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SDS-based DNA extraction method of Sediment

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We use this protocol and it's working

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Abstract

This method presents an optimized protocol of SDS-based DNA extraction method for sediment samples.
The extracted DNA can be used for metagenomic research.

Materials

Materials
MGIEasy Stool Microbiome DNA Extraction Kit (940-000122-00, MGI Tech)
Magnetic bead purification (N411-03, Vazyme) or Column purification (47014, QIAGEN)

Supplies:
- Pipette tips (assorted volumes)
- Tubes

Equipment:
- Micropipettes, various volumes
- Microcentrifuge
- Vortex
- MGISP-NE384, MGI Tech
- Magnetic rack

Sample preparation

1 Sample preparation

1.1 10 g sediment samples were firstly split into aliquots of 1 g to adapt the 2 mL centrifuge tubes and high-speed centrifuge.

1.2 After removal of the supernatants all aliquots of each sample were combined.

DNA Extraction

2 DNA Extraction

2.1 10 mL lysis solution ([M] 0.1 Mass Percent NaH2PO4, [M] 0.1 Mass Percent Na2HPO4, [M] 0.1 Mass Percent EDTA, [M] 0.1 Mass Percent Tris-HCl, [M] 1.5 Mass Percent NaCl, [M] 1 % (v/v) CTAB) were added to each sample.

2.2 The samples were shaken for 00:05:00 5m

2.3 100 µL lysozyme ([M] 100 % (v/v)) and 100 µL proteinase k ([M] 20 % (v/v)) were added to the sample.

2.4 Incubation at 37 °C for 00:30:00 . 30m

2.5 870 µL [M] 20 % (v/v) SDS was added to the sample, followed by incubation at 65 °C for 02:00:00 2h

Chloroform extraction

3 Chloroform extraction

3.1 The samples were centrifuged for 00:10:00 at 10000 x g, 25°C, and collect supernatant to a new 2 mL centrifuge tube. 10m

3.2 The supernatant was extracted twice with equal volume mixture of phenol, chloroform, and isoamyl alcohol (25:24:1 v/v/v).

Isopropanol precipitation

4 isopropanol precipitation

4.1 DNA was precipitated overnight at -20 °C with 0.6× volume of isopropanol and 0.1× volume of 3M NaAc (pH 5.2)

4.2 The precipitated DNA was washed twice with [M] 70 % (v/v) 70% ethanol, and finally resuspended in 100 µL TE buffer (pH 8).

DNA Purification 26m 30s

5 Two different methods, column purification (step 6) or magnetic bead purification (step 7), were employed to purify the manually extracted DNA.

6 Column purification (47014, QIAGEN)

6.1 Load DNA sample onto an MB Spin Column and centrifuge at 15.000 x g for 00:01:00 . 1m

6.2 Discard the flow-through and repeat step 6.1 to ensure that all of the lysate has passed through the MB Spin Column.

6.3 Carefully place the MB Spin Column into a clean 2 ml Collection Tube. Avoid splashing any flow-through onto the MB Spin Column.

6.4 Add 500 µL of Solution EA to the MB Spin Column. Centrifuge at 15.000 x g for 00:01:00 . 1m

6.5 Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.

6.6 Add 500 µL of Solution C5 to the MB Spin Column. Centrifuge at 15.000 x g for 00:01:00 . 1m

6.7 Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube.

6.8 Centrifuge at up to 16.000 x g for 00:02:00 . Carefully place the MB Spin Column into a new 1.5 ml Elution Tube. 2m

6.9 Add 100 µL of Solution C6 to the center of the white filter membrane.

6.10 Centrifuge at 15.000 x g for 00:01:00 . Discard the MB Spin Column. 1m

7 Magnetic bead purification (N411-03, Vazyme)

7.1 Mix the DNA Clean Beads thoroughly. Add of 2X volume of DNA Clean Beads to each sample tube. Mix with a vortexer until all beads are suspended.

7.2 Incubate at Room temperature for 00:05:00 . 5m

7.3 Centrifuge the tube(s) briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully remove and discard the supernatant. 5m

7.4 While keeping the tube(s) on the magnetic rack, add 500 µL of 80% ethanol to each tube to wash the beads and tube wall. Wait for 00:00:30. Carefully remove and discard the supernatant. 30s

7.5 Repeat step7.4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.

7.6 Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

7.7 Remove the tube(s) from the magnetic rack and add 50 µL of TE Buffer to elute the DNA. Mix with a vortexer until all beads are suspended.

7.8 Incubate at Room temperature for 00:05:00. 5m

7.9 Centrifuge the tube briefly and place on the magnetic rack for 00:05:00 until the liquid is clear. Carefully transfer 48 µL of supernatant to a new 1.5 mL centrifuge tube. 5m