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 × 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 spilled, clean with suitable laboratory detergent and water. If the spilled 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.

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.

Place **columns** and **microcentrifuge tubes** on a rack in rows organized as follows:

- **row 0:** Lysing Matrix A sterile screw cap tube.
- **row 1:** QIAshredder columns (lilac-colored columns supplied).
- **row 2:** 1.7 mL microcentrifuge tubes.
- **row 3:** DNeasy® columns (white columns supplied).
- **row 4:** 1.7 mL tubes with lids cut off (if necessary) or 2 mL collection tube.
- **row 5:** 1.7 mL tubes for the DNA extracts clearly labeled with the sample ID and date of extraction.

Columns and **microcentrifuge tubes** should be labeled on the **top of the lid and on the side**.

Citation
Abad Z.G. and Bienapfl J.C. 2019. Qiagen DNeasy Plant Mini Kit extraction procedure for oomycetes and fungi. Document control number: SOP-PID-02.01 *from* Abad, Z.G., Burgess T., Bienapfl J.C., Redford A.J., Coffey M., and Knight L. 2019. IDphy: Molecular and morphological identification of *Phytophthora* based on the types. USDA APHIS PPQ S&T Beltsville Lab, USDA APHIS PPQ S&T ITP, Centre for *Phytophthora* Science and Management, and World *Phytophthora* Collection. http://idtools.org/id/phytophthora/index.php <date you accessed site>

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☐ 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. Add **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.