Earth Microbiome Project (EMP) DNA extraction protocol

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Abstract

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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)

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- (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.

Next Steps

- 16S rRNA Amplification Protocol
- 18S rRNA Amplification Protocol

Before start

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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- C3 – 200ul/well
- C4 – 650ul/well
- C5 – 500ul/well
- C6 – 100ul/well

Protocol

Step 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.

Step 2.

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

DURATION
00:01:00 : Centrifugation

Step 3.

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

Step 4.

Add 0.1 to 0.25 grams of soil sample or sample swab.

NOTES

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.

Step 5.

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

AMOUNT
750 µl : PowerSoil®-htp Bead Solution

Step 6.

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

TEMPERATURE
60 °C : Heating solution

NOTES

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Solution C1 contains SDS. If it gets cold, it will precipitate. Heating at 60°C will dissolve the SDS. Solution C1 can be used while it is still warm.

Step 7.

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

AMOUNT
60 µl : Solution C1

Step 8.

Place sealed plates in 65°C water bath for 10 min. DO NOT SUBMERGE THE PLATES.

TEMPERATURE
65 °C : Water bath
DURATION
00:10:00 : Water bath

Step 9.

Place PowerSoil®-htp Bead Plate between the aluminum plate adapters and securely fasten to the 96 Well Plate Shaker.

Step 10.

Shake at speed 20 for 20 minutes.

DURATION
00:20:00 : Shaking

NOTES

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

Step 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.

AMOUNT
250 µl : Solution C2
DURATION
00:06:00 : Centrifugation

NOTES

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

Step 12.

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

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Step 13.

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

NOTES

The supernatant may still contain some particles.

Step 14.

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

TEMPERATURE
4 °C : Incubation
DURATION
00:10:00 : Incubation

Step 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.

AMOUNT
200 µl : Solution C3
DURATION
00:06:00 : Centrifugation

NOTES

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

Step 16.

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

Step 17.

Avoiding the pellet, transfer the entire volume (600 µl depending on sample type) of supernatant in Plate #1 to Plate #2.

Step 18.

Apply Sealing Tape to Plate #2 and centrifuge at room temperature for 6 minutes at 4500 x g.

DURATION
00:06:00 : Centrifugation

Step 19.

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

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Step 20.

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

Step 21.

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

TEMPERATURE
4 °C : Incubation
DURATION
00:10:00 : Incubation

Step 22.

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

DURATION
00:06:00 : Centrifugation

Step 23.

Carefully remove and discard Sealing Tape from Plate #3.

Step 24.

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

Step 25.

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.

AMOUNT
650 µl : Solution C4
DURATION
00:06:00 : Centrifugation

Step 26.

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

Step 27.

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

AMOUNT
650 µl : Solution C4

NOTES

It is safe to stop the protocol at this step and store the samples covered with Sealing Tape at 4°C.

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Make sure to briefly centrifuge the plate to collect any condensate on the plate seal after overnight storage.

Step 28.

Pipet samples “up and down” to mix.

Step 29.

Place Spin Plate onto Plate #6.

Step 30.

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

Step 31.

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

DURATION
00:05:00 : Centrifugation

Step 32.

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

Step 33.

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

GOTO
Repeating steps 30-32 -> go to step #30

Step 34.

Place the Spin Plate back on Plate #6.

Step 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.

AMOUNT
500 µl : Solution C5-D

Step 36.

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

DURATION
00:05:00 : Centrifugation

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Step 37.

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

Step 38.

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

DURATION
00:06:00 : Centrifugation

Step 39.

Discard the flow through.

Step 40.

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

Step 41.

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

AMOUNT
100 µl : Solution C6

Step 42.

Let C6 sit on the filter for 10 minutes at room temperature before final centrifugation step.

DURATION
00:10:00 : C6 sit on filter

Step 43.

Centrifuge at room temperature for 7 minutes at 4500 x g.

DURATION
00:07:00 : Centrifugation

Step 44.

Remove Centrifuge Tape and discard.

Step 45.

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

NOTES

Prolonged storage at 4°C will result in the evaporation of eluted DNA. We recommend storing DNA

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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.

Warnings

Please wear gloves at all times.

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

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