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SPOT DNA Extraction from 142mm A/E 1µm Filters

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

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Abstract

Reference: Lie et al, 2013. Small-scale temporal and spatial variations in protistan community composition at the San Pedro Ocean Time-series station off the coast of southern California. *Aquatic Microbial Ecology* 70, 93-110.

Materials

All the supplies needed for 1 sample.

	A	B
	50 ml falcon tube	4
	0.25 M EDTA, pH8	1.984 ml
	1M Tris, pH 8	1.24 ml
	2.5 M NaCl	6.096 ml
	10% SDS	1.24 ml
	Autoclaved Milli-Q water	7.44 ml
	500 ul of 0.5 mm beads	1
	20 ml syringe	1
	10% CTAB	2 ml
	100% EtOH (200 proof, molecular grade)	58.75 ml
	10.5 M ammonium acetate	6.25 ml
	Oak Ridge tube	2
	1X TE buffer	1100 ul
	2 ml non-lobind tube	plenty
	phenol	1600 ul
	chloroform:Isoamyl alcohol	1600 ul
	1.5 ml lobind tube	2

Troubleshooting

Before start

Preparation (prepare all solutions with DI water, then autoclave before use):

1. 0.25 M EDTA, pH 8
2. 1 M Tris (Trizma base), pH 8
3. 2.5 M NaCl
4. 10% CTAB (hexadecyltrimethylammonium bromide)
5. 10.5 M ammonium acetate

Exception!

6. 10% SDS (w/v). Prepare with autoclaved DI water. DO NOT AUTOCLAVE!

Day1

1 Prepare a 70°C water bath.

Note

Microwave water to heat faster, then cover the water bath with foil.

2 Prepare a razor blade, forceps, and a metal slab.

Note

Keep the metal slab in the fridge before use, and put ice packs under the metal slab while using it. By doing so, you can keep the metal slab cold as much as possible and thus minimize decay.

3 Cut the 142mm A/E filter into pieces on the metal slab and place it into a 50 ml falcon tube.

Note

The 142 mm A/E filters cannot be opened while frozen. It would help if you thawed filters before cutting them, but don't leave them thawed longer to prevent decay. (The decay is much less once stuff is in lysis buffer and heated to 70°C)

4 Add 6.2 ml of 2X lysis buffer and 500 µl of 0.5 mm zirconia/silica beads.

Note

To prepare 200 ml of 2X lysis buffer

	A	B	C
		Volume	Final concentration
	0.25 M EDTA, pH 8	32 ml	40 mM
	1 M Tris, pH 8 (Trizma base)	20 ml	100 mM
	2.5 M NaCl	8 ml	100 mM
	10% SDS (w/v)	20 ml	1%
	Autoclaved Milli-Q water	120 ml	

Note

Prepare beads in 0.5 ml tubes. This way you can prepare a bunch and directly measure the volume by marks on 0.5 ml tubes.

5 Bead-beat (vortex) for 1 min and then place in 70°C water bath for 7 min. Repeat this step 4 times.

6 Spin at 3901 rcf in the swinging bucket rotor (#4250) on Beckman Allegra at 20°C for 2 min.

7 Transfer the lysate to an Oak Ridge tube (labeled with “a”)

8 Add 6.2 ml of 2X lysis buffer on the filter (still in the original 50 ml falcon tube) and repeat step 5 again to increase yield.

Note

No need to add beads since there are plenty in tubes.

9 Transfer the lysate to a new Oak Ridge tube (labeled with “b”) with a 20 ml syringe.

Note

Make sure you have the tube under the syringe to catch drips.

Note

Transfer the lysate and the filter to a syringe.

Note

A syringe will allow you to squeeze as much lysate out of the filer as possible.

10 Ideally, "a" and "b" Oak Ridge tubes both have 6.2 ml of lysate, but you may get more lysate from "b". In this case, pour "b" lysate to "a" to equalize the volume.

11 Add 1 ml of 10% CTAB (hexadecyltrimethylammonium bromide) and 2.8 ml of 2.5 M NaCl.

Note

	A	B	C
		Volume	Final concentration
	Lysate	6.2 ml	
	10% CTAB	1 ml	1%
	2.5 M NaCl	2.8 ml	0.7 M

12 Incubate in a 70°C water bath for 10 min.

13 Add 28 ml of ice-cold 100% EtOH (200 proof, molecular grade) and 3 ml of 10.5 M ammonium acetate.

14 Invert to mix thoroughly.

15 Store at -20°C overnight.

Day2

16 Spin at 24,000 g / 13,000 rpm in the swinging bucket rotor on Beckman Avanti JE or Beckman
Sorvall at 4°C for 2 hrs.

> Note
>
> The swinging bucket rotor pellets the DNA at the very bottom of the tube. Do not use a fixed-angle rotor or DNA will be smeared along the side.

17 Gently pour out supernatant and dry pellet upside-down in the fume hood for at least 2 hours.

> Note
>
> Keep supernatants in labeled 50 ml falcon tubes at -20°C before you quantify DNA concentration and make sure everything is fine.

18 Resuspend the pellet in 500 µl sterile 1X TE buffer for 2 hours at 37ºC.

19 Transfer to 2 ml non-LoBind tubes.

20 Add 500 µl phenol (bottom layer). Mix several times by gentle inversion.

Note

The shelf life of phenol is ~6 months with equilibration buffer added and stored at 4°C. If acidic, DNA will not be in the aqueous layer. DO NOT use the phenol that is >6 months old.

Note

Phenol is covered with a buffer so pipette from the bottom layer of the container.

Note

Check that phenol is equilibrated to pH 8.0 every time before using it:
Pipet a small amount of phenol into a microcentrifuge tube, add an equal amount of water, mix by inversion, spin to separate layers, and test the water with pH paper.

21 Spin for 2 min at 12000 rpm (13,000xg).

22 Remove the phenol (bottom layer) but leave the interface behind.

Note

The interface is the line between organic and inorganic phases

Note

Keep phenol layer in labeled 2ml non-Lobind tubes at -20°C before you quantify DNA concentration and make sure everything is fine.

23 Add 300 µl phenol and 300 µl chloroform:Isoamyl alcohol to the aqueous phase.

24 Mix several times by gentle inversion. Then spin for 2 min at 12000 rpm (13,000xg).

25 Remove the bottom layer but leave the interface behind.

Note

Keep the bottom layer in labeled 2ml non-Lobind tubes at -20°C before you quantify DNA concentration and make sure everything is fine.

26 Add 500 µl chloroform:Isoamyl alcohol.

27 Mix by gentle inversion. Then spin for 2 min at 12000 rpm (13,000xg).

28 Transfer the TOP layer (YOUR DNA) to a new 2ml non-LoBind tube. LEAVE THE INTERFACE BEHIND. This top layer is your DNA!!!

29 Add 1375 µl ice-cold 100% EtOH (200 proof, molecular grade) and 125 µl 10.5M ammonium acetate.

30 Mix by inversion 5-10 times.

31 Store at -20°C overnight.

Day3

32 Spin at 20,000 g on Beckman Avanti JE at 4°C for 2 hrs.

33 Decant and air-dry the pellet in a bio-safety hood for 2 hrs.

Note

Keep supernatants in labeled tubes at -20°C before you quantify DNA concentration and make sure everything is fine.

34 Resuspend the pellet in 30-50 µl sterile 1XTE buffer.

Note

Resuspend the pellet in 50 µl sterile 1XTE buffer for surface and DCM samples.
Resuspend the pellet in 30 µl sterile 1XTE buffer for 150m, 500m, and 890m samples.

35 Vortex and quick spin twice. Then, incubate at 40 °C for 1 hr.

36 After re-suspension, immediately quantify using PICO Green (Invitrogen).

37 If "a" and "b" both get good yields, pool "a" and "b". Then transfer half of the total volume to a 1.5 ml LoBind tube for archiving, and transfer another half to a 1.5 ml LoBind tube for working stock.