Biology 324 **Moyer/Leaf**
Winter 2000 **Lab #5 – Genomic DNA Isolation**

This laboratory is designed to provide you with the basics for generating genomic DNA from environmental samples to be used as the template for PCR. The following protocol has been provided by Mo Bio laboratories for use with their UltraClean Soil DNA Isolation Kit (Cat. # 12800-50).

Please wear gloves at all times!

1. To the 2 ml Bead Solution tubes provided, add 0.25 to 1.0 g of soil, sediment, or environmental sample.

2. Gently vortex to mix.

3. Add 60 µl of **Solution S1** and invert once to mix.

4. Secure tubes horizontally on a flat-bed pad with tape and vortex at maximum speed for 10 minutes. Alternatively, a bead beater may also be used. Note: If a bead beater is used, beat 1 minute at 3800 rpm (lowest setting) then on ice for 1 minute (repeat twice more). **Alternatively, after adding Solution S1, vortex 3-4 seconds, then heat to 70°C for 5 minutes. Vortex 3-4 seconds. Heat another 5 minutes. Vortex 3-4 seconds. This alternative procedure will reduce shearing but may reduce yield.**

5. Make sure the 2 ml tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 30 seconds.

6. Transfer the supernatant to a clean microcentrifuge tube (provided).
Note: With 0.25 g of soil and depending upon soil type, expect 400 to 450 µl of supernatant. Supernatant may still contain some soil particles.

7. Add 250 µl of **Solution S2**, Vortex 5 seconds. Incubate at 4°C (on ice) for 5 min.

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

9. Avoiding the pellet, transfer 450 µl of supernatant to a clean microcentrifuge tube (provided).

10. Add 900 µl of **Solution S3** to the supernatant and vortex 5 seconds.

11. Load about 700 µl into spin filter and centrifuge at 10,000 x g for 1 minute. Discard the flow through and add the remaining supernatant to the spin filter and centrifuge at 10,000 x g for 1 minute. NOTE: A total of two loads for each sample processed are required.

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

13. Discard the flow through.

14. Centrifuge again for 1 minute.

15. Carefully place spin filter in a new clean tube (provided). Avoid splashing any Solution S4 onto the spin filter.

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

17. Centrifuge 30 seconds.

18. Discard spin filter. DNA in the tube is now application ready. No further steps are required. We recommend storing DNA frozen (-20°C). Solution S5 contains no EDTA.

19. Size and estimate concentration of genomic DNA by running 5 to 10 µl of sample on a minigel with Hi-Lo markers of known concentration.

20. Proceed to PCR of specified gene fragment.