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Parallel DNA+RNA extraction from freshwater samples using the Quick-DNA/RNA Microprep Plus Kit and Zymo-Spin II-µHRC Filters (Zymo Research)

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Note: maximum of 11 samples + extraction control possible at a time

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DNA extraction, RNA extraction, Freshwater sample, Microbial community, Quick-DNA/RNA Microprep Plus Kit

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Access to a -80°C freezer
Microcentrifuge
Bead beater –> recommendation from Zymo Research is a Vortex-Genie unit with a 24 Microtube holder
ZymoBIOMICS Quick-DNA/RNA Microprep Plus Kit
Zymo-Spin II-µHRC Filters
100% EtOH (1.5 mL per sample + extraction control)
Eliminase (or another RNase-eliminating reagent)
11 microtube racks (1x Qubit tubes; 1x 2 mL tubes + samples + extra tubes; 2x 1.5 mL tubes; 3x columns; 4x collection tubes) + 1 rack for falcon tubes + 1 ice rack
Timer

Preparation (all one day in advance):
Note: 1 set refers to 1x the number of samples + extraction control
- Clean whole extraction hood with bleach, EtOH, Eliminase (this order)
- Clean microtube and falcon racks in the same way and put them in the hood
- Clean pipettes, tip boxes (1x small, 1x medium, 3x large), and hood waste the same way
- Label tubes and columns in the hood and close them after labelling (no detailed labels necessary unless specified)
  - 1 set 2 mL tubes
  - 1 set Zymo-Spin ICXM columns + collection tubes (labelled “DNA”)
  - 1 set Zymo-Spin IC columns + collection tubes (labelled “RNA”)
  - 9 more sets of collection tubes (4 sets labelled “DNA”, 6 sets labelled “RNA”)
  - 2 sets 1.5 mL tubes (1 set labelled “DNA”, 1 set labelled “RNA”)
  - 2 sets Zymo-Spin III u-HCR Filter columns + collection tubes (1 set labelled “DNA”, 1 set labelled “RNA”)
  - 2 sets 1.5 mL tubes with detailed labels (1 set labelled “DNA”, 1 set labelled “RNA”)
  - 2 sets Qubit tubes (1 set labelled “DNA”, 1 set labelled “RNA”) + 4 additional Qubit tubes for standards (2 RNA + 2 DNA)
  - 2 additional 1.5 mL tubes for DNase-mix preparation + 2 additional 5 mL tubes for Qubit solution preparation (RNA + DNA)
- Put 100% EtOH and 5 mL tubes in falcon rack
- UV-sterilize everything overnight

Procedure

1 Place filters/bead tubes (thawed) in Microtube holder on Vortex-Genie, vortex for 40 min at max speed (never more than 18 tubes at a time, because >18 tubes will slow vortexing and create inaccurate results)

2 Centrifuge bead tubes for 1 min at 13,000 xg and room temperature

3 Transfer as much of the supernatant as possible into 2 mL tubes (ideally don’t transfer any beads)

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4 Add 1 volume of DNA/RNA Lysis Buffer to samples and vortex Note: because we transferred all the supernatant from bead tubes (instead of 400 µl as specified in the protocol), the samples are too large to carry out the next steps in one go. The next steps have to be repeated, so keep all columns/tubes/mixes etc. The first and second rounds are indicated in brackets.

5 Transfer 800 µl (first round)/rest (second round) of samples into Zymo-Spin ICXM in a collection tube and centrifuge for 30 sec at 13,000 xg and room temperature. SAFE THE FLOW-THROUGH

6 Transfer Filter columns into a new collection tube

7 Add 1 volume of 100% EtOH (800 µl (first round)/600 µl (second round)) to flow-through and mix well (pipette 10x up and down)

8 Transfer sample into Zymo-Spin IC columns in a collection tube and centrifuge for 30 sec at 13,000 xg and room temperature. (Note: Only 800 µl can be transferred at a time, so this step has to be repeated; repetition can be done right away by transferring the rest of the sample into the same columns in a new collection tube and repeating the centrifugation)

9 Transfer filter columns into a new collection tube Repeat steps 5-9 using the same columns.

10 Remove all collection tubes and respective racks that are not needed anymore from the hood

11 DNase treatment:

11.1 Prepare DNase Reaction Mix: for number of samples + extraction control + 1 buffer, mix 35 µl DNA Digestion Buffer with 5 µl DNase I

11.2 Wash all columns with 400 µl DNA/RNA Wash Buffer and centrifuge for 30 sec at 13,000 xg and room temperature

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11.3 Transfer all columns in new collection tubes

11.4 Add 40 µl DNase I Reaction Mix directly to the column matrix

11.5 Incubate columns at room temperature for 15 minutes

12 Add 400 µl DNA/RNA Prep Buffer to columns and centrifuge for 30 sec at 13,000 xg and room temperature

13 Transfer all columns in new collection tubes

14 Add 700 µl DNA/RNA Wash Buffer to columns and centrifuge for 30 sec at 13,000 xg and room temperature

15 Transfer all columns in new collection tubes

16 Add 400 µl DNA/RNA Wash Buffer to columns and centrifuge for 2 min at 13,000 xg and room temperature to ensure complete removal of wash buffer

17 Transfer one column at a time into a 1.5 mL tube (no detailed labels) and add 22.5 µl ZymoBIOMICS DNase/RNase-Free Water directly to column matrix, let stand for 5 minutes, and centrifuge for 30 sec at 13,000 xg and room temperature to elute DNA/RNA in water. (Note: only 8 open 1.5 mL tubes fit into 24-Microcentrifuge at a time)

18 Place Zymo-Spin II u-HRC Filter columns in new collection tubes and add 600 µl ZymoBIOMICS HRC Prep Solution. Centrifuge for 3 min at 8,000 xg and room temperature ◊ “prepared filter columns”

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19 Transfer one prepared filter column at a time into a 1.5 mL tube (detailed labels), transfer eluted DNA/RNA into the prepared filter column, and centrifuge for 3 min at 16,000 xg and room temperature. (Note: only 8 open 1.5 mL tubes fit into 24-Microcentrifuge at a time)

20 Aliquot 2.5 µl of each final sample into Qubit tubes for concentration measurement

21 Clean an ice rack with bleach, EtOH, Eliminase, and put final DNA/RNA samples on ice

22 Carry out Qubit measurement with 2 µl of aliquoted samples

23 Store final samples at -80°C

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