Perplexing Puddles

"Do They Contain Life?"

Science Focus: Constructing Evidence-Based Arguments

Lesson #	Lesson Title	Suggested Time
1	Can Life Exist Nearly a Mile Underground?	55 minutes
2	Extremophiles: What is an Extremophile?	55 minutes
3	Extremophiles: It's Classified!	55 minutes
4* **	Biofilms: Are they alive?	55 minutes
5	Biofilm Communication Can Biofilms Send Text Messages?	55 minutes
6	Biofilm Organization Taking a Microscopic Look	55 minutes

***Agar plates will need to be poured in advance. Once the plates have been inoculated you will need to plan for 5-14 days of observation.*

Perplexing Puddles- 9-12 (Life Science)

This unit highlights a NASA-funded research project at Sanford Underground Research Facility called *Life Underground*. During this unit, students will consider what attributes define life, and then use those ideas to hypothesize what life nearly one mile underground might be like if it exists. Students will also gather information about the microenvironments in Sanford Underground Research Facility and consider the likelihood of life in such extreme conditions. Students will learn about extremophiles, culture an everyday biofilm, and observe the biofilm to draw conclusions about the potential for life underground.

Perplexing Puddles

Do They Contain Life?

It is difficult to fathom that nearly a mile underground life can and does exist. In fact, Sanford Underground Research Facility is home to a whole host of organisms called biofilms. These biofilms metabolize various substances found in the rock walls and accumulated underground water. The strange sources of nutrients, coupled with variable levels of oxygen and temperatures cause these biofilms to be classified as extremophiles.

Extremophiles are organisms that thrive in conditions that would be fatal to most organisms. Extremophiles can be viewed in the thermal pools of Yellowstone, in polar ice caps, and in Sanford Underground Research Facility. These extremophiles, for many scientists, are simply perplexing puddles. In Sanford Lab, the extremophiles are being studied by a variety of university researchers and even NASA.

Biofilms are a collection of bacterial cells that secrete an extracellular polymeric substance (EPS). This substance helps the biofilm drastically increase its likelihood of survival. The EPS can surround multiple species of bacteria, therefore creating communities of bacteria, such as the plaque that forms on your teeth.

Biofilms can communicate using cell-to-cell communication techniques, known as quorum sensing. While in the biofilm, these bacterial cells can send out signals that are received by receptor proteins on the surrounding bacteria cells. The signals can be species specific or non-species specific.

At the 4850’ level of the Sanford Lab, biofilms are often growing in puddles that form as surface water makes its way underground. Some of the water underground is infiltration from rainfall or snowmelt. However, there is also fracture water that has been isolated from the surface for thousands of years. These biofilms are found in various colors and textures, similar to what is seen in Yellowstone National Park.

Perplexing Puddles

Standards Addressed in the Unit

HS-LS2-2 Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales.
HS-LS2-8 Evaluate the evidence for the role of group behavior on individual and species' chances to survive and reproduce.

Science and Engineering Practices

1. Asking questions (science) and defining problems (engineering).
2. Developing and using models.
3. Planning and carrying out investigations.
4. Analyzing and interpreting data.
5. Using mathematics and computational thinking.
6. Constructing explanations (science) and designing solutions (engineering).
7. Engaging in argument from evidence.
8. Obtaining, evaluating, and communicating information.

A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas (NRC 2011)

Perplexing Puddles

Materials List

➢ **Teacher-Provided**

Lesson	Materials Needed
1	➢ Materials to set up interactive notebooks ● *Perplexing Puddles* PowerPoint ● Pre-assessment handouts ● 4 oz jar sets (A,B,C) ● Active dry yeast ● Granulated sugar ● Sand ● Fizzing antacid tablet ● Thermometer ➢ Warm water (60-70 degrees F) ➢ Small beakers or graduated cylinders –for water ➢ Computer, projector, and speakers
2	● Pictures of Earthly Extremophiles ➢ Computer, projector, and speakers
3	● Extremophile Trading Cards ● Perplexing Puddles Cards (pictures from Sanford Lab)
4	➢ Green leaves (living) one per student or pair of students ➢ Agar plates ○ Erlenmeyer Flask or Beaker (500 ml) ○ Gram scale ○ Distilled water ○ Graduated cylinder (to measure water) ○ Hot plate or microwave ○ Aluminum foil ➢ Permanent markers w/fine tip ● Pencils with erasers ● PTFE (Teflon) tape for sealing petri plates after inoculation ● Computer, projector, and speakers ● Vials for collecting biofilm samples in the field (optional) ● Sample spatula for collecting biofilm samples in the field (optional) ● Poly inoculating loops ● Glass slides, cover slips and plastic pipettes
5	➢ Computer, projector, and speakers ● *Quorum-sensing signals control when bacteria turn deadly* Science Daily September 18, 2014 ● *Stop The Microbial Chatter* Nature July 2014 or computers for each student to read articles online. ● Transparency grids –one per lab group

6	➢ Microscopes ➢ Computer, projector, and speakers ● Prepared stained slides ● Transparency grids-one per lab group ● *Identifying and Characterizing* PowerPoint ● *Perplexing Puddles* PowerPoint ● Post-assessment handouts ➢ Bleach ➢ Zip top bags

Important Note	
Science Notebooks Anchoring Event	**You may wish to have students set up their Science Notebooks prior to the first lesson.** As students develop and refine their understanding of science concepts, it is helpful for them to reflect on and record what they have learned. A science notebook provides them with a record of their questions, ideas, experiences, and evidence. The science notebook can also be used by the teacher to assess the development of student understanding. Science notebooks may be a printed booklet prepared by the teacher, or a spiral notebook; though bound composition notebooks are preferred. Interactive Science Notebook support material can be found at the end of lesson 1. The anchoring event (Perplexing Puddles) is designed to provide students with a puzzling and engaging phenomenon for which they can come to a deeper understanding over the course of the unit. Do not attempt to explain this phenomenon to students. Allow them to wonder, reflect, and make sense of it on their own.

Lesson 1	Can Life Exist Nearly a Mile Underground?
Overview Standard(s) & Objectives	**Overview** In this lesson, students will investigate the properties of life. They will also gather information about the microenvironments in Sanford Underground Research Facility and consider the likelihood of life in such extreme conditions. **Standards** HS-LS2-2 Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales. **Objectives** ● Form a 'working' definition of life. ● Establish the properties of life. ● Make an inference about the possibility of life in Sanford Lab. **Advanced Preparation Requirements** ✓ *Agar plates will need to be poured prior to this lesson, please see page 28 for directions.* ✓ *Students will need to collect green (living) leaves for an activity in lesson four.* ✓ *The Properties of Life investigation requires advanced preparation of the samples. See directions following this lesson.* ✓ *Interactive Notebooks are encouraged and need to be set up prior to this lesson. Please refer to the Guide following this lesson.*
Background Information	**What is life?** What properties must exist for us to say that something is alive? What distinguishes a rock from the moss that grows on it? ● All life has order or is organized. It is composed of small structures known as cells. These cells carry out the basic processes necessary to sustain life. The cells are arranged into structures, which also carry out specific functions. ● All life uses energy. Organisms have the ability to capture and utilize energy; this is known as metabolism. ● All life responds to its environment. Organisms have the ability to respond to changes that occur in their environment. ● All life reproduces. Organisms have the ability to reproduce. Simple life forms, such as bacteria, reproduce by dividing and making almost exact replicas of themselves. More complex organisms use genetic material from two organisms (parents).

	• All life grows and develops. Organisms develop in ways determined by heredity. It is important to note that objects may have some of these properties yet not be a living organism. For example, fire uses energy, can grow, and responds to its environment (such as when it spreads rapidly in response to winds), but fire is not a living thing.
Materials	• Interactive notebooks • Perplexing Puddles PowerPoint • Pre-assessment handouts • Task Cards • 5 mL Metric scoops • 4 oz jar sets- **teacher prepared** • Yeast • Granulated Sugar • Sand • Fizzing Antacid tablet • Metric measuring spoons • Thermometer • Warm water (40 - 50°C) • Small beakers or graduated cylinders –for water • Video: Budding Yeast (run time 32 seconds)
Prior Knowledge	Students should recognize that all organisms require food/energy, grow and develop, and have the ability to reproduce.
Launch (20 minutes)	
Engagement and Communication of Student Expectations	**DO NOT REVEAL TO STUDENTS THAT LIFE/BIOFILMS ARE FOUND IN SANFORD LAB** Show the Perplexing Puddles PowerPoint Presentation. * *The cage video is extremely long. Continue with the presentation when you feel that it is appropriate.* Leave the picture from slide 15 on the screen, then ask students to complete the *unit pre-assessment*. Explain to students that in order to determine if life exists, they should first be able to define what properties are necessary for life. Place students in groups of 3-4 and have them brainstorm properties of life. The properties of life should be based on their experiences and not necessarily related to observations that they made from the PowerPoint. Ask them to explain why that property is required. Allow the students to work for 5-7 minutes. Remind students to record their ideas in their interactive notebooks. (the properties of life).

	As a class, share ideas about properties required for life. Record each group's properties on the board. Then narrow down the responses to the criteria for life this class will refer to during the properties of life investigation. Form a 'working' definition of the term life.
Explore (25 minutes)	
Procedure 	Explain to students that each team has been given a set of samples. No one knows for sure if they contain life. During the investigation, they should make careful observations and check for indications of life. **Reminder:** Students should add the same amount of water to each of the jars during the investigation. **Conduct the Properties of Life Investigation** (Task cards in kit) 1. Observe the three jars (A, B, and C). Draw a model of each jar in your science notebook and record any observations you make that might help you determine if life does, or does not, exist in the jars. 2. Add one tablespoon, or 15 mL, of sugar to each jar. Once again make observations and update your model if necessary. Record any evidence that might help you determine whether or not life exists in the jars. 3. Using a small beaker, or graduated cylinder, add enough warm water to each jar so that the water just covers the sand. Make observations and update your model. 4. Wait about five minutes and then add another tablespoon, or 15 mL, of sugar to each jar. Make your final observations and then determine which jar(s) you believe contain life, and which do not. 5. Write a paragraph in your science notebook reflecting upon the investigation and use evidence to support your claim- which jar(s) do and do not contain life. **If the Follow Up activity (optional) is not going to be utilized, have students dispose of the jar contents, clean, and dry the jars. It is recommended to strain the contents if you are concerned about getting sand in the drain.**
Questions	**Driving Question** ● What are the fundamental criteria for indicating life? ● Given these criteria, could life occur- or be maintained- underground in Sanford Lab? Why or why not? **Probing Questions**

	• What makes a living thing different from a non-living thing? • What are the properties of life? • How can life be detected? • Which sample(s), if any, contain life? What is your evidence?
Summarize (10 minutes)	
Communicate	**If you are going to incorporate the (optional) follow up extension, do not reveal/confirm which jar contains life.** Otherwise, reveal to students that jar B (the yeast jar) contains life. Instruct students to relate each property of life to the behaviors of yeast in their science notebooks. *Possible Responses:* • All life is composed of cells- the yeast is made of cells. • All life has order or is organized- the yeast cells all look similar, have similar organization. • All life uses energy- the yeast cells consumed the sugar in the solution. • All life responds to its environment- the presence of a gas/bubbles indicates that a chemical reaction (a response) is taking place • All life reproduces- all yeast can produce more yeast. All life grows and develops- yeast cells bud off and then grow in size – you may want to show students this under the microscope by placing a drop of the yeast solution from the jar on a microscope slide. Show video entitled Budding Yeast (run time 32 seconds) https://www.youtube.com/watch?v=iOvrq6ssy2Y Video clip is also available on the USB drive included in the kit.
Terminology and Concepts to Solidify	**Metabolism**- the sum of the chemical reactions that take place in an organism **Budding**- an asexual form of reproduction in which the offspring is an outgrowth of the parent **Homeostasis**- the set of internal conditions that are maintained by an organism
Connection to Big Ideas (Phenomena)	*Structure and Function of Organisms* All living organisms, from the smallest bacterium to the largest whale, share certain properties of life. These properties are used to determine if a sample of matter is alive.
Assessment	(Thinking back to the PowerPoint.) • Given the criteria for life, could life occur- or be maintained- underground in Sanford Lab? Why or why not? Ask students to support their claim in their science notebook. Rubric to grade the notebook is available in the print master.

Follow up (Optional)	If you opt to extend this activity, students need to design a procedure (record in their science notebooks) to establish which jar contains life. Each lab team will choose only one jar to test. Students will need to determine, in advance, which property, or properties will be tested for. (i.e., reproduction, or use of energy)

A Teacher's Guide to the Interactive Notebook

If you already have a science notebook, or journal, that students use during the class please continue to use that format. If you do not require a notebook for your classroom, try using an Interactive notebook for the duration of this unit.

The interactive notebook should be kept in a composition, or spiral, notebook. The front side of the first piece of paper in the interactive notebook should be designated as a table of contents. The table of contents will be created as the pages of the notebook are created. Following the table of contents, all pages should be numbered, and the dates recorded.

The next front side page should be the title page. For this unit students should create a page that reads Perplexing Puddles. Students are free to decorate this page any way they chose- they may even choose to utilize both the left and right-hand side pages.
Beginning with the backside of the second sheet of paper the students begin to make entries into their interactive notebook.

- Student output goes on left-hand pages.
- Input from other sources belongs on the right-hand pages.

At the end of the unit students will write a summary of what they learned during the unit. You may also have them select two items that represent their best work. Students should provide the dates of their chosen entries and then explain why they chose these two items, what they learned from these two items, and how these items reflect scientific thinking or skills. Students must use evidence to defend their choices and

Left Side – Output-Student Directed	**Right Side – Input- Teacher Directed**
RAP: Review and Preview or Reflect and Process Used for: Organizing new information in creative formats Interacting with information from the right page Lab Reflection questions Warm-ups Express opinions and feelings Explore new ideas Mind maps, Venn diagrams, Drawings, Terminology, Analogies, Creating Questions, Wanted posters, etc.	WOW – Words of Wisdom Used for: Class Notes Discussion Notes Handouts with new info Lab Procedures Answering questions from text Sample Problems Terminology/book definitions Movie, video notes

should not provide emotional responses such as “that is my best drawing” or “that activity was a lot of fun”.

In the print masters a rubric and score sheet has been included for your use. The interactive notebook should be graded at the end of the unit. It is encouraged, but not required that your students first grade their notebook using the rubric and then you the teacher also grade the notebook to allow students to reflect upon their detail and effort.

Encourage your students to share with their notebooks during parent teacher conferences, or other instances when they have special visitors in the classroom.

Advanced Preparation: Properties of Life Investigation

Materials

- 3 small jars per lab team (A, B, C)
- Sand
- Active yeast
- Granulated sugar
- Fizzing antacid tablets (pulverized)
- Warm water between 60° and 70° F
- Small beaker or graduated cylinder

Each lab team will require 1 set of jars

1. Place approximately 2 tablespoons, or 30 mL, of sand in each jar. (A, B, C)
2. In the jar labeled A- add nothing.
3. In the jar labeled B- add one teaspoon, or 5 mL , of dry active yeast.
4. In the jar labeled C -add 1/2 pulverized antacid tablet.

Investigation Directions

1. Observe the three jars (A, B, and C) and in your interactive notebook draw a model of each jar and any observations you make that might help you determine if life does, or does not, exist in the jars.

2. Add one tablespoon, or 15 mL, of sugar to each jar. Once again make observations and update your model. Record any evidence that might help you determine whether or not life exists in the jars.

3. Using a small beaker or graduated cylinder, add enough warm water to each jar so that the water just covers the sand. Make observations and update your model.

4. Wait about five minutes and then add another tablespoon, or 15 mL ,of sugar to each jar. Make your final observations and then determine which jar(s) you believe contain life, and which do not.

5. Write a paragraph in your interactive notebook reflecting upon the investigation and use evidence to support your claim as to which jar(s) do and do not contain life.

If the Follow Up activity (optional) is not going to be utilized, have students dispose of the contents, clean and dry the jars.

Jar Results

Jar A: No reaction or changes throughout the demonstration.

Jar B: Should have bubbling shortly after the warm water is added. Once sugar is added a second time you should expect to have an increase in the bubbles.

Jar C: The jar should bubble until the antacid dissolves. When the additional sugar is added there should not be any change in the bubbling of the jar, if any bubbling remains.

Adapted from NASA Astrobiology Science Learning Activities for After School.

Lesson 2	Extremophiles: What is an Extremophile?
Overview Standard(s) & Objectives	**Overview** During this lesson students will become familiar with extremophiles. In Sanford lab, the conditions can be very extreme; therefore, if life were to be found in these environments it would be classified as extremophiles. This lesson will help students understand what makes an extremophile different than most organisms in terms of the conditions that they need in order to survive. **Standards** HS-LS2-2 Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales. **Objectives** ● Describe various locations, both on earth and in outer space, that contain extremophiles. ● Hypothesize what conditions might be present in Sanford Lab and whether or not those conditions could support extremophiles.
Background Information	The term extremophile depends on what kind of organism you are. For example, it may seem extreme for humans to thrive in an environment that has both high temperature and humidity, it would be the ideal environment for pathogenic bacteria. Extremophiles are organisms living in conditions that are often considered less than ideal for others of the same type. Frigid temperatures, high acidity, temperatures near boiling, lack of sunlight, and sulfur rich areas are just a few of the conditions in which some extremophiles thrive. Extremophiles were first discovered just 40 years ago in the hot springs of Yellowstone National Park. Since their discovery, scientists around the world have worked to find how extremophiles might be useful to humans, and how they might harm humans. Keep in mind, that with 1 exception, all of the underground environments are non-photosynthetic. Organisms underground rely on the oxidation of inorganic chemicals (energy sources). The exception is a tiny area near a door that has the lights on all the time. Green algae/diatoms have colonized here. Astrobiologists are particularly interested in studying extremophiles, as many organisms of this type are capable of surviving in environments similar to those known to exist on other planets.
Materials	● Student interactive notebooks ● Pictures of Earthly Extremophiles (From NASA Astrobiology Institute)

	• Computer, projector, and speakers- or a computer for each student
Prior Knowledge	From lesson one, students need to be familiar with the properties of life. Students also need to be familiar with conditions that are necessary for life to thrive.
Launch (15 minutes)	
Engagement and Communication of Student Expectations	Give small groups *Earthly Extremophiles photos*. (Do not give groups the large extremophile examples card, yet) Ask students to determine if the pictures contain life, and if so, what would the conditions of life be? Create a list of those conditions on the board. Ask students what is similar and what is different between these pictures and the pictures taken in Sanford Lab. Students should record these differences in their interactive notebooks. A Venn Diagram is a recommended type of entry. The details on the examples card may be helpful to groups at this point.
Explore (25 minutes)	
Procedure	1. Visit the extremophile tutorial from Learn Genetics, either individually or as a class. http://learn.genetics.utah.edu/content/astrobiology/environments/ 2. As students watch each clip they need to complete a graphic organizer in their interactive notebook. Please refer to the template at the end of this lesson to set up the graphic organizer. **Teacher Tip** If time or computers are limited, the class could be divided into groups and the activity could be completed as a Jigsaw Activity. Each group could complete a couple of the locations and then share their responses with the rest of the class.
Questions	**Driving Question** • What conditions make an environment suitable for extremophiles? **Probing Questions** • Why is it important that we study extremophiles? • What do you think the conditions in Sanford Lab are like, and why? • What conditions exist in Sanford Lab that could allow for the presence of extremophiles?
Summarize (15 minutes)	
Communicate	Give students two minutes to make a list in their interactive notebooks of places where they are likely to find extremophiles in their everyday lives. Have each student find a partner, give each partner group two minutes to share their lists with each other. Each student should update his or her list to match the partner's list as well as brainstorm more places. Have each set of partners join another set of partners. Each group of four works to update their list and then brainstorm any new places.

	Have each group of four briefly share a part of their list with the rest of the class. See which group has the most entries and/or the most unique set of entries.
Terminology and Concepts to Solidify	**Extremophile**- an organism that thrives in conditions that would be fatal to most organisms **-phile** – the suffix that means to have an attraction to or love for something
Connection to Big Ideas (Phenomena)	*Structure and Function of Organisms* Organisms have specific structure, which allows them to survive in their environment. Each species' structure is suited for the environment in which it lives. *Interdependent Relationships in Ecosystems* Extremophiles have roles in the environment, which are vital to the ecosystem.
Assessment	Ask the students to write an entry in their science notebook stating if they feel the conditions in the Sanford Lab are suitable for extremophiles. Ask the students to provide evidence (temperature, lack of sunlight, etc.) for their reasoning.
Follow Up (Optional)	Students should turn in lab procedures to the teacher. The teacher should check to ensure that requested materials are available and that lab procedures are safe and complete.

Suggested Graphic Organizer

Location or Organism Name	Conditions	What the Extremophile Thrives on	Could this be Present in the Sanford Lab	Justification for Presence or Absence in Sanford Lab
Tardigrades				
Yellowstone National Park				
Great Salt Lake				
Cueva de Villa Luz				
East Pacific Rise				
Pitch Lake				
Atacama Desert				
Laguna Desert				
Sea Ice				
McMurdo Dry Valleys				
Europa				
Mars				
Titan				

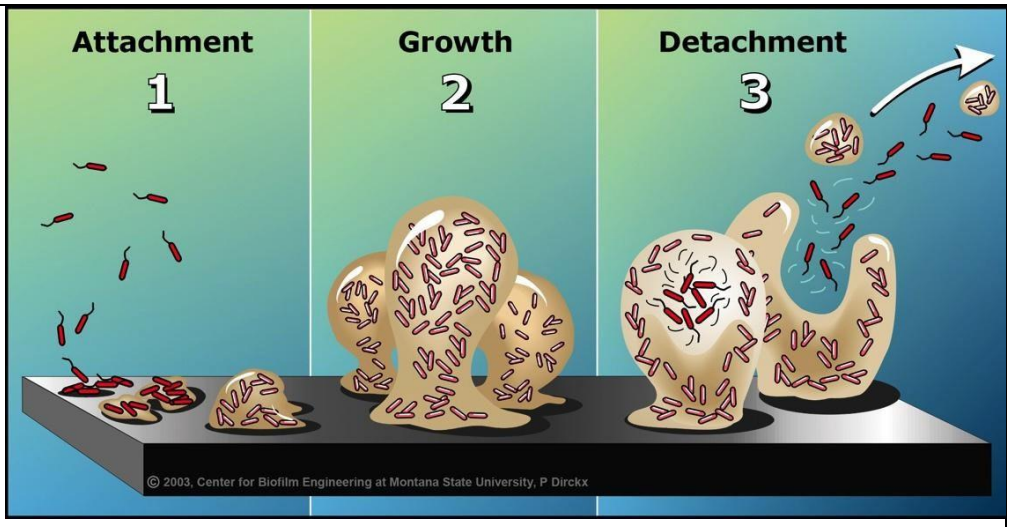
This is found as a print master at the end of the unit, if you desire, students can simply fill in a paper copy that can be taped into their science notebook.

Lesson 3	Extremophiles: It's Classified!
Overview Standard(s) & Objectives	**Overview** During this lesson, students will become familiar with various types of extremophiles and how they are classified. Students will be looking at extremophile cards and determining their classification. Using pictures of the perplexing puddles, students will hypothesize whether those perplexing puddles could be a potential habitat for extremophiles. **Standards** HS-LS2-2 Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales. **Objectives** ● State characteristics used to classify extremophiles. ● Explain how organisms depend on their environments and their structures to survive. ● Hypothesize whether the puddles present in Sanford Lab can support extremophiles.
Background Information	Extremophiles are classified by what substance, or substances, they metabolize and/or the conditions that they require to thrive. For an example, acidophiles are organisms that live in an acidic environment and thermophiles live at extreme temperatures. Astrobiologists are particularly interested in studying extremophiles, as they can help us understand what organisms may exist in conditions present on other planets. If you would like more information on the classification of extremophiles, or need an additional article for students to read, Montana State University has a great article http://www.montana.edu/msse/documents/Rothchild02.pdf
Materials	● Student interactive notebooks ● Extremophiles trading cards ● Perplexing Puddle Cards (pictures from Sanford Lab)
Prior Knowledge	Students should be familiar with the basics of classification. Students should understand that living organisms within an ecosystem interact with one another and with their environment.
Launch (10 minutes)	
Engagement and Communication of Student Expectations	**Class Discussion** ● How do you think extremophiles are classified? ● What conditions make an environment suitable for extremophiles? ● Can you determine anything about the extremophile using visual observation alone?

	● Do all extremophiles have the same environmental requirements? ● How do organisms make adaptions in extreme environments?
Explore (35 minutes)	
Procedure	1. Hand out Extremophile Trading Cards. 2. In small groups, students will work through each of the cards and make a list of the various extremophiles and the ability of the extremophile in their science notebook. Example: Acidithiobacillus thiooxidans –acidophile Cupriavidus metallidurans – metallophile 3. Have the students record each type of classification in their interactive notebooks and then write a definition as to what they think each type of classification stands for. For example, acidophile loves acid.
Questions	**Driving Question** ● How do you think extremophiles are classified? **Probing Questions** ● How have extremophiles adapted to live in places where life rarely exists? ● Can you determine anything about the extremophile using visual observation alone? ● Do all extremophiles have the same environmental requirements? ● Which classifications of extremophiles might be found in the Sanford Lab? Why do you think so? ● How do organisms make adaptions in extreme environments?
Summarize (10 minutes)	
Communicate	Hand out the *Perplexing Puddles* cards. Have students look over the cards and, in their interactive notebooks, write an entry about what they think might be in each of the puddles. Have students postulate whether the puddles are different. If so, why? Ask students to share their responses and lead students to the conclusion that, if life exists in these puddles, it must be extremophiles.
Terminology and Concepts to Solidify	**Extremophile**- an organism that thrives in conditions that would be fatal to most organisms. **-phile** – the suffix that means to have an attraction to or love for something
Connection to Big Ideas (Phenomena)	*Structure and Function of Organisms* Organisms have specific structure, which allows them to survive in their environment. Each species' structure is suited for the environment in which it lives. *Interdependent Relationships in Ecosystems* Extremophiles have roles in the environment, which are vital to the ecosystem.

Assessment	The student notebook entry of what they think may be living in the puddles and why. Students should be able to state that the organisms are extremophiles and provide justification.
Follow Up (Optional)	Students can begin setting up their experiments to test to see if the contents of their chosen jar (from day one) contain life.

Lesson 4	Biofilms, Are They Alive?
Overview Standard(s) & Objectives	**Overview** Students will culture a biofilm and then predict how the biofilm will grow on the plate. (Students may collect biofilms in the community.) **Standards** HS-LS2-8 Evaluate the evidence for the role of group behavior on individual and species' chances to survive and reproduce. **Objectives** ● Culture a biofilm. ● Predict biofilm growth. **Advanced Preparation Requirements** ✓ *Agar plates will need to be poured in advance. See teacher notes following this lesson.* ***Important:*** *Once the plates have been inoculated you need to plan for five to 14 days of observation.*
Background Information	Biofilms are communities of microorganisms, typically bacteria, that live in aqueous environments. The *Perplexing Puddles* cards show pictures of biofilms living in Sanford Lab. Since the conditions underground in Sanford Lab are not typically conducive to life, these biofilms are considered extremophiles. Many of these biofilms thrive in dark conditions, in the presence of salts and other metals found in rock of Sanford Lab. A biofilm is any group of microorganisms in which the cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilms are classified as communities and within a puddle there could be several distinct biofilms representing different organisms including bacteria, archaea, fungi and algae. There are many interesting biofilms underground at Sanford Lab. The NASA funded Life Underground research project is studying the organisms living in some of these biofilms.



AN INTRODUCTION TO THE BIOFILM LIFE CYCLE:

1) Free-floating, or planktonic, bacteria encounter a submerged surface and within minutes can become attached. They begin to produce slimy extracellular polymeric substances (EPS) and to colonize the surface.

2) EPS production allows the emerging biofilm community to develop a complex, three-dimensional structure that is influenced by a variety of environmental factors. Biofilm communities can develop within hours.

3) Biofilms can propagate through detachment of small or large clumps of cells, or by a type of "seeding dispersal" that releases individual cells. Either type of detachment allows bacteria to attach to a surface or to a biofilm downstream of the original community.

Graphic from Montana State University

Materials

- Student interactive notebooks
- Variety of green leaves (one per student or pair of students)
  For best results, avoid leaves with hard shiny surfaces.
- Petri Plates containing agar (one per student or pair of students)
- Pencil with an eraser
- Permanent markers with fine tip
- PTFE (Teflon) tape for sealing petri plates after inoculation
- Vials (if biofilm samples are collected from the field)
- Spatulas (if biofilm samples are collected from the field)
- Poly Inoculating loops (to inoculate agar plates, if biofilm samples are collected from the field)

Teacher Tip

While an incubator is optional, information about culturing and streaking plates, as well as building your own incubator are below.
https://www.umsl.edu/microbes/files/pdfs/streakplates.pdf

	https://www.umsl.edu/microbes/files/pdfs/Incubator.pdf
Prior Knowledge	Students should be familiar with basic lab procedures for collecting samples and preventing cross-contamination.
Launch (10 minutes)	
Engagement and Communication of Student Expectations	Watch the Biofilm Sci Show Biofilm: A New (Gross) Thing to Worry About (3 min and 51 seconds). Students should take notes in their interactive notebooks. (The video is available on the USB drive) Hank, the narrator, talks very quickly. Pause the video intermittently to give students a chance to get caught up on their notes. **Also please note that Cystic Fibrosis is a genetic disorder and is not an infectious disease.** Please make it clear to students that Hank is referring to the bacteria that are common in a person who is suffering from Cystic Fibrosis.
Explore (35 minutes)	
Procedure	1. Label your agar plate with name and date. 2. Inoculate the plate by placing a leaf on the agar. With the eraser end of a pencil, gently press the leaf into the agar. Try to get the entire surface area of the leaf to imprint on the agar. 3. After making the impression, carefully lift the leaf away from the media and discard. Note: a clear imprint of the leaf should be visible on the surface of the agar. 4. Close and seal the agar plate with the PTFE tape. Store the inverted plates in a warm place in the classroom or in an incubator at room temperature. SAFETY: Once sealed, the Petri plate should not be opened. Many types of bacteria are expected to grow, and not knowing what all of them are, it is best to avoid direct contact. Students should wash hands with soap after inoculation of the plates. 5. Students will draw a model of their plate, at time of inoculation, in their science notebooks. Students should include as many details as possible, type of leaf (if known), whether an upper or lower leave surface was imprinted, the types of observations they expect make, what data to collect and results expected.
Questions	**Driving Question** ● Are biofilms a population, community, or ecosystem? What evidence supports your choice? **Probing Questions** ● How do biofilms give an advantage? ● How could you determine if the biofilm is a population, community, or an ecosystem? ● Can the biofilms grow indefinitely, or will they eventually stop growing? Why?

Summarize (10 minutes)	
Communicate	Ask students to state how the classroom biofilms are different than the biofilms growing in the Sanford Lab. Discuss that biofilms are not always extremophiles. Discuss the differences in the conditions between the cultures in the Petri plates and the biofilms found underground. (Students should indicate that the cultured biofilms are not extremophiles.) Are biofilms a population, community, or ecosystem? What evidence supports your choice? Finally, Students will create a model in their interactive notebooks to show what they think their plate will look like after 24 hours, 48 hours, and 72 hours. Tell students that their models must include some of the properties of life.
Terminology and Concepts to Solidify	**Culture-** the cultivation or growing of cells in the lab setting **Aseptic technique**- a method used to prevent contamination of a culture or sample **Inoculate** – to add cells or organisms to a culture or medium **Planktonic-** bacteria that exist independently or are not a part of a biofilm **Organism**- an individual or contiguous living system **Population**- a group of the same species living together **Community**- groups of different populations that are living in the same area **Biofilm**- a film of bacteria living together such as the plaque on your teeth Biofilm organisms are microscopic and are not visible in the pictures as individuals.
Connection to Big Ideas (Phenomena)	*Structure and Function of Organisms* A biofilm forms a community that functions much like that of a multicellular organism. A biofilm can reproduce and send signals to other cells within the biofilm. *Interdependent Relationships in Ecosystems* Biofilms respond to their environment by metabolizing substances in the environment. Biofilms consist of several different species of bacteria and fungi, therefore making the biofilm a community.
Assessment	Evaluate the student's predictions on biofilm growth. Their models should indicate that the biofilms will grow to cover the plate (reproduce) and extract nutrients from the agar.
Follow Up (Optional)	Students should check their lab results and see if they can determine if life exists in the jar that they have chosen to test. Additional Follow up: Students could also chose different conditions to grow their biofilms from this lesson, such as temperature, amount of light, media, etc. Graph variables.

Pouring Agar Plates (Directions for 30 Plates)

Materials:

- Nutrient Agar
- Distilled Water
- Aluminum Foil
- Hot Plate/Microwave
- Erlenmeyer Flask – 1000 mL or larger
- Graduated Cylinder -1000 mL or larger
- Scale
- Sterile Petri Plates (30-50)

Directions:

1. Mix the nutrient agar as directed on the bottle. Typically you will mix 20-22 grams of agar with distilled water brought up to one liter in volume. **Make sure that all equipment has been cleaned and sterilized.**
2. Pour the agar solution into an Erlenmeyer flask (1000mL or larger).
3. Place a piece of aluminum foil over the mouth of the flask; leave a space for the hot air to escape.
4. Heat the solution on a hot plate until it boils. *Do not leave the flask unattended, once the gar begins to boil it quickly boils over.* If preferred, the flask can be heated in the microwave at 30 second intervals swirling the flask in between each interval until the agar comes to a boil.
5. Wait until the agar solution has cooled slightly. You want the solution to remain liquid, but not hot to the touch.
6. Pour the agar to create a thin layer on the bottom of the Petri plate. A depth of 2-3 millimeters is recommended.
7. Allow the plates to cool and then store the plates inverted (on their lids) until ready to inoculate.

Collecting Biofilm Samples (in the field)

Materials

- Sample spatula
- Small vial or other container that will prevent leakage
- Gloves

Directions

1. If collecting from an aqueous source, place a bit of the liquid in the vial before adding biofilm.
2. Using a clean sample spatula, scrape the biofilm and place the biofilm into vial.
3. Place the vial in a refrigerator if not used immediately to inoculate plates.

Inoculating Agar Plate (field sample)

Prepare a Safe and Sterile Workspace

1. Be familiar with all laboratory rules and safety precautions to be taken when working with microorganisms. Regardless of biohazard classification, all materials that come in contact with microorganisms are considered infectious waste and must be decontaminated prior to disposal. Follow safety guidelines in accordance with those provided by institutional Environmental Health and Safety departments, setting up appropriate waste receptacles for immediate and proper disposal of potentially contaminated materials (biohazards).
2. Sterilize all instruments, solutions, and media prior to using them for plating procedures.
3. Clear away all materials cluttering your work area on the laboratory bench.
4. Clean work area with disinfectant to minimize possible contamination.
5. Arrange all the supplies needed for the procedure on the laboratory bench near the sterile field. Make sure all the materials are properly labeled. Organizing the work area to maximize work efficiency and avoid unnecessary movements will minimize the exposure time of experimental materials to airborne contaminants.
    - Place agar plates or Petri dishes to your left.
    - Arrange cell cultures, tubes, flasks and bottles in the center of the bench.
    - Loosen the caps of tubes, flasks and bottles so they can be easily opened with one hand during subsequent manipulations.
6. Wash hands thoroughly with antiseptic soap and warm water before handling microorganisms.

Streak Plate Procedure: Isolation of Bacterial Colonies Using the Quadrant Method

With the streak-plate procedure, a mixture of cells is spread over the surface of a semi-solid, agar-based nutrient medium in a Petri dish such that fewer and fewer cells are deposited at widely separated points on the surface of the medium and, following incubation, develop into colonies. The quadrant method for isolating single colonies from a mixture of cells will be described here.

1. Streak-plating can be accomplished with a number of different instruments. A metal loop can be re-used multiple times and is utilized for streak-plating routine laboratory strains. Many research scientists prefer using disposable, pre-sterilized wooden sticks or flat toothpicks for streak-plating. These are an inexpensive alternative to disposable plastic loops and can be especially useful when working with an environmental sample such as soil that likely contains spore-forming bacteria.

- Disposable pre-sterilized sticks and toothpicks need not be flamed with a Bunsen burner thus preventing unnecessary aerosolization of spore-forming bacteria and cross-contamination of laboratory surfaces or agar plates with spores.

2. Label around the edge of the bottom (not the lid) of an agar plate with at least your name, the date.
    - The plates must be completely dry without condensation on the lid and pre-warmed to room temperature prior to streak-plating. If the plates are stored at 4 °C, remove them several hours or even the day before. Spread them out in small, staggered stacks of no more than 2-3 plates and allow them to dry.
3. The sample from which the streak-plate will be inoculated could be either a suspension of cells in broth or an existing colony from another agar plate. To begin, it is recommended that only one sample be used to inoculate a single plate. To save time and materials, a single plate can be used for multiple samples but only once you become proficient at streaking one sample on a plate.
4. For the first quadrant, the sample is picked up and spread over about one-quarter of the surface of the medium using a rapid, smooth, back-and-forth motion. Lift the bottom half of an inverted plate from the bench. Move the loop, stick, or toothpick back and forth many times across the agar surface from the rim to the center of the plate.
5. After completing the first quadrant, invert and set down the plate back into lid on the bench. Dispose of the stick or toothpick.
6. Turn the Petri dish 90 ° to streak the second quadrant. Lift the bottom half of an inverted plate from the bench then touch the loop, stick or toothpick to the first quadrant near the end of the last streak. Using the back-and forth pattern, cross over the last half of the streaks in the first quadrant then move into the empty second quadrant. Invert and set down the plate back into lid on the bench once the second quadrant is filled.
    - The loop, stick or toothpick should never go back into the first half of the streaks in the first quadrant where most of the original inoculum was deposited.
    - A new plastic loop, wooden stick or toothpick should be used for each quadrant.
7. Repeat step #6 twice for the third and fourth quadrants.
    - Be sure to dispose of the loop, stick or toothpick between each quadrant.
    - Avoid going into the first quadrant when streaking the fourth quadrant.
8. Incubate the plate upside down so condensation that accumulates on the lid does not drip on the colonies.

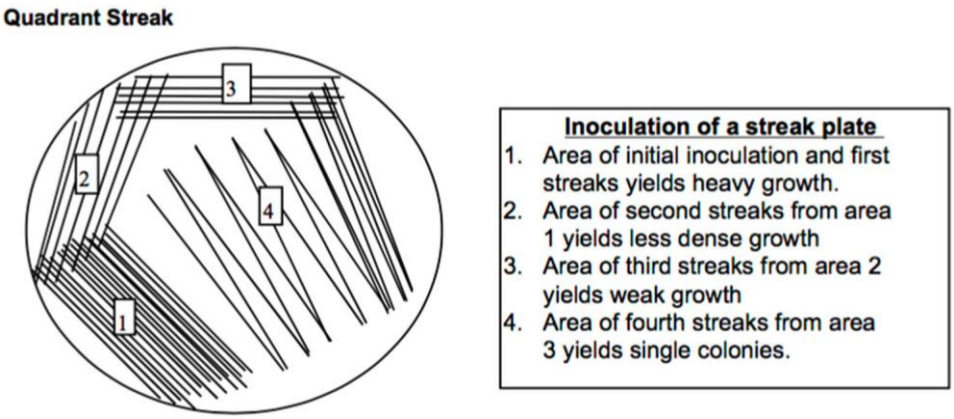
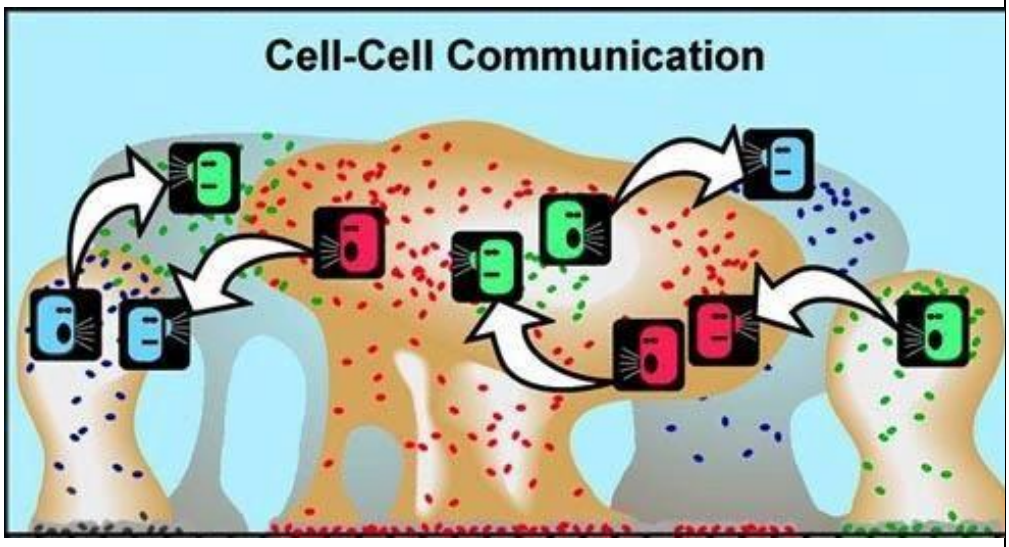
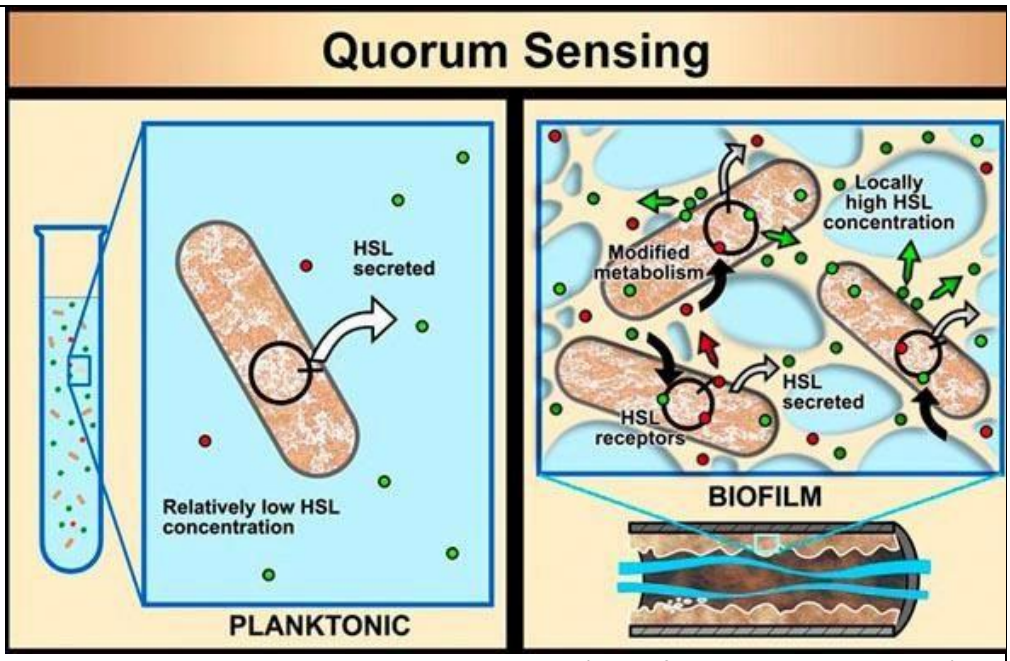


Image from the Department of Biology University of Missouri-St. Louis.

9. Seal the Petri plate with PTFE tape.

Lesson 5	Can Biofilms Send Text Messages?
Overview Standard(s) & Objectives	**Overview** In this lesson, students will learn how bacteria in biofilms communicate, via quorum sensing, so that the biofilm act along the lines of a multicellular organism. **Standards** HS-LS2-2 Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales. **Objectives** ● Define quorum sensing and explain how quorum sensing is used in a biofilm.

	• Determine the area of biofilm growth that has occurred on the Petri plate.
Background Information	Quorum sensing occurs when bacteria in the biofilm send a chemical messenger to surrounding bacteria in the biofilm to elicit a specific response (cell-to- cell communication). The bacteria can send out species-specific messages to be interpreted only by bacteria of the same species, or general messages that can be interpreted by many different species in the biofilm. This process of messaging allows for all the bacteria in the biofilm to carry out the same behavior at the same time, similar to cell-to-cell communication in multicellular organisms. Homoserine lactones (HSLs) are a class of signaling molecules involved in bacterial quorum sensing.  In the cartoon above, various species of bacteria are represented by different colors. Bacteria can produce chemical signals ("talk") and other bacteria can respond to them ("listen") in a process commonly known as cell-cell communication or cell-cell signaling. This communication can result in coordinated behavior of microbial populations. (Courtesy, MSU-CBE.)

	 Though planktonic cells secrete chemical signals (HSLs, for homoserine lactones), the low concentration of signal molecules does not change genetic expression. Biofilm cells are held together in dense populations, so the secreted HSLs attain higher concentrations. HSL molecules then re-cross the cell membranes and trigger changes in genetic activity. (Courtesy, MSU-CBE.)
Materials	• Student interactive notebooks • Computer, projector, and speakers • Grid transparencies, one per lab team • Copies of articles for every student or computers for every student to read the articles online • Stop the Microbial Chatter (Nature Magazine) http://www.nature.com/nature/journal/v511/n7510/full/511493a.html • Quorum-sensing signals control when bacteria turn deadly (for basic readers) https://www.sciencedaily.com/releases/2014/09/140918205902.htm • Video: TED Talk- How Bacteria Talk https://www.youtube.com/watch?v=KXWurAmtf78
Prior Knowledge	A biofilm community can be formed by a single bacterial species, but in nature biofilms almost always consist of rich mixtures of many species of bacteria, as well as fungi, algae, yeasts, protozoa, other microorganisms, debris and corrosion products.
Launch (10 minutes)	
Engagement and Communication	Ask students “Can biofilms communicate? If so, what might that look like?” Allow time to discuss this idea.

of Student Expectations	Students should record their ideas and thoughts about how bacteria might communicate, or why they do not need to, in their interactive notebook.
Explore (20 minutes)	
Procedure	1. Show the TED Talk How Bacteria Talk by Bonnie Bassler. (18 min and 11 seconds) Students should take notes in their interactive notebooks. Pause the video if needed to help students catch up. (The video is available on the USB drive)
Questions	**Driving Question** ● Why is communication within the biofilm essential to the survival of the biofilm? **Probing Questions** ● Why should studies of biofilms at Sanford lab continue? ● Why is it difficult for humans to overcome antibiotic resistance in terms of bacteria communication? ● Why is the ability to communicate within the biofilm detrimental to humans in terms of disease? **Teacher Tip** Help students to understand that the signaling molecule is also known as a ligand. Each ligand is specific to its receptor and each receptor elicits a specific response. Similar to a key that will only unlock a specific door.
Summarize (25 minutes)	
Communicate	Students silently read the following article: Stop the Microbial Chatter (Nature Magazine) If you have basic readers ….Quorum-sensing Signals Control When Bacteria Turn Deadly Students should take notes in their interactive notebooks. Ask students to write an entry in their interactive notebook describing any limitations to their biofilms as they are growing in a Petri plate rather than in nature. Have students place the transparency over the Petri dish and count the number of squares that contain growth and record any observations they have about the growth of the biofilm in their interactive notebooks.
Terminology and Concepts to Solidify	**Quorum Sensing**- a method of communication between bacteria that enables the coordination of group-based behavior based on population density. **Cellular Signaling**- a system of communication that governs the activities and coordinates the cells actions **Homoserine Lactones-** a class of signaling molecules involved in bacterial quorum sensing (bacterial hormones) **Signal Molecule** - molecules that are released from one cell and move to the receptor protein of another cell **Receptor Protein**- a protein on the cell membrane that receives a signal molecule from outside of the cell

	Ligand- a molecule that binds to a receptor found on a cell
Connection to Big Ideas (Phenomena)	*Structure and Function* Biofilms are equipped with structures that allow for the release of signaling molecules and also receptors that can then receive signals from other bacteria cells in the biofilm.
Assessment	Student entry in the interactive notebook stating whether or not they think that the biofilm growing on their Petri plate can or cannot communicate. If students believe that the biofilm can communicate have the students draw a model as to how the communication would occur on agar. If students do not think that the biofilm can communicate have the students state the limitations to the communication amongst the biofilms. The entry will be graded using the rubric used at the end of the unit.
Follow Up (Optional)	Students are to write a conclusion in their interactive notebook stating whether they accept or reject their hypothesis from their inquiry of life in the jars.

Lesson 6	Taking a Microscopic Look
Overview Standard(s) & Objectives	**Overview** In this lesson students will be viewing a stained sample biofilm culture under the microscope. Students will also tie together all pieces of this unit and determine what life, if any, might exist in Sanford Lab. **Standards**

	HS-LS2-2 Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales. **Objectives** ● Observe biofilm cells. ● Determine if a biofilm is a community, population, or organism. ● Make a hypothesis about the possibility of life in Sanford Lab. ● Reflect upon and summarize their learning over the course of this unit.
Background Information	Biofilm communities in nature almost always consist of rich mixtures of many species of bacteria, as well as fungi, algae, yeasts, protozoa, other microorganisms, debris and corrosion products. Some biofilms are composed of a single species and therefore would be a population. Depending upon the growth of the Petri plates students can conclude that the biofilm is either a community or a population. The bacteria prefer to live in the biofilm as opposed to planktonic (as individual cells). As a biofilm the bacteria can communicate with other bacteria and demonstrate a safety in numbers lifestyle.
Materials	● Student interactive notebooks ● Grid transparencies, one per lab team ● Microscopes ● Biofilm slides that are pre- stained ● Perplexing Puddles PowerPoint ● Post-Assessment Handout ● Computer, projector, and speakers ● Video- *Making Sense of How Life Fits Together* ● *Identifying and Characterizing* PowerPoint ● Bleach ● Zip top bags
Prior Knowledge	Students need to know how to use a microscope properly.
Launch (10 minutes)	
Engagement and Communication of Student Expectations	Show students the TED Talk *Making Sense of How Life Fits Together* http://ed.ted.com/lessons/making-sense-of-how-life-fits-together-bobbi-seleski (4 min and 52 seconds). Students should take notes in their interactive notebooks. Pause when needed, to allow time for students to catch up. Ask students at which level of organization they think the biofilms belong by using evidence from this unit.
Explore (35 minutes)	

Procedure	1. Students will once again count the growth on their biofilms using the grids. Students should record the number of boxes with growth in their interactive notebooks. 2. Have students look at the prepared slides. Explain to students that the slides are samples from the Sanford Lab. Students should make sketches of what they see in their interactive notebook. NOTE: Do Not use oil immersion. 400x magnification should be sufficient to view cell shape and arrangement. The biofilm cells are the smaller circles on the slide. The larger irregular shapes are diatoms and other organisms that live in the biofilm as well. 3. As a team, discuss what level of organization occurs in the biofilm based upon what students saw in the Petri plates and on the slide. Evidence should indicate that biofilms are communities and in some cases populations. **Optional Extension** You can continue to grow the plates and have the students continue to record the squares of growth until the plate has reached a bacterial lawn or carrying capacity. Students could predict what day they believe their plate will reach carrying capacity. Students can also graph their data from the growth to determine that the growth is exponential growth.
Questions	**Driving Question** • Is a biofilm a community, population, or organism? • Is it possible that some of the life in Sanford Lab is a biofilm, why or why not? **Probing Questions** • What level of organization is a biofilm? • What are the advantages to living in a biofilm? • Why are scientists interested in biofilms?
Summarize (10 minutes)	
Communicate	Ask the students: **Is it possible that some of the life in Sanford Lab is a biofilm, why or why not?** Why would NASA have a strong interest in the biofilms found in the Sanford Underground Lab? Share the following with the students: *The NASA Astrobiology Institute team has taken advantage of a unique opportunity to explore the subsurface ecosystems in Sanford Lab. They postulate that if life exists, or ever existed, on Mars or other planetary bodies in our solar system, evidence there would most likely be found in the subsurface. Therefore, if they can learn more about the life that exists nearly one mile underground at Sanford Lab, they may also learn more about the extraterrestrial life.*

	Biologic Dark Matter If we had more sophisticated equipment, what might we be able to learn about our samples? Scanning electron microscopes, a fully equipped genetics lab? (PowerPoint *Identification and Characterization*) **Post Assessment** Reopen the *Perplexing Puddles* PowerPoint (side 15) Students should complete the post assessment independently. Remind them to include information learned over the course of this unit. **Dispose of cultures properly** • Always destroy the microorganisms before disposing of dishes in the trash. **Take the proper safety precautions.** Before you attempt to dispose of the petri dishes, you need to take the proper safety precautions. Although most of the bacteria grown will not be hazardous, large bacteria colonies may pose more of a risk - so you will need to kill them before disposal using household bleach. • Protect your hands from the bleach by wearing rubber gloves, protect your eyes with plastic goggles and protect your clothes by wearing an apron. **Pour bleach into the petri dishes.** Open the petri dish and carefully pour a small amount of bleach on top of the bacteria colonies, holding the dish over a sink. This will destroy the bacteria. • Be very careful not to let any of the bleach touch your skin, as it will burn. • Place the disinfected petri dish into a zip top plastic back and dispose of the bag in the trash.
Terminology and Concepts to Solidify	**Planktonic-** bacteria that exist independently or are not a part of a biofilm **Organism**- an individual or contiguous living system **Population**- a group of the same species living together **Community**- groups of different populations that are living in the same area
Connection to Big Ideas (Phenomena)	*Interdependent Relationships in Ecosystems* Biofilms are composed of communities of microorganisms living together in the EPS.
Assessment	(Post-Assessment handout) After the research is done, the next step is to get published!

	Write a scientific article that addresses properties of life, extremophiles, biofilms in the Sanford Lab, how biofilms participate in a community, and how they are able to communicate with each other.
Follow Up (Optional)	Students share their findings from the inquiry lab with other members or lab teams in the class.

Print Masters

Unit Pre-Assessment

Name _____ Period _____

You've just returned from the 4850 level at Sanford Underground Research Facility, write a media press release describing your most recent discovery.

Details should include information about *Who* made the discovery, What the discovery is, *Where/When* the discovery was made, and of course *Why* is this news worthy? Use evidence to support claims.

****

FOR IMMEDIATE RELEASE

Perplexing Puddles Found in Sanford Lab!

(Lead, SD) –

Task Card

Properties of Life Investigation

Purpose: The purpose of this lab is to determine if life exists in any of the three jars.

Materials: 3 jars (containing a sample), sugar, warm water, small beaker or graduated cylinder, tablespoon or metric spoons, your interactive notebooks.

Procedure:

1. Observe the three jars (A, B, and C) and in your interactive notebook draw a model of each jar and any observations you make that might help you determine if life does, or does not, exist in the jars.

2. Add one tablespoon, or 15 mL, of sugar to each jar. Once again make observations and update your model. Record any evidence that might help you determine whether or not life exists in the jars.

3. Using a small beaker or graduated cylinder, add enough warm water to each jar so that the water just covers the sand. Make observations and update your model.

4. Wait about five minutes and then add another tablespoon, or 15 mL, of sugar to each jar. Make your final observations and then determine which jar(s) you believe contain life, and which do not.

5. Write a paragraph in your interactive notebook reflecting upon the investigation and use evidence to support your choice as to which jar(s) do and do not contain life.

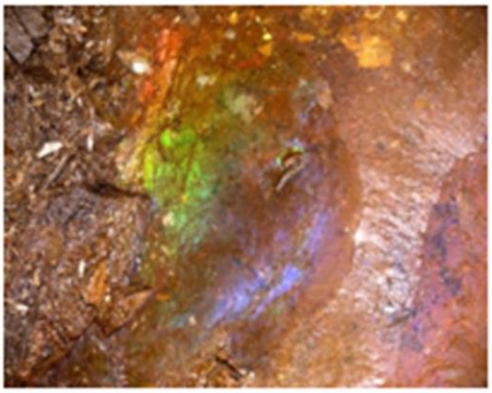
Extremophile Tutorial Graphic Organizer

Location or Organism Name	Conditions	What the Extremophile Thrives on	Could this be Present in the Sanford Lab	Justification for Presence or Absence in Sanford Lab
Tardigrades				
Yellowstone National Park				
Great Salt Lake				
Cueva de Villa Luz				
East Pacific Rise				
Pitch Lake				
Atacama Desert				
Laguna Desert				
Sea Ice				

McMurdo Dry Valleys				
Europa				
Mars				
Titan				

Perplexing Puddles Cards

- Found in a puddle, floating on the surface
- Thick like pudding or heavy cream
- Bubbles do not emit an odor when popped



- Found in a puddle, floating on the surface
- Looks like an oil slick, but breaks into chunks when disturbed
- Iridescence changes with angle of light and therefore, the rainbow colors cannot always be seen
- Chunks slowly float back together if left undisturbed
- A rust colored substance found nearby is similar to the puddle with bubbles

- Found on the rock wall
- It is a filmy, chalky substance
- There is a yellow color over a white under layer

800 Level biofilms- 55-60 degrees F (12-15 degrees C) with 100% humidity

Unit Post-Assessment

Name _____ Period _____

After the research is done, the next step is to get published!

Write a scientific article that addresses properties of life, extremophiles, biofilms in the Sanford Lab, how biofilms participate in a community, and how they are able to communicate with each other.

Most importantly, make sure the reader recognizes how this research can help scientists in their search for life in a variety of unexplored environments.

Remember to use evidence from the unit to support your claims.

****

Pre and Post Assessment Scoring Rubric

Criteria	Score of 5	Score of 3	Score of 0
Student indicates that life in Sanford Lab exists as a biofilm.	Student clearly indicates that life is present, in the form of a biofilm, and uses evidence from the unit to support their response.	Student indicates that life is present, but not necessarily in the form of a biofilm, evidence is weak or missing.	Student does not indicate that life is present or in the form of a biofilm.
Student indicates that life in Sanford Lab are classified as extremophiles.	Student clearly indicates that life in Sanford Lab are extremophiles using evidence from the conditions underground to support their response.	Student indicates that life in Sanford Lab are extremophiles, but weak or no evidence is used to support their response.	Student does not indicate that life in Sanford Lab are a type of extremophile.
Student indicates that biofilms are communities of organisms.	Student clearly indicates that biofilms are communities and uses evidence from the unit to support their response.	Student indicates that biofilms are communities, but evidence is weak or missing.	Student does not indicate that biofilms are communities.
Student indicates how this discovery helps scientists in their search for life in a variety of unexplored environments.	Student uses evidence to show that these discoveries are crucial in the search for life in in a variety of unexplored environments.	Student uses little evidence to show that these discoveries are crucial in the search for life in a variety of unexplored environments.	Student does not use evidence or fails to mention that these discoveries are crucial in the search for life in in a variety of unexplored environments.

Interactive Notebook Assessment Scoring Rubric

Criteria	Score 5	Score 3	Score 1	Score 0
Table of Contents	Table of contents is correct and up to date.	Table of contents has a few errors.	Table of contents contains several errors and/ or is lacking entries.	A table of contents is missing.
Title Page	The unit contains a title page featuring the title for the unit and questions about the unit topic.	The unit contains a title page for the unit but does not contain questions about the unit.	The unit contains a title page for the unit that does not clearly explain the topic covered in the unit.	The unit title page is missing.
Entries	All entries are dated and labeled. Entries are from a variety of sources or activities and are through, detailed, and numerous.	All entries are dated and labeled. Entries lack variety of sources or activities or are not detailed, thorough, or lack in number.	Entries are not all dated and/or labeled. Entries are few and provide little detail.	No entries are made.
Models	Models are numerous as well as through and clearly demonstrate the how, why, of the phenomena and makes predictions through detailed annotations.	Models are numerous as well as clear and demonstrate how and why of the phenomena but does not make predictions.	At least one model is present but is lacking in explanation of the how and why of the phenomena and fails to make predictions.	A model is not present.
Unit Summary	Summary includes two of the unit's best entries and why they are the best entries. The summary gives a detailed summary of what was learned in the unit beyond what was shared through the best entries as well as what is still unclear. The summary also gives possible answers to the question(s) recorded on the title page.	Summary includes two of the unit's best entries and why they are the best entries. The summary gives a somewhat detailed summary of what was learned in the unit beyond what was shared through the best entries as well as what is still unclear. The summary also gives possible answers to the question(s) recorded on the title page.	Summary does not include two of the unit's best entries and why they are the best entries. The summary gives a somewhat detailed summary of what was learned in the unit beyond what was shared through the best entries as well as what is still unclear. The summary does not give possible answers to the question(s) recorded on the title page.	No summary is present.

Interactive Notebook Student and Teacher Scoring Rubric

Name:		Unit:
	My Score	Teacher Score and comments
Table of Contents		
Title Page		
Entries		
Models		
Unit Summary		

Name:		Unit:
	My Score	Teacher Score and comments
Table of Contents		
Title Page		
Entries		
Models		
Unit Summary		

Chloroflexus aurantiacus
"Color-changer, green to orange"

Domain: Bacteria

Habitat: Hot springs around the world, including Yellowstone National Park.

Energy Source: Mixotroph (phototroph and chemotroph). Mainly uses light energy from the sun. In the dark, can use chemical energy from inorganic compounds (sulphur and hydrogen).

Challenges to Life: Heat, UV exposure, changing levels of light and oxygen

Adaptation: Can do both photosynthesis (like plants) and respiration (like animals), allowing it to survive a range of light and oxygen levels. It is green during photosynthesis and orange during respiration.

Talent: Thermophile. Thrives in temperature range of 35–70°C (95–158°F). Among the first organisms on early Earth to do photosynthesis.

Application: Studying this organism helps researchers learn about the evolution of photosynthesis.

Deinococcus radiodurans
"Terrible berry, survives radiation"

Domain: Bacteria

Habitat: Widespread, including deserts, hot springs, high mountains, polar regions and animal gut.

Energy Source: Chemotroph. Uses chemical energy from organic compounds.

Challenges to Life: Dehydration, cold, acidic pH, radiation, oxidative damage

Adaptation: Can rebuild its genome, after radiation breaks it into hundreds of fragments, using a unique DNA-repair mechanism. Produces antioxidants that protect proteins from radiation damage.

Talent: Radioresistant. Survives short doses of radiation up to 5,000 Gy. Grows under constant radiation levels of 50 Gy per hour. By comparison, a dose of 5 Gy kills humans.

Application: Research may lead to more efficient clean-up of nuclear waste, as well as new vaccines and cancer treatments.

Pyrococcus furiosus
"Raging fireball"

Domain: Archaea

Habitat: Hydrothermal vents in ocean floor; first discovered near volcanic islands in Italy.

Energy Source: Chemotroph. Uses chemical energy from organic compounds.

Challenges to Life: Heat, pressure, changes in oxygen levels when vent fluids contact sea water

Adaptation: A protein called reverse gyrase helps maintain DNA structure in extreme heat. High temperatures tend to unzip double-stranded DNA. Reverse gyrase twists DNA so much it can't unravel.

Talent: Hyperthermophile. Thrives in extremely hot temperatures. Grows best at 100°C (212°F), reproducing once every 37 minutes.

Application: Helping researchers genetically engineer plants that can grow in hot climates. Adding a gene to another plant made it able to grow in much higher temperatures.

Halobacterium salinarum
"Breathing bug that disregards salt"

Domain: Archaea

Habitat: Salty lakes around the world, including Great Salt Lake, Utah.

Energy Source: Chemotroph. Uses chemical energy from organic compounds.

Challenges to Life: Lack of water, temperature extremes, high salt, radiation, toxic chemicals

Adaptation: Special pumps in the the cell balance salt concentrations. The pumps keep salt levels inside the cell equal to salt levels outside the cell, preventing water loss and dehydration.

Talent: Halophile. Can grow in extremely salty water, 10 times saltier than the ocean.

Application: Researchers study halophiles to learn more about the important role they play in salt lake ecosystems, which support millions of migrating birds.

Psychrobacter arcticus
"Cold bug from the poles"

Domain: Bacteria

Habitat: Very cold places, including permanently frozen soil and pockets of salt water trapped in sea ice.

Energy Source: Chemotroph. Uses chemical energy from organic compounds

Challenges to Life: Cold, darkness, high salt, starvation, dehydration, limited space.

Adaptation: Special enzymes modify lipids to keep the cell membrane flexible in cold temperatures. Also, since cold temperatures slow down chemical reactions, proteins in the cell tend to be flexible and more active.

Talent: Psychrophile. Can grow at extremely cold temperatures, as low as -10°C (14°F).

Application: Researchers study cold-tolerant bacteria to learn whether life might exist on icy worlds like Mars and the moons of Jupiter.

Picrophilus torridus
"Burning paint"

Domain: Archaea

Habitat: Acidic environments, including hot springs and volcanic steam vents

Energy Source: Chemotroph. Uses chemical energy from organic compounds.

Challenges to Life: Acidic pH, high temperature.

Adaptations: Acid-resistant cell membrane acts as a barrier to protons, keeping the inside of the cell at a more neutral pH of 4.6. Uses difference in proton concentration between inside and outside the cell to generate energy. Smallest genome of any known organism minimizes the work of DNA repair.

Talent: Acidophile. Grows best at pH 0. Most life lives between pH 5 and 8; water has a pH of 7.

Application: Acid-resistant enzymes could clean up toxic mine sites, or be used as food supplements since the enzymes remain active in stomach acid.

Thellungiella salsuginea

"Saltwater cress"

Domain: Eukaryote (Plant – Angiosperm)

Habitat: Salty environments, including salt flats in northern Canada and seashores of eastern China.

Energy Source: Phototroph. Uses light energy from the sun.

Challenges to Life: High salt, cold temperatures, nutrient-poor soils, drought, flooding, toxic metals, short growing season

Adaptation: Roots have specialized ion transporters that keep out salt (sodium and chloride) but let in other nutrients (potassium). Vacuoles hold sodium away from the cytoplasm to prevent cell damage.

Talent: Halophile and psychrophile. Can grow and reproduce in extremely salty soil and sub-zero temperatures.

Application: Research is helping scientists engineer crop plants that can grow in harsh climates and soil conditions. A gene from this organism made cotton and corn plants more tolerant to salt and drought.

Antarctic Ice Fishes

(25+ species)

Domain: Eukaryote (Animal – Chordate)

Habitat: Southern Ocean surrounding Antarctica.

Energy Source: Chemotroph. Eats krill, small crustaceans and other fish.

Challenge to Life: Cold temperature

Adaptation: Antifreeze proteins keep their blood from freezing. They lack red blood cells, thinning their blood so that it flows better at low temperatures. They absorb oxygen through scaleless skin.

Talent: Psychrophile. Can thrive in very cold water, 1.5 to -1.8 °C (28 to 35°F).

Application: Antifreeze proteins are used to prevent ice crystal formation in popsicles and ice-cream, as well as to preserve human tissue for transplantation.

Tardigrade

"Little water bear" (1,150+ species)

Domain: Eukaryote (Animal)

Habitat: Wet environments, including hot springs, deep-ocean sediments, glaciers, fresh-water lakes, tropical forests, even the moss in your own backyard.

Energy Source: Chemotroph. Eats plants and bacteria.

Challenges to Life: Dehydration, extremely hot and cold temperatures

Adaptations: In a hibernation-like state called cryptobiosis, cells lose almost all water and stop all metabolism. A sugar molecule, trehalose, protects membranes and proteins from breaking.

Talent: Can survive more extremes (temperature, pressure, radiation, dehydration) than any other organism. They can even survive outer space.

Application: Research is helping us learn how organisms can survive without water. Understanding death-like cryptobiosis could allow us to reversibly stop our metabolism for long-distance space travel.

Acidithiobacillus thiooxidans

"Sulfur-breathing, acid-loving rod"

Domain: Bacteria

Habitat: Sulfide-rich, underground cave systems in Mexico, Italy and elsewhere.

Energy Source: Chemotroph. Uses chemical energy found in inorganic compounds (hydrogen sulfide).

Challenges to Life: Extreme pH, darkness, limited food resources, low oxygen, toxic gases

Adaptation: Builds a "snottite" to concentrate its acidic waste products. Snottites are too acidic for most other organisms, reducing competition for nutrients. Ion pumps raise the pH inside the cell, making it less acidic than its surroundings.

Talent: Acidophile. Grows best at pH 0–1. Most life lives between pH 5 and 8; water has a pH of 7.

Application: Researchers study snottites to learn more about early Earth environments, cave formation or expansion, and the possibility of life on other planets—underground.

*Cupriavidus metalliduran*s

"Metal enduring"

Domain: Bacteria

Habitat: Soils rich in natural metals or metal waste from mines and processing plants.

Energy Source: Chemotroph. Uses chemical energy from inorganic compounds.

Challenges to Life: Toxic chemicals

Adaptation: Metal-binding proteins convert metal dissolved in liquid into solid metal. Solid metal is pushed out of the cell through channels in the cell membrane, called heavy metal exporters.

Talent: Metallophile. Thrives in places contaminated with toxic heavy metals.

Application: Researchers hope to use the microbe's genes as biosensors, tools that would help industries more easily locate gold for mining. This organism may also be useful in cleaning up areas contaminated with heavy metals.

Riftia pachyptila

"Giant tube worm"

Domain: Eukaryote (Animal – Annelid)

Habitat: The deep sea, near geyser-like vents on the ocean floor, miles below the surface.

Energy Source: Chemotroph. Uses chemical energy from organic compounds.

Challenges to Life: Extreme pressure, darkness, low nutrients, toxic chemicals and extreme temperatures (vent water can be over 300°C / 572°F)

Adaptations: Through symbiosis with bacteria, converts vent chemicals into food. How this and other organisms survive crushing water pressure is unknown. High pressure changes the activity of some genes, but the functions of these genes are unknown.

Talent: Piezophile. Thrives in high pressure miles below the sea surface.

Application: Enzymes from this organism could be useful in industry because they function at high temperature and high pressure.

Examples of Earthly Extremophiles

Cold – The McMurdo Dry Valleys in Antarctica are some of the coldest, driest deserts on Earth, with average annual temperatures of -20°C (-4°F) and less than 10 centimeters (4 inches) of precipitation a year. Scientists have found bacteria in liquid water pockets embedded about twelve feet deep in "solid" lake ice. Some of these bacteria use chemical nutrients from particles of dirt in the ice and use energy from sunlight for photosynthesis.

Hot – Large concentrations of microbes thrive in Yellowstone National Park's Grand Prismatic Springs, a hot spring with water temperatures up to 90°C (188°F). Other hot springs in Yellowstone are extremely acidic, yet are home to many different kinds of bacteria and microbes. Many of these microbes use chemical nutrients in the waters and energy from sunlight for photosynthesis.

Deep underground – Scientists have discovered bacteria living in groundwater 5 kilometers below the surface in deep gold mines of the Witwatersrand Basin in South Africa. These microbes thrive in cavities and cracks in rocks. Scientists are also are investigating life within and below permafrost in northern Canada.

Bottom of the sea – Scientists have found abundant life clustered around hydrothermal vents on the ocean floor, including bacteria, mussels, clams, shrimp, and giant tubeworms that can reach ten feet in length. Water pouring out of the vents in the complete darkness thousands of feet under the surface of the sea can reach temperatures of 113-120°C (235-248°F). The high pressures keep the water from boiling. Bacteria use chemicals in the vent's water, primarily hydrogen sulfide, as their energy source instead of sunlight. Other creatures survive by eating the bacteria or each other.

High Acidity – The water in the Rio Tinto in southwestern Spain is very acidic, a result of chemical reactions between the water, and iron and sulfur minerals in the ground. The river has a deep red color, like wine, because of iron dissolved in the water. Microbes living in the water use chemical reactions with iron and sulfur minerals to generate the energy they need. Products from these metabolic reactions contribute to the low pH in the environment. Many algae and fungi also live in the acidic waters.

Perplexing Puddles Refurbishment Inventory

Lesson	*Quantity*	*Item*
All	1	Facilitator Binder w/ USB
	1	Shipping Tote
	25	Sample Spatulas
	50	Sample Containers
	50	Inoculating Loops
	144	Glass Slides (2 Boxes)
	100	Coverslips
	25	Disposable Pipettes
Lesson 1	8	Task Cards
	8 sets	4oz Jars (A, B, C)
	8	5mL Scoops
	1 Jar	Active Yeast
	` Container	Granulated Sugar
	Container	Sand
	14	Fizzing Antacid Tablets
	1	Thermometer
	`1 Set	Metric Spoons
Lesson 2	8 Sets	
Lesson 3	8 Sets	Extremophile Trading Cards (12/set)
	8 Sets	Perplexing Puddle Cards(3/set)
Lesson 4	Bottle	Agar
	100	Petri Dishes
	8 Rolls	PTFE (Teflon) Tape
Lesson 5	26	Article: Quorum-Sensing
	16	Article: Stop the Microbial Chatter
	13	Transparency Grids
Lesson 6	Multiple	Stained Slides

NOTICE: THE HEADER/FOOTER AND REVISION HISTORY OF THIS ATTACHMENT IS NOT INCLUDED IN THE PRINTED DELIVERABLE.ANY BLANK PAGES ARE LEFT SO INTENTIONALLY.

Revision History

Rev	Date	Section	Paragraph	Summary of Change	Authorized by
01	2/27/2024	NA	NA	Initial Release	CCR 911