HMW DNA Extraction for Filamentous Fungi (optimized for Discula destructiva)

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- **Protocol based on the Qiagen MagAttract HMW DNA Kit**
  - https://www.qiagen.com/us/resources/resourcedetail?id=3e331b6c-0742-483b-b74f-a7be2d4c1116&lang=en
- **Materials for homogenization:**
  - Mycelium (ideally 100-150 mg per extraction)
  - Autoclaved mortar and pestle
  - Liquid nitrogen
  - Autoclaved fine silica
  - 2 mL screw cap conical tubes
  - Sterilized small spatula (to scrape mycelium into tube after grinding)
- **Reagents for lysis:**
  - 5% (w/v) Yatalase solution: diluted in HPLC water
    - 30 uL of 5% Yatalase solution required per extraction
    - Important: make double what you need because bubbles make pipetting difficult i.e. make 60 uL for 1 extraction even though only 30 uL is required
    - 60 uL 5% Yatalase (for 1 extraction) = 3 mg Yatalase powder & 60 uL HPLC water

Homogenizing fungal mycelium

1. Add liquid nitrogen to a sterilized mortar and pestle so everything cools and allow it to evaporate.
2. Add sterilized fine silica (the smallest amount possible) to the mortar. IMPORTANT: adding too much silica will result in all the lysis buffer being absorbed in the subsequent lysis steps and improper lysis of cell walls.
3. Place mycelium into mortar and add more liquid nitrogen to freeze mycelium.
4. Gradually break up mycelium into chunks as liquid nitrogen evaporates, grinding to a fine powder once the liquid nitrogen fully evaporates.
5. Quickly add more liquid nitrogen to keep ground mycelium frozen, and repeat grinding for a 2nd time. At this point, you should have a fine, homogeneous powder that you can then scrape into a 2mL screw cap conical tube using a spatula. If not, repeat for a 3rd time, but try to not over-homogenize as this will result in degradation of DNA.
6. Place the tube containing the homogenized mycelium into liquid nitrogen until use or in the -80 freezer if it needs to be stored for the next day or longer.

5% (w/v) Yatalase solution preparation (do before lysis)

1. Calculate how much Yatalase solution is required for your extractions using the above formula.
2. Weigh out Yatalase powder (Yatalase is kept at 4°C until needed) in a mini weigh boat
3. Add the Yatalase powder into a 1.5 mL or 2 mL tube.
4. Pipet HPLC water into tube and lightly vortex until all powder is dissolved.

Lysis of fungal cell walls

1. To each 2 mL tube containing the homogenized mycelium, add 1 mL of ATL buffer (provided in kit) and 30 uL of 5% (w/v) Yatalase solution (diluted in HPLC water). The yatalase is a fungal-specific cell wall degrading enzyme used to help break up the chitin and beta glucans in the cell wall.
2. Gently invent tubes several times to mix the tissue with the Yatalase solution.
3. Place tubes onto a thermomixer (37℃ & 1,000 RPM) for 1 hour. Gently invert every 15 minutes to help ensure that the mixture stays homogeneous.
4. After 1 hour passes, remove tubes from the thermomixer and increase the thermomixer temperature to 56℃.
5. To each tube, add 90 uL of proteinase K solution (20 ug/uL) and 15 uL of RNase A (100 mg/mL).
6. Place tubes onto the thermomixer (56℃ & 1,000 RPM) for another hour.
7. Remove tubes from the thermomixer, and set the thermomixer to 20℃.
8. Centrifuge (10℃ & 20,000 G) for 5 minutes to pellet debris. You may need to centrifuge more than once if there is remaining debris at the top.
9. Remove tubes from the centrifuge, and transfer lysate to a new 2 mL microcentrifuge tube, making sure not to pick up any debris. You should be able to transfer ~400-450 uL of lysate-if not, just make sure the lysate is clean.

You can now move onto step 2 of the "Manual Purification of HMW gDNA from Fresh or Frozen Tissue" protocol of the MagAttract HMW DNA Kit. Important: Double volumes are being used throughout the remaining protocol since the starting amount of lysate is double.

1. Add 8 µL of RNase A (100 mg/mL) to the lysate. Mix by gently pulse-vortexing and incubate for 2 minutes at room temperature (20-22℃).
2. Add 300 µL Buffer AL to the sample. Mix by gently pipetting up and down a couple of times.
3. Add 560 µL Buffer MB to the sample.
4. Add 80 µL of MagAttract Suspension G to the sample. Ensure that the magnetic particles are fully resuspended by gently inverting until a homogenous mixture is formed.
5. Insert the tubes into the tube holder of the MagAttract Magnetic Rack (make sure hinges are pointing towards the center of the holder) and place onto the thermomixer (20℃ & 1,000 RPM) for 3 minutes.
6. Remove the tube holder from the thermomixer and place it onto the magnetic base. Wait until the beads are fully pelleted (1-5 minutes).
7. With the tube holder still on the magnetic base, remove all supernatant into a waste beaker. IMPORTANT: DO NOT DISTURB PELLET and remove supernatant completely.
8. Remove the tube holder from the magnetic base and add 1,400 µL of Buffer MW1 directly onto the pellet.
9. Place the tube holder back onto the thermomixer (20℃ & 1,000 RPM) for 2 minutes.
10. Remove the tube holder from the thermomixer, and place the tube holder back onto the magnetic base. Wait until the beads are fully pelleted (~1 minute).

11. With the tube holder still on the magnetic base, remove all supernatant into a waste beaker. IMPORTANT: DO NOT DISTURB PELLET and remove supernatant completely.
12. Repeat steps 8-11 for a 2nd MW1 wash step.
13. Remove the tube holder from the magnetic base and add 1,400 µL of Buffer PE directly onto the pellet.
14. Place the tube holder back onto the thermomixer (20℃ & 1,000 RPM) for 2 minutes.
15. Remove the tube holder from the thermomixer, and place the tube holder back onto the magnetic base. Wait until the beads are fully pelleted (~1 minute).
16. With the tube holder still on the magnetic base, remove all supernatant into a waste beaker. IMPORTANT: DO NOT DISTURB PELLET and remove supernatant completely.
17. Repeat steps 13-16 for a 2nd PE wash step.
18. Ensure that there are no traces of Buffer PE remaining by using a P20 pipet or a vacuum aspirator. Traces of wash buffer will carry over to DNA elution.
19. IMPORTANT: DO NOT PIPET WATER DIRECTLY ONTO THE BEAD PELLET! With the tube holder still on the magnetic base, carefully rinse the particles with 1,400 µL of HPLC water (distilled or nuclease free water will also work) by pipetting on the tube wall opposite of the pellet.
20. With the tube holder still on the magnetic base, incubate for 1 minute at room temperature (20℃) and remove all supernatant into a waste beaker.
21. Repeat steps 19-20.
22. Remove the tube holder from the magnetic base and add 100 uL of Qiagen Elution Buffer (EB) directly to the pellet.
23. Place the tube holder onto the thermomixer (20℃ & 1,000 RPM) for 3 minutes.
24. Remove the tube holder from the thermomixer, and place the tube holder back onto the magnetic base. Wait until the beads are fully pelleted (1-5 minutes).
25. Very slowly pipet the supernatant aka the Qiagen Elution Buffer that contains the HMW DNA to a new 1.5 mL microcentrifuge tube labeled “Elution A” and any other desired identifiers for your sample. (You can totally get away with standard pipet tips here for HiFi-aiming DNA if you pipet very slowly. For longer read applications like Nanopore, you would need to buy wide-bore tips or cut P200 tips with a razor.) You can stop here and perform DNA QC, or you continue with the additional elutions below to try to maximize the amount of DNA.
26. Add 100 uL of Qiagen Elution Buffer (EB) to each tube containing the magnetic beads and gently tap to resuspend the magnetic beads.
27. At this point, you have 2 option for how to incubate:
    a. Incubate at room temperature (20℃) for 1-2 hours with occasional tapping to resuspend the beads, or use a slow end-over-end tube mixer (4-5 rotations/minute).
    b. Alternatively, store tubes at 4℃ overnight and bring them to room temperature (20℃) the next day with gentle tapping to resuspend.
28. After either extended incubation, place the tube holder onto the thermomixer (37℃ & 1,000 RPM) for 7 minutes.
29. Remove the tube holder from the thermomixer, and place the tube holder back onto the magnetic base. Wait until the beads are fully pelleted (1-5 minutes).

30. Very slowly pipet the supernatant aka the Qiagen Elution Buffer that contains the HMW DNA to a new 1.5 mL microcentrifuge tube labeled “Elution B” and any other desired identifiers for your sample.
31. Add 50 uL of Qiagen Elution Buffer to each tube containing the magnetic beads and gently tap to resuspend the magnetic beads.
32. store tubes at 4°C overnight and bring them to room temperature (20°C) the next day with gentle tapping to resuspend.
33. After the extended incubation, place the tube holder onto the thermomixer (37°C & 1,000 RPM) for 10 minutes.
34. Remove the tube holder from the thermomixer, and place the tube holder back onto the magnetic base. Wait until the beads are fully pelleted (1-5 minutes).
35. Very slowly pipet the supernatant aka the Qiagen Elution Buffer that contains the HMW DNA to a new 1.5 mL microcentrifuge tube labeled “Elution C” and any other desired identifiers for your sample.
36. You may now run QC on the extracted DNA using Nanodrop, QuBit, or TapeStation (recommended).
37. Additional polysaccharide clean-up may be required after DNA extractions. Please reference “240506_Pooling and precipitation protocol_Niece.S(UTennessee)”.