Modified Experienced User Protocol (download complete protocol from http://www.mobio.com/pages/support-protocols.html)

Please wear gloves at all times

1. Add 15 ml of **PowerBead Solution** to a **PowerBead Tube**. **These tubes will now be referred to as PowerMax® Bead Solution Tubes.**
2. Add 1-2g of in liquid nitrogen homogenised mycelium (using a mortar) to **PowerMax® Bead Solution Tube**. Vortex briefly.
3. Check **Solution C1.** If **Solution C1** is precipitated, heat the solution at 60°C until the precipitate has dissolved. Add 1.2 ml of **Solution C1** to the **PowerMax® Bead Solution Tube** and vortex vigorously for 5 minutes.
4. Place the tubes in a water bath set at 65°C for 25 minutes and vortex every 5 minutes for 5 minutes.
5. Centrifuge tubes at 2500 x *g* for 3 minutes at room temperature.
6. Transfer supernatant to a clean **Collection Tube** (provided). The supernatant may still contain some
soil particles and color.
7. Add 5 ml of **Solution C2** and invert twice to mix. Incubate at 4°C for 10 minutes.
8. Centrifuge tubes at 2500 x *g* for 4 minutes at room temperature.
9. Avoiding pellet, transfer supernatant to a clean **Collection Tube** (provided).
10. Add 4 ml of **Solution C3** and invert twice to mix. Incubate at 4°C for 10 minutes.
11. Centrifuge tubes at 2500 x *g* for 4 minutes at room temperature.
12. Avoiding pellet, transfer supernatant to a clean **Collection Tube** (provided).
13. Shake to mix Solution C4. Add 30 ml of **Solution C4** to supernatant and invert twice.
14. This step requires three centrifugations. First, fill **Spin Filter** with solution from Step 13. Centrifuge at 2500 x *g* for 2 minutes at room temperature. Discard flow through and add second volume of supernatant to same **Spin Filter** and centrifuge at 2500 x *g* for 2 minutes at room temperature.
Discard flow through. Repeat until entire volume has been processed.
15. Add 10 ml of **Solution C5** to **Spin Filter** and centrifuge at 2500 x *g* for 3 minutes at room temperature. Discard flow through.
16. Centrifuge **Spin Filter** at 2500 x *g* for 5 minutes at room temperature.
17. Carefully place **Spin Filter** in a new **Collection Tube** (provided). Avoid splashing **Solution C5** onto **Spin Filter**.
18. Add 5 ml of sterile **Solution C6** to the center of **Spin Filter** membrane and centrifuge at 2500 x *g* for 3 minutes at room temperature.
19. Discard **Spin Filter**. The DNA in the tube is now ready for any downstream application.
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We recommend storing DNA frozen (-20°C). **Solution C6** does not contain EDTA.

Concentrating the DNA

The final volume of eluted DNA will be 5 ml. The DNA may be concentrated by adding 0.2 ml of 5M NaCl and inverting 3-5 times to mix. Next, add 10.4 ml of 100% cold ethanol and invert 3-5 times to mix. Centrifuge at 2500 x *g* for 30 minutes at room temperature. Decant all liquid. (If sterile DNA is desired, wash the DNA pellet with 70% cold ethanol. Be sure not to disturb the pellet.) Remove residual ethanol

in a speed vac, desiccator, or ambient air. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.

RNase treatment

Comment from Mo BIO technical support :

In our experience, there is really no need to perform an RNase digestion. We have compared qubit versus nanodrop readings using the PowerSoil chemistry and the readings are very similar, meaning nanodrop readings are not inflated by the presence of contaminating RNA when compared to qubit readings. Solution C4 used to bind, the nucleic acid to the silica is geared toward recovering high molecular weight DNA, so any RNA should also be mostly washed through.
If you feel that RNase is still necessary, you could include or introduce the RNase up front and add it in after the addition of the bead solution and then proceed with the protocol.