7	Transfer 100 µL of Soil Dilution #1 to a microtube. Then add 400 µL of sterile water.	

		Vortex for 5 seconds. Label this microtubes “Soil Dilution #2”.	
	8	Using proper technique to open the plate, pipette 100 µL of Soil Dilution #2 into the center of TSA Dilution #2. Using a sterile cell spreader, spread the solution evenly over the surface of the plate. Discard the spreader appropriately.	
	9	Repeat the above step for the LB Dilution #2 and MA Dilution #2 plates using a new sterile cell spreader each time.	
	10	Bring all six culture plates to the front bench. We will incubate them for one week at 25°C.	
	11	Do not discord the original soil sample. Bring it to the front bench for storage.	
	12	Now discard the soil solution and dilutions.	

| TB2 | Taxonomic Biodiversity - Analysis |

Objectives

- Determine if taxonomic biodiversity is different for the soil samples.

Overview

Our plates were incubated for one week at 25°C to allow for the growth of microorganisms on different types of media. We will visually analyze and evaluate the results from different plates.

Protocol

Throughout the laboratory manual, the “completed” column of boxes on the left in all the protocols are for the group to check off the steps as they are performed. This will help the group keep track of where everyone is together in the experiments. The “progress check” column on the right in all the protocols are questions and notes to consider as the experiments are being performed. Please make sure to include all the relevant information from this column and elsewhere in the research notebooks.

Graphic summary

Researchers and scientists often use schematic diagrams to represent information. Such a summary provides a visual representation to help the reader to quickly gain an overview on the protocol, as well as some salient details. Producing such a summary can also help us develop a deeper understanding of the protocol. In some cases, we will create graphic summaries to help us prepare for laboratory work, and we may refer back to these summaries in the laboratory as references.

Data table

Number of characteristics of colonies

Plate	Number of colonies	Description of colonies
TSA Dilution #1		
TSA Dilution #2		
LB Dilution #1		
LB Dilution #2		
MA Dilution #1		
MA Dilution #2		

Make sure to write down all the relevant data collected in this protocol. Afterwards, this information needs to be transferred to the shared data sheet. Measurements (numbers) must be entered into computer spreadsheets without units (letters) to perform calculations. **Record this information in the research notebook.**

Counting and observing colonies on plates

Completed	Step	Protocol	Progress check
	0	For safety, the instructional team has sealed the plates with parafilm. Do not remove the parafilm.	
	1	Look at each of the plates and write down what you see: number of colonies, color, shape, characteristics, etc.	What type of descriptions would be useful to include here?

| FB0 | Functional Biodiversity |

Functional biodiversity is a measure of the variability in how organisms within an ecosystem perform specific functions (e.g. metabolism, motility, respiration, etc.). We will characterize the diversity of carbon sources utilized by the microbial community within our soil samples and calculate common indicators of biodiversity: richness (S), the Shannon Diversity Index (H), and evenness (E).

Resources

- Community diversity
- Measuring biodiversity

| FB1 | EcoPlate™ Preparation |

Objectives

- Initiate an experiment to study carbon source utilization by microbial community in soil samples.

Overview

Functional biodiversity is a measure of the variability in how organisms within an ecosystem perform specific functions, e.g. metabolism, motility, respiration, etc. In this experiment, we will characterize the diversity of carbon sources that are utilized by the microbial community within our soil samples.

To examine carbon source utilization, we will use EcoPlate™, which is a specialized multi-well plates containing 31 different carbon sources commonly used by soil microbes. Each carbon source is present in three wells within the plate, allowing us to simultaneously measure carbon source utilization by soil microbes as well as E. coli (a well-characterized microorganism) and sterile deionized (DI) water as controls. As microbes use the carbon source present within a well, they begin to respire, which turns a dye, also contained within the well, purple. A dark purple well indicates high utilization of the carbon source within that well. Color development within the EcoPlate™ wells will be determined by measuring the change in absorbance at 590 nm (A590) that occurs between the day the plates are prepared and one week later

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
B	B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
C	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
D	D1	D2	D3	D4	D1	D2	D3	D4	D1	D2	D3	D4
E	E1	E2	E3	E4	E1	E2	E3	E4	E1	E2	E3	E4
F	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
G	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4
H	H1	H2	H3	H4	H1	H2	H3	H4	H1	H2	H3	H4

Blank:

A1: Water

Carboxylic Acids:

F2: D-Glucosaminic Acid

A3: D-Galactonic Acid γ-Lactone

B3: D-Galacturonic Acid

C3: 2-Hydroxy Benzoic Acid

D3: 4-Hydroxy Benzoic Acid

E3: γ-Hydroxybutyric Acid

F3: Itaconic Acid

G3: α-Ketobutyric Acid

H3: D-Malic Acid

Amino Acids:

A4: L-Arginine

B4: L-Asparagine

C4: L-Phenylalanine

D4: L-Serine

E4: L-Threonine

F4: Glycyl-L-Glutamic Acid

Carbohydrates:

B1: Pyruvic Acid Methyl Ester

G1: D-Cellobiose

H1: α-D-Lactose

A2: β-Methyl-D-Glucoside

B2: D-Xylose

C2: i-Erythritol

D2: D-Mannitol

E2: N-Acetyl-D-Glucosamine

G2: Glucose-1-Phosphate

H2: D, L-α-Glycerol Phosphate

Polymers:

C1: Tween 40

D1: Tween 80

E1: α-Cyclodextrin

F1: Glycogen

Amines:

G4: Phenylethyl-amine

H4: Putrescine

Dilutions

The ability to accurately and precisely perform dilutions is an essential laboratory skill. Demonstrating mastery of this skill can be helpful in securing an undergraduate research experience or internship.

Instructions for diluting a solution can be given in various formats. For example, the instructions may say to make a 1:10 dilution, to make a 1/10 dilution, to dilute a sample 10-fold, or to use a dilution factor of 10. In all of these cases, we will mix 1 “part” of the sample with 9 “parts” of the diluent, which is frequently water or a buffer. The word “part” here simply refers to a unit of measurement, for example, a µL or mL. Note: The 1:10 terminology is very confusing. It would be incorrect to mix 1 part of the sample with 10 parts of the diluent, which will actually result in a total of 11 parts!

Protocol

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Graphic summary

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Part A: Practice plate

Completed	Step	Protocol	Progress check
	1	Obtain a practice EcoPlate™.	
	2	Taking turns with all members of the team, practice pipetting 100 µL of water into each well within the plate. Observe how even the levels of water are within the wells and refine toward consistently pipetting 100 µL.	Determine which micropipette is appropriate to use for 100 µL. Record this information.

Part B: Sterile DI water (columns 1-4)

Completed	Step	Protocol	Progress check
	1	Trade in your practice plate for an unused and sterile EcoPlate™. Do not set up samples in the practice plate!	Are sterile DI water samples the experiment or positive or negative control? Explain.
	2	Label the slanted side of the EcoPlate™ with section-team (e.g. B02-C). Do not label the lid. To read the absorbance, light will pass through the plate (and the lid) in the spectrophotometer.	Why do you think we should not label the lid?
	3	Add 100 µL of sterile DI water to each well in columns 1-4. Water should be added to a total of 32 wells.	

Part C: E. coli culture (columns 5-8)

Completed	Step	Protocol	Progress check
	1	Obtain a saturated culture of E. coli. A culture in which cells have grown to their maximum density is called “saturated”.	Are E. coli samples the experiment or positive or negative control? Explain.

	2	Dilute 500 µL of the E. coli culture with 9.5 mL of sterile DI water. Measure E. coli with a micropipette and the sterile DI water with a serological pipette.	How would you call this dilution? What would xx be in this 1:xx dilution terminology?
	3	Add 100 µL of diluted E. coli culture to each well in columns 5-8. Dilute culture should be added to a total of 32 wells.	

Part D: Suspension of soil microbes (columns 9–12)

Completed	Step	Protocol	Progress check
	1	Retrieve the stored soil sample for this functional biodiversity protocol.	Are soil samples the experiment or positive or negative control? Explain.
	2	Uncap the tube. Using a serological pipette, add 14.5 mL of sterile DI water to the tube and cap tightly. This is the first step in a two-step or serial dilution.	Recall that there is 0.5 g of soil in the tube. Assuming that 1 g is equivalent to 1 mL, how would you call this dilution? What would xx be in this 1:xx dilution terminology?
	3	Vortex at maximum speed for 5 minutes to re-suspend the microbes present in the soil.	
	4	Dilute 500 µL of the soil suspension with 14.5 mL of sterile DI water. This is the second step in a two-step or serial dilution. Instead of a two-step dilution, we could have diluted the original 0.5 g sample in 449.5 mL of water to achieve the same final concentration.	How would you call this second dilution? What would xx be in this 1:xx dilution terminology? What is the final dilution after this two-step serial dilution? Why did we not do one dilution with 0.5 g and 449.5 ml?
	5	Shake to thoroughly mix the suspension.	

	6	Wait 2 minutes for the soil particles to settle to the bottom, leaving the microbes in the suspension above.	
	7	Add 100 µL of the final soil suspension to each well in columns 9-12. Final diluted soil should be added to a total of 32 wells.	
	8	Verify that the fluid level is uniform across the wells, i.e. each well having 100 µL in it. All 96 wells in the plate should have fluid at the same level. If not, consult with an instructional assistant!	

Part E: EcoPlate™ baseline reading and incubation

Completed	Step	Protocol	Progress check
	1	To get rid of any bubbles that will interfere with the absorbance readings, ask an instructional assistant to spin the EcoPlate™ in a plate centrifuge.	
	2	Observe as the instructional assistant uses a plate spectrophotometer to read the absorbance of each well at 590 nm (A590). Make this measurement with the lid off.	
	3	Record the absorbance readings in research notebooks and shared datasheet.	
	4	Cover the EcoPlate™ using plastic wrap and place it in a sealed container with a wet paper towels lining the bottom.	
	5	Incubate the EcoPlate™ at 25°C for 1 week.	

| FB2 | EcoPlate™ Analysis |

Objectives

- Characterize diversity of carbon sources utilized by microbiomes in soil samples

Overview

Our experiment is designed to characterize the diversity of carbon sources utilized by the microbial community within our soil samples. We incubated soil samples in specialized multi-well plates called EcoPlate™, which contains 31 different carbon sources of 5 different types: carbohydrates, carboxylic acids, amines, amino acids, polymers. As microbes use a carbon source present within a well, they respire and turn a colorless indicator purple. A dark purple well indicates high utilization of the carbon source within that well. Well color is measured by the absorbance at 590 nm (A590).

In the previous week, baseline A590 is measured; this week, the A590 is measured again. We calculate the change in absorbance that occurred during the one-week incubation. An increase in absorbance with time indicates that the soil microbes utilized the carbon source within that well. As experimental controls, we will also measure A590 for wells incubated with E. coli, a relatively well-characterized microorganism, and sterile deionized water. In this experiment, substantial color development or change in A590 with incubation time will be defined as 0.25 units. Color development at or below this threshold will be considered negligible. Color development above this threshold indicates that the soil microbes used the carbon source present in the well.

Protocol

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Graphic summary

Researchers and scientists often use schematic diagrams to represent information. Such a summary provides a visual representation to help the reader to quickly gain an overview on the protocol, as well as some salient details. Producing such a summary can also help us develop a deeper understanding of the protocol. In some cases, we will create graphic summaries to help us prepare for laboratory work, and we may refer back to these summaries in the laboratory as references.

Excel functions

=IF(): A function that performs a logic test and then assigns one value if the logical test is True and another value if the logical test is False. The syntax is =IF(logical_test, value_if_true, value_if_false). Useful symbols for logical test are >, <, =, <=, and >=. Use quotation marks around the value_if_true and/or value_if_false if they are text rather than numerical. Empty quotation marks (i.e. “”) will result in an empty cell. Examples: =IF(B2>B3,“Win”,“Loss”); =IF(F11>B15,1, “”)

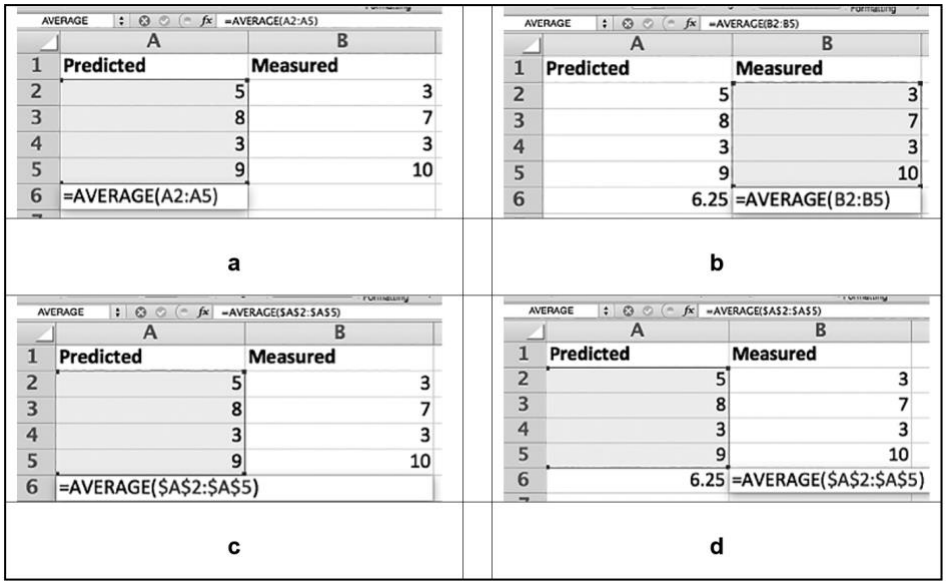
=AVERAGE(): A function to calculate arithmetic mean. The syntax is =AVERAGE(argument_#1, argument_#2, …). Example: =AVERAGE(22,67,F12), where 22 and 67 are values, and F12 references the value within cell F12.

=SUM(): A function to add all the arguments within parentheses. The arguments can be numeric values, references to cells, or a combination of both. The syntax is =SUM(argument_#1, argument_#2, …). Example: =SUM(1,5,A5)

What if we need to include many cells within a single column or row? Instead of listing them all out individually such as “=SUM(A1,A2,A3,A4)”, we can use the colon feature. Simply write the first and last cell in our function with a colon in between to capture all values “=SUM(A1:A4)”

$: A dollar sign is used to designate fixed references in Excel. Include a $ before the column/letter and/or row/number within a formula that you want to cut and paste into other cells without changing the cells referenced within the formula. Example: =A5/$A$1

Relative and fixed references: To average values in a column, use the =AVERAGE() function. Notice below that each cell used for the calculation is referenced by a row number and column letter. In this example, the value calculated in A6 will be the average of the values in cells A2, A3, A4, and A5. Notice that Excel highlights the cells used for a calculation. If we select cell A6, copy, and then paste into cell B6, the new formula now references the four cells directly above B6 and the value calculated in B6 will be the average of the values in cells B2, B3, B4, and B5.



Notice from the example above, by default, Excel defines cells referenced in a formula relative to the cell containing the formula. Therefore, if we copy and paste a formula into a neighboring cell, the cells referenced within the formula will change. The above example uses relative references.

In many cases, it is helpful to use fixed references rather than relative references. A formula containing a fixed reference will always refer to the same cell(s) regardless of which cell contains the formula. To create a fixed reference, include a $ before both the column/letter and row/number. Notice that in this case, when we select A6, copy, and paste it into cell B6, the highlighted cells used for the calculation remain the same. In other words, the formula contains fixed references. It is also possible to fix only the column/letter (e.g., $A2:$A5) or the row/number (e.g., A$2:A$5).

Part A: EcoPlate™ reading after incubation

Completed	Step	Protocol	Progress check
	1	Retrieve EcoPlate™ from sealed container, remove the plate's lid, and read the A590.	
	2	Record absorbance readings in research notebook and shared data sheet.	

Part B: Analysis of sterile DI water samples (columns 1-4)

Completed	Step	Protocol	Progress check
	1	Visually inspect these wells and confirm that there is not substantial color development. Record this observation.	
	2	Look at the corresponding absorbance data and confirm that there are no readings above 0.25. In this protocol, we consider A590 below this threshold to be negligible.	
	3	If there are one or more absorbance >0.25 readings, discuss within the team and decide how this should affect your interpretation of the soil sample data (columns 9-12). Consult with an instructional assistant as needed.	How would you interpret one positive well in columns 1-4? Many positive wells?

Part C: Analysis of E. coli samples (columns 5-8)

Completed	Step	Protocol	Progress check
	1	Visually inspect these wells and confirm that there is substantial color development in most of the wells. Record this observation.	
	2	Look at the corresponding absorbance data and confirm that there are multiple readings above the 0.25 threshold.	

	3	If there are no positive readings, discuss within the team and decide how this should affect your interpretation of the soil sample data. Consult with an instructional assistant.	How would you interpret no positive wells in columns 5-8?
	4	Look at absorbance data for E. coli across plates regardless of soil samples. Are the readings similar? Record this observation.	

Part D: Analysis of soil samples (columns 9-12)

Completed	Step	Protocol	Progress check
	1	Visually inspect these wells and confirm that there is substantial color development in some of the wells. Record this observation.	
	2	Divide into two pairs to analyze the soil sample data in parallel. After you are done, check your calculations with those by the other half of your group for consistency.	
	3	Download a blank template Excel file.	
	4	Enter the “before incubation” soil data from last week into the table in the spreadsheet. These data will be automatically rearranged in Column C based on the type of carbon source in the corresponding EcoPlate™ well.	
	5	Enter the “after incubation” soil data from this week in the table. These data will be automatically rearranged in Column D.	
	6	There are two contributions to absorbance change. Microbes use: (1) the carbon source provided by the EcoPlate™ and (2) carbon sources naturally present in the soil. The	

		contribution of (2) to the overall absorbance change is estimated using the blank well.	
	7	In Column E, calculate the absorbance change that occurred during incubation in each well. Write an equation for how you would perform this calculation in simple math terms. Based on your answer and the data sheet, write the Excel function you would use to calculate Column E.	Describe in your own words how and why you would perform this calculation. How does this step fit in with the overall description in Step #6?
	8	In Column F, change the negative values in Column E to 0 using the =IF() function in Excel. The =IF() function takes three inputs: logic statement, true value, false value. Negative values are likely the results of variability in measurements based on instrumental precision. As negative values are illogical in the biological sense in this case, we are simply converting them to 0 for the purpose of calculations.	Describe in your own words how and why you would perform this calculation. How does this step fit in with the overall description in Step #6?
	9	In Column G, calculate absorbance change due to use of each of the 31 carbon sources in the EcoPlate™ by subtracting the absorbance change in the blank well (A1 soil) from the absorbance change in each of the other 31 wells. We will use the term Well Color Development to indicate absorbance change due to use of a carbon source. You will need to use a fixed reference in the formula. Recall that using "$" in an Excel formula defines a fixed reference.	Describe in your own words how and why you would perform this calculation. How does this step fit in with the overall description in Step #6?

	10	In Column H, set each value in Column G that is below the predetermined threshold of 0.25 to 0. By doing this we are excluding from the analysis any absorbance changes that are not biologically relevant. Use the =IF() function in Excel to return 0 if < 0.25.	Describe in your own words how and why you would perform this calculation. How does this step fit in with the overall description in Step #6?
	11	When the two halves of your group have completed the calculations, check to make sure that you obtained the same numbers in Column H. If the numbers are not the same, troubleshoot the Excel spreadsheets to identify and correct the errors.	
	12	Report your data from Column H on the shared data sheet after checking for accuracy and consistency in previous step.	
	13	Check your calculations using another group's data. To do so, follow these steps: o Make a copy of your Excel file. o Pick another group's data and enter their "before incubation" and "after incubation" data in the spreadsheet (steps 3 and 4). Calculations in the spreadsheet should update with the new data. o Compare the numbers in Column H from your calculations vs. the ones posted in the shared datasheet by the other team. o The numbers from both sets of calculations should be the same. If they are not the same, consult with the other	

		group and troubleshoot the Excel spreadsheets to identify and correct errors. ○ If the numbers from both sets of calculations are the same, go talk with the other group and congratulation each other!	
	14	When all the groups have entered their data into the shared data sheet, download it as an Excel file so that you can analyze the data independently for other tasks.	

Part E: Generating figure and calculating statistics

Completed	Step	Protocol	Progress check
	1	Generate a 100% stacked bar graph to represent the distributions of the sum of color development of carbon source categories. Sort data to group the rows by soil samples. Create a 2 × 5 table (two soil samples by five carbon sources). Sum color development of each carbon source category for each soil sample. Generate the stacked bar graph.	
	2	Use a chi-square calculator (example) to determine a p-value for the comparison.	

| FB3 | Functional Biodiversity Calculations |

Objectives

- Quantify functional biodiversity by calculating common indicators of biodiversity: richness (S), the Shannon Diversity Index (H), and evenness (E)

Overview

Functional biodiversity is a measure of the variability in how organisms within an ecosystem perform specific functions (e.g., metabolism, motility, respiration, etc.). Recall that previously you characterized carbon source utilization by the microbial community within soil samples with an EcoPlate™ experiment. In this exercise you will use the EcoPlate™ data to calculate common indicators of biodiversity: richness (S), the Shannon Diversity Index (H), and evenness (E).

Richness: A common measure of biodiversity within a community. Species richness is the number of different species in an environment. In the context of the EcoPlate™ experiment, functional richness is the number of different carbon sources used by the soil microbes. Richness (S) is one way that scientists quantify and describe biodiversity within a community. Species richness is the number of different species in an environment. Similarly, in the context of the EcoPlate™ experiment, functional richness is the number of different carbon sources used by the soil microbes. Richness can be calculated by summing the number of wells with substantial color development. In this experiment, substantial color development (change in A590 with time) will be defined as 0.25. Color development at or below this threshold will be considered negligible. Color development above this threshold indicates that the soil microbes used the carbon source present in the well.

Shannon Diversity Index (H): A composite measure of biodiversity within a community that depends on both evenness and richness. The more diverse the community, the higher the H value. H is a single number that encompasses richness (S) and evenness (E). While H was originally developed to characterize species diversity, we can use H to characterize functional biodiversity of carbon source utilization. In the context of the EcoPlate™ assay, evenness refers to the variability of color development across the wells. H is defined as: $-\Sigma pi \times \ln(pi)$, where pi is the ratio of color development in one well compared to the sum of the color development in all the wells and ln is the natural log.

Evenness: A common measure of biodiversity within a community. Species evenness characterizes how similar in abundance different species within a community are. For example, a community with 5 ants and 4 butterflies is more even than a community with 5 ants and 1 butterfly. In the context of the EcoPlate™ experiment, functional evenness characterizes how similarly soil microbes utilize different carbon sources. For example, microbes that use a similar amount of arginine and xylose (i.e., resulting in wells of similar purple color) demonstrate more functional evenness than microbes that use a lot more arginine (i.e., dark purple) than xylose (i.e., light purple). Evenness can have a value between 0 (completely uneven) and 1 (completely even). We can calculate evenness (E) by removing the richness (S) component from the Shannon Diversity Index (H). Specifically, to calculate E, divide H by ln(S). Note that ln(S) is the theoretically possible maximum value of H given a certain number of carbon sources in the system. The resulting evenness value should be between 0 (completely uneven) and 1 (completely even).

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=AVERAGE(): A function to calculate arithmetic mean. The syntax is =AVERAGE(argument_#1, argument_#2, …). Example: =AVERAGE(22,67,F12), where 22 and 67 are values, and F12 references the value within cell F12.

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Part A: Richness (S)

Completed	Step	Protocol	Progress check
	1	Divide into two pairs to analyze the soil sample data in parallel. After you are done, check your calculations with those by the other half of your group for consistency.	
	2	Open Excel file with your EcoPlate™ data.	
	3	Recall that the values in Column H are the biologically relevant absorbance (>0.25). In Column J, indicate whether or not there was	Describe in your own words how and why you would perform this calculation. How

		a biologically relevant absorbance change from use of each of the EcoPlate™carbon sources. Specifically, assign a value of 1 (1=Yes) to positive absorbance changes and a value of 0 (0=No) otherwise. Use the =IF() function in Excel to return 1 if > 0 and to otherwise return 0.	does this step fit in with calculations toward the overall idea of richness?
	4	Calculate richness by summing the values in Column J. Make sure to use an Excel function instead of manually adding the cells or the numbers in the cells.	Describe in your own words how and why you would perform this calculation. How does this step fit in with calculations toward the overall idea of richness?

Part B: Shannon Diversity Index (H)

Completed	Step	Protocol	Progress check
	1	Sum the values in Column H. Make sure to use an Excel function instead of manually adding the cells or the numbers in the cells.	Describe in your own words how and why you would perform this calculation. How does this step fit in with calculations toward H?
	2	In Column K, calculate pi by dividing each value in Column H by the sum of the values (calculated in the step above). pi is the ratio of well color development in one well to well color development in all the wells.	Describe in your own words how and why you would perform this calculation. How does this step fit in with calculations toward H?
	3	Sum the 31 pi values and check that they add up to 1.	Why should the values add up to 1?
	4	In Column L, calculate pi × ln(pi) for all the carbon sources that have a value of 1 in the	Describe in your own words how and why you would

		richness column. Use the =IF() function in Excel to return pi × ln(pi) if the value in Column J=1 and to return “” nothing if not.	perform this calculation. How does this step fit in with calculations toward H?
	5	At the bottom of Column L, calculate the Shannon Diversity Index by multiplying the sum of all pi × ln(pi) values by -1.	Describe in your own words how and why you would perform this calculation. How does this step fit in with calculations toward H?

Part C: Evenness (E)

Completed	Step	Protocol	Progress check
	1	Recall that the Shannon Diversity Index (H) is a composite measure of richness (S) and evenness (E). We can calculate E by removing the S component from H. Divide H by ln(S).	Describe in your own words how and why you would perform this calculation. How does this step fit in with calculations toward E?
	2	Check that the value is between 0 and 1.	Why should the value be 0-1?

Part D: Checking calculations and recording data

Completed	Step	Protocol	Progress check
	1	Check all your calculations against those done by the other half of your group. Both results should be the same. If not, work together to figure out where the errors have occurred and correct them. Consult an instructional assistant as needed.	
	2	Check your calculations using another group’s data. To do so, follow these steps: a. Make a copy of your Excel file.	

		b. Pick another group's data and enter their "before incubation" and "after incubation" data in the spreadsheet (steps 3 and 4). Calculations in the spreadsheet should update with the new data. c. Go talk to the other group. Compare the numbers for S, H, and E from your calculations vs. the numbers they have. d. The numbers from both sets of calculations should be the same. If they are not the same, consult with the other group and troubleshoot the Excel spreadsheets to identify and correct errors. e. If the numbers from both sets of calculations are the same, go talk with the other group and congratulation each other!	
	3	Record the richness (S), Shannon Diversity Index (H), and evenness (E) in the research notebook and shared data sheet.	

| GB0 | Genetic Biodiversity |

In this experiment, we will characterize the diversity of microbes within our soil samples by sequencing a specific gene, the 16S ribosomal RNA gene. The 16S gene is a commonly used “molecular barcode” for identifying prokaryotic organisms.

The first step in this process is to extract all the DNA from each soil sample. Next, we will create billions of copies of each 16S gene present in our DNA extract using a process called polymerase chain reaction (PCR). After PCR we will have a solution containing a mixture of 16S genes from (theoretically) every prokaryotic microbe that was present in the soil sample. That mixture of 16S genes will be sequenced using an Illumina MiSeq next generation sequencing instrument that we have in the laboratory. The data that we get will be analyzed to test if the phyla present are different for the soil samples taken from near native and invasive plants. Finally, we will quantify genetic biodiversity by calculating richness (S), the Shannon Diversity Index (H), and evenness (E).

| GB1 | DNA Extraction |

Objectives

- Purify genomic DNA from the population of microbes in soil samples
- Measure concentration of genomic DNA in the final solution

Overview

We will use the Qiagen DNeasy PowerSoil Pro Kit to extract genomic DNA from the soil samples we previously froze. The purpose of storing the samples at -20°C was to maintain the composition of microbial communities as they are in the wild. The overall extraction and purification process will be to lyse the cells present in the soil samples, separate the cell's genomic DNA from large contaminants in the soil by passing the lysate through a filter, separate the DNA from small contaminants using a column that DNA preferentially binds to, elute the DNA from the column, and finally remove residual inhibitors using a specialized filter. After DNA extraction and purification, we will determine DNA concentration and purity of DNA in the soil samples using a compact spectrophotometer called NanoDrop. DNA concentration is proportional to the amount of light with a wavelength of 260 nm that the sample absorbs. The concentration of contaminating protein is proportional to the amount of light with a wavelength of 280 nm that the sample absorbs.

The protocol uses a centrifuge to separate solid components in a mixture (e.g. soil) from solubilized components (e.g. DNA) and to drive solutions through various filters and columns. The centrifugal force exerted on a sample within a centrifuge depends on both the speed of rotation (measured in revolutions per minute or RPM) and the radius of the centrifuge. It is important to record protocols in terms of the desired centrifugal force (expressed relative to the acceleration due to gravity or g) rather than RPMs because RPM alone is a meaningless quantity. It is unfortunate that centrifuges typically require the user to program RPMs rather than the desired centrifugal force, which is the relevant physical parameter. For convenience, the centrifuges in the laboratory have stickers converting RPM into g for the radius of the centrifuges used in the laboratory.

Protocol

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Graphic summary

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Important Notes

- Arrange tubes symmetrically in the centrifuge, using a counterbalance as needed
- Do not remove the blue inserts/adapters. They are used for the PCR tubes.
- Do not throw away any tubes until an IA has collected the purified DNA.

Part A: DNA extraction

Completed	Step	Protocol	Progress check
	1	The protocol is for one PowerBead Pro Tubes with 250 mg of soil. Each group will process 2 samples, so the centrifuge can be balanced. Make sure to arrange tubes symmetrically in the centrifuge, using a counterbalance as needed.	Why do you think it is important that tubes with equivalent samples are arranged symmetrically in the centrifuge? What do you think will happen otherwise?
	2	Do not throw away any tubes until an IA has collected the purified DNA.	Why might this be an important safeguard within the protocol?

	3	In a previous week, 250 mg of soil sample was added to a PowerBead Pro Tube. Retrieve the tube.	
	4	Add 800 µL of Solution CD1 to this tube. This solution helps disperse soil particles and solubilize cell membranes.	
	5	Vortex briefly to mix.	
	6	Attach your PowerBead Pro Tube (containing the soil and CD1 solution) horizontally on the vortex adapter and vortex at maximum speed for 10 minutes.	What do you think is happening to the microbial organisms in this step? How might it help with DNA extraction?
	7	Centrifuge PowerBead Pro Tube at 15,000 g for 1 minute. Centrifugation is a process in which components of a mixture are separated by density through the application of centripetal force.	
	8	Being careful to avoid the pellet, transfer the supernatant to a clean 2 mL microcentrifuge tube. You should expect to have 500-600 µL of supernatant, which may still contain some soil particles. The supernatant is the solution that sits above the pellet after centrifugation. The pellet is the solid residue that forms at the bottom of a tube after centrifugation.	Do you think the genomic DNA from microbes is in the pellet or the supernatant? Why?
	9	Add 200 µL of solution CD2 to the tube with the supernatant and vortex for 5 seconds. This solution precipitates common contaminants that are not genomic DNA.	
	10	Centrifuge at 15,000 g for 1 minute.	

	11	Being careful to avoid the pellet (which has contaminants), transfer up to 700 µL of the solution to a clean 2-mL microtube. You will likely get 500-600 µL, which is plenty.	Do you think the genomic DNA from microbes is in the pellet or the supernatant? Why?
	12	Add 600 µL of solution CD3 to the supernatant you just transferred and vortex for 5 seconds. CD3 is a salt solution that changes the chemistry so that DNA binds to the column in the next steps. A column is a device used to separate one or more components in a solution. The column used in this experiment preferentially binds to DNA (i.e. DNA will adsorb to the column), allowing the DNA to be separated from contaminants.	
	13	Load a total of 650 µL of the material from the previous step onto an MB Spin Column. The DNA is now on the membrane. Centrifuge at 15,000 g for 1 minute.	
	14	Discard the flow-through by removing the spin column from the collection tube, dumping the collection tube out, and placing the spin column back in the collection tube.	Where is the genomic DNA from microbes in this step? Why is it safe to discard the flow-through here?
	15	Repeat the previous 2 steps. Load any CD3 solution left onto the same MB Spin Column. If you do not have that much, load as much as possible. Centrifuge at 15,000 g for 1 minute.	
	16	Carefully remove the MB Spin Column from the collection tube and place it into a clean 2-	Where is the DNA from microbes in this step? Why?

		mL collection tube. Avoid splashing any of the flow-through onto the MB Spin column.	
	17	Add 500 µL of Solution EA to the MB Spin Column. Centrifuge at 15,000 g for 1 minute. This EA solution is a wash buffer that removes proteins.	Where is the DNA from microbes in this step? Why?
	18	Discard the flow-through and put the MB Spin Column back into the same 2-mL collection tube.	
	19	Add 500 µL of solution C5 to the MB Spin Column. Centrifuge at 15,000 g for 1 minute. This C5 solution is an ethanol wash that removes excess salt.	Where is the DNA from microbes in this step? Why?
	20	Discard the flow-through and throw away the used collection tube.	
	21	Now place the MB Spin Column into a new 2-mL collection tube. Centrifuge at 16,000 g for 2 minutes.	Where is the DNA from microbes in this step? Why?
	22	Carefully remove the MB Spin Column from the collection tube and place it into a new 1.5- mL elution tube.	Why do you think we need to place the spin column into a new tube?
	23	Add 50 µL of solution C6 to the center of the white filter membrane, without touching the membrane with your micropipette. This C6 solution is an elution buffer that washes the DNA off the membrane into the solution. Elution is the process that will release the DNA from the column. A buffer is a solution that maintains a stable pH.	

	24	Let the elution buffer sit for 1 minute on the membrane.	
	25	Centrifuge at 15,000 g or max speed for 1 minute. Discard the MB Spin Column and keep the elution tube! This is your DNA!	Where is the DNA from microbes in this step? Why?
	26	Label the tube with “genomic DNA” and your section and group.	

Part B: Concentration of genomic DNA samples

Completed	Step	Protocol	Progress check
	1	Add 1.5 µL of DNA sample to the NanoDrop pedestal.	
	2	Record the DNA concentration (ng/uL) and the 260/280 ratio in research notebook and the shared data sheet.	
	3	For the next step, you will determine if it is appropriate to combine the 2 DNA samples. Do both of your genomic DNA samples from your lab group have concentrations over 50 ng/ul? If so, combine the 2 tubes of eluted genomic DNA. If one or both of your genomic DNA concentrations is less than 25 ng/ul, or if the A260/A280 ratio of your sample is very far from 1.8, then do not combine the tubes. Check with an instructional assistant.	What does a A260/A280 ratio of 1.8 mean in this case?
	4	Measure concentration of the combined DNA sample on the Nanodrop and record concentration and 260/280 ratio in research notebook and shared data sheet.	

| GB2 | 16S Gene Amplification |

Objectives

- Use polymerase chain reaction (PCR) to amplify 16S gene present in genomic DNA extracts

Overview

In the previous experiment, we extracted and purified genomic DNA from a mixture of the many different organisms present in our soil sample. We will now use polymerase chain reaction (PCR), a method where scientists take advantage of the natural mechanism by which cells replicate their DNA, to make many copies of a specific gene within the genomic DNA. 16S is the specific gene that we will amplify in this experiment. 16S is important because it can be used to identify prokaryotic microbes within our soil sample. Typically, in PCR, most of the components will be mixed together in a Master Mix. In our case, this includes a high-fidelity DNA Polymerase called Q5, nucleotides, buffer, and the appropriate primers.

Protocol

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Graphic summary

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Data table

Components of each PCR sample

Tube	PCR1: no template	PCR2: E. coli colony	PRC3: 200 ng genomic DNA	PRC4: 200 ng genomic DNA
2× master mix	22 µL	22 µL	22 µL	22 µL
Water	28 µL	28 µL	___ µL	___ µL
DNA template	---	1 small colony	___ µL (200 ng)	___ µL (200 ng)
Total volume	50 µL	50 µL	50 µL	50 µL

16S amplification by PCR

Completed	Step	Protocol	Progress check
	1	You will add 200 ng of genomic DNA to each of the two PCR samples. Based on the concentration of your genomic DNA, how many µL will you add? If your concentration is over 200 ng/µl, add 1 µl. Enter the volume in research notebook and data table above. Check your calculation with other members of your group and an instructional assistant before setting up the reactions.	
	2	Further check within your group to determine if each of the four PCR samples (data table above) is an experimental sample or a positive or negative control. Record this information in research notebook.	
	3	Label the lid of 4 PCR tubes (which are smaller than standard microtubes) with section, -group, “-PCR,” and number (e.g.	

		B02-C-PCR1). If you label the sides of the tubes, the writing will come off during PCR.	
	4	Put the labeled tubes, water, and Master Mix on ice. The genomic DNA sample should already be on ice.	
	5	Set up the 4 reactions on ice as follows: ○ Add master mix to tubes PCR1-PCR4. Note that the 2 needed primers have already been added to the master mix. ○ Add the appropriate amount of MQ water to tubes PCR1-PCR4. ○ Add one E. coli colony to tube PCR2 by gently touching a small pipette tip to a small single colony within the plates provided by an instructional assistant, being careful not to poke the underlying agar. Transfer the colony to tube PCR2 by swirling the tip around in the mixture. ○ Add the appropriate volume of DNA template to tubes PCR3 and PCR4.	
	6	Store the unused genomic DNA at -20°C in case you need to repeat the PCR.	
	7	Place PCR tubes in centrifuge, using adapters that hold narrow PCR tubes firmly in place. Arrange tubes symmetrically in centrifuge, using counterbalance as needed.	Why do you think it is important that samples are arranged symmetrically? What do you think will happen otherwise?
	8	Centrifuge by pressing and holding the “short” button for about 5 seconds to drive fluid to the bottom of the tubes.	

	9	Place the tubes on ice until all groups have finished. Instructional assistants will run the following PCR protocol in the thermocycler: 1×: 3 min 98°C 35×: 45 sec 98°C, 60 sec 53°C, 90 sec 72°C 1×: 10 min 72°C, forever 4°C	What do the different temperatures do in each of the three repeating PCR steps?

| GB3 | Agarose Gel Electrophoresis |

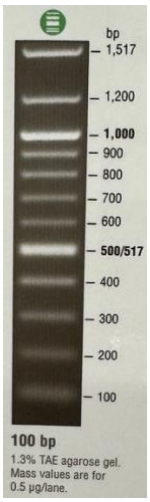
Objectives

- Use agarose gel electrophoresis to confirm that 16S gene was amplified in the PCR reactions

Overview

We previously performed PCR to amplify 16S gene from prokaryotes in our soil samples. Recall that 16S gene is sufficiently different across species, making it a useful molecular barcode for identifying organisms. Here, we will use agarose gel electrophoresis to confirm that PCR ran successfully.

Agarose gel electrophoresis is a method used to separate DNA fragments by size. The agarose gel acts as a molecular sieve. As negatively charged DNA is pulled through the gel toward a positive electrode, smaller DNA fragments move more quickly through the gel compared to larger fragments.



We will perform electrophoresis on our PCR products and use a 100 bp DNA ladder. Known fragments in the DNA ladder will separate by size, providing a visual guide to estimate the size of our PCR products. We will compare the position of the PCR products to the ladder to verify the presence of 16S.

Two different types of dyes are used during agarose gel electrophoresis. Loading dye is visible with the naked eye and can be any color or a combination of colors. Loading dye is mixed with the sample, in our case the PCR product, to help visualize the sample as we pipette the sample onto the gel. The master mix we used to perform PCR contains yellow and blue loading dyes, making the PCR product appear green. As the gel runs, the yellow and blue dyes separate from each other to allow us to monitor the progress of the sample as it moves through the gel. Specifically, the yellow dye moves fast through the gel, and the blue dye moves slowly through the gel. Our PCR product is expected to move at an intermediate speed. It is important to recognize that the DNA in the PCR product does not bind to the loading dye and is thus invisible to the naked eye at this point in the experiment.

The second type of dye used in gel electrophoresis is a DNA-binding dye. The specific DNA-binding dye we will use is called SYBR® Safe. An instructional assistant will add SYBR® Safe to the agarose solution prior to the start of class. SYBR® Safe intercalates between the stacked bases of the DNA. When this occurs, the SYBR® Safe will fluoresce at a visible wavelength when illuminated with UV light. Adding SYBR® Safe to the agarose gel allows us to view the PCR product on the gel (when illuminating with UV light) and to compare the location of the PCR product with the location of the DNA fragments with known sizes contained within the DNA ladder and separated by electrophoresis.

In summary, agarose gel electrophoresis is used to visualize DNA fragments separated by size. DNA ladder is a mixture of fragments of known size commonly used as a standard in gel electrophoresis.

Additional resources

- JoVE Science Education Database. Basic Methods in Cellular and Molecular Biology: DNA Gel Electrophoresis. Journal of Visual Experimentation, Cambridge, MA (2023). https://www.jove.com/v/5057/dna-gel-electrophoresis-concept-procedure-and-applications

Protocol

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Part A: Setting up the gel

Completed	Step	Protocol	Progress check
	1	Assemble a gel box: ○ Orient the base of the gel box so that the negative terminal (black) is on the left. DNA samples will flow from left to right towards the positive (red) terminal. ○ Insert gel tray into base. Make sure to line up the "+" and "–" symbols. ○ Insert black baffles on each side of gel tray by pressing them firmly but gently in place. Baffles define border of the gel. They are shaped like a wedge and will only fit in one orientation. Inserting baffles with too much force makes it difficult to remove them and may damage the gel. ○ Insert the comb (with the side designed to create 10 sample wells) into the notch on the negative side in your gel tray. Make sure the comb is slightly above the tray surface and facing away from the baffles.	Why do you think DNA samples will move from the negative terminal to positive terminal?
	2	Take a flask containing 50 mL of 2.0% agarose in buffer from the 65°C water bath. The agarose solution also contains SYBR® Safe, which allows visualization of the DNA bands after separation on the gel.	
	3	Using a Pasteur pipette and rubber bulb, seal the junction between the baffles and the gel tray with a small amount of agarose.	Why do you think it is important to seal the junction with a small amount of agarose first?

	4	Slowly pour remaining agarose into gel tray (in the center of the gel box). Allow gel to solidify at least 15 min.	
	5	Gently pour ~50 mL of buffer into gel box and then carefully remove the comb and baffles.	
	6	Gently pour ~200-250 mL of buffer into gel box. Make sure buffer covers gel completely.	

Part B: Running the gel with samples

Completed	Step	Protocol	Progress check
	1	Retrieve genomic DNA sample from previous experiment and put the sample on ice.	
	2	Label 4 small microtubes as follows: PCR1, PCR2, PCR3, PCR4. Add 5 µl of the appropriate PCR reaction to each tube.	
	3	Add 1 µl of 6× sample buffer to each of the above tubes to a total of 6 µL.	
	4	Identify Lane 1 in the gel. Samples will be loaded into the lanes indicated below: Lane 1: no sample Lane 2: 10 µL of 100-bp DNA ladder Lane 3: 5 µL of PCR no template Lane 4: 5 µL of PCR E. coli Lane 5: 5 µL of PCR from soil sample Lane 6: 5 µL of PCR from soil sample	
	5	Load samples into the appropriate lane: a. Load sample into a pipette tip.	

		b. Gently position the tip within the appropriate well while being careful not to disrupt the integrity of the well. c. Slowly eject the sample into the well; do not eject to the second stop because this will produce bubbles in the gel. d. Remove the tip before releasing plunger.	
	6	Place the lid on the gel box, connect the leads to a power source, and run the gel at 160 V for 30 min.	What results do you expect for each lane? Why?
	7	With the help of an instructional assistant, image the gel under UV light to verify the presence of the 16S PCR product at ~400bp.	
	8	Include the gel image into the research notebook and shared data document.	

* When cleaning and rinsing gel box after use do not touch the wires as they are very fragile. Please turn gel box upside down on a paper towel to air dry

| GB4 | Purification of 16S PCR Product |

Objectives

- Use magnetic beads to purify the 16S PCR product

Overview

We will use magnetic beads to clean up our PCR product. We will use magnetic beads, a common technology for DNA purification. The magnetic beads are conjugated with specific chemistry that will bind DNA. When we place the tube with our sample on a magnet stand, all of the beads with bound DNA will be pulled to the side of the tube along the magnet, and we can remove the supernatant and wash the DNA with ethanol. Finally, we will elute the cleaned DNA off of the beads. After clean-up, we will measure DNA concentration of purified products using NanoDrop.

Additional resources

Agencourt AMPure XP: https://www.beckmancoulter.com/wsrportal/techdocs?docname=B37419

Protocol

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Part A: Purifying 16S PCR product on a column

Completed	Step	Protocol	Progress check
	1	Make sure to save appropriate aliquots of PCR products for gel electrophoresis!	Why is it important to save samples for other experiments?
	2	Perform only on PCR products from soil metagenomic DNA. Do not carry out this purification on PCR products from E. coli or the no-template control samples.	Why do you think we should only perform clean-up procedures on PCR products from soil metagenomic DNA?
	3	Identify the correct PCR products to use. Set aside the other PCR products and continue protocol only with the appropriate tubes!	
	4	One pair in the group will cleanup PCR3, the other pair will cleanup PCR4. Place all tubes into an appropriately sized tube holder on the bench. Make sure all tubes are labeled consistently so that everyone in the group can understand.	
	5	Gently vortex the tube of magnetic beads to resuspend any particles that have settled.	
	6	Add magnetic beads to the PCR tube with PCR product. The volume of beads solution is 1.8 x the volume of PCR product. For example, if there is 45 µL of PCR product, then 1.8 x 45 µL = 81µL of magnetic beads.	What is the actual volume of your PCR product? What volume of magnetic beads should you use?
	7	Mix beads and PCR product thoroughly by pipetting 10× up and down to the first stop. Do not vortex! The color of the mixture should appear homogeneous after mixing.	Where are the PCR products in this step? Why?
	8	Let the tube sit for 5 min.	
	9	Place the PCR tube on the magnet stand and let it sit for 2 minutes. Make sure the	Where are the PCR products in this step? Why?

		solution is clear and that a visible clump of beads is present on the side of the tube before proceeding to the next step. This step will separate the magnetic beads and bound DNA from the rest of the solution.	
	8	With the tube still on the magnet stand, remove all the supernatant from the tube and discard into waste beaker. Do not touch the magnetic beads with the pipette tip. It is okay to leave a few µL of supernatant behind to prevent the beads from being drawn out with the supernatant. If you accidentally aspirate the beads, just pipette everything back into the tube and wait another 2 min for the solution to become clear again.	Where are the PCR products in this step? Why?
	9	With the tube still on the magnet stand, add 200 µL of 70% ethanol into the tube. A PCR tube holds exactly 200 µL, so if the tube is going to overflow, use 190-195 µL instead.	
	10	Allow the tube to sit for 30 seconds. Remove the ethanol and discard it in waste beaker.	Where are the PCR products in this step? Why?
	11	Repeat the previous 2 steps of adding and removing ethanol with incubation time.	
	12	Allow the tube to sit on the magnet stand for 30 seconds. This will ensure more ethanol evaporates. Do not allow the tube to dry much longer, as beads may crack.	Where are the PCR products in this step? Why?
	13	Remove the tube from the magnet stand, then add 40 µL of DI water to the tube. This solution level should be high enough to	Where are the PCR products in this step? Why?

		contact all the beads. If not, add 10 µL more to top up to 50 µL.	
	14	Mix the water and beads by pipetting 10× up and down to the first stop. Do not vortex.	
	15	Let the tube sit in a tube holder on the bench for 2 min. The DNA should now separate from the magnetic beads.	
	16	Place the tube back on the magnetic stand and let sit for 1 min. The beads will separate from the solution.	Where are the PCR products in this step? Why?
	17	Transfer the supernatant to a 1.5-mL microtube. There should be ~40 µL of supernatant. Label the tube with section, group letter, and “Cleaned DNA” (ex. B02-C-cleaned DNA). Do not throw this away, this is your cleaned up DNA.	

Part B: Measuring concentration of PCR products

Completed	Step	Protocol	Progress check
	1	Add 1.5 µL of PCR to NanoDrop pedestal.	
	2	Record the DNA concentration (ng/uL) and the 260/280 ratio in research notebook and the shared data sheet.	
	3	For the next step, you will determine if it is appropriate to combine the 2 PCR samples. Do both of your PCR samples from your lab group have similar concentrations over 10 ng/µl? If so, combine the 2 tubes. If one or both tubes are less than 10 ng/µl, or if the A260/A280 ratio is very far from 1.8, then do	What does a A260/A280 ratio of 1.8 mean in this case?

		not combine the tubes. Check with an instructional assistant.	
	4	Measure concentration of the combined DNA sample on the Nanodrop and record concentration and 260/280 ratio in research notebook and shared data sheet.	

| GB5 | Genetic Biodiversity |

Objectives

- Analyze 16S sequence data from soil samples

Overview

This week, we will analyze 16S sequence data from our soil samples. The data are already grouped into different bacterial phyla. We will compare the distribution of bacterial phyla using statistics and also determine richness, Shannon diversity index, and evenness of bacterial phyla in soil samples.

Protocol

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Part A: Comparing phyla distributions

Completed	Step	Protocol	Progress check
	1	Divide into two pairs to analyze the soil sample data in parallel. After you are done, check your calculations with those by the other half of your group for consistency.	

	2	Sum the number of sequences in all the phyla for each soil sample.	Would you consider this to be richness? Why or why not?
	3	Sum across soil sample(s) to calculate total number of sequences within each phylum.	
	4	Filter and sort the data largest to smallest by the new sum column created in the last step. We will continue with data for the top 10 most abundant phyla only.	
	5	Create a stacked bar graph to display the data of the top 10 most abundant phyla comparing between soil samples.	
	6	Report and analyze all data in research notebook.	

Part B: Calculating diversity measurements

Completed	Step	Protocol	Progress check
	1	Divide into two pairs to analyze the soil sample data in parallel. After you are done, check your calculations with those by the other half of your group for consistency.	
	2	Calculate richness (S) by counting the total number of phyla in the first soil sample. We can do this using the =COUNTIF() function. The syntax is =COUNTIF(range,“logic_test”). We should set up the logic test to ensure that we are not counting any zero values.	Why do we count the cells instead of summing the values to calculate richness? Why do we not count the cells with zero values in this calculation?
	3	Repeat this calculation for other soil sample(s) using the autofill function instead of re-entering the formula by hand.	

	4	For Shannon diversity index H, calculate pi by dividing the number of sequences in each phylum by the total number of sequences to determine the proportion of the total represented by that phylum.	What is represented by the pi value in Shannon diversity index H calculations?
	5	Calculate pi × ln(pi) for each phylum. Use can use an =IF() formula to prevent error values from appearing with cells that have zero values in the original phyla data.	In your own words, describe the rationale for using natural log and pi to account for evenness in a population.
	6	Calculate Shannon H by summing all pi × ln(pi) values and then multiplying -1.	In your own words, describe the rationale for summing all the values to account for richness in a population.
	7	Repeat the calculations for other soil sample(s) using the autofill function as much as possible instead of re-entering formulas.	
	8	For evenness, divide Shannon H by ln(S).	In your own words, describe how the formula H / ln(S) would result in evenness.
	9	Confirm calculations with others in the group and also with other groups in the lab section.	
	10	Report all data in research notebook.	

Part C: Microbiome Community Analysis

	1	Read through the provided Microbiome Analysis of our sequenced data across a span of several quarters.	In your own words, what is the analysis showing you?
	2	Take a closer look at the Beta Diversity Analysis. In your lab notebook, describe the data for the first ten pairwise comparisons.	What does a larger number versus a small number mean in distance?

	3	The Principal Coordinate Analysis takes the distances in Beta Diversity Analysis to visualize the data. Based off the PCoA graph describe what conclusions you can make? Include this in your lab notebook and writing assignment.	What is this data showing? What do the individual shapes and where they are placed in the graph mean?
	4	The PERMANOVA is a statistical test used to compare groups based on multiple variables. Based off the statistical test include what the P-value was and make a conclusion.	
	5	The Heatmap is another visualization of the sequenced bacterial phyla. Describe some main conclusions in your lab notebook based off the data.	
	6	Based on all these analyses, what can you conclude regarding the microbial diversity in both soils. Include this in your lab notebook and writing assignment.	How "confident" are you of this conclusion?

| PS0 | Process Skills |

Process skills are sometimes also known as professional skills, practical skills, workplace skills, transferable skills, soft skills, etc. Reflection and feedback are both important for process skill development and are key components in undergraduate education.

We will conduct group reflections and feedback using rubrics for process skills. In particular, we will work on interpersonal communication and teamwork within groups. Each of these process skills, there are multiple dimensions of reflections and feedback that we will consider together.

| PS1 | Interpersonal Communication |

Completed	Step	Protocol	Progress check
	1	Open the reflection and feedback rubric.	
	2	Discuss as a group how you have conducted communication based on the dimensions highlighted in the rubric. Consider your ratings, observable characteristics, and suggestions for improvement for each.	
	3	If you have given yourselves 5 (effectively) for any of the dimensions, return to the rubric and further consider how you can improve. Look at the observable characteristics and suggestions for improvement.	
	4	Enter your group's ratings, observable characteristics, and suggestions for improvement for each dimension on the shared spreadsheet in the cloud.	
	5	Ask your lab section instructional assistant to look over your responses and to provide further feedback.	
	6	Enter their comments and feedback on the shared spreadsheet in the cloud.	
	7	Complete the final reflection on Canvas as a group together with additional discussion.	
	8	Include a brief summary of this entire activity in your research notebook.	