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DNeasy PowerSoil Pro for Filters Extractions

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Protocol status: Working
We use this protocol and it's working

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Abstract

DNA Extraction protocol for low input (50ml cultures) filtered seawater.

Prep

1 Always wear gloves when doing molecular work - clean gloved hands with 70% etOH often and every time you touch anything not already on the molecular bench (e.g., hair, face, clothes, the fridge ...)

Remember to use filtered pipette tips for all molecular work

2 Set Mini-Incubator Temperature to 65ºC

3 Clean all surfaces and utencils (dissecting scissors and forceps) with 70% etOH

4 Fill ice bucket

5 Tube Prep per Sample (set out this many tubes for each sample)
1 x Power Pro Bead Tube
1x MB Spin Column *(do not put spin columns in heated incubator)*
3× 2 ml Microcentrifuge Tube
1 × 1.5 ml Elution Tube

(Do not reach hands all the way into the bags, lay bags flat and work tubes towards opening, then only take the tubes closest to the opening - key to avoid skin contact with inside surfaces of the bag)

6 Get 1 petri dish for every 2 samples (use bottoms and lids for cutting filters)

7 Put **CD3** in 65ºC incubator

8 Grab **CD2** from the fridge

Extraction 20m

9 Randomly select samples and place on ice - Multiples of 8

(try not to do all replicates for a given treatment in one extraction round)

10 Add 800 µl of **CD1** to all bead tubes being used

Put **CD1** back in the box.

11 Remove first sample from ice, and let thaw briefly if necessary (when they first come from –80 they will be very crunch, this is only necessary for the first few samples, they will thaw over ice)

Remove filter from tube with sterile forceps and use clean dissecting scissors to cut the filter into thin strips over a sterile petri dish. Put little pieces of filter into the bead tube so that all pieces are submerged in buffer. Label bead tube with sample number from lid of tube that the filter came out of - save the filter tube to record full sample information later.

Clean dissecting scissors and forceps with ethanol between samples, use clean petri dish bottom or top for each sample.

12 Put all bead tubes with shredded filters in the mini incubator at 65ºC for 10 minutes. 10m
00:10:00
(reminder: do not put spin columns in heated incubator)

Remove **CD3**

while incubating, label all tubes from step 4 with sample numbers

13 Move bead tubes to the vortex adapter (white plate with projections to secure tubes) on the Vortex Genie 2- place tubes so cap is towards center, makes sure tubes "click" into place, try to balance the tube placement as if it was a centrifuge 10m

Vortex all for 10 minutes on high 00:10:00

14 Move bead tubes to the centrifuge rotor (balanced!) - put centrifuge rotor in centrifuge and centrifuge for 1 minute at 15,000xG 15,000 x g, 00:01:00

15 Set the Blue 1ml/1000µl pipettor to 1000µl and remove all liquid from bead tube by pressing the filter fragments against the wall of the tube first and move liquid to clean 2ml microcentrifuge tube (with matching sample number), then put tip down into the beads and remove any remaining liquid, transfer to same microcentrifuge tube (some bead pick-up is OK, but excessive bead pick-up can clog the pipette tip)

Discard bead tube

16 Move microcentrifuge tube to the centrifuge rotor and centrifuge for 1 min at 15,000xG
15000 x g, 00:01:00

17 Avoiding pellet (white film on bottom side of tube, sometimes some beads) - move 600 µl supernatant to a clean 2ml microcentrifuge tube with matching sample name.

Discard previous microcentrifuge tube.

18 Use green pipettor with green tips to add 200µl of **CD2** to each microcentrifuge tube, vortex for 5seconds (ish) and move to centrifuge.

This will make the sample become white and cloudy.

Put **CD2** back in the **refrigerator**

19 Centrifuge microcentrifuge tubes for 1 min at 15,000xG 15000 x g, 00:01:00

20 Avoid pellet (obvious white gelatin-looking substance at bottom of tube) and move 600µl supernatant to a clean 2 ml microcentrifuge tube with matching sample number.

Discard previous microcentrifuge tube.

21 Give **CD3** a gentle swirl (no shaking, it will get foamy!) and add 600µl to each sample

CD3 is a salt solution, when added you will see density gradients

Vortex 5 seconds - until solution is homogenized

Put **CD3** back in the box.

22 Add 650 of sample to the top of the MB spin column with matching sample name
**Pipette slowly to avoid bubbles and splash back

23 Centrifuge MB spin columns for 1 min at 15,000xG 15000 x g, 00:01:00

24 Gently remove the spin column from the collection tube, hold spin column while dumping filtrate into the garbage, return spin column to collection tube and place back in tube rack

** be careful to not touch sides of spin column, as this could contaminate samples

25 Move remaining sample (~550µl, but leave the pipette set to 650) to top of spin column

Discard previous microcentrifuge tube.

26 Centrifuge MB spin columns for 1 min at 15,000xG 15000 x g, 00:01:00

27 Move spin columns to new, clean collection tubes (a.k.a. waste tubes in box, 2 ml tubes without caps, can be clear or opaque grey)

28 Add 500 µl of solution **EA** to top of each spin column

Put solution **EA** back in the box

29 Centrifuge MB spin columns for 1 min at 15,000xG 15000 x g, 00:01:00

30 Discard flow-through by gently removing MB spin column from collection tube and empty the collection tube into the garbage. Place MB spin column back into the same collection tube.

Be careful not to touch the sides of the spin column

31 Add 500 µl of **CD5** to tops of spin columns

Put **CD5** back in the box.

32 Centrifuge MB spin columns for 1 min at 15,000xG 15000 x g, 00:01:00

33 Move spin columns to new clean collection tubes (a.k.a. waste tubes in box, 2 ml tubes without caps, can be clear or opaque grey)

34 Centrifuge MB spin columns for **2 minutes at 16,000g** 16000 x g, 00:02:00

This step dries the column (the collection tubes will look mostly empty after centrifuging)

35 Move spin columns to 1.5 ml elution tubes (these have caps, the elution tube caps stay open ...)

36 Pipette 50µl (green pipettor with green tips) of **CD6** directly onto the center of the white silicate region of the spin column, but DO NOT touch the spin column filter. It is especially important to make sure that solution does not end up on plastic rim at the edge of the filter column.

Put **CD6** back in the box.

37 Place Spin columns in elution tubes into centrifuge rack with elution tube caps pointing inward.

Centrifuge for 1 min at 15,000g (don't forget to change the time and speed back).

15000 x g, 00:01:00

38 Remove spin columns and close elution tube caps (double check numbers match), discard spin column and put elution tube into aluminum cold rack and then place cold rack either on ice or in the fridge

- aluminum cold racks live in the fridge
- if ice is melted, drain the excess water into the sink. (Refill if necessary, or keep samples in the fridge)
- This is a good break point (if needed)! Samples can hang out on ice or in fridge for up to an hour.

QC - Qubit 1x dsDNA HS Assay

39 Take out:

- Qubit Flex Assay Tube Strips (8-tube strips, look like PCR tubes, but optically optimized for Qubit), 1 strip for each standard, and 1 tube / sample (for 16 samples, 2 strips)
- Run standards once a week
- Plastic reservoir for multipipetting - in white cardboard box in glass cabinet
- Qubit 1x dsDNA HS Assay working solution - opaque bottle in cabinet
- Qubit 1x dsDNA HS Assay Standards in box in fridge

40 (number of standards + samples) * 200 + 100 = µl of working solution to put into plastic reservoir

Use blue pipette and repeat 1ml as necessary

41 Use 200µl 8-channel multipipettor to put 190µl working solution into standard tubes and 195µl of working solution into sample tubes.

Resue tips if careful (green tips)

If not running standards, move to step 43.

42 If running standards, use 200µl repeater pipettor to put 10µl of standard 1 into all 8 standard 1 tubes and 10 µl of standard 2 into all eight standard 2 tubes.

Put pipette tip into working solution to ensure standard leaves the tip (surface tension!)

Change tips between standards (green tips)

43 Use red pipettor to add 5µl of sample to corresponding sample tubes. Do samples in order and label the start and end number of samples on the strip tab.

44 Vortex all strips

45 Quick spin all strips

46 Incubate in the dark for 2 minutes

If standards were used, return them to the fridge.

47 Open 1x dsDNA HS Assay program on Qubit instrument.

Select whether you are running standards or not (check date last run).

Before loading samples, ensure that there are no bubbles in the tubes.

48 If running standards, load standard 1 strip into the instrument and run sample. Ensure that the correct number of tubes is selected.

Once complete, load the standard 2 strip into the instrument and run sample. Again, ensure that the correct number of tubes is selected.

A standard curve should be established.

49 If not running standards
Put 1st strip into machine and select run samples.
Check that the right tubes are selected for measurement, that the units are correct (ng/µl), and that the volume is correct (5µl).

Run samples
Record values.
Select Add samples
Put 2nd strip into machine
Check that the right tubes are selected
Run samples.
Record values.

Recording and clean-up

50 Label sample tubes with experiment name

Put tubes into the extraction box in the -80ºC freezer in the numbered space in the box

51 Empty ice bucket and let it dry.

Put kits away and wipe everything down with 70% ethanol

52 Enter values into designated experiment spreadsheet.

PCR

53 Take out:

- Forward and reverse 16S Illumina primers from the -20ºC freezer and allow to thaw.
- KAPA HiFi HotStart from the -20ºC freezer and allow to thaw.
- PCR tube strips (use a single tube for 1 sample, a strip of 8 tubes for 8 samples, sets of 32 for 32 samples, etc.)
- 1.5 ml DNA Lobind tube for Master Mix (label MM)
- Extraction samples from the -80ºC freezer and allow to thaw.

54 To make the Master Mix, pipette in order:

- (1 µl forward primer) x (sample number+1) = forward primer volume
for example, 9 µl forward primer 8 samples
- (1 µl reverse primer) x (sample number+1)
- (12.5 µl KAPA HiFi Hotstart) x (sample number+1)

Mix by pipette

55 Once thawed, flash centrifuge extraction samples.

56 Use 200µl repeater pipettor to put 14.5 µl of Master Mix into all PCR tubes.

57 Pipette 10.5 µl of sample into the corresponding PCR tube using the grey VWR pipette with red pipette tips. Mix sample and Master Mix by pipette, change pipette tip for each sample.

58 Ensure that the lids are secure and quick spin all PCR tubes (if doing 32 samples, place 32-well adhesive seal on top of all samples, ensure that each well is secure, flash centrifuge).

59 Open thermal cycler and load blue rack. Try to center samples in the middle, and use empty tube strips on either side to balance if necessary.

60 Select *set up run* on the screen. Select *open method* and choose the method called *kapa hifi 16s.* The screen will display the procedure with durations and temperatures for the different stages. The volume should say 25 µl.

61 Click *next* and *start run.* Watch the screen until the procedure reaches "stage 2" to ensure that it did not stop running.

62 Clean all surfaces with 70% ethanol. Return extraction samples to the -80ºC freezer, and return KAPA Hifi Hotstart and Illumina primers to their designated bags/boxes in the -20ºC freezer