RNA Extraction Protocol for Filamentous Fungi (optimized for *Discula destructiva* and *Juglanconis japonica*)

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- **Materials for RNA extractions:**
  - Approximately 100 mg fungal mycelium
  - MN Bead Tubes Type C (Macherey-Nagel)
  - TRIzol™ Reagent (Invitrogen)
  - Phasemaker™ Tube (Thermo Scientific)
  - RNase Inhibitor (Applied Biosystems)

1. Pipet 1 mL of TRIzol™ Reagent (Invitrogen) into a MN Bead Tube Type C for every samples.
2. Collect fungal tissue for each sample and place into separate MN Bead Tubes Type C.
3. Homogenize tissue by vortexing 3,200 RPM for 20 minutes. This vortexing time may be able to be decreased depending on release of RNA/final concentration and quality of RNA.
4. Centrifuge the homogenate at 24 °C and 17,700 × *g* for 5 minutes.
5. Transfer 900 µL of the lysate to a new 1.5 mL microcentrifuge tube, ensuring no debris is transferred.
6. To the lysate, add 200 µL of chloroform and vortex 3 times at 3,200 RPM or until fully homogeneous.
7. Pipet the entire volume into a Phasemaker™ Tube and invert up to 10 times to distribute the polymer within the Phasemaker™ Tube.
8. Centrifuge this Phasemaker™ Tube at 24 °C and 17,700 × *g* for 10 minutes.
9. While that centrifuges, get a new 1.5 mL microcentrifuge tube and add 500 µL of 100% isopropanol.
10. After centrifugation, pipet 600 µL of the top aqueous layer into the new 1.5 mL microcentrifuge tube containing isopropanol.
11. Invert tubes up to 10 times to form a homogeneous mixture, and centrifuge at 24 °C and 17,700 × *g* for 10 minutes to pellet the RNA.
12. Discard the supernatant and air dry the RNA pellet for approximately 10 minutes or until the smell of isopropanol no longer lingers.
13. Wash the RNA pellet by adding 1 mL of 75% ethanol and gently inverting several times to ensure sufficient washing. Make sure not to dislodge the pellet.
14. Centrifuge the tube at 24 °C and 17,700 × *g* for 10 minutes.
15. Discard the supernatant and air dry the RNA pellet for approximately 10 minutes or until the smell of ethanol no longer lingers.
16. To the RNA pellet, pipet 2 µL of RNase Inhibitor and elute the total RNA in 100 µL of DEPC-Treated Water.

17. RNA concentration and quality can now be assessed using the TapeStation High Sensitivity RNA ScreenTape.
18. Store total RNA at -20 °C until used for library preparation and RNA sequencing.