DNA Extraction
(with Qiagen *DNeasy Plant Kit*)

1. To dried mycelium, add:

   400 µl of AP1

   2 µl of Rnase A
2. Grind solution with power drill and vortex to mix.
3. Incubate the mixture for 10 min at 65ºC. Mix 2-3 time during incubation by inverting tube.
4. Add 130 µl of Buffer P3 to the lysate, mix and incubate for 5 min on ice.
5. Centrifuge the lysate for 5 min at 13,000 rpm.
6. Transfer clear liquid to PURPLE QIAshredder Mini Spin Column placed in a 1.5 ml microcentrifuge tube. (The cap may need to be cut off so the tube will fit in the centrifuge.) Centrifuge for 2 min at 13,000 rpm.
7. Transfer the flow through fraction from the above step to a new tube and discard the Purple Spin Column. There should be about 400 µl.
8. Add 1.5 the volumes of Buffer AW1 to the cleared lysate and mix by pipetting.

   Ex. 400 µl → 600 µl of AW1 buffer
9. Pipet 500 µl of the solution to the CLEAR Dneasy Mini Spin Column in a 1.5 ml microcentrifuge tube (with the cap cut off). Centrifuge for 1 min at 8000 rpm. Discard flow through.
10. Repeat Step 9 with remaining sample. Discard flow-through portion and collection tube.
11. Place the Clear Spin Column in a new 1.5 ml tube, add 500 µl of Buffer AW2 to the Spin Column. Centrifuge for 1 min at 8000 rpm.
12. Discard flow-through, add 500 µl of Buffer AW2 to Column and centrifuge for 2 min at 13,000 rpm. Centrifuge again for 2 min at 13,000 rpm to make sure there is no alcohol residual.
13. Transfer the Spin Column to a new 1.5 ml microcentrifuge tube (to be used to store the DNA, so label the cap), pipet 50 µl of Buffer AE directly into the DNeasy membrane.
14. Incubate for 5 min at room temperature and then centrifuge for 2 min at 13,000 rpm.