Aug 22, 2018

Working

Earth Microbiome Project (EMP) high throughput (HTP) DNA extraction protocol

Forked from EMP DNA Extraction Protocol

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Earth Microbiome Project

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EXTERNAL LINK

http://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/dna-extraction-protocol/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Marotz, Clarisse, et al. "DNA extraction for streamlined metagenomics of diverse environmental samples." *BioTechniques* 62.6 (2017): 290-293.

EMP_HTP_DNA_Extraction_Protocol_v07112018.docx

GUIDELINES

QIAGEN® MagAttract® PowerSoil® DNA KF Kit (384) (Optimized for KingFisher)

The Knight lab has transitioned to the QIAGEN® MagAttract® PowerSoil® DNA KF Kit (384) (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 3-4 hours.

The protocol is followed as QIAGEN® recommends, with an added 10-minute water bath incubation at 65°C after the Lysis buffer addition, and retention of the 10-minute incubation at 4°C after lysate addition to IR Solution.

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.

QIAGEN® MagAttract® PowerSoil® DNA KF Kit (384)

The QIAGEN® DNeasy® PowerSoil® Kit (formerly: MoBio PowerSoil DNA Isolation Kit; QIAGEN® catologue: 12888-100) is still used if KingFisher instrumentation is not available, or if the sample set contains less than 48 samples.

Items included in the extraction kit (for one 96 well extraction)

- (1) Bead plate
- (2) 1.0ml collection plates
- Sealing Tape
- 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™ 1000 ul 8-channel pipette with extended length filtered tips (Rainin™ RT-LTS-A-1000µL-/F/X-768/8, catologue: 30389223).
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 presterilized reservoirs for pipetting steps carried out by epMotion® liquid handlers.
4. When setting up the mechanical lysis, it is important that the plate not rub against any surfaces of/in the plate shaker.
5. Make sure that the alpha-numeric grid is in the same orientation across all the plates.

Next Steps

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

SAFETY WARNINGS

Please wear gloves at all times.

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

BEFORE STARTING

Please wear at least the minimum required personal protective equipment.
Ensure that all necessary kit components are available as well as user-supplied consumables.
Clean all working surfaces, pipettes, and pens to remove DNA contamination.

General Safety

1 **Wear gloves at all times while handling materials related to this protocol.**

Mindfully change gloves when and where appropriate to limit contamination; please wear the proper personal protective equipment (PPE) and perform all work in accordance with the institution's biological and chemical hazard control plans.

Plating Samples

2 The first step of the genomic DNA (gDNA) isolation/extraction is to add sample material to the desired wells of the 96-well PowerBead® DNA Plate(s), Garnet.

3 Remove nuclease and nucleotide contamination from work surfaces and instruments prior to starting using an appropriate solution, such as RNase AWAY™ (Thermo Scientific™ catalogue: 700511), followed by wiping with 70% to 100% molecular biology grade ethanol to remove additional contaminants.

This is the way that all surface and instrument, other than the KingFisher™ Flex (follow manufacturer documentation), cleaning steps are carried out for EMP KingFisher™ HTP gDNA extractions by the Knight Lab at University of California San Diego.

4 Remove the PowerBead® DNA Plate (Bead Plate) from the QIAGEN® MagAttract® PowerSoil® DNA KF Kit (384), and centrifuge for 1 minute at 2500 x g to pellet the garnet beads prior to sample addition.

00:01:00 Centrifugation

5 Remove the Square Well Mat from the Bead Plate and set aside in a sterile location.

The Square Well Mat will be put back on to the Bead Plate after the samples have been added.

6 Add samples to Bead Plate:

Soil Material: 0.1 to 0.25 grams per well
Swabbed Material: 1 swab head per well
Liquid Material: 250 µl or less per well

7 This is an appropriate stopping point, or continue directly to the next step.
Ensure that you continue to retain the Square Well Mat in a sterile fashion.

If stopping, use the Square Well Mat to reseal the loaded Bead Plate, and store at -20°C until ready to proceed. It is important to note the orientation that the Square Well Mat has been placed back on top of the sample-containing Bead Plate. This is easiest done by using a laboratory grade permanent marker, such as VWR® Lab Marker (VWR® catalogue: 52877-310), to write directly on the Square Well Mat denoting where well A1 is.

Sample Lysis

8 Clean all work surfaces and instruments with RNase AWAY™ reagent (Thermo Scientific™ catalogue: 700511), wipe dry, and repeat with 70%-100% ethanol, wipe dry. Turn on a water bath to 65°C.

9 In a sterile reservoir, add 400 µl RNase A Solution to 75 ml of PowerBead® Solution (Bead Solution) for every 96-well plate that will be processed.

400 µl RNase A Solution (25 mg/ml)

75 ml PowerBead® Solution

PowerBead® Solution contains guanidinium thiocyanate (CAS: 593-84-0, less than 10% w/w); handle this reagent with care, and dispose of as hazardous chemical waste in accordance with all institutional and local regulations.

RNase A Solution is also a hazardous chemical mixture (ribonuclease, CAS: 9001-99-4, less than 10% w/w), and should be disposed of properly.

RNase A Solution is stable for approximately 1 year at room temperature, 25°C. For longer storage, it is recommended that you store the RNase A Solution at 2°-8°C. The Knight Lab at UC San Diego currently uses this solution at room temperature.

COMMENTS

Thorben Sieksmeyer Nov 6, 2018 01:10 PM
edited on Nov 6, 2018 01:15 PM

Why is the RNase needed and how much should you use if you are working with the collum based PowerSoil Kit and not the plate one? And what about using Proteinase K?
Thanks in advance

10 Add 750 µl of Bead Solution/RNase A Solution to each well of the Bead Plate(s).

750 µl Bead Solution/RNase A Solution

11 Check the bottle(s) of SL Solution, Lysis buffer. If precipitate is visible, heat at 60°C until dissolved.

SL Solution contains SDS (CAS: 151-21-3, less than 10% concentration w/w), which can precipitate if cold. Heating at 60°C will dissolve the SDS; SL Solution can be used while still warm.

60 °C **Water Bath**

00:00:00 **Until precipitate dissolves**

12 Add 60 µl of SL Solution to each well. Secure the Square Well Mat (retained during sample addition to Bead Plate) tightly to the plate.

60 µl **SL Solution**

Ensure that there is a complete seal of every well in order to prevent sample cross-contamination and/or loss. It is often necessary to use both gloved hands and a plate-sealing roller.

13 Place sealed Bead Plate(s) in 65°C water bath for 10 minutes. DO NOT SUBMERGE THE PLATE(S).

65 °C **Water Bath**

00:10:00 **Water Bath**

During incubation, or prior to starting, fill an ice container that is large enough to accommodate the Bead Plate(s) with enough ice to surround the Bead Plate(s).

14 Remove excess water from the Bead Plate(s), and make sure that all wells are still fully sealed.
Place the Bead Plate(s) between two Adapter Plates (QIAGEN® catalogue: 11990) and securely fasten to a 96-well Plate Shaker (such as, QIAGEN® TissueLyser® II; QIAGEN® catalogue: 85300).

Most Plate Shakers are designed to process two plates at once. If this is the case, it is important to balance the Plate Shaker. If working with two Bead Plates, simply attach each Bead Plate to a station on the Plate Shaker. If you only have one Bead Plate to affix to the Plate Shaker, attach the sample-containing Bead Plate to one station, and a spare/empty PowerBead® DNA Plate to the second station as a balance.

15 Shake at speed 20 Hz for 20 minutes.

00:20:00 **Shaking**

It is important to make sure that the Adapter Plates, holding the Bead Plates, are properly situated in the Plate Shaker and tightly fastened. No parts should rub against the Plate Shaker during operation if attached properly.

16 Centrifuge Bead Plate(s) at room temperature for 6 minutes at 3220 x g (or 4500 x g depending on centrifuge).
While centrifuging, or during Bead Plate shaking, add 450 µl IR Solution (Inhibitor Removal Technology® Solution) to each well of a clean/empty Collection Plate (1 ml) (provided in kit), and cover with clear Sealing Tape (provided in kit).

00:06:00 **Centrifugation**

450 µl **IR Solution to clean Collection Plate**

Lysate Transfer and Aliquoting

17 Sufficiently clean the work surface and a multichannel pipette that is capable of transfering volumes up to 1000 µl.

The tips used with the multichannel pipette must be able to fit in the round wells of the Collection Plate. The Knight Lab uses Rainin™ tips (catalogue: RT-1000F).

18 Remove the lysate-containing Bead Plate(s) from the centrifuge, and carefully remove and discard the Square Well Mat.

If working with more than one Bead Plate, only uncover and work with one at a time.

19 Remove the Sealing Tape from the IR Solution-containing Collection Plate.
Transfer 640 µl, or less, lysate from each well of the Bead Plate to the Collection Plate, and mix gently by pipetting up and down 4 times.

640 µl Bead Plate lysate to IR-Collection Plate

The transferred lysate may contain some particulate matter.

20 Apply a new Sealing Tape to the lysate/IR-containing Collection Plate (repeat process if working with a second Bead Plate).
Incubate Collection Plate(s) at 4°C for 10 minutes.

4 °C Incubation

00:10:00 Incubation

21 Centrifuge lysate/IR Collection Plate(s) at 3220 x g for 6 minutes.

00:06:00 Centrifugation

22 Remove Sealing Tape from lysate/IR Collection Plate(s).

23 Transfer entire volume of supernatant (~ 850 µl), avoiding pellet, to a new/sterile 1 ml Collection Plate (Collection Plate #2). Discard the used Collection Plate(s).

Some pellet material will likely be transferred to the new Collection Plate(s) #2.

850 µl supernatant

24 Apply new Sealing Tape to Collection Plate(s) #2, and centrifuge at 3220 x g for 6 minutes.

00:06:00 Centrifugation

25 Remove the Sealing Tape from Collection Plate(s) #2, and transfer 450 µl of supernatant to a clean KingFisher™ Deep Well 96 Plate.
Transfer the remaining supernatant, 400 µl, to a second KingFisher™ Deep Well 96 Plate. Discard Collection Plate(s) #2.

450 µl supernatant

400 µl remaining supernatant

This is an appropriate place to stop. If stopping, seal the KingFisher™ Deep Well 96 Plates with plate sealing foil, not Sealing Tape from kit, and store at 4°C overnight. Do not store longer than 1 day.

ClearMag® Reagent Aliquoting

26 For each 96 well plate processed, aliquot 500 µl ClearMag® Wash Solution to each well of 3 clean KingFisher™ Deep Well 96 Plates, and 100 µl Solution EB, Elution buffer, to each well of 1 clean KingFisher™ 96 KF Microtiter (200 µl) Plate.

500 µl ClearMag® Wash Solution

100 µl Solution EB, Elution buffer

65 µl Solution EB is used for low biomass sample types.

27 For each 96 well plate processed, suspend 2 ml ClearMag® Zorb Reagent in 45 ml of ClearMag® Binding Solution in a clean reservoir. Pipette up and down thoroughly to evenly disperse the magnetic beads in solution.

2 ml ClearMag® Zorb Reagent

45 ml ClearMag® Binding Solution

The beads will settle quickly, mix thoroughly right before addition to sample.

28 For each 96 well plate processed, add 47 ml of ClearMag® Binding Solution to a separate, clean, reservoir.

47 ml ClearMag® Binding Solution

29 Add 470 µl ClearMag® Zorb Reagent/ClearMag® Binding Solution to each well of one sample lysate containing KingFisher™ Deep Well 96 Plate.

470 µl ClearMag® Zorb Reagent/ClearMag® Binding Solution

30 To the remaining KingFisher™ Deep Well 96 Plate(s) containing lysate, add 470 µl ClearMag® Binding Solution to each well.

470 µl ClearMag® Binding Solution

Loading the KingFisher™ Flex™ Purification System

31 Initiate the 'KF_Flex_MoBio_PowerMag_Soil_DNA' program on the KingFisher™ Flex™ robot. Ensure that the protocol is set to utilize both sample lysate aliquots.

Depending on the version of BindIt™ Software operating on the KingFisher™ Flex™ Purification System, the "KF_Flex_MoBio_PowerMag_Soil_DNA" script may need to be downloaded and transferred to the KingFisher™ Flex™ machine.
Currently, the robotic script for the MagAttract® PowerSoil® DNA KF Kit can be found by visiting the following site, opening the SDS/Protocols tab, and selecting the "KingFisher Flex" option under the "Robotic Scripts" header:
https://mobio.com/products/dna-isolation/soil/powermag-soil-dna-isolation-kit.html

https://mobio.com/products/dna-isolation/soil/powermag-soil-dna-isolation-kit.html

If using two aliquots of the sample lysate for purification, as in this EMP protocol, add an additional sample binding step after the first (before the first wash step) in the BindIt™ Software, and transfer the modified protocol to the robotic platform.

32 Follow the onscreen prompts to properly load the KingFisher™ Flex™. The loading order should be: the tip comb, elution plate, ClearMag® Wash Solution filled plates, lysate with ClearMag® Binding Solution plate (Bind 2), and lysate containing ClearMag® Binding Solution/ClearMag® Zorb Reagent plate (Bind1).

Running the KingFisher™ Flex™ Purification System

33 The selected KingFisher™ Flex™ program will execute itself once the final plate is added and "Start" is pressed. The program takes approximately 65 minutes to complete, and requires no user intervention.

Unloading the KingFisher™ Flex™ Purification System

34 When the KingFisher™ Flex™ program finishes, remove the gDNA containing elution plate, and seal this with an appropriate storage seal (not Sealing Tape from kit).

35 Follow the onscreen prompts to cycle through each station on the KingFisher™ Flex™ deck.

Dispose of all liquids from plates as hazardous chemical waste (the gDNA elution plate should already be removed and appropriately sealed), and discard the emptied plates. The gDNA is now ready for downstream applications.

COMMENTS

Amandine Avouac May 20, 2019 02:44 PM

Hello, really good protocol, thanks for it. I was wondering what is the nature of the wash solutions, which chemicals are in it ? And same, what is the composition of the elution buffer which is supposed to make ADN neutral ? Thanks for your answers, I'm a student in molecular biology so I'm still learning and I want to understand and know what is inside the solutions I use ! :)

Janet Williams Feb 1, 2019 01:50 AM
edited on Feb 1, 2019 01:50 AM

In Step 13, there is a note to fill an ice container large enough to accommodate the Bead Plate. When is this used?
Thanks,
Janet