SPOT DNA Extraction from 142mm Durapore 0.22µm Filters

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Abstract

Reference: Fuhrman et al, 1988. Extraction from Natural Planktonic Microorganisms of DNA Suitable for Molecular Biological Studies. AEM 54(6), 1426-1429

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Guidelines

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Supplies:
a) Dry ice
b) 15 ml falcon tube – 1 per filter
c) Oak Ridge tube – 1 per filter; Nalgene #3119-ÃÂÃÂÃÂ0050 (polypropylene)
d) 2 x 2ml NON lo-ÃÂÃÂÃÂbind tube/filter for 1st and 2nd DNA precipitation steps (Day 2 steps 4, 14)
e) 2 x 1.5ml loâÃÂÃÂÃÂbind tube/filter (Day 3, step 6)
f) 1 x PCR tube/filter for 2ng/µl dilution
g) pH paper, range 5.5âÃÂÃÂÃÂ9

Reagents: Make all solutions with autoclaved 0.02µm filtered MilliQ water and autoclave.
a) 10X STE: 100mM Tris-Cl (pHâÃÂÃÂÃÂ8.0), 1M NaCl, and 10mM EDTA). Dilute to 1X STE.
b) 10% SDS: do NOT autoclave.
c) 10.5M NH_4OAC
d) 100% Ethanol (200 proof, molecular grade)
e) 1X TE, pH 8.0: 10mM Tris-ÃÂÃÂÃÂCl (pH 8.0) and 1mM EDTA
f) Phenol, pH 8
g) SEVAG aka chloroform: isoamyl alcohol
h) Phenol: chloroform: isoamyl alcohol, pH 8 (optional)

Day 1 Note: **Process in sets of 4 samples through step #9 and spin all together.

Reference:
Fuhrman et al, 1988. Extraction from Natural Planktonic Microorganisms of DNA Suitable for Molecular Biological Studies. AEM 54(6), 1426-ÃÂÃÂÃÂ1429

Protocol

Day 1

Step 1.

Prepare a boiling water bath; this MUST be a rolling boil.

NOTES

Jed Fuhrman 10 Aug 2015

Microwave water to heat faster, then put on hot plate, cover with foil, and boil.

Jed Fuhrman 10 Aug 2015

Using a full 1-2L beaker is recommended to prevent heat loss when you add samples.

Jed Fuhrman 10 Aug 2015

**Process in sets of 4 samples through step #9 and spin all together.

Day 1

Step 2.

Place 50ml falcon tube with 142mm filter on dry ice.

NOTES

Jed Fuhrman 10 Aug 2015

Filter MUST be kept totally FROZEN at all times to crush the filter.

Day 1

Step 3.

Using the bottom of an untouched STERILE 15ml polypropylene falcon tube as a pestle, vigorously crush the 142mm filter while in the 50ml falcon until it has been pulverized and the filter material is BELOW the 10ml line.

NOTES

Jed Fuhrman 10 Aug 2015

Only handle the 15ml falcon by the top lid, and use the conical base as the pestle.

Day 1

Step 4.

Add 9mls of 1X STE directly to the crushed filter pieces.

Day 1

Step 5.

Vortex for 10 seconds.

DURATION

00:00:10

Day 1

Step 6.

Add 1ml of 10% SDS drop-wise while swirling.

NOTES

Jed Fuhrman 10 Aug 2015

It is important to MIX SDS into STE buffer.

Day 1

Step 7.

Spin briefly in Eppendorf 5810R centrifuge at 15-25ºC by spinning up to 4000 rpm (3220xg) and immediately stopping.

Day 1

Step 8.

Place in rolling, boiling water bath for 2 minutes.

DURATION

00:02:00

Day 1

Step 9.

Spin at 4000 rpm (Eppendorf 5810R) for 10 minutes at 15ºC to separate filter and debris.

DURATION

00:10:00

NOTES

Jed Fuhrman 10 Aug 2015

This MUST be 15ºC (or room temp) because at lower temperatures SDS will precipitate, and you will lose DNA along with it.

Day 1

Step 10.

Add 3mls of 10.5M NH4OAC to an Oak Ridge tube for each filter.

Day 1

Step 11.

Pour supernatant (only) from 50ml falcon tube into Oak Ridge tube (38ml capacity).

Day 1

Step 12.

Use 1ml pipette to transfer any remaining supernatant to Oak Ridge tube: 0.5-1ml.

Day 1

Step 13.

Remove any large filter pieces using STERILE 1ml serological pipette to reach the bottom and drag the filter pieces up the sides.

NOTES

Jed Fuhrman 12 Aug 2015

(or with wide bore plastic pipette tip)

Day 1

Step 14.

Add 28mls of 200 proof (100%) EtOH (molecular grade).

Day 1

Step 15.

Invert to mix thoroughly.

Day 1

Step 16.

Store at -20°C overnight.

DURATION

18:00:00

Day 2

Step 17.

Pellet DNA in Sorvall RC5-B at 4ºC using the HB-4 rotor by spinning at max speed of 13,000RPM (27,900xg) for at least 2 hours.

DURATION

02:00:00

NOTES

Jed Fuhrman 10 Aug 2015

Sorvall takes ~30 minutes to cool to 4-10ºC and gauge may only read ~11000 RPM

When finished for the day, turn off, let warm to room temp, and wipe out any liquid with Kimwipe.
Jed Fuhrman 10 Aug 2015
Note: this 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.

Day 2

Step 18.
Gently pour out supernatant and dry pellet upside-down in fume hood for at least 2 hours.

DURATION
02:00:00

Day 2

Step 19.
Resuspend in 500ul 1X TE (pH 8.0) for 2 hours at 37ºC.

DURATION
02:00:00

Day 2

Step 20.
Transfer to 2ml non-LoBind tube.

Day 2

Step 21.
Add 500µl phenol.

NOTES
Jed Fuhrman 10 Aug 2015
Shelf life is ~6 months with equilibration buffer added and stored at 4ºC. If acidic, DNA will not be in the aqueous layer. Phenol is covered with buffer so pipette from the bottom of the container and blow out the pipette before pulling up.
Jed Fuhrman 12 Aug 2015
Check every time (once per day) that phenol is equilibrated to pH 8.0.
TO TEST pH: either a) test the equilibration buffer with pH paper (range 5.5-9) or b) pipet small amount of phenol into microcentrifuge tube, add equal amount of water, mix by inversion, spin to separate layers, and test water with pH paper.

Day 2

Step 22.
Mix 5 times by gentle inversion.

Day 2

Step 23.
Spin 2 min at 12000 rpm (13,000xg) to separate organic and inorganic phases (Beckman, beige)

DURATION
00:02:00

Day 2

Step 24.
Remove and discard bottom (phenol) layer. LEAVE INTERFACE.

NOTES
Jed Fuhrman 15 Aug 2015
INTERFACE is the line between organic and inorganic phases

Day 2

Step 25.

Add 300µl phenol and 300µl SEVAG (CHCl3:IsoamylOH 24:1 v/v) to aqueous phase.

NOTES

Jed Fuhrman 11 Aug 2015

Alternatively, add 600µl of pre-
mixed phenol:chloroform: isoamyl (25:24:1). Check it is at pH=8 before use.

Day 2

Step 26.

Mix by gentle inversion 5 times.

Day 2

Step 27.

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

DURATION

00:02:00

Day 2

Step 28.

Remove and discard bottom layer. LEAVE INTERFACE.

Day 2

Step 29.

Add 500µl SEVAG (CHCl3:IsoamylOH 24:1 v/v).

Day 2

Step 30.

Mix by gentle inversion.

Day 2

Step 31.

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

DURATION

00:02:00

Day 2

Step 32.

Remove TOP layer (YOUR DNA) and transfer to new 2ml non-LoBind tube.

LEAVE INTERFACE BEHIND. This top layer is your DNA!!!

Day 2

Step 33.

Add 125µl 10.5M NH4OAC. Mix by inversion 5-10 times.*

NOTES

Jed Fuhrman 12 Aug 2015

*You want to add 0.25 volumes of 10.5M NH_4OAc and 2.2 volumes Ethanol for a final concentration of 2.5M NH_4OAc and 70% Ethanol. We assume that extract volume is 500µl. If your volume differs, adjust amounts of NH_4OAc and ethanol accordingly. For 500ul: 25µl NH_4OAc: 0.25 x 500 = 125µl. 1375µl EtoH: 2.2 (500+125) = 1375µl

Day 2

Step 34.

Add 1375 µl ice-cold 100% EtOH (200 proof, molecular grade).*

NOTES

Jed Fuhrman 12 Aug 2015

*You want to add 0.25 volumes of 10.5M NH_4OAc and 2.2 volumes Ethanol for a final concentration of 2.5M NH_4OAc and 70% Ethanol. We assume that extract volume is 500µl. If your volume differs, adjust amounts of NH_4OAc and ethanol accordingly. For 500ul: 25µl NH_4OAc: 0.25 x 500 = 125µl. 1375µl EtoH: 2.2 (500+125) = 1375µl

Day 2

Step 35.

Mix by inversion 5-10 times.

NOTES

Jed Fuhrman 11 Aug 2015

Small bubbles may appear, this is ok.

Day 2

Step 36.

Precipitate overnight at -20ºC.

DURATION

18:00:00

Day 3

Step 37.

Spin at max speed (approximately 13,750RPM, 12,535xg) in Beckman Microfuge E with **horizontal** rotor at 4ºC (cold room) for 30 minutes to pellet most DNA in the center/bottom of tube.

DURATION

00:30:00

Day 3

Step 38.

To pellet the rest of the DNA, spin the same tube for at least 90 minutes at 14,000 rpm (20,800xg) at 4ºC (Eppendorf 5810R) .

DURATION

01:30:00

Day 3

Step 39.

Gently pour off supernatant.

Day 3

Step 40.

Dry pellet for at least 2 hrs by leaving open and upside-down.

DURATION

02:00:00

Day 3

Step 41.

Resuspend 5m and CMAX samples in 50µl of 1X TE and 150m, 500m, and 890m samples in 30ul 1X TE for 2 hours at 37ºC.

DURATION

02:00:00

Day 3

Step 42.

Transfer all re-suspended extract to 1.5ml **Eppendorf LoBind DNA tube**.

Day 3

Step 43.

Aliquot 10µl of extract to an additional 1.5ml **Eppendorf LoBind DNA tube** for archiving.

Day 3

Step 44.

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

NOTES

Jed Fuhrman 11 Aug 2015

To be within range of the picogreen standard curve (05-20ng), you may need to dilute the extracts if from an initial sample volume of 10-20L seawater: 1:30 for 5m, DCM and 1:10 or 1:15 for deeper depths.

Day 3

Step 45.

Prepare 2ng/µl dilution for a working stock using no more than 1µl of extract.

Day 3

Step 46.

Store dilution, extract, and archive at -80ºC

NOTES

Jed Fuhrman 11 Aug 2015

Archive DNA should NOT be in the same freezer as MAIN stocks.

Jed Fuhrman 11 Aug 2015

Use the 2ng/µl extract for downstream ARISA PCR, etc.

Warnings

Day 1 Note: **Process in sets of 4 samples through step #9 and spin all together.