Worcester Polytechnic Institute

Introduction to Synthetic Biology Curriculum

Introduction Activities

Alex Turland, Corbyn Lamy, Michael Giroux, Chloe LaJeunesse,
Kayla DeSanty, Shawna Henry, and Natalie Farny
10/17/2014

The following is a curriculum we developed for a 1.5-hour workshop designed to teach 5th -7th graders about synthetic biology. It was taught to 30 girls at the "Women in Science" summer camp at WPI on July 14th, 2014. Teaching notes for the curriculum are in italics.

What is "Synthetic Biology"?

What do you think of when you think of these two words? Shout out as many words as you can think of, and we'll put them on the board.

Likely responses for synthetic might include: fake, man-made, unnatural, machines, comes from a factory or a store, made up, not real

Likely responses for biology might include: nature, life, alive, things that move, things that grow, cells, animals, plants

Those are many great answers! And all of them are part of what synthetic biology is. As scientists, one way we can describe synthetic biology is the process of creating or changing a living being (a plant, animal, or even a single cell) to do something that we want it to do, or make something that we want it to make.

Often, we use bacteria for this purpose – can anyone describe what bacteria are?

Field responses – we assume that by grade 5 most students would at least have heard of bacteria before even if they don't know exactly what it is. We also anticipate that most students would have been exposed to the general concept of a cell. If this is not the case you may need to give the students more background on cells and bacteria.

Bacteria are very small, single-celled living beings. Bacterial are very important in our environment. They are found almost everywhere in nature. Although some types make you sick, most bacteria are completely harmless, and some of them are very good for our health. And it turns out that they grow very fast and very easily, so it is easy for scientists to grow them in the lab and get them to do things or make things.

So, what do you think bacteria could do that is useful to a human? Let's think for a minute… Think about a product you might like to make, or a problem in the world you might want to solve. Do you think bacteria could do it? Let's try to finish this sentence: Wouldn't it be cool if there were bacteria that _____. Use your imagination, and think of anything at all!

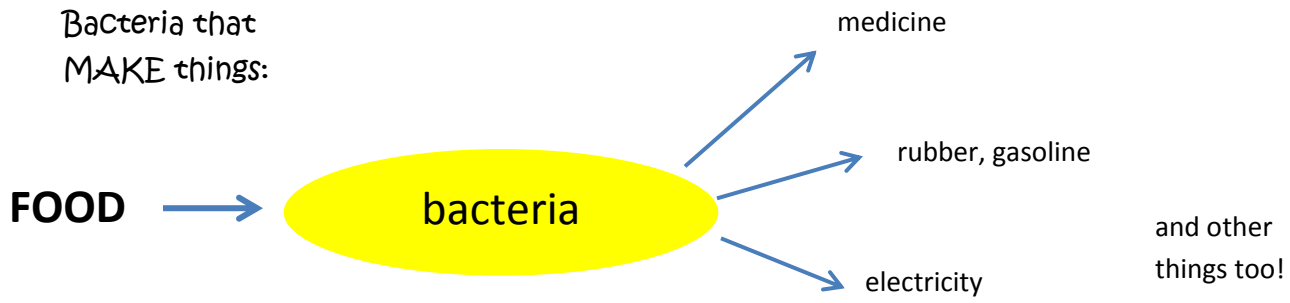
Give some example here to get their minds going…. Wouldn't it be cool if there were bacteria that glowed like Christmas lights and you could decorate your house with them? … that tasted like bubblegum and you could eat them? … that cleaned up pollution in the water or the air? Distribute the worksheet (Appendix 1), and give the students some time to think and write down an answer. We let them chat with their neighbors to come up with fun responses. Field some or all of the responses.

These are fantastic ideas, and as crazy as they might seem, synthetic biologists have already done some of the things that all of you came up with!

Here are some ideas of things that bacteria can make that have already been done by synthetic biologists:

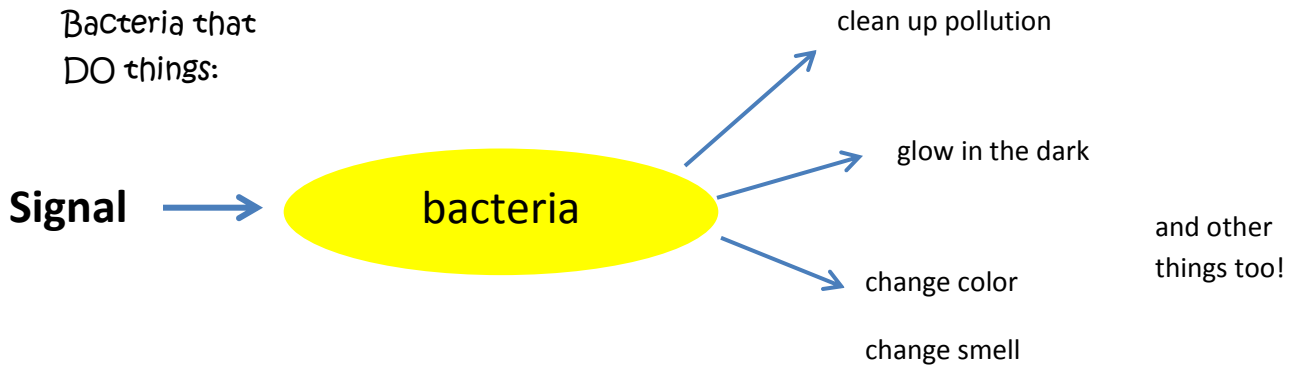
For example, if you give bacteria a certain type of food to eat, they can turn that food into things like medicine to cure diseases, they can make useful products like rubber or gasoline, and they can even generate electricity that you can use to run lights and computers. These are just some examples, and there are many more!

Sketch the following on the board as you describe the example above:



So what about some examples of things that bacteria could DO? Well, synthetic biologists have created a number of different bacteria that can do things that we want them to do. We can give them a signal (that tells them to "start") and they can do things like clean up pollution in the water or soil, glow in the dark, or change different colors when exposed to light (like film inside of old cameras!). The can even change the way they smell to make them smell like other things, like fruit!

Sketch the following on the board as you describe the examples above:



So, how do we get bacteria to do things we want them to do, or make things we want them to make?

In order to do this, we need to change the bacteria's DNA. Has anyone ever heard of DNA? (*show of hands*). Can anyone give a description of what DNA is? (*field responses*).

A good way to think of DNA is as an instruction manual. Let's say you got a bicycle for your birthday. That bicycle probably comes in a box that has a whole lot of pieces – big pieces and small pieces – and it also comes with some paper that has the instructions for putting the bicycle together in the right order so that it's safe to ride it. If you put the pieces together in the wrong order, it might fall apart while you are riding it and you could get hurt. So, it's really important to read the instructions carefully!

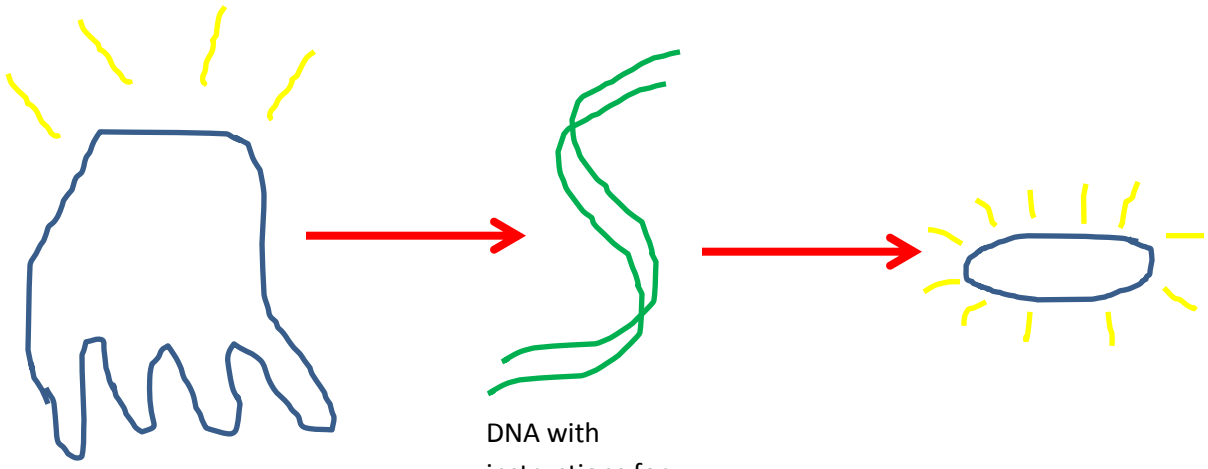
DNA is like the instructions that a cell uses to build an organism in the right order.

Every living thing on earth has DNA in its cells. And every living thing has a slightly different version of DNA that provides the instructions on how to build that organism. Human cells have human DNA that provides the instructions for making a human being. Bacteria cells have bacteria DNA that provides instructions for making a bacteria cell.

Since all living cells have DNA, we can sometimes move DNA between different organisms to get them to do things we want them to do. The glow-in-the-dark bacteria are a great example. Have you ever seen those jellyfish at the aquarium that glow when you shine a special purple light on them? Or the scary fish in "Finding Nemo" that has the flashlight on the end of its tentacle that it uses to lure in smaller fish? Many types of sea creatures have DNA that allows them to glow in the dark.

So, if we want bacteria to glow in the dark, synthetic biologists can work in the lab to take out the part of the DNA from the jellyfish that has the instructions for glowing in the dark, and move that piece of DNA into the bacteria so that the bacteria can glow in the dark also! Pretty cool, huh?

Sketch a jellyfish and arrow to a bacterium on the board as you describe the examples above (something similar to below):



In today's workshop, we will do three activities to learn about synthetic biology:

First, we will take a closer look at what DNA is, and how it works like a secret code that contains the instructions for building a cell. You will be building your own model of a DNA molecule, and then decoding it with your friends!

Second, we will see an example of a method synthetic biologists use to help them transfer DNA between organisms in the lab. You will try using the equipment yourselves, and will take a part of the experiment home with you!

Third, we will see how bacteria can DO something useful for us using synthetic biology. We have created some bacteria that can sense pollution in the water and can warn us that water is not safe to drink by producing different smells. You will smell the bacteria samples and try to figure out what type of pollution is in the water!

Activity 1: DNA Paper Puzzle

Ahead of Time Preparation:

1. *Print and cut out the DNA pieces from the attached PDF (DNA Pieces.pdf.) You may need to print different numbers of each page, as there is different numbers of pieces on each page (28 S, 42 P, 32 A, 32 T, 28 C, 32 G). There should be 6 of each piece for each child if each child is to make a standard 3 base-pair codon), so print and cut out the appropriate number of pieces. Using different colors for each type of piece makes the model a lot more interesting for the kids.*
2. *Make sure you have lots of scotch tape.*
3. *Create a template codon of DNA using the desired number of base pairs. This will be the same thing the kids will build and serve as an example to guide them. It should look something like this:*

Day of:

Pass out a set of 6 S pieces and 6 P pieces to each seat so they will be there when the children sit down. Make a pile of pieces for each type of nucleotide in the middle of the table.

DNA is often referred to as "the code of life". Today you will build a model of DNA using paper and we will show you how it translates into life. You may have seen a picture of DNA before, in a shape called a double helix. It is usually shown as a ladder that is twisted around the middle. The model we have today is still a ladder shape, but is not twisted.

Hold up the template so all of the kids can see.

All DNA is made out of two parts: the inside that holds the information and the outside that holds the inside together, called a backbone. The backbone is made of a special kind of sugar (S) and the parts that keep them held together (P) are called phosphate groups, but you don't really need to know that right now. It is easiest to start by building this backbone. There should be 3 S's and 3 P's on each side. Make sure that one side goes up and the other side goes down.

At this time, the kids should assemble their backbones (S and P) using scotch tape. Use this time to help the kids and answer questions.

Okay, now that you have your backbones ready, it's time to fill it with code. The inside of DNA is made of things called nucleotides, which can be combined in any way as long as the A's and T's go together and the C's and G's go together. As you can see, the both pairs are cut so they only match up with each other. Your backbone has room for 3 pairs, so now you can pick whatever you want and tape them in.

Again, use this time to assist the kids and answer questions. This should take a lot less time than the first construction step.

Now, the reason we had you make a piece of DNA that's 3 pairs long is because that's how the bacteria 'reads' the genetic code-- it splits the DNA in half down the middle and reads it in threes. What you just made is called a 'codon', and it's like a word in DNA-language. Depending on what they 'say', these

codons can be used like instructions to make different pieces of proteins, which is what cells are made of. By combining thousands and thousands of codons, DNA makes up huge set of instructions that tell cells how to make their own parts and do certain things, or even build a whole body like yours.

Optional:

Print out a DNA Codon Table and let the kids look up what they 'spelled' with their chosen base pairs.

Activity 2: Rainbow Gel

Ahead of Time Preparation:

1. *Create the different mixtures of food coloring. See Appendix 2 for protocol.*
2. *Create the number of practice gels needed for your group. See Appendix 2 for protocol.*
3. *Create the number of rainbow gels needed for your group and set up the gel electrophoresis chamber. See Appendix 2 for protocol.*

Day of:

Make sure when bringing the group into the lab, everyone has on proper safety attire (long pants, closed-toed shoes, gloves, and safety goggles.) If not, allow them some time to put the proper attire on, and explain to them that we are protecting ourselves and it is important to be safe when working in a lab.

Hey everyone! Remember the example about getting the piece of DNA from the jellyfish so we could make bacteria glow? Well in order to do that in the lab, we use a process called gel electrophoresis to separate out the piece of DNA that we are looking for. We will tell you all about that later, but first, have any of you seen or used a micropipette before?

Hold up micropipette you intend to use. Likely response will be no.

Don't worry if you haven't seen a micropipette before, we are going to teach you about them and how to use them too! Micropipettes are a tool that we use in lab to move very small volumes of liquid.

Depending on your audience, you may want to go into more detail, about how different pipettes have different ranges of liquid that they are capable of dispensing. For our audience (5^{th}-7^{th} graders), just the basic information was sufficient.

Ideally, at this point, you would hand each girl their own pipette so they could practice with you while you demonstrate. However, we had a limited number of pipettes and only demonstrated to them and then helped them individually when they actually used the pipettes later.

This is how you properly hold a micropipette (*show them where to place their hands).* To take up the liquid and to push it out, we push this button here on top using our thumbs (*push the button on top).* In order to use the pipettes, we first need to put a tip on them. (*Demonstrate how to put a tip on the pipette).* Then, we push down on the button until we feel it come to its first stop. (*This concept seemed confusing to the girls, a lot of them didn't really feel the first point of resistance, so it is important to make sure that they all understand what you are referring to).* Then, we place our tip into the liquid and slowly release the button. (*Demonstrate*) Once the button has been completely released, we can pull the pipette out of the sample of liquid and move it where we want.

Today, we are going to be loading our liquid samples, which are different mixtures of food coloring, into the gel. We made some food coloring samples for everyone to use ahead of time (*hand each person their sample of mixed food coloring)*. At the next station, you will practice the technique of loading your

sample into a well using a practice gel. (*Point the students towards the next station where the practice gels are waiting with pipettes and tips.)*

Now we are going to practice loading our samples into a gel. (*Ideally, you should have 2 practice gels being used at a time, and each practice gel should be supervised by an instructor. Take the first two volunteers to their respective practice gels.)* Just like before, we are going to push the button down, lower the tip into our food color sample and slowly release the button. (*Provide help if necessary)* Now, you should pull the pipette out of food coloring and move your sample to a well. (*Using a pipette on hand, show them how it is done first).* When loading the well you want to put your pipette tip over the middle of an empty well and slowly lower the tip so it is just barely breaks the surface of the water. (*The girls may have trouble seeing the wells; it is helpful to guide the tip just a little so they aren't missing. Some of the girls may also stab the gel too forcefully, so stress that the tip should barely hover over the well.)* Once your tip is over the well, push gently on the button on the pipette and the liquid will fall into the well. Then, gently remove the pipette. (*Have them practice loading 2 wells before moving on*, *sometimes more practice is needed, but just guide them along.)* Good! Now that you have practiced loading we are going to have you try loading your sample into a real gel! (*Move those that have practiced to the real gel).*

At the actual gel, the person helping the children load the samples should be prepared to guide the tip of the pipette to the correct well. It is important for the samples to be loaded well so that the girls can see what colors were in their mystery mixture and so they can bring home their own lane. Only 2uL per well is necessary for good results, and have the girls skip lanes between their samples so it is easier to cut out their lanes at the end of the activity. If one person is unable to correctly load their sample, just do it for them afterwards so they can still have something to bring home.

Now we are ready to load our samples in the real gel. I will help you just a little because this part can be tricky. (*Children load sample while you help make sure the pipette tip is in the well)* Excellent! Now we can run the gel and see what happens! (According to the protocol provided in Appendix 2, run the gel at 180V for 8-10 minutes).

While the gel is running answer any questions the children have so far and then explain to them what occurs during gel electrophoresis. Keep in mind, the information you provide in the next section is dependent on the age group of your audience.

Do you want to know what is happening to your food coloring samples in the gel right now? Okay! First, let's talk about what the gel is made of. The gel is made of a seaweed extract, called agarose, and water. Once it's made, it has a texture kind of like jello, but don't worry you will be able to feel it when you take your piece home with you! In the gel, there are a bunch of little holes, of different sizes. It helps to think of the gel like a maze of holes. We then turn electricity on and run it through the gel, which causes the molecules in the samples (in this case food coloring) to move towards the far end of the gel. However, they can only move through the maze of holes in front of them. So, the small molecules are tiny and can fit through the holes easily, which means they can move fast. The bigger molecules are too large, and they can't fit through all the tiny holes as easily. So, they move a lot slower than the tiny little molecules.

Right now, your food coloring samples have molecules of all different sizes, because the different colors are made of different sized molecules. So, the smaller molecules of one color are moving faster than the larger ones of another color. When the gel is done running, there will be bands of the different colors on the gel, because some colors move faster than others. (*Ask if there are questions so far, and if so expand explanations accordingly. Let the children look at the gel to see how their colors have started to separate)* Isn't that cool, your colors have already started to separate!

That is how we separate the bigger pieces from the smaller ones! Normally, we use gel electrophoresis for DNA. Do you want to see an example of a gel that we ran using some samples of DNA? (*Show blown up picture of gel, found in Appendix 3, or use your own gel example.)* As you can see, there are these bright bands, which are from the DNA. This lane all the way on the left, it is called a ladder. It acts like a ruler so we can tell the sizes of the pieces of DNA that we are testing. Can any of you guess which one of these bands is the smallest? (*Wait for response, and help if necessary.)* Can any of you guess which of these bands is the largest? (*Wait for response, and help if necessary.)* If we wanted to get the piece of DNA from the jellyfish that makes it glow, we would just need to know what size of DNA we were looking for. Then, we can cut out that piece of DNA from the gel and use it!

Your gel should be done now, so let's cut out your piece and put it into a bag so you can guess what colors are in your mystery food coloring mixture and then take it home with you! (*Cut up each person's lane and then put it into a bag and give it to the person. Make sure the bags are labeled with a warning label, similar to that shown in Appendix 4. Have the four original vials of food coloring available, with labels so the children know what color choices they have to choose from. Have each child guess what colors they have, and then explain to them whether they were right or wrong and explain why. The red food coloring we used separated into two different reds, so make sure to explain that to the children.)*

We hoped you all enjoyed using gel electrophoresis to find what colors were in your mystery food coloring sample! Next, you are going to another lab to see an example of what bacteria can do when we alter it using synthetic biology! Thanks!

Activity 3: Smell Test

Bacteria can be engineered to produce certain compounds in response to pollutants in water. If the compounds that are produced have distinct smells, pollution can be detected easily. For this activity, you will use two strains of bacteria. One strain will produce a banana odor if the water is contaminated with heavy metals such as arsenic or cadmium. The other strain will produce a wintergreen smell if the water is contaminated with antibiotics such as tetracycline. When heavy metals and antibiotics are not present in the water, the bacteria will be able to produce a banana or wintergreen odor. Thus, safe water will smell stinky like a plain bacterial culture.

An in depth explanation of the two bacterial strains and two different sniff test protocols can be seen in Appendix 2.

In this activity, there are water samples from Muddy Pond, Logan Lake, Crayfish Creek, and Rabbit River. There are two tubes for each of the four bodies of water. Each of the tubes contains the two strains of bacteria that are able to produce the banana odor and the wintergreen odor. In addition to the sample tubes, there are also six control tubes. The six control tubes are made up of two tubes that have plain, stinky bacteria, two tubes with bacteria that smell like bananas, and two tubes with the bacteria that smell like wintergreen. It is your job to determine what the four bodies of water are polluted with by sniffing the samples. You can refer to the control tubes at any time if you are having trouble determining what a particular water sample smells like. Good luck and remember to fill out the worksheet as you go!

Before the students begin the sniff test, talk about lab safety. Demonstrate wafting to assure that no one stick his/her nose directly into the sample and tell students that it is unacceptable to drink the samples. Also, make it clear that the sniffing the samples will not harm them because pathogens are dangerous pollutants are not being used in the experiment.

The worksheet corresponding to this activity can be found in Appendix 5.

Appendix 1: "Wouldn't it be cool if" Activity Worksheet

Wouldn't it be cool if there were bacteria that _____

_____

_____

_____

_____

Wouldn't it be cool if there were bacteria that _____

_____

_____

_____

_____

Wouldn't it be cool if there were bacteria that _____

_____

_____

_____

_____

Appendix 2: Rainbow Gel Protocols

Creating Mystery Food Coloring Samples

1. Gather as many 1.5mL Eppendorf's as you will need for your group.
2. Label each tube and record which colors you put in each, creating a key that shows which tube has which colors (this will be useful when the children later try to guess what their colors were).
3. Drop a few drops of the desired colors into each tube. If your colors are not in a container with a dropper, just micropipette ~50 uL of each color into the tubes.
4. Add ~ 50 uL of 50% glycerol, to assist with the loading of the colors into the gel.
5. Vortex the samples to ensure the colors and the glycerol are thoroughly mixed.

Preparing the Practice Gels

1. Measure out 50mL of water using a graduated cylinder.
2. Weigh out 0.5g of agarose.
3. Add the water and agarose to a small flask and swirl gently.
4. Microwave the solution for 60 seconds.
5. Carefully remove the flask (using heat resistant gloves) and swirl.
    - If agarose has not completely dissolved, microwave for an additional 20 seconds and swirl.
6. Using aluminum foil, make 2 rolls of equal thickness to use as holding racks for the combs.
7. Obtain a petri dish.
8. Obtain three 8-well combs and adjust to the correct depth for your petri dish.
9. Once the solution has cooled enough to pour, pour the solution into the petri dish and place the aluminum holders on the edge and rest the combs on top of them, with enough space in between the combs to allow room for the wells.
10. Once the gel solidifies (it will become slightly opaque) remove the combs, remembering to pull straight up so as to not damage the wells
11. Repeat for the appropriate number of gels for your group.

Preparing the Rainbow Gels

This procedure produces gels with 16 wells which are appropriate for groups no larger than 8.

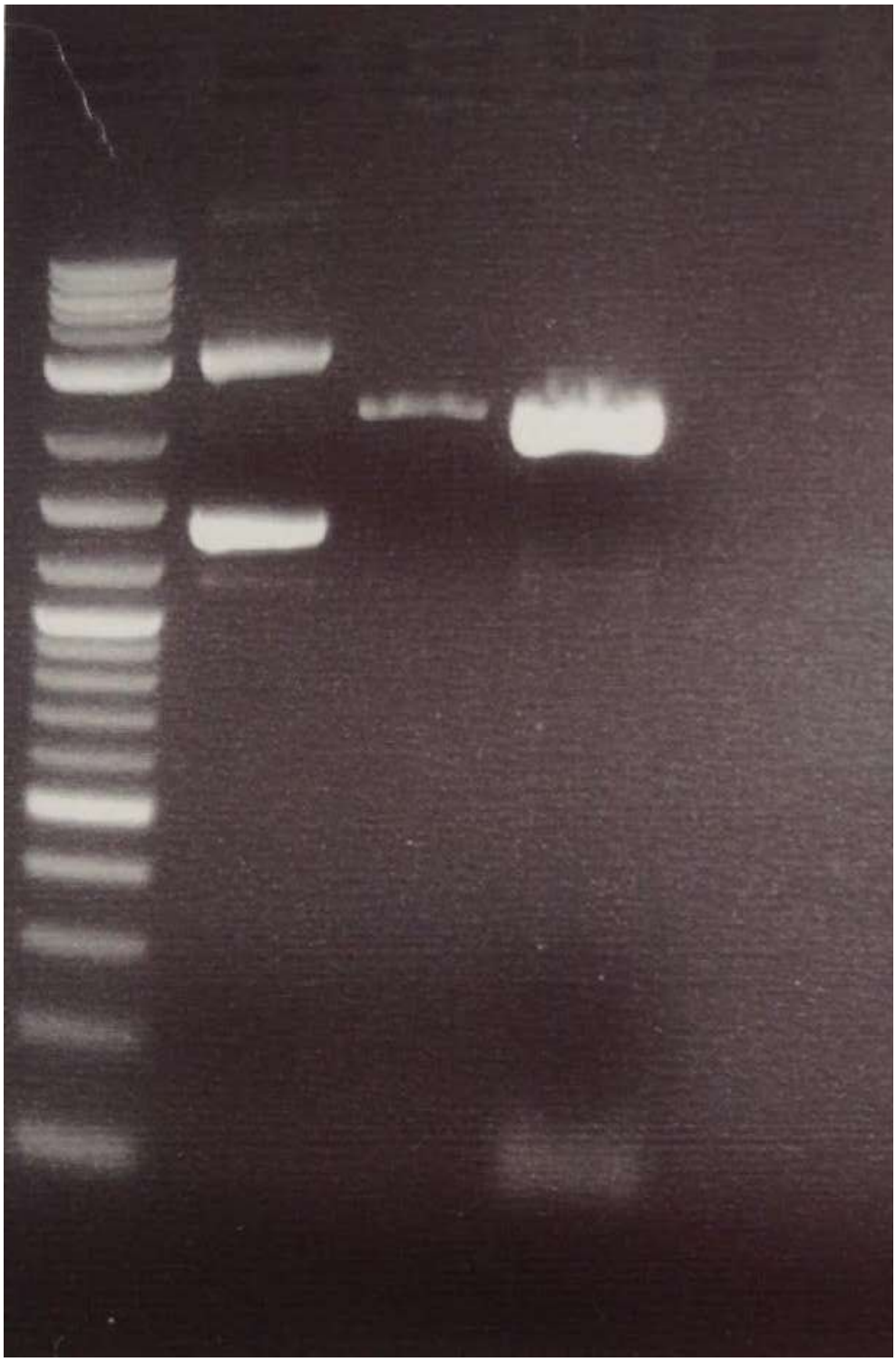
1. Measure out 50mL of water using a graduated cylinder.
2. Weigh out 0.5g of agarose.
3. Add the water and agarose to a small flask and swirl gently.
4. Microwave the solution for 60 seconds.
5. Carefully remove the flask (using heat resistant gloves) and swirl.
    - If agarose has not completely dissolved, microwave for an additional 20 seconds and swirl.
6. Using either tape or a clamp prepare the gel plate.

7. Obtain two 8-well combs.
8. Once the solution has cooled enough to pour, pour the solution into the prepared gel plate.
9. Place one 8-well comb about 1cm from the end of the gel and the second near the middle of the gel.
10. Once the gel solidifies (it will become slightly opaque) remove the combs, remembering to pull straight up so as to not damage the wells.
11. Repeat for the appropriate number of gels for your group.

Running the Rainbow Gel

1. Clean out the gel electrophoresis chamber with ethanol.
2. Place your rainbow gel into the chamber.
3. Fill the chamber with water so it covers the surface of the gel.
4. Have the children load their wells with their colors, making sure to skip wells between each sample.
5. Run the gel at 180V for 8-10 minutes.

Appendix 3: Example Gel



Appendix 4: Warning Label for Gel Bags

DO NOT EAT! Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash
DO NOT EAT! Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash
DO NOT EAT! Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash
DO NOT EAT! Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash
DO NOT EAT! Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash
DO NOT EAT! Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash	**DO NOT EAT!** Non-toxic; made from seaweed extract Discard in the normal trash

Appendix 5: Smell Test Protocols

****The full smell test protocol requires 3 days of preparation, whereas the shortened protocol only requires one overnight culture growing period.*

Full Protocol:

1. Using the Biobricks K1423007 and J45181 from the iGEM Parts Registry, perform two separate transformations according to the iGEM transformation protocol. The *E. coli* transformed with Biobrick K1423007 will convert isoamyl alcohol to isoamyl acetate in the presence of arsenic or cadmium, thus producing a banana odor. The *E. coli* transformed with Biobrick J45181 will convert salicylic acid to methyl salicylate, thus producing a wintergreen odor.

2. Prepare liquid cultures of the two new strains of *E. coli* by picking part of one colony off each plate and putting it in 25 mL of LB. Add 25 uL of the appropriate antibiotic to each liquid culture to prevent other bacteria from growing. (K1423007=chloramphenicol and J45181=ampicillin+kanamycin) Prepare an additional 25 mL culture of plain, non-transformed *E. coli* without any antibiotics. Allow the liquid cultures to grow in a 37---$^{=}$C shaker overnight for 16-20 hours.

3. In the morning measure the initial OD of the liquid cultures using a spectrophotometer. Dilute each liquid culture to an OD of 0.2 using liquid LB.

4. Obtain 14 glass test tubes. Number them 1-14 and add liquid cultures or LB to the test tubes according to the list below.

    1+2. 5 mL of the K1423007 culture

    3+4. 5 mL of the J45181 culture

    5-8. 5mL of the *E. coli* culture

    9+10: 2.5 mL of the K1423007 culture + 2.5 mL of the J45181 culture

    11-14: 5mL of LB

5. Add 5mM isoamyl alcohol and 10 uM cadmium nitrate tetrahydrate to tubes 1,2,9, and 10.

6. Add 2mM salicylic acid to tubes 3,4,9, and 10.

7. Add 5mM isoamyl acetate to tubes 11 and 12 and 5mM methyl salicylate to tubes 13 and 14. These four tubes, along with tubes 7 and 8, will act as controls during the sniff test.

8. Place tubes in a 37^{o}C shaker for 24 hours.

9. Label 14 plastic 15 mL conical tubes according to the list below:

    2 x Muddy Pond

    2 x Logan Lake

    2 x Crayfish Creek

    2 x Rabbit River

    2 x Stinky

    2 x Banana

    2 x Minty

10. Transfer the liquid in test tubes 1 and 2 to the Muddy Pond conical tubes.

11. Transfer the liquid in test tubes 3 and 4 to the Logan Lake conical tubes.

12. Transfer the liquid in test tubes 5 and 6 to the Crayfish Creek conical tubes.

13. Transfer the liquid in test tubes 9 and 10 to the Rabbit River conical tubes.

14. Transfer the liquid in test tubes 7 and 8 to the Stinky conical tubes.

15. Transfer the liquid in test tubes 11 and 12 to the Banana conical tubes.

16. Transfer the liquid in test tubes 13 and 14 to the Minty conical tubes.

17. Split up the conical tubes into 2 racks such that each rack has one of each tube.

18. Start the sniff test!

Shortened Protocol:

1. Grow a 50 mL overnight *E. coli* culture in an Erlenmeyer flask.

2. To make a 1:5 dilution, add 200 mL of LB to the overnight culture.

3. Label the 14 plastic 15 mL conical tubes.

    2 x Muddy Pond

    2 x Logan Lake

    2 x Crayfish Creek

2 x Rabbit River

2 x Stinky

2 x Banana

2 x Minty

4. Add 10 mL of the diluted liquid culture to each of the conical tubes.

5. In a fume hood, add 5.4 uL of isoamyl alcohol (5mM) to the 2 banana control tubes as well as the tubes containing water samples with "arsenic contamination."

6. In a fume hood, add 7.2 uL of methyl salicylate (5mM) to the 2 minty control tubes as well as the tubes containing water samples with "antibiotic contamination."

7. Split the samples and controls evenly among two racks and place each rack on a separate lab bench.

If there are more than 8 students, the sniff test may run more efficiently if another set of controls and sample tubes are prepared.

Appendix 6: Smell Test Worksheet

You are a synthetic biologist, and you have used your knowledge about DNA to create two types of bacteria:

Bacteria A can sense if there is arsenic in the water. When arsenic is present and bacteria are grown in that water, the bacteria will give off a smell of bananas.

Bacteria B can detect various types of medicines in the water. When medicines are present and bacteria are grown in that water, the bacteria will give off a minty smell.

Normally bacteria smell somewhat stinky, like rotten eggs. If there is no contamination in the water, the sample will be stinky.

Smell the "Control Sample" tubes using the wafting technique that your instructors have demonstrated. Then smell each of the four water sample tubes, and see if you can tell which water sources are contaminated, and which are safe to drink!

Remember: Stinky smell = clean water

Fruity/banana smell = arsenic pollution

Minty smell = medicine pollution

What's in the water? (Smell each sample and circle your response)

Muddy Pond:	safe to drink!	arsenic!	medicine!	both!
Logan Lake:	safe to drink!	arsenic!	medicine!	both!
Crayfish Creek:	safe to drink!	arsenic!	medicine!	both!
Rabbit River:	safe to drink!	arsenic!	medicine!	both!

Note: No real arsenic was used in this experiment