Version 6

Jul 27, 2021

Total Nucleic Acids Extraction from Soil V.6

In 1 collection

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SoWa RI Anaerobic and Molecular Microbiology (public)
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Citation: Roey Angel, Eva Petrova, Ana Lara-Rodriguez (07/27/2021). Total Nucleic Acids Extraction from Soil. https://dx.doi.org/10.17504/protocols.io.bwxcpfiw

ABSTRACT

The following protocol is intended for the simultaneous extraction of DNA and RNA (total nucleic acids, or TNA) from various soil and sediment samples. The protocol was designed based on two protocols published by Henckel et al. (1999) and Griffiths et al. (2000), with several critical modifications. Recently, we have added the option to include an ammonium aluminium sulfate salt for soils with high humic content (Braid et al., 2003). The result is a highly flexible and streamlined protocol, which delivers high yields of nucleic acids with quality suitable for all downstream molecular applications from most types of soil and sediment samples. Please cite Angel et al. (2012).

Henckel T, Friedrich M, Conrad R (1999). Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. Applied and environmental microbiology.

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ (2000). Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Applied and environmental microbiology.

Braid MD, Daniels LM, Kitts CL (2003). Removal of PCR inhibitors from soil DNA by chemical flocculation.. Journal of microbiological methods.

Angel R, Claus P, Conrad R (2012). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions.. The ISME journal.
https://doi.org/10.1038/ismej.2011.141

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PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Angel, R., Claus, P., and Conrad, R. (2012). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. ISME J 6, 847–862. doi:10.1038/ismej.2011.141.

COLLECTIONS

Total nucleic acids extraction, purification and cDNA synthesis from soil

KEYWORDS

DNA extraction, RNA extracion, soil, bead-beating, phenol-chloroform

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51908

PARENT PROTOCOLS

Part of collection
Total nucleic acids extraction, purification and cDNA synthesis from soil

GUIDELINES

- **RNA handling.** Since RNA is very sensitive to both chemical and enzymatic degradation, some precautionary measures should be taken. Commonly, DEPC treated water is used in RNA extraction protocols; however, please note that the substance is itself toxic and tends to break down to methanol when heated (e.g. by autoclaving). RNase free water (DEPC treated or not) can be purchased and is rather inexpensive. In addition, it is recommended to work with clean, preferably baked, glassware (3 h at 180 °C) for storing tubes, to clean surfaces, spatulas, pipettes and centrifuge parts with RNase eliminating solutions (such as RNase AWAY™), and to use fresh and clean reagents for the preparation of the various buffers and solutions (keep salt stocks separate from general chemical storage or label them as “RNA only” to avoid contamination). Do not autoclave tips or tubes; this is generally unnecessary for molecular work and might get them dirty or compromise the material. Instead, use tubes and tips marked as DNase/RNase free. Filter tips are preferred over standard tips. As a general rule, work with RNA should be quick, precise, and samples should be kept on ice, when possible. In our experience, this is much more important than any attempt to eliminate all RNases from apparatus and solutions. One should also bear in mind that soil samples contain far more RNases than any contamination that might exist in the buffers or the apparatus. Therefore, one should not worry too much about the purity of the extraction buffer and phosphate buffer. In contrast, the PEG Precipitation Solution, the Low TE Buffer, and the RNA storage solution are used to store extracted RNA for relatively long periods of incubation. Hence, extra care must be taken during their preparation to avoid contamination. When preparing the PEG Precipitation Solution, it is better to err on the side of precise concentration rather than risk contamination. To minimise the handling during this step (i.e. avoid transferring liquids between different flasks), simply weigh everything into a 250 ml DURAN Glass Bottle and slowly add water until the liquid reaches the 200 ml mark (after all solids have been soaked in the water), then shake the bottle vigorously and autoclave it. Prepare Low TE Buffer from pre-sold Tris and EDTA solutions (see Step 1.4).

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Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1**

RNase AWAY™ Spray Bottle, RNase in spray bottle; 475mL **Thermo Fisher Catalog #7002PK**

- **Choice of extraction buffer.** Using TNS buffer results in higher DNA and RNA yields and more intact rRNA. However, TNS tends to carry over significantly more humic substances with the nucleic acids compared to TNC buffer. We, therefore, recommend using TNS only for mineral soil and sediment samples (see Step 1.2).
- **Reducing the carryover of humic substances.** Adding aluminium salt in the form of $AlNH4(SO_4)_2*12H_2O$ to the phosphate buffer has been shown to decrease the concentration of humic substances in the extract and is recommended for very organic soils (see Step 1.1). For more detail see Braid et al., (2003).

Braid MD, Daniels LM, Kitts CL (2003). Removal of PCR inhibitors from soil DNA by chemical flocculation. Journal of microbiological methods.
http://10.1016/S0167-7012(02)00210-5

Aluminium ammonium sulfate dodecahydrate **Merck Millipore Sigma Catalog #1010310500**

- **Storing and working with phenol and chloroform.** Store phenol in a cool, dry, ventilated area away from sources of heat or ignition. Store separately from reactive or combustible materials and out of direct sunlight. Phenol will begin to oxidise once opened and should be used within a few weeks to a few months, depending on storage conditions and temperature. Older, oxidised, phenol solutions should not be used as they may cause “nicking” of the DNA. The phenol solution should contain an anti-oxidising agent (8-hydroxyquinoline) as an indicator (i.e., if the 8-hydroxyquinoline is oxidised, the phenol solution will turn a reddish colour).

Edward Moore, Angelika Arnscheidt, Annette Krüger, Carsten Strömpl, Margit Mau (2004). Section 1 - Isolation of Nucleic Acids. Molecular Microbial Ecology Manual.
http://10.1007/978-1-4020-2177-0_1

- **Use of phenol in the bead beating process.** Using phenol increases the yield up to 4 times compared with the phenol-free option (unpublished data), but also increases somewhat the carryover of humic substances. Still, considering the increased yield we recommend using phenol in the bead beating steps for all but the trickiest samples. If omitting phenol, double the volume of the extraction buffer and phosphate buffers added (to 0.9 ml in total).
- **Homogenizing the sample at a lower temperature.** MP Biomedicals offers an adaptor to their FastPrep homogeniser that allows sample homogenising at a lower temperature using dry ice. We would highly recommend using this adaptor, in particular, when processing samples in the presence of phenol (see above). From our experience, processing samples at a low temperature significantly reduces carryover of humic acids, helps to protect RNA from degradation and also the tubes from accidental overheating and leakage of phenol. **Use only one or maximum two tablespoons full of dry Ice because overloading the adapter with dry ice (especially when not using phenol) could cause the sample and buffer to freeze in during the bead beating process!**

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CoolPrep™ adapter for 24 x 2 mL tube holder on FastPrep-24
Sample homogeniser adapter
MP Biomedicals 116002528

- **LifeGuard Soil Preservation Solution.** If samples were stored in an RNA-preservation solution such as LifeGuard Soil Preservation Solution it is necessary to remove it before proceeding with RNA extraction. Centrifuge the sample at 10,000 rpm for 1 min and pipette the supernatant. Add 1 ml of PB (see Step 1.1), vortex the sample for 10 s to make sure the soil is washed by the buffer, and centrifuge it again at 10,000 rpm for 1 min. Remove the supernatant completely and proceed with normal extraction.

LifeGuard Soil Preservation Solution **Qiagen Catalog #12868-100**

- **Very dry soil samples.** For very dry soil (<10% WC) it is recommended to add some (up to 250 µl) PB pH 8.0 before starting the extraction (before the first bead beating processing). The reason is that in dry soils, some of the extraction buffer solution gets absorbed to the soil and cannot be recovered afterwards. This can lead to low recovery volumes from the bead-beating process, which could make it hard to separate the aqueous phase from the phenolic phase in the subsequent steps and eventually result in a reduced yield. After the first round of bead-beating, the soil is wet enough, and no further additions are required.

- **pH of the extraction buffers.** The pH of the phenol and the extraction buffers used in this protocol is set to 8.0. It has been shown that performing the extraction at around pH 5.0 significantly reduces the carryover of humic substances and is recommended for soils rich in organic material. Using such low pH, however, will also considerably decrease the amount of DNA yield (while supposedly not affecting RNA yield). Low pH extraction might therefore not be suitable if DNA is also to be analysed (particularly in a quantitative way). If opting for this option use the PB pH 5.8 in combination with water-saturated phenol. For more information see Mettel et al., (2010).

Carsten Mettel, Yongkyu Kim, Pravin Malla Shrestha, Werner Liesack (2010). Extraction of mRNA from Soil. Applied and Environmental Microbiology.
http://10.1128/AEM.03047-09

- **Number of bead beating processing repeats.** For most soil types (especially if they're not too clayish), single bead-beating processing should be sufficient to obtain enough NA for further applications. The following two repetitions in this protocol (see Step 8) is meant not only to increase the yield but also to reduce the bias associated with the susceptibility of different cell types to lysis. Feinstein et al., (2009) found that DNA extraction yields kept increasing even after the 6th bead beating repetition, and that community structure differed somewhat in each repetition.

Feinstein LM, Sul WJ, Blackwood CB (2009). Assessment of bias associated with incomplete extraction of microbial DNA from soil. Applied and environmental microbiology.
https://doi.org/10.1128/AEM.00120-09

- **Amount of crude extract to use for RNA purification.** This depends mostly on the colour of the crude extract, and its quantity and purity as measured spectrophotometrically. In addition, the decision should take

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into account the final volume of the RNA. Between 20-100 µl of total extract may be used depending on colour, purity and amount of NA.

- **Quantification.** It is not possible to precisely quantify total NA using a spectrophotometer (e.g. Nanodrop) since DNA and RNA require different multiplication factors and since the presence of co-extracted organic compounds from soil (humic substances) often obscure the measurement. More precise quantification of DNA and RNA can be obtained using specific fluorescent dyes (e.g. Invitrogen's PicoGreen™ and RiboGreen™). For more details see DNA quantification with PicoGreen and RNA quantification with RiboGreen. Quantification of a TNA solution using PicoGreen should only give the amount of DNA while using RiboGreen should yield twice the amount of DNA plus that of RNA. Thus, it is possible to quantify both DNA and RNA in a TNA solution without having to separate the two.

Quant-iT™ RiboGreen™ RNA Quantification
by Roey Angel,
Soil and Water Research Infrastructure
PREVIEW RUN

Qant-iT™ PicoGreen® dsDNA Quantification
by Roey Angel,
Soil and Water Research Infrastructure
PREVIEW RUN

- **Triple purification procedure.** Purification with phenol/chloroform and then chloroform should yield pure enough samples for most applications. In case the extract seems not clean enough, adding an additional phenol/chloroform purification step after the first one will ensure higher TNA purity while only minimally compromising the yield.

- **Processing multiple samples in parallel.** Depending on the centrifuge capacity at hand, this protocol can be used to extract up to 12 or even 15 samples in parallel. We do not recommend extracting more samples than that at a time due to handling difficulties.

MATERIALS TEXT

STEP MATERIALS

Potassium phosphate dibasic trihydrate **Merck Millipore** **Sigma Catalog #P9666** Step 1.1

Potassium phosphate monobasic **Merck Millipore** **Sigma Catalog #P9791** Step 1.1

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1** Step 1.1

Aluminium ammonium sulfate dodecahydrate **Merck Millipore** **Sigma Catalog #1010310500** Step 1.1

Lysing Matrix E 2 mL tube **MP Biomedicals Catalog #116914050-CF** Step 2

ROTI® Phenol **Carl Roth Catalog #0038.1** Step 3

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ROTI® Aqua-Phenol **Carl Roth Catalog #A980.1** Step 3

Dry Ice **Contributed by users** Step 4

ROTI® Phenol/ Chloroform/ Isoamyl alcohol **Carl Roth Catalog #A156.1** Step 9

ROTI® C/I **Carl Roth Catalog #X984.1** Step 12

Glycogen RNA grade **Thermo Fisher Scientific Catalog #R0551** Step 15

OneStep PCR Inhibitor Removal Kit **Zymo Research Catalog #D6030** Step 22

Genomic DNA Clean & Concentrator-10 **Zymo Research Catalog #D4011** In 2 steps

Trizma® base **Merck Millipore Sigma Catalog #93362** Step 1.2

Sodium chloride for molecular biology **Merck Millipore Sigma Catalog #S3014** Step 1.2

Hexadecyltrimethylammonium bromide **Merck Millipore Sigma Catalog #H6269** Step 1.2

Sodium dodecyl sulfate **Merck Millipore Sigma Catalog #3771** Step 1.2

Ethanol, Absolute, Molecular Biology Grade **Thermo Fisher Scientific Catalog #BP2818500** Step 1.4

Polyethylene glycol 8000 **Carl Roth Catalog #0263.1** Step 1.3

UltraPure™ 1M Tris-HCl pH 8.0 **Thermo Fisher Scientific Catalog #15568025** Step 1.5

UltraPure™ 0.5M EDTA pH 8.0 **Thermo Fisher Scientific Catalog #15575020** Step 1.5

Genomic DNA Clean & Concentrator-10 **Zymo Research Catalog #D4011** In 2 steps

SAFETY WARNINGS

Working with phenol and chloroform

Phenol is highly toxic for humans and animals and is also classified as mutagenic, teratogenic and potentially carcinogenic. Phenol is readily absorbed through intact skin and is highly toxic to cells. Cellular damage and death at the site of entry results in a chemical burn, which may be extremely serious. There can be a time delay between absorption of phenol, and the appearance of burn symptoms and phenol is a local anaesthetic which numbs sensory nerve endings; for both these

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reasons, phenol contamination may not be noticed until considerable absorption and damage has occurred. Phenol-chloroform may have even faster-penetrating ability than phenol alone, particularly towards glove materials. Besides the local toxic effect leading to burns, phenol also exerts systemic toxic effects on humans, which may lead to rapid poisoning if sufficient phenol is absorbed. Any exposure covering more than a few cm^2 of skin is potentially fatal and must be considered as a medical emergency.

Chloroform is toxic if inhaled or swallowed, can cause skin and eye irritation and is a suspected carcinogen.

Work with phenol and chloroform should only be done inside a fume hood. Always refer to the accompanying MSDS before working with any hazardous substance. Always wear gloves, eye protection and a lab coat when working with phenol and chloroform. Latex gloves should not be used with phenol. Disposable nitrile gloves are rapidly penetrated by phenol, but they can be used provided they are exchanged for fresh gloves immediately upon becoming contaminated. Double-gloving is recommended.

Handling spills

In case of a small spill (<25 ml), wipe the area using an LMW-PEG-soaked absorbent pad and then with water. Dispose of the wipes in a fume hood. If a spill occurs outside the hood. Larger spills should be handled by professionals.
In the event of skin contact, immediately remove contaminated clothing and wipe the area using an LMW-PEG-soaked absorbent pad until no phenol smell is noticeable. When LMW PEG is not available, flushing the exposed area with copious amounts of water for 15 minutes may be effective. In case of contact with eyes, immediately flush eyes with copious amounts of water for at least 15 minutes and subsequently obtain medical attention. All exposed persons should be removed from the area and seek immediate medical attention (subsequent to initial decontamination for skin/eye contact). In the event of ingestion, obtain immediate medical attention. Do not induce vomiting unless
directed to do so by medical personnel.

First Aid Kit

All labs utilizing phenol shall keep a first aid kit on hand containing:

- At least one-litre pharmaceutical grade polyethylene glycol (PEG), 300 or 400 molecular mass, e.g. Kollisolv® PEG 300 or 400 (Sigma Aldrich 91462-1KG or 06855-1KG). PEG 300/400 is a skin-safe, excellent phenol solvent.
- Laminate film gloves (Barrier®, Silver Shield®) for use by colleagues who are helping with decontamination. Do not put gloves on if your hand is already contaminated!
- Large cotton roll (e.g. VWR 470161-446).
- Wiping cloths (e.g. VWR 500030-610 or 500030-611).
- Selection of thick polyethylene bags for holding contaminated waste (e.g. large Ziploc® storage bags).
- Large squeeze-bottle of liquid hand soap (A squeeze bottle allows for faster application to the body than a pump dispenser).
- Copy of this SOP with the first aid section highlighted and copy of a phenol SDS from a reputable supplier (e.g. Sigma Aldrich).

Storage and waste

- Phenol is a combustible acid. It must be stored so that it cannot come in contact with strong oxidizers (such as nitric acid and bromine) and strong bases (such as potassium hydroxide) because a violent reaction could result.
- Store below eye level to prevent injuries in case of a spill.
- Phenol waste should be placed in a container that is clearly labelled and has a securely sealed lid.

BEFORE STARTING

1. Prepare all buffers and solutions in advance (see Step 1).
2. Clean all surfaces and centrifuges with an RNase eliminating solution (e.g. RNAse Away).
3. If not using Lysing Matrix E tubes, prepare lysing tubes by pouring 0.7 g (one full PCR tube) of 0.1 mm glass beads to the screw top tube.

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4. For each sample prepare in a rack: 1 lysing tube, 4 standard 2 ml tubes and 2 nonstick 2 ml tubes.

Solutions for TNA extraction 4h

1

Prepare the following solutions for TNA extraction
Use clean and preferably baked glassware (make sure all non-glass components can withstand the high temperatures).

1.1 **One of the following phosphate buffers:**

Phosphate buffer ([M] 120 Milimolar (mM) , pH 8.0)

12.43 g K2HPO4•3 H2O (M.W. 228.22)

0.751 g KH2PO4 (M.W. 136.09)

500 mL RNase-free water

Dissolve the salts in RNase-free water and fill up to 500 ml. Autoclave.

Phosphate buffer ([M] 120 Milimolar (mM) , pH 5.8)

1.16 g K2HPO4•3 H2O (M.W. 136.09)

7.47 g KH2PO4 (M.W. 136.09)

500 mL RNase-free water

Dissolve the salts in RNase-free water and fill up to 500 ml. Autoclave.

Phosphate buffer + $AlNH_4$ (120 mM PB pH 8.0 + 0.1M $AlNH_4(SO_4)_2 \cdot 12H_2O$)

2.04 g AlNH4(SO4)2 · 12H2O (M.W. 453.33)

45 mL 120 mM PB pH 8.0

Dissolve in PB. Autoclave.

Store at **Room temperature**

Potassium phosphate dibasic trihydrate **Merck Millipore Sigma Catalog #P9666**

Potassium phosphate monobasic **Merck Millipore Sigma Catalog #P9791**

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1**

Aluminium ammonium sulfate dodecahydrate **Merck Millipore Sigma Catalog #1010310500**

1.2 **Either TNC or TNS**
TNC

15.76 g TRIZMA (M.W. 121.14)

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1.17 g NaCl

20 g CTAB (M.W 364.45)

200 mL RNase-free water

Dissolve the salts in RNase-free water and fill up to 200 ml. Autoclave.

TNS

15.76 g TRIZMA (M.W 121.14)

1.17 g NaCl

20 g SDS (M.W 288.38)

200 mL RNase-free water

Dissolve the salts in RNase-free water and fill up to 200 ml. Autoclave.

Store at **Room temperature**

Trizma® base **Merck Millipore Sigma Catalog #93362**

Sodium chloride for molecular biology **Merck Millipore Sigma Catalog #S3014**

Hexadecyltrimethylammonium bromide **Merck Millipore Sigma Catalog #H6269**

Sodium dodecyl sulfate **Merck Millipore Sigma Catalog #3771**

1.3 PEG precipitation solution (30%)

60 g PEG (M.W 7000-9000)

18.7 g NaCl

200 mL RNase-free water

Add ingredients to a graduated Duran bottle. Add water to fill up to 200 ml, shake vigorously by hand, autoclave and mix well while hot (solution turns milky when hot, but then turns clear when cooled to room temperature).

Store at **Room temperature**

Polyethylene glycol 8000 **Carl Roth Catalog #0263.1**

1.4 Molecular-grade ethanol solution (75%)

75 mL Absolute ethanol

25 mL RNase-free water

Store at **-20 °C**

Ethanol, Absolute, Molecular Biology Grade **Thermo Fisher Scientific Catalog #BP2818500**

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1.5 **Low-EDTA TE buffer**

500 µl Tris-HCl 1 M, pH 8.0 (Trizma)

10 µl EDTA 0.5 M, pH 8.0

50 mL RNase-free water

Prepare in a laminar-flow hood (to protect stocks), filter sterilise (0.2 µm) and autoclave.

Store at **Room temperature**

UltraPure™ 1M Tris-HCl pH 8.0 **Thermo Fisher Scientific Catalog #15568025**

UltraPure™ 0.5M EDTA pH 8.0 **Thermo Fisher Scientific Catalog #15575020**

Extraction 4h

2 2m

Weigh **0.4 g soil** (0.2-0.7 g) into a Lysing Matrix E tube, or a 2-ml screw-cap tube filled with 0.7 g (one full PCR tube) of 0.1 mm glass beads and place the tube **On ice** .

Lysing Matrix E 2 mL tube **MP Biomedicals Catalog #116914050-CF**

3 30s

Add **375 µl** of either PB pH 8.0, PB pH 5.8 or PB+Al, **125 µl** of TNC or TNS and **400 µl** of TE-saturated phenol (if using PB pH 8.0) or water-saturated phenol (if using PB pH 5.8). Alternatively, add **675 µl** of PB, **225 µl** of TNC or TNS, if not using phenol (see Guidelines and Warnings on which combination of solutions to choose.)

ROTI® Phenol **Carl Roth Catalog #0038.1**

ROTI® Aqua-Phenol **Carl Roth Catalog #A980.1**

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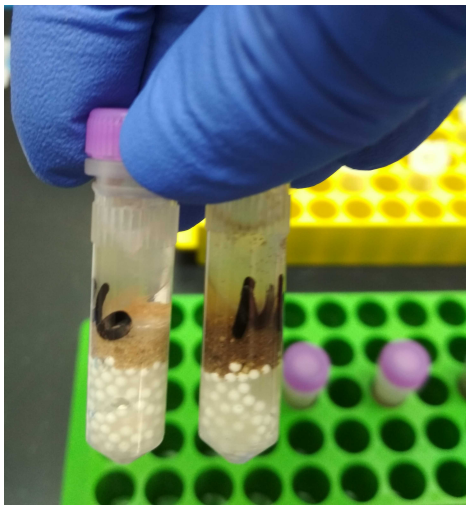

Lysing Matrix E tubes with soil and reagents prepared for the 1st bead beating round.

4 Immediately place the tube in a sample homogeniser and process for **00:00:30** at 6.5 m s^{-1}. We recommend using 2m the FastPrep-24™ sample homogenizer with the CoolPrep™ adapter for 24 x 2 ml **On ice (dry ice)** . If using the CoolPrep™ adapter make sure all slots are filled with either sample or empty tubes.

The bottom part of a CoolPrep FastPrep adaptor filled with the recommended amount of dry ice.

FastPrep-24™ 5G bead beating grinder and lysis system
Sample homogenizer
MP Biomedicals **116005500**

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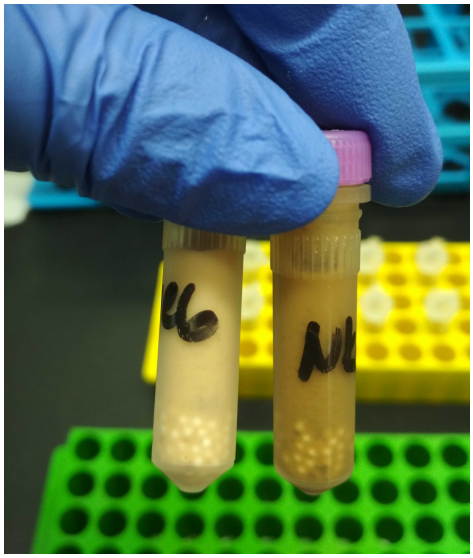
CoolPrep™ adapter for 24 x 2 mL tube holder on FastPrep-24
Sample homogeniser adapter

MP Biomedicals 116002528

Dry Ice **Contributed by users**

FastPrep-24™ 5G set up. The green-capped tubes are empty and are used to seal the CoolPrep adapter.



The samples right after bead beating.

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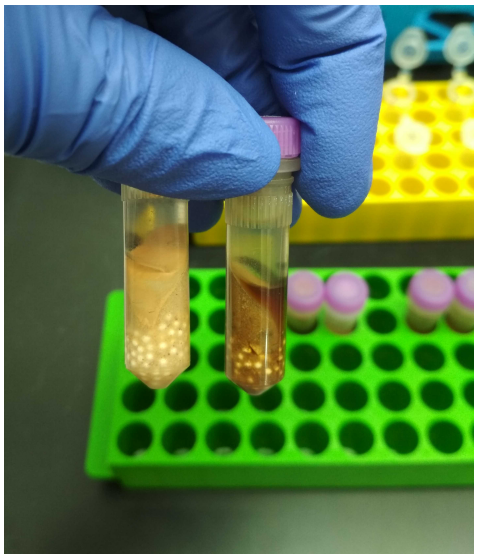
5 10s

Chill **On ice** for **00:00:10** (**not necessary if using the CoolPrep™ adapter**).

6 5m

Centrifuge at **14000 rpm, 15°C, 00:03:00** (centrifugation at RT is also possible).

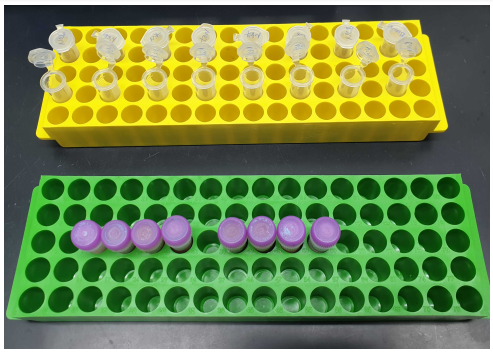


The samples after centrifugation.

7 5m

Transfer the entire liquid (aqueous and organic phases) to a fresh 2 ml tube by decanting or pipetting. tubes.

Your supernatant has two phases. An aqueous upper phase that should be transparent/translucent and a phenol phase that should be opaque. Make sure to pour both into the fresh 2 ml tube.



Tubes set up for 8 samples: A) Lysing Matrix E in a green stand (1 tube per sample). B) 2 ml tubes for collecting the supernatant after processing in a yellow stand (2 tubes per sample).

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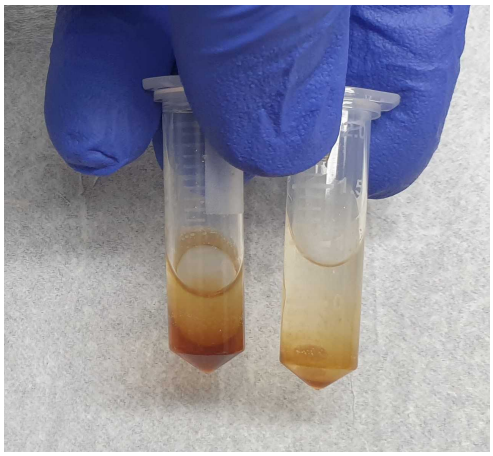


The collected supernatant at this stage.

8 Repeat **Steps 3-7** two more times using the same Lysing Matrix E tube. Be careful not to overfill the tube as this might cause phenol leakage during the sample homogenising process. If there is not enough space in the tube for all the reagents, decrease the phenol volume (down to 200 µl). For the second homogenising repetition use a fresh 2 ml tube to collect the supernatant in **Step 7**, while for the third repetition divide the supernatant between the two tubes from the previous repetitions to achieve equal volumes.

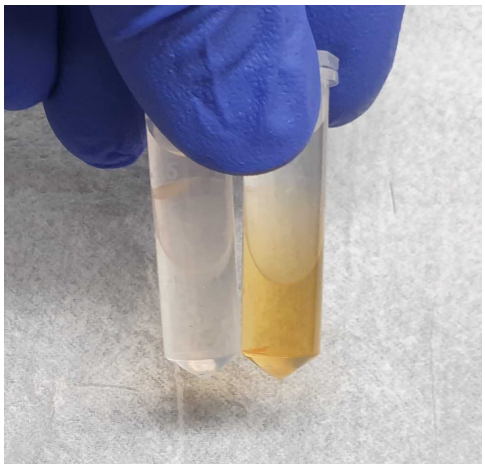
9

Add **800 µl phenol/chloroform/isoamyl alcohol 25:24:1** (or 1 volume) to each of the tubes containing the supernatant, to a maximum of 2 ml total volume in each tube.

ROTI® Phenol/ Chloroform/ Isoamyl alcohol **Carl Roth Catalog #A156.1**

10 5m

Mix the two phases , by hand or using a vortex. Centrifuge **14000 rpm, 15°C, 00:03:00** .



Following centrifugation, the liquid in each tube should separate into two phases.

Citation: Roey Angel, Eva Petrova, Ana Lara-Rodriguez (07/27/2021). Total Nucleic Acids Extraction from Soil. https://dx.doi.org/10.17504/protocols.io.bwxcpfiw

11 ⚠ 3m

Using a 1-ml pipette tip, carefully transfer the aqueous phase (the upper phase) from each tube to two fresh 2 ml tubes. Be careful not to touch or pipette the interphase or the organic phase (the lower phase)

If, by mistake, some of the interphase or organic phase were pipetted, simply return the liquid to the original tube and centrifuge it again.

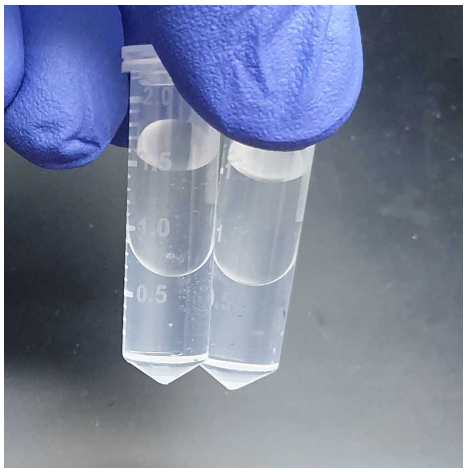
12 2m

Add **800 µl chloroform/isoamyl alcohol 24:1** (or 1 volume) to each tube.

ROTI® C/I **Carl Roth Catalog #X984.1**

13 5m

Mix the phases vigorously by hand or using a vortex. Centrifuge **14000 rpm, 15°C, 00:03:00** .



Following centrifugation, the liquid in each tube should separate into two phases.

14 ⚠ 3m

Carefully transfer the supernatant from each tube to a fresh 2 ml low-binding microcentrifuge tubes. At this point, you should have two low-binding tubes per sample.

If, by mistake, some of the interphase or organic phase were pipetted, simply return the liquid to the original tube and centrifuge it again.

Citation: Roey Angel, Eva Petrova, Ana Lara-Rodriguez (07/27/2021). Total Nucleic Acids Extraction from Soil. https://dx.doi.org/10.17504/protocols.io.bwxcpfiw

DNA LoBind Tubes
Microcentrifuge tubes
Eppendorf 0030108051

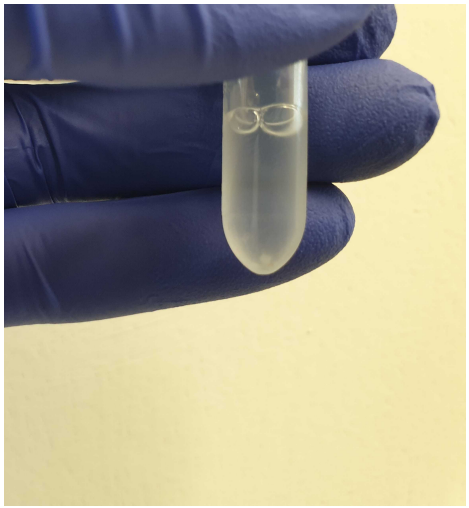
15 3m

Add to each tube **2 µl RNA-grade glycogen** and **1 mL PEG Precipitation Solution** (or twice the extract's volume).

Glycogen RNA grade **Thermo Fisher Scientific Catalog #R0551**

16 1h

Centrifuge **14000 rpm, 4°C, 01:00:00** .



A pellet should be visible at the bottom of the LoBind tube after centrifugation. The pellet should be white/opaque in colour. The size of the pellet will depend on the TNA content in a sample, but also on the amount of co-extracted contaminants.

17 5m

Decant the supernatant, briefly centrifuge or spin-down again to collect the drops and using a pipette remove as much as possible from the remaining precipitation solution. Be careful not to disturb the pellet.

18 2m

Add **1 mL ice-cold 75% EtOH** , invert the tube several times. Centrifuge at **14000 rpm, 4°C, 00:20:00** .

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19 5m

Remove the supernatant using a pipette, shortly centrifuge again to collect the drops and using a pipette remove as much as possible from the remaining ethanol. Be careful not to disturb the pellet.

20 10m

Leave the tubes open at **Room temperature** for approximately **00:05:00** (preferably under a flame, inside a laminar-flow hood, or inverted on a piece of kitchen paper, to prevent contamination) to evaporate the remaining ethanol. Alternatively, the pellets can be dried under a filtered stream of N_2, or in a speed-vac.

The pellets might not be completely dry at this point, but the remaining liquid should be pure water.

21 5m

Resuspend each pellet in **50 µl** to **100 µl Low-EDTA TE Buffer** and combine both subsamples into one of the non-stick tubes.

Purification

22

Remove co-extracted humic substances using OneStep™ PCR Inhibitor Removal Kit

This step is optional but very recommended.
OneStep PCR Inhibitor Removal Kit **Zymo Research Catalog #D6030**

22.1 1m Resuspend the resin in the column by brief vortexing (if the column is dry, add 100 µl of RNAse-free water prior to vortexing.)

22.2 4m

Loosen the cap by a quarter twist, place the column in a collection tube and centrifuge **8000 x g, Room temperature , 00:03:00**

22.3 30s

Place the column in a fresh 1.5 ml tube, pipette the entire TNA extract (up to 200 µl) on top of the resin.

Be careful not to disturb the resin with the tip

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22.4 2m

Centrifuge **8000 x g, Room temperature , 00:01:00**

22.5 Discard the column and retain the extract in the tube.

Quantification

23

Optional: load **5 µl** to **10 µl** of the total NA extract on an agarose gel to evaluate its quantity and quality using electrophoresis.

24 Quantify total NA extract using RiboGreen™ and PicoGreen™ (see Guidelines and Warnings) or only DNA using PicoGreen™.

SOWA Qant-iT™ PicoGreen® dsDNA Quantification
by Roey Angel,
Soil and Water Research Infrastructure
PREVIEW RUN

24.1 20m

Take out all reagents from the fridge and bring them to room temperature.
Take out the DNA samples from the freezer. DNA samples should be slowly thawed on ice.

Quant-iT™PicoGreen® dsDNA reagent is dissolved in dimethylsulfoxide (DMSO), which freezes below 19 °C. The reagent must be completely thawed before using it by bringing it to room temperature. After the reagent thawed, it is advisable to briefly vortex the tube to make sure it is adequately mixed and to spin it down in a centrifuge or a mini centrifuge.

Quant-iT™PicoGreen® dsDNA reagent is light sensitive and should be protected from light at all times.

Quant-iT™ PicoGreen™ dsDNA Assay Kit **Invitrogen - Thermo Fisher Catalog #P11496**

24.2 2m

Citation: Roey Angel, Eva Petrova, Ana Lara-Rodriguez (07/27/2021). Total Nucleic Acids Extraction from Soil. https://dx.doi.org/10.17504/protocols.io.bwxcpfiw

Prepare 22 ml 1X TE buffer by pipetting 1.1 ml of 20X TE buffer into 20.9 ml of nuclease-free water into a sterile and nuclease-free 50 ml tube.
Mix by inverting the tube several times.

1.1 mL 20X TE buffer

20.0 mL nuclease-free water

24.3 2m

For high-range quantification:

Dilute the DNA-standard stock solution (λ DNA 100 ng μl^{-1}) to a final concentration of 2 ng μl^{-1} by mixing 10 µl λ DNA-standard stock solution with 490 µl 1X TE buffer.

10 µl λ DNA-standard stock solution

490 µl 1X TE buffer

For low-range quantification:

Prepare a 40-fold dilution of the 2 ng μl^{-1} DNA-standard work solution by mixing 5 µl of the 2 ng μl^{-1} DNA-standard work solution with 195 µl 1X TE buffer to yield a 0.05 ng μl^{-1} DNA-standard work solution.

5 µl diluted DNA-standard solution

195 µl 1X TE buffer

24.4 If needed, prepare a dilution of each sample in 1X TE buffer so that the reading will be within the dynamic range.

It is advisable to run samples in duplicates for a more accurate quantification

24.5 2m

Prepare PicoGreen® work solution: 9950 µL 1X TE buffer + 50 µL PicoGreen® into a sterile and nucleic-acids free 50 ml tube. Mix and protect from light.

9950 µl 1X TE buffer

50 µl PicoGreen®

24.6 10m

Prepare the following standard mixture in the first two columns of the black, sterile, 96-well plate:

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Assay version	Diluted DNA std. (µl)	1X TE buffer (µl)	Final DNA amount (ng)
High-range (1-200 ng µl-1)	100	0	200
Use 2 ng µl-1 standard	50	50	100
	10	90	20
	1	99	1
	0	100	0
Low-range (50 pg µl-1 - 5 ng µl-1)	100	0	5
Use 0.05 ng µl-1 standard	50	50	2.5
	10	90	0.5
	1	99	0.05
	0	100	0

96-well microtiter plate

Nunc **265301**

black, flat bottom

24.7 Pipette 99 µl of 1X TE buffer in the remaining wells. 5m

99 µl 1X TE buffer

Tip: use a mechanical or electronic dispenser during this step and step no. 9 to speed up the work.

Multipette E3

Eppendorf **4987000010**

electronic dispenser

24.8 Pipette 1 µl of the unknown DNA samples in the remaining wells. 10m

1 µl of DNA sample

Use either a diluted sample in case the concentration is expected to be higher than the dynamic range limit or

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larger volume in case the concentration is expected to be below the detection limit.

24.9 Pipette 100 µL of the PicoGreen® work solution in each well, including the standard and unknown sample wells. 2m

100 µl PicoGreen work solution

24.10 5m

Protect the 96-well plate from light and incubate for 2-5 min at room temperature.

00:02:00

24.11 5m

Place the plate in a plate reader and measure the fluorescence according to the following parameters:

Excitation	~480 nm
Emission	~520 nm
Integration time	40 s
Lag time	0 s
Gain	Optimal
Number of flashes	10
Calculated well	highest standard
Shaking	5 s

It is also possible to set the gain to a fixed value (e.g. 100). If the fluorescence values of the standard drop over time this could indicate damage to the reagents or the DNA standard.

Synergy 2
absorbance microplate reader
BioTek **Synergy2**

24.12 10m

Plot the measured fluorescent values of the standard samples against their known concentrations and fit a linear curve using linear regression. Make sure that the coefficient of determination (R2) is close to 1 (typically > 0.99). Calculate the DNA concentrations in the unknown samples using the slope and intercept parameters of the linear equation. Output values you obtained are in ng μl^{-1}, assuming 1 µl of each sample was used.

Do not forget to account for any dilutions when calculating the concentration of the DNA in the unknown samples.

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Storage

25 The extract can be used directly as a DNA template for downstream applications. We recommend aliquoting **10 µl** to **100 µl** as a work-template to be stored at **-20 °C** , in order to minimise freeze-thaw cycles.

Genomic DNA Clean & Concentrator-10 **Zymo Research Catalog #D4011**

26 Depending on quality and quantity, aliquot **10 µl** to **100 µl** for RNA purification.
Proceed to Purification of RNA from a DNA/RNA Extract.

Purification of RNA from a DNA/RNA Extract
by Roey Angel,
Soil and Water Research Infrastructure
PREVIEW RUN

26.1

Prepare the following mixture in a 1.5 ml tube:

1. **10 µl** to **42 µl** of TNA extract (**1 µg** to **3 µg** of DNA).
2. **5 µl TURBO DNase buffer 10x**
3. **1 µl RNaseOUT**
4. **1 µl 0,1M DTT**
5. **1 µl Turbo DNase** per up to **2 µg DNA**
6. Complete to **50 µl** with RNase-free water

TURBO™ DNase (2 U/µL) **Thermo Fisher Scientific Catalog #AM2238**

RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #10777019**

USB Dithiothreitol (DTT) 0.1M Solution **Thermo Fisher Scientific Catalog #707265ML**

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #T143.1**

26.2 30m

Incubate at **37 °C** for **00:30:00** .

Step 26.2 includes a Step case.
Extended digest

Citation: Roey Angel, Eva Petrova, Ana Lara-Rodriguez (07/27/2021). Total Nucleic Acids Extraction from Soil. https://dx.doi.org/10.17504/protocols.io.bwxcpfiw

step case

Extended digest

If this procedure still leaves out undigested DNA (for example due to the presence of inhibitors), increase the incubation time (to 40–60 min) and add another equal dose of DNase half-way through.

26.3

Add **250 µl Binding Buffer** .

GeneJET RNA Cleanup and Concentration Micro Kit **Thermo Fisher Scientific Catalog #K0841**

26.4

Add **300 µl absolute ethanol** .

Ethanol, Absolute, Molecular Biology Grade **Thermo Fisher Scientific Catalog #BP2818500**

26.5 1m

Transfer the mixture to the Gene JET RNA Purification Micro Column preassembled with a collection tube. Centrifuge the column for **14000 x g, Room temperature , 00:01:00** . Discard the flow-through. Place the GeneJET RNA Purification Micro Column back into the collection tube.

26.6 1m

Add **700 µl Wash Buffer 1** (supplemented with ethanol) to the GeneJET RNA Purification Micro Column and centrifuge for **14000 x g, Room temperature , 00:01:00** . Discard the flow-through and place the purification column back into the collection tube.

GeneJET RNA Cleanup and Concentration Micro Kit **Thermo Fisher Scientific Catalog #K0841**

26.7 1m

Add **700 µl Wash Buffer 2** (supplemented with ethanol) to the GeneJET RNA Purification Micro Column and centrifuge for **14000 x g, Room temperature , 00:01:00** . Discard the flow-through and place the purification column back into the collection tube.

GeneJET RNA Cleanup and Concentration Micro Kit **Thermo Fisher Scientific Catalog #K0841**

26.8 1m

Repeat step 7.

undefined

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26.9 2m

Centrifuge the empty GeneJET RNA Purification Micro Column for an additional **14000 x g, Room temperature , 00:02:00** to completely remove residual Wash Buffer.

This step is essential to avoid residual ethanol in the purified RNA solution. The presence of ethanol in the RNA sample may inhibit downstream enzymatic reactions.

26.10 Transfer the GeneJET RNA Purification Micro Column into a clean 1.5 ml Collection Tube tube.

26.11 1m

Add **10 µl** to **20 µl RNA storage solution** or nuclease-free water to the GeneJET RNA Purification Micro Column. Centrifuge for **14000 rpm, Room temperature , 00:01:00** to elute the RNA.

THE RNA Storage Solution **Thermo Fisher Scientific Catalog #AM7000**

26.12 Discard the purification column. Use the purified RNA immediately in downstream applications or store at **-20 °C** or **-80 °C** until use.

For prolonged storage (more than 1 month), storage at **-80 °C** is recommended.

27 If the removal of RNA is necessary, follow up with RNaseH treatment and purification using e.g. Genomic DNA Clean & Concentrator.

Genomic DNA Clean & Concentrator-10 **Zymo Research Catalog #D4011**

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