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FUNDIS DNA Extraction V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

A quick and easy DNA extraction protocol for fungal tissue.



Adding Extraction Buffer and Thermal Cycling

30m

- 1 Tissue samples will arrive in batches of 96 in individually-capped 8-strip PCR tubes in a PCR tube rack. First you will spin the tubes down for  00:00:10 . 10s
- 2 Next, clean your area with a DNA destroying solution, or you can use 70% isopropyl rubbing alcohol, but be aware that it will not destroy DNA. It is not recommended to do your DNA extraction in a location where you also work with PCR products.
- 3 Next, arrange your area so that all of your supplies are within easy reach. I typically put the tubes to be samples to my left, then I have an empty PCR tube rack in front of me and another one to my right. Directly behind the tube rack in front of me, I have a microcentrifuge tube rack with 2 1.5mL tubes filled with autoclaved 2.5mm ball bearings. I also keep a pair of tweezers in one of the holes of the rack with the tines facing up. Next to that on the right, I have my  10 mL reagent reservoir, and on the left, a box of  100 μ L pipette tips.
- 4 Put on a fresh pair of gloves that fit snug around your finger tips. Pour  2-3 mL of ES extraction buffer into the reagent reservoir.
- 5 Take a strip of tubes from the rack on the left and move it to the middle rack, then open each of the tube lids carefully, ensuring that no tissue flies out or sticks to your glove. 
- 6 Using a  5-50 μ L 8-channel pipettor equipped with  100 μ L pipette tips, add  15-30 μ L of ES extraction buffer to each tube by carefully holding the pipette tips **above** the tube openings so that the tips do not make contact with the sample. This way we can use the same row of tips for many samples. How much buffer you add depends on the amount of tissue to be extracted. You will dilute this volume later, so do not use more than  30 μ L . If you are concerned that you may have contaminated the tips, discard them and get new tips. The samples are precious and cannot be replaced, the tips can be bought on ebay.  
- 7 Using the tweezers, drop 1 ball bearing into each tube in the strip being careful not to contaminate the tweezers. If 2 balls accidentally go into 1 tube, it is totally fine. Don't attempt to retrieve any of the ball bearings from inside the tubes. 
- 8 Close the lids and move the strip to the empty rack on the right in the correct position, and repeat this  [go to step #5](#) until all tubes have been processed.
- 9 Using a vortexer set to the maximum setting, vortex each strip of tubes for up to  00:00:10 making sure each tube in the strip gets shaken well. 10s



- 10 Spin down the tubes in a microcentrifuge for 00:00:05 5s
- 11 Incubate in a thermal cycler at 95 °C for 00:15:00 followed by a hold at 12 °C 15m
to bring the temperature back down. Your DNA is now extracted and you can pause and store the tubes at -20 °C until you are ready to proceed. II

Diluting

10m

- 12 If you paused after the previous step, remove your tubes from the freezer, bring up to room temperature, and spin down for 00:00:05 in a microcentrifuge. If you are continuing directly from the last step, you do not need to spin the tubes down as long as they do not have liquid at the top. 5s
- 13 Clean your work area with a DNA destroying cleaner if possible and set up 2 empty PCR tube racks, a reagent reservoir, a 50-300 µL 8-channel pipette and p200 pipette tips. Fill the reagent reservoir with 10 mL of DS (Dilution Solution containing [M] 10 millimolar (mM) Tris, [M] 100 micromolar (µM) EDTA, [M] 0.3 Mass Percent BSA)
- 14 Make sure you change your gloves before the next step.
- 15 As during the initial extraction, move 1 strip of tubes to an empty PCR tube rack and carefully open the lids making sure not to splash or get any liquid on your gloves. If you contaminate your gloves, change them for clean ones.
- 16 Using the multichannel pipette, transfer 75-125 µL of DS to each tube. The volume you choose will depend on how much ES extraction buffer you used. This is aiming for a 1:5 extract to dilution ratio. This does not need to be exact. Do not add more than 125 µL of DS to the tube no matter what as this will cause the tube to overflow. Add the DS by holding the pipette well above the openings of the tubes and try to aim the liquid into the tubes. Avoid dispensing with too much force to prevent splashing. If some of the DS does not make it into the tube, it is not a big deal. If a lot of it misses the tube, you can single-channel some in. This is meant to save pipette tips, but the most important thing is to avoid cross-contamination so if you are concerned, switch for new tips. These samples are precious, the tips are not.



- 17 Store diluted extractions at for future use, or use them immediately for PCR and then store them at