Molecular Methods in Microbial Ecology

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Tuesday  10/25/11  Introduction, Extraction of DNA from Winogradsky columns
                     Run DNA products on gel

Thursday  10/27/11  Lecture on PCR, Prepare PCR reactions

Tuesday  11/01/11  Analyze PCR results, Lecture on other molecular methods

Reading


- MoBio UltraClean Soil DNA Kit Extraction protocol

Day 1: Introduction, DNA Extraction, and Run DNA products on gel

We’ll sample sediment from your Winogradsky columns and extract the genomic DNA from all of the biological organisms in the sample. To do this, we’ll use a common DNA extraction kit that lyses the cells by mechanical “bead beating” (exactly what it sounds like) and further goes on to separate the nucleic acids from the other cell constituents using a spin column. Look at your GC results and think about the genes we are going to try to amplify (methanogenesis, sulfate reduction, methane oxidation) to guide your decision on where in the column you want to sample from. For example, did you have a large peak of methane somewhere? Perhaps you could choose that to target methanogens.

1. Pick a depth section of your Winogradsky column.

2. Remove ~1.0 gram of sediment from your chosen depth section with a 5 ml pipet and put it into a labeled microcentrifuge tube (between the 0.1 and 0.5 ml marks).

3. Spin for 2 min at 8,000 x g. Carefully remove the surface water from the sample via pouring.

4. Transfer the remaining sediment to the MoBio Extraction tube containing the bead solution (labeled) using a spatula.

5. Follow MoBio UltraClean Soil DNA Kit Extraction protocol.
MoBio UltraClean Soil DNA Kit Extraction protocol

Please wear gloves at all times!

1. To the 2ml **Bead Solution** tubes provided, add 0.25 - 1gm of soil sample (you already did this).

2. Gently vortex to mix.

3. Check **Solution S1**. If precipitated, heat solution to 60°C until dissolved before use.

4. Add 60μl of **Solution S1** and invert several times or vortex briefly.

5. Add 200μl of **Solution IRS** (Inhibitor Removal Solution).

6. Secure bead tubes horizontally on a flat-bed vortex and secure with electrical tape. Vortex at maximum speed for 10 minutes. Make sure your vortexer doesn’t walk away!

7. Centrifuge tubes at 10,000 x g for 30 seconds. DO NOT EXCEED THIS SPEED.

8. Transfer the supernatant to a clean 2 ml collection tube (label). Expect between 400 to 450μl of supernatant. Supernatant may still contain some particles.

9. Add 250μl of **Solution S2** and vortex for 5 sec. Incubate 4°C for 5 min (in the fridge).

10. Centrifuge the tubes for 1 minute at 10,000 x g.

11. Avoiding the pellet, transfer entire volume of supernatant to a clean 2 ml collection tube (label).

12. Shake to mix **Solution S3** before use. Add 1.3 ml of **Solution S3** to the supernatant and vortex for 5 seconds. The high volume of solution will touch the rim- be careful!
13. Load approximately 700μl onto a spin filter (label) and centrifuge at 10,000 x g for 1 minute.

14. Discard the flow through and add the remaining supernatant to the spin filter and centrifuge at 10,000 x g for 1 minute. Repeat until all supernatant has passed through the spin filter.

15. Add 300μl of **Solution S4** and centrifuge for 30 seconds at 10,000 x g.

16. Discard the flow through.

17. Centrifuge again at 10,000 x g for 1 minute.

18. Carefully place spin filter in a new clean 2 ml tube (label). Avoid splashing any Solution S4 onto the spin filter.

19. Add 50μl of **Solution S5** to the center of the white filter membrane.

20. Centrifuge at 10,000 x g for 30 seconds.

21. Discard the spin filter. DNA in the tube is now ready for next steps. Load 10 ul into another (labeled) microcentrifuge tube for the gel, as described below. Remaining 40 µl goes in freezer for PCR on Thursday.
Day 1, Part II: Visualize DNA by Electrophoresis

When you extract DNA from a community of microbes, you will ideally have a representation of the genomic DNA from all of the microbes living in the sample community. What “good” genomic DNA looks like on a gel is a blobby smear – but a pretty specific kind of blobby smear: one that remains near the wells of the agarose gel and is darkest on top, lightest as it trails down the gel. This means the majority of the DNA is still in large, long strings and we didn’t shear it much in the extraction process. You MUST wear gloves at all times during this procedure.

1. Add 5µl of 6x loading buffer to your tube with 10 µl of your DNA. The loading buffer contains glycerol to keep the DNA sample in the well, rather than floating up in the buffer, as well as a blue dye to make tracking the progress of the sample easier as it moves through the gel.

2. Load the sample DNA/dye mixture into a well of a 1% agarose gel made with a salt TAE buffer (Tris Acetic acid-EDTA).

3. The agarose gel also contains Ethidium Bromide, which intercalates into the DNA and fluoresces under UV light. This allows you to see your DNA within the gel. It is a carcinogen, so do not touch this with any exposed skin.

4. I will load 4µl of nucleic acid marker to the first well of the gel. This marker contains DNA fragments of known sizes and is a “ladder” (or ruler) against which you can compare your DNA to estimate size.

5. We’ll place the gel in a gel-running bed containing more TAE buffer. Cover goes on, wires are attached to power supply. When an electric current is run through the buffer (and hence through the porous gel), the negatively charged DNA will migrate from the negative (black) side towards the positive (red) side. If you were to run a gel long enough, the smaller DNA would eventually exit the gel and end up in the buffer on the positive side of the gel bed. Our gels will run at 100mV for ~45 minutes.