

Name: _____

MICROBIOME SCIENCE YOUTH WORKSHOP

Biology and the Built Environment Center

University of Oregon

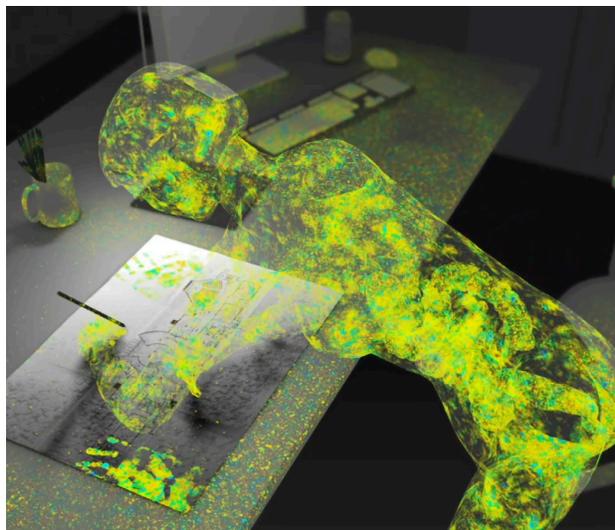
March 12, 2016

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WELCOME!



Welcome to the Biology and the Built Environment (BioBE) Center! We want you to have fun exploring the diversity of microbial life and learning more about the techniques we use to identify and characterize bacteria “in the wild” of our homes, schools and offices.

Although humans in the developed world spend 90% of their lives in enclosed buildings, we know relatively little about the biology of the built environment. Buildings are complex ecosystems that house trillions of diverse microorganisms interacting with each other, with humans, and with their environment.

Recent advances in microbial genomics offer the potential to significantly advance our understanding of the built environment “microbiome” – the totality of microbial cells, their genetic elements, and their interactions indoors. To realize this potential, the BioBE Center is training a new generation of innovators and practitioners at the **architecture-biology interface**. The vision of this national research center, funded by the Alfred P. Sloan Foundation, is to develop a hypothesis-driven, evidence-based approach to understand the built environment microbiome. Our goal is to optimize the design and operation of buildings to promote both human health and environmental sustainability, with an emphasis on green healthcare design.

On behalf of the BioBE Center and our volunteers,



Clarisse Betancourt-Román
BioBE Research Assistant



Dr. Roxana Hickey
BioBE Postdoctoral Fellow



Dr. Jessica Green
BioBE Director

SCHEDULE

12:00pm- 12:45pm	Group lunch at Pacific Hall, room 327 (Clarisse will be downstairs to meet with the group)
12:45pm- 1:15pm	Welcome to the BioBE Center, Dr. Jessica L. Green
1:15pm- 1:30pm	SAIL Program and future opportunities for students at UO, Ms. Laura Fernandez
1:30pm- 4:30pm	Science and architecture activities (Pacific Hall rooms 301, 303 and 103)
4:30pm- 5:00pm	Wrap-up /final group discussion (Pacific Hall room 327)

OVERVIEW OF ACTIVITIES

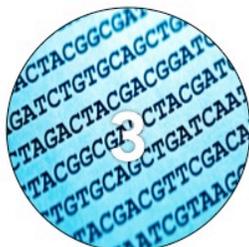
Today you will participate in four 45-minute activities designed to increase your understanding of the importance of microbes in the environment (to be completed in any order). Have fun!



What are microbes?



How do we get DNA from cells?



How do we identify microbes with DNA?



How are microbes influenced by architectural design?

ACTIVITY 1: MICROBIAL DIVERSITY IN POND SCUM

INTRODUCTION

The diversity of life becomes self-evident in the microscopic world and has fascinated individuals since the first glances at it through the microscopes of Antonie van Leeuwenhoek. You can explore this world of numerous single-celled and multi-cellular organisms. One can explore the questions of what is living/non-living, how are cells organized, what are the size limitations of life, how do microbes move, sense their world, interact with other microbes and the world around them. Fresh water is teeming with living organisms.

Vocabulary

Biofilm	Biofilms form when bacteria attach to surfaces exposed to water, and begin to excrete a slimy, glue-like substance
Protozoan	Single celled organism such as an amoeba that can move and feed on organic compounds of nitrogen and carbon
Demoslide	Combination collection/storage/and microscopic observation tubes for Protozoa and other cell cultures
Motile	Having the ability to move
Biota	A collective term for the animal and plant life of a region
Eukaryote	An organism possessing a nucleus and numerous other membrane-bound organelles
Eutrophic	Water source rich in nutrients and organic material that promotes a proliferation of life, with some microorganisms growing in excess, using up the available oxygen

OBJECTIVE

Understand microbial form and diversity in freshwater samples.

MATERIALS

- Glass microscope slides and cover slips
- Prepared demoslides
- Plastic pipettors
- Water samples from different sources
- Kimwipes
- Colored pencils

Safety Considerations

- Glass slides may be slippery and may break. If breakage occurs, please tell your instructor so the broken glass can be properly disposed of and is not a danger to others.
- Place glass slides in red biohazard bins when finished.
- After the activity it is very important to **wash your hands** with soap and water.
- Listen to the instructions about how to use a compound light microscope.

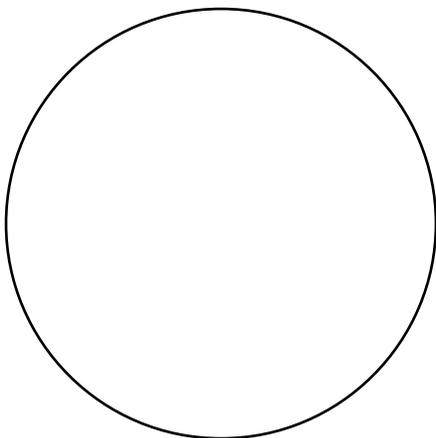
PROCEDURE*Biofilm and demoslides observation*

1. Observe two prepared samples from a biofilm (if available) or demoslides of a water sample using the 20X and 40X objectives. Write and draw your observations below. Describe color of objects and motility, if noted.

Prepared slide #1

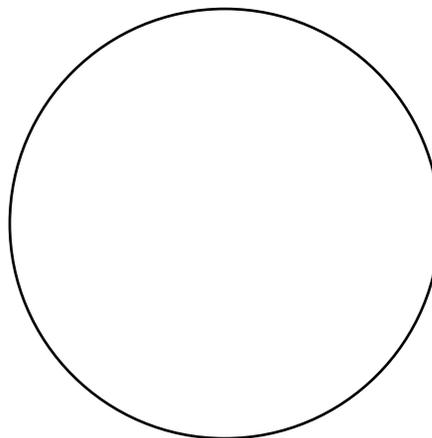
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*Water slide*

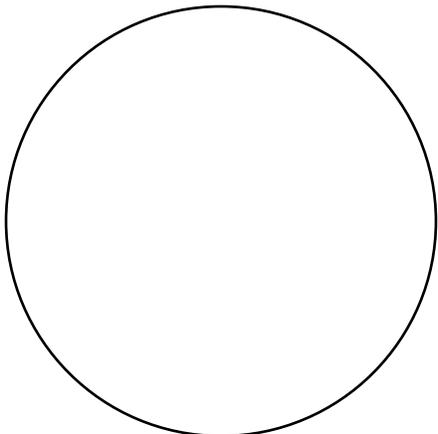
Pond water can be full of life! However, only a small amount of it is visible to the naked eye.

2. Observe your sample for macroscopic organisms (those that are visible to the naked eye).
3. Take a dropper and remove some water from near the interface of the water and mud.
4. Place a small drop onto a glass microscope slide using a plastic pipettor. Gently drop on a cover slip on top of the drop (instructor will demonstrate). If excess water is present blot with a Kimwipe prior to placing on stage of the microscope.
5. Look around the slide using low power (4X objective). Observe around the edges of debris present on the slide.
6. If you find interesting creatures try moving the objective lens up to the next higher power and observe the additional detail. If you are unsuccessful finding anything with your first slide, try making another. Draw your observations in the spaces below.

Student slide #1

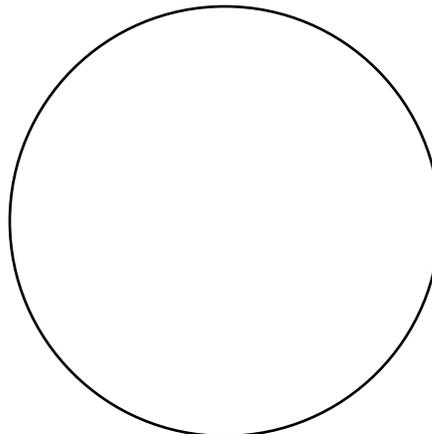
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**Student slide #2**

Magnification: _____

Source: _____



7. Observe the microbes as you have with the other protocols, this time try to determine how they make a living. In particular try to determine their food source.
8. Use different sources of water. Try to find some from pristine, undisturbed water sources, others from a pond or lake that may receive a heavy load of organic material, termed a eutrophic environment, and perhaps others that have been polluted. Record this in the “Other slide” area above.

QUESTIONS

1. Which type of sample had the most organisms (greatest number of organisms)?
2. Which type of sample had the most diversity (greatest number of types)?
3. Do all your water samples contain the same organisms? Explain.
4. What may happen to the number and diversity of microorganisms throughout the year in an aquatic environment?
5. Make a list of three (3) factors that could influence a water environment. Explain how each would impact the biota.

ACTIVITY 2: EXTRACTING DNA FROM STRAWBERRIES

INTRODUCTION

When a scientist needs a source of DNA (deoxyribonucleic acid), it must be extracted from the cells that are being studied. In this exercise you will extract DNA from strawberries rather than microbes. This exercise allows to see the chromosomal DNA!

Chromosomal DNA is the blueprint for an organism determining what that organism will look like. DNA contains all the genetic information that forms the basis of inheritance and directs protein synthesis. The strawberry is a eukaryotic plant cell containing the chromosomal DNA inside the **nucleus**. In order for one to be able to extract and visualize the DNA, one must have a large quantity of DNA inside the test tube. This means that the DNA must be extracted from a large number of cells. A single strawberry contains many cells and is a good sample for DNA.

What are the basic steps for extracting DNA from eukaryotic cells such as a plant cell? The entire process can be divided into four basic steps. There are special procedures and reagents that are used to accomplish each of these steps. The **four steps** include: **disrupting the cell wall**, **lysing the cytoplasmic membrane** (also called the plasma membrane), **lysing the nuclear envelope**, and **separating out the DNA** from the rest of the cellular material.

- Step 1** Like other plant cells, the strawberry cell contains a **cell wall** composed of cellulose located outside of the plasma membrane. The cell wall can be disrupted by either **physical** (e.g. freeze-thaw or mechanical beating) or **enzymatic** means (e.g. proteolytic enzymes added artificially or made naturally by the strawberry).
- Step 2** The process of “breaking open” the cell is referred to as “**lysing the cell**”. Once the cytoplasmic **membrane** is lysed the nucleus is released.
- Step 3** Since the **nuclear envelope** surrounds the DNA, this membrane must also be broken open to completely release the DNA. Lysing the plasma membrane and the nuclear envelope can be accomplished together since both are membranes with a similar composition of lipids and proteins. **Detergents** are useful reagents that assist with disrupting the membranes.
- Step 4** Following the lysing of the plasma and nuclear membranes, a **cell lysate** (extract) has been generated which contains the released DNA along with all the other cellular components. The next step is to **separate** out the DNA from all of the other molecules of the cell. The DNA is **precipitated** and thereby separated by using ethanol and salt, which react with the DNA causing it to become insoluble. Following the addition of the ethanol and salt, the DNA precipitates and appears as a stringy white substance.

OBJECTIVE

Understand how DNA is extracted from cells prior to analysis of the genetic code.

MATERIALS

- 1 glass test tube
- 1 plastic test tube with lid
- 1 test tube rack
- 1 glass beaker
- 1 piece of cheesecloth and a rubber band
- 1 wooden stick
- 1 tube of DNA extraction buffer
- 2-3 plastic pipettors
- Ice-cold ethanol (get this when you need it)

PROCEDURE

Lysing the cells and nuclei

1. Obtain one clear plastic bag, 1-2 strawberries, two test tubes (one glass, one plastic), test tube rack, beaker, piece of cheesecloth, wooden stick, and the DNA extraction buffer.
2. Place 1-2 strawberries inside the plastic bag. Use your hands to **gently** mash the bagged strawberries for 1 minute. *This will begin to mechanically break open the cells.*
3. Add the DNA extraction buffer to the bagged strawberry. Press the bag so that the air is removed and then seal the bag. Using your fist, gently mash the strawberry in the extraction buffer for 1 minute. *This will continue to lyse the cells and to break open the nuclei. The extraction buffer contains shampoo (a source of detergent), salt, and water.*
4. Place the cheesecloth onto the beaker to make a filter. Filter the strawberry extract by pouring it onto the cheesecloth and allowing the liquid material to enter into the beaker.
5. **Once the beaker is about 1/4 filled, stop.** Remember to close your zip lock baggie before setting it down and to the side. You may now remove the cheesecloth. *The filtration step is necessary to trap the larger cellular materials (broken cell walls, disrupted membrane, organelles) onto the cheesecloth. The DNA extract is filtered into the test tube.*
6. Transfer approximately 2 mL of the filtrate from the beaker into a test tube.

Precipitating and spooling the DNA

7. Obtain a squirt bottle containing ice-cold ethanol. Gently add the cold ethanol to the cell extract by slowly dripping the ethanol down the side of the tube. The ethanol should layer on top of your filtered cell extract. You should be able to see *two distinct layers*. The ethanol will be on the top and the cell extract below. Watch at the interface of these two layers. *The ethanol will assist in precipitating the DNA. You should begin to see white stringy material (mucous like) forming at the interface of the ethanol and extract. This is DNA.*
8. As you are observing the interface, insert a wooden stick through the ethanol layer until it reaches the interface of the ethanol and extract. Place the rod just below the interface and

twirl or turn the rod. The DNA should attach to the rod and as you twirl it the DNA will wind around the rod. As the DNA winds around the rod it may appear something like white cotton candy.

9. Wash your hands and clean up your laboratory bench putting all materials in the proper place. You may now discard the strawberry extract in the bag into the garbage.

QUESTIONS

Prior to the DNA extraction activity, answer the following:

1. Draw an example of a eukaryotic plant cell. Where is the DNA located in the cell? Label and mark the location of the DNA inside your cell.

After performing the DNA extraction activity, answer the following:

2. Following your DNA extraction, what did the DNA look like? Describe what you observed after you added the ethanol to the tube:
3. In this exercise you extracted DNA from cells of the strawberry. What makes the strawberry a good choice to use for the DNA extraction?
4. Each step on the procedure aided in isolating DNA from other cellular materials. Match the procedure with its function.

A. Filter strawberry slurry through cheesecloth	___	Precipitate DNA from solution
B. Mash strawberry with salty/soapy solution	___	Separate components of the cells
C. Initial smashing of strawberries	___	Break open the cells
D. Addition of ethanol to filtered extract	___	Break up proteins and dissolve cell membranes

ACTIVITY 3: BACTERIAL IDENTIFICATION VIRTUAL LAB

INTRODUCTION

The purpose of the lab is to familiarize you with the science and techniques used to identify different types of bacteria based on their DNA sequence. Not long ago, DNA sequencing was a time-consuming, tedious process. With readily available commercial equipment and kits, it is now routine. The techniques used in this lab are applicable in a wide variety of settings, including scientific research and forensic labs.

Basic steps

- Prepare a sample from a patient and isolate whole bacterial DNA.
- Make many copies of the desired piece of DNA.
- Sequence the DNA.
- Analyze the sequence and identify the bacteria.

The piece of DNA used for identifying bacteria is the region that codes for a small subunit of the ribosomal RNA (16S rRNA). We will refer to this piece as 16S rDNA. Different bacterial species have unique 16S rDNA sequences. The identification relies on matching the sequence from your sample against a database of all known 16S rDNA sequences.

OBJECTIVE

Understand how DNA sequence data is generated and analyzed to identify bacteria.

PROCEDURE & QUESTIONS

Go to <http://www.hhmi.org/biointeractive/explore-virtual-labs> (this should already be open for you). Scroll down and click on **The Bacterial Identification Virtual Lab**. Maximize the screen if you wish. Click to **Enter the Lab**. (Click the window on the left-hand side of the screen to enter the lab.) As you enter the lab, follow the instructions in the **left-hand graphics window**. Using the information in the **Notebook** window on the right, answer the following questions.

PART 1: SAMPLE PREPARATION

1. As the pathology lab technician, what is your task in this virtual lab?

2. Extracting DNA involves which initial step?

3. Why are the proteolytic enzymes necessary?

4. Why do you then need to inactivate the proteolytic enzymes and how do you do it?

5. After removing the enzymes, why do you spin the sample in the centrifuge?

6. Answer the following:
 - a. What is the pellet?

 - b. What is the supernatant?

 - c. Where is the DNA?

PART 2: PCR AMPLIFICATION

Go on to Part 2 and work through the PCR steps. Be sure to read the information in the notebook, including "What is PCR?"

7. What does "PCR" stand for and what is the purpose of PCR?

8. Fill in the blanks summarizing the process of PCR.

Step 1: _____ at 95°C to separate double-stranded DNA.

Step 2: _____ at 60°C to bind primers to single-stranded DNA.

Step 3: _____ at 72°C to copy the strand and produce double-stranded DNA.

9. What are primers? Why is a primer added?

10. Once the primers bind, what occurs next?

11. What is missing in the negative control tube?

12. What is present in the positive control tube that is not in the negative control tube?

Now run the PCR. Be sure to watch the virtual lab animation before proceeding to the following questions.

13. How many copies of the desired DNA have been synthesized after 30 cycles?

PARTS 3-5: PCR PURIFICATION, SEQUENCING PREPARATION & DNA SEQUENCING

These sections demonstrate how PCR products from Part 2 are cleaned and prepared for DNA sequencing. You should work through the virtual lab animations and understand what is happening at each step, but you do not need to answer specific questions. To watch PCR and DNA sequencing in action, check out these cool videos: <https://www.youtube.com/watch?v=-7GK1HXwCtE> and <https://www.youtube.com/watch?v=2KoLnIwoZKU>

PART 6: DNA SEQUENCE ANALYSIS

Click on “Learn about the science behind sequence matching.”

14. What is the ultimate goal of the sequence matching analysis?

15. What is “homology”?

16. What is BLAST and how is it used?

Click to go back to Part 6 and proceed through the instructions in the right-hand notebook window.

- **Hint:** “Command-A” will select all the data in the pop-up window, “Command-C” will copy it, and “Command-V” will paste it into the NCBI website (large box at the top of the BLAST search page).
- Follow the steps listed on the page and be patient. BLAST data can take a while to search.
- When the BLAST results appear, scroll down below the color key to the significant alignments, and then go back to the virtual lab window (left) and follow the instructions.

17. What is the scientific name of the bacterium you sequenced?

18. Write a brief description of this bacterium in the space provided. Draw a picture if you can find one. (**Hint:** search Google or Wikipedia for relevant information)

STUDENT WORKBOOK

After completing **Sample A**, perform DNA sequence analysis on **three** of the other five samples. Write in the letter of the samples you choose, the scientific name of the bacterium (after doing a BLAST search), and a brief description of each.

Sample Letter	Scientific Name of Bacterium	Brief Description

BUT WAIT! NOT ALL BACTERIA ARE PATHOGENS, RIGHT?

That's right! In fact, most of the bacteria found in and on our bodies are good for us. We refer to them as "commensals," normal members of a healthy human microbiome. Watch the following TED-Ed video online (<http://ed.ted.com/lessons/you-are-your-microbes-jessica-green-and-karen-guillemin>) and answer the following questions:

1. List two specific ways bacteria in our guts work to break down food for nutrients.
2. Name two factors that may influence the membership of the gut microbial community, making each person's microbiome unique.
3. What can happen when the gut microbial community has less variety of bacteria or only a few different types of "workers"?

