Qiagen DNeasy Plant Mini Kit Extraction Procedure for Oomycetes and Fungi
(Adapted from the brochure provided by the vendor)

Important points before starting:
1. Perform all centrifugation steps at room temperature (15-25°C).
2. Rotor size for the centrifuge is 6 cm fixed. Convert the listed RPM to x g if a different rotor size is used.
3. Preheat thermomixer (water bath or heating block) to 65°C.
4. Preheat (65°C) 100 μL of Buffer AE for each sample to be processed in a 1.7 mL microcentrifuge tube.
5. Warm Buffer AP1 to 37-42°C (to dissolve any precipitate).
6. Buffer AP1 may develop a yellow color upon storage. This does not affect the procedure.
7. Ensure that the correct volume of ethanol (96-100%) has been added to Buffers AW2 and AW1.
8. Do not heat Buffer AW1 after ethanol has been added.
9. Use decontaminated microcentrifuge tubes and tube openers.

Buffer AW1 contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. Discard the Buffer AW1 with the lysate flow-through into a 50 mL conical tube (or other appropriate container) and store with the hazardous waste materials.

Prepare and label all necessary microcentrifuge tubes and columns in advance
1. Prepare the Lysing Matrix A sterile screw cap tubes by adding a second sterile ceramic bead and write the label on the side of the tubes. This is important because the writing can be erased during disruption.
2. Place columns and microcentrifuge tubes on a rack in rows organized as follow:
   • 0 row The Lysing Matrix A sterile screw cap tube.
   • 1st row The QIAshredder columns (lilac colored columns supplied).
   • 2nd row 1.7 mL microcentrifuge tubes.
   • 3rd row DNeasy® columns (white columns supplied).
   • 4th row 1.7 mL tubes with lids cut off (if necessary) or 2 mL collection tube.
   • 5th row 1.7 mL tubes for the DNA extracts clearly labeled with the sample ID and date of extraction. Columns and microcentrifuge should be labeled on the top of the lid and on the side.

Citation

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Revision: Original
Effective Date: 1/21/2016
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1. Transfer 100-200 mg of mycelia into a **Lysing Matrix A** sterile screw cap tube. Add **600 μL Buffer AP1** and **6 μL RNase**, disrupt/homogenize 6.0/40sec in sample preparation system (i.e. MP Bio, Qbiogene FastPrep-24 Instrument, USA).

2. Centrifuge the tubes for 10-15 sec at 2,000-3,000 rpm. (i.e. **Centrifuge 5418**, Eppendorf, US)

3. Incubate in the Thermomixer (i.e. **Thermomixer, Eppendorf, US**) at 65°C, shaking at 700 rpm x 15 min.

4. Centrifuge the tubes for 15-30 seconds at 2,000-3,000 rpm.

5. Pipet **195 μL of Buffer P3** to the lysate in each tube. Vortex briefly or invert each tube 2-3 times.

6. Incubate for 5 min on ice to precipitate denatured proteins and cell wall components.

7. Centrifuge samples for 5 min at 14,000 rpm.

8. Pipet **600 μL of the lysate supernatant** into a labeled **DNeasy QIAshredder Mini Spin Column** (lilac) in a 2 mL collection tube (supplied). *Use a new disposable paper mat.

9. Centrifuge for 2 min at 14,000 rpm. Discard the column and keep the flow-through in the collection tube.

10. Transfer **450 μL of the flow-through fraction** from step 9 to a new 1.7 mL microcentrifuge tube without disturbing the pellet on the bottom of the collection tube.

11. In 675 μL (1.5x vol.) of **Buffer AW1** to each 450 μL sample & mix by slowly pipetting up and down.

12. Transfer **650 μL of the solution mixture** to the **DNeasy® Mini Spin Column** (white) in a 2 mL collection tube (keep remaining volume of the solution mixture for step 14).

13. Centrifuge for 1 min at 8,000-10,000 rpm. **Keep the column.** Discard the flow-through solution into the hazardous waste container (e.g. 50 mL conical tube).

14. Repeat steps 12 & 13 with the remaining portion of the solution mixture from step 11. Discard the flow-through solution (hazardous) and **keep the column.** (The total solution mixture volume is usually more than 650 μL so it is necessary to apply the sample in 2 subsequent loads).

15. Place the DNeasy® Mini Column in a new 2 mL collection tube (supplied).

16. Add **500 μL of Buffer AW2** to the column and centrifuge for 1-2 min at 8,000-10,000 rpm. **Keep the column.** Discard the flow-through solution into the hazardous waste container and replace the column onto the same collection tube.

17. Add another **500 μL of Buffer AW2** to the column and centrifuge for 2 min at 14,000 rpm. **Keep the column** and discard the flow-through solution, and then replace the column onto the same collection tube.

18. Centrifuge columns again 1-2 minutes at >8,000 rpm without additional buffer. This will dry and collect any additional flow-through.

19. Place the column in a new 1.7 mL microcentrifuge tube.

20. Pipet **100 μL Buffer AE** (at 65°C) onto the membrane in the column to elute your DNA. *Pipet the Buffer AE directly onto the membrane, but do not touch the membrane with the pipet tip.

21. Incubate for 5 min at room temperature.

22. Centrifuge for 1 min at 8,000-10,000 rpm. Discard the column and keep the flow-through solution (this is the sample DNA). Store at 4°C for immediate use (only) or at -20°C for long-term storage.