How do we isolate DNA from the microorganisms that live in the soil?

There are a number of different methods to use. Some methods separate the living cells away from the soil first and then use methods to break open (lyse) the cells. Other methods lyse the cells while the soil is still present. In either case, once the cells are broken open, a centrifugation step then pellets all of the cell debris, leaving molecules like proteins and nucleic acids in solution (referred to as the supernatant). This solution is then processed further to separate all of the proteins away from the nucleic acids, leaving only DNA and RNA in the supernatant. There are enzymes that can then be added to the nucleic acid solution that will remove the RNA, so that the solution only contains DNA. The kit that you will use in this lab uses a tiny filter to which only DNA adheres, and all of the remaining molecules pass through the filter and are discarded. The filter is washed and then the DNA is removed (eluted) from the filter by a specific buffer.

Methods

1. Find the bag with the sample of soil that you placed on your nutrient agar plate last week. Tilt the bag so that water drains to one side. Then take a sample out of the drier side. Weigh out 0.8 g of the soil on a 3X5 card using a toothpick to help slide small amount off of your spatula.

Put a bead solution tube in your cute little microcentrifuge tube rack. Using a marking pen, label the cap you’re your group’s code. Place the soil in the bead solution tube that has been labeled with your group’s code. Wipe the toothpick and use it to slide small bits of soil off your card and into the tube.

The bead solution is a buffer that will split up the soil particles and begin to remove the humic acids from your sample. Completely decayed organic material is called humus, because it is filled with many carbon-containing molecules called humic acids.

2. With the cap screwed on the tube, mix by finger-vortexing (flicking the side of the tube with your finger for 30 seconds. If the cap is on tight, you may shake the tube to try to break put the soil clods. This will thoroughly mix your sample with the bead solution.

3. Check to see if solution S1 is a clear liquid. If not, see your instructor about what to do next. If the solution is clear with no white material in it, then set your micropipettor to 60 use a clean tip and measure 60 μl of S1. Add this volume to your bead tube, screw on the cap and finger vortex again for 30 seconds to mix. Discard pipette tip into the refuse beaker.

Solution S1 contains a detergent called SDS (sodium dodecyl sulfate). This detergent helps to lyse the cells by breaking down the phospholipid bilayer of the cell membrane.
4. Set your micropipettor to 200, use a clean tip, and measure 200\(\mu l\) of the solution labeled IRS (Inhibitor Removal Solution). Add to your bead tube and cap tightly. Discard the pipette tip.

This solution removes humic acids and other compounds present in the soil that might interfere with Polymerase Chain Reaction (PCR). We will be using your DNA in a PCR reaction next week.

5. Make sure that your bead tube is labeled with your group code and tightly capped. The next step will involve vortexing using a special vortex machine. This step lasts for 10 minutes; so attach your tube and wait for other groups to attach their tubes before starting the machine. While waiting for other and for the vortexing to finish, READ AHEAD.

This step is the second way that the cells will be lysed. By shaking the beads they collide with the cells and break them open. This isolation method uses chemical lysis (SDS) and mechanical lysis.

6. After 10 minutes of vortexing, you will now centrifuge the tubes at a speed of 10,000 rpm. Centrifuges must be balanced when they spin. Your tube should weigh precisely the same as any other group’s tube because you have added the same amount of soil and reagents to it. That means that the tubes will be balanced with each other. What would you need to do if you only had one tube? ________________________________

How would you centrifuge three tubes at one time? ____________________________________________

You will centrifuge your tubes for 1 minute. This centrifugation step will remove the soil, the humic acids, the beads, and the cell debris by making a pellet at the bottom of the tube. Your DNA will be left in solution above the pellet, along with other molecules like RNA and proteins. The solution is referred to as the supernatant.

7. Set your micropipettor to 200, get a clean tip, and measure 200\(\mu l\) of the supernatant, and transfer the supernatant to a clean microcentrifuge tube. Your tube should have about 450 \(\mu l\) of supernatant in it. Transfer all of that to the new labeled microcentrifuge tube, but be careful not to put the tip in the pellet or transfer the pellet into the new tube. Discard your bead tube.

8. Use the 1000 (blue) micropipettor, set it to 250, get a clean tip, and measure 250\(\mu l\) of Solution S2. Add it to your to the tube with the supernatant. Discard the pipette tip. Cap the tube and finger vortex for 30 seconds.

S2 contains a reagent that will precipitate the proteins from the solution.

9. Make sure caps of your tube is firmly sealed. Place the tube in the microcentrifuge tube rack (at 4\(^\circ\) C) and incubate at 4\(^\circ\) C for 5 minutes. Centrifuge your tube for 1 minute at 10,000 rpm.
10. Set your micropipettor to 200. Hold your tube against a white surface to find the pellet. Get a clean tip, and transfer as much of the supernatant as possible to a clean (labeled) microcentrifuge tube. Do NOT place the tip in the pellet or remove the pellet. You will transfer about 600 to 700 μl of supernatant.

You are removing your DNA from the pellet containing additional humic acids, soil, and the proteins from the cells.

11. Set the 1000 (blue) micropipettor to 650 μl and add a total of 1300 μl of Solution S3 to the supernatant. Be careful as this volume will be the maximum that you can place in your tube. Close the cap tightly and finger vortex for 30 seconds.

Solution S3 has salts in it that will interact with the DNA and allow it to bind to the filter.

12. Get a spin filter from your instructor and place it in a clean (labeled) tube. Set your blue micropipettor to 700 and withdraw 700 μl from your supernatant and pipet it into the spin filter tube. Do NOT touch the spin filter with your micropipette tip. Close the cap and centrifuge at 10,000 rpm for 1 minute. Remember that the centrifuge must be balanced before it “takes off”. Pour off the liquid flow-through as demonstrated by your instructor. Repeat this step until you have processed all of the supernatant. This will mean doing this three times, pouring off the liquid each time.

This step takes the DNA out of solution and attaches it to the membrane because of the salts present in S3. All of the other molecules move through as liquid flow through. This includes RNA.

13. Set your blue micropipettor to 300, use a clean tip, and measure 300 μl of solution S4. Add this to the spin column and centrifuge for 1 minute at 10,000 rpm. Discard the pipette tip.

This solution contains ethanol and is like a wash or rinse solution that cleans the DNA. It does not remove the DNA from the filter.

14. Discard the flow-through from the tube after the centrifugation.

15. Centrifuge your tube again for 1 minute to remove all traces of the wash solution.

16. Take out a new microcentrifuge tube, label it, and place the spin filter in it. Set your micropipettor to 50 μl, get a clean tip, and measure 50 μl of Solution S5 to the filter. Do not touch the filter with your tip. Aim the tip at the middle of the filter so that all the liquid goes onto the filter, not the sides of the tube. Allow the tube to sit in the microcentrifuge rack for 3 minutes.

S5 is the buffer that will remove the DNA from the filter (it’s called the elution buffer).

17. Centrifuge your tube at 10,000 rpm for 1 minute.
18. Discard the spin filter. You have a tube with _____ µl of solution with DNA. This DNA corresponds to the chromosomes from all of the microorganisms present in your soil sample. In order to visualize the DNA, we will do a procedure called agarose gel electrophoresis.

What is agarose and what is gel electrophoresis?

Agarose is a substance that forms a solid matrix with pores or spaces in it that allow molecules such as proteins, RNA and DNA to move through it. The agarose comes as a powder and is mixed with a buffer solution. The buffer solution has ions in it that have the potential to carry a charge and thereby allow an electric current to move through the gel. The buffer and the agarose are heated to mix thoroughly and then allowed to cool to form a solid gel. We apply a device called a comb to make spaces in the solid gel to hold our DNA samples. The DNA needs to stay in the space (called a well) until we are ready to apply an electric current. We accomplish this by mixing our DNA with a set of dyes (to allow you to visualize the samples in the well and as they electrophorese and a heavy sugar solution that holds our sample in the well. The electrophoresis apparatus has the same buffer in it as the buffer that was used to make the gel. That means an equivalent potential to carry charge through out the electrophoresis apparatus. We apply an electric current using a power supply (normally for agarose gels we measure in terms of volts applied). DNA has an overall negative charge and it will move toward a positive pole for that reason. Our samples are loaded in such a way that the DNA will move through the holes in the gel in that direction. In addition to each of your DNA samples we will place a DNA marker in one lane of the gel. This marker DNA is a control for our electrophoresis. Even if none of us has been successful in our DNA isolation today, this DNA should electrophorese, and show us that the electrophoresis was successful.

After listening to instructions regarding the gel and the electrophoresis, you will select someone to load your sample on the gel. This person is now ready to practice loading the gel just with the loading dye after seeing a demonstration from your professor. While they practice, you should:

19. Set a micropipetter to 5µl and remove 5µl of your solution. Cap the tube tightly. We will store it in the freezer for use in next week’s lab.

20. Take your pipetter with the 5µl of your DNA solution to the side counter and add it to the bubble of loading dye provided by your instructor.

21. Make sure that you are loading your sample into the correct lane as designated on the board.

Electrophoresis will move the chromosomal DNA through the agarose matrix in the direction of the positive electrode. DNA as a molecule is invisible. We have added a stain to the gel that will allow us to see the DNA with the use of a ultraviolet illuminator. Electrophoresis of large pieces of DNA such as chromosomes takes quite some time. We will photograph the gel and have a picture of it ready for next lab.