NAME_____

Ladies in the Lab
Saturday, October 13, 2018

Time	Event	Staff Note
10:00-10:10	Arrival and check in. Meet your Lab Leader! Make a name tag. We will do a brief introduction to iGEM and our team before heading to the third floor for lab activities. Food, bags, stuff etc. should stay in room 1111	Check for closed toed shoes, no nuts in the lunch bag, photo release form completed, RSVP required. Lab Leaders will work with Session Leaders for check in. Session leaders leave when intro starts to prep your labs. Lab Leaders should have their rosters. KEEP THEM
10:10-10:55	Session 1 Bigs in Phage 1 12 in Phage 2 Littles in Forsyth	
10:55-11:40	Session 2 Bigs in Phage 2 12 in Forsyth Littles in Phage 1	
11:40-12:05	Wash hands. Move back to 1111 to enjoy our sack lunches with our lab groups	We have some families in different age groups who may want to connect
12:05-12:50	Session 3 Bigs in Forsyth 12 in Phage 1 Littles in Phage 2	
12:50-1:00	Pick up time	Grab your original sign in/roster. As students check out, mark them off of your sheet. Make sure kids go with with right parents.

The order is designed to first cater to our bigs, then out 12's then out littles get what is left.

Phage 1 – Understanding DNA; Gel electrophoresis and DNA extraction

Phage 2 – Observing bacteria; microscopes with premade slides and creating your own bacteria to take home

Forsyth – Working with bacteria; bacterial art, plating isolated colonies, gram stains. Students must wear gloves, coats, hair back and goggles$_{maybe}$.

My Role_____ Call location: ISC 1111

Call time for Lab Leaders is 9:40 Call time for Lesson Leaders is 9:30 Call time for others is 9:40

Forsyth Lab – Littles

PPE – Full

Learning objectives – What are isolated colonies? Our Synthetic Bacteria! Basic identification intro

Prep for littles – ONLY ONE Bunsen burner should be on, NOT at a table with students

Intro:

Have them put on PPE

What do we do in our labs? Explain that we have some synthetic bacteria for them to work with that grows green and pink! We will get to do some bacteria art today, then practice some microbiology techniques and then do some gram stains to start identifying bacteria.

BACTERIAL ART

Talk about how we edited the bacteria and how to plate gently

1. Write your name on the rim of your plate
2. Grab a sterile loop
3. Dip into a bacteria tube and GENTLY draw on the agar side of the plate
4. If you would like to add another color repeat but using the other side of your loop DO NOT DOUBLE DIP

PLATING ISOLATED COLONIES

When we are working with bacteria we sometimes want to grow it and want to be sure we can get samples that are 100% identical. We can do this because bacteria replicate asexually. BUT, when they are in a tube its impossible to pull out just one bacteria. So we work to plate isolated colonies. We can do this with streaking (demonstrate streaking on white board) or we can use beads to spread out a thin thin layer of bacteria so the colonies that grow don't touch and we know they were all started by just one little cell. We will use dye to evaluate your technique.

1. Give every student a plate
2. Have them add 1-2 drops of colored water (it STAINS) they can do mixed or same colors
3. Give them 4-6 beads and have them go crazy
4. Collect the beads (you will need to rinse them potentially between groups if we run low
5. Give each student a transfer paper to CAREFULLY put over their plate
6. Have them evaluate how well they did – are there big pools they missed?
7. They can take the plate home

GRAM STAINS – **MUST BE IN FULL PPE DOUBLE CHECK**

1. Grab and label a slide
2. Ad a very very small drop of water to your slide SO SO SO TINY if you need to use a loop to take a dab of water
3. Snap a sterile loop in half give half to a friend and take a streak of bacteria from one of the plates, *Bacillus subtilis* (+) and *Escherichia coli* (-), smear the bacteria onto the slide, the smear should be about a dime size
4. **LEADERS** take the slides and heat fix the bacteria (pass the slide through the flame for 1 second)
5. Cover the smear with several drops of crystal violet and let it stand one minute
6. Pour off the stain and rinse very gently with water, until it stops running purple, drain excess water
7. Cover the smear with iodine and let sit for 1 minute
8. Pour of the iodine, rinse very gently with water until it runs clear, drain excess water
9. Tip the slide on its side and add several drops of alcohol to the upper end of the slide. Let the alcohol flow over the smear drop by drop until the run off is mostly clear **quickly rinse with water to prevent over decolorizing**
10. Drain excess water and cover the smear with Safranin O stain let stand 1 minute

11. Wash slide again with water, drain and blot away excess water until dry
12. View under microscope **DON'T FORGET OIL ON OIL EMERSION**

Gram negative cells will be pink, gram positive will be purple

Forsyth Lab - Bigs

PPE – Full

Learning objectives – What are isolated colonies? Our Synthetic Bacteria! Basic identification intro

Intro:

Have them put on PPE

What is synthetic biology? What do we do in our labs? How have we created GFP or RFP bacteria? Transformation and CRISPR-Cas systems. Explain that we have some synthetic bacteria for them to work with today and that after working with that, we will shift to some more general microbiology techniques.

BACTERIAL ART

Students have already heard how we have created these bacteria so mingle, answer questions and remind them of the importance of the selective media plates they are plating on.

5. Write your name on the rim of your plate
6. Light up the Bunsen burners and sterilize your loops
7. Dip into your desired color and draw GENTLY on the agar side of the plate
8. Sterilize your loop again
9. If you would like to add another color repeat steps 3 & 4, otherwise turn off the burner, replace the loop and close your plate – plates grow for 24 hours, photos will be emailed

PLATING ISOLATED COLONIES

When we are working with bacteria we sometimes want to grow it and want to be sure we can get samples that are 100% identical. We can do this because bacteria replicate asexually. BUT, when they are in a tube its impossible to pull out just one bacteria. So we work to plate isolated colonies. We can do this with streaking (demonstrate streaking on white board) or we can use beads to spread out a thin thin layer of bacteria so the colonies that grow don't touch and we know they were all started by just one little cell. We will use dye to evaluate your technique.

8. Give every student a plate
9. Have them add 1-2 drops of colored water (it STAINS) they can do mixed or same colors
10. Give them 4-6 beads and have them go crazy
11. Collect the beads (you will need to rinse them potentially between groups if we run low
12. Give each student a transfer paper to CAREFULLY put over their plate
13. Have them evaluate how well they did – are there big pools they missed?
14. They can take the plate home

GRAM STAINS – **MUST BE IN FULL PPE DOUBLE CHECK**

One major distinction in bacteria classification is gram positive and gram negative. Gram positive cells have large peptidoglycan cell walls, gram negative cells have very thin peptidoglycan walls.

1. Grab and label a slide
2. Ad a very very small drop of water to your slide SO SO SO TINY if you need to use a loop to take a dab of water
3. Snap a sterile loop in half give half to a friend and take a streak of bacteria from one of the plates, *Bacillus subtilis* (+) and *Escherichia coli* (-), smear the bacteria onto the slide, the smear should be about a dime size. Technically dental tartar should have both positive and negative, students may want to scrape off their teeth tartar and plate that
4. Turn on Bunsen burners
5. Heat fix the bacteria (pass the slide through the flame for 1 second)
6. Cover the smear with several drops of crystal violet and let it stand one minute
7. Pour off the stain and rinse very gently with water, until it stops running purple, drain excess water
8. Cover the smear with iodine and let sit for 1 minute

9. Pour of the iodine, rinse very gently with water until it runs clear, drain excess water
10. Tip the slide on its side and add several drops of alcohol to the upper end of the slide. Let the alcohol flow over the smear. Drop by drop until it runs mostly clear, **quickly rinse with water to prevent over decolorizing**
11. Drain excess water and cover the smear with Safranin O stain let stand 1 minute
12. Wash slide again with water, drain and blot away excess water until dry
13. View under microscope **DON'T FORGET OIL ON OIL EMERSION**
14. **Gram negative cells will be pink, gram positive will be purple**

PHAGE LAB 1

PPE - None

Learning objectives – Identify DNA as the blueprint for life. Observing DNA in two ways.

Ask students what they know about DNA. What does it do? Where do we find DNA, emphasize it is in our skin and hair cells? Explain that DNA is unique; your DNA is not the same as my DNA. DNA is what tells a person to be a person and a berry to be a berry. But it's so small, it's really hard for us to see DNA. There are two ways we like to see DNA, we can use a technique called gel electrophoresis, and we can extract it from strawberries.

START WITH GEL ELECTROPHROESIS

Explain how gel electrophoresis works to separate big and little pieces of DNA using a semi-permeable membrane and electric charges. DNA is negatively charged so it runs to red (positive) and is pushed away from negative charge. Talk about the ball pit, what happens if you throw a big man in a ball pit? A little kid? A cat? A mouse? A marble?

1. Practice Pipetting, practice getting your dots the right size – you can let them practice pipetting a few drops of colored liquid onto paper towels.
2. Let each student load two colors into a gel – if there are extra wells they can load those as well. Load 10 ul at a time (pre-set pipettes) they can add up to two colors safely, sometimes 3 is ok. Have them label on tape what wells they loaded and label the box.
    a. **Bigs only – Explain forensic uses of DNA particularly paternity and ID samples at crime scenes. Talk about how we break up the DNA into a bunch of small pieces so we can compare it.**
    b. **Glove Up! Let the bigs load real DNA and ladder. It is important to know which has EtOH and which gels do not for this group.**
3. Gels will be taken downstairs to be run – you can send the group down to look at their gels at the end of this session.

While the gels run…

DNA EXTRACTION

1. Give every student a berry – have them remove the green top and put it into the bag
2. DESTROY THE BERRY – as they destroy it, talk about how cells hold their DNA inside them and so it is protected by a membrane and that Eukaryotes (like us and strawberries) have an extra membrane to protect their DNA and hold it together in their cells. To get to the DNA, we have to break that membrane, to do that we use lysis buffer
3. Let students measure out 50 ml of lysis buffer, and then add it into their baggies. They should try not to make too many bubbles as they mix up their baggies.
4. Have them write their names on a 50ml falcon tube and set it up in the rack with the lid off
5. Have them fold their coffee filters and put them into their falcon tubes
6. Have them SLOWLY pour their strawberry goop into the filter (we are aiming for 20-25 ml) Discuss how we are removing all of the membranes and strawberry mush surrounding the DNA, but that the DNA is small and can fit through the filter
7. Get the ethanol from the freezer, let every student add 25-30 ml until they hit the 50 ml point on the tube
8. Using stir rods have students scoop out their DNA gobs. They can take the DNA home.

DONE – Send students to BEL to see their gels.

Phage Lab 2 - Littles

PPE – None

Learning objectives – Explore bacteria structure & observe the limited morphology

THESE ARE BIG INSTRUCTIONS

Intro: We have been or will be working with bacteria today, a lot of the time we see them as big clumps, but sometimes we want to see what they look like as individuals. So today we will be using microscopes to see some of our different bacteria friends and then we will have a chance to build a bacterial friend for you to take home.

OBSERVING BACTERIA

There are 4 major morphologies (shapes)for bacteria, Cocci (circles), diplococcal (fused circles), Bacillary (rods) and Helical (spiral). The second major physical difference is flagella (used for movement) bacteria can have; one, a bundle at one end, one at each end and bacteria all over the membrane. We will be able to see the shapes of the bacteria using our light microscopes today, we won't get to see a lot of detail like the flagella or pili that exist for that we would need electron microscopes. **LEADERS please watch so so closely, especially for oil emersion!! you may have to help them get everything into focus** Turn on our microscopes and insert a slide

1. Adjust it so it looks like the bacteria is right under the light and observe through the smallest lens (4X)
2. Once the bacteria is in focus shift to the 10X
3. Again fix the bacteria into focus and move to the 40X
4. Repeat step 4, **add oil** and shift to the oil emersion 100X
5. What shape bacteria have you found?
6. Give students a chance to look at a couple bacteria

CREATING BACTERIA TO TAKE HOME –

Now that we have seen the major morphologies we can create our bacteria friends!

1. First, pick a color for your bacteria, bacteria come in different colors because of what they have inside their membrane (chlorophyll is a good example) or because we have stained them to see them better.
2. Let's pick a morphology or shape for our bacteria. Remember you can be Cocci (a sphere like a base ball), Diplococcus (like the number 8), bacillary or rod like (like a baby carrot) or helical/spiral (like curly pasta or twisty ribbon)
3. Now let's give our bacteria some flagella so he/she can move around! Pick how many flagella you would like your bacteria to have and where they should go
4. Now we can add pili/fimbria the bacteria uses these to stick to it's environment, your bacteria doesn't have to have these
5. Finally, let's pick what your bacteria can eat! Bacteria can eat all kinds of things, some eat glucose, fructose or galactose, these are all you and I like. But some bacteria eat things like oil, some eat human waste, some eat rocks. What would you like your bacteria to eat? Go ahead and draw what your bacteria would eat in his lunch box.

Phage Lab 2 - Bigs

PPE – None

Learning objectives – Explore bacteria structure & observe the limited morphology

THESE ARE BIG INSTRUCTIONS

Intro: We have been or will be working with bacteria today, a lot of the time we see them as big clumps, but sometimes we want to see what they look like as individuals. So today we will be using microscopes to see some of our different bacteria friends and then we will have a chance to build a bacterial friend for you to take home.

OBSERVING BACTERIA

There are 4 major morphologies (shapes)for bacteria, Cocci (circles), diplococcal (fused circles), Bacillary (rods) and Helical (spiral). The second major physical difference is flagella (used for movement) bacteria can have; one, a bundle at one end, one at each end and bacteria all over the membrane. We will be able to see the shapes of the bacteria using our light microscopes today, we won't get to see a lot of detail like the flagella or pili that exist for that we would need electron microscopes. Today we will use light microscopes **Leaders please make sure students don't forget oil for the emersion**

7. Turn on our microscopes and insert a slide
8. Adjust it so it looks like the bacteria is right under the light and observe through the smallest lens (4X)
9. Once the bacteria is in focus shift to the 10X
10. Again fix the bacteria into focus and move to the 40X
11. Repeat step 4**, add oil** and shift to the oil emersion 100X
12. What shape bacteria have you found?
13. Give students a chance to look at a couple bacteria

CREATING BACTERIA TO TAKE HOME –

Now that we have seen the major morphologies we can dive a little deeper. Bacteria are prokaryotic organisms and structurally simpler than the Eukaryotic cells in you and me. Let's start by picking the morphology for our bacteria, pick which ever you like best.

6. First, lets add a membrane to our cell, membranes and cell walls give the bacteria structure and help protect if from the outside world. It is semi permeable and lets only certain things through.
7. On this membrane, the bacteria may have pili or fimbriae, these are used for adhesion so the bacteria can stick to their environment, specialized pili are used for bacterial conjugation (this is bacteria sex – decide if your group is old enough). Add if you'd like
8. The bacteria may also have some of those flagella for moving, add if you would like
9. Now, Prokaryotes (like bacteria) do not have a nucleolus like Eukaryotic (our) cells do. Their DNA is not surrounded by a membrane; it is loose in a nucleoid region. Let's add that now. We are just going to add it directly into the cytoplasm. That's the jelly like material on the inside of cells. For now we can pretend the glue is our cytoplasm, holding all of our bacteria parts
10. If this bacterium has taken up extra DNA from the environment, another bacterium or from a transformation (if they have been edited!!!) it will have a round piece of DNA, a plasmid in the cytoplasm
11. Finally, to process that DNA into protein, our bacteria has to have lots of ribosomes, go ahead and add that now.