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Supplementary information

Extracting DNA from ocean microplastics: a method comparison study

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Protocol 1: GentraPuregene Tissue kit (Qiagen)

Adapted from Gentra® Puregene® Handbook – Qiagen, https://www.qiagen.com

Preparation

Prepare sterile scooper

Provide pipettes and pipette tips

Clean centrifuge

Clean Vortex

Sterilize beads

Decontaminate Bead-Beater

Aliquot:

- 100% isopropanol
- 70% EtOH
- RNase A
- Proteinase K (10U/µl)

Dilute Ready-lyse enzyme to 1000U/µl

Separate mortars, pestles and liquid nitrogen

Set water baths/incubators to required temperatures

Protocol

1. Add 1ml Puregene Cell Lysis solution to 2ml tubes.
2. Place whole or ground-up (liquid nitrogen) plastic fragments in tube.
3. Add 10µl Ready Lyse Lysozyme (diluted to 1000U/µl)to each tube and invert 25 times.
4. Incubate at 37° for 30 min.
5. Add 5µlProteinase K to each tube and mix gently.

6. Add ~0.4gof sterile 0.1mm zirconium beadsto tubes (for liquid nitrogen-ground plastics, skip steps 6 and 7).
7. Bead-beat for 90 seconds using bead-beating apparatus.
8. Incubate at 80˚C for 5 min.
9. Add 4µlRNAse A and mix by inverting the tube 25 times.
10. Incubate at 37˚C for 30 min, then ice for 5 min.
11. Add 250µlGentra Systems Protein Precipitation Solution.
12. Vortex vigorously for 20 sec and place tube on ice for 5 min.
13. Centrifuge at 13-16,000 x *g* for 3 min to precipitate protein (and beads) and transfer supernatant to a fresh tube.
14. Repeat spin and transfer a defined quantity of supernatant to a fresh tube containing 750µl 100% Isopropanol. Invert to mix.
15. Centrifuge at 13-16,000 x *g* for 5 min. to pellet DNA.
16. Discard supernatant and add 750µl 70% EtOH. Invert several times.
17. Centrifuge at 13-16,000 x *g* for 3 min.
18. Discard supernatant and allow DNA pellet to dry (to evaporate EtOH).
19. Re-suspend in 40µl 65°C Gentra Systems DNA rehydration buffer.

Protocol 2: MP Fast Spin kit (MP Biomedicals)

Adapted from MP BIOMEDICALS FastDNA™ SPIN KIT Protocols, http://www.mpbio.com/

Preparation

Prepare sterile scooper

Provide pipettes and pipette tips

Clean centrifuge

Clean Vortex

Decontaminate Bead-Beater

Dilute Ready-lyse enzyme to 1000U/µl

Separate mortars, pestles and liquid nitrogen

Set water baths/incubators to required temperatures

Protocol

1. Place whole or ground-up (liquid nitrogen) plastic fragments in Lysing Matrix A tube.
2. Add 1.0 ml of Cell Lysis Solution (in this case CLS-TC).
3. Add 10µl Ready Lyse Lysozyme (diluted to 1000U/µl) to each tube and invert 25 times.
4. Incubate at 37° for 30 min.
5. Bead-beat for 4 min using bead-beating apparatus (for liquid nitrogen-ground plastics, skip step 5).
6. Centrifuge at 14,000 x *g* for 5-10 min to pellet debris.
7. Transfer supernatant (600 – 700 µl) to a 2.0 ml microcentrifuge tube and add an equal volume of Binding Matrix. Invert to mix.
8. Incubate with gentle agitation for 5 minutes at room temperature on a rotator.
9. Centrifuge at 14,000 x *g* for 10 sec to pellet Binding Matrix. Discard supernatant.
10. Add 500 µl prepared SEWS-M and gently re-suspend the pellet using the force of the liquid from the pipet tip.
11. Transfer the re-suspended Binding Matrix to a SPIN Module (SPIN filter and Catch Tube). Centrifuge at 14,000 x *g* for 1 min. Discard contents of Catch Tube and replace.
12. Centrifuge a second time at 14,000 x *g* for 1 min and replace the Catch Tube with a collection tube.

13. Elute DNA by gently re-suspending Binding Matrix above the SPIN Filter in 40 µl of DES (DNase/Pyrogen-Free Water). Incubate for 5 minutes at 55˚C in a heat block or water bath.
14. Centrifuge at 14,000 x *g* for 1 min to bring eluted DNA into the collection tube. Discard the SPIN Filter.

<u>Protocol 3: MoBioPowersoil kit (MOBIO Laboratories)</u>

Adapted from PowerSoil® DNA Isolation Kit Protocol- MO BIO Laboratories, https://mobio.com/

Preparation

Prepare sterile scooper

Provide pipettes and pipette tips

Clean centrifuge

Clean Vortex

Decontaminate Bead-Beater

Dilute Ready-lyse enzyme to 1000U/µl

Separate mortars, pestles and liquid nitrogen

Set water baths/incubators to required temperatures

Check Solution C1. If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.

Shake to mix Solution C4 before use.

Protocol

1. To the provided tubes add either plastic fragments or ground-up plastics. Gently vortex to mix.
2. Add 60 µl of Solution C1 and invert several times or vortex briefly.

3. Add 10µl Ready Lyse Lysozyme (diluted to 1000U/µl) to each tube and invert 25 times.
4. Incubate at 37° for 30 min.
5. Bead-beat for 7 minutes using bead-beating apparatus (for liquid nitrogen-ground plastics, skip step 5).
6. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature.
7. Transfer the supernatant to a clean 2 ml collection tube.
8. Add 250 µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 min.
9. Centrifuge the tubes at room temperature for 1 min at 10,000 x g.
10. Avoiding the pellet, transfer up to, but no more than, 600 µl of supernatant to a clean 2 ml collection tube.
11. Add 200 µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 min.
12. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.
13. Avoiding the pellet, transfer up to, but no more than, 750 µl of supernatant into a clean 2 ml collection tube.
14. Shake to mix Solution C4 before use. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 seconds.
15. Load approximately 675 µl onto a Spin Filter and centrifuge at 10,000 x *g* for 1 min at room temperature. Discard the flow through and add an additional 675 µl of supernatant to the Spin Filter and centrifuge at 10,000 x *g* for 1 min at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x g for 1 min at room temperature.
16. Add 500 µl of Solution C5 and centrifuge at room temperature for 30 sec at 10,000 x *g*.
17. Discard the flow through.
18. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.

19. Carefully place spin filter in a clean 2 ml collection tube. Avoid splashing any Solution C5 onto the Spin Filter.
20. Add 40 µl of Solution C6 (contains no EDTA) to the center of the white filter membrane.
21. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
22. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application.

Protocol 4: MoBioPowerbiofilm kit (MOBIO Laboratories)

Adapted from PowerBiofilm® DNA Isolation Kit Protocol- MO BIO Laboratories, https://mobio.com/

Preparation

Prepare sterile scooper

Provide pipettes and pipette tips

Clean centrifuge

Clean Vortex

Decontaminate Bead-Beater

Dilute Ready-lyse enzyme to 1000U/µl

Separate mortars, pestles and liquid nitrogen

Set water baths/incubators to required temperatures

Warm Solution BF1 prior to use at 55°C for 5-10 min. Use Solution BF1 while still warm. Check Solution BF4 and warm at 55°C for 5-10 min if necessary. Solution BF4 can be used while still warm.

Protocol

1. Add solution BF1 to screw cap tubes containing beads.
2. Place either plastic fragments or ground-up plastics to screw cap tubes.
3. Add 10µl Ready Lyse Lysozyme (diluted to 1000U/µl) to each tube and invert 25 times.
4. Incubate at 37° for 30 min.
5. Add 100 µl of Solution BF2. Vortex briefly to mix.
6. Incubate the bead tube at 65°C for 5 min.
7. Bead-beat for 4 minutes using bead-beating apparatus (for liquid nitrogen-ground plastics, skip step 7).
8. Add 100 µl of Solution BF3 and vortex briefly to mix. Incubate at 4°C for 5 min.
9. Centrifuge the tube at 13,000 x *g* for 1 min at room temperature.
10. Add 900 µl of Solution BF4 and vortex briefly to mix.
11. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x *g* for 1 min. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.
12. Place the Spin Filter basket into a clean 2 ml collection tube.
13. Shake to mix Solution BF5 before use. Add 650 µl of Solution BF5 and centrifuge at 13,000 x *g* for 1 min at room temperature.
14. Discard the flow through and add 650 µl of Solution BF6 and centrifuge at 13,000 x *g* for 1 min at room temperature.
15. Discard the flow through and centrifuge again at 13,000 x *g* for 2 min to remove residual wash.
16. Place the Spin Filter basket into a clean 2 ml collection tube.
17. Add 40 µl of Solution BF7 (contains no EDTA) to the center of the white filter membrane.

18. Centrifuge at 13,000 x *g* for 1 min.
19. Discard the Spin Filter basket. DNA is now ready for downstream analyses.

Protocol 5: Phenol:Chloroform

Preparation

Prepare sterile scooper

Provide pipettes and pipette tips

Clean centrifuge

Clean Vortex

Sterilize beads

Decontaminate Bead-Beater

Aliquot Phenol, PCI (phenol-chloroform-isoamyl alcohol 25:24:1)

and Chloroform(15 ml)

Prepare: 70% EtOH

Proteinase K (10U/µl)

Lysozyme (1000U/µl)

Extraction buffer (see Table 1)

Separate mortars, pestles and liquid nitrogen

Set water baths/incubators to required temperatures

Protocol

1. Add 500µl extraction buffer to sterile 2ml tube.
2. Place whole or ground-up (liquid nitrogen) plastic fragments in tube.
3. Add 5µl proteinase K (10U/µl) and incubate at 50ºC for 30 min, and shortly cool down.

4. Add 10µl Lysozyme (1000U/µl), and incubate at 37ºC for 30 min.
5. Add~0.4g of 0.1mm sterile zirconium beads to tubes (for liquid nitrogen-ground plastics, skip steps 5 and 6).
6. Bead-beat for 10 min on a vortex.
7. Place the tubes in a 70ºC water bath for 30 min.
8. Add 500µl Phenol to the tubes accordingand mix end-over-end (~10x).
9. Spin for 10 minutes at 4500 x *g* in centrifuge.
10. Remove the aqueous phase by pipetting into a clean tube.
11. Add 500µlPCI and mix end-over-end (~90x).
12. Spin for 10 min at 4500 x *g*in centrifuge.
13. Remove the aqueous phase by pipetting into a clean tube.
14. Add 500µl Chloroform and mix end-over-end (~10x).
15. Spin the sample for 10 min at minimal 4500 x *g*in centrifuge.
16. Remove the aqueous phase by pipetting into a clean tube.
17. Add 2 volumes ice cold (-20ºC) ETOH and 0.05 volumes of 5M NaCl and mix end-over-end (~10x).
18. Incubate the solution at -20ºCovernight.
19. Centrifuge the solution for 20 min at maximum speed (20,000x*g*).
20. Discard the ETOH supernatant by decanting, but save the supernatant, and wash the pellet using 700µl70% ETOH and mix end-over-end.If pellet is visible, detach by flicking.
21. Centrifuge the solution for 20 min at maximum speed (20,000x*g*). Dry the tube containing the DNA pellet (0.5-4 h).
22. When the ETOH has evaporated add 40µl of 10mM Tris-HCL pH8 or 10mM Tris-1mM EDTA pH8 to dissolve the DNA.

23. Check if the pellet has dissolved and apply 5µlto an electrophoresis gel to evaluate DNA quality and quantity.

Table 1. Extraction/lysis buffer – stock solutions

Component	**Concentration (mM/%)-stock**	**Concentration (mM/%)-working solution**	**Diluting factor stock to working solution**
Tris-HCL pH8	100mM	10mM	10x
Na_2EDTA pH8**	250mM	25mM	10x
SDS***	10 v/v%	1 v/v%	10x
NaCl	5M	100mM	50x

Table 2. Chemicals needed for extraction

Component	Large working solution 24ml
Tris-HCL pH8*	2.4ml
EDTA pH8*	2.4ml
SDS*	2.4ml
NaCl*	480µl
Zirconium beads	Add later
Adding PCR-H_2O To final volume / extr.	~16.4ml
Phenol pH7.9	-
PCI pH7.9	-
Chloroform	-
ETOH 100% (v/v)	-
NaCl 5M	-
ETOH 70% (v/v)	-

*Molecular Grade chemicals

**Use preferably Na_2EDTA or Na_4EDTA as it is easier to dissolve than NaEDTA

***Sodium dodecyl sulfate