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Modified Nucleic Acid Extraction Protocol for Humic-Rich Permafrost Peat Samples

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We use this protocol and it's working

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Abstract

Slight modifications to the manufacturer's protocol (Qiagen #12966-10, now discontinued and replaced with #12988-10) enabled the successful extraction of high-quality DNA and RNA from permafrost peat samples. Integrating the DNA and RNA purification steps immediately after the extraction process helped streamline the workflow, resulting in consistent quality nucleic acids suitable for downstream sequencing and analysis. This improved approach optimizes the extraction process, ensuring more reliable and efficient results for permafrost peat studies.

Guidelines

To improve the chances of maintaining RNA integrity, perform the extraction using RNase-free practices. Be meticulous in cleaning your workspace, pipettes, and reagents with RNase-decontamination solutions such as RNaseZap. Ensure that all tubes and reagents are RNase-free and handle samples in a sterile environment using proper personal protective equipment (PPE). Additionally, minimize sample handling time at room temperature and work quickly to reduce the risk of RNA degradation.

Materials

- **DNeasy PowerMax Soil Kit**

Supplier: Qiagen, Catalog No. **12988-10**

- **Glass Bead, 0.1 mm**

Supplier: Qiagen, Catalog No. **13118-400**

- **Solution RD1 (PM1)**

Supplier: Qiagen, Catalog No. **26000-50-1**

- **Solution RD2 (C3)** – Proprietary buffer for precipitation, part of a kit.

Supplier: Qiagen, Catalog No. **12888-100-3**

- **Solution RD3 (PD4 or PM3)** – Proprietary binding buffer.

Supplier: Qiagen, Catalog No. **26000-50-3**

- **Solution RD4 (C5)** – Proprietary wash buffer.

Supplier: Qiagen, Catalog No. **12955-4-5D**

- **Solution RD5 (Nuclease-free water)**

Supplier: Thermo Fisher Scientific, Catalog No. **10977035**

- **Ethanol (100%)**

Supplier: Fisher Scientific, Catalog No. **BP28184**

- **Phenol:Chloroform:Isoamyl alcohol (PCI) (25:24:1, pH 8.0)**

Supplier: Thermo Fisher Scientific, Catalog No. **AM9720**

- **Sodium Acetate (3M, pH 5.2)**

Supplier: Thermo Fisher Scientific, Catalog No. **AM9740**

- **TE Buffer**

Supplier: Thermo Fisher Scientific, Catalog No. **AM9849**

- **RNaseOut**

Supplier: Thermo Fisher Scientific, Catalog No. **10777019**

- **DNase I (RNase-free)**

Supplier: Fisher Scientific, Catalog No. **50-100-3290**

- **RNase A (10 mg/ml)**

Supplier: Thermo Fisher Scientific, Catalog No. **EN0531**

- **Chloroform**

Supplier: Sigma-Aldrich, Catalog No. **C2432**

- **Linear Acrylamide (5 mg/ml)**

Supplier: Thermo Fisher Scientific, Catalog No. **AM9520**

Safety warnings

PCI is toxic and needs to be handled in the fume hood with proper PPEs. Handle with care.

Before start

To improve the chances of maintaining RNA integrity, perform the extraction using RNase-free practices. Be meticulous in cleaning your workspace, pipettes, and reagents with RNase-decontamination solutions such as RNaseZap. Ensure that all tubes and reagents are RNase-free and handle samples in a sterile environment using proper personal protective equipment (PPE). Additionally, minimize sample handling time at room temperature and work quickly to reduce the risk of RNA degradation.

Total nucleic acids extraction

3h

1 Preheat the water bath to 60 ˚C. If the phenol:chloroform:isoamyl alcohol (PCI) (25:24:1, pH8.0) is refrigerated, move from 4 ˚C to the fume food to warm to room temperature (RT). CAUTION: PCI is toxic and needs to be handled in the fume hood with proper PPEs. Handle with care.

2 Warm solution PM1 in the pre-heated water bath for 15 min. Meanwhile, clean the workspace and pipettes with RNaseZap to remove RNAse contamination.

3 Add 8 to 10 g of peat slurry (i.e., the peat preserved in Lifeguard) to the 50-ml bead tube (which is part of the kit). Note: in the PowerMax manufacturer's protocol, if Lifeguard is used on a sample, it is recommended to spin down the samples and remove it before adding the bead tube. However, we observed residual nucleic acids in the removed Lifeguard, and found good extraction efficiency when keeping Lifeguard in the slurry, so we keep it in; it is possible that the diluting effect of porewater in these often-saturated samples mitigates any negative impact on extractions of keeping Lifeguard in.

Safety information

PCI is toxic and needs to be handled in the fume hood with proper PPEs. Handle with care.

4 In the fume hood, add 15 ml PM1 to the sample-containing bead tube, then add 5 ml PCI. Shake to mix. (Note, with PCI, be sure to take reagent from the lower organic layer).

5 Ensure cap firmly tightened, and place the tube securely onto the vortex adapter of the Vortex-Genie 2 mixer inside the fume hood. Vortex at maximum speed for 10 min.

6 Centrifuge at 4,500 x g for 6 min at RT. Transfer the aqueous phase of supernatant to the new 50 ml tube. Record the volume (about 18-24 ml).

7 Add 1/3 volume (of the transferred supernatant) of C3 (about 6-8 ml), invert four to six times to mix. Place tubes on ice for 5 min.

8 Centrifuge at 4,500 x g for 6 min at RT. Transfer supernatant into two 50 ml tubes (about 10-12.5 ml each).

9 Add 1 volume of the transferred volume of PM3 (about 10-12.5 ml) and 1 volume of absolute ethanol (10-12.5 ml). Invert four to six times to mix.

10 Transfer the sample to the column tube. Centrifuge at 4,500 x g for 2 min at RT. Discard the flow-through and repeat till all liquid has been processed.

11 Add 16 ml of C5 to the column, and centrifuge at 4,500 x g for 2 min at RT. Discard the flow-through.

12 Add 16 ml absolute ethanol to the column, and centrifuge at 4,500 x g for 2 min at RT. Discard the flow-through. Repeat this wash step one more time.

13 Dry the column by an additional centrifuge at 4,500 x g for 8 min at RT.

14 Place the column in a new 50 ml tube. Leave the cap off for 10 min in the fume hood to allow remaining ethanol to evaporate.

15 Add 5 ml nuclease-free water to the column, sit the tube at RT for 1 min, then centrifuge at 4,500 x g for 2 min at RT. After the nucleic acids are eluted, discard the column.

Ethanol precipitation of total nucleic acids

10m

16 Add 1/10 volume of 3M sodium acetate (pH 5.2) (5 ml), swirl to mix.

17 Add 2.5-3 volume of cold absolute ethanol (kept at -80 ˚C freezer) (bring up to about 20 ml). Invert four to six times to mix.

18 Leave the tubes at -80 ˚C overnight for total nucleic acids precipitation.

Note

The protocol can be paused here.

Recover and concentrate the total nucleic acids

3h

19 Pre cool the centrifuge to 4 ˚C, take out the samples from -80 ˚C to let it thaw a little bit, and take out PCI to let it get back to RT in the fume hood (kept in dark).

20 Centrifuge the sample at 4,500 x g at 4 ˚C for 35 min. Carefully decant the supernatant.

21 Wash the pellet with 5 ml cold 70 % ethanol (kept at -20 ˚C freezer). Centrifuge at 4,500 x g at 4 ˚C for 15 min. Decant the supernatant.

22 Invert tubes on kimwipe on a cleaned surface in the fume hood to air dry the pellet (takes about 15 min).

22.1 Meanwhile, preheat the heat block to 37 ˚C, and take out reagents from -20 ˚C and 4 ˚C and put on ice for the following DNA and RNA recovery steps. Make a 1 % agarose gel for nucleic acids quality check (optional).

23 Resuspend the total nucleic acids pellet in 105 µl of TE buffer. Pipet up the TE buffer to wash the tube wall (especially the button tapered part) and flick the tube to mix.

24 Aliquot to two 2 ml tubes: 35 µl for DNA recovery and 65 µl for RNA recovery. What's left is for running the agarose gel to check the integrity of the total nucleic acids (optional).

DNA and RNA recovery

40m

25 RNA recovery- DNase treatment.

25.1 Make a master mix for the DNase treatment.

	A	B	C
	Item	Volume (µl)	Master Mix for 6.5 reactions (µl)
	10x DNase buffer	10	65
	RNaseOut	2	13
	Nucleic acids	65	-
	DNase	1	6.5
	ddH2O	22	143
	Total	100	-

25.2 Aliquot 35 µl of the master mix to RNA recovery tube. Pipette up and down to mix.

25.3 Incubate at 37 ˚C (heat block) for 25 min.

26 DNA recovery- RNase treatment

26.1 Add 1 µl of RNase A (10 mg/ml) to each DNA recovery tube. Flick to mix.

26.2 Incubate at 37 ˚C for 20 min.

PCI cleanup

30m

27 Add 414 µl and 350 µl of TE buffer to the DNA recovery tube and the RNA recovery tubes respectively to make the sample volume to 550 µl each.

28 Add 1 volume of PCI (550 µl) to the samples. Shake vigorously for 15 s to mix then let sit at RT for 2 min.

29 Centrifuge at 18,000 x g for 3 min at RT. Carefully transfer the top aqueous layer to a new 2-ml tube (about 500 µl).

30 Add 1 volume of chloroform to the samples. Shake vigorously for 15 s to mix then let sit at RT for 2 min.

Safety information

Chloroform is toxic and needs to be handled in the fume hood with proper PPEs. Handle with care.

31 Centrifuge at 18,000 x g for 3 min at RT. Carefully transfer the top aqueous layer to a new 2-ml tube (about 450 µl).

Ethanol precipitation of total nucleic acids

40m

32 Add 1/10 volume of 3M sodium acetate (pH 5.2) (about 45 µl).

33 Add linear acrylamide (5 mg/ml) to a final concentration of 10-20 µg/ml (about 1.5 µl). Flick to mix.

34 Add 2.5-3 volume of cold 100 % ethanol (kept at -80 ˚C freezer) (about 1.3 ml). Invert to mix.

35 Incubate at -80 ˚C for 30 min.

Note

NOTE: The protocol can be paused here.

Recover and concentrate DNA and RNA

1h 30m

36 Pre cool the microcentrifuge to 4 ˚C, and take out the samples from -80 ˚C.

37 Centrifuge the sample at 18,000 x g at 4 ˚C for 20 min. Decant the supernatant.

38 Wash the pellet with 1 ml cold 70 % ethanol (kept at -20 ˚C freezer). Centrifuge at 18,000 x g at 4 ˚C for 15 min. Decant the supernatant.

39 Invert tubes on a kimwipe on a cleaned surface in the fume hood to air dry the pellet (takes about 10 min).

40 Resuspend the DNA pellet and RNA pellet in 50 µl and 25 µl with TE buffer respectively. Wash the wall and flick to mix.

Note

Elution volume is changeable depending on the nature of the sample biomass content.

41 Store the DNA and RNA at -80 ˚C.