DNA Isolation Protocol

1) Get microcentrifuge tube and weigh it
2) Allow sample to thaw on ice
3) If the sample contains a high moisture content, it will be better to remove the water to obtain a higher DNA yield
4) Weigh about 0.5 g of sample in a microcentrifuge tube
5) Centrifuge at 10,000 g's for 1 minute
6) Remove supernatant
7) Reweigh microcentrifuge tube, obtain weight of pellet, ideally 0.25-0.3 grams.
8) Repeat steps 3-6 until sample pellet weight is 0.25-0.3 grams
9) Break up pellet at bottom of tube using a pipette tip or spatula
10) Add ALL contents of Bead Solution Tube to Microcentrifuge tube containing the sample
11) Gently vortex to mix.
12) **Check Solution S1.** If Solution S1 is precipitated, heat solution to 60°C until dissolved before use.
13) Add 60µl of Solution S1 and invert several times or vortex briefly.
14) Incubate at 70° C for 10 minutes
15) Add 200µl of Solution IRS (Inhibitor Removal Solution). Only required if DNA is to be used for PCR.
16) Secure bead tubes horizontally using the Mo Bio Vortex Adapter tube holder for the vortex (cat.13000-V1. Call 1-800-606-6246 for information) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See alternative lysis method for less DNA shearing).

17) Make sure the 2ml tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.

18) Transfer the supernatant to a clean microcentrifuge tube (provided).

19) **Note**: With 0.25gm of soil and depending upon soil type, expect between 400 to 450µl of supernatant. Supernatant may still contain some soil particles. Discard tube with soil pellet.

20) Add 250µl of Solution S2 to the supernatant and vortex for 5 sec. Incubate 4°C for 5 min.

21) Centrifuge the tube for 1 minute at 10,000 x *g*

22) Avoiding the pellet, transfer entire volume of supernatant to a clean microcentrifuge tube (provided).

23) Add 1.3 ml of Solution S3 to the supernatant (careful, volume touches rim of tube) and vortex for 5 seconds.

24) Load approximately 700µl onto a spin filter and centrifuge at 10,000 x *g* for 1 minute. Discard the flow through, 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. **Note**: A total of three loads for each sample processed is required.

25) Add 300µl of Solution S4 (ethanol) and centrifuge for 30 seconds at 10,000 x *g*

26) Discard the flow through.

27) Centrifuge again for 1 minute

28) Repeat steps 24-26 two times

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

30) Add 50µl of Solution S5 to the center of the white filter membrane.

31) Incubate at room temperature for 2 minutes

32) Centrifuge for 30 seconds

33) Discard the 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