EMP DNA Extraction Protocol

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Works for me dx.doi.org/10.17504/protocols.io.nutdewn

Earth Microbiome Project

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National Oceanic and Atmospheric Administration (NOAA)

EXTERNAL LINK

ATTACHMENTS
EMP_DNA_extraction_protocol_version_4_13.doc

GUIDELINES

**MoBio PowerMag Soil DNA Isolation Kit (Optimized for KingFisher)**

The Knight lab has transitioned to the PowerMag Soil DNA Isolation Kit (Optimized for KingFisher). We have validated a variety of sample types to ensure reproducibility when compared to MoBio PowerSoil Extraction Kit. This transition occurred to increase efficiency and reduce DNA extraction time from 6-8 hours to 2.5-3 hours.

The protocol is followed as MoBio recommends, with an added 10-minute water bath at 65°C after step 7.

The new kit can be implemented on the epMotion using a magnetic bead plate adapter for the epMotion. However, it does not reduce the amount of time the extraction takes by a significant amount: tests showed that the new kit on the epMotion took longer than the old kit. Comparison showed both the old and new kit performed well on the epMotion.

**PowerSoil-htp 96 Well Soil DNA Isolation Kit**

The MoBio PowerSoil DNA Isolation Kit is still compatible if KingFisher instrumentation is not available.

**Items included in the extraction kit (for 1-96 well extraction)**

- (1) Bead plate
- (1) Spin plate (filter)
- (1) 0.5ml collection
- (4) 1.0ml collection plates
- (2) 2.0ml collection plates
- (1) Microplate (DNA elution)
- Sealing Tape
- Centrifuge Tape
- Elution Sealing Mat
- Labeled solutions

**Important Considerations**

1. Normal diameter 1 ml pipet tips are too large for some of the pipetting steps. To get around this problem we use a Rainin 300 ul 8-channel with filtered tips (Rainin #SR-L300F) and a Rainin 1000 ul 8-channel with extended length filtered tips (Rainin #RTS-L1000XF).
2. Make sure that all of the necessary consumables and reagents are in place before you start the extraction. Remember, each pipetting step will require 1 box of 96 tips per plate.
3. We use individually wrapped reagent reservoirs and expose them to UV light for 30 minutes prior to usage.
4. In Step 10, it is important that the plate not rub against any surfaces in the shaker.
5. Make sure that the alpha-numeric grid is in the same orientation across all the plates. On a few occasions we...
have noticed that the sticker is not always in the same position on the plate.

**SAFETY WARNINGS**

Please **wear gloves at all times**.

Please refer to the SDS (Safety Data Sheet) for hazard information.

**BEFORE STARTING**

1. Clean all surfaces and pipettors to remove DNA
2. Label Plates
   - Plate #1 – 1ml collection plate
   - Plate #2 – 1ml collection plate
   - Plate #3 – 1ml collection plate
   - Plate #4 – 1ml collection plate
   - Plate #5 – 2ml collection plate
   - Plate #6 – 2ml collection plate
3. UV Sterilize and Label reservoirs
   - Bead solution – 750ul/well
   - C1 – 60ul/well
   - C2 – 250ul/well

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**MATERIALS**

<table>
<thead>
<tr>
<th>NAME</th>
<th>CATALOG #</th>
<th>VENDOR</th>
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</thead>
<tbody>
<tr>
<td>PowerMag Soil DNA Isolation Kit (Optimized for KingFisher)</td>
<td>2700-4KF</td>
<td>Mobio</td>
</tr>
<tr>
<td>PowerSoil-htp 96 Well Soil DNA Isolation Kit (if KingFisher instrumentation is not available)</td>
<td>12955-4</td>
<td>Mobio</td>
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<tr>
<td>DNA decontaminating solution (DNAaway, 10% bleach, etc.)</td>
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<tr>
<td>Large volume 8-channel pipette tips</td>
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<tr>
<td>100% Molecular grade ethanol</td>
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<tr>
<td>Sterile reagent reservoirs</td>
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<tr>
<td>Water bath set to 65°C</td>
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<tr>
<td>Ice bath</td>
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<tr>
<td>Swing bucket centrifuge capable of 4500 x g</td>
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<tr>
<td>Plate shaker with 4 metal plate adapters. This protocol uses the 96 well plate shaker (catalog #11996) listed on the MoBio website.</td>
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</tbody>
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**Citation:** Donna Berg-Lyons, Chris L. Lauber, Greg Humphrey, Luke Thompson, Jack A. Gilbert, Janet K. Jansson, Rob Knight (04/14/2018). EMP DNA Extraction Protocol. https://dx.doi.org/10.17504/protocols.io.nutdewn

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1 **BEFORE THE FIRST USE ONLY, Solution C5-D must be prepared.** Add an equal amount of 100% Ethanol to Solution C5-D (for the 4 prep kit = 120 ml, or for the 12 prep kit = 360 ml). Mix well. Put a check mark in the “ethanol added” box on the bottle cap label.

2 Centrifuge Bead Plate for 1 min at 2500 x g to pellet the beads.

3 Remove the Square Well Mat from the PowerSoil®-htp Bead Plate and set aside.

4 Add 0.1 to 0.25 grams of soil sample or sample swab. This is an appropriate stopping point and you can store the PowerSoil®-htp Bead Plate at 4°C covered with the Square Well Mat. This is the most time consuming step of the protocol. Care must be taken to avoid cross contamination between sample wells.

5 Add 750 µl of PowerSoil®-htp Bead Solution to the wells of the PowerSoil®-htp Bead Plate.

6 Check Solution C1. If Solution C1 has precipitated, heat solution at 60°C until the precipitate has dissolved.

7 Add 60 µl of Solution C1. Secure the Square Well Mat (from step 3) tightly to the plate.

8 Place sealed plates in 65°C water bath for 10 min. DO NOT SUBMERGE THE PLATES.
9. Place PowerSoil®-htp Bead Plate between the aluminum plate adapters and securely fasten to the 96 Well Plate Shaker.

10. Shake at speed 20 for 20 minutes.

00:20:00 Shaking

It is important that the plate not rub against any surfaces in the shaker.

11. Centrifuge at room temperature for 6 minutes at 4500 x g. While centrifuging, aliquot 250 µl of Solution C2 into each well of Plate #1 and cover with Sealing Tape.

00:06:00 Centrifugation

The Sealing Tape can be re-used when centrifuging Plate #1 in step 14 if handled carefully.

250 µl Solution C2

12. Remove and discard the Square Well Mat from the Bead Plate.

13. Carefully remove the Sealing Tape from Plate #1 and transfer the supernatant (~400-500 µl) from the Bead Plate to Plate #1 and pipette up and down 4 times.

The supernatant may still contain some particles.

14. Re-apply the Sealing Tape to Plate #1. Incubate at 4°C for 10 minutes.

4 °C Incubation

00:10:00 Incubation

15. Centrifuge Plate #1 at room temperature for 6 minutes at 4500 x g. While centrifuging, aliquot 200 µl Solution C3 into each well of Plate #3, then cover with Sealing Tape.

The Sealing Tape can be re-used when centrifuging Plate #3 in step 16 if handled carefully.

00:06:00 Centrifugation

200 µl Solution C3

16. After centrifugation, carefully remove and discard Sealing Tape from Plate #1.

17. Avoiding the pellet, transfer the entire volume (~600 µl depending on sample type) of supernatant in Plate #1 to Plate #2.
Apply Sealing Tape to Plate #2 and centrifuge at room temperature for 6 minutes at 4500 x g.

Carefully remove Sealing Tape from Plate #2 and Plate #3.

Avoiding the pellet, transfer the entire volume of supernatant (~600 μl) from Plate #2 to Plate #3 and pipette up and down 4 times.

Re-apply Sealing Tape to Plate #3. Incubate at 4°C for 10 minutes.

Centrifuge at room temperature for 6 minutes at 4500 x g.

Carefully remove and discard Sealing Tape from Plate #3.

Avoiding the pellet, transfer the entire volume of supernatant (~750 μl) to Plate #4.

Apply Sealing Tape to Plate #4 and centrifuge at room temperature for 6 minutes at 4500 x g. While centrifuging, add 650 μl of Solution C4 to Plate #5.

Avoiding any residual pellet, transfer up to 650 μl of supernatant in Plate #4 to Plate #5.

Add a second 650 μl (1300 μl C4 total) aliquot of Solution C4 to each well of Plate #5.

It is safe to stop the protocol at this step and store the samples covered with Sealing Tape at 4°C. Make sure to briefly centrifuge the plate to collect any condensate on the plate seal after overnight storage.

Avoiding any residual pellet, transfer up to 650 μl of supernatant in Plate #4 to Plate #5.

Add a second 650 μl (1300 μl C4 total) aliquot of Solution C4 to each well of Plate #5.

Pipet samples “up and down” to mix.

Place Spin Plate onto Plate #6.
30 Load approximately 650 μl from Plate #5 into each well of the Spin Plate and apply Centrifuge Tape.

31 Centrifuge at room temperature for 5 minutes at 4500 x g.

32 Discard the flow through and place the Spin Plate back on Plate #6. Carefully remove and discard the Centrifuge Tape.

33 Repeat steps 30-32 until all the supernatant has been processed. Discard the final flow through.

34 Place the Spin Plate back on Plate #6.

35 Confirm that ethanol has been added to Solution C5-D (see step 1). Add 500 μl of Solution C5-D to each well of the Spin Plate. Apply Centrifuge Tape to the Spin Plate.

36 Centrifuge at room temperature for 5 minutes at 4500 x g.

37 Discard the flow through and place the Spin Plate back on Plate #6.

38 Centrifuge again at room temperature for 6 minutes at 4500 x g.

39 Discard the flow through.

40 Carefully place the Spin Plate onto the Microplate. Remove Centrifuge Tape from the Spin Plate and discard.

41 Add 100 μl of Solution C6 to the center of each well of the Spin Plate. Apply Centrifuge Tape.

42 Let C6 sit on the filter for 10 minutes at room temperature before final centrifugation step.
43 Centrifuge at room temperature for 7 minutes at 4500 x g.

**Centrifugation**

44 Remove Centrifuge Tape and discard.

45 Cover wells of Microplate with the Elution Sealing Mat provided. DNA is now ready for any downstream application. No further steps are required.

Prolonged storage at 4°C will result in the evaporation of eluted DNA. We recommend storing DNA frozen (-20°C or -80°C). Solution C6 does not contain EDTA. To concentrate the DNA see the Hints and Troubleshooting Guide provided in the MoBio protocol.