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Dry Tissue sampling and DNA extraction with NEB monarch Kit

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Tissue Sampling and Physical tissue disruption

1 Flame sterilize a pair of fine-tip forceps in 70% ethanol

2 With the sterilized forceps remove and weigh 10-15mg of Hymenium tissue into a bead bashing tube with a conical bottom (our bead beater cannot hold flat bottomed tubes)

*Record the exact weighed amount of sampled tissue in the DNA extraction google sheet

3 fill out a tissue sampled sheet for the specimen and place it back into the collection box. If possible place the tissue sampled from along with the form into a small plastic bag separate from the rest of the specimen.

4 Place 1 [3mm] ball bearing and 8 [1.5mm] into the bead bashing tube with the sampled tissue

5 Place tubes into Bead bug and shake for 30 seconds at 350 speed

350 rpm, 00:00:30

6 Repeat step 5.

*if the sample is not a fine powder after a second shake, you may repeat shaking a third time.

Chemical lysis and incubation with NEB monarch Genomic DNA kit reagents 30m

7 add 400ul of Monarch tissue lysis buffer to the tube with powdered sample

8 add 10ul of Monarch ProK to sample tube

9 Spin down briefly so liquid and tissue are not stuck on the side of the tube. Place on ThermoMixer at 56C @1350RPM for 30 minutes. 30m

1350 rpm **56 °C** **00:30:00**

9.1 ⊛

Optional

If tissue or taxa are particularly recalcitrant, a final concentration of 3ug/ml Trichoderma enzymes can be added to the tissue and lysis buffer and incubate at room temperature on the ThermoMixer BEFORE ProK for 30 minutes

25 °C **00:30:00**

10 Centrifuge at max speed for 5 minutes

00:05:00

11 Pipette supernatant into a new low-retention 1.7ml centrifuge tube.

12 add 4.5ul of NEB RNAse enzyme to sample tube. Incubate on ThermoMixer at 56C and 1350RPM for 15 minutes.

Also, place ~1ml of NEB elution buffer (EB) into a centrifuge tube and incubate at 56C, this will be used at a later step.

00:15:00 **56 °C** **1350 rpm**

13 Centrifuge at max speed for 5 minutes.

00:05:00

GDNA spin column and Elution

14 Place 300ul of supernatant into anew tube. Add 600ul (2x volume of supernatant) of NEB GDNA binding buffer.

15 Pulse vortex each sample tube for ~10 seconds or until the sample is sufficiently homogenized.

16 Add 600ul of Sample/Binding buffer solution to GDNA binding column from NEB monarch kit

17 

Spin for 3 minutes at 1000g (xg and rcf are the same).

This step binds the GDNA to the column

1000 x g **00:03:00**

18 

Immediately spin at max speed for 1 minute.

This step clears all the buffer from the column

00:01:00

19 Empty or replace the collection tube, then repeat steps 16 through 18 for the remainder of the sample/binding buffer solution from step 15

20 Replace the binding column into a new collection tube. Pipette 500ul GDNA wash buffer onto column and centrifuge at maximum speed for 1 minute.

00:01:00

21 Dumb excess buffer from the collection tube and repeat step 20, increase spin time to 1 minute and 30 seconds.

00:01:30

22 Place the column into a low-retention 1.7ml centrifuge tube. Add 100ul of 56C elution buffer (EB) from step 12 onto the column. Let incubate on the bench for 1-2 minutes.

00:02:00 **25 °C**

23 Centrifuge sample at maximum speed for 1 minute to elute DNA.

DNA quality control, recording and storage

24 Take samples to Nanodrop. Check that the sensor is clean if it is not, place 5ul DNAse free water onto the sensor, and wipe clean with a kimwipe.

25 Blank the nanodrop with 2ul of EB from step 12.

26 Wipe liquid from sensor and pipette 2ul sample onto the sensor and hit "Measure".

*make sure you wipe the liquid off of the sensor with a kimwipe every time you measure a new sample or blank and again once finished.

27 Record these measurements into the google doc for each sample:

[ng/ul]: Tells us the concentration of our genomic DNA extract

Total ng: This is the total mass of our extract (calculate as ng/ul * 100)

260/280: Tells us the purity of our sample (DNA ~ 1.8, RNA ~2). a value of ~1.8-2.2 is considered usable

260/230: Tells us the cleanliness of our sample. Clean samples are generally ~2.0-2.2. This value may not be critical if you are using this sample for PCR.

Notes: anything you noticed about the spectra curve (also note if it looks normal) from the nanodrop or anything you notice about the sample itself.

28 Place a tough tag on top of the sample tube. Record the catalog number (include institution code if it is not already part of the catalog number) on the tough tag as well as on the side of the tube.

29 Place the recorded sample into a sample box and store it @ -4C (The cold room).