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Modified ZymoBIOMICS™ DNA/RNA Miniprep Kit for parallel extraction of DNA and RNA from peat soil

Forked from Standard ZymoBIOMICS™ DNA/RNA Miniprep Kit for parallel extraction of DNA and RNA

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We are still developing and optimizing this protocol

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Abstract

Modified ZYMO DNA/RNA miniprep extraction protocol written in a user friendly way to reduce in-process-decisions and process time. Sample type is organic soil, peat, from a degraded rich fen

This protocol includes modifications to tackle clogged filters.

- Samples were centrifuged for 45 seconds instead of 30 seconds in first pass through filters as 100 ul to 200 ul sample remained trapped above filter with standard protocol

Guidelines

Follow standard precautions for working with environmental samples and RNA i.e. clean/sterilise with 70% ethanol and 10 to 30 min UV if available, further remove DNA and RNases from work environment and equipment using a cleaner of your choice. Work in a designated RNA/pre-PCR area or cabinet. Wear a face mask if a risk of sneezing or coughing exists and you don't have a cabinet. Wear latex or nitrile gloves and change often.

Materials

ZymoBIOMICS™ DNA/RNA Miniprep Kit **Zymo Research Catalog #**R2002

KIT PROVIDED:
solutions and buffers:

DNA/RNA Shield **Zymo Research Catalog #**R1100-50

DNA/RNA Lysis Buffer **Zymo Research Catalog #**D7001-1-50

DNA/RNA Prep buffer **Zymo Research Catalog #**D7010-2-50

DNA/RNA Wash Buffer (concentrate) **Zymo Research Catalog #**D7010-3-24

ZymoBIOMICS™ DNase/RNase-Free Water **Zymo Research Catalog #**D4302-5-50

ZymoBIOMICS™ HRC Prep Solution **Zymo Research Catalog #**D7010-2-50

enzymes:

DNase I Set **Zymo Research Catalog #**E1010 or #E1009-A

both ##SKU have 250U DNase I and come with 4 ml DNA Digestion Buffer

tubes, filters, columns:

ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) **Zymo Research Catalog #**S6012-50

Spin-Away Filter (yellow) **Zymo Research Catalog #**C1006-50-F

Zymo-Spin™ IIICG Column (green) **Zymo Research Catalog #**C1006-50-G

Zymo-Spin™ III-HRC Filter **Zymo Research Catalog #**C1058-50

Collection Tubes **Zymo Research Catalog #**C1001-500

USER PROVIDED:
consumables:

- pipette tips
- Ethanol 70 % for cleaning
- sharpie
- freezer boxes

Fisherbrand™ Nitrile Indigo Disposable Gloves PPE Cat III **Thermo Fisher Catalog #**17182182

Kimberly-Clark™ Kimtech™ Science Precision Wipes **Thermo Scientific Catalog #**10180601

Thermo Scientific™ Snap Cap Low Retention Microcentrifuge Tubes **Thermo Fisher Catalog #**11569914

Thermo Scientific™ Snap Cap Low Retention Microcentrifuge Tubes **Thermo Scientific Catalog #**11579914

Invitrogen™ Nuclease-Free Water (not DEPC-Treated) **Invitrogen - Thermo Fisher Catalog #**AM9932

Ethanol, Absolute (200 Proof), Molecular Biology Grade, Fisher BioReagents™ **Fisher Scientific Catalog #**16606002

Thermo Scientific™ RNase AWAY™ Surface Decontaminant **Thermo Fisher Scientific Catalog #**14-754-34

equipment:
- mass balance for weighing tubes/samples
- Vortex
- Micro-centrifuge
- Freezers -80 °C
- Freezer -20° C
- optional choice between Nanodrop, gel electrophoresis, Qubit, TapeStation, and/or BioAnalyzer to characterise quantity, purity, and quality of extracted nucleic acids

Equipment

NanoDrop™ 2000	NAME
Microvolume Spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-2000	SKU
https://www.thermofisher.com/order/catalog/product/ND-2000	LINK

Equipment

2100 Bioanalyzer	NAME
Fragment Analysis system	TYPE
Agilent	BRAND
G2939BA	SKU
https://www.agilent.com/en/product/automated-electrophoresis/bioanalyzer-systems/bioanalyzer-instrument/2100-bioanalyzer-instrument-228250	LINK

Equipment

FinnPipette	NAME
F2	TYPE
ThermoScientific	BRAND

Equipment

MP Bio FastPrep 24	NAME
bead beating grinder	TYPE
MP Bio	BRAND
116004500	SKU
https://www.mpbio.com/us/fastprep-24-classic-instrument-1-each	LINK

Before start

- Before starting you will need to clean and de-contaminate work space and equipment where possible

- Add ethanol to **DNA/RNA Wash Buffer** (step 1.2) (once per kit)
- Reconstitute DNase and aliquot (step 1.3), freeze unused aliquots at - 20°C (once per kit)
- Aliquot provided water into 1.5 ml or 2 ml tubes
- Cut pipette tip ends off from one box of 1000 ul tips to transfer slurry (step 2)

- Perform all steps at room temperature unless specified

Pre-prep

46m

1

20m

Note: if you have a micro-centrifuge with 24 places, you can process maximum 12 samples per batch because steps 10 to 16 process DNA and RNA simultaneously, ie 12 samples x 2 (DNA + RNA) = 24

1.1

- If samples are frozen at -80°C in DNA/RNA Shield, retrieve now and place on ice to thaw during preparation (see step 2). Remember to check and vortex periodically during set-up.
- Retrieve DNase from freezer if already reconstituted and place on ice to thaw

1.2 (If not already done) Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml DNA/RNA Wash Buffer concentrate

3m

- this only needs to be done once per kit

1.3 (If not already done) Reconstitute lyophilized DNase I with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots @ -20°C

2m

- this only needs to be done once per kit

1.4 Prepare DNase Master Mix and store on ice until needed:

5m

	A	B	C	D	E	F
	sample no.	1	6	8	12	24
	DNase I (ul)	5	30	40	60	120
	DNase dgstn buffer (ul)	75	450	600	900	1800

1.5 Pipette 400 µl of DNA/RNA Lysis Buffer into one nuclease-free tube (not provided) for each sample to be processed (see step 4). Label with sample ID

3m

1.6 Pipette 800 µl of 95-100% ethanol into one nuclease-free tube (not provided) for each sample to be processed (see step 7). Make sure cap is tightly closed. Label with sample ID and 'RNA'

3m

1.7 for each sample:

10m

- Prepare and label a further 4 tubes with sample ID including 2 with 'RNA' and 2 with 'DNA'.
- Set up and label 5 collection tubes with sample ID including 2 with 'DNA', and 2 with 'RNA'

- Place a yellow filter in one collection tube labelled DNA
- Place a green column in one collection tube labelled RNA
- Place HRC filters in two collection tubes with one each labelled DNA and RNA

SAMPLE LYSIS

55m

2 Sample thaw and transfer protocol: 10m

- For liquid nitrogen flash frozen samples transfer 0.25 g sample to a ZR BashingBead Lysis Tube and add 750 µl DNA/RNA Shield to lysis tube, cap tightly and vortex briefly, also vortex briefly during thaw 2 or 3 more times.

OR

- For samples collected in DNA/RNA Shield, vortex briefly every five minutes while thawing on ice (may take 30 minutes or longer). Set pipette to 800 µl, vortex briefly or invert, and transfer 750 µl of slurry (using a pre-cut pipette tip) to a ZR BashingBead Lysis Tube and cap tightly.

3 40m

1. Label side of BB Lysis Tube with sample ID as lid labels wear off during bead beating
2. Bead beat on MP Bio FastPrep for 60 seconds at 6.5 m/s, then
3. rest for 5 minutes.
4. Repeat four more times for a total of 5 minutes bead beating

4 5m

1. Centrifuge samples 16 000 x g for 30 seconds and
2. transfer 400 µl of the supernatant to pre-prepared tube with lysis buffer (see step 1.5), and
3. mix well by inversion. (sample volume is now 800 ul)

DNA

8m

5 5m

1. Pour or pipette the sample into a Spin-Away™ Filter (yellow) in a collection tube and
2. centrifuge 16 000 x g for **45** seconds.

6 Transfer the Spin-Away™ Filter (yellow) containing DNA into a new Collection Tube and store until ready to proceed with step 10. **SAVE the flow through for step 7! 3m

RNA

35m

7 5m

1. Transfer flow-through (800 ul) from step 6 to pre-prepared tube with same volume of ethanol, close cap tightly and mix well by inversion. This contains RNA, sample volume is now 1600 ul.

8 10m

1. Transfer 700 ul of the RNA mixture into a prepared Zymo-Spin™ IIICG Column (green) in a Collection Tube
2. centrifuge 16 000 x g for **45** seconds.

3. Discard flow through.
4. Repeat steps 8.1 to 8.3 until all RNA solution has passed through the green column.

9

1. Add 400 µl DNA/RNA Wash Buffer to each green column
2. centrifuge 16 000 x g for **45** seconds
3. discard the flow-through
4. Add 80 ul DNase I Master Mix (step 1.4) to green column
5. incubate @ room temp for 15 minutes

20m

Parallel DNA and RNA processing

43m

10

1. Add 400 µl **DNA/RNA Prep Buffer** to the yellow filter / green column
2. centrifuge 16 000 x g for 30 seconds
3. Discard the flow-through.

5m

11

1. Add 700 µl DNA/RNA Wash Buffer to the yellow filter / green column
2. centrifuge 16 000 x g for 30 seconds
3. Discard the flow-through.

5m

12

1. Add 400 µl DNA/RNA Wash Buffer to the yellow filter / green column
2. centrifuge for **2 minutes** 16 000 x g to ensure complete removal of the wash buffer
3. Carefully transfer the column into a tube (not provided)
4. discard tube and flow-through

5m

13

1. Add 100 µl ZymoBIOMICS™ DNase/RNase-Free Water directly to the yellow filter / green column matrix
2. incubate for 5 minutes
3. centrifuge 16 000 x g for 30 seconds to elute DNA and RNA from the respective yellow filter / green column
4. You can pause here (store in fridge, on ice, or freeze), but make sure sample is thawed and RT before proceeding

8m

14

1. add 600 µl ZymoBIOMICS™ HRC Prep Solution to a Zymo-Spin™ III-HRC Filter in a Collection Tube
2. Centrifuge at **8,000 x g for 3 minutes**, discard collection tube and flow through
3. Transfer prepared III-HRC Filter to a new tube (not provided)

5m

15

1. Transfer the eluted DNA and RNA (step 13) into a prepared Zymo-Spin™ III-HRC Filter
2. centrifuge at exactly **16,000 x g for 3 minutes**

5m

16

1. Discard filter, close tube lid, place on ice
2. Pipette 3 to 10 ul of each sample to new tubes for purity, quantity, and quality testing
3. Place actual samples in -20°C or -80°C freezers for storage.
4. Optionally make 5 ul and 10 ul aliquots for downstream testing and processing to reduce contamination and degradation risk.

10m