E.Z.N.A. Fungal DNA Extraction Mini-Prep.

1. **Add a pinch of sand** to each tube along with **200 µl of buffer FG1.** Let sit for 5 min then another 10 min at **-20°C to soften the tissue**.
2. **Remove tubes from -20°C**, let thaw for 5 min, then **grind the tissue with a micropestle** until all chunks/pieces are homogenized.
3. **Add 600 µl buffer FG1**.
4. **65°C for 10 min**. Mix 2X.
5. **180 µl buffer FG2.** Vortex.
6. 5 min on ice.
7. **Centrifuge 10 min @ 10K *g*.** BALANCE and HINGES UP ALWAYS!
8. **Transfer 700 µl / 600 µl supernatant to new tube.** Do not transfer any tissue!
9. **0.7 vol (480 µl / 420 µl) isopropanol**. Vortex.
10. **Centrifuge 2 min @ 10K *g*.**
11. **Remove supernatant.** Do not disturb DNA pellet (if visible). Invert on paper towel to let dry for 1 min.
12. **300 µl ddH_20 heated to 65°C.** Vortex.
13. **4 µl RNase A.** Vortex.
14. **150 µl buffer FG3 + 300 µl 100% ETOH.** Vortex.
15. **Transfer** to HiBind Mini Column and supplied collection tube.
16. **Centrifuge 1 min @ 10K *g*.** Discard filtrate.
17. 750 µl DNA Wash Buffer.
18. **Centrifuge 1 min @ 10K *g*.** Discard filtrate.
19. Repeat steps 17 and 18.
20. **Centrifuge 2 min @ 10K *g*.** To remove all liquid.
21. **Transfer Mini Column to new 1.5µl microcentrifuge collection tube.** Let sit for 5-10 minutes so the wash buffer can evaporate.
22. **50 µl Elution Buffer heated to 65°C.** (Optional: let sit for 3-5 minutes).
23. **Centrifuge 1 min @ 10K *g*.**
24. Repeat step 22-23 one more time.

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2. **Remove tubes from -20°C**, let thaw for 5 min, then **grind the tissue with micropestle** until all chunks/pieces are homogenized.
3. **Add 600 µl buffer FG1**.
4. **65°C for 10 min**. Mix 2X.
5. **180 µl buffer FG2.** Vortex.
6. 5 min on ice.
7. **Centrifuge 10 min @ 10K *g*.** BALANCE and HINGES UP ALWAYS!
8. **Transfer 700 µl / 600 µl supernatant to new tube.** Do not transfer any tissue!
9. **0.7 vol (480 µl / 420 µl) isopropanol**. Vortex.
10. **Centrifuge 2 min @ 10K *g*.**
11. **Remove supernatant.** Do not disturb pellet. Invert on paper towel to let dry for 1 min.
12. **300 µl ddH_20 heated to 65°C.** Vortex.
13. **4 µl RNase A.** Vortex.
14. **150 µl buffer FG3 + 300 µl 100% ETOH.** Vortex.
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16. **Centrifuge 1 min @ 10K *g*.** Discard filtrate.
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24. Repeat step 22-23 one more time.