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## DNA extraction from water/soil: 50-50-50 buffer-chloroform/phenol method

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1 Works for me dx.doi.org/10.17504/protocols.io.8yphxvn

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### ABSTRACT

This protocol is derived from the "DNA extraction from water: 50-50-50 buffer-chloroform/phenol method" designed by The Department of Environmental Sciences from the University of Toledo, Ohio. With slight alterations in the steps described, the protocol can be used for both soil and sediment samples, as well as processed water samples where microbes have been concentrated onto a filter membrane. The original protocol from the University of Toledo has been attached for reference. An overview of the bead-beating technique as well as other manual DNA extraction and purification methods can be found in Miller *et al.* (1999).

### THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Miller, D. N., J. E. Bryant, E. L. Madsen and W. C. Ghiorse, 1999 Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl Environ Microbiol 65: 4715-4724.

  
DNA extraction water.pdf

### MATERIALS TEXT

Extraction Buffer (pH 8.0): 50 mM NaCl, 50 mM Tris-HCl (pH 7.6), 50 mM EDTA, 5% SDS (autoclave to sterilise)  
Dithiothreitol 1M  
Phenol (Tris-saturated, pH 8.0, refrigerated)  
Chloroform:isoamyl alcohol (24:1, refrigerated)  
Chloroform  
Sodium acetate solution 3 M (pH 5.2, sterile)  
Isopropanol  
70% Ethanol (filter sterilised, 0.2 um, ice cold)  
Microcentrifuge tubes 2 mL (sterile)  
Glass microbeads (0.10 - 0.11 mm)  
Forceps and scissors (sterile)  
Petri dish for cutting filter membranes (sterile)

### BEFORE STARTING

- This protocol begins with nucleic acid extraction. Please ensure that environmental samples have been processed appropriately before commencing. Steps of water filtration can be found in the uploaded pdf.
- For low biomass/DNA samples, it is recommended to increase the extraction buffer pH to 9.5.
- Store 70% ethanol in a freezer until needed in step 12 to ensure it is ice cold for pellet washing.
- Step 10 requires a refrigerated centrifuge set to 10°C. If possible and to save time, ensure that both a room temperature and 10°C centrifuge are available before commencing with the protocol.

## Sample preparation

### 1 Membrane filters

In an aseptic environment, such as a laminar flow hood, use sterile forceps to place a membrane filter (containing concentrated bacteria) into a petri dish. With flame-sterilised scissors, cut the membrane filter into small pieces ( $\sim 0.5 - 1 \text{ cm}^2$ ). Place approximately 8-10 pieces of the membrane filter into a 2 mL microcentrifuge tube.

### Soil or sediment

Weigh out approximately 0.8 grams of soil or sediment and place into a 2 mL microcentrifuge tube containing 0.4 - 0.5 mL glass microbeads. Ensure the microcentrifuge tube has a locking lid or screw cap to prevent the lid opening accidentally during bead beating steps.

## Nucleic acid extraction

- 2 Add 1 mL of extraction buffer to the microcentrifuge tube containing the sample.
- 3 Add 1  $\mu\text{L}$  of 1 M dithiothreitol into the lid of the microcentrifuge tube.
- 4 Close tube lids and place the tubes into a zip-lock bag to avoid leakage. Secure the bag to a vortex using tape so the tubes are lying flat. Vortex the samples for 10 - 12 mins at approximately 3000 rpm.
- 5 Remove tubes from the zip-lock bag and invert briefly to ensure no soil/sediment or membrane filter pieces are stuck in the lid. Centrifuge microcentrifuge tubes for 3 mins at 14,000 x g.

## Nucleic acid purification

- 6 After centrifugation, decant the supernatant into a sterile 2 mL tube by careful pouring. Do not include any pieces of filter membrane in the new tube.
- 7 Determine the volume of the extract in each tube. Add half of this volume of both phenol and chloroform:isoamyl alcohol to each tube and vortex until the solution appears milky. Centrifuge for 3 mins at 14,000 x g or until the phases are visibly separated. The upper aqueous phase contains the DNA. Using a sterile pipette tip, transfer the upper aqueous phase into a sterile 2 mL tube.
- 8 Determine the volume of the extracted upper aqueous phase, and extract again with an equal volume of chloroform. Centrifuge as above and transfer the new aqueous phase to a sterile 2 mL tube.

## Nucleic acid precipitation

- 9 Determine the volume of the extract. Add 0.1 volumes of sodium acetate solution 3 M and 0.7 volumes of isopropanol. Mix the solutions by inverting the tube several times. Do not vortex.
- 10 Precipitate the DNA by centrifugation for 30 mins at 14,000 x g in the 10°C refrigerated centrifuge.
- 10.1 The appearance of the resulting pellets may range from transparent to dark in colour, depending on the amount of organic acids co-extracted with the DNA.
- 11 Carefully discard the supernatant by aspirating the isopropanol. Ensure that the precipitated pellets do not detach from the side of the tube.

- 12 Wash the pellet by adding 0.5 mL of ice cold 70% ethanol and invert the tube gently to ensure that the ethanol contacts all surfaces inside the tube. Re-pellet the DNA a second time (as above) and centrifuge for 5 min at 14,000 x g in the 10°C refrigerated centrifuge. Optimization of this protocol has shown that centrifugation at cool temperatures (10-15°C) will result in better pellet formation and stability. Thus, the pellets are larger (containing more DNA) and will stick to the sides of the tube, which makes aspirating the alcohol easier. Remove the ethanol as above and be careful not to aspirate the pellet. Allow the pellet to dry for 2-5 mins.

#### Nucleic acid resuspension

- 13 Resuspend the pellet by adding 50 uL of DNase/RNase-free water and mix by gently flicking the tube until the pellet dissolves. Do not use nanopure or DI water for this purpose, as these sources are not clean enough.
- 13.1 If pooling of samples is required, resuspended extractions may be combined at this stage and concentrated through techniques such as ethanol precipitation.
- 14 If further clean-up steps are required to remove residual proteins and humic extracts, clean DNA with commercial kits or PVPP columns.
- 15 Ensure the extracted samples are properly labelled and store at -20°C.



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