**Biodiversity Project: instructions**  
**BIOL 1615, fall 2021**

**Purpose:** We can relatively easily observe and quantify the alfalfa diversity in the Science Garden as well as the animal diversity that is associated with the alfalfa in the Science Garden. But how about the hidden biodiversity, like the microbes that live in the alfalfa's plant tissue? The purpose of this authentic course-based research experience is to: 1) observe, quantify, and describe aspects of bacterial endophyte diversity in the leaves across the alfalfa populations in the Science Garden, and 2) collaboratively produce a scientific manuscript (i.e., lab report) at the “intermediate” level as described in the final lab report grading rubric.

**Background information and research questions:** As symbionts, bacterial endophytes constitute part of a diverse and ecologically important plant microbiome. Biologists have found that endophyte communities differ in composition among biomes, host species, and genotypes, and they exhibit a wide range of host specificity and often enhance plant health. The total number of estimated endophyte species is in the millions, but many have yet to be characterized, and more work is needed to understand which endophytes occur together and how they are genetically related. Further, less is known about bacterial endophytes in leaves relative to roots (Afzal et al., 2019).

You will ask and answer the following research questions:
1) How does bacterial species diversity in the leaves of alfalfa vary among the plant populations grown in the common environment of the Science Garden?
2) How does plant population affect the relative abundance of (at least) four focal bacteria (of your choice)?
3) What is known about these (at least) four focal bacteria and how are they related to each other?

While the research questions are chosen for you, your group will have a unique take on the project, as you will refine questions 2 and 3 based on your observations and interests. This project will work like a cooking show. We will sample leaves, culture microbes, and extract DNA from the garden together, but you will analyze last year’s (2020) DNA sequence data.

**Due Date:** You will work on this project for the rest of the semester. **By the end of class, the week of November 29, you will turn in a group lab report** that describes the entire scientific process.

**Requirements:** Your report will follow the format of manuscripts in the scientific journal *Ecology*. Therefore, your audience is the general scientific community. Download and use the provided lab report template; see link in Canvas. You should also look at peer-reviewed published articles for examples of good lab reports.
- Double-spaced, 12-point font, Times New Roman, numbered pages
• Sections in the following order:
  o Title
  o Authors
  o Introduction section: essential and relevant background information (with cited sources), the research questions, the reason for doing the research (purpose), and predictions.
  o Methods sections (tissue collection, sterilization, and plating; microscopy methods; molecular methods, statistical analysis): sufficient information to allow a practicing biologist to repeat your work.
  o Results section: concise description of the outcomes of data analysis (without interpretation) that references the tables and figures.
  o Discussion section: answers to all three research questions based on the data, includes limitations and the significance of the results.
  o References section: a list of references that were cited in the text, in alphabetical order and in APA citation format.
  o Acknowledgements section: a short paragraph thanking the funding source and the non-authors who helped with the project.
  o Tables section: tables in the order they are referenced in the text; each requires a caption above the table.
  o Figures section: figures in the order they are referenced in the text; each requires a caption below the figure.

Timeline: You will perform parts of the project and complete a draft of sections of this lab report each week. You will receive feedback about the quality of your drafts from your GTA along the way. You are expected to incorporate their comments and show the progress you have made in your final draft. Here is an overview of each week of the project, but Canvas houses all of the details.

1. **Week of Oct 4.** Your group will brainstorm specific research questions 2 & 3 after: 1) making sure you understand all the project requirements, 2) exploring the scientific literature for what is already known, 3) exploring the list of taxa available for analysis.
   • For **Wk6-inclass**, you will write a draft of your Title, Authors, Introduction (background information, research questions, project justification, and predictions), Acknowledgements, and References sections.

2. **Week of Oct 11.** Your group will conduct methods for this project on one assigned plant in the garden: tissue collection, sterilization, and plating.
   • For **Wk7-inclass**, you will submit a draft of the first section of the Methods of your lab report (tissue collection, sterilization, and plating).

3. **Week of Oct 18.** Your group will conduct methods for this project on one assigned plant in the garden: DNA extraction.
   • For **Wk8-inclass**, you will submit a draft of the first part of the third section of the Methods of your lab report (molecular methods).

4. **Week of Oct 25.** Your group will review a part of the methods for this project that was already completed for you: PCR and DNA sequencing.
   • For **Wk9-inclass**, you will submit a draft of the rest of the third section of the Methods of your lab report (molecular methods).

5. **Week of Nov 1.** Your group will conduct methods for this project on one assigned plant in the garden: microscopy.
• For **Wk10-inclass**, you will submit a draft of the second section of the Methods of your lab report (microscopy), including a figure.

6. **Week of Nov 8.** Your group will perform all the data analyses required to answer your research questions.
   • For **Wk11-inclass**, you will submit a draft of the Results section of your lab report, including all relevant tables and figures.

7. **Week of Nov 15.** Your group will interpret all the results produced the week before to ultimately answer the research questions based on data, and you will put the results in a larger context.
   • For **Wk12-inclass**, you will submit a draft of the Discussion section of your lab report, which will include at least three paragraphs (conclusions based on the data, limitations, and implications).

8. **Week of Nov 29.** You will spend this class session giving and receiving peer feedback on your lab report (**Wk14-inclass**) to help you prepare your **final group Biodiversity Project lab report**.
   You will submit the final group lab report by the end of class.
   • Your final group lab report will be graded using a published, validated grading rubric for scientific lab reports (Timmerman et al., 2011). You should see this grading rubric again in other science courses at USU. We do not expect you to write a lab report at the “expert” level in this introductory biology course, but your future instructors will. Thus, you will receive full credit on this assignment by meeting at least the “intermediate” level criteria for all sections of the lab report.

**Supplemental resources:**
• Visit the **Science Writing Center (SWC)** individually to receive feedback on a section of your group’s lab report to share with your group and use to create the final version of your group lab report. See Canvas for the steps you need to follow to receive one extra credit point on your lab report.
  o usu.edu/science/swc/
• For extra help finding relevant sources to reference in your lab report, you can request to meet with **Steph Crowell, your biology librarian**.
  o steph.crowell@usu.edu; (435) 797-2165

**References:**
Biodiversity project: Introduction
Week 6 of BIOL 1615, fall 2021

Objectives: In this lab you will be able to: 1) gather background information from the scientific literature, 2) make observations about the taxonomic data available to you for the biodiversity project, 3) present the information in a lab report, and 4) collaborate with your group members.

Equipment:
- A link to the Biodiversity Project lab report template is in Canvas
- A scientific review article about bacterial endophytes, downloadable from Canvas
- Links to Scopus and Google Scholar for searching the scientific literature are in Canvas
- A link to a guide for citing sources in the text and in an end-of-lab-report References section is in Canvas
- The taxonomy dataset: the link to the RStudio Cloud Week 8 project is in Canvas

Background information and methods will follow the format of the parts of the lab report you will write this week.

1) You will need a title for your group lab report. You can wait to come up with this until after you have written the draft of your introduction section. This way your title will reflect your group’s unique take on this project.

Title ideas:

2) Who should be an author on your group lab report, and who should instead be listed in the Acknowledgements section?

Today, it is unlikely that a scientist would be the only author of a paper. For example, the average number of authors on articles in the New England Journal of Medicine rose from slightly more than one in 1925 to more than six in 1995 (Columbia University, n.d.). As research has become more complex and multidisciplinary, the need for many different types of experts to perform studies has increased.

But who should be considered an author? Some say that being accountable for the entire content of an article should be a minimal responsibility for an author whose name is on a paper. Others say that, given the multifaceted nature of research, one person might not be able to take full responsibility. Some feel that a clinician who provided the blood samples for a study, without which the research could not have been done, should be an author. Others feel that the clinician should receive an acknowledgment. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship (Columbia University, n.d.).

Authorship vs. Acknowledgement ideas:

3) You will gather background information to put the research question in context. You will spend the rest of the semester going through the process of asking and answering these related research questions:

1. How does bacterial species diversity in the leaves of alfalfa vary among the plant populations grown in the common environment of the Science Garden?
2. How does plant population affect the relative abundance of (at least) four focal bacteria (of your choice)?
3. What is known about these (at least) four focal bacteria and how are they related to each other?
Where do we begin? During class you will be assigned and read a section of Afzal et al. (2019) to help your group better understand what bacterial endophytes are, what is known about them, and what the scientific community still doesn’t know about them. Take notes below as you read and digest the article. The notes will come in handy when you write the first paragraph of the lab report introduction.

Take home message from the section of the review article I read:

The most interesting things my group members learned from the article:

4) **Make observations** while exploring the bacterial taxonomic data from 2020, to help you come up with your unique take on this project (research questions 2 and 3).

For making observations to inform the emphasis of your group project, some reminders about how biologists organize life on Earth might be helpful. A “tree of life” can be constructed to illustrate when different organisms evolved and to show the relationships among different organisms. In Figure 1, from a single point, the three domains of Bacteria, Archaea, and Eukarya diverge and then branch repeatedly.

![Phylogenetic Tree of Life](image)

**Figure 1.** The three domains of life: Bacteria, Archaea, and Eukarya (from OpenStax College, 2013).

Taxonomy is the science of naming and grouping species to construct an internationally shared classification system. This system uses a hierarchical model. A hierarchical system has levels and each group at one of the levels includes groups at the next lowest level, so that at the lowest level each member belongs to a series of nested groups. In the most inclusive grouping, scientists divide organisms into three domains: Bacteria, Archaea, and Eukarya. Within each **domain** is a second level called a **kingdom**. Each domain contains at least one kingdom. Within kingdoms, the subsequent categories of increasing specificity are: **phylum, class, order, family, genus, and species** (from OpenStax College, 2013). Any one of these levels generally can be referred to as a **taxon**.
You will choose at least four bacteria taxa in the 2020 dataset to focus on in this project. Your choice must be justified, and better yet, grounded in what is already known about bacterial endophytes, as found in the scientific literature (e.g., Afzal et al. 2019). To make your choice, you will look through the pre-uploaded 2020 bacterial taxonomy dataset (“Taxa16S_2020.csv”) in the RStudio Cloud Week 6 project, using the pre-uploaded R script as a guide.

The 2020 bacterial taxonomy dataset has 36,918 rows of data. Each row is called an amplicon sequence variant (ATV). This means that each member (row) of the dataset has a unique DNA sequence. A putative taxonomic identification has been assigned to each ATV; you will learn more about this assignment process in Week 11. Further, this means there can be several representatives of the same species in this dataset. We have the following recommendation when making your choice:

- The dataset is organized from most abundant ATV to least abundant ATV. We recommend choosing more abundant ATVs and avoiding the ATVs at the bottom of the dataset.
- Some taxa in the dataset have obscure names, like “MBNT15”, as opposed to others, like “Desulfo bacterot”. We recommend avoiding the ATVs with the obscure names.

One you make your choice, keep track of the IDs.

IDs and putative taxonomic identification of our chosen ATVs:

Note: also in Week 11, you will receive all the rest of the data you need to answer the three research questions, including the DNA sequence for each ATV so that you can learn more about your chosen taxa with BLAST searches (like we did in Week 4).

5) Gather more background information for the unique take on your project.

Scientific articles are complete descriptions of new scientific research written by experts in the field. Scientific articles are the final step in the scientific process as they disseminate results. Dissemination is important because it keeps scientists in check and allows for a replication of scientific work. These articles are published in journals and are the way scholars communicate their findings with each other and the public. You probably have textbooks and a whole list of assigned readings for your other courses. Which might make you ask yourself, "ugh, why do I have to read complicated scientific articles on top of this?" Scientific articles are:

- current. Unlike your textbook which might be several years old, scientific papers give you the opportunity read cutting edge research the moment it is published. They allow you stay up to date on current trends in scientific research and advances in the field.
- contain data. Textbooks and news articles summarize research, whereas scientific articles contain the complete study - including summaries of raw data. This is important if you need exact results for your own research. These articles are written by the members of the research team who share their methodology which should enable you to replicate their study and validate results.
- peer-reviewed. Scientific articles are written by experts in the field and go through a peer-review process. This means other experts in the field have reviewed the study prior to publication to confirm the article's scientific quality. This helps ensure the articles are grounded by evidence. How to tell if an article is peer-reviewed? Many databases provide icons to help you determine if an article is academic (peer-reviewed), a book, a newspaper, etc. Look for an academic journal icon or use database tools to limit your results to only peer-reviewed articles. Alternatively, you can google the title of the journal the article was published from to find out whether it is peer-reviewed.
- relevant. Scientific research articles take a deep dive into narrow scientific topics. Your textbook might introduce a topic briefly but by locating scientific articles you can find more in-depth and current
research on a specific topic.

In Biology, a great option for finding scientific articles is Google Scholar. It’s easy to search, just like Google. Another great option for finding research is the database Scopus. Scopus can be a little tricky to search but here are some helpful tips. Your GTA will demonstrate this live during class:

- Refine your document search by searching for certain keywords only in the article title instead of in the article title, abstract, and keywords.
- Limit your document search to recent articles, like in the last ten years.
- Refine your search results by choosing “article” under document type.
- You can search within your results to narrow your search even further.
- In your search results, if a title sounds interesting, click on it and read through the abstract. This brief section at the beginning of the article will provide you with a concise summary of the author’s research, results, and interpretations. If the article still sounds relevant to your project, click the “Article Linker” button on the top of the page to find a full-text version of the article.

But how should you read a scientific article once you have one that sounds relevant to your project? This can be tricky because scientific articles are often long and full of complicated procedures and terminology. There is a way to read scientific articles efficiently. You might also find it helpful to have a dictionary at the ready to look up terminology you are unfamiliar with.

- Step 1. Read the discussion section. This section summarizes important results and gives conclusions based on the data. It also mentions how the results fit into the context of the field.
- Step 2. Read the introduction section. This will briefly explain the background of the research and why it was conducted.
- Step 3. Read the results section. Pay close attention to the tables and graphs. These are quick summaries of the findings of the study (the data the authors collected and the sample sizes they used).
- Step 4. Read the methods section. This tells you how the authors approached answering their research questions.

Notes about the relevant sources we found and corresponding information:
6) **Cite sources** you use information from to write your introduction. Whenever you summarize or paraphrase information that is not your own, you need to give credit to the source of the information, both in the text and in a References section towards the end of your report. In Biology, sources are cited in a certain way to aid the reader. We will use APA citation guidelines. In class you will use the example guidelines below (for three or more authors) to practice citing a source in the APA format, both as an in-text citation and a Reference section citation. However, see the link in Canvas for APA citation guidelines for all the details.

**Example in-text citation, APA format:**
(Kernis et al., 1993)
Kernis et al. (1993) suggest...

**Example reference section citation, APA format:**

7) In-class assignment. You are now ready to use all your notes and the Biodiversity Project lab report template to **complete a draft of the following sections of the lab report: title, authors, introduction, acknowledgements, references.**

You will receive credit for completing a draft of each of the following. The quality will not be assessed until the final lab report. However, the more effort you put in now, the more meaningful feedback you will receive from your GTA. Save your document as a pdf and upload it to Canvas. Your GTA will provide timely feedback to help you make improvements on your lab report.

- Title (1 pt)
- Authors (1 pt)
- Introduction part 1 (1 pt)
- Introduction part 2 (1 pt)
- Introduction part 3 (1 pt)
- Cited sources within the introduction in APA format (1 pt)
- Acknowledgements (1 pt)
- Cited sources in the References section in APA format (1 pt)

**References:**
Biodiversity project: Methods: Tissue collection, sterilization, and plating  
Week 7 of BIOL 1615, fall 2021

Objectives: In this lab you will be able to: 1) perform tissue collection, sterilization to remove epiphytes, and plating for microbe culturing, 2) present the information in the methods section of a lab report as a biologist would, and 3) collaborate with your group members.

Pre-lab read through: Highlight the parts of the methods that a trained biologist would need to know to replicate our methods, as opposed to the parts of the methods that are common knowledge or practice (like putting on a lab coat). Your lab report methods section will emphasize these relevant details.

Lab safety: Personal protective equipment (PPE) is required before you start step 1 of the methods: lab coat, safety googles, and disposable gloves. You should apply a little ethanol on your gloves throughout these methods to keep them sterilized. Only after equipment clean-up are laptops allowed at the group tables.

Equipment:
Plant tissue collection:
- Whirl-pak with label
- Sharpie
- Small bottle of ethanol
- Forceps

Plating for microbe culturing:
- Clorox spray and paper towels
- 1 sprig alfalfa from assigned plant
- Small beaker
- Ethanol
- Forceps and suture scissors
- Small empty petri dishes
- 0.1% NaClO (the active ingredient in bleach)
- Sterile water
- Conical tube for transporting sterile water
- Biosafety cabinet
- MEA plate
- Parafilm
- Sharpie
- Growth chamber set to 30°C

Sterilization for next week’s DNA extraction:
- Clorox spray and paper towels
- 1 spring alfalfa from assigned plant
- Small beaker
- Ethanol
- Forceps and suture scissors
- Large empty petri dishes
- 0.1% NaClO (the active ingredient in bleach)
- Sterile water
- Conical tube for transporting sterile water
- Balance and weighing paper
- 2 ml safe-lock microcentrifuge tube
- Sharpie
- -20°C freezer

Methods:
1) Group plant assignment. At the beginning of class, your GTA will assign your group one plant in the Science
Garden to work with. You will follow this week’s methods with this plant and extract the DNA from its leaf endophytes next week. Your assignment comes with a DNA #. Record the plot #, plant ID and DNA # below.

My group’s assigned Science Garden plant ID & plot #:
My group’s DNA #:

Add the correct label to your whirl-pak – it needs to match your GTA’s datasheet. For example, in the label “SG-1-VUH14-”, the 1 in “SG-1” needs to match your assigned plot number, and the “VUH14” needs to match your assigned plant number in that plot. Add your section number, group number, and DNA # to the label with the provided sharpie.

2) Plant tissue collection. Before going to the Science Garden, locate your plant on the map (Figure 1). Bring with you to the Science Garden: your lab manual, the small bottle with ethanol, forceps, and your labeled whirl-pak. All group members should be present, but only one or two group members need to perform the tissue collection methods.

![Science Garden map](image)

**Figure 1.** Science Garden map.

a. Once you are at your assigned plant in the Science Garden, dip your forceps into the bottle of ethanol to sterilize them.
b. With the sterilized forceps, take two sprigs (end of branch with leaves) total from the plant, each including at least 15 leaflets. Add the sprigs to the whirl-pak.

To use the whirl-pak: tear off the top of the bag along the perforation. Please discard this top piece of plastic in a trash can. Use the pull tabs on each side of the bag to open (sometimes a little pull on the bottom of the bag can help when opening). Put the sample into the bag, leave space at the top for closing. Pull the ends of the wire to close the bag. Hold the bag by the wire ends, whirl the bag three complete revolutions to form a seal. Bend the wire ends over onto the bag to complete the closing.

c. Return to the classroom. Sterilize your group table with Clorox spray and paper towels.

The next two major steps of the lab manual, plating for microbe culturing and sterilization for upcoming DNA extraction, are similar. One of your collected sprigs will be used for plating for microbe culturing, and the other will be used for sterilization for upcoming DNA extraction. Any leftover tissue from the two sprigs need to be saved in your whirl-pak – we will store the leftovers in a freezer long-term.

3) Plating for microbe culturing. Do this step in the biosafety cabinet to help you minimize contamination. Do not stick your head inside as seen in the pre-lab video. Two people should be able to work in the biosafety cabinet at a time.

a. Sterilize the surface of your plant tissue to remove the epiphytes (microbes on the outside of the plants).

- Fill a beaker with enough ethanol to cover the blades of your suture scissors. Rest your scissors and forceps (tip down) in the beaker to sterilize them and keep them sterile between uses.
- Rinse one alfalfa sprig gently under the tap for 30 seconds to remove surface debris.
- Separate the two halves of the small petri dish (top from bottom). You will cut leaf material into one half of the petri dish. Cut five segments that are about 2-5 mm (use the provided ruler to see what 2 x 2 mm looks like – it’s small!). Try to make sure the five segments are similarly-sized. Transfer any pieces that do not fall into the dish to the dish using your sterile forceps. Return the forceps to the beaker when finished.
- Fill the half of the petri dish containing your plant segments with 0.1% NaClO (sodium hypochlorite). Let them soak for two minutes. Make sure all segments are covered (not floating) by poking them down with your sterile forceps.
- After two minutes have passed, transfer your segments from the NaClO into the empty half of your petri dish using sterile forceps. Cover them with sterile water and let them soak for two minutes. Again, make sure the segments are covered and not floating. Try to minimize the amount of time your segments are exposed to the air.

b. Culture the endophytes.

- After two minutes in the sterile water, you will start transferring your five plant segments onto the nutrient agar of your culture plate, one at a time. The agar contains 2% malt extract, which creates an optimal environment for microbe growth. To start, remove one segment from the sterile water using sterile forceps. Do not wave it in the air to dry or pat it dry on paper towels—this can introduce contaminates and undo the surface sterilization you just performed.
- Open the lid of your MEA (malt extract agar) plate just enough to gently press the plant segment onto the agar (the lid of your dish should never be completely removed). The plant segment should stick to the agar so that it doesn’t move but be careful not to puncture or gouge the agar. If you do puncture/gouge the agar, the agar will dry out relatively quickly; consider starting over if your GTA has an extra plate. Try to make sure that the entire surface of the segment is touching the agar. Replace the lid in between plating segments to minimize the chance of air contaminants getting in.
- Repeat four times so there are a total of five segments on your plate. Plate them in a way that maximizes
the amount of space between each segment (Figure 2) so you can differentiate between endophytes growing from different segments in a few weeks.

- Seal the perimeter of your dish (where top and bottom meet) with parafilm. Flip the dish upside-down (agar side up) and mark each segment with a small number in sharpie (Figure 2). Then, add to the perimeter of the bottom of the dish: lab section #, group #, DNA# (not shown in Figure 2).

![Figure 2](image.png)

**Figure 2.** Number each plant segment, like this, on the bottom of your petri dish.

- You will incubate your dish at 30°C for up to three weeks. Between now and Week 10, your GTA will keep an eye on your microbe growth. Once your GTA sees a lot of endophytes growing on your dish, they will store the dish at 4°C for you, to prevent overgrowth.

- **Clean-up:** Pour your NaClO and ethanol down the drain with water from the facet. Be careful not to spill the NaClO when walking it to the sink. Rinse your small petri dishes and beakers with lots of water, dry them with paper towels, and return them to your tray. Clean your workspace with Clorox spray and paper towels. Any leftover tissue from the alfalfa sprig needs to be saved in your whirl-pak – we will store this in a freezer long-term.

4) **Sterilization for next week’s DNA extraction.** You will sterilize the leaf tissue surface to remove the epiphytes (microbes on the outside of plants). This way, you will only extract DNA from the leaf endophytes next week.

- Fill a beaker with enough ethanol to cover the blades of your suture scissors. Rest your scissors and forceps (tip down) in the beaker to sterilize them and keep them sterile between uses.
- Set up the four large petri dishes (tops and bottoms separated). The first one needs 0.1% NaClO (sodium hypochlorite). The last three dishes need sterile water.
- Rinse one alfalfa sprig gently under the tap for 30 seconds to remove surface debris.
- Use a classroom balance. These balances are sensitive; please give them space while they are working. Make sure the balance is measuring in mg before proceeding. Add a new piece of weighing paper to the balance and tare it (zero out) so that you only weigh the leaves you add. Use the sterile forceps and scissors to remove leaves, one at a time, from one alfalfa sprigs. Remove the number of leaves it takes to have 45-55 mg of plant tissue. Tell your GTA how many leaves you ended up having to use and how much they weighed so they can add it to their spreadsheet.

**# of leaves we used:**

**Exact weight of leaves (in mg):**

- Add the leaves to the dish with 0.1% sodium hypochlorite. Soak the leaves in the solution for 30 seconds.
- Using the sterile forceps, rinse each leaf in the series of sterile water dishes.
- Add all the leaves to the provided 2 ml safe-lock microcentrifuge tube. Use the sharpie to clearly label the top and side of the tube with your DNA#. Give it to your GTA to store in the freezer for next week.
- **Clean-up:** Pour your NaClO and ethanol down the drain with water from the facet. Be careful not to spill the NaClO when walking it to the sink. Rinse your large petri dishes and beaker with lots of water, dry
them with paper towels, and return them to your tray. Dispose of the weighing paper in a trash can. Clean your workspace with Clorox spray and paper towels. Any leftover tissue from the alfalfa sprig needs to be saved in your whirl-pak – we will store this in a freezer long-term.

5) Return all equipment to its original organization. Give your whirl-pak with leftover alfalfa tissue to your GTA to store in the freezer long-term. If you borrowed safety goggles, clean them with ethanol and a kimwipe at the cleaning station. Thank you!

5) In-class assignment. You are now ready to use a laptop, this lab manual, and the Biodiversity Project lab report template to complete a draft of the following subsection of the Methods section of your lab report: tissue collection, sterilization, and plating methods. Emphasize the relevant details for replicating these methods and leave out details that would be common knowledge to a trained biologist.

You will receive credit for completing a draft of each of the following. The quality will not be assessed until the final lab report. However, the more effort you put in now, the more meaningful feedback you will receive from your GTA. Save your document as a pdf and upload it to Canvas. Your GTA will provide timely feedback to help you make improvements on your lab report.

- A subject heading and subheading for this methods section of your lab report (1 pt)
- Description of how plant tissue was collected (1 pt)
- Description of how the plant tissue was sterilized and plated for endophyte culturing (1 pt)
- Description of how the plant tissue was sterilized for DNA extraction (1 pt)

6) If you borrowed a class laptop, delete your files. Clean the laptop lightly with isopropyl alcohol as instructed at the cleaning station. Thank you!
**Biodiversity project:** **Methods: Molecular methods (DNA extraction)**

Week 8 of BIOL 1615, fall 2021

**Objectives:** In this lab you will be able to: 1) perform DNA extraction from plant tissue, 2) present the information in the methods section of a lab report as a biologist would, and 3) collaborate with your group members.

**Pre-lab read through:** Highlight the parts of the methods that a trained biologist would need to know to replicate our methods, as opposed to the parts of the methods that are common knowledge or practice (like putting on a lab coat). Your lab report methods section will emphasize these relevant details.

**Lab safety:** Personal protective equipment (PPE) is required before you start step 1 of the methods: lab coat, safety googles, and disposable gloves. Only after equipment clean-up are laptops allowed at the group tables.

**Equipment for in-class DNA purification:**
- Plant tissue sample from Week 7 (will have been mechanically disrupted before class)
- Chemical aliquots
  - 400 ul Buffer AP1
  - Your GTA will dispense 4 ul RNase A for you
  - 130 ul Buffer P3
  - 800 ul Buffer AW1 (you will need 675 ul of this)
  - 500 ul Buffer AW2
  - 500 ul Buffer AW2
  - 100 ul Buffer AE
- Assortment of microcentrifuge tubes in a tube rack
  - 1 QIAshredder spin column with collection tube
  - 1 DNeasy spin column with collection tube
  - 1 collection tube by itself (does not have a lid)
  - 2 empty 2-ml microcentrifuge tubes (does have a lid)
- 100-1000 ul pipet
- 100-1000 ul tips
- Waste container
- Heating block set to 65°C
- Vortex
- Bucket of ice
- Microcentrifuge
- Sharpie

**Background information:** For extracting DNA from your leaf tissue sample, you will use supplies and chemicals from a DNeasy Plant Mini Kit made by a company called QIAGEN. This kit provides a fast and easy way to purify DNA from plants and microbes. The procedure will provide pure total DNA (genomic, mitochondrial, and chloroplast) for reliable polymerase chain reaction (PCR; see Week 9) in approximately one hour. DNA purified with this kit is up to 40 kb in size, with fragments of 20-25 kb predominating. DNA of this length denatures completely in PCR and shows the highest amplification efficiency. The DNeasy membrane ensures complete removal of all inhibitors of PCR (Qiagen, 2006).

There are two main steps of this DNA extraction protocol: mechanical disruption of plant tissue and DNA purification. The disruption of plant tissue will be completed by the lab coordinator before your class session. Complete and quick mechanical disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation. The lab coordinator will use a TissueLyser II machine for this because it provides convenient, simultaneous disruption and homogenization of multiple samples (48 total) through high-speed shaking in plastic tubes with 3 mm stainless steel, tungsten carbide beads. Disruption will be performed in two 1-minute high-speed (30 Hz) shaking steps. The position of the tubes within the adapter set will be reversed.
in between shaking steps. For optimal operation, the TissueLyser II will always be balanced. The beads are reusable.

DNA purification is the part of the DNA extraction process you will perform with your group during class. Your mechanically disrupted plant material will be lysed by addition of lysis buffer and incubation. RNase A in the lysis buffer will digest the RNA in the sample. After lysis, proteins and polysaccharides are salt-precipitated. Specifically, these cell debris and salt precipitates are removed by centrifugation through a QIAshredder spin column. The preparation of a cleared lysate is essential to prevent clogging of the DNeasy spin column in the following step. Purified DNA is eluted from the DNeasy spin column using a buffer called Buffer AE. Buffer AE is 10mM Tris-Cl, 0.5 mM EDTA, pH 9.0. The pH of Buffer AE is optimal for DNA elution from the DNeasy membrane.

In-class methods:
1) Locate all the materials you need (see equipment list on pg 1). You will notice your disrupted leaf tissue and a stainless steel bead in the 2-ml microcentrifuge tube you set up last week. When you are done using this tube, you will give it back to your GTA so that we can retain, clean, and reuse the stainless steel bead. Thanks!

2) You will receive training on how to properly pipet a certain volume using sterile techniques in the Week 8 video and in class. Also, Figure 1 is a good reminder of proper pipet usage. You will use a new pipet tip for each step. Discard used pipet tips in your waste container.

![Figure 1](http://www.fao.org/docrep/005/ac802e/ac802e05.htm)

Figure 1. Only use the first stop to suck up liquids, and only use the second stop to dispense liquids.

3) Add 400 ul Buffer AP1 to the tube with your disrupted leaf tissue.

4) Ask your GTA to pipet 4 ul RNase stock solution into this tube with your disrupted leaf tissue. (Do not mix Buffer AP1 and RNase A before use.)

5) Close the lid and vortex the tube with your disrupted leaf tissue vigorously for about 10 seconds.

6) This step lyses the cells. Incubate the tube with your disrupted leaf tissue in the heating block set to 65°C for 10 minutes. Mix it 2-3 times during incubation by inverting the tube.

7) This step precipitates detergent, proteins, and polysaccharides. Remove the tube from the heating block and add 130 ul Buffer P3 to the tube (i.e., to the lysate). Vortex for a few seconds. Then, stick the tube on ice for 5 minutes.
8) Remove the tube from ice and give it to your GTA for centrifugation. Your GTA will balance the microcentrifuge with all 6 tubes in the class and centrifuge the tubes for 5 minutes at 20,000 x g (14,000 rpm).

9) After centrifugation, you will remove the supernatant with your pipet (see Figure 2), approximately 530 ul, and add it to the QIAshredder Mini spin column (lilac) placed in its collection tube (Figure 3). Label the top with your unique DNA#.

![Figure 2](Image from eppendorf.com.)

**Figure 2.** The supernatant is the liquid that remains after centrifugation. You will pipet this out of the tube without disturbing the pellet at the bottom. (Image from eppendorf.com.)

![Figure 3](Image from eppendorf.com.)

**Figure 3.** The QIAshredder Mini spin column (lilac) in its collection tube.

10) Give this new labeled tube set-up (QIAshredder Mini spin column + collection tube) to your GTA for centrifugation. Your GTA will balance the microcentrifuge with all 6 tubes in the class and centrifuge the tubes for 2 minutes at 20,000 x g (14,000 rpm).

11) After centrifugation, pipet the supernatant (Figure 2) in the collection tube, approximately 450 ul, into a new empty 2-ml microcentrifuge tube. Label the top with your DNA#. You can put the used QIAshredder Mini spin column and its collection tube (Figure 3) in the waste container.

12) Add 675 ul of Buffer AW1 to the 2-ml microcentrifuge tube you are working on (i.e., to the cleared lysate). Mix by pipetting.

13) Pipet 650 ul of the mixture from step 12, including any precipitate that may have formed, into the DNeasy Mini spin column placed in its collection tube (Figure 4). Label the top with your unique DNA#.

![Figure 4](Image from eppendorf.com.)

**Figure 4.** The DNeasy Mini spin column in its collection tube.
14) This step binds your DNA to the filter in the spin column for washing it. Give this new labeled tube set-up (DNeasy Mini spin column + collection tube) to your GTA for centrifugation. Your GTA will balance the microcentrifuge with all 6 tubes in the class and centrifuge the tubes for 1 minute at 6,000 x g (~8,000 rpm).

15) After centrifugation, pour the flow-through liquid that is in the collection tube into your waste container. You will reuse the collection tube in the next step.

16) Repeat steps 13 and 14 with your remaining liquid sample. Discard the flow-through and collection tube into the waste container. You will use the DNeasy Mini spin column in the next step.

17) Place the DNeasy spin column into a new collection tube (in your tube rack). Add 500 ul Buffer AW2.

18) Give this labeled tube set-up (DNeasy Mini spin column + collection tube) to your GTA for centrifugation. Your GTA will balance the microcentrifuge with all 6 tubes in the class and centrifuge the tubes for 1 minute at 6,000 x g (~8,000 rpm).

19) After centrifugation, discard the flow-through and reuse the collection tube in the next step.

20) Add 500 ul Buffer AW2 to the DNeasy Mini spin column.

21) Give this labeled tube set-up (DNeasy Mini spin column + collection tube) to your GTA for centrifugation. Your GTA will balance the microcentrifuge with all 6 tubes in the class and centrifuge the tubes for 2 minutes at 20,000 x g (14,000 rpm) to dry the membrane. It is important to dry the membrane of the DNeasy Mini spin column, because residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over to the last step (elution).

22) After centrifugation, remove the collection tube carefully so that the column does not come into contact with the flow-through, because that will result in carryover of ethanol. Discard the flow-through and collection tube. Transfer the DNeasy Mini spin column to a new empty 2-ml microcentrifuge tube. Pipet 100 ul of Buffer AE onto the DNeasy membrane (without touching the membrane). Close the lid and let this sit for 5 minutes at room temperature.

23) This step elutes the DNA (removes it from the filter in the spin column). Next, give this labeled tube set-up (DNeasy Mini spin column + 2-ml microcentrifuge tube) to your GTA for centrifugation. The lid of the 2-ml microcentrifuge tube does not need to close over the DNeasy Mini spin column lid. Your GTA will balance the microcentrifuge with all 6 tubes in the class and centrifuge the tubes for 1 minute at 6,000 x g (~8,000 rpm).

24) After centrifugation, discard the DNeasy Mini spin column. Close the lid of the 2-ml microcentrifuge tube. Label the top and side of the tube clearly with your DNA#. Give this labeled tube to your GTA for storage in the classroom freezer (-20°C) for future PCR and sequencing.

25) Empty waste container into a classroom trashcan and rinse it with water at a classroom sink. Return all equipment to its original organization. If you borrowed safety goggles, clean them with ethanol and a kimiwipe at the goggles cleaning station. Thank you!

26) In-class assignment. You are now ready to use a laptop, this lab manual, and the Biodiversity Project lab report template to complete a draft of the beginning of the following subsection of the Methods section of your lab report: molecular methods. Emphasize the relevant details for replicating these methods and leave out details that would be common knowledge to a trained biologist.

You will receive credit for completing a draft of each of the following. The quality will not be assessed until the final lab report. However, the more effort you put in now, the more meaningful feedback you will
receive from your GTA. Save your document as a pdf and upload it to Canvas. Your GTA will provide timely feedback to help you make improvements on your lab report.

- A subheading for this methods section of your lab report (1 pt)
- Description of the mechanical disruption of plant tissue (1 pt)
- Description of DNA purification (1 pt)

27) If you borrowed a class laptop, delete your files. Clean the laptop lightly with isopropyl alcohol as instructed at the cleaning station. Thank you!

Reference:
Objectives: In this lab you will be able to: 1) articulate the general processes of gel electrophoresis, PCR and next-generation sequencing, 2) present the project details in the methods section of a lab report as a biologist would, and 3) collaborate with your group members.

Pre-lab read through: Highlight the parts of the methods that a trained biologist would need to know to replicate our methods, as opposed to the parts of the methods that are common knowledge or practice. Your lab report methods section will emphasize these relevant details.

Background information and methods:

In June 2021, Lauren Lucas took some of the extracted DNA from 177 Science Garden leaf tissue samples (sampled in the fall of 2020): 40 uL at approximately 10 ng/uL. She shipped this overnight on ice to a company called CD Genomics in Shirley, NY (https://www.cd-genomics.com). They performed:

1) DNA quality control (QC) procedures that included gel electrophoresis,
2) 16S DNA fragment library preparation via polymerase chain reaction (PCR) and the associated QC procedures,
3) And next-generation sequencing (NGS) with an Illumina platform.

Below you will find more general and specific details about each major step.

1) DNA QC

In general, gel electrophoresis separates DNA molecules based on charge and size (Figure 1). DNA is negatively charged and is repelled by the negative electrode (cathode) and attracted by the positive electrode (anode) when an electric current is applied across the gel. It separates because different lengths of DNA move through the gel matrix at different rates. Longer fragments move more slowly than shorter fragments. The gel is stained with a chemical such as ethidium bromide to visualize the band pattern. A molecular mass ruler is used to estimate the size of the band(s). The number of bands in the ruler and the size of each of those bands is known.

![Loading a sample into the gel](Image from Bio-Rad Laboratories, Inc.)

Specifically, in June 2021, CD Genomics completed QC and 16S amplification. First, they verified the presence of DNA from each sample with gel electrophoresis, using a 1% agarose gel that was run at 100V for 40 minutes. All samples passed this step of QC.

2) PCR and QC

PCR, polymerase chain reaction, was developed by Kary Mullis in 1983 and transformed molecular
biology into a multidisciplinary research tool. PCR had an impact on four main areas of biotechnology: gene mapping, cloning, DNA sequencing, and gene detection. PCR is now used as a medical diagnostic tool to detect specific mutations that may cause genetic disease, in criminal investigations and courts of law to identify suspects on a molecular level, and in the sequencing of the human genome. Prior to PCR, the use of molecular biology techniques for therapeutic, forensic, pharmaceutical, or medical diagnostic purposes was not practical or cost-effective. The development of PCR technology changed these aspects of molecular biology from a difficult science to one of the most accessible and widely used tools in genetic and medical research.

PCR produces exponentially large amounts of a specific piece of DNA from trace amounts of starting material (template). The template can be any form of double-stranded DNA, such as genomic DNA. A researcher can take trace amounts of DNA from a drop of blood, a single hair follicle, or a corn chip (for example) and use PCR to generate millions of copies of a desired DNA fragment. In theory, only a single molecule of double-stranded template DNA is needed to generate millions of copies. Prior to the development of the PCR technique, it would have been impossible to do forensic or genetic studies with a minute sample containing only a few molecules of source DNA. The ability to amplify a precise sequence of DNA to a sufficient quantity that a researcher can analyze and manipulate is the true power of PCR.

Before a region of DNA can be amplified, one must identify and determine the sequence of an area of DNA upstream and downstream of the region of interest. These areas are then used to determine the sequences of oligonucleotide primers that will be synthesized and used as starting points for DNA replication. Primers are needed because DNA polymerases can only add nucleotides to the end of a preexisting chain. The DNA polymerase used in PCR must be a thermally stable polymerase because the polymerase chain reaction cycles between ~59°C–94°C. The thermostable DNA polymerase (Taq) used in PCR was isolated from a thermophilic bacterium, Thermus aquaticus, which lives in high-temperature steam vents such as those found in Yellowstone National Park.

Two new template strands are created from the original double-stranded template during each complete cycle of the strand synthesis reaction. This causes exponential growth of the number of template molecules, i.e., the number of DNA strands doubles at each cycle. Therefore, after 30 cycles there will be $2 \times 10^{30}$ times more copies than at the beginning. Once the DNA of interest has been sufficiently amplified, it can be visualized using gel electrophoresis. This allows researchers to determine the presence or absence of the desired PCR products.

PCR involves a repetitive series of cycles, each of which consists of template denaturation, primer annealing, and extension of the annealed primer by Taq DNA polymerase (Figure 2). Following DNA extraction, the template DNA, oligonucleotide primers, thermostable DNA polymerase (Taq), the four deoxynucleotides (dNTPs: A, T, G, C), and reaction buffer are mixed in a single micro test tube. The tube is placed into the thermal cycler. These thermal cyclers contain an aluminum block that holds the samples and can be rapidly heated and cooled across wide temperature differences. The rapid heating and cooling of this thermal block is called temperature cycling or thermal cycling. The first step of the PCR temperature cycling procedure involves heating the sample to ~94°C. At this high temperature, the template strands separate (denature). This is called the denaturation step. The thermal cycler then rapidly cools to ~59°C to allow the primers to anneal to the separated template strands. This is called the annealing step. The two original template strands may reanneal to each other, or compete with the primers for the primers' complementary binding sites. However, the primers are added in excess such that the primers actually outcompete the original DNA strands for the primers' complementary binding sites. Last, the thermal cycler heats the sample to ~72°C for Taq DNA polymerase to extend the primers and make complete copies of each DNA strand. This is called the extension step. Taq polymerase works most efficiently at this temperature. Two new copies of each complementary strand are created. There are now two sets of double-stranded DNA (dsDNA). These two sets of dsDNA can now be used for another cycle and subsequent strand synthesis. At this stage, a complete temperature cycle (thermal cycle) has been completed.
Specifically, for each of the 177 DNA samples from fall 2020 of the Biodiversity Project, CG Genomics amplified the gene region called 16S V5-V7, with the 16S primers listed in Table 1. The 16S ribosomal RNA gene region codes for 16S rRNA, which is the component of the 30S small subunit of a prokaryotic ribosome that binds to the Shine-Dalgarno sequence. This gene is used for reconstructing bacteria phylogenies, due to its slow rate of evolution ("16S", 2020). The chosen primers also help discriminate against organellar contamination (Giangacomo et al., 2020). All PCRs were carried out in 30 μL reactions with:

- 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs)
- 0.2 μM of forward and reverse primers
- about 10 ng template DNA

A unique barcode (index) was also added to each of the 177 samples during PCR, and the barcode (index) was internal to the sequencing primers. Thermal cycling consisted of:

- initial denaturation at 98 °C for 1 min,
- followed by 30 cycles of:
  - denaturation at 98 °C for 10 s,
  - annealing at 50 °C for 30 s,
  - elongation at 72 °C for 60 s,
- and finally, 72 °C for 5 min.

Table 1. Primers used by CD genomics to amplify 16S. “F” refers to the forward primer, “R” refers to the reverse primer.

<table>
<thead>
<tr>
<th>Primer name and direction</th>
<th>Sequence of the primer</th>
<th>Citation for the primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>799F</td>
<td>5'-AACMGGATTAGATACCCKG-3’</td>
<td>Chelius &amp; Triplett (2001)</td>
</tr>
<tr>
<td>1193R</td>
<td>5'-ACGTCATCCCCCACCTTCC -3’</td>
<td>Bodenhausen et al. (2013)</td>
</tr>
</tbody>
</table>
After PCR, equal parts of 1× buffer and PCR were used to operate electrophoresis on 2% agarose gels for detection (80V, 40 min). All 177 samples passed this PCR QC inspection via gel electrophoresis, meaning they had enough amplified DNA for library construction and sequencing. **Figure 3** shows an image of one of the three gels they ran on our PCR product; notice the band illuminated at each well of the gel.

![Figure 3: Amplification results for 93 of our samples.](image)

**Information for Honor’s lab section 001 only:**
ITS, internal transcribed spacer, is an approximately 600 base-pair locus frequently used in species-level systematics for fungi. The ITS1 region was amplified for all 177 samples using the specific primers ITS1-1F-F (5'-CTTGGTCATTTAGGAAAGTAA-3'; Grades & Bruns, 1993) and ITS1-1F-R (5'-GCTGCGTTCTTGCATGC-3'; White et al., 1990). All other PCR and sequencing methods were the same as those carried out for the bacterial endophytes, except the bright main band of approximately 350 bp was extracted from the gel for each sample, as opposed to 300 bp for 16S (see below).

### 3) DNA sequencing with an Illumina platform (NGS methods)

Sanger sequencing is a technique developed by Frederick Sanger in the 1970's (Brooker et al., 2008). In this technique, one strand of a DNA fragment (up to 1,000 nucleotides long) is used as a template for synthesis of a nested set of complementary fragments; these are then analyzed to yield the sequence (**Figure 4**). Specifically, each new strand starts with the same primer and ends with a dideoxynucleotide (ddNTP), which is a modified deoxynucleotide (dNTP). Incorporation of a ddNTP terminates a growing DNA strand because it lacks a 3’–OH group, the site for attachment of the next nucleotide. Because each type of ddNTP is tagged with a distinct fluorescent label, the identity of the ending nucleotides of the new strands, and ultimately the entire original sequence, can be determined. Sanger sequencing is still widely used today for routine, small-scale sequencing jobs. But “next-generation sequencing” (NGS) techniques have been developed that do not rely on chain termination; instead, hundreds of thousands of fragments (up to 1,000 nucleotides long) are sequenced in parallel. Strands of each fragment are synthesized, one nucleotide at a time, and a chemical technique enables electronic monitors to identify in real time which of the four nucleotides is added. In class, you will further articulate how NGS works. You can add your notes below:
In July 2021, CD Genomics completed library preparation and 16S sequencing. The bright main band of approximately 300 bp was extracted from the gel for each sample. All extracted PCR products were mixed (pooled) at the same concentration and then gel-purified with a Qiagen Gel Extraction Kit (Qiagen, Germany). According to the manufacturer’s suggestion and the added barcode (index), TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) without PCR was used to generate the sequencing libraries. The 16S DNA fragment libraries were then evaluated with the Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system to assess final concentrations and purity. CD Genomics did not share these QC results with us.

CD Genomics used an Illumina sequencing machine called a Novaseq run in PE250 mode. They sequenced 250 bp from each end of each amplified fragment (i.e., we have the forward and reverse strand). These fragments mostly overlap; thus, most PCR fragments were less than 500 bp.

CD Genomics ran a 16S sequencing QC report. A sample of this report is shown in Table 2. The raw data was transferred to the Center for High Performance Computing at the University of Utah via secure, password protected FTP.
Table 2. A sample of the 2020 16S sequencing QC report. Raw PE is the number of paired-end reads (forward and reverse). Base is the number of bases (nucleotides) sequenced. Avglen is the average sequence length (in each direction – for forward and for reverse). Q20 = % of bases with base quality score > 20; this corresponds with an error probability of 0.01 or less. Q30 = % of bases with base quality score > 30; this corresponds with an error probability of 0.001 or less. GC% = percent of bases that were G or C.

<table>
<thead>
<tr>
<th>Sample name (Plot-plant)</th>
<th>Raw PE</th>
<th>Base</th>
<th>Avglen</th>
<th>Q20</th>
<th>Q30</th>
<th>GC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-APLL7</td>
<td>65,949</td>
<td>16,112,448</td>
<td>377</td>
<td>98.59</td>
<td>95.47</td>
<td>53.34</td>
</tr>
<tr>
<td>5-AWFS2</td>
<td>77,479</td>
<td>18,217,729</td>
<td>377</td>
<td>98.46</td>
<td>95.1</td>
<td>53.78</td>
</tr>
<tr>
<td>2-VIC25</td>
<td>90,524</td>
<td>19,761,363</td>
<td>377</td>
<td>98.5</td>
<td>95.25</td>
<td>53.72</td>
</tr>
<tr>
<td>3-AWFS7</td>
<td>108,795</td>
<td>25,383,134</td>
<td>378</td>
<td>98.77</td>
<td>95.91</td>
<td>53.43</td>
</tr>
<tr>
<td>6-VIC2</td>
<td>91,201</td>
<td>20,540,278</td>
<td>377</td>
<td>98.66</td>
<td>95.65</td>
<td>53.09</td>
</tr>
<tr>
<td>6-VIC8</td>
<td>93,151</td>
<td>21,341,002</td>
<td>377</td>
<td>98.53</td>
<td>95.36</td>
<td>53.66</td>
</tr>
<tr>
<td>2-VUH16</td>
<td>96,345</td>
<td>21,300,354</td>
<td>376</td>
<td>98.58</td>
<td>95.48</td>
<td>54.1</td>
</tr>
<tr>
<td>3-APLL8</td>
<td>57,997</td>
<td>13,634,518</td>
<td>377</td>
<td>98.63</td>
<td>95.59</td>
<td>54.1</td>
</tr>
</tbody>
</table>

In-class assignment. You are now ready to use a laptop, this lab manual, and the Biodiversity Project lab report template to complete a draft of the rest of the following subsection of the Methods section of your lab report: molecular methods. Emphasize the relevant details for replicating these methods and leave out details that would be common knowledge to a trained biologist.

You will receive credit for completing a draft of each of the following. The quality will not be assessed until the final lab report. However, the more effort you put in now, the more meaningful feedback you will receive from your GTA. Save your document as a pdf and upload it to Canvas. Your GTA will provide timely feedback to help you make improvements on your lab report.

- Description of the DNA QC methods (1 pt)
- Description of the library preparation via PCR and associated QC methods (1 pt)
- Description of the NGS methods (1 pt)

If you borrowed a class laptop, delete your files. Clean the laptop lightly with isopropyl alcohol as instructed at the cleaning station. Thank you!

References:
16S ribosomal RNA. (2020, October 16). In Wikipedia.


**Biodiversity project:** **Methods: Microscopy & Figure 1**  
**Week 10 of BIOL 1615**

**Objectives:** In this lab you will be able to: 1) perform microscopy to observe and generally characterize the endophytes you’ve cultured since Week 7, 2) present the project details in the methods section and Figure 1 of a lab report as a biologist would, and 3) collaborate with your group members.

**Pre-lab read through:** Highlight the parts of the methods that a trained biologist would need to know to replicate our methods, as opposed to the parts of the methods that are common knowledge or practice. Your lab report methods section will emphasize these relevant details.

**Lab safety:** Personal protective equipment (PPE) is required before you start step 1 of the methods: lab coat, safety googles, and disposable gloves. Wearing a mask this week will keep you safe from microbe allergens and COVID-19.

**Equipment for making Figure 1b:**
- 1 prepared slide for microscopy practice
- 1 new microscope slide
- 1 new coverslip
- 1 Sharpie
- 1 bottle of ethanol
- 1 reusable plastic pipette/dropper
- 1 disposable loop (for sampling a bacterial endophyte)
- 1 forceps (for sampling a fungal endophyte)
- 1 beaker with staining rails
- Methylene blue stain
- 1 spray bottle of distilled (DI) water
- 1 box of kimwipes
- Leica compound light microscope
- Classroom laptop (start in Mac mode; if you must switch to PC mode during class, restart the laptop and press and hold the Option key as the laptop begins to start)
  - Microscopy software: Leica Acquire in Mac mode; Leica LAS EZ in PC mode
- For clean-up:
  - Biohazard disposal for culture plates and used loops only.
  - Slide disposal box for used coverslips and slides only.
  - Funnel and carboy for Methylene blue stain waste.
  - All other trash (like used gloves) need to go in a trashcan.
  - Remove all photos from the classroom laptop when you are done.
  - Isopropyl alcohol and cloth for gently cleaning classroom laptop.
  - Clorox spray and paper towels for cleaning group table.

**Background information and methods:** Culturing endophytes is a great way to reveal the hidden biodiversity inside plant tissues. Using the steps below, you and your group members will characterize the endophyte diversity you’ve cultured from your leaf tissue since Week 7. But the main limitation of the culture-dependent approach to describing endophyte diversity is that unculturable species and some slow growing or weakly competitive species may not be isolated (e.g., Dissanayake et al., 2018). To overcome the shortcomings of the culture-dependent approach, we will ultimately describe the patterns of biodiversity found in the Science Garden using next-generation sequencing methods to characterize both culturable and unculturable endophytes.

**Step 1**) Count the number of different endophyte entities on your culture plate and describe the morphology of each entity in Table 1 below. You shouldn’t have to open the petri dish for this; instead, view the endophyte growth through the bottom of the dish. Note: You will not include this Table 1 in your lab report, but you will write about your observations in the Methods: Microscopy section.
First, consider whether you have bacteria or fungi growing on your plate. When classifying bacteria and fungi, morphological features are relevant. Phenotypically distinct-looking colonies are formed by various species of bacteria and fungi. The size, shape, texture, color, and margins of the colonies vary. On agar media, bacteria emerge as oily spots, whereas fungi develop as powdery or fuzzy mats. Bacterial colonies are a mass of bacterial cells on a dense medium, isolated from a single bacterium -- all bacteria are genetically similar within the colony and may be considered a clone. For bacteria species, the color of the colony can vary, from white or buff to violet or purple. The bacterial colonies may have a smooth, glistening, rough, dull, or rugged surface (wrinkled) (Figure 1; Microbiology Society, 2021). Their texture can be butyrous (buttery), viscid (sticks, difficult to get off), brittle/friable (dry, breaks apart) or mucoid (sticky, mucus-like). On the other hand, fungal hyphae can run across the solid media, and the colors of the hyphae and the fungal spores can vary among the fungal species (CD Genomics, 2021).

![Figure 1. Bacterial colony morphology. Colony morphology is a method that scientists use to describe the characteristics of an individual colony of bacteria growing on agar in a petri dish, and it can be used to help to identify them. (Image from Microbiology Society.)](image)

<table>
<thead>
<tr>
<th>Morphological description of bacterial entities</th>
<th>Morphological description of fungal entities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 1. Observations of different endophyte entities on our culture plate for DNA#: _______.

**Step 2)** Photograph the endophyte diversity on your culture plate.

With the underside of your plate face-up, take a clear photo of your entire plate with a cell phone to emphasize the diversity of endophytes on your plate. Save this photo for making Figure 1a of your lab report.

**Step 3)** Select and prepare a slide of one endophyte on your culture plate. The methods are slightly different for bacteria and fungi; follow the appropriate methods below.

- **If you choose a bacterial endophyte:**

  Many bacteria genera and species look similar. That makes identifying the members of a microbial community difficult. Researchers look for differences at a particular gene, called 16S, which is common to most bacteria, to identify bacteria to genera or species, but some progress can be made towards identification by looking at their morphology under a microscope. You’ll try your best, using the visual guide below from Yang et
al. (2016), to make an identification based on morphology (**Figure 2**):

<table>
<thead>
<tr>
<th>Morphological Feature/Shape</th>
<th>Example Organism(s)</th>
<th>Functional Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curvature</td>
<td><strong>Caulobacter crescentus</strong>, <strong>Vibrio cholerae</strong>, <strong>Vibrio parahaemolyticus</strong></td>
<td>Cell curvature enhances surface colonization in aquatic environments with moderate flow.</td>
</tr>
<tr>
<td>Helical</td>
<td><strong>Helicobacter pylori</strong>, <strong>Campylobacter jejuni</strong>, <strong>Spirochetes sp.</strong>, <strong>Spirochaeta aurantiaca</strong>, <strong>Legionella interroga</strong></td>
<td>Helical shape increases torque, which can enhance speed and facilitate escape from viscous solutions.</td>
</tr>
<tr>
<td>Waves</td>
<td><strong>Spirochetes sp.</strong>, <strong>Borrelia burgdorferi</strong>, <strong>Treponema pallidum</strong></td>
<td>Flat-wave morphology allows alternative motility in gel-like media: a) wriggling, b) lunging, c) translocating</td>
</tr>
<tr>
<td>Heterogeneity in Shape</td>
<td><strong>Caulobacter crescentus</strong>, <strong>Helicobacter pylori</strong>, <strong>Mycobacterium tuberculosis</strong></td>
<td>Asymmetric growth and division can contribute towards directional motility, daughter cells with differing cell fates, and the formation of heterogeneous populations with varying susceptibility to antibiotics and other stresses. Increase/enhance successful adaptation to different host environments.</td>
</tr>
<tr>
<td>Filamentation</td>
<td><strong>Legionella pneumophila</strong>, <strong>Streptococcus pneumoniae</strong>, <strong>Uropathogenic Escherichia coli</strong></td>
<td>Evade phagocytosis-mediated killing (harder to engulf long filaments). Filaments can promote slow, ligand-mediated uptake and invasion. Increase/enhance attachment to host surfaces/cells. Promote/facilitate the development of antibiotic resistance (promote/enhance the emergence of beneficial mutations in response to stress).</td>
</tr>
<tr>
<td>Size Minimization</td>
<td><strong>Moraxella catarrhalis</strong>, <strong>Neisseria meningitidis</strong>, <strong>Salmonella typhimurium</strong>, <strong>Streptococcus pneumoniae</strong></td>
<td>Cell size minimization helps to avoid complement-mediated killing by the host. Mucosa-associated bacterial lineages show size minimization from rods to cocci.</td>
</tr>
<tr>
<td>Swarm Cell Differentiation</td>
<td><strong>Pseudomonas aeruginosa</strong>, <strong>Proteus mirabilis</strong>, <strong>Vibrio parahaemolyticus</strong></td>
<td>Surface sensing via rotational inhibition of a polar flagellum elicits genetic reprogramming events that can lead to cell elongation and expression of factors which promote swarming motility.</td>
</tr>
<tr>
<td>Developmental Cycles</td>
<td><strong>Uropathogenic Escherichia coli</strong>, <strong>Chlamydia sp.</strong>, <strong>Rickettsia sp.</strong></td>
<td>Morphological changes associated with developmental cycles provide a mechanism for bacterial pathogens to grow intracellularly, subvert host immunity and facilitate subsequent rounds of invasion and infection. Chlamydia sp. and Rickettsia sp. developmental cycles are not pictured here.</td>
</tr>
<tr>
<td>Bacterial Appendage Number &amp; Placement</td>
<td><strong>Borrelia burgdorferi</strong>, <strong>Campylobacter jejuni</strong>, <strong>Helicobacter pylori</strong></td>
<td>Flagella placement determines distinct modes of motility and morphological characteristics crucial for nutrient and niche acquisition.</td>
</tr>
<tr>
<td>Stalks or Prosthecae</td>
<td><strong>Asticcacaulis bigrosothecium</strong>, <strong>Asticcacaulis eccentricus</strong>, <strong>Caulobacter crescentus</strong></td>
<td>Stalks increase overall surface area and can allow for enhanced nutrient acquisition. Stalk elongation allows bacteria to physically separate from competitors.</td>
</tr>
<tr>
<td>Fimbriae or Pili</td>
<td><strong>Neisseria meningitidis</strong>, <strong>Uropathogenic Escherichia coli</strong></td>
<td>Pili can facilitate a number of different functions depending on how many are present: a) competence (single pilus), b) self-aggregation (two pili), c) crossstalk with host cells (dashed lines) (three or more pili)</td>
</tr>
</tbody>
</table>

**Figure 2.** Examples of bacterial morphological variation and functional consequences from Yang et al. (2016).
You will follow these steps below for making your microscope slide with a bacterial endophyte sample:

1. Draw a circle in Sharpie on the underside of one slide.
2. Using the plastic pipette/dropper, place a drop of ethanol in the circle.
3. With a disposable loop, pick a small amount of your sample.
4. Mix your sample gently with the loop in the drop of ethanol and spread it within the perimeter of the circle.
5. Allow to air dry completely. This will take about 5 minutes.
6. Once dry, place the slide on the staining rails over the beaker.
7. Add 2-3 drops of methylene blue stain to the circle, ensuring it covers the entire circle.
8. Let sit for 1 minute.
9. While holding the slide at an angle over the beaker, use the DI water spray bottle to gently rinse the slide. Do not spray directly into the circle. Rather, let the water run down the slide until clear. Collect all rinse water in the beaker.
10. Gently apply the coverslip to circle.
11. Blot excess water from the sides and back of slide with a kimwipe.
12. Clean-up your group tray. Using the provided funnel, add your Methylene blue stain waste to the designated carboy. Keep the remaining ethanol in the bottle for the next lab section. Rinse forceps and dropper in the sink, dry them with paper towels, then return them to the tray. Return the tray to the classroom cart.

- If you choose a fungal endophyte:

In the past decade, molecular analyses have helped clarify the evolutionary relationships between fungal groups, although there are still areas of uncertainty (Reece et al., 2014). Figure 3 presents a simplified version of one hypothesis. There are three phyla but four groups of fungi: phylum Zygomycota, the zygomycetes; phylum Ascomycota, the ascomycetes; phylum Basidiomycota, the basidiomycetes, and phylum Deuteromycota, known as the imperfect fungi. Several other groups that historically have been associated with fungi, such as the slime molds and water molds, now are considered to be protists, not fungi. The three phyla of fungi are distinguished primarily by their sexual reproductive structures. In the zygomycetes, the fusion of hyphae leads directly to the formation of a zygote, which divides by meiosis when it germinates. In the other two phyla, an extensive growth of dikaryotic hyphae may lead to the formation of structures of interwoven hyphae within which are formed the distinctive kind of reproductive cell characteristic of that particular group. Nuclear fusion, followed by meiosis, occurs within these cells. The imperfect fungi are either asexual or the sexual reproductive structures have not been identified.

![Figure 3](image.jpg)

**Figure 3.** The four major groups of fungi. The imperfect fungi are not a true phylum, but rather mainly a collection of fungi in which sexual structures have not been identified (figure made by McGraw-Hill, Inc.).

You will look for morphological differences in the structure of the hyphae (the branching filaments that make up the mycelium, or vegetative part, of a fungus). Ascomycetes and basidiomycetes have septate hyphae; their filaments are partitioned by cellular cross-walls called septa (**Figure 4a**). The structure of these septa varies across taxa. By contrast, in zygomycetes, the cells are coenocytic, with no distinction between individual cells.
Rather, the filaments are long and tubular, with a cytoplasm lining and large vacuole in the center (Figure 4b).

**Figure 4.** Ascomycetes and basidiomycetes have septate hyphae, whereas zygomycetes’ hyphae are coenocytic (figure made by Pearson Education, Inc.)

You will capture the morphological details (hyphae structure, specifically) of your sampled fungus (for example, see Figure 5), to try to identify it to a major fungus group(s) using the guidelines in Figure 4.

**Figure 5.** These coenocytic hyphae indicate the fungus is in the phylum Zygomycetes. Note: this image is missing a scale bar.

You will follow these steps below for making your microscope slide with a fungal endophyte sample:

1. Draw a circle in Sharpie on the underside of one slide.
2. Using the plastic pipette/dropper, place a drop of ethanol in the circle.
3. Sterilize the forceps in the bottle of ethanol.
4. With sterilized forceps, pick a small amount of fungus from the base of your sample (to ensure you sample hyphae).
5. Mix your sample gently with the forceps in the drop of ethanol and spread it within the perimeter of the circle.
6. Allow to air dry completely. This will take about 5 minutes.
7. Once dry, place the slide on the staining rails over the beaker.
8. Add 2-3 drops of methylene blue stain to the circle, ensuring it covers the entire circle.
9. Let sit for 1 minute.
10. While holding the slide at an angle over the beaker, use the DI water spray bottle to gently rinse the slide. Do not spray directly into the circle. Rather, let the water run down the slide until clear. Collect all rinse water in the beaker.
11. Gently apply the coverslip to circle.
12. Blot excess water from the sides and back of slide with a kimwipe.
13. Clean-up your group tray. Using the provided funnel, add your Methylene blue stain waste to the designated carboy. Keep the remaining ethanol in the bottle for the next lab section. Rinse forceps and dropper in the sink, dry them with paper towels, then return them to the tray. Return the tray to the classroom cart.

**Step 4) Review how to properly use a compound light microscope.**

There are two types of microscopes in the classroom, a stereoscope and a compound light microscope. A stereoscope (Figure 6) is a binocular microscope (or “dissecting microscope”) that magnifies at a relatively low power for viewing three-dimensional, opaque objects, such as flowers, insects, mineral specimens, fossils, coins, or really anything (Mahan, 2017). Generally, the magnification of a stereoscope is between 20x and 50x, and specimens are lighted from above. The three-dimensional image is produced by two objective lenses and eye pieces. **You will not use a stereoscope in class this week.**
Figure 6. A stereoscope (a.k.a. dissecting scope) (photo by Leica Microsystems, Inc.).

You will use a compound light microscope in class this week. A compound light microscope (Figure 7) might have a binocular (two eyepieces) or monocular head, and it magnifies at a much higher power than a stereoscope. It's designed for viewing small cells, or thin sections of organs or tissues placed on a glass slide. The specimens are thin enough that light can pass through them from below. Magnifications of microscopes generally range from 40x to 1000x, and there is one objective lens (the lens above the specimen) used at a time. Usually there are several objective lenses that can be rotated into place above the specimen for viewing with varying magnification. The compound light microscope will help you view and image the structure of your endophytes.

First, practice using a compound light microscope (Figure 7) with a prepared slide, because it takes practice to master microscopy. Always keep the microscope covered with a bag when not in use. Always carry the microscope neck (a.k.a. arm) with one hand and have your other hand under the base. The objective lens provides the primary magnification, which is multiplied by the ocular lens (10x) to calculate the total magnification. The objective lenses are: 1) 4x scanning (usually red), 2) 10x Low power (usually yellow), 3) 40x High power (usually blue). Avoid the 100x (“oil immersion”) lens, as using it might crack the slide. To focus the image, start with the scanning lens (4x) and the stage in the uppermost position (lens not touching slide). Secure the slide on the stage with the specimen holder. Slowly lower the stage with the coarse focus knob until the slide is visible. Use the fine focus knob to focus the image completely. Then switch to the lens of the next highest magnification (low power, 10x). Little adjustment will be needed to focus the image. Then switch to the lens of the next highest magnification (high power, 40x). Again, little adjustment will be needed to focus the image. Adjusting the illumination (light intensity) sometimes can vastly improve the image. When finished, remove and return the prepared slide and return to the scanning lens (4x) with the stage in the uppermost position.

Figure 7. Labeled parts of a compound light microscope.

Step 5) Use a compound light microscope to view and image your endophyte slide to use as Figure 1b in your lab report.

Remove gloves when touching the classroom laptop. Use your group’s assigned Leica compound light microscope with a USB cable plugged into your group’s assigned classroom laptop. On the microscope, you will need to turn the main power switch on, then turn the camera on separately. Press the USB symbol on the side of the microscope gently if it is not already illuminated with a green light. Open the Leica Acquire software on the classroom laptop (in Mac mode).
Recall the proper way to get a slide in focus, starting with the lowest objective lens (4x) first. Stop at the 40x lens. Play with the light adjustment before taking your picture.

- In camera mode of Leica Acquire, you can change where your photo will be saved (at the bottom of the window: Folder > change folder > Desktop).
- Take the photo with the red camera icon. You will be asked which magnification you are using. Choose the magnification that matches the magnification on the smaller zoom knob. In picture mode, click on the thumbnail of your photo at the bottom of the window. Modify your photo by adding a scale bar. You can save your photo with the annotations you have made, using the merge icon.
- If you cannot get Leica Acquire in Mac mode to work, you can switch to PC mode and use Leica LAS EZ instead. When restarting the laptop, hold down the Option key.
- Tell your TA if it seems like your microscope needs cleaned; we use special equipment for microscope cleaning.

**Step 6** Clean-up. Please pay close attention to the details of the clean-up procedure for this week.

Place your coverslip and slide in a slide disposal box. Dispose of your culture plate and the loop (if used) in the biohazard disposal bin. Return the microscope to your group’s section of the cabinet. Clean your table with Clorox spray and paper towels. You can now remove your PPE. Be sure to throw used gloves in a trash can.

**Step 7** Create Figure 1 for your lab report with the two photos you now have.

You may need to edit (rotate and crop) your whole-plate photo (e.g., you can do this in the Preview app on a Mac). Insert the first photo into your lab report google doc. Resize the photo appropriately. Click on the photo and choose the “Wrap text” icon. Now, when you insert the second photo, you can position it to the right of the first photo. This way, both photos are a part of Figure 1. Write “Figure 1” and an informative caption below the side-by-side photos, and make sure to briefly refer to both photos in the caption. See Figure 8 for an example.

![Figure 1](image)

**Figure 8.** An example of Figure 1 for the biodiversity lab report (however, a scale bar is missing in the second photo).

Next, delete any files you added to the class laptop and “empty the trash”. Clean the laptop lightly with isopropyl alcohol as instructed at the cleaning station. Thank you!

**Step 8** In-class assignment.

You are now ready to use a laptop, this lab manual, and the Biodiversity Project lab report template to complete a draft of the Methods section of your lab report: microscopy and the Tables & Figures section:
Figure 1. Emphasize the relevant details for replicating the methods and leave out details that would be common knowledge to a trained biologist.

You will receive credit for completing a draft of each of the following. The quality will not be assessed until the final lab report. However, the more effort you put in now, the more meaningful feedback you will receive from your GTA. Save your document as a pdf and upload it to Canvas. Your GTA will provide timely feedback to help you make improvements on your lab report.

- A subheading for this methods section of your lab report & a Tables & Figures heading (1 pt)
- Description of the compound light microscopy methods (1 pt)
- Description of the diversity and morphology of endophytes you cultured (1 pt)
- A reference to Figure 1 in the microscopy methods section (1 pt)
- An explanation for why we will not rely on culturing endophytes to ultimately describe endophyte diversity in the Science Garden (1 pt)
- Figure 1 (a. with whole-plate photo, and b. at least one compound light microscope photo with a scale) with informative caption (1 pt)

References:


Biodiversity project: Statistical Methods, Results, & Tables and Figures
Week 11 of BIOL 1615

Objectives: In this lab you will be able to: 1) perform statistical analysis to answer the three research questions, 2) present the project details in the methods section, results section, and tables and figures section of a lab report as a biologist would, and 3) collaborate with your group members.

Pre-lab read through: Highlight the parts of the methods that a trained biologist would need to know to replicate our methods, as opposed to the parts of the methods that are common knowledge or practice. Your lab report methods section will emphasize these relevant details.

Equipment:
- Three datasets for research Qs1&2: the link to the RStudio Cloud Week 11 project is in Canvas
- The DNA sequences for research Q3: downloadable from Canvas
- The BLAST website for research Q3: the link is in Canvas
- Biodiversity Project lab report template: the link is in Canvas

Background information and methods: In October 2021, Dr. Zach Gompert used the 25,969,391 16S forward sequences and the 25,969,391 reverse sequences from CD Genomics and the DADA2 (version 1.16.0) bioinformatics pipeline in R (version 4.0.2) to identify 16S unique sequence variants and associate each sequence variant with a taxonomic identification (Callahan et al., 2016). He first filtered and trimmed the forward and reverse sequences, removing sequences with more than two expected errors or more than 0 ambiguous bases, and trimming bases with quality scores less than two. He then used the ‘learnErrors’ function to estimate error rates directly from the data. Next, identical sequences were clustered using the ‘dada’ function while accounting for possible errors based on the estimated error rates. The program tries to cluster exactly matching sequences, but also allows for errors such that two 'exactly matching' sequences could differ by a base or two if the estimated error rate is not too low as they could still be exactly matching. He then merged corresponding forward and reverse reads with ‘mergePairs’ and removed chimeric reads pairs with ‘removeBimerDenovo’. This gets rid of read pairs that appear to belong to different operational taxonomic units (OTUs) or amplicon sequence variants (ATVs); we will refer to them as ATVs to specify that these are really sequence variants and that the connection between sequence variants and taxonomic units can be nebulous. This resulted in 60,833 unique ATVs.

Zach next assigned putative taxonomic identifications to each ATV based on the curated SILVA database (silva_nr99_v138_train_set.fa.gz). Where possible, species-level taxonomy was assigned with SILVA species assignment database (silva_species_assignment_v138.fa.gz). He then removed likely plant organellar ATVs by dropping those with taxonomic assignments of “Mitochondria” or “Chloroplast”. (Note: Chloroplast genomes contain 16S and often amplify with the same primers used to amplify microbial 16S DNA fragments. Consequently, to avoid obtaining many chloroplast sequences and thus fewer bacterial sequences, the extracted DNA can be treated with molecular reagents that either digest specific sequences found at very high concentration (such as the chloroplast fragments) or that prevent chloroplast sequences from being PCR amplified. These approaches will not remove all chloroplast 16S sequences but will enrich for bacterial 16S (relative to chloroplast 16S) during library preparation.) This left us with 36,918 ATVs.

Lastly, he estimated the relative abundance of each ATV in R using a Dirichlet-multinomial model; the estimate is based on the number of sequences assigned to each ATV for each plant sample but accounts for the fact that rare microbes may have been missed in our sequencing effort (i.e., not seeing a microbe in a sample doesn’t mean it wasn’t there).

Honor’s Lab only: Here are the details you need about the ITS sequences for the fungal endophytes; all other details are the same for 16S and ITS. There were 17,517,070 ITS forward sequences and 17,517,070 reverse sequences from CD Genomics. There were 6,693 unique ITS ATVs after using ‘removeBimerDenovo'
but before removing organellar ATVs. After removing organellar ATVs, this left us with 4,873 ATVs.

Before we get into the details of statistical analysis, let’s first think about how the six populations of alfalfa in the Science Garden are genetically related to each other (see figure to the left). You might want to talk about the information in this paragraph in the introduction or discussion of your lab report (we will work on the discussion section in Week 12). Specifically, this is a UPGMA (Unweighted Pair Group Method with Arithmetic mean, which is a hierarchical clustering method) tree from pairwise FST. Do you remember making a phylogenetic tree during Week 4? This tree differs from the one you made in that: 1) Zach used 107,496 variable nucleotides distributed across the genome (all eight alfalfa chromosomes), as opposed to the one chloroplast gene you used to make your tree, and 2) this tree is constructed based on the relative allele frequency differences between pairs of populations (as measured by FST). The population VIC (Victor, ID) is the most different from the rest. The overall FST (i.e., overall difference in allele frequencies among the populations) is 0.014 out of 1; a 1 would mean the populations are completely genetically differentiated. This overall FST is relatively low and is explained by there being several cases where individuals from a population are more closely related to individuals from another population than they are to individuals from their own population. This complexity reflects the sources of the seeds used to plant this garden; remember, the seeds were taken from wild (“feral”) alfalfa that escaped cultivation. Thus, for example, seed stock used near Alpine, WY (ALP) is relatively closely related to the seed stock used around Davis, CA (APLL), overall. Even though the alfalfa populations do not differ much genetically, they could still differ at specific genes that affect the microbes they harbor. This would especially be true if these genes are under selection and thus contribute to local adaptation of the alfalfa populations.

Now it is time for you to actively participate in data analysis. The rest of this lab manual is dedicated to the steps you will take to answer the three research questions. These steps are organized by the three research questions.

**Q1: Does bacterial species diversity in the leaves of alfalfa vary among the plant populations grown in the common environment of the Science Garden?**

First, it’s customary to start by describing the taxonomy data – this will feel familiar, as we will use some of the functions we ran in RStudio Cloud in Week 6. At the beginning of your Results section, you should state the total number of sequences we received from the 177 plant samples (see above). Then, state the total number of ATVs for data analysis (see above).

**The total number of sequences we received from the 177 plant samples:**
**The total number of ATVs for data analysis:**

Note: In a manuscript, it’s customary to state which version of R you used. The version is stated when you open the RStudio Cloud project for the first time. You will use the data in “Taxa16S_2020.csv” and the ‘unique’ and ‘length’ functions in R to describe the number of unique phyla, classes, orders, families, and genera our ATVs were distributed across.

**The version of R we used:**
**The number of unique phyla:**
**The number of unique classes:**
**The number of unique orders:**
**The number of unique families:**
**The number of unique genera:**
You will use the ‘barplot’ function to make a plot to visually show readers how many taxa there are in each unique phylum. This is your second required figure – we’ll refer to this as **Figure 2**. (Note: In the Discussion section you’ll work on in **Week 12**, you can compare these numbers and distribution to what others have found in similar studies, to put your project in a larger context.) Remember to number tables and figures in order of appearance/reference in your lab report.

Next, we will explicitly answer Q1. We need the R package called VEGAN for this. The VEGAN package provides tools for descriptive community ecology, like basic functions for diversity analysis. It is customary to report which version of R packages you used. This information will show up after you install and load the package.

**The version of the VEGAN package we used:**

You will need to use a different, new data table for answering Q1: “bacteriaAbundEst_2020-plantIDs.csv”. In this file, each plant has its own row, and each unique ATV has its own column. ATVs are numbered, and this number corresponds to the first column, “ID” in “Taxa16S_2020.csv”. And, each numerical cell of this data table is the estimate (see above) of the proportion of microbes from a given ATV in a given plant. As such, each row in this data table will sum to 1. To be clear, this data table tells us about the **relative abundances** of microbes from ATVs across the Science Garden plants. To look at the pattern of bacterial endophyte **diversity** in the Science Garden, we need to calculate species diversity from these relative abundances. We will use a common, simple estimate of species diversity called the Shannon index ($H'$; “**Diversity index**”, 2020):

$$H' = - \sum_{i=1}^{R} p_i \ln p_i$$

In this equation, $p_i$ is the proportion of individuals belonging to a taxon in a dataset; in other words, it is the values in our “bacteriaAbundEst_2020-plantIDs.csv” dataset. A diversity index like this provides more information about community composition than simply species richness (the number of species present) because it takes the relative abundances of different species into account. In other words, it provides important information about rarity and commonness of a species in a community. Specifically, the Shannon index accounts for both abundance and evenness of the species present.

Now, you will use the VEGAN function called ‘diversity’ and make a new data matrix to house the diversity estimates. Start by estimating species diversity across the entire Science Garden.

**Mean Shannon index for the entire garden:**

Then, similar to **Week 5**, you will calculate the means and standard errors for the ATV diversity across alfalfa populations in the garden, and you will put these values in a table for your lab report, which you will refer to as **Table 1**.
Table 1. Mean Shannon index (+- standard error) for leaf bacterial endophytes (ATVs) across alfalfa populations (Pop). Population abbreviations stand for (in order of appearance): Alpine, WY; north of Davis, CA; west Reno, NV; Bonneville Shoreline Trail in Logan, UT; Victor, ID; Verdi, NV.

<table>
<thead>
<tr>
<th>Pop</th>
<th>Diversity</th>
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<tbody>
<tr>
<td>ALP</td>
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<td>APLL</td>
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<td>AWFS</td>
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<td>VIC</td>
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<td>VUH</td>
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Then, you will perform an ANOVA to help you quantify the variation across populations versus within populations. You may need to perform a subsequent Tukey’s HSD to figure out which populations are significantly different from one another. You can also use Tukey’s HSD to look at differences among the plots in the Science Garden, if you had a significant p-value for the plot effect.

ANOVA Pop p-value:
ANOVA Plot p-value:

If applicable, Pop Tukey’s HSD results (the Pop differences that are significantly different from 0):
& Plot Tukey’s HSD results:

Q2: Does plant population affect the relative abundance of my focal bacteria?

In the RStudio Cloud project, you will use the data in “bacteriaAbundEst_2020-plantIDs.csv” to calculate the mean (and standard error) of the relative abundance of each of your chosen bacteria found in the sampled leaf tissue in each of the alfalfa populations. Add these means and standard errors to your Table 1:

Table 1. Mean Shannon index (+- standard error) for leaf bacterial endophytes (ATVs) across alfalfa populations (Pop), followed by mean relative abundance (+- standard errors) for the focal bacteria in the dataset (referred to by ATV ID number). Population abbreviations stand for (in order of appearance): Alpine, WY; north of Davis, CA; west Reno, NV; Bonneville Shoreline Trail in Logan, UT; Victor, ID; Verdi, NV.

<table>
<thead>
<tr>
<th>Pop</th>
<th>Diversity</th>
<th>ATV#</th>
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Then, you will perform an ANOVA for each of your chosen bacteria, but with a different dataset, “bacteriaAbundEst_logit_2020-plantIDs.csv” – it is not appropriate to run an ANOVA on proportion data, so here we provide you the data that has been logit-transformed. Specifically, the logit transformation is the log of the proportion divided by one minus the proportion:

$$\text{logit}(p) = \log\left(\frac{p}{1-p}\right)$$

If your population-level p-value is significant (equal to or less than 0.05), you will want to perform a Tukey’s HSD to help you think about which specific populations are different from each other.

Notes about each ANOVA performed (Pop and Plot p-values and Tukey’s results) to include in the Results section of your lab report:
**Q3: What is known about my focal bacteria? How are they related to each other?**

You will not use RStudio Cloud to answer this question. Instead, you will use some of the methods you followed in Week 4; you will use BLAST and make a Neighbor-joining tree in GenBank. You will need “Sequences16S_2020.fasta” from Canvas – you should be able to open this document in Microsoft Word.

**BLAST**

GenBank is the National Institute of Health (NIH) genetic sequence database, an annotated collection of all publicly available DNA sequences. You will use BLAST (Basic Local Alignment Search Tool) to search for sequences that are related to the sequence you input. A simple analogy about what BLAST is doing is the sticky note and the library. Say you have a three-word phrase written on a sticky note. Your job is to go to the school library, look for every book that has that three-word phrase, and write down the exact page number and name of every book you find. Next, you must search for every book that has the three-word phrase, even if the spelling is not perfect. You must keep doing this until you find every last book that has a part of the three-word phrase. Your last chore is to put all the names and page numbers of the books you found in order, from most to least similar to the original phrase. That is essentially what BLAST is doing in a few seconds.

More specifically, see the steps in the BLAST algorithm in the figure to the left (Kerfeld & Scott, 2011). The BLAST algorithm begins by fragmenting the query sequences into “words” (16-256 nucleotides or 2-3 amino acids), and from each word creating a set of acceptable “synonyms” that represent possible changes in sequence due to mutation. Words and their synonyms are scored with respect to how well they match the query sequence, based on substitution matrices from curated alignments of gene/protein families. The words that match sufficiently well to have a score above a set threshold value are carried forward to compare to all the sequences in the database being searched for homologs (i.e., related by descent from a common ancestral DNA sequence). All the sequences in the database are then scanned for the presence of these words; sequences carrying two matches within a preset distance from each other (which suggests a conserved region shared by both query and subject sequences) are set aside until the entire database has been scanned. This “short list” of subject sequences is then carried forward by extending the alignment outward from the words to determine whether the match between the query and subject sequences extends beyond the local match between the subject sequence and the word. Initial “rough” alignments are extended without gaps to verify that the sequences match beyond the word hits. If the threshold score for the “ungapped” alignment is high enough that it suggests that the two sequences are indeed homologs, a second alignment is undertaken in which gaps are allowed to optimize the alignment. The sequences retrieved after these steps are referred to as the “subject” sequences. The extent of the sequence similarity between the subject and query sequences is reported as a raw score, S, which is calculated...
from the alignment of the two sequences by assigning the scores from each pair of nucleotides in the alignment. For more details about how S is calculated, see Kerfeld & Scott (2011). Gaps are spaces introduced into either the query or subject sequence to optimize alignments. It is assumed that a gap corresponds to either an insertion or deletion mutation event. Alignments are extended position by position with concomitant scoring until the match falls below a threshold score, at which point it is terminated. It is important to note that alignment between the query and subject sequences does not have to cover the full length of the two sequences.

Larger databases are more likely to include sequences with matches to the query that are due to chance, not homology. E-values are the number of subject sequences that can be expected to be retrieved from the database that have a bit score (similar to S) equal to or greater than the one calculated from the alignment of the query and subject sequence, based on chance alone. E-values for subject sequences that are very similar to the query sequence will be quite small and are widely used as a means to assess the confidence with which one should claim the subject sequence(s) and the query sequence as homologs. Researchers tend to draw an arbitrary line below which they consider E-values to provide convincing evidence that two sequences are homologs (e.g., E = 0.00001). It is informative to scrutinize this assumption, as we cannot “prove” whether the sequences are homologs. Other approaches (e.g., protein structure, gene context) might be used to strengthen or refute such an assertion.

So, why are you using the taxonomy and hits from BLAST and not just what you have already from “Taxa16S_2020.csv”? Answer: you will likely get a longer sequence (which is better for making a phylogenetic tree). Plus, it is a bigger database than SILVA, so you might get more detailed (species-level) taxonomy. As you perform BLAST searches for your focal bacteria (ATVs), you will add information to what we will refer to as Table 2.

Table 2. What is known about the focal bacteria, according to SILVA and Genbank databases, followed by information obtained through internet searches.

<table>
<thead>
<tr>
<th>ATV ID</th>
<th>Taxon (SILVA)</th>
<th>Genbank description</th>
<th>BLAST Accession</th>
<th>BLAST E-value</th>
<th>Isolation source (Genbank) &amp; additional info</th>
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1) Find the DNA sequences of your chosen ATVs in the “Sequences16S_2020.fasta” document. Your sequences are in FASTA format; they start with a title (which starts with a “>”) and a hard return. Copy and paste the sequences you need into a new document for easy access. You will also eventually need an “outgroup” when making your NJ tree, a different sequence included in “Sequences16S_2020.fasta” (more on this below).

2) Go to the website https://blast.ncbi.nlm.nih.gov/Blast.cgi. Select “Nucleotide BLAST” since we are using DNA sequences (sequences of nucleotides).
3) Copy and paste one DNA sequence into the text box, including the title (the “>ID_1” or “>seq0-3” part, for example), like this:

![BLAST Screen Shot](image)

Limit your search to bacteria sequences. Under Choose Search Set, type “bacteria” into the text box next to Organism, and choose “bacteria (taxid:2)”.

4) Click the blue BLAST button. Your search may take a few seconds. When the page of results loads, click the link for “Back to Traditional Results Page”.

5) Look under Graphic Summary. This figure tells you the number of results you have. Red bars represent the top matches to your query sequence and how well they aligned. In this example, all results aligned perfectly to the query sequence:

![Graphical Summary](image)

Scroll down to the section titled “Sequences producing significant alignments”. The taxa in the list that appear below this section are those with sequences identical or most similar to your query DNA sequence. The most similar sequences are listed first, and as you move down the list, the sequences become less similar to your DNA sequence. Choose the first result in the list; record relevant information from the description, the Accession, and the E-value in your Table 2. Then, click on the Accession link to find more information about it.

6) Repeat Step 3 until you have “blasted” all of your chosen DNA sequences. Fill out Table 2 as you go.
Neighbor-joining tree

You will also use NIH's website to make your neighbor-joining (NJ) tree. It uses a clustering algorithm and is based on genetic similarity. Refer to the Week 4 lab manual for how to interpret phylogenetic trees, in general.

7) Replace each of your DNA sequence names (e.g., ID_1”) with a more meaningful one based on the information from your Table 2. Note: there cannot be spaces in your new name.

8) Go back to the nucleotide BLAST page and click the box to choose the option to “Align two or more sequences.” A second text box will then appear.

- Start with an outgroup. An outgroup is an organism not belonging to the group whose evolutionary relationships are being investigated. For example, if your chosen bacteria are all in the same genus, choose an “outgroup” sequence that is not in that genus. Copy your chosen “outgroup” sequence, including the “>” symbol and the name, and paste it into the first text box.

- Copy the rest of your DNA sequences, including the “>” symbol and the name, and paste them all into the second text box, one after the other separated by hard returns.

- Choose “Somewhat similar sequences (blastn)” from the Program Selection menu.

- Click the link “Algorithm Parameters” to adjust the following BLAST settings:
  - Uncheck the “Short queries” box.
  - Change the “Word size” to 7 in the drop-down menu.
  - Uncheck the Filter box for “Low complexity regions.”
  - Uncheck the Mask box for “Mask for lookup table only.”

- Click the blue BLAST button. When the page of results loads, click the link for “Back to Traditional Results Page”.

- Click the link for the “[Distance tree of results]” after “Other reports”.

9) Change the “Tree Methods” settings to make this a NJ (neighbor-joining) tree. You can change the format of your phylogenetic tree (under Tools>Layout) so that it is “slanted”, similar to the trees we have practiced.
interpreting in Week 4. You can save your tree as a pdf (under Tools>Download) for converting to a jpg, then uploading into your lab report as Figure 3. Or you might want to use screen capture software to select your tree for making an image. Make sure you can read all names on your tree before finalizing it.

**Note:** If you receive a server error when you click “Distance tree of results”, clear your browser's cache/cookie/history and try again. If that doesn't resolve the issue, use https://mafft.cbrc.jp/alignment/server/ instead.

- Paste all sequences into one text box. Change “uppercase / lowercase” from amino acid to “same as input”. Submit.
- Look at the alignment on the next page. Then choose the phylogenetic tree button.
- Do not change the default settings and choose “Go!”.
- Choose “view tree on Phylo.io”

In-class assignment: You are now ready to use all your notes and the Biodiversity Project lab report template to complete a draft of the following sections of the lab report: **Methods: Statistical analysis, Results, Tables & Figures.**

Remember, results are simply your findings. A results section of a scientific paper is strictly for narrating your findings, without trying to interpret or evaluate them. This is done using text, tables, and figures. If you found a significant p-value, this value should be included in your results. Speculating why a difference among means exists, however, belongs in the discussion section – we will write this next week. You may have seen a “discussion” section in a scientific research paper. Discussion means interpreting your results and trying to explain what they mean.

You will receive credit for completing a draft of each of the following. The quality will not be assessed until the final lab report. However, the more effort you put in now, the more meaningful feedback you will receive from your GTA. Save your document as a pdf and upload it to Canvas. Your GTA will provide timely feedback to help you make improvements on your lab report.

- Appropriate headings or subheadings for these new sections of your lab report (1 pt)
- Statistical analysis methods: a description of the treatment of raw sequence data already performed for you (1 pt)
- Statistical analysis methods: a description of the statistics used to answer Qs1&2 (1 pt)
- Statistical analysis methods: a description of the statistics used to answer Q3 (1 pt)
- Results: descriptive statistics with reference to Figure 2 (1 pt)
- Results: Q1 species diversity results with reference to Table 1 (1 pt)
- Results: Q2 focal bacteria abundance results with reference to Table 1 (1 pt)
- Results: Q3 BLAST and Neighbor-joining tree results with reference to Table 2 and Figure 3 (1 pt)
- Table 1 with caption (1 pt)
- Table 2 with caption (1 pt)
- Figure 2 with caption (1 pt)
- Figure 3 with caption (1 pt)

If you borrowed a class laptop, delete your files. Clean the laptop lightly with isopropyl alcohol as instructed at the cleaning station. Thank you!

**References:**
Biodiversity project: Discussion
Week 12 of BIOL 1615

Objectives: In this lab you will be able to: 1) discuss the significance of your results as well as limitations and future directions, 2) present the project details in the discussion section of a lab report as a biologist would, and 3) collaborate with your group members.

Equipment:
- Week 12 pre-lab video for an example lab report discussion section: see Canvas
- Biodiversity Project lab report template: the link is in Canvas

Background information and methods:
You will write three main sections of the Discussion section of your lab report:

1) The answers to your research questions based directly on your results. This section will include comparing your findings to your original predictions and to what other (published) studies have found, to ultimately highlight the significance of your findings.

Ideas from Lauren’s example lab report discussion section in the pre-lab video:

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Ideas my group has in class before we start writing:

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2) Limitations of the data or project design and corresponding implications.

Ideas from Lauren’s example lab report discussion section in the pre-lab video:

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Ideas my group has in class before we start writing:

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3) Potential future directions of the project and corresponding implications.

Ideas from Lauren’s example lab report discussion section in the pre-lab video:

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Ideas my group has in class before we start writing:

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In-class assignment: You are now ready to use all your notes and the Biodiversity Project lab report template to complete a draft of the following sections of the lab report: Discussion.

You will receive credit for completing a draft of each of the following. The quality will not be assessed until the final lab report. However, the more effort you put in now, the more meaningful feedback you will receive from your GTA. Save your document as a pdf and upload it to Canvas. Your GTA will provide timely feedback to help you make improvements on your lab report.

• Answer the research questions as best you can, given your results (1 pt)
  • Address whether your results support your original predictions (1 pt)
  • Compare your results to other studies in the scientific literature (1 pt)
• Discuss any limitations (1 pt)
• Address what you would do in the future to follow-up on this project (1 pt)

If you borrowed a class laptop, delete your files. Clean the laptop lightly with isopropyl alcohol as instructed at the cleaning station. Thank you!
Biodiversity project: Peer-review & final lab report
Week 14 of BIOL 1615

Objectives: In this lab you will be able to: 1) use a grading rubric to provide constructive feedback for your peers, 2) incorporate feedback received to polish your final lab report, and 3) collaborate with your group members.

Equipment:
- A complete draft of your group lab report
- The final lab report grading rubric
- Your peers’ lab report to review, delivered via Canvas: Wk14-inclass
- Previous lab manuals and resources to use as references during your peer review

Background information: There is background information about the scientific peer-review process below. You will receive additional training about ethics associated with the peer-review process via the pre-lab video. Take notes as you watch the pre-lab video:

- A brief history:
- The process:
- The responsibilities of a peer-reviewer:
- What are the potential problems with peer review?
  - Criticisms:

- But the peer-review benefits outweigh the costs

- New approaches:

  When you hear that research is “peer-reviewed” this usually refers to the fact that it has been published in a peer-reviewed journal. There is a formalized process of peer review prior to publication, which acts as a quality control filter (Novella, 2008). Typically, the journal editor will give a submitted paper to a small number of qualified peers – recognized experts in the relevant field. The reviewers will then submit detailed criticism of the paper along with a recommendation to reject, accept with major revisions, accept with minor revisions, or accept as is. It is rare to get an acceptance as is on the first round. The editor also reviews the paper and may break a tie among the reviewers and add their own comments. The process, although at times painful, is quite useful in not only checking the quality of submitted work but improving the quality. A reviewer, for example, may point out prior research the authors did not comment on, or may point out errors in the paper which can be fixed.

  It is important to realize that not all peer-reviewed journals are created equal. Journals range from prestigious to small or obscure. The most prestigious journals have tons of submissions and can pick and choose the most relevant or important studies. Small or obscure journals have a harder time getting world-class experts to review their submissions. Even at the best journals, the process is only as good as the editors and reviewers, who are people who make mistakes. A busy reviewer may give a cursory read through a paper that superficially looks good but miss subtle mistakes. Or they may not take the time to chase down every reference or check all the statistics. In addition, peer reviewers have biases. They may be prejudiced against studies that contradict their own research or their preferred beliefs. They may therefore bias the published studies in their favored direction and may be loath to give a pass to a submission that would directly contradict something they have published. For these reasons editors often allow authors to request or recommend reviewers, or to request that certain people not be asked to be reviewers. Sometimes an editor will specifically use a reviewer that the authors request not be used, thinking they may be trying to avoid legitimate criticism. The process generally works and is certainly better than having no quality control filter.

  Once published, papers are then read by the broader scientific community. Here, scientists can pick over a study, dissect the statistics and the claims, bring to bear knowledge from related areas or other research, and thereby are more likely to find problems than the few pre-publication reviewers. Arguments can be tested in the unforgiving arena of the scientific community, weeding out bad arguments and honing others.

  During class this week, your group will provide constructive feedback regarding a peer group's lab report to help them communicate as clearly as possible in their final, submitted version. It is important to give firm but friendly feedback and deliver it in an effective way (Padula, 2015). Here are four pieces of advice for doing so:
1) Try to avoid being tired when conducting your peer review.

2) List the positives and the negatives and keep sarcastic comments to yourself.

3) Give concrete examples and advice. No one likes to be told “this is unclear” without getting the context they need to understand why.

4) Don't be afraid to seek support. If you're not sure if the project was conducted in the best manner, don't hesitate to read outside sources (e.g., the Purdue OWL APA website) and ask others for guidance, or recommend the peer group seeks guidance from the GTA.

**Methods:** Before class, your GTA manually assigned one of your group members as the leader-for-the-day as well as which group's lab report your group will review. Towards the beginning of class, your GTA will share this information. It is the leader-for-the-day who will ultimately upload your group's draft lab report pdf, click on the provided Canvas rubric, and leave the written comments on the Canvas assignment for the other group, as well as share the feedback you receive on your group's lab report.

1) When instructed towards the beginning class, the leader-for-the-day will upload a pdf of your draft lab report to the Canvas assignment “Wk14-inclass”. Then, they will refresh the page until they receive the other group's lab report to read. After waiting a few minutes, you can let your GTA know if you haven't received it and they will help you. Then, the leader-for-the-day will download the received pdf and share it with their group members so that each group member can read it on their own computer.

2) Each group member will then read the other group's lab report. Consult the final grading rubric for each lab report section as you go and chose the appropriate score for each rubric category. Make notes below while doing so; these notes will help you and your group members craft your written feedback to your peer group. Spend the majority of your time as a peer-reviewer evaluating the overall writing quality.

**Important notes:**
- Your scores will not affect the group’s grade in this course; instead, your scores will help the group make their final edits.
- You will not be penalized for not being in the "expert" category on the final draft of your research proposal.
- Reference the lab report assignment guidelines document, the lab report template, and the Purdue OWL: APA website as needed (see the Week 6 page for links to these).

**Individual notes while reading my assigned peer group’s lab report:**

Introduction: Accuracy/relevance:

Introduction: Context:

Methods: Tissue collection, sterilization, plating:

Methods: Microscopy methods (and results, Figure 1):

Methods: Molecular methods (DNA extraction, PCR, next-generation sequencing):
Methods: Statistical analysis:

Results: Q1 (and Figure 2, Table 1):

Results: Q2 (and Table 1):

Results: Q3 (and Table 2, Figure 3):

Discussion: Conclusions based on the data:

Discussion: Limitations of design:

Discussion: Implications of research:

References:

**Writing Quality:**
- Overall, do all parts of the lab report fit together?
- Or is there too much repetition across sections? Or does the Introduction sound like it's from a different report than the Discussion? If either of these is the case, what can the group do specifically to fix this?

3) When all group members are done reading and making notes, come to a group consensus about the score for each criterion of the rubric as well as the helpful written comments you will leave to help them improve their lab report. Then, the leader-for-the-day will click on the rubric scores and save it and submit comments to the other group via the Canvas assignment “Wk14-inclass”.

To receive full credit on this “Wk14-inclass” assignment, your group's leader-for-the-day needs to save the chosen score (rating) for each criterion on the provided rubric (1pt total) and submit effective feedback in a comment to the peer group (1pt total).

4) Afterwards, your group's leader-for-the-day will need to share the score and comments your group received from the other group. You will have the rest of class time to edit your final lab report based on your GTA's suggestions along the way, your peers’ suggestions from this review, and any comments received from the SWC (if applicable). One group member needs to submit your final lab report to the "Lab report" assignment in Canvas by the end of class.

**References:**